

Application Note SC-XRD 392

## Thaumatococcus S-SAD

### In-House Sulfur-SAD Phasing of Thaumatococcus daniellii

#### Introduction

The crystal structure of Thaumatococcus daniellii was determined by sulfur-SAD phasing methods using data collected on a D8 VENTURE diffraction system (Figure 1). The D8 VENTURE features a new CMOS detector, the PHOTON 100. Using one crystal, a complete data set was collected to 1.65 Å in less than 3 hours. The experimental phases derived from the anomalous signal of the sulfur atoms allowed 95% of the protein backbone to be traced.

#### Protein and crystal data

- 207 residues, 16 Cys, 1 Met
- Space group:  $P4_12_12$
- Unit cell:  $a=b=57.9 \text{ \AA}$ ,  $c=149.9 \text{ \AA}$
- Crystal size:  $0.12 \times 0.12 \times 0.25 \text{ mm}$



Figure 1. D8 VENTURE with TXS microfocus rotating anode, HELIOS MX optics, KAPPA goniometer and PHOTON 100 detector.

Component	Feature
Turbo X-ray source with Helios MX optics	High intensity X-Ray source, Very stable beam
KAPPA goniostat	High precision mechanics, Enhanced flexibility during data collection (true high multiplicity data)
PHOTON 100 CMOS detector	High sensitivity for detecting weak reflections

Table 1. Instrument requirements for in-house S-SAD

## Experimental data

### Data collection

A single crystal of Thaumatin was flash-cooled at 100K. Data were collected on a Bruker D8 VENTURE diffraction system. The 12 fold overall multiplicity of the data was much lower than normally required for S-SAD phasing. The D8 VENTURE features a new TXS HB microfocus rotating anode generator equipped with HELIOS-MX optics, KAPPA goniostat and the new PHOTON 100 detector (Table 1). The TXS is directly mounted to the goniometer base allowing down-stream alignment which minimizes systematic errors. The combination of the TXS HB source, flexible KAPPA goniostat, CMOS detector and the PROTEUM2<sup>(1)</sup> software suite made it possible to obtain a high-quality 1.65 Å dataset within a few hours.

### Data processing

The data sets were integrated and scaled using SAINT<sup>(1)</sup> and SADABS<sup>(1)</sup> respectively. XPREP<sup>(1)</sup> software was used for space group determination and for data analysis. (Table 2).

### Structure determination

The sulfur substructure was determined using XM (SHELXD)<sup>(2)</sup>. With the resolution truncated at 3.5 Å, XM found 16 sulfur sites (15 Cys and 1 Met). Density modification and phase extension out to 1.65 Å was then carried out with XE (SHELXE)<sup>(3)</sup>. The refined heavy atom sites output after the initial round of density modification were used in the final cycles to produce experimental phases. The polyaniline model autotraced by XE was 95% complete containing 202 of the 207 residues present in the known thaumatin structure. Electron density for many of the side chain atoms was clearly visible in the final map (Figure 2).

### Conclusion

The crystal structure of Thaumatin was successfully solved by SAD phasing methods using data collected on a D8 VENTURE system. Because of the very weak anomalous signal from

Data collection and processing statistics	
Resolution (Å)	1.65 (1.70 - 1.65)
Exposure time (sec/deg)	20
Total measurement time (hrs)	2.3
$R_{int}$ (%)	5.6 (37.2)
$R_{p.i.m.}$ (%)	1.73 (12.5)
I/s(I)	30.5 (5.4)
Completeness (%)	99.7 (97.1)
Multiplicity	11.2 (7.0)

Table 2. Data collection and processing statistics

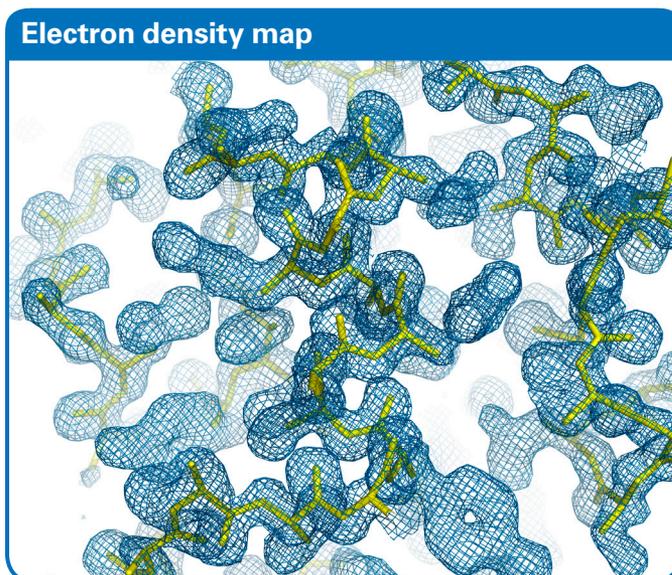


Figure 2. Experimental electron density map contoured at  $1\sigma$ . The main chain atoms traced by XE are shown in yellow.

native sulfur, typically multiplicities on the order of 40 are necessary for successful S-SAD phasing<sup>(4)</sup>. The successful phasing from a relatively low multiplicity data set in this case highlights the very high quality of the data. The combination of the new high-brilliance TXS microfocus rotating anode, KAPPA goniostat, PHOTON 100 detector and PROTEUM2 software allow high-quality S-SAD data to be collected in the home lab.

### References

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4. Douth, J., Hough, M.A., Hasnain, S.S. & Strange, R.W., (2012). *J. Synchrotron Rad.* **19**, 19-29.

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