Optimization of macromolecular anomalous diffraction analyses

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Analyses from multi- and single-wavelength anomalous diffraction (MAD and SAD) experiments have produced more than 80% of all *de novo* macromolecular crystal structures obtained so far in the 21st century, and SAD alone accounted for more than 70% of those reported in 2013 [1]. Nevertheless, at least until recently, there has been poor representation from challenging SAD analyses with weak signals and/or noisy data, such as pertain particularly when at low-resolution (d_{min} > 3.5 Å) or for only-light-atom (Z ≤ 20) native molecules [2]. Conventional MAD experiments and many SAD studies are performed at resonance, where anomalous scattering effects can be maximized; however, the sharpness of typical resonant features challenges the energy resolution delivered by many synchrotron monochromators, leading to sub-optimal experiments. We are striving to improve instrumentation and experimental designs for optimized on-resonance SAD/MAD experiments and for optimized native SAD experiments, necessarily only approaching resonance. Several light elements (Z = 15-20; P, S, Cl, K, Ca) that are commonly found in biological structures can be effective scatterers for SAD phasing when low energy (long wavelength) x-rays are used. Anomalous diffraction signals from light elements increase as the x-ray energy is lowered; however, adverse effects from x-ray absorption, scattering-angle expansion and radiation damage also increase, and these effects complicate the optimization. Using microcrystals helps to address absorption, and the consequent impact of radiation damage can be mitigated by combining data from multiple crystals [3]. Lowered x-ray energy also facilitates elemental identification for light elements (Ca, K, Cl, S, P, Mg and Na) through f″ scattering factor refinements [4]. We have suggested that native SAD analyses could rival the present-day dominating successes from Se K-edge analyses of selenomethionyl proteins [5]. New beamlines will enhance this prospect and provide other benefits for anomalous diffraction analyses. At NSLS-II, we have developed the NYX microdiffraction beamline for resonance experiments at energy resolution $\Delta E/E = 7 \times 10^{-5}$ and for off-resonance experiments down to 5 keV (currently 6.5 keV).

References

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