Springer Theses

Recognizing Outstanding Ph.D. Research

More information about this series at http://www.springer.com/series/8790

Aims and Scope

The series "Springer Theses" brings together a selection of the very best Ph.D. theses from around the world and across the physical sciences. Nominated and endorsed by two recognized specialists, each published volume has been selected for its scientific excellence and the high impact of its contents for the pertinent field of research. For greater accessibility to non-specialists, the published versions include an extended introduction, as well as a foreword by the student's supervisor explaining the special relevance of the work for the field. As a whole, the series will provide a valuable resource both for newcomers to the research fields described, and for other scientists seeking detailed background information on special questions. Finally, it provides an accredited documentation of the valuable contributions made by today's younger generation of scientists.

Theses are accepted into the series by invited nomination only and must fulfill all of the following criteria

- They must be written in good English.
- The topic should fall within the confines of Chemistry, Physics, Earth Sciences, Engineering and related interdisciplinary fields such as Materials, Nanoscience, Chemical Engineering, Complex Systems and Biophysics.
- The work reported in the thesis must represent a significant scientific advance.
- If the thesis includes previously published material, permission to reproduce this must be gained from the respective copyright holder.
- They must have been examined and passed during the 12 months prior to nomination.
- Each thesis should include a foreword by the supervisor outlining the significance of its content.
- The theses should have a clearly defined structure including an introduction accessible to scientists not expert in that particular field.

Kiyotaka Akabori

Structure Determination of HIV-1 Tat/Fluid Phase Membranes and DMPC Ripple Phase Using X-Ray Scattering

Springer

Author
Kiyotaka Akabori
Carnegie Mellon University
Pittsburgh, PA, USA

Supervisor
Dr. Stephanie Tristram-Nagle
Department of Physics
Carnegie Mellon University
Pittsburgh, PA, USA

ISSN 2190-5053 ISSN 2190-5061 (electronic)
Springer Theses
ISBN 978-3-319-22209-7 ISBN 978-3-319-22210-3 (eBook)
DOI 10.1007/978-3-319-22210-3

Library of Congress Control Number: 2015950468

Springer Cham Heidelberg New York Dordrecht London

Printed on acid-free paper

Springer International Publishing AG Switzerland is part of Springer Science+Business Media (www.springer.com)

Supervisor's Foreword

In physics, a graduate student has many, diverse fields of study from which to choose for the Ph.D. thesis. These fields range from astrophysics and cosmology, to computational physics, to high-energy experiment or theory, to quark interaction experiment or theory, to condensed matter theory or experiment. Biological physics is another, more recent choice in many physics departments. Biological physics has several subfields, such as biomembrane structure, dynamics and organization (both experiment and theory), folding and unfolding of single protein, DNA and RNA molecules, collective vibrational modes in biomacromolecules, transport and rheology in biopolymer gels, and interaction and structure of supramolecular assemblies. The two focuses of the current thesis are both in the area of biomembranes. Why study biomembranes? Membranes play a central role in both the structure and function of all cells, plant and animal. Membranes not only define compartments, they also determine the nature of all communication between the inside and the outside. In addition, most of the fundamental biochemical functions in cells involve membranes at some point, including such diverse processes as prokaryotic DNA replication, protein biosynthesis, protein secretion, bioenergetics, and hormonal responses. Although biomembranes have been studied since the beginning of the twentieth century, there are many mysteries that remain: precise structure and physical properties of single component lipid membranes, the reason for the large diversity of lipids in biomembranes, and the details of complex interactions or proteins and peptides with biomembranes. The techniques developed by physicists are uniquely suited to probe these underlying questions. Two interesting reference books in this field are *Biomembranes, Molecular Structure and Function*, Robert B. Gennis, Springer, 1989, New York, and *Lipids & Membrane Biophysics*, Faraday Discussions, Vol. 161, Royal Society of Chemistry, 2013, Cambridge, England.

