MS02.04.05 DATA REDUCTION AND SCALING FOR MAD
(WITH SOME COMMENTS ON PHASE DETERMINATION).
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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 (NEWLSLC) 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 MADRB estimates the MAD parameters, F, 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 refinements 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 further enhanced by a "moving SAD" approach which follows its own version available from the author (email: afr@bilbo.bio.purdue.edu).

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MS02.04.06 DESIGNER LABELS: USEFUL TOOLS OR JUST ANOTHER FAD?
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At the present time the only crystallographic methods available 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.

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: Selenium-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.

Four distinct types of antifreeze peptides (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 anti-parallel strands and one sheet of two anti-parallel strands; with the triple-stranded sheets forming a β-sandwich.

We have crystallized type III AFPS in six 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 Youngnam 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 unpar-

tially an homologue to E. coli DNA polymerase I which has long been

used for DNA replication study [Lawyer et al., 1993]. The crystal struc-
ture of Taq DNA polymerase could be useful as a substitute for DNA

replication study of E. coli DNA polymerase I. The structure determina-
tion 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 re-

placement is sometimes utilized to provide phase information. This re-

port 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 Coelho1,2,

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Multiwavelength anomalous data collected at ESRF, BL-19, were

used to solve the structure of desulfoferrodoxin (DFX), isolated from the

sulphate reducing bacteria Desulfovibrio vulgaris strain H277 (DSM 4415).

This non-heme iron protein is a 13.4 kDa monomer with 125 residues

and a separation in three oxidation states. The crystals of the fully oxidized

iron protein is a 13.4 kDa monomer with 125 residues.

The two midpoint redox potentials for

Lehmann in 1991 [1].

The iron atom positions were determined

from the crystal space group R32 (a=112.5Å, c=63.2Å, Z=1)

0.109 Å for each data set. In the real space, the resolution is less than 4 Å,

the multiplicity is around 4.5 and the completeness is greater than 96%.

The iron atom positions were determined from an anomalous

daughter 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.

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earch 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 rigorous appli-
cation of MAD at the K-absorption edge of sulfur. The diffraction data

were collected at three different wavelengths near the K-absorption edge

of the sulfur containing aminoacids (5.02 Å) 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 crystalization 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 (Phyloxybato) and 10% ethyleneglycol. A special sample

holder was developed for maintaining the humid atmosphere of the pro-

tein crystal at temperature of ~30°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 P212121 [3]. The complete-

ness of the data set is 90% at 3 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 100 unique reflections shows many of

the vectors connecting the sulfur atoms. In the first step towards phas-

ing the Bragg reflections it was observed that anomalous dispersion of

the disulfide bridges is anisotropic.


(1993)


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PS02.04.12 ENVELOPE DETERMINATION IN MACRO-

MOLECULAR CRYSTALLOGRAPHY BY THE MASC

METHODE. M. Ranumm, W. Sheppard, R. Kahn, R. Fourme, L.M. Li de La Sienna,

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The Multiwavelength Anomalous Solvent Contrast (MASC) is a way to

produce a physical contrast variation in a macromolecular crys-
tal. 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 determi-

nation of the molecular envelope and low resolution phases [Fourme et


Ammonium selenate was added to the mother liquor of crystals of two

proteins (P64k from the outer membrane of Norisiera meningitoidea 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 (O(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 ESTI-

MATES AND ANOMALOUS SCATTERING MODELS

FROM THE MULTIWAVELENGTH ANOMALOUS

DIFFRACTION (MAD) OF NATIVE PROTEIN METAL

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A strategy is presented for refining anomalous scattering models and

calculating macromolecular phases from multivave-

length 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 er-

rors, applying stereochemical restraints, taking into account more

than one type of anomalous scatterer, and partly compensating for

inhomogeneities in the vectors connecting the sulfur atoms. For the

sulfite reductase hemoprotein (SirHb), relative weights for MAD and multi-
