Serial, In-situ, Low Energy and Large Complex Crystallography at EMBL@PETRAIII beamlines

Gleb Bourenkov

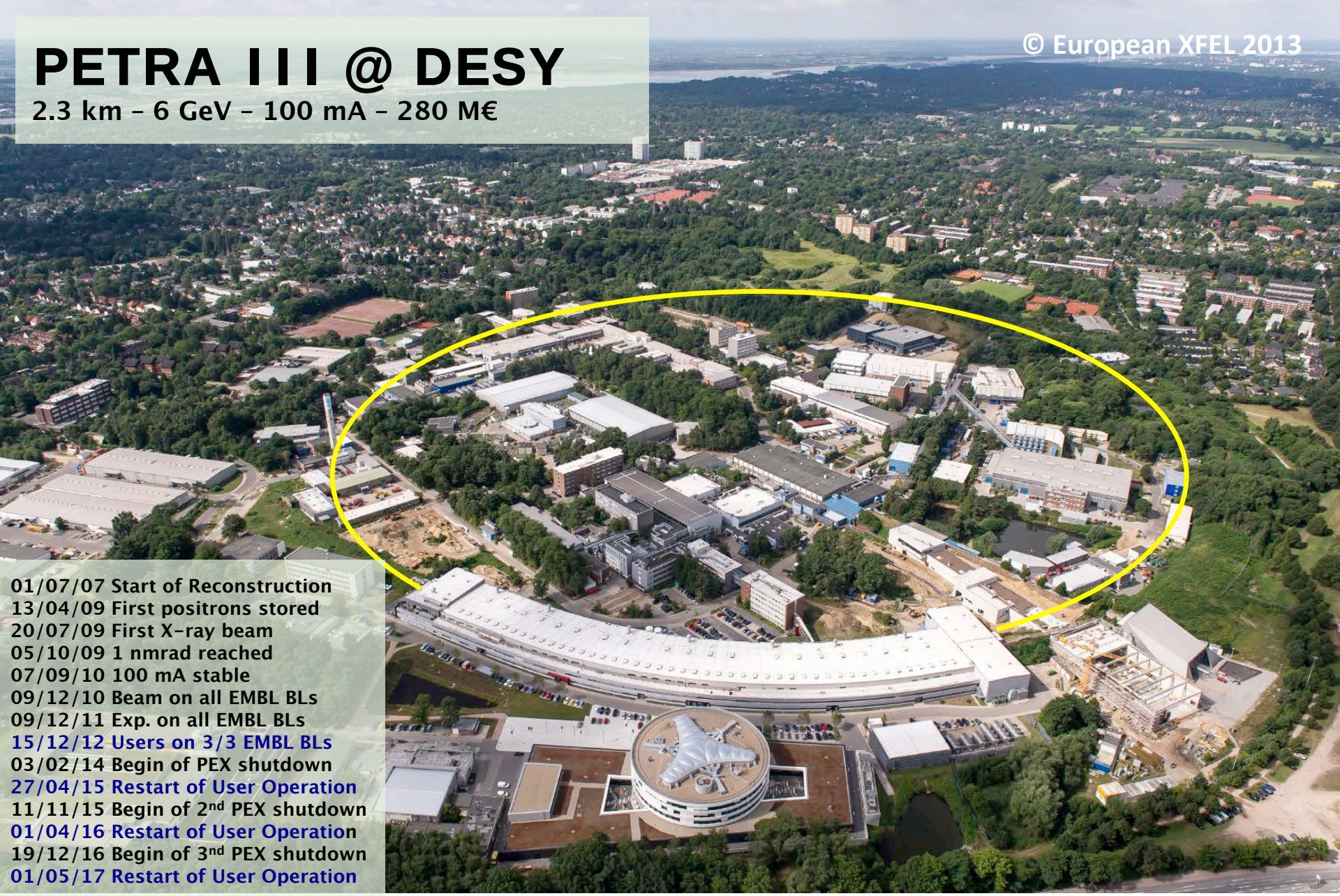
EMBL Hamburg

Beyond the black boxes, Strasbourg
08.10.2016

EMBL

PETRA III @ DESY

2.3 km – 6 GeV – 100 mA – 280 M€



01/07/07 Start of Reconstruction
13/04/09 First positrons stored
20/07/09 First X-ray beam
05/10/09 1 nmrad reached
07/09/10 100 mA stable
09/12/10 Beam on all EMBL BLs
09/12/11 Exp. on all EMBL BLs
15/12/12 Users on 3/3 EMBL BLs
03/02/14 Begin of PEX shutdown
27/04/15 Restart of User Operation
11/11/15 Begin of 2nd PEX shutdown
01/04/16 Restart of User Operation
19/12/16 Begin of 3nd PEX shutdown
01/05/17 Restart of User Operation

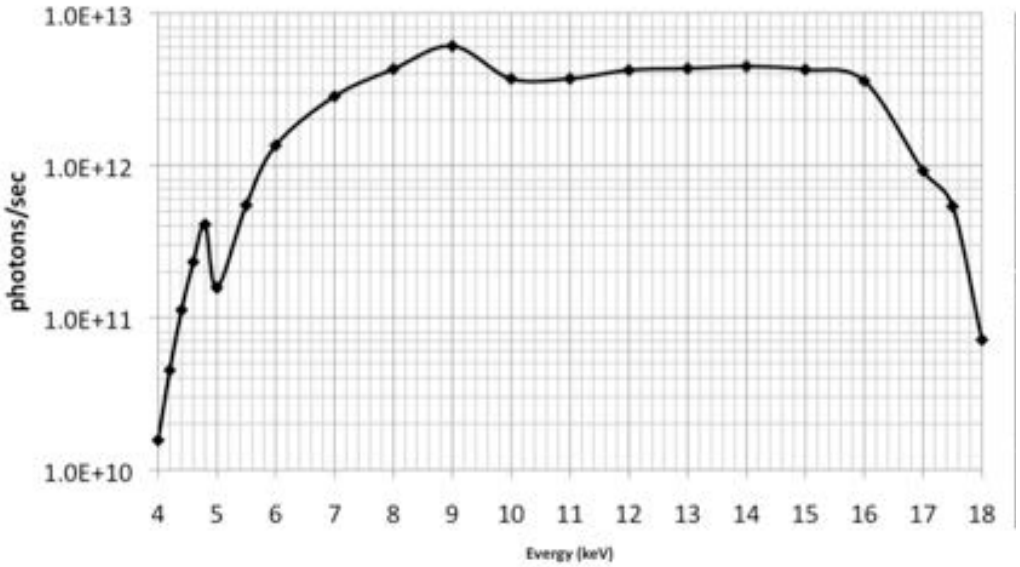
P13

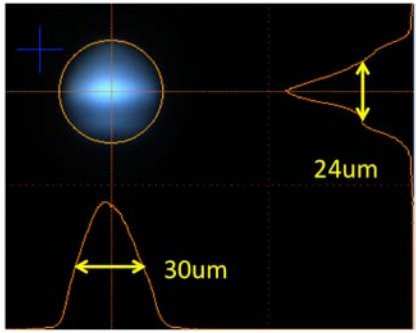
4-15 keV, 25 x 30 µm focus

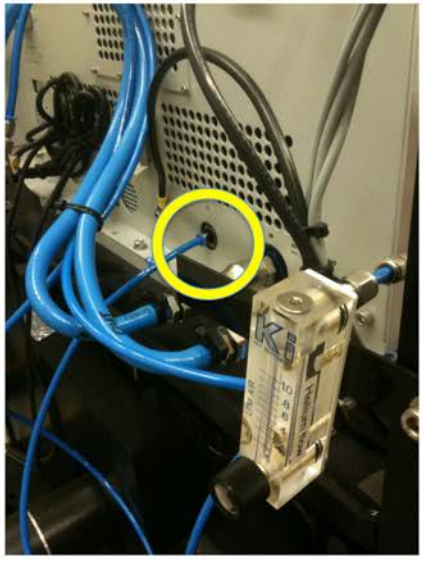
P14

7- 35 keV, 4 x 5 µm focus
rapid switch to large high flux beam

Low energy setup at P13



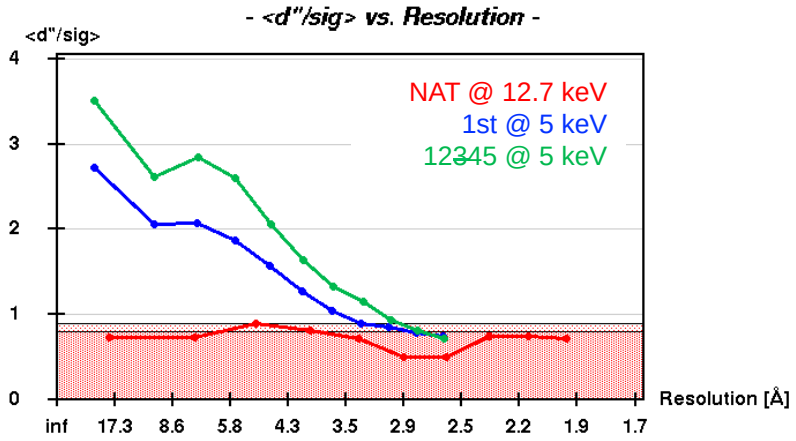


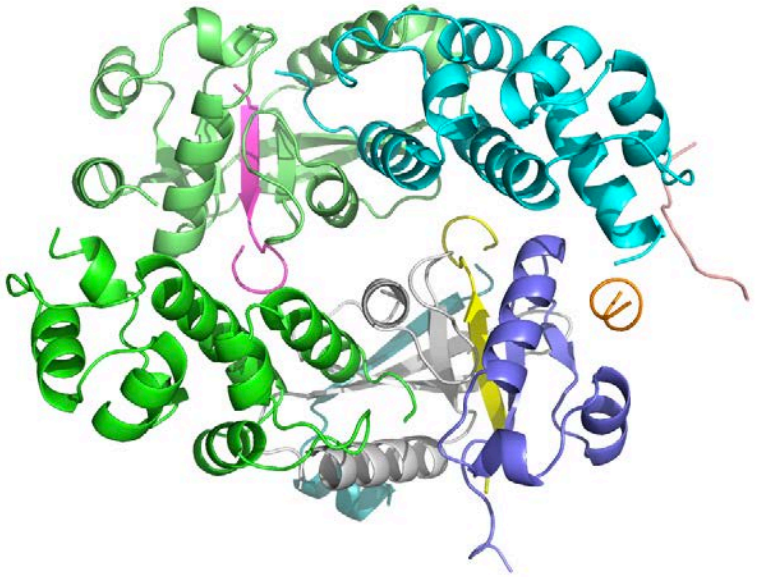


P13: S-SAD at 5 keV

- 600 a.a. residues including 18 sulphurs
- <ΔF/F> @ 5 keV: 1.8%

- Data collection started: 18:22 hrs
- Native finished 20:06 hrs
- Traced: 20:30 hrs





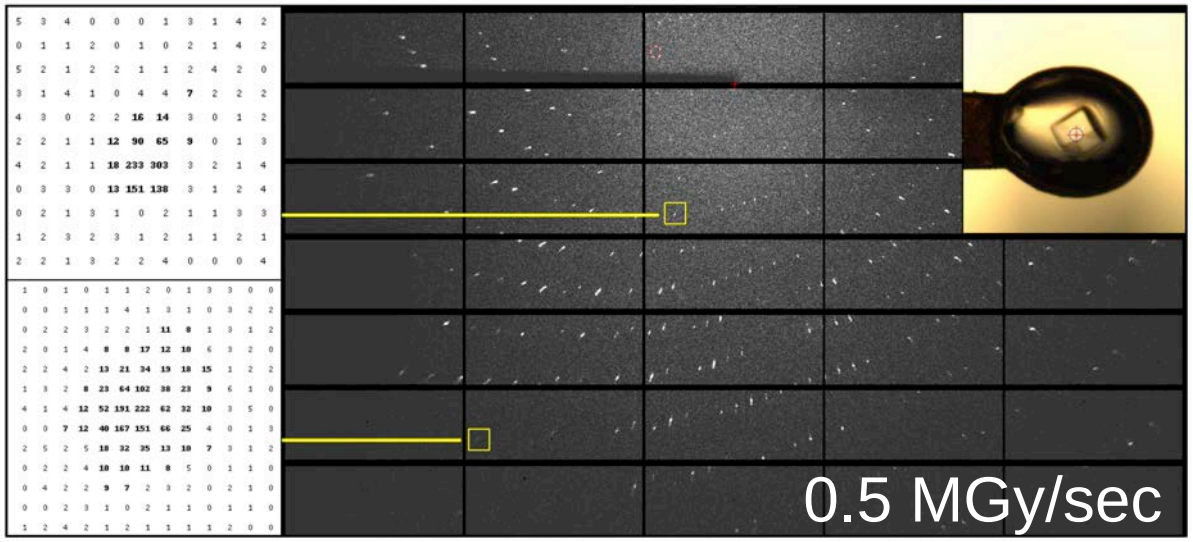
Michele Cianci

Low exogenous background

Backrgound
phot/pix/mm

1.7 ± 0.1

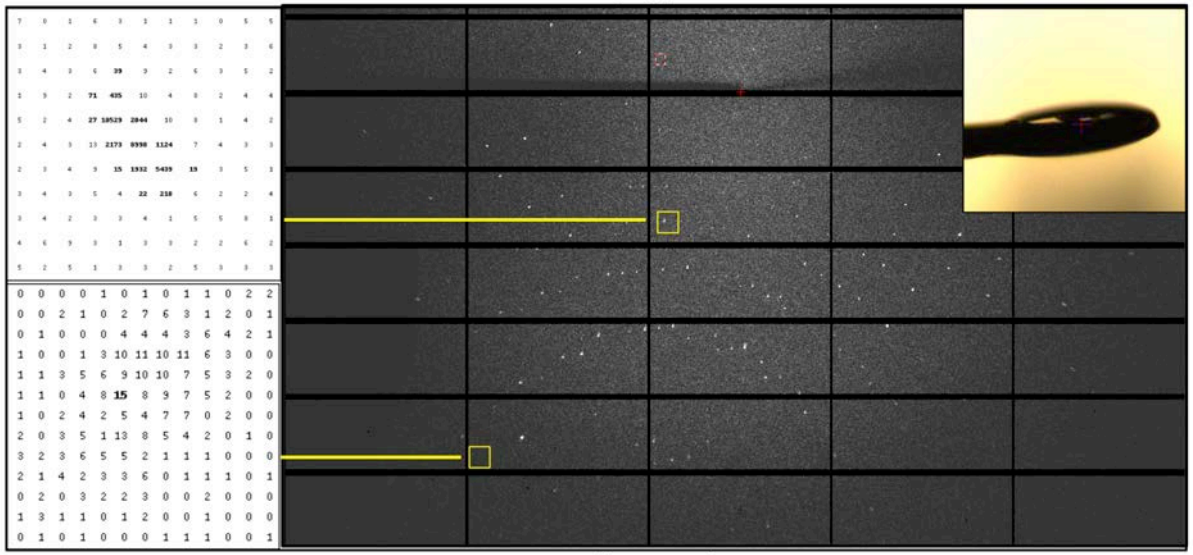
0.13 ± 0.03



(top)

