Keynote Lectures

[KN8] Multi-crystal native SAD analysis of macromolecular structure.

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Structure determinations for biological macromolecules that have no known structural antecedents typically involve the incorporation of heavier atoms than those found natively in biological molecules. Currently, selenomethionyl proteins analyzed from single-or multi-wavelength anomalous diffraction (SAD or MAD) data predominate for such de novo analyses. Naturally occurring metal ions such as zinc or iron do often suffice in MAD or SAD experiments, and sulfur SAD has been an option since first demonstrated with crambin 30 years ago; however, SAD analyses of only-light-atom (Zmax ≤ 20) structures have not been common. We have now devised robust procedures for enhancing the signal-to-noise in measurements of anomalous diffraction by combining data collected from several crystals at a lower than usual x-ray energy. Tests were devised to assure statistical equivalence of all accepted crystals. In initial studies at 7 keV, we solved five native SAD structures using this multi-crystal approach; later applications at 6 keV have produced three additional novel structures. These native SAD structures had up to 52 anomalous scatterers and 1200 ordered residues per asymmetric unit and they were determined at resolutions from 2.3 Å to 3.2 Å. Besides the expected protein sulfur atoms, we typically found additional light anomalous scatterers, which we identified as Ca, Cl, S, P or Mg by f″ scattering-factor refinements. We suggest that multi-crystal SAD analyses can provide truly routine structure determinations for generic native macromolecules. Synchrotron beamlines optimized for low-energy x-ray diffraction measurements will facilitate such direct structural analysis.

Keywords: anomalous diffraction; multiple crystals; sulfur SAD