(Mini-)Kappa Diffractometers on EMBL MX Beamlines @ PETRAIII

Gleb Bourenkov
EMBL Hamburg

MADaC 2015, SOLEIL

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EMBL@PETRAIII MX Beamlines

P13

4-15 keV,

30 µm focus

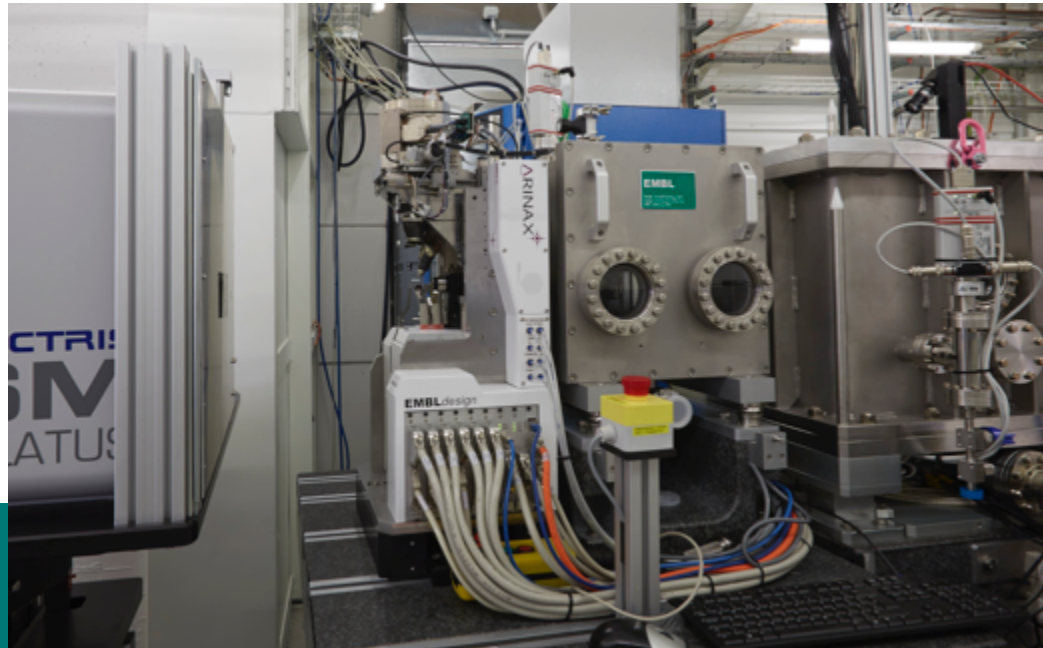
10 µm mini-beam option

P14

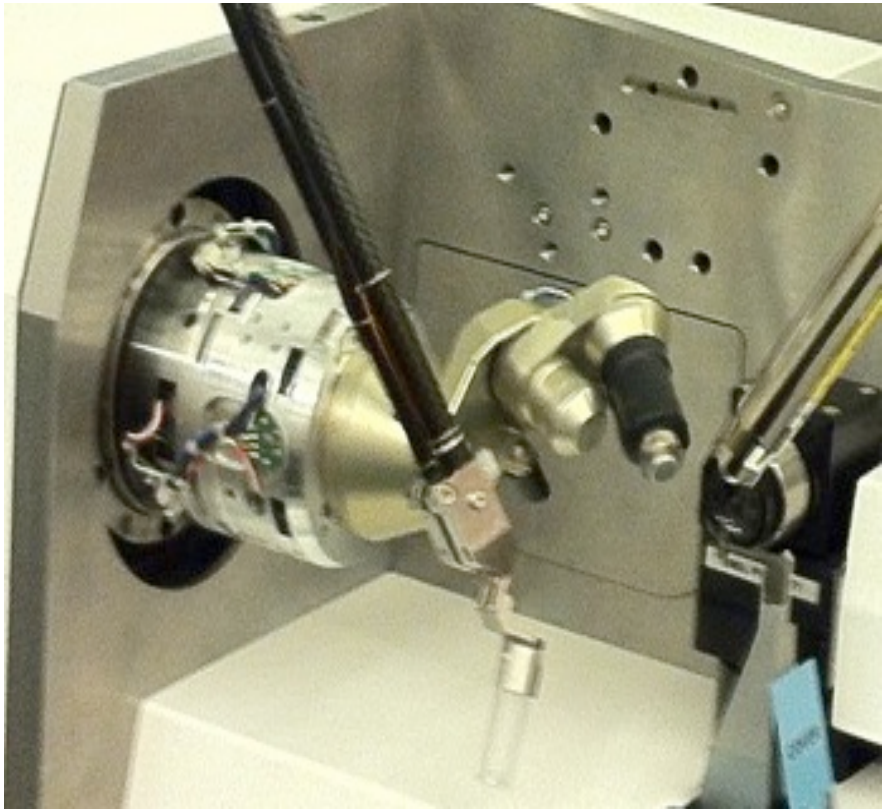
6-25 [35] keV

4 x 5 µm focus

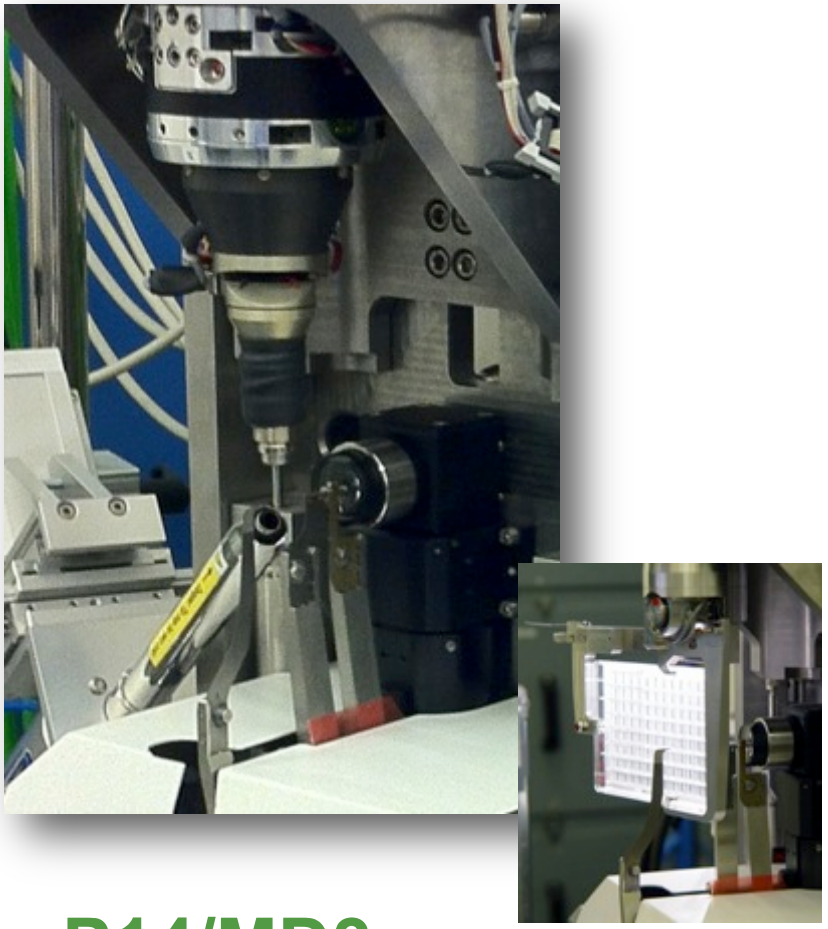
Large top-hat parallel beam option



Persistently installed Mini-Kappa heads



P13/MD2



P14/MD3

