the oriented CD results. TisB oligomerization in lipid bilayers was investigated using MD simulations. The results for dimerization of TisB in DMPC bilayers is supported by MD.

Interfacial Protein-Lipid Interactions II

2511-Pos Board B281
Conformational Dynamics of Membrane-Bound α-Synuclein in a Highly Mobile Membrane Mimetic
Joshua V. Vermaas, Emad Tajkhorshid. University of Illinois at Urbana-Champaign, Urbana, IL, USA.

α-Synuclein is a widely studied unstructured protein that plays an important role in pathophysiology of Parkinson’s and Alzheimer’s diseases through its aggregation, a process closely connected to its binding to and restructuring of the cellular membrane. Thus the dynamics of the membrane-bound form of the protein is highly relevant to unravel the molecular mechanism of its involvement in these diseases. In order to characterize the dynamic range of conformations accessible to membrane-bound α-Synuclein, molecular dynamics offers a potentially powerful method, owing to its detailed atomistic and dynamic description of the semi-liquid environment of biological membranes. Nonetheless, the applicability of the method is hampered by the slow diffusion of lipid molecules when described atomistically relative to simulation accessible timescales. We have developed and employed a novel membrane representation (termed DMM—a highly mobile membrane mimetic) with enhanced lipid dynamics and without compromising atomic details, which was used to perform 10 independent simulations of binding of α-Synuclein to bilayers composed of a mixture of PS and PC lipids. In all simulated systems, α-Synuclein spontaneously binds to the membrane without the application of an external force. Rather than converging to a single structure, these simulations capture an ensemble of diverse, highly dynamic structures of α-Synuclein in the presence of the membrane. While the observed conformational diversity can be attributed primarily to two highly flexible regions, namely near the turn and the region bracketed by critical Gly residues. Not only do the resulting ensemble of membrane-bound structures reflect the horseshoe-shape of the original structure, but also show similarities to a proposed linear configuration of the protein. The observed structural diversity suggests that in its membrane-bound form, α-Synuclein exists in an equilibrium between the horseshoe and linear conformations that have been also postulated experimentally.

2512-Pos Board B282
Role of the Cyclization in De Novo Design of Antimicrobial Peptide Mimics
Konstantin Andreev 1, Marija Kosutic 1, Andrey Ivankin 1, Mia Huang 2, Joshua V. Vermaas 1, Emad Tajkhorshid. University of Illinois at Urbana-Champaign, Urbana, IL, USA.

Cyclization is an important tool for the design of antimicrobial peptides. Herein we present results of a study aimed to establish how the cyclization affects the ability of cyclic N-substituted glycine oligomers (peptoids) to disrupt selectively bacterial, but not mammalian, cell membranes. Lipid monolayers at the air/liquid interface composed of LPS or DPPC/Cholesterol 60/40 mixed film was used to mimic the mammalian plasma membrane. Interactions of cyclic and linear peptoids with model lipid membranes were investigated using constant-pressure insertion assays, phase microscopy (EFM), and synchrotron X-ray reflectivity (XR) and grazing incidence X-ray diffraction (GIXD). Insertion assays show that both cyclic and non-cyclic peptoids readily incorporate into the bacterial, but not mammalian, membrane mimics. Moreover, their insertion into the bacterial membrane mimics was accompanied by rapid deterioration of the structural order in the lipid acyl chains. Electron density profiles across the film, derived from XR data, demonstrate that both peptoids penetrate into the hydrophobic core of DPPC more efficiently than that of LPS, which might be due to a difference in packing of the hydrophobic core. Nevertheless, our data indicate that, despite these similarities, the mechanisms of action of cyclic and linear peptoids on bacterial membranes are different.

