MX applications at a low-emittance PETRAIII ring

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EMBL-Hamburg

14.06.2017

Round beams workshop SOLEIL

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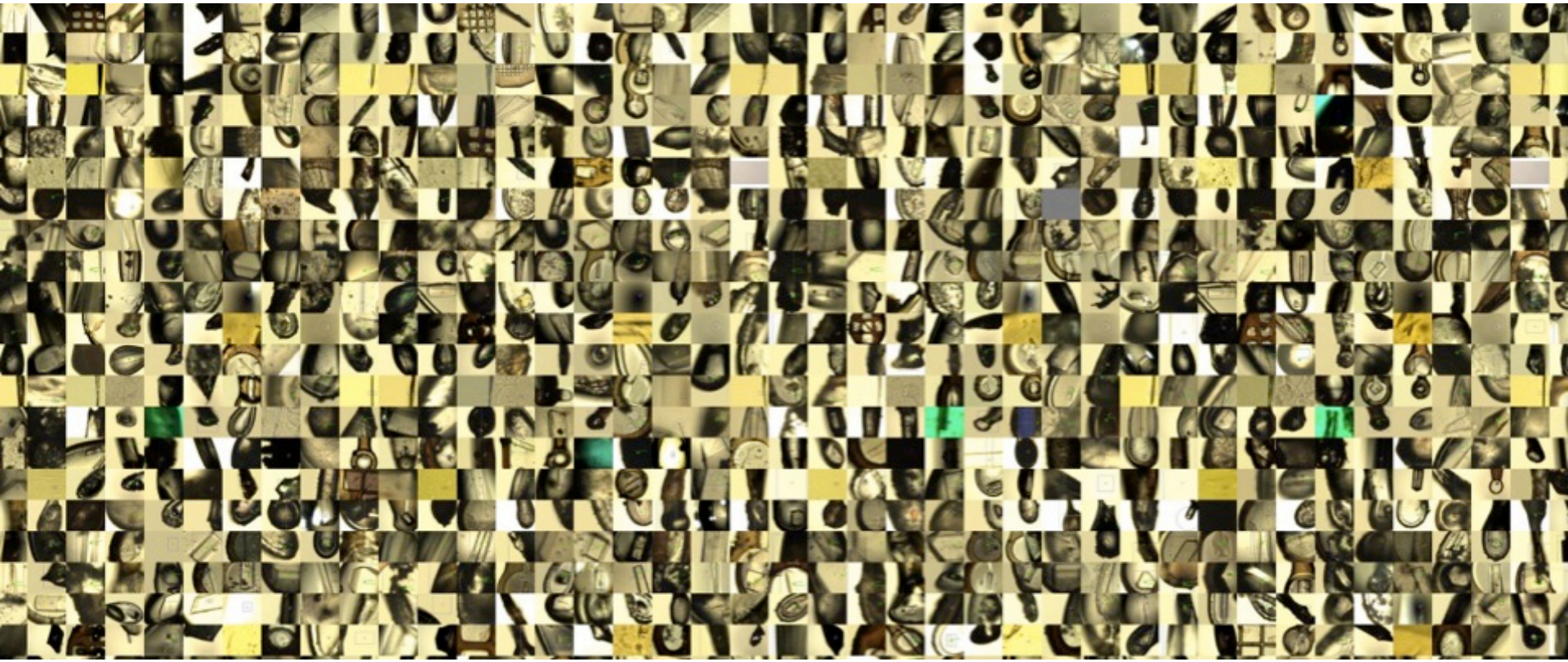
Macromolecular crystal diversity



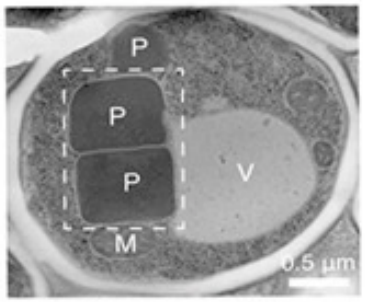
Synchrotron beams are set up to match:

linear dimensions and shapes

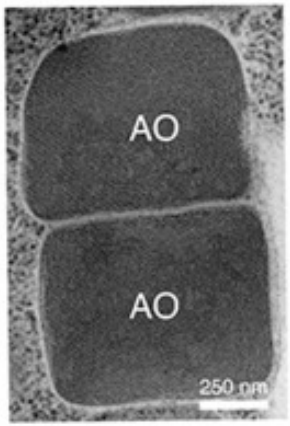
angular dimensions/spacing of Bragg diffraction maxima

while tolerable time of experiment is a few munutes per sample

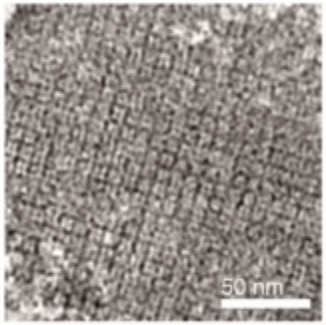
Crossing the length scales



(a)

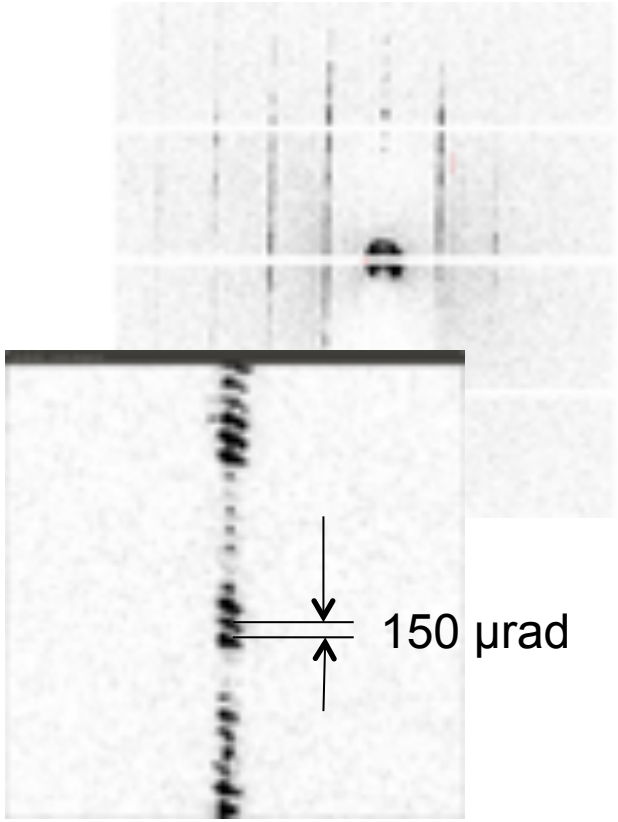


(b)



Alcohol Oxidase crystals in yeast peroxisome
Jakobi et al. IUCrJ. 2016 3, 88–95.

Space group $I2_13$
Period a=230 Å



Space group $P6_{1?5}$
Period c = 5520 Å

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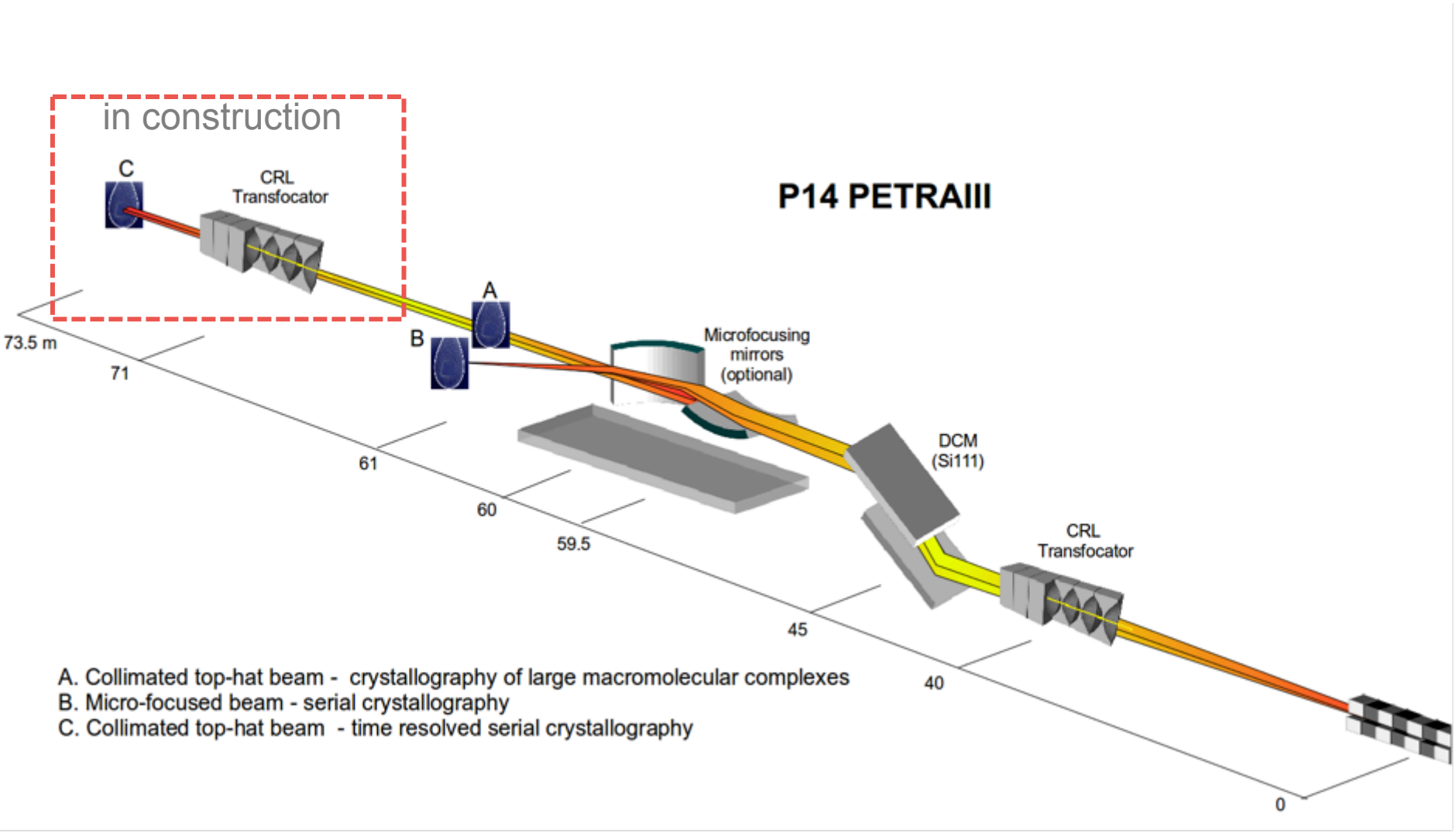
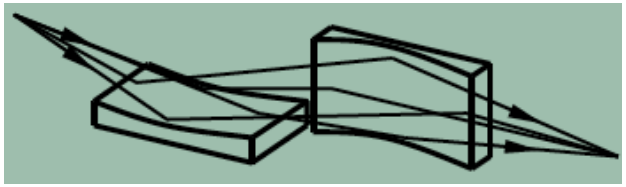
P14 Schema


