MS3-O5 Experimental phasing with serial crystallography at XFEL and synchrotron radiation

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X-ray crystallography is a powerful method to determine biomacromolecular structures at atomic resolution. However, there are still many challenging targets like membrane proteins, which are often reluctant to produce large well diffracting crystals. Serial crystallography is an emerging method for microcrystals. It has been first established at XFEL and known as serial femtosecond crystallography (SFX). After the success of SFX at XFEL, serial crystallography was imported to synchrotron radiations (SR). Fast read-out detectors and high-flux microbeam beamlines have made synchrotron serial crystallography (SSX) practical option.

Phase problem has been the critical problem in crystallography. While nowadays most structures are solved by the molecular replacement, there is still much demand for *de novo* structure determinations. SAD, the most popular experimental phasing method, utilizes the small anomalous difference (< ~5%) and therefore highly accurate data is essential. Barends *et al.* [(2014) *Nature*, **505**, 244-247] proved SAD method worked with SFX, but it required an enormous number of diffraction patterns. An efficient *de novo* phasing method is highly demanding.

Here we present the successful demonstration of experimental phasing with serial crystallography. We used microcrystals of mercury-bound luciferin-regenerating enzyme (LRE), a soluble protein, as a first sample. SFX datasets were collected at BL3, SACLA and processed using CrystFEL. SSX datasets were collected by raster-scanning of cryoloops (similar protocol to Gati *et al.* [(2014), *IUCrJ*, **1**, 87-94]) at BL41XU, SPring-8 and processed using CrystFEL or XDS as random snapshots. At SR, crystals can be rotated during X-ray exposure, which is potential advantage over XFELs. We studied the effect of crystal rotations and larger rotations per image to a certain degree resulted in higher anomalous signal and better statistics with the same number of images. In both SFX and SSX cases, the selection of diffraction images based on the data processing statistics was proven to be effective. We will discuss the data processing method using SAD-ability or anomalous signal as a data quality indicator.

Keywords: serial crystallography, SFX, SAD, phasing, data processing

MS4 New developments in phasing and refinement

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MS4-O1 Refinement without a model

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Refinement is a problem of optimising the parameters of a model against observed data. When the data are limited in number (for example due to low resolution) or of low quality, the refinement becomes poorly determined, which manifests itself in weak convergence and a multiplicity of local minima.

One way to address the problem is through more parsimonious parameterisations of the model. We explore the use of control points to represent the positions and thermal motion of whole groups of atoms within the structure. This approach has significant potential for refining a homologous structure into low resolution electron density (for example from electron cryomicroscopy). It also has potential for increasing the radius of convergence of molecular replacement, and for providing an alternative characterisation of the domain motions, currently represented by TLS parameters or the generation of ensembles.

Keywords: refinement, model free, control points