

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

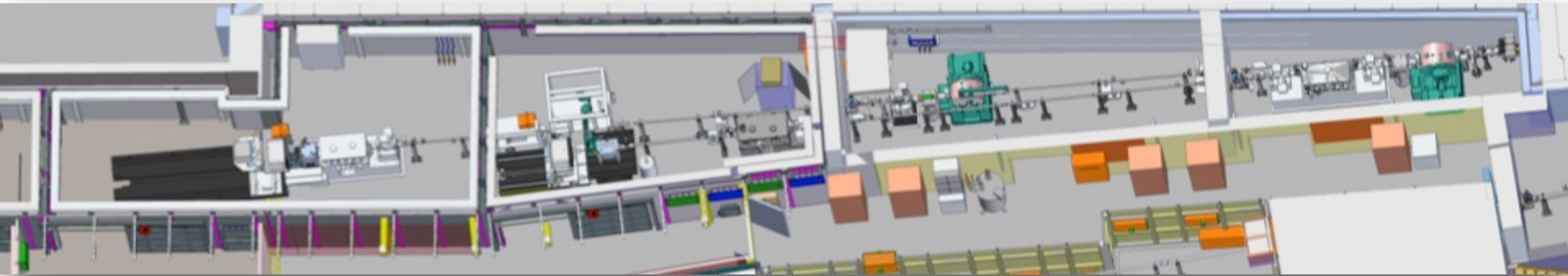
Gleb Bourenkov
EMBL Hamburg

MADaC 2015, SOLEIL

EMBL



EMBL@PETRAIII MX Beamlines



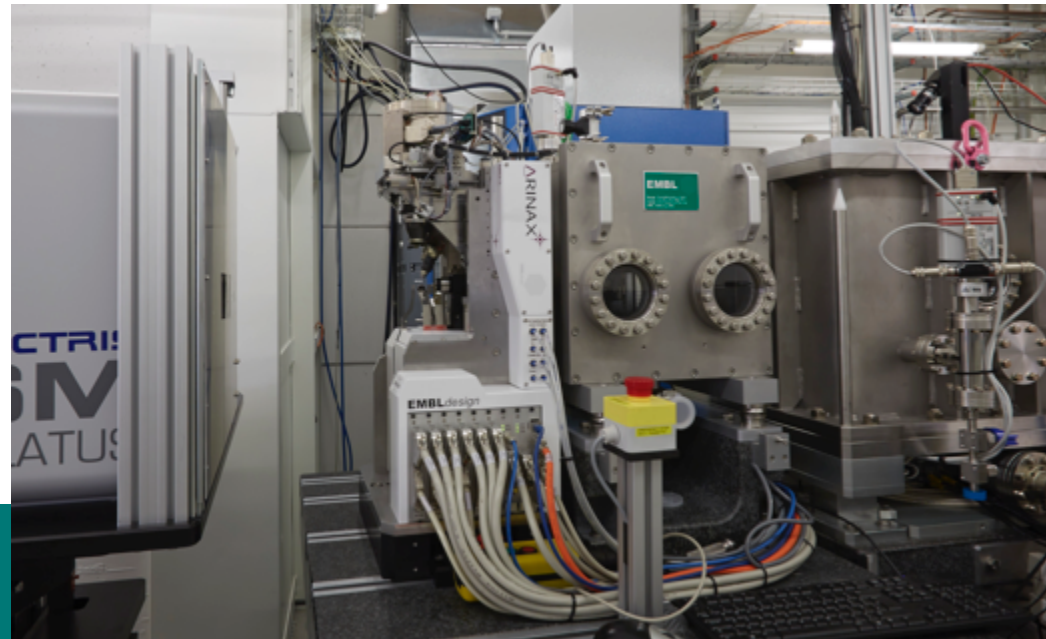
P13

4-15 keV,
30 μm focus
10 μm mini-beam option

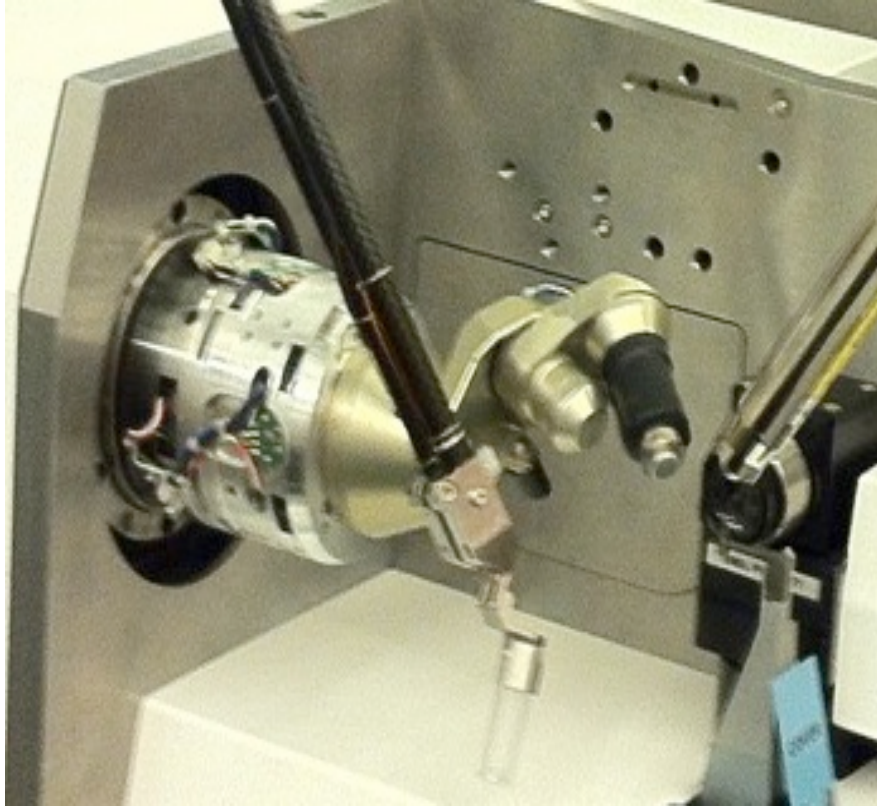


P14

6-25 [35] keV
4 x 5 μm focus
Large top-hat parallel beam option



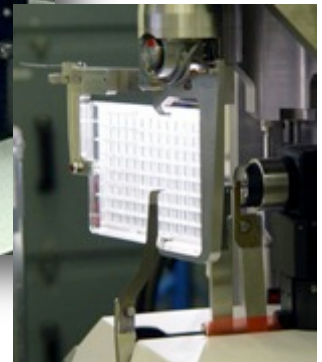
Persistently installed Mini-Kappa heads



P13/MD2

