the determinants of water permeation and the effect of the lipid or polymer membrane on channel function, the development of specific water flow inhibitors, the design of artificial water channels and aquaporins for the use in industrial water filtration applications rely on accurate ways to quantify water permeabilities (P_f). A commonly used method is to reconstitute membrane channels into large unilamellar vesicles (LUVs) and to subject these vesicles to an osmotic gradient in a stopped-flow device. Fast recordings of either scattered light intensity or fluorescence self-quenching signals are taken as a readout for vesicle volume change, which in turn can be recalculated to accurate P_f values (Horner at al., *Science Advances* 1, e1400083, 2015; Hannesschläger et al., *Sci Rep* 8, 8516, 2018). By means of computational and experimental data, we discuss the pros and cons of using scattering versus self-quenching experiments or subjecting vesicles to hypo- or hyperosmotic conditions. In addition, we explicate the influence of the LUVs size distribution and remaining detergent after protein reconstitution on P_f values. We point out that results such as that the single channel water permeability (p_f) depends on the membrane matrix or on the direction of the applied osmotic gradient may be direct results of the measurement and analysis procedure.

663-Pos

Studying Weak Macromolecular Interactions by Sedimentation Velocity of Highly Concentrated Solutions

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Sedimentation velocity has unique potential to study weak interactions at high concentrations due to the strongly size-dependent macromolecular sedimentation coefficients. Due to the difficulty in describing nonideal sedimentation, previous sedimentation velocity approaches were limited to tracer configurations where the main solution components still sediment but remain invisible, thereby limiting quantitative interpretation. To address this problem, we have recently introduced a mean-field approximation to explicitly account for hydrodynamic interactions in the sedimentation of polydisperse concentrated solutions. For the first time, it enables unraveling macromolecular size-distributions of highly concentrated nonideal solutions, while simultaneously measuring nonideality coefficients that report on the macromolecular distance distribution in solution. Observing and modeling sedimentation profiles of concentrated solutions allows quantifying weak interactions that produce relatively long-lived complexes, transient complexes, and subtle attractive and repulsive interactions manifest in the solution structure. Previous examples include the study of bovine serum albumin up to 50 mg/ml, and gamma-crystallins up to 80 mg/ml with mM dimerization constants. Here we describe the application to characterize weakly self-associating IgG. We will report on ongoing work that aims at extending the concentration limits further, by utilizing higher-order hydrodynamic interaction terms, fabricating specialized sample holders through 3D printing, and optical techniques to reveal distortions from high refractive index gradients.

664-Pos

Developing a pH-Jump Chemical Triggering Method for Time-Resolved Diffraction in Bacterial HMG-CoA Reductase

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HMG-CoA reductase (HMGR) in Pseudomonas Mevalonii uses Mevalonate as a carbon source and converts it into 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) using the substrate Coenzyme A and cofactor NAD. The mevalonate pathway is critical for the survival of multiple gram-positive bacteria making HMGR a novel target for antibacterials. Previous studies propose that this enzyme's reaction mechanism involves the production of intermediates Mevaldehyde and Mevaldyl-CoA with structural evidence only for Mevaldyl-CoA. Using time-resolved crystallography we can observe structural changes along the reaction pathway and detect intermediates. By understanding the reaction mechanism in more detail, we can design inhibitors with higher specificity. Time-resolved techniques require a triggering system to achieve temporal control over the enzymatic reaction. To develop a pH-based trigger, we measured the effect of pH on the enzyme's activity in the crystallization environment. The enzyme's pH profile in crystallization conditions shows significantly reduced activity with increasing activity at alkaline pH. This is attributed to the presence of precipitant, ammonium sulfate. This was also observed outside the crystallization environment and with other ammonium salts. A strong correlation was observed between the dissociation constant of the ammonium salts and inhibition. Using this information, we developed a chemical triggering method in which we exchange the crystal constituents with an ammonium acetate buffer and introduce the ligands at crystallization pH with no enzymatic activity. To initiate the enzymatic reaction, these crystals are transferred to a higher pH for different time-periods before being flash-frozen. Structures generated from these crystals showed the formation of Mevaldyl-CoA and the release of HMG-CoA / NADH. They also captured movement in regions associated with catalytic site formation and product release. Currently, we are using this triggering system to collect data at faster timescales and at multiple time-points in individual crystals.

