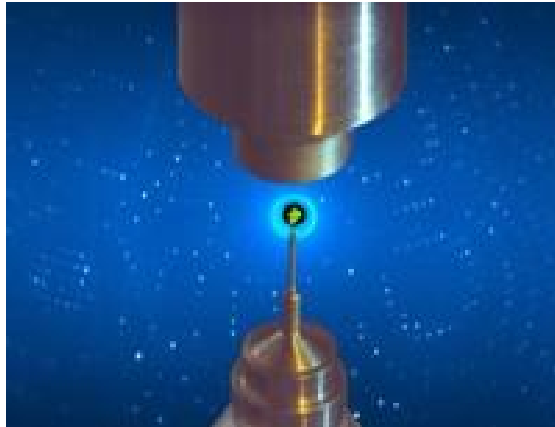
MLZ Conference 2021: Neutrons for Life Sciences



Report of Contributions

Contribution ID: **1** Type: **invited talk**

Neutron capture produced radioisotopes for diagnostics and therapy - opportunities and challenges.

Wednesday, June 9, 2021 9:50 AM (20 minutes)

Radioisotopes are an indispensable tool for diagnostics and therapy in nuclear medicine. As a rule of thumb, proton rich isotopes are most efficiently produced by accelerators, whereas neutron rich isotopes are more efficiently produced by neutron capture reactions. Here, we will focus on the latter process. Inherent to the purpose of nuclear medicine, most of these isotopes are short-lived and therefore cannot be produced on stock.
In the last 10 years, dramatic changes appeared in the supply and applications of these isotopes. Most notably, 99Mo99mTc, the most used isotope for nuclear diagnostic techniques, has suffered from unreliable supply. As the same process is utilized for the production of 131I, its supply is also hampered. On the other hand, isotopes like 177Lu used for targeted cancer therapy can now be produced carrier-free, thereby increasing its production efficiency and allowing hospitals to keep the unavoidable radioactive wastage to a minimum. Yet another application of increasing importance are poly-L-lactic (PLLA) microspheres containing radioactive 166Ho in the GBq range for selective internal radiation therapy of liver cancer.
The presentation will briefly discuss new routes towards minimizing radioactive waste for the production of radioisotopes and will give insight into very new applications of neutron-produced isotopes, such as the targeted therapy of up-to-now incurable metastases of prostate or neuroendocrine tumors.

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Session Classification: Drug design and delivery

Track Classification: Drug design and delivery

Contribution ID: **2** Type: **Poster**

The impact of specific drug molecules on lipid bilayers

Wednesday, June 9, 2021 1:00 PM (20 minutes)

We have investigated the impact of the drugs benzocaine and propanolol on a lipid bilayer formed by L-alpha-phosphatidylcholine. The methods used were neutron reflectivity, grazing incidence small angle neutron scattering, small and ultra small angle neutron scattering. On the one hand, we observed a membrane stiffening and a stalk formation for benzocaine. On the other hand, disordered bilayers (lamellar powders) and highly curved structures were found in the presence of propranolol. In this way we hope to explain diseases when high doses of drugs are applied to humans, and what mechanisms could underlie in general when drugs are applied at normal doses.

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Presenter: FRIELINGHAUS, Henrich (JCNS)

Session Classification: Poster Session

Track Classification: Biological membranes, surfaces and interfaces

Contribution ID: **3** Type: **Talk**

Treating cancer with neutrons –Options for Boron Neutron Capture Therapy

Wednesday, June 9, 2021 10:10 AM (20 minutes)

Despite all advances in cancer treatment, there are still clinical situations in which a locoregional tumor is fatal. More efficient local therapies are mandatory. Boron Neutron Capture Therapy (BNCT) has the potential of such a modality to overcome radiation resistance in certain tumors or to re-irradiate local recurrences after high-dose radiotherapy.
BNCT is based on the 10B(n,α)7Li reaction producing 2 particles with high biological effectiveness in killing cells and short range in tissue (approx. the diameter of one cell). If such reactions can be selectively triggered in tumor cells, a "cell-surgery"results: single tumor cells invading normal tissues are destroyed without damaging surrounding healthy structures.
BNCT was proposed as early as 1936, but so far, no large-scale clinical trials were possible to prove the usefulness of the method for cancer therapy. This was mainly because BNCT required research reactors, mostly located far from a hospital and not always available for patient care.
Recently this situation is changing with the advent of accelerator-based neutron sources. First projects have started at hospitals in Japan and similar projects are underway in other countries.
In this presentation, BNCT development from an academic research project to a market-ready therapy is presented. The current BNCT projects worldwide and the efforts in Europe and Germany to develop and establish this modality will be shown.

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Session Classification: Drug design and delivery

Track Classification: Drug design and delivery

Contribution ID: **4** Type: **invited talk**

Neutron crystallography in the fight against COVID-19: Drug Design Targeting SARS-CoV-2 Main Protease

Thursday, June 10, 2021 3:30 PM (30 minutes)

COVID-19, caused by SARS-CoV-2, is a global health and economic catastrophe. The viral main protease (Mpro) is indispensable for SARS-CoV-2 replication and thus is an important target for small-molecule antivirals. Neutrons are an ideal probe to observe protonation states of ionizable amino acids at near-physiological temperature, directly determining their electric charges –crucial information for computer-assisted and structure-guided drug design. Our structures of Mpro collected at near-physiological temperatures revealed the reactivity of the catalytic cysteine, malleability of the active site, and binding modes of clinical protease inhibitors. Neutron crystal structures of ligand-free and covalent inhibitor-bound Mpro allowed direct observation of protonation states of all residues in a coronavirus protein. The catalytic Cys-His dyad exists in the reactive zwitterionic state, with both Cys145 and His41 charged, instead of the anticipated neutral state. Covalent inhibitor binding results in modulation of the protonation states, retaining the overall electric charge of the Mpro active site cavity. High-throughput virtual screening in conjunction with *in vitro* assays identified a non-covalent compound with micromolar affinity, used as a lead to design novel Mpro inhibitors. Our research is providing real-time data for atomistic design and discovery of Mpro inhibitors to combat the COVID-19 pandemic and prepare for future threats from pathogenic coronaviruses.

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Session Classification: Neutrons in the fight against virus diseases

Track Classification: Neutrons in the fight against virus diseases

Contribution ID: **5** Type: **Poster**

High-resolution neutron spin echo spectroscopy with the J-NSE "PHOENIX"at MLZ

Wednesday, June 9, 2021 2:40 PM (20 minutes)

Neutron spin echo (NSE) spectroscopy provides the ultimate energy resolution in quasi-elastic thermal and cold neutron scattering spectroscopy. In terms of Fourier-time (τ) –or equivalently in terms of the accessible energy (E) –high resolution means the extension of τ (respectively E) into to the regime of μs (neV). The recently upgraded Jülich neutron spin echo spectrometer J-NSE "PHOENIX"at MLZ with its superconducting, fringe-field compensated main precession coils and optimized field shape provides a broad Fourier time range relevant for thermally driven fluctuations in biology and soft matter [1]. The J-NSE "PHOENIX"meets the needs to look into the microscopic dynamics of soft- or biological matter. We present the results on the performance of the spectrometer after the refurbishment and some selected examples from the realm of soft matter dynamics and biology. Recent experiments comprise measurements with the J-NSE of protein domain fluctuations under physiologically relevant conditions [2], membrane dynamics in lipase containing microemulsions [3] or internal dynamics of microgel particles [4] which might be used as functional coatings or drug delivery systems.

[1] S. Pasini, et al., Rev. Sci. Instrum. 90, 043107 (2019)
[2] L. Balacescu, et al., Scientific Reports 10, 1570 (2020)
[3] S. Engelskirchen et al., Frontiers in Chemistry, 8, 613388 (2021)
[4] T. Kyrey, et al., Soft Matter, 15, 6536 (2019)

Primary author: HOLDERER, Olaf

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Presenter: HOLDERER, Olaf

Session Classification: Poster Session

Track Classification: Neutrons and complementary methods in biology

Contribution ID: **6** Type: **Poster**

Mucin thin layers on top of model membranes: a model environment for delivery

Wednesday, June 9, 2021 1:20 PM (20 minutes)

Mucus is a highly viscoelastic secretion, covering the epithelia surfaces of several body tracts. Its function and composition differ at different locations, but its general task is to protect tissues from dehydration, mechanical stress, and to act as barrier against microorganisms and toxic substances. Mucus is mainly composed of water, lipids, small proteins and nucleic acids, but its mechanical properties are due to high molecular weight glycoproteins, mucins, which can establish adhesive interactions with particulates. The development of mucosal drug delivery vehicles is a great challenge because little is known about the interactions between mucin and macromolecules: they can rapidly penetrate or establish prolonged contact with mucus, depending on their specific formulation. We worked on the development of model mucus environments to deepen the understanding of mucin interactions with polymers used in pharmaceutical formulations by complementary techniques. Beside SAXS and SANS in bulk, we applied QCM-D and neutron reflectivity on thin mucin layers. Further, we developed a bio-inspired complex model consisting in a mucin layer on top of a single supported membrane, structurally investigated by neutron reflection. Since complexation between mucins and biomacromolecules takes place close to cell membrane surface, our model is potentially predictive of the fate of nanodrugs intended to cross mucus and enter epithelial cells.
Rondelli V., et al.,Int.J.Mol.Sci.(2019)

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Presenter: RONDELLI, Valeria (Università degli Studi di Milano)

Session Classification: Poster Session

Track Classification: Biological membranes, surfaces and interfaces

Contribution ID: **7** Type: **Talk**

Fusion mechanisms of small extracellular vesicles with model membranes

Thursday, June 10, 2021 9:30 AM (20 minutes)

Extracellular vesicles (EVs) are a potent intercellular communication system. Such small vesicles transport biomolecules between cells and throughout the body, strongly influencing the fate of recipient cells. Due to their specific biological functions they have been proposed as biomarkers for various diseases and as optimal candidates for therapeutic applications. Despite of their extreme biological relevance, their mechanisms of interaction with the membrane of recipient cells are still hotly debated. We performed a multiscale investigation based on AFM, SAXS, SANS and Neutron Reflectometry to reveal structure-function correlations of purified EVs in interaction with model membrane systems of variably complex composition, to spot the role of different membrane phases on the vesicles internalization routes. Our analysis reveals a strong interaction of EVs with the model membranes and preferentially with the borders of protruding phase domains. Moreover, we found that upon vesicle breaking on the model membrane surface, the biomolecules carried by/on EVs diffuse in a way that departs from the expected simple fusion. Our approach has clear implications on the modulation of EVs internalization routes by targeting specific domains at the plasma cell membrane and, as a consequence, on EVs-based therapies.

F. Perissinotto, V. Rondelli et al., Nanoscale 2021

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Session Classification: Biological processes

Track Classification: Biological processes

Contribution ID: **8** Type: **Poster**

The Phase Behaviour of the Myelin Basic Protein

Wednesday, June 9, 2021 2:40 PM (20 minutes)

The Myelin Basic Protein (MBP) is an essential part of the myelin sheath in almost all vertebrates and, thus, contributes significantly to flawless signal conduction. Here, one of its key properties is the ability to perform a Liquid-Liquid Phase Separation (LLPS), the coexistence of highly concentrated protein phases within a diluted solution.
Microscopy experiments have indicated that a LLPS of MBP would occur upon the addition of Polyethylene glycol (PEG). By using contrast matched PEG in 100% D2O, USANS experiments (KWS-3 at MLZ) confirmed that the optically observed droplets originated from MBP condensates. The droplet size was determined to be in the low µm range, which is in good accordance with DLS measurements. Kinetic studies on the droplet growth pointed out that an equilibrium size was reached after only a few minutes. Furthermore, the investigations have shown that both coalescence and Ostwald ripening contribute to droplet expansion.
Neutron scattering experiments at KWS-2 revealed unfolding of the proteins as well as increasing size of MBP molecules upon the addition of PEG. As a complementary technique, CD spectroscopy was used which supported the previous finding.
It is concluded that variations of protein structure and the occurrence of a LLPS are related phenomena which affect each other. Hence, future examinations will cover this effect in more detail, as well as droplet growth kinetics of the earliest stages of a LLPS with improved temporal resolution.

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Presenter: GRAF VON WESTARP, Igor

Session Classification: Poster Session

Track Classification: Protein structure, function and dynamics

Contribution ID: **9** Type: **Poster**

The direct geometry cold chopper spectrometer TOFTOF

Wednesday, June 9, 2021 2:40 PM (20 minutes)

TOFTOF is a direct geometry disc-chopper time-of-flight spectrometer. A cascade of seven fast rotating disc choppers is used to prepare a monochromatic pulsed beam which is focussed onto the sample by a converging super-mirror section. The scattered neutrons are detected by 1000 3He detector tubes with a time resolution up to 50 ns. The detectors are mounted at a distance of 4 m and cover 12 m2 (or 0.75 sr). The high rotation speed of the chopper system together with a high neutron flux in the wavelength range of 1.4 -14 Å allows free tuning of the energy resolution between 3 meV and 2 μeV.
The fast neutron background is suppressed by the s-shaped primary neutron guide. This enables the investigation of weak signals. The existing linearly tapered neutron guide yields a beam spot size of 23x47 mm2. As alternative option a focussing guide can be used. This leads to an intensity gain up to a factor of 3 (wavelength dependent) on a sample area of 10x10 mm2.

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Presenter: WOLF, Marcell (TUM)

Session Classification: Poster Session

Track Classification: Neutrons and complementary methods in biology

Contribution ID: **10** Type: **Poster**

Linking genes and membrane lipid composition to insights of the antifungal mechanism of Amphotericin B provided by neutron reflectometry

Wednesday, June 9, 2021 1:40 PM (20 minutes)

Candida glabrata has been known as a non-pathogenic yeast found in healthy humans, but the number of infections caused by it has increased, making understanding its virulence an urgent task. In our multidisciplinary effort, we combine methods to produce *C. glabrata* strains with well-defined genetic modifications of virulence/resistance factors(1) with characterization of their membrane lipid composition and structure and interaction mechanism with the antifungal drug Amphotericin B (AmB). AmB, being a WHO essential medicine, has a broad spectrum and has been used against systemic fungal and parasitic infections. It is often a last line of defense, but despite its long track record, its mechanism of action and how resistance can evolve is not well understood. We use neutron reflection to study the AmB mechanism in model(2) and in membranes from *C. glabrata* strains with increased or decreased AmB resistance due to a defined change in gene expression. Hereby we have observed that resistance is related to increased AmB membrane insertion and decreased sterol extraction while high susceptibility to AmB correlates with a higher sterol extraction and lower insertion. Our integrative approach demonstrates how neutron techniques can provide insight into the molecular basis of antifungal activity and resistance with the aim to improve therapies, find better drugs and new drug targets.
1 Ishchuk et al. Front Microbiol, 2019. 10: 1679
2 Delhom et al. Nanomaterials, 2020. 10(12)

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Session Classification: Poster Session

Track Classification: Biological membranes, surfaces and interfaces

Contribution ID: **11** Type: **Poster**

LP3 and DEMAX

Wednesday, June 9, 2021 2:40 PM (20 minutes)

Proteins are of enormous importance to life on earth. They have a multitude of different functions in all organisms and can work as enzymes, gene regulators, structural components, transporters, and receptors. Most drugs act on proteins. The structures and mechanisms of proteins are therefore prominent topics in life science research.
Access to both state-of-the-art X-ray (MAX IV) and neutron sources (ESS) will increase the capacity for innovation in the life sciences. To enable efficient use of these unique and powerful facilities by Lund researchers, Lund University hosts the protein production platform, LP3 (www.lu.se/lp3). LP3 assists users with: 1) Recombinant protein production, 2) biophysical protein characterisation 3) High-throughput crystallization and structure determination, and 4) Stable isotope labelling and bio-deuteration of biological macromolecules.
Since 2016, the DEuteration and MAcromolecular Xtallization (DEMAX) platform of the ESS is co-localized with LP3. DEMAX and LP3 are coordinating in their efforts [1-4] to develop cost-effective production of deuterated proteins for macromolecular crystallography, enable crystallization of interesting proteins for neutron work, and for the production of labelled proteins/lipids for neutron reflectometry.

