dynamics of CFTR under control conditions and during PKC stimulation while simultaneously monitoring CFTR expression at the cell surface as total fluorescence intensity. During PKC stimulation, CFTR became aggregated into large (1 −2 μm diameter) structures on the plasma membrane called platforms. PKC induced a significant (1.5-fold) increase in CFTR surface expression and increased the CFTR confinement within platforms, as indicated by a decline in its confined diffusion coefficient from $0.011 \pm 0.001$ μm2/s to $0.0033 \pm 0.0002$ μm2/s. Pharmacological inhibition of ROS or acid sphingomyelinase (ASM) prevented the PKC-induced aggregation and tethering of CFTR. This suggests a scheme in which PKC stimulates the production of ROS, which activates ASM and ceramide synthesis, leading to the formation of platforms in the outer leaflet of the plasma membrane. Ceramide platforms may help stabilize CFTR in the membrane so that its surface expression and channel function are increased during secretagogue stimulation.

**633-Pos Board B413**

**Refinement and Evaluation of a CFTR Homology Model and Identification of Residues Controlling Channel Gating**

Gorman Stock[1], Guiying Cui[2], Nael A. McCarty[2], **James C. Gumbart**[3].
[1]Chemistry, Georgia Institute of Technology, Atlanta, GA, USA, [2]Pediatrics, Emory University, Atlanta, GA, USA, [3]Physics, Georgia Institute of Technology, Atlanta, GA, USA.

Cystic Fibrosis, which affects nearly 1 in 2,500 births in the Caucasian population, is caused by various mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) protein. CFTR is a member of the diverse ATP-binding-cassette (ABC) protein superfamily. While most ABC proteins are transporters, CFTR alone has evolved to function as an ion channel. Currently no crystal structure of full length CFTR exists, but due to its biological relevance, many CFTR homology models have been proposed. Using a recently published and experimentally supported model of CFTR, we have carried out all-atom Molecular Dynamics (MD) in an attempt to further characterize the dynamics of this recalcitrant protein. First, we refined the model utilizing MD flexible fitting (MDFF) and structural data from a 9-Å cryo-EM map of CFTR (Rosenberg et al., 2011). Next, equilibrium MD simulations of the model within a membrane were used to probe 3 physically relevant states: the apo state, the proposed semi-apo state (1 ATP bound), and the bound state (2 ATP bound). Initial results show a difference in dimerization in the nucleotide-binding domains (NBDs) between the three states. In other experiments, residues in CFTR's NBDs indicating sequence divergence from the canonical ABC protein sequence (suggesting that they may be involved in slowing the ATP hydrolysis rate, which would likely support longer open durations) were returned to the canonical sequence. Burst durations (in ms) measured for single-channels expressed in oocytes were WT-CFTR (683 ± 59), S573E-CFTR (427 ± 83)*, G576S/Y577A-CFTR (431 ± 46)*, (* = $p < 0.05$). These results suggest that CFTR's NBDs evolved in a manner that would support the shift from transporter to channel function. (Support: 5R01-DK056481).

**634-Pos Board B414**

**Solution NMR to Investigate Gating in the NaK Channel**

**Joshua Brettmann**, Andrew Meiburg, Katherine Henzler-Wildman.
Biochemistry and Molecular Biophysics, Washington University, St Louis, MO, USA.

The NaK channel is small bacterial non-selective cation channel that has proved to be an excellent model system to study basic biochemical and biophysical question concerning ion channels. NaK has been crystalized in the closed state and as an open channel using a truncated construct (NaKΔ19) missing the M0 helix. NaK shares a homologous architecture to tradition K+ channel pores such as KcsA, but it is non selective. However, small mutation in the selectivity filter can change NaK into a K+ selective channel. Solution NMR spectra of NaK solubilized in isotropic bicelles show superior spectral resolution, allowing for detailed solution NMR studies of NaK structure and dynamics in bicelles. Regulation of NaK is currently unknown, however the location of the amphipathic M0 helix on the membrane surface packed against two adjacent monomers makes it a likely contributor to channel gating. Preliminary work suggests that the full length NaK channel exists in at least two states in slow exchange at 45oC. Mutations to the M0 helix shift the equilibrium to a single population, supporting a role for the M0 helix in determining the structural and functional state of the channel. NaKΔ19 NMR spectra are also sensitive to addition of amino acids just before the M1 helix. This suggests that this region connecting the M0 helix to the M1 helix is important in determining NaK dynamics. Comparison of our results with functional studies highlights specific interactions between the M0 helix and the pore domain that determine the structural and functional state of the channel.