in construction
C
CRL
Transfocator
P14 PETRAIII
A
B
Microfocusing
mirrors
(optional)
DCM
(Si111)
CRL
Transfocator
73.5 m
71
61
60
59.5
45
40
0
A. Collimated top-hat beam - crystallography of large macromolecular complexes
B. Micro-focused beam - serial crystallography
C. Collimated top-hat beam - time resolved serial crystallography

Beamline P14 – microfocus mode



- FWHM Source
- Horizontal

  300 µm * 17 µrad
- Vertical

  12 µm (on a paper) x 5 µrad

  apparent V source size

  20 to 30 µm

  according to CRL(2:1)@10ms

- FWHM Focus (60:1)
- Horizontal

  7 µm * 1200 µrad

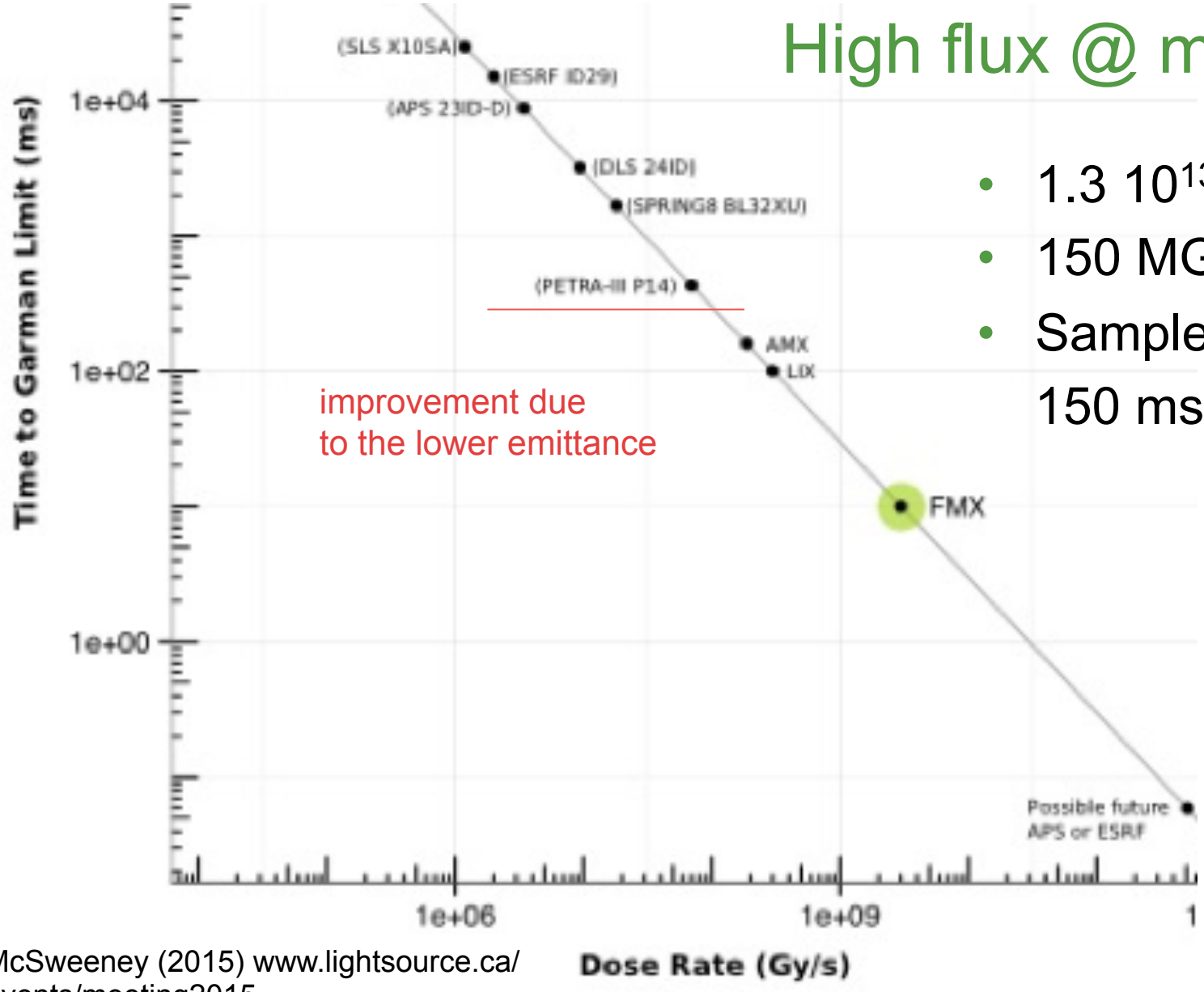
  emittance x 1.6

- Vertical (46:1)

  5 µm x 500 µrad

  <u>emittance x 40</u>

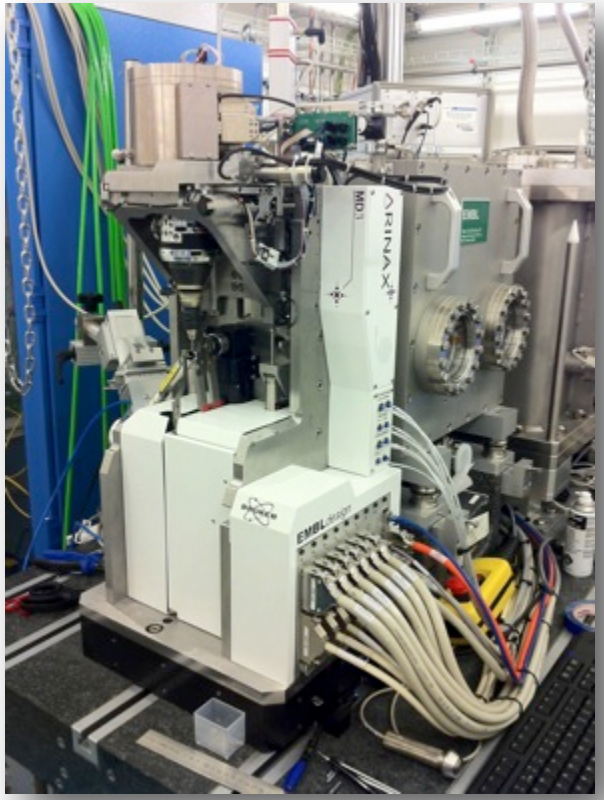
High flux @ micorfocus



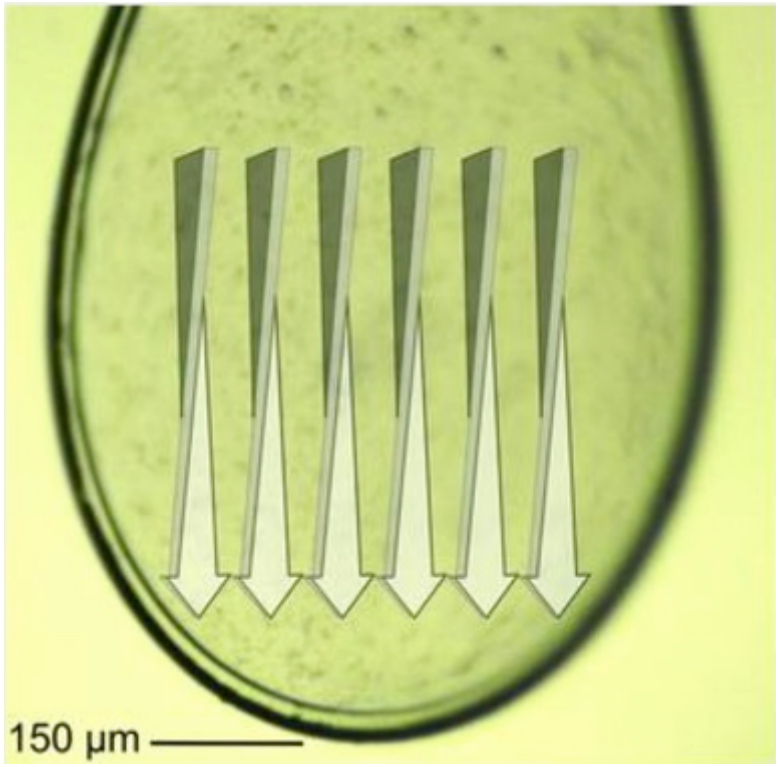
- 1.3 10^{13} Ph/second
- 150 MGy / sec
- Sample life time
  150 ms

