2574-Pos

Annexin V is a Sensor of Negative Plasma Membrane Curvature

Christoffer Dam Florentsen[1], Guillermo S. Moreno Pescador[1], Alexander K. Sonne[1], Jesper Nylandsted[2], Poul Martin Bendix[1].
[1]Niels Bohr Institute, University of Copenhagen, Copenhagen, Denmark, [2]Danish Cancer Society Research Center, Copenhagen, Denmark.

Motivated by important cellular repair mechanisms in mammalian cells, where the protein family of annexins play a pivotal role, we employ optical manipulation of plasma membrane (PM) vesicles to study dynamics of annexins within highly curved nanotubes.[1] Membrane curvature induced recruitment of annexins and lateral interactions between proteins surrounding the site of rupture may play an important role in molecular membrane repair processes.[2]

The PM comprises a mixture containing a plethora of different lipids and proteins. It is desirable to maintain this complexity in a model system. A biomimetic model system allows quantitative investigation of protein-protein and protein-membrane interactions whereas reconstitution and encapsulation of membrane proteins in synthetic vesicles is more problematic. We have developed an assay which allows optical manipulation of isolated PM vesicles containing cell expressed fluorescent proteins. PM vesicles are formed using blebbing agents[3] and isolated from cells. The cytosolic content from the mother cell leaks into the vesicle and allows investigation of internal membrane-protein interactions. We have verified that both cytosolic and transmembrane proteins are correctly located and oriented in the PM vesicles. Hence, these vesicles provide a realistic platform for studying membrane protein dynamics.

Optical manipulation of these PM vesicles shows that annexins ANXA4 and ANXA5 strongly sense negative membrane curvatures and become enriched within nanoscale tubes. Intriguingly, a closely related protein ANXA2 does not sense membrane curvature despite its closely related structure. The preliminary data also shows a remarkable difference in mobility within the protein family. Several annexin family members have been shown to form oligomers on the PM, when Ca^{2+} is abundant and this feature may explain the observed difference in mobility and possibly also variability in curvature affinity.[4] These findings could help understanding how annexins synergistically work together to facilitate PM repair.

2575-Pos

Substrate Inducedconformational Changes of Liposomebound Cytochrome C

Raghed Kurbaj, Bridget Milorey, Reinhard Schweitzer-Stenner.
Chemistry, Drexel University, Philadelphia, PA, USA.

The electron transfer protein cytochrome *c* can acquire peroxidase activity upon its interaction with cardiolipin, an anionic lipid and a major ingredient of the inner mitochondrial membrane. Thus far attempts to correlate peroxidase activities with protein conformation have not been very successful. The yet unanswered question arises whether guaiacol, a common substrate for determining peroxidase activity, causes structural changes of membrane bound cytochrome *c*. To address this issue conformational changes of the protein cytochrome *c* binding to liposomes of 20% 1,1',1,2'-tetraoleyolcardiolipin (TOCL) and 80% 1,2-deoleyol-sn-glycero3-phosphocholine (DOPC) in the presence of varying concentrations of guaiacol were determined by visible circular dichroism and absorption spectroscopy. We recently found that at slightly acidic pH (6.5) cardiolipin-bound cytochrome *c* exists as an ensemble of conformations including a population of high spin species. These high-spin species should be more peroxidase active than the cardiolipin-bound low-spin proteins, but our results from peroxidase assays suggest that the difference in peroxidase activity between the high and low spin cardiolipin-bound non-native proteins was indistinguishable. We measured changes of the positive Cotton band of the Soret band as a function of TOCL concentration in the presence and absence of different guaiacol concentrations at pH 6.5. We found that the addition of guaiacol changes the intensity of the Cotton band significantly at intermediate cardiolipin/protein ratios (15) while corresponding changes are small or negligible at low (5) and high cardiolipin/protein ratios (60). Based on an earlier modeling of spectroscopic response data we interpret these findings as indicating that substrate binding to the protein stabilizes the unfolded conformation of membrane bound proteins in a way that allows for an unfolding of the protein at lower cardiolipin concentrations. Similar measurements at pH 7.4 and additional peroxidase activity measurements are underway and will be presented at the conference.

2576-Pos

Membrane-Bound Structures and Associated Electron Transport Functions of Cytochrome C

Minh D. Phan[1], Keel Yong Lee[2], Hanyu Wang[1], James F. Browning[1], Sushil K. Satija[3], John F. Ankner[1].
[1]Neutron Scattering Div, Oak Ridge National Lab, Oak Ridge, TN, USA, [2]Wyss Institute for Biologically Inspired Engineering, Harvard University, Cambridge, MA, USA, [3]Center for Neutron Research, National Institute of Standards and Technology, Gaithersburg, MD, USA.

