Board B602

We investigate the incorporation of semiconducting polyelectrolytes, poly(2,5-methoxy-propyloxy sulfonate phenylene vinylene) [MPS-PPV], into ribonucleoprotein vaults—hollow barrel-like protein cages. They are the largest ribonucleoprotein found in higher eukaryotic cells. Although cellular function of these protein cages is unknown, its hollow cavity and subcellular localization suggest that they may be involved in nucleo-cytoplasmic transport. Their hollow capped-barrel shape is measured to be 40 by 70 nm. However, a mechanism for access to the vaults' interior has not been identified. By filling the cage interior with semiconducting polyelectrolytes, whose photophysics is strongly dependent on its environment, we can compare polymer conformation in these confined systems to conformation in solution using fluorescence spectroscopy and Small-angle X-ray Scattering (SAXS). The combined results from fluorescence measurement, fluorescence quenching studies, and SAXS measuments indicates that luminescent semiconducting polymers can be localized in of the vaults interior. And the results indicate that vaults can potentially be used as biologically synthesized nanocapsules for delivery and encapsulation applications.

1627-Pos Using Multiple Luminescent Probes To Report Molecular Mobility In Amorphous Solids

Rashmi S. Tiwari, Richard D. Ludescher

Rutgers University, New Brunswick, NJ, USA.

Board B603

The rates of physical change and chemical reaction in amorphous solid biomaterials are controlled in mysterious ways by the molecular mobility of the closely packed, hydrogen bonded matrix. In an effort to understand how mobility modulates these rates, we have developed a small library of polar luminescent probes that are sensitive to local dynamic properties of the matrix. This study has explored the possibility of using multiple probes simultaneously dispersed within an amorphous solid to investigate the mobility of different components (protein and carbohydrate, for example) with the matrix.

Phosphorescence emission spectra and time-resolved intensity decays were collected for each probe in binary mixtures of erythrosin B, vanillin, and tryptophan dispersed within thin films of amorphous sucrose. Data collected from these mixtures at a variety of temperatures were compared to data collected from sucrose films containing a single probe. Maximum emission energy and bandwidth were determined by fitting spectra to a log normal distribution function while lifetimes were determined using either a stretched exponential decay function (erythrosin B and tryptophan) or a sum of exponentials (vanillin).

Emission energies and lifetimes from the binary mixture of erythrosin B and vanillin probes were indistinguishable from data from films with individual probes, except for an anomalous increase in erythrosin delayed fluorescence in the mixture. Lifetimes from erythrosin B and tryptophan in the binary mixture were also similar to those in films with individual probes. Data from the tryptophan/vanillin mixture, however, differed from the single probe data due to the difficulty of selectively exciting tryptophan in the presence of vanillin. This research thus demonstrates the possibility of collecting mobility data on several components of a complex amorphous solid by simultaneous labeling with multiple probes.

Micro- and Nanotechnology Nanopores

1628-Pos Recent Developments Towards A Centralized Repository For Structural Nanobiology Data

Raul E. Cachau[1], Fernando D. Gonzalez-Nilo[2]

[1] *NCI-SAIC, Frederick, MD, USA*
[2] *CBSM, Universidad de Talca, Talca, Chile.*

Board B604

We developed a prototype repository and clearinghouse for the fast exchange of information among nanobiology practitioners, interested in the structural aspects of this emerging field. This repository acts as a prototype engine for analysis tools design and structure dictionary development. New forms of annotation and data integration must be developed to fully integrate structural and biological information. Our observations on data integration and pattern mining will be discussed in this presentation.

1629-Pos Nanopore Cheminformatics Analysis Of Aptamer-target Binding Strength

Angela Hernandez[1,2], Amanda Davis[2], Karen Thomson[1,2], Stephen Winters-Hilt[1,2]

[1] *University of New Orleans, New Orleans, LA, USA*
[2] *Research Institute for Children, Children's Hospital, New Orleans, LA, USA.*

Board B605

Aptamers are nucleic acids selected for their ability to bind to molecules of interest and may provide the basis for a whole new class of medicines. They are replacing antibodies as detection reagents due to several advantages: versatility, the creation of a lab-on-a-chip approaches, low detection limits, simpler reactions to perform, and due to the diversity and specificity of aptamer-target binding.

Two varieties of bifunctional 'pseudo-aptamer' (obtained by design, not by SELEX) are examined using a nanopore detector: the three-way dsDNA junction, and the linear dsDNA with bulge. According to our design, the blunt-ended extremity of the dsDNA molecule inserts itself within the alpha-hemolysin channel, producing sensitive, highly modulated, blockade signals, while the free extremity of the DNA molecule is designed to terminate in an

ssDNA overhang (or "sticky" end). The lengths of ssDNA overhangs examined in the binding (annealing) study ranged from one to several bases. Preliminary statistical analyses using hidden Markov models (HMMs) indicates a clear change in blockade pattern upon ssDNA binding to the captured aptamer's complementary overhang region. This is consistent with the hypothesis that significant conformational changes occur during the annealing/binding event. Eventually, the objective is to extend the ssDNA portion to be a well-studied, ~70 base, ssDNA aptamer, joined to the same bifunctional aptamer molecular platform. One such study in-process focuses on G-4 complex aptamers, particularly those that bind thrombin. A minor change to the Y-aptamer's 'base' may also be used to establish a force-spectroscopy platform on the dissociation kinetics of the G-4 complex, and thereby, indirectly, on the aptamer-thrombin dissociation kinetics. Nanopore-cheminformatics guided refinements SELEX-identified aptamers may eventually provide a means for more directed, aptamer-based, drug discovery.

1630-Pos Structural Analysis Of Nanoparticles Functional Building Blocks And Nanoscale Periodic Patterns

Nicole Keller, Ian McRae, Raul E. Cachau

Advanced Technology Program, NCI, SAIC-Frederick, Frederick, MD, USA.

Board B606

Nanobiology is a new discipline that integrates fundamental research in biology, physics, engineering and the biomedical sciences. The use of nanoparticles in the biomedical sciences opens the doors to the delivery of new, more potent, effective and better targeted agents to the cell, enabling the development of new strategies for improving human health. The peculiar nature and the novelty of nanoparticles' chemistry demand the proper characterization of the nanoparticles functional building blocks and the common patterns relating chemically diverse moieties. In this presentation we will analyze the structural patterns emerging in families of nanoparticles stored in a recently developed database (see Cachau & Gonzalez-Nilo, this meeting) and correlate their structural properties with biological data. The patterns found will be used to establish enhanced forms of annotation and improve the design of future generations of nanobioparticles.

1631-Pos Motive Principle of the Conservation of Differential on the Macroscale Clarifies Second Law Violations in Microstate

Nancy Beth Orr

Science Foundation for Public Policy, Palo Alto, CA, USA.

Board B607

Neurophysiological inferences empirically derived from in-vivo observation of time-dependent motion-analysis subjects were compared to data drawn from observational astronomy to yield a general *motive principle* observed to operate at both macro and micro scales.

The conservation of energy is proposed derived from the *conservation of differential* $E_s{=}K$ which includes four-dimensional quantized spacetime, and therefore the degrees of freedom necessary for the differential required of motion: and thus, for energy to exist. The motive principle of space-energy differential cannot be annulled: differential can only re-arranged. This axiom of first cause, paired with *equidynamics*, serves to clarify the transient statistical exceptions to the second law in microstates are not in violation of superceding principles.

While the Lagrangian equation is characterized by calculation of the path of least action, equidynamics proposes to also calculate the local *resolution of differential:* $\delta r{=}\Sigma\delta\Re$. This distinction is crucial in nanoscale interactions, non-covalent forces, non-equilibrium states and self-assembly of complexity because since *any difference* is potential for motion; equidynamics presumes; and so, better reveals greater variation of qualitative attributes casual to biophysical events.

Though the resolution of differential has a time arrow, *motion is non-preferential in direction of energy flow according to* $\Phi{=}\Re$ whereby any state is the summing of causally-interactive spatio-temporal-energy relationships; resulting in $\delta r{=}\Sigma\delta\Re$ which describes motion as non-preferential to higher or lower states. The only preference is that movement is toward the *equipoint* of system attributes.

The *second law of thermodynamics is proposed clarified* as follows: Entropy is ≥ 0 on average, *in a spatially expanding Universe system*. In gedanken experiments of a collapsing universe, with increasing spatial confinement, entropy is ≤ 0. Variations about the average are predicted and causal to both construction and dissolution of complexity.

