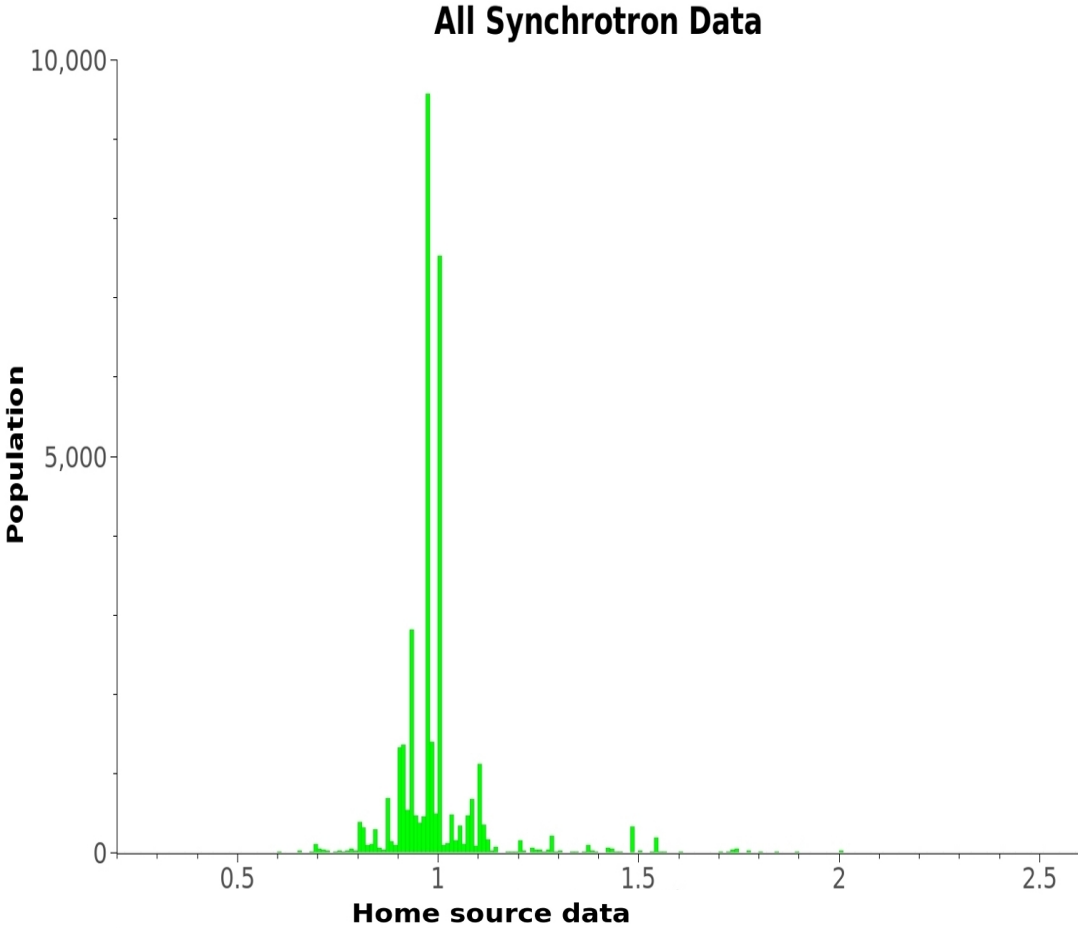
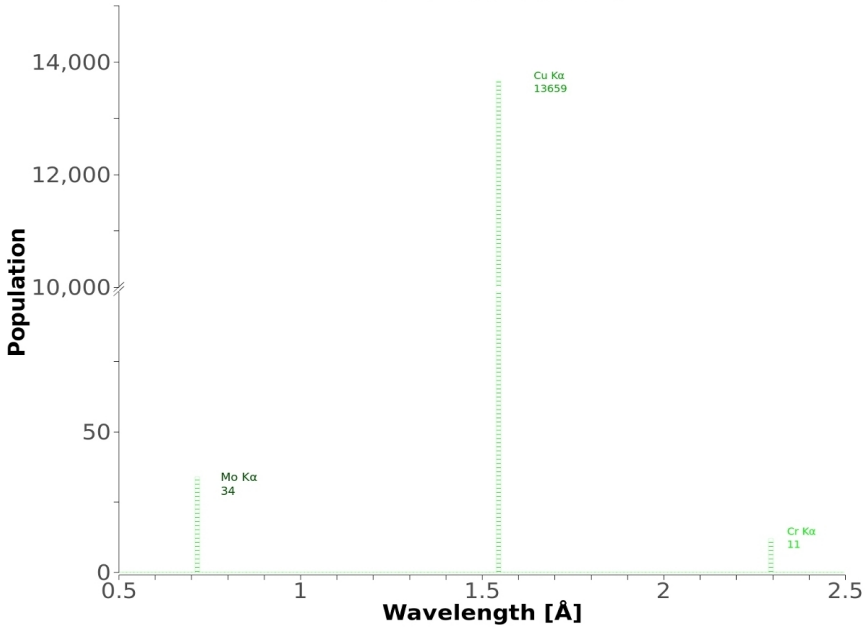
Macromolecular Crystallography at High(er) energy ?

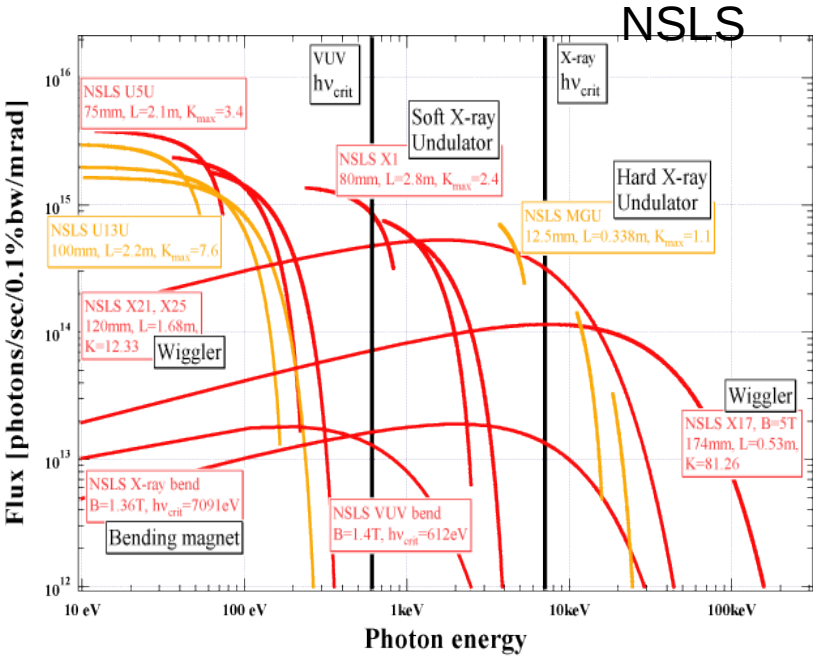
Beamline X6A Scientific Advisory Committee Meeting

Friday February 10 2012

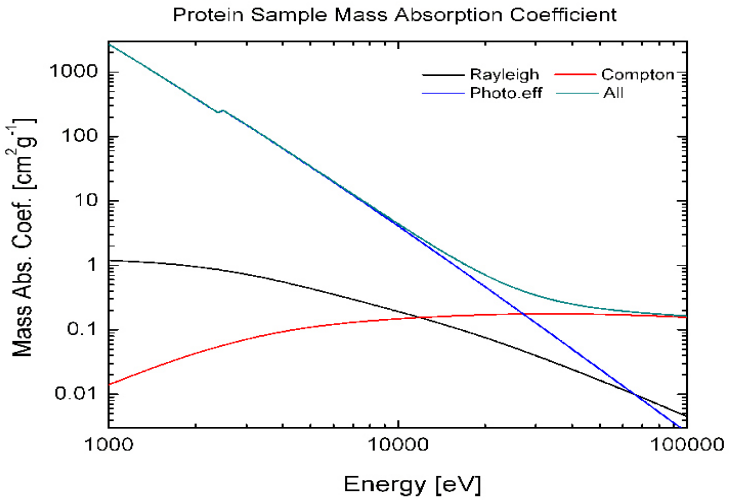
Jean Jakoncic


All Synchrotron Data
Population
10,000
5,000
0
0.5
1
1.5
2
2.5
Home source data


Population
14,000
12,000
10,000
50
0
Cu Kα
13659
Mo Kα
34
Cr Kα
11
0.5
1
1.5
2
2.5
Wavelength [Å]


NSLS
Flux [photons/sec/0.1%bw/mrad]
VUV
hν crit
X-ray
hν crit
Soft X-ray
Undulator
Hard X-ray
Undulator
NSLS U5U
75mm, L=2.1m, K max=3.4
NSLS X1
80mm, L=2.8m, K max=2.4
NSLS MGU
12.5mm, L=0.338m, K max=1.1
NSLS U13U
100mm, L=2.2m, K max=7.6
NSLS X21, X25
120mm, L=1.68m,
K=12.33
Wiggler
Wiggler
NSLS X17, B=5T
174mm, L=0.53m,
K=81.26
NSLS X-ray bend
B=1.36T, hν crit=7091eV
Bending magnet
NSLS VUV bend
B=1.4T, hν crit=612eV
10 eV
100 eV
1keV
10keV
100keV
Photon energy

Why Higher Energies ?