A broad set of non-steroidal anti-inflammatory drugs is known to influence granulocin channel lifetimes in planar lipid bilayers. Therefore, we examined the influence of modestly supratherapeutic dosages (1 mM and 10 μM concentrations) of salicylic acid, acetylsalicylic acid, acetaminophen, ibuprofen, and diclofenac in the aqueous subphase on dipalmitoyl-PC pressure-area curves. We observed the following consistent, general patterns: a) transition from gas to liquid-expanded phase was initiated 20-40 A²/molecule higher than normal (i.e. 110-130 vs. 90); b) the incompressibility (dρ/dA) was similar or slightly higher than normal throughout the liquid-expanded phase compression; c) the shoulder in the ρ-A curve between 75 and 40 A²/molecule, which represents the slow conversion of liquid-expanded phase into liquid-condensed phase, is replaced by a continual rise in pressure; d) the final steep rise in pressure, which normally occurs at ~40 A²/molecule once conversion to the liquid-condensed phase is complete, is, in most cases, slightly postponed to a lower area/molecule, in which case it is then steeper. Controls without lipid showed no rise in pressure over the relevant surface area compression. We interpret these findings to imply that these NSAIDS all interact strongly with lipid head-groups to enhance condensation of the gas phase and homogenize the two liquid phases in the coexistence region. Supported by a Mentoring Grant from Brigham Young University.


2514-Pos Board B284
Optical Issues for the Rapid Determination of Surface Tension using Captive Bubbles
Hamed Khojoojin, Jim P. Goodarzi, Stephen B. Hall, Oregon Health & Science University, Portland, OR, USA.

Bubbles and droplets provide several advantages for studies on interfacial films, particularly at the very low surface pressures achieved by pulmonary surfactant in the lungs. The captive bubbles commonly used to study pulmonary surfactant, however, also suffer from a major disadvantage. The bubbles float against a concave surface molded into an agarose gel, which obscures the location of the upper air/water interface. Methods that calculate surface tension in real time use the height of the bubble, which requires the position of the upper surface. Experiments that use feedback to maintain constant surface tension, and fixed thermodynamic conditions, require measurements in real time. The studies reported here considered how a series of optical issues affect measurements of a bubble’s height and surface tension. As long as the imaged intensities remained within the dynamic range of the camera, total light intensity had no effect. Longer wavelengths were scattered less by the gel and surfactant suspensions, which increased their transparency. Misalignment of the camera and agarose gel relative to the gravitational frame of reference produced subtle changes but important errors. More collimated light generated narrower edges and more accurate dimensions, but darkened the concave surface of the gel, which further obscured the location of the air/water interface. With optimized optics, a single threshold intensity can accurately locate all sections of the interface. This interfacial grayscale allowed accurate measurements in real time that extended over the full range of surface tensions during compression of a solid film, and over a broad range of volumes during isobaric compression of a collapsing film.

2515-Pos Board B285
A Systematic Approach Towards Elucidation of the Mode of Action of a Bacterial Thermosensor
Joost Ballering 1, Larisa E. Cybulski2, Diego de Mendoza1, J. Antoinette Killian3. 1Universiteit Utrecht, Utrecht, Netherlands, 2Universidad Nacional de Rosario, Rosario, Argentina.

The Bacillus subtilis membrane harbors the temperature sensing and signaling protein DesK. At low temperatures it triggers expression of a desaturase, which introduces double bonds into pre-existing phospholipids, thereby regulating membrane fluidity. Recently it was discovered [1] that both sensing and transmission of DesK, which has five transmembrane segments, can be captured into one single chimerical transmembrane segment, the so-called ‘minimal sensor’. This simple system offers excellent perspectives to study the molecular detail of a biologically very important mechanism. As a first step we analyzed membranes of Bacillus subtilis grown at different temperatures with several physical techniques including 31P-NMR and Differential Scanning Calorimetry. We analyzed the membrane lipid headgroup and acyl chain composition and we identified transition temperature fluctuations related to the growth temperature. We found significant differences in membrane lipid composition and phase behavior for Bacillus subtilis membranes depending on growth temperatures. Furthermore the transmembrane segment of the minimal sensor was synthesized and incorporated in the Bacillus membranes. These
vesicles were analyzed with Tryptophan Fluorescence Spectroscopy to measure the position of the minimal sensor with respect to the membrane, which shows the switch behavior between high and low temperature of the minimal sensor. [1] Cybulski LE, Martin M, Mansilla MC, Fernandez A, de Mendoza D. Membrane thickness cue for cold sensing in a bacterium. Curr. Biol. 2010

2516-Pos Board B286
How does Thermosensor Desk Measure Membrane Thickness? Tryptophan Fluorescence and Mutagenesis Analysis
Larisa E. Cybulski1,2, Diego de Mendoza1, Joost Ballering1, Antoinette Killian1,2
1Instituto de Biología Molecular y Celular de Rosario, Rosario, Argentina, 2Biochemistry of Membranes- Chemistry Dept, University of Utrecht, The Netherlands, Netherlands, 3Biochemistry of Membranes, Chemistry Dept. Utrecht University, Utrecht, Netherlands.

