

Protein Crystallography GeniX^{3D} Cu High Flux



Application Note n° AN-G12

A b s t r a c t

X-ray beam delivery systems based on low power microfocus sources are being increasingly used in Macromolecular Crystallography (MX) because of their high performance, moderate price and low level of maintenance. These X-ray sources systems perform indeed as well or sometimes even better than traditional rotating anode generators with large source focus (see XENOCS Application Note AN-G8; or MAR Research Application Note from February 07 on www.xenocs.com).

The aim of this application note is to evaluate the new XENOCS X-ray beam delivery system : the GeniX 3D, and to compare it with the standard low power system the GeniX. The two systems have been compared in the context of macromolecular crystallography data collections where test samples of trypsin of different sizes have been used in a standard setup using a MAR345 image plate detector. GeniX 3D did provide better data quality for all tested crystals due to the higher useful flux and slightly smaller focal spot. This is particularly pronounced for small crystals (lower than 150 microns) where a factor of 2 in flux compared to the GeniX has been measured.

The evolution of low-power X-ray beam delivery systems for Macromolecular Crystallography

Data courtesy of Dr Hassan Belrhali, EMBL (European Molecular Biology Laboratory), Grenoble, France.

I n t r o d u c t i o n

The GeniX was introduced four years ago and is today accepted as a high performance system for protein crystallography in the laboratory with high reliability and low cost of ownership. A new low-power X-ray beam delivery system has been developed by XENOCS: the GeniX 3D platform. The GeniX 3D Cu High Flux, which is the model developed for protein crystallography was assessed at the European Molecular Biology Laboratory (EMBL) Grenoble Outstation and directly compared to the standard previous generation GeniX Cu VHF.

E x p e r i m e n t

Protocol

Three trypsin crystals (Leirois et al, 2006, figure 2) of sizes 200, 150 and 80 microns have been compared. Each crystal has been exposed to the GeniX and then to the GeniX 3D sources and highly redundant data set was collected at 100 K with identical exposure times.

Experimental set-up

The experimental set-up (figure 1) was composed of the microfocus source systems (GeniX or GeniX 3D), a MAR base equipped with a horizontal single-phi axis, 2 pairs of slits and related ionisation chambers and a MAR345 image plate detector used under its largest mode (345 mm diameter) and highest resolution (100 micron pixels). All samples were kept frozen during data collection using an Oxford 600 Cryogenic cooler and all slits apertures have been set to 1 mm² for all data collections (full beam illumination) in order to avoid mechanical bias.

X-ray beam delivery systems description

Table I summarizes the performances of the two low-power X-ray source systems tested. GeniX 3D Cu HF is using an X-ray source with a smaller focus size and an increased brightness compared to the previous GeniX Cu VHF. The two systems are coupled with optimal single-reflection FOX 3D multilayer optics. The optical mirror within GeniX 3D Cu HF possesses an increased solid angle of collection. Consequently the overall flux delivered by GeniX 3D is higher while the spot size is slightly smaller when compared to its GeniX equivalent as shown in Table I.

Table I : X-ray beam properties of the two low-power sources

System	GeniX Cu VHF	GeniX 3D Cu HF
Mirror type	FOX 3D 14_39	FOX 3D 8_30
Spot Size at focus (HxV μ m at FWHM)	190x190	175x175
Total Flux (when entire beam-path under vacuum - Mph/s)	300	430
Flux within 100 μ m ² at focal spot (with all system beam path under vacuum-Mph/s)	50	90
Divergence (HxV, mrad at FWHM)	5.4x5.4	~ 6x6

X-ray spot sizes and divergences of the two systems being equivalent, we initiated comparisons in terms of beam intensities and MX data collection performances.



Fig.1 : The GeniX 3D Cu High Flux beam delivery system installed at the EMBL Grenoble outstation.

Slits openings (H = V in mm)		GeniX Ionisation chamber counts		GeniX 3D Ionisation chamber counts (increase relative to GeniX)	
S1	S2	IC1	IC2	IC1	IC2
2	2	27.0	22.7	40.4 (+50%)	34.7 (+53%)
1	1	25.6	21.2	39.1 (+53%)	33.6 (+58%)
0.75	0.75	23.9	19.9	38 (+59%)	32.5 (+63%)
0.50	0.50	16.0	12.6	26.8 (+67%)	22.1 (+75%)
0.25	0.25	5.5	3.0	9.9 (+80%)	6.3 (+110%)

Table II: X-ray beam intensities measured in the ionisation chambers for the two source systems. The indicated (%) values represent the increase obtained with the GeniX 3D for every slit opening.