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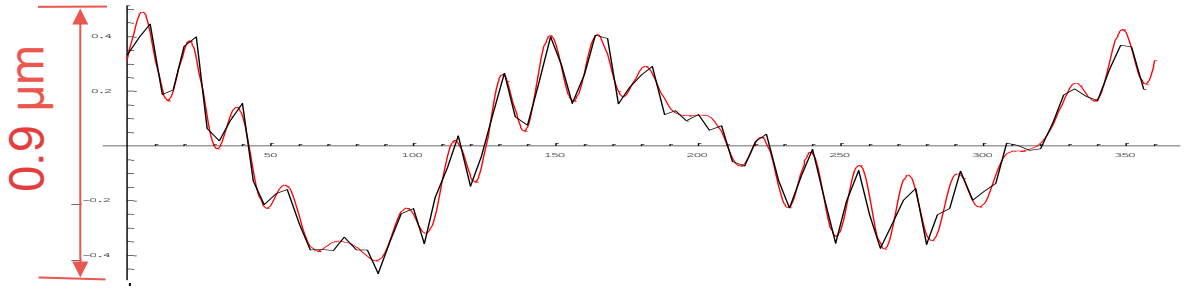
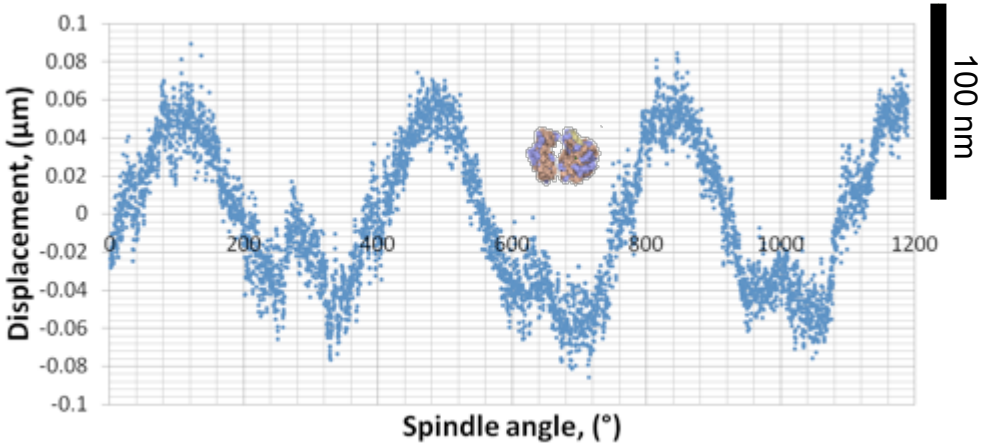
Measured sphere of confusion

- MD2 – **2.5 µm** with Kappa open, **0.95 µm** without Kappa



- MD3 – **0.20** µm independent on the Kappa pose



- off-center component: 50 nm
- rmsd from ideal: 16 nm

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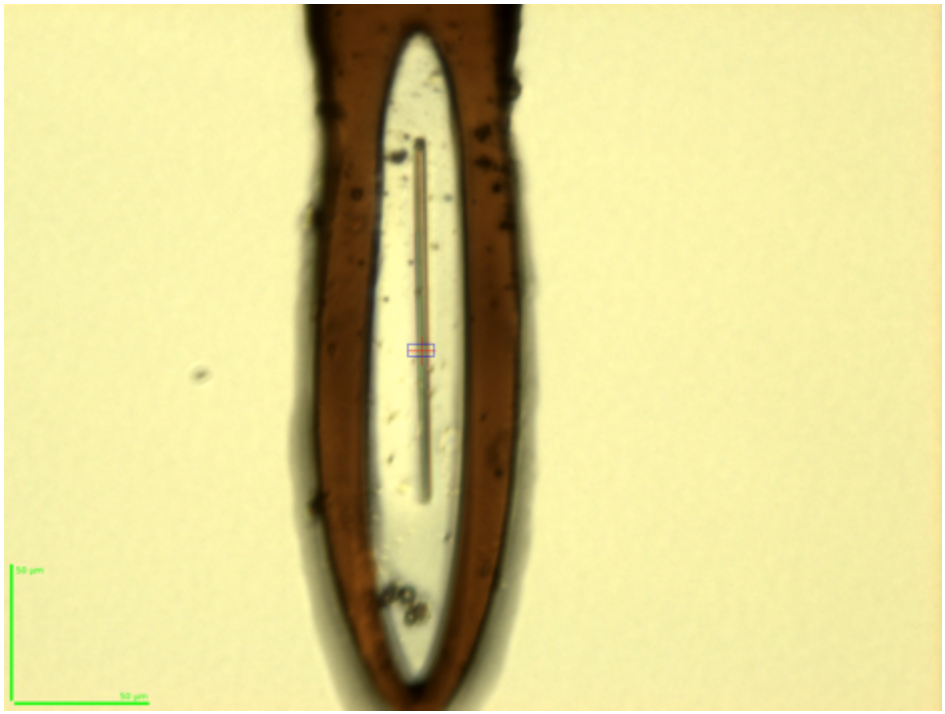
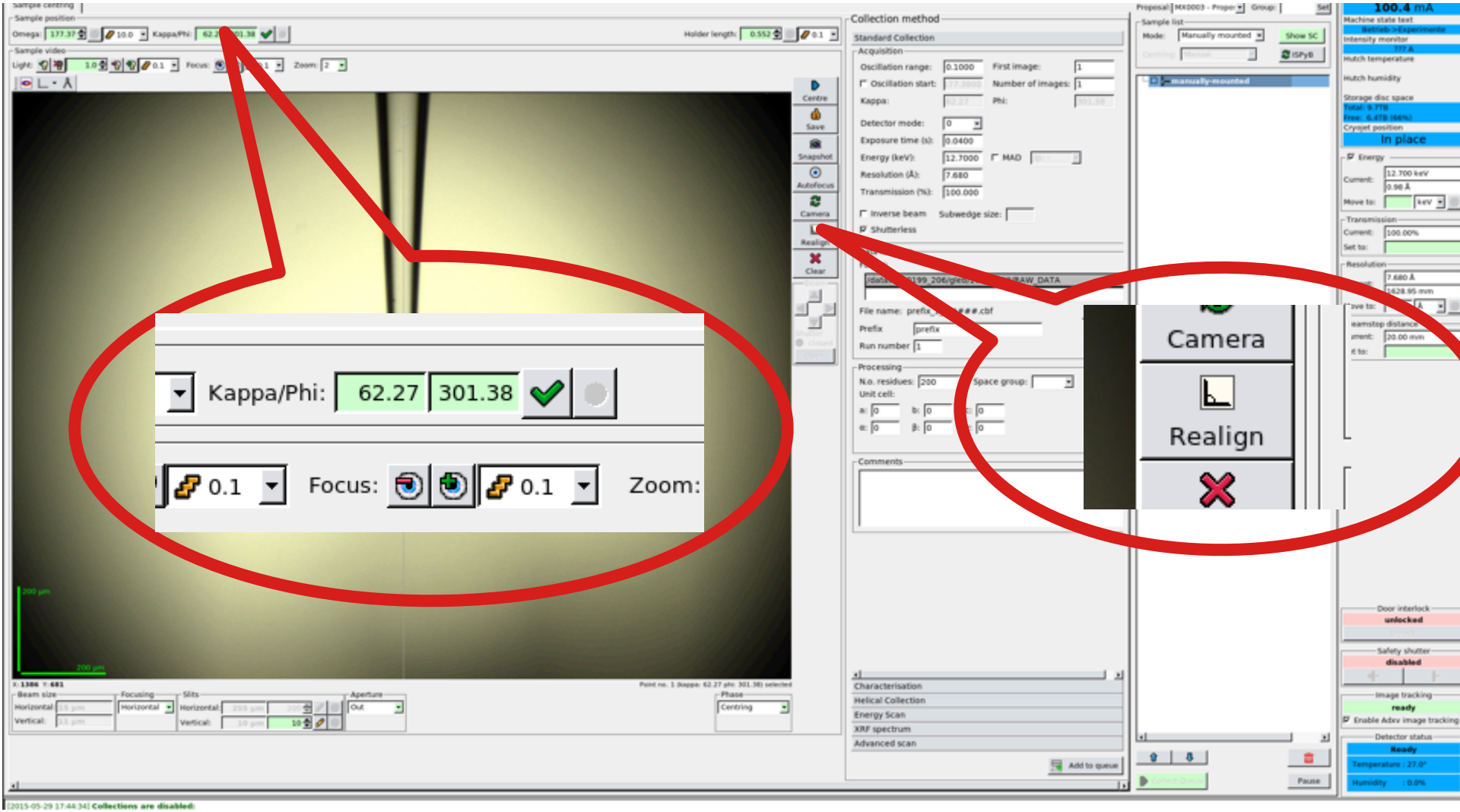
Shutterless helical scans - 4D scans on µ-sized needles with MD3



