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First soft x-ray cryocrystallography on riboflavin-binding protein (RfBP)

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The cryogenic MAD (multiwavelength anomalous dispersion) studies on the unknown structure of RfBP were done in the range of the sulfur K-absorption edge at 2.47 keV. Some technical improvements, like a focusing glass capillary system, new MWPC components with minimal absorption, and vacuum tight system (2x0.001 mbar) for diffraction measurements on biochemical crystals, were unavoidable to make the experiment at 5Å possible.

The mounting equipment for the very sensitive RfBP crystals played a decisive role by enclosing them in N_2 and He anaerobic surroundings, decreasing the absorption and protecting them simultaneously. The measurement temperature in vacuum was about -40°C.

The 8 disulfide bridges and the phosphoserine cluster with 8 P atoms are the native labels in each molecule on which anomalous scattering has been measured. The wavelength calibration was done with the absorption spectra of lyophilized protein and of single crystals. After application of the optical theorem and Kramers-Kronig relation, the spectra yield the dispersive f' and absorptive f'' terms of the sulphur scattering factor.

Data collections were performed at three different wavelengths. Because of the crystal radiation sensitivity and limited cryoconditions, the best data set had a 25% completeness in the resolution range of (50-4.7)Å. Indexing and integration were done with ADFILM and Partials (P.Schwager & K.Bartels). Internal data scaling at one wavelength was done in parallel with SCADAD (K. Bartels) and with SCALEPACK (Z. Otwinowski). Inter-wavelength scaling was carried out with SCALEIT (CCP4) and with our own program, taking the wavelength dependencies between f'' into account.

The results after the algebraic dispersion analysis with PHDIAN (M. Huetsch) will be connected with direct methods to determine the sulphur substructure in the biologically important molecule of RfBP.