665-Pos

Single Molecular Observation of AFP and Ice-crystal Dynamics in *Caenorhabditis elegans* by Time-Resolved X-ray Diffraction Measurements

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Antifreeze proteins (AFPs) can inhibit the ice-crystal growth, so are widely used to apply food, transportation, medical and other fields as freezing-preservation technique. However, knowledge regarding the dynamical property of AFP-ice interaction is limited. To observe the AFP-ice interaction *in vivo*, we generated the transgenic *Caenorhabditis elegans* expressing AFP at intestinal cells of worm, and monitored the AFP and ice-crystal motions by x-ray single molecular observations such as DXB (Diffracted X-ray Blinking).

DXB is unique method to monitor the internal motions of single molecules from the time-resolved x-ray diffraction images. DXB can perform at from synchrotron radiation to laboratory x-ray source using monochromatic x-ray. To monitor AFP internal-motions in living worm by DXB, gold nanocrystals were bound to AFP of intestinal cells specifically in *C. elegans*. The internal-motions of the wild-type AFP gradually decreased, whereas that of the AFP mutant increased, when the temperature was decreased at 0°C to −20°C. This AFP mutant has low-binding ability to ice-crystals. The wild-type AFP binds to ice-crystals stably, resulting that its motion is slow. On the other hand, the AFP mutant could not bind to ice-crystals, resulting that its motion is fast. In addition, we observed the diffraction rings from ice crystals below −10°C in each sample. The rotational motion of ice-crystals significantly differed between the AFP-expressing and wild-type animals. We also found that the size of ice-crystals in AFP-expressing groups is smaller than control groups by analyzing the diffraction pattern of ice-crystal. In this presentation, we will discuss about dynamical aspect of AFP-ice interaction in living animal.

666-Pos

DNA-Origami-Assisted Flow-Aligned Single-Particle Diffractive Imaging using XFEL Pulses

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Outrunning radiation damage, highly intense femtosecond pulses of X-ray free-electron lasers (XFELs) open up the possibility of structure determination of macromolecules to viruses at room temperature, by aggregating diffraction patterns recorded from uncrystallized single-particles. A key challenge in XFEL single-particle diffractive imaging (SPI) is to either constrain the orientation of the particle or to determine it from each of the very noisy weak diffraction patterns. Here we report a unique approach to address these challenges using structural DNA nanotechnology. A DNA-origami "rigid tail" is site-specifically attached to the macromolecule to flow-align it in a thin liquid jet, and also provides a strong holographic reference. In a proof-of-principle study, the computational design and production of the DNA-origami-target construct has been achieved and the experimental results obtained from the Linac Coherent Light Source (LCLS), USA show the alignment of the target single-particle, consistent with simulations, at extremely low concentrations

approaching single-molecule in the interaction region with the nanofocus hard X-ray laser beam, in a sum of as few as a thousand single-shots. The results open up the possibility of single-molecule diffractive imaging in solution with XFEL pulses. *Acknowledgements: The Human Frontier Science Program (RGP0010/2017).*

667-Pos

User Capabilities at the GM/Ca@APS Structural Biology Facility at the Advanced Photon Source

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The General Medical Sciences and Cancer Institutes' structural biology facility at the Advanced Photon Source (GM/CA@APS) operates a national user facility for crystallographic structure determination of biological macromolecules, with an emphasis on challenging, high-impact projects. The facility includes two undulator beamlines, 23ID-B and 23ID-D, that provide stable, intense X-ray beams of user-selectable size down to 5-micron diameter, an intuitive user interface for experiment control, and an automated pipeline for data processing. The beamlines are equipped with high capacity (288 samples) automounters and Dectris PADs allowing 100Hz data collection. This presentation will give an overview of recent developments at the beamlines, including advances in the implementation of serial crystallography experiments with viscous-jet or fixed target mounting systems. These efforts align well with the planned APS storage ring upgrade (APS-U) that increases the brightness of the X-ray beam by 100 to 1000 fold. Researchers who seek beam time for traditional crystallography or interested in pursuing novel structures via serial crystallography at GM/CA@APS are encouraged to contact us, https://www.gmca.aps.anl.gov/userprogram/applying.html, to help enable their experiments.
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668-Pos

