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
Paula J. Booth, Paul Curnow.
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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
Anthony Lee.
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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
William Dowhan.
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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
Hagan Bayley.
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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.
1. Holden MA, Needham D, Bayley H: Functional bionetworks from nanoliter water droplets. J. Am. Chem. Soc. 2007, 129:8650-8655.
2. Bayley H, Cronin B, Heron A, Holden MA, Hwang W, Syeda R, Thompson J, Wallace M: Droplet interface bilayers. Mol. BioSystems 2008:published ASAP, DOI: 10.1039/b808893d.
3. Hwang WL, Holden MA, White S, Bayley H: Electrical analysis of protein pore insertion and blockade in droplet interface bilayer networks. J. Am. Chem. Soc. 2007, 129:11854-11864.

1108-Symp
Predictive Computational Models Of Complex Biological Systems: Antiarrhythmics And Cardiac Tissue Dynamics
Colleen E. Clancy.
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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

pacing conditions, the model predicts that the Na+ channel blocker flecainide results in development of tachyarrhythmias. However, the same drug is predicted to exhibit antiarrhythmic effects on slow heart rate dependent arrhythmia triggers. Our results suggest that drug efficacy in arrhythmia treatment is drug and circumstance dependent and that a computational approach can be utilized to predict experimentally testable outcomes.

1109-Symp
Optogenetics: Development And Application
Karl Deisseroth.
Stanford University, Stanford, CA, USA.
Integrating microbial opsins and solid-state optics allows millisecond-precision bidirectional control of defined cell types in freely behaving mammals. Following the introduction of the microbial opsin genes ChR2 and NpHR to neurobiology, genomic strategies allowed the discovery and adaptation for neuroscience of a third major optogenetic tool, namely a cation channel (VChR1) with action spectrum significantly redshifted relative to ChR2, to allow tests of the combinatorial interaction of cell types in circuit computation or behavior. We also have developed genetic targeting tools for versatile use of microbial opsins with existing resources including cell type-specific promoter fragments or Cre-LoxP mouse driver lines suitable for a wide variety of neuroscience investigations, and developed integrated fiberoptic and solid-state optical approaches to provide the complementary technology to allow specific cell types, even deep within the brain, to be controlled in freely behaving mammals.

1110-Symp
Semiconductor Chips with Nerve Cells and Brain Tissue
Peter Fromherz.
Max Planck Inst Biochem, Martinsried, Germany.
The lecture describes the electrical interfacing of semiconductor devices with cultured neuronal networks and brain tissue in both directions. Individual capacitors and transistors on silicon chips are used as well as multi-transistor and multi-capacitor arrays fabricated by an extended CMOS technology [1]. On the biological side, three levels of interfacing are considered with recombinant ion channels, with individual nerve cells from snails and rats, as well as with rabbit retinae and slices from rat hippocampus. Particular attention is given to the mechanism of signal transduction between the electronic and ionic system that avoids electron exchange between semiconductor and electrolyte. In that respect, the Johnson noise is most useful tool to determine the seal resistance of cell-chip junctions. In the case of electronic stimulation of neuronal activity, a displacement current across electrolyte-oxide-semiconductor (EOS) capacitors gives rise an Ohmic current along the seal resistance and to a voltage change across the cell membrane that opens ion channels. In the case of electronic recording of neuronal activity, the current through ion channels gives rise to an extracellular voltage in the cell-chip junction that plays the role of a gate voltage on EOS field-effect transistors. In general, the interfacing of nerve cells from invertebrates (leech, snail) is simpler to achieve than the stimulation and recording of mammalian neurons because the capacitive and ionic currents are larger for larger cells. With cultured and acute brain slices, the interfacing refers to small groups of neurons. The implementation and understanding of neuro-electronic interfacing is a basis for applications in biosensorics, neurophysiology, neuroprosthetics and experiments that may lead to neurocomputers.
[1] P. Fromherz, Solid State Electronics 52 (2008) 1364.

