marXperts

Atomic resolution data collection with the PILATUS3 R 1M detector on a *metaljet* X-ray source

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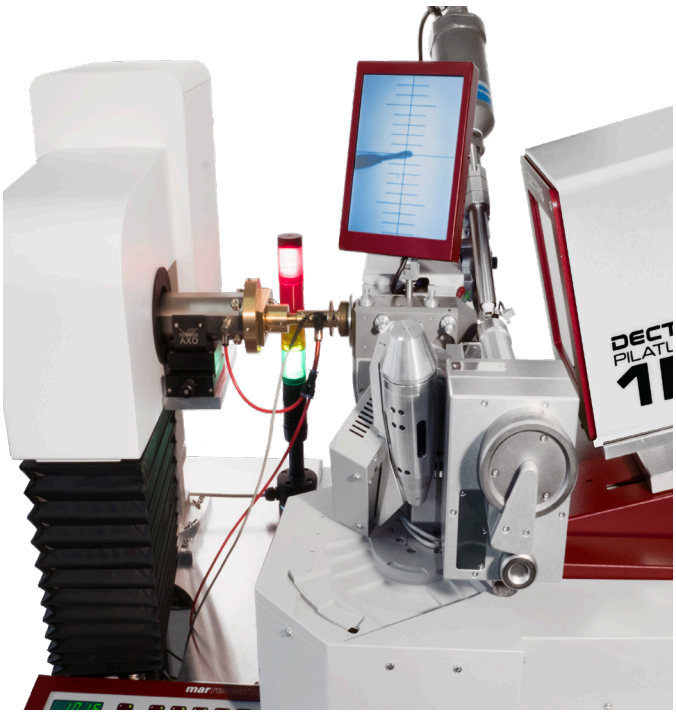
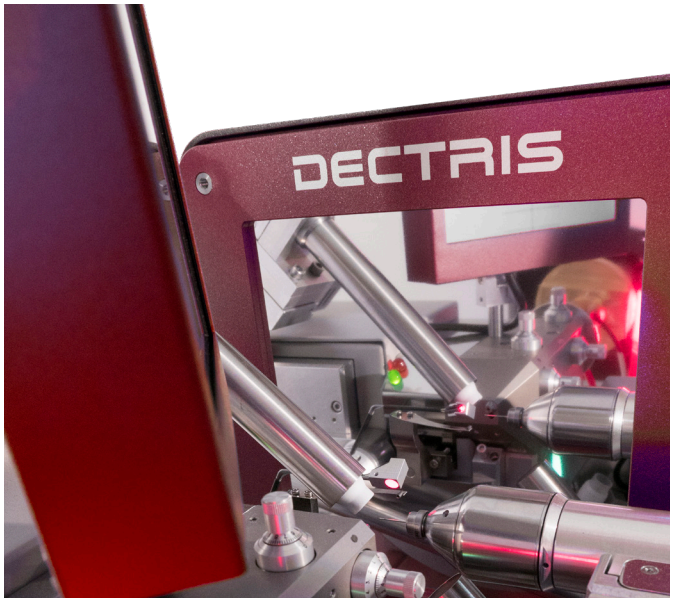
1. Introduction

Home X-ray sources have limited capabilities compared to synchrotron sources, in particular when it comes to collect data on very small protein crystals that require extreme brilliance of the X-ray beam. Generally speaking, the brilliance is the amount of X-ray photons per time unit in a given area. Since protein crystals often grow to sizes of only 20 microns or less, it does not help to use X-ray sources where the vast majority of the available X-ray photons don't hit the sample. Here is where the new MetalJet X-ray source invented and manufactured by Excillum in Sweden comes into play.



metaljet with PILATUS3 R 1M and mardtb

In the MetalJet technology the traditional metal anode - typically a copper wheel in the case of rotating anodes or a steady copper target for sealed tubes - is replaced by a liquid metal film, typically an alloy of Gallium and Indium. The liquid metal dissipates the heat generated by the electrons hitting the target much more efficiently than solid anodes. This allows for smaller focal points on the target (10-20 microns). This yields an X-ray source with a much higher brilliance than traditional X-ray generators. Effectively, the Excillum MetalJet D2+ running at 70 kV with 250 Watts is the MOST POWERFUL home lab X-ray source currently available. It is therefore the very best choice for in-house data collection even of very small protein crystals. The liquid metal alloy generates X-ray photons with an energy of 9.2 keV or 1.348 Angstroem.



