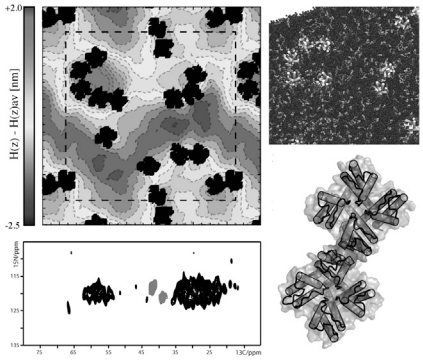
3174-Plat
Use-Dependent Activation of Kv1.2 Channel Complexes
Victoria Baronas, Brandon McGuinness, Yury Y. Vilin, Robin Kim, Runying Yang, Harley T. Kurata.
University of British Columbia, Vancouver, BC, Canada.
In excitable cells, ion channels are often challenged by rapid and repetitive stimuli. Ion channel responses to repetitive stimulation shape cellular behaviors by regulating the duration and termination of bursting action potentials. Therefore, we have investigated the regulation of voltage-gated potassium (Kv) channels under high frequency stimuli, with a particular focus on the canonical delayed rectifier Kv1.2. Previous reports have demonstrated a unique prepulse potentiation behavior that is observed in Kv1.2 channels expressed in mammalian cell lines. In this study, we demonstrate that this prepulse potentiation enables Kv1.2 channels to exhibit marked use-dependent activation, with trains of brief depolarizations causing dramatic increases in elicited current. This property arises from a stabilization of the channel open state in potentiated channels by ~2.5 kcal/mol, reflecting a 28 $\pm$ 1 mV leftward shift in activation V1/2 after channel potentiation, and a marked acceleration of channel activation. Importantly, Kv subunits can assemble into heteromeric channels, generating diversity of function and sensitivity to signaling mechanisms. We demonstrate that although other Kv1 channel types are not prone to the use-dependent activation observed for Kv1.2, this property is conferred to other Kv1 subunits when they co-assemble with Kv1.2 in heteromeric channels. Our observations suggest a unique role for Kv1.2 subunits as potential suppressive components of heteromeric Kv1 channels, and describe a novel mechanism of channel regulation that will influence channel activity during bursts of cellular electrical activity. These findings illustrate that the functional output of heteromeric Kv channels integrates the biophysical properties and signaling sensitivities of their component subunits. We highlight the importance of expanding biophysical studies of Kv channels to better understand interactions between different subunit types in heteromeric complexes.

3175-Plat
Functional Coupling in Nociceptive Sensory Neurons Between Ip3 Receptors and the Calcium-Activated Ano1(TMEM16A) Chloride Channel
Xin Jin[1], Shihab Shah[1], Yani Liu[2], Huiran Zhang[2], Meredith Lees[1], Zhaojun Fu[1], Jonathan D. Lippiat[1], David J. Beech[1], Asipu Sivaprasadarao[1], Stephen A. Baldwin[1], Hailin Zhang[2], **Nikita Gamper**[1].
[1]University of Leeds, Leeds, United Kingdom, [2]Hebei Medical University, Shijiazhuang, China.
Ca^{2+}-activated Cl- channels (CaCCs) are an important group of ion channels with diverse physiological roles whose molecular identity long time remained enigmatic. Members of the anoctamin (ANO) or TMEM16 family of proteins were recently identified as likely CaCC candidates. Thus, ANO1 (TMEM16A) mediates CaCC currents in epithelial and smooth muscle cells and in damage-sensing (nociceptive) sensory neurons. We report that ANO1 channels in small neurons from dorsal root ganglia (DRG) are preferentially activated by particular pools of intracellular Ca^{2+}. As demonstrated by patch-clamp and iodide imaging experiments, these ANO1 channels can be selectively activated by the G protein-coupled receptor (GPCR)-induced release of Ca^{2+} from intracellular stores, but not by Ca^{2+} influx through voltage-gated Ca^{2+} channels. Co-immunoprecipitation experiments and proximity ligation assay (PLA) suggested that this ability to discriminate between Ca^{2+} pools was achieved by the tethering of ANO1-containing plasma membrane domains to juxtamembrane regions of the endoplasmic reticulum. The ANO1-containing plasma membrane microdomains were assembled within lipid rafts and also contained GPCRs such as bradykinin receptor-2 and protease-activated receptor-2. As revealed by GST pull-down and peptide competition electrophysiology, interaction of the C-terminus and the first intracellular loop of ANO1 with IP3R1 (inositol 1,4,5-trisphosphate receptor 1) contributed to the tethering. Disruption of membrane microdomains by cholesterol extraction blocked the ANO1 and IP3R1 interaction and resulted in the loss of coupling between GPCR signaling and ANO1. The junctional signaling complex enabled ANO1-mediated excitation in response to specific Ca^{2+}signals rather than to global changes in intracellular Ca^{2+}.

