

MASSIF-1: Player piano dystopia or brave new world?

Matthew W. Bowler and Didier Nurizzo

29/03/2021

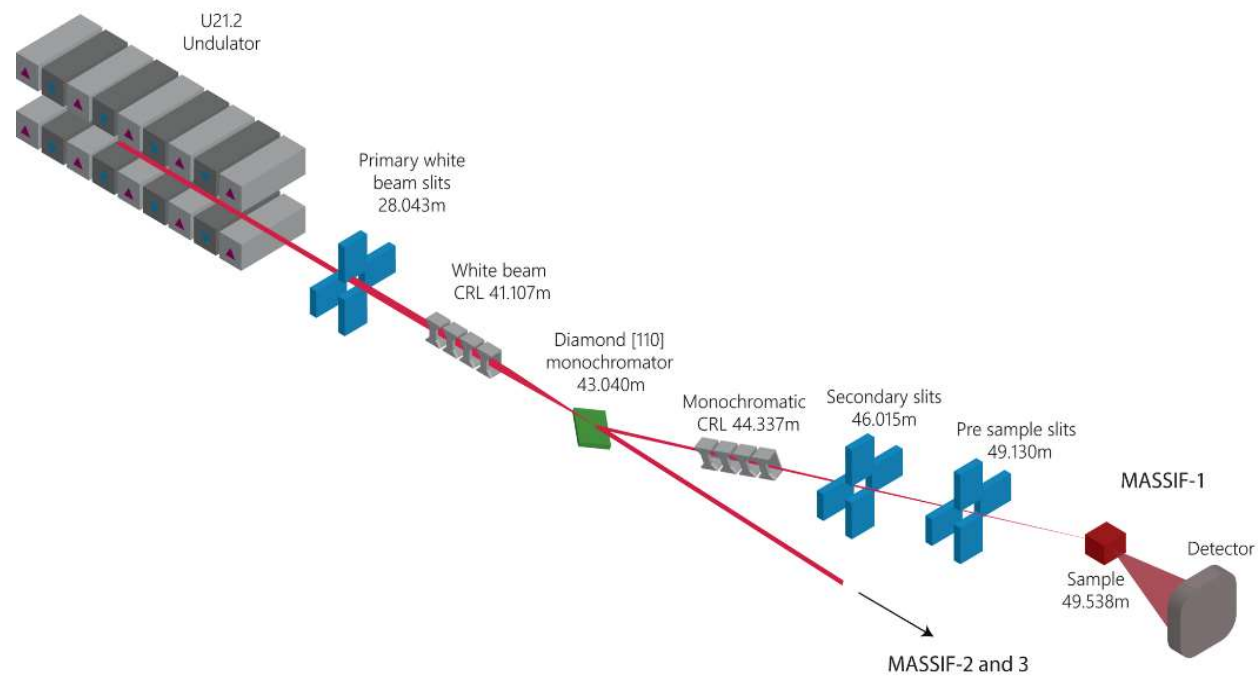


EMBL

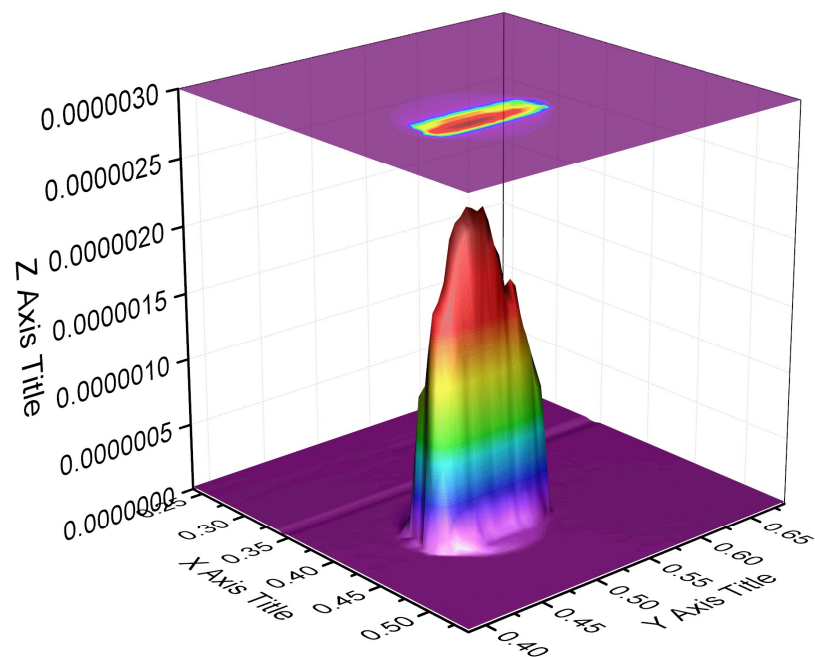




- Fully autonomous – **no user control**
- Arinax MD2s
- EMBL Flex SC – 368 sample capacity
- Pilatus3 2M



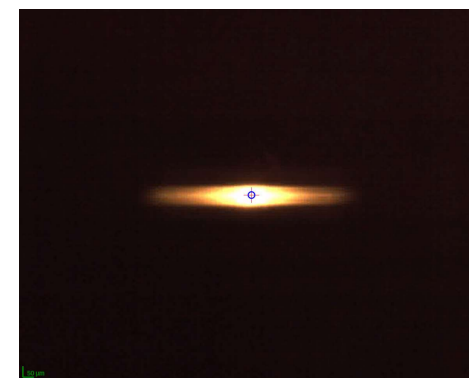
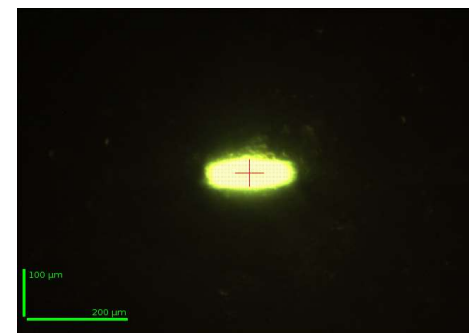
MASSIF1 post EBS