Detail of sample during data collection

In this study we demonstrate the amazing capabilities of a high-end in-house data collection system consisting of:

- the Excillum MetalJet D2+ X-ray source running at 70 kV with 250 W
- the DECTRIS PILATUS3 R 1M hybrid photon counting detector with an active area of 169 x 179 mm and a pixelsize of 172 microns. The R series is the laboratory version of the famous PILATUS3 detectors found at so many synchrotron beamlines today. The R series shares the same specs with the synchrotron series except for a reduced frame rate ("only" 5 frames/sec for the PILATUS3 R 1M).
- the mardtb goniostat 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.

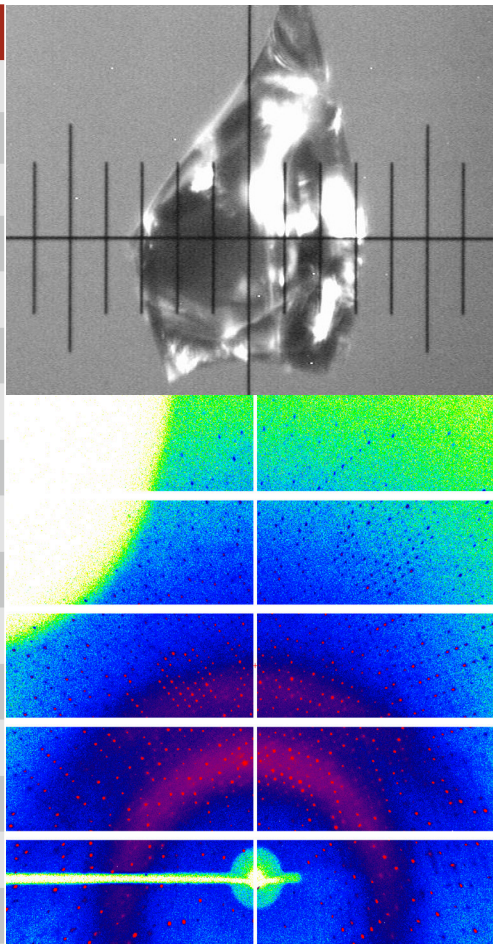
To obtain atomic resolution data of approx. 1.0 Ang., data were collected at a distance of 90 mm and a 2-theta angle of 30 degrees.

2. Trypsin

2.1 Data collection

Two data sets have been collected from a frozen crystal of bovine trypsin (courtesy of M. Perbandt, U. of Hamburg). The orthorhombic crystals diffract to atomic resolution (Liebschner, D., Dauter, M., Brzuszkiewicz, A., Dauter, Z., Acta Cryst. Sect. D 69: 1447-1462 (2013)). The crystal used here had a physical size of approx. 300 x 150 x 150 microns and a mosaicity of approx. 0.2^0. 2x1440 images were collected in shutterless operation mode with 2 seconds per image, one with a 2θ-offset of 30^0 and one without offset. The total data collection time was 2x720 minutes. Data were processed using XDS.

		Set 1	Set 2
Distance crystal-detector	**[mm]**	90	45
2-theta	**[deg.]**	30	0
Total PHI range	**[deg.]**	360^0	360^0
PHI/image	**[deg.]**	0.25^0	0.25^0
Number of images		1440	1440
Exposure time/image	**[sec]**	2	2
Total exposure time	**[min]**	48	48
Max. resolution	**[Ang.]**	1.00	1.2
# unique reflections		166607	122324
# measured reflections		380629	627886
Multiplicity		2.3	5.1
Completeness	**[%]**	74.0 (29.4)[1]	94.0 (69.2)[2]
Rsym[1]	**[%]**	1.7 (12.4)[1]	2.0 (6.2)[2]
Rmeas[1]	**[%]**	2.1 (17.4)[1]	2.2 (8.2) [2]
$\langle I/\sigma \rangle$[1]		26.5 (4.3)[1]	45.5 (10.0)[2]
SIG[1] ano		1.18 (0.70)[1]	1.47 (0.77)[2]



