Monday, 6th February 2012, MX winter WS - ESRF

EMBL@PetraIII P13-MX beamline for structural biology (and longer wavelengths…)

Michele Cianci

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Outline

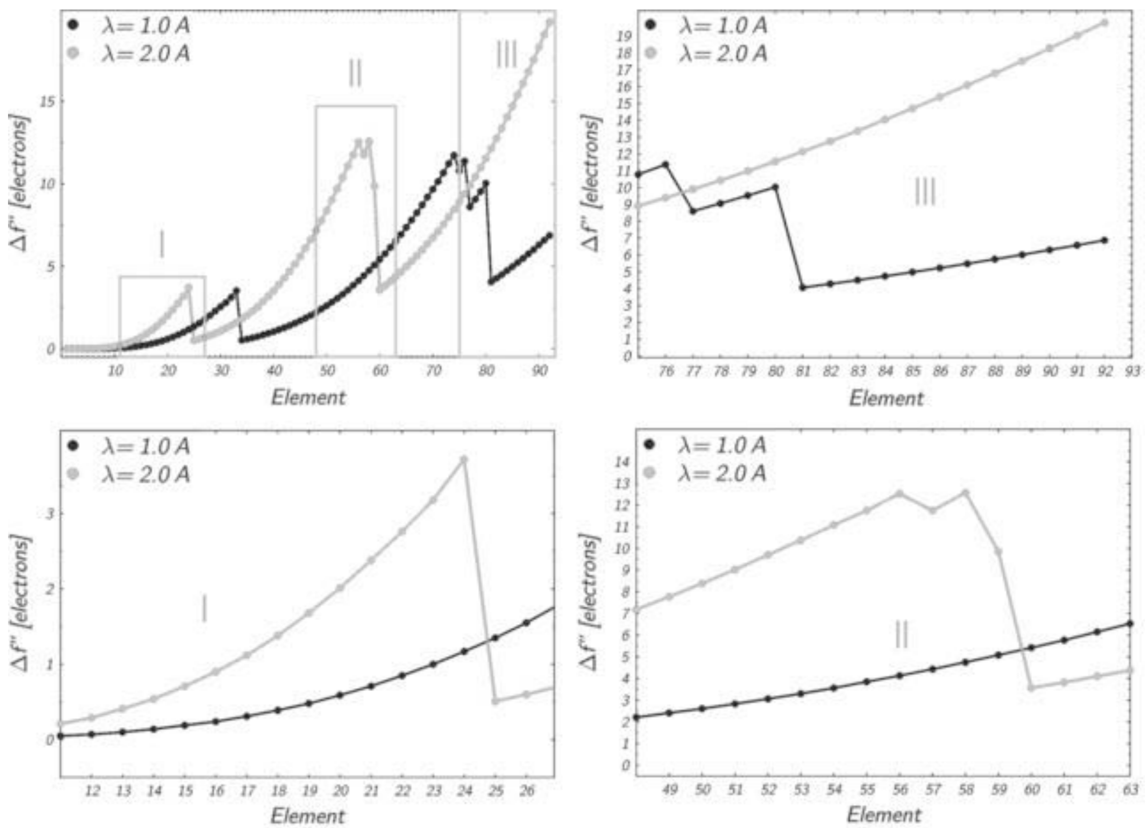
- General Introduction
- Brief summary of MX facilities at Petra III
- Preparation of P13 for longer wavelength work
- First results
- Conclusions

What are Softer or Longer X-rays?

Description	Wavelength range (Å)	Energy range (keV)
Short wavelengths (hard X-rays)	<0.7	>17.0
Normal wavelengths (normal X-rays)	0.7-1.5	8.0-17.0
Longer wavelengths (softer X-rays)	1.5-3.0	4.0-8.0
Long wavelengths (soft X-rays)	>3.0	<4.0

Softer and soft X-rays in macromolecular crystallography.
Djinovic-Carugo et al., *J. Synchrotron Radiation*. (2005) Vol. 12, page 410-419.

What's up with Softer or Longer X-rays?



Anomalous scattering length ([Delta]f'') values in units of electrons at [lambda] = 1.0 Å (black) and [lambda] = 2.0 Å (grey) for the first 92 elements (top left), for elements 11-27 (bottom left), 48-63 (bottom right) and 75-92 (top right).

Softer and soft X-rays in macromolecular crystallography.
Djinovic-Carugo et al., *J. Synchrotron Radiation*. (2005) Vol. 12, page 410-419.

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So what we can do with Softer X-rays or Longer wavelengths?

- Use stronger anomalous signal for difficult cases of phasing

- locate ions in biological structures and make sense of their activities

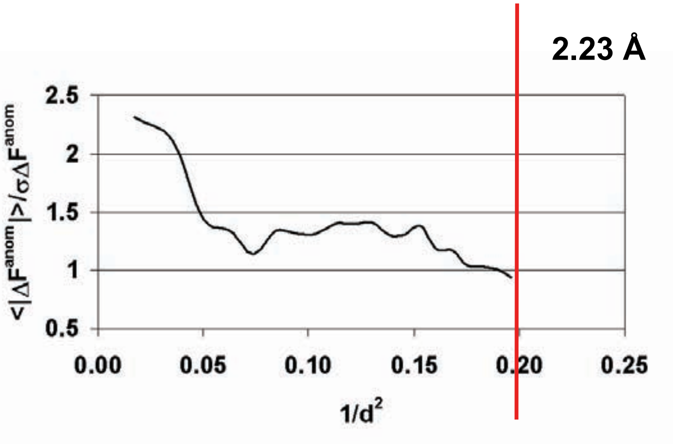
Ion location example

M Cianci, B Tomaszewski, JR Helliwell and PJ Halling, (2010) J. AM. CHEM. SOC., *132,* 2293-2300

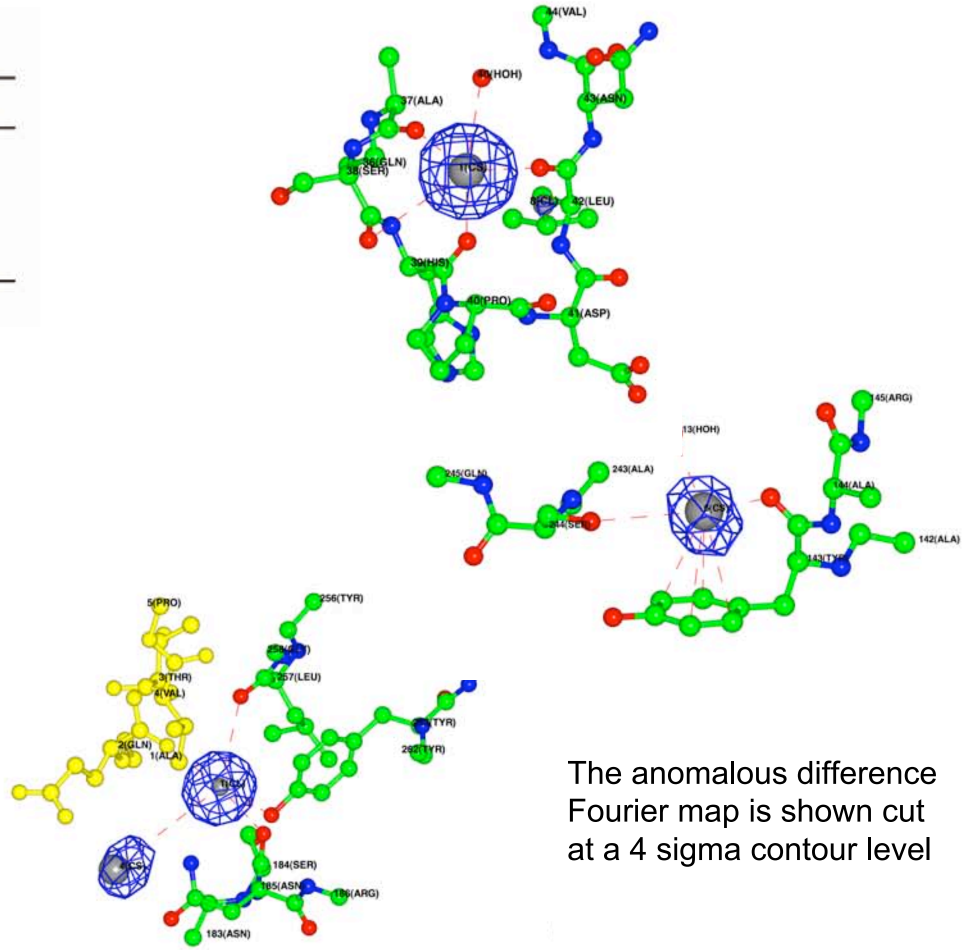
Table 1. Effect of Salt Soaks on Catalytic Activity of Subtilisin Crystals in Acetonitrile

added salt in soak solution	initial rate[a] (nmol min^{-1}mg^{-1})
None	2.4 ± 0.8
KCl	5.6 ± 2.6
Choline-Cl	10.8 ± 3.7
CsCl	13.7 ± 5.2

[a] Rates are shown as mean ± standard deviation.



Plot of $<|\Delta F_{anom}|>/\sigma\Delta F_{ano}$ *versus* $1/d^2$ for Cs-CAN where d is the interplanar spacing, for Cs-ACN. The diffraction anomalous signal is above one across the whole resolution range. Data collected at Cs L1 edge @2.167 Å.