1 Koruza et al. Crystals, 2018. 8(11)

2 Koruza et al. Arch Biochem Biophys, 2018. 645: 26

3 Koruza et al. Acta Crystallogr D Struct Biol, 2019. 75: 895

4 Koruza et al. J Struct Biol, 2019. 205: 147

Primary authors: KNECHT, Wolfgang (Lund University); FISHER, Zoe (European Spallation Source ERIC)

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Session Classification: Poster Session

Track Classification: Neutrons and complementary methods in biology

Contribution ID: **12** Type: **Poster**

Effects of glassy matrices on the protein-like dynamical transition of PNIPAM

Wednesday, June 9, 2021 2:40 PM (20 minutes)

Hydrated proteins undergo a dynamical transition (DT) at $T_d \approx$ 180-230 K. The transition is associated with the activation of protein dynamics on the ps-ns time scale, suitably detected by Elastic Incoherent Neutron Scattering (EINS). The DT has been also observed in other biomolecules and is deemed necessary for biological functionality. Surprisingly, a DT has been recently found in a non-biological system, i.e. poly(N-isopropylacrylamide) (PNIPAM). Despite its synthetic nature, PNIPAM is able to reproduce the complex solvent-macromolecule interactions at the basis of biological processes. The generality associated with the observation of the DT suggests a prominent role of the solvent. In proteins, the main features of the DT are critically affected by the presence of *stabilizing* compounds. The formation of a glassy stabilizing matrix inhibits the protein mobility by shifting T_d toward higher temperatures and by reducing the amplitude of associated protein motions. Interestingly, the constraining action on protein fast dynamics is thought to be related to the bioprotectant ability promoted by stabilizers over longer timescales, although the microscopic details of these processes are unclear. By means of EINS techniques, we exploited the bio-mimic behaviour of PNIPAM by investigating the fast dynamics of PNIPAM chains when in presence of water/stabilizer mixtures. As in proteins, we found a tight connection between polymer dynamics and characteristics of the solvent.

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Session Classification: Poster Session

Track Classification: Protein structure, function and dynamics

Contribution ID: **13** Type: **Talk**

Structural dynamics of substrate processing by the PAN-proteasome complex studied by TR-SANS

Wednesday, June 9, 2021 11:30 AM (20 minutes)

Crystallography and cryo-EM have provided new and exciting insight into protein substrate degradation by AAA+ ATPases and the PAN-proteasome system. However, direct structural information on the conformational changes of the working complex, as well as the respective substrate states and populations during the active unfolding and degradation process remain scarce.
Here, we apply time-resolved small angle neutron scattering (TR-SANS) to obtain structural insight into the respective components during the active process in solution. By combining solvent contrast variation and selective macromolecular deuteration with online fluorescence, we were able to obtain separate structural information on the conformational states of the protein unfoldase PAN and the GFP substrate during the active reaction on the sub-minute timescale [1, 2].
While PAN undergoes a reversible conformational contraction during the substrate unfolding process, GFP aggregates rapidly in the presence of PAN alone, but is being hydrolyzed very efficiently once the proteasome partner is added to the reaction.
TR-SANS is thus a very promising technique that can provide structural kinetics from individual partners in complex solution ensembles, impossible to separate by SAXS [3], and complementary to “static snapshots” from crystallography and cryo-EM.

[1] Ibrahim et al. (2017) Sci. Rep. 7, 40948
[2] Mahieu et al. (2020) Biophys. J. 119(2), 375
[3] Mahieu et al. (2020) EPJ Web of Conferences 236, 03002

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Session Classification: Protein structure, function and dynamics

Track Classification: Protein structure, function and dynamics

Contribution ID: **14** Type: **Poster**

X-ray and Neutron Small Angle Scattering study of the non structural proteins nsp10 and nsp14 from SARS-CoV-2

Wednesday, June 9, 2021 2:40 PM (20 minutes)

The outbreak of the coronavirus disease (COVID-19) caused by the coronavirus SARS-CoV-2 spread to every continent affecting the global health and economy. The first two open reading frame of the SARS-CoV-2 genome are translated into two polyproteins.These are cleaved into 16 non-structural proteins (nsp1-nsp16), which are essential for viral replication and transcription [1].Among these, nsp14 is a bifunctional protein.Its N-terminal domain possesses exoribonuclease (ExoN) activity and plays a proofreading role for prevention of lethal mutagenesis, whereas the C-terminal domain functions as a guanine-N7 methyl transferase (N7-MTase) for mRNA capping [2].The activity of the ExoN domain of nsp14 is significantly increased upon interaction with the nsp10 protein. The nsp10 is a single domain protein [3], which stimulates ExoN and the N7-MTase activity of the nsp14.
Within this context, SANS and SAXS experiments may play a key role to investigate structural and conformational features of the SARS-CoV-2 nsp10-nsp14 proteins at their physiological temperature and conditions.By using both techniques we could get information on the hydration shell of the proteins and the amount of the oligomeric species as well.With this goal in mind, we are going to perform a SEC-SAXS experiment (P12,Petra III,Hamburg) and a SANS experiment (KWS2,MLZ,Garching).
Ref.:
[1]J.Ziebuhr,Curr.Top.Microbiol.,287,57(2005)
[2]L.Eckerle,J.Virol.81,12135,(2007)
[3]A.Rogstam,Int.J.Mol.Sci,21,7375(2020)

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Session Classification: Poster Session

Track Classification: Neutrons in the fight against virus diseases

Contribution ID: **15** Type: **Poster**

Exploring dynamic processes in biological systems with SPHERES

Wednesday, June 9, 2021 2:40 PM (20 minutes)

The neutron backscattering spectrometer SPHERES (SPectrometer for High Energy RESolution) at MLZ is a third generation backscattering spectrometer with focusing optics and phase-space transform (PST) chopper. It covers a dynamic range of ± 31μeV with a high resolution of about 0.66μeV and a good signal-to-noise ratio. The instrument performance has been improved over the recent years by different measures. The intensity has been more than doubled by the upgrade of the PST chopper and the focusing guide. The signal-to-noise ratio can be significantly improved by employing the new background chopper.
SPHERES enables investigations on a broad range of scientific topics. It is in particular sensitive to the incoherent scattering from hydrogen and allows to access dynamic processes up to a timescale of a few ns. Therefore it is well suited to study dynamic processes in various biological systems. Selective deuteration allows for example to follow the mobility of water on the surface of proteins (e.g. Y. Fichou et al., PNAS 112, 6365 (2015)) or measure internal protein motions (e.g. A. Stadler et al., J. Phys. Chem. B 123, 7372 (2019)).

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Session Classification: Poster Session

Track Classification: Neutrons and complementary methods in biology

Contribution ID: **16** Type: **Talk**

Linking cell uptake to self-assembled block co-polymer nanoparticle morphology –small angle scattering studies

Thursday, June 10, 2021 5:20 PM (20 minutes)

In tandem with cell studies we have used SAXS and SANS to link nanoparticle structure and its relationship to drug content to provide new strategies for drug delivery nanoparticles as well provide insight into outstanding questions about the entry of particles into cells. Through carefully controlled polymerisation reactions self-assembled block co-polymers offer exquisite control over particle shape and surface characteristics. Small angle X-ray (SAXS) and neutron (SANS) scattering offer two similar and complementary techniques to characterise self-assembled particles used for drug delivery. There is no need for any special sample preparation and structure is evaluated in an environment very close to that of the physiological milieu.

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Presenter: GARVEY, Christopher (MLZ)

Session Classification: Drug design and delivery

Track Classification: Drug design and delivery

Contribution ID: **19** Type: **Poster**

Dynamical differences between polymorphs of lysozyme amyloid fibrils with different levels of cytotoxicity

Wednesday, June 9, 2021 2:40 PM (20 minutes)

Amyloid fibrils are self-assembled protein filaments, the deposition of which in tissues causes amyloidosis. Recently, much attention has been paid to polymorphism, where proteins form various amyloid fibrils that differ in structure and show different levels of cytotoxicity depending on fibrillation conditions. Since intramolecular motions in the fibrils are considered to play a crucial role in amyloidosis, it is important to understand the dynamical features of their polymorphs. In this study, we focused on the polymorphism of hen egg white lysozyme (HEWL), a model for studying lysozyme amyloidosis in human and prepared D2O hydrated powder samples of two HEWL amyloid polymorphs formed at pH 6 or 2, which are known to show distinct levels of cytotoxicity. We carried out the elastic incoherent neutron scattering (EINS) measurements on these samples using the IN13 spectrometer at ILL in France in the temperature range from 20 K to 310 K. The temperature dependences of the mean square displacements (MSDs) of atomic motions in the proteins were evaluated from the EINS spectra. It was found that whereas the MSD values are similar between the two fibrils at lower temperatures, the fibrils with higher toxicity show significantly larger MSDs at higher temperatures (> 270 K). This implies that the differences in the anharmonic, diffusive local motions are related to the differences in the level of cytotoxicity. At the conference, more detailed results will be presented.

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Session Classification: Poster Session

Track Classification: Protein structure, function and dynamics

Contribution ID: **20** Type: **Talk**

ANSTO's National Deuteration Facility (NDF): A Molecular Deuteration Platform for Characterisation Studies in the Life Sciences

Friday, June 11, 2021 9:50 AM (20 minutes)

The NDF at the Australian Nuclear Science and Technology Organisation (ANSTO) provides deuteration through both biological and chemical techniques for a diversity of molecules and applications. Molecular deuteration of organic compounds and biomolecules significantly increases options in complex structure function investigations by providing contrast and improved data resolution when using neutron techniques.
Along with capabilities for provision of variably deuterated proteins, NDF provides access to a range of deuterated lipids, unsaturated phospholipids (such as POPC and DOPC) and detergents. The availability of these custom complex deuterated molecules which are generally unavailable commercially, adds to the range of characterisation techniques possible across multiple research areas in the life sciences including drug discovery and vaccine development.
Match-out detergents can be utilised to determine membrane protein conformation in solution via small angle neutron scattering (SANS) and deuterated lipids can be employed to construct biologically relevant lipid matrices. Encapsulating various molecules within lipid nanoparticles (LNP) has garnered high interest during the worldwide COVID-19 pandemic with the development of RNA vaccines. Deuterating components of these LNP is essential for neutron techniques that can be used to study the stability and structure of these drug delivery vehicles.
An overview of the NDF capabilities will be provided in this presentation.

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Session Classification: Neutron and complementary methods in biology

Track Classification: Neutrons and complementary methods in biology

Contribution ID: **21** Type: **Poster**

Following the diffusive processes during a non-classical protein crystallization via neutron spectroscopy

Wednesday, June 9, 2021 2:40 PM (20 minutes)

Following molecular dynamics during the temporary evolution of kinetically changing samples is a major challenge. With recent developments of analysis frameworks, accessing the short-time self-diffusive properties of protein solutions by measuring specific energy transfers via neutron backscattering, kinetically changing samples can be investigated. The immobile fraction, determined by multi-dimensional fits of full QENS spectra, can be assigned to proteins in a gel-like state or in crystals [1]. Using time-dependent neutron spin echo measurements at different momentum transfers q, we access the collective diffusion during the crystallization process in different phases of the sample.
Here, we discuss the results of a recent protein crystallization study. CdCl2 induces a non-classical crystallization process [2,3] of BLG with a metastable intermediate phase. We investigated the short-time collective and self-diffusion of BLG by neutron spin-echo (IN11), FWS and QENS (IN16b), respectively, of the crystallization process for different sample conditions. Combining the different results, a consistent picture of the process can be obtained, which differs significantly from classical BLG crystallization induced by ZnCl2[1]. This implies a strong influence of seemingly subtle cation-specific effects on protein crystallization.
[1]C. Beck et al., Cryst. Growth Des. 2019
[2]A. Sauter et al., J. Am. Chem. Soc. 2015
[3]A. Sauter et.al., Faraday Discuss. 2015

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Presenter: BECK, Christian (Universität Tübingen)

Session Classification: Poster Session

Track Classification: Protein structure, function and dynamics

Contribution ID: **22** Type: **Poster**

Dynamics of apolipoprotein B-100 assessed by elastic incoherent neutron scattering

Wednesday, June 9, 2021 2:40 PM (20 minutes)

Protein dynamics is pivotal to fulfill protein function. Apolipoprotein B-100 is a giant monomeric protein with a fascinating dynamical history: it mediates the conversion from very low density lipoprotein (VLDL, ~50 nm) to low density lipoprotein (LDL, 22 nm). As a key-player in the cholesterol transport system, the protein is intimately linked to the development of atherosclerosis and cardiovascular diseases.

We here employed elastic incoherent neutron scattering (EINS) experiments to assess the local motions in isolated apo B-100 and compare it to those in VLDL and LDL complexes. All samples were lyophilized and hydrated with D2O to finally obtain approximately one single hydration layer around the protein. EINS scans were carried out from 20 to 315 K at the backscattering spectrometer IN13 (energy resolution of 8 µeV) at the Institut Laue Langevin (ILL), Grenoble, France. The mean-square displacements (MSD) as a measure of flexibility and the mean environmental force constant <k> to quantify structural resilience were calculated.

Our results suggest that apo B-100 with an MSD of 1.5 $Å^2$ at 310 K is a highly flexible protein. Apo B-100's force constant <k> is comparable to that of VLDL, whereas their values substantially differentiate from LDL. LDL shows a much higher molecular resilience and thus can be considered much more rigid than VLDL and apo B-100.

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Session Classification: Poster Session

Track Classification: Protein structure, function and dynamics

Contribution ID: **23** Type: **Poster**

Influence of NaCl on Phospholipid membranes

Wednesday, June 9, 2021 2:20 PM (20 minutes)

Understanding the structure and dynamics of phospholipid membranes is of paramount importance for biophysics, biology and medical sciences. Virtually every living organisms is comprised of several of those membranes that provide a variety of functions, ranging from the separation of volumes to more complex functions like nutrient or information transport across the membrane.
All those functions are linked both to the structure and dynamics of the membrane itself. In this contribution we present the combination of a well-studied model membrane of L-alpha-phostatidylcholine (SoyPC) and NaCl. We were able to determine the location of the ions, their influence on the structure of the membrane and subsequently how this change in structure impacts the dynamics of the membrane. This was done by means of neutron reflectometry, grazing-incidence small-angle neutron scattering (GISANS) and grazing-incidence neutron spin-echo spectroscopy (GINSES).
We were able to show that the ions will concentrate along the boundary of the headgroup-water interface, more or less pronounced as a function of concentration and temperature. This change we linked to a distinctive increase in mobility along the membrane surface with increasing concentration.
This information we can now use to better judge our model systems and differentiate in-vitro between salt-free and physiological conditions. Also, this allows further research on the impact of small ionic molecules on phospholipid membranes.

