composition of the liquid-disordered phase have a diameter below optical resolution. Direct imaging of the intrinsic contact angle formed by the membrane and the two phases in budded vesicles (PRL, 103:238103, 2009) is also hindered by the poor axial resolution in confocal microscopy. Here, we use a super resolution technique, stimulated emission depletion (STED) microscopy in both 2D and 3D mode combined with microfluidics to study these remarkable membrane morphologies. We first designed a microfluidic device which can dramatically increase the trapping efficiency of giant unilamellar vesicles (GUVs) and improve the solution exchange rate. Then, with a resolution less than 35nm from STED microscopy, we visualize the membrane nanotube structures with unprecedented detail, and compare the directly measured nanotube diameters with previously reported theoretical and experimental ones. Additionally, by manipulating the height of the microfluidic channels, we pinch and orient the budded vesicles to image the intrinsic contact angle with lateral STED resolution. These highly curved membrane structures imaged with super resolution microscopy will serve to deepen and expand our understanding of biomembranes. This work is part of the MaxSynBio consortium, jointly funded by the Federal Ministry of Education and Research of Germany and the Max Planck Society.

**515-Pos Board B285**

**Intrinsic Curvature Effects of Oxidized Lipids on Spatial Lipid Organization**

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The oxidized lipid, PGPC, is a curvature inducing lipid. It has distinctly different effects on the lipid organizations in POPC, DOPC, and DPPC bilayers that are observable by average Laurdan fluorescence generalized polarization measurements. These bilayer lipids themselves differ in their individual curvatures. Oxidized lipid- induced spatial lipid reorganization was investigated in model bilayer lipid membranes using Dynamic Light Scattering and Laurdan Fluorescence. Distinct differences were observed between the effects of the oxidized lipid, PGPC, on the three bilayer lipid membranes of POPC, DPPC, and DOPC. The OxPL, PGPC, increases the polarity of POPC and of the gel phase DPPC and decreases the polarity of DOPC and of the liquid phase DPPC. DLS experiments showed that mixed vesicles are formed by POPC / PGPC and by PGPC / gel phase DPPC. On the other hand DOPC / PGPC and PGPC / liquid phase DPPC separate into coexisting vesicles and micelles. The DLS and Laurdan fluorescence experiments interpreted together suggest that POPC mixes randomly with PGPC; but DOPC and DPPC do not. The different lipid organizations are hypothesized to be driven by intrinsic curvature differences between the mixing molecules.

**516-Pos Board B286**

**Vitamin E Bends Model Cell Membranes to Promote its Antioxidant Function**

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The primary role of vitamin E in membranes is to protect phospholipids from oxidation. A longstanding question has been whether there is a structural component in support of its function. Here we focus on the effect of vitamin E on the structure of model membranes with phospholipids containing PC and PE headgroups. Using solid-state $^2$H NMR spectroscopy, complemented by DSC, we compare the molecular organization of 1-palmitoyl-2-oleoyl-sn-glycerophosphatidylethanolamine (16:0-18:1PE, POPE) and 1-palmitoyl-2-oleoyl-sn-glycerophosphatidylcholine (16:0-18:1PC, POPC) mixed with increasing concentration of vitamin E. Somewhat akin to cholesterol, the general effect on both POPE and POPC is to broaden and depress the gel to liquid crystalline phase transition - vitamin E disrupts chain packing in the gel phase and restricts chain reorientation in the liquid crystalline phase. The spectra observed with POPE-$d_{31}$ consist of a superposition of two components at higher temperatures that we ascribe to vitamin E promoting a transition from lamellar ($L_\alpha$) to inverted hexagonal phase ($H_{II}$). In contrast, POPC-$d_{31}$ maintains $L_\alpha$ phase in the presence of vitamin E. The tendency to induce negative curvature indicated by the formation of $H_{II}$ phase suggests vitamin E would make membranes less permeable to free radicals.

**517-Pos Board B287**

**Single-Lipids Diffusion and Lipid Sorting at Nanoscale Curvature Sites**

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Membrane curvature may cause changes to the local effective membrane viscosity and induce a lateral sorting of membrane components. However, the relationships between curvature, diffusion, and lipid shape have not been established. We used single-particle tracking to observe fluorescently labeled lipids on nanoscale curvature sites in supported lipid bilayers (SLBs). Giant unilamellar vesicles (GUV) were burst over 100 nm diameter nanoparticles (NP) on glass coverslips. Membrane composition variations yielded minimal changes to the effects of curvature, including mixtures of POPC, lysoPC, DOPC, and DLPC. However, membrane curvature had a widely varying effect on different fluorescent lipids. Lipid head labeled TexasRed-DHPE diffused half of the speed on curvature site compared to lipid tail labeled TopFluorPC or TopFluorPIP$_2$ yet two time faster in flat lipid bilayer. The curvature affected these fluorescent lipids in drastically different ways. We hypothesize that diffuser tail properties affects diffusion at curvature sites more than head groups. Further head-labeled lipids and tail-labeled lipids will be studied to examine the generality of this observation. Additionally, the site of the fluorescent label affected the sorting of the fluorescently labeled lipid to the curvature site. Understanding and comparing the molecular sorting in lipid bilayers can further our understanding of the curvature sensing lipids properties such as tail saturate/ unsaturated preference and head group types. These studies will contribute to the greater biophysical understanding of membrane curvature.

