CORE
Metadata, citation and similar papers at core.ac.uk
Provided by Elsevier - Publisher Connector

866-Pos Board B646
A Two Nanopore System for Controlling DNA Motion
Tamas Szalay.
Applied Physics, Harvard University, Cambridge, MA, USA.
Two nanopores placed in sufficiently close proximity to capture single biomolecules can enable new studies of stretching and relaxation dynamics as well as sequence or structure information. To realize such a system, we have designed a hollow AFM-like chip geometry with an integrated nanopore that can be precisely positioned relative to a second nanopore fabricated in a flat membrane, using a combination of electronic and optical feedback. This approach has allowed us to probe single molecules of DNA interacting with both pores simultaneously and to progress towards exerting control on the DNA directly.

867-Pos Board B647
Ion Transport Through Synthetic Nanopores Deposited in Porous Manganese Oxide Wires
Timothy S. Plett[1], Trevor Gamble[1], Eleanor Gillette[2], Zuzanna Siwy[1].
[1]Physics, University of California, Irvine, Irvine, CA, USA, [2]Chemistry, University of Maryland, College Park, MD, USA.
Synthetic nanopores emerged as an important tool to understand ionic and molecular transport at the nanoscale. Properties of ion current passing through nanopores can reveal geometric as well as electrochemical characteristics of the structures. Ionic selectivity, for example, is indicative of the presence of surface charges, while ion current rectification indicates broken electrochemical symmetry in the form of patterned surface charge or geometry. In this study, we utilized synthetic nanopores to perform conductivity experiments on manganese oxide, a porous material whose electrical state can be modified. The measured ion current carried information on the effective size of the voids as well as the polarity of the surface charges. Membranes containing many pores, as well as single pores, were coated with a gold layer via sputter deposition, and then electrochemically deposited with manganese oxide wires. The gold layer extended inside the pores, in direct contact with the MnO_2 wires, which permitted electrochemical modification of the wires. Measurements of ionic current through the wires were performed immediately after deposition, after an initial reduction with lithium, and after discharging the wires. These measurements revealed that each electrical state demonstrated different conductivites and provide strong evidence that the material has been successfully altered inside the nanopores. Experiments performed at a range of electrolyte concentration indicate the voids' diameter of the porous MnO_2 is dependent on the oxidation state of the material.

868-Pos Board B648
Improved Protocol for the Hydrophobization of Glass Pipettes for Use in Patch-Clamp Experiments; Tera-Seals and Tenths of Fa Noise
Arturo Galván-Hernández, Iván Ortega-Blake.
Universidad Nacional Autónoma de México, Cuernavaca, Mexico.
Patch-clamp experiments are used to study a variety of electrophysiological responses such as single-channel recordings and channel kinetics. Although an effective and extensively used technique, there exist several problems when using it, for example leakage through the lipid-glass interface or even through the thin glass wall. It is also important that the bilayer is stable and long lasting. In this study we present an improved experimental protocol which is intended to alleviate the above mentioned problems. Borosilicate glass pipettes of 1 μm inner diameter (I.D.) are coated via immersion in poly-dimethylsiloxane (PDMS) and curing agent to be then cured with ultraviolet (UV) light. PDMS is bound to the surface of the glass via a chemical reaction accelerated by the UV light, thus rendering the surface of the glass micropipette hydrophobic. This hydrophobic surface produces better membrane patch seals, with seal resistances in the order of Tera-Ohms (10^{12} Ohms) and noise level of < 100 fA. Normally micropipettes of 1 μm, would not yield lipid bilayer (LB) by the tip-dip method. The I.D. of the micropipettes are measured using the bubble number method and the results show that the I.D. of the micropipettes does not vary significantly when treated, hence no blocking occurs. The results indicate that the proposed treatment is very convenient for improving the patch-clamp technique by increasing seal resistance, and therefore stability and the life-time of the patch, as well as eliminating the possibility of ionic leakage through the thin glass wall of the tip and finally, reproducibility is increased due to the possibility of using micropipettes of 1 μm to obtain LB.