3x3x100 µm crystal
Structure solved at 1.7 Å resolution

Kappa in MxCuBE



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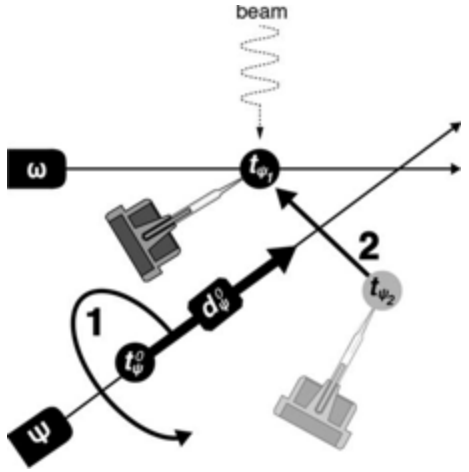
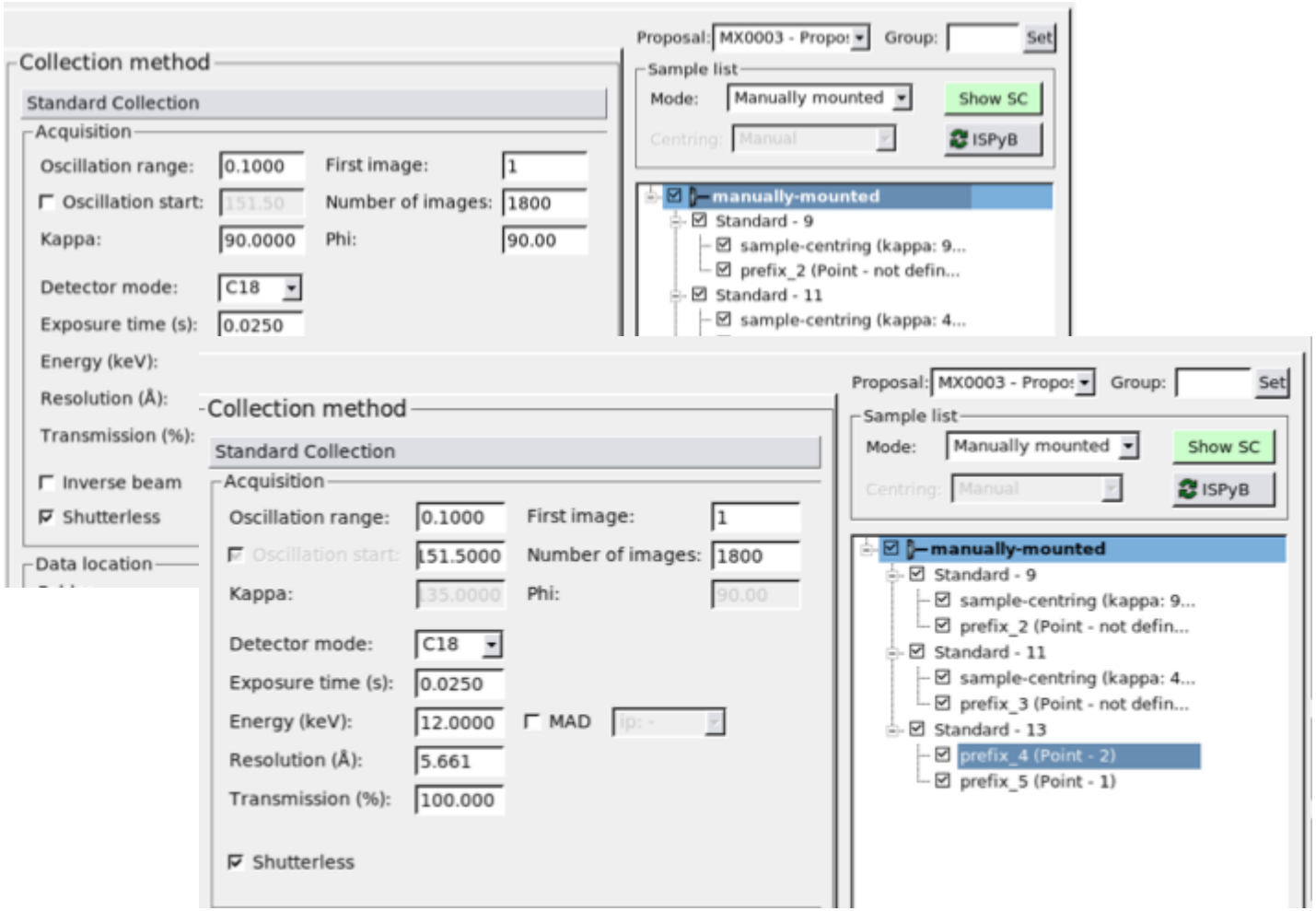
Centering corrections (Brockhauser et al, 2011)



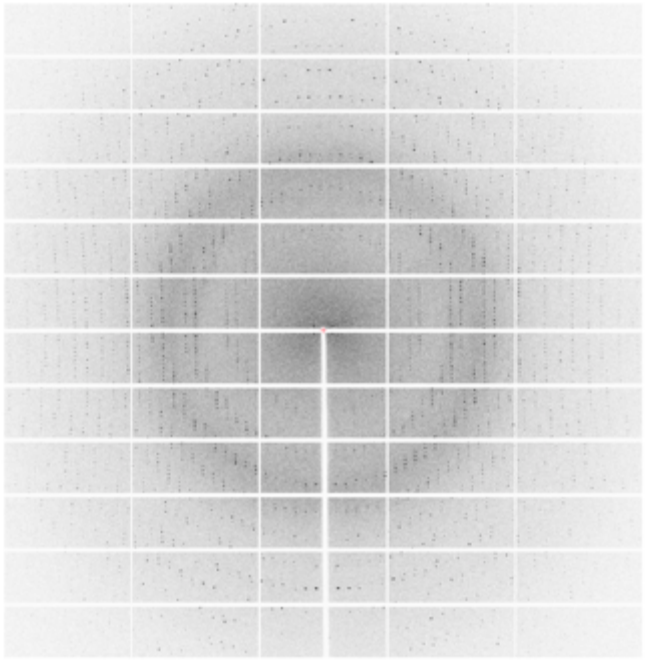
accuracy ~ 20 µm

Queued multi-orientation data collections in MxCuBE

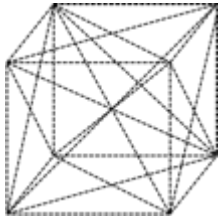


Goniometric calcs

Hs	Ks	Ls	Del	Kap	Phi	Omg	Hb	Kb	Lb
1	0	0	10	180	207	183	0	0	1
0	1	0	21	180	104	243	0	0	1
0	0	1	0	108	25	9	0	1	0
1	1	0	35	179	248	164	0	0	1
0	1	1	24	180	308	267	1	0	0
1	0	1	0	59	317	117	0	1	0
-1	0	1	31	180	10	315	0	1	0
0	-1	1	0	102	105	218	1	0	0
1	-1	0	4	180	152	216	0	0	1
1	1	1	8	180	273	130	-1	0	1
-1	1	1	39	180	157	251	0	-1	1
1	-1	1	0	45	205	329	-1	0	1



13 standard alignments



HKLs – direction to align with the spindle
Del – closest possible angle HKL to spindle
Kap, Phi – alignment angles
HKLb – directions to align with the spindle-beam plane at Omega=Omg

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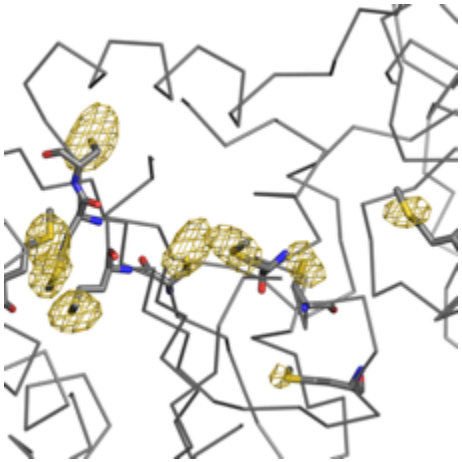
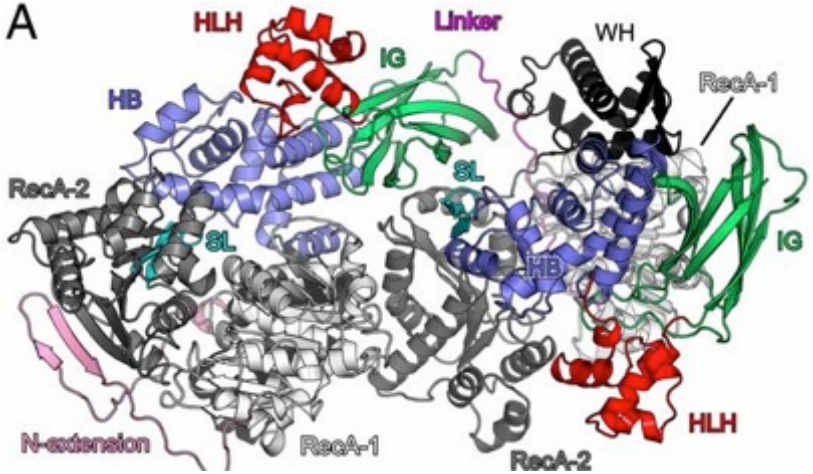
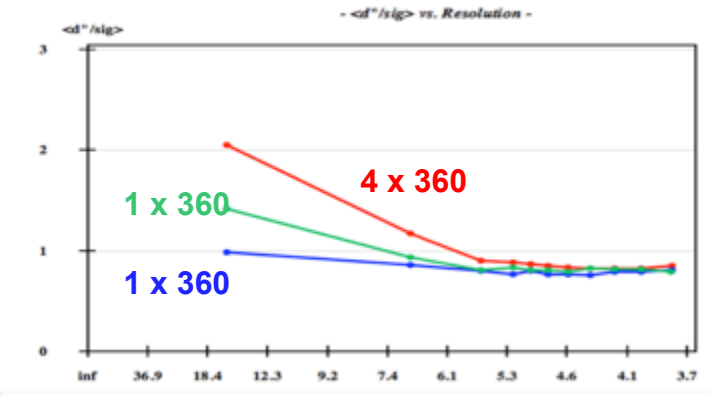
Santos K.F., Jovin S.M., Pena V., Luhrmann R., Wahl M.C. Structural basis for functional cooperation between tandem helicase cassettes in Brr2-mediated remodeling of the spliceosome.



