apparatus. Combined X-ray crystallographic and functional studies of DesK show that helical rotations in the central four-helix bundle modulate its association with the ATP binding domains. We propose that this signaling-induced transitional rotation provides a switching mechanism to stimulate the kinase or phosphatase activities in response to changes in the lipid environment. These results also provide a new insight into temperature-sensing mechanisms.

1104-Symp
Membrane Protein Folding: Insights Into Folding Transition States And Lipid Control Mechanisms
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General folding principles have emerged from studies on water-soluble proteins, but it is unclear how these ideas will translate to transmembrane proteins, which expose rather than hide their hydrophobic surfaces. We combine kinetic and thermodynamic studies of the reversible unfolding of bacteriorhodopsin to provide a definitive value for the reaction free energy and a means to probe the transition state. Our analyses show that the major unfolding step in the sodium dodecylsulfate-induced denaturation of bacteriorhodopsin involves loss of α-helical structure and proceeds with a large free energy change. Bacteriorhodopsin is folded into mixed detergent/lipid (CHAPS/DMPC) bicelles and once folded, is found to be kinetically very stable. The kinetics, together with studies of mutants, also give information on the transition state for this major unfolding step. The bicelles used in this work increase the stability of other membrane proteins. Alteration of the bicelle properties highlights the influence of certain bicelle parameters on stability. Further information on the lipid parameters that influence folding is gained from studies in lipid-bilayer vesicles.

1105-Symp
How Lipids Regulate Membrane Protein Function
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To what extent can our understanding of how water molecules interact with a water-soluble protein help us to understand how lipid molecules interact with a membrane protein? A first shell of water molecules is found covering the surface of a water-soluble protein, and water molecules are also found buried within the structure. Interactions of these water molecules with the protein help define its structure, and thus its function. Similarly, the surface of the transmembrane region of a membrane protein is covered with a first-shell of perturbed lipid molecules, referred to as the lipid annulus. Binding constants of lipids to these annular sites can be determined using a fluorescence quenching method, studying the quenching of the fluorescence of Trp residues in the protein by lipids with bromine-containing chains. Such studies show that the lipid annulus is heterogeneous - the mechanosensitive channel MscL, for example, contains a 'hot-spot' where anionic lipids bind with high affinity. Binding of anionic lipids to this hot-spot has a large effect on the flux through the MscL channel. Lipid molecules can also be found buried within the structure of a membrane protein, for example, at protein-protein interfaces in multimeric proteins. An example is provided by the homotetrameric potassium channel KcsA. The crystal structure of KcsA by MacKinnon shows an anionic lipid molecule bound at each monomer-monomer interface. Occupation of these sites by anionic lipid molecules is not required for tetramer formation, but is important for function. The open probability of the channel increases markedly with increasing anionic lipid content in the membrane, three of the four intersubunit binding sites having to be occupied by anionic lipid for the channel to be open.

1106-Symp
How Does a Membrane Protein Know What is In and What is Out? Lipids as Topological Determinants
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Primary functions of lipids are to define barrier properties of membranes and provide a scaffold within which membrane proteins are organized. Using a genetic approach to alter the phospholipid composition of the *Escherichia coli* coupled with biochemical approaches to monitor topological organization of membrane proteins, dependence of lactose permease (LacY) on phosphatidylethanolamine (PE) for proper orientation with respect to the plane of the membrane was determined. Assembly of LacY in the absence of PE results in topological inversion of its N-terminal half, which is largely reversed by post-assembly synthesis of PE. Replacement of PE by the foreign lipids phosphatidylcholine, monoglucosyl diacylglycerol, or diglucosyl diacylglycerol, which exhibit similar properties to PE, restores proper topology thereby supporting common functions for lipids with diverse structures. Topology of LacY in membranes lacking PE is dependent on a connecting flexible hinge region in order for the two halves of LacY to independently respond to the lipid environment. Final topology is determined after LacY exits the translocon by long-range and short-range interactions between the net charge of extra-membrane domains and the net charge density of the phospholipid bilayer surface. PE appears to dampen the translocation potential of acidic residues in normally cytoplasmic domains in favor of the cytoplasmic retention potential of basic residues. Thus a primary role for PE is to allow the presence of acidic residues in the cytoplasmic domains of membrane proteins for functional purposes without affecting protein topological. The topologies of two amino acid permeases (PheP and GabP) unrelated to LacY are also topologically sensitive to membrane lipid composition strongly indicating that lipid environment is a significant determinant of final topological organization of multiple membrane proteins. Supported in part by NIGMS R37-GM20478.

Symposium 10: New Frontiers in Biophysics

1107-Symp
Building And Controlling Networks Of Droplet Interface Bilayers
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One goal of synthetic biology is the manufacture of micromachines from simple parts. Such machines would be motile, able to generate, store and use energy, capable of sensing and carrying out computation, and able to take up substrates and convert them to products. We have found that aqueous droplets can be connected by lipid bilayers to form networks in a hydrocarbon environment [1]. We propose to use these networks for the construction of "soft" micromachines (or "prototissues", by analogy with efforts to build protocells). Proteins can be incorporated in to the bilayers of the networks [2]. Therefore, we expect that membrane proteins will play a role in the functioning of droplet-based micromachines, notably by allowing the droplets to communicate and exhibit emergent properties. Towards this end, we have engineered the staphylococcal alpha-hemolysin pore to endow it with a variety of capabilities. We have been able to alter the pore size, and its ion selectivity and rectification properties. We have also altered the pore so that it is regulated by chemicals, light and temperature. With these components, we have shown that droplet networks can behave like simple electrical circuits [3], be used to form tiny batteries [1] and respond to light [1]. With these subsystems in place, the manufacture of the proposed micromachines may soon be in the offing.
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1108-Symp
Predictive Computational Models Of Complex Biological Systems: Antiarrhythmics And Cardiac Tissue Dynamics
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Effective pharmacological treatment of cardiac arrhythmia is a long sought and, as yet, elusive goal. Poor efficacy and outcomes in treating arrhythmia with drugs is due, in part, to failure to accurately predict how drugs with implicitly complex pharmacodynamics affect multi-component interactive cardiac cells and tissues. For example, an assumption that drug block of voltage gated Na+ channels results in current reduction is much too simplistic. Rather, multiple factors including complex drug pharmacokinetics, pH dependence, voltage dependence, conformation-specific block and rate-dependent properties of drugs, as well drug interaction with the multiple mechanisms and triggers of arrhythmia must be considered for development of appropriate pharmacological intervention for arrhythmia management. Our goal has been to develop novel theoretical approaches through the construction of detailed representations of drug block in virtual cardiac cells and tissues. I will present a multi-scale computational approach to predict the effects of antiarrhythmic drugs that target cardiac Na+ channels. The models reproduce experimentally observed pharmacokinetics of drug channel interactions including dose-dependence and steady-state drug effects, and well as dynamic properties such as use- and rate-dependence and recovery from block. The drug-channel models are incorporated into computational representations of cardiac tissue to test potentially arrhythmogenic situations in which the models predict specific drugs to be proarrhythmic or antiarrhythmic. For example, under particular rapid