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Session Classification: Poster Session

Track Classification: Biological membranes, surfaces and interfaces

Contribution ID: **24** Type: **Poster**

Molecular bases of proteome adaptation to High Pressure in extremophilic Archaea

Wednesday, June 9, 2021 2:40 PM (20 minutes)

Proteome adaptation to high pressure in Archaea is still an open debate. Whole genome comparative studies could not identify a clear adaptation pattern, and HP adaptation is often considered as concomitant to another adaptation, for instance to high or low temperature.
Studies on whole cells of the near isogenic HP-adapted *T. barophilus* (T=85°C, p=400bars, piezophilic) and HP-sensitive *T. kodakarensis* (T=85°C, p=1bar, piezosensitive) highlighted the differences in proteome dynamics between these two species. The observed results are congruent with two major adaptive strategies, either structural differences, which would generate the difference in dynamics of the proteomes, or the existence of a protection mechanism to maintain proteome functionality under pressure stress.
We investigated the first hypothesis using EINS and QENS to unravel the dynamics of the protein *Phosphomannose Isomerase* from the two species. This approach allowed a detailed characterization of the dynamics of the two proteins without the complications of a whole-cell environment. Our results show that the dynamics of the protein from the piezophile is more stable on a time scale of 10-100 ps, and that large side-chain motions are even favoured by HP. In contrast, the protein from the piezosensitive species is destabilized by HP, becomes more rigid and its structure is likely affected. This is the first experimental demonstration of the effect of HP adaptation on the fast dynamics of proteins.

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Session Classification: Poster Session

Track Classification: Protein structure, function and dynamics

Contribution ID: **25** Type: **Poster**

ON THE INTERACTIONS BETWEEN LACTOFERRIN AND β-LACTOGLOBULIN: A SMALL-ANGLE NEUTRON SCATTERING STUDY

Wednesday, June 9, 2021 2:40 PM (20 minutes)

The protein-protein interactions, namely those between the cationic lactoferrin and the oppositely charged β-lactoglobulin have been the subject of many studies due to their appeal for different food and pharmaceutical applications. These two proteins show some peculiarities in terms of physicochemical properties and behaviour in solutions. Although the hetero-complexation of the proteins is mainly based on non-covalent interactions, a closer look at the interactions involving lactoferrin (LF) and β-lactoglobulin (BLG) reveals fairly unexpectedly behaviour based on a dominating charge regulation mechanism. Electrostatic complexes and even liquid-liquid phase separation have been observed for these proteins thus constituting model systems when examining the influence of experimental variables such as pH, temperature, etc.
Previously, we have shown that human LF exhibits a compact monomeric conformation in solution whilst BLG is predominantly in a dimeric conformation over a broad range of pH from 3 up to 9. With the aim of obtaining additional insights at the molecular level, here we report on the interactions studied by small-angle neutron scattering under temperature conditions and salt addition. Additionally, two different stoichiometric conditions have been regarded as 1:2 and 1:10 (LF:BLG). The results consistently displayed that the complexation between the LF and BLG occurs at pH 5.9 and the LF-2(BLG) hetero-complexes formation takes place.

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Session Classification: Poster Session

Track Classification: Protein structure, function and dynamics

Contribution ID: **26** Type: **Poster**

OBSERVING THE CONFORMATIONAL CHANGES OF HUMAN LACTOFERRIN USING SMALL ANGLE NEUTRON SCATTERING

Wednesday, June 9, 2021 2:40 PM (20 minutes)

Lactoferrin (Lf) is a non-heme protein known for its ability to naturally bind tightly Fe3+ ions in various physiological environments. Therefore, Lf has a significant role in the processes of iron regulation at the cellular level and organize the specific and non-specific immune response in the body.
The conformational changes within the protein structure caused by the iron-binding are continuously studied. Resolving the iron binding-release mechanism and study of physicochemical factors influenced by it will help gain a wider understanding of its biological and medical properties.
The presented study was addressed towards observing the conformation stability of human lactoferrin influenced by the temperature, ph effect and salt concentration in solution by small angle neutron scattering (SANS).
The SANS study allowed us to structurally differentiate between the iron-free Lf (apo-Lf) and iron-saturated Lf (holo-Lf) in solution [1]. The 3-dimensional models computed for both forms of human lactoferrin also show visible differences, both having a more compact conformation compared to the high-resolution structure.

[1] Anghel, L., Radulescu, A. & Erhan, R.V. Structural aspects of human lactoferrin in the iron-binding process studied by molecular dynamics and small-angle neutron scattering. Eur. Phys. J. E 41, 109 (2018). https://doi.org/10.1140/epje/i2018-11720-x

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Session Classification: Poster Session

Track Classification: Protein structure, function and dynamics

Contribution ID: **27** Type: **Talk**

Translocation of non-ionic synthetic polymers through lipid membranes

Thursday, June 10, 2021 2:20 PM (20 minutes)

We synthesized the non-ionic alternating amphiphilic polymers containing dicarboxylic acids as hydrophobic blocks and ethylene glycol oligomers as hydrophilic, and studied their properties. The lengths of the building blocks and their mass ratio allows tuning the amphiphilicity of the polymer chains in a wide range. As a consequence the polymers either dissolve in water as free chains or form micelles and gels.
We found that the water-soluble polymers with short hydrophobic and hydrophilic blocks can passively translocate through biological membranes. We studied translocation properties using Pulsed Field Gradient NMR and fluorescent microscopy. The results show that the translocation process consists of a relatively fast membrane saturation with the polymers and a slow release process. The translocation time varies from minutes to many hours depending on the polymer and lipid composition, polymer molecular weight and temperature. In order to understand the interaction of the polymers with lipid membrane and the translocation mechanism we performed Neutron Reflectometry and Small Angle Neutron Scattering experiments. The first measurements show that the polymers are located mainly in the hydrophobic membrane interior and cause thickness fluctuations of the tail region.
As the polymers do not show any damage of the membranes and are biodegradable, they might be interesting candidates for drug delivery applications.

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Presenter: KOSTYURINA, Ekaterina

Session Classification: Biological membranes, surfaces and interfaces

Track Classification: Biological membranes, surfaces and interfaces

Contribution ID: **28** Type: **Talk**

Neutron Scattering Experiments and Multi-Scale Simulations Reveal Dynamical Properties of the Bacterial Cytoplasm Near Cell-Death Temperature

Thursday, June 10, 2021 11:30 AM (20 minutes)

Daniele Di Bari^{1,2,3}, Stepan Timr⁴, Fabio Sterpone⁴, Alessandro Paciaroni¹, Judith Peters^{2,3}.

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Life on Earth exhibits an amazing adaptive capacity to a vast range of temperatures. While the molecular mechanisms underlying such adaptability are not yet fully understood, it has been proposed that the temperature of cellular death coincides with a catastrophic denaturation of the proteome. Here we combine incoherent quasi-elastic and elastic neutron scattering experiments with multi-scale molecular dynamics simulations to describe the dynamical features of the proteins in the *E. Coli* cytoplasm when approaching thermal denaturation, and to characterize distinct contributions to their pico- to nanosecond dynamics. Moreover, we test the validity of the Lindemann criterion —linking structural fluctuations and melting —in cell-like conditions.
Our results allow us to rationalize the existence of a specific dynamical regime revealed by neutron scattering experiments to be a general signature around cell-death temperature.

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Session Classification: Protein structure, function and dynamics

Track Classification: Protein structure, function and dynamics

Contribution ID: **29** Type: **Talk**

New insights into the interaction of Class II dihydroorotate dehydrogenases with ubiquinone in lipid bilayers as a function of lipid composition

Tuesday, June 8, 2021 10:50 AM (20 minutes)

The fourth enzymatic reaction in the de novo pyrimidine biosynthesis, the oxidation of dihydroorotate to orotate, is catalyzed by dihydroorotate dehydrogenase (DHODH). Enzymes belonging to the DHODH Class II are membrane-bound proteins that use ubiquinone as their electron acceptor. We designed this study to understand the interaction of an N-terminally truncated version of human DHODH (HsΔ29DHODH), a target for anti-inflammatory drugs, and wild-type bacterial DHODH from Escherichia coli (EcDHODH), with ubiquinone (Q10) in supported lipid membranes using neutron reflectometry (NR). NR allowed us to determine in situ, under solution conditions, how the enzymes bind to lipid membranes and to resolve the location of the Q10. We can show that EcDHODH binds more efficiently to simple bilayers consisting of POPC and TOCL than HsΔ29DHODH. Q10 is exclusively located at the center of all the lipid bilayers investigated, including more complex lipid mixtures mimicking either bacterial or mitochondrial membranes. Incorporation of Q10 into lipid bilayers also increases the efficiency of DHODH binding to the lipid bilayers, as shown by increased enzyme retention upon rinsing. We therefore show that the interaction between the enzymes located at the bilayer-water interface and the membrane is mediated by Q10. Our results highlight the importance of Q10 as well as lipid composition on enzyme binding and enzyme retention.

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Presenter: OROZCO RODRIGUEZ, Juan Manuel (Lund University)

Session Classification: Biological membranes, surfaces and interfaces

Track Classification: Biological membranes, surfaces and interfaces

Contribution ID: **30** Type: **Talk**

Protein short-time diffusion in polydisperse crowding

Wednesday, June 9, 2021 4:30 PM (20 minutes)

Knowledge of the protein tracer diffusion constitutes a key element to describe intracellular transport, which can be modeled by the self-diffusion in colloid systems. However, it is necessary to test the underlying assumption that neither the protein shape and size nor the polydisperse nature of the cytosol matter. We present a combined experimental and simulation study of the protein tracer diffusion in deuterated E.coli cellular lysate. Quasi-elastic neutron scattering accesses the short-time diffusion of immunoglubulin (IgG) in this lysate. Varying the mixing ratio and volume fraction of IgG and lysate, we observe that this diffusion only depends on the total volume fraction of macromolecules. Stokesian dynamics simulations confirm that when the tracer size agrees with the average size of the polydisperse lysate, these proteins are indeed slowed down similar to a monodisperse solution of the same volume fraction. In contrast, larger/smaller proteins diffuse slower/faster, respectively. IgG being close to this average size, we obtain a consistent picture of the diffusion from simulations and experiments. Ongoing investigations with different tracer proteins in lysate as well as binary mixtures of proteins support this colloid picture of the self-diffusion even in such complex polydisperse cell-like environments, which is promising for a future quantitative understanding of reaction pathways in biology. References: M.Grimaldo et al., J.Phys.Chem.Lett. and Q.Rev.Biophys.2019

Primary authors: GRIMALDO, Marco; LOPEZ, Hender; BECK, Christian (Universität Tübingen); ROOSEN-RUNGE, Felix; ZHANG, Fajun; OETTEL, Martin; BARRAT, Jean-Louis; SEYDEL, Tilo (Institut Max von Laue - Paul Langevin); SCHREIBER, Frank

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Session Classification: Protein structure, function and dynamics

Track Classification: Protein structure, function and dynamics

Contribution ID: **31** Type: **Talk**

Modelling the collective dynamics of membrane multilayers and complex membranes

Wednesday, June 9, 2021 5:10 PM (20 minutes)

The dynamical properties of lipid membranes play an important role in how cells, virus and organelles interact with the world around them. These properties arise from a complex interplay of forces over a wide range of time and length scales and, as such, are ideally suited for study with neutron spin echo (NSE) spectroscopy. Recently, the NSE technique has been extended to grazing incidence geometry, allowing in-plane collective motions to be investigated in lipid membrane multilayers. The results indicated the presence of a new type of collective oscillation not previously observed in soft-matter samples - the so-called surface mode phonon. Here, we introduce the model developed to interpret the results of these experiments.

The model is an extension into the time domain of work done by Romanov and Ul'yanov on the structural and dynamic properties of smectic films. By comparing the results of the NSE experiment and the output of the model for various realistic parameter sets, we examine how the model can be used to interpret grazing incidence NSE data. We also consider at how the model has been implemented and the effects that this has on the stability and validity.

Finally, we look at some of the ongoing molecular dynamics simulation work at JCNS-4 and how this fits in with the push to extend recent work on model phosphatidylcholine-based membranes to more biologically relevant natural membranes featuring a diverse range of lipid classes.

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Session Classification: Biological membranes, surfaces and interfaces

Track Classification: Biological membranes, surfaces and interfaces

Contribution ID: **32** Type: **Poster**

Quantum cascade laser-based infrared spectrometer combined with small angle neutron scattering for life science applications

Wednesday, June 9, 2021 2:40 PM (20 minutes)

Infrared spectroscopy serves as local probe reporting on specific vibrations in some side chains which are spectrally distant from the complicated infrared spectrum of a protein in solution. Here, infrared spectroscopy can give information on the fold of the protein and also follow aggregation phenomena. Small angle neutron scattering also reports on the global structure of proteins in solution and can give information on the shape of growing aggregates or folded proteins in solution.
In the framework of a BMBF-funded project, we would like to explore the capabilities of quantum cascade lasers for this combination of methods. Their advantage is superior gaussian beam characteristics and higher spectral density over the glow bar infrared light sources of the FTIR spectrometer. Their disadvantage is the more complicated pulsed mode of operation and the limited spectral width they can cover.
As a first scientific sample, the effect of temperature on protein aggregation and amyloid formation in insulin as a peptide hormone, secreted in ß-cells of the pancreatic islets was examined. For this purpose insulin was dissolved in a phosphate buffer, where the pH was adjusted to 2. In the following, the temperature was increased from 25 to 37 ℃ and IR spectra were recorded at different times. In the beginning, no change was observed in the spectra. But, after 100 min, a shift from 1655 to 1622 cm-1 appeared in the amide I region which can be due to amyloid formation of insulin.

Primary author: Dr DADFAR, Seyed Mohammad Mahdi

Co-authors: Prof. FITTER, Jörg; Dr STADLER, Andreas; Dr RADULESCU, Aurel; Dr SCHRADER , Tobias

Presenter: Dr DADFAR, Seyed Mohammad Mahdi

Session Classification: Poster Session

Track Classification: Protein structure, function and dynamics

Contribution ID: **33** Type: **Poster**

Development of DD Neutron Generator for life sciences and health

Wednesday, June 9, 2021 2:40 PM (20 minutes)

Neutrons techniques such as Neutron Small Angle Scattering (SANS), Neutron Macro-molecular Crystallography (NMX), Neutron Reflection (NR) are the popular non invasive probes in life sciences and health. All these neutronic instrumentation have been employed in studying the morphology of large molecules and macro-molecular complexes , the drug molecules and their interactions with the biological molecules and other unique structural information's.