The Bacillus subtilis histidine kinase DesK is a 5 transmembrane stretches- thermosensor suited to remodel membrane fluidity when temperature drops below 30 oC. We have recently designed a hybrid nanosensor with one transmembrane domain which is 5 times smaller than the parental protein. This chimerical protein fully retains, in vivo and in vitro, the sensing properties of the parental system and was called Minimal Sensor-DesK (MS-DesK). A recent paper (1) provides evidence that this perfectly simplified system could serve as a model to study a complex biological phenomena. Thus the MS-DesK is used here to study the conversion of a physical stimulus into a biological response that involves a change in the signaling transduction state. The N-terminus of TMS1 contains three hydrophobic aminocids near the lipid-water interface creating an instability hot spot. We show that this boundary sensitive residue controls the sensing and transmission activity by mutagenesis and in vitro reconstitution experiments. Accordingly, we hypothesize that membrane thickness is the temperature agent that determines the signaling state of the cold sensor by dictating the hydration level of the meta-stable hydrophobic spot. Tryptophan-labeled peptides corresponding to the sensor (functional and non-functional) transmembrane domain were synthesized and incorporated into membrane vesicles. Fluorescence spectroscopy and Circular Dichroism data of the peptides incorporated in liposomes of varying fatty acid chain length and different melting temperatures is presented. [1] Cybulski LE, Martin M, Mansilla MC, Fernandez A, de Mendoza D. Membrane thickness cue for cold sensing in a bacterium. Curr. Biol. 20: 1539-1544, 2010

2517-Pos Board B287
Characterization of Oxidized Phospholipid containing Reconstituted High Density Lipoprotein Particle
Subhabrata Kar, Rajan K. Tripathy, Mitul A. Patel, Abhay H. Pande. NIPER, S.A.S. Nagar, Mohali, India. Apolipoprotein A-I (apoA-I) is a major protein constituent of high density lipoprotein (HDL) which plays a prominent role in reverse cholesterol transport as well as other functions of HDL. Oxidative stress in many inflammatory conditions leads to peroxidation of phospholipids resulting in the formation of oxidized HDL particles. Effect of oxidized phospholipids on the properties of HDL is not well understood. In this study we have characterized the effect of oxidized phospholipids on the properties of reconstituted HDL particles. Reconstituted HDL particles containing varying amount of oxidized-PAPC were prepared by cholate dialysis method and purified using gel filtration chromatography. Purified HDL particles were used for characterization. Our results indicate that presence of oxidized-PAPC not only modifies the lipidic environment of HDL particles but also drastically alters the secondary structure and stability of bound apoA-I and induces significant change in global conformation as well as orientation of bound apoA-I. We acknowledge financial support from NIPER, S.A.S. Nagar, India.

2518-Pos Board B288
Probing the Activator and Hydrophobic Substrate Binding Sites of PTEN by 31P NMR
Yang Wei, Mary F. Roberts.
Boston College, Chestnut Hill, MA, USA.
PTEN (Phosphatase and Tensin Homolog) antagonizes the PI3K signaling pathway by dephosphorylating PI(3,4,5)P3 at the inositol C-3, thus suppressing cell proliferation. PI(4,5)P2, the product of dephosphorylation, activates this enzyme, possibly by binding to the N terminal region of PTEN (not visible in the crystal structure). A distinct hydrophobic pocket near the active site has also been identified (by MD simulations). We have used 31P NMR as a probe of the spatial location and functional role of these potential phospholipid binding sites. At 242.7 MHz, the linewidths of phosphodiester resonances of phosphoinositides that bind exclusively to the active site (PI, P(4)P) are broadbanded consistent with intermediate exchange. Estimated linewidths for the bound activator PI(4,5)P2 are smaller. Other amphiphiles can also be affinity purified PTEN in a specific fashion, for example, diC7-PC which is used as a matrix in assays. This ligand is likely to bind in the hydrophobic pocket identified by simulations. 13P high resolution field cycling NMR, with PTEN spin-labeled on the active site cysteine, was used to determine the distance between the unpaired electron and the phosphorus nuclei of the different bound lipids. Results of these relaxation studies indicate there are discrete sites for both substrate and activator lipids that are also separate from the region occupied by non-specific lipids such as diC7-PC (or a nonionic detergent such as Triton X-100). The distances derived are used to propose a model of PTEN regulation by the activator and matrix lipids.

