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Native-SAD Phasing at Synchrotrons: A Low-dose and High-redundancy Strategy

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Native biological macromolecules contain intrinsic light elements such as sulfur in proteins and phosphorus in nucleic acids. Native-SAD phasing utilizes the anomalous signals from light elements for de novo structure determination: first the substructure of anomalous scatterers is determined; phase evaluation for the entire structure then follows. Synchrotron beamlines are expected to be ideal instruments for native-SAD phasing due to their brilliant and energy-tunable x-rays. However, anomalous signals from light elements are typically very weak at x-ray energies accessible to most synchrotron beamlines. Efforts have been made to promote the utility of synchrotrons for routine native-SAD phasing with no requirement for heavy-atom incorporation. Our strategy is to limit the x-ray dose per crystal and to enhance the signal-to-noise ratio by increasing data redundancy through use of multiple crystals. We have devised a robust procedure and applied it for routine native-SAD analyses on real-life membrane proteins, protein-protein complexes, and recalcitrant proteins. Here we use these real-life case studies to illustrate our procedures in sample preparation, x-ray energy selection, data collection, data analysis and phasing.

Keywords: native SAD, synchrotron, redundancy