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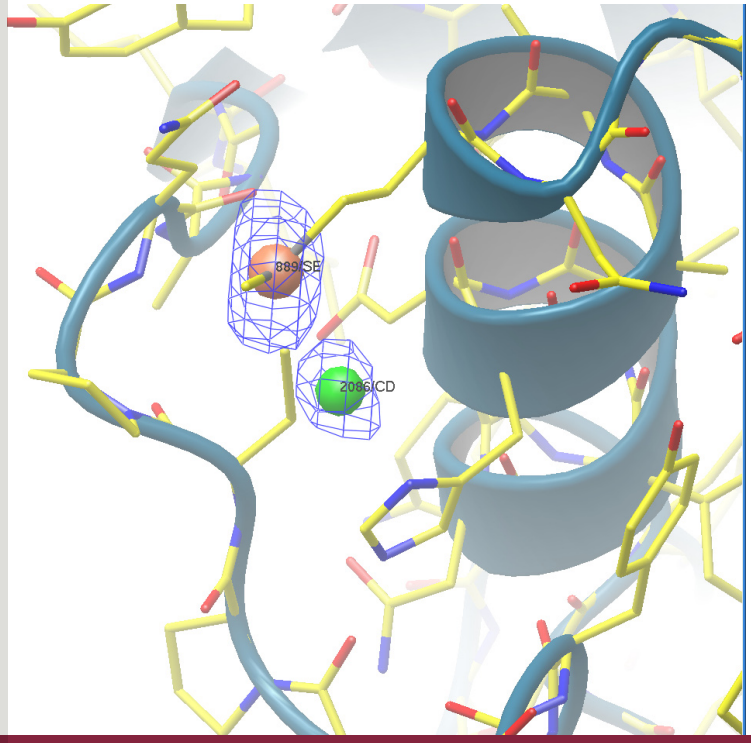

SAD phasing from data collected with the mar555 flatpanel detector

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Application Note 22Sep08

Introduction

Selenium is one of the few materials capable of converting X-rays directly into electrons. The mar555 flatpanel detector makes use of this unique property of selenium. With its highly sophisticated read-out system, the detector directly counts the X-rays where they hit the detector without taking the detour of converting them into visible light as other detectors like the CCD's and image plate scanners do. The result of this direct conversion is an unprecedented spatial resolution and improved signal-to-noise ratio.

In this study, we have used a mar555 detector system to collect SAD data from a frozen crystal of the feruloyl esterase domain of Xylanase10B (FAE). All data were collected at ESRF beamline ID23-2 that is operated at a fixed wavelength of 0.873 Ang. (14.2 keV). For maximum anomalous differences a wavelength close to 12.6 keV would have been optimal, so the used wavelength clearly is on the remote side. We systematically explored how many data were actually required to solve the structure from scratch.

Experiment

X-ray Detector System

Detector	mar555
Read-out time	1.25 sec
Active area	430 mm x 350 mm
Diagonal	555 mm
Pixel size	139 μ
Total number of pixels	2560 x 3072
Dynamic range	0 - 250.000
Total noise	750 e rms (= 6 X-rays @ 12 keV)

Crystal

Protein	Feruloyl esterase domain of Xylanase 10B[1]
PDB entry	1GKK[1]
Space group	P 2_1 2_1 2_1
Unit cell parameters	a=65.4 b=108.8 c=113.9
Amino acid residues	2 x 296 (dimer)
Anomalous scatterers	2 x 8 seleniums in methionines 2 x 5 Cd ions from crystallization buffer 2 x 1 phosphate ion from phosphorilated Ser
Size of crystal	1000μ x 100μ x 50μ
Mosaicity	< 0.1°

Data collection

mar555 @ ESRF ID23-2	
Distance	248 mm
Exposure time	1.0 sec / 1.0 deg.
Transmission	100 %
Energy	14.2 keV = 0.873 Ang.
Total no. of images	180
Multiplicity	3.82
High resolution limit	1.60 Ang.

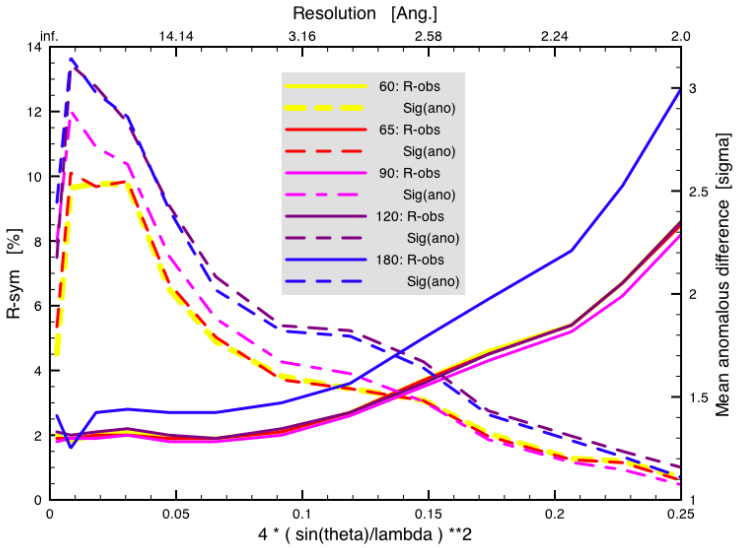

Figure 2: R-factors and anomalous signals for 60, 65, 90, 120 and 180 images
The dotted lines show mean anomalous differences (SigAno) as computed by XDS.

