BL13B1 蛋白質結晶學光束線備用新 Q-315r CCD 面積偵測器性能測試報告

Performance Test Report of the Backup Q315r CCD Area Detector Used at BL13B1 PX Beamline

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(一) 簡介

基因體醫學核心設施(Synchrotron Radiation Protein Crystallography Facility, SPXF)包含了兩座光束線(BL13B1 和 BL13C1)，配備兩台美國 ADSC 公司 Quantum 系列的 CCD (Charge Coupled Device)面積偵測器，其型號為 Q315 (BL13B1)和 Q210 (BL13C1)。

BL13B 為 MAD (Multi-wavelength Anomalous Dispersion) 實驗站，是目前解析新的蛋白質結構最主要的實驗技術。為了提高此實驗站的使用效率，在硬體上要盡可能使用最快最大的面積偵測器，因此使用當時世界上最快最大的 Q315 為 MAD 實驗的偵測器。若 Q315 發生故障，則會將 BL13C1 所使用之擁有同樣讀取速度的 Q210，安裝至 BL13B1，以維持其數據收集效率，BL13C1 則改用 Mar 345 IP 偵測器。Q210 曾兩度因 Q315 故障而安裝至 BL13B1，然而因為 Q210 (210mm 直徑)相較於 Q315 (315mm 直徑)偵測面積較小，嚴重影響 BL13B1 的數據收集效率，加上 Q315 故障檢修須寄回美國，來回需時約 3 個月，以一年 9 個月的用戶時間來計算，每故障一次會造成一年約三分之一的時間數據收集效率不佳，用戶抱怨連連，故中心決定使用 2008 年統籌款添購一台同樣大小性能更佳的 Q315r 面積偵測器，做為 BL13B1 的備份偵測器。

Q315r 於 2009 年 3 月運抵 NSRRC，利用 3 月儲存環停機時間安裝在 BL13B1，並於出光後進行性能測試，本報告將利用標準樣本(Insulin)的量測，來檢驗這台新的面積偵測器性能是否正常。

(二) ADSC Q-315r CCD 面積偵測器系統

下圖為此面積偵測器之照片

下表為 ADSC Quantum CCD 面積偵測器的一些特性參數：

Specification	Units	Quantum Q315	Quantum Q315r
Active Area	mm	315x315	315x315
# of Pixels	Quantity	6144 x 6144 37.75 Million	6140 x 6140 37.75 Million
Pixel Size	Microns	51 x 51	51 x 51
Spatial Resolution FWHM	Microns	90	90
CCD Type		Thomson 7899 (2K x 2K)	Atmel THX 7899 (2K x 2K)
Dark Current	e/pixel/sec	0.015	0.015
Read Noise Full Resolution	electrons	13.5	11.0
Dynamic Range Full Resolution		14100	18100
Readout Times: Full Resolution 2x2 Binned @430Khz	Seconds	1.1 330 ms @ 4 corner	0.9 250ms @ 4 corner

(三) 實驗方法及參數 (因專有名詞之故，此節將以英文撰寫)

A well-accepted evaluation of the quality of data from a given detector is to measure the peak heights in the anomalous difference Patterson for P6 myoglobin. A strong set of Patterson vectors arising from the Fe atoms in the heme group has been the metric for high quality data. Recently, it has been argued that the test is not sensitive enough because the δ f" term for Fe at CuKα is 3.204 electrons.

Z. Dauter, et al. have reported a method for solving structures of strongly scattering macromolecules using the anomalous scattering signal of sulfur. A major prerequisite for using this method is data of extremely high quality because the δ f" term for sulfur at CuKα is only 0.557 electrons. This method may become more widely used as a tool for checking the quality of data produced by modern area detectors since the signal is much weaker, and, as a result is a more demanding experiment.

I. Insulin Structure Determination by Sulfur SAD (Single-wavelength Anomalous Dispersion)

Successfully structure determination by sulfur SAD and high percentage sequence coverage by automatically model building represent extremely high data quality.

Incident X-ray Energy: 7 keV (where the δ f" term for sulfur is 0.721 electrons)

Defining Aperture Size: 200 μm
Crystal Size: 120 μm x 120 μm x 120 μm
Detector Distance: 132 mm
Oscillation Range: 180°
Frame Width: 0.5°
Exposure Time: 1 second

II. ADFM (Anomalous Difference Fourier Map) Calculation

Clear observation of anomalous scatters (sulfur) with extremely low δ f'' term of 0.244 electrons in the anomalous difference map indicates that the data is of the highest quality.
Incident X-ray Energy: 12.398 keV (where the δ f'' term for sulfur is 0.244 electrons)
Defining Aperture Size: 200 μm
Crystal Size: 120 μm x 120 μm x 120 μm (same crystal as previous experiment)
Detector Distance: 150 mm
Oscillation Range: 180°
Frame Width: 0.5°
Exposure Time: 1 second (with attenuation to 50% of original beam intensity)

