sensor. A reduced sensitivity of effector Th-cells towards oxidation is due to upregulation of Orai3 and of cytosolic antioxidants. The differential redox regulation of ORAI channels is a novel mechanism to tune Th-cell based immune responses during clonal expansion and inflammation.

1109-Plat
Comparative Analysis of Cholesterol Sensitivity of Kir Channels: Role of the Cytoplasmic Domain
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Kir channels are important in setting the resting membrane potential and modulating membrane excitability. A common feature of Kir2 channels and several other ion channels that has emerged in recent years is that they are regulated by cholesterol, a major lipid component of the plasma membrane whose excess is associated with multiple pathological conditions. Yet, the mechanism by which cholesterol affects channel function is not clear.
Here we show that in addition to Kir2 channels, members of other Kir subfamilies are also regulated by cholesterol. Interestingly, while similarly to Kir2 channels, several Kir channels are suppressed by an increase in membrane cholesterol, the function of others is enhanced following cholesterol enrichment. Furthermore, similarly to Kir2.1, and independent of the impact of cholesterol on channel function, we find that mutation of residues in the CD loop affect cholesterol sensitivity of Kir channels.
Among Kir2.1 CD loop residues, we have recently shown that the L222I mutation has the strongest effect on cholesterol sensitivity. This result is surprising since Kir2.2, which is as cholesterol sensitive as Kir2.1, already has an isoleucine at the corresponding position. Here we obtain further insight regarding the role of the cytosolic domain of Kir2 channels by examining mutations in adjacent cytosolic regions that also lead to loss of cholesterol sensitivity. In addition, we trace the source of the difference between Kir2.1 and Kir2.2 to a residue in the EF loop, N251, whose mutation to an aspartate reverses the effect of the L222I residue, and restores cholesterol sensitivity.
These findings suggest an indirect role of the cytosolic domain of Kir channels in regulating the effect of cholesterol on channel function and provide insight into the structural determinants of their gating mechanism.

1110-Plat
Molecular Mapping of An I_{KS} Channel Opener Reveals Crucial Interactions Between KCNE1 and the Kv7.1 Voltage Sensor Paddle
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Voltage-gated K^+ channels co-assemble with accessory subunits to form macromolecular complexes. In heart, assembly of Kv7.1 pore-forming subunits with KCNE1 auxiliary subunits generates the repolarizing K^+ current I_{KS}. We and others, recently suggested a strategic location of KCNE1 wedged close to helices S1 and S4 of two adjacent Kv7.1 voltage sensing domains (VSD) and nearby helix S6 of another Kv7.1 subunit. Here we show that the I_{KS} channel opener, diisothiocyanostilbene-2',2'-disulfonic acid (DIDS) acts on I_{KS} as a gating-modifier, thereby converting the time- and voltage-dependent channels into almost voltage- and time-independent currents. While DIDS activates Kv7.1, it does not affect Kv7.2. The two isothiocyanate functionalities are crucial for the potent activating effect of DIDS on $I_{KS,}$ since 4'-acetamido-4'-isothiocyanostilbene-2',2'-disulfonic acid (SITS) that has only one of these groups and 4,4'-dinitrostilbene-2,2'-disulfonic acid (DNDS), which lacks isothiocyanate groups and thus cannot form covalent bonds with amino acids, do not activate I_{KS} currents. Mutagenesis and modeling data indicate that DIDS activates I_{KS} by docking to an externally-accessible pocket, formed at the interface between the superficial N-terminal boundary of the KCNE1 transmembrane segment and the VSD paddle motif of Kv7.1. DIDS does not activate the channel complex formed by co-expression of KCNE1 and a chimeric Kv7.1 endowed with a Kv7.2 VSD paddle. DIDS binding at the Kv7.1 VSD-KCNE1 interface reveals that two lysine residues, K41 in KCNE1 and K218 in Kv7.1 S3-S4 linker are distant to about 10 [[Unable to Display Character: Å]]. Thus, KCNE1 affects Kv7.1 channel gating by closely interacting with the VSD paddle motif.

