International Workshop: Synchrotron and Neutron Scattering in Biomaterials and Soft Matter

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Book of Abstracts

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Hydration-induced phase transitions in surfactant and lipid films

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For several surfactant and lipid systems it is crucial to understand how hydration influences the physical and chemical properties. When humidity changes it affects the hydration degree by adding or removing water molecules. In many cases this process induces transitions between liquid crystalline phases. This phenomenon is of general interest for numerous applications simply due to that humidity variations are ubiquitous. Of particular interest are hydration-induced phase transitions in amphiphilic films, which in many cases appear as the frontier towards a vapor phase with changing humidity. Considering this, it is important to characterize the film thickness needed for formation of 3D liquid crystalline phases and the lyotropic phase behavior of this kind of films. In this work we study this issue by employing a recently developed method based on humidity scanning quartz crystal microbalance with dissipation monitoring (HS QCM-D) [1], which enables continuous scanning of the film hydration [2]. We investigate five surfactants films (DDAO, DTAC, CTAC, SDS, n-octylβ-D-glucoside) and one lipid film (monoolein) and show that HS QCM-D enables fast characterization of hydration-induced phase transitions with small samples. Film thicknesses range from tens to hundreds of nanometers and clear phase transitions are observed in all cases. It is shown that phase transitions in films occur at the same water activities as for corresponding bulk samples. This allows us to conclude that surfactant and lipid films, with thickness as low as 50 nm, are in fact assembled as 3D-structured liquid crystalline phases. Further, liquid crystalline phases of surfactant films show liquid-like behavior, which decreases the accuracy of the absorbed water mass measurement. On the other hand, the lipid monoolein forms more rigid liquid crystalline films allowing for accurate determination of the water sorption isotherm, which is also true for the sorption isotherms corresponding to the solid surfactant phases.

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Synergy of Flourescence Microscopy and Neutron Reflectivity in SLB studies

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Neutron reflectivity (NR) has proven a powerful tool to understand the nano-structure of supported lipid bilayers (SLBs) in the Z-dimension. Orthogonally, fluorescence microscopy (FM) has proven a powerful tool for investigating the structure of SLBs in the X- and Y-dimensions. It is thus interesting to note how little literature exists in which the two techniques are combined to characterize an SLB system. This is potentially due to the two instrumentation typically using different substrate materials. In this study we aim to bridge this gap and demonstrate how FM can be effectively interfaced with NR substrates. FM characterization of SLBs formed on common NR substrates (i.e., Silicon and quartz) which have been cleaned by a variety of methods will be presented and compared to the most commonly used FM substrate, borosilicate coverslips. Basic studies, such as these, allow the optimization of SLB quality for systems formed on NR substrates and aids our understanding of SLB formation from more complex vesicle compositions. Additionally, we hope to demonstrate the importance of utilizing FM as an orthogonal technique when designing NR experiments of SLB-based systems.

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Lipoprotein structure dependency on its lipid cargo and exchange dynamics

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Atherosclerosis is the leading cause of death in western society, its consequences of cardiovascular diseases such as strokes and heart attacks arise from the build-up of plaque in the blood from lipoprotein deposition onto cell membranes in the artery walls. Essential information regarding molecular mechanisms resulting in plaque build-up, is missing. Lipoprotein particles have long been used as biological markers to indicate the progression to atherosclerosis. Understanding these processes and determining the lipoprotein particle composition is fundamental in the diagnosis and treatment of the disease.
Small angle neutron scattering with selective deuteration studies will provide crucial information about lipoprotein structure and its dependence on the lipid cargo as well as information about lipid exchange kinetics between different lipoprotein forms and the cell membrane.

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FUNCTIONAL AND STRUCTURAL STUDIES OF CHOLESTEROL-DEPENDENT CYTOLYSINS

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FUNCTIONAL AND STRUCTURAL STUDIES OF CHOLESTEROL-DEPENDENT CYTOLYSINS

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ABSTRACT

Cholesterol-dependent cytolysins (CDCs) are a large family of β-barrel pore-forming toxins that are produced by Gram-positive bacteria. Pore formation is strictly dependent on the presence of membrane cholesterol, which functions as the receptor for most CDCs. The pore-forming mechanism of the CDCs is a multistep process that involves recognition and binding to the cholesterol-containing membrane, oligomerization of the soluble monomers on the target cell membrane to form a large complex and penetration into the membrane to become a transmembrane pore. We demonstrate the use of tethered bilayers (tBLMs) as a platform for functional and structural studies of membrane associated proteins by electrochemical and surface sensitive techniques. The reconstitution of the cholesterol-dependent cytolysins (CDC) as pneumolysin (PLY) from Streptococcus pneumoniae, vaginolysin (VLY) from Gardnerella vaginalis and pyolysin (PLO) from Trueperella pyogenes into tBLMs was followed in real-time by electrochemical impedance spectroscopy (EIS) and surface plasmon resonance (SPR). Changes of the EIS parameters of the tBLMs upon exposure to PLY, VLY and PLO monomer solutions were consistent with the dielectric barrier damage occurring through the formation of water-filled defects in bilayers. Complementary atomic force microscopy (AFM) and neutron reflectometry (NR) measurements revealed structural details of the membrane bound CDCs. In conclusion, tBLMs are a reliable and complementary method to explore the activity of CDCs in eukaryotic cells and to develop strategies to limit the toxic effects of CDCs.

KEYWORDS: cholesterol-dependent cytolysins, tethered bilayer membranes, electrochemical impedance spectroscopy, atomic force microscopy, surface plasmon resonance, neutron reflectometry

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Self-association of a highly charged, arginine-rich cell-penetrating peptide

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Small angle X-ray scattering (SAXS) measurements reveal a striking difference in intermolecular interactions between deca-arginine (R10) and deca-lysine (K10), two short, highly charged peptides. Comparison of SAXS curves at high and low salt concentration shows that R10 self-associates, while interactions between K10 chains are purely repulsive. The propensity of R10 to self-associate is heightened at low-to-intermediate ionic strengths indicating that the attraction between R10

molecules has an important electrostatic component. SAXS data is complemented by potentials of mean force between the peptides, calculated by means of umbrella sampling molecular dynamics (MD) simulations. Our combined SAXS and MD simulation study indicates that the solution behavior of R10 is governed by the interplay of two counteracting electrostatic interactions: the double layer force, and a short-range attraction between oppositely charged groups in the C-terminal residues. Self-association may enhance the bioavailability of R10, and our findings provide a possible explanation for the presence of an upper limit on the optimal number of arginine residues, observed for efficient cell membrane translocation.

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Neutron studies of new drug leads for the inhibition of cancer-related human carbonic anhydrase IX

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Human carbonic anhydrase IX (HCA IX) expression in aggressive tumors, is an indicator of metastasis and poor cancer patient prognosis. As such, HCA IX has emerged as an important cancer imaging, diagnostic, and therapeutic target. Efforts to develop specific inhibitors for HCA IX are troublesome due to the presence of 14 other HCA isoforms. It has been well established, that ligand (inhibitor) binding to a target protein is mediated through interactions that may include: H-bonding directly and/or through intervening waters, electrostatic interactions with charged or polar amino acid side chains, metal coordination, energetic changes through water displacement, aromatic stacking, or other hydrophobic interactions. Our goal is to apply a powerful combination of X-ray and neutron protein crystallography to the HCA IX system in order to observe the details of ligand binding that involves H atoms. Comparing neutron crystal structures of unbound and drug-complexes of HCA IX with saccharin (a recently identified lead compound, that demonstrates some HCA IX specificity), provides a unique opportunity to directly investigate how saccharin binds through H-bonding, the role of water displacement, and how the making/breaking of H-bonds modulate binding and provides isoform specificity. On the basis of two solved X-ray and neutron derived structures each, we can show the changes in the H-bonding network due to saccharin binding. It is expected this fine structural detail, that is unique to neutron crystallography, can then be used for rational drug design.

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Water-dispersible core-shell nanoparticles with tunable liquid crystalline internal structures studied by SAXS

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We have developed a procedure to reproducibly prepare water-dispersable core-shell nanoparticles that display a variety of liquid crystalline cores. The particles are made from symmetric or assymetric poly(acrylic acid-b-acrylamide) block copolymers, where the acrylic acid block is neutralized by an cationic alkyltrymethylammonium surfactant by a simple titration. Synchrotron small angle X-ray scattering (SAXS) has been used to identify the internal liquid crystalline structures, by the indexation of the Bragg peaks of each phase structure. The initially prepared particles display cubic or hexagonal core structures which agree with structures found in the aqueous surfactants used to produce them. Moreover, the core structure can be modified by mixing in long chain n-alcohols, either before dispersing the mixture in water, or by letting the alcohol diffuse into the core through water. Hence, the core strucutures can be tuned in order to produce additional lamellar, reverse hexagonal and reverse micellar internally-structured particles. By our procedure, we obtain nanoparticles displaying the reproducible colloidal and structural properties. The nanoparticle dispersions are kinetically stable for several months, as evidenced by measurements of hydrodynamic radii and zeta potentials with time. We have also performed time-dependent synchrotron SAXS measurements to monitor the structural transitions caused by the n-alcohol incorporation in the structured cores. We have studied the kinetics of the structural transitions as they evolve in a mixed aqueous dispersion, where initially alcohol-free nanoparticles are mixed with saturated aqueous solutions of the long-chain alcohols. The time period observed for transition from one phase to another phase, as well as the variation in the cell parameters of each phase, were investigated with the refinement of the SAXS data.

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Drug delivery applications of self-assembling lipid formulations

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Encapsulation of drugs into lipid-based liquid crystalline (LC) phases offers a broadly applicable approach for the in vivo stabilization and sustained release delivery of peptides and proteins as well as small molecule drug substances. This is exemplified by Camurus' FluidCrystal® Injection depot, an adaptive drug delivery system, combining ease of manufacturing, handling, and injection, with long acting release. The system exploits specific liquid mixtures of naturally occurring polar lipids and small amounts of solvents, which upon contact with minute quantities of aqueous tissue fluids self-assemble into reversed LC phases. The reversed hexagonal and micellar cubic phases and their mixtures are among most promising LC structures for drug delivery applications due to the anticipated good loading and encapsulation ability in the coexisting lipophilic and discrete aqueous nanodomains. The ability of these phases to resist phase changes on exposure to excess aqueous media is a further important property allowing their use as time-persistent reservoirs in drug delivery applications. Upon water uptake, for instance by absorption of interstitial water at the site of injection, the in vivo functional LC sustained release matrix is spontaneously generated; physically materialized as a stiff monolith with the active drug evenly enclosed. The resulting encapsulation of dissolved or dispersed active pharmaceutical ingredients provides a release duration from a small volume injection, which is tunable from days to months from a single injection.

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Anisotropic dynamics of magneto-responsive anisotropic colloids investigated with XPCS

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The transition for hard thin rods is an isotropic to nematic transition where the particles have orientational order but no positional order. With more elaborate anisotropic particle, in both shape and interaction, a multitude of different transitions and phases can be found. To be able to manipulate the colloidal particles such that a desired structure and/or phase behavior can be obtained is not only of fundamental scientific interest but also highly asked from practical applications. One way to do so is by subject the system to an external field. Many of the phases formed by anisotropic particles are by them self anisotropic. This can be characterized by scattering or microscopic techniques. It is not only the anisotropic structural and orientational aspects of these systems that are of fundamental interest but so are also the relaxation processes (the dynamics).
Anisotropic dynamics has been observed in ordered colloidal phases (crystalline or liquid crystalline) and in suspensions where the particles interact with an external field, e.g., gravity or shear. Controllable external fields can be used to "tune" the single particle properties as well as the inter-particle interactions. The anisotropic dynamic function, i.e. the wave vector (q) dependent diffusion D(q) at different orientations, of charged hematite prolate particles under the influence of magnetic field has been investigated with XPCS. Different aspect ratios, concentrations and magnetic field strengths have been investigated in order to probe different phase states. Together with the anisotropic D(q) we will present the anisotropic form, P(q), and structure, S(q), factor to link the dynamic response to the structural and orientational state of the system.

