Tuesday, June 27, Session III

L9

ULTRAFAST LASER SPECTROSCOPY AS A TOOL FOR STUDIES OF PROTEIN FUNCTION

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Time-resolved laser spectroscopy with high (< 100 fs) resolution is now a standard tool for studies of ultrafast processes in molecules, proteins, solid state materials and other systems. It is able to follow the key dynamical processes, such as energy and electron transfer, isomerization, bond breaking and formation and many others. Current state-of-art laser systems are able to deliver ultrashort pulses with time duration less than 20 fs in a broad spectral range extending from X-rays to mid-IR. Typically, in the transient absorption mode, which is probably the most common type of time-resolved experiment, the process of interest is initiated by exciting the sample by an excitation (pump) pulse that is, after a precise delay time, followed by a probe (typically broadband) pulse that monitors the changes in the sample caused by the excitation pulse. Here we will show a few examples how the time-resolved laser spectroscopy can be used to determine not only the function of proteins, but also how the recent advances of this technique, which includes time-resolved Raman spectroscopy or time-resolved X-ray diffraction, can relate structure and function of proteins.

L10

SINGLE MOLECULE FLUORESCENCE AND APPLICATIONS

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Fluorescence methods with single molecule sensitivity have been incredibly improved in the past two decades. These novel approaches have recently provided dynamic insights into the biomolecular mechanisms of molecular motors, ligand binding and protein–nucleic acid interactions. The sensitivity and precision of such *in vitro* fluorescence-based imaging techniques can be further enhanced through the use of nanostructures, such as zero mode waveguides. The increasing time-range and robustness of single molecule techniques, which emerge as a set of crucial tools for elucidating biological processes at utmost detail and accuracy will be demonstrated on our current research on SecYEG driven protein translocation.

L11

STATE-OF-ART BIOLOGICAL SMALL-ANGLE-SCATTERING AND NEW POSSIBILITIES ON FREE ELECTRON LASERS

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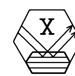
Biological Small Angle X-ray Scattering (SAXS) became one of the standard techniques in structural biology. As a solution scattering method SAXS does not rely on high quality protein crystals and is not limited in protein size and folding state. On the other side, SAXS cannot provide high resolution protein structures, however modern scattering data evaluation permits model building of macromolecular assemblies in the range down to 15 Angström. High quality SAXS data can be recorded at standard lab based SAXS devices, but the full advantage of solution scattering can be obtained at dedicated SAXS synchrotron beamlines. Using SAXS, environmental parameters can be altered and parameters such as temperature, pH-value and salinity adjusted to the protein activity. The most fascinating and challenging approach is the investigation of kinetics and dynamics of protein reaction. Starting from simply fast mixing experiments using protein and ligand solutions for investigation of protein kinetics, modern synchrotron beamlines allow as well pumped-probed experiments for exploring the dynamic of protein-protein interactions and



protein domain motions. Following this idea of investigation on biological systems "at work" new possibilities are provided by Free Electron Lasers. These upcoming high brilliance X-ray sources are able to record X-ray diffraction data in the fs time range, which is suitable to record protein dynamics in detail.

In this lecture the basics of biological SAXS will be explained and high end application of the SAXS method introduced. Some technical details of Free Electron Lasers will be given and future prospective applications of this method discussed.

1. Small Angle X-ray Scattering; Manfred Roessle, Dmitri I Svergun; 2013; Encyclopedia of Biophysics.
2. Versatile sample environments and automation for biological solution X-ray scattering experiments at the P12 beamline (PETRA III, DESY). Clement E Blanchet, Alessandro Spilotros, Frank Schwemmer, Melissa A Graewert, Alexey Kikhney, Cy M Jeffries, Daniel Franke, Daniel Mark, Roland Zengerle, Florent Cipriani, Stefan Fiedler, Manfred Roessle, Dmitri I Svergun; 2015; Journal of applied crystallography.
3. Potential for biomolecular imaging with femtosecond X-ray pulses; Nature 406, 752-757 (17 August 2000) Richard Neutze et.al.

Tuesday, June 27, Session IV

L12

DYNAMIC LIGHT SCATTERING, DLS, TO ANALYSE AND SCORE PROTEIN SOLUTIONS

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Dynamic Laser Light Scattering (DLS) is today a well established method to characterize bio-molecular solutions by analysing the dispersity of the suspension and as also recently reviewed by Minton [2016] DLS is the most powerful, highly adaptable and a widely used method to analyse the size distribution of various kinds of particles in solution, till now mostly measuring in cuvettes. Fields of application include size determination and quantification of macromolecules, viscosity determination of blood [Popov and Vitkin, 2016], optimizing solubility and homogeneity of biological samples, analysing dimensions and symmetry of particles [Schubert et al., 2015; Maes et al., 2015; Passow et al., 2015], determining the density of bacterial cultures [Loske et al., 2014], verification of pharmaceutical formulations [Fávero-Retto et al., 2013] support of three-dimensional *in vivo* imaging, time-resolved analysis of protein assembly or enzyme-catalysed reactions via monitoring changes of the particle size distribution [Georgieva et al., 2004; Yang et al., 2015; Liu and Falke et al., 2016] and monitoring different stages of crystallization reactions [Meyer et al., 2015; Schubert et al., 2017]. DLS is non-invasive and non-destructive and can be adapted to perform measurements *in situ* in a variety of sample containers, including very thin capillaries to monitor for example counter diffusion crystallization experiments [Oberthür et al., 2012]. In principle, the intensity fluctuations of coherent laser light scattered by particles in solution are recorded over time at a specified angle, correlated with itself after short time intervals and visualized as an intensity auto-correlation function (ACF) [Chu, 1970]. These intensity fluctuations, caused by Brownian motion of particles, are evaluated by algorithms such as CONTIN [Provencher, 1982] and allow to determine the diffusion coefficient of the particles in solution. Considering the viscosity and temperature, the Stokes-Einstein equation is used to calculate the hydrodynamic radius (R_H). DLS measurements were successfully used to analyse sample solutions in flow to analyse different stages of protein folding by Gast et al., in 1997 and a particular fiber optic DLS probe was applied by Leung et al. [2006] to characterize latex particles in flow, pointing at a variety of potential industrial applications to count and determine the size of particles for quality control. The application of DLS in a shear flow and in a microfluidic channel was mathematically described by Destremaut et al. [2009], taking the channel dimensions, shear rates, velocity profile of a Poiseuille flow and interferences of different Doppler shifts into account. The resulting theoretical approximation of an ACF with some geometrical restrains underlined that below a critical flow rate the ACF is dominated by Brownian motion of the scattering molecules. In summary, DLS techniques allow to verify the stability and homogeneity of samples in a very time-efficient way and are highly sensitive towards large (unspecific) aggregates of biological macromolecules. This qualifies DLS to be an excellent method for sample quality verification prior or during SAXS experiments. Details and examples will be presented.

Destremaut, F., Salmon, J.-B., Qi, L., & Chapel, J.-P. (2009). *Lab on a Chip*, **9**, 3289.

Fávero-Retto, M. P., Palmieri, L. C., Souza, T. A. C. B., Almeida, F. C. L., & Lima, L. M. T. R. (2013). *European Journal of Pharmaceutics and Biopharmaceutics*, **85**, 1112–1121.

Gast, K., Nöppert, A., Müller-Frohne, M., Zirwer, D., & Damaschun, G. (1997). *European Biophysics Journal*, **25**, 211–219.