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Biophysical methods: structure, dynamics and gorgeous images

Editorial overview

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Wah Chiu's research focus has been on the technology development of electron cryomicroscopy for subnanometer resolution structure determination of molecular machines in solution states. His group has pioneered many experimental and computational methodologies for reconstructing three-dimensional images from micrographs of single particles and their structural interpretations and annotations. These methodologies have been applied to uncover structure and function relationship in many molecular machines close to or over 1 MDa including the acrosomal bundle, herpes simplex virus type-1 capsid, rice dwarf virus, bacteriophages epsilon15 and P22, ryanodine receptor channel and GroEL. His research has been partially supported by NIH NCRR.

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Keith Moffat's research focuses on the development and implementation of techniques for time-resolved X-ray crystallography, which in favorable cases achieve 100 ps time resolution and excellent crystallographic resolution, better than 0.2 nm. He applies static and time-resolved crystallographic techniques to a variety of classes of signaling photoreceptors, sensitive to either blue or red light, with the goal of understanding the molecular mechanism of light-dependent signal transduction at the atomic level. He began his research career studying hemoglobin, which might well be thought of as the paradigm of a signaling molecule. The thermodynamic considerations underlying cooperativity in oxygen binding by hemoglobin and its sensitivity to for example pH and allosteric effectors also underlie signaling by photoreceptors.

As often turns out to be the case, articles commissioned and written completely independently of each other turn out to offer subthemes that span several disciplines. The articles in this issue offer thoughts on the advantages of collecting data on a single sample versus averaging over many samples; and if the latter, the mode of averaging to be employed given the likely levels of static or dynamic sample heterogeneity. They also offer examples of the combination of techniques, either directly in the case of combining X-ray and spectroscopic approaches, or indirectly in combining various spectroscopic approaches to a single class of macromolecules. All examine their scientific frontiers, perhaps most spectacularly in the case of the exceptionally large complexes now accessible to X-ray techniques, whose structures are then presented in talks and on the printed page in ways that challenge the frontiers of visualization and interpretation.

The technology in cryo-EM continues to evolve in all steps of the procedure from cryo-specimen preparation, data collection and processing to structure mining and archival. CCD cameras have been used successfully for cryo-EM data collection to subnanometer resolution but have not yet achieved the limit of microscope resolution (3–4 Å) for biological macromolecules. Faruqi and Henderson review the latest commercially available CCD cameras for cryo-EM data collection as well as those still under development. The later are fabricated by direct detection using either, hybrid pixel detectors or monolithic active pixel sensors or MAPS. They point out the potential advantages of these two new types of electron detectors, which are being explored by experimental physicists in light of their detective quantum efficiency, modulation transfer function, robustness and sensitivity to radiation damage, speed of readout and the number of pixels.

Helical filaments are a common form of macromolecular assemblage, which occurs either naturally or artificially. The classic, Fourier–Bessel methods of 3D electron microscopy approached the helical reconstruction of electron images of single filaments by assuming the filament to be a perfect helix. The single particle cryo-EM approach introduced by Egelman has been shown to be able to retrieve structures of single filaments without imposing the perfect helical symmetry assumption. This reconstruction method has now advanced to the stage where it can be used effectively to obtain structures that were previously impractical to determine by classical methods. Such approaches promise to yield structures of most of the biologically native filaments whose deviation from perfect helical symmetry is likely to reflect functional relevance.

Small angle scattering (SAS) has undergone a renaissance in biological applications because of the availability of dedicated, intense, synchrotron-based, X-ray beam lines and more sophisticated modeling tools for

analyzing the resultant data. The technique can be used to deduce the shape and size of macromolecular complexes or individual macromolecules of any size and under different chemical and biochemical conditions. More advanced analysis can even reveal the internal organization of molecular components in a large complex (for example, distinguish protein from RNA in ribosomal subunits), and provide data sufficiently accurate to enable atomic models of the individual components of the complex to be fitted into the SAS-derived model. SAS has thus become a method to effectively complement structural data from crystallography, NMR and cryoEM with flexible systems, particularly in cases where the models are ambiguous or there is missing density. Petoukhov and Svergun summarize the latest applications of this methodology to a broad spectrum of examples.

Currently, the Protein Data Bank mostly contains structures with tens of kDa per crystallographic asymmetric unit. Only in the last few years have a few structures of exceptionally large macromolecular complexes been solved, which exhibit no non-crystallographic symmetry and an asymmetric unit larger than 500 kDa. Examples include ribosomal subunits, RNA polymerase II and fatty acid synthase. As might be expected, structure determination of such complexes poses unique technical challenges in purification, crystallization, data collection, phase determination and ultimately, structure determination, interpretation and presentation (for the last, compare the article by Goddard and Ferrin). Mueller, Jenni and Ban describe a systematic approach to these challenges and present many innovative solutions to the problems which pervade all stages of the project. In an interesting sense their article is a throwback. In some respects it reads like a review written in the late 1960s of then-conventional protein crystallographic approaches to lysozyme-sized molecules, in which careful attention had to be paid to all aspects of the biochemistry, crystal order, signal-to-noise in data collection and the limitations of various phasing strategies. Attention must still be paid, but is now aided by modern purification techniques, by data collection at intense synchrotron beam lines using low-noise detectors, and of course by advanced algorithms encoded in software running on hardware that could only be dreamed of in the 1960s. An IBM 360/44, anyone?

