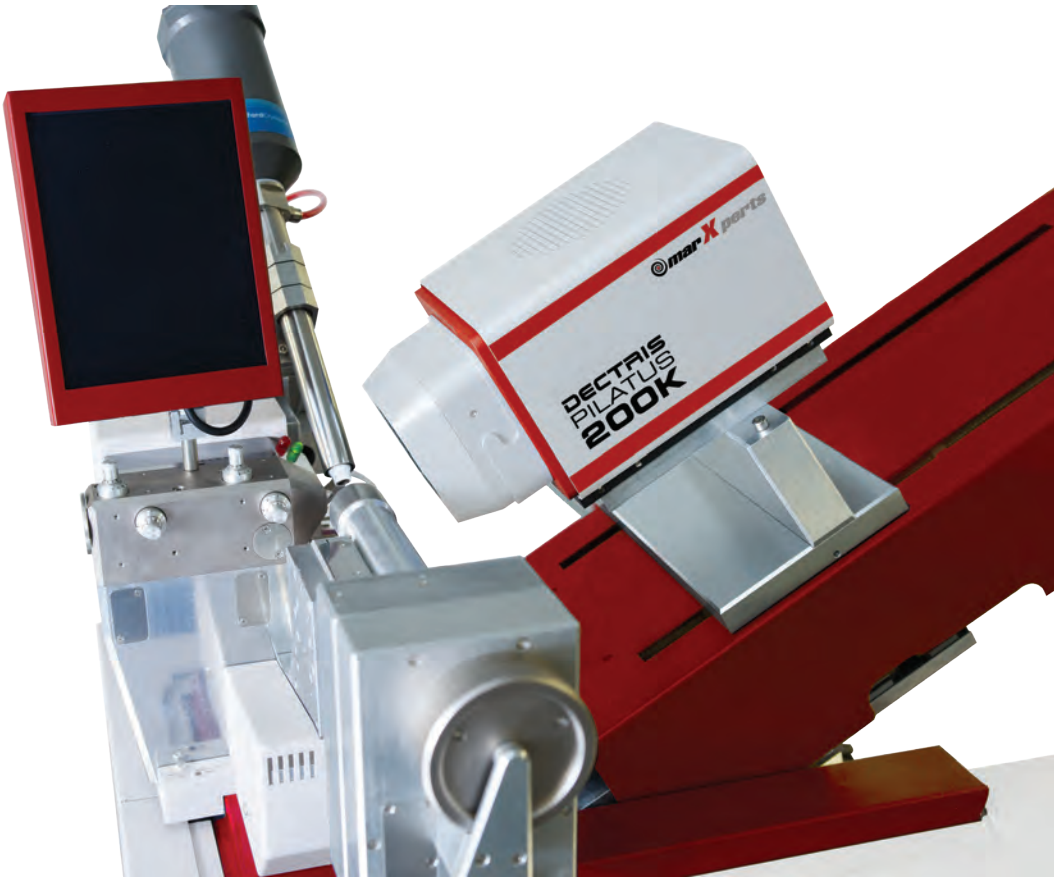

Sulfur-SAD phasing of lysozyme at room temperature with a PILATUS3 R 200K-A detector on a marµX 2G micro-beam generator

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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" 20 frame/second instead of 500. 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 200K-A detector mounted on a micro-focus sealed-tube generator, namely the marμX 2G 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.

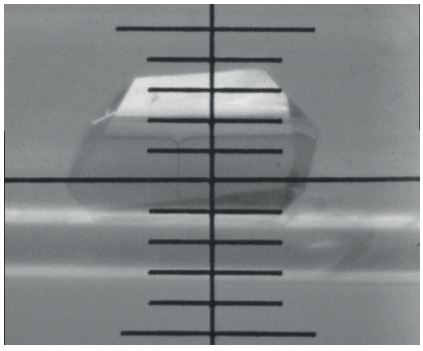
The PILATUS3 R 200K-A is the smallest of the series of DECTRIS detectors with an active area of only 84 x 70 mm (487 x 407 pixels with a pixelsize of 0.172 mm). To obtain a reasonable resolution of approx. 1.5 Ang., some data were collected at a 2-theta angle of 28 degrees. With the low divergence of the Incoatec source and the virtually inexistent point-spread of the detector, the detector-to-crystal distance could be kept small at 55 mm.

mardtb goniostat with Pilatus3 R 200K-A

Incoatec IμS source operated at 30 Watt

2. Data collection

Three data sets were collected from a lysozyme crystal. The crystal was **not** frozen but mounted conventionally in a capillary and data were collected at room temperature. Radiation damage in the course of the experiment has therefore been a certain issue. The crystal had a physical size of approx. 250 x 450 x 550 mm and a mosaicity of approx. 0.2^0. Data were processed using XDS.