**635-Pos Board B415**

**A New Class of Positive Gating Modulators of hKv3.2 Channels: Insights into the Mechanism of Action**

**Qiansheng Liang**[1], Giuseppe Alvaro[2], Charles Large[2], Manuel Covarrubias[1].
[1]Thomas Jefferson University, Philadelphia, PA, USA, [2]Autifony Therapeutics Limited, London, United Kingdom.

Neuronal $K_V3$ channels are implicated in neurological (tinnitus, hearing loss) and psychiatric (schizophrenia) disorders, and are potential therapeutic targets. Here, we investigated two new compounds (AUT3 and AUT5) developed by Autifony Therapeutics, which may act as specific modulators of this $K^+$ channel sub-family. Initial screening of heterologously expressed $K_V$ channels in *Xenopus* oocytes demonstrated that AUT5 acts as a relatively selective positive modulator of $hK_V3.2$. AUT5 (2 μM) induces a substantial parallel negative shift in the conductance-voltage relation ($\Delta V_{1/2} = -24.1 \pm 2.0$ mV, n = 9). However, 2 μM AUT5 has little to no effect on Kv1.2, Kv3.4, Δ28-Kv3.4 and Kv4.2. The positive modulatory effect on hKv3.2 is concentration-dependent with an EC50 = 2 – 4 μM. AUT3 also acts as a positive modulator of $hK_V3.2$, albeit the effect is ≥ 10-fold weaker. Toward investigating the mechanism of action, we examined tail current kinetics and found a dramatic slowing of deactivation with 2 μM AUT5 ($\tau_a = 24.7 \pm 6.2$ ms vs $6.5 \pm 1.1$ ms of control at −50 mV, n = 6). We observed similar positive modulation by AUT3 and AUT5 in mammalian cell lines (HEK-293 and stably transfected cells). We hypothesize that a direct interaction between AUT compounds and the channel's activation machinery favorably affects the energetics of $hK_V3.2$ activation gating. To test this hypothesis, we are currently investigating possible effects on voltage sensor movements, extending the screening to additional Kv channels, and searching for putative binding sites in the Kv3.2 protein.

**636-Pos Board B416**

**PKA Reduces the Rat and Human KCa3.1 Current, CaM Binding and $Ca^{2+}$ Signaling, which Requires Ser332/334 in the CaM-Binding C Terminus**

**Raymond Wong**, Lyanne C. Schlichter.
University of Toronto, Toronto, ON, Canada.

The $Ca^{2+}$-dependent $K^+$ channel, KCa3.1 (*KCNN4*/IK/SK4), is widely expressed and contributes to cell functions that include volume regulation, migration, membrane potential and electrical excitability. KCa3.1 is now considered a therapeutic target for several diseases, including CNS disorders involving microglial activation; thus we need to understand how KCa3.1 function is regulated. KCa3.1 gating and trafficking require calmodulin binding to the two ends of the CaM-binding domain (CaMBD), which also contains three conserved sites for Ser/Thr kinases. While cAMP protein kinase (PKA) signaling is important in many cells that use KCa3.1, reports of channel regulation by PKA are inconsistent. Here, I first compared regulation by PKA of native rat KCa3.1 channels in microglia (and the microglia cell line, MLS-9) with human KCa3.1 expressed in HEK293 cells. In all three cells, PKA activation with Sp-8-Br-cAMPS decreased the current, and this was prevented by the PKA inhibitor, $PKI_{14-22}$. Inhibiting PKA with Rp-8-Br-cAMPS increased the current in microglia. Mutating the single PKA site (S334A) in human KCa3.1 abolished the PKA-dependent regulation. CaM-affinity chromatography showed that CaM binding to KCa3.1 was decreased by PKA-dependent phosphorylation of S334, and this regulation was absent in the S334A mutant. Single-channel analysis showed that PKA decreased the open probability in wild-type but not S334A mutant channels. The same decrease in current for native and wild-type (but not S334A) expressed KCa3.1 channels occurred when PKA was activated through the adenosine A2a receptor. Finally, by decreasing the KCa3.1 current, PKA activation reduced $Ca^{2+}$-release-activated $Ca^{2+}$ (CRAC) entry following activation of metabotropic purinergic receptors.

