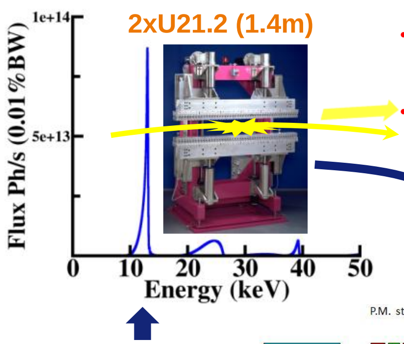
MASSIF-3: What can be done with a small beam and a very fast detector

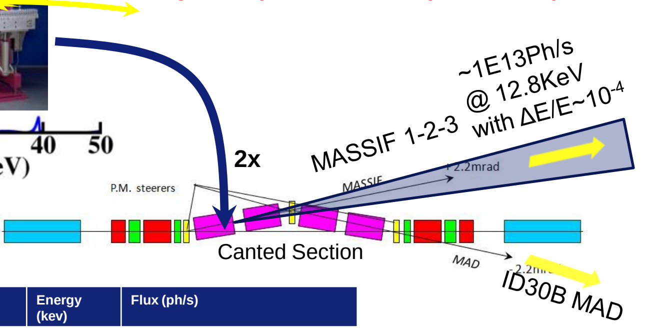
P. Carpentier, ESRF

ESRF Users Meeting 2015: Meeting of MX BAG Representatives and Beamline Staff,
9th February 2015

MASSIF-1-2-3 SOURCE

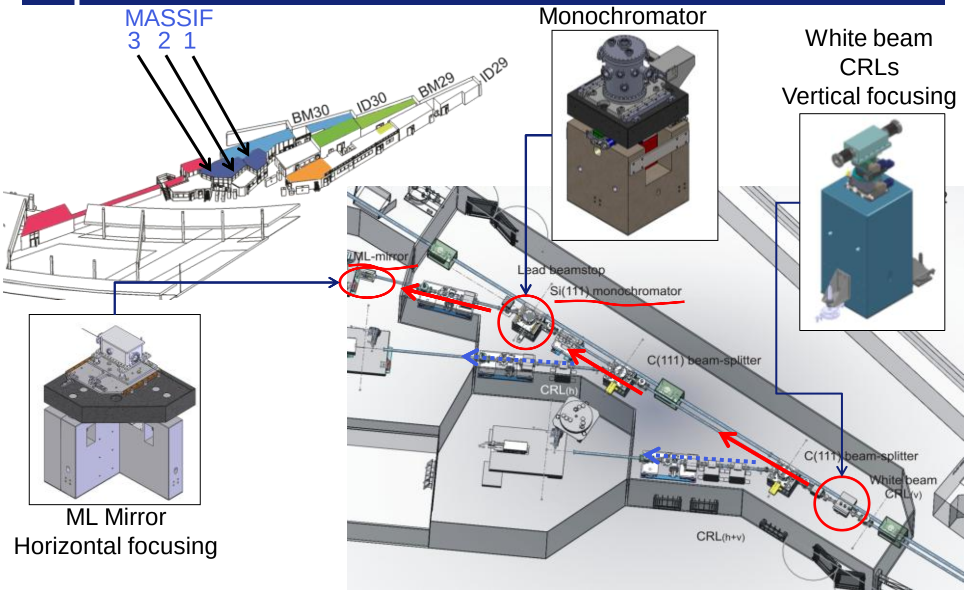


- Canted and Fixed E (no source tuning as ID23-2 ID30B MAD and MASSIF independent beamlines
- High Flux (@12.8keV ~ 10^{13}ph/s as ID29)



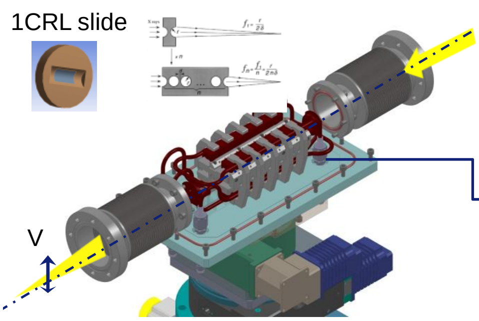
Beamline	Source	Energy (kev)	Flux (ph/s)
ID14-1/2	U23+U24.4+U35 (tandem ID14-4)	13.3	< 10^{12} for 0.005%BW
ID23-2	U20.2 (canted with ID23-1)	14.2	< 10^{12} for 0.01% BW
ID23-1	U35	MAD 6-20	~10^{12}
ID29	U35+ U21	MAD 6-20	~10^{13}
Massif	2xU21.2 (canted with ID30-B)	12.8	~10^{13} for 0.01%BW

