Protein-Lipid Interactions IV

3604-Pos Board B332
To be or not to be in Membrane Domains: Transbilayer Asymmetry and Sphingomyelin-Dependent Preferential Partitioning of the Acetylcholine Receptor
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A peptide GWALP23 (acetyl-GGALW5LALALALALALW19LAGA-amide) is a membrane anchor that is thought to be a part of its clustering mechanism. Previous studies from our group have shown that AChR lacks preference for Lo domains when reconstituted in sphingomyelin (SM), cholesterol (Chol) and POPC (1:1:1) model systems (Bermúdez et al., 2010). Here we study the effect on the possible Lo-preferential partitioning of purified AChR reconstituted in two different model systems (POPC:Chol, 1:1 and POPC:Chol:SM, 1:1:1) under: a) induced transbilayer asymmetry, resulting from addition of brain sphingomyelin (bSM) to the external hemilayer; and b) the presence of different pure SM species in the model membrane (bSM, 16:0, SM, 18:0-5 or 24:1- SM). AChR distribution was evaluated by fluorescence resonance energy transfer (FRET) efficiency between the AChR intrinsic fluorophores and Laurdan or dehydroergosterol fluorescence, and also by determining the presence of AChR in detergent-resistant and detergent-soluble domains (1% Triton X-100, 4°C). Both studies show that the induction of transbilayer asymmetry or the presence of 16:0-OH or 18:0-OH, as opposed to bSM or 24:1-SM, leads to a preferential partitioning of AChR in Lo domains. Thus, the localization of AChR in Lo domains strongly depends on the characteristics of the host lipid membrane.

3605-Pos Board B333
Formation of Giant Unilamellar Vesicles Containing Active Proteins
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Giant unilamellar vesicles (GUVs), composed of a phospholipid bilayer, are often used as a model for cell membranes. However the study of proteo-membrane interactions in this system is limited because the incorporation of integral and lipid-anchored proteins into the GUVs remains challenging. All pre-existing protocols either produce membranes with a very low protein density or are tailored to specifically support the inclusion of a particular protein. We recently developed a simple generic method to incorporate protein in GUVs. It does not require specific lipids or reagents, works in physiological conditions with high concentrations of protein, and the resulting proteo-GUVs can be micromanipulated. Moreover, our protocol is not limited to a narrow range of protein substrates; indeed we have already successfully incorporated two trans-membrane proteins and one lipid-anchored peripheral protein. These first proof-of-principle proteins present different types of challenges and thus demonstrate the versatility and utility of our method. TolC is an integral membrane protein and part of a heterotrimer, that together comprise a major multidrug efflux pump in E coli. The neuronal t-SNARE is a protein complex with a single transmembrane domain that mediates membrane fusion. Because of its propensity to aggregate t-SNARE is usually not functional after insertion in GUVs. To study lipidated proteins, we incorporated a modified form of the autophagy protein GABARAP L1, which we anchored it to the membrane via a cysteine-maleimide covalent bond. In each case, we verified that the proteins remain active after incorporation. We also verified their mobility by performing diffusion measurements via fluorescence recovery after photo bleaching (FRAP) experiments on micromanipulated GUVs. The diffusion coefficients are in agreement with previous data.

3606-Pos Board B334
Influence of a Central Tryptophan and of Cholesterol on the Properties of Defined Transmembrane Helical Peptides
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GWALP23 (acetyl-GGALW5LALALALALALW19LAGA-amide) provides a favorable host framework for investigations of the influence of guest amino acids, for example a third, centrally located, Trp residue, within the hydrophobic core of a well characterized transmembrane helix. It is crucial to note that the orientation and rotation of GWALP23 are sensitive to single-residue replacements, in part because the membrane-spanning helix exhibits only limited dynamic averaging of solid-state NMR observables such as the H quadrupolar splitting (Biophys. J. 101, 2939). We introduced a Trp residue into position 12 or 13 of GWALP23 (replacing either L12 or A13) and incorporated specific 2H-Ala labels within the helical core sequence. Solid-state 2H NMR spectra of GWALP23-W12 reveal that the peptide remains helical and retains a dominant preferred tilted transmembrane orientation with only a low extent of dynamic averaging, comparable to GWALP23 itself. Indeed, the tilt and dynamics of GWALP23-W12 are quite similar to the values observed for GWALP23 in DMPC bilayer membranes. We have analyzed the dynamics of the peptide helices using a modified Gaussian treatment as well as a semi-static treatment. The results indicate that a central Trp residue at position 12 does not appreciably perturb the properties of bilayer-spanning GWALP23. (By contrast, Arg-12 or Lys-12, when charged, induces multi-state behavior for GWALP23 in bilayer membranes [PNAS 110, 16921] ). Additionally, we are investigating the influence of cholesterol upon the properties of membrane-spanning GWALP23, GWALP23-W12 and GWALP23-K12.

