Application Note PX030

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PX030 - Using CrysAlis^{Pro} to tackle macromolecular non-merohedral twins

Introduction

Non-merohedral twinning is characterized by having two or more crystal lattices in different orientations for which the twin law does not belong to the crystal system or metric symmetry. This can be a property of the internal growth of a single crystal or the result of multiple, intergrown crystals. In any case, non-merohedral twinning of macromolecular crystals results in very chaotic diffraction patterns and overlapping reflections. These diffraction patterns can be a challenge to index and to integrate depending on your data collection and processing package. In fact, one is normally justified in discarding a twinned crystal in favor of another crystal that is not twinned. However, a twinned crystal might end up being the only or the best diffracting crystal available. With that situation in mind, here, we present the data collection and processing for a lysozyme sample with three lattices by using CrysAlis^{Pro} to illustrate an approach for tackling this problem.

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 CCP4[1]. A picture of the mounted crystal is shown in Figure 1. Data collection temperature was 100 K.

Table 1: XtaLAB SynergyCustom specifications.

X-ray source	FR-X
Operating power	45 kV x 66 mA = 2.97 kW
X-ray optic	Confocal VariMax VHF
Beam characteristics	FWHM = 100 µm Divergence = 10 mrad
Goniometer / Detector range	4-circle Kappa with telescopic 2θ arm / distance range of 35 – 210 mm
Detector **Active area** **Frame rate**	Hybrid photon counting HyPix-Arc 150° 77.5 x 121.8 mm² Up to 70 Hz

Pixel size	100 µm
Cooling	water-cooled

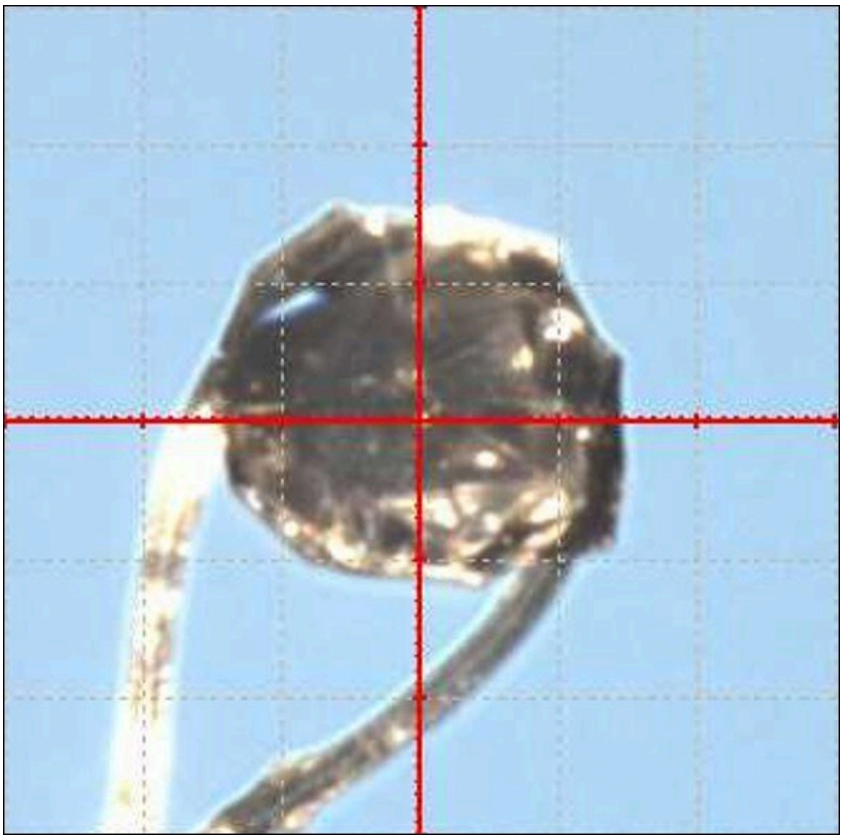


Figure 1: The crystal of lysozyme selected because it looked like several crystals intergrown. The gridlines are 0.1 mm apart.