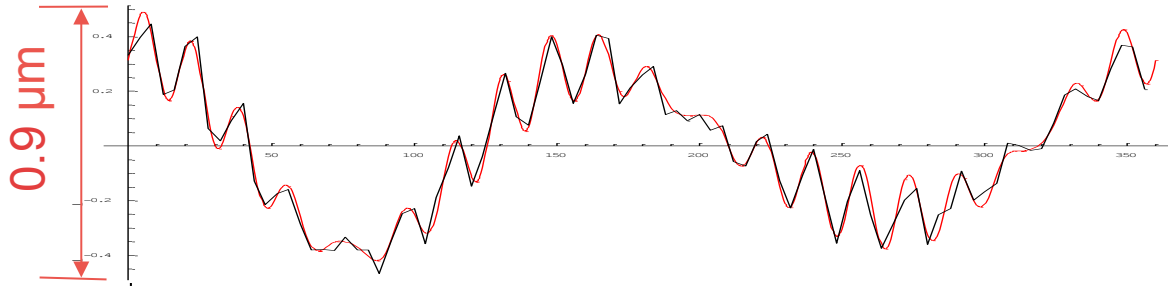


P14/MD3

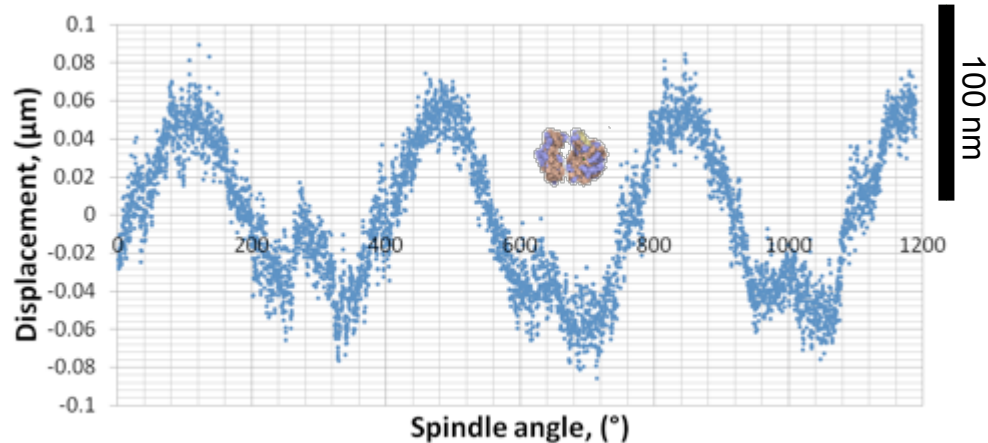


Measured sphere of confusion

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

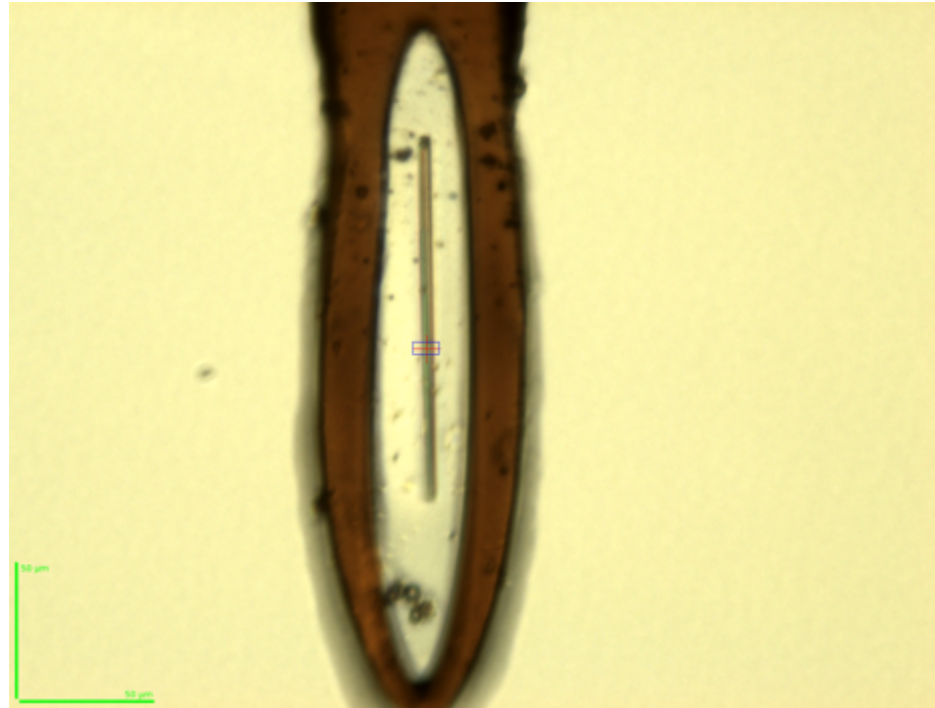


- MD3 – **0.20 μm** independent on the Kappa pose



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

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



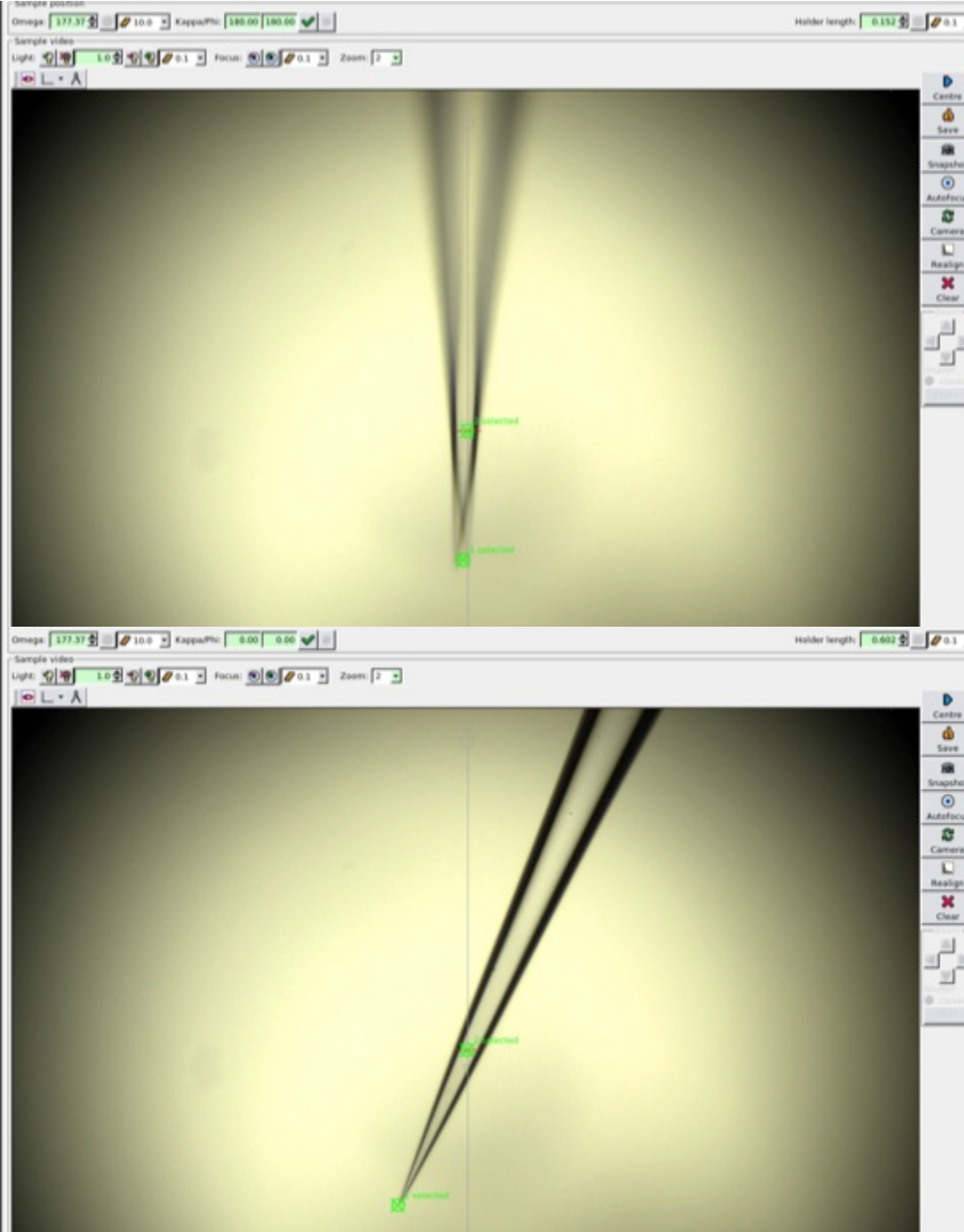
3x3x100 μm crystal
Structure solved at 1.7 Å resolution