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These studies are being carried out by neutrons produced  via nuclear  reactions  with  acceler
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In present work, we will try to look the feasibility of recently developing compact, controlled, safe, high flux neutron sources for numerous applications in life sciences. We will also discuss its limit and opportunities in all kinds of biological, health, research and development. Such as, these sources are already started acting as an alternative of conventional nuclear reactor/accelerator, by addressing the radio pharmaceutical nuclei production, boron neutron capture therapy, elemental composition of bone (specially Mn), non invasive radiography etc issues.

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Presenter: DUBEY, Rakesh (NeutronGate Oy)

Session Classification: Poster Session

Track Classification: Neutrons and complementary methods in biology

Contribution ID: **34** Type: **Talk**

Small-angle Neutron Scattering Studies of the Replicase Cofactor Nsp7/8 Complex from SARS-CoV-2

Thursday, June 10, 2021 4:20 PM (20 minutes)

Severe acute respiratory syndrome (SARS) is a viral infectious disease caused by the new coronavirus strain (CoV2). The SARS-CoV2 replication and transcription complex (RTC) is formed with at least 9 NSPs that are arranged into one functional assembly. The non-structural proteins (NSPs) Nsp7 and 8 are important components of this complex. Our overall aim was to investigate the structural basis of mechanism of binding and recognition of nucleic acids by Nsp7/8. First, we studied Nsp8 conformation alone and complexed with Nsp7 using small-angle neutron scattering (SANS). We produced a partially deuterated Nsp7/8 complex (dNsp7/Nsp8) and at the contrast match point of dNsp7 we singled out the scattering from bound Nsp8. The *P(r)* of dNsp7/8 has a bimodal shape indicating that the bound NSP8s are spatially separated in the complex. This shows that Nsp7 dramatically modifies Nsp8 conformation in the complex. Next, using the contrast match point of nucleic acids we single out the scattering of Nsp7/8 in complex with double stranded (ds) DNA and dsRNA substrates. The SANS profiles of Nsp7/8/DNA and Nsp7/8/RNA are best fit with a mixture of Nsp8/DNA (or RNA) and free Nsp7 dimers, supporting the dissociation of Nsp7/8 complex into smaller subunits. In contrast, Nsp8 alone did not bind the nucleic acids tested suggesting that Nsp7 is needed for Nsp8 to form a complex. Our results provide insight into SARS-CoV2 RTC that may be used to design novel therapeutic strategies for COVID-19.

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Presenter: LEITE, Wellington (Oak Ridge National Laboratory)

Session Classification: Neutrons in the fight against virus diseases

Track Classification: Neutrons in the fight against virus diseases

Contribution ID: **35** Type: **Talk**

Conformational Changes of IDP under Influence of Guanidinium Chloride: Integrative Approach using X-ray/Neutron Scattering and Single Molecule Spectrosopy

Wednesday, June 9, 2021 3:50 PM (20 minutes)

IDPs are identified by the presence of unfolded region due to relatively abundant polar residues content within its amino acid sequence. Together with other residues, IDPs exhibit not only high flexibility but also sensitivity to physico-chemical fluctuation such as pH, temperature, and ions concentration. For this reason, IDPs are involved in cellular processes such as DNA repair scheme and chromatin modification. In this project, we pursue a quantitative description of structure and dynamics of IDPs with different net charges: namely Prothymosin Alpha and Myelin Basic Protein. Here, we employed neutron spinecho spectroscopy (NSE) and small angle X-ray scattering (SAXS) to gain insight on the emergence of internal friction within the peptide and its conformational change as a function of Guanidinium Chloride (GndCl) concentration respectively. The experimental results obtained from SAXS shows contraction and expansion as measured by FRET. Similarly, from NSE data, we are able to extract the internal friction which is in good agreement with FCS results.

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Presenter: STADLER, Andreas (FZ Jülich)

Session Classification: Protein structure, function and dynamics

Track Classification: Protein structure, function and dynamics

Contribution ID: **36** Type: **Talk**

Towards time-resolved protein dynamics on nanoscopic scales

Wednesday, June 9, 2021 4:10 PM (20 minutes)

Protein function is realized by an interplay of molecular structure and dynamics. Although various methods have been established to study the evolution of macromolecular conformation after trigger events, the time-resolved evolution of protein dynamics on molecular length scales presents experimental challenges, in particular in the native environment in solution [1].
Here, we present a case study by quasi-elastic neutron scattering addressing the evolution of nanoscopic protein dynamics during thermal denaturation [2]. Using so-called elastic and inelastic fixed window scans, we obtain time-resolved information on the change of dynamics and the related dynamical confinement on nanometer length and nanosecond time scales. Upon heating, the dynamics first increase due to thermal activation, and then dramatically drop down upon unfolding and cross-linking. The slower dynamics is preserved when cooling back, due to the formed protein gels. The emerging picture thus includes a flexible motion of the protein network on nanosecond time scales, which is simply slowed down by the presence of the cross-linked neighbors.
The experimental approach allows to follow nanoscopic dynamics with a sampling time below one minute, which opens opportunities for dynamical changes e.g. driving protein assembly.
[1] M Grimaldo, F Roosen-Runge, F Zhang, F Schreiber, T Seydel, Quart.Rev. Biophys. 2019, 52, e7
[2] O Matsarskaia, L Bühl, C Beck, et al., Phys.Chem.Chem.Phys. 2020, 22, 18507-18517

Primary authors: MATSARSKAIA, Olga; BECK, Christian (Universität Tübingen); ZHANG, Fajun (University of Tuebingen); SEYDEL, Tilo (Institut Max von Laue - Paul Langevin); SCHREIBER, Frank (Uni Tübingen, Angewandte Physik); ROOSEN-RUNGE, Felix

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Session Classification: Protein structure, function and dynamics

Track Classification: Protein structure, function and dynamics

Contribution ID: **37** Type: **Poster**

Organisation and dynamics of hemoglobin within mammalian red blood cells studied with neutron scattering

Wednesday, June 9, 2021 2:40 PM (20 minutes)

The highly penetrative and non-ionizing nature of neutrons can provide an ideal probe of structure and dynamics in cellular systems in a near physiological context. While many cellular systems can be quite complex the red blood cell (RBC) provides a simple system in which useful quantitative information can be extracted. Investigations with SANS [1] and QENS [2] from cell suspensions and concentrated hemoglobin solutions have shown the power of the technique. Here we discuss the analysis of scattering from red blood cell and define a simple scattering problem where it is the intra-cellular solution of hemoglobin which provides the only resolvable component to the neutron scattering. Using small angle neutron scattering techniques we have probed the organization of the hemoglobin from the overall envelope of the globular tetramer to the organization imposed by the cell membrane of the red blood cell. Dynamics have been probed from internal motions within globular tetramer which reflect the mechanical elasticity, and with QENS and field gradient NMR technique to study translational dynamics within the RBC [3]. The physiological implications of these observations are discussed.

[1] C. J. Garvey et al, Euro. Biophys. 2004, 33, 589-595; A. M. Stadler et al., J. Phys. Chem. Lett. 2010, 1, 1805-1808.
[2] A. M. Stadler et al., BBA 2014, 1840, 2989-2999; A. M. Stadler, et al., J.R. Soc. Inter. 2012, 9, 2845-2855.
[3] K., Shou et al., R. Soc. Open Science 2020, 7, 201507.

Primary authors: GARVEY, Christopher (MLZ); STADLER, Andreas (FZ Jülich)

Presenter: GARVEY, Christopher (MLZ)

Session Classification: Poster Session

Track Classification: Protein structure, function and dynamics

Contribution ID: **38** Type: **Poster**

Studies of the localization of small molecules in self-assembled lamellae structures by neutron diffraction and molecular deuteration

Wednesday, June 9, 2021 2:40 PM (20 minutes)

The self-assembly of biological amphiphilic molecules, lipids, into lamellar structures, forms the basis of a selective transport barrier around the cell cytoplasm, the lipid bilayer. Neutron diffraction, when combined with molecular deuteration, provides an important high resolution tool in the understanding the localization of molecules in this structure. This paradigm, a description of the locus of a molecule within the bilayer structure, can provide important mechanistic insight into biological problems such as the mode of action of cryoprotectants, the interaction of cell penetrating peptides or the relationship between organization and barrier properties of skin lipids in inflammatory conditions. MIRA is a cold triple axis spectrometer, when used as a two-axis diffractometer, is well suited for the measurement of lamellar diffraction with low background. The use of MIRA in the context of the above examples is discussed.

Primary authors: GARVEY, Christopher (MLZ); GEORGII, Robert

Presenter: GARVEY, Christopher (MLZ)

Session Classification: Poster Session

Track Classification: Biological membranes, surfaces and interfaces

Contribution ID: **39** Type: **Poster**

Neutron crystal structure analysis of green fluorescent protein

Wednesday, June 9, 2021 2:40 PM (20 minutes)

Fluorescent proteins (FPs) have revolutionized the imaging technologies in biological science. A better understanding of the structure and function for FPs will help to develop new molecular designs to generate further practical devices. Recently, we reported neutron structural analysis of the green fluorescent protein (GFP) to show the characteristic protonation (deuteration) states of the chromophore and surrounding key residues with their hydrogen-bonding network, including water molecules, by neutron crystallography. The structure has a deprotonated hydroxyl group in the fluorescent chromophore. Also, the protonation states of His148 and Thr203, as well as the orientation of a critical water molecule in direct contact with the chromophore, could be determined. The results demonstrate that the deprotonated hydroxyl group in the chromophore and the nitrogen atom ND1 in His148 are charged negatively and positively, respectively, forming an ion pair. The position of the two deuterium atoms in the critical water molecule appears to be displaced slightly toward the acceptor oxygen atoms according to the obtained omit maps. This displacement implies the formation of an intriguing electrostatic potential realized inside the protein. Our findings provide new insights into future protein design strategies along with developments in quantum chemical calculations.

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Presenter: ADACHI, Motoyasu (National Institutes for Quantum and Radiological Science and Technology)

Session Classification: Poster Session

Track Classification: Protein structure, function and dynamics

Contribution ID: **40** Type: **Talk**

JCNS Deuteration Service: What can we do for life sciences?

Wednesday, June 9, 2021 5:30 PM (20 minutes)

Neutron scattering experiments involving soft matter materials often require specific contrast to observe different parts of the materials. In order to increase the availability of deuterium labelled materials, we are establishing deuteration support to MLZ users. Proposals for the deuteration support can now be submitted in combination with proposals for neutron beamtime.

Our main synthetic focus at JCNS-1 is in the area of polymer and organic chemistry. Anionic and controlled radical polymerization techniques allow the synthesis of various polymers with narrow molecular weight distributions. A typical example is poly(ethylene glycol) with specific degrees of deuteration in a large range of molecular weights (from oligomers to more than 200 kDA). The so obtained polymers can be functionalized afterwards to introduce diverse functional groups or be attached to molecules like proteins. We have recently started synthetic efforts towards the production of deuterated phospholipids and surfactants (e.g. Brij, Tween).

The presentation will cover the following topics: i) the contents of a deuteration proposal as well as its connection to the review process and ii) the synthetic expertise available at JCNS-1 with focus on relevant materials for life sciences.

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Presenter: FRUHNER, Lisa (JCNS-1, Forschungszentrum Jülich GmbH)

Session Classification: Biological membranes, surfaces and interfaces

Track Classification: Neutrons and complementary methods in biology

Contribution ID: **41** Type: **Poster**

Macromolecular Neutron Diffraction at the Heinz Maier-Leibnitz Zentrum

Wednesday, June 9, 2021 2:40 PM (20 minutes)

Neutron single crystal diffraction provides an experimental method for the direct location of hydrogen and deuterium atoms in biological macromolecules, thus providing important complementary information to that gained by X-ray crystallography. At the FRM II neutron source in Garching near Munich the neutron single crystal diffractometer BIODIFF, a joint project of the Forschungszentrum Jülich and the FRM II, is dedicated to the structure determination of proteins. Typical scientific questions address the determination of protonation states of amino acid side chains, the orientation of individual water molecules and the characterization of the hydrogen bonding network between the protein active centre and an inhibitor or substrate. This knowledge is often crucial towards understanding the specific function and behaviour of an enzyme. BIODIFF is designed as a monochromatic diffractometer and is able to operate in the wavelength range of 2.4 Å to about 5.6 Å. This allows to adapt the wavelength to the size of the unit cell of the sample crystal. Data collection at cryogenic temperatures is possible, allowing studies of cryo-trapped enzymatic intermediates. Some recent examples will be presented to illustrate the potential of neutron macromolecular crystallography.

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Co-author: SCHRADER, Tobias

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Session Classification: Poster Session

Track Classification: Neutrons and complementary methods in biology

Contribution ID: **42** Type: **Talk**

Life Science at the SNS Second Target Station

Tuesday, June 8, 2021 2:10 PM (20 minutes)

The second target station (STS) is a >$1Billion, Department of Energy project to be constructed at Oak Ridge National Laboratories Spallation Neutron Source (SNS). The STS will provide entirely new capabilities for studying a broad range of materials with neutron scattering and support a wide variety of users. The science capabilities provided by the instrument suite at the STS will complement those of the two existing DOE Office of Science neutron scattering user facilities at ORNL, the First Target Station (FTS) of the SNS, and the High Flux Isotope Reactor (HFIR). The STS will deliver the highest peak brightness of cold neutrons globally. Advances in neutron optics, instrumentation, and detectors will enable types of experiments to become possible and new systems to be studied with neutrons. The STS instrument systems group is currently working with the neutron community to determine an initial suite of eight neutron instruments with transformative new science capabilities via an instrument selection process. This talk will provide updates on the STS project's status and the next generation of neutron life science instrumentation.

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Presenter: Dr COATES, Leighton (Oak Ridge National Laboratory)

Session Classification: Neutron and complementary methods in biology

Track Classification: Neutrons and complementary methods in biology

Contribution ID: **43** Type: **Talk**

DEMAX: the DEuteration and MAcromolecular Xtallization platform of the ESS.

Friday, June 11, 2021 10:10 AM (20 minutes)

Neutron techniques, incl. small angle neutron scattering (SANS), reflectometry (NR), protein crystallography (NPX), benefit from the usage of deuterated molecules. Many neutron facilities provide deuteration support to users to facilitate better measurements and enable research not possible without deuterated materials. NPX requires large protein crystals of partially or fully deuterium-labeled proteins. The high neutron flux that ESS will deliver when fully operational will enable high throughput experiments with shorter measuring times and make it possible to study smaller and difficult-to-prepare (precious) samples. To support the community, the Deuteration and Macromolecular Crystallization Platform (DEMAX) is establishing methods for deuteration and crystallization in order to meet this need. DEMAX has chemistry, biology, and crystallization laboratories for producing deuterated molecules, incl. lipids, surfactants, proteins, monomers etc. The group also offers crystallization optimization and scale-up for proteins. Through regular beamtime access at MAX IV we collect room temperature X-ray data on protein crystals for users that do joint refinement. The DEMAX platform strives to increase the range of deuterated molecules available for neutron scattering. DEMAX is currently working on accepted proposals from the second pilot call for proposals and aims to publish a third call later this year.