1.5 ± 0.1

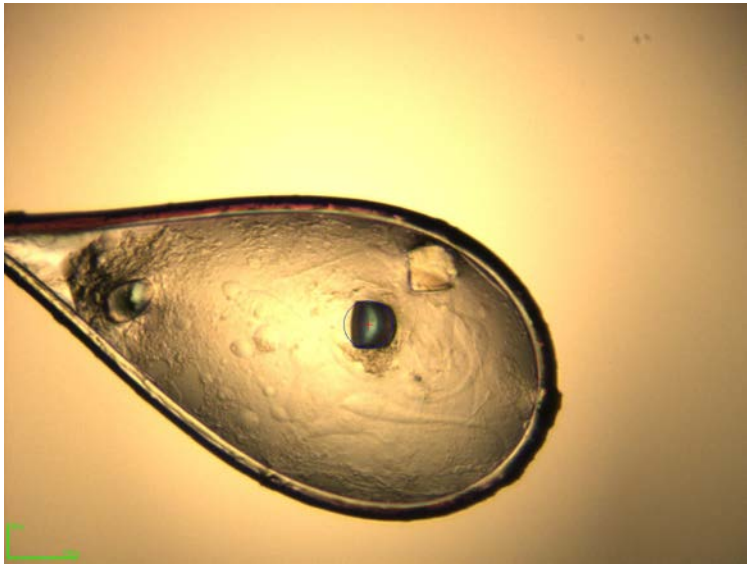
0.19 ± 0.04

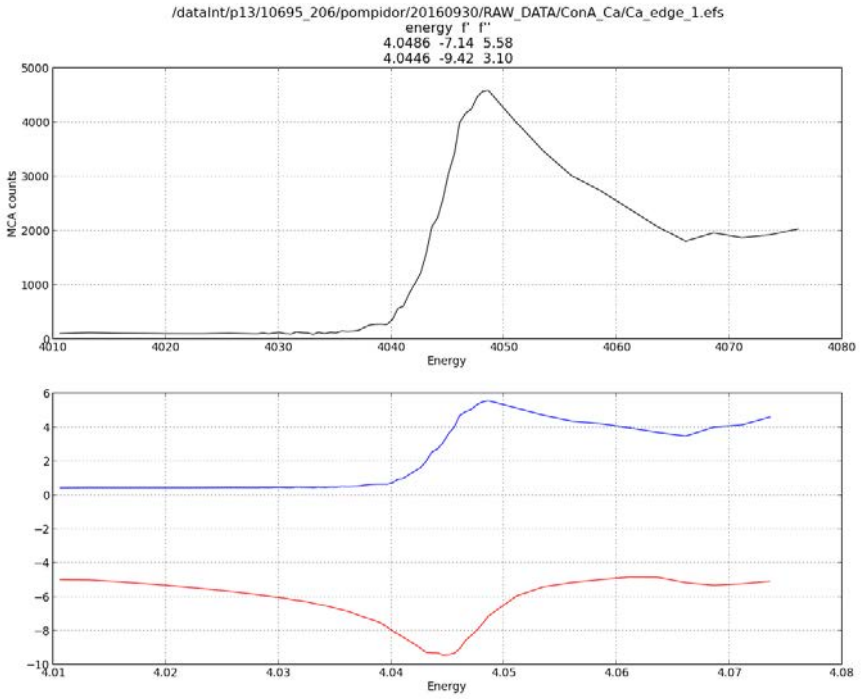


Data collection at low energy

Guillaume Pompidor

- Ca *K* absorption edge scan (4.05 keV)
- Concanavalin A crystal (70 x 70 x 20 μm^3)





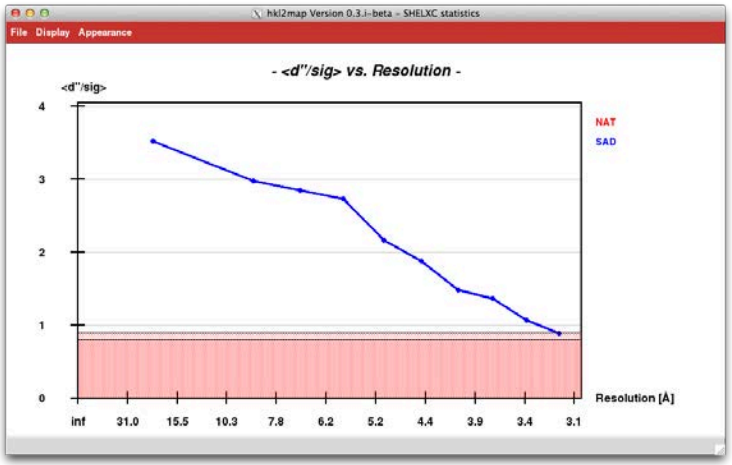
Data collection at low energy

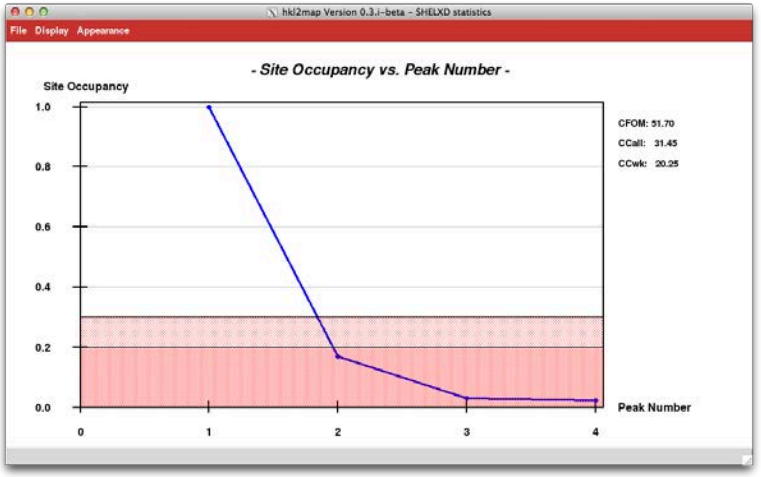
- Concanavalin A crystal (70 x 70 x 20 μm^3)
- 10 % transmission
- Data collection at the peak

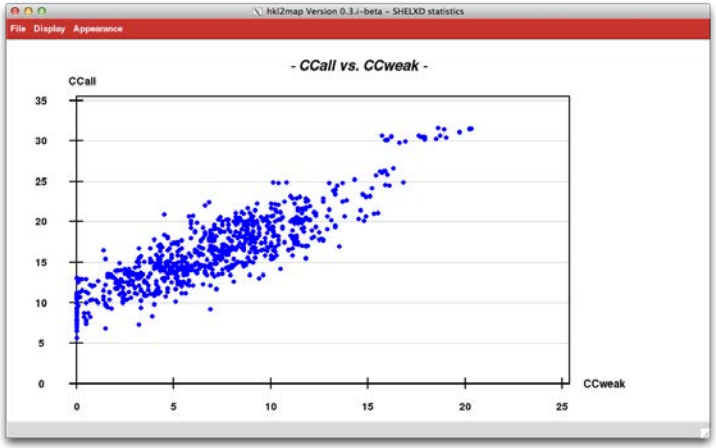
SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE >= -3.0 AS FUNCTION OF RESOLUTION

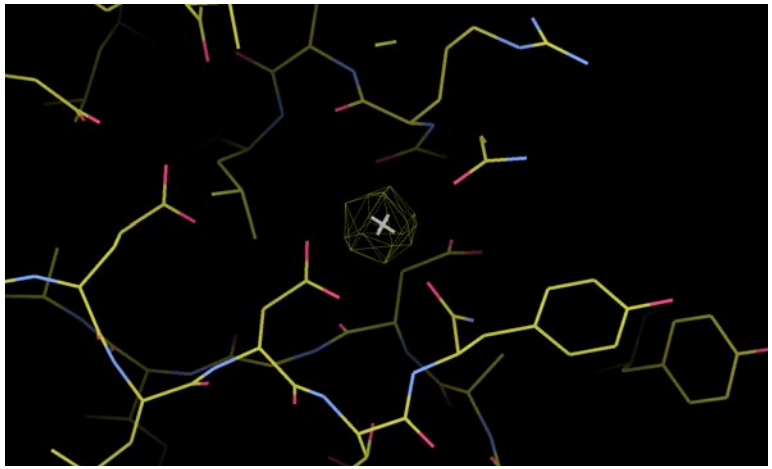
RESOLUTION LIMIT	NUMBER OF REFLECTIONS OBSERVED	UNIQUE	POSSIBLE	COMPLETENESS OF DATA	R-FACTOR observed	R-FACTOR COMPARED expected	I/SIGMA	R-meas	CC(1/2)	Anomal Corr	SigAno	Nano
9.20	2056	319	322	99.1%	5.2%	5.6%	2056	28.70	5.7%	99.7*	89*	3.349	118
6.54	3856	563	563	100.0%	6.4%	6.5%	3856	24.75	7.0%	99.7*	85*	2.919	238
5.35	4533	712	714	99.7%	8.1%	7.5%	4531	20.38	8.9%	99.4*	76*	2.289	317
4.64	5672	861	862	99.9%	8.2%	7.1%	5671	22.58	9.0%	99.4*	65*	1.931	384
4.15	5919	979	981	99.8%	8.5%	7.7%	5914	19.23	9.3%	99.2*	54*	1.573	449
3.79	6403	1069	1072	99.7%	9.5%	10.5%	6397	14.74	10.5%	99.0*	45*	1.212	488
3.51	6633	1164	1174	99.1%	11.4%	12.6%	6623	11.84	12.6%	98.3*	35*	1.000	535
3.28	6349	1206	1215	99.3%	15.9%	17.8%	6326	7.92	17.7%	97.5*	32*	0.879	545
3.10	6272	1245	1351	92.2%	22.9%	25.6%	6212	5.14	25.7%	95.0*	26*	0.865	546
total	47693	8118	8254	98.4%	9.7%	9.9%	47586	14.95	10.7%	99.2*	60*	1.475	3620

Data collection at low energy









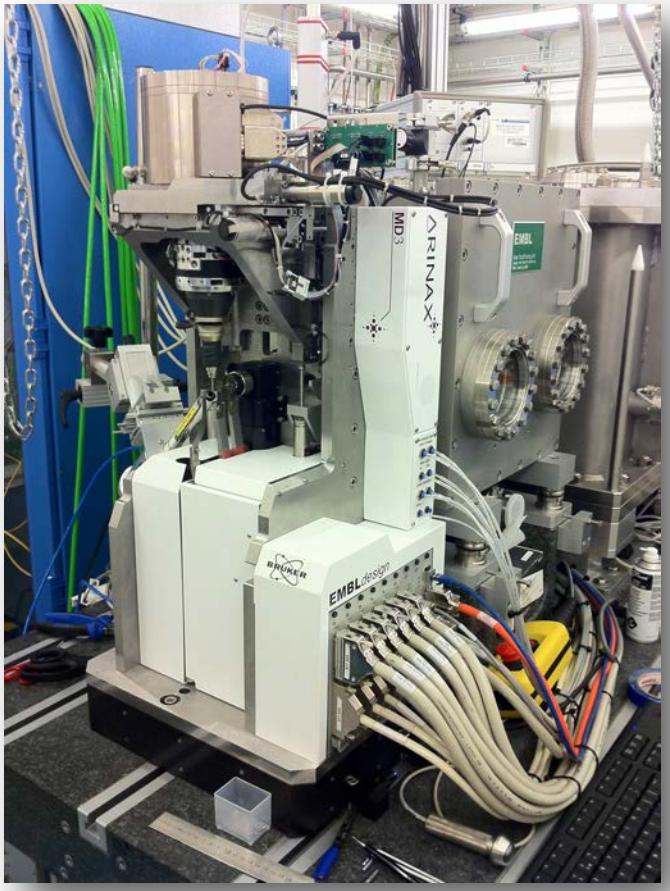
Anomalous difference map (>15 σ)

