MS1-P10 Application of on-chip room-temperature protein crystallography to visualize the dynamics of structural changes

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The visualization of conformational changes at high spatial and temporal resolution still remains an experimental challenge in structural biology. The time dimension is incorporated by triggering the reaction of interest in the crystal prior to X-ray exposure, and then collecting the diffraction patterns at different time delays. Serial crystallography is a powerful approach to collect complete diffraction data sets by measuring single diffraction pattern from single non-frozen crystals and a breakthrough for Time-resolved X-ray crystallography (TRX) experiments. However, very high crystal quantities are needed. Recent advances in diffraction compatible microfluidic systems offer new opportunities to position single microcrystals into the beam. Therefore only several hundreds of crystals are required to collect complete datasets. A semi-transparent microfluidic chip has been developed to conduct *in situ* pump-probe X-ray diffraction to experiments at time scales between 50 milliseconds to 1 second. We are applying this approach using a highly promising drug target candidate Thioredoxin from *Wuchereria bancrofti* (*Wb*Trx-1). The parasite *Wuchereria bancrofti* causes human lymphatic filariasis, a disease that affects approximately 130 million people worldwide. *Wb*Trx-1 has a molecular weight of 16 kDa, contains one disulphide bridge and acts as an antioxidant by ensuring the supply of reducing equivalents for many biological functions via thiol-disulphide exchange cycle. In a first step the time resolved S-S bond cleavage of *Wb*Trx-1 induced by UV radiation will be studied. This approach may be also convenient for solving the phase problem using Radiation Damage Induced Phasing (RIP) method. Details will be presented.

Keywords: Time-resolved X-ray crystallography (TRX), reaction kinetics, Radiation Damage Induced Phasing (RIP)

MS1-P11 The analysis of the mesh scan data for macromolecular data collection

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Developments in microfocus X-ray beamlines and fast shutterless data acquisition with a pixel-array detectors have enabled diffraction data collection from crystals exhibiting strong variation in diffraction quality, as well as from multiple very small crystals mounted in one sample holder. One of the core features of the protocol of such complex diffraction experiments is the ability to automatically recognise and rank protein diffraction on images collected during the low-dose 2D mesh scans. We will present novel methods and software programs which can score the quality of diffraction and identify positions and shapes of individual single crystals. Using a complex statistical analysis the program compares diffraction patterns measured at different spots of the mesh and detects whether they correspond to one or different crystal lattice, or to an overlap of multiple crystal lattices. Further, this information together with the estimates of diffraction quality is used by the program BEST[1] to design the optimal protocol of multi-crystal/multi-position data collection, considering radiation damage effects. The program can be also useful in the evaluation of synchrotron serial crystallography data[2]. The details of algorithm and results of the first test applications will be discussed.

References:

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