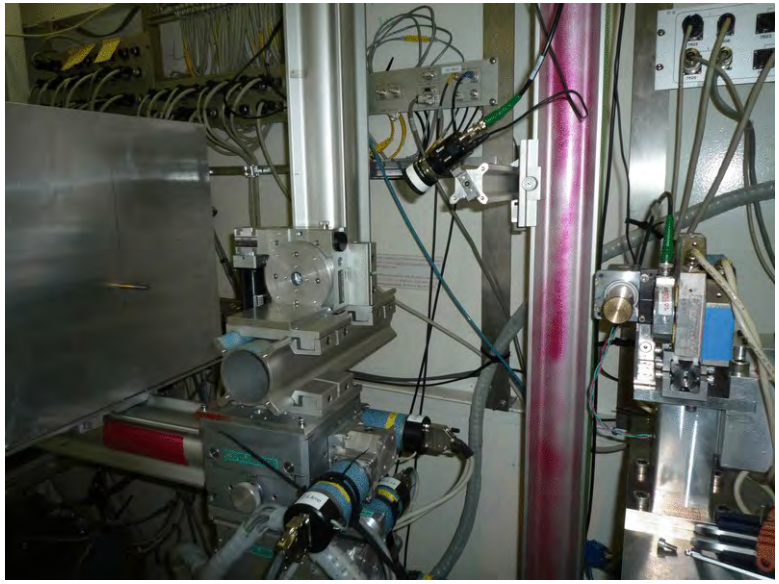
Wavelength Dependence of Radiation Damage to Macromolecules



Users' Meeting, Radiation Effects Workshop
05/21/2014

Jean Jakoncic, Photon Sciences Directorate, Brookhaven National Laboratory, Upton, NY.

Outline

Background Information / Motivations

Beamlines / Detectors

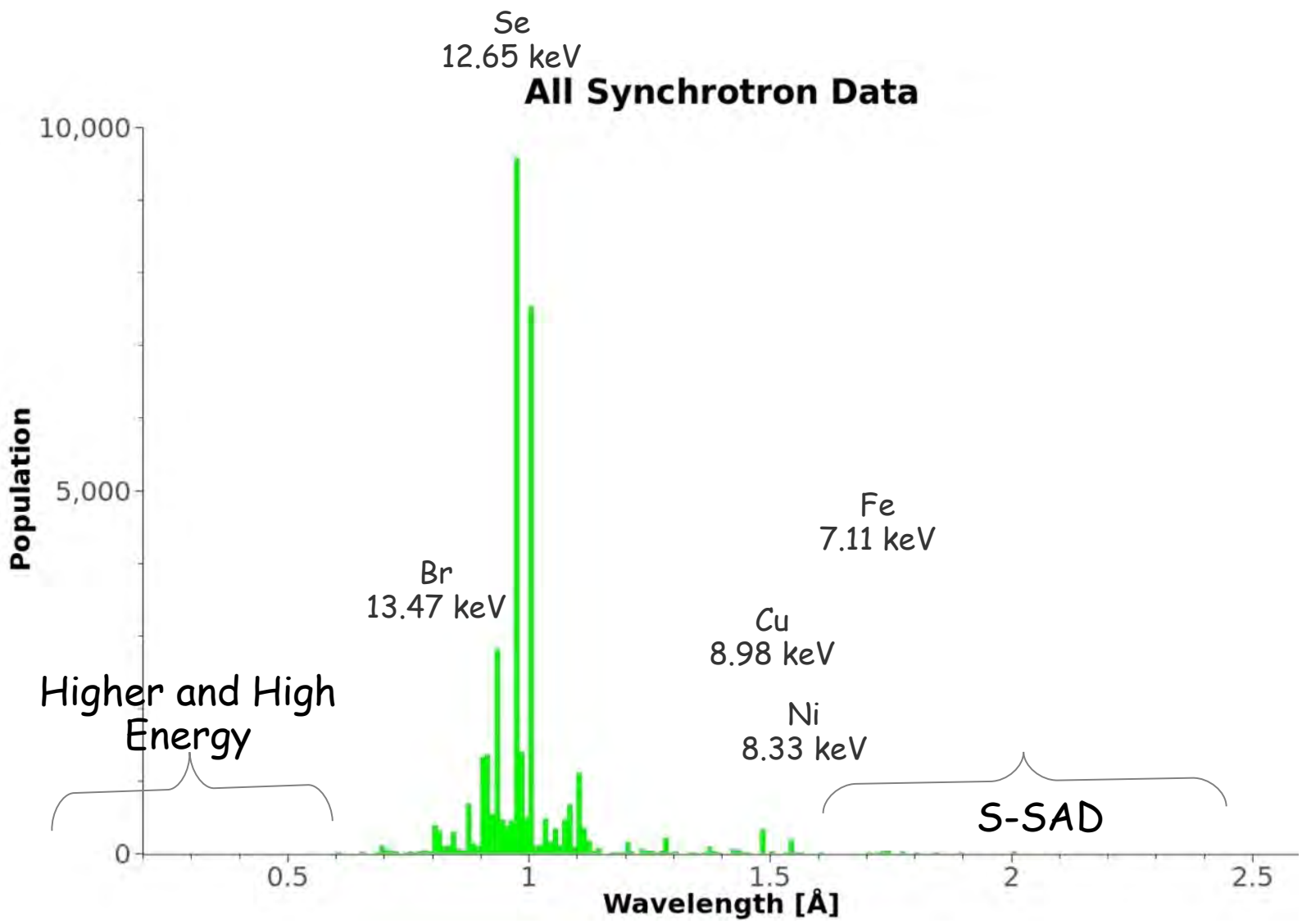
Experimental Results

Concluding Remarks

NSLS-II Opportunities

PDB Survey (2010)

Most MX Beamlines : 0.7 to 2 Å (17.5 and 6.5 keV)

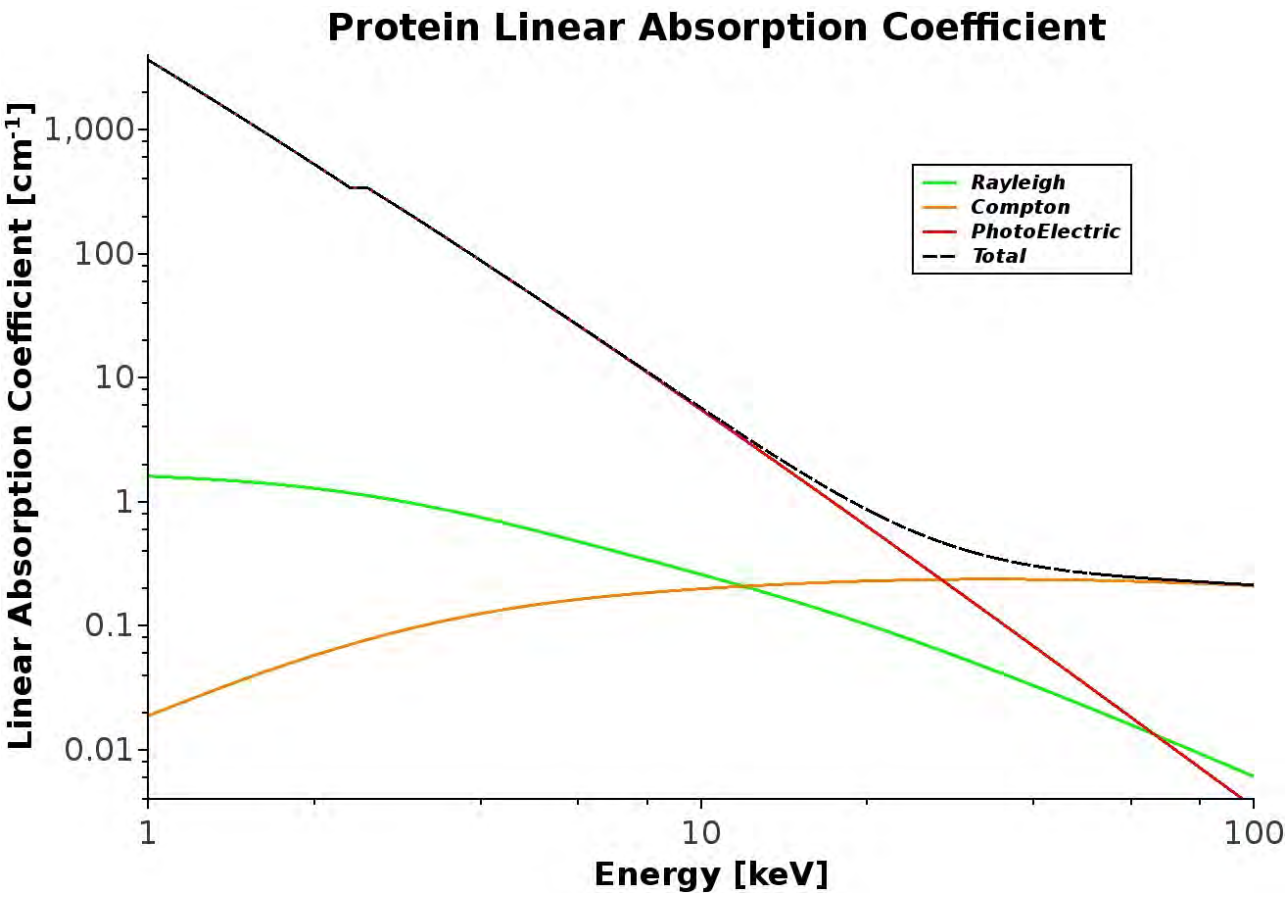


Most SR Structures at 1 ± 0.2 Å (12.6 ± 1 keV)

Higher Energy ?

Should there be an optimal energy ?

Photoelectric Cross Section Greatly Decreases with Increasing Energy. (Bad Ones)
Rayleigh Scattering Slowly Decreases with Increasing Energy. (Good Ones)
Compton Scattering ~ Constant. (Background and some energy; uncharacterized !)



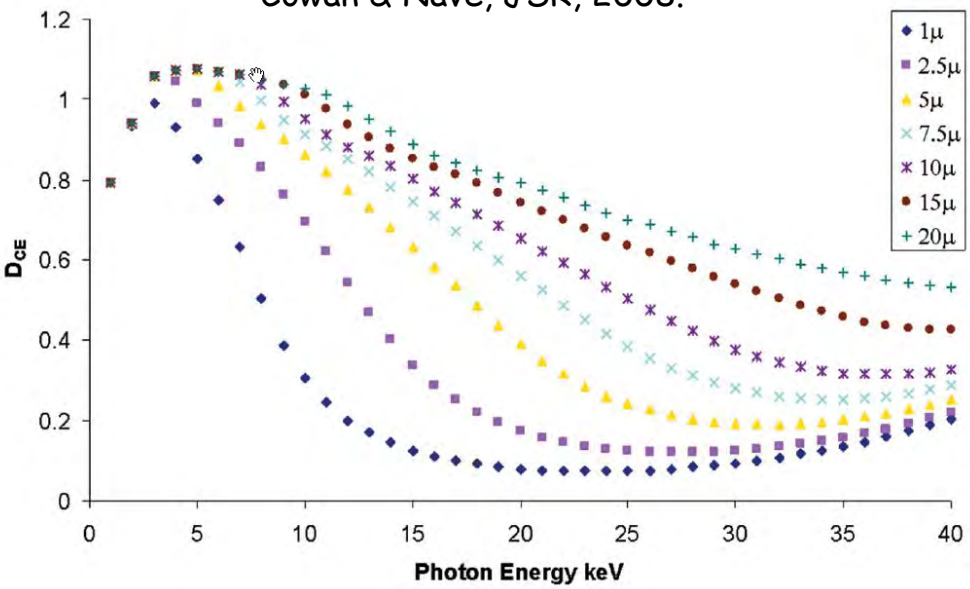
E (keV)	12.5	25
All (cm^{-1})*	3.25 (3.2)	0.62 (0.62)
Phot.Elec.& Compton Rayleigh	2.84 (87) 0.21 (7) 0.2 (6)	0.31 (50) 0.24 (38) 0.07 (12)

Linear Absorption Coefficients for a Protein sample.
* Number in () is the absorbed fraction for a sample 100 microns deep.

& Number in () is the corresponding fraction

Higher Energy ?

Cowan & Nave, JSR, 2008.



Ratio of deposited energy to scatter for crystals of different sizes.
Simulations take into account Compton scattering effects
and photo-electron escape
(Note: calculation for a small Bragg angle only) .

"a significant gain could be obtained for a wide range of crystal sizes in the 20-30 keV"
"provided detector efficiency remains optimized"

$$I = \frac{\lambda^3}{wV^2}\left(\frac{e^2}{mc^2}\right)V_{cr}I_0LPT|F(hkl)|^2$$

λ : wavelength ; w : velocity ; V : UC ; V_{cr} : crystal
L : Lorentz factor ; P : incident beam polarization
T : correction for sample abs ($e^{-\mu ad}$) ; I_0 : incident photon flux

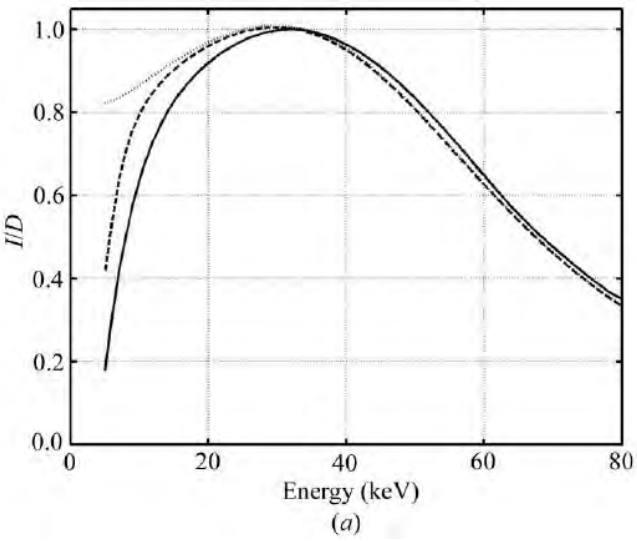
<u>Inelastic Mean Free Path of e- in water.</u>
(traveled distance between collision).
Energy deposited during each collision.
PE may escape medium.

