bulk pool in a time of a few microseconds. Combined with the chain dynamics of the protein, these collective dynamics account quantitatively for the observable proton and deuteron spin-lattice relaxation in protein systems including whole tissues. Of particular note is the importance of the internal water molecule dynamics rather then the water at the protein surface in making a significant and sometimes dominant contribution to the spin-lattice relaxation rate constants in the range of most magnetic imaging experiments.

Platform Q: Ligand-gated Channels

879-Plat The Free Energy Landscapes Governing Conformational Changes in a Glutamate Receptor Ligand-Binding Domain

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Ionotropic glutamate receptors (iGluRs) are ligand-gated ion channels activated by the agonist glutamate. The extracellular ligand-binding domain of these receptors responds to agonist binding by undergoing a conformational change that opens a cation-permeable channel in the postsynaptic neuron. iGluRs have been the subject of intense study, and a wealth of insight has been gained into the structure and dynamics of the ligand-binding domain. The energetic basis controlling the conformational response of the ligand-binding domain underlying iGluR function, however, has yet to be revealed. We computed the free energy landscapes governing the opening/closing of the GluR2 ligand-binding domain in the apo, DNQX-, and glutamate-bound forms using all-atom molecular dynamics simulations with explicit solvent, in conjunction with an umbrella sampling strategy. The apo S1S2 is found to easily access low-energy conformations that are more open than observed in X-ray crystal structures. A conformational free energy of 9 to 12 kcal/mol becomes available upon glutamate binding for driving the conformational changes in S1S2 associated with receptor activation. Features in the glutamate-bound S1S2 free energy landscape suggest a sequence of interactions in the cleft that correspond to different states of cleft closure. Small-angle X-ray scattering profiles calculated from computed ensemble averages were found to agree better with experimental results than profiles calculated from static X-ray crystal structures. A cluster of water molecules in the cleft may contribute to stabilizing the apo S1S2 in open conformations. Free energy landscapes were also computed for the glutamate-bound T686A and T686S S1S2 mutants, and the results elaborate on findings from experimental functional studies.

880-Plat *T*esting Mechanisms Of AChR Gating By Φ-value Analysis

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After binding agonists, diliganded neuromuscular acetylcholine receptors (AChRs) gate between C(losed) and O(pen) conformations. A number of specific αsubunit structural transitions have been proposed as being important in C ↔ O gating of cys-loop receptors, including the rotation of the extracellular domain (ECD) β-core, a *cis-trans* isomerization of an M2-M3 linker proline and the perturbation of a salt bridge between R209 (in pre-M1) and E45 (in loop L2). To test these proposals, we measured rate constants and Φ values from ∼**200** different α subunit mutant constructs of the salient and nearby residues (mouse $\alpha_2\beta\delta\varepsilon$, cell-attached, HEK cells, PBS, 22°C, + 70 mV pipette, 500mM ACh/20mM choline/5mM carbachol). The ECD residues K145 and Y127 have Φ-values of 0.96 and 0.77 respectively, which suggests that these residues move early in the gating reaction but asynchronously, so a rotation of the β-core ECD, if present, is not as a rigid body. In the M2-M3 linker, mutations of P272 and G275 produced functional AChRs, so the full isomerization of these backbone bonds is not essential for gating. Overall, the M2-M3 linker had an average Φ-value of 0.64. The putative salt bridge partners E45 and R209 have similar Φ-values (0.80 and 0.74, respectively), with other pre-M1 residues either showing no sign of movement (M207, Q208 and P211) or moving late (L210; Φ=0.36). Several results argue against the salt bridge hypothesis:

(i) E45R is a gain-of function mutation,
(ii) a number of uncharged R209 mutants produce functional AChRs, and positions 45 and 209 show little energetic interaction.

We think that there is no single 'on-off' switch for the channel gating, but that the conformational trajectory through the protein is complex, with energy spread across many residues and along broad boundaries.

881-Plat Using Molecular Dynamics Simulations To Study The Channel Gating Mechanism Of The Nicotinic Receptor

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We previously reported that in target molecular dynamics (MD) simulations with the nicotinic acetylcholine receptor, structural changes at the ligand binding-sites contributed to the channel pore wideness. We also showed in the normal MD simulation that hyperpolarized electric potentials prompted cations translocation through the channel and widened the pore also. Now, we performed target MD simulations including electric-field bias as the hyperpolarized potential, to illustrate how ligand-binding triggers the cations translocation and initiates the electrical current passing through the channel.

