LABNOTE IPDS 2T

HIGH-THROUGHPUT SULPHUR-SAD PHASING OF ELASTASE USING A STOE IPDS 2T DIFFRACTOMETER EQUIPPED WITH A XENOCS GENIX CU K_α MICROBEAM DELIVERY SYSTEM

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CHARACTERISTICS

Solving protein crystal structures by single-wavelength anomalous diffraction (SAD) from sulphur atoms has become a widely advocated technique in recent years[1]. This method has several appealing features: (1) Since it uses the anomalous scattering properties from sulphur atoms that are naturally present in protein molecules, the haphazard step of preparing heavy-atom derivatives is removed; (2) Data can be collected away from the absorption edge, using laboratory equipment operating either with Cr K_α[2] or Cu K_α[3] radiation. On the other hand, given that the anomalous scattering strength from sulphur atoms is rather weak at these wavelengths (f"=0.557 at Cu K_α), the data have to be collected with a very high degree of accuracy, completeness and redundancy[4]. Such measurements are therefore ideal to test the capabilities and performances of a laboratory diffractometer setup.

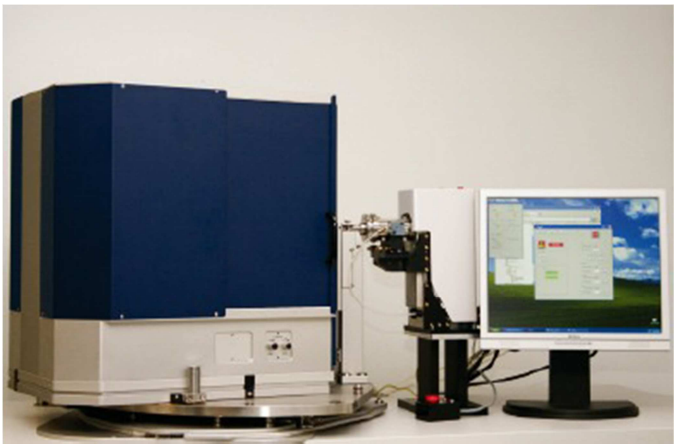

Fig.1: *The combined IPDS 2T - GeniX setup at EPFL*

The *GeniX* microbeam delivery system was operated at 50 kV, 1 mA. The circular image plate (scanned surface: 170 mm radius) was positioned at a distance of 150 mm from the sample with zero 2θ offset, thus allowing data to be collected at a maximum resolution of 1.89 Å. A highly redundant data set, consisting of two 0–180° omega-scans recorded at two different phi angles (0° and 90°) was collected. The data comprise a total of 360 frames, each corresponding to a 1° omega rotation and an exposure of 300 seconds. *Fig. 2* displays an example of a data frame. The frames were indexed and integrated with the *MOSFLM*[5] program. Data reduction (scaling and merging) was carried out with the program *SCALA*[6] from the *CCP4*[7] package. Details of data reduction statistics are given in Table 1.

The STOE image-plate system *IPDS 2T*, equipped with a *GeniX* microbeam delivery system manufactured by *XENOCS* (Fig. 1) was used to collect a highly redundant data set from crystals of porcine pancreatic Elastase, a 26 kDa protein of 240 amino acids (1826 nonhydrogen atoms) containing 10 sulphur atoms (8 Cys + 2 Met). The crystals belong to space group $P2_12_12_1$ with cell parameters 51.31 58.02 75.41 Å.

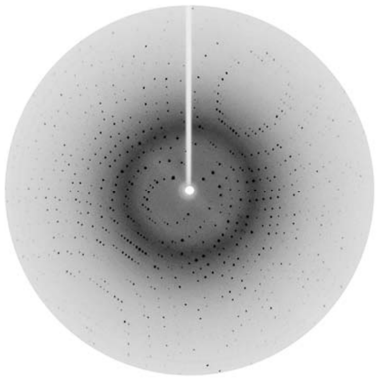

Fig.2: *Diffraction frame recorded on an elastase crystal. Scan width 1° (Omega), exposure time 300 sec. Crystal-Detector distance 150mm.*
Resolution at edge (170mm from center): 1.89 Å

The reduced data were then fed into the *autoSHARP*[8] software package, using the program defaults for all non-compulsory input parameters. Detection and refinement of sulphur atoms, SAD phasing and phase improvement by density modification, chain tracing, model building and refinement of the structure were carried out in a completely automatic fashion, without further user intervention. The final model consists of 237 residues (out of 240), refined to R=15.5% and R-free=21.2%.

As can be seen from Table 1, the data quality is indeed very high. Though the anomalous differences are small, the *SHELXD* heavy atom detection method, as implemented in *autoSHARP*, readily located 8 out of the 10 sulphur atoms. The two additional sulphurs were located by the automatic procedure for analysing residual maps. The SAD phases are of very good quality as is evidenced in fig. 3, where the experimental electron density map computed from SAD phases and after density modification, but prior to any model building, is displayed. The structural model of elastase, as retrieved from the PDB database, is superimposed to this map. As can be seen, the agreement is very good.

As a conclusion, the quality of the data produced by the combined *GeniX-IPDS2T* setup installed at *EPFL*-Lausanne is sufficiently good to solve a 26 kDa protein structure in a quasiautomatic fashion by sulphur-SAD.

