present in the membrane. We have performed contrast-matching small-angle neutron scattering (SANS) experiments to study the conformational changes of the glycosylated form of A1AT for different concentrations of the osmolyte poly(ethelene glycol) (PEG) and in the presence of two different lipid membranes: POPC and POPS. We also monitor the structural changes of the lipid vesicles in the presence of A1AT by SANS. Guinier fits were used as a first approximation to obtain the radius of gyration (Rg) of A1AT. Bragg peaks were used to study structural changes of lipid vesicles. We observed that the Rg of A1AT changes as a function of PEG concentration in solution and when in the presence of lipid vesicles. The deformations monitored through changed in A1AT Rg in the presence of lipid vesicles are compared to the deformations of the glycoprotein observed under osmotic pressure and to the structural changes observed in the lipid vesicles. [1] Petrusca, et al., JBC 2010.

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Pten Binding to Morphologically Heterogenous Membrane Environments

Arne Gericke[1], Katrice E. King[1], Brittany M. Neumann[1], Rakesh K. Harishchandra[1], Alonzo H. Ross[2].
[1]Worcester Polytechnic Institute, Worcester, MA, USA, [2]University of Massachusetts Medical School, Worcester, MA, USA.

PTEN (phosphatase and tensin homologue deleted on chromosome 10) is a tumor suppressor protein that dephosphorylates phosphoinositides at the 3-position of the inositol ring. PTEN's membrane association has been shown to be a complex process that involves binding to phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2] and phosphatidylserine [PS]. Recent evidence suggests that the presence of cholesterol promotes the formation of PI(4,5)P2 enriched domains. This study has three objectives: First, to investigate PTEN's binding to lipid systems with laterally heterogeneous membrane morphology. Second, to delineate the kinetics of PTEN lipid binding and activation using stopped-flow fluorescence measurements. Third, by using single molecule fluorescence microscopy determine the lateral diffusion of membrane bound PTEN on supported bilayer and how the binding is affected by laterally heterogeneous membrane environments. Overall the study aims to understand the dynamics of PTEN-membrane interactions at the molecular level.

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Characterization of an Intermediate for the Formation of the Transmembrane Helix of pHLIP Peptide

Francisco N. Barrera, **Alexander G. Karabadzhak**, Donald M. Engelman.
Yale University, New Haven, CT, USA.

The pHLIP® -pH (low) insertion peptide- binds to the surface of lipid bilayers as a disordered peptide at neutral pH. However, when the pH is lowered, pHLIP inserts across the membrane to form a transmembrane (TM) helix. Peptide insertion is reversed when the pH is raised above the characteristic pKa, which is 6.0 for POPC liposomes. Due to its unique properties pHLIP serves as a good model system to study the process of folding/insertion and exit/unfolding of a peptide into/from a membrane. We previously found that the process of insertion of pHLIP in POPC phospholipid bilayer occurs in several steps with different intermediates (Andreev et al 2010, PNAS). The number of intermediates correlates with the number of protonatable groups in pHLIP's sequence. For pHLIP, the pH-dependent insertion and folding processes are coupled, and single sigmoidal transition is observed. Interestingly, we have found that for two pHLIP variants (D14A and D25A where each aspartic residue in TM part is replaced by alanine), the pH curve shows two independent sigmoidal transitions, suggesting that the mutations stabilize an intermediate state along the membrane-attached to transmembrane pathway. We implemented circular dichrosism (CD), oriented CD, fluorescence, FRET to elucidate the nature of these intermediates. From our experiments we inferred that the intermediates correspond to a quasi-transmembrane state. We hypothesize that the intermediate emerges from the protonation of acidic residues at the peptide C-terminal end, and complete TM folding happens with the protonation of the aspartic acid residue located in the hydrophobic core of the membrane. These results further our understanding of formation of transmembrane helix during spontaneous process of insertion of a polypeptide in membrane.

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How Different Lipidic Mimetic Systems of *E. COLI* Membranes Affect Membrane Protein Reconstitution: A Steady-State Fluorescence Anisotropy Study

Mariana Ferreira[1], Silvia Lopes[1], Paula Gameiro[2].
[1]Requimte, Porto, Portugal, [2]Requimte, Faculdade de Ciencias da Universidade do Porto, Porto, Portugal.

