Application Note PX023

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PX023 - ClpS SAD phasing with 30 minutes of data collection on the XtaLAB Synergy-S

Introduction

Rigaku's XtaLAB Synergy-S is much more than a crystal screening instrument. It comes equipped with a powerful source, an ultrafast kappa goniometer, and a hybrid photon counting detector to enable fast data collections on macromolecular crystals. Here, we show a 30-minute data collection on an orthorhombic crystal of ClpS. The data are complete to 1.28 Å and the structure can be solved by single wavelength anomalous dispersion (SAD).

Experimental overview

Cryocooled crystals of ClpS were provided by the laboratory of Karl Schmitz (University of Delaware). The crystallization condition was 10% PEG3350, 7.5 mM $NiCl_2$, 0.1 M HEPES pH 7.5. The PEG3350 was increased to 40% before flash-cooling in liquid nitrogen. 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
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

The crystal was transferred from liquid nitrogen storage dewar to the goniometer using cryotongs. The crystal was kept at 100 K using an Oxford Cryosystems Cryostream 800. Data collection and processing were performed using CrysAlis^{Pro}, and file preparation and structure solution were performed using various programs of CCP4[1]. A large 0.25 mm x 0.3 mm x 0.4 mm crystal was used for data collection (Figure 1).

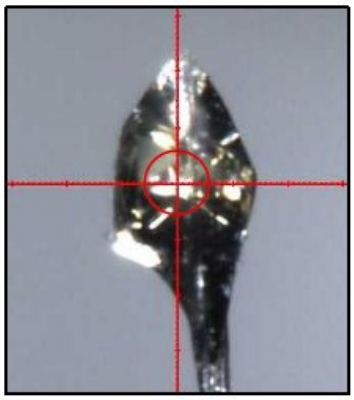

Figure 1: ClpS crystal. Grid spacing is 0.1 mm. Circle is 0.1 mm.

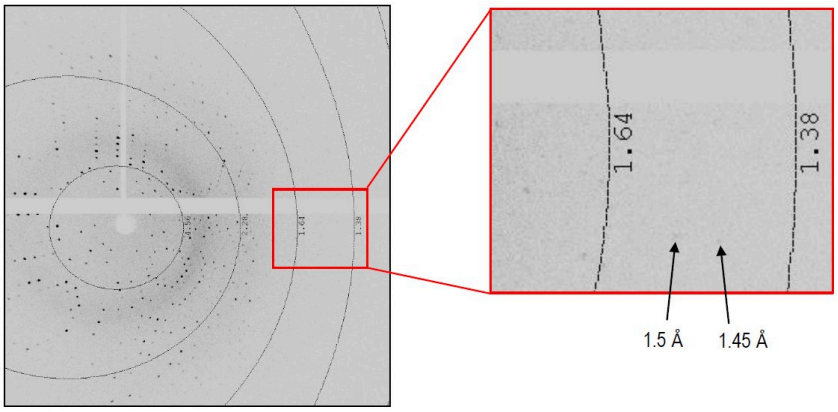

Figure 2: 0.8-second exposure per 0.25° rotation of the ClpS crystal at distance of 32 mm and 2θ of 22.8° on the HyPix-6000HE. The resolution rings from left to right are 4.56, 2.28, 1.64, and 1.38 Å. Zoomed panel shows weak, high resolution spots.

The ClpS crystal was screened using a crystal to detector distance of 32 mm and 0.8 second exposures per 0.25° rotation. The highest resolution diffraction spots were visible to ~1.45 Å (Figure 2). Autoindexing revealed a primitive, orthorhombic unit cell with a = 51 Å, b = 52 Å and c = 54 Å. Ten scans, calculated by the strategy algorithm of CrysAlis^{Pro}, were collected to achieve complete, 9-fold redundant data to 1.3 Å in 30 minutes (Table 2).

Table 2: Scans collected on the ClpS crystal.