McSweeney (2015) www.lightsource.ca/events/meeting2015

Serial Synchrotron Crystallography (2013)



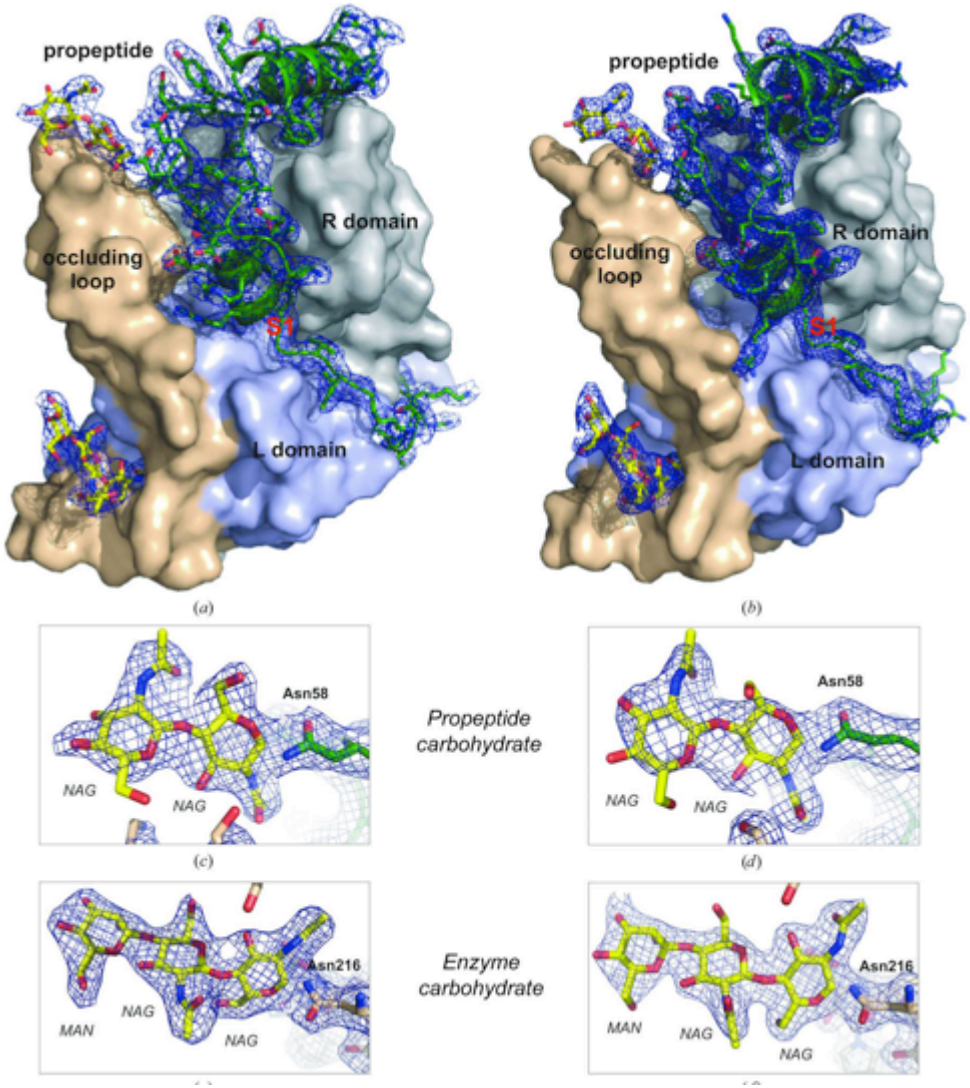
Presentation of frozen suspension of micro-crystals to a micro-beam using precision diffractomter

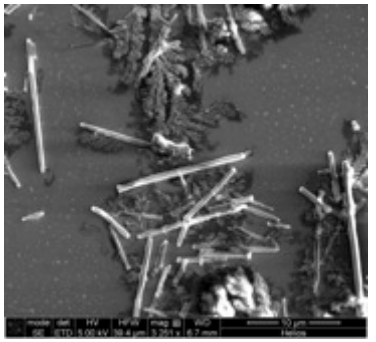


Data collection using **seialized helical scans**

PETRA III

XFEL





PETRA III	XFEL
Crystal Size	
10^7 unit cells [10 x less than smallest crystals used at synchrotrons before]	
Resolution	
2.8 Å	2.1 Å
Material used	
15 nl	10 ml
Result	
Models are identical within error	

Gati, Bourenkov, … Schneider, Redecke, Chapman (2014) IUCrJ 1:87

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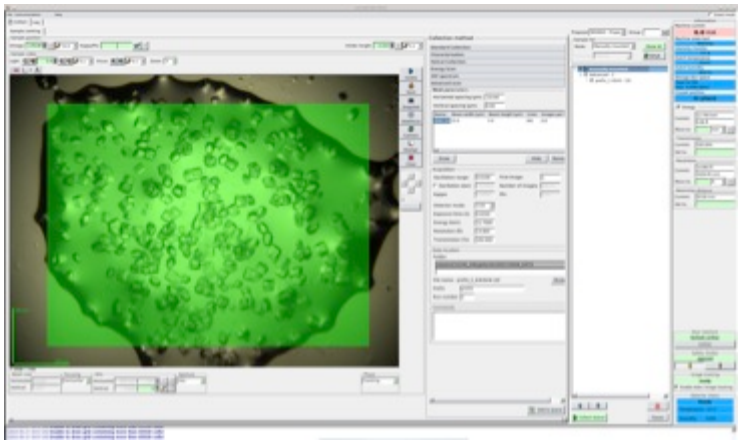
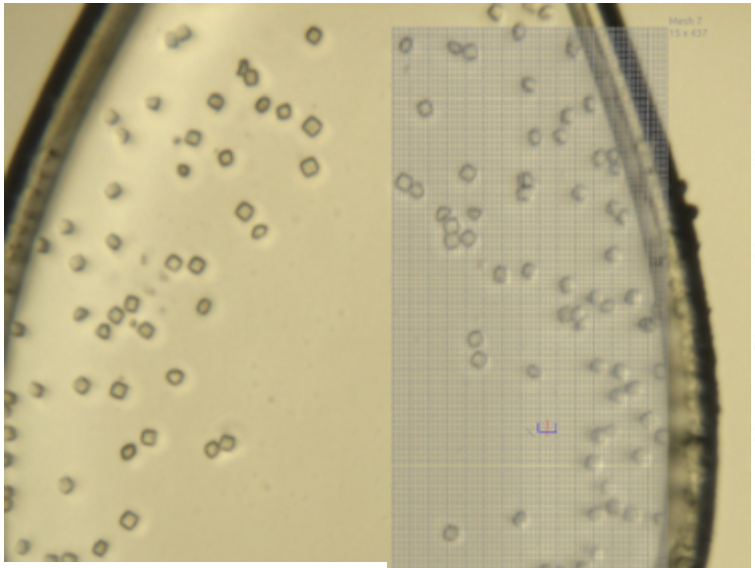
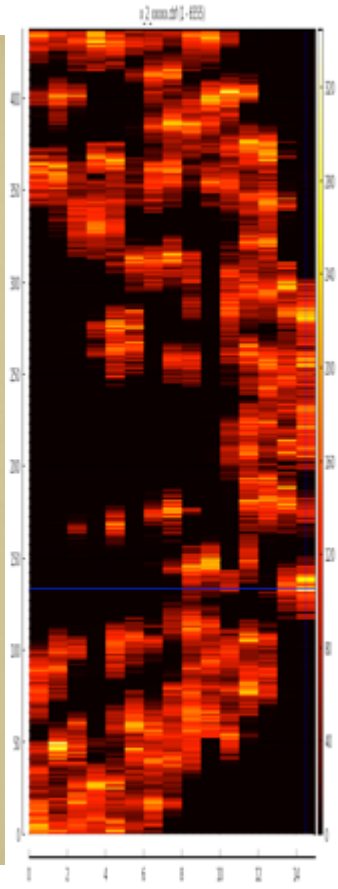
User-mode serial crystallography in crystralline suspensions: automated experiment in 3 min

