rounds of mutagenesis and selection of those sensors with the desired characteristics in a high-throughput (HT) manner i.e. by FACS. However, there is currently no similar HT technology for sorting libraries of fluorescent protein biosensors. Thus, we have developed a novel microfluidic platform designed for high-throughput screening based on FRET change upon analyte binding. Using this platform, we sort a library of fluorescent protein Zn^{2+} sensors, selecting for both amount of FRET response and binding affinity for Zn^{2+}. The device is shown schematically below. The microfluidic platform incorporates a laser diode-bar optical trap, two-channel fluorescence excitation and detection, and bright-field imaging. This technology has numerous potential applications due to the great versatility in selection parameters and its ability to sort based upon cellular perturbations.

2798-Plat
Single-molecule Studies of RNA Unzipping Kinetics Using Nanopores
Jianxun Lin[1], Ralf Bundschuh[2], Amit Meller[1].
[1]Biomedical Engineering Department and Physics Department, Boston University, Boston, MA, USA, [2]Departments of Physics and Biochemistry, The Ohio State University, Columbus, OH, USA.
The α-Hemolysin protein pore has been previously used to study the unzipping kinetics of DNA duplexes, taking advantage of its sub-2nm pore constriction, which permits single stranded nucleic acids but not double stranded structures. Here we present the first extensive nanopore study of RNA unzipping kinetics as a function of voltage and temperature, and compare it with DNA unzipping of molecules with identical primary sequence. Our studies reveal clear differences between RNA and DNA, having similar bulk melting properties, highlighting the nanopore ability to probe subtle differences in nucleic acids' free energy and their interactions with the pore. In addition, we find that RNA hybrids with different overhang sequences display large difference in both unzipping times and current blockage signals. These results may be interpreted in the context of strong interactions between poly-A tail and the pore. Our work sheds light on the mechanics of nanopore unzipping and its sensitivity to small differences in nucleic acid base pairing stability. We currently in the process of developing a theoretical model to explain these observations, which will also set the stage for further studies involving multiple hairpin structures of RNA, and to more complicated and interesting biological problems, involving RNA-helicase interactions.
1. Mathé, J., Visram, H., Viasnoff, V., Rabin, Y. & Meller, A. (2004) Nanopore unzipping of individual DNA hairpin molecules. Biophys J 87, 3205-3212.
2. Dudko, O., Mathé, J., Szabo, A., Meller, A. & Hummer, G. (2007) Extracting kinetics from single-molecule force spectroscopy: Nanopore unzipping of DNA hairpins. Biophys. J. 92, 4188-4195.

2799-Plat
Artificial Nanopores that Mimic the Transport Selectivity of the Nuclear Pore Complex
Tijana Jovanovic-Talisman[1], Jaclyn Tetenbaum-Novatt[1], Anna S. McKenney[1], Anton Zilman[2], Reiner Peters[3], Michael P. Rout[1], Brian T. Chait[1].
[1]The Rockefeller University, New York, NY, USA, [2]CNLS, Los Alamos National Laboratory, Los Alamos, NM, USA, [3]CeNTech, University of Muenster, Muenster, Germany.
Nuclear pore complexes (NPCs) act as effective and robust gateways to the nucleus, allowing only the passage of selected macromolecules across the nuclear envelope. NPCs are comprised of an elaborate scaffold that defines a ~30 nm diameter passageway between the nucleus and cytoplasm. This scaffold anchors proteins termed FG-nups, whose natively disordered domains line the passageway and form an effective barrier to the diffusion of most macromolecules(1). However, cargo-carrying transport factors overcome this barrier by transient binding to the FG-nups. To test whether nothing more than a passageway and a lining of transport factor-binding FG-nups are sufficient for selective transport, we designed a functionalized membrane that incorporates just these two elements. We demonstrate that this membrane functions as a nanoselective filter, efficiently passing transport factors (NTF2, Kap95, Kap121) and transport factor-cargo complexes (Kap95/IbbGFP, NTF2/RanGDP) that specifically bind FG-nups, whilst significantly inhibiting the passage of proteins that do not bind. We show that the selectivity is based on the strength of binding to the FG-nups and pore geometry, as it is in vivo(2). The data also supports our in silico prediction that competition between transport factors and nonspecific macromolecules enhances the selectivity of the NPC(3). This effect has not been a major feature of other models for nuclear transport. In summary, we show that our artificial system faithfully reproduces key features of trafficking through the NPC, including transport factor-mediated cargo import. Nano-devices of this kind are useful for assessing the significance of parameters that govern NPC gating, and have many potential applications including the purification of macromolecules from crude mixtures.
1. Alber, F. et al. Nature 450, 695, 2007.
2. Shulga, N. et al. J. Cell Biol. 149, 1027, 2000.
3. Zilman, A. et al. PLoS.Comput.Biol. 3, 1281, 2007.

