Sulfur-SAD phasing of hen egg lysozyme with Cu radiation obtained in-house with a Xenocs GeniX micro-beam generator

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Introduction

Hen egg lysozyme is a 129 residue protein that contains 8 cysteine residues that form 4 disulfide bridges as well as 2 methionine residues, i.e. in total 10 sulfur atoms per molecule. In addition, there are some bound chloride anions originating from the crystallization buffer. It has been shown[1] that phasing from the combined anomalous signal of sulfurs and chlorides is possible from highly redundant synchrotron data at a wavelength of 1.54 A. It has been mentioned that high redundancy (i.e. > 20) seems to be crucial for the success of the method.

We have repeated the experiment using a ***mar345dtb*** image plate detector system mounted on a very compact in-house X-ray source, the GeniX CU High Flux micro-beam generator manufactured by Xenocs.

Figure 1: Genix High Flux micro-beam generator

Experiment

X-ray Generator

The GeniX is a high brightness micro-beam generator (Figure 1) that is operated at 50 Watts (50 kV, 1 mA). The generator is coupled to a single reflection multilayer mirror that delivers a beam with physical dimensions in the focal spot of approx. 230 x 230 µm FWHM and a divergence of < 5 mrad (Figure 2). Despite the low power, the efficient coupling of the micro-beam generator with the Xenocs optic provides a high intensity beam with performance equivalent to that of traditional rotating anode generators, but with the benefit of lower facilities and maintenance requirements.

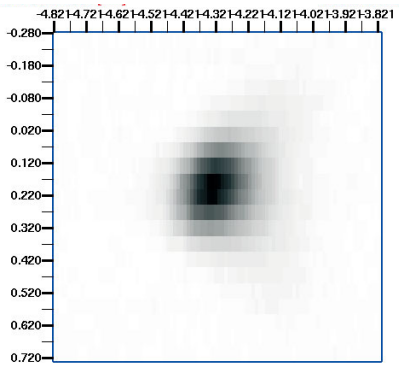

Figure 2: primary beam at focal distance in a 1 x 1 mm area as scanned by the dtb.

X-ray Detector System

The ***mar345*** image plate detector (Figure 3) is a very low noise image plate detector that delivers superb data quality. The scan times are as short as 34 seconds for a 180 mm diameter scan and only 66 sec for a 345 mm diameter scan. The pixel size is either 100µ or 150µ.

The "***desktop beamline***" (***dtb***) is a very powerful single axis goniometer system. A unique feature is the capability of automatically finding and optimizing the X-ray beam. It is also very helpful for characterizing the X-ray beam.

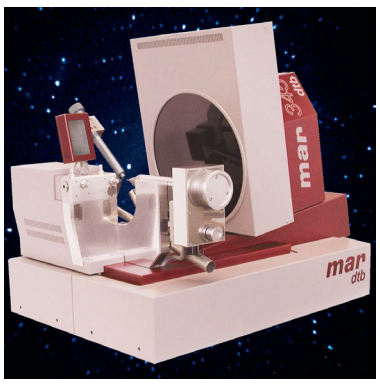

Figure 3: ***mar345*** image plate detector mounted on a "desktop beamline"

Crystal

Protein	Hen egg white lysozyme
Space group	P 4_3 2_1 2
Unit cell parameters	a=b=79.1 Ang. c=37.9 Ang.
Molecular weight	14 kDa (129 amino acid residues)
Anomalous scatterers	8 sulfurs in disulfide bridges (Cys-Cys) 2 sulfurs in methionines 7 chloride anions
Size of crystal	400µ x 300µ x 200µ
Mosaicity	< 0.15°

Figure 4: Lysozyme crystal used for data collection

Data collection

System	mar345dtb on GeniX High Flux
Generator power	50 Watt (50 kV, 1 mA)
Optics	Xenocs GeniX High Flux multilayer mirrors
Temperature	100 K
Distance crystal-detector	100 mm
Exposure time per image	120 sec
Total data collection time	21 h 35 min
Delta-PHI per image	1.0°
Detector read-out mode	345 mm diameter @ 150µ pixelsize
Total no. of images	372
Low resolution limit	55 Ang.
High resolution limit	1.55 Ang.

