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Editorial overview: Biophysical and molecular biological methods: Structure, dynamics, and single molecules

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Janet Smith received a PhD in Biochemistry at the University of Wisconsin-Madison and did postdoctoral research at the Naval Research Laboratory and at Columbia University. She joined the faculty of Biological Sciences at Purdue University and, in 2005, joined the University of Michigan. Her research investigates the function and structure of complex enzymes with multiple active sites and proteins of viral replication. As Scientific Director of the GM/CA beamline facility at the Advanced Photon Source, she has led the development of an advanced micro-crystallography capability.

New technologies for investigating the physical properties of biological macromolecules are improving the sensitivity of measurements, accessing shorter time scales, and examining with greater accuracy both populations and single molecules. All serve to bring biophysical measurements closer to the conditions and time scales of biological processes.

A sea change underway in determination of atomic-level structures of macromolecules and complexes is addressed in three reviews on X-ray crystallography and electron microscopy. In crystallography, new, high-speed, low-noise detectors have revolutionized data collection practice. This is combined with new statistical methods to evaluate data and determine diffraction limits and new methods for phase determination without recourse to specific chemical labels. Meanwhile, new microscopes and detectors together with improved methods for sample preparation have vastly expanded the resolution limit and reduced the macromolecule size that is accessible by electron cryo-microscopy (cryo-EM).

Better statistical methods for treatment of high-multiplicity data from multiple crystals together with improved methods to detect a weak native anomalous scattering synergize with new fast-readout, photon-counting detectors capable of providing low-noise data with blinding speed. Recently, Karplus and Diederichs introduced 'new' correlation coefficient statistics to more rigorously determine the diffraction limit from crystallographic data. The methods are being rapidly adopted, but with some confusion in the community. Their review clarifies the applicability of various statistical estimates of data quality, dependent on whether they are applied to individual measurements or to merged data. These methods are particularly important for high-multiplicity data, as needed for phase determination from a weak anomalous signal (*c.f.* Hendrickson and Liu). The review highlights examples of dramatic improvement in the quality of a refined model when high-resolution, weak data are included in crystallographic refinement.

Hendrickson and Liu review the state of crystallographic phase determination using the anomalous scattering of native atoms, specifically S in proteins and P in nucleic acids. Recently, they showed how a very weak anomalous signal can be detected in diffraction data from weakly diffracting crystals by combining data from many sample crystals. They describe applications of the new method to even more challenging problems — low-resolution, large structures — that illustrate its broad applicability and wide adoption. They also summarize advances in crystal handling, goniometry, and data processing that support the native SAD method and improve its chances of success.

In recent years, the world of biology has been treated to stunningly detailed structures of macromolecular complexes from cryo-EM. The recent leap to sub-nanometer resolution is the result of new high-speed, direct-electron detectors, improved reconstruction methods, and improved sample preparation methods. The high-resolution cryo-EM maps are obtained by iterative fitting of low-contrast, high-noise projection images of many thousands of individual particles to an improving EM reference model, and thus are highly susceptible to model bias. Determination of the true resolution of an EM map and assessment of the accuracy of particle orientations are critical to establishing the validity of an EM result. Rosenthal and Rubinstein review current methods for validation of EM maps and fitting atomic models, and summarize the challenges to reconstructing maps for smaller or flexible particles.

One of the most exciting scientific opportunities being exploited with the new free electron laser (FEL) sources is the ability to probe dilute metalloprotein samples in an undamaged state. The X-ray FEL pulses are short (<50 fs) and intense enough so that measurable spectroscopic or diffractive events can occur before the sample is changed by the radiation. This circumvents problems encountered in studies using synchrotron radiation and paves the way for spectroscopic and crystallographic visualization of many important metal centers. Kern *et al.* review accomplishments and controversies in the study of metalloproteins by the 'measure before modify' methods enabled by FEL radiation. The most exciting developments have come from the simultaneous measurement of X-ray spectra and crystallographic data from crystals of metalloproteins in which fast chemical changes are light-triggered.