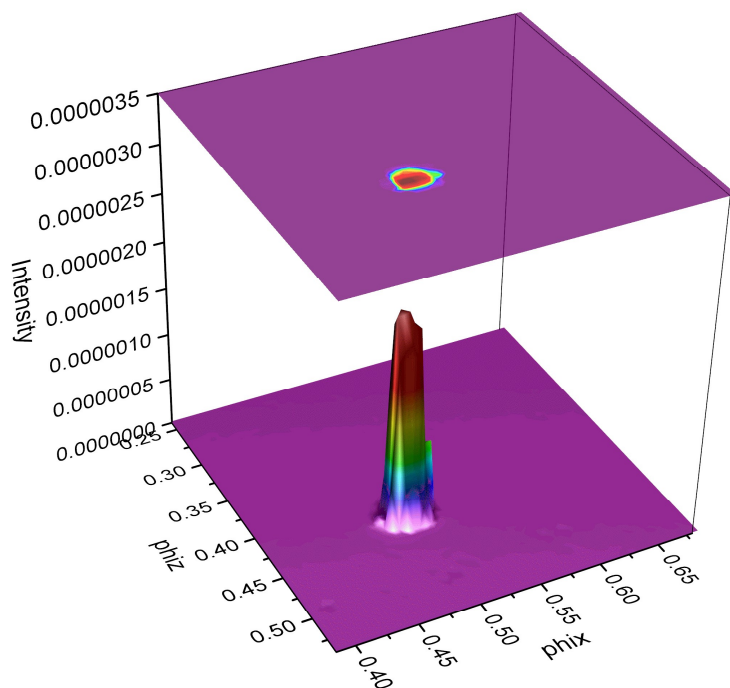
Size: $265 \times 65 \mu\text{m}^2$ FWHM
Flux: 3.1×10^{12} ph/s



Size: $130 \times 29 \mu\text{m}^2$ FWHM
Flux: 8×10^{12} ph/s



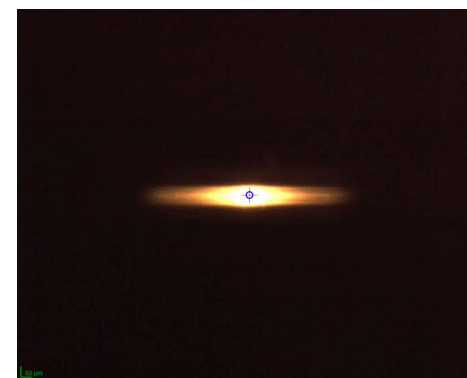
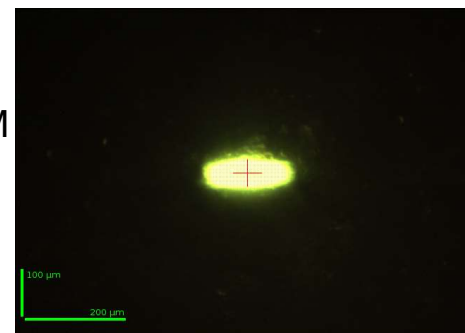
MASSIF1 post EBS



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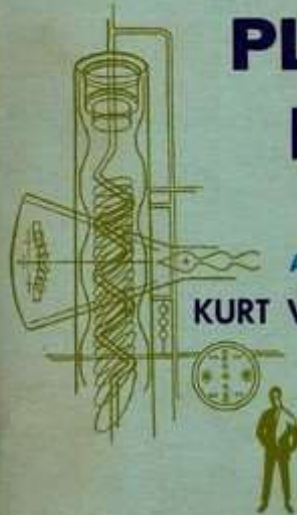
Size: $130 \times 29 \mu\text{m}^2$ FWHM
Flux: 8×10^{12} ph/s



America in the Coming Age of Electronics

PLAYER PIANO

A NOVEL BY
KURT VONNEGUT JR.



Fully automatic (unattended) data collection – what do we gain?

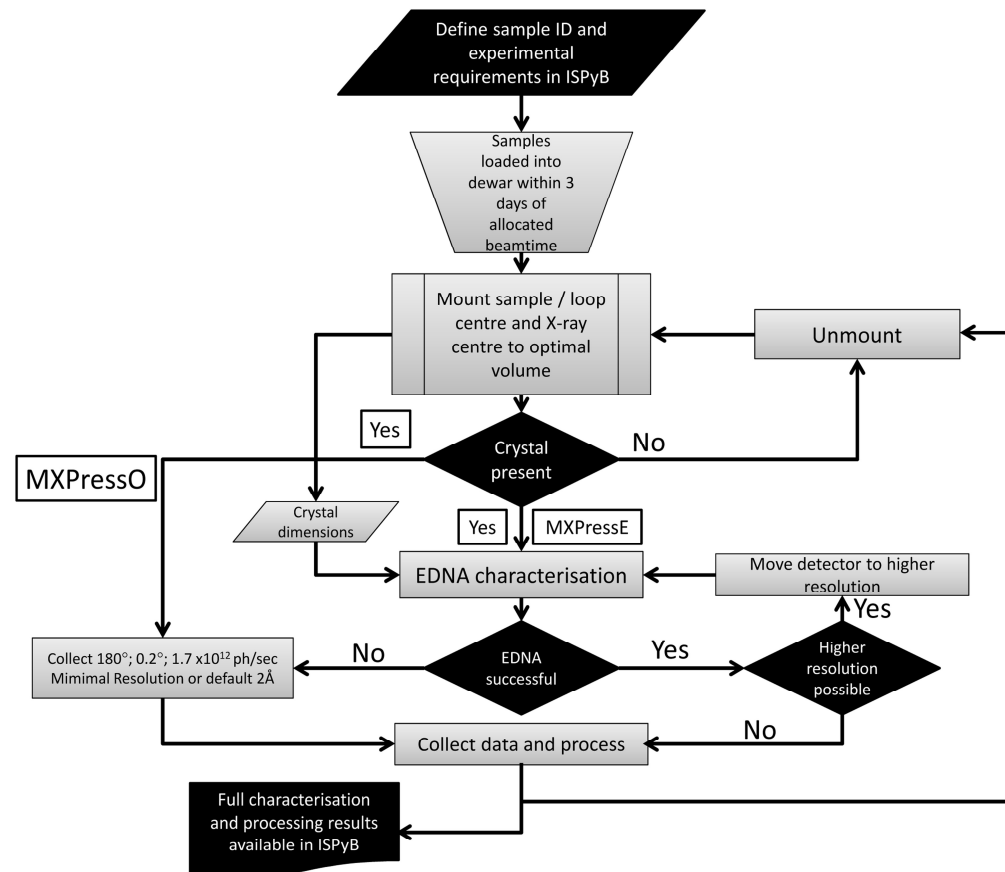
- 40,000 person hours for data collection and travel
- €1,070,400 in expenses and
- 625,845 kg of CO₂ 🌿

(data from @EU_Eurostat)

