Application Note PX022

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PX022 - Thaumatin S-SAD phasing with 2 hours of data collection on the XtaLAB Synergy-S

Introduction

We recently introduced a sealed-tube 4-circle diffractometer for small molecule and macromolecular crystallography, the XtaLAB Synergy-S. It features three new technologies in a compact cabinet that will fit into any home laboratory: microfocus PhotonJet-S sources (with Cu, Mo, or Ag targets), an ultrafast (10° per second) Kappa goniometer, and a HyPix-6000HE hybrid photon counting detector with 0.1 mm by 0.1 mm pixels. Here, we collected a two-hour data set on a thaumatin crystal to demonstrate the quality of data from the XtaLAB Synergy-S system using a protein crystal with an average size unit cell.

Experimental overview

T. daniellii thaumatin was purchased from Sigma and suspended in ultrapure, laboratory grade water to a concentration of 50 mg/ml. Crystals of thaumatin were grown at 18˚C from hanging drops consisting of 6 µL of 50 mg/ml thaumatin plus 4 µL of crystallization solution [24% (w/v) NaKTartrate, 15% (v/v) ethylene glycol, and 0.1 M BisTris Propane, pH 6.6] over a well containing 500 µL of crystallization solution. Prior to flash-cooling, each crystal was soaked for ~15 to 30 seconds in the above crystallization solution at 25% (v/v) ethylene glycol for cryoprotection. The XtaLAB Synergy-S system and detector specifications are listed in Table 1.

Table 1: XtaLAB Synergy-S specifications.

X-ray source	PhotonJet-S Cu source with continuously variable divergence slit Beam size = 100 µm
Operating power	50 kV x 1 mA = 50 W
Goniometer / Detector range	4- circle Kappa with telescoping 2θ arm / distance range of 30 – 250 mm
Detector Active area Readout time Pixel size Cooling	Hybrid photon counting HyPix-6000HE 77.5 x 80.3 mm Continuous (7 ns) 100 µm air-cooled

Data collection was performed using CrysAlis^{Pro}, processing was performed using XDS[1], and structure solution was performed using various programs through HKL-3000R[2]. A large (0.6 mm x 0.3 mm x 0.3 mm) thaumatin crystal was selected for data collection to illustrate the worst-case scenario for spot spread due to beam divergence (Figure 1).

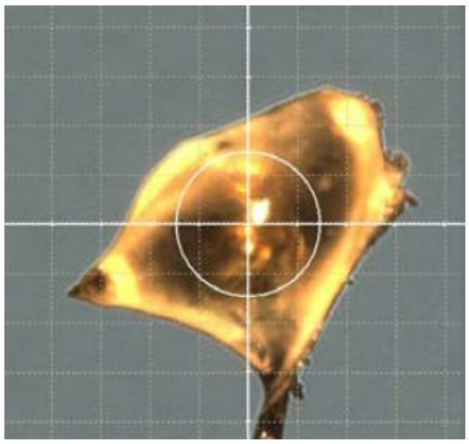

Figure 1: Thaumatin crystal. Grid spacing is 0.1 mm. Circle is 0.3 mm.

Results

The thaumatin crystal diffracted to beyond 2 Å and auto-indexing revealed a primitive, tetragonal unit cell a = b = 57 Å and c = 149 Å. Spot separation along the longest primitive cell edge was achieved using the full divergence of the X-ray beam and a detector distance of 60 mm (Figure 2).

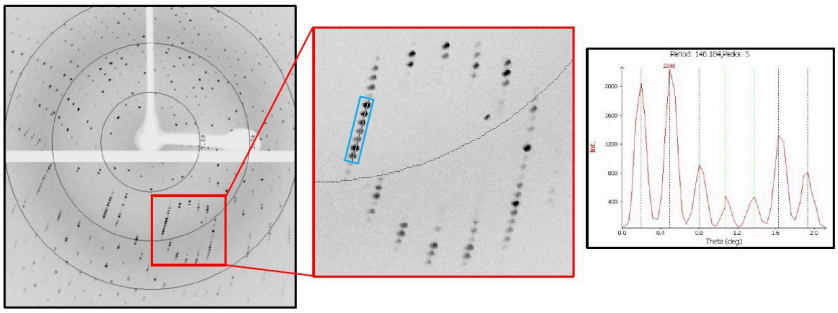

Figure 2: 20-second exposure per 0.2° rotation of the thaumatin crystal at distance of 60 mm on the HyPix-6000HE. The resolution rings are 7.09, 3.74, and 2.68 Å. Zoomed in panel shows lunes along the 150 Å cell edge. Peak separation and d-spacing of spots boxed in blue is shown in the plot at right.

Six scans, calculated by the strategy algorithm of CrysAlis^{Pro}, were collected to achieve complete, redundant data to 1.4 Å in 2 hours (Table 2).

Table 2: Scans collected on thaumatin.

