Biophysical techniques, such as single molecule fluorescence microscopy and FRET, single ion-channel patch clamping, and optical tweezers often yield data consisting of noisy dwells separated by discrete steps. When an underlying kinetic model can be assumed, steps may be located in a rigorous fashion using methods derived from the theory of hidden Markov models. In the absence of a kinetic model, such steps are often located by eye or with the assistance of heuristic fitting or filtering techniques. We present an objective, rigorous, model-independent method enabling identification of non-uniform steps present in such noisy data. Our method is based on the Schwarz Information Criterion, a test statistic enabling meaningful comparison between fits with differing numbers of parameters, and does not require the assumption of any underlying kinetic or state models. It is hence particularly useful for analysis of novel and poorly understood systems.

Platform AT: Membrane Proteins - II

1853-Plat The Outer Membrane Translocon for Nuclease Colicins: Higher Resolution Structure of the OmpF Porin

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The *E. coli* OM translocon for colicins E2/E3 consists of the high affinity receptor, BtuB [1, 2] and receptor-translocators, the OmpF or OmpC porin [2]. A complex containing colicin E3/BtuB/OmpF was cross-linked *in situ*. Channel activity of OmpF can be occluded by colicin E3, or its 83 residue N-terminal disordered translocation domain, T83. Occlusion is negated by the mutations, Asp5Ala and Arg7Ala, implying an electrostatic interaction with one or more critical carboxylate or basic residues in the constriction zone [3]. T83 was shown to bind/insert to/into OmpF at low, but not high ionic strength. A 1.6 Å crystal structure of OmpF was obtained by crystallization of OmpF in the presence of 1 M $MgCl_2$ and T83. T83 was not seen in the electron density. However, a hexa-aquo Mg^{2+} ion is seen that bridges between the two carboxylate residues, Asp113 and Glu117 in the L3 loop of the porin constriction zone, and is the major change in the OmpF electron density compared to that of the OmpF structures obtained at somewhat lower (2.2 Å) resolution [4]. The higher resolution results in the detection of more than 300 crystallographic H_2O molecules with a minimum in the water density at the position of the constriction filter.

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1854-Plat Real-time Visualization of the Assembly of Aquaporin-4 Isoforms into Orthogonal Arrays Revealed by Single Particle Tracking (SPT)

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Aquaporins (AQPs) are a family of integral membrane proteins whose function is to facilitate osmotically driven water transport across cell plasma membranes. AQP4 and AQP1 are the primary water channels expressed in the eye and central nervous system. AQP4 naturally exists in two isoforms, a full-length form (M1), and a shorter form (M23), which is truncated by 22 residues at the N-terminus, but otherwise identical to M1. Freeze-fracture electron microscopy has shown that M23, but not M1, forms large orthogonal arrays of particles (OAPs) in the plasma membrane of primary astrocytes and transfected cell lines. We tracked the membrane diffusion of AQP4 isoforms M1 and M23, and AQP1 labeled with quantum dots at an engineered external epitope at frame rates up to 91 Hz and over times up to 6 min (Crane and Verkman. *Biophys. J.* In Press). SPT in transfected primary astrocytes and COS-7 cells showed that >85% of M23 was highly restricted or immobile, with range <50 nm at 1s and diffusion coefficient D~7×10^{-11} cm^2/s, consistent with its expected confinement within OAPs. Under identical conditions, >70% of M1 exhibited Brownian diffusion, covering >400 nm in 1s, with D~5×10^{-10} cm^2/s. Likewise, >75% of AQP1 showed Brownian diffusion, with D~9×10^{-10} cm^2/s. Deletion of a C-terminal PDZ-binding domain in AQP4, as well as disruption of the actin skeleton had no effect on M23 diffusion, indicating that array formation is independent of cytoskeletal interactions. The diffusion of N-terminal deletion mutants of AQP4 were also investigated, as well as AQP4 isoforms following cellular treatments that modulate phosphorylation signaling pathways. SPT permits real-time visualization of AQP4 within OAPs in live cells. Our results indicate that an N-terminal motif in M1 is responsible for the disruption of OAPs.

1855-Plat A Novel Experimental Approach To Characterize Membrane Protein Interactions

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We have revisited the technique of fluorescence recovery to probe interactions between membrane proteins embedded within the same or in different membranes.

For this purpose we use a medium of model membranes with tunable intermembrane distance.

When the membranes are far apart, the only possible interactions occur between proteins embedded within the same bilayer, whereas when membranes get closer to each other, interactions between proteins embedded in opposite membranes may occur as well.

By screening the proximity conditions between two selected proteins, we determine both the arrangement of these proteins and their stoichiometry within the assembly.