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NANO-SCALE INFRARED IMAGING OF B-SHEET STRUCTURES IN SYNAPTIC JUNCTIONS OF CULTURED NEURONS ISOLATED FROM TRANSGENIC MICE, MODELS OF ALZHEIMER´S DISEASE

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INTRODUCTION:
Amyloid β is a class of aggregation-prone proteins, which may misfold into stable, β-sheet reach fibrils. Amyloid β is linked to the development of synaptic pathology in Alzheimer's disease (AD)1. However, a main question in the AD field is how amyloid β contributes to AD neuropathology? Up to now there is little evidence for β-sheet formation at the sub-cellular level of a neuron Our aim was to study β-sheet structures in synaptic junctions of AD transgenic neurons. We aim to understand

the mechanism by which amyloid β is involved in synaptic pathology in AD.
MATERIAL & METHODS:
To study β-sheet structures at sub-cellular level in AD neurons (APP/PS1) we used a new technique which combines scattering-scanning near-field optical microscopy and mid-infrared synchrotron radiation (IR s-SNOM). Scanning in nanometer proximity to the sample with the tip of an atomic force microscope (AFM) this new approach enables molecular vibrational spectroscopic imaging with nano-scale spatial resolution (~ 40 nm) in the full mid-infrared (1000-5000 cm-1) region. Moreover, synchrotron infrared spectroscopy imaging is a direct method to target specifically β-sheet structures in their native state since no sample processing, e.g. purificatin, concentration precedures or laballing with conformation specific antibodies nor dyes are required. In that way chemical structures that could be affected or lost during chemical processing remain in situ and contribute to the infrared spectrum2,3. IR s-SNOM experiments were done at Advanced Light Source, Lawrence Berkeley National Laboratory, USA.
RESULTS:
Using synchrotron-based infrared micro-spectroscopy imaging (Maxlab, Lund, Sweden and NSLS, Brookhaven, USA) we have studied the secondary structure of proteins in cultured neurons at the micro level. The analysis of protein secondary structure showed a significantly higher ratio of β-sheet in AD transgenic cultured neurons expressing AD mutant APP compared to wild-type neurons, suggesting that the abnormal (β-sheet reach) protein structure occurs within AD neurons. Here we report that using IR s-SNOM we imaged neurons at nanometer scale (neuronal synapses). Moreover, analyzing Amide I peak positions, which appeared to be shifted in AD transgenic neurons, we obtained structural information about β-sheet structures in those synaptic junctions.
DISCUSSION & CONCLUSIONS:
Our results show that β-sheet formation can initiate within AD transgenic neurons and their synapses. However, since identifying the neurotoxic agents is a top priority in the AD field, further experiments are required to to understand how β-sheet rich variants of proteins may propagate from one neuron to the next, seeding the misfolding and triggering aggregation on the way, and nano-scale infrared imaging could a useful tool in this study.
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Neutrons and Industry: How the ISIS Neutron Scattering Source Interacts with Industry

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Neutrons provide a powerful tool to solve a range of challenging industrial problems. Neutrons have specific advantages over other forms of radiation (X-rays and light) such as they have a high penetrating power, they are non-destructive, they have a magnetic moment and they scatter from materials by interacting with the nucleus of an atom. The final point is one of the most significant features of neutron scattering when studying multi-component systems, which are commonly used in industry. The neutron scattering power of atoms varies, randomly, from element to element and

isotope to isotope. This means that light atoms, such as hydrogen, can be distinguished from heavier atoms, such as metals. Also, importantly, this dependence allows isotopes of the same element to have substantially different scattering powers and so by altering the isotopic content of a sample (switching the solvent from H2O to D2O, for example) or by using isotopic substitution with a molecule (D for H, for example) specific areas of interest within the system under investigation can have their scattering power enhanced without appreciably changing the chemical properties of the sample.

At ISIS we collaborate with a wide range of companies from small and medium-sized enterprises (SMEs) to multinational corporations, with sectors including chemicals and plastics, healthcare, aerospace, transport, manufacturing, automotive and the energy industry.

In this talk, details on the beamlines most commonly used by our industrial partners will be given and several examples of how these beamlines have been utilised by industry will be shown. The ISIS access mechanisms available to industry, including the relatively new ISIS Collaborative R&D (ICRD) Programme, will also be discussed.

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Reversible glycan self assembled monolayers (rSAMs) for ultra-sensitive virus sensing via enhanced multivalent interactions

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Fast and reliable techniques for probing influenza viruses and its subtypes are not only needed for surveillance and clinical diagnosis, but also for screening of drug candidates and vaccines to catch up with the rapidly evolving influenza virus. Current diagnostic and surveillance tools that allow subtyping of influenza viruses have several drawbacks. They typically require the use of highly trained personnel and days of influenza virus amplification and testing. As presented in recent literature, non-labelling sensors using glycans is a promising area for ultra-sensitive influenza virus detection. The strong affinity between influenza viruses and its corresponding host is based on multivalent lectin-glycan interactions. The mechanisms behind these synergistic interactions are however not fully understood. The main challenge of glycan sensors is to develop planar and robust molecular architectures which mimic its native environment on the dynamic cellular membranes. The most studied planar system for glycans is self-assembled monolayer. The covalently bound glycans however precludes reusability and adaptability through lateral diffusion of layer components. Two dimensional fluidic alternatives such as the lipid bilayers are fragile and unstable in atmospheric conditions, which makes it unsuitable for robust biosensing.
We have exploited hydrogen bonded amidinium-carboxylate ion pairs to develop restorable and adaptable self-assembled monolayers (rSAMs)1 featuring sialic acid head groups to probe influenza viruses (Figure 1). Apart from being restorable by cycling the pH between 8 and 2, these surfaces displayed a femtomolar and nanomolar affinity for H5N1 influenza virus (KD = 6 x 10-15 M) and hemagglutinin (KD = 9 x 10-9 M) respectively. Surface techniques such as in situ ellipsometry, FTIR spectroscopy and atomic force microscopy were used to study the assembly and disassembly of the reversible self-assembled monolayers and high affinity adsorption of surface proteins and viruses.

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Neutrons and Industry: How the ISIS Neutron Scattering Source Interacts with Industry

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Studying soft matter surfaces and interfaces with in-situ and in-operando x-ray and neutron scattering

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Neutron Reflection

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Neutron reflection in combination with isotopic substitution is a non-invasive technique that gives direct information about the composition and structure of layers at certain flat surfaces, including air-water, silica-water, silicon-water, sapphire-water and modified versions of these surfaces. It has been used successfully to explore layers of e.g. surfactants (detergents), polymers, proteins at such surfaces, often obtaining quantitative information inaccessible to other techniques. Potentially more interesting is that mixtures of the above can also be explored, which leads to both interesting new surface structures and to quantitative information about mixing. The latter opens the possibility of predicting the surface properties of mixtures. The scope and limitations of the technique will be explored with examples of adsorption of surfactants, proteins, polymers and some of their mixtures.

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Metabolic plasticity as mediated by the dhurrin metabolon

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Cyanogenic glucosides are a class of specialized metabolites found in numerous crop plants with dhurrin being present in Sorghum bicolor. Dhurrin is formed from tyrosine in a pathway catalyzed by CYP79A1, CYP71E1, UGT85B1 and P450 oxidoreductase (POR).
The three membrane bound proteins are situated in the ER membrane and the biosynthetic enzymes are thought to organize in enzyme complexes facilitating channeling of toxic and labile intermediates.
Functional importance of identified protein-protein interactions and lipid environment was studied by reconstitution of the dhurrin pathway in proteoliposomes. UGT85B1 binding to liposomes was dependent of the presence of CYP79A1 and CYP71E1. Binding of the soluble UGT85B1 stimulated flux through CYP79A1 and increased channeling of the intermediates. Maximum channeling was obtained when 15% of the phospholipids in the liposomes were negatively charged.
In vivo studies based on transient expression in Nicotiana benthamiana demonstrated that all enzymes were active when expressed as eGFP fusion proteins and catalyzed efficient channeling of intermediates. Protein-protein interactions and channeling was studied in planta using fluorescence lifetime imaging microscopy, fluorescence correlation spectroscopy and metabolite analysis. We demonstrate that the enzymes catalyzing dhurrin biosynthesis are organized within dynamic metabolons enabling plants to adapt to environmental challenges. Using the styrene maleic acid (SMA) copolymer, discrete lipid particles (SMALPs) were excised from the ER membrane enabling

purification of the dhurrin metabolon by affinity chromatography and characterization by mass spectrometry based proteomics. A model for the organization of the dhurrin metabolon in multi-enzyme clusters is presented. This SMALP nanodisc approach may be generally employed for detergent free isolation of entire biosynthetic pathways organized within metabolons to identify enzymes catalyzing missing steps and to identify the presence of lipids stabilizing protein entanglement. Unfortunately the SMALPs are catalytically inactive. Reconstitution of the membrane proteins in classical nano discs provided a catalytic active system. Neuron reflectometry studies enabled monitoring of shifts in the conformation equilibrium of the POR enzyme and offers unique opportunities to study the structural dynamics of metabolons either isolated as SMALPS or reconstituted in nano discs.

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Molecular dynamics simulations and neutron reflectivity as an effective approach to characterize biological membranes and related macromolecular assemblies

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Refolding of SDS-unfolded proteins by non-ionic surfactants

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The strong and usually denaturing interaction between anionic surfactants (AS) and proteins/enzymes has both benefits and drawbacks: For example, it is in good use in electrophoretic mass determinations (SDS-PAGE) but limits enzyme efficiency in detergent formulations. Therefore, studies of the interactions between proteins and AS as well as non-ionic surfactants (NIS) are of both basic and applied relevance. The AS sodium dodecyl sulfate (SDS) denatures and unfolds globular proteins under most conditions. In contrast, it has been shown that the NIS octaethylene glycol monododecyl ether (C12E8) protects bovine serum albumin (BSA) from unfolding in SDS. Here, we investigate whether globular proteins unfolded by SDS can be refolded upon addition of C12E8 and dodecyl maltoside (DDM). Four proteins, BSA, α-lactalbumin, (αLA), lysozyme (LYZ), and β-lactoglobulin (βLG), were studied by small-angle X-ray scattering (SAXS) and both near- and far-UV circular dichroism (CD). All proteins and their complexes with SDS were attempted refolded by the addition of C12E8, while DDM was additionally added to SDS-denatured αLA and βLG. Except for αLA, the proteins did not interact with NIS alone. The addition of NIS to the protein-SDS samples, except aLA, resulted in refolding of the tested proteins and dissociation from surfactant micelles. It concluded that NIS competes with globular proteins for association with SDS, making it possible to release and refold SDS-denatured proteins by adding sufficient amounts of NIS. In the last part of the talk, recent data from synchrotron radiation SAXS in combination with stopped-flow techniques on the kinetics of unfolding and refolding will be shown.

