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Advancing Native-SAD Phasing at Synchrotron with 13 Real-life Case Studies

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The key step in elucidating de novo 3D X-ray structures relies on the incorporation of heavy elements into proteins or crystals. Selenomethionine incorporation or heavy metal derivatization are however not always possible and require additional efforts. Exploiting anomalous signals from intrinsically present elements like S, P, and Ca2+ from proteins and nucleic acids, as well as Cl-, SO42-, and PO42- from crystallization solutions, is therefore an appealing alternative. Such a method has been shown to be valid by collecting data from several crystals and combining them(1). Recent developments at macromolecular crystallography beamlines are however pushing the limits of what could be obtained out of a single crystal. Here we introduce a novel data collection routine for native-SAD phasing, which distributes tolerable X-ray life-doses to very high multiplicity X-ray diffraction data sets measured at 6 keV energy and at different crystal orientations on a single crystal. This allows the extraction of weak anomalous signals reliably by reducing both systematic and random measurement errors. The data collection method has been applied successfully to thirteen real-life examples including membrane proteins, a protein/DNA complex, and a large protein complex. In addition to de novo structure determination, we advocate such a data collection protocol for molecular replacement solvable structures where unbiased phase information is crucial in objective map interpretation and model building, especially for medium and low-resolution cases.

[1] *Liu, Q., Dahmane, T., Zhang, et al., … Hendrickson, W. A. (2012). Structures from anomalous diffraction of native biological macromolecules. Science (New York, N.Y.), 336(6084), 1033–7. doi:10.1126/science.1218753*

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