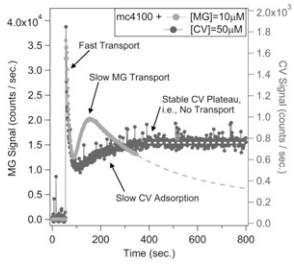
119-Plat
The Structure of the KtrAB Potassium Transporter
Ricardo Vieira-Pires, Andras Szollosi, João Morais-Cabral.
IBMC, Porto, Portugal.
The Trk/Ktr/HKT superfamily of ion transporters in bacteria, archaea, fungi and plants includes key transporters involved in osmotic regulation, pH homeostasis and resistance to drought and high salinity. These ion transporters are closely related to the superfamily of tetrameric cation channels, which includes potassium, sodium and calcium channels. A structural relationship between these superfamilies is observed both at the level of the membrane protein, with a similar 4-fold architecture and also at the level of the regulatory protein, that harbors RCK (regulate conductance of K^+) domains. Here we describe the crystal structure of a Ktr ion transporter from *Bacillus subtilis*, the KtrAB potassium transporter. The structure shows a homodimeric membrane protein, KtrB, assembled with a cytosolic octameric KtrA ring bound to ATP. Biochemical studies demonstrate that KtrA binds ATP and ADP and a comparison between the structures of the isolated full-length KtrA protein with ATP or ADP reveals a ligand dependent conformational change in the octameric ring. A 4-fold symmetrical KtrA ring is observed with ATP and a 2-fold symmetrical KtrA ring is observed with ADP. The asymmetrical conformational change of the KtrA regulatory ring together with its non-covalent interaction with the KtrB membrane protein, contrasts with what is described for ion channels, such as BK and MthK. Our study uncovers important differences between KtrAB K^+ transporter and ion channels, providing novel insights on the mechanism of regulation of the superfamily of ion transporters.

120-Plat
Sodium Coupling and Nucleoside Specificity of a Concentrative Nucleoside Transporter
Zachary Johnson, **Seok-Yong Lee**.
Duke University, Durham, NC, USA.
Concentrative nucleoside transporters (CNTs) are ion-coupled membrane transporters that utilize ion gradients to transport nucleosides and nucleoside-derived anticancer and antiviral drugs into cells. Humans have three subtypes of CNTs with varying nucleoside and nucleoside-drug specificity. The crystal structure of a CNT from *Vibrio cholerae* (vcCNT) in complex with uridine revealed the overall architecture, the nucleoside-binding site, and the putative sodium-binding site of this class of transporter [1].
In our follow-up studies, we have addressed two issues: the role of sodium in nucleoside transport and the substrate specificity of vcCNT. With regard to the role of sodium in nucleoside transport, the hypothesis that we gained from the structure is that sodium binding stabilizes the nucleoside-binding site thereby increasing its affinity for nucleosides. To test this structural hypothesis, we performed mutagenesis, isothermal titration calorimetry, and radioactive nucleoside uptake experiments. Our results are consistent with our structural hypothesis. To dissect the substrate specificity of vcCNT, we first observed that the nucleoside-binding site of vcCNT is very similar to hCNT3 (>90% sequence identity). As a result, our functional studies showed that vcCNT displays similar substrate specificity as hCNT3. To understand the structural basis of nucleoside and nucleoside-drug specificity, we have performed crystallographic and functional studies of vcCNT in complex with several different nucleosides and nucleoside-derived drugs. Our results provide a structural basis for the substrate specificity of vcCNT and hCNT3.
Reference
1. Zachary Johnson, Cheom-Gil Cheong, and Seok-Yong Lee, Crystal structure of a concentrative nucleoside transporter from *Vibrio cholerae* at 2.4 Å. *Nature*. 2012, Mar 11;483(7390):489-93.

