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Editorial overview — Biophysical methods: 'Seeing is believing'

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Complex cellular processes, upon which life as we know it depends, are essentially a chain of biochemical encounters involving proteins, nucleic acids, peptides, and smaller metabolites. To obtain a deeper understanding of biological systems, it is therefore essential to develop the ability to describe molecular mechanisms underlying these events at atomic resolution. Providing such 'visuals' along the trajectory of a biological cascade is the raison d'etre of the field of biophysics, which uses a range of physical, spectroscopic, and computational tools to achieve this goal. Given the importance of biophysics and its impact on biological and medical research, the current issue of the Current Opinions in Structural Biology is focused on exciting developments in molecular biophysics. In our choice of various new methods (inevitable owing to space limitations), we have tried to reflect an important recent shift in emphasis from static three-dimensional structures of complexes to a more dynamic view of molecular encounters, which are critical for biological function.

Traditional methods for obtaining high-resolution three-dimensional structures of proteins and nucleic acids include X-ray crystallography, cryo-electron microscopy (covered in a previous volume), and nuclear magnetic resonance (NMR) spectroscopy. Of these, the latter affords the important advantage of providing a molecular view in solution that permits the dynamic behavior of biomacromolecules to be probed. Combining this with the ability to measure structural and dynamic parameters for multiple molecular sites simultaneously, and on a range of biologically relevant timescales from picoseconds to seconds, NMR has become a powerful biophysical tool. Dyson and Wright demonstrate this for intrinsically disordered proteins (IDPs) and intrinsically disordered regions (IDRs) within structured proteins, both involved in cellular function and regulation as well as in protein misfolding and disease. Described are innovative NMR applications that provide insights into the complexity of IDP interactions with cellular targets and mechanisms of protein aggregation. Al-Hashimi and co-workers extend the utility of NMR methods, supported by computational techniques, to characterizing conformational ensembles of nucleic acids and their implications upon interactions with proteins in the context of regulation of gene expression. Hansen and co-workers introduce a method for following dynamics of protein side

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chains, a refreshing approach in light of the prevalent bias toward more easily measured backbone amide parameters and the evident importance of side chains to interactions at the surface of proteins. Somewhat counter-intuitively, NMR in the solid state (made possible by the introduction of ultra-fast magic-angle spinning) is emerging as a promising technique for investigating protein dynamics, and the contribution by Pintacuda and co-workers nicely summarizes the increasing number of systems for which this approach is applicable.

In contrast to NMR, fluorescence-based methods require chemical labeling and provide information for only a few locations simultaneously (up to three) in a protein, but fully compensate for this with excellent sensitivity (in which NMR is notoriously challenged), enabling them to accurately follow single molecules with good spatio-temporal resolution. Fluorescence methods come in several flavors, depending on the desired information and labeling scheme, and two main approaches are represented here. Ghosh and Enderlein focus on information that can be obtained from singly labeled biomolecules, avoiding the difficulties encountered when highly homogenous doubly labeled samples are required. Insights into molecular motions are obtained by following intensities in two orthogonally polarized planes, the distance between the dye and a quencher (usually a tryptophan residue), or by adding an information layer related to fluorescence lifetime of various species. Ha and coworkers extend single-molecule fluorescence resonance energy transfer, which follows the donor—acceptor distance between two dyes, to multicolor fluorescence resonance energy transfer measurements affording three simultaneous distance measurements or information on colocalization of three species. In both cases, applications to studying protein conformations and protein—protein/nucleic acid interactions are immediate and obvious.

