Membrane Dynamics & Bilayer Probes II

2185-Pos Board B204
Surfactants Alter Nanoparticle - Model Cell Membrane Interactions
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Due to their small size, nanoparticles (NPs) have the ability to penetrate pulmonary and vascular tissue, and as a result, are classified as potential human carcinogens. On the other hand, nanoparticle insertion into targeted cells can play a key role in drug delivery and gene therapy applications, prompting a need to more thoroughly characterize nanoparticle/membrane interactions. Polystyrene nanoparticles with modifications in surface functionalization and detergent conditions were introduced to a Langmuir phospholipid monolayer, a cell membrane outer leaflet model. Negatively charged (COO^- functionalized) detergent free nanoparticles solubilized a zwitterionic 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) monolayer held at constant, physiological pressure, removing material from the air/water interface to a greater extent than did positively charged (NH_3^+ functionalized) nanoparticles. To determine the role of lipid charge in nanoparticle/membrane interactions, negatively charged 1,2-dilauroyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (DLPG) and positively charged 1,2-dimyristoyl-3-trimethylammonium-propane (DMTAP) lipid monolayers were used. Nanoparticles of opposite charge than the membrane removed a larger percentage of the monolayer compared to like-charged particle/phospholipid systems. Vesicle leakage assays were run to determine applicability of these results to a more physiologically relevant bilayer system.
Ionic and non-ionic surfactants, typically present in nanoparticle solutions to limit aggregation, all showed significant surface activity and monolayer insertion which can be directly correlated to surfactant hydrophobicity. Adding surfactant to detergent-free nanoparticle solutions decreased the magnitude of monolayer solubilization compared to nanoparticles alone. To better understand nanoparticle-detergent-membrane interactions, either nanoparticles or detergents were introduced to the monolayer, and following a time lapse, the other component was introduced. Under various surfactant and salt conditions, dynamic light scattering and zeta potential measurements were used to estimate the amount of particle associated detergent and determine particle size and extent of aggregation. A model of detergent sequestration by the polystyrene nanoparticles explains these results.

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Size-Dependent Interaction between Gold Nanoparticles and Lipid Bilayer Membranes
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Metal nanoparticles, in particular gold nanoparticles (AuNPs) are of great interest in biomedical research, such as in diagnosis and therapeutics[1]. Recent studies indicate the size-dependent cytotoxicity of the AuNPs[2], however the means of membrane association and interaction are currently unknown. To better understand and optimize these novel compounds, their interactions with biological membranes need to be investigated. We used AuNPs stabilized by triphenylphosphine derivatives ranging in size from 1.4 to 15 nm size in bilayer experiments [3]. We observe size-dependent membrane association: AuNPs of 15 nm diameter generate ion-selective currents while smaller 1.4 nm particles show no such effects. We therefore presently examine intermediate AuNP sizes to find the size-limitation for these effects.
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2187-Pos Board B206
Study of Nanoparticle-Lipid Bilayer Interactions: Insights from Coarse-Grained Molecular Dynamics Simulations
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One of the main aims in the design of engineered nanomaterials with applications in medicine, such as diagnostic and therapeutic nanoparticles (NPs), is the ability of these materials to translocate across the human cells without damaging essential tissues. The entry point of a NP to a cell is the plasma membrane. Thus, the first step into assessing the NP cytotoxicity requires a thorough understanding of the NP-membrane interaction mechanism. Extensive Molecular Dynamics (MD) simulations and free-energy calculations were employed, providing insights into the significance of NP surface chemistry and cholesterol concentration of the membrane in the NP-membrane interplay. The MARTINI coarse-grained force-field as implemented in the GROMACS simulation package was employed to model NPs and investigate their interaction with a model lipid bilayer. NPs with a diameter of 3 nm and a surface comprising different patterns of hydrophobic and hydrophilic groups were designed. MD equilibrium simulations were carried out in order to gain insight into the mechanism of NP-membrane interaction. To evaluate the molecular energetics of this interaction, Potential of Mean Force (PMF) calculations, using the Umbrella Sampling technique, were also performed. Our results demonstrate that certain surface patterns, such as an ordered distribution of hydrophilic groups, alter the interaction mechanisms and change the molecular energetics of the NP-membrane interactions. Moreover, PMF calculations showed that an increase in the cholesterol concentration of the membrane leads to a higher energy barrier of NP translocation across the membrane. The results from the present study provide valuable information on possible links between NP surface characteristics and cholesterol concentration with specific interaction types with the cell membrane, and provide insights into the design of NPs with tailored functionalities, for example direct cellular entry.

