

Challenges and strategies for n-SAD phasing at longer X-ray wavelength

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Single-wavelength Anomalous Dispersion (SAD) is the most popular experimental phasing technique to determine X-ray structure in the field of structural biology. The data collection, in this method, is performed at the absorption edge of anomalous scatterers, which are either introduced in the crystal or natively present in the macromolecules. In case of native SAD phasing, which uses the weak anomalous scattering signals from light elements – such as sulfur, phosphorous, or any ions, naturally present in the macromolecules, the most suitable energy would be around 2.5 keV (or $\lambda = 5 \text{ \AA}$), above the sulfur K-edge. However, such low X-ray energy is not attainable at most of the current operational macromolecular crystallography beamlines. In addition, native SAD at such low energy comes with more challenges, caused by x-ray absorption due to crystal thickness, cryo-loop, solvent around the crystal, air, as well as detector efficiency. Thereby, an X-ray energy around 6 keV is considered as a good “compromise” between anomalous diffraction signal and absorption effect [1, 2, 3]. Here, we present the promises and challenges associated with native-SAD data collection at X-ray energy below 6 keV, in particular for X-ray absorption effects and optimum crystal size, using both test proteins and real-life examples.

[1] Liu et al. (2012) Science, 336, 1033-1037.

[2] Olieric et al. (2015) Acta Cryst, D72, 421-429.

[3] Weinert et al. (2015) Nat. Methods, 12, 131-133.

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