In-vacuum long-wavelength macromolecular crystallography – Adding new colours to the crystallographer's palette

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Solving the crystallographic phase problem by single wavelength anomalous diffraction (SAD) directly in the absence of a known 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. This technique, also referred to as native phasing, utilizes the increase of the anomalous signal from sulfur or phosphorus towards their K absorption edges which are around 5 Å and 6 Å , respectively. 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].

At Diamond Light Source, over the past years, the long-wavelength MX beamline I23 [2] has been designed and constructed and has recently started "friendly" user operation. 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. 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, the dedicated kappa goniometer, new conductive sample mounts and an air-lock system to transfer cryogenically cooled crystals into the large vacuum end station.

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, sulfur, 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.

Several structures have been solved using SAD phasing based on phasing information from phosphorus, sulfur, potassium, calcium, vanadium and other elements [3,4] and experiments making use of the unique in-vacuum setup for X-ray crystallography (e.g. absolute structure determination from small molecules, diffuse scattering) are currently in preparation. An overview on the project, the technical challenges and how they could be overcome and results from this novel instrument will be presented.

References

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