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**
**Kerstin Wagner**[1], Blanca Ramos-Cerrillo[2], Roberto P. Stock[2], Luis A. Bagatolli[1].
[1]University of Southern Denmark, Odense M, Denmark, [2]National Autonomous University of Mexico, Cuernavaca, Mexico.
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**
**Anna R. Chase**[1], Jefferson D. Knight[1,2], Joseph J. Falke[1].
[1]University of Colorado-Boulder, Boulder, CO, CO, USA, [2]University of Colorado-Denver, Denver, CO, USA.
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)$P_2$**
**Siddharth Shenoy**[1], Arne Gericke[2], Alonzo Ross[3], Mathias Lösche[1,4].
[1]Carnegie Mellon University, Pittsburgh, PA, USA, [2]Kent State University, Kent, OH, USA, [3]University of Massachusetts Medical School, Worcester, MA, USA, [4]NIST Center for Neutron Research, National Institute of Standards and Technology, Gaithersburg, MD, USA.
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**
**Roger E. Koeppe II.,**[1], Johanna M. Froyd-Rankenberg[1], Vitaly V. Vostrikov[1], Christopher D. DuVall[1], Denise V. Greathouse[1], Christopher V. Grant[2], Stanley J. Opella[2].
[1]University of Arkansas, Fayetteville, AR, USA, [2]University of California, San Diego, La Jolla, CA, USA.
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**
**Olivier Soubias**, Walter E. Teague, Kirk G. Hines, Klaus Gawrisch.
NIH, Rockville, MD, USA.
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

and changes in lipid-rhodopsin interactions by $^2$H NMR. The amount of MII formed after photoactivation was determined by UV/vis spectroscopy and the rate of transducin activation studied with a GTPγS-assay. At low rhodopsin concentrations (1/300 and lower) rhodopsin appears to be predominantly monomeric. At rhodopsin/lipid ratios higher than 1/300, the level of oligomerization increases in a highly cooperative fashion with concentration such that at physiological concentrations rhodopsin is mostly oligomeric. Protein function correlated tightly with rhodopsin oligomerization. Data on the influence of bilayer properties on the monomer - oligomer transition of rhodopsin and the rate of transducin activation will be presented.

**2862-Plat**
**Effects of Membrane Geometry on Voltage-Gated ion Channel Distribution Studied with a Model System**
**Sophie Aimon**, Gilman Toombes, Domanov Yegor, Patricia Bassereau.
Institut Curie, paris, France.
Voltage-gated ion channels are inhomogeneously distributed between the highly curved axons and distal dendrites and the relatively flat soma and proximal dendrites. To investigate the effects of membrane geometry on channel distribution and diffusion, we developed a model system based on membrane nano-tubes connected to cell-sized Giant Unilamellar Vesicles (GUVs). KvAP, a bacterial analog of eukaryotic Kv channels [1], was purified, fluorescently labeled and reconstituted into GUVs. Channel density and homogeneity in GUVs were quantified via confocal microscopy while patch-clamp was used to measure the activity of the reconstituted channels. To study the effect of membrane curvature, we pulled a membrane nano-tube from a GUV and could set the tube radius between 10 nm and 200 nm by varying the tension of the GUV membrane. The concentrations of channels in the tube and GUV were measured via confocal microscopy while diffusion was measured by tracking individual channels labeled with quantum dots. As the tube radius decreased, the channel concentration increased while the diffusion coefficient decreased. Results obtained with this model system should give insight into the diffusion of membrane proteins into and out of synaptic boutons.
[1] Ruta et al., Nature 2003, 422 : 180-185.

**2863-Plat**
**The Role of Cardiolipin Domains in Protein Localization in Bacterial Cells**
**Lars D. Renner**, Douglas B. Weibel.
University of Wisconsin Madison, Madison, WI, USA.
A central question in cell biology is how the spatial organization of machinery within the cell is established, maintained, and replicated in response to external stimuli. In Eubacteria, our understanding of the spatial and temporal organization of proteins is beginning to take shape. Many proteins localize to regions of rod-shaped bacterial cells that are characterized by a high intrinsic curvature (e.g. the poles). Recent data suggested that there are geometric cues for the localization of proteins and lipids in bacteria. We present data testing the hypothesis that membrane anisotropy at highly curved regions of the cell wall leads to protein localization. This research takes a top-down approach that focuses on a combination of in vivo and in vitro experiments with Escherichia coli cells. To study the response of lipids to the geometry of the cell wall in vitro, we have developed a technique for controlling the curvature of bacterial cells using microstructured polymers and quantitatively measuring the spatial localization of lipids in the resulting membranes. This approach allows to engineer an 'artificial' pole with a user-defined curvature into the E. coli inner membrane and to measure the spontaneous localization of lipids and polar proteins to this region of the cell. Using this approach we have determined that a critical radius of curvature of ~1.3μm-1 is required to drive the formation of cardiolipin-rich domains in the membrane. We have observed that the bacterial division protein MinD localizes to regions of high curvature and co-localizes with cardiolipin domains. Our data provide support for the curvature hypothesis as a general mechanism for regulating spatial organization in bacterial membranes. This research is expanding our understanding of Eubacteria and provides insights into the spatial and temporal dynamics of membranes relevant to cell biology.

