Wednesday Morning, October 17, 2007

Understanding Biointerphases and Magnetism with Neutrons Topical Conference

Room: 618 - Session NT+BI-WeM

Phospholipid Bilayers and Membranes

Moderator: M. Tanaka, University of Heidelberg

8:00am **NT+BI-WeM1 Tethered Bilayer Lipid Membranes in Biomedical Research: Lessons from Neutron Scattering**, ***M. Lösche***, *F. Heinrich*, Carnegie Mellon University, *D.J. McGillivray*, The Australian National University, *G. Valincius*, Institute of Biochemistry, Vilnius, Lithuania, *Y. Sokolov, J.E. Hall*, UC Irvine **INVITED**

Tethered bilayer lipid membranes (tBLMs) on solid supports hold potential to mimic biological membranes. Molecular-scale studies of the interactions of peptides and proteins with membranes provide ample opportunities in biophysical and biomedical research. Membrane stabilization by the proximity of a solid substrate provides resilience to the system, but has often at the same time introduced severe problems. A prerequisite, for example, for tBLM characterization by scattering and electrochemical techniques is a low defect density of the membrane. Only then is it possible to quantify minor structural and functional changes induced by, e.g., protein interaction with the membrane. We have optimized a membrane architecture on molecularly flat gold surfaces which meets all these challenges. Different lengths of the hydrophilic poly(ethylene glycol) (PEG) spacer that controls the structure of the inner monolayer leaflet provide highly hydrated sub-membrane spaces between 20 Å and 60 Å in thickness, as determined by neutron reflection. Such tBLMs may be composed of charged or zwitterionic lipids with various chain saturation, and can include cholesterol. The membranes are highly insulating and are routinely probed with electrochemical impedance spectroscopy (EIS). As an example for ongoing biomedical research we will discuss the interaction of soluble prefibrillar β-amyloid oligomers with tBLMs and compare the impact of the peptide on such membranes with that of a pore forming bacterial exotoxin, Staphylococcus aureus α-hemolysin.

8:40am **NT+BI-WeM3 Study of Fluctuation and Destabilization of Single Phospholipidic Bilayer by Neutron and X-ray Scattering**, ***T. Charitat***, CNRS-Université Louis Pasteur, France, *S. Lecuyer*, Harvard University **INVITED**

Supported bilayer are interesting model systems for biologist and present also fascinating physical properties. We investigate experimentally these dynamical properties on floating bilayer. First, the equilibrium structures of single and double bilayers are studied by neutron reflectivity. The submicronic fluctuation spectrum of a floating bilayer is determined by off-specular X-ray scattering: surface tension, bending modulus and, for the first time with this technique, inter-membrane potential. Using fluorescence microscopy, we show that this single bilayer can be completely destabilized leading to well control vesicles formation. Destabilization can occur either at the main gel-fluid transition of the lipids, and can be interpreted in terms of a drop of bending rigidity, or under an AC low-frequency electric field applied in the fluid phase. In that last case we also study the effect of the electric field at the molecular length scale by neutron reflectivity. In both cases, the destabilization leads to the formation of relatively monodisperse vesicles, which could give a better understanding of the formation mechanism.

9:20am **NT+BI-WeM5 Protein-induced Pores in Membranes Detected and Studied by Neutron Scattering**, ***H.W. Huang***, Rice University **INVITED**

Gene encoded antimicrobial peptides kill bacteria by forming pores in the bacterial membranes. Apoptotic protein Bax forms pores in the outer mitochondrial membrane to release the apoptosis-inducing factor cytochrome c from mitochondria. The evidence of pore formation in membranes is usually ion conduction or leakage. The structure of a pore in a fluid membrane is difficult to detect or measure by conventional methods such as electron microscopy. Neutron scattering is uniquely suited for such structural studies. We will show neutron scattering from membrane pores made by antimicrobial peptides, alamethicin, magainin, protegrin as well as by bee venom toxin melittin. Surprisingly, these peptides form two different kinds of transmembrane pores first detected by neutron methods.