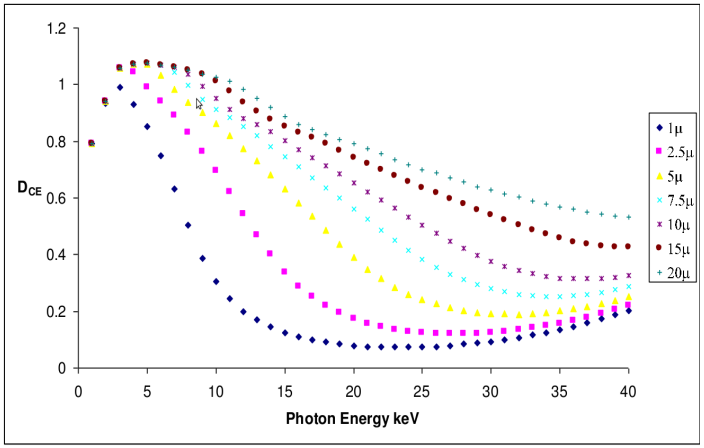
	12 keV	55 keV
Generic sample	2.4*/	0.2/
	2.2	0.03
Lyso Ho	8.3/	0.3/
	7.3	0.11

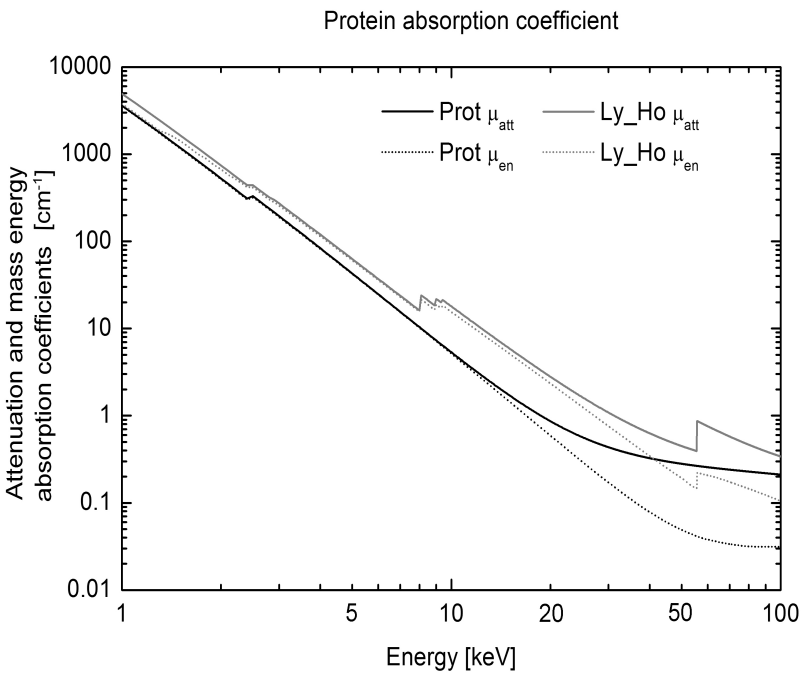
* mass attenuation / mass energy-absorption Coefficients in cm2/g

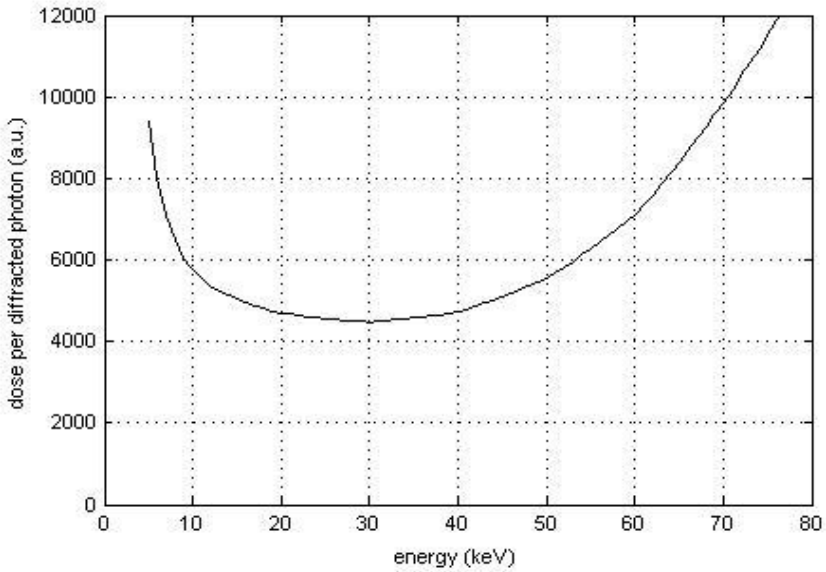
C. Nave

diamond

Include Photo-electron escape



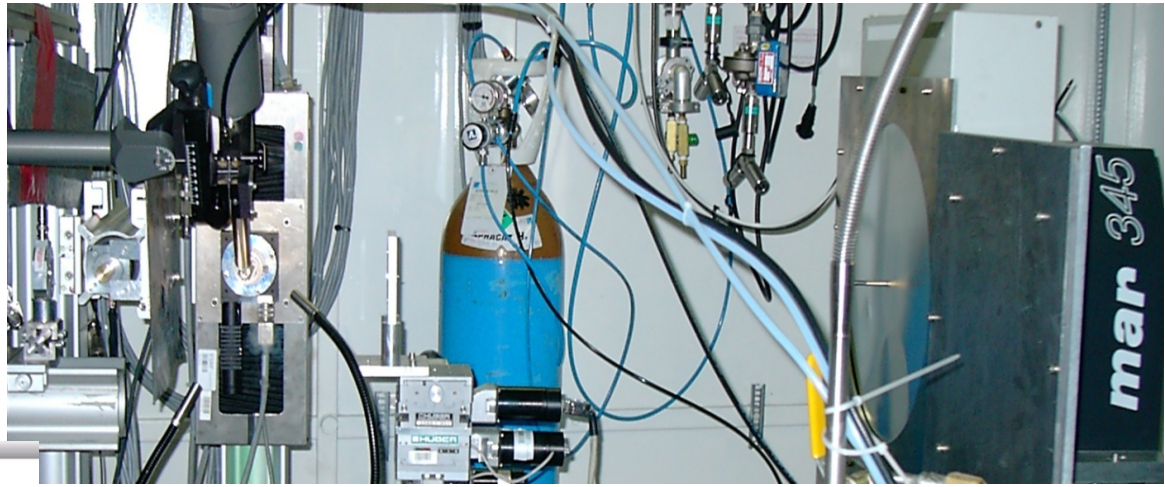


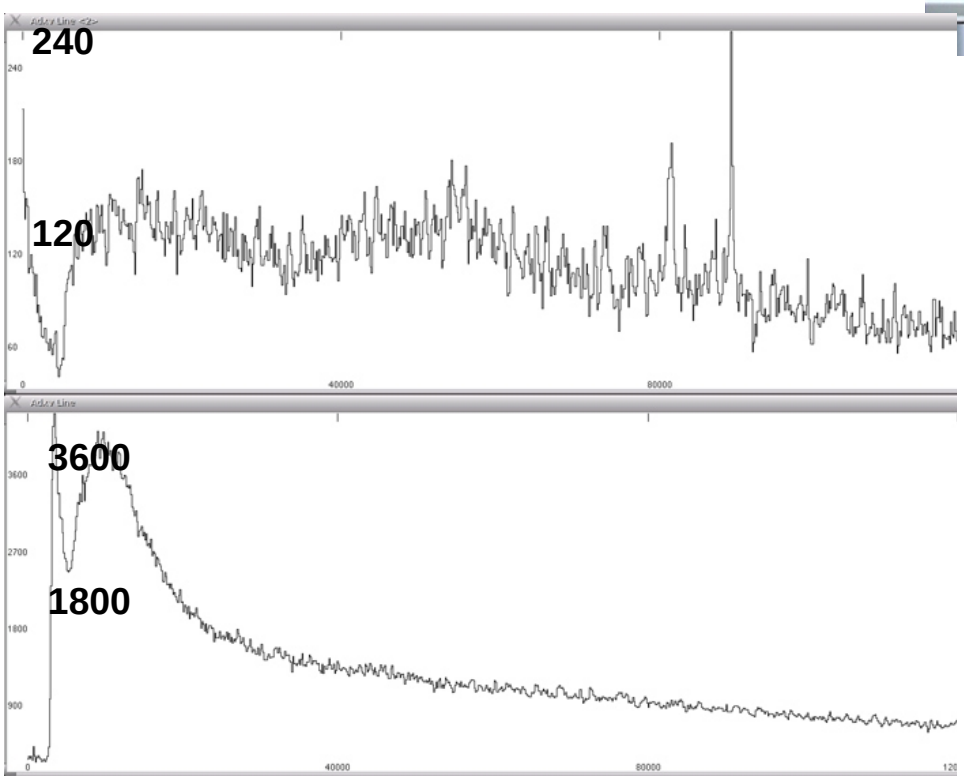


Calculated for a lysozyme protein crystal 100 um thick (at 1.5 A resolution)
V. Honkimaki

