# Platform: Ion Channel Regulatory Mechanisms

### 3169-Plat

**CFTR Clustering and Tethering in Ceramide-Platforms in Response to Post-Infection PKC Stimulation**

**Asmahan AbuArish**[1], Elvis Pandzic[1], Paul W. Wiseman[1],
John W. Hanrahan[2].
[1]Physics, McGill University, Montreal, QC, Canada, [2]Physiology, McGill University, Montreal, QC, Canada.

Cystic fibrosis transmembrane conductance regulator (CFTR), an anion channel located on the apical surface of epithelia, functions in fluid secretion and is essential for mucociliary clearance of bacteria from the lung. The loss of CFTR function leads to recurrent bacterial infections and inflammation, however the precise role of CFTR in host-pathogen interactions is not well understood. Here, we examine the effects of *Pseudomonas aeruginosa*(*Pa*) and the soluble bacterial effectors flagellin and LPS on the distribution and dynamics of CFTR on the plasma membrane of human bronchial epithelial primary cells. Both image correlation spectroscopy (ICS) and its derivative the k-space correlation spectroscopy (kICS) were used to quantitatively measure CFTR aggregation level and dynamical tethering in lipid rafts before and during *Pa* infection. Before infection, 30% of CFTR was found to partition into rafts and undergo slow transport dynamics ($D=8x10^{-3}m^2/s$). Acute exposure to *Pa* or its effectors stimulated the aggregation of lipid rafts into m-size platforms. This significantly increased the fraction of the confined CFTR (>50%), reduced its transport ($D=2x10^{-3}m^2/s$), and increased CFTR aggregation level (3-fold) due to its entrapment into these platforms. Intact lipid rafts, protein kinase C (PKC) activation and the increase in ceramide production by acid sphingomyelinase (aSMase) were essential for platforms formation, and for CFTR tethering and clustering post-infection. PKC activation alone (independent of infection) induced platform formation and increased CFTR stability and aggregation, revealing a central role for PKC in modulating the formation of ceramide-rich platforms from subresolution lipid rafts upon infection, an action mediated via aSMase activation. The formation of ceramide-rich platforms may be involved in bacterial internalization by host epithelial cells as suggested previously, or may stimulate CFTR function by bringing it into close proximity with receptors and their associated signaling molecules.

### 3170-Plat

**Orai1 Pore Mutations and Calcium-Dependent Inactivation**

**Franklin M. Mullins**[1], Michelle Yen[2], Richard S. Lewis[2].
[1]Pathology, Stanford University, Stanford, CA, USA, [2]Molecular & Cellular Physiology, Stanford University, Stanford, CA, USA.

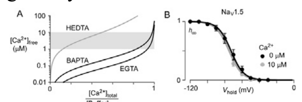
$Ca^{2+}$ entry through CRAC channels causes rapid $Ca^{2+}$-dependent inactivation (CDI) via interaction with a site located nanometers from the pore mouth. We previously found that mutations of W76 and Y80 in human Orai1 greatly alter the extent and kinetics of CDI (Mullins et al. 2009). Based on a recent crystal structure of a *Drosophila* Orai hexamer (Hou et al. 2012), the side chains of W76, Y80 and a ring of basic residues hypothesized to form a gate (R83, K87, R91) are all predicted to face the human Orai1 pore lumen. We mutated these residues to explore their contributions to CDI. STIM1 and variants of Orai1 were expressed in HEK cells, and the kinetics and extent of CDI were measured during 200-ms hyperpolarizations in 20 mM $Ca^{2+}$. $Ca^{2+}$-selectivity and magnitude of mutant currents were comparable to those of WT Orai1, indicating that observed changes in CDI were not likely due to changes in the local $[Ca^{2+}]_i$ near the pore.
At position 80, kinetics of CDI were affected by the size and charge of side chains: F was similar to Y (WT), while A, C, and S were significantly faster. Adding a positive charge (R or K) increased the speed further. These data suggest that Y80 limits the rate of inactivation by steric and/or hydrophobic interactions. The extent of inactivation was also influenced by substitutions: at position 80, $Y(WT) \approx F \approx A \approx C \approx S > K \approx R \approx Q$, while D, E, and W did not inactivate. Like W76E, Y80D, and Y80E, the R83E, K87E, and R91E mutations eliminated or markedly reduced CDI. Overall, CDI is disrupted by introduction of negatively charged side chains in the pore, but positive side chains permit or facilitate CDI. These effects suggest that CDI involves the interaction of an anionic species with basic pore-lining side chains in Orai1.

### 3171-Plat

**Conservation of Calmodulin Regulation Across Sodium and Calcium Channels**

**Manu Ben Johny**, Philemon S. Yang, Jacqueline Niu, Wanjun Yang,
Rosy Joshi-Mukherjee, David T. Yue.
Johns Hopkins University, Baltimore, MD, USA.