1632-Pos Surface Enhanced Raman Scattering (SERS) Substrates with Controlled Morphology and Architecture Created by Biomineralization

Bingbing Sun, Hong Shen

University of Washington, Seattle, WA, USA.

Board B608

Surface Enhanced Raman Scattering (SERS) is becoming a powerful vibrational spectroscopic tool for the detection, identification and differentiation of biological molecules. SERS substrates with high reproducibility, stability and significant Raman enhancement are desired for the increasing applications of SERS. Biomineralization is an elegant process, which has created a variety of nanostructured architectures in nature. In this study, by mimicking naturally occurring biomineralization, a process was developed to construct active SERS substrates. Silver nanostructures, which formed through the reduction of silver nitrite with sodium borohydride incorporated in the biominerals, adopted the controlled morphology and architectures of biominerals. UV-visible spectroscopy, scanning electron microscopy and energy dispersive spectroscopy were used to char-

acterize the substrates. SERS signals from rhodamine 101 adsorbed onto the biomineralized Ag substrates were acquired by a Renishaw InVia Raman Microscope. We have demonstrated that the composition of mineral solutions dictates the nanostructure size and architecture of substrates, resulting in differential Raman enhancements. Our results demonstrate that biomineralization is a very convenient and cost-efficient process for fabricating SERS substrates with high reproducibility and stability.

1633-Pos Electroporation Chip For Adherent Cells On Photochemically Modified Polymer Surfaces

Irene Frischauf[1], Michael Olbrich[2], Esther Rebollar[2], Christoph Romanin[1], Johannes Heitz[2]

[1] *Institute for Biophysics, Linz, Austria*
[2] *Institute for Applied Physics, Linz, Austria.*

Board B609

Spatially localized UV-surface modification of polymers is a powerful tool for the fabrication of high throughput microarrays. Here we photochemically modified the surface of polytetrafluoroethylene (PTFE) and ultra low adhesion (ULA) Petri dishes resulting in enhanced cell adhesion and proliferation at the modified areas. An excimer lamp (Xe2*) was employed as light source. Reactive gases include ammonia (NH3) and oxygen (O2). Photo-dissociated fragments of the reactive gases reacted either directly with the polymer surface or are deposited thereon. Newly incorporated chemical groups at the surface significantly increased the biocompatibility of the polymer and the adhesion of deposited metals. The latter was utilized to implement an electroporation setup suitable for adherent transfection of several biological cell types such as human embryonic kidney cells (HEK293). Thus, the metallic micro electrodes were directly deposited at the surface as polymer modification substantially improved metal adhesion. Electroporation parameters were optimized based on the transfection of enhanced green fluorescent protein (EGFP) to evaluate the potential of our electroporation chip for microarrays. This prototype PTFE electroporation microchip was compared with micro patterned ULA Petri dishes and with standard cell culture polystyrene (PS) Petri dishes. Transparent, working at low voltages with adherent cells, this electroporation chip substantially reduced the amount of sequential working steps usually required for transfection and subsequent assay methods. The electroporation microchip might have the potential for cost-efficient high density microarrays applicable in high throughput screening methods.

Supported by FWF: NSI-NBPF, BI-0102.

1634-Pos Reversal Potential Of Synthetic Conical Nanopores: Theory And Experiment

Javier Cervera[1], Antonio Alcaraz[2], Birgitta Schiedt[3], Reinhard Neumann[3], Patricio Ramirez[4]

[1] *Universitat de Valencia, Valencia, Spain*
[2] *Universitat Jaume I de Castelló, Castelló, Spain*
[3] *Gesellschaft für Schwerionenforschung (GSI), Darmstadt, Germany*
[4] *Universitat Politècnica de València, Valencia, Spain.*

Board B610

Membranes containing a single nanopore generated with track-etching techniques have emerged as promising materials to mimic biological ion channels. Synthetic nanopore membranes have also potential applications in electrically assisted single-particle detection, analysis and separation of biomolecules. They are more robust and allow easier control of the geometry than biological channels, and can readily be integrated into functional systems. We present here a detailed theoretical and experimental account of the reversal potential in synthetic conical nanopores. In these systems the fixed charge distribution is inhomogeneous and, as a result, the ionic selectivity is not only a property of the nanopore itself, but depends also crucially on the direction of the concentration gradient. Previous studies of current-voltage curves provide an adequate description of the conductive properties of the system, but give only indirect clues about how charge regulation is performed. The study of the reversal potential offers in this sense essential information. We present here a physical model for reversal potential in conical nanopores based on the Nernst-Planck and Poisson equations. The theoretical results are compared with experimental data obtained and good agreement is found using only one fitting parameter, the surface charge density, which is determined independently from the current-voltage curve of the nanopore.

1635-Pos Single-walled Carbon Nanotubes: Interactions and Assembly

Rudolf Podgornik[1], Rick F. Rajter[2], Adrian V. Parsegian[1], Roger H. French[3], Wai-Yim Ching[4]

[1] *NICHD/NIH, BETHESDA, MD, USA*
[2] *Department of Materials Science and Engineering, MIT, Cambridge, MA, USA*
[3] *DuPont Co. Central Research, Experimental Station, E400-5207, Wilmington, DE, USA*
[4] *Department of Physics, University of Missourîâ "Kansas City, Kansas City, MO, USA.*

Board B611

Promising as practical devices, alluring as model objects for theory, simple enough for description by precise wave functions, complicated enough to confound handling, nanotubes taunt us. Beginning with a description of the forces between nanotubes themselves, dominated by van der Waals attractions, this poster will describe a vast and varied range of forces within what seemed to be a set of cartoon-simple structures. The strength and the nature of the forces that guide assembly will be assesed by investigating the van der Waals - London dispersion interactions between a single-walled carbon nanotube immersed in water and interacting with three different objects: an optically isotropic planar substrate, an optically anisotropic planar substrate, and another single-walled carbon nanotube of identical chirality. These interactions were derived from ab initio optical properties and an appropriate formulation of

the Lifshitz theory. The effect of relative geometry and the relative separation on the magnitude of the dispersion interaction was investigated in detail. There emerges a learning strategy- through spectroscopy, through assembly with other materials, and through computation - to test whether these materials can be used to realize the expectations made of them.

1636-Pos The Assembly of Quantum Dots Arrays Using Actin Filaments as Templates

Lan Yao[1], Gregory Andreev[2], Oleg Andreev[1]

[1] *University of Rhode Island, Kingston, RI, USA*
[2] *University of California, La Jolla, CA, USA.*

Board B612

The fabrication of semiconducting and metallic nanowires is a significant challenge for the nanoscale electronic devices. One of the attractive ideas is the application of the self-assembling biomolecules as templates for fabrication of nanowires. Here we proposed to use F-actin for fabrication of semiconductor nanowires. We developed three methods of conjugation of quantum dots (QDs) to F-actin:

(i) EDC-induced conjugation of amino-QDs (Invitrogen, Inc.) to carboxyl groups at N-terminus of actin;
(ii) conjugation of amino-QDs to Cys residue at C-terminus of actin using bifunctional crosslinkers with NHS ester and maleimide active groups;
(iii) initial conjugation of carbohy-QDs with amino-phalloidin (it has high affinity to F-actin) and then incubation with F-actin.

The several QDs were tested, some of them made long and more uniform filaments than other, probably due to a compatibility of F-actin periodicity with the size of QDs. The obtained nanowires were visualized by fluorescence microscopy since QDs demonstrate a strong fluorescence with negligible photobleaching. The fluorescent properties of free QDs and attached to F-actin have been studied using fluorescence spectroscopy and fluorescence anisotropy imaging method developed in our laboratory. The data indicated that QD transition dipoles are likely circular (elliptical) dipoles rather than linear dipoles that are characteristic for the most of organic dyes. Our preliminary results showed the feasibility of fabrication of long and uniform semiconductor filaments by conjugation of QDs to F-actin.

This work was supported by URI foundation grant to O.A. The correspondence should be addressed to Oleg Andreev (andreev@-mail.uri.edu).

1637-Pos The Cytotoxic Effects of Quantum Dots on the Oocytes of *Caenorhabditis elegans*

Shyemaa Shehata, Cecile Fradin

McMaster University, Hamilton, ON, Canada.

