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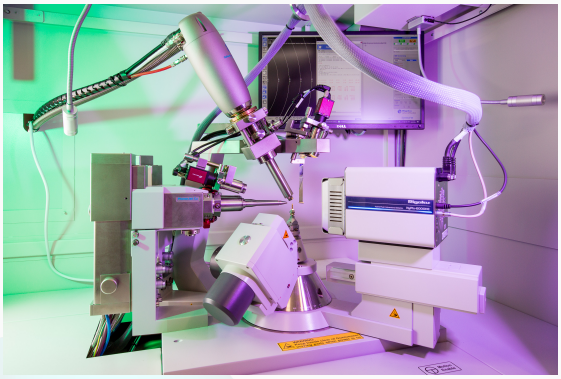

S-SAD phasing of HEWL using data collected on the Synergy-S

Introduction

- The XtaLAB Synergy-S is a microfocus sealed-tube 4-circle diffractometer for small molecule and macromolecular crystallography. Here, we collected diffraction data from a single cryo-cooled crystal of Hen Egg White Lysozyme (HEWL) to demonstrate Sulphur SAD phasing.

Experimental Overview

- 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 PHENIX-AutoSol[1]. A single crystal (~0.2 mm^3) was looped from its hanging drop (Figure 1), passed through Parabar 10312 (formerly known as Paratone N), then mounted directly on the goiniometer (Figure 2).

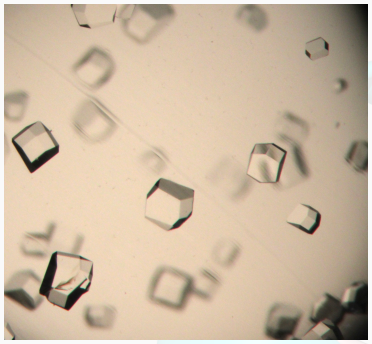

Figure 1: HEWL crystals grown in hanging drops. Mother liquor contains 5% NaCl, 100 mM Sodium Acetate pH 4.5.

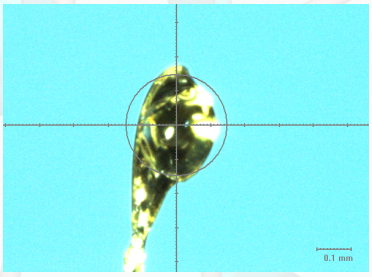

Figure 2: HEWL crystal. Circle is 0.3 mm. Crystal mounted in Paratone N as a cryoprotectant.

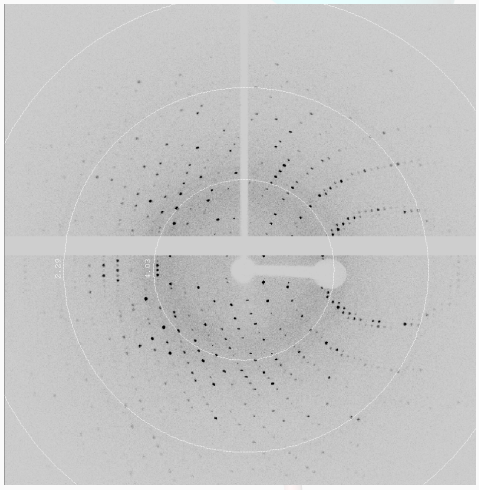

Figure 3: 1sec exposure per 0.15° rotation of the HEWL crystal at a distance of 33 mm. Diffraction extended to ~ 1.5Å resolution.

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 2Theta 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^2 Continuous (7 ns) 100 µm air-cooled

Table 1: XtaLAB Synergy-S specifications.

Results

- The HEWL crystal showed visible diffraction to ~1.5 Å (Figure 3). Auto-indexing revealed a primitive, tetragonal unit cell with a = b = 78.29 Å and c = 37.44 Å. To accurately measure the small anomalous differences due to the presence of Sulphur atoms, a dataset with a high degree of multiplicity was necessary.
- Data collection was calculated with the strategy algorithm of CrysAlis^{Pro} to achieve a complete, highly redundant data to 1.8 Å. In total, 2194 frames covering 329° of data split into 12 scans over 40 mins were collected.

