MILESTONE 24

Structures of membrane proteins

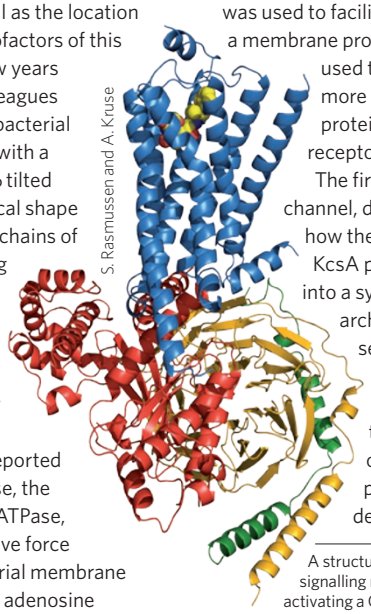
An estimated 20–30% of proteins in the human genome are membrane proteins, which mediate a broad range of biological processes. Obtaining X-ray crystal structures of membrane proteins has been extremely challenging; fewer than 500 unique membrane protein structures have been deposited in the Protein Data Bank.

The first high-resolution X-ray crystal structure of an integral membrane protein was reported in 1985, when Hartmut Michel and colleagues published the structure of a photosynthetic reaction centre. They observed a large hydrophobic surface that could interact with membrane lipids and 11 long α-helices that could span the lipid bilayer, as well as the location of functionally important cofactors of this multiprotein complex. A few years later, Georg Schulz and colleagues reported the structure of a bacterial porin, a membrane protein with a completely different fold: 16 tilted β-strands formed a cylindrical shape (a 'β-barrel'), with the side-chains of polar amino acids projecting into the centre of the pore. This architecture enables the side-chains to interact with water and solute molecules as they passively diffuse through the protein.

In 1994, Abrahams *et al.* reported the structure of an F_1-ATPase, the catalytic centre of the F_OF_1-ATPase, which uses the proton-motive force across the inner mitochondrial membrane to facilitate the synthesis of adenosine triphosphate. The authors proposed that the flow of protons through the F_O domain somehow causes the F_1 domain to rotate, which advances each of the three nucleotide-binding sites of the F_1 domain to the next step in the catalytic cycle.

The 1975 electron crystallography analysis of bacteriorhodopsin — an archaeal protein that uses light energy to export protons from the cell — by Henderson and Unwin provided the first structural identification of α-helices in membrane proteins. The X-ray crystal structure of this protein, reported in 1997, was the first time the lipidic cubic phase (LCP), a crystalline phase with a toothpaste-like consistency, was used to facilitate the crystallization of a membrane protein; LCP has since been used to obtain the structures of more than 45 unique membrane proteins, including G protein-coupled receptors (GPCRs).

The first crystal structure of an ion channel, determined in 1998, illustrated how the four identical subunits of the KcsA potassium channel assembled into a symmetric, inverted-cone-like architecture. The 12-Å-long selectivity filter comprises oxygen atoms from main-chain carbonyls at discrete locations; the distance between the oxygen atoms leads to the preferential coordination of de-solvated K^+.



S. Rasmussen and A. Kruse

A structural model of a GPCR (blue) with signalling molecule bound (yellow spheres), activating a G protein (red, gold, and green).

In 2007, two X-ray crystal structures of a GPCR, the human β2 adrenergic receptor, were published. The authors used an antigen-binding fragment that bound tightly to the GPCR or a T4 lysozyme insert to stabilize the protein for crystallization. Because many drugs elicit their biological effect(s) by binding to a GPCR, the structures of these and other GPCRs may be used to develop highly efficacious drugs with few side effects.

Technological advances (Milestone 25) and an infusion of funding have led to a surge of interesting membrane protein structures in the past few years. In the near future, crystallographers will probably obtain structures of more complex species — for example, heteromeric complexes of multiple membrane proteins or membrane proteins bound to cytosolic regulatory proteins and signalling partners. Although progress has been slow, it has been worth the wait: some of the most interesting structures from the past decade were of integral membrane proteins and even more exciting discoveries are right around the corner.

Joshua M. Finkelstein, Senior Editor, Nature

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