2800-Plat
Tunable Microfluidic Devices for Dynamically Controlling Sub-Cellular Environments
Nirveek Bhattacharjee, Albert Folch.
University of Washington, Seattle, WA, USA.
Microfluidic devices with the versatility of precisely controlling the immediate micro-environment of a single cell or a small population of cells have resulted in a paradigm shift in studying cellular development, local sub-cellular cell signaling, and ligand-activated dynamic cellular responses. In this paper, we report the development of multi-layered PDMS microfluidic devices that are dynamically tunable to provide on-demand, different spatio-temporally regulated micro-environments to *in-vitro* cultures of primary cells, like neurons and myotubes. The cell culture chamber was made in PDMS by exclusion-molding from a SU-8 master. The pneumatic actuator channel was molded separately, aligned and plasma-bonded on top of the cell-culture chamber. The roof of the cell-chamber contained elastomeric actuators designed to generate a dynamically-addressable, isolated compartment within the chamber. The separation of fluidic micro-domains was characterized with dyes. This tunable device can be used to (a) co-culture spatially separated cell populations, (b) dynamically separate axonal and somal compartments of a neuron, and (c) focally incubate subcellular regions of myotubes with extra-cellular biomolecules and ligands. We demonstrate time-lapse imaging and simulated models using the finite-element method, to show the dynamic tuning of the microfluidic channels, in the absence as well as in the presence of cells.

Platform AT: Unconventional Myosins

2801-Plat
An Ex Vivo Motility System Reveals the Cellular Roadmap for Myosin Motors
Crista M. Brawley, Ronald S. Rock.
The University of Chicago, Chicago, IL, USA.
Eukaryotic cells have a self-organizing cytoskeleton, where motors transport cargoes on these cytoskeletal tracks. We have demonstrated that myosin X can select for motility based on actin architecture (Nagy et al., PNAS:105(28), 2008). We hypothesize that any myosin motor may possess this ability of selection. To understand the sorting processes, we describe a system to observe single-molecule traffic patterns of multiple motor types in a cellular context. We followed the motility of class V, VI, and X myosins on preserved actin cytoskeletons from Drosophila S2 cells. From the trajectory maps, we find a radial actin architecture, but with a subset of filaments having the opposite polarity (barbed ends toward the cell center). We also see regional control of motility for all three motors, with several preferred regions (with varying velocity). Any of these features are not apparent from conventional in vitro motility assays using purified components. We further compared our S2 trajectory maps to maps of myosin V, VI and X created in Cos-7 and U2OS cells. These mammalian cell types display a complex actin architecture, revealed dramatically by the maps. Cos-7 cells have more isotropic actin than S2 cells and support motility of all three classes of myosins. U2OS cells do not support motility of class V and X very well. Although, myosin VI does make processive runs on U2OS cells, and these runs are specific for the stress fibers that span the length of the cells. Interestingly, the velocities for all three classes of myosins are similar for all cell types analyzed. This conclusion supports that myosin motility is selected for based on the actin architecture of cells, and we further hypothesize that the more dynamic the actin is the more myosin tracking that will occur.

2802-Plat
TEDs Site Phosphorylation Regulates Myosin I Motor Activity And Function In Fission Yeast
Sheran L. Attanapola, **Daniel P. Mulvihill**.
University of Kent, Canterbury, United Kingdom.
Myosins are evolutionarily conserved motor proteins that associate with actin to undertake a plethora of diverse cellular functions. Type I myosins are single headed monomeric motors which are involved in a range of motile and sensory activities in a variety of cell types. Myosin I's from lower eukaryotes have a conserved ''TEDs site'' motif within its motor domain. Phosphorylation of a conserved serine residue within this motif plays an important role in regulating the motor protein's activity. However, the mechanism by which TEDs site phosphorylation affects the motor activity and function of each myosin I remains unclear.

