s1.m3.p12 **Direct incorporation of experimental phase information in model refinement.** Pavol Skubák,[a] Garib N. Murshudov[b], and Navraj S. Pannu[a], [a]*Biophysical Structural Chemistry, Leiden Institute of Chemistry, Leiden University, The Netherlands, and* [b]*Structural Biology Laboratory, Department of Chemistry, University of York, UK. E-mail: p.skubak@chem.leidenuniv.nl*

Keywords: Automatic model building and refinement; Multivariate likelihood; Single wavelength anomalous diffraction

The incorporation of prior phase information in a maximum likelihood formalism has been shown to strengthen model refinement. However, the currently available likelihood refinement target using prior phase information has shortcomings: the refinement target considers experimental phase information indirectly and statically in the form of Hendrickson-Lattman coeffcients. Furthermore, the current refinement target implicitly assumes that the prior phase information is independent from the calculated model structure factor.

We have derived a multivariate likelihood function that overcomes these shortcomings and directly incorporates experimental phase information from a single wavelength anomalous diffraction (SAD) and single isomorphism replacement (SIR) experiments allowing for the simultaneous refinement of heavy atom and model parameters. We have implemented this function in the refinement program REFMAC5. The multivariate likelihood function used in conjunction with the automated model building procedures of ARP/wARP leads to a successful model building when current likelihood functions fail.

s1.m3.p13 **Obtaining high-quality experimental phases: results from MAD experiments on urate oxidase derivative crystals with Gd complexes.** Meike Stelter, Jean Vicat & Richard Kahn. *Institut de Biologie Structurale J.-P. Ebel CEA, CNRS, UJF, UMR 3075, 41 rue Jules Horowitz, F38027 Grenoble cedex 1, France. E-mail: Meike.Stelter@ibs.fr*

Keywords: Gd complexes; Anomalous diffraction; Macromolecular structures.

A new class of Gd complexes to obtain high-phasing-power heavy-atom derivatives for macromolecular crystallography was presented in a recent paper [1]. With each of the seven described complexes it was possible to solve the structure of urate oxidase from *Aspergillus flavus* by single-wavelength anomalous diffraction (SAD) phasing with data collected at the Gd L_{III} absorption edge [2].

We present results obtained with derivative crystals of urate oxidase and the complex Gd-DOTMA. Phases at 1.46 Å resolution were obtained by the multiple-wavelength anomalous diffraction (MAD) method. In the crystals the occupation of the major complex binding site was high enough to allow identifying the binding mode and building the model of the ligand molecule bound to the protein.

On the basis of this example a strategy for data collection, phasing and refinement was developed in order to optimize the use of experimental phase information to enhance the electron density corresponding to parts of the structure that are not yet modeled during the structure refinement, like ligands of the complexes even with rather low occupancies.

For subsequent data collection on other derivatives using synchrotron radiation we adopted the strategy of collecting one data set at the maximum of f" at the Gd LIII absorption edge (λ = 1.711 Å) where, due to technical constraints, data resolution is limited to about 2.7 Å and one data set at maximum resolution collected at a far shorter wavelength.

For derivatives with sufficient anomalous signal, phasing followed by a structure refinement taking into account the experimental phase information are performed in order to identify the binding mode of the complexes for derivatives with binding site occupancies high enough to allow to observe the ligand molecules in the refined electron density maps. For derivatives with lower binding site occupancies (often though it is still high enough to permit good phasing), binding site identification and possibly phasing let compare binding efficiencies and location of the different complexes as well as the protein residues involved in their binding.

Combining these different kinds of information may allow to identify possible systematic patterns and thus to predict the behavior of the different complexes with proteins of unknown structure, the fixation of the complexes depending on the nature of residues at the protein surface, and possibly on other factors such as the precipitating agent.

We are now working on derivative crystals of several test proteins (urate oxidase, hypothetical protein Yggv from *E. coli*, glucose isomerase from *Streptomyces rubiginosus*) with the different gadolinium complexes.

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[2] Girard, É, Stelter, M., Anelli, P. .L., Vicat, J. & Kahn, R (2003). *Acta Cryst.* D**59**, 118-126