1111-Plat
Kv Channel Modulation: Closed State Block of Benzocaine But Not of Bupivacaine
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Local anaesthetics (LAs) block action potentials mainly by blocking Na channels. They are generally assumed to preferentially bind to channels in inactivated and/or open state. Recently it has been suggested that they mainly bind to channels in intermediate closed states. This is based on the finding that LAs affect the currents time and voltage-dependently in voltage clamped channels; that they reduce the peak current more at low voltage steps than at high.
In previous studies on inactivating K channels we have concluded that LAs preferentially bind to channels in open state. In the present study we have reanalysed the effects of LAs on K channels with special reference to the new findings of closed state binding. We analysed the effects of bupivacaine and benzocaine on Kv3.1 and Shaker channels expressed in Xenopus oocytes. As shown previously bupivacaine induces a peaked current in both channel types. In accordance with the results on the Na currents bupivacaine reduced the peak less at +60 mV than at lower potentials. Nevertheless, a modelling analysis suggested that the results are explained by binding preferentially to open channels. In contrast, benzocaine did not induce a peak at any potential, but the early current was reduced more at low potentials than at high. The modelling analysis suggested that the effect is caused by binding to closed and open channels.
We thus conclude that bupivacaine and benzocaine blocks K channels differently; bupivaciane open state-dependently and benzocaine both open and closed state-dependently. We also conclude that a time and voltage-dependent block, similar to that reported for Na channels, with less inhibition of the peak current at high potentials than at low potentials, does not necessarily imply binding of channels in a closed state.

1112-Plat
Introducing Drug Action into Single-Cell Cardiac Models
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Drug development failures due to adverse cardiac effects cost the drugs industry millions of dollars every year. Many of these failures may be predicted through mathematical modelling of drug actions. In order to achieve this it is necessary to investigate the effectiveness of different ways of incorporating drug action into models. Five different single-cell cardiac models are studied with and without drug action. These comprise two rabbit models (Mahajan *et al.*, 2008; Shannon *et al.*, 2004) and three other species (ten Tusscher and Panfilov, 2006; Hinch *et al.*, 2004; Faber *et al.*, 2007). The L-type calcium channel regulation properties of the different models are compared, and their calcium-dependent and voltage-dependent inactivation properties are considered. It is found that the different models respond in very different ways to the introduction of drug action through a simple pore block with none of the models successfully reproducing experimental results for both drugs that are considered. It is therefore concluded that the kinetics of drug action on active and inactive channels must be included to better model the drug action. The differing responses of the models at different pacing frequencies and drug doses indicate that it is necessary to perform experiments at a range of frequencies and drug concentrations.

Platform S: Imaging & Optical Microscopy I

1113-Plat
Telomeres Diffusion Study Implies on A Self-Organization Mechanism of the Genome in the Nucleus
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The human genome contains tens of thousands of genes that are organized in chromosomes and packed in the nucleus of the cell. How can the chromosomes and DNA stay organized in territories without any compartmentalization? This order is sustained throughout the life cycle of a cell, a property that emerges as a key contributor to genome function, though its full extent is not yet known.
To address this question, we studied fluorescently-labeled telomeres diffusion in a broad time range of 10^{-2} - 10^{4} seconds by combining a few microscopy methods followed by comprehensive diffusion analysis [1]. We found that the telomeres follow a complex diffusion pattern never reported before. The diffusion of the telomeres was found to be anomalous (subdiffusive) at short time scales and it changes to normal diffusion at longer times.
The transient diffusion indicates that telomeres are subject to a local binding mechanism with a wide but finite time distribution.
We therefore suggest that local temporal binding mechanism leads to the maintenance of structures and positions in the nucleus without the need for actual

compartments. Such a mechanism has another advantage by providing flexibility. If telomere binding is switched off (e.g., by shortening the binding time), this will allow the nucleus to undergo architectural changes.