- 200 kDa
- 6 Å anomalous data
  6 keV (DCM de-tune)
  4 κ-settings
- >60 sulphur positions -





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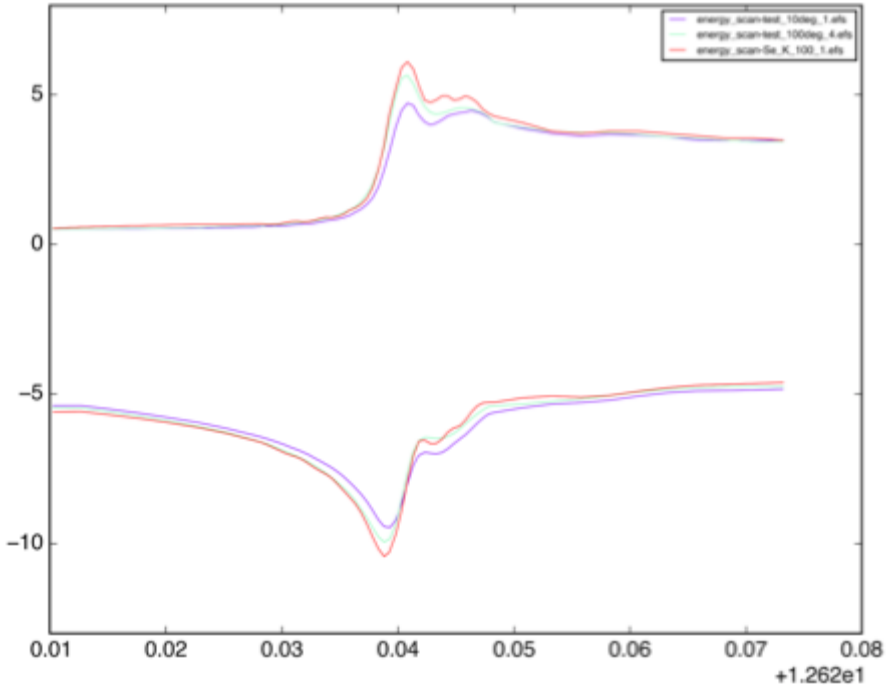
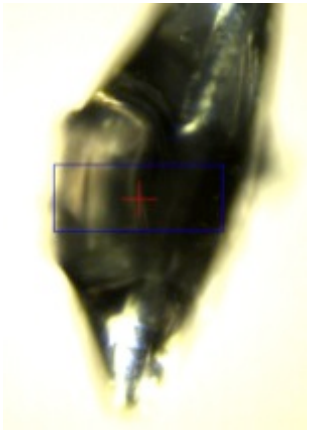
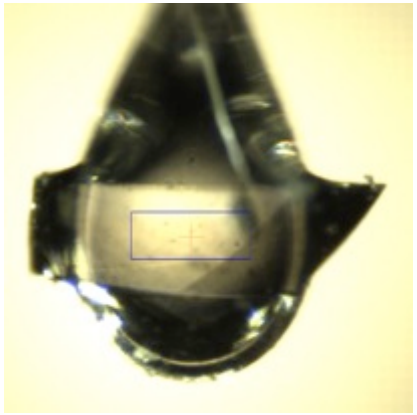
Anisotropic anomalous scattering with vertical spindle

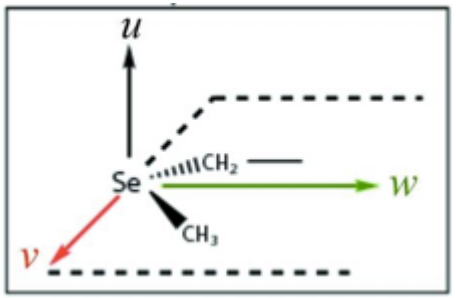
- Se K edge fluorescence scans of orthorhombic FAE crystal, 64 SeMets/Unit cell at different Omega angles

  U. Zander and D. De Sanctis



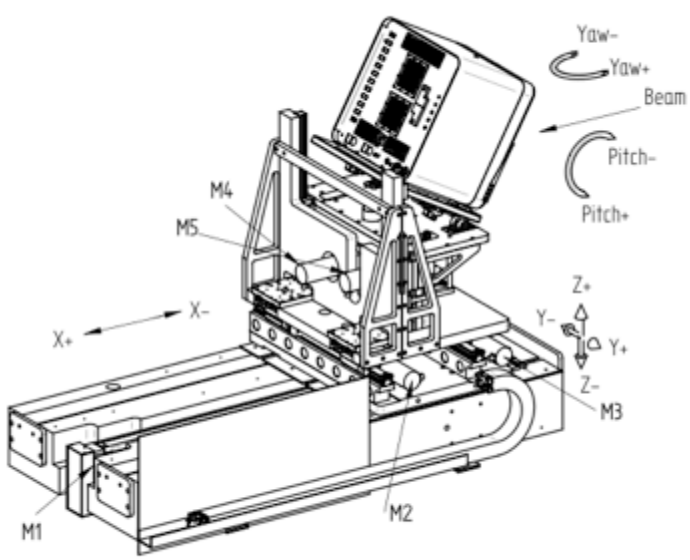






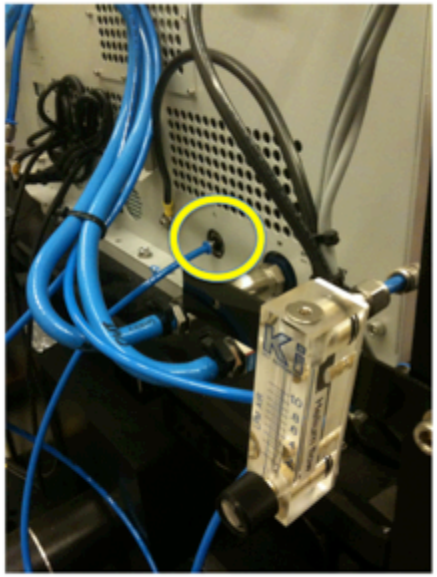
Schiltz & Bricogne, 2008

Low energies at P13

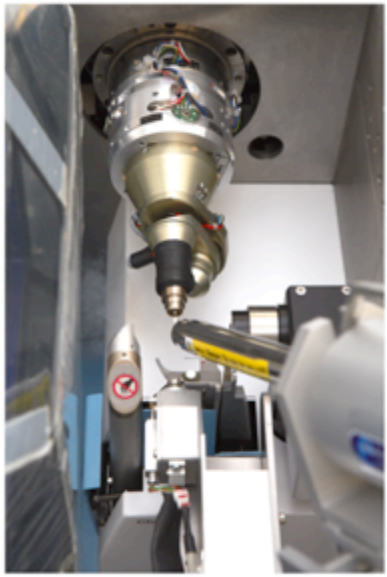


(a)

(b)



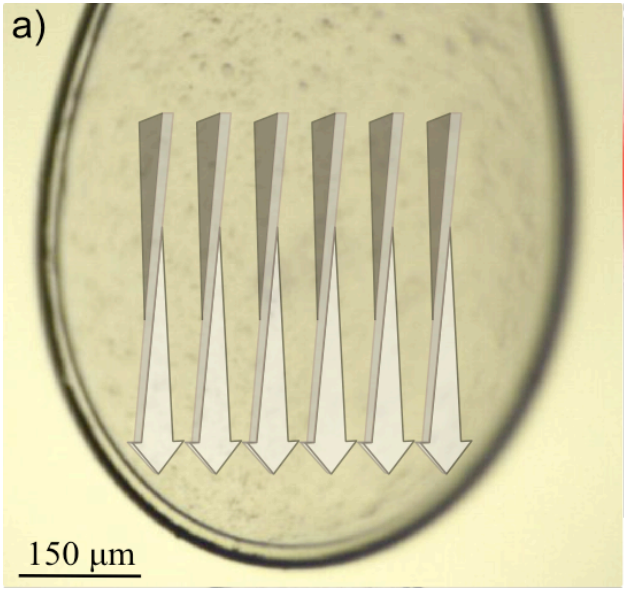
(c)

