each measurement. This knowledge is modeled by a multivariate normal distribution of the ratio of channels in each possible pair of starting and ending states, the later predicted by the kinetic model under test. By using Bayes theorem we calculate the posterior distribution that result after taking into account the current measurement and we calculate the partial likelihood of each measurement. The distribution of channels at the end of the measurements is then used to calculate the prior distribution of starting state-ending state pair of the next measurement interval.

We present a reliable approximation to the likelihood function that opens the door to several possibilities: a) estimate the kinetic parameters that best represent the experimental data with their error rates, b) to choose between alternative kinetic models and c) to optimize the experimental protocols.

1486-Pos Board B396

Effect of a Temperature Increase in the Non-Noxious Range on Proton-Evoked ASIC and TRPV1 Activity

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Acid-sensing ion channels (ASICs) are neuronal H+-gated cation channels, and the transient receptor potential vanilloid 1 channel (TRPV1) is a multimodal cation channel activated by low pH, noxious heat, capsaicin, and voltage. ASICs and TRPV1 are both expressed in sensory neurons. It has been shown that raising the temperature increases TRPV1 and decreases ASIC H+-gated current amplitudes. To understand the underlying mechanisms, we have analyzed ASIC and TRPV1 function in a recombinant expression system and in dorsal root ganglion (DRG) neurons at room and physiological temperature. We show that in this range, the temperature does not affect the pH dependence of ASIC and TRPV1 activation. A temperature increase induces, however, a small alkaline shift of the pH dependence of steady-state inactivation of ASIC1a, ASIC1b, and ASIC2a. The decrease in ASIC peak current amplitudes at higher temperatures is likely in part due to the accelerated open channel inactivation kinetics and for some ASIC types to the changed pH dependence of steady-state inactivation. The increase in H+-activated TRPV1 current at the higher temperature is at least in part due to a hyperpolarizing shift in its voltage dependence. ASIC and TRPV1 currents of DRG neurons are similarly regulated by temperature as the cloned channels, with the exception that the decrease in peak ASIC current amplitudes at 35°C is more pronounced in DRG neurons. The H+-evoked depolarization measured under current-clamp was significantly reduced at 35°C for a sub-population of ASIC channels, without however affecting the number of action potentials. Our study shows that the contribution of TRPV1 relative to ASICs to H+-gated currents in DRG neurons increases with higher temperature and acidity. Still, ASICs remain the principal pH sensors of DRG neurons at 35°C in the pH range >=6.

1487-Pos Board B397

The Role of the Cytoplasimic Domain in pH-Dependent Gating by the KCSA Channel

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Ion channels are membrane-spanning proteins that regulate ion flow across the membrane by responding to environmental changes. These changes are sensed by a sensor region, which then conveys the information to the pore region, which induces channel opening or closing. However, the mechanism for this conveyance remains unknown.

The KcsA channel is a representative potassium channel activated by protons (changes in pH). It is a tetramer with four homologous subunits, each of which has two transmembrane segments which form a transmembrane pore, an α-helix at the N terminus, and a cytoplasmic domain made of 35 amino acids at the C terminus. Studies have argued the region that senses protons and regulates gating is entirely within the transmembrane segments. However, we have found that the cytoplasmic domain may also be involved in these two functions.

Here, we show that the charged amino acids in the cytoplasmic domain, which makes up about half of this domain's amino acids, play an important role in pH-dependent gating. To investigate their effects, we made these amino acids neutral and measured gate activity. The mutant channel could be activated at what is normally inactive pH. This suggests that charges on the cytoplasmic domain generate electrostatic repulsion or attraction within the tetramer and influence KcsA channel activation in response to pH changes.

