Macromolecular assemblages
Machines and networks
Editorial overview
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Helen's research focuses on the structure and function of macromolecular complexes, using cryo-electron microscopy and image reconstruction, increasingly in combination with information from X-ray crystallography. Particular areas of interest are molecular chaperones, amyloid fibrils and membrane proteins.

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Axel Brunger's research focuses on the structure and function of proteins involved in neurotransmission, using X-ray crystallography, solution NMR spectroscopy and other biophysical techniques. Other interests include methods development in computational crystallography.

The tremendous success of structural biology in defining the structures of large and complex assemblies is transforming many areas of cell biology and biochemistry, as structural information helps to illuminate biological function. This progress depends on the preparation of multicomponent assemblies, intact or in parts, and their structural analysis by a combination of X-ray crystallography, NMR spectroscopy and electron microscopy. Even the notoriously difficult area of membrane protein structure is beginning to yield to the ingenuity of molecular biologists in overexpression and to efficient, automated screens to produce suitable crystals.

Many of these complexes can be considered as molecular machines or motors, in which a rotary or linear movement is used for motility, nucleic acid processing, folding or unfolding, or as transducers of light or chemical energy. The coordination of intercellular adhesion and cell motility relies on chains of interaction in membrane–cytoskeleton networks. Signalling pathways regulate these complex interconnections during development. The reviews in this section reflect these themes, with particularly impressive advances to report in the areas of membrane proteins and nucleic acid processing.

Replication and transcription

The first review, by Jeruzalmi, O'Donnell and Kuriyan (pp 217–224), covers recent progress on DNA polymerase processivity factors, the ring-shaped sliding clamps and the clamp loader complexes that open the rings and assemble them onto primed DNA. These structures suggest mechanical analogies, with a spring-loading mechanism proposed for the clamp loaders to assemble the rings of the sliding clamps around double-stranded DNA. Crystal structures of various sliding clamps from different species have clarified how the clamp interacts with the DNA polymerase.

Progress has also been made on structural studies of eukaryotic and bacterial transcription factors, and their complexes with promoter DNA. Burley and Kamada (pp 225–230) review recent structures of multiprotein complexes on promoter DNA. These structures, involved in initiating or inhibiting transcription, reveal how binding of the regulatory proteins can control access to and change the conformation of the DNA.

Folding and unfolding

Zhang, Beuron and Freemont (pp 231–238) discuss chaperone, protease, unfolding and disassembly activities by a variety of ring-shaped and other chaperones. The unfolding and disassembly systems are members of the large AAA+ ATPase superfamily, whose members also include clamp loaders and helicases. A common theme to this family appears to be unfolding, disassembly or unwinding, of either protein or nucleic acid structures. These functions are often mediated by multiple interactions between the chaperone and substrate protein, regulated by binding and hydrolysis of ATP in a cooperative action. In the case of the chaperonin GroEL, binding of ATP and

the co-chaperonin GroES forms an enclosed cavity that encapsulates the non-native substrate protein and facilitates its folding. Delivery of unfolded proteins to proteases by the Hsp100 family of unfoldases plays a major role in cellular regulation, and the disassembly properties of NSF (*N*-ethylmaleimide-sensitive factor) and p97 are used to recycle SNARE complexes in membrane vesicle fusion.

Membrane proteins

The spectacular recent progress in determining membrane protein structures is described by Byrne and Iwata (pp 239–243). This has been a particularly exciting period, with the first atomic structure of a G-protein-coupled receptor, bovine rhodopsin, the first complete ABC transporter, MsbA, the outer membrane protein TolC with its 140 Å long solvent tunnel, the sarcoplasmic reticulum Ca^{++}-ATPase and a chloride channel all being reported. The Ca^{++}-ATPase contains several cytoplasmic domains that undergo large movements, seen in a comparison of the Ca^{++}-bound state with a Ca^{++}-free structure determined by electron crystallography at lower resolution. Another very extended α-helical membrane transporter, the lipid flippase MsbA, is arranged as a surprising, V-shaped dimer. A major new impetus to drug design is provided by the long-awaited atomic structure of bovine rhodopsin. In addition to the packing of the seven transmembrane helices, which was roughly known from electron crystallography, an extracellular plug structure, blocking access to the chromophore site, and a transverse cytoplasmic helix are unexpected new features. The next big challenge is to understand the photoexcited state and the activation of the G-protein transducin. In many of these cases, we have just reached the beginning of the process to decipher their mechanisms of action.

A bacterial photosystem I complex (12 proteins and 128 cofactors) is described by Saenger, Jordan and Krauß (pp 244–254). The light-driven reactions of photosynthesis involve photosystems I and II, as well as cytochromes and other cofactors, in a complex electron transport chain accompanied by release of oxygen, proton transport and reduction of $NADP^+$. The photosystem I structure provides the basis for understanding the electronic and spectroscopic properties of the huge array of chromophores supported, and in some cases optically tuned, by the protein matrix. Impressively, the same group has also determined the structure of photosystem II, at somewhat lower resolution.

Membrane–cytoskeleton networks

Pokutta and Weis (pp 255–262) cover the complexes involved in cell adhesion and motility. These dynamic protein–protein networks are the targets of regulatory signals that control cell adhesiveness, important in the development of tissues. Recently determined structures of complexes involving cadherin–catenin, vinculin and ezrin/radixin/moesin have provided valuable insights into the mechanism of cell adhesion. For these structures, recurrent features are connectors and adaptors linking actin filaments to membrane adhesion complexes, regulated by phosphorylation. Modulation of the contacts in extensive interaction surfaces provides gradations in the strength of adhesion.

Fold recognition by single-particle cryo-electron microscopy and bioinformatics

Structure determination of very large (hundreds of kilodaltons to megadaltons) complexes is mainly the territory of cryo-electron microscopy, although X-ray crystallography is steadily advancing into this arena, notably with large protein–nucleic acid assemblies. The recent rapid development of single-particle analysis (three-dimensional structure determination from isolated complexes in vitrified solution) is reaching the level of resolution needed for fold recognition, at least in the favourable case of highly symmetric and rigid structures, such as icosahedral viruses. The lack of lattice constraints means that more conformations can be explored, although this often comes at the cost of lower resolution due to increased flexibility.

Chiu *et al.* (pp 263–269) have pioneered the use of bioinformatics tools to analyse cryo-electron microscopy maps at intermediate resolution (7–9 Å). There is a great need for tools to interpret such maps. In this resolution range, α-helical and β-sheet features can be recognised, although individual strands are not resolved. They have applied this approach to a 7 Å map of rice dwarf virus to illustrate the identification of secondary structure elements and the recognition of existing folds. Other approaches are being used in the interpretation of membrane protein density maps, in which sequence can be threaded through transmembrane helical features. Even tools for displaying such large complexes at intermediate resolution need development.

In summary, structural information on heterogeneous multiprotein complexes is beginning to emerge in a number of areas. These structures are snapshots of dynamic protein–protein network interactions, an important first step towards an understanding of the mechanisms of these molecular machines at an atomic level.