By reduction of their contact area, we were able to reduce the measurement noise sufficiently to perform single molecule translocation and even nanopore force spectroscopy experiments. We applied this technique to the translocation of DNA hairpin molecules, but also to a G-quadruplex DNA structure, which has not been characterized using nanopore force spectroscopy before. From the data, the unfolding rates of these DNA structures are extracted and compared with those obtained with other single molecule techniques.

3116-Pos

Studying Voltage Dependent Noise in Polymer and Solid State Nanopores

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Studying the noise properties of ion currents in nanopores can improve detection limits for nanopore sensors as well as give insight into behavior of transport at the nanoscale. We focused on the so-called 1/f noise that is observed in the low frequency regime of the ion current power spectra. We found that 1/f noise in single conically shaped nanopores in polymer films exhibits voltage-dependent noise properties, which are not observed for cylindrical pores. The current passing through the nanopore in the low conductance state shows equilibrium 1/f noise, similar to the noise observed in solid state nanopores. Equilibrium fluctuations are defined as the voltage independent power spectrum magnitude normalized by the current squared. The high conductance state causes the 1/f noise to increase exponentially with increased applied voltage, showing a non-equilibrium 1/f noise. Therefore we can switch between the equilibrium and non equilibrium behavior simply by adjusting the voltage. The current in the high conductance state is about 5 times higher then the current in the low conductance state but the noise at 1 Hz is over 100 times higher. Cylindrically shaped nanopores in polymer and solid-state films do not show current rectification and show equilibrium 1/f noise. We discuss these results and give a comparison of the nanopore noise in these various systems. We hypothesize that the non-equilibrium current fluctuations originate from structural fluctuations of flexible polymer pores. The hypothesis is tested by comparison of noise properties between polymer and silicon nitride pores studied at different electrolyte concentrations.

3117-Pos

Slowing DNA Translocation through Nanopores using Organic Salt Solutions

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One of the key challenges to nanopore DNA sequencing is to slow down DNA translocation. Here, we report that the translocation velocities of various DNA homo- and co-polymers through protein pores could be significantly decreased by using electrolyte solutions containing organic salts. Using a butylmethylimidazolium chloride solution instead of the commonly used KCl solution, DNA translocation rates on the order of hundreds of microseconds per nucleotide base were achieved. The much enhanced resolution of the nanopore coupled with the different event blockage amplitudes produced by different nucleotides permits the convenient differentiation between various DNA molecules.

3118-Pos

Electrophysiological Method for Quantification of the Number of phi29 DNA Packaging Nanopore in Planar Bilayer Membrane

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Bacterial virus phi29 uses one of the strongest DNA packaging nanomotors to package its micron-length genomes into a pro-capsid. After re-engineering, whether the DNA nano-motor can be used to pump drugs, DNA, RNA or other therapeutic molecules into specifically targeted cells represent a great challenge in nanomedicine. We have recently successfully embedded the connector, a core component of phi29 DNA package motor, into a planar bilayer membrane (BLM). Under an electric field, double-stranded DNA translocated through the connector channel.

The application of phi29 connector array as a stochastic sensor requires knowledge of the number of channels on each membrane. We herein report a method for precise counting of the number of channels on each membrane by electrophysiological approach. Generally, the number of channels is determined by the conductance of total channels and conductance per single channel. Using a derived empirical equation, we can calculate the conductance per single channel at any salt concentration using conductivity of respective conducting buffer. The total conductance of total channels can be measured by ionic current through all the channels under an applied ramp voltage. Comparison of calculated and true values established this as a feasible, reliable and reproducible approach.

3119-Pos

Ultrathin Nanopores for Nucleic Acid Analysis

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Over the past decade, synthetic nanopores in solid-state membranes have gained reputation as platforms for studying the biophysical properties of biopolymers. In this presentation, the precise fabrication of ultrathin nanopores and their utility for analyzing complex nucleic acid samples will be discussed, with major emphasis on their resolution capabilities for different biopolymers. The membrane thickness was found to play an important role on the signal obtained from different biopolymers. These findings are critical for developing ultrasensitive nanopore assays which profile biopolymer structure, important for genomics and other biophysical studies.

3120-Pos

Single Channel Sensing of dsDNA using the Membrane- Adapted Nanopore of phi29 DNA Packaging Motor

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The bacteriophage phi29 DNA-packaging motor, geared by six pRNA molecules, contains a truncated cone shape connector channel that is 3.6nm in diameter at its narrow end and 6nm in diameter at its wide end. This channel allows dsDNA to enter and exit the virus procapsid during virus maturation and infection, respectively. We modified the genes that code for the core of the phi29 DNA packaging motor in order to change the amino acid sequence of its protein for membrane incorporation. The modified connector was reconstituted into liposomes and fused into a planar lipid bilayer membrane. Distinctive current jumps were found for each connector insertion. The conductance of each connector channel was measured and found to be uniform (4.8nS in 1M KCl). The membrane embedded connector channel was found to be able to translocate dsDNA. The translocations were recorded as blockage events of the current. The blockages were equal for each of the individual channels, generating a clean, homogenous and uniform signal representing DNA translocation.