Beam intensities

The relative X-ray beam intensities have been measured with the MAR base ionisation chambers and are shown in Table II. GeniX 3D full beam intensity has been measured to be superior by almost 50% to the equivalent GeniX one (slit positions of 2x2 and 1x1 mm²). Below 1x1 mm² closure, the GeniX 3D beam intensity was increasingly superior to the GeniX one. At 0.25 x 0.25 mm² we recorded an intensity increase of respectively +80 and +110 % in the first and second ionisation chambers respectively in favour of the GeniX 3D beam. GeniX 3D beam intensity is therefore notably superior when considering smaller beams because of the reduced beam size and higher brilliance of this new platform, in agreement with Table I values.

Data collection and analysis

Three trypsin crystals (Leiros et al, 2006) of different sizes have been consecutively exposed to the GeniX beam and then to the GeniX 3D one and 180 degrees data collected in each case. For every crystal, the exposure time per frame was identical on the 2 sources. All crystals have been mounted with c-axis (long dimension) almost along rotation axis (figure 2.b), however avoiding a perfect alignment to ensure completeness of data collections. All images have been processed with the HKL2000 program suit (Z. Otwinowski and W. Minor, 1997).

Crystallographic data statistics are presented in Table III.

Crystal parameters remained identical as well as overall mosaicity demonstrating that (1) the crystal did not suffer from substantial radiation damages and (2) that the GeniX and GeniX 3D beam divergences are similar (confirming Table I values). If the overall completeness is close to 100 % as expected from 180 degree data collections, it is worth to note that the total number of reflections is always superior with GeniX 3D and this appeared to be more pronounced with the small crystal (+30% of data!). This should be correlated with GeniX 3D higher flux and therefore (I/σ) values that are systematically superior notably at high resolution and increasingly pronounced as samples are getting smaller (trypsin 3).

Both systems are providing excellent data quality as reflected by Linear R-factors and very good and stable c-values (not shown here).

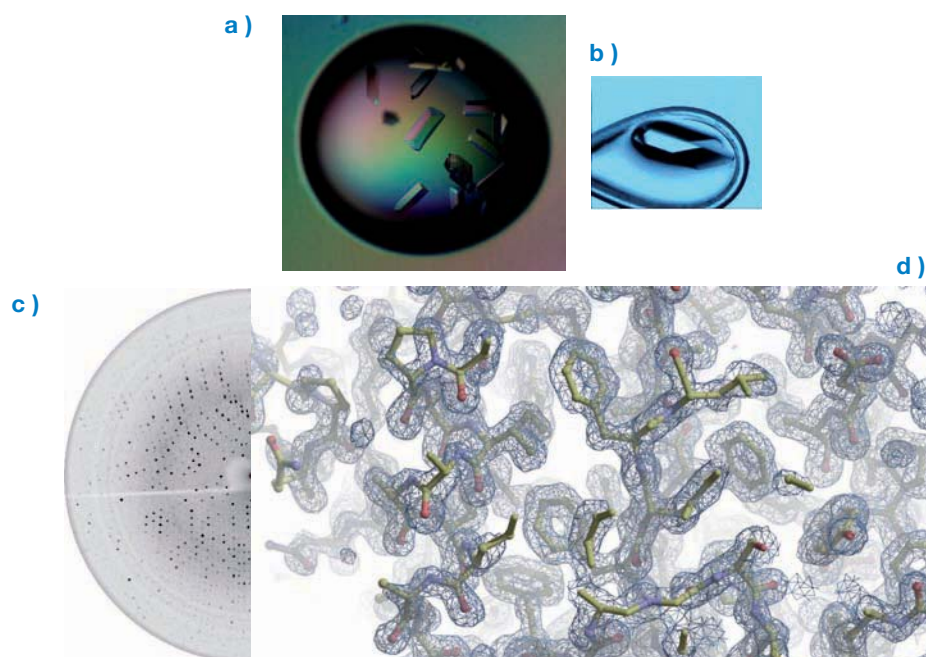


Fig. 2 : From the crystal to the structure. Photos represent from top left to bottom right : a) a drop b) a trypsin crystal in a loop c) a diffraction pattern measured with GeniX 3D d) an electron density map computed with the recorded data