Kappa in MxCuBE

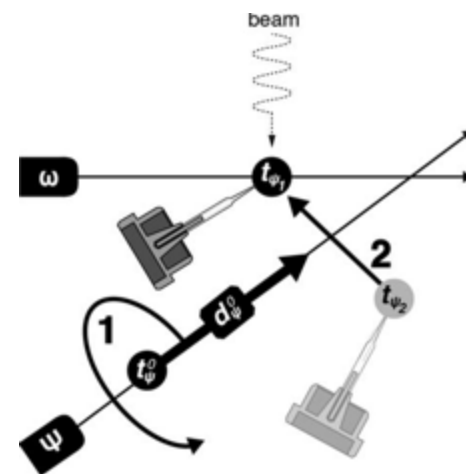
The screenshot displays the MxCuBE software interface, which is used for controlling the MX3000-3 Synchrotron. The interface is divided into several panels:

- Sample position:** Shows Omega (377.37) and Kappa/Phi (62.27 / 301.38). A red arrow points to the Kappa/Phi input fields.
- Sample video:** Shows a live video feed of the sample. A red circle highlights the Kappa/Phi input fields and the Focus/Zoom controls.
- Collection method:** Shows acquisition parameters such as Oscillation range (0.1000), Exposure time (0.0400), Energy (12.7000), Resolution (7.680), and Transmission (100.000).
- Camera/Realign:** A panel with a 'Camera' button and a 'Realign' button. A red circle highlights these buttons.
- Machine state:** Shows various machine parameters including Machine state text, Intensity monitor, Hatch temperature, Hatch humidity, Storage disc space, and Energy.

The Kappa/Phi input fields are highlighted with a red circle, and the Camera/Realign buttons are also highlighted with a red circle. A red arrow points to the Kappa/Phi input fields.



Centering corrections (Brockhauser et al, 2011)



accuracy $\sim 20 \mu\text{m}$

Prefix:

Run number:

Processing:

No. residues: Space group:

Unit cell:

a: b: c:

\alpha: \beta: \gamma:

Comments:

Beamstop distance:

Current:

Set to:

Queued multi-orientation data collections in MxCuBE

The image displays two overlapping windows from the MxCuBE software, used for configuring X-ray diffraction data collection. Both windows show the 'Collection method' set to 'Standard Collection' and the 'Acquisition' parameters.

Top Window Configuration:

- Proposal: MX0003 - Propo: [dropdown] Group: [dropdown] Set
- Sample list: Mode: Manually mounted Show SC
- Centring: Manual ISPyB
- Acquisition parameters:
 - Oscillation range: 0.1000 First image: 1
 - Oscillation start: 151.50 Number of images: 1800
 - Kappa: 90.0000 Phi: 90.00
 - Detector mode: C18
 - Exposure time (s): 0.0250
 - Energy (keV): [blank]
 - Resolution (Å): [blank]
 - Transmission (%): [blank]

Bottom Window Configuration:

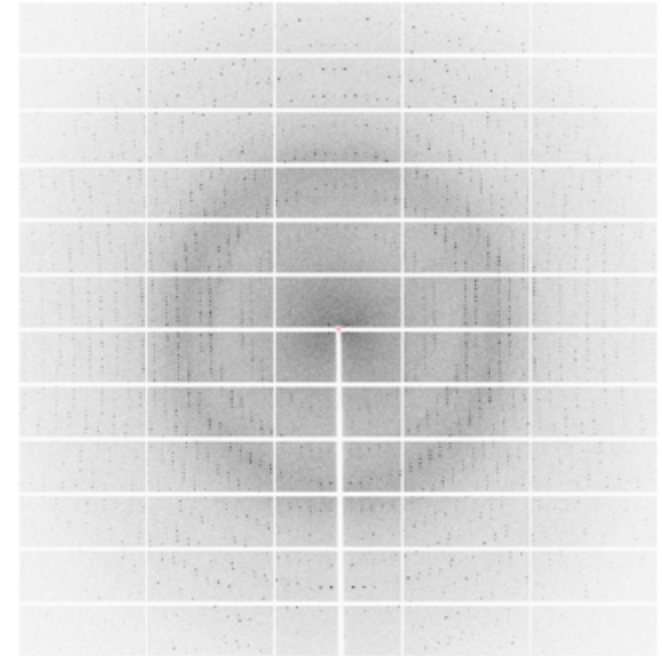
- Proposal: MX0003 - Propo: [dropdown] Group: [dropdown] Set
- Sample list: Mode: Manually mounted Show SC
- Centring: Manual ISPyB
- Acquisition parameters:
 - Oscillation range: 0.1000 First image: 1
 - Oscillation start: 151.5000 Number of images: 1800
 - Kappa: 35.0000 Phi: 90.00
 - Detector mode: C18
 - Exposure time (s): 0.0250
 - Energy (keV): 12.0000 MAD: [dropdown]
 - Resolution (Å): 5.661
 - Transmission (%): 100.000
- Additional options: Inverse beam, Shutterless, Data location

Sample List Hierarchy (Bottom Window):

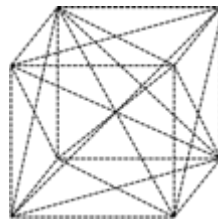
- manually-mounted
 - Standard - 9
 - sample-centring (kappa: 9...)
 - prefix_2 (Point - not defin...)
 - Standard - 11
 - sample-centring (kappa: 4...)
 - prefix_3 (Point - not defin...)
 - Standard - 13
 - prefix_4 (Point - 2)
 - prefix_5 (Point - 1)

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

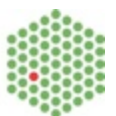


HKLs – direction to align with the spindle

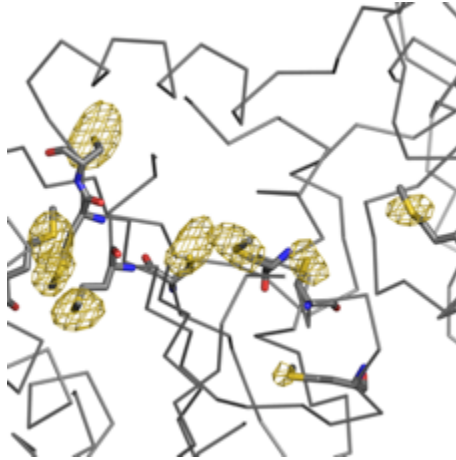
Del – closest possible angle HKL to spindle

Kap, Phi – alignment angles

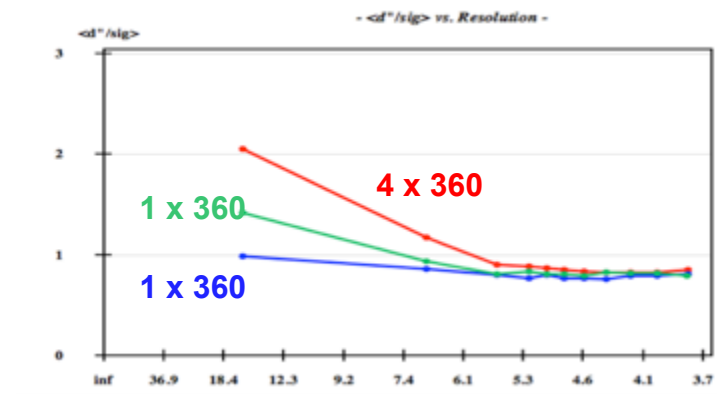
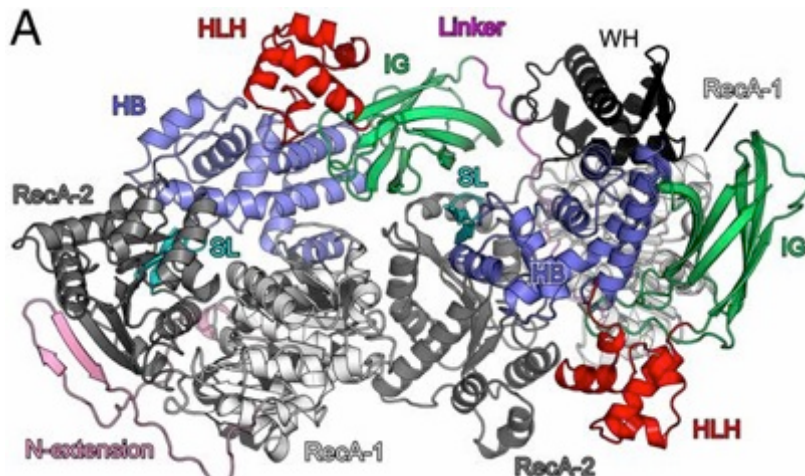
HKLb – directions to align with the spindle-beam plane at $\Omega = \text{Omg}$ EMBL



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.



