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9th European Biophysics Congress
Lisbon – Portugal – 13–17 July 2013

9th EBSA

European Biophysics Congress

July 13th – 17th 2013, Lisbon, Portugal

SPBf SOCIEDADE PORTUGUESA DE BIOFÍSICA

EBSA Biophysics in Europe

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9th EBSA European Biophysics Congress Lisbon (Portugal), July 13th – 17th, 2013

Organized by The Portuguese Biophysical Society.

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Welcome to the 9th European Biophysics Congress

Dear Colleagues,

On behalf of the Portuguese Biophysical Society and EBSA, it is our pleasure to welcome you to Lisbon, for the "9th European Biophysics Congress - EBSA2013".

EBSA 2013 results from four years of planning and organization, in interaction with national societies and congress chairs. Certainly your lively participation will be essential for attaining a high scientific level. The excellent conditions of the congress venue will foster a most pleasant atmosphere.

The biennial EBSA congresses are the flagship of this federation of learned societies, together with the EBSA Journal, the "European Biophysics Journal". In addition to the expected participants from 31 European countries, it is a great pleasure to also welcome biophysicists from 16 other nations outside the European area, with a significant participation of Asia, North and South Americas, and also the nearby African countries.

For this congress, all EBSA member societies were contacted in order to suggest speakers. Also for the first time, reduced fees were considered for participants members of any biophysical society, and we would like to stress the excellent links of EBSA and the Biophysical Society (USA).

The Portuguese Biophysical Society (www.spbf.pt) is a very active and young entity with 300 members, most of them being middle career or students, who attained international visibility and certainly it is a great pleasure for the Portuguese biophysicists to meet their friends from abroad at this western part of Europe.

The organization of a congress is always a major task, and this is still more complex in these times of such a troubled economic situation in Europe. Because of this, we would like to thank the speakers who accepted the invitation under significant restrictions of financial support.

Our thanks to the symposia chairs for the selection of speakers and the oral presentations, to the sponsors that contributed financially, to the "Societé Française de Biophysique" that allowed us to use the platform for abstracts submission, with

a special word to Éric Quiniou. We would like to extend our acknowledgements to "Turismo de Lisboa" and specifically Ana Mendes, for their support, and to FLAD (Luso-American Foundation).

A significant number of bursaries were provided by EBSA (60 grants) to European students, and the Biophysical Society (USA) also attributed 12 grants to students of any nationality.

And at last, we thank to the members of the Organizing Committee, with a special mention for the fantastic help of Marco Domingues, to the EBSA Executive Committee who followed this process during these four years, and among other duties collaborated in the process of poster awards, a special word to the continued support from Antony Watts for his permanent help along the congress preparation, Avanti for starting the European Award of an already reputed scientific prize, and Springer for the continuous support of these congresses.

Once the congress is finished, and you have been living these days by the banks of the Tagus River learning and teaching biophysics, we expect you to stay in Lisbon for some time. This city has a most beautiful urban geography, in addition to its fantastic light. And of course there is the Atlantic, the incredible fine arts and gastronomy, a very specific late gothic architecture, and why not seek out some translations of our beautiful literature and poetry?

And now, let's start working, and see you again at the next EBSA Congress in Dresden, Germany, on 18-22 July 2015.

Manuela Pereira

Manuel Prieto

The congress co-organizers

EBSA Welcome

Ladies and Gentlemen,

On behalf of the Executive Committee of the European Biophysical Societies' Association, I am pleased and honoured to welcome you at the 9th European Biophysics Congress in Lisbon.

This congress is the 6th since EBSA decided to organize congresses biennially. We are able to organize congresses more frequently since EBSA has a secure annual income from the European Biophysics Journal. Anthony Watts, the managing editor of the journal has done a magnificent job in the past several years. We are much obliged for his editorial activity and for his pivotal role in the Executive Committee. Last year we renewed the contract with Springer for another 6 years, thereby we are confident that we can continue our mission to support "Biophysics in Europe".

In addition to congresses, another main activity of EBSA is to organize schools and Biophysics Courses to engage and train younger scientists in the area. The 2nd EBSA School on Membrane Biophysics held in Lacanao, France in June 2012, was again a great success and heavily oversubscribed. EBSA supported the Croatian International School of Biophysics in Primosten, also in 2012, and we plan to contribute to the next school in 2014. In the past two years we awarded many bursaries to make the participation of young scientist possible at a number of relevant international meetings. Additionally, we supported several visits between European laboratories to outstanding young biophysicists.

We continue our tradition with the EBSA Young Investigators' Prize. This time we received 8 nominations from 6 member societies (Austria, France, Hungary, Italy, Spain and United Kingdom). In a very strong competition, the Executive Committee selected Dr. Michael Karl Sixt from Austria. He will receive €2000, an engraved medal and a certificate, and has been invited to present a plenary lecture at the Congress.

As a very new initiative, through the very generous contribution of Walt Shaw, CEO of Avanti Polar Lipids Inc. has established a biennial award – The Avanti

Polar Lipids / EBSA Award - to be given by EBSA. The award will be presented at the EBSA Congress to an investigator for outstanding contributions to our understanding of lipid biophysics. Several outstanding nominations arrived with the Executive Committee for this Award and Professor Félix M. Goñi Head of the Biophysics Unit (Joint Center of the Spanish National Research Council (CSIC) and the University of the Basque Country) is the first winner of this prestigious prize. The winner receives an honorarium of US$ 3,000 and will deliver a plenary lecture at the Congress.

The financial support from EBSA and Springer was pivotal in organizing this congress. This is the second time when 60 bursaries were offered for young talented colleagues to facilitate their participation. EBSA is important in supporting "Biophysics in Europe". Please be involved in the activities of EBSA, please attend the General Assembly on July 15, even if you are not the voting representative of your country.

As President, and on behalf of the Executive Committee of EBSA and the 32 Member Societies, I would like thank our hosts, the Portuguese Biophysical Society. Special thanks are due to Manuela Pereira (President, Portuguese Biophysical Society) and Manuel Prieto and to all the Members of the Organising and Scientific Committees, who have worked so tirelessly and conscientiously to make this congress a success. The members of the Executive Committee and the Secretary of EBSA, Antoinette Killian, also played an essential role in helping the organizers, and give their unreserved support to the local organizers.

I am confident that this Congress will yet again be a success, as were the previous congresses, and I am looking forward to meeting you at the 10th European Biophysics Congress to be held in Dresden in 2015 organized by the German Biophysical Society.

László Mátyus
President of EBSA

European Biophysics Journal – YOUR Journal

The European Biophysical Societies Association, EBSA, is supported by subscriptions from member societies together with a good income stream from the European Biophysical Journal (EBJ) published by Springer and for which EBSA holds the copyright. The Journal is therefore integral to the health of EBSA and the support of all its activities, including this congress.

EBJ has been growing significantly in recent years, now receiving 3 times more submissions than just 8 years ago – it is also now a monthly publication. Submissions are truly international in origin, and the developments in science in Asia are certainly reflected in our global visibility. Additionally, in 2011, EBJ published 121 papers (∼55% acceptance rate); >1900 citations for EBJ papers for the year; IF 2.139, and visibility and downloads per month numbers are significantly increasing (∼10,000 in 2010, ∼11,000 in 2011 and **>16,000 full downloaded articles on average per month in 2012**). In the face of competition from many new journals in this area, is holding its own position (continually in the upper 50%) very well.

With the retirement of Phillip Kuchel, Sydney, AU last year, Frances Separovic has been appointed as the new Editor in the Pacific Rim. Frances is head of the School of Chemistry, University of Melbourne, Australia, and works on membrane peptides, using a range of biophysical methodologies. She has extensive experience of biophysics, having been intimately involved with the Australian Society for Biophysical and the Biophysical Society (USA), of which she is a Fellow. Frances is also on the editorial board of Biochim. *Biophys. Acta – Biomembranes* and *Acc. Chem. Res.*

Frances Separovic

Thomas Pomorski

We also have editors in Europe (Thomas Pomorksi, Copenhagen, DK – Function and regulation of lipid pumps), and the USA (Paul Janmey, Philadelphia – cell adhesion and mechanics), all of whom are a great help in maintaining the Journal's international profile so effectively and efficiently.

Paul Janmey

The journal publishes papers in the field of biophysics, which is defined as the study of biological phenomena by using physical methods and concepts. Original papers, reviews and Biophysics letters are published. The primary goal of this journal is to advance the understanding of biological structure and function by application of the principles of physical science, and by presenting the work in a biophysical context.

Original papers, reviews and Biophysics letters reporting a distinctively biophysical approach at all levels of biological organisation will be considered, as will both experimental and theoretical studies. The criteria for acceptance are scientific content, originality and relevance to biological systems of current interest and importance. Principal areas of interest include:

- Structure and dynamics of biological macromolecules
- Membrane biophysics and ion channels
- Cell biophysics and organisation
- Macromolecular assemblies
- Biophysical methods and instrumentation
- Advanced microscopies and single molecule studies
- System dynamics

Your abstracts in this issue of EBJ are fully citable, and for future submissions, please see the link from (`http://www.ebsa.org/`). Springer kindly host a portal for EBSA and EBJ is freely available to all members of all adhering biophysical societies to EBSA.

Finally, Springer continues to be a very strong supporter of EBJ, EBSA and our congresses. We are indebted to the company, Sabine Schwartz and her staff for all their advice, input and highly efficient way in which they produce the Journal for us – please do visit Sabine and her colleagues on the Springer stand at the Congress.

EBJ is YOUR Journal; please do consider it first for your next publications.

Anthony Watts
Managing Editor
Oxford, U.K.

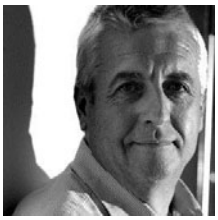
Michael Sixt – EBSA young researcher award

Michael Sixt studied human medicine at the University of Erlangen, Germany, and received his MD in 2002. It was his time as a clinical resident at the Dermatological Clinic Erlangen, which directed his interest to the migration of dendritic cells, a system he is still working on. He followed his previous supervisor, Lydia Sorokin, to Lund in Sweden, where he did a postdoc investigating the extracellular matrix of lymph nodes, and studying how this affects transport of antigens. He then moved to the Max Planck Institute of Biochemistry in Martinsried near Munich, were he started as a group leader in 2005. Since 2010 he has been working as an Assistant Professor at the Institute of Science and Technology (IST) Austria in Klosterneuburg, near Vienna.

Michael is an enormously creative and innovative scientist, who, throughout his young career, has been building bridges from his biomedical background to the neighboring research fields of physics, molecular biology and biochemistry. Recently he has particularly addressed how cytoskeletal dynamics and transmembrane force coupling are fine-tuned in migrating leukocytes. He found out that these cells can adapt their actin polymerization rate according to the adhesive nature of the substrate. The data provide a first explanation how cells that follow a soluble guidance cue can prioritize the soluble cue over the adhesive substrate. Recently, he developed new assays that allowed him to address directly the distribution of endogenous chemokines and their effect on leukocyte navigation. Using this approach he provided the first example of a functional chemokine gradient *in vivo*. Notably, he found that this gradient is not soluble but immobilized to heparan sulfate residues, which has far reaching consequences regarding the mode how these gradients are perceived and interpreted by the migrating cells. Michael makes a very worthy recipient of the EBSA Young Investigator's Award for 2013.

Gerhard Schütz, Vienna University of Technology, Austria

Félix M. Goñi – AVANTI award



Félix M. Goñi (San Sebastián, Spain, 1951) earned his MD in Navarra (1975) and became interested in Biophysics during summer courses at the Gulbekian Foundation (Oeiras, Portugal) under N. van Uden. His post-doctoral work under D. Chapman (Royal Free Hospital, London) involved studies of lipid-protein interactions in membranes. From 1984 he has been a full Professor of Biochemistry at the University of the Basque Country (Bilbao, Spain). His research deals with molecular interactions in membranes, with particular attention to lipids and detergents. In 1999 he founded the Unidad de Biofísica, a joint centre of the University of the Basque Country and the Spanish National Research Council (CSIC), and has been head of this institute from 2002. He has chaired the Publications Committee of FEBS (2006-2011) and is currently Chair of the International Relations Committee of the U.S. Biophysical Society. He was President of the Spanish Biophysical Society (1992-1998), who made him a Honorary Member in 2011.

Congresses of the

EUROPEAN BIOPHYSICAL SOCIETIES' ASSOCIATION

1st EUROPEAN BIOPHYSICS CONGRESS, 1971, BADEN, AUSTRIA
2nd CONGRESS, 1997, ORLEANS, FRANCE
3rd CONGRESS, 2000, MUNICH, GERMANY
4th CONGRESS, 2003, ALICANTE, SPAIN
5th EBSA / 15th IUPAB / SFB CONGRESS, 2005, MONTPELLIER, FRANCE
6th CONGRESS, 2007, LONDON, UNITED KINGDOM
7th CONGRESS, 2009, GENOA, ITALY
8th CONGRESS, 2011, BUDAPEST, HUNGARY
9th CONGRESS, 2013, LISBON, PORTUGAL

for your diary

10th CONGRESS, 2015, Dresden, Germany
July 18 – 22, 2015

—

11th congress will be in 2017 (see http://www.ebsa.org/ for venue and dates)

EBSA Bursaries

Awarded to:

Aekbote, Badri – *Szeged, Hungary*

Antosová, Andrea – *Kosice, Slovakia*

Artetxe, Ibai – *Leioa, Spain*

Barnoud, Jonathan – *Paris, France*

Bednarikova, Zuzana – *Kosice, Slovakia*

Borile, Giulia – *Padova, Italy*

Cehlar, Ondrej – *Bratislava, Slovakia*

Coceano, Geovanna – *Trieste, Italy*

Covino, Roberto – *Trento, Italy*

Cujová, Sabína – *Prague, Czech Republic*

Dasanna, Anil – *Toulouse, France*

Deák, Robert – *Budapest, Hungary*

di Carlo, Maria – *Palermo, Italy*

Duan, Chenxi – *Grenoble, France*

Durka, Kamil – *Lodz, Poland*

Efimova, Svetlana – *Saint Petersburg, Russia*

Elani, Yuval – *London, UK*

Garaiova, Zuzana – *Bratislava, Slovakia*

Ghazaryan, Narine – *Yerevan, Republic of Armenia*

Girych, Mykhailo – *Kharkiv, Ukraine*

Goncharova, Iryna – *Prague, Czech Republic*

Gráczer, Éva – *Budapest, Hungary*

Hadju, Kata – *Szeged, Hungary*

Hakobyan, Lilit – *Yerevan, Republic of Armenia*

Hikisz, Pawel – *Lodz, Poland*

Iarinca, Luiza – *Cluj-Napoca, Romania*

Iftemi, Sorana – *Iasi, Romania*

Istrate, Claudia – *Bucharest, Romania*

Jakubowski, Rafal – *Torun, Poland*

Jobin, Marie-Lise – *Pessac, France*

Klose, Daniel – *Osnabrück, Germany*

Kopecka, Miroslava – *Prague, Czech Republic*

Kozlowska, Justyna – *London, UK*

Kudryashova, Ksenia – *Moscow, Russia*

Kwiatkowska, Marta – *Lodz, Poland*

Lighezan, Liliana – *Timisoara, Romania*

Lima, Angelica – *Cachan, France*

Marcuello, Carlos – *Zaragoza, Spain*

Martinez, Denis – *Pessac, France*

Mathesz, Anna – *Szeged, Hungary*

Michelssens, Servass – *Leuven, Belgium*

Müller, Jochen – *Munich, Germany*

Nagy, Krisztina – *Szeged, Hungary*

Nord, Ashley – *Oxford, UK*

Novotna, Pavlina – *Prague, Czech Republic*

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Pytel, Edyta – *Lodz, Poland*

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Takáts-Nyeste, Annamária – *Budapest, Hungary*

Tetryakova, Tatyana – *Tiblisi, Georgia*

Tripon, Carmen – *Cluj-Napoca, Romania*

Wypijewska, Anna – *Warsaw, Poland*

Scientific Programme

SATURDAY, JULY 13th 2013 PAVILHÃO ATLÂNTICO
Rossio dos Olivais, Lote 2.13.01A, 1990 – 231 Lisboa
Web site : http://www.pavilhaoatlantico.pt

15.00-16.00 **Registration**

17.00-17.30 **Welcome Address**

17.30-18.15 **PLENARY LECTURE**
Lewis Kay, University of Toronto, Canada
Seeing the invisible by solution NMR spectroscopy
Chair: Antoinette Killian

18.30-19.30 **Welcome Reception**

SUNDAY, JULY 14th 2013 PAVILHÃO ATLÂNTICO
Rossio dos Olivais, Lote 2.13.01A, 1990 – 231 Lisboa
Web site : http://www.pavilhaoatlantico.pt

9.00-9.45 PLENARY LECTURE
Michael Sixt, EBSA Award Speaker, Institute of Science and Technology, Austria
Cytoskeletal mechanics of chemotactic leukocytes
Chair: Lázló Mátyus

09.50-12.30 1. BIOLOGICAL ELECTRON AND PROTON TRANSFER
Chairs: R. Louro and A. Konstantinov

Invited speakers

Ulrike Alexiev, Germany
Exploring the entrance of proton pathways in cytochrome c oxidase from P. denitrificans

Irene Díaz-Moreno, Spain
How redox proteins form transient complexes in photosynthesis and respiration

Cyrille Costentin, France
Proton-coupled electron transfers in phenol and tryptophan oxidations: An electrochemical approach

Short talks

Petra Hellwig, France
Study of the Fe-S vibrational modes in complex I by means of Raman, Far IR and electrochemistry

Ana Sofia Fernandes Oliveira, Portugal
Exploring the dioxygen diffusion pathways in aa3 Cytochrome c oxidases: Insights from MD simulations

Filipa Calisto, Portugal
Structural characterization of peripheral subunits from Alternative Complex III

09.50-12.30 2. CELL BIOPHYSICS AND SIGNALING
Chairs: J. Rino and L. Mátyus

Invited speakers

George Barisas, U.S.A.
Nanoparticle probes of molecular rotation on cell surfaces

T. W. J. (Dorus) Gadella, The Netherlands
Enhanced fluorescent proteins for FRET and for studying signaling across the membrane

Maria García-Parajo, Spain
Biophysics of leukocyte adhesion: nanoscale organization and dynamics of the integrin LFA-1

Short talks

Nils Petersen, Canada
Protein-protein-protein interactions in membranes measured by triple correlation of confocal images

Hetvi Gandhi, Germany
Early events in cytokine receptor mediated signaling

Joachim Piguet, Switzerland
Tracking NK1 receptor diffusion in the membrane of living cells. Roles of clathrin and cytoskeleton.

09.50-12.30 3. CHEMICAL AND SYNTHETIC BIOLOGY
Chairs: R. Eritja and A. Casini

Invited speakers

Wim Quax, The Netherlands
Turning Bacillus subtilis into a methylotrophic terpenoid synthesizing cell

Pier Luigi Luisi, Italy
Title to be announced

Gonçalo Bernardes, Portugal
Chemoselective transformations for bioimaging and targeted therapeutics

Short talks

Yuval Elani, U.K.
Manufacturing vesicles with internal bilayer partitions: a novel unit for synthetic biology

Vânia Brissos, Portugal
Stabilization of FMN-dependent NADPH:dye/quinone reductase from Pseudomonas putida by directed evolution

Sónia Mendes, Portugal
Catalytic and spectroscopic characterization of two bacterial dye-decolourising peroxidases

12.30-15.00 POSTER SESSION, LUNCH & EXHIBITS

15.00-15.45 **PLENARY LECTURE**
Viola Vogel, ETH Zürich, Switzerland
Mechanobiology: From molecular zippers to the recognition of nanotopographies
Chair: Erick Dufourc

16.15-18.30 4. MOLECULAR MOTORS
Chairs: M. Pereira and H. Grubmüller

Invited speakers

Jacek Czub, Poland
The mechanism of energy transmission in F1-ATPase as revealed by molecular dynamics simulations

Michael Börsch, Germany
Motors, gears and breaks of FoF1-ATP synthase monitored by single-molecule FRET

Julie Plastino, France
Membrane dynamics and cytoskeleton assembly in cell motility

Short talks

Ilja Küsters, Netherlands
Single molecule observation of protein translocation

Ashley Nord, U.K.
Stepping Behavior of Rotary Molecular Motors

Stefan Balint, Spain
Correlating cargo transport with the cytoskeletal network at high resolution

16.15-18.30 5. PROTEIN FOLDING, ASSEMBLY AND STABILITY
Chairs: E. Melo and A. Kolinski

Invited speakers

Anne Ulrich, Germany
Transport machineries in biomembranes that utilize electrostatic "charge zippers"

Mikael Oliveberg, Sweden
Prion-like aggregation in ALS

Martin Blackledge, France
Protein conformational dynamics and molecular recognition in folded and unfolded proteins by NMR

Short talks

Birgit Habenstein, Germany
Tau structure in paired helical filaments revealed by solid-state NMR

Karin Hauser, Germany
Fibril formation of polyglutamine repeats: a spectroscopic study

Cláudio Gomes, Portugal
Cross talks between amyloid-forming proteins in neurodegeneration

16.15-18.30 6. SYSTEMS BIOLOGY
Chairs: A. Salvador and J. M. G. Vilar

Invited speaker

Oliver Ebenhöh, U.K.
The role of mixing entropy in carbohydrate metabolism

Leonor Saiz, U.S.A.
Negative feedback and crosstalk in the Transforming Growth Factor β signaling pathway

Short talks

Marina Monteiro, Portugal
FRAP biophysical tool to probe nucleic acids-membrane ligand interactions in pDNA purification

Tomas Tokar, Slovakia
Bcl-2 family regulation of apoptosis by non-trivial decisioning

Jan Sielewiesiuk, Poland
Positive and negative feedback loops coupled by a common promoter

MONDAY, JULY 15th 2013 PAVILHÃO ATLÂNTICO
Rossio dos Olivais, Lote 2.13.01A, 1990 – 231 Lisboa
Web site : http://www.pavilhaoatlantico.pt

9.00-9.45 **PLENARY LECTURE**
Professor Sir Alan Fersht, FRS, Department of Chemistry, University of Cambridge, U.K.
Tumour suppressor p53: biophysics and drug discovery
Chair: Catherine Royer

09.50-12.30 7. CHANNELS AND TRANSPORTERS
Chairs: G. Soveral and N. Schmitt

Invited speakers

Rainer Schindl, Austria
Activation mechanism of the store-operated calcium channel complex STIM1 and Orai1

Teresa Giraldez, Spain
Gating ring motions underlying function of BK channels

Michael Pusch, Italy
CLC-5, an endosomal chloride – proton exchanger mutated in Dent's disease: a biophysical perspective

Short talks

Vicente Aguilella, Spain
SARS-CoV E protein ion channel characterization by tuning the protein and lipid charge

Gyorgy Panyi, Hungary
Locked-open activation gate impedes recovery from inactivation in Shaker K+ channels

Katsumi Matsuzaki, Japan
Influenza A virus M2 protein forms a dimeric channel in biomembranes

09.50-12.30 8. BIOMOLECULAR SIMULATION: SPANNING SCALES
Chairs: C. Soares and I. Vattulainen

Invited speakers

Markus Deserno, U.S.A.
Optimization of an elastic network augmented coarse-grained model to study CCMV capsid deformation

Luca Monticelli, France
Modeling the effect of nano-sized polymer particles on the properties of lipid membranes

Helmut Grubmüller, Germany
Atomistic simulation of single molecule experiments: Molecular machines and a dynasome perspective

Short talks

Gerhard König, U.S.A.
A hybrid quantum-chemical approach for free energy simulations.

Manuel Melo, Netherlands
Mixing and matching simulations at different resolutions

Luís Filipe, Portugal
Conformational determinants of peptidic tree-like molecules: insights from MD simulations

09.50-12.30 9. IMAGING AND BIOSPECTROSCOPY
Chairs: M. Prieto and P. Schwille

Invited speakers

Jens Michaelis, Germany
Mechanistic insight into eukaryotic gene expression from single molecule experiments

Catherine Royer, France
Quantifying protein interaction networks in live cells using fluorescence fluctuation microscopy

David Klenerman, U.K.
Single molecule studies of protein aggregates

Short talks

Axel Hochstetter, Switzerland
Tracing the microscopic motility of unicellular parasites

Maria Sarmento, Portugal
PI(4,5)P2 acts as a lipid calcium sensor in the presence of physiological calcium concentrations

Jacob Piehler, Germany
Dynamic submicroscopic signaling zones revealed by TALM and image correlation analysis

12.30-15.00 POSTER SESSION, LUNCH & EXHIBITS

14.30-14.45 ***Company talks***
NanoTemper Technologies

15.00-15.45 **PLENARY LECTURE**
Maria João Romão, Lisbon, Portugal
Unraveling new functions and modes of action of molybdenum-dependent enzymes
Chair: Helmut Grubmüller

16.15-18.30 10. MOLECULAR RECOGNITION AND NANOBIOPHYSICS
Chairs: P. Eaton and J. Piehler

Invited speakers

Vinod Subramaniam, The Netherlands
Getting a grip on alpha-synuclein amyloid oligomers - single molecule approaches

João Pedro Conde, Portugal
Lab-on-chip detection of biomolecules with integrated sensors

Daniel Müller, Switzerland
Quantifying and localizing interactions guiding the structural and functional properties of GPCRs

Short talks

Peter Jonsson, U.K.
Molecular nanomechanics and local stimulus of individual biomolecules on the surface of cells

Anny Slama-Schwok, France
Regulation of Nitric oxide synthases by fluorescent NADPH derivatives upon two photon excitation

Agata Szuba, Germany
Signal-Driven tethering system based on DNA-Origami linked to lipid bilayers

16.15-18.30 11. MEMBRANE STRUCTURE AND DOMAINS
Chairs: M. J. Moreno and F. Goñi

Invited speakers

Banafshe Larijani, U.K.
Effects of phosphoinositides and their derivatives on membrane morphology and function

Hans-Joachim Galla, Germany
Substrate turn and stimulated ATP-hydrolysis of the hABCC3 transporter show positive cooperativity

Jesús Pérez-Gil, Spain
Modulation of phase coexistance and biophysical activity in pulmonary surfactant membranes and film

Short talks

Liana C. Silva, Portugal
Ceramide activates endocytosis and forms ordered intracellular lipid domains in response to TNF-α

Sebastian Finger, Germany
The effect of cyclic antimicrobial hexapeptides on model bacteria membranes

Miglena I. Angelova, France
Local pH gradients induce polarization of Lo and Ld domains in GM1-containing giant vesicles

16.15-18.30 12. PROTEIN-NUCLEIC ACID INTERACTIONS
Chairs: A. Athanasiades and A. Wilkinson

Invited speakers

Anastassis Perrakis, The Netherlands
Binding J: molecular biophysics to understand the binding of a unique protein to a unique DNA base

Oscar Llorca, Spain
Transient geometries in nonsense-mediated mRNA decay (NMD) visualized by cryo-EM

Dagmar Klostermeier, Germany
RNA binding and unwinding by the T. thermophilus DEAD-box helicase Hera

Short talks

Beáta Vértessy, Hungary
Genomic integrity of virulence genes is preserved by a dUTPase-based molecular switch

Carina Monico, Italy
Protein-DNA interactions probed by Ultrafast Force-clamp Spectroscopy

Nicolas Fiszman, France
Eucaryotic translation at single molecule scale

18.30-19.30 EBSA General Assembly

TUESDAY, JULY 16th 2013 PAVILHÃO ATLÂNTICO
Rossio dos Olivais, Lote 2.13.01A, 1990 – 231 Lisboa
Web site : http://www.pavilhaoatlantico.pt

9.00-9.45 **PLENARY LECTURE**
Félix Goñi, Avanti Award Speaker, Universidad del País Vasco, Spain
A lifetime of oily games and greasy ministrations
Chair: Manuel Prieto

09.50-12.30 13. MATERIAL SCIENCE IN BIOPHYSICS
Chairs: J. C. Marcos and A. Turberfield

Invited speakers

Friedrich Simmel, Germany
Dynamical diversity of compartmentalized in vitro transcriptional oscillators

Thomas Scheibel, Germany
Processing of recombinant proteins for materials applications: About spider silk and more

Rui Afonso, Portugal
Young Biophysicist Award - Portuguese Biophysical Society
Nanotube-forming hydrophobic dipeptides: structure, properties and applications

Short talks

Badri L. Aekbote, Hungary
Optical tools for localized fluorescence enhancement and single cell studies

Arwen Tyler, U.K.
Tuning curvature in inverse micellar and bicontinuous cubic phases

Lea-Laetitia Pontani, U.S.A.
Specificity, flexibility and valence of DNA bonds guide emulsion architecture

09.50-12.30 14. PROTEIN-LIPID INTERACTIONS
Chairs: L. Loura and A. Killian

Invited speakers

Amitabha Chattopadhyay, India
Interaction of membrane cholesterol with G Protein-Coupled Receptors: A multidimensional approach

Lena Mäler, Sweden
Lipid interactions of glycosyltransferases

Daniel Otzen, Denmark
Liprotides: complexes between fatty acids and (partially denatured) proteins

Short talks

Yvonne Klapper, Germany
Lipid coated quantum dots as a model to study lipid-protein interactions via FCS

Hartmut Luecke, Spain
Structure, function & inhibitors of the pH-gated H. pylori urea channel essential for acid survival

Georg Pabst, Austria
Modulation of ion-channel activity by cholesterol and ceramide

09.50-12.30 15. NEUROSCIENCES
Chairs: A. Sebastião and M. Ferenczi

Invited speakers

Jean-Philippe Pin, France
Enlightening allosteric properties of metabotropic glutamate

Yasser Roudi, Norway
Inhibitory networks of grid cells

Karri Lamsa, U.K.
Rectification of glutamate receptors set cell type –specific plasticity rules in interneurons

Short talks

Andrea Mescola, Italy
Surface strategy for regulation and control of neural cell adhesion

Megan Oliva, Australia
Differential modulation of NaV1.1 and NaV1.2 sodium channels by the β1 auxiliary subunit

Daniele Arosio, Italy
Improving a GFP-based sensor to measure intracellular parameters like pH and ion concentrations

12.30-15.00 POSTER SESSION, LUNCH & EXHIBITS

14.30-14.45 ***Company talks***
GE Healthcare

15.00-15.45 **PLENARY LECTURE**
Petra Fromme, Arizona State University, Tempe (AZ), U.S.A.
Femtosecond crystallography: Dawn of a new era in structural biology
Chair: José Carrascosa

16.15-18.30 16. BIOLOGICALLY ACTIVE PEPTIDES
Chairs: M. Bastos and E. Dufourc

Invited speakers

Karl Lohner, Austria
Disruption of bacterial membranes based on membrane curvature generation by antimicrobial peptides

Miguel Castanho, Portugal
Unraveling a new actor in dengue virus-cell fusion

Huey Huang, U.S.A.
Process of inducing pores in membranes by melittin

Short talks

Annika Kopp, Germany
Biophysical investigations into the effect of antimicrobial peptides on bacterial membranes

Isabel Alves, France
How do a proapoptotic and a cell penetrating peptide work together to kill cancer cells?

Peter Judge, U.K.
Non-uniform changes in lipid order induced by the Membrane Targetting Sequence of the MinD ATPase

16.15-18.30 17. NEW AND NOTABLE
Chairs: L. Martins and A. Watts

Invited speakers

Michael Mayer, U.S.A.
Single protein characterization methods with nanopores

João Morais Cabral, Portugal
Structure and function of the KtrAB ion transporter

Mark Dodding, U.K.
Structural basis for kinesin-1: Cargo recognition

Short talks

Fabiola Gutierrez, Netherlands
Altering the torsional rigidity of proteins with surfactants

Francesca Munari, Germany
Conformational plasticity of the multi-domain Heterochromatin Protein 1β

Maximilian Richly, France
Investigating the Cell Membrane via Single Particle Tracking and Hydrodynamic Force Application

16.15-18.30 18. PROTEIN STRUCTURE AND FUNCTION
Chairs: C. Frazão and A. Ulrich

Invited speakers

Francesca Marassi, U.S.A.
Structural studies of membrane proteins in membrana

Ben Berks, U.K.
Moving folded proteins across membranes: structural analysis of the Tat protein translocase

Reinhard Grisshammer, U.S.A.
Structure of the agonist-bound neurotensin receptor NTS1

Short talks

Ana P. Batista, Portugal
Investigating substrate interaction on Type II NADH:quinone oxidoreductase from Escherichia coli

Roslin Adamson, U.K.
Probing GPCR-Gα interactions: A functional study by EM and SPR

Patrick Drücker, Germany
The Annexin A2 core domain is identified to induce membrane curvature

19.30-24.00 Conference Dinner

WEDNESDAY, JULY 17th 2013 PAVILHÃO ATLÂNTICO
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Web site : http://www.pavilhaoatlantico.pt

9.00-9.45 **PLENARY LECTURE**
Nynke Dekker, Kavli Institute of Nanoscience, TU Delft, The Netherlandss
Investigating transcription and replication at the level of single molecules and cells
Chair: Anthony Watts

09.50-12.30 19. INTRINSICALLY DISORDERED PROTEINS
Chairs: C. Gomes and Y. Levy

Invited speakers

Kresten Lindorff-Larsen, Denmark
Combining NMR and molecular simulations to study protein dynamics

Peter Tompa, Belgium
Supertertiary structural ensembles of proteins

Ben Schuler, Switzerland
Probing the polymeric properties of IDPs with single-molecule spectroscopy

Short talks

Ana-Cristina Sotomayor-Perez, France
Disorder-to-order transition in RTX proteins: Implications for toxin physiology

Volodymyr Shvadchak, Netherlands
Repeats in the α-synuclein sequence determine its conformation on membranes

Larisa Kapinos Schneider, Switzerland
Karyopherin binding induces conformational transitions in the intrinsically disordered FG domains

09.50-12.30 20. RNA STRUCTURE AND FUNCTION
Chairs: C. Arraiano and B. Vertessy

Invited speakers

Chirlmin Joo, The Netherlands
Defense against viral attack: single-molecule view on a bacterial adaptive immune system

Eric Westhof, France
RNA architectural modules, their detection in RNA Sequences and the assembly of large RNAs

Frederic Allain, Switzerland
Splicing and translation regulation by small RNA binding proteins

Short talks

Gilmar F. Salgado, France
Probing DNA G-quadruplex structures inside living cells using NMR spectroscopy

Francesco Colizzi, Italy
Symmetry and asymmetry in the unwinding of nucleic acids

Tobias Schmidt, Germany
Deciphering the RNA-binding complex NF90-NF45: complex formation facilitates RNA chaperone activity

09.50-12.30 21. BIOPHYSICS IN EUROPE (TEACHING, CAREER AND FUNDING)
Chairs: M. Castanho and C. Royer

Invited speakers

Jeremy Craven, U.K.
Keeping the physics in physical biochemistry teaching

Bertrand Garcia-Moreno, U.S.A.
Why, how, and whither biophysics?

David Pina, Belgium
Funding opportunities within the next European Framework Programme for Research and Innovation — Horizon 2020

Silke Schumacher, Germany
EMBL and EMBL's training activities

12.30-15.00 POSTER SESSION, LUNCH & EXHIBITS

14.30-14.45 ***Company talks***
Leica Microsystems

15.00-15.45 **PLENARY LECTURE**
Martin Hof, J. Heyrovský Institute of Physical Chemistry of the ASCR, Czech Republic
Hydration, mobility, aggregation and nanodomain formation in model membranes studied by fluorescence
Chair: Manuela Pereira

16.15-18.30 22. MOLECULAR BASIS OF DISEASE
Chairs: C. Rodrigues and R. Ariëns

Invited speakers

Albin Hermetter, Austria
Supra-molecular interactions of oxidized phospholipids in cells and lipoproteins

Thomas Gutsmann, Germany
Reconstituted micriobial lipid membranes as a tool in drug research

Javier Sancho, Spain
The LDL receptor: folding and binding events in function and in disease

Short talks

Ari Gafni, U.S.A.
Synergistic interactions of neuron-bound Alzheimer's Aβ40 and Aβ42: a single molecule study

Markus Rudolph, Switzerland
Structure of human α-2,6 sialyltransferase reveals mode of binding of complex glycans

Pascal Preira, France
Membrane dynamic organization of HIV co-receptors analyzed by Single Particle Tracking at the surface

16.15-18.30 23. SINGLE MOLECULE BIOPHYSICS
Chairs: N. Santos and Y. Engelborghs

Invited speakers

Peter Hinterdorfer, Austria
These IgGs are made for walkin': Random antibody movement on bacterial and viral surfaces

Marriano Carrión-Vázquez, Spain
Common characteristics in early amyloidogenesis: from single-molecules to therapy

Jasna Brujić, New York, U.S.A.
Novel analysis methods in force-clamp spectroscopy shed light on protein folding

Short talks

Niels Zijlstra, Netherlands
Aggregation conditions strongly influence the molecular composition of alpha-synuclein oligomers

Jörg Langowski, Germany
Live cell protein mobility and interaction maps by light sheet fluorescence correlation spectroscopy

Giovanna Coceano, Italy
Biomechanics study of cancer cells by optical tweezers and speckle microscopy

16.15-18.30 24. SUPRAMOLECULAR ASSEMBLIES
Chairs: J. Carrascosa and M. Rappolt

Invited speakers

Jose M. Valpuesta, Spain
The protein folding pathway: a coordinated network of molecular chaperones

Borislav Angelov, Czech Republic
Structural analysis of tetrahedral channel formation and hydration in cubosome nanoparticles

Giulio Caracciolo, Italy
Targeted drug delivery by nanoparticle-protein corona

Short talks

Antoine Loquet, Germany
Structure of a bacterial filament solved by solid-state NMRă: the type III secretion system needle

Maité Paternostre, France
Peptide nanotubes: structure and mechanism

Francesco Spinozzi, Italy
Quaternary structure of protein assemblies from small-angle x-ray and neutron scattering

19.00-21.00 **Farewell Cocktail**

Table of Contents

ABSTRACTS

This supplement was not sponsored by outside commercial interests. It was funded entirely by the publisher.

Abstracts

– Plenary Lectures –

O-1

Seeing the invisible by solution NMR spectroscopy

L. E. Kay
University of Toronto, Canada

Many biochemical processes such as ligand binding, enzyme catalysis, molecular recognition and protein folding proceed through the formation of functionally important intermediates that escape detection using traditional structural biology methods. A Nuclear Magnetic Resonance (NMR) approach for seeing such 'invisible' states will be described. A related problem is one where 'near invisible' systems, such as supra-molecular machines, are studied by solution NMR. New NMR approaches and applications that bring such systems into focus will be presented.

O-2

Cytoskeletal mechanics of chemotactic leukocytes

M. Sixt
Institute of Science and Technology, Am Campus 1, 3400 Klosterneuburg, Austria

The organizational principle of the immune system is based on high-speed cell motility. Accordingly, immune cells migrate up to 100 times faster than mesenchymal or epithelial cell types. One striking peculiarity of migrating leukocytes is, that they do not strictly rely on transmembrane adhesion receptors when crawling through three-dimensional environments but are able to directly transduce force by deformations of the cell body. Using quantitative live cell imaging we show that invasion of dense matrices and crawling over stiff surfaces relies on adhesion, while migration in the confined space of an interstitium does not and that leukocytes can shift back and forth between these modes without altering their proteome.

The force-generating module of leukocytes is exclusively based on actomyosin dynamics. But also here the cells show enormous plasticity and blocking contractility shifts the cells towards an entirely protrusive locomotion strategy, while dampening protrusion activates the blebbing mode. When interfering with actin nucleators we find that ablation of Arp2/3 activity at the leading edge abrogates actin branching and transforms the cells from roundish/amoeboid to an almost linear elongated cell shape. However, inhibiting actin branching merely blocked the cells response to directional cues, while actual speed was even accelerated.

O-3

Mechanobiology: From molecular zippers to the recognition of nanotopographies

V. Vogel
Laboratory of Applied Mechanobiology, Department of Health Sciences and Technology, ETH Zurich, Switzerland

The geometry by which mechanical forces pull on multivalent bonds determines their stability. Multivalent bonds can be strong if they are broken all at once, but they are weak if they are opened in a zipper geometry. This has been nicely demonstrated in AFM experiments and we thus asked whether the geometry-sensitive mechanical features of multivalent bonds can also be employed to engineer complex devices and how they are exploited by cells. First, we engineered cargo loading and unloading stations integrated onto microfabricated surfaces where molecular shuttles are propelled by the motor protein kinesin. Second, since topography sensing is one of the most fundamental processes that cells exploit to interact with their environments, we have grown highly flexible hairy silicon nanowires on micropatterned islands on otherwise flat glass surfaces. This allowed us now to visualize how filopodia exploit a zipper mechanism to probe the adhesion strength. Filopodia thus serve as a tool for cells to recognize surface topographies and they thereby steer fundamental cell functions such as cell adhesion, spreading, migration and division. Finally, some bacterial adhesins can recognize the mechanical strain of tissue fibers again by utilizing multivalent binding motifs.

O-4

Unraveling new functions and modes of action of molybdenum-dependent enzymes

M. J. Romão
REQUIMTE - Departamento de Química, CQFB, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal

Molybdenum-dependent (Moco) enzymes exist in all domains of life and their importance is exemplified by their ubiquity, their roles in metabolic diversity and in global geochemical cycles. Protein crystallography has had a major impact in the field of Moco-enzymes and it has allowed to identify unexpected co-factors and to discover new metal ligands that challenged earlier proposed functions of the individual active sites.

In the talk bacterial and mammalian Mo-dependent enzymes will be considered. Structural studies of bacterial formate dehydrogenases and nitrate reductases have highlighted how the detailed analysis of the enzyme active sites under different redox states can provide insight into novel reaction mechanisms, contradicting previously assumptions on the respective modes of action. Among mammalian Mo enzymes, studies with aldehyde oxidases (AOX) will be described. These are complex proteins characterized by a broad range substrate specificity, although their true physiological function is still to be unraveled. Very recently, it was recognized the emerging importance of the role of AOX in the metabolism of drugs and xenobiotics. We have solved the crystal structures of mouse and human AOX. The combination of crystallographic data with kinetic, mutagenesis and molecular docking studies have made a decisive contribution to understand the molecular basis of the rather broad substrate specificity of AOs. The crystal structures will allow to define in silico models for AO binding affinities of drugs under development, information of paramount importance in drug discovery.

Abstracts

– Plenary Lectures –

O-5

A lifetime of oily games and greasy ministrations

F. M. Goñi
Unidad de Biofísica (CSIC, UPV/EHU), 48080 Bilbao, Spain

(1976-1978): In my post-doctoral years in London, under D. Chapman, I studied the interaction of intrinsic proteins with membrane lipids, together with J.C. Gómez Fernández, and helped to describe that, against the predominant view at the time ("annular lipids") all lipids were freely exchanging in the plane of the membrane at the relevant time-scale of the enzyme turnover time. (1979-1989): After my return to Spain I worked on detergents and the mechanism of membrane solubilization. Original observations at the time included the ability of detergents to induce the lysis and reassembly (apparent fusion) of vesicles and the surfactant-induced release of vesicle contents at concentrations below those causing solubilization. (1987-2000): We were able to produce the first model system of catalytically-promoted membrane fusion, in which the catalyst was phospholipase C. (1994-present): At the instigation of my long-time associate Alicia Alonso we investigated the possible parallelism between phospholipase C/diacylglyceride production and sphingomyelinase/ceramide production, with the discovery that ceramide was not primarily a fusogen, as was diacylglycerol, but rather destroyed the bilayer permeability barrier. (1997-present): As a result of our studies on sphingomyelinases, Alonso and myself have developed a systematic series of studies on the physical properties of ceramides and related simple sphingolipids

O-6

Investigating transcription and replication at the level of single molecules and cells

N. Dekker
Department of Bionanoscience, TU Delft, The Netherlands

Single-molecule force and torque spectroscopy are very versatile techniques that allow us to shed light on genomic processes such as transcription and replication. In this talk, I will show how one can use these approaches to understand the mechanical properties of DNA and RNA. Then, I will highlight how we can study transcription by RNA-dependent RNA polymerases. We show how a very general approach that consists of parallel tracking to acquire hundreds of traces of individual RNA-dependent RNA polymerases transcribing RNA in real time, combined with an analysis method that can simultaneously probe different intermediate states visited by the polymerases, can readily elucidate the mechanochemistry of these enzymes. Lastly, as the true environment of all of these molecules is the living cell, I will demonstrate our ability to track replication inside bacterial cells, and discuss the implications of our observations on the dynamics of the replication fork.

O-7

Hydration, mobility, aggregation and nanodomain formation in model membranes studied by fluorescence

M. Hof
J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Dolejškova 3, CZ-18223 Prague 8, Czech Republic; Hof@jh-inst.

Fluorescence can be used in all kind of model membrane systems, such as monolayers, supported lipid bilayers, or unilamellar vesicles, as also in cells. Using a fluorescent reporter one can gain information on location, dynamics and polarity of the labelled system. Although recently super-resolution microscopy appeared, the combination of "conservative" techniques can still provide valuable information on questions in lipid membrane biophysics. Specifically, time-dependent fluorescence shift method[1] (for protein applications see presentation of M. Amaro), different variants of fluorescence fluctuation spectroscopy[2], and a Monte Carlo/Fluorescence Resonance Energy Transfer approach will be discussed[3]. From the application of those techniques three membrane topics will be addressed: Influence of monovalent ions ("Hofmeister"-series)[4–7], impact of truncated oxidized phospholipids[8–10] and dynamics and size of lipid nanodomains in model membranes[11].

1) Jurkiewicz Biochimie 2012 94 26; 2) Machán BBA-2010 *1798* 1377; 3) Šachl Biophys J 2011 101 L60; 4) Vácha JPC A 2009 113 7235; 5) Vácha JPC B 2010 *114* 9504; 6) Jurkiewicz BBA 2012 1818 609; 7) Pokorná Farad Discus 2013 160 341; 8) Beranova Langmuir 2010 *26* 6140; 9) Volinsky Biophys J 2011 101 1376; 10) Jurkiewicz BBA 2012 1818 2388; 11) Štefl Biophys. J 2012 102 9 2104

Abstracts

– Biological Electron and Proton Transfer –

O-8

Proton-coupled electron transfers in phenol and tryptophan oxidations: An electrochemical approach

C. Costentin
Université Paris Diderot, Sorbonne Paris Cité, Laboratoire d´Electrochimie Moléculaire, Unité Mixte de Recherche Université - CNRS No 7591, France

Association between single electron transfer and proton transfer in many reactions of electron transfer, radical chemistry and biochemistry is well a recognized phenomenon. There is some evidence that the two reactions might be concerted in many natural processes, notably in the reactions of Photosystem II. Coupling proton transfer to electron transfer entails an improvement of the driving force of the reaction. Two types of mechanisms may be followed; mechanisms in which the two reactions occur in a stepwise manner, with proton transfer first, followed by electron transfer (EPT) or, *vice versa*, electron transfer first, followed by proton transfer (PET) and a mechanism in which proton and electron transfer occur in a concerted manner (CPET). Only in the last case will the benefits of the additional driving force offered by the coupling with proton transfer be fully exploited. Electrochemistry, through techniques like cyclic voltammetry, can provide a quite effective access to CPET in terms of diagnosis and quantitative kinetic characterization. Several examples will be presented : phenol (mimicking tyrosine) and tryptophan oxidations with a particular emphasis on the role of the proton acceptor (water or external base).

O-9

How redox proteins form transient complexes in photosynthesis and respiration

I. Díaz-Moreno[1], B. Moreno-Beltrán[1], A. Guerra-Castellano[1], K. González-Arzola[1], J. M. García-Heredia[1], A. Velázquez[2], P. M. Nieto[3], M. Ubbink[4], A. Díaz-Quintana[1], M. Á. de la Rosa[1]
[1]Instituto de Bioquímica Vegetal y Fotosíntesis, cicCartuja, Sevilla, Spain, [2]Instituto de Biocomputación y Física de Sistemas Complejos (BIFI), Zaragoza, Spain, [3]Instituto de Investigaciones Químicas, cicCartuja, Sevilla, Spain, [4]Institute of Chemistry, Leiden University, The Netherlands

Protein complex formation is at least a two-step process in which the formation of a final, well-defined complex entails the initial formation of a dynamic encounter complex. The lifetime of the protein complex is determined by the dissociation rate. Highly transient complexes, with lifetimes on the order of milliseconds, exhibit moderate or low binding affinities, with dissociation constants in the μM–mM range. Electron transfer (ET) reactions mediated by soluble redox proteins exchanging electrons between large membrane complexes in photosynthesis and respiration are excellent examples of transient interactions. Here, experimental approaches based on dia and paramagnetic NMR spectroscopy are combined with NMR restraint- or charge-driven docking simulations to study the molecular recognition processes in ET complexes, using the cyanobacterial C*f*-Cc_6 interaction in photosynthesis and the plant Cc_1-Cc_6 adduct in respiration, as physiological model systems. Both ET ensembles exhibit optimal coupling between the redox centers although they might differ in their dynamic behavior. Needless to say that such an integrative methodology opens new perspectives in our understanding of the dynamic, transient adducts formed between proteins beyond the model systems herein analyzed.

O-10

Exploring the entrance of proton pathways in cytochrome c oxidase from P. denitrificans

K. Kirchberg[1], T.-Y. Kim[1], H. Michel[2], U. Alexiev[1]
[1]Freie Universität Berlin, Physics Department, Berlin, Germany, [2]Max-Planck Institute of Biophysics, Department of Molecular Membrane Biology, Frankfurt a.M., Germany

Cytochrome *c* oxidase (C*c*O), the terminal oxidase of cellular respiration, reduces molecular oxygen to water. The mechanism of proton pumping as well as the coupling of proton and electron transfer is still not understood in this redox-linked proton pump. Two proton transfer pathways have been suggested, originating at the N-(negative) side of the membrane with differential involvement in the redox cycle. We recently showed that the first H^+-uptake at the N-side of fully oxidized C*c*O coincides with single electron input into Cu_A at the opposite side of the membrane. This indicates long-range interactions and efficient H^+-uptake mechanisms, such as proton collecting antennae switched on by electron injection [1]. To further understand the mechanism of proton pumping single cysteine variants are employed [2]. They provide selective binding sites for various reporter molecules, such as fluorophores for conformational changes, pH-indicator dyes for protonation reactions, or polarity labels for sensing the local environment. A correlation between the differential involvement of the proton pathways and protein surface properties will be discussed.

[1] K. Kirchberg, H. Michel, U. Alexiev, *J Biol Chem* **2012**, *287*, 8187
[2] K. Kirchberg, H. Michel, U. Alexiev, *Biochem Biophys Acta* **2013**, *1827*, 276

O-11

Structural characterization of peripheral subunits from Alternative Complex III

F. G. Calisto, P. N. Refojo, M. M. Pereira
Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. Da República EAN, 2780-157 Oeiras, Portugal

Respiratory chains are composed of several membrane protein complexes enabling the transduction of energy from oxidoreduction reactions to the establishment of transmembrene difference of the membrane potential by charge translocation. Central to aerobic respiratory chains is cytochrome bc_1 complex, which presents quinol:cytochrome *c* oxidoreductase activity. We recognize the existence of an alternative complex, Alternative Complex III – ACIII, performing the same function as the bc_1 complex but structurally totally distinct. Besides four transmembrene proteins, ACIII contains three peripheral subunits, ActA and ActE are facing the periplasm but the orientation in relation to the membrane of the ActB remains unknown.

In this work we investigated the orientation of subunit ActB and its functional implications, taking advantage of ProteinaseK activity on isolated membrane vesicles from *R. marinus*. We observed that ActB was digested by ProteinaseK, while ActE was present at a constant level. As a control membranes were solublised with Triton X-100. In its presence ActE was also sensitive to ProteinaseK.

The orientation of ActB subunit towards the cytoplasm, in opposition to the other peripheral proteins which face the periplasm has strong repercussion in the operative mode of ACIII.

Abstracts

– Biological Electron and Proton Transfer –

O-12

Exploring the dioxygen diffusion pathways in aa3 Cytochrome c oxidases: Insights from MD simulations

A. S. F. Oliveira, J. M. Damas, A. M. Baptista, C. M. Soares
Instituto de Tecnologia Química e Biológica - Universidade Nova de Lisboa

Cytochrome c oxidases (CCOX) are members of the haem-copper oxidase superfamily and are the terminal enzymes of the respiratory chain. These proteins are membrane-bound multi-subunit redox-driven proton pumps, which couple the reduction of molecular dioxygen to water with the creation of a transmembrane electrochemical proton gradient.
Over the last 20 years, most of the CCOX research focused on the mechanisms and energetics of reduction and/or proton pumping and little emphasis has been given to the pathways used by dioxygen to reach the binuclear site. The main objective of this work is to identify possible dioxygen pathways in the reduced CCOX from *Rhodobacter sphaeroides*[1] using extensive Molecular Dynamics (MD) simulations. Our simulations allowed the identification of two possible dioxygen channels, whose entrances are both located in the membrane region. The first channel is a Y-shaped hydrophobic cavity with a constriction point near $F282_I$ and $W172_I$, and it corresponds to the oxygen pathway previously identified in the structure[2]. The second channel starts near the hydroxyl farnesyl tail of haem a_3 and ends near the $Y288_I$ (which is covalently linked to the $H284_I$ imidazole group).
[1]Qin et al. (2009) *Biochemistry* **48**, 5121-5130
[2]Svensson-EK et al. (2002) *J.Mol.Biol.* **321**, 329-339

O-13

Study of the Fe-S vibrational modes in complex I by means of Raman, Far IR and electrochemistry

M. Yegres[1], T. Friedrich[2], P. Hellwig[1]
[1]Laboratoire de bioélectrochimie et spectroscopie, UMR 7140, CNRS-Université de Strasbourg, France., [2]Institut für Biochemie und Org.Chemie, Albert-Ludwigs-Universität Freiburg, Germany

Complex I couples the electron transfer from NADH to ubiquinone with a translocation of protons across the membrane. It has an unusual L-shaped structure consisting of peripheral arm extending into the aqueous phase, that includes all know cofactors and a membrane part embedded in the lipid bilayer, where proton translocation takes place. There is evidence that the energy released by the redox reaction is transmitted by conformational changes of a 140 Å long amphipatic helix that is aligned to the membrane arm. It is proposed that the helix acts like a piston transmitting the energy from the peripheral arm to the membrane arm. Here we study on the action of the protein by means of the Fe-S vibrational spectroscopies in the low frequency range. These signals are sensitive to redox state, cluster ligation, hydrogen bonding and the conformation of bound cysteine residues. The spectra obtained from the *E. coli* complex I were compared to those of the soluble fragment and model compounds. In addition, labeling of the Fe-S clusters by ^{54}Fe was performed. A full assignment of the spectral features was performed for the oxidized, the electrochemically and the NADH reduced form giving clear evidence on the conformational changes responsible for proton translocation.

P-14

Monitoring redox activity of heme proteins by photochromic fluorescence resonance energy transfer

H. Bayraktar, S. Manioglu
KOC UNIVERSITY, Department of Chemistry, Istanbul, Turkey

The real time imaging of redox proteins is crucial for understanding their role in cellular signaling. In mitochondria, they are key regulators of cellular processes and different activity can be observed depending on the cell type. The current methods to measure the redox activity are limited due to the lack of genetically encoded probes. The activity of Cytochrome *c* (Cyt *c*), heme protein, is a regulator for apoptosis and the electron transfer. Here we present photochromic fluorescent energy transfer (pcFRET) method to measure the redox activity of Cyt *c*. We have previously used pcFRET as an ultrasensitive method measuring the photocycle of rodopsin membrane proteins. It is a general tool for turning absorbance changes into fluorescence. The Venus fluorescent fragment was ligated into the Cyt *c* expression vector with standard cloning methods. The expression of Cyt *c*-Venus in BL21 expression cells was demonstrated. The detection of pcFRET for the Cyt c oxidation in BL21 cells is currently being studied. The expression of Cyt *c*-Venus will be optimized in cells, we will investigate how frequently the oxidation state of Cyt *c* change. We will study the frequency change of redox signal depending on external stimuli.

P-15

investigation of charge translocation by the respiratory complex I reconstituted in liposomes

P. J. Castro, A. P. Batista, M. M. Pereira
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Respiratory complex I plays a central role in energy production by coupling electron transfer between NADH and quinone to ions translocation across the membrane, thereby establishing an electrochemical potential. This L-shaped enzyme consists of hydrophilic and membrane domains. The membrane domain includes seven hydrophobic subunits and the three largest subunits, NuoL, M and N (*E. coli* nomenclature), are homologous to each other and to Na^+/H^+ antiporter complex (Mrp) subunits. Previous studies indicate that complex I from *Rhodotermus marinus* transduces energy by two different processes: proton pumping and Na^+/H^+ antiporting.
This work aims at evaluating the ability of the isolated complex I to translocate H^+ and Na^+ across the membrane and to determine the stoichiometry of the process. In order to achieve our goal, the enzyme was purified from *Rhodotermus marinus* and incorporated into liposomes. The proteoliposomes were characterized by Dynamic Light Scattering (DLS). The existence of NADH:quinone oxidoreductase activity and the formation of membrane potential after addiction of the substrates proved that the incorporation was successful. H^+ and Na^+ translocation were monitored by fluorescence spectroscopy and ^{23}Na-NMR, respectively.

Abstracts

– Biological Electron and Proton Transfer –

P-16

Electron transfer with azurin at Au/SAM junctions in contact with the glass-forming environment

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Interfacial biological electron transfer under the condition of approaching the glass-transition threshold was explored by the method of rapid-scan protein film voltammetry. The gold-deposited alkanethiol SAM/azurin assemblies were placed, for the first time, in contact with a buffered protic ionic melt, choline dihydrogen phosphate ([ch][dhp]), containing less than two, or more (up to 15) water molecules per [ch][dhp]. The extra confinement of Az films within the semi-solid environment allowed for the essential alteration of the protein's conformational flexibility, directly controlling ET in a dynamical regime, that was further tuned through the temperature (273–353 K) and pressure (0.1–150 MPa) variations. As the glassy state was approached, the Marcus theory-based data analysis revealed: (a) the transitory traversing of a broad nonergodic ET zone, and (b) the ultimate breakdown of the medium's linear response motif (i.e., an increase in anharmonicity for the ET energy profiles).

P-17

The selectivity of type II NADH: quinone oxidoreductase for quinones – a docking study

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Type II NADH: quinone oxidoreductases is a flavoprotein that catalyzes the transfer of electrons from NADH to quinones. NDH-II has been detected in different microorganisms which produces different quinones . In *E. coli* the physiological quinone is ubiquinone, while *S. aureus* and *B. subtilis* synthesis menaquinone. Recently the high resolution structure of NDH-II of *S. cerevisiae* in complex with quinone, NAD and FAD was obtained. These studies provided a crucial piece of information on the conformation and arrangement of the enzyme and its substrates. However, the reaction and selection mechanism behind this interaction is still unknown.
What is the binding hotspot between quinone and NDH-II in the different organisms?
Are these hotspots related to quinone selection by the different NDH-II?
To unravel the interaction hotspots of NDH-II:quinone we setup an *in silico* docking calculation based on the recently published high resolution structures of NDH-II. This strategy uses a combination of homology modeling and docking experiments of different quinones interacting with NDH-II from different organisms. The differences between docking sites will shed light into the molecular selective determinants for different quinones by NDH-II.

P-18

Structure of the membrane protein menaquinol fumerate reductase from *Chloroflexus aurantiacus* at 3 Å

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The photosynthetic green filamentus bacteria *Chloroflexus aurantiacus* has a quinol:fumerate reductase (QFR) that reduces fumerate to succinate. As our study has shown the composition of ligands for the electron transfer process with FAD, three different iron-sulphur clusters, Fe_2S_2, Fe_4S_4 and Fe_3S_4 beside two hemes is well conserved with for example Wolinella succinogenes. All known protein structures of QFR are functional dimers, therefore initially we tried to solve the X-ray diffraction data in a dimer solution until we found with anomalous data(Fe edge) the convincing solution in a trimeric arrangement in the asymmetric unit. The sequence homology of the *Chloroflexus aurantiacus* QFR to the closest pdb entry from E. coli is for the big subunit A only 29 %, the ferredoxin iron sulphur binding subunit B 24% and the transmembrane subunit C without any significant identity to known structures. The current model has 3090 residues placed in the electron density at 3 A resolution. For each monomer the FAD, the three FeS clusters and two heme are found. Each C subunit has five transmembrane helices. Now with the preliminary structure the identifying process of the trimer forming residues has begun and shades a light on the evolution of this important membrane protein and its functional organization.

P-19

Role of proton motive force and ATPase activity in biohydrogen production by Rhodobacter sphaeroides

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In order to examine the role of proton motive force (PMF) or the proton H^+-ATPase in H_2 production by *R. sphaeroides*, isolated from mineral springs in Armenia, PMF and its components (the membrane potential ($\Delta\psi$) and the transmembrane pH gradient (ΔpH)) and ATPase activity were determined, and the effect of hydrogenase inhibitor diphenylene iodonium (Ph_2I), was examined. Under nitrogen limitation conditions $\Delta\psi$ was shown to be of -98 mV and the reversed ΔpH was +30 mV resulting in PMF of -68 mV. The addition of Ph_2I decreased $\Delta\psi$ to –70 mV in concentrations of 20 μM and higher; lower concentrations of Ph_2I had no valuable effect on $\Delta\psi$. The $[pH]_{in}$ determined by the quenching of fluorescence of 9-aminoacridine was effectively blocked by Ph_2I. The *R. sphaeroides* membrane vesicles demonstrated significant ATPase activity. The incubation of the vesicles in the presence of Ph_2I caused to marked inhibition in ATPase activity revealing concentration dependence effect. The 10-20 μM Ph_2I did not affect the ATPase activity, whereas 40 μM Ph_2I caused to marked inhibition ($\sim$2 fold) in ATPase activity.
The results point out a role of PMF and the H^+-ATPase in hydrogenase activity involved in H_2 production. Moreover, relationship of F_0F_1 with hydrogenase is suggested.

Abstracts

– Biological Electron and Proton Transfer –

P-20

Functional equivalence of as isolated and high energy metastable states of cytochrome c oxidase

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The current model of the proton pumping in cytochrome c oxidase (CcO) presumes the existence of a "high-energy" metastable $\mathbf{O_H}$ state. It has been suggested that there exist differences between ligation and protonation states of the catalytic site of $\mathbf{O_H}$ and the "resting" state of the oxidized CcO. Our previous study did not reveal differences either in the spectral characteristics (optical, EPR) or the kinetics of electron transfer to the catalytic site in these two forms of CcO. In this work, the reactions of as "isolated" fast form (**O**) and ($\mathbf{O_H}$) of the oxidized CcO with H_2O_2 have been investigated by stopped-flow method. The rate constants for the binding of H_2O_2 to the catalytic site are almost identical for both forms of the fully oxidized CcO. Using phenol red as a pH indicator we have found that a relaxation of $\mathbf{O_H}$ to **O** state and the binding of H_2O_2 to the catalytic site of both forms are not coupled with an apparent proton uptake or release. Our findings again indicate that there is no difference in the ligation and protonation states of the catalytic site of **O** and $\mathbf{O_H}$ of the oxidized CcO.

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P-21

Dynamic control for short-range biological electron transfer: insights from protein film voltammetry

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For the case of short-range biological electron transfer, under certain experimental conditions, the slowest conformational fluctuations of redox-active proteins may directly control the electron transfer rate, through the pre-exponential term of a kinetic equation. In contrast, the long-range ET is controlled by the donor-acceptor electronic coupling. Our approach implies special design of interfacial biomimetic assemblies, encompassing Au-deposited self-assembled monolayer films of variable thickness and head group composition, providing different interaction modes for the functionalized redox proteins (cytochrome *c*, azurin, myoglobin, etc.). Furthermore, the exterior manipulations by using highly viscous media such as aqueous glucose mixtures or water-doped protic ionic melts as a glass forming environment, further tuned by the variations of temperature and pressure, allows for the careful identification of a dynamically controlled regime of ET, along with the disclosure of novel non-ergodic and nonlinear medium response motifs.

P-22

Lab-on-a-chip tool for bioelectronic investigations

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We established a non-invasive method for the measurement of electric signals associated to membrane-coupled signal- and energy transduction phenomena of living cells. The method uses a pair of metal film electrodes picking up the signals. In microscopic dimensions, the electrodes observe events only from their close vicinity, so objects of micrometer scale (typically cells or thin tissues) can be monitored this way. Cell delivery can be controlled by microfluidic tools in a lab-on-a-chip measuring chamber, giving further access to microscopic observations. We applied the method to monitor fundamental cell physiological processes on different levels of organization (Chlamydomonas cells, neuro-epithelial tissues).

We expect our method to become a highly sensitive, versatile tool for the kinetic investigation of electric phenomena associated to transport phenomena across cellular and epithelial membranes. The financial support of Hungarian research grant KTIA-OTKA CK 78367 is gratefully acknowledged.

P-23

NMR studies of the bioenergetic metabolism of metal reducing bacteria

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Metal reducing bacteria are becoming the focus of research efforts to apply their unique metabolic potential in fields such as bioremediation of metal contaminated environments,and bioelectrosynthesis or energy production from cheap raw materials such as wastewater. These organisms have a unique organization of their bioenergetic redox chains,with ATP production that occurs across the cytoplasmic membrane coupled to the reduction of the solid-phase electron acceptor that occurs at the cell surface, in a process called extracellular respiration. *Shewanella oneidensis* MR-1 is a model Gram negative metal reducing bacterium, which has numerous multi-heme cytochromes that participate in electron transfer across the periplasm and that are displayed on the cell surface to reduce the solid-phase electron acceptors. This last process can proceed by direct contact or be mediated by small redox shuttles such as flavins. NMR spectroscopy was used to determine the protein-protein interactions that sustain electron flow across the periplasmic space during extracellular respiration and the protein-small molecule interactions that mediate indirect electron transfer between the cells and the solid-phase acceptors.

Abstracts

– Biological Electron and Proton Transfer –

P-24

Ion translocation by respiratory Complex I: The role of NuoL subunit

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Complex I is the least understood enzyme of the respiratory chain and its deficiencies have been implicated in several neurodegenerative diseases. It couples the oxidation of NADH and reduction of quinone to charge translocation across the membrane, contributing for the establishment of the membrane potential. The membrane arm contains Na^+/H^+ antiporter-like subunits (NuoL, M and N), which are homologous to Na^+/H^+ antiporters (Mrp), suggesting that these subunits may participate in charge translocation. Understanding the coupling of the enzymatic reaction to charge translocation is still a major question in complex I research. Crystallographic structural data showed the presence of a ∼110 Å long amphipathic helix part of the C-terminal of NuoL subunit, which may function as a coupling element.
In this work we investigate the role of NuoL. We used an *E. coli* mutated strain in NuoL, obtained from the 'Keio collection' and monitored the ion transport. Proton translocation was studied by quenching of ACMA fluorescence and ^{23}Na-NMR spectroscopy was used to investigate sodium transport. We observed that the presence of NuoL subunit is not essential for entire proton translocation by complex I, but is determinant for sodium transport.

P-25

Interaction mechanisms between plant GALDH and cytochrome c

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The flavoprotein L-galactono-γ-lactone dehydrogenase (GALDH) catalyzes the terminal step of vitamin C biosynthesis in plant mitochondria. Here we investigated the interaction between *Arabidopsis thaliana* GALDH and its natural electron acceptor cytochrome *c* (Cc). Using laser spectroscopy we observed that $GALDH_{SQ}$ oxidation by Cc follows a kinetic mechanism involving protein association, to form a transient bimolecular complex prior to electron transfer. The kinetic analysis of both $GALDH_{SQ}$ and $GALDH_{HQ}$ oxidation suggests that GALDH reduction by its carbohydrate substrate limits the overall rate of Cc reduction. ITC analysis showed that GALDH weakly interacts with both oxidized and reduced Cc. Chemical shift perturbations for ^{1}H and ^{15}N nuclei of Cc mapped the interacting surface of Cc to a single surface surrounding the heme edge. In summary, the results point to a relatively low-affinity GALDH/Cc interaction, similar for all partner redox states, directed by electrostatic complementary forces and involving protein-protein dynamic motions.

P-26

Inter- and intra-monomeric communication in the cytochrome bc1 complex as revealed by MD simulations

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The cytochrome bc_1 complex (bc_1) plays an important role in cell bioenergetics acting as a dimeric redox-driven proton translocase. Mitchell's Q-cycle in general describes mechanism of bc1 activity: ubiquinol is oxidized in the site Q_P of the cytochrome *b* subunit, whereby the two released electrons are transferred to the heme of cyt. c_1 (via the mobile "head" domain of the Rieske protein) and to a ubiquinone molecule in the Q_N site of cyt. *b*.
As it was discussed before [1] the ubiquinol reduction in the Q_N site should be coupled with the mobility of the Rieske "head" domain, while thermodynamics considerations [2] imply the coupling between the two "head" domains. However, the details of both the coupling events in bc_1 remain elusive. We performed equilibrium and metadynamics MD simulations of the bc_1 with different ligands to establish inter- and intra-monomeric coupling in bc_1 dimer while application of various computational techniques allowed us to track possible pathways for allosteric signal propagation.
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P-27

Molecular details of electron transfer in fumarate reduction by flavocytochrome c3

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Numerous redox enzymes catalyze two-electron processes, even though the connection from the protein surface to the active site is made through chains of redox cofactors that only exchange one electron at a time. The soluble fumarate reductase from the periplasmic space of *Shewanella* is one of these proteins. The three-dimensional structure showed that this protein contains a chain of four hemes, which interacts with the FAD catalytic centre that performs the obligatory two electron-two proton reduction of fumarate to succinate. In order to investigate the role played by the redox chain in the catalytic activity of this enzyme, transient kinetic studies of flavocytochrome reduction in the absence and in the presence of substrate were performed. The kinetic contribution of each heme for electron uptake and conduction to the catalytic centre was determined. This enabled the observation that the catalytically most competent states of the enzyme are those least prevalent in a quasi-stationary condition of turnover. Comparison of the results from fumarate reductases from two different organisms enabled the identification of the role of the redox properties of individual hemes in the modulation of the catalytic activity of the enzymes.

Abstracts

– Biological Electron and Proton Transfer –

P-28

Position of W104 in AppA BLUF domain revealed by fluorescence spectroscopy

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AppA is a BLUF protein which serves as a transcriptional anti-repressor found in *Rhodobacter sphaeroides*. Similarly to other flavoproteins the absorption of the photon is followed by an electron transfer cascade, but the same time the hydrogen-bond network around FAD undergoes to a reorganization which is crucial for the formation of the light adapted state of the protein.
There are several different X-ray structure for AppA, which show the position of W104 at a different place: in the original crystal structure from Anderson et al W104 was located closer to the flavin forming a hydrogen bond with Q63. A different – shorter length – AppA structure has shown a structure with W104 farer from the flavin partially exposed to the solvent.
In order to clarify the location of W104 we made several mutants and performed fluorescence anisotropy, fluorescence anisotropy decay and FRET measurements. Exchanging the tryptophan at W64 to phenylalanine caused that we could excite selectively the W104 tryptophan. For the dark state (dW64F) we observed a slower rotational correlation time than in a partially solvent exposed case (lW64F).

P-29

The monoheme cytochrome c subunit of alternative complex III from Rhodothermus marinus

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In *Rhodothermus marinus*' respiratory chain, quinol: electron acceptor oxidoreductase activity is performed by the alternative complex III (ACIII). This seven subunit complex is a member of a recently identified family of enzymes which catalyses the same reaction as the bc_1 complex, but is structurally unrelated to it. The ACIII contains 4 transmembrane subunits without redox cofactors, one cytoplasmic subunit with 4 iron-sulfur clusters and two periplasmic subunits containing C-type hemes. A structural and functional association between the ACIII and the caa_3 heme-copper oxidase (HCO) from *R. marinus* has been reported.
The monoheme cytochrome *c* subunit (mhc) was structurally and functionally characterized. For this purpose, a truncated form of its coding gene was cloned and expressed in *E.coli*. The UV-Visible spectra of the protein are characteristic of a low spin heme with a histidine-methionine-Fe coordination. By measuring the mhc: O_2 oxidoreductase activity of the caa_3 HCO, we conclude that mhc is the electron donor for the caa_3 HCO in its association with the ACIII.

P-30

Alternative complex III: a different architecture using known building modules

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Alternative complexes III (ACIII) form a recently identified family of enzymes with quinol:electron acceptor oxidoreductase activity that replace functionally cytochrome bc_1/b_6f. Gene clusters encoding ACIII are widespread in the Bacteria domain and are mostly present in genomes were the coding genes for the bc_1/b_6f complexes are absent. Furthermore, the gene clusters are frequently associated with those coding for oxygen reductases subunits. Biochemical and genomic analyses showed that ACIII is composed of six to eight subunits, most of which homologous to different proteins already observed in other known enzymatic complexes. In fact, ACIII can be seen as a combination of modules present in different enzyme families, namely the complex iron-sulfur molybdenum containing enzymes to which enzymes like polysulfide reductase and DMSO reductase belong to. With the increasing number of completely sequenced genomes, a larger number of gene clusters coding for ACIII were identified and an unanticipated diversity in gene clusters, both in terms of its constitution and organization was detected. The several unexpected gene arrangements brought new perspectives to the role of the different subunits of ACIII.

P-31

Bacteriorhodopsin and ATP-synthase create gradients in atto- to picoliter sized compartments

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The light-driven proton pump bacteriorhodopsin (bR) is found in the cell membrane of *Halobacterium salinarum*. The protein forms crystalline regions (purple membrane) consisting of hexagonally packed trimers. Upon light irradiation bR pumps protons out of the cytosol creating an electrochemical H^+-gradient that is used for ATP synthesis. This task is carried out by the ubiquitous protein ATP-synthase that produces ATP if the proton electrochemical potential is large enough to overcome the free energy of ATP hydrolysis. The protein is also capable of reversing the process, i.e. using ATP to create H^+-gradients.
Both proteins are studied to large extend in model systems. Here we present pore-spanning membranes (PSMs) as a new model, providing a system that consists of atto- to picoliter sized compartments covered by a lipid bilayer of desired lipid and protein composition. To obtain such a bilayer, bR and ATP-synthase are reconstituted into liposomes that are spread on porous substrates with defined pores. A pH-sensitive fluorescent dye entrapped in the compartments allows to monitor the proteins' H^+-pumping activity by means of fluorescence microscopy.

Abstracts

– Biological Electron and Proton Transfer –

P-32

Direct electrochemistry of gold-deposited self-assembled monolayers of L-cysteine

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We report on the gold-deposited self-assembled monolayer films (SAMs) of L-cysteine (Cys), which, when probed by the fast scan cyclic voltammetry (CV), exhibit a pair of well-defined waves of electron exchange in a quasi-reversible kinetic regime. The signal emerges in the course of successive cycling over the potential range of 0.4 to −0.4 V (phosphate buffers, pH 5.5 to 8). The formal redox potential of the Au/Cys electrode is pH dependent with a slope 44 mV/pH. The redox process, seemingly, is coupled to a proton translocation, involving the carboxylic group of Cys. Furthermore, we observed the electro-catalytic activity of Au/Cys electrodes toward the ascorbic acid decomposition. Based on these results we suppose that all the data reported for different proteins immobilized at Cys SAMs should be reconsidered very carefully, in particular when successive cycling over the potential range of 0.4 to −0.4 V was applied, aiming a disclosure of the proteins' voltammetric response.

Abstracts

– Cell Biophysics and Signaling –

O-33

Biophysics of leukocyte adhesion: nanoscale organization and dynamics of the integrin LFA-1

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Integrins are cell membrane adhesion receptors involved in morphogenesis, immunity, tissue healing and metastasis. A central, yet unresolved question regarding the function of integrins is how these receptors regulate their conformation and dynamic nanoscale organization on the membrane to generate adhesion-competent microclusters upon ligand binding. We exploit single molecule fluroescence approaches (nm accuracy & ms temporal resolution) to investigate molecular conformation, lateral organization and dynamics of the β_2-integrin LFA-1 on immune cells. Our results indicate that LFA-1 forms stable and non-mixable nanoclusters in the neighborhood of GPI-anchored protein nanodomains prior to ligand activation [1]. Mobility of LFA-1 depends on its conformational state and anchoring to the cytoskeleton [2,3]. Furthermore, lateral mobility resulted crucial for microcluster formation upon ligand binding and for stable leukocyte adhesion under shear stress conditions [3]. Our ongoing research centers on the role that mechanical stimuli (shear-stress and isotropic mechanical stretching) have on both activation and lateral mobility of LFA-1 on monocytes.

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O-34

Enhanced fluorescent proteins for FRET and for studying signaling across the membrane

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A screening method is reported that, in addition to fluorescence intensity, quantifies the excited state lifetime of a fluorescent protein, providing a direct measure for the quantum yield of the fluorescent protein. A library of cyan fluorescent protein (CFP) variants was screened eventually yielding the bright cyan fluorescent protein variant, mTurquoise2 which has a very high quantum yield of 0.93, and a seriously increased fluorescence lifetime of 4 ns. mTurquoise2 is the preferred donor to YFP variants (like mVenus or mCitrine) with an R0 of 5.83 nm.

We apply the different probes for the study of GPCR-triggered signaling across the membrane in single mammalian cells. A new Gq FRET sensor was made to monitor activation of the G protein, and downstream signaling events like generation of lipid-derived second messengers, PtdInsP2-dependent PLC relocalization, and RhoGEF activation with a variety of FRET-, ratio imaging- and TIRF-microscopic applications will be presented.

Goedhart J, von Stetten D, Noirclerc-Savoye M, Lelimousin M, Joosen L, Hink MA, van Weeren L, Gadella TWJ, Royant A. (2012) Structure-guided evolution of cyan fluorescent proteins towards a quantum yield of 93%. Nat Commun. 3:751.

O-35

Nanoparticle probes of molecular rotation on cell surfaces

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Rotation of membrane proteins is a sensitive measure of their aggregation state and their interactions with other membrane species. We have used nanoparticles including asymmetric quantum dots (QD) as non-bleaching probes of the rotation of individual cell surface proteins. Invitrogen QD655s conjugated to A2 DNP-specific IgE allow examination of slow rotation of the Type I Fcε receptor (FcεRI) on RBL-2H3 cells. For individual QDs in image pair sequences, we calculate the time-autocorrelation function for fluorescence polarization fluctuations (PFTAC). PFTAC decay extends well into the ms timescale, as implied by time-resolved phosphorescence anisotropy results. Treatment effects suggest that such slow decay may be a property of the membrane itself, perhaps reflecting large-scale fluctuations of mesoscale membrane regions. To examine PFTAC decays *faster* than imaging measurements permit, we have applied time-correlated single photon counting to individual QD fluorescence and analyzed data down to correlation times of 1ns. These measurements should thus include the 80μs hydrodynamic rotation of single FcεRI molecules, but significance of current data is limited by QD emission rates. Other probes are therefore being explored. Supported by NSF grant MCB-1024668.

O-36

Protein-protein-protein interactions in membranes measured by triple correlation of confocal images

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Protein-protein interactions have been measured successfully and quantitatively by fluorescence correlation spectroscopy in solution (Elson, BJ 101, 2855-2870 (2011)) and image correlation spectroscopy on cell surfaces (Kolin and Wiseman, Cell Biochem Biophys 49, 141-164 (2007)). These tools fail to provide information about about protein-protein-protein interactions. It has been known for some time that higher order moments or correlations contain the relevant information (Palmer and Thompson, BJ 52, 257-270 (1987); Heinze, Jahnz, and Schwille, BJ 86, 506-516 (2004)) but it is only recently that triple correlation functions of ternary complexes have been measured in solution (Ridgeway, Millar, and Williamson, J. Phys Chem B 116, 1908-1919 (2012) and PNAS 109, 13614-13619 (2012)). We demonstrate how complete and quantitative information for ternary complexes of membrane proteins can be obtained from three confocal images from each of three distinctly labeled proteins from a combination of image correlation spectroscopy, image cross-correlation spectroscopy, and image triple cross-correlation spectroscopy. This is illustrated with simulations and measurements of interactions between selected proteins. The simulation and analysis programs for these will be presented and will be available for interested parties.

Abstracts

– Cell Biophysics and Signaling –

O-37

Early events in cytokine receptor mediated signaling

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The cytokine Interleukin-4 (IL-4) is believed to induce heterodimeric IL-4 receptor (IL-4R) complexes at the surface of target cells. Here we study at the biophysical and cell biological level how these signals are transduced to the downstream transcription factor STAT6. We show that individual receptor subunits accumulate in a novel type of early sorting endosome stably anchored within the actin cortex (cortical endosomes), and characterize their trafficking by FRAP and quantitative imaging. In addition we found that pharmacological inhibition of receptor internalization blocks STAT6 activation. Endocytosis thus acts upstream of JAK/STAT signaling. Fluorescence cross-correlation spectroscopy (FCCS) analysis of IL-4R subunits in their native plasma membrane environment revealed that complex formation is indeed ligand induced, however the affinities for recruitment of a second receptor chain are comparably low. In addition, fluorescence lifetime imaging (FLIM) showed that the complexes preferentially form within the cortical endosomes, where the subunits are enriched. Thus, subcellular concentration of cytokine receptor subunits within signaling endosomes constitutes a novel, thermodynamic prerequisite for IL-4R signal transduction not yet described in other pathways.

O-38

Tracking NK1 receptor diffusion in the membrane of living cells. Roles of clathrin and cytoskeleton

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NK1 receptor is a very potent target for treatment of depression and attenuation of side effects in cancer therapy. In this work, we describe the impact of impaired endocytosis and cytoskeleton function on the receptor's mobility pattern in the plasma membrane of living cells.
We found that non-activated receptors diffuse freely and in confined domains, which are directly related to clathrin-mediated endocytosis (CME). Inhibitors of different steps of CME, lead to an accumulation of NK1R in confined membrane domains, and a diminution of NK1R mediated Ca^{2+} release.
Depolymerisation of actin and microtubules does not significantly modify freely diffusing receptors diffusion parameters, indicating absence of direct interaction of NK1R with the cytoskeleton. Nevertheless, actin depolymerisation triggers the apparition of fast receptors in circular domains in the absence of activation. This effect is correlated with the formation of cell membrane blebs. Microtubule depolymerisation also significantly increases the fraction of receptors slowly diffusing in domains. This increase in confinement is related to early endocytosis inhibition.
Our results point to the central importance of clathrin in NK1R membrane homeostasis and regulation of the receptor's activity.

P-39

Relationship between Bax and Bak distribution and apoptosis onset in U-87 MG cells upon HypPDT

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Apoptosis is a key process in the development and maintenance of tissue homeostasis, which is tightly regulated by balance between cell survival and damage signals. Our work focused on the intrinsic mitochondrial apoptotic pathway, where Bcl-2 family of proteins plays the major role. We were particularly interested in two pro-apoptotic players Bak and Bax, and investigated their role in apoptosis triggered by hypericin photodynamic therapy (HypPDT). We show the distribution of Bax and Bak in U-87 MG human glioma cells incubated with Hyp before and after PDT. One hour post HypPDT there is a significant Bax translocation into mitochondria, however our results indicate that in U-87 MG cells there are two populations of mitochondria. One, almost exclusively localized near the plasma membrane, contains Bax and Bak simultaneously, and the other which contains Bax only and is distributed throughout the cell. The different protein content and spatial distribution of these two populations suggest that they can play different roles in response to apoptotic stimuli. Further we investigated relationships between onset of apoptosis and either Bax translocation, or metabolic flux rate in U87 MG cells. Supported by EU grants (ITMS: 26110230013; 26220120040) and by APVV-0242-11.

P-40

The investigation of cellular adhesion in the context of external stimuli

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Cellular dynamics as observed in cell migration strongly depends on the adhesion to the underlying surfaces. Thereby, it is indisputable that cells respond to different substrates morphologies and mechanical properties by changing their cellular properties such as cell locomotion, adhesion, growth, differentiation and elasticity. Here, we investigated substrates with different physical and chemical properties varying in rigidity or porosity. By means of *single cell force spectroscopy* (SCFS) we investigated the adhesion strength of epithelial cells as a function of substrate properties. SCFS is a modified version of the *atomic force spectroscopy* (AFM) and is used to measure the strength of overall cell adhesion down to single-molecule levels. Concretely, cellular adhesion of the epithelial cell line MDCK II (Madin-Darby Canine Kidney) to PDMS (Polydimethylsiloxane) substrates with different elasticity and on normal Petri dish was investigated.

Abstracts

– Cell Biophysics and Signaling –

P-41

Effect of dinitrosyl iron complexes on mechanical properties of red blood cells

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Dinitrosyl iron complexes (DNICs) are generated in living systems endowed with the ability to synthesize nitric oxide. DNICs have every reason to be regarded as signaling agents responsible for regulation of biochemical and functional activity of blood cells. The aim of our study was to investigate the action of DNICs on the mechanical properties of red blood cells. DNICs were synthesized by A. Vanin method. Atomic force microscopy researches were carried out using atomic-force microscope NT-206 ("MicroTestMachines", Belarus) working in contact mode. The Young's modulus was calculated using the Hertz model describing the elastic deformation of the two bodies in contact under load. It was obtained that DNICs at concentration (50 nM-2.5 μM) caused a decrease of the elastic modulus of red blood cells, the largest effect was observed at concentration of 50 nM. These results are in good agreement with obtained changes in parameters of osmotic and acidic hemolysis of red blood cells treated with DNICs. The totality of the experimental data suggests that DNICs improve deformability of red blood cells that can be important for blood microcirculation.

P-42

Multispot multiphoton Ca2+ imaging in acute myocardial slices of CPVT hearts

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Rationale: Alterations in cardiomyocyte (CM) Ca^{2+} handling play a role in initiating and sustaining arrhythmias. **Aim:** We aimed to establish a multicellular cardiac model to investigate Ca^{2+} dynamics in CM obtained from mice harbouring a single a.a. mutation in the cardiac ryanodine receptor linked to inherited stress triggered arrhythmia. **Results**: Acute thick (450 μm) ventricular slices were obtained from young WT and CPVT mice. Slices were loaded with the Ca^{2+} indicator Fluo4-AM and imaged with a multispot multiphoton microscope (MMM) to optically monitor intracellular Ca^{2+} fluctuations during electrical pacing upon β-adrenergic stimulation. Heart slices from CPVT mice developed Ca^{2+} alternans and increased propensity to spontaneous diastolic Ca^{2+} release when adrenergically stimulated. These cell-wide Ca^{2+} waves originated typically from the same Ca^{2+} release hotspot. **Conclusions**: We developed a powerful close-to-*in vivo* model of Ca^{2+} imaging in acute heart slices that allows to observe several cells simultaneously in their own tissue environment. Moreover the concurrent high spatial and temporal resolution afforded by the parallel scanning in MMM can be exploited to assess subcellular Ca^{2+} dynamics in a wide tissue region.

P-43

Challenging cellular mechanics - tension homeostasis by surface area regulation

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This study describes a mechanical model to map elastic properties of epithelial cells. In contrast to the commonly used contact model devised by Hertz, we used an approach relying on a modified liquid droplet model that also takes the architecture of the apical membrane into account. Force spectroscopy comprising indentation and tether pulling experiments was applied to map the membrane's in-plane tension as a set-point for regulation and the available surface area at the same location. Evidence for the validity of our model is given by obtaining the same mechanical properties mainly independent from the geometry of the indenter. Thereby, we can show how cells quickly adapt to a mechanically challenging situation as experienced during migration, cell division or exposure to osmotic stress. Various stimuli target membrane reservoir availability, actomyosin-integrity, membrane-cytoskeleton attachment sites, and hydrostatic pressure to provoke a tension-driven response of the apparent area compressibility that can readily be transformed into the available membrane surface area. We found substantial changes in membrane tension and area compressibility modulus due to interfering with membrane-cytoskeleton attachment sites and available membrane surface area.

P-44

Single particle tracking reveals that amyloid aggregates alter the mobility of GM1 ganglioside

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Neuronal impairment in Alzheimer's disease (AD) is currently attributed to a complex cascade of events triggered by the interaction of amyloid oligomers, constituted primarily by Aβ1-42 peptide, with the plasma membrane. Amongst the variety of toxic mechanisms proposed, one involves the binding of amyloid species to GM1 gangliosides. GM1 takes part into the formation of membrane rafts, dynamic and specialized membrane microdomains responsible for the compartmentalization of cellular processes such as signalling and protein trafficking. The interaction with GM1 has been demonstrated to be a crucial factor also in mediating the aggregation and toxicity of other amyloidogenic proteins and peptides, such as amylin (also known as human islet polypeptide, hIAPP), whose aggregation is associated to the development of type II diabetes. Here we take advantage of single particle tracking (SPT) techniques to monitor in real-time in living cells the dynamics of GM1 following the binding of amyloid aggregates of Aβ1-42 and amylin to the plasma membrane. We demonstrate that a direct interaction takes place in vivo, heavily affecting the diffusion properties of a subpopulation of GM1 molecules. Our results might imply an additional mechanism of toxicity, where amyloid aggregates alter cellular processes dependent on membrane raft mobility and clustering.

Abstracts

– Cell Biophysics and Signaling –

P-45

Chelidonine interferes with the action of IL-6 in human uveal melanoma cells

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There is increasing evidence suggesting that IL-6 is involved in tumorigenesis. It can stimulate the proliferation of tumor cells as well as promote cell survival through the inhibition of apoptosis. Chelidonine, the major alkaloid component of *Chelidonium majus*, was reported previously to provoke cell death in a variety of tumor cells. One of its intracellular targets is the antiapoptotic Bcl-2 protein. Expression of Bcl-2 is upregulated by STAT3 activation, which is the major mechanism responsible for IL-6-mediated survival of tumor cells. Herein we studied the effect of chelidonine as well as its interference with the action of IL-6 in a human uveal melanoma (UM) cell line, OCM-3. Our flow cytometric cell proliferation assay demonstrated the antiproliferative effect of chelidonine. Combined analysis of PI exclusion/annexin V binding and DNA fragmentation revealed chelidonine-induced apoptosis of OCM-3 cells. Pretreatment of cells with even sublethal doses of chelidonine abolished IL-6-evoked STAT3 activation. Our findings indicate the possible use of chelidonine in the therapy of UM: it can either provoke cell death or – at lower doses – weaken the antiapoptotic machinery of tumor cells fuelled by IL-6, therefore sensitizing them for apoptosis.

P-46

Biological activity of extract of Actinida arquta leaves

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The aim of the reported study was to determine the polyphenol composition and biological activity of kiwi leaves extract. Plant extracts are known for their beneficial health and prophylactic effects. But the molecular mechanism of the interaction between an extract component and the living organism has not yet been explained. The health-boosting activity of the extracts is ascribed mainly to the antioxidant activity towards biological systems of polyphenolic compounds contained in the extracts.
The study comprised a quantitative and qualitative analysis of the extract from kiwi leaves, using the UPLC-DAD and UPLC-ESI-MS methods, it's antioxidant activity towards the erythrocyte membrane subjected to physicochemical oxidizing agents, and an examination of the extract's effect on the properties of the erythrocyte membrane.The results obtained have shown that the extract under study is rich in polyphenols, mostly flavonoids, inhibits membrane oxidation induced by UVB radiation, UVC and AAPH. It also causes a decrease in membrane fluidity and packing order of the polar heads of membrane lipids.
This work was sponsored by the Ministry of Science and Education, sci. project no. NN304 173840 and NN312 422340.

P-47

Red Blood Cell Membrane Properties Influence Shape Transition Critically

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Upon moderate acceleration in capillary flow red blood cells (RBC) transit from their biconcave discocyte rest shape to an axisymmetric parachute shape with an intermediate slipper-like shape. Those shape transitions have been described as continuous phase transitions and are governed on the one hand by membrane viscoelastic properties and on the other hand by the imposed flow forces. From a physiological point of view shape transitions reduce the slip velocity and by this lower flow resistance. In addition changing shapes supports tank-treading ability of the RBC membrane.
In our experiments we identified the slipper shape as a steady and not purely transient dynamic shape and define two critical velocities to characterize the discocyte to slipper and the slipper to parachute transition, respectively. Additionally we selectively increase the RBC membrane properties by applying formaldehyde and diamide, respectively. These modifications affect the critical velocities by an increasing number of cross-links in the cytoskeleton. Furthermore we use cholesterol to change the lipid area of the membrane. We then compare these model systems to real anemia such as hereditary spherocytosis. In several widespread diseases deformability is altered and causes critical symptoms because oxygen transportation efficiency is affected.

P-48

Modulation of cone photoresponse by whole-cell delivery of zGCAP3 and its monoclonal antibody

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The physiological function of guanylate cyclase-activating protein 3 (zGCAP3) was investigated in zebrafish cones by recording the effect on the photoresponse by cytosol injection of exogenous zGCAP3 and its monoclonal antibody. These proteins were delivered via whole-cell by an internal perfusion system coupled to a pressure-polished patch pipette. Whole-cell recordings had stable light sensitivity, dark current amplitude, response kinetics and light adaptation. The rising phase of the response to saturating flashes was particularly fast (current fell to 0 within ∼12 ms), while the recovery phase of the response to sub-saturating flashes was monotonic or biphasic suggesting the existence of two types of cones having similar spectral sensitivity. Injection of anti-zGCAP3 produced current fall to zero level in ∼5 min, and progressively slowing down kinetics of responses delivered on decaying current; however, control antibody gave similar results as anti-zGCAP3. Purified zGCAP3 did not alter the photoresponse, indicating that the target GC was already saturated with endogenous zGCAP.

Abstracts

– Cell Biophysics and Signaling –

P-49

Unraveling the role of neuron sub-cellular mechanical properties in Traumatic Brain Injuries

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During violent head shocks, neurons can be severely damaged, see their very complex interactions with extracellular matrix, other neurons and glial cells highly modified and their function impaired. Based on the assumption that cell deformation is the initiating event of Traumatic Brain Injuries (TBI), we investigate the mechanical properties of neurons at the sub-cellular level (soma vs axon).
In this way, we developed a new method to control and tune separately matrix stiffness, cell shape and protein type and density, which are major environment factors in neuron function and mechanotransduction. In addition, we developed a magnetic tweezers set-up to probe the neuron mechanical behaviour in response to a strain exerted at the sub-cellular level (axon vs soma).
Taking advantage of these techniques, we studied the mechanosensitivity of neurons deposited on laminin stripes on substrates of stiffness ranging from 3 to 500 kPa. We show that soma and axon structures behave differently when mechanically stressed but also that axon is the mechanosensitive part of the neuron. We explain these differences by differences in cytoskeletal organization and composition through pharmacological treatments and immunostaining experiments. These events can explain diffuse axonal injuries in TBI

P-50

Cytotoxic properties of ferrocenes in cancer cells are related to the generation of oxidative stress

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Bioorganometallic chemistry is a branch of science who carries out the synthesis of organometallic compounds which can be potential anticancer drugs, e.g. ferrocene derivatives. Chemical stability, easy chemical modification and strong redox properties of these compounds make them attractive potential chemotherapeutic agents.
The aim of this study was to investigate the effect of ferrocene derivatives (15 and 15CL) on the generation of reactive oxygen species (ROS) in HepG2 liver cancer cells and the role of ROS production in cell cytotoxicity. Oxidation-sensitive fluorescence dye H_2DCF-DA was used to measure ROS production. HepG2 cells were exposed to 20, 60, 100 μM of 15 and 15CL. After 0.5, 3 and 6 h of exposure, fluorescence was measured.
Ferrocenes caused the increased intracellular generation of ROS in a dose time-dependent manner. Pretreatment of cells with ROS scavengers N-acetyl cysteine (3mM) ??and vitamin E (50μM) decreased significantly ROS level. Our results show that the cytotoxic properties of the investigated ferrocene derivatives are related to their oxidative properties and ROS generation in cells. Greater effects were observed for CL-substituted derivative 15CL.

P-51

Nitroxides as modulators of oxidative stress generated by anticancer drugs

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Breast cancer is the most common cancer among women in Europe. The most effective therapy is based on the combined effects of doxorubicin and taxanes, which carries a number of undesirable side effects such as generation of reactive oxygen species (ROS) in normal cells, e.g. cardiomyocytes. Excessive generation of ROS causes oxidation of lipids, protein and DNA. In connection with this fact scientists are still searching for effective methods of adverse effect reduction. It is believed that nitroxides may exhibit this property.
The purpose of this study was to identify parameters related to oxidation of cellular components in breast cancer cells (MDA-MB-231) exposed to anticancer drugs doxorubicin and docetaxel and to evaluate the effect of pyrroline nitroxide derivative Pirolin on ROS generated by these drugs. After 1h -preincubation with PL, MDA-MB-231 cells were treated for 2h with IC_{50} concentration of DOX, DTX or DOX-DTX. Then the cells were incubated for 3, 12, 24, 42, 72 h in fresh medium and the resultant cell lysates were used for analysis of thiol groups and total antioxidant capacity.
We have found that Pirolin alone did affect the investigated parameters. However, preincubation of cells with Pirolin enhanced changes induced by doxorubicin.

P-52

Quercetin exerts its antitumoral effect while manifesting a strong antioxidant character

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We found previously that the flavonoid quercetin (QC) is a strong inducer of apoptosis in human leukemia Jurkat T cells and an activator of the ryanodine receptor/Ca^{2+} release channel. Here we show that exposure to QC for 1 h reduces significantly the mitochondrial level of superoxide anion and the cellular content of H_2O_2, and prevents the extensive increase in the H_2O_2 level induced by menadione. However, QC decreased clonogenic survival in a dose-dependent manner and reduced significantly the clonogenicity after exposure to menadione. By using dantrolene, a ryanodine receptor inhibitor, we found that inhibition of Ca^{2+} release protects cells against QC-induced cell death. In addition, QC manifested a biphasic effect on the mitochondrial transmembrane potential, by first inducing near complete depolarization of the mitochondria, which then entered a persistent hyperpolarized state. Menadione induced a gradual depolarization of the mitochondria, whereas the combination QC-menadione elicited a depolarization kinetic pattern that was intermediate between the two traces corresponding to the agents applied alone. **Acknowledgements**. This work was supported by a grant of the Romanian National Authority for Scientific Research, CNCS - UEFISCDI, project number PN-II-ID-PCE-2011-3-0800.

