control ionic conditions in the cell and energise osmotic potentials, secondary transport schemes and ionotropic signalling.
A surprising finding from the Na+,K+-ATPase structure was the docking of two conserved tyrosine residues at the C-terminus of the alpha subunit into the transmembrane domain, hinting that this was a previously unidentified regulatory element. Several mutations causing human neurological syndromes have subsequently been mapped to the C-terminal structure element, also clearly indicating that conservation of the structure is important for pump function.
Mutational analysis confirmed this and prompted our further analysis by electrophysiology and molecular dynamics simulations, which have shown a profound effect of the C-terminus on the electrogenic transport properties. We further propose that the C-terminal region forms a binding pocket that can be exploited for pharmacological intervention in cardiovascular and neurological disease.

1103-Symp
Alternating Access Mechanism of Glutamate Transporters
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In the central nervous system, glutamate transporters are responsible for the glutamate clearance following rounds of neurotransmission. They are molecular pumps, which utilizes the energy of pre-existing electrochemical gradients of ions to drive substrate uptake against steep concentration gradients. Sodium coupled aspartate transporter from Pyrococcus horikoshii, GltPh, is a homologue of the mammalian transporters and has served as a model system, within which to understand the molecular details of transport. The previously determined crystal structures of GltPh revealed the substrate and sodium binding sites located near the extracellular solution leaving the question of how they reach the cytoplasm unanswered. Recently, we have determined the crystal structure of a double cysteine mutant of GltPh, captured by cross-linking in a novel conformational state. In this state the substrate-binding sites are near the cytoplasmic surface of the protein. These findings suggest a novel and unexpected mechanism, by which GltPh and, by analogy mammalian glutamate transporters catalyze trans-membrane transport of their substrates.

1104-Symp
Alternating Access of the Maltose Transporter
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No Abstract.

Platform R: Channel Regulation & Modulation

1105-Plat
Photopharmacology: Controlling Native Voltage-Gated Ion Channels with Light
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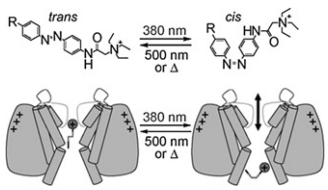
Optical control of proteins provides critical advantages for studying cell signaling and offers great promise in biotechnology and biomedical research. We have developed a series of photochromic ligands (PCLs) that target voltage-gated ion channels. They possess an azobenzene photoswitch connected on one side to a quaternary ammonium ligand (internal blocker for potassium, sodium and calcium channels) and on the other side to a variety of chemical groups. The azobenzene photoisomerizes between cis and trans configurations using different wavelengths of light, thereby repetitively turning on and off ion flow.
Alteration of the R group makes our approach very modular. First, increasing hydrophobicity allows better membrane penetration and therefore greater potency of the PCL. Second, PCLs with a charged R group require hydrophilic pathways to cross cell membranes and can be specifically targeted to cells expressing entry-route proteins. Third, selectivity for certain ion channels can be attained, allowing a more precise control over cellular excitability. Fourth, some PCLs act as cis blockers, offering the advantage of being silent in the dark. Finally, modifying the R group can be used to tune the spectral characteristics of the PCL, with potential interest for vision restoration.



1106-Plat
Receptor and Subunit Specificity in AKAP79/150 Actions On M-Type(KCNQ) K^+ Channels
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A-kinase-anchoring protein (AKAP)79/150 mediated PKC phosphorylation of M-type (KCNQ) channels is involved in M current (I_M) suppression by muscarinic M_1, but not bradykinin B_2 receptors (Hoshi *et al.* Nat. Cell Biol. 7:1066-73). In this study, we first explored the involvement of AKAP79/150 in muscarinic suppression of KCNQ currents by co-transfecting AKAP79 with KCNQ1-5 subunits in CHO cells stably expressing M_1 receptors. Expression of AKAP79 sensitized KCNQ2-5 and KCNQ2/3, but not KCNQ1, channels to suppression by the M_1 receptor agonist oxotremorine (oxo-M). Mutation of the PKC phosphorylation site on KCNQ4 (T553A) eliminated the effect of AKAP79, confirming the role of PKC. Co-transfection of wild-type, but not dominant negative, calmodulin abolished the effect of AKAP79 on KCNQ2/3 channels. We asked if purinergic and angiotensin suppression of I_M in superior cervical ganglion (SCG) sympathetic neurons involves AKAP79/150, since purinergic P2Y receptors depress I_M in SCG neurons via a similar mechanism to that of bradykinin, involving IP_3-mediated Ca^{2+} signals, whereas angiotensin AT_1 receptors depress I_M via a similar mechanism as M_1 receptors, by depletion of PIP_2. Transfection of ΔA-AKAP79, which lacks the A-domain necessary for PKC binding, did not affect I_M suppression by the purinergic agonist UTP (2 μM), nor by bradykinin (100 nM), but did reduce I_M suppression by oxo-M (1 μM) and angiotensin II (500 nM). We also tested association of AKAP79 with M_1, B_2, $P2Y_6$ and AT_1 receptors via FRET experiments on CHO cells under TIRF microscopy, which revealed weaker FRET between AKAP79 and $P2Y_6$ or B_2 receptors than for M_1 and AT_1 receptors. Our data suggest AKAP79/150 action generalizes to KCNQ2-5 subtypes, is disrupted by calmodulin, and is involved in angiotensin, but not in purinergic, suppression of neuronal M current. Supported by NIH grants R01 NS043394 and R01 NS065138.

1107-Plat
Potassium Channel Modulation by A Toxin Domain in Matrix Metalloprotease 23
Srikant Rangaraju[1], Keith Khoo[2], Zhiping Feng[2], George Crossley[3], Daniel Nugent[4], Ilya Khaytin[4], Michael Pennington[4], Raymond Norton[5], **K. George Chandy**[1].
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Peptide toxins found in a wide array of venoms block K+ channels causing profound physiological and pathological effects. Here, we describe the first functional K+ channel-blocking toxin domain in a mammalian protein. Matrix metalloprotease 23 (MMP23) contains a domain (MMP23TxD) that is evolutionarily related to peptide toxins from sea anemones. MMP23TxD shows close structural similarity to the sea anemone toxins BgK and ShK, and the domain blocks K+ channels in the nanomolar to low micromolar range (Kv1.6 > Kv1.3 > Kv1.1 = Kv3.2 > Kv1.4 in decreasing order of potency), while sparing other K+ channels (Kv1.2, Kv1.5, Kv1.7, KCa3.1). Full-length MMP23 suppresses K+ channels with a pattern of inhibition consistent with MMP23TxD activity. Our results provide clues to the structure and function of the vast family of proteins that contain domains related to sea anemone toxins. Evolutionary pressure to maintain a channel-modulatory function may contribute to the conservation of this domain throughout the plant and animal kingdom.

1108-Plat
Differential Redox Regulation of ORAI Channels: A Mechanism to Tune T-Cell Responses
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Phagocytes play an essential role in host defence against pathogens by generating reactive oxygen species (ROS). Effector T helper (Th) cells migrating to sites of infection will be exposed to this highly oxidative environment. Here we show how Th-cells respond and adapt to ROS. Oxidation affects different Ca^{2+}-signalling pathways essential for T-cell function. ORAI1 channels are inhibited with an IC_{50} of ~40 μM H_2O_2, but ORAI3 channels are insensitive. We identify cysteine (C195) of ORAI1, absent in ORAI3, as the major redox

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