β_1 adrenergic GPCR



Thanks to Tony Warne



Diffraction Plan entry	Definition	Default value
Protein acronym	Defines the protein that is registered with the ESRF safety group	Required field
Sample name	User defined unique identifier	Required field
Pin barcode	Barcode identifier	none
Experiment type	Define MXPressE / O / SAD / Score	MXPressE
Space Group	If present used for strategy calculation and autoproccessing	none
Pre-observed resolution	Resolution that the detector will be set to for mesh scans, characterisation images and default data collection	2.0 Å
Required resolution	Threshold resolution, samples below cutoff will not be collected	none
Radiation sensitivity	BEST input in case of highly radiation sensitive crystals	1
Required completeness	-	99%
Required multiplicity	-	4
Number of positions	For multiple crystals	1
Preferred beamsize	Select appropriate beamsize for crystals	50 µm

Diffraction Plan entry	Definition	Default value
Protein acronym	Defines the protein that is registered with the ESRF safety group	Required field
Sample name	User defined unique identifier	Required field
Pin barcode	Barcode indentifver	none

ExiMX Extended ISPyB for MX ESRF

Retrieved 5062 samples for selected proposal

Home Shipment Proteins and Crystals Prepare Experiment Data Explorer Offline Data Analysis SMS

search by protein acronym Log out mx415@mx415

Shipment

Name: AA123A Type: SPNE Beamline: #Sample Changer: Status:

⚠ Sample name field is mandatory and no special characters are allowed

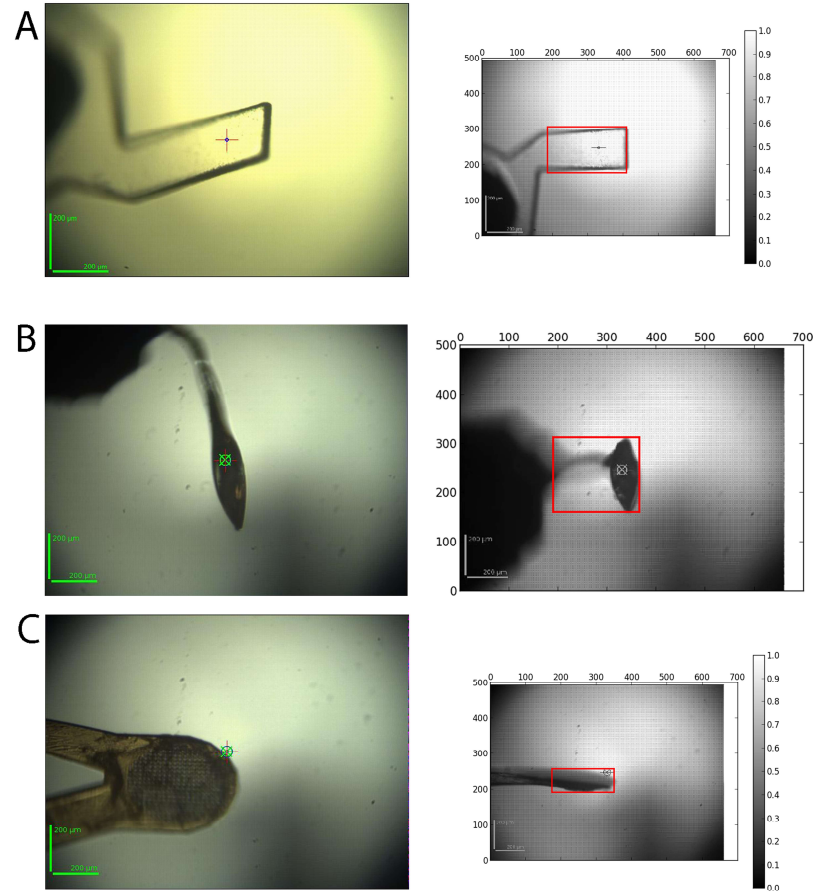
⚠ Protein + sample name should be unique for the whole proposal

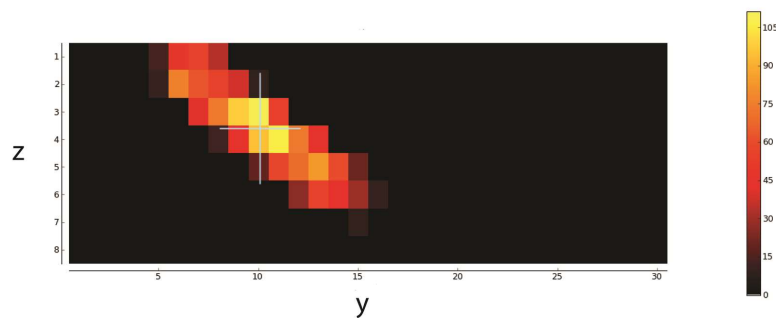
#	Protein Acronym	Sample Name	Pin BarCode	Crystal Form	Exp. Type	Aimed resolution	Required resolution	Beam Diameter	Number of positions	Aimed multiplicity	Aimed Completeness	Forced Space G	Radiation Sensitivity	Smiles	Tot Rot. Angle	Observed resolution	Comments
1	LYS	a1		Undefined	MXPressO	1.7							0.7	Ccfcococf	360		
2	LYS	a2		Undefined	MXPressO	1.7									360		
3	LYS	a3		Undefined	MXPressE_SAD	1.7											
4	LYS	a4		Undefined	MXPressE_SAD			20									
5	LYS	a5		Undefined	MXPressP				7	6							
6	LYS	a6		Undefined	MXPressE				3	6	80						
7	LYS	a7		Undefined	MXPressE												
8	THAU	x234		P41212 - (57.3 , 67.3 , 148.8 - 90 , 90)	MXPressE												
9	THAU	x235		P41212 - (57.3 , 67.3 , 148.8 - 90 , 90)	MXPressE	3.2									200		
10	THAU	x235		P41212 - (57.3 , 67.3 , 148.8 - 90 , 90)	MXPressE	3.2									200		

Required completeness	-	99%
Required multiplicity	-	4
Number of positions	For multiple crystals	1
Preferred beamsize	Select appropriate beamsize for crystals	50 µm

