Tuesday Morning, October 16, 2007

Understanding Biointerphases and Magnetism with Neutrons Topical Conference

Room: 618 - Session NT-TuM

Applications to Biological Materials and Soft Matter

Moderator: M. Lösche, Carnegie Mellon University

8:00am **NT-TuM1 Cold and Thermal Neutrons: Suitable Probes for Biomaterials**, ***J. Katsaras***, National Research Council of Canada **INVITED**

Neutrons are electrically neutral, subatomic elementary particles whose existence had been postulated by Ernest Rutherford and discovered by James Chadwick in 1932. With the exception of hydrogen, neutrons are found in all atomic nuclei, and free neutrons are unstable with a mean lifetime of ~ 900 s. Thermal and cold neutrons, used for the study of materials ranging from engineering to biomedical applications, are typically produced by reactor- or accelerator-based sources and are the result of interacting with a given temperature moderator (e.g., graphite, water, liquid deuterium). Compared to thermal neutrons, cold neutrons possess 5 - 20 times longer wavelengths and are preferred for the study of biological materials with inherently large unit cells. Unlike x-rays, neutrons interact strongly with nuclei and the strength of their interactions varies dramatically and non-monotonically from element-to-element across the periodic table. This statement applies equally to isotopes of the same element and has been used to great advantage by biologists and polymer scientists who typically study materials rich in hydrogen. The classic example is the isotopic substitution of ^{1}H for ^{2}H (deuterium), where one can accentuate, or nullify, the scattering from particular parts of a macromolecular complex by selective deuteration. Recently, we have used a number of neutron scattering techniques to study for example, the location of cholesterol in polyunsaturated fatty acid membranes (discovered that cholesterol can reside in unexpected places within the membrane!) and the distribution of water in aligned, self-assembled lipopolysaccharide bilayers isolated from the Gram-negative bacterium, Pseudomonas aeruginosa. These results provide insights as to how molecules penetrate and assemble in biologically relevant membranes, and will be the topic of discussion. Web Site: http://neutron.nrc-cnrc.gc.ca/people/katsaras/index.html.

8:40am **NT-TuM3 Small Angle Neutron Scattering Studies of the Counterion Effects on the Molecular Conformation and Structure of Charged G4 PAMAM Dendrimers in Aqueous Solutions**, ***W.-R. Chen***, Oak Ridge National Laboratory **INVITED**

The structural properties of generation 4 (G4) poly(amidoamine) starburst dendrimers (PAMAM) with an ethylenediamine (EDA) central core in solutions have been studied by small angle neutron scattering (SANS). Upon the addition of , SANS patterns show pronounced inter-particle correlation peaks due to the strong repulsion introduced by the protonation of the amino groups of the dendrimers. By solving the Ornstein-Zernike integral equation (OZ) with hypernetted chain closure (HNC), the dendrimer-dendrimer structure factor is determined and used to fit the experimental data, where is the magnitude of the scattering wave vector Q. Quantitative information such as the effective charge per dendrimer and the radius of gyration, R_G, at different pD values is obtained. The results show that only changes by about 4 % when the pD value varies from 10.25 to 4.97, and significant counterion association/condensation occurs, strongly mediating the inter-dendrimer interaction. The influence of interplay between counterions and molecular protonation of dendrimers imposes a strong effect on the dendrimer conformation and effective interaction. Although the change of is very small, careful analyses of the high data and fitting parameters indicate a possible internal structure change of a dendrimer when the amino groups are progressively charged.

9:20am **NT-TuM5 Neutron Reflection Study of Peptides and Proteins at Interfaces**, ***J.R. Lu***, *X.B. Zhao, F. Pan, M. Yaseen*, University of Manchester, UK **INVITED**

Neutron reflection has been deployed to study the adsorption of short designed peptides and proteins at the solid/water interface, focusing on the determination of the structure of the interfacial layer. The effects of surface hydrophobicity, the size and stability of globular proteins and the solution environmental conditions on the structure and composition of the adsorbed protein layers are examined and key features highlighted. The advantegeous features of neutron reflection are described.

