Application Note PX024

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PX024 - Lysozyme S-SAD phasing with two minutes of data collection on the XtaLAB Synergy-Custom

Introduction

A modern, home laboratory X-ray diffraction system with the latest in photon counting detector technology should deliver high-quality data in a short amount of time. Rigaku Oxford Diffraction currently promotes the XtaLAB SynergyCustom outfitted with a MicroMax™-007 HF rotating Cu anode, VariMax optics, ultrafast Kappa goniometer, and a HyPix-6000HE hybrid photon counting detector as the gold standard for a home laboratory X-ray diffraction system. To back up that claim, we present here the results of a 2.25-minute data collection on a 0.2 mm lysozyme crystal along with the sulfur SAD phasing of the data set.

Experimental overview

Lysozyme from chicken egg white (Sigma L6876) was suspended in 0.1 M NaOAc pH 4.4 to make a 40 mg/mL solution. Cryoprotected tetragonal crystals of lysozyme were grown at 18˚C from sitting drops consisting of 2 µL of lysozyme plus 2 µL of crystallization solution [7% NaCl, 0.1 M NaOAc pH 4.4, 18% ethylene glycol] over a well containing 400 µL of crystallization solution. All system specifications are listed in Table 1. Data collection and processing was performed using CrysAlis^{Pro}, and structure solution was performed using various programs through HKL-3000R[1]. A picture of the mounted crystal is shown in Figure 1. Data collection temperature was 100 K.

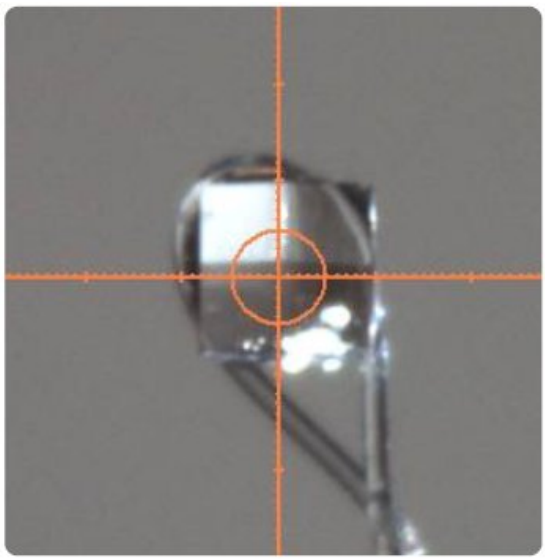

Figure 1: Lysozyme crystal. Grid spacing is 0.1 mm. Circle is 0.1 mm diameter.

Table 1: XtaLAB Synergy-S specifications.

X-ray source	MicroMax-007HF Cu
Operating power	40 kV x 30 mA = 1.2 kW
X-ray optic	Confocal VariMax VHF
Beam characteristics	FWHM = 100 µm, Divergence = 10 mrad (adjustable) Adjusted to 6 mrad here
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

Results

The lysozyme crystal diffracted to beyond 1.6 Å (Figure 2) and auto-indexing revealed a primitive, tetragonal unit cell a = b = 78 Å and c = 37 Å. The slit was adjusted to reduce the beam divergence to 6 mrad and reduce the size of the diffraction spots. Three scans, calculated by the strategy algorithm of CrysAlis^{Pro}, were collected to achieve complete, redundant data to 1.7 Å in two minutes (Table 2). The speed of data collection was 1.25° per second.

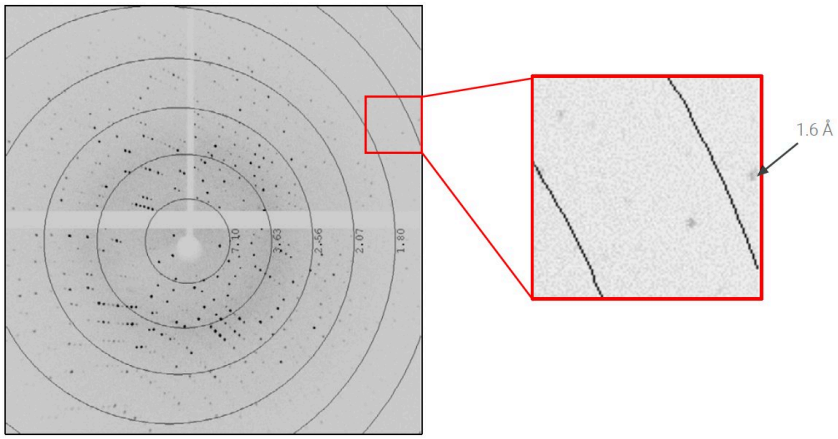

Figure 2: 0.2-second exposure per 0.25° rotation of the lysozyme crystal at distance of 35 mm.

