under physiological conditions. In our work, we focus on the influence of reactive aldehydes (RA) on uncoupling proteins (UCPs) which localized in the inner mitochondrial membrane. Previously, we have shown that while reactive aldehyde 4-hydroxy-2-nonenal (HNE) strongly potentiates the activation of UCPs in the presence of free fatty acid (FFA), it is unable to activate UCP directly [1]. We suggested that the observed effect is attributable to HNE binding to the amino acids: lysine, cysteine and histidine. To attain better mechanistic insight, we now compared the effect of two further RAs, 4-oxo-2-nonenal (ONE) and 4-hydroxy-2-hexenal (HHE). For this, we measured total protein content [2] of bilayers of different lipid composition, surface potential and membrane order parameter. The potency of RA to activate UCP1 increased in the order HHE-HNE-ONE. Phosphatidylethanolamine’s (PE) presence in the membrane was crucial for the RA effect to occur. We now propose that RAs enhance UCP1 activity (in the presence of FFA) by binding to PE’s amino group, which results in the formation of a PE adduct. The latter alters mechanic and electric bilayer properties, thereby inducing protein conformational changes. In tum FA-mediated proton transport accelerates.


1289-Pos Board B240
The Role of Membrane Context in the Interaction of Polyglutamine Peptides with Lipid Membranes
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Huntington’s disease is a dominant genetic neurodegenerative disorder associated with motor and cognitive decline, caused by a mutation in the polyglutamine (polyQ) repeat region near the N-terminus of the huntingtin (htt) protein. Expansion of the polyQ repeat above 35-40 repeats results in the disease that is characterized by inclusion body aggregates of mutated protein. The polyQ expansion in htt is flanked by a 17 amino acid N-terminal sequence (Nt17) and a proline-rich (polyP) region. To investigate the interaction between htt exon1 and lipid membranes, a combination of Langmuir trough techniques and vesicle permeability assays measuring calcein leakage were used to directly monitor the interaction of a variety of synthetic polyQ peptides with total brain lipid extract (TBLE) model membranes doped with specific membrane components. Our data suggests that the Nt17 domain plays a critical role in htt binding and aggregation on lipid membranes, and this lipid htt interaction is enhanced by the presence of the polyP domain. The exogenous addition of cholesterol, a primary neuronal plasma membrane component reduced in many Huntington’s disease models, to the TBLE monolayer caused a notable condensing effect in the membrane at low surface pressures. This resulted in reduced peptide insertion into lipid monolayers and decreased levels of induced vesicle permeability, though the effect does not scale linearly with cholesterol concentration. Results from parallel studies on htt-membrane interaction with sphingomyelin, a major component of membranes in the nervous system, will also be discussed.

1290-Pos Board B241
Pit(4,5)P2 Lipid Binding Induces a Reorientation of FGF2 Molecules Near Membrane Surface to Facilitate the Unconventional Oligomerization-Dependent Secretion Process as Revealed by a Combined FTIR/NMR/X-Ray Study
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Fibroblast growth factor 2 (FGF2) secreted by an unconventional mechanism has been suggested through a membrane phosphatidylinositol 4,5-bisphosphate (Pit(4,5)P2)-dependent process by involving FGF2 oligomerization and lipidic membrane pore formation (Steringer et al., J. Biol. Chem, 2012, 287, 27659), but the mechanism of lipid-induced oligomerization remains elusive. Herein, we demonstrate by a combined lipid monolayer/FTIR method that Pit(4,5)P2 binding to FGF2 induce a reorientation of FGF2 molecule near membrane surface to allow the exposure of hydrophobic surface responsible for FGF2 dimer formation. By using the structural information of FGF2/Pit(4,5)P2 complex obtained by NMR and FGF2 dimeric contact surface obtained by X-ray, we further illustrate a molecular mechanism responsible for lipid-induced FGF2 oligomerization process. The results not only provide a structural basis for FGF2 oligomerization near membrane surface, but also suggest a novel Pit(4,5)P2 - induced protruding, rather than insertion, process as a crucial event to trigger the proper orientation of FGF2 molecule for its oligomerizations.

1291-Pos Board B242
Membrane Shape Transition Mediated by Curvature-Inducing Proteins, Membrane Tension, and Macrocrowders
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Classical BAR (Bin/amphiphysin/Rvs) domains and their distant relatives, I-BAR domains, can bind to and reshape membranes in vitro and in vivo through their intrinsic “crecent-shaped” and “zellplatten-shaped” homomeric structure. However, the mechanisms by which plasma membrane intrusions induced by classical BAR domains, or extrusions induced by I-BAR domains, are barely studied. Here we used a GUV membrane shape assay to quantitatively investigate the key factors regulating BAR- and I-BAR-induced membrane shape transition processes. We found that the IMD-induced membrane curvature transition depends on protein density and membrane tension, as we found earlier for the endophilin N-BAR and full-length protein. Furthermore, we demonstrated that macromolecular crowding on the membrane surface significantly influences the BAR proteins’ ability to induce membrane curvature changes.