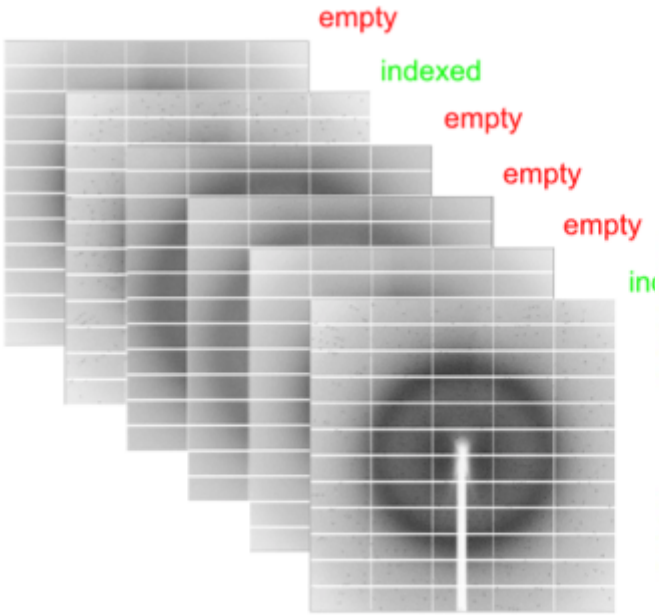


(d)

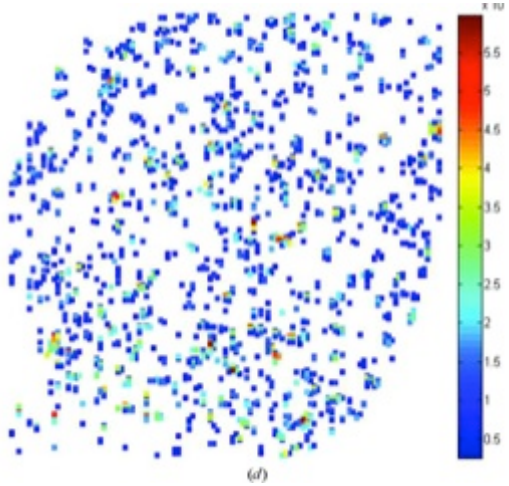
DECTRIS Pilatus 6MF with calibration tables for low energies
Helium cone
Pseudo 2Θ-arm

Serial Synchrotron Crystallography (SSX)

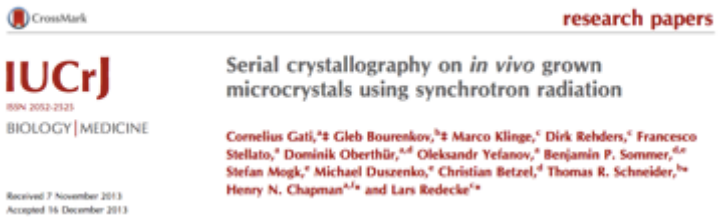




detector frames



- 30000 frames collected via series of parallel helical scans ± 60° rotation per line
- Each point exposed to the dose limit
- Processing by CRYSTFEL (White 2012, localization) and XDS integration
- 80 crystals contributed to final data set


CrossMark

research papers

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ISSN 2052-2525

BIOLOGY | MEDICINE

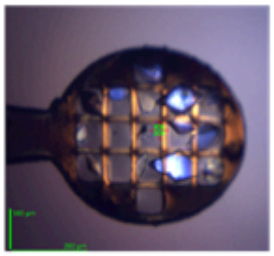
Serial crystallography on *in vivo* grown microcrystals using synchrotron radiation

Cornelius Gati, Gleb Bourenkov, Marco Klinge, Dirk Rehders, Francesco Stellato, Dominik Oberthür, Oleksandr Yefanov, Benjamin P. Sommer, Stefan Mogk, Michael Duszenko, Christian Betzel, Thomas R. Schneider, Henry N. Chapman and Lars Redecke

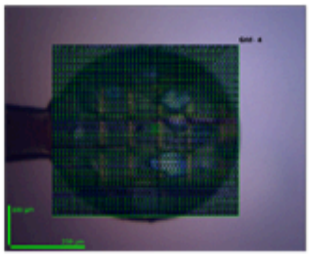
Received 7 November 2013
Accepted 16 December 2013

EMBL

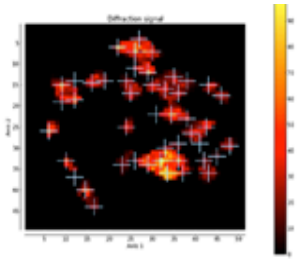
Automated multi-(μ)-crystal data collection



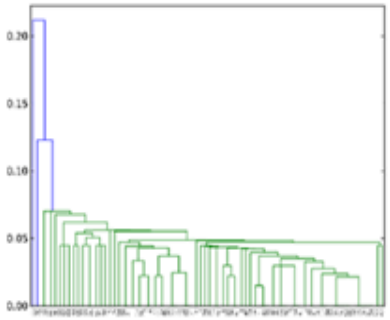
Crystals on a mesh loop



Mesh scan of sample



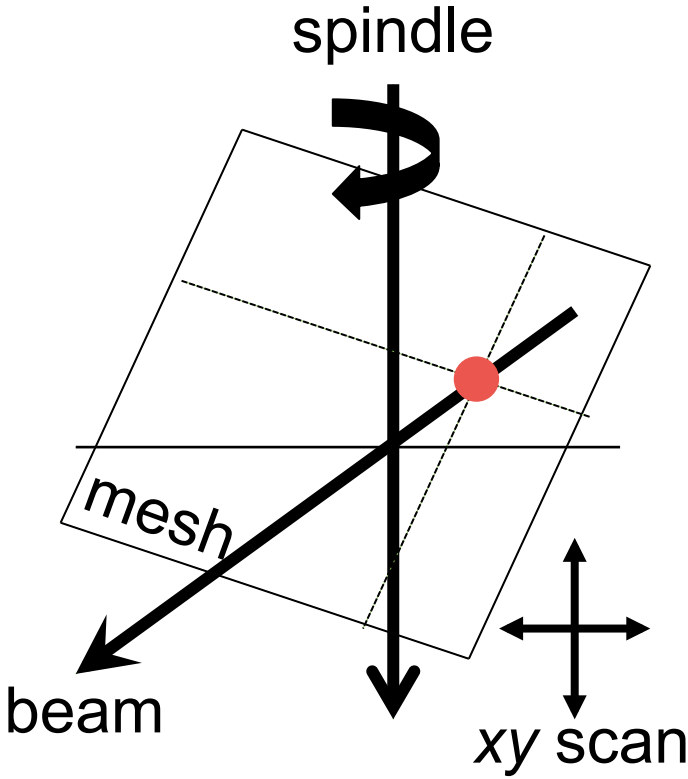
Automatic determination of crystal positions for series of partial data collections



Dataset combination based on hierarchical cluster analysis:

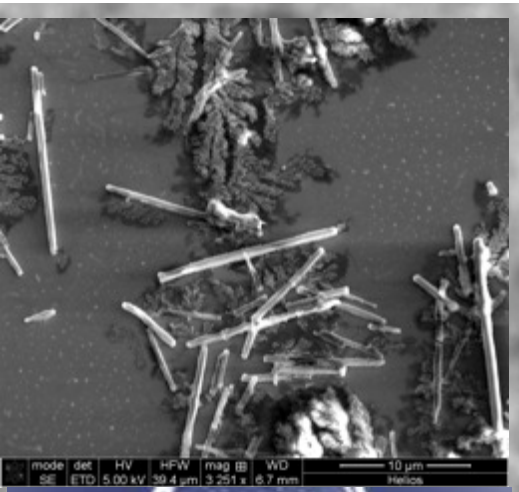
Structure solution

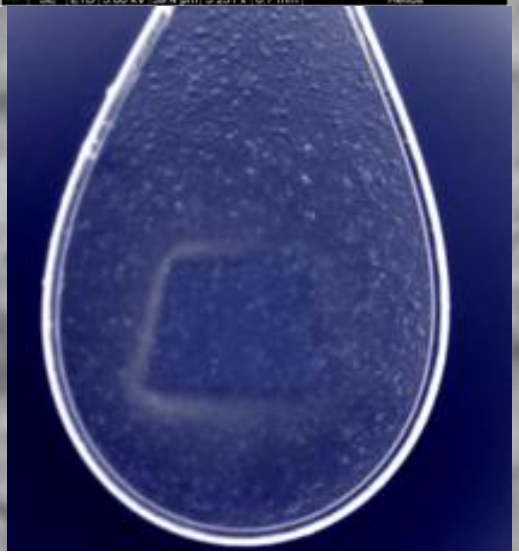
Zander, Bourenkov, Popov, …, Leonard (2015)

