

Biophysical methods

New approaches to study macromolecular structure and function

Editorial overview

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Significant advances in biophysical methods have been made during the past few years. Macromolecular X-ray crystallography has become more automated, cryo-electron microscopy of single particles has reached an unprecedented degree of resolution, new NMR spectroscopy and mass spectrometry techniques allow application to large complexes and computational techniques have been developed to analyze the flood of genomic and structural information. The coming years promise even more dramatic advances, in particular in the area of single-molecule imaging and manipulation.

Two reviews discuss experimental and computational advances in macromolecular crystallography that are stepping stones towards high-throughput structure determination and automation. Particular highlights are robotic screening and crystal mounting systems, as discussed by Stevens (pp 558–563), and automation of MAD (multiple wavelength anomalous diffraction) phasing, heavy-atom searching and chain tracing, as discussed by Adams and Grosse-Kunstleve (pp 564–568). Clearly, crystallization is still the major bottleneck. Hajdu (pp 569–573) discusses the intriguing possibility of high-resolution structural studies without the need for crystallization by using extremely intense X-ray pulses, many orders of magnitude more powerful than current X-ray sources, on single molecules. Theoretically, sufficient information for structure solution could be collected from such experiments before the specimen is destroyed by radiation damage.

The flood of genomic and structural information that is presently being obtained requires the development of novel computational techniques to analyze and interpret this information. Gerstein and Jansen (pp 574–584) review the utility of whole-genome expression experiments for bioinformatics. Computational cluster analysis of the results revealed insights about protein function, structure and localization.

The development of solution NMR has been dramatic over the past decade. Although early studies of proteins by NMR were limited to relatively small proteins,

advances in NMR pulse sequences, instrumentation and isotope labeling strategies have allowed application to proteins and protein complexes as large as 30 kDa and beyond. Goto and Kay (pp 585–592) review important labeling techniques. Of particular importance are the random incorporation of ^2H , selective amino acid labels and segmental sequence labeling techniques.

One major challenge in structural biology is the elucidation of the structure and function of membrane proteins. X-ray crystallography is playing an increasingly important role, but efforts to crystallize and solve the structures of membrane proteins are often extremely time-consuming, in many cases requiring years or decades for successful structure solution. Solid-state NMR provides an attractive alternative for the study of the structure and mechanism of membrane proteins. de Groot (pp 593–600) reviews the recent advances in this field.

Large macromolecular complexes and assemblies are often transient or dynamic in the cellular context. Some of these assemblies have been linked to particular diseases, such as Alzheimer's and prion-related diseases. Miranker (pp 601–606) reviews the utility of mass spectrometry to study macromolecular complexes. Significant advances have been made in the study of both noncovalent assembly and their assembly and disassembly in real time.

Many biological processes occur at water/membrane interfaces. Yet, our understanding of protein–membrane–water interactions is rather limited. A case in point is protein-induced membrane fusion as it occurs in cellular trafficking and compartmentalization, and in invasion of a viral pathogen into a host cell. Lentz *et al.* (pp 607–615) review current models of cell membrane fusion. Clearly, more studies and methodological advances are required to obtain a molecular understanding of these important biological processes.

Cryo-electron microscopy provides another way of studying macromolecular assemblies, including assemblies that are associated with biological membranes. Tao and Zhang (pp 616–622) review techniques that have been developed to study a broad range of samples, ranging from 500 kDa protein complexes to large subcellular organelles. These and other emerging methodologies will allow one to study macromolecular structure, function and dynamics on larger and more challenging systems than ever before. Ultimately, it is hoped to bridge the gap between biophysical studies of isolated macromolecules or complexes, and the complexity of cellular systems.