Protein crystal analysis: working with smaller crystals

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This application note aims to throw new light on the laboratory X-ray analysis of small protein crystals. Combining a small size source (70µm) and a *FOX2D CU 25_25P* one can still measure, in laboratory conditions, crystals as small as 80 x 30µm².

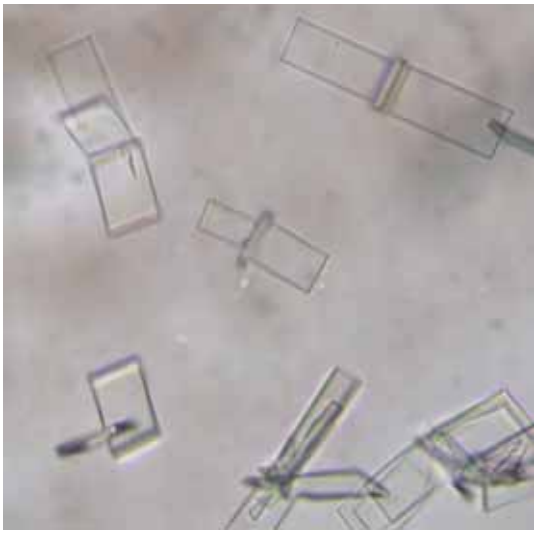
The presented results were obtained with a Micromax 007HF Cu rotating anode generator operating at 40 kV and 30 mA, equipped with a different *FOX2D* mirror on each side, *FOX2D CU 12_38P* and *FOX2D CU 25_25P*, respectively. Data was collected on a MAR345 image plate detector. The detector was operated in 345mm scan mode (mar2300). Experiments were performed successively on two small protein kinase crystals on both experimental setups.

Experiment

Diffraction patterns were collected for two small protein kinase crystals, crystal A of 100 x 40µm², and crystal B of 80 x 30 µm² on both experimental setups. Radiation damage can be significant for such small crystals, so this should be taken into account when considering the data. The diffraction pattern for crystal A was first collected on *FOX2D CU 25_25P* and then on *12_38*, while crystal B was first collected on *FOX2D CU 12_38P* then on *25_25.*

Both crystals diffract in C2 space group. A 200 degrees dataset was collected for each crystal on each setup, using identical parameters, meaning the exposure time, the crystal to detector distance, etc. (one image at every 1 degree, 5 minutes per frame). Data was processed using MOSFLM and SCALA programs.

Diffraction patterns were recorded for crystal A of 100 x 40 µm², on both experimental configurations. Results comparison of the different datasets is presented in the table below.



The biggest crystals on this image are about 80x40x5µm³, representative of the crystals we tested.

Crystal A: Size about 100 x 40 µm²
Crystal to detector distance: 220mm
(2.39Å maximum resolution)

	FOX2D CU 25_25P *(measured fist)*	***FOX2D CU 12_38P*** *(measured second)*
Max intensity	608	1682
Resolution	to 2.39 Å	to 2.39 Å
R_{sym}	7.4% (25.4%)	8.0% (28.1%)
R_{meas} (all I+ and I-)	5.0% (22%)	9.2% (32.8%)
Mean(I)/sd(I)	22.0 (5.0)	21.2 (4.4)
Completeness	96.5% (multiplicity 4.0)	98% (multiplicity 4.0)
	Wilson <B> 34	Wilson <B> 31

As one can see similar results were obtained on both experimental setups for crystal A. The slightly better data obtained with *FOX2D CU 25_25P* mirror configuration might be due to crystal radiation damage while measured first with *FOX2D CU 12_38P*.

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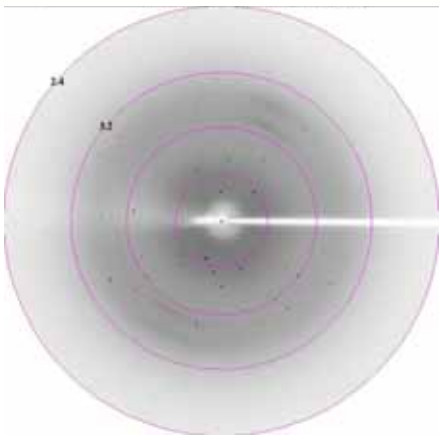
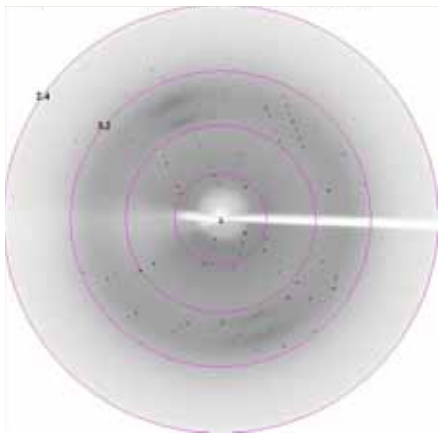
Application Note

