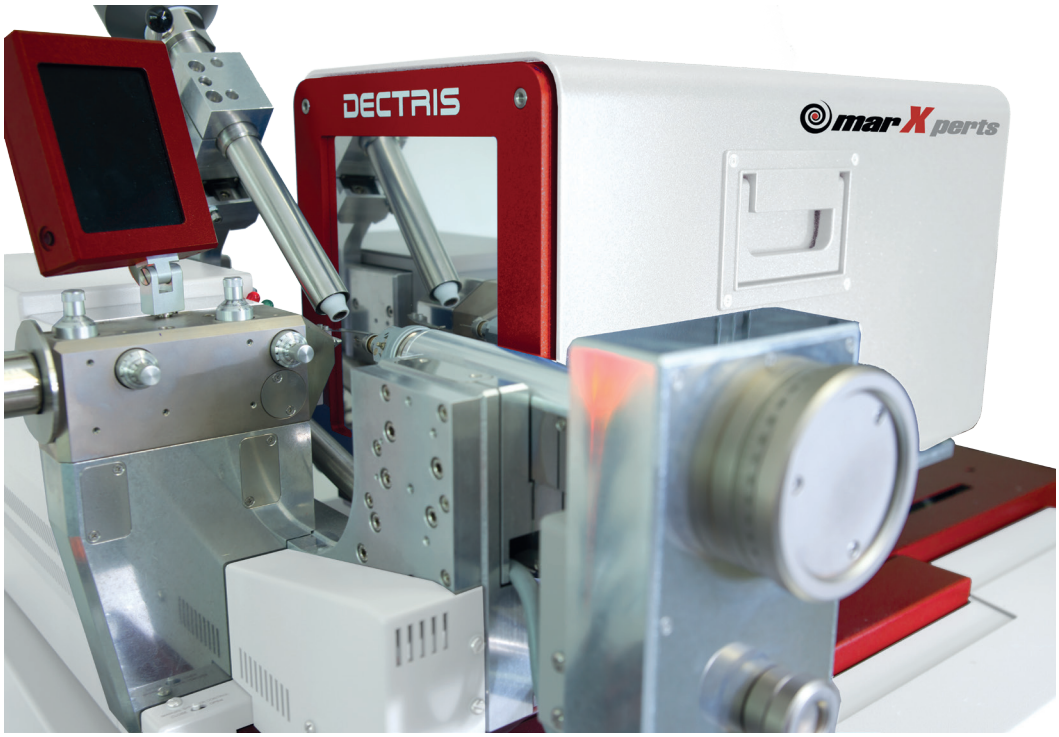

Sulfur-SAD phasing of lysozyme with a PILATUS3 R 1M detector on a marμX 2G micro-beam generator

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1. Introduction

The PILATUS3 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 frame/second for the 1M model. The so called “pixel detectors” have gained reputation for being excellent detectors and today they are, in fact, the very first choice for synchrotron beamlines. For conventional laboratory use, however, they are not yet that common.

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 feasibility of sulfur-SAD phasing with lysozyme using a Pilatus3 R 1M detector mounted on a micro-focus sealed-tube generator, namely the marμX2G system that consists of an Incoatec IμS source operated at 30 Watt (50kV/600 μA) and a mardtb ”desktop beamline” goniostat. This is an excellent test for the overall performance of the combination of all components.

The PILATUS3 R 1M is the largest of the laboratory series of DECTRIS detectors with an active area of 168 x 180 mm (981 x 1043 pixels with a pixelsize of 0.172 mm). In contrast to the smaller 200K and 300K detector versions, with the 1M detector it is usually possible to obtain high resolution data from a single data set without having to use a 2-theta offset. With the low divergence of the Incoatec source and the virtually inexistent point-spread of the detector, the detector-to-crystal distance could be kept small at 70 mm.