[1] Last shell in brackets: 1.10-1.0 Ang.
[2] Last shell in brackets: 1.27-1.2 Ang

Crystal and diffraction image
Shadow of cryo cooler in upper left corner

2.2 Structure solution & refinement

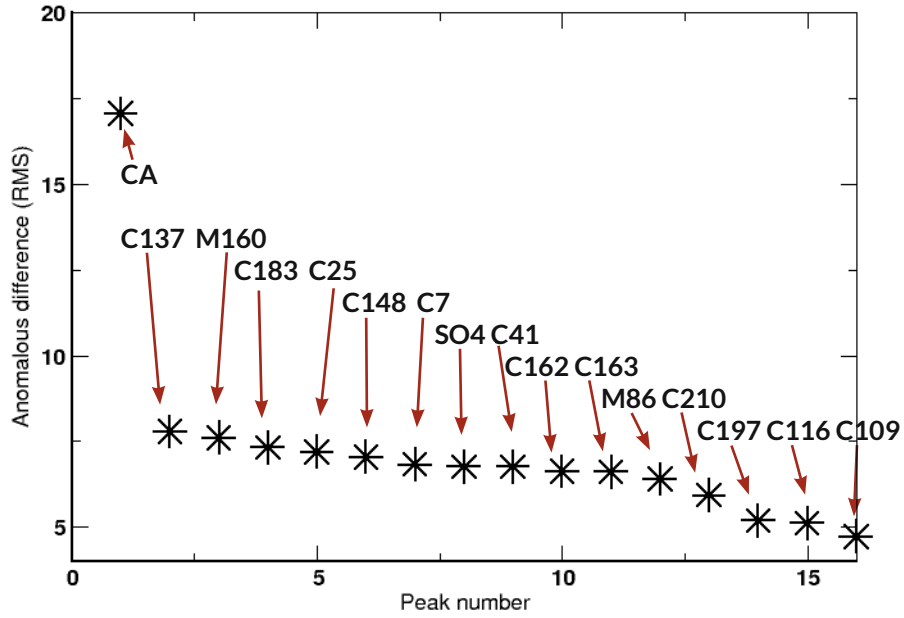
Program SHELXD was instructed to locate 14 anomalous scatterers: 12 sulfur atoms in CYS disulfide-bridges and 2 sulfur atoms from Met residues at 1.0 Ang. resolution. The best solution was used for phase calculation and further improvement by density modifications with program SHELXE.
The following results were obtained from SHELXD:

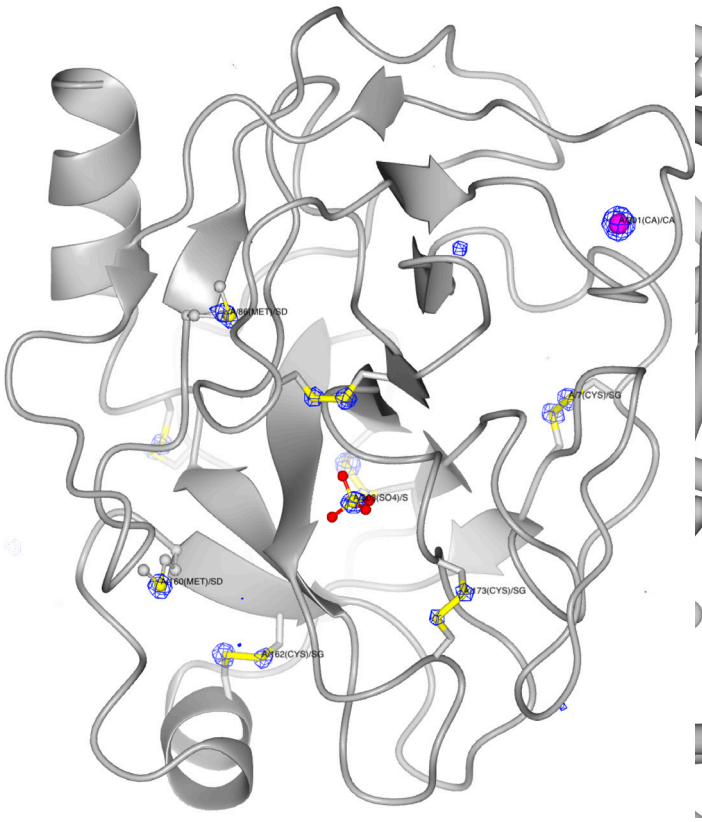
- CFOM/PATFOM: 67.8 / 10.36
- CC all/weak: 39.9 / 27.9