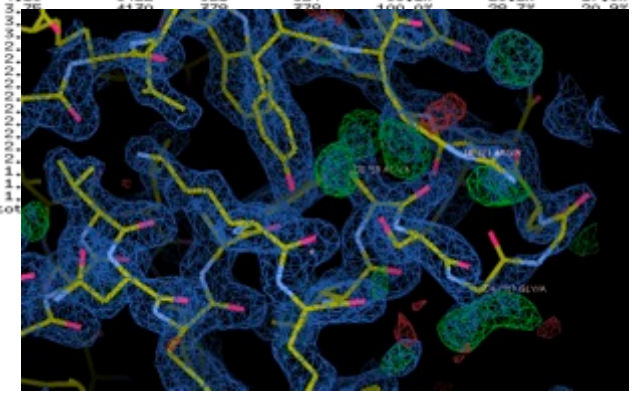






SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE >= -3.0 AS FUNCTION OF RESOLUTION

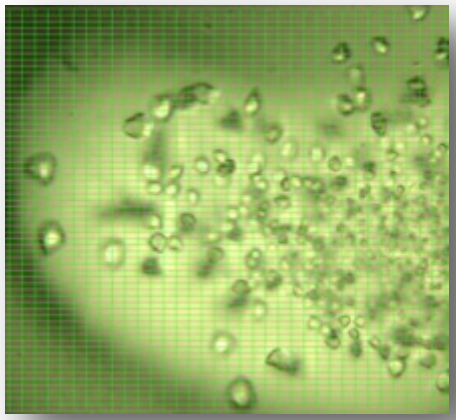
RESOLUTION LIMIT	NUMBER OF REFLECTIONS OBSERVED	UNIQUE	POSSIBLE	COMPLETENESS OF DATA	R-FACTOR observed	R-FACTOR expected	COMPARED	I/SIGMA	R-meas	CC(1/2)	Anomal Corr	SigAno	Nano
8.38	1202	145	149	97.3%	15.8%	12.1%	1195	18.17	16.8%	98.9*	6	1.245	58
5.93	2160	236	236	100.0%	24.0%	17.3%	2157	14.64	25.4%	96.6*	-6	1.005	135
4.84	3060	290	292	99.3%	26.2%	19.4%	3059	15.50	27.6%	97.7*	0	1.065	187
4.19	3422	321	324	99.1%	25.2%	17.8%	3416	15.72	26.5%	97.9*	3	1.095	225
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	4123	15.08	30.1%	98.3*	-3	1.057	268
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	4482	13.40	34.6%	97.6*	7	1.053	292
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	4838	11.15	41.6%	96.4*	6	0.907	337
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	5007	8.54	49.8%	91.8*	-3	0.846	354
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	5390	7.36	59.6%	93.9*	-4	0.761	377
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	5657	6.20	72.4%	91.9*	2	0.782	391
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	6078	5.31	87.7%	91.0*	2	0.704	438
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	6359	4.98	95.8%	88.1*	-4	0.701	442
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	6680	4.15	110.7%	82.8*	11	0.700	485
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	6923	3.60	129.6%	80.9*	4	0.674	496
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	7108	2.97	161.8%	83.6*	0	0.630	500
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	7291	2.67	182.9%	65.5*	0	0.632	526
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	7400	2.14	236.6%	61.9*	0	0.650	546
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	7501	1.72	248.6%	52.3*	6	0.630	555
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	5863	1.31	333.0%	33.1*	11	0.620	503
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	2632	0.87	372.2%	4.2	4	0.585	208
tot	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	103219	6.10	59.8%	97.3*	2	0.755	7323

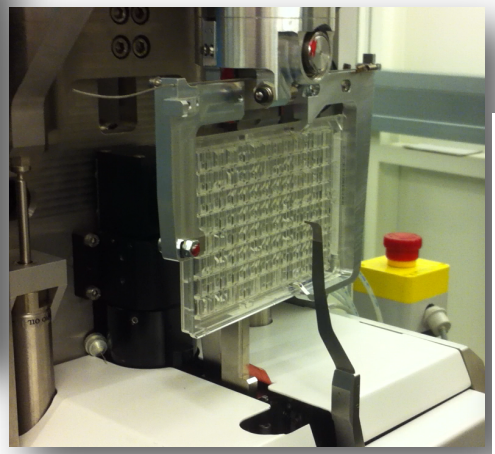


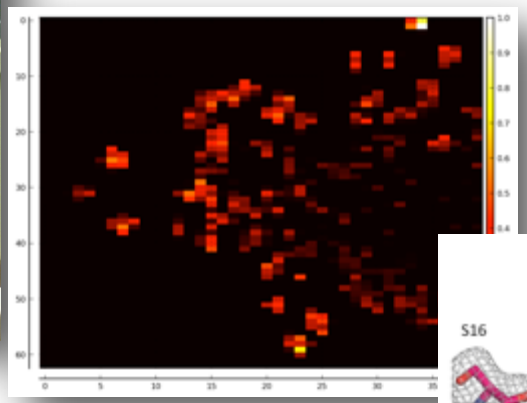
Definition of the R.O.I. and scan parameters

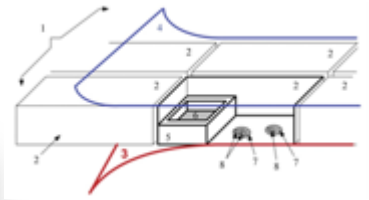
Heat plot for diffraction score generated in real-time

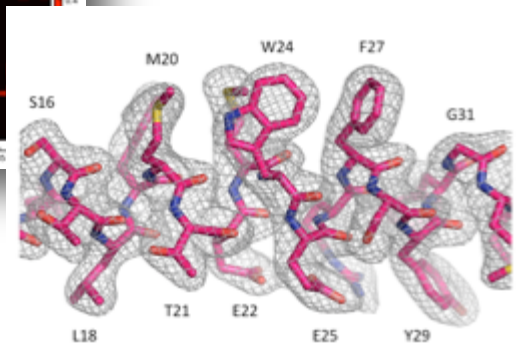
Serial crystallography *in-situ, mesophase crystallization*

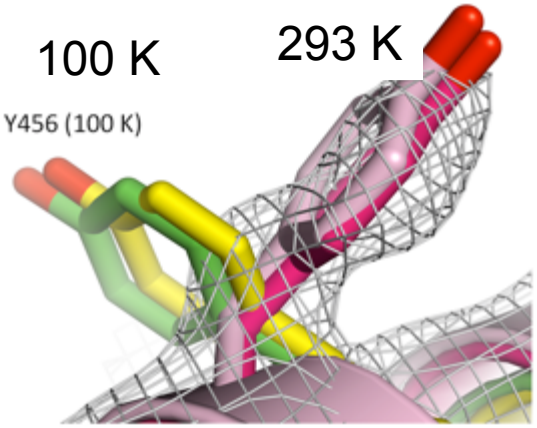








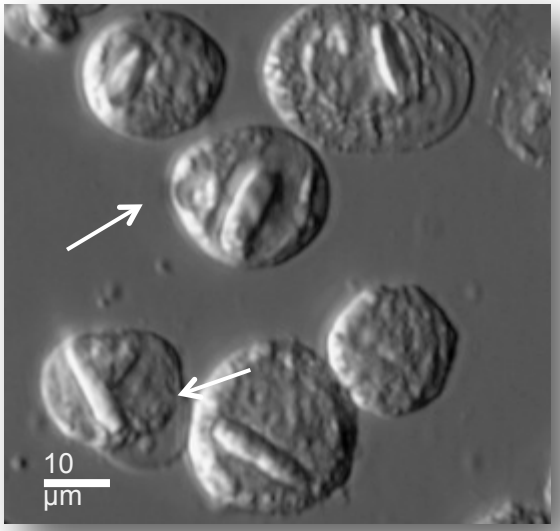


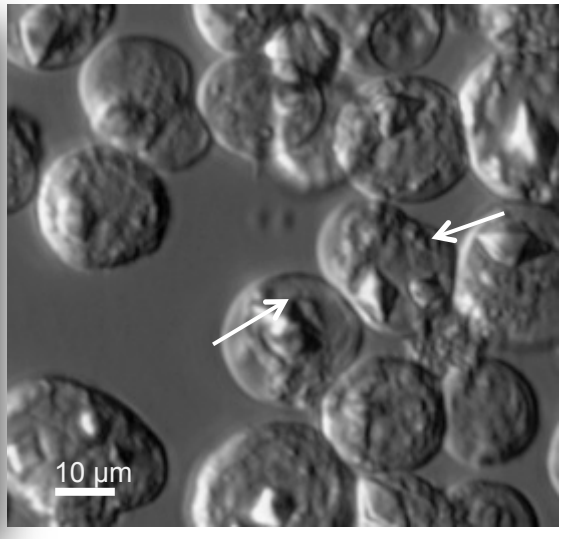


Proton-coupled oligopeptide transporter PepTSt (*Str. thermophilus*)

