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

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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

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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

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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

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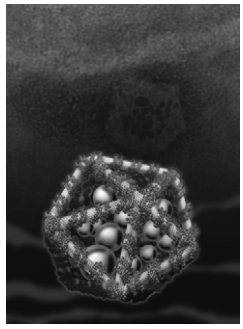
The construction of well-defined 3D architectures is one of the greatest challenges of self-assembly. Nanofabrication through molecular self-assembly has resulted in the formation of DNA polyhedra with the connectivities of cubes,tetrahedra, octahedra,dodecahedra,and buckminsterfullerene. DNA polyhedra 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

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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