pleckstrin homology (PH) domains are an important class of membrane targeting domains that specifically bind target phosphoinositides present at the surface of inner cell membranes. Aside from target lipid headgroupprecognition, the other protein-lipid interactions that occurduring membrane docking are not well defined. Currently, high-resolution structural characterization of protein-membrane interfaces is difficult to achieve while this information is crucial to a physical chemical understanding of reversible protein-membrane binding. In this study, site-directed spin-labeling and electron paramagnetic resonance (EPR) power saturation measurements were employed to determine membrane depth parameters for the PI(3,4,5)P3-specific GRP1-PH domain docked to synthetic bilayer membranes. A library of nitroxide spin-labeled PH domain mutants was generated using site-directed cysteine mutagenesis and disulfide coupling to a methanethiosulfonate spin label (MTSSL). Subsequently, membrane depth parameters were determined for each spin-labeled position in the membrane-docked state. The depth parameters were then used as constraints to model the angular orientation and depth of penetration that describes the membrane docking geometry. Our preliminary structural model identifies the membrane binding surface of GRP1-PH and characterizes its partitioning into the membrane bilayer. Ultimately, the results of this study will aid in understanding the molecular determinants of the electrostatic search mechanism this PH domain uses to rapidly find its rare target lipid on the plasma membrane surface. Supported by NIH GM063235 (J.J.F.).

2070-Pos

Sequence-Specific Stereomeric Environment in a DNA Duplex Revealed by a Nucleotide-Independent Nitroxide Probe

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In site-directed spin labeling, structural and dynamic information on a parent macromolecule is obtained by monitoring a covalently linked nitroxide radical using electron paramagnetic resonance (EPR) spectroscopy. Our group have developed a method of attaching nitroxide species, such as 1-oxyl-4-bromo-2,2,5,5-tetramethylpyrroline (R5a), to a specific nucleotide position within a target DNA or RNA sequence. The method relies on site-specific introduction of a phosphorothiate during the solid phase chemical synthesis of nucleic acids, and at each given labeling site the nitroxide is attached to one of two phosphorothioate diastereomers (Rp or Sp) in an approximately 50/50 ratio. We have recently reported that variations in DNA structural and dynamic features at the level of an individual nucleotide can be detected using R5a attached to mixed phosphorothioate diastereomers, in which an observed EPR spectrum is presumably a sum of those obtained from either diastereomer (Popova et al., Biochemistry, 2009, 48, 8540-50). In this work, we report X-band EPR spectra of R5a attached to purified Rp and Sp diastereomers at different sites within a B-form DNA duplex. Results are compared to those obtained with mixed nitroxide diastereomers, and advantages and limitations are discussed regarding the necessity of diastereomer separation when probing DNA local environment. Our work is a further step forward in developing a SDSL methodology that may provide a mean for studying structure and dynamics in large DNA molecules.

Nano-Materials

2071-Pos

Analysis of Postphotoactivation Scanning Diffusion Profiles for Multiple Species with Distributed Diffusion Coefficients

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Postphotoactivation scanning (PPS) is a method for quantifying diffusion coefficients of particles in simple and complex materials (e.g., hydrogels) over length scales ~100μm-mm (Geonnotti et al., 2008). Diffusing particles are labeled with caged fluorophore, and a slit-shaped region of sample is exposed to UV to generate a line of fluorescence. A high-resolution scanner quantifies intensity profiles as particles diffuse out from the fluorescent region over time; these are fit to the solution of the diffusion equation to obtain the diffusion coefficient. We use this technique to measure mobility of HIV-like liposomes. Here, we describe a novel method for analyzing PPS profiles for multiple diffusing species with a distribution (α) of diffusion coefficients (D). To determine $\alpha(D)$, we generated sets of diffusion profiles for a discretized range of D by numerically solving the diffusion equation using the experimental initial condition and assuming given D. We computed net diffusion profiles resulting from the sums of profiles with distribution α. We used an optimization scheme to deduce $\alpha(D)$ that minimized the squared difference of observed and computed profiles. The method was validated using simulated and experimental data. Experimental results for fluorescein diffusing in PBS (D 4.3×10^{-6} cm^2/s) are similar to literature values. We also measured diffusion coefficients of solutions of labeled ~100nm liposomes (D $3.2\pm2\times10^{-8}$ cm^2/s, n 4). We were able to resolve 2 distinct peaks in α corresponding to the D of liposomes and of free label. Measured values for diffusion coefficients of liposomes are similar to those predicted by Stokes-Einstein (D $3.9\pm1\times10^{-8}$ cm^2/s). We are using the technique to analyze interactions of HIV-like liposomes and anti-HIV antibodies. The method can be applied to describe diffusion of multiple species within complex materials. [Supported by Duke CFAR and NIH AI48103]