Data processing (XDS[2], SHELXC/D/E[3,4,5,6])

No. images	55	60	65	90	120	180
R_{merge}	3.2 (8.7)	3.2 (8.6)	**3.2 (8.5)**	3.1 (8.2)	3.3 (8.6)	4.5 (12.7)
R_{meas}	4.4 (11.8)	4.4 (11.7)	**4.4 (11.6)**	4.2 (11.2)	4.1 (11.0)	5.3 (14.8)
Sig_{ano}(XDS)	1.45 (1.11)	1.45 (1.11)	**1.44 (1.11)**	1.46 (1.07)	1.59 (1.16)	1.56 (1.11)
<I/σ>	16.4 (7.1)	16.5 (7.3)	**16.8 (7.5)**	19.2 (8.8)	22.1 (10.3)	21.8 (10.1)
Measurements	122.322	133.314	**144.291**	198.205	266.179	404.441
Unique hkl	74.597	80.219	**85.618**	100.703	102.868	105.709
Multiplicity	1.64	1.66	**1.68**	1.97	2.59	3.82
Completeness	70.3 (72.2)	75.6 (77.2)	**80.7 (82.2)**	94.9 (93.4)	97.0 (96.1)	99.6 (98.6)
SHELXD / SHELXE						
No. of images	**55**	**60**	**65**	**90**	**120**	**180**
CC all	14.9	14.4	**36.9**	40.0	43.3	45.5
(weak)	4.10	3.7	**12.6**	14.5	16.0	16.9
PATFOM	1.96	1.86	**4.18**	4.59	5.1	5.72
Contrast	0.09	0.09	**0.37**	0.79	0.80	0.81
(enantiomorph)	0.09	0.09	**0.07**	0.18	0.18	0.18
Map CC last shell	0.49	0.32	**0.80**	0.84	0.86	0.87
(enantiomorph)	0.35	0.28	**0.41**	0.44	0.38	0.38
Pseudo-free CC	32.7	29.4	**65.9**	70.2	71.5	72.8
(enantiomorph)	31.7	28.9	**27.4**	31.1	29.9	27.5

Last shell (2.10-2.00 Ang.) in parentheses

Structure solution and phasing

Program SHELXD was instructed to locate 26 anomalous scatterers: 16 seleniums and 10 Cd ions. The best solution was used for phase calculation and further improvement by density modifications with program SHELXE.

In order to assess the relationship between multiplicity of the data and success of automatic phasing, the available range of data of 180 degrees was split up in smaller ranges, always starting with image no. 1. The table above shows that the completeness with 55 images and 60 images, respectively, is not sufficient to give a clean solution. As little as 65 degrees of data with a multiplicity of < 1.7 already give the correct solution. The experimental map is of similar quality as the one obtained from processing 180 images (Figure 4). The automatic tracing of the experimental maps is straightforward.

