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Guest Editor's Introduction

New and emerging methods in membrane protein research

In this issue of the *Methods* journal, experts in the field of membrane biology present recent methodological advancements in the area of membrane proteins. Experimental protocols are presented for ensemble measurements on activity and/or structure of the proteins as well as their single-molecule analyses. The first four articles address the analysis of membrane proteins in the detergent-solubilized state. Mirjam Klepsch and colleagues report on an improved 2D electrophoresis method for the proteomic analysis of membrane proteins, which offers an alternative to the nowadays often used 2D-liquid chromatography methods. The mass spectrometric analysis of full-length analysis of membrane proteins has been a challenge for many years and still poses major hurdles compared to the analysis of water-soluble proteins. Guillaume Gabant and Martine Cadene present a series of approaches to determine the mass of these hydrophobic proteins and to analyze their stability, monodispersity and folding state by mass spectrometry. Edmund Kunji has taken the analysis of the hydrodynamic properties of membrane proteins by size-exclusion chromatography to its limits. Together with his coworkers, Kunji presents approaches to extract detailed information on the radius of the proteins at the midpoint of the membrane, the average radius, the Stokes' radius, and the excluded volume. A direct method to determine the mass of membrane proteins is presented by Dirk Jan Slotboom and co-workers, using SEC in combination with static light scattering. Next to sedimentation equilibrium centrifugation, this is the only method that can measure the molecular mass of a membrane protein without any assumption on the amount of detergent bound or the shape of the protein.

Derek Marsh has provided state-of-the-art methods to probe lipid-protein interactions by ESR, which can be used to determine the stoichiometry and selectivity of lipids with integral and peripheral membrane proteins or membrane bound peptides. Patrick Schulz and colleagues describe an assay for the characterization of electrogenic events in membrane transport proteins. Rather than a need for reconstituting the proteins into giant-unilamellar vesicles or black-lipid membranes, the method takes advantage of membrane vesicles or proteoliposomes that are adsorbed to a

solid-supported membrane. The method offers an alternative for measurements in vesicular structures that are not accessible for standard electrophysiology. Christoph Danelon and colleagues present protocols on recent electrical and optical techniques for investigating cellular signaling reactions in artificial and native membranes, immobilized on solid supports. These membranes are suited to record a few or even single ion channels by impedance spectroscopy.

Fluorescence measurements are dealt with in three papers. First, Ana Garcia-Seaz and Petra Schwille describe the theoretical basis of fluorescence correlation spectroscopy (FCS) and some practical implications for its application in membrane studies, e.g. estimation of concentrations, diffusion coefficients, molecular interactions and membrane partitioning. An extension of the FCS analysis is presented by Geert van den Bogaart et al., in a new method termed dual-color fluorescence burst analysis (DCFBA). DCFBA correlates the fluctuations in intensity of fluorescent labeled membranes and encapsulated (macro)molecules (labeled with second fluorophore) for each liposome diffusing through the observation volume. The analysis provides detailed information on molecule transport through membrane pores. An extension of the method can be used to estimate quantitatively the binding of proteins to liposomes and to membrane proteins. The tracking of single molecules in live cells is presented by Stefan Wieser and Gerhard Schuetz. Approaches are presented to localize fluorescent molecules and probe their trajectories in live cell plasma membranes.

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