Experiments B and B'

- ID15B at ESRF AMPW (asymmetric multipole wiggler)
- Energy 55 keV, 5 10^{11} phs-1 in 0.3 x 0.3 mm^2
- Detector MAR345





setup

D ~ 650 mm

T = 100 K

2 beam stop (@20 and 60 cm)

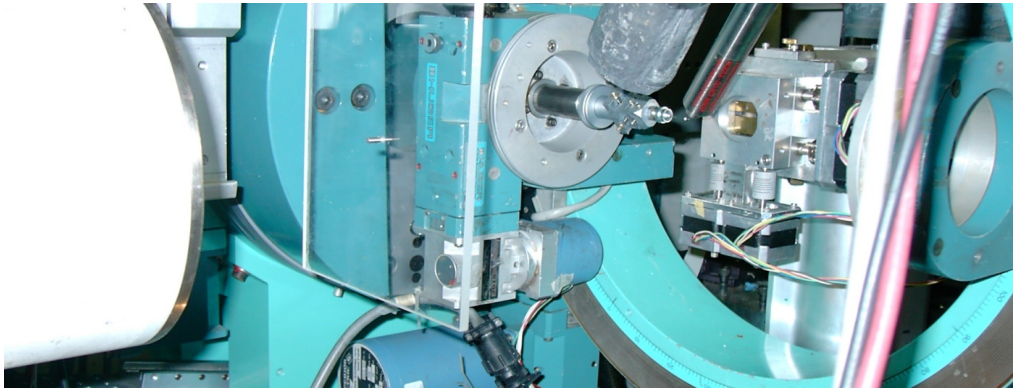
on 0.5 mm Al, 3.5% abs

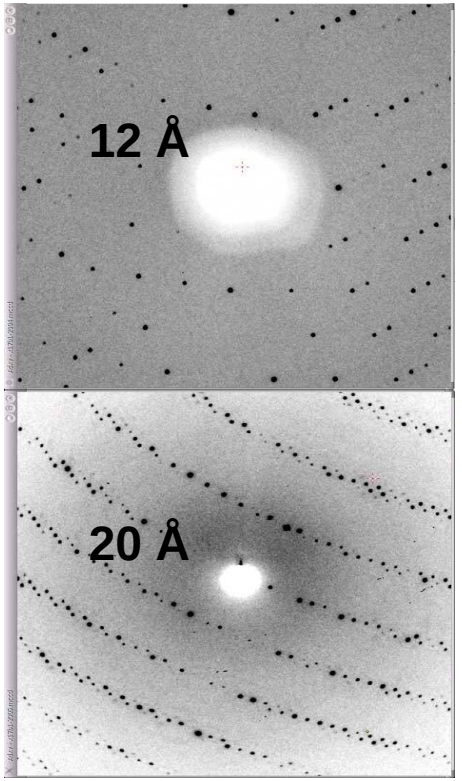
1st exp : 1 BS PB 6 mm D 25 mm L

2nd exp : + 1 BS PB 2 mm D 4 mm L

Experiments C and C'

- X17B1 at NSLS : superconducting wiggler
- Energy 55 keV : flux ~ 6 10^9 (0.3 x 0.3 mm2)
- Detector : MAR CCD 165 mm