The anomalous difference Fourier map is shown cut at a 4 sigma contour level

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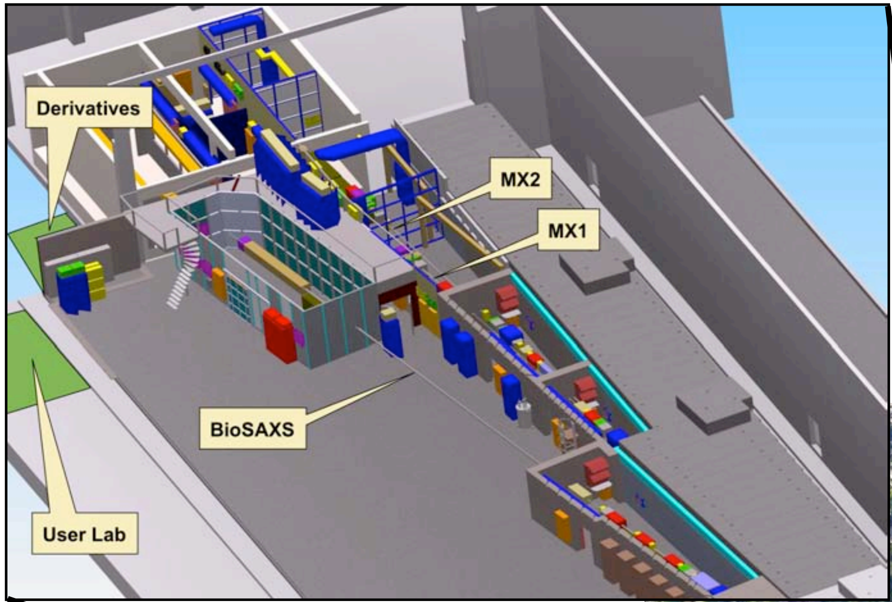
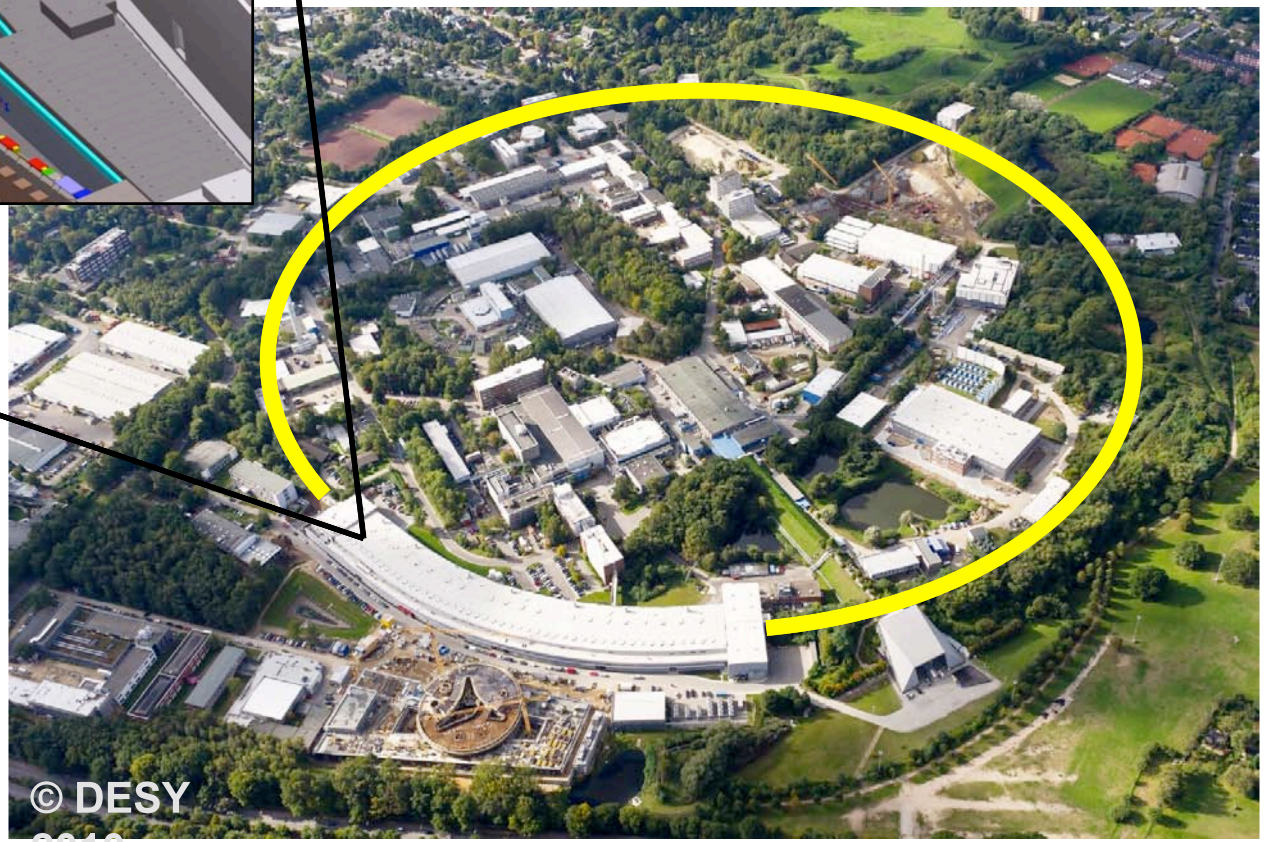
“We choose to go to 4 keV. We choose to go to 4 keV in this beamline and do the other things, not because they are easy wavelengths, but because they are longer, because that light will serve to collect and measure the best of our anomalous signal, because that’s the signal we are willing to phase with, one we are unwilling to waste, and one which we intend to study, and the others, too…”

PETRA III

6 GeV – 2304 m
100mA - 1nmrad





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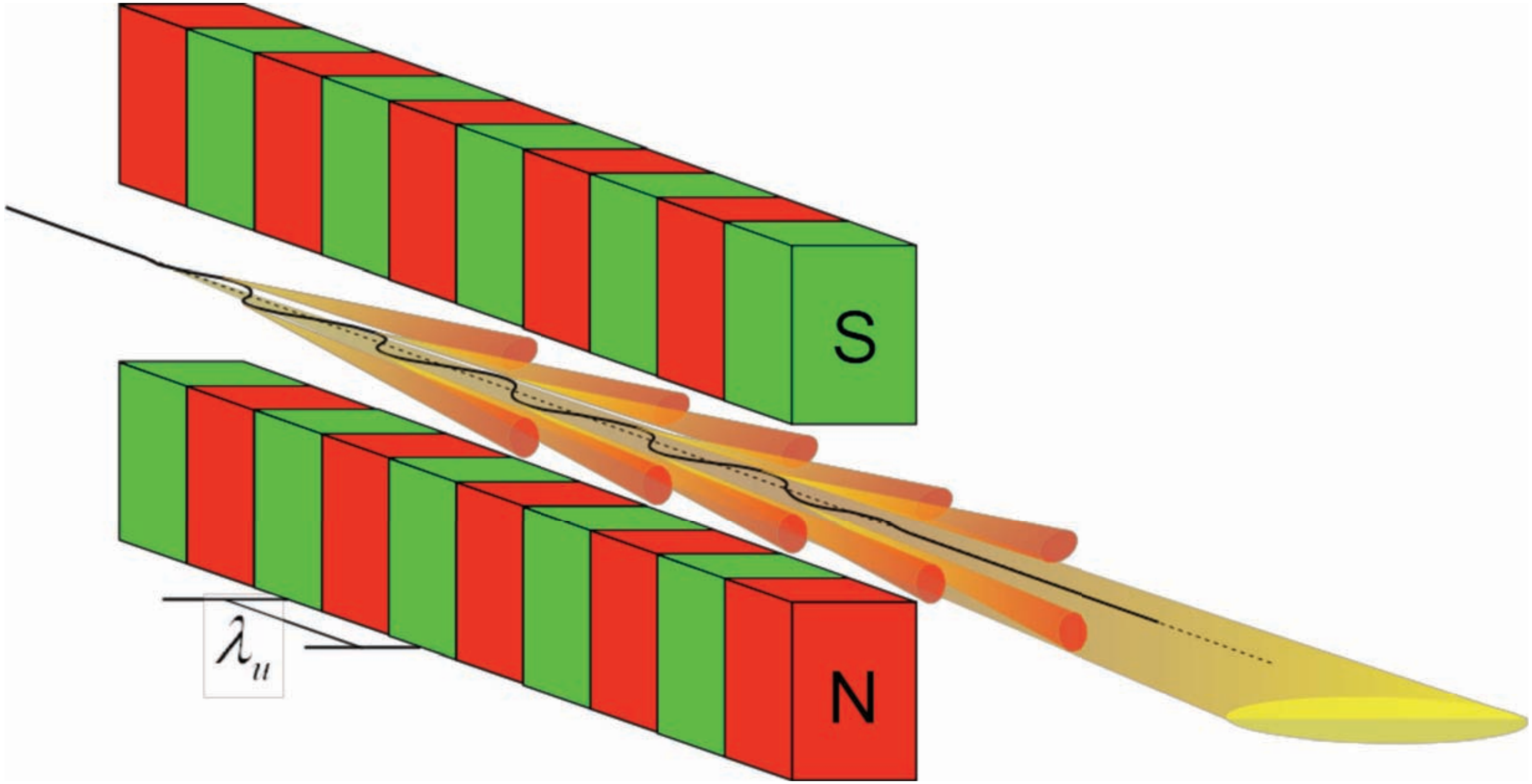
Undulator



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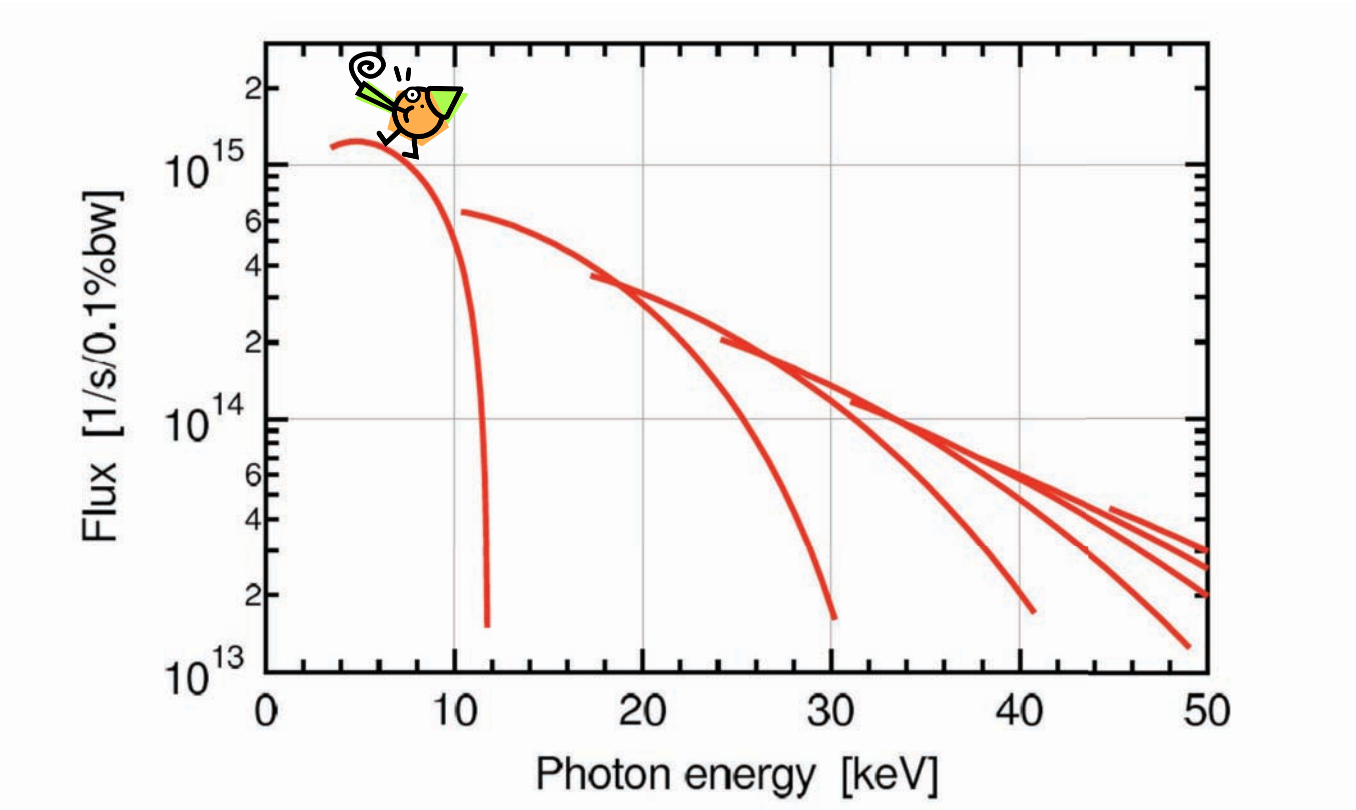
Output of our Undulator

… which we have been given…



Partial flux of the standard 5m Petra III undulator through an aperture of 1x1 mm^2 at 40m distance from the source.

