**197-Symp**
**Phase Separation of Multi-Valent Signaling Proteins**
**Michael K. Rosen**.
Department of Biophysics and Howard Hughes Medical Institute, UT Southwestern Medical Center at Dallas, Dallas, TX, USA.
Cells are organized on length scales from Angstroms to microns. However, the mechanisms by which Angstrom-scale molecular properties are translated to micron-scale macroscopic properties are not well understood. We have shown that interactions between multivalent proteins and multivalent ligands can cause liquid-liquid demixing phase transitions, resulting in formation of micron-sized liquid droplets in aqueous solution and micron-sized puncta on membranes. These transitions appear to occur concomitantly with sol-gel transitions to form large, dynamic polymers within the droplets/puncta. I will discuss how such transitions may control the spatial organization and biochemical activity of actin regulatory signaling pathways, and contribute to formation of PML nuclear bodies in the mammalian nucleus. Our data suggest a general mechanism by which cells may achieve micron-scale organization based on interactions between multivalent macromolecules.

## Platform: Voltage-gated Na Channels

**198-Plat**
**Structure/Function Insights into Eukaryotic Channel Blocker Binding Sites in a Prokaryotic Sodium Channel**
**Claire Bagnéris**[1], Claire E. Naylor[1], Paul G. DeCaen[2], David E. Clapham[2], David Pryde[3], B.A. Wallace[1].
[1]Birkbeck College, London, United Kingdom, [2]Harvard Medical School, Boston, MA, USA, [3]Pfizer Neusentis, Cambridge, United Kingdom.
Voltage-gated sodium channels are involved in electrical signalling in excitable tissues. They exhibit strong selectivity for sodium over other cations, thus enabling the cascade of events associated with action potentials. Mutations in these channels have been linked to a number of channelopathies such as epilepsy, cardiac arrhythmia, and chronic pain syndromes and therefore human sodium channels are targets for the development of local anaesthetics, antiepileptics, and other pore blocking drugs. Crucially, drugs which block eukaryotic channels have also been found to bind to and block bacterial sodium channels. Crystallographic, computational and electrophysiology experiments have been used to determine their functional effects and locations within the channel cavity. Their binding sites correspond closely to those previously predicted for these drugs in human sodium channels based on mutational studies, and may aid in the design of more specific and selective drugs.
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**199-Plat**
**Intracellular Calcium Attenuates Persistent Current Conducted by Mutant Human Cardiac Sodium Channels in Long-QT Syndrome**
**Franck Potet**, Thomas M. Beckermann, Jennifer D. Kunic, Alfred L. George.
Departments of Medicine and Pharmacology, Vanderbilt University School of Medicine, Nashville, TN, USA.
Mutation in the cardiac voltage-gated sodium channel $Na_V1.5$ can cause congenital long-QT syndrome type 3 (LQT3). Most $Na_V1.5$ mutations associated with LQT3 promote a mode of sodium channel gating in which some channels fail to inactivate, contributing to sustained or persistent sodium current ($I_{Na,P}$), which is directly responsible for delayed repolarization and prolongation of the QT interval. LQT3 patients have highest risk of arrhythmia during sleep or during periods of slow heart rate. During exercise (high heart rate), there is an increase in the steady-state intracellular free calcium (Ca) concentration. We hypothesized that higher levels of intracellular Ca may act in some way to lower arrhythmia risk in LQT3 subjects. We tested this idea by examining the effects of varying intracellular Ca concentrations on the level of $I_{Na,P}$ in cells expressing a typical LQT3 mutation, delKPQ. We found that elevated intracellular Ca concentration significantly reduced $I_{Na,P}$ conducted by delKPQ channels but not wild-type channels. $I_{Na,P}$ measured 200-ms after the peak transient current (expressed as % of peak current) for mutant channels was $2.6 \pm 0.4\%$ in low Ca and $0.6 \pm 0.1\%$ in high Ca ($p<0.001$). This attenuation of $I_{Na,P}$ in delKPQ expressing cells by Ca remained in the presence of KN-93 indicating it was not due to CaM kinase II activation ($2.2 \pm 0.2\%$ in low Ca and $0.9 \pm 0.3\%$ in high Ca; $p<0.001$). We conclude that intracellular Ca contributes to the regulation of $I_{Na,P}$ conducted by a LQT3 mutant and propose that, during excitation-contraction coupling, the increase of intracellular free Ca may contribute to suppression of mutant channel current and protect cells from delayed repolarization. These findings help explain the increased arrhythmia risk in LQT3 during slow heart rate.