[1] I. Bronstein, Y. Israel, E. Kepten, S. Mai, Y. Shav-Tal, E. Barkai and Y. Garini, Transient anomalous diffusion of telomeres in the nucleus of mammalian cells. Physical Review Letters **103**, 018102 (2009).

1114-Plat

Mapping Neuronal Connectivity Using Stochastic Optical Reconstruction Microscopy (Storm): The Brainstorm Project

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The human brain is a highly sophisticated circuit consisting of hundreds of billions of neurons that are interconnected by an even larger number of synapses. This dense network of neurons and their connections holds key information to understanding normal brain function and perhaps what underlies its disorders. Obtaining a physical map of the brain's connectivity, however, is highly challenging due to the small size and high density of neuronal processes within a given volume. Therefore, in order to generate a map of neuronal connectivity a technique that can provide high spatial resolution and molecular specificity is needed.

We are using 3D multi-color stochastic optical reconstruction microscopy (STORM) in order to trace neuronal networks in culture at high spatial resolution. In order to capture an entire network of connections, we are using an automated, motorized piezo stage to image large areas in x-y ($\geq$ 120x120 μm) as well as in z ($\geq$ 2 μm). With this technique we can not only outline neuronal morphology at 30 μm lateral and 50 μm axial resolution, but we can also image synaptic content with high molecular specificity and identify synaptic connections. These techniques will be greatly useful for generating connectional maps of neurons in the mammalian brain and help obtain a physical understanding behind brain function.

1115-Plat

Monitoring the [ATP]/[ADP] Ratio in Beta-Cells During Glucose Stimulated Insulin Secretion Using the Genetically Encoded Fluorescent Reporter Perceval

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Pancreatic beta-cells secrete insulin in response to elevated blood glucose levels. Glucose stimulated insulin secretion depends on glucose metabolism that produces ATP. The resulting increase in [ATP]/[ADP] ratio closes ATP-sensitive potassium (KATP) channels, which leads to membrane depolarization and opening of voltage-dependent Ca2+ channels. This causes an elevation of intracellular free Ca2+ and insulin exocytosis. Insulin is secreted in a pulsatile manner, which is thought to be regulated in part by oscillations in glucose metabolism. Such metabolic oscillations would also lead to oscillations in the [ATP]/[ADP] ratio and hence regulate KATP channel activity.

Oscillations in [ATP]/[ADP] ratio have been demonstrated using biochemical and luciferase assays, but neither approach allows measurements of such oscillations in single cells. Perceval is a recently developed fluorescent protein biosensor for [ATP]/[ADP] ratio, and it permits direct measurement of [ATP]/[ADP] ratios inside living cells. We use Perceval in combination with quantitative confocal and two-photon excitation microscopy for direct measurement of the [ATP]/[ADP] ratio in beta-cells during glucose stimulated insulin secretion. For this purpose we have developed an adenoviral vector to express Perceval specifically in the beta-cells of intact mouse islets. Dynamic changes in [ATP]/[ADP] ratio can be correlated with glucose metabolism (by simultaneous imaging of Perceval fluorescence and NAD(P)H autofluorescence) and with intracellular free Ca2+ levels (by simultaneous imaging of Perceval fluorescence and the calcium sensor, FuraRed). This data allows us to test hypotheses regarding the role of localized subcellular signaling complexes and putative microdomains of glucose metabolism, [ATP]/[ADP] ratio, and Ca2+ dynamics in the regulation of glucose stimulated insulin secretion.

