driving the peptide away from normal fibrillization and rather producing amorphous aggregates. The results of the peptide's morphological evolution and secondary structure changes revealed that some antibodies have the potential of preventing formation of parallel beta sheets structures, which may be an indication of Aß fibrillization preclusion. This work provides insights to understand the mechanistic effects in which antibodies alter the secondary structure of amyloid peptides modifying their fibrillization, critical for Aß plaque clearance in the brain of patients with AD.

Platform AK: Membrane Structure II

2194-Plat

Protein-Lipid Interactions are Determinants of Small and Large-Scale Membrane Domains

Hermann-Josef Kaiser, Daniel Lingwood, Ilya Levental, Kai Simons.
MPI-CBG, Dresden, Germany.

Sphingolipids and cholesterol exhibit preferential association that cells employ to compartmentalize their membranes and regulate protein function. Separation of artificial membranes into liquid-ordered (Lo) and liquid-disordered (Ld) phases is regarded as a common model for this compartmentalization. However, tight lipid packing seems to conflict with efficient partitioning of transmembrane (TM) proteins into the Lo phase. To assess membrane order as a component this organization we performed fluorescence microscopy with the membrane probe C-laurdan. We compared Lo-Ld phase separated model membranes with plasma membrane systems that exhibit inducible phase separation: giant plasma membrane vesicles (GPMVs) and plasma membrane spheres (PMS). Notably, only the latter support selective inclusion of TM proteins with the sphingolipid GM1 into one phase. We found comparable small differences in order between the separated phases of both biomembranes. Lateral packing in the ordered phase of GPMVs resembled the Lo domain of model membranes, whereas the GM1 phase in PMS exhibited considerably lower order suggesting that cholesterol-mediated tight lipid packing is insufficient to explain the coalescence of a TM protein-selective lipid phase as seen in the PMS. To further investigate basic interactions between TM proteins and lipids that might contribute to protein-selective phase behavior we analyzed the order of model membranes into which we integrated a TM peptide. We found that the peptide affected the order of bilayers in a cholesterol and acyl chain length-dependent manner. The results show that TM proteins influence their local lipid environment and thus have the potential to become determinants in the formation of membrane domains.

2195-Plat

N-3 Polyunsaturated Fatty Acid Incorporates Directly into Lipid Rafts to Disrupt Domain Clustering and MHC Lateral Organization of Antigen Presenting Cells

Benjamin Drew Rockett, Kristen Carraway, **Saame R. Shaikh**.
Brody School of Medicine, East Carolina University, Greenville, NC, USA.

N-3 polyunsaturated fatty acids (PUFA) are under clinical testing for the treatment of symptoms associated with inflammatory disorders such as cardiovascular disease. However, effective use n-3 PUFAs as nutraceuticals has been limited by a poor understanding of their molecular mechanisms. Here we addressed how the n-3 PUFAs eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids modified lipid raft and protein lateral organization of antigen presenting cells, whose function is suppressed by n-3 PUFAs. Quantitative fluorescence microscopy showed that DHA, but not EPA, relative to controls, diminished lipid raft clustering and increased their size. A significant amount of DHA incorporated directly into rafts without changing cholesterol distribution between rafts and non-rafts. Quantification of fluorescence co-localization images showed that DHA selectively altered the lateral organization of the major histocompatibility complex (MHC) class I protein. FRET microscopy measurements showed that DHA modified MHC class I clustering on a nanometer scale. Taken together, our findings are not in agreement with studies in model membranes on unsaturated fatty acids and lipid rafts. Therefore, we propose a new model, which reconciles contradictory viewpoints from biophysical and cellular studies, to explain how an unsaturated fatty acid modifies lipid rafts on several length scales. Our study provides mechanistic details by which DHA suppresses antigen presenting cell function, which allows us to more effectively use these fatty acids in the clinic.

2196-Plat

Detection of Domain Formation in a Subpopulation of Late Endosomes by Templated J-Aggregates

Gary Mo, Christopher M. Yip.
University of Toronto, Toronto, ON, Canada.