Abstracts

– Cell Biophysics and Signaling –

P-53

A fluorescence anisotropy study between gentamicin, kanamycin, amikacin and cell membrane or the liposomal membrane

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Aminoglycosides are hydrophilic, polycationic, amine-containing carbohydrates that are binding both to the anionic outer bacterial membrane and to anionic phospholipids in the cell membrane of mammalian renal proximal tubular cells.Aminoglycoside antibiotics, largely used in infections with Gram-negative bacteria, are known to induce a pronounced nephrotoxicity and ototoxicity. The mechanisms by which these antibiotics interact with cell membrane are not yet fully understood.Our aim was to study the modification of membrane fluidity induced by these antibiotics.We tested the effect of 3 aminoglycosides:gentamicin, amikacin and kanamicin on artificial (liposomes) and natural membranes (opossum kidney[OK] epithelia cells).Liposomes prepared from dimyristoyl-phosphatidylcholine (DMPC) mixed with cardiolipin, which mimick the heterogeneous charge composition of the natural cell membrane, were used.Membrane fluidity was assessed using fluorescence spectroscopy recordings on TMA-DPH labeled liposomes and OK cells.The fluorescence anisotropy of liposomes was increased at all temperatures (15-35^{0}C) in the presence of all antibiotics, especially kanamicin. In conclusion both membrane models and cell membrane showed a decreased membrane fluidity in the presence of the applied antibiotics.

P-54

Wharton Jelly human Mesenchymal Stem Cell migration on noisy nanograting

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Securing cell adhesion and polarization between biomaterials and cells, and driving the migration is one of key considerations for tissue engineering and regeneration. However, the cell–substrate interaction and the effects induced by topography has only been recently reported. Wharton Jelly human Mesenchymal Stem Cells (WJ-hMSC) are an attractive source to use in tissue engineering and tissue regeneration due to their ability to migrate, proliferate and differentiate. In this study we observed the cell morpho-functional properties induced by thopography by using nanopatterns composed by ridges and grooves with 500 nm width and 350 nm deep, enriched with an increasing percentage of noise, in order to break the anisotropy. This study demonstrates that the localization of FA on well-defined nanogratings is critically determined by the topographical variation present in the nanopattern and that, their features influence strongly the morfological cell properties and the migration features.

P-55

Cell mechanics in the context of confluent monolayers

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Epithelial cells are exposed to a number of mechanical challenges such as osmotic stress or forces exerted by the cytoskeleton or by the extracellular environment. The question arises how polar epithelial cells respond to stimulated interference in the context of a confluent monolayer. How do epithelial cells respond to changes in cortical and membrane tension by surface area regulation if challenged by diverse mechanical cues? We answer this question by provoking changes in tension using external stimuli directed towards the contractile actomyosin cortex (cytochalasin D, blebbistatin) and excess surface area by cholesterol extraction (MBCD). A combination of site-specific AFM-indentation experiments with membrane-tether pulling allowed us to simultaneously monitor changes in membrane tension and excess surface area at the same position. Generally, we observed that membrane tension is readily adjusted by sacrificing or producing excess surface area of the plasma membrane. We also found that isolated apical membranes from confluent MDCK II monolayers display identical mechanical properties as the apical side of living MDCK II cells in a confluent monolayer confirming that membrane mechanics in conjunction with cytoskeletal adhesion governs the elastic response.

P-56

Biological activity of selected anthocyanins toward the lipid and erythrocyte membrane

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Anthocyanins are flavonoids that endow the plants with colours. They possess antioxidant, antibacterial, antivirus properties; and are helpful in treating cancer. Though the spectrum of anthocyanins action is very broad, the molecular mechanism of their interaction with living organisms on the cell level has not yet been fully explained. The study comprised: *Oenin chloride* (Malvidin-3-O-glucoside chloride), *Kuromanin chloride* (Cyanidin-3-O-glucoside chloride), *Myrtillin chloride* (Delphinidin-3-O-glucoside chloride), which occur e.g. in fruit of bilberry and blackcurrant. In the study, it was determined the anthocyanins' antioxidant activity and location within the lipid and erythrocyte membrane, using the fluorimetric method with various fluorescent probes and observing the shapes of erythrocytes under a microscope.

The results obtained indicate that the anthocyanins perfectly protect the erythrocyte membrane against oxidation, locating mainly in the outer part of the membrane, significantly decreasing the packing order of the polar heads of lipids and inducing echinocytes. However, they do not affect membrane fluidity, and thus do not penetrate the membrane hydrophobic region.

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Abstracts

– Cell Biophysics and Signaling –

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Signaling pathways and messenger crosstalk in plants triggered by high and low temperatures

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The high and low temperatures trigger in plants the signaling processes coupled with formation of secondary mediators. In the present work the cytoplasm crosstalk among messenger systems of calcium ions, red light, cyclic GMP, H_2O_2 and NO is considered. The data are brought forward to show the changes in $[Ca^{2+}]_{cyt}$ are registered after cGMP introduction into plant cell, red light induces Ca^{2+} entry into the cytoplasm from outer space, NO increases guanylate cyclase activity and rises $[Ca^{2+}]_{cyt}$. Besides the messenger activity, under nanomolar concentrations NO itself acts in plant cell as a cytoprotector-antioxidant. A number of protein specific binding sites for cGMP were identified in the cytoplasm. Their affinities for cyclic mononucleotide are controlled by Ca^{2+}/calmodulin, GTP and red light. Two temperature-triggered signaling pathways in plant cell accompanying by $[Ca^{2+}]_{cyt}$ increase are considered. First one is developed directly just after temperature action and is provided by the opening Ca^{2+} channels and second one functions via NO and cGMP located in a pathway upstream of $[Ca^{2+}]_{cyt}$. The general scheme of signaling events in plant cell to the temperature action is discussed.

P-58

The effect of glyphosate, its metabolites and impurities on human acetylcholinesterase activity

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Glyphosate [*N*-(phosphonomethyl)glycine] is used all over the world to protect agricultural and horticultural crops and it's not safe as it had been considered before. Poisonings still pose a challenge and problems for toxicological investigations.

That is why we investigate the effect of the most popularly used pesticide: glyphosate, its metabolites: aminomethylphosphonic acid and methylphosphonic acid, impurities: *N*-(phosphonomethyl)iminodiacetic acid, *N*-methylglyphosate, hydroxymethylphosphonic acid and bis-(phosphonomethyl)amine on acetylcholinesterase (AChE) activity present in human erythrocytes membrane (*in vitro*). The analysis of noxious effects of metabolites and impurities seems to be very important to evaluate the toxicological risk that is exerted by these substances (EU regulations 1107/200/EC).

The erythrocytes were incubated with xenobiotics at concentrations of 0.01 to 5 mM for 1 and 4 h. Glyphosate, its metabolites and impurities were not able to inhibit AChE activity, in contrast to organophosphorous compounds. Statistically significant decrease in the activity of AChE (about 20%) were observed only for very high concentrations of these compounds (0.25–5 mM).

P-59

Bacterial chemotaxis in chemical gradients created in a flow-free microfluidic device

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Motility helps bacteria explore spatially heterogeneous environments. By chemotaxis bacteria constantly detect the concentration gradient of chemoeffectors and make „decisions" on the net direction of movement. The technology of microfluidics is suitable for precise manipulation of liquids in microscopic dimensions in creating devices to generate stable and well-controlled chemical gradients. We have fabricated and experimentally characterized a microfluidic device that creates temporally stable chemical concentration gradient in a flow-free environment. The majority of concentration gradient generating devices are based on mixing of chemical species by the help of laminar flow, our design is a novel conception eliminating the disturbance of fluid flow. Thus bacteria in our devices swim in a static but chemically heterogeneous environment. We have studied the chemotactic response of *E. coli* to several substances. We tested some well-known attractants and repellents, measured the effect of conditioned media, cell-cell signalling molecules and antibiotics. In this device we are able to observe the behavior of the culture for an extended period of time (∼24 h) therefore we can study small variations in chemotaxis in various chemical environments.

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Measurement of weak interactions between adhesion molecules by means of colloidal probe microscopy

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Weak interactions displayed on cell surfaces play a pivotal role in cell adhesion, cell development, wound healing, tumorgenesis, and tissue formation. Other important interactions comprise proteins in docking or fusion processes. In order to get more information about such processes, we have to mimic native cell-cell contacts and the contact between vesicles and cells. For this reason carbohydrate recognition and synaptotagmin - PIP_2 interaction processes were explored by colloidal probe microscopy. An *in situ* coupling of the carbohydrate to membrane-coated surfaces was employed to functionalize the solid supported lipid bilayer with sugar moieties and the dynamic strength of the homomeric self-association was measured as a function of calcium ions and loading rate. A deterministic model was used to estimate the basic energy landscape and number of participating bonds in the contact zone.

Abstracts

– Cell Biophysics and Signaling –

P-61

Bacterial sensor domain that recognizes its cognate signals in the monomeric state

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Bacteria respond to environmental signals by means of three different protein systems, the most complex of which, the chemoreceptor system, allows these organisms to mediate chemotaxis towards or away from particular components. We present here the characterization of PctA, PctB and PctC, three different amino acid chemoreceptors from the ubiquitous and human patogen bacteria *Pseudomonas putida*, by analytical ultracentrifugation (AUC), isothermal titration calorimetry (ITC) and differential scanning calorimetry (DSC), in combination with hydrodynamic modeling.
Enterobacterial responses towards aminoacid are mediated by chemoreceptors that possess a 4-helix bundle LBR. These domains are shown to stabilize and activate the receptor as dimers upon recognition of their cognate ligands. The ligand profile of the *P. aeruginosa* receptors was uncovered by ITC, and DSC studies showed that ligand recognition stabilizes PctA, PctB and PctC LBR's. However, unlike enterobacterial chemoreceptors, *P. aeruginosa*'s adopt a "double PDC" domain that maintains the monomeric conformation upon activation by their cognate aminoacids. AUC studies in combination with hydrodynamic models allowed us to confirm also the structural conformation predicted by homology modeling of these chemotaxis receptors.

P-62

Biophysical characterization of human mesenchimal stem cells

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Human mesenchymal stem cells (hMSC) are able to self renew or to be committed towards cells of various lineages, such as osteoblasts. For this reason they have acquired a promising role in the field of regenerative medicine, even if major limitations to their clinical application to repair bone defects exist due to the poor availability of cells and the time required to differentiate to a stage suitable for implantation. Physical stimuli, such as pulsed electromagnetic fields (PEMFs), have been widely used in orthopedics and, at the moment, PEMF therapy is approved for bone disorders. The mechanism through which PEMF promotes the formation of bone remains however elusive. Our activity focused on the *in vitro* characterization of the molecular mediators of the effects of PEMF treatment on osteogenic differentiation of hMSC. In particular, through a combined biochemical and biophysical approach, we concentrated on the role of calcium, highlighting a potential role of intracellular calcium stores in the PEMF-induced differentiation pathway.

P-63

Single cell adhesion on different multifunctional substrates: a new quantitative approach

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The attachment of cells to other cells plays a key role in many biological and pathological processes and the study of its properties is fundamental for the organization in multicellular organisms.
A better knowledge of the affections that influence the adhesion capability of cells in several pathologies, could enable the development of new therapeutical strategies.
We introduce a method that allows the investigation of cell adhesion at the single cell level providing the capability to test the adhesion of a single cell on multifunctional substrates. We applied single cell force spectroscopy (SCFS) on custom designed molecular patterns prepared by using a cantilever based technique and we tested the adhesion of Chinese Hamster Ovary cells and Human Embrionic Kidney cells on two polyelectrolytes that are widely used as adhesive factors for cells growth: Polyethylenimine and Poly-D-Lysine [1]. Our results confirm the hypothesis on the mechanism of adhesion promotion by protonated molecules. The technique here introduced could be extended to test the adhesion on an unlimited number molecular species and could be employed also in the study of cell-cell adhesion.
[1] Canale C., Petrelli A. et al., Biosensors and Bioelectronics (2013) in press (available on line)

P-64

Membrane binding and mobility of the A-kinase anchoring protein AKAP18δ

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AKAP18δ is a member of the A-kinase anchoring protein family of scaffolding proteins that orchestrates the acute regulation of body water balance. It is located on aquaporin-2 (AQP2)-bearing vesicles and binds both protein kinase A and phosphodiesterase to spatiotemporally determine cellular signaling events. We defined a hitherto unobserved mechanism through which AKAP18δ can anchor to membranes via fluorescence correlation spectroscopy and the use of different AKAP18δ variants. The affinity is exclusively provided by electrostatic attraction of amino acid residues, and although they are distantly located on the sequence, they concentrate in the tertiary structure to form a binding surface **(1)**. High-speed atomic force microscopy permitted us to directly "watch" single AKAP18δ molecules at sub-molecular and sub-second temporal resolution in Brownian motion on top of lipid bilayers. We propose that the sharp dependence of AKAP18δ's mobility on the membrane composition we observed contributes to the specific recruitment of the AKAP18δ-based signaling module to membranes such as those of AQP2-bearing vesicles.
(1) A. Horner, F. Goetz, R. Tampe, E. Klussmann, P. Pohl. 2012. Mechanism for targeting the A-kinase anchoring protein AKAP18d to the membrane. J. Biol. Chem. 287: 42495-42501

Abstracts

– Cell Biophysics and Signaling –

P-65

Signal Transfer within Transmembrane Protein Complexes Probed by FT-IR Difference Spectroscopy

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The communication within the biomembrane is essential for many vital processes in any cell but little understood on the atomistic level. In this work, we investigated the molecular basis of the signal transfer between two membrane proteins. The target was the light-sensitive transmembrane complex between sensory rhodopsin I (SRI) and its cognate transducer (HtrI) from the archaeon *Halobacterium salinarum*. The photoreceptor SRI belongs to the family of seven-transmembrane proteins which carry all-*trans* retinal as chromophore. In the cell membrane, SRI is tightly bound to a second transmembrane protein HtrI, to form a functional complex. The SRI/HtrI complex functions as a color-sensitive molecular machinery to trigger the movement of the flagellated cells towards favourable or away from harmful light conditions (phototaxis). The atomic structure of the SRI/HtrI complex has not been solved yet and, therefore details on the interaction which defines the binding site between receptor and transducer are missing. Here, we applied FT-IR difference spectroscopy on SRI / HtrI mutants to demonstrate that at the cytoplasmic-proximity of the membrane, Tyr210 of SRI and Asn53 of HtrI interact via a hydrogen bond which is crucial for signal transfer from the receptor to its transducer.

P-66

Raft Localization, Microdomain Confinement are Reversed During LH receptor Desensitization

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W examined the involvement of membrane microdomains during luteinizing hormone receptor (LHR) recovery from desensitization. Single particle tracking experiments showed that untreated individual LHR were confined within cell-surface membrane compartments (199 ± 17 nm) and associated with the bulk plasma membrane. After brief human chorionic gonadotropin (hCG) exposure, LHR remained for several hours desensitized to hCG challenge and were confined within smaller diameter (<120 nm) membrane compartments and associated with rafts. By 5 hours, when hCG challenge again resulted in elevated cAMP, unoccupied LHR were found in larger 169 ± 22 nm diameter membrane compartments and >90% of LH receptors were in the bulk plasma membrane. Thus, during recovery from LHR desensitization, LHR are located with rafts and confined within mesoscale (80-160 nm) cell-surface compartments. This may reflect hormone-driven translocation of receptors into rafts and formation there of protein aggregates too large or rigid to permit signaling. Once bound hormone is removed, receptor structures must dissociate before LHR can signal effectively in response to hCG challenge. Moreover, such larger protein complexes are more easily constrained laterally and so appear resident in smaller membrane compartments.

P-67

Ionizing irradiation causes rapid activation of K+ channels in A549 cells and lymphocytes

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The focus of the present work is to investigate the very early irradiation-induced cellular effects and potential entrance points into radiation induced signal transduction cascades. Because of their relevance in human physiology it is hence crucial to understand the sensitivity of ion channels to ionizing irradiation. Our data show that ionizing irradiation with x-ray as low as 0.1 Gy causes in the epithelial lung cancer cell line A549 an immediate increase in the conductance of two K^+ channels. This includes an activation of hIK type channels, e.g. channels, which are effective in cell cycle control. We found that the same stimulation of channel activity, which occurs after irradiation, was also induced in response to a treatment of A549 cells with H_2O_2 and upon elevation of the cytosolic Ca^{2+} concentration. The results of these experiments imply that irradiation generates in cells a rapid increase in reactive oxygen species and in the cytosolic concentration of Ca^{2+}. The data imply that irradiation could affect cell-cycle control by activating certain K^+ channels via a membrane hyperpolarization. In addition we find that the same type of channel is activated in resting lymphocytes after low-dose irradiation smaller 1 Gy x-ray.

P-68

Biophysical characterization of Neurospora crassa membrane and its sensitivity to Staurosporine

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N. crassa is a filamentous fungus widely used as a model multicellular eukaryote. Staurosporine (STS) is a drug used to induce programmed cell death (PCD) in various organisms. In *N. crassa*, STS up-regulates the expression of the ABC transporter ABC-3, which localizes at the plasma membrane (PM) and is responsible for STS efflux[1]. To understand the role of PM biophysical properties in *N. crassa* and STS-induced PCD, we studied 3 strains (wild type (wt), the ABC-3 deletion mutant and slime, devoid of cell wall). Our results show that *N. crassa* possesses a highly ordered PM probably with distinct types of ordered domains. As conidia grow away from a state of latency, ordered domains decrease. In the slime strain, STS induces PM reorganization, leading to increased global rigidity at the expense of ordered domains. An analogous relation has been observed for *Saccharomyces cerevisiae* mutant vs. wt cells[2]. STS has distinct effects on wt and ABC-3 mutant strain. Overall, our results suggest important biological roles of the PM biophysical properties in *N. crassa* and that ordered domains might be involved in the mechanisms of STS-induced PCD. Supported by FCT grant PEst-OE/QUI/UI0612/2011;[1]Fernandes *et al.*2011. Fungal Genet Biol,1130; [2]Aresta-Branco *et al.*2011.J.Biol.Chem,5043

Abstracts

– Cell Biophysics and Signaling –

P-69

Determination of relations between lipid peroxidation, antioxidant defence and trace elements in COPD

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An imbalance between oxidants and antioxidants is proposed in the pathogenesis of COPD. The present study aimed to assess oxidative stress dependent lipid peroxidation, antioxidant enzyme activities and the role of the trace elements in enzyme activities and relation of them with defense system molecules were also searched in patients with COPD. 26 patients with acute exacerbation and 26 controls were studied. SOD,CAT,GSH,MDA levels were determined. The role of the trace elements in enzyme activities and relation of them with defense system molecules were also searched. Serum concentrations of Fe , Cu and Zn were determined. MDA was found to be statistically higher but GSH,SOD and CAT enzyme activity lower in RBC of patient than controls. While decreased Fe and Zn but increased Cu concentrations were determined in patient than that of controls. Results of this study indicate,there are alterations in serum concentrations of trace elements in COPD patients, suggesting that they may play a role in the pathophysiology of this disease by virtue of their role in oxidative stress. We recommend further studies on the role of trace elements in the pathophysiology of COPD, their association with markers of oxidant/antioxidant status and on the clinical significance of their deficiency.

P-70

Cell stiffening and softening evoked by optical stress application

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The mechanical properties of living cells are largely determined by the cytoskeleton, a complex bio-polymer network consisting of filamentous actin, microtubules and intermediate filaments. Cell-mechanical properties are meant to be of great importance in all kinds of tissue development (e.g. cancer). Commonly described as viscoelastic material, cells should be time translation invariant (TTI) in there mechanical response to a transient stress.
As cells are continuously restructuring their cytoskeleton, time translation invariance does not need to be fulfilled. We investigated the mechanical reaction of single suspended cells to a transient stress by using the optical stretcher, an optical divergent dual beam trap. There was no time dependence found in their mechanical behavior, after application of a small stress. By increasing the transient stress over a certain point we observed the cells to strongly stiffen directly after stress application, followed by softening back to its original value and even further. Additionally we found the characteristic times describing this behavior to be cell type specific.

P-71

The studies of natural antioxidants for capacity to protect organism from cell damage

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Erythrocyte membranes contain many polyunsaturated fatty acid moieties and they are susceptible to various oxidative stresses. Natural antioxidants are extensively studies for their capacity to protect organisms and cell from damage induced by oxidative stress, the latter being considered a cause of ageing, degenerative diseases. In a previous work we obtained results that shown how plant extract can influents on some electrical properties of bilayer of lipid membranes (BLM). After adding the plant extract in KCl, NaCl, $CaCl_2$ membrane washing liquids the value of membrane resistance changed accordingly on two, four, one orders. Transport of those through BLM pores discharge the membrane potential and durations tends to cause a dielectric breakdown of the BLM in presence of that plant extract. We were following of our investigations and studied the kinetic changes of hemolys curve under influence of plant extract. It was measuring the RedOx potential and pH of plant extract and calculating the value of rH, which pointed the degree of oxidation of our example. The definite of inhibition activity of plant extracts by products of LPO testify about a stage changes in a free radical processes in during oxidative stress. These results were statistical processing with MatLab.

P-72

Multi-ion sensor system for real-time ion transport monitoring – new tool for investigating cystic fibrosis mechanism?

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Cystic fibrosis (CF) is the most common lethal genetic disease caused by disturbed ion transport within cells is. In CF abnormal ion transport leads to increased viscosity of the mucus layer and chronic infections often leading to patient death. There are many contradictory hypotheses of the ion transport through epithelial cell layer and available measuring techniques give only the information about the total current flowing across epithelial cell monolayer, without possibility of distinguishing particular ion (K^+, Na^+, Cl^-, $HCO_3{}^-$) contributions.
In this work novel potentiometric system for real-time determination of K^+, Cl^-, Na^+ ions and pH in cell monolayer is described. The constructed electrodes were integrated with reference electrode in one system allowing direct measurement of the ion concentration changes in both apical and basolateral face of the cell monolayer grown on porous support. The described sensor system was successfully applied to in vitro studies of ion fluxes in human bronchial cells 16HBE14o-. For the first time particular ion concentrations in apical and basolateral layers has been monitored.
The work was supported by Ministry of Science and Higher Education Grant No 1828/B/PO1/2010/39.

Abstracts

– Cell Biophysics and Signaling –

P-73

DC-SIGN glycosylation regulates receptor spatiotemporal membrane organization and endocytosis

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An evolving concept in cell membrane biology concerns the intimate relationship between cell membrane organization and cellular function. Glycosylation, a post-translation modification found in virtually all membrane proteins has been described to modulate receptor membrane organization and functions. However, the molecular mechanisms governing these processes are still unknown. We studied how the single *N*-glycosylation of the pathogen recognition receptor DC-SIGN affects its spatiotemporal membrane organization using a variety of single molecule approaches. DC-SIGN de-glycosylation was shown to decrease receptor membrane lateral diffusion. At larger spatial scales (μm scale), glycosylated DC-SIGN was corralled into particular membrane areas, which decreased receptor encounter with regions of transient confinement (TCZ). Dual-colour SPT measurements revealed that TCZ were mainly due to DC-SIGN association with clathrin. This was further confirmed by the co-localization of clathrin with gp120-coated beads bound to DC-SIGN. Overall, we show that DC-SIGN membrane organization, dynamics and antigen internalization are highly dependent on its glycosylation state, highlighting the crucial role of glycans in the modulation of membrane spatiotemporal arrangement.

P-74

Nanoscale EGFR separations in cells using fluorophore localisation confidence intervals

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The human epidermal growth factor receptor (EGFR) is a key target for anti-cancer therapeutics. EGFR activation is triggered by epidermal growth factor binding and involves dimer formation. It may also be mediated by extra- and intracellular domain conformational changes and by clustering within plasma membrane domains, both of which remain poorly understood. This is largely because current methods have insufficient resolution in cells to report changes in receptor structure or to distinguish EGFR dimers from confinement within lipid rafts or 'fences'. Distinguishing dimerisation from clustering depends on determining separations in the 10-80 nm range in cells. Our method quantifies individual separation confidence intervals and uses them to determine the number of separations present in single molecule fluorescence images and their values. In common with other single molecule separation fluorescence techniques, it relies on the positional shift of the centroid of a fluorescence spot when one of the two molecules in it photobleaches, but is better suited to measurements in the plasma membrane of intact cells. We have used it to investigate the conformation and supra-molecular organisation of wild-type EGFR and a range of EGFR mutants permanently expressed in mammalian cells.

Abstracts

– Chemical and Synthetic Biology –

O-75

Chemoselective transformations for bioimaging and targeted therapeutics

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Covalent protein modification is a key instrument in Chemical Biology: attachment of biophysical probes enable protein tracking and imaging; attachment of lipids to small therapeutic peptides extends their half-life in circulation; and protein conjugates are important biologic targets for the treatment of human diseases. In addition, proteins are modified after translation, increasingly significantly their structural complexity and functional capability. The use of efficient, complete, chemo- & regioselective methods in benign aqueous systems offers ways to redesign the structure and function of proteins of biological and therapeutic interest.
This abstract features examples of (a) precise and controlled methods for site-specific protein labeling *in vitro* and *in vivo*[1,2]; (b) development of chemically-defined, traceless therapeutic proteins for cancer therapy[3,4] and (c) controlled delivery of therapeutic CO using chemically-defined carbonyl metalloproteins[5,6].
[1] G. J. L. Bernardes *et. al.*, *J. Am. Chem. Soc.*, 2008, **130**, 5052-5053; [2] J. M. Chalker *et. al.*, *Acc. Chem. Res.*, 2011, **44**, 730-741; [3] G. J. L. Bernardes *et. al.*, *Angew. Chem. Int. Ed.*, 2012, **51**, 941-944; [4] M. Steiner *et. al.*, *Chem. Sci.*, 2013, **4**, 297-302; [5] C. C. Romão *et. al.*, *Chem. Soc. Rev.*, 2012, **41**, 3571-3583; [6] T. Santos-Silva *et. al.*, *J. Am. Chem. Soc.*, 2011, **133**, 1192-119.

O-76

Turning Bacillus subtilis into a methylotrophic terpenoid synthesizing cell

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Due to its amazing synthesizing capacity *B. subtilis* is the host of choice for the production of enzymes and vitamins representing 2 million tons of fermentation broth every year. Both classical mutagenesis and metabolic engineering have resulted in a wealth of genetic tools that are being used to build efficient cell factories for small molecules (riboflavin) and secreted enzymes. All industrial *B. subtilis* fermentations are glucose based fed-batch processes. A switch to methanol as substrate would not only stop the competition with the food value of sugars, but could also lead to a very significant cost reduction. Recently obtained knowledge on the C1-assimilation pathway from the related methylotrophic *B. methanolicus,* renders *B. subtilis* into an ideal starting point for a synthetic biology endeavour of creating a versatile methanol cell factory. C1- assimilation genes have been functionally expressed in *B. subtilis* resulting in the incorporation of methanol into the ribulose monophosphate pathway (RUMP). One of the end products of RUMP is pyruvate, which is in *B. subtilis* the starting compound for the methyl-erythritol phosphate (MEP) pathway leading to isoprene. On its turn isoprene is the general precursor for terpenoids, which form a functionally and structurally highly varied group of natural products including numerous medicines. In this study the effect of introducing several essential MEP pathway genes was investigated and it was shown that various carotenoid compounds novel to *B. subtilis* can be produced efficiently.

O-77

Stabilization of FMN-dependent NADPH:dye/quinone reductase from Pseudomonas putida by directed evolution

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Thermostability is a key parameter in bioprocesses involving enzymes. Consequently, improving the stability and understanding the mechanisms that confer enzyme stability is a major objective of many protein engineering studies. PpAzoR is a FMN-dependent NADPH:dye/quinone reductase from *Pseudomonas putida*, showing broad substrate specificity making it very attractive for bioremediation, however, its thermal stability represents a serious drawback with a half life of 13 min at 50°C. Therefore directed evolution approaches were followed in order to improve PpAzoR thermostability. A combined strategy of error-prone PCR and DNA shuffling was used to generate mutant libraries, which were screened to identify enzymes with increased thermostability. Five rounds of mutagenesis/recombination and screenings (≈ 10,000 clones) yielded 26 improved variants. Characterisation of the best hits and X-ray crystal studies provide a clear distinction between 1) mutant enzymes with an increase thermal unfolding temperature and thus with increased optimal temperature and 2) mutants that hardly suffer irreversible denaturation through aggregation. Therefore two sets of mutations were clearly identified, those that increase the thermal stability of the native state and those that prevent aggregation of the unfolded state.

O-78

Manufacturing vesicles with internal bilayer partitions: a novel unit for synthetic biology

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Vesicles serve crucial functional roles as models of artificial cells, as drug-delivery vehicles, and as
models to gain insights into the biophysical behaviour of membranes. To date, manufacturing strategies have focused on uni-compartment structures, resulting in vesicles with homogenous internal contents. This imposes certain limitations on their use as models of biological systems, which by their nature are not homogenous and have a complex spatio-dynamic organisation. We bridge this divide by fabricating networked multi-compartment vesicles, with spanning bilayers segregating the vesicle into distinct regions. These were generated by encasing multiple water-in-oil droplets with an external bilayer, using a process of gravity-mediated phase-transfer. We were able to exert control over the content of individual compartments, and could define the vesicle architecture. In addition, by inserting protein pores, material transfer between the compartments was achieved, and the possibility of inserting complex inter- and intra-vesicle network communication networks demonstrated. The membrane bound structures can be viewed as an enabling technology which allows the complex organization seen in real cells to be introduced into artificial ones for the first time.

Abstracts

– Chemical and Synthetic Biology –

O-79

Catalytic and spectroscopic characterization of two bacterial dye-decolourising peroxidases

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We have established catalytic and spectroscopic fingerprints of two recombinant DyP decolourising peroxidases from the soil bacteria *Bacillus subtilis* (BsDyP) and *Pseudomonas putida* (PpDyP). DyPs are a family of heme-containing peroxidases with wide substrate specificity and a high potential for biotechnological applications. The genes encoding BsDyP and PpDyP, belonging to subfamilies A and B, respectively, were cloned and heterologously expressed in *Escherichia coli*. The purified BsDyP is a single 48 kDa monomer (T_{opt} 20 – 30°C) whereas PpDyP is a 120 kDa homotetramer with a peculiar flat and broad temperature profile (T_{opt} 10-30°C). PpDyP exhibits higher activities and a wider scope of substrates, oxidizing with increased specificity high-redox anthraquinonic or azo dyes, phenolic, non-phenolic lignin units and manganese and ferrous ions. Our spectroscopic data suggest that subtle differences in the active sites might account for the distinct catalytic behaviour. In particular, resonance Raman spectroscopy reveals heterogeneous and distinct spin populations, with catalytically relevant 5-coordinated species being more abundant in PpDyP. Our results will guide future optimization of these biocatalysts towards their application in biodegradation and/or bioconversion processes.

P-80

CURVIGAMI – Minimal membrane curvature-inducing scaffolds

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Biological membranes are dynamic cellular barriers that suffer deformation and bending. Despite huge effort in identifying the general elements involved in membrane curvature, the physical-chemical basis of curvature induction is still poorly understood. Here we fill this gap by engineering a minimal curvature-inducing system. Due to its exclusive nanoengineering properties, DNA origami technology will be utilized to build minimal curvature-inducing scaffolds. This state-of-the-art technology enables the folding of long strands of DNA into nano-objects with defined shapes by using sequence-specific short DNA staples. Hybrid origami scaffolds with specific functional membrane-attachment groups bound at defined positions on the scaffolds will be produced. The interaction of our structures with lipid model systems will be studied and their capability of inducing membrane curvature evaluated. Fluorescence microscopy and atomic force microscopy methods will be give information on the extent, localization and forces involved in the interactions. At the end, this quantitative characterization of minimal membrane-inducing scaffolds will help understand the role of cooperativity in membrane deformation and the rules that govern the induction of membrane curvature.

P-81

Novel peptide biosurfactants for pharmaceutical formulation

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With the aim to develop biosustainable, environmentally-friendly surfactants for pharmaceutical and biomedical use, we have designed and synthesized peptidyl biosurfactants based on the dimeric plant defensin SPE10 (an antifungal isolated from *Pachyrrhizus* seeds). Molecular modelling studies were used to guide amino acid mutations in the defensin sequence such that the (disulfide-stabilised) folded peptides would possess distinct hydrophobic and hydrophilic surfaces and present as amphiphilic surfactants. Defensin and two engineered mutants (incorporating C-terminal His tags) were cloned into a modified pET-24b vector and transformed into *E. coli* Shuffle cells. Expression of the peptides in the whole cell lysates was demonstrated by immuno-blotting with an anti-His tag antibody and their identity confirmed by mass spectrometric analyses of the bands excised from SDS-PAGE gels.

P-82

Cytochrome P450 for the conversion of deoxypodophyllotoxin to podophyllotoxin

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Podophyllotoxin is a pharmaceutically highly relevant precursor for the anticancer drugs etoposide and teniposide. The natural sources of podophyllotoxin are limited and chemical synthesis is not economically feasible. The rhizomes of the abundantly growing plant *Anthriscus sylvestris* contain deoxypodophyllotoxin and in trace amounts podophyllotoxin.

The conversion of deoxypodophyllotoxin to podophyllotoxin was achieved by us with cytochrome P450 3A4, heterologously expressed in *E. coli* Dh5α. Cytochrome P450 3A4 is an external heme containing monooxygenase, which is mainly expressed in the human liver and gastrointestinal tract. Cytochrome p450 3A4 is responsible for detoxification and clearance of xenobiotics. Therefore this enzyme is capable of oxidizing a broad range of substrates. The spacious binding site has been shown to fit and metabolize at least 422 drugs.

Cytochrome P450 3A4 hydroxylates deoxypodophyllotoxin at the C-7-position to form stereoselectively the diastereomer of podophyllotoxin, epipodophyllotoxin, which can also be used as precursor for etoposide and teniposide.

Based on the crystal structure of cytochrome P450 3A4 (2.05 Å) a model was made for the docking of deoxypodophyllotoxin and epipodophyllotoxin into the enzyme. Future plans include the engineering of the enzyme towards higher efficiency.

Abstracts

– Chemical and Synthetic Biology –

P-83

The tumbleweed hub: characterisation and dynamics

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The Tumbleweed is a designed synthetic protein motor which has 3 dimeric coiled coils at its centre, which, when disulphide-bonded together, form a hub for the other motor components. This bottom-up approach to motor design allows us to exploit the complexity of protein building blocks, and through the process of building a synthetic motor we hope to gain insight on natural biological motors and their function. For this poster, the Tumbleweed's central hub is discussed.

We use FRET and other biophysical techniques, such as Circular Dichroism (for secondary structure and stability data) and Dynamic Light Scattering (for hydrodynamic diameters), to gain information on our coiled coil peptide hub system. FRET (Fluorescence or F?rster Resonance Energy Transfer) is highly dependent on the distance between the donor and acceptor molecules involved (the rate of energy transfer is inversely proportional to the separation to the 6th power), and hence is useful to look at the hub's dimensions, and variations in the separation between known locations in the system. This poster details the results we have gained using these techniques, and the conclusions we can draw from them about the structure and dynamics of the Tumbleweed hub.

Reference:
E H C Bromley et al., HFSP J. 3(3), 204-212 (2009)

Abstracts

– Molecular Motors –

O-84

Motors, gears and breaks of FoF1-ATP synthase monitored by single-molecule FRET

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Catalytic activities of enzymes are associated with elastic conformational changes of the protein backbone. F_oF_1-ATP synthase is a rotary molecular machine which catalyzes the formation of adenosine triphosphate (ATP). The *Escherichia coli* enzyme consists of a membrane-bound F_o motor where proton translocation through F_o drives a 10-stepped rotary motion [1]. An internal central stalk transduces the energy of this rotation to the F_1 motor where ATP is synthesized in an 120° rotary stepping cycle [2,3]. To prevent wasteful hydrolysis of ATP, F_oF_1-ATP synthase utilizes different autoinhibitory mechanisms including mechanical blocking of subunit rotation. These conformational changes can be monitored in real time by single-molecule Förster resonance energy transfer (FRET). The rotary mechanics of proton-driven F_oF_1-ATP synthase will be discussed and a single-molecule FRET approach to observe both rotations simultaneously in a triple-labeled single F_oF_1-ATP synthase[4] at work will be presented.

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[2] M. Diez et. al., Nature Struc. Mol. Biol. 11 (2004) 135-141.
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[4] S. Ernst et. al., J Biomed Opt 17 (2012) 011004.

O-85

The mechanism of energy transmission in F1-ATPase as revealed by molecular dynamics simulations

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FoF1-ATPase is a motor protein that synthesize ATP using the proton gradientas a free energy source. The proton flow through the membrane-embedded Fo generates the rotary torque that drives the rotation of the F1 asymmetric shaft. The energy of the rotating shaft is used by the catalytic subunit of F1 to synthesize ATP. The microscopic mechanism of this energy conversion is still not fully understood.

To investigate this mechanism we used atomistic MD simulations. Analysis of the rotational fluctuations revealed that the elasticity of the F1 shaft, as sensed by Fo, arises from two distinct contributions: the intrinsic elasticity and an effective potential imposed by the catalytic subunit. Separation of these two contributions provided a quantitative description of the dynamic coupling between the rotor and the stator and enabled us to propose a minimal model of the F1 energetics near the crystal structure resting state.

To directly study the energy transmission between the rotor and stator subunits of F1 during the catalytic cycle we employed a force-probe MD in which the central shaft is driven to rotate by externally applied torque. This approach allowed to propose the mechanism by which the rotating shaft induces a sequence of conformational changes at the active sites.

O-86

Membrane dynamics and cytoskeleton assembly in cell motility

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Many cell movements proceed via a crawling mechanism, where cytoskeleton assembly beneath the leading edge membrane pushes out the front of the cell to form a lamellipodia. The goal of our research is to study the interplay between membrane and cytoskeleton dynamics during motility. In a first part, we use a simple system of crawling cell motility, the *Caenorhabditis elegans* sperm cell, to probe the relationship between membrane tension and cytoskeleton dynamics, and find that increased tension enhances motility by increasing lamellipodia organization. We then turn to a simple model of cell motility *in vivo*, the ventral enclosure event of *C. elegans* embryogenesis, coupled with *in vitro* bead motility assays, to study how Ena/VASP proteins enhance lamellipodial protrusion. Based on results from this combined approach, we propose that VASP acts by retaining nascent filaments at the surface, thus potentially enhancing Arp2/3 complex activity, leading to an increase in actin network growth and motility. Overall these studies highlight how small alterations in the mechanical properties of the cell membrane or in cytoskeleton biochemistry have large effects on motility.

O-87

Correlating cargo transport with the cytoskeletal network at high resolution

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Intracellular transport plays an essential role on fundamental processes inside the cell. Motor proteins move cargo through the microtubule network to the required location of function. To understand how cargo-bound motors navigate and overcome the physical barriers set up by this complex network, we have developed a correlative imaging method based on single particle tracking and superresolution microscopy. This allows us to map the transport trajectories of cargos to individual microtubules with high spatiotemporal resolution. We use this method to study the behavior of lysosomes at microtubule–microtubule intersections. We conclude that microtubule intersections represent a source of hindrance for cargo transport that leads to long pausing periods when the separation is smaller than 100 nm.

These results give novel insights into the effect of the cytoskeletal geometry on cargo transport *in vivo* and have important implications for the mechanisms that cargo-bound motors use to maneuver through the obstructions set up by the complex cytoskeletal network. Furthermore, this method can also be used for a wide range of applications in biology, where putting dynamics into the context of nanoscale ultrastructural or molecular information is important.

Abstracts
– Molecular Motors –

O-88

Single molecule observation of protein translocation

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Bacterial protein secretion is mediated by the ATPase SecA that translocates proteins targeted for export through a protein-conducting membrane channel, termed SecYEG. The mechanism of protein translocation has so far mostly been addressed by conventional biochemical assays that involve ensemble averaging. Here, we present an *in vitro* protein translocation assay with single molecule sensitivity using surface-immobilized membrane vesicles. Using TIRF microscopy, formation of a specific protein translocation intermediate is observed by single molecule FRET. This assay represents a step towards the real time observation of single preproteins being translocated by the bacterial translocon.

O-89

Stepping Behavior of Rotary Molecular Motors

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A large number of biological processes are governed by the action of molecular motors, which convert chemical energy into mechanical motion. While the combination of structural data and single-molecule experiments have begun to uncover the nature and mechanisms underlying motor-protein function, achieving sufficient spatial and temporal resolution to resolve kinetics and discrete stepping behaviour of rotary motors remains a challenge. We are using a backscattering laser dark-field microscope to track gold nanoparticles attached to flagellar motors and F1-ATPase with sub-nanometer and microsecond resolution. We have found this setup to be useful for both in vitro and in vivo experiments due to low background scattering from cells and high back scattering from gold nanoparticles, allowing for arbitrary precision in localization, provided sufficient collected photons. We present preliminary data from the application of this technique in an attempt to characterise the ATP-binding, catalytic, and inhibited states of yeast F1-ATPase and the stepping behaviour of the bacterial flagellar motor.

P-90

Design and characterisation of a soluble variant of the flagella motor protein MotB

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The bacterial flagellum is a complex nanomachine that provides motility through its action as an ion driven screw propeller. The flagellum is an essential virulence factor of many bacteria, including *H. pylori*, where motility is required to initiate and maintain a robust infection of the human epithelial gastric cells resulting in diseases such as gastric cancer and duodenal ulcers. Motor rotation is governed by the stator complex, which includes motility proteins A and B ($MotB_2MotB_4$) and forms a proton conduction channel. An active stator MotB component anchors to the peptidoglycan layer and the cytoplasmic membrane simultaneously, *via* its C-terminal domain and the N-terminal transmembrane (TM) helix, respectively. A soluble chimeric variant of MotB was constructed by replacing the native transmembrane helical region with a GCN4 leucine zipper. Several techniques were used to characterise chimeric MotB including small angle X-ray scattering (SAXS). A full-length model of soluble MotB was derived from SAXS data using previously solved crystal structures of the C-terminal domain and the GCN4 zipper. This initial structural characterisation of the full-length protein will aid further understand the activation mechanism.

P-91

Investigating the coupling mechanism of complex I: Is a long amphipathic α-helix involved?

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Complex I plays a central role in energy transduction. It catalyzes the oxidation of NADH and the reduction of quinone, coupled to ion translocation across the membrane. The research on this enzyme has gained a new enthusiasm, especially after the resolution of the crystallographic structures of bacterial and mitochondrial complexes. Most attention is now dedicated to the investigation of the energy coupling mechanism.
In this work, we made a thorough investigation of complex I and group 4 [NiFe] hydrogenases and established a third member of this family of proteins: the energy-converting hydrogenase related complex. We observed that four subunits (NuoB, D, H and antiporter-like) are common to the 3 types of complexes and we have denominated these subunits as the universal adaptor.
We further explored the proprieties of the adaptor by investigating the structural characteristics of the antiporter-like subunit. We observed that the adaptor contains an antiporter-like subunit with a long amphipathic α-helix. The long helix is a common denominator that has been conserved through evolution. This should reflect a key role of such helix in the coupling mechanism of this family of enzymes. These findings are a step forward in the investigation of the coupling mechanism of complex I.

Abstracts

– Molecular Motors –

P-92

Kinesin KIFC1 actively transports bare double-stranded DNA

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Over the last years, exogenous DNA molecules have been used in gene and molecular therapy. These therapies imply the delivery of DNA into cells: at present, it is not known how these DNA molecules reach the cell nucleus. We used an *in-cell* single molecule approach to observe the motion of exogenous short DNA molecules in the cytoplasm of eukaryotic cells. Our observations suggest an active transport of the DNA along the cytoskeleton filaments. We developed an *in-vitro* motility assay, in which the motion of single DNA molecules along cytoskeleton filaments in cell extracts is monitored; we demonstrate that microtubule-associated motors are involved in this transport. Precipitation of DNA bound proteins and mass spectrometry analyses reveal the preferential binding of the kinesin KIFC1 on DNA. Cell extract depletion of kinesin KIFC1 significantly decreases DNA motion confirming the active implication of this molecular motor in the intracellular DNA transport. Moreover, using Fšrster Resonance Energy Transfer microscopy we showed the interaction between KIFC1 and DNA molecules.

P-93

Covalent cargo loading to molecular shuttles via copper-free "click chemistry"

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Molecular motors such as the motor protein kinesin are envisioned to drive future nanomachines. Powered by ATP consumption, kinesins actively propel microtubules as transporters in the well-established molecular shuttle system. In order to harness this biological transport system for the assembly of nanoscale hybrid devices, the development of adequate cargo loading strategies for molecular shuttles are an important prerequisite.
In this work, we introduce copper-free "click chemistry" represented by the strain-promoted azide-alkyne cycloaddition as a compelling cargo loading strategy. The bioorthogonal reaction is easy to implement in the motor protein driven transport of microtubules and has pronounced advantages over existing cargo loading strategies. In addition, it offers the possibility to combine with one or more of the existing approaches for multiple and orthogonal cargo loading.
Copper-free "click chemistry" is a most useful conjugation method for many kinds of motility assays especially for cargo loading on actively propelled microtubules to design nanoscale biosensors or assembly lines.

P-94

Simulations of neck linker modified and one head loaded kinesin

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Recently, Czovek et al. established a complete, thermodynamically consistent kinetic model for the two-headed homodimeric motor protein, kinesin. Computational simulations based on the model justified the crucial role of the conformational changes of the neck linkers (NLs, the peptide chains connecting the two motor domains to the stalk) in the directional movement and force-generation of conventional kinesin. The model was able to reproduce a large number of experimental data (speed, dwell time distribution, randomness, processivity, hydrolysis rate, etc.) astonishingly well under normal as well as under highly unphysiological conditions. Moreover, it enabled a more detailed deconvolution of the mechanochemical cycle than it is experimentally possible. Having such a powerful model, we have applied it to modified versions of the wild-type kinesin, and reproduced (i) the speeds, processivities, and ATP consumption rates of NL modified kinesin; and (ii) the force-velocity relationship of the one-head-pulled kinesin. The good agreement between the simulations and the experiments further justify the legitimacy of the model, which thus provides a detailed understanding of the experimental observations and the basic mechanism of the operation of kinesin.

P-95

Effect of phosphorylation on the motility of the biological nano motor Cin8

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Saccharomyces cerevisiaes Cin8, a member of the kinesin-5 family of motors that performs important functions in mitotic spindle dynamics such as spindle assembly and anaphase spindle elongation. Resent work has shown that Cin8 is a bidirectional motor and moves *in vitro* towards the minus-end of microtubules (MTs) and changes directionality as a function on ionic strength conditions and MT binding geometry (Gerson-Gurwitz et al., 2011). Previous work from our laboratory had also indicated that Cin8 is differentially phosphorylated during late anaphase at three cyclin-dependent kinase 1 (Cdk1) specific sites located in its motor domain. *In vivo,* this phosphorylation causes Cin8 detachment from the spindles, reduces spindle elongation rate and aids in maintaining proper spindle morphology (Avunie-Masala et al., 2011).
Here, we examined the motile properties of Cin8 by a single-molecule fluorescence motility assay. To study the effect of phosphorylation, we examined the activity of phosphorylation-deficient and phosphorylation-mimic mutant of Cin8. We found that addition of negative charge in the phospho-mimic mutant weakens the MT-motor interaction and affects the motile properties of Cin8. Results will be presented.

Abstracts

– Protein Folding, Assembly and Stability –

O-96

Protein conformational dynamics and molecular recognition in folded and unfolded proteins by NMR

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Proteins are inherently dynamic, exhibiting conformational freedom on timescales from picoseconds to seconds, implicating structural rearrangements essential for function. NMR is sensitive to conformational fluctuations occurring up to the millisecond and we have recently developed methods to quantitatively describe motions occurring in proteins on timescales from picoseconds to millisecond that can be used to study the role of protein dynamics in molecular recognition. [1–4]

Intrinsically disordered proteins (IDPs) represent extreme examples where protein flexibility plays a determining role in function. The development of meaningful descriptions of the behaviour of IDPs is a key challenge for structural biology.[5,6] We present approaches to determine structural behaviour in IDPs from NMR data, and apply these to describe the formation of dynamic complexes in viral IDPs.[7–9]

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2. Markwick et al *J.Am.Chem.Soc.* 131, 16968, 2009
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6. Jensen et al *J.Am.Chem.Soc.* 130, 8055, 2008
7. Salmon et al *J.Am.Chem.Soc.* 132, 8407, 2010
8. Ozenne et al *J.Am.Chem.Soc.* 134,15138, 2012
9. Jensen et al *PNAS* 108, 9839 2011

O-97

Transport machineries in biomembranes that utilize electrostatic "charge zippers"

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Membrane proteins are engaged in diverse transport processes of moving hydrophilic material across hydrophobic lipid bilayers, involving e.g. pore formation or more subtle catalytic mechanisms. We demonstrate here a new structural principle for the folding and self-assembly of membrane proteins, based on electrostatic interactions. In these so-called "charge zippers", long ladders of salt bridges form between amphiphilic transmembrane segments, running all the way across the lipid bilayer. The role of this functionally important structural motif will be illustrated for two case studies with pharmaceutical and biotechnological relevance: (i) the ***biofilm-inducing peptide TisB***, which enables the controlled passage of protons across bacterial membranes; and (ii) the ***TatA translocase***, which drives the export of fully folded proteins through a pore with variable diameter.

O-98

Cross talks between amyloid-forming proteins in neurodegeneration

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Protein misfolding and conformational changes are a cornerstone of neurodegenerative diseases involving formation and deposition of toxic protein oligomers. Although mutations favor protein aggregation, physiological factors such as labile metal ions within the cellular environment are play also a prominent role in aggregation processes [1]. Here I will explore our recent progresses on the analysis of amyloid formation by S100 proteins overexpressed in the brain in amyloid diseases (AD and ALS) under conditions mimicking the synaptic environment. These proteins form amyloid-like structures [2] characterized by the typical Thioflavin T fluorescence and FT-IR fingerprints. We have screened the effect of calcium and zinc, two major players in the chemical biology of the glutamatergic synapse, in the amyloidogenesis pathway, finding a strong dependence on the amyloid formation kinetics and morphology as inferred from TEM. Also, we have recently [3] elicited that S100A6, which is overexpressed in AD and ALS, is itself amyloidogenic and seeds SOD1 aggregation, shortening its nucleation process.

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O-99

Tau structure in paired helical filaments revealed by solid-state NMR

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Alzheimer's disease and other tauopathies are accompanied by the intracellular self-assembly of the protein tau into fibrils termed "paired helical filaments" (PHFs). Tau is associated to the microtubules and can, upon hyperphosphorylation, detach and assemble into PHFs, the major component of the diseased brain deposits. So far, atomic details on the structural basis of PHFs are missing due to the intrinsic insolubility and lack of long-range order of the assemblies, making studies by solution NMR and X-ray difficult. Here, we present a solid-state NMR spectroscopic study of PHFs from two variants of a truncated tau isoform (K19 wild-type and K19 C322A mutant), known to form PHFs comparable to those found in diseased brains (1).

The results on K19 wild-type and C322A mutant PHFs disclose an intermolecular disulfide bridge (DSB) formation and a DSB-dependant polymorphism in the core structure (2). Our results further reveal the rigid -structured core of both fibrils to comprise the same short stretch of 20-30 residues containing several -strands connected by short kinks. The long-range distances obtained from solid-state NMR unveil two different possible structures of Tau in PHFs.

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(2) Daebel V et al. *JACS* 2012, *134*, 13982

Abstracts

– Protein Folding, Assembly and Stability –

O-100

Fibril formation of polyglutamine repeats: a spectroscopic study

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The formation of β-sheets plays an important role in protein folding, but also in fibril formation due to their propensity for aggregation. PolyQ repeats are found in proteins associated with many neurodegenerative diseases and it is agreed that the length of the polyQ sequence is critical for inducing fibril formation, although the molecular mechanisms are still poorly understood. Polyglutamine (polyQ) peptides have been analyzed in dependence of the sequence lengths ($K_2Q_nK_2$ with n=10,20,30) by IR- and CD-spectroscopy. $K_2Q_{10}K_2$ reveals a temperature-stable random structure in a wide concentration range. Increase of the peptide length to $K_2Q_{20}K_2$ results in the formation of different β-structures, clearly seen in IR bands representative for intra- and intermolecular β-sheets. Intermolecular β-sheet and the aggregation tendency are significantly enhanced in $K_2Q_{30}K_2$. Time-resolved IR studies have been performed by a laser-excited temperature-jump technique. Rapid heating of the solvent is induced by a Raman-shifted Nd:YAG pulse and ns-to-μs peptide dynamics is monitored at single wavelengths using a quantum cascade laser tunable in the amide I region. The T-jump studies indicate different conformational dynamics for intra- and intermolecular β-structures.

P-101

Natural tetrahydrofuran derivatives reduce insulin amyloid aggregation

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The clearance of protein amyloid self-assemblies represents an attractive strategy to develop therapies for amyloidosis. Insulin amyloids are formed in the patient with diabetes type II at the sites of frequent insulin injections. We investigated anti-amyloid ability of natural tetrahydrofuran (THF) derivatives (W10-W14) using both the spectral and microscopic techniques. By ThT assay and atomic force microscopy it was found that structure of THFs is factor significantly affecting their depolymerizing activity. While diastereomeric mixtures of 2,3,4-trisubstituted THF W12-W13 display no disassembly activity, optically pure THF W10, W11 and W14 with substituent at C-5 are able to destroy insulin fibrils more effectively. The highest anti-amyloid activity of derivative L-*altro*-W14 in comparison with its *D-galacto* diastereomer is the most probably due to the advantageous configuration. The observed features make THF compound W14 of potential interest as a natural therapeutical agent targeting insulin-associated amyloidosis. (This work was supported by ESF 2622012033, 26110230061, APVV:0171-10, 0203-10, SK-RO-0016-12,VEGA 0181).

P-102

Influence of nanosphere size and structure on insulin amyloid aggregation

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Insulin fibrils are highly ordered protein aggregates associated with insulin localized amyloidosis. We have investigated the influence of two types of spherical nanospheres formed from polylactic acid dosed by Fe_3O_4 nanoparticles and these nanospheres modified by bovine serum albumin on insulin amyloid aggregation in vitro. The size of nanospheres was characterized by SEM and dynamic light scattering measurements giving hydrodynamic diameters of 60 nm and 196 nm, respectively. The anti-amyloidogenic activity of nanospheres was monitored by ThT assay and AFM. Our results suggested that magnetite nanospheres are able to inhibit insulin fibrillization and destroy the insulin amyloid aggregates. We have found that the inhibition was more effective for larger nanospheres than for smaller ones. The opposite dependence was observed for depolymerization-small nanospheres were more effective than larger nanospheres. We conclude that the extent of anti-amyloidogenic activity is affected by the size of nanospheres (due to steric inability to affect bonds involved in insulin fibrillization) and the presence of Fe_3O_4 nanoparticles. This work was supported by ESF 26110230061, VEGA 0181, 0041, APVV 0171-10 and VVGS 38/12-13.

P-103

Stabilization of bacterial luciferase and NADH:FMN-oxidoreductase in a gelatinous environment

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A coupled enzymatic system of luminous bacteria NADH:FMN-oxidoreductase-luciferase, widely used in bioluminescent analysis, gradually becomes inactive during storage and application *in vitro*. The aim of our work was to increase the stability of these enzymes by varying the gel-like gelatine and starch environment.

It was shown that in the presence of gelatin the temperature optimum of enzymes was 25?C, the same as that of the buffer solution, and in the presence of starch it was 33?C. Gelatin did not influence the thermal inactivation rate of the coupled enzymatic system, while starch reduced its denaturation rate. The starch environment decreased the thermal inactivation rate constant of 43?C 12 times. Thus starch is the better additive for improving the thermal stability of the enzymes.

The coupled enzymatic system surrounded by both gelatin and starch increased its resistance in an alkaline pH region and its optimum ionic force expanded to lower values.

Furthermore, a reagent composed of luciferase and oxidoreductase, immobilized in gelatine gel by dosing in drops and drying, was prepared. The active conformation of enzymes in the reagent is thus stabilized, so the enzymes remain stable when exposed to negative factors and long-term storage of more than two years.

Abstracts

– Protein Folding, Assembly and Stability –

P-104

Analysis of GPCR-Ligand Interaction by NMR: NPY and Y1R

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G protein-coupled receptors (GPCRs) form the backbone of cellular communication and allow cells to detect external signals, such as light or molecules, or to communicate with each other. With these tasks, GPCRs are involved in most physiological processes and have become of high interest for the research community as well as for the pharmaceutical industry. Unfortunately, molecular characterisation of these large transmembrane proteins is difficult. Solution nuclear magnetic resonance (NMR) spectroscopy offers the opportunity to study structural and dynamical aspects in the interaction of ligand and receptor. In our research, we are interested in the interaction between the neuropeptide Y (NPY) and one of its GPCR, the neuropeptide Y receptor type 1 (Y1R). The activity of this system plays an important role in the circuitry of energy homeostasis and in the onset of anxiety. The Y1R was produced recombinantly in *Escherichia coli* as inclusion bodies, solubilised in SDS, refolded and incorporated in DMPC/DHPC bicelles. Several differently ^{15}N-labelled NPY variants were synthesized by solid phase peptide synthesis and studied by NMR in the presence and in the absence of the receptor to map the amino acids involved in receptor binding, indicated by a change of their chemical shifts.

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Protein Folding Pathways with Realistic Atomistic Force Fields

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It is still under debate whether proteins fold through few well defined pathways or through a large multitude of independent ways. Answering these questions is made difficult by the fact that standard molecular dynamics (MD) simulations are often impracticable. The Dominant Reaction Pathway (DRP) approach permits to efficiently study the thermally activated conformational dynamics of biomolecules in atomistic detail and to characterize the folding pathways of a protein once. We first apply the DRP studying the folding pathways of the Fip35 WW Domain, a 35 amino-acids long protein. Performing all atom simulations, we can show that this small protein folds following only two pathways. Our results are compatible with ultra long MD simulations and consistent with experimental available data on the folding kinetics. We apply then our simulation scheme to a much more challenging task: performing an all-atom folding simulation of a 82 amino-acids long protein displaying a topological knot in its native conformation. We can portray the folding mechanism and identify the essential key contacts leading to the proper formation of this knot. We show that non native contacts can sensibly enhance the probability of correctly forming the knot.

P-106

Modeling of non-covalent complexes of the cell-penetrating peptide CADY and its siRNA cargo

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CADY is a cell-penetrating peptide spontaneously making non-covalent complexes with short interfering RNAs (siRNAs) in water. Neither the structure of CADY nor that of the complexes is resolved. We have calculated and analyzed 3D models of CADY and of the non-covalent CADY-siRNA complexes in order to understand their formation and stabilization. Data from the *ab initio* calculations and molecular dynamics support that, in agreement with the experimental data, CADY is a polymorphic peptide partly helical. We calculated and compared several complexes with peptide/siRNA ratios of up to 40. The initial binding of CADYs is essentially due to the electrostatic interactions of the arginines with siRNA phosphates. Due to a repetitive arginine motif (XLWR(K)), CADYs can adopt multiple positions at the siRNA surface. Nevertheless, several complex properties are common: an average of 14 ± 1 CADYs is required to saturate a siRNA. The 40 CADYs/siRNA that is the optimal ratio for vector stability always corresponds to two layers of CADYs per siRNA and the peptide cage is stabilized by hydrophobic CADY-CADY contacts. The analysis demonstrates that the hydrophobicity, the positive charges and the polymorphism of CADY are mandatory to make stable the CADY-siRNA complexes.

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Structure-based design of antimicrobial peptaibols bearing unnatural α,α-dialkylglycines

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Peptaibols are a class of natural antibiotics bearing a high content of natural α,α-dialkylglycines (α,α-dags), such as the α-aminoisobutyric (AIB), α,α-diethylglycine (DEG) and isovaline (IVA). The concept behind the antibiotic activity of peptaibols is associated with their ability to form pores in lipidic membrane bilayers, leading to leakage of cytoplasmic material, and, ultimately, to cell death. These pores are formed mainly due to the helical structure of peptaibols in apolar environments, which is thought to be induced by the steric constraints of α,α-dag residues.
In the present work we focus on two peptides from this class: zervamicin IIB and antiamoebin I. Zervamicin IIB and antiamoebin I are 16-residue peptides, with different α,α-dag contents. We perform a computational molecular dynamics study of antiamoebin and zervamicin analogs bearing non-natural α,α-dags, to understand the effect of these residues in the secondary structure, and their ability to restrain the conformational space of the peptides where they are inserted. The aim of this study is to propose novel antibiotic peptides that are structurally pre-organised and less prone to metabolic degradation in order to increase their activity.

Abstracts

– Protein Folding, Assembly and Stability –

P-108

Transthyretin aggregation into amyloid: molecular species, mechanism and kinetics

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Amyloidoses are clinical disorders caused by extracellular deposition of insoluble amyloid fibrils, derived from the aggregation of misfolded peptides or proteins which, under normal conditions, are soluble. Transthyretin (TTR), a 55 kDa homotetramer, is one of more than twenty human proteins that cause amyloidogenic diseases.
Several studies on TTR aggregation suggest that amyloid fibril formation is a multi-step process initiated by the dissociation of the tetramer to non-native monomeric intermediates that assemble into non-fibrillar and cytotoxic oligomers which further aggregate to form fibrils.
In order to understand the molecular basis of aggregation and also investigate new compounds able to inhibit/disrupt amyloid fibrils, we studied the kinetics of TTR aggregation using several spectroscopic techniques. Light Scattering and Transmission Electron Microscopy were used to characterize the molecular species present along the TTR aggregation pathway.
Our data shows that WT-TTR assembly from acid-unfolded monomers can be described as a 2-step process with a faster protein concentration-dependent first step and a second concentration independent step. Furthermore, the TTR amyloid pathway involves the accumulation of an intermediate composed of 8 to 10 monomeric units.

P-109

The internal friction of proteins determined by MD simulations

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The rates of protein conformational changes are usually not only limited by external but also internal friction, however, the origin and significance of this latter phenomenon is poorly understood. It is often found experimentally that a linear fit to the reciprocal of the reaction rate as a function of the viscosity of the external medium has a non-zero value at zero viscosity, signifying the presence of internal friction. To better understand this phenomenon, we have performed molecular dynamics simulations of a conformation change of tripsin, where we could separately control the friction of the surface and the interior of the protein. Here we present the results of our simulations, and also compare them to the experimental data obtained for the activation of tripsin.

P-110

An intermediate state for folding and aggregation elucidates amyloidogenicity of β2m Δn6

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One of the major components of amyloid plaques of patients with dialysis-related amyloidosis (DRA) is a cleaved variant of protein β2-microglubulin (hβ2m), ΔN6, which lacks six N-terminal residues. *In vitro* experiments showed that contrary to the wild-type form, ΔN6 is able to form amyloid fibrils at physiological pH. Furthermore, a mild acidification of the medium (pH 6.2), such as that occurring in the synovial fluid of DRA patients, strongly enhances fibril formation by ΔN6. Such behavior has been linked with a supposed ability of ΔN6 to populate one or more amyloidogenic conformers. We present a computational approach to investigate the *de novo* amyloid assembly by ΔN6. We show that deletion of the N-terminal hexapeptide triggers the formation of an intermediate state for folding and aggregation exhibiting an unstructured strand A detached from a well-preserved core. Strand A plays a pivotal role in dimerization by acting as a sticky hook. The detachment of strand A from the core is maximized at pH 6.2 making the identified amyloidogenic state the key player in ΔN6 aggregation at this pH value. Based on an analysis of the dimer interfaces we predict that residues Tyr10, His13, Phe30 and His84 may be important players in ΔN6 amyloid assembly.

P-111

pep14-23, a peptide drug lead against dengue virus, binds anionic lipids becoming α-helical

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Dengue virus (DENV) causes a mosquito-borne disease that affects millions, causing 20,000 deaths worldwide every year. With outbreaks occurring in tropical and increasingly in temperate regions and without effective therapies available, a better understanding of DENV life cycle is of utmost importance. DENV capsid (C) protein interaction with host lipid droplets (LDs) is essential for viral replication. We studied this interaction before [1,2], developing pep14-23, a patented peptide inhibitor of this interaction. In this study, we evaluated pep14-23 (and the corresponding C protein region) interaction with phospholipids. The combination of bioinformatics analysis with experimental tensiometry, circular dichroism and zeta potential data shows that pep14-23 binds anionic phospholipids, acquiring α-helical structure. Given LDs negative charge, pep14-23 inhibition of DENV C may involve a similar interaction mechanism with LDs phospholipids (which may also occur in the corresponding intrinsically disordered region of DENV C protein). This data may help to design future pep14-23 based therapies for DENV and related flaviviruses.

1. Carvalho *et al.*, 2012, J Virol, 86:2096
2. Martins *et al.*, 2012, Biochem J, 444:405

Abstracts

– Protein Folding, Assembly and Stability –

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The impact of room temperature ionic liquids on lysozyme amyloid fibrillization

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Accumulation of amyloid fibrils in tissues is a hallmark of various diseases and amyloids are also used as novel biomaterials. The direct amyloid self-assembly *in vitro* serves as useful tool for study the mechanism of amyloid fibrillization and helps to search active antiamyloidogenic drugs. Therefore, finding the effective conditions for amyloid fabrication is of great importance. Room temperature ionic liquids have been found to alter self-assembly of proteins. We have studied the effect of imidazolium-based ionic liquids (ILs) on kinetics of lysozyme amyloid formation at various temperatures by Thioflavin T assay, CD spectroscopy and AFM. We have found that the efficiency of ILs strictly depends on their concentrations and anion type. For 1-butyl-3-methyl-imidazolium acetate, the acceleration of lysozyme amyloid formation kinetics is observed at concentrations up to 30% (v/v) IL in 250 mM glycine buffer. At higher IL concentrations, the inhibition of lysozyme fibrillization was observed. We have found that lysozyme amyloid properties can be modulated by chemical structures of ILs. The work was supported by Slovak grant agency VEGA n. 0155, 0181 and APVV 0171-10 as well as ESF project 26110230061.

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Structure and dynamics of heat unfolded ribonuclease A investigated using neutron scattering

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Structure and dynamics play the key role in protein function, but roughly 30% of eukaryotic proteins are partially or even completely unfolded [1]. Nevertheless, intrinsically unfolded proteins are functional and involved in several biological processes. To get further insight into disordered structures and their dynamics, we use Ribonuclease A (RNase A) as a model system, as it is a well known protein denaturing reversibly upon heating. Additionally, the unfolding transition, as well as stability and protein-protein interaction are influenced by the pH. A detailed study of the structure and dynamics of RNase A at several pH values using Small Angle Neutron and X-ray Scattering (SANS, SAXS) as well as Neutron Spin Echo Spechtroscopy (NSE) and Circular Dichroism Spectroscopy is presented. The combination of these techniques allows us to observe large-scale internal dynamics of subdomains or of unfolded protein strands that operate on the same length scale as rotational diffusion. However, the timescale can be different and depends on the protein structure and internal interactions. [1] A. L. Fink, Current Opinion in Structural Biology 2005,15:35-41

P-114

Polymorphism of lysozyme amyloid fibrils and cell viability

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Lysozyme amyloid aggregates play important role in systemic amyloidosis associated with presence of protein deposits in various organs leading to the failure of the affected organs. Recent studies suggest that cytotoxicity of amyloid aggregates is due to generic structural properties common to oligomeric and certain fibrillar aggregates. We studied cytotoxic effect of lysozyme amyloid fibrils formed *in vitro* at two different experimental conditions causing diversity of fibril morphology. The fibril structural characteristics were evaluated by spectroscopic methods, atomic force microscopy and image analysis. The cytotoxic effect of both types of lysozyme amyloid fibrils on the renal LLC-PK1 cells was judged employing different methods. Our findings support the hypothesis that mature amyloid fibrils are actively involved in the cytotoxic processes. However, the inhibition of renal cell growth, promotion of the cell death and accumulation of the cell in late apoptotic/ necrotic stages significantly depended on the structural properties of studied fibrils. This work was supported by projects VEGA 0181, APVV-0171-10, SK-RO-0016-12, ESF 26110230061, grant of the Romanian National Authority for Scientific Research, CNCS–UEFISCDI, PN-II-RU-TE-2011-3-0204.

P-115

Amyloid aggregation triggers: hydrophobic surfaces, metal ions, intramolecular interactions, lipids

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Amyloid aggregates that form after conversion of peptides to structures rich in beta-sheets are related to various neurodegenerative diseases. Recently, beta-sheet formation as the early stage of amyloid formation is considered to be involved in neurotoxicity. Here, we demonstrate in a systematic overview how various factors enhance or inhibit beta-sheet formation. We identified the effect of interfaces on the onset of aggregation dominating over other factors in a set of tailor-made model peptides with defined secondary structure propensities and binding sites for metal ions. Various sophisticated surface-sensitive infrared and X-ray methods were complemented with molecular dynamics simulations. Transitions of a helical intermediate state to beta-sheet were characterized and manipulated. The delicate balance of stabilizing interactions with metal ions, intramolecular salt bridges, nematic order, hydrophobic interactions with surfaces and conformational energies as well as their relation to each other will be discussed. In conclusion, our study implies that transient air-water interfaces occurring during *in vitro* experiments may lead to amyloid seeding. We also show that minimal changes in the peptide sequence may reverse effects of metal ions or internal stabilizing interactions.

Abstracts

– Protein Folding, Assembly and Stability –

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Molecular structure changes of Aβ(1-16) peptide induce by transition metals

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It is generally accepted that metal ions play an important role in neurological pathologies. Metal ions were shown to enhance amyloid beta peptides aggregation or to induce a misfolding in their secondary structure that promotes fibril formation. In this work we used α-HL protein pores inserted into planar lipid membranes as model biological nanoreactors suited to study structural and functional details that govern the interactions manifested between peptide monomers and the interior of the pore lumen. Using this system we were able to investigate at the single molecule level the changes induced by either Cu^{2+} or Zn^{2+} complexation in the conformation of a truncated amyloid of human origin, $A\beta_{1-16}$. The experimental data suggests that the Zn^{2+} modulation of the human $A\beta_{1-16}$ peptide is less effective as compared to the Cu^{2+}. In addition, this study points out the potential of such nanopores as highly sensitive real-time conformation sensors. The differences between Zn^{2+} and Cu^{2+} binding may serve as a basis for understanding the role of metal-induced misfolding of amyloid peptide in AD pathogenesis.
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P-117

Brownian dynamics simulation of clathrin cage formation

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The processes of endo- and exocytosis are associated with the transport of nutrients, hormones and proteins in to and out of living cells. When these molecules enter a cell, they are collected and encapsulated in vesicles for further transport to a destination with the cell. Likewise, the products of organelles are encapsulated before being transported to the edge of the cell. The central protein in the formation process of these vesicles is clathrin.
Clathrins have three long legs that enable them self-assmeble into polyhedral cages. We investigate the formation and structure of clathrin cages by means of computer simulations. To achieve this, we have developed a highly coarse-grained patchy particle model by representing a clathrin protein as a rigid triskelion with interaction sites on the legs. To simulate their dynamics, we have implemented a Brownian Dynamics algorithm to describe their translational and rotational motion.
We present validation tests of the algorithm to show that both static and dynamic properties are consistent with theory. The model clathrins are observed to self-assemble into cages within several second. These cages are structurally similar to those observed by *in vitro* experiments.

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Minichaperone protect and reactivation of maltodextrin glucosidase enzyme against thermal stress

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The chaperonin GroEL plays an essential role in promoting protein folding and in protecting against mis-folding and aggregation in the cellular environment. Here we demonstrate that its isolated apical domain part known as mini-GroEL (21 kDa) was able to provide statistically significant and specific protection of MalZ, a 69 kda monomeric protein from E.coli, against thermal inactivation at stoichiometrical concentrations which was monitored by means of light scattering and measurement of enzyme activity. The thermally-stressed Malz at 50°C showed a complete loss of structure and was prone to aggregation. Mini-GroEL was able to bind to this state and suppress its aggregation, thereby preventing irreversible denaturation of the enzyme. Moreover, mini-GroEL is also able to promote refolding of protein after thermal denaturation. The mini-GroEl bound Malz exhibited native-like secondary and tertiary structure showing the interaction of mini with the MG state of the Malz was confirmed by intrinsic fluorescence measurements. 8-Anilinonaphthalene sulphonate (ANS) binding studies revealed the involvement of hydrophobic interactions in the formation of the complex. SDS/PAGE analysis confirmed that mini-GroEL had formed a soluble complex with MalZ after a period of thermal stress.

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Role of ionizable amino acid residues on α-helix conformational stability and folding dynamics

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Although the formation of α-helix represents one of the simplest scenarios in protein folding, it has been a subject of great interest in recent years. Recent experimental studies suggest that the stability of native helical conformations in amyloid-forming peptides and proteins, such as amyloid β-peptide (Aβ) and lung surfactant protein C (SP-C), play a crucial role in the aggregation behaviour of such proteins, reducing their propensity for amyloid fibril formation.
We performed time-resolved photoacoustic calorimetry (TR-PAC) experiments associated with a laser-pulsed pH-jump technique to examine the early folding dynamics and to identify the key factors governing the folding mechanism of an α-helical peptide model with 13 amino acid residues. In addition, CD and NMR techniques have been applied to obtain structural details. Our results have provided strong evidence that two pairs of side chain interactions stabilize the helical conformation: a salt bridge between Glu2 and Arg10, and a π-stacking interaction between His8 and Tyr12. Using a laser-pulsed pH-jump we induce destabilization on site-specific regions of the α-helix peptide, namely due to protonation of His8 and Glu2. Conformational and dynamics parameters, such as volume changes, enthalpy, and kinetics were obtained.

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– Protein Folding, Assembly and Stability –

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CABS-flex: a server for fast simulation of protein dynamics

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The CABS-flex server [1] implements CABS-model based protocol for the fast simulations of near-native dynamics of globular proteins. In this application, the CABS model was shown to be a computationally efficient alternative to all-atom molecular dynamics (MD) [2]. The simulation method has been validated on a large set of MD simulation and NMR ensemble data. Using a single input (user-provided file in PDB format), the server outputs an ensemble of protein models (in all-atom PDB format) reflecting the flexibility of the input structure, together with the accompanying analysis (residue mean-square-fluctuation profile and others). The ensemble of predicted models can be used in structure-based studies of protein functions and interactions. The CABS-flex server is freely accessible at http://biocomp.chem.uw.edu.pl/CABSflex

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P-121

Thermodynamical studies of the peptide containing MABA acid which promotes bends in proteins

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For the peptide Ac-DDATKT-NH_2 (Dag1), derived from the immunoglobulin binding protein G from Streptococcus, and its mutant (Ac-DD-(*MABA*)-KT-NH_2 [Dag1_M]), the pK_a values of the charged amino-acids groups present in the above-mentioned sequences were determined by potentiometric titration measurements in the temperature range from 25^oC to 50^oC. The temperature range for the phase transition of these peptides was determined by using differential scanning calorimetry. CD spectra were carried out in a temperature range of 10^oC-90^oC. Our earlier studies suggested that the presence of like-charged residues at the end of a short polypeptide chain composed of nonpolar residues can induce a chain reversal. To decrease mobility of peptide under study (Dag1) and ensure a better stability of its structure (better stiffness), the amino acids from turn region of Dag1 in the original sequences was replaced by rest of the meta-aminobenzoic acid (MABA), which played role of β-mimetic. Presented data show that the charged residues present in both sequences have a similar environment (similar conformational preferences) which suggest that some short peptide fragments excised from proteins can fold in aqueous solution into conformations with shape similar to that they assume in the parent protein.

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Mapping induced folding of viral nucleoproteins using SDSL combined with EPR spectroscopy

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Site-directed spin-labeling (SDSL) combined to Electron Paramagnetic Resonance (EPR) spectroscopy has emerged as a valuable tool for studying these conformational changes. In particular, SDSL has been shown to be well-suited to study induced folding events within intrinsically disordered proteins (IDPs) that are not readily amenable to X-ray crystallography. In SDSL method, a nitroxide side chain is introduced *via* cysteine substitution mutagenesis followed by modification of the unique sulfhydryl group with a specific nitroxyde reagent. Measurements of the EPR spectral properties of the label provide a wealth of information on its environment in the protein. This study aims at mapping the disorder-to-order transition of the nucleoprotein (N) of Henipah Viruses within the *Paramyxoviridae* family. The unstructured C_{term} region of the N protein, N_{tail}, interacts with its partner, P_{XD} (X domain of the phosphoprotein partner), through an α-helical induced folding. Comparison with the well-characterized Measles virus N_{TAIL}–P_{XD} system, allowed us to specify the structural models of Henipah viruses N_{TAIL}–P_{XD} complexes proposed previously.[1]

[1]M. Martinho et al, *J. Biomol. Struct. Dyn.* **2013**.

P-123

Conformational Selection Underlies Recognition of a Molybdoenzyme by Its Dedicated Chaperone

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Molecular recognition mechanisms between proteins are essential in many biological processes. Understanding the key role played by dedicated chaperones in metalloprotein folding and assembly requires the knowledge of their conformational ensembles. In this study, the NarJ chaperone dedicated to the assembly of the membrane-bound respiratory nitrate reductase complex NarGHI, a molybdenum-iron containing metalloprotein, was taken as a model of dedicated chaperone. The combination of two techniques *ie* site-directed spin labeling followed by EPR spectroscopy and ion mobility mass spectrometry, was used to get information about the structure and conformational dynamics of the NarJ chaperone upon binding the N-terminus of the NarG metalloprotein partner. The study of singly spin-labeled proteins revealed the location of the binding site. Inter-label distance measurements by pulsed double electron-electron resonance (DEER) techniques and ion mobility mass spectrometry experiments demonstrated the existence of several conformers and the selection of one of them upon complex formation. Taken together these results suggest that the recognition mechanism between the dedicated chaperone and the metalloprotein is governed by a conformational selection mechanism.

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– Protein Folding, Assembly and Stability –

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Human septins SEPT5-SEPT7-SEPT8 interact in a hetero-complex

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Septins are proteins belonging to the GTPases family, which are able to form hetero-oligomeric complexes and higher-order structures as filaments. Septins are involved in important cellular processes, such as cytokinesis and membrane trafficking, and some of them have been found to be involved in neurological disorders, as the Alzheimer's disease. The human genome encodes 13 septins which are divided into 4 subgroups based on sequence similarity. Since proteins from different subgroups interact among themselves to form filaments, many different combinations could be assembled, but only a few have been actually described so far. Based on our previous results of septin interactions from Yeast Two-Hybrid experiments, we have started biochemical and biophysical studies on putative heterocomplexos, particularly human septins 5, 7 and 8. In order to verify if those septins interact, a co-expression system was designed. SEPT7 was fused with His-tag and coexpressed with SEPT5 and SEPT8 in *E. coli*. All the septins were recovered after co-purification by nickel affinity chromatography, thus validating the interaction. The presence of human septins 5, 7 and 8 in the complex was confirmed by mass spectroscopy. Current efforts are aiming to check if this complex is able to form filaments.

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Replica-exchange molecular dynamics simulations of the amyloid-beta(16-22) fragments

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Folding and self-assembly of the 42-residue amyloid-β peptide (Aβ) are linked to Alzheimer's disease (AD). This self-assembly process is however difficult to observe experimentally. Simulations thus play an important role in overcoming this problem.
We have performed replica-exchange molecular dynamics simulations of the monomer and some oligomers of Aβ peptide fragments with the OPEP simulation program [2,3]. This program package is a coarse-grained model of protein. It consists a detailed representation of the backbone, modeled by its N, H, Cα, C', O atoms and in one bead or centroid for all side-chains.
Aβ normally makes the helix structures. However, it is believed that Aβ will form the sheet structure when they are gathered together, and this self-assembly process is the cause of AD. We focused on the points which sequences tend to aggregate, and we simulated in various fragments of this protein. This time, we will announce about the Amyloid-β(16-22) fragments which is a well-studied system numerically.
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P-126

Amyloid Disruption by Hypersonic Wave in Non-equilibrium Molecular Dynamics Simulations

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Amyloids are insoluble and misfolded fibrous protein aggregates and associated with more than 20 serious human diseases. Recently, there are some experimental reports that cavitation disrupts amyloid fibrils. However, it is still unknown how the cavitation or bubble in water disrupts the amyloid fibrils at atomic level. In order to answer this problem, we performed isobaric-isothermal molecular dynamics simulations of an amyloid-β oligomer in explicit water, which constitutes an amyloid fibril. Amyloid-β fibrils are known to be associated with the Alzheimer's disease.
We put twelve amyloid-β peptide molecules, 10169 water molecules, and twelve sodium ions as counter ions. The simulation was started from the experimentally-known amyloid oligomer structure in the amyloid fibril. To express supersonic wave, sinusoidal pressure was applied between -100 MPa and 300 MPa. When the pressure was decreased to a negative value of -100 MPa from a room pressure, a bubble formation was observed around the C-terminal region, the amino acid residues of which were hydrophobic. Even after the bubble size increased, the secondary structures of the oligomer were maintained. When the pressure was increased to a positive value, the bubble shrank, and the oligomer was disrupted.

P-127

Monitoring the early stages of protein fibrillation: time-resolved small-angle x-ray scattering studies

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Many human pathologies are associated with protein aggregation and fibrillation, hence the possibility to structurally describe this phenomena is a necessary condition to develop successfully therapy strategies. Small-Angle X-ray Scattering experiments raise the advantage that structural analysis can be performed on proteins directly in solution, without disturbing the intrinsic equilibria, and the use of synchrotron radiation enables to investigate the kinetic process from its very first stages, with a time resolution of milliseconds. Prefibrillar intermediates are a key issue in amyloid researches, because they show the highest cytotoxicity with respect to mature fibrils. However, the early aggregation process is still largely unknown, since it is very fast. We present the structural analysis of the very first aggregation stages of three proteins: a mutant apomyoglobin, the neuronal-specific septin-3 protein and the amyloid beta peptide. The achieved results demonstrate that SAXS can successfully monitor the first stages of amyloidogenic process, hence it can be also exploited to monitor effects of pharmaceutical agents in modifying or preventing the early amyloid aggregation patterns.

Abstracts

– Protein Folding, Assembly and Stability –

P-128

Studies on composition-dependent structure of neuronal and bio-inspired supramolecular assemblies using small angle x-ray scattering

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Understanding the nature of interactions between biological molecules enabling the assembly of supra-molecular structures is a central goal in biophysics. These structures are critical for a wide range of cellular functions, e.g. remodelling of the supra-molecular structure of cytoskeletal proteins complexes, which occurs during cell division, locomotion, intracellular trafficking and signal transduction.

The cytoskeleton comprises three negatively charged proteins, including filamentous-actin, intermediate filaments and microtubules. The supra-molecular assemblies of these cytoskeletal filaments are mediated, in vivo, by complex interactions between one or more types of cross-linking proteins and by unstructured regions radiating from the filament backbone.

The main objective of this project is to elucidate the nature of the structures and interactions in these supra-molecular assemblies. We aim to elucidate the dominant interactions between different subunits, their critical concentration for structure's integrity and the consequent structural alterations once this concentration is not met. We also aim at studying intermolecular interactions of interfilament proteins adsorbed onto various surfaces for new, highly functional, highly "tunable" smart materials and surfaces.

P-129

Characterization of HEWL fibrillation using the fluorescence properties of Alexa 488 and Nile Red

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Amyloid fibers contribute to the pathology of many diseases, including type II diabetes and Alzheimer's. Recent research has proposed that membranes containing acidic phospholipids can facilitate the initial steps of amyloid fibril assembly, by both amyloidogenic and non-amyloidogenic proteins. To elucidate this question, we have been focusing our studies on hen egg white lysozyme (HEWL) as a model non-amyloidogenic protein.[1] The conformational changes and fibrillation kinetics of HEWL in solution at pH 2.2 and 57 ^{0}C were monitored by following the variations in the fluorescence properties of both HEWL fluorescently-labeled with Alexa 488 (HEWL-A488) and using the extrinsic dyes Thioflavin T (ThT) and Nile Red (NR). The characteristic stages of HEWL fibrillation could be clearly identified by tracking the increase in HEWL-A488 fluorescence anisotropy over time. The general use of NR as an amyloidotropic dye was further confirmed by quantitatively measuring its binding to HEWL and insulin fibrils through fitting an associative model to their fluorescence anisotropy decays. Additional FRET studies are planned to clarify whether ThT and NR compete for the same binding site in HEWL fibrils. Supported by project PTDC/QUI-BIQ/099947/2008 FCT/Portugal.

[1]Melo *et al. J. Phys. Chem. B* **2013**, *117:*2906

P-130

Characterization and optimization of a novel protein refolding methodology

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Recently, a simple yet effective method to recover refolded and active proteins has been developed, based on the association of an anionic detergent (sodium dodecyl sulfate, SDS, known as denaturing agent) with an amphipathic diol solvent. Up to now, this cosolvent effect has been observed on both soluble and bacterial membrane proteins. None to say, it is crucial to have a clear picture of the physicochemical and molecular basis of these refolding processes. We have therefore used both experimental (intrinsic fluorescence and circular dichroïsm) and theoretical (coarse-grained and atomistic molecular dynamics) approaches to help understanding such intricate phenomena. We first start with the study of the detergent/cosolvent couple (by considering different alcohols molecules) and then investigate the protein/detergent/cosolvent complex (by using peptides as models for alpha- and beta- secondary structures). Such a study gives access to the nature and strength of the interactions between the molecules, and the system stability as a function of time. Original detergent/solvent pairs can be proposed in order to improve the efficiency of our refolding protocol, as well as to sharpen our understanding of its mechanism, paving the way to the full protein characterization.

P-131

Fibrillation of Human Serum Albumin at physiological pH is inhibited by oxidation

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Oxidative stress and amyloid fibrils formation have been suggested to underlie the loss of cellular function in pathologies like Parkinson's and Alzheimer's disease. The understanding of the temperate relationship between these two processes can be of great importance in revealing the molecular basis of neurodegeneration.

Here we show that oxidation of HSA induced by H_2O_2 inhibits amyloid fibrils formation. Structural properties and aggregation pathways of non-oxidized and oxidized HSA were studied by means UV-Vis absorption and fluorescence, CD, FTIR, light scattering and TEM. The mutual interactions of several mechanisms contribute to the formation of fibrils in HSA through a non-nucleated process being the aggregation pathway regulated by the balance between attractive and repulsive interactions in partially unfolded molecules. In the case of oxidized HSA, this equilibrium is altered by minute variations due to protein oxidation which causes changes in protein tertiary structure leading to protein compaction and resulting in increased stability and reduced association propensity of oxidized molecules. HSA forms thin and straight amyloid fibrils while Oxidized HSA forms curly and amorphous aggregates resulting from a slower process with different intermediate states.

Abstracts

– Protein Folding, Assembly and Stability –

P-132

Gas-phase, conformational dynamics hexa-coordinated globin proteins

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The coupling of fast, gas-phase separation techniques based on ion-neutral collisions and mass separation combined with theoretical calculations have shown unique strength in resolving the structure of isomers, conformers, and species of differing chemical class (derived from differences in functional groups, polarities, and atomic compositions). In the present talk, we describe the use of Trapped Ion Mobility Spectrometry coupled to Mass Spectrometry (TIMS-MS) and theoretical calculations for the study of conformational motifs in hexa-coordinated globin proteins. In particular, this study focuses on the comparison of the conformational space of native and engineered mutants as a way to better understand the ligand binding in the heme cavity. Inspection of the TIMS-MS results showed that multiple conformations are observed for each charge state (e.g., z = 8, 9 and 10). Comparison with the theoretical calculations showed that, the heme orientation in the heme cavity defines two major distinct conformational groups, which can be correlated with the results observed for z = 8 and z = 9; the higher number of conformations observed at z = 10 maybe a consequence of the coulombic repulsion between the amino acid side chains near the heme cavity.

P-133

Amino acids structural volume change upon photoinduced pH jump

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A major challenge in biophysical chemistry is the understanding of the mechanisms of protein folding: how an unstructured polypeptide chain can rapidly adopt a unique, densely packed, three dimensional structure. The charge state of the ionizable groups on the side chains of amino acids influence the polypeptide chain stability, determining protein structures. The amino acid protonation step constitutes the earlier event in acid-induced (un)folding processes in proteins. We describe the structural changes induced by a sudden pH jump on amino acids in aqueous solution at pH above their pKa values. A methodology in which a phototriggered acid generator deprotonates very quickly, resulting in a long-lived reversible pH-jump is associated with photoacoustic calorimetry. As the proton gradient protonates amino acid residues it produces distinct charged species. This induces the rearrangement of the structure of surrounding water molecules. Those structural changes are accompanied by a variation in the overall solution volume, inducing a pressure wave. Photoacoustic calorimetry allowed the determination of the enthalpy, kinetics and volume changes of the protonation of the amino acids mainly involved in the ionic equilibrium of proteins. We thank FCT: PTDC/QUI-QUI/099730/2008.

P-134

Structural investigation of the human septin 2/6/7/9 hetero complex

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Septins (SEPT) are conserved guanine nucleotide binding proteins which self-organize into heterofilaments which are involved in cytokinesis and a variety of cellular events. In humans 13 SEPT have been identified. The only crystal structure of a complex available is that of a hexamer SEPT2-SEPT6-SEPT7. Recent studies indicate that SEPT9 occupies the terminal position of this complex, leading to octamers. The genes corresponding to SEPT7-His/SEPT9 (residues 262-568) were cloned into the bicistronic vector pETDuet and SEPT2/ SEPT6 into pRSFDuet. Proteins were co-expressed in *E. coli*, co-purified by affinity chromatography and molecular exclusion. The resulting complex was confirmed by LC-ESI MS/MS after tryptic digestion. SEPT9 (residues 262-568) was subcloned into the pET28a (+) vector for crystallographic and biophysical studies. Circular Dichroism was performed to evaluate its stability on thermal unfolding in the presence and absence of nucleotides (GTP or GDP) and Mg^{2+}, suggesting that protein stability is ligand dependent. Isothermal Titration Calorimetry studies are currently being done to evaluate thermodynamic binding parameters of SEPT9 by nucleotides. Thus, the present study is expected to contribute to our knowledge of the SEPT biophysics and stability for nucleotides.

P-135

Proline-containing tripeptides are effective inhibitors of A-beta amyloid fibrillization

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Alzheimer's disease (AD) is the most frequent form of dementia among the elderly and is associated with the extracellular A-beta amyloid deposits in brain. One of the most straightforward approaches finding a cure is the targeting beta-amyloid fibrillization. In the present work we have focused on tripeptides with different binding affinity to A-beta, but with mutual ability to cross the blood-brain barrier. "*In silico*" screening of 8000 peptides resulted in selection of a number of tripeptides with significant binding affinity and ability to inhibit fibrillization. By both docking and MM-PBSA methods tripeptides containing Proline and aromatic amino acids were identified as potentially the most effective inhibitors. The inhibitory activity of the best fitted tripeptides (WWW, WWP, WPW, PWW) was investigated also experimentally using ThT fluorescence assay and AFM. In vitro experiments revealed IC_{50} values in micromolar range and, thus, fully confirmed theoretically predicted significance of Proline in tripeptide sequence. *This work was supported by 2011/01/B/NZ1/01622, ESF 26110230061, APVV 0171-10, VEGA 0181, VVGS 38/12-13.*

Abstracts

– Protein Folding, Assembly and Stability –

P-136

High pressure FTIR studies on allergens: searching for ways to produce hypoallergenic food

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Food allergy is an IgE-mediated immune disorder representing a health problem of great public concern. We are looking for the answer whether reduction or loss of IgE-binding can be achieved by denaturing pressure or combined pressure-temperature treatment. This would open the way to produce hypoallergenic food.
We performed Fourier transform infrared and tryptophan fluorescence spectroscopy to explore the p-T phase diagram of a fish allergen (Gad m 1). The phase diagram was found to be quite complex containing partially unfolded and molten globule states. A pressure of 500 MPa leads to a partially unfolded state at 27 °C. The complete pressure unfolding could only be reached at elevated temperature (40 °C) and pressure 1.14 GPa. A strong correlation was found between Ca^{2+}-binding and the protein conformation. The heat and the combined heat and pressure treated protein samples were tested with sera of allergic patients.
In case of the apple allergen (Mal d 1) we also studied the effect exerted on pressure stability by environmental factors (different pD, effect of sugar and ionic strength), which can be important for the stability of the protein in the apple. In all cases the allergen unfolded with a transition midpoint in the range of 150-250 MPa. The unfolding was irreversible.

P-137

ANS fluorescence on the stability of extracellular Glossoscolex paulistus hemoglobin with denaturant

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Glossoscolex paulistus giant extracellular hemoglobin (HbGp) has a molecular mass of 3.6 MDa. The present work focuses on the study of HbGp oligomeric stability in the presence of a cationic surfactant (DTAB) as well as urea and guanidine denaturants. Anilino-naphtalene sulfonate (ANS) is used as an extrinsic fluorescence probe. It has been used in recent years as a sensitive probe for protein unfolding studies. HbGp concentration was 0.1 mg/mL, while DTAB was in the range 0-25 mmol/L. Urea and guanidine were in the range 0-5 mol/L and sodium fosfate 30 mmol/L, at pH 7.0, was the buffer. Fluorescence was monitored through excitation at 295 and 350 nm. ANS in pure buffer has a very low emission centered at 510 nm. In the presence of 9 mmol/L DTAB, the emission increases 50-fold and the maximum emission wavelength shifts to 495 nm. Native HbGp also produces a 8-fold increase in ANS emission with a shift in maximum to 480 nm. The presence of urea or guanidine in HbGp solution does not enhance the ANS emission observed in native HbGp. This observation suggests that these denaturants do not promote an increase in accessibility of HbGp hydrophobic sites for the binding of the probe. Acknowledgments: CNPq and FAPESP Brazilian agencies for financial support.

P-138

Insights into the interaction of beta-2 microglobulin fibrils with serum amyloid p component

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Dialysis related amyloidosis (DRA) results in the deposition of amyloid in the joints causing pain and restricted mobility for sufferers. The major protein component of these amyloid deposits is fibrillar â$_2$-microglobulin (β_2m). Serum amyloid-P component (SAP) is a protein ubiquitously present in fibrillar deposits and is thought to play a key role in stabilising the fibrillar structures and preventing their clearance by the host's defences.
Using solid and solution-state NMR we investigated the structural transitions that result in the conversion of monomeric â$_2$m into its fibrillar form and identified sites involved in the interaction with SAP. Magic-angle spinning (MAS) correlation experiments have permitted the substantial assignment of the fibrils. 2D experiments of labelled fibrils in the presence of unlabelled SAP reveal significant changes in the spectral region corresponding to the fibril's glutamate sidechains. Modification of the glutamate sidechains of amyloid fibrils to remove their charge results in complete loss of binding between the fibrils and SAP, highlighting the essential nature of the fibril glutamate sidechains for binding with SAP.

P-139

Thermodynamic parameters of tissues collagens denaturation - DSC measurements

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Functioning of connective tissues is related to the integrity of their supporting framework of collagen. Understanding the relationship between the structure of molecules and the energetics of their stability is essential to understanding processes of the tissues development and degradation.
In the work thermodynamic stability and conformational transitions of collagens in different histological types of connective tissues were investigated using differential scanning calorimetry (DSC). The thermal transitions were studied in the temperature range 25^oC - 90^oC in in samples of ligament, cartilage, artery, skin, nerves and peritoneum, and 25^oC - 160^oC in bone .
To characterize the thermodynamic stability of a system and transition between the folded and unfolded state of the protein, the free energy and entropy change were calculated using enthalpy, transition temperature, and the heat capacity change obtained from DSC scans. Thermodynamic parameters of collagen unfolding were tissue dependent. Denaturation of the molecules in different tissues occurred at different temperatures and with different cooperativity and enthalpy of the processes. Results demonstrated that even the same type collagen molecule can exists in different thermal states in different tissues.

Abstracts

– Protein Folding, Assembly and Stability –

P-140

In vitro characterization of amyloid fibrils with novel aminobenzanthrone dyes

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Due to pathological and functional roles of amyloid fibrils, development of the effective fluorescent dyes for their detection and characterization seem to be of considerable significance to medicine and material science. The present study provides new insights into the complexation of the prospective amyloid reporters belonging to the class of aminobenzanthrone fluorescent dyes (BD), with lysozyme amyloid fibrils. The dye-protein binding parameters were found to differ for morphologically distinct lysozyme fibrils prepared either by organic solvent or acidic denaturation. This finding was interpreted in terms of the differences in the conformation of "steric zipper" determining the protofilament substructure of amyloid fibrils. Förster resonance energy transfer showed that BD and classical amyloid marker Thioflavin T (ThT) occupy distinct binding sites in the fibril structure, presumably, uncharged surface grooves or dry "steric zipper" interface. This assumption is corroborated by the fact that BD fluorescence display lower ionic strength dependence compared to ThT. It is concluded that BD can be used as complementary to ThT amyloid reporters suitable for fibril identification and structural description.

P-141

Inactivation of trimeric purine nucleoside phosphorylase: analytical ultracentrifugation studies

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Purine nucleoside phosphorylase (PNP, EC 2.4.2.1), the key enzyme in the purine salvage pathway catalyzes the reversible phosphorolytic cleavage of the glycosidic bond of purine nucleosides. This protein is at the centre of research and medical interest. The functional molecule of mammalian PNP consist of three identical monomers. It is already known that the wild type PNPs do not dissociate into active subunits, but the question if the monomers are intermediates of the PNP inactivation process is still open. Analytical ultracentrifugation with absorbance detection was used to answer this question. Simultaneous measurements of the activity decline and the presence of different PNP forms in solution show a correlation between the enzyme inactivation and the disappearance of trimers. At the same time, only the appearance of the species with mass much higher than that of the trimer was observed. We conclude that, at the time scale of an analytical centrifugation experiment, the monomers are not significantly populated intermediates of PNP inactivation, but the trimers probably aggregate into larger complexes.