Board B613

Recently, quantum dots have begun to be used as fluorescent probes for live cell imaging. Their photostability makes them suitable for tracking long term events which is not possible to do with fluorescent proteins, while their size allows them to be used for studying single molecule behavior. However, little is known of their toxic impact on cells. We are interested in understanding these cytotoxic effects, and chose *Caenorhabditis elegans* as a model organism. Quantum dots are introduced into these nematodes by microinjection. They are then taken up by already existing oocytes and are incorporated into any oocytes formed by the nematode after microinjection. After the oocytes are fertilized, their development is tracked over time. This is repeated for different quantum dot doses and the developing worms are monitored to detect any abnormalities. Confocal fluorescence images of the nematodes are also taken and used to quantify the uptake of the quantum dots into the oocytes. This provides further understanding of the dose dependency of the quantum dots on cytotoxicity. In addition, fluorescence correlation spectroscopy is being used to understand the dynamic behavior of the quantum dots in the oocytes. Together these results provide a picture of how quantum dots are taken up by cells, their behavior once in the cell and any subsequent cytotoxic effects that they may have.

1638-Pos Nanofluidic Diodes and Bipolar Transistors

Eric B. Kalman[1], Ivan Vlassiouk[1], Pavel Apel[2], Zuzanna S. Siwy[1]

[1] *University of California, Irvine, Irvine, CA, USA*
[2] *Flerov Laboratory of Nuclear Reactions, Dubna, Russian Federation.*

Board B614

There are well-known devices for controlling the transport of electrons, but very few control ions in a solution. Ionic rectifiers prepared on the basis of single conically-shaped glass nanopipettes and nanopores were the first examples of a system that could control ionic flow electrically without the necessity of introducing moving (mechanical) parts. The next step forward was made by preparing ionic diodes that function according to a similar mechanism as that of semiconductor diodes. We accomplished it by patterning the surface of single conical nanopores in polymer films with sub-10 nanometer openings so that there was a zone with positive charges and a zone with negative charges, similar to a PN junction. These rectifying devices are capable of rectifying ion currents with degrees of rectification reaching several hundreds and show much smaller off-state currents than found for the simple conically-shaped nanopores. Efforts were also made towards achieving even more precise control of the transport of ions and charged molecules by constructing ionic transistors. We developed the first bipolar ionic transistor that functions in a similar way to its semiconductor bipolar junction (BJT) counterpart. The device consists of a single double conical or hourglass-shaped nanopore in a polymer film with the small pore opening between 2 and 6 nm in diameter, again chemically modified so that there are zones of positive charge at the large pore openings, and negative charge near the small opening in the

pore center. We show we can chemically influence the electric potential in a sufficient manner to gate the ion current.

1639-Pos Ion Current Oscillations Caused by Femtoliter Volume Precipitation in a Nanopore

Matthew R. Powell[1], Michael Sullivan[1], Ivan Vlassiouk[1], Dragos Constantin[1], Olivier Sudre[2], Craig Martens[1], Robert S. Eisenberg[3], Zuzanna S. Siwy[1]

[1] *University of California, Irvine, Irvine, CA, USA*
[2] *Teledyne Inc, Thousand Oaks, CA, USA*
[3] *Rush Medical College, Chicago, IL, USA.*

Board B615

The fixed negative surface charges inside single conical polymer nanopores result in transport properties not encountered in micrometer-scale counterparts. A notable example of an effect caused by these permanent negative charges is the enhancement of ionic concentration inside the pore when compared to the bulk solution. The nanopores described here are created with the track etching technique resulting in a conical geometry having a large opening of hundreds of nanometers and a small opening of several nanometers. A new phenomenon is presented detailing an oscillating ionic current through our conical nanopore when a small amount of a divalent cation is added to a buffered monovalent ionic solution. An ionic current enhancement brought on by the superposition of the electric field from the fixed negative surface charges and the externally applied electric field causes a formation and redissolution of nanoprecipitates that temporarily block the ionic current through the pore. The frequency and character of these ionic current oscillations is regulated by the transmembrane potential and the chemistry of the nanoprecipitate. This oscillating system could be used as a model for studying nonlinear electrochemical processes and early stages of crystallization.

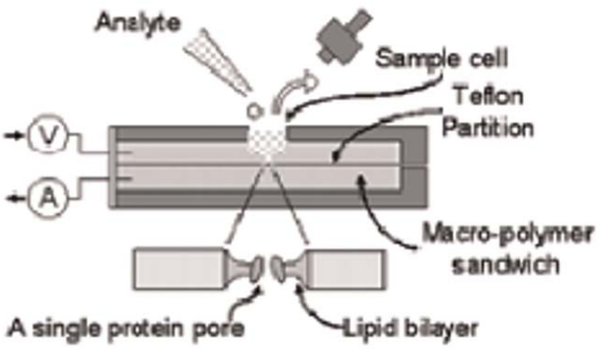
1640-Pos Biosensing On A Modular And Portable Chip Containing A Single Ion Channel

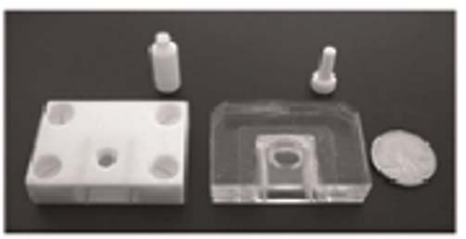
Ji Wook Shim, Li-Qun Gu

University of Missouri, Columbia, MO, USA.

Board B616

Engineered protein channels can be used as receptive probes for single-molecule biosensing. Future biosensors might be created from an array of target-specific ion channels in which each protein pore acts as a sensor element. One step toward this goal is to create a portable, durable, single protein channel-integrated chip device. We have developed a versatile, modular chip that contains a single ion channel. The core of the device is a long-lived lipid membrane sandwiched between two air-insulated agarose layers with gel *in situ*. A single protein pore embedded in the membrane serves as the sensor. This modular device is highly portable, allowing a single ion channel to continuously function following detachment from the instrument, independent transportation and storage. It is also highly durable, as evidenced by continuous observation of single channel dynamics for days. Once protein pores are installed, the chip becomes a robust sensor for real-time targeting such as detection of the second messenger IP3. This pluggable biochip could be incorporated into many devices, such as a micro-fluidic system, and be made into a micro-array for both biomedical detection and membrane protein research.





1641-Pos Detection of Clinically-Relevant Cryoglobulins using Resistive-Pulse Sensing

Erik C. Yusko, Jeff D. Uram, Robertson D. Davenport, Michael Mayer

University of Michigan, Ann Arbor, MI, USA.

Board B617

We present a nanopore-based assay that uses the resistive-pulse technique for detecting and analyzing the formation of aggregates from cryoglobulin proteins. Cryoglobulins are abnormal immunoglobulins present in blood that can aggregate and precipitate at temperatures below 37°C causing symptoms such as blood hyperviscosity, vasculitis, skin lesions, liver or spleen enlargement, joint pain, kidney ailments, and peripheral nerve damage. Current methods for detecting and analyzing cryoglobulins require incubating 5–10 mL of blood serum at 4°C for 3–7 days and observing the volume occupied by the precipitated cryoglobulins (cryocrit). The assay we are developing is designed to be rapid (minutes to hours), to require only micro liters of blood serum, and to function by monitoring the change in electrical resistance (resistive-pulse) when a cryoaggregate passes through a pore. Preliminary data from resistive-pulse analysis of blood serum from patients known to have cryoglobulin

proteins exhibited an increase in peak amplitude and frequency of the resistive-pulses when compared to blood serum from healthy controls. Unlike the current method of cryoglobulin detection, this technique provided the opportunity to study kinetics of cryoaggregate formation and the threshold temperature at which cryoaggregates begin to form. Pore-based resistive pulse sensing may therefore be a useful method for rapidly detecting the presence of cryoglobulins in a clinical environment, and the kinetics of aggregate formation as well as the threshold temperature for aggregate formation may correlate with clinical symptoms and lead to improved patient care.

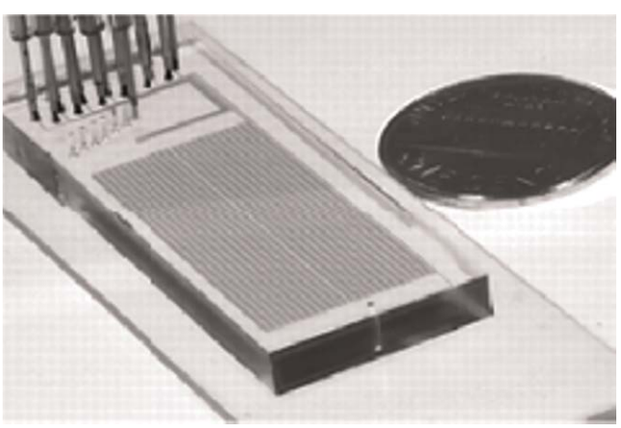
1642-Pos Pharmacological Inhibitors of a New Hepatitis C Target Discovered by Microfluidic Affinity Analysis

Doron Gerber, Shirit Einav, Paul Bryson, Ella H. Sklan, Menashe Elazar, Sebastian Maerkl, Jeffrey S. Glenn, Stephen R. Quake

Stanford University, Stanford, CA, USA.