The connector channel is larger than the previously studied ion channels, which could only let ssDNA pass. The robust property of the connector in ion and dsDNA tranlocation has extensive potentials in microelectromechanical sensing, microreactors, gene delivery, drug loading and DNA sequencing. Single molecule and low concentration sensing can be achieved using this membrane embedded connector system. Additionally, the available crystal structure of the connector protein makes it easy to modify the channel for specific applications and the established large scale purification procedure of the connector will facilitate its practice.

3121-Pos

Fingerpriting of DNA and RNA using the Channels of Bacteriophage phi29 DNA Packaging Motor

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Living systems contain a wide variety of nanomachines and highly-ordered structures of macromolecules that could serve as modules, tool boxes or building blocks in nanotechnology. The ingenious design of the bacteriophage phi29 DNA packaging motor with an elegant and elaborate channel has inspired its application for single molecule detection and sensing. The central component of the phi29 motor is the connector composed of twelve copies of the protein gp10, which form a dodecamer channel. The connector after incorporation into a lipid bilayer can serve as a detector for extremely sensitive, reliable, and precise sensing and fingerprinting of ions and macromolecules at the single molecule level (Nature Nanotechnology, in press). Double stranded and single stranded DNA can be electrophoretically driven through the channel in a concentration and voltage dependent manner. Information about the structure, length and conformational dynamics can then be deduced by their characteristic dwell time during translocation and by their relative percentage in current blockades. This protein nanopore system with explicit engineering capability has potential technological applications such as rapid DNA sequencing, gene therapy and controlled drug delivery.

3122-Pos

Inhibition of the Voltage-Gated Sodium Current and Opening of Nanopores By Ultra -Short Electric Pulses

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Exposure of mammalian cells to high-voltage, ultra-short electric pulses (USEP) leads to formation of membrane nanopores and alters multiple physiological processes, including function of voltage-gated channels. However, it is not known if USEP affect the channels directly, or the effects are mediated

by leak currents through nanopores and respective shifts in the transmembrane ion balance. We employed whole-cell patch-clamp to explore the effect of 300-ns USEP on voltage-gated sodium channels in neuroblastoma cells (NG108). We found that a single USEP could inhibit VG INa , with the threshold at about 1.8 kV/cm. Voltage-dependent activation and inactivation curves shifted to more negative membrane potentials: V0.5 of activation moved from -22.8 ± 0.2mV before USEP to -26.4 ± 0.6mV after it (mean $\pm$ s.e.), and V0.5 of inactivation changed from -65.9 ± 0.2mV to -72.2 ± 0.2, respectively; the slope factor did not change. Concurrently, USEP exposures induced a non-inactivating, voltage-sensitive inward current due to nanopore formation. The presence of 100μM Gd3+ in the bath buffer significantly reduced the nanopore current and also eliminated the inhibitory effect of USEP on VG INa. This finding suggests that USEP-induced inhibition of VG INa. and changes in its kinetic characteristics may be mediated by opening of nanopores and consequent alterations of the ion equilibrium.
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3123-Pos
Rectification of a Modified Nanofluidic Diode Dependent on the pH
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We study the affect of varying pH on the rectification of a nanofluidic diode. The diode is a conically shaped nanopore in PET or Kapton that has a distribution of charge along the surface. The distribution is such that there is a boundary where one side is positively charged and the other side is negatively charged. We also measure nanopores that have neutral and negative charges on either side of the boundary. The charges are modified by a 2 step chemical reaction using EDC/PFP for the 1st step and ethylenediamine or propylamine for the second step. Characterization of the nanopore was done by taking I-V curves from −5V to 5V with buffered solutions of KCl.

3124-Pos
Light-Induced Permeability Changes in Liposomes Containing Photo-Polymerizable Phospholipids
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We have designed a novel class of light-triggerable liposomes prepared from a photo-polymerizable phospholipid $DC_{8,9}PC$ (1,2- bis (tricosa-10,12-diynoyl)-*sn*-glycero-3-phosphocholine) and DPPC (1,2-Dipalmitoyl-*sn*-Glycero-3-Phosphocholine). Exposure to UV radiation (254 nm) for 0-45 minutes (25 ^{0}C) resulted in photo-polymerization of $DC_{8,9}PC$ in these liposomes and the release of an encapsulated fluorescent dye (calcein). Photopolymerization and permeability changes did not occur from UV-triggered Egg PC/$DC_{8,9}PC$ liposomes. We propose that phase separation and packing of polymerizable lipids in the liposome bilayer are major determinants of photo-polymerization resulting in the formation of local defects and/or lipidic pores in the liposome membrane. Differential Scanning Calorimetry show phase transition peaks at 36.8 ^{0}C and 41.6 ^{0}C, respectively, in liposomes composed of DPPC:$DC_{8,9}PC$ (9:1 mole ratio) indicating that the reactions occurred while these lipids are in the gel phase (25 ^{0}C). Our results indicate that $DC_{8,9}PC$ and DPPC molecules undergo de-mixing in the gel phase. This hypothesis is supported by Molecular Dynamics simulations that indicate separation of $DC_{8,9}PC$ and DPPC in the solid phase lipid bilayer. Cryo-electron microscopic images of the liposomes show major changes in liposome morphology after UV irradiation. When an appropriate tunable photo-sensitizer dye is included in the aqueous compartment of these liposomes, release of contents is triggered by excitation with a laser at the wavelength of the encapsulated dye. Inhibition of release in the presence of oxygen radical scavengers indicate that the mechanism of release involves chemical changes in $DC_{8,9}PC$ unrelated to photo-polymerization. The laser-mediated chemical modifications in $DC_{8,9}PC$ are being analyzed by MS, LC, GC and NMR. We are further developing these liposomes for undergo triggered release of chemotherapeutic agents (e.g. doxorubicin) and are testing their efficacy *in vitro* and *in vivo*.