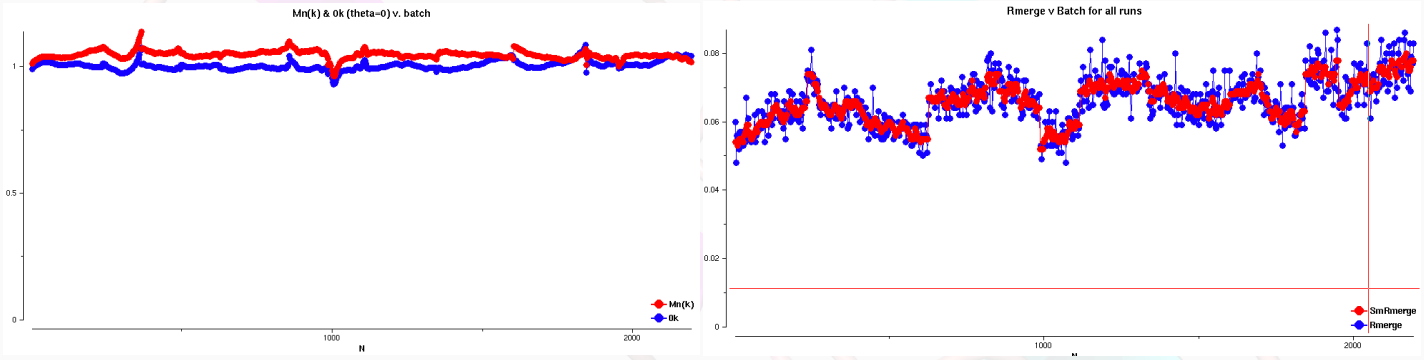

Figure 4: Output from AIMLESS showing Scales (L) and R_{merge} (R) vs frame number. Minimal radiation damage during the data collection is seen by the similar scale factors and R_{merge} throughout data collection.

Space group	$P4_32_12$
Unit cell lengths (Å) angles (˚)	78.29, 78.29, 37.44 90, 90, 90
Resolution (Å) (last shell)	20.58 – 1.8 (1.84 – 1.80)
Total # reflections	581467
Unique # reflections	11295
Completeness (%)	100 (100)
Completeness Anom (%)	100 (100)
Multiplicity	51.5 (25.5)
Multiplicity Anom	26.6 (12.6)
<I/σI>	59.7 (13.4)
$R_{p.i.m}$ (%)	0.9 (5.5)
$R_{p.i.m}$ Anom (%)	1.2 (7.6)
$CC_{1/2}$ (%)	100 (98.8)

Table 2: Crystal parameters and processing statistics for HEWL.

The data were integrated and merged to 1.8 Å with CrysAlis^{Pro} and then scaled using AIMLESS[2] (Figure4). This resulted in a 100% complete data set with an overall $R_{p.i.m}$ of only 0.9% (Table 2).

To locate the Sulphur substructure atoms, PHENIX-AutoSol[1] was used to look for the predicted 10 Sulphur atoms (8 from Cysteine and 2 from Methionine residues). A substructure solution containing 19 sites (FOM 45.9%) was found and was subsequently used for density modification. The resulting R-factor was 32%.
A model was automatically built from the experimentally phased electron density maps using PHENIX-AutoSol. This auto-built model contained 125 residues (out of 132), 174 waters, R_{work} = 21.3%, R_{free} = 23.6% and a map-model CC of 80%; (Figures 5 + 6).

The extra peaks in the anomalous difference Fourier maps were due to the presence of Chloride atoms present in the mother liquor. At the Cu Ka wavelength of 1.5418Å, Chlorine and Sulphur have an anomalous signal of 0.697e and 0.569e respectively.

