High speed and high resolution data collection with the EIGER R 4M detector on a mardtb goniostat

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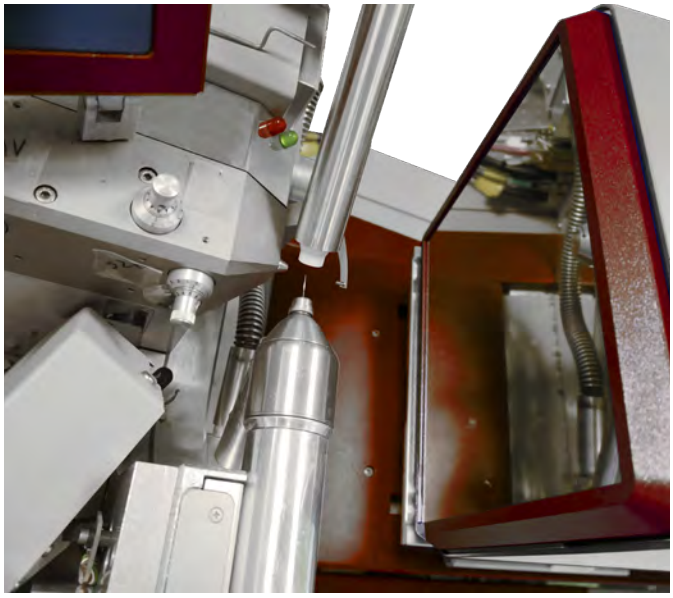
1. Introduction

The EIGER R detector series is the laboratory version of the hybrid photon counting detectors manufactured by DECTRIS. The R series is identical to the synchrotron series except for the frame rate which is “only” 5 frames/second instead of 750 for the 4M version. While the DECTRIS detectors today can be found on almost all protein crystallography beamlines at synchrotrons worldwide, they are not yet that common in home laboratories despite of the excellent price/performance ratio of the laboratory series of both EIGER and PILATUS3 detectors.

mardtb goniostat with EIGER R 4M

On a home X-ray source, detector speed is not really the time limiting factor for a data collection but rather the exposure time, i.e. the amount of X-ray photons required to obtain a useful signal. The usability of a detector on a home source therefore depends on its sensitivity. In this study, we demonstrate the capabilities of the EIGER R 4M detector mounted on a mardtb ”desktop beamline” goniostat sitting on a high brilliance Rigaku FR-E generator operated at 2 kW. The mardtb goniostat is the perfect instrument for this setup with its unique feature to automatically find and optimize the primary X-ray beam. This feature ensures that always the best possible beam hits the crystal and thus helps to keep exposure times as short as possible.



Detail of sample during data collection

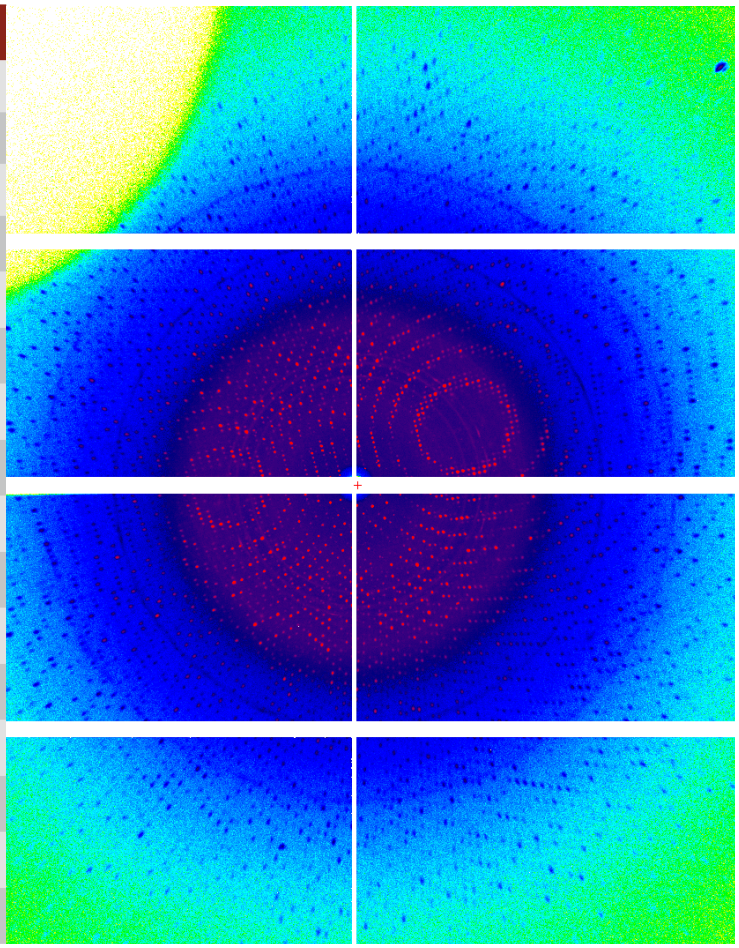
The EIGER R 4M is the largest version of the laboratory series of EIGER detectors with an impressive active area of 155x 165 mm (2070 x 2167 pixels) and a pixelsize 75 microns. To obtain high resolution data of approx. 1.5 Ang., data were collected at a distance of 50 mm without the need to move the 2-theta stage.