We also explore the dependence of the latter parameter on pH.

To validate the novel approach described herein, we first carry out the well-documented interaction between streptavidin and biotinated transmembrane peptides.

Next, we investigate the more complex interactions between proteins constitutive of a Pseudomonas aeruginosa efflux pump: MexA and OprM, and show that interactions occur only within a narrow range of intermembrane distance.

1856-Plat Protein Interactions Mediated by Lipid Membranes

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Membrane proteins can experience mutual interactions mediated by the lipid bilayer. These occur whenever local protein-imposed changes in some membrane property excite fields which extend beyond their point of imprint. One example is membrane curvature, another is lipid composition. Interactions result because the stresses associated with these fields in turn induce forces on other proteins. By using a combination of coarse-grained simulations and analytical theory we illustrate how such interactions manifest themselves and which consequences they have. For instance, the curvature fields emanating from local membrane curvers can drive their spontaneous aggregation and subsequent vesiculation even in the absence of any direct protein-protein binding. Such physical forces therefore complement and assist specific interactions in their task of controlling protein and membrane organization.

1857-Plat Subunit Structures Of *N*-methyl-d-aspartate Receptor Analyzed By Atomic Force Microscopy

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Glutamate is one of the most important neurotransmitter which mediates fast excitatory synaptic transmission via ionotropic glutamate receptors (iGluRs) in the central nervous system (CNS). Among iGluRs, *N*-methyl-D-aspartate receptor (NMDAR) is involved in learning and memory and also implicated in a number of disease and injury states. Despite its functional importance, little is known about the whole three-dimensional structure of NMDAR. Here, we demonstrate the structure of NR1 subunit-containing NMDAR purified from primary cortex neuron under the liquid condition as determined by atomic force microscopy (AFM). AFM image of NMDAR showed a diagnostic structure highly correspondent to it of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR) which had been previously determined by electron microscopy. In the control, NMDAR showed various conformations, which was thought to reflect the change of the two dimeric extracellular amino-terminal domain. Stimulation of NMDAR by glutamate/glycine markedly altered its conformation with larger separation of two dimeric N-terminal domains (NTDs). Whereas, MK801, a selective antagonist for NMDAR, prevented the agonist-induced separation of NTDs. Our present data implicate that such NTDs movement is an important action connected to the ion channel pore opening in NMDAR.

1858-Plat Biophysical Characterization of the Human Adenosine A_2a G-Protein Coupled Receptor

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G-protein coupled receptors (GPCRs) are a large family of integral membrane proteins, many of which have been identified as important pharmaceutical targets. Structural and conformational studies of these proteins are impeded by their relatively low abundance in native cells, low levels of expression in recombinant systems, and difficulties associated with their isolation and stabilization in membrane-mimetic environments. Here, we describe biophysical characterization of the human adenosine A_2a receptor (A_2aR). We overcame the obstacle of expression by producing A_2aR in a heterologous yeast system, purified the receptor using Ni-NTA chromatography, and reconstituted the receptors in mixed micelles of dodecylmaltoside, CHAPS, and cholesterol hemisuccinate. Typical yields were ~ 6 mg active A_2aR per liter of culture; activity was verified by ligand-binding assays.

We used CD and intrinsic fluorescence spectroscopies to monitor the conformation of purified A_2aR. CD spectra indicate that the protein is highly alpha-helical, as expected. A_2aR contains 7 tryptophans; intrinsic fluorescence spectra of the receptor indicate that these residues are in a highly non-polar environment. Heating the protein samples to 90°C led to an ~80% loss of alpha helical content, a substantial decrease of fluorescence intensity, and a small red shift of the fluorescence emission maximum. These changes, which were largely irreversible, are consistent with thermal denaturation of receptor secondary and tertiary structure. Binding of agonist (N^6-cyclohexylyadenosine) and antagonist (theophylline) did not induce any detectable changes in CD or fluorescence spectra of A_2aR, implying that conformational changes associated with these processes do not cause appreciable changes in secondary structure or in the global environment of the tryptophans. Thermal denaturation studies in the presence of both agonist and antagonist reveal slight increases in the midpoints of unfolding, suggesting that the ligand-bound form of A_2aR is more stable than the unliganded receptor.