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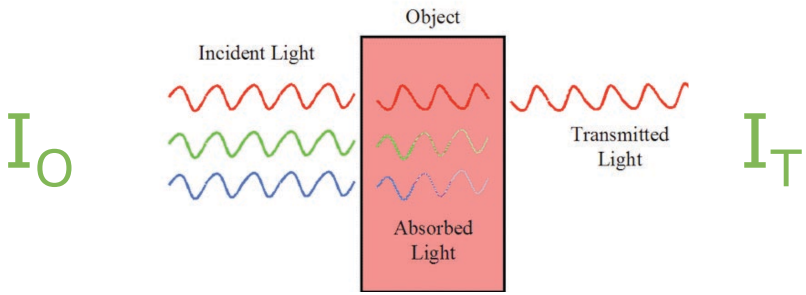
Transmission (I_T/I_O) of X-rays (%)



Energy (keV)	Air 100 mm, 1 atm, 295 K	Helium 100 mm, 1 atm, 295 K	Kapton 50 um	Lysozyme Crystal 50 um
12	96	99	98	98
8	89	99	95	94
6	75	99	90	87
4	39	99	69	63

www. http://henke.lbl.gov/optical_constants/

Crystal Lysozyme 1.43g/cm^3, $C_{613}H_{959}N_{193}O_{185}S_{10}$

MX beam lines specifications

Beamline	**MX1**	**MX2**
Main purpose	Wide range tunability *longer wavelength*	μ-focus special applications
Energy range	5(4)–17 keV	7-35 keV
Bandpass ΔE/E Si(111) **Si(311)** **MLM** **Full 1st**	**$< 2*10^{-4}$** **$< 5*10^{-5}$** – –	**$< 2*10^{-4}$** **$< 5*10^{-5}$** 1-2 % 5 %
Focus H / V	28 x 13 (30-100) μm	4 x 1 (1-2)[a] μm
Divergence H x V	0.2 mrad x 0.15 mrad	0.5 mrad x 0.3 mrad
Demagnification ratio H / V	1:12 / 1:15	1:40 / 1:30
Intensity, ph/s	$1*10^{13}$ – $3*10^{13}$ ph/s	$1*10^{13}$ ph/s
Pink beam[b] intensity, ph/s	n/a	~10^{16} ph/s

[a]with add-on optics

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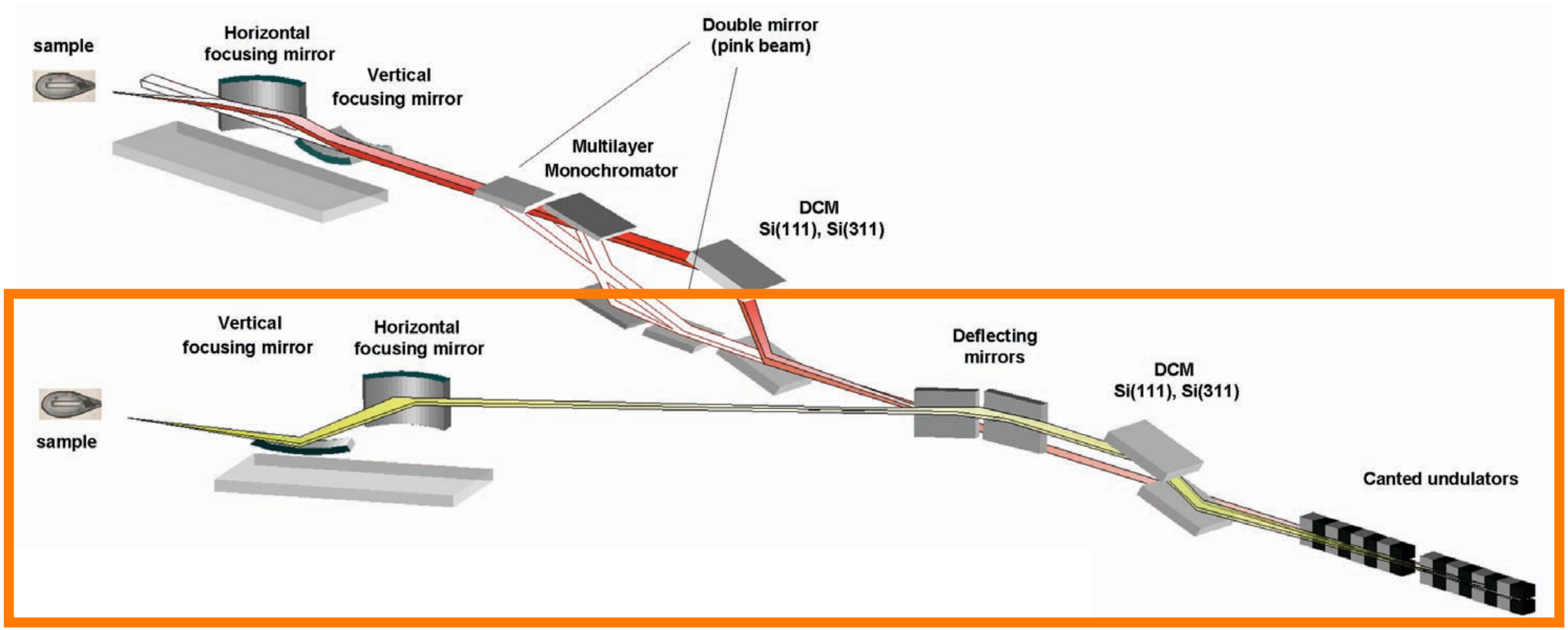

MX beamline layout

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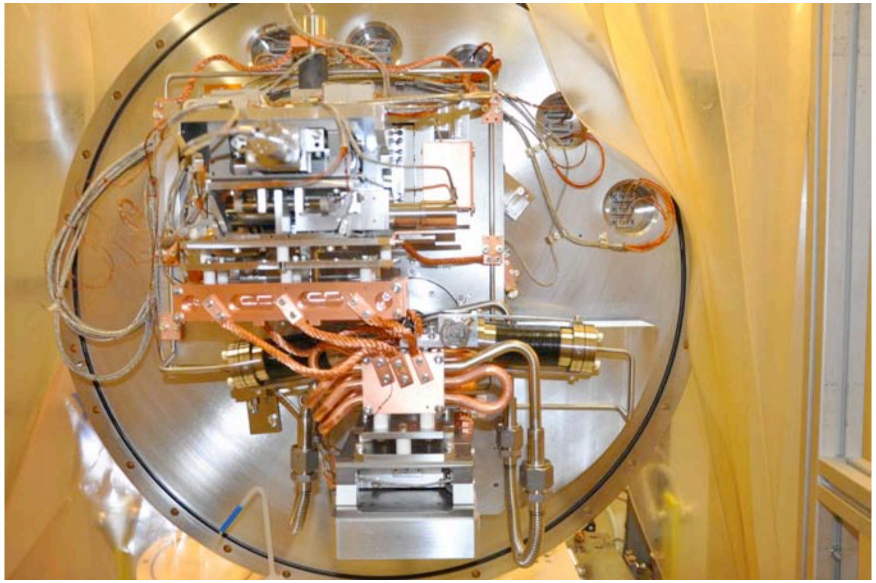
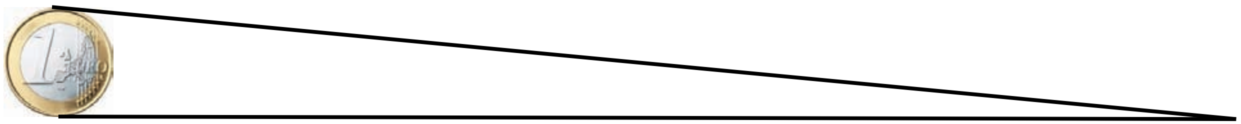
Preparation for longer wavelength - DCM

	EMBL MX1 specifications
Crystals	Silicon <111>,<311>
Range (Bragg angle)	working range from -3.0°-40.0°. Resolution < 0.04 arcsec (0.18 urad) Repeatability <0.1 arcsec. (0.5 urad)
Fixed exit	fixed exit
Scanning speed	Up to 1.0 degree/sec
Crystal cooling	Both crystals will be cryogenically cooled
Other	Piezo Roll and Pitch on the second crystal for intensity feedback



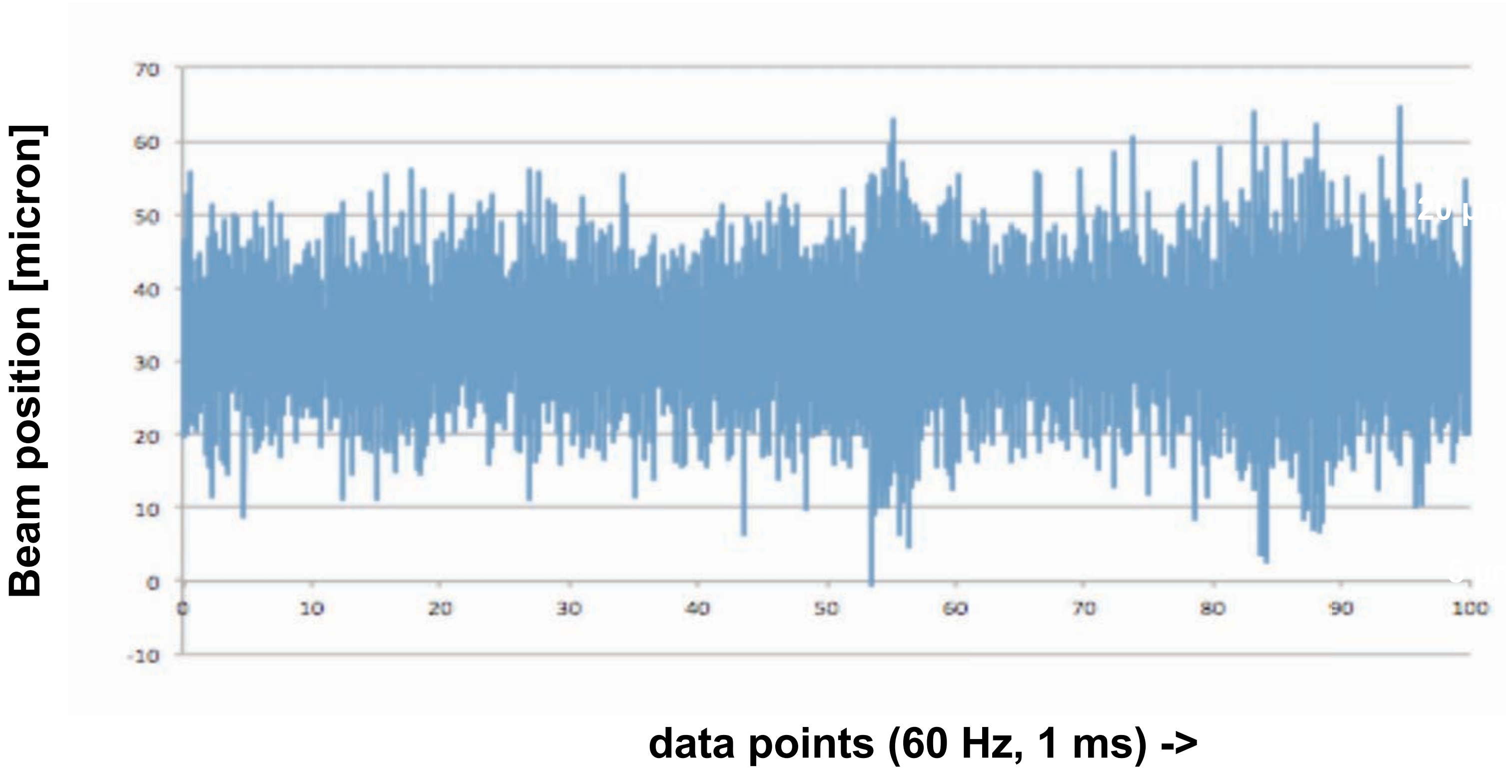
0.5 urad = 0.5 um/meter =



46.5 km

The beam (P14-MX2)

