Our combined electrophysiology and fluorescence microscopy approach is experimentally straightforward and enables rapid systematic investigation of the interactions between nanoparticles and the lipid components of the cell membrane. These model studies will aid in the rational design of safe nanoparticles -for drug delivery and subcellular labeling- that traverse the plasma membrane without adverse effects on membrane integrity.

2074-Pos

Surface Electrostatics and Lipid-Substrate Interactions of Nanopore-Confined Lipid Bilayers

Antonin Marek, Maxim A. Voinov, **Alex I. Smirnov**.
North Carolina State University, Raleigh, NC, USA.

Substrate-supported lipid bilayers serve many purposes: from acting as versatile models of cellular membranes to biotechnological applications including substrate functionalization and stabilizing membrane proteins in functional conformations. While adsorption and subsequent reorganization of phospholipid vesicles on solid substrates were studied in the past, the exact nature of physicochemical interactions between the lipids and substrate surfaces remain largely unknown. Here we employed recently synthesized pH-sensitive spin-labeled phospholipids - derivatives of 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol (PTE) with pH-reporting nitroxides that are covalently attached to the lipid's headgroup - to investigate surface electrostatics of nanotubular lipid bilayers confined in cylindrical nanopores. The lipid nanotubes were formed by self-assembling phospholipids inside ordered nanochannels of anodic aluminum oxide with pore diameters from 60 to 170 nm and diameter-to-pore length ratio of up to 1:1000. ^{31}P NMR confirmed formation of macroscopically aligned lipid nanotubes with just 1-2° mosaic spread from zwitterionic DMPC, anionic DMPG, and their mixtures. Interfacial potentials were measured by carrying out titration experiments and observing the protonation state of the nitroxide tag by EPR. For nanopore-confined DMPC:DMPG (1:1) bilayers the protonation equilibrium was shifted to more acidic values: when the single lipid bilayer was deposited per nanopore the pK_a of the nitroxide probe was shifted by (-0.91 ± 0.05) pH units but only by (-0.34 ± 0.05) when three bilayers per nanopore were present. Notably, the nitrogen hyperfine coupling constant for non-protonated nitroxides remained the same in all the samples indicating essentially the same interfacial dielectric environment. Thus, these shifts in pK_a must come from changes in the lipid bialyer surface potential that was estimated to increase by 52 ± 3 mV. EPR data on the lipid-substrate interface were combined with differential scanning calorimetry to elucidate effects of pore curvature, surface modification, and binding of antibacterial peptides on lipid-substrate interactions. Supported by DE-FG02-02ER15354.

2075-Pos

Reconstitution of Nanosized HDL Bearing Anti-Amyloid Flavonoids for Targeted Drug Delivery

Panupon Khumsupan[1], Chris E. Manansala[1], Vasanthy Narayanaswami[1,2].
[1]California State University, Long Beach, Long Beach, CA, USA, [2]Center for the prevention of obesity, Cardiovascular Disease & Diabetes, Children's Hospital Oakland Research Institute, Oakland, CA, USA.

High-density lipoproteins (HDL) are lipid-protein particles that are involved in transport of plasma cholesterol from peripheral tissues to the liver, a process called reverse cholesterol transport. In humans a subclass of HDL contains apolipoprotein E (apoE), an anti-atherogenic protein. ApoE serves as a ligand for the low-density lipoprotein (LDL) receptor family of proteins. Our objective is to employ reconstituted HDL containing recombinant human apoE3 as a vehicle to transport and target curcumin, an anti-amyloid and anti-inflammatory flavonoid, to the cells lining the blood brain barrier. Curcumin metabolites are far less potent than curcumin; therefore it is important to deliver active curcumin at inflammatory or amyloid aggregation sites. To achieve this, HDL was prepared by reconstituting palmitoyloleoylphosphatidylcholine, cholesterol, and human apoE3. Non-denaturing polyacrylamide gel electrophoresis of the reconstituted HDL indicates the molecular mass and diameter of the particles to be ~600 kDa and ~17nm, respectively. Curcumin was incorporated by direct addition into reconstituted HDL particles by incubation at 37oC for 6 h. We exploited the inherent fluorescent property of curcumin to determine its presence within the reconstituted HDL. A dramatic shift of the wavelength of maximal fluorescence emission of curcumin was noted from ~550 nm in dimethylsulfoxide or aqueous buffer or Triton X-100 micelles to ~490 nm in HDL. In addition, an enormous enhancement in fluorescence emission intensity was noted in curcumin-containing HDL. These observations indicate that curcumin has partitioned efficiently into apoE3-containing HDL. Partitioning of curcumin does not significantly alter the particle integrity. In conclusion, we report that curcumin can be packaged into apoE containing HDL particles. Its presence in the context of a lipoprotein complex bearing apoE offers the potential for its delivery across the blood brain barrier in an active form for treatment of Alzheimer's disease.

