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Editorial overview

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Robert M. Stroud has devised novel approaches to resolve mechanisms in biochemistry at the level of atomic structure and mechanism. He focuses on understanding membrane proteins and targeting of their synthesis. He determined mechanisms of many enzyme paradigms and uncovered new principles for drug design aimed at modulating their action.

He was elected a fellow of the Royal Society of Medicine (UK) in 1992, and member of the National Academy of Sciences (US) in 2003. He was elected president of the US Biophysical Society in 1986 and a founding Fellow of the society in 2000.

At the Stroud Lab scientists seek to understand molecular mechanisms of certain key biological pRocesses, as well as signal transduction between processes at the level of protein structure, dynamics and mechanism.

Gerhard FX Schertler

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Gebhard Schertler was born in Dornbirn, Austria and studied Chemistry and Biochemistry at the University of Innsbruck, Austria. From 1984–1989 he did a PhD at the Max-Planck Institute for Biochemistry in Munich with Dieter Oesterhelt and Hartmut Michel. Major fields of study were in molecular biology, biophysics, and biochemistry of membrane proteins and membrane protein crystallisation. He switched to the field of G Protein-Coupled Receptors as a Research Associate in the Division of Structural Studies at the MRC Laboratory of Molecular Biology in 1989. He has moved to Switzerland and has been appointed as a full professor for structural biology at the ETH in Zurich in 2010. He is heading the Department of Biology and Chemistry and the Biomolecular Research Laboratory at the Paul Scherrer Institute in Switzerland. He is developing nano-diffraction methods with synchrotron radiation and free electron lasers. His laboratory is investigating receptor activation and the signalling complexes of GPCRs with a multidisciplinary approach.

This is a time of critical increases in understanding membrane protein structures and their mechanisms in biology. As the first structures of membrane proteins determined by X-ray crystallography from rich natural sources, bovine rhodopsin, bovine aquaporin from eye lens and proteins from mammalian tissues, the advent of cloning and expression opened a broader horizon and first gave rise to predominantly prokaryotic membrane proteins expressed in a prokaryotic host. Eukaryotic proteins present greater challenges as their normal synthesis proceeds through cellular pathways inside the cell, processed in the endoplasmic reticulum and Golgi apparatus. Thus, as the number of membrane proteins is now increasing exponentially and primarily represented by prokaryotic species, the numbers of eukaryotic membrane proteins is steadily increasing, building toward the opportunities to understand targets of pharmacological significance. It emerges that different classes of receptors, transporters, pumps or channels often yield to crystallization using specialized techniques that serve to fix particular kinds of dynamical properties associated with transport or signaling. In this issue we sought to bring together a series of timely reviews that summarize several of the generally applicable technologies, alongside others that have so far gained special advantage for certain membrane protein classes.

A steady stream of methodological improvements and a wealth of empirical experience with the expression purification and crystallization of particular membrane protein classes are driving a growing output of new and very exciting insights into membrane protein structures and their mechanisms. Recent highlights include the increasing use of lipids in the lipid-detergent-protein micelles used for crystallography, and the use of thermal methods of optimizing stability of ligated states or alternate constructs of membrane proteins. In addition there are important breakthroughs in the determination of alpha helical membrane proteins in lipidic or detergent environments using solution NMR techniques. Progress in solution NMR has led to the first complete assignment and structures of helical membrane proteins up to the sizes of the EmrE dimer in solution, and to more rapid ways of determining a three-dimensional backbone structures. Careful sample preparation is at the center of this new and successful development (Nietlispach *et al.*).

The first applications of the extremely high intensity pulsed bursts from the X-ray free electron lasers (XFELs) to femtosecond nano-crystallography of membrane proteins have become a reality, raising the promise for structures from even smaller crystal samples as outlined here by Fromme and Spence. Theoretical calculations and simulations of the dynamic nature of proteins are starting to play an important role in the interpretation of membrane protein structures and in the design of experiments

necessary to test structure function hypotheses. These developments are making membrane protein structural biology of transporters, receptors and channels one of the most active and exciting areas in modern biology that is becoming increasingly important for pharmaceutical science and industry.

The increasing experience with expression and characterization of membrane proteins in detergent solution for the first time makes structural genomics approaches on integral membrane proteins look more feasible and are successful with prokariotic membrane proteins, outlined by Love *et al.* whereas large-scale efforts for eukaryotic ones are still in their infancy. Nevertheless, rapid progress with eukaryotic G-Protein Coupled-Receptors (GPCRs), using conformationally stabilized GPCRs, and with functionally expressed GPCRs fusion partners, might open the possibility for high throughput approaches for this large and important class of eukaryotic membrane proteins.

An increasing number of beta barrel outer membrane and mitochondrial membrane protein structures with increasingly sophisticated functions are being determined. These, and the machinery that catalyses folding of these integral membrane proteins are summarized here by Buchanan *et al.* Respiratory and photosynthetic membrane protein assemblies of often many partners were at the beginning of the success story of membrane protein structural Biology. A recent highlight reviewed here by Sazanov *et al.* is about the first key membrane protein enzyme of the respiratory chain that plays a central role in cellular energy production. This review outlines the nature of the structure of many subunits, and the areas that are still question marks for future analysis of this critical complex. As a mitochondrial enzyme it is involved in reactive oxygen species, and triggering apoptosis and so is recognized in many human neurodegenerative diseases, as well as ageing. As one of the biggest membrane protein complexes the structure determination and the manifold of insights it reveals are amazing (Sazanov *et al.*).

The last few years have seen dramatic growth in the understanding of GPCR structures. The first structures of partial and full agonistic ligands bound to the receptor have been obtained and the first structures in an active conformation competent to bind the G protein have been obtained. Different strategies have been developed for stabilization of flexible loops in class 1 GPCRs. These include mutagenesis, truncation of flexible regions, insertion of other stable entities, and use of antibody fragments, or other binding proteins as summarized by Standfuss *et al.* The impact of the new structures has enabled a molecular dynamics approach to understand the signaling by GPCRs as outlined by Filizola.
Crystallization within lipidic phases, leading to so-called type 1 crystals of membrane protein within lipid bilayers, has transitioned from a boutique method applicable to colored photoactive haloarchaeal proteins, to a robust method that can be applied to non-colored, mammalian GPCRs and is presented here by Cherezov. The method has been automated such that only minute amounts of protein are needed for crystallization trials. With new developments in the synchrotron beam lines microcrystallography, crystallography applied to crystals in the range of 10 microns is yielding structures of membrane proteins grown by the LCP method and other methods.

Fragments of binding partners are increasingly useful in generating more thermo-stable and crystallizable complexes of membrane proteins, so increasing the area available for building the lattice, and ordering often flexible regions that serve as the targets for selective binding, or stabilizing particular structural states of a dynamic trajectory in the membrane protein. There are several available schemes beyond the normal monoclonal antibody generation. In one such example, Camelid single chain antibodies, now also termed 'nanobodies' are proving valuable in this role. They increase the hydrophilic surface of the complex with the target protein for lattice formation, and can be selected to trap a particular structural state of a membrane protein (Kobilka *et al.*)