869-Pos Board B649
Multiscale Diffusion Measurements in Biological Gels Using Photoactivatable Fluorescent Nanoparticles
Joshua C. Kays[1], Benjamin S. Schuster[1], Daniel B. Allan[2], Justin Hanes[3], Robert L. Leheny[2].
[1]Biomedical Engineering, Johns Hopkins University, Baltimore, MD, USA, [2]Physics, Johns Hopkins University, Baltimore, MD, USA, [3]Ophthalmology, Johns Hopkins University School of Medicine, Baltimore, MD, USA.
Diffusion through biological gels is crucial for effective nanoparticle drug delivery. In this work, we develop a method to measure diffusivity over a large range of length scales – from tens of nanometers to tens of microns – using photoactivatable nanoparticle probes. Nanoparticles composed of block copolymers of poly(lactic-co-glycolic acid) (PLGA) and polyethylene glycol (PEG) were synthesized and confirmed to possess dense PEG coatings that resist bioadhesion, and these particles were conjugated with caged rhodamine to make them photoactivatable. Using confocal microscopy, we activated a region of these particles with a brief, targeted exposure to UV light, uncaging the rhodamine and causing the particles to become fluorescent. We observed their collective diffusion over tens of minutes and tens of microns, thus obtaining a measure of their diffusivity. This technique is complementary to traditional multiple particle tracking (MPT); it extends the range over which particle motion can be directly observed. We confirmed the accuracy of this technique with reference to MPT measurements and the known diffusivity of particles in water. We applied the method to a model fibrin gel system, and found that both our method and MPT measurements show an immobile fraction of particles and mobile fraction that diffuses over long scales. Finally, we examined nanoparticle diffusion in sputum collected from cystic fibrosis patients, and we measured particle diffusion over distances relevant to drug delivery in the lungs. Coupled with traditional MPT, this technique enables multiscale characterization of particle mobility in complex biological fluids

870-Pos Board B650
Behavior Response of Caenorhabditis Elegans to Physical Complex Stimuli in a Controlled Microfluidic System
Sunhee Yoon[1,2], Hailing Piao[3,2], Zhongwei Wang[3,2], Insu Lee[3,2], Ga Lahm Park[3,2], Tae-Joon Jeon[1,2], Sun Min Kim[3,2].
[1]Department of Biological Engineering, Inha university, Incheon, Korea, Republic of, [2]Biohybrid Systems Research Center (BSRC), Incheon, Korea, Republic of, [3]Department of Mechanical Engineering, Inha university, Incheon, Korea, Republic of.
Caenorhabditis elegans (C.elegans) has great potential as a model organism because of its genetic homology with human. So various studies on human neural network in organism level have been studied with C.elegans. Furthermore, C.elegans respond clearly on a various stimuli including chemical gradient, temperature, electric field, light, and other chemical/physical stimuli. C. elegans live under the environment with complex combination of these stimuli but previous studies only have focused on the single stimulus conditions. Here, we developed a microfluidic system for analyzing the behavioral response of C.elegans to physical complex stimuli; temperature gradient and electric field. Also, we compared the responses of wild type (N2) and mutant worms (PR678, IK589, BC347, and CB78) with this device to show that the specific gene affects on the behavior of C. elegans. The developed device has a potential to be a tool for the behavior of C. elegans and to be applied for studying of human nerve system.

871-Pos Board B651
Powered DNA Logic Gates
Dominic Scalise, Rebecca Schulman.
Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD, USA.
Chemical computers process information at the molecular scale, facilitating the exploration of biological phenomena, and enabling bottom-up control over physical and biological materials. Synthetic DNA circuits are chemical computers noted for their scalability and ease of design. However, most existing DNA circuits cannot respond to changing input signals, because their reactants are consumed in the process of computing a single output product. Here we demonstrate a mechanism to continuously supply DNA circuits with fresh reactants while degrading old output products. This mechanism powers DNA based circuits to operate continuously in dynamic environments.

872-Pos Board B652
A Systematic Investigation to Determine the Optimal Lipid Coating for Nanopore-Based Sensing Experiments
Olivia M. Eggenberger[1], Brandon R. Bruhn[1], Michael Mayer[1,2], Haiyan Liu[1], Geoffray Leriche[3], Jerry Yang[3].
[1]Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA,
[2]Chemical Engineering, University of Michigan, Ann Arbor, MI, USA,
[3]Chemistry and Biochemistry, University of California San Diego, La Jolla, CA, USA.
Despite the importance of proteins, nanopore sensing has so far been mostly focused on single molecule DNA and RNA characterization. One factor that limited experiments with proteins were nonspecific interactions of proteins with the walls of synthetic nanopores. We showed recently, that nanopores with fluid coatings of phospholipid bilayers circumvented this problem. In

