Application Note PX021

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PX021 - I-SAD phasing of the HIV integrase core domain using data collected on the XtaLAB Synergy-S

Introduction

The XtaLAB Synergy-S is our microfocus sealed-tube 4-circle diffractometer for small molecule and macromolecular crystallography. It features three new technologies in a compact cabinet that will fit into any home laboratory: a 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 an overnight data set on a crystal of the HIV integrase core domain (IN) to demonstrate iodine SAD phasing on a real-world laboratory sample.

Experimental overview

A plate of IN crystals was generously provided by Professor Michael Parker and Dr. Michael Gorman from Bio21 Molecular Science and Biotechnology Institute at The University of Melbourne. The general preparation, purification, and crystallization of the IN protein was similar to previous description.[1] The IN protein was crystallized in condition 30 of the Qiagen NeXtal $AmSO_4$ Suite (0.2 M potassium iodide and 2.2 M ammonium sulfate). The XtaLAB Synergy-S system and detector specifications are listed in Table 1. Data collection and processing were performed using CrysAlis^{Pro} and structure solution was performed using various programs through the interface of HKL-3000R[2]. An IN crystal (0.3 mm x 0.2 mm x 0.2 mm) was looped from its crystallization plate and passed through Paratone N oil before flash cooling in liquid nitrogen (Figure 1).

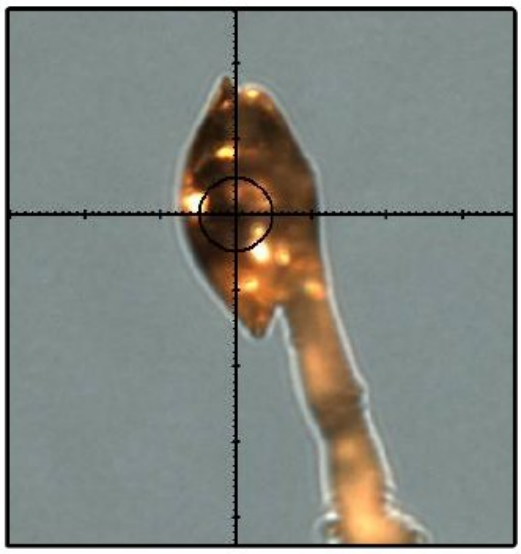

Figure 1: IN crystal. Circle is 0.1 mm.

Table 1: XtaLAB Synergy-S specifications

X-ray source	PhotonJet-S Cu source with continuously variable divergence slit
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

Results

The IN crystal showed visible diffraction to ~2.1 Å with visible ice rings, and auto-indexing revealed a primitive, tetragonal unit cell a = b = 46 Å and c = 140 Å (Figure 2).

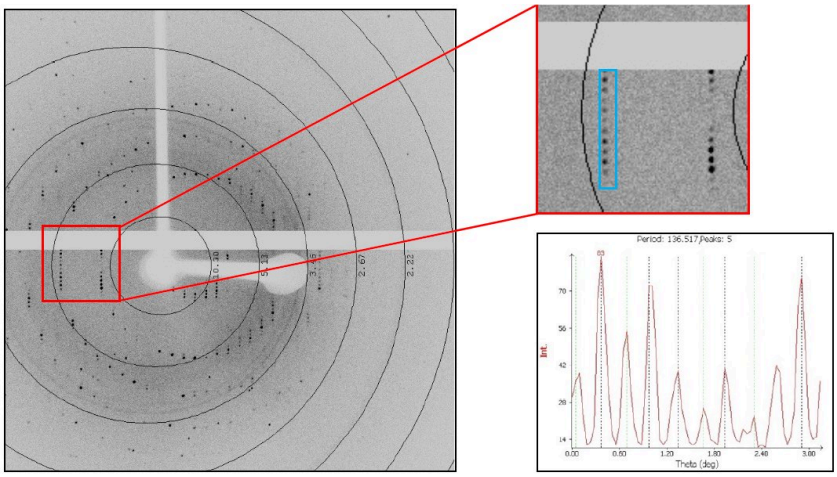

Figure 2: 35-second exposure per 0.15° rotation of the IN crystal at distance of 55 mm on the HyPix-6000 HE. The resolution rings are 10.3, 5.13, 3.46, 2.67, 2.22 and 1.94 Å. Zoomed in panel shows lunes along the 140 Å cell edge. Peak separation and d-spacing of spots boxed in blue is shown in the plot at the bottom right.