The first focus of the current thesis is Tat, the transactivator of transcription, an important protein for HIV-1 infection. Tat acts by enhancing the readout of HIV-1 RNA, through molecular interactions with the HIV-1 DNA. In order to carry out its function, Tat translocates across the T-cell's nuclear membrane, by relying on a highly positively charged, basic region of only 11 amino acids that can translocate through membranes without requiring energy. It is counterintuitive

that a highly charged molecule could not only translocate itself through the low dielectric medium of the hydrocarbon interior of lipid membranes but that it can also be engineered to pull larger, uncharged molecules through membranes as well. Many investigations have attempted to probe Tat's structure in membranes in order to understand its translocation through them. In this thesis, high intensity synchrotron X-rays were used to probe the molecular details of the structural and elastic interactions of Tat with membranes composed of several types of lipids. These experiments were directly compared with atomistic molecular dynamics simulations of Tat interacting with membranes. This comparison delineated the precise location of Tat in biomembranes and its effect on the membranes.

The second focus of the current thesis is the enigmatic ripple phase. While it is not of physiological importance, since it is primarily gel-like in character and since it only forms in single component membranes, it has been the subject of many theoretical and experimental physics papers as an example of a periodically modulated phase. Despite many systematic studies over the past three decades, molecular details of the structure were still lacking, which impeded theoretical understanding of its origin. In this thesis, Dr. Akabori used synchrotron X-rays to probe the ripple phase in the lipid DMPC, oriented onto a silicon wafer and hydrated through the vapor in a hydration chamber. These techniques allowed him to quantitate the degree and direction of chain tilt in both the major and minor arms of the corrugated, sawtooth pattern. A new model of the ripple phase emerged which will serve to motivate theoreticians to supply a driving force.

Pittsburgh, PA, USA Stephanie Tristram-Nagle
2015/5/29

Abstract

This thesis employs X-ray scattering to study the structure of two different stacked lipid membrane systems. The first part reports the effect on lipid bilayers of the Tat peptide Y_{47}GRKKRRQRRR$_{57}$ from the HIV-1 virus transactivator of transcription (Tat) protein. Synergistic use of low-angle X-ray scattering (LAXS) and atomistic molecular dynamics (MD) simulations indicated Tat peptide binding to neutral dioleoylphosphatidylcholine (DOPC) headgroups. This binding induced the nearby lipid phosphate groups to move 3 Å closer to the bilayer center. Many of the Tat arginines were as close to the bilayer center as the locally thinned lipid phosphate groups. Analysis of LAXS from DOPC, DOPC/dioleoylphosphatidylethanolamine (DOPE), DOPC/dioleoylphosphatidylserine (DOPS), and a mimic of the nuclear membrane indicated that the Tat peptide decreased the bilayer bending modulus K_c and increased the area per lipid, possibly facilitating Tat membrane translocation. Although a mechanism for translocation remains elusive, this study suggests that Tat translocates from the headgroup region.

The second study presents the structure of the asymmetric ripple phase formed by dimyristoylphosphatidylcholine. We determined the most detailed ripple phase structure by combining synchrotron LAXS and wide-angle X-ray scattering (WAXS) from highly aligned multilamellar samples. We derived three intensity corrections to calculate the X-ray form factors from the 52 measured reflections. The LAXS analysis provided a high-resolution two-dimensional electron density map. The ripple major arm was demonstrated to be consistent with the gel phase, and the major and minor arm structures were clearly different, supporting the coexistence of different molecular organizations. The minor arm electron density profile was qualitatively consistent with interdigitated chain packing previously proposed by MD simulations. Analysis of high-resolution near grazing incidence WAXS showed that major arm hydrocarbon chains were tilted parallel to the ripple plane by 18° with respect to the bilayer local normal, toward the next nearest neighbor similarly to the gel $L_{\beta F}$ rather than the $L_{\beta I}$ phase. By measuring the Bragg rod lengths in transmission WAXS, we determined that major arm chains in opposing leaflets were coupled. The LAXS and WAXS results together indicated

that chains in the major arm were shorter by 1.3 Å compared to the gel phase, suggesting a gauche-trans-gauche kink in the ripple major arm. In contrast to the LAXS analysis, the measured nGIWAXS was consistent with disordered chains in the minor arm similarly to the fluid L_α phase.