Beamline P14

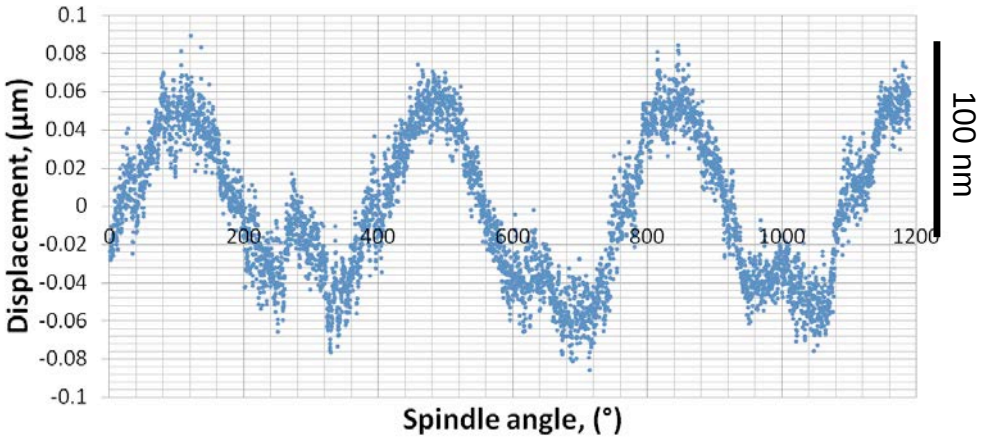
- Beam size in microfocus mode 5-6(h) x 4-5 (v)
- Flux 7 10^{12} ph/sec
- Peak dose rate **100 MGy/sec**

Crystal life time
~0.3 second at 100 K
~5 millisecond at room temperature

MD3: diffractometer with nanometer precision

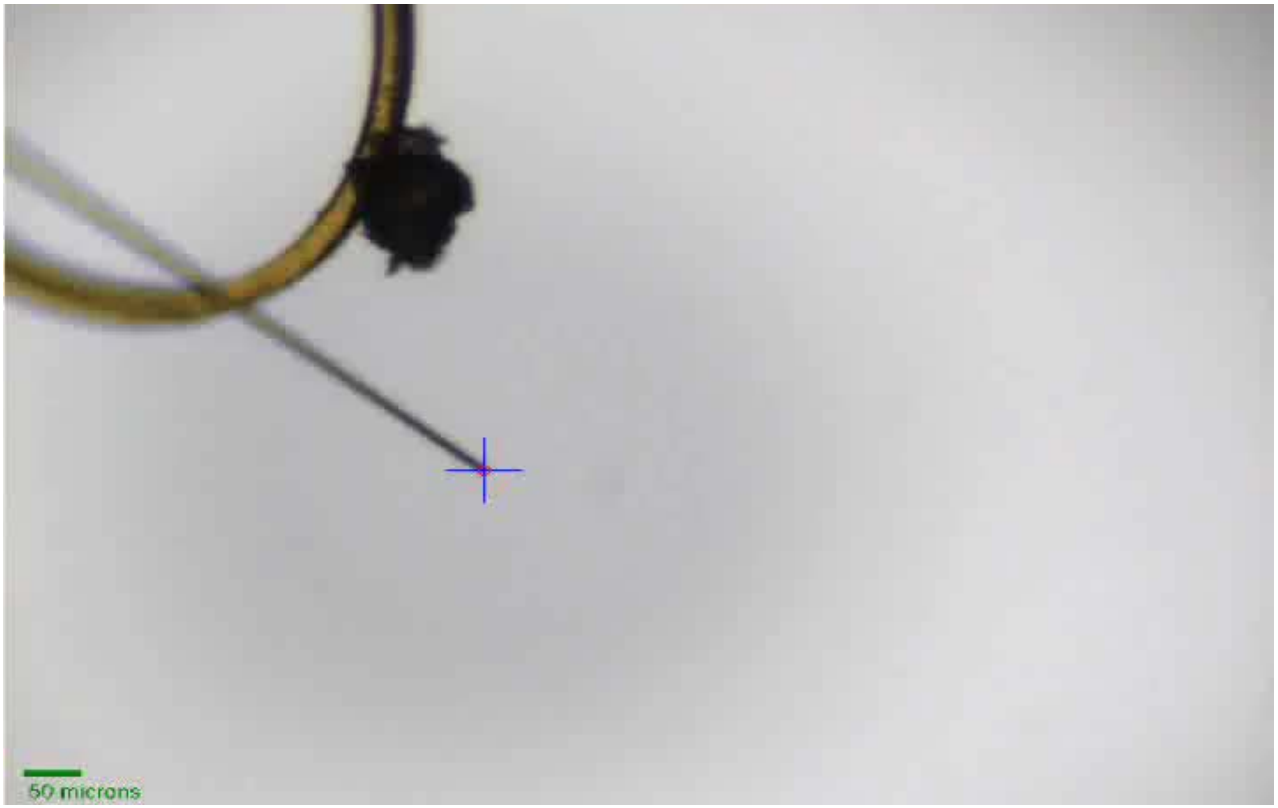


MD3
EMBL-ARINAX



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

Helical or '4D-Scan' (Florent Cipriani, Alexandre Gobbo, EMBL-GR)



- Synchronous movement of omega, centering table and alignment table (5 motors) to collect data along a long needle in a shutterless 'continuous helical scan'
- Here: carbon fibre 10 micron thick, 650 micron long.

P14: µ-crystals

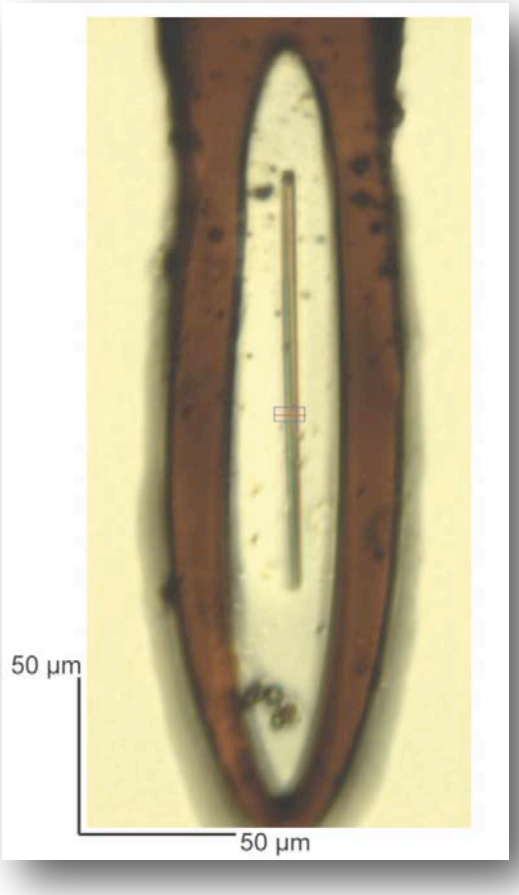
Group of M. Than, University of Jena

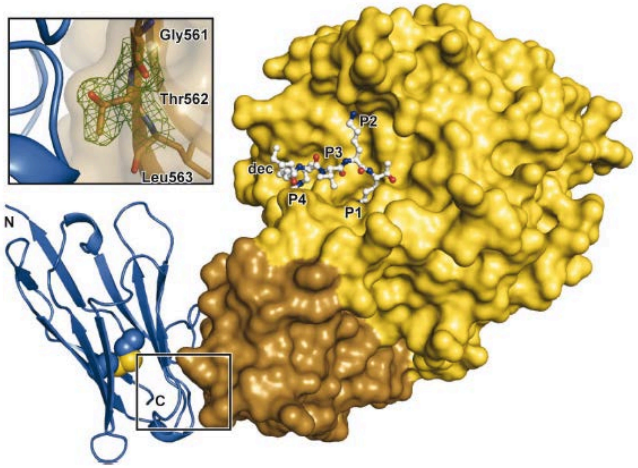
- Furin-antibody complex, 120 kDa
- 5 x 5 µm^2 beam
- X-ray centering in plane of loop
- 120 µm 180 deg. helical scan
  1500 frames -> 1 min total
- 30 MGy for the entire exposed region
  (deadly dose to entire sample in 1 min.)

- Exposed volume per frame:
  5 µm x 5 µm x 5 µm = 125 µm^3

- Diffraction to 2 Å
- Refinement to 2 Å: $R^{w/f}$ = 16.3 / 19.7

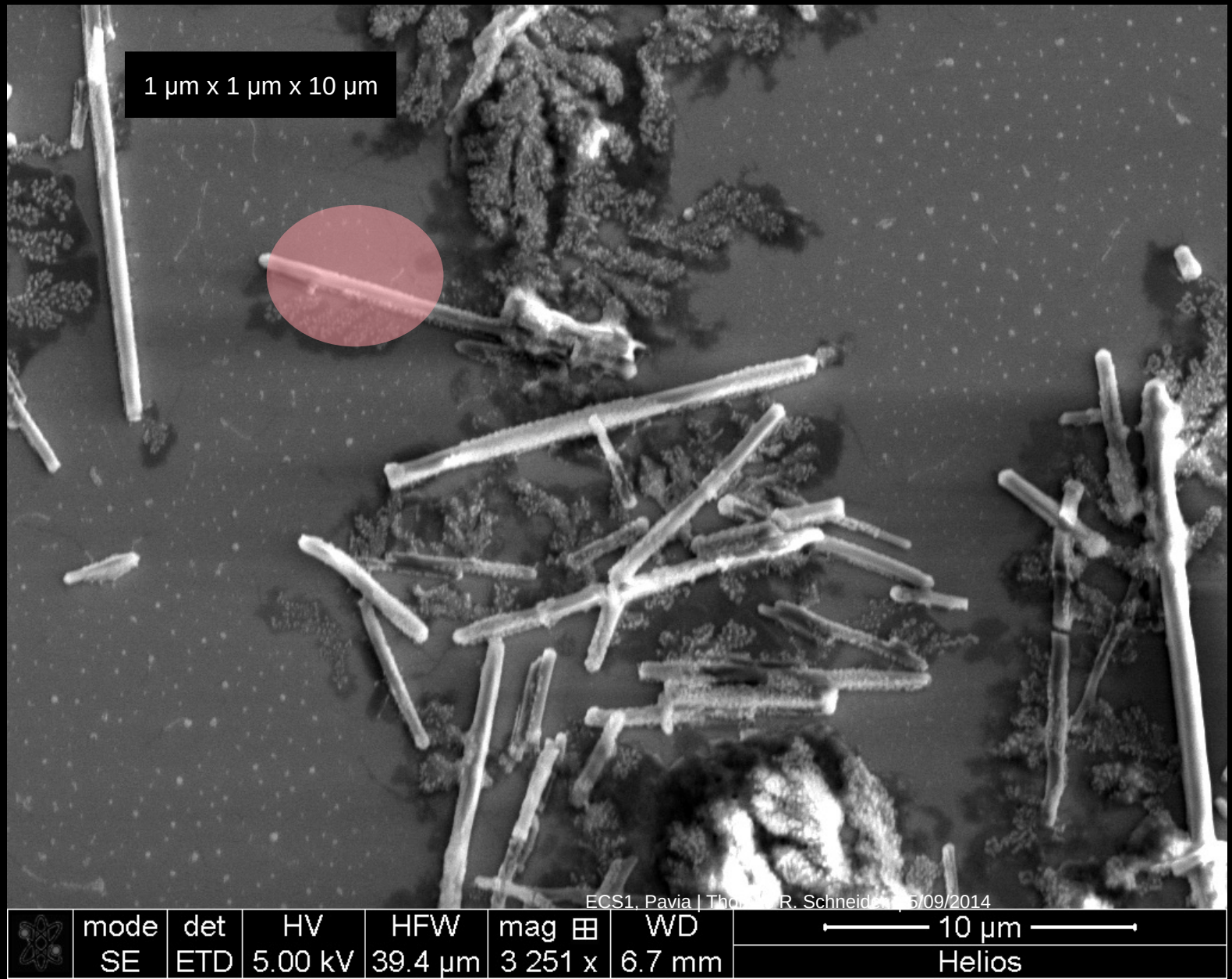
- Dahms, Creemers, Bourenkov, Brandstetter, Than
  Sci. Rep. ***6*** (2016), 340303



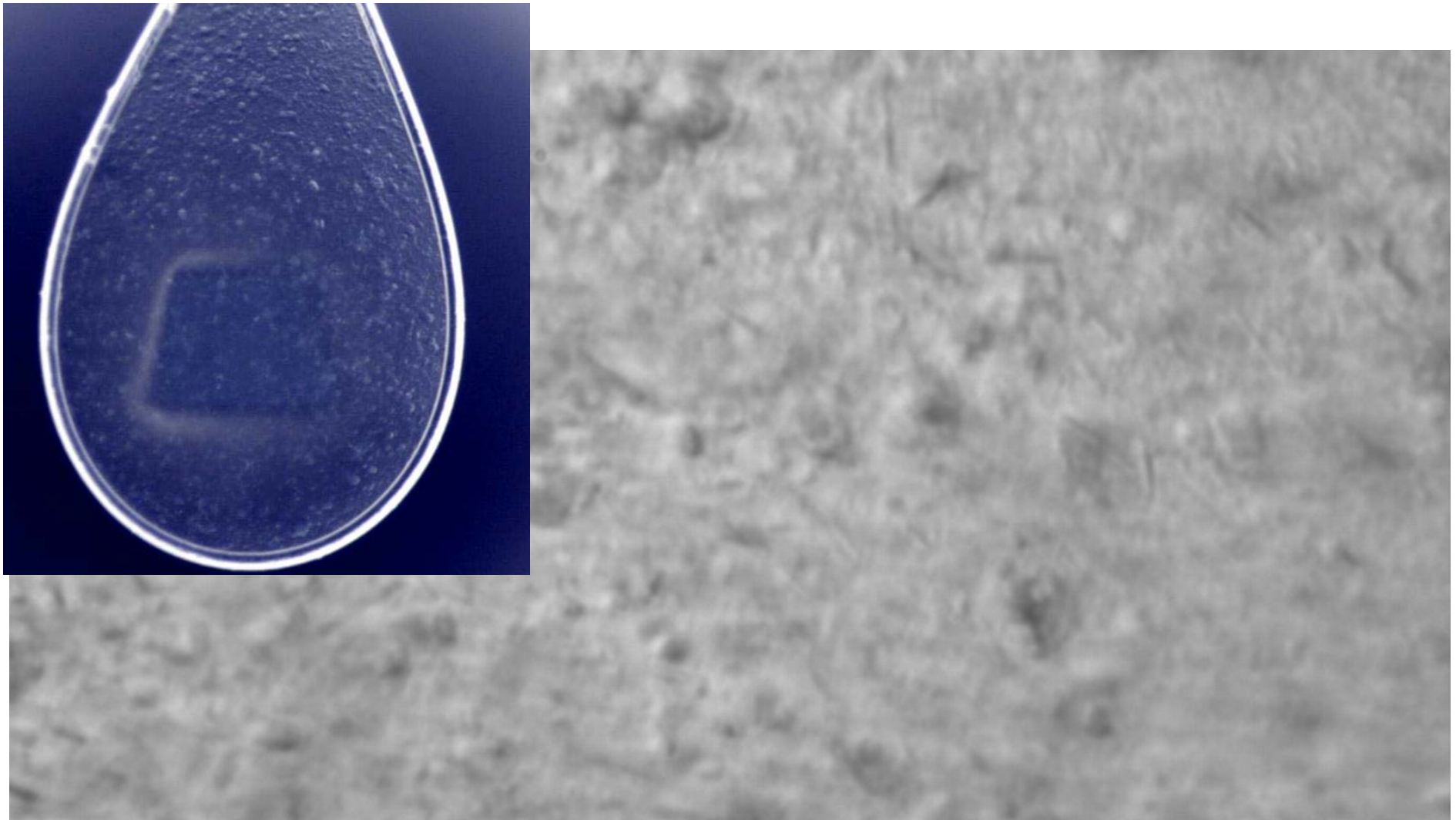


Cathepsin B

Redecke, L., ... Chapman, H. (2012): Natively Inhibited Trypanosoma brucei Cathepsin B Structure Determined by Using an X-ray Laser. Science 339:227 [4HWY:2.1 Å]