1859-Plat Fluorinated Surfactants as Chaperones for Insertion/Folding of Membrane Proteins

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The principal difficulty in experimental exploration of folding and stability of membrane proteins (MPs) is their aggregation outside of the native environment of the lipid bilayer. Recently we suggested circumventing this problem with the help of fluorinated nondetergent surfactants that act as chemical chaperones [Biochemistry 2006, 45:2629]. The ideal chaperone surfactant should satisfy the following requirements: 1) prevent aggregation of MP in solution; 2) have minimal perturbation of MP structure; 3) dissociate from MP during membrane insertion; 4) not partition into lipid bilayer. Here we compared how surfactants with hemifluorinated (HF-TAC) and totally fluorinated (F-TAC) hydrophobic chains of different length compare to this ideal. First, we demonstrate using FCS of dye-labeled F-TAC and HF-TAC that neither of them will bind lipid vesicles (LUV). Thus, unlike the detergents, fluorinated surfactants do not compromise vesicle integrity even at concentrations far exceeding their CMC. Second, we examined interaction of surfactants with two model MPs, diphtheria toxin T-domain (DTT) and annexin B12, known to insert into the bilayer at acidic pH. Site-selective labeling of DTT with fluorescent dyes indicate that the surfactants do not interact uniformly and concentrate in most hydrophobic patches. The observed reduction in the efficiency of FRET between acceptor-labeled (H)F-TACs and donor-labeled DTT upon addition of LUV indicates that the protein sheds the layer of surfactant during its bilayer insertion. CD measurements suggest that the presence of surfactants does not alter the structure of model MPs. The cooperativity of the thermal unfolding transition, however, is reduced by the presence of surfactants, especially above the CMC. The linear dependence of the enthalpy of unfolding of DTT on the surfactant concentration is encouraging for future application of (H)F-TACs to determining the stability of the membrane-competent conformations of other MPs.

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1860-Plat Influence of the Hydrophobic Segment of Diacylglycerol Kinase Epsilon on the Membrane Topology and Activity of the Enzyme

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Diacylglycerol kinase epsilon (DGKε) is unique among mammalian DGK isoforms in having a segment of hydrophobic amino acids. This hydrophobic segment is predicted by simple algorithms to be a transmembrane helix. However, previous studies showed that the N-terminus of DGKε is located on the cytoplasmic side of the plasma membrane, the same side that the remainder of the protein is located. Thus, the hydrophobic segment, comprising residues 20–40, is not a transmembrane helix. Further theoretical calculations using angular dynamics showed that the Pro residue within the hydrophobic segment favors a bent conformation and that its replacement by Ala causes the segment to become a transmembrane helix. We made a P32A mutation of DGKε having a FLAG tag at the N-terminus. To compare the membrane topology of DGKε with the P32A mutant, NIH 3T3 cells have been transfected with FLAG-DGKε and its P32A vectors. Comparison of permeabilized and non-permeabilized cells, by immunofluorescence microscopy using anti-FLAG primary antibodies showed that the N-terminus of the P32A mutant is exposed on the external side of the membrane, in contrast to the N-terminus of full-length FLAG-DGKε that is exposed to the antibody only in permeabilized cells. To produce more of the proteins they were expressed in COS-7 cells. Activity assay *in vitro* showed that this mutated form of DGKε had a lower K_m and a lower catalytic rate constant for 1-stearoyl-2-arachidonoyl-sn-glycerol, compared with the wild-type enzyme, but retained its specificity for substrates with polyunsaturated acyl chains. Our studies demonstrate that DGKε is a monotopic protein that can be converted into a bitopic protein by a single amino acid replacement. This mutation also has significant consequences on the catalytic properties of the protein.

Platform AU: Voltage-Gated Na Channels

1861-Plat Intracellular Ca^{2+} Can Directly Regulate $Na_V1.4$ Through An Efl Motif In C-terminus Of The Channel

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Intracellular Ca^{2+} regulates gating of both voltage-dependent cardiac sodium channels ($Na_V1.5$) and skeletal muscle sodium channels ($Na_V1.4$) through EF- hand like (EFL) and IQ motifs in C-terminus (CT). The role of upstream EFL motif in Ca^{2+} regulation of wild type $Na_V1.4$ current is uncertain. Increasing intracellular Ca^{2+} shifts the steady-state availability of $Na_V1.4$ in the depolarizing direction compared to the Ca^{2+}-free condition. However mutations in the EFL key residues with an intact IQ motif abolishes Ca^{2+} mediated channel regulation. We conclude that the Ca^{2+} binding to the EFL motif has a critical role in controlling Na current availability in skeletal muscle through direct binding of Ca^{2+}. Since both EFL and IQ motifs are associated with regulation of fast inactivation, we evaluate the role of EFL and IQ in the myotonia-associated mutant $Na_V1.4$-F1705I, which alters fast inactivation.

1862-Plat Relative Comparison of the Effective Gating Charge between Voltage Gated K- and Na-Channels

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