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Structural and dynamics studies of a truncated variant of CI repressor from bacteriophage TP901-1

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The CI repressor from the temperate bacteriophage TP901-1 consists of two folded domains, an N-terminal helix-turn-helix DNA-binding domain (NTD) and a C-terminal oligomerization domain (CTD), which we here suggest to be further divided into CTD1 and CTD2. Full-length CI is a hexameric protein, whereas a truncated version, CIΔ58, forms dimers. A linker separates the NTD and CTD, which hamper crystallization of CI. Thus, to obtained structural information of CI we employ complementary biophysical techniques together with the divide and conquer strategy. Hence, we solve the crystal structure of NTD, and reconstruct an ab initio envelope of CIΔ58 from small angle X-ray scattering (SAXS) data.
Furthermore, we performed nuclear magnetic resonance (NMR) and assigned the CIΔ58. From NMR we identify the dimerization region of CIΔ58 as CTD1 and determine its secondary structure to be helical both within the context of CIΔ58 and in isolation using circular dichroism spectroscopy. To our knowledge this is the first time that a helical dimerization domain has been found in a phage repressor. We also precisely determine the length of the flexible linker connecting the NTD to the CTD based on relaxation experiments measured with NMR. Using electrophoretic mobility shift assays and native mass spectrometry, we show that CIΔ58 interacts with the OL operator site as one dimer bound to both half-sites, and with much higher affinity than the isolated NTD domain thus demonstrating cooperativity between the two DNA binding domains.
Finally, using SAXS data and state-of-the-art ensemble selection techniques, we delineate the conformational space sampled by CIΔ58 in solution, and we discuss the possible role that the dynamics play in CI-repressor function.
Future experiments will include characterization of CTD1 and CTD2, but also to gain more structural information of CI and its interaction with DNA. Here it would be obvious to include small angle neutron scattering.

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SELECTIVE LABELING OF SIALIC ACID ON CANCER CELLS USING IMPRINTED FLUORESCENT CORE-SHELL PARTICLES

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Background: Sialic acid (SA) plays an important role for regulation of the innate immunity and as markers of the immune cells, but can be recognized by a variety of receptors. The expression of SA correlates with different disease states, such as various forms of cancer. Aim: We have investigated a novel strategy for specific fluorescence labeling of cell surface glycans terminating with SA, or Neu5Ac. Methods: We have developed SA imprinted core-shell nanoparticles equipped with nitrobenzoxadiazole (NBD) fluorescent reporter groups allowing environmentally sensitive fluorescence detection at convenient excitation and emission wavelengths. Results: We have recently shown that the SA nanoparticles stained prostate cancer cell lines in correlation with the SA expression levels. Here we show that cancer cell lines from blood, breast, skin and brain tissue display

different staining pattern for the fluorescent SA nanoparticles and for FITC-labeled lectin. Moreover, viable cells have been incubated with SA nanoparticles and staining pattern as well as internalisation will be shown. Conclusion: We have demonstrated an imprinting approach to produce tailor-made receptors that will allow an effective targeting of tumor glycan motifs for imaging and therapeutic applications.

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Neutron crystal structure determination of triose phosphate isomerase

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Hydrogen atoms account for approximately half of the atoms in any given protein. Moreover they play a crucial role in enzyme catalysis, protein-ligand and protein-drug interactions. However X-ray crystallography which is the most used method to obtain protein structures cannot unambiguously determine hydrogen positions. Neutron macromolecular crystallography (NMX) offers a unique approach for locating individual atoms by leveraging the neutron scattering properties of the hydrogen isotope deuterium (D). NMX can provide information on protonation states of active site residues in enzymes, H-bonding networks and orientation of solvent molecules. However production of deuterated proteins and growing large crystals of proteins is often the bottleneck in neutron crystallography.
In this project we aim to use NMX to collect neutron diffraction data and determine crystal structure of Triose phosphate isomerase (TIM). TIM is a key enzyme in glycolysis and it catalyzes the interconversion of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. However, the precise mechanism of this multistep reaction catalyzed by TIM is not fully elucidated. The objective is to determine the neutron crystallographic structure of TIM in complex with the inhibitor phosphoglycolohydroxamate – mimics of the elusive enediolate intermediate.
We have succesfully purified and co-crystalized deuterium labeled TIM with with reaction-intermediate analog PGH. Optimisations have been developed in order to increase crystal sizes, which is required for further data collection using NMX.

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pH triggered aggregation of an intrinsically disordered proline-rich peptide

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Disordered, proline-rich regions of natural proteins such as amelogenin have recently been found to play an important role in biomineralisation1. Short synthetic peptides that mimic the disordered

regions of such proteins may therefore be bound onto biomaterial surfaces to stimulate bone formation and regeneration.
To understand the behaviour of such biomolecules, two synthetic proline-rich peptides (Table 1) that have previously shown potential in inducing bone mineralisation2 were dissolved at pH 3-12 and analysed by high throughput small-angle X-ray scattering. In order to evaluate chain conformation, size and shape of the peptide aggregates, both model-independent analysis and geometrical body modelling were performed.
Table 1: Amino acid sequence of the two examined proline-rich peptides.
Peptide Sequence
P2 PLVPSQPLVPSQPLVPSQPQPPLPP
P5 PLVPSSPLVPCCPLVPCCPSPPLPP

While P2 maintained its disordered structure throughout the tested pH range, P5 formed aggregates at high pH (pH 8-12). Radius of gyration nearly doubled and over tenfold increase in the calculated molecular weight was observed in this pH region, indicating that several peptide chains clustered together into nanoparticles whose scattering profile best fitted a triaxial ellipsoidal model.
The main difference between the two examined peptides is the presence of cysteine (C) in the amino acid sequence of P5. The observed aggregation at high pH may therefore be explained by the formation of disulphide bonds which link several peptide chains together as the thiol groups of the cysteines become deprotonated with increasing pH.
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Cubosome and Hexosome Nanocarriers as Versatile Platforms for Drug Delivery

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Non-lamellar liquid crystalline nanostructures offer numerous advantages as attractive versatile and robust platforms for drug delivery and bio-imaging. They display nanostructures closely related to those observed in biological membranes and posess interesting characteristics. The attractiveness of this liquid crystalline formulation principle is linked to the nanostructural versatility, the compatiblity, digestiblity and bioadhesive properties of their main biologically relevant lipid constituents, and the capability of solubilizing and sustaining the release of amphiphilic, hydrophobic, and hydrophilic drugs. Two distinct strategies appear promising in the development of drug delivery applications: formation of ISASOMES (internally self-assembled 'somes' or particles) like cubosomes, hexosomes, and micellar cubosomes, and in situ formation of non- parenteral dosage forms with tunable nanostructures at the administration site. This contribution outlines the unique features of cubosomes and hexosomes and their potential utilization as promising platforms for delivering drugs and loading imaging probes and summarizes our recent studies including radiolabeling of hexosomes for in vivo experiments using an advanced NanoSPECT/CT scanner.

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Model membranes, living organisms and lateral membrane organization

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A small-angle view on macromolecular self-assembly: from supramolecular interactions to supramolecular structures

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ESS View on SasView: Small Angle Scattering data analysis within the SINE2020 project

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SasView is a well-established open source, collaboratively developed software for the analysis and the modeling of small angle scattering (SAS) data. The core functionality of SasView includes the fitting of model functions, pair-distance distribution function inversion and model-independent analysis. SasView provides a large collection of form and structure factors and with the recently introduced modularization allows for easy incorporation of user-defined models.
The European Spallation Source (ESS) has during the last years taken an active role in supporting SasView with the aim of providing it for ESS users from the start of operation.
To increase this effort and as part of the EU funded Horizon2020 project - SINE2020, ESS also employs two full time SasView developers. The aim of the project is to deliver inter-operable versatile, robust, reliable, maintainable and sustainable data analysis software that can be used by all the involved neutron scattering facilities (i.e. ESS, ILL, ISIS, LLB, MLZ, and PSI).
Here we present, how the SINE2020 project enables the development of new features, code refactoring, GUI re-design and optimization for faster analysis methods by use of GPUs. We also discuss an anticipated outcome of the project, which is a better user experience and make SasView a potential tool for live analysis of SAS data.

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LP3 – Lund Protein Production Platform

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Proteins are of enormous diversity and importance for life on earth. They have a multitude of different functions in all organisms, for example as enzymes, gene regulators, structural components, transporters, receptors, and energy converters. Most drugs act on proteins. It is therefore not surprising that the structure and mechanistic function of proteins are prominent topics in life science. Access to both state-of-the-art X-ray (MAX IV) and neutron sources (ESS) will increase the capacity for innovation in the life sciences, material research and physics. To enable efficient use of these unique facilities by Lund researchers, Lund University hosts the protein production facility, LP3.
LP3 can help with:
a. Recombinant protein production
b. High-throughput crystallization
c. Stable isotope labelling and bio-deuteration
(www.lu.se/lp3)

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Size dependent two-photon absorption cross-section of CsPbBr3 perovskite quantum dots

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All-inorganic colloidal perovskite quantum dots (QDs) based on cesium, lead and halide have recently emerged as promising light emitting materials. Their photoluminescence (PL) can be tuned across the entire visible spectrum, they have a high PL quantum yield and a low-threshold for lasing. CsPbBr_3 QDs have been also demonstrated as stable two-photon-pumped lasing medium. However, the reported two photon absorption (TPA) cross-sections for these QDs differ by an order of magnitude. Here we present an in-depth study of the TPA properties of CsPbBr_3 QDs with mean size ranging from 4.6 nm to 11.4 nm. By using femtosecond transient absorption (TA) spectroscopy we found that, for the 9 nm size QDs, the TPA cross-section is 1.8×10^5 GM (1 GM = 1×10^-50 cm^4 s/photon), and the cross section follows a power law dependence on QDs size with exponent 3.7±0.2. The empirically obtained power-law dependence of the TPA cross-section on QDs size, together with the quadratic dependence of the two-photon excited PL intensity on excitation intensity, suggests that the TPA process is mediated by a virtual state. The revealed power-law dependence and the understanding of TPA process will be valuable for developing high performance nonlinear optical devices based on CsPbBr_3 nanocrystals.
Because of the extraordinary optical properties and strong application potential, we plan to carry out more fundamental study about this type of perovskite QDs by using X-ray and neutron based techniques.

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Realizing the potential of research infrastructures

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Exploring protein association pathways with Small Angle X-ray Scattering

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Protein performs its biological functions by interacting with other proteins. Protein complexes, which are formed as a result of these interactions, consist of two or more components that associate along specific pathways - protein association pathways (PAPs). The association pathway from monomer to oligomer is critical in a range of biological processes and thus it is of a vital importance to elucidate both atomic-resolution structures of intermediates along the pathway as well as the structure of the final state. Although considerable progress has been made in using experimental and computational techniques to determine start and final structural states, we have a very limited understanding of what happens in between. Small Angle X-ray Scattering is a powerful method to study how proteins associate to form complexes but it is limited to obtaining overall shapes of

molecules - not atomic details. To overcome this problem we developed a method that combines a computational structural modeling (which delivers atomic-resolution structures) with experimental data (which provides information about the population of the different structural states). The method applies Bayesian probabilistic model to analyze SAXS data from mixtures of oligomeric species that are in equilibrium with each other. The method allows for a modeling large structural ensembles, it can be used to assess uncertainty of all modeling parameters and enables minimization of over-fitting using probabilistic concept called model evidence. We demonstrated that ensembles determined with this approach explain experimental data to a higher degree and are less prone to over-fitting than the current state-of-art methods used to analyze data.

