was explored via molecular dynamics simulations of multiple LH2 complexes in a membrane patch. LH2s from two species - Rb. sphaeroides and Rps. acidophila - were simulated to also allow investigation into whether the LH2s from species with spherical chromatophores have different curvature properties than those from species with lamellar chromatophores. We found little dependence of curvature on species, suggesting that the packing of the complexes, as well as the distribution of LH1s, may be the largest factors in determining membrane shape.

45-Plat Structural and Energetic Aspects of Membrane Binding by Ras GTPases:

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Ras GTPases become functionally active when anchored to membranes by inserting their lipid modified side chains. However, the mechanism of membrane insertion and the structure of the resulting complex remain elusive. Recently, the structure of the full-length H-ras protein in a DMPC bilayer has been characterized through modeling and molecular dynamics simulations. It was found that ras binding to membrane involves, in addition to the anchor, a direct interaction with the membrane phosphates of basic residues from either the linker or the catalytic domain. Two nucleotide-dependent modes of membrane binding were obtained. In the GTP-bound active state, Ras predominantly binds the bilayer via helix 4 and the anchor, whereas in the GDP-bound form the role of helix 4 is replaced by the linker. These results were confirmed by mutagenesis experiments. Furthermore, the local structure and dynamics of the bilayer was perturbed upon protein insertion and this perturbation is dependent on the insertion depth and backbone localization of the anchor, which in turn is modulated by the catalytic domain and the linker. Computation of the potential of mean force (PMF) for the transfer of the anchor into the bilayer resulted in a downhill profile. The insertion into the hydrophobic core produces ~30 kcal/mol gain in free energy. Combining these results, a mechanistic model for the activation state-dependent membrane-reorganizing effect of Ras, and a novel "balance model" for the regulation of H-ras membrane orientation and signal output, are proposed.

46-Plat OmpG: A Quiet Pore?

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Bacterial outer membrane proteins are structurally robust molecules with a β-barrel architecture. Thus they are good candidates for applications in biotechnology: for example, as components of biosensors. OmpG from E. coli is a non-specific monomeric pore that undergoes pH-dependent, voltage-driven and spontaneous gating. Single channel recording studies have shown that gating of OmpG causes transient current blockades and results in numerous spikes in the single channel recording that would interfere with the analyte signal. Thus these intrinsic activities of OmpG must be eliminated if it is to be effective as a biosensor.

The spontaneous gating of OmpG is particularly challenging to understand. We have focused our attention on trying to eliminate the spontaneous gating activity of OmpG. We have used Molecular Dynamics (MD) simulations of OmpG to identify regions of the protein implicated in spontaneous gating to design mutants intended to be resistant to this type of gating. Two approaches were adopted to enhance the stability of the open conformation; optimization of the inter-strand hydrogen bonding of the barrel and reducing the mobility of the loop (L6) implicated in gating. Characterization of the mutants by single channel electrical recording has shown that they are substantially quieter than wildtype OmpG. In the quietest mutant, gating activity is reduced by ~90 %.