		Set 1	Set 2	Set 3	Combined
Distance crystal-detector	**[mm]**	55	55	55	
2-theta	**[deg.]**	0	28	28	
Total PHI range	**[deg.]**	360^0	360^0	90^0	
PHI/image	**[deg.]**	0.1^0	0.5^0	0.1^0	
Number of images		3600	720	900	
Exposure time/image	**[sec]**	1	10	4	
Total exposure time	**[min]**	60	120	60	
Max. resolution	**[Ang.]**	2.4	1.6	1.6	**1.6**
# unique reflections		8631	30318	19076	**30398**
# measured reflections		48124	172492	43151	**324230**
Multiplicity		5.6	5.7	2.3	**10.7**
Completeness[1]	**[%]**	97.7 (87.3)	99.7 (99.1)	62.6 (43.9)	**99.8 (99.1)**
Rsym[1]	**[%]**	3.7 (4.8)	5.4 (43.8)	4.5 (37.0)	**4.6 (44.6)**
Rmeas[1]	**[%]**	4.1 (6.0)	5.9 (49.8)	5.5 (46.6)	**4.8 (49.2)**
$\langle I/\sigma \rangle$[1]		35.7 (16.0)	19.2 (3.3)	13.7 (2.7)	**28.0 (3.5)**
SIG^1_{ano}		1.16 (0.83)	0.91 (0.79)	0.97 (0.80)	**1.07 (0.78)**

[1] Last shell in brackets: 2.56-2.4 Ang for set 1, 1.71-1.60 Ang. for sets 2 and 3, respectively

3. Structure solution and refinement

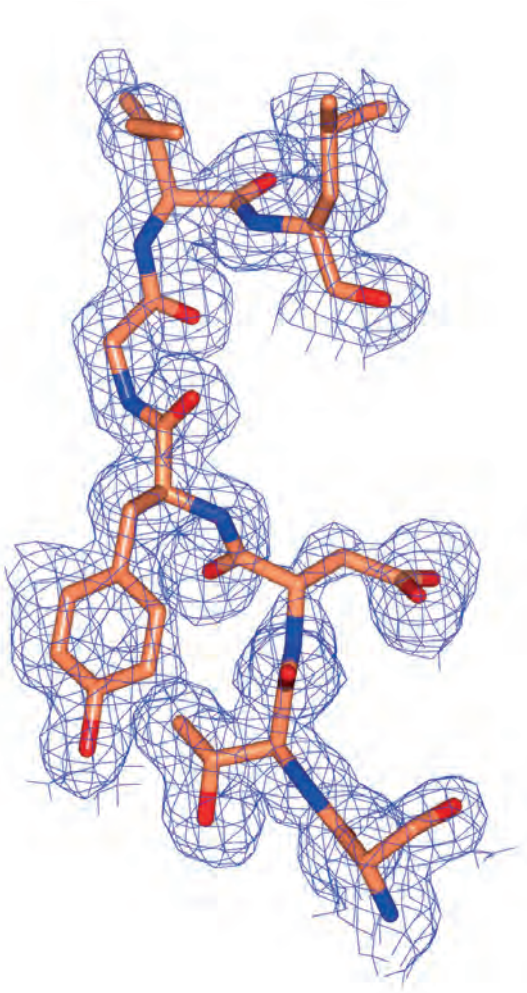
Program SHELXD was instructed to locate 17 anomalous scatterers: 10 sulfur atoms and 7 Cl ions at 1.8 Ang. resolution. The best solution was used for phase calculation and further improvement by density modifications with program SHELXE. The following results were obtained from SHELXD:

- PATFOM: 1.38
- CC all/weak: 37.9 / 16.6

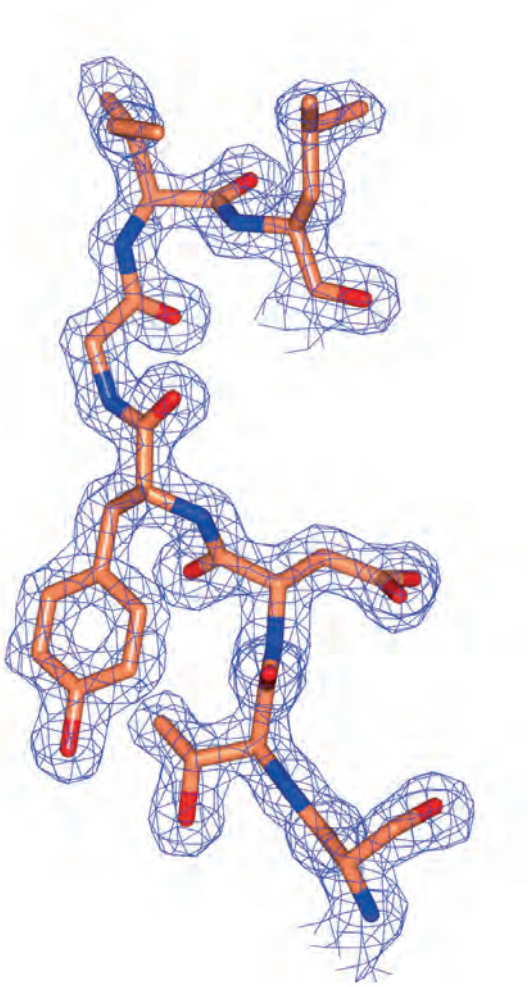
The following results were obtained from SHELXE:

- Contrast / enantiomorph: 0.48 / 0.32
- Pseudo Free CC / enantiomorph: 70.8 / 52.4

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 1.6 Ang. resolution that matches the published data.



Residues 50 to 56: initial SHELXE map



Residues 50 to 56: final 2Fo-Fc map

4. Conclusion

A total of 810 degrees of data collected from a single lysozyme crystal within just 4 hours on an in-house micro-focus generator yielded good enough data for ab initio structure solution. This was done despite of using a non-frozen crystal at room temperature and with the smallest of all DECTRIS pixel detectors, the PILATUS3 R 200K. To achieve higher resolution, we made use of the built-in 2-theta offset of the mardtb goniostat. 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.