3607-Pos Board B335
Regulation of K-RAS Membrane Association: Calmodulin Versus PDEδ
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K-Ras is a small GTPase that plays a critical role in human cancer cell biology. Selective membrane localization and clustering of K-Ras4B is mediated by its polybasic farnesylated C-terminus. The importance of the subcellular distribution for the signaling activity of K-Ras4B became apparent from recent in vivo studies [1]. PDEδ and the Ca2+-binding protein calmodulin (CaM) are known to function as potential binding partners for farnesylated Ras proteins, leading to a modulation of the signaling activity of K-Ras4B. The latest study of our group showed that PDEδ is not able to extract K-Ras4B from model raft membranes; instead, an effective delivery of PDEδ-solubilized K-Ras4B to the plasma membrane was proposed [2]. Compared to PDEδ, CaM exhibits additional interaction sites to the G-domain of K-Ras4B and was shown not to be required for the transport of K-Ras4B to the plasma membrane. Thus, it was suggested that calmodulin dissociates K-Ras4B from membranes [3]. However, the exact role of CaM in the intracellular localization and dynamics of K-Ras4B still remains elusive.

In the present approach, the influence of CaM on the interaction of GDP- and GTP-loaded K-Ras4B with anionic model raft membranes has been investigated by a combination of different spectroscopic and imaging techniques. The results suggest that binding of the acidic CaM to the polybasic stretch of K-Ras4B reverses its charge, leading to repulsion of the complex from anionic membranes. Since one farnesyl anchor alone is not sufficient to stably anchor Ras proteins to membranes, CaM would be able to dissociate K-Ras4B from plasma membranes, contrary to PDEδ.

References:

3608-Pos Board B336
Influence of Glutamic Acid Residues on the Properties of Model Membrane-Spanning Helices
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GWALP23 (acetyl-GGALW5LALALALALALW19LAGA-amide) is a constructive model peptide for investigations of single-residue effects on protein-lipid interactions and the properties of membrane-spanning helices (J.Biol. Chem. 285, 31723). GWALP23 has favorable properties in bilayer membranes because the peptide exhibits only limited dynamic averaging of NMR observables such as the H quadrupolar splitting or the 17N-H dipolar coupling (Biophys. J. 101, 2939). To investigate the potential influence of negatively charged glutamic acid side chains upon system properties, we have substituted a single Leu residue with Glu at different positions and incorporated specific 2H-Ala labels in the core of the single-Trp peptide Y'GWALP23 (see Biochemistry 51, 2044). Solid state 2H NMR experiments showed well defined H quadrupolar splittings for Y'GWALP23-E16 in the pH range from 4.0 to 9.0, suggesting that the peptide helix is well oriented in DOPC lipid bilayers. The E16-containing peptide seems to exhibit multi-state behavior at pH 10.9, in bilayers formed by ether-linked lipids, suggesting a pKa that is above pH 9 for the E16 side chain. The rather modest shift in the H quadrupolar splittings suggests that the orientation of the transmembrane peptide helix changes rather little at high pH. It is conceivable that the close proximity of E16 to W19 might perturb the orientation to the neutral peptide side helix and perhaps influence the pKa of E16. The molecular cousin having E14 instead of E16 shows multi-state behavior from pH 4.0 to pH 10.9 rendering pKa
determination enigmatic at this time. Some puzzles remain with respect to comparisons between E16 and E14. We are additionally investigating the peptide-lipid behavior when Glu is introduced in position 12, at the peptide center.