10:40am **NT-TuM9 Neutron Reflectivity and Scattering for Studying Biomolecules at Interfaces**, ***T. Nylander***, Lund University, Sweden **INVITED**

Neutrons are relatively more harmless to matter than X-rays and therefore they are particularly usefull to study biological material. Hydrogen scatters neutron very well and further more the scattering from hydrogen and deuterium is very different. Selective deuteration of one component and contrast matching of the aqueous solvent makes it possible to study one component at a time as the other can be made invisible. The potential of neutron scattering/reflectivity measurements will be discussed for two systems, lipid self-assembly and DNA compaction. There is an increasing demand for methods to study processes at the lipid-aqueous interface as the importance of lipids and lipid self-assembly structures as regulators for biological activity as well as for vehicles for drug delivery. Spreading of vesicles on surfaces are quite an established method to forming bilayers on hydrophilic surface, and spreading can be affected by the surface properties as well as properties that affect the stability of the vesicles. The full potential of non-lamellar liquid crystalline nano particles (LCPN) has only recently been realized, as improved methods and new lipid combinations have been introduced for producing biocompatible and structurally well-defined nano-particle dispersions of different phase structures. These include bicontinuous cubic, reverse hexagonal, and reverse cubic phases. Directly relevant for drug delivery systems is the fusion of these LCPN with a (model) membrane. Again numerous studies has been conducted on vesicle fusion, but very few on the interaction between non-lamellar LCNP and membranes. The compositional changes in the lipid bilayer will be monitored by neutron reflectivity using selective deuteration of the lipids and proper contrast matching. This has given us information on structural changes when the LCNP interacts with a surface and a bilayer. Our results show that interaction between the LCPN lipid components with the acyl chains of the bilayer, consistent with the formation of a mixed bilayer. As we observed a diffraction peak at high q after a certain time, we concluded that the interaction eventually lead to formation of multilayers on the surface. We are today fascinated of the supra-molecular structures formed by DNA packing in the cell nucleus and we are trying to understand to what extent packing regulates transcription/translation. The long-term objective of our work is to design a module for DNA packaging, where the packing and thereby the transcription can be switched on and off. This module would be a transcription competent synthetic analogue of a real cell nucleus. We are therefore study the relation between structure of the aggregate (using dynamic light scattering, small angle x-ray and neutron scattering, cryo-TEM), the degree of compaction (using fluorescence) and transcription/translation (using biochemical assays, including RNAse and luciferase production). It has been shown that DNA can be compacted by cationic lipids both in bulk and at interfaces. By using neutron scattering of DNA coated particles we have shown that the aggregate formed by CTAB when interacting with DNA is elongated and of the same structure on the surface as on the bulk.

11:20am **NT-TuM11 Roles of Carbohydrates in Fine Adjustment of Biological Interfaces**, ***M. Tanaka***, University of Heidelberg, Germany **INVITED**

In nature, cell-cell and cell-tissue contacts are madiated by various biopolymers based on carbohydrates. This paper describes some of our recent studies that physically model the active roles of carbohydrates in fine-adjustment of contacts at biological interfaces by specular and off-specular neutron scattering at defined relative humidity as well as in bulk water. The planar planar geometry of the biomembrane models containing oligo- and polysaccharides enable one to identify the in-plane and out-of-plane momentum transfers at various angles quantitatively, which can be used to determine the influence of carbohydrates on structural and mechanical properties of membranes.

Understanding Biointerphases and Magnetism with Neutrons Topical Conference

Room: 618 - Session NT-TuA

Magnetism

Moderator: M. Grunze, University of Heidelberg, Germany

2:00pm **NT-TuA2 Introduction to Magnetic Neutron Scattering**, ***S.E. Nagler***, Oak Ridge National Laboratory **INVITED**

Neutron scattering is arguably the most powerful experimental technique available for characterizing magnetic structures and excitations. This talk will provide a brief introduction to neutron scattering, with emphasis on its application to problems in magnetism. The talk is intended for scientifically literate non-specialists.