Platform E: Micro and Nanotechnology Nanopores

47-Plat Mica, Bioenergetics, and the Origin of Life

Helen Hansma

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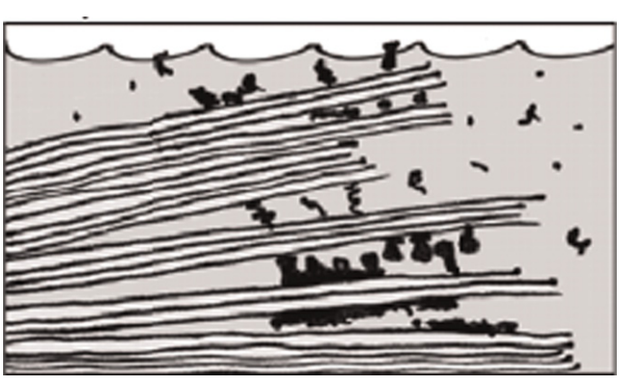
Life may have originated between mica sheets, which would have provided many many confined spaces with surprising similarities to cells, as well as a possible source of energy for the synthesis of biomolecules. Mica is a layered mineral with flexible sheets, 2nm thick. Mechanical energy is produced by the movement of mica sheets, in response to forces such as ocean currents. The energy of a carbon-carbon bond at room temperature is comparable to a mechanical force of 6 nN moving a distance of 1 Angstrom. Mica movements may have facilitated mechanochemistry. Mica is also a rich source of potassium ions, which bridge anionic sites between adjacent mica sheets. With a hexagonal grid of anionic sites spaced 0.5 nm apart on each mica sheet, the K^+ concentration between mica sheets is 100 mM when the mica sheets are ~0.7 nm apart; this is comparable to the K^+ concentrations in cell cytoplasms. Low entropy is characteristic of life; entropy is much lower between mica sheets than on a mineral surface or in a 'prebiotic soup.' The sketch shows 22 mica sheets (44 nm thick) populated by a variety of types of 'prebiotic molecules.'



48-Plat Precise Modulation of Chemical Concentrations over Cellular Microdomains: A Chemical Signal Generator

Brandon Kuczenski, William C. Messner, Philip R. LeDuc

Carnegie Mellon University, Pittsburgh, PA, USA.

Cellular signal transduction often involves responses to localized, transient variations in chemical concentrations. We demonstrate a method for controlling cells' chemical environments over a wide dynamic range with high speed and precision using closed loop pressure control. The interfacial planes formed between parallel laminar flow streams in a microfluidic device can be positioned by controlling the relative flow rates of the fluids. Our approach employs closed loop control of upstream reservoir pressure, which enables near-instantaneous adjustments to flow rates and offers much improved responsiveness over displacement-driven flow techniques.

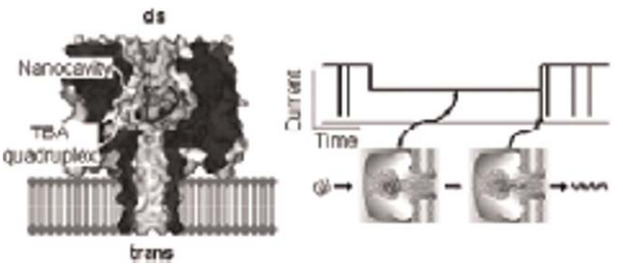
Under laminar flow conditions, mixing of heterogeneous parallel streams occurs by diffusion, resulting in a spatialization of chemical concentration across the width of the channel relative to the position of the interfacial plane. Thus, the concentrations of the interdiffusing chemicals at a fixed point can be modulated in time across the range of the concentration profile by adjusting reservoir pressures. This allows for arbitrary time-varying concentration "signals" to be delivered to a fixed point in the channel. The method is demonstrated by monitoring the concentration of cytosolic calcium within NIH-3T3 fibroblasts as the concentration of extracellular agonists is modulated. This work will provide insight into the response of cellular systems to spatiotemporal variations in their chemical environments.

49-Plat A Guest-Nanocavity Supramolecular System For Non-covalent Single-Molecule Manipulation

Ji Wook Shim, Li-Qun Gu

University of Missouri, Columbia, MO, USA.

α-hemolysin (αHL) has been used as a receptive single-molecule detector for various biosensing applications. A molecule that transports or interacts with the lumen of the β-barrel of αHL can produce changes in pore conductance that lead to identification and quantification of the target. Here, we report that the nanocavity (4.6 nm), another domain enclosed by αHL between the *cis* vestibule and β-barrel, can non-covalently encapsulate single guest molecules. We used the thrombin-binding aptamer (TBA) as the guest. This 15-base short DNA (GGTTGGTGTGGTTGG) must fold into a four-stranded complex, G-quadruplex, to facilitate its function as an inhibitor to thrombin clotting and as a biosensor for protein detection. By detecting conductance "signatures" we determined that the captured TBA sits in the bottom of the nanocavity, vibrating and frequently turning over in different orientations. Most notably, the TBA quadruplex spontaneously unfolds into a linear DNA sequence in the nanocavity and leaves the pore from the β-barrel, driven by voltage. This guest-nanocavity supramolecule becomes a non-covalent system for single-molecule manipulation and is useful for tracking folding and unfolding pathways, molecular conformation distributions and variations under changing conditions.