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Membrane interactions and antimicrobial effects of layered double hydroxide nanoparticles

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Layered double hydroxide (LDH) nanoparticles have the potential of being used as antimicrobial agents and as carriers of antimicrobial peptides (AMPs). As with many inorganic nanoparticles, membrane interaction and destabilization plays an important part of the mode of action and is critical for the successful use of LDH nanoparticles as therapeutics. Furthermore, when AMPs are used as treatment, they are often rapidly degraded with a corresponding activity loss if they have not been designed to be proteolytically stable. By combining AMPs with inorganic nanoparticles, it might be possible to reduce the damaging effects of such proteolytic inactivation.

To use LDH nanoparticles, as both antimicrobial agent and carriers of AMPs, will require a straightforward understanding of membrane interactions and the factors that determine them. To elucidate these factors, we have investigated the effect of particle size on LDH interactions with both bacteria- and mammalian mimicking lipid membranes as well interaction and antimicrobial effect of an AMP/LDH nanoparticles system. Taken together, these findings demonstrate a set of previously unknown nanoparticle behaviors, including dual mode killing/clearance of bacteria and cooperative LDH/AMP membrane activity, of potential therapeutic interest.

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Neutron and x-ray scattering approaches for interdisciplinary structural biology

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SANS study of the self-aggregation of alkylglycoside surfactants with oligomeric head-groups

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Tracking solvents in the skin - Molecular mobility of solvents, lipids and proteins in intact stratum corneum

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Solvents are commonly utilized in dermal and transdermal formulations, as well as in sanitary products and cleansers. Apart from dissolving active compounds, the solvent itself can penetrate the skin. The uptake of solvent can lead to changes in the molecular organization of skin lipids and proteins, which, in turn, may alter macroscopic properties of the skin, such as the elasticity, softness and barrier function.
The aim of the present study is to examine the molecular effects of ten different solvents on the outermost layer of skin barrier, stratum corneum (SC). We use polarization transfer solid-state NMR [1-2] on natural abundance 13C intact SC. From these experiments, we are able to monitor changes in molecular dynamics of the solvent molecules inside SC, enabling us to draw conclusions on interactions and partitioning of solvents in SC. Simultaneously, we obtain atomically resolved information on changes in molecular dynamics on the lipid and protein components SC induced by the addition of the solvents. In particular, we obtain resolved information on changes in the minor fraction of fluid SC components. SC differs from most other biological membranes in that the main fraction of both lipids and proteins are solid at ambient conditions, although the minor fluid fractions are considered crucial to its barrier and mechanical properties. The present NMR experiments provide simultaneous information on fluid and solid SC components with a molecular detail that have not been achieved with other methods. We previously applied the same approach to study the effects of moisturizers and penetration enhancers on intact SC [3-4].
All solvents investigated are incorporated in SC, influencing molecular mobility of both the solvent molecules and SC components. Our results show variations in solvent molecular dynamics, interactions between solvents and SC components and effects of solvents on SC lipids and proteins, depending on solvent identity and hydration conditions. The findings can be directly related to practical utilization of solvents in skin products. All solvents investigated fluidize SC lipids. Comparing the results obtained from different solvents reveals the essential role of water in the mobility of keratin proteins.

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SANS study of the self-aggregation of alkylglycoside surfactants with oligomeric head-groups

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The increased effort to preserve the environment has driven extensive research toward the identification of surfactants that are nontoxic, biodegradable, and synthetized from sustainable resources

(1). Alkylglycosides, which have a head-group consisting of one or several sugar moieties, promise to meet these demands. Alkylglycoside surfactants with functionalised oligomeric head group (>3 sugars) have recently proved possible to synthetize by enzymatic means (2,3). This novel class of surfactants has been specifically designed to ensure biocompatibility and controlled biodegradability, and hence lend themselves to applications within the area of in vivo controlled release (e.g. food additives).
Our study focused on a surfactant comprising a long alkyl chain, 16 carbons, and a long glucose chain, 8 glucose units, which is referred to as C16G8. Since the functionalities and possible applications of C16G8 can compete with the widely used Polysorbate 80, we investigated thoroughly the self-aggregation mechanism. We characterised the system with several techniques, such as light scattering, both static (SLS) and dynamic (DLS), NMR, SAXS and SANS. The complementary use of neutrons and x-rays was crucial to determine the structure of the aggregates, since the contrast between the glucose chain and the alkyl chain differs when probed with x-rays and neutrons.
We will discuss the effect of temperature and concentration on the size and shape of the aggregates and, furthermore, the effect of different anomeric configurations (4). The combination of these techniques allowed us to reveal the features of this novel sugar surfactant and build a fundamental knowledge required for identification and development of applications.

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Lipoprotein structure dependency on lipid cargo and exchange dynamics - Implications for atherosclerosis development

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Atherosclerosis and related cardiovascular disease constitute the leading cause of death in westernized societies [1]. In atherosclerosis, plaques of fat and fibrous elements accumulate in the arteries

leading to heart disease and stroke [2]. The levels of different plasma lipoprotein particles, low density lipoprotein (LDL), oxidized LDL (oxLDL), high density lipoprotein (HDL), lipids and cholesterol have been associated with the disease and are therefore currently being used as key clinical markers [3]. However, the impact that the apolipoprotein isoform, the apolipoprotein oxidation state, the lipid cargo and the presence of divalent ions have on the structure and stability of the lipoprotein particles is still not fully known. Together with their subsequent effects on lipoprotein interactions with blood vessel components these parameters need to be thoroughly investigated in order to understand the molecular mechanisms behind the initial events of plaque-build up. This can in turn allow for the development of novel strategies for the diagnostics and treatment of cardio-vascular disease. Here we use small-angle x-ray scattering and small-angle neutron scattering in combination with selective deuteration to provide novel information on both structure of the lipoproteins and the molecular exchange which occurs between lipoprotein particles and cell-membrane mimics. Focusing on the lipid exchange kinetics between both native HDL and LDL and liposomes made of "invisible" PC lipids [4] we show that the apolipoprotein plays a key role in enhancing lipid exchange. Furthermore, we present a novel structural model for LDL and the effects that temperature has on the particles overall structure as well as on the organization of the particles hydrophobic core and which now allows us to start relating the specific structural changes with the observed lipid exchange.

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Structure and dynamics at buried fluid interfaces

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Interfaces induce microscopic ordering of dense fluids, which in turn modifies their dynamic and thermodynamic properties [1]. The obvious experimental techniques for studying these phenomena are x-ray and neutron scattering, but experiments at buried fluid interfaces, i.e., fluid-fluid interfaces, solid-fluid inter-faces, or fluids confined between two solid surfaces at short separation, are very challenging. We have developed unique methodology for probing the microscopic structure and ensuing properties of confined fluids, based on x-ray scattering from colloid-filled micro- or nanofluidic containers. First, small-angle x-ray scattering (SAXS) probes the anisotropic pair structure in terms of the anisotropic structure factor [2], allowing experimental studies of inhomogeneous fluids at a fundamental and highly accurate level. Second, in the long-wavelength limit the anisotropic structure factor yields the confined fluid's density fluctuations [3], which are believed to be instrumental in order to understand the behavior of fluids (or water) in solvophobic (hydrophobic) confinement. Finally, simultaneous high-energy SAXS and x-ray photon correlation spectroscopy (XPCS) experiments provide means to study the connection between the fluid's anisotropic structure factor and its wave-vector-dependent collective diffusion coefficient [4]. These experimental results are essential in order to develop the theory of inhomogeneous fluids and to guide future work on chemically and biologically relevant systems.

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Can neutron reflectometry offer structural and compositional information about native supported cell membranes and biological nanoparticles?

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Biological nanoparticles such as extracellular vesicles, exosomes and also virions are generating a rapidly growing interest due to the key roles they play in various biological processes and because of their potential use as biomarkers in clinical diagnostics and as efficient carriers in drug-delivery and gene-therapy applications. Their full exploitation, however, depends critically on the possibility to detect and classify them into different sub-populations, tasks that in turn relies on efficient means to identify their unique biomolecular and physicochemical signatures. Due to their huge diversity, such information remains rather elusive and there is accordingly a need for new and complementary characterization and enrichment schemes that can help expanding the library of distinct features of biological nanoparticles. Fluorescence and light-scattering based analysis offer single nanoparticle resolution, and can be used to quantify optical density as well as molecular content based on labeling procedures. We recently developed a two dimensional flow nanometry concept that can add to this also information about their individual size, and potentially sorting of individual particles based on several combined features.[1] With this type of biological nanoparticles as hand, we have put significant effort into improved means to form planar supported lipid bilayers of high compositional complexity.[2] In the studies of these systems, we hope to be able to utilize neutron reflectometry (NR) to gain structural as well as compositional information not easily obtained by other means. While the planar and continues configuration of SLBs make them well suited for NR investigations, we are also interested in exploring the possibility to study interactions of biological nanoparticles with planar lipid bilayers, to thereby unravel information about the structure of the membrane-membrane interface formed when contact is made.[3]

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Multi-modal and high-resolution investigation of biodegradable Mg implants in bone

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Magnesium(Mg)-based metals are potentially unique in the field of orthopedic and cranio-maxillofacial surgery. Similarly to permanent metals, they offer load-bearing capacities. However, they can biodegrade over time, upon substitution by new healthy bone, which avoids the long-term complications and the need of implant removal through a second surgery. They have the potential to benefit a vast number of patients and have been extensively studied in the recent years. However, uncertainties persist on how Mg degradation occurs in the complex system of the living body and observations of uncontrolled degradation have hindered their wide access to the market.
One reason why Mg degradation in bone has not been fully elucidated yet is that most of the commonly available techniques lack sufficient resolution to depict the dynamic changes that the materials undergo once in bone.
In the present study we employed synchrotron-based micro-computed tomography (SRμCT), histology and chemical characterization by means of Electron Probe Micro Analysis (EPMA) to shed light on the degradation behavior of 3 Mg-based materials in vivo. We compared the bone response to the materials to that of Ti controls, which are known to become encapsulated by bone.
In brief, mini-screws made of Mg-2Ag, Mg-10Gd, Mg-4Y-3RE (nominal composition of 3 Mg alloys) and Ti were inserted in the tibia of rats, after ethical approval. The samples with surrounding bone were explanted after 1 and 3 months of healing. The explants had a diameter of 3.4 mm and were scanned in absorption mode at the beamline P05 operated by HZG at the storage ring PETRA III at DESY, with photon energy of 25 KeV for the Mg samples and 40 KeV for the Ti samples. Three-dimensional reconstructions of the SRμCT were obtained with "back projection of filtered projections" algorithm and had a final spatial resolution of 4.8 μm and an isotropic voxel size.
The 3D data-sets were segmented in relevant sub-volumes (implant – bone – degradation products) and quantitative analyses were performed. In particular, we calculated the degradation rates, which were similar among the 3 materials. On the contrary, the degradation behaviour was different and Mg-2Ag lost integrity too quickly. In addition, we measured the 3D bone-to-implant contact as parameter for bone integration and we found that Mg-10Gd and Mg-4Y-3RE yielded similar results than Ti, while Mg-2Ag was encapsulated by soft tissues.
After SRμCT, thanks to the non-destructive nature of this technique, the samples were prepared for histological sections. The registration of the histological and SRμCT slices produced new insight onto the degradation behaviour and the tissues that surrounded the samples. Chemical analysis of the histological slides gave information on the composition of the degradation products of Mg alloys and of the distribution of elements in the surrounding bone. The degradation layers were not made of Mg after 1 and 3 months of healing in bone, but they contained minerals coming from the body fluids as Ca, P and Na. They had also high content of C and O.
In conclusion, SRμCT enabled us to investigate the degradation behavior of Mg alloys at unprecedent spatial and density resolution. In addition, the multi-modal approach, which combined SRμCT, histology and chemical analysis on the same samples, was helpful to gain new knowledge on the degradation behavior of Mg in bone.