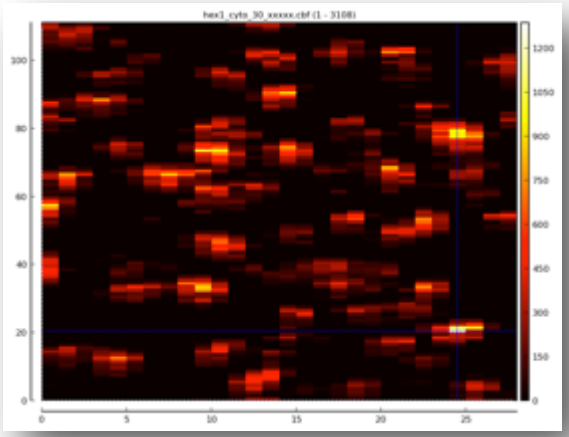
Schneider, Löw, Meijers
Marquez, Cipriani

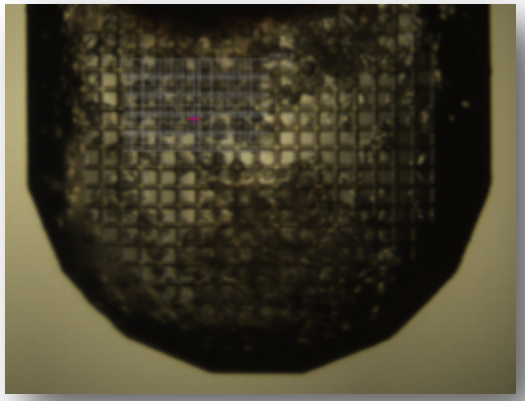
In-cellulo serial crystallography *in-vivo & cryo*