E (keV)	1	10	20	50	100
IMFP (nm)	3	25	40	60	100

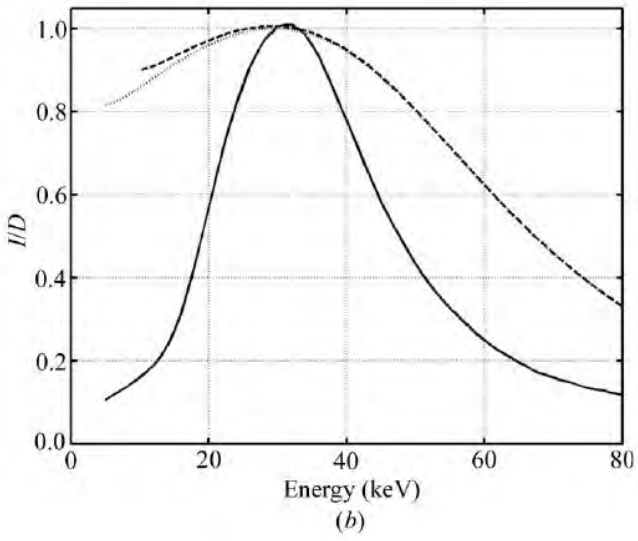
*TPP-2M NIST formula

Dose = μ_{en}/ρ.Fluence.t.E
Dose=1000.e.E.t.Fl.μ_{en}/ρ (in Gy)
(1 Gy= 1000 J/g)
e=1.6 10^{-19} J
E: energy in eV
t: total exposure time
Fl: fluence, ph/s/cm^2
μ_{en}/ρ: in cm^2/g

Higher Energy ?

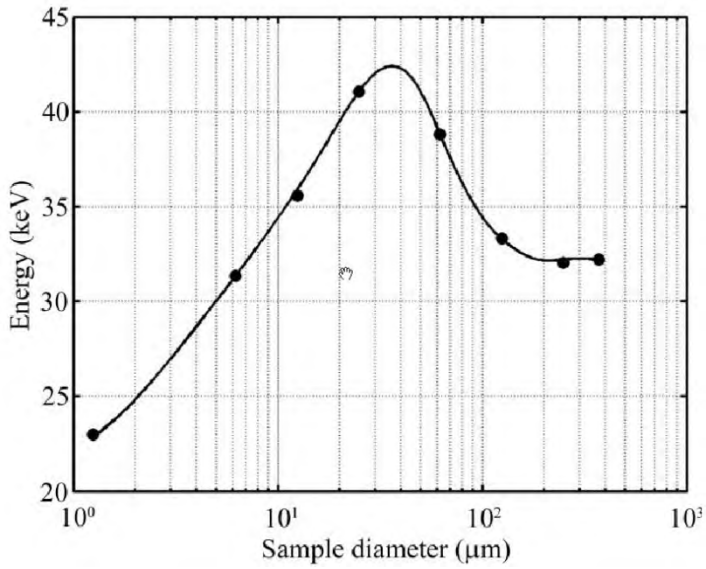


200 μm



5 μm

s of ratios I/D obtained by simulation (MC solid line) and of Ihkl/D calculated
equation (left) (dashed line) and with the high-energy approximation of
tion (right) (dotted line) Ratios are normalized to 1 at 33 keV.
urme et al, JAC 2012)



Photon Energy ensuring the highest simulated I/D ratio versus sample diameter (spherical sample R fully bathed in uniform unpolarized square beam R).
(Fourme et al.)

$$\frac{I_{hkl}}{D} = r_e^2 \frac{V}{\nu^2} \frac{d_{hkl}\rho}{\Delta\varphi \cos\theta} P \frac{A}{A'} |F_{hkl}|^2 \frac{\lambda^2}{E\mu_{en}},$$

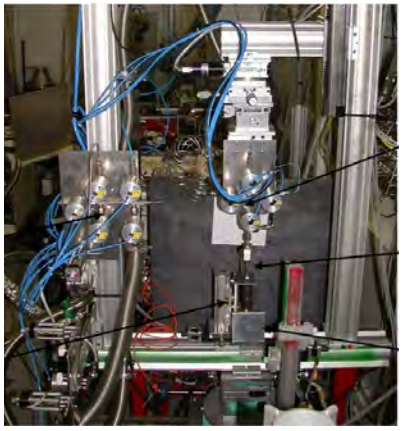
$$\frac{I_{hkl}}{D} \propto \frac{V}{E^3 \mu_{en}},$$

Beamlines / Detectors

There are no dedicated macromolecular crystallography at high energy (E > 30 keV). Experiments were performed at ID15B (ESRF) and X17B1 (NSLS), 2 multi-purpose High Energy Scattering Beamlines.

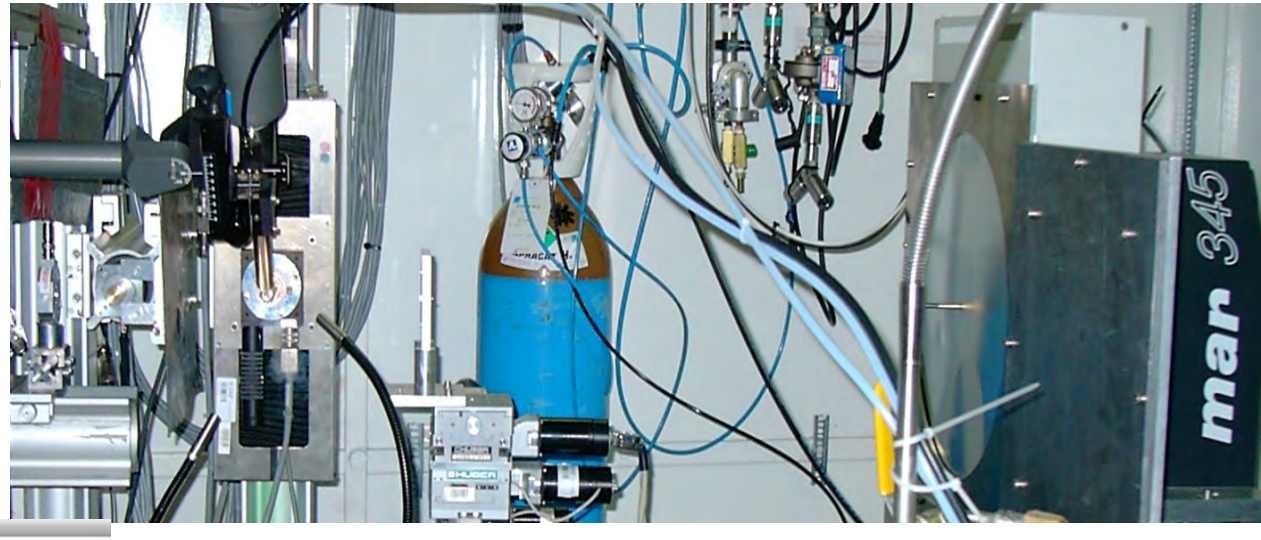
There "are" no dedicated "modern" large and fast area detectors for MX at High Energy. As a result, initial data were recorded on either "standard" CCD detector or Imaging Plate.

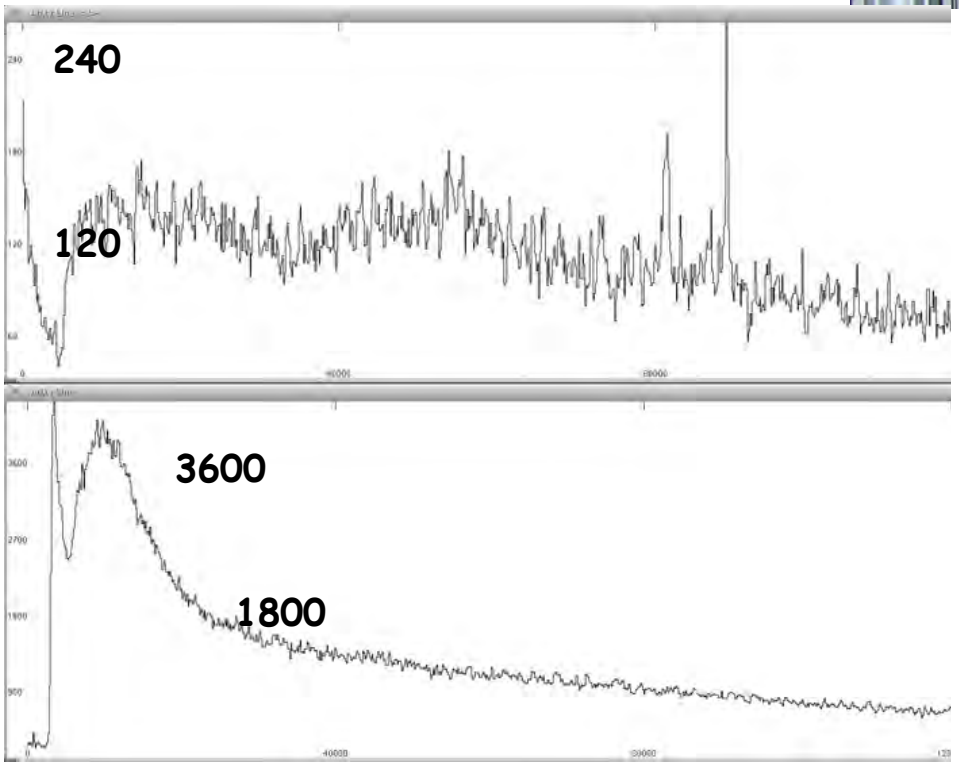
Beamlines/Detectors specs will be highlighted during the experimental results as they are Key components of the experiments.



Preliminary Experiments : ESRF ID15B

- ESRF AMPW (asymmetric multi-pole wiggler)
- Single Si(311) Bragg cylindrically bent mono.
- Energy 56 keV, 5 10^{11} phs^{-1} in 0.3 x 0.3 mm^2
- Detector MAR345 (max. eff. 30 %)
- *Data on a lyso. sample @RT before at 75 keV.*





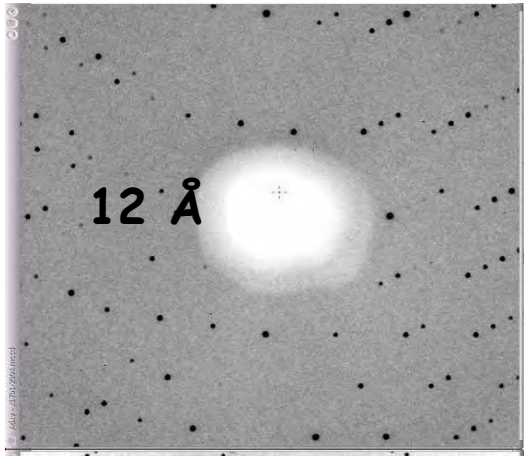
Final setup :
Detector ~ 650 mm
Temperature = 100 K
2 beam stop (@20 and 60 cm)
both on 0.5 mm Al plate (3.5% abs)

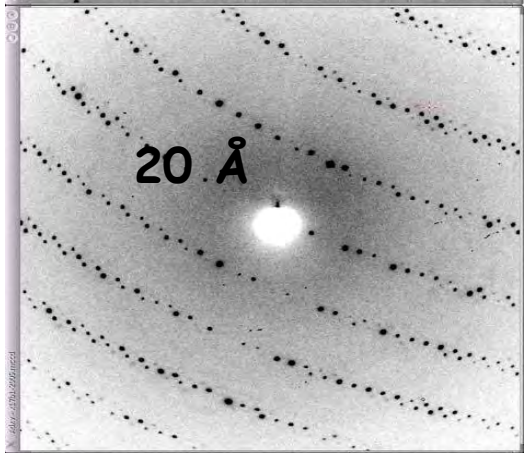
2nd exp : + 2nd BS Pb 2 mm D 4 mm L near sple.

1st exp : 1 BS Pb 6 mm D 25 mm L near det.

Preliminary Experiments : NSLS X17B1

- X17B1 at NSLS : superconducting wiggler
- Laue/Laue sagitally bent Si(311) mono.
- Energy 55.6 keV : flux ~ 6 10^9 (0.3 x 0.3 mm^2)
- Detector : MAR CCD 165 mm (max. eff. 15 %)