Crystal name	Trypsin 1		Trypsin 2		Trypsin 3	
Crystal size ($\mu\text{m} \times \mu\text{m} \times \mu\text{m}$)	200 x 200 x 600		150 x 150 x 600		80 x 80 x 600	
X-ray Source	GeniX	GeniX 3D	GeniX	GeniX 3D	GeniX	GeniX 3D
Cell parameters ($\text{\AA}, \text{\AA}, \text{\AA}$)	60.17 64.15	60.10 64.17	61.41 63.70	61.35 63.69	60.21 64.15	60.22 64.15
	69.97	69.95	68.91	68.90	69.83	69.91
Mosaicity ($^{\circ}$)	0.63	0.72	1.05	1.04	0.64	0.69
Total rotation range ($^{\circ}$)	180	180	180	180	180	180
Oscillation amplitudes ($^{\circ}$)	1	1	1	1	1	1
Number of spot recorded (% of increase versus GeniX)	98 741	104 552 (+6%)	110 690	116 330 (+5%)	85 328	112 003 (+31%)
Unique reflections	15 218	15 889	16 042	16 153	14379	15 956
Overall completeness (%)	>99	>99	>99	>99	>99	>99
Redundancy	6.5	6.6	6.9	7.2	5.9	7.0
(I/σ) low – high resolution (% of increase versus GeniX)	63 - 6	67-8.5 (+41%)	74-5.5	77-9.9 (+80%)	53-2.5	57-5.7 (+128%)
Resolution limits ($\text{\AA} - \text{\AA}$)	20-2.1	20-2.1	20-2.1	20-2.1	20-2.1	20-2.1
Linear Rf overall (low-high res) %	5.2 (2.3-17)	4.9 (2.9-16)	4.1 (1.8-18.3)	3.8 (2.0-17)	7.3 (2.8-26)	8.7 (2.7-42)
Exposure time (sec)	45	45	60	60	120	120

Table III: Crystallographic data statistics. 3 bovine trypsin crystals of various sizes have been used. All trypsin crystals used belonged to orthorhombic $P2_1 2_1 2_1$ space group and were large needle shaped. The crystal long axis was roughly oriented along the spindle axis during data collection.

Preliminary performance comparisons with a 1200 Watts rotating anode micro-source

Samples Trypsin 1, 2, and 3 have been afterwards exposed to a 1200 Watts rotating anode micro-source (Micromax 007 HF with Varimax HF optics) (data to be disclosed in a later publication). Exposure times have been set so that (I/σ) values at the edge of the detector were equivalent to the one obtained with the GeniX 3D source. Similar complete measurements have been then collected.

It was observed that in order to collect equivalent (I/σ) values at the edge of the detector, the exposure time used with the rotating anode source was only half the one we have set for the GeniX 3D Cu HF. The data qualities were slightly better with the rotating anode micro-source generator, probably due to the slightly lower beam divergence. But for this type of crystals the difference was not impacting the resolution capability.

C o n c l u s i o n s :

GeniX 3D delivers a beam which is 2 times more intense in small dimensions (lower than 150 microns) compared to the previous GeniX system. This is of particular interest in the Macromolecular Crystallography field where typical samples dimension are of 100 microns or smaller. GeniX 3D beam intensity stability and moderate divergence enable excellent crystallographic data to be recorded.

The achieved crystallographic performances when compared to the latest generation of microfocus Rotating Anode Generators for these Trypsin crystals turned out to be of comparable quality with measurement times only twice longer with GeniX 3D.

GeniX 3D Cu HF operating at 30 Watts is a compact, reliable, low-maintenance system providing good performances for MX applications. It turns out to be an excellent low-cost-of-ownership alternative to high power rotating anode generators for data collections, cryogenic condition optimizations and protein crystal screenings prior to synchrotron trips !

References:

Leiros, H.-K.S., et al. (2006) Acta Crystallogr., Sect.D 62: 125.

Z. Otwinowski and W. Minor, Methods in Enzymology, Volume 276: part A, p.307-326, 1997.