

and the individual bilayer leaflet thicknesses. Recently, protocols enabling the preparation and characterization of stress-free, free-floating asymmetric vesicles (aLUVs) via a cyclodextrin mediated exchange were developed [1]. Here, we report of aLUVs studies with gel and fluid phase bilayer leaflets using small- and wide-angle x-ray scattering (SWAXS), and small-angle neutron scattering (SANS). Together, these techniques allow for obtaining the structural details of the two membrane leaflets. No hydrocarbon chain correlation peak was observed in the wide-angle regime of DPPC/POPC aLUVs at room temperature, consistent with an earlier observation of the partial fluidization of gel DPPC through coupling to the inner, fluid POPC leaflet [1]. On the other hand, POPE/POPC aLUVs, below the melting temperature of pure POPE, showed a WAXS-peak typical of hydrocarbons packed in a 2D hexagonal lattice. This coupling between the two bilayer leaflets manifested itself as through a small increase in area per lipid. However, our studies also showed that the underlying energetic contributions are insufficient to break up the hydrogen bond-stabilizing network formed by PE headgroups.

This work is supported by the Austrian Science Fund FWF, Project No.P27083-B20 (to G.P.).

[1] Heberle, F. A., Marquardt, D., Doktorova, M., Geier, B., Standaert, R. F., Heftberger, P., Kollmitzer, B., Nickels, J. D., Dick, R. A., Feigenson, G. W. et al. (2016). *Langmuir*, 32(20), 5195(5200).

1107-Pos Board B175

Mitochondrial Membrane Organization in Regulation of Apoptosis

Artur Dingeldein¹, Jörgen Aden¹, Tobias Sparrman¹, Radek Sachl², Sarka Pokorna², Martin Hof², Gerhard Gröbner¹.

¹Chemistry, Biophysical Chemistry, Umeå, Sweden, ²J. Heyrovský Institute of Physical Chemistry, Prague, Czech Republic.

Programmed cell death (apoptosis) is an essential mechanism in life. Key regulators of the intrinsic mitochondrial apoptotic pathway are pro- and anti-apoptotic members of the Bcl-2 family who meet at the mitochondrion's surface - as defined by its outer membrane system - where they arbitrate a life or death decision. Our main aim is to address this molecular regulation mechanism occurring at this mitochondrial outer membrane (MOM) level. For this purpose we use the anti-apoptotic Bcl-2 protein itself which is an integral membrane protein, and its counterpart, the pro-apoptotic Bax protein, to address specifically the importance of the MOM system in the recruitment of Bax. We could show that damaged mitochondrial outer membranes - generated upon oxidative stress - increase the affinity and therefore the translocation of Bax towards these membranes dramatically, and even promote partial penetration of full-length Bax [1]. This process was characterized by a combination of solid state NMR spectroscopy, differential scanning calorimetry and CD spectroscopy [1]. More recently, we found by applying fluorescence based leakage assays that Bax under these conditions is already able to induce MOM pore formation without additional mediator proteins (e.g. tBid); albeit creating a pore size not sufficient for cytochrome c release [2]. Surprisingly, the Bax-induced leakage increased with the amount of oxidized lipid present without any detectable threshold neither for oxidized phospholipids (OxPL) nor Bax. And the leakage rate correlated with i) Bax to lipid ratio and ii) the oxidized lipid content. This observation of an OxPLs induced significant increase in Bax membrane affinity, challenged the traditional view of Bax activation. We therefore propose a model, where Bax in a dynamic equilibrium shuttles between cytosol and the membrane. Upon apoptotic stress this equilibrium shifts towards the membrane-bound state; a prerequisite for Bax induced permeabilization of the MOM and the final execution of cell death through the release of apoptotic factors from the mitochondrial intermembrane space.

[1] M. Wallgren, M. Lidman, Q. Dat Pham, .. G. Gröbner. The oxidized phospholipid PazePC modulates interactions between Bax and mitochondrial membranes. *BBA Biomembranes* 1818 (2012) 2718 - 2724.

[2] M. Lidman, Š. Pokorná, A.P.G. Dingeldein, T. Sparrman, .. G. Gröbner. The oxidized phospholipid PazePC promotes the formation of Bax pores in mitochondrial membranes. *BBA Biomembranes* 1858 (2016) 1288 - 1297.