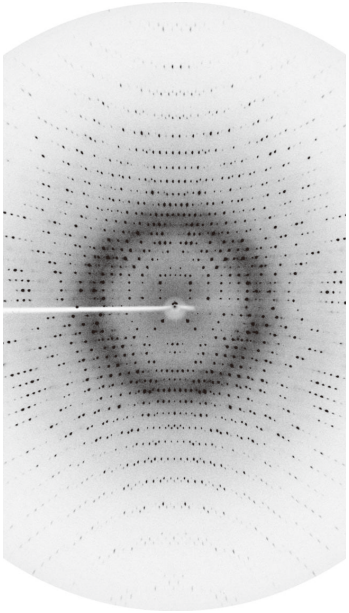

Figure 5: Lysozyme diffraction image

Data processing

Data were processed using the autoMAR program package (i.e. programs marProcess for data reduction, marPost for data merging and marScale for scaling).

Property	Overall	Inner shell	Outer shell
Low resol. limit	27.9	27.1	4.53
High resol. limit	1.55	1.57	1.55
R_{merge}	3.12	2.05	13.33
R_{meas}	3.24	2.13	14.47
R_{pim}	0.86	0.56	5.31
R_{ano}	1.5	1.7	3.5
$\langle I/\sigma \rangle$	13.8	27.9	4.2
Multiplicity	27.2	25.7	18.1
Anom. multiplicity	14.7	15.9	12.2
Anom. completeness	99.6	99.2	97.4

Structure solution and phasing

Program SHELXD[2,3,4] has been instructed to locate 17 anomalous scatterers: 8 sulfurs in disulfide bridges, 2 sulfurs from methionines and 7 chloride anions.
SHELXD located all of them. The best solution was used for phase calculation and further improvement by density modifications with program SHELXE[5]. The automatic tracing of the final experimental map shown in Figure 6 is very straightforward. It does not differ much from the density of the final model (Figure 7).

SHELXD	
High resolution limit	1.7 Ang.
CC all / weak	38.05 / 22.54
PATFOM	4.82
No. of located peaks	17

SHELXE	
High resolution limit	1.55 Ang.
No. of cycles	50
Contrast (enantiomorph)	0.378 (0.259)
Connectivity (enantiomorph)	0.906 (0.864)
Pseudo-free CC (enantiomorph)	72.13 (50.11) %

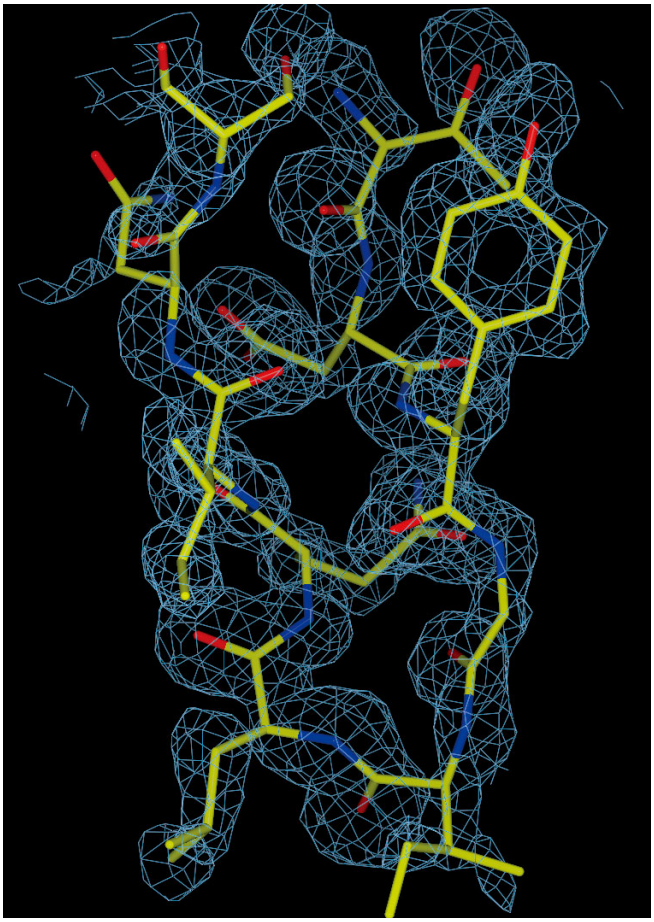

Figure 6: Electron density of experimental map. Phases from 17 anomalous scatterers after density modifications with SHELXE. Residues 51-60 shown here.