The following results were obtained from SHELXE:

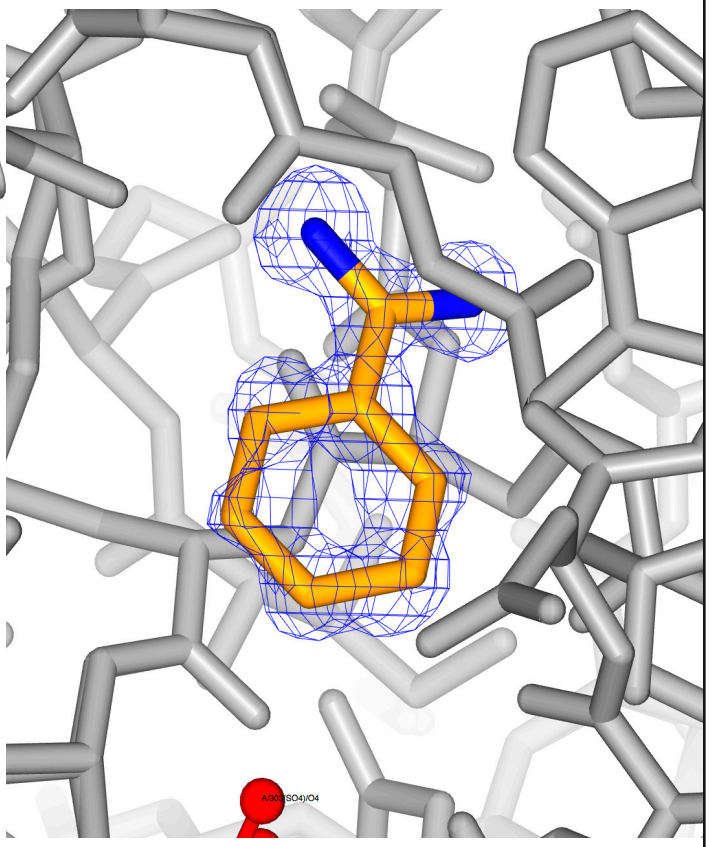
- Contrast / enantiomorph: 0.50 / 0.37
- Pseudo Free CC / enantiomorph: 85.2 / 55.2

The resulting experimental phases from SHELXE were used for chain-tracing from scratch using program arp/warp. The program automatically built 215 out of 223 residues and yielded a refined model at 1.0 Ang. resolution that matches the published data including a bound ligand benzamidine. With the phases of the refined model, an anomalous difference map was computed, that showed the highest peaks at bound calcium ion (17 σ) followed by 15 peaks above 3 σ from sulfurs contained in cysteins and methionines as well as a sulfate ion.





Anomalous differences > 4 σ from a 1.5 Ang. map
Atoms shown: calcium ion, sulfate and side chains of Cys & Met