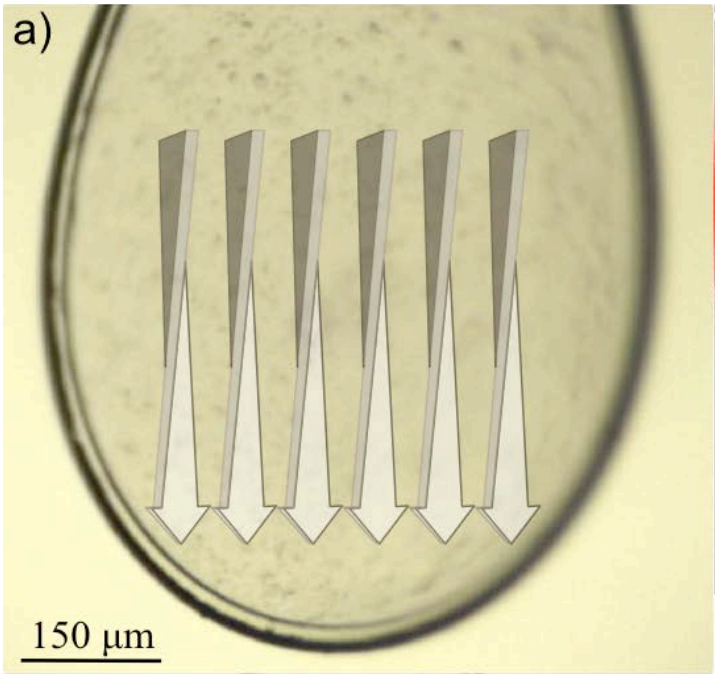


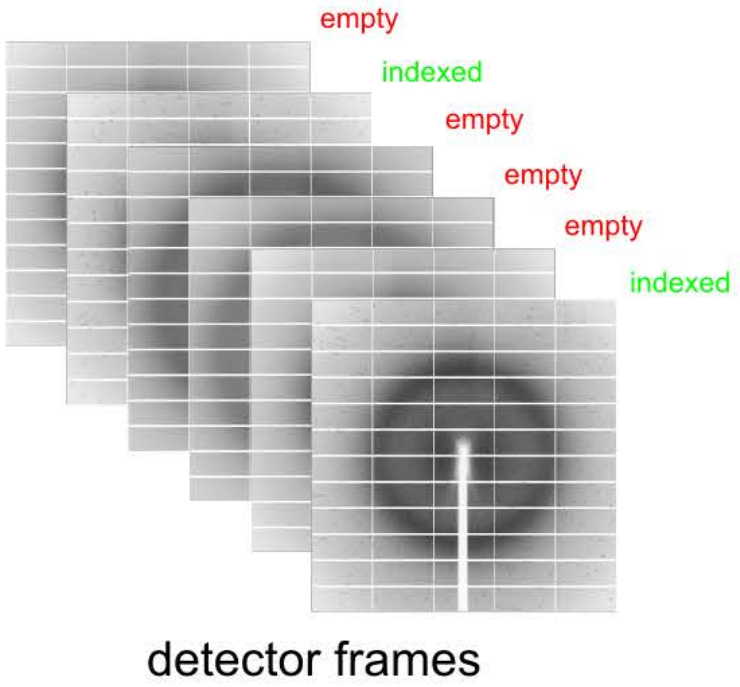
Cathepsin B suspension in a loop on P14 diffractometer



Cathepsin B diffraction image exposure dose 34 MGy

Serialized helical scanning data collection





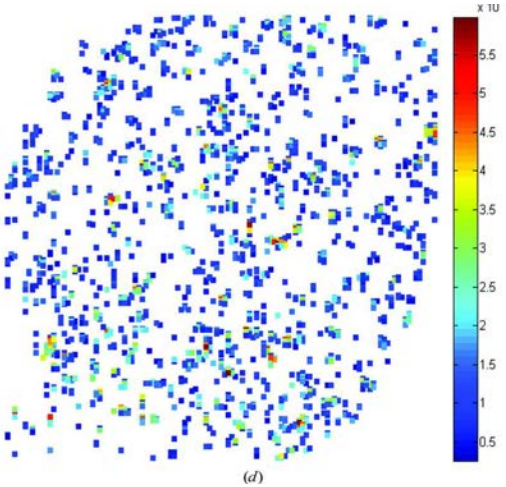
Series of frames acquired shutter-less during continuous motion of sample mount in such a way that each crystal passes through the beam while rotating by 1-2° and receiving its life-dose exposure

Scanned area 0.6x0.6 mm
Rotation range ± 45° per line
Per frame: 0.375° rotation, 2.5 µm translation
5 µm translation between lines

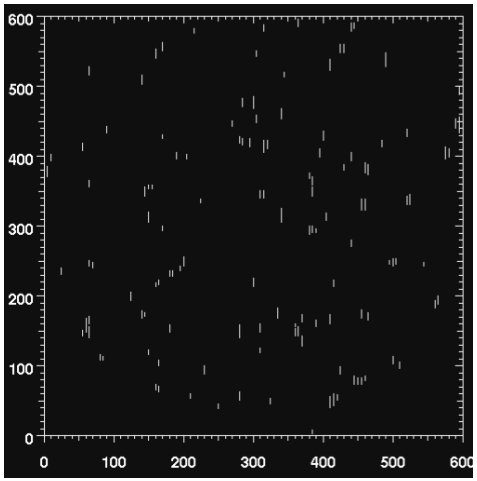
Cathepsin B data collection and processing

22800 frames recorded

2200 frames indexed (CRYSTFEL, White et al. 2012)
500 clusters of adjacent hits along scan direction (the same xtl hit more than once)
150 clusters successfully integrated/scaled (XDS, Kabsch 2010)
120 partial data sets (400 frames) merged with XSCALE
80 crystals contributed to the final data set



initial hits

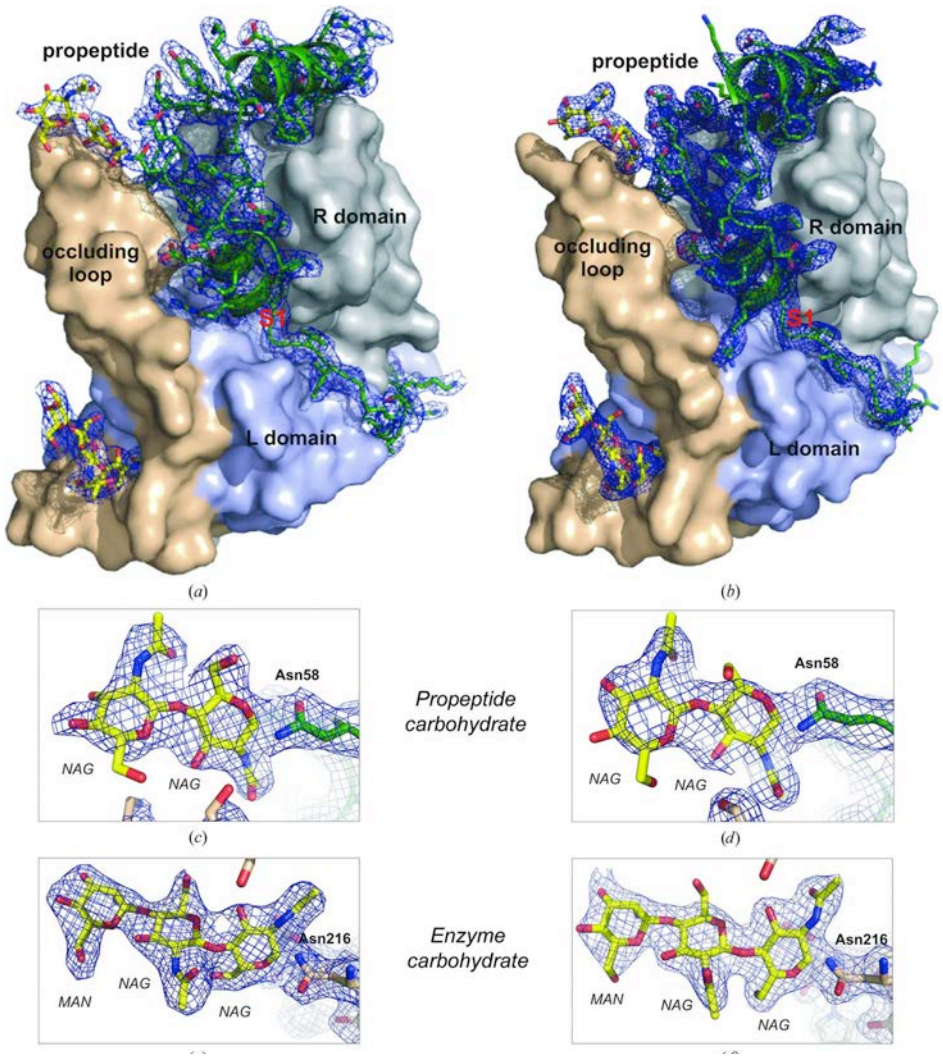


final data

$P4_122$ a=123.5 Å, c=54.3 Å
Resolution= [88.1-3.0 Å]
Completeness= 99.8%
Multiplicity= 12.3
$CC_{1/2}$= 0.99 (0.79 high-res)
<I/SigI>= 3.7 (8.9 low, 1.0 high)

Mol. Replacement + refinement
Rwork,free = 22.9, 26.1

PETRA III XFEL



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

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

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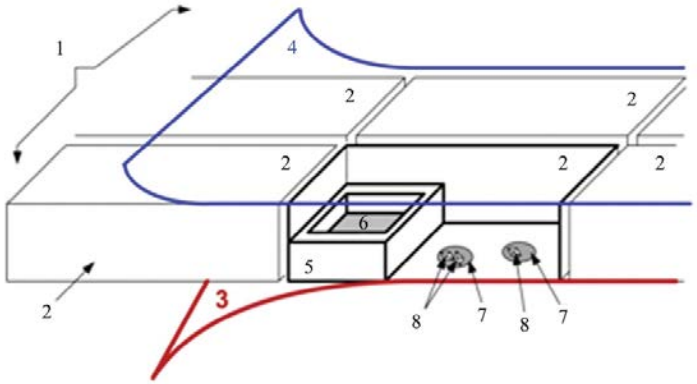
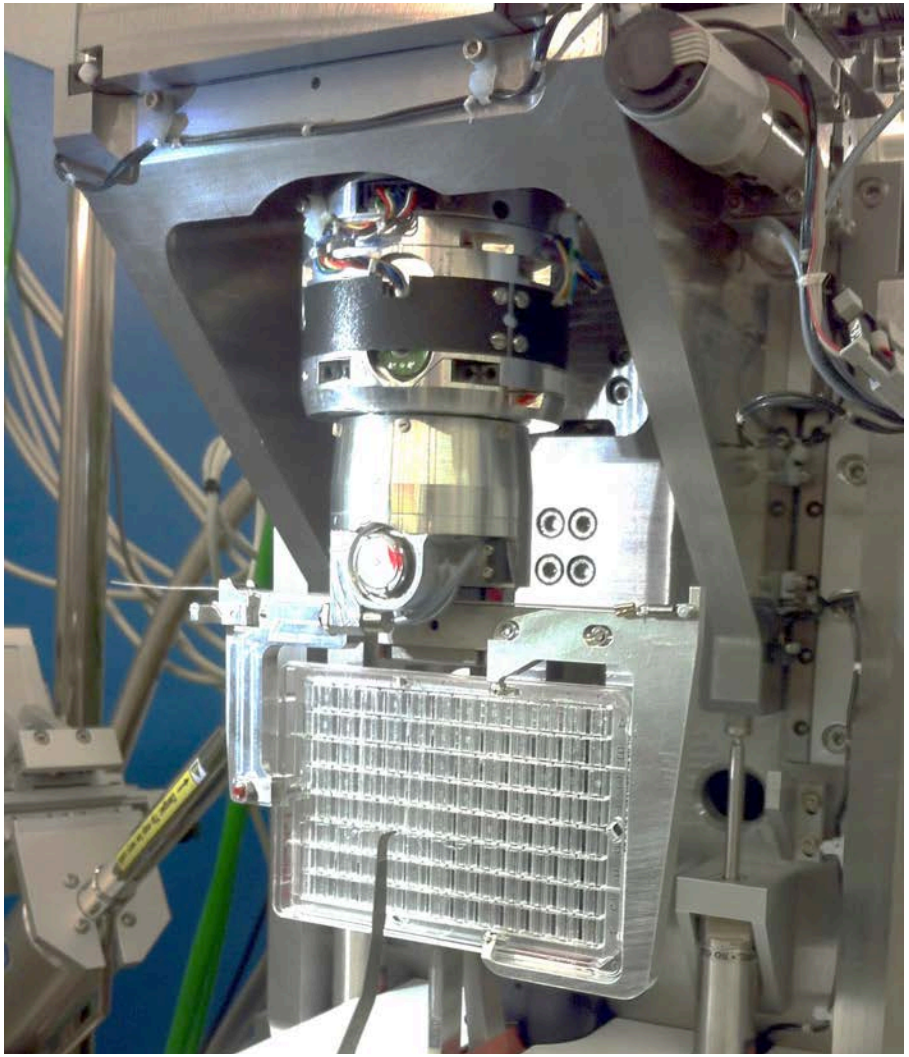
In-situ serial crystallography