50-Plat Automated Formation And Long-term Stabilization Of Freestanding Membranes

Jacob Schmidt

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

Membrane channel proteins are major targets of drug discovery and screening and recent work has also shown their potential as single molecule sensors. Conventional freestanding membranes housing these proteins can be problematic to form and are extremely fragile, limiting any technology using these membranes. We have been working to address these shortcomings through the development of techniques to stabilize membranes and automate their formation.

We have developed two approaches for membrane stabilization; hydrogel encapsulation/conjugation of membranes and the freezing of membrane precursors. In hydrogel encapsulation, membranes are created by forming a conventional freestanding lipid bilayer membrane which is then encapsulated by a hydrogel polymerized in situ. Using cross-linkable lipids membranes can be created that bond directly to the hydrogel matrix. These encapsulated membranes can withstand severe mechanical perturbation and are significantly longer-lived than their unencapsulated counterparts, resulting in lifetimes over ten days. Another approach to membrane preservation has been to utilize a high freezing point organic phase which can be frozen before the spontaneous process of bilayer formation is complete. In the frozen state, the membranes can withstand commercial shipping and be preserved indefinitely. Our initial work with automated membrane formation utilized a microfluidic device which extracted the organic solvent bringing together two lipid monolayers. Recently we have modified the inverted phase method of Funakoshi et al so that the fluid densities spontaneously drive membrane formation. This method requires only the creation and dispensing of fluid droplets, which is easily done robotically and in a high throughput manner. Preliminary work with this platform will

be presented as well as our efforts to apply it to high throughput screening of ion channel proteins.

51-Plat Nanopore Analysis of Biopolymers under Physiological Ionic Strengths

Meni Wanunu, Devora Cohen-Karni, Jason Sutin, Amit Meller

Boston University, Boston, MA, USA.

Nanopores are emerging tools for studying biopolymer properties at the single-molecule level, revealing information on nucleic acid structure and reporting on interactions with proteins. Since nanopore detection relies on measurements of the electrolyte current through the pore, nanopore studies have generally required high ionic strength solutions, typically 1 M KCl. However, many interactions between biomolecules are highly salt dependent, limiting the utility of nanopore experiments. In this presentation, we demonstrate that DNA translocation experiments ***can*** be performed under physiological ionic strengths by applying *asymmetric* salt conditions, where the "analyte" chamber and "waste" chamber are maintained at low and high ionic strengths, respectively. Surprisingly, these conditions dramatically enhance the sensitivity of the nanopore to DNA, while a negligible loss in the signal-to-noise ratio is observed. We provide a theoretical model to explain these observations, and demonstrate a direct application of this system for nanopore detection of the interactions between human genomic DNA fragments and transcription factors at the single-molecule level. These findings pave the way for a wide variety of biologically relevant single-molecule experiments using nanopores.

52-Plat Light Driven Fluid Pump along Arbitrary Paths

Franz M. Weinert, Dieter Braun

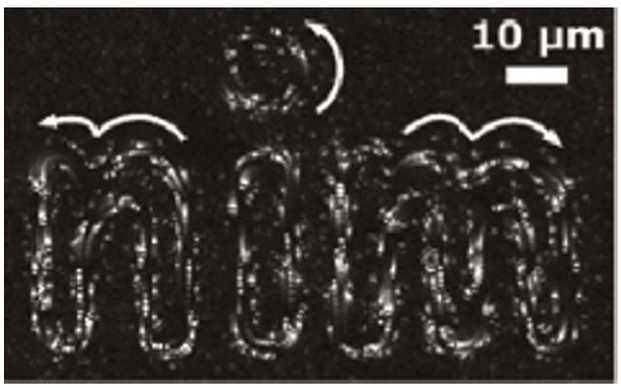
Ludwig Maximilian University, Munich, Germany.