3609-Pos Board B337
Influence of pH and Histidine Residues on Membrane-Spanning Helical Peptides
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Synthetic model peptides such as GWALP23 (acyetyl-GGALW1)LALALALA LALW2(LAGA-ami)de provide a useful host framework for investigations of the influence of polar amino acids, for example histidine residues, within the hydrophobic core of a transmembrane helix. Importantly, membrane-spanning GWALP23 is quite sensitive to single-residue replacements, in part because the transmembrane helix exhibits only limited dynamism averaging of solid-state NMR observables such as the 4H quadrupolar splitting (Biophys. J. 101, 2939). We inserted His residues into position 12 and/or 13 of GWALP23 (replacing either L12 or A13) and incorporated specific 4H-Ala labels within the helical core sequence. Solid-state 4H NMR spectra of GWALP23-H12 reveal a marked difference in peptide behavior between acidic and neutral pH conditions. At neutral pH, GWALP23-H12 exhibits a well-defined tilted transmembrane orientation in both DOPC and D LPC bilayer membranes. To prevent the acid catalyzed degradation of lipids, we employed ether-linked DOPC bilayers to observe the effect of low pH on the L12H mutant. Under acidic conditions GWALP23-H12 is highly dynamic and exhibits multiple states. Indeed, the multi-state behavior of GWALP23-H12, when His is charged between pH 1.5 and pH 3, resembles closely that of GWALP23-R12 at neutral pH (J. Am. Chem. Soc. 132, 5803). The dramatic change in the behavior of GWALP23-H12 indicates a pK value less than 3 for His near the center of a lipid bilayer. Investigations are in progress to chemically exchange the C2 imidazole hydrogen of His for deuterium in the peptide, toward a goal of enabling direct observation of the His ring by solid-state 4H NMR over a range of pH and buffer conditions.

3610-Pos Board B338
Molecular Insight for the Effect of Lipid Raft on Thrombospondin-1 and Calreticulin Interactions
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Thrombospondin-1 (TSP1) binding to calreticulin (CRT) on the cell surface stimulates association of CRT with LDL receptor-related protein (LRP1) to signal focal adhesion disassembly and engagement of cellular activities (J. Biol. Chem. 275:36358-68, 2000; J. Biol. Chem. 277: 37219-28, 2002). Study demonstrated that lipid rafts are necessary for TSP1-mediated focal adhesion disassembly (J. Biol. Chem. 279: 23510-16, 2004), but the molecular mechanism of the interaction is still unknown. In this study, we investigated the interactions of a lipid bilayer and a lipid raft with CRT and TSP1-CRT complex and their effects on the structural changes of CRT and TSP1-CRT complex via atomically detailed molecular dynamics simulations. The lipid bilayer was modeled as a 1-palmitoyl-2-oleoyl-sn-glycero-3phosphocholine (POPC) bilayer with 1152 lipids. The lipid raft was modeled as a bilayer of POPC lipids without cholesterol-mediated phase segregation. Additional studies show how lipid rafts affect the TSP1-CRT complex, both in terms of the conformation of TSP1 and the location of CRT on the cell surface. The results from this study provide molecular insight for the effect of lipid raft on TSP-CRT interactions and TSP-mediated focal adhesion disassembly.

