# NSSM´19

## 16th Nordic workshop on Scattering from Soft Matter

# Abstracts


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# Oral presentations

**Mapping the transport of fats by lipoproteins**

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The metabolism of lipids including cholesterol involves the production of lipid carrying particles known as lipoproteins. Lipoproteins are nano-emulsion like particles composed of fats and proteins (named apolipoproteins). The complexity of lipoproteins is great, with different compositions not only in terms of the amounts of the fat and proteic components, but also on the specific protein type and isoform. Specific apolipoproteins isoforms are known to mark an increased risk for developing atherosclerosis (and other amyloid/plaque forming diseases) where fat accumulation to form plaques occurs at the initial stages of this terrible disease. In this talk, I will present the efforts of my group to explore the role of lipid dynamics in their transport throughout the body by lipoproteins. We show the unique power of using small angle neutron scattering and neutron reflection as two complementary techniques to map the structural and compositional changes taken place at both lipoproteins and model membranes over time as lipid exchange and takes place. Contrast matching and deuteration are key to highlight the different aspects of these complex natural nanoparticles. In particular, the reproducible and controllable methodology we have developed so far allows to follow the kinetics of lipid exchange within the lipid monolayer stabilising lipoproteins and the model membranes as a function of the composition of the donor membrane. Our results that the good cholesterol (HDL particle) is better at removing lipids than the bad cholesterol (LDL particle) from the model membrane in agreement with the biological functions of these particles. Our most recent results show that HDL removes to a larger extent saturated than unsaturated fats, while the presence of as little as 10 mol% cholesterol dramatically decreases the ability of HDL to remove saturated fats. These effects occur despite the fact that our studies are performed at body temperature, where the membranes are expected to be in the fluid phase. By correlating the functional data with proteomics on the lipoproteins fractions studied, we will better understand how HDL is able to remodel atherosclerotic plaque. This in turn will empower designing better nanocarriers for HDL therapy.

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# Effects of oxidation on the physicochemical properties of polyunsaturated lipid membranes

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The exposure of biological membranes to reactive oxygen species (ROS) plays an important role in many pathological conditions such as inflammation, infection, or sepsis. ROS also modulate signalling processes and produce markers for damaged tissue. Lipid peroxidation, mainly affecting polyunsaturated phospholipids, results in a complex mixture of oxidized products, which may dramatically alter membrane properties. Here, we have employed a set of biophysical and surface-chemical techniques, including neutron and X-ray scattering, to study the structural, compositional, and stability changes due to oxidative stress on phospholipid bilayers composed of lipids with different degrees of polyunsaturation. In doing so, we obtained real-time information about bilayer degradation under *in situ* UV exposure using neutron reflectometry. We present a set of interrelated physicochemical effects, including gradual increases in area per molecule, head group and acyl chain hydration, as well as bilayer thinning, lateral phase separation, and defect formation leading to content loss upon membrane oxidation. Such effects were observed to depend on the presence of polyunsaturated phospholipids in the lipid membrane, suggesting that these may also play a role in the complex oxidation processes occurring in cells.

# Understanding blood cell stabilization – Effect of plasticizer on lipid monolayers

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Plastic bags are used to collect and store blood for transfusion purposes. The most common material used for these bags is polyvinylchloride (PVC) which is plasticized with di(2-ethylhexyl) phthalate (DEHP). DEHP is inherently toxic [1] and leeches out of the polymer matrix. However, DEHP has been shown to stabilize red blood cells and thereby increase the shelf life of stored blood [2,3]. We have studied how DEHP interacts with a lipid monolayer by utilizing a simple binary model system, consisting of the lipid 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and the plasticizer DEHP. This investigation has been performed primarily by surface pressure measurements, using a Langmuir trough, and by neutron reflectometry. Deuterated DEHP was synthesized and used in the reflectivity measurements to identify that component directly.

Monolayers of DMPC behave significantly differently when DEHP is introduced. First, a monolayer with high surface pressure is formed at larger areas when DEHP is present. Further, a plateau in the surface pressure becomes apparent as the DEHP content is increased in the monolayer. Following the isotherm studies, neutron reflectivity was measured at surface pressures above and below the plateau-region for four different compositions (0, 20, 40 and 60 mol% DEHP respectively) and in two different contrasts (deuterated DMPC + hydrogenous DEHP and hydrogenous DMPC + deuterated DEHP). Data could be well-fitted as uniform monolayers which suggests that there is intermixing at a molecular level. At 60 mol% initial DEHP content, DEHP content is reduced in the monolayer as the surface pressure increases. This is not the case for the other monolayer compositions which indicates that DEHP is squeezed out of the monolayer at high surface pressures when the content is too high. At least 25 mol% of DEHP remains at surface pressures of 30 mN/m. Data representing this are shown in Figure 1.

Given that the DEHP mixes with the DMPC in the monolayer, we conclude from the pressure-area isotherms that DEHP helps the formation of a more compact monolayer even at large surface areas.

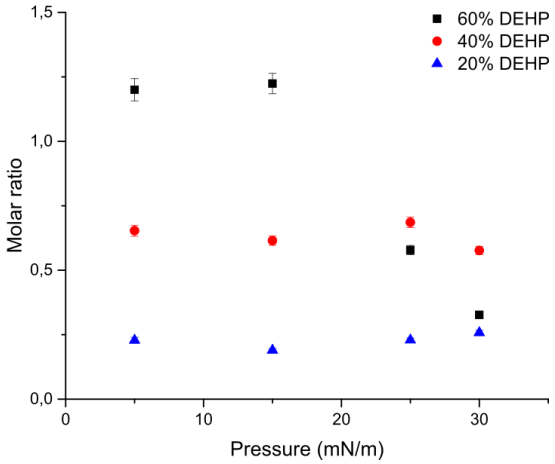


**Figure 1.** Molar ratio between DEHP and DMPC for different surface pressures as obtained from fitting reflectivity data.

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**The kinetic pathways of the solubilisation of lipid membranes by detergents seen by SAXS**

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Triton X-100 and sodium dodecyl sulphate (SDS) constitute some of the most widely used detergents in membrane protein studies. A more detailed understanding of the molecular interactions with the membrane could guide the development of better methods for protein purification and reconstitution from biological membranes. By using time-resolved small-angle X-ray scattering (TR-SAXS) and the stopped-flow technique, we have studied the effect of Triton X-100 and SDS on liposomes, vesicles consisting of bilayers of dipalmitoylphosphatidylcholine (DPPC)-phosphoplipids at 20 degrees Celsius. The ratio of Triton X-100 to lipid needed to completely solubilise the membrane was found to be very high at this temperature, and the process of solubilisation was resolved at timescale of minutes. Triton X-100 was also found to have other, long-time effects on the liposomes at the lower ratios, where the small-angle scattering (SAXS) data indicate the transition from unilamellar to multilamellar bilayer structure. This effect was not seen when using liposomes with a small amount of charged phospholipids, where the Triton X-100 instead seems to rearrange the liposome into cylinder-like structures. In contrast, SDS was found to merely insert into the bilayer at this temperature for both charged and uncharged liposomes. The results require further investigation and data modelling to understand the kinetics of the surfactants at this temperature, which will yield novel information to the field of membrane solubilisation, which has not been studied to any great degree using small angle scattering techniques before.

# 2D and 3D orientational imaging of bone and cartilage using small- and wide-angle X-ray scattering

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Bone and cartilage are hierarchical biological tissues, where many of their structural properties are related to *orientation* of the different constituents at the microscale. Conventional techniques used to study bone and cartilage, such as histology[1] and micro-CT[2] requires tedious sample preparation to provide contrast, nor is it possible to obtain orientational information. Using scanning small-angle X-ray scattering (sSAXS)[3] and X-ray diffraction computed tomography (XRD-CT)[4] we show that chemical and orientational information[5,6,7] from fine structural details of the epiphyseal growth cartilage and the subchondral bone (cf. Figure 1) of a porcine femoral joint can be resolved in 3D.

The combined information of these X-ray scattering techniques provides insights into cartilage mineralization and bone growth, and is a powerful tool to gain detailed data on the pathology of cartilage-bone diseases such as osteochondrosis and osteoarthritis.

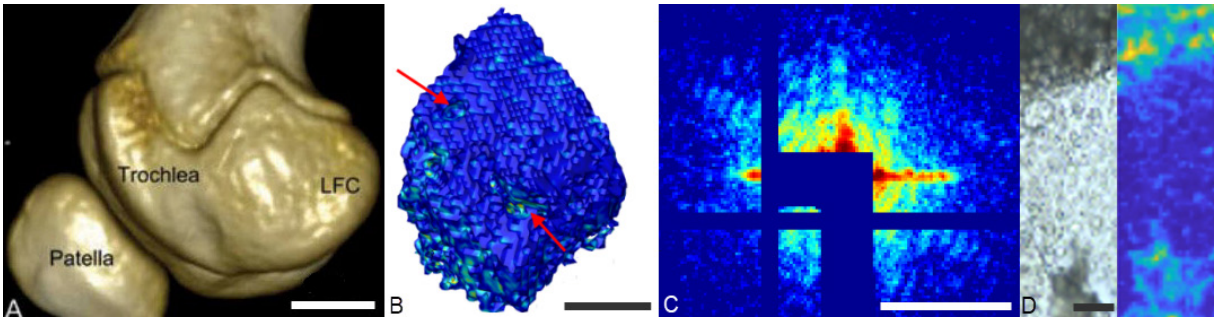


Figure 1: A) Volume-rendered CT image of the distal femur and patella from a Landrace piglet. Image adapted from [8]. Abbreviation: LFC., lateral femoral condyle. Bar is 1 cm. B) 3D XRD-CT tomogram of the mineralized cartilage and subchondral bone of a porcine medial femoral condyle. The red arrows point to holes in the bone-cartilage interface where cartilage canals pass through the interface. Bar is 1 mm. C) A SAXS pattern obtained from the bone-cartilage interface showing the anisotropic nature of the small-angle signal. Logarithmic color scale. Bar is 0.1 $nm^{-1}$ D) Left panel: Bright field micrograph of the bone-cartilage interface and a cartilage canal (bottom). Darker regions are bone and a cartilage canal, while the brighter region is cartilage. Bar is 100 µm. Right panel: Corresponding scanning SAXS map from the integrated scattered signal (whole detector region).

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## Small-angle scattering model for characterizing wood nanostructure and moisture behavior

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Small-angle neutron and x-ray scattering (SANS and SAXS) offer efficient tools for characterizing the nanoscale structure of wood. They are particularly sensitive to the cross-sectional dimensions and mutual organization of cellulose microfibrils having a diameter of 2-3 nm. The packing of the microfibrils is influenced by sorption and desorption of water and the structural changes caused by water can be observed with SANS and SAXS. However, the analysis of the scattering data to obtain quantitative information is challenging and currently no established way applicable to wood samples exists.

In this contribution, we present a model based on cylinders packed in a hexagonal lattice with paracrystalline distortion, which was used to successfully analyze SANS and SAXS data from wood samples under wet and dry states as well as during drying. The optimized fitting parameters reflected the changes in the nanostructure of wood during drying, which was particularly highlighted by a decrease of the interfibrillar distance from around 4 to 3 nm. A version of the fitting model compatible with the SasView software will be made publicly available in order to allow also non-specialist users to apply it in their data analysis.

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Prof. RAUTKARI, Lauri (Aalto University, Finland)
Prof. ÖSTERBERG, Monika (Aalto University, Finland)

# Grazing incidence small angle neutron scattering: Challenges and Opportunities

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Neutrons are characterised by a weak interaction for many engineering materials. As a result they can penetrate deeply into matter and are sensitive to light elements. If applied under grazing incidence beam geometry (see figure 1, right panel) neutrons can offer surface sensitivity and the opportunity to address relevant scientific challenges in connection with solid-liquid, like e.g. surface slip [1], or liquid-liquid boundaries. Reflectometry experiments probe the scattering length density along the normal of interfaces by analysing the specularly scattered intensity. Lateral fluctuations result in intensity scattered away from the specular condition. I will discuss the principles and peculiarities of grazing incidence scattering experiments [2]. One specific example, the self assembly of polymer micelles close to interfaces, is taken as a show case in order to introduce the scattering geometry and accessible length scales and the basic idea of the distorted wave Born approximation is lined out.

The low absorption and brilliance of neutrons beams imposes challenges on the feasibility of depth resolved GISANS measurements. On the example of a micellar surfactant solution we will analyse the limitations and opportunities of the method. For surface layers of micrometer thickness a clear difference in the structure can be resolved [3] and some qualitative depth information can be extracted [4]. However, it turns out that the signal emitted from a specific depth is always limited and as a result extracting specific information form well defined distances from an interface remains challenging [5]. In the future a combined approach of optimised instrumentation and sample design may allow to overcome this limitations by using reference layers and resonators.

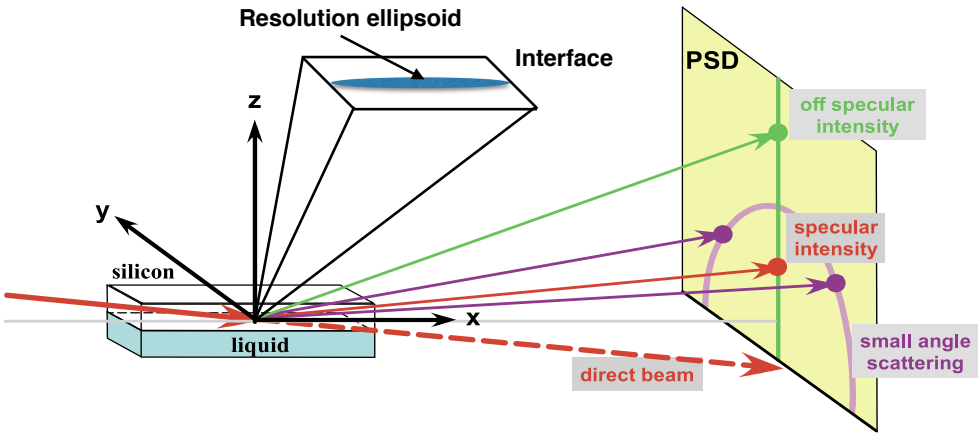


**Figure 1:** Scattering geometry for gracing incidence scattering experiments from the solid-liquid boundary [2]. The strongly anisotropic resolution offers the opportunity to address multiple length scales but imposes challenges with respect to the analysis of the data.

