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

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How does Thermosensor Desk Measure Membrane Thickness? Tryptophan Fluorescence and Mutagenesis Analysis

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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 hydrophilic aminoacids near the lipid-water interface creating an instability hot spot. We show that this boundary-sensitive motif 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 hydrophilic 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

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Characterization of Oxidized Phospholipid containing Reconstituted High Density Lipoprotein Particle

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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 characterized. 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 rHDL 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.

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Probing the Activator and Hydrophobic Substrate Binding Sites of PTEN by 31P NMR

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PTEN (Phosphatase and Tensin Homolog) antagonizes the PI3K signaling pathway by dephosphorylating $PI(3,4,5)P_3$ at the inositol C-3, thus suppressing cell proliferation. $PI(4,5)P_2$, 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 ^{31}P 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, PI(4)P) are broadened consistent with intermediate exchange. Estimated linewidths for the bound activator $PI(4,5)P_2$ are smaller. Other amphiphiles can also be affected by PTEN in a specific fashion, for example, diC_7PC which is used as a matrix in assays. This ligand is likely to bind in the hydrophobic pocket identified by simulations. ^{31}P 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 nonspecific lipids such as diC_7PC (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.

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Osh4 Membrane Binding through Molecular Dynamics

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Osh4 is an oxysterol 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 play a key role in this proteins 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-crease 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(4,5)P2 lipids in addition to PC/PE lipids. Systems were simulated using molecular dynamics for hundreds of 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-crease region, and was stabilized by penetration of a surface phenylalanine into the membranes hydrophobic core.

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Intermembrane Lipid Transfer is Facilitated by Mitochondrial Nucleoside Diphosphate Kinase D

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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