Abstracts

– Systems Biology –

O-142

The role of mixing entropy in carbohydrate metabolism

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The vast diversity of carbohydrates is generated by carbohydrate-active enzymes (CAZymes) accepting many different substrates and catalyzing numerous reactions. This promiscuity is in stark contrast to most enzymes active in central metabolism which catalyze exactly one or a very small number of reactions. The multitude of accepted substrates and catalyzed reactions makes CAZymes hard to characterize in classical enzymological terms. Equilibrium constants and Michaelis constants have no straight forward analogon for CAZymes.

We show how statistical thermodynamics can be employed to concisely describe and explain the action of CAZymes, where the mixing entropy of the reactants emerges as an important state variable of metabolic systems. We can thus correctly predict equilibrium distributions and explain their dependence on the initial conditions, which has not been possible with previous approaches. Experimentally verified stochastic simulations confirm the validity of our approach outside equilibrium.

Our proposed interpretation of polydisperse pools as statistical ensembles facilitates a new perspective to understand many enzymatic processes. We illustrate how entropy gradients are exploited constructively in vivo to establish a robust buffering and integrating metabolic function.

O-143

Negative feedback and crosstalk in the Transforming Growth Factor β signaling pathway

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The transforming growth factor β (TGF-β) signaling pathway transduce extracellular signals into transcriptional responses controlling key cellular processes, such as differentiation, proliferation, and apoptosis, through a network of receptors. Defects along of the pathway have been associated with a wide range of diseases, including developmental diseases and a variety of cancer types. Here, we examine the role of the negative feedback through protein products of transcriptional regulation by mediator SMAD proteins in the behavior of the network by analyzing a novel, detailed computational model of the pathway. The model includes macromolecular assembly, receptor trafficking and signaling, activation of two SMAD channels, nucleocytoplasmic shuttling of smad-complexes, and feedback through inhibitory SMADs. This computational model is able to accurately reproduce and explain experimental data in diverse cell types and our analysis uncovered the importance of negative-feedback-mediated crosstalking between channels in the TGF-β pathway. In addition, we identified key crosstalk points among pathways through literature mining approaches, by constructing a detailed ligand-receptor network for all the members of the TGF-β superfamily and mapping the interactions with other pathways.

O-144

FRAP biophysical tool to probe nucleic acids-membrane ligand interactions in pDNA purification

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Chromatographic techniques are powerful tools capable to purify plasmid DNA (pDNA) up to therapeutical grade to be delivered to patients to overcome genetic disorders or acquired diseases. Membrane-based hydrophobic interaction chromatography is a suitable approach to assure high pDNA quality standards. Adequate membrane adsorbers establish hydrophobic interactions between pDNA (or RNA) and matrix ligands (Raiado-Pereira, L., *et al.* 2010). Thus, membranes functionalized with DOPE, DSPA, DOPC or DLPA lipids were used as nucleic acids adsorbing matrices.

FRAP (fluorescence recovery after photobleaching), a biophysical technique used to study specific molecular interactions, can foster the characterization of key interactions in membrane chromatography. This work focus on the design of a FRAP method to mimic interactions with functionalized membrane adsorbers and pure nucleic acids (pDNA and RNA) using a confocal laser scanning microscope. This allowed the spatiotemporal monitoring of specific fluorophores on their movement through changing mobile phase toward membrane ligands. Results compare the molecular mobilities and immobile fractions of marker molecules in the vicinity of functionalized membranes.

O-145

Positive and negative feedback loops coupled by a common promoter

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Our hypothetic system consists of two genes which are transcribed simultaneously. Their transcription is regulated by two transcription factors, repressor and inductor. The process is going on when the promoter is free of the repressor and is bound with inductor. Proteins encoded in the regulated genes undergo many transformations with the transcription factors as end products. We represented the system by a set of ordinary differential equations. We investigated how evolution of the system depends on its parameters: rate constants, Hill coefficients of cooperativity and numbers of intermediate substances in the repressor and inductor loops. The characteristic equation, as well as conditions of saddle-node bifurcation, has been obtained in a general form. Qualitative feature of the system are determined by simple relations between repressor and inductor concentrations in equilibrium points. These relations in their turn determine corresponding relations between the parameters of the system. Conditions for Hopf bifurcation have been formulated in less general forms in some special cases. Oscillations can appear if Hill coefficient of the repression is enough higher than that of the induction and turnover time of the inductor loop is not to much shorter than that of the repressor loop.

Abstracts

– Systems Biology –

O-146

Bcl-2 family regulation of apoptosis by non-trivial decisioning

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One of the most important signaling checkpoints of apoptosis is the Mitochondrial Outer Membrane Permeabilization (MOMP), which is controlled by Bcl-2 family of proteins. Bcl-2 proteins are regulated by variety of incoming pro- & antiapoptotic signals and as a response to these, Bcl-2 proteins may initiate MOMP and thus allow apoptosis commitment.
Using computational modeling & simulations we found that interplay between the Bcl-2 family proteins form a regulatory network which can integrate a multitude of continuous inputs into single binary output. Particular Bcl-2 proteins may serve as a "toggles", up-/downregulation of whose can "switch on" the MOMP. We have discovered that Bcl-2 family performs pattern recognition - nontrivial behavior, often associated with neural networks and artificial intelligence.
We conclude that Bcl-2 proteins constitute molecular device, controlling apoptosis commitment in much more sophisticated manner than previously thought.
This work was supported by: Agency of the Ministry of Education of Slovak Republic for the Structural funds of the EU, Operational program Research and Development (SEPO II, CEVA), Slovak Research and Development Agency (APVV-0242-11) and Scientific Grant Agency of the Ministry of Education of Slovak Republic (VEGA-1/1246/12)

P-147

Proteasome inhibitor delivery by functionalized gold nanoparticles in pancreatic cancer cells

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Gold nanoparticles are promising vectors as anticancer drug delivery systems. According to the unique physical-chemical properties, these nanoparticles/anticancer drug are uptake by endocytic vesicles and are able to accumulate within the cancer cells via the enhanced permeability and retention (EPR) effect.
Our approach was based on pegylated gold nanoparticles (PEGAuNPs) as a delivery vehicle of a proteasome inhibitor (bortezomib, BTZ). Cytotoxicity and uptake studies of BTZ combined with PEGAuNPs were performed in a human pancreatic cancer cell line (S2-013).
S2-013 uptake of 38 nm PEGAuNPs showed the increase of BTZ internalization and diffusion in the cytoplasm. *In vitro* cytotoxicity studies demonstrated that PEGAuNPs internalized show no toxicity at concentrations up to 0.1 nM. Also, a significant inhibitory effect of BTZ was observed, more pronounced in presence of PEGAuNPs. These findings demonstrate that PEGAuNPs EPR effect in S2-013 cells. Also they will contribute to design and optimize the nanoparticles uptake for overcoming multidrug resistance (MDR) mechanisms and increase the selectivity of the anticancer drugs.

P-148

Changes in some biophysical and biochemical parameters in blood and urine of workers chronically exposed to low levels of benzene

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Benzene occurs naturally as a component of petroleum,or may be manufactured synthetically.It is found in the environment as a contaminant from both human activities and natural processes, posing serious bio-hazards.To study possible health hazards of benzene contamination, 250 males occupationally exposed to low levels of benzene in daily activity were compared to 65 healthy individuals of the same socio-economic standard. Benzene itself was not detected in blood or urine of all participants, but the levels of its metabolites; phenol and muconic acid, were higher in the blood and urine samples of benzene-exposed workers. The results also indicate that this group is under oxidative stress. The determined liver and kidney function tests showed non-significant deviation from controls. The hemolysis degree ,blood viscosity, RBCs aggregation and form factor were significantly deviated from normal. The deviation of the determined biochemical and biophysical parameters from normal may predispose such workers to a variety of health problems. Early correction of the oxidative stress, the hematological parameters and improvement of working environment are necessary to prevent their progress to more serious health problems, especially in children and young adolescents working under similar conditions.

P-149

An integrated systems biology approach reveals highly plastic responses to antimicrobial peptide challenge

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The ability to understand in detail how peptides comprising the innate immune response have evolved to kill bacterial pathogens is of fundamental interest and will inform the development of new antibacterial therapeutics. Many cationic antimicrobial peptides (AMPs) share a range of structural and physical features that have been linked to antibacterial activity and yet vary dramatically in their potency towards the same bacterial target. We hypothesised that a whole organism view of AMP challenge on *Escherichia coli* could provide a sophisticated, bacterial perspective of AMPs, with differing potency, to enable an understanding of how this is linked to their mode of action. We used a ^{1}H NMR metabolomic approach to characterise the effect on *E. coli* of challenge with four structurally and physically related AMPs; magainin 2, pleurocidin, buforin II and a designed peptide comprising D-amino acids only. Sub-inhibitory conditions, where these peptides nevertheless induced a bacterial response, were identified enabling electron microscopic and transcriptomic analyses. Although some common features of the bacterial response to AMP challenge could be identified, the metabolomes, morphological changes and the vast majority of the changes in gene expression were specific to each AMP. We show the antibacterial mode of action of AMPs can be accurately predicted by comparing ontological profiles generated by transcriptomic analyses. The response of *E. coli* to AMP challenge is highly plastic, with the bacteria capable of deploying a multifaceted response adapted to each AMP which depends more on mode of action rather than the physical properties of the AMP.

Abstracts

– Systems Biology –

P-150

Exploring energy and nitrogen metabolism in the Legume-Rhizobia symbiosis

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We have created a metabolic model for the model plant Medicago Truncatula. The model encompasses 1636 reactions with over 76% percent of the catalysing enzymes having genetic evidence. Our model is fully compartmentalised describing metabolic processes in the ER, Golgi, mitochondrion, peroxisome, plastid, vacuole and the cystosol. Moreover, our model is not only mass balanced, but also charge balanced. Based on this model we use flux balance analysis to investigate the changes of flux distributions depending on nitrogen sources, varying external pH and differences between free living plants and plants in symbiosis with rhizobia. For the latter study, we connected our network to a rhizobial model extracted from the Biocyc database, based on transport systems found in the literature. We found changes in amino acid transport, depending on the amount of energy required by the plant for maintenance.

P-151

The interplay between tRNA competition and decoding on mRNA translation elongation rate modulation

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There has been much discussion in the literature regarding the determinants of ribosomal decoding speed during the mRNA translation process. Computational results based on mechanistic models for ribosomal kinetics have predicted a major role for near-cognate and non-cognate tRNA binding competitive behavior on translation elongation rate (Fluitt et al., 2007; Zouridis and Hatzimanikatis, 2008). In contrast, a recent experimental study proposed the difference between Watson-Crick and non-Watson-Crick type of tRNA decoding as the main determinant of translation speed (Spencer et al., 2012). We model stochastically the translation process using a mechanistic model for ribosomal kinetics that accounts for both competitive tRNA behavior and Watson-Crick and non-Watson-Crick types of decoding. With this framework we identify the relative roles that tRNA binding competition and decoding interaction type play on translation elongation rate, and we explore the conditions that allow for optimal protein synthesis.

P-152

Uranium deposition in bones of wistar rats associated with skeleton development

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One of the pathway by which Uranium (U) can enter the food chain is through rock phosphate, used as source of phosphorus in the making of fertilizers and livestock feed supplements, as dicalcium phosphate (DCP). The prolonged utilization of such fertilizers causes absorption of substantial amounts of Uranium by plants, increasing this element in the human diet. DCP is extensively used in broilers diet, another important consumption item by humans. DCP can present concentrations of Uranim as high as 200 ppm. This radiobiological issue is a matter of great concern since 80% of incorporated U is accumulated in the skeleton. The uranyl radical ++UO2 produced in the gastrointestinal tract appears to mimic ++Ca. In this regard, we carried out an experiment with sixty female Wistar rats submitted to a daily intake of ration doped with Uranium from weaning to adulthood. Uranium in bone was quantified by the SSNTD technique, and bone mineral density (BMD) analysis performed. Uranium concentration as function of age exhibited a sharp rise during the first week of the experiment and a drastic drop of 70% in the following weeks. Data interpretation indicates that Uranium mimics calcium. Results from BMD suggest that radiation emitted by incorporated U could induce death of bone cells.

P-153

Melanocortin 4 receptor: in silico mutagenesis and docking studies

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MC_4R regulates feed intake and energy balance and plays an important role in control of feeding behavior. It is known at least 37 natural mutations of the MC_4R, which are associated with the development of obesity in humans. Important for agonists binding residues were also identified by alanine-scanning mutagenesis.

Here we report on *in silico* mutagenesis of previously constructed by us homology model of the MC_4R and future docking of these mutants with endogenous and synthetic agonists. Residues of two kinds, those natural mutations associated with obesity (7 residues) and important for agonists binding residues according to alanine-scanning mutagenesis data (12 residues), were subjected to *in silico* mutations. Then endogenous agonist α-MSH, its synthetic analog NPD-MSH and synthetic non-peptide agonist THIQ were docked to the constructed mutants and possible modes of their binding were analyzed. The docking results were compared with the literature data on the binding affinity and possible modes of binding of THIQ, α-MSH and NDP-MSH.

The data obtained can help to explain low binding affinity of the some natural mutants of MC4R which associated with obesity in humans. These data can also be useful in the design of new synthetic selective agonists for the MC_4R.

Abstracts

– Channels and Transporters –

O-154

Gating ring motions underlying function of BK channels

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In many neuron types, large conductance voltage- and calcium-dependent potassium channels (BK, hslo or KCa1.1) provide a mechanism to couple Ca^{2+} signaling to membrane potential. Inherited defects in BK channels function lead to seizure and epilepsy, indicating that this coupling mechanism is crucial to regulate neuron excitability in the healthy brain. In fact, the interaction between Ca^{2+} influx and BK activation is involved in numerous neuronal processes such as repolarization and hyperpolarization following the action potential (AP), dendritic Ca^{2+} spikes, and neurotransmitter release. A key feature to BK physiological role is that the channel's open probability is synergistically activated by transmembrane voltage and intracellular calcium. The voltage sensor resides within the transmembrane region of the channel, while Ca^{2+} binding is sensed by a large C-terminal intracellular region, where eight Regulator of Conductance for K+(RCK) domains form a "gating ring". Calcium binding to this region reduces the energy required to open the channel, but the exact mechanism underlying this process is still uncertain. Structural studies and a biochemical study using isolated gating rings suggest that Ca^{2+} binding expands the gating ring. The large movement of the gating ring would physically pull and open the gate located at the pore domain. In the present study we investigate the calcium and voltage-dependence of conformational changes in the intact human BK channel by patch-clamp recordings and simultaneous measurements of fluorescence energy transfer between CFP and YFP variants of the green fluorescent protein, inserted into three sites in the BK gating ring. Depending of the site studied, different movements are detected that differ in their Ca- and V-dependence. Here we show that Ca^{2+} binding produces large structural changes that are not obligatorily coupled to the opening of the pore.

O-155

CLC-5, an endosomal chloride -- proton exchanger mutated in Dent's disease: a biophysical perspective

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Mutations in CLC-5 lead to Dent's disease. CLC-5 is a Cl(-)/H(+) antiporter and a specific Glu (E211, "gating Glu") is essential for antiporter function as mutating it converts CLC-5 into a Cl- channel (Picollo & Pusch, Nature, 2005). A further Glu (E268, "proton Glu") is the intracellular entry point for H(+) (Accardi et al, JGP, 2005). Mutant E268A eliminates steady-state transport (Zdebik et al, JBC, 2008) but exhibits transient currents upon voltage steps (Smith & Lippiat, Faseb J, 2010). From the dependence of the transients of E268A on pH and [Cl], we conclude that they represent the movement of an intrinsic gating charge followed by the voltage dependent binding of extracellular Cl ions. We further found that the gating Glu mutation E211D abolishes stationary transport but displays transients which are shifted by 150 mV compared to those of E268A, identifying E211 as a major component of the charge movement. We suggest that that the initial events in the transport cycle are a movement of the gating Glu from the "external site" (Sext) to the "central site" (Scen) with accompanying displacement of a Cl- ion from Scen to the inside, followed by binding of an extracellular Cl ion into Sext. These biophysical insights increase our molecular understanding of CLC antiporters.

O-156

Activation mechanism of the store-operated calcium channel complex STIM1 and Orai1

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Store-operated Ca^{2+} channels (SOC) are used for ubiquitous signaling pathways in both electrically excitable and non-excitable cells, the most prominent being immune cells. The SOC channels are activated in response to cellular stimuli which deplete the endoplasmic reticulum (ER) of Ca^{2+}, a prerequisite event which opens exquisitely Ca^{2+} selective plasma membrane (PM) channels, augmenting cytosolic Ca^{2+} levels. The principal molecular components of SOC entry in many cell types include the ER-inserted STIM1 and the Orai1 PM channel subunits. Here we present the activation mechanism upon store-depletion; resulting in an oligomerisation of STIM1 proteins and a conformational reorientation that allows a physical interaction at ER-PM junctions with the Orai1 channels. The fluorescence resonance energy transfer (FRET) microscopy in combination with whole-cell patch-clamp recordings and side-directed mutagenesis enables to precisely monitor the time-dependent localization, interaction and activation of the store-operated STIM1/Orai1 channel complex. A novel FRET based approach identified high and low affinity coupling sites within the STIM1 and Orai1 proteins. Our results lead to a dynamic choreography of STIM1 and Orai1 for activation of store-operated calcium channels.

O-157

SARS-CoV E protein ion channel characterization by tuning the protein and lipid charge

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SARS coronavirus (CoV) envelope (E) protein forms voltage-independent ion channels with symmetric transport properties in planar lipid bilayers [C. Verdiá-Báguena et al., *Virology*, 432 (2012) 485]. Here, we provide new significant insights on the involvement of lipids in the structure and function of the CoV E protein channel on the basis of three series of experiments. First, reversal potential measurements at several pH conditions reveal the contributions to channel selectivity from ionizable residues of the protein transmembrane domain and also from the negatively charged groups of DPhPS lipid. Second, the change of channel conductance with salt concentration reveals two distinct regimes (Donnan-controlled electrodiffusion and bulk-like electrodiffusion) fully compatible with the outcomes of selectivity experiments. Third, by measuring channel conductance in mixtures of neutral DPhPC lipids and negatively charged DPhPS lipids in low and high salt concentrations we conclude that the protein-lipid conformation in the channel is likely the same in charged and neutral lipids. These series of experiments support the proteolipidic structure of SARS-CoV E channels and explain the key role of lipid charge in channel conductance.

Abstracts

– Channels and Transporters –

O-158

Influenza A virus M2 protein forms a dimeric channel in biomembranes

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The influenza A virus M2 protein has been considered to form an acid-activated, amantadine-sensitive, homotetrameric proton channel. We investigated the oligomerization of the protein in living CHO cells using the coiled-coil fluorescence labeling technique developed in our laboratory and FRET [1]. The protein formed a tetramer at pH 4.9, whereas it existed as a dimer at pH 6.0. The proton channel activity was determined with the pH-sensitive dye SNARF-4F. The intracellular pH was exponentially decreased when the extracellular pH was reduced. The rate constant per protein for the dimer was slightly larger than that for the tetramer. Alanine-scanning experiments showed that His37 was crucial to the channel activity. The addition of amantadine inhibited the tetramerization at acidic pH and the activity of the dimeric channel, suggesting that the drug binds to the dimeric form and blocks both the proton conductance and tetramerization. The amantadine-resistant S31N mutant forms a functional dimeric channel irrespective of pH and the presence of amantadine, indicating that Ser31 is important to amantadine binding. Taken together, the dimer is the minimal functional unit of the M2 channel contrary to the prevailing model.

[1] Kawano, K. et al., Anal. Chem. 85, 3454 (2013)

O-159

Locked-open activation gate impedes recovery from inactivation in Shaker K+ channels

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In the absence of N-type inactivation Shaker potassium channels display slow (C-type) inactivation. It has been shown earlier that Cd^{2+} traps the V476C *Shaker* channels in the open state, even at very negative voltages, by forming a metal bridge between a cysteine in one subunit and a native histidine (H486) in a neighboring subunit. The current experiments tested the hypothesis that locking the activation gate in the open configuration prevents recovery from inactivation. To address this hypothesis we compared the extent of recovery from inactivation for control conditions and in the presence of 20 μM Cd^{2+}. V476C/IR channels contained an alanine in position 449 to facilitate the entry of the channels into the slow-inactivated state. 2.0-s-long depolarizing pulses from a holding potential of -120 mV to +50 mV were applied and when applicable, 20 μM Cd^{2+} was added to the fully inactivated channels. Under control conditions the channels completely recovered from inactivation within 60 s, whereas upon Cd^{2+} application less than 10 % of the current recovered under identical conditions. The protonation of the interacting histidine prevented the Cd^{2+} modification. Our experiments suggest that the closure of the activation gate is essential for the recovery from slow inactivation.

P-160

Selectivity mechanism of FNT channels by potentials of mean force calculations

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The formate-nitrite transporter (FNT) family of membrane proteins is involved in the translocation of monovalent polyatomic anions, such as formate, nitrite and hydrosulfide, across the membrane in variety of microorganisms, among which a number of pathogens. Recently solved structures of five of its members reveal a pentameric protein organization sharing certain structural properties with aquaporins, which suggests a channel-like permeation mechanism. However, the selectivity mechanism of FNT channels and the molecular mechanisms involved in solute permeation remain poorly understood, including the protonation state (charged or neutral) of the solutes while permeating. Moreover, given the wide range of substrates that permeate FNT channels, it remains unclear how the preference for certain substrates has diverged among different members of the family. We use molecular dynamics simulations to compute potentials of mean force for full permeation events across FNT channels. Initial analysis of the FocA formate channel reveals a high permeation barrier for ions through the hydrophobic pore of the channel, pointing towards a selectivity mechanism involving neutral species.

P-161

Permeation and Regulation of Urea Transport in the Urea Transporter B

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Urea transporter B (UT-B) is a passive membrane channel that facilitates highly efficient permeation of urea. In red blood cells (RBC), while the major function of UT-B is to transport urea, it is assumed that this protein is able to conduct water. The crystal structure of a mammalian UT was reported recently as a homotrimer and each monomer contains a urea conduction pore. However, important transport characteristics, such as the regulation of urea transport and water permeability, have not been identified. To understand these transport properties of UT-B, we have performed functional assays on variant RBC characterized by the absence of UT-B or AQP1. From the osmotic and diffusional water permeability coefficients (Pf and Pd), we conclude that UT-B is as efficient as AQP1 in water transport. Interestingly, uptake studies using 14C-urea indicated that the rate of urea transport through UT-B pore was reduced in the absence of AQP1 and can be modulated by changing the osmolarity conditions. Overall, our data provide evidence that urea and water share a common pathway through UT-B pore leading to a potential competition between the two substrates.

Abstracts

– Channels and Transporters –

P-162

Reversible assembly of twin-arginine protein translocation sites

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The Tat translocation machinery transports folded proteins across the cytoplasmic membrane of bacteria and the thylakoid membrane of chloroplasts. How this transporter moves folded proteins of varying size across a membrane without significant ion leak is unknown, and is an area of active investigation. We demonstrate here that the Tat translocation site assembles on demand by substrate-induced association of the protein TatA, providing a mechanism to maintain membrane integrity between transport events. We imaged yellow fluorescent protein (YFP)-tagged TatA to investigate TatA-YFP complexes and used a custom contour-based spot counting algorithm to examine changes in cluster populations in the absence of proton motive force (PMF). Thus we could probe the assembly and disassembly of the TatA-YFP complex under different substrates and with the removal of PMF, in order to test which parts of the transport cycle occur unenergised. Subsequently we additionally investigated the oligomeric states of TatBYFP and TatC-YFP fusions, to examine the relative stoichiometry of TatA, TatB and TatC complexes using step counting frequency analysis of photobleaching traces.

P-163

Carbonic anhydrase II enhances activity of monocarboxylate transporters via direct interaction

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Carbonic anhydrase II (CAII), a ubiquitous cytosolic enzyme catalyzing the reversible hydration of CO_2, enhances transport activity of the proton-linked monocarboxylate transporters MCT1 and MCT4, while leaving transport activity of MCT2 unaffected. This interaction is independent of CAII catalytic activity, but is mediated by the enzyme's intramolecular H^+-shuttle His64. H^+ transfer between enzyme and transporter requires close proximity of the two molecules. This is achieved by direct binding of CAII to the transporter's C-terminal tail, with the acidic amino acid clusters E^{489}EE in MCT1 and E^{439}EE in MCT4 being crucial for direct binding and functional interaction with CAII. Introducing a putative CAII binding site into the C-terminal of MCT2 by exchanging the last seven amino acids of the MCT2 C-terminal with the corresponding amino acids of the MCT1 C-terminal (R^{483}DKESSI to P^{483}AEEESP) indeed enabled CAII to enhance transport activity of MCT2. Our results suggest that CAII forms a "non-catalytic transport metabolon" with MCT1 and MCT4, by directly binding to the C-terminal of the transporter to establish a H^+-shuttle between the two proteins, which stabilizes the H^+ gradient and enhances lactate flux across the cell membrane.

P-164

Interaction of ADWX-1 and COBA1 toxins with Kv1.1 potassium channel by using docking and MD methods

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Potassium channels play a critical role in limiting neuronal excitability. This study is based on Kv1.1-toxin system for drug design. First, we tried to dock Coba1 and ADWX-1 into the Kv1.1 channel by using HADDOCK which is known as one of the best docking programs. After docking, we used MD methods for equilibrium of potassium and toxin complexes and we compared these with the docking results for interacting residues. Finally, we used free energy molecular mechanic simulations to calculate the affinities of Coba1 and ADWX-1 which are adsorbed to Kv1.1. In all these studies, Coba1 toxin's ARG6, LYS10, ARG14, ARG18, LYS21, ILE23, ASN24, LYS28, and TYR30 residues play a key role in the interaction with Kv1.1 channel. But only LYS21 has remained in the same position in both, after docking and after MD simulations. Similarly for ADWX-1 toxin, LYS28 residue has shown the same stable situation in both cases, after docking and after MD simulations.

P-165

Sodium-Galactose Transporter: first steps of transport mechanism investigated by molecular dynamics

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Sodium-Galactose transporter (SGLT) is a secondary active symporter able to accumulate sugars like glucose/galactose into cells using the electrochemical gradient of Na+ across the membrane. This transport is thought to occur via an alternating-access mechanism in which the protein, switching from an outward to an inward-conformation, guarantees a correct uptake of sugar molecules important in intestinal absorption and renal reabsorption. In this study, using classical MD simulations and Bias Exchange Metadynamics, we would like to understand the molecular basis of the first steps of the transport cycle, the stabilisation of the Sodium ion in its binding site and the coupled mechanism of binding/dissociation of Sodium and Galactose. This knowledge and the understanding of the full transport mechanism could shed light for the development of therapeutic approaches.

Abstracts

– Channels and Transporters –

P-166

Minimal viral K+ channels as robust model systems for understanding structure/function correlations

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The channel proteins from Chlorella viruses are miniature versions of K^+ channels; in essence they represent the pore module of complex K^+ channels from eukaryotes. The combination of small size and robust function makes them good model systems for understanding basic structure/function correlates in K^+ channels. The channel Kcv_{NTS} counts only 82 amino acids per monomer and molecular dynamics simulations suggest that the channel is quasi fully embedded in the membrane bilayer. The intimate interaction between the channel protein and the surrounding bilayer offers the possibility to examine the performance of the channel in different experimental systems. We therefore performed single channel experiments in vertical and horizontal lipid bilayers as well in the membrane of native HEK-cell system. Furthermore we monitored channel conductance and gating in lipids with different head-groups and/or lipids with fatty acid chains of different length.

P-167

Potassium channel Kcv interaction with model membranes

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K^+- channels are transmembrane proteins abundant in virtually all biological systems. Although these have been subject to intense investigation, basic relationships between their structure and their function are not understood sufficiently. We study a viral K^+- channel, Kcv, which is the smallest potassium channel known to be expressed naturally in a eukaryotic cell in interaction with model membranes. Biochemical and functional evidence indicate that K^+- channels localize to lipid raft microdomains on the cell surface and that changes in membrane cholesterol can directly modulate ion channel function.
We present spectroscopic data revealing the structural changes induced by the presence of Kcv, on model biomimetic membranes as a function of membrane composition. Particular focus is given to mimic specialized lipid raft composition and their typical asymmetry in the two leaflets. WAXS measurements confirm that the protein insertion affects the average local order of the hydrophobic chains, likewise shifting the thermotropic behavior of the bilayer as also confirmed by DSC. Moreover, at the bilayer scale (SAXS) it is seen that the channel arrangement 'match' in the P_β' phase geometry. Significant results from neutron reflectivity on single floating bilayer will be presented.

P-168

An influence of neuroactive compounds on insect ion channels

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Sodium ion channels (Na_v) embedded in the cell membrane allows to change its dielectric properties. In excitable cells, they cause formation of action potentials [1]. The main goal of our study was to investigate the effect of neuroactive substance such as scorpion toxin LqhαIT on the activity of neuronal Na_v from *Periplaneta americana* cockroach. The nervous system of this organism is well known as a good model for studying the mechanisms of toxin activities [2]. Investigations were made using electrophysiological method double oil gap on single axon. To obtain detailed knowledge on molecular mechanisms of toxin binding molecular dynamics simulations were conducted. In the calculations our new model of the domain IV of the cockroach Na_v obtained by homology modeling was used [1] [3].
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P-169

The role of pH in the regulation of anionic transport in pollen

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Pollen tube growth is an well known example of apical polarized cellular growth and a representative model for many phenomena such as cellular growth, morphogenesis and developmental studies. Over the past decades it has been shown that pollen tube growth is underlined by multiple ionic fluxes, each with specific properties, distinct roles and unique spatial and temporal patterns. However, despite considerable effort, still not much is known about the molecular identity or regulation mechanisms of many of these putative transporters, in particular, the anionic transporters. In the past decade, our group has observed large chloride fluxes entering the shank of the tube, and its exit at the tip. We have also shown recently the presence of three populations of calcium-regulated anion currents in the membrane of the lily pollen protoplasts by means of the patch-clamp technique.
Here, we present novel data from patch-clamp experiments in pollen protoplasts demonstrating a strong regulation of these anionic currents by both internal and external pH. We are also developing a mathematical model to integrate the acquired data and test different hypothesis on to the nature of the channels/transporters responsible for the observed phenomena.

Abstracts

– Channels and Transporters –

P-170

Critical residues within membrane domains regulate the function and trafficking of NMDA receptors

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N-methyl-D-aspartate (NMDA) receptors mediate fast excitatory neurotransmission in the mammalian central nervous system (CNS). A growing body of evidence indicates that abnormalities in NMDA receptor function are associated with a number of neurological and psychiatric disorders. Thus, the precise understanding of the molecular mechanisms underlying the regulation of the number of surface NMDA receptors is critical for our knowledge of normal synaptic physiology as well as of the etiology of many human CNS diseases.
In this study, we investigated the mechanism by which the membrane (M) domains of both GluN1 and GluN2A-C subunits regulate the surface number of NMDA receptors using quantitative assays, biochemistry, microscopy and electrophysiology on cultured heterologous and cerebellar granule cells. We found that there are key amino acid residues within both the GluN1 (W636 and Y647/T648) and GluN2 (e.g. GluN2B-W635 and S645/Y646/T647) M3 domains that regulate the trafficking of the NMDA receptors to the cell surface, likely on the level of their ER processing, as well as the functional properties of NMDA receptors. We conclude that the M3 domains are critical structural and functional determinants of the NMDA receptor trafficking.
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The single channel water permeability of aquaporins

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Aquaporins (AQPs) facilitate water transport in all forms of life. Their reported water permeabilities p_f span a wide range: from three times the diffusion limit to three orders of magnitude below it. The exact molecular determinants of such diversity are unknown, mainly because the p_f values for one and the same protein are so broadly dispersed. For example, four independent molecular dynamics simulations envisioned that water transport through the aquaglyceroprotein GlpF (glycerol facilitator of E.coli) would be much faster than through channels exclusively transporting water, such as the orthodox aquaporins AQPZ (from E.coli) or human AQP1. However, three existing independent experimental studies all conferred the opposite result. To solve the conundrum, we now used (i) fluorescence correlation spectroscopy to count the number of proteins reconstituted per lipid vesicle and (ii) stopped flow measurements to determine p_f of these vesicles. We observed water movement through all three proteins AQP1, AQPZ and GlpF at roughly the same rate which suggests bulk water mobility.
Acknowledgments: The project was supported by grant P23466 of the Austrian Science Fund (FWF) to P.P.

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Binding of cisplatin impairs the function of Na+/K+-ATPase by binding to its cytoplasmic part

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Na^+/K^+-ATPase generates concentration gradients of sodium and potassium ions across the plasma membrane of all animal cells. These gradients are responsible for membrane transport and for electrical activity of nerve and muscle. Change of activity of this enzyme can influence these mechanisms and can potentially result in variety of diseases. Cisplatin is the most widely used chemotherapeutics in cancer treatment. However, treatment by the cisplatin has many side-effects such as hearing loss, neuropathies and acute renal failure. Gradient of sodium ions is essential for the correct functioning of the kidneys. According to our results on whole protein and on its large [C45] and small [C23] cytoplasmic loops, we can conclude that the cisplatin significantly inhibits activity of the sodium potassium pump. The next conclusion is that cisplatin binds to cysteine residues in the large cytoplasmic loop and the molecular mechanism of the inhibition might be related to cisplatin binding to Cys367.
Acknowledgement: This work was supported by Czech Ministry of Health NT11071-4/2012 , by Palacky University (PrF-0213-017) and by the Ministry of Education, Youth and Sports of the Czech Republic, grant No. CZ.1.07/2.3.00/20.0057

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The gating mechanism of the human aquaporin 5

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Aquaporins are protein channels located across the cell membrane with the role of conducting water or other small sugar alcohol molecules (aquaglyceroporins). The high-resolution X-ray structure of the human aquaporin 5 (HsAQP5) shows that HsAQP5, as all the other know aquaporins, exhibits tetrameric structure. By means of molecular dynamics simulations we analyzed the role of spontaneous fluctuations on the structural behavior of the human AQP5. We found that different conformations within the tetramer lead to a distribution of monomeric channel structures, which can be characterized as *open* or *closed*. The switch between the two states of a channel is a tap-like mechanism at the cytoplasmic end which regulates the water passage through the pore. The channel is *closed* by a translation of the His67 residue inside the pore. Moreover, water permeation rate calculations revealed that the selectivity filter, located at the other end of the channel, regulates the flow rate of water molecules when the channel is *open*, by locally modifying the orientation of His173. Furthermore, the calculated permeation rates of a fully *open* channel are in good agreement with the reported experimental value.

Abstracts

– Channels and Transporters –

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Molecular dynamics study on mechano-gating of the bacterial mechanosensitive channel MscL

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The bacterial mechanosensitive channel MscL is constituted of homopentamer of a subunit with two transmembrane inner and outer (TM1, TM2) helices and gated by tension in the membrane. TM1s line the ion permeable pore and the narrowest part around G22 is called gate. In TM2, some amino acids including F78 and F83 face the bilayer and play a role of mechanosensor. Our previous experimental study shows two mutant MscLs that are harder (F83N) or easier (G22E) to open than wild type (WT) but it remains unclear why the opening behavior of the mutants differs from that of WT. To address this question, we performed MD simulations to get insight into the detailed differences in the opening process among them. As a result, it was shown that F83N could not open the gate as widely as WT and some water molecules penetrated into the space between F78 and lipids. F78 is known to be a major tension sensor with strong interactions with lipids, thus F83N seems to be less effective mechanosensitivity due to a hydrophilic substitution of N83. G22E penetrated much more water molecules through the gate than WT. The gate region of WT is hydrophobic and no water penetration was observed. The substitution of E22 changed the environment of the gate to hydrophilic, which occurs much water penetration.

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The open pore of SecYEG does not show physiologically relevant ion selectivity

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The bacterial translocon SecYEG resides in the cytoplasmic membrane and either translocates secretory proteins from the cytoplasm to the periplasm or reconstitutes transmembrane proteins into the cytoplasmic membrane. In its open and unoccupied state, it is a large ionic channel with ∼0.5 nS conductivity under physiological conditions [1]. Unhindered proton flow through this channel would be lethal due to collapse of the transmembrane proton gradient.
To test the ion selectivity of SecYEG, we reconstituted it into lipid bilayers and measured the reversal potential under asymmetric salt. Both the channel that was activated by signal peptides and the plug deletion mutant showed a very modest preference for anions over cations (the permeability ratio is 4.1±1.6). We thus conclude that SecY is a deficient barrier even to K^+ ions, and the opened channel by itself cannot sustain the proton motif force across the cytoplasmic membrane.
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P-176

Molecular origin of VDAC selectivity towards inorganic ions

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The voltage-dependent anion channel (VDAC) forms the major pore of the outer mitochondrial membrane. Its high conducting open state features a moderate anion selectivity. We examined the translocation of small inorganic ions across VDAC using molecular and Brownian dynamics simulations together with continuum electrostatic calculations of the mouse VDAC wild-type and mutants to propose a molecular mechanism for VDAC selectivity. The analysis of the simulation trajectories indicates no distinct pathways for ion diffusion and no long-lived ion-protein interactions. It points to a pore region comprising the N-terminal helix and the barrel band encircling it as a major checkpoint of the ion transport through the channel. The calculated dependence of the ion distribution in the wild-type channel with the salt concentration is in very good agreement with the experimental observations and can be explained by an ionic screening of the distribution of the charged residues located in the pore. Altogether these results bolster the role of electrostatic features of the pore as the main determinant of VDAC selectivity towards inorganic anions [1,2].
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[2] E.-M. Krammer et al. (2013) *Biochim. Biophys. Acta* **1828**:1284.

P-177

A novel fluorescence system for potassium channel Kv1.1 and Kv1.3 ligand screening

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Voltage-gated potassium channels (Kv) are involved in a number of serious immune, neuronal and cardiac disorders. Efficient and selective Kv channel modulators have attracted attention as prospect therapeutic drugs for clinical use.
We present a new method for discovery and quantitative analysis of novel Kv1.1 and Kv1.3 potassium channel pore blockers. The method combines functional expression of target protein in cytoplasm membrane of *E.coli* and fluorescence detection of ligand-receptor interaction using confocal microscopy. We use chimeric proteins KcsA-Kv1.1 and KcsA-Kv1.3, which were constructed by transferring ligand binding site of Kv1.1 and Kv1.3 channels into respective region of the bacterial potassium channel KscA. Chimeric proteins keep the ability to bind well-known pore blockers of respective eukaryotic potassium channels.
The proposed method enables one to search for Kv1.1 and Kv1.3 ligands both among individual compounds and in complex mixtures of natural biologically active peptides. The assay was successfully applied to crude venoms and their fractions at all stages of venom separation. Novel high-affinity pore blockers of Kv1.1 and Kv1.3 channels were identified.

Abstracts

– Channels and Transporters –

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The Formate/Nitrite Transporter Family of Anion Channels

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The Formate/Nitrite Transporter family of integral membrane proteins comprises pentameric channels for monovalent anions that exhibit broad specificity for small anions such as chloride, the physiological cargo molecules formate, nitrite and hydrosulfide, and also larger organic acids. Three-dimensional structures are available for the three known subtypes, FocA, NirC, and HSC that reveal remarkable evolutionary optimizations for the respective physiological context of the channels. FNT channels share a conserved translocation pathway in each protomer, with a central hydrophobic cavity that is separated from both sides of the membrane by a narrow constriction. A single protonable residue, a histidine, plays a key role by transiently protonating the transported anion to allow an uncharged species to pass the hydrophobic barrier. Further selectivity is reached through variations in the electrostatic surface potential of the proteins, priming the formate channel FocA for anion export, while NirC and HSC should work bi-directionally. Electrophysiological studies have shown that a broad variety of monovalent anions can be transported, and in the case of FocA these match exactly the products of mixed-acid fermentation, the predominant metabolic pathway for most enterobacterial species.

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Biophysical characterization of AQP7 in adipocytes

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The plasma membrane aquaglyceroporin 7 is expressed in adipose tissue and there is evidence pointing towards its role in glycerol transport in adipocytes and a correlation between AQP7 deregulation and the development of obesity. However no direct assessment of the contribution of AQP7 to glycerol and water transport has been reported in adipocytes. This work aims to investigate the role of AQP7 expression on 3T3-L1 adipocyte membrane permeability to glycerol and water. In order to attain this goal, two stable cell lines were obtained from the wild type 3T3-L1: AQP7-knockdown and AQP7 overexpressing cells. Fluophore loaded cells were subjected to osmotic shocks either by the addition of mannitol (a non-permeable solute) or glycerol. By the means of this non-invasive technique it was possible to gain insight of individual cells response to the applied osmotic stimuli and ultimately assess the values of P_f and P_{gly} with minimal alterations to cell's environmental and physiological status. We observed that, unlike the human isoform, mice AQP7 does not seem relevant for water transport. Most importantly we were able to ascertain that both mice and human AQP7 are functional as glycerol channels, confirming the direct involvement of AQP7 in glycerol transport in these cells.

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Screening aquaporin modulators by heterologous expression in yeast

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Aquaporins (AQPs) are a highly conserved family of transmembrane proteins ubiquitous in nature. The 13 mammalian AQP isoforms (AQP0-12) are differentially expressed in many types of cells and tissues in the body and can be divided in two major groups: those strictly selective for water (orthodox aquaporins), and those that are also permeable to other small solutes including glycerol (aquaglyceroporins). They play fundamental roles in human physiology and pathophysiology, therefore AQP modulators are predicted to be of broad medicinal utility.
Although some compounds have been described as AQP inhibitors, few are considered suitable candidates for clinical development. Also there are numerous critical issues to be addressed before engaging in clinical studies, such as their selectivity as well as modulation mechanism. To assist these questions, we expressed mammalian AQPs in a *S. cerevisiae* strain deficient in endogenous aquaporins. All the AQPs were localized at the cell membrane and were functional. This yeast-system enables to access AQP function individually, and screen for AQPs' modulators, as well as to study AQP regulation.

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Coarse-grained MD simulation study focusing on the conformational change of MscL

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Mechanosensitive (MS) channels activate by sensing membrane tension. One of MS channels MscL is a homopentamer of a subunit with transmembrane inner and outer (TM1 and TM2) helices. TM1s including G22 line the ion/water permeable pore and TM2s including F78 face the bilayer to sense membrane tension. We performed an opening process of MscL using all-atom molecular dynamics (MD) simulations, but it was suffering from a short time limitation with excessive amount of membrane tension. In this study, coarse-grained (CG) MD simulations of wild type (WT), G22N GOF and F78N LOF mutant MscLs were performed to reproduce the opening process under more moderate conditions and to get insight into the detailed conformational change of TM1 and TM2 helices. Each CG model was constructed based on the equilibrated all-atom closed MscL. CGMD simulations for the opening of MscL were performed for 3-10 μs under the condition of 90 bar membrane tension.
As a result, G22N (F78N) was actually easier (harder) to open than WT and the whole size of the channel was different among WT, G22N and F78N MscLs, suggesting that both of the amino acid substitutions in the cytoplasmic side of TM1 (G22N) and in the periplasmic side of TM2 (F78N) can affect conformational changes of both TM1 and TM2 helices.

Abstracts

– Channels and Transporters –

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Functional characterization of mammalian AQP3 and AQP7 by heterologous expression in yeast

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Aquaporins (AQPs) are intrinsic membrane proteins that transport water (orthodox aquaporins) and in some cases other small solutes such as glycerol (aquaglyceroporins). In mammals, thirteen AQPs (AQP0-AQP12) are differentially expressed in diverse cells and tissues. In particular the aquaglyceroporins AQP3 and AQP7 are strongly expressed in skin epidermal cells and adipocytes respectively. Evidences that AQP7 is involved in obesity were found in AQP7 null mice who showed adipocytes hypertrophy and later in obese humans. In addition, AQP3 facilitates migration and proliferation of epidermal cells, suggestingan important role in skin carcinogenesis.
Our work aims to functional characterize these membrane channels by expressing mammalian aquaglyceroporins AQP3 and 7 in yeast. For that, we cloned cDNAs from mouse AQP3 and human AQP7 in an appropriate expression vector used to transform a *S.cerevisia*e strain defective in endogenous AQPs. Using GFP as expression reporter, the AQP3-7 cellular localization was determined by fluorescence microscopy. Water and glycerol transport activity of individual aquaporins will be easily characterized by stopped-flow fluorescence technique, as well as the screening of new compounds as activity modulators.

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Modifiers of the membrane dipole potential are promising synergists of antimicrobial agents

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The membrane dipole potential originates from the specific orientation of lipid and water dipoles at the membrane-solution interface. The adsorption of some electroneutral molecules, dipole modifiers, may lead to significant changes in the magnitude of the potential drop. The present study examines the effects of dipole modifiers on the ion channels of various origins reconstituted into artificial planar lipid bilayers. It was shown that some flavonoids, especially phloretin, enhanced membrane activity of antifungal lipopeptide syringomycin from *Pseudomonas syringae* and polyene macrolides from *Streptomyces*, amphotericin, nystatin and filipin; introduction of styrylpiridinium dye RH421 increased activity of antimicrobial lipopeptide surfactin from *Bacillus subtilis* and antibacterial peptides cecropins from *Hyalophora cecropia*. The observed effects may rather be attributed to influence of membrane dipole potential on the formation of lipopeptide and peptide pores and direct interaction of dipole modifiers with polyene/sterol complexes. The study was supported in part by RFBR (12-04-00948,12-04-33121), the Grant of the President of RF (#MK-1813.2012.4), the Program of the RAS «MCB» and the Russian State Contract #8119 (MES,FTP,SSEPIR).

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Tryptophan mutants of KcsA channel as a tool to study ion and lipid-protein interactions

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The K^+ channel KcsA from *S.lividans* has been used as a model system to attempt answering a fundamental question about ion channels: how to reconcile ion selectivity and rapid permeation, two apparently opposed phenomena. According to our thermal stability and fluorescence spectroscopy experiments on wild-type KcsA, K^+ and Na^+ antagonize each other in binding to the protein according to their relative affinity, potassium being more effective in stabilizing the protein against thermal denaturation. Likewise, these assays have been used to monitor phospholipid binding to KcsA, which also modulates channel function.
WT-KcsA intrinsic fluorescence emission derives from five tryptophan residues per monomer, two of them located at the intracellular membrane interface and the rest at the opposite site, close to the selectivity filter. In this work we show how site-directed mutagenesis of one of more of these aromatic residues serves to identify the residue(s) that senses the binding of either ions or lipids and the protein regions involved in the associated conformational changes.
Work partly supported by Spanish BFU2011-25920 and CSD2-2008-00005

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Effect of a gold-based compound on AQP3 permeability and cell proliferation of tumor cells

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Aquaporins (AQPs) are a family of transmembrane proteins present in all types of organisms involved in the transport of water and small solutes such as glycerol. They participate in a wide range of physiological functions, such as water/salt homeostasis, exocrine fluid secretion and epidermal hydration, and are associated with human diseases. Despite their enormous potential as targets for disease treatment, there are no reported AQPs inhibitors for clinical trials.
Recently, we identified a gold-based compound as inhibitor of human AQP3 with high potency and high selectivity [1]. The mechanism of selective binding to AQP3 is due to binding of Au (III) to a cysteine residue (Cys40), postulated by *in silico* methods. In this work we confirmed the mechanism of binding using HEK cells transfected with either AQP3 wild-type and mutant, in which Cys40 residue was replaced by Ser40 residue. Water and glycerol permeabilities were assessed to characterize the kinetics of AQP3 transport and the inhibitory effect of the gold-based compound in human melanoma with high AQP3 expression was correlated with inhibition of cell proliferation.
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Abstracts

– Channels and Transporters –

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Distribution of UCP2 and UCP4 correlates with a certain type of cell metabolism

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Despite intensive research, the functions of mitochondrial uncoupling proteins (UCPs) with the exception of UCP1 are still unknown. Even their expression at the protein level is disputable. We have previously shown that UCP2 transports protons similar to UCP1, is expressed in tissues and cells of the immune system at the protein level and is up-regulated in T-cells due to stimulation in vitro1,2. In our current work we reveal that UCP2 which is expressed in undifferentiated stem cells3, disappears during embryonic stem cells differentiation into neurons. In contrast, UCP4 expression starts simultaneously with the expression of neuronal markers, indicating its strong association with the nervous system. Surprisingly, neuroblastoma cells only contain UCP2. This implies that UCP2 may be a marker for highly proliferative cells that switch to aerobic glycolysis and are totally distinct to neurons. We propose that different UCPs (UCP1-UCP5) are abundant in definite cell types and may be an indicator for certain types of cellular and mitochondrial metabolisms.

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P-187

Mechanosensor and gate is tightly coupled in the bacterial mechanosensitive channel MscL

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The bacterial mechanosensitive channel MscL is constituted of homopentamer of a subunit with an inner (TM1) and an outer (TM2) transmembrane helix. The major issue on MscL is to understand the gating mechanism driven by tension in the membrane. We found that F78 in TM2 acts as a major tension sensor. Neighboring TM1s cross and interact with each other and G22 in TM1 forms a gate. Upon membrane stretch, the helices are dragged by lipids at F78 and tilted, accompanied by outward sliding of the crossings, leading to a gate expansion. We performed MD simulations of several MscL mutants to get insights into the relationship between the tension sensor F78 and the gate. The GOF mutant G22N shows spontaneous opening without membrane stretch and easier to open than WT, while the LOF mutant F78N cannot be opened even under strong membrane tension. To test whether the behavior of G22N is independent of the tension sensing at F78, the double mutant G22N/F78N MscL simulation was performed with and without membrane stretch, and found that G22N/F78N MscL did not begin channel opening in the both conditions, suggesting that the tension sensor and the gate of MscL is tightly connected and that the interaction between the tension sensor and lipids is essentially important for the MscL opening.

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Channel mining: Searching for virus channels in environmental probes

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Viruses, which infect the freshwater algae Chlorella or the seawater alga Micromonas, code for small membrane proteins with the charateristics of potassium channels. These channels have a monomer size of less than 100 amino acids and in their general architecture they represent the "pore modules" of all known potassium channels. This includes two transmembrane domains, a pore helix and the selectivity filter. The structural simplicity and functional robustness makes the small viral K+ channels very good models systems for understanding the molecular basis of channel function. To learn more about structure/function correlates in these simple channels we try to generate a large library of channel sequences. For this purpose we search for channel orthologes in environmental samples, in which viruses are abundant. The new channel sequences will be analysed and channel function will be tested in HEK293 cells with whole-cell patch-clamp measurements.

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ClyA nanopore as emerging platform for protein sensing and characterization

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Recently, we have introduced Cytolysin A from *Salmonella typhi* (ClyA) as the first biological nanopore that allows the investigation of natively folded proteins. The structure of the ClyA dodecamer is ideal for this task because proteins such as thrombin (37 kDa) or MDH (70 kDa) can be electrophoretically trapped between the cis entrance and the narrower trans exit and can therefore be interrogated for several minutes. Ionic currents through ClyA are so sensitive to the vestibule environment that blockades imposed by human and bovine thrombin can be easily distinguished. ClyA nanopores decorated with covalently attached aptamers have selectively captured and conveyed cognate protein analytes into their interiors, but excluded non-cognate proteins. Remarkably from the same purified monomers, we could isolate three nanopore types probably corresponding to the 12mer, 13mer and 14mer oligomeric forms of ClyA. In the lipid bilayers, 12mer and 13mer ClyA nanopores remained open in a wide range of applied potentials (from +90 mV to -150 mV), which allowed detailed investigation of the nanopores using thrombin as molecular caliper. Our results show that even sub-nanometer differences in the size of the nanopore affect greatly the recognition and translocation of protein molecules.

Abstracts

– Channels and Transporters –

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NanoFAST biochip – A method to study pore-forming proteins in nm-sized free-standing lipid bilayers

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We have developed a new technology platform to measure the translocation of fluorescent molecules through protein channels or transporters in free-standing nm sized lipid bilayers. The silicon-based chip contains thousands of 10 μm x 0.8 μm cylindrical cavities and a SiO_2 lid on top with openings in the range of 100 nm. Large unilamellar vesicles (LUVs) or proteo-LUVs can be spread directly on the hydrophilic surface while solvent-free, pore-spanning lipid bilayers are obtained (1).

The flux of fluorescent molecules through pore forming proteins into each single cavity can be read out by inverted fluorescence microscopy. The parallel time-resolved detection of three fluorescent signals enables us to monitor the kinetics of the transport substrate, a control dye and a lipid dye to monitor bilayer integrity at the same time. Hundreds of individual translocation curves can be processed, giving good statistics.

Here we present data on LUV spreading with cavity sealing efficiency, characterization of the free-standing bilayers and the selective transport of dyes through pore forming proteins.

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P-191

Atomic Force Microscopy provides new insight about the mechanism of ATP-binding to UPC1

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Uncoupling protein 1 (UCP1) is a mitochondrial membrane protein involved in non-shivering thermogenesis. Increased attention has been focused on this protein due to its putative involvement in the pathogenesis of obesity. UCP1 is activated by long-chain fatty acids and inhibited by purine nucleotides (PN). The discrepancy between low PN concentrations necessary to inhibit the protein-mediated proton transport and the much higher PN concentrations present in the cell remains unexplained. We previously demonstrated that protein inhibition is overridden when high potentials are applied at constant ATP concentrations[1]. In our current work, we used the topographic and recognition modes of AFM to analyse the ATP-protein interaction at the single molecule level. By using cantilever tips with ATP at different cross-linker lengths[2], we reveal that the distance between the ATP binding site and the lipid surface is 1.27 nm. Based on our results, we hypothesised that the ATP binding site can be accessed from both sides of the membrane. However, only the binding from the c-side would lead to a conformational change in the protein and to its inhibition.

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Abstracts

– Biomolecular Simulation: spanning scales –

O-192

Optimization of an elastic network augmented coarse-grained model to study CCMV capsid deformation

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The major protective coat of most viruses is a highly symmetric capsid that forms spontaneously from many copies of identical proteins. Structural and mechanical properties of such capsids, as well as their self-assembly process, have been studied experimentally and theoretically, including modeling efforts by computer simulations on various scales. Atomistic models include specific details of local protein binding but are limited in system size and accessible time, while coarse grained (CG) models can access to longer time and length-scales, but often lack specific local interactions. Multi-scale models aim at bridging this gap by systematically connecting different levels of resolution. In this talk I will report on our recent coarse-graining efforts of a particular virus, CCMV (Cowpea Chlorotic Mottle Virus), which has an icosahedrally symmetric capsid consisting of 180 identical protein monomers. The basic idea is to use atomistic data of small units (dimers) in order to construct and optimize a supportive elastic network undergirding a MARTINI-level CG model. I will show that this permits us to predict inter-protein conformational flexibility and properties of larger capsid fragments of 20 and more subunits. Furthermore, the model reproduces experimental (Atomic Force Microscopy) indentation measurements of the entire viral capsid.

O-193

Atomistic simulation of single molecule experiments: Molecular machines and a dynasome perspective

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Proteins are biological nanomachines which operate at many length and time scales. We combined single molecule„ x-ray crystallographic, and cryo-EM data with atomistic simulations to elucidate how these functions are performed at the molecular level. Examples include the mechanics of energy conversion in F-ATP synthase and tRNA translocation within the ribosome. We will further demonstrate how atomistic simulations enable one to mimic, one-to-one, single molecule FRET distance measurements, and thereby to markedly enhance their resolution and accuracy. We will, finally, take a more global view on the 'universe' of protein dynamics motion patterns and demonstrate that a systematic coverage of this 'dynasome' allows one to predict protein function.

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O-194

Modeling the effect of nano-sized polymer particles on the properties of lipid membranes

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Every year, tons of plastic litter end up in the oceans. Plastics are degraded slowly into small particles and ingested by wildlife. The presence of plastic microparticles in fish and marine wildlife is massive and well documented, but its impact on the food chain is not understood. The interaction between plastics and cells is mediated by cell membranes. How are cell membranes affected by plastics?
Here we focus on a plastic material of everyday use, polystyrene (PS). We use atomistic and coarse-grained molecular simulations to investigate the interaction between nano-sized PS particles and model membranes. We find that small enough PS particles permeate membranes easily, and partition to the lipid tail region. Polymer nanoparticles significantly modify structural, dynamic and mechanical properties of the membrane; e.g., diffusion slows down and membranes get softer. We then explore the effect of polymer chains on phase-separated ternary mixtures, featuring liquid disordered (Ld) and liquid ordered (Lo) domains. PS accumulates in Ld domains, and it stabilizes phase separation by altering domain composition and increasing the line tension. We suggest that plastic nanoparticles may affect cellular function by altering membrane properties and lateral organization.

O-195

Conformational determinants of peptidic tree-like molecules: insights from MD simulations

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Peptide dendrimers are tree-like molecules formed by alternating functional amino acids with branching diamino acids such as lysine.[1] Unfortunately these molecules have not yielded to structural characterization and little is known about their molecular-level structure. Computational methods seem to be an adequate tool to address these issues.
Herein we present a comprehensive structural characterization of peptide dendrimers using molecular simulation methods.[2] Multiple long molecular dynamics (MD) simulations were used to extensively sample the conformational preferences of several third-generation peptide dendrimers, including some known to bind aquacobalamin.
The results clearly show that a trade-off between electrostatic effects and formation of hydrogen bonds controls structure acquisition in these systems. Moreover, by selectively changing the overall dendrimer charge we are able to manipulate the exhibited behavior.
Our results are in accordance with the most recent experimental evidences and shed some light on the key molecular level interactions controlling structure acquisition in these systems.

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Abstracts

– Biomolecular Simulation: spanning scales –

O-196

A hybrid quantum-chemical approach for free energy simulations

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Free energy simulations are the most accurate computational method for determining free energy differences in biophysics. However, their reliability is limited by two factors: a) the need for correct sampling and b) the accuracy of the parameters in molecular modeling. Parametrization is especially problematic in drug design, where ligands often contain non-standard chemical groups. Here, we present a simple way to solve this problem by post-processing molecular dynamics simulations with quantum-chemical calculations. First, a molecular dynamics trajectory is generated to perform proper sampling of all relevant degrees of freedom. In a second step, the potential energies of each frame of the trajectory are evaluated with a QM/MM approach. Free energy differences are then calculated based on the QM/MM energies. Since all QM/MM post-processing steps are independent of each other, this approach is trivial to parallelize. Thus, highly parallel computer architectures can be employed with high efficiency, which allows us to perform the post-processing very rapidly. We illustrate this approach for the calculation of solvation free energies of amino acids and discuss the benefits and limitations of our method.

O-197

Mixing and matching simulations at different resolutions

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Computer simulations of molecular processes can be carried out at different levels of detail, exchanging accuracy for speed of calculation. To balance this approach and provide better accuracy at high simulation speeds multiscaling schemes were introduced – where a system is simultaneously treated at two or more levels of detail.

In this study we benchmarked the performance of different multiscaling schemes by dual-scale simulating a box of 320 hexadecane molecules with a united-atom fine grain (FG) model and the Martini coarse grain (CG) model. The performance of each scheme was compared regarding a) speedup in entropic convergence, and b) preservation of fine- and coarse-grain structural characteristics.

Three different schemes were compared – the temperature-scaling (TS), the mass-scaling (MS), and the multigraining (MG) schemes – differing in the way FG particles are kept from scattering when close to a full CG representation, and in the way temperature is coupled. TS provided the best compromise between a quick convergence of entropy (∼3.5-fold speedup) and the preservation of structural features. MS reached convergence with a comparable speedup, but at the cost of structural accuracy. Finally, MG preserved structure the best, but with very little speedup.

P-198

Transmembrane domain assembly of semaphorin co-receptors: coarse-grained MD simulations

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The class 3 semaphorins (Sema3s) are a sub-family of proteins involved in a variety of biological processes. Their mechanism of action requires the formation of specific transmembrane receptor complexes. Neuropilins bind the secreted class 3 semaphorins (Sema3A) with high affinity but require a member of the plexin family to form complexes able to activate downstream signal transduction cascades: neuropilins act as the ligand-binding subunit while plexins function as the signal-transducing subunit. Here, we address the role of neuropilin 1 and plexin A1 in Sema3A signaling by characterizing the assembly of their transmembrane domains (TMs) in DOPC and POPC lipid bilayers using coarse-grained simulations. The TMs spontaneously assemble to form homodimers and heterodimers with a very high propensity for a right-handed packing of the helices. Left-handed packing is also observed but with a very low propensity and only in the presence of the plexin A1 TM. Dimerization is driven by GxxxG motifs. PMF calculations predict the hierarchy of association of these intra membrane domains. Sema3 receptors represent major advances toward the design of therapeutic agents for cancer treatment and our results may contribute to therapeutic perspectives.

P-199

Multi-scale simulations of the influenza virus transmembrane peptide in lipid bilayers

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The influenza virus (IV) is responsible for worldwide flu outbreaks like the recent one due to H1N1. Infection starts with the binding of the virus membrane bound Hemagglutinin (HA) to sialic acid at the surface of the membrane of respiratory tract epithelial cells. The virus is then internalized into an endosome, where the low pH triggers conformational changes in HA, ultimately leading to membrane fusion. Here we report multi-scale simulation studies of the transmembrane peptide (TP) of HA, which is responsible for its membrane attachment and also shows evidences of playing a role in the last steps of the fusion process. The simulated TP is constituted by the cytosolic tail, the transmembrane domain and the ectodomain linker. To get the best from different resolutions we first self-assembled the three TP in a pure DMPC membrane with the fast coarse-grain MARTINI force field. The resulting conformations are then simulated with the united-atom GROMOS 54A7 force field, which provides a more realistic atomistic model for the interaction between the peptides and the membrane. We found that the TP trimer forms tightly packed helices of approximately 17 residues (for each peptide) with the aromatic residues flanking it, near the phosphorous.

Abstracts

– Biomolecular Simulation: spanning scales –

P-200

H2 diffusion and proton transfer pathways in a [NiFeSe] hydrogenase: a computational approach

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Hydrogenases (hases) are metalloenzymes able to catalyze the reversible reaction $H_2 \leftrightarrow 2H^+ + 2e^-$. The [NiFeSe] hases are a subgroup of the [NiFe] family with higher activities, but the molecular determinants responsible for this special property are still unknown. Here we investigate if the difference in activity is related with differences in the pathways that allow the transfer of H_2 and protons between the deeply buried active site and the protein exterior. To study H_2 diffusion we performed molecular dynamics simulations of a [NiFeSe] and [NiFe] hase, each with 100 H_2 molecules, using GROMACS and the GROMOS 43a1 force field. To investigate the proton transfer pathways we used a combination of Poisson-Boltzmann calculations and Monte Carlo simulations and a distance based network.

In the [NiFeSe] hase, we found an alternative channel, which is absent in the standard [NiFe] hases, that allows for the direct access of H_2 to the active site; this can be one of the reasons for the higher activities observed in [NiFeSe] hases. The proton transfer pathways of [NiFeSe] hases and [NiFe] hases are also different.

P-201

Dissolving fullerene in alkanes vs. lipid membranes

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C_{60} fullerene is a pure carbon cage molecule with a diameter of 1 nm and approximately spherical shape. Potential applications of fullerene range from energy production to medical imaging and drug delivery. Handling pristine fullerene is problematic because of its low solubility in most solvents, including alkanes. The few good solvents for fullerene are toxic aromatic compounds.

Experimental and simulation studies indicate that fullerene partitions inside lipid membranes. We hereby use coarse-grained molecular dynamics simulations to investigate fullerene aggregation in alkanes and in various lipid membranes. We find that fullerene aggregation is thermodynamically more favourable in alkanes. We use simplified models to interpret aggregation thermodynamics in terms of the differences between isotropic and substantially two-dimensional solvent systems. We find that solvent density and solvent-solvent interactions are key factors in determining fullerene aggregation behaviour. Surprisingly, acyl chain alignment does not affect fullerene aggregation significantly. Confinement in the membrane plane is important only for large clusters in thin membranes. We conclude that lipid bilayers are effective and biocompatible solvents for pristine fullerene.

P-202

Multiscale kinetic description of disease-related peptide assembly

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The aberrant aggregation of amyloidogenic proteins is one of the main factors for the onset of neurodegenerative diseases. One step towards understanding the disease cause is to elucidate the assembly mechanism of the proteins involved. Here we investigate the assembly processes of two short peptides derived from aggregating proteins: the polar GNNQQNY peptide from the yeast protein Sup35 and the KLVFFAE peptide with hydrophobic core and charged termini from the amyloid beta-protein. Our goal is to quantify differences in the assembly processes of the two peptides due to differences in their primary structures and extend the implications to the full length proteins. To this end we use a multiscale approach by combining coarse grained (Bereau et al. 2009) and all-atom MD simulations (Gromos96). The kinetic behavior of the peptides during assembly is described by transition networks between aggregation states and first passage time distributions to representative aggregation states. The aggregation states are defined in terms of oligomer size, H-bond pattern, alignment and beta content. The relationship between aggregation states within the free energy landscape is described by disconnectivity graphs.

P-203

Frontier residues lining globin internal cavities present specific mechanical properties

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The internal cavity matrix of globins plays a key role in their biological function. Previous studies have already highlighted the plasticity of this inner network, which can fluctuate with the proteins breathing motion, and the importance of a few key residues for the regulation of ligand diffusion within the protein. In this work we combine all-atom molecular dynamics and coarse-grain Brownian dynamics to establish a complete mechanical landscape for six different globins chain (myoglobin, neuroglobin, cytoglobin, truncated hemoglobin and chains and of hemoglobin). We show that the rigidity profiles of these proteins can fluctuate along time, and how a limited set of residues present specific mechanical properties that are related to their position at the frontier between internal cavities. Eventually we postulate the existence of conserved positions within the globin fold, which form a *mechanical nucleus* located at the centre of the cavity network, and whose constituent residues are essential for controlling ligand migration in globins.

Keywords: Globins, molecular dynamics, coarse-grain model, protein mechanics, cavity network.

Reference: Bocahut et al., *JACS* **2011**, 133, 8753-8761.

Abstracts

– Biomolecular Simulation: spanning scales –

P-204

FATSLiM: a new analytical tool dedicated to MD simulations of lipid membranes

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With the increase of both the size and the duration of the Molecular Dynamics (MD) simulations of membrane systems arises some issues related to the analysis of generated data. In order to overcome these difficulties, the development of a new tool began: FATSLiM ("Fast Analysis Toolbox for Simulations of Lipid Membranes") aims to analyze and extract physico-chemical data from MD trajectories of membrane systems. Focused on the properties of the lipid bilayers, FATSLiM is yet capable of extracting lipid bilayer thickness, area per lipid and lateral diffusion.
FATSLiM is also designed to be efficient in order to minimize the time required for analysis. Thus, only a few seconds are required to extract the thickness of the bilayer vesicle formed by a lipid includes about 3000. Generally, FATSLiM is often faster (up to 100 times) than the existing softwares.
To date, FATSLiM is also the only tool that can extract physico-chemical data from MD simulations of non-planar lipid bilayers. FATSLiM is then perfectly suited for the analysis of the effect of a protein (or other molecule) on the properties of a membrane.

P-205

Self-assembly of cysteine on Au(110)-(1×1): a first principles study

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We have studied the formation of the cysteine clusters on the relaxed Au(110)-1×1 surface by means of density functional-theory calculations within the framework of LCAO and norm-conserving pseudopotentials.
Because of the flexibility of the thiol side chain, cysteine exhibits 71 unique conformers, therefore differences in the molecule-surface interaction mechanism are determined by the orientation of the N and S atoms. We investigated the clusters formed by the cysteine rotational conformers. Due to their geometric structure, these cysteine conformers allow to different functional groups to interact with the metal surface. For each structure, we investigate the protonated and de-protonated forms. The total-energy optimization was accompanied by investigations of the electron transfer and changes in the electronic structure upon adsorption.
We pointed out a decreasing trend for the binding energy per molecule by increasing the cluster's size. We comment on this effect by using our results on the electronic density of states and the molecule-surface charge transfer. Also, the geometric parameters are discussed in this context.
Our results should be relevant to understand the physico-chemical properties of self-assembled cysteine monolayers and other higher coverage structures.

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Computational study of the conformational preferences of kyotorphin derivatives

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Kyotorphin (KTP) is an endogeneous dipeptide (L-Tyr-L-Arg) with analgesic activity when injected directly into the brain but unable to cross the blood–brain barrier (BBB). Several derivatives have been developed to improve the analgesic activity upon systemic administration. The amidation of KTP (KTP-NH_2) [Ribeiro *et al.*, *Br J Pharmacol*, 163, 964, 2011] and the grafting of ibuprofen in KTP-NH_2 (IbKTP-NH_2) [Ribeiro *et al.*, *Mol Pharmaceutics*, 8, 1929, 2011], result in highly analgesic forms that seem able to cross the BBB. In a previous work, molecular dynamics (MD) simulations were used to investigate the membrane-induced conformational changes in KTP [Machuqueiro *et al.*, *J Phys Chem B*, 114, 11659, 2010]. In this work, we performed MD simulations of KTP-NH_2 and IbKTP-NH_2 both in water and in membrane, to gain molecular insight into the effects of the chemical changes introduced in the derivatives. Afterwards, an extensive conformational analysis was done based on the energy surfaces on multidimensional representation spaces of the conformations. Here, we will focus on the results of this analysis that reveal the structural complexity such small molecules can exhibit, particularly in a membrane environment.

P-207

Constant-pH MD study on the pulmonary surfactant protein C misfolding: pH and deacylation effects

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Chloroform/methanol mixtures have proven to be very useful to mimic the membrane environment due to their preferential solvation effects and extensively used to study the misfolding of the surfactant protein C (SP-C). This small highly hydrophobic protein adopts a mainly helical structure while associated with the membrane, but misfolds into a β-rich structure under certain environmental conditions, eventually leading to the formation of amyloid aggregates associated with Pulmonary Alveolar Proteinosis (PAP). In order to understand the properties relevant for the SP-C loss of structure and consequent amyloid fibril formation in PAP, we have performed two sets of constant-pH MD simulations using a chloroform/methanol/water mixture and a dipalmitoylphosphatidylcholine (DPPC) bilayer. We have observed that the reduced ability of the solvent mixture for stabilizing the charged residues promotes intra-protein interactions that contribute to the loss of structure and formation of β motifs, which are not observed in the DPPC simulations. These contrasting results in the different sets of simulations call into question the suitability of this type of solvent mixtures to mimic a membrane environment and to understand the misfolding process under physiological conditions.

Abstracts

– Biomolecular Simulation: spanning scales –

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When Fat Means Thin: Nascent Lipid Droplets in POPC Membranes

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Lipid droplets (LD) are primary repositories of esterified fatty acids and alcohols in animal cells. These organelles originate on the lumenal or cytoplasmic side of endoplasmic reticulum (ER) membrane and are afterwards released to the cytosol.
In this talk, we report subatomistic microsecond-timescale simulations of LD formation in systems containing 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), triolein (TO), cholesterol (CHOL), and water. We found that (1) nascent LDs originated inside POPC bilayer and contained TO and CHOL; (2) CHOL poorly participated in LD formation, but was present at the POPC/TO interface; (3) unlike in water, the shape of LD inside POPC bilayer was planar with its dimension in the bilayer normal direction more than four times smaller than in the two other directions; (4) although containing more than 6,000 lipids (over 30 nm diameter), LD did not bud from the bilayer; (5) TO-rich systems rearranged into emulsion-type phase, but did not destruct POPC bilayer.
The presented research is the world's first attempt to describe ontogenesis and constitution of real-sized cholesterol containing lipid droplets using subatomistic precision.

P-209

Guiding molecular dynamics using solution X-ray scattering spectra

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Advances in solution X-ray scattering techniques (WAXS) offer the ability to probe transient changes in molecular conformations down to picosecond levels, [1] and at resolutions down to secondary structure levels where their fluctuations become apparent. [2] The spectra contain valuable distance information on the global morphology of the molecule – this can be translated into biasing potentials useful for driving molecular dynamics simulations. This link between solution spectra and molecular structure will be important in deriving and tracking protein conformations and understanding structure-function relationships.
We implement in GROMACS one such WAXS calculation/driving method based on the explicit solvent formulation of Park *et. al.*,[3] and present ongoing validation work. The ensemble-average spectra and forces can be calculated with minor effects on performance. We will discuss the physical interpretation of a WAXS potential, and demonstrate the ability to drive conformational changes on model systems such as lysozyme and LAO-binding protein folds.
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P-210

Accurate Prediction of Kinetics and Thermodynamics of a Peptide Model using μs-long MD Simulations

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cyc-RKAAAD is a short cyclic peptide known to adopt a stable single turn α-helix in water. Due to its simplicity and the availability of thermodynamic and kinetic experimental data, cyc-RKAAAD poses as an ideal model for evaluating the aptness of current molecular dynamics (MD) simulation setups to sample conformations that reproduce experimentally observed properties. Here, we extensively sample the conformational space of cyc-RKAAAD using μs-long MD simulations and, using Cartesian-coordinate PCA (cPCA), we construct its energy landscape, thus obtaining a detailed description of the helical and non-helical subensembles. The cPCA state discrimination, together with a Markov model built from it, allowed us to estimate the equilibrium constant of unfolding (1.257) and the relaxation time ($\sim$0.435 μs) at 298.15 K, which are in excellent agreement with the experimentally reported values (1.092 and 0.42 μs) [1]. Additionally, we compared this landscape with the ones obtained by REMD and bias-exchange metadynamics and discuss the sampling and computational gains achieved. Overall, modern simulations methods are shown suitable to explore the conformational behavior of peptide systems with a high level of realism.
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P-211

Role of many-body correlations in hydrophobic hydration

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The hydrophobic effect is commonly associated with the demixing of oil and water at ambient conditions and plays the leading role in determining the structure and stability of biomolecular assembly in aqueous solutions. On the molecular scale hydrophobic effect has an entropic origin.
Analyzing collective fluctuations in water clusters [1] we are able to provide a fundamentally new picture of hydrophobic effect based on pronounced many-body correlations affecting the switching of hydrogen bonds between molecules. These correlations emerge as a nonlocal compensation of reduced fluctuations of local electrostatic fields in the presence of an apolar solute. In the presence of the solute the maximized electrostatic noise is a result of nonlocal fluctuations in the labile HB network giving rise to strong correlations among at least up to four water molecules.
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Abstracts

– Biomolecular Simulation: spanning scales –

P-212

Torsion and curvature of FtsZ filaments

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FtsZ plays a crucial role in cell division. Experiments have shown that the proteins form long, flexible filaments; however, their dynamics and force generation mechanisms remain unclear. The use of simplified models in which the interaction parameters between monomers may be tuned to reproduce the structures observed experimentally has proved to be a fruitful approach [1]. Experimental progress suggests that torsion and anchoring of the filaments to the membrane may be relevant to understanding the global behaviour of these systems [2].
We present simulations for a lattice model that includes these two aspects, missing in previous models. Filament torsion and curvature compete with the preferred monomer orientation on the substrate and produce a complex phase diagram which can be experimentally tuned through the design of protein mutants with an specific anchoring to the membrane. The exploration of our model over the space of interaction parameters suggests the interaction among monomers has been biologically selected to be in the polymorphic region of the phase diagram, so that they may be induced to assemble structures under the effects of weak biochemical signals.
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P-213

Replica-permutation method for biomolecules as a better alternative to replica-exchange method

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Efficient sampling in the conformational space is necessary to predict the native structures of proteins. The replica-exchange method (REM) is one of the most well-known methods among the generalized-ensemble algorithms which realize efficient sampling in the conformational space for biomolecular systems. We had recently proposed a better alternative to the REM, the replica-permutation method (RPM) [1], in which temperatures are permutated among more than two replicas, while they are exchanged only between two replicas in REM. Furthermore, the Suwa-Todo [2] algorithm is employed in RPM instead of the Metropolis algorithm.
We will show the results of RPM applications to a double-well potential system, Met-enkephalin in a vacuum, and a C-peptide in explicit water in our presentation. These results will be compared with those of REM to see sampling efficiency of RPM.

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P-214

Focussing the computational microscope upon the cell envelope of E.coli: a molecular dynamics study

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Gram-negative bacteria such as *E.coli* are protected by a surprisingly complex cell envelope. The cell envelope is composed of membranes that form a protective barrier around the cells, and control the influx and efflux of solutes via various routes and mechanisms. To enable a comparative study of the *E.coli* inner and outer membranes, we have constructed molecular models of both membranes. Our atomistic-level models incorporate the heterogeneity of the various lipid types, including the lipopolysaccharide molecules of the outer membrane, the mixture of phospholipids in the inner membrane and some of the peptidoglycan of the periplasmic space. We have performed a series of simulations exploring how these various constituent components influence the structure and dynamics of the membrane. In particular, we have simulated the process of electroporation, which has revealed molecular-level insights into key differences in the behavior of the two E.coli membranes.

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Molecular basis of the stability of G-quadruplexes - molecular dynamics simulation study

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G-quadruplexes (G4) are secondary DNA structures in which four guanine residues are held in a plane by Hoogsteen bonds. G4 mainly occur at the ends of linear chromosomes (telomeres), terminating in a 3'-single-stranded overhang of TTAGGG tandem repeats. The formation of G4 in telomeres has been shown to decrease the activity of the enzyme telomerase, which is responsible for maintaining length of telomeres and is involved in the majority of all cancers. Thermodynamic stability of varius forms of G4, expressed as the unfolding free energy, was determined experimentaly, however the molecular basis of G4 structure stabilization has not been fully elucidated. The aim of our research was to determine the microscopic origin of the G4 thermodynamic stability and structual diversity. Using equilibrium molecular dynamics (MD), steered MD and free energy simulations we focused on comparing the properties of parallel, antiparallel and mixed G4 structures. By detailed thermodynamics analysis of folding substeps and decomposing the overall unfolding free energies into enthalpic and entropic contributions, we identified the possible driving forces governing the G4 folding process. In particular, the molecular mechanism of higher stability of the anti-parallel structures is suggested.

Abstracts

– Biomolecular Simulation: spanning scales –

P-216

The flexibility of fibrinogen is important to understand its adsorption behavior

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Fibrinogen (fg) is a large, dimeric blood protein, which plays a central role in blood clotting and immune response. If fg forms layers on the surface of external bodies, such as implants, inflammation and thrombosis can be the result. Because of these reactions, the behavior of fibrinogen near the surface of materials has represented an important research topic for several decades, prompting both experimental and computational studies. In some of these studies, it is assumed that fg can be modeled as a stiff dumbbell, bending slightly around its central region.
Here we present molecular dynamics simulations that reveal a different bending mechanism. We have simulated fg in full atomistic detail with explicit solvent. In the trajectories we can identify bending events that occur at a hinge point located in the coiled-coil regions of fg. Our simulations allow us to identify highly populated conformations of the hinge and demonstrate that the rest of the protein remains stiff. This implies a bending behavior that is qualitatively different from current models. We suggest a new model that replaces the single hinge at the central domain with one hinge per protomer. The model provides a qualitative interpretation of experimental results for the bending distribution of adsorbed fg.

P-217

Modelling protein-ion interactions in nonaqueous solvents

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The role of counterions in nonaqueous enzymology is a long-standing issue with numerous fundamental and technological implications. To gain a molecular insight into this subject, the X-ray structure of subtilisin soaked in CsCl and acetonitrile was determined[1]. Due to the limitations of the crystallographic analysis (crystal contacts, artificial electrostatic environment, lack of explicit dynamics, etc.), we pursued this characterization by simulating the protein and ions in acetonitrile solution[2].
Multiple molecular dynamics simulations, in different conditions, were performed and we observed that chloride ions tend to stay close to the protein surface, while cesium ions often move away. Similar ion distributions were found when sodium was used instead of cesium, validating the use of Cs^+ as a model for more physiological ions. However, Na^+ forms stronger interactions with Cl^-, decreasing the probability of protein-anion interactions, which may explain the experimentally observed cation-dependent catalytic rate. This work provided important contributions to the understanding of protein-ion interactions in non-conventional media and we are now studying other systems, to obtain a general picture.
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P-218

Probing energy transduction mechanisms in bacteriorhodopsin using electrostatic calculations

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Bacteriorhodopsin (bR) is a light-driven proton pump, and a well characterized model system for energy transduction. In response to luminous stimuli, bR transfers a proton from the cytoplasmic to the extracellular side of the membrane. This pumping of protons generates an electrochemical proton gradient across the membrane, which is known to affect the pumping rate of the protein. bR has been extensively studied both experimentally and computationally, although, among the different theoretical studies performed, only a few have considered the effect of a pH gradient.
We have modified our continuum electrostatics method in order to include a pH gradient, and observed the effect of this gradient on the titration behavior of bR, particularly regarding the residues which are known to participate in the proton transfer process.
bR was inserted in a DMPC lipid bilayer and simulated using molecular mechanics/dynamics (MM/MD) methods. All titratable sites of bR were considered to be connected to either the cytoplasmic or the extracellular side of the membrane, which were exposed to different pH values. We then analyzed the effect of the pH gradient on important residues by calculating their two-dimensional titration curves as function of the pH on each side of the membrane.

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T-20 and T-1249, HIV fusion inhibitors, interacting with model membranes: a molecular dynamics study

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Peptide fusion inhibitors (FI), such as T-20 (Enfuvirtide or Fuseon) or T-1249 interfere with human immunodeficiency virus (HIV) fusion of the virus envelope to the immune system cells, effectively inhibiting the process by binding to the protein machinery responsible by recognition and fusion. It was observed that both peptides interact with 1-palmitoyl-2-oleyl-phosphatidylcholine (POPC) (liquid disordered–ld) and POPC/cholesterol(1:1) (liquid ordered–lo) bilayers. Interaction of these fusion inhibitor peptides with both the cell membrane and the viral envelope membrane appears to be paramount for function. We addressed this problem with a molecular dynamics approach trying to ascertain the differences in the interaction of T-20 and T-1249 with ld and lo model membranes. To this effect, structural and dynamical parameters of the species involved are calculated and discussed. T-20 and T-1249 interactions with model membranes are shown to have measurable differences on all parameters analyzed. T-1249 interaction is generally stronger on all accounts.
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Abstracts

– Biomolecular Simulation: spanning scales –

P-220

QM/MM Implementation of fireball DFT code and first results

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We implemented a new QM/MM interface with the fireball DFT code[1] and the Amber molecular dynamics package. Fireball is a MD DFT code with excellent computational efficiency that, in combination with amber, allow us to study biomolecular systems using MD simulations. The QM/MM implementation is based in the sqm code[2] of amber: the electrostatic embedding is implemented like a Three-body interaction with the electronic structure and a coulombic interaction with the cores. The implementation have been tested in two models A) The study of the HOMO-LUMO energy gap in retinal for channelrhodopsin along 100 ps that describe the experimental difference in the wavelength absorption[3] due to the pH-driven change of the protonation state of Asp195. B) The study of the reaction that take place in the first step of RNA cleavage in RNase A[4] using umbrella sampling. In this study we characterize the conformational space for the two reaction coordinates with a potential mean force surface, describing both the reaction pathway and the energy of the transition state.

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P-221

Unraveling the structural determinants of the Oct4/Sox cooperativity by molecular dynamics

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Oct4 is a key regulator of stem cell pluripotency. Usually, it binds cooperatively to the DNA with Sox factors to regulate transcription. For instance, in pluripotent cells Oct4 binds cooperatively with Sox2 when the Oct4 binding site follows immediately the Sox site (canonical). To assist the second cell fate decision, it cooperates with Sox17 on a binding site where one base has been removed between the Oct and the Sox sites (compressed). Based on the structure of the Oct1/Sox2/canonical DNA complex, it was proposed that this difference is determined by the residue 57 in the Sox factors. However, experiments showed that although $Sox17^{E57K}$ behaves like Sox2, $Sox2^{K57E}$ does not behave like Sox17. Here, we built models of Oct4/Sox heterodimers bound to the canonical and compressed motifs. From molecular dynamics and MM/PBSA calculations, we propose that the residues 57 and 46 in the Sox factor determine the Oct4/Sox cooperativity. On the compressed motif, L46 from Sox17 packs tightly in a hydrophobic pocket of Oct4. On the other hand, E46 in Sox2 generates a less favorable Oct4/Sox interaction, explaining why $Sox2^{K57E}$ does not act like Sox17. Finally, we show by simulations and experiments that the cooperativity of the mutant $Sox2^{K57E/E46E}$ with Oct4 is similar to that of Sox17.

P-222

Computational biotechnology: design of selective ubiquitin

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Ubiquitin is an important signaling molecule. It is involved in a myriad of signaling pathways and binds a diverse set of receptors. Both NMR experiments and molecular dynamics simulations revealed that the global conformational ensemble of free ubiquitin covers all the conformations found in ubiquitin complexes, suggesting conformational selection as a dominant recognition mechanism.
Based on those findings a novel computational design strategy is proposed: rather than optimizing the binding interface, the dynamics will be fine tuned to achieve selective binding. Ubiquitin has two dominant global states, an open and a closed state. In native ubiquitin both states are evenly populated, and complexes can be formed with binding partners that require either of the two states. Modifying the dynamics, such that only one state is populated would result in a selective binding. An automated, thermodynamics free energy based computational screening approach is proposed to identify point mutations stabilizing ubiquitin in either of the two states. With this approach several promising mutations were identified. Those promising mutations are validated, by determining the free energy profile along the coordinate that describes the open-closed transition.

P-223

On applications of virtual atomic force microscope in studies of brain proteins

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The brain contains over 10**11 neurons and 10**14 synapses. Hundreds of different protein pairs govern proper development and functioning of neuronal networks. Among other factors mechanical stability of individual protein molecules or complexes contribute to brains activity. The data on important synaptic components, such as neurexins (NRXNs – related to autism), neuroligins (NLGNs -linking pre- and post-synaptic part of the synaptic cleft), contactins (CNTNs present in Ranvier nodes), cytokines (MCP1- immune response) and reelins (RELNs - regulation of neuronal migration) are emerging only recently due to advances in single molecules Atomic Force Microscopy. In this presentation we will show how out efforts in understanding of nanomechanics of these systems led to developing steered molecular dynamics based computer simulation protocols that help to interpret AFM spectra and to see these proteins at mechanical work.

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Abstracts

– Biomolecular Simulation: spanning scales –

P-224

Nanomechanics of proteins related to autism spectrum disorder

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Functioning of the brain is highly dependent on proper development and maintenance of neuronal networks. These processes require the assembly of a highly ordered proteins. Some of them, such as neurexins form synaptic complexes with neuroligins linking pre- and post-synaptic part of the synaptic cleft. Others, contactins contribute to proper functioning of Ranvier nodes and reelins plays a pivotal role in the regulation of neuronal migration and positioning in the developing brain [1]. Recent genetic studies indicate that the deletions or lack of these proteins may lead to severe diseases such as autism or schizophrenia [2-3]. In our studies we used all-atom and coarse-grained (CG) steered molecular dynamics (SMD) to understand the nanomechanics of these proteins. We have performed over 6000 SMD simulations using CG and all-atom approaches where the total time was 17 μs. We show that such an approach gives useful hints for understanding proteins stability and possible abnormalities induced by compromising their mechanical strength.

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P-225

Aβ-peptide and beta sheet breakers interaction. A computational study

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Short peptides (breakers) mimicking the 17-21 region of the $Abeta_{1-40}$ sequence customized with the substitution of hydrophobic with hydrophilic residues have been shown in *in vitro* experiments to be able to inhibit fibril formation by stabilizing the $Abeta_{1-40}$ alpha-helix structure.
We performed extensive Molecular Dynamics simulations of model systems comprising one $Abeta_{1-40}$ molecule in association with sets of chemically different breakers in water. We have examined the effect of three kinds of breakers on the folding propensity of the $Abeta_{1-40}$ peptide, i.e. the so-called Soto breaker (Ac-LPFFD-NH_2) and two variants thereof obtained with single residue substitutions (Tau-LPFFD-NH_2 and Ac-LPFFN-NH_2). A few interesting $Abeta_{1-40}$ structural modifications induced by the presence of breakers have been monitored and identified by comparing to what occurs in the peptide solubilization process in the absence of breakers. MD simulations show that the Ac-LPFFN-NH_2 breaker has the capability of stabilizing the $Abeta_{1-40}$ structure.

P-226

Electric field effects on EGF and bFGF ligand-receptor unbinding investigated by Steered MD method

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EGF and bFGF (Epidermal and basic Fibroblast Growth Factor) are signaling factors that control various aspects of morphogenesis, patterning and cellular proliferation. These ligands act through high-affinity transmembrane receptors with an intracellular tyrosine kinase moiety. In most systems the distribution of these peptides controls the differential behavior of the responding cells. EGF receptor signaling is known to participate in the control of the correct number of neurons. bFGF promotes outgrowth of brain neurons, and protects neurons against toxic processes [1].
In the close vicinity of membrane very high local electrical fields, up to 10^9 V/m may occur [2] and may affect protein function and structure [3].
In this study we present a series of the Steered Molecular Dynamics simulations of unbinding EGF and bFGF ligands from their native receptors (1IVO, 1EV2) in a range of external electric fields and a comparison with MD simulations performed without the electric field.

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P-227

Computational nano study of molecular mechanic methods and effect of IgG in alzheimer β-amyloid-peptid

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Amyloid β-peptid is found in found in an aggregated poorly soluble in senile or nervous plaques deposited in the brain of individuals affected by alzheimers disease.Conformational studies on these peptides in aqueous solution are complicated by their tendency to aggregate,and only recently NMR structures of Aβ-(1-40) & Aβ-(1-42) have been determind in trifluoroethanol or in SDS micelles.
All these studies hint to the presence of two helical regions,connected through a flexible kink,but it proved difficult to determine the length and position of the helical stretches with accuracy and,most of all,to ascertain whether the kink region has a preferred conformation.

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Abstracts

– Biomolecular Simulation: spanning scales –

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NBD-cholesterol fluorescent reporters in fluid phase bilayers: a molecular dynamics perspective

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Fluorescent probes are nowadays a well-known necessity in microscopic and spectroscopic studies of lipid bilayers. One important class of such probes is that of cholesterol (Chol) derivatives, including commercially available sterols labelled with a fluorescent NBD group at the 22 or 25 position (22-NBD-Chol and 25-NBD-Chol, respectively). Towards the understanding of these probes' behaviour we have carried MD simulations of either two Chol, 22-NBD-Chol or 25-NBD-Chol molecules, in fluid phase POPC bilayers. Analysis included molecular area and volume, membrane thickness, mass density profiles, location of selected atoms, tilt angles of sterol and lipid axes, deuterium order parameters of the POPC acyl chains, and dynamical sterol properties. Overall, our results suggest neither probe is efficient in mimicking Chol, as their orientation differs considerably from that of Chol, and their insertion disrupts the bilayers' structure and dynamics. Of both labelled species, 22-NBD is the least appropriate for use as a fluid phase Chol fluorescent reporter.
This work was funded by FEDER (COMPETE program) together with FCT – Fundação para a Ciência e a Tecnologia, project reference FCOMP-01-0124-FEDER-010787 (FCT PTDC/QUI-QUI/098198/2008). J. R. R. acknowledges a grant under this project.

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Simulation of pyrene in palmitoylsphingomyelin/cholesterol bilayers: a molecular dynamics approach

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Lipid rafts are membrane micro domains enriched in sphingomyelin and cholesterol. They play a part in regulating a variety of cellular events such as protein trafficking and signal transduction. A detailed knowledge of the behavior of these systems is required to thoroughly understand the functional role of membranes. Fluorescent probes are widely used to explore the structure and dynamics of model membranes. In this work, we address the properties of free pyrene probes inside phospholipid membranes and unravel their influence on membrane structural and dynamical properties. For this purpose, we have carried out an atomic-level molecular dynamics simulation of palmitoylsphingomyelin bilayers in the absence and in the presence of cholesterol, both systems with two or four pyrene probes at a temperature of 333 K. Parameters such as area per lipid, membrane thickness, order parameters and dynamic properties such as lateral diffusion, were assessed.
This work was funded by FEDER (COMPETE)/FCT, project reference FCOMP-01-0124-FEDER-010787 (FCT PTDC/QUI-QUI/098198/2008). P. D.S. acknowledges a grant under this project.

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Behaviour of NBD-headgroup labelled Phosphatidylethanolamines in POPC Bilayers: A Molecular Dynamics Study

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A homologous series of fluorescent phosphatidylethanolamines (diCnPE), labeled at the headgroup with a NBD fluorophore and inserted in POPC bilayers, was studied using atomistic molecular dynamics simulations. The longer-chained derivatives (n = 14, 16, 18) are commercially available, widely used fluorescent membrane probes. Properties such as location of atomic groups and acyl chain order parameters of both POPC and NBD-diCnPE, fluorophore orientation and hydrogen bonding, membrane electrostatic potential, lateral diffusion and rotational dynamics were calculated for all derivatives in the series. Compared to previously studied fatty amines NBD-Cn and acyl chain-labeled phospatidylcholines NBD-PC, the fluorophore in NBD-diCnPE locates in a similar region of the membrane (near the glycerol backbone/carbonyl region) but adopts a different orientation (with the NO2 group facing the interior of the bilayer).
The authors thank Advanced Computing Laboratory (UC), funding by FEDER (COMPETE)/FCT, project FCOMP-01-0124-FEDER-010787 (PTDC/QUI-QUI/098198/2008; LL, JPPR) and grant SFRH/BD/65375/2009 (FCT; HALF).

P-231

Recent development and application of all-atom molecular dynamics at constant pH

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Many biological processes such as enzyme catalysis, ATP synthesis and drug efflux are driven by protonation or deprotonation of specific sites. However, a detailed mechanistic understanding has been lacking due to the limited resolution of wet-lab experiments and limited accuracy of theoretical studies. I will discuss the recent development of the continuous constant pH molecular dynamics in all-atom representation, which has opened a door to accurate prediction of protonation states of biological systems and elucidation of dynamic processes coupled to proton relocation. Some recent applications will be highlighted, including ionization-coupled conformational dynamics of proteins and surfactant micelles as well as pH-dependent morphological transition of surfactant assemblies.

Abstracts

– Biomolecular Simulation: spanning scales –

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Modeling DNA compaction by the histone-like nucleoid structuring protein H-NS

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Bacteria organize their chromosomal DNA within a structure called the nucleoid, by employing nucleoid-associated proteins. The Histone-like Nucleoid Structuring protein H-NS can bridge DNA by binding to two separate DNA duplexes. Such an H-NS – DNA assembly forms by starting with the initial binding of an H-NS dimer to a specific nucleotide sequence, followed by additional H-NS dimers interacting with bound H-NS and binding to adjacent sites on DNA. Several nucleotide sequences have been identified to which H-NS binds strongly. The structure and dynamics of such an assembly are difficult to investigate experimentally. Molecular simulation can complement experiments by modeling the dynamical time evolution of biomolecular systems in atomistic detail. We used conventional molecular dynamics simulations to determine the stability of various conformations of H-NS. By using adaptive bias potentials in the metadynamics approach we were able to obtain insights into the mechanism of DNA binding by H-NS and preliminary estimates of the free energies related to H-NS binding to different nucleotide sequences. These studies are the first steps towards obtaining a high-resolution model for the formation of H-NS - DNA assemblies.

Abstracts

– Imaging and Biospectroscopy –

O-233

Single molecule studies of protein aggregates

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Small soluble protein aggregates are thought to play a key role in the initial development of neurodegenerative diseases, such as Alzheimer's and Parkinson's disease, but are difficult to study using conventional methods due to their low concentration and dynamic and heterogeneous nature. We have developed single molecule fluorescence based methods to detect and analyse the protein oligomers formed during an aggregation reaction, with time, and to study how these oligomers interact with the membrane of live neuronal cells. I will present recent work from our laboratory on beta amyloid, tau and alpha synuclein oligomers to show how such studies can provide new insights into both the aggregation pathway and also the molecular mechanism of cellular damage.

O-234

Mechanistic insight into eukaryotic gene expression from single molecule experiments

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Gene expresiion in eukaryotes is an extremely complicated and highly regulated process. By performing single molecule experiments it is possible to obtain dynamic mechanistic information about crucial steps in gene expression, such as the role of transcription factors [1], [2], the structure of the elongation complex [3], [4] and of the transcription initiation complex [5] as well as nucleosome structure and dynamics [6]. Of particular interest for structural models of transient complexes (such as the ones occuring in eukaryotic gene expression) is the so called Nano-Positioning system [1], [7], which combines the data from smFRET experiments and existing structural models with bayesian parameter estimation in order to obtain quantitative information.

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O-235

Quantifying protein interaction networks in live cells using fluorescence fluctuation microscopy

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Protein interactions are central to the control of all important biological functions. Quantifying these interactions is necessary for characterizing the mechanisms of action in functional regulation. In particular, deep understanding of these systems requires quantification in the context of live cells or even organisms. Recent progress in fluorescence fluctuation microscopy has allowed for measuring such interactions in live cells. I will present results on the live cell characterization of the mechanisms of regulation of gene expression by nuclear receptors and by bacterial repressors using such approaches. I will also highlight the use of these approaches to investigate the mechanisms of ribosome biogenesis in yeast and protein trafficking in neurons. In these cases we have achieved a spatial and temporal characterization of the interactions, stoichiometries, dynamics and biological noise in such systsems.

O-236

Tracing the microscopic motility of unicellular parasites

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The motility of unicellular parasites in mammalians seems very interesting, yet very complex. In a world, were inertia cannot be used for propulsion, in a world at low Reynolds numbers, most of our everyday strategies of self-propulsion do not work.

One genus of parasites that know their way around is the flagellate *Trypansome*, that is the causative agent of the sleeping sickness.

Using high-speed microscopy in combination with optical tweezers in microfluidic devices, new light has been shed on the motility of these parasites. Astonishingly, *Trypanosomes* express elaborate motility patterns if optically trapped. These results even can be used to measure the torque and power generated by their beating flagellum.