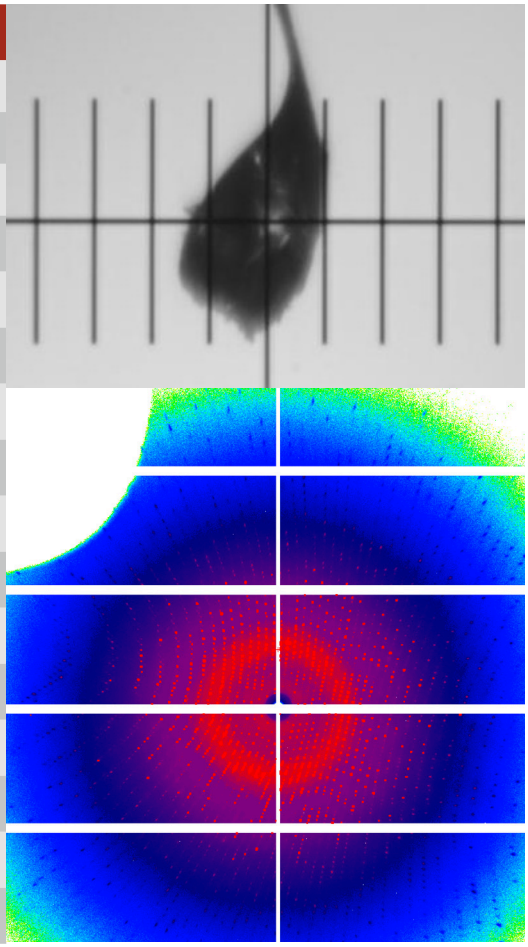
Final 2Fo-Fc map at 1.0 Ang.: details around benzamidine.
The phenyl ring comes in 2 slightly different orientations.

3. Lysozyme

3.1 Data collection

One data set has been collected from a frozen crystal of hen-egg lysozyme soaked with $YbCl_3$ (courtesy of G. Bourenkov, EMBL Hamburg). The tetragonal crystal had a physical size of approx. 150 x 100 x 50 microns and a mosaicity of approx. 0.2^0. 900 images were collected in shutterless operation mode with 58 seconds per image and a rotation of 0.5^0/image. Data were processed using XDS.

		Set 1
Distance crystal-detector	**[mm]**	50
2-theta	**[deg.]**	0
Total PHI range	**[deg.]**	450^0
PHI/image	**[deg.]**	0.5^0
Number of images		900
Exposure time/image	**[sec]**	58
Total exposure time	**[min]**	870
Max. resolution	**[Ang.]**	1.24
# unique reflections		60807
# measured reflections		767214
Multiplicity		12.6
Completeness	**[%]**	96.3 (65.4)[1]
Rsym[1]	**[%]**	3.5 (29.1)[1]
Rmeas[1]	**[%]**	3.7 (32.9)[1]
$\langle I/\sigma \rangle$[1]		38.6 (3.7)[1]
SIG^1_{ano}		1.75 (0.66)[1]



[1] Last shell in brackets: 1.27-1.24 Ang.