## Platform BD: Protein-Ligand Interactions

**2864-Plat**
**Protein Affinity Pattern Calculations using Protein-Fragment Site Identification by Ligand Competitive Saturation (SILCS)**
**E. Prabhu Raman**, Wenbo Yu, Alexander D. MacKerell Jr.
University of Maryland, Baltimore, MD, USA.
We demonstrate the applicability of a computational method, Site Identification by Ligand Competitive Saturation (SILCS) to identify regions on a protein surface with which different classes of functional groups interact. The method involves MD simulations of a protein in an aqueous solution of chemically diverse small molecules. In the present application, SILCS simulations are performed with an aqueous solution of 1 M benzene and propane to map the affinity pattern of the protein for aromatic and aliphatic functional groups. In addition, water hydrogens and oxygen serve as probes for hydrogen bond donor and acceptor functionality, respectively. The method is tested using a set of proteins for which crystal structures of complexes with several high affinity inhibitors are known. SILCS simulations are performed for these proteins and the affinity pattern is obtained as 3D probability distributions of fragment atom types on a 3D-grid surrounding the protein called ''FragMaps''. Good agreement is obtained between FragMaps of each type and the positions of chemically similar functional group in inhibitors as observed in the Xray crystallographic structures. For proteins for which inhibitor decoy sets are available, we demonstrate the statistical significance of the SILCS predictions by showing a significantly higher degree of overlap of the ligand atoms in the experimental conformation with the FragMaps. For a few test cases, we correlate the extent of overlap of the ligand functional groups with FragMaps to the experimental binding affinities. SILCS is also shown to capture the subtle differences in protein affinity across homologs, information which may be of utility towards specificity-guided drug design. Taken together, our results suggest that SILCS can recapitulate the location of functional groups of bound inhibitors, suggesting that the method may be of utility for rational drug design.

**2865-Plat**
**CHARMM Additive All-Atom Force Field for O-Glycan and N-Glycan Linkages in Carbohydrate-Protein Modeling**
**Sairam S. Mallajosyula**[1], Olgun Guvench[2], Alexander D. MacKerell Jr.[1]
[1]University of Maryland School of Pharmacy, Baltimore, MD, USA,
[2]University of New England College of Pharmacy, Portland, ME, USA.
The O-glycosidic and N-glycosidic linkages are important protein modifications in which oligosaccharides are linked to Ser/Thr and Asn residues, respectively. These linkages involve the anomeric carbon of the carbohydrates and the alcoholic side groups of Ser/Thr or the amide group of the Asn side chain. The O- and N-glycosidic linkages are ubiquitous in biological systems including glycoproteins like mucin, epidermal growth factor (EGF), domains of different serum proteins and Notch receptors, among many others, where the presence of the carbohydrate moiety is important for the biological functions of the proteins.
In an ongoing effort to develop the CHARMM all-atom additive carbohydrate force field we present and validate parameters that will enable the modeling of the O- and N-glycosidic linkages. The parameters represent an extension of the existing CHARMM carbohydrate and protein force fields.1-2 The target data for the optimization process included quantum mechanical (QM) potential energy scans of the torsions involved in the glycosidic linkages. Force field validations included comparison of the intermolecular geometries for the QM and crystal studies, comparison of the crystalline unit-cell properties and experimental NMR J-coupling constants. The optimized parameters were then used to rationalize the differences between the Ser and Thr O-glycan linkages using a Hamiltonian Replica Exchange protocol (HREX). We found that the solvent structure closely governs the linkage geometry due to the involvement of bridged waters between the carbohydrate and protein regions.
(1) Guvench, O.; Hatcher, E. R.; Venable, R. M.; Pastor, R. W.; Mackerell, A. D. J. Chem. Theory Comput 2009, 5, 2353-2370.
(2) Guvench, O.; Greene, S. N.; Kamath, G.; Brady, J. W.; Venable, R. M.; Pastor, R. W.; Mackerell, A. D. J. Comput. Chem. 2008, 29, 2543-64.

**2866-Plat**
**Kinetic Properties of the Two-State Model for Cooperativity**
Sargis Simonyan, **Nadja Hellmann**.
Institute for Molecular Biophysics, Mainz, Germany.
Cooperativity is a regulation mechanism of protein function which is defined by equilibrium binding properties, namely the shape of the oxygen binding curve. This sigmoid shape is the consequence of the existence of different conformations which differ in ligand and effector binding affinity.
Positive cooperativity also leads to characteristic behavior in the kinetics of ligand binding and dissociation. Oxygen dissociation from hemocyanins is typically ''auto-catalytic'' since the off-rate for oxygen is slower for the initial high affinity state than for the final low affinity state. The relative change of the off-rate at the beginning of the reaction compared to the final phase might serve as a measure for kinetic cooperativity. We compared the oxygen dissociation kinetics of 6 different arthropod hemocyanins. The modulation of the kinetics by allosteric effectors in most cases is what might be expected, leading to an increased rate for negative effectors and an decreased rate for positive effectors. A rather unexpected finding was the mostly linear change in apparent rate of dissociation with decreasing saturation degree. Simulations based on the MWC-model showed that the observed relationship between off-rate and saturation degree is typical for relatively slow conformational transitions and not-too-large allosteric equlibrium constants. It can be shown that under the conditions employed here (high cooperativity) the maximal increase in the off-rate is