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# Screening of protein interactions, aggregation pathways, higher order structures and stability with advanced laboratory SAXS

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Small Angle X-ray Scattering (SAXS) is a very fast technique for obtaining structural information on protein complexes in solution. With its high sensitivity to aggregation, the ability to study protein-protein interactions and determine the higher order structure, SAXS is a valuable tool for formulation development. Through structural, as well as aggregation and stability studies, it can greatly support optimization of immunogenicity and shelf-life.

Data acquisition and analysis within minutes also renders SAXS an excellent initial step to make quick decisions in the formulation development process. Being non-intrusive and performed in a native-like environment, SAXS is optimally suited for validation of protein folding and higher order structure obtained through high-resolution but intrusive characterization techniques such as crystallography and cryoEM.

With the introduction of its dedicated solution SAXS instrument, the BioXolver, Xenocs moves the sample handling technology previously only seen at synchrotrons (low volumes, high throughput and multimodal measurements) into the home laboratory. The BioXolver is equipped with a level of automation that enables users of any skill level to obtain high quality data and results within minutes.

Designed with the ability to perform large-scale screening and automatic mixing of multiple sample components immediately prior to the measurements, the instrument facilitates the study of biomolecular kinetics, previously not feasible in an automated fashion on laboratory SAXS instruments. By integrating computer vision technology, only 5 uL of sample are required and in-line UV/Vis absorption measurements enable automatic concentration determination on the exposed sample.

Additionally, the BioXolver is the first laboratory instrument with published and proven size exclusion chromatography (SEC)-SAXS capabilities[1], extending the capabilities of laboratory SAXS to more complicated samples which previously required synchrotron radiation.

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# MICROFOCUS SOURCES PLUS MULTILAYER OPTICS AND SCATTERLESS APERTURES FOR SAXS EXPERIMENTS IN THE HOME-LAB

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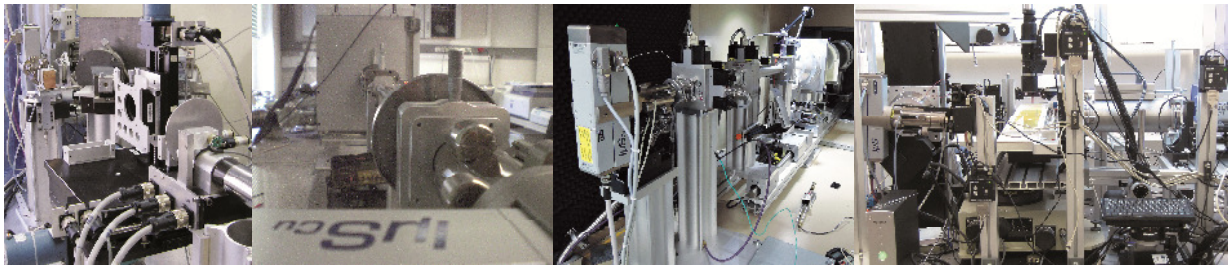
Nowadays, X-ray optical components, such as multilayer mirrors or scatterless apertures, are used as beam conditioning devices in nearly all state-of-the-art X-ray analytical equipment, either in the home lab or at synchrotron beamlines. We will be discussing pinholes, optics and their use in combination with high brilliance laboratory sources for SAXS applications.

Scatterless pinholes are usually made of oriented single crystals, such as Ge or Ta, and show a significant reduction of parasitic scattering commonly associated with conventional metal apertures. Therefore, such pinholes allow an improvement of X-ray analytical instruments as the number of necessary pinholes can be reduced. Further, the use of scatterfree pinholes enables a significant reduction of the background. This improves the data quality at low resolution which is beneficial for small angle scattering, as well as for crystallography applications. Our SCATEX pinholes are either made of Germanium for energies below 11.2 keV or of Tantalum for energies above 11.2 keV and are available with diameters ranging from 2 mm down to 20 µm and below. Therefore, these novel apertures are applicable to a wide range of different applications. We will be showing results about development and use of these pinholes.

Multilayer X-ray mirrors are widely used as monochromators and beam shaping devices. The so-called Montel optics consist of bent substrates with shape tolerances below 100 nm, upon which multilayers are deposited with single layer thicknesses in the nanometer range and up to several hundreds of layer pairs. The multilayers are designed with lateral thickness gradients within ± 1% deviation of the ideal shape. Very low shape tolerances below 100 nm and figure errors well below 5 arcsec are required for multilayer mirrors to ensure a superb flux density in combination with very high-brightness microfocus X-ray sources such as the Incoatec Microfocus Source IµS.

The air cooled IµS is available with Cr, Co, Cu, Mo, and Ag anodes. The implemented Montel optics form either a highly collimated beam with a low divergence (below 0.5 mrad) or a focusing beam with higher divergence (up to 10 mrad) and very small focal spots (diameter below 100 µm). Applications realized with an IµS are small-angle X-ray scattering, texture, stress analysis, µ-diffraction, single crystal diffraction to name but a few. In our presentation we will be presenting selected examples of IµS solutions for SAXS and GISAXS with ex- and also in-situ set-ups (see fig.). At modern synchrotron sources, an efficient use of the limited beamtime is an important issue. Compact easy-to-install microfocus sources can be an attractive supplementary equipment for high-end laboratories there as well.



Four systems with IµS (from left to right): 1) XRD/XRR setup in synchrotron optics lab at ESRF, Grenoble, France; 2) Bruker NANOSTAR with SCATEX in Vienna, Austria; 3) IµS and SCATEX Upgrade on a customized SAXS setup at Univ. Hamburg, Germany; 4) GISAXS setup with Dectris detector at Slovak Academy of Science, Bratislava, Slovakia

**Combined RheoSAXS Investigations using a Laboratory SAXS System**

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Using rheology the flow and deformation of a material can be characterized which enables to relate a material's molecular structure to its mechanical properties. By applying mechanical force, e.g. shear to a material, molecular assemblies may orient or crystallize.

Small-angle X-ray scattering (SAXS) can determine the size, shape, inner structure and orientation of nanosized materials. Relating the nanostructure of a material to its macroscopic mechanical properties requires *in-situ* characterization techniques such as rheology combined with SAXS. RheoSAXS experiments have so far been performed at synchrotron beam lines, mainly due to insufficient X-ray flux of laboratory X-ray sources and the lack of a dedicated RheoSAXS laboratory set-up.

In this contribution we present a novel and unique experimental setup for performing combined RheoSAXS studies in one go with the SAXSpoint 2.0 laboratory SAXS system. The integrated RheoSAXS module enables temperature-controlled rheological experiments with *in-situ* determination of shear-induced structural changes of nanostructured materials on a nanoscopic length scale (from approx. 1 nm to 200 nm) by small-angle X-ray scattering.

We will discuss selected combined rheology-SAXS studies of colloidal systems for investigating shear-induced structural changes which were performed with this optimized Rheo-SAXS laboratory setup.

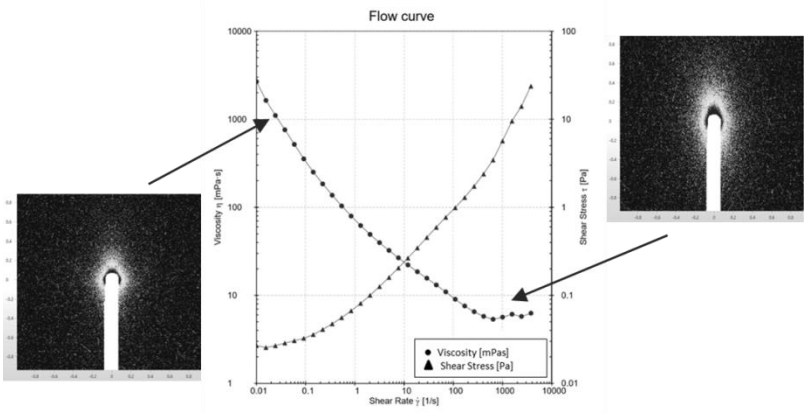


*Figure 1: Flow curve and scattering patterns of a combined rheological-SAXS measurement of dispersed graphite oxide particles exhibiting shear-thinning properties and shear-induced anisotropic scattering.*

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# LIQUID-METAL-JET X-RAY SOURCE FOR IN-SITU SAXS STUDIES IN THE HOME LABORATORY

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High-end x-ray scattering techniques such as SAXS, BIO-SAXS, non-ambient SAXS and GISAXS rely heavily on the x-ray source brightness for resolution and exposure time. Traditional solid or rotating anode x-ray tubes are typically limited in brightness by when the e-beam power density melts the anode. The liquid-metal-jet technology has overcome this limitation by using an anode that is already in the molten state.

We have previously demonstrated prototype performance of a metal-jet anode x-ray source concept [1-3] with unprecedented brightness in the range of one order of magnitude above current state-of-the art sources. Over the last years, the liquid-metal-jet technology has developed from prototypes into fully operational and stable X-ray tubes running in many labs over the world. Small angle scattering has been identified as a key application for this x-ray tube technology, since this application benefits greatly from high-brightness and small spot-sizes, to achieve a high flux x-ray beam with low divergence. Multiple users and system manufacturers have since installed the metal-jet anode x-ray source into their SAXS set-ups with successful results [4, 5]. With the high brightness from the liquid-metal-jet x-ray source, in-situ SAXS studies can be performed – even in the home laboratory [6, 7].

The influence of the size of the x-ray source and its distance to the x-ray optics on the divergence will be discussed, and how to minimize the divergence and maximize the flux in SAXS experiments targeted to specific applications.

This presentation will review the current status of the metal-jet technology specifically in terms of stability, lifetime, flux and brightness. It will also discuss details of the liquid-metal-jet technology with a focus on the fundamental limitations of the technology. It will furthermore refer to some recent SAXS and GISAXS data from users of metal-jet x-ray tubes.

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# RESOLVING THE KINETIC PATHWAYS FOR POLYELECTROLYTE COACERVATE FORMATION USING TIME-RESOLVED SAXS

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Complexes formed by coacervation of oppositely charged polyelectrolytes have recently attracted a large interest due to their possible applications in the biomedical field. Polyelectrolyte coacervates show promising properties to act as carriers providing a high drug loading and triggered release by external stimuli like pH or salt concentration. Understanding the kinetic processes involved in the coacervate formation is a crucial key in practical formulations and being able to selectively tailor and design polyelectrolyte coacervates as drug delivery systems.

The few studies on coacervate formation reported in the literature are mostly related to cocervates formed by homopolymers with an off-stoichiometric charge balance in order to provide the necessary colloidal stabilization in aqueous solutions [1,2]. Alternatively, diblock copolyelectrolytes carrying a charged and a neutral block can form stable core-shell coecervate systems. In this case, the coacervates are stabilised against aggregation and macroscopic phase separation by steric repulsion of the neutral polymer block in the coacervate shell. However, kinetic studies on the formation of core-shell coacervates are even more seldom [3] and miss structural information of the aggregates itself. Therefore studying the formation process with scattering techniques using X-ray or neutron probes where information of size, shape and internal structure of the aggregates can be studied, can significantly contribute to the understanding of mechanisms and kinetic pathways of coacervate formation.

In this work, the coacervation of anionic polyelectrolyte Poly(Sodium 4-Styrene Sulfonate) (PSSS) and cationic copolymer Poly(ethylene oxide)-block-Poly((Vinylbenzyl)Trimethylamonium chloride) (PEObPVBTA) was studied by time-resolved small-angle X-ray scattering (TR-SAXS). Mixing the polyelectrolytes in a stoichiometric 1:1 charge ratio resulted in the formation of stable spherical core-shell coacervates consisting of a central core of complexed PSSS and PVBTA blocks and a neutral PEO corona. Using a stopped-flow apparatus coupled to the synchrotron SAXS instrument, the whole formation kinetics of coacervates could be followed *in situ* from a few milliseconds. Our results show the formation of metastable large-scale aggregates immediately after mixing that subsequently rearrange into core-shell coacervates over time. While the initial cluster formation is very fast and completed within the dead time of the stopped-flow, the subsequent rearrangement becomes significantly slower with increasing molecular weight of the cationic PVBTA block. Interestingly, the overall kinetic process is essentially concentration independent, indicating that the rearrangement process is accomplished by a chain exchange mechanism.

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[3] J. Zhang *et al*, Phys. Chem. Chem. Phys. 16 (2014) 117-127.

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**To understand the antimicrobial activity of the salivary protein Histatin 5**

Marie Skepö
Theoretical Chemistry, Lund University, Sweden

Histatin 5 is a saliva protein that acts as the first line of defence against oral candidiasis caused by *Candida Albicans,* and it also possesses bactericidal effects. The antimicrobial activity has been ascribed to the high content of basic amino acids. Histatin 5 also participate in the formation of a protective layer on smooth tooth surfaces, and thereby prevent microbial colonization and stabilize mineral-solute interactions. It is established that various transitional metals, such as zinc, nickel, copper, and iron are intrinsically present in the saliva and it is hypothesized that the metal binding abilities of Histatin 5 plays an important role for the candidacidal mechanism. Histatin 5 binds zinc and copper and possesses definitive metal binding motifs for copper and nickel as well as for zinc. In this presentation I will discuss how we use computer simulations on both the atomistic and coarse-grained level in combination with experimental techniques such as scattering and surface adsorption techniques to achieve a molecular understanding of the systems of interest. Furthermore, I will present our latest results regarding Histatin 5 in solution, its interaction with multivalent ions, and the interaction with bilayers corresponding to model cell membranes. The possibility of using Histatin 5-spermidine conjugate as an anti-fungal drug will also be highlighted.

**From Tubes to Twisted Ribbons in a Self-Assembling Model Peptide System**

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Many peptides are known to self-assemble in aqueous solution into long fibrillar or twisted ribbon aggregates, and in some cases nanotubes are observed. In the short model peptide system $A_nK$, varying the length of the hydrophobic alanine chain, *n*, from short to long, one can observe both self-assembled hollow tubes (n=6) and twisted ribbons (n=8,10). The aggregates have been fully characterized, and simple thermodynamic models describing them have been constructed. The models take into account the various hydrogen bond deformations occurring when the peptides assume the different final aggregate structures. Comparing the models verifies the experimentally observed transition from tubes to twisted ribbons with increasing *n* in $A_nK$. This type of fundamental understanding of the aggregate structure and thermodynamics within self-assembling peptide systems can be helpful, both in tailoring novel biomaterials, but also to better understand certain diseases, where protein self-assembly plays a decisive role.

