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.