2792-Symp
Structural Studies on the Chromatin Remodeling Factor ISW1a
Timothy J. Richmond, Kazuhiro Yamada, Tim Frouws, Brigitte Angst, Kyoko Schimmele.
ETH Zürich, Zürich, Switzerland.
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.
1. V. K. Gangaraju and B. Bartholomew (2007). ''Dependency of isw1a chromatin remodeling on extranucleosomal DNA'', *Mol Cell Biol*, MCB.01731-06.
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
Tae-Joon Jeon, Jason Poulos, **Jacob Schmidt**.
University of California Los Angeles, Los Angeles, CA, USA.
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
Joshua Edel, Andrew deMello, Monpichar Srisa-art.
Imperial College London, London, United Kingdom.
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
Gary M. Skinner[1,2], Michiel van den Hout[1], Onno Broekmans[1], Cees Dekker[1], Nynke H. Dekker[1].
[1]Delft University of Technology, Delft, Netherlands, [2]The University of Arizona, Department of Physics and BIO5 Institute, Tucson, AZ, USA.
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
Giovanni Maglia, Andrew J. Heron, William L. Hwang, Matthew A. Holden, Ellina Mikhailova, Qiuhong Li, Steven Cheley, Hagan Bayley.
Oxford University, Oxford, United Kingdom.
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.

1. Holden MA, Needham D, Bayley H: Functional bionetworks from nanoliter water droplets. J. Am. Chem. Soc. 2007, 129:8650-8655.

2797-Plat
A Microfluidic Platform For High-Throughput Screening And Sorting Of Cells Based Upon FRET Response
Emily A. Gibson[1], Philip J. Dittmer[1], Kevin Dean[1], Ralph Jimenez[2], Amy E. Palmer[1].
[1]Dept. of Chemistry and Biochemistry, University of Colorado, Boulder, CO, USA, [2]JILA, NIST and University of Colorado, Boulder, CO, USA.
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