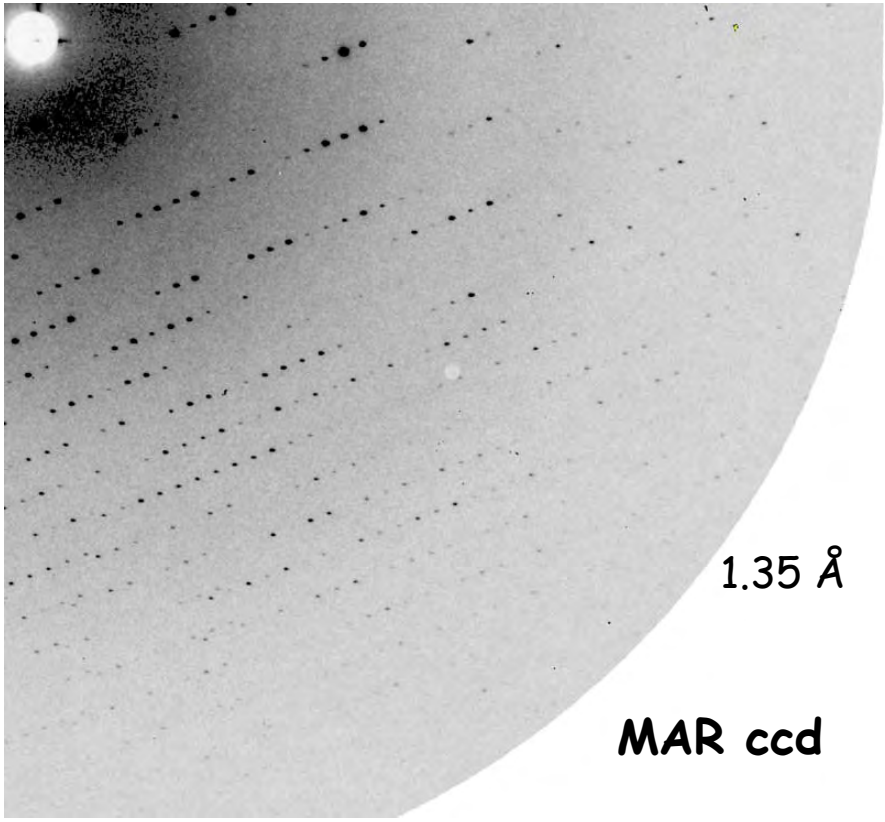


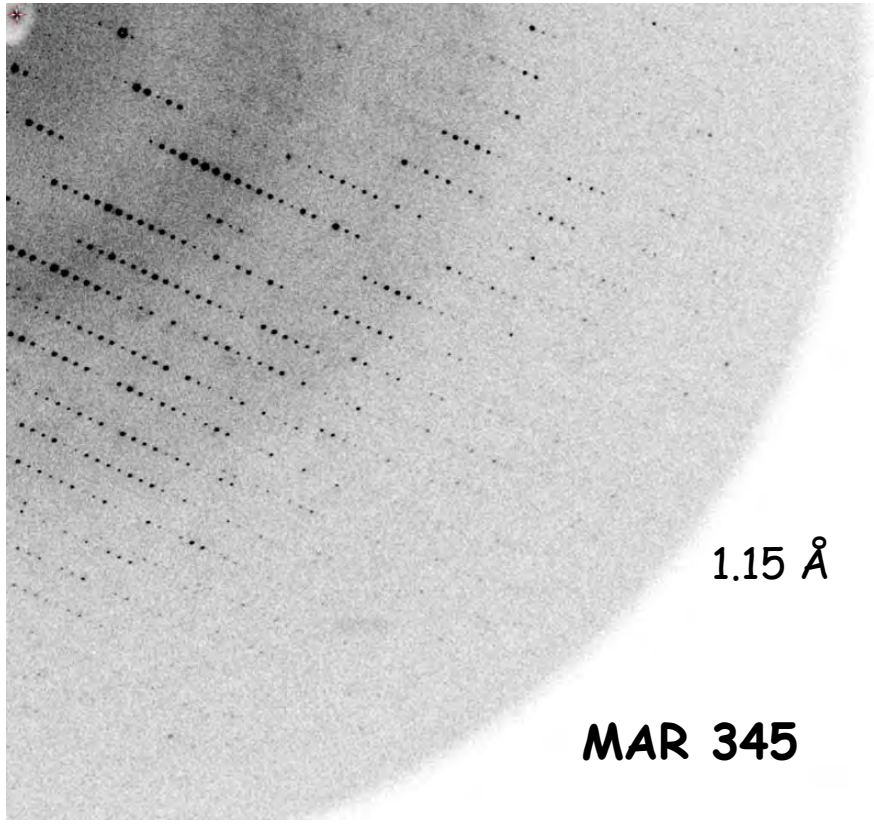
Final setup
Detector ~ 400 mm (165 mm in diameter)
Temperature = 100 K
1 beam stop at 10 cm on 3.2 mm Plex.(7% abs)

1st exp : 1 BS Pb 6.4 mm in diam. and 15 mm L

2nd exp : 1 BS Ta 3.2 mm in diam. and 15 mm L

Lysozyme (Ho K edge, 55.6 keV) SAD/MAD

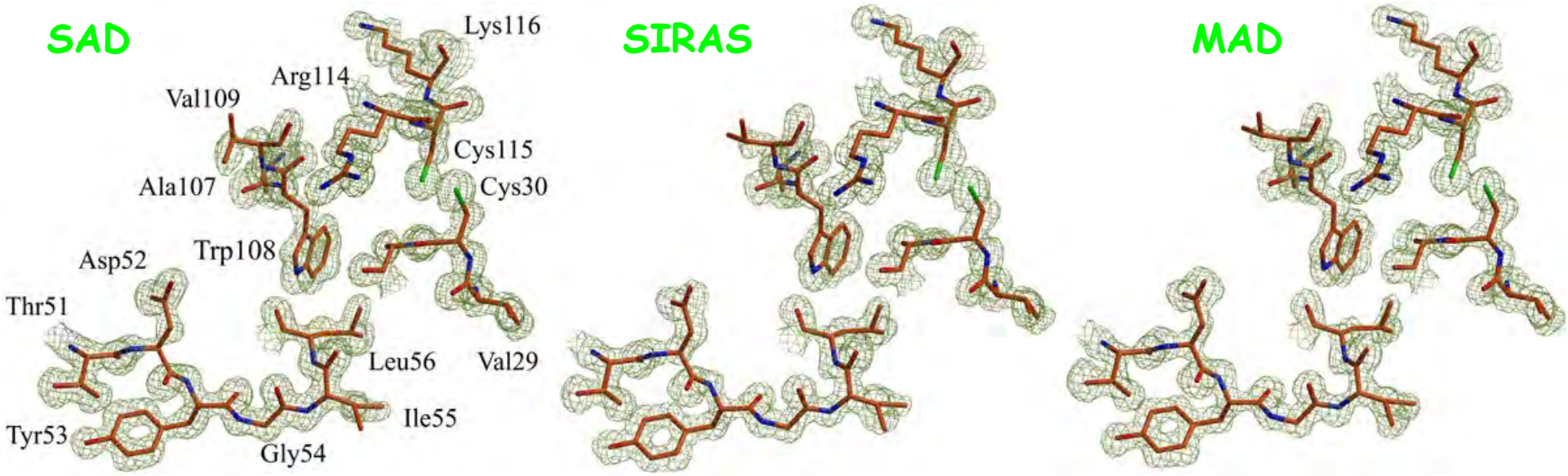




Beamline	NSLS X17B1	ESRF ID15B
Exp. Time (90°)	9000 s	360 s
Flux :	9 10^9 ph/s	2 10^{11} ph/s
Det. Max. eff. (%)	15	30

Jakoncic et al., Anomalous Diffraction at Ultra High Energy For Protein Crystallography, J.A.C. (2006)

Lysozyme, Ho K edge

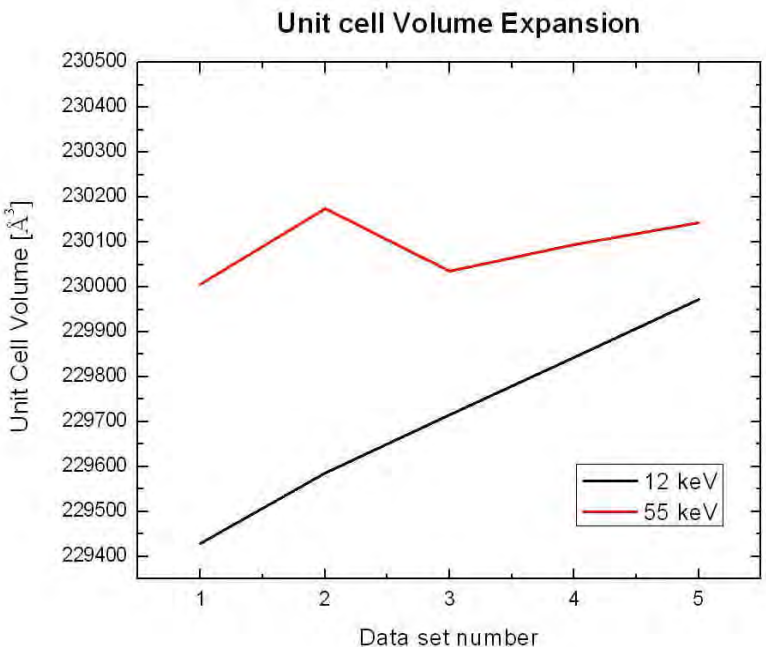


2 BPU.PDB 2CGI.PDB

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

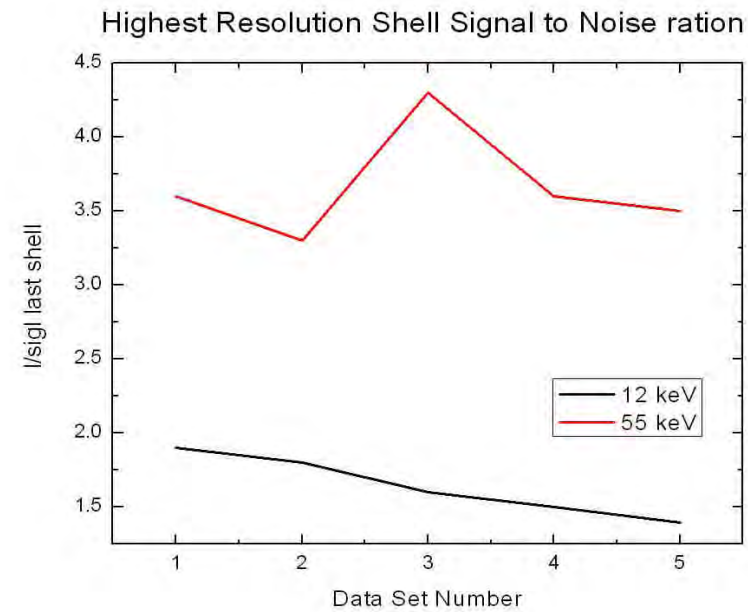
At ID15B, 900 degrees collected at the high energy remote of Ho K abs. edge; + 360 + 360 : ~1600 degrees collected in total.

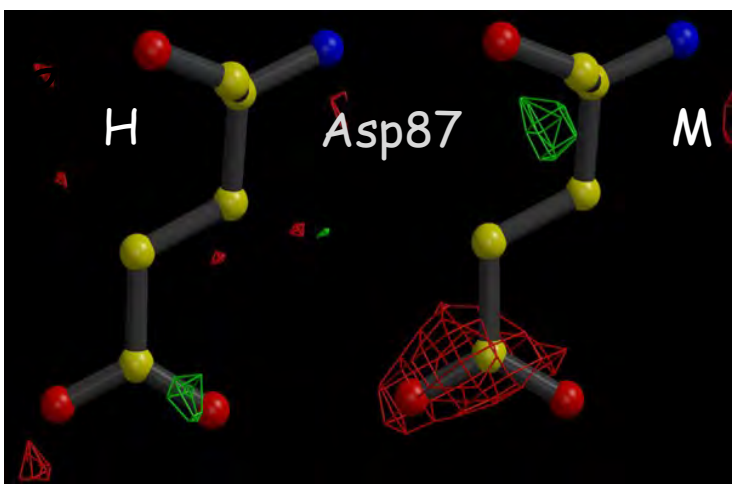
Radiation Damage ? 12.6 vs 55.6 keV



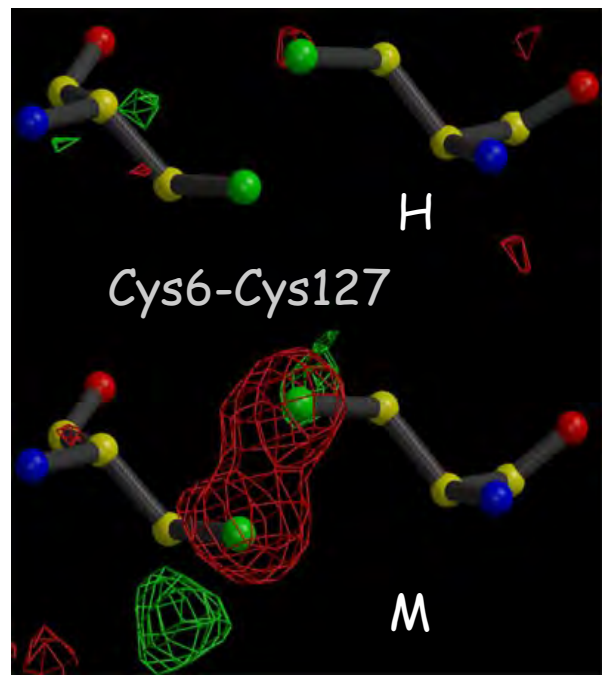
MAR345
@55.6 keV
Eff: 30 % max

Q210
@12.6 keV
Eff. 20 % max





Maps calculated with
Fobs of DS1 and DS5 (ds1 and ds9)



Methods: lyso. sample of same size exposed at 12 keV at beamline X6A with same beam size. Exposure time adjusted to achieve the same resolution (1.15 Å). 1600 degrees collected at 55 and 12 keV.
Each data set, consists on 90 deg. 5 data sets equally spaced processed. (90 deg every 180 deg)DS1 3 5 7 9
1-90; 180-270; 360-450; 540-630; 720-810; 900-990; 1080-1170; 1260-1350; 1440-1530.