Crystal Direct™ plate scanner mounted on MD3

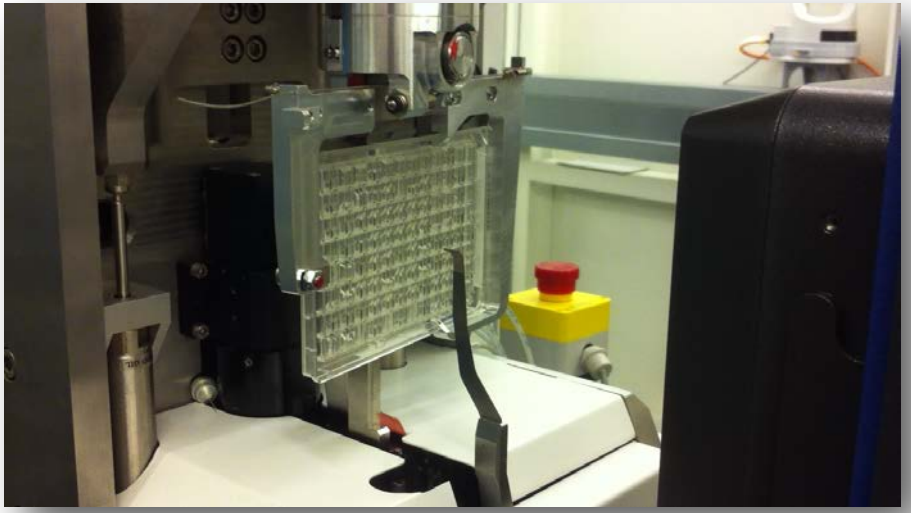


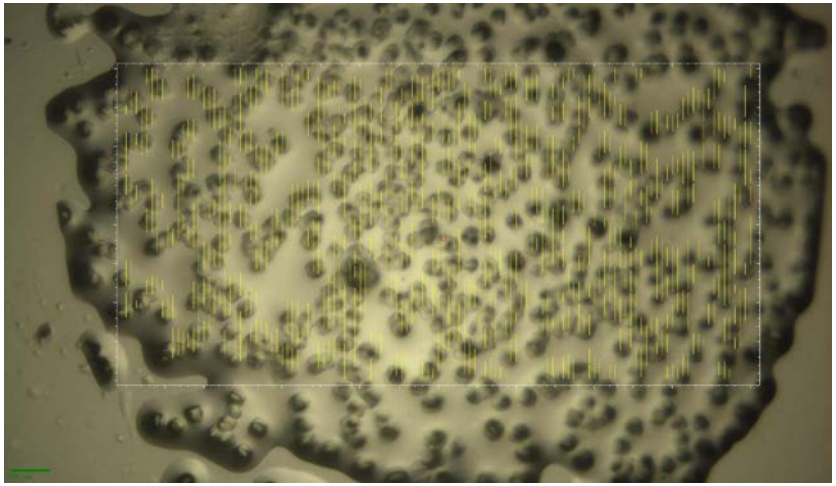
Cipriani, Marquez et al. (2012) 'CrystalDirect: a new method for automated crystal harvesting based on laser-induced photoablation of thin films.' Acta Cryst D68:1393.

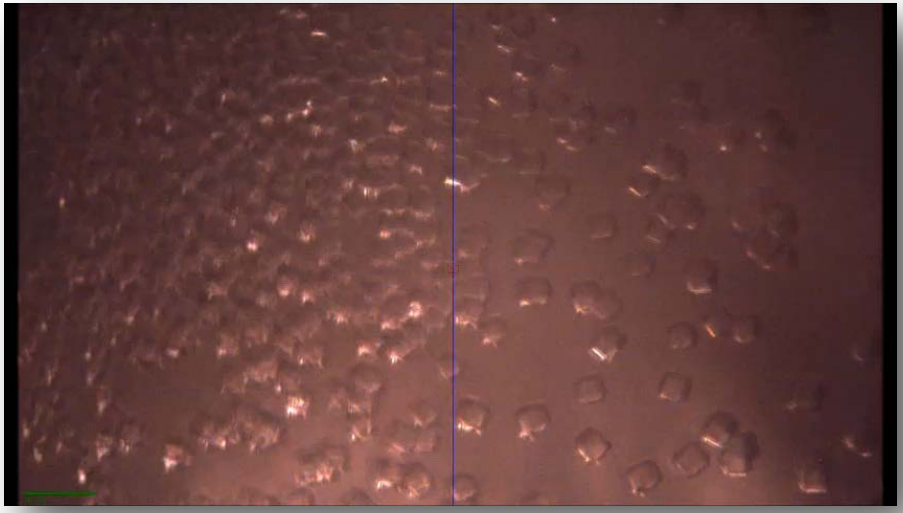


In situ data collection

- Continuous serial helical scans with a micro-beam on CrystalDirect plates





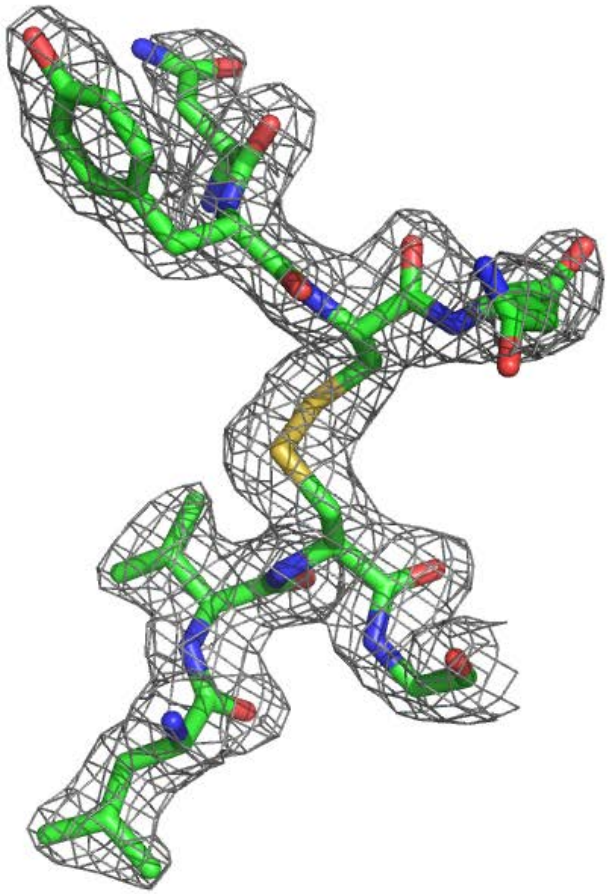


In-Situ SX data set

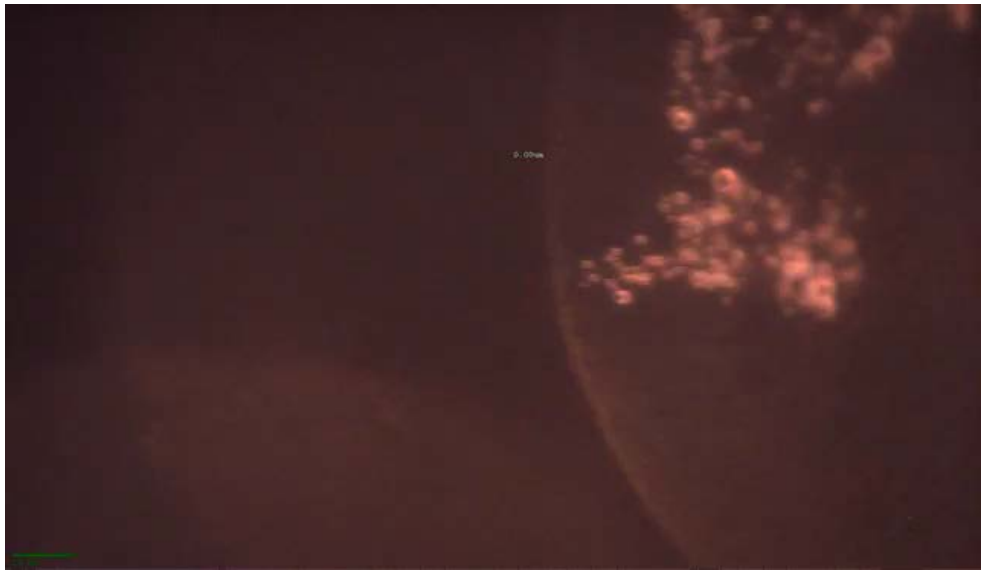
24000 frames measured
9000 diffraction images
1200 wedges indexed, 0.5-1.7°
990 wedges integrated/scaled

300 crystals

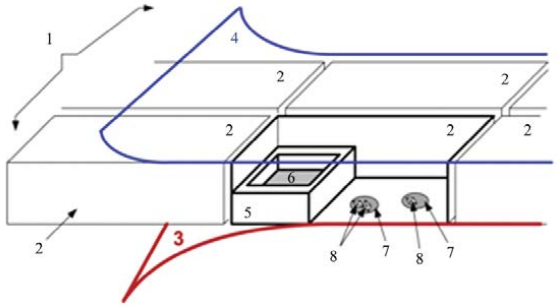
Resolution [67-2.03]
452237 reflections / 5259 unique
Multiplicity 86
$CC_{1/2}$=0.998 (0.85 high)
I/SigI=15 (25 low, 1.5 high)

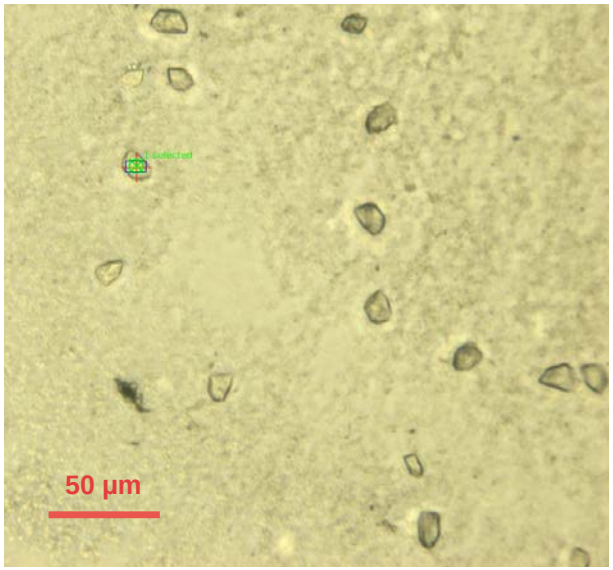


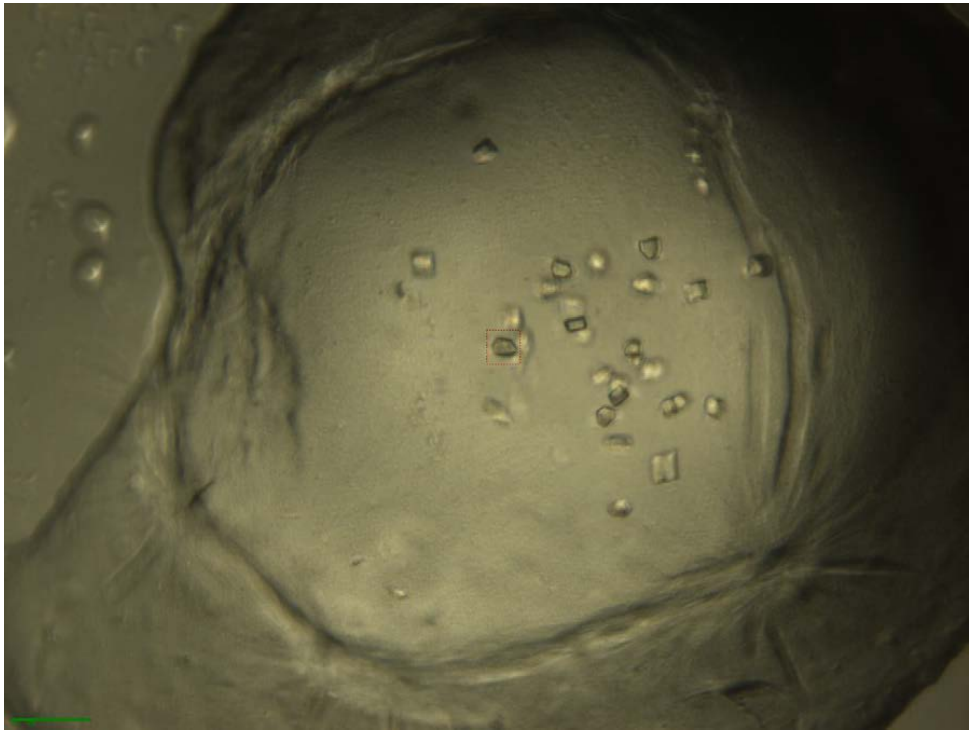
R/Rfree=0.143/0.184



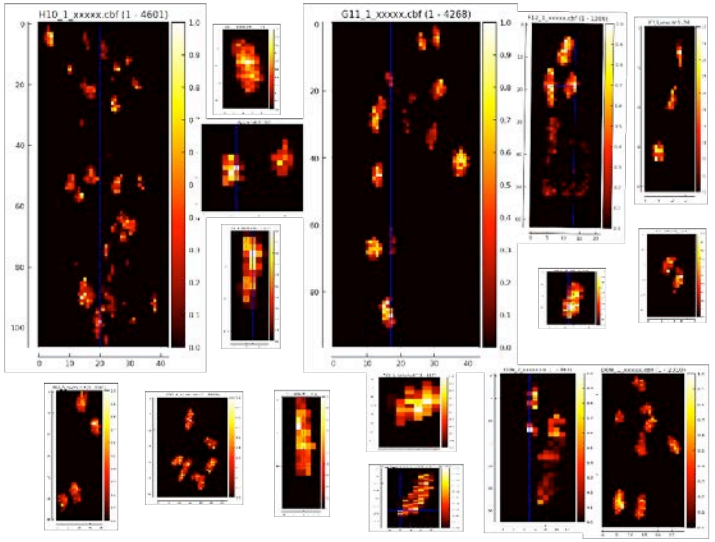
Lipidic Cubic Phase (LCP) crystallization in CrystalDirect plates







