

Single-wavelength anomalous dispersion phasing for Serial Millisecond Snapshot Crystallography

S. Botha^{a,b,*}, J. Martin-Garcia^{b,c}, H. Hu^{b,c}, U. Weierstall^{b,c}, M. Fuchs^d, W. Shi^d, B. Andi^d, J. Skinner^d, H. Bernstein^e, P. Fromme^{b,c}, N. Zatsepin^{a,b,*}

^a Department of physics, Arizona State University, Tempe, AZ 85287, USA

^b Biodesign Center for Applied Structural Discovery, Arizona State University, Tempe, AZ 85287, USA

^c School of Molecular Sciences, Arizona State University, Tempe, AZ 85287, USA

^d Energy & Photon Sciences Directorate, Brookhaven National Laboratory, Upton, NY 11973, USA

^e School of Chemistry and Materials Science, Rochester Institute of Technology, Rochester, NY, USA

* Author contacts: sbotha@asu.edu and nzatsepi@asu.edu

Serial femtosecond crystallography (SFX) is an up and coming method for room-temperature protein structure determination [1] and it has been shown that SFX data can be phased *de novo* [2]. This method of data collection has been adapted for synchrotron sources, termed serial millisecond crystallography (SMX) [3, 4, 5]. SMX substantially reduces radiation damage incurred by the individual protein crystals compared to conventional, oscillation data collection approaches, facilitating room-temperature structure solution from micrometer sized crystals at synchrotrons. However, *de novo* phase retrieval remains difficult and is rarely applied to serially-collected SFX and SMX data. Here, we present a systematic study of single-wavelength anomalous dispersion (SAD) for *de novo* phasing of SMX data. This study is aimed at determining optimal experimental parameters for data collection (e.g. energy, exposure, number of patterns), as well as establishing data metrics for successful SAD phasing of serial millisecond snapshot crystallography data.

Using the model system proteinase K, various SMX datasets were collected at the Frontier Microfocusing Macromolecular Crystallography beamline of NSLS-II at the Brookhaven National Laboratory. The anomalous signal strength was investigated along with the SAD phasing potential of the individual datasets. The protein crystals were injected into the X-ray interaction region in a free-standing lipidic cubic phase (LCP) jet for serial snapshot data collection. For native sulphur-SAD phasing, various exposure times and X-ray wavelengths ranging from 7.11 keV down to 5 keV were used. Furthermore, other anomalous scatterers, such as mercury, were also introduced into the crystals and datasets collected below, at and above the absorption edge.

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