2188-Pos Board B207
How Instantaneous Lipid Flows Influence Membrane Protein Diffusion
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Instantaneous lipid flows have previously been shown to exist within pure lipid bilayers using molecular dynamics simulations. The role of lipid flows and implications for membrane dynamics over a larger scale is yet to be explored. using HPC resources we capture the behaviour of ''crowded'' bacterial membranes by simulating large arrays (up to 256) of outer membrane proteins (OMP) in a 120nmx120nm patch of bilayer. We use a POPE:POPG 3:1 lipid mix and five different OMPs. Correlated lipid movement stretches over 10s of nanometres and is a function of system size which has important implications for simulations attempting to capture protein clustering. We show that these short lived correlated lipid movements are not suppressed by the addition of outer membrane proteins and play a role in both the rotational and translational diffusion of the proteins.

2189-Pos Board B208
Diffusion Effects in Cross-Linked Bilayers
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We investigate peptide interactions with model membranes and how they affect phase dynamics and mobility. Previously, we have studied lipid phase rearrangement due to cross-linking lipids in the headgroup position. Clustering and possible subanomalous diffusion inhibit domain coalescence and alter the conformation energy minimum. Building on this, our current efforts investigate peptide perturbations in lipid bilayers. We cross-link transmembrane peptides on the surface of lipid vesicles to examine the interactions between the helices, mimicking B cell receptor clustering. We conduct these studies by modifying the transmembrane portion of the mIgM receptor and incorporating the resulting peptide into the bilayer. We analyze these associations using microscopy, FRAP, FRET and CD spectroscopy.

2190-Pos Board B209
Measuring Lipid Membrane Viscosity using Rotational and Translational Particle Diffusion
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The two-dimensional fluidity of lipid bilayers enables the motion of membrane-bound macromolecules and is therefore crucial to biological function. However, lipid bilayer viscosity remains poorly quantified, largely due to the difficulty of relating the diffusion coefficients of membrane-associated tracer particles to the viscosity of the underlying membrane. We address this with a new technique for measuring lipid bilayer viscosity, in which determination of both the rotational and translational diffusion coefficients of tracer particles enables quantification of viscosity as well as the effective size of the tracers. Surprisingly, we find a wide distribution of effective tracer sizes, due

presumably to a wide variety of couplings to the membrane. We provide viscosity measurements for lipid bilayers with various compositions in the gel and liquid crystalline phases.

2191-Pos Board B210

Mc Model of Lipid Raft Protein Diffusion Matched to Live Cell Measurements with Controlled Chemical Perturbation Experiments

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Partitioning of certain type extra-cellular or cytosolic leaflet membrane proteins into functioning cholesterol stabilized domains within both leaflets of the membrane structure has long been shown by various studies facilitating different techniques. We have modeled how lipid-stabilized nano-domains influence the diffusion of some membrane proteins. By combining these simulations with non-perturbative experimental measurements of the diffusion behavior for chemically unperturbed and controlled perturbation cases of that diffusion, we were able to quantify the essential parameters describing the domains. The simulations feature four independent parameters, i.e. area fraction of the membrane occupied by domains, size of the domains, probability of the proteins to exit the domains, and effective protein diffusion within the domains. Kinetic Monte-Carlo modeling of proteins' association with membrane nanodomains has been performed over a selected region of the parameter space. Some of these parameters may be modulated at the single cell level during the time of a single experiment, allowing precise matching of all four parameters. We have used our non-perturbative imaging based FCS, bimFCS, to measure the diffusion of several membrane proteins from inner and outer leaflets of the cell-membrane over multiple length scales simultaneously. This allows extracting information of the domains which transiently trap the diffusing proteins.
We studied GFP-tagged GPI anchored proteins for the external leaflet and compared measurements of monomers and induced dimers on the same cells. For the intracellular leaflet, we studied Lyn-anchored GFP and used the Rapamycin induced cross-linking of the FKBP12 and Frb domains. We were able to successfully match experimental data with in-silico modeling by Kinetic Monte Carlo simulations, giving insight about sizes and area fraction of raft domains for each leaflet of the membrane, and also affinity of associated proteins with them.

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High-Speed Interferometric Scattering Microscopy of Receptor Mobility Reveals Anomalous Diffusion in Model Membranes

