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
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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
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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
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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
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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
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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