1488-Pos Board B398

Molecular Interactions Involved in KCSA pH Gating

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The bacterial potassium channel KcsA is gated by high concentrations of intracellular protons, allowing the channel to open at pH < 5.5. Replacing key ionizable residues from the N and C termini of KcsA with residues mimicking their protonated counterparts with respect to charge renders the channel open up to pH 9.0 (Thompson et al. 2008). We proposed that these residues function as the proton-binding sites. At neutral pH they form a complex network of inter- and intrasubunit salt bridges and hydrogen bonds near the bundle crossing, stabilizing the closed state. At acidic pH, these residues change their ionization state, thereby disrupting this network, favoring channel opening. While our previous work identified a network of residues involved in pH sensing, it did not rigorsously dissect the interactions that govern channel opening. To this end, we performed a series of single and pairwise mutations of the residues in the pH sensor. Using electrophysiology and X-ray crystallography we hope to gain a deeper insight into the mechanism of proton dependent gating in KcsA.

1489-Pos Board B399

Binding of Isoflurane to Glic Alters the Structure and Dynamics of the Protein

Dan Willenbring, Lu Tian Liu, Yan Xu, Pei Tang.

General anesthetics are thought to interact with proteins to produce anesthesia. Most of the experimental efforts to date have been focused on searching for discrete anesthetic binding sites. How exactly anesthetics work after the events of anesthetic binding is rarely explored. With the recent availability of two crystal structures for the bacterial Gloeobacter violaceus pentameric ligand-gated ion channel (GLIC), which is sensitive to a variety of general anesthetics, we performed multiple replica molecular dynamics simulations for more than 100 ns with and without the general anesthetic isoflurane. Multiple sites within the protein, primarily in the extracellular domain and at the interface of extracellular and transmembrane domains, provided favorable binding environments for isoflurane, showing little isoflurane translational displacement over the course of the simulations. Isoflurane caused changes to the structure and dynamics of GLIC when compared to the control simulation. Subunit-subunit interactions in the extracellular domain of the pentamer were disrupted, resulting in increased flexibility of the subunit pairs, as measured by Gaussian network model analysis. Salt-bridge linkages between and within the extracellular region of subunits containing isoflurane binding sites were altered by the presence of isoflurane, reflecting a change in the tertiary and quaternary structure of the channel. Changes were not isolated to the extracellular domain. The subunits containing more isoflurane binding sites also showed a greater change in tilt angle of the second transmembrane helices. Taken together, anesthetic binding at subunit interfaces can lead to changes in quaternary structure and global dynamics, causing allosteric changes in channel motional characteristics critical to gating. Supported by the NIH (R01GM56257, R01GM66358, R37GM049202, and T32GM075770), and the NSF through TeraGrid resources provided by PSC, NICS and TACC (TG-MCB050030N).

Acetylcholine Receptors

1490-Pos Board B400

Ligand-Induced Internal Molecular Dynamics of Nicotinic Acetylcholine Receptor Analysis by Diffracted X-Ray Tracking

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The nicotinic acetylcholine receptor (nAChR) is a pentameric ligand-gated ion channel in the central and the peripheral nervous system. The gating mechanism of nAChR is still unclear since the structure of nAChR in the presence of acetylcholine (ACh) has not been determined yet. Additionally, single molecular dynamics of nAChR in the presence of acetylcholine was not observed.

In this study, diffracted X-ray tracking (DXT) was applied for nAChR system. DXT is a method to track the X-ray diffraction spot from the gold nanocrystal labeled on an individual single protein and enables us to observe intermolecular dynamics of the protein in real time and real space. Dynamic twisting motions upon gating of KcsA, a pH sensitive potassium channel, were successfully revealed by the DXT [Shimizu *et al*. (2008). Cell *132*, 67-78]. At first step of new experiments, acetylcholine-binding protein (AChBP) was used as a model system of nAChR. AChBP is a structural and functional homologue of extracellular ligand-binding domain of nAChR. We investigated internal motions of AChBP by DXT in the absence and the presence of ACh, and found that ACh significantly activated the motion of AChBP. This result indicates that the ligand binding may initiate vigorous molecular fluctuations in AChBP, which was also confirmed by molecular elasticity measurement of AChBP with atomic force microscopy (AFM).

In order to apply the DXT for the full length of nAChR, technological innovations will be incorporated into the DXT, such as efficient preparations of gold nanocrystals and speedup of the time resolution of DXT (5-10 μs). Additionally, we can control the orientation of adsorbed nAChR. The improvement of our technology and experimental results will be discussed.