High Throughput Scattering Beamline for Life Sciences at NSLS-II

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Life Sciences X-ray scattering (LiX) beamline at NSLS-II is designed and developed to provide high throughput scattering experiments in structural biology. Static solution scattering experiments are fully automated, with sample loading, data collection and processing all performed without any user intervention. At a given time, up to 360 samples in 20 different sample holders can be loaded into a sample storage box. A six-axis robot transfers individual holders to the solution scattering module, whereby data is collected on the fly while the sample flows through a flow cell to mitigate radiation damage. The flow cell is made up of 3 channels aligned vertically and each channel is automatically moved into the beam depending on sample position. Within the sample holder, there are two rows of sample positions, each connected to a single channel within the flow cell. This setup allows for concurrent operations whereby the sample is loaded in one cell, as the second cell is primed and ready for a subsequent measurement. Sample-buffer subtraction is carried out using the reference water peak intensity. To obtain the water measurement, wide angle data collection is needed (~2.0A). In order to do so, LIX uses three x-ray Pilatus detectors which collects data simultaneously and stitches it together to cover a q range from 0.005 $Å^{-1}$ to 3.0 $Å^{-1}$. Furthermore, to investigate heterogeneous systems, size exclusion chromatography (SEC) can be performed utilizing a Shimadzu HPLC system connected in-line with the middle flow channel of the flow cell. This allows for a seamless transition between standard SAXS measurements and SEC-SAXS.

669-Pos

Recent Developments at the Beamline for Biological Small Angle x-ray Scattering BL4-2 at SSRL

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The small-angle x-ray scattering station BL4-2 at the Stanford Synchrotron Radiation Lightsource (SSRL) is a permanent experimental station dedicated to structural biology and biophysics, providing state-of-the-art experimental facilities for structural studies on nucleic acids, proteins, protein assemblies, virus particles, biological fibers, lipid membranes and their complexes. The beamline features a pinhole camera with adjustable sample-to-detector distance and a large area pixel array detector (Pilatus3 X 1M) with a 500 Hz image frame rate.
Available at the beamline is a range of specialized sample environments for a variety of different SAXS experiments, including a fully automated high-throughput sample delivery robot for static solution scattering on biological macromolecules, including an integrated data reduction and analysis pipeline. For aggregation prone protein samples or weakly bound complexes a size-exclusion chromatography (SEC) setup can be directly connected to the SAXS instrument allowing in-line SEC-SAXS experiments to provide the highest sample purity for the SAXS data collection. Furthermore a temperature controlled flow-cell enables measurements of fluid samples over a wide range of temperatures (0° to 100°C).
In addition to the static solution scattering measurements we also provide state-of-the-art instrumentation for time resolved experiments on weakly scattering biological systems in the millisecond time scale and above. Our stopped-flow device has been optimized to substantially reduce the required sample volume in order to allow time-resolved measurements on systems that are difficult to produce in larger amounts.
Here we will discuss recent technological developments and present some of the recent scientific results obtained at the beamline.

Posters: Molecular Dynamics I

670-Pos

A Versatile Lambda-Dynamics Module for GROMACS

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Understanding the function of biomacromolecules by means of atomistic simulations is complicated by titratable sites which occur in different forms whose charge distribution differs, e.g., due to protonation, reduction or small-molecule binding. This variability is often crucial for molecular function and interactions and thus has to be included for a realistic description of conformational variability. One way to include chemical variability in molecular dynamics (MD) simulations is λ-dynamics. Here, we are developing a λ-dynamics module for the open source MD package GROMACS.
In λ-dynamics, interconversion between discrete, physical site forms like the protonation forms of a titratable aminoacid is enabled by connecting these forms via continuous λ-variables, thus adding a variable charge distribution as crucial physical detail neglected in standard MD at the expense of introducing unphysical intermediate states. The population of these intermediates is controlled via a novel bias potential which explicitly punishes the occurrence of unphysical states. While avoiding overrepresentation of unphysical intermediates, this adjustable bias potential still ensures sampling of all physically relevant configurations. An optimal tradeoff is achieved between sampling quality (staying close to the physical states) and efficiency (frequent interconversion between site forms).
Our λ-dynamics module improves over existing implementations by enabling variable sites a) to binding any number of different ligand types instead of just protons and b) having any number of binding forms accounting for more than two binding forms, tautomerism and coupled binding of protons, electrons and possibly other ligands. These capabilities are paramount for a realistic description of titratable sidechains and oft complicated biological cofactors or drug molecules. Our module also enables multiple different λ-dynamics variants within the same MD code thus allowing for a direct comparison of the λ-dynamics variants in terms of sampling and computational cost.

671-Pos

The Role of Rapid Protein Dynamics in Artificial Enzyme Design

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The successful use of directed evolution to improve a computationally designed enzyme into a series of improved protein catalysts motivated us to