**637-Pos Board B417**

**Cardiac Sodium Channel: Activation by CaM Involves a NaV1.5-NaV1.5 Interaction**

Sandra B. Gabelli[1], Agedi Boto[1], Victoria Halpernin[2], Mario A. Bianchet[1], Federica Farinelli[3], Srinivas Aripirala[1], **Jesse B. Yoder**[1], Jean Jakoncic[4], Gordon F. Tomaselli[3], Mario Amzel[1].
[1]Biophysics and Biophysical Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD, USA, [2]Physiology, Johns Hopkins University School of Medicine, Baltimore, MD, USA, [3]Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA, [4]National Synchrotron Light Source, Brookhaven National Laboratory, Upton, NY, USA.

Voltage-gated sodium channels (NaV) are integral membrane proteins, part of a macromolecular complex that is central to signaling in the heart and other excitable tissues. Regulation of the essential functions of the channel

are complex and may differ among tissue-specific isoforms nevertheless, mechanistic understanding of the molecular regulation of the channel is beginning to emerge. We and others have demonstrated the importance of the carboxyl terminus (CT) in the regulation of the channel. The CT is a hot spot for mutations that produce inherited cardiac arrhythmias, myotonias, epilepsy and autism. In the case of NaV1.5, the cardiac channel, mutations of critical structural motifs in the CT (including an EF hand-like motif and an IQ motif) result in disease conditions such as Brugada and LQT syndromes. Also, altered NaV channel trafficking and function with consequent intracellular Na+ overload contributes to the development of dilated cardiomyopathy. The structure of the CT of the Nav1.5 channel in complex with calmodulin (CaM), determined to 2.9 Å resolution, shows that many of the mutations associated with disease states occur at CTNav1.5-CaM interfaces. Based on this structure a mechanism for the transition to the non-inactivated state of the channel is proposed.

**638-Pos Board B418**

**Calmodulation of Voltage-Gated Calcium Channels by Blue Light**

**Jacqueline Niu**[1], Manu Ben-Johny[1], Paul J. Adams[2], David T. Yue[1].
[1]Biomedical Engineering, Johns Hopkins University, Baltimore, MD, USA, [2]Biology, Kwantlen Polytechnic University, Surrey, BC, Canada.

Delivering custom-shaped signaling inputs to dissect biological networks has been a coveted goal of engineering-minded biologists, keenly aware of the advantages of analyzing electronic circuitry with a signal generator. We have recently devised a rapamycin-based chemical-dimerization strategy to produce step-like increases in the concentration of calmodulin (CaM) at the cytoplasmic mouth of L-type $Ca^{2+}$ channels. This tactic revealed that CaM binding to channels induces two effects: a large increase in peak open probability ($P_O$), and the ability to undergo $Ca^{2+}$-dependent inactivation (CDI) (Adams *et al* (2014) *Cell*, **in press**). Yet, the rapamycin system is comparatively slow ($t_{rise\text{-}half}$ ~30 s) and irreversible. Here we explore the use of a light-based dimerization system from *Arabidopsis* (CRY2 and CIB1, Kennedy *et al* (2010)) to deliver CaM more rapidly and reversibly upon activation by blue light. To evaluate functionality with $Ca^{2+}$ channels, HEK293 cells expressing Ltype Cav1.3 channels were endowed with CIB1 targeted to the membrane and CRY2 glycine linked to a dominant-negative $CaM_{1234}$, where mutations eliminate $Ca^{2+}$ binding and presumably render this construct a constitutive apoCaM. On dimerization with blue light, CDI was indeed attenuated, confirming functionality with $Ca^{2+}$ channels. More telling, blue-light exposure left peak currents unchanged, arguing that $CaM_{1234}$ in fact acts just like apoCaM. Next, we expressed a variant of $Ca_V1.3$ (MQDY) with diminished apoCaM binding at baseline, and changed the CIB1 payload to wild-type CaM. Reassuringly, blue-light exposure increased both peak current and CDI by ~20% (5/6 responding cells), consistent with enhanced MQDY binding to CaM upon blue-light recruitment. Intriguingly, full enhancement occured in the very first current evoked after blue-light illumination (<20 s); CaM exchange with these channels thereby occurs on an even faster timescale. In all, light-based recruitment promises a powerful tool for investigating CaM modulation of membrane proteins.