Results

A single lysozyme crystal that looked like several intergrown crystals was selected (Figure 1) and screened. To maximize the separation of reflections from multiple lattices, the divergence of the optic was reduced to ~1.5 mrad (~4.5% of maximum beam intensity) and the rotation width was reduced to a minimum of 0.2°. At least two lattices were present in the diffraction pattern (Figure 2). Two scans, calculated by the strategy algorithm of CrysAlis^{Pro}, were collected to achieve complete, 10-fold redundant data to 1.5 Å in 10 minutes (Table 2). Note that the crystal rotations are 0.1° and that the goals for completeness and redundancy were only applied to the first lattice (twin component) indexed.

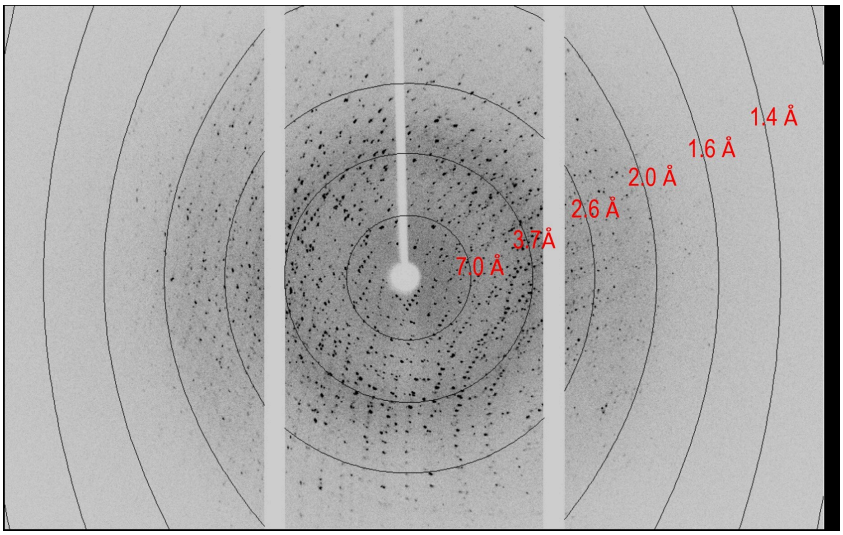


Figure 2: A 3-second exposure per 0.2° rotation of the LY crystal at distance of 41 mm and 2θ of -0.63°.

Table 2: Scans collected on lysozyme at 41 mm.

Scan #	φstart	φend	Total rotation	Δφ	ω	k	2θ	# img	Exposure per image	Total Exposure
1	2°	117°	88°	0.1°	-35.8°	-20°	-0.59°	1150	0.3 s	5 m 45 s
2	120°	191°	51°	0.1°	-35.8°	-20°	-0.59°	710	0.3 s	3 m 33 s
										9 m 18 s

When the entire data set was auto-indexed and twin finding turned on, three lattices were found representing approximately 48%, 31%, and 14% of the found peaks (with 7% unindexed). The data were reduced using the multi-crystal option so that a separate, unmerged mtz file would be generated for each lattice. The final ratios of the twin components to each other was 0.41, 0.32, and 0.27. In addition, the reflection files for all three lattices were merged using the Proffit merge tool, then scaled together, and then exported to an unmerged mtz file. All four mtz files were run through AIMLESS[2] and the resulting scaling statistics are reported in Table 3. Based on the <I/σI> and the $CC_{1/2}$ in the highest resolution shell, lattice 1, 2, and 3 were scaled to resolutions of 1.5 Å, 1.5 Å, and 1.6 Å, respectively. Each data set is fully complete with ~10-fold redundancy overall and in the highest shell. Lattice 1 appears to be the best statistically with the strongest <I/σI> and the lowest R_{merge}. The statistics for all data merged achieve a slightly better high-resolution limit of 1.45 Å with increases in redundancy and R_{merge}.

Table 3: Statistics from data processing and structure refinement.