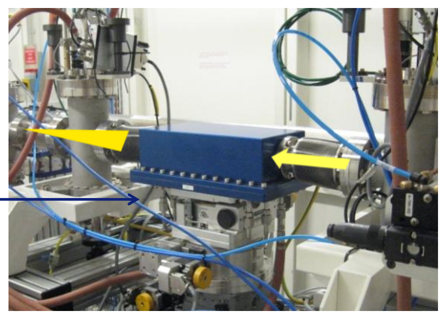
MASSIF-3, LOCATION, OPTICS, DESIGN

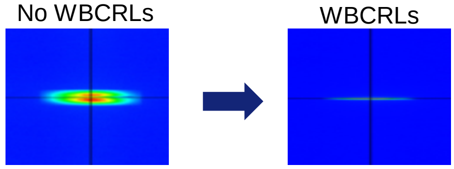


Simplicity, stability, reduce number of optical elements to adjust

MASSIF-3, H-FOCUSING WB-CRLS



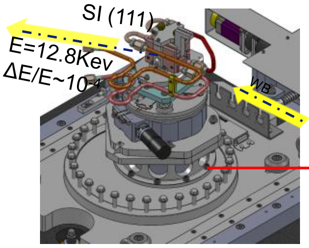


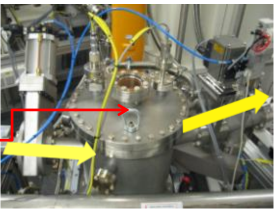


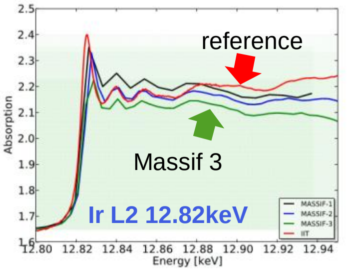
Focusing of beam @ the last beam viewer ~1.5m from the sample position

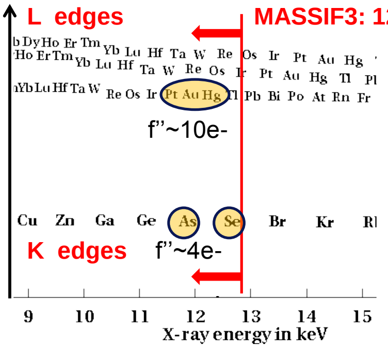
WBCRL, Extremely stable no need to be retuned
but vertical defocusing not possible

MASSIF-3 BEAM ENERGY





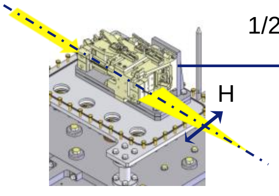


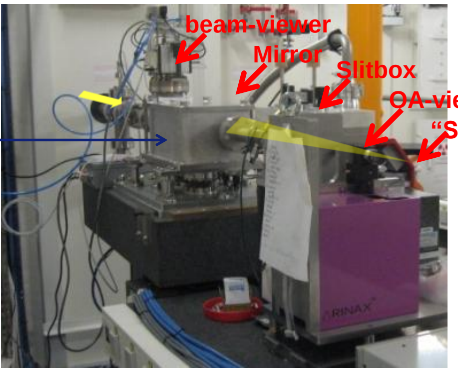


- Calibration of the Si (111) mono L2 abs edge of Ir foil @ 12.812keV (ΔE/E~10^{-4}), fast, easy and accurate ~1eV
- Stable, need to be controlled and eventually recalibrated ~each month
- Same concept as ex-ID14-1-2-3
  E > Absorption edge around 12 KeV widely use for phasing protein crystals
  L edges f'' ~ 10e- (as Pt, Au, Hg…)
  K edges f'' ~4e- (as Se,As…)