D_{55keV}^{DS}= 0.1 MGy (total: 0.9)

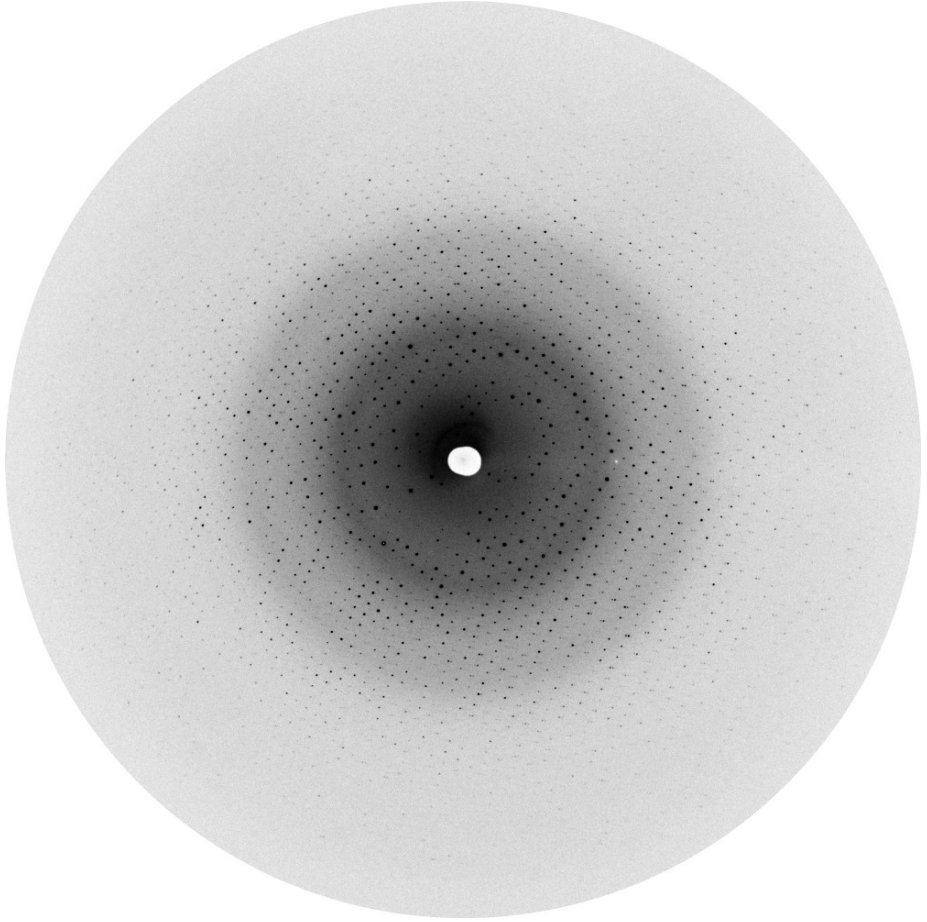
D_{12keV}^{DS}= 0.28 MGy (total:2.5)

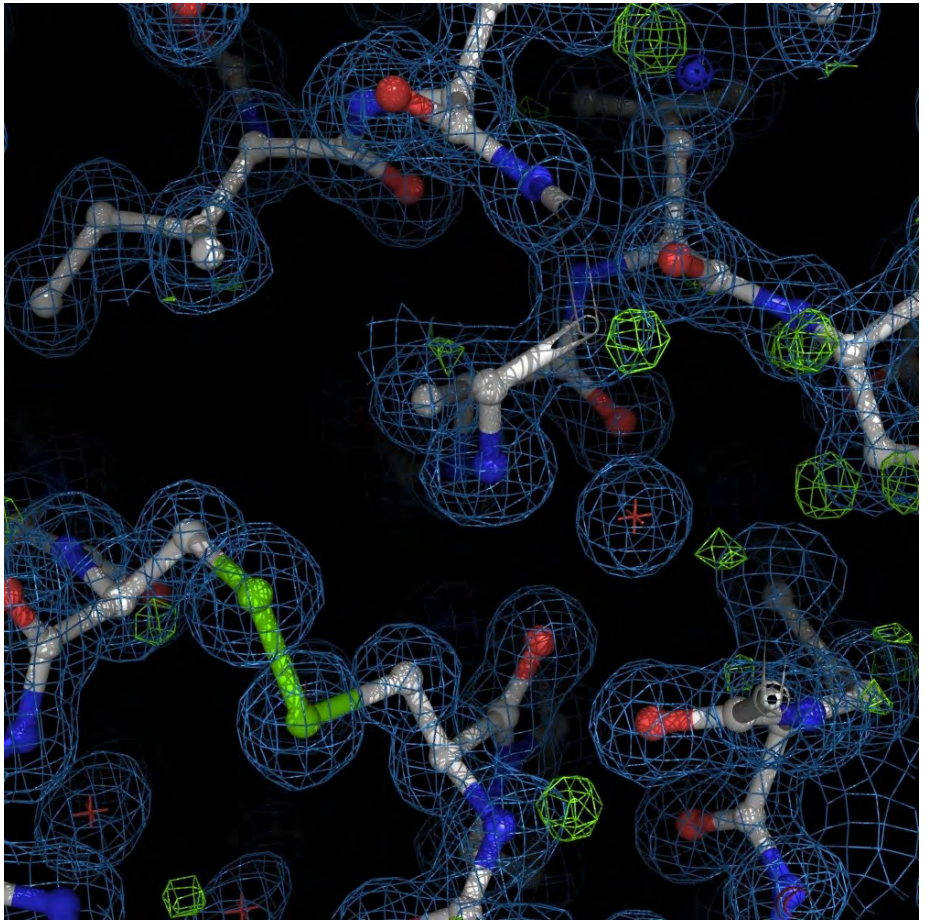
Thaumatin @ 56 keV

Higher energy : Higher resolution
(sample limited, not instrument limited, low diffracting angles ….)

ergy / Wavelength : 56 keV / 0.2214 Å **(MAR345 30 % max eff.)**
x : ~ 10^{11} ph.s^{-1} (300 μm slits)
. time : 20 s (0.25°), 400 frames | Total time : 8000 s **(0.13 MGy)**
tector mode / cycle : 3000 (30 cm diameter, 100 µm pix) / 87 s
e to high Dyn. Rge. Only one data set collected.

Detector distance : 670 mm
Resolution : 20.00-1.10 Å | Mosaicity : 0.3°
Rmerge : 5.7 %
Tetragonal Thaumatin (Asherie et al, 2009)
2VI4.PDB (R/Rfree:12.5/14 %)





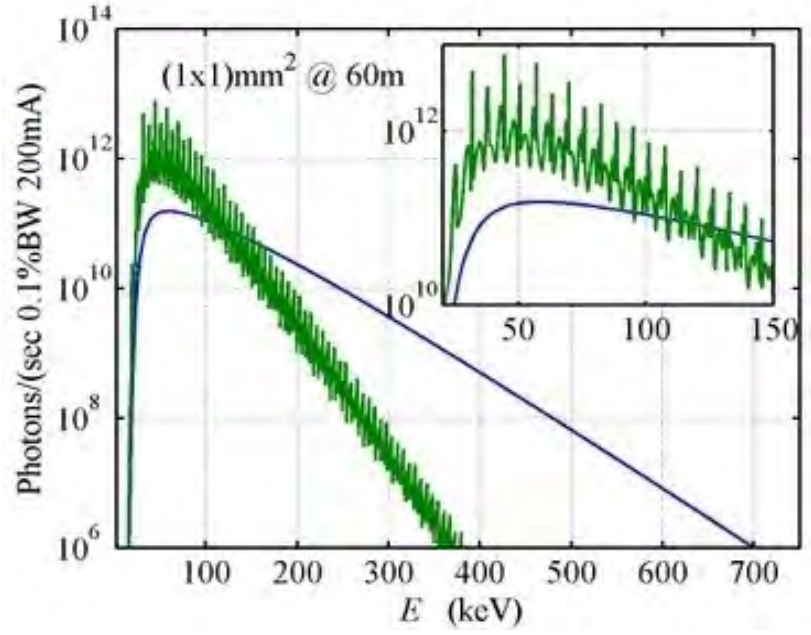
"High Throughput" 2008 @ ID15B

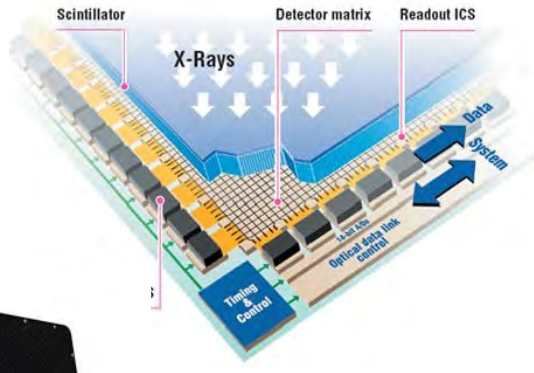
- E= 56 keV ; 0.2214 Å
- U22 undulator > 10^{12} ph/s
- PIXIUM 4700 Detector

CsI:Tl scintillator on amorphous Si diode array

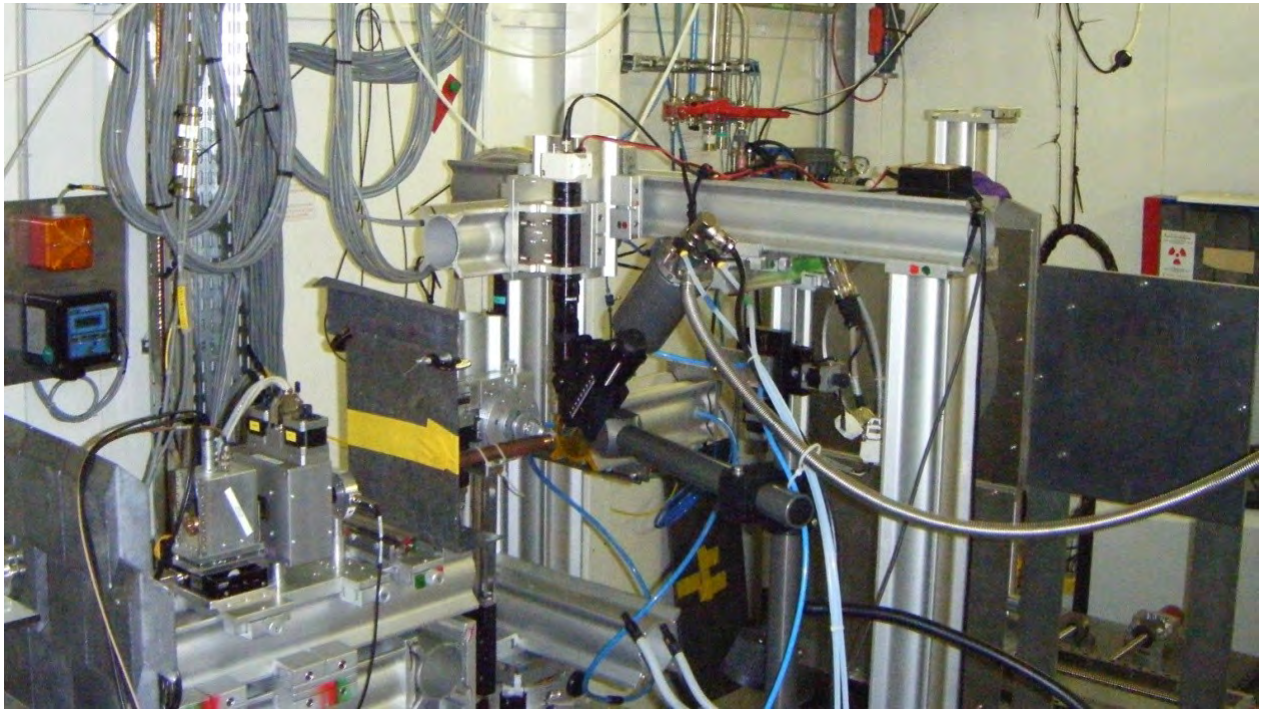
Detector efficiency @56 keV : 85 % max.)

<u>0.4 sec. per frame mode only</u>





Digital flat panel
medical imager
Indirect detection
Angio./Fluo./Radio.

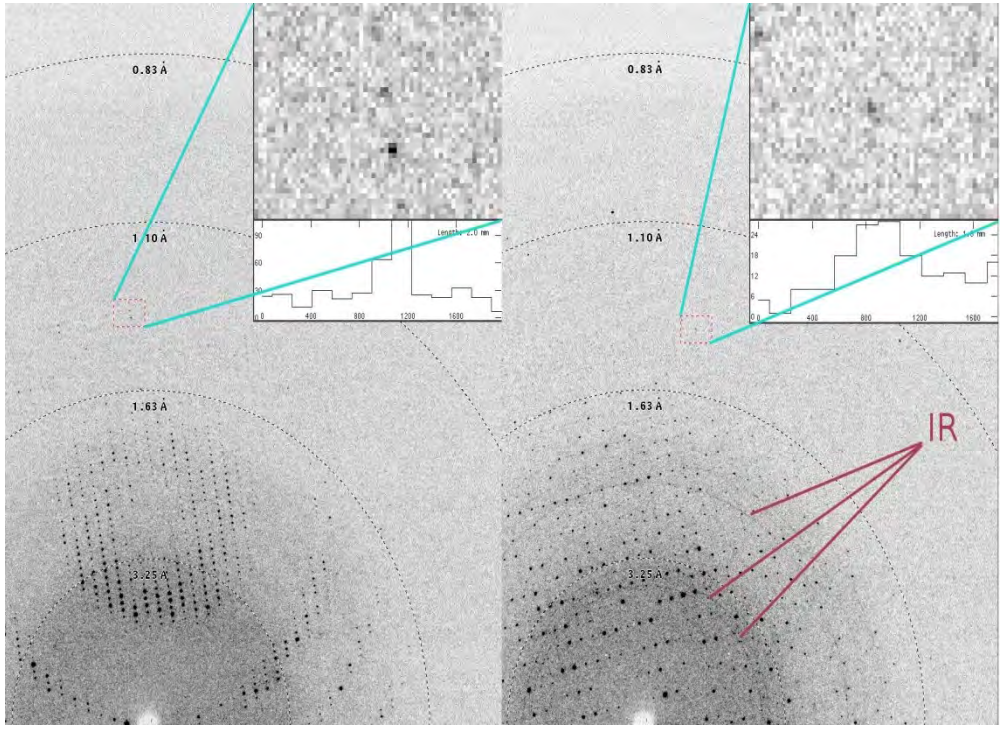


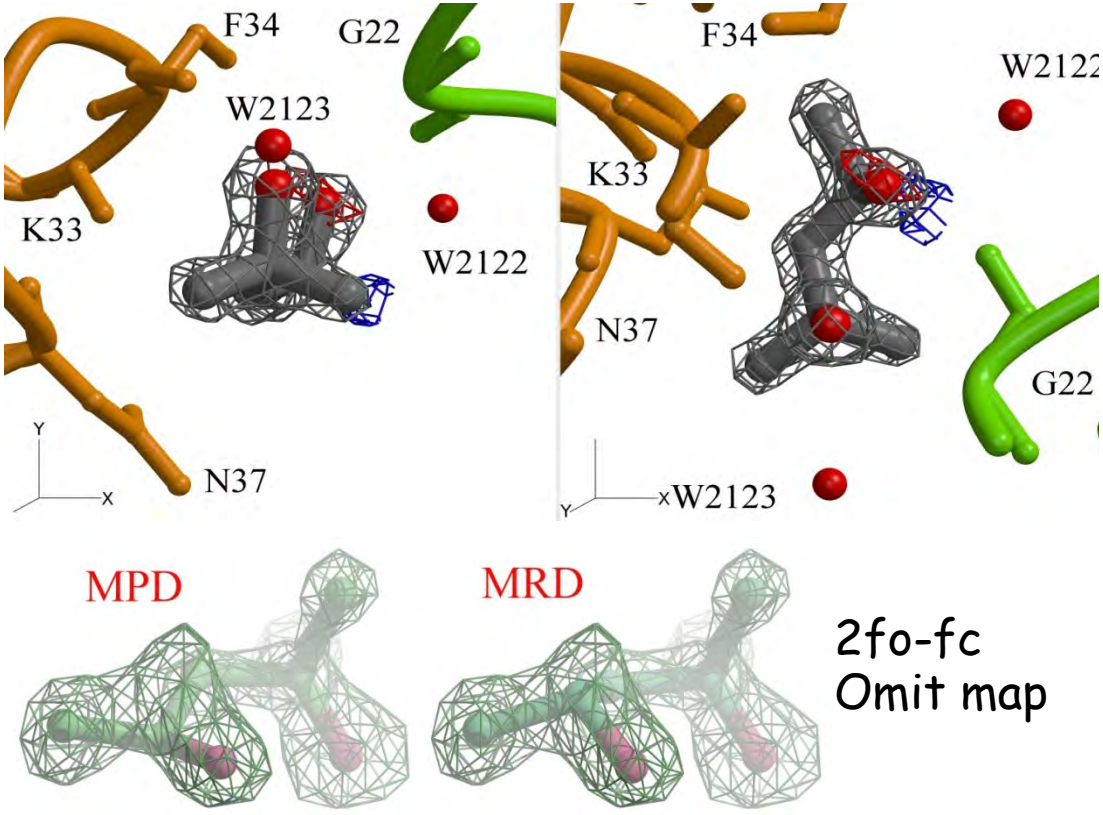
Lysozyme with MPD at 56 keV

- Lysozyme / MPD (2 overlapping passes, some lost images due to detector control)
- 0.4 sec / 0.5 °. 180 frames total (72 seconds total !! < MAR 1 image) **<u>0.03 MGy</u>** ?
- High throughput achievable (despite det. trigger)

Resolution : 20.00-1.50 Å | Mosaicity : 0.5°

Rmerge : 6.2 % | PDB : 2X0A | R/Rfree : 15 / 20 %





2fo-fc
Omit map

Lysozyme crystallized in 65 % racemic MPD.
MPD unambiguously assigned as R-MPD.

