MAD & MIR - Macromolecular Phasing

MS02.04.01 REFINEMENTS OF HEAVY-ATOM AND PROTEIN PARAMETERS AGAINST A HIGHLY ACCURATE MAD DATA SET. Axel T. Brunger*, F. Temple Burling*, Kevin M. Flaherty+, William I. Weis+. *Howard Hughes Medical Institute and Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06520, USA, +Department of Structural Biology, Stanford School of Medicine, Stanford University, Stanford, California 94305, USA

A highly accurate and complete multi-wavelength anomalous scattering (MAD) data set was obtained previously at 1.8 Å resolution from a crystal of a Yb^{3+}substituted subtilisin fragment of mannose-binding protein A (F.T. Burling, W.I. Weis, K.M. Flaherty & A.T. Brunger, Science 271, 72-77, 1996). By keeping the lack-of-closure expressions for both Bijvoet mates separate, a realistic probabilistic description of the MAD experiment was implemented. This description was formulated in the crystallographic computing language of the new version of X-PLOR (a pre-release will be available soon). This language allows one to chose between a variety of refinement methods (including conjugate gradient minimization and simulated annealing) against several target functions with restraints or constraints. Refinements of heavy-atom parameters and protein parameters were performed against the observed MAD phase probability distributions using a maximumlikelihood target function. Differences between these and conventional residual-based refinements will be discussed.

MS02.04.02 THE ANALYTICAL APPROACH OF PHASING BY MULTIWAVELENGTH ANOMALOUS DIFFRACTION (MAD). Hao Wu & Wayne A. Hendrickson, 630 W. 168Th Street, Dept. Of Chemistry, Columbia University, New York, NY 10032

The *de novo* phasing of macromolecular crystal structures by MAD relies on the mathematical relationships among the structure factors of both Friedel mates at particular wavelengths. The least-squares solutions to these equations give rise, among other parameters, to the structure factor amplitudes of the anomalous scatterers alone which are optimal for locating the anomalous scattering centers by either Patterson synthesis or direct method. The desired phase angles for the crystal can then be determined explicitly or represented by phase probability distributions using the Hendrickson-Lattman coefficients.

The MADSYS package, which was developed over the years to perform analytical MAD phasing, was recently implemented to maximize automation and self-containment while improving performance. The sampling in derived structure factor amplitudes should also give better phase probabilities.

MS02.04.03 TREATMENT OF MAD DATA AS A SPECIAL CASE OF MIR. V. Ramakrishnan[1,2], V. Biou[2], F. Shu[2], and R.M. Sweet[2], Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, UT 84132 and [2]Biology Department, Brookhaven National Laboratory, Upton, NY 11973

We show that MAD data can be treated as a special case of MIR, and phased using a variety of popular MIR phasing programs. In this approach, data for one of the wavelengths is considered the "native" data, and the others are considered "derivatives". The dispersive differences between wavelengths are equivalent to isomorphous differences. Bijvoet differences at each wavelength are also included in the phasing, resulting in "derivatives" that have both isomorphous and anomalous differences. In the case of the C-terminal domain of initiation factor 3, three wavelength MAD data were collected on crystals of the selenomethionyl protein. All the MIR phasing programs tried gave easily interpretable maps that had excellent map correlation coefficients with the final structure. We also discuss our experience with a number of issues such as which wavelength should be considered the reference or "native" data set, how additional MIR phase information from heavy-atom derivatives should be included with MAD data, etc. Results on data where MAD alone failed to produce interpretable maps will also be discussed.

MS02.04.04 SHARP :MAXIMUM-LIKELIHOOD REFINEMENT OF HEAVY-ATOM PARAMETERS IN THE MIR AND MAD METHODS. Éric. de La Fortelle and Gérard. Bricogne, MRC-LMB, Hills Road, Cambridge CB2 2QH, England.

The problem of estimating heavy-atom parameters (esp. occupancies) from acentric reflexions in the MIR method has a long history of difficulties, and a conceptually satisfactory solution allowing bias-free refinement of all parameters (including the lack of isomorphism) has only recently been obtained by a recourse to the method of maximum-likelihood estimation [1,2]. The situation is essentially identical in the case of MAD phasing. The maximum-likelihood method needs to be invoked to exploit incomplete phase information in a heavy-atom parameter refinement while preventing that information from biasing the results.

We have designed and written from scratch a computer program –SHARP (Statistical Heavy-Atom Refinement and Phasing) – that fully implements the maximum-likelihood approach. It can refine simultaneously the scale factors, a parametrised model for the lack of isomorphism and all heavy-atom parameters from MIR and MAD data, or any mixture of them. The program performs in conformity with theoretical requirements on simulates data, and all tests on measured datasets have shown satisfactory results, outperforming the classical program MLPHARE[2]. The likelihood function has also been used as a detection tool, especially to plot residual Fourier maps and probe for minor sites.

Special care has been taken to make the program user-friendly. A self-explanatory graphical user interface has been set up, based on hypertext tools, so that SHARP can be run from anywhere through an Internet connection. The use of the software and interface will be demonstrated.

The next developments will include automatic heavy-atom detection, validation of further model hypotheses (new sites etc.) on a fraction of the data unused in the refinement, and a two-way connection to program BUSTER for further phase improvement, e.g. by mode permutation.

References

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[3] Bricogne, G. (1993). *Acta Cryst.* D49, 37-60.