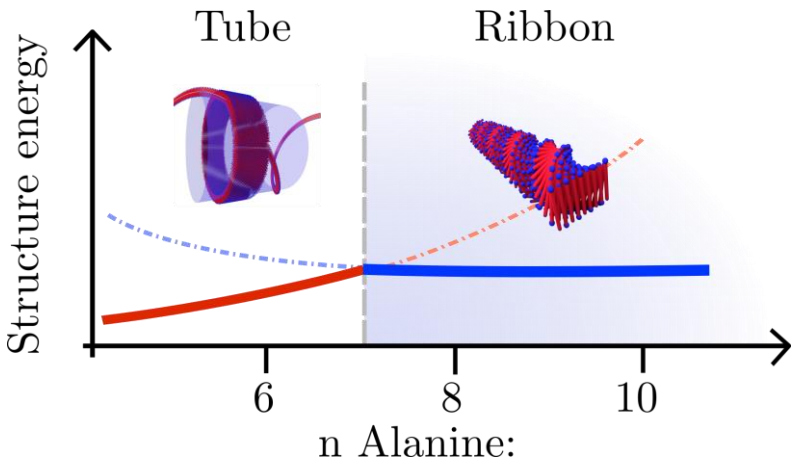


*Figure 1. A schematic representation of the total structure energies as a function of total alanine content in the AnK model peptide system.*

# A deeper look into the self-assembly process of phospholipid nanodiscs

*Lise Arleth[a], Nicholas Skar-Gislinge[a], Nicolai Tidemand Johansen[a] and Rasmus Høiberg Nielsen[a,b]*
[a]Niels Bohr Institute, University of Copenhagen
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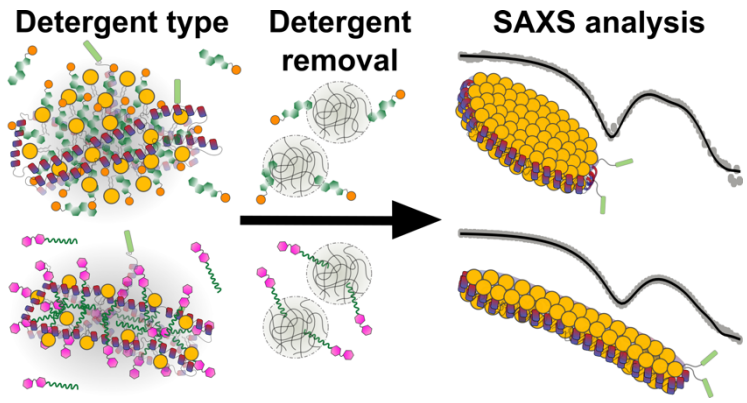
This talk will present and discuss a SAXS study of what we believe are the governing factors in the self-assembly process of phospholipid nanodiscs. The talk is based on very recently published work.[1]




**Abstract:** Phospholipid nanodiscs have quickly become a widely used platform for studies of membrane proteins. However, the molecular self-assembly process that ultimately should place a membrane protein inside a nanodisc is not well-understood. This poses a challenge for a successful high yield reconstitution of general membrane proteins into nanodiscs. In the present work, the detergent mediated self-assembly process of POPC-MSP1D1 nanodiscs was carefully investigated by systematically modulating the reconstitution parameters and probing the effect with a small-angle x-ray scattering analysis of the resulting nanodiscs. We confirm that the discs can be underloaded with lipids when using a too low POPC:MSP1D1 ratio during the reconstitution, but they cannot be overloaded showing that the MSP belt defines an upper limit. We find, somewhat surprisingly, that the speed of the reconstitution process does not affect the final outcome, this is in contrast to the result for liposome reconstitution. Finally, we find that there is a large effect of the choice of detergent used in the reconstitution process. A significantly increasing POPC:MSP1D1 stoichiometry of the formed nanodiscs was observed as the reconstitution detergent type is changed in the order: Tween80, DDM, Triton X-100, OG, CHAPS, Tween20 and Cholate but with no simple correlation to the characteristics of the detergent. All together this point towards that the POPC to MSP1D1 ratio of the POPC-MSP1D1-detergent mixed pre-particles that are formed before detergent removal, is the main determining factor for the shape and stoichiometry of the finally formed discs. The study also emphasizes that the detergents optimal for solution storage and crystallization of membrane proteins, in particular DDM, should not be used alone for nanodisc reconstitution.

[1] **A comprehensive study of the self-assembly of phospholipid nanodiscs: What determines their shape and stoichiometry?** Skar-Gislinge, Johansen, Høiberg-Nielsen and Arleth, Langmuir, 2018, 34(42), 12569-12582.

# IDPs vs. the forces of evil: Finding the biological function of Keif

**Stephanie Jephtah**

**Lund University, Sweden**

Knowing your enemy is essential in war, and this also applies in the war against bacterial infections. In order to develop new antibiotics, it is necessary to not only understand the antibacterial agents, but also the bacteria it is supposed to work against. Magnesium transporter A (MgtA) is a protein found in the cell membrane of *S. typhimurium* and *E. coli*. Recent studies have discovered that the N-terminus of MgtA (amino acid 1-33, from hereon called Keif) is intrinsically disordered, but the benefit of this unstructured part is not yet clear. Thus, the aim of this study is to figure out how the intrinsic disorder of Keif contributes to the biological function of MgtA. Initial bulk studies of Keif have been performed using small-angle X-ray scattering (SAXS) in combination with both all-atom molecular dynamics (MD) simulations and coarse-grained Monte Carlo (MC) simulations. Comparisons between experiment and simulations have indicated that the simulation models are appropriate to use as complementary tools to study the peptide. Investigation of surface interactions have also been done using QCM-D, which showed strong adsorption and interesting magnesium ion concentration dependence. While the study has yielded fascinating results, more research is still needed to deduce the biological function of Keif. Ongoing and future studies includes neutron reflectivity (NR) in addition to further SAXS and simulation studies. By combining all these different methods, we hope to gain a holistic understanding of the biological function of Keif in MgtA, which in the future might lead to new effective antibiotics.

# Sulfobetaine-Phospholipid Interactions and the influence of sub-phase ions

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The majority of surfactants are produced from petrochemical sources and can be harmful or polluting to the environment. Here we present some investigations into novel sulfobetaine surfactants which are known to have lower toxicity and greater biocompatibility than traditional detergents. Despite the technological relevance of these materials, many of their fundamental structural and physicochemical properties are yet to be investigated in detail. The structure of sulfobetaine surfactants is analogous to that of phosphocholine phospholipids; they are also zwitterionic but the positions of the charges are reversed relative to the alkyl tails (see Figure 1). Recent studies of ion binding to sulfobetaine surfactants have hinted at intriguing differences from phospholipids [1] in that monolayers are unaffected by cations in solution. Further, a Langmuir isotherm study has shown that mixed monolayers of sulfobetaines and phosphocholine lipids deviate from ideal behavior [2]. Here we will present X-ray and Neutron reflectometry results that show that the structure of monolayers of SB3-18(2), a di-chain sulfobetaine is modified by the specific binding of iodide ions, effectively the inverse of similar behavior seen for Calcium interactions with phospholipids [3], see Figure 1 (top). Further we have investigated the structure of mixed sulfobetaine-phospholipid monolayers. The data shown in Figure 1 (bottom) indicates that the monolayer structure of mixtures is closer to that of pure DMPC than that of the single chained sulfobetaine SB3-18. We will present a summary of our results so far and also introduce some preliminary results demonstrating that sulfobetaines are able to form vesicles and polymer-stabilized nanodiscs. Together these results demonstrate the analogous but importantly distinct behavior of these surfactants and has significant implications for our understanding of such systems.

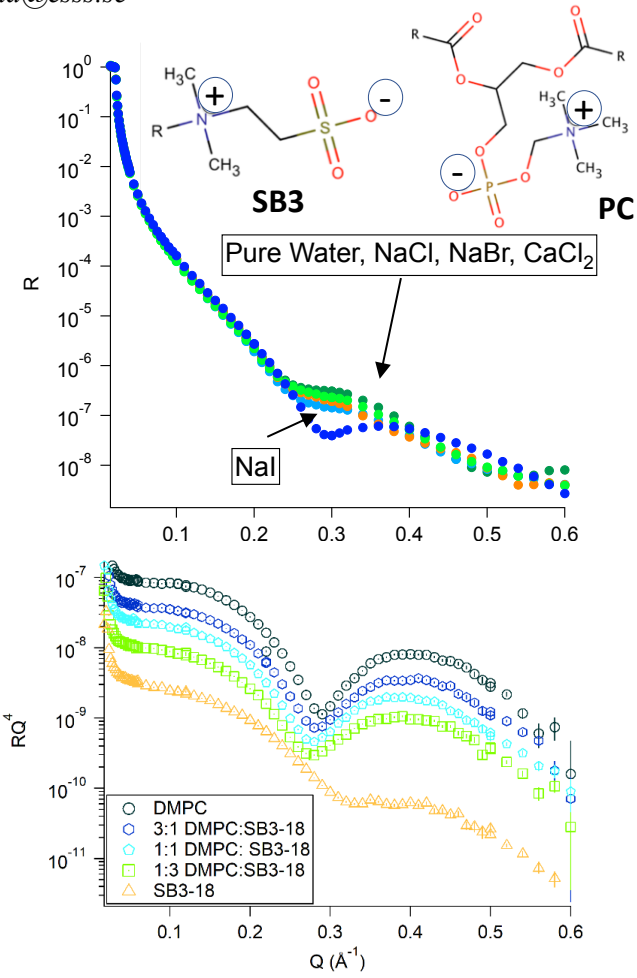


*Figure 1* X-Ray Reflectivity data for (top) SB3-18(2) monolayers on sub-phases containing different ions at 50mM and 30mNm$^{-1}$ (bottom) DMPC and SB3-18 mixtures at 35mNm$^{-1}$

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## SPHERICAL HARMONICS EXPANSION SHOW CHAIN RETRACTION

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The mechanical properties of polymer melts under deformation are well described by the tube model developed in the 1970s by de Gennes and Doi and Edwards. In the tube model, the restricted motion of a given chain due to its neighbors is modeled as though the chain is confined to a tube. However, the validity of the tube model for rapid deformations compared to the molecular relaxation was recently questioned [1]. The authors proposed a new analysis method for anisotropic 2D small angle (neutron) scattering data for uniaxially extended polymer melts expanding the data in spherical harmonics to ease the separation of the isotropic and anisotropic contributions. Using this method, they searched for the signature of chain retraction, explained below, during relaxation after deformation which is a cornerstone of the tube model. The signature was absent in their experimental data and that lead them to conclude that chain retraction either does not occur or that it is shielded by some other non-linear effect not yet included in the tube model.

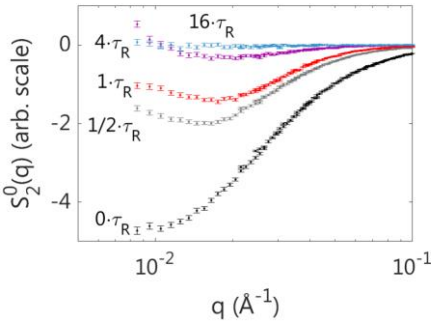


Figure 1: Expansion coefficient, $S_2^0(q)$ for the leading anisotropic contribution to the 2D data followed thorugh relaxation. That the dip shifts towards larger $q$ during $t \leq \tau_R$ show that relaxation of molecular stretching and orientation are decoupled.

However, we employ the same analysis also on small angle neutron scattering data for a polystyrene melt of about a factor of five lower molar mass macroscopically stretched more than a factor of ten more, and we do in fact see the proposed signature in the harmonics namely that the minimum in the expansion coefficient of the leading anisotropic contribution to the scattering pattern shifts towards larger $q$ as the relaxation times approaches the Rouse time, $\tau_R$, as can be seen in Fig. 1. We therefore conclude that the relaxation of the molecular stretching and orientation are decoupled, or in other words that the chain retracts, which supports the tube model.

Chain retraction arises during relaxation if the deformation is fast enough to not only orient the chains but also stretch them. The hypothesis of the tube model is that the relaxation of the stretching of the molecule happens on a fast time scale, $\tau_R$, through a Rouse-like process whereas the relaxation of the orientation happens on a much slower timescale through reptation. That these timescales are well separated causes the molecule to shrink in all dimensions within a timeframe up to $t \approx \tau_R$ after deformation preserving its shape, and only for $t > \tau_R$ does it relax back to its equilibrium shape.

**Acknowledgements**
NordForsk, Danish Council for Independent Research, DanScatt, ANSTO, PSI.

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[2] Z. Wang *et al*, Phys Rev X 7, 031003 (2017)

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# Small-angle neutron scattering and *in situ* UV/Vis absorption spectroscopy

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[b] School of Chemistry and CRANN, University of Dublin, Trinity College, College Green, Dublin 2, Ireland
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The ability to observe chemical processes simultaneously with small-angle scattering is highly desirable for a deeper understanding of biological and soft matter systems. This is particularly crucial for dynamic systems, to ensure sample stability, purity, chemical conditions, and for where it is not possible to perform further measurements or characterisation on intermediate states *ex situ*. However, while such environments are becoming almost mainstream at large scale X-ray sources, they remain rare at neutron facilities.

Consequently, as a part of a larger sample environment development project at KWS-2 at the Jülich Centre for Neutron Science, which already includes *in situ* dynamic light scattering and Fourier infrared spectroscopy, we have recently developed a set-up for *in situ* UV/Vis absorption spectroscopy and light irradiation. A proof-of-concept investigation was undertaken to provide mechanistic and kinetic insights into the photoisomerization and self-assembly of a novel photoresponsive azobenzene-containing surfactant (AzoPS), see Fig. 1. It was shown that the incorporation of spectroscopy with SANS allows the scattering profile, and hence micelle shape, to be correlated with the extent of photoisomerisation in real-time. This combined UV-Vis/SANS approach could be extended to various other systems to allow monitoring of their self-assembly process, where the only requirement is the presence of a characteristic absorption spectrum.