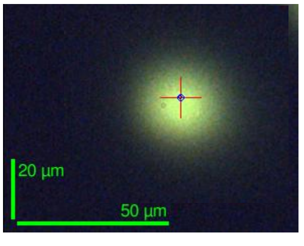
MASSIF-3, V-FOCUSING FOCAL SPOT

Setup for beam commissioning July 2014





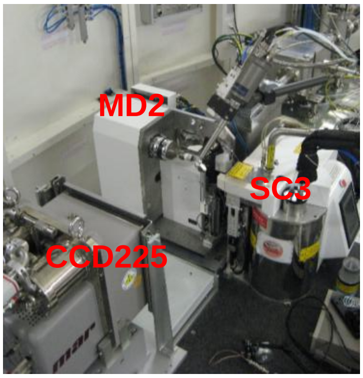
1/2 KB Mirror, horizontal focusing
Multilayer 170 x 30 mm^2
Si substrate + $[Ru/B_4C]_{70}$ layers



Beam @ sample position:
12.812KeV, ~15x15µm^2 ,~1.5x10^{13}ph/s
Position relatively stable
need to be check and eventually re-adjusted on the daily basis

MASSIF-3, EXPERIMENTAL SETUP

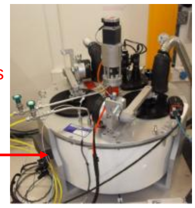
Current Setup

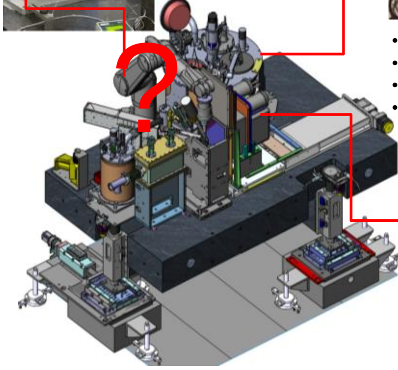


- minidiffractometer MD2 120º/1s
- sample changer SC3
- detector MarCCD 225
- **MxCube** + new control software ***e-motion***

Future Setup

- SOC: 2 µm < µbeam
- free rotation
- fast & accurate few rot/s
- shutterless collection
- Fast d2scan





- 240 SPINE samples
- 3 independent ports
- barcode reading
- unipuck potentiality

Eiger 4M (Feb 15)

MASSIF-3, DETECTOR FOR SMALL BEAM AND HIGH INTENSITY

	Marccd225	Marcd HS	Pilatus 2M	Pilatus 6M	Eiger 4M
Area	225mm x 225mm	225mm x 225mm	254mm x 289mm	424mm× 435mm	155mm × 163mm
Nb pixels	3072 x 3072	2880 x 2880	1475 × 1679	2463 × 2527	2070 × 2167
Pixel Size	73µm	78µm	170µm	170µm	75µm
Shutterless Frame rate	NA	10f/s	250/f/s (100)	100f/s	200f/s -> 750f/s
Readout	1s	1ms	0.95ms	0.95ms	3µs
Noise	$10e^-$/pixel + $0.01e^-$/pixel/s	$8e^-$/pixel + $0.003e^-$/pixel/s	None (0/1Ct)	None (0/1Ct)	None (0/1Ct)

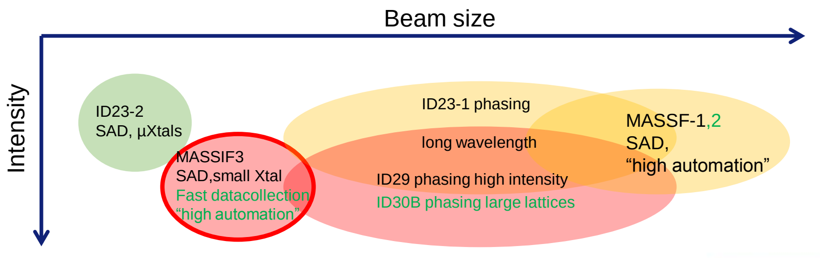
Good special resolution (large unit cell)
But read out noise

> 100Hz, 25% of photons are lost

- MARCCD 225 provisional detector
- Eiger 4M (end February 2015): small pixel size, no intrinsic noise, continuous reading, 200frames/s → 750 frames/s, but divergence + 4M → limitation for large unit-cells !