Crystal and diffraction image
Shadow of cryo cooler in upper left corner

3.2 Structure solution & refinement

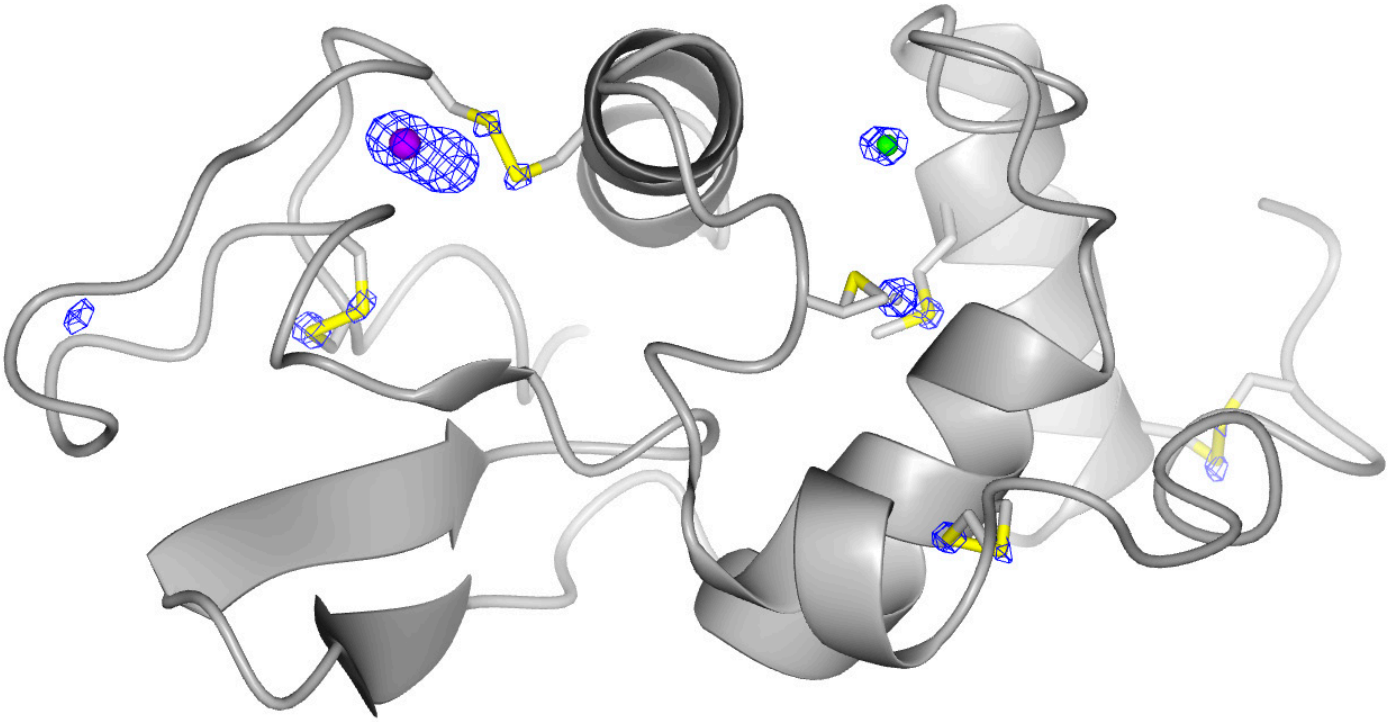
Program SHELXD was instructed to locate 10 anomalous scatterers: 8 sulfur atoms in CYS disulfide-bridges and 2 methionine sulfurs at 1.24 Ang. resolution. The best solution was used for phase calculation and further improvement by density modifications with program SHELXE.
The following results were obtained from SHELXD:

- CFOM/PATFOM: 50.1 / 4.59
- CC all/weak: 31.7 / 18.2

The following results were obtained from SHELXE:

- Contrast / enantiomorph: 0.45 / 0.33
- Pseudo Free CC / enantiomorph: 77.3 / 47.7

The resulting experimental phases from SHELXE were used for chain-tracing from scratch using program arp/warp. The program automatically built all residues and yielded a refined model at 1.24 Ang. resolution that matches the published data including a bound $YbCl_3$ molecule. With the phases of the refined model, an anomalous difference map was computed at 1.5 Ang., that showed the highest peaks at the bound Yb ion (24 σ) followed by peaks from Met105 (8σ), 2 peaks from solvent (presumably chlorine ions) at 7 σ and peaks from sulfur atoms in Cys76 (6 σ), Cys80 (6 σ), Cys115 (6 σ), Met12 (5 σ), Cys64 (5 σ), Cys6 (5 σ), Cys30 (5 σ) and Cys127 (4 σ).



The shown map is an anomalous difference map with at 1.5 Ang. with peaks above 5 σ. The by far largest peak belongs to an $YbCl_3$ molecule (where only the Yb atom is shown). All other peaks belong to sulfurs from 4 disulfide bridges and 2 methionine residues as well as 2 chlorine atoms from the solvent.