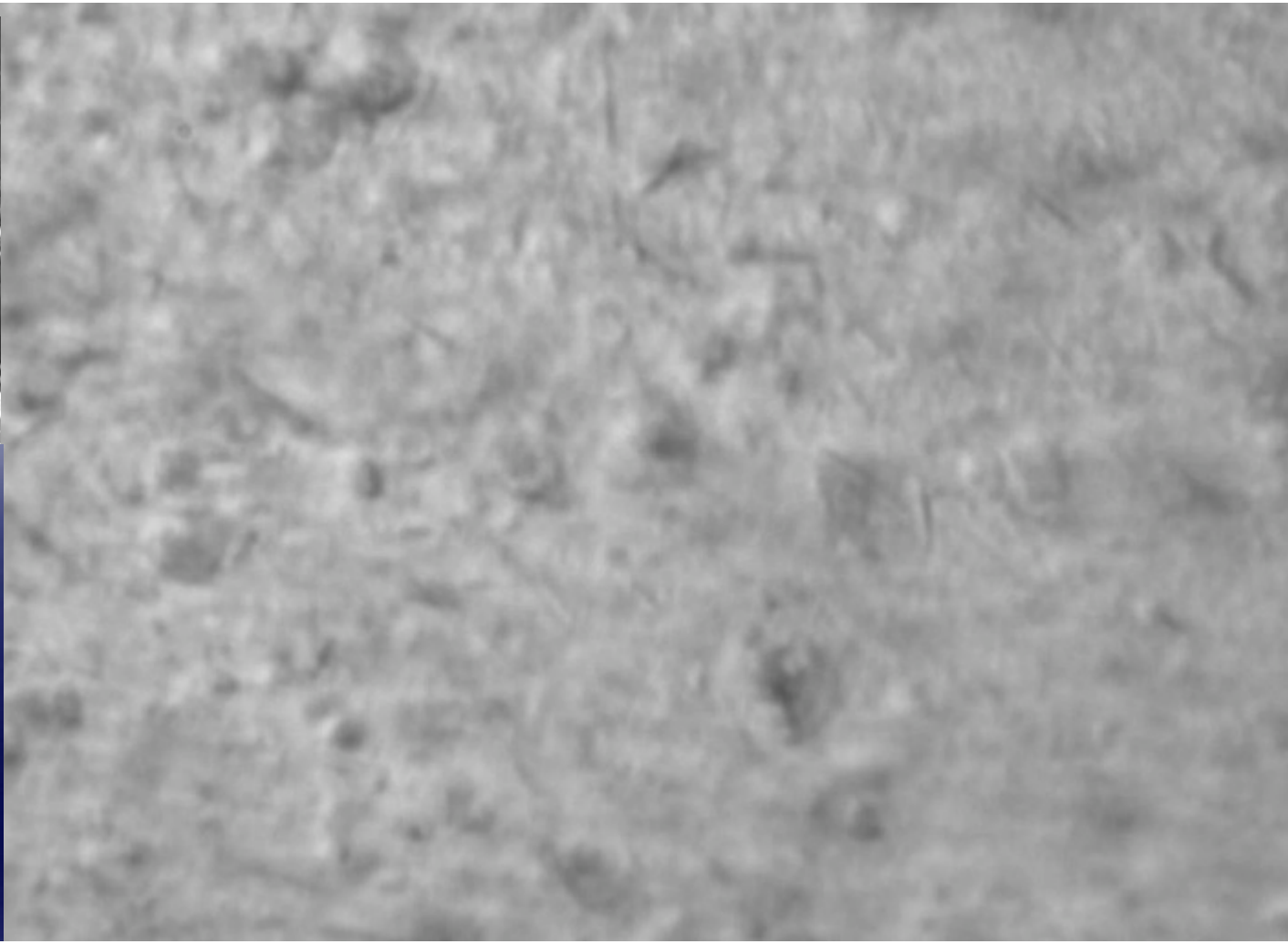


mesh alignment to the xy scan plane

Cathepsin B crystal suspension in aligned cryoloop viewed with P14 on-axis microscope

