2792-Symp
Structural Studies on the Chromatin Remodeling Factor ISW1a
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DNA in eukaryotic cells is organized hierarchically in chromatin. The vital life processes of DNA transcription, replication and repair, and the pathological progression of cancer and viral infection occur in the context of chromatin. Nucleosomes, the fundamental repeating units of chromatin, are actively positioned along DNA by ATP-dependent, chromatin remodeling factors. We have solved the X-ray structure of the ISW1a(ΔATPase) remodeling factor from the yeast, *S. cerevisiae*. ISW1a is a member of the ISWI family of remodeling factors and spaces nucleosomes *in vivo* to a repeat of ~165 bp. Cryo-electron microscopy image analysis of ISW1a(ΔATPase) bound to nucleosomes suggests the regions of interaction between it and nucleosomes. Combined with solution data [1] and the modeled structure of the ATPase domain (homology with Sso [2] and Rad54 [3]), a tentative mechanism for nucleosome remodeling is proposed.
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2. H. Durr, C. Korner, M. Muller, V. Hickmann and K. P. Hopfner (2005). ''X-ray structures of the Sulfolobus solfataricus SWI2/SNF2 ATPase core and its complex with DNA'', *Cell* **121,** 363-73.
3. N. H. Thoma, B. K. Czyzewski, A. A. Alexeev, A. V. Mazin, S. C. Kowalczykowski and N. P. Pavletich (2005). ''Structure of the SWI2/SNF2 chromatin-remodeling domain of eukaryotic rad54'', *Nat Struct Mol Biol* **12,** 350.

Platform AS: Micro & Nanotechnology: Nanopores

2793-Plat
Shippable And Indefinitely Storable Lipid Bilayer Precursor
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Although artificially reconstituted lipid bilayers have been used for over four decades for basic scientific research, to host engineered pore proteins for sensing applications, and to measure drug/ion channel interactions, their wider application is limited by a number of factors including fragility, short lifetime, and manual formation at the time and place of use. We have recently developed a new approach in which the self-assembly of lipid bilayers formed using the Mueller-Rudin method (Mueller et al. Nature 194, 979 (1962)) can be reversibly halted through freezing. (Jeon et al. Lab Chip 8, 1742 (2008)) When frozen before bilayer self-assembly is complete, the bilayer precursor is sufficiently robust to withstand shipping and indefinite storage. After thawing, the process of self-assembly resumes and results in a lipid bilayer membrane indistinguishable from one formed conventionally. In this way, we eliminate the need for the bilayer solution to be prepared at the time and place of use by a skilled operator. Instead, the bilayer precursor may simply be thawed prior to use. We will present our work with this platform and discuss our efforts to create membrane arrays with this technology and their application to high throughput screening of ion channels.

2794-Plat
High-Throughput DNA Assays Using Picoliter Reactor Volumes
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The online characterization and detection of individual droplets at high speeds, low analyte concentrations, and perfect detection efficiencies is a significant challenge underpinning the application of microfluidic droplet reactors to high-throughput chemistry and biology. Herein, we describe the integration of confocal fluorescence spectroscopy as a high-efficiency detection method for droplet-based microfluidics. Issues such as surface contamination, rapid mixing, and rapid detection, as well as low detections limits have been addressed with the approach described when compared to conventional laminar flow-based fluidics. Using such a system, droplet size, droplet shape, droplet formation frequencies, and droplet compositions can be measured accurately and precisely at kilohertz frequencies. Taking advantage of this approach, we demonstrate a high-throughput biological assay based on fluorescence resonance energy transfer (FRET). By attaching a FRET donor (Alexa Fluor 488) to streptavidin and labeling a FRET acceptor (Alexa Fluor 647) on one DNA strand and biotin on the complementary strand, donor and acceptor molecules are brought in proximity due to streptavidin-biotin binding, resulting in FRET. Fluorescence bursts of the donor and acceptor from each droplet can be monitored simultaneously using separate avalanche photodiode detectors operating in single photon counting mode. Binding assays were investigated and compared between fixed streptavidin and DNA concentrations. Binding curves fit perfectly to Hill-Waud models, and the binding ratio between streptavidin and biotin was evaluated and found to be in agreement with the biotin binding sites on streptavidin. FRET efficiency for this FRET pair was also investigated from the binding results. Efficiency results show that this detection system can precisely measure FRET even at low FRET efficiencies.

2795-Plat
Probing the Structural Properties of RNA using Solid-State Nanopores
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Solid-State Nanopores have been used extensively to investigate the physical properties of DNA. As a result, we are on the verge of using these nanopore devices as local-force probes for investigating DNA structure and protein/DNA interactions. However, DNA is not the only biopolymer with an important role within the cell.
Here we show, the first observations of RNA molecule translocation through a solid-state nanopore. We have recorded the translocations of hetero-polymeric double-stranded A-RNA and homo-polymeric single strands of RNA through a solid-state nanopore device.
We compare the relative blockage currents of these molecules to the well-characterized values obtained for B-DNA, which we use here as a calibration tool. In this way, we have been able to discern differing polymer diameters of 2 - 3 nm, with sub-nanometer resolution. Such high resolution measurements demonstrate that small differences in polymer thickness, e.g. due to the binding of a protein, can be readily detected using our solid-state nanopore device.

2796-Plat
Electrical Communication In Droplet Interface Bilayers Networks
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The engineering of nanoscale biochemical systems to process electrical information is appealing not only from the standpoint of miniaturization, but also because interfaces of biological computational devices with biological systems may be better tolerated than interfacing with traditional electronics. We have shown that aqueous droplets in a hydrophobic environment can communicate through proteins inserted into bilayers that form spontaneously between the droplets. Networks of droplet interface bilayers (DIB) have been used to build tiny batteries and sense light (1). We have now engineered the α-hemolysin protein nanopore to work as a diode over a wide range of ionic strengths. Networks of DIB with the incorporated protein-diode were used to build micro-devices that can process electrical information and function as a current limiter, a half-wave rectifier and a full-wave rectifier.

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2797-Plat
A Microfluidic Platform For High-Throughput Screening And Sorting Of Cells Based Upon FRET Response
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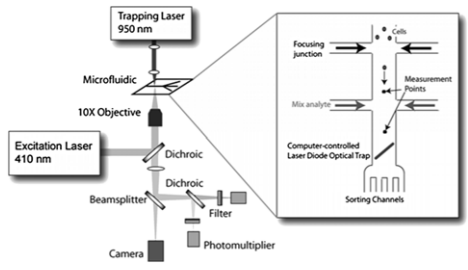
Fluorescent protein biosensors are powerful tools for real-time quantitative measurements of specific analytes in cells. These sensors have been used in mammalian cells to provide insight into signaling processes, as well as identify the cellular perturbations associated with disease and cellular targets of drug-based therapeutics. Development of new fluorescent proteins typically involves



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
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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.
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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
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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.
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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
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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
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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
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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.