(四) 性能測試結果 (因專有名詞之故，此節將以英文撰寫)

I. Insulin Structure Determination by Sulfur SAD (Single-wavelength Anomalous Dispersion)

A total of 360 images of 0.5° oscillation angle and 1 second exposure time data were collected at 7 keV (where the δ f'' term for sulfur is 0.721 electrons) and processed with HKL2000. The overall R_{sym} is 4.6 % out to a maximum resolution of 2.12 Å with an average redundancy of 20.0, $<I/\sigma(I)>$ of 33.1, completeness of 100.0 % and $\chi 2=1.017$.

These data were then imported to CCP4i package for structure determination. Substructure was successfully determined by SHLEX C/D/E. Phase angles were calculated by Phaser and then optimized by DM. 94% of the sequence were automatically build by ARP/wARP that indicates good quality of data. Coot was used for further manual model building. By refmac5, R factor and R_{free} of final insulin model were refined to 21.05% and 21.11%, respectively.

II. ADFM (Anomalous Difference Fourier Map) Calculation

Using the same crystal, a total of 360 images of 0.5° oscillation angle and 1 second exposure time data were collected at 12.398 keV (where the δ f'' term for sulfur is 0.244 electrons) and processed with HKL2000. The overall R_{sym} is 4.0 % out to a maximum resolution of 1.4 Å with an average redundancy of 21.1, $<I/\sigma(I)>$ of 21.5, completeness of 100.0 % and $\chi 2=1.062$.

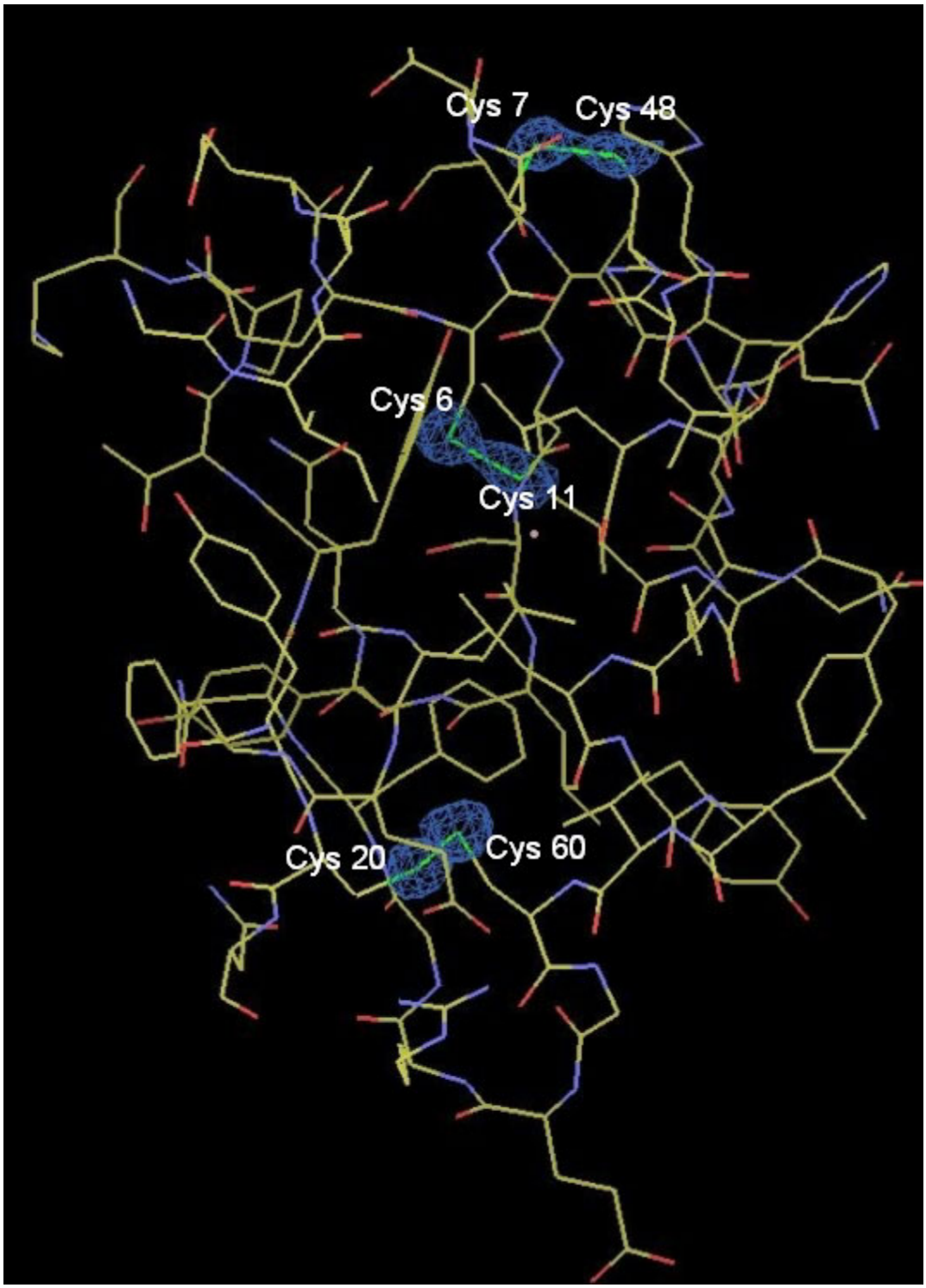
These data were imported into CCP4i package. Molecular replacement was performed by Molrep using final insulin model from previous SAD experiment. By refmac5, R factor and R_{free} of final insulin structure were refined to 20.49% and 20.40%, respectively. A difference Fourier map with the coefficients ΔFanom,φcalc-90° was calculated and the peak heights were extracted. The positions of each sulfur atoms are clearly marked by a significant peak. Features such as this are not possible unless the data is of the highest quality.