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

•IR due Mounting / un-mounting samples x times
Jakoncic et al., submitted.

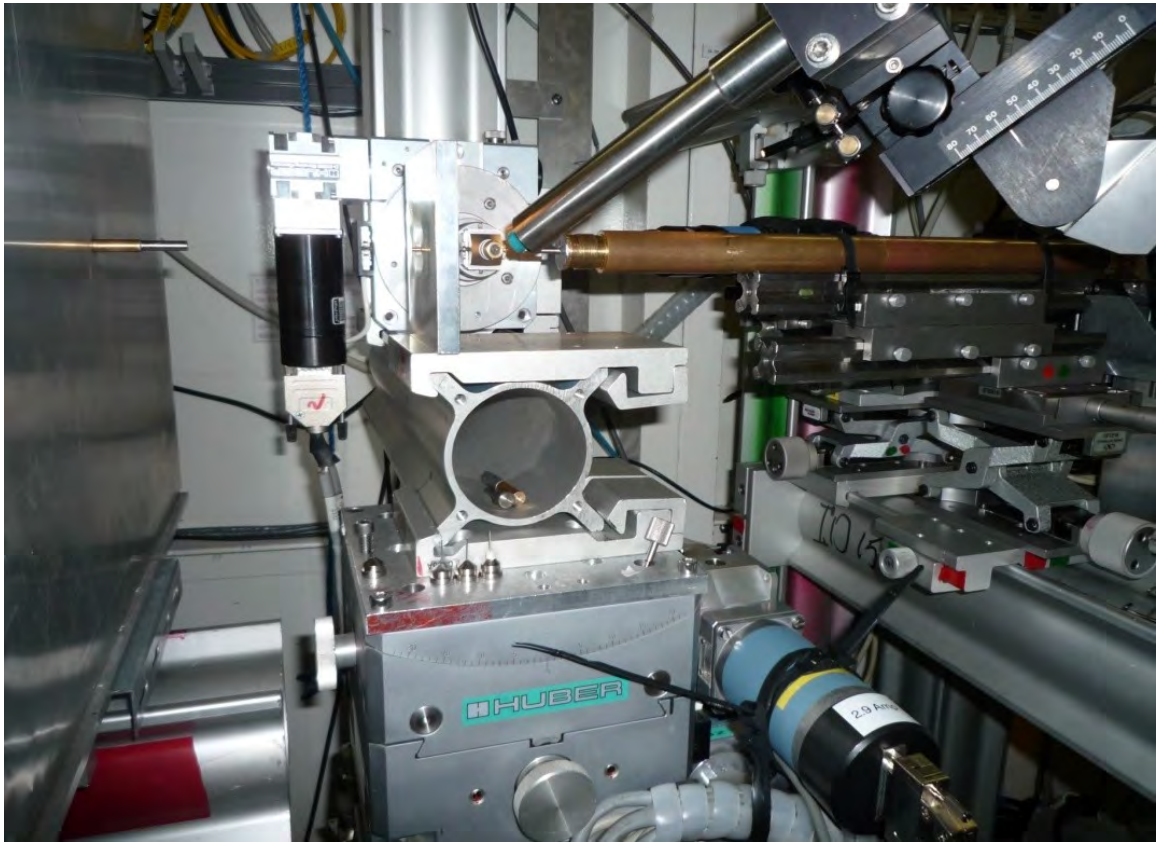
Low Dose MX @ 38 keV (ID15 side)

- ID15 H axis 38 keV, 0.32 Å, … 10^{12} ph/s
- "Improved" beamstop (+ W rod) (previous experiment with V axis and one BS far)
- Wat. cooled Mono upgrade

  0.4 mm cracked > 2mm (bandwidth : 10 -> 40 eV)

  10^{12} phs^{-1} : 0.2 mm slits
- G. Cingolani (2 HR DS)
- S. Gabelli (1 HR DS + 3 HA DS @Ba)
- E. Rudino-Pinera (3 DS on Cu enzymes RD)

- Multiple Room Temp. data collection (JJ)
- 1 Proteinase K co-crystallized in TeO_6(JJ)



2 beam stop (no mirror for harm. Reject.)
Det. Distance ~ 38 cm.
Det. Max. Eff. ~ 60 %)

Proteinase K @ 37.8 keV : SAD Te

Investigate phasing at E > 30 keV (ave. optimized energy from simulations*).

Te K edge in the proper E range (20-40) - Te-Met (*Steinbacher et al., 1998*)

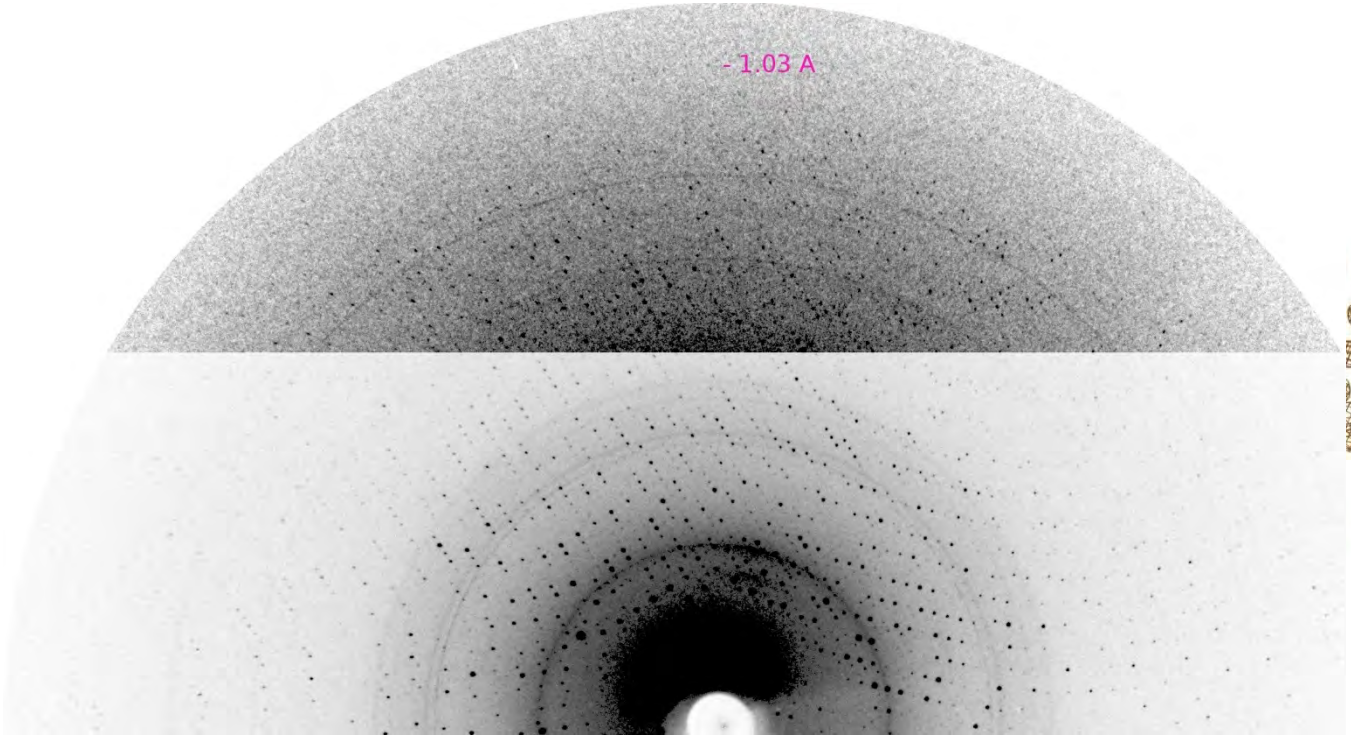
Selenate (JJ)

ProtK: 279 amino acids long - Co-crystallized in TeO_6

Structure solved SAD (far high energy remote of Te K edge (31.8 keV)

Data collected at **37.8** keV (maximize detector efficiency Ba; **60 % max**).

SAD structure solved at 1.05 Å resolution (0.12 ° mosa., 99 % comp. 9.9 % Rm) **0.08 MGy**



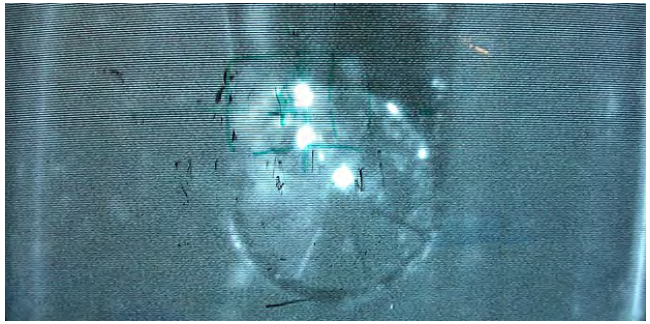
*(Fourme et al., 2012)

4FON.PDB

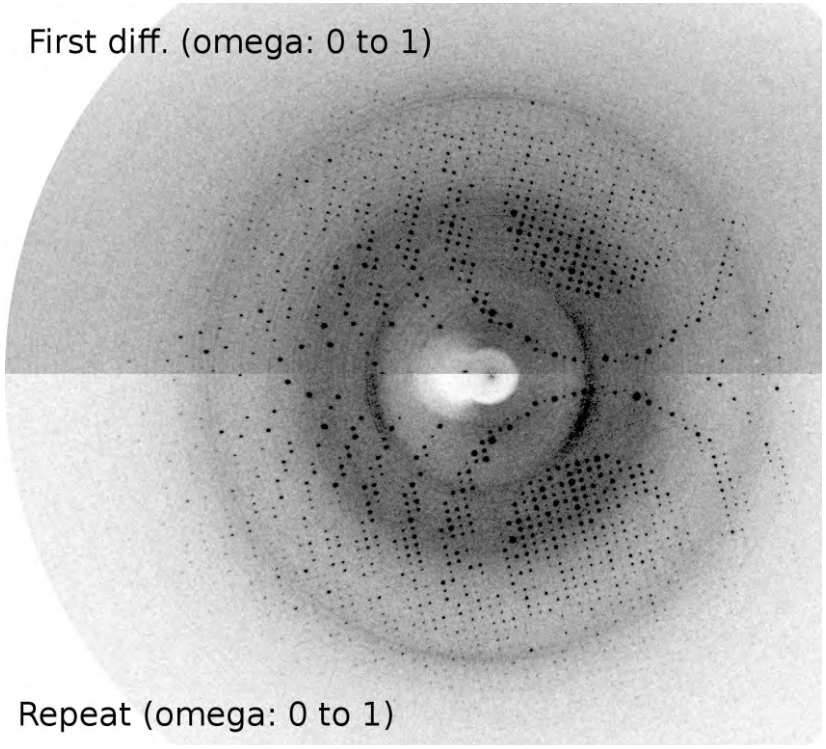
Proteinase K @ 37.8 keV @ Room Temperature

ProtK: 279 amino acids long. VDHD, 0.4 M SO_4, pH 6.5

Structure solved by MR at 37.8 keV (maximize detector efficiency Ba; **eff 60% max**).



72 frames collected (2s, 1 deg)
Mosa : 0.05 deg.
11.00-1.6 Å ; 99.5 % ; Multi. : 5.9
I/sI: 11.1 (1.50)
Rm: 12.8 % (crystal motions)
Flux : 1.1 – 1.3 10^{11} ph.s^{-1}

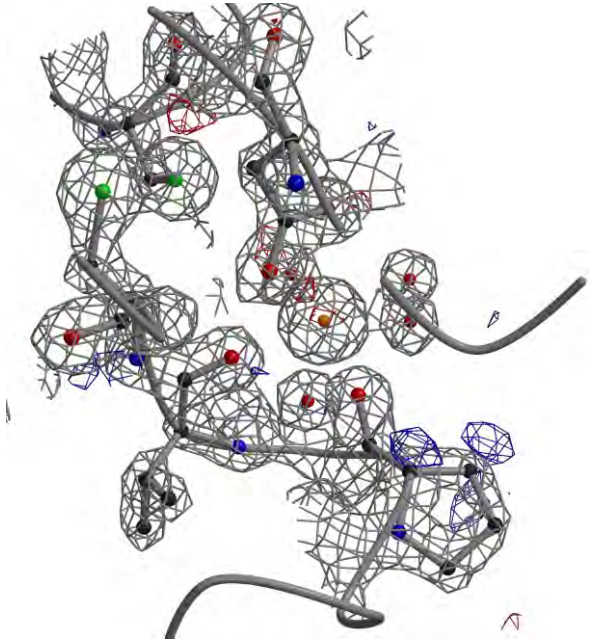


μ_{en}/ρ = 0.0552 $cm^2.g^{-1}$
Dose = <u>0.04 MGy</u>

Sample moved ..
+ 15 % higher Is.