We introduce a microfluidic device for real-time analysis of the impact of drugs such as 2-Deoxy-*D*-glucose (2-DG) onto the *Trypanosomes* on the cellular level. Within seconds the drug reversibly paralyses the parasites. By semi-automated image analysis we physically quantify the effect of 2-DG as a *sleeping pill* for those causing the sleeping sickness.

Abstracts

– Imaging and Biospectroscopy –

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PI(4,5)P2 acts as a lipid calcium sensor in the presence of physiological calcium concentrations

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The localized enrichment of $PI(4,5)P_2$ in the plasma membrane at particular sites and timings is essential for the regulation of several cellular functions. During Ca^{2+}-triggered exocytosis, $PI(4,5)P_2$ is known to interact and co-segregate with specific synaptic proteins. The role of Ca^{2+} in this process is still not well understood. Ca^{2+} has already been shown to induce $PI(4,5)P_2$ clustering at non-physiological concentrations of Ca^{2+} and/or $PI(4,5)P_2$, or within membranes under high surface pressure. Here, we aimed to understand if physiological $[Ca^{2+}]$ are able to modulate $PI(4,5)P_2$ lateral organization. Using several different approaches which included information on fluorescence quantum yield, polarization, spectra and diffusion properties of a fluorescent derivative of $PI(4,5)P_2$ (TopFluor(TF)-$PI(4,5)P_2$), we show for the first time that Ca^{2+} promotes $PI(4,5)P_2$ clustering in bilayers at physiological concentrations of both Ca^{2+} and $PI(4,5)P_2$. The data is consistent with an average cluster size of $\sim$15 $PI(4,5)P_2$ molecules. Additionally, calcium mediated $PI(4,5)P_2$ clustering was more pronounced in liquid ordered (l_o) membranes. These results suggest that $PI(4,5)P_2$ functions as a lipid calcium sensor in the plasma membrane.

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Dynamic submicroscopic signaling zones revealed by TALM and image correlation analysis

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By orthogonally labeling the type I IFN receptor subunit IFNAR1 and IFNAR2 expressed at endogenous level with monofunctional quantum dots, the dynamic formation of the individual IFN ternary complex was probed by dual color single molecule fluorescence imaging over extended time period. By tracking and localizing individual receptor subunits, TALM (Tracking And Localization Microscopy) images were obtained with spatial resolution of $\sim$15 nm together with temporal resolution ranging from 0.5 ms to seconds. Image correlation analysis of such TALM images was implemented to quantitatively explore the temporal and spatial dynamics of the receptors and cytosolic effector protein. Long-term tracking of individual ternary complexes was established based on time-lapse particle correlation and pair correlation of TALM (pcTALM). To this end, the life-time of the signaling ternary complex over 15 s was confirmed, as well as a submicron confinement of the ternary complex in plasma membrane where transient recruitment of the cytosolic effector protein STAT2 was observed. Our results highlight the role of microcompartmentation for the assembly and stability of signaling complexes in the plasma membrane.

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Direct observation of supported lipid bilayer formation with interferometric scattering microscopy

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Supported lipid bilayers (SLB) are commonly used to study processes associated with or mediated by lipid membranes. The mechanism by which SLBs form is a matter of debate, largely due to the experimental difficulty associated with observing the adsorption and rupture of individual vesicles. Here, we use interferometric scattering microscopy (iSCAT) to directly visualize membrane formation from nanoscopic vesicles in real time. We observe a number of previously proposed phenomena such as vesicle adsorption, rupture, movement and a wave-like bilayer spreading. By varying the vesicle size and the lipid-surface interaction strength, we can rationalize and tune the relative contributions of these phenomena. Our results are in agreement with a model where the interplay between bilayer edge tension and the overall interaction energy with the surface determine the mechanism of SLB formation. The unique combination of sensitivity, speed and label-free imaging capability of iSCAT provides exciting prospects not only for investigations of SLB formation, but also for studying assembly and disassembly on the nanoscale with previously unattainable accuracy and sensitivity.

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Therapeutic protein encapsulation in isasome type lipid nanocarriers

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Advances in protein nanoencapsulation have led to studies of nanostructured lipid particles as multicompartment carriers. Nanostructured formulations for therapeutic protein delivery include internally self-assembled (isasome) particles, which display inner structure of nanochannels. They are formed by fragmentation of liquid crystalline phases of hydrated nonlamellar lipids using amphiphilic dispersing agents. Defining an appropriate delivery strategy for therapeutic proteins, based on isasome carriers, requires knowledge of their hierarchical organization, which determines the loading properties. The purpose of our work was to reveal the structural features of protein-loaded isasome nanocarriers by means of high resolution small-angle X-ray scattering and cryogenic transmission electron microscopy. The obtained results demonstrate that the effects of entrapped a-chymotrypsinogen A and brain-derived neurotrophic factor are concentration dependent. This conclusion is in agreement with previous studies showing that guest species smaller than the mesophase periodicity of the lipid carriers are confined within the aqueous channels and influence the lipid hydration, whereas larger proteins are expelled and partition at grain boundaries of subdomains formed in the carriers.

Abstracts

– Imaging and Biospectroscopy –

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Non-invasive in vivo optical measurements of blood oxygenation

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Abnormal blood gases can result from various pathological conditions such as metabolic acid-based imbalances, ventilatory failure, enzymatic deficiencies, hemoglobin M disease, drug-induced toxicities, carbonmonoxide poising and various metabolic conditions. The objective of this study was to demonstrate the feasibility of assaying for the oxyhemoglobin, methemoglobin and deoxyhemoglobin via noninvasive measurements of optical reflection and transmission. The values of diffuse reflectance and transmission were measured on ten subjects (6 Male, 38±7 years; 4 female, 26±2 years) to determine the hemoglobin constituents in earlobe blood. Diffusion approximation model (1DA) was used to calculate linear absorption and scattering coefficient from reflection and transmission data. The accuracy of 1DA results was tested by model Monte Carlo calculations. By using differential absorption and scattering technologies, three or four hemoglobin moieties can be determined non-invasively. In this methodology, one fitting constant is required for the three component assay compared to fitting constants for the two component assay with pulse oximeter. Therefore the present approach is used to improve on the current oximeters.

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Collagen fibrosis quantified by non linear optical microscopy to study cardiac disease progression

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Non-linear optical microscopy, in particular the combination of Two-Photon Excitation microscopy (TPE) with Second Harmonic Generation (SHG) signal, has been shown to be a powerful tool for non-invasive imaging of thick specimen (ChenX et al. NatProtoc 2012). No sample staining is needed because numerous endogenous fluorophores can be excited by a TPE mechanism and non-centrosymmetric structures such as collagen can generate SHG (WilliamsR. et al. BiophysJ 2005). Recently these two techniques have been promisingly used in diagnosis of many diseases (LacombR. et al. BiophysJ 2008). Here we implement TPE-SHG microscopy to study increased fibrosis induced by cardiac diseases. We propose a 3D quantitative analysis to measure fibrosis in tissue from a rat model of heart failure post myocardial infarction (LyonAR. et al. PNAS 2009). We observe a 3-fold increase in collagen fibrosis 4 weeks after infarction which is further increased up to 5-fold at 20 weeks (CaorsiV. et al. PlosOne 2013). A different spatial distribution is observed providing insight on the morphological effect of disease progression. Further use of SHG combined with polarization analysis will allow gaining more structural information, particularly on fibril size and packing order in healthy and diseases tissues.

P-243

Surface-proximity enhanced resolution imaging

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When placing a fluorescent molecule close to a metal film, its fluorescence lifetime changes due to the energy transfer from the molecule's excited state into surface plasmons of the metal. This is similar to Förster Resonance Energy Transfer (FRET), where the energy of an excited donor molecule is transferred into the excited state of an acceptor molecule. The coupling between the excited molecule and a metal film is strongly dependent on the molecule's distance from the metal. In the past, we have used this effect to localize, with nanometer accuracy, tubulin molecules above a metallic surface. Here, I will present an extension of this method, which is named surface-proximity enhanced resolution imaging, and which we have used for mapping the basal membrane of live cells with an axial accuracy of ∼3 nm. The method is easy to implement and does not require any change to a conventional fluorescence lifetime microscope; it can be applied to any biological system of interest, and is compatible with most other superresolution microscopy techniques which enhance the lateral resolution of imaging.

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Validation of the biological activity of a new engineered recombinant collagen

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Recombinant proteins are produced by biomolecular engineering and aim to overcome the biological activities of its natural counterparts, as an alternative in regenerative medicine fields, especially in the hemostasis area. Natural collagens are microfibrillar proteins with a triple helix as molecular building block, which is deemed necessary for aggregating the platelets and therefore the blood coagulation. The conformation, the structure and the activity of recombinant collagens remain still unexplored, and new analytical tools and processes need to be developed for characterizing this new family of proteins.

The morphology was investigated by atomic force microscopy, circular dichroism and surface-enhanced Raman scattering; the activity was quantified by ELISA and aggregation tests. We developed plasmonic sensors integrated in a microfluidic platform to measure the affinity in arterial flow conditions, faithfully replicating the first step of the coagulation process.

We show that engineered recombinant collagens form a triple helix but remain in nodular nanostructures. Nonetheless, the biological activity was found to be of the same order as native collagens.

Abstracts

– Imaging and Biospectroscopy –

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Amyloid-like aggregation of RNA polymerase σ^{70} subunit: an AFM study

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σ^{70} subunit of RNA polymerase (RNAP) plays crucial role in transcription initiation. Its mechanism implies binding of σ^{70} subunit with core enzyme of RNA polymerase followed by formation of holoenzyme RNAP. We have revealed factors (mainly concentrations of mono- and divalent cations) bringing to self-interaction of σ^{70} subunits and formation of linear amyloid-like fibrils. Morphology of fibrils was characterized by atomic force microscopy (AFM). AFM results have also shown the influence of the cationic surroundings on the ability of σ^{70} subunit to form fibrils. Three genetic mutants of σ^{70} subunits, devoid the whole or part of 1.1 region, were used to elucidate the role of N-terminus in aggregation. Obtained data allowed us proposing a model for such aggregation based on a mechanism of a domain swapping. This system may be also used as a model for study amyloidosis.
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Time resolved fluorescence emmision spectroscopy of amphotericin B

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Amphotericin B (AmB) is a lifesaving polyene antibiotic used widely to treat deep-seated mycoses. Both the pharmaceutical effectiveness as well as toxic side effects depend on molecular mechanism that govern organization the of the drug.
Fluorescent spectroscopy and particularly fluorescence lifetime is a technique, applied in the present work, which appears to be particularly sensitive to detect and distinguish molecular organization forms of the drug. The results of the analyses show that in all of the systems studied, the drug appears in a different spectral forms, interpreted as monomeric, dimeric and aggregated. Esspecialy the specific molecular aggregates can be responsible for ionophore activity and for destabilization of biomembranes, leading to cell death and for the toxic side effects of the drug.
This work was supported by National Science Centre (grant # N N202 112340) and partially as a part of the project "Molecular Spectroscopy for BioMedical Studies", which was financed by the Foundation for Polish Science within the TEAM program.

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Nanoscale infrared imaging of lipoprotein multilayers

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The precise imaging of biomolecular entities contributes to an understanding of the relationship between their structure and function. However, the resolution of conventional infrared microscopic imaging is diffraction limited and does not exceed a few micrometres. Atomic force microscopy, on the other hand, can detect infrared absorption down to the sub-micrometer level. In the present report, we demonstrate that for multi-bilayer lipid samples containing the plant photosynthetic antenna complex LHCII, the resolution of this latter technique can be as high as 20 nm.
This study was a part of the project "Molecular Spectroscopy for BioMedical Studies", which was financed by the Foundation for Polish Science within the TEAM program, by National Science Centre of Poland (grant no. N N202 112340) and by the Swiss National Science Foundation (grant no. 206021-139080).

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Fluorescent analysis of viscous microenvironment impact upon bioluminescent reaction of bacteria

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Viscous media are the simplest models of intracellular environment for enzymes. Earlier it was found that glycerol, sucrose, gelatin and starch affected the intensity of bacterial bioluminescence *in vitro* and enzymes thermostability. Possible mechanism is alteration of conformation states of enzymes and its substrates. So the study aimed to find conformational changes of the components of bacterial bioluminescent reaction in viscous media.
Steady-state and time-resolved fluorescence of flavin mononucleotide (FMN), nicotinamide adenine dinucleotide (NADH), bacterial luciferase and NAD(P)H:FMN-oxidoreductase was studied in media containing glycerol, sucrose, potato starch and gelatin.
No effect on the energy of excited states of FMN was found. Fluorescence anisotropy of the nucleotides indicated the variations of apparent volumes of these molecules in viscous media that can result in steric hindrance during interaction with enzymes. Thermally induced conformational transitions of enzymes were studied from their intrinsic fluorescence. The conformational transition of luciferase was found to start at 40°C and not complete up to 70°C. The patterns obtained gave explanation for the mechanisms underlying the thermal inactivation of bioluminescence reaction observed in modified media.

Abstracts

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A flexible laboratory small- and wide-angle X-ray scattering (SWAXS) system for biophysical and medical analytics

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Small- and wide-angle X-ray scattering (SWAXS) is an important method for the characterization of nanostructures present in biological and pharmaceutical materials, e.g. proteins, lipids and others. SWAXS produces complementary information to other characterization methods like electron microscopy, X-ray crystallography or NMR and therefore contributes to a better understanding of complex biological processes. We report about a new and flexible SWAXS laboratory system and its broad possibilities for bio analytical research. The system features full automatization for fast analysis of multiple solid and liquid samples. Examples of SAXS analysis of proteins and protein complexes with a special focus on non-destructive measurements of sensitive samples as well as high-resolution studies of large protein structures are presented. In addition, SAXS and in particular WAXS studies of solid-state powder and granulate investigations of pharmaceutical formulations are shown[1]. These studies include the determination of binary mixtures structural analytics of self-assembling behavior of different formulations as well as monitoring temperature-dependent structural changes.

[1]A. Hodzic *et al* A system for analyzing granulates for producing a pharmaceutical product. Patent available from the World Intellectual Property Organization (WIPO), WO/2011/095364, 2011.

P-250

3D structure of human telomeric repeat sequence with 2,4,6-Triarylpyridine [1] derived ligand by NMR

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G-rich DNA sequences can adopt unusual structures called G-quadruplexes (G4). G4 are constituted by the stacking of tetrads, which are established by the association of four guanines. The human telomeric sequence formed by repetitive nucleotide sequences (T_2AG_3) at each end of chromosomes, is an important example of an intramolecular G4 [2]. Targeting the human telomeric sequence with G4 ligands aiming to inhibit its interaction with telomerase or interfere with telomeric functions has been described as a logical strategy to develop anti-cancer drugs. Nowadays, only a few 3D structures of G4–ligand complexes are available and more information is needed to understand the binding mechanism which can be important for rational drug design. The results show that $d[AG_3(T_2AG_3)_3]$ retains much of its 3D structure in presence of ligand. The largest changes in the structure upon ligand binding are located in a region formed by the bases G3, G4, T5, T6 and A19. CD melting studies have shown a 10 °C shift in stabilization when five to one moles of ligand and G4 were used respectively. From chemical shift mapping, we can observe two possible binding sites located in groove regions. These results corroborate with unrestrained docking experiments.

[1] *Smith N et al, OBC , 2011.* [2] *Patel DJ, Structure, 1993.*

P-251

Protein-monolayer interactions investigated by fluorescence microscopy and correlation spectroscopy

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Lipid bilayers are composed of a wide variety of lipids and proteins which can lead to spatial heterogeneity and formation of domains, so-called 'lipid rafts', which play an important role in cell signaling.

Model membrane systems have become invaluable tools to investigate these specific features of cellular membranes. Although a variety of experimental assays does exist, many of them are rather complicated in their preparation and difficult in their practical realization.

Here, we use a new simple miniaturized monolayer assay combined with confocal fluorescence microscopy and fluorescence correlation spectroscopy (FCS).[1] This approach allowed us to investigate morphology and lipid fluidity of the monolayer and to correlate these factors with protein binding. Particularly, we studied the influence of phase separation on Cholera toxin β and Streptavidin binding to lipid monolayer.

Our results show that the affinities of Cholera toxin β and Streptavidin to the monolayer depend on lipid surface density. Moreover, FCS measurements indicate a correlation between higher protein binding and increased lipid diffusion.

References:
1. Chwastek G. and Schwille P. (2013) *ChemPhysChem* DOI: 10.1002/cphc.201300035

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Analysis of protein assemblies from fluorescence data by superresolution microcopy

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Superresolution microscopy shows unrecognized fluorescence signal patterns. Interpretation requires dedicated strategies: 1) empirical analysis of spatial intensity values; 2) inverse approaches converting signals into objects; and 3) decomposing signal patterns into spatial modes. We use STED microscopy data from ryanodine receptor (RyRs) Ca^{2+} release channels.

Ventricular myocytes (VM) underwent RyR2 immunofluorescence labeling and STED microscopy. For signal analysis of RyR2 signal patterns, we established multi-scale strategies using wavelet decomposition of images into spatial modes to identify dominant scales and patterns. Distinct scales were used to represent inter-cluster spacing between segregated assembly structures versus intra-cluster patterns from individual protein super-structures. For object-based analysis, RyR2 cluster sub-structures were identified using a multi-step thresholding procedure. As result, we identified sub-structures of individual RyR clusters as discrete objects of variable sizes with a typical spacing ranging from 78 to 128 nm (IQR range).

Our data suggest common building principles of membrane protein assemblies for complex signal patterns of RyR2 clusters. Building blocks may enable local control mechanisms of highly localized Ca^{2+} signals.

Abstracts

– Imaging and Biospectroscopy –

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Modulating tension in pore spanning membranes

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Membrane tension is involved in many cellular processes of the plasma membrane e.q. endocytosis, exocytosis, cell motility and division. Adhesion of membrane to the cortical actin cytoskeleton is one source of tension in cellular membranes. It is believed that cells modulate their tension when needed by changing the structure of the cytoskeleton or the cytoskeleton-membrane interaction.
Here we present a model system which allows to modulate tension in planar free standing membranes as a mimic of cellular plasma membranes.
Membranes spanned over a highly ordered pore-array are a suited model system for the cell membrane as they are taking the influence of an underlying network into account. Spreading giant unilamellar vesicles on top of self-assembled-monolayers (SAMs) results in solvent free pore-spanning membrane patches. The SAM is composed of thiol mixtures, which modulate the hydrophilicity of the substrate's surface. This way the membrane tension is directly modulated as a function of SAM composition and can be probed by means of force spectroscopy.

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A Single Molecule Study of Toll-Like-Receptor 4 Structure and Signalling

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In this study, we use a single molecule fluorescence approach to image the reorganisation on the surface of live cells of individual fluorescently labelled Toll-like receptor 4 (TLR4) molecules during signalling. TLR4, a key membrane protein in the innate immune system, is involved in the recognition of microbial pathogens, by detecting the presence of the lipopolysaccharide (LPS) component of exogenous Gram-negative bacteria.
Single molecule tracking experiments will be described that allow us to follow changes in the diffusion of TLR4 and its oligomerisation state over a period of 30 minutes following addition of LPS. These studies provide new insights into how the TLR4 receptor is organised on the cell surface and cooperatively reorganises on binding LPS to trigger downstream signalling and modulate the immune response.

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Plasmonic-based instrument response function for time-resolved fluorescence

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We investigated plasmonic platforms to target ultra-short fluorescence and accurate instrumental response function in a time-domain spectroscopy and microscopy. The interaction of metallic nanoparticles with nearby fluorophores resulted in the increase of the dye fluorescence quantum yield, photostability and decrease of the lifetime parameter. The properties of platforms were applied to achieve a picosecond fluorescence lifetime (21 ps) of erythrosine B, used later as a better choice for deconvolution of fluorescence decays measured with "color" sensitive photodetectors. The response functions were monitored on two photo-detectors; microchannel plate photomultiplier and single photon avalanche photodiode as a Rayleigh scattering and ultra-short fluorescence. We demonstrated that use of the plasmonic base fluorescence standard as an instrumental response function results in the absence of systematic error in lifetime measurements and analysis.

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Imaging fusion of single vesicular stomatitis virus with supported lipid bilayers

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Fusion of enveloped viruses with cellular membranes is mediated by viral glycoproteins in response to binding to cognate receptors and/or low pH. Accumulating evidence suggests that additional cellular factors, including lipids, can strongly modulate the fusion process. Here, we used retroviral particles pseudotyped with the Vesicular Stomatitis Virus (VSV) G protein and imaged single virus fusion with dextran-supported lipid bilayers. Incorporation of diffusible fluorescent labels into the viral membrane and the viral interior allowed observation of lipid mixing (hemifusion) and content transfer (full fusion) mediated by VSV G at low pH. Lipid compositions containing anionic lipids, phosphatidylserine (PS) or bis(monoacylglycero)phosphate (BMP), greatly enhanced the efficiency of hemifusion and permitted full fusion, contrary to bilayers of zwitterionic lipids. Kinetic analysis showed that the lifetime of a hemifusion intermediate was significantly shorter for BMP-containing compared to PS-containing bilayers. The strong enhancing effect of BMP, a late endosome-resident lipid, on VSV fusion is consistent with the model that this virus initiates fusion in early endosomes, but releases its core into the cytosol after reaching late compartments.

Abstracts

– Imaging and Biospectroscopy –

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1O2 luminescence and mitochondrial autofluorescence after illumination of Hyp/mitochondria complex

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A study of hypericin (Hyp) interaction with mitochondria (mito) as well as the time-resolved measurement of singlet oxygen ($^1O_2$) formation and annihilation after illumination of Hyp/mito complex is presented. High concentration of Hyp leads to its aggregation inside the mito and the relative population of the monomeric form of Hyp decreases concomitantly. Production of $^1O_2$ in mito after illumination of the complex is characterized by a rise lifetime of $\sim 8\ \mu$s and shows saturation behaviour with respect to Hyp concentration. Our results confirm that only the monomeric form of Hyp is able to produce its excited triplet state, which consequently leads to $^1O_2$ production. An influence of photoactivated Hyp on mito respiration chain was evaluated by monitoring of time resolved NAD(P)H fluorescence.
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Chiroptical study of bilirubin interaction with model membranes: influence of serum albumin

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Bilirubin, the end product of the heme catabolism, was found to have numerous positive and negative effects in the human organism; its adverse effect on cell membranes and membrane proteins among them. As it is thought that the neurotoxic effect of bilirubin is caused by its enantiodiscimination on the membrane, we examined the interaction of bilirubin with model membranes using the techniques sensitive to the chiral structure: vibrational and electronic circular dichroism spectroscopies. Since serum albumin is the main transport protein of bilirubin in the mammalian body, its influence on the bilirubin-model membranes interaction was explored.
The spectral study helped to identify the lipid functional groups, which were significantly influenced by the interaction. It also enabled to observe the time development of the system. It was found, that the membrane composition strongly influenced the enantioselective binding of bilirubin on the membranes. However, obtained results indicate that the interaction is governed not only by the membrane composition but also by the physicochemical conditions.
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Characterization of Curli A Production on Living Bacterial Surfaces by Scanning Probe Microscopy

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Curli are adhesive surface fibers produced by many Enterobacteriaceae, such as *Escherichia coli* and *Salmonella enterica*. They are implicated in bacterial attachment and invasion to epithelial cells. In this study, atomic force microscopy (AFM) was used to determine the effects of curli on topology and mechanical properties of live *E. coli* cells. Young's moduli of both curli-deficient and curli-overproducing mutants were significantly lower than that of their wild-type strain, while decay lengths of the former strains were higher than that of the latter strain. Surprisingly, topological images showed that, unlike the wild-type and curli-overproducing mutant, the curli-deficient mutant produced a large number of flagella-like fibers, which may explain why the strain had a lower Young's modulus than the wild-type. These results suggest that the mechanical properties of bacterial surfaces are greatly affected by the presence of filamentous structures such as curli and flagella.

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Discovering new frontiers in single particle tracking with interferometric scattering microscopy

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Single particle tracking (SPT) has become a popular tool in the life sciences thanks to its ability to study the dynamics of heterogeneous systems at the nanoscopic level. However, one of the long-standing limitations of SPT is the trade-off between imaging speed, localisation precision, and the size of the reporter particle. Here, we apply interferometric scattering microscopy (iSCAT) to two ubiquitous model systems that benefit from the unprecedented speed, sensitivity and localisation precision of the technique. On the one hand, we report on the dynamics of short strands of DNA below the persistence length (150 bp) via the tethered particle motion assay with imaging speeds as high as 50,000 Hz and with a localisation precision down to 1 nm – previously inaccessible due to the size of the reporting bead. On the other hand, we present the results of tracking small gold particles (< 40 nm) on supported lipid bilayers in which diffusion can be characterised over more than four orders of magnitude in time. The simple experimental implementation together with the promise for smaller label sizes, 3D imaging capability, and high-speed, demonstrate the potential of iSCAT of becoming a premier tool for studying nanoscopic dynamics at exceptional imaging speeds and sensitivity.

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Crossing the border towards deep UV time-resolved microscopy of native fluorophores

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Based on recent advances in fiber amplified laser technology and ultrasensitive detection, we present a novel approach to extend time-correlated single photon counting (TCSPC) into the deep ultraviolet (UV). By using 266 nm excitation, direct access is granted to the native fluorescence of biomolecules originating from appropriate chromophoric groups. We present label-free FLIM of cells where the aromatic amino acids tryptophan and tyrosine within the proteins become visible. As a benchmark, FCS with organic fluorophores in the deep UV is shown. Another application of time-resolved fluorescence microscopy in the deep UV includes microfluidics which enables label-free identification of various aromatic analytes in chip electrophoresis. Fluorescence decay curves are gathered on-the-fly and average lifetimes are determined for different substances in the electropherogram to identify aromatic compounds in mixtures. Based on the time-correlated single photon counting the background fluorescence can be discriminated resulting in improved signal-to-noise-ratios. Microchip electrophoretic separations with fluorescence lifetime detection can be performed with protein mixtures emphasizing the potential for biopolymer analysis.

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Long Stokes shift red fluorescence protein simplifies intracellular chloride and pH measurements

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Chloride and protons play important closely related roles in diverse cellular functions; as a consequence, alterations in their homeostasis lead to several human diseases. In spite of its importance, only a few techniques are so far available to measure intracellular chloride.
Here, we develop a new biosensor for measuring simultaneously real time optical detection of pH changes and chloride fluxes in live cells through fluorescence imaging. Our new biosensor is constituted by a chimeric construct linking the pH- and chloride-sensing green element E^2-GFP with LSSmKate2. LSSmKate2 is a red fluorescence protein, chloride and pH insensitive, characterized by a long Stokes shift ($\sim$140 nm), a photophysical property that allows the innovative application of the sensor by using only two excitation wavelengths (cf. three excitation wavelengths required by ClopHensor) as LSSmKate2 and E2GFP can be excited at the same wavelength.
Furthermore, our new sensor enables by means of ratio imaging analysis the construction of pH and chloride maps for proper application in vivo.

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Combined full-field fluorescence and optical coherence microscopy

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Full-field optical coherence microscopy (FF-OCM) is a recent optical imaging technology based on low-coherence interference microscopy, achieving $\sim 1\mu$m spatial resolution in semi-transparent samples. It has been successfully applied to three-dimensional imaging of various biological tissues at cellular-level resolution.
A dual modality imaging system is presented [1], capable of providing simultaneously FF-OCM and fluorescence images, thus allowing the use of target-specific fluorescent probes to identify molecular components of subcellular structures. The FF-OCM system, based on a Linnik-type interference microscope, uses a halogen lamp and a high-dynamic area camera (maximum of sensitivity at 800 nm). The fluorescence system uses a 488 nm CW laser and is coupled to FF-OCM by dichroic beam splitters. Optical sectioning is achieved in FF-OCM owing to the coherence gating property of the light source, and by structured illumination for fluorescence imaging.
This system can be used in conjunction with a biopsy procedure with minimal preparation. The images obtained provide complementary information and could enhance the accuracy of disease diagnosis or any biomedical applications based on tissue morphology and biochemical properties.
[1] Makhlouf et al, Optics Letters, 37, 1613 (2012)

P-264

Raman spectroscopy for DNA analysis of Lemna minor and Cryptocoryne lutea under the impact of Na^+Cl^-

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Raman scattering of light had been used to extend knowledge about the influence of toxic concentrations of NaCl (0.1;0.5;1M) on nuclear DNA (extracted from *Lemna minor*, *Cryptocoryne lutea* leaves). The analysis of Raman spectra supported the existence of plant DNA structural specificities in the interaction and stability of DNA molecule secondary structure. During the experiment, the information of Raman spectrum indicate that the interaction of Na^+Cl^- ions with DNA molecule is mainly through the phosphate groups of DNA molecule with negligible change of the DNA spectra at high concentration of NaCl in both plants. Spectra were collected at range of 1750cm^{-1} to 350cm^{-1}. Data obtained using 514.5 and 785.0nm excitation. Observed DNA molecular changes in the Raman spectra bands of PO_2^- interaction in region 1750cm^{-1}-1100cm^{-1}, band at 1065cm^{-1} assigned to the (PO_2^-)group. The Raman bands are suggests that Raman scattering of light can be used to evaluate the impact of adverse factors on aquatic plants This work has been supported by the ESF within the Project "Support for the implementation of doctoral studies at Daugavpils University"Agreement Nr. 009/0140/1DP/1.1.2.1.2/09/IPIA/VIAA/015

Abstracts

– Imaging and Biospectroscopy –

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Raman spectroscopy as a tool for dental fluorosis characterization

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Dental fluorosis is an irreversible condition due to excessive consumption of fluorides. The incidence of fluorosis has increased worldwide. The objective is to characterize the changes in tooth enamel affected by fluorosis, using Raman spectroscopy. Two teeth were analyzed, one healthy and one with severe fluorosis, 20 spectra were obtained and averaged for each specimens. Four regions were identified: 400-500 cm^{-1} (ν_2, OPO bending), 500-620 cm^{-1} (ν_4, OPO bending), 962 cm^{-1} (ν_1, symmetric stretching) and 980 1100 cm^{-1} (ν_3, asymmetric stretching) corresponding to the phosphate group vibrations. The most intense peak in the spectra of both teeth was located at 962 cm^{-1}. In the healthy tooth, the intensity average ratio I_{962}/I_{580} was 2.72 (SD 0.47), whereas for the tooth with fluorosis it was 3.41 (SD 0.38) ($p < 0.0001$). The average ratio I_{962}/I_{440} was 3.62 (SD 0.44) for healthy tooth and 3.97 (SD 0.58) for the tooth with fluorosis ($p < 0.042$). A significant difference was observed also in the pattern of intensities in the region of the vibration modes ν_2. The enamel showing severe fluorosis presented significantly differences in the vibrational modes ν_1, ν_2, ν_3 and ν_4 compared with the health enamel. Raman spectroscopy can be a useful tool in the diagnosis of dental fluorosis.

P-266

Molecular biophysics of membrane-active peptides: from mono molecular interactions to amyloidogenesi

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The understanding of protein structure, assembly and function relationships at biomolecular interface is critical in underpinning the mechanisms governing amyloid beta-induced neurotoxicity. In the search for methods to study the interaction of peptides and proteins with biological membranes linear dichroism (LD) spectroscopy has emerged as a powerful technique. LD relates shear-aligned phospholipid membranes with conformation and binding geometries of membrane-bound proteins and peptides in solution phase. This presentation will highlight how LD spectral features are used to interpret and differentiate membrane-binding mechanisms of membrane-active peptides. The information gained is unique in that it can provide a rapid and accurate answer to mechanistic aspects of biological function at the molecular level, which is otherwise accessible only by resource costly high-resolution approaches (e.g. NMR or crystallography). Therefore, this solution-based technique holds particular promise in the development of high-throughput approaches in providing solutions to a variety of healthcare problems including bacterial cross-resistance, viral infectivity and amyloidogenesis

P-267

Engineering and characterization of GFP-based biosensor for pH-chloride intracellular measurement

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ClopHensor, a new ratiometric GFP-based biosensors, is a powerful tool for non-invasive pH and chloride quantification in cells. E^2GFP is the ClopHensor chloride-sensitive element. E^2GFP dissociation constant of about 50 mM (at pH=7.3) makes it ideal for quantifying physiological chloride concentration. Unfortunately, chloride affinity of E^2GFP strongly depends on pH values in solution: precise chloride measurements require pH quantification and thus the use of three different excitation wavelengths.

Here, we present a detailed study of E^2GFP-V224L-H148G, selected among several GFP variants with improved sensing capabilities. Chloride affinity of E^2GFP-V224L-H148G was measured (Kd = 14.8 +/-0.8 mM at pH=5.2), moreover its emission spectra displayed two distinct emission peaks at 480 and 520 after excitation at 415 nm. Fluorescence emission spectra also displayed a clear isosbestic point at 496 nm, indicating the presence of two species in equilibrium in the pH range from 4.8 to 8.5. While being chloride independent, the 520-to-496 (nm) ratio showed high dynamic range and pK_a value of 7.28 +/-0.04, centered in the physiological pH range.

We exploited these properties to measure pH using a single excitation wavelength in vitro; applications to living cell samples will also be discussed.

P-268

Probing the mesh formed by the semirigid polyelectrolytes

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We correlated conformation and dynamics of the semirigid polyelectrolytes deoxyribonucleic acid (DNA) and hyaluronic acid (HA) in the semidilute regime, across a broad concentration range (10^{-3} - 10^{2} g/L). The polyelectrolyte mesh size $(bn)^{-1/2}$ (b,n are the monomer size and concentration) is commonly taken as synonymous and/or equal to de Gennes correlation length ξ [1]. We performed small-angle X-ray scattering on HA and DNA (range 3-130 g/L), to complement our previous dielectric spectroscopy study[2] (range 0.01-5 g/L) in order to provide reference values for ξ. Then, we directly probed DNA and HA mesh, by employing fluorescence correlation spectroscopy to measure the diffusion coefficient of fluorescently labeled DNA probes[3].

For the salt-free solutions we found that the DNA or HA mesh size has to be 2-3 times larger than the fragment for these to start to diffuse freely (as they would in a dilute solution). For a tighter mesh, but still larger than the fragments, the coefficient is only half the free diffusion value. Conversely, fragments show the free diffusion coefficient as if there is no mesh when placed in DNA or HA solution in 10 mM buffer.

[1] J.Combet et al. Macromolecules 2005 [2] S.Tomic et al. Phys.Rev.E 2007 [3] K.Salamon et al. Macromolecules 2013

Abstracts

– Imaging and Biospectroscopy –

P-269

Antifungal defensin Psd1 increases membrane roughness and promotes apoptosis in Candida albicans

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*Ps*d1 is a defensin, isolated from *Pisum sativum* seeds, previously shown to have a strong interaction with fungal-specific membrane components [1]. *Candida albicans* is an important human pathogen, causing oral, genital and systemic opportunistic infections, which are especially relevant clinically in immunocompromised patients, such as HIV-infected individuals. We tested the effects of this antimicrobial peptide, comparing it with the antifungal drugs amphotericine B and fluconazole, at the minimal inhibitory concentration (MIC) and at a 10-fold higher concentration. By atomic force microscopy (AFM) imaging we assessed morphological changes on *C. albicans* cells. SYTO-9 and propidium iodide allowed us to image live and dead cells by confocal microscopy and to quantify their ratio. Our results show that, with increasing incubation times and *Ps*d1 concentrations, there is an increased cell death and surface roughness, with the appearance of apoptotic features, such as membrane blebs, cell size alterations, membrane disruption and leakage of cellular contents. Thus, we were able to visualize the action of *Ps*d1 against a relevant fungal human pathogen, aiming at its possible use as a natural antimycotic agent.

[1] Gonçalves *et al.* (2012) *Biochim Biophys Acta* 1818:1420

P-270

Cytoprotective bile acids are high affinity ligands for the apoptotic protein BAX

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Hydrophilic bile acids (such as ursodeoxycholic acid, UDCA) can inhibit apoptosis in both hepatic and non-hepatic cells. The mechanism associated with this effect seems to be related with the blockage of a series of processes that converge on mitochondrial damage. Bax is a pro-apoptotic member of the Bcl-2 family that is involved in pore formation on mitochondrial membranes. Submicellar concentrations of cytoprotective bile acids have been shown to modulate Bax translocation to mitochondria, suggesting that these molecules could interact directly with the protein. In this study, our objective was to evaluate the affinity of bile acids to recombinant Bax protein, making use of fluorescence methodologies. Here, we show that cytoprotective bile acids bind to recombinant Bax protein with significantly higher affinity than apoptotic bile acids. Notably, the binding site for UDCA seems to be located in a hydrophobic pocket of the protein. This interaction could be responsible for the disruption of Bax translocation to the mitochondrial outer membrane in the presence of UDCA.

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P-271

Interaction of cytotoxic and cytoprotective bile acids with membranes of living cells

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Deoxycholic acid (DCA) is an apoptotic bile acid at submillimolar concentrations, while ursodeoxyhcolic acid (UDCA) prevents apoptosis in the same concentration range. The mechanisms that trigger these opposite signaling effects are still unclear. We have recently shown that these bile acids exhibit low partition to cholesterol-rich (liquid ordered,l_o) membranes, and that DCA and other apoptotic bile acids partially disrupt the ordering of lipid model membranes by cholesterol in liquid disordered membranes (l_d).

Using fluorescence microscopy methodologies, we show that fluorescent derivatives of DCA and UDCA are present at very low concentrations in the plasma membrane of both HEK293 and hepatocyte living cells, possibly as a consequence of low partition of bile acids to cholesterol-rich membranes. Additionally, both cytotoxic and cytoprotective unlabeled bile acids have no effect on the fluidity of the plasma membrane at apoptotic concentrations. However, fluorescent derivatives of bile acids are found significantly enriched in the mitochondrial membrane of hepatocytes. These results suggest that the modulation of apoptosis by bile acids is not the result of modulation of plasma membrane structure and are likely associated with mitochondria damage/protection.

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P-272

Origin of A0, A1 and A3 conformational substates of carbonmonoxy myoglobin

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Temperature dependence of infrared absorption spectra of complex of myoglobin with carbon monoxide (MbCO) showed that MbCO has at least three conformational substates, characterized by different spectra of infrared absorption in the region of C-O absorption. However, structures of these substates were unknown.

At the same time recent results of the high-resolution X-ray study of MbCO showed presence of three different substructures of MbCO.

To check if these sub-structures correspond to the substates observed in the infrared spectra we performed DFT quantum chemical calculations of the MbCO active center with its closest distal environment, which correspond to each of the refined sub-structures. These calculations revealed the dependence of vibrational frequency of the coordinated C-O ligand on the changes in the structure of the heme environment. The calculations showed, that the observed different X-ray structures correspond to the A_0, A_1, and A_3 substates. It was also shown that electronic structure of different parts of the heme environment notably depends on the electrostatic interactions between them. This conclusion questions reliability of results of the standard molecular dynamics approach to determination of the structure and dynamics of the heme environment.

Abstracts

– Imaging and Biospectroscopy –

P-273

Chiroptical methods as a potential tool for clinical diagnosis of colon cancer

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Colon cancer is frequent form of cancer with high mortality due to the fact that almost half of the colon cancers are detected at an advanced stage. Unfortunately, well-established recent clinical procedures have low reliability and sensitivity at an early-stage of the disease. Therefore, our new approach is based on using chiroptical methods – electronic circular dichroism (ECD) and Raman optical activity (ROA) – combined with Raman and IR spectroscopies to investigate the human blood plasma with the aim to find new specific spectral markers for minimally-invasive clinical diagnosis of the disease. For the spectral measurement, we used thawed blood plasma samples from healthy controls and the colon cancer patients with minimal sample treatment. The obtained spectra were evaluated by chemometric methods, which successfully discriminate the blood samples of healthy controls and the colon cancer patients.

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P-274

Cationic fluorescent polymeric thermometers with the ability to enter into yeast and mammalian cells

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Intracellular temperature influences on the diverse cellular reactions, and vice versa. Hence, an accurate measurement of intracellular temperature should contribute to biological, industrial and medical applications. Previously, we established a method for intracellular thermometry based on a fluorescent polymeric thermometer (FPT), but it required a microinjection technique to enter the thermometer into cells. In this study, we tried to introduce FPT into yeast cells, but their rigid cell wall and small size did not allow the microinjection. Thus, we newly developed various kinds of cationic fluorescent polymeric thermometers (CFPTs). Among them, we found several useful CFPTs, which showed spontaneous and rapid (within 10 min) entry into yeast cells and subsequent stable retention in their cytoplasm. The fluorescence intensity of a CFPT, NN-AP2.5, in yeast cells at 35 °C was 2.2-fold higher than that at 15 °C, and the evaluated temperature resolution was 0.09-0.78 ^{o}C between these temperatures. Interestingly, these CFPTs could also readily enter and function in mammalian cells as well. Taken together, our novel new method using CFPTs enabled a practical intracellular thermometry in a wide range of cells without a difficult microinjection procedure.

P-275

Monitoring the reconstitution of membrane proteins by fluorescence correlation spectroscopy

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Liposomes are commonly used for studying the function of membrane proteins in general and membrane transporters in particular. However, appropriate conditions for the reconstitution of a membrane protein have to be determined on a case-by-case basis. The preparation of high quality proteoliposomes can be expedited by methods that allow a fast characterization of the nature and composition of particles formed during reconstitution trials. Fluorescence correlation spectroscopy (FCS) allows to monitor the sizes of diffusing particles and thereby distinguishes micelles, liposomes and aggregates in homogeneous and inhomogeneous samples. Dual-color fluorescence cross-correlation spectroscopy (dcFCCS), an extension of FCS, additionally analyzes the co-localization of protein and lipid in the diffusing entities. FCS and FCCS require only microliter quantities of dilute solutions and short acquisition times of just a few minutes, making these techniques an excellent addition to current methodologies used to aid membrane protein reconstitution. As proof-of-principle, we have used FCS and dcFCCS to guide the reconstitution of a multidrug resistance membrane transporter into liposomes. Functional reconstitution was subsequently verified by a transport activity assay.

Abstracts

– Molecular Recognition and Nanobiophysics –

O-276

Lab-on-chip detection of biomolecules with integrated sensors

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Detection of bioanalytes (such as DNA, proteins, cells, metabolic products) is a central aspect in medicine, food safety, environmental control, etc. We will start by describing our research in two miniaturized transducer concepts. The first involves the use of thin-film silicon photodiodes as optical/electrical transducers and the second involves the use of the measurement of streaming currents in micro/nanochannels as a surface charge/electrical transducer. These transducers are integrated with molecular recognition elements able to specifically capture the bioanalyte of interest. We will discuss the strategies that we use for this integration, and how the characteristics of the biosensor relate to the sensitivity of the detection. In addition, we will briefly describe on-going research integrating these sensors in cell-chips.

To take full advantage of miniaturization, it is crucial in addition to address two other issues: (i) fluidic handling from sample to sensor; (ii) consideration of the interfering effects of the chemically and physically complex biological sample matrix. We will use our work on the detection of a toxin (ochratoxin A, OTA) produced by fungi that contaminate several sources of food and drink as a case study of an integrated lab-on-a-chip analytical system.

O-277

Getting a grip on alpha-synuclein amyloid oligomers - single molecule approaches

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Early oligomeric aggregates of human alpha-synuclein are implicated in interactions with essential cellular components leading to toxicity. Very little is known about the molecular details of these aggregate species. We have developed a method that uses sub-stoichiometric labeling, that is, only a fraction of the monomers contain a fluorescent label, in combination with single-molecule photobleaching to determine the number of monomers per oligomer (Zijlstra et al., *Angew Chem Int Ed Engl 51*, 8821–8824, 2012). The number of bleaching steps gives the number of fluorescent labels per oligomer. Knowing the exact label density, that is, the fraction of labeled monomers at the start of the aggregation, we can correlate the number of fluorescent labels per oligomer to the total number of monomers. Using this method, we can determine the composition, probe the distribution in the number of monomers per oligomer, and investigate the influence of the fluorescent label on the aggregation process.

O-278

Quantifying and localizing interactions guiding the structural and functional properties of GPCRs

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Understanding how molecular interactions contribute to the functional state of G protein coupled receptors (GPCRs) is of particular importance because they mediate most of our physiological responses, and act as therapeutic targets for a broad spectrum of diseases. This talk highlights how single-molecule force spectroscopy (SMFS) can be employed to characterize the intra- and intermolecular interactions of GPCRs embedded in their physiologically relevant membrane and exposed to physiological relevant conditions. The positional accuracy of SMFS localizes these interactions to structural regions of the GPCR whereas the sensitivity of SMFS quantifies their stabilizing interaction forces. To approach the kinetic, energetic and mechanical properties of the structural regions dynamic SMFS probes their stability over a wide range of loading rates. These parameters provide insight into the energy landscape that describes the functional and structural properties of the GPCR. Selected highlights exemplify the application of SMFS to characterize how inter- and intramolecular interactions change structural and functional properties of GPCRs in relation to their functional state (ligand binding), diseased state (mutation), or lipid environment such as cholesterol.

O-279

Molecular nanomechanics and local stimulus of individual biomolecules on the surface of cells

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Nanopipettes can be used to locally deliver and manipulate molecules on the surface of cells and cell membrane mimics. We use this to measure intermolecular forces between different membrane proteins and to stimulate TRPV1 ion channels in sensory neurons. Using supported lipid bilayers (SLBs) we measured the lateral force vs distance between various proteins expressed on the surface of T cells, information that might help in understanding how different proteins organize in the membrane of a living cell. This was performed using nanopipettes to trap and move the proteins laterally in the SLB. Here a liquid flow through the pipette creates a localized force field that traps the molecules below the tip of the pipette. Stimulation of TRPV1 channels, involved in the sensation of heat and pain in the body, was achieved by delivering the molecule capsaicin out of the pipette, using pressure or voltage to control the rate of delivery. This resulted in single cell information about the open probability of TRPV1 at different concentrations of capsaicin, as well as the number of channels per cell. In addition, I will also discuss how stimulation of individual receptors can be used to map their distribution on the cell surface, and how to trap membrane proteins on the surface of live cells.

Abstracts

– Molecular Recognition and Nanobiophysics –

O-280

Signal-Driven tethering system based on DNA-Origami linked to lipid bilayers

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Considerable work has been put into building DNA-origami structures for a variety of applications and into linking oligonucleotides to lipid membranes. By bringing these systems together, we devised a 'novel biological tethering system based on a controllable DNA origami box coupled to a vesicle'. The system comprises a hexagonal DNA origami box that is attached to a lipid vesicle by cholesterol-modified oligonucleotidic 'anchor strands'. Opening of the DNA-origami box can be controlled by the binding of specific ligands to aptamer locks. Upon opening, several single-stranded DNA 'catcher strands' are exposed. These strands are complementary to 'receiver strands' linked to target species present in solution. Consequently, these target species bind to the DNA-origami box only in the presence of a signal establishing a signal-driven tethering system. Our system may be used as a signal-driven targeted drug delivery system in which drugs or compounds encapsulated in vesicles are delivered to specific targets. Other potential applications include vesicle fusion by membrane destabilization, using the system to 'fish' for a specific target in solution and forming highly ordered vesicle networks which may be extended to artificial tissue.

O-281

Regulation of Nitric oxide synthases by fluorescent NADPH derivatives upon two photon excitation

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We report the structure-based design and synthesis of new NADPH derivatives able to turn on and off the activity of NO-synthase (NOS), upon two-photon excitation. These derivatives called nanotrigger NT1 and nanoshutter NS1, target the NADPH site of NOS by a nucleotide moiety mimicking NADPH linked to a conjugated chromophore with non-linear absorption properties. By introducing a donor-donor substituent in NT1, an ultrafast electron injection to FAD turns on NOS catalysis. In contrast, NOS catalysis was inhibited by NS1 that could not provide reducing equivalents to the protein. NS1 became fluorescent once bound to NOS while free NS1 was not fluorescent in aqueous medium. NS1 co-localized with endothelial NOS (eNOS) in living endothelial cells. Thus, NS1 constitutes a new class of eNOS probe with two-photon excitation in the 800-950 nm range. NT1 allows spatio-temporal control of NO formation in endothelial cells and holds a great potential for improvement in endothelial dysfunctions.

Li *et al*, PNAS (2012), 109:12526.
Beaumont *et al*, ChemBioChem (2009), 10:690.

P-282

2D fusion assay based on membrane-coated microspheres

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Membrane fusion is a key process in life to merge two separate lipid bilayers. A major goal in neurophysiology and research of enveloped viruses is to understand and control the biology and physics of membrane fusion and its inhibition. This poses an experimental challenge on the realization of fast and reliable assays that allow to identify the different stages of membrane-membrane interaction with a minimal use of fluorescent labels. Here, we establish a new 2D assay, which captures and quantifies membrane fusion and its inhibition. The assay is based on membrane-coated microspheres that allow optical inspection of membrane-membrane interaction in 96-well-plates and unequivocal assignment of the various stages of fusion such as docking and membrane merging initiated by molecular recognition events without extensive labeling. The assay allows investigating a large number of interaction partners in a quasi-native environment by automated image analysis on a single particle basis. Imaging can be carried out with a conventional optical microscope. Membrane fusion driven by heterodimeric coiled-coil formation as a proof of concept using fusogenic K- and E-peptides provided comparable results as typical liposome assays, however, with additional information on docking efficiency.

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BINDING KINETICS DETERMINED FROM THE FREQUENCY REPONSE TO THERMAL FORCING

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Recent studies show that in addition to thermodynamic parameters (among which the equilibrium constant K), both association and dissociation rate constants, k_- and k_+, have to be considered when evaluating ligand therapeutic efficiency. Hence, we propose an on-chip technique enabling drug screening: chemical dynamics can now be measured with only 2 μL of reagents, i.e. 100 times less than with SPR or stopped-flow. No grafting or flow is required, which simplify procedures. Our method is here demonstrated on a model system made of two complementary oligonucleotides, one of them bearing a fluorescent tag quenched upon pairing. Sample temperature is modulated by Joule heating, which yields rate constant oscillations and thus concentration variations. The associated fluorescence sinusoidal signal is detected by a PMT connected to a lock-in amplifier. Scanning ω then provides the amplitude and phase transfer functions characterizing the chemical system. After fitting, one obtains relaxation times τ between 1.53 and 0.27 s for average temperatures between 22.5 and 32.5°C. Next, knowing K thanks to a thermal denaturation experiment, we can extract k_+ and k_- from $K{=}k_+[P]/k_-$ and $1/\tau = k_+[P]{+}k_-$, where P is the unlabeled strand in excess. Arrhenius analysis even yields activation energies.

Abstracts

– Molecular Recognition and Nanobiophysics –

P-284

AFM based-force spectroscopy as a functional diagnostic nanotool for hematological diseases

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Glanzmann thrombasthenia (GT) is a rare hereditary hematological disease associated to the membrane glycoprotein $\alpha_{IIb}\beta_3$, the integrin receptor for fibrinogen in platelets. Using AFM based force spectroscopy, we have previously characterized at the single-molecule level and compared the binding of fibrinogen to human platelets and erythrocytes [1]. The aim of this study was to set AFM based-force spectroscopy as a functional diagnostic tool for hematological diseases. The interaction between fibrinogen and platelets from GT patients was studied using fibrinogen-functionalized AFM tips. Our results show that, if the patient has a mutation on the β_3 subunit-associated gene, a significant reduction both on the frequency of fibrinogen-platelet binding and on its force occur. When the GT patient has a homozygous mutation on the α_{IIb} subunit gene, a significant reduction of the frequency of the fibrinogen-platelet interaction arises, but not on its strength. The relation of these results with the clinical data demonstrates the applicability of AFM as a highly sensitive nanotool for the functional evaluation of the outcome of genetic mutations resulting in hematological diseases.

[1] Carvalho et al., ACS Nano, 2010, 4, 4609.

P-285

Ultra-thin membranes composed of cationic-anionic surfactant pairs

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Construction of self-assembled bilayer membranes requires a careful choice of compounds, whose delicate interplay between head group attraction and chain repulsion engenders a truly unique balance over a narrow temperature range. Nature's choice: phospholipid bilayers, are unsuitable for some nanotechnological applications owing to their relatively high cost and lack of robustness. We report the investigation of artificial bilayers composed of long-chained organic ions, such as dodecyltrime-thylammonium (DMA+) and perfluorooctaonate (PFO-). Various ratios of DMA/PFO surfactants result in bilayers of different stability, thickness, area per molecule, and density profiles. Our bilayers are ultra-thin: as low as 2 nm in thickness. To our best knowledge, we have not come across any bilayers which are so thin. We discuss further steps to utilize these surfactant bilayers as highly selective, salt-impermeable membranes.

P-286

Tagged hyperbranched rolling circle amplification for multiplex genotyping assays

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Single Nucleotide Polymorphisms (SNPs) are the most abundant type of variation in the human genome and consequently of utmost importance in clinical diagnosis.
The availability of large SNP databases has motivated the implementation of new strategies to rapidly, costlessly, and efficiently genotype SNPs in highly multiplexed and parallelizable assays.
We have developed a variation of hyperbranched rolling circle amplification (HRCA) allowing multiplexed assays and various detection schemes among which DNA microarrays.
Our technique, called tagged hyperbranched rolling circle amplification
(THRCA), allows to screen multiple SNPs in a single tube. The amplicons are recognized by a terminal ssDNA molecular tag generated during polymerization. The amplification is processed at a constant temperature around 60°C and generates its results in less than 30 minutes.
We present two hybridization strategies to detect THRCA molecules using a dual-color fluorescence-based detection. We apply THRCA to the detection of two bi-allelic SNPs on human gene GJB2.

P-287

Detection of Amyloid Marker Thioflavin T on Ag Nanoparticles by Surface-Enhanced Raman Scattering

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The vibrational characterization of the dye used for detection of amyloid aggregates Thioflavin T (ThT) was carried out by means of Raman and surface-enhanced Raman scattering (SERS). The Raman spectrum of ThT in aqueous solution correlates very well with the spectrum of ThT with the torsional angle between the benzothiazole (BT) and dimethylaminobenzene (DMB) moieties of 37° calculated using DFT calculations at the B3LYP/6-31+G** level. The SERS technique was very useful for the detection of two structures of ThT molecules in the vicinity of the metal nanoparticles: directly adsorbed onto the metal surface and molecules forming multilayers. The structure of ThT molecules in multilayers is similar to the structure of free ThT in aqueous solution. The molecules directly adsorbed on the metal surface actually correspond to the molecules with 90° torsional angle between the BT and DMB rings.

Acknowledgements This work has been supported by the Slovak Research and Development Agency (APVV-0242-11), Slovak Grant Agency VEGA (1/1246/12) and by the project CELIM funded by FP7 EU.

Abstracts

– Molecular Recognition and Nanobiophysics –

P-288

Ultrastable nanohubs from streptavidin for precise construction in biophysics

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The femtomolar affinity of streptavidin:biotin makes it one of the most common connections for nanobiophysics (e.g. DNA:protein, protein:surface/nanoparticle) and a paradigm in molecular recognition. But streptavidin is not without its limitations: the binding of four biotinylated ligands to a streptavidin tetramer can lead to aggregation. To overcome this we previously generated monovalent streptavidin, with one high affinity biotin binding site per tetramer. For many nanoassemblies it is desirable to have two biotin binding sites per tetramer, but there are different possible arrangements, leading to two biotin binding sites either cis or trans. We have developed a strategy to isolate each form, validated by crystallography, and find distinct DNA and protein target binding. Dissociation of biotin is limiting in certain applications (e.g. DNA amplification, single molecule imaging and force spectroscopy), which led us to develop traptavidin with 10-fold reduced biotin off-rate and similar precise control over subunit organization. We have used streptavidin and these novel variants to understand the limits of non-covalent molecular recognition and to enhance force stability at the cell:immunomagnetic bead synapse, for improved recovery of Circulating Tumour Cells.

P-289

Evaluation of functional features of immobilised enzymes using optical and electrochemical methods

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Free-label, specific and sensitive new devices used for toxic compounds detection are needed. Using devices, such biosensors, employing biomolecules as analytical tools offer advantages due to their specificity, selectivity and quick response for real-time analysis. Immobilised biomolecules usually show lower activity towards specific biomolecular interactions compared with free ones, thus biosensors stability and analytical performance depends on the immobilisation process used. In this work optimization of different immobilization methods of glucose oxidase and acetylcholinesterase are presented with the main purpose of enzymatic reactions characterization and used materials bioactivity. Surface Plasmon Resonance and Electrochemical Impedance Spectroscopy are used to characterise the functional features of immobilised biomolecules on gold electrode surface for enzymatic inhibitors as heavy metals ions, mycotoxins and pesticides.

Acknowledgments: The writing of this work has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 245199. It has been carried out within the PlantLIBRA project (website: www.plantlibra.eu). This report does not necessarily reflect the Commission views or its future policy on these areas.

P-290

Interaction of nanoparticles with biomembranes and lipid vesicles modified by artificial receptors

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Targeted drug delivery requires that the carriers recognize specific receptors at the cell surface and that the drug is taken up by the cells. The carriers should overcome two barriers – the glycocalix and the cell membrane. This complex process should be analysed by both model and real biological systems. Both approaches were applied. 1. We studied the interaction between polyamidoamine dendrimers (PAMAM) of fourth generation and large unilamellar lipid vesicles (LUV) containing the receptor - calix[6]arene (CX) that recognize amino groups on PAMAM. At PAMAM concentrations > 0.3 μM, the diameter of CX-modified LUV substantially increased. PAMAM reduced negative Zeta potential of LUV towards positive values. No changes were observed for LUV without CX. 2. We studied also the interaction of lipid-based nanoparticles grafted with 5-50 mol% polyethylene glycol (PEG) with prostate cancer cells (PC3). Using flow cytometry we showed that nanoemulsions with 5 mol% of PEG were taken up by the majority of PC3 cells. Increase in PEG content led to decreased cellular uptake. Intracellular integrity of the nanoparticles was studied with confocal microscopy and cell viability was analyzed.

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P-291

AFM evaluation of the influence of different fibrinogen mutations on its binding to erythrocytes

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Hemorheological parameters such as erythrocyte aggregation are affected by the increase in blood plasma fibrinogen levels. We previously characterized the single-molecule interaction between fibrinogen and a previously unknown $\alpha_{IIb}\beta_3$-related receptor on human erythrocyte membranes [1]. Our aim was to study the effect of different human fibrinogen mutants on the binding to this receptor. For that purpose, we used four different fibrinogen variants, specifically mutated on its putative recognition sites (αD97E, αD574E, αD97E/αD574E and γ'/γ'). By AFM based force spectroscopy measurements, we determined the force necessary to break the bond between each fibrinogen mutant and erythrocytes at the single-molecule level. Similar measurements were done with platelets. Changes on the fibrinogen-erythrocytes binding force and on the binding frequency were found for the different mutants. These results may lead to the identification of the fibrinogen amino acid residues sequences involved on the interaction with the erythrocyte membrane receptor, contributing also to the evaluation of the cardiovascular risk associated to fibrinogen mutations found *in vivo*.

[1] Carvalho *et al.*, ACS Nano, 2010, 4, 4609

Abstracts

– Molecular Recognition and Nanobiophysics –

P-292

Investigating nanoparticle internalization patterns by time-resolved colocalization analysis

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Designing nanomaterials for specific biological or medical applications requires the knowledge of their uptake mechanisms including endocytic cell entry routes, endosomal sorting and resulting intracellular pathways to control their fate, efficiency and toxicity. Time-resolved quantitative colocalization analysis is a method based on confocal fluorescence microscopy being well suited to characterize nano-sized materials with respect to their intracellular trafficking in a sophisticated manner.[1]
The capabilities of this method are demonstrated by studying the internalization pathways of two oppositely charged superparamagnetic maghemite nanoparticle species.
[1]C. Schweiger, R. Hartmann, F. Zhang, W. J. Parak, T. H. Kissel, P. Rivera_Gil, *Journal of Nanobiotechnology* **2012**, *10*, 28.

P-293

Development of Electrochemical Impedance Immunosensors as Point of Care Medical Diagnostic Tools

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In the last 30 years the efforts for the production of inexpensive, sensitive and reliable point of care medical diagnostic devices have constantly risen [1,2,3]. Micro- and nanotechnologies, automatized read-out systems and surface functionalization protocols have been developed, but still the application to complex biosamples remains a challenge [4].
In this work we developed electrochemical impedance immunosensors based on capacitance read-out for the detection of biomolecules in small sample volumes. We performed electrochemical impedance spectroscopy (EIS) measurements of DNA hybridization in electrochemical cells with microfabricated gold electrodes. We tested the time stability in two different setups with two microelectrodes, with and without a third, reference electrode. We demonstrated that the 3 electrodes setup was suitable to measure DNA hybridization kinetics, and for protein detection in biosamples through DNA-directed immobilization schemes.
[1] J.L. Arlett et al.; *nature nanotechnology;* (**2011**)
[2] Yan Xiao-Fei et al.; C*hin. J. of Anal. Chem.* (**2011**)
[3] V. Tsouti et al.; *Biosensors and Bioelectronics;* (**2011**)
[4] J.P. Tosar et al.; *Biosensors and Bioelectronics;* (**2010**)

P-294

Modified LDL-based drug delivery system: a new approach for effective targeted delivery

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Low-density lipoproteins (LDL) have proven to be useful vehicles for targeted delivery of lipophilic drugs to cancer cells. In this work we evaluate a hypothesis that protective coating of the LDL surface by modified dextran (Dm) will make this delivery system more efficient. In using photosensitizer hypericin (Hyp) as lipophilic drug model, we intend to show that such modification can reduce the interaction of the [LDL-drug] complex with other serum constituents, such as free lipoproteins, and consequently decrease the drug redistribution processes. We have also shown that the modification of LDL molecules by dextran does not inhibit their recognition by cellular LDL receptors and U-87 MG cellular uptake of Hyp loaded in LDL/Dm complex appears to be similar to that one observed for Hyp transported by unmodified LDL particles. This approach could lead to a construction of effective delivery system of hydrophobic drugs to cancer cells.
Acknowledgements: This work was supported by the SF of the EU (Contracts: Doktorand, NanoBioSens and SEPO II), by the contract APVV-0242-11, by the project CELIM funded by 7.FP EU, and by the International Program for Scientific Cooperation (PICS N°5398) from the CNRS.

P-295

Conformational Insights into Recognition Mechanism of O-Antigen Polysaccharides by Tailspike Protein

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Tailspike proteins (TSP) of bacteriophages recognize and cleave O-antigen (OAg) polysaccharides on the outer membrane of bacterial cells as a first step in phage infection. Understanding OAg recognition by TSPs is thus important for phage therapy against shigellosis.
We pursued a systematic investigation of OAg fragments of *Shigella flexneri* serogroup Y for up to 12 repetitions of its basic repeat unit (RU) by molecular dynamics (MD) and Monte Carlo simulations at different levels of description [1]. The global conformations of the oligosaccharides were sensitively influenced by the rhamnose (1-3) linkage which allows extreme, hairpin-like conformations. In this way, longer chains sampled a rich spectrum of configurations stabilized by intra-molecular hydrogen bridges.
100 ns MD simulations resulted in stable complex formation of the TSPSf6 with the 2RU octasaccharide at its equilibrium solution conformation, which are in agreement with experiment [2]. By studying a series of mutants, two residues are found to dominate recognition. By further studying longer fragments close to the binding site, we suggest that conformational selection must precede octasaccharide binding.
[1] Wehle M, *et.al*, *J Am. Chem. Soc.* 2012, 134, 18964.
[2] Müller J J, *et.al*, *Structure* 2008, 16, 766.

Abstracts

– Molecular Recognition and Nanobiophysics –

P-296

Gene vectors with the inclusion of anticancer drugs and metal nanoparticles

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The tendency of the last time is to construct multicomponent nanostructures on the base of biological macromolecules for the wide applications including anticancer therapy. Novel structures with metallic nanoparticles modifying properties of systems are in the center of interest due to the unusual physical properties and potential using in biotechnologies. Gene vectors on the base of DNA-polycation complexes with the inclusion of anticancer coordination compounds of platinum group metals and silver nanoparticles were produced and studied by the methods of viscometry, dynamic light scattering, circular dichroism, electronic spectroscopy, atomic force microscopy. Phase diagrams for DNA solutions with different concentrations of synthetic polycations were constructed. The influence of solution composition, ionic strength and pH on the formation of condensed DNA particles was examined. The role of structure, charge and length of polycation in gene vectors formation, the conditions for the incorporation of anticancer drugs and nanoparticles were regarded.

P-297

In situ quantification of signaling complex dynamics by live cell micropatterning

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Mechanistic understanding of signal transduction across the plasma membrane demands quantitative analysis of the protein-protein interaction involved in signaling complex formation and signal propagation by cytosolic effector proteins. For this purpose, we have developed generic tools based on micropatterned surface functionalization. To this end, we devised surface modification by photolithography and microcontact printing for in *situ* capturing of HaloTag fused proteins into micropatterns. Thus, functional micropatterning of the type I interferon (IFN) receptors within the plasma membrane of living cells was succeeded. Quantitative interaction dynamics of ligand binding, ternary complex formation, signal propagation to the associated Janus kinases, and to the cytosolic effectors STAT1&2 were achieved by single molecule and ensemble assays. Moreover, the interaction of IFN receptor with the negative feedback regulator USP18 was resolved in a quantitative manner. For analyzing cytosolic effector complexes, we developed nanobody micropatterns for efficient capturing of EGFP fusion proteins. Thus, the effector complex of STAT1 and STAT2 were directly captured in *situ* from lysed cells at different states of activation for quantitatively assessing the stoichiometry and stability.

P-298

Cap- and 4E-BP binding cooperativity in eIF4E by analytical ultracentrifugation and fluorescence

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Specific recognition of the mRNAs 5' terminal cap structure by the eukaryotic initiation factor eIF4E is a rate-limiting step in the cap-dependent translation. Each of a small 4E-binding proteins, 4E-BP1, 4E-BP2, and 4E-BP3, inhibits the translation initiation by competing with eIF4G initiation factor for the other eIF4E binding site. Cooperativity between the two binding centres is a subject of controversy in the literature. Moreover, the very values of the association constants of the binary and ternary complexes, composed of eIF4E, mRNA 5'cap, and 4E-BP, determined by fluorescence titration and SPR, seem equivocal. Here, the interaction of eIF4E with a cap analogue m^7GTP and with 4E-BP1 was characterized by analytical ultracentrifugation and fluorescence titration. Sedimentation velocity experiments showed formation of 1:1 stable complex of eIF4E and 4E-BP1. The affinity of 4E-BP1 for eIF4E increases tenfold in the presence of m^7GTP, $K_{as} = 6 \times 10^6$ $M^{-1}$for the *apo*-, and $K_{as} = 50 \times 10^6$ M^{-1} for the cap-bound, eIF4E. The association constant for m^7GTP binding to eIF4E, $K_{as} = 10^8$ M^{-1}, decreases twice upon prior incubation of eIF4E with 4E-BP1. Our results provided a deeper insight into the mechanisms underlying the regulatory processes at early stages of the mRNA translation.

P-299

Chiroptical properties of bilirubin bound to mammalian serum albumins

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Bilirubin (BR) is orange yellow pigment of bile, product of the heme metabolism. The main part of total pigment amount in mammals is bound to transport proteins, mainly to serum albumin. Although BR-serum albumin interactions are among the most studied serum albumin-ligand interactions, the location and chiroptical properties of binding sites are not yet clear. Chiroptical properties of BR bound to binding sites with high and low affinity of serum albumin in mammals were investigated in this work. Considering the optical activity of BR-serum albumin complexes, it is possible to study and characterize them by circular dichroism spectroscopy, which is a method inherently sensitive to the structure of chiral substances.
Also, mechanism of interaction between BR bound at different albumin binding sites with structure analogues of mutagens were studied by chiroptical spectroscopy which tightly connected with antimutagenic properties of bile pigment.
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Abstracts

– Molecular Recognition and Nanobiophysics –

P-300

Nanobiodevice that consists of an artificial lipid membrane suspended over a microwell array

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The combination of biological systems and semiconductor nanotechnology offers great potential for device applications, such as high throughput and highly sensitive bio-sensors. Our aim is to fabricate a nanobiodevice that consists of a suspended artificial lipid membrane with receptor proteins bound to it, to mimic a post synapse. We have succeeded in fabricating a microwell array sealed with a lipid bilayer on a Si substrate for analyzing ion channel activity. First, we introduced model proteins such as α-Hemolysin into the lipid membrane and observed Ca^{2+} ion transport through them from the change in the intensity of a fluorescent probe (fluo-4). Second, we also tried to control the growth of neurons on the nanobiodevice by modifying the substrate surface (morphology, coating, and/or electrostatic charges). The confinement of hydrogels in the microwells was proposed to improve the stability of the lipid membrane during neuron culture. Our naobiodevice will be a promising platform for controlling artificial synaptic connection and analyzing biological signal transduction.

P-301

Indirect optical manipulation of live cells with functionalized polymer microtools

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Cells can be trapped and manipulated with optical tweezers. However, due to the high laser intensity inside an optical trap, a cell is likely to get damaged by the trapping laser itself. Previous studies confirmed a dosage dependent viability for trapped cells. Therefore, even in traps with low laser power, a trapped cell will die over time.
To overcome this limitation we designed and fabricated two-photon polymerized microtools, which can be bound to target cells, and manipulated by holographic optical tweezers. The binding is achieved by functionalizing the surface of the cells with biotin molecules, and that of the microtools with biotin and streptavidin. Thus, when a holographically trapped microtool is brought to contact with a target cell, biotin-streptavidin-biotin bridges form between them, providing a force much greater than an optical trap can exert. Therefore bound cells can be dynamically manipulated in 3D by rotating and translating the microtool with holographic optical traps.
With this indirect manipulation the target cell is separated from the trapping laser in space, thereby its exposure by the optical field can be greatly reduced.

P-302

Microfluidic single cell analysis with affinity beads

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The functioning of living cells relies on a complex cellular biochemical network. Important in this context are concentrations of cellular components like proteins and their changes in response to extracellular stimuli. Progress of understanding these central processes requires elucidation of the heterogeneity between individual cells. Chemical cytometry has been used as a valuable tool in single-cell analysis, but suffers from limited analytical sensitivity and low throughput. We demonstrate here that these problems can be overcome using micron-sized beads as intracellular af?nity probes, which are easily transferred into human cells via phagocytosis. Upon release from their phagosomes using endosome disrupting methods, beads coated with different specific capture agents are able to bind efficiently multiple cytosolic target analytes on their surface. For single cell analysis of bound analytes, an optically trapped bead is extracted from its host cell in a microfluidic device. Non fluorescent analytes can be targeted with a detectable secondary capture agent to be ultimately quantified. Compared to classical chemical cytometry, our method has the potential to reach high analytical sensitivity and reasonable throughput for single cell analysis in many biomedical fields.

P-303

Thermodynamics of anticancer drug lead binding to target proteins by thermal shift assay

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The lead discovery in pharmaceutical design is based on selecting the highest affinity compound that binds and inhibits the target protein function. However, compounds with similar affinities often have very different enthalpic and entropic contributions due to the enthalpy-entropy compensation phenomenon. Isothermal titration calorimetry (ITC) is a method of choice for the determination of lead compound observed and intrinsic equilibrium binding enthalpy, entropy, and the Gibbs free energy. However, the thermal shift assay (ThermoFluor) is a more-general method and avoids the narrow window of ITC K_d measurements.
Combination of the intrinsic enthalpies, entropies, and the Gibbs free energies together with the crystal structures of compounds bound to target protein isoforms provide the direction of optimization of the compound binding affinity and selectivity towards the desired enzyme isoform. Compounds are mapped in the direction of increasing functional groups to correlate with the increments in the intrinsic thermodynamic binding parameters. The structure-thermodynamics correlations are used for the design of drug-like lead compounds with desired binding properties to carbonic anhydrases, heat shock protein 90 (Hsp90), and several epigenetic therapeutic targets.

Abstracts

– Membrane Structure and Domains –

O-304

Effects of phosphoinositides and their derivatives on membrane morphology and function

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One of the fundamental problems in the study of membrane function and morphology is that the roles of proteins and lipids are usually investigated separately. Contrary to current doctrine giving proteins sole responsibility for shaping endomembranes, our recent findings demonstrate that a neutral lipid, diacylglycerol, is an *in vivo* modulator of organelle morphology in mammalian cells and echinoderm embryos, indicating a fundamental conserved function. To elucidate the complex role of diacylglycerol we employed a chemical biology approach to manipulate acutely endogenous diacylglycerol levels in cellular sub-compartments. Acute and inducible diacylglcerol depletion in the ER and nuclear envelope, result in failure of the nuclear envelope to reform and the reorganisation of the ER at cytokinesis. Live cells depleted of this neutral lipid divide without a complete envelope surrounding their chromosomes, and unless rescued by ectopic diacylglycerol, die soon thereafter. To date the key functional role of diacylglycerol has been considered to be as a second messenger, we attribute two new and conserved functions to diacylglycerol: a structural role in organelle shaping for which proteins alone are insufficient, and a role in localised extreme membrane curvature required for fusion.

O-305

Modulation of phase coexistance and biophysical activity in pulmonary surfactant membranes and film

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Pulmonary surfactant is a lipid-protein complex, which stabilizes the respiratory surface of lungs. The composition of surfactant has evolved to contain similar amounts of saturated and unsaturated phospholipids, taking the structure of surfactant layers near a critical ordered/disordered phase coexistence. Such structure is particularly well adapted to simultaneously sustain appropriate dynamics and mechanical stability.
Physiological amounts of cholesterol shift the structure of surfactant membranes from a gel/fluid into a liquid-ordered/liquid-disordered type of coexistence. Interestingly, exacerbated proportions of cholesterol abolish the ability of surfactant films to sustain very low surface tensions. The study of surfactant from mammals with variable body temperatures reveals that cholesterol is finely tuned depending on temperature, associated to changes in the content of the specific surfactant lipopeptide SP-C. In fact, SP-C can revert deleterious effects of cholesterol, and it also shifts the phase diagram of cholesterol-containing ternary lipid membranes towards phases with less cholesterol, indicating that the combined action of cholesterol and SP-C could play a role to fit the properties of surfactant to specific environmental constraints.

O-306

Transport and ATPase activity of the human ABCC3 transporter show positive cooperativity

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Human ABC transporters are involved in a multitude of physiological processes including multi-drug resistance. Despite their medical relevance, little is known about the mechanism of ATP hydrolysis and substrate translocation. We investigated the kinetics of ATP hydrolysis and substrate translocation for the human multi-drug resistance related protein 3 (hMRP3, ABCC3) with the focus on the cooperative interactions between the two ATP hydrolysing nucleotide binding domains (NBDs) and the two substrate translocating transmembrane domains (TMDs) of the functional protein in reconstituted large unilamellar vesicles (LUVs).
Upon stimulation with transport substrates, ATPase activity of hMRP3 increased from a basal level by a factor of three showing positive cooperativity with a Hill coefficient of $n = 2$. While we found different affinity constants for all tested substrates, the Hill coefficient and v_{max} seem to be substrate independent. This supports the idea that substrate-induced cooperativity of the two NBDs in ABCC3 is mediated by the TMDs and not by direct interaction between substrate and NBDs.
Further, we investigated the ATP driven uptake of substrates into the lumen of hMRP3-containing LUVs and found substrate translocation to be a cooperative process again with the same k_M value that has been found for the APT activity and a Hill coefficient of $n \approx 2$ for all tested substrates. This leads us to the assumption that the two TMDs of ABCC3 act in a highly synchronized manner during substrate translocation.

O-307

The effect of cyclic antimicrobial hexapeptides on model bacteria membranes

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The rising antibiotic resistance accompanied with decreasing numbers of novel antibiotics challenges the current pharmaceutical research to find enduring active antimicrobial agents. Antimicrobial peptides (AMPs) as evolutionary ancient molecules to control the coexistence of microbes and complex multicellular organisms are such promising agents. Peptide induced lipid demixing attracted attention as a possible initial step in the mechanism of the antimicrobial action of AMPs. In this study we tried to identify the boundary conditions of anionic lipid clustering in different model membranes after peptide binding. We determined the thermotropic properties of binary and ternary mixtures of phosphatidylethanolamines, cardiolipin, and phosphatidylglycerols, with and without bound cyclic peptides containing arginine, phenylalanine and tryptophane.
DSC, ITC, and ATR-IR spectroscopy was used to study binding to lipid bilayers. Peptide interactions with lipid monolayers were investigated by IR-reflection-absorption-spectroscopy and epifluorescence microscopy.
The results show that not only the number of charges but also the amino acid sequence and the lipid mixing behavior influences the ability of peptides to induce membrane domain formation.

Abstracts

– Membrane Structure and Domains –

O-308

Ceramide activates endocytosis and forms ordered intracellular lipid domains in response to TNF-α

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Ceramide (Cer) is an important sphingolipid involved in the regulation of several cellular processes. The mechanism by which Cer regulates these processes is thought to be associated with the biophysical changes that this lipid promotes on cell membrane properties. However, studies regarding the impact of Cer generation on the biophysical properties of cell membranes were still missing. In the present study we show that increase in the levels of Cer by treating the cells with TNF-a and/or bacterial sphingomyelinase induced profound changes in membrane properties through the formation of highly-ordered Cer-enriched domains. We further show that these domains exist in intracellular vesicles that colocalize with endocytic markers. Moreover, we provide evidence for Cer-mediated activation of macropinocytosis and clathrin-mediated endocytosis. Our results link the biophysical changes induced by Cer to important cell processes and emphasize the existence of Cer-enriched vesicles with distinctive biophysical properties that might function as intracellular signaling platforms.

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O-309

Local pH gradients induce polarization of Lo and Ld domains in GM1-containing giant vesicles

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The effect of an external local pH gradient on raft-like Lo domains in model membranes was studied on giant unilamellar vesicles (GUV) made of PC, SM, cholesterol and the ganglioside GM1, [1]. The spatial pH gradient, generated by local microinjection of acid, promotes segregation of domains and polarization of the GUV. Lo domains within an Ld phase accumulate to the basic side. Ld domains within an Lo phase accumulate to the acidic side. This is not observed without GM1 or with asialo-GM1 and thus is related to GM1 protonation. Laurdan fluorescence experiments on LUVs show an increase in lipid packing below pH 3-4, due to abolishment of repulsion between GM1 molecules after protonation. It is much higher for Ld vesicles than for Lo vesicles. The tendency of Ld phase to segregate towards the acidic side of a spatial pH gradient might be thermodynamically explained by its increased packing as well as the resulting decrease of bilayer thickness mismatch with the Lo phase induced by low pH. Such effects might play a role in cellular polarization processes which involve lateral pH gradients due to segregation of NHE1 H+ exchanger.

[1] Staneva G, Puff N, Seigneuret M, Conjeaud H, Angelova MI (2012) Langmuir 28 16327–16337.

P-310

Importance of mitochondrial membrane potential at cellular response generated against chemicals

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The alteration of mitochondria membrane potential ($\Delta\Psi$m) and permeability causes release of proapoptotic proteins as cytochrome c and Smac-DIABLO and so death process enters into an irreversible stage. Therefore, the changes of $\Delta\Psi$m are an important apoptotic marker.Lipophilic, cationic florescent dye rhodamine 123 easily passes through cell membranes and accumulates in mitochondria, most negatively charged organelle in cell. Depolarization of $\Delta\Psi$m decreases accumulation of rhodamine 123 in this organelle and thus changes at $\Delta\Psi$m can be determined relatively by measuring florescence intensity. In this study, we used methotrexate (MTX) as a cytotoxic drug for breast cancer cells (MCF-7) and with $\Delta\Psi$m analysis we determined that MTX could lead cells to apoptosis at lower concentrations when MCF-7 cells arrested at different phases of cell cycle. We used both flow cytometric and immunofluorescence methods for $\Delta\Psi$m analysis. Consequently we suggest MCF-7 cells arrested at G2/M and G1/S phases of the cell cycle by plant extracts as genistein and mimosine becomes more sensitive to MTX and with an application like this MTX could kill cancer cells at lower concentrations and MTX damage to normal cells could be reduced to minimum.

P-311

Molecular crowding effects on the structure and dynamics of a lipid bilayer

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The cytoplasm of cells is an aqueous medium with a high concentration of small molecules and molecular assemblies where a significant fraction of the water is involved in solvation and does not behave as bulk water. This particular type of water may affect the structure and dynamics of solutes being generally described as molecular crowding effects. In this work we have evaluated the effect of a non-reducing sugar (trehalose) as an approximated model of cell cytoplasm molecular crowding, in the viscosity and phase behavior of POPC, DMPC/DSPC and SpM/CHOL membranes (at different lipid molar ratios). The effects at the membrane interface and hydrophobic core were characterized via the temperature dependence of fluorescence anisotropy and lifetimes of NBD-DMPE and TMA-DPH, respectively. It is observed a significant increase in the phase transition interval indicating stabilization of phase coexistence by 1M trehalose. This effect is particularly relevant for membranes with coexistence of liquid-disordered and solid phases. The results obtained with the fluorescence probes were complemented with DSC experiments.

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Abstracts

– Membrane Structure and Domains –

P-312

Effects of trimethoxy catechin-gallate on the structural properties of phosphatidylcholine membranes

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A synthetic trimethoxy derivative of a green tea polyphenol, 3-O-(3,4,5-trimethoxybenzoyl)-(–)-catechin-3-gallate (TMCG) has showed high antiproliferative activity against malignant melanoma. The hydrophobic nature of TMCG suggests that the interaction with membranes would be important to understand the molecular mechanism of its activation by tyrosinase, and its potential capacity to modulate membrane related processes. We look into the interactions of TMCG with a model system composed of dipalmitoylphosphatidylcholine membranes by using different biophysical techniques. Differential scanning calorimetry shows broad complex thermograms untill a 0.25 molar fraction of TMCG is reached where a cooperative peak at 24°C is present. At high TMCG concentration a gel phase immiscibility is found suggesting the formation a phospholipid-TMCG complex. TMCG at high concentration shows a drastic reduction in the interlamellar repeat distance of the phospholipid. The hydrocarbon chain conformational disorder is increased and the carbonyl region shows an increase in the hydrogen bond pattern of the phospholipid. The above evidence supports the idea that TMCG incorporates into the phosphatidylcholine bilayers and produces structural perturbations which might affect the function of the membrane.

P-313

Polarity properties of lipid bilayers formed by sphingomyelin-based binary mixtures

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Sphingomyelin (SM) is a major constituent of eukaryotic membranes. Its mixtures with cholesterol (CHOL) and other phospholipids (*e.g.* POPC, DOPC) are important for comprehending the plasma membrane properties, yet they are less studied. Lipid bilayers provide a complex media whose polarity is mainly determined by water penetrating into the bilayer (forming a polarity gradient), though the influence of dipoles from phospholipids (*e.g.* –PO, –CO, –OH) and the double bond of CHOL cannot be neglected. CHOL derivatives are interesting tools to verify the influence of double bonds in the polarizability effects. Pyrene fluorescence was used to access an equivalent polarity in the ordered section of lipid bilayers. For egg-SM/CHOL mixtures, higher CHOL amounts leads to substantial changes in the thermal behavior and polarity values. Egg-SM/POPC and egg-SM/DOPC show different behaviors depending on the glycerolipid proportion. Adding 7-dehydrocholesterol (7DHC) or cholestanol (DCHOL) to egg-SM bilayers showed the same tendency detected upon mixing higher amounts of CHOL.

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P-314

Cholesterol and palmitoylceramide interactions in gel bilayers: a study using transparinaric acid

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The lifetimes of *trans*-parinaric acid (tPA) have been widely used to study the molecular order and lateral heterogeneity of lipid bilayers. In this work, we have used the lifetimes of tPA to investigate the effect of cholesterol (Chol) and/or palmitoylceramide (pCer) when incorporated into liposomes made of either dipalmitoyl phosphatidylcholine (DPPC) or palmitoylsphingomyelin (pSM). At room temperature, tPA lifetimes showed that Chol had a disordering effect on both DPPC and pSM bilayers, whereas pCer had an ordering effect. When both Chol and pCer where incorporated an intermediate situation showing the effect of both lipids could be seen. This behavior was further characterized by measuring tPA lifetimes over increasing temperatures, which allows monitoring the melting of the gel phase (gel-to-fluid transition). The ternary mixtures showed a single melting profile, which again showed contribution from both Chol and pCer. These results agree with recent work in our lab showing that incorporation of both Chol and pCer into gel bilayers of DPPC or pSM could give rise to a homogeneous lamellar gel phase with unique properties. These data become relevant in the context of sphingolipid signaling and membrane platform formation.

P-315

Correlating laurdan fluorescence lifetime and generalized polarization in lipid membranes

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Laurdan is a solvatochromic dye widely used in sensing the lipid packing of both model and biological membranes. Generalized polarization (*GP*) assesses the shift of the fluorescence emission maximum from about 440 nm (blue channel) in condensed membranes to about 490 nm (green channel) in more fluid membranes. The fluorescence lifetime of Laurdan is rarely used in spite of its high potential in sensing the changes in the environment. In this study, we used both *GP* values and fluorescence lifetimes in the two channels to characterize giant unilamellar vesicles (GUV) with different lipid composition. The results indicate a strong correlation between *GP* and the lifetimes recorded in the two channels for different lipid phases. Unexpectedly, the studies reveal that both *GP* and fluorescence lifetimes depend on the angle between the orientation of the linear polarization of the excitation light and the position on the circumference of the GUV. This angular variation depends on the lipid phase and the emission band and can be interpreted in terms of different conformers of Laurdan.

Abstracts

– Membrane Structure and Domains –

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Optically-triggered high-voltage spark-gap switch-based system for cell nanoporation

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The system for electroporation of cells with nanosecond-duration pulses of strong electric field is presented. It consists of the electric pulse generator based on spark-gap switch and a coaxial cuvette with the volume of 30 μl and 1-mm distance between the electrodes. The spark-gap is optically triggered by a 0.45-ns duration and 1-mJ energy laser pulse (wavelength 1062 nm). A 75-Ohm impedance transmission line with a 1:100 attenuator and the 6-GHz wideband real-time oscilloscope were used to monitor the pulse. The system can generate near-perfect square-wave pulses (rise and fall times <0.5 ns) with the duration from 10 to 90 ns and the maximal amplitude of 12.5 kV. The main advantage of the system is the ability to generate single pulses with the amplitude and duration precisely set in advance.
The system was tested on human erythrocytes. The fraction of electroporated cells was determined from the extent of hemolysis after long (20–24 h) incubation in 0.63% NaCl solution at 4 ^{o}C. The dependence of the fraction of electroporated cells on the amplitude of the electric field pulse was determined for pulses with the duration from 10 to 95 ns. For the 95 and 40-ns duration pulses, the amplitude required to electroporate 50% of cells was 11 and 60 kV/cm respectively.

P-317

Structural properties of lipid rafts in biomembranes: a molecular dynamics simulation study

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A number of experimental studies show the existence of highly ordered lateral domains in biomembranes rich in sphingomyelin (SM) and cholesterol. These domains, called functional lipid rafts, take part in a variety of processes such as membrane trafficking, signal transduction, and regulation of the activity of membrane proteins.
In this work we have created three independent systems - all of them consist of water, SM, cholesterol and POPC lipids in ratio 1:1:2. The first system is mixed lipids randomly inserted in the water box, the second is bilayer without formed raft and the last with SM and cholesterol raft in the center of biomembrane. The aim of the research is to understand how raft can be formed and its evolution during the time.
The MD simulations were carried out using the program Gromacs, version 4.5. Such properties of the membrane were measured: lateral diffusion of the lipids, area per lipid, order parameter, thickness and density profiles. We showed that raft always have the higher thickness than POPC bilayer, SM lipids have crystal phase and cholesterol plays crucial role in formation of the raft. Also we found out that two monolayers of the raft are asymmetric with respect to each other.

P-318

Dynamic membrane structures at high pressure

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Lipid membrane dynamics and micromechanics are vitally important for a wide range of cellular processes including mediating protein activity, signaling, material transport and apoptosis. Pressure has a key role to play both in the study of these fundamental membrane properties and in the biology which they support.
We have recently developed a new platform for high pressure and pressure-jump microscopy, tailored for experiments on soft-matter and biological systems. This has led to a series of exciting studies of the pressure dependence of key micromechanical membrane parameters and membrane structural dynamics.
Of particular interest are our recent experiments imaging dynamic lateral phase separation in model membranes under high pressure: Using fluorescence microscopy, we have been able to image the formation and evolution of membrane microdomains in giant unilameller vesicles triggered by rapid application of high pressure. We have also made the first measurements of the bending rigidity of hydrated lipid membranes under pressure using high speed video microscopy to image the thermal fluctuations of giant unilamellar vesicle model membranes contained in our high pressure microcopy system.

P-319

Effect of amphiphile dipole moment in solubility and partition to membranes

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The dipole potential of a membrane is oriented with the positive pole in the bilayer center and the negative at the headgroup region. This electrostatic potential is much larger than usual transmembrane potentials, generating a strong local electrical field. In this work we studied the interaction between two amphiphiles, a Rhodamine (RG-C_{14}) and Carboxyfluorescein (CBF-C_{14}) derivative, and membranes. Both probes are attached to a 14 carbon alkyl chain but have a dipole moment which is different in magnitude and orientation. The relative partition coefficient (Kp_{rel}) between a POPC bilayer and membranes with distinct lipid composition was measured and rationalized in terms of membrane dipole potential and amphiphile dipole moment. A strong increase in Kp_{rel} with the membrane dipole potential was attained for RG-C_{14} (opposite orientation of dipole moment and membrane dipole potential) while little effect is observed for CBF-C_{14} (same orientation of dipole moment and membrane dipole potential). The aggregation of the fluorescent amphiphiles in different membranes was also studied and RG-C_{14} showed an increase in aggregation with the membrane dipole potential. The transmembrane location of the amphiphiles was also evaluated from fluorescence anisotropy and lifetimes measurements.

Abstracts

– Membrane Structure and Domains –

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Niemann-Pick disease type C: sphingosine accumulation affects membrane biophysical properties

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Niemann-Pick type C1 (NPC1) is a rare inherited disorder associated with mutations in the NPC1 gene, encoding a large transmembrane protein whose loss of function leads to lipid accumulation, including cholesterol and sphingolipids, in the late endosomal/lysosomal compartments. The mechanism under this complex process is not well understood, but it is known that lysosomal storage of sphingosine (sph) is an early event in NPC1 pathogenesis. In this study, we address the impact of sph in the biophysical properties of models of plasma (PM) and lysosomal (LM) membranes as a tool to understand the implications of sph cellular accumulation. Using fluorescence spectroscopy we show that sph accumulation leads to the formation of sph-enriched gel domains. These domains are easily formed in PM as compared to LM models where higher sph concentrations (or lower temperatures) are required. In PM models, sph is mainly neutral whereas in LM models, the positive charge of sph leads to electrostatic repulsion, reducing shp ability to form gel domains. Thus, formation of sph-enriched domains in cells might be mainly a charge driven process. Acknowledgments: Fundação para a Ciência e Tecnologia, PT: PTDC/QUI-BIQ/111411/2009, PEst-OE/QUI/UI0612/2011, SFRH/BD/88194/2012, C*ompromisso para a Ciência*

P-321

Biophysics of a novel surfactant secreted from stem cells differentiated into pneumocyte-like cells

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Lung surfactant is a lipid-protein complex lining the alveolar spaces, where it reduces surface tension at the air-liquid interface. Surfactant is synthesized and secreted by type II pneumocytes into the respiratory surface as membranous particulate entities termed lamellar body particles (LBPs). Once reaching the interface, LBPs disintegrate to form the surface active film.

Since established surfactant-producing cell lines are not available at present, we have developed a new cellular model based on the differentiation of placental stem cells to pneumocyte-like cells (PLCs). PLCs express surfactant markers and are able to generate and secrete surfactant-like complexes.

In the present study we have tested packing, hydration and adsorption properties of the lipid-protein complexes secreted by PLCs. Exocyted material from PLCs manifests a noticeable similitude with the secretion product of pneumocyte primary cultures, exhibiting a comparable structural organization in the form of highly packed and dehydrated phospholipid membranes, which display a notable surface activity even in presence of inhibitory agents. These findings reveal that PLCs could be a promising cell model to investigate surfactant biogenesis and secretion.

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Hybrid Polymer-Lipid Vesicles as Biomimetic Membranes: Towards the Control of Lipid Distribution

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In the field of biomimetic structures and drug delivery, polymersomes (vesicles obtained by the self-assembly of block copolymers) have been studied extensively over the past decade. The modulation of their membrane properties is a key to exploit all their potentialities.

We present an approach which consists in blending phospholipids and copolymers to obtain hybrid vesicles and take advantage of both properties of polymer and lipid membrane. Using lipids of different melting temperature and copolymers of different architecture and molecular weight, we were able, using epifluorescence, confocal microscopy and differential scanning calorimetry, to evidence the formation of hybrid Polymer/lipid Giant Unilamellar Vesicles whose membrane structuration and stability can be controlled by the polymer/lipid molar composition, fluidity of the lipid phases and thickness of the polymer membrane. Especially, we are able to formulate polymersomes presenting micrometric lipid domains or vesicles in which lipids are homogeneously distributed or possibly structured in "nano" domains. The membrane properties of these new kinds of vesicular structures are actually under investigations as well as a fine determination of the role of each component on the hybrid polymer/lipid membrane structure.

P-323

Evaluating lateral partitioning of DPH in DMPC/cholesterol bilayers by fluorescence anisotropy

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Steady-state fluorescence anisotropy ($< r >$) eventually varies with changes in rotational correlation time of a fluorophore. So, it is effectively suited to quantify the fluorophore lateral partition between liquid-ordered/liquid-disordered coexisting phases in lipid bilayers, provided that the proper experimental conditions are fulfilled toward the sensitivity/accuracy of measurements (D. Marsh. (2010) *BBA* **1798**, 688). The partition equilibrium of DPH (1,6-diphenyl-1,3,5-hexatriene) − a non-polar fluorescent probe totally inserted inside the ordered section of bilayers − was accessed by steady-state anisotropy, using lipid bilayers containing binary mixtures of DMPC/cholesterol. This mixture is especially suitable, since the region of phases coexistence is established on its phase diagram. We are able to detect DPH partitioning in DMPC/cholesterol MLV, from 5 to 20 mol % of cholesterol (independent of fluorescence lifetime effects), which is a region of phases coexistence slightly shifted from the well-known phase diagram. We discuss the results and pertinences based on the fluorescence sensitivity and DPH molecular structure.

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Abstracts

– Membrane Structure and Domains –

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Unraveling Nystatin Molecular Action Mechanism: the Influence of Membrane Composition and Properties

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Nystatin (Nys), a naturally occurring polyene antibiotic, has strong antifungal activity, but elevated cytotoxicity towards mammalian cells. Nys targets the plasma membrane (PM) of sensitive organisms forming ion channels, possibly due to Nys-sterol interactions and/or preference for ordered membrane regions. The existence of ergosterol-free sphingolipid-enriched gel domains in yeast cells led us to hypothesize that Nys pore formation might be governed by the presence of gel phase in the PM of fungal cells. Fluorescence spectroscopy studies using liposomes composed of a fluid lipid and different gel-domain forming lipids (sphingomyelin (SM) or DPPC) show that Nys has stronger partition from the aqueous medium into gel-enriched membranes particularly containing SM. However, the distribution of membrane-associated Nys species among gel and fluid domains is markedly different in the presence of DPPC. Formation of Nys aggregates with long fluorescence lifetime (Nys active species) depends on the number of Nys molecules located within the gel phase. We conclude that Nys partition and aggregation depend on both lipid -type and -phase and are enhanced by the presence of DPPC gel phase.

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P-325

The role of lipid membrane in amyloid fibril formation and toxicity in Alzheimer's disease

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Alzheimer's disease is a progressive neurodegenerative disease associated with amyloid fibril formation in the brain. It is now accepted that the cytotoxicity is a result of the non-specific interaction of toxic soluble amyloid oligomers with the surface of plasma membrane. We used atomic force microscopy (AFM), atomic force spectroscopy (AFS), frequency modulated Kelvin probe microscopy (FM-KPFM), Langmuir-Blodgett monolayer technique and Surface Plasmon Resonance (SPR) to study effect of membrane structure and composition on binding of amyloid-β (1-42) peptide and fibril formation. We show that cholesterol induces electrostatic domains in lipid membrane which creates a target for amyloid binding. Hormone melatonin, which regulates and maintains the body's circadian rhythm, has been shown to be protective against AD, but molecular mechanism of this protection is not understood. We show that melatonin and cholesterol have the opposite effects of the lipid membrane properties which, in turn, affect amyloid binding to the lipid membrane.

E.Drolle, R.Gaikwad, Z.Leonenko, *Biophys. J.*, 2012, 103: L27-L29; F.Hane, E.Drolle, R.Gaikwad, E.Faught, Z.Leonenko, *J. of Alzheimer's Disease,* 2011, 26:485-494.

P-326

Effect of melatonin and cholesterol on the structure of DOPC and DPPC lipid membranes

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The cell membrane plays an important role in amyloid toxicity - amyloid oligomers can interact with lipid membrane, inducing various defects that are toxic to the cell. Membrane composition may affect its interaction with amyloid peptides. Both melatonin and cholesterol have been linked to amyloid toxicity, with melatonin having been shown to have a protective role against amyloid toxicity, however the underlying molecular mechanism of this protection is still not well understood. We used small-angle neutron diffraction from oriented lipid multilayers, small-angle neutron scattering from unilamellar vesicles and molecular dynamics simulation experiments to elucidate the structure of DOPC and DPPC model membranes determine the effects of melatonin and cholesterol. We find cholesterol and melatonin to have opposite effects on lipid membrane structure: the incorporation of melatonin results in membrane thinning, in stark contrast to the increase in membrane thickness induced by cholesterol. The fluidity and the state of disorder of the membrane are significantly increased in the presence of melatonin. These different effects of cholesterol and melatonin may help to understand their relation to amyloid toxicity.

