Sulfur-SAD at 15000 eV? Optimize Your Phasing Experiment in Time and Space

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Successful Sulfur SAD experiments are difficult due to a very small anomalous signal. Bijvoet ratio for tetragonal lysozyme at 7112 eV (Fe edge) is only 1.02% (10 Sulfurs, 7 Chlorides). That ratio drops down to only 0.46% at 15000 eV. In reality it is even lower due to partial occupancies for some of the Chloride atoms. To test the effect of different data collection timing protocols, sulfur-SAD experiments were performed at 15000 eV at SSRL's BL11-1, equipped with Pilatus 6M PAD detector. The time domain was explored as the dose was kept constant (707 Gy per image) with rotation of 0.1 degree/image and three different attenuations and rotation speeds. Initially a very slow rotation was tried - 100 seconds per 1 degree. Then a fast data collection was tried over the same crystal volume with rotation speed of 5 seconds per 1 degree. Finally a third dataset with an intermediate rotation speed was collected over the same volume with a rotation speed of 15 seconds per 1 degree. All three experiments produced successful S-SAD solution at 15000 eV. The accuracy of the data correlates well with the rotational speed. Slower data collection was better. The dataset that used the slowest rotation speed required about 1/3 of the data redundancy as the fastest dataset. Slowing the data collection while keeping the absorbed dose under 2 MGy, could solve structures with even lower Bijvoet values. While the presented experiments are at high energy, the lessons learned could be applied at the most useful energies for phasing for Sulfur or other elements. Such approach is particularly useful for phasing of radiation sensitive samples in low quantities, which may not survive long enough to achieve the required redundancy under usual data collection protocols. A slower approach is a reasonable compromise between accumulated redundancy required for successful phasing and time required for the experiment. Hardening the sample holder increases the diffraction data accuracy and reproducibility.