R/Rfree=15.8/18.4 %

Highest RT protK.
No damage observed



4B5L.PDB

*Very few PK samples tested. Original high energy experiment with lysozyme crystals at room temperature.

ILK-PINCH Complex (T. Boggon, Yale U.)

E=13.5 keV (APS) ADSC Q315

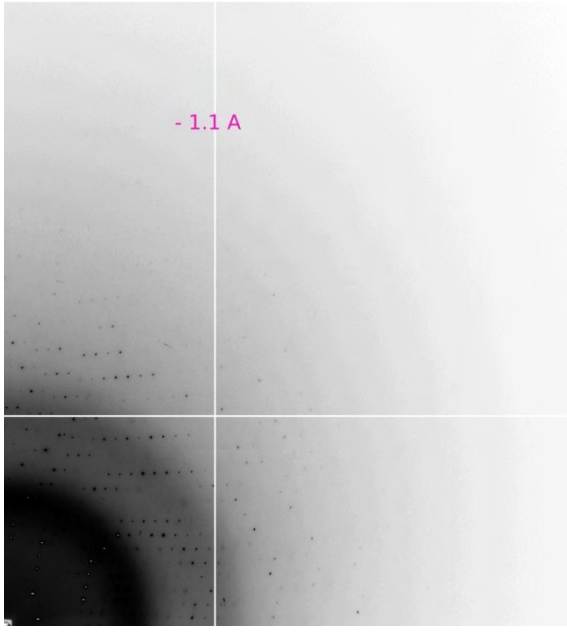
Exp.1.5 s ; 220 frames 1 deg
30-1.2 Å , Rm = 6.1 % ; Compl. 94.2 %
I/sI : 16 (1.5) , Red. 2.5. ~Mosa.: 0.8°

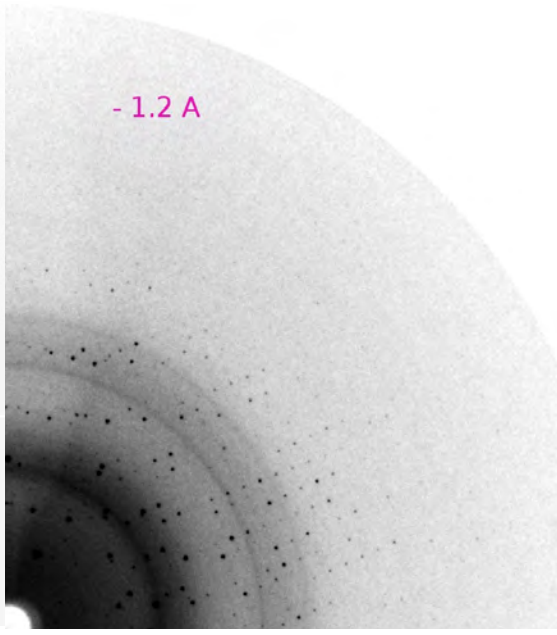
(LR data : 0.5 sec / deg d = 250)

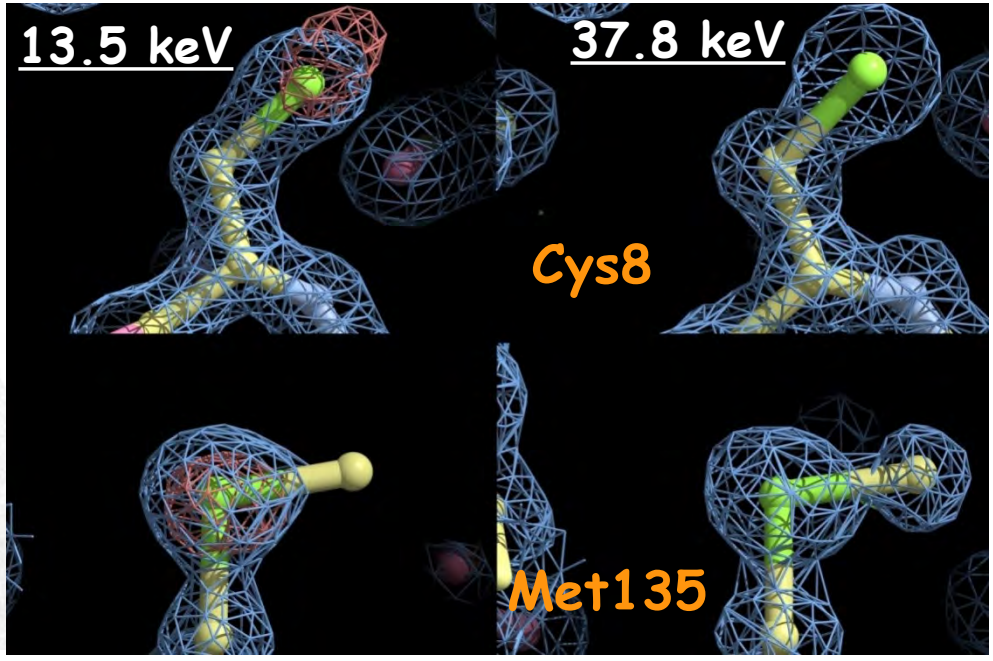
E=37.8 keV (ID15) MAR345

Exp.40-60s ; 400 frames 0.5 deg
20-1.2 Å. Rm: 5.7 % ; Compl.: 99.3 %;
IsI: 14.2 (1.3). ~Mosa.: 0.6°

P_1 structure	low + high pass	High (2 DS prior)
Resolution (Å)	40.00-1.20	20.0-1.20
R/R_{Free} (%)	17.5/19.8 [4HI9]	14.4/16.8 [4HI8]
B factor ($Å^2$)	28.7	16.8
Total exposure time (s)	330	20000
Flux (ph/s)	5.10^{11}	$3.5\ 10^{10}$
Beam size (μm^2)	100 x 100	150 x 150
Dose (MGy)	~6 (only high-pass)	1.0
Wavelength (Å)	0.9197	0.328
μ_{en} (cm^2/g)	1.7	0.065
Detector max. eff. (%)	80*20	55





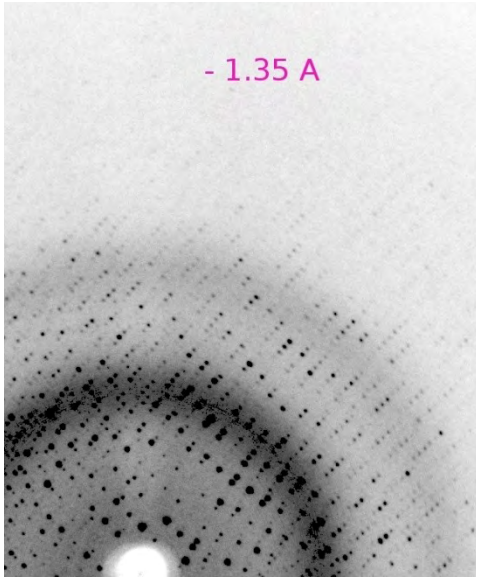


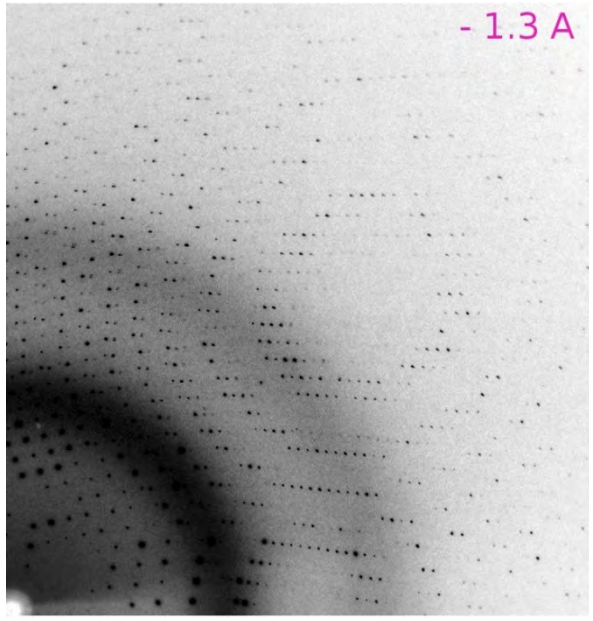
(Si(111) Laue; Vert. Rot. Axis; macro:
fixed dose/image, mono theta realigned every 40 images.

Bacteriophage Tail Needle Knob (G. Cingolani, T. Jeffeson U)

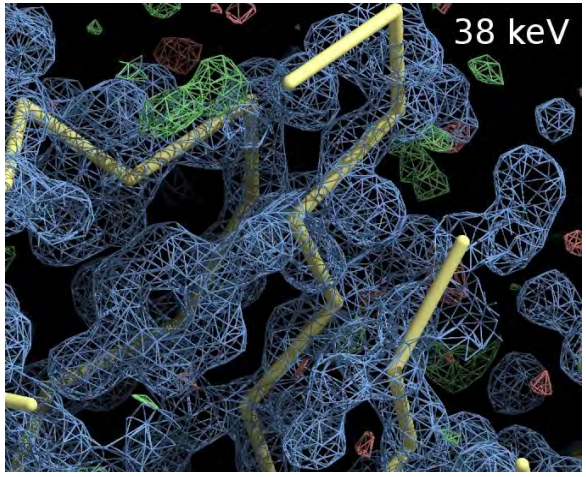
E= 38 keV (ID15) ;MAR345 (1800)
D=260 ; 300 frames 0.4 deg. V axis
Stats: 20-1.4 Å ; 99.9 % ; Rm: 7.8 %
I/sI : 17 (2) ; Mult. : 5 ; ~mosa.: 0.6°

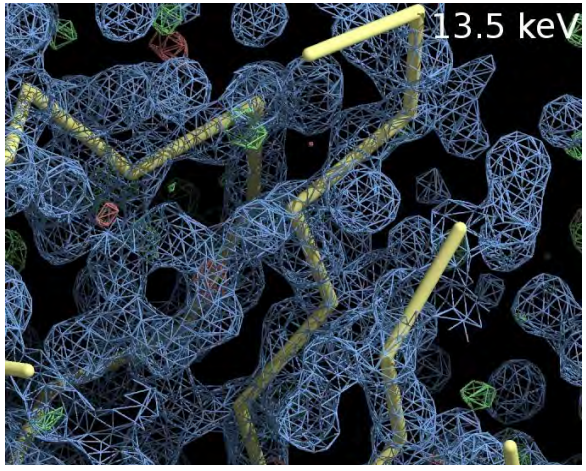
13.5 keV (F1@CHESS); ADSC Q270 ;
D=170 ; 240 frames 0.5 deg
Stats: 20-1.4 Å ; 93.3 % ; Rm: 10.1 %
I/sI : 27 (11) ; Mult. : 5.1; ~mosa: 0.4°





	High Energy	Medium Energy
λ (Å) / E (keV)	0.328 / 37.8	0.918 / 13.5
Total exp. time (s)	4500	240
Flux ph/s (0.2 mm)	$5.6\ 10^{10}$	$3\ 10^{11}$
Dose (MGy)	0.2	0.5
μ_{en} (cm^2/g)	0.053	1.2
R factor (%)	13.8 (16.4)	12.3 (14.8)
Wilson B factor ($Å^2$)	9.6	10.0



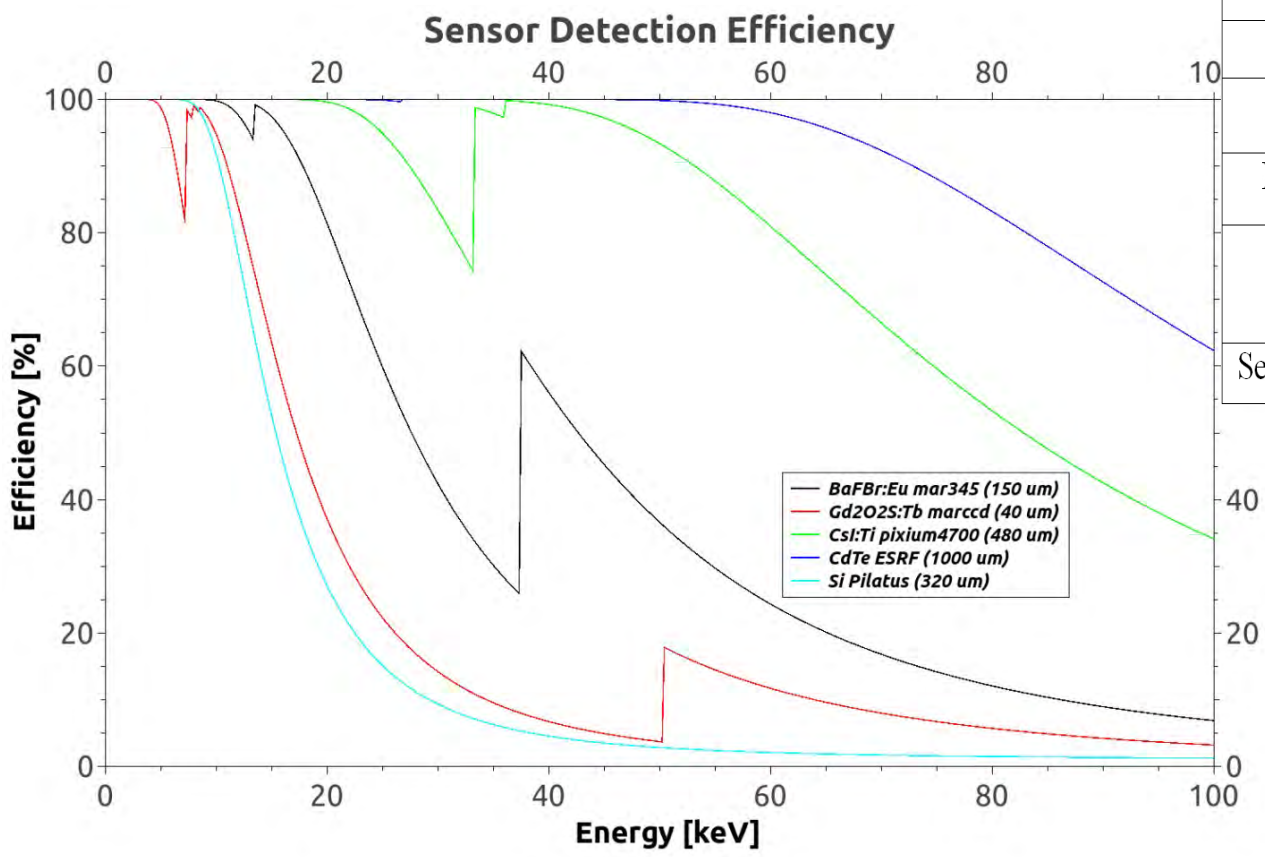


$P2_12_12_1$: 60 90 90 90 90 90; 52 kDa ; 150 amino acid long; trimer.