Scan #	ω start	ω range	Δω	φ	κ	2θ	No. img	Time / img	Total exposure
1	-58°	61°	0.25°	-152°	105°	-23.35°	244	0.8s	3m 15.2s
2	-48°	91°	0.25°	118°	86°	-23.35°	364	0.8s	4m 51.2s
3	-32°	81°	0.25°	-10°	41°	-23.35°	324	0.8s	4m 19.2s
4	2°	26°	0.25°	-120°	77°	22.8°	104	0.8s	1m 23.2s
5	2°	26°	0.25°	-180°	77°	22.8°	104	0.8s	1m 23.2s
6	2°	26°	0.25°	120°	77°	22.8°	104	0.8s	1m 23.2s

7	-1°	55°	0.25°	-60°	-99°	22.8°	220	0.8s	2m 56s
8	-51°	79°	0.25°	-150°	-19°	22.8°	316	0.8s	4m 12.8s
9	-28°	72°	0.25°	0°	-77°	22.8°	288	0.8s	3m 50.4s
10	-93°	45°	0.25°	60°	-178°	-23.35°	180	0.8s	2m 24s
									29m 58s

Table 3: ClpS processing statistics.

Space group	$P2_12_12_1$
Unit cell (Å)	51.5, 52.2, 54.7
Resolution (Å) (last shell)	24.2-1.28 (1.30-1.28)
Total # reflections	360644
Unique # reflections	38669
Completeness (%) (last shell)	99.8 (97.2)
Multiplicity (last shell)	9.3 (4.7)
<I/σ(I)> (last shell)	22.0 (1.9)
R_{merge} (%) (last shell)	4.9 (75.1)
R_{meas} (%) (last shell)	5.2 (84.2)
R_{pim} (%) (last shell)	1.6 (36.5)
$CC_{½}$ (last shell)	1.0 (0.63)
R / R_{free} (%)	12.9 / 16.3

The resulting data were integrated and scaled to a limit of 1.28 Å where the last shell had 97% completeness, a suitable mean signal-to-noise ratio of 1.9, and a high $C_{½}$ of 0.63 (Table 3). This resulted in a 99% complete data set with an R_{merge} of 5%. The data had a small but measurable anomalous signal above the level of noise (0.8) to ~2 Å as reported by SHELXC[2] (Table 4). Using the Matthews coefficient, the solvent content was estimated at 39% with two molecules of ClpS in the asymmetric unit. Therefore, the potential anomalous scatterers were the 4 sulfurs per ClpS chain (only in Met residues) and any ordered Ni ions from the crystallization condition. To try to locate the 8 sulfur sites and phase the data, SHELXD[2] was run to 2.2 Å for 4000 trials and the best solution had a CC_{All} = 31.0, CC_{Weak} = 16.8, CFOM = 47.8, and 11 sites with high occupancy. The 11 sites (8 sulfurs and 3 Ni ions shown in Figure 3) were used in phasing to 1.28 Å with the PHASER SAD pipeline (PHASER[3], PARROT[4], BUCCANEER[5]). The resulting model was subjected to several rounds of adjustment in COOT[6] and refinement with REFMAC[7] to give an R = 12.9% and an R_{free} = 16.3%. A total of 156 residues were modeled, residues 5 to 80 in chain A and 1 to 80 in chain B (Figure 3).

Table 4: Anomalous signal to noise ratio per resolution shell.

Shell	**Mean anomalous signal to noise**
37.75 – 6.86	1.27

6.86 – 4.22	1.25
4.22 – 3.18	1.11
3.18 – 2.60	0.93
2.60 – 2.22	0.88
2.22 – 1.96	0.89
1.96 – 1.76	0.84
1.76 – 1.60	0.78
1.60 – 1.47	0.77
1.47 – 1.37	0.75
1.37 – 1.28	0.75