- Beam position is very stable:
  - rmsd 1.5 µm @ <10 Hz
  - rmsd 7 µm @ > 30 Hz
  - N.B. 60 m from the source, 20m from the DCM



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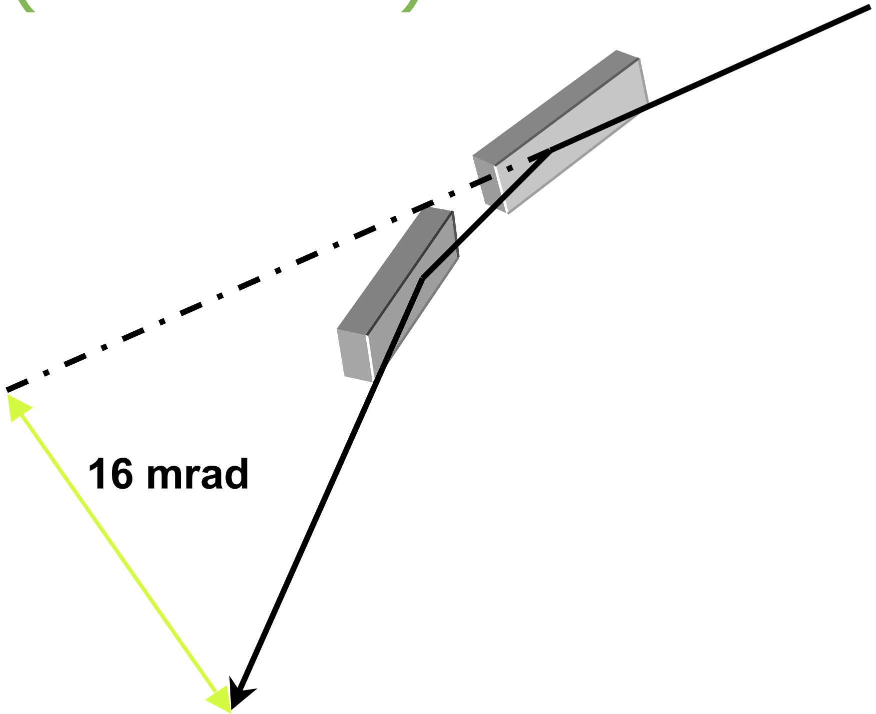
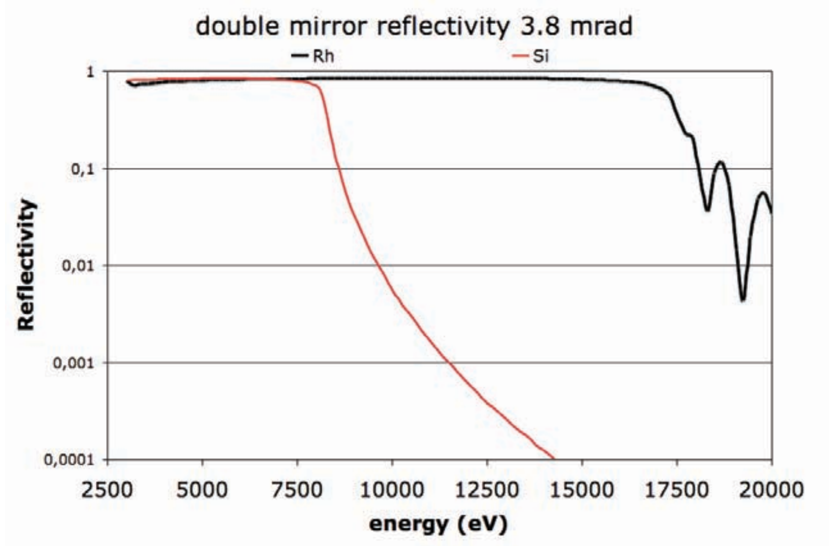
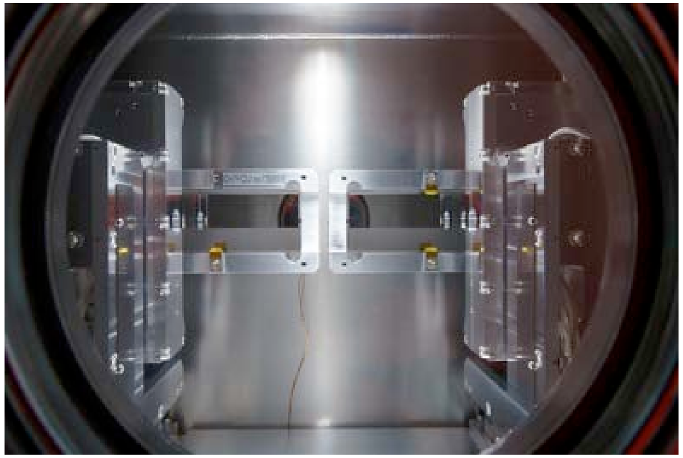
Preparation for long wavelength – HDM (… and KB)







Advantages:

•Compact solution
•Temperature stable
•No moving parts when changing energy

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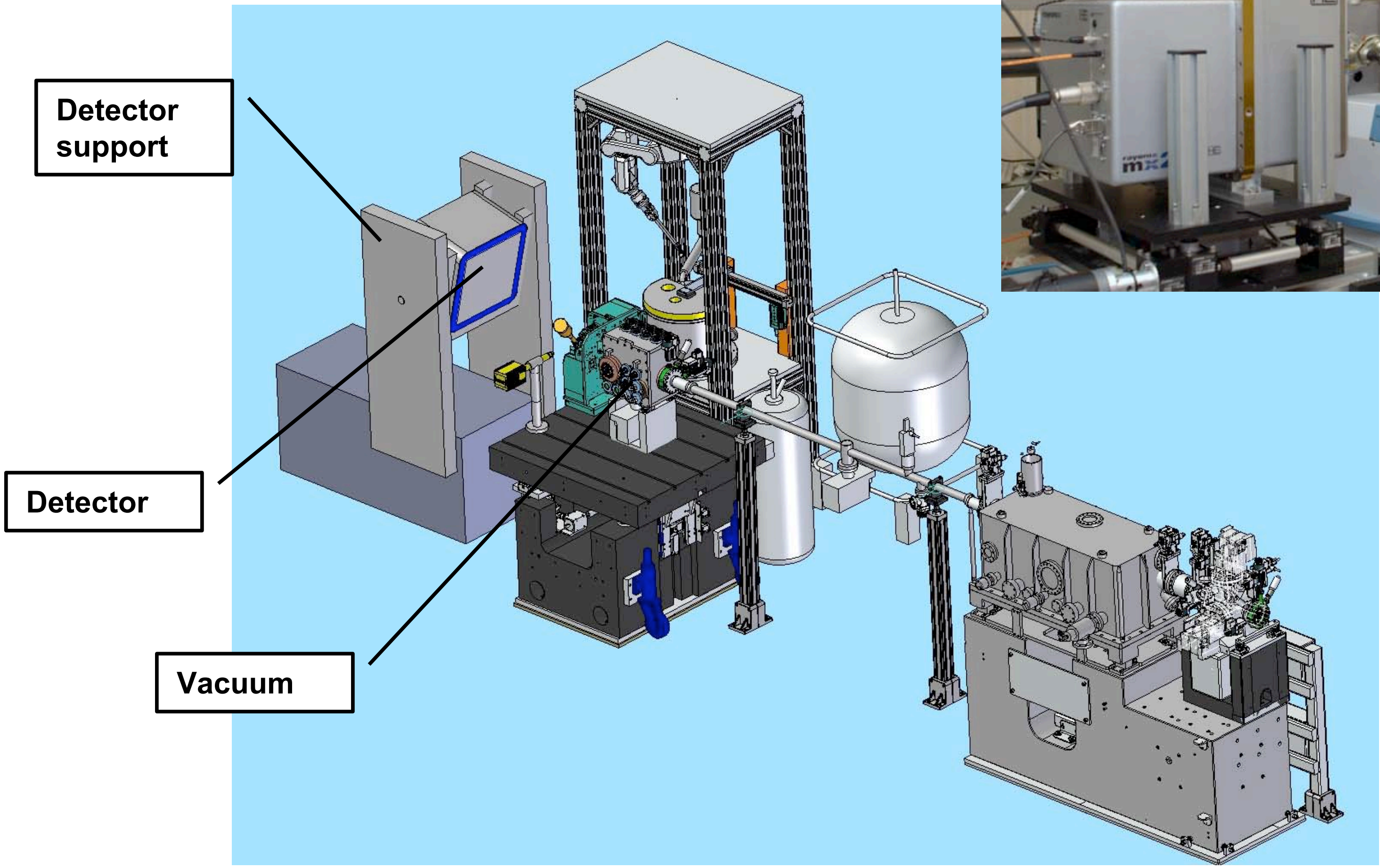
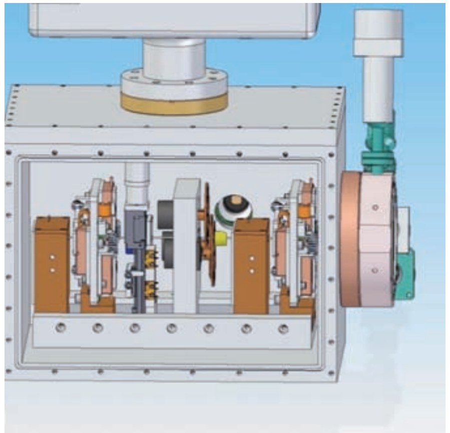
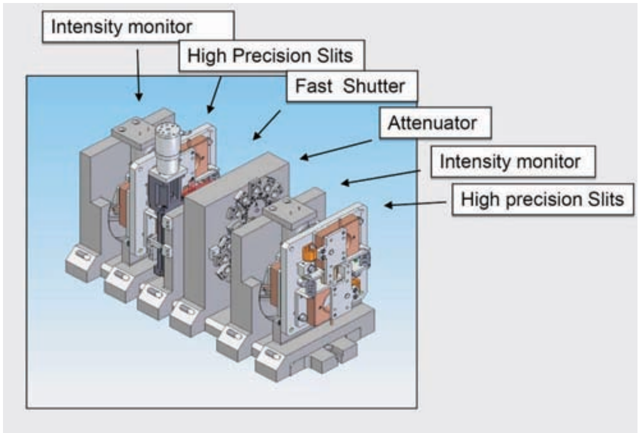
Preparation for long wavelength - Experimental Station

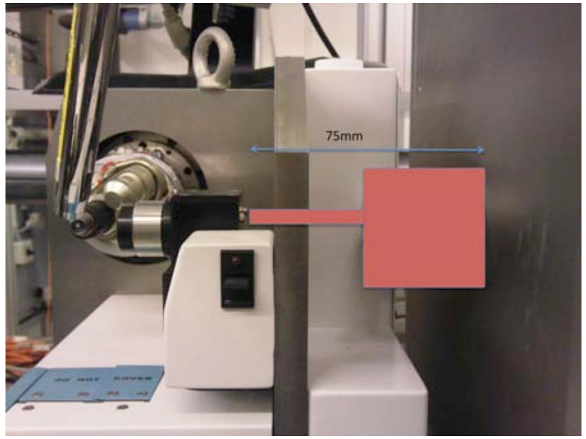


Preparation for longer wavelength – Beam Conditioning Unit

- Compact & modular.
- Vacuum ~ 10-6 mbar.
- Monochromatic applications – i.e. no cooling needed.