addition, anchoring proteins to lipid anchors slowed down protein translocation through nanopores and enabled determination of parameters such as the shape, volume, and dipole moment of individual non-spherical proteins. To slow down the translocation time of lipid-anchored proteins further, we have recently formed highly viscous coatings from archaea-inspired lipids. These synthetic lipids are composed of two hydrophilic head groups connected by a long hydrophobic chain; each head group is also attached to an acyl chain that spans half of the membrane thickness. As these lipids contain -in one molecule- the components of a typical lipid bilayer, a single layer is sufficient to form a stable membrane. Experimentation with many lipid coatings, including a variety of archaea-inspired lipids, has revealed differences in translocation of protein, bilayer stability and event frequency. Every bilayer composition tested has been designed to optimize each of these characteristics.

873-Pos Board B653

The Effect of Inter-Particle Interactions on Heating Efficiency in Magnetic Nanoparticle Hyperthermia: An Experimental Model

Matthew D. Bausch, Benjamin Evans.
Physics, Elon University, Elon, NC, USA.

Magnetic nanoparticle hyperthermia offers new potential for treatment of certain cancers through local heating or ablation of cancerous tissues. The primary goal in developing agents for hyperthermia is generally to maximize the heating efficiency of an ensemble of magnetic nanoparticles. In this context, heating efficiency is defined as the power absorbed per gram of magnetic material (Specific Absorption Rate, SAR). In concentrated nanoparticle solutions, inter-particle interactions begin to have a significant effect on heating efficiency. However, this effect is not well-described in either experimental or theoretical studies, and so it remains a significant barrier to progress in the field. In particular, experimental studies often do not control for aggregation of the nanoparticles, which can dramatically affect local concentrations.

We present here measurements of SAR vs. concentration for an aggregate-free formulation of magnetite nanoparticles. Using a previously-reported magnetic-nanoparticle/silicone composite material, we are able to produce homogeneous suspensions of nanoparticles in concentrations ranging from 0 to 350 mg/mL. This allows us to measure the effect of increasing nanoparticle concentration on SAR independently of clustering behavior. Measurements of SAR taken in a 300 kHz field show a local maximum at concentrations on the order of 10 mg/mL. Below this concentration, increasing SAR indicates that inter-particle interactions contribute to heating; above this concentration the reverse is evident. This experimental data contributes to a fundamental understanding of collective behavior in magnetic nanoparticle hyperthermia, which may inform the development of new materials for this application in the future.

874-Pos Board B654

Threading Immobilized DNA Molecules Through Solid-State Nanopores

Harpreet Kaur[1,1], Santoshi Nandivada[1], Changbae Hyun[1], Tao Huang[1], Min Xiao[1], David McNabb[2], Jiali Li[1].
[1]Physics, University of Arkansas, Fayetteville, AR, USA, [2]Biological Sciences, University of Arkansas, Fayetteville, AR, USA.

In pursuit of manipulating and controlling DNA translocation speed in voltage biased solid-state nanopores, we designed and constructed an apparatus that can control a DNA molecule moving speed and measure the ionic current change when the DNA is captured and released from the nanopore. The probe tip's position is sensed and controlled by a tuning fork based force sensor and a nano-positioning system. Using this apparatus, we demonstrate DNA translocation rate of >100μs/base or <1nm/ms in silicon nitride nanopores. This rate is 1000 times slower than free DNA translocation through solid-state nanopores reported previously, which could provide enough temporal resolution to read each base on a tethered DNA molecule. We show results of this apparatus for measuring controlled translocations of ds/ss hybrid DNA molecules through solid state nanopores. The ds/ss hybrid DNA is a 48.6 kb double-stranded λ DNA with 1kb single-stranded DNA ligated to one end, and biotinlated oligo ligated to the other end for attaching the hybrid DNA to a probe tip. We further compare the controlled translocation with free translocation of these hybrid DNA molecules through solid state nanopores.

875-Pos Board B655

Combining Microfluidics and Fluorescence to Quantify the Timing of Viral Release

Rachel N. Hanson, Christina K. Chan, Emily A. Anderson,
Jolene L. Johnson.
Physics, St. Catherine University, St. Paul, MN, USA.

Release of viral particles from a host cell is an important step in the viral life cycle. This has been well studied in a variety of viral systems, but quantitative data on the exact timing of viral release and potential cell-to-cell variations is still needed. This data could shed light on the underlying mechanisms that affect the timing and rate of viral production. In this poster, we explain the experimental setup that will allow us to gather this data using viral like particles produced in the HIV-1 test system.