More biophysical methods are described in this volume, testifying to the wealth of experimental options under development and in use. Small-angle/wide-angle X-ray scattering (SAXS/WAXS) measurements provide information on the shape of biomacromolecules owing to their correlation with pairwise distances between adjacent atoms. Anfinrud and co-workers demonstrate how time-resolved SAXS/WAXS can report on structural effects of rapid temperature or pH jumps, allowing investigations of folding/unfolding processes, as well as phenomena of solution oligomerization, aggregation, and interaction between biomolecules. Robinson and co-workers use advanced mass spectrometry, a well-established method in determining the assembly of multicomponent protein complexes, to take aim at membrane-embedded proteins, whose size and sample preparation challenges often defy structural methods. Focusing on bacterial drug efflux systems, a promising therapeutic target, H-/D-exchange results and antibiotic-dependent dimerization exemplify how mass spectrometry can be applied for the discovery of new and desperately needed antibiotics. Less known and quite intriguing is the method suggested by Otzen, Buell, and Jensen, using flow dispersion during laminar flow in microfluidic systems to provide populations of molecular sizes in various phases, leading up to applications for detecting biomolecular interactions.

Computational approaches cannot be overlooked in an age when our ability to simulate even the most complex systems is increasing at a surprising rate. Docking of two binding partners — the process of finding their most stable and probable binary complex — is a time-honored aim of structural studies owing to its obvious implications for drug discovery, especially in a high-throughput setting. Bonvin and co-workers describe how available

experimental and bioinformatics data, in particular pertaining to molecular shape and sequence coevolution, respectively, can be incorporated into and streamline docking algorithms. Another computational application covered by Orengo and co-workers, aimed at enhancing our characterization of protein functional space, describes software tools and machine learning approaches for classification of protein functional families, identification of specificity-determining positions, and prediction of functional sites.

In addition to these methodological developments, biophysical studies are now being targeted at systems of increasing complexity. Biomolecular phase separation phenomena — biophysics *par excellence* — have been extensively explored in recent years owing to their importance in physiology and disease on the one hand and engineering applications on the other hand. Positioned somewhere between solution and solid phases, such systems often require a combination of methods for proper characterization. Fawzi, Mittal and co-workers present an integrated approach combining NMR, optical spectroscopy, and computational tools, all adapted to the unique conditions within the condensed phase, to characterize motions and molecular interactions that give it its macroscale properties. Emmanouilidis, Jeschke, Allain and co-workers focus on phase-separating RNA-binding proteins, for which dispersed and condensed phases impact regulation of RNA processing. They show what information can be obtained from NMR and electron paramagnetic resonance (EPR) experiments and how these can be combined to understand the structure and dynamics of this phase separation process. Another complex system drawing great attention is aggregation-prone (or amyloid) proteins, where a key consideration is the kinetics of transformation between monomeric, oligomeric, and fibrillar species as determinants of pathological outcome. Linse describes approaches for obtaining rate constants, correlated to energy barriers, and equilibrium constants, correlated to population distributions of these species, and the implications of the "scaling-up" of such observations to the *in vivo* setting. Undoubtedly the most complex system considered here, the cellular environment, is addressed by Gruebele and Pielak. In-cell NMR and time-resolved fluorescence microscopies are used to provide a real-time view of protein behavior in the most biologically relevant environment. This approach offers a new view of the surfaces of proteins, a collective set of attributes termed "quinary" structure, and its role in controlling protein motions, interactions with other cellular components, and, ultimately, function.

From a bird's-eye view of these 15 contributed articles, two important precepts emerge. First, the range of methodologies presented here vary in their applicability to a given research question, and molecular size, affinity range, expected timescale, and environment are all essential considerations. Correct choice of method is therefore imperative. Second, the majority of these reviews (and works cited within) use a combination of two or more methods to obtain the desired biophysical characterization of the system under study, and it is our prediction that this integrative trend will intensify in future research. Overall, this issue presents cutting-edge biophysical research inexorably pushing forward possibilities of identifying, observing, and characterizing biological processes. This is attributable to new methodologies, as well as application of established ones to increasingly challenging systems. We are confident that this collection will assist in the design and execution of new experiments enhancing our ability to 'see' biological events — and consequently more strongly 'believe' in the ensuing structural conclusions we aim for as a community.

Conflict of interest statement

Nothing declared.

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