**200-Plat**
**Single-Particle Tracking Palm of Nav1.6 in Hippocampal Neurons Demonstrates Unique Subcellular Diffusion Landscapes**
**Elizabeth J. Akin**[1], Kristen Brown[1], Sanaz Sadegh[2], Aubrey V. Weigel[2], Jean-Baptiste Masson[3], Diego Krapf[2], Michael M. Tamkun[4].
[1]Cell and Molecular Biology, Colorado State University, Fort Collins, CO, USA, [2]School of Biomedical Engineering, Colorado State University, Fort Collins, CO, USA, [3]Institut Pasteur, Paris, France, [4]Biomedical Sciences, Colorado State University, Fort Collins, CO, USA.
The Nav1.6 isoform is one of the major Nav channels of the central nervous system. In addition to its AIS localization, this isoform is found at a lower density throughout the somatodendritic region of the neuron, where it is thought to contribute to the back-propagation of the action potential. Due to the low number of somatodendritic Nav channels, immunocytochemistry is not sensitive enough to detect these channels and instead electron microscopy is required to visualize them. Thus, the dynamics and diffusive behavior of these channels have never been previously observed in real time. To visualize this behavior, we have utilized a Nav1.6 construct tagged with the photoswitchable fluorophore, Dendra2 (Nav1.6-Dendra2). This construct was transfected into cultured rat hippocampal neurons and imaged via TIRF microscopy. We combined single-particle tracking with photoactivated localization microscopy (sptPALM) such that we tracked only a small subset of Nav1.6-Dendra2 molecules at any given time. A steady-state density of active fluorophores was maintained via a low-power activation laser and the trajectory of each molecule was determined using an automated detection and tracking algorithm. We typically obtained statistics of tens of thousands of trajectories in each cell with high spatial and temporal resolution. Then these trajectories were used to obtain a diffusivity map across the neuronal surface using a Bayesian inference scheme. Consistent with previous observations, this method showed that AIS Nav1.6 channels are stably anchored, presumably to AnkyrinG. In contrast, Nav1.6 channels in the somatodendritic region showed both diffusive behavior and periods of transient confinement within specific membrane regions, thus creating small membrane clusters. Interestingly, we found large, micron-size regions of membrane completely devoid of Nav1.6, which implies that the channel is physically excluded from these domains.

**201-Plat**
**β4 Modulates $Na_V1.2$ Toxin Pharmacology**
**John M. Gilchrist**[1], Samir Das[2], Filip Va Petegemn[2], Frank Bosmans[1,3].
[1]Department of Physiology, Johns Hopkins University, Baltimore, MD, USA, [2]Department of Biochemistry and Molecular Biology, and the Life Sciences Institute, University of British Columbia, Vancouver, BC, Canada, [3]Solomon H. Snyder Department of Neuroscience, Johns Hopkins University, Baltimore, MD, USA.
The voltage-gated sodium ($Na_v$) channel signaling complex contains up to four distinct beta-subunits (β1-β4) that influence the functional properties of the pore-forming alpha-subunit. While investigating whether beta-subunits also influence ligand interactions, we found that β4 can drastically alter the response of the neuronal $rNa_v1.2a$ isoform to spider and scorpion toxins that target paddle motifs within $Na_v$ channel voltage sensors. In conjunction with the β4 crystal structure, we utilized the altered sensitivity to a tarantula toxin as a tool to probe the interaction between $rNa_v1.2a$ and β4. As a result, we identified $^{58}$Cys as an exposed residue that when mutated eliminates the influence of β4 on $rNa_v1.2a$ toxin pharmacology. In addition, we exploited β4-induced alterations in toxin sensitivity to probe the interaction between $rNa_v1.2a$ and a β4 mutant that mimics a β1 mutation implicated in epilepsy (C121W). We found that although this mutant still folds and traffics to the membrane, its interaction with the $Na_v$ channel is lost. The principles emerging from this work: *1)* help explain tissue-dependent variations in $Na_v$ channel pharmacology; *2)* enable the mechanistic interpretation of beta-subunit-related disorders; and *3)* provide new opportunities to design molecules capable of correcting aberrant beta-subunit behavior.

**202-Plat**
**Distinct Voltage Sensor Gating of Cardiac $Na_V$ Channels**
**Zoltan Varga**[1], Angela R. Schubert[1], Alexandra B. Asaro[1], Jianmin Cui[1], Mark A. Zaydman[1], Colin G. Nichols[2], Jonathan R. Silva[1].
[1]Biomedical Engineering, Washington Univ. in St. Louis, St. Louis, MO, USA, [2]Cell Biology and Physiology, Washington Univ. in St. Louis School of Medicine, St. Louis, MO, USA.
The channel-forming α subunit of voltage-gated $Na^+$ ($Na_V$) channels contains four domains (DI-DIV) each with six membrane-spanning segments (S1-S6). Voltage-clamp fluorometry (VCF) allows the tracking of the fourth charged segment (S4) in each domain with a site-directed fluorophore, which reports voltage-dependent changes in local protein conformation. We have created four novel channel constructs that enable us to study the voltage sensor