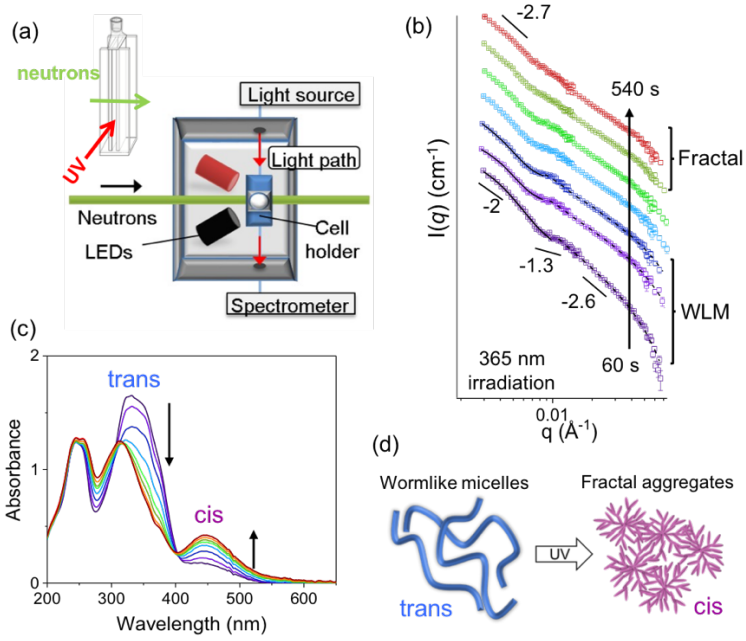


**Figure 1**. Self-assembly behaviour of AzoPS in $D_2O$ (0.2 mmol $L^{-1}$) upon *trans-cis* photoisomerisation. (a) Schematic representation of the SANS/spectrometer set-up. (b) SANS scattering profiles as a function of UV irradiation time. (c) *In-situ* UV-Vis absorption spectra upon UV-irradiation. (d) Schematic representation of the wormlike micelle to fractal aggregate transition.

**Reference**: E. A. Kelly, J. E. Houston, R. C. Evans, *Soft Matter*, 2018, DOI: 10.1039/c8sm01948g

# STIMULI RESPONSIVE NEMATIC AND SMECTIC LIQUID-CRYSTALLINE ORDERS IN SUSPENSIONS OF COLLOIDAL ELLIPSOIDS STUDIED BY SAXS

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[b] *Physical and Colloid Chemistry, Debye Institute for Nanomaterials Science, Utrecht University, Utrecht, The Netherlands*

We report a Small-Angle X-ray Scattering (SAXS) study of field-induced colloidal self-assembly of core-shell magnetic ellipsoids. Being made up of hematite cores and silica shells, these particles align in a direction perpendicular to the external magnetic field. For particles with smaller aspect ratio, $\rho = 2.8$, we observe four different self-assembled phases - oriented glass, smectic, nematic and para-nematic, as a function of the sedimentation-induced variation of the local particle concentration. An interesting peak shape was observed with highly anisotropic tails in the longitudinal direction (along the smectic periodicity) that can be rationalized by the formation of a Bragg cylinder. The application of an external field freezes one of the rotational degrees of freedom; promoting non-centrosymmetric interparticle interactions and thereby confining the particles in 2D, which is turn leads to the formation of the Bragg cylinder in Fourier space. For particles with larger aspect ratio, $\rho = 3.7$, we observe a para-nematic phase along with a nematic one.

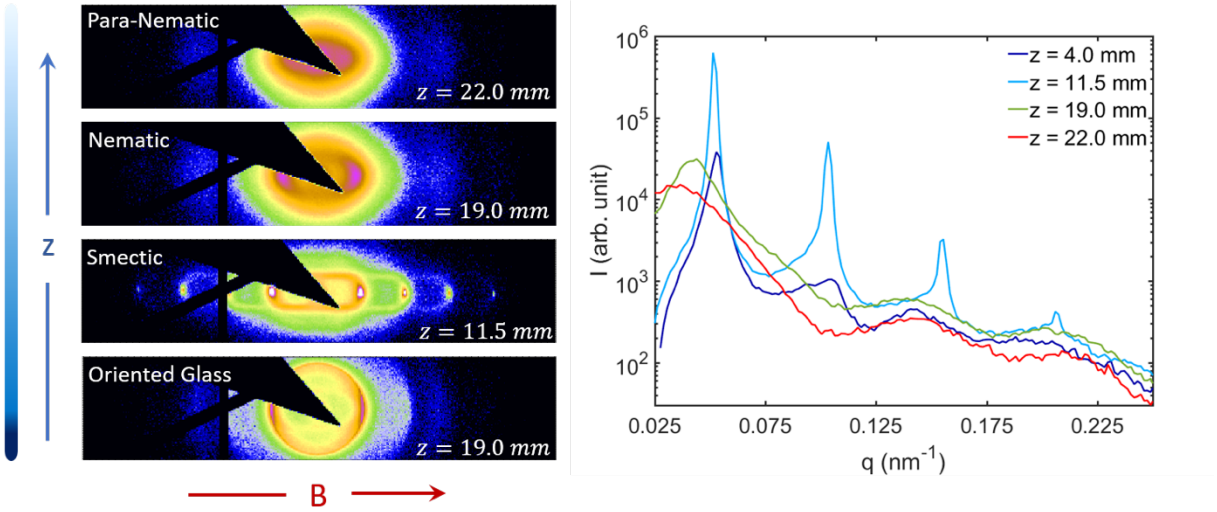


Figure 1: Left panel shows the 2D diffraction patterns of various self-assembled phases at different heights in the capillary in the presence of an external magnetic field of 550mT for $\rho = 2.8$ while the right panel represents the corresponding intensity profile along the direction of the field.

# Poster presentations

# LIPOSOMAL FORMULATIONS FOR TREATMENT OF FABRY DISEASE

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Fabry disease results from a mutation in the α-Galactosidase A (GLA) gene that causes buildup of sphingolipids in the body. This can cause a series of problems including life-threatening complications. Fortunately, the disease can be treated and current treatment involves intravenous injection of GLA in the patient. Without encapsulation of GLA, the treatment has a number of drawbacks including low GLA stability, high immunogenicity and low bioavailability, which in the end causes high medicine prizes. Liposomes that can encapsulate GLA are therefore being developed and have shown good results for encapsulation effect and stability. The liposomes are formed of several different lipid components and targeting ligands are being incorporated in the structures to improve targeting and bioavailability. The difference in the liposome composition can cause changes in size, polydispersity and structure and characterization of different liposome formulations is therefore crucial for better understanding the impact of liposome composition. We are using small-angle X-ray scattering (SAXS) to characterize the liposomal structures. To model the data we have used a crystalline model [1] with a finite amount of layers and disorder between layers, together with only three Gaussians to describe the cross-section profile of the lipid bilayer (Fig. 1). Unbound and encapsulated GLA contributes to the total scattering and is included in the model as a background on absolute scale so that the amount of GLA in the sample can be estimated. With the model we can get information on the bilayer thickness, degree of multi-lamellar structure, bilayer ordering and the amount of GLA in solution. Estimations of GLA concentrations from SAXS fits with those obtained with other methods and the amount of multi-lamellar structure is similar to that seen with cryo-TEM showing that the SAXS data can give us some information on the structure, composition and polydispersity of the samples. To complement SAXS we use static and dynamic light scattering to get estimates of the overall liposome sizes as well as information on the polydispersity. All this information will be used to develop new nanoliposome drug formulations which will hopefully prove better candidates for treatment of Fabry Disease and potentially other disease were liposomes can be a used as a successful drug delivery system.

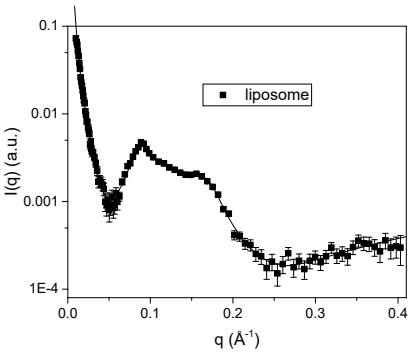


Figure 1: SAXS data of a liposomal drug formulation with a model fit.

**Acknowledgements**
This project has received funding from the European Union's Horizon 2020 research and innovation programmer under the grant agreement No 720942.

**References**
[1] A. Guinier, 1963, Freeman, San Francisco

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# The effect of 3D-printed micromixers on the properties of peptide-loaded microgels

Bruno C. Borro [1*], Adam Bohr [1], Saskia Bucciarelli [2], Johan Peter Boetker [1], Camilla Foged [1], Jukka Rantanen [1], Martin Malmsten [1,3]

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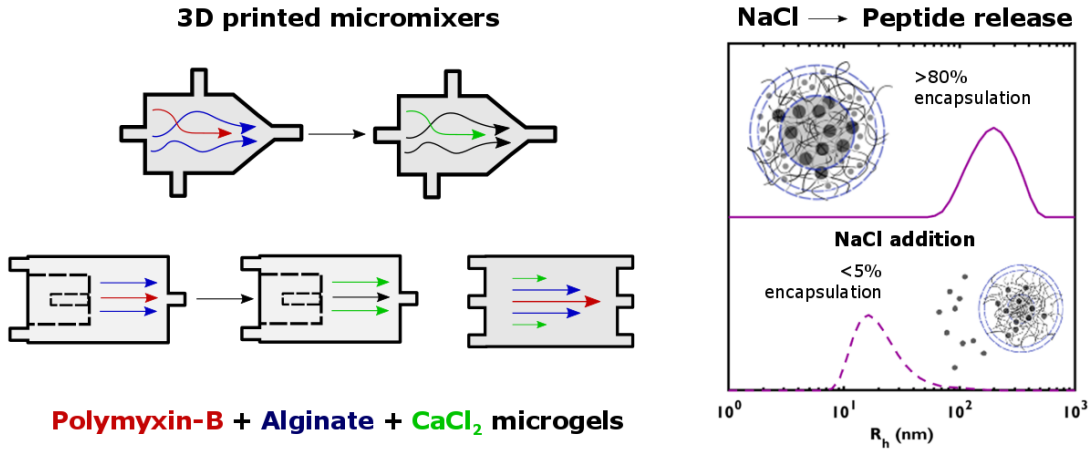
In an effort to contribute to the research in scalable production of polymeric delivery systems loaded with antimicrobial peptides (AMPs), we here investigated effects of three-dimensional (3D) printed micromixer designs on microfluidic particle generation. For this purpose, 3D printing was applied to manufacture micromixers with three different geometric designs, which were used to prepare $Ca^{2+}$-cross-linked alginate microgels loaded with the AMP polymyxin B in a continuous process. Based on fluid dynamic simulations, the flow patterns in the micromixers were designed to be either *(i)* turbulent with chaotic disruption, *(ii)* laminar with convective mixing, or *(iii)* convective with microvortex formation. The physicochemical properties of the microgels prepared with these micromixers were characterized using photon correlation spectroscopy, laser-Doppler micro-electrophoresis, small-angle x-ray scattering, and ellipsometry.




**Graphical abstract**: Microfluidics-based self-assembly of peptide-loaded microgels: Effect of 3D-printed micromixer design

Particle size and compactness were found to depend on micromixer geometry: The smallest and most compact particles were obtained by preparation involving microvortex flows, while larger and more diffuse microgels were formed upon laminar mixing. Polymyxin B was localized in the particle interior and caused particle growth with increasing peptide loading. $Ca^{2+}$-induced cross-linking of alginate, in turn, resulted in particle contraction. The peptide encapsulation efficiency was higher than 80% for all investigated micromixer designs, and the smallest particles, generated by microvortex-mediated self-assembly, displayed the highest encapsulation efficiency. Ellipsometry results for surface-immobilized microgels, as well as peptide encapsulation data, demonstrated electrolyte-induced peptide release. Together these findings demonstrate that microfluidics using 3D-printed micromixers offers promises for the continuous manufacturing of AMP-loaded microgels. Although the micromixer combining turbulent flow and microvortices was the most efficient, all three micromixer designs were found to mediate self-assembly of small microgels displaying efficient peptide encapsulation. This demonstrates the robustness of employing 3D-printed micromixers for microfluidic assembly of AMP-loaded microgels during continuous production.

# MICROFOCUS SOURCES PLUS MULTILAYER OPTICS AND SCATTERLESS APERTURES FOR SAXS EXPERIMENTS IN THE HOME-LAB

**J. Wiesmann, J. Graf, U. Heidorn, F. Hertlein**
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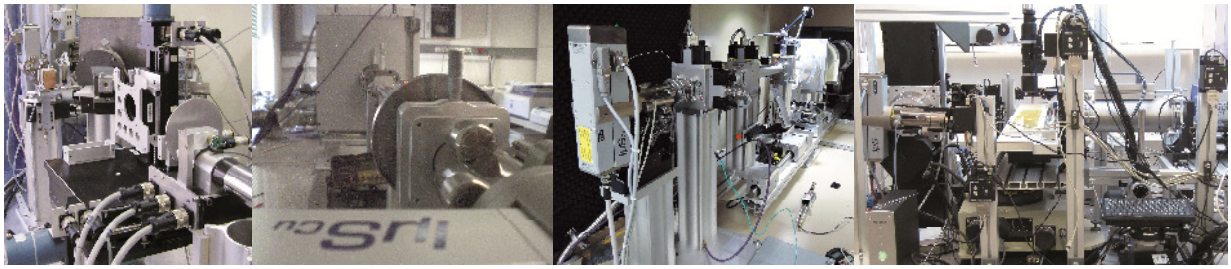
Nowadays, X-ray optical components, such as multilayer mirrors or scatterless apertures, are used as beam conditioning devices in nearly all state-of-the-art X-ray analytical equipment, either in the home lab or at synchrotron beamlines. We will be discussing pinholes, optics and their use in combination with high brilliance laboratory sources for SAXS applications.

Scatterless pinholes are usually made of oriented single crystals, such as Ge or Ta, and show a significant reduction of parasitic scattering commonly associated with conventional metal apertures. Therefore, such pinholes allow an improvement of X-ray analytical instruments as the number of necessary pinholes can be reduced. Further, the use of scatterfree pinholes enables a significant reduction of the background. This improves the data quality at low resolution which is beneficial for small angle scattering, as well as for crystallography applications. Our SCATEX pinholes are either made of Germanium for energies below 11.2 keV or of Tantalum for energies above 11.2 keV and are available with diameters ranging from 2 mm down to 20 µm and below. Therefore, these novel apertures are applicable to a wide range of different applications. We will be showing results about development and use of these pinholes.