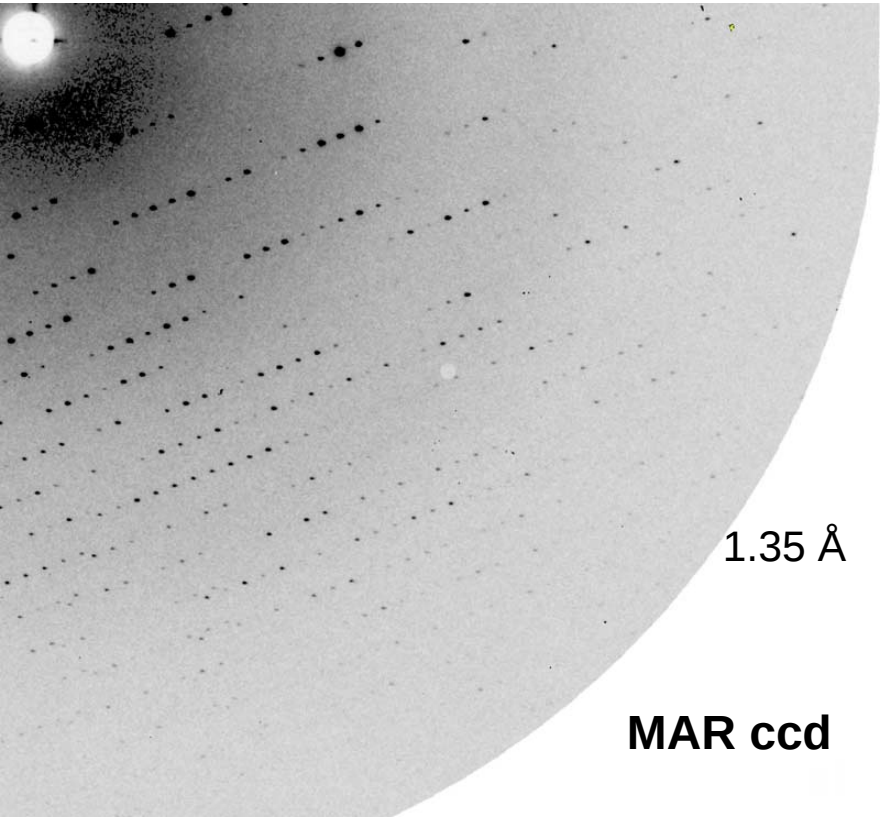


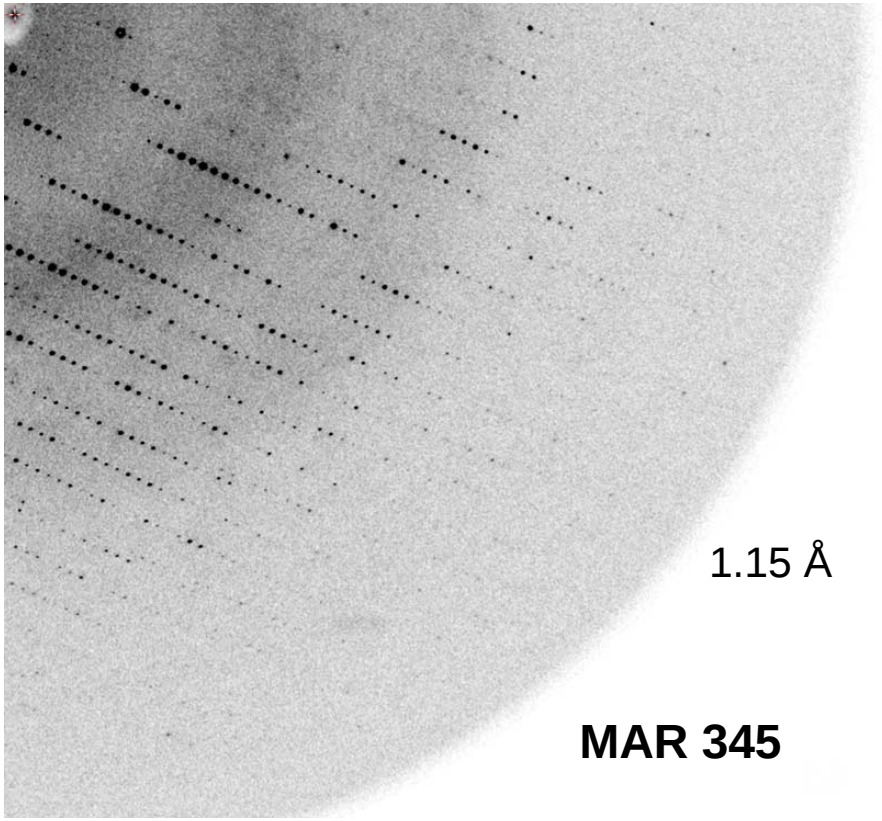


1st exp : BS Pb 6.4 mm in diam and 15 mm L
2nd exp : BS Ta 3.2 mm D and 15 mm L

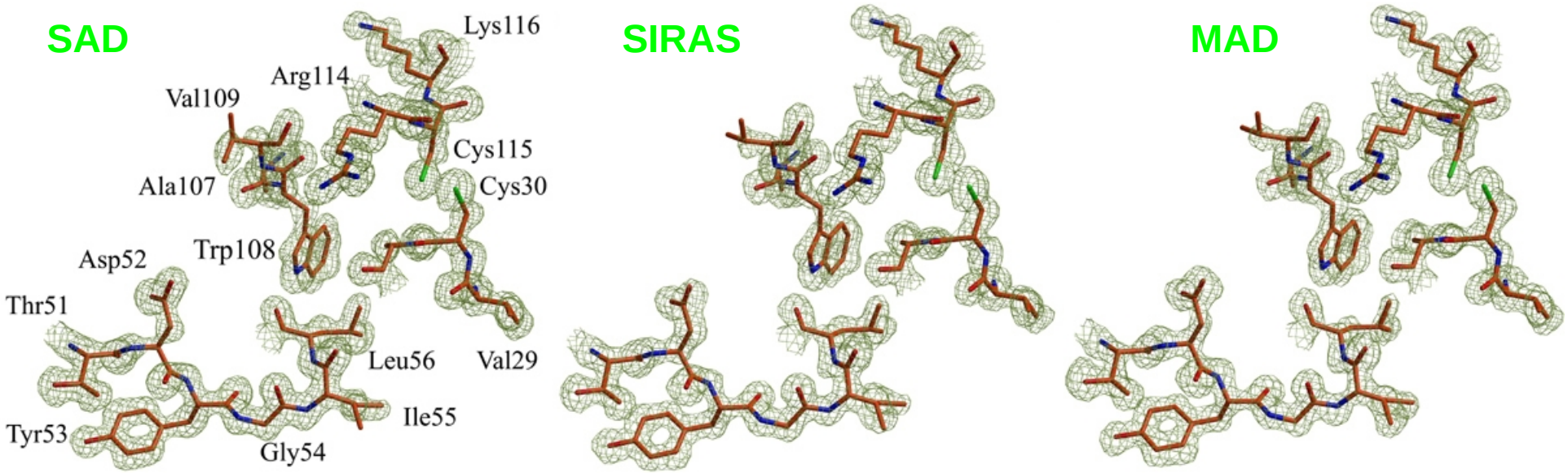
setup
D ~ 400 mm
T = 100 K
1 beam stop at 10 cm on 3.2 mm Plex., 7% abs.

Results





Results



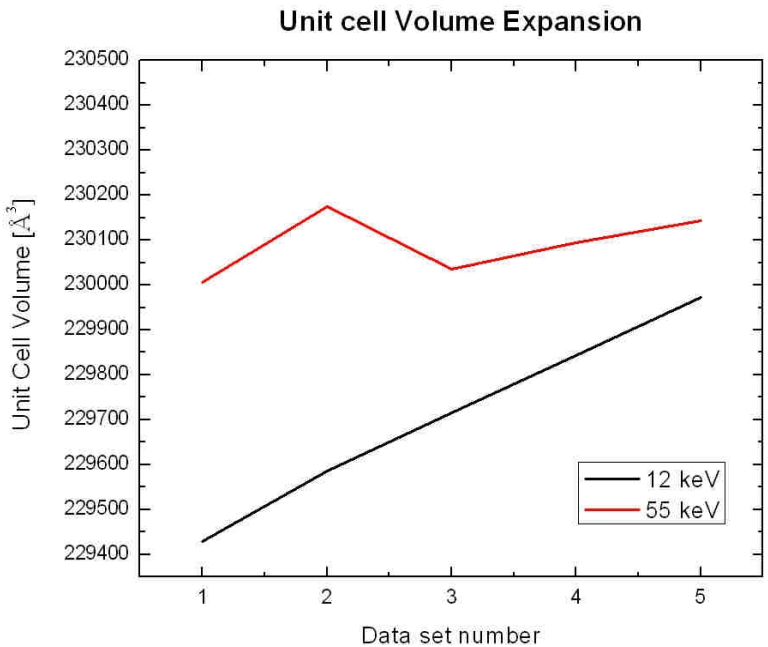
Beamline		X17B1	ID15B	
Method		SAD_{pk}	$SIRAS_{rm}$	MAD_{+nat}
shelxe	connectivity	0.89	0.88	0.89
	fom	0.75	0.79	0.81
	mapCC*	0.89	0.8	0.83
Arp	Res built (AA / wat)	124 / 162	128 / 156	127 / 162
	R / R_{free} (%)	24 / 27	21 / 25	20 / 23

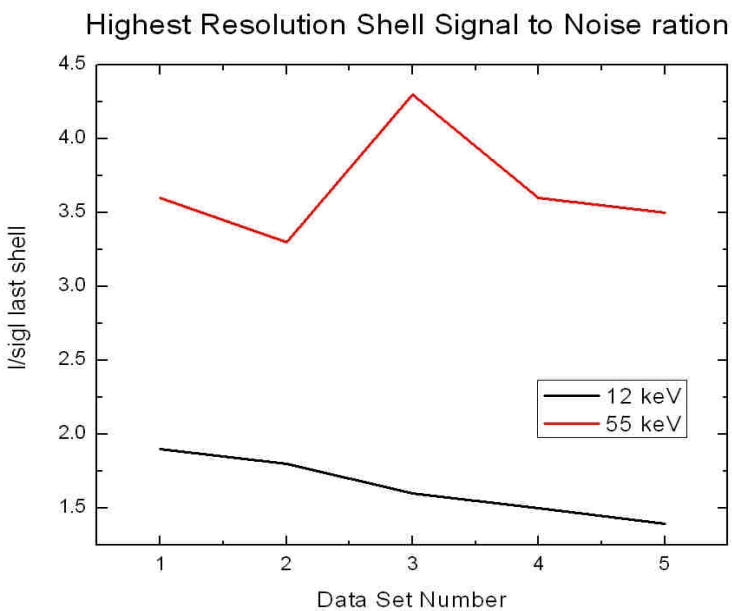
Results

	ID15B				X17B1			
HEWL	Native	Holmium			Native	Holmium		
Data	Native	Peak	Remote	Inflection	Native	Peak	Remote	Inflection
Wave. (Å)	0.2227	0.2229	0.2201	0.2227	0.2227	0.2229	0.2201	0.2227
Resolution Limits (Å)	**25.00-1.40 (1.45-1.40)**	**20.00-1.25 (1.29-1.25)**	**22.00-1.25 (1.29-1.25)**	**20.00-1.25 (1.29-1.25)**	**30.0-1.35 (1.40-1.35)**	**30.0-1.35 (1.40-1.35)**	**30.0-1.35 (1.40-1.35)**	**30.0-1.45 (1.50-1.45)**
Complet.(%)	99.9 (100)	98.9 (100)	99.2 (100)	99.0 (100)	**97.5 (97.3)**	99.2 (92.4)	100 (100)	100 (99.8)
I/σ(I)	29.1 (5.00)	21.0 (3.5)	26.2 (4.3)	21.0 (3.5)	14.9 (2.7)	24.9 (2.1)	35.4 (3.7)	19.2 (2.3)
R_{merge}* (%)	9.2 (48.4)	10.3 (60.0)	9.8 (63.5)	9.5 (61.4)	8.8 (44.2)	7.3 (75.3)	6.7 (73.7)	7.9 (69.3)
Mosaicity (°)	0.15	0.25	0.25	0.25	0.1	0.46	0.47	0.46
Total reflections	453777	453706	748407	455949	191991	321800	565274	177785
Multiplicity	10.4	7.4	12.2	7.4	4.0	6.5	11.5	4.5
#Frames	240	180	360 * 2.66	180	90	180	360	120

At ID15B, peak and inflection data collected first, then 2 times 360 degrees and 240 degrees collected at the remote energy (1320 º).
1320 degrees (11 times 120) collected on similar sample (size, cryo, resol., condition) at 12.5 keV.
DS 4, 6, 8, 9 and 11 used for analysis.