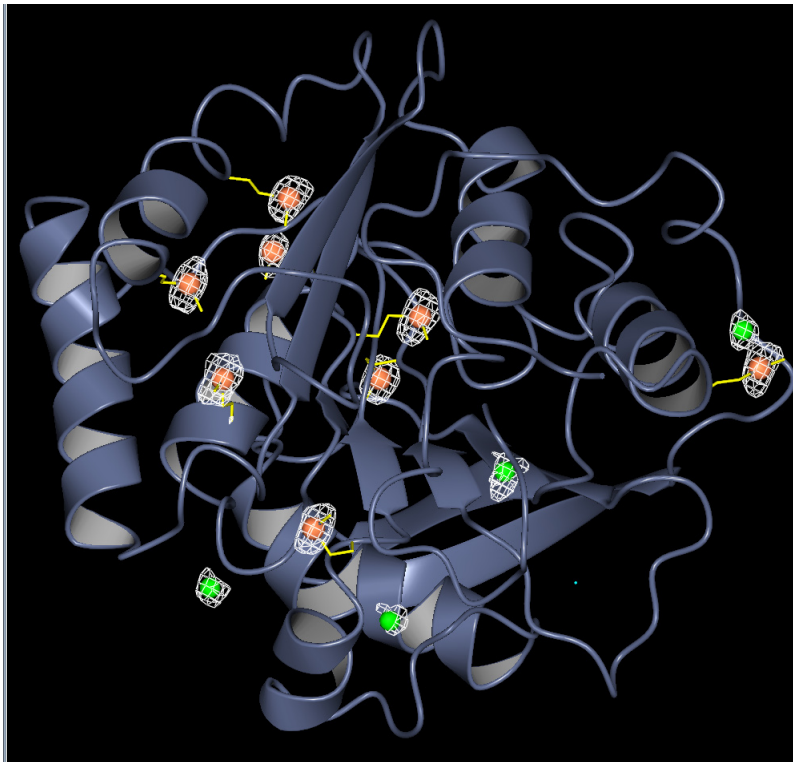

Figure 3: Anomalous difference Fourier map after 65 images
65 images have been used to obtain anomalous differences and experimental phases. The map shows all density > +3 σ (white). Cd ions are drawn in green, Se atoms in coral and methionine residues in yellow. For sake of clarity, only molecule A of the dimer is shown. The 3 highest peak in the map are from Se-atoms of residues MSE 869 1024, 1031, respectively (all 15 σ)

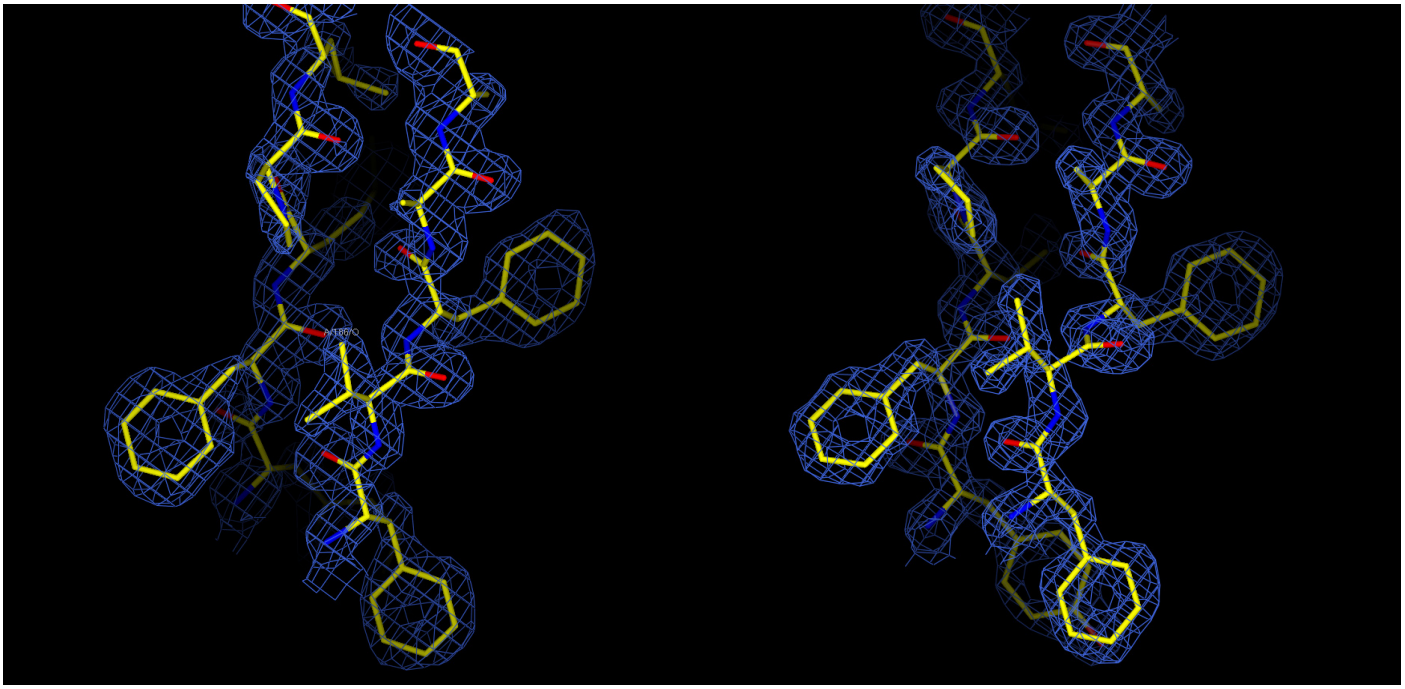

Figure 4: Experimental maps after 65 and 180 images
A typical section of the map shows residues ranges 973 to 977 and 1009 to 1013 located in the central beta-sheet.Contours are drawn at 1.1 σ. The figure on the left hand side uses experimental phases after SHELXE from 65 images, the figure on the right hand side uses phases after processing 180 images. The differences are negligible.

Conclusion

The SAD experiment with the mar555 flatpanel detector yield protein phases of impressive quality. An amazingly low value of only 65 degree of data with 80% completeness and a very low multiplicity of 1.7 are sufficient to solve a large protein structure from scratch.

Acknowledgements:

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