121-Plat
Real-Time Observation of Molecular Transport across Biological Membranes with Non-Linear Optical Spectroscopy and Fluorescence Microscopy
Michael J. Wilhelm[1], Yajing Wu[1], Christian Spahr[1], Joel B. Sheffield[2], Grazia Gonella[1], Hai-Lung Dai[1].
[1]Temple University, Department of Chemistry, Philadelphia, PA, USA, [2]Temple University, Department of Biology, Philadelphia, PA, USA.
Molecular transport in biological systems occurs predominantly at the cellular membrane. Real-time observation of transport therefore demands a surface sensitive technique, capable of discriminating against an otherwise dominating bulk background signal. Towards this end, we have employed time-resolved second-harmonic scattering (SHS) to characterize the adsorption, as well as the transport efficiency and kinetics, of cationic dyes through living bacterial cells. SHS stems from a second-order non-linear optical response. In the presence of strong electric fields, coherent signal is produced whenever SHS-active molecules adsorb to surfaces or interfaces. For systems containing dual-surfaces, in close proximity, adsorption of SHS-active molecules to both sides results in coherent signal cancellation. Transport through membranes, as monitored by SHS, is characterized by a signal rise and decay directly proportional to the rates of adsorption and transport, respectively. The figure below depicts the dramatically different transport dynamics of malachite-green and crystal-violet in E.coli(mc4100). Both dyes rapidly transport through the outer membrane whereas only MG, well-known to stain even the outer coating of endospores, is observed to penetrate the cytoplasmic membrane. SHS results will be discussed and compared to complementary bright-field microscopy experiments.



Symposium: From Molecules to Tissues: How Forces are Transduced in Biology

122-Symp
The Function of Myosin-10 In Vitro and Inside Filopodia
Yasuharu Takagi[1], Rachel E. Farrow[2], Gregory I. Mashanov[2], Neil Billington[1], Thomas G. Baboolal[3], Christopher Batters[4], James R. Sellers[1], Michelle Peckham[3], **Justin E. Molloy**[2].
[1]NHLBI, NIH, Bethesda, MD, USA, [2]MRC NIMR, London, United Kingdom, [3]University of Leeds, Leeds, United Kingdom, [4]Ludwig-Maximilians-University, Munich, Germany.
Myosin-10 is an actin based motor that localizes to actin rich areas within the cell and carries cargos including netrin receptors and β integrins. Using transient kinetic and single molecule assays, a number of groups have shown that myosin-10 can act as a processivemotor, capable of translocating along actin as a single molecule. However, both the mechanical and kinetic parameters are quite diverse amongst different groups (Kerber & Cheney 2011, J. Cell Sci. **124**:3733-3741 and references therein). Negative stain electron microscopy and single particle image processing performed using a truncated myosin-10 construct (1 - 940 a.a.) containing a leucine-zipper at the C-terminal end confirmed dimerization of the molecule with a distance between motor domains of ~50 nm. When bound to F-actin, the myosin-10 lever arm makes a shallower angle with respect to the filament axis than seen for acto-myosin-5 or acto-myosin-2. *In vitro* actin gliding assays showed myosin-10-HMM moved F-actin at ~410 $nm.s^{-1}$ similar to the speed of GFP-tagged, intact myosin-10 we observed moving within filopodia of live mammalian cells (~600 $nm.s^{-1}$) using TIRF microscopy. Optical trapping experiments revealed the average work-stroke size was ~17 nm with a single actomyosin-10 stiffness of ~0.4 $pN.nm^{-1}$ and an ADP-release limited detachment rate of ~13 s^{-1}. In most raw data traces, we observed unitary displacements, however at low [ATP] (<500 nM) and low optical trap stiffness (<0.005 $pN.nm^{-1}$) we observed staircase-like interactions with an average movement of ~35 nm between steps; a behavior characteristic of a processive molecular motor. We will discuss these measurements in the context of structure, mechano-chemical coupling and the functional significance of this motor in the living cell.

123-Symp
Actin as a Tension Sensor
Edward Egelman, PhD[1], Vitold Galkin[2], Albina Orlova[2].
[1]BMG, University of Virginia, Charlottesville, VA, USA, [2]University of Virginia, Charlottesville, VA, USA.
An unsolved puzzle has been why the sequences of all eukaryotic actins have been so exquisitely conserved over large evolutionary distances, but the bacterial actin-like proteins show no such sequence conservation, and have diverged so much that many are as different from each other as they are from eukaryotic actin. We can show that actin filaments exhibit large amounts of cooperativity in structural states, as well as allosteric relationships within the subunit. Interestingly, some of the most dramatic allosteric couplings involve elements in actin that are not present in the bacterial actin-like proteins, such as the N-terminus, the C-terminus, the DNase I-binding loop, and the "hydrophobic plug". We suggest that these insertions provide for the extraordinary properties of actin, allowing actin filaments to form highly organized structures such as muscle sarcomeres, stereocilia of the inner ear, microvilli, stress fibers, etc. In contrast, the bacterial ParM protein forms a very different filament than F-actin, which accounts for why that filament behaves very differently: it shows dynamic instability, and the growth at the two ends is very