Detector Considerations

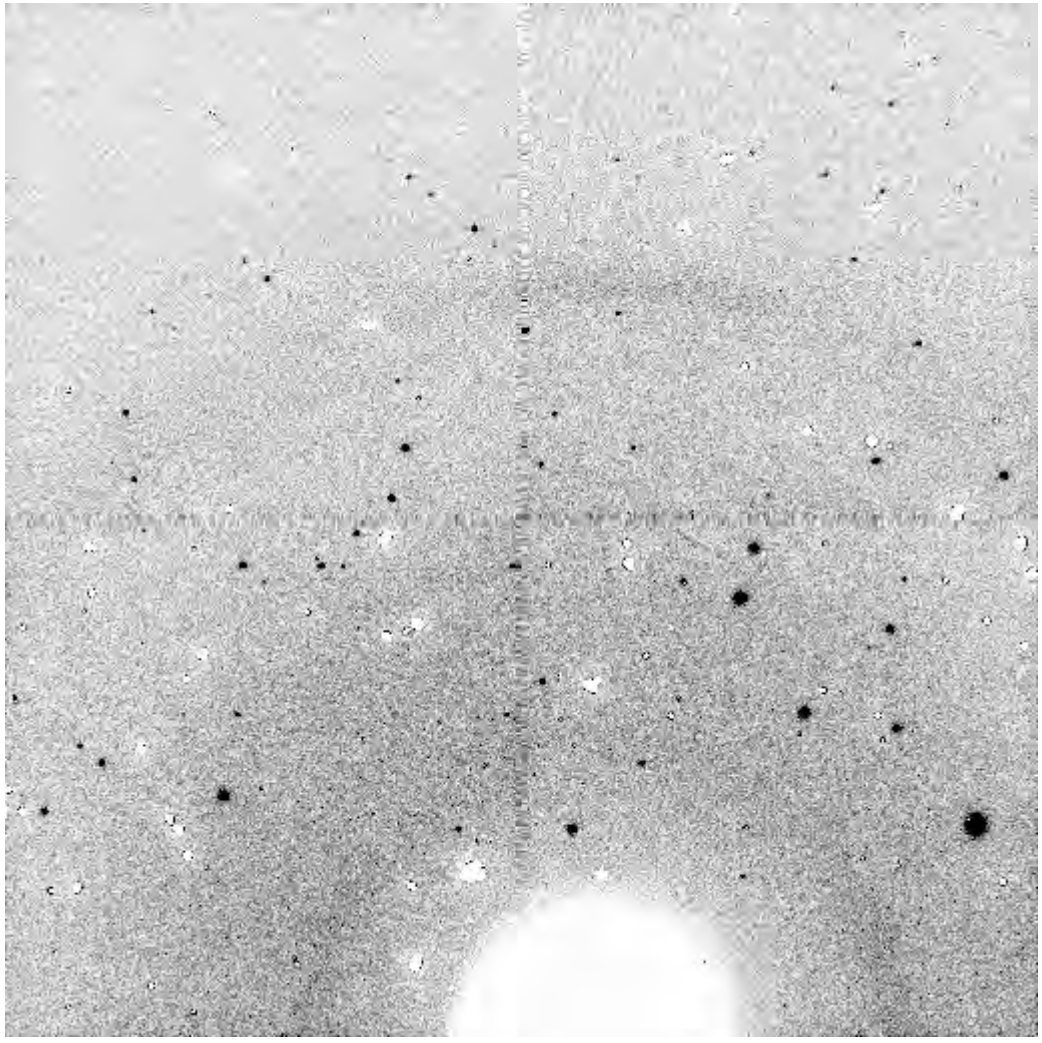
- Scintillator screen FOT CCD (MAR CCD)
- Automatic image plate (MAR 345)
- Image intensifier (FreLon)
- CsI:Tl scintillator on aSi (PIXIUM)

	MARCCD	MAR345	PIXIUM
Dimension (mm)	165 (D)	345 (D)	400 x 300
Pixels	4k x 4k	From 1k x 1k To 3k x 3k	2600 x 1920
PSF (pix.)	2.5	2.8	1
Speed (Hz)	< 0.1	< 0.02	7.5
Dyn. Range	45000	120000	16000
Dead Time (s)	~ 10	~ 35-110	~ 0.1
Pixel size (µm)	40 (80 bin)	100 or 150	143
Sensor	Gd2O2S:Tb FO CCD	BaFBr:Eu Laser readout	CsI:Ti on aSi TFT
Sensor Thick. (µm)	40	200	0.5



CdTe frames from ProtK at RT

Maxipix detector chip
512 x 512 pixels (2 x 2 modules)
55 microns pixels; 28 x 28 mm^2 area
CdTe Sensor
E=38 keV (0.328 Å)
PSF 1 pix
CdTe thickness ~ 1 mm
0.3 ms readout time
Count rate : 2.10^5 cts/pixel/s
350 Hz.
~ dyn. Range: 11810 /pix/img (chip)



0.01 deg for 0.5 sec
Distance : 130 mm from sample
Resolution at the top left corner : 1.35 Å
Sample previously irradiated for ~ 720 s
Loss of diffraction after ~ 1400 s of exp.
No shutter used and RT
2 10^{11} ph/s through 0.1 x 0.1 mm^2

Dose (700 s) : 0.5 MGy

Detector also tested at 73 keV (weak diff ….)

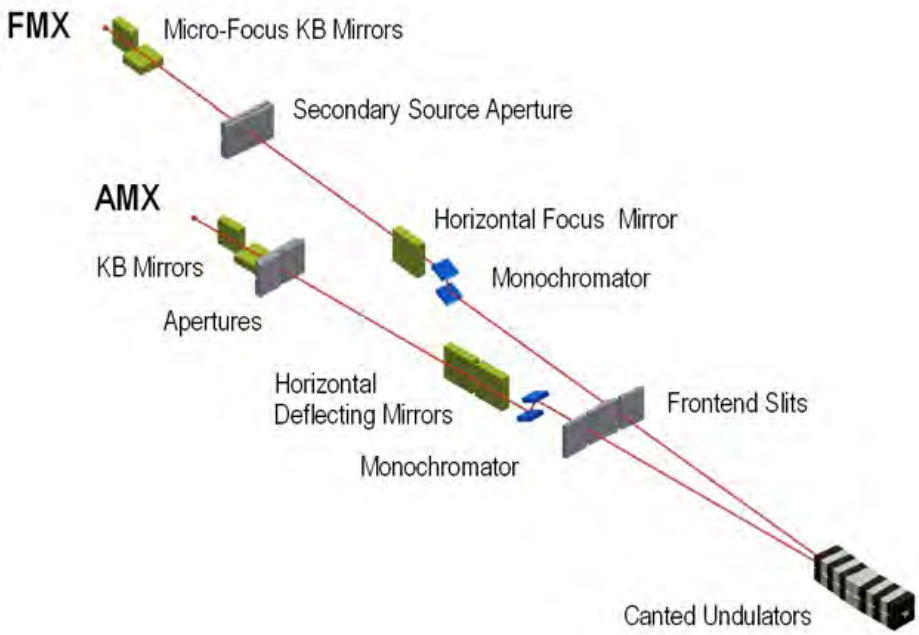
Concluding Remarks
Possible Improvements

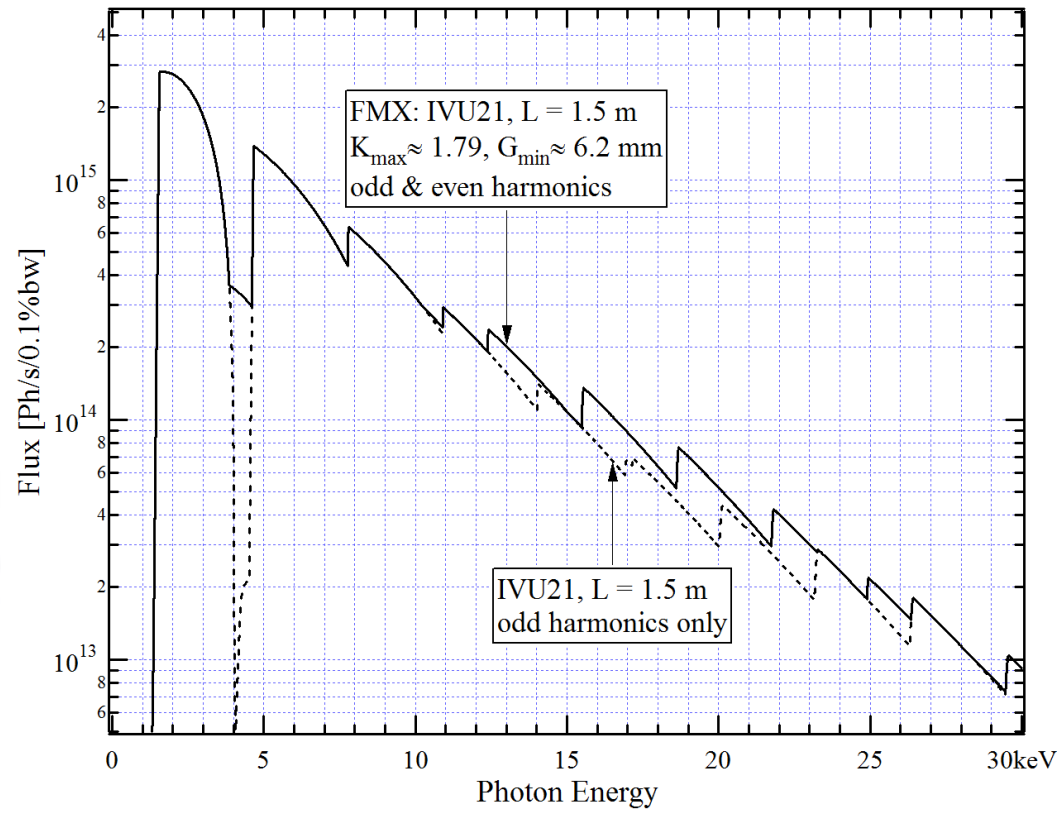
- x samples tested, RT, SAD, MAD, Ho, Te.. : NO Radiation Damages effects observed (all data collected at low dose).

- XPD/ID15A : large area CsI:Tl detector (Perkin Elmer) : test longer exposure time, on weakly diffracting samples and ultra high resolution protein crystallography (sample needed …)
- ID15-side/FMX: MX mini-diffractometer (rotation + 1st beamstop (XYZ) + sample visu. (XY)+ scatter shield + cryo (MD2 ?)
- Possibility to test a larger CdTe detector ….. 15x15 cm would be great for the purpose (small pixel size, and small scattering angle)
- Need a dedicated detector : efficient, large, high DR. fast …

- **NSLS-II applications: XPD / FMX ?**

High Energy MX@FMX (E < 30 keV)





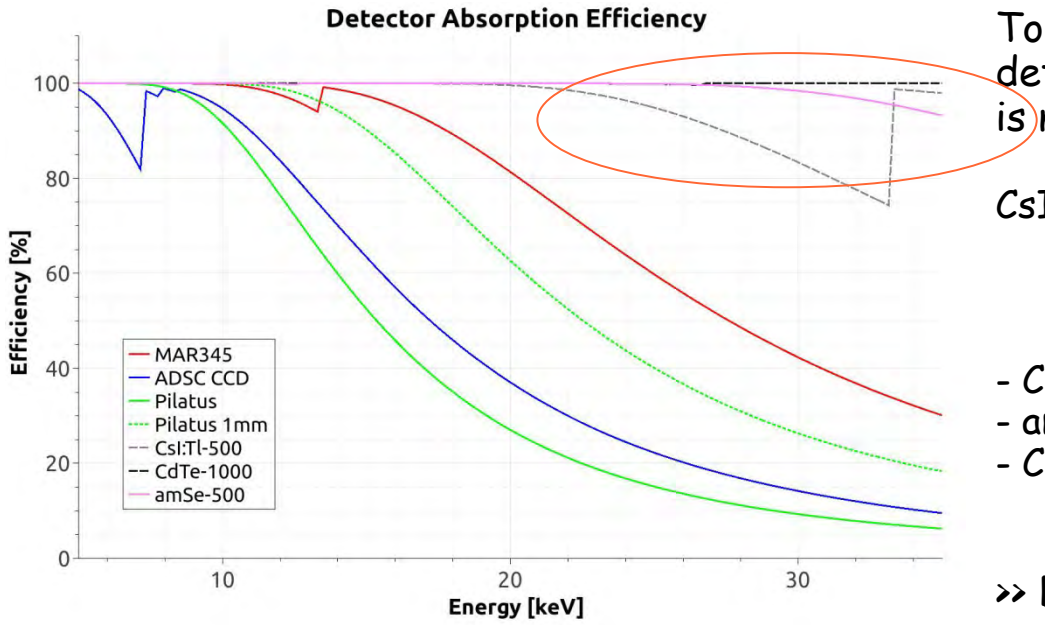
FMX : 5-30 keV (1-20 μm); 10^{13} ph/s @ 12.7 keV
AMX : 5-18 keV (5-100 μm); 2 10^{13} ph/s @ 12.7 keV

Mono : DCM Vert. cryo-cooled Si(111).
H Focusing mirror
Pt coated KB mirror pair (reflectivity > 0.8 @ 30 keV@2 mrad) [1 Å and 0.1 μrad]

FMX beamline performance

Energy (keV)	12.5	15	20	30
Total flux from U21 (10^{13} ph.s^{-1})	22	15	5.5	1
Single mirror efficiency*	0.95	0.95	0.93	0.84
Overall focusing efficiency (HFM + KB)	0.85	0.85	0.8	0.6
Estimated flux at sample (10^{12} ph.s^{-1})	10	7	2.3	0.3

FMX "High Energy" Area Detector



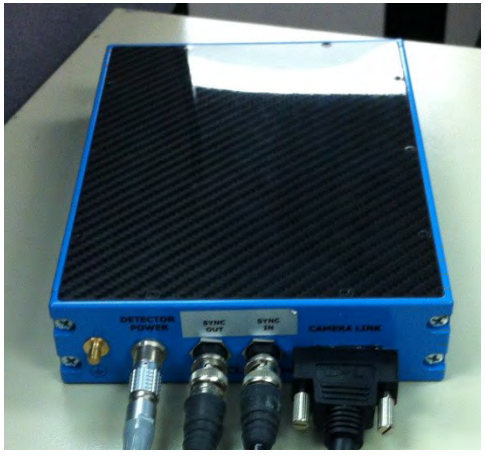
To fully take advantage of MX@ HiEn, a dedicated detector with significantly higher efficiency (at 30 keV) is needed.

