Reviews arising from the 2017 conference of the Australian Society for Biophysics and the Japanese Society for Biophysics

Cristobal G. dos Remedios¹

Received: 12 September 2018 / Accepted: 18 September 2018 / Published online: 24 September 2018

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This issue contains 20 articles from contributors at the joint symposium of the Australian Society for Biophysics (ASB) and the Biophysical Society of Japan (BSJ) held at the University of Technology Sydney in November 27–29, 2017. For a small society, the ASB regularly “punches” above its weight. The next meeting of the ASB will again be a joint conference, this time in conjunction with the Asian Biophysics Association to be held in Melbourne December 2–6, 2018 at the University of Melbourne. The programmed symposium themes will include Biophysics and Medicine, Membrane Biophysics, Structural Biology, Nanobiophysics, and Imaging and Super-resolution Microscopy.

The Australian Society for Biophysics, Bob Robertson Award

Dr. Michael Parker from the St Vincent’s Institute for Medical Research received the 2016 Bob Robertson Award and medal for his work on pore-forming, cholesterol-dependent cytolysins (CDCs). These are a family of pore-forming toxins that punch holes through the cell membranes of eukaryotic cells, and function as bacterial toxins and virulence factors in Gram-positive bacteria. CDCs are secreted as soluble, stable monomeric proteins that bind to cholesterol-rich membranes where they assemble into ring-shaped pore-forming complexes that undergo a structural change that drives a large pore through the membrane, potentially causing the lysis of the target cell. Understanding just how CDCs transition from a discrete monomer to a complex, membrane-spanning protein machine is an ongoing challenge. While many of the details have been revealed, there are still questions that

remain unanswered. The authors review the current knowledge of the structure of the secreted monomers, and show how they interact with target membranes to form complete pores.

Macromolecular structure and function

Scattered light is a particular problem in CD spectroscopy

The appointment of Dr. Alison Rodger to the chair of Molecular Sciences at Macquarie University was a boost for biophysics in Australia. Her revue addresses a practical problem involving corrections to linear dichroism (LD) spectra for scattered light in flow systems involving turbid liposome samples. Currently there is no easy way to deal with these artifacts. LD spectroscopy is used to determine binding modes of proteins and small molecules (peptides) to liposomes. Equations for a modified Rayleigh-Gans-Debye approximation for the turbidity (scattering) are available but have not been implemented. Scattering calculations require methods that estimate liposome integrity (volume loss and changes in orientation) during the LD experiment where they are subjected to shear (flow) stresses. Dr. Rodger and her colleagues provide practical advice on how to estimate these parameters using the fluorescence intensity of low concentrations of (< 1 mM) calcein.

SAXS as a screening tool

Dr. Po-chia Chen and colleagues compare the use of small-angle X-ray scattering (SAXS) to other structural methods. They review the recommend guidelines for screening protocols that are tailored to available X-ray sources. SAXS is a good screening tool for several reasons. It requires minimal amounts of sample and is not costly to set up. Other advantages include its ability to

✉ Cristobal G. dos Remedios
cris.dosremedios@sydney.edu.au

¹ The University of Sydney, Sydney, New South Wales, Australia

detect binding thermodynamics, and its ability to provide structural information needed to distinguish between agonists and inhibitors. However, the serial nature of SAXS measurements limits the number of titrations that can be achieved in a day. The review collates the essential information needed to conduct SAXS, and they predict it will be increasingly used as a screening method.