1116-Plat

Multiple Components Mapping of Live Tissue by Phasor Analysis of Fluorescence Lifetime Imaging

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In fluorescence lifetime microscopy (FLIM) of live tissues a major issue is the assignment of autofluorescence to specific molecular components and their interactions within the physiological context. Here we use the phasor approach to fluorescence lifetime imaging to analyze complex decays in a live tissue. The tissues used were seminiferous tubules from the testes of wild type mice or mice expressing GFP from an Oct4 transgene. Lifetime images were acquired in the time domain and analytically transformed in the phasor representation. By examination of the clustering of the phasors we identified different molecular components: auto fluorescence, GFP, collagen and retinol. Each chemical species was identified and categorized by its specific location in the phasor plot. This phasor fingerprint reduces the importance of knowing the exact lifetime distribution of the fluorophores and emphasizes the contribution of the species to the signal. To better identify specific tissue components we also used spectral imaging and second harmonic generation microscopy. Linear combinations in the same pixel of molecular species were recognized and their relative fraction was calculated and mapped. The analysis of the fluorescence decay with higher harmonics of the phasor plot separates different molecular components that have the same location in the phasor plot at one harmonic but arise from different lifetime distributions. The phasor approach to lifetime imaging in live tissue provides a unique and straightforward method for interpreting complex decays in terms of molecular features by identifying fluorophores and obtaining functional maps of their relative concentration. This method has the potential to become a non invasive tool to characterize the local microenvironment and monitor differentiation and diseases in label-free live tissues. Work supported by NIH-P41 P41-RRO3155 and P50-GM076516, NIH RO1 HD49488, NIH PO1 HD47675, CIRM RC1-00110 PD.

1117-Plat

Ultra-High Resolution Imaging of the Dynamic Nature of Post-Synaptic Molecules

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The spatial and temporal regulation in the composition of the postsynaptic membrane of synapses participates in the different forms of synaptic plasticity that trigger the cellular processes of memory formation, consolidation, and retrieval. Neurotransmitter receptors move rapidly in and out of synapses by lateral diffusion. This mobility is crucial to control the number of receptors present at a given synapse. Thus, the equilibrium between the synaptic and extra synaptic AMPA receptor number is crucial in controlling basal transmission and synaptic plasticity. This balance is regulated by the subunit composition of these receptors and by the interaction of intracellular scaffold proteins. However, how the trafficking of receptors and the scaffolding molecules in and out synapse is controlled remains unknown. Here we attempt to determine the relative distribution and trafficking properties of AMPA receptors and various scaffold proteins at unprecedented spatial ($<$ 40nm) and temporal resolution ($>$50 Hz) using a variety of novel ultra-high resolution fluorescence imaging approaches. We combine Single Particle Tracking (SPT) and Photo Activation Localization Microscopy (PALM) to map trajectories at the level of individual molecules. Here we describe the implementation of a multimodal microscope along with the development of a new dedicated analysis for single molecule segmentation and tracking. Furthermore we will discuss the application of SPT-PALM experiments on living neurons. With this novel approach, we expect to comprehend the motilities of receptors or scaffolding proteins when they traffic between the submicron sized molecular zones of dendritic spines. The combination of this type of detection and analysis will provide the information from thousands of discrete trajectories from a single cell with which it would be possible to appreciate finer details of versatile molecular mechanisms pertinent in the functioning of an excitatory synapse.

1118-Plat

Optical Recording of Electrical Activity of Cortical Layer 2/3 Pyramidal Neurons Using A Genetically-Encoded Voltage Probe

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Voltage-Sensitive Fluorescent Protein 2.3, VSFP2.3, is a genetically-encoded probe of membrane voltage using fluorescence resonance energy transfer (FRET) between a pair of cyan (CFP) and yellow (YFP) fluorescent proteins to convert voltage-activated motions of a voltage sensor domain from *Ciona intestinalis* voltage-sensitive phosphatase (Ci-VSP) into a differential voltage dependent fluorescence signal. To evaluate the utility of VSFP2.3 as a probe of electrical activity of neurons in intact brain tissue, we performed targeted whole cell current clamp and simultaneous optical recordings from L2/3 pyramidal