MASSIF-3, ESRF MX BEAMLINES

Beamline	Beam size h.v (µm)	Flux (Ph/s)	Energy (KeV)	Detector	
ESRF MASSIF3	15x15	1.5E13	12.8	Mar CCD 225, Eiger 4M	SAD Mini-focus fast detector Automation
ESRF MASSIF-1	100x100 (200-50)	2E12	12.8	Pilatus 2M	SAD Automation
ESRF ID23-2	7x5	1E12	14.2	Pilatus 2M	SAD Microfocus
ESRF MASSIF-2	100x100 (200-50)	2E12	12.8	?	SAD Automation
ESRF ID23-1	45x30	2E12	6-20	Pilatus 6M	MAD
ESRF ID29	50-30	1E13	6-20	Pilatus 6M	MAD High flux
ESRF ID30-B	200-20	1E13	6-20	Pilatus 6M	MAD Low divergence



MASSIF-3 VS MINI- MICRO-FOCUS BEAMLINES

Beamline	Beam size h.v (µm)	Flux (Ph/s)	Detector
ESRF MASSIF3 Fixed λ	15x15	1.5E13	Mar CCD 225, Eiger 4M
ESRF ID23-2	7x5	1E12	Pilatus 2M
SLS PX1 MAD	15x5	2E12	Pilatus 6M
APS 23ID MAD	5x5, 1x1	5E10, 3E9	Pilatus 6M
APS 23IDE MAD	5-10-20	1E13	Pilatus 6M
APS 24IDE MAD	5-100	2E12	CCD ADSC 315
DLS I24 MAD	5x5	1E12	Pilatus 6M
Spring8 BL32XU MAD	1x1, 10x10	6E10, 4E12	CCD Rayonix 225
Soleil PX2 MAD	5x10	1E12	CCD ADSC 315
PETRA III MX2 MAD	1x5	5E12	Pilatus 6M
Australian MX2 MAD	20x10	1E12	CCD ADSC 315

+ projects …

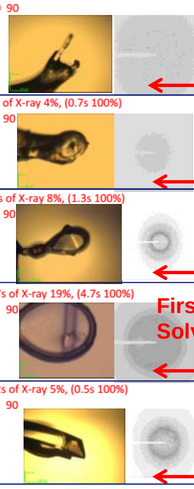
- Few micro- mini-focus beamlines for MX (but increasing number)
- Massif 3: high flux (1E13Ph/s), intermediate focusing (micro/mini-focus)
- ESRF Fixed λ, simplicity, stability, (MASSIF-3) Se-edge
- Fast detector 200Hz (1 month), 750Hz (in 6 months)
- Second generation of robotics for samples to come
- Upgrade phase II, flux increase, h-beam size << 10µm

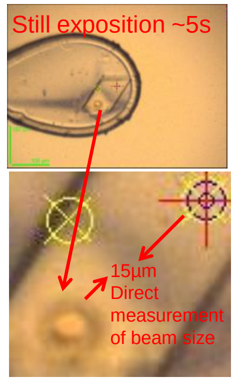
OPERATION, DATA-COLLECTIONS ON MASSIF-3

- **54 shifts 01-08-2014 to 28-02-2015, 152 shifts 1-03-2015 to 31-07-2015**
- **1st structure solved, Lysozyme 1.4Å, 24/11/14**
- **1st official user, EMBL MX1633, 11/12/14**

Some examples of in-house datacollections XDS statistics

	RESOL.	COMPL.	R-FACT	I/SIGMA	SigAno	Equivalent collection time, X-ray 100%
FAE-SeMet P212121	0.1s/frame, 224 frames, 0.6deg, 22.4s of X-ray 10%, (2.24s 100%) UNIT CELL 64.5 107.9 112.4 90 90 90					
	8.29	98.00%	3.80%	23.79	1.967	
	...	...	...	...	...	
	2.8	98.40%	48.80%	2.25	0.789	
	total	99.30%	13.90%	8.12	0.999	2.2s of X-ray 100%
Thaumatin P41212	0.1s/frame, 76 frames, 1.45deg, 7.6s of X-ray 4%, (0.7s 100%) UNIT CELL 57.0 57.0 150.9 90 90 90					
	8.6	99.60%	5.00%	27.78		
	...	...	...	...		
	2.91	99.90%	48.40%	5.2		
	total	99.90%	14.10%	14.65		0.7s of X-ray 100%
ProtK P43212	0.1s/frame,160 frames, 0.95 deg, 16s of X-ray 8%, (1.3s 100%) UNIT CELL 67.5 67.5 101.1 90 90 90					
	3	99.20%	3.60%	54.57		
	...	...	...	...		
	1	50.50%	23.00%	3.05		
	total	91.40%	5.40%	24.2		1.3s of X-ray 100%
New strcuture CMD P41212	0.1s/frame,237 frames, 0.4 deg, 23.7s of X-ray 19%, (4.7s 100%) UNIT CELL 89.3 89.3 141.3 90 90 90					First new structure Solved CMD 16/12/14
	7.84	99.50%	2.60%	38.68		
	...	...	...	...		
	2.64	98.10%	55.10%	2.11		
	total	99.60%	7.20%	13.26		"4.4s" of X-ray 100%
trypsin P41212	0.1s/frame,102 frames, 1.1 deg, 10.2s of X-ray 5%, (0.5s 100%) UNIT CELL 89.3 89.3 141.3 90 90 90					
	4.48	99.20%	2.40%	44.98		
	...	...	...	...		
	1.5	95.40%	67.50%	2.03		
	total	98.90%	5.90%	16.11		0.5s of X-ray 100%