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Biocatalytic reduction of oxygen catalyzed by human ceruloplasmin

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Biocatalytic reduction of oxygen catalyzed by human ceruloplasmin

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Ceruloplasmin (Cp) is a copper containing enzyme with ferroxidase activity, that oxidises ferrous iron Fe(II) into ferric iron Fe(III) with concomitant reduction of oxygen directly to water [1]. Even though a ferroxidase activity has historically been considered as a primary role of Cp, it is capable to oxidize a wide range of structurally unrelated substrates due to its unique structure and copper content [2]. Few attempts were made earlier to achieve the bioelectroreduction of oxygen catalyzed by commercially available ceruloplasmin. These attempts were either completely unsuccessful [3-4] or almost negligible bioelectrocatalytic currents only in the presence of a redox mediator were obtained [5]. The aim of this study is to use the purified enzyme from human serum for the construction of oxygen sensitive biosensors and biocathodes of implantable biofuel cells, if the bioelectroreduction of oxygen will be observed.

We have shown that the commercial preparation of human ceruloplasmin did not exhibit a ferroxidase activity, i.e. neither increase in open circuit potential (OCP) values nor oxygen uptake on Clark electrode were observed. Thus, we purified ceruloplasmin from human serum. In the current research OCP measurements in human blood and in buffer as well as oxygen concentration measurements on Clark type electrode, in the presence of different substrates, were used to compare the catalytic activities of the commercially available preparation and purified ceruloplasmin. As the commercially available enzyme appeared to be catalytically inactive, further electrochemical investigations were carried out with purified biocatalyst. Bioelectroreduction of oxygen was observed on carbon nanotube modified graphite electrodes (GR/CNTs) in the presence of mediator by using cyclic voltammetry. However, the obtained current densities are only in μA cm-2 range [6]. Amperometric studies showed a minor bioleletroreduction of oxygen on GR/CNTs electrode even in the absence of mediator. Continuation of this work will be focused on further investigations of Cp activity towards certain substrates as well as accurate determination of catalytic constants in order to understand the bioelectrocatalytic inertness of this complex enzyme.

Acknowledgements: This work was supported financially by the Swedish Research Council (project 2013-6006) and by the Russian Science Foundation (project 14-14-00530).

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Cancer Cell Response of Cubosomes and Hexosomes

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Background: Cubosomes and hexosomes are aqueous dispersions of liquid crystalline particles enveloping internal nanostructures of inverted type 3-D bicontinuous cubic and 2-D columnar hexagonal phases, respectively [1]. These nanostructured dispersions are attractive as drug nanocarriers owing to their unique properties. The optimal utilization of these soft nanocarriers underscores the need of assessing the potential toxicity and investigating their cellular response. Despite their potential applications, the number of studies on the biological evaluations of these nanocarriers is still limited.

Aim of the study: The main objective of this work was to focus on understanding the effect of the lipid composition and the stabilizer type on the cellular uptake and potential cellular toxicity of a series of cubosomes and hexosomes based on the binary mixtures of phytantriol (PHYT) or monoolein (MO) lipids with oleic acid (OA).

Methods: The morphology and the internal liquid self-assembled nanostructures of these lipidic aqueous dispersions were investigated by Transmission Electron Cryo-Microscopy (Cryo-TEM) and synchrotron small angle X-ray scattering (SAXS). Live-cell imaging was used to investigate the cellular uptake and intracellular fate of the cubosomal and hexosomal nanoparticles upon exposure to two different cell lines: H1299 and MCF-7. The effects of variations in the lipid composition and/or concentration on the cellular response were investigated. In addition, relevant toxicity studies were performed by flow cytometry.
Results and discussion: The results reveal that four different hexosomes based on PHYT/OA at different weight ratios induce a severe toxic cellular response. This response was correlated to the magnitude of uptake by the H1299 and MCF-7 cells. There is distinguishable effect of varying the lipid composition of these samples on the cellular response. Further investigations were performed on dispersions containing MO/OA at different weight ratios. Clearly, the replacement of PHYT by MO leads to a significant decrease in the cellular toxicity. However, the uptake of MO/OA in both cell lines induces clear morphological changes in both cell lines. They were visualized as vesicular structures in the live-cell microscopy. These vesicular structures are identified as lipid droplets using fluorescence microscopy.
Conclusions: Cubosomes and hexosomes based on the binary PHYT/OA mixture and stabilized by the triblock copolymer F127 are toxic to cells; however this toxicity is tuned by altering lipid composition and content, such as substituting PHYT with MO. The mechanism contributing to cell toxicity is not yet clear, which calls for in detail vitality studies to fully understand the cellular response to these soft lipidic nanoparticles.

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A new chemical deuteration laboratory at ESS

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A new chemical deuteration laboratory is being established at ESS. Funded by the EU as a SINE2020 (Science and Innovation with Neutrons in Europe in 2020) project, and in conjunction with other members of the 'Deuteration Network' (ISIS, ILL and FZJ, with ANSTO as an observer member) the deuteration laboratory at ESS, Lund, Sweden, aims to provide the European neutron scattering community with novel deuterated molecules to facilitate neutron scattering studies. The scope and aims of the initiative will be discussed.

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Probing the structure and dynamics of HSA using neutron scattering

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To fully understand the function of proteins it is necessary to consider both their structure and dynamics. Using a combination of small-angle neutron scattering, neutron spin-echo and backscattering spectroscopy we have studied the internal motions in the protein human serum albumin (HSA). These techniques have previously been shown to be very powerful tools for probing the slow, internal dynamics in biological samples.

HSA is a protein abundant in blood, and plays a key role in the transport of compounds such as nutrients, hormones and drugs. Here we show that it is possible to detect the changes in the internal dynamics that occurs upon binding of small molecules such as heme and myristic acid under physiologically realistic conditions.

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Structural characterization of fouling-resistant polymer brushes clarifies fouling resistance mechanisms

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Poly(ethylene glycol) (PEG) coatings have long been a benchmark for antifouling coatings in biomedical devices, and for increasing the circulation lifetime of drugs and delivery vehicles. However, limited chemical stability restricts its use for long-term applications, or in harsh environments. In clinical use, PEG also shows adverse side effects, such as complement activation and dose-dependent clearance. This has stimulated research in alternative, but more chemically resistant and bioinert

polymers, with equally good antifouling properties.
While there has been significant progress in responsive, or otherwise 'smart', antifouling coatings, many applications will, for the foreseeable future, have to rely on 'passive' coatings, depending solely on the intrinsic fouling-resistant properties of the polymer. A widely used strategy for this is to prepare polymer brushes with stable backbones with side-chains based on PEG, or otherwise endowing PEG-like properties. We demonstrate that the antifouling properties of such polymer brushes are thickness-dependent in a non-trivial manner, and that there is an optimum thickness for the fouling resistance. We aim to clarify the reasons for this dependence, and to understand how variations in physicochemical properties influence the antifouling performance. Our primary tools for this are quartz-crystal microbalance investigations of structure and viscoelasticity, and neutron reflectometry for structural characterization of hydrated polymer films.

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Different expression levels of glycans on leukemic cells - a novel screening method with molecularly imprinted polymers (MIP) targeting sialic acid

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Background
Sialic acid (SA) is normally expressed on the cell membranes and is located at the terminal position of the sugar chains. SA plays an important role for regulation of the innate immunity and function as a markers of the cells and can be recognized by a variety of receptors. The level of SA expression is increased on metastatic cancer cells. The availability of specific antibodies against SA is limited. We have recently presented a novel method for specific fluorescence labeling of SA molecular imprinted polymers (MIP).

Aims
The aim of this study is to analyze SA expression on leukemic cells by using SA-MIP.

Methods
We have performed an extended screening of SA expression by using SA-MIP and included four different chronic lymphocytic leukemia (CLL) cell lines, conveniently analyzed by flow cytometry and fluorescence microscopy.

Results
SA expression was detected in four cell lines at different levels and the SA expression were verified with lectin-FITC. Higher expression of SA in the more aggressive CLL cell lines was observed.

Conclusion
These results show that SA-MIP can be used as a plastic antibody for detection of SA using both flow cytometry and fluorescence microscopy. We suggest that SA-MIP can be used for screening of different tumor cells of various stages, including CLL cells.

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A COMPARATIVE STUDY ON EFFECTS OF GLYCOLS ON PERMEABILITY AND DISTRIBUTION OF THE MODEL DRUG METRONIDAZOLE IN SKIN

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ABSTRACT

Stratum corneum (SC) is an obstacle in dermal drug delivery and thus increasing SC permeability is often a prerequisite for successful delivery of hydrophilic compounds, macromolecules and conventional drugs for new therapeutic indications. Though the primary barrier to percutaneous absorption lies within stratum corneum (typically 80%) diffusion within the viable tissue, metabolism and transport by interstitial fluid will also influence the bioavailability of compounds in specific skin compartments. Products for topical drug delivery contain dozens of components for various reasons. Co-solvents are primarily included to increase drug solubility in the vehicle, which however does not necessarily facilitate a higher bioavailability. They may nevertheless enhance drug penetration by affecting SC barrier properties and in some cases help to accumulate drugs in specific parts of the skin.
The objective of the current study was to investigate the potential of introducing two common non-volatile co-solvents, propylene glycol and hexylene glycol, to increase the capacity of the stratum corneum reservoir aiming for increased bioavailability of topically administered drugs in viable epidermis and dermis. We approach this problem by combining in vitro diffusion methodology, microtome cryo-sectioning and analytical techniques with small angle X-ray diffraction and two photon microscopy.

KEYWORDS

Topical drug delivery, metronidazole, two photon microscopy, small angle X-ray diffraction, in vitro diffusion, skin

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When thrown into the wonderful mix of constraints and desires, there is a product and a customer…

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Synchrotron radiation aids structure based inhibitor development - towards novel antibiotics

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Molecular transport in lipid membranes: lipid exchange and translocation processes investigated by neutron scattering

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High-resolution structure, interactions, and dynamics of self-assembled biomolecular complexes

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Introducing new industrial users to large scale X-ray and neutron facilities

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Small-angle x-ray and neutron scattering – complementary approaches for understanding colloid and interface behavior of systems relevant for technical applications of enzymes

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Citrem-phospholipid lamellar and non-lamellar liquid crystalline nano-assemblies for immune-safe drug delivery

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Drug delivery applications of self-assembling lipid formulations

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Multi-modal and high-resolution investigation of biodegradable Mg implants in bone

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Can neutron reflectometry offer structural and compositional information about native supported cell membranes and biological nanoparticles?