We optically drive fluids on the microscale. The fluid follows the paths of a moving warm spot created by an infrared laser scanning microscope. We are able to pump water with a velocity of 150 μm/s and a resolution of 3 μm. The method aims at pumping signalling molecules in cell culture or the manipulation of fluid flow inside living cells.

The pumping mechanism is based on thermal expansion and the nonlinear combination with the temperature dependence of viscosity. Calculations based on the Navier-Stokes equation confirm the experiments. An analytical solution for the pump velocity is derived.

Our approach differs fundamentally from conventional methods of controlling liquid flow, allowing a whole new range of applications. We give several examples. We pump nanoparticles along millimeters through a gel and create a dilution series of biomolecules by aliquotation and mixing.

Furthermore, we accumulate DNA in free solution by a combination with thermophoresis. The optical non-contact control of fluid flow expands the microfluidic paradigm into previously inaccessible regimes of closed fluid flow, remote flow control in chambers hosting extreme conditions and tiny fluid volumes.

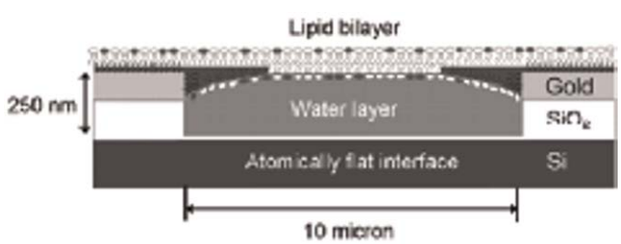


53-Plat Freestanding Lipid Bilayers Assembled Near An Atomically Flat Silicon Interface And Characterized By Fluorescence Interference Contrast Microscopy

Prasad V. Ganesan, Steven G. Boxer

Stanford University, Stanford, CA, USA.

When a supported phospholipid bilayer is assembled on a flat silicon/silicon dioxide wafer, the distance of fluorophores associated with the bilayer from the wafer surface can be measured with high precision using fluorescence interference contrast (FLIC) techniques. However, it is difficult to incorporate functional, mobile cell membrane proteins into supported bilayers. We show that lipid bilayers can be assembled spanning micron-sized 'wells' etched into a wafer substrate, so that the bilayers are near (within hundreds of nanometers) but not in contact with the wafer surface. The wells are etched through thin films of gold and thermally grown silicon dioxide, and each exposed layer is functionalized with an appropriate surface chemistry. The lipid can be added either dissolved in decane or as vesicles; freestanding bilayers form spontaneously, with the lipid associating with the modified gold layer. The bilayers formed are slightly curved, and the nature of the curvature can be quantified using FLIC. This platform promises to provide an environment for fluorescently labelled membrane proteins similar to that of traditional black lipid membranes, while also allowing for direct measurement of conformational changes by interferometry.



54-Plat Biofunctional Micropatterned Surfaces to Study Individual LFA-1&ICAM-1 Interactions in Living Cells

R. Diez-Ahedo[1], D. Normanno[1], O. Esteban[1], B. Joosten[2], C. G. Figdor[2], M. F. Garcia-Parajo[1]

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[2] *NCMLS-Nijmegen Center for Molecular Life Sciences, Nijmegen, The Netherlands.*

