have observed that KL4 containing monolayers demonstrate an increased tolerance to repeated compression and expansion due to a softening in folding collapse behavior caused by direct interactions with POPG. This change in folding dynamics leads to increased monolayer reversibility due to almost complete reincorporation of folds upon expansion. We will discuss the potential role of KL4 in lowering the resistance to in-plane shear in POPG containing monolayers in the context of the overall importance of collapse mode in establishing robust and reversible synthetic model lung surfactant.

2857-Plat
Exploring Supramolecular Aspects of the Effect of Sphingomyelinase D On Sphingomyelin-Containing Membranes
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Lipid-modifying enzymes play a vital role in the regulation of lipids as mediators of cell function. At the same time, the activity of these enzymes is highly affected by the lipid membrane structure. These processes at lipid membranes can be observed in situ through the application of different biophysical techniques. Thus, we are investigating a spider venom enzyme termed sphingomyelinase D (SMD). SMD hydrolyses sphingomyelin (SM) into ceramide-1-phosphate (Cer-1-P). While SM is an integral constituent of many cell membranes, Cer-1-P occurs in very low concentrations and is suggested to be a novel lipid second messenger. At present, the physiologically relevant mechanism following Cer-1-P formation by SMD is incompletely understood, but possibly related to the modulation of membrane properties.
Our results show a strong dependency of SMD activity on the phase state of the substrate. SMD is two orders of magnitude more active towards fluid- than gel-phase liposomes. The presence of cholesterol evens out this difference in activity at an intermediate level. The effect of SMD on fluid-phase giant unilamellar vesicles (GUVs) is observed by confocal fluorescence microscopy. GUVs composed of lauroyl-SM show a macroscopic domain formation and/or shrinking and buckling accompanied by the multiple formation of membrane tubes. GUVs composed of egg-SM display a beveling of the membrane and the formation of caps (outside curvature) approx. three days after the addition of SMD. Which membrane morphology evolves is likely a question of enzyme kinetics vs. the dynamics of lipid reorganization.
GUVs of raft-like mixtures exhibit a single homogenous phase after the addition of SMD. The consequences of SMD activity and Cer-1-P formation on cellular systems are currently being examined. This will indorse the correlation between enzymatic activity and membrane structure influencing the regulation of physiological processes.

2858-Plat
Protein-Lipid Interactions Shaping the Electrostatic Membrane Search of a Pleckstrin Homology Domain
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The membrane-targeting domains of peripheral proteins play an important role in mediating cell signaling events originating at the plasma membrane. The pleckstrin homology (PH) domain is the most common membrane targeting domain, and many PH domains specifically recognize membrane-bound PIP lipids. Recently, the representative PH domain of the General Receptor for Phosphoinositides (GRP1 PH) has been found to use an electrostatic search mechanism requiring anionic background lipids of the plasma membrane to more rapidly and tightly bind the rare phosphatidylinositol-3,4,5-phosphate (PIP_3) lipid second messenger. The contributions of the seven basic residues on the GRP1 PH membrane-proximal face to the protein-lipid interactions that occur during electrostatic searching were investigated. Point and double mutants of the isolated Grp1 PH domain were purified with alanine replacing each of the seven basic residues. For each mutant domain, the relative affinities for phosphatidylinositol-(3,4,5)-trisphosphate (PIP_3) were determined in the presence and absence of anionic background lipids. While the wild-type PH domain displays a ~10-fold enhanced affinity for PIP_3 in the presence of anionic background lipids, this enhancement is significantly decreased in the point and double mutant PH domains possessing the R322A and K279A mutations. Thus far, the results suggest that while most basic residues interact with the membrane at a detectable level, the protein-lipid interactions between basic residues R322 and K279 and the membrane are most crucial to electrostatic searching. Additional experiments are in progress to determine the specificity of these protein-lipid interactions, and the effect of mutations on membrane binding kinetics.