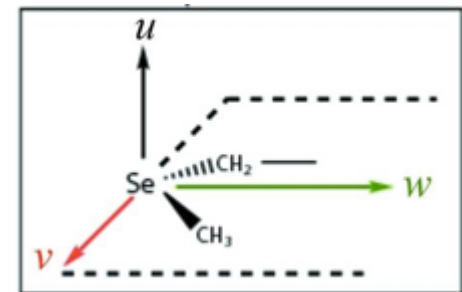
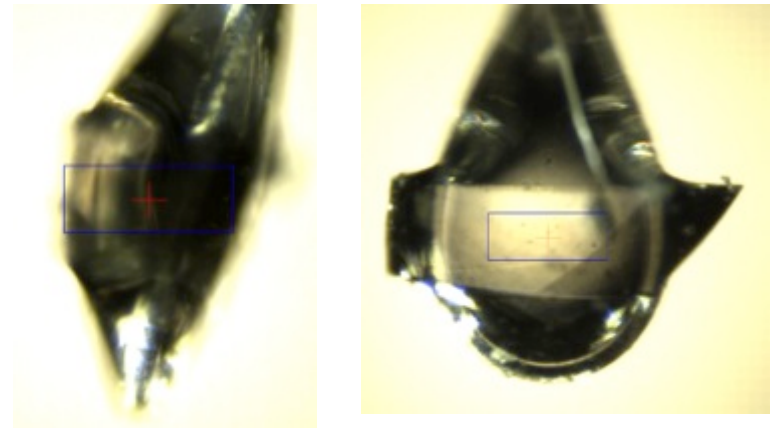
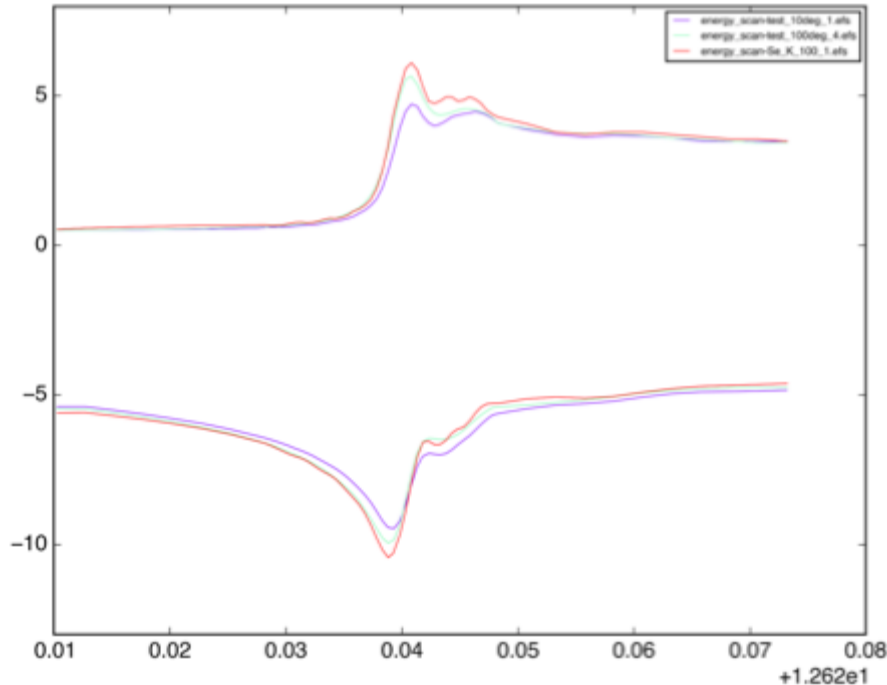
- 200 kDa
- 6 Å anomalous data
6 keV (DCM de-tune)
4 κ -settings
- >60 sulphur positions -



Anisotropic anomalous scattering with vertical spindle

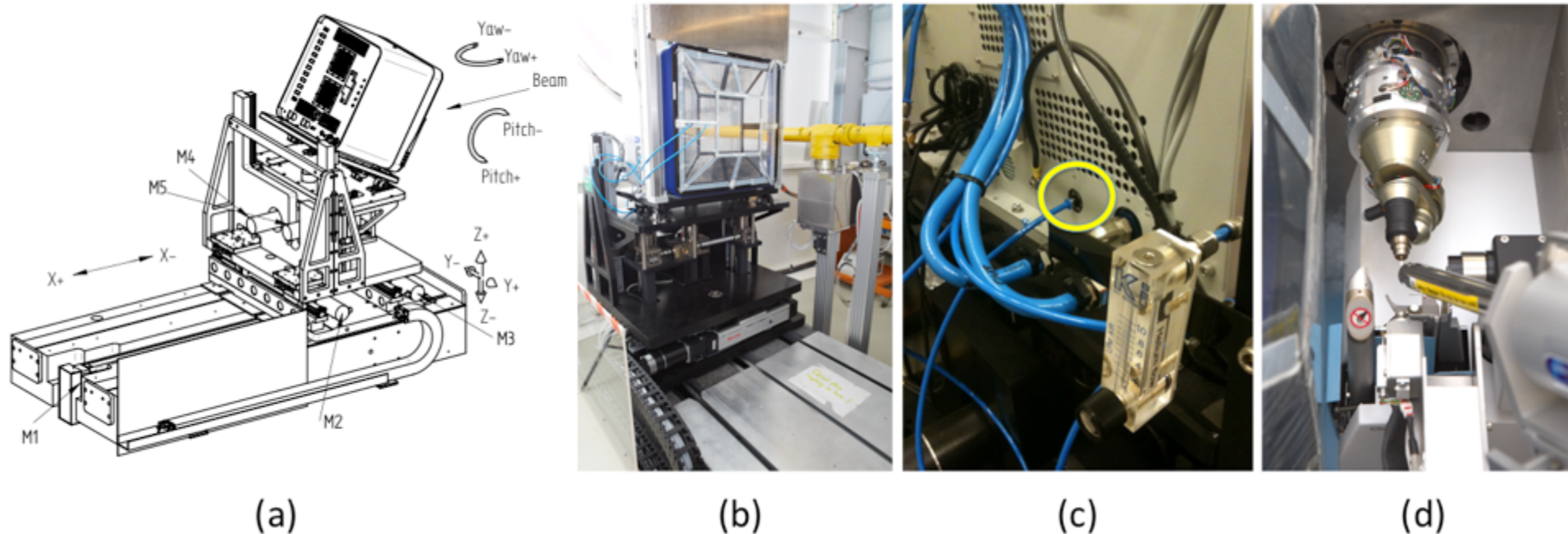
- Se K edge fluorescence scans of orthorhombic FAE crystal, 64 SeMets/Unit cell at different Omega angles

