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)

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,