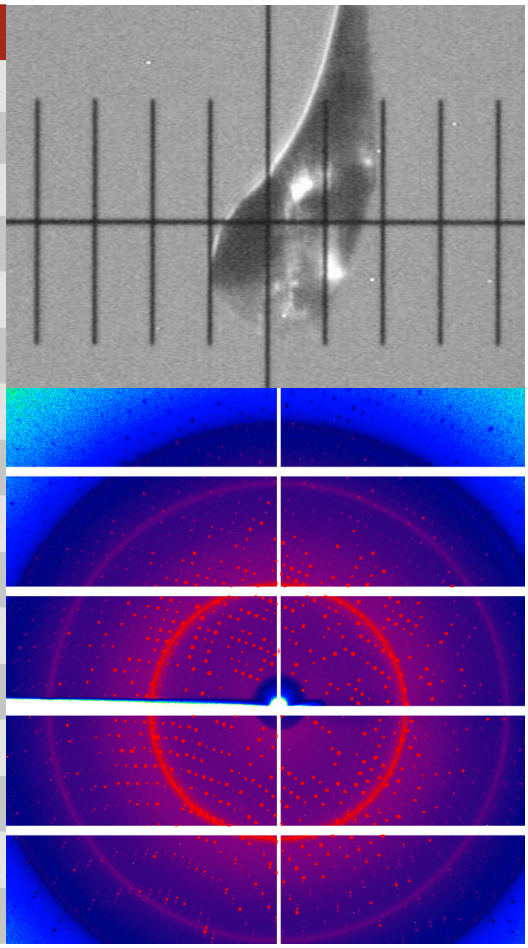
In order to judge the minimum amount of data required to solve the structure, instead of 900 images covering 450^0, a subset of only 140 images corresponding to only 70^0 of data already gave the correct solution. For this subset of data, the total multiplicity was 2.1.

4. Thaumatin

4.1 Data collection

One data set has been collected from a frozen crystal of thaumatin (courtesy of G. Bourenkov, EMBL Hamburg). The tetragonal crystal had a physical size of approx. 100 x 50 x 30 μm and a mosaicity of approx. 0.2°. 1080 images were collected in shutterless operation mode with 30 seconds per image and a rotation of 0.5°/image. Data were processed using XDS.

		Set 1
Distance crystal-detector	**[mm]**	100
2-theta	**[deg.]**	0
Total PHI range	**[deg.]**	540°
PHI/image	**[deg.]**	0.5°
Number of images		1080
Exposure time/image	**[sec]**	30
Total exposure time	**[min]**	540
Max. resolution	**[Ang.]**	1.7
# unique reflections		53997
# measured reflections		776770
Multiplicity		14.4
Completeness	**[%]**	99.6 (98.6)[1]
Rsym[1]	**[%]**	3.5 (16.6)[1]
Rmeas[1]	**[%]**	3.3 (13.2)[1]
<I/σ>[1]		52.8 (11.7)[1]
SIG[1]$_{ano}$		1.07 (0.62)[1]



[1] Last shell in brackets: 1.81-1.7 Ang.