Multilayer X-ray mirrors are widely used as monochromators and beam shaping devices. The so-called Montel optics consist of bent substrates with shape tolerances below 100 nm, upon which multilayers are deposited with single layer thicknesses in the nanometer range and up to several hundreds of layer pairs. The multilayers are designed with lateral thickness gradients within ± 1% deviation of the ideal shape. Very low shape tolerances below 100 nm and figure errors well below 5 arcsec are required for multilayer mirrors to ensure a superb flux density in combination with very high-brightness microfocus X-ray sources such as the Incoatec Microfocus Source IµS.

The air cooled IµS is available with Cr, Co, Cu, Mo, and Ag anodes. The implemented Montel optics form either a highly collimated beam with a low divergence (below 0.5 mrad) or a focusing beam with higher divergence (up to 10 mrad) and very small focal spots (diameter below 100 µm). Applications realized with an IµS are small-angle X-ray scattering, texture, stress analysis, µ-diffraction, single crystal diffraction to name but a few. In our presentation we will be presenting selected examples of IµS solutions for SAXS and GISAXS with ex- and also in-situ set-ups (see fig.). At modern synchrotron sources, an efficient use of the limited beamtime is an important issue. Compact easy-to-install microfocus sources can be an attractive supplementary equipment for high-end laboratories there as well.



Four systems with IµS (from left to right): 1) XRD/XRR setup in synchrotron optics lab at ESRF, Grenoble, France; 2) Bruker NANOSTAR with SCATEX in Vienna, Austria; 3) IµS and SCATEX Upgrade on a customized SAXS setup at Univ. Hamburg, Germany; 4) GISAXS setup with Dectris detector at Slovak Academy of Science, Bratislava, Slovakia

# Probing the Structure of Lipid Bilayer and their Interaction with Indolicidin using Small Angle X-ray and Neutron Reflectivity methods

Josefine Eilsø Nielsen[1], Victoria Ariel Bjørnestad[1], Abdullah Lone[2], Håvard Jenssen[2], Tania Kjellerup Lind,[3] Marité Cardenas[3] and Reidar Lund[1]

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Antibiotic resistance is one of the biggest threats to global health, according to WHO. AMPs seem to be able to evade much of the bacterial resistance mechanisms and are therefore promising candidates for future antibiotics. Instead of blocking specific biochemical pathways as most available antibiotic agents today, most AMPs act physically on the cytoplasmic membrane itself.[1-2] The precise microscopic mechanism for the disturbance of the membrane has not fully been proven but several theories has been suggested including membrane deformation and pore formation. Here we have used state of the art neutron and x-ray scattering techniques to investigate the microscopic mechanism of action of AMPs with model bacterial membranes. SAXS measurements on a model peptide, Indolicidin together with lipid vesicles has shown that Indolicidin interacts with the membrane. Based on analysis of the results we can see that the peptide does not seem to affect the structure of the bilayer significantly but situates in the interface between the lipid head group and the tail in the outer leaflet in the bilayer.[3] This causes and slight alteration in the lipid chain packing as seen by calorimetry but does not lead to any significant membrane thinning or similar. This is further confirmed by Neutron Reflectivity and Atomic Force Microscopy on planar supported bilayers.[4] Combining these techniques has given us a new important insight into how the peptide interact with the bilayer. Rather than supporting any specific structural model, we speculate that the mechanism is compatible with the disordered model proposed by Wimley.[5]

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# Modelling human stratum corneum with commercially available lipids

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## Background

Stratum corneum (SC) constitutes the main barrier for drugs diffusing through the skin, while also protecting the body from harmful chemicals and infection, and preventing trans-epidermal water loss. The skin barrier is commonly described by the "brick and mortar model" (van Smeden and Bouwstra. 2016), with the corneocytes being the "bricks", and the lipid matrix between the corneocytes forming the mortar. The lipid matrix is thought to be the main transportation pathway through the skin barrier. It is a complex mixture of mainly free fatty acids, ceramides and cholesterol, in a roughly equimolar ratio, assembling into two distinct phases; the long and short periodicity phases (LPP and SPP, respectively) (van Smeden and Bouwstra. 2016). The exact composition of lipids plays an important role in the proper formation of LPP and SPP, drug penetration over the skin, and skin barrier function. Lipid composition is often dysregulated in skin conditions such as atopic dermatitis and psoriasis (van Smeden and Bouwstra. 2016).

## Aim

The aim of this project is to develop physical models of the SC formed of commercially available lipids by spincoating lipid mixtures onto solid supports, in order to mimic the molecular structure of native SC lipid matrix. The structure and interactions of the supported lipid film with drug compounds will be characterised and compared to total extract of pig SC using neutron reflectometry, ATR-FTIR and QCM-D measurements.

## Methods

ATR-FTIR: Attenuated total reflection (ATR) Fourier transformed infrared (FTIR) spectroscopy allows deposition of a lipid layer onto a prism, and the subsequent study of functional group environment as a function of temperature (phase transitions) or as a result of lipid multilayer interactions. Studying the SC lipid models with ATR-FTIR will allow us to compare their phase behaviour, lipid multilayer interactions with various drug compounds, and potential subsequent structural lateral changes to the lipid film.

Neutron Reflectometry: Neutron Reflectometry provides insight into the structure perpendicular to interfaces, making it possible to investigate the internal structure of the lipid multilayer at physiologically relevant conditions.

QCM-D: Quartz crystal microbalance with dissipation (QCM-D) quantitatively measures changes in film thickness and rigidity without the use of external probes. Changes in molecular structure of the lipid multilayers under different conditions are also possible to assess through changes in dissipation, which relates to rigidity of the supported lipid film. It is also possible to expose the lipid multilayers to various molecules of interest to study binding to the SC lipids.

# Diffusion and Arrest of Ellipsoidal Particles in the Presence of an External Field

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In addition to the usual gas, liquid, crystal and glassy states found for spherical particles, anisotropic particles are known to exhibit a rich phase behavior. While the majority of the existing studies addresses structural properties, dynamic behavior of anisotropic particles is a relatively lesser explored avenue. Here we present the results from a systematic investigation of the collective dynamical properties of ellipsoidal colloids as a function of the volume fraction measured with differential dynamic microscopy (DDM) and X-ray photon correlation spectroscopy (XPCS) to probe different length scales of the system. We show that with both techniques it is possible to overcome the roadblock caused by the opacity of the samples. We combine our dynamics results with small-angle X-ray scattering and rheological measurements to relate the collective short and long time diffusion coefficients with the structural correlations and the evolution of the zero shear viscosity as the system approaches an arrested state. We find that the short time diffusion coefficient scales with the inverse of the structure factor [fig.1(a), (b)] while the long time diffusion coefficient varies as the inverse of the zero shear viscosity [fig.1(c), (d)]. As the particles are made up of magnetic hematite cores and silica shells, we moreover use an external magnetic field to control the orientational degrees of freedom of the particles to investigate the field effects on their anisotropic dynamics. We find that at high field the anisotropy in dynamics increases with an increase in concentration [Fig. 1(e)].

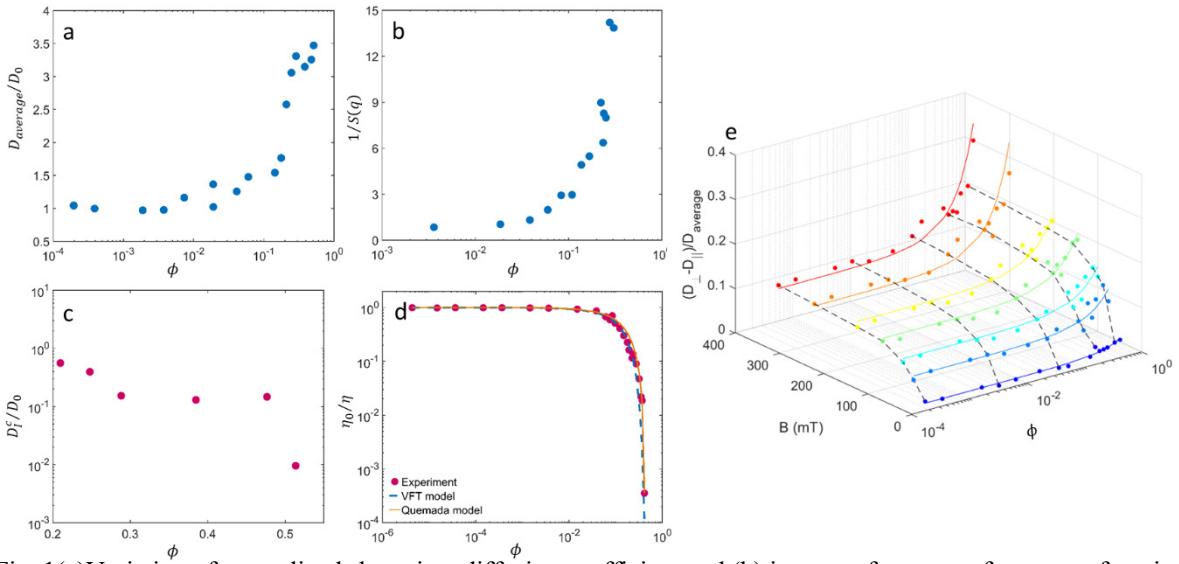


Fig. 1(a)Variation of normalized short time diffusion coefficient and (b) inverse of structure factor as a function of volume fraction (φ). (c) Variation of normalized long time diffusion coefficient and (d) normalized inverse zero shear viscosity as a function of φ. (e) Variation of the anisotropy in the short time diffusion coefficient in presence of an external magnetic field.

# LoKI - Broad Band Small-Angle Neutron Scattering at the ESS

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The European Spallation Source (ESS) is a long pulse 5 MW spallation source currently being built in Lund, Sweden, aimed at delivering a suite of world-leading, multi-disciplinary instrumentation. LoKI is one of two small-angle neutron scattering (SANS) beamlines, designed with the needs of the soft matter, materials and bio-science communities in mind. The trend in all these fields is towards complexity and heterogeneity. These factors are manifested both spatially and temporally, and therefore the high flux ($1.1 \times 10^9$ n $s^{-1}$ $cm^{-2}$ at sample position), small beam sizes and wide simultaneous Q-range ($10^{-3}$ to 2 $Å^{-2}$) available at LoKI, will make it optimal for performing structural and time-resolved studies.

The instrument itself consists of 3, 5 and 8 m collimation sections with the sample positions 22.5 m from the source. Using the full ESS pulse of 2.86 ms and two pairs of choppers will provide a wavelength band of 7 Å at 14 Hz or 17.5 Å at 7 Hz, whilst maintaining reasonable momentum resolution (10-30%). Combining these configurations with large area detector arrays made of stacked boron-coated straws, will provide large solid-angle coverage with good efficiency, thus, making maximal use of the flux available from the source. In the future, the small beam sizes/high flux available at LoKI are expected to make scanning and microfluidics routine, the wide simultaneous Q-range will benefit dynamic structures, such as structures under shear, and its high flux and excellent signal-to-noise ratio will greatly improve the study of weakly scattering biological samples, and enable “single-shot” kinetic measurements in sub-second time scales.

**Kinetics of Unfolding and Refolding of BSA using Anionic and Nonionic Surfactants**

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Globular proteins like bovine serum albumin (BSA), lysozyme and β-lactoglobulin can be unfolded at neutral pH by the anionic surfactant Sodium Dodecyl Sulfate (SDS). Complexes are formed, in which intact SDS micelles are decorated by the partly unfolded protein. Regardless of the native secondary structure of the proteins, they have been shown to adopt a secondary structure consisting mainly of α-helices. Addition of the nonionic surfactant Octaethylene Glycol Monododecyl Ether (C12E8) to the SDS-protein complexes facilitates the refolding of protein back to its native state. This contribution will present the modeling and analysis of data recorded on BSA at the European Synchrotron Radiation Facility (ESRF) in Grenoble. Here, the kinetics of the unfolding and refolding processes has been studied by a combination of stopped-flow techniques and synchrotron radiation based Small-Angle X-ray Scattering (SAXS). The processes have been followed with millisecond time resolution. For BSA, only small changes can be observed during the unfolding process, possibly because the protein already consists of mainly α-helix. Moreover, these small changes take place within the first second after mixing of BSA and SDS. A core-shell model has been used for modelling the unfolding process. In contrast, our preliminary analysis of the refolding process suggests that it can be modelled by linear combinations of components such as native protein, protein-SDS complex and $C_{12}E_8$-micelles.

**Interactions between Anionic Surfactants and Polymeric Micelles: Stability and Solubilisation kinetics**

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The kinetic processes involved in mixtures of surfactants and block copolymer micelles are not well understood. However, it is commonly known that surfactants exhibit rather fast equilibration kinetics, in the order of micro- to milliseconds, while polymers are much slower, in the order of minutes to months. In this contribution, we will present a study of the stability and solubilization kinetics of block copolymers micelles upon addition of sodium dodecyl sulphate (SDS) using small angle X-ray scattering (SAXS) and time resolved neutron scattering (TR-SANS). We compare the ability of the surfactant to dissolve and form mixed micelles with two amphiphilic polymers; poly(ethylene propylene)-poly(ethylene oxide) (PEP-PEO) and end-capped PEO ($C_{28}$-PEO). While the kinetics of $C_{28}$PEO occurs on time scales on the order of minutes-hours on ambient temperatures, that of PEP1-PEO20 is known to be frozen on practical time scales. Addition of SDS to PEP1-PEO20 shows close to no change, even after extended period of time. However, upon addition of SDS to $C_{28}$PEO5 we observe a dissolution over hours and formation of mixed micelles. Time-resolved SAXS of addition of SDS to $C_{21}$PEO5 shows a fast dissolution and formation of mixed micelles over seconds. Furthermore, the kinetics of formation of mixed micelles is seen to accelerate with the amount of added surfactant.

# NanoMAX: A 2D Compact SAXS Instrument

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A new 2D Kratky SAXS system has been developed. This is a compact system with many unique features, including modularly designed sample attachments, enabling quick change for different applications; easily configurable system settings, empowering users to set up the instrument most suitable to their application needs, and user customizable software, allowing users to configure the instrument to their own unique hardware requirements. The system utilizes some of the most advanced technologies in X-ray sources, optics and hybrid photon counting detectors. Combined with this unique design principle, the system delivers performance that features high intensity, completely eliminated parasitic scattering, high resolution (low $q_{min}$) and high angular resolution with the added capability of collecting data from SAXS region to WAXS region with automated sample to detector distance change.

