View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

3578-Pos Board B306

Actin Filaments Attachment to the Plasma Membrane Cause the Formation of Ordered Lipid Domains in Live Cells

Parham Ashrafzadeh, Jelena Dinic, Ingela Parmryd.
Medical Cell biology, Uppsala University, Uppsala, Sweden.

The aim of this study was to investigate the relationship between ordered plasma membrane nanodomains and actin filaments using di-4-ANEPPDHQ and laurdan together with the reagents that affect actin filament dynamics in live Jurkat and primary T cells. The degree of lipid packing can be quantified using polarity sensitive membrane dyes such as laurdan and di-4-ANEPPDHQ. These two dyes display a red shift in their emission peaks for membranes in ld phase relative to lo phase. Laurdan is uncharged and can easily flip between two leaflets of the plasma membrane and we demonstrate that it reports equally on the two leaflets of the plasma membrane. Di-4-ANEPPDHQ, on the other hand, has got two positive charges and we show that it cannot flip between the two leaflets. Lowering the number of potential attachment points for actin filaments in the plasma membrane by decreasing the level of phosphoinositides led to a decreased proportion of ordered membrane domains. Stabilizing actin filaments using jasplakinolide increased the fraction of ordered membrane domains while disrupting actin polymerization using latrunculin B had the opposite effect. Importantly, the altered dynamics of intracellular actin were reflected in the outer leaflet of the plasma membrane. Membrane blebs are not connected to actin filaments and had a lower fraction of ordered domains than the rest of the plasma membrane. The fraction of ordered domains increased when either lipid raft or non-lipid raft markers were patched. Interestingly, this increase was strongly correlated with an increase of polymerized actin filaments at the plasma membrane. Our study suggests that actin filaments attachment to the plasma membrane leads to the formation of ordered domains shifting the focus from lipid-lipid to lipid-protein interaction in lipid raft manifestation.

3579-Pos Board B307

Study of Protein Diffusion in Defective Erythrocyte Membrane

He Li, **George Lykotrafitis**.
Department of Mechanical Engineering, University of Connecticut, Storrs, CT, USA.

In the hemolytic disorders of hereditary spherocytosis (HS) and hereditary elliptocytosis (HE), the red blood cells (RBCs) are abnormal. In HS, membrane proteins that facilitate the vertical interactions between the lipid bilayer and the spectrin cytoskeleton are defective, resulting in RBC membrane loss and the spherical shape of the erythrocytes. In HE, membrane proteins that support the horizontal interactions within the spectrin cytoskeleton are defective, resulting in loss of the elasticity of the spectrin network and the elliptic shape of the erythrocytes. Several attempts have been made to experimentally correlate the severity of the disease in each case with higher diffusivity of band-3 proteins located in the RBC membrane. However, the experimental results are inconclusive. To better understand them, input from simulations is needed. Because of the complexity of the system however, the implementation of atomistic simulations is not feasible.

In this work, we introduce a two-component, coarse-grain molecular dynamics erythrocyte membrane model that simulates explicitly the phospholipid bilayer, membrane proteins, and the cytoskeleton by coarse-grain particles. The particles represent the actin junctions, spectrin, glycophorin, immobile band-3, mobile band-3 and aggregation of lipids. The proposed model allows us to study the diffusion of band-3 particles in the healthy RBC membrane and in HS and HE RBC membranes. We can observe the hop diffusion of band-3 proteins between the corrals formed by the cytoskeleton. In addition, we measure the band-3 diffusion in the membrane with proteins defects in the vertical and horizontal interactions, respectively. The measured diffusion coefficients demonstrated that spectrin content is the major determinant of the lateral diffusion of band-3. Direct comparison with experimental results is very promising.

3580-Pos Board B308

Dynamic Behavior of the Active and Inactive State of Adenosine A2A Receptors

Sangbae Lee, Supriyo Bhattacharya, Nagarajan Vaidehi.
The City of Hope, Duarte, CA, USA.