U. Zander and D. De Sanctis



Schiltz & Bricogne, 2008

Low energies at P13

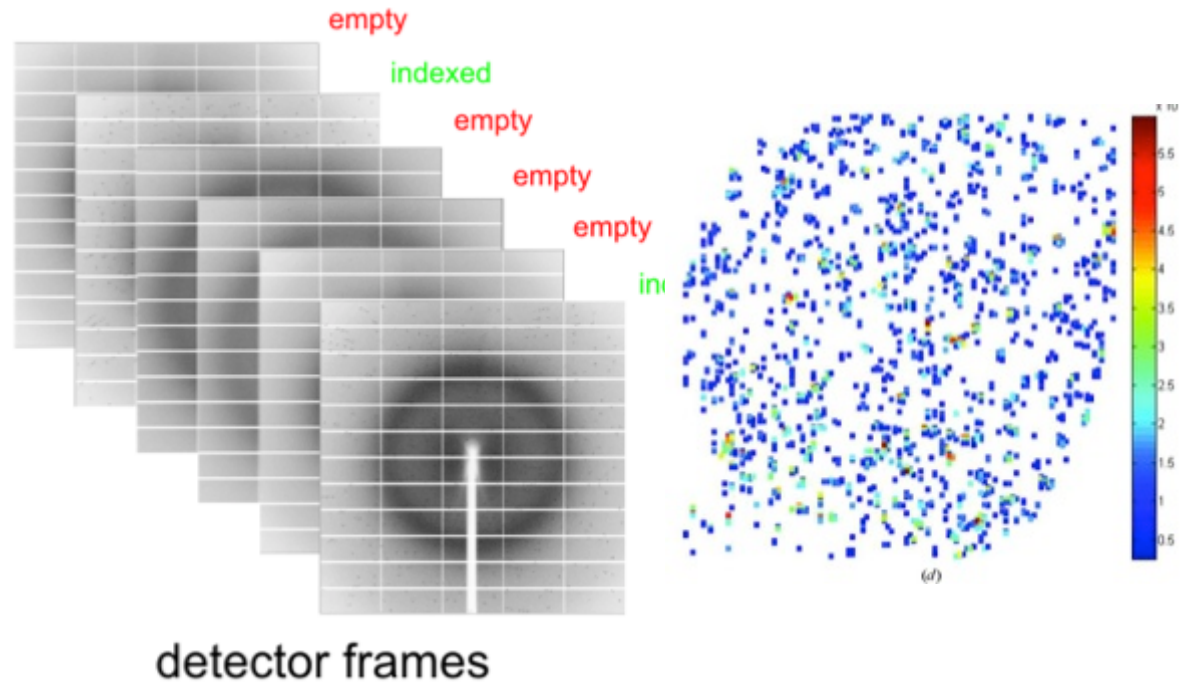
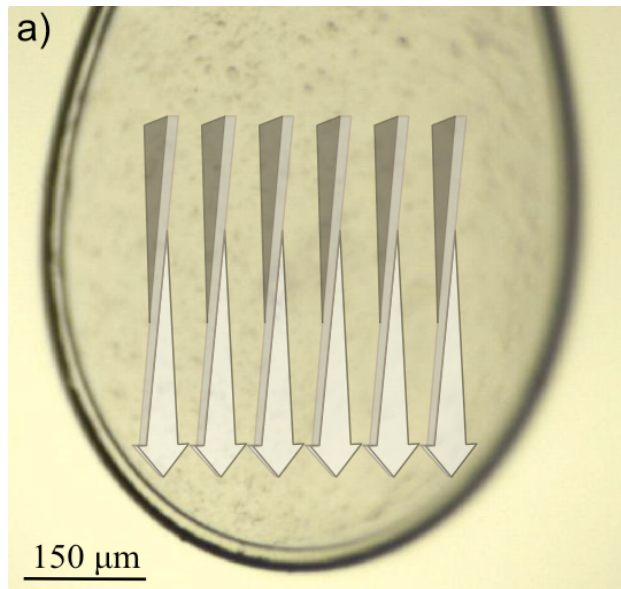


DECTRIS Pilatus 6MF with calibration tables for low energies

Helium cone

Pseudo 2 Θ -arm

Serial Synchrotron Crystallography (SSX)



- 30000 frames collected via series of parallel helical scans $\pm 60^\circ$ 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



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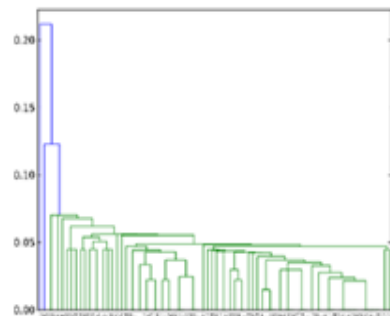
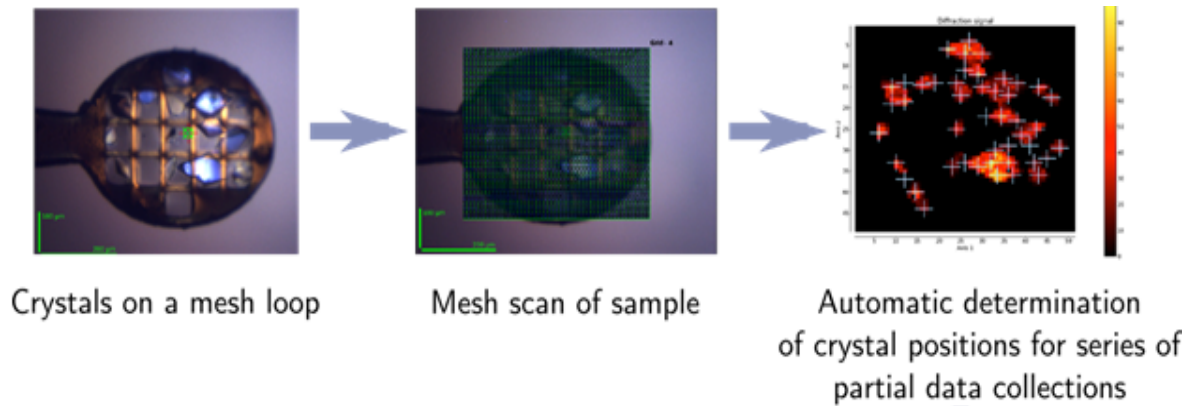
Received 7 November 2013
Accepted 16 December 2013

research papers

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

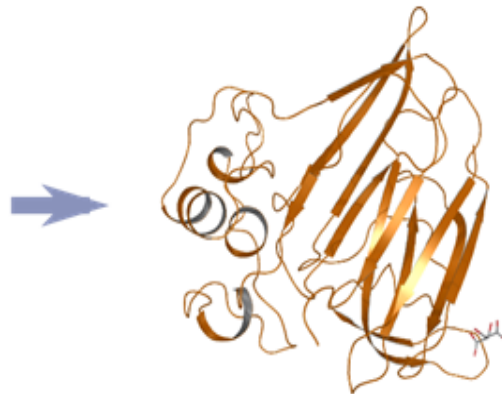
Cornelius Gati,^{1*} Gleb Bourenkov,^{2,3*} Marco Klinge,⁴ Dirk Rehders,⁵ Francesco Stellato,⁶ Dominik Oberthür,^{4,6} Oleksandr Yefanov,⁶ Benjamin P. Sommer,^{6,7} Stefan Mogk,⁸ Michael Duszynski,⁹ Christian Betzel,⁴ Thomas R. Schneider,¹⁰ Henry N. Chapman^{11,12} and Lars Redecke^{1*}

Automated multi-(μ)-crystal data collection

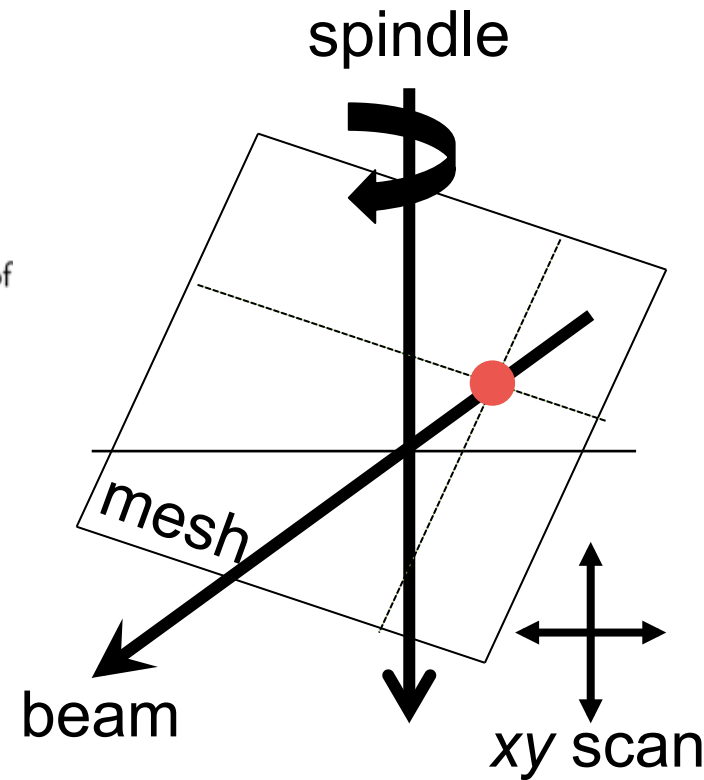


Dataset combination based on hierarchical cluster analysis:

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

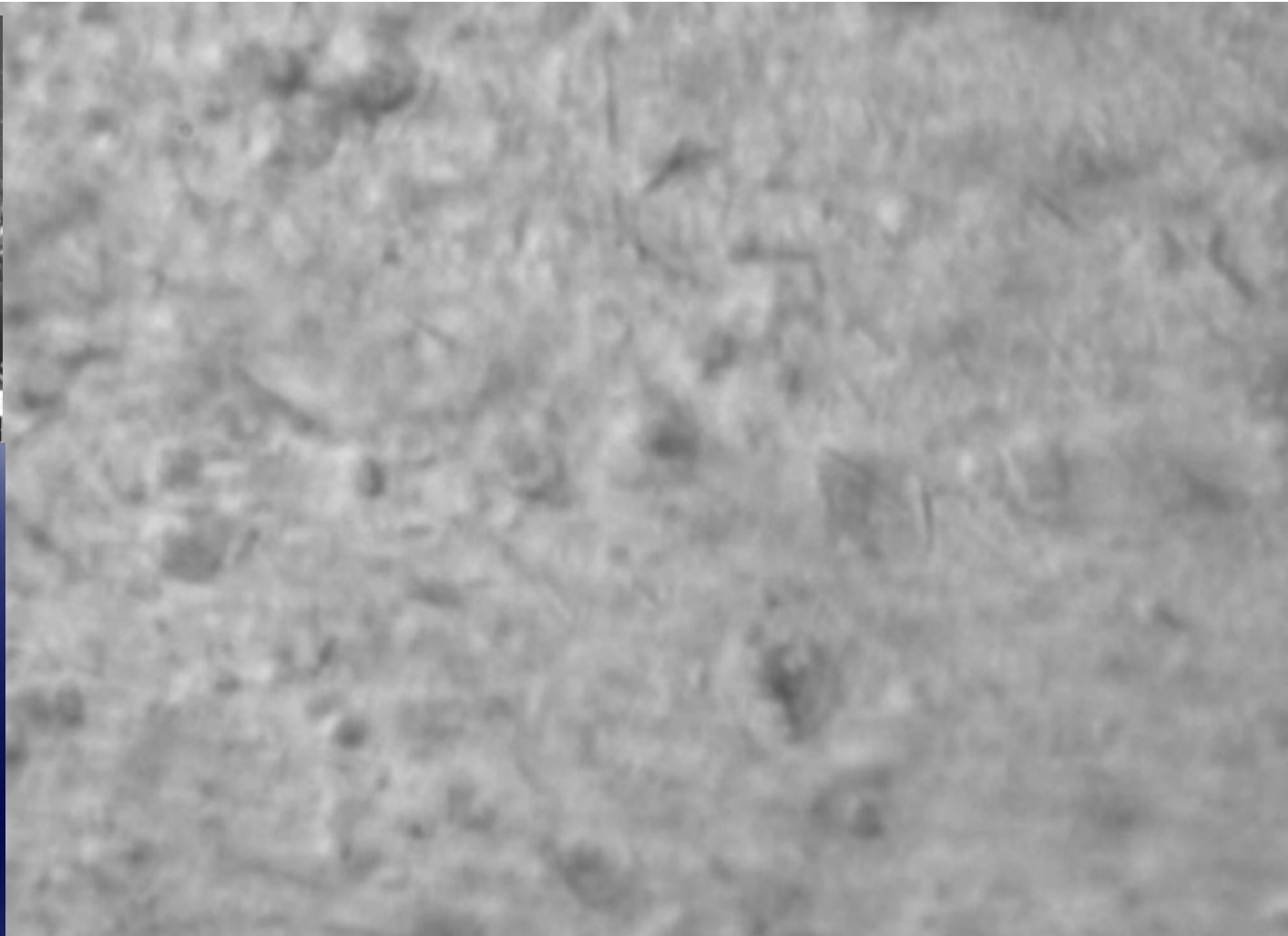
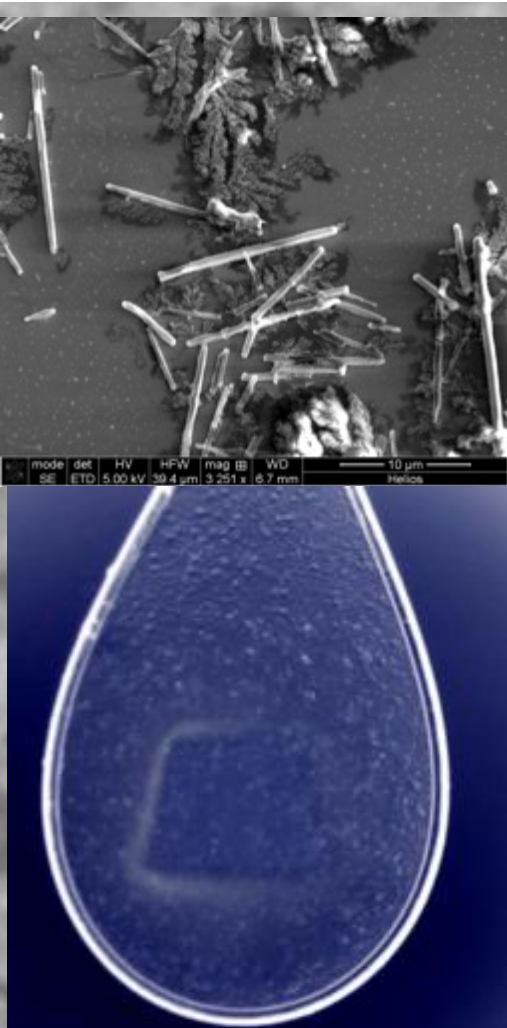