Quantum biology and light-harvesting systems

The main focus of non-trivial quantum mechanical effects in biology is on the potential to use “quantum biology” and energy transfer processes in photosynthetic light-harvesting systems. Ultrafast laser spectroscopy has uncovered coherent oscillations or “quantum beats”. These persist for 10^2 fsec and are the virtual “signatures” for quantum transport phenomena. In this two-part review, Dr. Paul Curmi and his colleagues explain the language and basic quantum mechanical phenomena that underpin quantum transport in open systems such as light harvesting and photosynthetic proteins, including the photosystem reaction center. Coherence effects are considered in detail and they define factors such as delocalized excitations (or excitons), entangled states, and coherent transport. Critical factors for understanding coherence processes include the time scales and length scales of energy transport. They analyze the role proteins play in maintaining chromophore systems, and they use spectroscopic techniques, (energy transfer dynamics) to describe “quantum beats” with reference to coherent phenomena in light harvesting. Part 2 reviews energy transfer processes in light-harvesting systems. Most long-lived coherent phenomena are vibrational or vibronic (where coherent excitation transport occurs within a protein complex). In contrast, transport between proteins is probably incoherent. Whether evolution played a role in selecting these non-trivial quantum phenomena may be an unanswerable question, because dense packing of chromophores leads to strong coupling and hence nontrivial quantum phenomena. However, evolution may have optimized the light-harvesting systems either for high chromophore density or for the ensuing quantum effects as these are inextricably linked and cannot be switched off.

Fluctuation theorem and neuroscience

The fluctuation theorem is a nonequilibrium statistical physics theorem that describes entropy production in nonequilibrium states. It has been used to estimate the driving power of motor proteins from fluctuation in their motion. In this review, Dr. Kumiko Hayashi uses the fluctuation theorem in experiments on motor proteins where force measurement is a central issue. She first introduces the

application of the fluctuation theorem for measuring torque produced by the rotary motor protein F1-ATPase that drives flagellar rotation. Then she extends this application to a recent trial estimating the force generated during cargo transport in vivo by the microtubule motors, kinesin, and dynein. Elucidation of the physical mechanism of such transport motors is important, especially for neurons where deficits in cargo transport are implicated in neuronal diseases. Finally, Dr. Hayashi discusses the potential for the fluctuation theorem as a new technique in the field of neuroscience.

The inventor of “Mechanomedicine”

In the years since the term mechanobiology was first introduced, research ranging from basic biology to medical research has been conducted from the perspective of mechanobiology. Dr. Keiji Naruse and his colleagues coined the term “mechanomedicine” which focuses on the fields encompassing the pathology and treatment of diseases. It is based on the application of mechanobiology to fields such as the respiratory and cardiovascular systems. Physical factors, such as contraction and relaxation, require feedback from mechanosensors in tissues that are important for maintaining homeostasis. Loss of homeostasis leads to pathology. Thus, in this review, the authors provide an overview of mechanomedicine by introducing several mechanosensitive channels including a particular type of mechanosensor they discovered in the cardiovascular system. They describe stretchable, three-dimensional cell culture scaffolds on which peptides self-assemble, and highly motile sperm sorter using a technique based on microfluidic mechanics; and a device used to promote the development of fertilized ova.

DNA origami

Dr. Shelley Wickham reviews the field of structural DNA nanotechnology where base-pairing drives the self-assembly of nanostructure. In recent years, it has rapidly expanded in complexity and functionality. DNA nanostructures (origami) can be made in arbitrary 3-dimensional shapes and used as a scaffold for many other functional molecules. Recently, these areas have merged to produce “switchable” DNA nanostructures that change state in response to their environment. The authors review these nanostructures for use as molecular actuators that can be triggered by local chemical changes, and external actuators triggered by light, electric or magnetic fields. The latter is a relatively a new area that allows remote control of nanoscale devices. The author discusses recent applications of switchable DNA nanostructures to perform functions such as opening a capsule to deliver molecules to a target cell. She also discusses some challenges and future directions

for achieving the technological complexities required to translate them to living cells.

Membranes, ion channels, and receptors

The effects of hydronium ions on membranes

Dr. Charles Cranfield and his colleagues presented the combined results from two recent studies showing how hydronium

ions (H_3O^+) modulate the structure and ion permeability of phospholipid bilayers. Briefly, molecular dynamics (MD) simulations support a model where the hydrogen bonding capacity of H_3O^+ for carbonyl and phosphate oxygens can explain the origin of the pH-induced changes in lipid packing in phospholipid membranes. These molecular-level studies are an important step towards a better understanding of the effect of pH on biological membranes. The figure below shows MD simulations of phospholipid packing bonding and in the presence of hydronium ions, water and hydroxyl ions.