Acknowledgments

I would like to thank my advisors Profs. John F. Nagle and Stephanie Tristram-Nagle for giving me an opportunity to work in the field of biophysics, for guiding my research, and for supporting me as a research assistant.

I especially thank Michael S. Jablin for a number of exciting discussions and projects we shared. Despite our differences, he always respected my opinions and helped me shape my ideas.

I thank Profs. Angel E. García, Robert M. Suter, and Frank Heinrich for serving on my Ph.D. committee.

Thanks to Dr. Kun Huang for providing us with many simulation results.

I thank Prof. Christian D. Santangelo for giving me a chance to work on a theoretical problem.

I thank my parents for allowing me to study at UCSB and financially supporting me. Many thanks to my wife for the countless moments of joy and laughter, supporting my Ph.D. study with great meals every day, and being a wonderful mother. And to Shyn, I am so proud to be your father.

Contents

Notations

A	ripple amplitude 65, 103
$A(\theta)$	absorption factor 93
$\mathbf{a_1}$	monoclinic unit cell vector 66
$\mathbf{a_2}$	monoclinic unit cell vector (ripple wavevector) 66, 65
A_L	area per lipid 20, 46
a	overall scaling factor in SIMtoEXP 24
$\mathbf{a}$	orthorhombic unit cell vector 131
$\mathbf{b}$	orthorhombic unit cell vector 131
B	bulk modulus 15
χ^2	goodness of fit 22, 24
χ	angle measured from the meridian on a CCD detector 94
c_i	amplitude of the volume probability distribution 17
CH	methine group 17
CH_2	methylene group 17
CH_2+CH	methylene and methine group combination 17
CH_3	terminal methyl group 17
Δq_r	instrumental resolution in q_r 78
Δq_z	instrumental resolution in q_z 78
$\Delta\omega$	range of ω over which a sample is rotated 87
D	lamellar repeat distance 28, 65, 66
D-spacing	lamellar repeat distance, D 14
D_HH	head-to-head distance 32
$\langle D_\mathrm{phos} \rangle$	phosphorus-phosphorus distance averaged over a bilayer 49
D_phos	phosphorus-phosphorus distance in the suppressed region 25
D^0_phos	phosphorus-phosphorus distance in the unperturbed region 25
D_PP	PC-PC, phosphate-phosphate, or phosphorus-phosphorus distance 32
f_1	ratio of the minor to the major arm electron densities 103

f_2	ratio of the kink region to the major arm electron densities 103
$F(\mathbf{q})$	X-ray form factor 15
$F^{\text{sim}}(q_z)$	simulated X-ray form factor 24
F_{hk}	X-ray form factor for the (h,k) reflection 90
F_{hk}^{ori}	F_{hk} for the oriented sample 101
F_{hk}^{un}	F_{hk} for the unoriented sample 101
$F_C(\mathbf{q})$	contour part of the X-ray form factor 103
$F_T(\mathbf{q})$	transbilayer part of the X-ray form factor 103
γ	ripple oblique angle 65
(h,k)	ripple phase Miller indices 86
H_{Tat}	Tat cylinder height 25
$I(\mathbf{q})$	measured intensity 15
I_{hk}^{obs}	observed intensity for the (h,k) reflection 89
K_{c}	bending modulus 15
λ	X-ray wavelength 72
λ_r	ripple wavelength 65, 66
μ	X-ray absorption length 79
ω	beam incident angle 14
ϕ	angle measured from the equator or in-plane chain tilt direction 83, 133
Φ_{hk}	X-ray phase factor for the (h,k) reflection 101
P_i	volume probability distribution 17
ψ	chain tilt angle with respect to the z direction 66
$\mathbf{q}$	X-ray momentum transfer vector 15
q_x	in-plane q-space coordinate perpendicular to the beam direction ... 84
q_y	in-plane q-space coordinate 84
q_z	q-space coordinate perpendicular to the substrate 15, 84
q_r	azimuthal in-plane q-space coordinate; $q_r = \sqrt{q_x^2 + q_y^2}$ 15
q_{hk}^r	q_r value of the (h,k) reflection 86
q_{hk}^z	q_z value of the (h,k) reflection 86
$\rho(x,z)$	ripple electron density map 101
$\rho(z)$	bilayer electron density profile normal to the membrane plane 15
$\rho_{\text{H}i}$	electron density of the i^{th} headgroup Gaussian in the 2G hybrid model 104
ρ_{M}	electron density of the terminal methyl Gaussian in the 2G hybrid model 104
$\rho_{\text{Head}}(x)$	electron density profile along the headgroup positions 107
ρ_{W}	average water electron density 24
R_{Tat}	radius of Tat cylinder 25
R_2	outer radius of the boundary region 26
R_3	outer radius of the unperturbed region 27