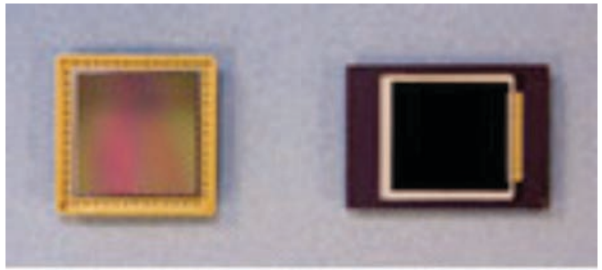


Preparation for longer wavelength: Rayonix 225HE

- Back illuminated chip with twice the quantum efficiency of standard front-illuminated chips
- P thickness 20 um (vs. 40 standard)

Model	mx225	mx225HE	
Mode	n/a	Slw	Fast
# pixel	3072 x 3072	3072 x 3072	
PSF FWHM, micron	100	100	
PSF 1%, micron	~500	~500	
Signal-to-noise(1)	0.6	3.2	1.5
Readout time (sec)	1.0	3.5	1.0

1) Signal-to-Noise : Electrons per photon / Read noise



MX standard (left) and HE (right) CCD chips

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First results

24/11/2011: First beam in the experimental hutch – Safety Checks passed

29/11/2011: New calibrations for DCM and Undulator

02/12/2011: Installation and optical alignment for the BCU

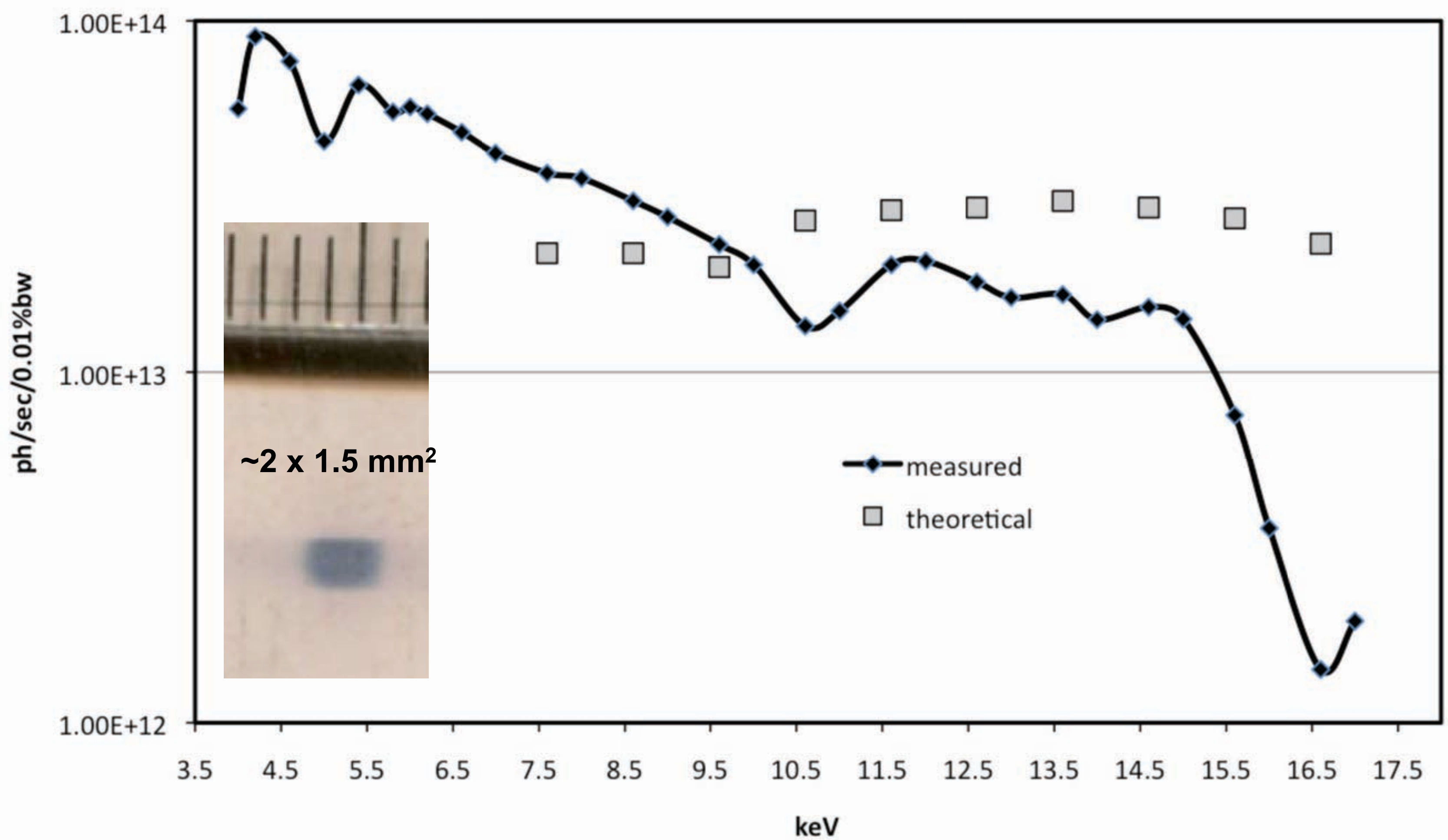
10/12/2011: Beam through the BCU

12/12/2011: First diffraction image collected on a insulin crystal

13/12/2011: First data collection on a insulin crystal at λ=1.541 Å and successful phasing on S anomalous signal; data collections at various wavelengths.

20/12/2011: shutdown!!!

P13: Total photon flux at 100 mA ring current with Si-111



- > 10^{13} ph/sec between 4 and 10 keV

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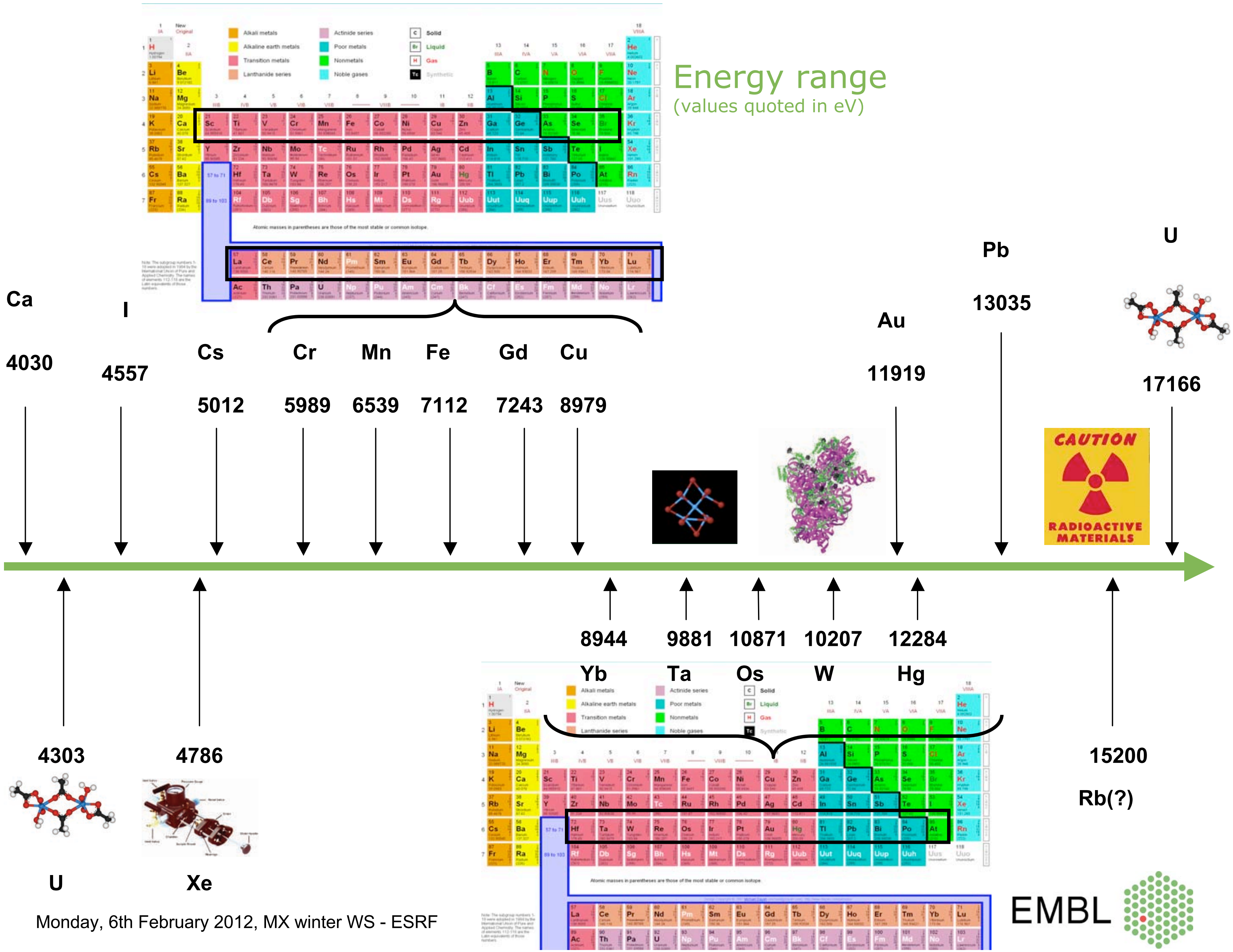
Energy range

(values quoted in eV)



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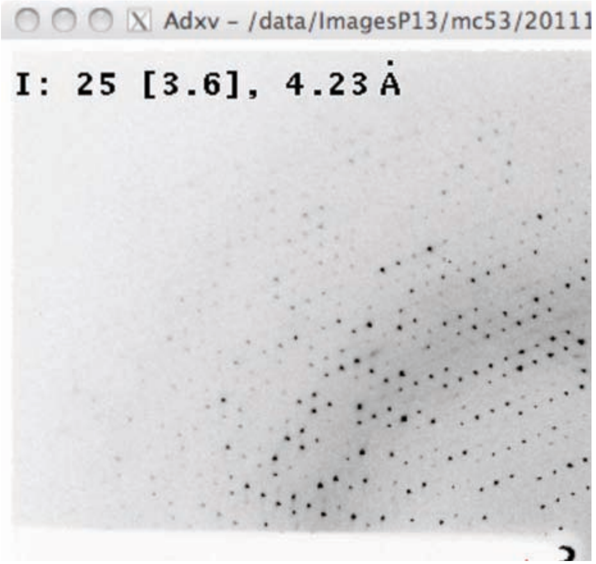
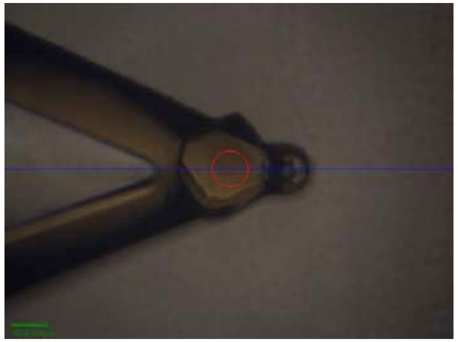
Derivatization Laboratory, (11 m^2)

44 elements
152 derivatives

Data collection at 12.6keV = λ 0.984 Å



Beam size 50um Ø
Xtal-to-detector distance 200 mm
Exposure time 5 sec/degree
999 degrees collected



SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE >= -3.0 AS FUNCTION OF RESOLUTION