2519-Pos Board B289
Osh4 Membrane Binding through Molecular Dynamics
Brent Rogaski, Jeffrey D. Klaus, University of Maryland, College Park, MD, USA.
Osh4 is an oxyester binding protein (OSBP) homologue found in yeast that has been implicated in the intracellular transport of sterols between membranes. It has been proposed that Osh4 acts as a lipid transport protein, capable of transferring a single sterol residue from the endoplasmic reticulum to the plasma membrane. Phosphoinositides (PIPs) are thought to bind to Osh4’s surface and function in a key role in this protein’s functionality. Blind docking techniques were used to probe the Osh4 surface and identify potential binding conformations. Model ligand compounds for phosphatidylcholine (PC), phosphatidylserine (PS), and two PIP (PI[4,5]P2 and PI[3,4,5]P3) head groups were docked against several conformational snapshots of the Osh4 proteins surface to determine possible regions favorable to interactions with membrane lipids. The PIP models frequently docked to a lysine-rich region on an exposed portion of the proteins β-barrel (β-crescent region). This surface, along with the front and distal binding surfaces proposed from experimental cross-linking studies, were aligned with the surface of model membranes. Three model membranes were used containing: 1. PC/PE lipids, 2. PC/PE lipids with one PI[4,5]P2 residue per leaflet, and 3. PS and PI[3,4,5]P2 lipids in addition to PC/PE lipids. Systems were simulated using molecular dynamics for 1 to 2 nanoseconds. The Osh4 protein bound to the membrane in all cases where acidic residues were present in the membrane. The Osh4 protein did not bind to the membrane with only PC/PE lipids over the course of ~1μs of MD simulation. Additionally, the bound membrane/protein conformation was largely the same regardless of the starting orientation of Osh4. This conformation featured charge-charge interactions between the protein and the membrane on several surface loops surrounding the β-crescent region, and was stabilized by penetration of a surface phenylalanine into the membranes hydrophobic core.

2520-Pos Board B290
Intermembrane Lipid Transfer is Facilitated by Mitochondrial Nucleoside Diphosphate Kinase D
Malgorzata Tokarska-Schlüter1,2, Andy Amoscato3, Yulia Y. Tyuna1, Sanciote Ramirez Rios1,2, Raquel F. Epand4, Richard M. Epand4, Marie-Lise Lacombe5, Uwe Schlatterner1,2, Valerian E. Kagan5,6.
1LBFA, Joseph Fourier University - Grenoble 1, Grenoble, France, 2Inserm, U1055, Grenoble, France, 3Center for Free Radical and Antioxidant Health, Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA, USA, 4Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, ON, Canada, 5Inserm, UMR_S 938 equipe 10, UMPC University Paris 06, Paris, France. Mitochondria-specific cardiolipin (CL) is mostly confined to the site of its biosynthesis, the inner mitochondrial membrane. Some CL remodeling occurs in the ER and thus implies the necessity of CL trafficking across mitochondrial membranes. The involved mechanisms are unknown. However, we have recently identified mitochondrial nucleoside diphosphate kinase (NDPK-D) to be capable in vitro to simultaneously bind two CL-containing membranes and to facilitate transfer of model lipids between them. This property relies on the symmetrical hexameric structure of NDPK-D which exposes positively charged residues that bind with high affinity to anionic phospholipids, in particular CL. In this work, we have studied the role of NDPK-D for mitochondrial membrane asymmetry in vivo. We used HeLa cells that are devoid of immuno-detectable amounts of NDPK-D to stably express wild-type and membrane-binding incompetent mutant forms NDPK-D. Analysis of purified mitochondrial inner and outer membranes by electro-spray ionization mass spectrometry revealed that the presence of NDPK-D wild-type, but not of membrane-binding incompetent mutant, leads to a partial collapse of CL asymmetry. These changes are CL-specific as the distribution of phosphatidylcholine remains unchanged in both HeLa cell lines. The pattern of different CL species