CsI:Tl, amSe and High Energy scintillators

- CCD, least noisy, slow, costly and small, $$$$ (16 bit)
- amSi, large, fast, most noisy + lag $$ (14 bit)
- CMOS, med-size, fast, noisy $$ (14 bit)

>> Det. Of choice here CMOS: cost, performances.

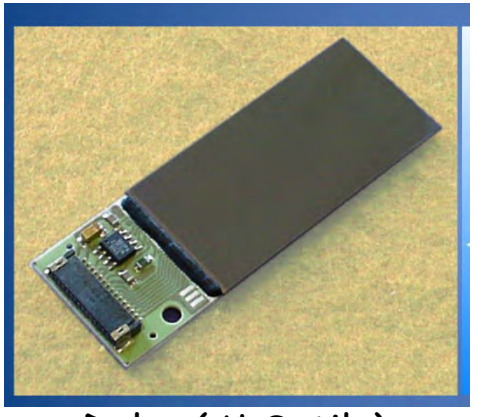
Option 1 : DEXELA 1512 (LAG < 1 %)
Option 2 : DALSA Xineos 1515
Option 3 : existing PE detector (large, fast but lag (~8 % at first frame)
Option 4 : Refurbish a CCD with CsI:Tl or high energy scintillator.



Dexela 1512 (cm)

Perkin Elmer1621 (")

Dalsa Xineos 1515 (cm)

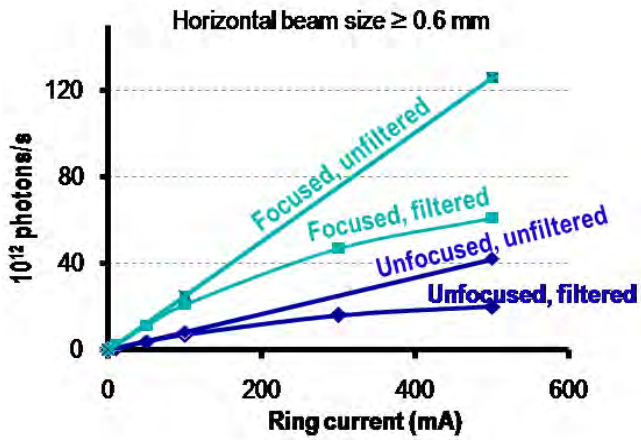


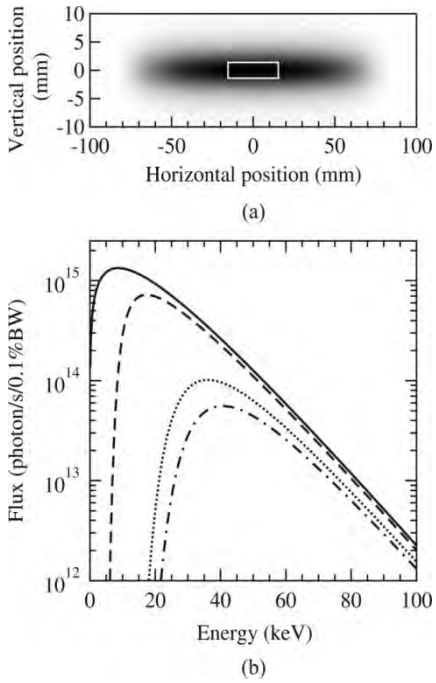
Dalsa (ALS, tile)

High Energy MX@XPD (E > 30 keV)

Damping Wiggler Source (DW100)

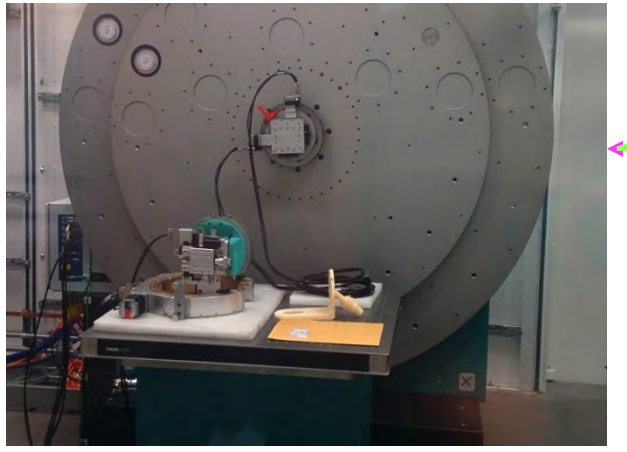
Energy range (keV) : 30-70
E. resolution : ~0.001 (@HiFlux mode)
Beam size (@sple) (mm2) ~ 0.75 * 0.1
Flux at sample @30-40 keV (ph/s) : > 10^{13}
(forMX exp : 0.1x0.1mm^2 (flux > 10^{12} ph.s^{-1}))
Detector: amSi TFT diode array + 0.5 mm CsI:Tl





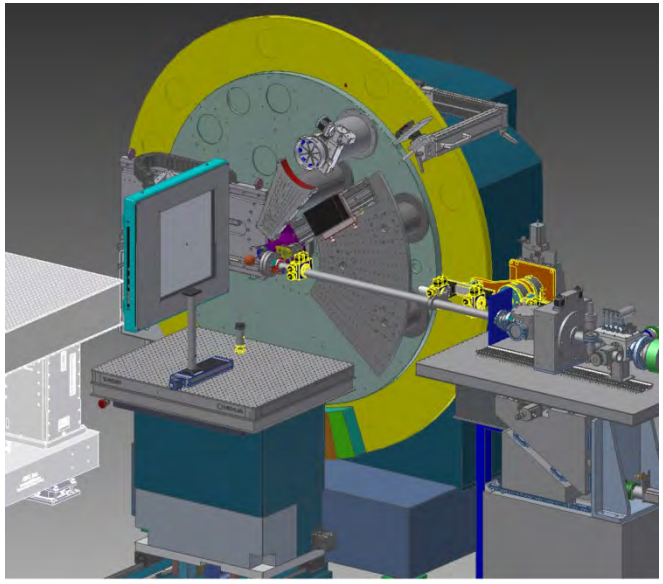
ddition of :
$_2$ cryo-stream, 2 beamstop (prim. Sec) OAM (FOV < 1 mm)
nd we'll be GOOD TO GO !

Perkin Elmer XRD1621



XPD Expmt hutch

OAM

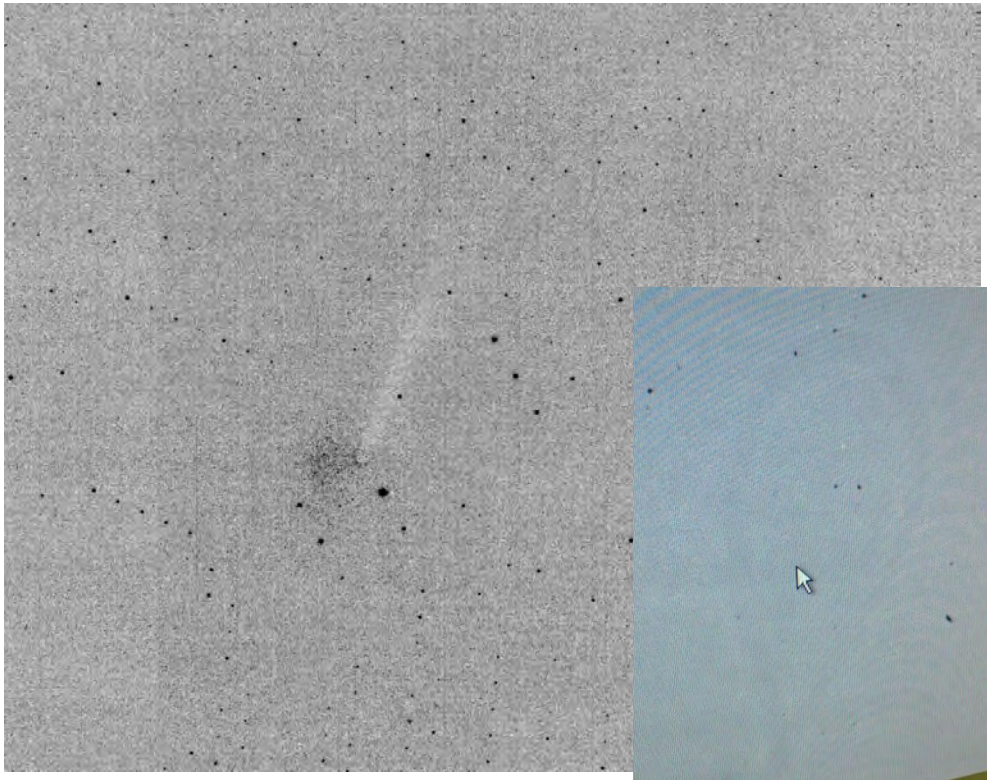


Preliminary Evaluation of DEXELA 1512

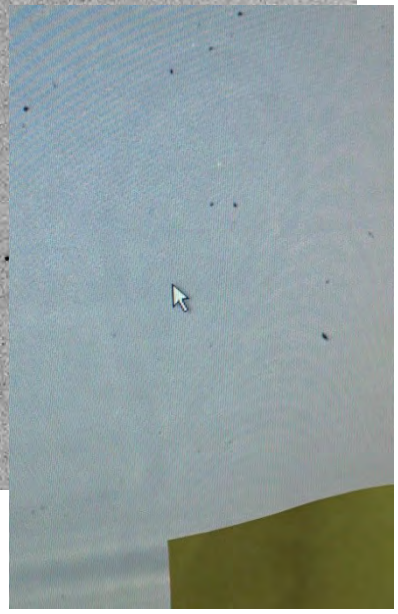
CMOS sensor 15x12 cm coupled to Fiber Optic Plate and 0.2 mm CsI:Tl scintillator
1536 x 1944 pixels 75 microns pixel pitch 14 bit counter up to 56 FPS.

Detector tested at : 15 keV, 20 keV, 39 keV; 67 keV (X6/X9; X13; X7; X17).

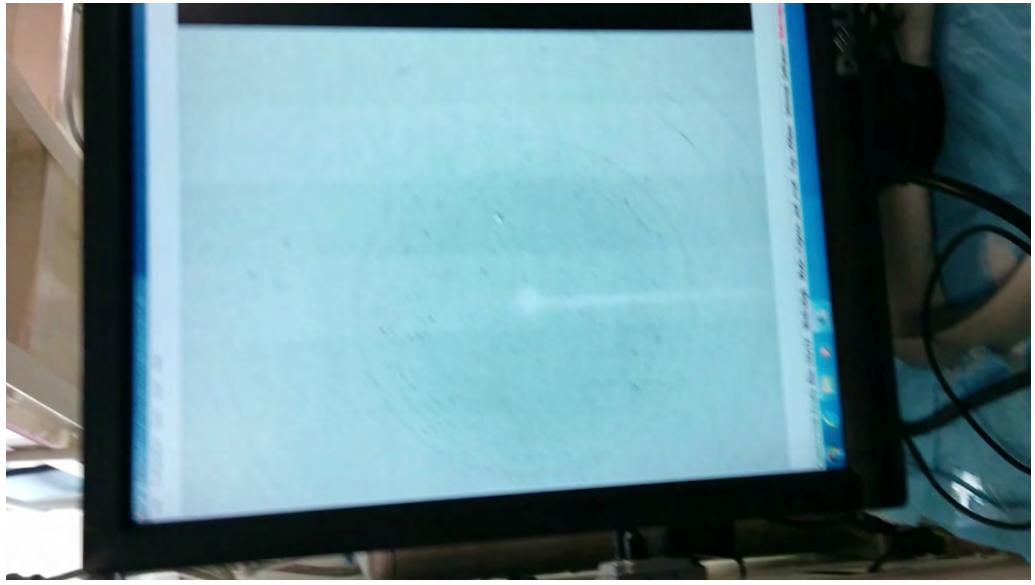
Detector performs well (in terms of sensitivity/speed). (compare to existing PE XRD detectors).
(100 % vs 90 % at 38 keV and 70 % vs 40 % at 67 keV for 0.2 mm CsI vs 0.48 mm CsI)
Noisy for low signals and tend to heat (need extra cooling; larger devices have fans).
Used the proprietary control software (allow for single shots and somewhat sequences).
Protein sample tested at X6 and X13.



X13B 20.16 keV
5s/frame; 1 deg.
Sample diffracted to 1.8 Å.



Align. Of sample using X-ray !!!



First Diffraction from Protein Crystal.
X6A, 50 FPS at 15 keV.
Poorly frozen sample (slow rotation).

Acknowledgments

X6A, NSLS and ESRF Staff

Collaborators

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M. Ruat and C. Ponchut (DETECTOR@ESRF)

Samples/Collaborations (High Energy, Selenate)

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Nilda Alicea-Velazquez, Amy Stiegler andTitus Boggon (Yale University)
Neer Asherie (Yeshiva University)
Gino Cingolani (Thomas Jefferson University)