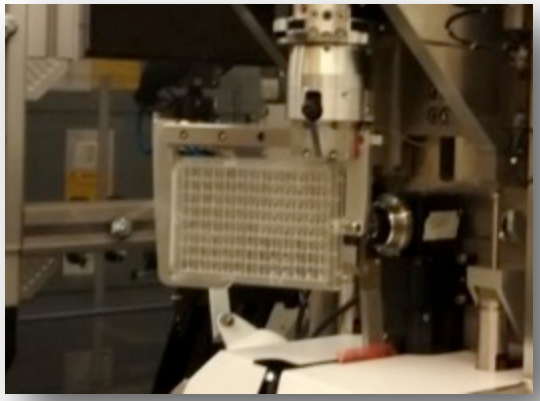




Insect cells with crystalline recombinant protein

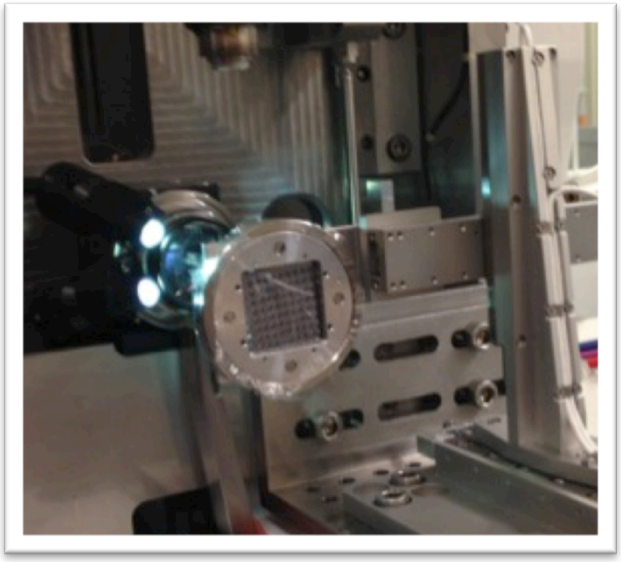


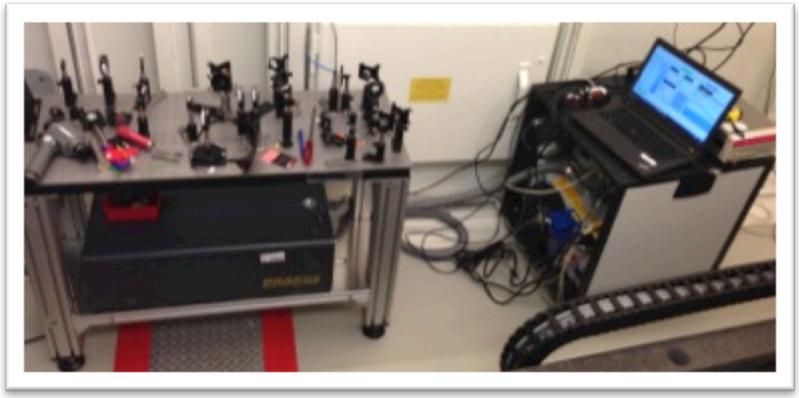




Redecke, Rudolph, Schönherr

Pump-probe serial crystallography on P14



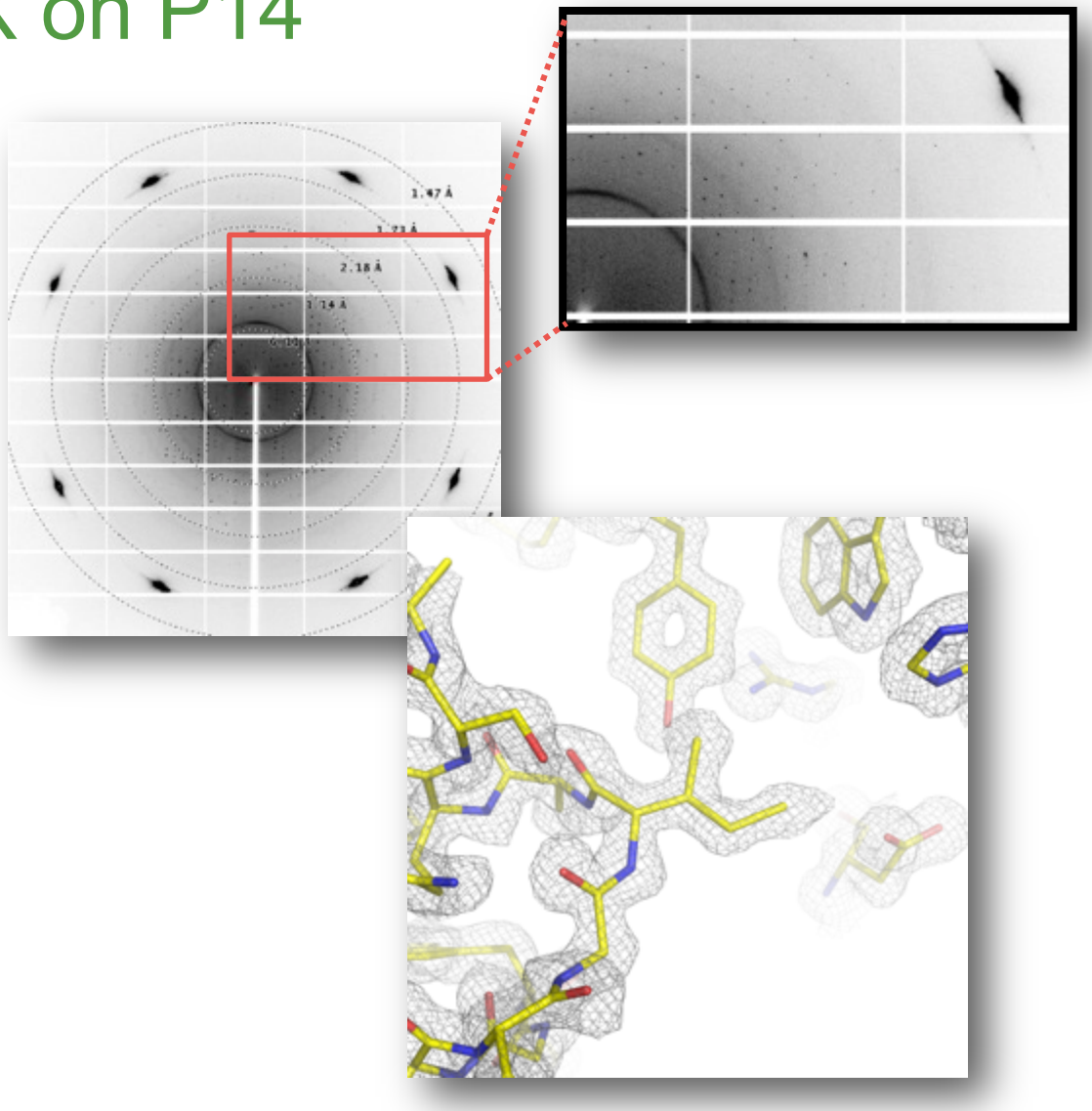


- M-Chip (25000 holes)
- 30 Hz -> 40 – 60000 frames/hr

Eike Schulz, Rike Müller-Werkmeister, Dwayne Miller, MPISDM Hamburg

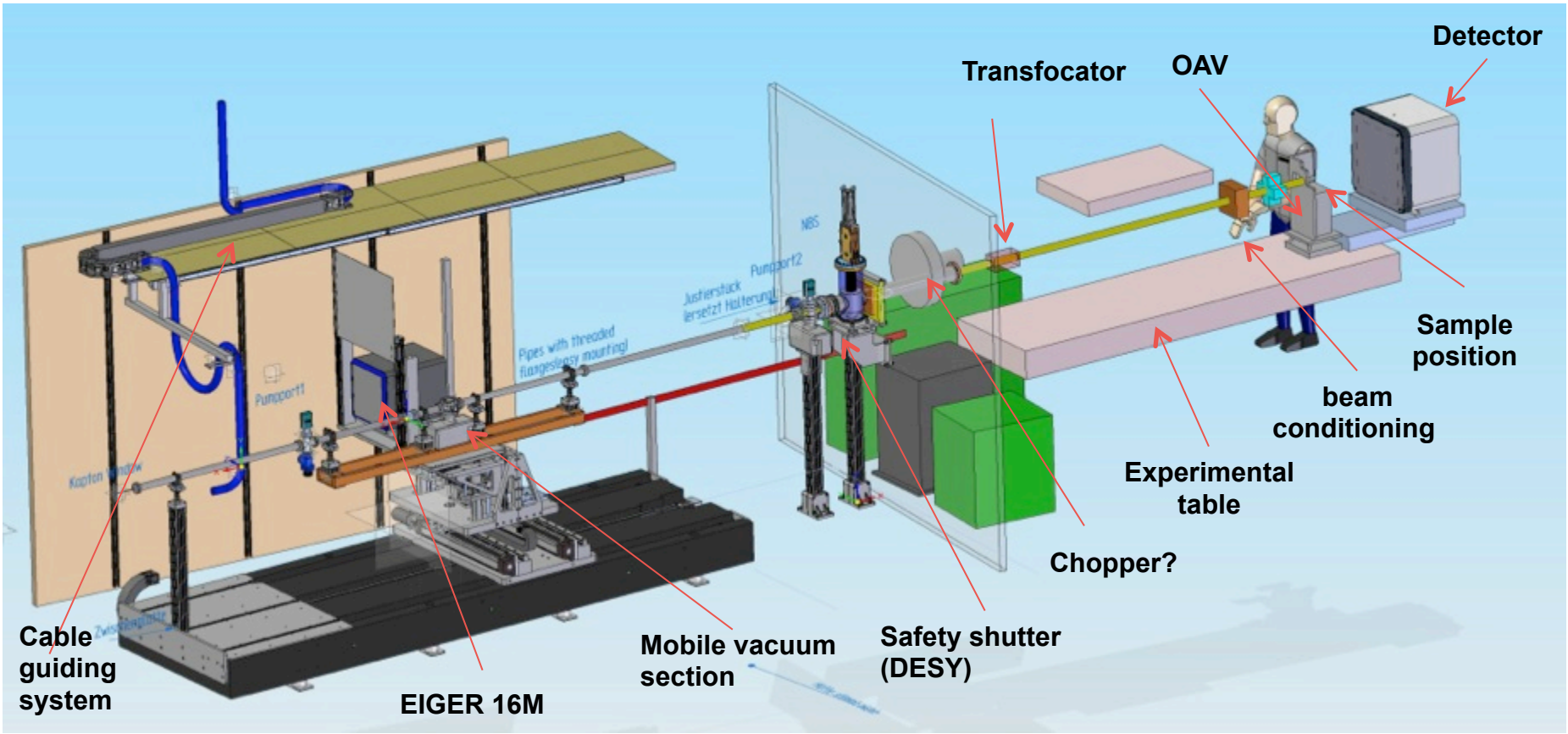
Time-resolved SSX on P14

- first successful experiments in 2016
- enzyme substrate un-caging
- high resolution



Eike Schulz, Rike Müller-Werkmeister,
Dwayne Miller, MPISDM Hamburg

Beamline extension with dedicated time-resolved SSX with a permanent laser installation



EMBL

Scaling the serial micro-crystallography

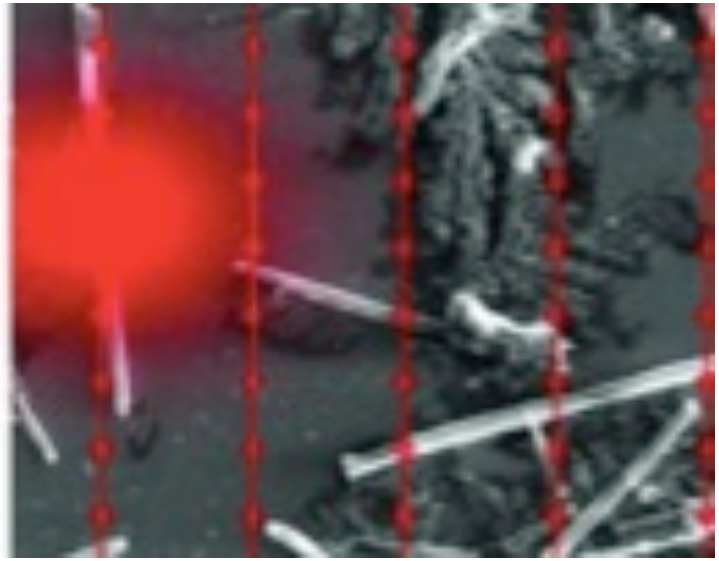
- Flux density is a current limitation
  150 ms life time *vs*
  1.5 ms frame cycle of a standard detector

- 100 fold smaller beam area translates into 10 fold speed up and 10 fold background reduction (CatB example).

- All instrumentation and methods are in place, no mechanics issues etc

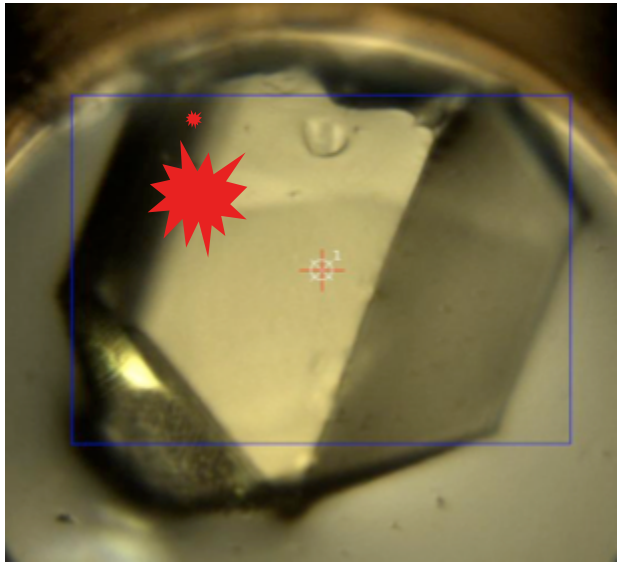
- Needs both smaller horizontal emittance *and* coherence-preserving optics

Tailoring beam to the sample



10 µm

40 kDa
10^7 unit cells



100 µm

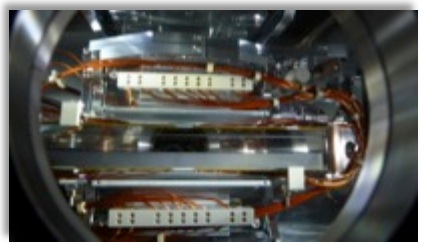
1 MDa
10^{12} unit cells

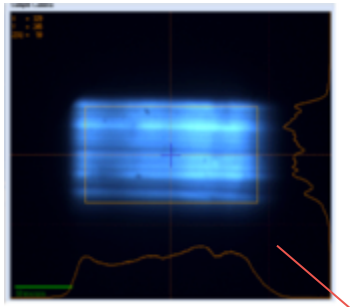
"microfocus"