**518-Pos Board B288**

**Cardiolipin-Induced Phase Separation in Biomimetic Mitochondrial Membranes and Cardiac Vesicles is Dependent on Cardiolipin Concentration and Acyl Chain Composition**

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Cardiolipin (CL) is critical for inner mitochondrial membrane (IMM) biophysical organization and protein activity. It is hypothesized that localized domains of CL form within the IMM to regulate the formation and function of respiratory proteins complexes. However, it is poorly understood how mitochondrial CL domains are organized. To address this, we used biochemical and biophysical techniques to study CL microdomains. We first used classical detergent extraction to isolate CL microdomains. Studies with three different detergents, at varying concentrations, showed increased non-specific solubilization of all mitochondrial phospholipids and protein. This demonstrated that CL microdomains couldn't be isolated via detergent extraction methods. Subsequently, we studied CL microdomains by constructing, and quantitatively imaging, giant unilamellar vesicles (GUVs) modeling the IMM. In addition, we constructed giant mitochondrial vesicles (GMVs) from isolated rodent cardiac mitochondrial phospholipids. Results from imaging experiments indicated that in the absence of protein, the physiochemical properties of lipid-lipid interactions were not sufficient for formation of phase separated microdomains. However, reconstitution of cytochrome c at differing concentrations induced strong morphological changes concomitant with formation of distinct phase separated structures that were driven by a decrease in phospholipid packing and increase in the membrane elasticity modulus. Lastly, we studied the role of CL concentration and acyl chain composition on membrane domain organization and phase separation with cytochrome c. Decreased CL concentration, as observed in several metabolic diseases, prevented morphological phase separation. Similarly, the incorporation of docosahexaenoic acid (DHA) into CL, also associated with differing metabolic disorders, prevented phase separation due to differences in cytochrome c-acyl chain cofactor interactions. Altogether, our data demonstrate that CL microdomain organization is driven by lipid-protein interactions and likely exists in specific regions of high membrane curvature and protein concentration.

**519-Pos Board B289**

**Impact of Phospholipid Acyl Chain Length Mismatch on Sterol Affinity and Lateral Segregation**

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Lateral segregation into ordered and disordered domains may occur when mixing phospholipids (PLs) with high and low gel-fluid transition temperatures. Cholesterol is known to influence this lateral domain formation in model

membranes, which likely resembles the formation of cholesterol rich nanodomains in biological membranes. In previous studies the sterol affinity difference between unsaturated and saturated PLs was found to influence the ability of cholesterol to promote formation of ordered domains.
Our aim was to study the formation of ordered domains when there is an acyl chain length mismatch between unsaturated PCs and SM before segregation. Preliminary sterol partitioning results suggested that the sterol affinity for the di-monounsaturated-PC bilayers correlated with acyl chain length. Sterol affinity for palmitoyl-sphingomyelin (PSM) in these PC bilayers was also examined. 2H nuclear magnetic resonance (NMR) spectroscopy results showed that the acyl chain order of deuterated PSM in binary bilayers correlated with the acyl chain length of the di-monounsaturated-PC included in the bilayer. Lateral segregation was explored using time resolved fluorescence of *trans*-parinaric acid. To compare to what degree cholesterol influenced lateral segregation we determined the amount of saturated PLs required to form ordered domains in fluid bilayers containing 0 or 20 mol% cholesterol.
General observations about SM and cholesterol interaction with unsaturated mismatched acyl chain length PCs, and how it leads to formation of ordered domains will be presented.

**520-Pos Board B290**
**Are Vitamin E and PUFA Driven Together by Choleterol? Computer Simulation Studies**
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Vitamin E (α-tocopherol) is the principal antioxidant in cell membranes and is believed to protect PUFA (polyunsaturated fatty acids) from oxidation. Since α-tocopherol is in low concentration in cell membranes, generally less than 0.1 mol%, co-localization with PUFA-containing phospholipids would be advantageous to protect them from oxidative damage. We hypothesize that cholesterol, ubiquitous in the plasma membrane of animals, drives α-tocopherol and PUFA-containing phospholipids together. All-atom, umbrella sampling molecular dynamics (MD) simulations that were run on bilayers composed of SOPC (1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine) and SOPC with cholesterol (50 mol%) demonstrate that the presence of cholesterol reduces membrane affinity for α-tocopherol. With the use of coarse graining (CG) methods, a new model for α-tocopherol was created to better mimic the physical properties of the branched phytyl side chain. This model was then employed in CG simulations on a bilayer composed of α-tocopherol mixed with SM (sphingomyelin), cholesterol and polyunsaturated PDPC (1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine) that separates into SM-rich/cholesterol-rich (raft) and PDPC-rich/cholesterol-poor (non-raft) domains. The results of these studies will be presented.

