photon emission from single molecules. This technique enabled us to dissect the spatial relationships between the neuronal SM protein Munc18-1 and the SNARE proteins syntaxin-1 and SNAP-25 at nanometer scales. Strikingly, we observed syntaxin-1 and SNAP-25 extensively associating with Munc18-1. Rescue experiments with syntaxin-1 mutants revealed that Munc18-1 recruitment to the plasma membrane depends on the Munc18-1 binding the N-terminal peptide of syntaxin-1, and occurs through interactions with the open conformation of syntaxin-1 that is permissive to SNARE-complex assembly. Our results, corroborated by biochemical and physiology experiments, provide unexpected insights into the functional association of SM and SNARE proteins on the neuronal plasma membrane, suggesting a general mechanism by which recruitment of an SM protein to an on-pathway SM-SNARE tri-partite complex sets the stage for membrane fusion reactions. Our high-resolution imaging approach additionally provides a novel framework for investigating interactions between the fusion machinery and other sub-cellular systems in situ.

1004-Symp
Contribution of Potassium Channels and Calcium-Activated Chloride Channels to Neuronal Signaling
Lily Jan.
Univ of California, San Francisco, San Francisco, CA, USA.
In a long-term collaboration with Yuh Nung Jan, we have taken the approach of starting our ion channel studies with molecular identification so that we can study one type of ion channel at a time. Recent studies of the contribution of potassium channels and calcium-activated chloride channels to neuronal signaling will be presented.

Platform: Voltage-gated K Channels: Gating

1005-Plat
Voltage-Dependent Gating in MthK K^+ Channels occurs at the Selectivity Filter
David J. Posson, Jason G. McCoy, Crina M. Nimigean.
Weill Cornell Medical College, New York, NY, USA.
The determination of how ion channels open and close is crucial for understanding their physiological roles and for potential therapeutic modulation, but locating the gate within channels is not trivial. For voltage-gated Kv channels, a primary activation-gate formed by a helical bundle crossing near the intracellular pore entrance has been established. For several types of ligand-gated channels (CNG) and large-conductance Ca^{2+}-activated K^+ (BK) channels however, the K^+ selectivity filter has been proposed to act as the conduction gate. We investigated the location of the voltage-dependent gate for the prokaryotic MthK channel, a BK channel homologue lacking voltage-sensor domains, using quaternary ammonium (QA) blockers as state-dependent probes. Intracellular QA blockers bind within an aqueous pore cavity between the putative intracellular-facing gate and the selectivity filter. Thus, these blockers ideally probe the gate location: an intracellular gate will only allow binding when open, whereas a selectivity filter gate will always allow binding. A kinetic analysis of tetrabutylammonium block of single MthK channels during gating determined that the voltage-dependent gate is located above the QA binding site in the pore. X-ray crystallographic analysis of the MthK pore with tetrabutylantimony confirmed QA binding immediately below the selectivity filter, unequivocally placing the voltage-dependent gate within the selectivity filter, akin to the C-type inactivation gate in eukaryotic K^+ channels. In addition, state-dependent binding kinetics suggested that the selectivity filter inactivation led to conformational changes within the cavity and intracellular pore entrance, suggesting an allosteric connection between cytoplasmic domains and selectivity filter.

1006-Plat
Voltage Sensor Trapping in the Relaxed State
Shobana Sundaram, **Carlos A. Villalba-Galea**.
Virginia Commonwealth University, Richmond, VA, USA.
The voltage sensitive phosphatase from Ciona intestinalis (Ci-VSP) has constituted an intriguing model for the study of the dynamic of voltage sensing domains (VSD). Four of the five arginines in the fourth (S4) segment of the VSD function as charges sensing the difference in electrical potential across the membrane. The voltage-driven movement of the S4 segment towards the extracellular space triggers relaxation which is characterized by a shift in voltage dependence for the movement of charges to more negative values. The mechanism for relaxation remains unclear. However, it is thought to encompass the rearrangement of the VSD to satisfy the new position of the S4 segment following activation. In this view, changing the membrane potential from negative to positive voltages drives the VSD from the resting to the active state. As the S4 segment moves, the VSD gains potential energy, part of which is dissipated during a voltage-independent transition leading the VSD to the relaxed state. Replacement of the fourth arginine to a histidine (R232H) causes the VSD of Ci-VSP to display a ''pump-like'' behavior, which differs from the ''transporter-like'' behavior observed in the mutant R371H of Shaker. The cycling of this ''pump'' is driven by relaxation. Furthermore, we found that the net sensing charge of the mutant R232H reversibly seemingly decreases over 60% during this process. We concluded that after relaxation, the histidine in position 232 is deprotonated and ''trapped'' within the VSD without net charge. Similar observations are made using Molecular Dynamics simulation of the Ci-VSP voltage sensor bearing the mutation R232H. We propose electrically driving the VSD back to the resting state is inefficient after neutralizing the histidine R232H. Thus, recovery from relaxation last several seconds, diverging in two orders of magnitude the recovery observed with the native arginine.