3176-Plat
Ion Channel - Ion Channel Interaction at Atomic Resolution
Elwin van der Cruijsen, Koen Visscher, João Rodrigues, Alexandre Bonvin, Marc Baldus, **Markus Weingarth**.
Utrecht University, Utrecht, Netherlands.
Multiple lines of experimental evidence highlight the lateral patchiness of bacterial and eukaryotic cellular membrane surfaces, suggesting membranes in which proteins and lipids are organized in heterogeneous domains. This corroborates the notion that membrane signaling proteins like ion channels are assembled in supramolecular clusters, in which channel gating is coupled, possibly to achieve an optimal response to a single stimulus. How clustering occurs, the composition of the clusters and how the stimulus may be transferred between channels remains largely unknown, let alone at atomic resolution. Here we have studied clustering of ion channel KcsA in complex E. coli membranes at near atomic resolution using solid-state NMR experiments, biochemical methods and extensive multi-copy channel simulations. In particular, we analyze the influence of the membrane composition on channel function1,2.



1. Weingarth, M. et al., JACS, 2013, 135, 3983.
2. Van der Cruijsen, E. et al., PNAS, 2013, 110, 13008.

Platform: Neurons: Modeling, Synaptic Transmission, and Optogenetics

3177-Plat
Using a Cell Model to Study the Effect of Cholesterol on Exocytosis
Neda Najafinobar[1], Lisa Mellander[2], Mike E. Kurczy[1], Ann Sofie Cans[1].
[1]Chalmers University of Technology, Gothenburg, Sweden, [2]University of Gothenburg, Gothenburg, Sweden.
Exocytosis is an essential cellular process in neuronal communication. This cellular function involves vesicle fusion and vesicle content release of neurotransmitter molecules. In the quest to determine the molecules affecting exocytosis, simplified model systems such as artificial cells are valuable tools. The models allow us to study the effect of different components such as various lipids on the process of exocytosis in a controlled manner. In this study the effect of cholesterol on membrane dynamics for exocytosis has been investigated. Membrane lipids including cholesterol provide a platform for carrying out and regulating exocytosis. Although cholesterol is a major component of membrane and certainly affects exocytosis, the overall biochemical and biophysical properties are not been fully understood.
Two different artificial cell models have been applied in this work. The first cell model is based on pure lipid composition that provides total control of all the components in the system. The second cell model is constructed from cell plasma membrane from PC12 cells. This model gives the advantage of working with a system that is very much like a real cell and has almost all the components of the cell membrane including membrane proteins.
Kinetic information of single vesicle release of dopamine is recorded using carbon fiber amperometry in both models. In the former model we have shown that adding cholesterol to the system slows the kinetics of the release in a concentration dependent manner. In the latter model, we show that depleting cholesterol from the membrane enhances the kinetics of the events. The result from the latter model not only validates the result from pure lipid-liposome system but also indicates the importance of lipids in the presence of all the membrane components including proteins.

3178-Plat
BDNF Modulates Presynaptic Functions at a Central Synapse
Maryna Baydyuk, Xinsheng Wu, Jiansong Sheng, Liming He, Ling-Gang Wu.
NINDS/NIH, Bethesda, MD, USA.
Our brain function relies on the communication between neurons, a process called synaptic transmission. Brain derived neurotrophic factor (BDNF), a member of the neurotrophin family, regulates synaptic transmission in many brain areas and thus may critically influence brain function. The synaptic effect of BDNF may contribute to its well-known roles in regulation of neuronal survival, synapse development, and synaptic plasticity. Despite these important roles, the basic mechanism by which BDNF affects synaptic transmission remains poorly understood. Previous studies suggest that BDNF acts on transmitter release at nerve terminals, the presynaptic component of the synapse. However, the mechanism by which BDNF regulates transmitter release is unclear. In this study we investigate the role of BDNF in presynaptic function by electrophysiological recordings at the calyx of Held nerve terminal. The calyx of Held is a glutamatergic synapse in the auditory brainstem with a large nerve terminal, which allows direct presynaptic patch-clamp recording and