**L-03** **Monday, 13.06., $17^{00}$ - $17^{40}$**

## Long-wavelength macromolecular crystallography – MAD or SAD?

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Over the past years technical developments such as single-photon counting detectors and improved stability from synchrotron sources and Beamline equipment have led to an increased number of protein and nucleic acid structures being solved by experimental phasing techniques at longer wavelengths around λ = 2 Å [1]. Single wavelength anomalous diffraction (SAD) utilizes therein the increase of the anomalous signal from sulphur or phosphorus towards their K absorption edges which are around 5 Å and 6 Å, respectively. Solving the crystallographic phase problem directly in the absence of a know protein model similar to the one under investigation and without additional labelling of the protein or nucleic acid has the potential to become the method of choice for phasing macromolecular crystals.

At Diamond Light Source, over the past years, the long-wavelength MX Beamline I23 [2] has been designed, constructed and is currently being commissioned. The Beamline differs radically from the existing well developed and established MX beamlines. To eliminate air absorption, the complete Beamline is operated in vacuum, including the sample environment and the detector.

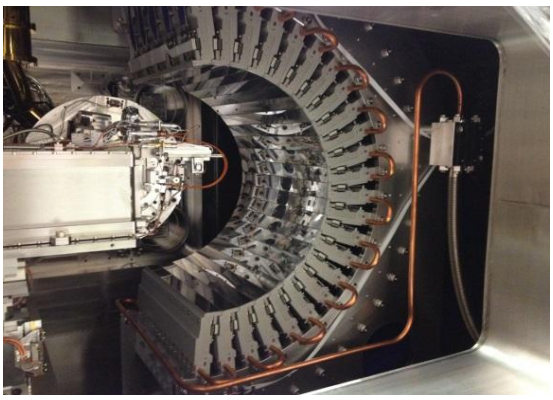

*Figure 1*: Pilatus 12M detector in I23 vacuum vessel.

Several technical issues had to be addressed, leading to a variety of pioneering new developments, like the large in-vacuum semi-cylindrical Pilatus 12M detector (Fig.1) and the dedicated kappa goniometer. The Beamline covers a wavelength range from 1.1 to 5.9 Å (2.1 – 11.5 keV) which allows accessing several K absorption edges of biological relevance like phosphorus, sulphur, chlorine, potassium and calcium, elusive on other MX beamlines. Apart from experimental phasing experiments, anomalous contrast can be used to identify and distinguish these light atoms in the electron density and use their positions to help model building at low resolution.

First data has been successfully collected and several structures have been solved using SAD phasing based on phasing information from phosphorus, sulphur and calcium. At the moment the data quality at wavelengths larger than 3.1 Å is affected by the increased sample absorption and most of the experiments have been performed around this wavelength with an anomalous signal approximately twice as high as at 2 Å. Significant amount of work will be needed to correct for the absorption effects to obtain data quality of similar quality as obtained at shorter wavelengths.

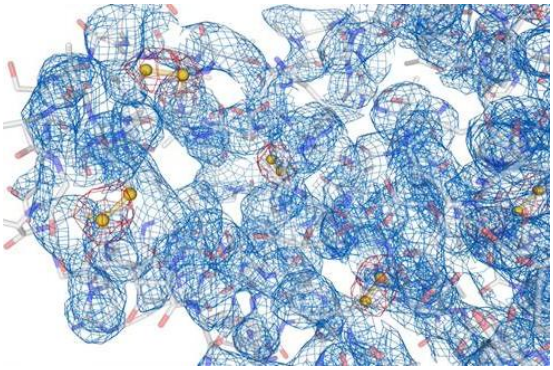

*Figure 2:* Electron density map (blue $2F_o$-$F_c$, 1 sigma) and anomalous difference Fourier map (red, 5 sigma). Thaumatin model (PDB code 4ZG3) superimposed.

Nevertheless, first data around the sulphur K-edge from the test protein thaumatin could be collected and processed with subsequent successful structure determination based on anomalous differences. Figure 2 shows the electron density map after experimental phasing from data collected at a wavelength of 4.96 Å (E = 2.5 keV) at a maximum resolution of 3.2 Å, limited by the size of the detector.

The presentation will focus on the underlying design ideas of the new long-wavelength in-vacuum Beamline, discuss first results and give an outlook on potential MAD experiments around the edges of sulphur and phosphorus.

[1] J. Rose, B.-C. Wang, M. Weiss, *IUCrJ* **2** (2015) 431.
[2] A. Wagner, R. Duman, K. Henderson. V. Mykhaylyk, *Acta Cryst.* D**72** (2016) 430.