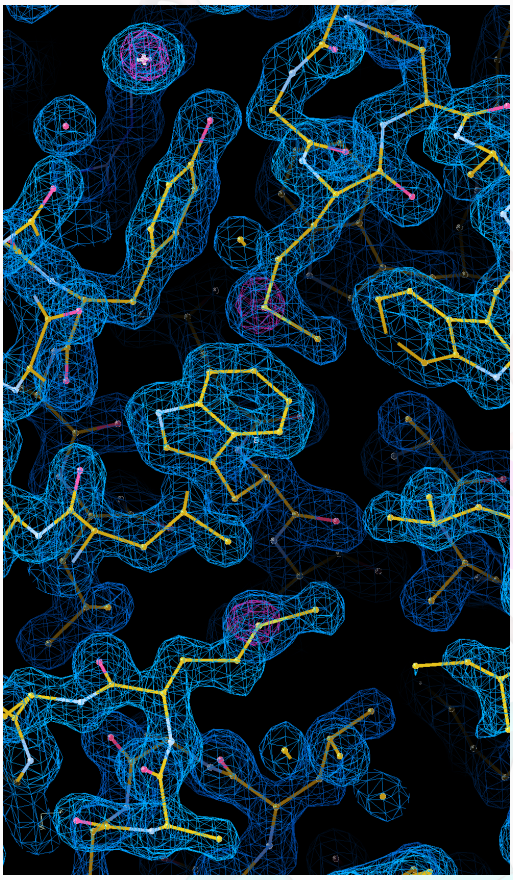

Figure 6: Electron density maps showing anomalous difference peaks in purple at 4σ. Figure generated using COOT[3].

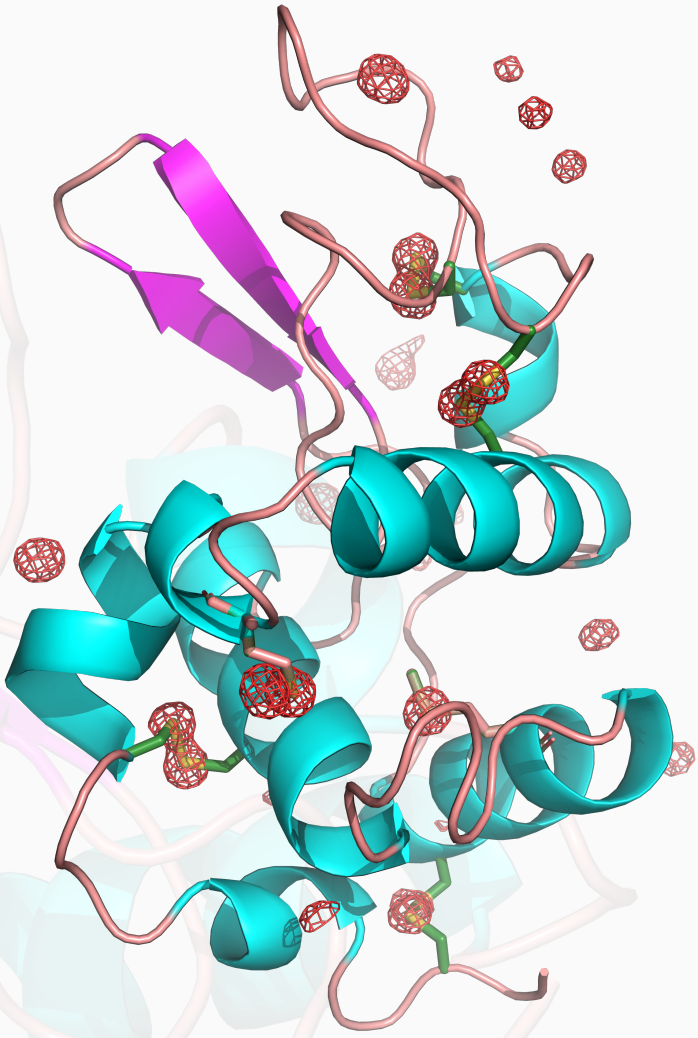

Figure 5: Cartoon of the auto-built HEWL structure showing anomalous difference peaks in red at 4σ. Peaks are due to the Sulphur and Chlorine atoms. Figure generated using PyMol[4].

Conclusion

- The combination of a sealed tube Cu X-ray source (PhotonJet-S Cu) with a direct photon counting HyPix-6000HE detector together with a sophisticated data collection and processing program (CrysAlis^{Pro}) enabled a well-diffracting sample like HEWL crystals to yield a complete, highly redundant data that can be solved by S-SAD phasing in ~ 40mins.

References

1. Grosse-Kunstleve R.W. and Adams P.D. (2003) Acta Cryst. D59, 1966-1973.
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