1292-Pos Board B243
Establishing the Synergy of Forces Governing TIM3 Binding to Lipid Membranes
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T cell/transmembrane immunoglobulin mucin protein 3 (TIM3) is an integral membrane protein on the plasma membranes of phagocytes and other cells that participates in the recognition of surface-marked cells by the innate immune system. It recognizes its targets by specifically binding phosphatidylserine (PS) on their surfaces. Thus, a detailed characterization of the TIM3/PS interaction is key to understanding its function. Our group has recently identified the binding orientation of TIM3 to lipid membranes, leading us to hypothesize a synergy of different forces in play in the TIM3/PS interaction. Here we describe the hydrophobic insertion and electrostatic attraction as two other forces, in addition to the well-known calcium-mediated binding to PS at a pocket on the protein surface. We have further quantified these three forces in terms of dissociation constants in different large unilamellar vesicle systems, using fluorescence spectral shift as the tool. Our quantitative analysis has led to the surprising discovery of a three- to four-fold affinity enhancement effect of minute amounts of the negatively charged phosphatic acid (PA) are present in the membrane. Study of this PA enhancement effect has been extended to TIM1 and TIM4, two other members of the TIM protein family, and the results will be discussed.

1293-Pos Board B244
Unraveling the Dual Role of Surfactant Protein A at Atomic Detail
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Surfactant Protein A (SP-A) plays an important role in pulmonary innate immunity by recognizing canonical patterns on microbial surfaces. It protects the lungs from infection by recognizing the lipid component (lipid A) on gram negative bacterial surfaces, and by helping to initiate various clearance mechanisms. SP-A is also known to aggregate dipalmitoylphosphatidylcholine (DPPC), the major constituent of lung pulmonary surfactant, to form tubular myelin, a highly structured form of surfactant lipids and proteins. It is unclear how SP-A can bind to both DPPC and lipid A for two very different functions. To determine the dual role of SP-A, we performed crystallographic and mutational analyses as well as all-atom molecular dynamics (MD) simulations. We found several critical binding features for lipid binding that involve cation-pi interactions and hydrogen bonds. MD simulations revealed that
1294-Pos Board B245
Identifying the Choline-Cation Tyrosine-PI Interactions of an Amyloid Tau He1, Hanif M. Khan2, Cedric Graufel3, Nathalie Reuter4, Anne Gershenzon5, Mary F. Roberts1.

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Transient binding of amphipathic proteins to membranes can be mediated by phospholipid cation / tyrosine π interactions. Bacillus thuringiensis phosphatidylinositol-specific phospholipase C (BtPI-PLC) is a secreted virulence factor that targets GPI-anchored proteins in the outer surface of eukaryotic plasma membranes, a bilayer rich in choline-containing lipids. BtPI-PLC has a plethora of tyrosine residues around the surface of the α/β-barrel, and molecular dynamics (MD) simulations suggest that choline headgroups and these Tyr residues form short-lived cation-π complexes. To investigate these interactions, BtPI-PLC was site-specifically spin-labeled and high resolution field cycling 31P NMR relaxometry was used to quantify the effect of the spin-labeled BtPI-PLC variants on phosphatidylcholine (PC) and phosphatidylmethanol (PMe, used as the surrogate substrate) in small vesicles. The paramagnetic relaxation enhancement at very low fields (<0.04 T) confirmed the existence of two moderately long-lived sites for PC binding - one near the active site and the other on the barrel rim quite removed from the active site. The distances extracted were consistent with the two major sites of PC binding suggested by the simulations. That the discrete phospholipid sites detected by NMR represent cation-π interactions is shown by measuring the binding affinity for BtPI-PLC variants in which the unnatural amino acid 3,5-difluorotyrosine has been site-specifically substituted for Tyr, thus reducing the strength of cation-π interactions. These binding studies coupled with changes in enzyme activity provide a detailed and quantitative picture of how PC interactions with this PI-PLC influence its transient binding and cleavage of PI in membranes.

1295-Pos Board B246
Membrane Interaction of Amyloid-Beta Peptide Induces Spontaneous Membrane Invagination
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One of the hallmark characteristics of Alzheimer’s disease (AD) is extracellular plaques, in which the 42aa Amyloid beta peptide (Aβ 1-42) is the main component. Aβ 1-42 is produced at cholesterol-rich regions of neuronal membranes by endoproteolysis of the parental amyloid precursor protein and is secreted into the extracellular space. Although Aβ 1-42 accumulates extracellularly, neurons internalize the Aβ 1-42 peptide which could contribute to disease progression and which may be the first step to both cytotoxicity and propagation of misfolded Aβ between cells.