882-Plat Crystal Structure Of A Cyclic Nucleotide Regulated Channel

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Cyclic nucleotide regulated channels are 6 TM channels with a C-terminal cyclic nucleotide binding domain that play important role in sensory systems and cardiac function. There are three families of cyclic nucleotide regulated channels: the hyperpolarization -activated cyclic nucleotide dependent gated (HCN) channels, the cyclic nucleotide gated (CNG) channels and the more recently defined family of prokaryotic cyclic nucleotide regulated channels, which includes the MlotiK1 channel (1,2).

I will present the 3.1 Å crystal structure of the MlotiK1 channel. A comparison of this model with the voltage-gated channel Kv1.2 structure provides new insights about channel gating. I will also present and discuss possible models for the mechanism of ligand-induced activation in this channel.

References

1-. Clayton, G. M., Silverman, W. R., Heginbotham, L., and Morais-Cabral, J. H. (2004). Structural basis of ligand activation in a cyclic nucleotide regulated potassium channel. Cell *119*, 615–627.
2-. Nimigean, C. M., Shane, T., and Miller, C. (2004). A cyclic nucleotide modulated prokaryotic K+ channel. J Gen Physiol*124*, 203–210

883-Plat Gated Access to and Voltage-dependence within the Pore Of Cyclic Nucleotide-Gated (CNG) Channels

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By opening and closing the ion permeation pathway or pore, ion channels regulate ion fluxes across cell membranes. In CNG channels, when this gate is closed, it substantially reduces cation permeation, but does not prevent the access of pore blockers (i. e., tetracaine and quaternary ammonium) to the inner cavity located below the selectivity filter (SF) suggesting that the gate is not at the intracellular end of the pore. Here we tested the hypothesis that the SF is the gate region. We took advantage of silver's (Ag^+) capability to bind irreversibly with single cysteines substituted along the SF. By using electrophysiological measurements from inside-out patches, we found that intracellularly applied Ag^+ can effortlessly access the inward facing end of the SF in both open and closed channels, corroborating that the gate is not intracellular. Strikingly, a position known to be in the lining of the extracellular side of the SF was modified by Ag^+ ions in open, but remarkably low modification in closed channels. The modification rates at this position, but not to the inward facing end, correlate with the relative open channel probability. Our observations indicate a state dependent pattern consistent with gated access at the middle of the selectivity filter. We also tested the voltage-dependence of the modification rates along the pore in open channels. Interestingly, Ag^+ applied intracellularly shows no voltage-dependence accessibility to the inner cavity. While Ag^+ access to the extracellular side of the SF shows stronger voltage-dependence compare to the inward facing end, suggesting that the electrical field drops along the SF. In conclusion, the SF in CNG channels constitutes the primary gate of the pore and concentrates the membrane electrical field in the open conformation favoring ion conduction.

884-Plat Gated Access to the Pore of P2X Receptor Channels

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P2X receptor channels are cation channels that are gated by extracellular ATP. The trimeric architecture makes them unique among the ligand-gated channels. To date, seven mammalian subunits have been cloned ($P2X_1$-$P2X_7$). Each subunit is predicated to have two transmembrane (TM) helices flanking a large extracellular domain that contains the ATP binding site, with the NH_2 and COOH termini on the intracellular side of the membrane. In this study we used the substituted-cysteine accessibility method to explore which parts of the two TMs of the $P2X_2$ receptor channel line the pore and to gain insight as to the location of the gate. Residues in TM1 and in TM2 were individually mutated to cysteine on the background of a channel in which three cysteine residues (one in TM2 and one in each intracellular tail) were mutated to threonine. The rates of MTSET reactivity with introduced cysteine residues were examined in either closed or open state. The results thus far show that only one residue in TM1 is modified by MTSET, while in TM2 seven residues are modified, consistent with TM2 making substantial contribution to lining the pore. The pattern of state-dependent reactivity of cysteines substituted in TM2 and the implication for the location of the gate will be discussed.

885-Plat A Mechanism for pH dependent Gating In Kcsa

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Activation gating in KcsA is elicited by proton binding neat the inner helical bundle (IHB), leading to a widening of the permeation pathway. The molecular entities responsible for proton sensitivity have remained elusive, limiting our understanding of the mechanism underlying channel activation. We have employed a multidisciplinary approach that include site directed mutagenesis, electrophysiological techniques, radiotracer bulk assays, electron paramagnetic resonance spectroscopy (EPR) and X-ray crystallography to dissect the molecular basis of KcsA proton-dependent gating. Here, we show that the proton sensor is self-contained within KcsA structure since we were able to detect opening at the IHB by EPR both in the absence (detergent micelle) and in the presence of a lipid membrane (with and without negative lipid required for channel functioning). C-terminal truncation in KcsA renders fully functional channels, as judged by single channel analyses and spectroscopic evaluation, suggesting that the KcsA pH sensor must be contained at the intracellular part of either transmembrane segment TM1 and/or TM2. Extensive mutagenesis analysis revealed that proton dependent gating in KcsA is driven by a complex set of electrostatic interactions between R117 and E118 at the bottom of the TM2 and by a group of residues lying at the N-terminal part of TM1; H25 being the most important. By combining charge neutralizations at both regions of the channel, we have

created an constitutively open-channel even at pH 9. Using Fab-assisted crystallization methods we have "trapped" the structure of this mutant in a partially open state. Although the activation gate appears to be in the early stages of opening, full opening appears to be inhibited by lattice forces.

