Protein-Lipid Interactions I

258-Pos Board # B132
Binding of NAP-22, a Calmodulin-Binding Neuronal Protein, to Raft-Like Domains in Model Membranes
Tapan K. Khan[1], Bing Yang[1], Nancy L. Thompson[1], Shohei Maekawa[2], Richard M. Epand[3], Ken Jacobson[1]
[1]Univ. of North Carolina at Chapel Hill, 108 Taylor Hall, CB #7090, Chapel Hill, North Carolina 27599, [2]Kobe-University, Rokkodai, Nada-ku, Kobe, 657-8501 Japan, [3]McMaster University, Hamilton, Ontario, Ontario L8N 3Z5, Canada
The cholesterol-binding protein NAP-22 is a major component of the detergent insoluble low-density fraction of rat brain. In this study, we found, using fluorescence microscopy, that native NAP-22, but not a demyristoylated form, binds to cholesterol-rich raft-like domains in planar supported monolayers and remains bound after non-ionic detergent extraction. NAP-22 also protects the cholesterol-rich domains during extraction by methyl-β-cyclodextrin. The lateral mobility of this protein is much lower than other raft components. This study suggests that NAP-22 binding may be employed to image cholesterol-rich regions, such as caveolae/rafts on the plasma membrane of cells. NAP-22 binding may modulate their functions. Studies of the interaction of NAP-22 with living cells are now ongoing. (Supported by NIH GM41402 (KJ), NSF MCB-0130589 (NTL) and MT-7654 (RE)).

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Differentiation Between Pore Formation vs. Membrane Disruption by Lytic Peptides using Surface Plasmon Resonance (SPR)
Niv Papo[1], **Yechiel Shai**[1]
[1]Weizmann Institute of Science, Rehovot, 76100 Israel
Lytic membrane active peptides are used in the defensive and offensive systems of all organisms. We utilized SPR to study the interaction between lytic peptides and both hybrid monolayers (HPA sensor chip) and lipid bilayers (L1 sensor chip). We investigated peptides that represent two major families: (i) The bee venom, melittin, and the shark repellent, pardaxin, as models of non cell-selective peptides that form transmembrane pores via the "barrel-stave" mechanism; and (ii) Magainin and a diastereomer of melittin (four amino acids were replaced by their D-enantiomers), as models of bacteria-selective peptides that act via the "carpet" mechanism. SPR allows differentiating between two major steps: membrane binding and membrane insertion. The pore forming peptides bind to PC/cholesterol several hundred fold better than those that act via the carpet mechanism, mainly because they insert into the inner leaflet (2/3 of the binding energy), whereas the others remains on the membrane surface. On the other hand, the ~100-fold stronger binding of the bacteria-selective peptides to PE/PG compared to PC/cholesterol resulted only from electrostatic attraction to the outer leaflet of the negatively charged headgroups. These results clearly differentiate between the two general mechanisms: pore formation and a detergent-like effect (carpet mechanism), in agreement with their biological function.

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Topographical Organization of the N-Terminal Segment of Lung Pulmonary Surfactant Protein B ($SP\text{-}B_{1\text{-}25}$) in a Phospholipid Bilayer as Determined by Fluorescence Quenching
Yudong Wang[1], Murali K Rao[1], Eugene Demchuk[1]
[1]NIOSH, 1095 Willowdale Rd., Morgantown, West Virginia 26505
The location and depth of each residue on the phospholipid bilayer (PB) was determined by fluorescence quenching with synthesized single residue substituted peptides that were reconstituted into DPPC-enriched liposomes. The single residue substitutions in peptides were either aspartate or tryptophan. Aspartate was subsequently labeled with the NCD-4 fluorophore. The spin-labeled compounds, 5-DSA, 7-DSA, 12-DSA, CAT-16 and CAT-1, were used in quenching experiments. Our observations indicate that residues 1-6 are located at the surface of PB; residues 7-9 are embedded in PB; residues 10-22 are involved in an amphipathic α-helix with its axis somewhat parallel to the surface of PB; residues 23-25 reside at the surface. Effects of the inter-molecular disulfide bond formation in the $SP\text{-}B_{1\text{-}25}$ dimer were also investigated. The data suggest that hydrophobic sides of the amphipathic helixes face each other forming a hydrophobic domain. The environment-specific conformational liability in this hydrophobic domain may explain the key impact of SP-B on the phospholipid transport from bi- to mono-layer and in modulating the cell inflammatory response during the respiratory distress syndrome conditions.

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A Generic Model for the Thermodynamics and Structure of Lipid Membranes Containing Integral and Peripheral Proteins.
Hergen Schultze[1], Reiner Kree[1]
[1]Georg-August-University Goettingen, Bunsenstr. 9, Goettingen, 37073 Germany
Our model is based on a two-state lattice-gas for the lipid layer and the hard-hexagon model for the proteins. Both models for itself are well known in statistical physics. The coupling of the hard hexagons with the lattice gas and the extension to more complex shapes are used to study generic thermodynamical and structural effects of biomembranes. The lattice-gas model is well established to describe the main transition of a pure lipid layer or the mixing behaviour of a membrane with two sorts of lipids. The hard-hexagon model is used to describe the adsorption of disc-like particles, which we interpret as proteins. The coupling leads to a system of integral or peripheral proteins respectively. The model is generalised to arbitrary shapes and interactions (soft cores and binding sites). We perform Monte-Carlo Simulations to calculate the thermodynamics and to study the phase diagram. We observe the formation of protein aggregates depending on the protein-lipid interaction and compare the results to analytical approximations done in previous work. Models of this type may serve as a starting point to investigate the structure and dynamics of proteins in biomembranes.

262-Pos Board # B136
Deformation of Giant Unilamellar Vesicles by Molecular Motors
Martijn VanDuijn[1], Gerbrand Koster[1], Marileen Dogterom[1]
[1]FOM Institute for Atomic and Molecular Physics (AMOLF), Kruislaan 407, Amsterdam, 1098 SJ Netherlands
Some biological membranes, such as those from the endoplasmic reticulum, form complex tubular networks inside the cell. Several studies suggest that the formation of these networks depends on the interaction of motor proteins with the cytoskeleton. An experimental model system was developed to study the extraction of lipid membrane tubes from giant unilamellar vesicles. Membrane tubes were formed upon the incubation of kinesin-coated vesicles on a network of immobilized microtubules. Formation of tubes correlated with the amount of motors used, down to a threshold concentration under which no tubes were formed.

The tension in the vesicle membrane was changed, either with buffers of different osmolarities, or by the addition of pore-forming drugs. In both cases, relaxation of vesicles resulted in more extensive tube formation, whereas tenser vesicles yielded fewer tubes.

The data suggest that several independent motors can join forces to form a membrane tube. The dependence on the kinesin density and the membrane tension can be understood by a theoretical model in which motor proteins dynamically associate into clusters.