combination with a previously available variant, show folding kinetics that is in correspondence with the results obtained from autocorrelation analysis of single-trajectory full-atom molecular dynamics simulations for a similar mutant. Closer investigation of existing all-atom computational data suggests that helix 2 of lambda 6-85 is involved in a short-lived off-pathway trap, which is in agreement with experimental data. Our work demonstrates that a match between fast protein folding experiments and molecular dynamics simulations can be extended to several reaction coordinates to obtain experimental confirmation of deviations from two-state folding behavior even for very simple folding reactions. As computation becomes more affordable, it will be possible to simulate both the new probes and any mechanistic deviations that insertion of the probe causes in experiment.

1747-Plat
Photobleaching and Stability of Red Fluorescent Proteins
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Fluorescent proteins (FPs) are used routinely to visualize structural organization and protein dynamics. Compared with other mutants, there is a large demand for red FPs (RFPs) due to the higher transparency of cells and tissue at longer wavelengths. However, the application of RFPs is limited by the increased susceptibility to photobleaching [1]. A possible mechanism for this may be a decrease in structural stability of the beta-barrel, leading to dark state conversion of the chromophore or oxygen access. To characterize the relation between photostability and structural stability, we use temperature-dependent terahertz (THz) time-domain spectroscopy [2]. Temperature dependent terahertz absorbance measurements were made between 80-270K as a function of photobleaching for mCherry, TagRFP-T and mOrange2 RFP samples. We find that: 1) the absolute THz response follows the thermal stability, as defined by the melting temperature; 2) the protein dynamical transition temperature [3] also follows the thermal stability; 3) the thermal stability increases with photobleaching and 4) the photostability does not follow the thermal stability. The THz sensitivity to thermal stability is verified by CD measurements. The higher stability with photobleaching is surprising, but could possibly be a driving force toward the photobleached state. Our result provides additional insight into photobleaching mechanism, and introduces a way to estimate the qualities of FPs.

Platform: Voltage-gated K Channels II

1748-Plat
Emerging Role for KCNQ1 in Ischemia-Induced Neuronal Death
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The slowly activating component of the delayed rectifier potassium current, IKs, composed of KCNQ1-KCNE1 channel subunits, is an important contributor to normal cardiac repolarization. Emerging evidence indicates that KCNQ1 subunits are also expressed in brain, but its precise physiological role in neuronal function has not been investigated. Potassium channels are key players in ischemia-induced death of hippocampal CA1 pyramidal neurons which, in turn, leads to impaired cognition. REST, a gene silencing transcription factor, is up-regulated in global ischemia and contributes to neuronal death. Recently we showed that REST is enriched at the promoter of the KCNQ1 channel and that KCNQ1 mRNA and protein expression are increased in post-ischemic CA1 neurons. Additionally, functional KCNQ1 channels were identified in cultured neurons using KCNQ1-specific inhibitors Chromanol 293B and JNJ-303 and induction of oxygen-glucose deprivation (OGD) induced a marked increase in KCNQ1 currents (~34%, $p > 0.02$), evident at 48 h post-ischemia. To assess a possible role for KCNQ1 in ischemia-induced neuronal death, we induced OGD in dissociated hippocampal neurons in the absence and presence of Chromanol 293B (100 μM) and treated with propidium iodine. Inhibition of KCNQ1 markedly diminished cell death, assessed at 24 h (21%, $p > 0.002$) and 48 h (28%, $p > 0.001$) suggesting that an up-regulation of KCNQ1 channels may contribute to ischemia-induced neuronal death. To examine a possible role for REST in ischemia-induced up-regulation of KCNQ1, we performed single-locus ChIP which revealed enrichment for REST by ~2-fold and a decrease in trimethylation of lysine 27 on histone 3 (H3K27me3), an epigenetic mark of gene repression at the KCNQ1 promoter, consistent with the increase in KCNQ1 expression after ischemia. Our findings reveal, for the first time, a role for REST in KCNQ1 expression in response to ischemic insults.

1749-Plat
Spectroscopic and Biochemical Studies of TRIP8b Regulation of HCN Channels
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TRIP8b, an accessory subunit of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, alters both cell surface expression and cyclic nucleotide dependence of these channels. The mechanism through which TRIP8b exerts these dual effects is still poorly understood. Besides binding the terminal three residues of HCN channels, TRIP8b also binds directly to the cyclic nucleotide-binding domain (CNBD). A small central portion of TRIP8b, termed $TRIP8b_{core}$, is involved in this interaction. Binding of $TRIP8b_{core}$ to the CNBD dramatically reduces the effects of cAMP on the channel. Using spectroscopic and biochemical techniques, we sought to understand how and where TRIP8b binds to the CNBD and how it reduces the cyclic nucleotide dependence of HCN channels. To closely examine the binding of $TRIP8b_{core}$ to the CNBD, we used double electron-electron resonance (DEER) at Q-band frequencies to study conformational changes in the soluble CNBD of HCN2 in the presence of TRIP8b and cAMP. We show that the overall structure of the TRIP8b bound conformation of the CNBD closely resembles the apo state. In addition, we use DEER between the CNBD and $TRIP8b_{core}$ and nuclear magnetic resonance (NMR) to localize the binding site for TRIP8b on the CNBD. Finally, to understand the mechanism of TRIP8b inhibition of cAMP regulation of HCN channels, we performed binding studies of cAMP and TRIP8b on the CNBD and developed a multi-state mathematical model to explore 1) whether cAMP and TRIP8b can bind simultaneously to HCN channels, 2) whether TRIP8b reduces the affinity for cAMP binding, and 3) whether TRIP8b reduces the effect of cAMP on HCN channels by preventing the structural rearrangements associated with channel opening.

1750-Plat
Epilepsy Related Slack Channel Mutants Lead to Channel Over-Activity by Two Different Mechanisms
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So far, 12 sodium-activated potassium channel (KCNT1, Slack) genetic mutants had been found in severe early-onset epilepsy patients by whole genome sequence. However, the biophysical properties change of these mutated Slack channels and the underlying mechanisms had not been fully investigated. In this abstract, we first measured the KD value of sodium sensitivity of these mutant channels by inside-out patch. We found that two mutants (R409Q and R455H) on the RCK1 domain and one mutant (Y775H) on the RCK2 domain decreased the KD value of sodium sensitivity. Furthermore, we found that the double mutant (R455H/R409Q) on RCK1 domain can decrease KD value of sodium sensitivity further, reflecting these mutants allosterically regulated sodium affinity of Slack channel. In addition, electrophysiology and molecular simulation reflected the mutant Y775H may directly facilitate sodium binding of Slack channel. Second, single channel recording data revealed that all mutants lead to over-activity of Slack channel even if they did not change the sodium sensitivity. We set up a two-step activation model to explain the gating mechanism change and concluded that these mutant channels lead to channel over-activity can be categorized as two different mechanisms.

1751-Plat
Quantum Calculations Show a Water Column in a Potassium Ion Channel Pore, and its Role in Gating and Conduction
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A continuous water column is shown to exist in the pore of a potassium channel. Quantum calculations (HF/6-31G*) were performed on the Kv1.2 channel (pdb:2A79/3Lut), giving the structure of the pore with 50 water molecules. Calculations were done with 0, 1, or 2 ions. The protein from the PVPV intracellular gating region to the entrance to the selectivity filter, including the tyrosine of the TVGYG sequence (≈14.1 Å) was included, with water extending slightly past the region. Results show how the ion(s) restructure(s)