3125-Pos
Label-Free Immunoassay Based on Functionalized Nanopipette Probes
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Nanopipette technology is capable of detecting and functional analyzing biomolecules based on difference on their size, shape and electrical charge. This unique label-free biosensor is inexpensive, easy to fabricate and versatile. It gives a fast and real time output even in small reaction volume (attoliters). At this point, the nanopipette size and geometry, together with the surface chemistry preparation for attachment of a biomarker, antibody or protein probe was optimized by both experiments and modeling to result in detectable signals by the nanopipette. In this phase, the goal of the surface chemistry procedure was to prepare nanopipette tip in a way that only controlled amount of the surface is functionalized and used for probe attachment. Preliminary experiments are demonstrating the sensitivity and selectivity of the technique with specific proteins targeting HPV as well as environmental toxins. These results prove that nanopipettes functionalized with appropriate molecular recognition elements can be used as HPV/toxin sensors. A highly sensitive nanopipette probe can be precisely positioned, unlike other nanosensing technologies, at any subcellular region of a single living cell with submicron accuracy using a micromanipulator. This approach uses a movable sensor on an attached cell, in contrast to a fixed sensor detecting responses from floating cells. The functionalized nanopipette paves the way for in vivo immunoassay down to the single cell level.
Reference:
S. Umehara, M. Karhanek, R. W. Davis and N. Pourmand, PNAS, 2009, 106, 4611.

3126-Pos
The Nano-Scale Secret of Biological Secretion for Adhesion
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We discovered, by employing the atomic force microscope and transition electron microscopy, that 1) ivy and marine mussels secrete nanoparticles for surface permanent adhesion, whose force is significant larger than the force generated by gecko for reversible surface adhesion; and 2) Sundew secretes nanoparticles, which are cross-linked with muco-polysaccharide for high elastic adhesive for prey trapping. The nanoparticle-based adhesion mechanism has important implications for engineering surface adhesive materials and devices for biomedical applications.
Adhesion in nature has been the focus of intense study over the past few years. Nevertheless, research in this field has primarily concentrated on understanding the chemical aspects of adhesion. While scientists have been able to determine some of the molecular structures present in the adhesives secreted by surface affixing biological systems, such as mussels and barnacles, the fundamental adhesion mechanisms used by these systems are still unknown. This research focuses on the nano-scale morphological similarities of adhesive materials secreted from marine mussels, barnacles and ivy. We have discovered that marine mussels secrete large amounts of adhesive materials in the form of nanoparticles for surface adhesion. This is in keeping with our previous work, which indicated a similar phenomenon for ivy. Both studies concur with earlier research on marine barnacles, polychaetes and sea stars. Taken together, these results indicate that nanoparticles are used by natural, biological systems to increase surface adhesion.
We recently extended the study to Sundew and observed that nanoparticles secreted from the Sundew tentacles form scaffolds by cross-linking with munopolysaccharide. The secreted material is highly elastic and has been effectively used by the sundew for trapping prey.
The ivy nanoparticles have been isolated from the secretion using SEC-HPLC. Physical properties have been further characterized and will be discussed in details through this talk.

Biotechnology & Bioengineering II

3127-Pos
A Novel Approach for Efficient Photosynthetic Hydrogen Production
Nathan Nelson.
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Despite its enormous complexity, the plant PSI is arguably the most efficient nano-photochemical machine in Nature. It emerged as a homodimeric structure containing several chlorophyll molecules over 3.5 billion years ago, and has perfected its photoelectric properties ever since. Based on the structure of cyanobacterial and plant PSI together with the information gained from recently discovered PSI encoded by marine viruses we suggested a holistic solution for reasonable and efficient photosynthetic hydrogen production. Essentially what separates photosynthesis and respiration is the unique soluble cytochrome recognition by cytochrome oxidize and PSI. We think that the virus eliminated it by the introduction of PsaJ-F fusion protein. This generates a novel photorespiration that can be operated under anaerobic conditions providing there is an electron acceptor available. Thus practically we can utilize it for hydrogen production from organic material where the electron is donating by PSI > -0.6 V instead of NADH -0.34 V where PSII is inactivated and that way to separate for the first time in photosynthetic organism oxygen and hydrogen production in the light. Thus our system utilizes cyanobacteria engineered to have a novel