Adenosine A_{2A} receptors belong to the class A G protein-coupled receptor (GPCR) and are drug targets for the treatments of Parkinson's disease and cardiac ischemia. A_{2A} receptor has been thermostabilized with specific mutations and crystallized in the inactive and active conformations. The crystal structures do not provide a complete picture of how the mutations thermostabilize the specific conformational state of the receptor. Using MD simulations, we investigated the differences in the stability and energy landscape of mutant and wild type in the active and inactive state in the presence/absence of agonist and antagonist ligand. Our results show that the mutations lead to enthalpic stabilization of the receptor. Stabilization resulting from mutations is due to the formation of inter-helical hydrogen bonds that improves the transmembrane domain packing. Such mutations also alter the conformations of the neighboring residues to improve the interhelical packing. The active mutant shows less movement of the TM6 with respect to TM3 than the wild type receptor in this conformation. This TM6 movement signifies the activation process in GPCRs. While this restricted movement of the thermostable mutant is advantageous in purification and crystallization of active mutant, it could be the reason why these mutants are not efficient in activating the G proteins.

3581-Pos Board B309

Protein-Induced Membrane Shape Instability: Dynamics and Membrane Tension Dependence

Zheng Shi, Tobias Baumgart.
Chemistry, University of Pennsylvania, Philadelphia, PA, USA.

Cellular membranes undergo constant shape remodeling involving the formation of highly curved structures. However, the physical and molecular mechanisms governing the transition of membrane shapes are still not clear. Here, we seek to demystify this problem by quantifying the dynamics of membrane tubulation induced by the binding of peripheral proteins. A recently developed single giant unilamellar vesicle (GUV) transfer method is utilized to record the association of fluorescence labeled-proteins onto single GUVs as well as the simultaneous changes in GUV shape. Endophilin, a BAR domain-containing protein, is chosen due to its well-know role in promoting the formation of transport vesicles during clathrin-mediated endocytosis.

During endophilin-membrane association, we observe membrane tubulation when protein density on the relatively flat GUV reaches a critical level, as marked by a sudden decrease of GUV membrane area. The critical protein density is found to increase with membrane tension, which is accurately controlled through micro-pipette aspiration. Furthermore, the roles of lipid composition are investigated. PE lipids which provide more lipid packing defects in the bilayer are found to reduce the protein density required for initiating membrane tubulation. The negatively-charged PS lipids, while playing essential roles in determining the membrane binding rates and affinity of endophilin, do not alter the membrane instability criterion.

The observed membrane instability criterion as a function of both membrane tension and critical protein density, agrees well with a theoretical curvature instability model. In this model, membrane-bound diffusive proteins, which can be treated as two-dimensional van der Waals gas, couple with the local out-of-plane fluctuations of the bilayer to drive the planar membrane unstable. The results give new insights into the dynamics of biological membranes and support a mechanism in which membrane tension is used to regulate dynamic transport and signaling processes in cells.

Membrane Fusion II

3582-Pos Board B310

Insights into the Lateral Organization and Molecular Order of Lipid Mixtures that Mimic the HIV-1 Membrane by Multiphoton Fluorescence Microscopy

Nerea Huarte, Pablo Carravilla, José L. Nieva, Jose Requejo-Isidro.
Biochemistry and Molecular Biology, Biophysic Unit, Leioa, Spain.

Compositional domain structure of the HIV membrane determines its virulence. Moreover, anti-gp41 broadly neutralizing antibodies recognize their epitopes in the context of the viral lipid membrane. The lipid composition has been proposed to modulate neutralizing epitope accessibility, and, therefore, understanding at the molecular level the factors governing the membrane organization of this pathogen may contribute to the development of unprecedented vaccines. Lipidomic analyses support the resemblance between the HIV envelope and raft-like membrane macroassemblies. However, the mole percentages of SPM and Chol (17 and 46 %, respectively) actually suggest that this compositionally heterogeneous membrane may lie close to the boundary between Lo/Ld co-existence and pure Lo phase. Here, we use two-photon microscopy of Laurdan-labeled giant unilamellar vesicles as a means to quantify the contribution of individual lipid species to the molecular order measured in HIV membrane mimics. In addition, to test local modulation of order by protein, we assess the effect exerted by peptides derived from the gp41 sequences that insert into the viral envelope.