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Session Classification: Neutron and complementary methods in biology

Track Classification: Neutrons and complementary methods in biology

Contribution ID: **44** Type: **Poster**

Relationship between the protonation state of the substrate and the absorption spectrum in a mutant of the enzyme that synthesizes a photosynthetic pigment

Wednesday, June 9, 2021 2:40 PM (20 minutes)

PcyA reduces biliverdin IXα (BV), a heme degradation product, in a ferredoxin-dependent manner, to synthesize phycocyanobilin, which plays an important role in photosynthesis and biological photoresponse. PcyA is a unique enzyme that sequentially reduces D-ring vinyl group and A-ring vinyl group of BV in a site-specific manner. In this study, Ile86 located near the important amino acid Asp105, which has been thought to be a proton donor to BV, was substituted (I86D mutant). Interestingly, the absorption spectrum of the complex of PcyA mutant I86D and BV shows a significantly high absorption peak at730 nm as compared with the wild type PcyA and BV complex. Its absorption maximum is presumed to be derived from BVH+ with protons added to BV by PcyA in the first step reaction, but hydrogen atoms cannot be identified even by high-resolution X-ray crystal structure analysis. Therefore, we have been engaged in neutron crystallography to visualize the structure of BVH+ and the protonation state of amino acids in PcyA.
As a result of the neutron crystal structure analysis, we could observe the state of BVH+ protonated by BV reduction. This result was consistent with the results of spectroscopic and computational studies. Both Asp 105 and mutated Asp 86 were protonated and formed hydrogen bonds. We were able to identify the hydrogen-bonding network of the active site which could not be identified by high-resolution X-ray crystal structure analysis.

Primary authors: Prof. UNNO, Masaki (Ibaraki University); Mr IGARASHI, Keisuke (Ibaraki University); Dr HAGIWARA, Yoshinori (National Institute of Technology, Kurume College); Dr SUGISHIMA, Masakazu (Kurume University); Dr WADA, Kei (University of Miyazaki); Prof. FUKUYAMA, Keiichi (Osaka University); Mr IKEDA, Atsushi (Ibaraki University); Dr KUSAKA, Katsuhiro (Ibaraki University); Dr YANO, Naomine (Ibaraki University); Dr JOUTSUKA, Tatsuya (Ibaraki University); Dr OSTERMANN, Andreas (Technische Universität München)

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Session Classification: Poster Session

Track Classification: Protein structure, function and dynamics

Contribution ID: **45** Type: **Poster**

Mechanism of disaccharide-induced protein stabilization from neutron scattering and modeling

Wednesday, June 9, 2021 2:40 PM (20 minutes)

Proteins are an important component in many medical and food products, and the long-time properties of these products are directly dependent on the stability of their proteins. To enhance this stability it has become common to add disaccharides in general, and trehalose in particular. However, the mechanisms by which disaccharides stabilize proteins and other biological materials are still not fully understood, and therefore we have used neutron diffraction and quasielastic neutron scattering (QENS) in combination of molecular modeling to investigate the stabilizing role of the disaccharides trehalose and sucrose on myoglobin. Our aim was to enhance the general understanding of the role of disaccharides and to obtain specific insights into why trehalose exhibits extraordinary stabilizing properties. The diffraction results show that both disaccharides are preferentially excluded from the protein surface, but that this effect is more pronounced for trehalose than sucrose. Hence, the disaccharide molecules are generally not affecting the protein by direct interactions. Instead, the QENS and modeling results show that the protein dynamics is slowed down by a slowing down of the protein hydration water, as a result of the "slaving mechanism" . Since the water dynamics and protein motions are slower in the trehalose solution, the results explain the more efficient stabilizing effect of trehalose on proteins.

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Presenter: SWENSON, Jan (Chalmers University of Technology)

Session Classification: Poster Session

Track Classification: Protein structure, function and dynamics

Contribution ID: **46** Type: **Talk**

High-resolution structure studies of NADH-cytochrome b5 reductase

Wednesday, June 9, 2021 10:50 AM (20 minutes)

NADH-cytochrome b5 reductase (b5R) on endoplasmic reticulum membrane in mammalian liver cell plays a variety of roles concerning lipid unsaturation and xenobiotic metabolism. b5R transfers electrons from two-electron carrier of NADH to one-electron donor of cytochrome b5. In the redox cycle of b5R, a hydride transfer from NADH to oxidized FAD and deprotonation from the reduced FADH take place in b5R. Therefore, high-resolution structure analyses including the information about hydrogen atoms and valence electron densities are required for understanding molecular mechanisms of the b5R redox reaction. High-resolution X-ray crystal structures were previously determined using the oxidized form of b5R (M. Yamada et al., J. Mol. Biol., 2013; K. Takaba et al., Sci. Rep., 2017). In this work, the neutron crystal structures of the oxidized form of b5R were determined including hydrogen atoms of solvent molecules, and the X-ray crystal structures of the reduced form of b5R were determined including hydrogen atoms of the NADH cofactor. Recently, neutron diffraction data sets of the reduced form have been collected at BIODIFF of FRM II. The neutron structure analysis of the oxidized form clearly shows the hydrogen-bonding network from the FAD cofactor to the protein surface. The X-ray structure analysis of the reduced form reveals the NAD+ and NADH bound states using wildtype and T66V mutant. These structural features indicate a proton transfer pathway from FAD to the protein exterior.

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Presenter: Dr HIRANO, Yu (National Institutes for Quantum and Radiological Science and Technology)

Session Classification: Protein structure, function and dynamics

Track Classification: Protein structure, function and dynamics

Contribution ID: **47** Type: **Poster**

KWS-2 –the Extended Q-Range High-Flux SANS Diffractometer for Life Sciences

Wednesday, June 9, 2021 2:40 PM (20 minutes)

KWS-2 represents a classical pinhole SANS diffractometer where, combining the conventional mode using different neutron wavelengths and detection distances with the focusing mode using MgF2 lenses, a wide Q-range between 1 x 10-4 and 1 Å-1 can be explored. The high neutron flux, comparable with that of the world leading SANS instruments, which is supplied by the neutron delivery system (cold source, selector, guides) and the multi-MHz detection system enable high intensity measurements and time-resolved studies with ms time resolution. Using choppers and TOF data acquisition the wavelength resolution can be tuned between 1 and 20% and inelastic-free measurements are enabled, thus data with reduced incoherent background from highly protonated samples. A broad range of in-situ complementarities (FT-IR, UV-Vis, DLS) are available or in development for providing additional information at local molecular level or monitoring the sample quality/composition during the SANS investigation.
The instrument is designed for the study of complex morphologies and structural correlations over the mesoscopic length scale in the fields of soft condensed matter, chemistry, and biology. To optimize the use of the beam-time and to expand the instrument performance, robotics for a continuous supply of the instrument with samples and wide-angle scattering capabilities are in development.
The instrument concept and its relevant performance for studies in the life sciences field will be presented.

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Presenter: RADULESCU, Aurel (Forschungszentrum Jülich GmbH, Jülich Centre for Neutron Science at MLZ)

Session Classification: Poster Session

Track Classification: Neutrons and complementary methods in biology

Contribution ID: **48** Type: **Poster**

Hydrogen bonding network running through protein kinase investigated by neutron crystallography

Wednesday, June 9, 2021 2:40 PM (20 minutes)

Casein kinase II (CK2), is a serine / threonine kinase ubiquitously distributed among eukaryotic cells, is known to be involved in the cell cycle and cell survival and proliferation. CK2 is one of the drug target proteins, because the relationship between CK2 over-expression and carcinogenesis and cancer metastasis has been pointed out. We aimed to elucidate the hydrogen bonding network involved in the catalytic reaction of CK2 and to obtain the knowledge of conformation of the hydrogen atom and hydration structure effective for the development of inhibitors. In this study, we prepared large crystals for neutron diffraction experiments of wild type CK2α (catalytic subunit). The large crystal of about 2 mm3 was obtained by the macro seeding method. After the dialysis of the obtained crystal against deuterium solvent, a neutron diffraction data were collected at FRMII BioDIFF(100 K) to resolution of 1.90 Å. Then, X-ray diffraction experiment at PF BL-5A was collected using the same crystal to resolution 1.10 Å for the joint refinement. As a result of neutron crystallography, an interesting hydrogen bonding network running through CK2α was found. This discovery can be expected to contribute to the development of an anticancer drug with a new inhibitory mechanism.

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Session Classification: Poster Session

Track Classification: Protein structure, function and dynamics

Contribution ID: **49** Type: **Poster**

Monitoring in vitro human digestion of model food, a plant protein gel, using SANS"

Wednesday, June 9, 2021 2:40 PM (20 minutes)

Proteins are essential macronutrients in the human diet, being fundamental in body structure and functions. The protein digestibility depends not only on their composition but also on food structure, which in turn can be influenced by different types of processing.
We monitored degradation kinetics of the structure during simulated gastric and intestinal digestion, and analyzed its impact on digestibility. As a model solid-like food (the form in which most proteins are ingested) we used plant protein gels - from rapeseed (napin and cruciferin). The gels were synthetized by heat-treatment of solutions at various concentrations and pHs.
Different techniques were used. We will focus here on SANS, on 1cm size samples as in real digestion bolus, but which need to be homogeneous. We make a link with rheological measurements. SAXS (LLB and SWING-SOLEIL synchrotron) gives additional information at the same q values but not the same size of samples,enabling inframillimetric access to the spatial gradient. We also used UV fluorescence imaging (DISCO) at intermediate gel size (20-200 microns).
The state of gelled proteins defines both their intrinsic digestibility and the gel structure. To separate the two, we propose to introduce the protein inside a gel of a different species, polygalacturonane (issued from pectin), not digestible by human enzymes. First results on betalactogloblin show we can use contrast matching.
We may discuss other techniques available via MLZ programs.

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Session Classification: Poster Session

Track Classification: Biological processes

Contribution ID: **50** Type: **Talk**

The structure of KRas at the membrane –simulation and experiment.

Tuesday, June 8, 2021 11:40 AM (20 minutes)

KRas4B is a membrane-anchored signaling protein and primary target in cancer research. Predictions from molecular dynamics simulations have previously shaped our mechanistic understanding of KRas signaling but disagree with recent experimental results from neutron reflectometry, nuclear magnetic resonance, and thermodynamic binding studies [1]. We compare this body of biophysical data to back-calculated experimental results from a series of molecular simulations that implement different subsets of molecular interactions. Our results show that KRas4B approximates an entropic ensemble of configurations at model membranes, which is not significantly affected by interactions between the globular G-domain of KRas4B and the lipid membrane. These findings promote a model of KRas, in which the G-domain explores the entire accessible conformational space while being available to bind to effector proteins [2].

[1] Van, Q. N. et al. Uncovering a membrane-distal conformation of KRAS available to recruit RAF to the plasma membrane. Proc National Acad Sci 117, 24258–24268 (2020)
[2] Heinrich, F., Van, Q.N., Jean-Francois F., Stephen A.G., Lösche M., Membrane-bound KRAS approximates an entropic ensemble of configurations. Biophys. J. (2021), under review

Primary author: HEINRICH, Frank (Carnegie Mellon University)

Presenter: HEINRICH, Frank (Carnegie Mellon University)

Session Classification: Biological membranes, surfaces and interfaces

Track Classification: Biological membranes, surfaces and interfaces

Contribution ID: **51** Type: **Talk**

Integrative approach to structure of huge protein complex in Kai-clock protein system

Wednesday, June 9, 2021 11:10 AM (20 minutes)

Kai-clock system is one of the simplest biological clocks: the system is composed with only three proteins, KaiA, KaiB and KaiC, and they repeat association-dissociation with 24-hrs period as follows, A2+B4+C6 → A2C6+B4 → A2+B6C6 → A12B6C6 → A2+B4+C6 → ⋯. To understand this system, it is essential to reveal the complex structures in every phase. For this purpose, we have been focused on the largest complex, A12B6C6, which could appear at the turning point from the association phase to the dissociation phase. Basic structure of A12B6C6 was reported with cryo-EM observation by J. Snijder, et al.. However, the reported structure missed the N-term domains of KaiA. Here, we introduced the whole structural model of A12B6C6 in solution.
To analysis this huge complex in solution, we utilized two state-of-art solution scattering techniques, SEC-SAXS and SEC-inversed Contrast Matching-SANS (SEC-iCM-SANS). In addition, we combined computational modeling and molecular dynamics simulation to build three-dimensional structure and to clarify its dynamics. We should note that the iCM-SANS selectively observing the KaiA protomers in the complex played crucial role in the analysis. The detailed will be shown in the presentation.

Primary author: SUGIYAMA, Masaaki (Kyoto University)

Presenter: SUGIYAMA, Masaaki (Kyoto University)

Session Classification: Protein structure, function and dynamics

Track Classification: Protein structure, function and dynamics

Contribution ID: **52** Type: **Talk**

Combining small-angle scattering with computational modelling to reveal structural details of Hepatitis B virus

Thursday, June 10, 2021 4:40 PM (20 minutes)

The genetic material of viruses is typically protected in an icosahedral capsid, which is primarily assembled from over a hundred subunits of the same protein in a spontaneous self-assembly process. Similar highly efficient assembly processes are ubiquitous in biological systems, and viral capsids in particular present a unique platform to exploit for therapeutic advances in the targeted cellular delivery of cargo packaged within the capsid. Our research aims to provide a more detailed understanding of how this precise viral capsid protein assembly process occurs from a pool of single building blocks, and specifically how the RNA is incorporated into the capsid. Here, we present results from small-angle neutron scattering (SANS) experiments using contrast variation to reveal the final assembled structural organization of both the protein and nucleic acid components from recombinant Hepatitis B virus (HBV) capsid protein and a synthetically prepared RNA containing the capsid protein binding domain. Time-resolved small-angle x-ray scattering (SAXS) experiments were also used to determine the HBV assembly pathway in the presence and absence of RNA. We employed Bayesian statistics-based computational methods to extract kinetic parameters of assembly and the overall size and shape of the dominant structural intermediates from the SAXS data. The developed framework can be extended to other hierarchical assemblies in biology.

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Presenter: POTRZEBOWSKI, Wojciech

Session Classification: Neutrons in the fight against virus diseases

Track Classification: Neutrons in the fight against virus diseases

Contribution ID: **53** Type: **Talk**

Neutron structures of Leishmania mexicana triosephosphate isomerase complexes with reaction intermediate mimics shed light on the proton shuttling steps

Tuesday, June 8, 2021 5:10 PM (20 minutes)

Triosephosphate isomerase (TIM) is a key enzyme in glycolysis that catalyses the interconversion of glyceraldehyde-3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP). This simple reaction involves the shuttling of protons mediated by protolysable side chains. The catalytic power of TIM is thought to stem from the ability to facilitate the deprotonation of a carbon next to a carbonyl group to generate an enediolate intermediate. The enediolate intermediate is believed to be mimicked by the inhibitor 2-phosphoglycolate (PGA) and the following enediol intermediate by phosphoglycolohydroxamate (PGH). We have determined the neutron structure of Leishmania mexicana TIM with both inhibitors and performed joint neutron-X-ray refinement followed by quantum refinement. The structures show that in the PGA complex, the postulated general base Glu-167 is protonated, while in the PGH complex it remains deprotonated. The deuteron is clearly localized on Glu-167 in the PGA–TIM structure, suggesting an asymmetric hydrogen bond instead of a low-barrier hydrogen bond. The full picture of active site protonation states allows us to investigate the reaction mechanism with density functional theory calculations.