similar. While biochemists have typically focused on how small molecules, pH, and other proteins modulate the activity of a protein of interest, it is clear that mechanical forces can play a large role. We provide some new insights into the mechanical properties of F-actin, and suggest how actin can act as a tension sensor in many cell biological systems. In contrast to the long held view that F-actin is almost inextensible, we show how subdomain 2 of actin cooperatively and allosterically modulates both the bending and stretching stiffness of F-actin. Further, we show that the ability of actin-binding proteins to change actin's structure depends upon the intrinsic plasticity and cooperativity of actin.

124-Symp
Rupture and Contraction of Crosslinked Actin Networks by Myosin Motor Activity
Gijsje Koenderink.
FOM Institute AMOLF, Amsterdam, Netherlands.
Self-organized contractile arrays of actin filaments and myosin motors drive cell division, migration, and tissue morphogenesis. Biophysical studies have provided detailed mechanistic insights into the mechanisms of force production by individual motor molecule. However, it is not well understood how motors and actin filaments collectively self-organize into force-generating arrays. It is for instance poorly understood how network connectivity (or crosslinking) influences active contractility. We addressed this problem by reconstituting cell-free model systems from purified actin, myosin, and actin crosslinking proteins. By studying motor-driven activity over a broad range of network connectivities, we discovered that myosin motors contract actin networks into clusters that exhibit a scale-free distribution of sizes, characteristic of a critical state. Surprisingly, this critical behavior occurs over a broad range of network connectivities. To explain this robustness, we performed simulations of contractile networks taking into account network restructuring: motors reduce connectivity by promoting crosslink unbinding. We demonstrate that this coupling between activity and connectivity drives initially well-connected networks to a critically connected state. This model provides new avenues to understand contraction and rupture phenomena occurring during cell and tissue morphogenesis.

125-Symp
Shape Changes Induced by Actin Dynamics and Contraction
Cécile Sykes.
Curie Institute, Paris Cedex 05, France.
In order to unveil generic mechanisms of cell movements, we design stripped-down experimental systems that reproduce cellular behaviours in simplified conditions. Actin-based motility is mimicked using beads or oil droplets placed in an appropriate in vitro system that contains the actin machinery. Cortices of cells and their contractility are mimicked using liposomes covered with actin filaments that are straight or growing in branches, in the presence of myosin. We find that the efficiency of contraction or motility depends on the concentrations of proteins, on the length of the filaments, and on the strength of their attachment to the liposome membrane. Moreover, the mechanics of bio-mimicking liposomes can be characterized using tube pulling experiment, and we will present an unexpected result, that membrane dynamics is not only affected by the presence of the cytoskeleton, but to a large extend by membrane composition and liposome preparation.

Platform: Other K Channels

126-Plat
Functional Modulation of Cardiac ATP-Sensitive Potassium Channels by Nitric Oxide via Intracellular Signaling
Dai-Min Zhang[1], Yongping Chai[1], Jeffrey R. Erickson[1], Joan Heller Brown[2], Donald M. Bers[1], **Yu-Fung Lin**[1].
[1]University of California, Davis, Davis, CA, USA, [2]University of California, San Diego, La Jolla, CA, USA.
ATP-sensitive potassium (K_{ATP}) channels are crucial for stress adaptation in the heart. Nitric oxide (NO) has been shown to stimulate cardiac K_{ATP} channels; however, the mechanistic details remain poorly understood. Here we sought to delineate the intracellular mechanism responsible for NO modulation of sarcolemmal K_{ATP} ($sarcK_{ATP}$) channels in ventricular cardiomyocytes. Cell-attached patch recordings were performed in combination with pharmacological, genetic and biochemical approaches. Bath application of the NO donor NOC-18 increased the single-channel activity of Kir6.2/SUR2A (*i.e.*, the cardiac-type K_{ATP}) channels in transfected HEK293 cells, which was abolished by selective suppression of cGMP-dependent protein kinase (PKG), extracellular signal-regulated protein kinase (ERK)1/2, Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), and reactive oxygen species (ROS) (hydrogen peroxide H_2O_2 in particular), respectively. Importantly, NO donors potentiated function of $sarcK_{ATP}$ channels preactivated by the channel opener pinacidil in adult rabbit ventricular myocytes, through destabilizing the longest closed state and facilitating opening transitions, and the potentiation was nullified when PKG, calmodulin, CaMKII or ERK1/2 was inhibited. Exogenous H_2O_2 also stimulated ventricular $sarcK_{ATP}$ channels in intact cells in an ERK1/2- and CaMKII-dependent manner. Genetic ablation of CaMKIIδ, the predominant cardiac CaMKII isoform, diminished PKG stimulation of mouse ventricular $sarcK_{ATP}$ channels (compared with wild-type controls). Kinase activity and Western blot assays further supported that NO-PKG activation augmented CaMKII activity in ventricular myocytes, which was mediated by ERK1/2. Collectively, we demonstrate that NO stimulates ventricular $sarcK_{ATP}$ channels via a cGMP/PKG/ROS(H_2O_2)/ERK/calmodulin/CaMKII signaling cascade that alters channel gating. This novel signaling pathway may control cardiac excitability and mediate, in part, cytoprotection against ischemia-reperfusion injury, by opening myocardial K_{ATP} channels.