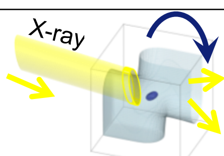
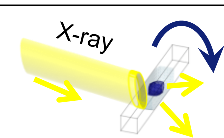
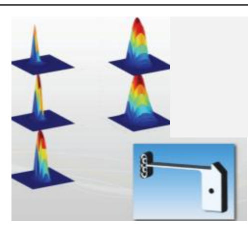
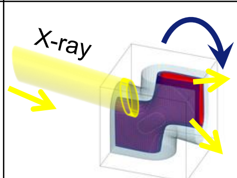
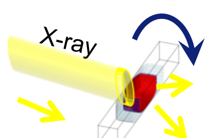




Full X-ray beam datacollections time 0.2-3s

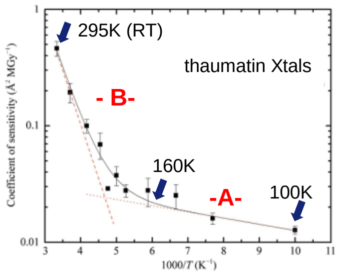
DOSE RATE ON MASSIF-3

Typical protein crystals, different sizes, Datacollections over 100°, during 1, 5s,
In 15x15 µm² X-ray beam @ 1.5x10¹³ph/s, Dose isosurfaces @ 0.1, 20 and 30 MGy.

RADDOSE 3D	100x100x100µm³	10x10x100µm³	Future apertures
1s	 ADW Dose: ~5MGy	 ADW Dose:~12MGy	
5s	 ADW Dose: ~25MGy	 ADW Dose:~60MGy	Aperture, cleaning or resizing the beam down to 5µm (Gaussian ~1x10¹²Ph/s) Complementarily with future ID23-2, 1µm beam

Datacollection time 0.2-3s (750 frames/s of 0.1°) with 100% X-ray beam on Massif-3 ⟶ take full advantage of the Eiger detector

RADIATIONS DAMAGE 100K, RT

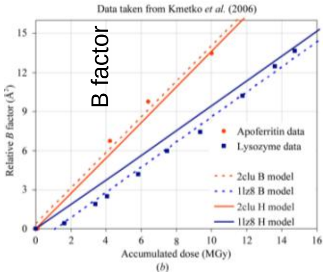


100K → 295K Radiation damage sensitivity 100x
Warkentin *et al* (2010) Acta Cryst D

-A- at cryogenic temperature e⁻ highly mobile, e⁻ diffusion mainly responsible damages 100K → 160K

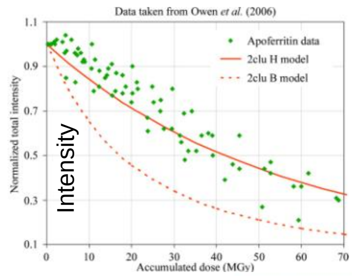
- B- T > 160K OH• become mobile, OH• diffusion responsible for RT damages Owen et al (2012) Acta Cryst D

100K → Native (N) —K1→ Disordered (D) —K2→ Amorphous (A)