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Presenter: OKSANEN, Esko (European Spallation Source ESS ERIC)

Session Classification: Protein structure, function and dynamics

Track Classification: Protein structure, function and dynamics

Contribution ID: **54** Type: **Talk**

Strong Adverse Contribution of Conformational Dynamics to Streptavidin–Biotin Binding

Wednesday, June 9, 2021 3:30 PM (20 minutes)

Upon protein ligand binding, changes of conformational entropy occur in protein and hydration layer. In an experimental study, we investigated the relevance of conformational entropy for the binding of biotin to the protein streptavidin. Using QENS, we investigated changes in conformational entropy between the ligand-free and the ligand-bound state. We compared the internal dynamics of streptavidin before and after biotin binding. Thermal Diffusion Forced Rayleigh Scattering (TDFRS) was used to gain information on the hydration layer.
QENS results show that the conformational entropy of streptavidin reduces upon biotin binding. TDFRS results and extrapolation from QENS data and literature data indicate an increase in hydration layer entropy upon biotin binding. This indicates that the hydration layer plays an important role in stabilising the binding of biotin to streptavidin. The change in conformational entropy per residue upon biotin binding to streptavidin is of the same order of magnitude as that in protein folding processes. This indicates that a significant change of conformational entropy occurs upon biotin binding to streptavidin. This is remarkable, since no significant structural change occurs upon binding. The internal dynamics show that the residues of free streptavidin perform a jump-diffusive motion in the picosecond timeframe, while the more rigid complex does not show jump-diffusive motions. I will discuss how different biotin saturations affect this.

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Session Classification: Protein structure, function and dynamics

Track Classification: Protein structure, function and dynamics

Contribution ID: **55** Type: **Poster**

Neutron Diffractometer for Protein Crystallography at Cold Neutron Beam Line of JRR-3

Wednesday, June 9, 2021 2:40 PM (20 minutes)

The elucidation of the protein-protein interaction, especially among membrane proteins and protein complexes, is one of the most important research fields in life science. Such proteins have large molecular weights, and the lattice lengths of their crystals have large values. Cold neutrons contribute to improve the difficulty in separating Bragg peaks from those crystals. JRR-3 has three cold neutron beamlines in the beam hall facility, in which neutron guides have been recently upgraded to supermirror guides (m=3). The neutron intensity gain by the upgrading has also been estimated.
A diffractometer, to be installed at the cold neutron beamline, will be equipped a feature to choose a wavelength appropriate for data collection from crystals with a large unit cell. While BIX-3 and BIX-4 diffractometers with the Neutron Imaging Plate detector are now located in the reactor hall of JRR-3, this detector is more suitable to be used in the beam hall with a lower gamma-ray background. As the effectively usable angular divergence is limited for the single crystal diffraction method, neutron beam within the divergence of 1.0 degree at the cold beamline was simulated by the McStas program. From the calculation the peak wavelength of the spectrum at C1-3 beam port (a candidate port for the installation) was shifted from 0.4 nm to 0.29 nm, and the gain was one order of magnitude at the wavelength of 0.29 nm by the upgrading. The performance of the diffractometer will be discussed.

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Session Classification: Poster Session

Track Classification: Neutrons and complementary methods in biology

Contribution ID: **56** Type: **Talk**

Neutron Crystallography of the carbon fixing enzyme Rubisco

Tuesday, June 8, 2021 5:30 PM (20 minutes)

Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) is responsible for photosynthetic CO2 fixation and is the most abundant enzyme on earth. The carbon atoms in every organism in the entire biosphere have passed through the reaction cycle at the active site of a Rubisco enzyme at some point in time. In the photosynthetic CO2 reduction reaction catalysed by Rubisco, atmospheric CO2 is fixed to a sugar phosphate and the product, 3-phosphoglycerate, is used to build the organic molecules of life. Rubisco is present in most autotrophic organisms including plants, algae, photosynthetic bacteria and cyanobacteria. Both land-based and marine Rubisco are important - oceanic phytoplankton are estimated to provide around 45% of global net primary production.
We have obtained large, well-ordered crystals of spinach Rubisco and performed the first neutron diffraction experiments on crystals of Rubisco from spinach (spinacia oleracea) at IMAGINE (HFIR), MLZ's Biodiff and MaNDi (SNS). The crystals screened to date have scattered to resolutions of up to 2.1 Å at Biodiff.
Detailed mechanisms have been proposed for how the Rubisco-catalysed reactions may proceed based on high-resolution X-ray structures. While these studies agree on "the big picture", they differ significantly in details such as the movement of electrons and protons. Neutron diffraction may help to visualize protons directly, and this information will be invaluable for efforts to improve carbon fixation catalysis.

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Session Classification: Protein structure, function and dynamics

Track Classification: Protein structure, function and dynamics

Contribution ID: **57** Type: **Talk**

Structural characterization of mRNA - lipid nanoparticle upon pH changes: a SANS study

Wednesday, June 9, 2021 9:30 AM (20 minutes)

Therapeutic treatments based on the production of proteins by delivering messenger RNA (mRNA) represent a versatile approach. Lipid nanoparticles (LNPs) are promising vehicles for mRNA delivery and are formed by a cationic ionizable lipid (CIL), DSPC, cholesterol (Chol) and a pegylated (PEG) lipid. Even though some LNPs for small interference RNA (siRNA) delivery were recently FDA approved, and vaccines against SARS-CoV-2 based on mRNA-LNPs have been developed and given emergency approval in the last months, there are still concerns about the safety profile of LNPs. In addition, it is not clear how to improve their efficacy following endocytosis. It is suggested that there is a pH change from 7.4 in the extracellular region, to 6.5 in early endosomes, 5.5 in late endosomes and 4.5 in lysozomes. Moreover, the release of siRNA from LNPs occurs within 5-15 min of endocytosis, which implies that LNPs must be designed to escape early endosome compartments at pH 6.5. A good understanding of the physical and chemical characteristics of the LNPs under study is necessary to progress from pre-clinical testing.
We employed small angle neutron scattering (SANS) to investigate the LNP structure and the distribution of components in the LNPs at pH values mimicking the endosomal compartment for 3 different LNP compositions. For the 3 formulations, the LNP core-shell structure was disrupted suggesting that a redistribution of the components occurs upon lowering the pH.

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Session Classification: Drug design and delivery

Track Classification: Drug design and delivery

Contribution ID: **58** Type: **Talk**

Endocytosis across scales

Thursday, June 10, 2021 2:40 PM (20 minutes)

Clathrin-mediated endocytosis is a crucial cell biology process allowing internalization of many cell-surface proteins, and other cargo, in eukaryotes. Clathrin-coated vesicles (CCVs) are assembled with their cargo at the plasma membrane, then transport to the early endosome inside the cell. A CCV consists of a clathrin scaffold coating a lipid vesicle, in which the cargo is embedded, linked by adaptor proteins that are associated with effectors of CCV assembly, stability and disassembly. It has recently been determined that a single adaptor protein AP2 is sufficient to initiate and drive clathrin-coated bud formation on appropriate membranes, enriched in PtdIns(4,5)P2.
In vivo, AP2 interacts solely with one leaflet of the cellular membrane. Therefore, an alternative valid model system is to explore clathrin assembly on a flat lipid surface (in our case, Langmuir monolayers and solid-supported bilayers). This allows us to probe the system with a set of state-of-the-art characterization methods typical of soft matter and physical chemistry, including neutron reflectometry, interfacial tensiometry and rheology. We thus have been able to analyse the first stages of CCV assembly by using cargo embedded in a lipid monolayer/bilayer.
We show here, in particular, the influence of AP2, and subsequently the clathrin scaffold, on the composition, structure and mechanics of the complex layer that self-assembles in stages.

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Session Classification: Biological membranes, surfaces and interfaces

Track Classification: Biological membranes, surfaces and interfaces

Contribution ID: **59** Type: **Poster**

Protein and water dynamics at the atomic level

Wednesday, June 9, 2021 2:40 PM (20 minutes)

Despite of the pivotal role that hydrogen (H) atoms play in protein biological function, and the fact these comprise approximately 50% of all protein atoms, their observation through X-ray diffraction remains elusive. Conversely, neutron diffraction data at resolutions better than 2.5 Å allows the determination of H positions, providing unique insight to the catalytic mechanisms of enzymes. Additionally, neutron diffraction is uniquely suited for the study of atomic thermal motion, as routinely done for small molecules, since neutrons scatter from atomic nuclei, while X-rays interact with electrons.
Our study focuses on the description of protein and water structure and dynamics, through neutron crystallography. Perdeuterated hen egg-white lysozyme (D-HEWL) was produced recombinantly with the aim of growing crystals of several mm3. Complete neutron diffraction datasets were collected at D19, Institute Laue-Langevin, to a resolution of 1.0 Å on D-HEWL crystals at both 100 and 298 K.
The D-HEWL 298 K neutron structure provided a clear and complete picture of lysozyme's active site in its active state (room temperature and pH close the enzyme's optimal pH). The unambiguous assignment of H positions allowed the determination of the orientations of protein residues and waters molecules, and of the protonation states of the catalytic residues. Furthermore, atomic motion is analyzed based on anisotropic ADPs obtained from neutron and X-ray diffraction data at both 100 and 298 K.

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Session Classification: Poster Session

Track Classification: Protein structure, function and dynamics

Contribution ID: **60** Type: **Poster**

Elucidating Melittin selectivity using complex cell membrane models

Wednesday, June 9, 2021 2:40 PM (20 minutes)

Cancer is one of the major threats to our health on a global scale. In order to battle these diseases while maintaining the quality of life for patients it is important to find anticancer drugs with a high selectivity for the target cancer cell. Melittin, a peptide found in Honey bee venom has long been known for its antimicrobial effects. Later studies have also shown Melittin to be effective against several types of cancer cells and recently it was discovered that it can selectively target aggressive forms of breast cancer over healthy cells. Melittin act by binding to the lipid membrane that surround the cells. Our approach is to study Melittin's interaction with specific lipid components in models of cancer cell membranes in order to understand the peptide's selectivity for cancer cells over the healthy cell of the body. We do this by a combination of neutron scattering and computer simulation techniques. Developing new methods for extracting and purifying deuterated lipids from cell cultures enables us to tailor the cell membrane lipid composition to specifically probe the differences in Melittin's interaction with models of cancer cells and healthy cells. Combining the experimental results with computational simulations, we aim to obtain a detailed picture of how different lipids influence Melittin's selectivity and potency against cancer cell membranes.

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Session Classification: Poster Session

Track Classification: Drug design and delivery

Contribution ID: **61** Type: **Talk**

Phytochrome function: structural changes and protonation dynamics

Thursday, June 10, 2021 10:10 AM (20 minutes)

Phytochromes are red/far-red photochromic biliprotein light sensors that act as master regulators of plant development. They regulate the expression of ~20% of all plant genes, controlling for example germination, pigment production, stem extension and flowering time. Phytochromes are also known in prokaryotes, Cph1 from *Synechocystis*, for example, providing a valuable model for structure/functional studies. De/reprotonation of the chromophore, neighboring histidines and waters is important in both photon absorption and the subsequent photocycle. Following numerous successful X-ray diffraction, solid-state NMR and vibrational spectroscopic studies, we are interested in exploiting neutron diffraction (ND) to investigate protonation dynamics and simultaneously avoid radiation damage in 3D studies of the molecular action mechanism. We have proof-of-principle that phytochrome structure studies can be extended to ND and that Cph1 holoprotein can be deuterated effectively. We hope that it will be possible to generate appropriately deuterated crystals to allow ND at better than 2 Å resolution with low incoherent scattering, and thereby the 3D structure including the positions of functionally-important hydrogens/protons to be solved at near-atomic resolution and with minimal radiation damage. The project will thus provide important, unique information regarding the role of protonation dynamics in phytochrome function in particular and protein function in general.

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Session Classification: Biological processes

Track Classification: Biological processes

Contribution ID: **62** Type: **Talk**

Life sciences with neutrons at IBR-2 reactor

Tuesday, June 8, 2021 3:50 PM (20 minutes)

Currently, the scientific park of research instruments at the high flux pulsed reactor IBR-2 in FLNP JINR (Dubna, Moscow Region, Russia) consists of a complex of 15 neutron spectrometers for condensed matter studies, including 8 diffractometers, 3 reflectometers, 1 small-angle neutron scattering spectrometer, 2 inelastic neutron scattering spectrometers, and 1 spectrometer for neutron radiography and tomography. The User Program is successfully running for a suite of IBR-2 spectrometers. About 30% of the total number of submitted proposals for experimental time is for SANS instrument –YuMO spectrometer.
In recent years, in addition to traditional studies in the field of life sciences (membranes, peptide-membrane interaction, polymer complexes, etc.) there is a significant increase in interest in the study of biohybrid complexes of various nature on the YuMO spectrometer.
The report will provide an overview of the most interesting results of practical importance in the field of medicine and agriculture.

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Session Classification: Life Sciences at Russian Neutron Sources

Track Classification: Life Sciences with neutrons in Russia

Contribution ID: **63** Type: **Talk**

Not just a fluidifying effect: omega-3 phospholipids induce formation of non-lamellar structures in biomembrane

Thursday, June 10, 2021 2:00 PM (20 minutes)

Polyunsaturated omega-3 fatty acid docosahexaenoic acid (DHA) is found in very high concentrations in a few peculiar tissues. DHA was proposed to affect the function of the cell membrane and related proteins through an indirect mechanism of action, based on the DHA-phospholipid effects on the bilayer structure. Most studies have focused on its influence on lipid-rafts, neglecting the effects on liquid disordered phases that constitute most of the cell membranes.
By combining NR, cryo-transmission electron microscopy, SANS, DLS and EPR, we characterize liquid disordered bilayers formed by the naturally 1-palmitoyl-2-oleoyl-snglycero-3 phosphocholine and different contents of a di-DHA glycero-phosphocholine, 22:6-22:6PC, from both a molecular/microscopic and supramolecular/mesoscopic viewpoint. We show that, below a threshold concentration of about 40% molar percent, incorporation of 22:6-22:6PC in the membrane increases the lipid dynamics promoting the membrane deformation. Notably, beyond this threshold, 22:6-22:6PC disfavours the formation of lamellar phases, leading to a phase separation consisting mostly of small spherical particles that coexist with a minority portion of a lipid blob with water-filled cavities. From a molecular viewpoint, the polyunsaturated acyl chains tend to fold and expose the termini to the aqueous medium. We propose that this peculiar tendency is a key feature of the DHA-phospholipids making them able to modulate the local biomembranes morphology

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Session Classification: Biological membranes, surfaces and interfaces

Track Classification: Biological membranes, surfaces and interfaces

Contribution ID: **64** Type: **Poster**

Temperature-induced structural changes of PNIPAM-from milliseconds to minutes

Wednesday, June 9, 2021 2:40 PM (20 minutes)

Structural changes at the intra- and interchain level induced by the phase transition of poly(N-isopropylacryl amide) (PNIPAM) can be tracked in real-time by time-resolved small-angle neutron scattering (tr-SANS). PNIPAM is one of the most commonly and extensively studied thermoresponsive polymer due to lower critical solution temperature (LCST) in water that occurs at the physiologically relevant temperature. Block copolymer micelles and polymer microgels have been used as drug carriers and as microreactors for enzymatic reactions. For drug release and enzyme reactions, the kinetics of the transport across the micellar core and shell region depends on the local mobility of the core-shell region. The control of time-response is of critical importance for applications of responsive polymers. A general and fundamental understanding of the volume phase transition kinetics is still lacking. The aim of the present study is investigation of the collapse kinetics of PS-PNIPAM micelles by temperature jump above LCST using TR-SANS, light scattering and cryo-electron microscopy. The thermal responsiveness was followed over a broad timescale from the early-stage collapse of polymer in milliseconds to slow growth of aggregates in minutes. Using different experimental methods for homopolymers or micellar block copolymers we got comparable results indicating a common multistep scheme. Thus we concluded a general mechanism for PNIPAM polymers behavior depending on the temperature.