Outer membrane proteins (Omp) in bacteria, porins, have an important role on antibiotic translocation trough bacterial membranes. OmpF is one of the most important *E. coli* porins in the permeation of beta-lactam and quinolone antibiotics. Moreover, these antibiotics might penetrate the bacterial outer membrane through porins or through the lipid/protein interface. We focused on the characterization of *E. coli* membranes, once this knowledge is crucial for studies of antibiotic translocation and useful to understand and counteract bacterial antibiotic resistance, one of the biggest concerns of nowadays. Steady-state fluorescence anisotropy is a good technique to detect changes in lipid environments through the use of fluorescent probes. We used two fluorescent probes, DPH and TMA-DPH (which provide information from the core and from the interface regions of the bilayer, respectively), and determined the temperature dependence of these two anisotropy probes, incubated or incorporated into POPE/POPG (0.75:0.25), POPE/POPG/Cardiolipin (0.67:0.23:0.10) and *E. coli* total lipid extract liposomes (LUVs) and OmpF proteoliposomes. POPE/POPG and POPE/POPG/Cardiolipin mixtures are conventionally used to mimic the *E. coli* membranes, while the *E. coli* total lipid extract is a natural system. In this study we determine the phase transition temperatures characteristics of each membrane mimetic system and still prove the correct insertion of the protein in the membrane. When comparing the transition temperatures obtained for each mimetic system, our study suggest that the ternary lipid mixture is a better system to mimic the *E. coli* membranes than the binary system.

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The Shape and Free Energy of a Lipid Bilayer Surrounding a Membrane Inclusion

Richard O. Tjörnhammar, Olle Edholm.
Theoretical Physics, Stockholm, Sweden.

Membrane inclusion interactions are studied within the scope of continuum theory. We show that the free energy functional for the membrane thickness can be rewritten as a constant times a dimensionless integral. For cylindrical inclusions, the resulting differential equation gives a thickness profile that depends on the radius of the cylinder and one single lipid property, a correlation length that is determined by the ratio of the thickness compressibility and bending moduli. The solutions decay in a non-monotonic fashion with one single observable minimum. A solution for planar geometry may either be explicitly constructed or obtained by letting the radius of the cylinder go to infinity. In dimensionless units the initial derivative of the thickness profile is universal and equal to $-1/\sqrt{2}$. In physical units, the derivative depends on the size of the hydrophobic mismatch as well as the membrane correlation length and will usually be fairly small but clearly non-zero. The line tension between the protein inclusion and a fluid phase membrane will depend on the hydrophobic mismatch and be of the order of 10 pN (larger for the gel phase). This results in free energy costs for the inclusion that will be up to tens of kJ/mol (in the fluid phase)

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Molecular Insights into the Recognition of Cellular Membrane Geometry

Richard L. Gill, Jr.[1], Jean-Philippe Castaing[2], Kumaran S. Ramamurthi[2], Fang Tian[1].
[1]Department of Biochemistry and Molecular Biology, The Pennsylvania State University, Hershey, PA, USA, [2]National Cancer Institute, NIH, Bethesda, MD, USA.

Many essential cellular processes such as endocytosis, vesiculation, organelle synthesis and cell division require transient membrane deformations. Recently, it was discovered that the activity of some proteins (e.g. human ArfGAP1) dramatically increases with the curvature of the membrane bilayer, and that membrane curvature could serve as a geometric cue for the subcellular localization of some proteins (e. g. SpoVM and DivIVA). In addition, the entry of some bacteria and virus into human cells also requires alteration of membrane shapes. Membrane geometry is increasingly viewed as a critical component for creating microenvironments for membrane fusion and fission, protein localization, trafficking and signaling. This underscores the importance of understanding the molecular mechanisms responsible for the generation, recognition, maintenance, and regulation of membrane architecture.

SpoVM, a 26-residue peptide, was recently found to recognize and preferentially localize to the slightly curved outer surface of the forespore (diameter of curvature, R, ~1 mm) during *Bacillus subtilis* spore development. However, little is known about how this is accomplished. SpoVM was predicted to exist as a straight, amphipathic α-helix that shallowly inserts into the membrane surface. However, using solution NMR, we have found that the SpoVM molecule adopts a loop-helix structure and that the helix deeply embedded into bicelles. This model has provided new structural insights into SpoVM function. We are extending this study with model membranes that are similar in curvature and lipid composition to that of the *B. subtilis* forespore using solid-state NMR.