SAXS systems generally fall into design categories that use either pinhole or Kratky collimation systems to reduce or eliminate parasitic scattering. The NanoMAX is a modernized 2D Kratky system that eliminates data corrections required of traditional Kratky systems with the added benefit of offering compactness and superb flux for samples when compared to standard pinhole systems.


The NanoMAX system configured with a microfocus sealed tube system.
The NanoMAX can also be configured with alternative X-ray sources, such as a rotating anode or liquid metal jet (LMJ) sources.

Laboratory space is a precious and expensive resource leaving researchers to favor high quality, compact systems where possible. The NanoMAX design satisfies this criterion well in that the system size is approximately 1 m long whereas traditional pinhole systems usually require 3 m system lengths or longer. The NanoMAX system can be installed on a variety of X-ray sources, including the open port of a rotating anode, and has the advantage that higher flux is delivered to the sample in a much shorter camera length compared to pinhole SAXS systems. Most importantly, the NanoMAX incorporates features and hardware to support SAXS data collection for both isotropic and anisotropic scatterers with a very wide q-ranges of 0.0043 $Å^{-1} < q < 3.5$ $Å^{-1}$.

## CONCLUSIONS

In the presentation, the presented results will illustrate the range of capabilities of the NanoMAX system and its compatibility for a broad range of samples types and experiments. Additionally, the system offers a versatile range of sample stages to allow measurement for powders, liquids, solids whether they exhibit isotropic or anisotropic scattering.

# UNFOLDING OF OVALBUMIN BY SDS AND REFOLDING BY ADDITION OF NONIONIC SURFACTANTS

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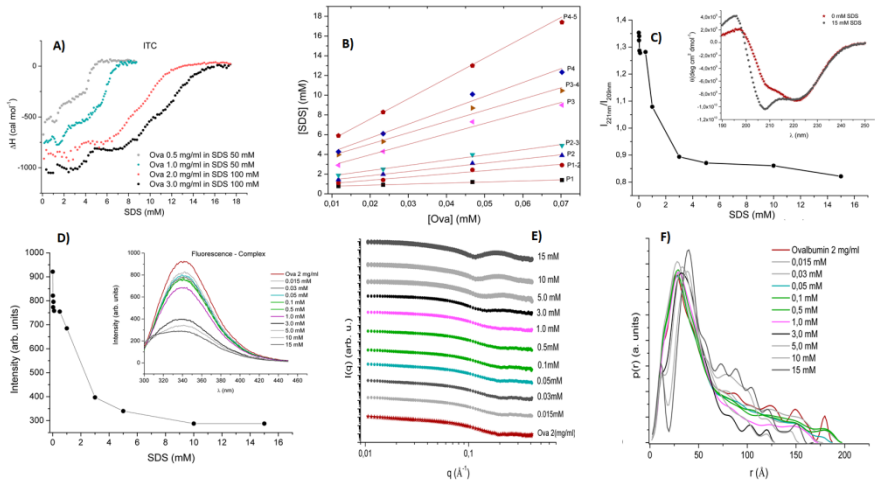
Complexes formed by Ovalbumin (Ova) unfolded by the surfactant Sodium Dodecyl Sulfate (SDS) and the subsequent refolding of the protein by the nonionic surfactant Octaethylene glycol monododecyl ($C_{12}E_8$) have been investigated. To study the complex formed, Isothermal Titration Calorimetry (ITC), Fluorescence, Circular Dichroism (CD) and Small-Angle X-ray Scattering (SAXS) were used.




Panel A) in figure 1 shows the ITC curves for different concentrations of Ova (0.5, 1, 2 and 3 mg/ml) titrated with SDS (50 and 100 mM). The negative enthalpy reveals an exothermic process of the system and the similar behavior for different protein concentrations shows that the Ova interacts with SDS in a ratio dependent manner and allows binding stoichiometry to be obtained. Panel B) shows the results for different transitions from ITC and gives the free SDS concentration from the y intercept and the number of SDS bound to the protein as the slope. Panel C) shows far–UV CD data of the SDS-Ova system. Data in red are for the pure protein, which contains a large amount of β-sheet secondary structure. Data in black are for the unfolded protein with 15 mM of SDS and agree with a large α-helical content. Panel D) shows the fluorescence results for different concentrations of SDS (from 0 to 15 mM). The intensity in arbitrary units is decreasing as the [SDS] is increasing. Panels E) and F) show SAXS results for the SDS-Ova complex for eleven different concentrations (from 0 to 15 mM) of SDS in a 2 mg/ml protein solution. The results show an evolution of a minimum in the three last curves (5, 10 and 15 mM) of SDS. According to the pair distance disitrbuition function, $p(r)$, the complex dereases in size (from 180 to 125 Å) and the oscilation at short distances in the curves in black and grey suggest that a core-shell structure is formed when the concentration of SDS is above 3 mM. We can see the system evolve into a form of core-shell, where the protein decorates SDS micelles.

The results of ITC, far-UV CD, Fluorescence and SAXS together provide important data for a tentative model of the protein-surfactants interactions and complex formation. Currently, we are working on refolding of Ova by addition of nonionic surfactant to the SDS-Ova complexes.

**Acknowledgements**

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# A SETUP FOR LASER-PUMP X-RAY-PROBE TIME-RESOLVED SOLUTION SCATTERING EXPERIMENTS AT THE COSAXS BEAMLINE.

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The CoSAXS beamline at MAX IV is a multi purpose SAXS beamline currently under construction. CoSAXS will offer a high X-ray flux, with a large fraction coherent flux [1]. The beamline will feature an Eiger2/Pilatus3 detector system for simulataneous SAXS/WAXS detection with a high readout frequency. CoSAXS will offer several sample environments to the user community. One of these environments will enable the users to study non-equilibrium processes where the sample has been perturbed by a laser pulse. The initial configuration of this setup will offer the possibility to study time-resolved structural changes following a laser induced temperature jump. This makes it possible to study for example folding/unfolding of proteins as well as phase transitions in lipidic systems. It will also be possible to interface other lasers, making it possible to study for example signal transduction in photoreceptor proteins [2,3]. The typical time-resolution will be in the millisecond range, but even micro- or sub-microsecond resolution can be achieved. This resource is planned to become available to users during 2020.

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# COSAXS, THE UPCOMING SCATTERING BEAMLINE AT MAX IV LABORATORY TO STUDY STRUCTURE AND DYNAMICS OF NANOSTRUCTURES

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The first small angle scattering beamline at the MAX IV laboratory, namely CoSAXS, is progressing toward the first commissioning X-ray experiments in 2019.

The undulator and all optical components has been y installed and mechanically tested in 2018, the SAXS vacuum vessel has started the production at the factory in October 2018 and the components and experimental table at the experimental hutch are in an advanced state of design.

Specifically, the SAXS flight tube will be a 17 m long, 1 m diameter chamber with working vacuum pressures of 0.01 mbar and two in vacuum 2D detectors in simultaneous SAXS/WAXS detection mode (see figure). The SAXS detector, an Eiger2 4M model, presents the latest technology in hybrid pixel x-ray detectors with high spatial resolution, increased count rate, fast frame and triggering capabilities compatible with Time Resolved SAXS (millisecond to microsecond time resolution) and XPCS experiments. The expected available q-range of the beamline is projected to be $6 \times 10^{-4} < q < 6$ Å$^{-1}$ (from 1 μm to 1 Å d-spacings).

From the first day of operation, the basic configurations for solid and solution SAXS experiments at the beamline will be provided, including high throughput BioSAXS capabilities. Of major interest to the project is the development of a modern set of sample environments, together with the user community. The new cell for combined X-ray scattering/spectroscopy experiments, microfluidics projects and stand-alone devices (like stop-flow, heating stages and Rheometers) adapted to the beamline capabilities are presented.

First commissioning experiments and expert (friendly) user activities are planned for 2019. The first external users call is expected in early 2020.

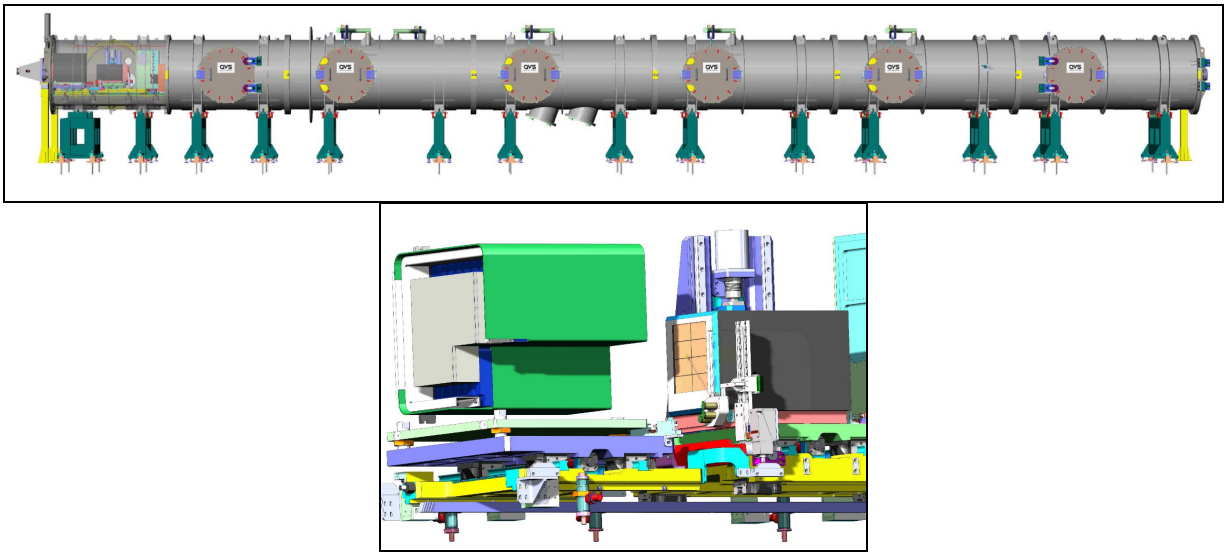

Figure 1: (top) CoSAXS Vacuum Vessel design; (bottom) L-shaped customized WAXS detector and SAXS detector design at its closest position inside the vacuum vessel. A users friendly software will determine optimal signal/noise and positioning of the SAXS detector.

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## In situ x-ray scattering used to track pore formation in FeOOH nanorods

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Thermal-induced dehydroxylation of the paramagnetic lepidocrocite (γ-FeOOH) produces the ferromagnetic maghemite (γ-Fe2O3). In this study, in situ x-ray scattering (SAXS/WAXS) was used to observe the temporal evolution of the crystal structure, porosity and morphology of iron oxide nanorods, during this procedure. The samples were heated up to 350 °C with several different heating rates, ranging from 1 °C/min to 130 °C/min, in order to see how the heating rate can be correlated to the characteristics of the formed pores.

The experiments were performed at the cSAXS beamline at SLS. The high flux and time resolution of cSAXS coupled with the simultaneous acquisition with both SAXS and WAXS detectors enabled the observation of both the temporal evolution of the crystal structure and particle porosity, since a large q range was covered. The SAXS data has been modelled by considering the scattering from pores embedded in the fractal arrangement of the lepidocrocite rods, forming the matrix.

The data indicate the formation of pores upon heating the lepidocrocite, followed by a subsequent growth of the pores. Furthermore, the behavior of the pore-formation seems to be affected by the heating rate. This is in line with previous results obtained by means of electron microscopy and surface adsorption measurements, which revealed that the pores are typically around 3 nm and that the amount and size are strongly dependent on the heating rate and final temperatures.

# Membrane interactions of nanoclay particles as carriers of antimicrobial peptides

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Membrane interactions are critical for the successful use of inorganic nanoparticles as antimicrobial agents and as carriers of, or co-actives with, antimicrobial peptides (AMPs). In order to contribute to an increased understanding of these, we investigated the effects of particle size and charge on nanoclay interactions with both bacteria-mimicking, mammalian-mimicking lipid membranes as well as Gram-negative and Gram-positive bacteria.

The first project focused on cationic charged layered double hydroxide nanoparticles (LDH) and the effect of particles size (42-208 nm) [1]. It was found that with decreasing particle size increased binding to bacteria-mimicking membranes, extractions of anionic lipids and resulting membrane destabilization was seen. This also translated into size-dependent synergetic effects with the antimicrobial peptide LL-37. Due to possible strong interactions with anionic lipopolysaccharide and peptidoglycan layers, direct membrane disruption of both Gram-negative and Gram-positive bacteria is suppressed. However, LDH nanoparticles cause size-dependent charge reversal and resulting flocculation of both liposomes and bacteria, which may provide a mechanism for bacterial confinement or clearance.

A follow up study on nanoclays was conducted focusing on the membrane interactions of anionic charged laponite nanoparticle in the absence and presence of LL-37. Because of the net negative charge of laponite nanoparticles, LL-37 loading capacity increases with increasing charge contrast. Peptide binding to laponite nanoparticles was demonstrated to occur primarily at the outer surface of the nanoparticles in a largely helical conformation, causing charge reversal, effects again increasing with pH.

Bare as well as peptide-loaded laponite nanoparticles caused bacteria flocculation of *E. coli*, originating from the interaction of laponite with bacterial lipopolysaccharide (LPS), present in the outer *E. coli* membrane. This effect seems to be limited to Gram-negative bacteria as laponite did not cause flocculation of *Bacillus subtilis* (*B. subtilis*), nor did it bind to lipoteichoic acid in such bacterial membranes.

As such, the present investigation reports on several novel phenomena by demonstrating that size of the nanoparticle, in this case of LDH nanoparticles, affects membrane interactions and that nanoparticle charge does not invariably control membrane destabilization and bacteria flocculation, by identifying the ability of anionic laponite nanoparticles to effectively flocculate Gram-negative bacteria through LPS binding, the latter offering a promising approach for confinement of infection and inflammation caused by such pathogens.

