

Protein Crystallography GeniX Cu High Flux



Application Note n° AN-G8

Abstract

The GeniX Cu High Flux beam delivery system was compared to a traditional rotating anode generator for protein crystallography applications.

The results obtained with the GeniX compare favorably with those obtained using the rotating anode generator. Considering the much lower cost and maintenance requirements of the GeniX, these results make the GeniX a very attractive alternative to rotating anodes for protein crystallography.



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

Protein crystallography with the GeniX CU High Flux beam delivery system

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

Introduction

At the European Molecular Biology Laboratory (EMBL) Grenoble Outstation the performance of the GeniX Cu High Flux was assessed for protein crystallography and, in particular, compared to a traditional rotating anode system.

Experiment

The experimental strategy was to carry on data collections from identical crystals with 2 different sources:
a) the GeniX beam delivery system : 50 Watts: 50 kV and 1 mA and
b) the EMBL Grenoble rotating anode generator (RAG) : SIEMENS MX18XHF-SRA, 200 μm source, 3 kW, 40 kV–75 mA.

In order to perform a proper relative calibration, the sources were equipped with identical XENOCS FOX optics and have been alternatively interfaced to the MAR single phi-axis base, equipped with a MAR345 image plate detector.

We have used 2 test crystalline samples:

- a single crystal of Porcine Pancreatic Elastase (PPE) as an example of a large crystal ($> 200 \mu\text{m}$)
- a Bovine Trypsin (BT) crystalline sample as an example of a small crystal ($< 100 \mu\text{m}$). The diffraction patterns collected were processed with the MOSFLM and SCALA programs of the CCP4 program suite.

Crystals were frozen at 100 K using an Oxford Cryosystem cooler and alternatively exposed to the 2 sources with identical experimental conditions (see Table I). A complete and highly redundant data set has been collected for each experiment (see Table II and III).

Table I : Source and diffractometer parameters

SOURCE

Source type	Rotating Anode Generator	GeniX
Voltage	40 kV	50 kV
Current	75 mA	1 mA
Power	3000 Watts	50 Watts
Source size	200 μm x 200 μm	60 μm x 60 μm
Optic type	FOX 2D	FOX 2D

DIFFRACTOMETER

Detector	Mar345
Area	345 mm diameter
Pixel size	100 μm
Phi axis	Horizontal
Slit apertures (slits 1, slits 2)	1.5 x 1.5 mm ² ; 1.2 x 1.2 mm ²

Table II : Experimental results with a large Elastase crystal

Crystallographic parameters

	RAG (3kW, 40KV, 75mA)	GeniX (50W, 50kV, 1mA)
Resolution limits	33.0 Å - 2.0 Å	33.0 Å - 2.0 Å
N. of reflections	103091	103198
N. of unique reflections	14558	14961
Completeness	94.7 %	95.0 %
Multiplicity	7.1	6.7
Mean (I)/sd(I)	45.5	33.5
Rmerge	2.8 %	3.5 %
Rpim	1.7 %	2.1 %
Fractional Partial Bias	-0.009	-0.004

Crystal parameters

Enzyme	Porcine Pancreatic Elastase (PPE)
Crystal size	300 μm x 300 μm x 1000 μm
Space Group	P2 ₁ 2 ₁ 2 ₁
Cell parameters	49.6 Å, 57.6 Å, 75.1 Å
Delta phi/image	1°
Overall oscillation	180°
Exposure time/frame	120 sec
Mosaicity	0.30°

Table III : Experimental results with a small Trypsin crystal

Crystallographic parameters

	RAG (3kW, 40KV, 75mA)	GeniX (50W, 50kV, 1mA)
Resolution limits	34.0 Å - 2.0 Å	34.0 Å - 2.0 Å
N. of reflections	101422	102908
N. of unique reflections	15036	15067
Completeness	97.0 %	97.5 %
Multiplicity	6.7	6.8
Mean (I)/sd(I)	32	25
Rmerge	4.3 %	5.4 %
Rpim	2.6 %	3.2 %
Fractional Partial Bias	-0.008	-0.009

Crystal parameters

Enzyme	Bovine Trypsin (BT)
Crystal size	80 μm x 80 μm x 300 μm
Space Group	P2 ₁ 2 ₁ 2 ₁
Cell parameters	54.4 Å, 58.3 Å, 66.9 Å
Delta phi/image	1°
Overall oscillation	180°
Exposure time/frame	300 sec
Mosaicity	0.25°

The ratio between the RAG power to the GeniX one is of 60. If we take the source size into account, the power density ratio is of 5.4. However, despite this substantial difference, it is remarkable that the crystallographic statistics issued from the 2 systems, using the same sample and the same exposure time, are similar as evidenced by the overall [I/Sigma(I)] ratios of 1.35 and 1.28 for the large and small crystal experiment respectively.

As a consequence, the crystallographic quality factors are in both cases of similar values. The Rmerges were of 2.8 % versus 3.5 %, for the large crystal case and of 4.3 % versus 5.4 % for the small crystal, with the RAG and the GeniX sources respectively. The data sets being collected with similar redundancies, the Rpims do follow the same trend.

The mosaicity of the crystals were modest: 0.30° and 0.25° for the elastase and trypsin samples respectively. In both systems the residual divergence of the X-ray beam has been determined to be lower than 0.1°. The median spot size extracted by MOSFLM for both crystalline samples is in both cases of 11x12 pixels. Hence, we can extrapolate that the GeniX system would permit data collection of a crystalline sample with a 220 Å cell parameter aligned along the Phi-axis, at 1.9 Å resolution.

Conclusion

GeniX definitively proves to be a very interesting alternative to the traditional rotating anode in the protein crystallography applications. Indeed this micro-source operating at 50-watts low power provides comparable crystallographic results as a 3 kilowatts rotating anode generator, both equipped with a FOX2D multilayer optic. One could even postulate that the GeniX system might be superior to a RAG equipped with Yale mirrors or multilayer optics based on the double reflection principle.

Hence, GeniX beam intensity is comparable to the one delivered by classical rotating anodes but with a very compact, silent, robust and stable system. Straightforward integrated in a pre-existing instrumental environment, the GeniX has low maintenance and operational costs and offers a user-friendly environment.

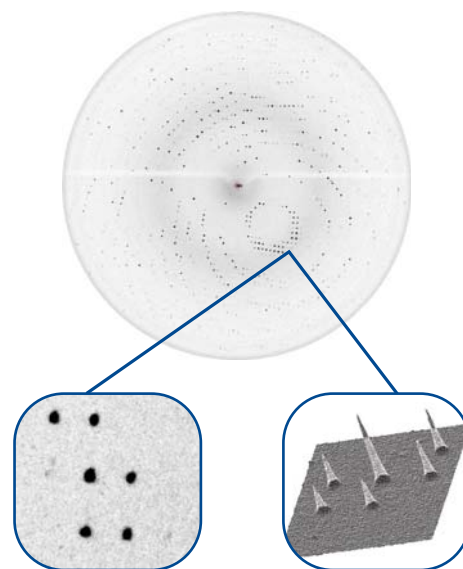


Fig. 2 : Example of an elastase crystal diffraction pattern produced with the Genix system. A zoomed central area where no copper-Kβ contamination is detectable. The diffracted spots present regular Gaussian profiles.

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