**639-Pos Board B419**

**Pegylated Cholesterol and Methyl-Beta-Cyclodextrin are Modulators of L-Type Calcium Channel Current and Decrease Membrane Capacitance in Vascular Smooth Muscle Cells**

**Rikuo Ochi**, Sachin A. Gupte.
Pharmacology, New York Medical College, Valhalla, NY, USA.

Loading of pegylated cholesterol (PEG-cholesterol) by pretreatment of several hours decreases current density and augments voltage-dependent inactivation of L-type Ca channel current (ICa,L) and depletion of cholesterol by similar long pretreatment with methyl-beta-cyclodextrin (MbCD) increases the ICa,L density in A7r5 aortic smooth muscle cells. On the other hand, dehydroepiandrosterone (DHEA), a cholesterol-derived steroid hormone, rapidly induces voltage-dependent inhibition of ICa,L. If endocytosis and exocytosis of CaV1.2-containing membrane vesicles are involved in these modulations, they should induce a change in membrane capacitance (Cm). Cm could be affected also by a change of thickness and dielectric constant of lipid bilayer. Here we studied acute effects of PEG-cholesterol, MbCD and DHEA on Cm of isolated bovine coronary artery smooth muscle cells by whole-cell patch clamp technique. Ramp steps of 5 or 10 ms were applied repetitively before, during and after application of the modulators. Control Cm was $18.3 \pm 4.5$ pF (mean ± SD.). PEG-cholesterol and MbCD induced gradual and small decrease of Cm and their washout decelerated the rate of the decrease. Cm after 10 min of wash-in normalized by the initial value was: control without modulator, $1.01 \pm 0.02$ (mean ± SD.); 1 mM PEG-cholesterol, $0.96 \pm 0.02$ ($p<0.01$, compared with control); 10 mM MbCD, $0.90 \pm 0.05$ ($p<0.0001$); 0.1 mM DHEA, $1.00 \pm 0.03$. Since the change of Cm is small, it little affects qualitative conclusion of the modulation of ICa,L density obtained by the pretreatment experiments with PEG-cholesterol and MbCD. The small but significant decrease of Cm reflects structural changes of the lipid bilayer which may be involved in the modulation of ICa,L by PEG-cholesterol and MbCD.

**640-Pos Board B420**

**NMDA Receptor Inhibition of L-Type Calcium Channels via ER Calcium Depletion and Activation of STIM1 in Cultured Hippocampal Neurons**

**Philip J. Dittmer**, Mark L. Dell'Acqua, William A. Sather.
Pharmacology, University of Colorado School of Medicine, Aurora, CO, USA.