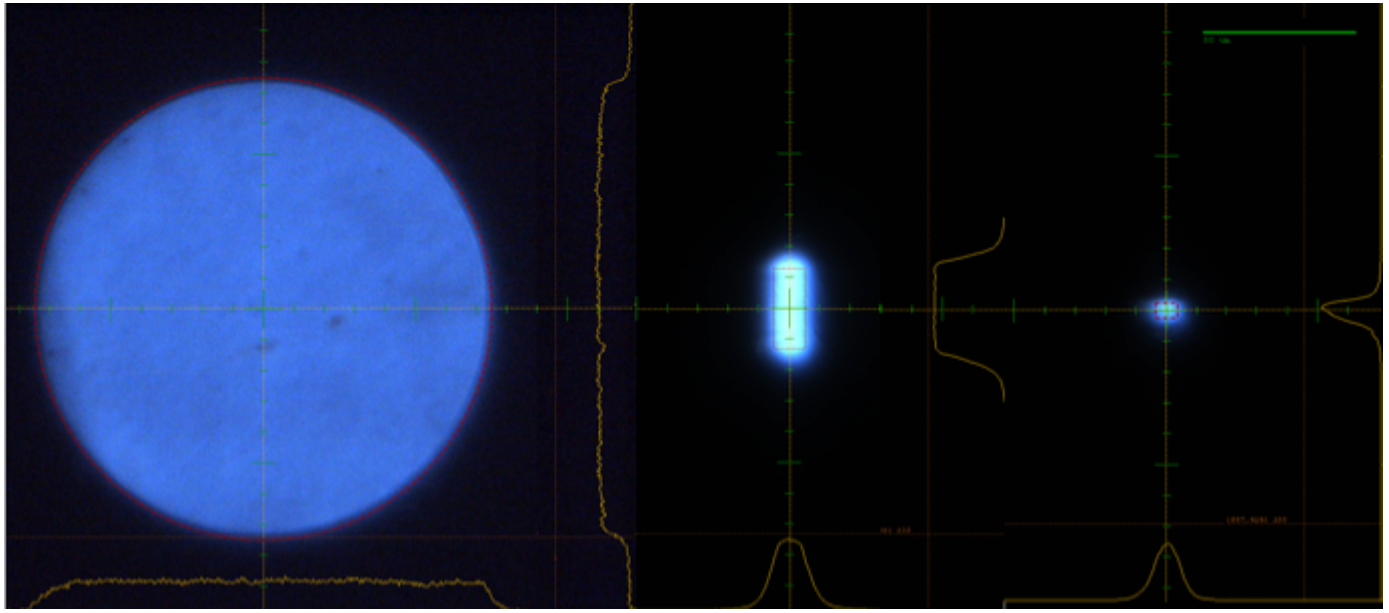


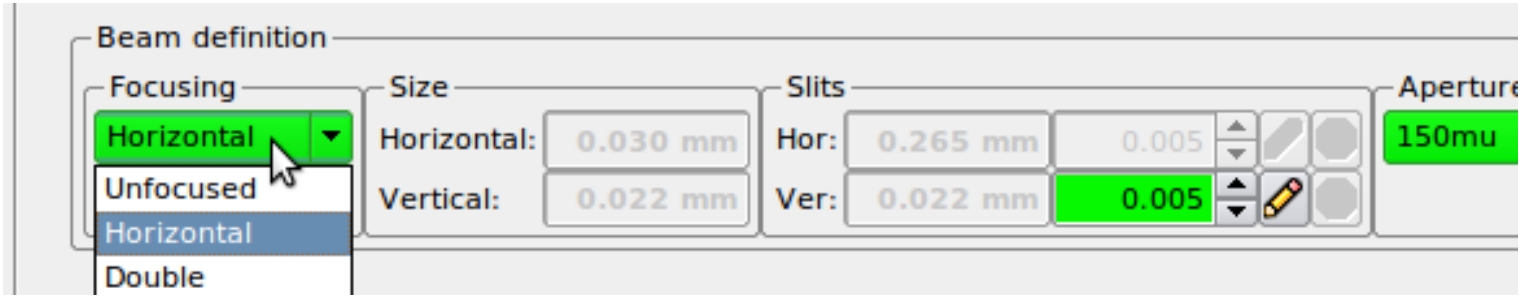




EMBL

Matching the beam to the crystal
Unfocused – Hor/Ver focused – Double focused

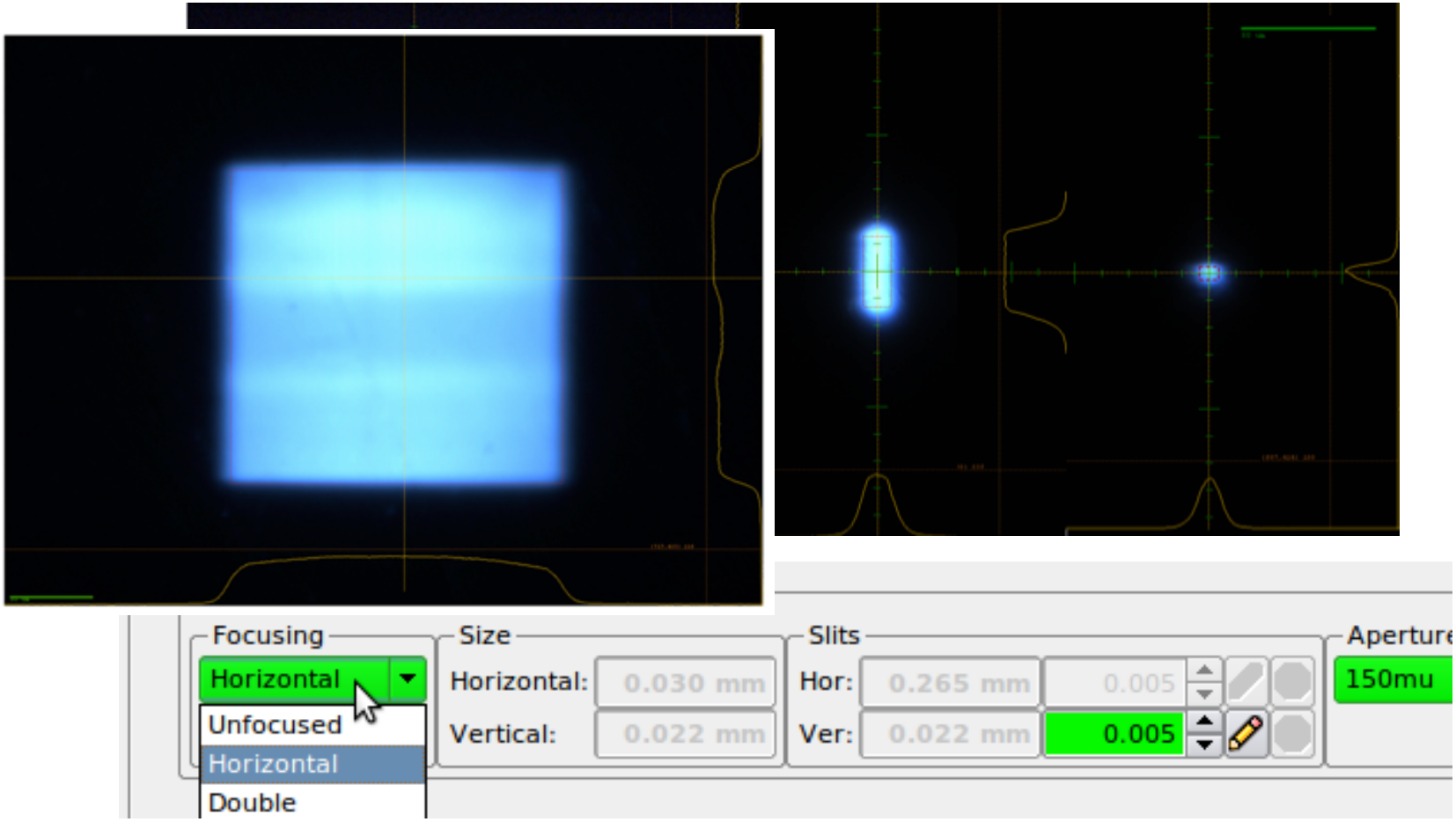




5 to 500 µm in 20 seconds

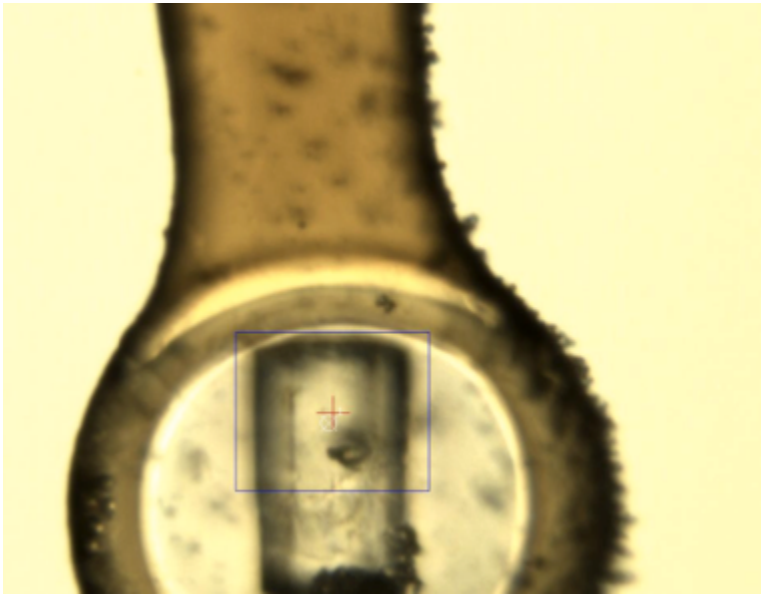
EMBL

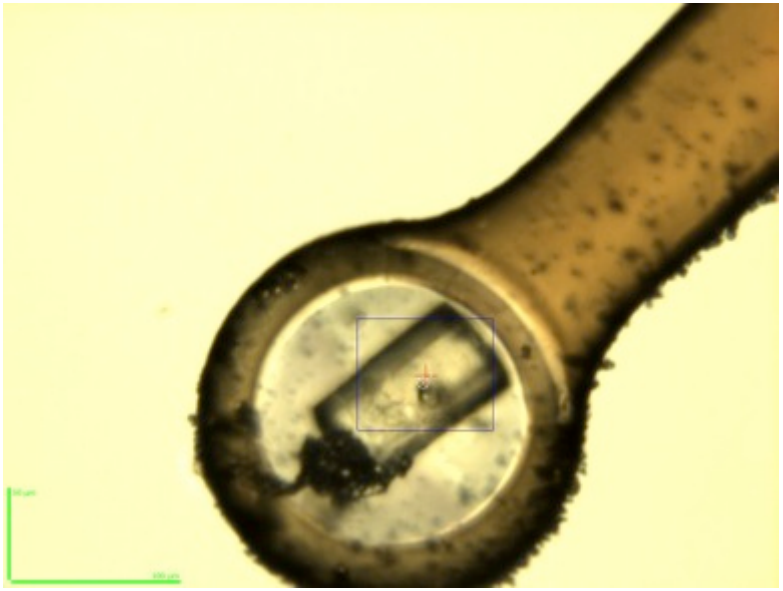
Matching the beam to the crystal
Unfocused – Hor/Ver focused – Double focused