1007-Plat
Structural Mechanism of Voltage-Dependent Gating in an Isolated Voltage-Sensing Domain
Qufei Li[1], Sherry Wanderling[1], Marcin Paduch[1], David Medovoy[1], Carlos Villalba-Galea[2], Raymond Hulse[1], Benoit Roux[1], Anthony Kossiakoff[1], Eduardo Perozo[1].
[1]University of Chicago, Chicago, IL, USA, [2]Virginia Commonwealth University School of Medicine, Richmond, VA, USA.
The voltage-sensing domain (VSD) is a common scaffold responsible for the transduction of transmembrane electric fields into protein motion. They play an essential role in the generation and propagation of cellular signals driven by voltage gated ion channels, voltage sensitive enzymes and proton channels. All available VSD structures are thought to represent the activated conformation of the sensor due to the overall structural similarities and the mid-point of the voltage dependence of activation curves. Yet, in the absence of a resting state structure, the mechanistic details of voltage sensing remain controversial. The voltage dependence of the VSD from Ci-VSP (Ci-VSD) is dramatically right shifted, so that at 0 mV it presumably populates the putative resting state. We have determined crystal structures of the Ci-VSP voltage sensor in both active (Up) and resting (Down) conformations, between which the S4 undergoes a ~5 Å displacement along its main axis with an accompanying 55-90° rotation resembling the basic helix-screw mechanism of gating. In the process, the gating charges change position relative to a ''hydrophobic gasket'' that electrically separates intra and extracellular compartments. This movement is stabilized by an exchange in countercharge partners in helices S1 and S3, for an estimated net charge movement of ~1 e_o. EPR spectroscopic measurements confirm the limited nature of S4 movement in a membrane environment. These results provide an explicit mechanism of voltage sensing in diverse voltage dependent cellular responses.

1008-Plat
Tracking S4 Movements by Metal-Ion Bridges
Jakob Renhorn, Fredrik Elinder.
Department of Clinical and Experimental Medicine, Linköping, Sweden.
Electrical signalling in excitable cells depends on voltage-gated ion channels which open and close in response to changes in membrane potential. The voltage-gated ion channel consists of a central ion-conducting pore domain surrounded by four voltage-sensor domains (VSDs). The VSDs sense changes in membrane potential and confer this information to the pore domain. The fourth segment (S4) of each VSD carries several positively charged residues which gives the VSD its gating ability. S4 must traverse outwards through the membrane electric field in order for the channel to open. The open-state structures of both K and Na channels are known at atomic level through x-ray crystallography. In a previous investigation (Henrion et al., 2012, PNAS 109:8552-8557) we described four closed molecular configurations of a VSD based on 20 engineered metal-ion bridges, Rosetta modelling and molecular dynamics. A subset of these interactions was used to generate a detailed model of the intermediate conformations during VSD gating. Our results suggested that S4 slides >12 Å along its axis during gating. Whether or not S4 continues to move after channel opening and during inactivation is not clear. Therefore, in the present study, we used the same technique, with double cysteine mutations and Cd ions, to explored molecular rearrangements in the activated state. Mutated Shaker K channels were expressed in Xenopus oocytes and studied by the two-electrode voltage-clamp technique. We found several new metal ion bridges suggesting possible S4 movements in the activated state.