Data collection



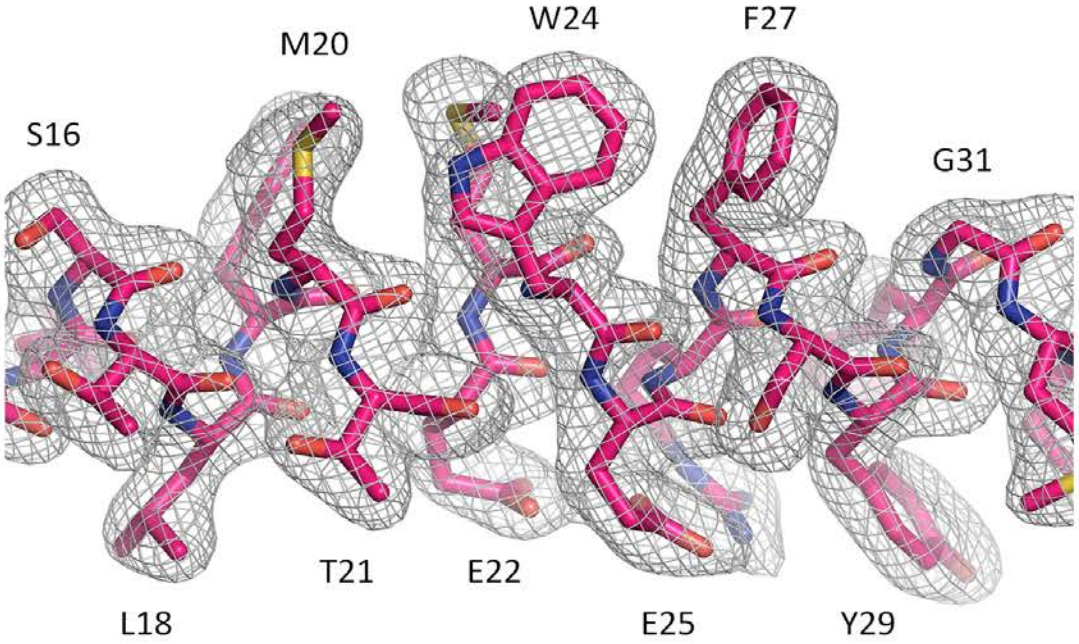
- Proton-coupled oligopeptide transporter PepTSt (*Str. therm.)*
- 19 of 96 wells in a plate contained xtls
- Define ROIs (1 to 11 per well)
  - 0.4 x 0.4 mm^2 with hundreds of crystals down to 36 x 48 μm^2 with few crystals
- Queue for execution in MxCuBE.

- **66 ROIs – 958 helical scans - 36013 frames in 100 min.**

- DOZOR used to identify frames with >10 spots
- 5290 frames in 497 wedges (min three frames) integrated with XDS

PepTSt In-situ Structure

- ~1.3 M reflections
- 23105 uniques to 2.50 Å
- R_w / R_f = 21.9 / 23.5 %



Massive multi-crystal SAD phasing at 6.5 keV

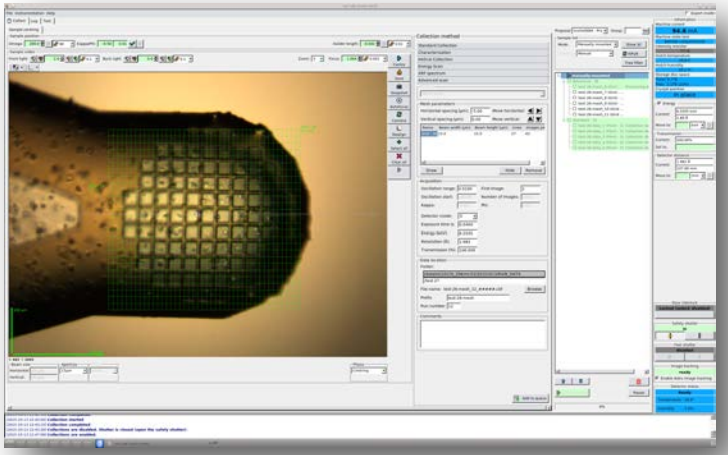
Michele Cianci

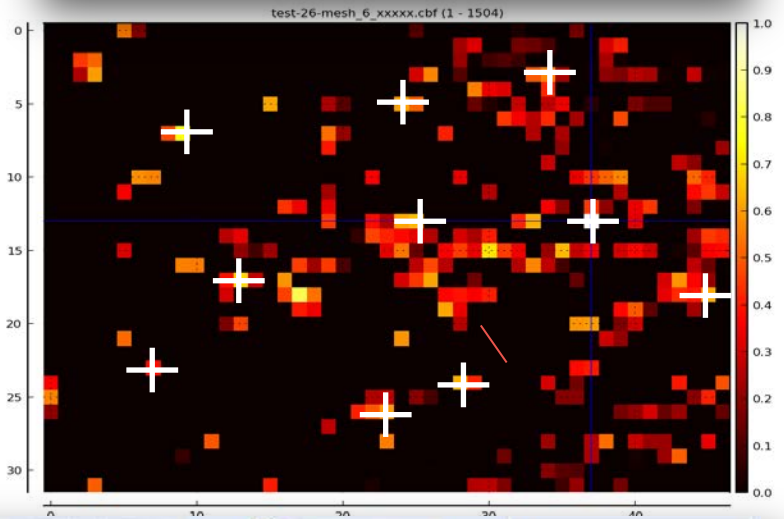
Concanavalin A, 237 a.a. 1 Mn 1 Ca
Crystal diimensions < 15 µm

28 meshes x 1500 frames
1 min per mesh

300 best crystals x 10 deg. data

85 data sets merged

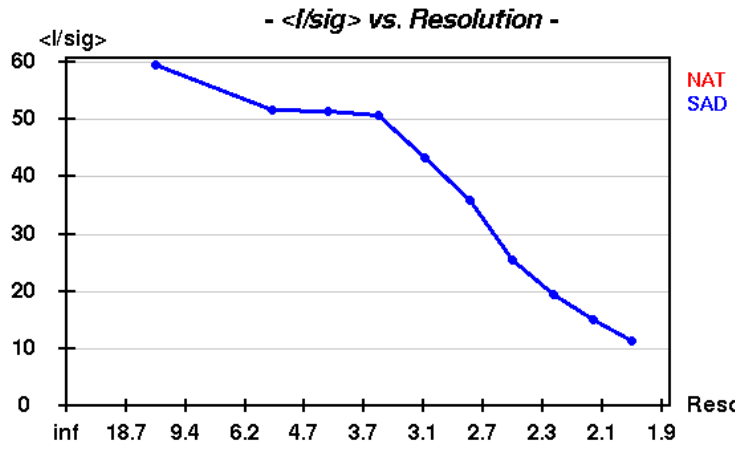


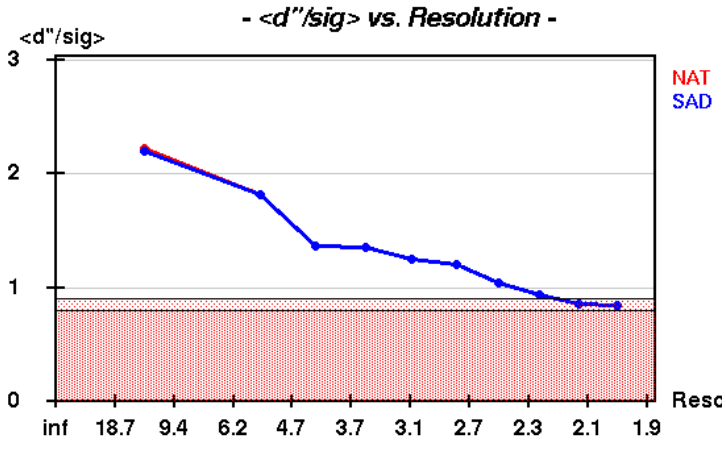


Data merged from 85 data sets of 10°each

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

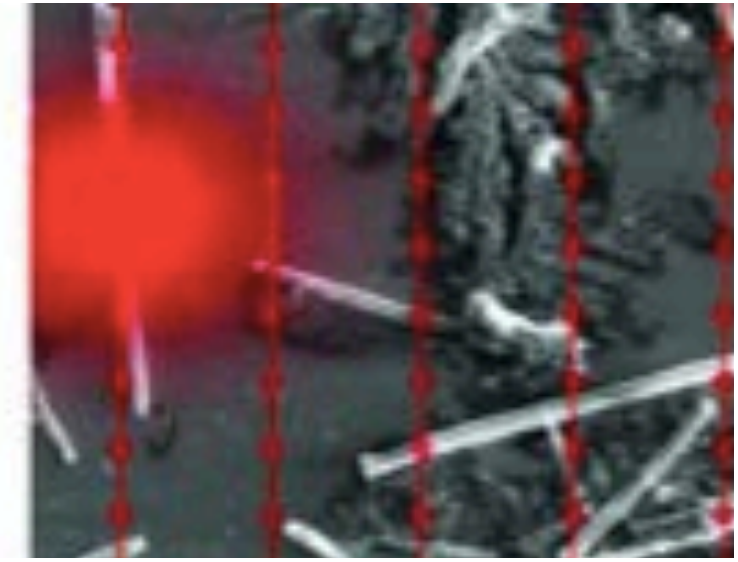
RESOLUTION LIMIT	NUMBER OF REFLECTIONS OBSERVED	UNIQUE	POSSIBLE	COMPLETENESS OF DATA	R-FACTOR observed	R-FACTOR expected	COMPARED	I/SIGMA	R-meas	CC(1/2)	Anomal Corr	SigAno	Nano
8.63	6778	379	382	99.2%	8.7%	8.7%	6778	47.15	9.0%	99.7*	72*	2.129	142
6.10	10968	712	712	100.0%	8.9%	9.5%	10968	37.35	9.2%	99.8*	65*	1.697	309
4.98	14790	898	898	100.0%	8.9%	9.6%	14790	38.25	9.2%	99.9*	41*	1.303	399
4.32	18163	1064	1064	100.0%	8.7%	9.3%	18163	40.28	9.0%	99.9*	45*	1.315	483
3.86	19178	1209	1209	100.0%	9.9%	10.0%	19178	35.06	10.3%	99.8*	42*	1.347	560
3.52	20537	1380	1380	100.0%	10.3%	10.4%	20537	32.35	10.6%	99.8*	36*	1.270	639
3.26	22892	1431	1431	100.0%	11.5%	11.5%	22892	29.99	11.8%	99.7*	31*	1.176	668
3.05	26028	1603	1603	100.0%	12.6%	12.6%	26028	27.17	13.1%	99.7*	33*	1.209	751
2.88	26282	1652	1652	100.0%	14.0%	14.6%	26282	22.51	14.5%	99.7*	26*	1.089	778
2.73	25333	1783	1783	100.0%	16.8%	17.3%	25333	18.27	17.4%	99.2*	20*	1.021	843
2.60	28177	1855	1855	100.0%	18.9%	19.8%	28177	16.69	19.6%	99.4*	18*	0.993	880
2.49	28483	1955	1955	100.0%	20.8%	21.8%	28483	14.31	21.6%	99.2*	12*	0.901	930
2.39	28429	2015	2015	100.0%	22.4%	23.7%	28429	12.77	23.3%	99.1*	16*	0.914	959
2.31	25556	2087	2088	100.0%	23.6%	24.9%	25556	11.18	24.7%	99.1*	11	0.883	994
2.23	27243	2192	2193	100.0%	25.8%	27.1%	27243	10.45	27.0%	98.7*	6	0.816	1047
2.16	27318	2230	2230	100.0%	29.6%	31.4%	27318	9.11	30.9%	98.4*	9	0.820	1066
2.09	25719	2360	2360	100.0%	32.0%	33.6%	25719	7.85	33.6%	98.0*	4	0.828	1132
2.03	26119	2395	2395	100.0%	36.2%	38.6%	26118	6.90	38.1%	97.7*	6	0.811	1148
1.98	24225	2474	2474	100.0%	37.2%	40.7%	24224	6.01	39.3%	97.9*	5	0.777	1187
1.93	16627	2416	2492	97.0%	42.0%	46.8%	16531	4.25	45.4%	92.0*	4	0.761	1083
total	448845	34090	34171	99.8%	13.7%	14.2%	448747	16.91	14.2%	99.8*	19*	0.985	15998