2. Data collection

One data set has been collected from a frozen crystal of copper nitrite reductase (courtesy of Svetlana Antonyuk, U. of Liverpool). The cubic crystals diffract to atomic resolution (Horrell, S., Antonyuk, S.V., Eady, R.R., Hasnain, S.S., Hough, M.A., Strange, R.W., Iucrj 3: 271-281 (2016)). The crystal used here had a physical size of approx. 100 x 50 x 50 microns and a mosaicity of approx. 0.2^0. 120 images were collected in shutterless operation mode with 15 seconds per image. The total data collection time was 30 minutes. Data were processed using XDS.

		Set 1
Distance crystal-detector	[mm]	50
2-theta	[deg.]	0
Total PHI range	[deg.]	60^0
PHI/image	[deg.]	0.5^0
Number of images		120
Exposure time/image	[sec]	15
Total exposure time	[min]	30
Max. resolution	[Ang.]	1.57
# unique reflections		72675
# measured reflections		241439
Multiplicity		3.3
Completeness[1]	[%]	93.5 (89.3)
Rsym[1]	[%]	2.3 (10.3)
Rmeas[1]	[%]	2.6 (12.7)
$\langle I/\sigma \rangle^1$		31.5 (9.2)
SIG^1_{ano}		1.03 (0.72)

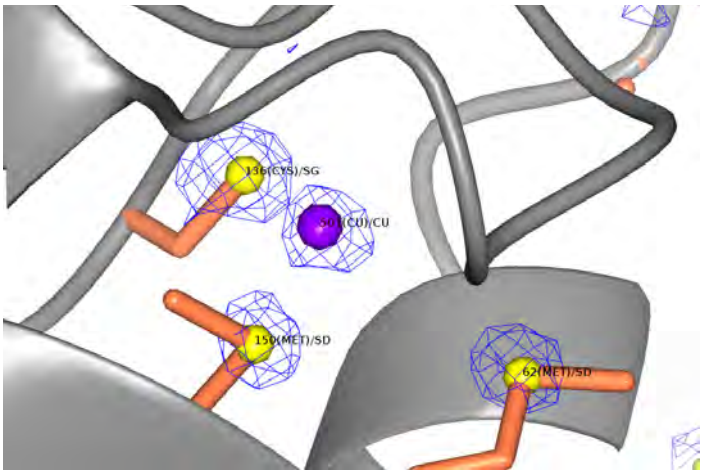
[1] Last shell in brackets: 1.63-1.57 Ang.