Although controlling the self-assembly of molecules to form specific structural motifs is important for the design of materials with unique optical or electronic properties, the growth of these assemblies also provides useful information regarding their local environment. We have previously reported the formation of templated J-aggregates of an organic chromophore on model supported planar bilayers [Langmuir, 25, 10719], finding that the spectral characteristics of the J-aggregates reflected the structure of the nucleating lipid bilayer. Here, we describe how the structure of membrane domains on internal organelles in live cells can be inferred from the presence of specific J-aggregate forms. Cell lines expressing GFP-conjugated wild-type Rab5a or Rab7, GTPase markers of early and late endosomes, respectively, were treated with dyes and studied using confocal microscopy and fluorescence spectroscopy. Remarkably, while we did not observe any significant co-localization of the GFP-Rab5a marker and J-aggregates, there was clear evidence that the J-aggregates were present within late endosomes labeled with GFP-Rab7. Close inspection revealed that the J-aggregates were confined to smaller vesicles within the lumen of the late endosome. These in vitro results were compared with J-aggregates formed on multi-component planar phospholipid bilayers mimicking the lipid compositions of late endosomal and mitochondria. Correlated confocal fluorescence and atomic force microscopy revealed that a lipid enriched only in late endosomes was responsible for the formation of these J-aggregates. Live cell imaging suggested that these structures were present during an early phase of late endosome maturation, after Rab5 conversion but prior to acidification.

2197-Plat

Micropatterning of Plasma Membrane Proteins to Analyze Raft Localization in Living Cells

Julian Weghuber[1], Stefan Sunzenauer[1], Mario Brameshuber[1], Stefan Wieser[1], Lawrence Rajendran[2], Gerhard J. Schuetz[1].
[1]Universität Linz, Linz, Austria, [2]ETH Zurich, Zurich, Switzerland.

We have developed an assay for quantitative analysis of the interaction between a fluorescently marked protein (prey) and a membrane protein (bait) using microstructured surfaces covered with biotinylated ligands (antibodies) targeted against the bait. The proof-of-concept was demonstrated for the interaction between CD4, a major co-receptor in T-cell signalling, and Lck, a protein tyrosine kinase essential for early T cell signalling. Here we present improvements and a more precise characterization of the method as well as the applicability of the assay for the analysis of protein interactions within lipid rafts in the inner and outer leaflet of the plasma membrane. We stably expressed fluorescently labelled raft and non-raft proteins in the human T24 cell line as prey proteins and determined the degree of interaction with the antibody-targeted bait proteins CD59 (GPI-anchored protein, raft marker) and CD71 (Transferrin-receptor, non-raft marker), respectively. We found strong interaction of CD59 with putative raft markers including various GPI-GFP constructs, the inner-leaflet associated proteins Lck and Flottilin1 and a Pleckstrin-Homology domain fused to GFP. Importantly, we did not find interaction of CD59 with CD71-GFP and other potential non-raft proteins. When CD71 was used as the bait protein we did not find interaction with the putative raft markers. While the detected absence of CD71 from and the presence of CD59 in lipid rafts confirm current knowledge, it is still very unclear if a lipid-raft dependent coupling of proteins and certain especially negatively charged lipids across the plasma-membrane bilayer exists. Thus, our micropatterning assay will be of great interest to address this question.

2198-Plat

Differentiating Lipid Phase Domains in Cells using Fluorescence Lifetime of DiI

Hari S. Muddana, Tristan Tabouillot, Homer H. Chiang, Peter J. Butler.
Pennsylvania State University, University Park, PA, USA.

Dynamic heterogeneous distribution of lipid phases is thought to be an important means by which lipids modulate cellular biology. However, information about these domains is not accessible using conventional optical microscopy because their size (10-100nm) is well below the diffraction limit. Fluorescence lifetime (FL) of variable chain length di-alkyl carbocyanine dyes (DiIs) has been proposed to reflect lipid phase, but verification that fluorescence readouts reflect lipid phase in cells is lacking. Thus, we used time-correlated single photon counting techniques to test the fidelity with which photophysical properties of DiI-C12 and DiI-C18 report lipid order in cells. DiI FL increases with increased solution viscosity (1 to 70 cP) were independent of acyl chain length, demonstrating that lifetime reports chromophore headgroup local viscosity. Sensitivity of FL of DiI to membrane order was evaluated in giant unilamellar vesicles (GUVs) composed of DOPC (liquid-disordered), DOPC:Chol (liquid ordered), DPPC:Chol (liquid ordered), and DPPC (gel phase). FL of DiI increased significantly with increase in membrane order, and correlated well with a decrease in diffusion coefficient. Phase partitioning of DiI-C12 and

DiI-C18 was characterized in phase-coexistent GUVs composed of DOPC:DPPC:Chol (2:2:1). Short chain DiI-C12 and long chain DiI-C18 partitioned to liquid-disordered and liquid-ordered membrane phases, respectively. FL of DiI-C18 stained cells (1.47 ± 0.49 ns) was higher compared to DiI-C12 stained cells (1.26 ± 0.12 ns), indicating that DiI-C18 and DiI-C12 partitioned into liquid-ordered and liquid-disordered phases respectively. In conclusion, FL of DiI is a sensitive indicator of membrane fluidity and different chain length DiI's partition to different membrane phases both in model and cell plasma membranes.