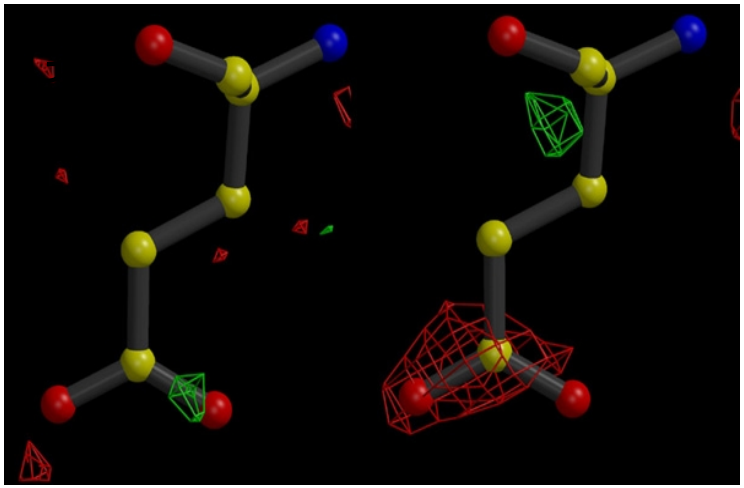
Radiation Damage ?

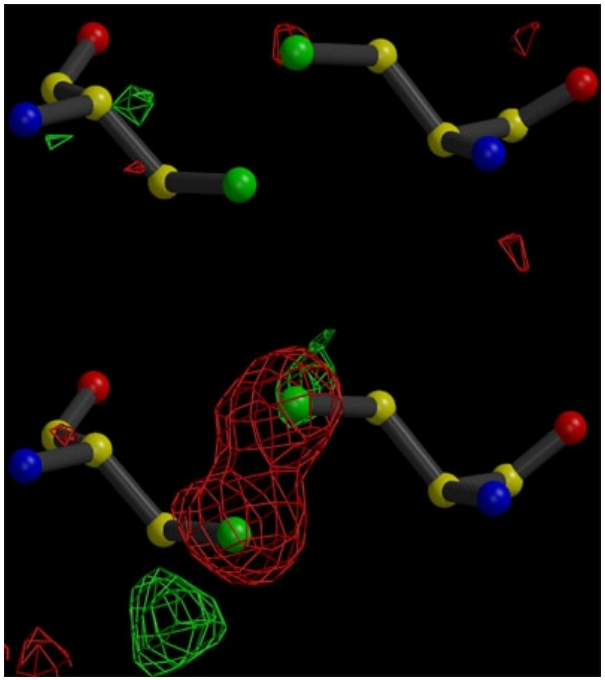




Asp87

Maps are calculated with Fobs of DS1 and DS5)



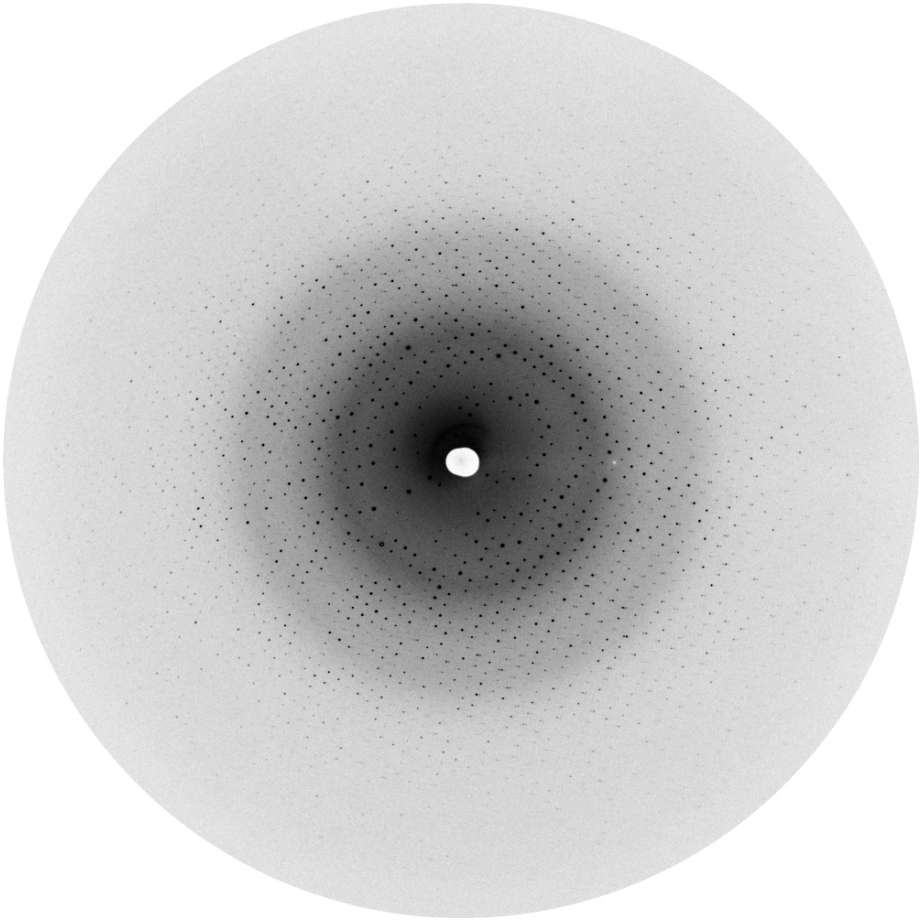


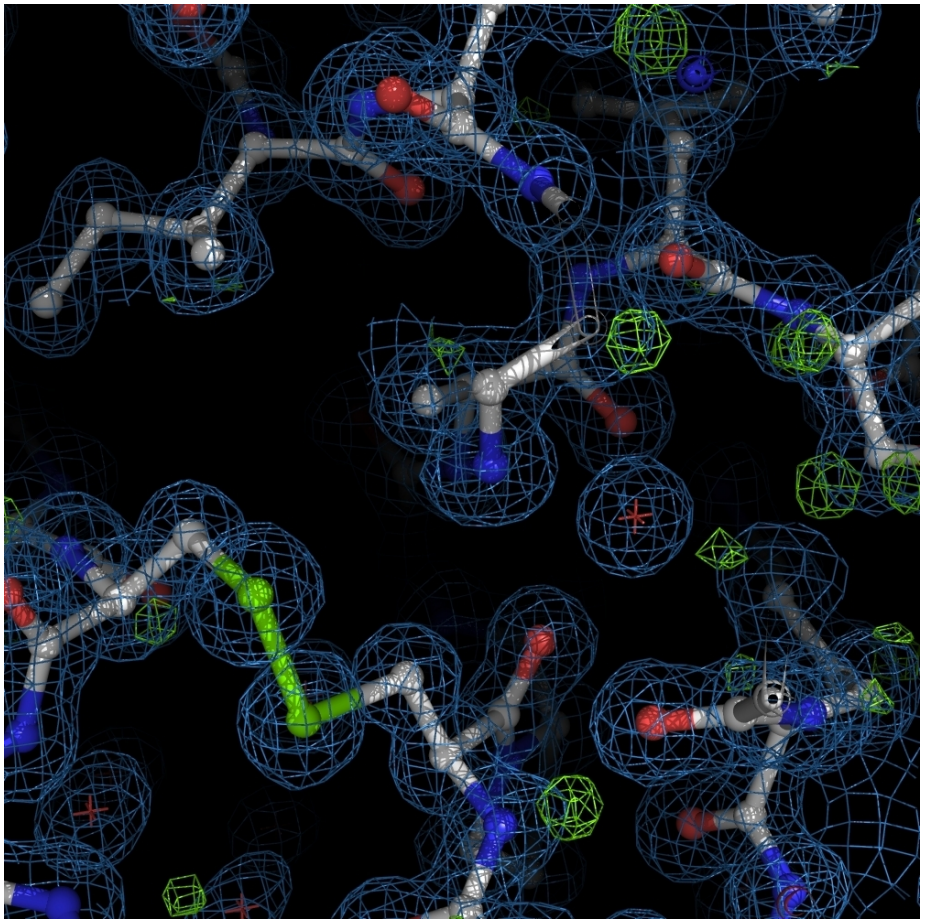
Cys6-Cys127 S-S bridge

Experiment D (2007 ID15B)