The sole class I myosin in the fission yeast, Schizosaccharomyces pombe, Myo1 is required to promote polymerisation of cortical actin patches, as well as to regulate lipid organisation and endocytosis. We have identified the serine residue within the motor domain of Myo1, which corresponds to the TEDs site. Using a phosphospecific antibody we have established that this conserved serine is phosphorylated in vivo within fission yeast. Mutating this serine within the Myo1 protein to either alanine or aspartic acid has revealed that its normal phosphorylation plays a crucial role in regulating the protein's affinity for actin and ability to function within the cell. Live cell imaging of these strains indicate Myo1 TEDs site phosphorylation is required for Myo1 to recruit to dynamic non-motile foci at the cell surface. In addition and unregulated phosphorylation can lead to inappropriate association with actin filaments, which is normally inhibited by tropomyosin. We also present data which illustrate the important role this phosphorylation event plays in Myo1's functions during actin organisation, endocytosis and lipid raft distribution.

2803-Plat
How Do Myosin VI and Myosin Va Navigate Intersections And Cooperate On Actin Tracks While Transporting Cargo In Vitro?
M.Y. Ali[1], Kathleen M. Trybus[1], David M. Warshaw[1], H.L. Sweeney[2].
[1]University of Vermont, Burlington, VT, USA, [2]University of Pennsylvania, Philadelphia, PA, USA.
Myosin Va (myoVa) and myosin VI (myoVI) are processive molecular motors that transport cargo on actin tracks in opposite directions. We have shown that myoVa can effectively maneuver through an in vitro cytoskeletal model system composed of actin filament intersections and Arp2/3 branches (Ali et al. 2007). Here we challenge Quantum dot (Qdot)-labeled expressed myoVI with actin filament intersections and observed that myoVI maneuvers through intersections with the following statistics: 38% turned left or right with equal probability; 28% crossed over the intersecting actin filament; 34% terminated their run. The myoVI cross over probability is twice that of myoVa suggesting that the range of the myoVI leading head's diffusional search may be longer than myoVa. Similar to myoVa, myoVI has significant flexibility allowing it to turn at intersection angles up to 155°. When multiple myoVI were attached a Qdot, the turning probability increased to 53% whereas the cross over probability decreased to 15%. MyoVa and myoVI may be colocalized to the same cargo in vivo and to determine how these oppositely directed motors might interact during cargo transport, we attached both motors in a 1:1 ratio to a Qdot. We observed two types of movement associated with these myoVa/myoVI-labeled Qdots. A given Qdot would move in both the plus- or minus-end direction for periods of time at velocities appropriate for the specific motor, suggesting that myoVa and myoVI take turns transporting the Qdot. Other Qdots moved continuously but at velocities suggesting that both motors are simultaneous interacting with actin and undergoing an effective ''tug of war.'' These studies may help characterize how actin-based motors deliver their cargo through the complex actin network.

2804-Plat
Smy1p: An Orphan Kinesin Finds a Home
Alex R. Hodges, Carol S. Bookwalter, Elena B. Krementsova, Kathleen M. Trybus.
University of Vermont, Burlington, VT, USA.
Long distance cargo transport in budding yeast is carried out not by kinesin, but along actin cables by two non-processive class V myosins, Myo2p and Myo4p. Overexpression of Smy1p, a kinesin-related protein, rescues the temperature sensitive *myo2-66* mutant yeast strain, which is defective in Myo2p transport.[1] The mechanism by which a kinesin family protein rescues actin-based transport is unknown, but does not require microtubules.[2] To address this question, we expressed Smy1p and Myo2p in insect cells and characterized them *in vitro*. Smy1p does not move microtubules in an ensemble motility assay, and is not an active motor. Using total internal reflection fluorescence microscopy (TIRFM), we find that Smy1p does not bind strongly to microtubules, but diffuses along them in the presence or absence of ATP. Surprisingly, Smy1p also binds to and diffuses along actin-fascin bundles. This binding is ionic strength-dependent, indicating the interaction is electrostatic in nature. When a single Myo2p is attached to a quantum dot cargo, the complex does not move processively on actin bundles. However, when several Smy1p molecules are attached to the quantum dot in addition to a single Myo2p, the complex supports continuous, unidirectional movement. 46% of moving quantum dots run to the end of the actin bundle, with run lengths greater than 10 microns observed. We hypothesize that Smy1p acts as an electrostatic tether, keeping the quantum dot bound to actin after Myo2p undergoes its powerstroke. We propose that overexpression of Smy1p rescues the *myo2-66* mutant by enhancing the binding of cargo to actin. A similar mechanism likely contributes to transport in wild-type cells when both Smy1p and Myo2p are present on the same cargo.

