

# Xcalibur PX Ultra SAS Phasing of Insulin

at York Structural Biology Laboratory

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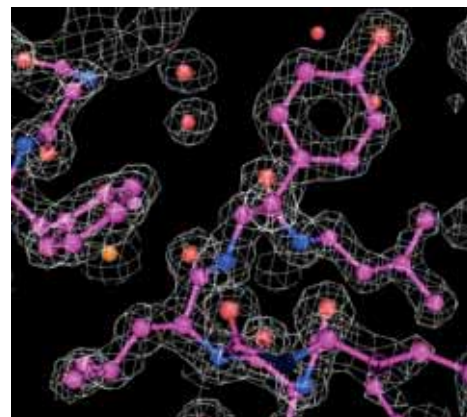


Figure 1. A section of the electron density map calculated using the phases. FOM and DM.

The Xcalibur PX Ultra was used to collect highly redundant data from cubic Insulin in order to demonstrate protein structure phasing using the sulphur anomalous signal. Native cubic Insulin crystallises in the space group  $I2(1)3$  with cell edges of 77.86 Å and contains 6 cysteine residues in 3 disulphide bridges.

Data were collected using the 4-circle kappa geometry and the Onyx CCD in a 'straight-on' (theta 0) position. 2 Å data were collected as five runs totalling 1814 images with an exposure time of 20 sec per frame (2x20 sec correlated pair) and a scan width of 0.5° in omega.

Data were indexed and integrated using MOSFLM and scaled and merged using SCALA in the CCP4i interface. [1,2].

ALL 6 sulphur positions were readily located using the 2 Å data and SHELXD. The 5 runs of data were used, however, the sulphur sites could also be located using only the first two runs. The sulphur parameters were then refined using mlphare (CCP4).

At this stage a second shell of data was collected to a resolution of 1.5 Å with the Onyx CCD detector offset by -20° in theta. Data were collected as a single run totalling 238 images of exposure time 150 sec per frame and a frame width of 0.5° in omega.

The 1.5 Å and 2 Å data were combined and the program DM (CCP4) was used to perform density modification, extending the phases to 1.5 Å resolution.

The electron density map was calculated using the phases, FOM and DM. The resultant map was found to be of very high quality and comparable to the final refined map obtained from synchrotron data (see figures 1 and 5).

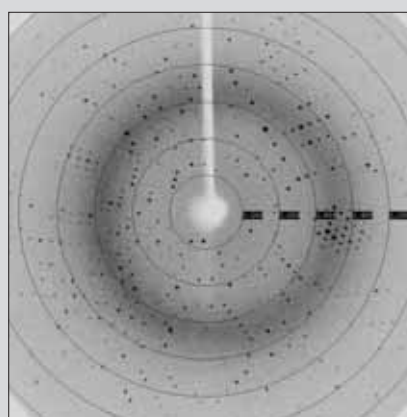


Figure 2. A 20 sec image taken at zero theta.

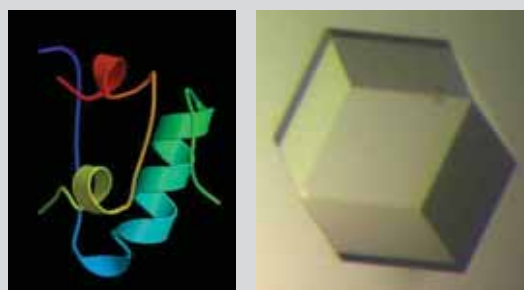


Figure 3. An Insulin crystal.

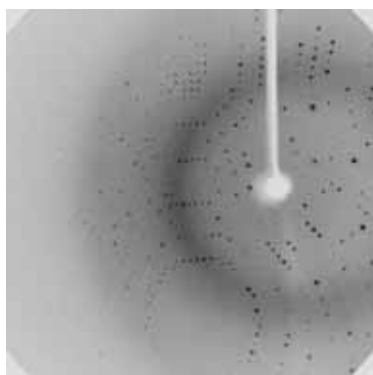


Figure 4. A 150 sec image taken at  $-20^\circ$  theta offset.

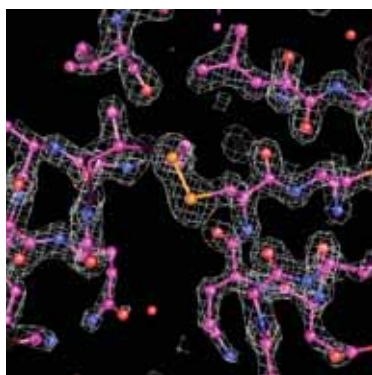


Figure 5. A section of the electron density map showing the di-sulphide bridges.

<b>System</b>	<b>Xcalibur PX Ultra</b>
Power	2.2 kW (max possible 3 kW)
Detector distance	80 mm (Range 60 – 150 mm)
Optics	Enhance Ultra (incorporating multi-layer optics)
Temperature	120 Kelvin

<b>Data set</b>	<b>2.0 Å</b>	<b>1.5 Å</b>
Exposure time	20 sec	150 sec
R merge	0.041 (0.184)	0.043 (0.342)
Mean I/sd(I)	110.1 (10.6)	26.6 (1.9)
Completeness	99.2 (95.0)%	94.9 (67.5)%
Multiplicity	75.6 (7.5)	5.7 (2.0)
Anomalous completeness	97.3 (81.8)%	-
Anomalous multiplicity	39.7 (4.0)	-
Total no. of observations	409851 (5623)	69206 (2505)
Total no. of unique	5420 (745)	12092 (1234)
Low resolution limit	31.78 (2.11) Å	31.78 (1.58) Å
High resolution limit	2.00 (2.00) Å	1.5 (1.5) Å
Two theta setting	$0^\circ$	$-20^\circ$
Oscillation range	$0.5^\circ$	$0.5^\circ$
Total data collection time	24 hrs 11 mins	20 hrs 21 mins

## References

- 1 MOSFLM (Leslie, A.G.W., (1992), Joint CCP4 + ESF-EAMCB Newsletter on Protein Crystallography, No. 26)
- 2 "The CCP4 Suite: Programs for Protein Crystallography". Acta Cryst. D50, 760-763

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