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protein-domain interactions is essential to understand cell functionality, and yet today we cannot distinguish between the thousands of possible lipid domains, nor is there a method to determine with which domains a protein associates with.
In this research I have developed a new methodology which determines in vitro the lipid composition a protein has a high affinity to. The method utilizes a liposome library composed of more than 250 liposomes, each made of a different lipid composition. The library has the largest and most diverse composition of lipid liposomes ever made. The affinity of proteins to each liposome is studied by advanced microfluidics and image processing, developed at Harvard. http://youtu.be/f7OvnE0g-Uo Using this method we characterize the binding specificity of toxins, peptides, trans-membrane domains and viral proteins to lipid domains.
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2617-Pos Board B309
Direct Visualization and Quantification of Doc2B-Mediated Membrane Fusion
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The C2 domains of the Doc2b protein are known to play a crucial role in regulating exocytosis through the coupling of calcium signals to secretory events. The exact mechanism, however, remains debated: Doc2b either inhibits SNARE-mediated membrane fusion at low calcium concentrations or it directly enhances membrane remodelling events at elevated calcium concentrations.
Using a novel combination of optical trapping and fluorescence microscopy, we have addressed this issue by monitoring membrane-membrane interactions between two optically trapped micrometer-sized lipid-coated polystyrene beads. We initially brought the beads in close proximity to allow any protein-lipid and lipid-lipid interactions to take place and then retracted one bead while continuously measuring the force of the interaction between the two beads. The lipid coating on the beads was fluorescently labelled, enabling simultaneous imaging of lipid remodelling and measurement of forces associated with this process.
The results show that in the presence of Doc2b, phosphatidylserine and calcium, a micrometers-long membrane stalk forms between the two individual membranes which is stable on a timescale of multiple minutes. Lipid mixing was observed, thus providing unambiguous evidence for an active role of Doc2b in membrane remodelling. The interaction forces between the two lipid-coated beads displayed a broad range up to 150 pN when a membrane stalk was formed.
We conclude that Doc2b contributes to SNARE-dependent exocytosis by enhancing the probability of membrane stalk formation during local calcium elevations. In the near future, we aim to use a fluorescently labelled variant of Doc2b to determine the number of proteins bound needed to mediate the interaction and the position of the Doc2b protein with respect to the membrane stalk.

2618-Pos Board B310
The Role of Salt in Mitochondria: Returning Cytochrome C to its Native State After Its Dissociation from Cardiolipin Containing Membranes
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Cytochrome c is a well-researched bio-molecule, however, over the past decade, research on cytochrome c has shifted, driven by the necessity to understand this protein's role in triggering apoptosis. Very recent works by the Kagan group and others have shown that the protein is primed for this function by gaining peroxidase activity while still bound to the inner membrane surface of mitochrondra. Liposomes with anionic phospholipids have been used as model system for the inner mitochondrial membrane to which cytochrome c binds in vivo. In order to check whether cytochrome c - liposome interactions cause any irreversible structural changes to the protein, we subjected different protein-liposome mixtures to ultracentrifugation and measured the fluorescence and CD spectra of the proteins in the respective supernatants as well as of the corresponding protein-lipid complexes, in the absence and presence of 100 mM NaCl. Our data indicate that cyt c remains in a non-native conformational state after dissociation from liposome binding sites. Upon the addition of salt, however, the protein switches back to its native conformation. We propose that the liposome bound state of the protein contains a substantial fraction of what Pletneva and coworkers describe as an extended conformation of cytochrome c. After dissociating from the liposome but due to the positively charged surface of the protein, the repulsive forces between positive charges particularly in the N- and C-helix keep cyt c in this extended, partially unfolded state. The addition of NaCl neutralizes these charges, thus allowing the protein to return to its native state. Our data suggest that without the presence of NaCl in the inner membrane space of mitochondria cytochrome c would stay in a frustrated, misfolded state.

2619-Pos Board B311
RI-Dom, a Cell-Penetrating Peptide. Interaction with DNA and Membranes
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riDOM (retro-inverso-dioleoyl-melittin) is a cell penetrating peptide with excellent transporter properties for DNA. It is a chimeric molecule where ri-melittin is fused to dioleoylphosphoethanolamine. The physical-chemical properties of riDOM in solution and in the presence of DNA, heparan sulfate and lipid membranes were investigated with spectroscopic and thermodynamic methods. riDOM in solution forms nanoparticles with a diameter of ~13 nm, composed of about 220 - 270 molecules. riDOM binds tightly to DNA with a microscopic binding constant of $5 \times 10^7\ M^{-1}$ and a stoichiometry of 12 riDOM per 10 DNA base pairs. In the complex the DNA double strand is completely shielded by the more hydrophobic riDOM molecules. Sulfated glycosaminoglycans such as heparan sulfate are also linear molecules with a negative charge. riDOM binding to heparan sulfate on cell surfaces can therefore interfere with DNA-riDOM binding.. riDOM nanoparticles bind efficiently to neutral and charged phospholipid bilayers. When dissolved in the membrane riDOM nanoparticles dissociate and form transient pores. The binding of cationic riDOM nanoparticles to negatively charged membranes consists of an initial electrostatic attraction to the membrane surface followed by a hydrophobic partitioning into the bilayer interior. ζ-potential titrations yield an effective riDOM charge of $z_{riDOM=2}$. The Gibbs free energy of binding is −8.0 to −10.0 kcal/mol which corresponds to the partition energy of a single fatty acid. Half of the hydrophobic surface of the lipid moiety with its 2 oleic acyl chains is therefore involved in a tight lipid-peptide interaction. This packing arrangement guarantees a good solubility of riDOM both in the aqueous and in the membrane phase. The binding enthalpy is small with $\Delta H^0_{riDOM} \sim -6.0$ to −1.0 kcal/mol and riDOM binding is hence entropy-driven.

2620-Pos Board B312
Interactions of the Kindlin Family Pleckstrin Homology Domains with Model Membranes Containing Zwitterionic Lipids and Phosphatidyl Inositol Phosphates
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Kindlins are a family of proteins which act in synergy with talin to stimulate the inside-out activation pathway of integrin receptors. Integrins are cell surface receptors that play a key role in various signal transduction pathways and are involved in many diseases such as thrombosis, inflammation and cancer. There is sequence similarity between the kindlin and the talin head domain but the kindlins have an additional pleckstrin homology (PH) domain. This PH domain is believed to be important for localization of kindlins to the cell membrane and for integrin activation, although the details of its membrane interactions (e.g. with PIP_2 lipids) remain unknown. In this study, we use the known NMR and crystal structures of the PH domains of the three kindlin isoforms to examine their association with different lipid bilayers containing zwitterionic lipids and phosphatidyl inositol phosphates at both atomistic and coarse-grained resolution using a multiscale simulation approach. Our results suggest a novel model for the kindlin PH/lipid encounter and subsequent interactions. In particular, all the PH isoforms associate with the PIP_2 lipids in the membrane via a highly positively charged loop and mutations of positive residues in this loop perturb the orientation of the PH domains relative to the lipid bilayer. We also demonstrate that reduction of the PIP_2 concentration in the lipid bilayer reduces the association of the PH domains with the membrane. Clustering of PIP_2 lipids in the bilayer leaflet adjacent to the protein is seen when the kindlin PH domains interact with charged lipid headgroups.

2621-Pos Board B313
Cell Membrane Composition Affects GPCR Aggregation
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Recent experimental studies demonstrate that membrane composition is altered in certain human disorders. Modifying membrane composition (e.g. varying the