Shell Lower limit	Upper Angstrom	Average I	Average error	Average stat.	Norm. Chi**2	Linear R-fac	Square R-fac	Average Redundancy	Average I/error
30.00	4.56	18345.7	327.5	78.9	0.969	0.038	0.044	19.5	
4.56	3.62	24387.5	430.5	78.6	0.952	0.046	0.054	18.2	
3.62	3.17	14729.7	264.2	57.3	1.071	0.051	0.058	18.7	
3.17	2.88	8530.2	122.9	39.8	1.094	0.046	0.052	20.4	
2.88	2.67	5992.5	84.6	34.3	1.039	0.044	0.050	20.6	
2.67	2.51	4689.8	72.6	31.2	1.009	0.048	0.052	20.7	
2.51	2.39	3887.2	60.6	30.4	1.008	0.050	0.055	20.5	
2.39	2.28	2979.6	48.4	28.0	1.000	0.055	0.058	20.6	
2.28	2.20	2519.7	45.2	28.5	0.988	0.062	0.063	20.5	
2.20	2.12	2109.2	43.8	28.8	1.034	0.075	0.077	20.2	
All reflections		8975.3	152.8	44.0	1.017	0.046	0.052	20.0	33.1

HKL2000 statistics of data collected at 7 keV

Shell Lower limit	Upper Angstrom	Average I	Average error	Average stat.	Norm. Chi**2	Linear R-fac	Square R-fac	Average Redundancy	Average I/error
30.00	3.02	20498.9	215.3	47.2	1.151	0.031	0.038	21.4	
3.02	2.39	6076.7	74.8	24.4	1.084	0.037	0.041	20.4	
2.39	2.09	3328.8	62.0	19.8	1.104	0.061	0.067	20.5	
2.09	1.90	1815.9	39.0	16.5	1.185	0.074	0.074	20.6	
1.90	1.76	1045.7	24.5	14.5	0.984	0.082	0.081	21.4	
1.76	1.66	625.3	16.7	13.9	1.034	0.101	0.090	21.4	
1.66	1.58	445.0	16.0	14.4	1.024	0.138	0.125	21.4	
1.58	1.51	326.7	15.5	15.0	1.074	0.191	0.173	21.5	
1.51	1.45	245.7	16.3	15.9	0.999	0.257	0.229	21.4	
1.45	1.40	182.5	17.1	16.9	0.987	0.353	0.317	21.3	
All reflections		3552.9	50.6	20.0	1.062	0.046	0.040	21.1	21.5

HKL2000 statistics of data collected at 12.398 keV



Anomalous difference Fourier of insulin using the coefficients ΔFanom,φcalc-90°

Atom	Peak Heights	
	BL13B1 Q315 (old)	BL13B1 Q315r (new)
CYS 6	13.20	13.77
CYS 60	12.08	12.99
CYS 20	11.60	11.65
CYS 11	10.83	11.41
CYS 7	8.31	9.37
CYS 8	7.68	7.86

Peak heights, I/s, for the insulin anomalous difference Fourier

(五) 結論

由上列標準樣本(Insulin)的數據分析顯示，這台 ADSC Q-315r CCD 面積偵測器，可以成功的收集極微弱的異常散射信號，並解析出蛋白質結構，繞射數據品質極高，性能優良可放心使用。