Three scans, calculated by the strategy algorithm of CrysAlis^{Pro}, were collected to achieve complete, redundant data to 1.9 Å in 15 hours (Table 2).

Table 2: Scans collected on the IN crystal

Scan #	ω start	ω range	Δω	φ	κ	2θ	No. img	Time/img	Total exposure

1	-47°	145.05°	0.15°	-98°	35°	11.598°	967	35s	9h 24m 5s
2	-4°	45°	0.15°	120°	-99°	-12.145°	300	35s	2h 55m
3	-55°	46.05°	0.15°	150°	77°	-12.145°	307	35s	2h 59m 5s
									15h 18m 10s

The data were integrated and scaled to 2.05 Å with CrysAlis^{Pro} and then rescaled using AIMLESS[3] (Table 3). This resulted in a nearly 100% complete data set with an R_{merge} of 6%.

Table 3: Crystal parameters and processing statistics for IN

Space group	$P4_32_12$
Unit cell lengths (Å)	45.6, 45.6, 139.6
Resolution (Å) (last shell)	23.81–2.05 (2.11–2.05)
Total # reflections	97275
Unique # reflections	9943
Completeness (%) (last shell)	100 (100)
Completeness Anom (%) (last shell)	100 (100)
Multiplicity (last shell)	9.8 (6.9)
Multiplicity Anom (last shell)	5.2 (3.7)
<I/σI> (last shell)	24.9 (2.2)
R_{merge} (%) (last shell)	5.8 (86.8)
R_{merge} Anom (%) (last shell)	4.8 (81.4)
$CC_{½}$ (%) (last shell)	100 (80.1)

There was a large anomalous signal in the data, likely due to the iodide ions present in the crystallization conditions (Figure 3).

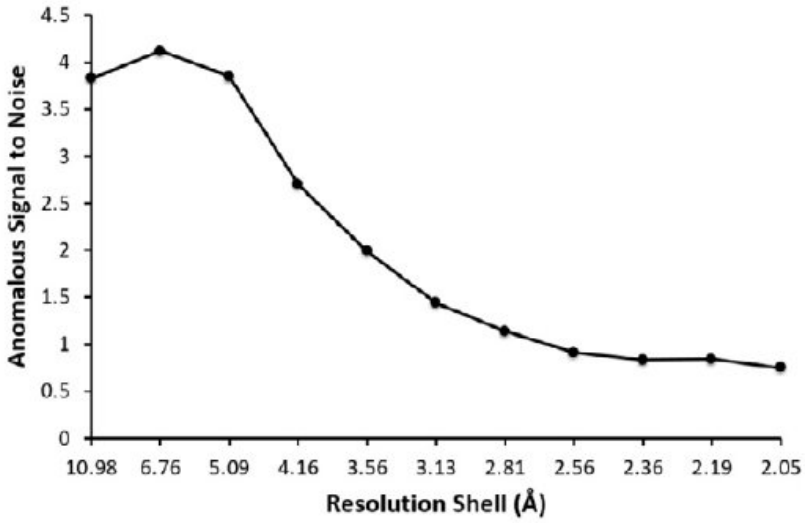

Figure 3: Anomalous signal to noise plot from SHELXC. Noise level cutoff shown as dotted red line.

To locate the iodide substructure atoms, SHELXD[4] was run for 1000 trials looking for five iodides with a resolution cutoff of 2.5 Å. A substructure solution with six sites (CC_{All} = 40.1, CC_{Weak} = 21.4, PATFOM = 10.18) was used for phasing with SHELXE to 2.05 Å. The Figure of Merit after density modification with DM[5] was 0.84. A partial model was automatically built by ARP/wARP[6] and it was further adjusted by hand in COOT[7], waters and six iodide ions were added, and the resulting model was refined using REFMAC[8] (R = 24.8% and R_{free} = 31.1%; Figure 4).

Figure 4: Cartoon of IN structure showing anomalous difference peaks in cyan at 4 rmsd.

Conclusion

The perfect combination of a modern sealed tube Cu X-ray source (PhotonJet-S Cu) with the most sensitive detector (HyPix-6000HE) and a sophisticated data collection and processing program (CrysAlis^{Pro}) exists today in the XtaLAB Synergy-S. Given a strong anomalous scatterer like iodine and a well-diffracting regular sample like the IN crystal, an

overnight data set with the XtaLAB Synergy-S yields complete, redundant data that can be solved by SAD phasing—even with ice rings present in the data.

References

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