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 $\{|G(\mathbf{h})|\}$ 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 Institiute, 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-

morphous replacement (MIR) phases were determined by matching histograms of electron density. Accurate metal cluster geometries and the associated errors in atomic positions can be determined from refinement against anomalous differences using normal scattering phases from a refined structure. When applied to MAD data collected on SiRHP, these methods confirmed the 4Fe-4S cluster asymmetry initially observed in the refined 1.6 Å resolution structure and resulted in a MAD-phased, experimental, electron-density map that is of better quality than the combined MAD/MIR map originally used to solve the structure.

PS02.04.14 SIMPROT: A PROGRAM IN DEVELOPMENT FOR CRYSTAL STRUCTURE DETERMINATION OF PROTEINS. F.R. Seljee, R. Peschar and H. Schenk, Laboratory for Crystallography, Amsterdam Institute for Molecular Studies (AIMS), University of Amsterdam, Nwe. Achtergracht 166, 1018 WV Amsterdam, The Netherlands

An overview will be presented of the Direct Methods program SIMPROT that is designed to deliver ab initio an initial model of a protein structure if isomorphous data sets are available, e.g. from SAS, MAD or SIR experiments. Basically, SIMPROT follows the same methodology as employed in the Direct Methods software package SIMPEL [1]. However, is has been suitably modified in accordance with the difference structure factor formalism that has been developed recently [2], [3], [4].

The latter formalism is based on using the difference between two isomorphous structure factors as variable in the derivation of the Joint Probability Distributions upon which Direct Methods are based. It has been shown that this approach leads to accurate phasesum invariant estimates. A graphical overview of SIMPROT is given and some preliminary results will be discussed.

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Structure Determination Using Powder Data

MS02.05.01 SUPRAMOLECULAR STRUCTURES FROM HIGH RESOLUTION POWDER DIFFRACTION. R. E. Dinnebier, Lehrstuhl fuer Kristllographie, University of Bayreuth, 95440 Bayreuth, Germany

Over the past few years, the feasibility of determining crystal structures ab initio from powder diffraction data has been steadily improved. Although a number of complicated inorganic crystal structures have been solved by this method, very little has been done in the field of supramolecular structure determination, namely for organic- and organometallic compounds. Assuming the material itself is well crystallized, the use of Synchrotron radiation is necessary to get a resolution as high as possible over the entire angular range of the powder pattern. Besides the higher resolution, advances in the computational aspects of the problem are also crucial for structure determination. Especially the development of more sophisticated grid search techniques of molecular fragments, considering geometrical and physical aspects of the crystal, is an important step forward in finding the right solution. The structure solutions presented here stand for some of the most complicated organic and organometallic structures which have ever been solved ab inito from high resolution powder data. They include the high and the low temperature phase of the Ru-sawhorse dimer $[Ru_2(O_2PMe_2)_2CO)_4]n$ (1), the industrial important (Kolbe-Schmitt-synthesis) phenolates C_6H_5OA (A= K, Rb, Cs) (2), C_6H_5OK $2(C_6H_5OH)$ (2), C_6H_5OK $3(C_6H_5OH)$ (2), Na-Para-Hydroxy-Benzoate $NaC_7H_5O_3$ (2), base free alkaline-cyclo-pentadienide C_5H_5A (A=Li,Na,K,Rb) and the triclinic low temperature form of $C_{60}Br_{24}(Br_2)_2$ (3). In addition to well known techniques such as direct methods, difference Fourier synthesis, Patterson maps and Rietveld analysis, the newly proposed pseudo-atom method proved to be a very efficient tool to solve all structures containing well defined molecular fragments. In the case of $C_{60}Br_{24}(Br_2)_2$ the orientation of the well defined $C_{60}Br_{24}$ molecules in a triclinic distorted fcc lattice could be found unambiguously by maximizing of nearest neighbor distances. The structural motive of the high temperature form of the Ru-sawhorse dimer was found by conventional direct methods. The similarity criterion for the low temperature phase resulted in a restricted 4-dimensional grid search.

It can be shown that the precision is comparable to that achievable with single crystal techniques, and, therefore, allows for the interpretation of binding mechanism and reactions. Nevertheless, much more work is required in developing these structure solution methods further.

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MS02.05.02 SOLUTION AND REFINEMENT OF DRUG STRUCTURES FROM POWDER DATA. Kenneth Shankland, ISIS Facility, Rutherford Appleton Laboratory, Chilton, Didcot, Oxon OX11 0QX, U.K.

The vast majority of pharmaceuticals used in everyday life are moderately sized organic compounds. Usually, their molecular structures are known from single crystal X-ray experiments. However, polymorphism (which is frequently found in pharmaceuticals) often makes the determination of a particular crystal form very difficult. In such cases, obtaining a structure solution from powder data is an attractive option, but one which presents a considerable challenge given that most of the compounds of interest will crystallise in low symmetry space groups with large unit cells, leading to complex diffraction patterns with lots of peak overlap. Model building is usually precluded due to conformational flexibility in the molecules, so *ab initio* methods offer the best hope of a solution.

Computational strategies such as standard direct methods, combined maximum entropy / log-likelihood gain and optimal extraction of structure factors will be discussed, but a particular emphasis will be placed on using sample preparation and data collection strategies to maximise the chance of obtaining a structure solution.

MS02.05.03 ZEOLITE STRUCTURE DETERMINATION FROM POWDER DATA: COMPUTER-BASED INCORPORATION OF CHEMICAL INFORMATION. R.W. Grosse-Kunstleve, L.B. McCusker &;Ch. Baerlocher, Laboratory of Crystallography, ETH Zentrum, CH-8092 Zurich.

Many technologically and industrially important materials, including zeolites, are synthesized and used in polycrystalline form. Since the crystal structures of such phases often determine their useful properties, it is essential that methods to study their structures are available.

The FOCUS method, which incorporates the use of chemical information into the structure determination process, has been developed. FOCUS combines automatic Fourier recycling (using