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Session Classification: Poster Session

Track Classification: Drug design and delivery

Contribution ID: **66** Type: **Talk**

Development of SEC-SANS for life science applications at ISIS TS2

Thursday, June 10, 2021 11:10 AM (20 minutes)

In-line size exclusion chromatography (SEC) is now routinely offered by a number of small-angle x-ray scattering (SAXS) instruments at synchrotron light sources, particularly those which focus on protein solution scattering, being pioneered on high flux undulator sources. There has recently been a successful demonstration of in-situ SEC-SANS [1, 2] at a reactor neutron source. Here, we demonstrate the feasibility of a SEC-SANS system on the time of flight SANS2 beamline. The test system consisted of a commercial Agilent HPLC and autosampler, connected to Shodex/Superdex columns, the output of which then flows through an adapted quartz cuvette. The cuvette includes 3-D printed inserts with a channel structure optimised to ensure the optimum flow of liquid within the exposure cell. Here, we will present results from a number of systems, including proteins, liposomes and membrane proteins in copolymer nanodiscs, demonstrating the feasibility and utility of SEC-SANS on a pulsed source for classical bioSANS and more generic biophysical systems of interest. We will discuss our thoughts for optimising the technique on a time of flight SANS instrument, including equipment design, such as the use of multiple binary pumps and valves, flow cell limitations through to data reduction and processing.

[1] Jordan et al, J. Appl. Cryst, 2016, 49, 2015-2020 [2] Tidemand Johansen et al, Acta Cryst D Structural Biology, 2018, D74, 1178-1191

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Session Classification: Protein structure, function and dynamics

Track Classification: Protein structure, function and dynamics

Contribution ID: **67** Type: **Talk**

Nanoscale morphology of thermoresponsive double hydrophilic block copolymers in aqueous solutions: impact of block length asymmetry and temperature effects

Thursday, June 10, 2021 5:40 PM (20 minutes)

Double hydrophilic block copolymers can self-assemble in water, leading to scaffolds with strong potential in favor of controlled encapsulation of pharmaceutics. We recently demonstrated that thermoresponsive double hydrophilic poly(N-isopropylacrylamide)-block-poly(oligo ethylene glycol methyl ether acrylate) (PNIPAM-b-POEGA) block copolymer self-assemblies in water can successfully encapsulate indomethacin, a hydrophobic anti-inflammatory drug. By complementing small angle neutron scattering (SANS) with Fourier transform infrared (FTIR) spectroscopy measurements, we explore the influence of temperature as external stimulus and polymer block length asymmetry on chemical bond vibrations and nanoscale assemblies of PNIPAM-b-POEGA block copolymers in aqueous solutions. By SANS, we identify transformations from spheres to fractal core-shell morphologies in the asymmetric block copolymer with the largest PNIPAM block length, while core-shell morphologies are formed for the symmetric diblock copolymer. For both symmetric and asymmetric systems, the evolution of the amide I band upon heating reflects that solvent-polymer interactions are still favorable even at the highest temperatures. Moreover, our results suggest that double hydrophilicity correlates to absence of critical behavior in the vicinity of the cloud point.

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Session Classification: Drug design and delivery

Track Classification: Drug design and delivery

Contribution ID: **68** Type: **Poster**

In situ light scattering techniques at neutron instruments at the MLZ

Wednesday, June 9, 2021 2:40 PM (20 minutes)

Biological samples often show a sufficiently broad spectral range where light absorption does not play a dominant role. This enables in situ sample control using dynamic and static light scattering techniques. Many biological samples undergo a slow aggregation process during the comparatively long neutron data collection times. If the aggregates are staying few in number and/or if their form factor has decayed enough in the relevant q-range, the neutron measurement can be continued. If not, a fresh sample should be used. Dynamic or static light scattering as an in situ technique offers an elegant way to get information on sample aggregation state with an update rate on the order of minutes during the neutron beam time.
Candidates for neutron instruments to be equipped with an in situ light scattering set up are small angle scattering, spin echo, time-of-flight and backscattering instruments operating sample environments near or at room temperature. We routinely provide in situ dynamic light scattering with one fixed scattering angle at the instrument KWS-2 at MLZ to interested users. For the Jülich spin echo spectrometer J-NSE we have developed a temperature-controlled sample environment which includes two laser colours and three light scattering angles. This not only enables dynamic but also static light scattering at six different q-values. This contribution discusses the experiences made with these in situ set ups and looks into future developments and improvements.

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Presenter: SCHRADER, Tobias

Session Classification: Poster Session

Track Classification: Neutrons and complementary methods in biology

Contribution ID: **69** Type: **Talk**

Antibacterial toxin binding to receptor lipids revealed by neutron reflection

Tuesday, June 8, 2021 10:30 AM (20 minutes)

Escherichia coli bacteria secrete colicins, a class of antibacterial proteins to kill closely related competing strains. This relies on the surprising ability of these toxins to cross the Gram-negative outer membrane (OM), a robust, impermeable, asymmetric lipid bilayer comprising an outer leaflet of lipopolysaccharide (LPS) and an inner leaflet of phospholipid. Colicins attach to their target cells by interacting with specific outer membrane protein receptors but colicin N (ColN) attaches to the core oligosaccharide of LPS. Here, we identify an exposed loop region at the end of the R-domain which is critical for ColN toxicity. Mutants which showed reduced in vivo toxicity also displayed lower binding to LPS in vitro, confirming the correlation between toxicity and LPS recognition. Using neutron reflectometry and in vitro models of the OM, we show that the inner core oligosaccharide of LPS must be exposed for colicin N to bind specifically. Since such exposure naturally occurs next to the outer membrane protein receptor for colicin N (OmpF), the combined results suggest how this non-canonical LPS-binding region may guide the initial steps of ColN OM-translocation.

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Session Classification: Biological membranes, surfaces and interfaces

Track Classification: Biological membranes, surfaces and interfaces

Contribution ID: **70** Type: **Talk**

Temperature-induced reorganization of influenza A nucleoprotein complex

Thursday, June 10, 2021 10:50 AM (20 minutes)

Two influenza A nucleoprotein variants (wt: G102R; and mutant: G102R and E292G) were studied with regard to macro-molecular interactions in oligomeric form (24-mers). The E292G mutation has been previously shown to provide cold adaptation. Molecular dynamics simulations of these complexes and trajectory analysis showed that the most significant difference between the obtained models was distance differences between nucleoprotein complex strands. Influenza virus nucleoprotein complexes were isolated from strains bearing the corresponding NP amino acid substitutions. The isolated complexes were characterized by transmission electron microscopy and differential scanning fluorimetry (DSF). Presence of the E292G substitution was shown by DSF to affect nucleoprotein complex melting temperature. Using small-angle neutron scattering (SANS), the supramolecular structures of isolated complexes of these proteins was studied at temperatures of 15, 32, and 37℃. SANS data show that the structures of the studied complexes (mutant or normal proteins with RNA) at elevated temperature differ from the rod-like particle model and react differently to temperature changes. The data suggest that the mechanism behind cold adaptation with E292G is associated with a weakening of the interaction between strands of the ribonucleoprotein complex and, as a result, the appearance of inter-chain interface flexibility necessary for complex function at low temperature.

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Session Classification: Protein structure, function and dynamics

Track Classification: Protein structure, function and dynamics

Contribution ID: **71** Type: **Poster**

Mechanisms of action for the supramolecular drugs: neutron study

Wednesday, June 9, 2021 2:40 PM (20 minutes)

Many low molecular weight compounds and peptides are capable of forming supramolecular complexes. In the form of such complexes, the molecules are capable of multicenter cooperative binding to target proteins. It is advisable to study these complexes using small-angle scattering methods in combination with molecular dynamics modeling in the free diffusion approach.
When studying the mechanism of interaction of a triazavirin drug with polypeptides by neutron small angle scattering methods in combination with molecular dynamics, it was shown that the drug molecules are capable of forming linear supramolecular complexes and altering the quaternary structure of proteins (Shvetsov et al. 2018)(Zabrodskaya et al. 2018)(V. V. Egorov et al. 2017).
Supramolecular amyloid-like peptide complexes are capable of specific effects on the secondary structure of the protein, which can be used to create a new class of antiviral drugs, as was shown using small-angle neutron scattering and time-resolved x-ray scattering(Zabrodskaya et al. 2017)(V. V. Egorov et al. 2013)(Matusevich et al. 2015).
The interaction of supramolecular complexes formed in lipid membranes with receptors can be used to modulate cell signaling, including the creation of immunomodulating drugs that affect T cells. The effect of complexes on the chromatin structure can be used to create a new class of drugs - epigenetic regulators that affect gene expression(Lebedev et al. 2019).

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Session Classification: Poster Session

Track Classification: Drug design and delivery

Contribution ID: **72** Type: **invited talk**

Combining NMR, SAXS and SANS in integrative structural biology to study dynamics and allostery in protein complexes

Friday, June 11, 2021 9:00 AM (30 minutes)

Most eukaryotic proteins are comprised of multiple structural domains connected by linkers of variable length and rigidity. We combine solution NMR spectroscopy and small angle scattering (SAXS, SANS) with crystallography and cryo-EM in integrative structural biology approaches to study the conformational dynamics of multidomain proteins and the roles of the connecting linkers. Studies with multidomain RNA binding proteins (RBPs) and the multidomain chaperone Hsp90, will be discussed.
The molecular functions of multi-domain proteins often rely on dynamic structural ensembles and can be controlled by population shifts between inactive and inactive conformations. This is not visible in static structures. The domains in these proteins are often connected or flanked by intrinsically disordered regions, where posttranslational modifications can further modulate the molecular interactions to regulate the biological activity. Integrative structural biology combining solution techniques, especially NMR spectroscopy, can help to unravel the molecular recognition, dynamics and regulation of protein complexes.

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[2] Jagtap, PKA et al. Angew. Chemie 58, 9429–9433 (2019).
[3] Kooshapur, H. et al. Nature Commun 9, 2479 (2018).
[4] Mackereth CD et al. Nature 475, 408–411 (2011).

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Session Classification: Neutron and complementary methods in biology

Track Classification: Neutrons and complementary methods in biology

Contribution ID: **73** Type: **Talk**

Changes in chromatin organization induced by macromolecules and protein complexes as a possible mechanism for epigenetic regulation

Thursday, June 10, 2021 9:50 AM (20 minutes)

An important question in the study of chromatin packing is to identify the functional significance of its structure and dynamics. The integral properties of chromatin packing as a polymer chain are extremely important for understanding the mechanisms of interaction between distant chromatin regions, the formation of loops, and, ultimately, the formation and dynamics of topologically associated chromatin domains - genome regions associated in space and having similar characteristics of transcriptional activity. The self-organization and evolution of such domains occurs under the influence of a complex of biochemical (post-translational modification of histone proteins, nucleosome dynamics, interaction of chromatin with specific nuclear proteins), and chemical (charge interactions, macromolecular crowding) factors.
Organization of genetic material in eukaryotes exhibit two-phase fractal property of chromatin during interphase, variability in its structure parameters in a number of cell types, and strong effect of macromolecular crowding on both large-scale hierarchy and small-scale nucleosome arrangements, thus providing the experimental basis for the studies chromatin organization throughout its structural hierarchy. Under certain conditions, the chromatin structure as observed by SANS can be influenced by non-nuclear proteins and protein complexes. These changes may manifest an important element in epigenetic regulation mechanisms.

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Session Classification: Biological processes

Track Classification: Biological processes

Contribution ID: **74** Type: **Talk**

Complementary Methods: Molecular Dynamics and SANS

Friday, June 11, 2021 9:30 AM (20 minutes)

The SANS method is often used to study various types of biological systems. However, in the common case, SANS gives only a limited view on the structure of biomacromolecular complexes in solution. In this case, SANS can be used in conjunction with other methods, such as molecular modelling and molecular dynamics. Molecular modelling methods are also commonly used to study biomacromolecular systems in solution. The combination of these two complementary methods in some cases allows you to get a fully atomic idea of the structure of the biomacromolecular complex in solution.
The use of SANS and molecular dynamics methods requires a direct comparison of SANS data and molecular dynamics data. In this case, the correct accounting of the solvent contribution remains an important factor. We used the method we developed for calculating SANS[1] and NSE curves along MD trajectories. We used this method for a number of multicomponent biological systems, such as complexes RecA :: ssDNA, RecA :: RecX :: ssDNA[2], various RNP complexes from influenza A virus[3] and glycoprotein complexes such as Glucoamilase from Asp. Awamori. In some cases, the use of this combined approach makes it possible even to find new complexes in solution or shed light on some interesting effects with observable via SANS data parameters.
[1] Shvetsov A.V. et.al. doi:10.1134/S1027451013060372
[2] Shvetsov A.V. et.al. doi:10.1016/j.febslet.2014.01.053
[3] Shvetsov A.V. et.al. doi:10.1080/07391102.2020.1776636

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Session Classification: Neutron and complementary methods in biology

Track Classification: Neutrons and complementary methods in biology

Contribution ID: **75** Type: **Talk**

Retrieving Myelin/Nerve Fibers in a Brain Section by Small Angle Scattering

Tuesday, June 8, 2021 1:30 PM (20 minutes)

The investigation of fiber distribution and myelin orientation in the brain has received increasing attention in recent years, as the 3D fiber structure reveals the connectivity of the axonal network (connectome) that is necessary to understand dysfunctions of the brain [1]. Hence, we adapt scanning small angle neutron/x-ray scattering (sSANS/sSAXS) to map an entire brain section of a mouse and to investigate the microstructural insights of the anatomical regions in the tissue [2-3]. We extract the orientation and spatial distribution of the nerve fibers and determine their degree of orientation in the section. Moreover, we quantify the orientation of myelin sheaths and their assembly from the myelin Bragg peaks across the section. Finally, we illustrate the potential of scanning SAS by comparing the result with the fiber orientation maps (FOM) of 3D polarized light imaging (PLI) in the same brain [4-5]. In the future, the scanning neutron/x-ray can serve as an alternating technique for imaging other biological tissues.
[1] Y. Shi et al., Molecular Psychiatry 22, 1230-1240 (2017). [2] H. Inouye et al., PLOS One. 9, e100592, (2014). [3] M. Georgiadis et al., NeuroImage, 204, 116214 (2020). (2015). [4] M. Axer et al., Front. Neuroinform., 5, 34 (2011). [5] S. Maiti, S. Förster et al., (submitted).

