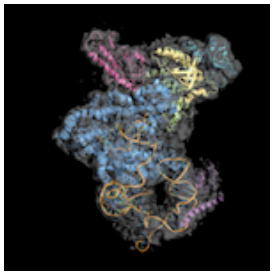

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Keynote 3 - Advanced Methods to prepare, detect and score nano-sized Crystals & towards a better Understanding of the Nucleation-Process

Monday 19 November 2018 17:05 (40 minutes)

At modern micro-beam synchrotron (SR) and Free-Electron-Laser (FEL) beamlines micro-sized crystals are preferred and mandatory for serial diffraction data collection. Therefore, new, advanced and reliable methods to prepare, detect and score 3D micro- and nano-sized crystal suspensions, most suitable for X-ray diffraction experiments need to be established in time. Further, a better understanding of the nucleation process is of fundamental importance to grow crystals of desired dimensions.
Experimental data propose that during crystallization biomolecules pass through a nucleation intermediate. However, till now the nucleation process is discussed in theory and experiment differently (1), and till now no clear and unambiguous information is available. In order to obtain more insights about the process and to obtain supporting evidence for the tow-step nucleation mechanism (2) and theory we investigated the nucleation process and early crystallization events for various proteins, applying complementary biophysical methods (3,4,5).
We particular applied in situ dynamic light scattering (6) , small-angle X-ray scattering and transmission electron microscopy experiments. The data we will present strongly support the existence of a two-step mechanism of nucleation. However, the early process is governed by the formation of liquid dense clusters as initial step, followed by the transition to higher order assemblies inside these clusters (7). After crystal nuclei have formed they continue to grow in size. The desired size for SFX experiments is preferably in the upper nanometer or lower micrometer regime. This guides to a strong demand to develop and establish new methods to analyze, score and optimize protein nano- and micro-crystal suspensions for serial crystallography. To support and facilitate this demand we recently designed and constructed a particular microscope based setup, based on detecting second harmonic generation (SHG) signals of crystalline particles in sample suspensions. This method and setup enhances the already available signal sensitivity to such extend that detection of relative small crystals and crystals with higher symmetry, known to produce rather weak signals, is now possible and distinguishing between amorphous and crystalline particles is possible.
Further, the instrument is equipped with additional channels, which are capable to detect the third harmonic generation signal as well, and the signal of three-photon excited UV-fluorescence.
All in parallel provide most and complementary information(s) about the crystalline state of the sample suspension. Details and experimental data will be presented.

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