but it is also important in studying DNA replication because it is apparently an homologue to *E. coli* DNA polymerase I which has long been used for DNA replication study (Lawyer *et al., 1993).* The crystal structure of Taq DNA polymerase could be useful as a substitute for DNA replication study of *E. coli* DNA polymerase I. The structure determination of Taq DNA polymerase was initiated. The crystals of intact Taq DNA polymerase were grown at 22°C by the hanging drop method. X-ray diffraction pattern breaks down a crystal structure into discrete sine waves in Fourier series. The original shape of an object in the form of electron density may be represented as the sum of those sine waves with varying amplitudes and phases in three dimensions. The molecular replacement is sometimes utilized to provide phase information. This report will describe phase determination to solve the crystal structure of Taq DNA polymerase by the molecular replacement.

PS02.04.10 MAD PHASING USED IN THE STRUCTURE DETERMINATION OF DESULFOFERRODOXIN. Ana Coelho[1,2], Pedro M. Matias[1], Maria A. Carrondo[1,3], Vilmos Fülöp[4], Ana Gonzalez[5] and Andy Thompson[6]. [1]ITQB, Universidade Nova de Lisboa, 2780 Oeiras, Portugal; [2]Universidade de Évora, 7000 Évora, Portugal; [3]IST, Universidade Técnica de Lisboa, 1000 Lisboa, Portugal; [4]LMB and OCMS, University of Oxford, Oxford OX1 3QU, UK; [5]ESRF, BP-220, 38043 Grenoble Cedex France; [6]EMBL Grenoble Outstation, BP-156, 38042 Grenoble Cedex France

Multiwavelength anomalous data collected at ESRF, BL- 19, were used to solve the structure of desulfoferrodoxin (DFX), isolated from the sulphate reducing bacteria *D. desulfuricans* ATCC 27774. This non-heme iron protein is a 13.4 kDa monomer with 125 residues and two iron centres. The two midpoint redox potentials for this protein (4 and 240 mV) permit its separation in three oxidation states. The crystals of the fully oxidized form belong to space group R32 (a=112.5Å, c=63.2Å, Z=1). The MAD method was tried due do the failure in finding suitable heavy atom derivatives to be used with the MIR method. The crystal used for data collection was frozen and mounted with the c axis perpendicular to the spindle. Data were collected at 3 wavelengths near the iron absorption edge and scaled against a data set collected at 1.09Å. For each data set the R_{merge} is less than 4%, the multiplicity is around 4.5 and the completeness is greater than 96%. The iron atom positions were determined from an anomalous difference map and used for phase refinement, giving a figure of merit of 0.7 at 2.8 Å. The electron density maps obtained were improved by solvent flattening before model building. Refinement is in progress.

PS02.04.11 MULTIPLE ANOMALOUS DISPERSION AT THE K-ABSORPTION EDGE OF SULFUR WITH BOVINE TRYPSIN. Sigrid Stuhrmann, Klaus S. Bartels, Heinrich B. Stuhrmann, GKSS-Research Center, D-21502 Geesthacht, Germany

Bovine trypsin is a serine protease which has six cystines and two methionines. The biochemistry and the structure of the protease is well known. It is therefore a good candidate for a first more rigourous application of MAD at the K-absorption edge of sulfur. The diffraction data were collected at three different wavelengths near the K-absorption egde of the sulfur containing aminoacids (5.02Å) at the beamline A1 of HASYLAB (Hamburg). The anomalous dispersion is not obscured by the absorption due the sulfate ions of the mother liquor. The feasibility of protein crystallographic studies at wavelengths near the K-absorption edge of sulfur had first been shown with hen egg white lysozyme by M. Lehmann in 1991[1].

The crystallization method adapted from Bartunik et al. [2] was further improved for cryocooling under special conditions. The best cryoprotectant for the trypsin crystal was a buffer containing 80% of a synthetic sugar (Phytohistol) and 10% ethylenglycol. A special sample holder was developed for maintaining the humid atmosphere of the protein crystal at temperature of -80° C in an evacuated environment.

The bovine trypsin crystals have the orthorhombic unit cell a=54.9 Å, b=58.5 Å, c=67.6 Å and the space group $P2_12_12_1$ [3]. The completeness of the data set is 90% at 5 Å resolution and 15% in the resolution shell of 5 to 3 Å. Considerable changes had to be made in the program FILM in order to index reflections collected on four area detectors. The difference patterson map based on 1000 unique reflections shows many of the vectors connecting the sulfur atoms. In the first step towards phasing the Bragg reflections it was observed that anomalous dispersion of the disulfide bridges is anisotrophic.

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PS02.04.12 ENVELOPE DETERMINATION IN MACRO-MOLECULAR CRYSTALLOGRAPHY BY THE MASC METHOD. M. Ramin, W. Shepard, R. Kahn*, R. Fourme, I.M. Li de La Sierra, G. Grübel+, A. Thompson+, A. Gonzales+ & M. S. Lehmann+$, LURE, Université Paris-Sud, Bât. 209d, 91405 Orsay Cedex, France, *IBS J.-P. Ebel, 41 Avenue des Martyrs, 38027 Grenoble, France, +ESRF, BP220, 38043 Grenoble Cedex, France, $ILL, Avenue des Martyrs, 38042 Grenoble Cedex, France

The Multiple wavelength Anomalous Solvent Contrast (MASC) is a way to produce a physical contrast variation in a macromolecular crystal. This variation is obtained by tuning the X-ray wavelength near an absorption edge of an anomalous scattering species randomly dispersed in the mother liquor. MASC is, in principle, applicable to the determination of the molecular envelope and low resolution phases [Fourme *et al*. (1995) *J. Synchrotron Rad*. 2, 36-48]

Ammonium selenate was added to the mother liquor of crystals of two proteins (P64k from the outer membrane of *Neisseria meningitidis*) and xylose isomerase).Data at 3-4 wavelengths near the selenium K-edge were collected from cryocooled crystals, using undulator radiation at the ESRF ('Troika' beam line) and an imaging plate detector. Results regarding the extraction of the moduli of the Fourier coefficients of the macromolecular envelope {|G(h)|} and their phasing will be presented. Problems encountered during the set up of this new method will be discussed.

PS02.04.13 IMPROVED PHASES, PHASE ERROR ESTIMATES AND ANOMALOUS SCATTERING MODELS FROM THE MULTIWAVELENGTH ANOMALOUS DIFFRACTION (MAD) OF NATIVE PROTEIN METAL CLUSTERS. Brian R. Crane and Elizabeth D. Getzoff, Department of Molecular Biology, The Scripps Research Instiiute, La Jolla California, 92037

A strategy is presented for refining anomalous scattering models and calculating macromolecular phases from multiwavelength anomalous diffraction (MAD) of native protein metal clusters. This procedure, incorporated in the program MADPHSREF, refines an anomalous scattering model directly against Bijvoet and dispersive differences while making likelihood estimates of errors, applying stereochemical restraints, taking into account more than one type of anomalous scatterer, and partly compensating for inherent correlations between lack-of-closure expressions. Probabilistic rejection of aberrant observations, re-evaluated before each refinement cycle, improved refinement convergence and accuracy compared to other less flexible rejection criteria. MADPHSREF allows the facile combination of MAD phase information with phase information from other sources. For the sulfite reductase hemoprotein (SiRHP), relative weights for MAD and multiple iso-