886-Plat Structure of Acid-sensing Ion Channel

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Acid-sensing ion channels (ASICs) are ligand-gated ion channels activated by the simplest ligand protons. These voltage-independent sodium channels belong to the epithelial sodium channel/degenerin family of ion channels and are implicated in perception of pain, ischemic stroke, mechanosensation, learning and memory. Here we report the crystal structure of a deletion mutant of ASIC1 from chicken at 1.9 Å resolution and low pH state. The receptor is a homotrimer with a chalice-like shape and is composed of short intracellular amino and carboxyl termini, 2 transmembrane helices per subunit, glycosylated extracellular domains rich in disulfide bonds, and bound chloride ions. The extracellular domain contains grooves enriched in acidic residues and carboxyl-carboxylate pairs within 3 Å, suggesting that at least one carboxyl group bears a proton. Electrophysiological studies on aspartate-to-asparagine mutants confirm that these carboxyl-carboxylate pairs are involved in proton sensing. A disulfide-rich 'thumb' domain lies between the acidic residues and the transmembrane pore, coupling the binding of protons to the opening of the ion channel and demonstrating that proton activation involves long-range allosteric conformational changes.

Platform R: Self Assembled Session: Membrane Dynamics and Biological Function

887-Plat Membrane-active Peptides and Drugs: Kinetic and Equilibrium Experiments

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One of the difficulties in membrane research concerns how to reconcile the results of equilibrium experiments with that of corresponding kinetic experiments. Take the example of membrane-active antimicrobial peptides. One can study the peptide-lipid interactions by examining the physical properties of the peptide-lipid mixtures. With a high degree of precision and reproducibility, we have detected and measured the effect of membrane thinning by peptides, peptide orientation change with concentration, pore formation in fluid membranes, and, more recently, reconstructed the electron density image of a pore from X-ray anomalous diffraction. But pore formation in cell membranes caused by water-soluble peptides occurs as a kinetic process. Typical kinetic experiments are performed with a vesicle suspension, for example measuring the content leakage when peptides are introduced into the suspension. How does one compare the kinetic experiments with the effects seen in peptide-lipid mixtures? In this talk, I will present a solution by the experiment of individual giant unilamellar vesicles (GUVs). We will see that the responses of individual GUVs depend on the poorly understood physics of defect, therefore they are not individually reproducible, contrary to the equilibrium experiments. Nevertheless the responses of individual GUVs reveal information not obtainable by suspension experiments, and they are consistent with the equilibrium results. We will also see this new method can distinguish different membrane-active effects between an antimicrobial peptide and an amphiphilic drug.

888-Plat Influence of Small Drugs on Permeablility and Fluctuation Lifetimes of Membranes

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It has long been known that lipid membranes become more permeable in their chain-melting regime. Unfortunately, examples that document such phenomena are rare and the data quality is often poor. In this work, we monitored membrane vesicle permeation by fluorescence correlation spectroscopy (FCS) and showed that the permeation of fluorescence dyes through such membranes follows the heat capacity profiles of the lipid system. This is due to the known coupling of the heat capacity with the elastic constants that determine the magnitude of the work necessary to create a transient pore. Since anesthetic molecules are able to change the lipid melting profiles they should influence their permeability in a predictable manner. Here, we confirmed these predictions using our FCS permeation experiment. Addition of octanol to lipid membranes is shown to have a significant effect on the permeability, depending on the state of the membranes. Further, when using black lipid membranes (BLM) one finds that permeation events for ions through the membranes appear in quantized steps that resemble those found for ion channel proteins. Both, current intensities and open lifetimes are quite similar to those found for such proteins.

Here we show that the open lifetimes are related to the overall permeability. Using pressure-perturbation calorimetry we found that relaxation processes are proportional to the heat capacity profile. Within the melting transition relaxation processes are slow. The application of anesthetics (and other drugs) has a predictable influence on the relaxation times. Since the pore formation is a consequence of density fluctuations, the relaxation time scales are closely coupled to pore open times.

As a result, we find a self-consistent coupling of membrane permeability and the open lifetimes of lipid pores, and a coherent and predictable effect of anesthetic and other drugs.

889-Plat Additive-induced Domain Formation In Membranes

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