Biological membrane channels and pore-forming proteins display a level of sophistication in managing molecular scale transport that is typically unmatched by inorganic analogs, and efforts to develop nanopores that approach the transport efficiency of biological molecules often run into fabrication or synthetic difficulties. A different approach is exploiting nanomaterial scaffolds that provide near-atomic level of control over the structure and surface properties of these structures. Carbon nanotubes provide an especially interesting system for such studies due to their inherently smooth hydrophobic pore walls that support extremely high transport rates, which are comparable to biological channels. We describe the preparation of nanotube-based membrane nanopores, their assembly into biological membranes, and single-channel measurements of molecular transport in these assemblies.

2802-Plat
Controlled Envelopment of Magnetic Particles within Liposomes using a Custom-Built Multi-Layer Magnetic Microfluidic Device
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Liposomes can be magnetically triggered to release chemicals by enacing magnetic particles in an organized and predetermined fashion (e.g. linear array). Here we demonstrate a controlled arrangement of magnetic particles within liposomes using a custom-built microfluidic device. The multi-layer magnetic microfluidic device consists of two thin bottom layers containing low-melting point (117°C) 52% In - 48% Sn solder and a third top layer devoted to the input of lipids and magnetic particles. The microchannels containing the solder are electrically isolated and arranged in a lattice pattern; the top layer contains one channel, which is wide enough to span this solder lattice. To facilitate installing the solder into the channels, we reduce the surface free energy by first injecting a solution of 3-mercaptopropyltrimethoxysilane in acetonitrile and allowing it to dry. By controlling the current in the solder lattice we can non-invasively corral magnetic particles contained in the top channel. By applying the magnetic field from the device's microcontroller, we are able to control the movement of magnetic particles within the channels. This system allows for the study of molecular transport in biological systems with high throughput and minimal destruction of the sample.

2803-Plat
Probing the Mechanical Coupling of the Cell Membrane to the Nucleus with Vertical Nanopillar Arrays
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The structure of the nuclear envelope is crucial for many cell signaling processes, from signal transduction to migration and motility, yet little is known about how the nucleus senses and responds to the cell exterior. Previous studies of the mechanical properties of the nucleus rely on invasive techniques like micropipetting, and often neglect the participation of the cytoskeleton and extracellular membrane, as they require isolation of the nucleus from the cell. In order to properly interrogate this process, we need a technique that can measure the response of the nucleus in intact cells as they function normally. Through previous studies performed by group on the interface between cells and nanostructures, we observed that the nuclear envelope of adherent cells deforms in response to the structure of the culture surface. Taking advantage of this response, we fabricated vertical nanopillars of various diameters, heights, and pitch to observe the nuclear deformation under different stress to the cell membrane. This platform allows us to explore the variation in nuclear mechanics, and its effects on cell behavior, among cell types and disease models in living cells.
Protein structure determination by X-ray crystallography can be time-consuming and expensive. Several important classes of proteins, such as membrane proteins and those containing intrinsically disordered regions, are severely underrepresented in the PDB because they are difficult to crystallize. However, there are other sources of information about protein structure, including as experiments (e.g. cross-linking experiments, solid state NMR, EPR), bioinformatics (e.g. secondary structure predictions, homologous proteins), and evolution (e.g. residue-residue contacts predicted from coevolution). These types of information share two properties. First, the information can be sparse. For example, an experiment may give us a few residue-residue contacts, but we may know little about the rest of the structure. Second, this information can contain errors and ambiguities. Predictions from bioinformatics may have errors and experimental results sometimes lack resolution and can be ambiguous. A successful method must be able to reliably integrate such sparse and noisy data into a reasonably accurate structural model. We are developing an algorithm, called MELD: Modeling with Limited Data, that can deal with such data within a sound statistical mechanical framework. I will outline how the method works and present several examples of results obtained using MELD, including structural models constructed from limited solid-state NMR data and protein structures modeled from evolutionary contact predictions.