RESOLUTION LIMIT	NUMBER OF REFLECTIONS OBSERVED	UNIQUE	POSSIBLE	COMPLETENESS OF DATA	R-FACTOR observed	R-FACTOR expected	COMPARED	I/SIGMA	R-meas	Rmrgd-F	Anomal Corr	SigAno	Nano
5.84	22667	410	415	98.8%	3.5%	4.2%	22667	125.26	3.5%	0.6%	85%	2.090	172
4.14	41060	727	727	100.0%	4.1%	4.2%	41060	127.27	4.2%	0.6%	82%	1.660	329
3.38	54007	955	955	100.0%	4.5%	4.5%	54007	114.35	4.6%	0.7%	67%	1.480	443
2.93	68276	1128	1128	100.0%	5.7%	5.5%	68276	92.22	5.8%	1.0%	43%	1.204	531
2.62	81034	1287	1287	100.0%	8.8%	8.6%	81034	66.65	8.9%	1.6%	14%	0.980	610
2.40	88945	1392	1392	100.0%	13.6%	13.7%	88945	46.81	13.7%	2.5%	-5%	0.849	660
2.22	98163	1543	1543	100.0%	22.3%	23.0%	98163	31.18	22.4%	4.2%	1%	0.785	740
2.08	102203	1635	1635	100.0%	32.6%	34.3%	102203	21.76	32.9%	6.3%	-6%	0.766	782
1.96	85202	1716	1780	96.4%	57.1%	61.2%	85179	10.44	57.6%	17.6%	0%	0.779	807
total	641557	10793	10862	99.4%	7.4%	7.5%	641534	56.49	7.4%	2.7%	16%	1.018	5074

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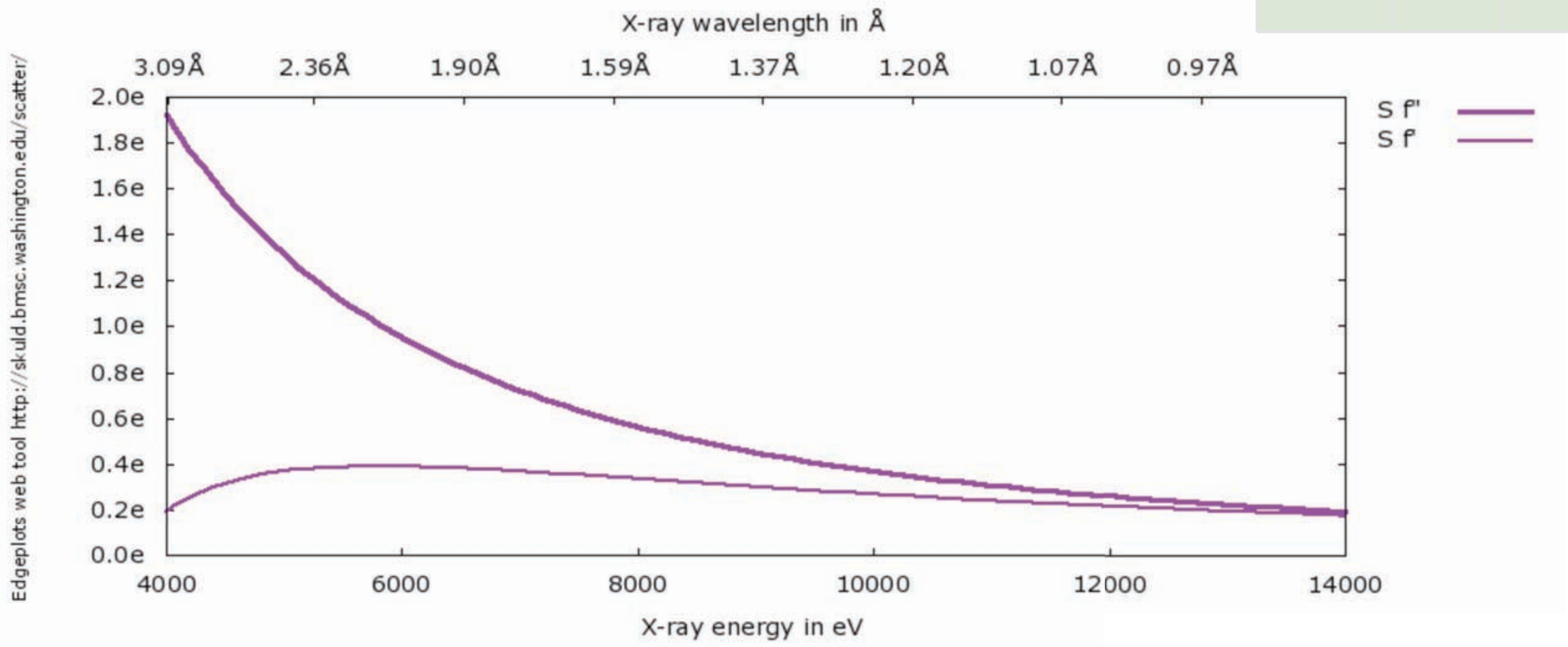
Zn-free Insulin (G. Sheldrick, BIOXHIT)

- Expected signal for 6 S in 532 atoms at 12.6 keV:

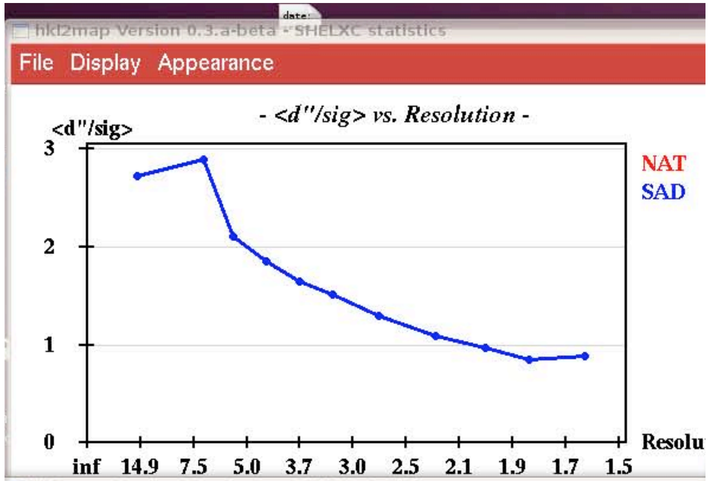
$$\frac{\langle \Delta F^{\pm} \rangle}{\langle F \rangle} = \left(\frac{2N_A}{N_P} \right)^{1/2} \frac{f_A^{"}}{Z_{eff}} = \left(\frac{2 \cdot 6}{451+81} \right)^{1/2} \frac{0.24\ e^-}{6.7\ e^-} = 0.0054$$

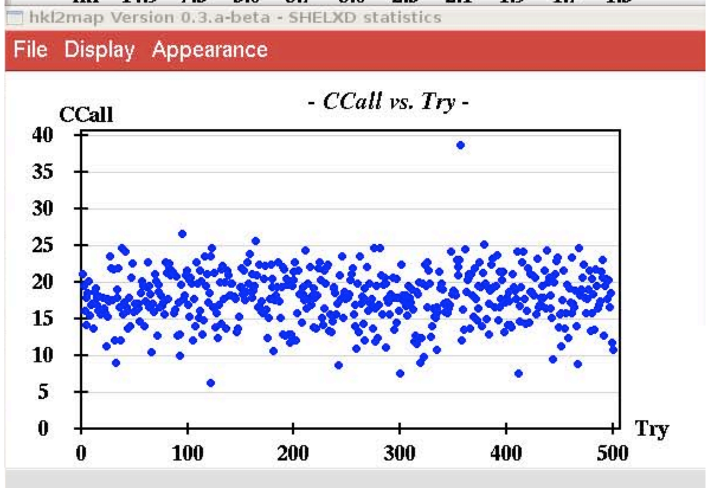
E[eV]	λ [Å]	f"[e-]	<df/f>
12.6	0.98	0.24	0.54
11.0	1.12	0.31	0.69
8.0	1.55	0.55	1.23
6.0	2.07	0.95	2.13
4.6	2.70	1.53	3.42

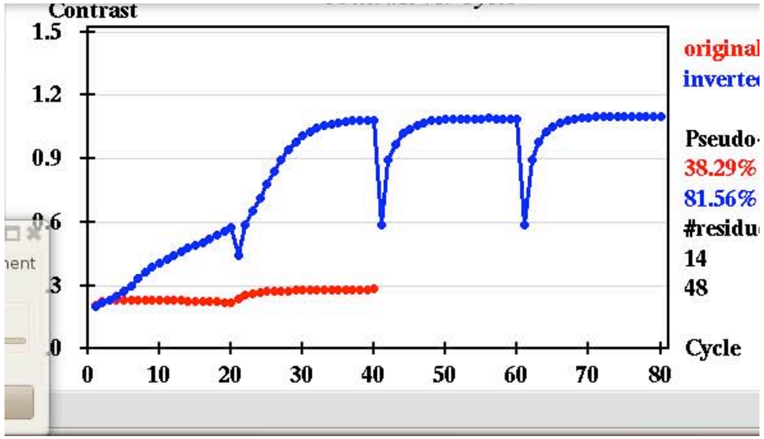


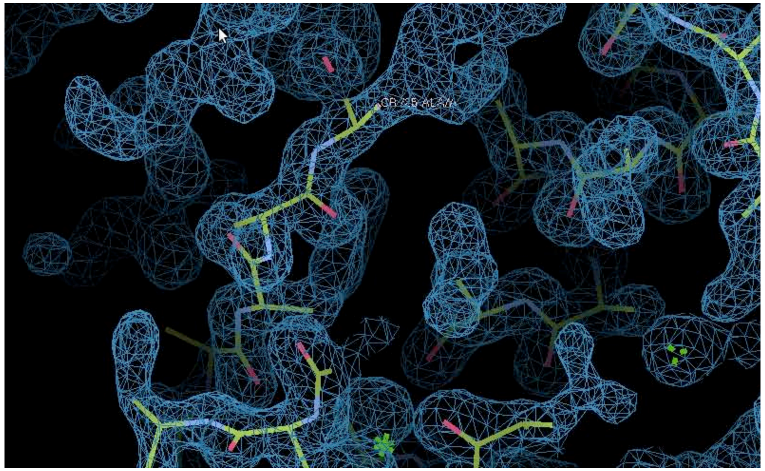
Hendrickson & Teeter (1981) Nature 290:170 Ethan Merritt's web-site

Phasing steps









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Data collection

So we tried:

Data collection at 12.6keV = λ 0.984 Å

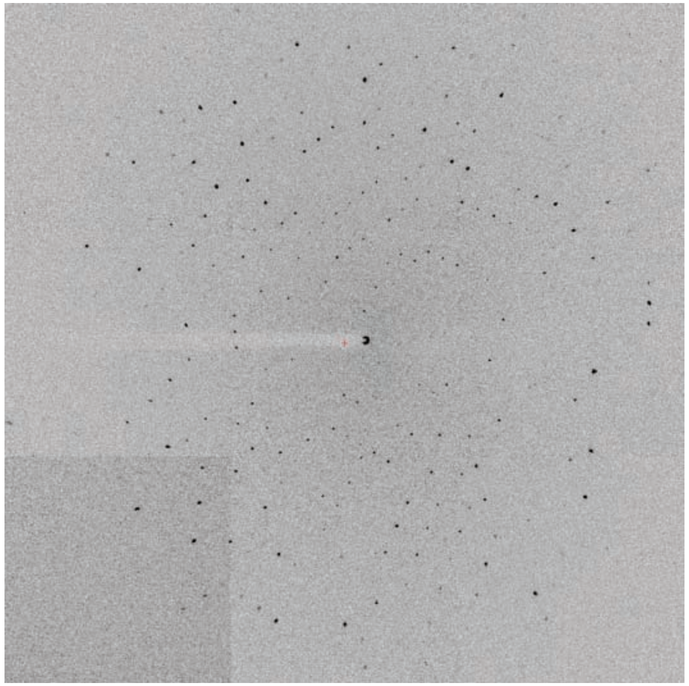
Data collection at 8keV = λ 1.541 Å

Data collection at 5keV = λ 2.069 Å

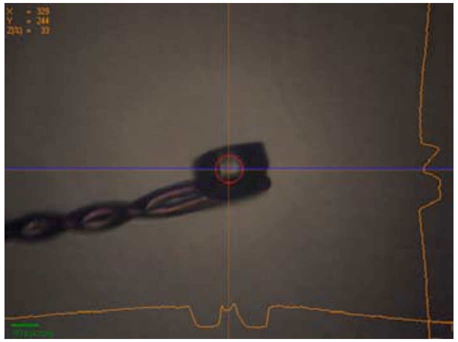
Data collection at 5.5keV = λ 2.5 Å

All of them gave clear phasing solutions

Data collection at 4.6keV = λ 2.69 Å



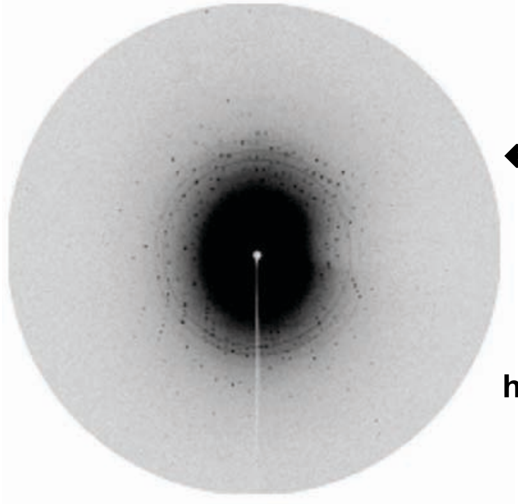
Beam size 50um Ø
Xtal-to-detector distance 100 mm
Exposure time 45 sec/degree
360 degrees collected



SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE >= -3.0 AS FUNCTION OF RESOLUTION

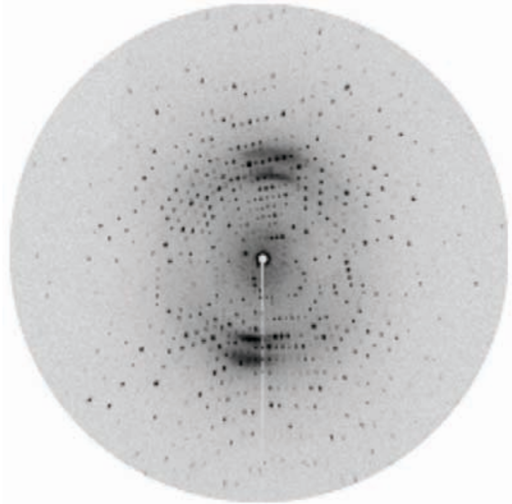
RESOLUTION LIMIT	NUMBER OF REFLECTIONS OBSERVED	UNIQUE	POSSIBLE	COMPLETENESS OF DATA	R-FACTOR observed	R-FACTOR expected	COMPARED	I/SIGMA	R-meas	Rmrgd-F	Anomal Corr	SigAno	Nano
9.16	2088	109	109	100.0%	2.5%	3.4%	2088	85.59	2.6%	0.8%	98%	6.294	41
6.52	3770	190	190	100.0%	3.7%	4.0%	3770	68.76	3.8%	1.2%	98%	6.433	81
5.34	4844	248	248	100.0%	4.3%	4.3%	4844	66.70	4.4%	1.4%	95%	5.078	110
4.63	5583	288	288	100.0%	4.3%	4.4%	5583	65.10	4.4%	1.3%	88%	3.694	131
4.14	6203	327	327	100.0%	4.1%	4.2%	6203	66.92	4.2%	1.4%	88%	3.244	149
3.78	6602	354	354	100.0%	4.8%	4.9%	6602	56.86	5.0%	1.6%	72%	2.040	163
3.50	7390	400	400	100.0%	6.9%	6.6%	7390	42.55	7.1%	2.8%	56%	1.733	186
3.28	7434	421	421	100.0%	10.4%	10.6%	7434	27.67	10.7%	4.1%	55%	1.369	198
3.09	2296	397	454	87.4%	17.3%	19.6%	2273	8.53	19.0%	15.2%	23%	0.962	161
total	46210	2734	2791	98.0%	5.4%	5.5%	46187	48.19	5.5%	3.1%	83%	2.775	1220

A visual comparison

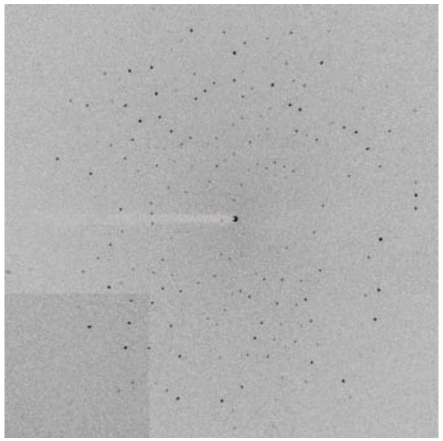


🡐 in air atmosphere (left)

helium atmosphere (right) 🡒



Diffraction images collected at 2.60 Å wavelength from a crystal of porcine pancreatic elastase at the same sample-detector distance (36 mm) and the same exposure time at the XRD1 beamline at Elettra.
Djinovic-Carugo et al., *J. Synchrotron Radiation*. (2005) Vol. 12, page 410-419.



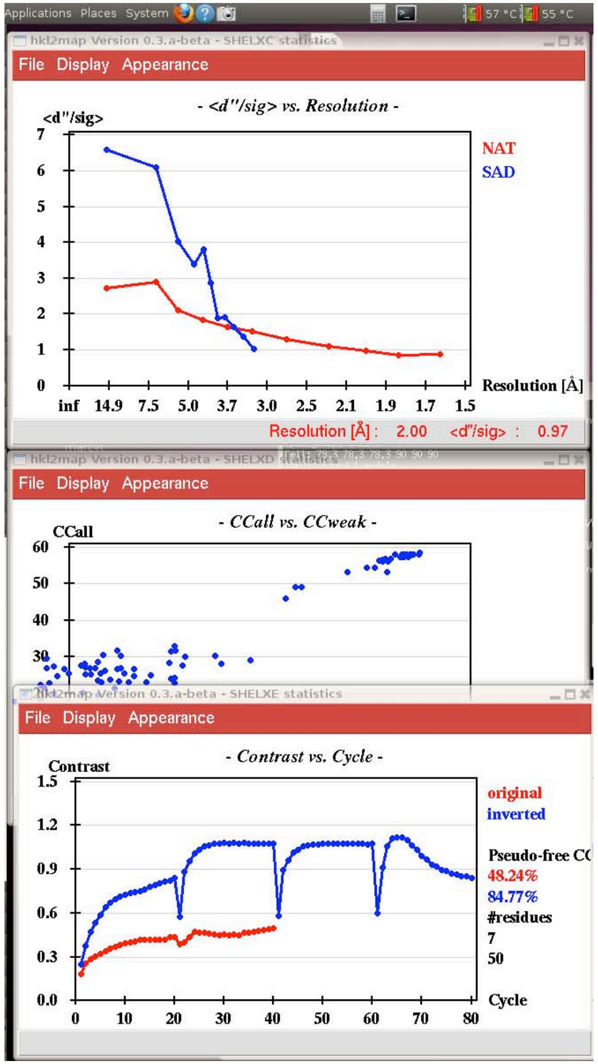
🡐 in air atmosphere (left)

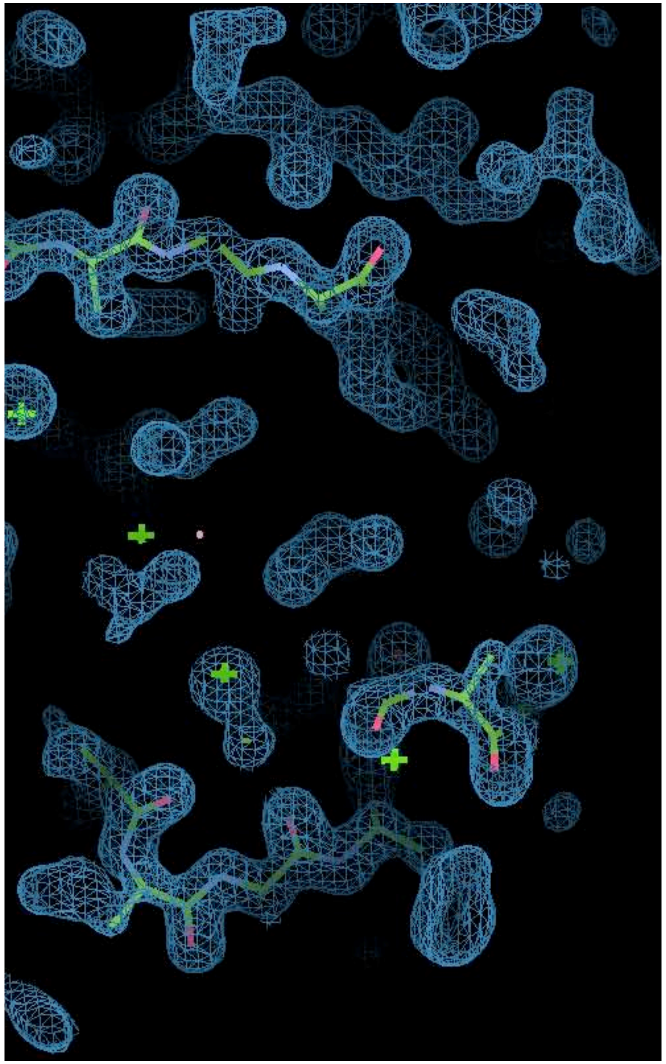
helium atmosphere (right) 🡒

?

Diffraction images collected at 2.69 Å wavelength from a crystal of insulin at the sample-detector distance (100 mm) at P13 beamline at Petra III.