1108-Pos Board B176

Neutron Scattering to Study Membrane Systems: From Model Membranes to Living Cells

Jonathan D. Nickels, Sneha Chatterjee, Christopher Stanley, Shuo Qian, Xiaolin Cheng, Dean A.A. Myles, Robert Standaert, James Elkins, John Katsaras.

Oak Ridge National Lab/Univ. of Tennessee, Knoxville, Oak Ridge, TN, USA.

The existence and role of lateral lipid organization in biological membranes has been studied and contested for more than 30 years. Lipid domains, or

rafts, are hypothesized as scalable compartments in biological membranes, providing appropriate physical environments to their resident membrane proteins. This implies that lateral lipid organization is associated with a range of biological functions, such as protein co-localization, membrane trafficking, and cell signaling, to name just a few. Neutron scattering techniques have proven to be an excellent tool to investigate these structural features in model lipids, and more recently, in living cells. I will discuss our recent work using neutrons to probe the structure and mechanical properties in model lipid systems and our current efforts in using neutrons to probe the structure and organization of the bilayer in a living cell. These efforts in living cells have used genetic and biochemical strategies to generate a large neutron scattering contrast, making the membrane visible. I will present our results showing *in vivo* bilayer structure and discuss the outlook for this approach.

1109-Pos Board B177

Investigation of Domains in Mixtures of High-Melting Phospholipids, POPC, and Cholesterol

Matthew H. Raymonda, Paulo Almeida, Antje Pokorny.

UNC Wilmington, Wilmington, NC, USA.

We used fluorescent resonance energy transfer (FRET) measurements of large unilamellar vesicles to investigate domain formation in lipid mixtures composed of 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) and equimolar portions of cholesterol (Chol) and an ordered, high-melting phospholipid: N-palmitoyl-sphingomyelin (SM), porcine brain sphingomyelin (BSM) or 1,2-dipalmitoyl-phosphatidylcholine (DPPC). Mixtures with different concentrations of POPC were examined as a function of temperature. The FRET results are interpreted to indicate that at 25°C ordered and disordered lipid domains exist. As the temperature is increased to 40°C the area of domains of liquid ordered phase appears to decrease. When the temperature increases to 60°C the liquid disordered phase is the primary phase of the entire vesicle. All three mixtures show the same trends in domain size between the liquid disordered and liquid ordered phases. These FRET measurements are used for comparison when performing Monte Carlo simulations of these mixtures. This work has been supported by NSF grant CHE-1464769.

1110-Pos Board B178

Lipid Phase Heterogeneity and Size Dependence in Quarternary Lipid Bilayer System: A Coarse-Grained Molecular Dynamics Study

Shushan He, Lutz Maibaum.

Department of Chemistry, University of Washington, Seattle, WA, USA.

The spatial organization of phospholipids and sterols in cellular membranes is believed to play an important role in membrane function and cell activities. Both recent experimental and simulation studies have suggested various possible heterogeneous spatial distributions of lipids and cholesterol, including the formation of nanoscale lipid domains, in multi-component bilayer systems. In this study, we use coarse-grained Molecular Dynamics simulations to study the phase behavior of quarternary lipid mixtures as a function of membrane composition. We focus on quantifying the phase composition of heterogeneous membranes using model facilitated statistical approaches, as well as identifying the nature of lipid heterogeneity by measuring signatures in observables such as partial density correlation functions, as well as local lipid density distributions and their dependence on system size.

1111-Pos Board B179

Quantifying Protein and Lipid Accumulation at Sites of Membrane Curvature

Michelle K. Knowles, Aml Alnaas, Carrie Moon, Alan Weisgerber.

Chemistry and Biochemistry, University of Denver, Denver, CO, USA.

Protein and lipid organization is tightly regulated on the cellular plasma membrane. This process is driven by intermolecular interactions and leads to nanoscale domain formation where specific biochemical reactions can occur. One feature of membranes that plays a less obvious role is the shape of the lipid membrane. This aspect is difficult to measure in living cells, making biochemical assays a useful tool for quantifying curvature dependent sorting. In our work, we use nanoscale patterning to create substrates that introduce curvature into supported lipid bilayers with the goal of separately assessing the role of shape and chemical composition on the organization of proteins and lipids. To create substrates, nanoparticles are deposited onto glass surfaces prior to lipid bilayer formation. Confocal and total internal reflection fluorescence microscopies, combined with colocalization, single molecule imaging and tracking methods, are used to assess protein and lipid accumulation at regions