	Lattice 1	Lattice 2	Lattice 3	All merged
Unit cell	79.0 Å, 79.0 Å, 37.0 Å	78.8 Å, 78.8 Å, 37.0 Å	78.8 Å, 78.8 Å, 37.0 Å	78.8 Å, 78.8 Å, 37.0 Å
Resolution (last shell)	25.6–1.50 Å (1.53–1.50 Å)	27.0–1.50 Å (1.53–1.50 Å)	27.0–1.60 Å (1.63–1.60 Å)	27.0–1.45 Å (1.48–1.45 Å)

Completeness (last shell)	99.9% (100%)	100% (100%)	100% (100%)	100% (100%)
Redundancy (last shell)	10.4 (9.2)	10.3 (9.3)	10.5 (9.9)	30.6 (26.6)
Total reflections	200157 (8655)	198566 (8648)	168300 (7722)	651557 (28016)
Unique reflections	19332 (941)	19233 (931)	15977 (777)	21290 (1053)
<I/σI> (last shell)	11.1 (1.3)	6.8 (1.0)	6.9 (1.1)	11.1 (1.5)
$CC_{1/2}$	0.99 (0.55)	0.99 (0.43)	0.99 (0.51)	0.99 (0.59)
R_{merge} (last shell)	15.2% (142.7%)	22.3% (158.6%)	23.8% (173.1%)	26.0% (270.6%)
R_{meas} (last shell)	16.0% (151.1%)	23.5% (167.9%)	25.1% (182.5%)	26.5% (275.9%)
R_{work}	15.0%	15.3%	15.5%	14.0%
R_{free}	19.9%	20.5%	21.3%	18.0%

Before solving any structure, the Free R flag from lattice 1 was transferred to the other three data sets so that all structure refinements would use the same Free R set. Then, the model from PDB id 2LYZ (with solvent, ligands, and alternate conformations removed) was refined against each data set using Refmac[3]. Each structure was finished with several rounds of manual adjustment in Coot[4] and refinement with Refmac. The last refinements were carried out using anisotropic B factors. Figure 3 shows that each structure was nearly indistinguishable from the next. There were no significant artifacts observed in the electron density maps. The R factors for each of the three individual structures of lysozyme were similar, but the R factors for the structure from the merged data was markedly better (by ~2% in Rf_{ree}).

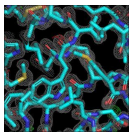
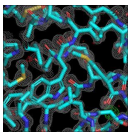
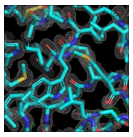
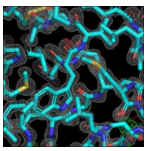
Lattice 1	**Lattice 2**	**Lattice 3**	**All combined**
			

Figure 3: Final structures for four data sets. View is centered on Trp28. Maps shown are 2Fo-Fc in gray (@2 rmsd), positive Fo-Fc in green (@3 rmsd), and negative Fo-Fc in red (@-3 rmsd).

Conclusion

Protein crystals with multiple lattices, or non-merohedral twins, are easily handled by using CrysAlis^{Pro} for data collection and processing. The X-ray beam divergence and the rotation width must be minimized by the experimenter at the time of crystal screening to minimize overlap of diffraction spots and give the best chance for resolving each lattice. As our results here show, a three-component twin is a straightforward and manageable problem. However, the difficulty of

separating individual diffraction patterns will scale with the size of the unit cell. Once the individual lattices are processed and assessed, merging the data sets together should be attempted. As we found here, there may be benefit in both diffraction resolution and final R factors of a structure when the data sets are merged.

References

1. Winn M.D. et al. (2011). *Acta Cryst.* D67, 235-242.
2. Evans P.R., Murshudov G.N. (2013). *Acta Cryst.* D69, 1204-1214.
3. Murshudov G.N., et al. (2011). *Acta Cryst.* D67, 355-367.
4. Emsley P., Lohkamp B., Scott W.G., Cowtan K. (2010). *Acta Cryst.* D66, 486-501.

Related products

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.