inactivation process in WT channels. To investigate the structural changes that underlie the unusual behaviour of this mutant, we performed a series of MD simulations of the pore domain of WT hKv1.5 and T480A. Analysis of the trajectories shows that T480A affects the stability and flexibility of the filter region and the surrounding pore loop. These results show that residue T480 (located outside the pore region that determines the integrity of the selectivity filter) affects the stability of the filter and influences C-type inactivation.

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Free Energy Landscape for the Inactivation of the KcsA Potassium Channel

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The potassium ion channel, KcsA, gates the passage of ions through cell membranes in response to a change in pH. Recent experimental results have demonstrated the existence of two gates in KcsA: an intracellular gate and a gate at the selectivity filter. Lowering the pH opens the intracellular gate allowing ions to pass. After a period of time, however, the channel inactivates by constricting the selectivity filter and impeding the flow of ions even though the bottom gate remains open. We have used path-based molecular dynamics simulations to probe the detailed mechanism of this phenomenon by finding dynamical pathways to inactivation in KcsA. We have computed free energies and rates of inactivation that agree with recent experimental results. We also provide a molecular rationalization for the coupling between the opening and closing of the lower gate and the inactivation of the selectivity filter.

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Influence Of The Kcsa C-terminal Domain In The Coupling Between Activation And Inactivation Gates

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Truncation of KcsA C-terminal domain (CTD) been reported to impair ion channel activity 1. However, we have shown that a KcsA lacking the CTD (KcsA-ΔCTD) is capable of catalyzing pH-dependent rubidium influxes 2.To investigate the functional and structural roles of KcsA CTD in channel gating, we have studied pH-dependent structural changes of the activation gate by EPR and Fluorescence spectroscopy in full length (FL) and ΔCTD KcsA. Proton-dependent macroscopic currents of KcsA-ΔCTD inactivated faster and deeper when compared to the FL channel. Additionally, single channel analysis showed that at steady state KcsA-ΔCTD has an open probability Po not higher than ~ 0.001, about one order of magnitude lower than FL-KcsA. Recently, by solving a family of KcsA-ΔCTD open structures we have proposed the mechanism by which the activation gate is allosterically coupled to the selectivity filter. As a result, we have hypothesized that a larger opening at the activation gate in KcsA-ΔCTD is directly correlated with an enhancement in the rate of inactivation. Distances estimated by fluorescence resonance energy transference (FRET) indicates that KcsA-ΔCTD activation gate opened to a larger extent than that in FL-KcsA, thus strengthening the coupling between activation and the collapse of the selectivity filter. Our x-ray structures of closed and open FL-KcsA in addition to the KcsA-ΔCTD in the open conformation are in agreement with a mechanistic model where the larger the opening at the activation gate the deeper inactivation at the selectivity filter.

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3393-Pos Board B440

On the Structure-Function correlates of Ion Occupancy and modulation of C-type Inactivation

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KcsA, a proton activated K channel, has served as an archetypical K pore providing molecular insights into understanding selectivity, ion-permeation, gating and pore-blocking. A recent set of crystal structures describing the mechanism of C-type inactivation in this channel now allows for an understanding this mechanism at atomistic level. Our results show that KcsA inactivation is strongly coupled to the opening of the activation gates and is modulated by the amount and direction of current passing through the channels. As also implicated by studies in eukaryotic channels, C-type inactivation in KcsA involves an intimate interplay between the selectivity-filter region and permeant-ions. This study attempts at further understanding this close association between the ion and filter by correlating high resolution structures with macroscopic and single- channel functional data. We have obtained several KcsA crystal structures of the closed and the open mutant channel, in the presence of different permeant ions (K+, Rb+, Cs+ and NH4+) and blockers (Ba2+ and TEA). These structures reveal different ion occupancies depending upon the nature of the permeant ion, blocker and the extent of channel opening. Analyzed in the light of extensive functional evidence, these results uncover several important features of the interplay between ion interactions and the evolution of C-type inactivation.

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Nanoplasmonic Fluorescence Enhancement Applied to Study of Ion Channels

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A metal nanoparticle can act as an antenna capable of increasing both the excitation rate and quantum yield of a fluorophore in close proximity to the nanoparticle. These properties thus enhance the fluorescence yielding an increase in brightness and decrease of the photobleaching rate - a highly useful tool in fluorescence studies of membrane proteins from the macroscopic to single molecule level. We have applied this technique to image purified membrane proteins in supported bilayer. The biological sample is purified KcsA K-channels labeled with TMR-6-M in the bundle crossing and reconstituted as proteoliposomes. We have also studied the membrane fluorophore DiI C18 in supported bilayer as a control. Among the many approaches towards fabrication of effective nanoparticles, we have synthesized spherical silver nanoparticles of ~100 nm diameter coated with a thin SiO2 outer layer (Ag@SiO2). We have explored different size particles and various SiO2 thicknesses to find experimental conditions for optimal fluorescence enhancement. The SiO2 layer provides protection from chemical attack, acts as a spacer layer to avoid direct metal-fluorophore quenching, and allows surface functionalization. We have conjugated silica-coated silver particles to glass coverslips via polylysine (PL) in order to achieve a high-density silver nanoparticle monolayer. We record fluorescence from the labeled ion channels in an inverted TIRF microscope configuration imaged with a high-speed EMCCD camera. KcsA proteoliposomes are added to an Ag@SiO2-PL coverslip surface to rupture as supported bilayer patches for single molecule imaging. DiI liposomes were used in the same way. The KcsA-TMR and DiI samples show enhancement of at least 4-fold and 10-fold, respectively, compared to the same sample without nanoparticles. These results demonstrate the utility of this technique in fluorescence studies of ion channels or other membrane proteins. Supported by NIH 1R21MH078822 & 1F31NS054532.

3395-Pos Board B442

KcsA Gating Explored with Quaternary-Ammonium Blockers

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The bacterial potassium channel KcsA, an archetypal K^+ channel pore, is proposed to close at an intracellular constriction. The inner helices form a bundle crossing that separates the intracellular solution from a large, hydrated internal vestibule within the pore domain (Doyle et al. Science, 1998). This vestibule has been shown to be the receptor-site for open-channel blockers such as quaternary-ammonium ions in KcsA and other voltage-gated potassium channels (Armstrong and Hille, JGP, 1972; Holmgren et al. JGP, 1997; Zhou et al. Nature, 2001; Lenaeus et al. NSMB, 2005; Yohannan et al. JMB, 2007). Since KcsA is gated by intracellular protons, it is predicted that pH will dramatically alter the accessibility of channel blockers to the vestibule. We are exploring the state-dependence of channel block by quaternary-ammonium ions using steady-state single channel recording of the non-inactivating KcsA E71A channel (Cordero-Morales et al. NSMB, 2006). Preliminary results indicate a profound state-dependence, with TBA blocking kinetics and percent block changing dramatically as a function of channel open probability. We will compare these results with blocking data for a pH-insensitive KcsA mutant we previously reported (Thompson et al. PNAS, 2008). These results demonstrate that the pH-sensor of KcsA operates to gate ion access to the vestibule.

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Stability And Conductance Assessment Of A Putative Low-k+ Inactivated State Of The Kcsa Channel

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Potassium channels constitute a large family of proteins, notably involved in the regulation of the activity of excitable cells. The channels partly exert that function by varying their conductance through a mechanism known as C-type inactivation: Shortly after the activation of K+ channels, their selectivity filter stop conducting ions at a rate that depends on various stimuli. This