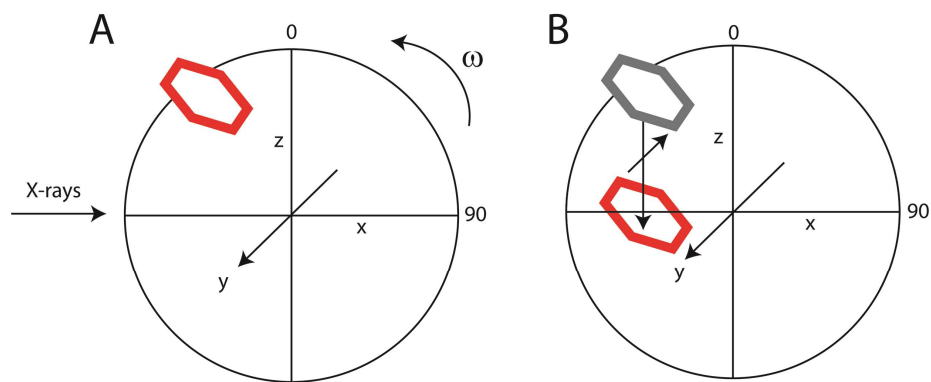




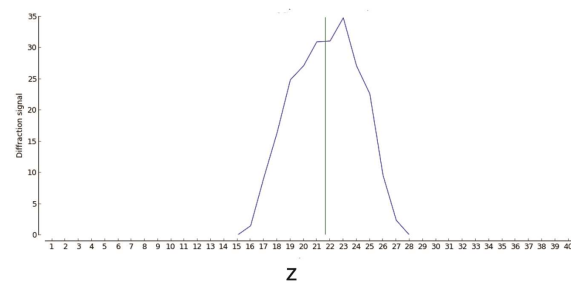
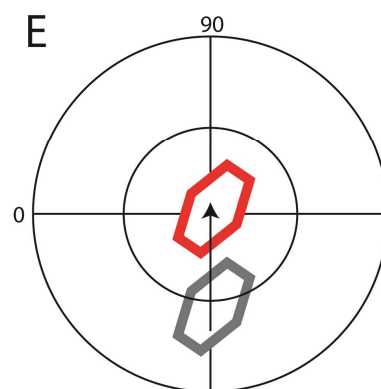
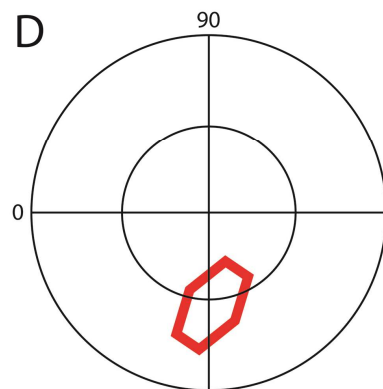




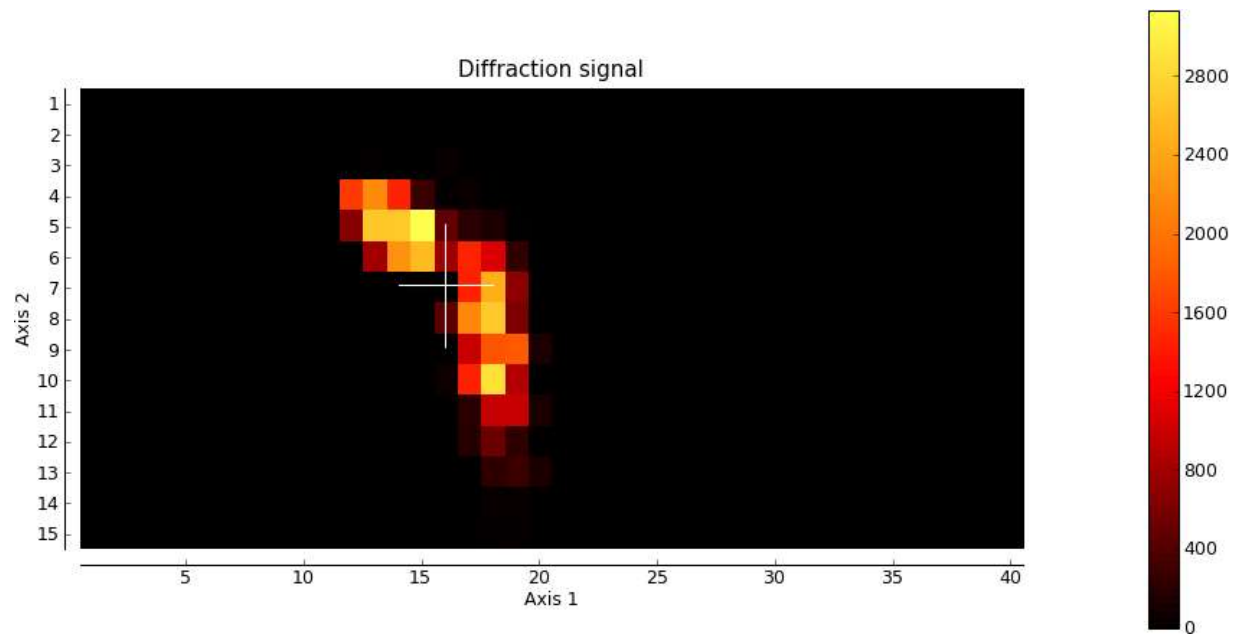
Software routines locate crystals and centre to best volume.



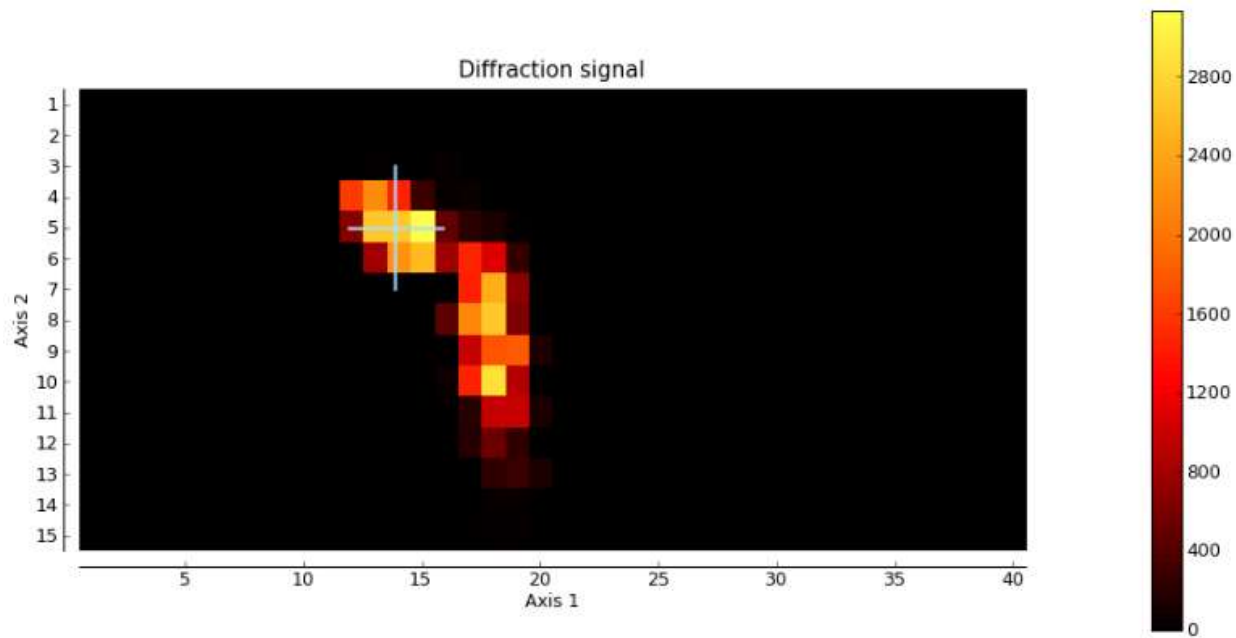
Characteristics such as beam size and flux as well as crystal volume lead to highly optimised data collection