Interaction with membrane bilayer is likely the first step in the molecular mechanism of neuronal Aβ 1-42 uptake. Therefore, we studied interaction of Aβ 1-42 with the lipid bilayer in a giant unilamellar vesicles (GUVs) model system. We found that the Aβ 1-42 bound to the lipid bilayer and, after a lag phase induced invagination of the membrane into small vesicular structures. Only early oligomeric structures of Aβ and stabilized the negative curvature of membrane, whereas both monomeric and fibrillar forms of the peptide were unable to induce or sustain membrane invagination.

Our results suggest that Aβ may facilitate vesicle formation in lipid bilayer membranes, which may be a factor in cellular Aβ internalization but may also hint at a possible physiological function of the Aβ peptide at the synaptic membrane.

1296-Pos Board B247
The Novel Inhibitor “Anle145c” Efficiently Inhibits Fibril Formation of Islet Amyloid Polypeptide (IAPP) and uses Distinctly Different Modes of Action in the Absence and Presence of Membranes
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Amyloid formation in the pancreas by islet amyloid polypeptide (IAPP) is closely associated with type-2 diabetes. Compelling evidence indicates that membranes play a crucial role in contributing to IAPP amyloid formation and that IAPP amyloid formation leads to cell membrane disruption [1]. Since both IAPP amyloid formation and membrane damage are considered perilous to the pancreatic beta-cells, their inhibition may be an effective strategy for the prevention and/ or treatment of the disease. Here, we studied the interaction between a novel amyloid inhibitor “anle145c” [2] and IAPP in the absence and presence of model membranes. Our results suggest that addition of anle145c to IAPP inhibits IAPP fibril growth even at sub-stoichiometric concentrations, both in the absence and presence of membranes. Anle145c also reduces membrane damage induced by IAPP but does not induce membrane leakage by itself. Most notably, anle145c shows a strong membrane interaction, resulting in a distinctly different mode of inhibition than in the absence of membranes. We present a model in which anle145c interacts with oligomeric IAPP species in solution, but with nonomeric or early oligomeric IAPP species in the presence of membranes.


1297-Pos Board B248
Influence of Sequence and Lipid Type on Membrane Perturbation by Human and Rat Amyloid β-Peptide (1-42)
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The hallmark characteristics of plaque formation and neuronal cell death in Alzheimer’s disease (AD) are caused principally by the amyloid β-peptide (Aβ). Current research focuses on understanding the interactions between Aβ and neuronal cell membranes, given the relationship between membrane perturbation and neurotoxicity. Aβ sequence and lipid composition are essential variables to consider when elucidating the impact of the biological membrane on Aβ sequence and the effect of Aβ on membrane integrity. Atomistic molecular dynamics simulations testing two Aβ sequences (Human Aβ(1-42), (HAB) and Rat Aβ(1-42) (RAβ)), five lipid types, and totaling 9 μs in simulation time, were performed in order to explain the effect of these variables on membrane perturbation. All metrics used to assess membrane perturbation agree in such that it can be concluded that HAB and RAβ contribute to membrane perturbation by causing a more rigid, gel-like lipid phase. The presence of cholesterol in a model raft membrane was found to moderate the amount of perturbation caused by HAB and RAβ. Differences between HAB and RAβ were seen based on lipid headgroup charge and hydrogen bond capacity. The position of arginine in the N-terminal region was determined to be the mediating factor in these differences in lipid affinity and disruption between HAB and RAβ. Overall, this work rationalizes the influence of sequence and lipid type on Aβ-membrane interactions, providing mechanistic insight into the etiology of AD.

Membrane Receptors and Signal Transduction II

1298-Pos Board B249
Effect of Thanatophoric Dysplasia Type I Mutations on Fibroblast Growth Factor Receptor 3 Dimerization
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Thanatophoric dysplasia type I (TDI) is a lethal human skeletal growth disorder with a prevalence of 1 in 20,000 to 1 in 50,000 births. TDI is known to arise due to five different mutations, all involving the substitution of an amino acid with a cysteine in fibroblast growth factor receptor 3 (FGFR3). Cysteine mutations in receptor tyrosine kinases have been previously proposed to induce receptor cross-linking in the unliganded state, thus emulating the effect of ligand and leading to receptor overactivation. Here, we characterize the effect of three TDI mutations, Arg248Cys, Ser249Cys, and Tyr373Cys, on FGFR3 dimerization in mammalian membranes, in the absence of ligand. We demonstrate that the mutations lead to modest stabilization and structural perturbations of the FGFR3 dimers. Based on available FGFR crystal structures, we argue that the effects of these mutations cannot emulate the effect of the ligand, thus challenging the current understanding of the molecular interactions that underlie TDI.