P-327

Influence of ectoin on the structural organization of natural and artificial tear fluid lipid layer

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We investigate the influence of Ectoin on the structural organization of the natural and artificial tear fluid lipid layers (ATFLL) using surface activity analysis and topographical studies. The natural meibomian lipids exhibit a continuous pressure-area isotherm without any phase transitions. In the presence of ectoin, the isotherm is expanded towards higher area per molecule implying decreased interaction between the lipid molecules. The AFM scans show presence of fiber like structures in the natural meibomian lipid film. In the presence of ectoine, droplet-like structures are observed which are hypothesized to be triacyl glycerols excluded from the lipid film. ATFLL illustrate the fluidizing effect of ectoine on the lipid films where the pressure-area isotherms are expanded in the presence of ectoin. With the addition of a triacyl glycerol to the mixture of DPPC and Chol-Palmitate, we observed the formation of similar drop-like structures in the presence of ectoine as in the case of natural meibomian lipid films. Consequently, the hypothesis explaining the exclusion of tri/di acyl glycerol from the meibomian lipid film in the presence of ectoine in the subphase is confirmed which lead us to a model describing the fluidizing effect of ectoine on meibomian lipid films.

Abstracts

– Membrane Structure and Domains –

P-328

Effects of modifiers of the membrane dipole potential on sphingolipid containing bilayers

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Here we studied the effect of flavonoids (phloretin, quercetin) and styryl dyes (RH237, RH421) on the magnitude of dipole potential (Vd) of bilayers simulating mammalian membranes, containing cholesterol and sphingomyelin (I), and fungi, enriched of ergosterol and phytosphingosine (II). The changes in ionophore steady-state membrane conductance to estimate ΔV_d of bilayers made from equimolar mixiture of DOPS, DOPE and sterol with 20 mol% of sphingolipid content after two-side addition of flavonoids (up to 80μM) and styryl dyes (up to 7.5μM) to a bathing solution (0.1M KCl pH7.4) were measured. Phloretin adsorption reduced Vd of I and II type bilayers on 93±13 and 76±14 mV, respectively. Quercetin induced alteration of Vd was –62±8 mV independent of membrane composition. Styryl dyes were equally effective for I type bilayers (130±9 mV), while RH237 and RH421 increased Vd of II type membranes on 120±9 and 85±6 mV, respectively. Role of lateral organization of these membranes and distribution of dipole modifiers between liquid-ordered and liquid-disordered lipid domains is discussed. The study was supported in part by RFBR (12-04-00948,12-04-33121), the Grant of the President of RF (MK-1813.2012.4), the Program of the RAS «MCB» and the Russian State Contract #8119 (MES,FTP,SSEPIR).

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Coarse-grained modeling of supported and tethered bilayers

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Modern Bio-Nanotechnology uses artificial biomimetic membranes for a variety of applications including drug-delivery, bio-sensing, bio-compatible coating etc. The fundamental building block for such systems are lipid bilayers. Here we present a multiscale molecular modeling investigation of supported and tethered biomembranes in connection to various support platforms. In computer simulations these systems have been studied only rarely up to now although the capabilities of cyber-engineering are tremendous. We present systematic studies on different length scales of the changes that a support and tethering to it inflicts on a phospholipid bilayer using molecular modeling. We characterize the density and pressure profiles as well as the density imbalance induced by the support. We determine the diffusion coefficients and characterize the influence of corrugation of the support. We also measure the free energy of transfer of phospholipids between leaflets using the coarse-grained Martini model. Additionally different support characteristics such as curvature and roughness are addressed.

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Location and fluorescent behavior of NBD-labeled fatty amines in POPC:Chol and SpM:Chol bilayers

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A homologous series of fluorescent NBD-labeled fatty amines of varying alkyl chain length, NBD-C_n, inserted in POPC and *N*-palmitoyl sphingomyelin bilayers, with 50 mol% and 40 mol% cholesterol, respectively, was studied using atomistic molecular dynamics (MD) simulations. The results are compared with MD data in pure POPC (Filipe HAL et al, JPCB **2011**, *115*-10109) and with experimentally determined fluorescence results. For all amphiphiles in both bilayers, the NBD fluorophore locates at the glycerol/phosphate region with the NO_2 group facing the water, in a more external position than in pure POPC. This shallower location of NBD agrees with lower fluorescence quantum yields and smaller ionization constants. For n $\geq$ 14, the amphiphiles show significant mass density near the bilayer midplane resulting from interdigitation with the opposite bilayer leaflet. However, this effect is less pronounced than in POPC. The effects of these amphiphiles on the structure and dynamics of the host bilayer were found to be relatively mild.
The authors thank Advanced Computing Laboratory (UC), funding by FEDER (COMPETE)/FCT, project FCOMP-01-0124-FEDER-010787 (PTDC/QUI-QUI/098198/2008; LL) and grant SFRH/BD/65375/2009 (FCT; HALF).

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Sulfurated naphtoquinones modifications on lipid membranes

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Quinones are structures present in many naturally occurring compounds, e.g. 1,4-naphthoquinones like Vitamin K, doxorubicin, etc. are among the examples of this vast class of chemicals used in the treatment of bleeding, lymphoma, carcinoma, etc. Only one sulfurated naphthoquinone was found in nature but many were synthesized and proved to be potent antibacterial and antifungal agents. Furthermore, several thionaphthoquinones have been recently synthesized because of their interesting spectroscopic properties and also as attractive organic dyes due to their high solubility in organic solvents. Their red color in the solid state also leads to applications as organic nonlinear optical materials.
New naphthoquinones and hydroquinones, bearing alkyl side chains that match the
phospholipids POPC and POPE, were synthesized in order to investigate their interactions with lipids. It was observed that, in general, these additives destabilize the lipid bilayer and induce less organized structures with higher amount of curvature. Moreover, cubic phases, not normally observed in the pure lipids when fully hydrated, were detected. Coexistence of lamellar phases was interpreted as a consequence of microsegregation of the components in the mixtures.

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– Membrane Structure and Domains –

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Phytochemical composition-dependent interaction of Rosaceae fruit extracts on the lipid membrane associated with their bioactivity

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Epidemiological research has suggested that the consumption of foods rich in flavonoids and other functional ingredients correlates with a lower risk of many oxidative stress-related diseases. The effects of polyphenols were exclusively explained by their binding to or interference with enzymes, receptors and other biomolecules occuring in lipid membrane environments. Therefore, the bioactivity of polyphenols may be attributed to their common mode of action on the membrane. The purpose of this work was to determine the ability *in vitro* of extracts from fruits of *Rosaceae* family to inhibit lipid peroxidation in liposomes and cyclo-oxygenase activity; and to demonstrate whether those abilities are correlated with a modification of the bilayers and the chemical composition of the extracts. We examined the effect of the extracts on membrane fluidity and their specific binding to the liposome. It was analyzed also how the anthocyanins, flavonols and flavanols content in the extracts effected their activities. The correlation between biological activity of the extracts and the degree of association with the membrane and it's fluidity modification indicates the significance of the interactions with membrane. Work supported by NCN for research in the years 2010-2013, grant N N312 263638.

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NMR of biomembranes demonstrate that grape polyphenols protect membrane lipids from oxidation

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Preventing lipid oxidation is a major issue to preserve human health from pathologies such as cancers or cardiovascular diseases. Polyphenols supplied by foods are thought to protect cell membranes from lipid oxidation damages. Using liquid-state NMR, we determined the antioxidant activity of four red wine polyphenols, catechin, C, epicatechin, EC, epicatechin gallate, ECG, and epigallocatechin gallate, EGCG, on a biomembrane model. The latter known as isotropic bicelles is composed of saturated and unsaturated phospholipids containing linoleic acid, a common target for oxidation in biological membranes. The kinetics of membrane lipid oxidation with or without tannins was studied by following the area decrease of the bis-allylic methylene group resonance, as an indicator of lipid oxidation. The tannins exhibit an antioxidant activity at 50 μM, a concentration between those found in blood (<1μM) and red wines (<500μM), in a descending order: EGCG>ECG>EC>C. The higher the antioxidant activity, the greater the number of phenolic hydroxyl groups on the tannin structure. Furthermore, the antioxidant activity increases with the tannin concentration until reaching a *plateau*. Very interestingly, a synergetic effect was demonstrated in the presence of the four tannins all together.

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Pore spanning membranes as a model system for the selective generation of membrane curvature

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Several processes such as fission, fusion, cell division and cell movement involve the generation of curvature and changes in membrane shape. This rearrangement in shape can be achieved by scaffolding proteins or insertion of transmembrane helices. A useful tool to study and quantify the influence of single parameters of these biological processes are artificial model membrane systems. A model system which mimics the biological cell membrane very well are pore spanning membranes (PSMs). Those PSMs are generated on top of a porous substrate, have two accessible aqueous compartments and consist of solid supported and free spanning areas. They provide the opportunity to investigate biological processes in a defined environment with high statistics. Using pore spanning membranes it could be shown that osmolality differences between the cavities induce membrane evaginations in a reversible manner: with increasing osmolality gradient the cavity volume also increased. The produced evaginated pore spanning membranes were treated with an ENTH domain, a curvature sensing respectively curvature inducing domain, to show its binding and to investigate its activity. ENTH treatment resulted in increasing evaginations and fission of vesicles.

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Interaction of antimicrobian peptide OK1 with model membranes as observed by optical microscopy

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Studies of the action mechanisms of Ocellatin (OK1) were conducted using GUVs of different phospholipid compositions. The peptide induces the formation of pores and, occasionally, disruption. The results showed that the pore formation efficiency depends on the GUVs composition. In particular, the peptides act to a higher extent on surface charged vesicles. In this study, we observe the effect of 500 nM OK1 on GUVs containing POPC:POPG (9:1), POPC:POPG (8:2) and POPC:POPG (1:1). The results evidence the formation of pores in the negative charged bilayer followed by contrast lost at *ca.* 10 minutes of peptide contact in the outer solution, whereas the same effect takes place two-fold slower for GUVs composed just by POPC. Contrarily, GUVs containing cholesterol (Chol) – (POPC:Chol – 9:1) display a quite different scenario when interacting with OK1. In fact, this peptide does not cause any significant change in the Chol-containing membrane features. This study revealed that, certainly, the interaction and peptide binding must be driven by electrostatic mediated by hydrophobic forces. Further, it seems that Chol may inhibit such an effect, probably due to changes in the fluidity of the bilayer.
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Abstracts

– Membrane Structure and Domains –

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How do oxidized lipids behave in Langmuir monolayers and Langmuir-Blodgett films ?

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Oxidation of unsaturated lipids promotes important changes in the lipid packing or phase separation in cellular membranes or membrane models. Here, we study by Atomic Force Microscopy (AFM) the behavior of oxidized lipids in Langmuir-Blodgett (LB) films by using two strategies : either following the evolution of LB films of POPC (palmitoyl-oleoyl-phosphatidylcholine) naturally ageing in contact of atmospheric oxygen, or incorporating a known amount of a defined oxidized derivative of POPC (ALDO-PC) in POPC monolayers before their LB transfer.
AFM images of naturally ageing POPC LB films show the appearance of small circular domains after 2 days of exposure to air. These domains (not observed if the samples are kept under vacuum) are characterized by a higher thickness (+0.8 nm) as compared to the intact POPC regions, likely due to a reversal of the oxidized chain which is more polar that intact hydrophobic chains. In the second case, surface pressure measurements show that ALDO-PC induces a slight expansion of the mixed monolayers, suggesting that they are rather homogenous. This hypothesis is confirmed by their smooth and homogenous AFM images. Finally, these results confirm that oxidation in POPC LB films occurs locally in areas presenting likely a looser packing or a defect.

P-337

Deformable biphenyl liposomes: characterization by ss-NMR and perspectives in structural biology

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One of the challenges of structural biology is to understand the function of bio-molecules within the biological membrane. A new membrane model from a lipid: TBBPC, dispersed in water has been developed The presence of a biphenyl unit on one of its chains confers a positive magnetic susceptibility anisotropy to the molecule, resulting in an oblate deformation of liposomes under high magnetic fields. ^{31}P and ^{2}H ss NMR have been performed to characterize this deformation by varying different parameters predicted by Helfrich: the magnetic field strength (B), the elasticity, the vesicle size and the temperature. Biphenyl vesicles dynamics have also been studied deciphering order and orientational aspects. The oblate deformation of these fluorescent liposomes is persistent outside B, which was validated by optical and electronic microscopies and X-rays diffraction. The use of deformable biphenyl liposomes as a mimic of biomembranes is now studied by ^{15}N ss-NMR by inserting into them ^{15}N labeled Surfactin or Pf1 coat protein

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Advancing high resolution structural analysis of lipid membranes using a genetic algorithm

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We adapted a high resolution method for joined refinement of small angle x-ray and neutron scattering data of unilamellar vesicles to the more general case of multilamellar vesicles (MLVs) formed by zwitterionic phospholipids. By using a genetic algorithm the new technique is capable of retrieving the positions and volume distributions of quasi-molecular groups within phospholipid bilayers from x-ray data only. The analysis was tested on a series of saturated and unsaturated phospholipids and binary mixtures with cholesterol. Our results are in good agreement with previous reports using a simultaneous analysis of neutron and x-ray data. For example, the positions of hydrophobic groups as well as of headgroup fragments were shifted further away from the bilayer center in the presence of cholesterol as a result of the well-known bilayer condensation by cholesterol. Finally we show that the structural information on hydrophobic groups can be further improved upon the additional analysis of neutron scattering data due to the higher contrast.

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New HIV fusion inhibitors LJ001 and JL103 act by modifying viral lipid membrane properties

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It was recently reported that a new aryl methyldiene rhodanine derivative, named LJ001, and oxazolidine-2,4-dithione, named LJ103, act on the viral membrane of enveloped viruses, such as HIV-1, avoiding its fusion with the target cell membrane [1]. The aim of the present work was to clarify the mechanism of action of these new viral entry inhibitors, focusing on the change that these molecules induce on membrane properties. Fluorescence spectroscopy assays show that both molecules insert in lipid membranes, but at different depths. Using DPH, TMA-DPH and Laurdan fluorescence emission, as well as surface pressure measurements, we observed that LJ001 and JL103 increase the membrane rigidity and surface pressure. Singlet oxygen production was assayed using 9,10-dimethylanthracene and the ability to impair membrane fusion was evaluated by FRET. The results indicate that the production of singlet oxygen by LJ001 and JL103 occurs and induces several changes on membrane properties, such as an increase in its rigidity and an ordering effect on the polar head groups. The end result is the inhibition of the formation of the fusion pore necessary for cell infection.

[1] *Vigant et al. (2013) PLoS Pathog. 9:e1003297*

Abstracts

– Membrane Structure and Domains –

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Structural, thermodynamic and kinetic properties of bolaamphiphile membranes

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In the present study, we investigated the polymorphism and its time-dependence of a new series of bolaamphiphile molecules based on N-(12-Betainylamino-dodecane)-octyl-β-D-Glucofuranosiduronamide Chloride. To obtain six members of this series, the length of the main bridging chain and the lateral chain were varied in order to modify the hydrophilic-lipophilic balance. Another chemical modification was to introduce a diacetylenic unit in the middle of the bridging chain to study the influence of the π-π stacking on the supramolecular organization of these molecules. Dry bolaamphiphiles self-organize in supramolecular structures such as lamellar crystalline structure, L_c, lamellar gel structure, L_β', lamellar fluid structure, L_α, and lamellar isotropic structure, L. Thermal hysteresis of these structures, following phase transitions, are investigated by small-angle and wide angle X-ray scattering. Once the thermal cycle is accomplished, the system remains in the kinetically stabilized undercooled high-temperature phase at ambient temperature. Subsequently, the kinetics of the relaxation to the thermodynamically stable phase at the same temperature is followed. This relaxation is exceptionally slow on the order of hours or days.

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Lipid bilayers containing sphingomyelins and ceramides of varying N-acyl chain lengths

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The thermotropic properties of aqueous dispersions of sphingomyelins (SM) and ceramides (Cer) with N-acyl chains varying from C6:0 to C24:1, either pure or in binary mixtures, have been examined by differential scanning calorimetry, finding complex endotherms. In some cases, e.g. C18:0 SM, atomic force microscopy revealed coexisting lamellar domains made of a single lipid. Partial chain interdigitation and metastable crystalline states were deemed responsible for the complex behavior. SM:Cer mixtures (90:10) gave rise to bilayers containing separate SM-rich and Cer-rich domains. In vesicles made of more complex mixtures (SM:PE:Chol, 2:1:1), it is known that sphingomyelinase degradation of SM to Cer is accompanied by vesicle aggregation and release of aqueous contents, but domain separation was not observed by confocal microscopy. Vesicle aggregation occurred at a faster rate for the more fluid bilayers, according to differential scanning calorimetry. Contents efflux rates measured by fluorescence spectroscopy were highest with C18:0 and C18:1 SM, and in general those rates did not vary regularly with other physical properties of SM or Cer. In general the individual SM and Cer appear to have particular thermotropic properties, often unrelated to the changes in N-acyl chain.

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Investigation of membrane fusion as a function of lateral membrane tension

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Membrane fusion is an omnipresent process for transmitting molecules, proteins and lipids between cells and cell compartments. Proteins of the SNARE family (soluble *N-ethylmaleimide-sensitive factor attachment protein* receptors) are necessary to bring the membrane of a small vesicle and a cell membrane into close contact to promote fusion processes. We devised model systems based on small peptides forming coiled coil complexes to mimic the native situation of SNARE assembly. With these model systems we investigate the fusion of lipid membranes as a function of mechanical parameters such as membrane tension and curvature to seek a better understanding of fusion processes in eukaryotic cells where the plasma membrane is attached to the cytoskeleton generating a defined tension.
Here we use a force microscopy and spectroscopy to address this question. Increasing membrane tension is induced by applying an external force to a giant vesicle or solid supported membrane. By applying a defined external force it is possible to adjust the membrane tension and quantify fusion with small vesicles.

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Protein partitioning in liquid-ordered (Lo) / liquid-disordered (Ld) domains

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The lack of transmembrane proteins partitioned in the current lipid-only models for membrane rafts calls for close scrutiny of raft mimetics. Using small angle X-ray scattering (SAXS) and molecular dynamic simulations (MD), we determined structural and elastic parameters (spontaneous curvature, bending rigidity, Gaussian curvature modulus) for coexisting Lo/Ld domains in ternary mixtures of dioleoylphosphatidylcholine/dipalmitoylphosphatidylcholine/cholesterol and dioleoylphosphatidylcholine /distearoylphosphatidylcholine/cholesterol. Substituting these values into theoretical calculations, yields the energy penalty upon insertion of transmembrane proteins into Lo and Ld phases and consequently the preferred partitioning in one of these domains. We discuss our findings for different geometric protein shapes.

Abstracts

– Membrane Structure and Domains –

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Mapping local organization of cell membranes using excitation polarization resolved fluorescence

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In cell biology, structural organization of proteins and lipids are direct signature of molecular interaction mechanisms which play a determining role in biological functions such as cell shape maintenance and motility, vesicular trafficking or signalling. A fully excitation polarization resolved fluorescence microscopy imaging, relying on the use of a tuneable incident polarization and a non-polarized detection, is able to study new regions on complex and heterogeneous molecular organization of cell membranes. With this technique we probe the molecular orientational order of the lipid reporter di-8-ANEPPQ in the plasma membrane of labeled COS-7 cells. We find that lipid packing is affected by cholesterol depletion, and reflects the strong interplay between the cell plasma membrane and its nearby cytoskeleton. We also reveal morphological changes, like membrane patches formation, at the sub-diffraction scale, due to pharmacological perturbation of the actin cytoskeleton.
This technique can evidence, in a non-invasive optical way, local organization in cell membranes, complementarily to existing methods such as generalized polarization (GP).

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Giant unilamellar vesicles from films of agarose and lipids display hindered mechanical response

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Giant Unilamellar Vesicles (GUVs) are suitable membrane model. Producing these vesicles by the classical electroformation method is often limited by lipid composition and requires low ionic strength. The recent method for producing GUVs from films of agarose and lipids circumvent both undesired issues, although the effect of agarose on GUVs is not known. Here, we study the effect of residual agarose on the mechanical response of GUVs. Electric pulses are applied to induce vesicle deformation and opening of macropores. The relaxation dynamics (relaxation time of deformation, τ_{relax}, and pore closure time, T_{pore}) of the GUVs are used to characterize the mechanical response of electroformed vs. agarose-formed GUVs. The presence of residual agarose on the GUVs alters the mechanical response of GUVs: Both τ_{relax} and T_{pore} show a much broader distribution of values towards slower dynamics. These hindered responses are correlated with the amount of residual agarose. Drastic morphological transformations occur, including very long-lived pores, increased membrane permeability, and expulsion of a polymer network through the macropore. Thermal treatment above agarose melting transition basically recovers the mechanical properties found for electrofrmed GUVs. Financial Support: FAPESP.

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Monte Carlo simulations of lateral organization of lipid mixtures using WroSIM

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Lateral organization of lipid molecules in biomembranes exhibits heterogeneities which are believed to have impact on physiological membrane properties. Interactions between lipids can lead to creation of nanodomains which may affect signalling and vesicular trafficking. However, mechanisms of lipid domains formation remain controversial, mostly due to the fact that creation of domains occurs at the molecular level and is hard to be probed experimentally. Molecular Dynamics simulations also cannot fully deal with lipid domain formation due to a limited computational power, which limits membrane size possible to simulate. One of computational methods capable of dealing with the lateral lipids organization are Monte Carlo (MC) simulations. Recently developed algorithms, such as Massive Parallel Kawasaki Kinetics (MPKK) [1], provide computationally effective approaches to Monte Carlo simulations of lipid membranes by employment of GPU-based computations. This allows for up to 50 times faster simulations with regard to previously applied sequential algorithms. We present WroSIM - a lipid membrane simulation package employing both traditional MC and the MPKK method that utilizes GPU architectures.
[1] Lis, M., et al. *Journal of Chemical Theory and Computation* 8.11 (2012): 4758-4765.

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Does cardiolipin addition produces superior bacterial membrane model systems?

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Steady-state fluorescence anisotropy and dynamic light scattering were used to determine thermotropic properties of bacterial membranes lipid model systems. Different lipid proportions of PE:PG:CL were used in order to mimic bacterial membranes from different strains. Cardiolipin inclusion as a third lipid component of any PE:PG mixture considerably changes the system properties and the main transition temperatures obtained are undoubtedly cardiolipin dependent. Additionally AFM experiments were performed and results show that even at small concentration cardiolipin produces important changes not only in the membrane thermotropic properties, but also in the bilayer structure. Studies of the interaction of moxifloxacin and enrofloxacin with these model systems also show that cardiolipin absence greatly influence the conclusions obtained. Preliminary circular dichroism results of an *E. coli* membrane protein, OmpF, reconstituted in different model system membranes, with and without cardiolipin, also point out for its influence on proteins conformation. These results show that cardiolipin incorporation in membranes model system can have a significant impact on membrane properties and its addition should be considered when aiming to construct model system of bacterial membranes.

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– Membrane Structure and Domains –

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Effects of anti-tumor lipid drugs on the packing and lateral organization of yeast membrane extracts

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Synthetic anti-tumour lipids are a class of drugs derived from lysophosphatidylcholine that are found to be both cytotoxic and cytostatic, and to exert these effects preferentially in tumour cells. Where lysophosphatidylcholine is metabolized in vivo, structural differences in the drugs confer lipase and acyltransferase resistance, making them more stable. While the mode of action is not fully understood, it is believed that these drugs accumulate preferentially in lipid rafts leading to breakdown of these membrane structures. Lipid rafts are important sites for signal transduction and membrane trafficking, therefore this disruption has impacts on cellular functions at various levels. In this study Saccharomyces cerevisiae cells were treated with edelfosine, miltefosine or perifosine and the cell membranes were extracted using a modified Folch method. Surface pressure – area isotherms and Brewster angle microscopy were used to compare the effects of each drug on lipid packing and lateral domain organization of the yeast extracts. Results show changes in the film behaviour of extracts of drug treated yeast membranes, suggesting alterations in packing. In addition Brewster angle microscopy reveals changes in the size and shape of domains in drug treated yeast extracts.

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2D kinetics in fluid bilayers of pyrene-lipids studied by nitroxide quenching and excimer formation

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Fluorescence quenching of py10-PC by the spin probe 10-DOXYL-PC in fluid bilayers was examined by combined steady-state and lifetime techniques. Our findings are in excellent agreement with the theoretical predictions by a kinetic formalism specific for fluorescence quenching processes occurring in two-dimensional (2D) media [Razi Naqvi *et al.* (2000) *J. Phys. Chem. B* 104, 12035]. However, for py6-PC quenching by 5-DOXYL-PC, large deviations are observed, the quenching efficiency appearing higher than predicted theoretically, but increasing the collisional distance allows for a very good approach to the experimental values.
Excimer formation of py10-PC and py6-PC in fluid bilayers is in accordance with the theoretical predictions, until probe molar ratios of 2 and 4 mol %, respectively. Above these values, experiments display a quenching efficiency lower than theory. Molecular dynamics have shown increased ordering and tight packing in the py10-PC vicinity and interdigitation of pyrenyl group into the apposed bilayer leaflet, accounting for diminished lateral diffusion dynamics of both probes.
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Stearoyl-phytoceramide gel domains as a model of ordered domains in plants and fungi cell membranes

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Phytoceramide is the backbone of major sphingolipids (SLs) in fungi and plants. Its sphingoid base, phytosphingosine, differs from that usually found in mammals by the addition of an hydroxyl group to the 4-ene, which may be a crucial factor for the different properties of membrane microdomains among those organisms. Recently, SLs hydroxylation in animal cells emerged as a key feature in several physiopathological processes. Hence, the study of fungal SLs provides a means for clarifying those questions. In this work, binary mixtures of N-stearoyl-phytoceramide (SPhCer) with palmitoyloleoylphosphatidylcholine (POPC) were studied. Steady-state and time-resolved fluorescence of multilamellar vesicles, atomic force microscopy on supported lipid bilayers and confocal microscopy of giant unilamellar vesicles were employed. As for other saturated ceramides, gel domains start to form with just ∼5 mol% SPhCer at 24°C, excluding (though to a lesser extent) probes such as DPH. However, SPhCer gel-enriched domains in coexistence with POPC-enriched fluid present additional complexity, since their properties (maximal order, shape and thickness) change at specific POPC:SPhCer molar ratios. Supported by FCT, Portugal grants PTDC/QUI-BIQ/104311/2008, PEst-OE/QUI/UI0612/2011 and SFRH/BD/64442/2009

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Interaction of core-shell polymeric nanoparticles with Giant Vesicles as a model for cell membranes

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Our project's aim is to investigate the applicability of novel nanoparticles (NPs) for drug delivery to the skin, specifically targeting inflammatory diseases. Core-shell polymeric nanoparticles below 50 nm were synthesised, as this small size is capable of penetrating the *stratum corneum (SC)*. The polystyrene core, prepared by emulsion polymerization, contains a surface-bound iniferter (benzyl dithiocarbamate), which when irradiated with UV light enables the generation of new monomer shells by control/living radical polymerization. A series of monomers (neutral, anionic, and zwitterionic) were then grafted on the core producing a library of NPs with various surface charges. The physico-chemical properties of the NPs were characterized by dynamic light scattering, zeta potential measurements and transmission electron microscopy. Giant unilamellar vesicles (GUVs) between 1 – 5 μm were used as model cell membranes due to their comparative size and structure. The affinity of the different NPs for biomimetic membranes with different lipid composition was investigated using surface plasmon resonance analysis. Generalised polarisation and anisotropy spectroscopies as well as confocal microscopy were employed to elucidate the NPs interaction and internalisation within the GUVs.

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Photo irradiation on model membrane: micropipette study of phospholipids oxidation in micro vesicles

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Cell membranes contain unsaturated lipids which may be degraded by action of singlet oxygen ($^1O_2$). In biological media such effects culminate in cell death. Methylene blue (MB) is an oxidizing agent due to high quantum yield of $^1O_2$ production when exposed to irradiation. We evaluate the damage on model membranes of POPC and DOPC giant vesicles as a result of lipids oxidation due to MB photo irradiation. Applying the micropipette technique and recording the vesicles response to small suction pressure under the light of phase contrast and fluorescence microscopy, it was possible to determine the rate of area increase of lipids as a result of peroxidation by $^1O_2$. For POPC superficial area increase was 12% and for DOPC 19% on the average. Damage caused by irradiating small concentrations of MB results in lower degree of peroxidation along with two distinct rates of area increase as function of time. The results suggest a competitive process of hydroperoxidation followed by fragmentation of the lipids acyl chains. The fragmentation leads further to permeability increase of the vesicles with upsurge of small and large micropores on the membrane. We have found an ideal pressure in the micropipette where micropores open and reseal on the membrane avoiding disruption of the vesicles.

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Influence of lipid peroxidation on membrane susceptibility to electroporation

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The phenomena resulting in increased permeability of lipid membranes caused by pore creation in lipid bilayers and biological membranes under the influence of electric field is called electroporation. This method finds its application in biology, biotechnology and medicine. It is used mainly to the cell-cultures transfection and direct delivery of drugs. Therefore, it is necessary to identify factors affecting efficiency of electroporation. One of them may be the presence of lipid peroxidation products in the membrane. The studies of the significance of lipid peroxidation products on electroporation parameters were carried out on flat bilayer lipid membranes (BLM) using the voltoamperometric and chronopotencjometric methods. To identify the oxidation products, infrared spectroscopy ATR-FTIR, H1-NMR and gas chromatography combined with mass spectrometry GC-MS were used. The presence of lipid peroxidation products in the membrane cause a drop in the value of specific capacity (mainly by increasing the thickness of the membrane) and the increase in the value of the resistance of BLM. Observation of electric parameters of BLM showed that the presence of lipid peroxidation products in membrane reduces the susceptibility of membranes to electroporation (higher values of breakdown voltage).

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A model lipid bilayer system for the investigation on cell-penetrating peptides

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Cell-penetrating peptides (CPPs) and antimicrobial peptides (AMPs) are short cationic peptides that are part of the innate immune system by all living organisms and thus have high therapeutic potential. While CPPs are able to transport cargo to a target cell by translocating it through the plasma membrane, most AMPs show antimicrobical potential by disrupting membranes or membrane penetration followed by targeting cytoplasmic compounds. In any case, interaction with the lipid bilayer is crucial. For most peptides the exact mechanism is unclear and subject of current scientific research. For this, suitable lipid bilayer model systems are needed. We developed a model lipid bilayer system based on a porous solid support that separates aqueous compartments and allows us to monitor changes in membrane permeability. The porous support of the model lipid bilayer is based on either anodic aluminium oxide (AAO) or silicon nitride. Model membranes are prepared by spreading giant unilamellar vesicles (GUVs) onto the support. Membrane pore formation in absence or presence of AMPs can be monitored through the entrapment or exclusion of dye by fluorescence microscopy. Additionally, changes in ion current across the membrane can be evaluated by scanning ion-conductance microscopy (SICM).

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Oscillatory phase separation in giant lipid vesicles induced by transmembrane osmotic gradients

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Using fluorescence microscopy, we show that hypoosmotic gradients equilibrate across suspended lipid bilayers via a series of specific membrane transformations, which repeat periodically throughout the dissipation of the gradient. Sugar-loaded giant unilamellar vesicles (GUVs) inflate when submerged in water, eventually leading to membrane rupture. Surprisingly, solute efflux occurs through microscopic, transient pores with ms life times, rather than GUV lysis. Pore healing allows for new swell-burst cycles - with gradually slower cycle kinetics, due to the declining strength of the osmotic differential. In phase separating lipid mixtures, we can indirectly visualize membrane tension fluctuations via domain size dynamics; tension promotes domain coalescence, resulting in large domains prior to pore formation. After membrane healing, domains rapidly disperse into sub-resolution dimensions. This remarkable mechanism is an autonomous self-regulatory response, in which an external osmotic perturbation is managed by coordinated physical mechanisms, allowing membrane compartments to survive hypoosmotic chock. This negative feedback loop of sensing and regulation by a purely synthetic system, suggests a primitive form of quasi-homeostatic regulation of broad importance in biology.

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A bacterial monorhamnolipid alters the biophysical properties of DEPE model membranes

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The interaction of a monorhamnolipid (monoRL) produced by *P. aeruginosa* MA01 with dielaidoylphosphatidylethanolamine (DEPE) membranes has been studied. Incorporation of monoRL into DEPE shifts the temperature of the L_β-to-L_α and the L_α-to-H_{II} phase transitions toward lower values. DSC indicates the coexistence of lamellar and hexagonal-H_{II} phases at 60°C, at which pure DEPE is lamellar, i.e., monoRL facilitates formation of the hexagonal-H_{II} phase in DEPE, and destabilizes the bilayer organization. The phase diagram indicates a near-ideal behavior, with better miscibility in the fluid phase than in the gel phase. As indicated by FTIR spectroscopy, incorporation of monoRL into DEPE shifts the frequency of the CH_2 symmetric stretching band to higher wavenumbers, both below and above the main gel to liquid-crystalline phase transition temperature. Examination of the C=O stretching band of DEPE indicates that monoRL/DEPE interactions result in an overall dehydration effect on the polar headgroup of DEPE. These results are discussed on the light of the possible role of rhamnolipids as bilayer stabilizers/destabilizers during cell membrane fluctuations events.

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Brazilian propolis: comparison of antioxidant activities and interactions with lipid bilayers by FCS

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Propolis, a product resulting from the collection of resinous compounds processed by bees, has a broad spectrum of preventive actions and diseases treatment, especially antimicrobial, anticancer and antioxidant activities. It is known that the resinous compounds that bees collect in vegetation, such as terpenoids, flavonoids and cafeic acids, are closely linked to the therapeutic action and affect the properties of biological membranes of target cells. In this study, we measured the antioxidant activity of propolis collected by four bee species (one type was collected by Africanized bees specie and the others by Indigenous species) by optical absorption and electron spin resonance (ESR) experiments, using scavenging assays of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, showing that the propolis of indigenous bees specie has a greatest potential to scavenging the radical and inhibit its oxidant action. We also investigated the interaction of propolis with model membranes, by fluorescence techniques, including fluorescence correlation spectroscopy. We concluded that the resins have affinity for lipid bilayers of DMPC (zwitterionic vesicles) and DMPG (anionic vesicles), and, from this interaction, the antioxidant action may be active against lipid peroxidation in cell membranes.

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Fluorescence spectroscopy and microscopy to re-evaluate the properties of sphingolipids domains

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The aim of this study is to provide further insight about the interplay between important signalling lipids and to characterize the properties of the lipid domains formed by those lipids in membranes containing distinct composition. To this end, we have used a fluorescence spectroscopy, confocal and two-photon microscopy and a stepwise approach to re-evaluate the biophysical properties of sphingolipid domains, particularly lipid rafts and ceramide (Cer)-platforms. With this strategy we were able to show that, in binary mixtures, sphingolipids form more tightly packed gel domains than those formed by phospholipids with similar acyl chain length. In more complex lipid mixtures, the interaction between the different lipids is strongly dictated by the Cer-to-cholesterol (Chol) ratio. The results show that in quaternary phospholipid/sphingomyelin/Chol/Cer mixtures, Cer forms gel domains that become less packed as Chol is increased. These results suggest that in biological membranes, lipid domains such as rafts and ceramide platforms, might display distinctive biophysical properties depending on the local lipid composition at the site of the membrane where they are formed.

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Interaction of selected anthocyanins with lipid bilayers

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It was studied the effect on model lipid membranes of compounds from the anthocyanins group: Cyanidin-3-O-glucoside chloride (*Kuromanin chloride*), Delphinidin-3-O-glucoside chloride (*Myrtillin chloride*) and Malvidin-3-O-glucoside chloride (*Oenin chloride*). Model membranes were formed of DPPC, egg phosphatidylcholine (eggPC) and lipids extracted from erythrocytes. The interaction of anthocyanins with lipids was studied using the differential scanning calorimetry (DSC), infrared spectroscopy (ATR IR) and fluorimetrically. The calorimetric measurements indicate that the compounds studied do not cause changes in the main phase transition temperature DPPC, only a small change in the pretransition – the most changed was *Kuromanin chloride* and the least *Oenin chloride*. The results obtained with the ATR IR method did not show changes in the alkyl chain region, only a small shift of bands from the phosphate groups for *Kuromanin chloride* and *Myrtillin chloride*. At the choline group level a change was observed for *Kuromanin chloride* only. Distinct changes are, however, caused by anthocyanins in the polar part of the lipid membranes, which was evidenced by fluorimetric examination.

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GM1 Concentration Effect on the Lateral Phase Separation in the PC/SM/Chol Bilayers

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The components of biological membranes appear to be nonuniformly distributed laterally. Among the various types of membranes domains, the most documented are the so-called lipid rafts that are assumed to undergo biologically important size-modulations from nanorafts to microrafts. Model membrane systems containing Lo domains coexisting with an Ld phase, are important tools for the modeling of lipid rafts. In particular, the factors affecting Lo domain size and the search for Lo nanodomains as precursors in Lo microdomain formation are subjects of intense investigation. In the present work, the effect of ganglioside GM1 concentration on the lateral phase separation in the PC/SM/Chol bilayers was studied by fluorescence microscopy and fluorescence spectroscopy. GM1 above 1 mol % hinders the formation of micrometer-scale Lo domains but Laurdan GP measurements and Gaussian spectral decomposition suggest that Lo/Ld phase separation at a submicrometric level still occurs. The Lo/Ld phase separation temperature increases with GM1 content. This indicates that GM1 could act as a linactant that "arrest" Lo domain growth through a decreasing effect on the line tension between phases and thereby stabilize Lo nanodomains. Such an effect could be relevant to size-modulation of rafts in vivo.

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Damage of erythrocytes structure in CAD. Protective effect of atorvastatin and rosuvatsatin

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The aim of this study is to determine the changes in structure and function of erythrocytes in patients with coronary artery disease (CAD) and the impact of statin therapy on improvement determined parameters. The study involved 30 patients with CAD after myocardial infraction within 6 months, at the age of 62,5±6,8 years. One group have taken atorvastatin in dose 40 mg/day, second – 40 mg/day rosuvastatin. Control was healthy individuals in appropriate age.
The results show, increased level of lipid peroxidation (13%), total cholesterol level (19%) and decreased fluidity membranes of erythrocytes (14% in subsurface layers and 7% in deeper layers), in patients with CAD. Monthly treatment atorvastatin resulted in reduction of lipid peroxidation (25%) and rosuvastatin contributed significantly to decrease of lipid peroxidation (14%) and increase membrane fluidity of erythrocytes in subsurface layers (11%). No significant changes was observed in –SH group and total cholesterol levels.
Summary, CAD caused disorders of erythrocytes structure and statin therapy improves the value of determined parameters. These results suggest that rosuvastatin has more potent antioxidant.

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Fluorescent cholesterol analogues dehydroergosterol and cholestatrienol in lipid bilayers

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Fluorescent techniques are indispensable tools in membrane biophysics, making the understanding of fluorescent reporters' behaviour a necessity. Cholesterol (Chol) is an ubiquitous component of many biological membranes. Although Chol is not fluorescent, a large variety of fluorescent sterols, either bearing an extrinsic fluorescent label or exhibiting intrinsic fluorescence, are commercially available and have been widely used. The latter include dehydroergosterol (DHE) and cholestatrienol (CTL). To clarify the extent to which these fluorescent probes mimic Chol's behaviour (location, orientation, dynamics) in membranes as well as their effect on host bilayer structure and dynamics, simulations of POPC bilayers with sterol proportions ranging from 0 to 50% were carried out. Overall, both probes are adequate Chol analogues, especially CTL, whose structure resembles that of Chol more than DHE does. Additionally, their insertion does not significantly disturb the host bilayers' structure and dynamics.
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Investigation of polyenes/membrane interaction to understand their mode of action

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Polyenes are a large family of well-know antifungal molecules with a lactone ring. It is admitted by the scientific community that the mode of action of polyenes against microorganisms and their toxicity against mammalian cells are due to a strong interaction with sterols (ergosterol and cholesterol for fungal and mammalian cells, respectively) which causes the permeabilization of cells' membranes.
However, all polyene molecules present specific behaviours: their efficiency against fungi and their toxicity for mammalian cells are different, even if their structure is very similar. Thus, the mechanism of action of polyenes is still under consideration.
To unravel this mechanism, we studied the interactions between polyenes and model membranes. We used Langmuir monolayers combined with micro- and nano-scale imaging techniques to characterize interactions between polyenes and several lipids. We also used fluorescent probes to determine the influence of polyene on the organization of lipids in liposomes. Our results revealed particular interactions with specific phospholipids that could explain the specific targeting of fungal membranes compared to mammalian ones and the differences observed in the mode of action of these polyenes.

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Photophysical properties and photodynamic activity of a novel menthol–Zn phthalocyanine conjugate

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The synthesis, photophysical properties, and photodynamic activity of the lipophilic tetramenthylsubstituted zinc phthalocyanine (ZnMintPc, main regioisomer {2,9,16,23-tetrakis{[(1S,2R,5S)-5methyl-2-(1-methylethyl)cyclohexyl]oxy } phthalocyaninato}zinc(II)) are described. This compound was synthesized under cyclotetramerization conditions by reaction of substituted phthalonitrile prepared from 4nitrophthalonitrile with natural l-menthol. This new phthalocyanine presented remarkable structural and photophysical features with potential application in photodynamic therapy (PDT) involving hydrophobic membrane regions. This photosensitizer was incorporated in micelles of 12 different surfactants. Solubilization or aggregation in the lipophilic compartment of the micelles was assessed by UV–vis absorption and photoluminescence spectroscopies. Singlet oxygen was produced by irradiation of the micelles with light from red LEDs, peaked at 635 nm, and the quantum yields were obtained using 1,3diphenylisobenzofuran (DPBF) as probe. Two micellar systems, more speci?cally Brij 30 and Pluronic F-127, displayed excellent ability to generate singlet oxygen. Photobleaching rates of the ZnMintPc incorporated into the micelles were also obtained. The results demonstrated the lipophilic ZnMintPc is suitable for PDT applications and suggest the use of Pluronic F-127 in the formulation of adequate drug delivery systems for this photosensitizer.

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Enzymatic digestion on the surface of single biomimetic membranes

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To assess the structure of complex biomembranes the use of asymmetric model systems is rare,due to the difficulty of realizing artificial membranes with defined heterogeneous composition and applicable for non-average structural investigation.We developed for the first time an experimental model bearing forced membrane asymmetry in the form of single floating bilayer,prepared by Langmuir-Blodgett Langmuir-Schaefer techniques,then investigated by X-ray and Neutron Reflectivity.We tested the asymmetric phospholipid/cholesterol/ganglioside model system.In fact gangliosides in real membranes reside in the outer layer,which has been invoked to provide the structural basis for the third-dimension static deformation of membrane portions.Also cholesterol transverse disposition may assume specific arrangement in asymmetric domains.Gangliosides of different species differ for the number and arrangement of sugars in the hydrophilic heads.Different Gangliosides have different packing characteristics,giving different properties to the membrane they are included in.The potentialities of the techniques used,revealing the cross profile of the membranes,allowed us to follow the structural effects brought by a soluble enzyme digesting the sugar heads of gangliosides in single floating membranes.

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Interaction of new active Ruthenium anticancer agents with lipid bilayers

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Membrane lipids are potential targets for therapeutic interventions, enhancing the selectivity of drugs to cancer cells and providing a possible new mechanism of action for prospective drugs. Ruthenium complexes are a proven and promising effective alternative to platinum-based drugs that are currently used in cancer chemotherapy. Their mechanism of action is currently subject of intense research. We are interested in the role the cell membrane may play on the mode of action of these prospective metallodrugs. In this work we investigate the interaction of lipid bilayers as a model for the cell membrane with three Ru-complexes found active against human tumor cells. We present our results on three complexes, their synthetic precursor and ligands. The intrinsic fluorescence of a subset of compounds/ligands (spectral shift, anisotropy, lifetime) demonstrated that two of the Ru complexes have strong interactions with fluid phase lipids, and which ligand is promoting the interaction. Absorption spectroscopy, zeta potential and dynamic light scattering data were used to prove a similar interaction with the membrane for the non-fluorescent Ru-complex. Acknowledgements: FCT (PTDC/QuiQui/101187/2008, PTDC/QuiQui/118077/2010, PEst-OE/QUI/UI0100/2011, PEst-OE/QUI/UI0612/2011, Ciência2008).

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Modulating the phase separation in pore-spanning lipid bilayers

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Cells are capable of controlling the process of raft formation by changes in lipid composition and certain proteins e.g. the cortical actin cytoskeleton. Studying the segregation of artificial lipid bilayers containing cholesterol, sphingolipids and and phospholipids into liquid-ordered (lo) and liquid-disordered (ld) domains offer insights into these processes. Pore-spanning lipids bilayers (PSLBs), consisting of a planar membrane tethered to a solid support and freestanding membrane areas separating two aqueous compartments. They offer a way to investigate how lipid-substrate and lipid-protein interactions alter the behaviour of lipid domains. Lateral organization of the membrane can be probed by fluorescence microscopy whereas scanning ion-conductance microscopy is capable of contact-free topography mapping with nanometer resolution. Preparing phase-separated PSLBs on porous silicon nitride substrates results in submicron-sized lo domains which can be modulated by e.g. cholesterol extraction and the multivalent binding of Shiga Toxin's subunit B to its glycosphingolipid receptor Gb3. Results show that PSLBs, combining long time stability and high lipid/domain dynamics, are a superior model system to study processes at the plasma membrane.

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Simulations of the rupture of liposomes near solid surfaces

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The behavior of lipid membranes near solid surfaces has a great significance both in medicine and in technology. In spite of the widespread use and study the theoretical analysis is rather scarce. Our main goal here is to understand the process during which membrane vesicles first adhere to solid surfaces, then rupture (or go through transient ruptures) due to the mechanical tension induced by the adhesion (between the membrane and the surface and two adjacent membrane areas), and finally spread along the surface forming a supported lipid bilayer. In our theoretical description we simultaneously consider the dynamics of spontaneous pore opening and closing; volume loss via leakage through the pores; and the advancement of the adhesion fronts. All these processes are supposed to follow an overdamped dynamics and are coupled to each other through membrane tension.
Here we identify under which conditions the dynamics leads to the formation of hat shaped geometries. We found that the most determining parameters with respect to the shape and dynamics of a vesicle are the drag coefficient along the adhesion front and the line tension along the pore. Using numerical simulations we could conclude that a hat shaped geometry occurs in a rather narrow range of parameters.

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Glusosylceramide effect on membrane properties: Impact on Gaucher Disease

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GlcCer is an ubiquitous GSL involved in cell maintenance and survival. For instance, impairment of the lysosomal machinery involved in GlcCer degradation leads to its accumulation in the lysosome (Lys), a hallmark of Gaucher disease (GD). The biology and biochemistry of this disease was extensively studied, but little attention was given to the biophysical consequences of GlcCer accumulation, which likely contribute to Lys malfunctioning. Our results show that GlcCer promotes strong alterations on the biophysical properties of model membranes, namely formation of GlcCer-enriched gel domains and membrane tubules. In cell membranes with increased levels of GlcCer, as in mutant fibroblasts from GD type I, there is a global decrease in cell membrane fluidity. Moreover, GlcCer-induced alterations on membrane biophysical properties are pH dependent. Our results highlight the impact of increased GlcCer levels on membrane biophysical properties, further suggesting that alterations on membrane properties might be one of the mechanisms contributing to GD pathology.
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Lipid domain formation and membrane shaping by C24:0-ceramide

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Ceramides are an important group of sphingolipids involved in the regulation of several cellular processes. The mechanism by which ceramides exert their biological action is yet not fully known, but evidence suggests is related with profound alterations in membrane biophysical properties. The purpose of the present study was to characterize the biophysical impact of the very long acyl chain and asymmetric C24:0-Ceramide in a fluid model membrane. To accomplish this goal fluorescence spectroscopy, confocal microscopy and surface-pressure area studies were performed in different types of model membranes. In monolayers, C24:0-Ceramide displayed condensed type behavior. A gradual transition from a liquid expanded to a liquid condensed phase at higher surface pressures was observed in the mixed POPC/C24:0-Ceramide monolayers for ceramide concentrations above 10 mol%. Fluorescence spectroscopy studies showed that the solubility of C24:0-Ceramide in the fluid POPC is very low driving the formation of highly-ordered gel domains. Confocal microscopy studies have further revealed that C24:0-Ceramide forms two types of gel domains with distinct properties. In addition, C24:0-Ceramide promotes strong changes in the shape of the vesicles, including domains with sharp edges and tubule-like structures.

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Tracking NK1 receptor diffusion in the membrane of living cells. Effect of receptor activation

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The tachykinin receptor 1 (NK1R) is a 7TM receptor (GPCR) involved in numerous functions of the nervous system, particularly in nociception, inflammation and emesis. In this work, we combine the high stability of quantum dots and multi-parameter analysis to describe the mobility of NK1R in the plasma membrane of living cells and the impact of the found mobility patterns on the function of the receptor.
Two mobility modes of the NK1R co-exist in the basal state: (i) freely diffusing receptors with diffusion coefficients around 0.023 μm2/s, and (ii) receptors diffusing in confined membrane domains of 150 - 600 nm sizes with a broad distribution of diffusion coefficients.
After activation with the natural agonist substance P, two phases appear. In the first 10 - 30 seconds, the population of freely diffusing receptors strongly decrease and a third immobile receptor population appears with diffusion coefficient <10-3 μm2/s and mobility parameters describing immobile individuals.
30 minutes later the population of freely diffusing receptors increases again and a fourth population of fast diffusing receptors appears in circular domains. This population shows high diffusion coefficients >0.1 μm2/s, close to ideal diffusion in lipid bilayers, and stable nanometer-sized symmetrical domains.

Abstracts

– Membrane Structure and Domains –

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Electroformation of GM1-containing GUVs under physiologically relevant conditions

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Giant unilamellar vesicles (GUVs) are generally formed in low ionic strength conditions. However, preparing GUV under physiologically relevant conditions is important especially when investigating peptide/membrane interactions since peptide properties highly depend on ionic strength. Up to now, several articles reported on GUV formation under physiologically relevant conditions. Nevertheless, the electroformation protocol still needs to be optimized for each particular lipid composition, buffer conditions and experimental setup. Our future aim is to investigate amyloid beta (A-beta) peptide interaction with ganglioside (GM1) containing GUVs, which is motivated by an interest in the membrane-related mechanisms of Alzheimer's disease. Here we report on electroformation of GUVs made from natural lipid mixtures mimicking external neuronal membrane: PC, SM, Chol, and GM1. The formations have been done using pH 7.4 buffer containing 140 mM NaCl under electric field from about 200 mV to 700 mV during several hours at around 200 Hz with osmolarity compensation. We found that osmotic pressure strongly affects to the growth of GUVs. That allowed us to study lo/ld phase behavior of GM1 containing artificial membranes mimicking neuronal plasma membranes at physiologically relevant conditions.

P-373

Photo-oxidation of unsaturated lipidsă: measure of membrane structure and properties

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Lipid oxidation plays a central role in the life of eukariotic cells, for example in mitochondrial respiration, cell signaling and cell apoptosis. Besides, oxidation of living tissues is at work in Photodynamic Therapy (PDT) as a clinical treatment for various pathologies; in that case, a photosensitive molecule is brought into contact with the targeted tissues and an appropriate illumination induces the oxidation process. One known pathway for living organism oxidation is based on the generation of singlet oxygen, a highly reactive species that reacts efficiently with double bonds present on phospholipid hydrocarbon chains.
We study DOPC and POPC giant vesicles decorated by a new amphiphilic chlorin photosensitizer. By combining a quantitative fluorescence analysis of the photosensitizing process with a micropipette control of the vesicle area, we show that the full hydroperoxidation of the lipids can be achieved, and we measure precisely the associated molecular changes in terms of apparent area increase per molecule. We also show that a fully hydroperoxidized membrane keeps its mechanical integrity, and measure the reduction of the membrane elastic modulus associated to such an oxidation.

P-374

Effects of sugar stereochemistry on lyotropic mesophases of branched-chain synthetic glycolipids

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Synthetic branched-chain glycolipids such as 2-hexyl-decyl-α-D-glucopyranoside, 2-hexyl-decyl-β-D-glucopyranoside, 2-hexyl-decyl-α-D-galactopyranoside and 2-hexyl-decyl-β-D-galactopyranoside may be used as model systems to help understand the consequences of chain-branching for the structure and stability of cell membranes. Furthermore, they allow the effects of the stereochemistry of the sugar headgroup on the phase behavior to be assessed. The binary phase diagrams of these lipids in water were investigated by polarizing microscopy and X-ray diffraction. Although the four compounds are chemically very similar, they showed different thermotropic mesophases due to subtle changes in their stereochemistry at the anomeric and epimeric centres. In excess water, all four glycolipids formed inverse bicontinuous cubic *Ia3d* (Q_{II}^{G}) and *Pn3m* (Q_{II}^{D}), and/or inverse hexagonal H_{II} phases over a wide temperature and concentration range, showing their potential as materials for novel biotechnological applications such as drug-delivery and *in meso* protein crystallization.

P-375

Local micro-partition coefficients govern solute permeability of cholesterol-containing membranes

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The permeability of lipid membranes for metabolic molecules or drugs is routinely estimated from the solute's oil/water partition coefficient. However, the molecular determinants that modulate the permeability in different lipid compositions have remained unclear. Here, we combine scanning electrochemical microscopy and molecular dynamics (MD) simulations to study the effect of cholesterol on membrane permeability, as cholesterol is abundant in all animal membranes. For hydrophobic solutes, we find a bell-shaped dependence on cholesterol concentration [Chol], whereas the permeability for hydrophilic solutes monotonously decreases with [Chol]. The simulations indicate that cholesterol does not affect the diffusion constant inside the membrane. Instead, local partition coefficients at the lipid head groups and at the lipid tails are modulated oppositely by cholesterol, explaining the experimental findings. Structurally, these modulations are induced by looser packing at the lipid head groups and tighter packing at the tails upon the addition of cholesterol.

Abstracts

– Protein-nucleic Acid Interactions –

O-376

Binding J: molecular biophysics to understand the binding of a unique protein to a unique DNA base

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Base J was discovered in the DNA of pathogenic protozoa, such as *Leishmania,* where it replaces a fraction of base T, mostly in the telomeres. Recently, we showed that a non-telomeric, fraction of J is acts as a transcription termination signal.
JBP1 recognizes J-DNA through a small domain, DB-JBP1, with ∼10,000 preference over normal DNA. DB-JBP1 has a "helical bouquet" fold with a "ribbon" helix encompassing a single residue (Asp525) responsible for specificity towards J-DNA. Mutational analysis and H/D-exchange MS data, allowed to construct a model of DB-JBP1 bound to J-DNA, validated by SAXS.
Analysis of the pre-steady state kinetic data of the binding of JBP1 to J-DNA, showed a second order rate constant of 70 $\mu M^{-1}sec^{-1}$ and that JBP1 binds to J-DNA in a two-step reaction. As the second (slower) step in binding is concentration independent, we inferred that JBP1 undergoes a conformational change upon binding to DNA. This notion of a conformational change upon J-DNA binding was confirmed by SANS, showing that the shape of JBP1 is more elongated in complex with DNA.
The conformational change upon DNA binding may allow the hydroxylase domain of JBP1 to make contact with the DNA and hydroxylate T's in spatial proximity, resulting in regional introduction of base J into the DNA.

O-377

Transient geometries in nonsense-mediated mRNA decay (NMD) visualized by cryo-EM

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Nonsense-mediated mRNA decay (NMD) is a post-transcriptional surveillance mechanism that degrades aberrant mRNAs containing premature translation termination codons (PTCs) and coding for truncated proteins. In humans, NMD is stimulated when a stop codon is present upstream of an exon-junction-complex (EJC). A crosstalk between the stalled ribosome and an EJC, mediated by several proteins that interact with the ribosome and the EJC forming transient complexes, regulates the NMD response. The molecular mechanisms to discriminate normal and aberrant translation termination events are not completely understood.
I will describe our research on the structure of several large and transient complexes that participate in NMD solved using cryo-electron microscopy (cryo-EM). We have studied the structural basis for three events during NMD: (a) the regulation of UPF1 phosphorylation by the SMG1 kinase; (b) the structure of a complex containing factors UPF1, UPF2, UPF3 bound to the EJC, an NMD intermediate, and (c) we have determined the structure and conformational flexibility of two AAA+ ATPases that associate with NMD factors and whose activity is required for NMD. Collectively these findings reveal the role of protein scaffolds and large conformational changes in the regulation of NMD.

O-378

RNA binding and unwinding by the T. thermophilus DEAD-box helicase Hera

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DEAD-box helicases catalyze the unwinding of RNA duplexes in an ATP-dependent reaction. The RNA unwinding activity is provided by a helicase core formed by two flexibly linked RecA-domains. Additional domains mediate binding of RNA, nucleotide binding or partner proteins, or assist in duplex separation. The DEAD-box helicase Hera from *Thermus thermophilus* consists of a helicase core, a dimerization domain and an RNA binding domain (RBD). Hera is a bona fide helicase that unwinds short RNA duplexes in an ATP-dependent reaction. By combining mutational analysis, NMR chemical shift perturbation experiments and X-ray crystallography, we have dissected the mode of RNA binding to the Hera RBD. The RBD contacts two distinct recognition elements in the RNA substrate: The core region of the RBD binds single stranded RNA, and the C-terminal tail contacts the stem of a nearby hairpin. Adjacent double-stranded regions can then be presented to the Hera helicase core for unwinding. This scenario is consistent with a function of Hera as a general RNA chaperone.

O-379

Eucaryotic translation at single molecule scale

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Using single molecule total internal reflection fluorescence microscopy, we observed translation of short messenger RNA (mRNA) strand by single eukaryotic ribosomes. The initiation is done through an Internal Ribosome Entry Site (IRES) from the Cricket Paralysis Virus (CrPV). The ribosome-mRNAs complexes are fixed to a microscope coverslip through the mRNA, and mRNAs are located thanks to fluorescently labelled oligonucleotides hybridized to them downstream start codon. Because of the ribosome helicase activity, the double strand formed by the oligonucleotide and the mRNA is opened while the ribosome translates this region of the mRNA. Two different oligonucleotides are hybridized at two different places on the mRNA. Thus, the consecutive loss of the fluorescence signal of both oligonucleotides allows us to measure the translation speed distribution of single ribosomes. Moreover, the departure time of these oligonucleotides give evidence of the influence of a CrPV IRES initiation on the kinetics of translation. This experiment opens the door to the study of eukaryotic translation at the single molecule level.

Abstracts

– Protein-nucleic Acid Interactions –

O-380

Protein-DNA interactions probed by Ultrafast Force-clamp Spectroscopy

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Ultrafast force-clamp technique allows the probing, under controlled force, of protein-DNA interactions with unprecedented temporal and spatial resolution. Short- and long-lived bimolecular interactions, ranging from ∼100 μs up to hundreds of seconds, can be detected as well as subnanometer conformational changes occurring upon bond formation. The application of our method to the study of lactose repressor-DNA interactions showed two kinetically well-distinct populations of interactions, representing strong specific interactions with DNA-binding sites (called operators) and a fast scanning of the protein along non-cognate DNA sequences. Our results demonstrate the effectiveness of the method to study the sequence-dependence affinity of DNA-binding proteins along the DNA molecule and the effects of force on a wide range of interaction durations, including ∼μs timescales not accessible to other current single-molecule methods. Therefore, this improvement in time resolution provides important means for the investigation of the long-puzzled mechanism of target search on DNA and possible conformational changes of the protein occurring upon target recognition.

O-381

Genomic integrity of virulence genes is preserved by a dUTPase-based molecular switch

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Transfer of virulence genes in Staphylococci is under control by repressor proteins (e.g. Stl) and was recently reported to be activated by helper phage dUTPases (1). dUTPases hydrolyse dUTP, thereby sanitizing the nucleotide pool, producing the dTTP precursor dUMP and preserving genomic integrity (2). Here we investigate the molecular mechanism of the dUTPase-induced gene expression control. We find that helper phage dUTPase (3) removes the Stl protein from its cognate DNA by direct binding to the repressor. The architecture of the dUTPase:Stl complex is revealed by biophysical techniques (EMSA, SEC, mass spectrometry, calorimetry, native gel, etc). The capability of trimeric dUTPase to exert de-repression is paralleled with drastic inhibition of dUTPase enzymatic activity in the dUTPase:Stl complexes. Unlike in small GTPases, hydrolysis of the substrate nucleoside triphosphate (dUTP in this case) is required prior to the interaction with the partner (Stl repressor in this case). Hence, expression of virulence genes only occur if dUTP is cleared from the nucleotide pool, a condition promoting genomic stability of the virulence elements.
References: (1) Tormo-Mas, Nature 465:779-82; (2) Vertessy, Acc Chem Res. 2009 42:97-106; (3) Leveles, Acta Crystallogr Sect F 2011 67:1411-3.

P-382

Probing the Physical Properties of a DNA-Protein Complex Using Nanofluidic Channels

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Nanochannels are an important tool to investigate single DNA molecules, both from a fundamental polymer physics perspective as well as in e.g. optical mapping techniques. While equally relevant, less effort has been made on studying DNA-protein complexes. A main reason for this is that most proteins stick to the channel walls. We have overcome this by coating the channels with a lipid bilayer.
RecA is a protein involved in homologous recombination and DNA repair that forms helical filaments on DNA. Nanofluidic funnels allow probing of the extension of a single filament at different channel dimensions, which in turn enables determination of physical parameters such as persistence length. For RecA filaments on dsDNA we obtain a persistence length of 1.15 ± 0.30 μm.
By forming the RecA filament on a circular template we are able to study bending of the filament in nanoconfinement. The filaments can be strongly bent without causing permanent damage. In the narrow end of the nanofunnel, the bending energy corresponds to >10kT.
Importantly, we perform the experiments in solution without attaching the DNA or protein to any surfaces or "handles". This means that we will be able to do the same kind of analysis on any DNA-protein complex, such as chromatin extracted from cells.

P-383

Activation enhances DNA-binding of RXR but not the number of binding sites as shown by FCS/ChIP-seq

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The Retinoid X Receptor (RXR) is a key component of transcriptional regulation by heterodimeric nuclear receptors. However its mobility and diffusion properties in the nuclei of single cells are not understood. We characterized the nuclear dynamics of RXR during activation. Experiments covered various time frames and resolutions. At the whole-genome level, agonist treatment increased the DNA-binding probability of RXR, but not the number of binding sites as shown by chromatin immunoprecipitation sequencing. For single-cell studies we used HeLa cells expressing GFP-RXR. Stripe-FRAP revealed an overall slowing down of receptor motion upon activation. FCS and 2D SPIM-FCS showed the coexistence of a fast and a slow receptor population with diffusion times in the 1-100 ms range. Ligand treatment induced an immediate and reversible transition to the slow state. This transition occurred even for RXR lacking its DNA-binding domain. This indicates that the ligand induced conformational switch can lead to increased DNA-binding via the partner receptor in the heterodimer. Coexpression of mCherry-RAR resulted in a similar transition to the slow state as ligand binding. Coactivator recruitment was indispensable for the transition.

Abstracts

– Protein-nucleic Acid Interactions –

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Addressing the role of individual residues in the Nucleophosmin-G-quadruplex DNA interaction

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Nucleophosmin (NPM1) is an ubiquitously expressed protein, mainly localized at nucleoli, that plays a key role in several cellular functions, including ribosome biogenesis, centrosome duplication, and response to stress stimuli. In acute myeloid leukemia more than 50 mutations map to the terminal exon of the *NPM1* gene and result in the destabilization of the NPM1 C-terminal domain and in the aberrant and stable translocation of the protein from nucleoli to the cytoplasm.
Previously we have shown that NPM1 C-terminal domain binds G-quadruplex DNA and we have analysed the complex formed with a G-quadruplex sequence derived from the c-MYC promoter by NMR methods. We have shown that the NPM1 terminal three-helix bundle binds the G-quadruplex DNA at the interface between helices H1 and H2, through electrostatic interactions with the G-quadruplex phosphate backbone. Furthermore, we have shown that a 17-residue lysine-rich sequence at the N terminus of the three-helix bundle is disordered and, although necessary for high affinity binding, does not participate directly to the contact surface in the complex.
Here we explore the role of individual residues in the nucleophosmin G-quadruplex interaction. Critical residues belonging either to the unstructured tail (aa225-241), or to the three-helix bundle (aa242-294) of the nucleophosmin C-terminal domain, were mutated and the effect of mutations on the binding kinetics was assessed by surface plasmon resonance.

P-385

The structure of complexes of nuclear proteins HMGB1 and H1 with DNA modified by cisplatin

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Cisplatin (cis-DDP) is one of the most successful and widely used anti-tumor drugs. Many cellular components can interact with cisplatin, but its main target is DNA. Cis-DDP form stabile adducts on DNA resulting in changes in DNA structure. Some nuclear proteins such as non-histone proteins HMGB1/2 and linker histone H1 recognize and bind specifically to these DNA regions. Using Principal Component Analysis we obtained rate constants of the reaction of DNA with cis-DDP. The analysis of CD spectra of DNA-protein complexes showed that the structure of DNA-platinum adducts in complex with H1 or HMGB1 is different from the structure of DNA-H1 and DNA-HMGB1 complexes. Analysis of the protein CD band shows that the interaction of HMGB1 with DNA-cis-DDP complexes results in changing in the protein secondary structure. The presence of H1 causes the formation of DNA "cross-links", while in the case of HMGB1 protein the amount of protein-protein interactions decreases due to selective bending HMGB1 to platinum adducts on DNA.
The authors appreciate the financial support from the Ministry of Education and Science of the Russian Federation Part of the work was performed with the financial support of the Russian Foundation for Basic Research (12-08-01134).

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Histone-specific changes in nucleosome architecture revealed by selective acetylation and spFRET

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Epigenetic regulation of nucleosome structure through histone acetylation and DNA methylation is a key mechanism to control genetic accessibility. We explore the structure-defining properties of selective histone tail acetylation and DNA methylation in a combined bulk FRET - single molecule FRET assay by 1) performing multiplexed bulk FRET to determine nucleosome stability against salt-induced dissociation and 2) single molecule FRET experiments at quasi-bulk conditions to map the structural heterogeneity within the sample. Additionally, nucleosome dynamics are investigated by species-selective Fluorescence Correlation Spectroscopy (FCS).
While H3-acetylated nucleosomes were significantly less stable than non-acetylated nucleosomes, acetylation of histone H4 stabilized nucleosomes against dissociation and counteracted the effect of H3-acetylation. Single molecule FRET revealed that, regardless of the extent of acetylation, nucleosome dissociation is initiated by a transition of nucleosomes into an intermediate conformation, which is suppressed at higher nucleosome concentration. H4-acetylated nucleosomes undergo the transition at higher ionic strength than H3-acetylated and non-acetylated nucleosomes, further confirming its structure-stabilizing properties.

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Computational study of interactions between amino acids and nucleobases in aqueous solvent

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The genetic code table provides a universally conserved link between mRNAs and cognate proteins. However, a question which still remains open is why a given nucleotide triplet codes for a particular amino acid? Recently, a potential connection between physicochemical properties of codons and cognate amino acids, i.e. of mRNA and cognate protein sequences was explored[1]. The study showed that mRNA coding-sequence pyrimidine content strongly correlates with the average propensity of protein sequences to be solubilized by pyrimidine mimetics (protein polar requirement), hinting at the possibility of complementary binding between these two biopolymers. To further examine potential interactions between proteins and their mRNAs on a microscopic level, molecular dynamics simulations were employed to obtain structurally- and energetically-derived scales of amino-acid solubility in nucleobase-water solutions. These were then used to obtain proteome-wide correlations between average sequence properties of mRNAs and cognate proteins and to compare them with the ones observed using the polar requirement scale. This allowed us to further explore the mRNA-protein complementarity hypothesis and probe the limits of its validity.[1]Hlevnjak *et al.*(2012) *Nucl Acids Res*40(18):8874

Abstracts

– Protein-nucleic Acid Interactions –

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Models of DNA interaction with Ruthenium Compounds Containing Biological Active Ligands

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DNA binding with the organometallic ruthenium complexes with quinolone antibacterial agents ofloxacin, [(η6-p-cymene)RuCl(O,O-oflo)] 3 2.8H2O (1 3 2.8H2O), Ru-oflo, nalidixic acid [Ru(η6-cymene)Cl(O,O-nalidixicato)], Ru-Nal phenantroline, [Ru(η6-cymene)Cl(phen-Ph2)]Cl, Ru-phe as well as DNA interaction with oflo-, nal- and phen- ligands were studied by spectroscopic methods [electronic, fluorescence, and circular dichroism (CD)], atomic force microscopy (AFM), viscometry and gel-electrophoresis. The modeling of DNA complexes with ruthenium compounds was carried out. The calculation of the structures of the compounds were conducted in three phases. First, the structures of the ligands were calculated. Then the structure of (Ru-cym)Cl3. molecule was obtained. Furthermore, using the program HyperChem v 7.0, two of three chlorine atoms in the molecule (Ru-cym)Cl_3 were replaced by the calculated ligands. After that steps more precise calculation of the structures of ruthenium complexes were done. Quantum mechanical calculations were performed in the software package Firefly v. 7.1 G by the Hartree - Fock method. DNA complexes with ruthenium compounds were modeling with docking procedure.

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Immobilization-free picomolar Interaction Studies Using Microscale Thermophoresis

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The analysis of bio-molecular interactions, such as protein-protein, protein-nucleic acid or protein-small molecule, not only helps to develop therapeutics or diagnostics techniques, but it also provides important insights into cellular processes. Here we present a novel instrument to analyze picomolar to millimolar affinities of biomolecular interactions, which is based on the method Microscale Thermophoresis (MST). MST analyzes the directed movement of molecules in optically generated microscopic temperature gradients. This thermophoretic movement is determined by the entropy of the hydration shell around the molecules. Almost all interactions and also any biochemical process relating to a change in size, charge and conformation of molecules alters this hydration shell and is thus detectable by MST. Here we show examples of antibody antigen interactions with picomolar affinities as well as interaction directly measured in cell lysate.

P-390

Modeling the bent PC-23RSS DNA based on FRET data and molecular dynamics simulations

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During V(D)J recombination a RAG1/2 protein machinery assists the assembly of gene segments coding for variable regions of IG light and heavy chains. The process involves the formation of a paired complex (PC) between two DNA sequences - 12RSS and 23RSS - followed by the elimination of the DNA region between them. During this process, RAG1/2 machinery first has to bring the 12- and 23-RSS regions close in space and bend them tightly in order to fulfill the spatial constraints imposed by V(D)J recombination.

We present here a model of the 23RSS region built starting from 15 FAM/TAMRA FRET pairs. In a first step a coarse grained bend was generated by progressively imposing unequal constraints on opposite sides of the DNA structure which minimally affect its local parameters. In the second step, the model was refined by imposing the FRET distance constraints while progressively relaxing the constraints used in DNA bending.

In support of the tight 23RSS bending comes the fact that its end to end distance decreases from 217Å in its linear form to ∼77Å in the presence of RAG machinery.

The model was validated by further FRET experiments which confirmed that the energy transfer could be increased or decreased by placing fluorophores on the concave and convex sides of the bent respectively.

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FRET, FCCS and MD-modeling reveal homodimerization of the Fos transcription factor in live cells

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Fos and Jun transcription factors play a role in cell proliferation, differentiation, apoptosis and oncogenesis. They function as homo- or heterodimers that bind to AP-1 (activator protein-1) regulatory elements in the promoter and enhancer regions of numerous mammalian genes. Whereas purified Jun can also form homodimers, less stable than the heterodimer, earlier in vitro studies showed that Fos was mainly monomeric. One of the reasons for the instability of Fos homodimers is the repulsion between the negatively charged side chains of the leucine zipper.We have previously confirmed by fluorescence crosscorrelation spectroscopy that Fos and Jun formed stable complexes in HeLa cells and described their C terminal using FRET and molecular dynamic modeling. Here we demonstrate that Fos can also homodimerize in live cells and estimate their K_d. FRET efficiency between full length Fos-CFP and Fos-YFP increased with increasing acceptor-to-donor ratio up to E∼4%. Truncation of the C termini by 165 AA brought the dyes closer together resulting in E∼10%. FCCS corroborated stable association. MD modeling also indicated that stable homodimers could be formed. Our results introduce Fos homodimers as a new form of AP-1, possibly a new oncogenic form in Fos-overexpressing tumors.

Abstracts

– Protein-nucleic Acid Interactions –

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Interaction of a triazoloacridone derivative C1305 with telomeric DNA - through molecular dynamics

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Human telomeres are structures that consist of double stranded DNA terminal sequence and protein complex known as shelterin. Telomeric 'TTAGGG' DNA sequence that repeats through 2-20 thousands base pairs is capped by a number of proteins among which are telomeric-repeat binding factors 1 and 2(TRF1 and TRF2). TRFs recruit other proteins to form shelterin, which prevents activation of DNA damage response by the DNA termini. It was shown that loss of shelterins trigger rapid DNA damage response, particulary harmfull to tumour cells, which results in apoptosis. Experimental studies have shown that C-1305 - a triazoloacridone derivative - intercalates into G-rich DNA sequences. In our research on molecular details of TRFs DNA recognition, we are especially interested in specificity of C1305 intercalation into DNA and its impact on the DNA structure.
Using equilibrium molecular dynamics (MD), steered MD and free
energy simulations, we analyze how DNA is affected by the presence of C-1305, which was previously shown by affiliated group to selectively cause TRFs dissociation from telomeric DNA and to induce fast DNA damage response.

P-393

Crystallization of ORF112 from Cyprinid Herpes virus 3 in complex with DNA

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ORF 112 is a protein of fish herpes viruses that is homologous to the pox viral inhibitor of antiviral responses E3L and thus a protein likely important for the pathogenicity of this virus family. The aim of the project was the crystallization of ORF112 from Cyprinid Herpes virus 3 in complex with DNA. In order to achieve it, the protein was expressed in an *E.Coli* expression system and was purified in homogeneity. After that, the complex between the protein and an oligonucleotide double-stranded $T(CG)_7$ was formed and different crystallization screens were performed. Crystals were obtained in one of the tested conditions and this result was reproducible. The crystals are being optimized and, in the future, they will be analysed by X-ray, in order to obtain the atomic structure.

P-394

Mechanisms of TRF1/TRF2 binding properties and DNA sequence recognition: a Molecular Dynamics study

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Telomeres are protein-DNA complexes that play a crucial role in maintaining chromosomal termini in linear chromosomes. They act as stabilizing and recognition factors, solving the end-replication problem and preventing the chromosomes from ligation by cell's own repair mechanisms. Since they undergo gradual shortening during cell proliferation, certain cells produce telomerase - an enzyme able to elongate the DNA termini; this is also the case in many types of cancer. Disruption of telomeres may elicit apoptosis, which makes them a potent target for chemoterapeutics.
Human telomeric DNA consists of numerous repeats of tandem 5'-TTAGGG-3' sequences, which are recognized by two homologous Myb-like shelterin domains, called telomere recognition factors: TRF1 and TRF2. The study was aimed at determinig the molecular mechanisms of these domains' DNA binding and sequence recognition. Despite their high similarity, they are believed to govern different functions in stabilizing the telomeric structure, and a comprehensive MD analysis of their affinity towards DNA could elucidate these differences. Thus, a free-energy profile calculation and detailed examination of binding mechanism is carried out in order to draw conclusions concerning functional properties of both proteins.

P-395

Single-molecule fluorescence studies of the enzymatic activity of primase RepB'

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This work aims to elucidate the replication mechanism of the bacterial plasmid RSF1010 by investigating the complementary DNA synthesis on a short DNA sequence known as single-strand initiator A (ssiA) that serves as a starting point for the plasmid's duplication. Functional interactions between the template DNA oligomer ssiA and RepB', a primase encoded by the plasmid, were investigated by means of fluorescence spectroscopy, gel electrophoresis and single-molecule total internal reflection fluorescence microscopy (TIRFM). As a prerequisite for TIRFM studies biotinylated template DNA was immobilized onto glass cover slides. A silanization protocol for defined densities of binding sites was developed based on varying concentrations of triethoxysilanes 3-aminopropyltriethoxysilane (APTES) and 3-triethoxysilylpropionitrile. Subsequent crosslinking of the amine moieties of APTES and lysine residues of streptavidin via glutaraldehyde allowed binding of biotin-ssiA. Enzymatic activity was investigated by PicoGreen binding to single ssiA oligomers after interaction with RepB' and polymerase. The single-molecule fluorescence time traces exhibit distinct fluorescence intensity levels that could be assigned to the native, double-stranded primed and elongated ssiA template.

Abstracts

– Protein-nucleic Acid Interactions –

P-396

Fluorescent quantification of Gibbs free energy of cap binding to DcpS reveals DcpS-cap interactions

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Decapping Scavenger (DcpS) enzyme regulates mRNA metabolism, competing with other cap-binding proteins for short cap species arising in the mRNA 3'→5' decay. We aimed to find DcpS-cap interactions crucial for the ligand binding. Several methylenebis(phosphonate), imidodiphosphate and phosphorothioate cap analogs modified in the cleavage site were selected as resistant to DcpS-mediated hydrolysis. Using time synchronized fluorescence titration we determined their association constants (K_{AS}) and Gibbs free energies of binding (ΔG^0). Methylation of 2'O or 3'O position of 7-methylguanosine, or extension of the triphosphate chain increased ΔG^0, pointing the important role of ribose hydroxyls and proper alignment of the phosphate chain in complex formation. Cap analogs missing the second nucleoside exhibited higher ΔG^0 than their dinucleotide counterparts, suggesting that the second nucleoside stabilizes cap-binding. The identification of cap's functional groups evoking efficient binding to DcpS provides an insight into the mechanism of DcpS catalysis.

P-397

Structural and functional characterisation of Ms1

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Ms1 (Myocyte stress 1) is a muscle specific protein, implicated in the development of cardiac muscle hypertrophy. In rat models of induced left ventricular hypertrophy, Ms1 expression is increased 3 times after applying stress signal and before early response genes are expressed. It suggests that Ms1 can act as a very important stress sensor that correlates external signals with a cellular response, however its complete mode of action needs to be further elucidated.
In this work, we present a new evidence that Ms1 can be involved in gene expression regulation through a direct DNA-binding, We have solved the NMR solution structure of Ms1 and it contains winged-helix-turn helix domain (WHTH) - a common domain present in transcription factors which mediates specific protein-DNA interaction. Using SELEX we identified a DNA motif bound by Ms1 and the specificity of this interaction was confirmed using mutagenesis, EMSA, NMR and fluorescence. Furthermore, using NMR, we have mapped the DNA-binding site of Ms1 and it confirmed the involvement of helix 3 in the specific DNA-binding, what is characteristic for transcription factors containing WHTH domain. Additionally, we will present cellular localisation studies of Ms1 and factors that affect its nuclear localisation.

Abstracts

– Material Science in Biophysics –

O-398

Nanotube-forming hydrophobic dipeptides: structure, properties and applications

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Dipeptides with hydrophobic side-chains crystallise in a peculiar fashion, different from the layered structure of larger peptides with hydrophobic side-chains. Instead, hydrophobic dipeptides form head-to-tail hydrogen bonds, creating tubular helices. The inside of these helices is often empty, constituting one-dimensional nanotubes.

The nanotubes have dimensions ranging from 3 to 10 Å, with varying degrees of helicity. They can be hydrophobic or hydrophilic, with the hydrophobic ones proving to be excellent molecular hosts for small guest molecules. Despite the limited space, molecules diffuse through the nanotubes quickly and freely, being easily adsorbed/desorbed. These properties are attributed to the flexibility of the crystalline framework. They also display surprisingly high thermal and chemical resistance, making them extremely interesting materials for several adsorption and adsorption-based separation processes.

O-399

Processing of recombinant proteins for materials applications: about spider silk and more

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Proteins reflect one fascinating class of natural polymers with huge potential for technical as well as biomedical applications. One well-known example is spider silk, a protein fiber with excellent mechanical properties such as strength and toughness [1]. During 400 million years of evolution spiders became outstanding silk producers. Most spider silks are used for building the web, which reflects an optimized trap for flying prey. We have developed biotechnological methods using bacteria as production hosts which produce structural proteins mimicking the natural ones [2]. Besides the recombinant protein fabrication, we analyzed the natural assembly processes [3] and we have developed spinning techniques to produce protein threads closely resembling natural silk fibers. In addition to fibers, we employ silk proteins in other application forms such as hydrogels, particles or films with tailored properties, which can be employed especially for materials applications [4].

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O-400

Dynamical diversity of compartmentalized *in vitro* transcriptional oscillators

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Encapsulation of biochemical reaction networks is a central step for the creation of cell-like reaction containers, which potentially alters their dynamics in a complicated and effectively unpredictable manner. We here demonstrate this by encapsulating a previously developed, synthetic in vitro transcriptional oscillator system [1,2] into microemulsion droplets with volumes ranging from tens of femtoliters to several picoliters. Within thousands of droplets, large variations in amplitude, frequency, and damping of the oscillations are observed. While, in general the diversity of dynamical behaviors increased for smaller droplets, the degree of diversity depended on the position of the operating point of the oscillator within its phase space. Similar to partitioning effects arising in biological cell division the observed variability could be mainly attributed to statistical variations in the concentrations of the oscillator species rather than to the stochasticity of chemical kinetics for small molecule numbers. Variability generated by compartmentalization poses a major challenge for cell-scale molecular engineering and the realization of artificial cell-like reaction compartments. In the future it will be interesting to explore which synthetic circuits provide better or worse performance in the presence of such variability, and to develop strategies for either active error-reduction or increased noise resilience.

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O-401

Optical tools for localized fluorescence enhancement and single cell studies

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There is an increasing interest in functionalized complex microstructures for micro-and nanotechnology applications in biology. We introduce such micrometer-sized 3D tools that can be actuated in optical tweezers system with six degrees of freedom to manipulate biological objects without optically damaging them. The tools are made of SU-8 photoresist by two photon polymerization which is capable of making practically any arbitrarily-shaped 3D structures with submicrometer features. Functionalization of these tools is a major improvement in their biological applicability. We functionalize them with two goals in mind: first, protein coatings enable their use in specific microtool-cell or microtool-protein interactions and second, gold nanoparticle coatings aim their application in localized and targeted metal-enhanced fluorescence studies. The surface functionalization chemistry, single cell manipulation and ability to enhance florescence will be presented.

*R*eference: B. L Aekbote, J. Jacak, G. J Schütz, E. Csányi, Z. Szegletes, P. Ormos, L. Kelemen. 'Aminosilane-based functionalization of two-photon polymerized 3D SU-8 microstructures'. *European Polymer J* 48 (2012) 1745–1754.

Abstracts

– Material Science in Biophysics –

O-402

Specificity, flexibility and valence of DNA bonds guide emulsion architecture

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We develop micron-sized emulsions coated with specific DNA sequences and complementary sticky ends. The emulsions are stabilized with phospholipids on which the DNA strands are grafted through biotin-streptavidin interactions. Grafting DNA onto the liquid interfaces of emulsions leads to exciting new architectural possibilities due to the mobility of the DNA ligands on the surface. We produce two complementary emulsions: the first one is dyed in red and functionalized with S sticky ends; the second, dyed in green, displays the complementary S' sticky end. Mixing those emulsions reveals strong, reversible and specific adhesion between the complementary emulsions due to the short-range S-S' hybridization. The fluidity of the binders leads to diffusive adhesion patches, which allows the bound droplets to rearrange throughout the packing structure. We quantify the strength of adhesion between two complementary emulsions and build a theoretical framework that quantitatively describes the increase in the patch size with droplet radii, DNA concentration and the stiffness of the tether to the sticky-end. This emulsion system opens the route to directed self-assembly of more complex structures through distinct DNA bonds with varying strengths and controlled valence and flexibility.

O-403

Tuning curvature in inverse micellar and bicontinuous cubic phases

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Non-bilayer phases are biologically relevant for e.g. during membrane fusion.Several complex 3D lyotropic phases are known, but for decades only one example was found of a structure based upon a complex close packing of inverse micelles – an Fd3m cubic phase.We reported the discovery[1] of a novel lyotropic phase (spacegroup $P6_3/mmc$) whose structure is based upon a hexagonal close packing of identical inverse micelles.These ordered micellar phases respond differently to hydrostatic pressure[2] compared to the bicontinuous cubic phases which swell with pressure.
We have engineered highly swollen bicontinuous cubic phases by increasing bilayer stiffness,inducing electrostatic interactions and applying hydrostatic pressure.By doing so,we exceeded the predicted maximum lattice parameter swelling from calculations which suggest that thermal fluctuations should destroy such phases larger than 30 nm.[3]
To understand events such as membrane fusion, understanding the processes governing phase transitions, the intermediates formed and the transition mechanism are vital.We have investigated lamellar to non-lamellar transition kinetics using time-resolved pressure jump XRD studies.
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[3]*J. De Phys. II*,**2**,425 (1992)

P-404

Quantitative XRD analysis of the Dehydration-Hydration performance of (Na+, Cs+) exchanged smectite

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This works aims at characterizing the link between dehydration–hydration behavior, charge location and the ionic radius, in the case of dioctahedral smectites. For that, a natural montmorillonite and beidellite, with different charge location (respectively di and tri-octahedral), are selected. The exchange process is directed using Na^+ and Cs^+ cations. The hydration hysteresis is investigated "in situ" as a function of relative humidity condition rates. All samples are studied using quantitative XRD analysis. This method allows us to determine the structural parameters obtained from the theoretical Mixed Layer Structure used to fit experimental XRD patterns. For both Na^+ and/or Cs^+ exchangeable cations, an increase of hydration heterogeneity degree for the tetrahedral substituted smectite layer is noted and the position of exchangeable Cs^+ cation induce a homogeneous hydration trend which is interpreted by a new interlamellar space organization.

P-405

Nanostructures and drug distribution within lipid carriers revealed by super-resolution microscopy

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Nanostructured lipid carriers (NLC) are a smart formulation principle for poorly soluble drugs with a matrix consisting of a blend of a solid and a liquid lipid. The nanostructures are important for the *in vivo* performance of the particles. Until now, due to the lack of suitable methods, it was not possible to investigate these nanostructures and only theories about their internal structure exist. Here, a method to investigate the shape, inner structure and drug distribution within NLC was developed. A combination of single particle tracking (SPT) and super-resolution fluorescence microscopy based size determination was applied. Fluorescent molecules were used as drug mimetics within the liquid lipid and as affinity stain to determine the size and shape of the NLC in aqueous solution. Single particle data from drug mimetics reveal two subpopulations with different confinement lengths, indicating confined areas for the fluid phase that are much smaller than the average size of the NLC itself with about 160 nm. In summary, a spherical outer shape with a drug loaded liquid core was found for the NLC investigated.
(1) Kim TY, Uji-i H, Möller M, Muls B, Hofkens J, Alexiev U(2009) Biochemistry 12:3801; (2) Kirchberg K, Kim TY, Haase S, Alexiev U (2010) Photochem Photobiol Sci 9:226

Abstracts

– Material Science in Biophysics –

P-406

The role of structural architecture of Bis-quat gemini surfactant-based complexes in gene delivery

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The conformational flexibility provided by the spacer and by the double hydrocarbon chains to cationic gemini surfactants has been shown to confer them properties of successful non-viral gene delivery systems, with low cytoxicity and high nucleic acid protection. In this work, two families of *bis-quaternary* gemini surfactants, represented by the general structures $[C_mH_{2m+1}(CH_3)_2N^+(CH_2)_sN^+(CH_3)_2C_mH_{2m+1}]2Br^-$ and $(nSer)_2N5$, were used to prepare cationic gene carriers. An extensive study on the transfection efficiency, cytotoxicity and physical properties of pDNA complexes formed by each gemini surfactant, alone or in combination with helper lipids (cholesterol and DOPE), was performed. The most efficient complex formulations in transfecting HeLa cells were those containing gemini surfactants that displayed a phase transition in aqueous dispersions close to the physiological temperature. We hypothesize that pDNA complex structural architecture influences membrane interactions and, then, the success in overcoming biological barriers to gene delivery. Therefore, a biophysical approach emerges as a strategy to implement the rational design of efficient gene delivery systems.

P-407

Amyloid fibers formation using different peptides: A comparative approach

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Amyloid fibers, often associated with several human degenerative diseases (such as Alzheimer's and Parkinson's diseases), may also have physiological roles, having even been suggested as potential novel biomaterials [1]. In general, amyloid fibers share a common β-sheet rich architecture that is behind their exceptional stability, mechanical strength and resistance to degradation, rendering them excellent nanomaterial candidates [1]. The potential to form amyloids (and other protein/peptide aggregates) can be predicted from the peptide amino acids sequence [1,2]. Here, we used four amyloidosis models, consisting on different amyloid peptide sequences (STVIIE, QVQIIE, ISFLIF and GNNQQNY) to evaluate, by atomic force microscopy (AFM), circular dichroism (CD) and Fourier Transformed Infra-Red (FTIR) spectroscopy under different conditions, which type of amyloid species would be formed (namely, amyloid oligomers, protofibrils or fibrils) at different times of incubation (24 h, 72 h and 2 weeks). AFM, CD and FTIR data, taken together, indicate the peptide STVIIE as the most reproducible and amenable peptide for developing amyloid-based nanotechnology approaches.

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2. Maurer-Stroh et al., 2010, Nat Methods, 7:237

P-408

Carbon nanotube as functional matrix for bacterial photosynthetic reaction centers

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Photosynthetic reaction center protein (RC) purified from Rhodobacter sphaeroides R-26 purple bacterium was immobilized on $–NH_2$ and -COOH functionalized and non-functionalized carbon nanotubes (CNTs) and the optical and electric properties of the complex was investigated. The RC binding was proved by electron microscopy and atomic force measurements. The kinetics of the absorption change after single saturating flash excitation shows that the RCs remain active in the complex for several weeks. If the CNT/RC complex was bound to transparent conductive electrode a photocurrent was measured in a specially designed electrochemical cell. Light induced conductivity of the complex was also measured in a dried complex. The special electronic properties of our CNT/RC complexes open the possibility for several directions new generation applications in optoelectronics, e.g. in microelectronics or energy conversion.

P-409

Purple bacterial pigment-protein complexes as photovoltaic material for the generation of DC and AC

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Reaction centres and RC/light harvesting 1 (RC-LH1) complexes from *Rhodobacter sphaeroides* have been interfaced with electrodes for the generation of photocurrents. Unmodified RCs can be interfaced directly with gold electrodes, producing high photocurrents despite the absence of an intervening functionalised self-assembling monolayer to protect the protein from the metal, and to orient the protein at the electrode surface. RCs or RC-LH1 complexes sandwiched in a cavity between a transparent conducting glass electrode and a platinum back electrode produce a conventional direct photocurrent in response to continuous illumination and a novel alternating current in response to discontinuous illumination. The magnitude of the open circuit voltage of such cells can be boosted by over 30-fold by manipulation of the electrolyte. Variation of the material for the counter electrode, variation of the electrolyte, or the use of inhibitors, allows either the AC or DC output of the cell to be isolated. The symmetry of the AC output from RC-LH1 complex-based cells can be enhanced by employing a back electrode comprising superhydrophobic carbon nanotubes. The prospects for boosting the current and voltage output and stability of such protein-based photoelectrochemical cells are discussed.

Abstracts

– Material Science in Biophysics –

P-410

Aliskiren loaded PLA nanospheres: preparation, physicochemical characterization and in vitro release

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Hypertension is a global public health issue and approximately 7.1 million deaths per year are attributed to it. Aliskiren (ALIS) is very effective renin inhibitor used to treat high blood pressure. However, the limiting factor in clinical praxis is the relatively low bioavailability of ALIS. One of the various possibilities how to increase ALIS bioavailability is nanoencapsulation of ALIS.
Polymer nanospheres (NPs) created by poly(lactic acid) (PLA) were used for drug encapsulation. Characterization of the prepared ALIS loaded nanospheres has been accomplished by the routine methods such as SEM, DLS, DSC, FTIR, UV/VIS and fluorescence spectroscopy. The preparation with 5% w/w theoretical loading of ALIS which provided a drug content of 3.1% w/w and drug entrapment of 36%, good morphological features and a relatively high NPs recovery of 78% and particle size of 235 nm was selected as the optimal starting formulation for *in vitro* studies. Drug release from NPs appears to consist of two components which an initial rapid release followed by a slower exponential stage.
(This work was supported by grants ESF 26110230061, APVV 0742-10, 0171-10, VEGA 0041 and 0181).

P-411

Photocurrent generated by photosynthetic reaction centers/carbon nanotube/ITO bio-nanocomposite

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Different preparations and experimental conditions are used to find the most efficient energy converting systems in nanotechnology. Different biological systems and various inorganic matrices (e.g. indium tin oxide, carbon nanotubes, silicon nanostructures) are used in different laboratories. Reaction center proteins (RC) purified from purple bacterium *Rhodobacter sphaeroides* were bound successfully to functionalized multiwalled carbon nanotubes immobilized onto the surface of ITO in our studies. Electron microscopy and AFM images, flash photolysis and conductivity have shown that RCs can be bound effectively to the carbon nanotubes. A special electrochemical cell was designed for measuring the photocurrent generated by this composite. Several hundreds of nA photocurrent was measured with fully active RCs which was sensitive to the conditions that fulfil conditions of the RC photo turnover.

P-412

Protein-based high-speed all-optical logic

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The principle of all-optical logical operations utilizing the unique nonlinear optical properties of a protein was demonstrated by a logic gate constructed from an integrated optical Mach-Zehnder interferometer as a passive structure, covered by a bacteriorhodopsin (bR) adlayer as the active element. Logical operations were based on a reversible change of the refractive index of the bR adlayer over one or both arms of the interferometer. Depending on the operating point of the interferometer, we demonstrated binary and ternary logical modes of operation. Using an ultrafast transition of the bR photocycle (BR-K), we achieved high-speed (nanosecond) logical switching. This is the fastest operation of a protein-based integrated optical logic gate that has been demonstrated so far. The results are expected to have important implications for finding novel, alternative solutions in all-optical data processing research.

P-413

Continuous extraction of plasmids in aqueous two-phases systems for biomedical applications

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Recent years have witnessed an increased effort to develop novel methods for nucleic acids purification. This has been mainly motivated by the expected developments of molecular therapies (DNA vaccination and gene therapy) and molecular diagnostics. In both cases simple, efficient and economic methods are needed to obtain in large or small-scale adequate samples of purified genetic material. Although most methods are chromatography based, aqueous two-phase systems (ATPS) have also received some attention. These systems are obtained by mixing in water two polymers or a polymer and a salt, which above critical concentrations form two distinct phases that enables the separation of compounds from complex mixtures. Their main advantages include ease of scale-up, low cost, non-toxicity of the reagents, high capacity and possibility of continuous operation.
In this work the continuous purification of nucleic acids in ATPS was studied using a model plasmid molecule in polyethylene glycol – sodium citrate systems. Complete removal of RNA and protein contaminants from a bacterial lysate was obtained in a multistage operation. Continuous extraction on microfluidic devices is underway and will be presented.

Abstracts

– Material Science in Biophysics –

P-414

Phototransformation of BCD180 at its interaction with nano-organized systems under visible light

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Bicyanine dyes (BCD) have favorable spectral characteristics to be used in Photodynamic Therapy, a treatment medical that consists in the introduction of a photosensitizer in an organism with subsequent irradiation, causing a curative action. It is important to study the phototransformation of these dyes, because they can lose their activity or can form toxic photoproducts under the action of visible light, and the interaction with biological systems can alter the mechanisms of photoreactions. We investigated the phototransformation of BCD180 at its interaction with SDS micelles and DNA by optical absorption spectra as a function of irradiation time. BCD180 is transformed under visible light, with the disappearance of its main absorption band (633 nm). Another absorption band (554 nm) associated with the photoproduct generation was formed. It was also phototransformed. The interaction of BCD with SDS and DNA was confirmed by changes in absorption spectra. Irradiation of BCD in the presence of SDS or DNA causes BCD phototransformation that is lower as compared with homogeneous solution, indicating a protection effect of interaction of BCD with both compounds. The molecular oxygen increases the phototransformation rate due to the formation of singlet oxygen at the BCD excitation.

P-415

Engineering purple bacterial photoreaction centres for photo-device applications

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Reaction centres power much of biology by transducing the energy of sunlight into a flow of electrons through a linear or cyclic electron transfer chain. There is burgeoning interest in the interfacing of reaction centres of various types to man-made electrodes in order to exploit their innate highly-efficient photochemical charge separation for applications in photovoltaics, photocatalysis, biosensing and molecular-scale computing. One major challenge in this work is binding of the protein to the electrode in a controlled, stable and densely-packed manner for the generation of optimal currents. In this work we outline the use of protein tags to bind reaction centres to specific electrode materials with a defined orientation, and to induce the formation of oligomeric assemblies of reaction centres in solution and on surfaces.

P-416

A molecular dynamics study of free and steered adsorption of BSA over graphene

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Albumin, the most abundant plasma protein in mammals, is responsible of a manifold of vital functions. A better understanding of its adsorption over different surfaces would have a high impact on areas ranging from medicine to biochemical engineering [1,3]. Here, we study the bovine serum albumin (BSA), which is the most widely used in experiments due to its low cost and its similarity to the human albumin. Recently it has been shown that graphene can be an optimal candidate to be used as an implant material [3]. Therefore the study of adsorption of BSA molecules on graphene will be of interest for addressing the use of graphene as biocompatible material.

Here we report a molecular dynamics (MD) study of the free and forced adsorption of BSA over graphene. The simulations were carried out using the AMBER force-fields [2] and explicit solvent. This allows us to address several open questions: mechanisms behind the adsorption; role of the water molecules in the adsorption; the most favorable adsorption orientation. Furthermore, we demonstrate that BSA does not denature during adsorption if the solvent is explicitly included, at variance of recent findings [3].