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Studying soft matter surfaces and interfaces with in-situ and in-operando x-ray and neutron scattering

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The investigation of soft matter nanostructures at surfaces, interfaces and in thin films requires dedicated analytical techniques, which provide information from a molecular to a mesoscopic scale, to account for the complexity of the systems. Whereas imaging techniques can give detailed information about real space structures, these methods render difficulties in probing buried nanostructures. In particular, in case of kinetic changes of the nanostructure the use of in-situ or in-operando x-ray and neutron scattering techniques is a very powerful approach. To enable detection of nanostructures at surfaces, interfaces and in thin films the use of reflection geometry has proven to be advantageous. Instead of transmission of the x-ray or neutron beam, a grazing incidence set-up is chosen. The combination of grazing incidence small and wide angle x-ray scattering (GISAXS and GIWAXS) allows for an in-depth structure characterization.1-4 The crystalline structure is probed with GIWAXS and the mesoscale structure (nanometer to micrometer) is determined with GISAXS. Due to the high brilliance of synchrotron sources a high time resolution can be achieved in in-situ and in-operando studies, which in turn allows for the investigation of complex processes such as thin film preparation via printing or via spray coating. By the use of neutron special contrast conditions can be established which are beneficial for e.g. detection of water in soft matter samples. Different examples will be presented to demonstrate the present possibilities.

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Synchrotron radiation aids structure based inhibitor development - towards novel antibiotics

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Infectious diseases have had an immense impact on evolution and have shaped the often twisting path in human history. These events are not necessarily closed in the relics of the past; the spread of antibiotic resistance is still one of the major challenges that the world faces in the 21st century. Pseudomonas aeruginosa and other gram-negative pathogenic bacteria present incurable infections as a serious problem not witnessed at the end of the 20th century. Mycobacterium tuberculosis on the other hand is carried as dormant infection in approximately one-third of the global population and leading to the occurrence of drug-resistant bacteria in about 20% of the cured patients. In the past effective drugs mostly came from natural products present in plants, bacteria and fungi and the main discoveries were due to coincidence. Rational target based development strategies of novel drugs that include antibiotics are based on established mechanisms of action and validated targets. When possible this approach is strengthened by high resolution structures of target ligand complexes. Target validation by gene knock-outs identifies typically 200-300 genes / proteins as essential in bacterial genomes. These potential targets must also be adapted to biochemical or biophysical assays used in screening campaigns for small molecules that interfere with their function or activity. Characterized inhibitors from these screens may define a class of compounds that are good candidates for improvements. Determination of the high resolution x-ray structures of target-ligand complexes provide extremely useful information on the binding mode of such molecules and can direct the design process to improve the properties of the initial inhibitors. Examples from structure based inhibitor development will be shown targeting fatty acid biosynthesis in Pseudomonas aeruginosa, as well as cysteine biosynthesis and cell wall remodeling machinery in Mycobacterium tuberculosis.

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Model Membranes, Living Organisms and Lateral Membrane Organization

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Biological membranes are the active boundary between cells and their surrounding environment. Membranes are sophisticated and dynamic assemblies that perform a diverse array of functions, including selective transport, localization, communication and recognition, to name a few. It is also widely accepted that the plasma membrane is laterally heterogeneous, containing nanoscopic regions enriched in certain types of lipids, whose physical properties differ from the surrounding lipids [1]. These functional domains have come to be known as rafts, and have been implicated in

a wide range of cellular functions including signal transduction, drug uptake and interactions with pathogens. We have used different neuron scattering techniques to study the lateral organization of membranes and their associated dynamics [2-4]. More recently, we have studied the plasma membrane of the Gram-positive bacterium Bacillus subtilis. Data from model and living systems will be presented, including evidence for the existence of lipid nanodomains in a living prokaryote system, implying that lipid organization is a feature that has likely evolved over time, eventually becoming an integral property of biological membranes.

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High-resolution structure, interactions, and dynamics of self-assembled biomolecular complexes

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Using SAXS, in combination with Monte Carlo simulations, and our unique solution x-ray scattering data analysis program, we resolved at high spatial resolution, the manner by which wtSV40 packages its 5.2kb circular DNA about 20 histone octamers in the virus capsid. This structure, known as a mini-chromosome, is highly dynamic and could not be resolved by microscopy methods (Nucleic Acid Research, 41, 1569, 2013). Using time-resolved solution SAXS, stopped-flow, and flow-through setups the assembly process of VP1, the major caspid protein of the SV40 virus, with RNA or DNA to form virus-like particles (VLPs) was studied in msec temporal resolution. By mixing the nucleotides and the capsid protein, virus-like particles formed within 35 msec, in the case of RNA that formed T=1 particles, and within 15 seconds in the case of DNA that formed T=7 particles, similar to wt SV40. The structural changes leading to the particle formation were followed in detail (J. Am. Chem. Soc. 134, 8823, 2012). More recently, we have extended this work to study the assembly of HBV virus-like particles.

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Residual stress and hydrogen effect on Ti-6Al-4V alloys produced by Electron Beam Melting

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Need for production of affordable and personalized orthopedic implants with net/near-net shaped parts made with a strong and durable material which have comparable properties as human tissues, such as bones made solid free form (SFF) production techniques which commonly known as additive manufacturing or “3D printing is an very interesting. The so called Electron Beam Melting (EBM) is one of the popular techniques used for this purpose. Thanks to high strength-to-weight ratios, good fatigue and corrosion resistance and biocompatibility of Ti and its alloys (e.g., Ti–6Al–4V), Ti based materials, especially a Ti–6Al–4V often used as a structural materials in medical and aerospace industry. To ensure performance in critical applications in the medical device, implant, and in airframe

applications, a clear understanding of the effects of processing on microstructure and mechanical properties of Ti–6Al–4V manufactured by EBM is needed. The EBM process is complex and the results depend upon different settings of the system, such as beam current, beam size, scan speed, and scanning direction / strategy. Each set of processing parameters provides a somewhat different built environment, resulting in different microstructures, hydride formability and potentially different states of residual stress. As both residual stress and hydrides are known to deteriorate the material mechanical properties significantly, the general aim of this project is to gain better understanding of the influence of EBM parameters on these.

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INTRODUCING NEW INDUSTRIAL USERS TO LARGE-SCALE X-RAY AND NEUTRON FACILITIES

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A wide range of experimental techniques for structural studies of anything from materials to biological systems down to atomic length scales are available at various international large-scale X-ray and Neutron facilities. They are in many cases mainly exploited by highly specialized academic researchers. A large, unexploited potential for an expanded user community outside of academia exists in industrial research settings. To promote an increased industrial use, various efforts must be made to overcome the barriers, which include limited knowledge about the opportunities, and lack of specialized expertise (especially for smaller companies).

Following the heavy Danish investments in the European Spallation Source (ESS) in Lund (Sweden), the Capital Region of Denmark and the University of Copenhagen (through the faculty of Science and the faculty of Health and Medical Sciences) have supported the initiative NXUS (Neutron and X-ray User Support). NXUS acts as a mediator between experiment facilities and non-expert users in industry, hospital research, and academia, providing expertise in data acquisition and analysis within the technique of small-angle scattering (SAXS and SANS). NXUS is research-based and is anchored at the Niels Bohr Institute, University of Copenhagen.

After an initial pilot phase of three years, a new national industrial portal for industrial use of large-scale facilities (LINX – Linking Industry to Neutrons and X-rays) was established in early 2016. This is initially based on 3 Universities and 15 initial industrial partners.

Selected cases will be presented along with conclusions from the pilot phase and an introduction of the new LINX portal.

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Computer Simulation Study of dsDNA: Melting and Bubble Formation

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Melting of DNA is the temperature-induced separation of the two strands in the double helical structure. It usually starts in regions rich in the weaker A-T base pair, causing the formation of *bubbles*, i.e. regions where the strands are separated [1]. Since local openings of the DNA helix are fundamental for certain biological processes, studies of bubble formation can provide insight in such processes. In this study we investigate if a simple, coarse-grained model can describe the DNA melting process and especially the bubble formation. Also, we simulate scattering data to predict what additional information can be provided by SAXS. The results, obtained by canonical Monte Carlo simulations, show that an alternating sequence exhibits a melting curve very similar in shape to experimental melting curves. Tri-block sequences melt stepwise, so that a bubble is formed. Simulated scattering data provides information about changes in shape and size upon melting.

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Lipid-based liquid crystals as drug delivery vehicles for antimicrobial peptides

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Resistance to traditional antibiotics is a rapidly increasing problem that in the future could make infections impossible to treat and bring the state of medical care back to the pre-antibiotic era from the beginning of the last century. Antimicrobial peptides (AMPs) have a huge potential as new, sustainable therapeutics against infectious diseases as they are less prone to induce high-level resistance due to their fast and nonspecific mechanism of action. One challenge of using AMPs as pharmaceuticals is their limited stability during storage as well as after administration, which dramatically reduces their efficiency. These challenges can be overcome with novel formulation strategies.

In this work, lyotropic liquid crystalline (LC) structures consisting of cubic glycerol monooleate/water and hexagonal glycerol monooleate/oleic acid/water have been examined as carriers for AMPs. These LC structures have capability of solubilizing both hydrophilic and hydrophobic substances as well as being biocompatible and biodegradable. Both bulk gels and discrete dispersed structures, i.e. cubosomes and hexosomes have been studied. Three AMPs named AP114, DPK-060 and LL-37 have been investigated with respect to phase stability of the LC structures and antimicrobial effect. Characterization of the LC structures was performed using small-angle x-ray scattering (SAXS), dynamic light scattering, ζ-potential, and cryogenic transmission electron microscopy (Cryo-TEM) and peptide loading efficacy by ultra-performance liquid chromatography (UPLC). The antimicrobial effect of the LCNPs was investigated in-vitro using minimum inhibitory concentration (MIC) and time-kill assay.

The most hydrophobic peptide (AP114) was shown to induce an increase in negative curvature of the cubic LC system. The most polar peptide (DPK-060) induced a decrease in negative curvature while LL-37 did not change the LC phase at all. The hexagonal LC phase was not affected by any of the AMPs. Moreover, peptides AP114 and DPK-060 loaded cubosomes showed preserved antimicrobial activity, whereas LL-37 loaded particles displayed a loss in its broad spectrum bactericidal properties. AMP loaded hexosomes showed a reduction in antimicrobial activity.

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Structure of Trehalose-Water Solutions by Neutron Diffraction and EPSR Modelling

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The di-saccharide trehalose has been extensively studied in recent years due to its superior ability to stabilize biological macromolecules in extreme conditions. A more fundamental understanding of the molecular properties of trehalose in solutions may lead to improved preservation techniques, such as improved food and medication storage, or successful cryopreservation of human organs for transplantations.

In this work we have studied the structural properties of trehalose-water solutions using neutron diffraction, with six different isotope compositions, and Empirical Potential Structure Refinement (EPSR) modelling. Using this approach,we have been able to answer important questions regarding how trehalose interacts with water and thereby perturb the hydrogen bonded network structure of water. The results show that the water structure is significantly perturbed by the presence of trehalose, and that water forms extensive hydrogen bonding with trehalose (about 10 hydrogen bonds per trehalose molecule, depending on the used definition of a hydrogen bond), primarily via the hydroxyl groups of trehalose, and particularly with the hydroxyl groups on the methyl groups. This finding is in strong contrast to a previous neutron scattering study of the same system [1]. Another important finding is that the trehalose molecules are homogeneously dispersed in the water with no preference of forming clusters, which are commonly observed in molecular dynamics (MD) simulations of the solution. The insights we have gained from this study are helpful for understanding the extraordinary stabilizing effect of trehalose on proteins.