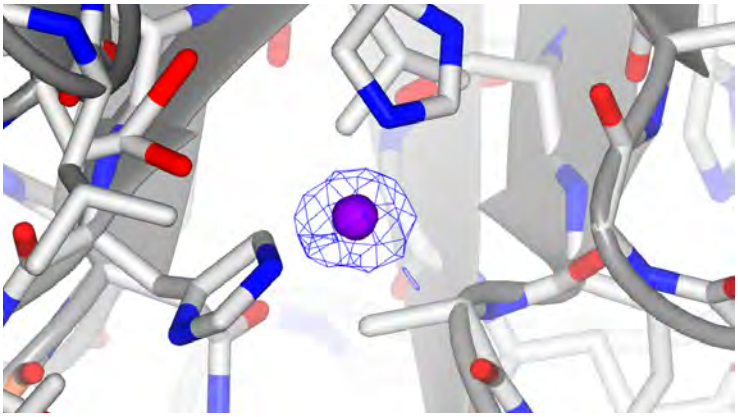
Diffraction image, shadow of cryo cooler in upper left corner

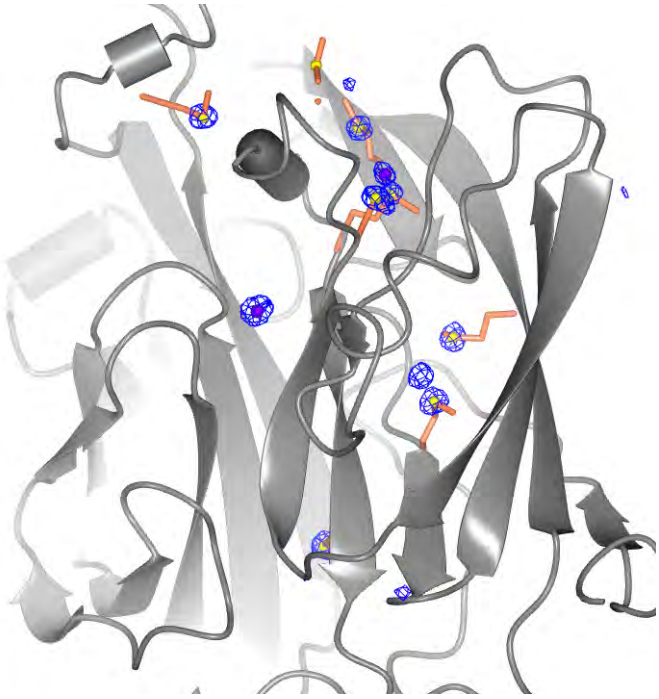
3. Structure refinement

The structure of copper nitrite reductase contains 2 copper ions. One is deeply buried inside the protein, the other one more accessible to solvent. Besides the copper atoms, the protein with 334 amino acid residues contains 9 methionines and 1 cystein residue. We attempted to solve the structure by locating the sulphurs and copper ions using program SHELXC/D but did not succeed. We therefore proceeded to conventional structure refinement departing from PDB entry 5I6K and ended with a structure refined to an overall R-value of 14.1% to 1.57 Ang. resolution that includes 6 NO_2-molecules as ligands and approx. 150 water molecules. The phases from the refined model were taken to compute an anomalous difference map. The largest positive peak in the map belongs Cys 136 SG with a signal of +13σ, followed by the signal of the copper atom approx. 2.5 Ang. away from the sulfur of the cystein with a signal of 9.7σ. The next 10 highest peaks belong to the other copper ion and the sulfurs of the methionine residues, all with signal around 5σ. This finding clearly indicates the outstanding quality of the data set and suggests that the ab initio structure solution might have worked with a data set with higher redundancy.



Anomalous difference peaks around copper 501 (purple, 9.8σ), Cys 136 (13σ), Met 62 (5.5σ) and Met 150 (5.2σ)


Anomalous difference peaks around copper 502 (purple, 5.8σ)


Anomalous difference peaks >5σ in protein, Met and Cys side-chains in salmon, sulfurs in yellow, coppers in purple

4. Conclusion

A total of **60 degrees** of data collected from a single crystal of copper nitrite reductase within just **30 minutes** on an in-house micro-focus rotating anode generator yielded exceptionally good data with very good anomalous signals. While the data just did not seem to be sufficient for ab initio structure solution the results suggest, that this should have worked by collecting a data set with higher redundancy. It has been shown elsewhere (e.g. application note AN260107 by marXperts at http://www.marxperts.com/pdf/mar.AN260107.pdf) that redundancy can play a critical role. Still, the data collected here show the amazing power of the EIGER R 4M detector combined with a mardtb goniostat. In conjunction with a micro-focus rotating anode generator or - much better - an Excillum Metaljet X-ray source you are looking at a truely magnificent data collection system with ultimate performance.