Mora-Rey and Wilmot describe recent advances in integrating optical and X-ray measurements, particularly in a synchrotron X-ray beam line, such that the relationship between diffraction data and the chemical states of the protein manifest in their optical properties can be correlated. Integration can be versatile; optical measurements include UV/visible absorption, front face fluorescence, Raman and IR spectroscopies. When a microspectrophotometer is carefully integrated into the beam line, it allows the study of for example a photoreduced product of a

protein in a crystal, or assurance that data from multiple crystals are in a desired, well-defined chemical state before the protein is photoreduced by the intense X-ray beam. Using spectroscopic signals, it also becomes possible to monitor crystal growth, to identify the location of tiny crystals picked up on the loop for data collection, and to use radiation damage with confidence for phase determination from a combination of undamaged and radiation-damaged crystals.

Visualization is a critical but often overlooked step in the discovery process in any structural biology method. Imaging technologies have advanced to the point where they can reveal various structural details at length scales from cells to organelles to atoms. It is a daunting task to display all this structural information either at once or in parallel. How can one examine the trees in detail in the context of a forest? Which exactly are the trees? Are the individual species of tree of interest, or does this lie more in the branches, leaves and leaf veins of a particular species for the biological problem at hand? The developers in visualization come from background in biology, informatics, visualization and computer science, and are motivated by different considerations. New tools continue to be emerged from various developers, but the challenge remains to disseminate these tools to a broad user community in a seamless manner, that builds on existing, widely-adopted tools yet offers obvious advantages over them. Goddard and Ferrin review the latest tools in biological structure visualization at various levels of complexity, and propose the need to unify a common standard format and map coordinate systems so that the data and software can be easily shared and experimented with.

Subramaniam, Bartesaghi, Liu, Bennett and Sougrat review the recent progress in using electron cryo-tomography to reconstruct the 3D structure of a large biological assembly from a series of images taken on a single sample at different specimen tilt angles. This approach contrasts with single particle reconstruction methods, in which single images are taken on a series of samples at random orientations. This method is particularly useful to study assemblies, which either exist in multiple conformations or are difficult to reconstruct with standard single particle cryo-EM methods. They point out the current limitations of this methodology including radiation damage and consequent lower resolution, wedge effects arising from the inability to sample all tilt angles, and the availability of standardized, robust image processing software. They demonstrate the application of this approach to large virus particles. In some cases, different structures have been reported from seemingly identical structures determined in different laboratories. However, future developments in data collection and processing will evolve electron cryo-tomography into a powerful structural tool for studying large assemblies and cells, a tool that will fill the resolution gap in the 10–2 nm range.

Clore, Tang and Iwahara review the use of paramagnetic relaxation enhancement (PRE) in NMR-based structure and dynamics determinations. This approach obviously needs either an intrinsic paramagnetic center such as Mn^{2+} , or the introduction of a site-specific spin label. The latter is usually flexibly connected to the macromolecule of interest and hence the distances between the paramagnetic center and individual protons have to be modeled by an ensemble of conformations for the center. In the slow exchange regime, information that powerfully constrains structure determination can be obtained; and in the fast exchange regime, information on dynamic processes and the existence and even the properties of low-population intermediates can be inferred. In contrast with Nuclear Overhauser Effect measurements, PRE can extend over long range (up to 3.5 nm), cross peaks are easy to assign, and hundreds of distances between the paramagnetic center and protons are revealed simultaneously—always provided that the PRE effects are measured accurately and precisely. Examples feature binding of small proteins to specific and non-specific sites on cognate DNA.

Solid-state NMR techniques continue to advance as the review by McDermott and Polenova details, with examples drawn very much from an enzymological perspective. In the solid state, Michaelis complexes and intermediates in membrane-associated enzymes are

accessible, including such features as active site compression suggestive of substantial strain, short/strong hydrogen bonds, and unusual vibrational or NMR spectral signatures. PRE is also applicable in the solid state (compare the review by Clore and co-workers) and has revealed, for example, proton locations up to 2.0 nm from Co^{2+} in matrix metalloprotease2.

The primary photochemical processes in photoreceptors are all extremely fast, certainly sub-ps and extending to fs, yet the photoreceptors typically undergo subsequent structural reactions that can occur over time scales up to s. So, how do these ultrafast physical and chemical processes ultimately drive the much slower biological processes? Kennis and Groot review recent applications of ultrafast spectroscopies such as UV-vis, fs IR and the relatively new and powerful fs stimulated Raman to photoreceptors. The last offers excellent time resolution in the fs range with high spectral resolution $\sim 15\text{ cm}^{-1}$, but is just beginning to be more widely applied. They emphasize those photoreceptors that contain flavins as the chromophore and (in distinction to better-studied, retinal-containing proteins such as bacteriorhodopsin) do not undergo isomerization but rather, light-induced electron redistribution, that leads to rearrangement of hydrogen bonds in the vicinity of the flavin and ultimately leads to covalent bond formation between the flavin and a nearby cysteine residue.