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# Salt-induced Morphological Transitions of Sodium Dodecyl Sulphate micelles studied by SAXS: Effect of Ionic Strength and Cation

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The surfactant sodium dodecyl sulfate (SDS) is one of the most studied in soft matter science due to its many applications and its ability to aggregate into a wide variety of interesting and dynamic micellar structures. The morphology of the micelles can change with the surfactant type and conditions such as temperature and solvent composition. Despite over a century of research, there are still kinetic pathways of morphological transitions that are not fully understood. Herein, we investigate morphological transitions in aqueous solutions of SDS in the presence of different types of salts (NaCl, KCl, $MgCl_2$ and $CaCl_2$) at different concentrations using synchrotron radiation small-angle X-ray scattering (SAXS). Previous literature suggest that "worm-like" micelles are formed by fusion of globular and cylindrical micelles at high salt concentrations in a way similar to step-like polymerization. The goal here is to better understand the effect of ionic strength and other nature of the cation on these transitions.

# Structural and functional characterization of PilA from freshwater cable bacteria

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Cable bacteria are unique and intriguing electric lifeforms that very efficiently conduct electrons along centimeters distances. They were discovered after observing that it is possible to measure currents over centimeter distances in marine sediments. In these sediments cable bacteria feed on hydrogen sulfide in the anaerobic depths of the sediment by releasing electrons, which travel along the cable to ultimately react with oxygen at the aerobic surface.
The enigma of how these astonishing organisms conduct electrons along their filaments is still unresolved.
Recent proteomics, transcriptomics and genomic studies have pinpointed a set of proteins that could be the components of the conductor machinery, one of them, PilA, is a pilin like protein. We hypothesize that PilA could act as conduits by forming fibers which would constitute the main conductive components of the filaments. This hypothesis is strengthened by the observation that in *Geobacter* bacteria, pilin proteins polymerize to form micrometers long pili that have been proven to conduct currents. To validate the basis of our hypothesis, we want to test if also PilA from cable bacteria can oligomerize to form fibers. Therefore, we will try to induce oligomerization of the purified recombinant protein. To monitor the status of the protein in solution we intend to use SEC for quick screening and SAXS for a more accurate analysis of the protein oligomeric state.

# Structural and functional studies of the *D. alkaliphilus* PilA

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The *Desulfovibrio alkaliphilus* is capable of producing e-pilis, comprising of the Type IV major subunit protein, PilA. These PilA or e-Pili proteins are currently of high importance and interest in the field of Electromicrobiology. The *Geobacter sulfurreducens* has been used as host organism for testing various PilA genes to verify them as conductive e-pili. One of these PilA genes is from the *D. alkaliphilus*. Unlike the *G. sulfurreducens* the *D. alkaliphilus* PilA contains a so-called head domain, which is a feature for many other PilA molecules which has also been shown to be conductive.
We have successfully purified recombinant full-length *D. alkaliphilus* PilA which will be used for several functional and structural studies e.g. *in vitro* polymerization in effort of measuring the conductive properties of PilA filaments in an isolated environment not affected by other cell components such as cytochromes. Furthermore, the structure will be described by the use of a combination of X-ray crystallography and cryo-EM to elucidate the atomic structure and function of PilA filaments and in particular pinpoint the orientation of aromatic residues which might directly facilitates the electron transport. Moreover, the polymerization process will be investigated and described using small-angle X-ray scattering.

# The STING of a Fruit Fly

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It is crucial for animals to protect themselves against virus infections. In mammals, cytokines named interferons is pivotal in combating infecting viruses. The expression of interferons is induced by proteins called pattern recognition receptors (PRRs) and they respond to conserved pathogen associated molecular patterns (PAMPs). For insects, it is believed that the protection against RNA viruses is governed almost exclusively by RNA interference. Transcriptional responses induced by PRRs in *D. melanogaster* are only characterized for bacterial and fungal pathogens. Our resent work show that the protein STING is essential for a transcriptional response against certain RNA viruses in *D. melanogaster*. STING is well known in mammalian immunity where it is an adaptor between the PRR cGAS and the transcription factor IRF3. cGAS recognizes dsDNA in the cytosol and produces a cyclic dinucleotide (CDN) that activates STING which activates IRF3 and induces the expression of interferons. STING in mammals have also been shown to respond to membrane fusion events and RNA viruses via uncharacterized mechanisms. The aim of this study is to characterize the activation mechanism for STING and the downstream pathway in *D. melanogaster*. Since IRF3 is absent in insects and interferons first arose in vertebrates, *D. melanogaster* can potentially reveal more ancient functions of STING that might be conserved in mammals. To determine the activation mechanism, we have purified recombinant *D. melanogaster* STING in order to test binding to different CDNs and solve the structure by x-ray crystallography. We will also investigate the activation of STING by different pathogens and PAMPs in *D. melanogaster* cells and describe the STING driven transcriptional response.

# SELF-ASSEMBLY INDUCED 3D PATTERNING AT THE WATER-AIR INTERFACE STUDIED WITH NEUTRON REFLECTIVITY AND AFM

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Among the several compounds protecting the outermost surface of mammalian hair, the branched fatty acid 18-methyl eicosanoic acid (18-MEA, Figure 1 top left) is the most abundant and the most intriguing as the precise function and role of positioning of its isopenultimate methyl branch is still debated. A suggested reason for its structure is the decrease in melting temperature without the introduction of a double bond inherently prone to oxidation. In addition, a decreased packing density compared to the straight chain analogue has been proposed to better correspond to the limited number of binding sites on the underlying protein matrix.

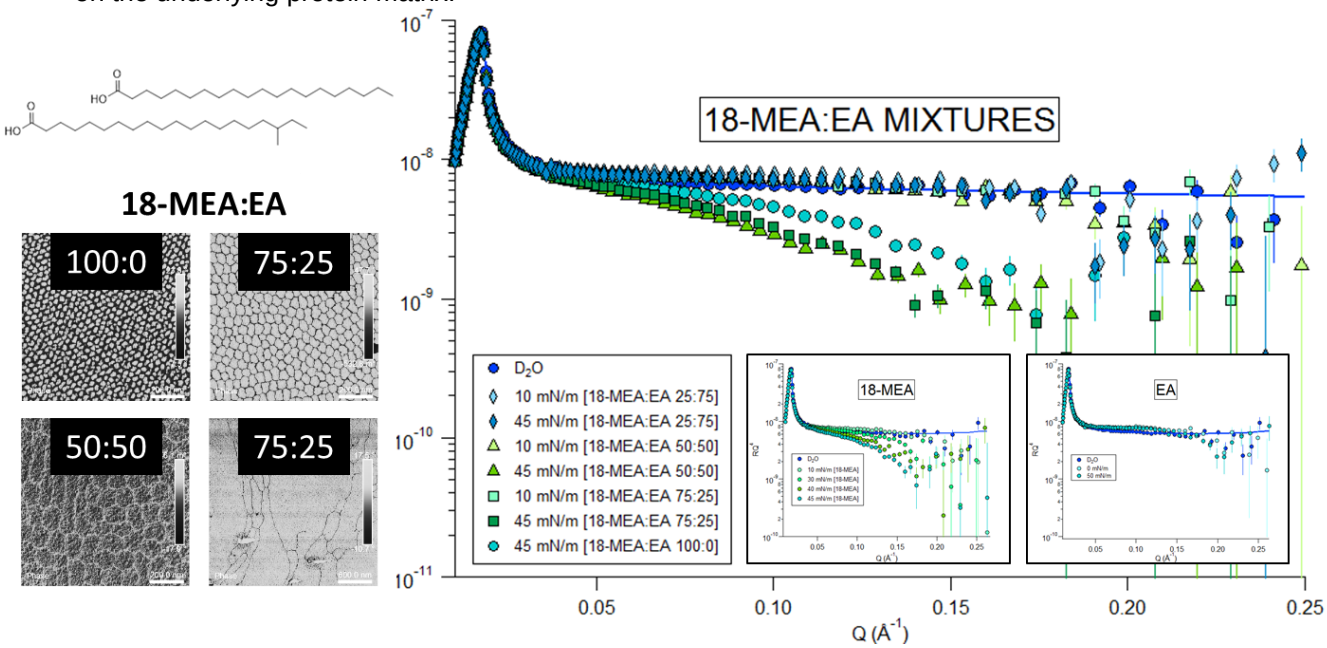


**Figure 1:** Neutron reflectometry on water-air interface of pure and mixtures of 18-MEA and EA at varying surface pressures. AFM imaging of deposited mixtures of 18-MEA and EA on silicon wafer.

In this study, 18-MEA and mixtures with its straight chain analogue eicosanoic acid (EA Figure 1 top) have been studied to understand their self-assembly behaviour at the water-air interface and thus give insight to the importance of the methyl branch on chain packing. As a first approximation of the structure on the water-air interface the Langmuir-Blodgett method was used to deposit monolayers on solid substrates. Depositions at different surface pressures were characterised by atomic force microscopy (AFM). This representation of the water surface showed domain formations with varying size depending on the ratio of the two fatty acids, as shown in Figure 2. No domains were observed for pure EA monolayers. To study the system in situ at the water-air interface, neutron reflectivity (NR) was employed at the FIGARO beamline at ILL. At high surface pressures, the results suggest a curvature of the liquid interface driven by chain packing constraints of the branched fatty acids. The extent to which the interface shows out of plane fluctuations is strongly influenced by the ratio of branched fatty acid in the monolayer.

This 3D ordering of the water-air interface due to self-assembly factors allows the possibility of using the liquid-air interface for templating purposes, as well as revealing the biological function of the studied molecules.

# ON THE VARIATION OF MEMBRANE PROPERTIES AS DEPENDING ON LIPID CHIRALITY

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Bio-synthesis exhibits a dedicated preference in chirality: the ingredient molecules of the bio-machinery that were selected in biological evolution mostly consist of only one enantiomer, apparently depending on the functional role of the molecular unit. The lipid system, the major compound of all biomembranes, is an example of this. The glycerol backbone with the fatty acid chains is in all L-α-form, and as a result the hydrophilic-hydrophobic membrane interface is a chiral interaction platform.
Lipid chirality was established as the origin of the liquid-condensed domains in monolayers, with their shapes varying upon the detailed composition, and their crystallographic symmetry being different for the enantiomers, their mixtures, and the racemic system. Adsorption at the interface is thus most probably sensitive to chirality and may have a significant biological impact for unspecific bilayer binding: the adsorption and insertion of a small chiral compound may vary depending on its symmetry. This effect may propagate into the chain region, changing the state there.
First results involving membranes from mixtures of the enantiomers of DPPC as a test system will be presented. Studies include physico-chemical properties of the chiral lipid membrane, its structure, and its dynamics. Methods applied were calorimetry, neutron diffraction, and quasielastic neutron scattering. The final results from the project will hopefully provide fundamental insight on the chiral system as is, and also serve as a benchmark tool for future studies on chiral recognition at bilayer surfaces, namely the interaction of chiral drugs with biomembranes.

# Tuning the alignment of cellulose nanocrystals and lepidocrocite hybrids using magnetic field

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The possibility to tune the alignment of cellulose nanocrystals (CNC) and lepidocrocite (LPC) nanorods, individually and in combination has been investigated. Contrast-variation small-angle neutron scattering, polarized optical and scanning electron microscopy have been used to examine the system. The measurements were performed for pure nanoparticles (CNC and LPC) as well as for their mixed state in CNC and LPC ratio 10:1 and 100:1. It is found that in individual states CNC and LPC align perpendicular and parallel to the field, respectively whereas a distinct character is observed in the mixed state. For the samples (10:1), two different field regimes were observed where CNC are found to be aligning parallel and perpendicular to the file at low and high magnetic fields, in the influence of the lepidocrocite. On the other hand, for the samples (100:1), partial alignment of the LPC perpendicular to the field (preferred orientation of the CNC) and partial along the filed. Overall, the present studies suggest the possibility to control the switching of the orientation from one preferred direction to other to a complete randomization.

# Small-angle scattering of interpenetrating polymer networks (IPNs) as medical devices with reduced risk of infection

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A common material for urinary catheters is a hydrophobic polymer, silicone elastomer. The properties of silicone make it well-suited for producing medical devices; it has favourable mechanical properties and is chemically inert. However, this hydrophobic surface makes it prone to the adhesion of bacteria and subsequent rapid formation of biofilms. The bacteria that grow in biofilms tend to be resistant to antibiotic treatment, which is a serious problem. Device-associated infections present a real challenge in modern medicine, and therefore, generating materials that resist bacterial attachment and biofilm growth is a worthwhile development for reducing the number of infections.

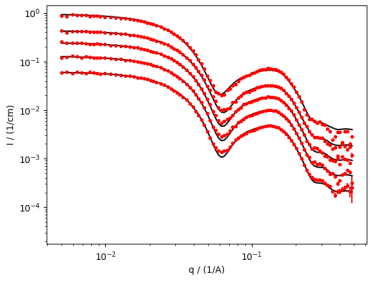
To reduce the adhesion of bacteria and the risk of infection, at BioModics, we have produced silicone catheters and medical devices that are functionalised by the inclusion of a hydrophilic hydrogel interpenetrating polymer network (IPN). The hydrophilic polymer network is introduced by treating the hydrophobic silicone with supercritical carbon dioxide (at elevated but easily accessible temperature and pressure). This expands the silicone network, and then hydrophilic monomers are introduced to react and form an IPN within the silicone network. This hydrogel not only reduces the risk of infection, but it also has the potential to act as a drug delivery mechanism. The IPN act as a reservoir for hydrophilic small molecules that can be suspended and then controllably released at site from the IPN-impregnated silicone.

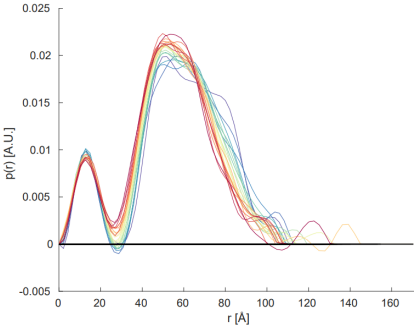
The release properties are dependent on the morphology of the IPN. However, it is a challenge to get insights to the micro structure of the IPNs. Therefore, at the University of Copenhagen, we have performed small-angle scattering measurements (with both X-rays and neutrons) to investigate the distribution of the polymer molecules within the IPN. X-ray measurements are sensitive to differences in electron density, and they primarily revealed the structure of the inorganic filler in the silicone. Neutron measurements are sensitive to isotopes, and by introducing water or heavy water ($D_2O$) to the IPN, we were able to observe the distribution of water and the hydrophilic domains. We will discuss how studies on these nanostructured materials perform as promising future medical devices and how the structure-property relationships arising from scattering measurements are assisting optimisation of the materials for the future.