Board B618

Effective therapies are urgently needed against Hepatitis C virus (HCV), a major cause of viral hepatitis. We used *in vitro* protein expression and microfluidic affinity analysis to study RNA binding by the HCV transmembrane protein NS4B, which plays an essential role in HCV replication. We characterized the microfluidic system with HUR and HUD. Both bind RNA specifically. HUD has dissociation constants (Kd) of 23nM and 268nM to AU3 rich and mutanted motifs, similar to what was previously described in the literature. Next, we show that HCV NS4B binds RNA, and that this binding is specific for the 3′ UTR of the negative strand of the viral genome with Kd of ~3.5nM. NS4B RNA binding is a new target for drug discovery. Therefore, we performed a high-throughput microfluidic screen of a compound library and identified 18 compounds that significantly inhibited binding of RNA by NS4B. One of these compounds, Clemizole-hydrochloride, was found to have a significant *in vivo* antiviral effect on HCV RNA replication with little toxicity for the host cell. These results yield new insight into the HCV life cycle and provide a candidate compound for 1pharmaceutical development.



1643-Pos The Progeny Chambers: Tracking Lineages Of Cells In Lines Using Microfluidic Devices

Amy C. Rowat, Jeremy J. Agresti, David A. Weitz

Harvard University, Cambridge, MA, USA.

Board B619

Cells that are genetically identical show phenotypic variation, for example during differentiation, development, and response to environmental stress. How is non-genetic information passed on to progeny cells? To elucidate the mechanisms of non-genetic inheritance, we are developing tools to study lineages of single cells and their progeny. We trap single cells in long, thin chambers of a polydimethylsiloxane (PDMS) microfluidic device and culture them so they grow in a line. This makes it easy to follow lineages deriving from a single progenitor cell. These studies provide insights into heritable patterns of gene expression, and ultimately, into the role of epigenetic mechanisms in adaptation, development, and differentiation.

1644-Pos Viral Encapsidation of Nanoemulsion Droplets

Connie B. Chang, William M. Gelbart, Charles M. Knobler, Thomas G. Mason

University of California, Los Angeles, Los Angeles, CA, USA.

Board B620

Here we demonstrate the self-assembly of virus-like droplets (VLD) composed of virus protein coats encapsidating surfactant-stabilized nanoemulsion droplets. By varying the pH and ionic strength of the solution conditions, a variety of VLD structures have been formed and elucidated by transmission electron microscopy. These structures include nanoemulsion droplets encapsulated by single, double, and multiple coats of protein. We seek to understand the equilibrium dynamics and kinetics of the assembly process.

1645-Pos Electrical Signature of Surface Interaction on the Nanopipette Biosensor Tip

Senkei Umehara[1], Annika Branting[1,2], Nader Pourmand[1,3], Miloslav Karhanek[1]

[1] *Stanford University, Palo Alto, CA, USA*
[2] *Royal Institute of Technology, Stockholm, Sweden*
[3] *University of California Santa Cruz, Santa Cruz, CA, USA.*

Board B621

We have developed a nanopipette-based electrical biosensing platform, in which target molecules are probed by an ultrafine, electro-

lyte-filled glass electrode with a tip opening of approximately 50 nm in diameter. Its voltammetric response is clearly affected by

(i) molecular translocation through the nanoscale opening, and by
(ii) surface properties at the nanopipette tip.

We are now optimizing this nanopipette biosensor to achieve high specificity and sensitivity by chemically modifying the tip surface. A series of nanopipettes functionalized with different biological recognition elements will serve as specific probes for multiplexed detection.

Here we show the effect of surface modification on the ionic current flow, whose understanding facilitates a rational design for successful nanopipette functionalization. We measured and analyzed the voltammetric response of nanopipette electrodes at various stages of the functionalization steps: before modification, after poly-L-lysine (PLL) coating, and after conjugation of functionalizing molecules to the PLL polymer layer. When a symmetric external voltage was applied, the current flow was rectified differently depending on the polarity of fixed charges introduced to the tip surface. The role of those charges was further demonstrated by semiquantitative theories and multiphysics modeling. In addition, through continuous voltage-clamp measurements, we observed real-time changes in current amplitude after additions of molecules that were supposed to interact with the nanopipette tip surface. We interpret these changes as molecular binding events at the PLL-coated surface.

Successful functionalization is crucial for high specificity and sensitivity of the biosensor. Analyzing electrical signatures of nanopipettes and characterizing nanoscopic events at their tip region paves the way to applications using this biosensor platform.

1646-Pos Beta Lactoglobulin Measured in Solid-state Nanopores

Jiali Li[1], Brad Ledden[1], Brian Thomas[1], Jason Giurleo[2], Troy Messina[2], David Talaga[2]

[1] *University of Arkansas, Fayetteville, AR, USA*
[2] *Rutgers, The State University of New Jersey, Piscataway, NJ, USA.*

Board B622

Ionic current measured in nanometer-sized silicon nitride pores was used to characterize the protein Beta lactoglobulin (BLG) under different conditions. Ionic current blockage signals of BLG translocation in the nanopores in KCl solution at pH 7 with no denaturing agent, and with 5M and 8M Urea were measured and compared. The transient current blockage signals were analyzed in terms of the structures present along the folding pathway for BLG. BLG forms amyloid when incubated at 5M Urea and 37°C with a lag phase of 10–14 days. BLG translocations through the nanopores were measured on samples that were incubated for 0, 11, and 30 days. BLG translocations through the nanopores were also measured at pH 4.6 for different temperatures. These studies show that ionic current blockage signals generated by BLG protein translocation in silicon nitride pores could be used to characterize the BLG conformation changes and aggregation leading to amyloid formation.

1647-Pos Solid State Nanopore Measurement of DNA Hybridization

Ryan Rollings[1], Edward W. Graef[2], Jiali Li[1]

[1] *University of Arkansas, Fayetteville, AR, USA*
[2] *Southeast Missori State University, Cape Girardeau, MO, USA.*

Board B623

We report the on the use of nanometer sized solid state nanopores to electronically analyze DNA probe-oligonucleotide complexes and DNA hairpins. The DNA structures studied were electrophoretically driven through the pores and produce discrete current blockages that are characteristic of the DNA molecules translocating through them. We use 132-mer oligonucleotides engineered to accept identical 12-mer probes that are conjugate to three unique, equally spaced sites on the 132-mer oligonucleotides. We use the translocation of DNA hairpins to estimate the ability of the nanopore to spatially resolve the single and double strand sections of the hairpin and use these results to analyze the translocation of the more complicated probe-oligonucleotide complexes. We demonstrate discrete current blockages that are representative of the molecules studied and discuss progress towards detection of the position of hybridized probes along single strand DNA.

1648-Pos Unzipping DNA Hairpins using 2nm Solid-State pores

Ben McNally, Meni Wanunu, Amit Meller

Boston University, Boston, MA, USA.

Board B624

The unzipping dynamics of double stranded DNA and RNA molecules can reveal information on their structure and biological function. A number of single-molecule techniques, such as Optical Tweezers, Atomic Force Microscopy and Nanopores, have been used to study unzipping dynamics. In particular, nanopore experiments allow the sequential unzipping of DNA fragments since the force is applied locally on the molecules. However, to date nanopore unzipping experiments have been restricted to phospholipids embedded protein pores, such as α-hemolysin, limiting the range of forces and force ramps. Here we report, for the first time, on DNA unzipping studies using 2 nm pores fabricated in silicon nitride (SiN) membranes. We performed a systematic experimental study of the unzipping kinetics as a function of DNA hairpin length, temperature and voltage. We find that the unzipping time depends exponentially on the number of bases in the double-stranded hairpins. Our temperature dependent studies provide an estimate for the activation energies associated with nanopore unzipping. Our results are an important step in developing novel methods for rapid characterization of structured nucleic acids and DNA sequencing.

1649-Pos Translocation of DNA-Protein Structures Through Solid-State Nanopores

Stefan W. Kowalczyk, Ralph M. M. Smeets, Nynke H. Dekker, Cees Dekker

Delft University of Technology, Delft, The Netherlands.

Board B625

Nanometer-sized pores (*nanopores*) can be used as a probe to detect single biomolecules. DNA is driven through the constriction by an externally applied electric field. The passage of a single DNA molecule through the nanopore results in a temporal current blockade. The technique can be used to study the physics underlying polymer translocation, and has the potential of interesting device applications like DNA sizing, sequencing, and haplotyping (deducing the constituents of an individual chromosome).

