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Sulfur-SAD phasing and UV-RIP analysis on a single Cysteine bridge protein

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The advantage of using the anomalous signal of sulfur for phase determination is that only a single, well-diffracting crystal is needed and that a native structure will be obtained. Using long-wavelength S-SAD to a resolution of 1.9 Å we have determined the novel structure of an 89 residue protein with only 2 Cysteines fixed in a disulfide bridge. To the best of our knowledge, the Bijvoet ratio in our example is one of the smallest for which a successful structure solution by S-SAD has been reported. Data were collected on 3 different volumes of a single crystal at beamline 14.1. at BESSY II, Berlin [1], at a wavelength of 1.8 Å. At this wavelength the maximum resolution obtainable was 1.9 Å. The data were processed in space group I222 with a low resolution R-factor of 3.2% and a multiplicity of 17. Based on an anomalous correlation coefficient cut off at 30% the signal extends to 2.6 Å. The sulfur substructure was determined using AutoSol/HYSS [2] showing a total of four clear sulfur positions in the asymmetric unit with a resulting FOM of 0.27 and a BAYES Coefficient of 0.36. The crystal has a solvent content of 62% and the structure reveals a dimer and large solvent channels. Density modification lead to well-defined electron density maps for the protein and associated solvent molecules. This example demonstrates that S-SAD phase determination can work with as little as one S-atom per 45 amino acid residues. Additionally, we performed a UV-RIP (ultraviolet radiation damage-induced phasing) experiment in which a dataset was collected before and after irradiating the crystal with a hard UV laser. An isomorphous difference map shows the clear disruption of both disulfide bridges and we are currently working on combined phasing using both anomalous and isomorphous differences based on the S-SAD and UV-RIP data.

[1] *Mueller U., Darowski N., Fuchs M. R., et al. (2012): Facilities for Macromolecular Crystallography at the Helmholtz-Zentrum Berlin. J. Synchr. Rad. 19, 442-449.,* ***[2]*** *Adams, P.D., Afonine P.V., Chen V., et al. (2010): PHENIX – A Comprehensive Python-based System for Macromolecular Structure Solution. Acta Cryst. D66, 213-221.*

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