Preliminary experiments show that KCNA4 subunits, which can form hetero-tetrameric channels with KCNA2, are not sequestered by KCNA2(F233S). We are actively studying the interaction of KCNA2(F233S) with KCNA4 and KCNAB2 subunits, both known to facilitate KCNA2 expression.

1389-Plat
Structural Basis for Gating of the Two-Pore Domain K^+ (K_{2P}) Channels TASK-1 and TALK-2
Marcus Schewe[1], Elena B. Riel[1], Susanne Rinné[2], Wojciech Kopec[3], Jan Langer[1], Peter Lindemann[4], Björn C. Jürs[5], Marc Nazaré[4], Bert L. de Groot[3], Niels Decher[2], Thomas Baukrowitz[1].
[1]Institute of Physiology, University of Kiel, Kiel, Germany, [2]Institute for Physiology and Pathophysiology, University of Marburg, Marburg, Germany, [3]Biomolecular Dynamics Group, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany, [4]Department of Medicinal Chemistry, Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), Berlin, Germany, [5]MSH Medical School Hamburg - University of Applied Sciences and Medical University, Hamburg, Germany.
Two-pore domain K^+ (K_{2P}) channel activity is controlled by various stimuli that have been thought to finally converge at the selectivity filter (SF) gate. However, recent crystallographic studies have identified lower gates in TASK-1 and TASK-2 K_{2P} channels but pharmacological means to open these gates are currently unknown. Here, we report that TALK-2 K_{2P} channels also possess a lower gate by utilizing scanning mutagenesis, pore blocker analysis, permeant ion effects, chemical modification and MD simulations. Moreover, we report small molecule drugs and cellular lipids including long chain fatty acid esters (LC-CoAs) that open the lower gates in homomeric TASK-1, TALK-2 and TASK-1/TALK-2 heteromeric K_{2P} channels. Intriguingly, one of these openers is the pulmonary arterial hypertension (PAH) antidote ONO-RS-082 acting on PAH disease-gen TASK-1. Our results suggest that ONO-RS-082 open the lower gates by binding to the pore cavity when the lower gates are open which bears major implications for future design of PAH drugs. Finally, our results suggest a classification in the K_{2P} channel family with channels that exclusively utilize the SF gate (e.g. TREK-1/-2 and TRAAK), channels that exclusively utilize the lower gate (e.g. TASK-1) and K_{2P} channels with two functional gates (e.g. TALK-2 and TASK-2) highlighting the unusual diversity in structural gating mechanisms within this K^+ channel family.

Platform: Microtubule-associated Motors-Cytoskeleton-based Intracellular Transport

1390-Plat
The Mitotic Kinesin-6 KIF20A is an Unconventional Transporter and Network Builder
Fanomezana M. Ranaivoson[1], Amna Abdalla Mohammed Khalid[2], Steven Rosenfeld[3], Christoph F. Schmidt[4], Anne M. Houdusse[1].
[1]Structural Motility, Institut Curie, UMR CNRS 144, Paris, France, [2]Third Institute of Physics-Biophysics, Georg August University Göttingen, Göttingen, Germany, [3]Dept of Cancer Biology, Mayo Clinic, Jacksonville, FL, USA, [4]Department of Physics and Soft Matter Center, Duke University, Durham, NC, USA.
The kinesin-6 KIF20A critically participates in cell division and is a promising anti-cancer drug target. The mechanisms underlying its cellular roles remain elusive. We present here the first high-resolution structure of KIF20A as well as functional studies that provide insights into how this atypical kinesin works as a transporter and a microtubule organizer. While KIF20A has been described as a slowly processive transport motor, we find that it also has a remarkable capability to contract microtubules into open-porous networks. Structural insights from the KIF20A pre-power stroke conformation highlight the role of extended insertions in the motor domain in shaping the motor mechanochemical cycle. In particular, key functions are uncovered for the C-terminal neck linker, which is much longer than that of other kinesins. By elongating its mechanical element, this dimeric kinesin has acquired the ability to organize MTs into mesoscopic structures without losing its capacity to move on microtubules.