**521-Pos Board B291**
**Accurate Phase Separation of Complex Lipid Mixtures (DPPC/DOPC/CHOL) with a Refined Coarse Grained Martini Model**
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Ternary mixtures containing a high melting temperature lipid (such as dipalmitoyl-phosphatidylcholine, DPPC), a low melting temperature lipid (such as di-oleoyl-phosphatidylcholine, DOPC), and cholesterol (CHOL) form bilayers consisting of up to three different lipid phases. The lipid phases that can form are liquid-disordered (Ld), liquid-ordered (Lo), gel-like (Lb), or any combination of the three. The phase(s) present within these membranes are dependent on both temperature and the specific percentage composition of the three components within the membrane. These phases have been well mapped experimentally to construct detailed phase diagrams.
Previous efforts to use Molecular Dynamics (MD) simulations to reproduce the distribution of phases have proved somewhat challenging. This is either due to the size/timescale sampling restrictions of all-atom approaches, or some of the inherent limitations of Coarse Grain (CG) forcefields such as limited lipid parameters that were made to reproduce homogenous membranes, but can behave erroneously when in lipid mixtures.
In this work, the existing CG MARTINI DPPC and DOPC lipid parameters were iteratively optimized by fitting to extensive all-atom simulations run using the CHARMM36 forcefield. The parameters were tested not only to preserve structural and thermodynamics characteristics of homogenous lipid species, but also to properly reproduce experimental phase separation. Several iterations of the parameters were tested across the entire phase diagram, and at different temperatures. Our optimized parameters possess greatly improved fidelity to experimental phase properties. This approach can be applied to other, biologically relevant lipid mixtures.
This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344 and by Los Alamos National Laboratory under Contract DE-AC52-06NA25396. Release numbers: LLNL-ABS-739386.

**522-Pos Board B292**
**Lipid Domain Boundary as Universal Attractor**
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We show that boundary of liquid-ordered domains (rafts) works as the universal attractor for a wide variety of membrane minor components, such as various peptides and non-bilayer lipids. Using elasticity theory approach developed for lipid membranes, we show that these kinds of impurities tend to distribute to the narrow intermediate region at the liquid-ordered domain boundary. Moreover, redistribution of these components dramatically varies the morphology and size of liquid-ordered domains, which is achieved by changing the domain boundary energy. This effect was predicted theoretically and confirmed experimentally for the ganglioside GM1 [1].
Despite the low concentration, minor components play a crucial role in cell life: whether it amphipathic peptide, cancer-related ganglioside or fusion peptides sensible to raft boundary. Liquid-ordered lipid domains are assumed to be important actors in diverse cellular processes, mainly signal transduction and membrane trafficking. They are thicker than the disordered part of the membrane, thus compensating the hydrophobic mismatch between transmembrane proteins and the disordered lipid environment. That leads to the main cause of the boundary energy, a strained asymmetric structure of the boundary, which can be relaxed by the line-active components.
Therefore the found attractive activity of the domain boundary can explain the mechanisms and suggest the new pathways of the strong influence of the low concentration of membrane impurities on various physiological processes involving rafts. The work was supported by the Russian Science Foundation (project #17-79-20440)
1.Galimzyanov et al., Langmuir **33(14)** (2017).
2.Galimzyanov et al., Physical Review Letters **115.8** (2015).

**523-Pos Board B293**
**Interactions between Sterols and Phospholipids with Different Headgroups - Influence on Lateral Segregation**
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Cholesterol is an important modulator of the structure and function of mammalian cell membranes. The effect of cholesterol is mediated through the interactions between the sterol and the phospholipid components of the membrane. Recently, we have reported a correlation between the relative affinity cholesterol have for different phospholipid components and the degree to which the sterol can facilitate lateral domain formation. In the current project, the aim is to determine the affinity of cholesterol and other sterols for phospholipids with different headgroups, and to analyze how the measured affinities relate to the formation of lateral membrane structure. The affinity of the sterols for phospholipid bilayers is measured using different fluorescence based partitioning assays, and the formation of lateral domains in the bilayers is determined by measuring the fluorescence lifetimes of *trans*-parinaric acid as a function of lipids composition. The results from these experiments offer insights into the mechanisms driving the formation of lateral domain formation that will help us understand the processes that occurs in cellular membranes.

**524-Pos Board B294**
**Lipid Domains at the Plasma Membrane of Red Blood Cells: Organization and Involvement in Deformation**
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Erythrocytes are highly deformable cells that go through capillaries eight times-narrower than their diameter to deliver oxygen throughout the body. This deformability is linked to erythrocytes specific features such as their