DIRECT METHODS VERSUS SIR, MIR, SAD, MAD, SIRAS, MIRAS: THE FIRST APPLICATIONS

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Direct Methods solved in the practice the phase problem for small molecules, but did not play a central role for the evolution of the methods traditionally used in macromolecular crystallography. The joint probability distribution technique has been recently coupled with the ability of treating measurement and model errors, so making Direct Methods competitive with the traditional techniques used for dealing with SIR, MIR, SAD, MAD, SIRAS, MIRAS cases. We describe the first applications of Direct Methods to real cases: the new procedures show a high degree of automatism and efficiency.

Keywords: DIRECT METHODS MAD MIR

PRACTICAL ASPECT OF SULFUR SINGLE-WAVELENGTH ANOMALOUS SCATTERING PHASING

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Almost all known proteins contain sulfur. Thus; the use of sulfur as an anomalous phasing probe for protein structure determination has been investigated since the early 1980's. However; during the past two decades only a few de novo structures have been determined using this method; because sulfur's anomalous scattering signal is weak with Δ F' ranging from 0.124 to 1.42 e- over the tunable range of most synchrotron X-ray sources. Using third generation synchrotrons; recent CCD detector technology and special attention in data collection we have shown that the weak sulfur anomalous scattering signal can be recorded with the accuracy needed for successful de novo structure determination. Based on these successes we have developed data collection; data processing and phasing procedures for protein structure determination using sulfur single-wavelength anomalous scattering (SAS) data. Our results show that sulfur phasing can be applied to many crystal structure determinations if the correct experimental procedure is pursued. The practical aspects of this approach will be presented.
Work supported in part from the National Institutes of Health (GM062407); IMCA-CAT APS; Argonne national Laboratory; the Georgia Research Alliance; and The University of Georgia Research Foundation.

Keywords: **SULFUR PHASING, SAS DATA, ANOMALOUS SCATTERING**

α -D-GLUCURONIDASE: EXPERIENCES WITH A SAD EXPERIMENT IN SPACE GROUP *P*1

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Both selenomethionyl and native α-D-glucuronidase crystallize in the triclinic space group *P*1 with two molecules in the asymmetric unit. We carried out a single wavelength experiment at a wavelength chosen to maximize the f" contribution of the selenium. The intensity of the incident beam was attenuated in order to reduce crystal decay whilst also permitting data collection to 2.2 Å resolution. 750° of data were collected, then the crystal was rotated 90° perpendicular to the x-ray axis using the cryo-arc as part of the goniometer head, in order to sample a different orientation of the reciprocal space and a further 90° of data were collected. Finally, the crystal was replaced in its original position and a further 250° of data collected. The quality of both the scaling and the anomalous signal was only marginally improved when the second data pass from a perpendicular orientation was incorporated. We show that redundancy is the most important factor that impacts on the solution of the selenium substructure using snb. Indeed, the anomalous signal from a complete data set of only 180° (anomalous multiplicity of 1) was not accurate enough to solve the substructure whereas the data from 360° was sufficient. We also show that the quality of the final map was drastically improved by incorporating native data collected to 1.5 Å.

Keywords: **α D GLUCURONIDASE TRICLINIC SPACE GROUP SAD METHOD**

FINDING THE ANOMALOUS SCATTERERS FROM A MAD EXPERIMENT VIA DIRECT METHODS

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The method of joint probability distribution function has been applied to estimate the structure factor moduli of the anomalous scatterer substructure . The two-wavelength case has been examined first: the prior knowledge of the moduli $|F_1^+|$, $|F_1^-|$, $|F_2^+|$, $|F_2^-|$ is used to predict the value of $|F_{0a}|$, arising from the normal scattering of the anomalous scatterers. The method has been generalized to n - wavelengths, so as to meet the common experimental procedures of MAD techniques. The approach requires the prior knowledge of the experimental moduli $|F_i^+|$, $|F_i^-|$, i = 1, …,n , where n is the number of wavelengths used for the experiment. The advantages of the method are the following: a) the complete experimental information is used for the estimates (i.e., not only the anomalous differences but also the dispersive differences); b) no supplementary information is necessary (e.g. , the ignorance of the number of anomalous scatterers and of their occupancy factors is not critical); c) the procedure is fully automatic. A number of applications to real cases of interest will be shown.

Keywords: **PROBABILITY THEORY ANOMALOUS DISPERSION PROTEINS**