Application Note SC-XRD 508

Low Multiplicity Sulfur SAD phasing of Lysozyme

Shutterless data collection using the PHOTON 100

Introduction

Sulfur SAD phasing using Cu Kα radiation requires the collection of very accurate data. To improve the chances of success, sulfur SAD data sets are collected with a high multiplicity to improve the data quality. However, high multiplicity data sets can be time consuming and difficult to obtain if the samples are sensitive to radiation damage. The PHOTON 100 utilizes CMOS technology which allows for the implementation of advanced features such as shutterless operation which are not available for older imagers like CCDs or Imaging Plates. In shutterless mode, the sample is constantly exposed to X-rays and continuously rotated, which maximizes data acquisition efficiency and, even more importantly, eliminates mechanical jitter, resulting in superior data quality, faster.

Data collection

Data were collected at 100 K on a flash-cooled single crystal of HEW lysozyme using a Bruker D8 VENTURE diffraction system. The D8 VENTURE (Figure 1) features the microfocus IμS sealed-tube source equipped with HELIOS MX optics, KAPPA goniostat and the PHOTON 100 detector. The attributes of the D8 VENTURE make it ideal for dealing with small protein crystals (Table 1). A data set with an overall multiplicity of 8-fold was collected in 1.8 hours. The relevant statistics are shown in Table 2. Figure 2 shows an image of the crystal with after data collection. The yellow tint of the crystal indicated that the sample had experienced some radiation damage during the data collection.

Figure 1: D8 VENTURE featuring a PHOTON 100 detector, IμS microfocus source.

Data processing

The data collection and reduction was carried out using the PROTEUM2 software suite[1].The data were integrated and scaled using SAINT[1] and SADABS[1] respectively.

Figure 2. Lysozyme crystal after X-ray exposure.

Protein and Crystal

- 129 residues, 8 Cys, 2 Met
- Space group: $P4_32_12$
- Unit cell: $a=b=77.4$, $c=37.9$ Å
- Crystal size: 0.070 x 0.087 x 0.117 mm^3

Structure determination

XPREP[1] was used to generate anomalous difference coefficients and phase shifts. The sulfur substructure was determined using XM (SHELXD)[2]. With the resolution truncated at 3.0 Å, XM found 9 sulfur sites (8 Cys and 1 Met). Density modification and phase extension out to 1.78 Å was then carried out with XE (SHELXE)[3]. XE produced initial protein phases and a polyalanine model containing 109 residues. The initial XE phases produced a very interpretable map and were input into the 'Phase and Build' application in the PHENIX[4] software suite in order to complete the model. The polyalanine trace was used as a template for model building. PHENIX was able to build in 116 residues and assigned side chains for 110. A quick refinement of the initial model produced the electron density map shown in Figure 3. The missing residues included a 10 residue surface loop and 3 residues at the C-terminus. However, there was visible electron density in both regions which would have allowed the missing residues to be incorporated into the model but this was beyond the scope of this project. The entire process took about 3 hours to complete.

Electron density map

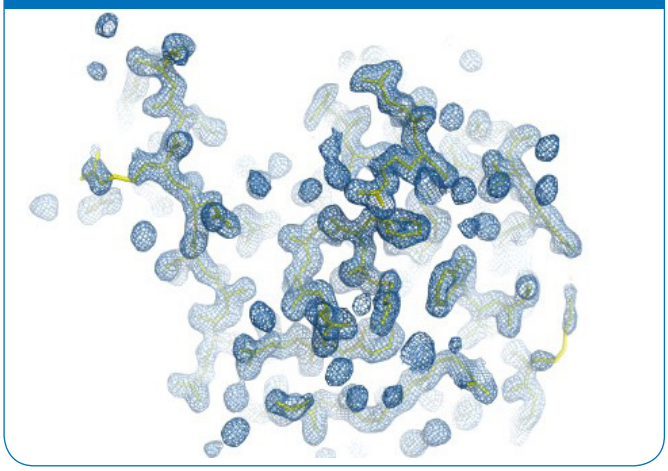

Figure 3: Electron density map after the initial model refinement contoured at 1σ. The resulting model is shown in yellow.

Conclusion

The successful phasing from a low multiplicity data set highlights the very high data quality that can be achieved with the D8 VENTURE. The shutterless method of data collection helps reduce systematic errors and produces more accurate data. The advanced features offered in the D8 VENTURE make it much easier to investigate more difficult projects.

Component	Feature
IμS X-ray source with HELIOS MX optics	High intensity source, very stable and focused beam, no maintenance
KAPPA goniostat	High precision mechanics, enhanced flexibility during data collection
PHOTON 100 CMOS detector	High sensitivity, shutterless operation

Table 1: Enhanced features of the D8 VENTURE

Data collection and processing statistics	
Resolution (Å)	1.78 (1.88 – 1.78)
Exposure time (sec)	12
Rotation angle (°)	0.2
Degrees collected (°)	110
Measurement time (min)	110
R_{int} (%)	6.87 (52.0)
$I/\sigma(I)$	34.2 (2.85)
Multiplicity	7.74 (7.1)
Completeness (%)	99.9 (99.8)

Table 2: Data collection and processing statistics

References

1. Bruker AXS (2014). PROTEUM2, Version 2014.5, Bruker AXS Inc., Madison, Wisconsin, USA.
2. Schneider, T.R. and Sheldrick, G.M., (2002). Acta Cryst. D58, 1772-1779.
3. Sheldrick, G.M., (2002). Z. Kristallogr. 217, 644–650.
4. Adams, P. D., et al, (2010). Acta Cryst. D66, 213-221.

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