Halobacterium Salinarum, has been studied by multidisciplinary approaches to reveal the molecular machinery of proton transfer and photocycle. For example, in order to study the functionally related structural change in M-state intermediate, different media with a high pH value have been used to extend the lifetime of M state. However, not much attention have been paid to the conformational changes that occur in the ground state, especially around the retinal binding pocket, due to local environment.
Here, the conformation changes to the bR ground state, especially around the retinal binding pocket, have been studied by solid-state NMR through chemical shifts and torsion angle measurements, combined with the light-induced kinetic and UV spectroscopies. All the experimental results have been discussed in the context of X-ray crystal structures, and possible mechanisms have been proposed.

1927-Pos Board B64
Stripping the CLC-ec1 Dimerization Interface: An Investigation into the Role of Van Der Waals Interactions in Membrane Protein Assembly
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What drives membrane proteins to fold and oligomerize in lipid bilayers? One possibility is that protein-protein interactions stabilize the assembled state. In membrane proteins, interfaces are typically lined by non-polar residues offering weak van der Waals interactions (VDW), yet a large network of these might confer strong stability. To investigate the role of side-chain VDW interactions on the free energy of membrane protein complex formation, we have made a library of mutations on the dimerization interface of the CLC-ec1 Cl-/H+ antiporter - a model system that we have developed to measure equilibrium dimerization in lipid bilayers. The interface is comprised of four alpha-helices lined by non-polar residues: I - F219, I220, I223, I227; H - L194, I197, I198, I201; P - L406, I409, I410, L413; Q - I422A, L423A, I426A, I430A, I434A. We systematically mutated each helix surface to alanine (all-ALA), stripping the interface of its VDW interactions. In all cases the protein expresses, is stable and upon reconstitution in membranes, shows Cl- transport activity comparable to wild-type CLC-ec1. We ran gel-filtration chromatography over the course of one week to screen the stoichiometry of the protein in detergent. All-ALA helix I, P & Q are stable dimers, however all-ALA helix H showed shifts to monomer directly after purification. Furthermore, single-mutant L194A was sufficient to shift the protein to a monomer-dimer mixture. Because helix H interacts with helix P, and all-ALA helix P is still dimeric, these results suggest that dimer stability cannot be explained by VDW interactions alone, at least in detergent. We are currently measuring the change in free energy of CLC-ec1 dimerization with these subtractive mutations to quantify the role of VDW interactions in membrane protein assembly.

1928-Pos Board B65
Sarcolipin-Mediated Regulation of SERCA by Computer Simulations
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The sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) is a transmembrane pump that, upon ATP hydrolysis, pumps Ca^{2+} against a concentration gradient from the cytosol to the sarco- or endoplasmic reticulum, thus terminating muscle contraction and priming the cell for the next excitation-contraction stimulus[1]. SERCA function in skeletal muscle cells is regulated by sarcolipin[2,3] (SLN), a 31 amino acid transmembrane peptide that inhibits SERCA by lowering its apparent Ca^{2+} affinity. By combining solid-state NMR and cross-linking experiments, we characterized the structure of the SERCA/SLN complex in its natural membrane environment in both Ca_2-E1:ATP and H_n-E2:ATP states of the catalytic cycle. In this work, we employ molecular dynamics computer simulations to investigate SERCA's mechanism of regulation by mapping the effects of SLN binding on SERCA's free energy landscape, sub-microsecond structural dynamics, and allosteric coupling between the transmembrane and cytoplasmic domains. Our calculations indicate that upon binding to SERCA, SLN increases its average tilt angle with respect the unbound state. The interaction between the two proteins results in a shift of the free energy basin of SERCA and in an altered coupling between its transmembrane and cytoplasmic domains, which may be responsible for the reduced activity of SERCA in its SLN-bound state.
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1929-Pos Board B66
Monitoring Apolipoprotein Binding to Single Lipoproteins
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Lipoproteins such as very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) are key players for cholesterol transport and fundamental to understanding the mechanisms of heart disease. Apolipoproteins bind to lipoproteins and their quantity and location have been proposed to be more significant indicators of heart disease than levels of good (HDL) or bad (LDL) cholesterol. We are developing a general experimental framework to study the interaction of apolipoproteins with individual lipoproteins in vitro. Specifically, we are studying apolipoprotein C-III (ApoCIII), which inhibits the breakdown of triglyceride-rich VLDL and is correlated with hypertriglyceridemia and atherosclerosis. To measure the quantity of ApoCIII on lipoproteins, we created a two-color system by labeling lipoproteins and ApoCIII with different fluorophores. Colocalization measurements were conducted via wide-field fluorescence microscopy using chambers with thickness of less than 1 μm. Lipoproteins and ApoCIII diffuse freely and are tracked simultaneously, avoiding potential artifacts from the use of surface attachments. ApoCIII binding on individual lipoproteins is quantified and exchange rates between free and bound are characterized. Of particular interest is to capture heterogeneous phenomena that are thought to occur in vivo, such as asymmetric binding distributions, which cannot be easily identified in bulk experiments.

1930-Pos Board B67
NMDA Receptor Transmembrane Domain: Structure and Mechanism of Ion Selectivity
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NMDA receptors (NMDARs) are iGluR subfamilies that are activated during synaptic transmission. The voltage dependence of NMDARs differentiates them from other iGluRs: at typical neuronal resting voltages, NMDAR channels are mostly blocked by Mg^{2+}, but when membrane voltage is depolarized, Mg^{2+} block is relieved, resulting in Ca^{2+} influx through NMDAR at postsynaptic sites. The mechanisms, by which NMDARs select Ca^{2+} for permeation over all other physiological ions, while binding Mg^{2+} and restricting its permeation, are not well understood.
Recently, partially resolved medium-resolution structures of an NMDAR tetramer were published. The structures tremendously improve our knowledge and understanding of the architecture and design of the NMDARs. Yet, one of the key structural features, namely the ion selectivity filter and parts of the pore region of the ion channel itself, are missing from these structures. This region of the protein has not been resolved also in the earlier high-resolution structure of an AMPAR, a closely related iGluR family member.
Previously a successful homology model of the NMDAR TMD using one of the potassium channel family members, NaK channel as a template, was shown to have good agreements with available experimental data [Siegler et. al., Nat Neurosci 15: 406–13]. Given recently released NMDAR structures and our previously developed homology model, we are now in position to propose a high resolution NMDAR TMD model that is based on a hybrid structure. We performed extensive molecular dynamics (MD) and targeted MD simulations of our NMDAR TMD domain model in lipid bilayer and water. We demonstrate that our proposed structure is stable in simulations and has a well-formed binding site for Mg^{2+} and Ca^{2+}.

1931-Pos Board B68
Elucidation of the Channel Activities of Gramicidin a in the Presence of Ionic Liquids (ILS) using Model Cell Membranes
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Ionic liquid(IL) is a salt in the liquid state below 100°C and have been considered as eco-friendly solvent that can replace organic solvent due to their unique