Mechanoreceptors and membrane gating

Dr. Boris Martinac is well known for his interest in mechanoreceptors. Here, he summarizes his views on how mechanical stimuli act on cellular membranes, and how they are linked to intracellular signaling events and downstream effectors. Mechanosensitive (MS) ion channels are the fastest primary mechano-electrical transducers known. They convert mechanical stimuli into meaningful intracellular signals on a submillisecond time scale. Much of our understanding of the biophysical principles that underlie and regulate the conversion of mechanical force into conformational changes in MS channels comes from studies based on MS channel reconstitution into lipid bilayers. Bilayer reconstitution methods have enabled researchers to investigate the structure-function relationships of MS channels, and to probe their specific interactions with their membrane lipid environment. This brief review focuses on close interactions between MS channels and the lipid bilayer and emphasizes the central role that the

transbilayer pressure profile plays in mechanosensitivity and gating of these fascinating membrane proteins.

Selectivity of glutamate selective receptors

Dr. Yoshi Nakayama is an Exchange Fellow with the Japan Society for the Promotion of Science (JSPS). He and his colleagues describe how membrane sensors on the bacterium, *C. glutamicum*, specifically detect the food additive monosodium glutamate (MSG) responsible for the fifth basic taste modality called “UMAMI”. Bacterial export of MSG is facilitated by opening mechanosensitive channels, but these are not simply “osmotic safety valves” to prevent bacterial rupture when they are osmotically shocked. They are actually “metabolic valves” that continuously and specifically release glutamate as part of a “pump-and-leak” mechanism. Opening the channel specifically facilitates the efflux of glutamate. A proposed gating mechanism is illustrated in the figure below.

Biophysics and cancer

Biophysics of cancer

Dr. Pierre Moens examines how two cytoskeletal proteins, cofilin and profilin, regulate the motility of metastatic cancer cells. These two proteins are key players in actin cytoskeleton. They play influence microRNAs, PI(4,5)P2 binding, pH, oxidative stress, and post translational modifications. The authors highlight similarities, complementarities and differences between the two proteins and discuss how their interaction affects actin filament dynamics.

Autoinhibition and release mechanisms of Ras effectors

In this review Dr. Ruth Nussinov and her colleagues review autoinhibition as an effective mechanism that guards proteins against spurious activation. Despite its ubiquity, the distinct organization of the autoinhibited states and their release mechanisms differ. Signaling is most responsive to the cell environment only if there is a small shift in the equilibrium is required to switch the system from an inactive (occluded) to an active (exposed) state. This underscores the challenge in pharmacological intervention to exploit and enhance autoinhibited states. Ras belongs to superfamily of small GTPases that, in general, are responsible for cell proliferation. Here, the authors review auto-inhibition and release mechanisms

at the membrane, focusing on three representative Ras effectors: Raf protein kinase; PI3K α lipid kinase; and NORE1A (RASSF5) tumor suppressor, and they highlight their ramifications to drug discovery. They further touch on Ras upstream and downstream signaling, Ras activation, and the Ras superfamily in this light, altogether providing a broad overview of the principles and complexities of autoinhibition.

MicroRNAs and breast cancer

Breast cancer, the second highest cancer in women worldwide, with mutations in three gene (ER-, PR, and HER 2) contributing 10–20%. Currently, chemotherapy remains the main treatment for early-stage breast cancer, as there is no approved targeted therapy for this subtype. MicroRNAs (miRNAs) play a key role in the post-transcriptional regulation of gene expression in almost all key biological processes including proliferation, differentiation, angiogenesis, migration, and apoptosis, and they play an important role in carcinogenesis. The review by Malla et al. focuses on the recent advances in miRNAs involved in breast cancer in terms of improved diagnosis, prognosis, and treatment of breast cancer and its subtypes. The review also focuses on the development, optimization of miRNA-based drug stability, improvement of miRNA delivery, and control of the off-target effects of miRNA therapeutics which may be proved to be a promising in the treatment of cancer.