The well known RecA protein plays an essential role in the central steps of recombination: the pairing and strand exchange of homologous DNA molecules. To accomplish its DNA strand exchange activities RecA polymerizes onto DNA to form a stiff helical nucleoprotein filament. Here we demonstrate nanopore translocations of RecA-DNA filaments and compare the induced current blockades to those resulting from translocations of bare DNA. We find a tenfold higher current blockade than for bare DNA. This can be understood because of the larger diameter of the RecA-DNA filament. In addition we show the fabrication of many kbp long ss/ds-DNA constructs. Such molecules can be used to limit binding of RecA specifically to the single-stranded regions. In this way we can use RecA proteins as local markers on the DNA. The results presented show great promise for the detection of local protein structures on DNA.

1650-Pos Fabrication And Integration Of Sub-5 nm Nanopores For Single Molecule Analysis

Birgitta Schiedt[1,2], Anne-Laure Biance[1,2], Jacques Gierak[1], Ali Madouri[1], Jérôme Mathé[2], Laurent Bacri[2], Lars Bruchhaus[3], Ralf Jede[3], Loic Auvray[2]

[1] *LPN/CNRS, Marcoussis, France*
[2] *MPI, Université d'Évry Val d'Essonne, Évry, France*
[3] *RAITH GmbH, Dortmund, Germany.*

Board B626

In recent years, nanopores have become an important tool to study the properties of single biomolecules in ionic solutions. This technique is based on the translocations of these molecules through a pore of comparable size, which are recorded as changes in the pore's conductance. Among the various topics that can be studied with this technique are e.g. DNA unzipping or protein denaturation.

Various types of nanopores are nowadays used for these experiments, including biological, chemical and solid state pores. Here we present a way to fabricate sub-5 nm pores in 20–100 nm thick SiC membranes by direct drilling with a focussed ion beam. The mid-term stability of these pores was also investigated, revealing that this depends on the aspect ratio of the pores. In order to obtain a user-friendly setup allowing low-noise measurements, these membranes, which usually contain more than one nanopore to allow the choice of a specific size, have to be carefully integrated into an electrolytic cell. For this purpose, the membranes are fixed on a piece of Pyrex containing a micrometer sized aperture, which is used to select the desired pore and at the same time reduces the area of the membrane which is exposed to the liquid, thus reducing the capacitance and the noise of the system. The transport properties of test biological molecules through these nanopores will also be discussed.

1651-Pos Effect of Strand Orientation on Blockage Currents of Single-Stranded DNA Immobilized Within a Biological Nanopore

Kunal K. Mehta, Robert F. Purnell, Jacob J. Schmidt

University of California, Los Angeles, Los Angeles, CA, USA.

Board B627

Nanopores such as the pore protein alpha-Hemolysin (aHL) offer the capability of sensing a large variety of biological molecules with a high degree of sensitivity and selectivity. This capability is particularly attractive for DNA sequencing applications because it could enable inexpensive and rapid sequencing at the single-molecule level. To establish low-noise baseline conductances, we have studied immobilized polyhomonucleotide ssDNA strands inside the aHL channel, which allows a low measurement bandwidth. The ssDNA is electrophoretically driven into the aHL pore but is prevented from complete translocation by the presence of a biotin-streptavidin termination which cannot fit into the aHL. In our previous work with this system, the 3′ end of the ssDNA was free and could enter the pore. Here, we report the results of an extension of this work in which the 5′ end is free to enter the pore. We report high resolution blockage currents from immobilized polyhomonucleotide strands of adenine, cytosine, and thymine inserted with the 5′ end first, compare them with strands inserted with the 3′ end first, and comment on the feasibility of ssDNA sequencing based on these results.

1652-Pos Computational Study of Hydrocarbon Adsorption in Nanoporous Metal-Organic Frameworks

Ilgeun Park, Rakwoo Chang

Department of Chemistry, Kwangwoon University, Seoul, Republic of Korea.

Board B628

The adsorption properties of hydrocarbons in nanoporous metal-organic framework (MOF) materials are studied using a grand canonical Monte Carlo (GCMC) simulation method. The adsorption isotherm of hydrocarbon in IRMOF-1 and three other frameworks with slightly modified side chains shows a different behavior

depending on the system pressure and the hydrocarbon length. We attribute this behavior to the competition between enthalpic and entropic effects due to the interaction between adsorbate and adsorbent. The results also showed a good agreement with experimental results.

1653-Pos Facilitated Diffusion inside Nanopores: a Chemical Model of the Nuclear Pore Complex

Yaron Caspi, David Zbaida, Michael Elbaum

Weizmann Institute, Rehovot, Israel.

Board B629

The nuclear pore complex (NPC) serves as the gateway to the cell nucleus. The transport of proteins and RNAs is coordinated through the central channel of the NPC. The mechanism is one of receptor-mediated transport, where a signal-bearing cargo molecule is recognized by a specific receptor that facilitates its diffusion through the pore. The NPC thus maintains high degrees of both versatility and selectivity in the molecular cargoes that can pass through. The exact physical mechanism for the selective passage of receptors and receptor-cargo complexes is yet unknown, but all evidence points to low-energy interactions with unstructured phenylalanine-glycine repeat domains among the nucleoporins lining the NPC channel.

In order to examine the chemical feasibility of facilitated diffusion inside the pore we have constructed a simple chemical mimic of the nuclear pore that retains some essential features. Track-etch polycarbonate membranes were grafted with poly-N- isopropylacrylamide (pNIPAM), a well-known hydrogen bond forming polymer. Free pNIPAM polymers diffused through the pores much faster than smaller dextrans. Moreover, we showed that oligo-DNA coupled to carrier pNIPAM "receptors" diffused across the grafted membranes significantly faster than bare DNA that remained uncoupled to the carrier. Hence we were able to reconstruct artificially a possible mechanism for the macromolecular selectivity of the nuclear pore, using relatively simple chemical components.

1654-Pos Micropatterned Ligand Arrays To Study Spatial And Regulatory Mechanisms In FceRI Signaling

Alexis J. Torres, David Holowka, Barbara Baird

Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY, USA.

Board B630

Spatial targeting and membrane compartmentalization are common mechanisms of regulation in receptor mediated signaling. However the tools for studying these dynamic interactions are limited. Recently, we established the use of patterned lipid bilayers that contain specific ligands as a tool for visualizing spatial distribution of signaling molecules, and this approach also provides new insights into the structural and functionally relevant membrane components. We are investigating fundamental mechanisms in immune cell signaling, specifically IgE receptor (FcεRI) signaling in mast that is involved in allergic responses. We use standard photolithography and the polymer lift-off method to microfabricate surfaces with patterned lipid bilayers. DNP-cap-PE within the fluid lipid bilayers bind and cross-link anti-DNP IgE bound to FcεRI on the surface of RBL mast cells, thus activating signaling events in these cells.

Previously, we showed that early signaling components such as Lyn kinase selectively co-redistribute with IgE-FcεRI that are clustered specifically over the patterned ligands. Currently we are studying the dynamics of actin cytoskeleton rearrangement that accompany FcεRI mediated activation, and we have identified possible new adapter proteins that may be involved in linking F-actin to other signaling molecules. We found that F-actin and actin binding proteins such as vinculin and paxillin are recruited to the clustered receptor sites and this local recruitment may be mediated by interactions with Lyn kinase. Further biochemical characterization revealed a role for paxillin in the negative regulation of FcεRI receptor signaling. Micropatterned ligand arrays combined with more standard biochemical and biophysical analysis are proving valuable for untangling the complex reorganization of the actin cytoskeleton during FcεRI receptor signaling.

1655-Pos Nanopore-based Kinetics Analysis Of Individual Antibody Interactions

Eric W. Morales[1,2], Iftekhar Amin[1,2], Amanda Davis[1], Karen Thomson[1,2], Alexander Stoyanov[1], Stephen Winters-Hilt[1,2]

[1] *Research Institute for Children, New Orleans, LA, USA*
[2] *University of New Orleans, New Orleans, LA, USA.*

Board B631

Antibodies have undergone extensive study and manipulation since their discovery but much still remains unresolved regarding their dynamics, conformational changes and functionality. Nanopore detection with the highly stable α-Hemolysin channel provides a new approach to study these characteristics at the single molecule level. Due to the channel's 1–2 nm scale inner diameter, only pA ionic currents thread their way through the channel's unique geometry, along with polypeptide chains, ssDNA, and portions of dsDNA. Depending upon their structure, portions of many biomolecules can be captured and held electrophoretically by the channel's chalice like shape to produce lengthy, reproducible, blockade signals ("signatures" – as observed previously in DNA hairpin studies.