Energy / Wavelength : 55 keV / 0.2214 Å
Flux : ~ 10^{11} ph.s^{-1}
Exposure time : 20 s (0.25°), 400 frames total | Total time : 4200 seconds
Detector mode / cycle : 3000 (30 cm diameter, 100 µm pix) / 87 s

Detector distance : 670 mm
Resolution : 20.00-1.10 Å | Mosaicity : 0.3°
Rmerge : 5.7 %
Tetragonal Thaumatin (Asherie et al, 2009)





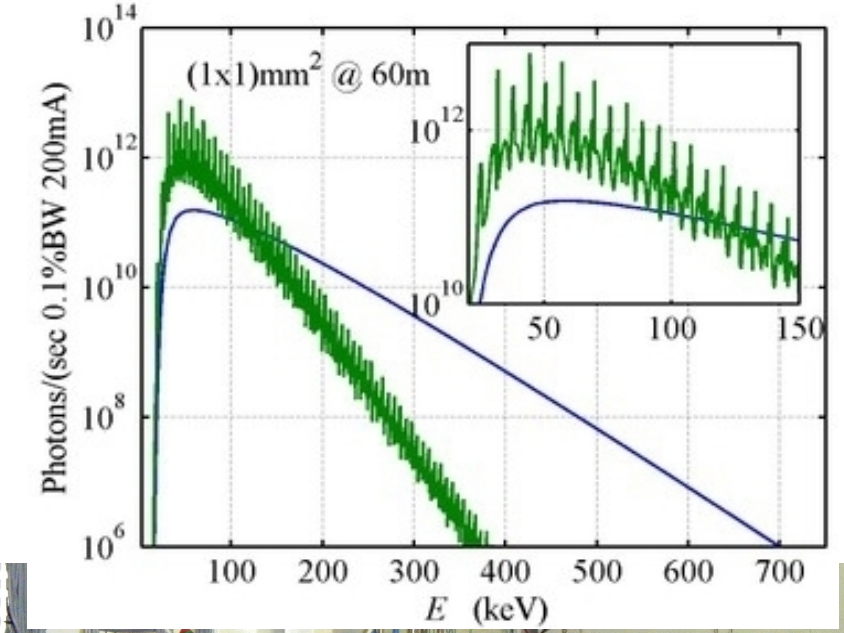
Other samples tested, Xe and Hg, but none did diffract .. Need pre-screening

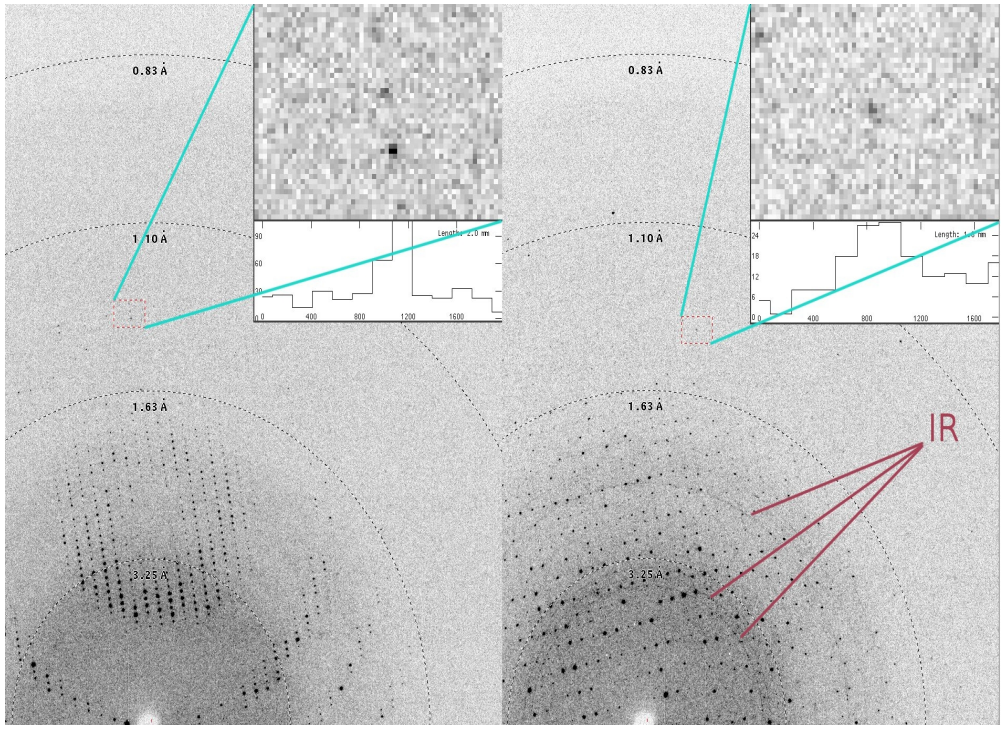
Experiment E (2008 ID15B)

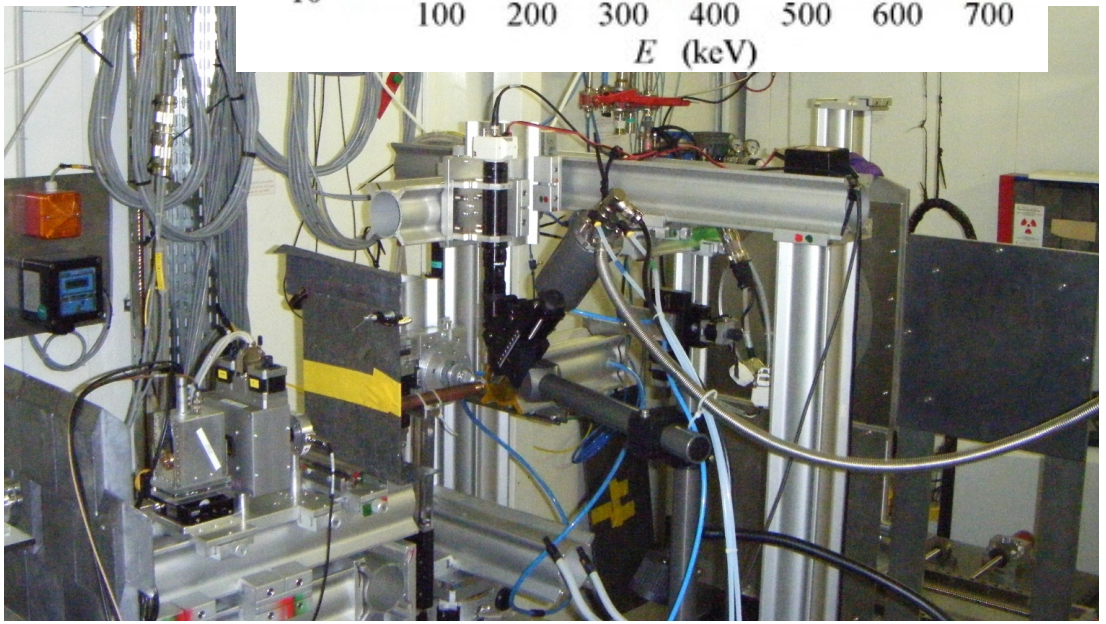
- E= 55 keV 0.2214 Å
- U22 undulator 10^{12} ph/s+ Pixium detector
- Lysozyme / MPD
- 0.4 sec / 0.5 °. 180 frames total (72 seconds)