Lymphocyte function-associated antigen-1 (LFA1;αLβ2) is a leukocyte specific integrin transmembrane protein that mediates migration across the endothelium and within tissues. It also takes part of the immunological synapse by binding with high affinity to its ligand ICAM-1. We have recently shown that integrin mediated adhesion depends not only on receptor occupancy but also on its nano-cluster organization on the cell membrane. In order to get deeper insight on the mechanisms that control and regulate LFA-1 clustering we have fabricated large areas of biofunctional micropatterned surfaces containing ICAM-1 ligands using soft-lithography. Homogenous ICAM-1 regions (from 10μm-1μm) have been achieved over 1cm^2 areas, with variations in ICAM-1 density below 9%. THP-1 cells (monocytic cell line) expressing LFA-1 have been stretched over the patterned surfaces and the diffusion of labeled LFA-1 in the ICAM-1 regions has been followed in time using single molecule sensitive TIRF microscopy. Single molecule fluorescence trajectories show an increase of intensity on individual fluorescent spots as well as reduction of its mobility consistent with selective recruitment of individual LFA-1 clusters on the ICAM-1 patterned areas. Fluorescent spots on the non-patterned areas (BSA coated) show on the other hand random diffusion. Surprisingly, *on-off* blinking and large intensity fluctuations were observed on the LFA-1&ICAM-1 bound (immobile) clusters compared to the randomly diffusing LFA-1. We are currently performing statistical analysis on the *on-off* times to correlate the binding kinetics of LFA-1&ICAM-1 with receptor-ligand density. These studies will shed light on the role of affinity *vs.* avidity of LFA-1 to its ligand ICAM-1.

Symposium 3: Membrane Protein Structure: Freed from the Lattice

55-Symp Solid-state NMR

Marc Baldus

Max Planck Inst Biophys Chem, Gottingen, Germany.

Solid-state NMR (ssNMR) offers structural insight into the formation of molecular complexes for a wide range of molecular sizes and binding affinities. In our contribution we will discuss recent methodological progress in probing structure, topology and complex formation in membranes. In addition, we will show how to follow protein activation/inactivation in membrane-embedded ion channels and seven-helix receptors by multidimensional ssNMR.

56-Symp Insights Into Disulfide Bond Formation In The Periplasm

John Bushweller, Yunpeng Zhou, Tomasz Cierpicki

University of Virginia, Charlottesville, VA, USA.

Insights into Disulfide Bond Formation in the Periplasm.

We have used solution NMR methods to study the structure and function of the integral membrane enzyme DsbB. The proteins DsbA and DsbB work in concert to mediate the formation of disulfide bonds in the periplasm of E. coli. DsbA is a soluble protein that functions as the primary oxidant for proteins in the periplasm whereas DsbB is an integral membrane protein that reoxidizes DsbA and is itself reoxidized by various quinones. Mutations of DsbA in pathogenic bacteria are avirulent, suggesting this pathway may be a useful target for the development of novel antibiotics.

In order to quench extensive conformational exchange seen in the spectra of wildtype DsbB, we have employed an inter-loop disulfide bonded species, referred to as DsbB(CSSC), which is a reaction intermediate in the mechanism of DsbB. Using a combination of backbone dihedral angle restraints, a limited number of NOE constraints, pramagnetic relaxation effect (PRE) restraints, and an extensive set of dipolar couplings, we have determined the backbone structure of DsbB(CSSC) in DPC micelles to high-resolution. To our knowledge, this represents the first solution structure of a bona fide α-helical membrane protein with more than 2 TM helices. The structure shows the predicted 4 TM helices as well as an additional N-terminal helix. Examination of the two periplasmic loops, which are critical for enzymatic function, reveals that the region of the protein which binds to DsbA has increased mobility, likely mimicing the typically unfolded substrates of DsbA. NMR chemical shifts for the inter-loop disulfide bond suggest a highly polarized disulfide bond primed for disulfide exchange. We have also been able to determine the binding site for ubiquinone on DsbB and shown the importance of residues lining this binding site for function.

57-Symp Potassium channels conduct and regulate potassium flux across the cell membrane

Roland Riek

The Salk Institute, La Jolla, CA, USA.

Potassium channels conduct and regulate potassium flux across the cell membrane. Several crystal structures and other biophysical studies of tetrameric ion channels have revealed many of the structural details of ion selectivity and gating. A narrow pore lined with four arrays of carbonyls is responsible for ion selectivity, whereas a conformational change of the four inner transmembrane helices is involved in gating of the channel. Here, we studied full-length KcsA, a prototypical K+ channel, by solution-state

Nuclear Magnetic Resonance Spectroscopy (NMR) at open, closed and intermediate states. These studies elucidate that at least two conformational states occur both in the selectivity filter and near the C-terminal end of the inner transmembrane helice 2 (TM2). In