Long-wavelength crystallography – Exploiting anomalous dispersion from light elements

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Macromolecular crystallography is a very well established method in structural biology, responsible for the majority of structures in the Protein Data Base (PDB). Most structures nowadays are determined using data from synchrotron beamlines. These beamlines typically operate in a wavelength range from 0.8 to 2 Å.

Anomalous dispersion is an effect which leads to intensity differences between reflections hkl and –h-k-l, so called Friedel pairs. This signal is typically small, but increases towards absorption edges of elements present in the crystal structure. The anomalous signal can be exploited to solve the crystallographic phase problem either by SAD (single-wavelength anomalous diffraction) or MAD (multi-wavelength anomalous diffraction) techniques. The most commonly used element utilised for this purpose is selenium with an absorption edge at a wavelength of around 0.98 Å. It is based on substituting the amino acid methionine with seleno-methionine. This works very well for bacterial expression systems, but can become laborious, expensive or even not feasible for expression systems from higher organisms. An alternative is using the intrinsic anomalous signal from sulphur present in the two amino acids cysteine and methionine. With the absorption edge of sulphur at a wavelength of 5.02 Å, however, these so-called native SAD experiments are very demanding, as for longer wavelengths the diffraction angles are increasing as is the absorption from the sample, sample mounts and even air. At the moment only two dedicated beamlines to allow operation at wavelengths longer than 3 Å exist, BL-1A at Photon Factory and I23 at Diamond Light Source.

Long-wavelength X-rays are not only useful for native phasing experiments. With the absorption edges of Ca, K, Cl, S and P becoming accessible, anomalous contrast can be utilised to either assist model building at low resolution or identify these light elements unambiguously.

I will present the latest status of the I23 beamline at Diamond, discuss the basic ideas of native phasing and anomalous contrast, discuss the challenges arising when operating at wavelengths beyond 3 Å and show recent results from experiments covering a range of wavelengths all the way to the phosphorous edge at 5.78 Å.