$\sigma_{\mathrm{H}i}$	width of the i^{th} Gaussian in the 2G hybrid model	104
σ_{M}	width of the terminal methyl Gaussian in the 2G hybrid model	104
σ_F	uncertainties on X-ray form factors	100
σ_I	uncertainties on observed intensities	98
σ_i	volume probability distribution width for molecular component i	17
$S(\mathbf{q})$	X-ray structure factor	15
T	temperature	14
θ	chain tilt angle or X-ray scattering angle	73
V_{HL}	lipid headgroup volume	20
V_{Tat}	Tat volume	13, 25
V_{L}	lipid volume	20
w_s	sample width along the beam direction	73
ξ_{M}	ripple major arm tilt angle	107
ξ_{m}	ripple minor arm tilt angle	107
x_{M}	projected length of the major arm	66, 103
x_{Tat}	Tat mole fraction	28
$z_{\mathrm{Head}}(x)$	headgroup z positions	107
z_i	center of the volume probability distribution for molecular component i	17
z_{phos}	phosphorus z position in the suppressed region	26
z^0_{phos}	phosphorus z position in the unperturbed region	27
z_{Tat}	Tat position measured from the bilayer center	31, 41, 46
z_{guan}	guanidinium peak position	52
$Z_{\mathrm{H}i}$	center of i^{th} Gaussian in the 2G hybrid model	104

Acronyms

CCD	charge coupled device	14
CD	circular dichroism	11
CG	carbonyl/glycerol	17
CHESS	Cornell High Energy Synchrotron Source	71
CPP	cell penetrating peptide	9
DLPC	dilauroylphosphatidylcholine	65
DMPC	dimyristoylphosphatidylcholine	1
DOPC	dioleoylphosphatidylcholine	2, 66
DOPE	dioleoylphosphatidylethanolamine	2
DOPG	dioleoylphosphatidylglycerol	66
DPPC	dipalmitoylphosphatidylcholine	2, 67
EDP	electron density profile	17
FWHM	full width half maximum	25, 78
GUV	giant unilamellar vesicle	9
HC	hydrocarbon chain	17
HIP	hexafluoroisopropanol	12
LAXS	low angle X-ray scattering	11, 14
LUV	large unilamellar vesicle	9
MD	molecular dynamics	4
MLV	multilamellar vesicle	12
nGIWAXS	near grazing incidence wide angle X-ray scattering	69
NMR	nuclear magnetic resonance	11
PC	phosphatidylcholine or phosphate/choline	1, 2, 9, 17, 66
PE	phosphatidylethanolamine	2, 9, 66
PF	phase factor	110
PG	phosphatidylglycerol	2, 9, 66
POPC	palmitoyloleoylphosphatidylcholine	67

PS	phosphatidylserine	2, 9
Tat	transactivator of translation	3
TFA	trifluoroacetate	13
TFE	trifluoroethanol	12
THC	Tat-in-hydrocarbon-chain model	19
THG	Tat-in-headgroup model	18
tWAXS	transmission wide angle X-ray scattering	69
WAXS	wide angle X-ray scattering	14