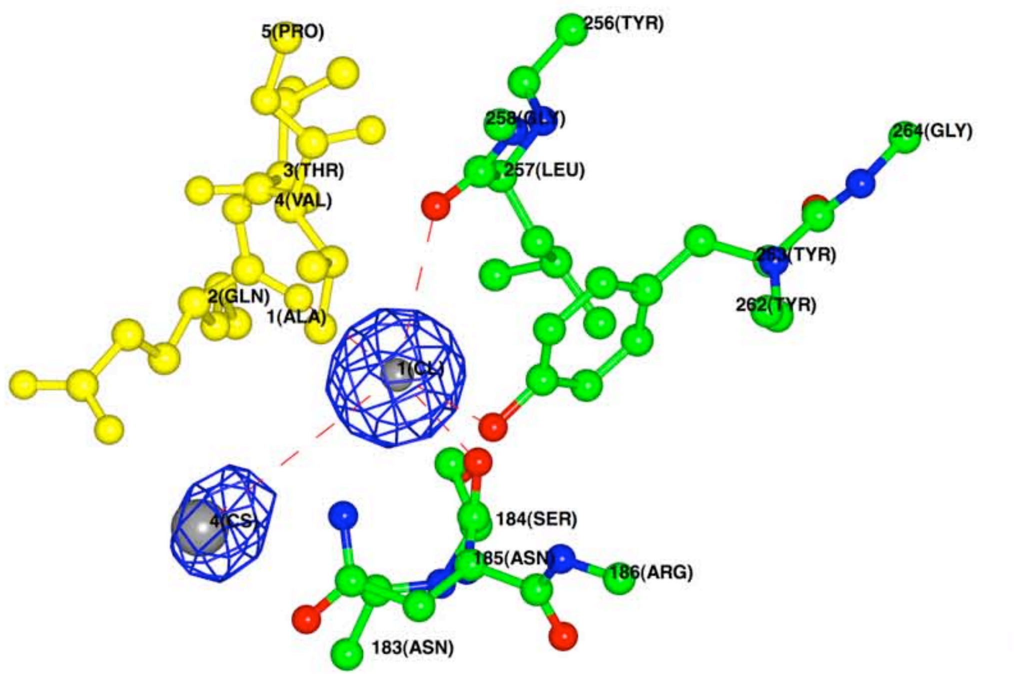
Phasing steps





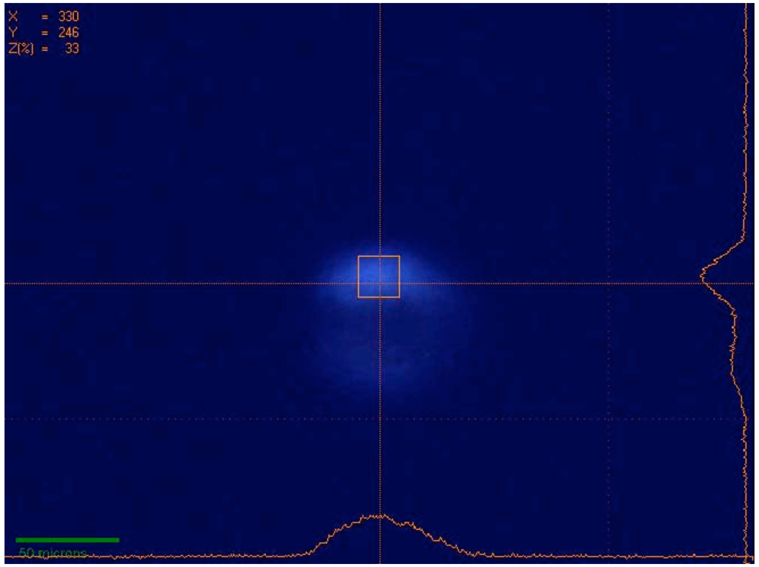
Energy range

(values quoted in eV)



Cl	K	Ca		Cs	Cs	Cs	Cr
k	k	k		L-III	L-II	L-I	K
2822	3607	4038	**4.6**	5012	5359	5713	5989

Small beam by slitting down the beam



λ = 0.984 Å
Beam size 20 x 20um^2 nominal

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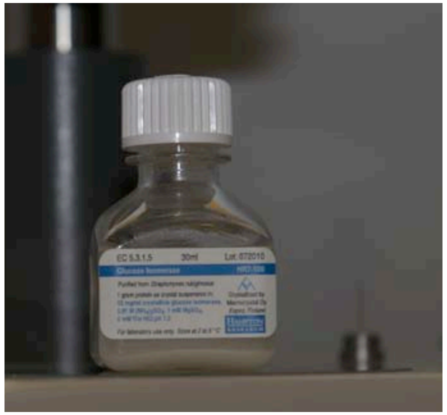
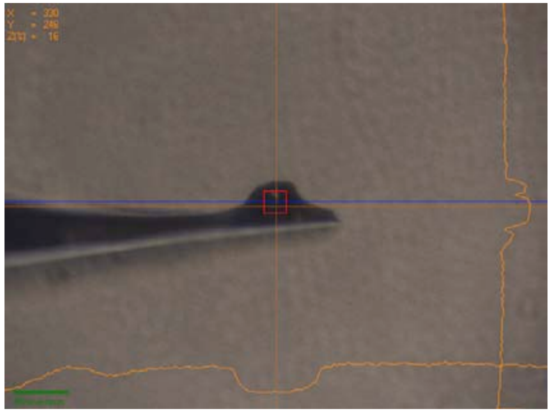
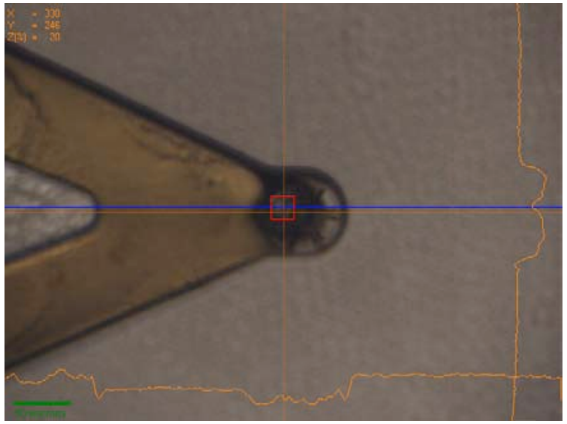
Small beam data collection on GI





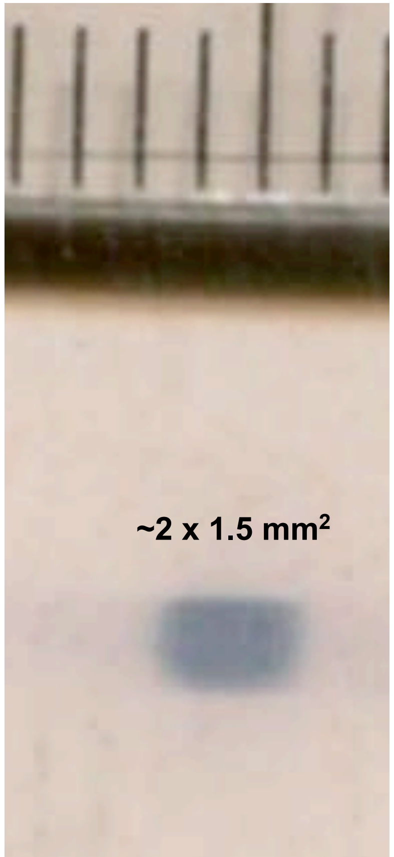


Crystal size ~ 30 x 30 x 30 um^3 , 90 degrees collected, Exposure time 15sec/degree

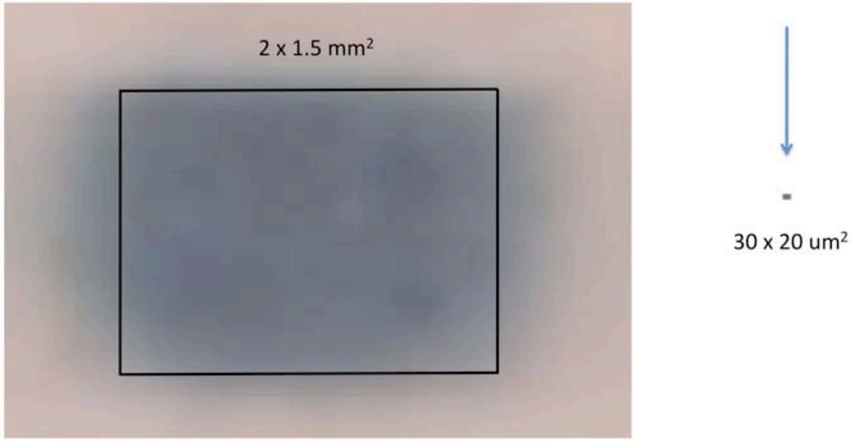
SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE >= -3.0 AS FUNCTION OF RESOLUTION

RESOLUTION LIMIT	NUMBER OF REFLECTIONS OBSERVED	UNIQUE	POSSIBLE	COMPLETENESS OF DATA	R-FACTOR observed	R-FACTOR expected	COMPARED	I/SIGMA	R-meas	Rmrgd-F	Anomal Corr	SigAno	Nano
6.40	4249	935	1047	89.3%	5.5%	5.7%	4214	21.82	6.3%	5.2%	-11%	0.751	644
4.54	7938	1685	1806	93.3%	7.7%	7.5%	7876	18.33	8.7%	7.0%	-7%	0.793	1221
3.71	9997	2128	2249	94.6%	8.0%	7.4%	9932	19.40	9.1%	7.9%	-5%	0.856	1534
3.21	11992	2552	2669	95.6%	10.7%	10.3%	11929	14.95	12.2%	11.5%	-2%	0.835	1822
2.87	13526	2898	2993	96.8%	17.0%	16.9%	13454	10.08	19.4%	19.9%	-4%	0.777	2052
2.62	14952	3223	3303	97.6%	23.2%	23.5%	14868	7.79	26.5%	24.9%	-3%	0.782	2263
2.43	16219	3509	3581	98.0%	31.4%	32.2%	16146	5.90	35.9%	35.6%	-4%	0.764	2441
2.27	17333	3775	3829	98.6%	37.5%	38.1%	17246	4.97	42.8%	44.3%	-4%	0.758	2598
2.14	17747	3966	4097	96.8%	45.3%	45.4%	17606	3.94	51.7%	55.4%	-8%	0.735	2617
total	113953	24671	25574	96.5%	17.1%	17.0%	113271	9.73	19.5%	23.2%	-5%	0.780	17192

Beam Size at 56.25m from the U29



Beam Size with KB mirrors



EMBL

What next?

Instrumentation:

focus size with KB system down to 20 um
New detector support with 2theta swing

Goniometry:

Mini Kappa - commissioning

X-ray fluorescence detector:

Amptek 123 - installation commissioning

Extended energy range:

Extend to softer x-rays (2.7 – 3.0 Å) for experiment (how do we measure the flux?)
Helium cone

Tunability:

17-5 for routine experiments

Summary and Future outlook:

application	parameter	MX1
Highly brilliant and highly stable beam,	**Main purpose**	Petra III, 3(1/2)rd generation light source, low emittance 1 nmrad
Wide range tunability, broad spectrum of experimental phasing methods *in crystallo* spectroscopy	**Energy range**	5(4)–17 keV
Wide range tunability *in crystallo* spectroscopy	**Bandpass $\Delta E/E$**	Si(111) - $2*10^{-4}$ Si(311) - $5*10^{-5}$
Matching the beam to the size of the crystal	**Focus H / V**	28 x 13 μm (10-5 μm with collimation) (100 μm defocussed)
Large unit cells, low mosaicity crystals	**Divergence H x V**	0.2 mrad x 0.15 mrad
Fast data collection, small beam	**Intensity, ph/s**	$1*10^{13}$ – $3*10^{13}$ ph/s

MX1 is not only a beam line for soft x-rays… low divergence, small beam size and high photon flux will allow to collect data at any energy from small samples with large unit cells.

Thanks

- Michele Cianci (MX1)
  Gleb Bourenkov (MX2)
  Manfred Roessle (BioSAXS)
- Stefan Fiedler & Team
- Christoph Hermes & Team
- Florent Cipriani & Team
- MAATEL

- Johannes Schmidt & Cyber Group
- Matthias Wilmanns
- Rob Meijers & Team
- Admin HH & HD
- Scientific Advisory Board
- Friendly Users

- DESY
- ESRF
- BMBF