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CORRELATED SINGLE-WAVELENGTH ANOMALOUS DIFFRACTION PHASING AND REFINEMENT

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Recently, there has been a resurgence in phasing using the single-wavelength anomalous diffraction (SAD) experiment - data from a single wavelength in combination with density modification techniques have been used to solve structures, even with a very small anomalous signal. Furthermore, SAD can be favorable to a multi-wavelength anomalous diffraction (MAD) experiment in a case where a crystal exhibits radiation decay during the course of a MAD experiment.

Currently, to refine the anomalous substructure and phase a SAD data set, conventional techniques employ a least squares function either on the Bijvoet differences or the Bijvoet/Friedal pairs. These equations neglect some of the important correlations that occur in a SAD experiment. Indeed, since data from a SAD experiment comes from the same crystal and share the same model of anomalous scatterers, explicitly accounting for these correlations may improve results further. Here, a novel formulation for SAD phasing and refinement employing multivariate statistical techniques is presented. The equation developed accounts explicitly for the correlations from the observed and calculated Friedal mates in a SAD experiment. Furthermore, the function derived requires only a one dimensional numerical integration. The correlated SAD equation has been implemented and test cases performed on real diffraction data have revealed significantly better results than the currently most used programs in terms of correlation with the final map and producing more reliable phase probability statistics.

Keywords: PHASING, SINGLE-WAVELENGTH ANOMALOUS DIFFRACTION, MULTIVARIATE STATISTICS

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THE 160 SELENIUM ATOM SUBSTRUCTURE OF KPHMT

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We present the largest successful application of selenomethionine MAD reported to date: the crystal structure of the decameric *E.coli* enzyme ketopantoate hydroxymethyltransferase (KPHMT), with 160 ordered selenium atoms and 560 kDa of protein in the asymmetric unit. Despite small (<150 μm), irregular, weakly diffracting (<3.2 Å) crystals, the substructure was solved by SAD combined with Direct Methods, using a 20-fold redundant "peak" dataset. SnB[1] produced the first correct solution after 2600 computing hours, and phases from SHARP[2] and Solomon[3] produced traceable maps, even before 20-fold NCS averaging. Subsequent analysis revealed that data redundancy was critical for success; on the other hand, speed and success rate vary considerably between direct methods programs. Apart from a favorable ratio of selenium to scattering matter, the procedure was quite general, suggesting that this is still a long way from the practical upper limit of applicability, if that exists.

References

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Keywords: SELENOMETHIONINE MAD DIRECT METHODS

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ENHANCEMENTS IN AUTOSHARP AND SHARP, WITH APPLICATIONS TO DIFFICULT PHASING PROBLEMS

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The current release (1.4.0) of SHARP [1] has been incorporated into a set of scripts called 'autoSHARP' which extend in both the upstream and downstream directions the initial coupling of SHARP to the SOLOMON density modification program. Upstream, autoSHARP can perform data checking and scaling, and heavy-atom detection; downstream, it can carry out density modification with automatic optimization of solvent flattening parameter and choice of hand, and launch the ARP/wARP map interpretation and model building protocol [2]. Several fully automatic structure determinations with autoSHARP will be reported, some of them in connection with the exploration of novel experimental phasing possibilities based on iodine binding and on combinatorial counter-ion replacement.

In parallel, we have completely redesigned and rewritten all the numerical internals of SHARP, including the computation of log-likelihood's and their derivatives, the marginalization over trial structure factors for the reference dataset, the application of the chain rule, and the choice of optimization algorithm. Speed gains in excess of an order of magnitude have been achieved, at the same time as gains in robustness and in the quality of the results. New features have been added, such as combined residual (log-likelihood gradient) maps and a modified representation for the final phase information. These new capabilities are being subjected to intensive tests on large and challenging structural problems, whose results will be presented.

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Keywords: MAD PHASING SAD PHASING SHARP PHASING PROGRAM

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STRUCTURE DETERMINATION OF THE EXTRACELLULAR DOMAIN OF THE LDL RECEPTOR: A NON-TRIVIAL CASE OF MAD PHASING

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We have solved the structure of the extracellular domain of the LDL receptor at 4 Å resolution. A combination of molecular replacement with a known fragment and a MAD experiment at the tungsten edge was used to solve the structure.

The structure determination was non-routine, in that the asymmetric unit contained 31 tungsten atoms arranged in clusters. The presence of so many anomalous scatterers in crystals of the 85 kDa protein, generated a tremendous anomalous signal in diffraction experiments using energies at the tungsten edge and above. Though in principle good for phasing, such a large anomalous signal proved in practice problematic for data processing and the structure determination as well. In addition, the poor quality of the crystals, together with their radiation sensitivity, rendered structure determination non-trivial.

We would like to describe the methods used that ultimately lead to the structure determination of this intriguing protein.

Keywords: LDL RECEPTOR, CHOLESTEROL METABOLISM, MAD PHASING