Cytochrome c (Cytc) mediates the electron transfer between the complexes involved in mitochondrial respiration, which is coupled with the ATP synthesis. A decline in this biological process leads to many bioenergetic diseases, such as heart failure, aging, and Barth syndrome. Cytc is anchored to the membrane by cardiolipin (CL), a unique lipid in the inner mitochondrial membrane. The interaction between CL and Cytc regulates the binding states of Cytc to the membrane and, therefore, regulates the electron transport function of Cytc. This work aims to resolve the structures of CL-Cytc interactions and corresponding electron transport function of Cytc. X-ray and neutron reflectivity data show that pathological conditions involved CL's exceeded concentration and remodeled CL acyl chains facilitate a hydrophobic interaction between Cytc and CL. On the other hand, electrostatic interaction is maintained in healthy conditions where CL's concentration is within a physiological range. Finally, capacitance-voltage profiling data prove that the hydrophobic interaction between Cytc and CL inhibits the electron transfer activity of Cytc, shedding light on a failure pathway of mitochondrial ATP synthesis.

2577-Pos

PS Membrane Asymmetry Influences the Folding and Insertion of a Transmembrane Helix

Haden L. Scott[1], Frederick A. Heberle[2], John Katsaras[3], Francisco N. Barrera[4].
[1]The Bredesen Center for Interdisciplinary Research and Graduate Education, Univ Tennessee, Knoxville, TN, USA, [2]Neutron Sciences, Oak Ridge National Lab, Houston, TX, USA, [3]Neutron Sciences Directorate, Oak Ridge National Laboratory, Oak Ridge, TN, USA, [4]Biochemistry & Cellular and Molecular Biology, University of Tennessee, Knoxville, TN, USA.

The plasma membrane (PM) has an asymmetric distribution of lipids between the inner and outer leaflets of its bilayer. A lipid of special interest in eukaryotic cells is the negatively charged phosphatidylserine (PS). In healthy cells, PS is actively sequestered to the inner leaflet of the PM but can redistribute to the outer leaflet when the cell is damaged or at the onset of apoptosis. However, membranes contain also proteins. Marginally hydrophobic membrane proteins contain acidic residues in their transmembrane sequence and can experience topological transitions after membrane association. The pH low insertion peptide (pHLIP), which undergoes a topological reorientation and inserts into the membrane at low pH, as its name implies, is a well-characterized model for studying these transitions. Although it is known that the inclusion of PS in symmetric vesicles affects the membrane insertion process of pHLIP by lowering the pH midpoint of insertion, it is unclear how PS asymmetry affects these topological transitions. Here, we studied pHLIP's topology using freely-floating asymmetric phosphatidylcholine (PC)/PS vesicles with PS enriched in the inner leaflet. We found that the protocol to create asymmetric vesicles had to be modified due to the inclusion of PS. We used Annexin V labeled with an Alexa 568 fluorophore as a new way of quantifying PS asymmetry. For pHLIP, membrane insertion was affected by the surface charge difference between bilayer leaflets because of the asymmetric distribution of charged lipids. We thus conclude that lipid asymmetry can have consequences for the behavior of membrane-associated proteins. A corollary is that model studies using symmetric bilayers to mimic the PM may fail to capture important details of protein-membrane interactions.

2578-Pos

Capturing Dynamic Transporter-Lipid Interactions

Argyris Politis.
Chemistry, King's Coll London, London, United Kingdom.

Membrane proteins are dynamic biomolecules responsible for many critical cellular functions. Transporters, a subclass of membrane proteins, play crucial roles for maintaining adequate conditions for life by moving diverse biomolecules across the biological membrane. Lipids surrounding transporters play key roles in ensuring correct protein function. Accumulating evidences suggest that transporters function as complexes with their surrounding membrane lipids. Despite advances on understanding membrane protein-lipid interactions, our knowledge of the role of lipids in mediating protein dynamics remains limited. Here, we showcase how membrane lipids impact on the oligomeric state and conformational dynamics of transporters. We show using native mass spectrometry on a eukaryotic purine symporter that specific lipids binding to the protein modulate the formation of its oligomeric states. Mechanistically, such lipid modulation is obtained through specific interactions of structural lipids with the protein, which has a stabilising effect in the protein interface. We also show using the emerging and powerful method of hydrogen-deuterium exchange mass spectrometry (HDX-MS) that specific lipid-protein interactions modulate the conformational dynamics of the homologous transporters LacY and XylE from the major facilitator superfamily (MFS), the