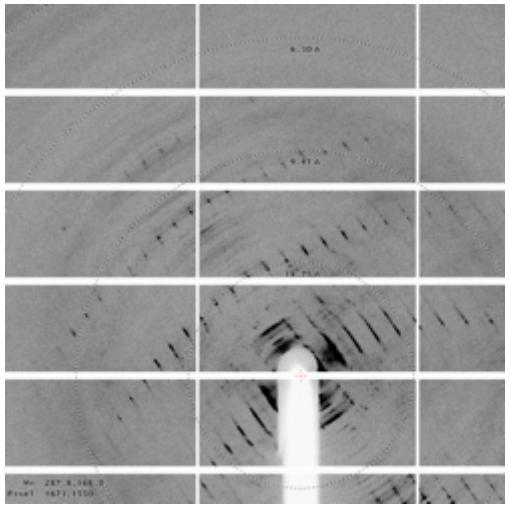
5 to 500 µm in 20 seconds

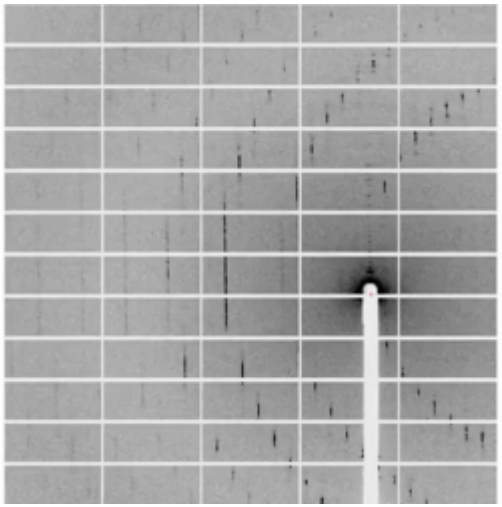
Matching crystals to the beam

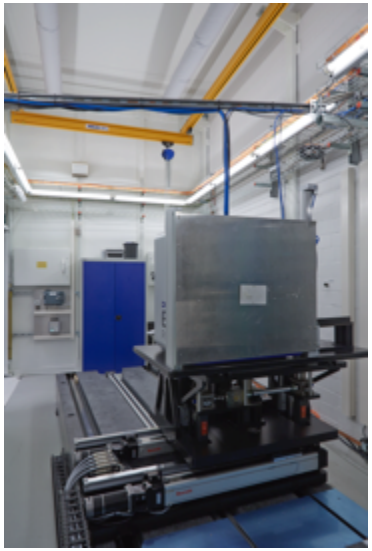




Very large unit cells



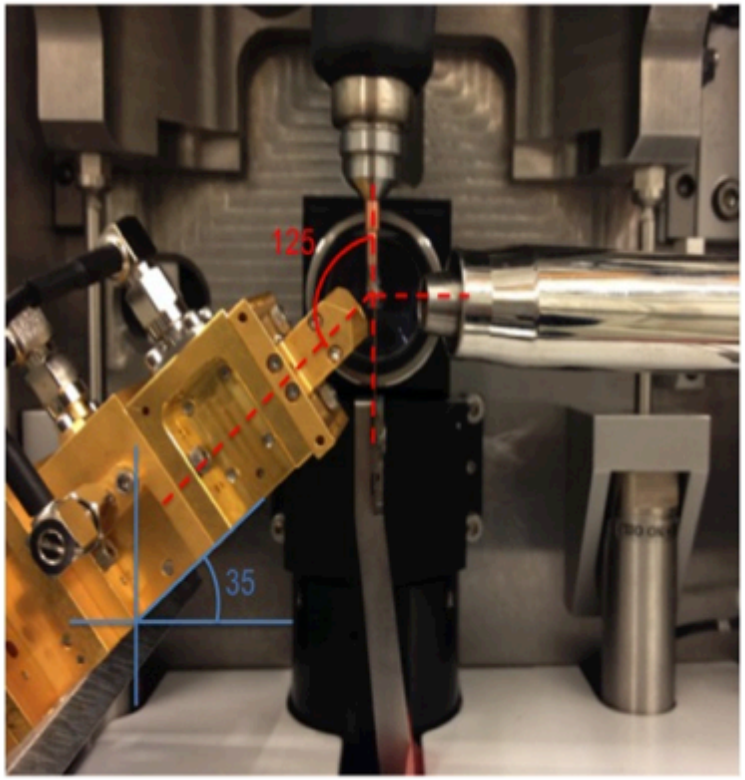


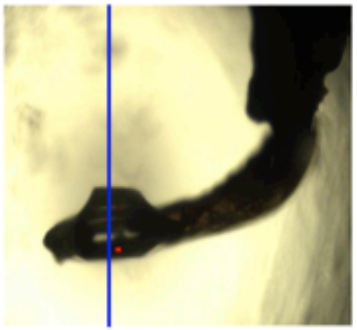


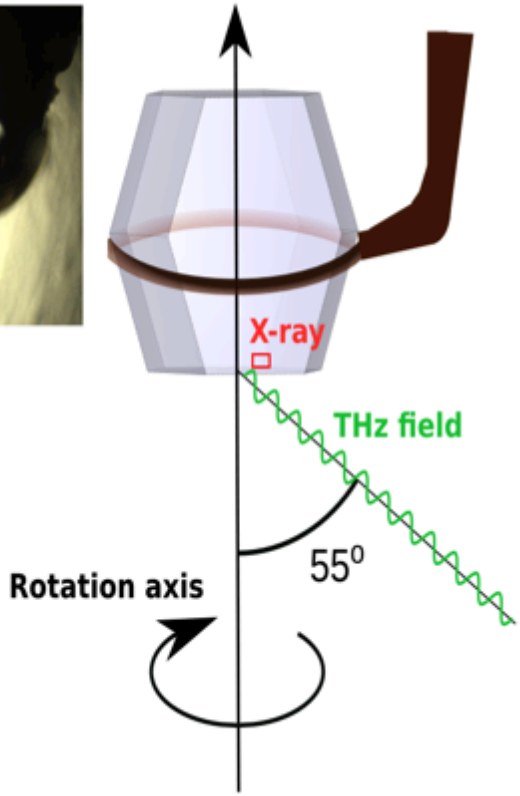
- Longest primitive cell dimension : 1850 Å
- Resolution 4.6 Å / 400 diffraction orders

- Parallel beam
- Mighty detector stage

Skimming geometry



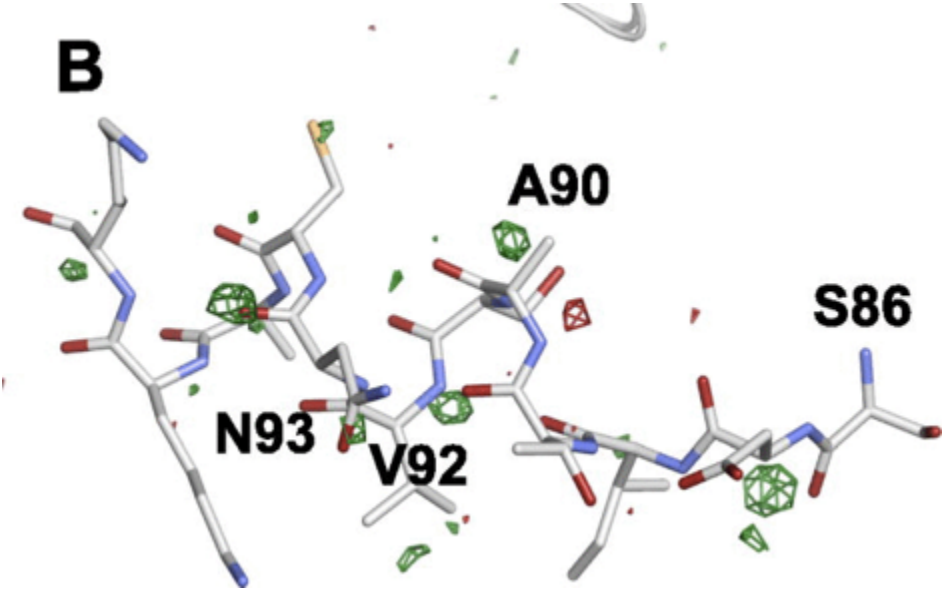




EMBL

Terahertz radiation induces non-thermal structural changes associated with Fröhlich condensation in a protein crystal

Ida V. Lundholm,[1,a)] Helena Rodilla,[2,a)] Weixiao Y. Wahlgren,[1] Annette Duelli,[1] Gleb Bourenkov,[3] Josip Vukusic,[2] Ran Friedman,[4] Jan Stake,[2] Thomas Schneider,[3] and Gergely Katona[1,b)]



3 sigma = 0.005 $e^-/Å^3$

Data collection:
Laser pulses at ½ frame rate
Superfine slicing – 0.005 deg.
odd/even data sets
7 lysozyme xtls

R_{merge} (%) [a, b]	9.4 (74.0)	9.4 (76.3)
$CC_{1/2}$ [a]	100.0 (99.1)	100.0 (99.1)
$\langle I/\sigma(I) \rangle$ [a]	75.6 (10.5)	75.6 (10.1)
Completeness (%)	91.1 (66.7)	90.9 (65.2)
Redundancy	197.0	197.0
Refinement		
No. reflections	11 830	11 829
R_{work}/R_{free} (%) [c]	16.6/19.1	16.6/19.0
No. atoms (protein)	1042	1042

Struct. Dyn. 2, 054702 (2015)

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  EMBL-Hamburg
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  Hamburg/Lübeck Universities
- Florent Cipriani, Alexadre Gobbo, Jeremy Sinoir
  Sandor Brokhauser, Andrew McCarthy
  EMBL-Grenoble
- Uli Zander, Sasha Popov, Igor Melnikov
  Christoph Müller-Dieckmann, Gordon A. Leonard
  Olof Svensson, Daniele De Sanctis ESRF Grenoble
- Ida Lindholm, Gergely Katona, University of Gothenburg
- Guenter Schneider, Karolinska, Roentgen-Angstroem Cluster