s1.m3.p14 **Direct Measurement of Structure-Factor Phases on Several Protein Structures.** Andrew A. Stewart and Qun Shen, *Cornell High Energy Synchrotron Source (CHESS), Cornell University, Ithaca, NY, 14853, USA. E-mail: aas52@cornell.edu*

Keywords: Reference Beam Diffraction; Direct Methods; Synchrotron Radiation

We report the measurements of triplet phases on several typical protein crystals using the reference-beam diffraction technique. The technique is based on a simple modification of the standard oscillating-crystal diffraction technique, which allows for the direct experimental measurement of triplet phase interference profiles. The alignment of a Bragg reflection so as it is continuously excited through out the experiment allows the collection of large numbers of triplet-phase interference profiles simultaneously on an area detector. Recent advances include the automation of the data collection procedure, and studies of more typical protein structures such as Thrombin and Thaumatin. We will present the measured interference profiles and discuss future prospects.

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s1.m3.p15 **Away from the edge: SAD phasing from the sulfur anomalous signal measured in-house with chromium radiation.** Richard Tyrrell[a], J. D. Ferrara[a], C. Yang[a], J. W. Pflugrath[a], H. Kawasaki[b], H. Yamazaki[b], A. Yamano[b], [a]*Rigaku/MSC, Inc., 9009 New Trails Drive, The Woodlands, TX 77381,* [b]*Rigaku Corporation, 3-9-12 Matsubara-cho, Akishima-shi TOKYO 196-8666 JAPAN. E-mail: rt@rigakumsc.co.uk*

Keywords: Sulfur SAD phasing; Cr radiation

Anomalous scattering with soft X-ray radiation opens new possibilities in phasing for macromolecular crystallography. Anomalous scattering from sulfur atoms collected on an in-house chromium radiation source (λ = 2.29 Å) was used to phase the X-ray diffraction data from thaumatin (22 kDa), trypsin (24 kDa) and glucose isomerase (44 kDa) crystals. The contribution to the anomalous term, Δf"=1.14 e^-, from sulfur for Cr Kα radiation is doubled compared to that for Cu Kα radiation, Δf"=0.56 e^-. For thaumatin and trypsin, the direct methods programs RANTAN or SHELXD successfully found sulfur positions using data sets with resolution limited to 3.5 Å. The statistical phasing program SHARP was able to produce interpretable electron density maps using the sulfur anomalous signal alone at a low resolution (~3.0 - 3.5 Å). For glucose isomerase, SOLVE/RESOLVE was used to automatically solve and build the structure using 3.0 Å data.

Much less data, that is lower redundancy, is required for this sulfur SAD phasing procedure compared to the highly redundant data reported in the sulfur SAD phasing procedure with Cu Kα radiation [1, 2].

Furthermore, Cr Kα radiation can also improve the strength of the anomalous signal of many other elements in macromolecules, like selenium, calcium, zinc, and phosphorus, because of increased Δf". This experimental study shows using Cr Kα radiation from an in-house rotating anode X-ray generator can provide sufficient phasing power from sulfur anomalous signals for routinely phasing protein diffraction data. In addition, the longer wavelength of Cr Ka radiation can greatly increase the in-house ability to resolve large unit cells and enhance the diffraction power for small crystals. These indicate Cr Kα radiation may be a good alternative to Cu Kα radiation for an in-house source.

We will discuss the solution of the structures of several novel proteins that have proven intractable by the conventional methods of Se-MAD and heavy derivatization using Cr enhanced anomalous scattering.

Finally, we will discuss improvements in instrumentation that provide yield the enhanced anomalous scattering information more quickly than that used in our original work [4].

[1] Yang, C & Pflugrath, J.W. (2001) *Acta Cryst*. D*57*, 1480-1490.
[2] Debreczeni , J. É., Bunkóczi, G., Ma, Q., Blaser, H. & Sheldrick, G. M. (2003) *Acta Cryst*. D**59**, 688-696.
[3] Ramagopal, U.A., Dauter, M., Dauter, Z. (2003) *Acta Cryst*. D**59**, 1020-1027.
[4] Yang, C, Pflugrath, J. W., Courville, D. A., Stence, C. N., & Ferrara, J.D. *Acta Cryst*. (2003). D**59**, 1943-1957.