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From contrast variation to phasing Bragg reflections using anomalous dispersion of phosphorus and sulfur: crystallographic studies of small ribosomal subunit 30S from *Thermus thermophilus* and bovine β-trypsin

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Multiwavelength anomalous dispersion converts sulfur and phosphorus into useful labels in macromolecular crystallography. As the K-edges of these elements are at wavelengths between 5 and 5.8 Å, the technique of x-ray diffraction has to be modified in such a way that it copes with the reduced penetration depth of soft x-rays in any kind of matter. Special attention was given to the sample environment, which had to be cooled and kept transparent for soft x-rays. Larger wavelengths mean also larger scattering angles. These were covered by four area detectors at $-49° < 2\theta < 117°$.
The method was subjected to a test by using single crystals of bovine trypsin. There are 6 cystines and 2 methionines per trypsin molecule. Data collected from frozen crystals (T = -60°C) at 3 wavelengths near the absorption edge of these sulfur atoms were used to phase 1000 unique reflections, the mean phase deviation of phases from the calculated ones being 70°.
Trypsin in a buffer containing 2.5 M ammonium sulfate also offers the possibility of contrast variation at the K-absorption edge of sulfate, 10 eV away from the previous edge. This aspect plays a prominent role in the investigation of 30S subunit from Thermus thermophilus (T30S) at wavelengths near the K-absorption edge of phosphorus [1]. The 1515 phosphorus atoms contrast the 16S rRNA sufficiently. Diffraction data were taken up to a resolution 40 Å at 3 wavelengths while the sample was kept at -115°C. It is interesting to note that the intensities of low order reflections from T30S is comparable to trypsin at 5 Å and also to that taken at 1.7 Å. The analysis of the diffraction data from T30S starts from the model determined by neutron scattering. The experiments were performed at HASYLAB in collaboration with the Max-Planck Research Unit for Ribosomal Structure.

[1] S. Stuhrmann, M. Hütsch, C. Trame, J. Thomas, H.B. Stuhrmann *J. Synchrotron Rad.* **2** 83-86 (1995).