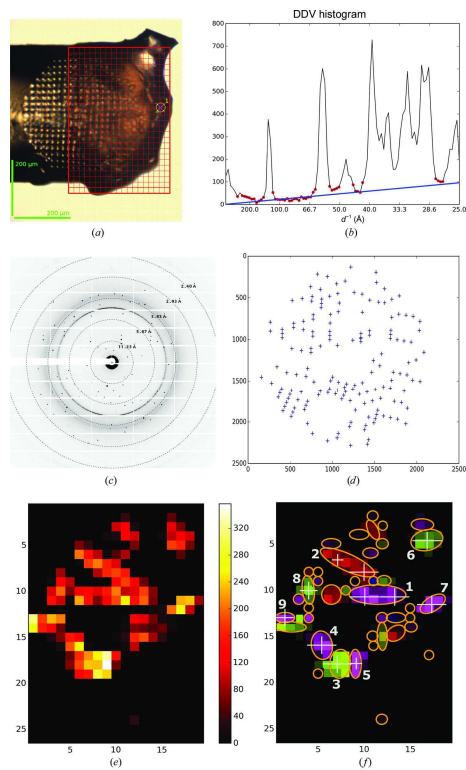


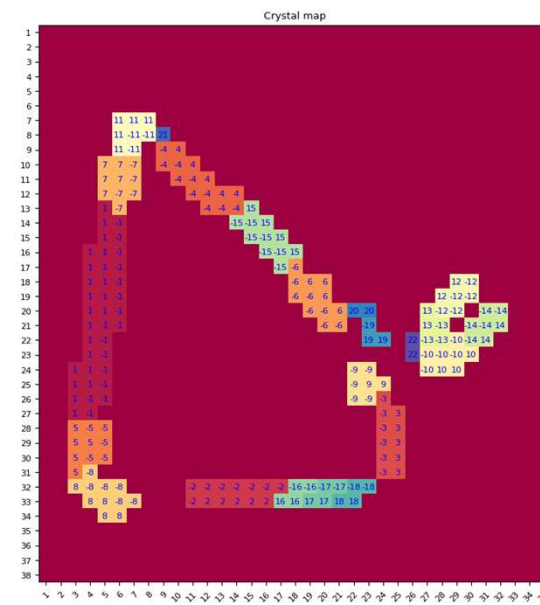
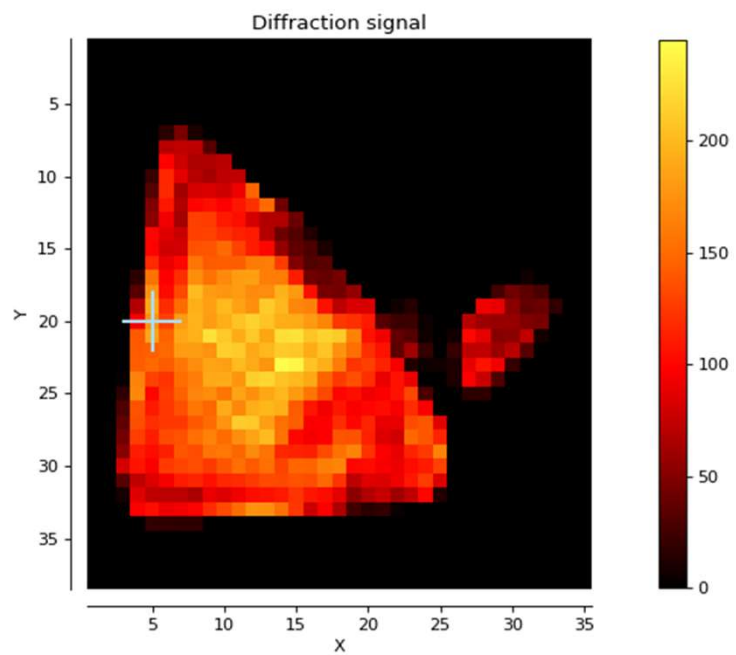
COG determination

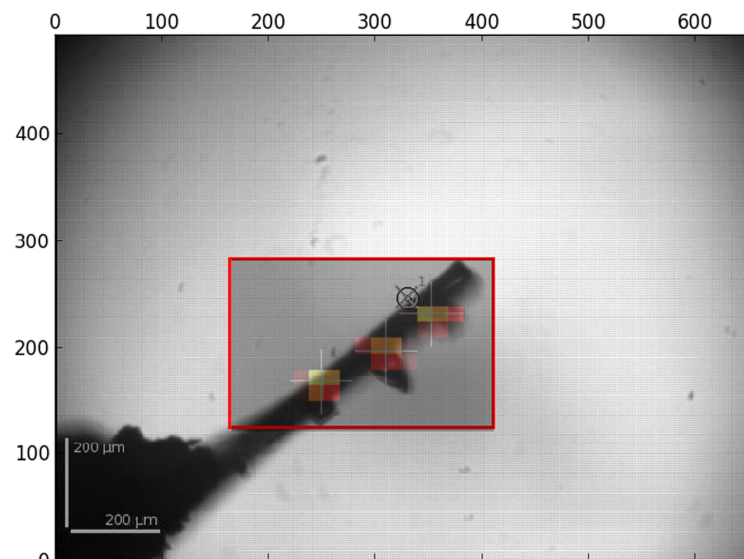
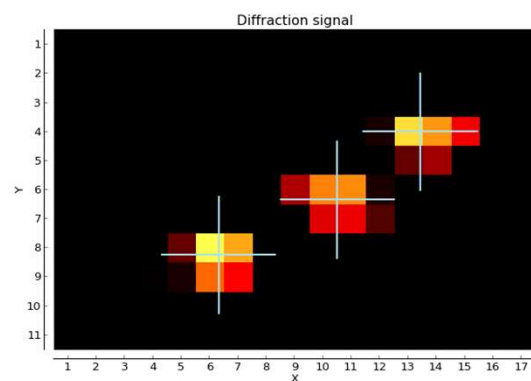
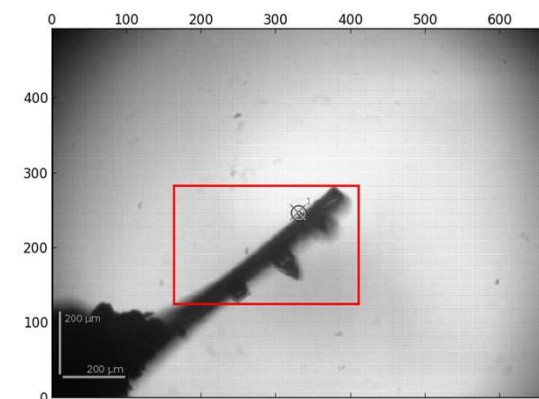