2:40pm **NT-TuA4 Polarized Neutron Reflectometry and Diffraction on Magnetic Thin Film Structures**, ***F.R. Klose***, Oak Ridge National Laboratory **INVITED**

In this presentation, I will review applications of polarized neutron reflectivity and diffraction in regard to magnetic thin film research. Polarized reflectivity is an ideal tool for investigating vector magnetization profiles in thin film systems with a vertical depth-resolution of a few monolayers. The method has been used for many years, for example, to demonstrate oscillatory exchange coupling in magnetic multilayers, the effect which is causing giant magneto-resistance. Recently developed polarized "off-specular / diffuse" scattering methods also allow investigations of lateral (in-plane) magnetic correlations on length-scales between 1 nm and 100 μm. High-angle magnetic neutron diffraction is an extremely powerful technique for investigating atomic-scale antiferromagnetism in thin films. The latter is very important in regard to the exchange bias effect which is used in magnetic storage technology. Recent research results on Fe-Pt based films for magnetic recording applications will be presented.

4:00pm **NT-TuA8 Opportunities for Neutron Scattering in Thin Magnetic Films for Sensor Technology.**, ***M.L. Plumer***, Memorial University of Newfoundland, Canada **INVITED**

With the continued demand for ever smaller and faster magnetic sensors based on thin-film technology the requirements for deeper understanding of the relevant processes involved continue to grow. Simple modeling methods based on Maxwell's equations, and simple experimental techniques that measure only bulk magnetic properties, served the industry well for the latter part of the twentieth century but are no longer adequate research tools for the engineering of the nanometer magnetic devices of today and tomorrow. This talk will review some of the detailed knowledge of both static and time-dependent behavior of interacting magnetic grains within films and multilayers that can be gained through micromagnetic simulations based on the Landau-Lifshitz-Gilbert (LLG) equations. Opportunities for the use of a variety neutron scattering techniques to measure such detailed equilibrium and dynamic properties will be discussed.

4:40pm **NT-TuA10 Nanostructures and Ordering Phenomena in Magnetic Colloids Probed by Small Angle Neutron Scattering**, ***A. Wiedenmann***, Hahn-Meitner-Institut Berlin, Germany **INVITED**

Small Angle Neutron Scattering (SANS) allows fluctuations of density, composition and magnetization to be analysed simultaneously on a nanometers length scale. This non-destructive technique was used to characterise magnetic colloids which are of growing interest for advanced medical applications. Such "Ferrofluids" consist of nanosized magnetic particles coated by nonmagnetic organic surfactants and dispersed in carrier liquids. Isotope contrast variation combined with the newly developed technique of polarised neutrons ("SANSPOL") allowed size distributions, compositions and magnetic moments of magnetic core-shell particles and magnetic aggregates to be evaluated precisely beside non-magnetic micelles of similar sizes.[1] In concentrated Ferrofluids an unconventional pseudo-crystalline ordering has been monitored by SANS resulting from strong field-induced inter-particle correlations.[2-3] New stroboscopic techniques have been developed which allowed the dynamics of ordering and realxation to be studied in a time range similar to that of X-ray photon-correlation spectroscopy.[4]

[1]Wiedenmann, A., Kammel, M., Heinemann, A., Keiderling, U. J. Phys. : Condensed Matter: 18 (2006) S2713-2736

[2]Wiedenmann, A., Hoell, A., Kammel, M. , Boesecke, P. Phys Rev. E 68 (2003) 031203

[3]Klokkenburg, M., Erné, B. H., Meeldijk, J.D., Wiedenmann, A., Pethukov, A.V., Philipse, A.P, Physical Revue Letters 97, 185702 (2006)

[4]Wiedenmann, A., Keiderling, U., Habicht, K., Russina, M., Gähler, R., Physical Review Letters 97, 057202 (2006)

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