# Global Fitting of Multiple Data Frames from SEC-SAXS to Investigate the Stucture of Covalently Circularised Nanodiscs

Abigail Barclay, Michelle Fridlund, Nicolai Johansen,
Martin Cramer Pedersen, Lise Arleth
*Niels Bohr Insitute, University of Copenhagen*

In the past couple of decades nanodiscs have been shown to be stable carriers for phospholipid bilayers[1] and can be employed as a platform for functional and structural studies of membrane proteins.[2] The recent improvement in new generation nanodisc production yield and stability comprise circularisation through covalently linking C- and N- termini of membrane scaffold proteins, induced by the *eSrt* enzyme.[3] In the present study a previously acquired synchrotron size-exclusion chromatography small-angle X-ray scattering data set was employed for the low-resolution structural analysis of three covalently circularised nanodisc samples. Scattering profiles of nanodiscs in solution were examined with the help of molecular-based mathematical modelling. Our investigation resulted in an enhanced experimental data fitting with the `WillItFit` framework. This involved developing a new model, specifically for SEC-SAXS analysis, where several data sets from the same sample can be fitted simultaneously with user-defined global and fraction-specific parameters. The preliminary structural results were consistent with previous studies of similar nanodisc constructs[4] and provide a solid model along with the strenghten confidence in further structural studies of membrane proteins contained by presented nanodiscs.

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## SWELLING OF THIN GRAPHENE OXIDE FILMS STUDIED BY IN SITU NEUTRON REFLECTIVITY

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Permeation of multilayered graphene oxide (GO) membranes by polar solvents is known to correlate with their swelling properties and amount of sorbed solvent. However, quantitative estimation of sorption using standard (e.g., gravimetric) methods is technically challenging for few nanometers thick GO membranes/films exposed to solvent vapors. Neutron reflectivity (NR) was used here to evaluate the amount of solvents intercalated into the film which consists of only ~31.5 layers of GO. Analysis of NR data recorded from the GO film exposed to vapors of polar solvents provides information about change of film thickness due to swelling, amount of intercalated solvent, and selectivity in sorption of solvents from binary mixtures. A quantitative study of GO film sorption was performed for $D_2O$, d-methanol, ethanol, dimethyl sulfoxide (DMSO), acetonitrile, dimethylformamide (DMF), and acetone. Using isotopic contrast, we estimated selectivity in sorption of ethanol/d-methanol mixtures by the GO film. Estimation of sorption selectivity was also performed for $D_2O$/DMF, $D_2O$/DMSO, and $D_2O$/acetonitrile binary mixtures. Sorption of polar solvents was compared for the thin GO film, micrometer thick free standing GO membranes, and graphite oxide powders.

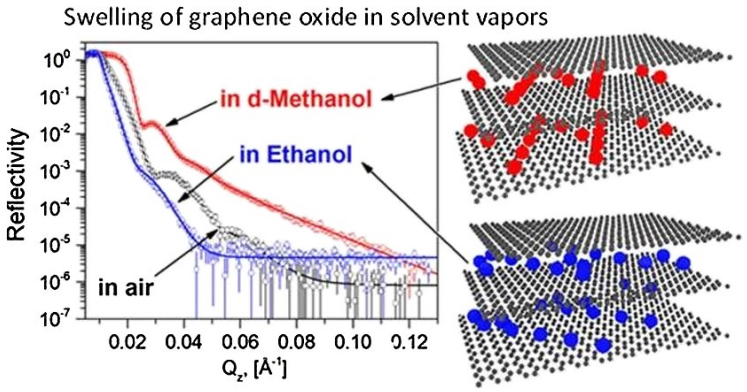


**Figure 1:** Using NR of the GO film for evaluation of selectivity in sorption from the binary vapor mixture. Reflectivity plot: black - GO film in air, blue - GO film intercalated by ethanol, red - GO film intercalated by d-methanol; solid lines represent best-fit model curves.

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# Unfolding and Partial Refolding of an Industrial Cellulase from the SDS-Denatured State: From β-sheet to α-helix and back

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Enzymes are used in many applications including food manufacturing, cosmetics, medication, and detergent formulations. Detergent formulations also contain ionic and nonionic surfactants as essential ingredient. It is well known that ionic surfactants such as sodium dodecyl sulfate (SDS) can denature proteins. Furthermore, it has earlier been shown that the nonionic surfactant octaethylene glycol monododecyl ether ($C_{12}E_8$) can refold certain globular proteins, which have been unfolded by SDS [1]. In this study, the cellulase Cel7b from *Humicola insolens* used in the detergent industry, which mostly contains β-sheet secondary structure, was studied. The unfolding using SDS and subsequent refolding using $C_{12}E_8$ and α-cyclo dextrin (α-CD) was investigated using near- and far-UV circular dichroism (CD), small-angle X-ray scattering (SAXS), isothermal titration calorimetry (ITC), and activity measurements. The results show that at low SDS concentrations Cel7b forms large aggregates due to neutralization of the protein charge by the anionic SDS molecules. CD shows that the protein adopts a-helical secondary structure, in the complexes with SDS. Cel7b could be partially refolded using $C_{12}E_8$; the secondary structure was almost completely restored to mainly β-sheet structure, whereas the tertiary structure was partially restored. Activity measurements show that enzymatic activity is partially restored at high concentrations of $C_{12}E_8$. However, SAXS measurements reveal that upon addition of $C_{12}E_8$, the sample becomes aggregated as is observed for samples with low amounts of SDS. This suggests that SDS and $C_{12}E_8$ form free mixed micelles, but as the system approaches charge neutralization the electrostatic interactions of Cel7b cannot be compensated for by the mixing entropy of SDS and $C_{12}E_8$ in mixed micelles. Attempts to refold using α-CD also resulted in aggregation at high amounts of α-CD.

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# Effects of co-assembly and core crystallization on the structure of n-alkyl-poly(ethylene oxide) micelles

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Molecular self-assembly is indispensable in the fabrication of nano-structures for various applications and in many cases the assembly is driven by partial crystallization.[1] Here we ask the fundamental question: How is the assembled micellar structure affected by crystallinity? For fundamental understanding we use an archetypical model system, n-alkyl-poly(ethylene oxide) ($C_n$-PEO), which forms spherical micelles in water. Application of small-angle neutron and X-ray scattering (SANS/SAXS) allows us to elucidate the micellar morphology. Core crystallization is unambiguously verified by a combination of differential scanning calorimetry, densiometry and wide-angle X-ray scattering. Contrast matching experiments on partially deuterated $C_{28}$-d,h-PEO indicate an anisotropic core shape, likely due to alkane packing restrictions. By mixing polymers with different n-alkyl block length, we are able to vary the melting point and thus control crystallization and aggregation behavior. We find that the aggregation number of the co-assembled micelles exhibits the same scaling laws as single-component micelles [2]. Likewise the melting point depression of the micellar cores follows a Gibbs-Thomson law as observed for pure n-alkyl-PEO systems [3]. In the presentation, we will provide an overview of the effects of core crystallinity, confinement and co-assembly on the structure and thermodynamic properties of n-alkyl-PEO micelles.

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**Applying X-rays and neutrons to understand a bacterial virulence factor**

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Bacteria use different strategies to survive in the environment and to invade host organisms. Of high importance is the ability of bacteria to adhere to surfaces and form biofilms. This requires specialized proteins with specific affinity for relevant biomolecules, such as carbohydrates or glycosylated proteins.

We are interested in a protein colonization factor that mediates the interaction between bacteria and chitin as well as *N*-acetylglucosamine-containing carbohydrates. We plan to study the structure and interactions of this bacterial virulence factor by a range of methods, including X-ray and neutron crystallography, Small-Angle X-ray Scattering (SAXS) and Small-Angle Neutron Scattering (SANS), and neutron reflectometry. Using neutron crystallography, SANS and neutron reflectometry on the protein can reveal the position of hydrogens within the protein and its ligand, greatly aiding the understanding of the molecular interactions.

In order to get strong coherent scattering from the protein for the neutron crystallography and to get a strong contrast between chitin and the protein for SANS and neutron reflectometry studies, it is a necessity to perdeuterate the protein, since deuterium scatters neutrons substantial better than hydrogen. We have managed to develop a protocol for the deuteration of the protein, that gives a good yield and a good purity. We are working on optimizing the protocol further and to use the protein for neutron studies.

For neutron reflectometry studies we also need very smooth chitin layers, we are working on generating these using spin coating and Langmuir-Blodgett deposition.

Understanding the structure and function of bacterial virulence factors is crucial for developing more sophisticated methods to fight bacterial pathogens, by for instance designing better vaccines.

# The effect of the relative permittivity and the temperature response on the structural properties and swelling of $Na^+$-, and $Ca^{2+}$-montmorillonite

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The thermodynamical and structural properties of charged colloids are strongly affected by the valency of the counterions, the solvent, as well as the temperature. With monovalent counterions, the electrostatic interaction between the particles is repulsive, whereas for multivalent counterions, the interaction can be attractive due to electrostatic ion-ion correlation effects.

Here we will present results regarding the structural properties, and the intracrystalline swelling of the natural clay minerals $Na^+$-, and $Ca^{2+}$-montmorillonite (Na-, and Ca-mmt) as an effect of decreasing the relative permittivity of the solvent,[1] and for the temperature response by mixing counterions.[2] For this purpose we have used the experimental techniques; small angle X-ray scattering, and osmotic pressure measurements, in combination with the continuum model utilizing coarse-grained molecular dynamics simulations, and Monte Carlo simulations of two parallel surfaces corresponding to two clay platelets.

It was found that it is possible to tune the electrostatic interactions to obtain a transition from a repulsive to an attractive system for Na-mmt by decreasing the relative permittivity of the solvent, i.e. from water to ethanol. This because when increasing the ethanol concentration, the Bjerrum length increases, and hence, the attractive ion-ion correlation forces are enhanced. For the Ca-mmt system, a non-monotonic behavior of the intracrystalline swelling as function of ethanol concentration was captured experimentally, where an increase in the osmotic pressure, and hence, an increase in *d*-spacing was found at low concentrations, indicating that short-ranged interactions dominate the system. Theoretically, the non-monotonic behavior could not be captured with the continuum model, probably due to the limitation that the electrostatic interactions solely enters the Hamiltonian via the Bjerrum length.

Moreover, it was found that the electrostatic interactions alone can give a positive, negative, or constant osmotic pressure response with temperature, depending on the monovalent/divalent counterion ratio. The increase in osmotic pressure with temperature, which occurs at a low fraction of divalent counterions, can be understood from the DLVO-theory. The origin of the opposite behavior can be explained by the enhanced attractive electrostatic ion-ion correlation interactions with temperature. Our theoretical predictions of the temperature response are in qualitative agreement with the experimental data, giving a further indication that our models can explain the underlying physics. The theory presented here is general and predicts that the temperature response for all types of charged colloids can be controlled by mixing counterions of different valence, if the interactions in the system are dominated by electrostatics.

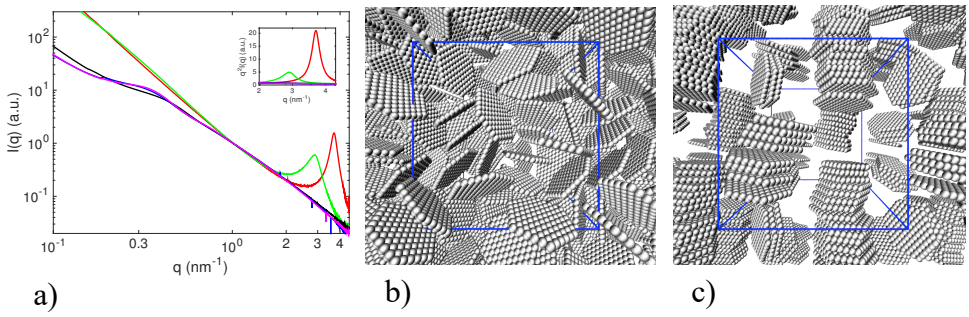


**Figure 1.** a) SAXS spectra for Na-mmt at 0 (magenta), 40 (black), 60 (green), and 95 (red) wt% ethanol, where the Bragg peaks represent tactoid formation in the system. Illustrative configurations obtained from the molecular dynamics simulations of Na-mmt with b) 0, and c) 100 wt% ethanol.

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# Monitoring Blocks' Structural Changes Upon Encapsulation and Release of Nanoparticles and Drugs

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Nanotheranostic polymeric carriers are promising candidates to deliver hydrophobic anti-cancer drugs to the tumor site and monitor treatment efficacy. In this study, a targeted nanocarrier made of multifunctional superparamagnetic iron oxide nanoparticles (SION) and Pluronic P123 employed to carry on paclitaxel (PTX) and magnetic resonance imaging. The structural properties of the system were investigated using small angle neutron scattering (SANS) techniques. Firstly, changes in the micelle structure upon addition of PTX to the solutions were studied. At higher PTX concentrations, stronger scattering intensities at low Q were observed, an indication of attractive interactions between micelles and formation of larger aggregates. Characterization of core/shell structure of micelles at higher temperatures showed same behavior of addition hydrophobic PTX. Accordingly, SANS scattering increased at low Q. The presence of the drug in micelle structure leads to the larger core, corona, and aggregation number. At higher temperature due to dehydration of hydrophilic PEO and more significant influence of hydrophobic interaction of PPO which is associated with a decrease in the CMC. In comparison to 34ºC, aggregation number and size of spherical micelles became large at 50ºC. Addition of drug resulted in the alteration of micelles' structure. However, the presence of SION with the size of >3 nm did not influence the micelle structure. Contrast matching techniques were used to investigate block changes in pluronic micelles. Evidently, the appearance of larger micelles coincides with high polydispersity. It might be due to the aggregation of SION or incorporation of drugs. Cryo-TEM images indicated that the size of SION will affect the overall structure of the DDS. It in turns leads to complexation of heat dissipation behavior of encapsulated SION and calls for comprehensive studies of the effect of magnetic field on the DDS. SANS experiment is a useful technique to reveal detailed in situ structural change as a necessary step for the formulation of nanocarriers in the context of nanomedicine and targeted drug delivery.