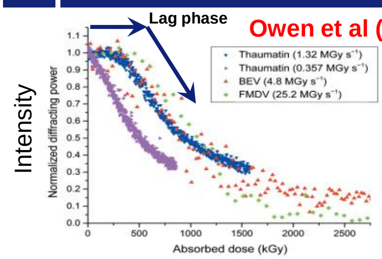


100K

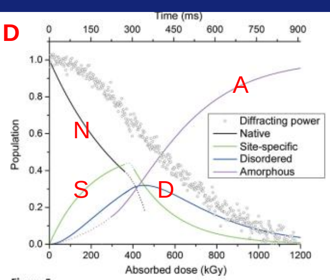
$$I = I_0 \exp\left(-\frac{B\sin^2\theta}{\lambda^2}\right)$$



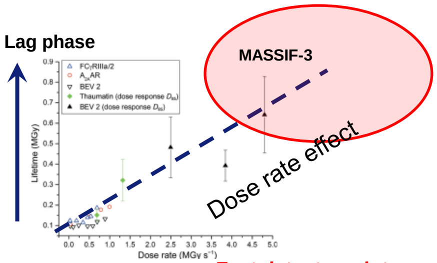
ROOM TEMPERATURE RADIATION DAMAGES



Owen et al (2014) Acta Cryst D



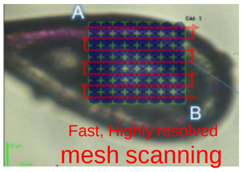
Native (N) —K0→ Site damage (S) —K1→ Disordered (D) —K2→ Amorphous (A)

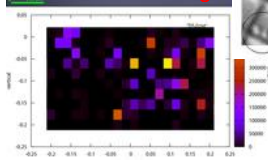


Room temperature using Humidity controller HC1

Fast detector + intense beam on MASSIF-3:
Data-collection time 0.2-3s, dose rate 10 Mgy/s → outrunning radiation damages at RT feasible

DATACOLLECTION OF MULTIPLE MICROCRYSTALS



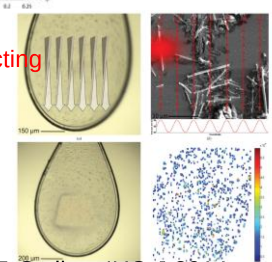


- Intense micro-beam = 15µm beam,10^{13}ph/s
- Eiger detector ~ 750 frame/s
- diffractometer ~ fast 2D displacement
- Computing images ?

- Fast and accurate 2D mesh scanning (few µm size Xtals, 100µmx100µm mesh, 10000frames in <1 minutes)
- Locate multiple micro-Xtals in the loop
- Collect a wedge of few degrees on each Xtal
- Merging and processing multiple wedges

collecting

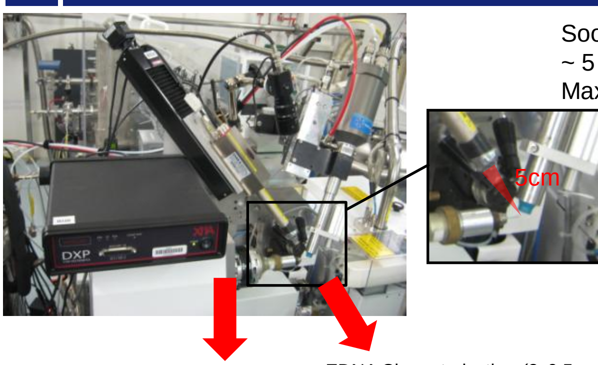
RT serial in Xtal dops



F. Stellato IUCrJ 2014

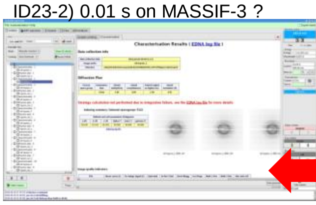
MASSIF-3 small intense beam and fast detector ideal for the development of cryogenic and room temperature serial crystallography (talk U. Zander)

X-RAY FLUORESCENCE CHARACTERIZATION

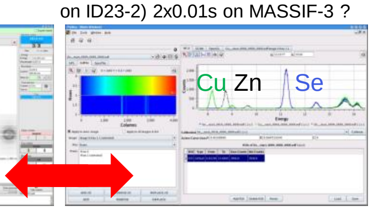


Soon: XRF: Xia Mercury + Ketek
~ 5 cm from the sample
Max rate 0.01s / spectrum

XRF Characterization (0.5s on ID23-2) 0.01 s on MASSIF-3 ?