Minisymposium 2: Microtubular Motors: Structural and Functional Diversity

1111-MiniSymp
Direct Observation of Individual Kinesin Head Motions
Nicholas R. Guydosh, Steven M. Block.
Stanford University, Stanford, CA, USA.
Optical-trapping assays for kinesin typically involve attaching a bead to the common stalk of the protein, whose motions report the average position of the molecule. Individual displacements produced by the separate heads therefore remain unresolved. We developed a novel assay for tracking a single head of Kinesin-1 while under controlled loads, by attaching a bead to one of the two head domains via a short (70 bp) DNA tether. This assay can directly report binding of the tethered head to the microtubule. Under hindering loads, we observed steps of ~16 nm, as anticipated for heads moving in a hand-over-hand walk. Under assisting loads, we observed large jumps (>16-nm) in displacement at the start of step dwells, as load pulled the rear head forward beyond its partner head by ~4 nm. Torque generated between the points of head attachment of the DNA and the neck linker tends to rotate the head, explaining this 'overshoot' feature. The durations of overshoots depend on the ATP concentration, implying that ATP binding to the new rear head allows the front (DNA-linked) head to rotate back to its normal orientation and bind the microtubule. To directly test whether one head is free to diffuse about its bound partner head between steps, we applied rapidly oscillating (hindering and assisting) loads to kinesin during stepping and measured the time-dependent difference between forward and rearward displacements of the bead. The magnitude of this signal varied between two discrete values, corresponding to those intervals when the bead-linked head adopted bound and unbound states. The existence of an unbound state disfavors models where one head docks against its partner between steps. We conclude that internal strain, generated whenever both heads bind the microtubule, is responsible for gating the kinetic cycle to ensure kinesin processivity.

1112-MiniSymp
The ATP State of a Mitotic Kinesin-5 Bound to Microtubules
Andrew Bodey[1], Masahide Kikkawa[2], **Carolyn Moores[1]**.
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The mitotic spindle is essential for faithful cell division. It is built from microtubules and is orchestrated by many proteins, including members of the kinesin superfamily. Kinesin-5 motors are essential for mitosis in many organisms and are involved in formation and maintenance of spindle bipolarity. Kinesin-5s share some properties with other kinesins including the ability to move - albeit slowly - towards the plus ends of microtubules. However, kinesin-5s have a number of unique properties, and are also of interest for cancer treatment because kinesin-5-specific small molecule inhibitors have been identified and are in clinical trials.
Outstanding mechanistic questions about kinesin-5 motors relate to their interaction with microtubules. We set out to understand this interaction using cryo-electron microscopy and image processing. Cryo-electron microscopy is uniquely suited to this goal since microtubules are too large and heterogeneous to be studied by other structural techniques. Using the motor domain from Klp61f (the Drosophila kinesin-5), we imaged microtubules bound by the motor in an ATP-like state and calculated the structure of the complex at ~10Å resolution. At this resolution, we are able to see the density associated with most α-helices in both the motor and the microtubule and visualise the motor in a tight-binding, AMPPNP conformation. The docked tubulin structure shows an excellent fit to our map, but available kinesin-5 crystal structures do not match the conformation of the motor in our maps, indicating that microtubule binding induces a conformational change in the kinesin-5 motor. Thus, calculation of kinesin-microtubule structures are essential for revealing the precise mechanism by which motors use energy from ATP and microtubule binding to generate force. Our structure also provides insight into the mechanisms by which anti-cancer drugs elicit their therapeutic effect.

1113-MiniSymp
LIS1 Converts Dynein to a Persistent-force State: A Molecular Model to Explain Lissencephaly
Michael D. Vershinin[1], Richard J. McKenney[2], Steven P. Gross[1], Richard B. Vallee[2].
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Cytoplasmic dynein is involved in diverse tasks such as cargo transport along microtubules, cell division and nuclear migration. We have used biochemical and biophysical tools to examine the regulatory roles of two proteins which are crucial parts of the dynein pathway: NudE and LIS1. LIS1 is the causative gene for the developmental brain disease, lissencephaly, which is associated with dynein-dependent defects in cell migration and division. NudE is a dynein-LIS1-interacting protein also implicated in brain development and mitosis. Using laser trapping of beads coated with dynein and its associated factors at the single-molecule limit we find that NudE reduces the frequency of motor-microtubule binding events and inhibits motor travel. In contrast, LIS1 dramatically extends dynein's force generating state. LIS1 alone binds transiently to dynein, but NudE recruits LIS1 to form a stable ternary complex with persistent force-generating activity. These results have important biological implications. LIS1 is known to participate in dynein functions that involve transport of very high loads, such as nuclear movement through the embryonic brain (Tsai, Bremner, Vallee, 2007, Nat. Neurosci. 10:970). Our results strongly suggest that it is this aspect of LIS1 function that makes the developing brain uniquely sensitive to decreased LIS1 expression in human lissencephaly. Supp. by grants GM47434, HD40182, and GM070676.