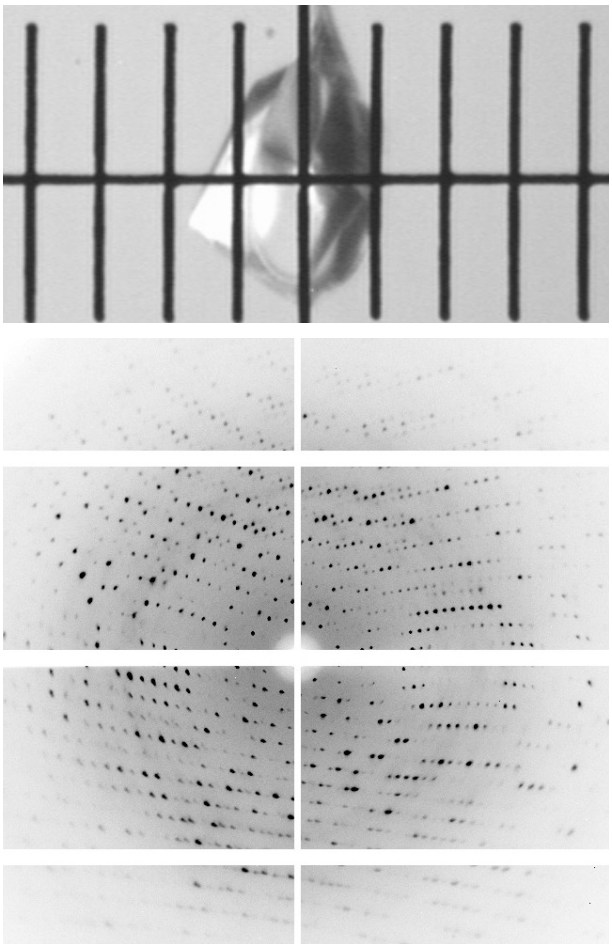
mardtb goniostat with Pilatus3 R 1M

Incoatec IμS source operated at 30 Watt

2. Data collection

One single data set was collected from a small frozen lysozyme crystal. The crystal had a physical size of approx. 250 x 150 x 100 mm and a mosaicity of approx. 0.5^0. The exposure time was 15 sec per 0.1^0. A total of 450^0 of data have been collected. The detector was mounted such that the rotating axis of the mardtb goniostat was parallel to the vertical boundary between 2 detector modules in order to mimimize the blind regions of the detector.
Data were processed using XDS.

		Set 1
Distance crystal-detector	[mm]	70
2-theta	[deg.]	0
Total PHI range	[deg.]	450^0
PHI/image	[deg.]	0.1^0
Number of images		4500
Exposure time/image	[sec]	15
Total exposure time	[min]	1125
Max. resolution	[Ang.]	1.7
# unique reflections		24976
# measured reflections		308596
Multiplicity		11.6
Completeness[1]	[%]	98.7 (99.4)
Rsym[1]	[%]	4.7 (40.7)
Rmeas[1]	[%]	4.9 (45.4)
$<I/\sigma>^1$		35.3 (3.8)
SIG^1_{ano}		1.28 (0.82)



[1] Last shell in brackets: 1.84-1.70 Ang.