	Overall	Inner shell	Outer shell
Low resolution limit / Å	27.09	27.09	1.98
High resolution limit / Å	1.87	5.93	1.87
Rmerge	0.047	0.025	0.141
Rmeas (within I+/I-)	0.050	0.027	0.152
Rmeas (all I+/I-)	0.050	0.029	0.151
Rpim (all I+/I-)	0.018	0.010	0.057
Rpim (all I+/I-)	0.013	0.008	0.041
Fractional number partial bias	-0.003	-0.008	0.003
Total number of observations	257953	8637	29515
Total number of unique reflections	18621	687	2246
Mean I/sd (I)	44.2	73.9	18.6
Completeness (%)	97.4	99.1	82.1
Multiplicity	13.9	12.6	13.1
Anomalous completeness (%)	97.1	99.8	81.0
Anomalous multiplicity)	7.3	7.5	6.8s

Table 1: Data reduction statistics

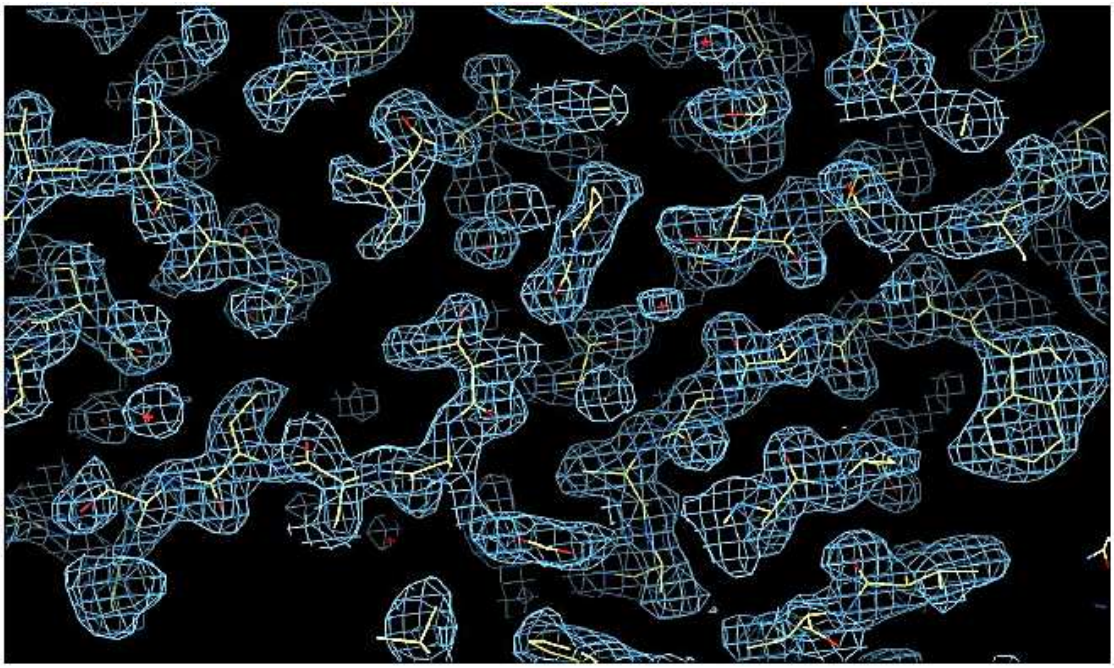

Fig.3: Experimental electron density map computed from Sulphur SAD phases and after density modification, but prior to any model building. The structural model of Elastase, as retrieved from the PDB database is superimposed to this map. As can be seen, the agreement is very good.

[1] Dauter, Z., Dauter, M., La Fortelle, E., Bricogne, G., & Sheldrick, G. M. (1999). Can anomalous signal of sulfur become a tool for solving protein crystal structures? *J. Mol. Biol.* **289**, 83–92.

[2] Yang, C., Pflugrath, J. W., Courville, D. A., Stence, C. N. & Ferrara, J. D. (2003). Away from the edge: SAD phasing from the sulfur anomalous signal measured in-house with chromium radiation. *Acta Cryst.* D**59**, 1943–1957.

[3] Lemke, C. T., Smith, G. D. & Howell, P. L. (2002). S-SAD, Se-SAD and S/Se-SIRAS using Cu Ka radiation: why wait for synchrotron time? *Acta Cryst.* D**58**, 2096–2101.

[4] Sarma, G. N. & Karplus, P. A. (2006). In-house sulfur SAD phasing: a case study of the effects of data quality and resolution cutoffs. *Acta Cryst.* D**62**, 707–716.

[5] Leslie, A.G.W., (1992). Recent changes to the MOSFLM package for processing film and image plate data. In Joint CCP4 + ESF-EAMCB Newsletter on Protein Crystallography, No. 26.

[6] Evans, P. R. (1993). Data reduction. In Proceedings of CCP4 Study Weekend, 1993, on Data Collection & Processing, pp. 114–122.

[7] Collaborative Computational Project, Number 4 (1994). The CCP4 Suite: Programs for Protein Crystallography. *Acta Cryst.* D**50**, 760–763.

[8] Vonrhein, C., Blanc, E., Roversi, P. & Bricogne, G. (2006). Automated Structure Solution with autoSHARP. In *Macromolecular Crystallography Protocols*, Volume 2 Structure Determination, S. Doublié (ed.) Humana Press, Totowa (NJ).