Resolution : 20.00-1.50 Å | Mosaicity : 0.5°

Rmerge : 6.2 % | PDB : 2X0A | R/Rfree : 14.6 / 19.9 %





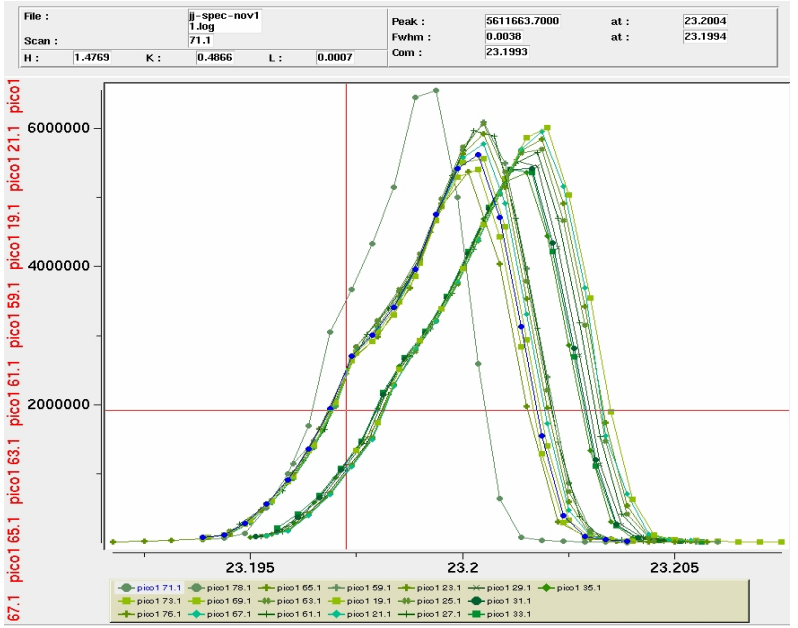


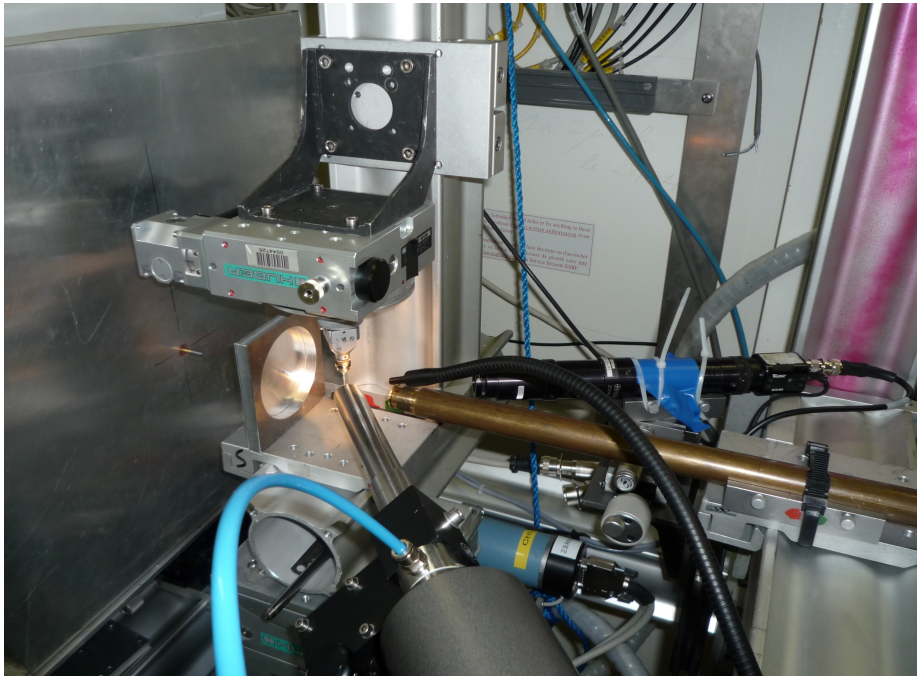
Small molecule single crystal diffraction, J. Heliwell, photosystem II tested (weak),

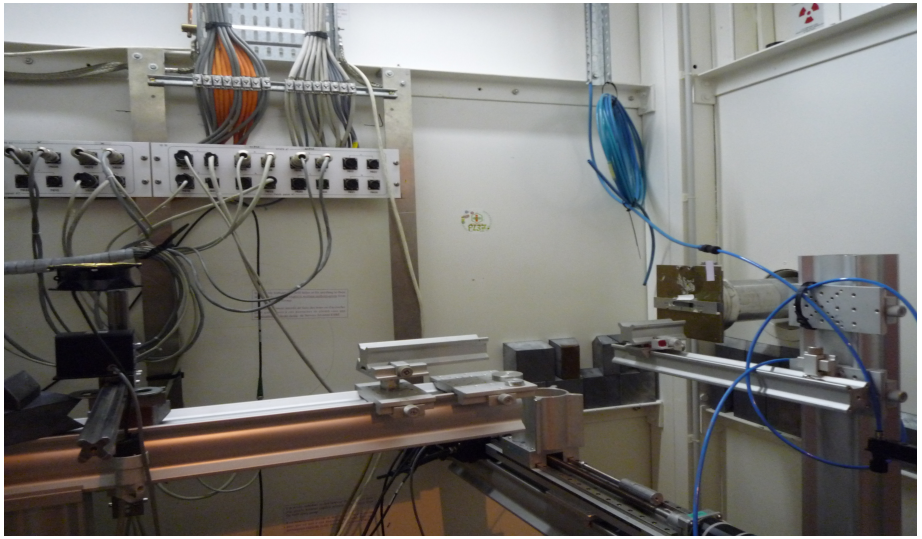
FrelOn + image intensifier tested to compensate for the detector triggering issue.

Experiment F (ID15C 2010)

- ID15C : 38 keV (0.32 Å) : !! above Ba K edge
- Mar345 (ESRF loan), on vertical axis
- Single Si(111) crystal mono : heat load !!
- Beam drifts ….. no BPM/feedback ..+ transfocator ..
- Samples tested : G. Cingolano (2 DS), T. Boggon. (4DS)
- All data collected as “good” as medium energy data
- Proteinase K : 0.9 Å (4 DS)
- Setup needs improvement (drift – rotation axis – detector- bckr)

