of monocytes. Association of LBP with distinct membrane domains is shown in analysis of DRM membrane fractions from primary monocytes. Studies on HEK293 cells and human macrophages demonstrate that LBP-binding to host cell membranes occurs independently of the TLR4-receptor and is involved in the uptake and intracellular transport of LPS.
Conclusions: In addition to its function in serum, LBP is also abundant in the cytoplasmic membrane and in intracellular compartments of monocytes and macrophages. Cell interaction, localization and intracellular LPS transport by LBP occur independent of TLR4, suggesting a new role of LBP in intracellular LPS-trafficking and signalling.
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1277-Pos Board B254
General Steric Trapping Strategy Reveals an Intricate Cooperativity Network in the Intramembrane Protease GlpG under Native Conditions
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Membrane proteins are assembled through balanced interactions among protein, lipids and water. Studying their folding while maintaining the native lipid environment has been a necessary but challenging. Here we present a set of methods for analyzing key elements in membrane protein folding, including thermodynamic stability, compactness of the unfolded state and unfolding cooperativity under native conditions. The methods are based on steric trapping which couples unfolding of a doubly-biotinylated protein to binding of monovalent streptavidin (mSA). We further advanced this technology for general application by developing versatile biotin probes possessing spectroscopic reporters that are sensitized by mSA binding or protein unfolding. By applying those methods to an intramembrane protease GlpG, we elucidated a widely unraveled unfolded state, subglobal unfolding of the region encompassing the active site, and a network of cooperative and localized interactions for maintaining the stability. These findings provide crucial insights into the folding energy landscape of membrane proteins.

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In Cell Footprinting Coupled with Mass Spectrometry for the Structural Characterization of a Membrane Protein
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Footprinting methods coupled with mass spectrometry have been used in recent years to analyze protein-protein and protein-ligand interactions and regions of protein conformational change. The footprinting method fast photochemical oxidation of proteins (FPOP) utilizes hydroxyl radicals to oxidatively modify solvent accessible sites in proteins. We have extended the use of FPOP as an in cell method where we can directly probe the structure of proteins in their native cellular environment. In cell FPOP would be especially useful for the study of membrane proteins owing to the difficulty in purifying and solubilizing these proteins for in vitro studies. We have used in cell FPOP coupled with mass spectrometry to identify the lipid binding region of the adaptor protein angiomotin (Amot). Amot can control cell shape and migration through its membrane binding ability. Identifying the lipid binding regions of Amot will aid in understanding its role in membrane trafficking. By oxidatively modifying MC7 cells transfected with membrane bound Amot and cells transfected with the S175A Amot mutant, which is localized in the cytosol, we can determine the lipid binding region from the changes in solvent accessibility that occurs upon lipid binding. High resolution mass spectrometry is used to identify oxidatively modified residues and for quantitation of oxidation levels. This study demonstrates the efficacy of in cell FPOP as a method for analyzing membrane proteins.

1279-Pos Board B256
Computational and Experimental Studies of Lipid-Protein Interactions in Biomemrane Function
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Integral membrane proteins constitute more than 30% of the human genome. Substantial lipid-protein interactions contribute to the structure and function of membrane proteins such as GPCRs and viral membrane ion channels. We address how physical properties of the membrane as well as positive charge on the lipid head group modulate protein activation. The flexible surface model (FSM) describes physical properties of the lipid environment that effect membrane protein activation [3]. According to the FSM, elastic coupling of the membrane lipids to integral membrane proteins define lipid-protein interactions through a balance of curvature and hydrophobic forces. Using UV-visible spectroscopy, FTIR spectroscopy [2], and molecular dynamics (MD) simulations, we discovered that lipid bilayer properties like head group and chain length modulate membrane protein functioning by influencing the conformational states. Using rhodopsin, a canonical G-protein-coupled receptor (GPCR) as a prototype, we show the effect of positively charged membrane lipid head group on rhodopsin activation using UV-visible spectroscopy and MD simulations. We discovered rhodopsin reconstituted in membrane lipids with a positively charged head group like DOTAP shifts the equilibrium to active Meta-II state. We propose a mechanism where membrane lipids with positively charged head group interact with Glu134 present on lipid-protein interface breaking the salt bridge with Arg135 in the conserved ERY motif. The interaction between Glu134 and the positively charged head group stabilizes the active Meta-II state. Our study gives insight on the role of membrane lipid-protein interactions in rhodopsin activation mechanism which can ultimately be extended to influenza virus membrane ion channels and other integral proteins. [1] A.V. Struts et al. (2015) Meth. Mol. Biol. 1271, 133-158. [2] E. Zaitseva et al. (2010) JACS 132, 4815-4821. [3] M.F. Brown (2012) Biochemistry 51, 9782-9795.

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Analysis of Unfolding of Apolipoprotein E Offers Insights into Lipid Binding Mechanism
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Apolipoprotein E (apoE) is a cholesterol transport protein that plays a critical role in lipid homeostasis. It is composed of a series of amphipathic α-helices: H1-H4 in the N-terminal (NT), and C1-C3 in the C-terminal (CT) domain. In humans, those bearing the APOE ε4 allele are at a higher risk for developing heart disease and Alzheimer's disease than those with APOE ε3 allele. The goal is to understand isoform specific differences in behavior between apoE3 and apoE4. The objectives are to compare their unfolding behavior at the sub-domain level, and, to determine the order of helix unfolding. Recombinant apoE bearing single Cys at defined locations were generated for labeling with fluorophores. Chemical-induced unfolding was monitored by circular dichroism and fluorescence spectroscopy. Fluorescence polarization measurements were carried out to determine changes in probe mobility. With apoE3 NT domain, helices H3 and H4 unfold with a significantly lower mid point of denaturation compared to H1 and H2, whereas with the CT domain, helix C3 offers the least resistance to unfolding compared to C1 and C2. Studies with the intact protein suggest a biphasic unfolding for apoE3, where the two domains unfold independently, and a cooperative unfolding for apoE4 possibly due to interaction between helices in the NT and CT domains. Taken together, our studies suggest the order of unfolding of helices: C3-C1/C2-H3/H4-H1/H2. We propose that apoE3 follows a similar order of lipid binding, based on the rationale that the ease of unfolding is reflective of the ease of lipid binding, with less structured segments seeking stability by lipid interaction. Our data are significant because understanding isoform-specific differences at the molecular level allows us to obtain insights into the physiological behavior of apoE in lipid metabolism.