2076-Pos

Identification of the Membrane Interactome Using Nanodisc Phospholipid Particles

Catherine S. Chan, Xiao X. Zhang, Huan Bao, Leonard J. Foster, Franck Duong.
University of British Columbia, Vancouver, BC, Canada.

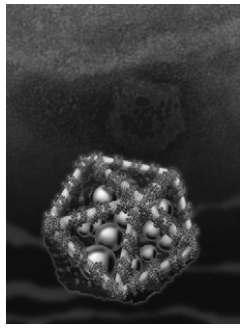
Membrane proteins have mostly been excluded in proteomic and interactomic studies due to the inherent difficulties in dealing with their highly hydrophobic properties. Nanodiscs have aided in the study of membrane proteins by overcoming many disadvantages arising from the use of detergent micelles and liposomes. These nanoparticles consist of a phospholipid bilayer circumscribed by an amphipathic scaffold protein, generating a soluble yet near-native environment for biophysical and biochemical studies of inserted membrane proteins. Nanodisc particles in combination with SILAC (stable isotope labeling by amino acids in cell culture) were used to study the membrane interactome for both protein-protein and lipid-protein interactions. Cultures grown in media containing an essential amino acid (arginine) that is either 'heavy-C^{13}' or 'light-C^{12}' labeled were used as prey in pull down assays containing immobilized nanodiscs as bait, followed by LC/MS-MS analysis. A quantitative proteome fingerprint based on the ratio of heavy versus light peptides of identified proteins was used to separate true interactors from contaminants. The well-characterized bacterial SecYEG and SecYEGDFyajC complexes reconstituted in nanodiscs were used as model systems to study protein-protein interactions. For lipid-protein interactions, dioleoyl-*sn*-glycero-3-[phosphor-*rac*-(1-glycerol)] (DOPG) and *E. coli* total lipid-reconstituted empty nanodiscs were used to isolate and identify acidic lipid-binding proteins.

2077-Pos

Icosahedral DNA Nanocapsules by Modular Assembly

Shabana Mehtab, Dhiraj Bhatia, Yamuna Krishnan.
National Centre for Biological Sciences - TIFR, Bangalore, India.

The construction of well-defined 3D architectures is one of the greatest challenges of self-assembly. Nanofabrication through molecular self-assembly has resulted in the formation of DNA polyhedra with the connectivities of cubes,tetrahedra, octahedra,dodecahedra,and buckminsterfullerene. DNA polyhedra could also function as nanocapsules and thereby enable the targeted delivery of entities encapsulated from solution. Key to realizing this envisaged function is the construction of complex polyhedra that maximize encapsulation volumes while preserving small pore size. Polyhedra based on platonic solids are most promising in this regard, as they maximize encapsulation volumes. We therefore constructed the most complex DNA-based platonic solid, namely, an icosahedron, through a unique modular assembly strategy and demonstrated this functional aspect for DNA polyhedra by encapsulating gold nanoparticles from solution.



2078-Pos

Computational Design of an RNA Nanoparticle Consisting of a Three-Way Junction and pre-miRNAs

Shu-Yun Le, Taejin Kim, Bruce A. Shapiro.
NCI-Frederick, Frederick, MD, USA.

Recent research on RNA-based regulation, RNAi, and development of human diseases has shed light on the huge potential of miRNAs as novel therapeutic agents for medicine. It has been suggested that a successful tissue-targetable nucleic acid delivery system has to overcome the problem of poor stability of nucleic acids in biological media. To solve this problem we rationally designed a pre-miRNA-nanoparticle-mediated RNA delivery system by integrating computer modeling, miRNA regulatory function and RNA structure versatility. It has been well documented that the initial product of a miRNA, the pri-miRNA, is transcribed in the nucleus. It forms a highly stable stem-loop structure that is processed to form the pre-miRNA by the RNase III enzyme, Drosha. The stable pre-miRNA stem-loop structure of 60-70 nt is transported to the cytoplasm and is then processed into a short double stranded fragment by dicer. Finally the miRNA duplex is unwound to form a ~22-nt single stranded mature miRNA which is associated with the RISC complex. In this study, we present a computational design of a synthetic, highly stable superstructure made from an RNA junction that can accommodate multiple