Experimental biophysics

Dynamic force spectroscopy, single molecule analysis, and super-resolution microscopy

The characterization of cellular activities at the molecular level requires imaging systems with high spatial and temporal resolution as well as ultra-sensitivity and low cell toxicity. In this review, Qian (Peter) Su discusses the use of nanotools (dynamic force spectroscopy, single molecule analysis, and super-resolution microscopy) to elucidate the interactions between inter-cellular networks and intracellular components, both of which are key to understanding adhesion, trafficking, inheritance, and division. The figure below puts their review into spatial perspective.

Thoracic aortic aneurism and epigenetic factors

Dr. Stefanie Portelli and colleagues present a carefully researched review of aortic aneurisms. Thoracic aortic

aneurysm (TAA) (dilatation of the wall of the aorta) is a clinically silent phenomenon that develops in patients with adverse remodeling of the aortic wall. It is familial in about 30% of cases, and accounts for 30% of all TAAs. Although medical therapy slows the progress of the dilatation, most patients will require surgery. Early surgery carries a small (1–5%) risk that significantly increases in cases of emergency intervention. The authors review the genes responsible for altered proteins involving the extracellular matrix, vascular smooth muscle, and transforming growth factor (TGF) signaling. Epigenetic factors are now recognized to influence the phenotype of many genetically triggered conditions. The authors review includes: (1) non-coding RNAs (miRNAs, lncRNAs); (2) nine genes involved in DNA methylation; and (3) modification of histones (positively charged proteins associated with DNA) that genetically trigger TAA. These factors are interesting because of their potential for therapeutic manipulation.

New ways to test and identify toxic chemicals in waste water

A report published in 2011 showed that the fluorescence intensity of SYBR- Green 1 (SG1), bound to native double-stranded DNA is reduced in proportion to the presence of toxic heavy metal ions and other chemical toxicants. The mechanism of this potential sensor was not fully understood but it was assumed that toxicants altered the helical structure of DNA, causing a proportional release of SG1 and therefore a decrease in fluorescence. This DNA-dye test had similar sensitivities to tests using microorganisms, but its physicochemical basis remained obscure. Here, Dr. Kanellis uses his chemistry expertise to analyze the chemical and biophysical basis for both the toxicity and the specificity of metal binding to

DNA and RNA. An accompanying Short Communication reviews the sensitivities and specificities of nucleotide-based and other biophysical tests currently used in assessing chemical toxicities of water samples.

Laser-induced breakdown spectroscopy

Dr. Vivek Singh reviews how laser-induced breakdown spectroscopy (LIBS) has significantly enhanced research in the biological science. LIBS is optical emission spectroscopy that uses light emitted from a plasma (gas) generated when a high power laser beam vaporizes solids, liquids, or gases. In recent years, interest has grown rapidly, and now extends to the identification of biological species, particularly into microbes. This interest derives from its ability to

identify diagnostic molecules, and has led to applications in agriculture, environment analysis, medicine (infectious diseases), forensic sciences, and a range of other fields in biology. LIBS provides a system for elemental analysis that surpasses the sensitivity of more traditional techniques and assays (bacterial morphology, ELAIS, FISH, PCR, MALDITOF, and Raman spectroscopy). LIBS can rapidly perform elemental composition analysis without the need for special specimen preparation. LIBS can detect a specific

bacterium when others are present. This has enhanced its use as a diagnostic technique for bacteria, molds, yeasts, spores, and even viruses known to cause infectious diseases. It can identify microorganisms in agriculture and the food processing industry by identifying bacteria such as *Salmonella enteric serovar typhimurium* in food. In the mining and environmental remediation industry, it can discriminate between inter- and intra-site differences in soil bacteria taken from mining sites, and can assess soil quality.