FOX2D CU 25_25P focusing optics

A CRYSTAL on *12_38*
Max intensity : 1682



A CRYSTAL on *25_25*
Max intensity : 608



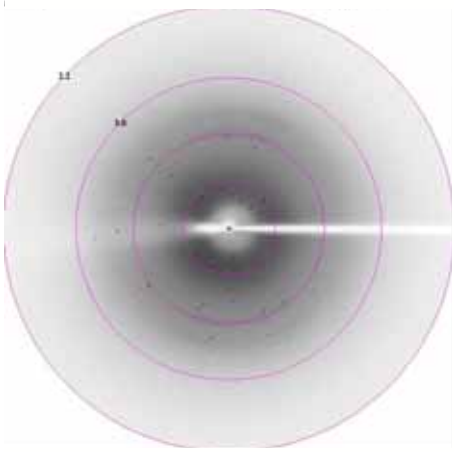
A second crystal of kinase protein was measured on both experimental set-ups. A small crystal (80x30µm²) was chosen which would not be expected to diffract on usual laboratory X-ray installations.

Crystal B: Size about 80 x 30 µm²
Crystal to Detector distance: 200mm
(2.2Å maximum resolution)

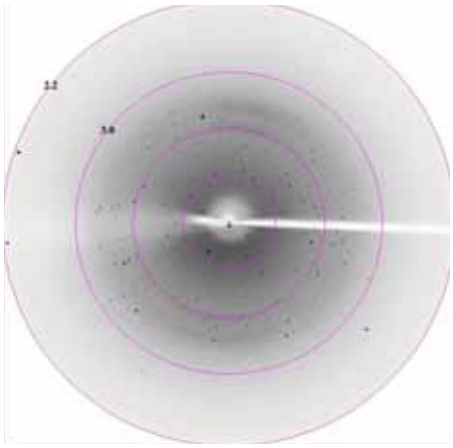
Measurements were possible with the *FOX2D CU 25_25P* configuration. Diffraction patterns were too noisy and spots too weak to be properly integrated in the *FOX2D CU 12_38P* configuration.

	FOX2D CU 25_25P (measured second)	*FOX2D CU 12_38P (measured first)*
Max intensity	684	259
Mosaicity	~2.0	undefined
Resolution	to 2.95 Å	Spots to 3Å, useful only to about 6Å (very noisy diffraction)
R_{sym}	13.8% (31.3%)	-
R_{meas} (all I+ and I-)	16.4% (37%)	-
Mean(I)/sd(I)	5.2 (2.4)	-
Completeness	99.9% (multiplicity 3.4)	-
	Wilson <B> 34	-

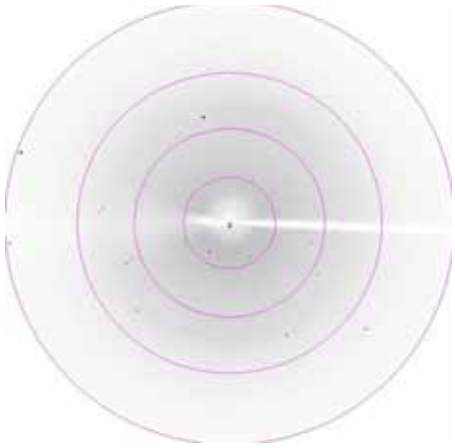
B CRYSTAL on *12_38*
Max intensity : 684



B CRYSTAL on *25_25*
Max intensity : 259



B CRYSTAL on *25_25*
contoured as on 12_38



Conclusion

Previous diffraction experimentations on more traditional homelab sources (300µm rotating anode generators) highlighted the efficiency of the *FOX2D CU 25_25P* for crystals of about 100-150µm size.
The present results reveal that exploitable datasets can be collected even with very small crystals (as 30µm) when a *FOX2D CU 25_25P* optic is combined with microfocus rotating anode generator (70µm). Use of this Xenocs mirror represents a big step in the use of laboratory X-ray installations for crystals screening. Experiments can be performed on small size crystals previously considered to diffract only when measured on synchrotron beamlines.
The *FOX2D CU 12_38P* gives a high flux beam approximately 3 times larger than the x-ray source, while the *FOX2D CU 25_25P* gives a very bright beam approximately the size of the source. For crystals of size comparable to or smaller than the x-ray source, the *FOX2D CU 25_25P* tends to give the better results, while for crystals larger than the source-size the *FOX2D CU 12_38P* will typically give better results.