MS02.04.05 DATA REDUCTION AND SCALING FOR MAD (WITH SOME COMMENTS ON PHASE DETERMINATION). Alan M. Friedman, Dept. of Biological Sciences, Purdue University, West Lafayette, IN 47907

During our work on the crystal structure determination by MAD of the lac repressor core fragment and the phage T4 gene 32 protein: ssDNA complex, we found it necessary to develop a new software package for MAD data reduction, scaling and phase determination. This package, called MADPRB, is derived from the package of Hendrickson, and has been employed in the successful structure determinations above and subsequently in other structures. In many cases MADPRB succeeded where other packages failed. We will discuss several of these cases and describe the principles behind data reduction and scaling in MADPRB.

The scaling program of MADPRB (NEWLSC) starts with a set of unreduced hkl's and integrated intensities from individual observations that have been collected to yield Bijvoet pairs of measurements at several wavelengths that are matched for absorption and decay. Using a moving box local scaling algorithm, the matched measurements are scaled so as to reduce the errors in the Bijvoet and dispersive anomalous differences. In order to do this the user need only input the relationship between matched Bijvoet pairs (e.g. inverse beam). From this the program understands the data collection geometry and automatically scales each observation with its matched mates and sorts all the data into sets for phase determination. The utility of this scaling in reducing errors will be demonstrated.

I will also make some comments about phase determination for MAD. I will suggest that the best method of phase determination requires calculating phases from the matched sets of observations, rather than by "pseudo-MIR" approaches which merge the raw data. This strategy has been adopted in MADPRB. Phase determination in MADPRB occurs in two passes. In the first pass, the program MADRBE estimates the MAD parameters, Fa, Fz and delphi by a modified "algebraic method." Several modifications improve the stability of the estimation for weak and/or poor data. The first pass of phase determination is completed by heavy atom refinement and total phase calculation as in the original Hendrickson package. In a second pass, a new program (BAYESFA) determines phases once the heavy atom parameters are refined. The programs are under further evolution, while the current version is available from the author (email: afried@bilbo.bio. purdue.edu)

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MS02.04.06 DESIGNER LABELS; USEFUL TOOLS OR JUST ANOTHER FAD? Neil Q. McDonald+, Structural Biology Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX; +Department of Crystallography, Birkbeck College, P.O. Box 123, Malet Street, London WC1E 7HX, UK

At the present time the only crystallographic methods able to derive ab initio phase estimates for medium to large sized proteins require the incorporation of a few electron dense or alternatively, anomalous scattering centres into a protein. Such atoms must modify the observed structure factors to the extent that their positions can be identified allowing phase estimates of the protein to be obtained.

Various techniques have been described to introduce labels into a protein or protein complex, examples include (1) direct chemical modification (2) protein engineering and (3) in vivo labelling of recombinant proteins. In some cases the incorporation of such labels has been developed to solve a specific structural problem. Other approaches are emerging as being more generally applicable.

I will review current methods for protein labelling with an emphasis on multi-wavelength anomalous scattering. I shall focus on chemical modification and incorporation of selenomethionine referring to specific examples in my laboratory.

PS02.04.07 STRUCTURE SOLUTION OF AN INTEGRAL MEMBRANE PROTEIN: NOVEL DERIVITIZATION METHODS. S.M. Prince, M.Z. Papiz*, G. Mcdermott, A.M. Hawthornthwaite-Lawless*, A.A. Freer, R.J. Cogdell~, N.W. Isaacs. Dept.'s of Chemistry and ~Biochemistry, University of Glasgow, Glasgow, G12 8QQ, UK. and; *CLRC Daresbury Laboratory, Daresbury, Warrington, WA4 4AD, UK.

The structure of the LH2 complex from purple bacteria was pursued using a number of novel derivitization techniques. The crystal structure of this integral membrane complex revealed a highly symmetrical molecule of nine subunits possessing C9 molecular symmetry, the C9 axis being coincident with the 3-fold axis of the R32 spacegroup. A number of factors obstructed or confused standard MIR techniques. These include; the small extent of the polar surface of the molecule, the alignment of the molecule with the crystallographic 3-fold - resulting in multiple sites with the same z fractional coordinate and the high pH (9.3) at which crystals were stable. These compromised; derivative binding, Patterson solution, and heavy atom salt solubility.

A number of techniques were attempted to form isomorphous heavy atom derivatives including: Seleno-methionine labelling and the exchange of bacteriochlorophyll co-factors for Pd substituted analogues. The method which finally resulted in a phase set used traditional heavy atom salt soaks with a additional stage. The second stage simply employed differing binding site dynamics or differing heavy atom salt chemistry to partially 'back-soak' away a subset of sites. This resulted in a considerable gain in isomorphism and difference Patterson maps trivial to solve.

The presentation will describe these derivitization methods, and successive manipulations demonstrating the quality of the phase set obtained.

PS02.04.08 STRUCTURE OF A TYPE III AFP PHASED BY THE ANOMALOUS SIGNAL OF A SINGLE IODINE ATOM USING CUKα RADIATION. [1]Yang, D.S.C., [1]Bubanko, S., [1]Xue, Y.Q., [1]Seetharaman, J., [2]Hew, C.L., [3]Fletcher, G.L. and [1]Sicheri, F.. [1]Department of Biochemistry, McMaster Univeristy, Hamilton, Ontario L8N 3Z5 Canada,; [2]Res. Inst., Hosp. for Sick Children, Toronto, Ontario M5G 1X8, Canada; [3]Dept. Ctr., Memorial University of Newfoundland, St.Johns, NFLD A1C 5C7 Canada

Four distinct types of antifreeze polypeptides (AFPs) have been isolated from polar marine fish, all of which act to inhibit ice growth through direct adsorption to the ice lattice. The α-helical structure of type I AFP has been solved and its mechanism is currently under investigation. A solution structure of a type III AFP has been determined by 2D NMR spectroscopy. It differs from type I AFP in that this structure consists of two sheets of three antiparallel strands and one sheet of two antiparallel strands; with the triple-stranded sheets forming a β-sandwich.

We have crystallized type III AFPs in three different crystal forms. Extensive molecular replacement (MR) studies using the NMR derived structure failed to yield a solution. We have recently crystallized an iodotyrosine derivatized AFP in a fourth crystal form. The Iterative Single Anomalous Scattering (ISAS) procedure was applied to determine the structure. Anomalous signal from four iodine atoms and four fold noncrystallographic symmetry averaging were used successfully to phase the structure which consists of 280 residues in the asymmetric unit. Details of data collection, processing and structure refinement will be presented along with a discussion of the MR effort.

PS02.04.09 AN APPLICATION OF GENETIC ENGINEERING FOR SOLVING THE CRYSTAL STRUCTURE OF TAQ DNA POLYMERASE. Youngsoo Kim, The Yeungnam University, Kyungsan, South Korea 712-749

Taq DNA polymerase from *Thermus aquaticus* has been shown to be very useful in the polymerase chain reaction method, which is being used for amplifying DNA. Not only is Taq DNA polymerase highly useful in commercial value for the polymerase chain reaction application,

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-