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.