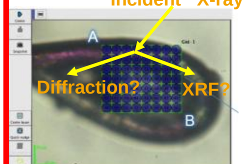


EDNA Characterization (2x0.5s on ID23-2) 2x0.01s on MASSIF-3 ?



Diffraction + XRF Simultaneous collection

Fast XRF detector
+
Fast X-ray detector

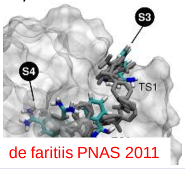


Simultaneously Identify Xtals and potential heavy atoms (2 mappings)

TIME RESOLVED CRYSTALLOGRAPHY

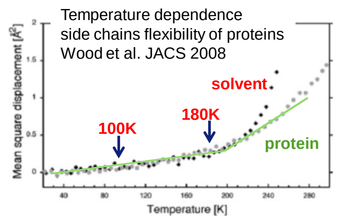
Enzymes, function mechanism

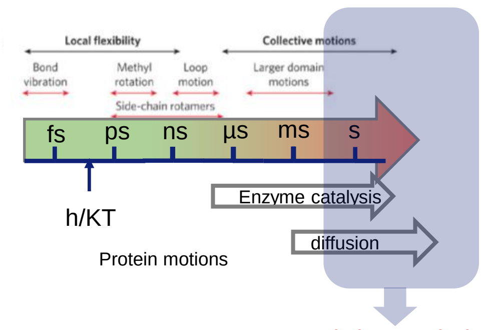
Dynamics



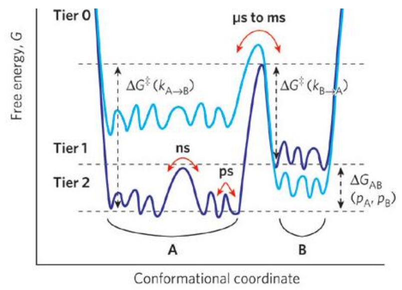
de faritiis PNAS 2011

Structures





K. Henzler-Wildman & D. Kern Nature 2007



Structural change during protein functioning

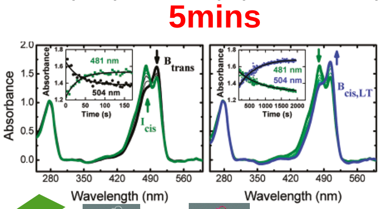
Time domain sub-"1s" accessible by crystallography using Eiger detector ?

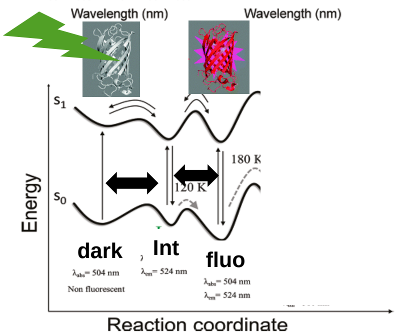
TIME RESOLVED CRYSTALLOGRAPHY IN THE PA-FP PADRON

532nm activation

Dark(trans) ⟷ **Int**(cis) ⟷ **Fluo**(cis)

5mins





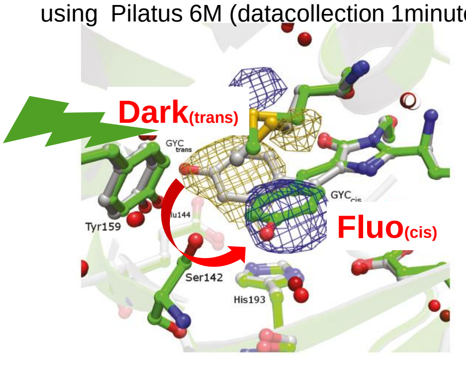
Faro at al. JACS 2011

Life time of intermediate I_{cis} ~ 5mins at 100K

Time resolved crystallography

Required to reveal the structure

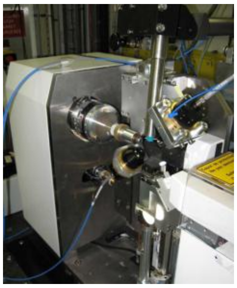
using Pilatus 6M (datacollection 1minute)



Time resolved crystallography using Eiger detector a step forward to the 1s time domain, for wider range of applications at 100K and RT.

ON-LINE MICRO-SPECTROPHOTOMETRY ON MICROCRYSTALS ?

Microspectrophotometer on MASSIF3/MD2 ?