1. SH Lillie and SS Brown (1992), *Nature* **356**, 358-61.
2. SH Lillie and SS Brown (1998), *JCB* **140**, 873-83.

2805-Plat
The molecular basis for bundle selectivity of myosin X
Stanislav Nagy, Ronald S. Rock.
University of Chicago, Chicago, IL, USA.
Eukaryotic cells organize their contents through trafficking along cytoskeletal filaments. When presented with many apparently similar alternatives within the cortex, it is important to understand if and how myosin motors identify the few actin filaments that lead to their correct destinations. Recently we showed that myosin X, an actin-based motor that concentrates at the distal tips of filopodia, selects the fascin-actin bundle at the filopodial core for motility. Myosin X, while poorly processive on single actin filaments, takes long processive runs on actin filaments tightly bundled by fascin. Such a fascin bundle is the precise actin structure to which myosin X motors localize in vivo. Using single molecule optical trapping experiments we have determined the step size of this motor to be 17 nm, which is nearly half of the 36 nm pseudo-helical actin repeat required for motors to be processive on single actin filaments. These results indicate that straddling two filaments within a bundle stimulates this motor's function. Our initial model attributed this motility to the short lever arms of myosin X, consisting of 3 IQ repeats rather then the six found in the processive myosin V. To test this, chimeras were constructed where the heads, the IQ domains and the post IQ sequence (containing the coiled-coil dimerization domains) of Myosin V and Myosin X were used to create six combinatorial constructs. Single molecule fluorescence studies of these constructs revealed that the post IQ region and not the short lever arm of this motor is the main contributor to its unique selectivity. This result provides remarkable insight into the ability of nature to fine-tune myosin motors to serve their specific functions in the cell.

2806-Plat
Watching 'ankle' action of myosin V
Katsuyuki Shiroguchi[1], Harvey Chin[2], Eiro Muneyuki[3], Enrique M. De La Cruz[2], Kazuhiko Kinosita Jr.[1].
[1]Waseda Univ., Tokyo, Japan, [2]Yale Univ., New Haven, CT, USA, [3]Chuo Univ., Tokyo, Japan.
Myosin Va is an actin-based linear molecular motor that 'walks' in discrete ~35-nm steps following a ''hand over hand'', alternating site mechanism in which the two feet ('heads' or 'motor domains') switch between leading and trailing positions along the actin. Previously, we have reported that the lifted foot has access to a next forward binding site by combination of its rotational Brownian motion through a flexible joint at the leg('neck' or 'lever arm')-leg junction and forward movement of the joint by lever action of the landed leg. Our purpose here is to understand how the lifted foot binds to the leading site on actin. To step successfully, the 'toe' of the lifted foot should point down to let the actin binding site of the foot be properly oriented relative to actin. Meanwhile, in two-foot binding posture, the trailing leg which is dissociated by ATP-binding corresponds to the toe-up position. Therefore, we characterize ankle action with ATP-binding in the absence of actin. To observe the ankle action under an optical microscope, we fixed the leg of monomeric myosin Va on a substrate, attached beads to the foot and visualized orientation of the duplex. When triggered by UV flash of caged ATP, the beads swing 60-100 degrees, maintain this position for tens of seconds, then relax back to the original angle position. This cycle is observed repeatedly and only by UV flash of caged ATP. Thus, we clearly showed that myosin Va adopts (at least) two stable angles depending on the nucleotide state, which suggests that the toe of the lifted foot points down before binding to actin. Lastly, the observed swing supports ''swinging lever arm'' model which is generally believed to be a common mechanochemical mechanism of a conserved catalytic motor domain of myosin family.

2807-Plat
A Branched Kinetic Pathway Facilitates Myosin Va Processivity
Chong Zhang[1], Neil Kad[2], David M. Warshaw[1].
[1]Univ Vermont Col Med, Burlington, VT, USA, [2]University of Essex, Essex, United Kingdom.
Myosin Va is a double-headed molecular motor capable of long distance cargo transport. How the heads coordinate their enzymatic and mechanical cycles during processive movement is still unclear. Previously, we reported that myosin Va utilizes two kinetic pathways (Baker et al., 2004; Kad et al., 2008). Here we challenge a Qdot-labeled myosin Va HMM in the TIRF assay under various substrate conditions. Increasing [ATP] >1mM, [ADP] >1mM, or [Pi]=40mM reduces run lengths. These run length and associated velocity data confined an analytical model of myosin Va's unloaded multipathway