"regular" MX beamline

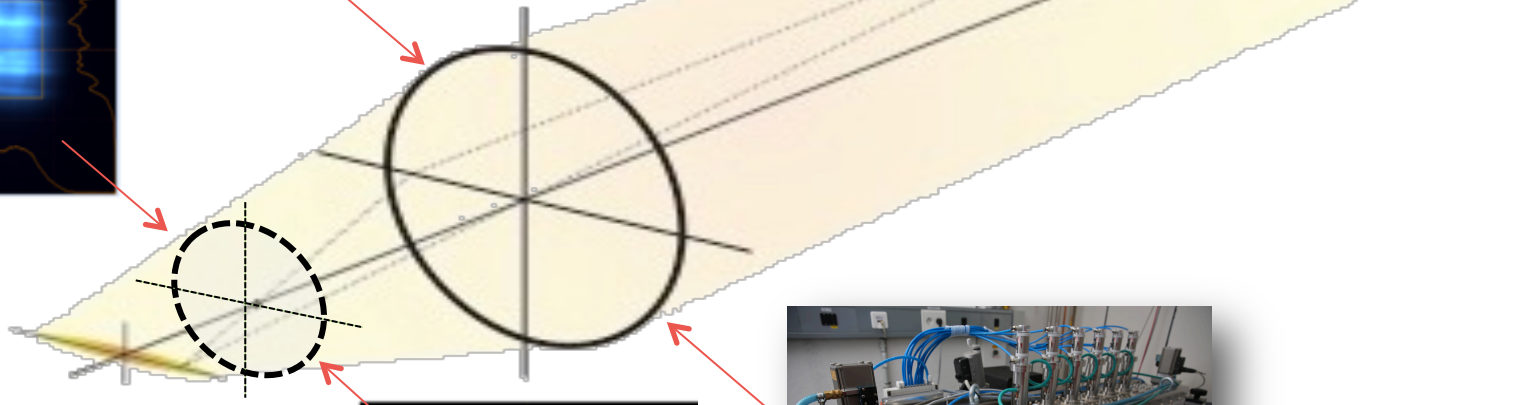
High flux density in a large area

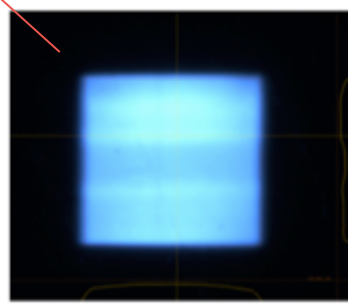
with mirrors



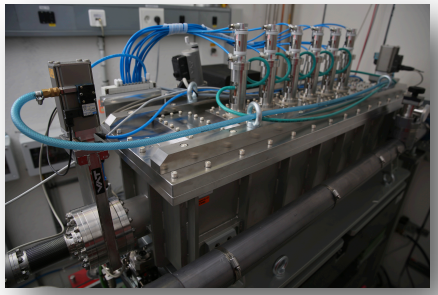


300 x 20 μm²



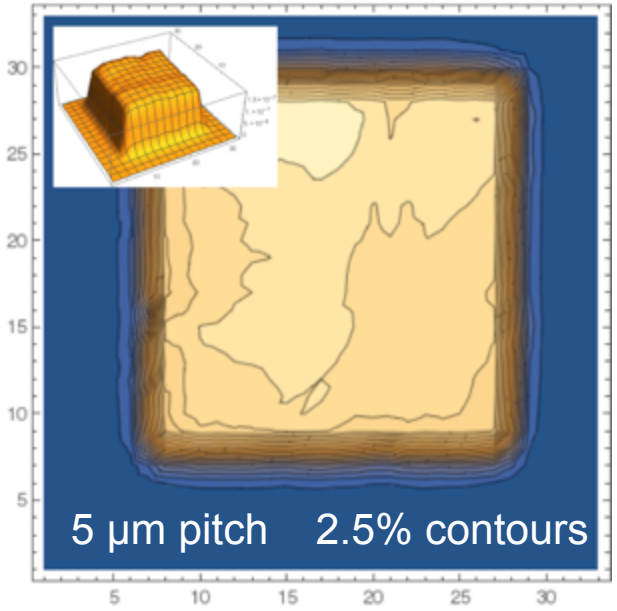


2 10¹³ ph/sec
in 200 x 200 μm²



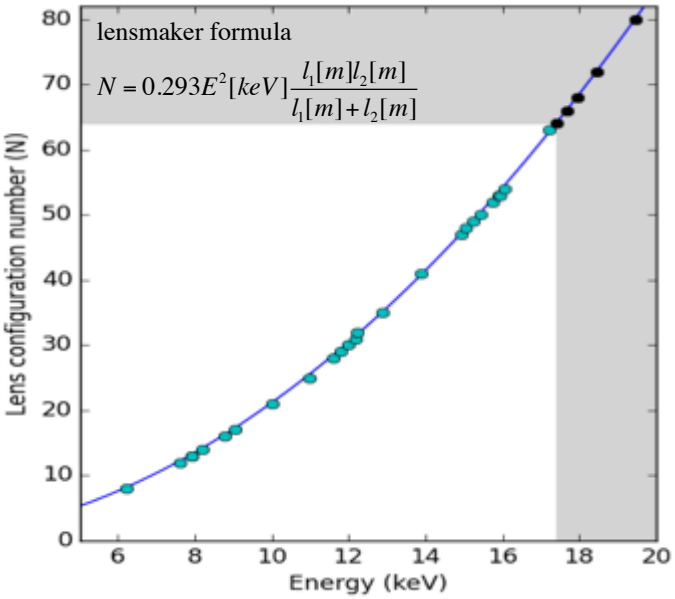
with CRLs

Top-Hat beam

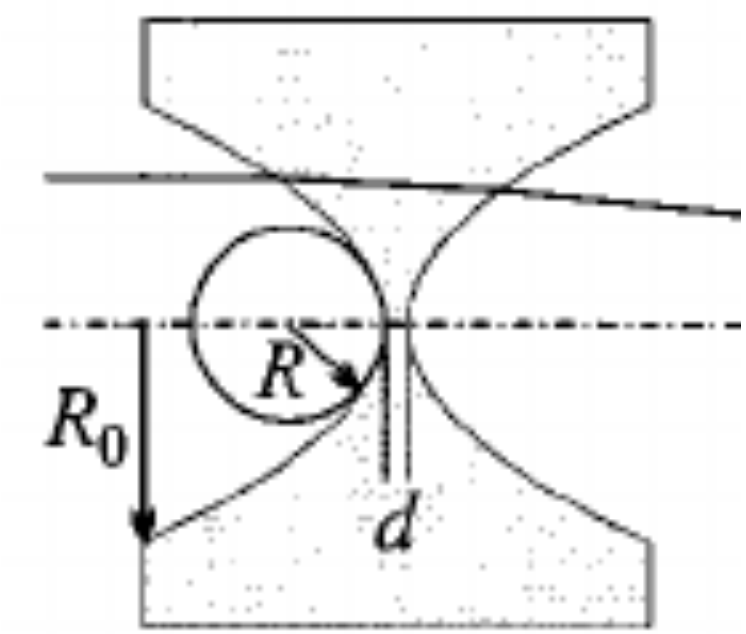


<2% intensity r.m.s.d.

Full energy range



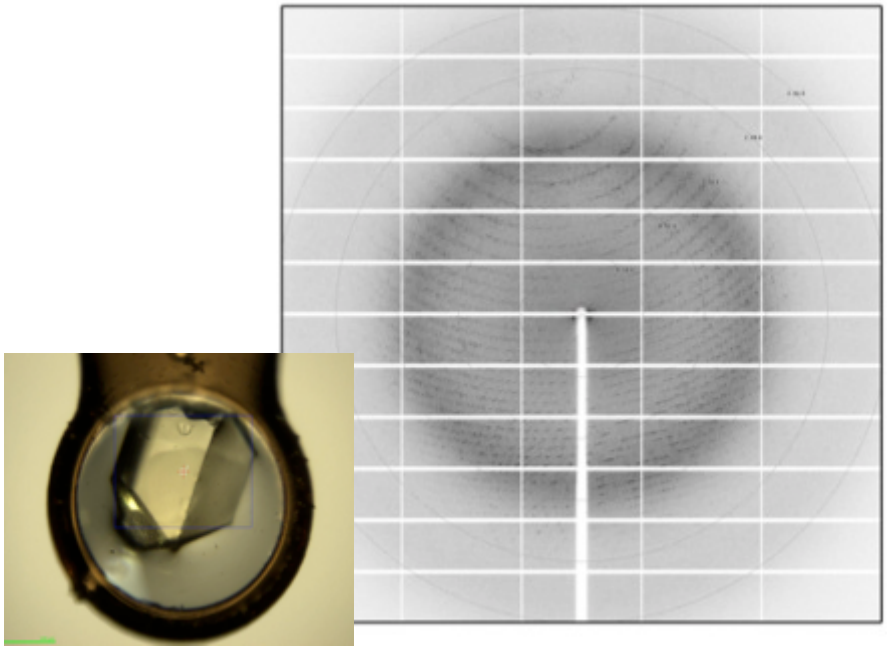
full PETRAIII beam is accepted by the lens



Be, R=500 μm R_0=650 μm

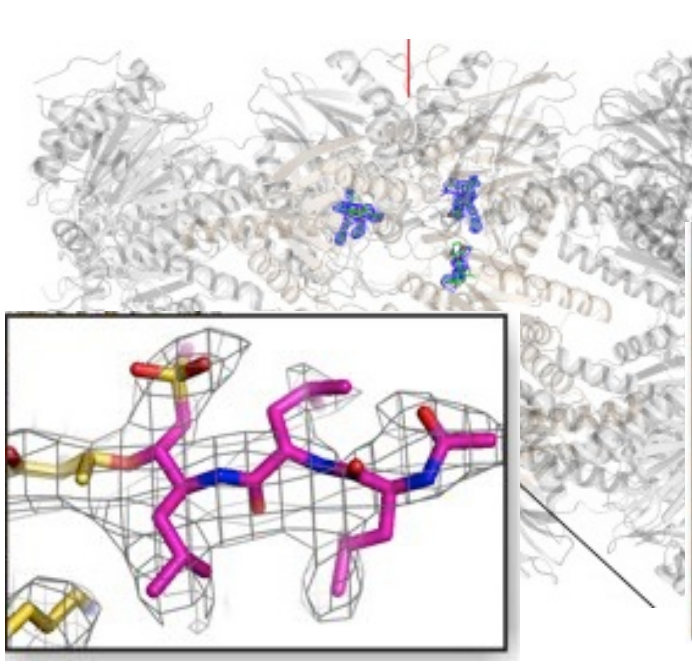
EMBL

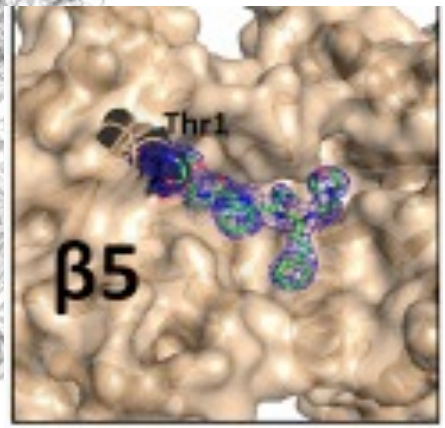
Tailored beam: application example Human 20S Proteasome – key target for tumor grows restriction

