3045-Pos Board B253

Mechanisms Governing Protein Clustering and Shape Changes in Biological Membranes

Sunil Kumar Palakurissi Balagopal[1], Sreeja K.K.[2].
[1]IIT Palakkad, Palakkad, India, [2]University of Pennsylvania, Philadelphia, PA, USA.

A characteristic feature of eukaryotic cells is the variety of membrane bound organelles, distinguished by their unique morphology and chemical composition. Despite the differences in membrane composition across organelles, ramified, tubular or sheet-like shapes are generic large scale morphologies observed in internal membranes, which suggest involvement of common underlying principles. While there is detailed knowledge of the molecular processes involved in membrane remodelling at short scales, our understanding of the underlying physical principles governing large scale morphogenesis is still rudimentary.

One common aspect is that membranes are subject to the action of curvature sensing and curvature generating proteins which modulate local membrane shape and lipid composition. Even at relatively small values of surface coverage these proteins can cluster through to a variety or processes and trigger morphological changes which are important for singling.

Another important feature, specific to organelle membranes, especially those in the trafficking pathways, is that they are subject to and driven by a continuous flux of membrane bound materials, on time scales comparable to membrane relaxation times.

Here we present Monte Carlo Simulation models that tests the effect of the above processes on the morphology of the membranes. We find that the steady state shapes obtained as a result of such active processes, bear a striking resemblance to the ramified morphologies of organelles in vivo, pointing to the relevance of nonequilibrium fission-fusion in organelle morphogenesis.

We show that, due to membrane curvature and composition interactions, the curvature-inducing membrane-nematogens can aggregate spontaneously, even at low concentrations, leading to a variety of membrane morphologies such as tubular and the sheet conformations. Strong lipid-protein interaction can result in fast protein clustering indicating a route to a lipid mediated signal amplification.

3046-Pos Board B254

Interplay of Curvature Sensing and Generation Mediated by Peripheral Membrane Proteins

Sachin Krishnan Thekke Veettil[1], Sovan Lal Das[2,3],
Sunil Kumar Palakurissi Balagopal[1,4].
[1]Department of Physics, IIT Madras, Chennai, India, [2]Department of Mechanical Engineering, IIT Kharagpur, Kharagpur, India, [3]Department of Mechanical Engineering, IIT Kanpur, Kanpur, India, [4]Department of Physics, IIT Palakkad, Palakkad, India.

Curvature sensing refers to the ability of proteins to bind onto membranes depending on their local curvature. As it offers a method for protein localization independent of the chemical composition, curvature sensing plays an essential role in many of the fundamental cell biological processes. Several classes of proteins such as the BAR-domain family and dynamin have been identified as capable of sensing curvature. Once bound, these proteins can alter the local shape of the membrane through curvature generation mechanisms like scaffolding, hydrophobic insertion, steric repulsion etc. Such mechanisms are believed to be responsible for stabilizing highly curved regions of intracellular membrane compartments. These two processes - curvature sensing and curvature generation - occur simultaneously and influence one another. While various mechanisms have been proposed for curvature sensing as well as curvature generation, the interplay between them is not well understood.

In this work, we employ grand canonical Monte Carlo simulations and mechanical-thermodynamical mean field models to address the above problem. In the simulations, membrane is modeled as dynamically triangulated surface and the local protein concentration using a scalar field. Using these techniques, we quantify the interdependence of curvature sorting and curvature generation. Specifically, we look at how the physical parameters such as the protein intrinsic curvature, the membrane tension, and protein-protein interaction influences the binding affinity and morphology of the membrane. As a limitting case, we obtain adsortion isotherms and curvature sorting curves for a non-deformable vesicle. The curves obtained using MC simulations matches well with analytical predictions from the mean field model for protein adsorption on vesicles. Our results conforms with observations in experiments using Single Liposome Curvature (SLiC) assays and membrane nanotubes pulled from Giant Unilamellar Vesicles (GUVs).

3047-Pos Board B255

Structural Lipids Stabilise Functional Oligomers of the Eukaryotic Purine Symporter UapA

Euan Pyle[1], Antreas Kalli[2], Zoe Hall[3], Bernadette Byrne[4], **Argyris Politis**[5].
[1]Chemistry, King's College London and Imperial College London, London, United Kingdom, [2]Leeds Institute of Cancer and Pathology, University of Leeds, Leeds, United Kingdom, [3]Chemistry, University of Cambridge, Cambridge, United Kingdom, [4]Chemistry, Imperial College London, London, United Kingdom, [5]Chemistry, King's College London, London, United Kingdom.