We have found that, in cultured hippocampal neurons, NMDA receptors (NMDAR) inhibit voltage-gated L-type $Ca^{2+}$ channels through $Ca^{2+}$ release from the endoplasmic reticulum (ER) and subsequent engagement of STIM1 with L channels. Here we employed laser spot photo-uncaging of glutamate near single dendritic spines combined with fluorescence $Ca^{2+}$ imaging to investigate the relationships between stimulus frequency (glutamate uncaging) and $Ca^{2+}$ release from the ER. Measurements of cytosolic $Ca^{2+}$ were made with the genetically-encoded $Ca^{2+}$ indicator, RGECO1, and simultaneously, measurements of $[Ca^{2+}]_{ER}$ made with a genetically-encoded $Ca^{2+}$ indicator targeted to the ER, D1ER. Using a laser pulse duration of 2 ms to photo-uncage glutamate near a single spine, simultaneous measurements of $Ca^{2+}{}_{cyto}$ and $[Ca^{2+}]_{ER}$ revealed that stimulation at 1 second intervals (1 Hz) for 60 seconds triggered a rapid rise in $Ca^{2+}{}_{cyto}$ followed by release of $Ca^{2+}$ from the ER. Spine stimulation at 6 second intervals (0.167 Hz) for 60 seconds elicited a large cytosolic $Ca^{2+}$ transient, but no significant $Ca^{2+}$ release from ER stores. At various stimulus frequencies, pharmacological analysis using the L-type $Ca^{2+}$ channel blocker, nimodipine, uncovered a direct correlation between the magnitude of the nimodipine-sensitive, L-channel $Ca^{2+}$ transient and $Ca^{2+}$ release from the ER. Together, these results suggest that even though the lowest frequency stimulation is capable of generating a cytosolic $Ca^{2+}$ signal, the NMDAR $Ca^{2+}$ signal must contain the necessary details to activate L-channels by membrane depolarization and integrate with the L-channel $Ca^{2+}$ signal to regulate $Ca^{2+}$-induced $Ca^{2+}$ release in a single dendritic spine. If this frequency dependence holds true for the activation of STIM1 to regulate L-channel, this work will have important implication in how we think about channel regulation.

**641-Pos Board B421**

**Defining Post as a Modulator of STIM1 Function during T Cell Activation**

**Christina Go**, Robert Hooper, Joseph Kedra, Jonathan Soboloff.
Biochemistry, Temple University, Philadelphia, PA, USA.

Upon engagement of the T cell receptor, InsP3 is produced, triggering the release of $Ca^{2+}$ from ER $Ca^{2+}$ stores. The resulting ER $Ca^{2+}$ store depletion is sensed by STIM1, which relocalizes to ER-plasma membrane junctions to activate $Ca^{2+}$ channels and inhibit Plasma Membrane $Ca^{2+}$/ATPase (PMCA)-mediated $Ca^{2+}$ extrusion, thereby enabling sustained increases in cytosolic $Ca^{2+}$ that drive the process of T cell activation. Partner of STIM1 (POST) is a recently identified STIM1 adaptor protein with multiple binding partners, including STIM1, PMCA and several other transporters, pumps and exchangers. POST is a multi-pass transmembrane protein found in the ER and PM predicted to have 10 transmembrane domains. To assess this predicted topology, we used fluorescence protease protection assays, finding that POST is actually a 9 transmembrane-containing protein with a cytosolic N-terminus and a luminal/extracellular C-terminus. To assess the dynamics of STIM1/POST-mediated control of PMCA activity, we utilized colocalization and FRET. Upon T cell activation, POST migrated and co-localized with both STIM1 and PMCA4 to the immunological synapse. Interestingly, FRET between POST and both proteins was observed, yet FRET between STIM1 and PMCA4 could not be detected. Finally, POST knockdown inhibited activation-dependent inhibition of PMCA4-mediated $Ca^{2+}$ clearance. These studies provide new insight into the topology and dynamics of POST/STIM1/PMCA4 interactions during T cell activation. Given its numerous targets, further investigations into POST function may reveal additional roles for POST in the T cell activation process.

**642-Pos Board B422**

**The Sigma1 Receptor Competes with STIM1 to Bind Orai1 to Regulate Store Operated Calcium Entry (SOCE)**

**Shyam Srivats**, Dilshan Balasuriya, Mathias Pasche, Robert Vistal, Colin W. Taylor, Mike J. Edwardson, Ruth D. Murrell-Lagnado.
Pharmacology, University of Cambridge, Cambridge, United Kingdom.

The Sigma1 receptor is an ER chaperone protein targeted to mitochondrial associated ER membrane regions (MAMs). An increase in its expression