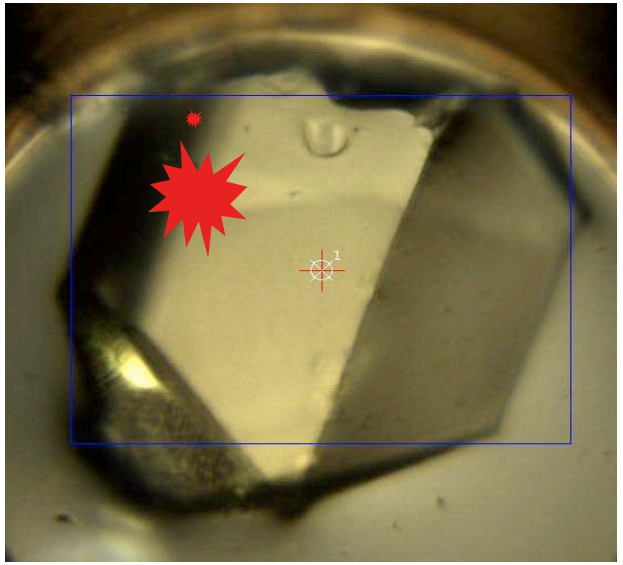
Complexes

Range of crystal dimensions in synchrotron crystallography



10 µm

40 kDa
10^7 unit cells



100 µm

1 MDa
10^{12} unit cells

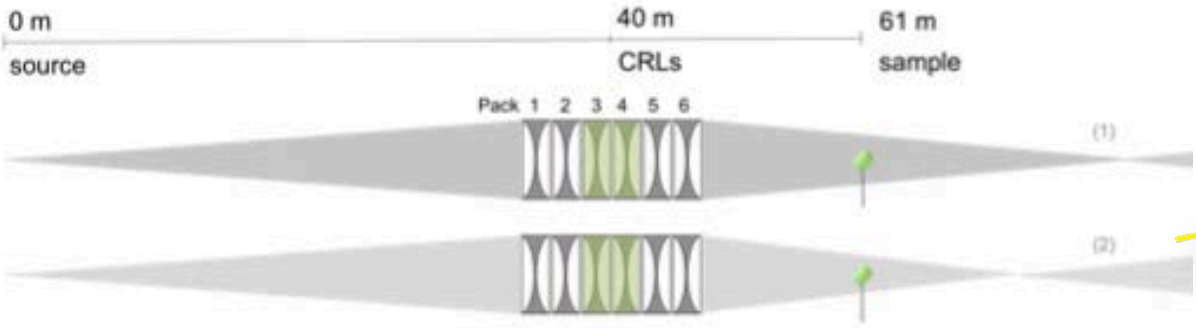
"microfocus"

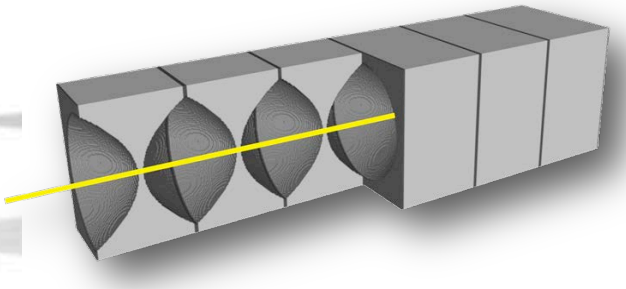
"regular" beamline

Large beam wins on large crystals of large complexes

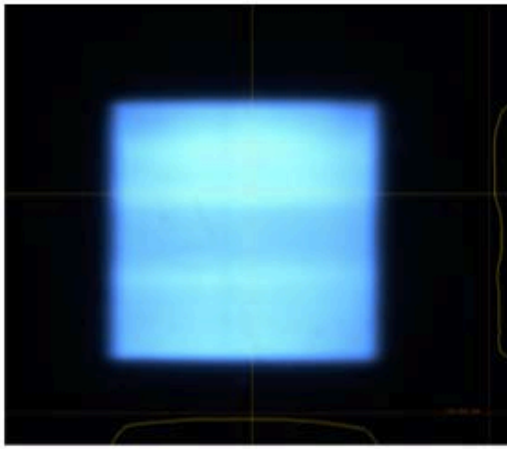
- High degree of intra-molecular disorder
- Short correlation lengths
- Crystal lattice is uniform over long distances *on average*

High flux density in large area: collimating CRLs P14/PETRAIII



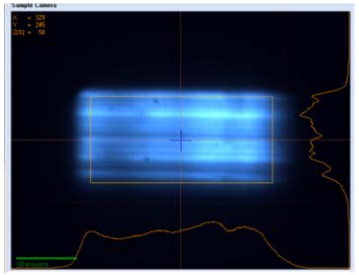


compound refractive lenses

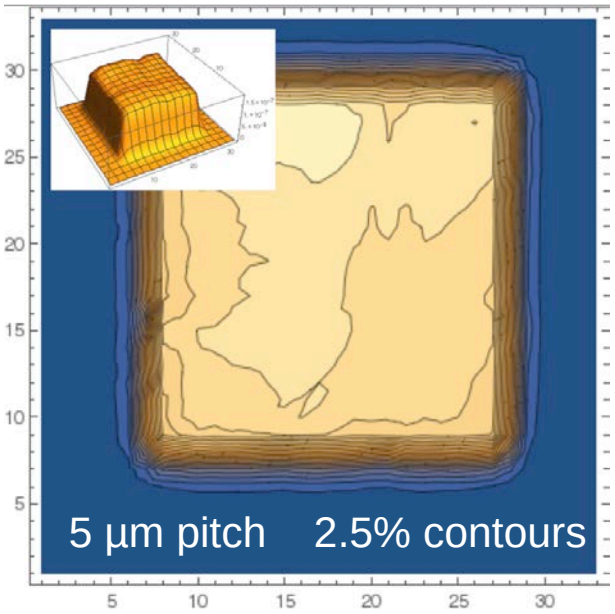


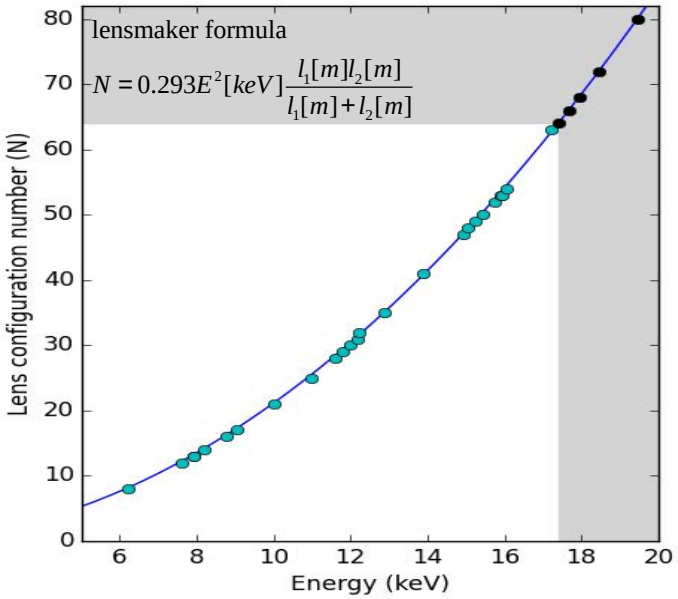
2x10^13 photons in 200x200 µm²

for comparison, the beam collimated by X-ray mirrors:



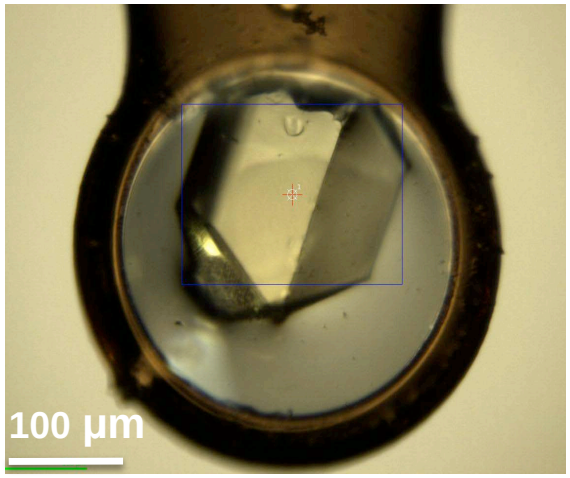
Top-Hat beam

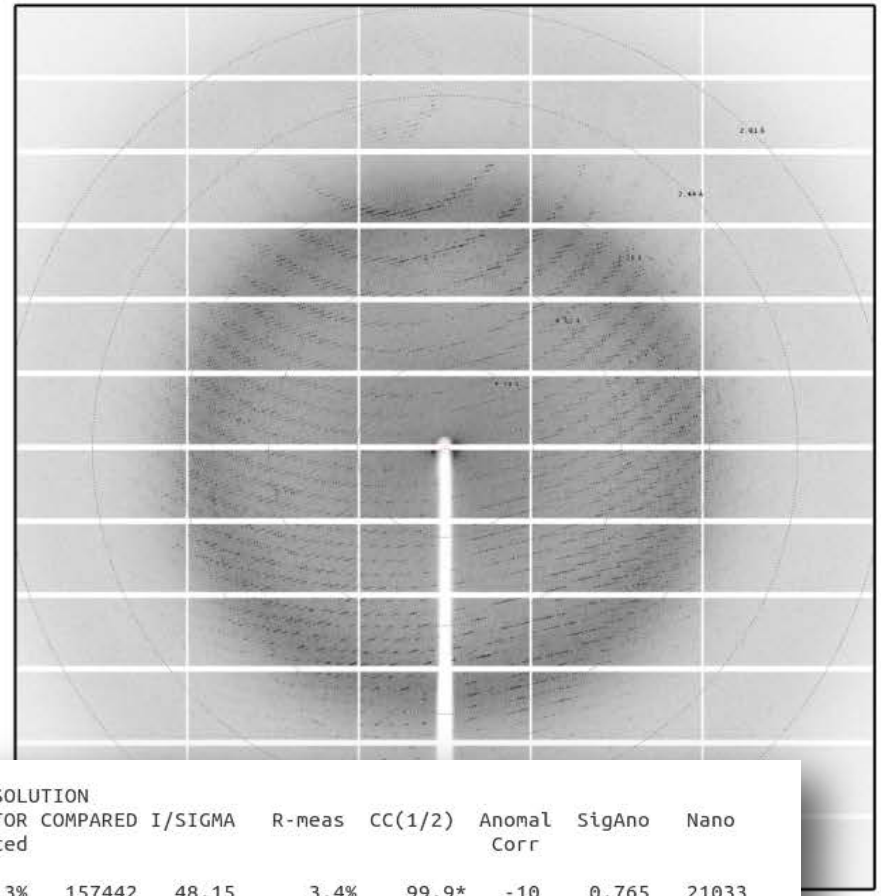




<2% intensity r.m.s.d.

Human 20S proteasome diffraction data



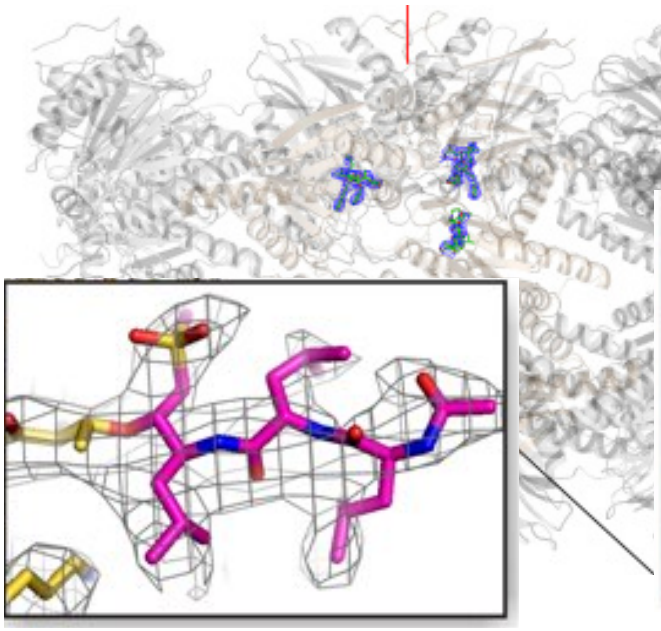


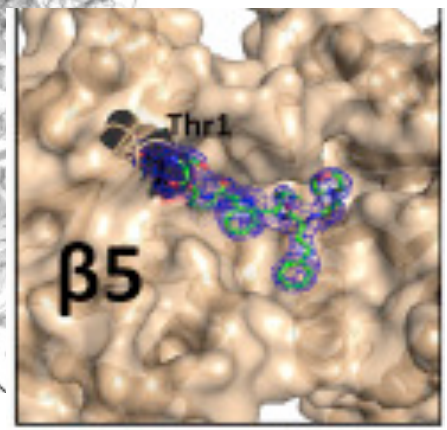
3 min data collection
Dose 15 MGy

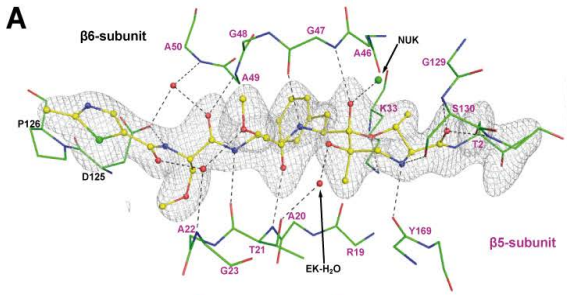
SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE >= -3.0 AS FUNCTION OF RESOLUTION