pre-miRNAs. A three-way junction building block, which was obtained from the RNAjunction database, was attached to three human let-7 pre-miRNAs. This highly stable RNA nanoparticle is expected to enable the binding of the dicer protein for the efficient cleavage of the pre-miRNA in the cytoplasm. As a result, the mature let-7 miRNAs can enter into the targeted cell cytosol, and be protected from degrading interactions while performing its specific regulatory functions.

2079-Pos

Surface-Charge Influences Voltage-Dependent Pore Formation in Lipid Membranes by Quantum Dots

Srujan Kumar Dondapati[1], Gerhard Baaken[1,2], Ali Zulqurnain[3], Feng Zhang[3], Wolfgang J. Parak[3], Jan C. Behrends[1].
[1]Laboratory for Electrophysiology and Biotechnology, Department of Physiology, University of Freiburg, Freiburg, Germany, [2]Laboratory for Chemistry and Physics of Interfaces, Department of Microsystems Engineering, Freiburg, Germany, [3]Department of Physics, Philipps University of Marburg, Marburg, Germany.

Semiconductor nanocrystals, such as quantum dots (QDs) have many biomedical applications. It is of great interest to understand the mechanism by which these nanoparticles cross the cell membrane. Here, QDs, such as cadmium selenide / zinc sulfide core shell (CdSe/ZnS) nanoparticles are shown to interact with lipid bilayers painted on picoliter microelectrode cavities and to produce rapid current bursts with half widths in the range of tens of microseconds. These bursts are voltage dependent and are observed in both polarities. The voltage-dependence of the burst frequency is strongly influenced by external solution parameters like pH and salt valency, charge of the lipids, bilayer size and also nanoparticle size and charge. Correlating these findings with results of dynamic light scattering (zetasizer), we present evidence that electrostatic interactions play a pivotal role in generating the current bursts. Future experiments will focus on optical characterization in order to obtain more insight into the mechanism of interaction.

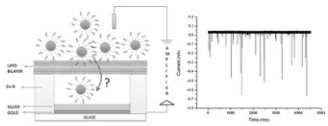


Figure 1. (A) Scheme of the measurement set up. (B) Current bursts measured across the bilayer formed over 10 um electrode in the presence of QDs at - 200 mV.

2080-Pos

Retrieval of a Metabolite from Cells with Polyelectrolyte Microcapsules

Deborah Studer[1,2], Raghavendra Palankar[1], Sebastian Springer[1], **Mathias Winterhalter**[1].
[1]Jacobs University Bremen, Bremen, Germany, [2]EPFL, Lausanne, Switzerland.

To monitor cellular processes in individual cells, it is an important goal to measure the concentrations of intracellular components in real time, and to retrieve them for analysis. We report here the use of functionalized polyelectrolyte microcapsules as intracellular sensors for in vivo reporting. When capsules were loaded with streptavidin and introduced into Vero fibroblasts by electroporation, they were initially inaccessible to cytosolic biotin-fluorescein (BF), but after several hours, they bound and accumulated BF. Our work demonstrates the utility of polyelectrolyte microcapsules for intracellular sensing and suggests that they can autonomously escape from an endocytic compartment, making them ideal carriers for intracellular investigations.

2081-Pos

Monofunctional Quantum Dot Probes for Single-Molecule Imaging

Samuel Clarke[1], Fabien Pinaud[1], Assa Sittner[1], Geraldine Gouzer[1], Oliver Beutel[2], Jacob Piehler[2], Maxime Dahan[1].
[1]ENS Paris, Paris, France, [2]Universität Osnabrück, Osnabrück, Germany.