2809-Plat
Real Rotamers using Real-Space Correlation Coefficients
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Protein side-chains occur in discrete low-energy, conformations known as rotamers. Rotamers can be elucidated by analysing chi angle distributions in high-quality protein models. A given side-chain’s rotamericity is calculated based on its position in multidimensional, chi-angle distributions, not by a simple distance from a rotamer-library member. Classical FRET, however, has been constrained by fluorophore pairs with long chemical linkers, large sizes, and long R0 values (30 to 60 Å). Here, we report that transition metal ion FRET (tmFRET) can be used in a sensitive, rapid, highly parallel screen, to overcome the limitations of classical FRET. The distances generated through this screen for the protein Maltose Binding Protein (MBP) match distances from backbone dipeptide and ternary complex with N-acetyl-D-glucosamine and beta-maltose interface demonstrating that the binding sites for glycans and fatty acids (PAMP-binding site) whereas the fatty acids occupy the cleft formed at A-B diffusion channel formed by B and D molecules. The co-complexed structures indicated that the glycan moieties which contain PAMPs bind at C-D interface with alternating A-B and C-D contacts. This leads to the formation of multiple subsites contributed by molecules A, C, and D and a supporting diffusion channel formed by B and D molecules. The co-complexed structures indicated that the glycan moieties which contain PAMPs bind at C-D interface (PAMP-binding site) whereas the fatty acids occupy the cleft formed at A-B interface demonstrating that the binding sites for glycans and fatty acids are independent of each other. Similarly, the binary complex with the muramyl dipeptide and ternary complex with N-acetyl-D-glucosamine and beta-maltose revealed that these are accommodated in different sub-regions of the PAMP-binding site. This indicates that the PAMP-binding site is capable of accepting different kinds of PAMP’s exhibiting varying specificities. It thus appears that the mode of binding of CPGRP-S essentially involves the interactions with the bacterial cell wall surface molecular patterns rather than cell membranes leading to the sequestration of the bacteria. Hence, CPGRP-S may be a protein antibiotic which may not suffer from bacterial resistance.

2812-Plat
Structural and Biochemical Characterization of a New Zincin Protease, NleC, an Enteropathogenic Escherichia Coli Type III Secretion System Effector Responsible for Cleaving NF{kappa}B Subunit Rela
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Utilizing the virulence machine termed the Type III Secretion System (T3SS), pathogenic-proteobacteria, such as enteropathogen O157:H7 Escherichia coli, inject virulent effector proteins into host cells. These effectors subvert host cell physiology, conveying a selective advantage to the bacterium. With x-ray crystallography, we determined the structure of the Sakai strain O157:H7 E. coli effector NleC, revealing a unique member of the Zincin zinc protease family. NleC is known to abrogate the inflammatory response of host cells by targeting NF{kappa}B subunits. While the presence of a zinc-coordinating aspartate places NleC in the Aspzincin subfamily, the active site sequence is unique, placing it in the metalloprotease group. NleC, an Enteropathogenic Escherichia Coli Type III Secretion System, New Delhi, India. Short peptidoglycan recognition protein (PGRP-S) is a member of the innate immune system which provides the first line of defense to hosts against invading microbes. PGRP-S recognizes conserved motifs called pathogen associated molecular patterns (PAMPs) present in microorganisms but absent in host. We have determined the structure of camel peptidoglycan recognition protein (PGRP-S) with various PAMPs like peptidoglycans, lipopolysaccharide, lipoateichoic acid, mycolic acid and five different fatty acids. The native structure revealed the presence of four crystallographically independent molecules A, B, C and D in the asymmetric unit. The buried surface area calculations indicated two stable contact regions, A-B and C-D which corresponded to opposite faces of the protein molecule resulting in the formation of a linear chain with alternating A-B and C-D contacts. This leads to the formation of multiple subsites contributed by molecules A, C, and D and a supporting diffusion channel formed by B and D molecules. The co-complexed structures indicated that the glycan moieties which contain PAMPs bind at C-D interface (PAMP-binding site) whereas the fatty acids occupy the cleft formed at A-B interface demonstrating that the binding sites for glycans and fatty acids are independent of each other. Similarly, the binary complex with the muramyl dipeptide and ternary complex with N-acetyl-D-glucosamine and beta-maltose revealed that these are accommodated in different sub-regions of the PAMP-binding site. This indicates that the PAMP-binding site is capable of accepting different kinds of PAMP’s exhibiting varying specificities. It thus appears that the mode of binding of CPGRP-S essentially involves the interactions with the bacterial cell wall surface molecular patterns rather than cell membranes leading to the sequestration of the bacteria. Hence, CPGRP-S may be a protein antibiotic which may not suffer from bacterial resistance.