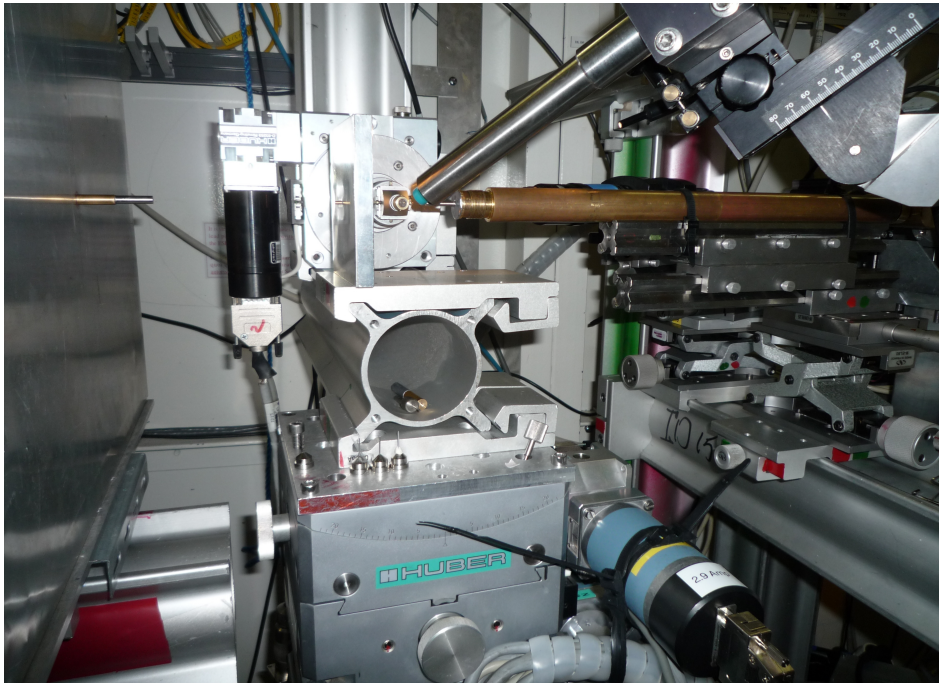


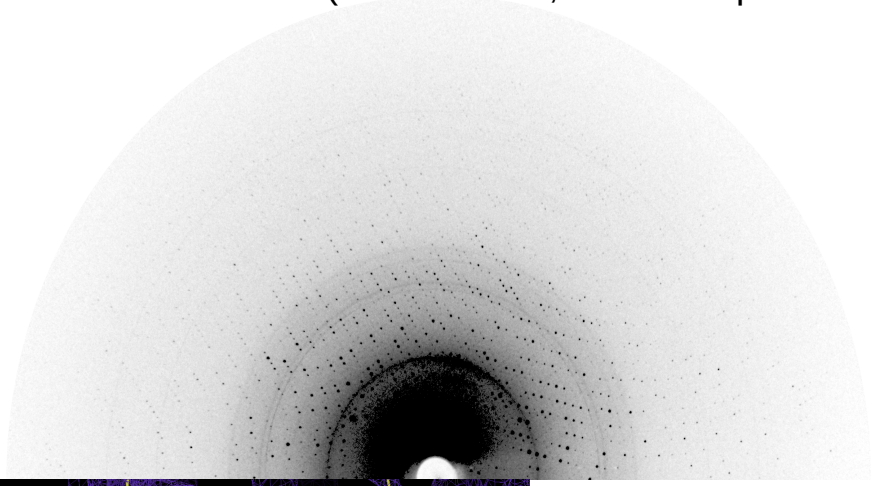


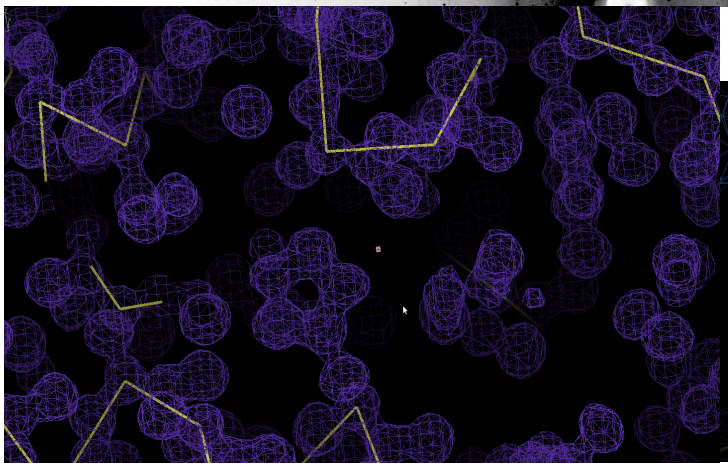


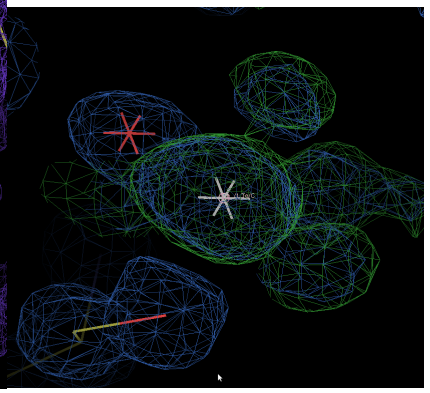
Experiment F' (ID15C 2011)

- ID15C H axis 38 keV … 10^{12} ph/s 'improved beamstop'
- E. Rudino-Pinera (3 DS on Cu enzymes), G. Cingolani (2 High Res DS), S. Gabelli (1 High Res DS + 3 HA DS)
- Multiple Room Temp. data collection
- 1 Proteinase K co-crystallized in TeO6 .. SAD structure solved at 1.05 Å resolution (0.12 º mosa., 99 % comp. 9.9 % Rm)









Detector Consideration

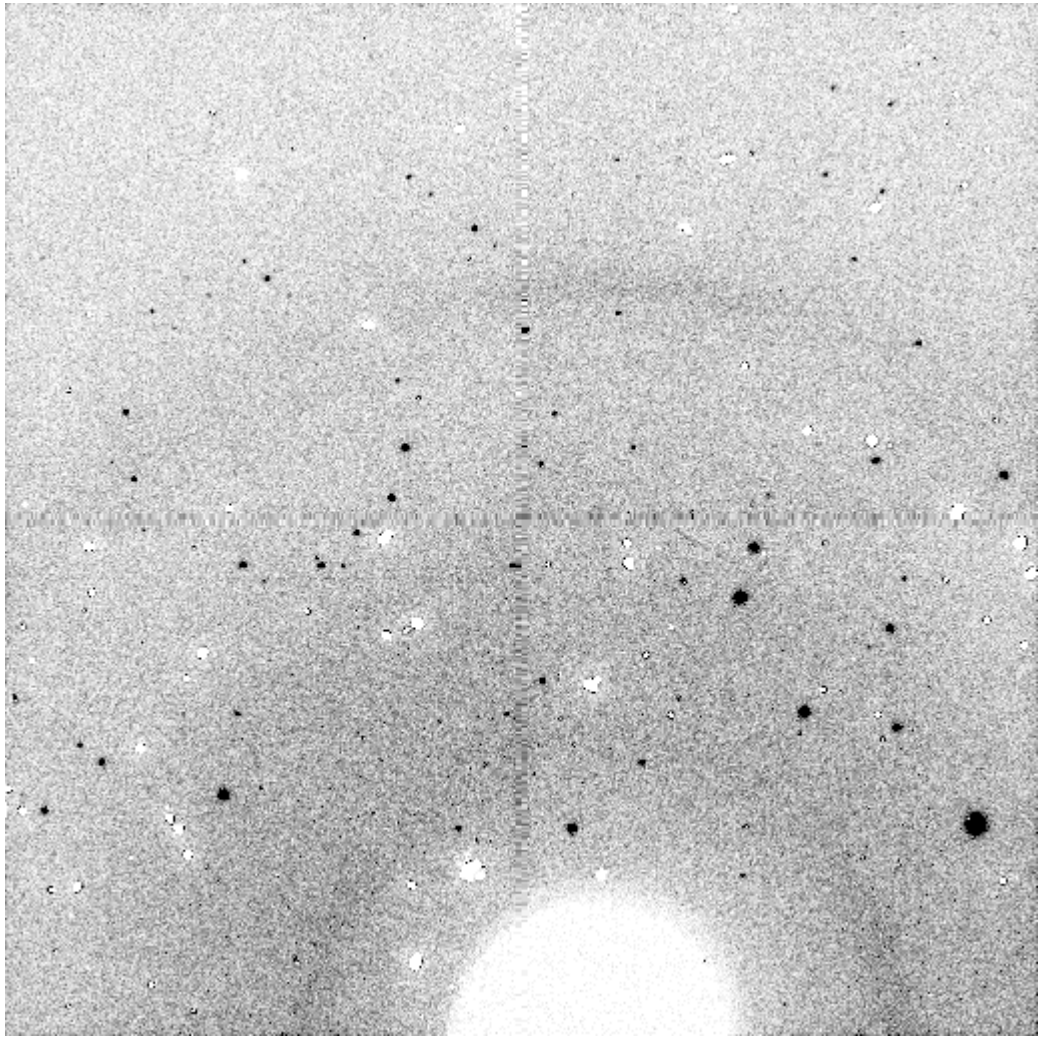
- Tested so far :
- Scintillator screen FOT CCD
- Automatic image plate
- Image intensifier
- CsI direct conversion to visible on aSi

	MARCCD	MAR345	PIXIUM
Area	165 mm (D)	345 mm (D)	400 x 300 mm2
Pixels	4k x 4k	1k x 1k to 3k x 3 k	2600 x 1920
PSF	2.5 pix	2.8 pix	1 pix
Speed	< 0.1 Hz	< 0.02 Hz	7.5 Hz
Dyn. Range	45000	120000	16000
Dead Time	10 s	50-11-s	0.1 s
Other	-80 C	x modes RT	RT ish 2x bin (30 Hz) Water
Cost	> 100 K	~ 100 K	< 100 K
Pixel size	40 um	100 or 150 um	143 um
Sensor	Gd2O2S:Tb FO CCD	BaFBr:Eu Laser readout	CsI on aSi
Sensor Thick.	40 um	200 um	0.5 mm

CdTe frames from ProtK at room T

Maxipix detector
CdTe Sensor (AcroRad)
512 x 512 pixels (2 x 2 modules)
55 microns pixels
28 x 28 mm^2 total area
E=38 keV
ProtK at 295 K
0.01 deg for 0.5 sec
Resolution at the top left corner : 5 A
Sample previously irradiated for ~ 720 s
Loss of diffraction after ~ 1400 s of exp.
No shutter used

Other detector specs.
PSF 1 pix
CdTe thickness < 1 mm
0.3 ms readout time
Count rate : 2.10^5 cts/pixel/s
350 Hz.



Future Improvements

- ID15C : BPM and feedback
- ID15A : large area CsI digital imager (GE ?)
- ID15 : MX mini-diffractometer (rotation +, 1er beamstop (XYZ) + sample visu. (XY)+ scatter shield + cryo mount all on the existing table ….
- Possibility to test a larger CdTe detector …..

Acknowledgment

T. Busalps (ESRF)
J. Daniels (ESRF)
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V. Honkimaki (ESRF)
C. Ponchut (ESRF)
V. Stojanoff (NSLS)
D.P. Siddons (NSLS)
Z. Zhong (NSLS)

ESRF and NSLS STAFF
ID15 STAFF

Dose estimation

For similar crystal size and shape, beam size, experimental setup the dose is proportional to :

$$\left(\frac{\mu_{en}}{\rho}\right) * Flux * E * T$$

Flux : fluence at the sample, photons / sec ;

E : energy (J) ; T : exposure time / data set (s)

(μ_{en}/ρ) : mass energy-absorption coefficient ($cm^2.g^{-1}$)

	ID15B	X17B1	X6A
E (J)	$8.91\ 10^{-15}$	$8.91\ 10^{-15}$	$1.92\ 10^{-15}$
Flux ($ph.s^{-1}$)	$1.8\ 10^{11}$	$9\ 10^{9}$	$5\ 10^{10}$
T(s) per data set (90°)	360	9000	360
($\mu_{en}/$ ▪) (cm^2g^{-1})	0.17	0.17	7.3
D*	~100	~120	~400