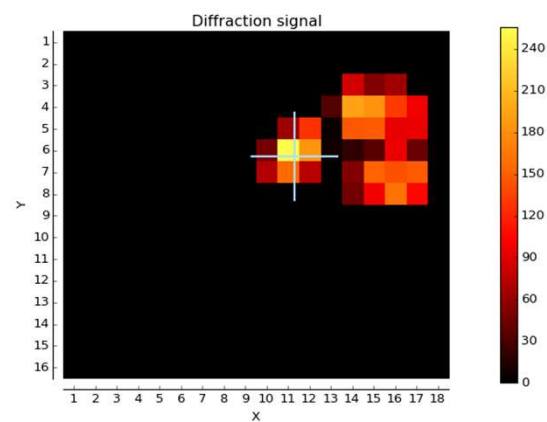
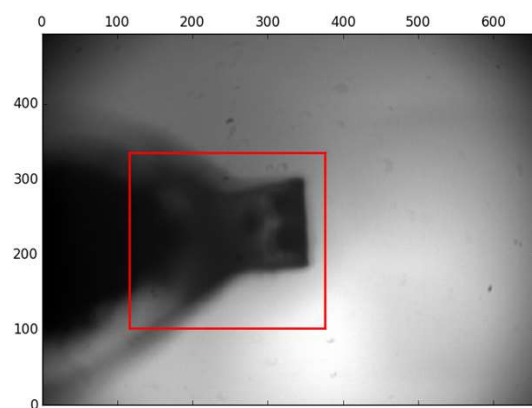
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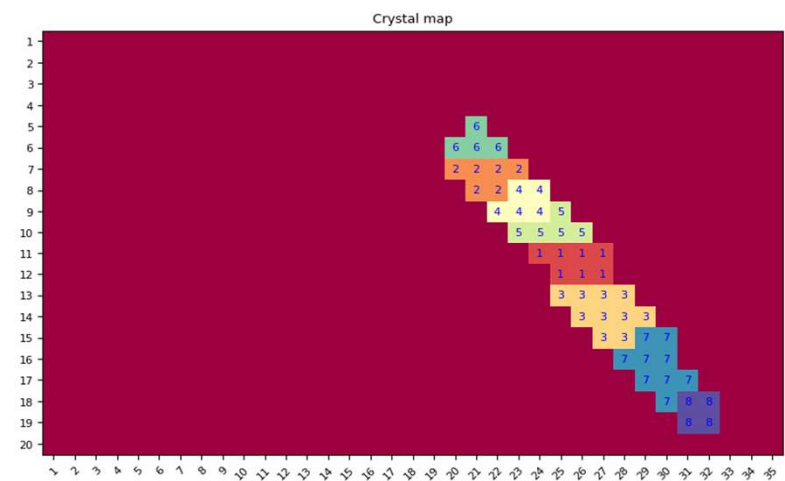
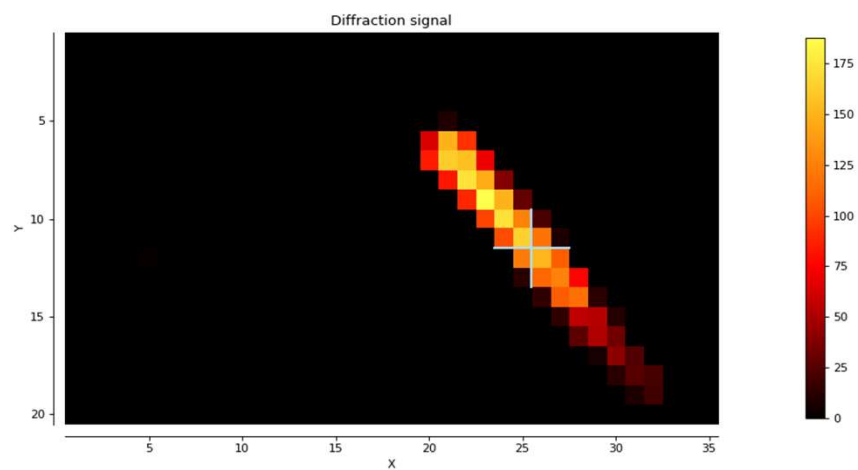