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Chemical characterization of TiO2@DNA nanohybrids with X-ray photoelectron spectroscopic (XPS)

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The utilization of DNA in electronic device such as micro and nano sensors in recent years has increased. High throughput micro array sensors in biotechnology applications and medical diagnosis, MEMS and NEMS opens up new windows for utilization of DNA based sensors. Although, there are limitations in construction of stable electronics devices based on DNA, even under the best of circumstances, DNA is constantly subjected to chemical modifications. Successful application of DNA in electronics devices is conditional on precise characterization of absorbed DNA onto substrate. In current study we present processing and characterization of novel mesoporous TiO2@DNA nanohybrid electrode for use in sensor and supercapacitor. X-ray photoelectron spectroscopic (XPS) was used to elucidate in detail the chemical composition of absorbed DNA and dopamine onto TiO2.

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Novel design of neutron reflectivity experiments to study structure-force relation in soft interfaces

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Many cellular processes are governed by specific inter-membrane interactions involving structural rearrangement, leaflet adhesion, protein incorporation and vesicles release. The extremely large variety of chemical functionality and the nature of non-specific forces (electrostatics, hydrogen bonding, solvation and steric interaction, dispersion forces), in addition to the specific interaction involved in biological systems, make this field of study extremely interesting but also very challenging. In particular, the possibility of correlating structural features with molecular interactions is limited by the scarce number of techniques which are suitable to this purpose. In fact, such a technique should satisfy some important requirements: i) non-invasive and non-destructive probe; ii) access to buried interfaces in complex liquid environment, iii) achievement of molecular resolution. Neutron reflectometry is capable to combine these fundamental aspects, and therefore it permits to study inter-membrane interaction in biological systems and the related processes.

We aim to design a novel experimental procedure and a dedicated sample environment to study the correlation between structure and intermolecular forces in membranes of biological relevance. Our starting point is the simplest case of a phospholipid monolayer decorated with end-grafted PEG chains at the oil-water interface, which mimics the leaflet of a membrane with intercalated proteins. This monolayer is brought in proximity of a solid substrate to investigate the structural changes arising from the molecular interactions and under a controlled pressure applied from the oil phase (Figure 1b). The presence of the solid substrate (Figure 1a) is fundamental to overcome limitation encountered with soft interfaces in liquid environments, like undesired interfacial curvature, beam adsorption and incoherent scattering.

The knowledge gained on this very simple model will serve as the base for the study of more complex systems, where not only grafting density and polymer chain length, but also the system geometry and components (bilayers, proteins) can be changed (Figure 1c). Our work represents the proof of a new way of conceiving the study of inter-membrane interactions by neutron reflectometry.

The project has been granted within the Röntgen-Ångström cluster.

![Figure 1 - Design of the neutron reflectometry experiment and possible systems under study][1]

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Citrem-phospholipid lamellar and non-lamellar liquid crystalline nano-assemblies for immune-safe drug delivery

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Non-lamellar liquid crystalline aqueous nanodispersions, known also as ISAsomes (internally self-assembled 'somes' or nanoparticles), are gaining increasing interest in drug solubilisation and bio-imaging, but they often exhibit poor hemocompatibility and induce cytotoxicity. This limits their applications in intravenous drug delivery
and targeting. Using a binary mixture of citrem and soy phosphatidylcholine (SPC) at different weight ratios,we describe a library of colloidally stable aqueous and hemocompatible nanodispersions of diverse nanoarchitectures (internal self-assembled nanostructures). This engineered library is structurally stable in human plasma as well as being hemocompatible (non-hemolytic, and poor activator of the complement system).
By varying citrem to lipid weight ratio, the nanodispersion susceptibility to macrophage uptake could also be modulated. Finally, the formation of nanodispersions comprising internally V2 (inverse bicontinuous cubic) and H2 (inverse hexagonal) nanoarchitectures was achieved without the use of an organic solvent, a secondary
emulsifier, or high-energy input. The tunable binary citrem/SPC nanoplatformholds promise for future development of hemocompatible and immune-safe nanopharmaceuticals.

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Small-angle x-ray and neutron scattering – complementary approaches for understanding colloid and interface behavior of systems relevant for technical applications of enzymes

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Successful application of enzymes for catalyzing processes in applications ranging from biomass conversion to fabric cleaning and care in laundry rely on good understanding of a multitude of colloid and interface phenomena. Systems of interest include concentrated liquid enzyme formulations and reactions where colloidal substrates are undergoing compositional changes due to enzymatic activity. We shall discuss mechanistic learning on such systems and how small-angle x-ray and neutron scattering investigations may come into play.

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Structural characterization of the intrinsically disordered saliva protein Histatin 5: A combined SAXS and Monte Carlo simulation study

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For more than 30 years, a coarse-grained model based on the primitive model, in combination with Monte Carlo simulations, have been used to model polyelectrolytes and polyampholytes under various conditions. Sometimes this model is also refered to as the bead-necklace model.

Our aim is to apply this model for intrinsically disordered proteins, and verify the simulation results by experiments. As model proteins we are using the salivary protein Histatin 5, a 24 AA long peptide, which has a strong fungicidal property [1]. In vitro, it has been found that this fungicidal action is strongly dependent on ionic strength [2, 3]. Conformational properties of Histatin 5 are considered to be of importance.

We would like to present results showing good agreement between the scattering curves for Histatin 5 obtained from SAXS and the simulations. At high salt concentration, the protein behaves as a neutral polymer, and at low salt concentration, a repulsive peak is obtained at low q. In the latter regime, it is the net charge of the protein that is of importance for the intermolecular interaction, charge distribution plays a minor role. Preliminary results also indicate that the peptide conformations are dependent on pH (in the salivary pH range) and the prescence of divalent ions. This indicates that electrostatic interactions indeed are important for the Histatin 5 bulk structure.

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SAXS study of structure and phase behaviour of pig gastric mucin at different temperatures and hydration levels

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One essential function of mucous gel is to protect the mucosa from dehydration. Mucus properties respond quickly to changes in ambient conditions, and when the relative humidity (RH) of the surrounding atmosphere decreases it undergoes a transition from elastic to glassy state. According to our previous calorimetric studies [1], this mucin glass transition occurs at an RH between 60-70%. Here, hydration and temperature-induced changes in pig gastric mucin (PGM) in a wide concentration range were studied using small angle X-ray scattering (SAXS). This work demonstrates three ranges of the scattering vector q corresponding to different fractal dimensions in PGM solutions. Such scattering can originate from PGM fiber-like structures that adopt random coil conformation in dilute solutions. Starting from about 20 wt% PGM, three peaks are clearly visible in the scattering pattern and they become more pronounced at intermediate concentrations, indicating structuring in the mucin system at lower levels of hydration. In strongly and fully dehydrated mucin, where the system is in a glassy state, these peaks do not appear. The SAXS data show that the structural changes at about 80 wt% of mucin at 25℃ correspond to a mucin glass transition, in agreement with our previous calorimetric results [1].
Temperature-induced changes in the phase behavior of mucin were observed at about 60-70℃ at

intermediate levels of hydration. Here the single main peak becomes double, indicating formation of a different structure at elevated temperatures. These results are in a good agreement with polarized light microscopy and DSC data. Obtained SAXS data are used to complete the PGM phase diagram.

Acknowledgements: The MAX IV - laboratory in Lund (Sweden) is acknowledged for providing time to run SAXS measurements. Authors are grateful to Drs. Tomas Plivelic and Sylvio Haas for technical assistance when running X-ray experiments at MAX IV / beamline I-911. Malmö University, Biofilms – Research Center for Biointerfaces, the Knowledge Foundation (KK-stiftelsen), the Gustav Th Ohlsson Foundation and the InterReg ESS & MaxIV: Cross Border Science and Society are thanked for financial support.

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Mucoadhesion - A Prerequisite or a Constraint in Nasal Drug Delivery?

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Mucoadhesion - A Prerequisite or a Constraint in Nasal Drug Delivery?

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The nasal cavity offers an attractive route for systemic drug delivery. Administration is easy with a rapid onset, first-pass metabolism can be avoided and there is a potential possibility to circumvent the blood-brain barrier. One major problem with nasal administration, however, is to circumvent the effect of mucocilliary clearance and prolong duration of an applied formulation at the site of action. The most common way to accomplished this is by adding various polymers to the formulation to induce interactions with the mucosa. However, this also lowers the water activity of the formulation and imposes a water gradient over the mucosa, which potentially induces a mucosal response affecting the barrier structure and properties.
The aim of this project was to determine if a nasal formulation with low water activity, that favours mucoadhesion, would also induce a mucosal response detrimental to drug absorption. We approached this problem by performing drug permeability studies ex vivo in flow-through diffusion cells with porcine nasal mucosa at 32℃. Donor formulations comprised Xylometazoline HCl dissolved in PBS pH 7.4 and PEG 1500 were used to adjust the water activity in these formulations from 1 to 0.8 (=65% PEG)1. Drug solubility was determined to match the thermodynamic activity of the drugs in the alternative formulations.
Our results show that a water gradient can be used to regulate drug flux over nasal mucosa, similar to what has previously been shown with oral mucosa and skin1, 2. We have also shown that drug permeability over nasal mucosa is much higher than over oral mucosa and skin (nasal > oral > skin)1,2. If mucoadhesion is achieved through water sorption by the applied formulation this mechanism will counteract drug uptake. The present data shows the importance of understanding water sorption and how it affects drug transport in nasal drug delivery systems.

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Transparent electrodes for biofuel cell applications

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Recent advances reached in nanotechnology have made technically possible to fit electronics in contact lenses. The continuous monitoring of some compounds, such as blood glucose, can help to increase the quality of life of diabetes patients. Contact lenses are minimally invasive, and the so-called smart electronic contact lenses can offer real-time, non-traumatic bio-sensing. As the name implies, electronic circuitry relies on electric power, and even though the power needed for a micro and nano-electronics is minimal, some power is required [1]. Thus, the development of micro or nano-scale power generator devices is needful and can be achieved through biofuel cells that generate electric power from chemical energy using different biofuels, such as glucose, ascorbate, and dopamine [2]. Here we present macro-scale, nanostructured, enzymatic electrodes including transparent bio-devices, which can be used as energy power source. The designed electrodes include indium tin oxide (In2O3/SnO2, ITO) as a conductive support, which is optically transparent in a broad range of wavelengths and it has good electric conductivity [3]. By adding a thin film of ITO nanoparticles, the real surface area can be significantly increased. In our case, ITO transparent and nanostructured electrodes are used to design high-performance capacitive CDH based anodes and BOx based cathodes for biofuel cells due to high enzyme loading and high double-layer capacitance of nanoparticle modified ITO surfaces. Thus, we believe this is a promising approach to develop biofuel cells for electronic contact lens applications.

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Adsorption of atherosclerotic lipoproteins to supported lipid bilayers

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Atherosclerosis is a major contributor to global morbidity and mortality with 31 % of all deaths in 2008 linked to cardiovascular disease [1]. It is has been shown that various lipoprotein particles in the blood play an important role in the development and subsequent rupture of atherosclerotic plaques [2, 3]. Current best practise for diagnosis and further monitoring of the progress of atherosclerosis is by measurement of the ratio of high (HDL) to low (LDL) density lipoproteins in the blood, with

high proportions of HDL showing a protective effect on the body [4]. In this work we aim to study the interaction of lipoprotein particles with cell membrane mimics using scattering methods that are especially sensitive to the adsorption, exchange and uptake of lipids from bilayers.