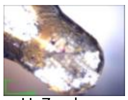
- **For spectrocopic measurements:**
  VM ~ 2Å/Da , 1000mg/ml
  10 µm Absorption ~ 2.5 at 280nm (lysozyme xtals) → absorption spectra
  Microcrystals (thin plates, needles) are perfect.

- **For diffration experiments:**
  Micro-crystals requires high doses (**ADWD ~ 10MGy**)
  for collecting complete datasets. Produce unwanted radiation induced structural/spectroscopic modifications.
  Microcrystals are problematic.

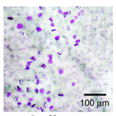
- **Solution: distribute the radiation damages over needles, plates or multiple µ-crystals ?**
  Composite datasets, sub-wedges of 10°, or helical datasets
  apparent ADWD of datasets **~1MGy**

A. Royant ,
CFP thin needle Xtal,
Cryobench ESRF

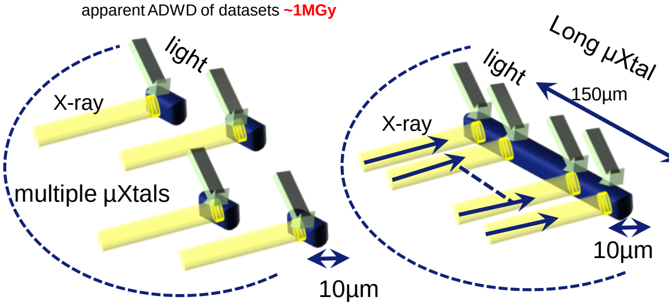
Multiple BR µXtals,



U. Zander



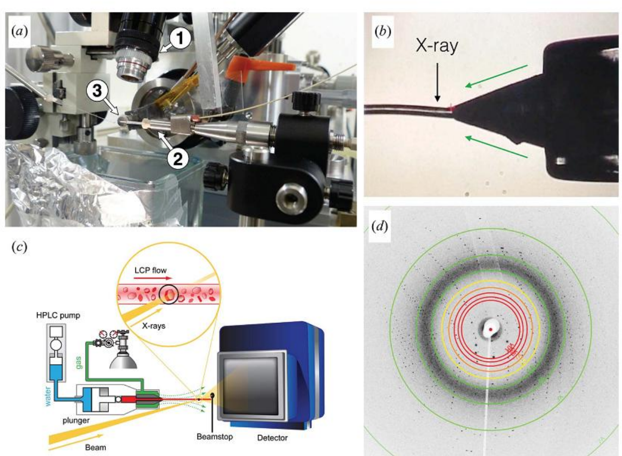
M. Caffrey ,
Faraday Discuss., 2007



Few 10 µXtals, dadatsets of 10°,
time resolved crystallography 100 ms time domain?

TIME RESOLVED CRYSTALLOGRAPHY MILLISECOND TIME SCALE

Small beam fast detector on ID13:
The ultimate experimental setup on ID13, P. Nogly et al. IUCrJ December 2014
Injector, 10000 x µXtals, 1 frame for some crystals
time resolved crystallography 1-10ms, limit for the Eiger detector 4M



SUMMARY

- MASSIF-3, fixed wavelength (12.81keV near Se edge), stable micro-focus beam 15µm, high flux (10^{13} ph/s) Minidiffractometer + sample changer SC3 + MARCCD detector, users mode since 11/12/14 for classical µ-crystallography
- Fast detector Eiger 4M, end of April 2015 (200frames/s ⟶ 750frames/s)
- 2nd generation of robotics to be decided …
- Setup will open new experimental opportunities:
  - ⟶ Fast datacollections, screening/evaluation at high rate
  - ⟶ Room temperature crystallography
  - ⟶ Serial crystallography
  - ⟶ Time resolved crystallography

PEOPLE INVOLVED

Gordon Leonard , Christoph Mueller-Dieckmann, Sean McSweeney (now NSLS-II)

Matthew Bowler	Antonia Beteva	David von Stetten
Carole Clavel	Hugo Caserotto	John Surr
David Flot	Fabien Dobias	Julien Soudarin
Nicolas Guichard	Thierry Giraud	Pascal Theveneau
Gordon Leonard	Matias Guijarro	
Andrew McCarthy	Mario Lentini	
Didier Nurizzo	Werner Schmid	

And many others …