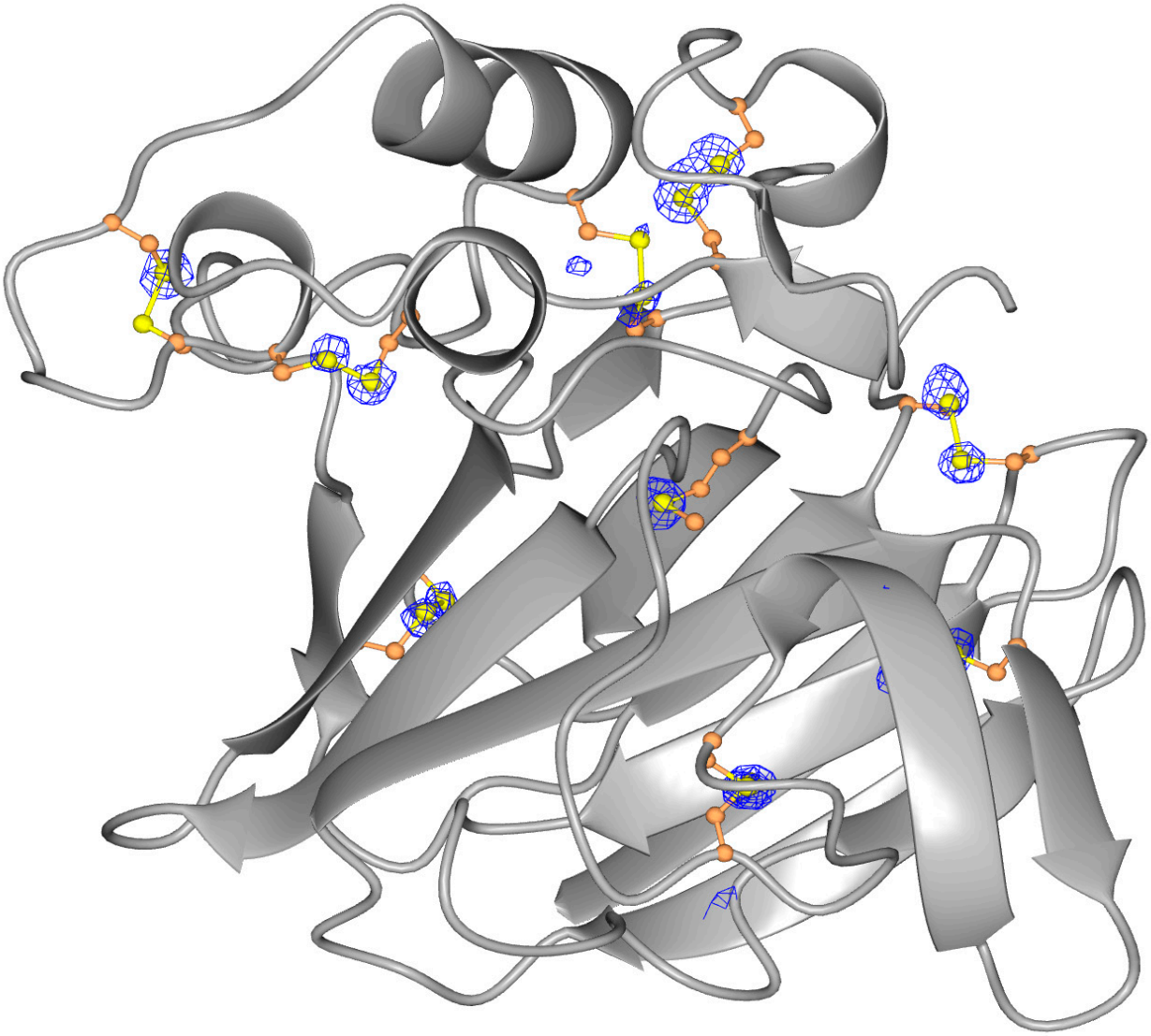
Crystal and diffraction image

4.2 Structure solution & refinement

Program SHELXD was instructed to locate 17 anomalous scatterers: 16 sulfur atoms in 8 disulfide-bridges and 1 sulfur in a methionine residue at 2.35 Ang. resolution. The best solution was used for phase calculation and further improvement by density modifications and chain tracing with program SHELXE.
The following results were obtained from SHELXD and SHELXE:

- CFOM/PATFOM: 38.1 / 1.51
- CC all/weak: 23.7 / 14.4
- CC for partial structure agains native data: 42.4 %

The resulting experimental phases from SHELXE with the best main-chain trace allowed for fully automatic model building in program arp/warp. With the phases of the refined model, an anomalous difference map was computed at 1.7 Ang., that showed the highest peaks from sulfurs in Cys145 (9 σ), Met112 (7.5σ), Cys134 (7.6 σ), Cys9, Cys77, Cys56, Cys149, Cys204 (all above 6 σ), Cys193, Cys66, Cys71, Cys158 (all above 5 σ), Cys164, Cys126, Cys121 and Cys177 (3-5 σ).



The shown map is an anomalous difference map with at 1.7 Ang. with peaks above 3 σ. The largest peak belongs to Cys145.

5. Conclusion

The combination of an Excillum MetalJet X-ray source with a DECTRIS hybird photon counting detector - here the PILATUS3 R 1M - is currently the most powerful X-ray diffraction equipment for the home lab. It yields ultra-high quality data comparable to those from synchrotron sources in very short time - even on very small crystals. The short wavelength of 1.34 Ang. allows for collecting data at atomic resolution with large surface detectors like the PILATUS3 R 1M or the EIGER R 4M detector.