Recently, it has been shown that the optical properties of quantum dot (QDs) nanoparticles enable novel experiments at the single molecule level in live cells, thereby opening new prospects for the understanding of cellular processes. One difficulty with these experiments is that the complex biological environment imposes stringent design requirements on QD probes, necessitating the development of smaller, low valency and more biocompatible QDs. In this work, we present our efforts towards minimizing the size and controlling the surface functionality of QDs. We show that an engineered peptide surface coating and a purification method based on gel electrophoresis are sufficient to produce compact monofunctional QDs covalently conjugated to streptavidin (SAV), biotin and antibodies. To prove the monofunctionality of the QD-SAV probes, we apply novel single-molecule assay following complexation of the QDs with a fluorescent dye biotin derivative. Counting the photobleaching steps of the fluorescent dye gives us direct access to the number of binding sites present on the QD surface. We then apply these QD probes to the targeting and tracking of individual biotinylated membrane proteins expressed in living HeLa cells. We analyze the diffusion properties of these membrane proteins and compare the measurements to those obtained using commercially available QD probes. Overall, these monofunctional QD probes should be useful for studying a wide-range of biophysical phenomena, down to the single molecule level in live cells.

2082-Pos

Quantification of Functional Binding Sites Per Quantum Dot

Holly N. Wolcott, Eric C. Greene.
Columbia University, New York, NY, USA.

Antibody conjugated quantum dots have become increasingly useful in the study of proteins both *in vitro* and *in vivo* and are particularly valuable in single molecule experiments due to their narrow emission spectra and photostability. Despite their wide range of uses, it has been difficult to determine the number of functional binding sites per quantum dot. Previous studies focused on the characterization the total number of antibodies conjugated to quantum dots rather than the quantification of functional antibodies per quantum dot. Understanding the later is especially important to interpretation of data obtained in single molecule experiments using quantum dot labeled proteins. Additionally, since the number of functional binding sites per quantum dot may vary depending on the antibody conjugation method and also between quantum dot preparations, it is necessary to develop a simple and rapid method to test this experimentally. In this study, we use a direct read-out from the protein itself to determine the average number of proteins bound per quantum dot. By using radio-labeled enhanced green fluorescent protein (EGFP) constructs conjugated to common affinity tags used for protein labeling, we will quantify the number of functional binding sites per quantum dot and the specificity of quantum dot labeling *in vitro*. This method will be extremely useful in the interpretation of data obtained using quantum dot labeled proteins.

2083-Pos

Quantitative Study of the Protein Corona on Engineered Nanoparticles

Xiue Jiang[1], Carlheinz Röcker[1], Feng Zhang[2], Wolfgang J. Parak[2], **G. Ulrich Nienhaus**[3,4].
[1]University of Ulm, Ulm, Germany, [2]University of Marburg, Marburg, Germany, [3]Karlsruhe Institute of Technology, Karlsruhe, Germany, [4]University of Illinois, Urbana-Champaign, IL, USA.

Nanoparticles are finding a rapidly expanding range of applications in research and technology, finally entering our daily life in medical, cosmetic or food products. Colloidal inorganic nanoparticles rendered water soluble by highly ordered organic shells hold great promise as powerful tools for applications in biotechnology and biomedicine. However, their ability to invade tissues, cells and even subcellular compartments may result in biological hazards if nanoparticle incorporation and migration within the body cannot be tightly controlled.

As yet, little known about the detailed mechanisms by which objects on the nanoscale interact with living organisms. Upon incorporation via the lung, gut or skin, nanoparticles become exposed to biological fluids containing dissolved biomolecules, especially proteins. Quantitative studies of the interactions between nanoparticles and biomolecules, which depend on the nature of the nanoparticle surface, are still scarce, and even less is known as to how such a 'protein corona' affects nanoparticle uptake by living cells.

Here we have employed fluorescence methods, especially FCS, to investigate the adsorption of human serum albumin and (apo-)transferrin on polymer-coated FePt nanoparticles of ~5 nm radius. Both proteins form a monolayer on the surface of these nanoparticles and bind with micromolar affinity. We have also studied the effect of the protein corona on the nanoparticle uptake behavior by HeLa cells (1, 2).

1. Röcker, C. et al., Nature Nanotechnology 4, 577-580 (2009).
2. Jiang, X. et al., J. R. Soc. Interface, published online, doi:10.1098/rsif.2009.0272.focus

2084-Pos

Nanocandles: Developing Optical Probes for the Cell Interior

Lindsey Hanson, Chong Xie, Yi Cui, Bianxiao Cui.
Stanford University, Stanford, CA, USA.

As knowledge of the bulk behavior of biological systems continues to grow, there is an increasing demand for knowledge of cellular processes at the single-molecule level. This presents a unique challenge, a combination of the dynamic nature of the system and the inability to modulate the concentration of