Table 2: Scans collected on lysozyme at 35 mm

Scan #	**ω_{start}**	**ω_{end}**	**Total rotation**	**Δω**	**φ**	**κ**	**2θ**	**# img.**	**Exp. per image**	**Total exp.**
1	-80°	8°	88°	0.25°	-171°	-59°	-7.34°	352	0.2s	1m 10s

2	-11°	40°	51°	0.25°	-90°	-99°	6.403°	204	0.2s	41s
3	-11°	21.5°	32.5°	0.25°	60°	-99°	6.403°	130	0.2s	26s

Table 3 shows the results of data processing with CrysAlis^{Pro} followed by scaling to 1.6 Å with AIMLESS[2]. This resulted in a 97.5% complete data set with an R_{merge} under 4% and an overall multiplicity of 8.5. To locate the 10 sulfur sites and phase the data, SHELXD[3] was run for 1000 trials and the best solution had a CC_{All} = 24.0, CC_{Weak} = 10.0, PATFOM = 1.31, and 14 sites with high occupancy (some of the sites are chloride ions). The 14 sites were used in phasing to 1.7 Å with SHELXE, refinement of sites and phase improvement with MLPHARE[4], and density modification and phase extension to 1.6 Å with DM[5]. The Figure of Merit after density modification was 0.81. The phases were passed to ARP/wARP[6] for three cycles of automated model building, which produced an initial model containing 129 aa. This model was manually adjusted in Coot[7] and refined for 10 cycles with Refmac5[8], which gave an R = 15.3% and an R_{free} = 17.9%. Figure 3 shows the model and maps.

Table 3: Crystal parameters and processing statistics for lysozyme

Space group	$P4_32_12$
Unit cell (Å)	78.8, 78.8, 36.8
Resolution (Å) (last shell)	26.27 – 1.6 (1.63 – 1.60)
Total # reflections	132191
Unique # reflections	15466
Completeness (%) (last shell)	97.5 (75.9)
Multiplicity (last shell)	8.5 (2.8)
<I/σ(I)> (last shell)	34.3 (6.3)
R_{merge} (%) (last shell)	3.8 (15.7)
$CC_{½}$(last shell)	100 (96.8)
R / R_{free} (%)	15.3 / 17.9

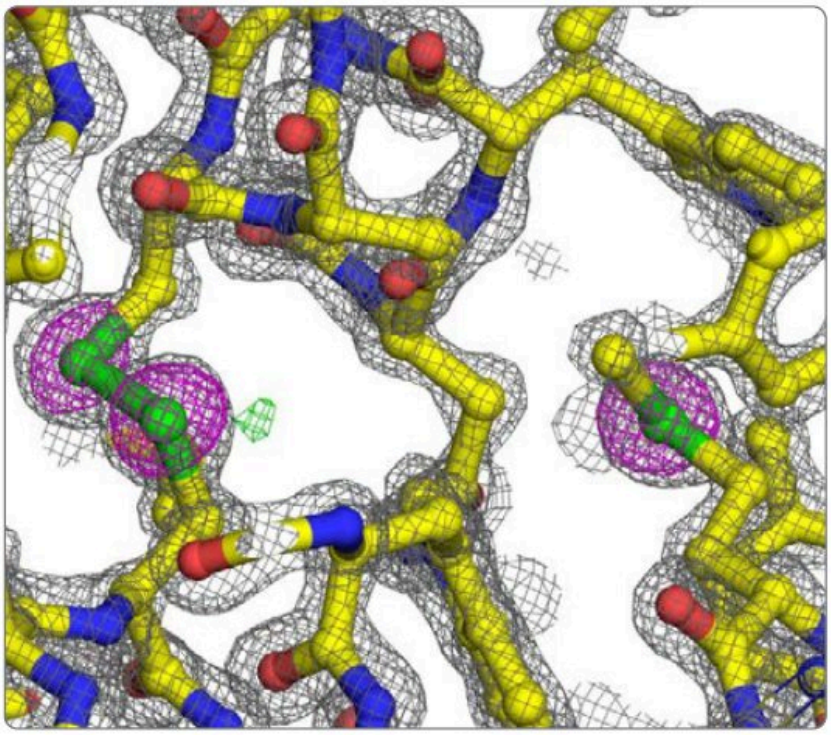

Figure 3: Detail of lysozyme refined model. 2Fo-Fc (grey mesh @ 2 rmsd), Fo-Fc (green and red meshes @ 3 and -3 rmsd, respectively), and anomalous difference (magenta mesh @ 5 rmsd) electron density maps. The disulfide between Cys 115 and 30 is on the left and Met 105 is on the right.

Conclusion

The crystal structure of hen egg white lysozyme was solved by S-SAD phasing to 1.6 Å using a data set collected in 2.25 minutes on the XtaLAB SynergyCustom system with a MicroMax-007 HF source. 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

HyPix-6000HE

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

VariMax

Single wavelength Confocal Max-Flux (CMF) optics for singl e crystal diffraction

XtaLAB SynergyCustom

A bespoke, extremely high-flux diffractometer with custom enclosure and the flexibility to utilize both ports of the rotati ng anode X-ray source.

MicroMax-007 HF

Microfocus rotating anode X-ray generator