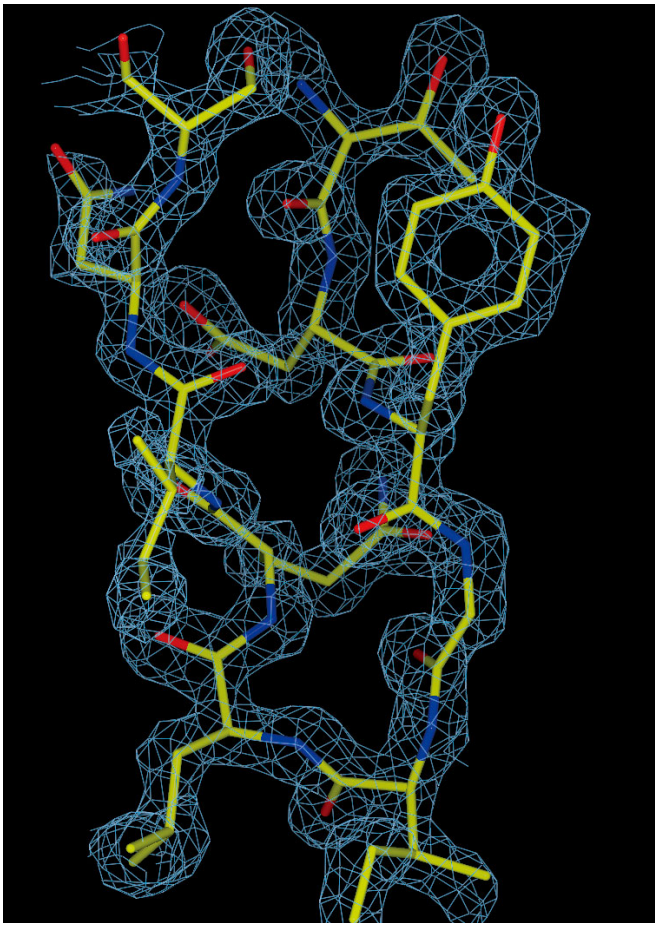

Figure 7: Final 2Fo-Fc map after refinement with REFMAC5.

Phasing from fewer data

In order to assess the relationship between multiplicity of the data and success of automatic phasing, the available range of data of 372 degrees was split up in smaller ranges, always starting with image no. 1. Phasing was tried to 1.7 Ang. resolution using SHELXD/E. The table indicates that the completeness with 30 images and 60 images, respectively, is not sufficient to give a clean solution. Amazingly, as little as 90 degrees of data with a multiplicity of < 10 already give the correct solution. All anomalous scatterers are correctly located and the experimental map is of similar quality as the one shown in Figure 6.

No. of images	30	60	90	120	150	180	360
Multiplicity	3.2	4.5	**6.6**	8.8	11.0	13.2	**26.3**
Completeness	67.2	95.5	**99.4**	99.4	99.4	99.8	**99.9**
CC all	17.4	13.0	**25.4**	29.0	33.0	33.8	**38.1**
(weak)	10.4	6.3	**13.7**	16.4	19.5	19.0	**22.5**
PATFOM	4.92	1.89	**2.07**	1.60	3.06	3.19	**4.65**
Contrast	0.296	0.289	**0.408**	0.400	0.390	0.392	**0.378**
(enantiomorph)	0.281	0.279	**0.315**	0.281	0.261	0.263	**0.257**
Connectivity	0.799	0.865	**0.912**	0.909	0.908	0.910	**0.908**
(enantiomorph)	0.790	0.865	**0.870**	0.850	0.852	0.848	**0.860**
Pseudo-free CC	49.9	54.1	**70.5**	65.5	66.6	70.2	**71.8**
(enantiomorph)	49.8	57.6	**58.1**	53.5	51.2	53.7	**57.4**

Conclusion

The combination of a Xenocs GeniX High Flux micro-beam generator and a marCCD image plate detector and goniometer system is a relatively low cost complete X-ray setup capable of delivering extremely good data in relatively short time. Due to the high photon flux and the small size of the beam in the focal spot the system can be particularly useful for analyzing even small crystals of sizes < 100 µm. It can be used for screening crystals as well as for collecting complete data sets and allows for ab-anitio structure determination with methods like sulfur SAD phasing.

References:

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