Structure solution



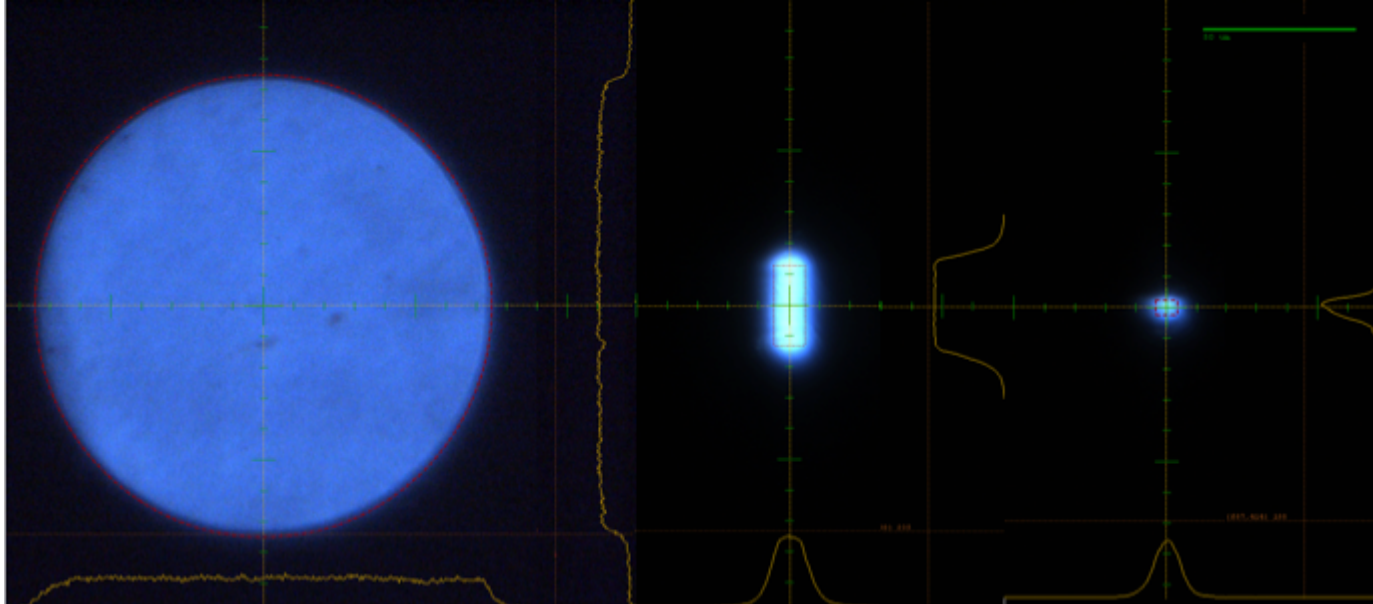
mesh alignment to the xy scan plane

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



Matching the beam to the crystal

Unfocused – Hor/Ver focused – Double focused



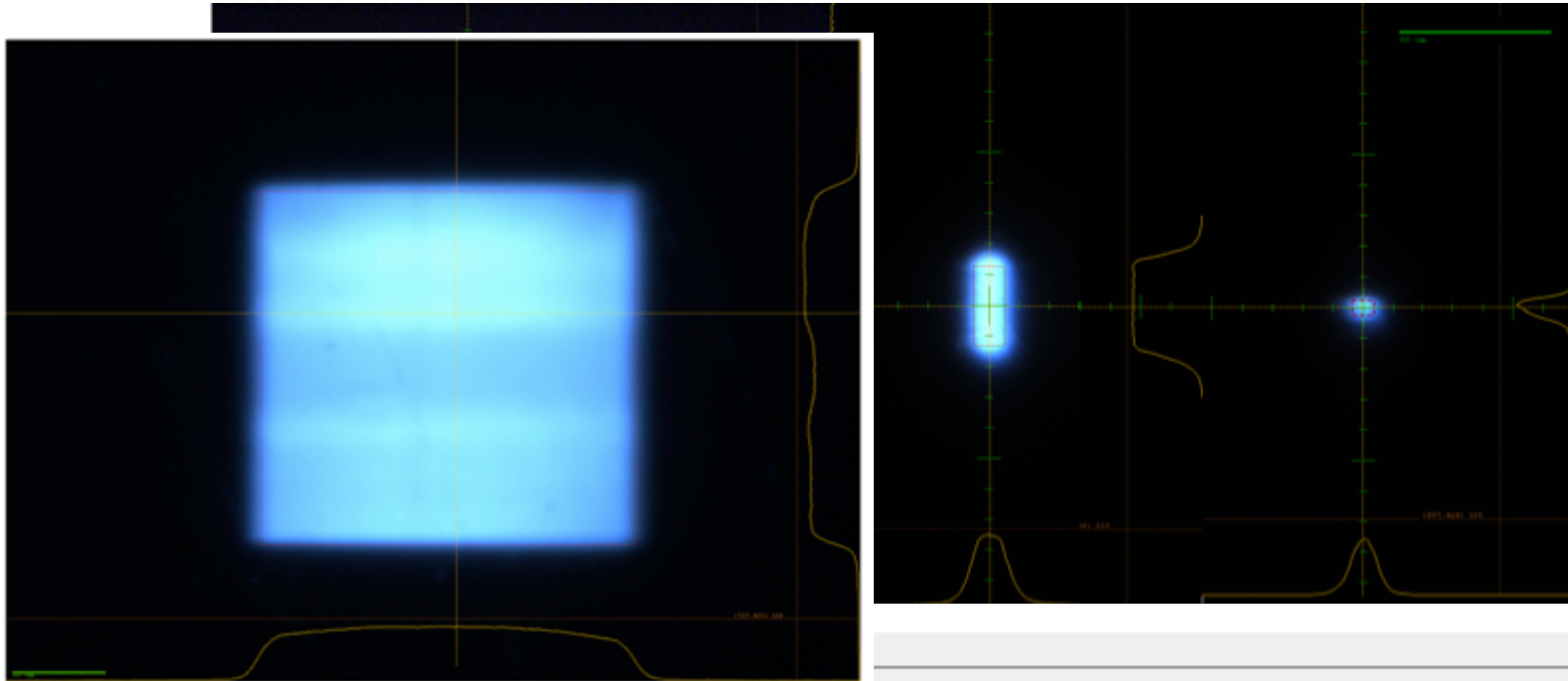
Beam definition

Focusing	Size	Slits	Aperture
Horizontal	Horizontal: 0.030 mm Vertical: 0.022 mm	Hor: 0.265 mm 0.005 Ver: 0.022 mm 0.005	150μm

5 to 500 μm in 20 seconds

Matching the beam to the crystal

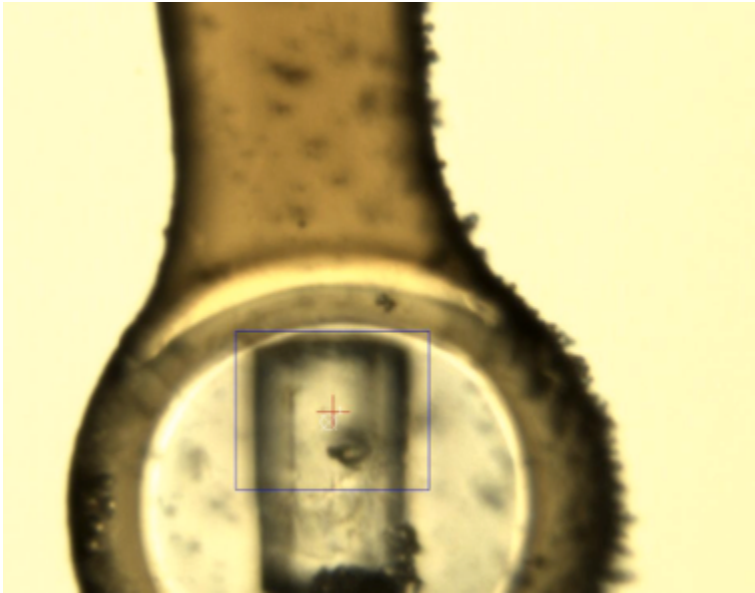
Unfocused – Hor/Ver focused – Double focused