UapA from *Aspergillus nidulans* is a eukaryotic membrane transporter that mediates proton-dependent uptake of xanthine and uric acid. To date, the role of lipids in stabilising eukaryotic transporter oligomers remains unclear. We use native mass spectrometry (MS) in conjunction with molecular dynamics (MD) simulations and in vivo studies to investigate the role of interfacial lipids in stabilizing UapA oligomers. We show that UapA exists primarily as a dimer and that it binds two lipid molecules. Using lipidomics we identified three classes of phospholipids: phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI) co-purified with UapA. Next we delipidated the UapA and subjected it to native MS analysis. Interestingly, delipidation resulted in dissociation of the UapA dimer into individual protomers. Subsequent addition of PI or PE reformed the UapA dimer and enabled recovery of its bound lipids, suggesting a key role of PI and PE lipids in the stabilisation of the dimeric interface. MD simulations allowed predicting a putative lipid-binding site near the UapA dimer interface. Mutational analyses further confirmed that this lipid-binding site is essential for the quaternary structure and function of UapA.

Concluding, we performed the first in-depth investigation of the role of lipids in the oligomerisation and function of a eukaryotic transporter. Importantly, we identified specific lipids crucial for maintaining UapA transporter in a functional dimeric state. Overall, the hybrid strategy used here, provide the enticing prospect to allow investigation of the function of lipid binding to a wider range of membrane-bound proteins.

3048-Pos Board B256

Investigating Structural Properties of *Pseudomonas Aeruginosa* Exou Toxin Upon Interaction with Liposome and Nanodisc Bilayers by EPR Spectroscopy

Tzvia I. Springer, Samantha Kohn, Jimmy Feix.
Biophysics, Medical College of Wisconsin, Milwaukee, WI, USA.

Pseudomonas aeruginosa (*P. aeruginosa*) is a Gram-negative opportunistic pathogen that commonly infects the lungs of cystic fibrosis (CF) patients and severe burn victims. One of the four type III secreted effector proteins of *P. aeruginosa* is ExoU, a bacterial phospholipase A2 (PLA2) enzyme evolved to utilize host ubiquitin (Ub) and ubiquitinated proteins for enzymatic activation, leading to the destruction of host membranes. ExoU has three major domains: a catalytic domain, a linker domain, and a four-helix bundle located in the C-terminus. Sequence and structural homology of the four-helix bundle are weak across various genus and species, suggesting unique structural and functional features that may play a role in the molecular mechanism of activation. Previous work identified regions within helix IV of the four-helix bundle that are necessary for both activation and localization of ExoU to the lipid bilayer, and suggested a synergistic mechanism of activation involving ubiquitin and membrane binding. We are interested in understanding conformational changes in ExoU upon membrane binding in the presence and absence of ubiquitin. We hypothesize that the C-terminal four-helix bundle maintains essential intramolecular interactions with the catalytic domain that are necessary for enzymatic activation. In this study, site-directed mutagenesis, continuous wave (CW) EPR spectroscopy, CW power saturation, and double electron-electron resonance (DEER) were used to investigate the structure and membrane localization of helix IV in the presence of LUVs and nanodiscs. Results show that ubiquitin binding facilitates membrane penetration by helix IV, and demonstrate the utility of nanodisc bilayers for the study of protein-membrane interactions.

Supported by NIH grant GM114234.

3049-Pos Board B257

Investigating the Conformational Dynamics and Membrane Interaction Near the Catalytic Serine of Exou Upon Interaction with Diubiquitin and Membranes by EPR Spectroscopy

Samantha Kohn, Tzvia Springer, Jimmy Feix.
Biophysics, Medical College of Wisconsin, Milwaukee, WI, USA.

Pseudomonas aeruginosa (*P. aeruginosa*) is a Gram negative, opportunistic pathogen that is a rising health concern as an ESKAPE pathogen resistant to multiple antimicrobial agents. *P. aeruginosa* manipulates the host response by translocating effector proteins into the host cell via a type III secretion