Two-dimensional infrared spectroscopy (2D-IR) is emerging as a powerful new way of accessing biomolecular dynamics on very short timescales. While the traditional focus in the field of vibrational spectroscopy had been on small molecules, developments over the past two decades have demonstrated that multidimensional IR techniques (conceptually closely related to those in NMR) are very powerful for investigating the conformational dynamics of small peptides down to the picosecond regime. Hamm and colleagues provide a snapshot of the latest developments, which show that the approach starts to be applicable not only to peptides, but also to larger proteins. They further outline the challenges and possible solutions on the way to making 2D-IR a broadly used technique for studying the functional dynamics of proteins.

Data that reveal the ligand and oligomer state of protein complexes with stunning clarity have been obtained lately using native mass spectrometry (MS). These accomplishments are the result of new gentle electrospray ionization methods that leave complexes intact and mass spectrometers with high mass accuracy over a large mass range. Sharon and Horovitz review recent results from native MS, including their own study of the GroEL chaperonin, and explain how complex questions about mechanisms of allostery can be addressed directly with experiments in favorable cases. One of the most exciting aspects of native MS is the ability to identify the components of a mixed population.

Complementing the information from MS with an independent readout that reports on the conformational state of macromolecules has been a long-standing challenge. Recent developments show remarkable progress in combining MS with fluorescence-based methods, especially Förster resonance energy transfer (FRET) and quenching. Czar and Jockusch summarize these advances; for example, how mass selection in combination with monitoring fluorescence lifetimes increases sensitivity to an unprecedented level and enables the important step from peptides to proteins. These techniques offer a new way of probing macromolecular structure and dynamics in the gas phase, which will be essential for testing under which conditions the solution structure is retained; they also provide a new opportunity for investigating the role of solvation for macromolecular stability and conformation.

The final three contributions in this issue focus on new trends in single-molecule methods and illustrate the impressive progress that has been made in terms of sensitivity, throughput, and scope. The two dominant pillars of single-molecule investigations of biomolecules are force and optical spectroscopy. After 20 years of rapid development, both are now firmly established components of biophysical research, but exciting progress continues to be made on the methodological side. Woodside and colleagues point out how the increasing sensitivity of optical tweezers and the use of theoretical concepts and advanced analysis tools have opened vast new territory. Examples are biomolecular systems that require sensitivity in the low piconewton regime, such as intrinsically disordered proteins, or the unprecedented details of folding and misfolding reactions that are being revealed. Processes of increasing complexity, such as interactions with molecular chaperones, protein aggregation, or ribosomal frame shifting are also starting to be addressed.

The strength of single-molecule techniques is their ability to resolve structural and dynamic heterogeneity by studying molecules individually. However, especially for force spectroscopy, throughput is often a limiting factor. Dekker and colleagues focus on new approaches to overcome this limitation and summarize how ideas such as parallel flow-stretch, arrays of optical traps based on nanophotonic standing waves, or magnetic tweezers combined with camera-based imaging enable multiplexing of force measurements on single molecules. As a result,

highly improved statistics are available, and even very rare events become accessible to more systematic study. However, the resulting flood of data also poses new challenges and opportunities for data analysis, which require innovative solutions, some of which are outlined in the review.

A shortcoming of many single-molecule techniques is their limitation to an *in vitro* setting. With the recent explosion of the field of cellular biophysics, often with a focus on the mesoscopic scale, the goal of linking the detailed and quantitative molecular picture obtained *in vitro* with the situation *in vivo* is becoming increasingly important. A method that is particularly suitable for closing this gap is single-molecule FRET. Sustarsic and Kapanidis outline the experimental challenges, such as fluorescence labeling, the reproducible delivery of molecules into cells, or the limited statistics available in live cells, and show how recent advances have started solving these problems. Both in bacterial and eukaryotic cells, quantitative single-molecule measurements are becoming feasible, and the increasing complexity of systems and the broad range of timescales accessible are promising a bright future for a quantitative understanding of biomolecular dynamics *in vivo*.

In summary, improvements in biophysical methodology — ranging from high-resolution structure determination to versatile types of spectroscopy — provide insights of increasing depth; on a range of timescales of increasing breadth; and on systems of ever-increasing complexity. We hope that this collection of accounts not only enables researchers in the field to keep abreast of the latest developments but also conveys the excitement of the new opportunities that arise from them.