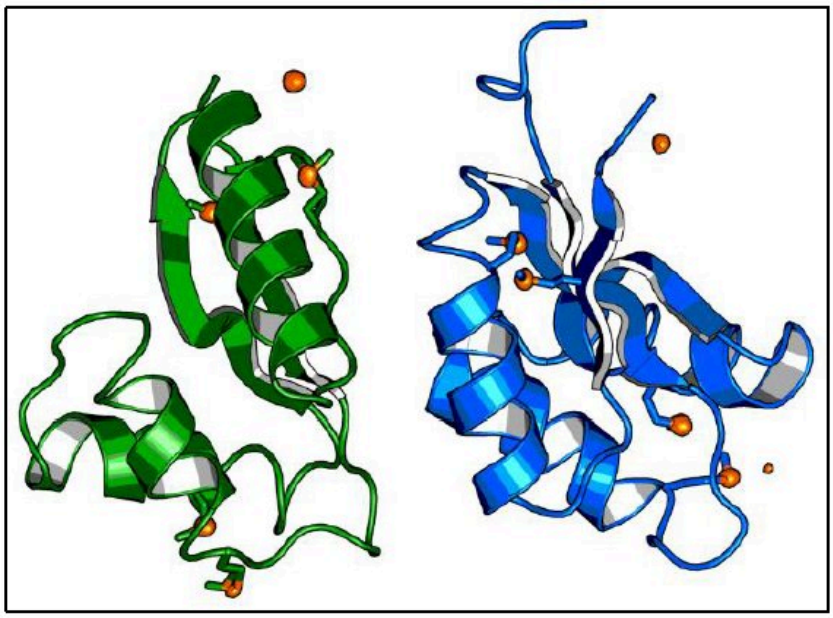

Figure 3: Cartoon ribbon of the 2 molecules of ClpS in the asymmetric unit (chain A is green and chain B is blue). All 8 Met residues are shown in stick representation. The anomalous difference map (orange) is contoured at 5 rmsd, revealing 11 peaks.

Results

Interestingly, the three well-ordered Ni^{2+} ions identified by their anomalous peaks appear to promote intermolecular interactions between crystallographic symmetry mates of ClpS. The Ni^{2+} ions were coordinated in octahedral geometry with water molecules and at least one side chain (a His or Glu) in two different molecules of ClpS (Figure 4). Given that ClpS functions as a monomer in solution, the contacts appear to be a result of the crystallization process.

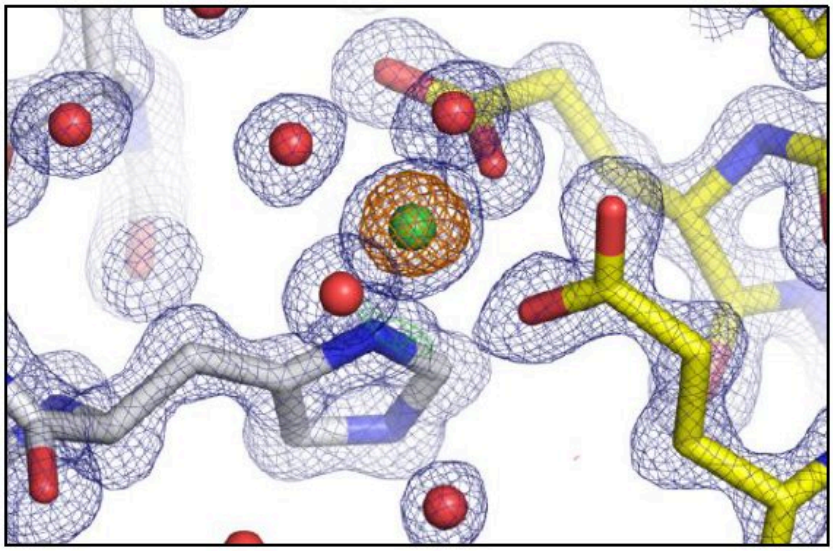

Figure 4: 2Fo-Fc (blue mesh @ 2 rmsd), Fo-Fc (green and red meshes @ 3 and -3 rmsd, respectively), and anomalous difference (orange mesh @ 5 rmsd) electron density maps. Strongest Ni^{2+} ion peak is shown at center.

Conclusion

A high-resolution, 1.28 Å data set was collected on a ClpS crystal in just 0.5 hrs using the XtaLAB Synergy-S. The data were of superior quality as evidenced by the data processing statistics and even more so by the success of SAD phasing—despite a small anomalous signal and multiplicity of less than 10 overall. Clearly, a modern microfocus sealed tube diffractometer can be the workhorse instrument for the home laboratory.

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.