127-Plat
Awakening Loss-of-Function KATP Channel Mutants with an Engineered 'Forced Gating' Approach
Jenny B.W. Li, **Harley T. Kurata**.
University of British Columbia, Vancouver, BC, Canada.
Regulation of inwardly-rectifying potassium channels by intracellular ligands couples membrane excitability to important signaling cascades and metabolic pathways. A significant barrier to understand coupling mechanisms between ligand binding and gating is that many functionally important channel motifs are highly sensitive to mutagenesis, resulting in a loss of function phenotype. We have developed a 'forced gating' approach that rescues function in electrically silent channel mutants, enabling characterization of channel motifs that are otherwise intractable to electrophysiological recording. This approach involves substitution of a glutamate in the hydrophobic Kir channel bundle crossing (F168E mutation in Kir6.2), generating channels that are pH sensitive and open upon alkalization, due to mutual repulsion of introduced negatively charged side chains in the channel gate. We have implemented this 'forced gating' approach in mutagenic scans of the Kir channel slide helix and G-loop, two motifs proposed to play a role in ligand dependent gating of Kir channels. Both motifs are also highly sensitive to mutagenesis, with alanine mutations causing nearly complete loss-of-function at 7/20 slide helix positions, and 8/13 G-loop positions. Without exception, expression of silent mutants on the Kir6.2[F168E] background permitted activation of functional channels in alkaline pH, and measurement of kinetics and potency of ATP inhibition. Our results highlight an essential 'aspartate anchor' (Kir6.2 residue D58) that bridges the slide helix and multiple interacting residues in the cytoplasmic domain. Disruption of the highly conserved 'aspartate anchor' uncouples the transmembrane and cytoplasmic domains, reducing ATP sensitivity of Kir6.2 far more than any other G-loop or slide helix mutants. These findings indicate a central role for the 'aspartate anchor' in coupling ligand binding to Kir gating, and also emphasize the potential general utility of this 'forced gating' method to study loss-of-function channel mutants.

128-Plat
Distant Cytosolic Residues in Kir Channels Control Channel Gating and Modulation by Cholesterol and PI(4,5)P_2
Avia Rosenhouse-Dantsker[1], Sergei Noskov[2], Huazhi Han[1], Scott K. Adney[3], Qiong-Yao Tang[3], Aldo A. Rodríguez-Menchaca[3], Gregory B. Kowalsky[1], Vasileios I. Petrou[3], Catherine V. Osborn[1], Diomedes E. Logothetis[3], Irena Levitan[1].
[1]University of Illinois at Chicago, Chicago, IL, USA, [2]University of Calgary, Calgary, AB, Canada, [3]Virginia Commonwealth University, Richmond, VA, USA.
In recent years, cholesterol has been emerging as a major regulator of ion channel function. Channels regulated by cholesterol include the Kir2 channels subfamily of constitutively active, strongly inwardly rectifying K^+ channels that set the resting membrane potential and modulate membrane excitability. Yet, the mechanism by which cholesterol affects channel function is unclear.
We have previously shown that Kir2 channels are suppressed by the elevation of membrane cholesterol and enhanced by cholesterol depletion. We thus hypothesized that cholesterol modulates the function of Kir2 channels by