HIV-1 is an important and extensively studied retrovirus. Transfecting cells with only the structural protein Gag produces viral like particles (VLPs). The budding and release of these VLPs is similar to viruses produced from cells transfected with the full viral genome. There is a temporal distribution in the release of viral like particles from transfected cells, but the exact distribution and its cause are not fully known. We are developing an experimental setup that combines microfluidics and fluorescence to determine these.

Here, we summarize previous microfluidic designs and elaborate on the design fabrication and testing process specific to the adherent mammalian cells needed to produce VLPs. These adherent cells are grown inside a PDMS/glass based microfluidic device. They are transiently transfected with HIV-1 Gag-YFP. A very slow flow of medium is established and a narrow channel section is observed downstream from the cell colony. A CCD camera is used to detect each VLP and a histogram of observed VLP vs. post transfection time is made. We explain the testing of our detection system limits using fluorescent beads as a model. Finally, we discuss the design and initial testing of microfluidic devices for single cell VLP production studies.

876-Pos Board B656

Single Molecule Characterization of Cholera Toxin and its Interaction with Gm1 Gangliosides Using Lipid-Coated Nanopores

Anirudh Vinnakota[1], Brandon R. Bruhn[1], Erik C. Yusko[2], Michael Mayer[1].
[1]Department of Biomedical Engineering, University of Michigan - Ann Arbor, Ann Arbor, MI, USA, [2]Department of Physiology & Biophysics, University of Washington, Seattle, WA, USA.

Characterization of resistive pulses from the translocation of particles through lipid-coated nanopores has previously been used to determine the size, charge, and ligand affinity of proteins. Here, we coat the nanopores with a lipid bilayer to prevent non-specific adsorption, concentrate a protein of interest on the bilayer surface, and slow the translocation times of proteins through the pore so that events may be effectively time-resolved and detected. This study aims to characterize cholera toxin and the binding kinetics of the multivalent interaction with GM1 gangliosides embedded in the lipid coating. Understanding the parameters of the GM1 and cholera toxin interaction on a single molecule basis may provide additional information about the pathophysiology of vibrio cholerae, which is known to attack GM1 dense cells in the intestinal epithelia. By monitoring the frequency of translocation events of the protein-ligand complex as a function of time, it is possible to measure the binding kinetics of the interaction between cholera toxin and the GM1 lipid anchors. Thus far, we have determined the shape, volume, and charge of the membrane-associated protein in addition to the effective on-rate of binding under varying concentrations of the GM1 ligand.

877-Pos Board B657

Improved Protocols for Dielectric Breakdown Nanopore Biosensors

Christopher Tow, **Jacob K. Rosenstein**.
Brown University, Providence, RI, USA.

Solid-state nanopores are attractive single-molecule biosensors but commonly suffer from low fabrication yield, poor repeatability, and weak signal-to-noise ratios as compared to biological protein-derived pores. Recent demonstrations of nanopore fabrication by dielectric breakdown in high electric fields offer a promising alternative to established methods which rely on electron microscopes or ion beams. Breakdown-derived nanopores can be fabricated on-demand in standard electrolytes, without need for vacuum systems or harsh chemicals. However, the stochastic and nonlinear nature of electrical breakdown introduces new challenges for achieving consistent sensor quality. We will present progress towards more reliable in situ formation of small-diameter solid-state nanopores, using self-limiting protocols that improve yield and avoid the runaway growth of nanopores in high electric fields.

878-Pos Board B658

Fabrication of Sub-20 NM Nanopore Arrays in Membranes with Embedded Metal Electrodes at Wafer Scales

Yanxiao Feng[1], Deqiang Wang[1], Jingwei Bai[2], Sung-wook Nam[1], Hongbo Peng[1], Robert Bruce[1], Lynn Gignac[1], Markus Brink[1], Phil Waggoner[3], Chao Wang[1], Mike Guillorn[1], Stanislav Polonsky[1], Ajay Royyuru[1], Satyavolu Papa Rao[1], Gustavo Stolovitzky[1].
[1]CIGIT.CAS, Beibei District, Chongqing, China, [2]Illumina, San Diego, CA, USA, [3]Ion Torrent by life Technologies, Guilford, CT, USA.

We introduce a method to fabricate solid-state nanopores at sub-20 nm diameter in membranes with embedded metal electrodes across a 200 mm wafer with CMOS compatible semiconductor processes. Multi-layer (metal-dielectric)