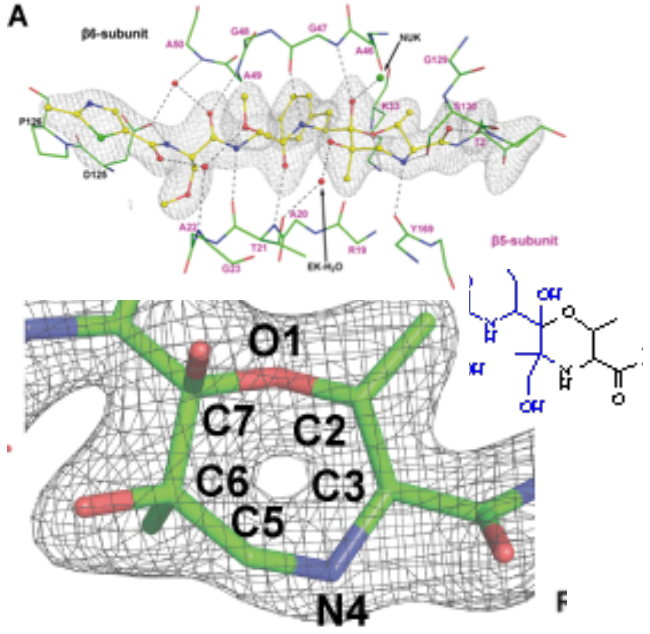


50000 atoms
3 min experiment -
10^{15} photons – resolution 1.8 Å
10^{7} data points (Bragg reflections) - 6 10^{5} unique

Human 20S Proteasome: EM and Crystallography







CryoEM
3.5 Å

MX
2.9 Å

MX
1.9 Å

- **Left:** P. C. da Fonseca, E. P. Morris, Cryo-EM reveals the conformation of a substrate analogue in the human 20S proteasome core. *Nat Commun* **6**, 7573 (2015).
- **Middle:** W. Harshbarger, C. Miller, C. Diedrich, J. Sacchettini, Crystal structure of the human 20S proteasome in complex with carfilzomib. *Structure* **23**, 418-424 (2015).
- **Right:** Schrader, Hanneberg, Schneier, Stark, Mata, Tittmann, Bourenkov, Chari, The inhibition mechanism of human 20*S* proteasomes enables next-generation inhibitor design *Science* **353** (2016)

Core Mediator structure at 3.4 Å extends model of transcription initiation complex

Kayo Nozawa[1], Thomas R. Schneider[2] & Patrick Cramer[1]

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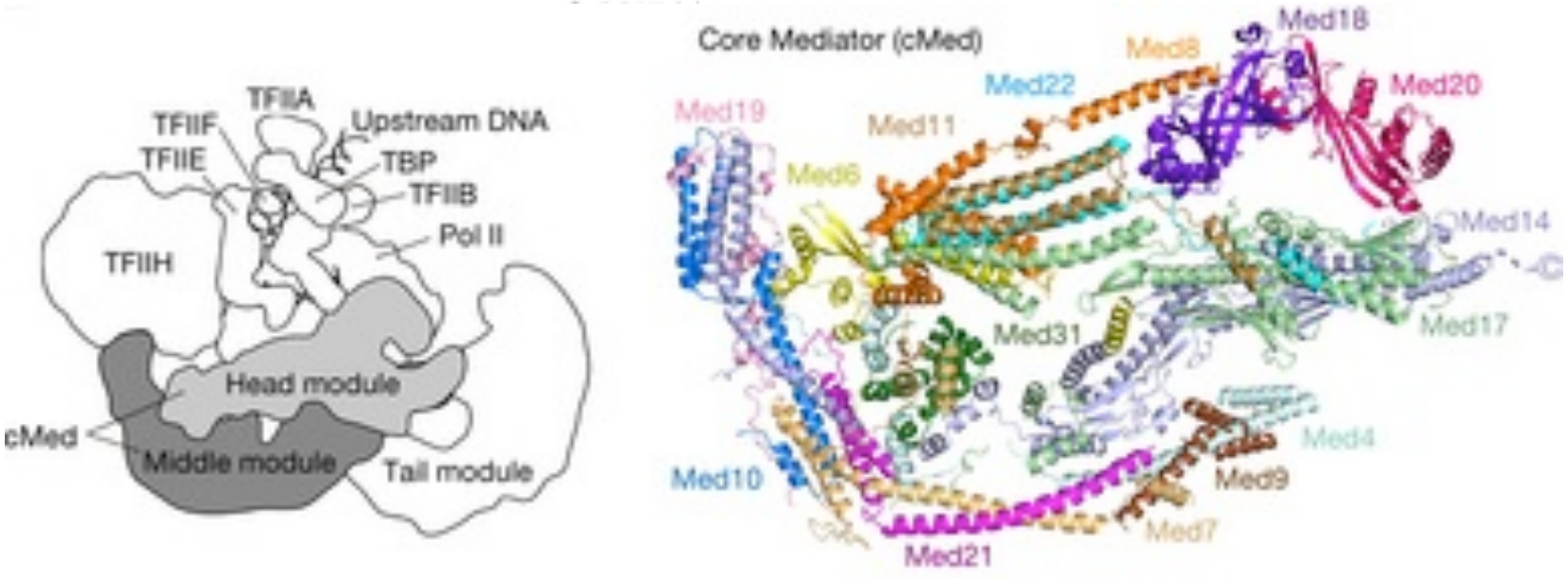
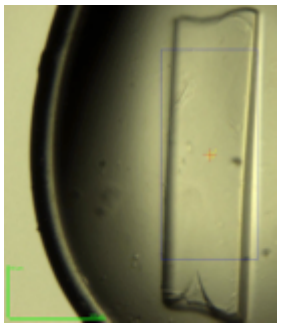
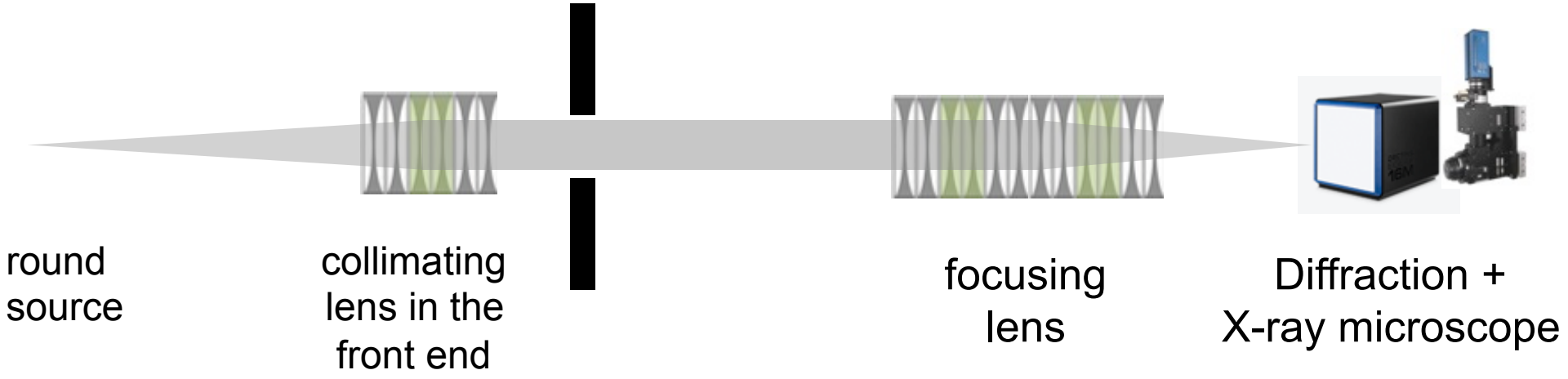




a PILATUS 6M detector, and on beamline PX1 at the Swiss Light Source in Villigen, Switzerland, using an EIGER 16M detector. Resolution higher than 4 Å was obtained at EMBL beamline P14 in Hamburg, Germany, using a homogeneous X-ray beam of 200 µm × 200 µm obtained via compound refractive lenses. A sulfur-single wavelength anomalous diffraction (S-SAD) data set

Ideal MX beamline?



- the beam is collimated to a maximum usable size (<~300 µm) -> no flux loss on the focusing lens
- which is used refocuse down to a (high-)-sub-1µm (diifraction limited, focal distance >~ 1m, divergence ~300 µrad)
- Stepwise adjustable top-hat beam (few µm step), in a full range of dimensions. Suitable for anomalous diffraction.

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- Anatoly Snigirev, Kaliningrad University
- Beamline Users: Lars Redecke, Dwayne Miller, Kayo Nozawa, and others