membrane mimics composed of zwitterionic (PC) and negatively charged (PG) phospholipids, major lipid components of erythrocyte and bacterial cell membranes, respectively. Microcalorimetry showed that RTD-1 and oRTD-1 did not affect the phase behavior of PC liposomes, while in PG liposomes both peptides induced new phase transitions above the chain melting transition of the lipid. The shape and fraction differed between both peptides, depending also on their concentration, which will be discussed in terms of their antimicrobial activity.

1859-Pos Board # B232
A MINIMAL AMINO ACID LENGTH REQUIREMENT OF INSERTION DETERMINANT OF APOCYTOCHROME C FOR THE INTERACTION WITH ITS N-TERMINAL
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The molecular mechanism of apocytochrome c, the precursor of cytochrome c, import and export through the mitochondrial outer membrane remains unknown. Spontaneous insertion of apocytochrome c is believed to involve protein-lipid interaction. We reported previously that a segment of 21 amino acids in the middle of cytochrome c (a.a. 68-88) plays an important role in determining the membrane transport process of apocytochrome c, by spontaneous penetrating and facilitating the penetration of the amino-terminus of cytochrome c into the lipid membrane. To further identify the key residues involved in this transport process, shorter peptide sequences of a.a. 68-88 were synthesized, and their interaction with the amino terminus of cytochrome c (a.a. 1-19) were assayed by measuring the changes in surface tension in the lipid monolayer formed at the air-water interface. We found that a 9 amino acid peptide (a.a. 81-88) retain the capability of interacting with the amino terminus (a.a. 1-19) of cytochrome c, which can enhance the penetration of the amino-terminus into the lipid monolayer. This result provided further support to our proposed pushing-over model for the membrane translocation of apocytochrome c.

1860-Pos Board # B233
Theoretical Analysis of Tether Formation in Electromotile Membranes
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Cochlear outer hair cells contain a protein called prestin that effects voltage-dependent changes in cell length. Two competing models have been developed that couple conformational change in prestin to either 1) area changes or 2) nanoscale curvature changes. Current experimental evidence can not distinguish between the two models. However, the recent application of tether formation experiments to outer hair cells provides an experimental scenario in which nanoscale curvature changes can be distinguished from in-plane area changes. Current thermodynamic models of tether formation predict that the length of a tether depends upon the pulling force (F) and the bending stiffness of the membrane. We have further developed these models to include the free energies associated with active area and curvature changes. This extended thermodynamic model predicts that the equilibrium tether length (L_t) and radius (R_t) will depend upon the applied voltage and the mode of electromotility. Specifically, voltage-induced changes in curvature are postulated to translate the F vs. R_t curve, while changes in membrane area will alter the shape of the curve. The model can be applied to experiments in which tethers are formed under imposed voltage from outer hair cells and prestin-transfected HEK to determine the mechanism of action of prestin.

1861-Pos Board # B234
Neuropeptide Y?Phospholipid Membrane Interaction Detected by Fluorescence and EPR Methods
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Neuropeptide Y (NPY) is one of the most abundant peptides in the central nervous system of mammalians. NPY acts by binding to at least five G-protein coupled receptors. Three spin-labelled NPY analogues containing the nitroxide group of the amino acid TOAC (2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid) as a paramagnetic probe were synthesized by solid-phase peptide synthesis. The analogues showed a marked selectivity for different receptor subtypes. To determine the rotational correlation time of the spin probes, electron paramagnetic resonance measurements were performed in solution and in the presence of liposomes. This allowed us to evaluate the backbone dynamics of the different parts of the NPY molecule in the free and membrane bound states. The results of these studies showed that NPY interacts with liposomes by using the C-terminal α-helix while the N-terminal tail retains a flexibility.

1862-Pos Board # B235
Effect of the pore region of a transmembrane ion-channel on the physical properties of a simple membrane
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The effect of membrane proteins and peptides on their surrounding lipids is crucial for the structure, dynamics, and function of complex biological membranes as well as the interplay between membrane proteins and their environment. Here, we present a study of the influence of the transmembrane pore region of an ion-channel on the physical properties of a phospholipid bilayer. We performed multinanosecond molecular dynamics simulations of the pore-forming aggregate of a-helical transmembrane peptides inserted in a simple lipid bilayer at similar conditions as those of the recent NMR experiments [Opella, S.J., et al. (1999) Nature Struct. Biol., 6, 374-379]. The results obtained are compared with simulations of a pure lipid bilayer membrane. Our analysis reveal that the peptides affect the properties of the lipid bilayer in several ways: (1) the bilayer thickness increases, (2) the number of gauche defects of the hydrocarbon chains decreases, (3) the dynamics of the lipid molecules is slowed down, and (4) the orientational distribution of the lipid headgroup dipole moments becomes broader (more disordered). Interestingly, the pore does not affect the two different sides of the membrane in the same way.

1863-Pos Board # B236
Role of the Structural Flexibility of Different Regions of the Apolipoprotein Molecule, Apolipophorin-III, in Lipid Binding
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Apolipophorin-III is an exchangeable apolipoprotein. In the lipid-free state the apolipoprotein is monomeric and has a compact five-helix bundle structure. Binding to a lipid surface requires the relaxation of one or more of the helices to allow the interaction of the hydrophobic surface of the helices with the hydrocarbon core of the lipid surface. The role of the structural flexibility of different pairs of á-helices (helices 1-5 and helices 3-4) and the loops connecting helices 2-3 and 4-5 on the lipid binding activity of the protein was investigated by disulfide mediated helix tethering experiments. The ability of oxidized and reduced disulfide mutants to interact with lipids was investigated by their ability to form discoidal lipoproteins upon interaction with DMPC multilamellar liposomes or with DMPC cholate micelles. The disulfide mutant of apolipophorin-III tethering the helices 1 and 5