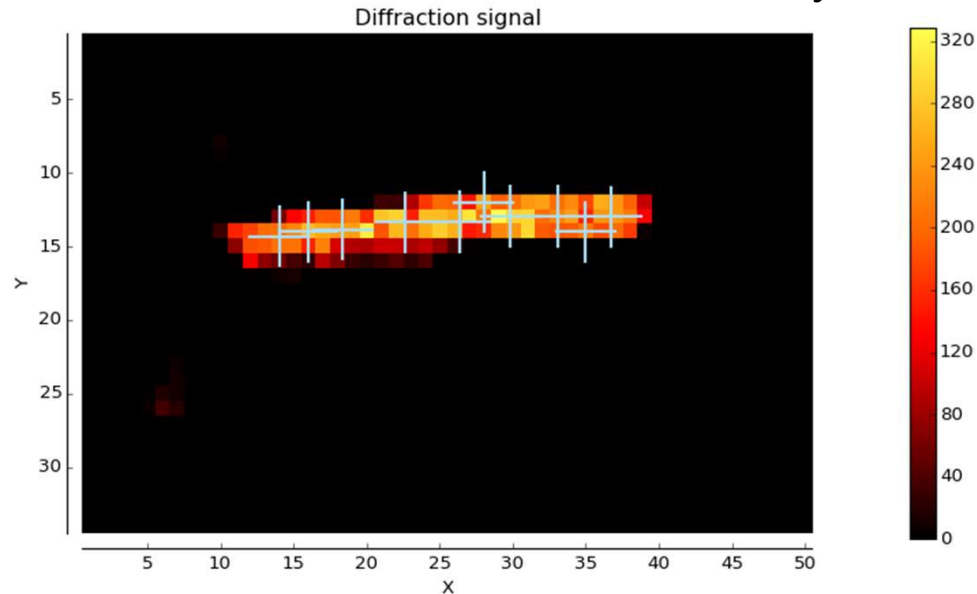




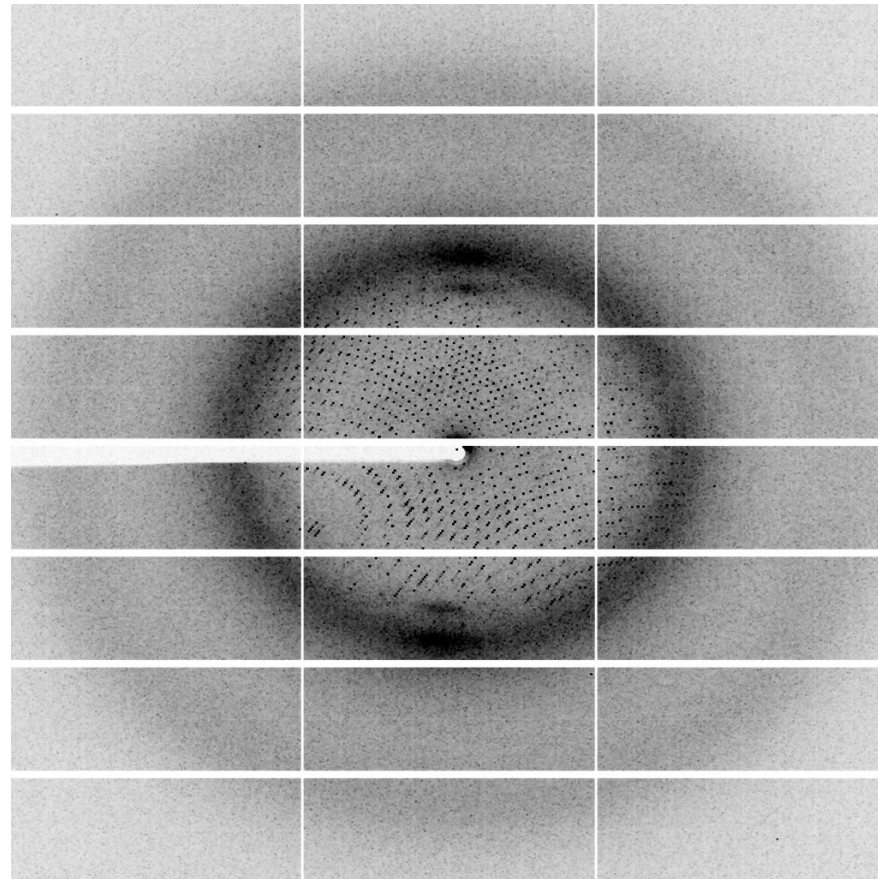




Pseudo-helical data collection – first automated helical workflow that accounts for crystal variability



- Full 'normal' data set the subsequent helical
- Auto-peak selection or user defined
- Stringent thresholding – regions within 30%
- SAD option available