3. Structure solution and refinement

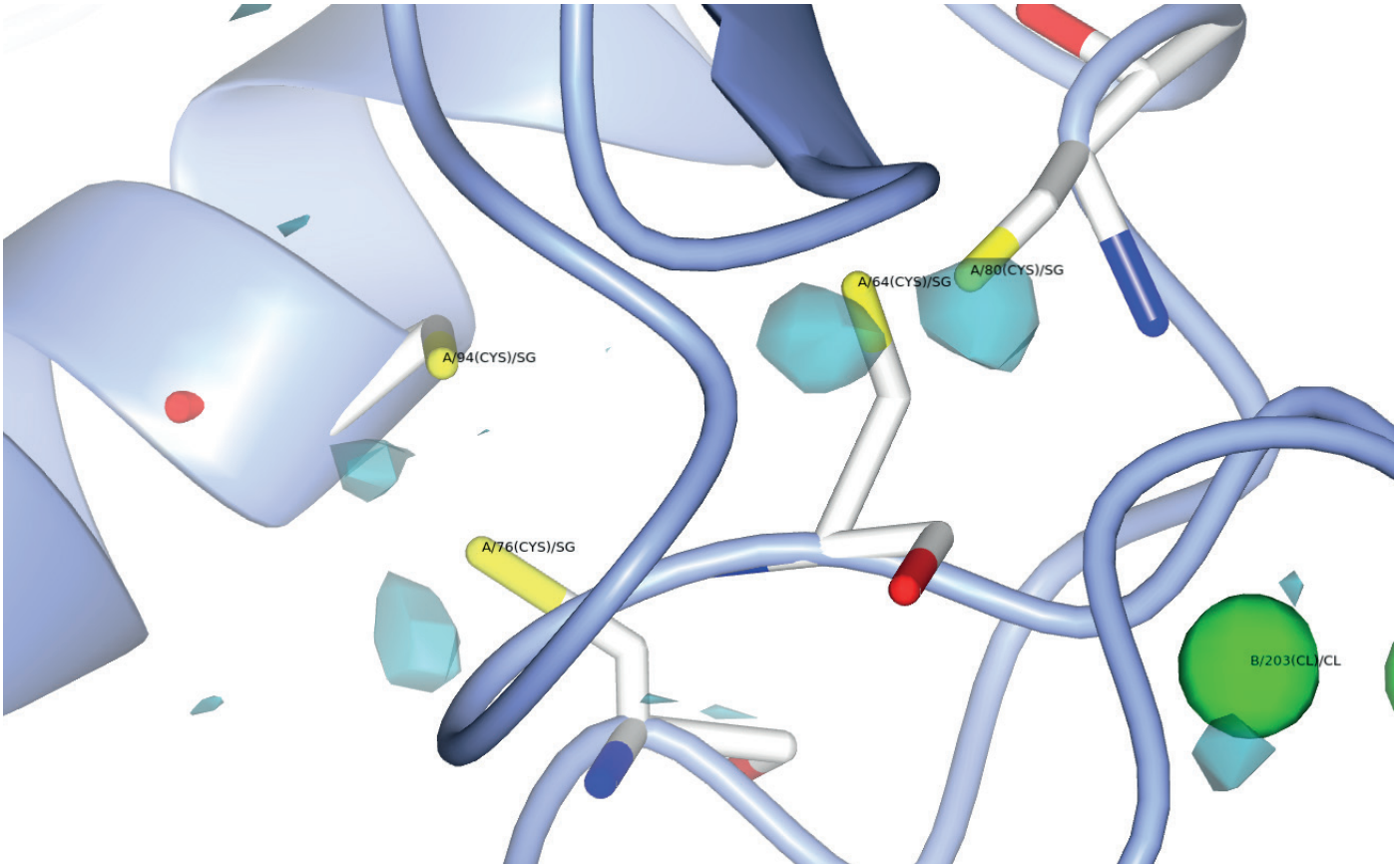
Program SHELXD was instructed to locate 17 anomalous scatterers: 10 sulfur atoms and 7 Cl ions at 1.8 Ang. resolution. The best solution was used for phase calculation and further improvement by density modifications with program SHELXE. The following results were obtained from SHELXD:

- PATFOM: 1.36
- CC all/weak: 35.4 / 17.9

The following results were obtained from SHELXE:

- Contrast / enantiomorph: 0.48 / 0.32
- Pseudo Free CC / enantiomorph: 65.4 / 53.7

The resulting experimental phases from SHELXE were used for chain-tracing from scratch using program arp/warp. The program automatically built all 129 residues and yielded a refined model at 1.7 Ang. resolution that matches the published data. With the phases of the refined model, an anomalous difference map was computed, that showed the highest peaks at the sulfur atoms of Met 105 (6.9 σ), the disulfid bridge between Cys 64 and Cys 80 (6.3 σ), the Chloride ion near Tyr 23 OH (5.7 σ) and the disulfide bridge betwwn Cys 76 and Cys 84 (4.5 σ). Water molecule #3 from PDB-structure 1LZ8 near Asn 65 ND2 shows a large anomalous difference of 4.5 s, suggesting that this peak corresponds to another chloride ion rather than a water molecule.



Anomalous difference map with final phases. The ligh blue dots are positive anomalous peaks around 5 sigma at the disulfide bridges Cys 64 - Cys 80 and Cys 76 - Cys 96. Another large anomalous peak is near the (green) chloride ion.

4. Conclusion

A total of 450 degrees of data collected from a single lysozyme crystal on an in-house micro-focus generator yielded good enough data for ab initio structure solution. Due to the size of the detector, there was no need to collect several data sets with 2-theta offsets which is mandatory for the much smaller 200K and 300K detectors. Just one data set was sufficient. This study hence nicely demonstrates the amazing capabilities of the entire setup consisting of a very fast and efficient detector, an easy-to-use goniostat and a small but very powerful home source.
In summary, the PILATUS3 R series of detectors are ideally suited for fast crystal screening experiments as well as for complete data collections with the aim of solving and refining protein structures in a home lab.