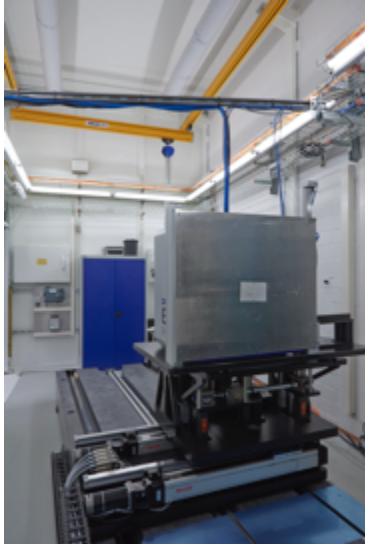
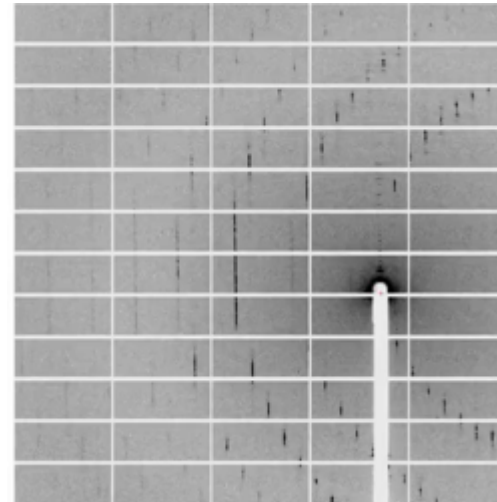
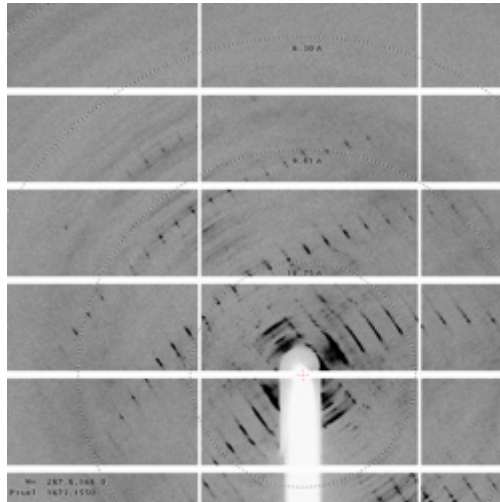
Focusing	Size	Slits	Aperture
Horizontal	Horizontal: 0.030 mm	Hor: 0.265 mm 0.005	150mu
Unfocused	Vertical: 0.022 mm	Ver: 0.022 mm 0.005	
Horizontal			
Double			

5 to 500 μm in 20 seconds

Matching crystals to the beam

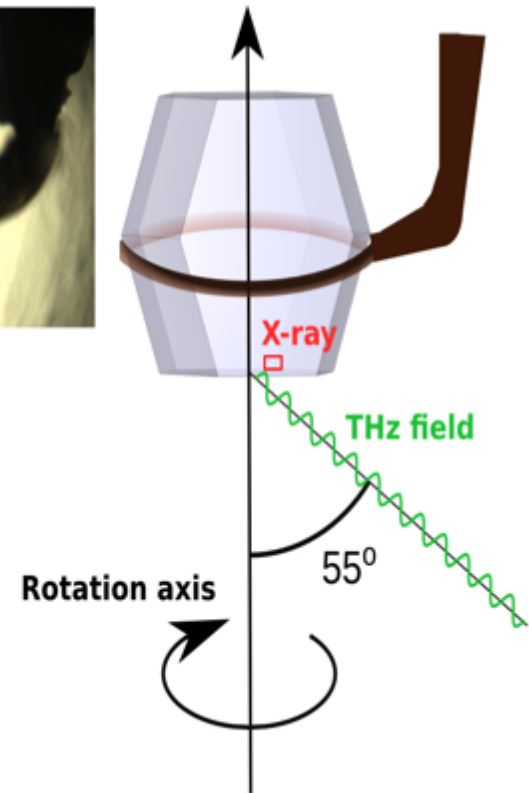
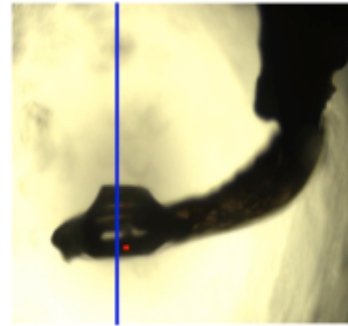
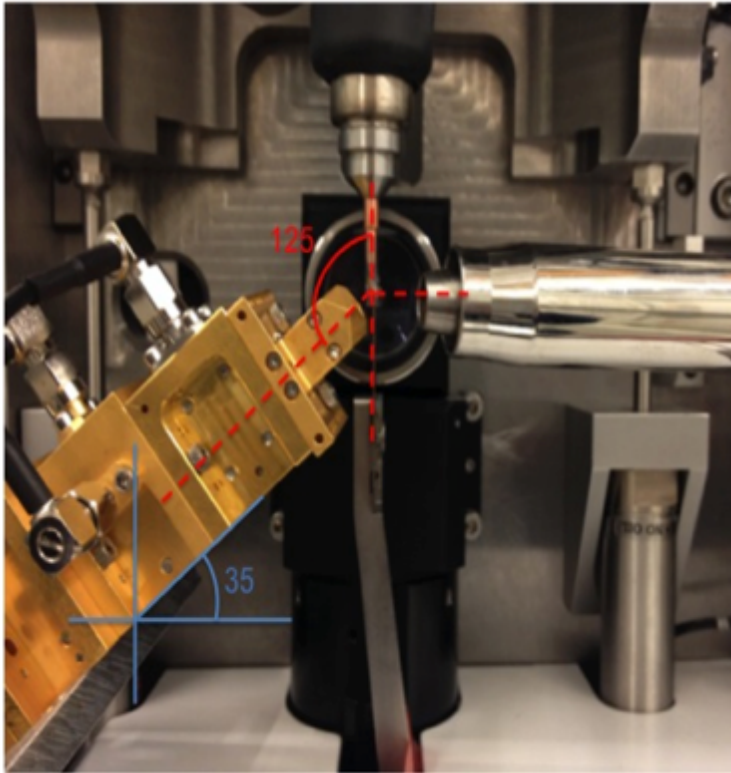


Very large unit cells



- Longest primitive cell dimension :
1850 Å
- Resolution 4.6 Å / 400 diffraction orders
- Parallel beam
- Mighty detector stage

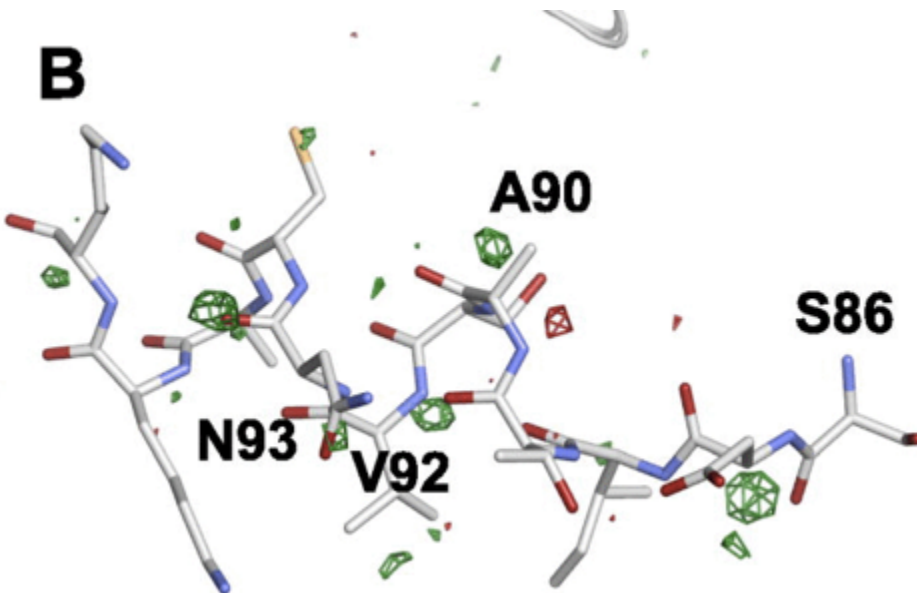
Skimming geometry



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)}

Data collection:
Laser pulses at 1/2 frame rate
Superfine slicing – 0.005 deg.
odd/even data sets
7 lysozyme xtls



3 sigma = 0.005 e-/Å³

R _{merge} (%) ^{a, b}	9.4 (74.0)	9.4 (76.3)
CC _{1/2} ^a	100.0 (99.1)	100.0 (99.1)
⟨I/σ(I)⟩ ^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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Hamburg/Lübeck Universities
- Florent Cipriani, Alexandre Gobbo, Jeremy Sinoir
Sandor Brokhauser, Andrew McCarthy
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Christoph Müller-Dieckmann, Gordon A. Leonard
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- Ida Lindholm, Gergely Katona, University of Gothenburg
- Guenter Schneider, Karolinska, Roentgen-Angstroem Cluster