We present evidence of different types of blockades signals associated with the same antibody, and hypothesize that these signal classes are associated with the different forms of capture of that molecule. The nanopore-capture "epitopes" of the observed molecules are hypothesized to result from the variety of peptide loops, glycosylizations, and carboxy terminii, that are present.

We show discernible change in antibody capture blockades upon introduction of antigen, suggesting that the state of antibody/antigen binding can be tracked indirectly by this means. To explore this further, DNA hairpin linked antibodies were made, anchoring the carboxy terminus of the Fc region, for which many fewer capture

classes were observed (the capture by the charged DNA attached region having much higher affinity for nanopore interaction). DNA-hairpin event transduction complexes may provide an incredibly versatile biosensing augmentation to any nanopore detector platform.

The effect of chaotropic agents on binding affinity are studied as part of an effort to obtain disassociation constants for monoclonal IgG antibodies. We describe how nanopore event-transduction approaches may eventually provide a direct analysis of the complex conformational negotiations that occur upon binding between proteins.

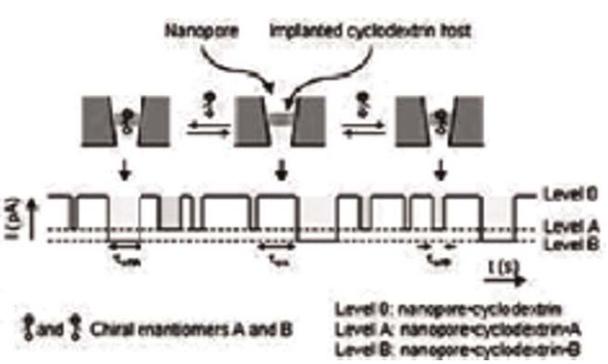
1656-Pos Pharmaceutical Detections With A Molecular Adapter Non-covalently Implanted In A Hand-held Nanopore

Changlu Gao, Li-Qun Gu

University of Missouri, Columbia, MO, USA.

Board B632

Molecular-scaled pores are critical for ultra-sensitive single-molecule detection, DNA sequencing, nanochemistry and controlled delivery. Single nanopores have been fabricated in solid substrates, through ion beam sculpting, electron beam lithography and ion-track etching. These high-tech manufactured nanopores are advantageous, due to their variable pore size and their ability to provide a broader choice of molecules and environments. However, their fabrication requires special materials, expensive facilities and experienced operators, which limit large-scale utilization in many laboratories. Our goal was to find a means of fabricating a robust, low-noise hand-held nanopore-terminated probe with molecular-scaled dimensions for various single molecule manipulations using low-cost techniques and resources readily on hand. Here, we describe the creation of such a nanopore on the terminal of a silica capillary pipette using the programmable pull-seal-etch technique. This nanopore exhibits extremely low noise (I_{rms}=1.2–1.8 pA, comparable to that of protein pore), and can be made as small as 1 nm, so that a single cyclodextrin (1.5 nm) can be implanted in the pore lumen. The trapped cyclodextrin acts as a molecular adapter, enabling the nanopore probe to discriminate single molecules of chiral pharmaceuticals in the mixture.



1657-Pos Probing RNA Unzipping Kinetics Using a Protein Nanopore

Jianxun Lin[1], Barak Akabayov[2,3], Irit Sagi[2], Amit Meller[1]

[1] *Biomedical Engineering Dept. & Physics Dept., Boston University, Boston, MA, USA*

[2] *Department of Structural Biology, Weizmann Institute for Science, Rehovot, Israel*

[3] *Biological Chemistry & Molecular Pharmacology, Harvard Medical School, Boston, MA, USA.*

Board B633

The unzipping kinetics of DNA hairpins, using the α-Hemolysin protein pore, has been extensively studied in the past few years.[1] In contrast, little work has been reported on RNA unzipping using a nanopore, despite its biological relevance. Here, we present for the first time an extensive study of RNA unzipping using protein pores. We have studied the unzipping kinetics of a series of simple, single hairpin RNA molecules at 5 different temperatures under a constant force, and compare it to the unzipping kinetics of DNA molecules with identical sequences. Our results show remarkable differences between RNA and DNA unzipping that can not be probed using bulk techniques. This work sheds light on the mechanics of nanopore unzipping and its sensitivity to small differences in hairpin stability. It also sets the stage for further studies involving multiple hairpin structures of RNA, and to more complicated and interesting biology problems, such as RNA-Helicase interactions.

References

1. Mathe, J., H. Visram, V. Viasnoff, Y. Rabin and A. Meller (2004) Nanopore unzipping of individual DNA hairpin molecules. Biophys. J. *87*, 3205–3212.

1658-Pos Patterned Cellulose Nanofibrils For Single Molecule Studies Of Cellulase Activity

Jose M. Moran-Mirabal, Navaneetha Santhanam, Harold G. Craighead, Larry P. Walker

Cornell University, Ithaca, NY, USA.

Board B634

The sustained rise in oil prices has increased the attractiveness of biologically derived fuels as an alternative to fossil fuels for transportation. Because cellulose is one of the most abundant biological compounds on earth, and an integral component of all plants, cellulosic ethanol has emerged as a strong biofuel candidate. In the extraction of fermentable sugars from cellulosic biomass, a key bioprocess is the enzymatic hydrolysis of cellulose into glucose monomers and dimers. Cellulases are the essential enzymes that catalyze the hydrolysis of the -1-4-glycosidic linkage in cellulose. Although there has been extensive research on cellulases, they still represent an area of opportunity to improve the economics of ethanol production. In particular better understanding on the processivity of individual or mixtures of soluble enzymes is essential in the development of strategies to liberate fermentable sugars from

plant biomass. We have developed a method to immobilize nanofibrils of bacterial microcrystalline cellulose (BMCC) onto a solid substrate. Through micropatterning of a polymer mask, we are able to selectively expose areas of a glass substrate to cellulosic material. Subsequent lift-off of the polymer layer removes the excess of BMCC, revealing patterns of single cellulose strands adsorbed to the solid glass substrate. The patterned BMCC nanofibrils can then be exposed to soluble enzymes, allowing the study the processive activities of single enzymes. Through total internal reflection fluorescence microscopy we have characterized the binding characteristics of three different cellulases to patterned BMCC. We have also studied the diffusive behavior of the cellulases in presence of the patterned cellulose through fluorescence correlation spectroscopy. The results presented shed light on the hydrolysis mechanisms and rates for cellulases on crystalline cellulose.

1659-Pos Poly(amidoamine) Dendrimer Binding, Pore Formation, And Supramolecular Structure With Phospholipids

Christopher V. Kelly, Pascale R. Leroueil, Blake W. Erickson, James R. Baker, Ioan Andricioaei, Bradford G. Orr, Mark M. Banaszak Holl

University of Michigan, Ann Arbor, MI, USA.

Board B635

The binding of poly(amidoamine) (PAMAM) dendrimers to phospholipids is examined through experimental and theoretical approaches. The formation of membrane pores on supported lipid bilayers on mica is observed with nanometer resolution with atomic force microscopy (AFM). The molecular structure of the dendrimer-lipid complexes is examined with isothermal titration calorimetry (ITC), dynamics light scattering (DLS), and all-atom molecular dynamics (MD) simulations. ITC and MD provide energetic information on the dendrimer-lipid complexes while AFM, DLS and MD provide structural information. Variations in dendrimer generation, dendrimer termination, lipid type, and lipid phase are explored. These combined experimental and theoretical approaches address the assorted lipid and nanoparticle properties that are relevant to nanoparticle targeted drug delivery and nanoparticle toxicity.

As example knowledge that can be gained through all-atom MD simulations, the binding of poly(amidoamine) (PAMAM) dendrimers to (dimyristoylphosphatidylcholine (DMPC) are explored. Upon binding to gel phase lipids, dendrimers remain spherical, have a constant radii of gyration, and approximately one quarter of the terminal groups in close proximity to the lipids. In contrast, upon binding to fluid phase bilayers, dendrimers flatten out with a large increase in their asphericity and radii of gyration. Although over twice as many dendrimer-lipid contacts are formed on fluid vs. gel phase lipids, the dendrimer-lipid interaction is only 20% enthalpically stronger. This stronger binding to fluid vs. gel phase lipids is driven by the hydrophobic interactions between the inner dendrimer and lipid tails. Results such as these address the assorted lipid and nanoparticle properties that are relevant to nanoparticle targeted drug delivery and nanoparticle toxicity.