10:40am **NT+BI-WeM9 Using Neutron Spectroscopy to Study Collective Dynamics of Biological and Model Membrane Systems**, ***M.C. Rheinstadter***, University of Missouri-Columbia **INVITED**

The spectrum of fluctuations in biomimetic and biological membranes covers a large range of time and length scales, ranging from the long wavelength undulation and bending modes of the bilayer with typical relaxation times of nanoseconds and lateral length scales of several hundred lipid molecules, down to the short-wavelength, picosecond density fluctuations involving neighboring lipid molecules. New developments and improvements in neutron scattering instruments, sample preparation and environments and, eventually, the more and more powerful neutron sources open up the possibility to study collective excitations, i.e. phonons, in artificial and biological membranes. The goal of this project is to seek relationships between collective dynamics on various length scales on the one hand, and macroscopic phenomena such as trans-membrane transport, pore opening, and membrane fusion on the other hand. The combination of various inelastic neutron scattering techniques enlarges the window of accessible momentum and energy transfers - or better: accessible length and time scales - and allows one to study structure and dynamics on length scales ranging from the nearest-neighbor distances of lipid molecules to length scales of more than 100 nm, covering time scales from about 0.1 ps to almost 1 μs. The fluctuations are quantified by measuring the corresponding dispersion relations, i.e. the wave vector-dependence of the excitation frequencies or relaxation rates. Because biological materials lack an overall crystal structure, in order to fully characterize the fluctuations and to compare experimental results with membrane theories, the measurement must cover a very large range of length and time scales. By using multiple instruments, from spin-echo to triple-axis spectrometers, we have successfully probed these fluctuations over the desired range of length and time scales.[1-5]

[1]M.C. Rheinstädter, C. Ollinger, G. Fragneto, F. Demmel and T. Salditt, Phys. Rev. Lett. 93, 108107, 1-4 (2004).

[2]Maikel C. Rheinstädter, Wolfgang Häußler and Tim Salditt, Phys. Rev. Lett. 97, 048103, 1-4 (2006).

[3]Maikel C. Rheinstädter, Tilo Seydel, Franz Demmel and Tim Salditt, Phys. Rev. E 71, 061908, 1-8 (2005).

[4]Maikel C. Rheinstädter, Tilo Seydel and Tim Salditt, Phys. Rev. E 75, 011907, 1-5 (2007)

[5]Maikel C. Rheinstädter, Tilo Seydel, Wolfgang Häußler and Tim Salditt, J. Vac. Soc. Technol. A 24, 1191-1196 (2006).

11:20am **NT+BI-WeM11 The Coupling between Hydration-Water and Protein Dynamics as Studied by Neutron Scattering**, ***M. Weik***, IBS, CEA-CNRS-UJF, France **INVITED**

The dynamics of proteins is influenced by motions of water molecules at the protein-solvent interphase. However, details about the dynamical coupling remain to be elucidated. Neutron scattering is particularly well-adapted to study macromolecular motions on the ns-ps time scale and their coupling to hydration-water dynamics. Indeed, elastic incoherent neutron scattering is sensitive to hydrogen/deuterium isotope labelling with the scattering cross-section of hydrogen being about 40 times larger than that of deuterium. Consequently, studying a completely deuterated protein hydrated in H_2O gives access to the dynamics of hydration water. Conversely, an identically prepared sample of hydrogenated protein hydrated in D_2O yields information on protein dynamics only, thus enabling a direct comparison between hydration water and protein motions. We studied the coupling between hydration-water and protein dynamics in a biological membrane (purple membrane (PM)) and a soluble, globular protein (maltose binding protein (MBP)) by measuring mean square displacements of hydrogen atoms in the temperature range from 20 to 300 K. Hydration-water in both PM and MBP undergoes a dynamical transition at 200 K, evidenced as a break in atomic mean square displacements as a function of temperature (Wood, Frölich, Plazanet, Kessler, Moulin, Härtlein, Gabel, Oesterhelt, Zaccai & Weik, unpublished results). In the case of PM, this dynamical transition corresponds to the onset of long-range translational diffusion of water molecules as evidenced by neutron diffraction.[1] When atomic mean square displacements of hydration-water molecules become as large as those of protein atoms, a dynamical transition appears at 250 K in PM and at 230 K in MBP. Our results shed new light on the coupling between hydration-water and protein motions and suggest that they are coupled at room temperature, yet decoupled at cryo-temperatures.

[1]Weik, M., Lehnert, U. and Zaccai, G. (2005) Liquid-like water confined in stacks of biological membranes at 200 k and its relation to protein dynamics. Biophys J., 89, 3639-3646.

Authors Index

Bold page numbers indicate the presenter