2199-Plat
Imaging of Mobile Stable Lipid Rafts in the Live Cell Plasma Membrane and their Involvement in Cellular Signaling During Heat Shock
Mario Brameshuber[1], Julian Weghuber[1], Verena Ruprecht[1], Imre Gombos[2], Ibolya Horváth[2], László Vigh[2], Hannes Stockinger[3], Gerhard J. Schuetz[1].
[1]University of Linz, Linz, Austria, [2]Hungarian Academy of Sciences, Szeged, Hungary, [3]Medical University of Vienna, Vienna, Austria.
The plasma membrane has been hypothesized to contain nanoscopic lipid platforms, also termed lipid or membrane rafts. Based on biochemical and cell biological studies, rafts are believed to play a crucial role in many signaling processes. However, there is currently not much information on their size, shape, stability, surface density, composition and heterogeneity. We present here a method which allows for the first time the demonstration that single rafts diffuse as stable platforms in the live cell plasma membrane. Our method senses rafts by their property to assemble a characteristic set of fluorescent marker-proteins or lipids on a time-scale of seconds. The special photobleaching protocol TOCCSL (Thinning Out Clusters while Conserving Stoichiometry of Labeling1) was used to reduce the surface density of labeled mobile rafts down to the level of well-isolated diffraction-limited spots, without altering the single spot brightness. The statistical distribution of probe molecules per raft was determined by single molecule brightness analysis. For demonstration, we used the consensus markers Bodipy-GM1, a fluorescent lipid analogue, and glycosylphosphatidylinositol-anchored monomeric GFP. For both markers we found cholesterol-dependent association in the plasma membrane of living CHO and Jurkat T cells in the resting state, indicating the presence of small, mobile, stable rafts hosting these probes. We further applied the technology to address structural changes in the plasma membrane during fever-type heat shock: at elevated temperatures mGFP-GPI homo-association disappeared, accompanied by an increase in the expression of the small heat shock protein Hsp27.
1. Moertelmaier, M., Brameshuber, M., Linimeier, M., Schütz, G. J. & Stockinger, H. Thinning out clusters while conserving stoichiometry of labeling. Appl Phys Lett 87, 263903 (2005).

2200-Plat
Cholesterol and Phosphatidylinositol 4,5-Bisphosphate Synergistically Affect Endothelial Biomechanics
Zhongkui Hong[1], Paul Hampel[1], Marius C. Staiculescu[1], Irena Levitan[2], Gabor Forgacs[1].
[1]University of MIssouri-Columbia, Columbia, MO, USA, [2]University of Illinois-Chicago, Chicago, IL, USA.
Membrane cholesterol induces the formation of cell membrane microdomains that are enriched with acidic lipids such as phosphatidylinositol 4,5-bisphosphate (PIP2). PIP2 regulates a number of cellular processes by serving as cross linker between the membrane and cytoskeleton, and by association with actin-bonding proteins as cofilin, gelsolin, profilin, α-actinin, MARCKS, filamin, etc. The objective of this study is to clarify how the co-operative action of cholesterol and PIP2 impacts the biomechanics of endothelial cells. We measured the stiffness of bovine aortic endothelial cells (BAEC) by membrane indentation and tether extraction using AFM at different cholesterol level and PIP2 conditions. BAECs were transfected with Pleckstrin homology domain of phospholipase C (PH-PLC), which can sequester PIP2. Transfection with PH-PLC did not alter the force needed to elongate a tether-nanotube (i.e. tether force), but it significantly increased cell stiffness. These results imply that though PIP2 in BAEC does not act as a direct cross-linker between the plasma membrane and cytoskeleton, it is an efficient regulator of the cytoskeletal architecture. To further elucidate the role of PIP2 in endothelial cells, we enriched BAECs with exogenous PIP2 at different cholesterol levels. Enrichment with exogenous PIP2 led to the increase in membrane stiffness in cholesterol depleted BAEC. However no changes in stiffness were observed in control BAEC at normal cholesterol level. Confocal imaging showed that under normal cholesterol level most of the exogenous PIP2 delivered into BAEC was localized in discrete membrane domains and perinuclear patches. However, in cholesterol depleted BAEC the exogenous PIP2 was mostly homogeneously dispersed across the cell. On the basis of these findings we conclude that cholesterol affects BAEC's biomechanical properties by regulating the cellular localization and metabolism of PIP2, and thus by remodeling the cytoskeleton and its interaction with the plasma membrane.

