MS2 Development of new types of sample preparation (both XFEL & synchrotrons)

Chairs: Jörg Standfuss, Gwyndaf Evans

MS2-O1 Enabling time-resolved structural studies of biological macromolecules

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In order to fully understand the mechanisms of biological processes, time-resolved methodologies that allow us to comprehend how function is linked to changes in molecular structure are required. Time-resolved X-ray crystallography provides a means of directly visualising structural rearrangements associated with function. Although time-resolved crystallography is a powerful tool it has not been widely applied to biomacromolecules, due to both the lack of beamlines where such experiments can be done as well as to the challenge of fast and uniform reaction initiation. To address these challenges we have developed a new multiplexing data collection method, based on the Hadamard transform, to make sub millisecond time-resolved data collection possible at standard macromolecular crystallography beamlines. We are also developing a suite of photochemical and rapid mixing tools for reaction initiation.

Keywords: time-resolved crystallography, rapid mixing, photocaging

MS2-O2 Structure determination of a membrane protein with data collected from micro-crystals in lipidic cubic phase at room temperature in low background CrystalDirectTM crystallization plates.

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Crystallization using the lipidic cubic phase (LCP) methodology in many cases is a decisive step towards obtaining high-resolution structural information for membrane proteins. However, harvesting crystals from LCP setups is notoriously difficult and frequently results in damaged or destroyed crystals. Furthermore, cryo-protection can be difficult, and data collection at cryogenic temperatures is often hampered by the LCP-matrix becoming opaque upon flash-cooling preventing optical crystal centering.

To overcome the above problems, we have grown crystals of a membrane transporter protein, $PepT_{st}$ (491 residues; Lyons et al. 2014), in CrystalDirect™ plates and collected *in situ* diffraction data at room temperature on the EMBL beamline P14 at PETRA III (DESY, Hamburg). The 5 µm micro-focus beam of P14 in combination with the low X-ray background of the CrystalDirect™ plates allowed to acquire diffraction data to 2.5 Å resolution from crystals with typical dimensions of 10 µm and embedded in LCP. Employing the serial helical scan data collection strategy (Gati, Bourenkov et al. 2014) as implemented in the high-precision MD3 diffractometer (ARINAX, Grenoble, France), 66 serial helical scans were performed on 19 crystallization wells delivering a total of 36013 frames containing more than 2 million reflections in 2 hours of beamtime.

The reflections were integrated, scaled, and merged into a 98% complete data set (space group $C222_1$) containing 23105 reflections to 2.5 Å. Structure solution by molecular replacement and refinement using standard methods delivered high-quality electron density maps and a high-quality model (R_{work}/R_{free} = 0.219/0.235) for the crystal structure of $PepT_{st}$ at room temperature.

Gati, Bourenkov et al. (2014)Lyons et al. (2014) EMBO Rep. 15(8):886-93