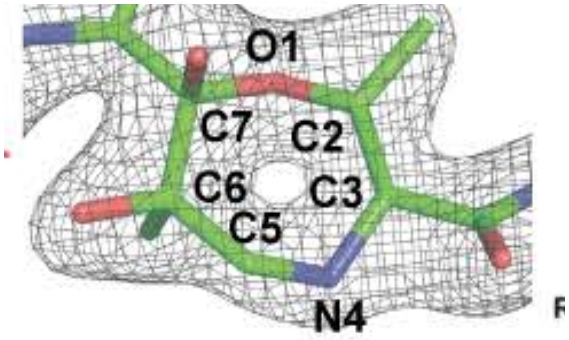
RESOLUTION LIMIT	NUMBER OF REFLECTIONS OBSERVED	UNIQUE	POSSIBLE	COMPLETENESS OF DATA	R-FACTOR observed	R-FACTOR expected	COMPARED	I/SIGMA	R-meas	CC(1/2)	Anomal Corr	SigAno	Nano
5.46	157498	24829	24896	99.7%	3.1%	3.3%	157442	48.15	3.4%	99.9*	-10	0.765	21033
3.87	294500	43706	43748	99.9%	3.4%	3.5%	294442	45.92	3.7%	99.9*	-12	0.740	39692
3.16	388827	56306	56353	99.9%	4.4%	4.5%	388805	35.03	4.8%	99.9*	-9	0.755	52328
2.74	456732	65777	65788	100.0%	8.3%	8.3%	456709	20.05	9.0%	99.6*	-4	0.775	60948
2.45	506328	75125	75179	99.9%	16.0%	16.1%	506255	11.12	17.4%	98.7*	-2	0.785	69504
2.24	554525	81083	81186	99.9%	29.3%	29.4%	554353	6.44	31.7%	95.9*	0	0.785	75248
2.07	626777	91705	91788	99.9%	53.7%	54.0%	626639	3.60	58.1%	87.7*	-1	0.762	84766
1.94	643839	93389	93462	99.9%	104.1%	104.6%	643733	1.88	112.6%	64.8*	0	0.725	86892
1.83	681229	100871	100985	99.9%	203.9%	205.4%	681020	0.92	221.0%	31.9*	0	0.668	90898
total	4310255	632791	633385	99.9%	8.4%	8.6%	4309398	13.35	9.1%	99.9*	-2	0.747	581309

Human 20S Proteasome: EM and Crystallography









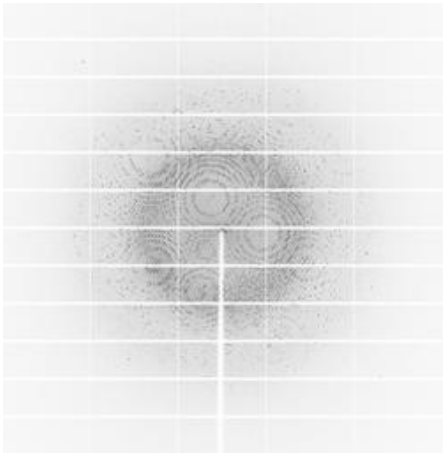
CryoEM
3.5 Å

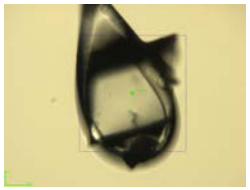
MX
2.9 Å

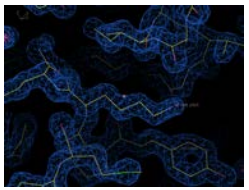
MX
1.9 Å

- **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)

Acetoacetate decarboxylase from *Clostridium Acetobutylicum*







$P2_12_12_1$ a=104 b=172 c=376 Å

2x dodecamer in assymetric unit
== 47000 atoms

Resolution 1.5 Å
256 diffraction orders

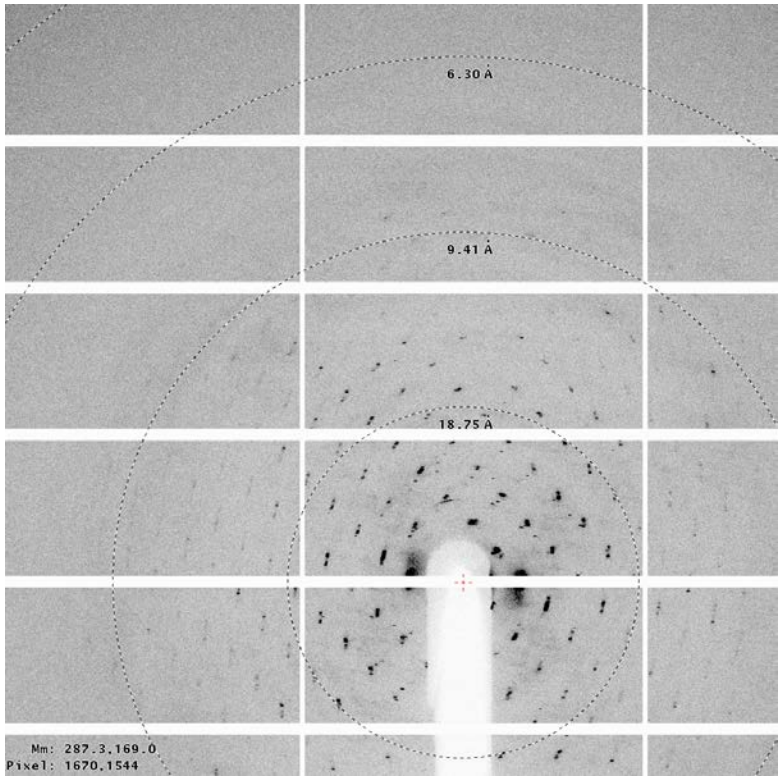
Data collected in 3 minutes

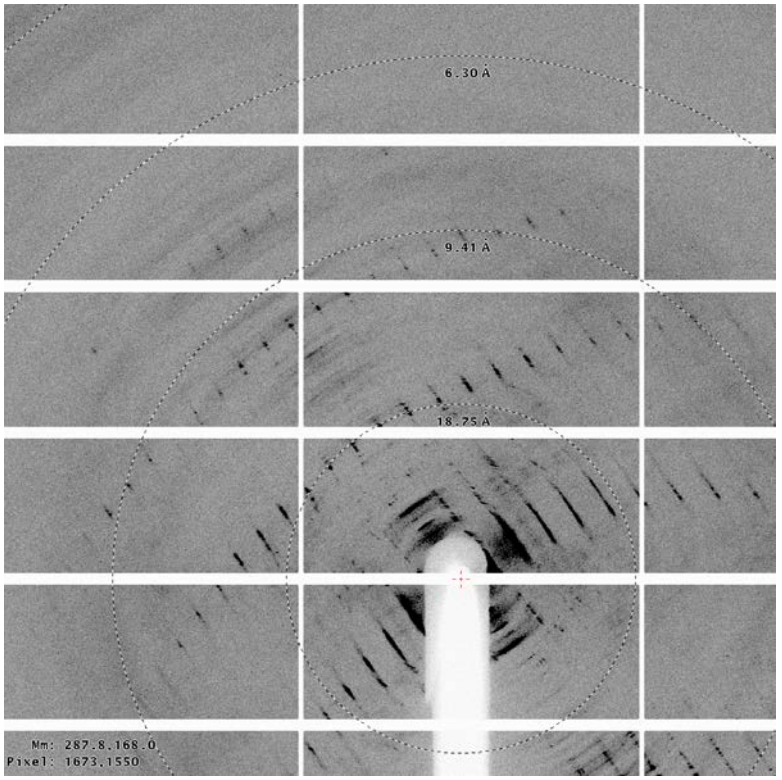
SUBSET OF INTENSITY DATA WITH SIGNAL/NOISE >= -3.0 AS FUNCTION OF RESOLUTION

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
4.47	517360	41480	41519	99.9%	3.9%	4.0%	517353	57.78	4.1%	99.9*	-19	0.737	36729
3.16	944778	73887	73905	100.0%	5.0%	4.5%	944769	50.74	5.2%	99.9*	-18	0.783	69127
2.58	1245686	95010	95022	100.0%	8.6%	7.7%	1245675	31.94	9.0%	99.8*	-13	0.790	90088
2.24	1482306	112101	112115	100.0%	13.4%	13.0%	1482299	21.86	13.9%	99.6*	-9	0.764	107237
2.00	1635415	126749	126760	100.0%	20.8%	21.4%	1635400	14.42	21.7%	99.0*	-8	0.744	121806
1.83	1680578	139968	139981	100.0%	38.0%	41.7%	1680566	7.88	39.7%	96.7*	-5	0.725	134621
1.69	1767570	151974	151987	100.0%	74.4%	83.4%	1767542	3.93	77.8%	87.3*	-5	0.708	146609
1.58	1806561	163088	163105	100.0%	127.8%	145.2%	1806507	2.07	134.1%	63.3*	-4	0.676	157214
1.49	1665271	164325	173380	94.8%	199.9%	232.0%	1663121	1.24	210.6%	34.7*	-2	0.642	151893
total	12745525	1068582	1077774	99.1%	12.3%	12.7%	12743232	14.69	12.9%	99.9*	-7	0.719	1015324

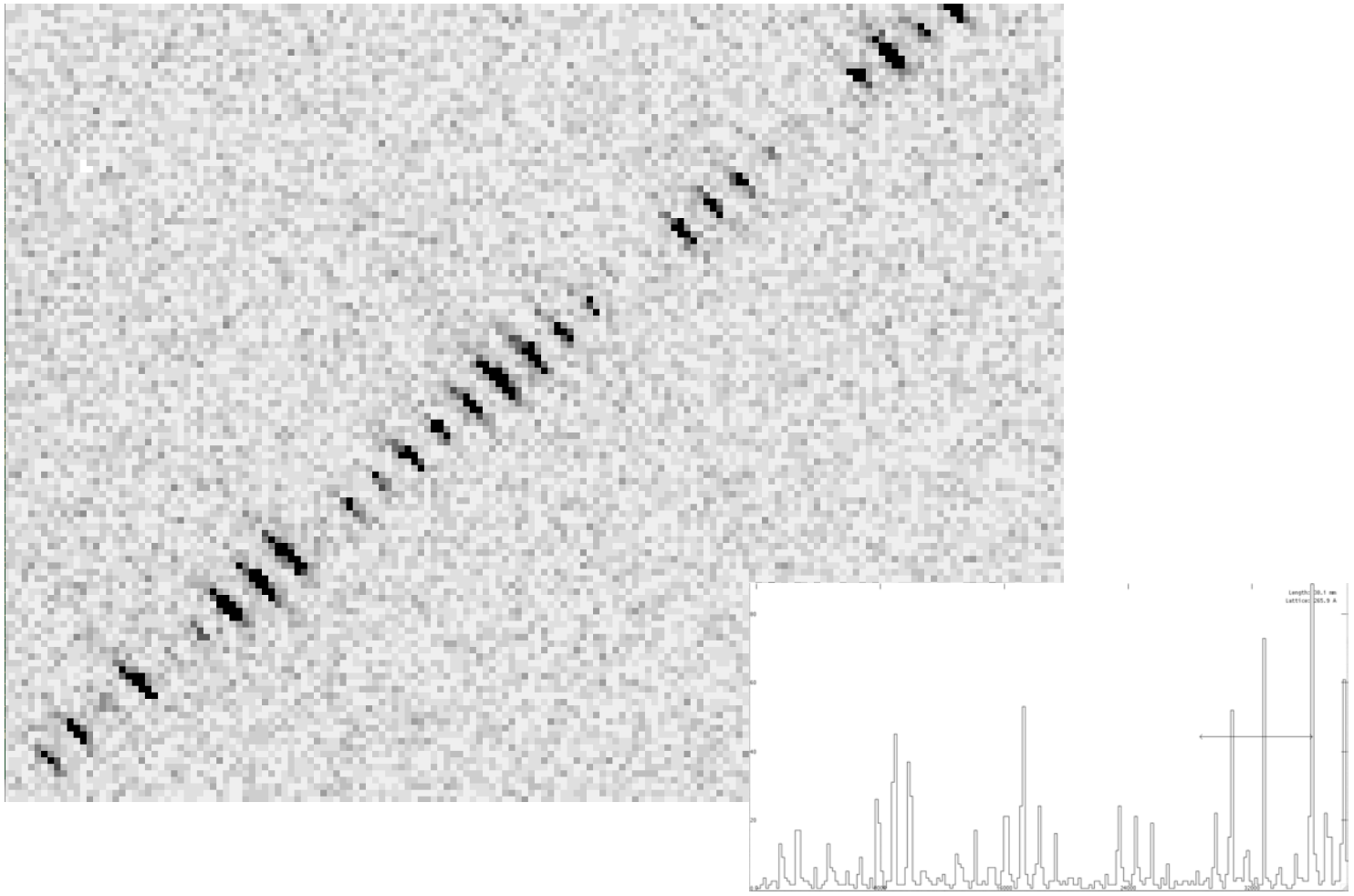
Kai Tittmann, Goettingen

Large unit cell example





1850 Å unit cell resolved



1850 Å / 4.6 Å = 400 diffraction orders

Summary

- Higher anomalous signals at low energies down to 4 keV
- Automated multi-crystal methods
  - crystal volumes down to 100 μm^3
- Cryo- and *In-situ*- Serial Synchrotron Crystallography (SSX)
  - crystal volumes down to 10 μm^3
- Standard options at P14 beamline
  - full-dose Cryo SSX - 10-90 min/mount
  - *In-situ* – 5 min/well
  - mesh&collect – 10 min/mount
- Large, parallel beams for large complexes
- Chemical details at atomic level on large complexes
- Extremely large unit cells can be resolved

Acknowledgements

- Thomas R. Schneider + Team
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- Valentin Gordeliy + Team
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