2201-Plat
KcsA Redistribution Upon Lipid Domain Formation in Supported Lipid Bilayers and its Functional Implications
Heiko M. Seeger[1], Andrea Alessandrini[1,2], Paolo Facci[1].
[1]CNR-INFM-S3 Natl.Center on nanoStructures and bioSystems at Surfaces, Modena, Italy, [2]Department of Physics, University of Modena and Reggio Emilia, Modena, Italy.
In a recent study we showed that the melting behavior of Supported Lipid Bilayers (SLBs) can be influenced by ionic strength and the preparation temperature [1]. By changing these parameters we could control the coupling between the two bilayer leaflets obtaining a coupled or decoupled melting behavior. Hence, we could provide evidence that the SLB model system is also suited for the study of lipid/protein interactions which had been questioned in the past. Further, we investigated the mutual interactions between the bilayer forming lipids and the pH-gated K^+ channel KcsA. In particular, we studied the melting behavior of the SLB and the ion channel distribution by temperature controlled atomic force microscopy (AFM) in liquid. We induced the formation of solid ordered domains in SLBs made of POPE:POPG 3:1 and KcsA. We found that the KcsA proteins were excluded from the solid ordered regions. Further, the ion channels tended to accumulate at the domain boundaries or they clustered in the liquid disordered phase [2]. This behavior is in agreement with what is expected from the hydrophobic matching principle. In addition using voltage clamping with temperature control we obtained that the lateral reordering of both the proteins and lipids results in changes of KcsA functionality. Functional modifications include both the protein's opening life times and ion channel conductance, displaying a biphasic behavior.
[1] Seeger et al., Biophys.J. (2009) 97:1067
[2] Seeger et al., submitted.

Platform AL: Cell Motility & Mechanics

2202-Plat
Mesoscopic Model of Actin-Based Propulsions
Jie Zhu, Alex Mogilner.
UC Davis, Davis, CA, USA.
In order to study movements of actin-propelled beads, we use stochastic simulations of 'in silico' actin network, in which microscopic individual actin filaments undergoing nucleation, elongation, attachment, detachment and capping are embedded in nodes-and-springs viscoelastic network representing macroscopic actin gel. Our study shows that the combined effects of macroscopic elastic deformation and microscopic ratchet are crucial for explanation of the observed force-velocity relations and orientations of the actin-propelled ellipsoidal beads.

2203-Plat
The Effects of Filament Aging and Annealing on a Lamellipodium Undergoing Disassembly by Severing
Paul J. Michalski, Anders E. Carlsson.
Washington University, St. Louis, MO, USA.
We construct a simplified model of a lamellipodium and use a numerical simulation to study its properties as it disassembles by filament severing. The growing lamellipodium is modeled as a 2D or 3D periodic lattice of crosslinked actin filaments. At each time step a new layer of actin filaments is added at the membrane, and existing filaments are severed stochastically. After each time step each filament is tested to determine if it remains in the connected network, defined as those filaments that are connected to the membrane by an unbroken path of filaments. Disconnected sections of the network are assumed to diffuse away rapidly and are removed after each time step. Filament aging, due to hydrolysis and other mechanisms such as association with actin binding proteins, is modeled by including several different filament chemical states, with stochastic transitions between the states. Filament annealing is included by allowing vacant sections of the network to grow new filaments off of existing filaments.
The properties of the model are studied as functions of the severing and annealing rates, as well as the number of states. We find that the network width of the multistate model is proportional to the sum of the average lifetimes of the states, and is well modeled by a simple kinetic theory. The edge of the growing network becomes sharper as either the number of states is increased or the dimensionality is increased. Annealing increases the average length of the network, and we find that the network length diverges at a critical annealing rate. These conclusions are robust to the presence of disorder and to changes in the topology of the network.