1391-Plat
Long-Range Coupling between Kinesins Regulates Collective Motor Behavior
Shane A. Fiorenza[1], Sithara Wijeratne[2,3], Meredith D. Betterton[1], Radhika Subramanian[2,3].
[1]Department of Physics, University of Colorado Boulder, Boulder, CO, USA, [2]Department of Genetics, Harvard Medical School, Boston, MA, USA, [3]Department of Molecular Biology, Massachusetts General Hospital, Boston, MA, USA.
Coupling of motor proteins within large arrays drives muscle contraction, flagellar beating, chromosome segregation, and other biological processes. Short-range interaction mechanisms of motor coupling, such as steric crowding and mechanical linkage, have been well-studied. However, coupling mechanisms that act at longer length scales remain largely unexplored. Here we report evidence of long-range motor-motor coupling on microtubules at picomolar protein concentration, where short-range motor interactions are unlikely. We find that the human kinesin-4 Kif4A changes processivity and velocity of other motors on the same microtubule in a dose-dependent manner, even at low density. Similar changes in Kif4A motility can be induced by kinesin-1 motors on the microtubule. A micron-scale attractive interaction potential between kinesin motors is sufficient to recreate the experimental results in a kinetic Monte Carlo simulation. A model in which the long-range interaction alters not just the binding-unbinding kinetics but also modifies the motor mechanochemical cycle better fits the data. Given the long length scale of interaction, the microtubule is a natural medium to couple motors, possibly via motor-induced lattice deformation. These results suggest a paradigm in which the microtubule lattice, rather than being merely a passive track, is a dynamic medium responsive to binding proteins to enable new forms of collective motor behavior.

1392-Plat
Engineered Icosahedral Protein Cage as a Flexible Model System to Study Properties of Multi-Motor Transport of Viral Cargoes
Somaye Badieyan, Michael P. Andreas, Tobias W. Giessen, Michael A. Cianfrocco.
University of Michigan, Ann Arbor, MI, USA.
Native cellular cargos and invading pathogens such as viruses take advantage of microtubule motor proteins to transport within the crowded cellular cytoplasm. How is the activity of different numbers and types of microtubule motor proteins bound to cargos coordinated to enable transport? While previous studies on membrane cargoes and linear DNA origami have established insight into differences in transport behaviors, there remains no established paradigm for rigid viral cargoes. To provide a flexible system to enable the study of viral-like cargoes, we engineered an icosahedral bacterial encapsulin nanocompartment to serve as a viral-like cargo onto which we can attach microtubule motor proteins. We chose encapsulins given their 1) rigid icosahedral structure; 2) alternative higher-ordered symmetries; 3) ability to package 'cargo'. We engineered the encapsulin to include 1) tagged subunits on the outside shell for binding motor proteins or cargo adaptors and 2) fluorescent proteins as 'cargo', to construct a fluorescently labeled viral-like cargo for motor attachment.
Using the motor-decorated encapsulin system, we determined the motile properties for the transport of fluorescently labeled encapsulin on microtubules in vitro. We compared the motile behaviors of encapsulins bound to human cytoplasmic dynein and/or human kinesin-1 (directly or through a cargo adaptor, e.g. BicD2 or FEZ1). These experiments showed increased on-rates and run-lengths for high surface coverage (~100 motors/encapsulin) compared to low surface coverage (~5 motors/encapsulin). We found that increased motor protein coverage led to extremely long run lengths without significant changes in velocity for both dynein and kinesin-1.
This work establishes a new model system for the flexible study of multi-motor transport for rigid viral-like cargoes. Future work will focus on benchmarking motile behaviors for multi-motor-bound encapsulins with a particular focus on bidirectional transport.

1393-Plat
Pathogenic Mutations in the Chromokinesin KIF22 Disrupt Anaphase Chromosome Segregation
Alex F. Thompson[1], Patrick R. Blackburn[2,3], Jane B. Lian[4], Eric W. Klee[3], Jason Stumpff[1].
[1]Molecular Physiology & Biophysics, University of Vermont, Burlington, VT, USA, [2]Department of Pathology, Cytogenetics, St. Jude Children's Research Hospital, Memphis, TN, USA, [3]Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA, [4]Biochemistry, University of Vermont, Burlington, VT, USA.
Mutations in the kinesin motor KIF22 (Kid) dominantly cause the skeletal developmental disorder spondyloepimetaphyseal dysplasia with joint laxity-leptodactylic type (SEMDJL2). Published analyses of SEMDJL2 patients identified mutations in proline 148 and arginine 149 of the motor domain alpha-2 helix as causative of disease pathology. These were predicted to be loss of function mutations that inactivate KIF22. We report the identification of a patient with a point mutation in the coiled-coil rather than motor domain of KIF22 and are investigating whether pathogenic mutations R149Q (motor domain) and V475G (coiled-coil domain) affect the function of KIF22 in mitosis. KIF22 uses plus end-directed motility and direct binding to chromosome arms to contribute to chromosome congression and alignment. Surprisingly, KIF22 with pathogenic mutations is capable of generating forces to move