2859-Plat
Binding Affinities of WT and H93R PTEN to Lipid Membranes Containing PS and PI(4,5)P_2
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PTEN is a phosphatidylinositolphosphate (PIP) phosphatase frequently mutated in human cancer [1]. By lowering PI(3,4,5)P_3 levels in the plasma membrane, it functions as an antagonist to PI3-kinase in the regulatory circuit that controls cell proliferation and survival. wt PTEN has only weak affinity to zwitterionic phosphatidylcholine (PC) membranes but a strong interaction with anionic lipids. Its C2 domain was shown to bind in a Ca^{2+} independent manner to phosphatidylserine (PS) and phosphatidylglycerol (PG), whereas a short N-terminal domain binds specifically to PI(4,5)P_2 [2,3]. H93R PTEN is an autism related mutant which has decreased phosphatase activity [4].
Using Surface Plasmon Resonance (SPR), we characterized the affinity of wt and H93R PTEN to tethered bilayer lipid membranes (tBLMs) that contain PC and PS, PC and PI(4,5)P_2, and PC, PS and PI(4,5)P_2. As compared with wt PTEN, we find that the H93R mutation is sufficient to cause significant changes in the protein's association with lipid membranes. H93R PTEN has a stronger affinity to membranes containing PS than wt PTEN. PI(4,5)P_2 enhances the apparent binding constant for both proteins and leads to intriguing binding kinetics of the protein to the membrane. The binding of either protein to membranes containing both PS and PI(4,5)P_2 shows a biphasic behavior, suggesting two independent binding sites. This supports the hypothesis of non-competitive binding of the protein to PS and PI(4,5)P_2 [5]. We estimate and compare the amount and the thickness of the adsorbed protein layers which is further investigated in neutron reflectivity experiments.
1) Shaw et al., Nature (2006) 441, 424-430
2) Lee et al., Cell (1999) 99, 323-334
3) Das et al., PNAS (2003) 100, 7491-7496
4) Redfern et al., Protein Science (2010) 19, 1948-1956
5) Redfern et al., Biochemistry (2008) 47(7), 2162-2171

2860-Plat
A Proline Kink in GWALP23
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GWALP23 (acetyl-GGALW^{5}LALALALALALW^{19}LAGA-ethanolamide) is a proven model membrane-spanning peptide (see JACS 130, 12584) that moves "beyond" WALP family peptides by employing, for the purpose of interfacial anchoring, only one tryptophan residue on either end of a central alpha-helical core sequence. Because of its systematic behavior in lipid bilayer membranes of differing thickness (see JBC 285, 31723), we utilize GWALP23 as a framework for introducing guest residues within the transmembrane sequence. For example, we have incorporated a central proline residue to give acetyl-GGALW^{5}LALALAP^{12}ALALALW^{19}LAGA-ethanolamide. We have synthesized the resulting GWALP23-P12 with selected ^{2}H and ^{15}N labels for solid-state NMR spectroscopy, to enable analysis of the peptide orientation and segmental tilt in oriented lipid bilayer membranes using combined (^{2}H)-GALA and (^{15}N/^{1}H)-PISEMA methods. In DMPC bilayer membranes, the peptide segments N-terminal and C-terminal to proline are tilted substantially with respect to the bilayer normal, by about 34°-40° and 27°-29° (± 6°), respectively, with a proline-induced kink angle of 20°-23°. The proline places restrictions on the dynamics of both segments. As has been described previously for GWALP23, the C-terminal helix ends before Ala-21.

2861-Plat
Rhodopsin - Rhodopsin Oligomerization in Model Lipid Bilayers - Functional Implications
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We studied rhodopsin oligomerization as a function of rhodopsin concentration and lipid composition and related oligomerization to shifts in rhodopsin function. In the rod outer segment disks of the retina, rhodopsin is densely packed in phospholipid bilayers with a high content of polyunsaturated acyl chains. In model membranes, increasing rhodopsin packing density was linked to a shift in the metarhodopsin-I (MI)/metarhodopsin-II (MII) equilibrium towards MI as well as to lower rates of MII formation. We reconstituted rhodopsin into various phosphatidylcholine bilayers at rhodopsin/lipid ratios ranging from 1:1,000 to 1:70 and followed rhodopsin oligomerization by cross linking of rhodopsin