Voltage-gated Na and $Ca^{2+}$ channels comprise two major ion-channel superfamilies supporting divergent biology and biophysics. Yet, the carboxyl tails of these channels exhibit remarkable homology, hinting at a long-shared and purposeful module. If homologous tail domains elaborated functions of like correspondence, the common origins of such a module could be assured. Indeed, dynamic interactions between calmodulin and tail domains of $Ca^{2+}$ channels elaborate robust and recognizably similar forms of $Ca^{2+}$ regulation. However, years of Na channel research have only revealed subtle and variable $Ca^{2+}$ effects, with divergent mechanisms. These studies employed BAPTA or EGTA to statically elevate intracellular $Ca^{2+}$ to 1-10 micromolar, a range well above their dissociation constants (**A**). With $Ca^{2+}$ appropriately buffered using HEDTA (**A**), no discernible $Ca^{2+}$-dependent shift in steady-state inactivation of Na channels was observed (**B**), thus intensifying the discordance between homology and function and casting doubt on prior structure-function studies. However, using $Ca^{2+}$ photouncaging, we find that these dissimilarities in Na channels are only apparent. Indeed, $Ca^{2+}$ regulatory function and mechanism are fundamentally conserved across channel superfamilies, thus substantiating the preservation of an ancient $Ca^{2+}$-regulatory design featuring calmodulin as a modulator throughout much of living history.




### 3172-Plat

**Discovery and Characterization of a Distinct Cyclic Nucleotide Binding Pocket in HCN Channels**

**Anna Moroni**[1], Marco Lolicato[1], Annalisa Bucchi[1], Cristina Arrigoni[1],
Stefano Zucca[1], Marco Nardini[1], Katie Simmons[2], Marco Aquila[1],
Frank Schwede[3], Gerhard Thiel[4], Martino Bolognesi[1], Dario DiFrancesco[1].
[1]University of Milan, Milan, Italy, [2]University of Leeds, Leeds, United Kingdom, [3]BIOLOG Life Science Institute, Bremen, Germany, [4]TU-Darmstadt, Darmstadt, Germany.

Direct binding of cAMP to the cardiac pacemaker channels mediates largely the autonomic regulation of the heart rate. Binding of cAMP to the canonical binding site in the CNBD of HCN channels, promotes quaternary changes in the C termini that, in turn, affects the transmembrane domain of the channel facilitating pore opening. In the crystal structure of the C-linker + CNBD portion of HCN4, we found a second and yet unknown binding pocket that can be occupied by cyclic nucleotides. Occupancy of this pocket prevents cAMP modulation of the current in HCN4 and in the native current If, leading to heart rate reduction in mouse SAN myocytes. Compound 11, a structurally unrelated molecule selected by docking, reproduces this effect with higher affinity and pinpoints this pocket as a promising drug target. We are currently working at the mechanism by which the distinct binding site disrupts the allosteric pathway of cAMP from the CNBD to the pore.

### 3173-Plat

**Membrane Asymmetry: Key to Phosphoinositide (4,5)-Bisphosphate Regulation of TRPV1**

**Anastasiia Stratiievska**[1,2], Eric N. Senning[1], Marcus D.D. Collins[1],
Carmen A.A. Ufret-Vincenty[1], Sharona E. Gordon[1].
[1]University of Washington, Seattle, WA, USA, [2]Bogomoletz Institute of Physiology, International Center for Molecular Physiology, Kyiv, Ukraine.

The signaling lipid phosphoinositide 4,5-bisphosphate ($PI(4,5)P_2$) is known to regulate the activity of many ion channels. For TRPV1 there is general agreement that $PI(4,5)P_2$ is important for regulating activity, but whether as an activator or inhibitor remains a topic of debate. Here, we show that $PI(4,5)P_2$ can activate or inhibit TRPV1 activity, depending on whether it is asymmetrically localized to the intracellular leaflet of the plasma membrane or whether it is present in both leaflets. When $PI(4,5)P_2$ was localized to its physiological site, the intracellular leaflet of the plasma membrane, we confirmed that it activated TRPV1. In contrast, we observed an inhibition of TRPV1 activity when we introduced dic8-$PI(4,5)P_2$ or BODIPY-labeled $PI(4,5)P_2$ to the extracellular leaflet of outside-out excised patches so that, in combination with endogenous $PI(4,5)P_2$ in the intracellular leaflet, localization of $PI(4,5)P_2$ was no longer asymmetric. Interestingly, calibrations of the mole fraction of $PI(4,5)P_2$ with isothermal titration calorimetry showed that much higher concentrations of $PI(4,5)P_2$ in the extracellular leaflet were required for inhibition ($\geq 3$ mole%) compared to the concentrations of $PI(4,5)P_2$ in the intracellular leaflet that produced activation (<0.1 mole%). In patch-clamp fluorometry experiments, application of BODIPY-labeled $PI(4,5)P_2$ onto outside-out excised patches revealed that $PI(4,5)P_2$ incorporation into the membrane is needed for its inhibitory effect. The opposite effects of $PI(4,5)P_2$ on TRPV1 activity may reconcile the activation of TRPV1 by $PI(4,5)P_2$ in physiological conditions with inhibition of TRPV1 by $PI(4,5)P_2$ reported in a reconstituted system(Cao et al., 2013). Our results also underscore the importance of membrane asymmetry, especially in functional studies of membrane proteins.