High and low density lipoproteins are aggregates of lipophilic cholesterol esters and triglycerides encapsulated by a lipid monolayer and apolipoproteins. The two classes differ in density, apolipoprotein and absolute component amounts. HDL is often known as the 'good cholesterol' due to its ability to clean up and remove cholesterol from fatty deposits, whereas LDL is considered 'bad cholesterol' as it has a tendency to deposit cholesterol to the arterial wall in the form of atherosclerotic plaques [5-7]. We have studied the interaction of lipoproteins with supported lipid bilayers using a number of surface sensitive techniques including, neutron reflection and QCM-d. Through the combination of neutron reflection with deuterated and hydrogenated supported lipid bilayers it is possible to monitor adsorption of the lipoproteins as well as the exchange and uptake from the lipid bilayer. Results show that exchange is greater with LDL whereas HDL shows a high level of lipid removal from the bilayer. Moreover, the extent of lipid exchange and lipid removal was also dependent on the charge of the supported lipid bilayer and the co-addition of LDL and HDL. These results highlight the role of lipoprotein particle type on their ability to exchange and deposit/remove lipids from model cellular membranes, as well as the existence of competitive processes between these particles and the model membrane.

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Synchrotron imaging of soft tissue biopsies

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Phase-contrast x-ray imaging has recently been proven to give improved contrast in soft tissue samples. In particular with highly brilliant synchrotron radiation, superior images can be obtained: Very good contrast with resolution ranging from micrometers to a few tens of nanometers. This is an excellent tool for studying three-dimensional morphology in many different fields of science.

With grating based interferometry phase-contrast imaging can be achieved at virtually any source, including Neutron sources, Synchrotron sources and ordinary x-ray tubes.

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Trapped in a crystal: Towards a new crystallographic method for structural determination of biomolecules

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X-ray crystallography is the most used method to obtain atomic resolution structures of proteins that allow us to understand their functions in the cell. A drawback is that not all proteins can grow crystals, which is a requirement for this technique. We aim to overcome that problem by trapping proteins into pores of already existing crystals of the tryptophane repressor protein from *E.coli* (TrpR). TrpR crystallizes in 30 -35 % isopropanol solution in a domain swapped conformation, forming an infinite network through the crystals which leads to a high stability of the crystals (Lawson et al. 2004). The structure of the domain swapped TrpR (PDB: 1MI7), solved in space group P6122, reveals large, straight solvent channels of 60-70 Å diameter width alongside the 61-screw axis that penetrate the crystal. The channels are large enough to accommodate small proteins or other biomolecules of up to ~30 kDa size. Soaking experiments with colored, small proteins such as cytochrome C and Texas Red® labelled calmodulin, added in lyophilized form to the TrpR crystals containing drops, show that the TrpR crystals stain within 1-2 h (Sprenger et al., unpublished observation). We are currently analyzing X-ray diffraction datasets of TrpR crystals with soaked calcium binding protein calbindin-D28K (CB). To constrain the orientations of CB we use constructs that contain cysteine residues at the surface that can be used to crosslink the protein to the N-terminal cysteine of TrpR that faces the channels. Furthermore, we replaced the bound Ca2+ of CB with Lanthanum that allow us to obtain anomalous diffraction datasets. The structure solutions for CB containing TrpR using MR show distinct changes of the electron density inside the channels compared to TrpR crystals without soaked protein. The results are indicating that the proteins are inside the channels contribute to the Braggs diffraction.

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The effect of pH and salt on the structure and molecular mobility of stratum corneum

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The uppermost horny layer of the skin, the stratum corneum (SC), serves as a protective barrier for terrestrial life. In dry and humid condition, this barrier experiences enormous osmotic shock and responds accordingly – the so-called responding membrane. To investigate the effect of hydration influence on the SC lipid and protein components at different pH, we performed natural abundance 13C solid-state nuclear magnetic resonance (ssNMR) experiments. The experiments were carried out on intact SC and isolated corneocytes (protein components) and provide molecularly detailed information on the dynamics of SC lipid and protein components. The effect of salts (mono and divalent cations) was also investigated, as they are part of natural moisturizing factor (NMF), a group of small polar hygroscopic molecules present in the skin and prevents skin from severe drying. We hypothesized that NMF components may be extracted from the SC upon washing in water, leading to changes in the molecular mobility in SC components, which in turn may affect the SC barrier properties. As NMF functions retaining molecular mobility in SC components similar to hydrated

skin also at dehydrated conditions [1], we later challenge this hypothesis by supplementing several classes of NMFs to the water treated SC and corneocytes. We show that hydration at different pH enhance mobility of the SC lipid and protein components. This effect seems slightly pronounced at pH 4.0 compared to pH 7.4. The addition of salt reduces the molecular mobility of the SC, which is in contrast with other NMF compounds, e.g. urea and glycerol. Small angle X-ray diffraction studies provide further insights on the structure of SC and corneocytes: hydration at various pH affects the corneocytes envelop and keratin structure while the SC lipid lamellar structure seems unaffected. These results provide a new molecular understanding on how the SC and corneocytes mobility can be tweaked by varying pH and would be beneficial in future research.

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Molecular dynamics simulations and neutron reflectivity as an effective approach to characterize biological membranes and related macromolecular assemblies

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In combination with other spectroscopy, microscopy, and scattering techniques, neutron reflectivity is a powerful tool to characterize biological systems. Specular reflection of neutrons provides structural information at the nanometer and subnanometer length scales, probing the composition and organization of layered materials. Currently, analysis of neutron reflectivity data involves several simplifying assumptions about the structure of the sample under study, affecting the extraction and interpretation of information from the experimental data. Computer simulations can be used as a source of structural and dynamic data with atomic resolution. In this talk, a tool to compare the structural properties determined by neutron reflectivity experiments with those obtained from molecular simulations will be presented. This tool allows benchmarking the ability of molecular dynamics simulations to reproduce experimental data, but it also promotes unbiased interpretation of experimentally determined quantities. Two application examples will be presented to illustrate the capabilities of the new tool.

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When thrown into the wonderful mix of constraints and desires, there is a product and a customer…

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As scientist by training, I found the first years in product development difficult when managers didn't want to make use of all data, when the best solution was not chosen.

Or was it? Was perhaps the thermodynamically stable formulation not the wanted one, when all parameters were weighed?
Now, after some more years I've learned to start with the customer and see how science can give her what she wants. And I'm not speaking about my customer; I'm speaking about my customer's customer. The end user.

To be able to make advanced techniques interesting for industry we have to take fundamental steps away from the techniques. We have to understand the target profile of the product.

To be able to make it interesting enough for industry to invest in, we have to take fundamental steps away from thinking "industry" and thinking "human being working at an industry". If it is an investment to be made – someone might be investing a career by doing so – take some time and think about them.

My talk will be about how we at CR Competence try to balance still being scientists while extending our understanding of industry, business and human beings.

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Molecular transport in lipid membranes: lipid exchange and translocation processes investigated by neutron scattering

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The phospholipid bilayer is the basic structural motif of most biological membranes. As such, many biological processes occur within or in the proximity of the cell membrane, and therefore, interest in the properties and behavior of lipids in membranes is considerable. For example, it is found that in nature the lipid distribution across the inner and outer leaflet of cell membranes is asymmetric [1] and this asymmetry plays a prominent role in processes like cell fusion, activation of the coagulation processes and the recognition and removal of apoptotic cells by macrophages. Therefore there is great interest in studying the factors determining lipid movement across membranes as well as the resulting lipid mapping in the membrane, both of which are far from being understood and characterized.
In the literature it is found that there are big discrepancies in the timescale of the occurrence of lipid flip-flop in model bilayer systems, partly due to the fact that these measurements were based on the indirect observation of the process [2,3]. However, with the sub-nanometer spatial resolution of neutron reflectometry, it is possible to directly obtain lipid composition differences in the leaflets of a bilayer, and in particularly resolve them for times scales as short as a few minutes.
Starting from these results we extensively studied, by neutron reflectometry, temperature and time dependence of the structure of lipid bilayers looking for traces of structural asymmetry and consequent relaxation towards an equilibrated symmetric bilayer.
We discovered that the structure of an asymmetric reconstituted lipid bilayer spontaneously relaxes, on a subsecond time-scale, if the lipids are in the fluid phase i.e. in biological relevant conditions [4].
The same results were confirmed by the monitoring the time- and temperature-dependence of the structure of a solid supported lipid bilayer exposed to a solution of isotopically labeled vesicles [5].
In this case, lipid interbilayer exchange was shown to be the time limiting process, while lipid intrabilayer movement (flip-flop) was too fast to be visualized within the experimental acquisition time.
The exchange process was characterized by an Arrhenius-like behavior and the activation energy of the process was found to be concentration-independent. The combination of the two results offers a novel point of view on the characteristics of inter- and intra-bilayer rearrangement processes.

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High-resolution structure, interactions, and dynamics of self-assembled biomolecular complexes

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Refolding of SDS-unfolded proteins by non-ionic surfactants

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Metabolic plasticity as mediated by the dhurrin metabolon

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Structural and dynamics studies of a truncated variant of CI repressor from bacteriophage TP901-1

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Probing the structure and dynamics of HSA using neutron scattering

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Synchrotron imaging of soft tissue biopsies

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Lipoprotein structure dependency on lipid cargo and exchange dynamics - Implications for atherosclerosis development

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Neutron Reflection

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Realizing the potential of research infrastructures

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Delivering the full potential of MAX IV – Sweden's so far most ambitious infrastructure project, will require efforts and collaboration by many parties and on many different levels. Not only do one need to secure that the MAX IV suite of beamlines matches the needs of academia and industry but also that they have the relevant methods implemented, are equipped with the right sample environment and that the best analysis tools are available to convert raw data to scientific insights. Furthermore, scientist needs to be trained and re-trained to make the best use of the available and rapidly developing instruments, methods and analysis tools. In my talk I will elaborate on what I see as the key steps that are being taken or needs to be taken to optimise the value of MAX IV for its users and funders.

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A structural and functional investigation of Ribonucleotide reductase Class III in Bacillus cereus

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All known cellular life forms to date store their genetic information as DNA by utilising an arrangement of four different nucleotides. It is well known that ribonucleotide reductase (RNR) is responsible for the rate-limiting step of DNA synthesis by producing deoxyribonucleotides from its ribonucleotide precursors. Because RNR is vital for DNA synthesis it is an important target for anticancer, antibacterial and antiviral agents. The RNRs are divided into three classes, consisting of one catalytic unit and a radical initiator unit. They all initiate ribonucleotide reduction via a transient protein-based thiyl radical, by

abstracting hydrogen from the 3ÅLposition of the ribose ring, but they differ in metal cofactors, radical chemistry and their response to oxygen. The pathogenic bacterium Bacillus cereus uses both class Ib and class III RNR. The RNR class III is only active during strict anaerobic conditions due to an oxygen sensitive glycyl radical in the catalytic unit (NrdD) and an iron-sulphur cluster, which is inactivated by oxygen on the radical initiator unit (NrdG). The focus of this project is to understand both the activation of NrdD by NrdG, and the re-activation of NrdG by a more general redox network in the model organism Bacillus cereus .We are currently expressing and establishing purification procedures for the NrdD and NrdG followed by crystallisation trial. There are, at present, no structures of NrdG from any organism. To understand the activation of NrdD, a combination of structural and spectroscopic studies will be performed in addition to binding and kinetic studies. This knowledge should give new insight into class III RNR activation, and confirm or contribute to a reviewing of the current reaction mechanism of this class of RNR.

References

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