Scan #	ω start	ω range	Δω	φ	κ	2θ	No. img	Time/img	Total exposure
1	-136°	186°	0.15°	-180°	0°	-37.8°	1240	2s	41m 20s
2	55°	37.95°	0.15°	84°	-171°	36.9°	253	2s	8m 26s
3	60°	64.05°	0.15°	-150°	178°	36.9°	427	2s	14m 14s
4	-27°	27°	0.15°	-97.7°	112°	36.9°	180	2s	6m

5	-119°	118.05°	0.15°	30°	-57°	-37.8°	787	2s	26m 14s
6	-58°	108°	0.15°	-30°	-19°	-37.8°	720	2s	24m
									2h 14s

Table 3 shows the results of data processing. The data were scaled to 1.4 Å treating the Friedel pairs as different reflections because the high redundancy allowed it. This resulted in a 100% complete data set with an R_{merge} under 10%. To locate the 17 sulfur sites and phase the data, SHELXD[3] was run for 500 trials and the best solution had a CC_{All} = 25.1, CC_{Weak} = 11.4, PATFOM = 0.93, and 14 sites with high occupancy. The 14 sites were used in phasing to 1.5 Å with SHELXE, refinement of sites and phase improvement with MLPHARE[4], and density modification and phase extension to 1.4 Å with DM[5]. The Figure of Merit after density modification was 0.87. From the anomalous difference Fourier, 2 additional sulfur sites were picked up and the phasing process repeated. The phases were handed to ARP/wARP[6] for 2 cycles of automated model building which produced an initial model containing 205 aa. This model was manually adjusted in Coot[7] and refined for 10 cycles with Refmac[8] which gave an R = 15.0% and an R_{free} = 16.2%. Figure 3 shows the model and maps near R53.

Table 3: Crystal parameters and processing statistics for Thaumatin with Friedel pairs unmerged.

Space group	$P4_12_12$
Unit cell (Å)	57.8, 57.8, 149.8
Resolution (Å) (last shell)	20-1.4 (1.44-1.40)
Total # reflections	698447
Unique # reflections	95481
Completeness (%) (last shell)	99.9 (100)
Multiplicity (last shell)	7.3 (5.2)
<I/σ(I)> (last shell)	19.6 (1.7)
R_{merge} (%) (last shell)	7.3 (88.8)
$CC_{½}$ (last shell)	99.9 (57.7)
R / R_{free} (%)	15.0 / 16.2

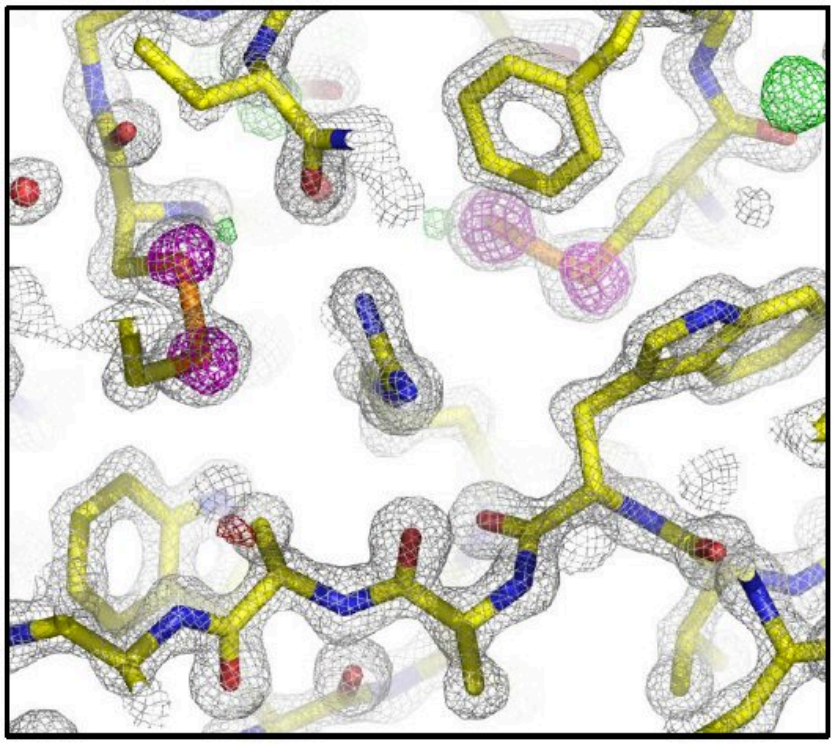

Figure 3: 2Fo-Fc (grey mesh @ 2 rmsd), Fo-Fc (green and red meshes @ 3 and -3 rmsd, respectively), and anomalous difference (magenta mesh @ 5 rmsd) electron density maps. R53 is in the center flanked by two disulfide bonds.

Conclusion

The crystal structure of *T. daniellii* thaumatin was solved by S-SAD phasing to 1.4 Å using a data set collected in 2 hours on the XtaLAB Synergy-S system. This solution was possible in such a short time because of the high flux and brilliance of the system combined with the speed, accuracy, and precision of the ultrafast goniometer and HyPix-6000HE detector.

References

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Related products

CrysAlis^{Pro}

User-inspired data collection and data processing software for small molecule and protein crystallography.

HyPix-6000HE

Extremely low noise detector based on direct X-ray detectio n technology.

XtaLAB Synergy-S

Our most popular diffractometer for Chemical Crystallograp hy and Mineralogy, configured with either single or dual mic rofocus sealed tube X-ray sources and an extremely low noi se direct X-ray detection detector.