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The diffusive behavior of membrane proteins has been reported to change with the timescale of the measurement. High-speed single-particle tracking studies have revealed that it is Brownian on time scales less than 100 μs, confined from 1 to 10 ms, and Brownian at longer times (>10 ms).[1] This behavior has been interpreted as hop diffusion between submicron compartments in the plasma membrane. Due to the fundamental difficulty in achieving simultaneously high spatial and temporal resolution, these results have relied on a single experimental approach. Furthermore, attempts to use a complementary fluorescence technique to study anomalous diffusion have not reproduced the hop diffusion model.[2]
Here we use interferometric scattering microscopy (iSCAT)[3] to track the motion of the GM1 ganglioside receptor binding the B subunit of cholera toxin using 40-nm gold nanoparticle labels, in both supported lipid bilayers (SLBs) and droplet hydrogel bilayers (DHBs).[4] In SLBs, in single trajectories containing >200,000 frames and providing simultaneous 10 μs temporal and sub-nm spatial precision, we observe a shift from Brownian diffusion at early time scales (30 - 100 μs) to confined diffusion (100 μs - 10 ms) back to Brownian diffusion (>10 ms). In contrast, GM1 diffusion in DHBs is Brownian at all timescales. These data suggest that the presence of a structural support on one side of the bilayer is sufficient to produce anomalous diffusion, even for diffusers that do not penetrate the membrane.

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2193-Pos Board B212

Investigation of Temperature Induced Mechanical Changes in Supported Bilayers by Reconstructed Atomic Force Microscopy Tapping Forces

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Membrane mechanics, such as rigidity, adhesion, and fluidity are known to play an important role in the regulation of many biological and cellular processes. These properties can be drastically affected by changes in temperature; increasing temperature can enhance membrane permeability and susceptibility whereas decreasing temperature is known to restrict membrane movement and the intake of essential nutrients. Temperature also plays a key role in determining the phase of a membrane; evidence of nanoscale rearrangement of lipids into raft-like domains has been found at physiological temperatures. Rafts are thought to be a site of importance for signal transduction and membrane protein trafficking. There is even a growing association between rafts and the development of neurodegenerative diseases. Thus, it is evident that temperature has a critical role in the maintenance of membrane biology. In this work, patches of total brain lipid extract bilayer containing 30% exogenous cholesterol were formed in phosphate buffered saline and examined using atomic force microscopy while the temperature was systematically increased from 28-40°C. Tapping mode atomic force microscopy (TMAFM) and scanning probe acceleration microscopy (SPAM) were used to obtain topographical and mechanical information of the sample, respectively. SPAM is relatively new technique that offers the advantage of quickly obtaining mechanical information, while maintaining nanoscale spatial imaging resolution, due to its basis in TMAFM. The principle of SPAM is that the noisy deflection signal is captured during TMAFM imaging and reconstructed to reveal the time-resolved forces between the cantilever tip and the sample surface. These tip/sample forces can be correlated with surface properties such as rigidity and adhesion. With SPAM it is also possible to construct harmonic images, thus providing further insights on subtle surface characteristics.

2194-Pos Board B213

Red Blood Cell Membrane Fluctuations and their Mechanisms: Passive Versus Active

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Red blood cells (RBCs) are soft and flexible biconcave discs, which are able to pass through capillaries with diameters several times smaller than the RBC size. The RBC deformability also results in noticeable membrane fluctuations, which must be correlated with RBC membrane properties. However, it is still under debate whether RBC membrane fluctuations are simply passive thermal undulations or whether a red cell also experiences active fluctuations which are driven by a metabolic activity or other cell processes. We will present direct evidence that the RBC undulations are not solely passive thermal fluctuations, which has been obtained from a set of different experiments and simulations using a high spatiotemporal resolution: from 10 microseconds to several seconds in time and up to 20 nanometers in space. Experimental results show a violation of the fluctuation-dissipation theorem (FDT) for freshly prepared RBCs indicating the existence of active processes. However, the FDT is satisfied for starved cells demonstrating that the membrane fluctuations are passive when the energy supply is absent. Experiments also show a considerable change in the fluctuation amplitudes for fresh and starved cells. Subsequently, we perform simulations which fully mimic and quantify the experiments. We are able to quantitatively extract RBC membrane properties including shear elasticity, bending rigidity, and membrane viscosity. Furthermore, we test several models for active fluctuations, which mimic different possible mechanisms including spectrin network remodeling, ion pumps, and change in the spontaneous membrane curvature. Simulation results agree well with experimental data and suggest that several processes mentioned above may contribute to active RBC fluctuations. We will discuss which processes are more likely to take place.

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Combined Stopped-Flow and Electrophysiological Experiments Suggest Direct Sodium Channel Inhibition by Model Fluorobenzene Anesthetics

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General anesthetics are often proposed to affect membrane properties through interactions with the lipid bilayer. Though recent studies have shown that inhaled anesthetics can interact more specifically with certain membrane proteins such as ion channels. For example, volatile anesthetics inhibit voltage-gated sodium channels to reduce neurotransmitter release. Fluorobenzenes (FBs), once considered for clinical use, were abandoned due to their flammability and toxicity but are still valuable model anesthetics for investigating the molecular mechanisms of anesthetics. We examined the properties of four FB compounds, 1,2-DiFB, 1,4-DiFB, 1,3,5-TriFB and HexaFB on lipid bilayer