[1] Curr. Opin. Biotechnol. 7 (1996) 72.
[2] J. Am. Chem. Soc. 117 (1995) 5179.
[3] Langmuir 2011, 27, 12938.

P-417

Biosensing properties of Au loaded mesoporous silica nanospheres coated with lipid bilayers

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We have developed a simple synthetic route to achieve the synthesis of gold loaded radial mesoporous silica nanoparticles (Au-MsNPs). These nanoparticles were synthesized in a one step procedure fully compatible with basic conditions required for the preparation of monodispersed nanospheres. These Au-MsNPs were characterized by transmission and scanning electron microscopy, Energy Dispersive X-ray analysis and N_2 adsorption. Metallic Au-nanoparticles embedded in pore channels were responsible for plasmonic activity. Au-MsNPs were then coated with phospholipid bilayers in order to design a biofunctional device with plasmonic properties for biosensing. The supported lipid bilayers were obtained after incubation of Au-MsNPs particles with different lipid vesicles. The coating efficiency was investigated by zeta potential, agarose gel electrophoresis and cryo-transmission electron microscopy. Different model systems have been investigated: direct adsorption of bovine serum albumin or molecular recognition events between a biotin receptor (integrated in the supported lipid bilayer) and avidin. Overall the results demonstrate the plasmonic sensitivity of the bare or the coated lipid bilayer Au-MsNPs (Veneziano et al. 2012).

Abstracts

– Material Science in Biophysics –

P-418

Polycarbonate polymers as novel antimicrobial agents

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Though the discovery of antibiotics lead to the extermination of many bacterial diseases, their overuse caused some bacteria to become resistant. Research into novel antimicrobials is thus crucial. One promising avenue are antimicrobial polycarbonate polymers but the mechanism of their action remains poorly understood.

Molecular dynamics simulations were used to study the dynamics of polycarbonate polymers in water solutions. Three monomers were designed using amino acids as templates. By using those monomers ten different polymer molecules were generated and studied either as one, two or three polymer molecules. MD simulations were ran for 50-ns at NPT conditions.

Our results indicate polymer molecules in water solution fold in a manner similar to proteins regardless of the polymer composition and number of polymer molecules. The folding process is fast and confirms the experimentally observed polymer aggregation, which makes the characterization difficult. Further simulation studies are on the way to determine the polymer-bacterial membrane interactions.

Abstracts

– Protein-Lipid Interactions –

O-419

Interaction of membrane cholesterol with G Protein-Coupled Receptors: A multidimensional approach

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G-protein coupled receptors (GPCRs) are the largest class of molecules involved in signal transduction across membranes. The serotonin$_{1A}$ receptor is an important member of the GPCR superfamily and is implicated in the generation and modulation of various cognitive, behavioral and developmental functions. Earlier work from our laboratory showed that membrane cholesterol is required for ligand binding activity, and G-protein coupling of serotonin$_{1A}$ receptors. Interestingly, recently reported crystal structures of GPCRs have shown structural evidence of cholesterol binding site(s). We reported the presence of cholesterol recognition/interaction amino acid consensus (CRAC) motifs in the serotonin$_{1A}$ receptor. Our recent results utilizing coarse-grain molecular dynamics simulations to analyze the molecular nature of receptor-cholesterol interaction offer interesting insight in cholesterol binding site(s) in the receptor. We showed utilizing homo-FRET that the serotonin$_{1A}$ receptor is constitutively oligomerized, with the possibility of higher order oligomers of the receptor. Progress in deciphering molecular details of the nature of GPCR-cholesterol interaction in the membrane would lead to better insight into our overall understanding of GPCR function in health and disease.

O-420

Liprotides: complexes between fatty acids and (partially denatured) proteins

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HAMLET (human alpha-lactalbumin made lethal to tumor cells) has highlighted the remarkable properties of complexes formed between alpha-lactalbumin and oleic acid. Recent work has demonstrated that this may be a more widespread feature of (often partially denatured) proteins and lipids. We have shown that the complex formed between equine lysozyme and oleic acid is able to transfer oleic acid to phospholipid vesicles with partial regain of native structure, indicating that the protein in a HAMLET-like complex serves as a (partially) reversible cargo transporter for lipids (JMB 398, 351). I will discuss our recent findings on the biophysical properties of such complexes. It is possible to convert bovine serum albumin (which binds fatty acids and other hydrophobic components very tightly in the native state), as well as several other proteins found in plasma and milk, to oligomeric hemolytic protein:lipid complexes. Structural analysis based on Small Angle X-ray Scattering suggests that protein-lipid complexes have a generic build-up consisting of a fluid hydrophobic core (mainly oleic acid) surrounded by a protein shell that forms a loose corona. The protein-lipid ratios vary in different complexes depending on the initial protein-lipid ratio, suggesting that it is possible for the complexes to optimize structure according to the amount of available lipid.

O-421

Lipid interactions of glycosyltransferases

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The finely tuned interactions between glycoslytransferases and lipids regulate enzymatic activity of the enzymes. They sense bilayer properties such as curvature, packing and headgroup charge. Here we discuss lipid-interacting properties of two enzymes, a monoglucosyltransferase from *Acholeplasma laidlaawi* (*al*MGS), and a digalactosyltransferase (*at*DGD2) from *Arabidopsis thaliana* (*at*DGD2).
*al*MGS catalyses the addition of a glucose to diacylglycerol, producing monoglucosyldiacylglycerol (GlcDAG). We have shown that lipid binding is related to a helical segment that contains a high number of positively charged amino acid residues. We also demonstrated by a combination of *in vivo* and biophysical studies that when *al*MGS is introduced into *E.coli*, it binds selectively to anionic lipids. This acts as a signal for the production of all lipids in the cell.
*at*DGD2 is responsible for synthesis of GalGalDAG under phosphate stress conditions in plants, and we have shown that this enzyme also binds selectively to anionic lipids. To study the influence of the galactolipids GalDAG and GalGalDAG on enzyme properties, we developed novel membrane mimetics, fast-tumbling bicelles, with these lipids and their use in studies of *at*DGD2 is discussed.

O-422

Lipid coated quantum dots as a model to study lipid-protein interactions via FCS

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In this investigation, we studied the interactions of human serum proteins and lipid coated quantum dots (lipid QDs). A detailed understanding between lipid surface and serum proteins is crucial to design improved drug delivery system, where medication is often encapsulated within lipid coating to avoid immune responses. With our strategy, we use the fluorescence from the quantum dot and can reliably study different interactions between lipids and proteins without introducing fluorescent marker on either interacting partners. To prepare lipid QDs, we used oleylamine-stabilized CdSe/ZnS as the core and coated the surface with different amphiphilic lysophospholipids and steric acid. The lipid encapsulation and the homogeneity of purified lipid QDs were verified by the Förster Resonance Energy Transfer, electron microscopy and fluorescence correlation spectroscopy (FCS) measurements. Hydrodynamic radii of the particle were measured by FCS as a function of the protein concentration, which can be used directly to calculate the binding constant. As a proof of principle, we demonstrate our label free measurement using Annexin V and lipid QDs with different concentrations of phosphotidylserine lipids.

Abstracts

– Protein-Lipid Interactions –

O-423

Structure, function & inhibitors of the pH-gated H. pylori urea channel essential for acid survival

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Half the world's population is chronically infected with *Helicobacter pylori*, causing gastritis, gastric ulcers and gastric adenocarcinoma. Its proton-gated inner membrane urea channel, *Hp*UreI, is essential for survival in the acidic environment of the stomach. The channel is closed at neutral pH and opens at acidic pH to allow the rapid access of urea to cytoplasmic urease, producing NH_3 and CO_2 that neutralizing entering protons and thus buffering the periplasm to a pH of roughly 6.1 even in gastric juice at a pH below 2.0. The crystal structure of *Hp*UreI reveals six protomers assembled in a hexameric ring surrounding a central bilayer plug of ordered lipids. Each protomer encloses a channel formed by a twisted bundle of six transmembrane helices. The bundle defines a previously unobserved fold comprising a two-helix hairpin motif repeated three times around the central axis of the channel, without the inverted repeat of mammalian-type urea transporters. Both the channel and the protomer interface contain residues conserved in the AmiS/UreI superfamily, suggesting the preservation of channel architecture and oligomeric state. Predominantly aromatic or aliphatic side chains line the entire channel and define two consecutive constriction sites in the middle of the channel.

O-424

Modulation of ion-channel activity by cholesterol and ceramide

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Ceramide, formed via hydrolysis of sphingomyelin by sphingomyelinase is known to cause the formation of gel phases which coexist with fluid domains. Using x-ray scattering in combination with osmotic stress, we determined membrane thickness and the membrane elastic parameters (bending rigidity, spontaneous curvature, Gaussian curvature modulus) for the coexisting lipid domains in four-component mixtures of sphingomyelin, palmitoyloleoyl phosphatidylcholine, cholesterol and ceramide. Experimental results were coupled theoretically to the opening probability of funnel-shaped and hourglass-shaped ion-channels located in either domain via the lateral pressure concept. Depending on protein geometry we found significant shifts of the conformational equilibrium of the ion channels either toward open or closed states. These changes are only modest in the presence of cholesterol, indicating a protective role of cholesterol by stabilizing membrane function.

P-425

Morphologies and dynamics of membrane interaction of COPII proteins

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The transport of cargo molecules from the Endoplasmatic Reticulum to the Golgi apparatus of eucaryotic cells is mediated by COPII-coated vesicles. This coat consists of the minimal components Sar1 (a small GTPase), the inner coat protein complex Sec23/24 and the outer coat complex Sec13/31. Further factors involved in COPII-transport are the guanine nucleotide exchange factor Sec12 and cargo proteins that are recruited by Sec24.
We examine the functional and morphological aspects of the GTPase activity of Sar1 and membrane interaction of COPII proteins by *in vitro* reconstitution using purified yeast proteins in combination with artificial membrane systems. Here we show the influence of individual COPII components and several protein variants on the GTPase activity of Sar1. Comparison between GTP hydrolysis and membrane interaction in presence and absence of different effectors gives useful provides on the initial steps of Sar1 recruitment and COPII vesicle formation.

P-426

N-BAR domain-induced remodeling of membranes

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Proteins of the Bin/Amphiphysin/Rvs (BAR) domain superfamily regulate membrane remodeling processes for cargo shuttling between cellular compartments. BAR domains are dimers of antiparallel helix bundles that accommodate differently shaped membranes. They assemble into regular protein arrays providing scaffolds for the generation of curved membrane domains that may subsequently undergo fission. The N-terminal BAR (N-BAR) domains have been proposed to bend membranes by insertion of amphipathic helices, representing an important factor in clathrin-mediated endocytosis (endophilin N-BAR) or generation of T-tubules in muscle cells (human N-BAR). It is an open question whether membrane remodeling and fission result from amphipathic helix insertion or whether amphipathic insertion serves as a mechanism for protein enrichment, resulting in protein crowding effects.
Our study deals with morphological aspects of the human N-BAR domain induced remodeling of membranes. As artificial model membranes we used vesicles of different size and composition. The effect of different curvatures and lipid composition on the binding of N-BAR was followed by negative staining electron microscopy. In addition, the insertion of N-BAR into lipid monolayers was studied using the film balance technique.

Abstracts

– Protein-Lipid Interactions –

P-427

Interactions of Rubber particle proteins REF and SRPP with membrane models

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The hevea (*Hevea brasiliensis*) latex contains two major proteins named Small Rubber Particle Protein (SRPP) and Rubber Elongation Factor (REF). They are both localized on the membrane of rubber particles. Genes encoding for REF and SRPP proteins have been cloned and various isoforms identified, but their real functional role has never been uncovered. We purified both proteins and discovered that REF displays aggregation properties. In a previous study, we characterized REF as an amyloid, whereas SRPP has mainly characteristics of an alpha-helical protein [Berthelot et al. (2012). *PLoS One* **7**:e48065].
In the present work, we investigated the interaction of both proteins with different membrane models. We combined various biophysical methods (PM-IRRAS/ellipsometry, ATR-FTIR, solid state NMR, PWR) to elucidate their interactions. SRPP shows less affinity than REF to the membranes but displays a kind of "covering" effect on the lipid headgroups without disturbing the membrane integrity. Contrarily, REF demonstrates higher affinity with a change in its aggregation properties. The amyloid nature of REF, which we previously reported, is not favored in presence of lipids. REF may bind and insert into the membranes.

P-428

Membrane interaction of disease-related Dynorphin A variants

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Spinocerebellar ataxia (SCA) is a family of neurological disorders characterised by a loss of muscle control, coordination and/or balance, caused by degeneration of parts of the central nervous system.
Recently, two mutations in the gene coding for the neuropeptide Dynorphin A (DynA) have been causatively linked to a form of SCA. These mutations result in two peptides, R6W-DynA and L5S-DynA, that have radically different cell toxicity properties, and also behave very differently in leakage studies, with R6W-DynA being much more potent in both these assays.
In the study presented here, we have investigated the membrane-interaction properties of the two DynA variants with circular dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopy, in a solution of isotropic bicelles.
Our results show that R6W-DynA has larger structural content than L5S-DynA, and that these tendencies are amplified in the presence of a lipid bilayer. R6W-DynA also associates stronger, and is more deeply buried in the bilayer than L5S-DynA.
Taken together, we suggest that the results presented here may partly explain the differences in cell toxicity of these disease-related neuropeptide variants.

P-429

Investigating how lipids modulate G protein coupled receptor (GPCR) function

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The molecular details of how membrane proteins work are poorly understood because of their instability out of the membrane environment, which indeed is essential for their correct function and greatly limits sample preparation for structural studies.
Ironically, the importance of the lipid influence in membrane protein activity is generally neglected. In this project we are investigating how lipids interact with the neurotensin receptor 1 (NTS1), a relevant mammalian G protein coupled receptor (GPCR). Previous studies have shown that the ability of NTS1 to bind its ligand, to oligomerise and/or to activate G proteins (which set a cascade of processes) is greatly affected by the composition of the lipid bilayer.
Here we use electron spin resonance (ESR) and spin labelled lipids to explore how lipids affect the oligomeric state of NTS1 and to determine whether some lipid species bind with greater affinity than others to the receptor. This information will be important for understanding how lipids modulate GPCR activity, which may provide new venues to design drugs that target these lipid-modulating mechanisms.
Oats and Watts, Curr Opin Struct Biol, 2011
Oats *et al.* Biochim Biophys Acta, 2012
Inagaki *et al.* J Mol Biol, 2012

P-430

Complete and reversible unfolding of an α-helical membrane protein

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The question of how an unordered polypeptide chain assumes its native conformation is one of the greatest challenges in molecular biophysics. Chemical denaturants have been used successfully for *in vitro* folding studies of soluble proteins and β-barrel membrane proteins, whereas denaturant-induced unfolding of α-helical membrane proteins is usually incomplete and irreversible. For instance, a combination of nuclear magnetic resonance and optical spectroscopy has revealed that urea is not able to abolish the secondary and tertiary structure of the α-helical membrane protein Mistic when the protein is solubilized in lauryl dimethylamine oxide micelles.
By contrast, we have found that Mistic can be unfolded completely and reversibly from micelles composed of alkyl maltosides or alkyl glucosides. As revealed by automated circular dichroism spectroscopy and techniques typically used in β-barrel membrane protein unfolding, Mistic unfolds reversibly following a two-state equilibrium that exhibits the same unfolded state irrespective of the detergent. This allows for a direct comparison of the folding energetics in different membrane-mimetic systems and contributes to our understanding of how α-helical membrane proteins fold as compared with β-barrel membrane and water-soluble proteins.

Abstracts

– Protein-Lipid Interactions –

P-431

How does the cholesterol influence the interactions of amyloid peptides with membranes?

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Alzheimer's disease and type II diabetes mellitus are characterized by the accumulation of fibrillar deposits in tissues, composed of the amyloid peptide Aβ and the islet amyloid polypeptide IAPP, respectively. It has been shown that the interaction of these peptides with membranes plays a critical role in the evolution of the diseases. The role of cholesterol, an important component of membranes, remains unclear. In this context, we investigate the effect of cholesterol on the peptide aggregational and conformational behaviour as well as amyloid peptides-membrane interactions.
The kinetics of fibrillation and the fibrils morphology were studied by ^{1}H NMR spectroscopy, circular dichroism, ThT fluorescence and electron microscopy. The results suggest that the cholesterol does not play a significant role in peptide aggregation, with comparable kinetics. Consistent with this, our TEM reveals similar fibrils morphology in the absence and in the presence of cholesterol. Currently, we are studying the barrier and binding properties of membrane using membrane leakage assays and ITC. Our preliminary results show that cholesterol slightly slows down membrane damage induced by amyloid peptides, suggesting somehow a protective effect.

P-432

Alpha-synuclein fibrillization plays essential role in membrane disruption

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α-synuclein aggregation is involved in the pathogenesis of Parkinson's disease. The prevailing hypothesis on amyloid toxicity involves membrane disruption by oligomeric species. However, isolated α-synuclein oligomers only transiently disrupt the membrane and vesicles remain intact. We studied synuclein aggregation in giant unilamellar vesicles and observed dramatic membrane disintegration and incorporation of lipids into fibrillar aggregates. Aggregation of α-synuclein in the presence of calcein filled vesicles shows synchronous leakage of dye with fibrillization. The evolving picture suggests that fibrillization and membrane disintegration during the aggregation process may be more relevant for toxicity than permeabilization by stable oligomeric species.

P-433

Alpha–synuclein–artificial lipid bilayer interaction

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Intrinsically Disordered Proteins (IDPs) are characterized by the lack of a well-defined 3D structure and show high conformational plasticity. Their structure depends tremendously on the local environment and confinement, and may accommodate several unrelated conformations. Among IDPs, α-synuclein (AS) is gathering a growing interest since the formation of aggregates of this protein in dopaminergic neurons is implicated in the development of Parkinson's Disease (PD) but its function and role is still poorly understood. Consistent with a specific role of the protein in the neurotransmission, it was observed a specific binding with neuronal membrane by fluorescent binding assays. In this context, we developed a novel method to deposit lipid bilayers with different lipids compositions on surfaces, to be used as model system to investigate AS membrane interaction. We used atomic force microscopy (AFM) in liquid physiological environment for the precise control of the lipid composition, promoting and highlighting the formation of lipid rafts, specialized microdomains particularly important in the neurotransmission and cell signalling. The role of cholesterol in lipid raft formation and the interaction between lipid rafts and AS will be discussed.

P-434

Phosphatidylcholine Membranes in Denaturing Conditions and Binding of Partially Denatured Proteins

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The interaction of partially folded proteins with lipid membranes is relevant both to important intracellular processes such as initial folding after protein synthesis as well to toxicity in protein misfolding diseases. In an attempt to better understand generic non-native conformations we have performed neutron reflectivity experiments to observe changes in the interfacial structure of phosphatidylcholine model bilayers upon incubation with protein in guanidine hydrochloride solutions. Two model proteins – bovine insulin and lysozyme have been used to investigate this behavior at the lipid-solution interface.
We have observed that both membrane phase and solution conformation play roles in determining the surface action of the protein on the bilayer. Correlation between conditions which have been previously observed to eliminate the lag phase for formation of amyloid fibrils leads us to believe that adsorption of non-native species could either play a protective role or is an initial step in membrane damage through a heterogeneous nucleation pathway.

Abstracts

– Protein-Lipid Interactions –

P-435

Interaction of the antimicrobial peptide gomesin with model membranes

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Gomesin is a potent cationic antimicrobial peptide isolated from the Brazilian spider *Acanthoscurria gomesiana*. The interaction of gomesin with vesicles composed of a 1:1 mixture of zwitterionic (POPC) and anionic (POPG) phospholipids is studied with isothermal titration calorimetry (ITC). In parallel, light scattering and optical microscopy are used to assess peptide-induced vesicle aggregation. The ability of gomesin to permeabilize the membrane is examined with fluorescence spectroscopy of the leakage of carboxyfluorescein (CF). Zeta potential measurements were performed to probe the vesicle surface potential as gomesin binds to the membrane. Vesicles coated with PE-PEG lipids are also investigated to assess the influence of peptide-induced vesicle aggregation in the activity of gomesin. A surface partition model combined with the Gouy-Chapman theory is put forward to fit the ITC results. The interaction of gomesin with anionic membranes is exothermic, enthalpy-driven and is virtually always accompanied by vesicle aggregation and CF leakage. The results point to a strong interaction of gomesin with the membrane surface, causing membrane rupture without a deep penetration into the bilayer core. Financial Support: FAPESP, INCT-FCx.

P-436

Calcium ions and anionic detergents – enigmatic mediators of phospholipase D

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Cleaving phospholipids to phosphatidic acid, the ubiquitous enzyme phospholipase D (PLD) plays a pivotal role in many cellular processes. Although PLDs from different origins share a highly conserved so-called HKD motif, which is essential for catalysis, their activity is modulated by effectors such as Ca^{2+} ions or sodium dodecylsulphate (SDS) in different ways. In this study we compare the effects of these two effectors on the activity of α-type PLD from white cabbage and of PLD from *Streptomyces* sp. In contrast to the plant PLD, the microbial enzyme lacks the Ca^{2+} -binding C2 domain. A micelle-based activity assay containing phosphatidyl-*p*-nitrophenol, SDS, and Triton X-100 (1:1:8, by mole) demonstrated that the presence of Ca^{2+} ions in millimolar range is essential for the plant PLD, whereas the activity of PLD from *Streptomyces* sp. is dramatically reduced with increasing Ca^{2+} ion concentrations. The variation of the SDS concentration also showed strong effects on both enzymes. The analysis of the micellar substrate by dynamic light scattering disclosed strong changes of the hydrodynamic radii of the micelles as the Ca^{2+} or SDS concentrations are changed. A correlation between the morphology of the micelles and the PLD activity is suggested.

P-437

Membrane protein organization and long-range lipid-protein interactions

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The lateral organization of biological membranes is of great importance for their function, with the formation of diverse structures such as functionally specialised membrane regions, super-complexes or transient signalling complexes. We find from a physico-chemical analysis that membrane mediated long range interactions are of considerable importance in the structuring of biological membrane and the formation of specific organizations. To date hydrophobic mismatch and membrane curvature effects have been highlighted as possible mediators of such long range interactions between membrane proteins. Here we investigate using molecular dynamics simulations how lipids can be perturbed by various membrane proteins and estimate the role of these perturbations in driving association. Finally we suggest that a general lipophobic effect, that implicitly incorporates both hydrophobic mismatch and curvature effects, may be of considerable importance in the folding and assembly of membrane proteins, analogous to the role of the hydrophobic effect in soluble protein folding and assembly.

P-438

Side chain protonation regulates lipid protein interactions in rhodopsin: A time-resolved FTIR study

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Membrane proteins are vital for cellular signalling. Their function originates in structural transitions of transmembrane and extramembraneous domains. The latter experience aqueous and hydrophobic solvation forces, respectively. We use time-resolved FTIR spectroscopy and static fluorescence measurements to study how the interfacial solvation balance affects protein structure. In transmembrane peptides derived from the bovine photoreceptor rhodopsin, a prototypical G protein-coupled receptor (GPCR), protonation of a conserved cytosolic site in helix 3 (E134) couples hydration to local structure by side chain partitioning at the water lipid interface [1]. Vice versa, the side chain charge affects structural changes induced within seconds by altered interfacial water potential. Opsin mutants containing amino acid replacements at position 134 exhibit similar structural responses to transient changes in water potential. Thus, the conserved carboxyl in GPCRs is a proton-controlled hydration site regulating the partial entry of water at the protein lipid interface which contributes to the free enthalpy decrease upon receptor activation.

Reference:

[1] S. Madathil et al. *J Biol Chem* **2009**, *284*, 28801-9

Abstracts

– Protein-Lipid Interactions –

P-439

Anionic lipid membrane-induced changes in GAPDH: towards "amyloid-like" fibril formation

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Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a homotetrameric enzyme nowadays revealed as a moonlighting protein, has been proposed to form "amyloid-like" fibrils in the presence of acidic membranes.[1,2] To better understand this protein-lipid interaction, a detailed study has been carried out by characterizing the protein structural and dynamical properties and possible formation of "amyloid-like" fibrils in the presence of lipid membranes. GAPDH-lipid membrane interaction presents a slow kinetics at room temperature as revealed by the changes in the intrinsic protein fluorescence properties over time and apparent fibril formation kinetics from ThT binding assays. GAPDH partition coefficients determined for POPC:POPG (3:7 and 7:3) vesicles increased with the proportion of negatively-charged lipid in the membrane. GAPDH was also labeled with two different fluorophores, Alexa488 and BODIPY-FL. Steady-state and time-resolved fluorescence measurements, as well as FRET experiments of GAPDH-fluorescent conjugates exhibited a complex behavior in the presence of lipid vesicles, reflecting the coupled oligomerization/lipid-interaction equilibria displayed by this protein.
Supported by project PTDC/QUI-BIQ/099947/2008 FCT/Portugal. [1] Zhao *et al.* **2004** *Biochemistry* 43: 10302. [2] Cortéz *et al.* **2010** *FEBS Lett* 584:625.

P-440

Membrane perturbation of erythrocyte ghosts induced by Macrovipera lebetina obtusa venom

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Introduction: Macrovipera lebetina obtusa (MLO) is the most important poisonous snake in Armenia. A specific toxin was not identified in the venom of this snake and they have no real toxins in the venom (like three-finger toxins of Elapidae) but they form complexes with other non-enzymatic proteins to achieve higher efficiency through synergy.
Ghosts are post-hemolytic residues of red blood cells. It is generally assumed that these residues are devoid of intracellular structure and consist primarily of the cell membrane. Hence ghosts are widely used in the study of composition, structure, and function of the red blood cell membrane.
Method: *Erythrocyte ghosts formation* Human blood was collected in sterile heparinized cannula from healthy female volunteers between 25-35 years of age. Erythrocyte membranes were obtained by the method of Dodge, Mitchell and Hanahan. The erythrocyte ghosts were visualized with ANS fluorescent probe. Images were collected on an epi-fluorescent microscope FM320-5M.
Results: The erythrocyte ghosts were deformed after adding the MLO venom. They shrink within 15-30 seconds, and pull in. This work was done to compare these results with the results obtained after the interaction of the venom with phospholipid vesicles (GUVs) which is absolutely different.

P-441

The segregation of phosphatidylserine by the C2 domain of PKCα is enhanced by the presence of PIP2

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PKCα is a classical isoenzyme that is activated by second messengers, namely the increase in Ca^{2+} concentration in the cytoplasm of the cell and the appearance of diacylglycerol in the membrane. The translocation of classical PKCs (cPKCs) to the plasma membrane is mediated by the C1 and C2 domains. It has been shown that the C2 domain of PKCα binds to the membrane through two sites one specific for Ca^{2+} which acts a bridge with anionic phospholipids (with preference for phosphadidylserine) and by another site specific for PIP_2. We have found that the C2 domain of PKCα induces fluorescence self-quenching of NBD-PS in the presence of Ca^{2+}, and especially when PIP_2 also present, which is interpreted as the demixing of phosphatidylserine and PIP_2 from a mixture of this phospholipid with phosphatidylcholine. An independent proof of the phosphatidylserine demixing effect given by the C2 domain was obtained by using ^{2}H-NMR, which led to a shift of the transition temperature of deuterated phosphatidylcholine as a consequence of the addition of the C2 domain, especially again in the presence of PIP_2. The formation of these domains may explain, at least partially, the well known activation of the enzyme when it binds to membranes containing phosphatidylserine and PIP_2.

P-442

Interactions of short cationic peptides with bilayer membranes

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Antimicrobial peptides and cell penetrating peptides often contain basic amino acids like lysine and arginine. Due to their positive net charge they can selectively interact with bacterial membranes. The initial interaction of these peptides with membranes is influenced by their charge and hydrophobicity.
To study the interaction of cationic peptides with model membranes of phosphatidylglycerols (PG), oligomers containing 5 positively charged lysines or arginines were used. The length, hydrophobicity and charge distribution of the peptides were altered due to insertion of spacers of uncharged amino acids, e.g. glycine, alanine and 4-aminobytyric acid. Differential Scanning Calorimetry was used to investigate the influence of the peptides on the phase transition of DPPG vesicles. We found that the shift of the phase transition temperature depends on the peptide concentration, and that peptides with amino acids with a more hydrophobic side shifts the phase transition to higher temperature. Temperature dependent ATR-IR measurements reveal for some peptides bound to DPPG membranes a transition from aggregated β-strands to unordered structures upon heating.

Abstracts

– Protein-Lipid Interactions –

P-443

Collective insertion behavior of influenza fusion peptides in model membranes

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Influenza haemagglutinin (HA) is responsible for binding to cells and the fusion of the viral and endosomal membranes. The HA peptide consists of three identical subunits, which contain two polypeptide chains (HA1 and HA2). The N-terminal of the HA2 subunit contains the 20 amino acid Fusion Peptide (FP). During the viral infection process, a decrease in the local pH level causes an extensive conformational rearrangement of each HA0 that reveals the individual FP, which is then inserted in the membrane of the healthy cell.
The mechanism of the insertion of the FP into the membrane of healthy cells and the structure of the peptide once it is inserted has been the focus of a significant amount of experimental and simulation studies. In this presentation, I will present the results of some recent work in which we have studied the insertion of the FP into model lipid membranes using coarse-grain molecular dynamics simulations. Additionally, I will present our model of a trimer of FP. We have used this trimer model to study the collective insertion of numerous trimers and then determining the peptide-peptide and peptide-lipid interactions that govern this insertion as well as the structural changes in the lipid membranes that occur as a result of this insertion.

P-444

Aβ variants'interaction with membranes

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Interactions between amyloid-β peptides and biological membranes are a key point in understanding Alzeimer disease. Previous studies have linked the toxic character of amyloids and their ability to interact with membranes [Berthelot et al., Biochimie, 95 (2013) 12-19]. Aβ's mutants were generated in earlier study, and their toxicity in yeast investigated [D'Angelo et al., Dis Model Mech. 6(1) (2013) 206–216]. Correlation between toxicity and peptides secondary structure was demonstrated using ATR-FTIR spectroscopy. In this study, amyloid-β peptide single mutants with variable toxicity were tested for their capacity to interact with membrane models. We performed calcein leakage experiments and plasmon waveguide resonance (PWR) to evaluate the effect of the peptides on the integrity of various membranes (phospholipids PC and PG). ATR-FTIR spectroscopy was used to determine the structure of amyloids interacting with membranes. All results converge toward the idea that the most toxic peptides characterized by an antiparallel β-sheet structure have a strongest interaction with membranes than other less toxic peptides, this interaction being specific of the lipid composition.

P-445

Studies on interactions of cecropin B with model lipid membrane composed of DMPC

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The increasing resistance of bacteria and pathogens on antibiotics becomes a substantial therapeutic problem and leads to urgent demands for new substances with a broad spectrum of activity. Cecropins, a group of antimicrobial peptides, were found in the innate immune system of insects and mammals and exhibit a strong antimicrobial activity on both Gram-positive and Gram-negative bacteria with no lytic action on eukaryotic cells. Cecropin B composed of 35 amino acid residues exerts the highest antibacterial activity among all cecropins and it can also lyse cancer cells.
To find out more about the details of cecropin B–lipid interactions we examined model lipid membrane composed of 1,2-mistyroyl-*sn*-glycero-3-phosphoholine (DMPC), one of the main components of mammalian membranes. We verified the action of cecropin B on DMPC membrane at the air-water interface using Langmuir trough. Lipid bilayer was transferred on solid support using the combination of Langmuir-Blodgett and Langmuir-Schaefer techniques and the peptide-lipid interactions were studied by means of Electrochemical Impedance Spectroscopy and Atomic Force Microscopy. Experimental results were supported by molecular modelling that enabled determination of detailed structure of cecropin B and its interfacial behaviour.

P-446

Biophysical studies of a new toxic peptide from Helicobacter pylori IsoA1/AapA1 genomic cluster

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Toxin-Antitoxin (TA) systems are present in almost all bacterial chromosomes; these regulatory systems are composed by two genes coding for a toxin and an antitoxin molecules. Among the TA systems, the type I system is characterized by a peptidic toxin which translation is inhibited by a non-coding sRNA.
Following the transcriptome of *Helicobacter pylori*, it was possible to establish that this human pathogen presents a major regulation at transcriptomic level, leading to the discovery of a new TA type I system; the AapA1/IsoA1. The AapA1 gene codes for hydrophobic peptide, which encompasses a predicted α-helix motif between TRP10 and LEU28. It also possesses a global positive charge of +7: MAT**K**(**H**)G**K**NSW**K**TLYL**K**ISFLGC**K**VVVLL**KR**. In this study we present the structure of A1 peptide obtained from NMR spectroscopy. Biophysical studies allow us to determine its membrane localization on *H. pylori*. We found out that PepA1 induces high rates of *H. pylori* cell mortality and erythrocyte cellular lysis. Additionally we tested the affinity of PepA1 for different membrane models using Plasmon Waveguide Resonance spectroscopy, evidencing a strong affinity in the order of 25 nM.

Abstracts

– Protein-Lipid Interactions –

P-447

Cytoskeletal interaction forces characterized by means of atomic force microscopy

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The cortical cytoskeleton is an essential mechanical component within cells. It regulates fundamental processes including cellular division, motility and shape. In order to investigate the basic mechanical behavior of filamentous actin (F-actin) a model system has to be established. The membrane attachment of F-actin is mimicked preparing pore spanning membranes (PSMs) containing a F-actin receptor lipid. Subsequently we are able to bind an overlying F-actin network to the PSMs. Either F-actin was bound via electrostatic interaction to the PSM or via the physiological linker protein ezrin to a PIP_2 containing PSM. These different immobilization strategies provide a versatile strategy to look at cytoskeletal attachment. Elucidating the mechanical properties of these distinct systems indentation experiments are performed by atomic force microscopy (AFM). This characterization leads to specific lateral tensions of the PSMs referring to F-actin covered and non-covered membranes. We also plan to investigate whether F-actin bundling proteins have an influence on the mechanical behavior of the PSMs. Furthermore we will analyze the viscoelastic behavior of PSMs induced by F-actin binding.

P-448

Interaction of the GTPase Sar1 with Artificial Membranes

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The transport of cargo molecules from the Endoplasmic Reticulum to the Golgi apparatus of the cell is mediated by COPII-coated vesicles. COPII recruitment starts with the binding of Sar1. Upon activation by GTP, Sar1p inserts an amphipathic helix into the membrane, presumably leading to an expansion of the proximal membrane leaflet and a curvature change of the bilayer.
We examine the functional and morphological aspects of the membrane interaction of Sar1p by *in vitro* reconstitution using purified yeast proteins in combination with liposomes as artificial bilayers. The change in curvature by the insertion of Sar1 amphipathic helix in the bilayer of GUVs is evident from confocal microscopy. Qualitative biochemical assays show that Sar1 also binds to small vesicles of the size that is adopted by COPII coated vesicles *in vivo* and *in vitro*. We are examining this binding of Sar1 to SUVs in a more quantitative manner by dual color fluorescence cross-correlation spectroscopy.
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P-449

A 3-step model for the lipid efflux induced by seminal plasma protein A1 from sperm membranes

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The bovine seminal plasma contains phosphocholine-binding proteins, which associate to sperm membranes upon ejaculation. These Binder-of-Sperm (BSP) proteins then induce a phospholipid and cholesterol efflux from these membranes. We determined physical and chemical parameters controlling this efflux by characterizing the lipid extraction induced by BSP1, the most abundant of BSP proteins in bull seminal plasma, from model membranes with different composition. The modulation of BSP1-induced lipid extraction from membranes by their chemical composition and their physical properties brings us to propose a 3-step extraction mechanism. First, the protein associates with membranes via specific binding to phosphocholine groups. Second, BSP1 penetrates in the membrane, essentially in the external lipid leaflet. Third, BSP1 molecules solubilize a lipid patch coming essentially from the outer lipid leaflet, without any lipid specificity, to ultimately form small lipid/protein auto-assemblies. The stoichiometry of these complexes corresponds to 10-15 lipids per protein. These findings contribute to our understanding of the mechanism by which BSP1 modify the lipid composition of sperm membranes, a key event in sperm capacitation.

P-450

In vitro investigation on the interaction of human islet amyloid polypeptide with model membranes

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Aggregation of islet amyloid polypeptide (IAPP) in human pancreatic islets is involved in the pathogenesis of type-II diabetes. Membranes are likely to play an important role in the toxic effect of IAPP in vivo, because fibril formation is catalyzed by membranes and the formation of IAPP fibrils, or its oligomeric intermediates can result in membrane permeabilization. Therefore, finding an effective anti-amyloid compound that can inhibit IAPP fibrillogenesis or its interaction with the membranes would be a vital strategy to fight type-II diabetes. Here, we investigated the IAPP-membrane interaction using full length (37 amino acids) IAPP as well as fragments corresponding to N-terminal part of IAPP (1-19), that interacts efficiently with membranes [1] and the amyloid forming region of IAPP (20-29). We used synthetic model membrane systems to test the interaction between IAPP and membrane lipids to analyze how these interactions are affected by various anti-amyloid compounds. In particular, we used (i) ThT fluorescence and EM to monitor fibril formation in the absence and presence of membranes, (ii) monolayer studies to monitor peptide insertion, and (iii) calcein leakage assays to check the effects of peptides on vesicle integrity.
[1]Lucie et al., (2010) BB Acta: 1805-11

Abstracts

– Protein-Lipid Interactions –

P-451

Structure and binding to Phosphorylated Phosphoinositides of the RGD1-RhoGAP domain of S.cerevisiae

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Lipid binding to proteins is driven by hydrophobic and electrostatic interactions, mediated through their acyl chains and headgroups. Among lipids, Phosphorylated Phospho-Inositides (PIP) regulate essential biological processes at the membrane-cytosol interface, such as membrane dynamics in cell division. We focus our attention on the Rgd1 protein involved in polarized growth and mitosis in yeast. PIPs not only bind to Rgd1p, but also regulate its activity and cellular distribution. Rgd1p is composed of a F-BAR domain and a Rho GTPase activating protein (RhoGAP). To understand the effect of PIPs on Rgd1p, a solution NMR study has been performed on its RhoGAP domain. A complete resonances and secondary structure assignment has been achieved on the $^{15}N^{13}C$ labeled protein. The 3D structure has been elucidated with residual dipolar couplings and NOEs. Titration studies have been performed.with $PI(4,5)P_2$. The binding site involves a non conserved region of the RhoGAP family. A solid state ^{2}H and ^{31}P NMR study has been performed for deciphering the protein effect on membrane dynamics. This NMR study of the RhoGAP domain should allow us to understand at the residue level the regulation of the Rgd1p activity by PIP.

P-452

Tracking membrane-driven protein oligomerization using fluorescence lifetime and homo-FRET studies

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It has been proposed that anionic lipid membranes can promote "amyloid-like" fiber formation of several non-amyloidogenic proteins, *e.g.* lysozyme (Lz) [1]. To obtain information about the factors that govern the formation of these structures, Lz interaction with anionic lipid vesicles was studied using both steady-state and time-resolved fluorescence techniques. The biphasic variation of the mean fluorescence lifetime of Lz fluorescently-labeled with Alexa 488 (Lz-A488) as a function of the surface coverage of the liposomes was quantitatively described by a three-state model. This cooperative model assumes that monomeric Lz molecules partition into the bilayer surface and reversibly assemble into oligomers with k subunits ($k \geq 6$) [2]. The global fit to the data was done using the partition coefficients previously determined by FCS [3] and by taking into account electrostatic effects by means of the Gouy-Chapman theory. Finally, the oligomer stoichiometry was further narrowed down to $k = 6 \pm 1$ by homo-FRET measurements, which takes into account the binomial distribution of fluorescently-labeled monomers among the oligomers. Supported by project PTDC/QUI-BIQ/099947/2008 FCT/Portugal.
[1] Zhao et al. **2004** *Biochemistry* 43: 10302
[2] Melo et al. **2013** *J. Phys. Chem. B* 117*:* 2906
[3] Melo et al. **2011** *Biochim. Biophys. Acta* 1808: 2559

P-453

Biophysical features of electrostatically-driven lipid-protein fibers

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Anionic lipid membranes have been proposed to trigger "amyloid-like" fiber formation of several non-amyloidogenic proteins, *e.g.* lysozyme.[1] Here, the structural properties of the mixed lipid-protein fibers formed upon lysozyme interaction with POPC:POPS 80:20 LUVs at a low L/P molar ratio were studied in detail. Using complementary time-resolved FRET measurements, at the single-fiber (FLIM-FRET) and macroscopic (bulk) level, it is shown that these fibers display a multilayer structure, in which predominantly oligomeric lysozyme[2] is sandwiched between adjacent lipid bilayers. Additionally, FRAP measurements showed that both lipids and lysozyme display a slow lateral diffusion in these mesoscopic structures, due to extensive membrane surface crowding[2] and/or protein confinement between cross-bridged bilayers. Furthermore, 2PE Laurdan generalized polarization revealed that the formation of these fibers is accompanied by extensive membrane surface dehydration. Finally, IR measurements support that anionic lipid membranes cannot generically trigger "amyloid-like" fiber formation of lysozyme, since these supramolecular assemblies do not exhibit a rich β-sheet structure. Supported by FCT.
1. Zhao *et al.*, *Biochemistry*, **2004,** *43*, 10302.
2. Melo *et al. J. Phys. Chem. B*, **2013**, *117,* 2906.

P-454

Cholesterol effects on stability and intracellular processing of melanosomal membrane proteins

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Malignant melanoma is one of the most insidious types of cancer. Melanosomal proteins members of the TRP-family are enzymes modulating the amount / quality of melanin. TRPs are also melanoma markers and immunological targets, so understanding their structure and function is crucial for their use in diagnosis and therapy. TRPs are type I membrane proteins, with an N-terminal catalytic domain in the lumenal side of the membrane, followed by a transmembrane (TM) segment and a C-terminal cytosolic short domain. Despite high sequence similarity, TRPs display different processing and trafficking along the secretory pathway and different responses to cholesterol-blocking agents, as shown by *in vitro* assays. To understand the structural basis of differences in interaction with membranes, we use molecular dynamics simulations of TM segments embedded in lipid bilayers, in the presence and absence of cholesterol. Our study is one of the few simulation studies on the importance of cholesterol for TM type I protein stability and trafficking. We discuss simulation data in correlation with experimental results and the possible impact on melanoma progression and therapy.
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Abstracts

– Protein-Lipid Interactions –

P-455

Phospholipids regulate the voltage-dependence and selectivity of plant VDAC

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VDAC channel constitutes the major transmembrane protein of the mitochondrial outer membrane, and is a key element in the regulation of solute exchange between mitochondria and cytoplasm. We recently showed that sterol-VDAC interactions significantly affect the function of VDAC purified from bean seeds (Phaseolus coccineus)[1, 2]. The present study further explores the lipid-VDAC interactions with a special emphasis on the role of phospholipid headgroup. VDAC was reconstituted in a planar lipid bilayer formed from either DOPC, DOPE or a DOPE/DOPC mixture. The main findings of this study are: (a) DOPE is essential for the channel gating at salt concentration prevailing *in vivo* and the voltage-dependence is inhibited when DOPE is replaced by DOPC (b) a 2% DOPE/DOPC mole fraction restore the VDAC gating, (c) VDAC selectivity but neither the single channel conductance nor the gating parameters is modulated by the degree of methylation of DOPE. Residues that form specific interactions with POPE but not POPC and therefore alter the ion selectivity depending on the lipid vicinity around VDAC were identified using comparative modeling and molecular dynamics.

[1] Mlayeh L, Chatkaew S, Léonetti M, Homblé F (2010) Biophys J **89**: 2097 [2] Homblé F, Krammer E-M, Prévost M (2012) BBA **1818**: 1486

P-456

Investigation of VapA, a pore forming tool of Rhodococcus equi to survive inside the phagosome

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Phagosomes arise in the course of phagocytosis of pathogens by professional phagocytes. The phagosome maturation, a series of 'kiss-and-run' processes with lysosomes and endosomes leads to the formation of phagolysosomes and finally to the destruction of the ingested pathogens. *Rhodococcus equi* is part of a group of bacteria that have developed strategies to survive within phagosomes – for *R. equi* by arresting the phagosome maturation in an early, prephagolysosomal state. Thereby the prevention of fusion processes and of the uptake of the proton pumping v-ATPase into the phagosomal membrane and a changed permeability of the phagosomal membrane are caused by the virulence-associated protein A (VapA) which is expressed in the membrane of *R. equi* [1]. This study focusses on the interaction between VapA and membrane systems reconstituted with different phospholipid mixtures. Different permeabilisation processes of processed and unprocessed forms of VapA were compared with electrophysiological measurements on freestanding membranes. Fluorescence spectroscopic measurements could reveal a lipid specificity of the interaction processes which were imaged and further analyzed by atomic force microscopy.

References:

[1] Giguere, S. et al., Infection and Immunity 67, no. 7, 3548-3557, 1999

P-457

Hemolysis of erythrocytes induced by Lys-substituted derivatives of gramicidin A

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Polycationic gramicidin A (gA) derivatives were shown to form unselective pores in lipid membranes and facilitate protein translocation into eukaryotic cells, regardless of the position of the polycationic cluster in the amino acid sequence (Stoilova et al. 2008 Biochim.Biophys.Acta 1778:2026). The present work demonstrated that introduction of a charged group near the N-terminus of gA altered its ability to disturb barrier properties of red blood cell membranes. The hemolytic activity of the Lys-substituted peptides essentially depended on the lysine position in the gA sequence: [Lys2]gA < [Lys5]gA < [Lys1]gA < [Lys3]gA. The Glu-substituted gA derivatives did not cause hemolysis. According to the data on osmotic protection, one can suggest that the formed pores were heterogeneous in size, as partial protection was attained with sucrose and raffinose, while nearly complete protection was exerted by PEG2000 and PEG4000 having larger molecular diameter. The osmotic protection was more pronounced at lower peptide concentrations suggesting that the size of the peptide-induced pores depended on the concentration of the peptide. The hemolytic efficacy of the Lys-substituted peptides correlated with their ability to induce leakage of carboxyfluorescein from liposomes.

P-458

Brucella membrane lipids and their role in antimicrobial peptide resistance

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Interactions between antimicrobial peptides (AMP) and bacterial membranes can be analysed by using reconstituted lipid membranes. Investigations with purified total lipids are limited and the activity of AMPs on Gram-negative membranes has been studied on few genus of the γ-*Proteobacteria* (*Proteus*, *Salmonella*, *E.coli*), that are quite sensitive. The brucellae are α-*Proteobacteria* that cause brucellosis, an important zoonosis. They are imperfectly detected by the innate immune system. The outer membrane of brucellae is of critical importance in this strategy. In addition to the lipopolysaccharide, phosphatidylcholine, aminolipids and long acyl chains are factors underlining AMP resistance. Fluorescence Resonance Energy Transfer Spectroscopy showed that more peptide could intercalate into *Brucella* reconstituted membrane, compared with the sensitive strains. Differences in fluidity and phase transition temperature (Tc) were observed, even without peptide addition. In contrast to *Proteus*, *Salmonella* and *E.coli* that had a Tc around 35°C, that of *Brucella* was around 25°C. Since *Brucella* membrane presented particularities and different behaviour, it can be a model of intrinsic resistance that has not been explored thus far, being applicable to other pathogens.

Abstracts

– Protein-Lipid Interactions –

P-459

Spectroscopic studies of the antimicrobial activity of PuroindolineB from wheat

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PuroindolineB (PinB) is a soluble antimicrobial protein which occurs naturally in wheat endosperm, thought to play an important role in seed defence against bacterial and fungal pathogens. The stability and antimicrobial properties of the puroindoline family have identified them as potential antibiotic compounds. PinB binds to anionic model membranes via a positively charged loop, which penetrates into the interface region of phospholipid bilayers. The effect of PinB on lipid dynamics has been investigated by solid state NMR and EPR spectroscopy and a naturally occurring mutation in the tryptophan-rich loop region (W to R) leads to an altered lipid interaction of PinB with lipids. Deuterium solid state NMR of head group deuterated phosphoglycerol (POPG-d2) shows differences in the quadrupolar splittings, which directly relate to head group dynamics upon binding of PinB. EPR studies on membrane packing (TEMPO partitioning) and dynamics (SASL spin labels) of PinB containing DPPG:DPPE membranes suggest that both mutant and wild type PinB disrupt the packing of the lipid head group region in gel phase lipids. Differences in the depth of membrane penetration of the wild type and mutant version of PinB are likely to impact on the potential mode of action of the proteins in vivo.

P-460

Scramblase 1: cholesterol affinity of the transmembrane domain and activity modulation by ceramide

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Human Phospholipid Scramblase 1 (hPLSCR1) is an endofacial plasma membrane protein whose function was discovered in processes of apoptosis. It can exist in a palmitoylated form in plasma membrane caveolae-like lipid rafts, and in a non-acylated form in the nucleus, where it will play the role of a transcription factor. We have worked with both the recombinant protein and peptides derived from the transmembrane domain (TMD) to examine insertion and affinity for specific lipid mixtures in model membranes. We have found a high affinity for lipid compositions giving rise to liquid ordered (lo) domains. The possible attraction of TMD for cholesterol has also been explored. The human scramblase family contains a CRAC domain sequence ([L/V]-[X](1-5)-[Y]-[X](1-5)-[R/K]) at the C-terminus of the TMD, but in the first member of the family the consensus sequence is not fully conserved. We aimed at determining the exact affinity of scramblase for cholesterol to better understand the implication of palmitoylation in the scramblase trafficking to cholesterol-rich domains, and the role of its TMD in the accommodation in this ordered region, a presumed affinity inherited with the rest of hPLSCR family members. We also intended to clarify the ceramide modulation of the protein activity.

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The effect of membrane lipid composition on interaction of tryptophan rich plant defense proteins

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Increased pathological resistance to conventional antibiotics has led to an interest in the use of antimicrobial cationic peptides as a novel family of antibiotics. Their selectivity towards different cells is as a result of differences in lipid membrane composition, charge and fluidity. In *E.coli* the zwitterionic headgroup Phosphatidylethanolamine (PE) dominates the lipid composition of the membrane however there is also a large amount of the anionic headgroup Phosphatidyl-*rac*-Glycerol (PG).

The aim of this research has been to investigate the interaction between cationic tryptophan-rich proteins and model lipid membranes, of varying polar headgroup ratio and fluidity related to the *E.coli* inner membrane. This process has enabled us to identify the role of each lipid constituent in controlling protein binding. We have studied wild-type Puroindoline-b and a mutant Puroindoline-b that features a Trp to Arg point mutation within the lipid-binding domain. Our results show that this point mutation alters the cell membrane selectivity of the protein and mode of action. To this end we have used the lipid monolayer model in conjunction with biophysical techniques to investigate protein penetration, adsorption and changes to the lateral lipid structure.

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Lipid binding characteristics of collybistin

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Collybistin is a brain-specific guanine nucleotide exchange factor (GEF) out of the Dbl protein family and is located at the postsynaptic membrane. The protein contains a Dbl homology domain (DH) followed by a Pleckstrin homology domain (PH) at the C-terminal end. In addition there is a SRC homology 3 domain (SH3) at the N-terminus. The PH domain is known to be an important regulation factor for the cellular localization of the protein by binding to membrane phosphoinositides.

Phosphorylated phosphoinositoles (PIP, also called phosphoinositides) play a role in membrane trafficking and lipid signaling. They are located at different cellular compartments. In this study the binding of collybistin to different PIPs is analyzed with the full length protein and an isoform lacking the N-terminal SH3 domain to determine the influence of the proposed SH3/PH domain interaction.

To quantify the protein-PIP interaction, we generated PIP-containing hybrid membranes on a hydrophobically functionalized gold surface. By means of the surface plasmon resonance (SPR) technique, the specific interaction of different membrane confined PIPs and collybistin is monitored as a function of protein concentration in a time resolved and label free manner.

Abstracts

– Protein-Lipid Interactions –

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Interaction of the antimicrobial peptide Esculentin 1b (1-18) with model membranes

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Esculentin 1b (1-18) [Esc(1-18)] is a synthetic peptide with the first 18 amino acids from Esculentin 1b and showed antimicrobial activity. The most common mode of action of antimicrobial peptides (AMPs) is the formation of a pore in the membrane of the microorganism, making it permeable. Our focus is to understand the mode of action of [Esc(1-18)] against liposomes composed of POPC/POPG in different molar ratios. For this purpose, several techniques were employed: fluorescence measurements of the leakage of carboxyfluorescein (CF) entrapped in vesicles, isothermal titration calorimetry (ITC), and optical microscopy of giant unilamellar vesicles (GUVs). The fluorescence studies showed that the ability of [Esc(1-18)] to promote CF leakage from vesicles increases with the POPG molar ratio. Moreover, the kinetics rate of leakage is rather slow and dependent on the molar ratio of the negatively charged lipid. The interaction of this peptide with POPG membranes gives rise to endothermic peaks at 25°C, with $\Delta H \cong 0.6$ kcal/mol of peptide that suggests pore formation. Optical microscopy showed that [Esc(1-18)] induces permeabilization of the membrane of GUVs composed of pure POPG and 1:1 POPC/POPG. Financial support: FAPESP, CNPq and INCT-FCx.

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Alpha-synuclein oligomers distinctively permeabilize model plasma and mitochondrial membranes

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Alpha-synuclein (aS) oligomers are increasingly considered to be responsible for the death of dopaminergic neurons in Parkinson's disease (PD). The toxicity mechanism of aS oligomers likely involves membrane permeabilization. Even though it is well-established that aS oligomers bind and permeabilize vesicles composed of negatively charged lipids, little attention has been given to the interaction of oligomers with bilayers of physiologically relevant lipid compositions. We demonstrate that aS binds to bilayers composed of lipid mixtures that mimic those of plasma and mitochondrial membranes. Circular dichroism experiments indicate that binding induces conformational changes in both oligomeric and monomeric aS. The membrane leakage that results from oligomer binding to physiologically relevant membranes differs from that observed for simple artificial model bilayers. Instead of inducing fast content release, oligomer binding increases the permeability of artificial mitochondrial membranes resulting in a slow loss of content. Oligomers are not able to induce leakage in artificial plasma membranes even after long-term incubation. The results suggest that the mitochondrial membrane is the most likely target of oligomer-induced damage in PD.

P-465

Activation of ezrin: synergism of PIP2 interaction and phosphorylation

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Ezrin is a plasma membrane-cytoskeleton linker that binds F-actin if it is switched from an inactive 'dormant' conformation to an active form. Several means of regulation of ezrin's F-actin binding activity have been described including phosphorylation of threonine-567 and binding of PIP_2. The relative contributions of these events towards activation of the protein and their potential interdependence are still under investigation. In this presentation, I will discuss the contributions of PIP_2 binding and phosphorylation of ezrin on its ability to bind F-actin using two in vitro assays. I) Fluorescence microscopy on ezrin-decorated solid supported membranes showed that, dependent on the PIP_2 binding and the 'phosphorylation' state, the ezrin capability of binding actin filaments differs. II) By means of colloidal probe technique, the apparent maximal adhesion force and the work of adhesion of the ezrin-F-actin interface was unraveled. Interactions of different ezrin mutants with surface immobilized F-actin were investigated by an AFM in the absence and presence of PIP_2. The results of both assays reveal that there is a synergism between 'phosphorylation' and PIP_2 binding leading to the conformational switch from the dormant to the active, F-actin binding state.

P-466

Membrane binding regulates an intramolecular interaction of the Spir-2 actin nucleator

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Polymerization of actin monomers into filaments is initiated by actin nucleation factors. Interaction of mammalian Spir-2 and mammalian formin Fmn2 was shown. This interaction is mediated by the N-terminal KIND domain of Spir and the Formin-Spir-Interaction motif (FSI) of Fmn-2. A C-terminal FYVE domain allows targeting of Spir to vesicular membranes, as seen on Giant Unilamellar Vesicles (GUVs). Spir solely binds to negatively charged GUVs, but without any obvious preference for a distinct lipid. Intriguingly, we uncovered that the FYVE domain does not only interact as a membrane binding motif, but also as a protein-protein-interaction module. Using Fluorescence cross-correlation spectroscopy, we revealed a hitherto unknown intramolecular interaction of the Spir N-terminal KIND domain and the C-terminal FYVE domain that is released upon binding of the FYVE domain to membranes. Competition experiments revealed overlapping interfaces of the intramolecular Spir FYVE:KIND-complex and the trans-regulatory Fmn-FSI:Spir-KIND complex. We propose a model in which the autoregulated interaction of the FYVE:KIND-domain is released upon membrane binding and allows binding of the Fmn-FSI to Spir-KIND, thereby establishing the functional Fmn:Spir actin nucleator complex at the membrane.

Abstracts

– Protein-Lipid Interactions –

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Protein-membrane interaction: molecular dynamics simulation of ASIC1 in lipid bilayer

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The acid-sensing ion channel 1 (ASIC1) is a neuronal cationic ion channel activated by protons. It comprises of a 6 α-helices transmembrane (TM) region and a large extracellular domain (ECL) with a complex architecture. The channel pore spans along its 3-fold axis of symmetry, but other ion passage pathways are available, like trough the three fenestrations found at the interface between the membrane and extracellular medium. Considering the last hypothesis, we performed a computational study of lipid dynamics at the interface with ASIC1. 100 ns molecular dynamics (MD) simulations with periodic boundary conditions were performed on ASIC1 embedded in a 200x200 Å DPPC bilayer consisting of 1220 lipid molecules. To avoid artifact effects due to the interaction of the membrane with image ECLs, the system was hydrated with 196634 water molecules. Control simulations were performed on only the TM region of ASIC1 in a similar membrane and on a plain DPPC bilayer. The membrane becomes considerably thinner near the TM region of ASIC1 (∼21Å). We identified 194 DPPC molecules which strongly interact with ASIC1 during the entire simulation. 111 of these are located on the extracellular side of the bilayer, emphasizing the influence of the ECL on the membrane.

P-468

Voltage- and calcium-dependent translocation of the CyaA toxin across a tethered lipid bilayer

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We report the design of novel biomimetic membrane model and its use to characterize *in vitro* the translocation process of bacterial toxin, the adenylate cyclase (CyaA) from *Bordetella pertussis*. The membrane was assembled over a calmodulin (CaM) layer and exhibits the fundamental characteristics of a biological membrane separating two *cis* and *trans* compartments. SPR was used to monitor the membrane interaction of the CyaA toxin, while the activation of the catalytic activity of CyaA by the tethered CaM was used as a probe of its translocation across the bilayer. Translocation of the CyaA catalytic domain was found to be strictly dependent upon the presence of calcium, and upon application of a negative trans-membrane potential, in good agreement with prior studies done on eukaryotic cells. These results demonstrate that CyaA does not require any eukaryotic components to translocate across a membrane, and suggest that CyaA is electrophoretically transported across the bilayer by the transmembrane electrical field. To our knowledge, this work constitutes the first *in vitro* demonstration of protein translocation across a tethered lipid bilayer. This biomimetic assembly opens new opportunities to explore the molecular mechanisms of protein translocation across biological membranes.

P-469

Investigation on membrane interactions and the structure of ghrelin

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Ghrelin is a stomach-derived 28 amino acid peptide with pivotal functions in energy homeostasis and growth by activating the growth hormone secretagogue receptor 1a (GHS-R1a). The structure of ghrelin is highly conserved. Posttranslational octanoylation at the Ser3 is obligatory for full activity. The peptide is poorly structured in solution but shows helix formation in the presence of TFE and SDS. Since ghrelin addresses a transmembrane GPCR, we aim to characterize the structural and dynamical properties of the peptide backbone as well as the octanoyl moiety bound to lipid vesicles. We synthesized ghrelin peptides with varying $^{13}C/^{15}N$ labeled amino acids covering 16 out of 28 residues and a peptide with a perdeuterated octanoyl chain. We have studied the membrane integration of the lipid modification of ghrelin by recording ^{2}H NMR spectra of vesicles containing perdeuterated DMPC and with and without associated ghrelin and a spectrum of the perdeuterated octanoyl chain in the presence of the membrane. ^{13}C NMR spectra under magic angle spinning conditions and measurements of the motional averaged dipolar couplings allowed the determination of backbone torsion angles and molecular dynamics, indicating a highly flexible confirmation of the membrane-attached N-terminus.

P-470

Glycolipid clustering and lipid reorganization induced by bacterial toxin binding to model membranes

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It is well known that lipid membrane properties change as a function of composition and phase state, and that protein-lipid interactions can induce changes in the membrane's properties and biochemical response. Here, grazing incidence x-ray diffraction (GIXD) was used to investigate molecular level changes in lipid organization induced by toxin binding to receptors in a lipid monolayer. Specific and multivalent binding of either cholera or shiga toxins to their respective receptors (GM1 and Gb3) served to cluster the glycosphingolipid components within model membranes. The resulting perturbations to lipid order were studied as a function of membrane composition and surface pressure and compared between the two toxins. As an example, at low monolayer surface pressures cholera binding perturbed the lipid order such that the molecules were no longer close packed, creating topological defects and lipid-protein domains with orientational texture. Cell surface micro-domains exhibiting this type of lipid order may promote membrane bending and serve as nucleation sites for tubule formation in clathrin independent endocytosis of toxins.

Abstracts

– Protein-Lipid Interactions –

P-471

Interactions of elastin-like polypeptides with model membranes on GUVs

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Abstract :
We evaluate in giant vesicle systems the lipid bilayer permeability to a new class of elastin-like polypeptides designed to penetrate the membrane of living cells upon a temperature trigger. Membrane translocation by the peptides is investigated as a function of penetrating amino acid content both in the presence and absence of peptide self-assembly.
We found not only that self-assembled bilayers of DOPC are impermeable to ELPs, but also that they are good substrates to selectively bind the peptides according to their molecular structure or self-assembled state. Our results point to a subtle solution of the passive/active penetration controversy: By passively binding the appropriate peptides, the membrane matrix is able to control the effective number of molecules that can be actively taken up by the cellular mechanisms.

P-472

Probing the interactions between a glycosyltransferase alMGS and lipids by phosphorus-31 NMR

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We have studied the interaction between a glycosyltransferase *al*MGS and lipids by using ^{31}P NMR. *Al*MGS is a monotopic glycosyltransferase from *Acholeplasma laidlawii*, synthesizing the nonbilayer-prone glycolipid monoglucosyldiacylglycerol. Previous studies have shown that *al*MGS is sensitive to lipid head-group charge and curvature stress. To explore the interaction between the enzyme and lipids, we used bicelle systems made up of long-chained lipids purified from *E.coli* cells and dihexanoylphosphatidylcholine (DHPC). The ^{31}P NMR spectra of bicelles after adding *al*MGS show that peaks for all the lipid phosphates shift and become broader. In addition, the phosphatidylglycerol (PG) and cardiolipin (CL) peaks shift more than phosphatidylethanolamine (PE), indicating a preferential interaction. ^{31}P spin-lattace relaxation rates for each lipid phosphate in the absence and the presence of *al*MGS show no evident differences. However, the spin-spin relaxation rates increase after addition of *al*MGS. The results suggest that *al*MGS can bind to bicelle and that it interacts with the negatively charged lipids PG and CL stronger than with PE. Meanwhile, an additional sharp peak was observed for the bicelle samples upon adding *al*MGS, indicating that smaller structures than bicelles were induced.

Abstracts
– Neurosciences –

O-473

Rectification of glutamate receptors set cell type –specific plasticity rules in interneurons

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Cortical inhibitory neuron network comprises anatomically and physiologically diverse GABAergic interneuron types. Glutamatergic synapses onto some interneurons express synaptic long-term potentiation (LTP) and –depression (LTD). The synaptic plasticity is induced by repetitive firing of glutamatergic afferents but induction patterns and mechanisms vary between interneurons.
We investigated LTP and LTD in excitatory glutamatergic synapses onto identified hippocampal interneurons *in vitro* in acute slice preparation and *in vivo* in urethane-anesthetized rat. We utilized gramicidin-perforated current clamp recordings in slices and juxtacellular spike probability and delay measurements *in vivo*. All cells were labeled for anatomical analyses.
Plasticity rules are specific to distinct interneuron types and fundamentally similar *in vitro* and *in vivo*. One form resembles LTP that occurs in pyramidal neurons which depends on N–methyl–d aspartate receptors and is triggered by coincident pre- and postsynaptic activity. The other depends on Ca^{2+} influx through glutamate receptors that preferentially open when the postsynaptic neuron is at rest or hyperpolarized. We suggest that the cell type-specific plasticity in interneurons plays a role in hippocampal function.

O-474

Enlightening allosteric properties of metabotropic glutamate receptors

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Metabotropic glutamate (mGlu) receptors are key modulators of synaptic transmission, regulating both pre- and postsynaptic processes. These receptors are more complex proteins than any other GPCRs, being composed of two identical subunits, each composed of three main domains: a venus flytrap domain (VFT, where glutamate binds) connected to a heptahelical domain (7TM, where positive allosteric modulators (PAM) bind) through a cystein-rich domain (CRD). Whereas agonists act by stabilizing a closed conformation of the VFT, how VFT closure leads to G protein activation remains unclear, but likely results from allosteric transitions within such a multidomain protein complex.
Using innovative technologies, we recently illustrate how these complexes proteins work to activate heterotrimeric G proteins. We show that a movement of the VFT lead to a relative movement of the 7TMs, and as a consequence, the activation of only one of these able to activate G proteins.
Taken together, these data illustrate the coordinated allosteric transitions that link agonist binding into the VFT clef, to G protein activation in such dimeric GPCR complexes.

O-475

Inhibitory networks of grid cells

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Grid cells are neurons in the medial entorhinal cortex where the firing fields of each neuron forms a hexagonal lattice tessellating the environment that
a rat or a mouse navigates (Hafting et al, Nature 2005). They are believed to be an important part of the spatial navigation system in mammals but no satisfactory theory still exists about the neural basis of their unique spatial selectivity. I will describe recent work on modeling the formation of grid cells through an inhibitory continuous attractor network and the predictions that the model makes. I will also describe how this model combined with a previously proposed model based on adaptation (Kropff & Treves, Hippocampus, 2008) can explain some developmental features of the grid cells.
Bonnevie T, et al, Nat. Neuro., 2013
Couet J. et al, Nat. Neuro., 2013

O-476

Improving a GFP-based sensor to measure intracellular parameters like pH and ion concentrations

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Improving a GFP-based sensor to measure intracellular parameters like pH and ion concentrations
Our recently proposed genetically-encoded ClopHensor opened the way for simultaneous ratiometric estimation of intracellular concentrations of Cl and pH without perturbation of cell functioning. ClopHensor is based on a GFP variant, E2GFP, containing a specific chloride binding site. We exploited various strategies to tailor biosensor properties, facilitate the use and extend the applicability range. Site specific mutations were designed to reduce pH dependence. Random libraries were generated and screened for specific spectroscopic properties. The simultaneous detection of intracellular pH and chloride concentration in living cells and Cl homeostasis in living brain will be shown. Applicability to 2-photon excitation microscopy, range of validity and accuracy of time-lapse measurements will be discussed.

Abstracts

– Neurosciences –

O-477

Surface strategy for regulation and control of neural cell adhesion

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The obtainment of 2D ordered neural network and their coupling to recording device such as MEAs can be exploited in the investigation of neural signal propagation. The primary limitation of a conventional MEA is its difficulty in accurately tracking and investigating the response of specific, individual neurons *in vitro* because the cells grow randomly on the surface of the MEA chip. Neuronal patterns can be achieved through the control of the local cellular environment at the micron level. Here, using lithography technique we reproduced gold geometrical microstructures on different substrates (Si_3N_4 and glass) and we developed a two-step chemical functionalization to control precise and exclusive positioning of the neural somata onto gold microregion. Growth of long-term low density neural network was obtained; to test their functionality the same procedure was transferred onto high density MEAs and the effect of the surface functionalization on the cell-to-electrode coupling was investigated. Different techniques were used to confirm the functionalization including water contact angle (WCA), atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS); we also performed immunofluorescence and SEM imaging to get deeper insight on the structure of neural network.

O-478

Differential modulation of NaV1.1 and NaV1.2 sodium channels by the β1 auxiliary subunit

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Purpose: Voltage-gated sodium channels are composed of 1 α and 2 β subunits. Different α-subunits are expressed in excitatory (NaV1.2) and inhibitory neurons (NaV1.1). Understanding the differential effects of β-subunits is important for understanding how they modulate neuronal function.
Methods: A Nanion patchliner was used to analyse α:β combinations transiently expressed in HEK293T cells.
Results: When co-expressed with $Na_V1.2$, β_1 caused a depolarising shift in voltage-dependence of inactivation, larger time constants of inactivation and more rapid recovery from inactivation. β_2 caused a further modulation with a hyperpolarising shift in voltage dependence of inactivation, shorter time constants of inactivation and slower recovery from inactivation. In contrast β_1 did not significantly modulate with $Na_V1.1$ function although addition of β_1 and β_2 slowed recovery from inactivation.
Conclusion: We observed differential effect of β_1 on $Na_V1.2$ and $Na_V1.1$. This has implications for disorders such as epilepsy, where knowledge of neuron type specific dysfunction caused by β_1 mutation is important for understanding disease mechanisms.

P-479

Nano-volume drop patterning of neural networks: a rapid assay for neuronal connect-ability

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The ability of neurons to extend projections and to form physical connections among them (i.e., ÒconnectabilityÓ) is altered in many neuropathologies. The quantification of these alterations is an important read-out to investigate pathogenic mechanisms and for development of neuropharmacological therapy, but current morphological analysis methods are very time-intensive.
We present and characterize a novel approach to quantify neural connections. The approach is based on the development of confined neural networks on discrete patterns of adhesion protein spots characterized by controlled inter-spot separations of increasing distance (from 40 μm to 100 μm), adsorbed in an adhesion repulsive layer. Under these conditions, the connect-ability of wild type neurons (WT) is shown to be strictly dependent from the inter-spot distance, and can be rapidly documented by simple optical read-outs. Moreover, we apply our approach to identify connect-ability defects in neurons from an animal model of Di George syndrome (i.e., schizophrenia), by comparative trials with WT preparations.
The results demonstrate the sensitivity and reliability of this novel on-chip-based connect-ability approach and validate its use for the rapid assessment of neuronal connect-ability deficits in neuropathologies.

P-480

Anacardic acid as potential acetylcholinesterase inhibitor

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Alzheimer's disease is the leading cause of dementia among people over 65 years of age. The cholinergic hypothesis is a line therapy based on increasing the level of acetylcholine by reversible inhibition of the enzyme acetylcholinesterase (AChE) therefore promoting an improvement in the patient's cognitive profile. This paper aims to propose anacardic acid derivatives as possible candidates to AChE inhibitors through molecular modeling. In total, twenty molecular structures with different functional groups were designed taking into account the possible interactions between these functional groups and enzyme active sites. For each molecule, a conformational analyze was done in a way to identify the more stable conformers. A single point was taken for the equilibrium geometry for each compound and several electronic properties important for the molecular recognition by the enzyme, like HOMO, HOMO-1, LUMO, LUMO+1, GAP and atomic charges, were calculated using the hybrid functional B3LYP and and basis set 6-311 + G (2d, p). Geometric and lipophilicity properties were also were determined for each compound using QSAR. The principal components analysis reveals that some of these compounds are correlated to donepezil, a drug with known biological activity, for a specific group of molecular descriptors. This correlation is shown more expressive when a solvatation model, in this case PCM, is included in the *ab initio* calculations.

Abstracts

– Neurosciences –

P-481

Study of the amino acid residues involved in the inhibitory action of steroids on the NMDA receptor

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N-methyl-D-aspartate (NMDA) receptors are glutamate gated ion channels involved in excitatory synaptic transmission, synaptic plasticity and excitotoxicity. Their activity can be influenced by endogenous ligands, including neurosteroids. We used electrophysiological and molecular biological techniques to identify amino acid residues on NMDA receptor that are important for the effect of 20-oxo-5β-pregnan-3α-yl sulfate (PAS).
Our previous results support the idea that it is the membrane domain of the NMDA receptor that is important for PAS effect. In experiments we performed a series of point mutations in the TM1 and TM3 membrane domains of the NR1 and NR2B subunit. Results show that out of 51 mutations in the TM1 and TM3 at both subunits, 10 substitutions for alanine increased the PAS inhibitory potency and 2 mutations reduced the potency.
The results of our experiments indicate that amino acid residues do not form an obvious biding site. We interpret the reduction of the PAS inhibitory effect that mutations allow the steroid to leave the central ion channel cavity, which steroid entered directly from the lipid membrane.
Detailed understanding of the mechanism of action of steroids on NMDA receptors has therapeutic importance for the development of drugs with neuroprotective effect.

P-482

Barriers in the brain: Confinement as barrier for lateral diffusion on crowded membranes

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The Brownian motion of membrane bound proteins on the surface of a biological membrane is strongly influenced by the shape of the membrane. These geometrical effects, together with crowding and jamming, hinder the lateral diffusion of particles on the surface of a crowded membrane. In this project we consider the lateral motion of receptors on the surface of dendritic spines, which are membrane structures, composed of a bulbous head and a thin neck. Experiments have shown that their shape is related to their physiological function and more specifically we will show that geometrical confinement and crowding helps sustain gradients in concentrations of receptors for very long time-scales.
This diffusion problem is addressed numerically, using Brownian dynamics, and analytically, using the concept of Mean First Passage Time. Both the numerical and analytical results show that for spine-like structure, the diffusion of the receptors is increasingly hindered for decreasing neck size of the spine. Besides the geometrical effects, our numerical simulation provides novel insights in crowding effects for interacting particles on curved surfaces. Both these geometrical and crowding effects make it possible to confine receptors to their functional domain for very long times.

P-483

Conformational dynamics associated with the ligand migration in hexa-coordinate globins

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Neuroglobin and cytoglobin are hexa-coordiante globin with protective function in vertebrate organisms. Both proteins exhibit a globin fold with unique structural futures including large internal cavities and an internal disulfide bridge. To understand the ligand migration mechanism and the role of distal pocket residues and the internal disulfide bond in tuning the affinity of hexa-coordinate globins for exogenous diatomic ligands , we have employed transient absorption spectroscopy and photoacoustic calorimetry to probe dynamics and energetics of structural changes associated with the CO migration between the heme distal pocket and surrounding solvent. Our data indicate a distinct mechanism of ligand migration in both proteins. Specifically, in neuroglobin the long hydrophobic channel provides an efficient pathway for the ligand escape from the protein matrix whereas in cytoglobin the disulfide bond controls the ligand migration pathway through remodeling of the internal cavities.

P-484

Modulation of GABAA by Clobazam and Diazepam in the presence of Furosemide and Zn2+

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Nowadays the most widely used compounds in clinical practice as sedatives, hypnotics, ansiolytics, muscle relaxants and anticonvulsants are the benzodiazepines. These express their activity via $GABA_A$ receptors, which are ligand-gated channels and represent the most important inhibitory receptors of the Central Nervous System. As we already know, $GABA_A$ consists of a pentameric subunit receptor, with different subunit classes and corresponding type of action. In this study we demonstrate the enhancement of the activity on native $GABA_A$ receptors of two important benzodiazepines: the 1,5 benzodiazepine Clobazam and the 1,4 benzodiazepine Diazepam in the presence of Furosemide and Zn^{2+}. It has already been demonstrated that Furosemide blocks the receptors with the α_6 subunit and that Zn^{2+} blocks the incomplete receptors. The $GABA_A$ receptor populations studied were those of rat cerebellar granule cells in culture. The drug effects were evaluated for the two different $GABA_A$ receptor populations present in these neurons, one mediating phasic inhibition and the other mediating tonic inhibition. For these experiments we used the patch clamp technique in the whole cell configuration.

Abstracts

– Neurosciences –

P-485

The Role of TAOK2 in Brain Development

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TAO protein kinases are known to activate mitogen-activated protein kinase pathways, interact with the cytoskeleton and to regulate plasticity. We believe that deletion of TAOK2 might particularly contribute to the pathology of autistic patients in the known chromosomal microdeletion of 16p11.2, a mutation that carries substantial susceptibility to Autism Spectrum Disorders. In our studies we have found that TAOK2 is activated through Nrp1-Semaphorin 3A signaling and once activated leads to JNK1 (c-Jun NH2-Terminal Kinase) phosphorylation. Down-regulation of TAOK2 is responsible for immature basal dendrite development and axon projection deficits. Also, overexpression of constitutively active MKK7-JNK1 rescues both defective phenotypes in the mouse cortex. Our findings support the hypothesis that underdeveloped neuron morphology contributes to the disconnection of brain regions that may underlie the autistic phenotype. Our studies continue pursuing a detailed structural analysis of the TAOK2 regulatory domain.

P-486

SNARE mediated membrane fusion on pore-spanning membranes

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Membrane fusion is a crucial step for many biological processes. One of the most common examples for the fusion of vesicles with a target membrane is the highly controlled release of neurotransmitter at the synaptic cleft. It is presumed, that Nethylmaleimide sensitive factor attachment protein receptor (SNARE) proteins constitute a minimal membrane fusion machinery which drives fusion by the formation of a coiled-coil complex. The exact mechanism of this process is investigated by various experimental techniques of which many focus on vesicle-vesicle fusion in bulk solution. In contrast to that we want to establish a fusion assay on pore-spanning membranes at a single vesicle level utilizing confocal laser scanning microscopy (CLSM).

The pore-spanning membranes are prepared by spreading of giant unilamellar vesicles (GUVs) on porous silicon nitride substrates functionalized with thiols after gold coating. By applying large unilamellar vesicles (LUVs), single SNARE mediated fusion events, indicated by Förster resonance energy transfer (FRET) upon lipid mixing, can be monitored via CLSM with a time resolution of 50ms.

Abstracts

– Biologically Active Peptides –

O-487

Unraveling a new actor in dengue virus-cell fusion

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Dengue Virus (DENV) infects 390 million people/year. We focused on the role of DENV capsid (C) protein in virus-endosome fusion and viral genome transfer to the cytosol using spectroscopic techniques. DENV C protein was able to translocate across cell membranes – supercharged protein – carrying a ssDNA oligonucleotide or a Green Fluorescence Protein (GFP)-encoding plasmid through a physical mechanism. GFP expression showed that C protein-mediated nucleic acid translocation is effective and functional. Two peptides corresponding to the putative RNA- or membrane-binding domains of the DENV C protein have the ability to transport nucleic acids into cells. The membrane-binding domain has the ability to translocate anionic lipid bilayers, at variance with the RNA-binding domain, revealing its dominancy on the translocation activity of DENV C protein. This work enlightens a new potential biophysical role of the capsid proteins of the viruses of the *Flaviviridae* family during cell infection, serving as natural transfection agents for the viral genome.

O-488

Process of inducing pores in membranes by melittin

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Melittin is a prototype of the ubiquitous antimicrobial peptides that induce pores in membranes. It is commonly used as a molecular device for membrane permeabilization. At nanomolar concentrations, melittin induces transient pores that allow transmembrane conduction of atomic ions but not leakage of glucose or larger molecules. At micromolar concentrations, melittin induces stable pores allowing transmembrane leakage of molecules up to tens of kDa, corresponding to its antimicrobial activities. Despite extensive studies, aspects of molecular mechanism for pore formation remain unclear. To clarify the mechanism, one must know the states of melittin-bound membrane before and after the process. By correlating experiment of giant unilamellar vesicles with that of peptide-lipid multilayers, we found that melittin bound on the vesicle translocated and redistributed to both sides of the membrane before the formation of stable pores. Thus the initial states for transient and stable pores are different, which implies different mechanisms at low and high peptide concentrations. To determine the lipidic structure of the pore, the pores in peptide-lipid multilayers were induced to form a lattice and examined by anomalous X-ray diffraction. The electron density distribution of lipid labels shows that the pore is formed by merging of two interfaces through a hole. The molecular property of melittin is such that it adsorbs strongly to the bilayer interface. Transient pores and stable pores can be viewed as the lipid configurations a bilayer adopts to accommodate its excessive interfacial area, under asymmetric and symmetric condition, respectively.

O-489

Disruption of bacterial membranes based on membrane curvature generation by antimicrobial peptides

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Nowadays, we face a worldwide re-emergence of infectious diseases and a rapid increase in pathogenic bacteria that are multi-resistant to commercially available antibiotics. Hence the WHO ranked antibiotic resistance as a priority disease and urged the development of antibiotics based on novel mechanism. Antimicrobial peptides, short amphipathic molecules, which mostly act on cytoplasmic bacterial membrane, represent promising candidates. Several models have been suggested to explain the membrane activity of these peptides. We proposed that these models are "special cases" within a generic phase diagram describing the morphological plasticity of peptide/lipid supramolecular assemblies (B. Bechinger & K. Lohner, BBA 1758(2006)1529).
Since bacteria regulate their lipid composition in a narrow window close to a lamellar to non-lamellar phase boundary, we have focused on the ability of antimicrobial peptides to induce bicontinuous cubic phases in model membranes rich in negative curvature lipids such as phosphatidylethanolamine. Generation of saddle-splay membrane curvature can release the high stored elastic energy in cytoplasmic membranes and may have implications for various mode of action such as pore formation, membrane blebbing or disruption at high peptide concentration. This observation has been included in the generic phase diagram.

O-490

How do a proapoptotic and a cell penetrating peptide work together to kill cancer cells?

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The proapoptotic peptide KLA was coupled to the CPP penetratin by a disulfide bond to allow for KLA cellular internalization and intracellular release. The effects of both KLA-Pen and the two peptides alone were investigated in human cell lines. In cancer cells, KLA-Pen had a strong impact on mitochondria tubular organization instantly resulting in its aggregation (live microscopy), while none of the peptides alone had this effect. Mitochondria in healthy cells were not affected by the conjugate. KLA-Pen selectively induced cell death in cancer but not healthy cells. To understand the mode of action of KLA-Pen in mitochondria and its selectivity towards cancer cells, its interaction with model membranes mimicking the two systems was investigated using DLS, calorimetry, plasmon resonance, CD and ATR-FTIR. The studies indicated that asides from electrostatic interactions, lipid interactions of KLA and KLA-Pen are highly influenced by membrane fluidity. Moreover, such parameters (electrostatics, membrane fluidity) influence the oligomerization state of the peptide strongly affecting its action mode. KLA-Pen may exert its deleterious action by the formation of pores with an oblique orientation in the membrane and establishment of important hydrophobic interactions.

Abstracts

– Biologically Active Peptides –

O-491

Non-uniform changes in lipid order induced by the Membrane Targetting Sequence of the MinD ATPase

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Amphipathic helices are commonly used as anchor domains to allow peripheral membrane proteins to interact with lipids via both hydrophobic and ionic bonds. The 11-residue Membrane Targeting Sequence (MTS) of the bacterial MinD ATPase protein binds strongly to the surface of anionic model membranes without perturbing the bilayer morphology. A combination of atomistic and coarse-grained MD simulations with EPR and solid state NMR spectroscopy has been used to study the changes in lipid dynamics across a range of molecular timescales which occur on MinD-MTS peptide binding. Non-uniform changes in lipid dynamics are induced by the insertion of the peptide into the membrane, including an increase in headgroup rotational correlation time and disordering of the lower half of the lipid acyl chains in close proximity to the peptide. Lipid demixing and the formation of domains which are stable on the microsecond timescale, are induced primarily by electrostatic interactions between the peptide and lipid. The high affinity of the MinD-MTS sequence for the membrane is determined by a balance of both entropic (lipid disordering) and enthalpic (non-covalent bond formation) forces, with implications for the design of membrane-active peptide sequences.

O-492

Biophysical investigations into the effect of antimicrobial peptides on bacterial membranes

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Antimicrobial peptides (AMPs) as part of the innate immune system are the first defense barrier against bacterial infections. Synthesized or activated upon need, they are present in epithelial surfaces, e.g. of the colon, skin and lung. Interestingly, those peptides evolved in parallel to bacterial resistances but are still an effective defense mechanism against infections. The development of innovative therapeutic agents to combat resistances requires an analysis of their origin. We analyzed the resistance induction probability of a fragment of the human AMP cathelicidin in comparison to conventional antibiotics. The results indicate the peptide as a promising candidate for drug development. With this knowledge we tried to improve the bactericidal activity of this peptide with a genetic algorithm, based on biological experiments. To characterize the interaction of a selection of new peptides, biological and biophysical techniques as SYTOX green assays and FRET were performed and compared with other AMPs. Furthermore, we established a new fluorescence approach using nanopores (nanoFAST chips, Nanospot GmbH) to visualize membrane peptide interactions over a huge amount of free-standing membranes.

P-493

The membrane action mechanism of novel antimicrobial peptide COD isolated from the venom of bee

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Antimicrobial peptides (AMPs) due to their unique mechanism of action are considered as promising alternatives to conventional antibiotics. The 18 amino acid residues peptide named COD which we isolated from the venom of wild bee *Colletes daviesanus* belongs to the category of cationic α-helical amphipathic AMPs. It possesses potent activity against various strains of bacteria and low haemolytic activity. In this study focused on the mechanism of action of COD and its synthetic analogs, we found out that these AMPs permeated both outer and inner cytoplasmic membrane of *E. coli*. This was determined by the measurement of changes in the fluorescence of fluorescent probe 1-N-phenylnaphtylamine and released of cytoplasmic β-galactosidase when peptides interact with bacterial cell membrane. In addition, the interaction of CODs with phospholipid liposome model mimicking *E. coli* cell membrane lead to leakage of calcein entrapped in liposome but no calcein leakage was observed from the liposomes mimicking membranes of erythrocytes. That is in good agreement with low hemolytic activity of COD and its analogs. These results suggest that COD peptides are specific against bacterial cell membrane and their killing mechanisms involved membrane perturbation.

P-494

Conformational plasticity of the cell-penetrating peptide SAP

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The cell-penetrating peptide SAP [$(VRLPPP)_3$], designed as an amphipathic poly-proline helix II (PPII), was suggested to self-assemble into regular fibrils that are relevant for its activity. We have shown [Angew Chem 120:5849] that a conformationally restricted amino acid at the position of Pro11 promotes the PPII conformation in SAP.
Here, we have analyzed the structure of SAP in the membrane-bound state by solid-state ^{19}F-NMR, which revealed, besides PPII, other structures. The conformational equilibria were studied and actively shifted by imposing selected substituents at Pro11. A series of rigid 4,5-methanoprolines (MePro) and other Pro analogues was used as structural probes and labels for NMR. We found that the configuration of cyclopropane in MePro governs the coil-to-PPII equilibrium in solution. By CD and ^{19}F-NMR we examined the slow kinetics of SAP binding and dependence on membrane composition. The peptide does not bind to *gel*-lipids, and it aggregates only in the raft-like bilayers. No self-association could be detected in solution, nor in *fluid* bilayers. When bound, SAP resides on the bilayer surface in a non-aggregated state, either in an extended (β) conformation or as a PPII, but these conformations are pre-formed in solution and are not induced by the membrane.

Abstracts

– Biologically Active Peptides –

P-495

Peptide induced demixing in PC/PS lipid mixtures

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The impact of the antimicrobial and antitumor peptide MP1 (IDWKKLLDAAKQIL-NH2) on lipid mixtures of phosphatidylcholine(PC) and phosphatidylserine(PS) was assessed by differential scanning calorimetry (DSC), pressure area isotherms and giant unilamellar vesicles (GUV) visualization by fluorescence microscopy. MP1 activity in cancer cell cultures is believed to be due to the presence of PS in these cells. Thermograms of POPC:DPPS multilamellar vesicles (MLV) in different molar fractions of DPPS showed that the system is miscibility at the composition 7:3. At this composition the peptide induced a decrease of the gel-to-liquid crystalline phase transition temperature suggesting lipid demixing consistent with the formation of dense fluorescence regions in GUVs. The effect of the peptide co-spread with lipids on compression isotherm of DMPC, DMPS and mixed DMPC/DMPS monolayers showed that the expanded-to-compressed liquid plateau is suppressed giving rise to a second plateau with the increase of the peptide concentration. These results indicate that the peptide interacts preferentially with DMPS containing monolayers. These different experimental approaches indicate that MP1 induces a phase rich in pure PC and another phase rich in peptide and PS.
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P-496

The effect of antibacterial peptide L1A on lipid phase transition

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L1A (IDGLKAIWKKVADLLKNT-NH_2) is a potent Gram negative antibacterial and less efficient to Gram positive. We have studied its effect on the phase transition of mixed lipid systems (POPE:DOPG, POPE:TOCL and DPPC:POPG) by differential scanning calorimetry (DSC) and (POPE:DOPG and POPC:POPG) by phase contrast (PCM) and fluorescence microscopies (FM). Thermograms of pure mixed lipids MLV, showed that the phase transition temperatures T_m=18, 13 and 35^{o}C for 3POPE:1DOPG, 3POPE:1TOCL and 4DPPC:1POPG. The addition of peptides to the lipid film before MLV formation the T_m increased 7, 7 and 5^{o}C respectively suggesting induction of lateral phase separation and stabilization of the gel phase. Phase contrast and fluorescence microscopy visualization of GUV, prepared with peptides added to lipid film before electroformation, showed black stains suggesting lateral phase separation. Visualization of POPE:DOPG GUVs obtained by spontaneous formation showed that the addition of peptides to the GUVs induced budding and gradual loss of phase contrast without vesicle rupture. These results are in agreement with the selectivity of this peptide to Gram negative bacteria and that lateral phase separation could play important role in its biological activity.
Support FAPESP and CNPq

P-497

Improvement of the HIV fusion inhibitor C34 activity by membrane anchoring and enhanced exposure

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It was recently demonstrated that the combination of cholesterol-tagging and dimerization of HIV fusion inhibitor peptide C34, using a PEG moiety as spacer, resulted in an increase of the antiviral potency and in an extension of the *in vivo* half-life of two new anti-HIV molecules: HIVP3 (C34-PEG_4-cholesterol) and HIVP4 ($[C34\text{-}PEG_4]_2$-cholesterol). The aim of the present work was to evaluate the interaction of these molecules with membrane model systems and human blood cells. Membrane partition, dipole potential and pressure assays indicate that HIVP3 and HIVP4 interact preferentially with cholesterol-rich liquid ordered phase membranes mimicking biological microdomains, known as lipid rafts. Interaction of peptides with erythrocytes and peripheral blood mononuclear cells showed that HIVP3 and HIVP4 are able to interact with the membrane of both blood cells in the same extension as another C34 derivate,C34-cholesterol.However, the pocket binding domain (PBD) of both new C34 derivates seems to be more exposed to aqueous environment than on C34-cholesterol. Altogether, the data indicate that the synergic effect between a membranotropic behavior and an enhanced exposure of PBD of the inhibitors result in a more efficient blocking of HIV entry.

P-498

The polyclonal antibodies as a new pathway in fight with amyloidoses

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Generation of amyloid deposits by many different peptides and proteins is associated with amyloid diseases. Our project aims at finding a natural inhibitor of formation of amyloid fibrils, which are formed by human cystatin C (hCC) L68Q variant and leads to death of patients caused by intracerebral hemorrhages (HCHWH-I). There are no reports on molecules inhibiting the fibril formation. Designing and synthesis of potential drugs (monospecific antibodies) to fight with this disease by inhibition of hCC fibrillization is the subject of this studies.
In order to obtain antibodies directed against, an antigen must be introduced, against which immunological response will be triggered in the form of monospecific antibodies. hCC molecule cannot be used as an antigen, because it is a physiological protein. Therefore we decided to introduced hCC fragments to a rabbits and checked if they trigger immunological response, shown as production of specific antibodies against hCC. The results turned out to be succeeded, we obtained antibodies interacting with hCC molecule. We would like to identify hCC epitopes for rabbit antibodies and checked whether the antibodies obtained by immunization of rabbits are able to suppress the process of fibrillization of hCC.

Abstracts

– Biologically Active Peptides –

P-499

Peptide lipidation by acyl transfer from lipids

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Acyl transfer from phospholipids to membrane-active peptides has been demonstrated using MS, LC-MS and LC-MS^n methods. Peptides lipidated in this manner include melittin, magainin II, PGLa, penetratin, LAK_1 and LAK_3. This transfer is a consequence of the innate reactivity of each peptide toward lipids and occurs in the absence of enzyme catalysis. Melittin is the most reactive peptide, with transfer occurring in a range of conditions of salt (from water to physiological concentrations), temperature (20 ¡C to 37 ¡C) and peptide to lipid ratio (P:L = 1:100 to 1:5). Acyl transfer is promoted by the inclusion of negatively charged lipids (PS and PG) in PC membranes, with transfer observed from both PS and PG components, alongside PC. Magainin II is less reactive than melittin, but is nevertheless lipidated in a broad range of conditions. The other peptides are the least reactive, with lipidation found under some, but not all, experimental conditions. These experiments demonstrate that peptide and protein lipidation should be expected to occur *in vivo*, with significant consequences for biological activity and protein turnover. It should also be considered when analyzing data from peptide- and protein-lipid systems accumulated over long time periods *in vitro*.

P-500

Structure-activity relationship studies of gomesin analogues containing tryptophan residue

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Gomesin (Gm) peptide has a broad antimicrobial activity, which gives it great interest as a target molecule for drug development. However, a strong lytic activity against pathogenic organisms is closely associated with toxic effects to the host, for example, hemolysis. In this study, we analyzed four Gm analogues, [Trp^1]-Gm, [Trp^7]-Gm, [Trp^1, Ser^{2,6,11,15}]-Gm and [Ser^{2,6,11,15}, Trp^7]-Gm, in an attempt to elucidate the role of the structure of these peptides on their antimicrobial actions. Therefore, tests were made in the growth inhibition of bacteria and fungi. Compounds were incubated with different concentrations in order to determine their MICs. Their hemolytic activities were evaluated against human erythrocytes. Spectroscopic analyses were also employed (circular dichroism and fluorescence) to monitor Gm analogues structural changes in water and sodium dodecyl sulfate (SDS). As expected, circular analogues ([Trp^1]-Gm and [Trp^7]-Gm) assume the same behavior that native Gm, which was not observed, in most conditions, for the linear peptides (with serine residues). Based on these data, it was possible to draw an important relationship between structure/activity for these peptides. And, this knowledge will serve as a support for further analysis of the mechanism of Gm action.

P-501

Computational methods to predict peptide orientation in membranes: LAH4 as a stringent test case

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Knowledge of peptide orientation in the membrane is essential to discriminate between different pore formation mechanisms by antimicrobial peptides (AMPs). Molecular dynamics (MD) simulations can provide this information, but their reliability is affected by the limited length of the trajectories and by force field approximations. To overcome time-scale problems, computational methods such as the "minimum-bias"[1,2] approach or potential of mean force (PMF) calculations can be performed, with either all-atoms (AA) or coarse-grained (CG) force-fields. In this work we assessed the reliability of these methods, by applying them to a very stringent test case. NMR experiments demonstrated that the amphipathic LAH4 AMP, which contains four His residues, is bound to the membrane surface at acidic pH, while it assumes a transmembrane orientation at neutral pH.[3] "Minimum-bias" AA and CG simulations and PMF calculations were performed with different force fields. Our preliminary results highlight the sensitivity of these simulations to force-field parameters, and indicate that some commonly adopted approaches fail to correctly predict peptide orientation in the membrane. [1] *Biophys.J.*,2007,**92**,903; [2] *J.Pept.Sci.*,2009,**15**,550; [3] *Biophys. J.*,2010,**99**,2507.

P-502

Design and mechanism of dengue virus capsid-cell penetrating peptides for nucleic acid delivery

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Cell-penetrating peptides (CPP) can be used to deliver different therapeutic molecules. Two novel CPP, pepR and pepM, were designed based on two domains of the dengue virus capsid protein, and their ability to deliver nucleic acids into cells was studied. Translocation studies performed by confocal microscopy, at 4°C and 37°C, revealed that pepR and pepM use distinct entry routes: pepM translocates membranes directly, while pepR uses an endocytic pathway. To further study these events, a methodology was developed to monitor and quantify the translocation kinetics of both peptides by time-resolved flow cytometry. Additionally, the molecular basis of the peptide-mediated translocation was explored by quantifying peptide-nucleic acid and peptide-lipid interactions. pepR and pepM bind ssDNA at the same extent, and partition studies revealed that both peptides bind preferentially to anionic lipid membranes, adopting an -helical conformation. Fluorescence quenching studies showed that pepM is completely inserted in the lipid bilayer, in contrast with pepR. Altogether, this work shows that: 1) time-resolved flow cytometry is suitable to attain detailed insight on cell-entry processes, and 2) dengue virus capsid peptides are good templates to design novel CPP for delivery strategies.

Abstracts

– Biologically Active Peptides –

P-503

Biofilm formation by different Candida albicans variants and their antifungal agents'susceptibility

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Biofilms represent the most common type of microbial growth in nature and are crucial to the development of clinical infections. Candidiasis associated with intravenous lines and bioprosthetic devices is problematic, since these devices can act as substrates for biofilm growth. In the present work *Candida albicans* biofilm formation was studied in the absence and in presence of a defensin, *Ps*d1 [1], and two current antimicotic drugs: amphotericine B and fluconazole. Three *C. albicans* variants were studied, one of them a mutant deficient in glucosylceramide synthase enzyme, conferring resistance to *Ps*d1 antifungal action. Differences in the biofilm formation were encountered for the variants studied. Atomic force microscopy (AFM) images showed that during the biofilm growth a structured mesh of fungal cells is formed with 1.2-1.5 μM height (single cell have 500-600 nm). The action of the three antifungal agents was evaluated both in terms of inhibition of the biofilm formation and disruption of previously formed biofilm.

