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.

Georgieva, D., Koker, M., Redecke, L., Perbandt, M., Clos, J., Bredehorst, R., … Betzel, C. (2004). *Biochemical and Biophysical Research Communications*, **323**, 1278–1286.

Liu, J., Falke, S., Drobot, B., Oberthuer, D., Kikhney, A., Guenther, T., … Raff, J. (2017). *European Biophysics Journal*, **46**, 77–89.

Leung, A. B., Suh, K. I., & Ansari, R. R. (2006). Applied Optics, **45**, 2186–2190.

Loske, A. M., Tello, E. M., Vargas, S., & Rodriguez, R. (2014). *Archives of Microbiology*, **196**, 557–563.

Maes, D., Vorontsova, M. A., Potenza, M. A. C., Sanvito, T., Sleutel, M., Giglio, M., & Vekilov, P. G. (2015). *Acta Crystallographica. Section F, Structural Biology Communications*, **71**, 815–822.

Meyer, A., Dierks, K., Hussein, R., Brillet, K., Brognaro, H., & Betzel, C. (2015). *Acta Crystallographica Section F Structural Biology Communications*, **71**, 75–81.

Minton, A. P. (2016). *Analytical Biochemistry*, **501**, 4–22.

Oberthuer, D., Melero-García, E., Dierks, K., Meyer, A., Betzel, C., Garcia-Caballero, A., & Gavira, J. A. (2012). *PLoS ONE*, **7**, e33545.

Passow, C., Hagen, B. ten, Löwen, H., & Wagner, J. (2015). *The Journal of Chemical Physics*, **143**, 44903.

Popov, I., & Vitkin, A. (2016). *Journal of Biomedical Optics*, **21**, 17002.

Provencher, S. W. (1982). *Computer Physics Communications*, **27**, 229–242.

Schubert, R., Meyer, A., Baitan, D., Dierks, K., Perbandt, M., & Betzel, C. (2017). *Crystal Growth & Design*. 17: 954-958.

Yang, Y., Heo, P., Kong, B., Park, J.-B., Jung, Y.-H., Shin, J., … Kweon, D.-H. (2015). *Biochemical and Biophysical Research Communications*, **465**(4), 864–870.

L13

ADVANCED *IN SITU* LIGHT SCATTERING APPLICATIONS

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Investigation of protein three dimensional structures based on recent methods, such as nuclear magnetic resonance spectrometry, (NMR), small-angle X-ray scattering (SAXS), cryo-electron microscopy (cryo-EM) and X-ray crystallography, relies on the absence of protein aggregates. Therefore sample preparation plays a crucial role for the successful application of all these methods. Since each step of sample preparation implies the risk to create aggregates, verification of the aggregation state after each step enables fast identification of weak points and contributes significantly to optimize sample preparation protocols. Such an analysis tool is dynamic light scattering (DLS) which has already many applications (Berne & Pecora, 1976). Key features of DLS analysis tools is a minimal sample volume, ease of use and usefulness of output. DLS applied *in situ* fulfills these requirements.

In this lecture *in situ* DLS measurements on sample aliquots of ~1 µl in multiwell plates (hanging drop, sitting drop or under oil) will be demonstrated, using a fully automated instrument. In order to demonstrate the capabilities of this device, model protein radius distributions will be measured. Measurements will be applied on aliquots of protein solutions as examples of intermediates from the daily lab work taken from purification steps e.g. concentration increasing. Another application is the analysis of crystallizing samples, before and after precipitant addition. Signal interpretation allows determination of aggregation or nucleation indicating the position in the phase diagram (Vekilov, 2010). Determination of micelle sizes is also easily feasible. This may be used to identify protein detergent complexes after addition of membrane proteins. In addition, a built-in camera allows to observe all wells, this means the instrument can be used as an imaging system as well (Meyer 2014). In combination with a UV light source it is even possible to distinguish protein from salt crystals by using the intrinsic fluorescence of proteins containing tryptophan.

1. Berne, B. J. & Pecora, R. (1976). *Dynamic Light Scattering* New York: John Wiley & Sons Inc.
2. Meyer, A., Dierks, K., Hussein, R., Brillet, K., Brognaro, H., Betzel, Ch. (2014). Acta Cryst. F71, 75-81.
3. Vekilov, P. G. (2010). *Nanoscale 2*, 2346-2357.

L14

HIGH-RESOLUTION PROTEIN STABILITY ANALYTICS USING NANODSF

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The fluorescence of tryptophans in a protein is strongly dependent on its close surroundings. By following changes in fluorescence, chemical and thermal stability can be assessed in a truly label-free fashion. The dual-UV technology by NanoTemper allows for rapid fluorescence detection, providing an unmatched scanning speed and data point density. This yields ultra-high resolution unfolding curves which allow for detection of even minute unfolding signals. Furthermore, since no secondary reporter fluorophores are required, protein solutions can be analyzed independent of buffer compositions, and over a concentration range of 150 mg/ml down to 5 ěg/ml. In addition, information on protein aggregation can be recorded in parallel, providing insight into colloidal stability of the sample. Therefore, nanoDSF is the method of choice for easy, rapid and accurate analysis of protein folding and stability, with applications in membrane protein research, protein engineering, formulation development and quality control. The presentation will cover biophysical concepts of the technique showing benefits of the nanoDSF technology platform, and will be followed by specific examples of nanoDSF applications towards various experimental systems.

Tuesday, June 27, Session V

PL2

INTEGRATED STRUCTURAL BIOLOGY: GENERAL CONCEPTS AND CASE STUDIES

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L15

AN INTEGRATIVE STRUCTURAL APPROACH TO RNA:PROTEIN INTERACTIONS

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The detailed characterization of biomolecular interactions requires the combination of different biophysical methods. The concept of integrative structural biology will be illustrated by the structural and functional study of ubiquitous RNase P enzymes, which catalyze the 5' maturation of pre-tRNAs. For a long time it has been thought that all RNase P were ribozymes. However, a novel kind of RNase P composed of proteins only, called PRORP for "Protein-only RNase P" was first discovered in human mitochondria, then described in Arabidopsis thaliana (1,2). The latter possesses three PRORP homologs: PRORP1 located in mitochondria and chloroplasts, PRORP2 and PRORP3 in the nucleus.

We developed an integrative approach in order to gain an insight into Arabidopsis PRORP enzymes and their mode of action (3). The affinity constant between a minimal tRNA substrate and a catalytically inactive PRORP2 enzyme was first determined by microscale thermophoresis (MST), ultracentrifugation and calorimetry (ITC), and shown to be in the micromolar range. A combination of mutagenesis and affinity measurements helped define the respective importance of individual pentatricopeptide repeats (PPR) of PRORP2 for RNA binding. A comparison of the crystal structure of PRORP2 and of solution structures of the enzyme and its complex with a pre-tRNA obtained by small angle X-ray scattering (SAXS) indicated that PRORP2 undergoes structural changes to accommodate its substrate. A dedicated SAXS setup was implemented to stabilize the complex during analysis. Altogether this study reveals the structural diversity and plasticity of protein-only RNase P enzymes.