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Session Classification: Neutron and complementary methods in biology

Track Classification: Neutrons and complementary methods in biology

Contribution ID: **76** Type: **Talk**

Structure of SARS-CoV-2 papain-like protease PLpro reveals a framework for antiviral inhibitor design

Thursday, June 10, 2021 4:00 PM (20 minutes)

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) papain-like protease (PLpro) is essential for the virus replication. PLpro has the additional function of removing ubiquitin and ISG15 (Interferon-stimulated gene 15) from host-cell proteins to aid coronaviruses in their evasion of the host innate immune responses. PLpro is thus an excellent drug target for a two-fold strategy to develop antiviral compounds that both inhibit viral replication and strengthen the immune response of the host. To provide a structural framework for efficient screening of inhibitor compounds, we expressed, purified and crystallized PLpro. The crystals are stable, reproducible, have a high solvent content of 66% suitable for soaking experiments and diffract to a high resolution of 1.5 Å. Bioinformatics analysis of the active site region based on the PLpro crystal structure coordinates showed interestingly high similarities to the proteasome active site and we screened 37 proteosome inhibitors by soaking and co-crystallization experiments. The PLpro crystals complexed with these compounds diffracted in the resolution range of 1.5 Å-2.5 Å and structural efforts to identify new antiviral compounds to combat the coronavirus spread will be presented.

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Session Classification: Neutrons in the fight against virus diseases

Track Classification: Neutrons in the fight against virus diseases

Contribution ID: **77** Type: **Poster**

Understanding the reaction mechanism of chlorite dismutase: Characterizing protonation states of turnover-associated amino acid residues using neutron cryo-crystallography

Wednesday, June 9, 2021 2:40 PM (20 minutes)

Chlorite dismutases (Clds), are heme b-dependent oxidoreductases that are catalysing the degradation of toxic chlorite to harmless chloride and molecular oxygen. This catalytic function turns Clds into interesting enzymes for bioremediation. The enzyme CCld (from Cyanothece sp. PCC7425) was studied extensively with regard to its biochemical and biophysical properties and represents a perfectly suited model for mechanistic studies. It exhibits the Cld-typical pH-dependent chlorite degradation activity and becomes irreversibly inactivated at basic pH. This was thought to be due to the presence of a conserved arginine residue in close proximity to the heme. Literature proposes that this amino acid residue possesses a shifted pKa value of the side chain, thereby being mainly deprotonated above pH 7 which correlates very well with the enzyme's activity profile. However, results obtained from neutron crystallography experiments with hydrogenated CCld crystals, after hydrogen/deuterium exchange, showed a still protonated arginine even at pD 9.4. To prove the obtained data and expand our knowledge, the planned neutron crystallography experiments with perdeuterated CCld crystals are expected to give more detailed informations on the protonation state of potentially turnover associated amino acid residues. Neutron cryo-crystallography should be performed to catch short-lived reaction intermediates of CCld. The results are highly important for interpretation of mechanistic data of CCld.

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Session Classification: Poster Session

Track Classification: Protein structure, function and dynamics

Contribution ID: **78** Type: **Talk**

XFEL and Neutron Diffraction Studies allow Determination of the Binding Environment of an Iron Binding Protein

Tuesday, June 8, 2021 1:50 PM (20 minutes)

The major iron uptake mechanism in bacteria is mediated by ABC transporters of the FutA type. We study iron uptake and homeostasis in highly adapted marine cyano-bacteria that are of high geochemical significance for global net carbon fixation. We study FutA substrate binding domains that bind iron in the Fe(III) oxidation state. FutA proteins also have an intracellular function in protection against photo-oxidative stress, then binding iron in the Fe(II) oxidation state.
Observation of iron complexes in X-ray crystallography is hampered by photoreduction of ferric Fe(III) to ferrous Fe(II), complicating study of the iron binding mechanism. We characterised photoreduction at room and cryogenic temperatures using dose-controlled X-ray crystallographic experiments and used single crystal, multi-crystal and serial crystallography data collection strategies with rotating anode, synchrotron or XFEL sources.
While the XFEL experiment serves as a "zero dose"reference point, we compare this experiment to a neutron diffraction experiment that avoids inherent photoreduction. The neutron experiment gives additional information and derives the protonation state of amino acids involved in metal coordination. Neutron diffraction data collection was carried out at BIODIFF using a single fully deuterated crystal sample produced by buffer exchange. Together with the X-ray crystallographic structures we can now define switches that allow FutA to bind Fe(III) and Fe(II) iron species.

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Presenters: TEWS, Ivo (University of Southampton); Dr HOUGH, Mike (Essex University)

Session Classification: Neutron and complementary methods in biology

Track Classification: Neutrons and complementary methods in biology

Contribution ID: **79** Type: **invited talk**

Neutrons reveal (some of) the secrets of heme peroxidases

Tuesday, June 8, 2021 4:40 PM (30 minutes)

We have used the ability of neutron crystallography to locate hydrogen atoms in our studies to investigate the mechanisms of the heme peroxidases cytochrome c peroxidase and ascorbate peroxidase. In order to do this we have cryo-trapped the labile intermediates known as Compound I and Compound II in crystals. A key question about these intermediates has been the protonation states of the ferryl oxygen atom. This should be indirectly resolvable from the Fe-O bond length, and this could be determined by X-ray crystallography. However, photoreduction makes this challenging, as these highly oxidised Fe(IV) intermediates are particularly sensitive to the direct and indirect reducing consequences of X-rays. In solving the structures by neutron crystallography we revealed that assumptions about the charge state of the active site histidine need to be revised. These results will be presented in the context of complimentary enabling spectroscopic and XFEL investigations. The protonation states of the residues in the pathway of proton-coupled electron transport in ascorbate peroxidase was also examined, showing that the arginine side chain can exist in the neutral form within the enzyme.

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Session Classification: Protein structure, function and dynamics

Track Classification: Protein structure, function and dynamics

Contribution ID: **80** Type: **invited talk**

Biological Research in Russia: Neutron Research as Essential Part of a Multidisciplinary Approach

Tuesday, June 8, 2021 3:30 PM (20 minutes)

Improving the parameters of biological macromolecules for usage in biomedicine and biotechnology requests the knowledge of the exact molecular mechanism of action of such biomolecules. Structural biology and dynamic studies of biological macromolecular complexes became essential part of such research. In addition to most abundant studies, where static structure of a protein is solved by the X-ray crystallography, other complementary methods, such cryo-electron microscopy, molecular dynamics simulation and small-angle scattering methods are used to receive additional information. Neutron studies in modern biology covers important field, allowing for observe structural features of extra-large proteins, nucleic acids and ribonucleoprotein complexes in their native state in solution. Despite the obvious deficiency of modern neutron research infrastructure for biological research in Russia, there is high level of expertise accumulated in a number of research centers of Russia. In this report most actual results of application of neutron methods for biomedicine and biotechnology will be summarized.

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Session Classification: Life Sciences at Russian Neutron Sources

Track Classification: Life Sciences with neutrons in Russia

Contribution ID: **81** Type: **invited talk**

Structural Investigation of Lipid Nanoparticles is key for Successful mRNA Delivery

Wednesday, June 9, 2021 9:00 AM (30 minutes)

Lipid nanoparticles (LNPs) constituted by a cationic ionizable lipid and helper lipids as cholesterol, phospholipids and poly(ethylene glycol) lipid as stabilizer have made mRNA therapeutics a reality. 2 mRNA-based vaccines against SARS-CoV-2 have received emergency authorization by many regulatory agencies using LNPs as delivery vehicle. We investigated the structure of mRNA-containing LNPs to understand how this relates to their transfection efficacy. Small angle X-ray and neutron scattering were fundamental to prove that LNPs have a disordered hexagonal internal structure in the presence of mRNA, independently of their size. Additionally, we found that the phospholipid DSPC and cholesterol are localized mainly at the LNP surface. Knowing their lipid distribution allowed us to vary their size and surface composition in order to increase protein production. This improvement is most likely related to the ability of LNPs to fuse with early endosome membranes. Another important consideration is the formation of a protein corona at the LNP surface upon administration and in particular Apolipoprotein E (ApoE), which is responsible for fat transport in the body. We found that binding of ApoE to LNPs induces a redistribution of the lipid across the particle, which can impact endosomal escape. Our findings highlight how neutrons can guide us in the rational design of nanomedicines for gene therapy with improved bioperformance.

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Session Classification: Drug design and delivery

Track Classification: Drug design and delivery

Contribution ID: **82** Type: **invited talk**

Mechanical plasticity of the ECM directs branch elongation in human mammary gland organoids

Tuesday, June 8, 2021 1:00 PM (30 minutes)

Epithelial branch elongation is a central developmental process during branching morphogenesis in diverse organs. This fundamental growth process of large arborized epithelial networks is accompanied with huge structural reorganizations of the surrounding Extracellular Matrix (ECM), which is well beyond its mechanical linear response regime. Here, we report that epithelial ductal elongation within human mammary organoid branches relies on an intricate tension-driven feedback mechanism, which is based on the non-linear and plastic mechanical response of collagen. Specifically, we demonstrate that collective motion of cells within the branches generates tension that is strong enough to induce a plastic reorganization of the surrounding collagen network which results in the formation of mechanically stable collagen cages. Such matrix encasing in turn directs further tension generation, branch outgrowth and plastic deformation of the matrix. The identified mechanical feedback loop model sets a framework to understand how mechanical cues can direct organogenesis.

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Session Classification: Neutron and complementary methods in biology

Track Classification: Neutrons and complementary methods in biology

Contribution ID: **83** Type: **invited talk**

Protein Dynamics in Complex Environments

Tuesday, June 8, 2021 11:10 AM (30 minutes)

After an introduction into strategies for controlling the time-averaged behavior of aqueous protein solutions in the bulk at near interfaces, we discuss their dynamical behavior.
First, this concerns the impact of temperature, salt concentration, protein concentration and other control parameters on the diffusion in equilibrium.
Second, we study how the dynamics in these complex systems changes if phase transitions are triggered. Examples are aggregation phenomena, network formation upon denaturation, phase separation, such as liquid-liquid phase separation, or crystallization. An important challenge in this context is the separation of the relevant time scales, and, under favorable circumstances, the separation of kinetics and dynamics.
We attempt a holistic discussion of the broad range of length and time scales, and we emphasize the need of using several complementary techniques to cover these, as well as the associated practical challenges.
Finally, we comment on models and theories suitable for a comprehensive explanation of these phenomena.
Invaluable contributions by numerous collaborators are gratefully acknowledged.
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Session Classification: Biological membranes, surfaces and interfaces

Track Classification: Biological membranes, surfaces and interfaces

Contribution ID: **84** Type: **Poster**

Dynamics of IDP Histatin 5 probed by QENS and compared with simulation

Wednesday, June 9, 2021 2:40 PM (20 minutes)

Intrinsically disordered proteins (IDPs) adopt a wide variety of conformations in solution, without a distinct equilibrium structure. Here, we investigate the dynamics of IDPs, using the antimicrobial saliva protein Histatin 5 as model. A suitable technique for this purpose is quasi- elastic neutron scattering (QENS), which through the incoherent scattering probes the self- diffusion of particles on biologically relevant length- and timescales. Here, focus is on the center-of-mass diffusion, considering dynamics with respect to temperature and self- crowding effects. The diffusion obtained is a convolution of translational and rotational diffusion, but implicit relations between these are known. Therefore, atomistic molecular dynamics simulations previously performed are analyzed to compare with the experimentally achieved results., providing further insight into the dynamical properties of IDPs and how these are affected by self-crowding and temperature.

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Session Classification: Poster Session

Track Classification: Protein structure, function and dynamics

Contribution ID: **85** Type: **invited talk**

Neutron Scattering Experiments Under (in-situ) Illumination

Thursday, June 10, 2021 9:00 AM (30 minutes)

The structure-dynamics-function relationship in proteins remains a field of great scientific interest. Photoactive proteins form a specific class, whose function can be activated by illumination. Two prototypical examples are the Orange Carotenoid Protein (OCP) and bacteriorhodopsin (BR). As to the first, photodamage of the photosynthetic apparatus of cyanobacteria in the case of excess light is prevented by a protection mechanism called non-photochemical quenching (NPQ). This process requires OCP as a light-sensitive effector. OCP is photosensitive and undergoes a pronounced structural change to its active state under illumination with blue light, but a high-resolution crystal structure of the active state is still elusive. We have used small angle and quasielastic neutron scattering (SANS and QENS, respectively) in the dark and under steady-state illumination achieving a turnover of more than 90% to investigate structural and dynamical changes of OCP during its activation [1,2]. In contrast, BR is famed for its light-induced photocycle, which makes it the first choice for the development of time-resolved (TR-) QENS experiments [3]. It will be shown, that TR-QENS permits investigations of protein dynamics in specific functional states on microsecond to millisecond timescales.

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Session Classification: Biological processes

Track Classification: Biological processes

Contribution ID: **86** Type: **Poster**

Diffusive-like motions in a solvent free myoglobin-polymer hybrid revealed by neutron scattering and MD simulations

Wednesday, June 9, 2021 2:40 PM (20 minutes)

Dancing water molecules on the surface of soluble proteins provide the essential lubricant for macromolecular function. Surprisingly, polymers attached to protein surfaces have been reported to replace hydration water and bring inactive dry proteins back to life (Perriman et al. (2010) Nat Chem 2, 622; Gallat et al. (2012) JACS 134, 13168). The mechanism behind polymer-assisted protein motions has remained elusive, however.
We have now combined elastic and quasi-elastic neutron scattering on TOFTOF and SPHERES (MLZ, Garching) and molecular dynamics simulations to shed light on the motions animating proteins and polymers in a water-free myoglobin –polymer hybrid (Schirò et al. (2021) Phys Rev Lett 126, 088102). Separating them was possibly by selectively masking the signal from either the polymer or the protein, using specific deuteration. Surprisingly, the polymer exhibits diffusion like motions that appear to substitute for hydration-water translational motions. Even if this substitution keeps dry proteins biologically active, certain dynamical modes are suppressed in the protein, possibly explaining the generally observed decrease in activity when hydration water is substituted by polymer coating. The study suggests ways to fine-tune polymer properties so that the decrease of protein activity can be minimized. This will be particularly important to rationally design protein-polymer hybrids for specific biotechnological applications, such as in medicine and cosmetics.

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Session Classification: Poster Session

Track Classification: Protein structure, function and dynamics

Contribution ID: **87** Type: **invited talk**

64 years of cutting-edge research with neutrons in Garching –How science and politics are intertwined

Thursday, June 10, 2021 1:00 PM (1 hour)

History of FRM II & MLZ

Presenter: PETRY, Winfried (FRM II - TUM)

Session Classification: History of FRM II & MLZ