[1] Gonçalves *et al.* (2012) *Biochim Biophys Acta* 1818:1420

P-504

The enhanced membrane interaction of a cell penetrating peptide in the presence of anionic lipids

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CPPs are able to deliver a large variety of macromolecules through both endocytotic and non-endocytotic pathways but the molecular requirements for an efficient internalization are not fully understood. RW16 (RRWRRWWRRWWRRWRR) is a CPP derived from Penetratin and has been shown to be a good non toxic CPP with anti-tumor activities. Herein, we describe its membrane interaction, insertion and perturbation with lipid model membranes of varied composition using different biophysical techniques: calorimetry, DLS, ^{31}P-NMR, fluorescence spectroscopy and CD. Depending on the lipids and on its aggregation state, the peptide behaves differently upon membrane contact. As a monomer the peptide interacts strongly with zwitterionic and anionic lipids with a dissociation constant in the nanomolar range while in the oligomeric form it interacts poorly with zwitterionic membranes but highly with anionic lipids (Jobin *et al.*, BBA 2013 1828:1457-1470). This enhanced interaction and perturbation observed with anionic lipids is interesting considering the reported potential of this peptide in selectively affecting the motility and intracellular actin-remodeling activity of tumor cells and knowing that cancer cells membranes are particularly more anionic than healthy cells.

P-505

Quantitative single-vesicle analysis of antimicrobial peptide-induced leakage

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Although the research field of antimicrobial peptides has attracted considerable scientific attention in the past decades, the microbicidal mechanisms of antimicrobial peptides still remain elusive. One of the keys to a more profound comprehension of the function of these peptides is a deeper understanding of their interactions with phospholipid membranes. In this study, the membrane-permeabilizing effects of antimicrobial peptides were scrutinized by combining two biophysical techniques. Confocal fluorescence microscopy to visualize leakage from individual surface-immobilized lipid vesicles was combined with fluorescence correlation spectroscopy to quantify leakage from a bulk collection of lipid vesicles in aqueous solution. Quantitative correlation between the two techniques was achieved through a detailed experimental protocol. The potential of combining the two techniques was tested using three canonical antimicrobial peptides: melittin, magainin 2, and mastoparan X. The results demonstrate an unprecedented level of insight into the molecular processes governing antimicrobial peptide-induced permeabilization of phospholipid membranes.

P-506

Membrane Interaction and Cellular Uptake of Antisecretory Peptide AF-16

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More than 1.5 million children die every year due to diarrhea. Access to safe drinking water could save many children's lives but in some cases immediate medical treatment is needed. Unfortunately, the drugs used today are not efficient enough and there is a need of better medications.
Antisecretory Factor (AF) is a protein with the ability to prevent hyper secretion of body fluids which occurs e.g. in diarrheal diseases. The amino acid sequence responsible for this antisecretory effect of AF has been identified. A 16 aa long peptide (AF-16) containing that sequence has been shown to exhibit both antisecretory and anti-inflammatory effects. In addition to treat diarrhea, AF has also been shown to cure other diseases caused by hyper secretion of body fluids. Although much is known about the positive physiological effects of AF, little is known about the molecular mechanism behind these effects.
We are currently studying the membrane interactions of AF-16 with calorimetry and spectroscopy methods and the cellular uptake of the peptide using confocal microscopy, in order to understand how it enters cells. The results, together with information about the intracellular interactions of the peptide, could lead to revelation of the mechanism behind the antisecretory activity.

Abstracts

– Biologically Active Peptides –

P-507

Interaction of fengycin with stratum corneum mimicking model membranes: a calorimetry study

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Fengycin, a lipopeptide produced by B. subtilis, exhibits remarkable antifungal properties making it an excellent candidate for topically treating localised dermatomycoses. The aim of this study was to further investigate the interaction of fengycin with *stratum corneum* (SC) model membranes. For this purpose, multilamellar lipid vesicles (MLVs), with a lipid composition mimicking that of the SC, was prepared and characterized by differential scanning calorimetry (DSC). The critical micelle concentration of fengycin was also determined at physiological skin conditions. The interaction of fengycin with SC-mimicking MLVs was investigated by isothermal titration calorimetry (ITC) and DSC. The results showed that changes in lipid phase behaviour as a function of temperature considerably affect the interaction of fengycin. At 40 °C and below, fengycin induces exothermic changes in lipid structures. Less-ordered lipid domains became more-ordered with its effect. At 60 °C, endothermic interaction enthalpies are observed, which could arise from "melting" of remaining solid domains enriched in high- melting lipids that without fengycin melt at higher temperatures.

P-508

Surface and lipid interaction properties of novel rhamnolipids to explain their eliciting activity

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Among glycolipidid biosurfactants, rhamnolipids hold a prominent position because of their interesting biological properties such as antimicrobial, antiphytoviral, zoosporicidal and plant defense elicitor activities [1-3]. It is generally recognised that these activities must be related to the interaction of these molecules with constituents of biological membranes [4] but the molecular mechanisms are not fully understood.
Novel rhamnolipids differing by one structural trait were synthetized by an enzymatic or a chemical pathway [5]. Their eliciting activity on the tabacco cells was evaluated by oxidative burst induction measurement. Their surface properties and their interaction with model membranes representative of the plant cell membranes were studied in relation with their structure. A clear correlation between the biological and biophysical properties exists. It provides insight about the mechanism governing the perception of rhamnolipids at the plant cell surface.
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P-509

Kinetic uptake profiles of cell penetrating peptides in lymphocytes and monocytes

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Nucleolar targeting peptides (NrTPs) are a novel family of cell-penetrating peptides (CPPs), derived from to the rattlesnake toxin crotamine, shown to internalize and deliver cargos into different cell types. In this study, we address NrTP kinetics of translocation into peripheral blood mononucleated cells (PBMCs) by flow cytometry. The kinetic profiles for each peptide are concentration-independent but significantly different among NrTPs, pointing out for the amino acid sequence importance. On the other hand, the same peptide behaves differently in lymphocytes and monocytes, suggesting differences in entry mechanism, which in turn reflect diversity in cell functionality. Uptake results obtained at 4C or using chemical endocytosis inhibitors support the importance of non-endocytic mechanisms in the cellular internalization of NrTP1 and NrTP5, while confirming endocytosis as the main mechanism of NrTPs entry. NrTPs (especially NrTP6) are an excellent intracellular delivery tool, with efficient internalization and no toxicity. **This work validates NrTPs as potential therapeutic tools for, *e.g.*, cancer or inhibition of viral replication. Furthermore, it establishes a new comparative and quantitative method to test CPP efficiency.**

P-510

Membrane-perturbing effects of antimicrobial peptides: a systematic spectroscopic analysis

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Antimicrobial peptides (AMPs) exhibit a strong activity against a wide range of microorganisms, mainly by perturbing the permeability of bacterial membranes through the formation of pores. However, AMPs effects on membrane properties probably extend beyond pore-formation. We performed a systematic spectroscopic analysis of the effects on membrane structure and dynamics of two very different AMPs: the cationic PMAP-23, which creates pores according to the "carpet" model, and alamethicin, which forms "barrel-stave" channels. By using fluorescence anisotropy measurements on liposomes comprising probes localized at different depths in the bilayer, we measured peptide effects on membrane fluidity and order. Peptide-induced perturbation of lateral mobility and domain formation were determined by several methods. All experiments were compared with liposome-leakage measurements: while for PMAP-23 all membrane-perturbing effects are correlated with the vesicle leakage process, alamethicin does not significantly influence membrane dynamics at the concentrations in which it forms pores. Surprisingly, in all cases the most significant peptide-induced effect is a reduction in membrane fluidity.

Abstracts

– Biologically Active Peptides –

P-511

Membrane interaction of tryptophan-arginine peptides with dual function

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Cell-penetrating peptides (CPPs) and antimicrobial peptides (AMPs) are two classes of membrane active peptides with several properties in common. Whereas CPPs have the ability to cross cellular membranes and deliver macromolecular cargo, AMPs have appeared a promising alternative to antibiotics. The challenge today is to combine knowledge about the different membrane active peptides to design peptides with membrane-specific actions, either as transporters or as growth inhibitors. Therefore our goal is to better understand the mechanistic details of how these peptides interact with cellular membranes. We have previously shown that number and position of the amino acid tryptophan in the peptide sequence affect both cellular uptake and antibacterial properties. To further investigate the origin of these variations, we have studied the induced changes caused by peptide binding to model membranes of different compositions, using the surface sensitive technique Quartz Crystal Microbalance with Dissipation monitoring. Our results indicate that the tryptophan position in the peptide sequence indeed affects the membrane interaction and that the effect is dependent on the membrane composition. These results give further information on how to regulate the action of membrane active peptides.

P-512

Conformation and activity of the antimicrobial peptide maculatin 1.1 depends on membrane lipids

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Understanding the structure-activity relationship is required for the development of antimicrobial peptides (AMP) with enhanced specificity against bacterial lipid membranes. The affinity for a particular membrane lipid composition is often assumed to be the governing mechanism, but AMP often attack charged membranes mimicking the bacterial envelope almost as efficiently as neutral and cholesterol-containing membranes mimicking eukaryotic cells. For instance, maculatin 1.1 is a 21 residue cationic AMP secreted from the skin of Australian tree frogs that acts against Gram positive bacteria but also has appreciable haemolytic activity. The peptide secondary structure was determined by CD spectroscopy in unilamellar vesicles and oriented bilayers using a range of phospholipids and was strongly influenced by lipid chain length and saturation. Dye release experiments using binary lipid mixtures in a competitive environment were performed to determine the AMP mechanism and affinity towards a particular lipid composition. The results indicate that a distinct structure of maculatin 1.1 is not essential for lytic activity and support a pore mechanism which is regulated by the membrane lipid composition.

P-513

Influence of cecropin A-melittin antimicrobial peptides on POPE/POPG lamellar phase periodicity

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New strains of infectious agents resistant to known antibiotics are increasingly appearing, an alarming situation as the antibiotics discovery is not keeping pace with this reality. In recent years, antimicrobial peptides (AMPs) have emerged as an interesting alternative. They are present in almost all living organisms as part of their immune system. Although in most cases they are thought to act primarily by membrane disruption, by a variety of mechanisms, the concomitant presence of internal targets has also been proposed. In this work we have studied the interaction of three AMPs, a cecropin A-melittin hybrid (CA(1-7)M(2-9)) and two lysine N(epsilon)-trimethylated analogs (K6 and K7), with model membranes of phosphatidylethanolamine (POPE) and phosphatidylglycerol (POPG) by Small Angle X-ray Diffraction (SAXD). Our results show that the addition of peptides to POPE/POPG (3:1) system lowers dramatically the periodicity (d) of the liquid crystalline lamellar phase, whereas the same parameter decreases only slightly in the gel phase. Further, the smaller periodicity observed in the presence of peptides is similar to the one measured for pure POPE.

P-514

nanoFAST biochips: a new and fast method to assay membrane permeation of cell penetrating peptides

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Nanospot GmbH has developed a biochip platform for the direct measurement of transport of fluorescent molecules across free-standing membranes. We use a simple centrifugation protocol to spread giant unilamellar vesicles (GUV) from different lipid mixtures onto micro-porous silicon chips and afterwards apply the molecule under investigation. The transport is measured in hundreds of cavities in parallel by fluorescence imaging, generating a multitude of individual transport kinetics. Additional control dyes in different fluorescence channels are used to check membrane integrity and lipid localization in each cavity.

Here we present the permeation kinetics of three cell penetrating FITC-labeled oligoprolines (1). Despite their structural similarity and slow permeation rates (few molecules per second and μm^2), we could resolve kinetic differences between the molecules and prove that neither of them forms pores big enough to release control dye during permeation.

References:

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Abstracts

– New and Notable –

O-515

Single protein characterization methods with nanopores

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Synthetic and biological nanopores can be used for fundamental and applied studies of individual biomolecules in high throughput. By measuring resistive current pulses during the translocation of single molecules through an electrolyte-filled nanopore, this technique can characterize the size, conformation, assembly, and activity of hundreds of unlabeled molecules within seconds. Inspired by the olfactory sensilla of insect antennae, we demonstrate that coating nanopores with a fluid lipid bilayer considerably extends the capabilities of nanopore-based assays. For instance, coating nanopores with different lipids allows fine control of the surface chemistry and diameter of nanopores. Incorporation of mobile ligands in the lipid bilayer imparts specificity to the nanopore for targeting proteins and introduces control of translocation times for targeted proteins based on their net electric charge. Most recently, we explored the potential of this technique for determining the affinity constant of a protein-ligand interaction, monitoring the kinetics of binding of this interaction, characterizing the aggregation state of Alzheimer's disease-related amyloid peptides, as well as determining the molecular shape, dipole moment and rotational diffusion constant of individual proteins.

O-516

Structure and function of the KtrAB ion transporter

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In bacteria, archaea, fungi and plants the Trk/Ktr/HKT ion transporters are key components of osmotic regulation, pH homeostasis and resistance to drought and high salinity. These ion transporters are functionally diverse: they can function as Na^+ or K^+ channels and possibly as cation/K^+ symporters. They are closely related to potassium channels both at the level of the membrane protein and of the cytosolic regulatory domains. We have determined the crystal structure of a Ktr K^+ transporter, the KtrAB complex from *Bacillus subtilis*. This structure shows the dimeric membrane protein, KtrB, assembled with a cytosolic octameric KtrA ring bound to ATP. Functional assays reveal that ATP is an activating ligand while ADP is not. A comparison between the structure of KtrAB-ATP and the structures of the isolated full-length KtrA protein with ATP or ADP reveals a ligand dependent conformational change in the octameric ring and provides new insights to the mechanism of activation in this ion transporter.

O-517

Structural basis for kinesin-1: Cargo recognition

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Transport on microtubules by the kinesin and dynein families of molecular motors is required for many cellular functions and plays a key role in many pathological processes ranging from neurodegenerative conditions such as Alzheimer's disease to viral and bacterial infections such as HIV-1 and *Salmonella*. Despite this, we lack a structural understanding of the interface between molecular motors and the cargoes they carry. Structural information on how motors recognize their cargoes is required for a molecular understanding of this fundamental and ubiquitous process.

For kinesin-1, the tetratricopeptide repeat (TPR) domain of the light chains (KLCs) can recognise 'tryptophan-acidic' motifs that are found in a number of its cargoes. Here we present the crystal structure of the TPR domain KLC2 in complex with a peptide harboring a kinesin-1 binding 'W-acidic' motif derived from SKIP, a critical host determinant in *Salmonella* pathogenesis and a regulator of lysosomal positioning.

Structural data together with biophysical, biochemical and cellular assays allow us to propose a framework for intracellular transport based on the binding by kinesin-1 of W-acidic cargo motifs through a combination of electrostatic interactions and sequence-specific elements, providing for the first time, direct molecular evidence of the mechanisms for kinesin-1:cargo recognition.

O-518

Altering the torsional rigidity of proteins with surfactants

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Non-ionic surfactants are widely used in protein biosensing to improve sensitivity and specificity in immunoassays. However, surfactants can potentially alter protein conformation, ligand-receptor affinity and thereby assay performance. In practice, the surfactant concentration in assay buffers is an empirical compromise that is reached without design rules based on molecular understanding. Recently we have developed a torsion profiling technique[1] based on magnetic particles to measure the mechanical properties of individual ligand-receptor pairs. Using a rotating magnetic field, we apply a controlled torque to a protein pair sandwiched between a functionalized magnetic particle and a substrate, and thereby determine its torsion constant. This method is suited to study the influence of surfactants on individual ligand-receptor pairs in the presence of different concentrations of surfactants. Our data show an increased rotational flexibility of individual proteinG-IgG pairs with increasing concentration of the surfactant Tween-20. These results demonstrate that the mechanical integrity of the protein pair is compromised.

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Abstracts

– New and Notable –

O-519

Conformational plasticity of the multi-domain Heterochromatin Protein 1β

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Heterochromatin Protein 1 (HP1) is a central player in epigenetics as it is involved in the process of heterochromatin formation, a fundamental mechanism of genome regulation in eukaryotes.

While structures of the two domains chromo (CD) and chromoshadow (CSD) are available (1,2,3), a description of the more functionally relevant full-length protein is still missing. Here, we provide a comprehensive characterization of the structure and dynamics of the full human HP1beta. HP1beta is highly flexible and populates an extended ensemble. NMR results reveal local motions within CD and CSD that can facilitate the recognition of different binding partners. Notably, structural propensities in CD provide a competent binding interface to form the intermolecular beta-sheet with methylated histone H3 (4).

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O-520

Investigating the Cell Membrane via Single Particle Tracking and Hydrodynamic Force Application

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We investigate the potential felt by membrane receptors inside microdomains via tracking of rare-earth doped luminescent nanoparticles and Bayesian inference analysis. The potential felt by peptidic toxin receptors in lipid rafts is well described by a second-order polynomial potential, possibly due to an inhomogeneous lipid and protein distribution [Türkcan et al., Biophys. J. 2012] In contrast, the potential experienced by transferrin receptors in cytoskeleton-delimited microdomains is localized at the border of the confinement domain. Using the inference approach, we extract the hopping energy, i. e. the barrier height, between adjacent microdomains[Türkcan et al., PLoS One 2013]. We also investigate the interaction of lipid rafts with the cytoskeleton via a hydrodynamic drag force applied on the labelling nanoparticle[Türkcan et al., Patent WO010811A1, 2012]. The receptors are displaced, with their confining raft platforms, over distances comparable to the cell size against elastic barriers. Amount and stiffness of the encountered barriers indicate that they are part of the actin cytoskeleton.

Abstracts

– Protein Structure and Function –

O-521

Moving folded proteins across membranes: structural analysis of the Tat protein translocase

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The Tat (twin arginine translocation) pathway exports folded proteins across the cytoplasmic membrane of bacteria. It is required for many important bacterial cellular processes and for the virulence of most bacterial pathogens. The Tat system is conserved in plant chloroplasts where it is essential to form the photosynthetic apparatus.
The Tat system has a particularly challenging and unusual task because it has to provide a transmembrane route large enough to allow the passage of structured macromolecular substrates of different sizes, and up to 6nm in diameter, while at the same time maintaining the membrane ion seal. How this achieved is currently unknown.
I will report on our recent progress in determining structures of the integral membrane proteins that form the Tat protein translocation site and discuss the implications for the mechanism of Tat transport.

O-522

Structural studies of membrane proteins in membrana

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The physical and chemical properties of lipid bilayer membranes and integral membrane proteins are highly connected. Structure determination of membrane proteins in a membrane environment eliminates the potential for distorting structure, dynamics and function and enables functional and ligand binding studies to be performed in a natural setting. NMR spectroscopy is compatible with structure determination of membrane proteins in membranes at physiological conditions. Advances in sample preparation, instrumentation, NMR experiments and effective computational methods, enable very high-resolution solid-state NMR spectra to be obtained from isotopically labeled membrane proteins in lipid bilayers and high quality structures of membrane proteins to be determined in membranes. Orientation restraints are particularly useful for solid-state NMR structural studies of membrane proteins since they provide not only information about three-dimensional structure, but also information about protein orientation in the membrane. We describe recent results obtained for a family of bacterial outer membrane proteins implicated in cell adhesion and pathogenesis.
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O-523

Structure of the agonist-bound neurotensin receptor NTS1

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Neurotensin (NT) is a 13 amino acid peptide that functions as both a neurotransmitter and a hormone through activation of the neurotensin receptor NTS1, a G protein-coupled receptor (GPCR) signaling preferentially through Gq. We have solved the structure at 2.8 Å resolution of NTS1 in an active-like state, bound to NT_{8-13}, the C terminal portion of NT responsible for agonist-induced activation of the receptor. Because wild-type NTS1 is unstable and thus not amenable to crystallization, we used alanine-scanning mutagenesis to stabilize NTS1 and to select for an active-like conformation in the presence of agonist, which combined with the bacteriophage T4 lysozyme fusion strategy and lipidic mesophase crystallization, resulted in diffracting crystals. The agonist binding pocket is located at the extracellular receptor surface. The peptide agonist binds to NTS1 in an extended conformation with the C-terminus oriented towards the receptor core. The NTS1 structure bears many hallmark features of an active-like receptor conformation such as an outward-tilted transmembrane helix 6 at the cytoplasmic surface and key conserved residues in positions characteristic for active GPCRs. Our findings provided for the first time insight into the binding mode of a peptide agonist to a GPCR.

O-524

Probing GPCR-Gα interactions: A functional study by EM and SPR

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The G protein-coupled receptor (GPCR), neurotensin receptor type 1 (NTS1), is pharmacologically important and activated by the tridecapeptide hormone, neurotensin (NT), initiating a cascade of interactions through G proteins to effect cellular responses. A novel DNA-nanotechnological approach for preparing samples for transmission electron microscopy (TEM) has been used to study NTS1 and $G\alpha_{i1}$, both separately and complexed, to a functionalised 2D DNA lattice[1]. NTS1 has also been functionally reconstituted in nanodiscs. A 20 Å resolution cryo electron crystallography structure of the DNA template alone has been solved. The interaction of Nanogold¨-labelled $G\alpha_s$ and $G\alpha_{i1}$ with detergent-solubilised and nanodisc-reconstituted NTS1 has been observed using EM. Additionally, we have used surface plasmon resonance (SPR) to investigate detergent-solubilised and nanodisc-reconstituted NTS1-NT interactions and to understand better the kinetics of NTS1-$G\alpha_{i1}$ and NTS1-$G\alpha_s$ interfacial interactions. The affinity of NTS1 for its ligand was determined to be 1-2 nM and ∼10 nM for NTS1-$G\alpha_s$ coupling. Ongoing work is designed to extract structural and quantitative data from the interaction.

Abstracts

– Protein Structure and Function –

O-525

Investigating substrate interaction on Type II NADH:quinone oxidoreductase from Escherichia coli

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Type II NADH dehydrogenases are membrane associated proteins that serve as alternative to complex I in catalysing electron transfer from NADH to quinone in the respiratory chain. Recently, the crystallographic structure of this enzyme was solved. Nevertheless, most questions regarding NDH-II functional mechanism are unanswered, such as the interaction with the substrates (NADH and quinones) and the way that FAD is reduced by NADH and oxidized by the quinone. In this work we investigated conformational changes in NDH-II from *Escherichia coli* due to interactions with its substrates. In order to identify the structural elements/motifs involved in such interactions, surface enhanced infrared absorption (SEIRA) spectroscopy was applied. Changes were observed upon interactions with NADH and with different quinones. Interactions with ubiquinone analogue promoted most pronounce conformational changes. The involvement of Trp and Glu/Asn residues in the binding of NADH and ubiquinone is suggested. A comparative study of the differences between ubiquinone and menaquinone bindings was performed. Implications on the catalytic mechanism of NDH-II are discussed.

O-526

The Annexin A2 core domain is identified to induce membrane curvature

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Comprehensive knowledge is gathered about the members of the Annexin protein family. Especially Annexin 2 (AnxA2) is involved in cellular signaling pathways, related to endo-/ exocytotic events on chromaffin granules and early endosomes and plays a key role in membrane dynamics. Recently it has been associated to Hepatitis C virus replication.
The cytosolic AnxA2 and its intra-cellular ligand S100A10 (p11) form a hetero-tetrameric complex, which is located at the inner leaflet of the mammalian plasma membrane and linked via calcium to acidic phospholipids. Membrane dynamics and micro domain formation putatively is mediated through a highly conserved protein core domain.
Here we extend this knowledge by probing the AnxA2 core domain, lacking residues 1-32 of the N-terminus in AnxA2, on giant unilamellar vesicles (GUVs), showing micro domain formation, membrane interconnection and a new, unknown appearance of intra-luminal vesicles (ILVs). We report on an *in vitro* system by Confocal Laser Scanning Microscopy (CLSM) the ILV formation, which is solely mediated through the AnxA2 core domain and identify domains rich in phosphatidylinositol(4,5)-biphosphate and cholesterol. Membrane affinity measurements are substantiated by Quartz Crystal Microbalance (QCM-D).

P-527

Transient fluorescent shifts elucidate enzymes´ active site dynamics-function relationships

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Enzymes' activity and substrate specificity are strongly connected to the active site surroundings and properties. We report a fluorescence method that specifically senses hydration and mobility in the active site vicinity of the dehalogenase (DhaA) enzyme. DhaA is labeled selectively at the tunnel mouth region leading to its active site, which allows the characterization of this biologically relevant area by fluorescence methods. Monitoring "time-dependent fluorescence shifts"[1–3] yields information on hydration and mobility of the dye's microenvironment at this particular site of DhaA. Both parameters are sensitive to the architecture of the tunnel mouth and vary substantially between different DhaA mutants. The role of both hydration and mobility in the enantioselectivity of designed DhaA enzymes will be discussed[4]. Moreover, organic co-solvents' influence on hydration and mobility at DhaA's tunnel mouth and its connection with observed enzymatic activity changes is also presented[5].
1-R. Jimenez, et al., *Nature 1994 (369) 471 ;*2-J. Sykora, et al., *Langmuir 2002 (18) 571 ;*3-A. Jesenska, et al., *JACS. 2009 (131) 494 ;*4-J. Sykora, et al., S*ubmitted ;*5-V. Stepankova, et al., *ChemBioChem 2013 (14) 890*

P-528

Structural analysis of dengue virus capsid protein and its interaction with lipid membranes

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Dengue virus (DENV) causes the major human arbovirosis, for which no vaccine or specific treatment is available. In previous studies we have shown that the interaction between DENV capsid protein (C) and cellular lipid droplets (LDs) is essential for viral replication and depends on the disordered N-terminal region of C protein. This work aimed to get insight on the position of C protein N-terminal region by determining its molecular envelope using small angle X-ray scattering (SAXS); and to evaluate intrinsic parameters of C protein binding to large unilamellar vesicles (LUVs) using isothermal titration calorimetry (ITC). SAXS experiments showed that C protein radius of gyration (Rg = 22,52 A°) is higher than that of the theoretical molecular envelope (Rg = 17,56 A°). This result was supported by the 3D analysis, which allowed the prediction of the position of the disordered N-terminal in DENV C protein structure. ITC experiments revealed a non-hydrophobic interaction between C protein and 1-palmitoyl-2-oleoyl phosphatidylserine LUVs. The results altogether provided new insights on C protein three-dimensional structure and on the biophysical properties of the C protein/lipid membranes interactions, open new possibilities to disrupt this essential interaction for DENV replication.

Abstracts

– Protein Structure and Function –

P-529

HIV-1 protease double mutants analysis by NMA indicates convergence to flexibility of the wild type

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The emergence of drug resistant mutations to the selective pressure exerted by antiretrovirals, including protease inhibitors (PIs), remains a major problem in the treatment of AIDS. During PIs therapy, major mutations are selected reducing both affinity for the inhibitors and the viral replicative capacity compare to protease wild type (wt). Additional mutations cancompensate for this reduced viral fitness. For investigate this phenomenon from the structural changes viewpoint, we combined MD and NMA to analyze the variations of the C-alpha flexibility and h-bond formation of wild type, single and double mutants of HIV-1 PR. Flexibility behavior of the double mutants was significantly closer to the wt than of the related single mutants. All single mutants showed a significant alteration in h-bond formation in comparison to wt. Most of the significantly changes occur in the border of flap/cantilever region. All the double mutants considered have their h-bond formation significantly altered in comparison to their respective base mutant with a probable effect that their flexibility pattern becomes more similar to wt. This methodology can be applied for investigate the structural effects of a large number of mutations in studies involving pathogen drug resistance and fitness.

P-530

Stability analysis of protein kinases

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Protein kinases are involved in regulating diverse and essential cellular processes, and their dysfunction is implicated in many diseases, making kinases key drug targets. Experiment and computational simulations have shown that kinases are often inherently dynamic and flexible molecules. Here we use a recently-developed theoretical method based on graph theory — stability — to analyse the multi-scale structural organisation and dynamics of kinases, and to predict the effects of mutations and interactions. Results are presented initially for the cyclin-dependent kinases (cdks), which are key regulators of the mammalian cell cycle and potential therapeutic targets for cancer. The cdks are highly conserved in sequence and structure, especially in their active sites, and it has traditionally been difficult to explain the differences in function between them and to design selective inhibitors. Our results include predictions for potential loss-of-function mutations, as well as dynamic information that may be relevant to currently unanswered questions such as the loss of function of the analog-sensitive cdk2 mutant. We also consider other kinase families, including the src family kinases, and the histidine protein kinases in fungal plant diseases and fungicide resistance.

P-531

Bound water plays a central role in ligand binding and function in the Catalase enzyme

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Molecular recognition is central to protein function but observing the chemical details of ligand-binding to proteins remains a major challenge. Important questions arise that relate to the role that structural dynamics play within the structure-function relationship and the involvement of bound water molecules. We report studies of the active site processes involved in the inhibition of the Corynebacterium glutamicum catalase enzyme by nitric oxide using a combination of techniques sensitive to both static structure and ultrafast dynamics. X-ray crystallography reveals a chain of hydrogen-bonded bound water molecules that interact with the NO ligand and the protein scaffold, while ultrafast two-dimensional infrared spectroscopy (2D-IR) experiments reveal that this interaction results in a dynamically-restricted, 'tight,' active site. The combination of bound water and restricted dynamics is in marked contrast to structurally-similar haem proteins that possess no peroxidase functionality. Our results demonstrate that these water molecules are central to molecular recognition of NO and the peroxide substrate and an important feature of the catalase mechanism.

P-532

A derivative of the natural compound kakuol affects DNA relaxation of topoisomerase IB

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Topoisomerases IB are anticancer and antimicrobial targets whose inhibition by several natural and synthetic compounds has been documented over the last three decades. Here we show that kakuol, a natural compound isolated from the rhizomes of *Asarum sieboldii,* and a derivative analogue are able to inhibit the DNA relaxation mediated by the human enzyme. The analogue is the most efficient one and the inhibitory effect is enhanced upon pre-incubation with the enzyme. Analysis of the different steps of the catalytic cycle indicates that the inhibition occurs at the cleavage level and does not prevent DNA binding. Molecular docking shows that the compound preferentially binds near the active site at the bottom of the catalytic residue Tyr723, providing an atomistic explanation for its inhibitory activity.

Abstracts

– Protein Structure and Function –

P-533

Structure and dynamics of the proline-rich microtubule binding motif on the neuronal protein tau

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The Alzheimer's disease-associated protein tau is a typical representative of intrinsically disordered proteins (IDPs). Under physiological conditions, tau associates with microtubules and regulates their dynamics, whereas during the progression of neurodegeneration tau dissociates from microtubules, misfolds and creates highly insoluble deposits. The insights into the structural propensities of tau regions responsible for microtubule binding are therefore very important. The monoclonal antibody Tau5 was used as surrogate tau protein binding partner to investigate the properties of tau-microtubule binding hotspot. The affinity and binding enthalpy and entropy of its interaction with full length and truncated tau variants were evaluated from the kinetic measurements obtained with the surface plasmon resonance. To obtain a X-ray structure of this region of tau, the antibody Fab fragment was crystallized with 30 amino acid long tau peptide Gly(201)-Arg(230) and the structure was solved to the 1.69 Å resolution. 13 residues from the tau peptide can be modeled in the complex structure that reveals several important features for which only propensities were previously observed by NMR. *Acknowledgement. This work was supported by the Slovak Research and Development Agency No. LPP-0038-09*

P-534

Structural evidence for a two-regime photobleaching mechanism in a reversibly switchable fluorescent

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Reversibly switchable fluorescent proteins are largely used in super-resolution optical imaging techniques. However, photobleaching events that eventually occur after several on-off cycles in RSFPs have become the crucial limiting parameter of these techniques.
IrisFP is the first fluorescent protein which combines both the photoswitching and photoconvertion properties, which makes IrisFP a good candidate as biomarker for super-resolution imaging. In our recent work, we have identified a two-regime photobleaching mechanism in the photoswitchable fluorescent protein IrisFP. A redox-dependent photobleaching pathway was found to take place when the power density of the exciting light is typically that of a standard PALM experiment. The highly conserved glutamate 212 undergoes a decarboxylation. Such modifications result in a disordered chromophore and a rearrangement of the H-bond network around it. A second photobleaching pathway, oxidation-dependent, was evidenced at much lower power density. This pathway involves the sulfoxidation of two key residues close to the chromophore: M159 and C171.

P-535

Probing G protein-coupled receptor dimerisation by FRET and DEER

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G protein-coupled receptors (GPCRs) are the largest class of eukaryotic membrane proteins, and are of great pharmaceutical interest, with approximately 40% of drugs targeting GPCRs. It has been shown that GPCRs can form oligomers in phospholipid bilayers both *in vivo* and *in vitro*[1].
Neurotensin receptor 1 (NTS1) is one of few GPCRs that can be produced in *E. coli* in an active state, and has been implicated in conditions such as schizophrenia and Parkinson's and postulated as a biomarker for various cancers. NTS1 has been shown to dimerise in lipid bilayers[2], and though a crystal structure of NTS1 in detergent was recently published[3], there is still no structural data on the receptor and its dimer in a membrane environment.
We are using Förster resonance energy transfer (FRET) and double electron-electron resonance (DEER) to obtain structural information on NTS1. Fluorescence or nitroxide spin probes are attached to engineered cysteines on the transmembrane helices. By measuring intradimer distances between the probes on each monomer, we aim to produce a model of the dimeric structure in a more native lipid environment.
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P-536

Intramolecular diffusion of gas inhibitors in Ni-Fe hydrogenase as viewed through EPR spectroscopy

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Hydrogenases are enzymes that reversibly catalyze the oxidation of molecular hydrogen into protons and electrons in microorganisms. Due to their promising applications in the field energy, these systems have been extensively studied for many years. A special attention was devoted to understand the inhibition processes of these enzymes, especially with O_2, in light of their potential use for biohydrogen production and biobattery [1-2].
In Ni-Fe hydrogenases, hydrophobic channels connecting the buried active site to the protein surface have been revealed by crystallography. These channels enable the intramolecular diffusion of gases (H_2 or inhibitors as O_2, CO or NO) which constitute a crucial step for the inhibition mechanism [2]. By using small diatomic paramagnetic compounds, we have investigated by EPR the interaction mechanism of the Ni-Fe *D. fructosovorans* enzyme with these inhibitors. Thanks to the different EPR signals given by the metal centers, we evidence their different sensitivity to the deleterious effect of NO, and show that the diffusion of O_2 within the enzyme can be followed by studying the spin-lattice relaxation property changes of the Ni-Fe cofactor.
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[2] Liebgott et al., Nat. Chem. Biol. 2010

Abstracts

– Protein Structure and Function –

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New insights in the translocation mechanism of ciprofloxacin revealed by fluorescence quenching

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Fluoroquinolones, antibiotics with a large spectrum of action against Gram negative and some Gram positive bacteria, might penetrate the bacterial outer membrane through porins or through the lipid/protein interface. OmpF is one of the most important porins of Gram negative bacteria involved in the translocation of fluoroquinolones trough the bacterial membrane. OmpF assumes the conformation of homo-trimer, whose monomers have two fluorescent tryptophan (Trp) residues, one located at the trimer interface (Trp^{61}) and the other at the lipid/protein interface (Trp^{214}). Thus, we proceeded to study the interaction of Ciprofloxacin, a second generation fluoroquinolone, with OmpF *E. coli* total extract proteoliposomes. Our study was carried out with proteoliposomes of native protein and two OmpF mutants (which lack one of each Trp, substituted by Phenylalanine). The association of Cpx with OmpF was determined by the quenching of the intrinsic protein fluorescence in the presence and absence of iodide and acrylamide (two quenchers that provide information about the Trp surrounding environment), under physiological conditions (T=37°C; pH 7.4). The membrane mimetic systems used were characterized by DLS and fluorescence anisotropy.

P-538

Structure/function studies of vanadium iodoperoxidase from the bacterium Zobellia galactanivorans

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In marine organisms, vanadium dependent haloperoxidases (vHPO) are involved in the production of halo-metabolites and classified in three groups (chloro-, bromo- and iodoperoxidase) according to the most electronegative halide that they oxidize. Whereas vHPO have highly analogue active sites, the molecular bases of their halide specificity are still unknown. In the team, we have solved the first structure of a vHPO specific to iodide, identified from the marine bacterium *Zobellia galactanivorans* (ZgIPO). Through a directed mutagenesis approach, residues in the active site have been targeted to modify the halide specificity of ZgIPO. The results of biochemical and steady-state analysis of the recombinant mutant enzymes are compared to those obtained with the wild type enzyme. In parallel, the electronic environment of the vanadium was determined by X-ray absorption spectroscopy for the native and reactive intermediate forms of ZgIPO and show modification in the coordination sphere of the V compared to other vHPO. These biochemical and structural studies will contribute to the understanding of the mechanisms of halide specificity in vHPO.

P-539

Fluoroquinolone complexes as metalloantibiotics: a fluorescence spectroscopy study

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Fluoroquinolones are antibacterial agents used currently against a wide variety of infections. Due to their extensive use, there has been an increasing threat of bacterial resistance to fluoroquinolones, which led to the need to improve existing antimicrobial drugs and/or develop new ones. Pushing forwards the concept that metal complexes could be an alternative to conventional drugs, as novel derivatives of fluoroquinolones, the study of fluoroquinolones–copper–phenanthroline complexes has become an increasingly field. These complexes seem to show high affinity towards DNA as well as nuclease activity towards plasmid, genomic and internucleosomal DNA. Our work focuses on biological and biophysical characterization of these complexes using a systematic and multidisciplinary molecular approach that can help to improve our understanding of the parameters that govern their translocation through bacterial membranes. Biological studies were performed in several bacterial strains that have a series of porin mutants which lack one or several major outer membrane proteins (OmpF, OmpC, etc). Based on these biological experiments, the *in vitro* biophysical study of complex-porin interactions using purified porins in proteoliposomes has been performed using fluorescence spectroscopy.

P-540

Transient kinetics show isomerisation steps in the kinetic pathway of Isopropylmalate Dehydrogenase

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3-Isopropylmalate dehydrogenase (IPMDH) is an essential enzyme in the leucine biosynthesis pathway of bacteria, yeast and plants. Because this pathway is absent in human, IPMDH may constitute a potential target for inhibition against pathogenic bacteria. Thus, detailed knowledge of the catalytic mechanism of IPMDH, including enzyme kinetics, are required. To identify the rate limiting step(s) of IPMDH catalysed reaction, time courses of NADH production were followed by stopped flow (SF) and quenched flow (QF). A burst phase of the NADH formation was shown by QF, indicating that the rate limiting step occurs *after* the redox step. The kinetics of protein conformational change(s) induced by the complex of Mg IPM were followed using a FRET signal formed by the Fluorescence Resonance Energy Transfer from protein tryptophan(s) to the bound NADH. A reaction scheme was proposed by incorporating the rate constant of a fast protein conformational change (possibly domain closure) derived from the separately recorded time-dependent formation of FRET. The limiting step of the steady state rate seems to be another slower conformational change (domain opening) that allows the product release.

Abstracts

– Protein Structure and Function –

P-541

Impacts of the neuronal tropomyosin isoform TMBr-3 on actin dynamics

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The microfilament system underlies a great diversity of cellular processes, including cell motility, intracellular transport, contractility, cell size and shape, cytokinesis and transformation. Structurally, microfilaments are composed of actin, mostly associated with tropomyosin (TM), a lateral binding coiled-coil protein. Both proteins exist in several isoforms, which assemble into many distinct microfilament subcompartments with specific composition. TMs are present in all eukaryotic cells and display a surprising variability: in mammals four genes are known, that produce about 25 protein isoforms *in vivo*. We found that the neuron specific TMBr-3 isoform bound F-actin with low affinity. It decreased the rate of actin filament depolymerization induced by dilution; in contrast, it did not change the polymerization kinetics of G-actin alone or accelerated by Arp2/3 and VCA. These results suggest, that the binding rate and the azimuthal position around the actin filament may be different for TMBr-3 than for skeletal muscle tropomyosin.

P-542

Calcium binding domain is essential for the Bmh1 dependent catalytic trehalase activity of Nth1

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The activity of neutral trehalase Nth1 from the yeast *Saccharomyces cerevisiae* is regulated by PKA phosphorylation, calcium and Bmh1 (yeast 14-3-3) protein binding. We used HDX-MS to study the structural changes of pNth1 upon Bmh1 and/or calcium binding. Four one-point mutations in the Nth1 calcium binding domain were prepared and tested for catalytic trehalase activity and Bmh1 binding (analytical ultracentrifugation, native gel electrophoresis). Far and near UV-CD spectroscopy was used for verifying the changes of both secondary structure of individual Nth1 mutant proteins and their tertiary structure upon Bmh1-binding. Our experiments revealed that residues D114 and D125 are crucial for the correct catalytic activity of Nth1. Supported by Grant P207/11/0455 and Research Project 644313.

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P-543

Sedimentation analysis and enzymatic characterization of the complex between 14-3-3 protein and ASK1

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ASK1 (Apoptosis signal-regulating kinase, MAP3K5) is a key molecular trigger in JNK or p38 dependent signaling pathways. Its activation in the presence of reactive oxygen species, anticancer drugs or during immune responce induces apoptosis and so far it has been connected with several neurodegenerative or cardiovascular diseases, diabetes and cancer development. The activation is tightly regulated by number of inhibitory/activating mechanisms. The 14-3-3 protein has been identified as one of the most important physiological regulator. It binds to phosphoserine 966 keeping the kinase inactive. It was shown that ASK1 is activated after dephosphorylation and disociation of 14-3-3 as a response to exposure to reactive oxygen species. The molecular mechanism of this interaction is still unknown.

Here we report the sedimentation velocity analysis of the complex between 14-3-3 and recombinant kinase domain of ASK1. We estimated stochiometry of the comlex as well as apparent Kd. We also performed basic enzymatic characterization which confirmed that kinase domain of ASK1 is inhibited directly by 14-3-3.

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P-544

Mechanistic studies of the Ras-superfamily by time-resolved FTIR spectroscopy

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The Ras protein is mutated in 30% of human tumors. Ras acts as a switch, transmitting a growth signal in an active GTP-bound form and turning the signal off in an inactive GDP-bound form. The switch-off is accomplished by GTP hydrolysis, which is catalyzed by Ras and can be further accelerated by GTPase activating proteins (GAPs). Mutations which inhibit hydrolysis cause severe diseases including cancer. We investigated the reaction of the Ras GAP protein-protein complex by time-resolved FTIR spectroscopy by means of caged-GTP.[1]

Further, we optimized an ATR-FTIR system based on a germanium crystal to perform stimulus induced difference spectroscopy of monolayers without the surface enhance effect. We synthesized linker molecules which allow for stable attachment of proteins via tags.[2] In the flow thorough system protein-ligand, protein-protein and protein-drug interactions can be investigated. Alternatively a model membrane can be attached to the germanium and membrane anchored proteins such as Ras can be investigated in its natural environment. We found that N-Ras dimerizes at a POPC model membrane.[3]

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Abstracts

– Protein Structure and Function –

P-545

Non-equivalent binding properties of carbohydrate binding domains of human galectin 4

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Galectins are members of the lectin family that is characterized by its affinity for β-galoctosides. They are widely distributed in normal and neoplasic cells of different organisms and are involved in a great diversity of cellular mechanisms such as cell-surface glycans interactions, cell adhesion and apoptosis. The malfunction of galectins is associated with tumor progression and human carcinogenesis, which has turned galectins into a very promising tool for cancer diagnosis and treatment. Human galectin-4 (HGal-4) is a tandem-repeat with two carbohydrate recognition domains (CRD-I and II). Here, we present studies concerning the response of HGal-4 and of its domains to the presence of different carbohydrates. Circular dichroism was used to monitor conformational changes and thermal stability of the proteins in the absence/presence of different carbohydrates. CD results showed that the presence of carbohydrates does not alter the protein structure, although increasing its thermal stability. Intrinsic Trp fluorescence was used to look for structural alterations in the carbohydrate binding site. Overall the results obtained so far suggest that each domain responds differently depending on the sugar type and, consequently, indicate a non-equivalent role in protein function.

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Interaction between the neuronal SL21 protein and calmodulin

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SL21 is a neuronal protein with a calmodulin-binding and microtubule-stabilizing activity. We have used a 44 residue peptide of SL21 protein, with microtubule binding activity. Our absorption, emission and circular dichroism measurements indicate that the peptide's tertiary structure is determined by the aromatic amino acids Trp, Tyr and Phe bound into chiral complexes. We also found that the peptide's secondary structure is thermally stable and depends on concentration, whereas the tertiary structure changes with temperature. Using the software packages CDPro and DICHROWEB, we obtained the percentages of the secondary structure elements: 55.1 % β-sheets, 18.75 % turns, 15.2 % unordered regions and 10.85 % α-helices. Using the ExPASy server, we also made a bioinformatics investigation of the peptide, obtaining results in agreement with experimental data. Electron microscopy showed that the SL21 peptides associate into clusters. Isothermal titration calorimetry measurements indicated that calmodulin binds to the SL21 peptide with a low affinity, in Ca^{2+}-independent manner.

P-547

Transition between PrPC and PrPSc studied using normal mode analysis and excited molecular dynamics

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In this work, we propose a new protocol to study the conformational transition between the PrP^C and its isoform PrPSc using Normal Modes Analysis, and carrying out Molecular Dynamics simulations inwhich the normal mode motions are kinetically activated. This is achieved through a script used withthe CHARMM program in which different random linear combinations of low frequency modes aregenerated, and which are kinetically excited by assigning velocities along them. The normal modes are first calculated for the structure of PrP^C (PDB_ID:1HJM). The MD simulations for PrP^C are carried out at different temperatures in the normal mode space, and at room temperature in the Cartesian space, within a water box at constant temperature and constant pressure conditions. A series of simulations with different excitation energies in the normal mode space were carried out and statistically analyzed. The initial results showed an increase of the length of $\hat{I}^2$-sheet which corresponds to the conformational transition between the PrP^C and PrP^{Sc}. These results showed that the simulation method that we developed could be used for studying such conformational changes very efficiently.

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Formation of helical structures in homopolymer chains using the multi-canonical MC simulation

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We study the coil-helix transition in homopolymer chains using the multi-canonical Monte Carlo simulation. The method allows to obtain a flat histogram in energy space and visit all possible energy states. We employed the potential proposed by Kemp and Chen [Phy. Rev. Lett. 81, 3880(1998)] for the helix formation and investigate the thermodynamic and structural properties of the homopolymer chain. We are particularly interested in the behavior of the chain at low temperatures and for longer sizes above N=51.

Abstracts

– Protein Structure and Function –

P-549

Determinants of selective group reduction in the TNT-bound xenobiotic reductase B from P. putida

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Xenobiotic reductases of the OYE family of flavoproteins have been shown to reduce the nitroaromatic explosive TNT. Most have nitroreductase activity, reducing the nitro side groups to hydroxylamine derivatives. Xenobiotic reductase B has the additional capacity to reduce the aromatic ring of TNT to form non-aromatic Meisenheimer complexes, which is associated with nitrite release, with important implications in TNT degradation. It is crucial to determine which structural elements interact with the substrate to give either one product (hydroxylamine derivatives) or the other (Meisenheimer complexes). Residues around the active site of XenB are involved in the functional differences between the family members, and the TNT-bound crystal structure, together with the structures of relevant mutants also reported, has helped to elucidate this. This study was supported by the Consolider-Ingenio del MEC 2010 CSD2007-00005, the Comisi—n Mixto CSIC(Espa–a) - FCT (Portugal) 20094PT0044, the Spanish Ministry of Economy and Competitiveness BIO2010-17227, and the Portuguese Science and Technology Foundation through grants PEst-C/EQB/LA0006/2011 and SFRH/BPD/30142/2006.

P-550

Allosteric transitions in the NO-receptor guanylate cyclase revealed by time resolved spectroscopy

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The mammalian nitric oxide receptor guanylate cyclase and other NO-sensors and NO-binding heme proteins were studied by time-resolved absorption spectroscopy. The triggered event is the cleavage of Fe-NO or Fe-CO bond to the heme iron and the response of the heme is recorded in a time-range which encompasses twelve orders of magnitude from 1 picosecond to 1 second. The transient spectra allowed to identify intermediate species corresponding to allosteric states of guanylate cyclase whereas the transitions are identified in kinetics from 1 ps to 1 s. We have assigned all structural transitions, including rebinding of the proximal His, the formation of 6-coordinate heme-NO, the breaking of the proximal His-Fe bond and the allosteric structure change in the entire protein. For five NO-binding heme proteins, we have demonstrated by transient Raman spectroscopy that the release of NO from the heme-iron does not induce an instantaneaous motion of the central Fe atom of the heme. The motion of Fe is retarded (by 6 ps to 40 ps depending on the heme protein) because of allosteric constraints exerted by the protein on the proximal histidine.

Yoo et al. (2012) *ACS Chem. Biol.* **7**, 2046.
Yoo et al. (2012) *J. Biol. Chem.* **287**, 6851.
Kruglik et al. (2010) *Proc. Natl. Acad. Sci. USA.* **107**, 13678.

P-551

Physiological and Biochemical insights into the E. coli cytochrome c peroxidase

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During infection, pathogenic microorganisms are frequently exposed to oxidative stress due to reactive oxygen species (ROS) generated by host defense mechanisms, e.g. superoxide anion, hydrogen peroxide and hydroxyl radicals. It has been shown that bacterial cytochrome c peroxidases (CCPs), usually diheme *c*-type enzymes, catalyze the reduction of hydrogen peroxide to water and play a key role in virulence in pathogenic bacteria, protecting these microorganisms against ROS.

The *yhjA* gene from *E. coli* encodes a putative CCP, which is proposed to be attached to the cytoplasmic membrane, and has the distinct feature of containing three instead of two *c*-type heme-binding motifs, with the extra heme being located at the N-terminus. A homology search shows that this gene has a high occurrence in the genome of pathogenic bacteria (*Salmonella* and *Yersinia*), but its physiological function remains unknown. The CCP gene was cloned and the protein homologous expressed for further biochemical characterization. In order to gain further biological insights we have characterized the growth of knockout mutants of *yhjA* and other genes related to oxidative stress, in the presence/absence of stress reagents, in aerophilic and microaerophilic environments.

P-552

Interaction between human protein S100A9 and the parasite Schistosoma mansoni's protein MEG14

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MEG14 is a protein encoded by a micro-exon gene (MEG) from the parasite *S. mansoni*, a causative agent of schistosomiasis. The MEGs are capable to produce a pool of variant secreted proteins by alternative splicing of micro-exons. Previous studies using the protein MEG14 as bait against a library of human leukocytes in yeast two-hybrid assay showed that S100A9 protein is a potential partner for protein MEG14. It has been previously described that S100A9 protein plays a role in the modulation of human immune system. This interaction was confirmed in a "Pull Down" experiment. In order to obtain further information about this interaction, the technique of Isothermal Titration Calorimetry was used and the resulting thermogram indicates that the interaction between the proteins is an exothermic process. The binding curve shows that the process has positive entropy and negative enthalpy suggesting that this reaction is entropically driven. The data indicated an affinity constant at the order of micro molar ($K_d = 2.10^{-6}$) and a stoichiometry of 1:1. Further experiments are being performed to confirm and understand how this interaction occurs. This data might provide better understanding of host-parasite relationship and provide insights about the modulation of immune system from humans.

Abstracts

– Protein Structure and Function –

P-553

Dielectric properties and hydration of human and bovine serum albumin complexes with chlorophyllin

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Water is known to play an important role in the stabilization of protein-ligand interaction. The water molecules can serve as bridges between protein and ligand, and interact with other water molecules to form a network of hydrogen bonds. Such water networks are essential for the protein-ligand complex to be stable and are important for any site-directed drug design strategies.
The interaction between chlorophyllin, a potent inhibitor of experimental carcinogenesis, and human and bovine serum albumins was investigated using differential dielectric method. This method allows studying both dielectric characteristics and hydration of the compounds under investigation.
We show that for all complexes the process of complex formation is accompanied by changes of dielectric parameters. We also present calculations of the hydration of proteins and their complexes with ligand, which were carried out basing on the model concepts. We found that the extent of hydration for all complexes is not equal to the sum of hydration of their components taking into consideration their relative concentration. It indicates that redistribution of free and bound water molecules accompanies formation of complexes. The possible causes of effects observed are discussed.

P-554

THz spectroscopy studies on proteins: exploring collective modes of amyloid fibrils

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The application of THz spectroscopy to biological samples is subject of renewed interest since it promises to give important information on protein dynamics by probing delocalised vibrational modes that depend on the overall structure. THz absorption spectroscopy provides a sensitive tool for probing proteins fast hydration water dynamics (ps range).
Based on the importance of the relationship between protein dynamics, involving side-chains and backbone fluctuations, and protein function and in the light of the functional relevance of amyloidal aggregation, it is of utmost importance to study the dynamical behaviour of proteins in their aggregated state.
We present here a THz study of Concanavalin A (ConA) amyloid fibrils in comparison with the native and the amorphous aggregated state. The study of ConA aggregates is of interest for a range of scientific applications spanning from medicine to biophysics.
Our results give significant information on the coupling between protein motions and hydration water in the different states, remarking the dependence on the structural features of samples of the spanning network of H-bonds between water molecules in the hydration shell. A structural characterization of the aggregates probed by mid IR and Raman spectroscopies is also presended.

P-555

Study of the cohesin-dockerin interaction and its role in the C. thermocellum cellulosome assembly

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Cellulosomes are elaborate nanomachines, capable of degrading efficiently two abundant polymers, cellulose and hemicellulose. The complex assembles through the high-affinity cohesin-dockerin interaction, whose specificity allows the incorporation of cellulases and hemicellulases onto a molecular scaffold. The spatial proximity of the enzymes and the enzyme-substrate targeting are responsible for the high efficiency of this multienzymatic complex. Both cellulosome assembly and cell-surface attachment are mediated by the cohesin-dockerin interaction which has an inherent spatial flexibility that may contribute also to the cellulosome catalytic synergy.
The nine cohesin domains present in *C. thermocellum* Cellulosome-integrating protein (CipA) display significant differences at the level of their primary structure, especially the first, second and ninth cohesins (present in the extremities of the scaffoldin) which are the most divergent, comparatively to the cohesins present inside the protein. By non-denaturing acrylamide gels and isothermal titration calorimetry, the dockerins were shown to have different specificities towards the various cohesins from the CipA scaffoldin. The different specificities may confer quaternary flexibility to the cellulosome, contributing to its high catalytic efficiency.

P-556

Subnanosecond resolution to discover ultrafast transient states of molecules. Regional core facility

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In our region, studies on molecular state reorganisations in the subnanosecond time regime was not available untill now. The recent developement of a new transient absorption spectrometer permits us to reveal the kinetics of structural or electronic state reorganisations following the processes through absorbance change measurements.
The technique is well known, the realised spectroscopic systems vary from one to another instrument in some technical parameters. Our system has a 100 fs pulsed laser pumping (Spectra Physics Mai Tai laser, @850-1050 nm) that results in a final subpicosecond time resolution at 1 kHz repetition rate (SpitFire Ace amplifier). After continuum generation, the short pulsed white light is transmitted through the sample either in pumped or not pumped states. The corresponding light intensity distributions read from a fast Andor Newport CCD array are used to calculate the absorbance differences using a NI Labview software that serves as the system controller, data aquisition and basic data analysis software.
Our system is used to reveal further information on the role of the tryptophanes in the electron donor chain of the photolyase and AppA proteins, known light sensitive proteins including a BLUF (blue light sensing using FAD) domain.

Abstracts

– Protein Structure and Function –

P-557

Global protein motions in the allosteric regulation of CRP/FNR family transcription factors

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Allostery is a fundamental process by which ligand binding to a protein alters its activity at a distinct site. There is growing evidence that allosteric co-operativity can be communicated by modulation of protein dynamics. We provide a foundational theory for how allostery can occur as a function of low frequency dynamics without a change in protein structure. We have generated coarse-grained models that describe the protein backbone motions of the homodimeric CRP/FNR family transcription factors, Catabolite Activated Protein (CAP) and GlxR. We demonstrate that binding the first molecule of cAMP ligand modulates the global normal modes resulting in negative co-operativity for binding the second cAMP ligand without a change in mean structure. The theory makes key experimental predictions and these are validated through an analysis of variant proteins by a combination of structural biology and isothermal calorimetry. A quantitative description of allostery as a free energy landscape revealed a protein 'design space' that identified the key inter- and intramolecular regulatory parameters that contribute to CRP/FNR family allostery.

P-558

Vanadium compounds as prospective therapeutics: X-ray structure of protein adducts

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In the last years, the presence of vanadium and vanadium complexes in biological systems has been studied due to their possible physiological roles as insulin-enhancer and as anticancer and anti-parasitic agents [1].
Among these actions, the potential benefits of vanadium compounds as oral insulin substitutes for the treatment of diabetes are especially studied. Several investigations have focused on the development of new and better insulin-enhancing compounds as well as on understanding the mechanism of their action. The role of the coordinated ligands is particularly relevant since these should be able to improve the absorption and possibly the transport and uptake of vanadium to the cells, reducing the dose necessary for producing an equivalent effect [1].
Our aim is to characterize structurally several V compounds interacting with human serum transferrin (hTf), its putative physiological carrier, responsible for its in vivo transport and distribution. X-ray structures of hen egg white lysozyme (HEWL) and hTf (at 1.3 Å and 3.6 Å resolution respectively), have already been obtained bound to some $V^{IV}O(carrier)_n$ complexes. Conformational changes on these adducts will be addressed using different techniques.
[1] Mehtab, S. *et al*, *J Inorg Bio,* **2013**, *121*, 187-195.

P-559

Structural rearrangements occurring on HCN2 CNBD domain upon cAMP binding

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Hyperpolarization-activated cyclic nucleotide-regulated (HCN 1-4) channels are the molecular determinants of the Ih/f current and control neuronal excitability and pacemaking [1].
HCN channels are dually activated by membrane hyperpolarization and binding of cAMP to their cyclic nucleotide binding domain (CNBD) [1], which is constituted by an eight-stranded antiparallel ß-roll, preceded by the A a-helix and followed by the B and C a-helices. The CNBD is connected to the channel transmembrane region through a six-a-helix (A'-F') folded domain called the C-linker [2]. The C-linker/CNBD of human HCN2 in complex with cAMP was crystallized and its structure solved [2]. However, the structure of the cAMP-unbound form of this protein is not yet known. Using NMR we determined an atomic-level model for the 3D structure of the human HCN2 D'-F'(C-linker)/CNBD in the cAMP-unbound form. This achievement provides insights into the structural rearrangements that occur on the CNBD domain upon cyclic nucleotide binding and that eventually lead to cAMP-modulation of the channel activity.
[1] Robinson & Siegelbaum (2003) Annu Rev Physiol **65**:453-480 [2] Lolicato M et al (2012) J Biol Chem **286**(52):44811-20

P-560

Quinone interaction on Type II NADH dehydrogenase - molecular selectivity for different substrates

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Type II NADH: quinone oxidoreductase (NDH-II) catalyzes the same reaction as respiratory Complex I, but does not directly contribute to the establishment of the membrane potential. The mode of interaction of NDH-II with the substrates, the nature and localization of the binding sites for different types of quinones remains unknown.
NDH-II is a promising target for the development of new drugs against pathogenic bacteria, since this protein is absent in humans, so understanding the structure/function relation, mainly the determinants of specificity for different substrates, will help searching efficient treatments against infectious diseases.
Therefore, the main purpose of this work is to investigate protein/ligand interaction. We expressed NDH-II from two organisms, *Escherichia coli* and *Staphylococcus aureus*, and performed fluorescence spectroscopy studies to determine substrate dissociation constants (KD) taking advantage of the tryptophan intrinsic fluorescence.
We observed that the substrates showing the lowest KD values were the quinones with a longer side chain and hypothesize that it helps orienting the substrate to the binding site. Enzymatic kinetic assays were also done to elucidate the catalytic mechanism.

Abstracts

– Protein Structure and Function –

P-561

Structure and dynamics of the human iron-binding protein Lactoferrin in solution

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Lactoferrin is an iron-binding protein with antimicrobial activity as part of the innate immune system. It consists of two domains, each with a iron(III)-binding site located in a cleft. The reversible iron-binding is supposedly connected with a conformational change of the clefts [1]. Our study aimed to elucidate the link between iron-binding, conformational change and domain dynamics in solution. A combined approach of small angle neutron scattering (SANS) for structural characterization and neutron spin echo spectroscopy (NSE) to elucidate the dynamic properties of different binding states was undertaken. The data of the SANS experiments were compared with calculations from 3D structures (crystallography and homology). The result proves that the binding site clefts are closed when occupied and open otherwise. The evaluation of the NSE data was based on the methodology by Biehl et al [2] involving the normal modes of deformation. It was found that the dominant internal dynamics are independent of the conformation, with relaxation times on the 50ns scale and displacements of <0.7nm. The prevalent motions are overdamped relative movements of the main domains like stretching and twisting. [1] Anderson et al., Nature, 1990 [2] Biehl et al., Soft Matter, 2011

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Efficient assembly of nanoliter crystallization screens with handheld motorized pipette

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High-throughput crystallization screening in multiwell format revolutionized the field of X-ray crystallography. However, reliable assembly of nanoliter drops has required the use of robotics. We have developed a protocol for the reproducible manual assembly of nanoliter-sized protein vapour-diffusion crystallization trials in a 96/192-drop format. The protocol exploits the repetitive pipetting mode of motorized handheld pipettes and needs no additional expensive instrumentation. Determination of precision of pipetting crystallization solutions with different viscosity proved that the handheld pipetting is comparable to robotics. We have tested the reproducibility and robustness of the protocol by repeated crystallization of an antibody Fab fragment in sitting drop on 96-well plate. We believe that the method could be useful especially for small crystallographic groups since it needs no additional expensive instrumentation [1]. We envisage that the use of motorized handheld pipettes would confer similar advantages also in the screening of crystallization conditions for organic molecules.
Support: Slovak Research and Development Agency grant No. LPP-0038-09 and VEGA grant No. 2/0163/13.
[1] Skrabana, R., Cehlar, O. & Novak, M. (2012). J. Appl. Cryst. 45, 1061–1065.

P-563

Investigation of the dynamics of ion translocation subunits from respiratory complex I

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Complex I is part of the respiratory chain and, therefore, a key protein in energy production. The high resolution structure of the entire complex was recently determined, but its catalytic mechanism is still poorly understood. Respiratory Complex I is a L-shaped protein, formed by a hydrophilic and a membrane domains. The charge translocation machinery is incorporated in the membrane domain and includes three large homologous antiporter-like subunits: NuoL, M and N. NuoL has 14 conserved transmembrane (TM) helices, with two antiparallel repeats of 5 TM each (half-channels), which are typical of secondary transporters. It has been accepted that the coupling ion in Complex I is the proton. Recently we observed also Na^+ transport in Complex I from some bacteria, in a Na^+/H^+ antiporter manner. This raised new questions regarding the transport by the subunits from Complex I. Our goal is to investigate the dynamics of H^+ and Na^+ transport in the NuoL subunit.
In this work, we study the structural antiparallel repeats of NuoL. Different constructs, carrying the two repeats together or separately, were expressed in *E.coli* and different biophysical techniques are used to understand the dynamics of that antiporter-like subunit.

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Structural determinants of superoxide reduction

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Superoxide radical $O_2^{\cdot -}$ is the univalent reduction product of molecular oxygen, known to be involved in a variety of cell toxicity mechanisms. While aerobes contain several antioxidant defence systems, such as superoxide dismutases, anaerobes and microaerophiles may depend only on the recently discovered superoxide reductases to keep oxygen toxic species below poisonous thresholds. Superoxide reductases are 14 kDa mononuclear iron proteins and are classified according to the number of iron centres, as 1Fe-SOR (neelaredoxins-Nlrs) or 2Fe-SOR (desulfoferrodoxins). SORs have a catalytic non-heme iron centre coordinated in a square-pyramidal geometry to four histidines in the equatorial plane, and a fifth axial position occupied by a cysteine. In the SOR resting state (oxidized form) a glutamate residue is present as sixth ligand, completing an octahedral geometry. The active center of SORs is present in a domain common to both 1Fe- and 2Fe-SORs. Our research focuses on the molecular mechanism of 1Fe-SORs, and aims to understand the role of the conserved key glutamate and lysine residues, proposed to return the enzyme to its oxidized resting state and to direct superoxide to the active site, respectively. Herein we present the crystal structures of two wild-type SORs.

Abstracts

– Protein Structure and Function –

P-565

Studies of natural polyphenols as potential epidermal growth factor receptor inhibitors

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Epidermal Growth Factor Receptor (EGFR) is a membrane receptor with an intracellular tyrosine kinase activity, involved in processes like cell proliferation, migration and differentiation. Interest in EGFR increased strongly following the finding that its excessive signaling, due to overexpression or mutations, interrupts the equilibrium between cell growth and apoptosis, leading to the development of solid tumors. EGFR has therefore become a key target of the pharmaceutical industry.
Our studies focus on investigating structure-function relationships of natural polyphenols as novel EGFR inhibitors. We selected a set of polyphenol molecules on the basis of their structural similarity with curcumin, known to inhibit EGFR activity. Their effects were studied in different environments: in solution, in biomimetic membranes and in human tumor cell lines. The specificity of their action on the tyrosine kinase or on the ligand binding domains of EGFR was determined. For curcumin, we observed a higher inhibition level in membrane environments than in solution. This result suggests a dual action mode: curcumin acts directly on the tyrosine kinase domain, but also on membrane fluidity, thus impacting EGFR motility, which is crucial for its activation.

P-566

Serial femtosecond crystallography at free electron lasers and synchrotrons

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The method of serial femtosecond crystallography (SFX) merges thousands of single crystal diffraction patterns to get the three-dimensional structure of the protein. Our group and collaborators developed SFX to exploit the intense, femtosecond X-ray pulses offered by Free Electron Lasers (FELs). The FEL pulses allow overcoming conventional radiation damage limits and so crystals too small for synchrotron radiation sources can therefore be used for SFX. One of the methods that gives many naturally small crystals is *in vivo* crystallization in cells. The application of the SFX technique to *in vivo* grown Cathepsin B crystals allowed us to solve the first new high-resolution biological structure with X-ray FEL pulses (L.Redecke *et al.* Science 339: 227-230), which was named by *Science* magazine as one of the top-ten breakthroughs of 2012.
Here we describe the basics of SFX, the application of the SFX technique to the Cathepsin B crystals and present some ideas and preliminary data that show how it is possible to partially extend the SFX methodology to synchrotron radiation. The experiments were carried in collaboration with CFEL DESY, Hamburg University, Arizona State University, the Max Planck Institute for Medical Research, SLAC, Gotheburg University, and Uppsala University.

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Virtual screening against a TB transcriptional regulator

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Mycobacterium tuberculosis, the causative bacteria of TB, was responsible for 1.4 million deaths in 2011. An estimated 8.7 million people were diagnosed with a new infection in the same year, with multi-drug resistant (MDR) and extensively-drug resistant (XDR) forms of TB on the rise. Treatment of TB relies upon four front-line drugs to which resistance has been steadily increasing. Ethionamide (ETH) is a second-line pro-drug in TB treatment, the bioactivation of which can be increased by repressing the regulator EthR; this has prompted considerable interest in synthetic EthR inhibitors. A series of synthetic inhibitors has been optimised which are capable of boosting ETH activity five-fold for the same dose. However, only a tenth of potential drugs entering clinical trials reach the market. Therefore, it is important to cultivate multiple leads. Using virtual screening in the docking software GOLD, research is ongoing to find a new lead compound for inhibition of EthR. Study of the available EthR protein-ligand crystal structures informs the docking protocol, which has been used to screen libraries of commercially available compounds.

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Kinetic characterization of Ca^{2+}-ATPase inhibition by tri-*n*-butyltin(IV) chloride

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Organotin compounds, such as tri-*n*-butyltin(IV) chloride (TBT), are widespread toxicants which disrupt different functions in living organisms. TBT, as a lipophilic compound, interacts with lipid membranes and membrane proteins. The inhibition of the calcium ATPase from sarcoplasmic reticulum membranes by TBT was studied to solve the kinetic mechanism of inhibition. It was found that the ATPase inhibition could not be reverted in a large time scale (up to 95 minutes), moreover, an excess of TBT over enzyme did not fully inhibit the ATPase activity, therefore it was concluded that TBT irreversible inhibited the enzyme while the effective TBT concentration decreased. The residual ATP hydrolysis activity was measured at different TBT concentrations with time, and the protective effect of different calcium concentrations on the TBT inhibition was also determined. The simplest kinetic mechanism to successfully explain all the observations and the kinetic behavior was found to be a single irreversible step of the inhibitor binding to the enzyme accompanied with a first-order inhibitor inactivation.

Abstracts

– Protein Structure and Function –

P-569

Isolation and characterization of a Danio rerio redox protein: the protein disulfide isomerase

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Protein Disulfide Isomerase (PDI) is an endoplasmic reticulum membrane protein. As a chaperone, PDI catalyses the formation of disulfide bonds and as a carrier, it interacts with thyroid hormones as a T_3-binding protein. The thyroid system plays a key role in various biochemical and physiological processes. When happening during the development, its alteration by endocrine disruptors (EDC) such as polychlorinated biphenyls or triclosan (TCS), causes permanent morphological and neurobehavioral deteriorations. Indeed, the massive use of EDC in household and healthcare products coupled to their release in aquatic environments exposes the thyroid system to serious damages from widely unknown nature. In this context, PDI is seen as a potential protein involved in these processes, as suggested by its expression pattern modification during a toxic exposure stress in many "omics" studies. The objective of the present work is to understand the molecular mechanisms of endocrine disrupting activity of TCS and other pollutants on *Danio rerio* PDI. Therefore, we first isolate its sequence to overproduce it, purify and characterize its structure and function. In parallel, molecular modeling studies are used to obtain its 3D structure and simulate the molecular interactions between TCS and PDI.

P-570

Understanding transport of iron cation in acetylacetone dioxygenas from Acinetobacter johnsoii

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Diketone dioxygenase, Dke1, a functional homotetramer with Fe(II) in each monomeric subunit catalyzes O_2-dependent cleavage of acetylacetone. We have studied impact of the outer shell residues on iron transport. The hydrophilic residues Glu98, Arg80 and Tyr70, which form a hydrophilic gate to the active site cavity are crucial for efficient O_2 reduction. Substitution by alanine, and in the case of Glu98, glutamine, leads to a 50–100-fold decrease in O_2 reduction rates. Experiments showed that these single point mutations leads to faster metal transport through Dke1, and Fe(II) depletion changes from biphasic to monophasic. In order to rationalize experiments we performed a series of molecular dynamics (MD) simulations. Simulations with Fe(II) placed in the active site revealed that the most stable is the native protein complex, while the retention time of the metal ion in the proper coordination is the shortest in the Glu98Gln variant, where, in only one of four subunits iron remained in the active site after 5 ns of MD simulations. The results enabled us to trace the possible iron ion paths and revealed the role of Glu98 in the metal ion shuttle. Finally, the simulations revealed vicinity of Glu98 and Glu11 as an alternating metal binding site.

P-571

Effects of Simvastatin on the structure and function of kidney brush border membrane macromolecules

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Simvastatin is one of the lipophilic statins,lipid lowering drugs. The pleiotropic effects of this drug on renal system has been shown with recent studies. However these results are contradictory. The current study assessed the effects of simvastatin on the structure and function of molecules of kidney brush border membrane (BBM) by Attenuated Total Reflectance-Fourier Transform Infrared spectroscopy and chemometric analysis.

Serum physiologic and 50 mg/kg/day simvastatin were given orally to control and simvastatin treated rats for 1 month. Macromolecular alterations with simvastatin treatment were determined from the analysis of spectral bands in both groups. To determine drug-induced the secondary structure alterations in proteins artificial neural network (ANN) analysis was applied. To distinguish both groups cluster and principal component analysis (PCA) were used.

Simvastatin treatment led to a decrease in the concentration of saturated and unsaturated lipids which can be related an increase in lipid peroxidation. A reduction in protein amount and membrane fluidity was also observed. A reduction in beta sheet and an increase in random coil content were obtained with drug treatment. Based on these alterations both groups were successfully discriminated.

P-572

Quantification of nanomolar binding of copper ions to proteins

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Copper or other divalent metal ions are vital for many proteins to assume their native structure or fulfil their biological function, and may play a key role in the regulation of such proteins. In studying interactions of copper and similar ions with proteins, a range of difficulties need to be addressed carefully before reliable quantitative information can be obtained. Among these are the poor solubility of free copper ions, their interactions with buffer components and other cosolutes, as well as their rather unspecific low-affinity binding to protein moieties. Here, we present how combined microcalorimetric protocols can be used to extract thermodynamic information characterizing all of these linked equilibria with the aid of global analysis. This approach is exemplified for the E1 region of the amyloid precursor protein (APP) as well as for its N-terminal growth factor-like domain (GFLD), which selectively binds copper ions with nanomolar affinity even in the absence of the previously described canonical copper ion binding domain of E1.

Abstracts

– Protein Structure and Function –

P-573

Thermodynamics of 4-substituted-2,3,5,6-tetrafluorobenzenesulfonamides binding to carbonic anhydrase isozymes

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Carbonic anhydrases (CAs) are metalloenzymes that catalyze carbon dioxide hydration and participate in the regulation of acid-base balance, and ion transport in many tissues and organs. The enhanced activity or expression of different CAs is associated with various diseases such as glaucoma, epilepsy, antiobesity, and cancer. There are 12 catalytically active CA isoforms in humans, which are distributed in many tissues/organs. As the differences between the active sites of the 12 catalytically active human CAs are very subtle, CAs have proved to be a challenging drug target for the development of isozyme specific inhibitors.
In this work, the series of 4-substituted-2,3,5,6-tetrafluorobenezenesulfonamides were synthesized as inhibitors of CAs. The binding affinity to CA I, II, VII, XII, and XIII was measured by the thermal shift assay (TSA) and isothermal titration calorimetry (ITC), and inhibition was determined by the stop-flow CO_2 hydration assay. Here, we present intrinsic thermodynamic parameters of binding that differ from the experimentally observed parameters. Crystal structures of CA II, XII, and XIII bound with the fluorinated compounds provided the structural details of inhibitor binding. The inhibitors could be developed further into drug lead compounds.

Abstracts

– Intrinsically Disordered Proteins –

O-574

Combining NMR and molecular simulations to study protein dynamics

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All-atom molecular dynamics simulations provide a vehicle for capturing the structures, motions, and interactions of biological macromolecules in full atomic detail. Such simulations have, however, been limited both in the timescales they could access and in the accuracy of computational models used in the simulations. I will begin by presenting briefly how progress has been made in both of these areas so that it is now possible to access the millisecond timescale, and how we have been able to parameterize relatively accurate energy functions. I will then present recent results that highlight how such long-timescale simulations have been used to provide insight in to the structural dynamics of proteins.
NMR spectroscopy has played a central role in these developments by providing the ability both to validate and to improve simulations. I will present results on how we have used NMR spectroscopy to validate simulations of disordered states of proteins. I will end by presenting a new method for how NMR and simulations can be integrated to provide a highly accurate description of the structural dynamics of dynamical proteins.

O-575

Probing the polymeric properties of IDPs with single-molecule spectroscopy

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Single-molecule spectroscopy provides new opportunities for investigating the structure and dynamics of unfolded and intrinsically disordered proteins (IDPs). The combination of single-molecule Förster resonance energy transfer (FRET) with nanosecond correlation spectroscopy, microfluidic mixing, and related methods can be used to probe intramolecular distance distributions and reconfiguration dynamics on a wide range of time scales, and even in heterogeneous environments. In view of the large structural heterogeneity of these systems, a description in terms of polymer physical principles is often a useful way of conceptualizing their behavior. I will provide examples ranging from the influence of amino acid composition, charge interactions, temperature, and macromolecular crowding on the structure and dynamics of unfolded proteins and IDPs.

O-576

Supertertiary structural ensembles of proteins

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Intrinsically disordered proteins and complex multidomain proteins are characterized by a dynamic
ensemble of conformations that cannot be unequivocally described by traditional static terms of structural biology. These states of proteins are critical in understanding their function at the atomic level, which will eventually lead to extending the structure-function paradigm to establish "unstructural biology" as a new field (1). The functional importance of structural complexity necessitates new standards and protocols for their description of structural ensembles termed "supertertiary" structure in the case of very large proteins composed of a combination of folded and disordered elements (2). We will outline the development of a new database (pE-DB) that is designed to hold structural ensembles of proteins, and through a few examples (PSD95, CBP) current experimental efforts to describe structural complexity at the supertertiary structural level.
1) Tompa, P. (2011) Unstructural biology coming of age. *Curr. Opin. Struct. Biol.* 21, 419-25.
2) Tompa, P. (2012) On the supertertiary structure of proteins. *Nature Chem. Biol.* 18, 597-600

O-577

Karyopherin binding induces conformational transitions in the intrinsically disordered FG domains

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Nuclear pore complexes (NPCs) regulate the selective exchange of macromolecular cargoes across the nuclear envelope. Access is limited to cargo-carrying nuclear transport receptors (e.g. karyopherin-β1, Kapβ1), which interact with several intrinsically disordered Phe-Gly (FG)-repeat domains (i.e. FG-domains) that pave the central pore [1]. Otherwise, the FG-domains collectively impose a steric barrier against the passage of non-specific macromolecules greater than 40 kDa. Using a novel surface plasmon resonance technique, we directly correlate conformational changes of surface-tethered FG domains of Nup62, Nup153, Nup214 and Nup98 to multivalent Kapβ1-FG binding interactions (i.e., binding avidity) *in situ* as a function of FG domain surface density [2, 3]. This behavior shows to vary between different FG-domains and likely depends on the FG-domain sequence and composition. The differential FG binding responses provide insight as to how Kapβ1 functions as an integral constituent of the NPC central channel that optimizes the translocation speed of a wide variety of cargoes.
[1] Peleg O., Lim R.Y.H., *(2010) Biol Chem 39, 719–730.*
[2] Schoch R. L., Lim R.Y.H. (2013) Langmuir, **DOI:** 10.1021/la3049289
[3] Schoch R. L., Kapinos L.E., Lim *(2012) Proc Natl Acad Sci USA 109, 16911–16916*

Abstracts

– Intrinsically Disordered Proteins –

O-578

Repeats in the α-synuclein sequence determine its conformation on membranes

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α-Synuclein (αSyn) is a140 amino acid (aa) amyloidogenic protein implicated in Parkinson's disease. Its physiological functions are yet unclear, but are believed to be connected to the interaction with synaptic vesicles or membranes of other organelles. We used the fluorescence of 13 single tryptophan mutants of αSyn to determine the immersion of different positions of the protein into lipid membranes. Our results support a previously reported 3/11 helical conformation of membrane-bound αSyn determined by the presence of 11 aa repeats in the sequence, but also point to the presence of a flexible break at residues 52-54 between two helical regions. Residues 89-100 are likely disordered but interact with membranes. Deletion of the 4 aa linker between repeats does not significantly affect αSyn membrane binding but strongly decrease the protein aggregation and fibril formation propensity. We believe that the 11 aa repeats in the sequence play a key role in αSyn's ability to switch between a helical conformation on membranes and β-sheets in fibrils.

O-579

Disorder-to-order transition in RTX proteins: Implications for toxin physiology

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Ligand-induced disorder-to-order transitions play a key role in the biological functions of many proteins that contain intrinsically disordered regions. This trait is exhibited by RTX (Repeat in ToXin) motifs found in more than 250 Gram-negative pathogenic bacteria. We investigated several RTX polypeptides derived from the CyaA toxin. We showed that the RTX polypeptides exhibit the hallmarks of intrinsically disordered proteins in the absence of calcium: they adopt pre-molten globule conformations and exhibit a strong time-averaged apparent hydration, due in part to the internal electrostatic repulsions between negatively charged residues, as revealed by the measured mean net charge. Calcium binding triggers a strong reduction of the mean net charge, dehydration, compaction, folding and stabilization of the RTX proteins. We propose that the intrinsically disordered character of the RTX proteins may facilitate the uptake and secretion of virulence factors through the bacterial secretion machinery. These results support the hypothesis that the folding reaction is achieved upon protein secretion and, in the case of proteins containing RTX motifs, could be finely regulated by the calcium gradient across bacterial cell wall.

P-580

NMR study of the interaction mechanisms of intrinsically disordered WH2 domains with actin

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WH2 repeats are a family of intrinsically disordered proteins (IDPs) involved in actin cytosqueleton remodelling. These short domains, isolated or repeated in various actin binding proteins display a low sequence identity and a large panel of functions. For instance, thymosin-ß4 sequesters G-actin into a pool of monomers whereas the first domain of Ciboulot (CibD1) promotes polarized actin assembly.
All WH2 domains fold similarly upon actin binding. They form an extended interface along actin, with an amphipatic N-terminal helix followed by an extended central strand and a more dynamic C-terminal region.
Chimeras of Tß4 and CibD1 differing in only four residues in their central region were designed, that exhibited opposite functions of sequestration or of promotion of actin assembly. Combined X-ray crystallography, NMR and SAXS studies showed that these opposite functions are linked to the level of dynamics of the C-terminal region, which can be controlled by the formation of a stabilizing intermolecular salt bridge involving residues in the central region of the chimera.
As a first step to gain insight into the folding upon binding mechanism of these functionally different IDPs, we investigated their conformational behavior free in solution by NMR.

P-581

ThT influences Aβ(1-40) aggregation process

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Here we present an experimental study of the small peptide Aβ(1-40) aggregation process performed at pH 7.4 in quiescent conditions. Our data show that, in our experimental conditions, the aggregation process takes place only in presence of ThT. We performed a set of measurements as a function of the dye concentration and temperature, keeping constant Aβ(1-40) concentration. Aggregation kinetics were monitored by means of Rayleigh Scattering and ThT Fluorescence. Fourier Transform Infrared Spectroscopy and two photon excitation fluorescence microscopy were also used to characterize secondary structure and morphology of the aggregates. Our data clearly show that Aβ(1-40) aggregation process is strongly affected by ThT addition. In presence of the dye the process is characterized by at least two phases. In the first step small oligomers (which bind ThT) are formed and represent an activated state/precursor for fibril growth (second phase). Importantly, Aβ(1-40) does not undergo aggregation in absence of ThT. At constant peptide concentration, the kinetics rates of the process increases at increasing dye concentration.

Abstracts

– Intrinsically Disordered Proteins –

P-582

MD simulations of intrinsically disordered proteins with replica-averaged chemical shift restraints

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Molecular dynamics simulations represent a powerful method for exploring the conformational space of folded proteins. However, the success has so far been limited when the method is applied to intrinsically disordered proteins, a situation that can be attributed to force field inaccuracy and sampling inefficiency. To address the issue, we have developed a strategy to combine the chemical shift information with molecular dynamics simulations for characterizing the structural ensembles corresponding to intrinsically disordered proteins. This method is based on the CamShift protocol for calculating the chemical shifts from inter-atomic distances and to calculate forces that minimize the deviations between experimental and calculated chemical shifts. We have used chemical shifts as these NMR parameters are most convenient for the study of intrinsically disordered proteins, since they, at least in principle, contain information about the structure and dynamics of the molecules. To further enhance the sampling efficiency, the method of well-tempered ensemble metadynamics approach with parallel tempering (PT-WTE) is added to the protocol. The capability of the protocol is demonstrated with in the case of the fragment F4 of tau (tauF4 = tau[Ser208-Ser324]).

P-583

Thioflavin T binding to fibrillar variants of apolipoprotein A-I N-terminal fragment

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Apolipoprotein A-I (apoA-I) is amenable to a number of specific mutations associated with hereditary systemic amyloidosis. However, the structural characterization of amyloid fibrils of apoA-I is still lacking. Amyloidogenic properties of apoA-I are determined mainly by its N-terminal fragment which is also the predominant form of apoA-I in amyloid fibril deposits. The present study was undertaken to elucidate the nature of interactions between an amyloidogenic variant of N-terminal fragment of apoA-I (1-83/G26R/W@8) and the most prominent amyloid marker Thioflavin T (ThT). Quantitation of the results of double fluorimetric titration revealed that ThT associates with apoA-I fibril sites embracing ~ 5 protein monomers with the Gibbs free energy change of *ca.* -37 kJ/mol. ThT quantum yield was found to increase upon fibril binding by more than three orders of magnitude, attaining the value of ~ 0.6. A β-strand–loop–β-strand structural model of apoA-I fibrils has been proposed, with tentative ThT binding sites being located in the groove between Thr16 and Tyr18 residues. Preliminary verification of this model has been performed with Rosetta molecular modeling suite.

P-584

Using single molecule techniques to find out how the major protein associated with Parkinson's disease

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The pathological hallmark of Parkinson's disease is the presence of insoluble protein deposits in the brain, which are formed when specific protein molecules misfold and aggregate into highly ordered fibrils. In Parkinson's disease, the deposits are primarily made up of alpha-synuclein, a protein whose major function is not fully known. Rather than the fibrils themselves being toxic, evidence now points towards the smaller, soluble oligomers formed in the initial stages of the process as being the culprit. It is vitally important to characterise these oligomers and determine how they are formed, and more importantly, how they damage neurons. The population of toxic oligomers is highly heterogeneous and is present in much lower concentrations than either monomeric or fibrillar alpha-synuclein. It is therefore necessary to use single-molecule techniques to study the processes involved in their formation and conversion into the less toxic fibrils.

P-585

New paramagnetic spin label for the study of structural transitions in proteins by EPR spectroscopy

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Site-Directed Spin labeling combined with Electron Paramagnetic Resonance (SDSL-EPR) is a powerful technique to get dynamics and structural information on proteins. It is based on the grafting of a nitroxide radical at a chosen position of a protein. EPR spectra reflect the mobility of the label and are thus very sensitive to reveal structural transitions. With the aim of enlarging the potentials of the technique, we develop and characterize new spin labels. In particular, the available spin labels are limited by their poor spectral diversity (3-lines spectra) which precludes the study of multiple sites simultaneously. This work is focused on the characterization of a new label: the 2-maleimido-proxyl-phosphorylated bearing a phosphorus nucleus in the environment of the radical leading to a 6-lines spectrum. The C-terminal part of the nucleoprotein of the Measles virus, which is an intrinsically disordered domain, was taken as a model protein able to undergo an induced folding in presence of its partner. A comparative study between the classical and the new label was carried out. The results show that the new label is able to report structural changes, thus opening new ways in the applications of SDSL-EPR.

Abstracts

– Intrinsically Disordered Proteins –

P-586

Ultrastructure-derived physiological and pathological functions of the disordered TPPP/p25

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Intrinsically disordered proteins are surprisingly common in the genome of eukaryotes carrying out important biological functions, many of them are tightly linked to the development of neurodegenerative diseases. The recently identified disordered Tubulin Polymerization Promoting Protein (TPPP/p25) modulates the dynamics and stability of the microtubule system and plays crucial role in the myelination of oligodendrocytes, however, at pathological conditions it is enriched in human brain inclusions in colocalization with α-synuclein characteristic for Parkinson's disease and other synucleinopathies. The extended unstructured segments of TPPP/p25 are localized at the N- and C-terminals straddling a flexible region, which is involved in physiologically and pathologically relevant macromolecular interactions (tubulin, α-synuclein, β-amyloid) and ligand binding. The interactions resulting in structural alterations with functional consequences: GTP promotes the dimerization of TPPP/p25, while its interaction with α-synuclein/β-amyloid leads to aggregation. Dimerization of TPPP/p25 favours its microtubule-related physiological functions, while the less structured monomers might be involved in the formation of pathological interactions leading to the etiology of neurological disorders.

P-587

Structural and dynamical effects of amyloid β phosphorylation at serine 26

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Aggregation of amyloid β (Aβ) peptide into oligomers and fibrils is a key step in pathogenesis of Alzheimer's disease (AD). In late-onset sporadic AD, promotion of Aβ aggregation by posttranslational modifications may contribute to AD pathogenesis. Phosphorylation of Aβ at Ser8 has been shown to favor its cytotoxic aggregation. Here we use NMR spectroscopy and molecular dynamic (MD) simulation to investigate how phosphorylation at the second serine, i.e. Ser26 modulates Aβ's structure and dynamics. The NMR results indicate that introduction of a phosphomimetic at position 26 diminishes Aβ tendency to form β-hairpins and decreases the mobility of peptide backbone around the site of modification. The replica-exchange MD data reveal that the phosphate group at Ser26 interferes with formation of a fibril-specific salt bridge between Asp23 and Lys28. The phosphorylation-induced changes are discussed in connection with the important role of conformational rearrangement in this region for progression of Aβ aggregation.

P-588

Structural insights into Notch1 ligands – protein targets in breast cancer

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Cell-to-cell communication is required for many biological processes and one of the most common systems utilized by a wide range of eukaryotes is the Notch-signalling pathway. High level expression of Notch receptors and ligands, and their increased activation in several breast cancers and early precursors, place Notch signalling as a key player in breast cancer pathogenesis.Studies have shown that the Notch ligand Delta-like-1 (Dll1) expression is undetectable in normal breast tissues, but moderate to high expression has been detected in breast cancer.
Our aim is to generate different ligand protein constructs and, using phage display technology, function-blocking antibodies specific to human Dll1. Structural insights of the Dll1 protein alone and different Ab-ligand protein:protein complexes will be obtained by X-ray crystallography. The only structural models of Notch pathway proteins, Jag2 and Notch1, show intrinsically disordered proteins, clearly suggesting Dll1 to have similar secondary structure disorder features.
We aim to further characterize the Dll1 constructs and the Ab-ligand complex interaction using techniques such as DSF, CD spectroscopy, Native-PAGE and SPR.

P-589

Single molecule fluorescence approach to investigate alpha-synuclein oligomer formation

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Parkinson's disease is one of the most diffused neurodegenerative diseases but the molecular mechanism leading to neuronal death is still unknown. Two lines of evidence link Parkinson's disease to alpha-synuclein: the first is the presence of amyloid like fibrils of the protein in patients' brains; the second is the discovery that three point mutations on alpha-synuclein gene and the gene triplication itself cause autosomal dominant early onset forms of the disease. Recently, several papers demonstrated that oligomers originated during fibril formation process are toxic for cells, making them a possible culprit for neuronal depletion. Given that oligomers constitute less than 1% of species present in solution during aggregation, a single molecule fluorescence method was developed to overcome difficulties regarding their low abundance, transient nature and heterogeneity. The evolution of oligomeric species in solution was recorded for the wild type protein and the early onset Parkinson's disease mutants. Insights gained from these experiments will contribute to the understanding of the molecular pathway leading to alpha-synuclein fibril formation, helping to unravel the etiopathogenesis of Parkinson's disease. (L Tosatto is recipient of a grant PAT - Marie Curie COFUND Actions)

Abstracts

– RNA Structure and Function –

O-590

Splicing and translation regulation by small RNA binding proteins

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RRMs are the most common types of RNA recognition modules, being present in about 1% of all human proteins. They are a typical βαββαβ fold although N- and C-terminal extensions of these domains have been observed. We have recently characterized the NMR structure of two RRM proteins bound to RNA, namely SRSF1 (previously known as ASF/SF2) and CPEB (Cytoplasmic polyadenylation element binding protein) which are an alternative-splicing factor and a regulator of translation, respectively.
The structure of both proteins bound to RNA present unusual features. SRSF1 contains a so-called pseudo-RRM which mediates sequence-specific recognition using almost exclusively via its α-helix 1 while the beta sheet surface of the RRM which is the common RNA binding surface in RRM is not involved in RNA recognition. In CPEB, the two RRMs from a V-shape surface in the free form which is used to bind the RNA in its center. The fold is unusual with several additional secondary structure elements. RRM1 binds the 5'end of the RNA while RRM2 binds only the 3'-terminal nucleotide. This binding arrangement is unprecedented among RRM-RNA structures.
These structural findings reinforce the idea that the mode the RNA binding of RRM is still highly variable and unpredictable. Functional data in support of these structural findings will be presented.

O-591

Defense against viral attack: single-molecule view on a bacterial adaptive immune system

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Bacteria maintain different strategies to protect the cell against invading foreign DNA. In a recently discovered adaptive immune system, fragments of foreign DNA are integrated into a loci on the bacterial genome, known as CRISPR. Short CRISPR-derived RNAs (crRNAs) are incorporated into the CRISPR-associated complex for antiviral defence (Cascade) and guide the complex's search for the DNA of returning invaders. Cascade must recognize both a "seed" sequence and an immediately adjacent PAM sequence in order for successful targeting of the foreign DNA. The mechanism and structural dynamics of this target recognition and binding process, however, are not well understood. Here we report a single-molecule FRET-based assay to monitor in real time the target recognition and binding process of Cascade. There is directionality to the base-pairing process between the crRNA and the target DNA. Upon binding, pairing occurs first over the seed region and then proceeds through a region further downstream on the target sequence. This suggests a mechanism for target recognition wherein the seed/PAM region is identified first, followed by a distinct kinetic intermediate, perhaps a structural transition, before subsequent target pairing can occur.

O-592

RNA architectural modules, their detection in RNA sequences and the assembly of large RNAs

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RNA architecture can be viewed as the hierarchical assembly of preformed double-stranded helices defined by Watson-Crick base pairs and RNA modules maintained by non-Watson-Crick base pairs. RNA modules are recurrent ensemble of ordered non-Watson-Crick base pairs. Such RNA modules constitute a signal for detecting non-coding RNAs. Through systematic comparisons between homologous sequences and x-ray structures, followed by automatic clustering, the sequence diversity in recurrent RNA modules was characterized. These data permitted the construction of a computational pipeline for identifying known structural modules in single and multiple RNA sequences in the absence of any other information. Any module can be searched. The pipeline can be used for 2D structure refinement, 3D model assembly, and for searching and annotating structured RNAs in genomic data. A first blind experiment in RNA three-dimensional structure prediction has been performed. The goals are to assess structure prediction techniques and to evaluate their relative strengths and limitations. The results give potential users insight into the suitability of available methods for different applications and facilitate efforts in the RNA structure prediction community in their efforts to improve their tools.

O-593

Symmetry and asymmetry in the unwinding of nucleic acids

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The forming and melting of complementary base pairs in RNA and DNA duplexes are conformational transitions required to accomplish a plethora of biological functions. Using fully atomistic simulation we have shown that RNA unwinding occurs by a stepwise process in which the probability of unbinding of the base on the 5' strand is significantly higher than that on the 3' strand [Colizzi and Bussi JACS, 2012]. The asymmetry in the RNA unwinding dynamics is compliant with the mechanism of helicase activity shown by prototypical DEx(H/D) RNA helicases and could allow deciphering the basis of the evolutionary pressure responsible for the unwinding mechanism catalyzed by RNA-duplex processing enzymes. In this spirit and from a broader standpoint, here we use a topology-based coarse-grain model to compare and characterize the mechanism of unwinding for both DNA and RNA. The (a)symmetric behavior of the 3'- and 5'-strand could be related to the (bi)directionality observed in molecular machineries processing nucleic acids.

Abstracts

– RNA Structure and Function –

O-594

Probing DNA G-quadruplex structures inside living cells using NMR spectroscopy

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We have used multidimensional NMR spectroscopy to probe G-quadruplexes (G4) structures inside living cells. G-quadruplexes are unusual nucleic acid structures made of stacked guanine quartets stabilized by an assembly of Hoogsteen hydrogen-bonded imino protons. Telomeres and some gene-promoter regions are rich in guanines sequences that adopt G-quadruplex structures under *in vitro* conditions. Is then reasonable to use G4 structures as potential anticancer therapeutic targets, and several lines of evidence indirectly points out roles in key biological processes such as cell regulation. However, direct evidence of G4 existence *in vivo* is scarce. Using SOFAST-HMQC type-spectra we probed a G-quadruplex canonical model made of $^{15}N\{d[TG4T]_4\}$ inside living X. laevis oocytes. The observations lead to conclude that the cytoplasmic environment preferably induces a unique conformation that most resembles the one found *in vitro* under KCl conditions. We also show that specific ligands targeting G-quadruplexes can be studied by NMR directly inside living cells, opening new venues to study ligand binding discrimination in physiological relevant conditions with atomic detailed information.

O-595

Deciphering the RNA-binding complex NF90-NF45: complex formation facilitates RNA chaperone activity

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The nuclear factor 90 (NF90) belongs to the dsRNA-binding protein family and forms a heterodimeric complex with the nuclear factor 45 (NF45). The heterodimer is implicated to participate in RNA metabolism emphasizing mRNA stabilization. NF90-NF45 is also involved in the life cycle of several viruses, *e.g.* hepatitis C virus (HCV), which reflects its putative role as a host factor of viral amplification and in innate immune response during viral infections. Here, we report a first biophysical characterization of the recombinant proteins purified from inclusion bodies. The heterodimer NF90-NF45 could be reconstituted. Several properties of the complex such as mutual thermodynamic stabilization of structural elements present in both proteins, improved RNA binding by NF90 and facilitation of a highly efficient RNA chaperone activity could be compared to the NF90 monomer. A detailed characterization of this activity gave rise to a novel reaction profile correlation plot unraveling the role of the three different RNA binding motifs of NF90 in the course of the catalyzed reaction. Further, this chaperone activity determined the catalytic efficiency of the HCV RNA-dependent RNA polymerase *in vitro* using the native origin of viral replication as substrate.

P-596

Comparison of different methods to extract RNA from cardiac tissue for miRNA profiling by qRT-PCR

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Despite the growing interest in cardiac miRNA expression profiling, having high quality and yield in RNA extraction from cardiac tissue is still challenging. We compared different methods of tissue homogenization and total RNA extraction from pig cardiac tissue aimed at miRNAs expression profiling. Small biopsies of right atrial appendages were obtained from pig hearts and treated according to four different protocols: no homogenization (P1) and homogenization by manual (P2) or automatic (P3 and P4) methods, followed by Proteinase K digestion (PKD) except in P4. Total RNA was extracted using *miRNeasy mini kit*, assessing RNA yield and quality by Nanodrop. cDNA synthesis and qRT-PCR were performed using *TaqMan MicroRNA Assay*. Homogenization was crucial to obtain high yield of pure total RNA. Automatic methods displayed higher yield (0.27 μg RNA/mg tissue in P3) than manual (0.06 μg RNA/mg tissue in P2), with better performance without PKD step (0.38 μg RNA/mg tissue in P4). RNA from P4 was suitable for miRNA expression profiling, as demonstrated by qRT-PCR on miRNA 21 and 29.

These results suggest the efficacy of an automatic homogenization to extract RNA suitable for miRNA expression profiling.