2072-Pos

Mucus Rheological Properties Altered by Functional Nanoparticles

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Multi-functionalized nanoparticles (NPs) have recently been extensively explored for their potential in novel drug delivery and nanomedicine applications. Functionalized NPs based local drug delivery across the mucosal epithelia has gained much interest.

Despite reports confirming cellular nanotoxicity effects, possible health hazards resulted from mucus rheological disturbances induced by NPs are underexplored.

Accumulation of viscous, poorly dispersed and less transportable mucus that could result in improper mucus rheology and dysfunctional mucociliary clearance are typically found to associate with many respiratory diseases such as asthma, cystic fibrosis (CF) and COPD (chronic obstructive pulmonary disease). Whether functionalized NPs can alter mucus rheology and its operational mechanisms are not resolved. Here we show for the first time that positively-charged functionalized NPs can effectively induce mucin aggregation and hinder mucin gel hydration. These NPs significantly increase the size of aggregated mucin approximately 30 times within 24 hrs. EGTA (ethylene glycol tetraacetic acid, 2 mM) chelation of indigenous mucin crosslinkers (Ca^{2+} ions) was unable to effectively disperse NPs-induced aggregated mucins. We also found that positively-charged NPs can significantly reduce the swelling kinetics and hydration of newly released mucus. Our results have demonstrated that positively charged functionalized NPs can serve as effective crosslinkers hindering mucin disaggregation and dispersion resulting in potential dysfunctional mucociliary clearance and health problems. This report also highlights the unexpected health risk of NP-induced change in mucus rheology and possible mucociliary transport impairment on epithelial mucosa. In addition, our data can serve as a prospective guideline for designing nanocarriers specific for mucosal epithelia drug delivery applications.

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2073-Pos

Silica Nanoparticles Permeabilize Lipid Bilayers

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Potential toxic effects of synthetic nanoparticles are of great public concern. Presently, the impact of nanoparticles at a cellular level is assessed by adding nanoparticles to cell cultures and subsequent evaluation of particle uptake by confocal fluorescence microscopy and of cell viability by conventional (fluorescence) assays. Cytotoxic effects of nanoparticles have been reported but a correlation between nanoparticle properties (e.g. size, shape, surface chemistry) and cell viability remains elusive. However, cellular uptake of nanoparticles is almost universally observed. Membrane translocation of nanoparticles is generally considered to be an active process, requiring the presence of receptors that mediate encapsulation of the nanoparticles into an intracellular vesicle, from which the particles may or may not escape into the cytosol.

Using electrophysiological methods we have demonstrated that spherical silica nanoparticles, under development for intracellular drug delivery, are able to permeabilize protein-free lipid bilayers as a function of size and surface charge. Single channel-like conductances, similar to those induced by membrane-disrupting β-amyloid peptides, are observed for rigid sterol-containing bilayers. For more fluid bilayers of DOPC the conductance gradually increases until the bilayer disintegrates, which has also been observed for cytotoxic amyloid oligomers. The most disruptive nanospheres were shown by confocal fluorescence microscopy to accumulate at the bilayer surface, and we demonstrated that a fraction of these particles translocate across the lipid bilayer, suggesting that passive uptake of nanoparticles may contribute to cellular uptake.

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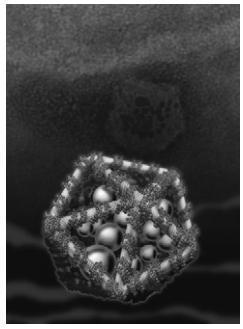
The construction of well-defined 3D architectures is one of the greatest challenges of self-assembly. Nanofabrication through molecular self-assembly has resulted in the formation of DNA polyhedra with the connectivities of cubes,tetrahedra, octahedra,dodecahedra,and buckminsterfullerene. DNA polyhedra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