1660-Pos Storable And Transportable Lipid Bilayer Membrane Precursor

Tae-Joon Jeon, Jason L. Poulos, Jacob J. Schmidt

University of California, Los Angeles, Los Angeles, CA, USA.

Board B636

Planar lipid bilayers play an important role in electrophysiological studies and are also of interest technologically as highly sensitive chemical sensors. However, the range and scope of the applications possible using an artificial membrane platform is limited due to the short lifetime and fragility of the 5 nm thick lipid bilayers. Significant recent activity has been made to devise techniques to create more reliable and robust artificial membranes. To improve membranes' stability, several different tethering methods have been employed. Soft and porous materials, such as hydrogels, have been also used to improve membranes' mechanical stability. However, those approaches do not result in membranes mechanically stable enough to transport or store indefinitely. Here we present a new approach addressing these problems using a solid membrane precursor, immobilized and preserved reversibly in a robust state through freezing. A membrane precursor was prepared from 1,2-Diphytanoyl-sn-Glycero-3-Phosphocholine lipids dissolved in a high freezing point solvent such as hexadecane. The membrane precursor was spread over a hydrophobic orifice as is typical. Prior to the spontaneous process of thinning to a bilayer, the membrane precursor was cooled to 4C or −20C, freezing the organic phase. Since the membrane precursor is frozen, it can be indefinitely stored and even transported. We have demonstrated its transportability by shipping membrane precursors via a commercial carrier. The membrane precursors were shipped approximately 120 miles (192 km) over 3 days and successfully formed membranes upon thawing. These membranes were able to support the measurement of channel proteins at the single molecule level. The membrane precursors are stored frozen, such that the evaporation is extremely slow, suggesting that this state can be maintained indefinitely. We have observed storage of membrane precursors for over 30 days, resulting in biologically functional membranes.

1661-Pos Construction of Biomimetic Membranes in a Sandwich Design for Transmembrane Protein Insertion

Jesper S. Hansen[1,2], Mark Perry[1,2], Claus Hélix Nielsen[3,1]

[1] *AQUAporin ApS, Lyngby, Denmark*
[2] *Memphys, University of Southern Denmark, Odense, Denmark*
[3] *Quantum Protein Center, Technical University of Denmark, Lyngby, Denmark.*

Board B637

Research in biomimetic membranes has attracted much attention, because transmembrane proteins (ion channels, transporters and receptors) account for approximately 30% of all proteins in nature. Transmembrane proteins can be chemically modified or genetically engineered, giving unprecedented control over membrane binding and transport properties of living cells.

In order to study transmembrane proteins under controlled circumstances they must be embedded into a matrix mimicking their natural environment *in vivo*. In general membrane spanning proteins retain their biological function when incorporated into artificially made membranes. This has opened new vistas for novel transmembrane protein-based nanobiotechonological applications. Scientific and commercial interests include molecular biology studies of cell adhesion and ligand-receptor interaction studies, screening platforms for drug discovery, development of nanobiosensor devices, immuno-assays and bioremediation platforms in environmental biology.

Our general aim is to explore novel ways of creating reproducible and stable biomimetic membranes in sandwich structures. Furthermore, to develop methods for controlled incorporation and distribution of transmembrane proteins into such encapsulated biomimetic membranes. The specific objective is to produce composite membranes incorporated with aquaporin water channel proteins for industrial water filtration applications.

1662-Pos Immune Cell Response To Antigen Stimulation As Observed In The Plasma Membrane Through Nano-optical Apertures

Paul Zhu, Jose M. Moran-Mirabal, Alexis Torres, Jinmin Lee, Barbara Baird, Harold Craighead

Cornell University, Ithaca, NY, USA.

Board B638

Due to their optical properties, Zero-Mode-Waveguides (ZMWs) have been used for single molecule fluorescence correlation spectroscopy (FCS) at micromolar concentration [1]. They have also been used to observe cell membrane events with high spatial resolution [2]. Recently, they were used to study plasma membrane invagination into sub-optical wavelength holes [3]. We are examining RBL mast cells sensitized with anti-2,4-dinitrophenyl (DNP), and in this study, we focus on membrane reaction to antigen stimulation as observed through ZMWs. Our initial fluorescence probes are DiIC12 that labels the plasma membrane and a GFP conjugate of Lyn kinase that is anchored to the inner leaflet of the plasma membrane and phosphorylates IgE receptor as an early event in stimulated transmembrane signaling.

We observe the plasma membrane invaginate into ZMWs with real time fluorescence imaging. A solution containing DNP-antigen was then added to the incubation well, triggering an immune response from the cells. We quantitatively analyze the cellular response as it occurs in or near the plasma membrane by measuring the dynamics of fluorescently labeled components before and after introduction of DNP- antigen. In initial experiments we find slight decrease of the number of ZMWs containing fluorescence, indicating possible retraction of membranes from the apertures when cells are stimulated.

References

[1]. Levene, M., Korlach, J., Turner, S., Foquet, M., Craighead, H., Webb, W. (2003). Zero-Mode Waveguides for Single-Molecule Analysis at High Concentrations. Science *299*: 682–686.

[2]. Edel, J., Wu, M., Baird, B., Craighead, H., (2005), High Spatial Resolution Observation of Single-Molecule Dynamics in Living Cell Membranes, Biophysical Journal, *88*, L43–L45.

[3]. Moran-Mirabal, J., Torres, A., Samiee, K., Baird, B. , Craighead, H., (2007), Cell investigation of nanostructures: zero-mode waveguides for plasma membrane studies with single molecule resolution, Nanotechnology *18*, 195101

1663-Pos Confined Brownian Motion studied by Optical Trapping Interferometry

Sylvia Jeney[1], Branimir Lukić[1], Jonas A. Kraus[2], Thomas Franosch[2], László Forró[1]

[1] *Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland*

[2] *Arnold Sommerfeld Center for Theoretical Physics (ASC) and Center for NanoScience (CeNS), München, Germany.*

Board B639

The dynamic behavior of a single colloidal particle in water confined by an optical trap and a plain surface is investigated at time scales where the inertia of the surrounding fluid plays a significant role. A weak optical trap with interferometric position detection allows monitoring a single micron-sized sphere with a spatial resolution better than 1 nm and a temporal resolution on the order of microseconds.

First, we quantify the influence of the confinement created by the harmonic potential of the optical tap on the particle's velocity autocorrelation, mean-square displacement and power spectral density. We find that they are in excellent agreement with the theory for a Brownian particle in a harmonic potential that accounts for hydrodynamic memory effects, which states that the transition from ballistic to diffusive motion is delayed to significantly longer times than predicted by the standard Langevin equation. This delay is a consequence of fluid's inertia, introducing a backflow on the particle's fluctuations. At longer times the motion of the particle is dominated by the trapping potential.

By identifying the time below which the particle doesn't "feel" the potential, we can exclude the existence of free diffusive motion as usually assumed in common optical trapping experiments.

Second, the particle is brought close to a hard surface and we observe how the subtle interplay of surface confinement and hydrodynamic backflow changes the decay of the particle's velocity autocorrelations from a slow $t^{-3/2}$ to a much faster power-law $t^{-5/2}$.

These findings show that the temporal resolution of Optical Trapping Interferometry can be extended down to time scales where the nature of the fluid influences diffusion, bringing the long discussed idea of using a Brownian particle as a local reporter of the dynamics of complex biological fluids one step further.

1664-Pos Hydrogel-stabilized Lipid Bilayers Through Covalent Membrane And Substrate Conjugation

Tae-Joon Jeon[1], Noah Malmstadt[2], Jason L. Poulos[1], Jacob J. Schmidt[1]

[1] *University of California, Los Angeles, Los Angeles, CA, USA*
[2] *University of Southern California, Los Angeles, CA, USA.*

Board B640

We have created long-lived and mechanically robust lipid bilayer membranes by covalently conjugating them *in situ* to a PEG-DMA (polyethylene glycol dimethacrylate) hydrogel. Diphytanoylphosphatidylethanolamine lipids were modified to attach a crosslinkable C=C functional group at the lipid head by a straightforward chemical synthesis with N-acryloxysuccinimide. Membranes were made from a mixture of this lipid with Diphytanolphospatidylcholine at a 1:20 ratio. The hydrogel conjugated membranes (cgHEMs) are extremely long-lived and have an exceptional mechanical stability. In typical laboratory conditions, the cgHEM remained intact over 11 days with gigaohm resistances. The cgHEM could also withstand severe mechanical perturbation. Fluorescence microscopy observations of the membrane's solvent annulus suggest that the long-term stability of membranes may be attributable to stabilization of the solvent reservoir. The membrane fluidity was also demonstrated by FRAP (fluorescence recovery after photobleaching) experiments and channel protein activities. The freestanding planar lipid bilayers can also be stabilized by employing a novel functionalized substrate. In this work, we have functionalized glass surfaces with a bind-silane (3-Methacryloxypropyltrimethoxysilane), enabling the covalent attachment of the encapsulating hydrogel to the glass surface with photo-polymerization initiation. The hydrogel consequently provides a mechanical support to the membrane, as well as to the solvent reservoir. Membranes supported in this way lasted over 12 days with giga-ohm resistances. We will present these experimental results as well as future directions toward further stabilization of the planar lipid bilayer membranes.