P-597

Nearest neighbor parameters for RNA from metadynamics simulations

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Nearest neighbor rankings are used to predict the stability of nucleic acid double strands. Such data for RNA may explain ribosomal frameshifting, a feature programmed e.g. in HIV, which is a way to compact genetic material. Several sets of such parameters have been determined by experimental (SantaLucia et al. Biochemistry 1998) and quantum chemical studies (Svozil et. al. J.Phys.Chem.B. 2010). As far as we know, no attempt has been made to obtain these parameters from atomistic simulations. The reason for the lack of such works is that free energy calculations are computationally too demanding as they require the entire free energy surface of the system to be properly sampled. With state of the art techniques, such as metadynamics, it is possible to efficiently sample the part of the phase space which is relevant for our purposes. This work is aimed at reconstructing free energy landscapes describing the melting of a set of double stranded RNA molecules in aqueous salt solution by metadynamics using a tailored, non-discrete version of path collective variables (Branduardi et. al. J. Chem. Phys. 2007) and to obtain the nearest neighbor parameters for RNA. Besides free energies the hydrogen bonding patterns and the sugar-pucker states of the RNA molecule are also analyzed.

Abstracts

– RNA Structure and Function –

P-598

Understanding dynamical differences of Hepatitis B-virus apical stem-loop using MD simulation

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Hepatitis B-virus replication begins with the binding of reverse transcriptase to the apical stem-loop (ASL) of epsilon, a conserved element of RNA pregenome. NMR studies have shown that human and duck ASLs have similar secondary and tertiary structures but show differences in their capping loop regions (human pseudo-triloop (UGU) and duck tetraloop (UGUU)) and flexibility. To understand the influence of sequence on the structure flexibility, we performed atomistic molecular dynamics simulation in explicit solvent (100 ns) using CHARMM force field. This allowed us to access at microscopic level the structural and dynamical properties of the systems. The dynamics was investigated at global and local levels. We have calculated NMR order parameters for C-H vector reorientations in sugar and base of nucleotides and found a relation between dynamical behavior of C-H vectors and the backbone and glycosidic dihedral angles.

Our simulation shows that base pair mutation (A-U → C-G) in the stem of duck ASL at position 3 and 6, reduces the flexibility of the system. We conclude that the difference in the flexibilities between the hairpins is due to the difference in stem sequence rather than the different sequence of loop regions.

Abstracts

– Biophysics in Europe (teaching, career and funding) –

O-599

Why, how, and whither biophysics?

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Biophysics emerged as a recognizable field and with a name of its own in the late 1940's. It evolved in response to the need to examine biological systems in rigorous quantitative and physical terms. 65 years after it began, biophysics is now widely recognized as the central organizing discipline of the post-genomic era. However, as important as biophysics has become, and rich as life at the interface between biology/physics/chemistry/mathematics /computation can be, biophysics faces challenges. Many institutions continue to struggle with the organization of departments of biophysics. There is also no consensus of how students should be trained for biophysics research. The problems have been exacerbated by the diversification of biophysics into sub-fields such as quantitative, systems, or computational biology, each requiring specialized training. I will share ideas from discussions at Johns Hopkins about the important role of biophysics in a modern science curriculum, the emerging opportunities in the field, challenges in training at the graduate and undergraduate level, and in the effective organization of research units with the depth, breadth and flexibility required to address the exciting but challenging research opportunities ahead.

O-600

EMBL and EMBL's training activities

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The European Molecular Biology Laboratory (EMBL) is the only European intergovernmental organisation for the life sciences. EMBL has 5 missions: 1) perform outstanding basic molecular biology research; 2) offer vital research infrastructure and services to its member states; 3) train scientists, students and visitors at all levels; 4) develop new instruments and methods and actively engage in technology transfer; and 5) take a leading role in the integration of like science research in Europe. Over the years, the Laboratory has established a number of highly successful training activities. The internal training consists of the International PhD Programme and the Postdoctoral Programme, including the interdisciplinary EIPOD programme. The added value of the programmes will be indicated by testimonials. The Visitor Programme offers scientists at all career stages the opportunity to associate with the laboratory for a period of study, reflection and exposure to ongoing research. The portfolio also includes a comprehensive schedule of courses, conferences and workshops including a wide range of bioinformatics courses organised by the European Bioinformatics Institute (EMBL-EBI).

Abstracts

– Molecular Basis of Disease –

O-601

Supra-molecular interactions of oxidized phospholipids in cells and lipoproteins

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Oxidized phospholipids (oxPL) are components of oxidized LDL. They contribute to the atherogenicity and the toxicity of this particle in vascular cells. Here we report on the exchange of the fluorescently labeled oxPLs 1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphocholine (POVPC) and 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phospho-choline (PGPC) between their physiologically relevant carriers (albumin and oxidized LDL) and cell membranes, the uptake of these lipids and the identification of their primary molecular targets in cultured macrophages. The molecular and supramolecular interactions of the OxPl depend on the small structural differences between their oxidized *sn*-2 acyl chains. POVPC contains an aldehyde group which may undergo covalent Schiff base formation with the free amino groups of proteins and aminophospholipids, whereas PGPC contains a carboxy group and only physically interacts with other biomolecules. The proteins covalently linked to fluorescent POVPC were identified by mass spectrometry. They are involved in cell signaling, apoptosis, transport and stress response. The respective polypeptides as well as the OxPl-tagged aminophospholipids represent the primary molecular targets of OxPl and potential platforms for toxic oxPL signaling in vascular cells.

O-602

Reconstituted micriobial lipid membranes as a tool in drug research

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Bacterial infections are still one of the major threats to human health worldwide. Bacterial pathogens frequently cause severe diseases not only as primary agents, but also subsequent to pathologies caused by other agents. This fact is due, at least in part, to the increasing occurrence of bacterial resistance to antibiotics. In turn, the spread of antibiotic-resistant clones is greatly enhanced by the improper use of antibiotics and by the massive application of antibiotics in animal husbandry.
The function of lipids and lipid membranes and their interaction with peptides and proteins is very important in the context of infections. We use a number of biological and biophysical techniques to characterize microbial and human immune cell membranes and in particular reconstituted membranes mimicking their natural examples. The goal is to establish and to use model membranes which are simple enough to allow a molecular analysis of the underlying mechanisms and to be as close to the natural system to allow a prediction of their biological behavior. The activity of different natural as well as synthetic Host Defense Peptides (HDP) on microbial and human immune cell membranes will be demonstrated.

O-603

The LDL receptor: folding and binding events in function and in disease

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The LDL receptor (LDLR) is a key membrane protein organized in discrete folding domains and responsible for the uptake of cholesterol-rich circulating lipoproteins. The LDLR binds LDLs, internalizes the cargo and releases it in endosomes for degradation before returning to the cell surface to fetch more LDL. The details of each of these steps are controversial. We have combined biophysical studies, including protein stability determinations, protein folding and ligand binding kinetics, to show the following:
1. Binding of LDL and β-VLDL occurs at the convex face of the extracellular binding modules and could imply a chelating effect.
2. The stability of the binding modules and the affinity of their complexes are debilitated in endosomal conditions (low pH and low [Ca^{++}]) compared to extracellular conditions.
3. The affinity of the β-propeller domain of the LDLR for the ligand binding domain is also decreased at low [Ca^{++}].
The compatibility of these findings with several mechanisms proposed for the LDLR functional cycle will be discussed.
On the other hand, loss of function mutations in the LDLR cause Familiar Hypercholesterolemia, which affects 1 in 500 individuals. We will show how MD simulations of LDLR modules can be used to predict the phenotype.

O-604

Synergistic interactions of neuron-bound Alzheimer's Aβ40 and Aβ42: a single molecule study

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Two amyloid_β (Aβ) peptides, Aβ40 and Aβ42, feature in the neuronal loss associated with Alzheimer's disease. While Aβ40 is dominant in healthy brains, the amyloid deposits in patients' brains are enriched in Aβ42 suggesting synergy in association of the two peptides. The nature of the Aβ40-Aβ42 interaction on the neuronal membrane, and its significance in the etiology of the disease, are largely unknown. In the present work fluorescence lifetime imaging microscopy (FLIM) and single molecule fluorescence resonance energy transfer (sm-FRET) were combined to characterize mixed Aβ40/ Aβ42 oligomers on the surface of primary hippocampal neurons and to identify novel synergistic interactions between these two peptides. While in its unmixed state each peptide was found to only form monomeric/dimeric species on the membrane, mixed Aβ40/Aβ42 samples readily formed larger oligomers, exclusively by the addition of Aβ42 onto preexisting Aβ40 oligomeric ÒseedsÓ. Our on-cell studies highlight the important role that interactions between the two Aβ peptides play in the evolution of neuron-bound oligomers in the brain, and support the notion that the known increase in the fraction of Aβ42 with aging may underlie the creation of neurotoxic mixed aggregates.

Abstracts

– Molecular Basis of Disease –

O-605

Structure of human α-2,6 sialyltransferase reveals mode of binding of complex glycans

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Human β-galactoside α-2,6 sialyltransferase I (ST6Gal-I) establishes the final glycosylation pattern of many glycoproteins by transferring a sialyl moiety to a terminal galactose. Complete sialylation of therapeutic immunoglobulins is essential for their anti-inflammatory activity and protein stability but difficult to achieve *in vitro* due to limited activity of ST6Gal-I for some galactose acceptors. No structural information on ST6Gal-I that could help to improve the enzymatic properties of ST6Gal-I for biotechnological purposes is currently available. We describe the crystal structure of human ST6Gal-I, which allows rationalizing the inhibitory activity of cytosine-based nucleotides. ST6Gal-I differs from related sialyltransferases by several large insertions and deletions that determine its regio- and substrate specificity. A large glycan binds to the active site in a catalytically competent orientation, representing the general binding mode of any substrate glycoprotein. Comparison with a bacterial sialyltransferase lends first insight into the Michaelis complex. The results support an S_N2 mechanism with inversion of configuration at the sialyl residue and suggest substrate-assisted catalysis with a charge relay mechanism that bears conceptual similarity to serine proteases.

O-606

Membrane dynamic organization of HIV co-receptors analyzed by Single Particle Tracking at the surface

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The HIV infection requires the sequential interaction of multiple receptors at the plasma membrane of the host cell. Firstly, the gp120viral Env protein interacts with a CD4 receptor present at the surface of the target cell. Then the same gp120 bind to coreceptors, either CCR5 or CXCR4 for R5- and X4-virus, respectively. By means of FRAPrv on HEK cells stably expressing CD4 and/or CCR5, we observed that CD4-CCR5 interaction involves multiple CCR5 molecules per CD4 and induces a confinement into μm-sized domains of both receptors. This interaction may correlate with compartimentalisation of CD4 and CCR5 within the plasma membrane.To elucidate the role of the dynamic organization of the HIV co-receptors in the infection process, we analyzed the diffusion of CD4 and CCR5 receptors by Single Particle Tracking at the surface of T lymphocytes using Quantum Dots functionalized with antibodies recognizing the native receptors. We investigated the influence on receptors diffusion of the temperature, the addition of gp120 and the binding of Maraviroc , an antagonist ligand of CCR5 used as a therapeutic agent. Further work is now in progress to explain the predominance of R5 viruses in the early stages of the HIV infection by using affinofiles cells. It consists of a CD4 and CCR5 dualinducible cell line approach to quantify the receptor usage pattern and efficiency of Env as a function of CD4 and CCR5 expression.

P-607

Finding combinatorial biomarkers for type 2 diabetes in the CAMD database

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Large biomedical databases are available publically or semi-publically to help scientists who work in biomarker discovery for major diseases. The Coalition Against Major Diseases (CAMD) database of the Critical Path Institute, Tucson, Arizona, USA, (http://c-path.org), contains carefully gathered laboratory, genetic and genomic data. Our aim is to identify previously unknown relations in CAMD data that could lead to new biomarkers for type-2 diabetes mellitus. A *combinatorial biomarker* is a set of data attributes that are derived from databases to identify association rules among two or more data items such that when an association rule holds it theoretically indicates higher probability of current or future development of a certain disease or condition.

The concept of combinatorial biomarkers appeared around 2010, and numerous authors simply use the term in the following sense: If – say – the high concentration of all of the molecules A, B and C characterizes well a certain condition X, then they say that {A,B,C} is a combinatorial biomarker of condition X.

The main novelty of our approach that we are looking not only for classical association rules, but we also allow more complex conditions, e.g.,:

$$(\text{attribute}_1 \text{ OR } \text{attribute}_2) \text{ AND } \text{attribute}_3 \text{ imply } (\text{diabetes marker}_1)$$

P-608

Biophysical Analysis of Protein Complexes and Membrane Proteins in Low Molecular Weight Lead Finding

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Nowadays more and more protein complexes and membrane proteins are in the focus of drug discovery as attractive targets to identify new low molecular weight (LMW) substances. To prove the direct interaction of LMW ligands with a potential drug target, a broad spectrum of biophysical methods is applied. High molecular weight, difficult handling and stability of these target proteins make their direct biophysical characterization very demanding in regard to sensitivity, specificity and higher throughput. Here we discuss examples how biophysical methods can support early lead finding to analyze interactions between LMW ligands and complexes/membrane proteins. Differential scanning fluorimetry and differential static light scattering are well established methods to determine apparent melting/aggregation temperature of soluble proteins. The analysis of a detergent-solubilized G-protein coupled receptor using both methods and the characterization of LMW antagonists is shown. Affinity-selection mass spectrometry is used to investigate a protein complex/ligand interaction in regard to (i) binding yes/no (ii) specificity and (iii) stoichiometry. These cases demonstrate the strength of biophysical methods to analyze non-covalent interactions between LMW ligands and complex drug targets.

Abstracts

– Molecular Basis of Disease –

P-609

Dengue virus capsid protein interacts specifically with very low density lipoproteins

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Dengue virus (DENV) infects millions of people worldwide. With no specific treatment available, understanding its replication mechanisms is highly required to identify future therapeutic targets. In this study, using AFM-based force spectroscopy, DLS, NMR, and computational studies, we show that DENV capsid protein (C) binds specifically to very low density lipoproteins (VLDL) but not to low density lipoproteins (LDL). DENV C-VLDL binding is similar to DENV C interaction with lipid droplets (LDs), host intracellular structures essential for viral replication [1]. As on the DENV C-LDs binding, previously characterized by us [2-4], DENV C-VLDL interaction is K+-dependent, involves DENV C intrinsically disordered N-terminus, and is inhibited by pep14-23, a novel peptide drug lead against DENV [3,4]. As perilipin 3 (DENV C target on LDs [2]) is structurally similar to the VLDL protein ApoE, this protein may be the DENV C ligand on VLDL, enabling lipoviroparticles formation. The inhibition of this process may potentially be used as target on DENV life cycle inhibition.
References: [1] Samsa et al. (2009) PLoS Pathog 5:e1000632; [2] Carvalho et al. (2012) J Virol 86:2096; [3] Martins et al. (2012) Biochem J 444:405; [4] Patent no. WO2012159187

P-610

Nucleosomal histones in neutrophils at patients with different types of COPD

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The purpose of research was studying of nucleosomal histones in neutrophils at patients with mixed (emphysematous and bronchial) and bronchial types of COLD. The composition of nucleosomal histones and histone H1 was examined in neutrophil's lyzats following the protocol of Markusheva L. et al. (2000). Patients were divided into 2 groups. 20 patients with mixed type of COLD (moderate severity, exacerbation, respiratory insufficiency of grade 2) were included in first group. Control group consisted of 20 subjects. 15 25 patients with bronchial type of COLD (moderate severity, exacerbation, respiratory insufficiency of grade 2) were included in second group. As compared to control ones the statistically significant change of the ratio of H1, H2A, H2B, H3 and H4 histones in neutrophils in patients with mixed type of COLD. The decreasing of histone H1 and sum fraction of histones Í2À, Í3 and Í4 was observed in neutrophils of patients with bronchial type of COLD as compared to control subjects and first group patients. Significant violations range nucleosomal histones and histone H1 in neutrophils of patients with bronchical type of COLD should be considered as a negative predictive setting, leading to a higher rate of progression of lung fibrosis.

P-611

Concanavalin A fibrils formation from Coagulation of Long-lived "Crinkled" Intermediates

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Understanding the early events during the amyloid aggregation processes is crucial to single out the involved molecular mechanisms and for designing *ad hoc* strategies to prevent and reverse amyloidogenic disorders. Here, we show that, in conditions in which protein is positively charged and its conformational flexibility is enhanced, Concanavalin A leads to fibril formation *via* a non-conventional aggregation pathway. By different techniques (LS, CD, SAXS, Fluorescence and Confocal Microscopy) we highlight the formation of an on-pathway long-lived intermediate and a subsequent coagulation of such "crinkled" precursors into amyloid-like fibrils. In particular, the possibility to generate a long-lived intermediate open the way to new strategies to induce more stable *in vitro* on-pathway intermediate species depending by the initial conformational flexibility of the protein. This will allow isolating and experimentally studying such transient species, often indicated as relevant in neurodegenerative diseases, both in terms of structural and cyto-toxic properties.

P-612

QM/MM study of HCV NS3/NS4A protease with its main substrates: from the structure to the kinetics

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We present a theoretical study of the reaction of the hepatitis C virus (HCV) NS3/NS4A protease with its main natural substrates (NS5A/5B, NS4B/5A, and NS4A/4B peptide junctions). This protease plays a key role in the HCV cycle because it is involved in the viral replication process inside the infected cell. The development of inhibitors of the NS3/NS4A protease has been and still is the main strategy in the fight against HCV. We applied a QM/MM technique which combines an accurate quantum method to describe the active site and a classical force field to take into account the effect environment where the reaction occurs.
The SCC-DFTB method with the CHARMM22 force field have been applied here as QM/MM method to describe:

- The ***potential energy surfaces*** and the ***minimum energy pathways***
- The ***free energy surfaces*** and ***potential mean force pathways,*** where T=300 K is considered
- The ***rate constants*** calculated by means of the TST adapted to be applied on enzymatic reactions

This study furnishes a satisfactory comparison with the experimental information available and suggests considering the barrier structures along minimum energy path as a useful starting point to guide the synthesis of a new type of inhibitors following a Transition State Analogues strategy.

Abstracts

– Molecular Basis of Disease –

P-613

The charge of red blood cells at COPD patients

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The aim of the work was to study the RBCs membrane charge at patients with different severity of COPD. Patients were divided into 2 groups. 18 patients with COPD, moderate severity bronchial form, exacerbation, respiratory insufficiency of grade 2 were included in 1-hd group. 15 patients with COPD, severity bronchial form, exacerbation, respiratory insufficiency of grade 2 were included in 2-hd group. The 3-hd group consisted of 32 healthy ones. The registration of the charge balance based on monitoring pH changing of RBCs under hyperthermic exposure. RBCs were incubated at rising temperature from 37 to 58°C (20 minutes); after that incubation at constant temperature (58°C) during 20 minutes was continued. It was found the significant difference in temperature-dependent responses of RBCs obtained from healthy ones and COPD patients. The monitoring of patient's RBCs response to hyperthermia showed decline of Δ pH linearly with temperature increasing (from 37 to 58°C). Under incubation of RBCs at 58°C it was found Δ pH alterations to have the look of a parabola. In spite of similar trends the degree of Δ pH change was more significant in RBCs of the 2-hd group patients. Our results demonstrated apparent failure of the adaptation mechanisms of RBCs to hyperthermic exposure.

P-614

Substrate dependent conformational changes of catalytic loop in *M. tuberculosis* tyrosyl-tRNA synthetase

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Tyrosyl-tRNA synthetase from *M. tuberculosis* (*Mt*TyrRS) is an enzyme that catalyzes the attachment of tyrosine to cognate $tRNA^{Tyr}$. *Mt*TyrRS is not able to cross-recognition and aminoacylation of human cytoplasmic $tRNA^{Tyr}$, therefore this enzyme may be a promising target for development of novel selective inhibitors as new antituberculosis drugs.
In order to study the conformational mobility of *Mt*TyrRS active center we have performed 100ns molecular dynamics (MD) simulations of enzyme and its complexes with L-tyrosine, ATP, tyrosyl-adenylate, and SB219383 inhibitor. The crystalline structure of *Mt*TyrRS dimer was used to complete the missing residues in loops. Different enzyme-substrate complexes of *Mt*TyrRS were built by superposition with bacterial TyrRS crystal structures. All MD simulations were performed using GROMACS software at Ukrainian National Grid using the MolDynGrid virtual laboratory services (http://moldyngrid.org/).
It was found that the catalytic loop fluctuated between open and semi-open conformations in the unliganded state or in the complex with L-tyrosine. After ATP binding at the active site this loop adopted semi-open conformation interacting with ligand. In the presence of tyrosyl-adenylate or SB219383 inhibitor the loop adopted closed conformations.

P-615

Study of the beetroot antioxidant interaction with cell membranes using a biomimetic approach

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Betalains are a class of natural pigments widely used as a food colorant. In addition to their powerful antioxidant properties, recent studies have reported their preventive role against cancers and their anti-proliferative properties on some human tumor cells. But despite these outstanding properties, the action mode of betalains has not been studied. Given that the betalains seem to exhibit their antioxidant activity in biological lipid environments, we aimed to characterize their action mode on the cell membranes. We focus on the water-soluble betanin which is the major betalain contained in red beetroots. This pigment was isolated from fresh beetroots. Its purity was guaranteed by several purification steps ended by a preparative HPLC. The interaction of the betanin with membrane was investigated using biomimetic lipid bilayer models. The versatility of these structures and the possibility of coupling a wide large panel of biophysical techniques, such as confocal microscopy, anisotropy, general polarization and surface plasmon resonance spectroscopies, allowed us to determine the betanin binding characteristics, its penetration depth in the lipid bilayer and its interaction consequences on the membrane properties as the lipid order, the membrane fluidity and permeability.

P-616

Oxidative modification of blood serum proteins and in multiple sclerosis

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Multiple sclerosis (MS) has been demonstrated to involve oxidative stress and augmented
glycoxidation. In this study, several markers of protein oxidative damage and glycoxidation have been compared in 13 relapsing remittent MS (RRMS) patients without immunomodifying treatment, 5 patients in clinical relapse, and clinically stable patient groups treated with interferon β 1a (18), β 1b (20) and mitoxantrone (9). The glycophore content and Amadori products were increased in not treated patients RRSM patients and the level of protein carbonyl groups were increased in patients treated with mitoxanthrone while the levels of protein tryptophans, thiol groups and thioredoxin were decreased in non-treated patients, with respect to the control group (18 subjects). Protein fructosylation, kynurenine and N-formylkynurenine were increased in RRMS patients without immunomodifying treatment while the level of advanced oxidation protein products (AOPP) was increased in all patients without except for patients treated with interferon β 1a, β 1b. Results of this study confirm the occurrence of protein oxidative and glycoxidative damage in MS and show that spectrophotometric and fluorimetric markers of this damage may be useful in monitoring oxidative stress in the course of therapy of MS.

Abstracts

– Molecular Basis of Disease –

P-617

Local beta-sheet formation in 153-156delVKQV mutant of human TyrRS associated with CMT disease

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Certain mutations in human TyrRS (*Hs*TyrRS) lead to Charcot-Marie-Tooth disease (CMT) – a group of heterogeneous inherited disorders that are characterized by degeneration of peripheral nerve fibers. Currently, three heterozygous missense mutations (G41R, E196K, K265N) and one *de novo* deletion (153-156delVKQV) in *Hs*TyrRS were identified in patients with CMT disease.
Since 3D structures of all 4 CMT *Hs*TyrRS mutants are still unknown, we performed computational modeling of mutant proteins structures using Modeller v9.7 software. Molecular dynamics (MD) simulations were carried out using GROMACS 4.0.7 (FF G53A6). All MD simulations and analysis of trajectories were performed using the MolDynGrid virtual laboratory services (*http://moldyngrid.org*).
In general, structures of *Hs*TyrRS mutants revealed less relaxed states with higher values of RMSD and higher values of gyration radii. The melting of H9 helix (T141-A148) and subsequent partial melting of H11 helix were observed in 153-156delVKQV mutant TyrRS. A novel beta-sheet formation was observed in S145-V152 region for 5-65 ns time interval.
Hence, the CMT-causing mutations in *Hs*TyrRS could be understood in terms of long-range structural effects on the dimer interface and local beta-sheet formation in CP1 region.

P-618

Divalent Cations dependence of the fibrinogen binding to its receptor on human erythrocytes

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The glycoprotein $\alpha_{IIb}\beta_3$ is the platelets integrin receptor for fibrinogen. Its activation is Ca^{2+}-dependent. Our previous studies showed that fibrinogen binding to its receptor on human erythrocytes is also impaired when calcium ions are removed from the medium [1]. From this point, we intended to study the influence of two additional divalent cations, $Mg^{2+}$and Mn^{2+}, and EGTA (a calcium chelator) on the interaction of fibrinogen with erythrocytes. By atomic force microscopy (AFM) based force spectroscopy, we determined the force necessary to break the bond between fibrinogen and erythrocytes, at the single-molecule level, as well as the binding frequency of this process. For the sake of comparison, similar measurements were done in parallel with platelets. Our results revealed that erythrocytes are more prone to bind fibrinogen in the presence of Mg^{2+} than with Ca^{2+} or Mn^{2+}. A higher binding with Mg^{2+} relative to Mn^{2+} was also observed for the fibrinogen-platelet interaction, but both with a lower strength than in the presence of Ca^{2+}. Therefore, the presence of magnesium ions seems to be the most relevant for the activation of the poorly characterized erythrocytes receptor for fibrinogen.
[1] Carvalho *et al.* (2010) *ACS Nano*, 4, 4609

P-619

FTIR and Resonance Raman studies on the coordination of Aβ16 with Cu(II) and Zinc (II)

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There is a broad interest in protein/peptide aggregation, since they represent a common feature of several degenerative diseases. An example is the amyloid beta (Aβ) peptide that plays a crucial role in Alzheimer's disease (AD); Cu-ions have been proposed to be linked to the aggregation cascade of the Aβ peptide and to be involved in the generation of ROS. Zn ions are eventually able to prevent this generation. The strongest binding sites for these metal ions are found in the N-terminal portion; that is why the truncated Aβ portion (1-16) is a valuable model for the study of the molecular basis of this disease.
For the coordination of Cu(I) and Cu(II) we studied the reorganization of the Aβ16-Cu-peptide upon redox reaction with electrochemically induced FTIR difference spectroscopy. For the Zn(II) we examined the coordination through FTIR and Resonance Raman. Different labeled samples and small model compounds have been analyzed. The complexes were prepared at different pH (6.8 and 8.9) simulating the two major complexes found at physiological pH. The data reveals that Cu binding involves Asp1, His6, His13 and His14. Changes in coordination upon reduction are found. The comparison between the Cu (II)-Aβ and the Zn(II) Aβ points towards different coordinations of the histidines.

Abstracts

– Single Molecule Biophysics –

O-620

Common characteristics in early amyloidogenesis: from single-molecules to therapy

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By using single-molecule force spectroscopy we have studied the monomer of most representative neurotoxic proteins and discovered that all of them adopt a rich collection of structures which was found to be correlated to cellular toxicity and neurodegeneration. We also found that an anti-amyloidogenic pharmacological agent (QPB1 peptide) was able to reduce this conformational polymorphism and amyloidogenesis in expanded poly-Q tracts, α-synuclein and the Sup35NM prion. The therapeutic potential of QBP1 was previously demonstrated in animal models for Huntigton's disease. This polyvalent drug also reduces the formation of the hyper-mechanostable conformers we detect in neurotoxic proteins and, based on their association to disease development, we propose they (or their precursors) may be the ones that may trigger the disease by a mechanical blockade of the molecular motors of the protein recycling machinery. These hyper-mechanostable structures (or their precursors) may serve as a target for prevention, treatment, and early diagnostics of these diseases. Our work opens the door to understand the molecular mechanism that triggers the toxicity of neurotoxic proteins. This may allow elucidating the primary cause of these diseases, a critical step for prevention, diagnostics and therapy.

O-621

Novel analysis methods in force-clamp spectroscopy shed light on protein folding

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Protein unfolding and refolding trajectories under a constant stretching force are manifestations of the underlying molecular processes in the end-to-end length. In the case of ubiquitin, I27 and NuG2 protein, the distribution of unfolding times at a given force is best fit with a stretched exponential function, while the collapse from a highly extended state to the folded length is well captured by simple diffusion along the free energy of the end-to-end length. Nevertheless, the estimated diffusion coefficient of ∼100nm2s-1 is significantly slower than expected from viscous effects alone, possibly because of the internal degrees of freedom of the protein. The free energy profiles give validity to a physical model in which the multiple protein domains collapse all at once and the role of the force is approximately captured by the Bell model.

O-622

These IgGs are made for walkin': Random antibody movement on bacterial and viral surfaces

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Immunoglobulins are key for the immune system. Via their Fab arms IgGs can bind two neighboring epitopes resulting in higher avidity and slower dissociation as compared to monovalent Fabs. By using the atomic force microscope we demonstrate that IgG molecules do not remain stationary on surfaces of regularly spaced epitopes but exhibit "bipedal" random walking. Their mobility depends on symmetry and spacing of the antigens; monovalent Fabs do not move. We identified steric strain as the main reason for short-lived bivalent binding. Upon collision, the randomly walking antibodies form transient clusters. Such aggregates might serve as docking sites for the complement system and/or phagocytes.

O-623

Biomechanics study of cancer cells by optical tweezers and speckle microscopy

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Characterizing the mechanical properties of cancer cells in connection with the cytoskeleton organization is important to understand better the migration mechanisms and to find new markers for cancer diagnosis and therapeutics.
We analysed the viscoelastic properties of three breast cell lines (MDA-MB-231, MCF-7, HBL-100) characterised by different metastatic potential, by the meaning of Optical Tweezers (OT) and Speckle Microscopy (SM).
OT were employed to extract tethers from the cell membrane and measure the pulling force versus tether elongation [1]. From these measurements we extracted three viscoelastic parameters. Tether stiffness and membrane rigidity of the cells with high metastatic potential were lower than for the other cell lines, while viscosity showed an inverse tendency.
SM is based on the analysis of the speckle formed by cells when illuminated by a tilted laser beam. Speckle dynamics reflects the thermal vibration of the cell, which is linked to its stiffness. SM has been originally proposed by our group for fast diagnosis of malaria [2] making available the analysis of thousand of cells per minute.
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Abstracts

– Single Molecule Biophysics –

O-624

Live cell protein mobility and interaction maps by light sheet fluorescence correlation spectroscopy

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The interior of a cell is a highly crowded environment. For proteins to fulfill their function, they must move through this "sticky tangle" of macromolecules to their target, either by active transport or by passive diffusion. These processes are central to cellular function, and for understanding them one requires techniques that can image the motion of proteins inside a living cell.

Here we describe a fluorescence correlation spectroscopy (FCS) setup that allows recording real-time mobility images of fluorescent molecules. Earlier studies used FCS in confocal microscopy, which allowed for high time-resolution but only for single-position measurements. We extended FCS to imaging using a selective plane illumination microscope (SPIM) with a high-speed EMCCD camera. Our setup allows fluorescence cross-correlation spectroscopy (FCCS) in two-dimensional images of live cells by two excitation lasers and dual-view optics. First applications of this system include mobility maps of oligomeric fluorescent proteins (FP), as well as two-colored FP dimers and the transcription factor system AP-1.

O-625

Aggregation conditions strongly influence the molecular composition of alpha-synuclein oligomers

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Growing evidence suggests that alpha-synuclein oligomeric aggregates are key players in the onset and progression of Parkinson's disease. However, very little is known about the molecular details of these aggregates.

For large protein aggregates, such as alpha-synuclein oligomers, it is very difficult to determine the number of monomers that form an oligomer using conventional techniques. We developed a method that uses sub-stoichiometric labeling in combination with single-molecule photobleaching to determine the number of monomers per oligomer [1]. By using the exact label density, we can link the number of fluorescent labels per oligomer to the total number of monomers. Using this combination of techniques, we are even able to distinguish multiple distinct species present in the same sample and determine their respective compositions.

For oligomers formed under high concentrations of alpha-synuclein, we find a single, well-defined species while for oligomers formed under the addition of dopamine, we find two distinct species. Although there are significant differences in the molecular composition of the oligomers formed under specific preparation conditions, the oligomers still have a well-defined composition.

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P-626

Comparing the dynamics of RNA and DNA hairpins by single molecule force measurements

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Hairpins are fundamental secondary structures consisting of a double-helical stem and a single-stranded loop and are formed by both DNA and RNA. Hairpin formation is of central importance to several mechanisms of molecular biology, as regulation of gene expression and translation. Hairpins also are a model system for studying the self-assembly of nucleic acid chains. We used a high-precision double optical tweezers setup to compare DNA and RNA hairpins with respect to their dynamics of unfolding and refolding under an external load.

Two different hairpin structures were studied, both as a DNA and as an RNA molecule. The two hairpins exhibit the same 13 basepair stem and a loop of 10 and 18 nucleotides, respectively. Significant differences are observed between DNA and RNA. The forces needed to unfold the hairpins are 40-50 % lower for the DNA than for the RNA hairpins. Hysteresis between unfolding and refolding curves is more pronounced for RNA than for DNA. Imposing a constant end-to-end distance, spontaneous force flips are observed for the wide-loop hairpin DNA, but are absent for the equivalent RNA structure. For narrow-loop hairpins, flipping between the folded and unfolded states occurs with both DNA and RNA.

P-627

Closure of DNA denaturation bubbles coupled to chain elasticity

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The physical understanding of biological processes such as Transciption requires the knowledge of dsDNA physics. The notable thermodynamic property of dsDNA is denaturation in which dsDNA is unwound into two ssDNAs via bubbles at its melting temperature. The dynamics of denaturation has been studied at the base-pair (bp) scale, ignoring chain degrees of freedom. Here, we consider *thermalized* or *pre-equilibrated* denaturation bubbles. Altan-Bonnet *et al*. measured the closure times of denaturation bubbles of length 18 bps at room temperature and found very long timescales in the 20-100 micro seconds range. To explain physical mechanism behind these very long timescales, we use simple coarse-grained models and Brownian dynamics. We show that the closure occurs via two steps: The first step consists of a fast zipping of the initial bubble until it reaches a meta-stable state due to large bending and twisting energy stored in the bubble**.** The closure of the meta-stable bubble occurs either via rotational diffusion of the side arms, or bubble diffusion along the chain or local thermal activation, depending on the DNA length and chain elastic moduli. We show that the physical mechanism behind these long timescales is therefore the dynamical coupling between base-pair and chain degrees of freedom.

Abstracts

– Single Molecule Biophysics –

P-628

MET receptor dimerization studied at the single-molecule level

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The receptor tyrosine kinase MET is involved in vertebrate development and plays an important role during tissue regeneration. Furthermore, it was found that MET is the target of different infectious bacteria, amongst them *Listeria monocytogenes* that induces bacterial uptake through the surface protein internalin B (InlB) and causes human listeriosis. However, many details of Met activation are still unclear.
With single-molecule fluorescence microscopy techniques we study activation and association of MET through InlB. In particular, we use single-molecule photobleaching to elucidate the mechanism of receptor activation. Therefore, single-cysteine mutants of the ligand were generated to obtain fluorescently labeled molecules with defined stoichiometry. Our studies reveal partially preformed MET dimers and an increase in receptor dimerization upon InlB binding.

P-629

Investigation of the pH stability of avidin and newly developed avidin mutants with the AFM

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The great stability of several avidin proteins over a wide pH range, particularly when combined with biotin, have been studied qualitatively in the last fifty years. In the present study, a more detailed investigation is made by performing molecular recognition studies, using AFM force spectroscopy. The applied measuring principle enables the investigation of forces and dynamics of the interaction between the proteins and a corresponding ligand (biotin), during a pH treatment and with different loading rates. Therefore, the ligand is coupled via a bifunctional PEG-crosslinker on the outer AFM tip apex, whereas the receptor is immobilized on the probe surface. By repeatedly approaching and withdrawing of the tip in z-direction, receptor-ligand complexes are formed and released. If this experiment is repeated at different pulling speeds (loading rates) and pH values, the energy landscape and the pH stability of the receptors can be examined. The measurements have been clearly shown that the three examined proteins are stable over a wide pH range. Moreover chimericavidin does not offer the pH stability on single molecule level as expected. All in all, the three proteins open the possibility for more applications, like for e.g. surface sensors, which are exposed extremes of pHs.

P-630

In vivo characterization of bacterial repressors using 2-photon fluorescence fluctuation microscopy

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Bacillus subtilis is a Gram positive soil bacterium able to rapidly adapting its metabolism depending on the nutrients available in the environment. In particular, its adaptation to different carbon sources which can be assimilated either through glycolysis or gluconeogenesis, is controlled by two transcriptional repressors CggR and CcpN. In order to characterize these two repressors in vivo, we are using highly sensitive and quantitative two-photon fluorescence fluctuation methods, specifically the two-photon scanning number and brightness (2psN&B) analysis and raster imaging correlation spectroscopy (RICS). We record very rapid scans of immobilized bacterial cells expressing the fluorescent protein fusions under inducing or repressing conditions. N&B analysis of the fluorescence fluctuations at each pixel over 50 scans allows for the direct measurement of the concentration and molecular brightness of the fluorescent proteins, providing information on the expression level and oligomerization state of the repressors. RICS analysis allows for the comparison of the diffusion properties of the fluorescent proteins, related to the fraction of repressor molecules bound or not to DNA. Our preliminary results indicate very different in vivo behavior for the CggR and CcpN repressors.

P-631

Discrimination of protein receptors through quantitative adhesion force maps

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Atomic Force Microscopy (AFM) is the only technique able to measure topography and intermolecular forces of biomolecules mimicking the physiological conditions with nanometer resolution. Working in the force based AFM Jumping mode (JM) is possible to take images without damaging soft samples. JM produces topography and tip-sample maximum adhesion images. Working in a repulsive regime applying very low forces the adhesion images become molecular recognition maps. Using this methodology it is possible to discriminate between avidin and streptavidin single receptors on hybrid samples. The adhesion maps obtained with biotin-PEG-tips showed features identified as avidin molecules, in the range of 40–80 pN, meanwhile streptavidin molecules gave 120–170 pN at the working conditions. Adhesion maps of enzymatic ferredoxin-$NADP^+$ reductase samples imaged with ferredoxin and flavodoxin functionalized tips were also obtained. Molecular recognition maps showed a high homology with topography features when FNR was attached in an oriented manner facing up the interacting surface [PEDS 11, 715-23, 2012]. This evidences that repulsive JM allows identifying biomolecules through the intermolecular specific force being very efficient when protein molecules are immobilized in an oriented manner.

Abstracts

– Single Molecule Biophysics –

P-632

Single molecule imaging of localisations, dynamics and stoichiometry of a regulated enhancer-binding protein in live Escherichia coli cells

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Bacterial enhancer-dependent transcription systems support major adaptive responses and offer a singular paradigm in gene control analogous to complex eukaryotic systems. Here we present new mechanistic insights into the control of one membrane stress responsive bacterial enhancer dependent system. Using millisecond single-molecule fluorescence microscopy of live Escherichia coli cells we determined the localisations, diffusion dynamics and stoichiometries of complexes of the bacterial enhancer-binding ATPase PspF during its action at promoters as regulated by inner membrane (IM) interacting negative controller PspA. We establish that (i) a stable repressive PspF-PspA complex is located in the nucleoid, transiently communicating with the IM via PspA, (ii) PspF as a hexamer stably binds only one of the two *psp* promoters at a time suggesting that *psp* promoters will fire asynchronously and (iii) cooperative interactions of PspF with the basal transcription complex influence dynamics of the PspF hexamer-DNA complex and regulation of the *psp* promoters.

P-633

Multimerization state of von Willebrand Factor determined by TIRF and AFM

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Von Willebrand Factor (VWF) is a blood glycoprotein that plays a crucial role in blood coagulation. Thanks to its shear flow sensitive structure VWF responds to shear by expansion to an elongated form, thereby exposing particular binding sites for collagen and the platelet receptor GPIb [1]. Thus VWF promotes adhesion of platelets to the injured vessel wall as well as platelet aggregation [2,3].

VWF exists as a multimer with a variable number of dimeric subunits. Smaller multimers are more resistant to shear forces than larger ones and subsequently binding sites for their ligands are not as readily exposed [4,5]. The size distribution of VWF in its native and diseased state is therefore of great importance for VWF functionality. We quantify the size distribution of recombinant VWF-eGFP using a combination of Total Internal Reflection Fluorescence (TIRF) and Atomic Force Microscopy (AFM).

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P-634

Assessing how cationic intercalators affect DNA using nanofluidic channels

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YOYO-1 is the traditionally used dye in fluorescence microscopy and is known from bulk studies to stain DNA heterogeneously. We characterize heterogeneous staining on the single DNA molecule level using nanofluidics and show that a DNA sample with substoichiometric amounts of YOYO added contains molecules ranging from a low degree of YOYO bound to fully saturated with dye. We also demonstrate how heterogeneous staining can be used to investigate how YOYO affects the properties of nanoconfined DNA in a time efficient manner. When YOYO intercalates it extends the DNA contour but also decreases the overall charge of the DNA complex. Our results suggest that these effects are of different importance at the different ionic strengths investigated. Finally, we can extract information about native DNA with high accuracy. We believe that the principle of intentionally using a heterogeneous sample will be generally applicable on studies of ligands and proteins binding to DNA.Additionally, we demonstrate a one-step method for mapping of single DNA molecules in nanochannels using netropsin - an AT specific minor groove binder - together with YOYO-1. We believe that this is a promising future method for example to identify pathogenic bacteria in a fast manner from extremely small samples.

P-635

Direct observation of lipid flux from single HDL particles into biomembranes

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High density lipoprotein (HDL) is a main carrier of cholesterol in the blood stream. Hitherto, the mechanisms how lipids flow from lipoproteins into the cellular plasma-membrane are far from being understood: it remains elusive whether receptors directly influence lipid efflux or keeps the lipoprotein particle attached to the plasma-membrane.

In this study we provide a mechanistic understanding of the cargo exchange process between HDL and biomembranes. The interaction between HDL and membranes was investigated with force spectroscopy and high speed atomic force microscopy; the transfer of single cargo molecules was directly visualized using a combined and simultaneously operating fluorescence and force microscope. In particular, we compared the transfer of the fluorescently labelled lipids DiI, and Bodipy-labelled cholesterol and cholesteryl-ester. Experimental evidence points to the fact that i) cargo transfer requires contact; ii) only amphiphilic cargo is transferred; iii) upon contact the particle incorporates into the hydrophobic core of the bilayer, which was abolished at increased membrane cholesterol levels. Live cell experiments confirmed the data obtained on the synthetic systems.

Abstracts

– Single Molecule Biophysics –

P-636

Biophysical properties of VWF in single molecule force spectroscopy

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Von Willebrand factor (VWF) is a huge multimerizing protein playing a key role in hemostasis. VWF binds to the injured vessel wall (collagen), recruits platelets and probably leukocytes to the site of injury and binds factor VIII. Sites for collagen binding as an initial event are located in domains A1 and A3. We performed Molecular Recognition Force Spectroscopy (MRFS) measurements of VWF's specific binding domains to relevant substrates so as to classify the forces and the dynamics of these interactions. We tested several sample preparation methods. When collagen was adhered to different surfaces or bound via EGS-linker or Acetal-PEG-NHS-linker, it showed a high adhesive behavior and was therefore not usable for MRFS. However, with a sample preparation procedure using a PEG_{800}-diamine layer, unspecific adhesion between tip and sample was low. We quantified intermolecular forces, unbinding-length, binding probabilities, effective spring constants as well as x_β and k_{off} between collagen III on the sample surface and rvWF A1-A2-A3-His bound to the tip as well as between collagen VI (sample) and rvWF A1-A2-A3-His (tip) and collagen VI (sample) and rvWF A1-A2-His (tip).

P-637

Addressing nutrient and pheromone response via Pho85 pathway at single cell level in S.cerevisiae

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Cell cycle is a highly controlled process in which cyclins and cyclin-dependent kinases (CDKs) mainly regulate the progression to ensure accurate duplication of the DNA, morphogenesis and the adaptation to environmental conditions. Our interest lies in the G_1/S-Transition, called START. Once the cell passes this checkpoint it has to progress through the entire cell cycle. The main CDK for cell cycle regulation in budding yeast is Cdc28 which interact and is thereby regulated with different cyclins. Another CDK is Pho85 which also bind diverse cyclins. Interestingly the expression of some of its own specific cyclins is cell cycle regulated. Pho85 is on one hand involved in G_1/S transition. On the other hand it seems to regulate cell cycle and adaptation to environmental chances like nitrogen, phosphate availability and pheromone response. If the conditions are not adequate cells are arrested in G_1.
To get a better understanding of the regulation of the cell cycle we use single molecule methods on single cell level. We quantify the mRNA level of different cell cycle regulated genes with *in situ* fluorescence hybridization (FISH) to do statistic analysis. To quantify and localize GFP-labeled proteins under different nutrient condition we use microfluidic devices in living cell.

P-638

Structural variety of nucleosomes seen by spFRET

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Opening of the nucleosome structure is essential for accessing genomic DNA. To study the mechanism of this process, we monitor the distance between various fluorescently labeled positions on nucleosomes by single-molecule FRET. Nucleosomes were reconstructed from 170bp long DNA fragments and recombinant or natural histones. Selective parts of the DNA or histones were labeled by Alexa dyes. Nucleosome opening was mimicked by salt induced destabilization. The stability, as measured by the salt concentration at the opening transition midpoint, is lowest for yeast, followed by Xenopus and mouse. Octamers isolated from HeLa cells gave less stable nucleosomes than recombinant octamers. The Widom 601 DNA sequence builds much more stable nucleosomes, and the distribution of FRET efficiencies is narrower than for those reconstituted on 5S rDNA or MMTV-B sequences. The opening pathway through an intermediate state, as found for Xenopus histones, could be verified for the mouse and yeast systems, suggesting a general mechanism for accessing nucleosomal DNA.

P-639

Vibrational spectroscopy for determination of moisturizing agents'mechanism

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The analysis of the skin hydration and barrier properties are important to evaluate the effects of topically applied moisturizing agents. The latter include a large range of molecules with different chemical structures: acids, alcohols, esters, triglycerides, fatty acid derivative and a mix of long chain lipids. Those molecules can act as: occlusive, humectants, lipids modulating or by using a combination of those mechanisms. ATR-FTIR spectroscopy was used to investigate the penetration kinetics of different class of molecules across stratum corneum (SC). The lack of penetration determined the occlusive property. Raman spectroscopy and Classical least square analyses were then performed to evaluate their effect on water content and to highlight molecular changes on the SC. The modification in SC barrier function and protein structure could explain the lipid modulating effect and humectants effect of the applied molecules. Among studied molecules, only lactic acid penetrated and interacted with the SC lipids, thus improved both the barrier function and the bound water content. This approach could be used in the future to characterize the mechanism of action of new dermo-cosmetic agents and help to select an optimal structure-activity relationship.

Abstracts

– Supramolecular Assemblies –

O-640

Structural analysis of tetrahedral channel formation and hydration in cubosome nanoparticles

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Knowledge from nonequilibrium lipid polymorphism contributes to advancement in protein crystallization and nanoencapsulation techniques. Self-assembled lipid phases are studied as bulk media as well as after fragmentation into nanoparticles. Small-angle X-ray scattering (SAXS) provides evidence for their morphological structural transitions. At variance to micelle-to-vesicle transitions and micelles formation, nonequilibrium SAXS studies on lipid polymorphism are very few. Vesicle-to-cubosome transitions have been recently investigated. We predicted the early intermediates during a vesicle-to-cubosome transition and presented experimental evidence for their occurrence by cryo-transmission electron microscopy. We show that the tetrahedral packing of small unilamellar lipid nanovesicles can lead to the formation of nanochannels in cubosome liquid crystalline nanoparticles. Related studies, such as large nanochannels hydration in cubic lipid/water phases, are also discussed.

O-641

Targeted drug delivery by nanoparticle-protein corona

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Targeted drug delivery is actively researched as a means to prolong, localize, and protect drug interaction with the diseased tissue. When nanocarriers enter a biological fluid (e.g. human plasma), proteins and other biomolecules adsorb on the surface with result that the targeting ability of nanoparticle is severely compromised. The idea that the 'synthetic identity' of nanoparticles is markedly different from the biological one (i.e. after exposure to biological fluids) has many potential implications. In this context, a ground-breaking paradigm shift has been recently suggested: the surface of nanoparticle-based delivery systems can be manipulated to dictate, after injection in the human body, the selective binding of plasma proteins that are specifically recognized by receptors of malignant target cells. Here, we show that, after exposure to human plasma, the new biological identity of nanocarriers can be successfully exploited to target cancer cells via receptor specific ligands.

O-642

The protein folding pathway: a coordinated network of molecular chaperones

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Molecular chaperones are a group of proteins devoted to either assisting the folding of other proteins or to their degradation. The former they generally do it by protecting the aggregation-prone regions of these proteins, thus allowing them to reach their native conformation using the information encoded in their own amino acid sequences. As for the later, this is achieved by the interaction of the chaperone:substrate complex with certain factors (cochaperones) which direct the complex to the proteasome degradation pathway. In both processes, chaperones can work by themselves but in most cases their function is performed by the coordinated concourse of different chaperones, which form transient complexes, thus acting like "assembly lines" that make more efficient the protein folding and degradation processes.
The talk will be devoted to analyze some of these "assembly lines", a work that has been mainly carried out by electron microscopy, a technique suited for the study of transient protein complexes generated during the folding and degradation processes.

O-643

Structure of a bacterial filament solved by solid-state NMRă: the type III secretion system needle

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I will show that Solid-State NMR methodology (1-5) is able to reveal the supramolecular interfaces and ultimately the complete atomic structure of a protein filament. Our approach is demonstrated on the *Salmonella typhimurium* Type III Secretion System Needle, a filamentous assembly that mediates bacterial injection of effectors into the host cells. We present an atomic resolution model of the Needle in its native filamentous state (4). Additional data on an homologous needle from *S. flexneri* have allowed us to propose a common architecture for the Needle architecture (6).
(1) Loquet et al., *JACS* **2010**
(2) Loquet et al., *JACS* **2011**
(3) Loquet et al., *Acc. Chem. Res.* **2013**
(4) Loquet et al., *Nature* **2012**
(5) Habenstein et al., *J. Biolmol. NMR* **2013**
(6) Demers et al., Plos Pathogens **in press**

Abstracts

– Supramolecular Assemblies –

O-644

Peptide nanotubes: structure and mechanism

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Supramolecular self-assembly is an attractive pathway for bottom-up synthesis of novel nanomaterials. Since nanotechnology is mainly relying on size-dependent physical phenomena, the control of the monodispersity is required, but the possibility of tuning the size is also essential. Lanreotide is a dicationic octapeptide spontaneously forming long nanotubes but monodispersed in diameter[1,2]. Their structure, their mechanism of formation[3] and the role of the counterions and repulsive forces[4] have been studied. The curvature radius of the nanotube is fixed at a very early stage of the assembly, upholding the idea that molecular determinants are controlling the curvature radius. For diameter tuning, we based our strategy on a structural approach by modifying the size of a precise aromatic amino acid involved in close contacts between peptide[5]. We demonstrate that this approach indeed enable the tuning of the diameter of the nanotubes from 9 to 35 nm while keeping a strict monodispersity. We finally build a geometrical model that explains how a modification of a few Å of a single aromatic residue induces a 4-fold increase of the diameter. We further demonstrate the application of such strategy by the formation of composite (silica-peptide) nanotubes of various diameters[6].

O-645

Quaternary structure of protein assemblies from small-angle x-ray and neutron scattering

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The new QUAFIT method for determining the quaternary structure of protein assemblies by x-ray or neutron small-angle scattering data is presented (1,2). The method is based on the idea that asymmetric monomers, formed by rigid domains of known structure, possibly connected by flexible linkers of known sequence, are assembled according to a point group symmetry. Scattering amplitudes of domains and linkers are combined by means of a spherical harmonics expansion. In order to avoid any overlap among domains, the "contact distance" between two domains is determined as a function of orientation by a novel algorithm, based on a Stone's invariants. QUAFIT has been tested by studying the structure of hemocyanin from Octopus vulgaris, a protein that shows a hierarchical organization of monomers. In the first QUAFIT study, the structures of the decamer and of the dissociated "loose" monomer have been identified by analysing scattering curves in the most and the least aggregative conditions, respectively. Results are in very good agreement with the model derived from electron microscopy.
1) F. Spinozzi and M. Beltramini, Biophys. J., 103:511–521, 2012.
2) F. Spinozzi, P. Mariani, I. Mičetić, C. Ferrero, D. Pontoni, and M. Beltramini, PLOS one, e49644, 2012.

P-646

Chiral assemblies of silver(I) mediated base pairs by vibrational and electronic circular dichroism

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The origin of the bioactivity of silver compounds is currently unknown; possible ways of their interactions with a cell are speculated on. The fact that silver compounds do not destroy mammalian cells make them one of the most prospective agents in drug design. Interest in the application of Ag(I)compounds has led to the need for detailed knowledge of the mechanism of their action. One of the possible ways is its coordination to GC pairs of DNA, where Ag^+ ions form Ag(I)-mediated base pairs and inhibit transcription. In thus study, a systematic chiroptical study on Ag(I) interaction with nucleobases and its derivatives, RNA and DNA in solution is presented. Ag(I)-mediated pairs of nucleobases and their self-assembled structures were studied in 3.0-10.0 pH range by vibrational (VCD) and electronic circular dichroism(ECD). On the basis of the obtained data, in the first time the formation of the Ag(I)-mediated self-assembled species of cytidine with a structure similar to the quadruplex i-motif structure in DNA was observed at neutral and basic pH. The couplet oscillator model calculation was performed for interpretation of the obtained VCD spectra and their variation as a formation of the different assemblies of Ag(I)-mediated dimers of bases.

P-647

Long-range 19F-19F NMR distance measurements in membrane-bound peptides spanning two helix turns

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NMR presents a powerful tool to measure precise distances between two selective labels that are non-perturbingly incorporated into the molecules. However, the structural questions that can be addressed by NMR are limited by the typically low distance range that can be addressed with conventional isotopes (2H, 13C, 15N). In the highly dynamic environment of liquid-crystalline lipid membranes it is particularly challenging to carry out distance measurements due to the partial averaging of the underlying dipolar couplings. We present here highly sensitive and long-range 19F-19F distance measurements within the antimicrobial helical peptide PGLa, in which 11-12 Å are detected between residues of 7 positions apart, corresponding to two helical turns. Using an improved CPMG solid-state 19F-NMR experiment we were able to perform these measurements in liquid crystalline membranes in a mechanically aligned sample. This to our knowledge unprecedented determination of an 11-12 Å distance by NMR in fluid membranes allows to address new structural questions, such as the formation of dimers, which have been not accessible so far using non-invasive NMR labeling.

Abstracts

– Supramolecular Assemblies –

P-648

Tracing the transient conformational signal in bacterial phototaxis using SDSL-EPR spectroscopy

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In *Natronomonas pharaonis* a sensory rhodopsin II – transducer complex (SRII/HtrII) mediates negative phototaxis. The initial signal, a light-induced outward movement of receptor helix F, leads to a conformational change of transducer helix TM2. The mechanism underlying the signal propagation still remains unclear.
For the HAMP domain, the mechanism comprises two distinct conformational states which can be observed by two-component cw-EPR spectra at ambient temperatures exhibiting a thermodynamic equilibrium that can be driven by salt-, temperature- and pH-changes.
To trace the conformational signal and it's propagation throughout the elongated transducer, we follow transient changes by time-resolved cw- and pulsed-EPR spectroscopy and find transient spectral changes that correspond to the above shifts in the thermodynamic equilibrium and are in agreement with a shift towards a more compact state of the HAMP domain.
Elucidating this signal beyond the HAMP domain requires an activation scheme within the highly cooperative framework of hexagonal arrays formed by the trimers of SRII/HtrII dimers.

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Towards a structural model for the tubes formed by the bacteriophage SPP1 Major Tail Protein

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Most bacteriophages have long tails that serve for bacterial target recognition and genome delivery. These tails are built onto a baseplate complex that stimulates the nucleation of the tail tube structure. They are organised around a tape measure protein that regulates the tube length. They are mostly formed through the assembly of a unique protein, the Major Tail Protein. As the tail assembly and functional dynamics are central to the infection process by phages, elucidating the structure of phage tails remains a crucial challenge.
In this study, we take advantage of the possibility to form tubes from the recombinant SPP1 Major Tail Protein gp17.1 alone, and we follow, using solution-state NMR, solid-state NMR and EM, the structural changes experienced at the residue level by gp17.1 during tube formation. We show that monomeric gp17.1 is partially folded in solution and that its β-structure content increases during tube assembly. From the identification of intermolecular contacts within tubes, we propose a 3D model for the gp17.1 tube. We design a gp17.1 mutant capable of inhibiting native gp17.1 tube formation. Finally, we determine the 3D solution structure of the two SPP1 tail-completion proteins, and we discuss the binding of these proteins to the recombinant gp17.1 tubes.
This work was supported by the french ANR program and national infrastructure FRISBI program.

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In silico design and experimental validation of peptidic self-assembled monolayers

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Self-Assembled Monolayers (SAMs) are made of small molecules that chemisorb on specific surfaces and spontaneously assemble as an ordered layer. The recently developed peptide-based SAMs can be exploited in biological applications thanks to their intrinsic biocompatibility. In this study we use atomistic simulations to design the best amino acid sequence of peptidic SAMs to be used in biological applications. The investigated peptides feature a head-group (Cysteine, for thiolate bond formation with gold), a linker formed by a sequence of 1 to 4 amino acids (chosen between A,F,G,P,S,Y), a zwitterionic layer formed by an alternation of opposite charged residues (KE), and an integrin binding end group (RGD). In the first screening we test the formation of secondary structures in isolated peptides using Replica Exchange simulations and validating the results by Circular Dichroism. The most promising peptides are then simulated in SAM configuration on gold surface and analyzed in terms of secondary structure, tilt angle, lateral interactions formation, hydrophilicity, height and RGD exposure to solvent. The results show that the optimal peptide needs a linker of 3 residues of Proline or Phenilanaline and a layer of at least 4 KE pairs.

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Effect of ionic strength on end-tethered ssDNA molecules on gold surfaces

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DNA conformational and mechanical properties play a significant role in determining DNA-protein interactions and in the hybridization efficiency. Counterions differently screen the electrostatic charge carried by the DNA backbone affecting it curvature and flexibility. In order to improve the understanding of crowded cellular environments and for the realization of more efficient biosensors, it is important to analyze the collective response of DNA brushes to changes of ionic strength. We report here about an Atomic Force Microscopy (AFM) study of the ionic effects on single stranded (ssDNA) confined monolayers tethered on ultra flat gold surfaces. We realized via Nanografting, an AFM-based lithography, micrometer sized brushes of short ssDNAs with controlled, variable surface density, confined inside a biorepellent self-assembled monolayer (SAM). Varying concentration and salt species (NaCl, KCl, $CaCl_2$) inside the AFM liquid cell we monitored with high precision the corresponding ssDNA brush height variations. The results related to scaling law of the height, cations binding affinities and electrostatic screening effects will be discussed in the framework of theory of polyelectrolyte brush.

Abstracts

– Supramolecular Assemblies –

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Why are cholesterol-based cationic lipid/DNA complexes so efficient?

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The low transfection efficiency (TE) of CL-DNA complexes (lipoplexes) is due to the multiple intracellular barriers that they must overcome to deliver exogenous DNA into the cell nucleus of the host cell and enable its expression. Depending on the mode of cellular uptake, lipoplexes can be shuttled to lysosomes, recycled back to the plasma membrane, or escape from endosomes. The incorporation of cholesterol and cholesterol-derivatives in the lipoplex formulation has been shown to boost TE, but the precise mechanism through which this occurs still deserves further investigation. To this end, here we address the transfection mechanisms of cholesterol-containing lipoplexes. We used CLs made of the cationic 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) and the neutral dioleoylphosphocholine (DOPC), and we gradually replaced DOPC molecules by cholesterol. Employing structural studies by synchrotron small angle X-ray scattering, laser scanning confocal microscopy and TE measurements, we were able to elucidate the relation between efficiency and transfection mechanism of cholesterol-containing lipoplexes.

P-653

MHC and Lipid-Raft Proximity: Features of CD1d distribution in a B lymphocyte membrane

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Cluster of Differentiation 1 (CD1) represents a third member of the antigen presentation family. Unlike Major Histocompatibility Complex (MHC) proteins which present peptides, CD1 presents lipids as antigens to T cells. We examined the distribution of CD1d, a subtype of CD1 proteins, in the plasma membrane of a B cell with fluorescent labeling methods, including fluorescence resonance energy transfer (FRET). Significant FRET efficiency, indicative of co-existence, between CD1d and MHC I heavy chain (MHC I-HC), β_2-microglobulin (β_2m) and MHC II proteins was observed in the plasma membrane. Physical proximity of CD1d to lipid-rafts was also found in the membrane, however, the hallmark feature of detergent resistance was absent from these rafts. Interestingly, cholesterol depleting agents, methyl-β-cyclodextrin / simvastatin altered the membrane protein distribution but these chemicals had minimal effect on association between CD1d and GM_1 ganglioside. Additionally, co-localization study between CD1d, MHC I-HC, β_2m, MHC II and raft molecules indicated the possibility of multimolecular complexes of these proteins both inside and outside of rafts. Thus, in summary, our study suggests an intricate relationship of CD1d with MHC and GM_1 molecules on the plasma membrane of B cells.

P-654

Molecular architecture characterization of a new Acyclovir polymorph

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Structural investigation of a relatively wide range of solid pharmaceutical forms such as polymorphs, salts, hydrates/solvates or co-crystals is an essential part on the so called *drug development process*. It is already established that different solid forms of the same pharmaceutical active ingredient can exhibit different physical and chemical properties, such as solubility and bio-availability. The structural study of a new pharmaceutical solid form leads also to the elucidation of the crystal packing modes and the types of intra- and inter-molecular interactions.
This work is focused on determining the molecular architecture of a new anhydrous form of the antiviral Acyclovir. The practical innovative scheme proposed here consists on combining complementarily structure-elucidation techniques, with the main focus on solid-state NMR and molecular modeling.

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Π-stacking induced molecular self-association

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Self-assembly is a ubiquitous natural process due to which individual entities organize themselves spontaneously without any external instruction. The aromatic molecules self-associate mainly in π-π stacked structures with an aggregate size distribution determined by the self-association constants. The present contribution focus on one of the simplest molecular processes: the self-association of aromatic monomers into one-dimensional rod like molecular aggregates. A new generic model (DK – decreasing K model) able to predict the analytic expression for the equilibrium constants $K_{n,m}$ which govern the self-association of two aggregates with n and m monomers respectively has been obtained. The model predicts also the concentrations of free monomers, the concentrations of n-mers and the total concentration of aggregates.
^{1}H NMR experiments have been used to assess the applicability of the proposed model in a particular case: π-stacking self-association of ciprofloxacin hydrochloride in solution. The heat exchange, which accompanies the dissociation processes, was measured by isothermal titration calorimetry. The thermodynamic parameters have been obtained by a dedicated fitting protocol adapted to the recently developed DK self-association model.

Abstracts

– Supramolecular Assemblies –

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DNA – cationic liposomes supramolecular assemblies: the structure and transfection efficiency

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The interaction of DNA polyanion with a dispersion of cationic liposomes results in a formation of supramolecular assemblies of regular inner microstructure - lipoplexes. They serve as a delivery vectors for genetic material. Despite the fact that lipoplexes have been used for transfection, and commercial lipid formulations are available, their efficiency needs to be improved. We will discuss and compare structural variety and binding capacity for DNA of lipoplexes prepared from neutral phospholipids with positive charges created either by cationic gemini surfactants (CnGS) or by divalent metal cations. The binding capacity of lipoplexes for DNA is in the range 40-95 % depending on the system, as we derived from spectrophotometry. A small angle synchrotron X-ray diffraction (SAXD) and neutron scattering (SANS) were used to examine the microstructure of assemblies. Selected lipolexes have shown good transfection efficiency for plasmid pEGFP using HEK 293 cells.

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Bozdaganyan Marine, Moscow, Russian Federation
Braun Christian, Darmstadt, Germany
Brissos Vânia, Oeiras, Portugal
Brocca Paola, Segrate , Italy
Brooks Nicholas, London, U.K.
Brückner Bastian Rouven, Göttingen, Germany
Brujić Jasna, New York, U.S.A.
Buchoux Sébastien, Amiens, France
Buimaga-Iarinca Luiza, Cluj-Napoca, Romania
Byrne Sarah L., London, U.K.
Caillon Lucie, Paris, France
Calamai Martino, Sesto Fiorentino, Italy
Calisto Filipa, Oeiras, Portugal
Campos Sara, Oeiras, Portugal
Caorsi Valentina, London, U.K.

Caracciolo Giulio, Rome, Italy
Cardoso Ana, Coimbra, Portugal
Cardoso Renato, Coimbra, Portugal
Carrascosa José L., Madrid, Spain
Carreira Ana C., Santarém, Portugal
Carrión-Vázquez Mariano, Madrid, Spain
Carvalheda Catarina A., Oeiras, Portugal
Carvalho Ana Luisa, Caparica, Portugal
Carvalho Filomena, Lisboa, Portugal
Casini Angela, Groningen, Netherlands
Castanho Miguel, Lisboa, Portugal
Castro Bruno, Castelldefels, Spain
Castro Paulo, Oeiras, Portugal
Cehlar Ondrej, Bratislava, Slovakia
Cerrada Alejandro, Madrid, Spain
Chaban Vitaly, Odense, Denmark
Chattopadhyay Amitabha, Hyderabad, India
Chaudhary Himanshu, Enschede, Netherlands
Cheleski Juliana , Sao Carlos, Brazil
Chen Po-Chia, Goettingen, Germany
Chiarella Sara, Rome, Italy
Chikhirzhina Elena, Saint-Petersburg, Russian Fed.
Chopineau Joël, Montpellier, France
Christian Bergsdorf, Basel, Switzerland
Christine Deillon, Fribourg, Switzerland
Cisse Ismaïl, Paris, France
Claudia Mihaela Istrate, Bucharest, Romania
Cleri Fabrizio, Villeneuve d'Ascq, France
Coceano Giovanna, Trieste, Italy
Coelho Manuel, Porto, Portugal
Coelho Silvia, Porto, Portugal
Colizzi Francesco, Trieste, Italy
Conde Joao Pedro, Lisboa, Portugal
Corvaglia Stefania, Basovizza , Italy
Costentin Cyrille, Paris, France
Coutinho Ana, Lisboa, Portugal
Covino Roberto, Trento, Italy
Cowsik Sudha, New Delhi, India
Crevenna Alvaro, Munich, Germany
Crowet Jean-Marc, Gembloux, Belgium
Cruz Davide , Oeiras, Portugal
Csomos Istvan, Debrecen, Hungary
Čujová, Sabína, Prague, Czech Republic
Cyboran Sylwia M., Wrocław, Poland
Czub Jacek, Gdańsk, Poland
Dabrowski Marcin, Toruń, Poland
Damas João M., Oeiras, Portugal
Danielsson Jens, Stockholm, Sweden
Dante Silvia, Genova, Italy
Darvas Maria, Trieste, Italy
Dasanna Anil Kumar, Toulouse, France
de Almeida Zaida L., Coimbra, Portugal
de la Rosa Miguel Ángel, Sevilla, Spain
Deák Robert, Budapest, Hungary
Debrand Simon, Dijon, France
Deillon Christine, Fribourg, Switzerland
Dekker Nynke, Delft, Netherlands
Deleu Magali, Gembloux, Belgium
Den Otter Wouter, Enschede, Netherlands
Dennison Andrew, Grenoble, France
Dér András, Szeged, Hungary
Derényi Imre, Budapest, Hungary
Deserno Markus, Pittsburgh, U.S.A.
Deville Célia, Gif-sur-Yvette, France
Di Carlo Maria Giovanna, Palermo, Italy
Dias Pedro, Lisboa, Portugal
Díaz-Moreno Irene, Seville, Spain
Dietz Marina, Frankfurt, Germany
Dijkman Patricia, Oxford, U.K.
Do Santos Alvares Dayane, São José do Rio Preto, Brazil
Dodding Mark, London, U.K.
Dolidze Tinatin, Tbilisi, Georgia
Domingues Marco, Oeiras, Portugal
Domingues Tatiana M., São Paulo, Brazil
Dong Gang, Vienna, Austria
dos Santos Andreia, Lisboa, Portugal
Dressler Lars, Halle/Saale, Germany
Drew Alan, London, U.K.
Drolle Elizabeth, Waterloo, Canada
Drücker Patrick, Münster, Germany
Duan Chenxi, Grenoble, France
Duarte Afonso, Oeiras, Portugal
Dubrovin Evgeny V., Moscow, Russian Federation
Dudek Anita, Baczyna, Poland
Dufourc Erick, Pessac, France
Durka Kamil, Łódź, Poland
Dursun Nizam Korkut, Pessac, France
Dwivedi Mridula, Muenster, Germany
Eaton Peter, Porto, Portugal
Ebenhöh Oliver, Aberdeen, U.K.
Efimova Svetlana, St.petersburg, Russian Federation
Eggart Benjamin, Augsburg, Germany
Elani Yuval, London, U.K.
Enderlein Jörg, Göttingen, Germany
Engelborghs Yves, Leuven, Belgium
Eritja Ramon, Barcelona, Spain

Esquembre Tomé Rocío, Lisboa, Portugal
Etienne Emilien, Marseille, France
Fabriciová Gabriela, Kosice, Slovakia
Fahmy Karim, Dresden, Germany
Fairhead Michael, Oxford, U.K.
Faller Roland, Davis, U.S.A.
Farina Francesca, Paris, France
Farrotti Andrea, Rome, Italy
Fasoli Anna, Ferrara, Italy
Faustino André F., Lisboa, Portugal
Fedunova Diana, Kosice, Slovakia
Fernandes Andreia, Porto, Portugal
Fernandes Fabio, Lisboa, Portugal
Fernandes Oliveira Ana Sofia, Oeiras, Portugal
Fernandez-Lima Francisco, Miami, U.S.A.
Ferreira Mariana, Porto, Portugal
Fersht Alan, Cambridge, U.K.
Filipe Hugo A. L., Coimbra, Portugal
Filipe Luís, Oeiras, Portugal
Finger Sebastian, Halle/Saale, Germany
Finot Eric, Dijon, France
Fischer Jennifer, Jülich, Germany
Fiszman Nicolas, Palaiseau, France
Florescu Monica, Brasov, Romania
Ford Caroline, Shedfield, U.K.
Fournier Jean-Baptiste, Roscoff, France
Franquelim Henri G., Martinsried/planegg, Germany
Frazao Carlos, Oeiras, Portugal
Freire João Miguel, Lisboa, Portugal
Fromme Petra, Tempe, Arizona, U.S.A.
Fromme Raimund, Tempe, U.S.A.
Früh Susanna, Zürich, Switzerland
Fu Biao, Cambridge, U.K.
Funari Sergio, Hamburg, Germany
Gabrielska Janina, Wrocław, Poland
Gadella Theodorus, Amsterdam, Netherlands
Gafni Ari, Ann Arbor, U.S.A.
Galla Hans-Joachim, Münster, Germany
Galvão Rita, Oeiras, Portugal
Gameiro Paula, Porto, Portugal
Gandhi Hetvi, Dresden, Germany
Gansen Alexander, Heidelberg, Germany
Garaiova Zuzana, Bratislava, Slovakia
Garcia-Moreno Bertrand, Baltimore, U.S.A.
García-Parajo María, Castelldefels, Spain
Garnier Norbert, Orléans, France
Gautieri Alfonso, Milan, Italy
Gazova Zuzana, Kosice, Slovakia
Gean Julie, Pessac, France
Genest Monique, Orléans, France
Ghazaryan Narine, Yerevan, Armenia
Giani Matteo, Enschede, Netherlands
Giehring Sebastian, Münster, Germany
Giraldez Teresa, Sta Cruz De Tenerife, Spain
Girych Mykhailo, Kharkiv, Ukraine
Gleisner Martin, Göttingen, Germany
Gomes Bárbara, Gueda, Portugal
Gomes Cláudio, Oeiras, Portugal
Gómez-Fernández Juan Carmelo, Espinardo , Spain
Gomide Andreza , São Paulo, Brazil
Gonçalves Sónia, Lisboa, Portugal
Goncharova Iryna, Prague 6, Czech Republic
Goñi Félix, Leioa, Spain
Gonzalez de Prado Salas Pablo, Madrid, Spain
Graczer Eva, Budapest, Hungary
Grage Stephan, Karlsruhe, Germany
Grauby-Heywang Christine, Talence, France
Grevesse Thomas, Saint-Gilles, Belgium
Grisshammer Reinhard, Rockville, U.S.A.
Groftehauge Morten, Durham, U.K.
Grolmusz Vince, Kuwait City, Kuwait
Grubmüller Helmut, Goettingen, Germany
Grudzinski Wojciech, Lublin, Poland
Gruszecki Wieslaw, Lublin, Poland
Guarrasi Valeria, Palermo, Italy
Guedes Ana Filipa, Lisboa, Portugal
Guerra Gabriela, Santo Tirso, Portugal
Gulnov Dmitry, Krasnoyarsk, Russian Federation
Gutierrez Fabiola, Eindhoven, Netherlands
Gutierrez Fabiola, Eindhoven, Netherlands
Gutsmann Thomas, Borstel, Germany
Güven Celal, Adiyaman, Turkey
Habenstein Birgit, Goettingen, Germany
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Hajnic Matea , Wien, Austria
Hakobyan Lilit, Yerevan, Armenia
Hakobyan Nune, Yerevan, Armenia
Halstrick Isabelle, Zürich, Switzerland
Harmouche Nicole, Pessac, France
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Hellwig Petra, Strasbourg, France
Henry Sarah, Pessac, France

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Hikisz Pawel, Łódź, Poland
Hinterdorfer Peter, Linz, Austria
Hochstetter Axel, Basel, Switzerland
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Hollmann Axel, Lisboa, Portugal
Homble Fabrice, Brussels, Belgium
Horak Martin, Prague , Czech Republic
Horner Andreas, Linz, Austria
Horrocks Mathew, Cambridge, U.K.
Huang Huey, Houston, U.S.A.
Hub Jochen, Göttingen, Germany
Huličiak Miroslav, Olomouc, Czech Republic
Hunt Neil, Glasgow, U.K.
Ianeselli Luca, Trieste, Italy
Iftemi Sorana Elena, Iasi, Romania
Ilie Ioana, Enschede, Netherlands
Ionescu Diana, Bucharest, Romania
Itoh Satoru, Okazaki, Japan
Jacchetti Emanuela, Pisa, Italy
Jain Neha, New Delhi, India
Jakubowski Rafał, Toruń, Poland
Jancura Daniel , Kosice, Slovakia
Janosi Lorant, Cluj-Napoca, Romania
Janshoff Andreas , Goettingen, Germany
Jeftic Jelena, Rennes, France
Jeremias Hélia, Oeiras, Portugal
Jesus Catarina S. H., Coimbra, Portugal
Jirira Nakai, London, U.K.
Jobin Marie-Lise, Pessac, France
Jones Michael, Bristol, U.K.
Joniova Jaroslava, Kosice, Slovakia
Jonsson Peter, Cambridge, U.K.
Joo Chirlmin, Delft, Netherlands
Judge Peter, Oxford, U.K.
Juhaniewicz Joanna, Warsaw, Poland
Juneja Alok, Stockholm, Sweden
Kaliasniova Katsiaryna, Minsk, Belarus
Kaminski Gabriele, Cambridge, U.K.
Kanash Jura, Minsk, Belarus
Kang Yu, Potsdam, Germany
Kapinos Schneider Larisa, Basel, Switzerland
Kasyanenko Nina, Saint-Petersburg, Russian Fed.
Kay Lewis, Toronto, Canada
Keller Sandro, Kaiserslautern, Germany
Kellers Petra, Göttingen, Germany
Kerkour Abdelaziz, Pessac, France
Khalid Syma, Southampton, U.K.
Khandelia Himanshu, Odense, Denmark
Khmelinskaia Alena, Martinsried, Germany
Khoshtariya Dimitri, Tbilisi, Georgia
Kiametis Alessandra Sofia , Brasília, Brazil
Killian J. Antoinette, Utrecht, Netherlands
Kimura Hitomi, Nagoya, Japan
Klapper Yvonne, Karlsruhe, Germany
Klement Reinhard, Göttingen, Germany
Klenerman David, Cambridge, U.K.
Kleszczynska Halina M., Wrocław, Poland
Kliesch Torben-Tobias, Göttingen, Germany
Klose Daniel, Osnabrück, Germany
Klostermeier Dagmar, Muenster, Germany
Klyuyev Dmitry, Karaganda, Kazakhstan
Kmiecik Sebastian, Warsaw, Poland
Knyazev Denis, Linz, Austria
Knyght Ivana, London, U.K.
Koehler Melanie, Linz, Austria
Kogut Mateusz, Gdańsk, Poland
Köhler Stephan, Mainz, Germany
Koken Ergun Cem, Ayd£n, Turkey
Kollmitzer Benjamin, Graz, Austria
Koneracka Martina, Kosice, Slovakia
König Gerhard, Washington, U.S.A.
Konstantinov Alexander , Moscow, Russian Federation
Kopecka Miroslava, Praha 4, Czech Republic
Kopp Annika, Borstel, Germany
Korkut Dursun Nizam, Bordeaux, France
Kosek Dalibor, Prague 2, Czech Republic
Kotb Metwally, Alexandria, Egypt
Kötting Carsten, Bochum, Germany
Kotuniak Radosław, Warsaw, Poland
Kozenkov Pavel, Saint-Petersburg, Russian Federation
Kozlowska Justyna, London, U.K.
Kramer Corinna, Goettingen, Germany
Krammer Eva-Maria, Bruxelles, Belgium
Krausova Barbora, Prague, Czech Republic
Kress Alla, Marseille, France
Kristensen Kasper, København Ø, Denmark
Krobath Heinrich, Lisboa, Portugal
Krüger Daniela, Halle / Saale, Germany
Kubica Krystian, Wrocław, Poland
Kudryashova Ksenia, Moscow , Russian Federation
Kuhlmann Jan, Goettingen, Germany
Kukura Philipp, Oxford, U.K.

Kumagai Patricia, São Carlos, Brazil
Küsters Ilja, Groningen, Netherlands
Kusters Remy, Eindhoven, Netherlands
Kwiatkowska Marta, Łódź, Poland
Lachowska Martyna, Warsaw, Poland
Lafleur Michel, Montréal, Canada
Lamsa Karri, Oxford, U.K.
Langowski Jörg, Heidelberg, Germany
Larijani Banafshe, London, U.K.
Latty Sarah, Cambridge, U.K.
Le Breton Nolwenn, Marseille, France
Le Meins Jean-Francois, Pessac, France
Le Monnier Elodie, Montpellier, France
Lecomte Sophie, Pessac, France
Lehnart Stephan, Goettingen, Germany
Leone Maurizio, Palermo, Italy
Leonenko Zoya, Waterloo, Canada
Lighezan Liliana, Timisoara, Romania
Lindorff-Larsen Kresten, Copenhagen, Denmark
Lira Rafael, São Paulo, Brazil
Lis Mateusz, Wrocław, Poland
Llorca Oscar, Madrid, Spain
Lohner Karl, Graz, Austria
Lopes Silvia, Porto, Portugal
Loquet Antoine, Göttingen, Germany
Lorenz Chris, London, U.K.
Loura Luis, Coimbra, Portugal
Louro Ricardo, Oeiras, Portugal
Lousa Diana, Oeiras, Portugal
Luchowski Rafal, Lublin, Poland
Luecke Hartmut, Irvine, U.S.A.
Luecke Hartmut, Leioa, Spain
Lukacs Andras, Pecs, Hungary
Lumb Hayley, Durham, U.K.
Madeira Ana, Lisboa, Portugal
Maglia Giovanni, Leuven, Belgium
Magyar Melinda, Szeged, Hungary
Mahadeo Mark, Calgary, Canada
Makowska Joanna, Gdańsk, Poland
Mäler Lena, Stockholm, Sweden
Malho Maria Inês, Lisboa, Portugal
Manuel Miguel, Faro, Portugal
Marassi Francesca, La Jolla, U.S.A.
Marcos João Carlos, Braga, Portugal
Marcuello Carlos, Zaragoza, Spain
Marreiros Bruno C., Oeiras, Portugal
Martinez Denis V., Pessac, France
Martínez José Ángel, Logroño, Spain
Martinho Marlène, Marseille, France
Martins Ana Paula, Lisboa, Portugal
Martins Ivo, Lisboa, Portugal
Martins Jorge, Faro, Portugal
Martins Ligia, Oeiras, Portugal
Martins Do Canto António Manuel T., Évora, Portugal
Matagne André, Liège, Belgium
Materska Joanna Maria, Warsaw, Poland
Mathesz Anna, Szeged, Hungary
Matos Pedro, Lisboa, Portugal
Matos Silva Sabrina , São Carlos, Brazil
Matson Dzebo Maria, Gothenburg, Sweden
Matsuo Hidetoshi, Nagoya, Japan
Matsuzaki Katsumi, Kyoto, Japan
Matulis Daumantas, Vilnius, Lithuania
Mátyus László, Debrecen, Hungary
Maximilien Jacqueline, Compiegne, France
Mayer Michael, Ann Arbor, U.S.A.
Meddah Chahrazed, Oran, Algeria
Melo Ana, Lisboa, Portugal
Melo Eduardo, Faro, Portugal
Melo Eurico, Oeiras, Portugal
Melo Manuel, Groningen, Netherlands
Mendes Sónia, Oeiras, Portugal
Mendieta Moreno Jesús I, Madrid, Spain
Merino Felipe, Münster, Germany
Mertins Omar, Sao Paulo, Brazil
Mertins Omar, Sao Paulo, Brazil
Merzel Franci, Ljubljana, Slovenia
Mescola Andrea, Genova, Italy
Meyer Tim, Goettingen, Germany
Michaelis Jens, Ulm, Germany
Michielssens Servaas, Heverlee, Belgium
Miksovska Jaroslava, Miami, U.S.A.
Mikulska Karolina, Toruń, Poland
Milac Adina-Luminita, Bucharest, Romania
Minicozzi Velia, Roma, Italy
Miskovsky Pavol , Kosice, Slovakia
Miszkiewicz Joanna, Warsaw, Poland
Modrak-Wojcik Anna, Warsaw, Poland
Mohrmann Hendrik, Berlin, Germany
Money Victoria , Durham, U.K.
Monico Carina, Florence, Italy
Monteiro Marina, Lisboa, Portugal
Monticelli Luca, Paris, France
Morais Sinara , São Carlos, Brazil
Morais Cabral João , Porto, Portugal
Morante Silvia, Roma, Italy

Moreno Maria João, Coimbra, Portugal
Mori Matteo, Rome, Italy
Mósca Andreia, Lisboa, Portugal
Müller Daniel, Basel, Switzerland
Müller Jochen, Munich, Germany
Munari Francesca, Göttingen, Germany
Muravlyova Larissa, Karaganda, Kazakhstan
Mykuliak Vasyl, Kyiv, Ukraine
Nadova Zuzana, Kosice, Slovakia
Nagy Krisztina, Szeged, Hungary
Nakagawa Lima Angelica, Cachan, France
Nasir Mehmet Nail, Gembloux, Belgium
Navarro José A., Seville, Spain
Negrerie Michel, Palaiseau, France
Nehls Christian, Borstel, Germany
Neubacher Henrik, Goettingen, Germany
Nikas Periklis, Genova, Italy
Nishikawa Naohiro, Nagoya, Japan
Nkoua Ngavouka Maryse Dadina, Trieste, Italy
Nord Ashley, Oxford, U.K.
Novotna Pavlina, Prague 6, Czech Republic
Nowacki Laetitia, Compiègne, France
Nowak Wieslaw, Toruń, Poland
Nyberg Lena, Gothenburg, Sweden
Oelkers Marieelen, Göttingen, Germany
Ogi François-Xavier, München, Germany
Oglecka Kamila, Singapore, Singapore
Oh Yoojin, Linz, Austria
Okumura Hisashi, Okazaki, Japan
Oláh Judit, Budapest, Hungary
Oliva Megan, Melbourne, Australia
Oliveberg Mikael, Stockholm, Sweden
Oliveira Ruben, Braga, Portugal
Omarova Elena, Moscow, Russian Federation
Orban Jozsef, Pecs, Hungary
Orcia Debora, Sao Carlos, Brazil
Orekhov Philipp , Moscow, Russian Federation
Orlov Sergey, Prague, Czech Republic
Ortega Alvaro, Granada, Spain
Ortega Arroyo Jaime, Oxford, U.K.
Orthaus Sandra, Berlin, Germany
Ortiz Antonio, Murcia, Spain
Ortore Maria Grazia, Ancona, Italy
Ostroumova Olga S., Saint-Petersburg, Russian Fed.
Otzen Daniel, Aarhus C, Denmark
Oueslati Walid, Zarzouna-bizerte, Tunisia
P. Varela Ana Raquel, Lisboa, Portugal
Pabst Georg, Graz, Austria
Pacheco-Gómez Raúl, Birmingham, U.K.
Palace Carvalho Alfredo J., Évora, Portugal
Palacios Chaves Leyre, Borstel, Germany
Panyi Gyorgy, Debrecen, Hungary
Paquete Catarina, Oeiras, Portugal
Paredes Martínez José Manuel, Trento, Italy
Paternostre Maité, Gif-sur-Yvette, France
Pazin Wallance, Ribeirão Preto, Brazil
Peplowski Lukasz, Toruń, Poland
Pereira Mafalda, Oeiras, Portugal
Pereira Manuela, Oeiras, Portugal
Perez Katia Regina, São Paulo, Brazil
Pérez-Gil Jesús, Madrid, Spain
Perovic Iva, New York, U.S.A.
Perrakis Anastassis, Amsterdam, Netherlands
Perronet Karen, Palaiseau, France
Pesina Daryna, Kharkiv, Ukraine
Petecchia Loredana, Genova, Italy
Petersen Nils, Edmonton, Canada
Petjukevics Aleksandrs, Daugavpils, Latvia
Petrelli Alessia, Genova, Italy
Pfau Thomas, Aberdeen, U.K.
Pfeil Marc-Philipp, Oxford, U.K.
Piccirilli Federica, Palermo, Italy
Picquart Michel, Mexico, Mexico
Piehler Jacob, Osnabrück, Germany
Piguet Joachim, Lausanne, Switzerland
Pin Jean-Philippe, Montpellier, France
Pinheiro Benedita, Caparica, Portugal
Pinto Sandra, Lisboa, Portugal
Pinto Vieira Joana, Lausanne, Switzerland
Pires Ricardo A., Caldas Das Taipas, Portugal
Plastino Julie, Paris, France
Plochberger Birgit, Vienna, Austria
Poccia Dominic, Amherst, Ma, U.S.A.
Pohl Elena E., Vienna, Austria
Pohl Peter, Linz, Austria
Pontani Lea-Laetitia, New York, U.S.A.
Posada Itziar M. D., Bilbao, Spain
Posch Sandra, Linz, Austria
Pozzi Daniela, Rome, Italy
Prates Ramalho João Paulo, Vora, Portugal
Pregent Stive, Tel Aviv, Israel
Preira Pascal, Toulouse, France
Prieto Manuel, Lisboa, Portugal
Pruchnik Hanna, Wrocław, Poland
Puff Nicolas, Paris, France
Purushothaman Sowmya, London, U.K.

Pusch Michael, Genova, Italy
Pytel Edyta, Łódź, Poland
Quax Wim, Groningen, Netherlands
Quiniou Éric, Orsay, France
Radu Ionela, Berlin, Germany
Rafael Magalhães Pedro, Oeiras, Portugal
Rappolt Michael, Leeds, U.K.
Rasoolzadeh Reza, Kashan, Iran
Ravi Jascindra, Teddington, U.K.
Refojo Patrícia N., Oeiras, Portugal
Renart María Lourdes, Elche, Spain
Renaud Jean-Paul, Illkirch, France
Rezaie Ghaleh Nasrollah, Göttingen, Germany
Ribeiro Miguel, Oeiras, Portugal
Ricardo Joana, Lisboa, Portugal
Richly Maximilian, Palaiseau, France
Rino José, Lisboa, Portugal
Riske Karin, São Paulo, Brazil
Robalo João, Évora, Portugal
Robin Thierry-Johann, Compiegne, France
Rocca Francesco, Trento, Italy
Rodgers Thomas, Manchester, U.K.
Rodrigues Cecília, Lisboa, Portugal
Rodrigues Claudia, Lisboa, Portugal
Rodrigues Margarida, Lisboa, Portugal
Rodziewicz-Motowidło Sylwia, Gdańsk, Poland
Roess Deborah, Fort Collins, U.S.A.
Romão Maria J., Caparica, Portugal
Romero Obando María P., Quito, Ecuador
Rondelli Valeria Maria, Segrate, Italy
Rosário Ana Lúcia, Oeiras, Portugal
Roses Subiros Cristina, Girona, Spain
Rossi Claire, Compiègne, France
Rossi Giancarlo, Roma, Italy
Roth Bastian, Darmstadt, Germany
Roth Sebastian, Berlin, Germany
Roudi Yasser, Trondheim, Norway
Roussel Guillaume, Namur, Belgium
Roversi Daniela, Rome, Italy
Royer Catherine, Montpellier, France
Rudolph Markus, Basel, Switzerland
Ruggiero Neto João, São José Do Rio Preto , Brazil
Rupprecht Anne, Wien, Austria
Rydberg Hanna, Gothenburg, Sweden
Sacquin-Mora Sophie, Paris, France
Sadowska-Bartosz Izabela, Rzeszów, Poland
Saiz Leonor, Davis, U.S.A.
Salgado Gilmar F., Pessac, France
Salvador Armindo, Coimbra, Portugal
Samatey Fadel, Onna, Japan
Sancho Javier, Zaragoza, Spain
Sanders Michael, Didcot, U.K.
Sanderson John, Durham, U.K.
Sandhu Sandeep, Birmingham, U.K.
Santos Filipa, Lisboa, Portugal
Santos Marino, Sobreda, Portugal
Santos Nuno, Lisboa, Portugal
Santos Telma, Lisboa, Portugal
Santos Silva Maria Margarida, Oeiras, Portugal
Saponaro Andrea Cosimo, Milan, Italy
Saravanan Manikam Sadasivam, Utrecht, Netherlands
Saribal Devrim, İstanbul, Turkey
Sarmento Maria , Lisboa, Portugal
Sas Sasa, Asdsd, Albania
Saulis Gintautas, Kaunas, Lithuania
Savytskyi Oleksandr V., Kyiv, Ukraine
Sawada Yasuyuki, Nagoya, Japan
Schatz Michaela, Göttingen, Germany
Scheibel Thomas, Bayreuth, Germany
Schindl Rainer, Linz, Austria
Schmatko Tatiana, Strasbourg, France
Schmidt Tobias, Halle/Saale, Germany
Schmitt Nicole, Copenhagen N, Denmark
Schor Marieke, Edinburgh, U.K.
Schroder Andre, Strasbourg, France
Schuler Benjamin, Zürich, Switzerland
Schumacher Silke, Heidelberg, Germany
Schütte Ole Mathis, Goettingen, Germany
Schwamborn Miriam, Goettingen, Germany
Schwenen Lando, Goettingen, Germany
Schwille Petra, Martinsried, Germany
Scott Luis Paulo, Santo André, Brazil
Sebastião Ana, Lisboa, Portugal
Seddon John, London, U.K.
Seegers Christel, Groningen, Netherlands
Sena Filipa, Oeiras, Portugal
Sengupta Durba, Pune, India
Separovic Frances, Melbourne, Australia
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Shen Jana, Baltimore, U.S.A.
Shrestha Dilip, Debrecen, Hungary
Shutava Iryna, Uppsala, Sweden
Shvadchak Volodymyr, Enschede, Netherlands
Sielewiesiuk Jan, Lublin, Poland
Sill Clemens, Jülich, Germany

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Silva Liana C., Lisboa, Portugal
Silva Patrícia, Lisboa, Portugal
Silva Renata, Coimbra, Portugal
Silva Tânia, Porto, Portugal
Silva Nóbrega Cláudia, Caparica, Portugal
Simmel Friedrich, Garching, Germany
Šimšíková Michaela, Brno, Czech Republic
Siotto Fenja, Darmstadt, Germany
Siposova Katarina, Kosice, Slovakia
Sixt Michael, Klosterneuburg, Austria
Skrabana Rostislav, Bratislava, Slovakia
Slama-Schwok Anny, Jouy En Josas, France
Small Lara, Durham, U.K.
Smyrnova Daryna, Leuven, Belgium
Soares Cláudio, Oeiras, Portugal
Somkuti Judit, Budapest, Hungary
Soskine Misha, Leuven, Belgium
Sot Jesús, Leioa, Spain
Sotomayor-Perez Ana-Cristina, Paris, France
Sousa Cristiana, Oeiras, Portugal
Sousa Tânia, Lisboa, Portugal
Sousa Simões Joana, Oeiras, Portugal
Soveral Graça, Lisboa, Portugal
Spinozzi Francesco, Ancona, Italy
Stange Roland, Leipzig, Germany
Starok Marcelina, Compiegne, France
Stavrov Solomon, Tel Aviv, Israel
Stefanovic Anja, Enschede, Netherlands
Steinem Claudia, Goettingen, Germany
Stellato Francesco, Hamburg, Germany
Sturgis James, Marseille, France
Subramaniam Vinod, Enschede, Netherlands
Sukiasyan Astghik, Yerevan, Armenia
Sumitomo Koji, Atsugi, Japan
Surleac Marius, Bucharest, Romania
Swainsbury David, Bristol, U.K.
Szalóki Nikoletta, Debrecen, Hungary
Szpryngiel Scarlett, Stockholm, Sweden
Szuba Agata, Dresden, Germany
Tabak Marcel, São Carlos, Brazil
Takáts-Nyeste Annamária, Budapest, Hungary
Talián Gábor, Pécs, Hungary
Tatarkovič Michal, Prague, Czech Republic
Tatum Natalie, Durham, U.K.
Taylor Garrick, Oxford, U.K.
Telley Ivo, Oeiras, Portugal
Tenorio Cavalcante Guacyara, Sao Paulo, Brazil
Teruel José A., Murcia, Spain
Teufel Lotte, Berlin, Germany
Thomas Franziska, Martinsried, Germany
Thye Julie, Durham, U.K.
Tinti Emmanuel, Namur, Belgium
Tittel Janine, Martinsried, Germany
Tobiszewski Adrian, Gdańsk, Poland
Toczylowska-Maminska Renata, Warsaw, Poland
Tokar Tomas, Kosice, Slovakia
Tomić Sanja, Zagreb, Croatia
Tompa Peter, Brussels, Belgium
Tosatto Laura, Trento, Italy
Toth Katalin, Heidelberg, Germany
Trebacz Hanna, Lublin, Poland
Tremblay Dominique, Gatineau, Canada
Tretyakova Tatyana, Tbilisi, Georgia
Trigo Marquês Joaquim, Lisboa, Portugal
Tripon Carmen, Cluj-Napoca, Romania
Trusova Valeriya, Kharkov, Ukraine
Tsuji Toshikazu, Yokohama-shi, Kanagawa , Japan
Turberfield Andrew, Oxford, U.K.
Turcu Ioan, Cluj-napoca , Romania
Tyler Arwen, London, U.K.
Uchiyama Seiichi, Tokyo, Japan
Uhrikova Daniela , Bratislava, Slovakia
Ulrich Anne, Karlsruhe, Germany
Underwood Elizabeth, London, U.K.
Urbanska Marta, Dresden, Germany
Uzun Göçmen Semire, Hatay, Turkey
Valpuesta José, Madrid, Spain
Vámosi György, Debrecen, Hungary
Van Qui, Goettingen, Germany
Vargas Carolyn, Kaiserslautern, Germany
Vasile Ionut, Magurele, Ilfov, Romania
Vattulainen Ilpo, Tampere, Finland
Vellosillo Perceval, Madrid, Spain
Veneziano Rémi, Montpellier, France
Ventura Ana Ester, Vila Verde De Ficalho, Portugal
Vértessy Beáta, Budapest, Hungary
Vetri Valeria, Palermo, Italy
Veya Luc, Lausanne, Switzerland
Vieira Sara, Rome, Italy
Vilar Jose, Leioa, Spain
Vizsnyiczai Gaszton, Szeged, Hungary
Vogel Horst, Lausanne, Switzerland
Vogel Viola, Zürich, Switzerland
Volotovski Igor, Minsk, Belarus
Vortmeier Gerrit, Leipzig, Germany

Vreede Jocelyne, Amsterdam, Netherlands
Vuletic Tomislav, Zagreb, Croatia
Vus Kateryna, Kharkiv, Ukraine
Vyumvuhore Raoul, Chatenay-malabry, France
Warzecha Marek, Warsaw, Poland
Watanabe Chiho, Paris, France
Watkins Erik, Grenoble, France
Watts Anthony, Oxford, U.K.
Webb Stephen, Didcot, U.K.
Westhof Eric, Strasbourg, France
Wieczor Milosz, Gdańsk, Poland
Wielgus-Kutrowska Beata, Warsaw, Poland
Wilkinson Anthony, York, U.K.
Windschiegl Barbara, Münster, Germany
Woźniak Edyta, Wrocław, Poland
Wolf Alexander, Berlin, Germany
Wypijewska Anna, Warsaw, Poland
Wysmołek Paulina Maria, Warsaw, Poland
Xu Ximing, Paris, France
Ye Weihua, Stockholm, Sweden
Yegres Michelle, Strasbourg, France
Yin Daniel, Oxford, U.K.
Ying Liming, London, U.K.
You Changjiang, Osnabrueck, Germany
Zaleska Mariola, London, U.K.
Zhou Man, Goettingen, Germany
Zidar Jernej, Singapore, Singapore
Zijlstra Niels, Enschede, Netherlands
Zinn-Justin Sophie, Gif-sur-Yvette, France
Zubriene Asta, Vilnius, Lithuania