3611-Pos Board B339
Analysis of the Molecular Organization of Lipoprotein-Associated Apolipoprotein E, an Anti-Atherogenic Protein
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Human apolipoprotein E3 (apoE3) is a 299 residue exchangeable apolipoprotein that has the ability to exist in lipid-free and lipoprotein-bound states. It is composed of an N-terminal domain bearing LDL-receptor binding sites and a C-terminal (CT) domain bearing high-affinity lipid-binding sites; the latter is also responsible for mediating cellular cholesterol efflux. ApoE plays a crucial role as an anti-atherogenic agent in atherosclerosis by virtue of its ability to mediate reverse cholesterol transport by promoting cholesterol efflux from macrophages, a process that leads to the initial formation of nascent discoidal high-density lipoproteins (HDL). The objective of this study is to understand the molecular organization and conformation of apoE3 (1-299) and isolated CT domain (201-299) on reconstituted HDL (rHDL) and rHDL generated by macrophages. To accomplish this, we over-expressed and purified recombinant apoE3 and the CT domain bearing single Cys at selected sites. rHDL was prepared using unlabeled or pyrene-labeled single Cys variants and phospholipids, while nHDL was generated by exposing cholesterol-loaded 774 macrophages to unlabeled or labeled apoE bearing probes at defined sites. Lipid-associated apoE was obtained by density gradient ultracentrifugation. The relative organization of apoE molecules on rHDL and nHDL was followed by monitoring pyrene excimer fluorescence and by site-specific cross-linking studies, both employing microscale analysis of a coaxial proximity at ~10 Å distance. The appearance of excimer emission and cross-linked dimers regardless of the location of the single Cys is indicative of pairs of apoE3 molecules oriented parallel to each other on the HDL to form a dimer. Based on our results, we propose that apoE molecules circumscribe a bilayer of lipids adopting a belt-like conformation. Our study offers mechanistic details of apoE interaction with lipids, which aids in understanding its role in cardiovascular disease.

3612-Pos Board B340
Dual-Carbon Fluorescence Cross-Correlation Spectroscopy of Reconstituted Protein-Membrane Systems
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Lipoposomes are commonly used as model membranes for studying the function of isolated membrane proteins. They are also used for reconstituting large membrane-associated protein complexes, such as the protein coats of intracellular transport vesicles. Here, we show examples of how dual-carbon fluorescence cross-correlation spectroscopy (or, more generally, fluorescence fluctuation spectroscopy) can serve to optimize in vitro reconstitutions and unravel protein-membrane interactions:
(A) The reconstitution of detergent-solubilized, purified membrane proteins into liposomes is a prerequisite for a myriad of experimental studies, but experimental conditions generally have to be determined on a case-by-case basis. Fluorescence correlation spectroscopy (FCS) distinguishes micelles, liposomes and aggregates in homogeneous and heterogeneous samples based on their different mobilities. Dual-carbon fluorescence cross-correlation spectroscopy (deFCCS), an extension of FCS, additionally detects the co-localization of protein and lipid in these diffusing entities, facilitating the optimization process [1]. The principle is not limited to proteoliposome formation, but can be applied more generally to reconstitutions into small diffusing membrane entities, such as liposomes or nanodiscs.
(B) Studies of large, multi-subunit protein complexes, such as the COP II transport vesicle coat, require analysis of protein-lipid as well as protein-protein interactions. By virtue of their low perturbation, fluorescence techniques help optimizing complex reconstitution systems to obtain suitable experimental conditions for cryo-electron microscopy analysis of a complex structure [2], and helping expand the complexity of the reconstitution. The interaction of COP II components among each other and with model membranes are investigated.

3613-Pos Board B341
Divalent Cation- and Cholesterol-Induced Perturbation in Lipid Lateral Organizations and Polypoisoositide-Protein Interactions
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Phosphatidylinositol 4,5-bisphosphate (PIP2) controls many important cellular processes. The roles of divalent cations and cholesterol in regulating PIP2-mediated lateral membrane organization and protein interactions remain enigmatic at this time. Some puzzles remain with respect to comparisons between E16 and E14. We are additionally investigating the peptide-lipid behavior when Glu is introduced in position 12, at the peptide center.

3611-Pos Board B339
Analysis of the Molecular Organization of Lipoprotein-Associated Apolipoprotein E, an Anti-Atherogenic Protein
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Human apolipoprotein E3 (apoE3) is a 299 residue exchangeable apolipoprotein that has the ability to exist in lipid-free and lipoprotein-bound states. It is composed of an N-terminal domain bearing LDL-receptor binding sites and a C-terminal (CT) domain bearing high-affinity lipid-binding sites; the