1665-Pos Development of a Model System for Stable Incorporation of Selected Transmembrane Proteins into a Bio-mimetic Membrane Design

Mark E. Perry[1,2], Jesper S. Hansen[1,2], Claus H. Nielsen[1,3]

[1] *AQUAporin Aps, Kongens Lyngby, Denmark*
[2] *University of Southern Denmark,, Odense, Denmark*
[3] *Quantum Protein Centre, Kongens Lyngby, Denmark.*

Board B641

The main goal of this project is to develop a stable bio-mimetic membrane system into which aquaporins, which are naturally occurring transmembrane water channels, can be incorporated for highly effective water purification through their high selectivity and rapid turnover.

Our model system approach is to incorporate tailored lipid membranes into a multi-aperture hydrophobic partition, and then stabilize the system by sandwiching the partition between applicable nano-biotechnological encapsulation materials.

We currently work with the characterization of several nanoporous materials and surface modifications together with different lipid compositions. Specifically, we study surface modifications which meet the tethering requirements of a cushion intermediate layer between the encapsulation materials and the membranes with incorporated transmembrane proteins. This design will allow water flow and minimize close contact between peripheral parts of integrated proteins and the support thus preventing protein denaturing.

We have demonstrated that by choosing a proper lipid composition, hydrophobic partition material and encapsulation membrane our system is capable of sustaining a bio-mimetic membrane for extended periods of time (days to weeks). Thus it should feasible to incorporate selected transmembrane peptides and proteins for a variety of practical applications.

1666-Pos Fluorescence and Magnetic Detection of Hybridized DNA Assemblies on a Micro-Hall Device

Steven M. Hira, Khaled Aledealat, Peng Xiong, Stephan von Molnár, Geoffrey F. Strouse, P. B. Chase

Florida State University, Tallahassee, FL, USA.

Board B642

The development of a dual detection platform to probe and discriminate nucleic acid base-pairing events through a combination of fluorescent and magnetic signatures may significantly impact the performance and dimensions of biomedical sensing devices. Toward this aim, investigations on the selective and controlled assembly of DNA duplex formation onto a micro-scale Hall device will be addressed. The precise immobilization of target ssDNA onto micrometer size patterns allows for multiple sequences to be sensed on a single device platform. The biological assembly is composed of two distinct components. One is a streptavidin-coated and fluorescently co-labeled magnetic nanobead (50 nm – 350 nm diameter) pre-conjugated with biotinylated ssDNA reporter strands. The other component is a complimentary, thiolated ssDNA target strand that was selectively immobilized onto Au photolithography patterns on a SiO_2 substrate surface. The device has been intentionally designed to detect hybridization of reporter and target DNA sequences by redundant signatures of the nanobead: fluorescence using confocal microscopy, and magnetic using a micro-Hall cross, in which DNA duplex formation brings the nanobead close enough to the Hall cross to induce a voltage change by the Hall effect.

Support: NIH NIGMS GM079592.

1667-Pos Interactions of Nuclear Proteins to MBF Binding Site Measured by Surface Plasmon Resonance

Grace F. Mao, James Brody

University of California, Irvine, Irvine, CA, USA.

Board B643

MBF, a binding factor regulated by transcription to target genes with functions which are important for bud growth and DNA synthesis, acts early in the cell cycle. Comprehensive understanding of MBF binding site is important for elucidating the complex events at the beginning of cell cycle. In this project, nuclear proteins were extracted from Saccharomyces cerevisiae cells which were grown for different time intervals after synchronization. A flow cytometer called FACScan was employed to ensure the cell populations are at distinct timepoints in the cell cycle. Surface plasmon resonance (SPR) based sensor, the Texas Instruments Spreeta, was then used to measure the binding of MBF binding site from RNR1 gene when exposed to nuclear proteins extracted at different timepoints. Highest DNA-nuclear proteins binding occurred at 15–30 minutes after synchronization. All other timepoints show similar DNA-nuclear proteins binding. Thus, we conclude:

1. Spreeta can be utilized to measure DNA-protein interactions.
2. Measurements verify that expression peak for binding of nuclear proteins from different time intervals after synchronization to MBF binding site occurs at the end of G1/S transition.
3. Measurements also verify that MBF acts as a repressor in other cell cycle phases.

1668-Pos Temperature dependent ionic conductance of OmpF: Effect of the confinement

Catalin Chimerel[1], Soroosh Pezeshki[1], Ulrich Kleinekathöfer[1], Liviu Movileanu[2], Mathias Winterhalter[1]

[1] *Jacobs University Bremen, Bremen, Germany*
[2] *Department of Physics, Syracuse University, 201 Physics Building, Syracuse, NY, USA.*

Board B644

The temperature dependence of the ion conductance through the outer membrane channel OmpF of *E. coli* was measured. For this a single trimer has been reconstituted into a planar lipid bilayer. We varied the temperature from −5 to 82 C and the salt concentration from 0.1 to 4M. The main contribution to the conductance stems from the ohmic part determined by the bulk conductance of the electrolyte for the given salt concentration and temperature. However, a temperature and salt dependent contribution is attributed to confinement effects. To understand such effects molecular dynamics simulations were performed. We observed a temperature dependent ion pairing that is enhanced inside of the nanopore. This could explain differences in the ionic conductance in confined environments.

1669-Pos Intrinsic Peroxidase-like Activity Of Ferromagnetic Nanoparticles

Xiyun Yan

Institute of Biophysics, CAS, Beijing, China.

Board B645

Nanoparticles that contain magnetic materials, such as magnetite (Fe_3O_4), are particularly useful for imaging and separation techniques. Since these nanoparticles are generally considered to be biologically and chemically inert, they are typically coated with metal catalysts, antibodies or enzymes to increase their functionality as separation agents. Here, we report that magnetite nanoparticles in fact possess an intrinsic enzyme mimetic activity similar to that found in natural peroxidases, which are widely used to oxidize organic substrates in the treatment of wastewater or as detection tools. Based on this finding, we have developed a novel immunoassay in which antibody-modified magnetite nanoparticles provide three functions: capture, separation and detection. The stability, ease of production and versatility of these nanoparticles makes them a powerful tool for a wide range of potential applications in medicine, biotechnology and environmental chemistry.

Biotechnology & Bioengineering

1670-Pos Extracellular Matrix Stiffness Influences Behavioral Decisions in Adult Neural Stem Cells

Elena de Juan-Pardo, Dexter J. D'Sa, David V. Schaffer, Sanjay Kumar

University of California, Berkeley, Berkeley, CA, USA.

Board B646

Neurodegenerative disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease and others are devastating illnesses that result from the death of specific populations of cells in the central nervous system (CNS). Adult neural stem cells (ANSCs), which are present in the CNS throughout human life, have significant promise for protecting and regenerating tissue affected by such diseases. However, to date they have exhibited very limited differentiation in vitro into phenotypes that are attractive for neuroregeneration. Soluble factors known to control neural stem cell differentiation into neurons during development exert different effects on ANSCs; therefore, it is important to comprehensively explore alternate signals of the stem cell microenvironment that may exert effects on ANSC differentiation into target phenotypes. Here we explore the effect of mechanical cues from the extracellular matrix (ECM) on behavioral decisions of ANSCs. We created two-dimesnional polyacrylamide substrates with stiffnesses that span six orders of magnitude ($<$10 Pa to $>$100 kPa) as verified by micro- and macroscopic rheometry. We then covalently conjugated each substrate with the ECM protein laminin and cultured rat hippocampal ANSCs on these substrates in growth or differentiation media. We examined the relationship between ECM stiffness and the differentiation trajectories of ANSCs, measured by the expression of specific differentiation markers, cell morphology and multicellular architecture. Our results illustrate the importance of biophysical cues from the ECM in sculpting the development and assembly of ANSCs.