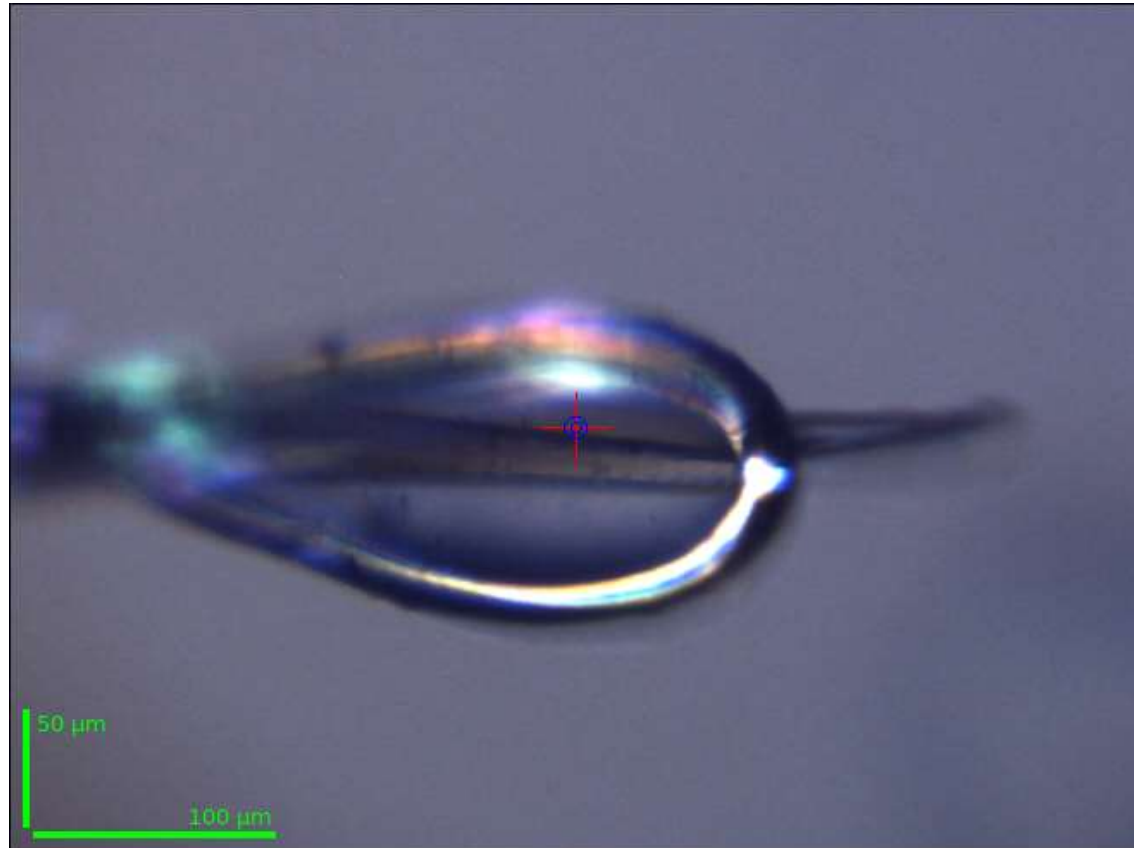


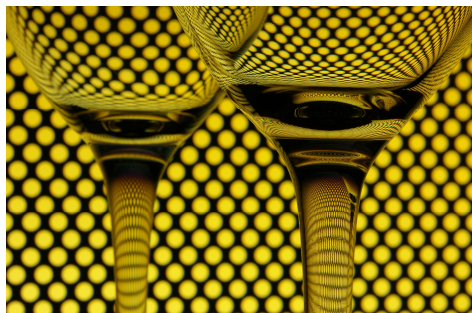
2 Å

Flu polymerase



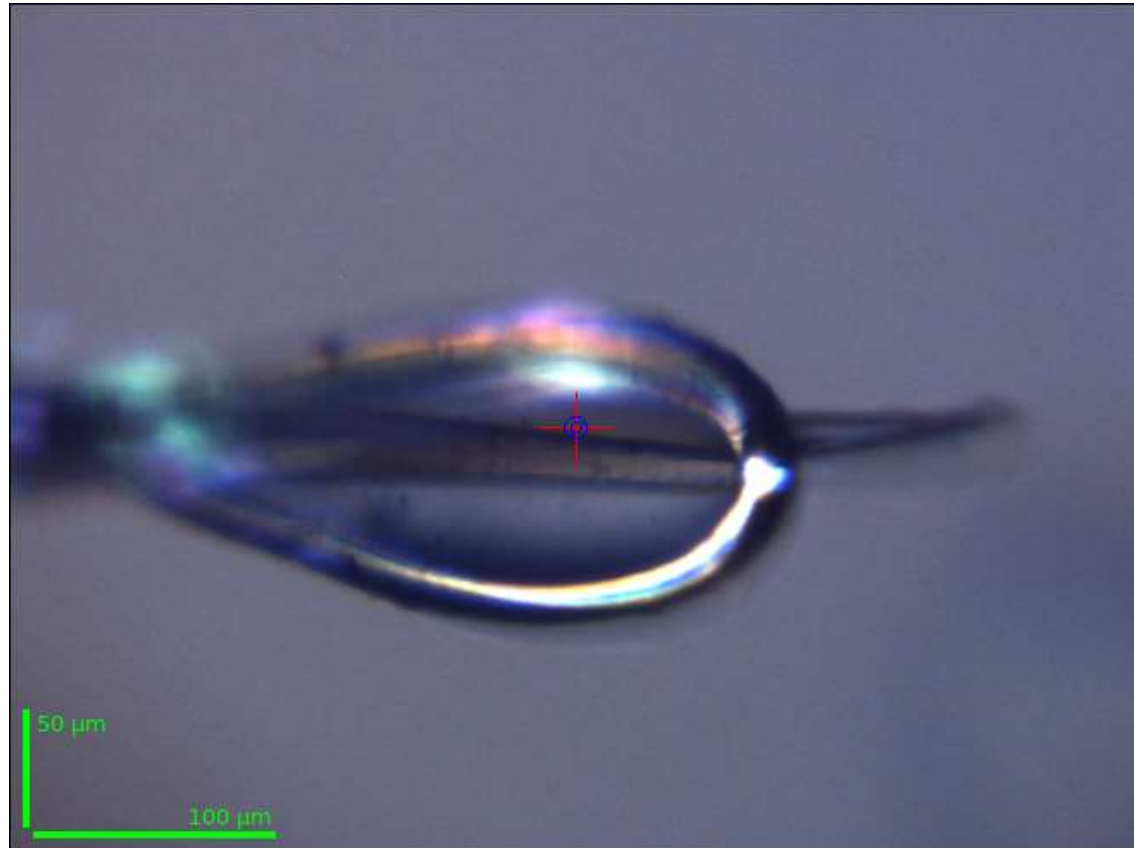
Flu polymerase

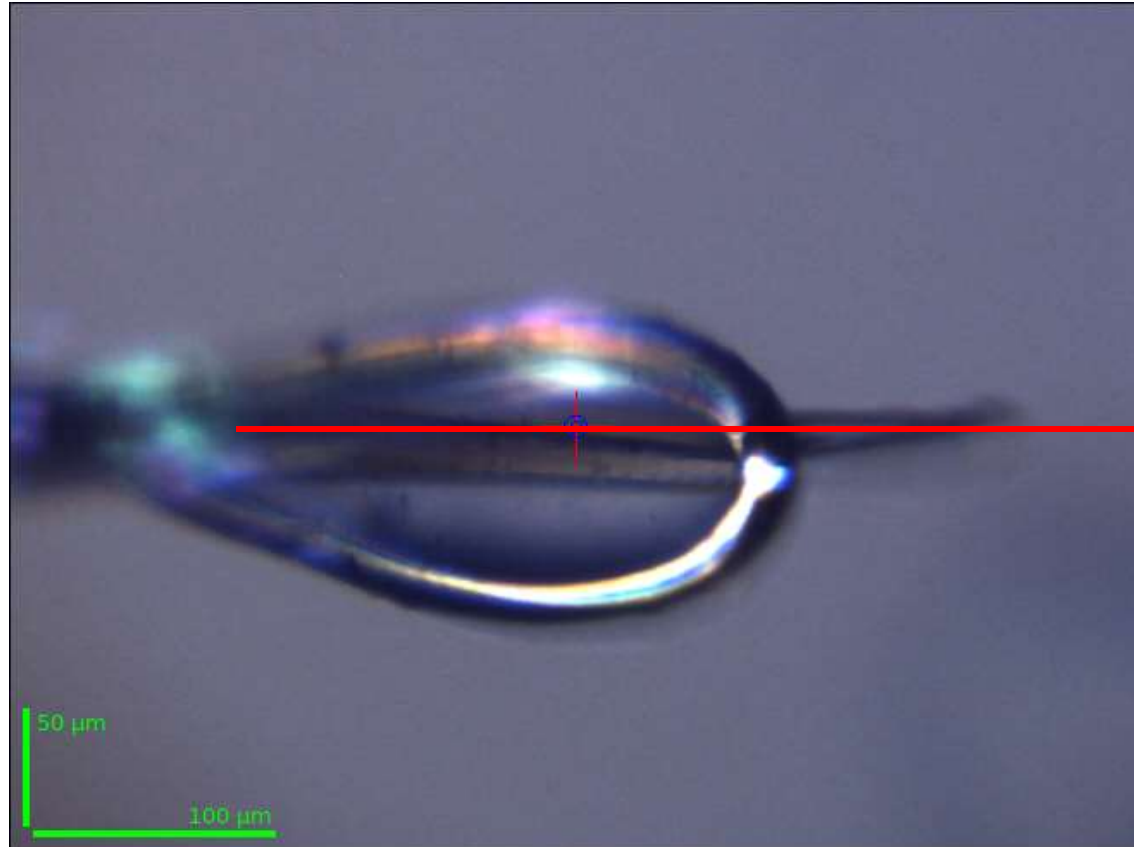






“Fata Morgana”



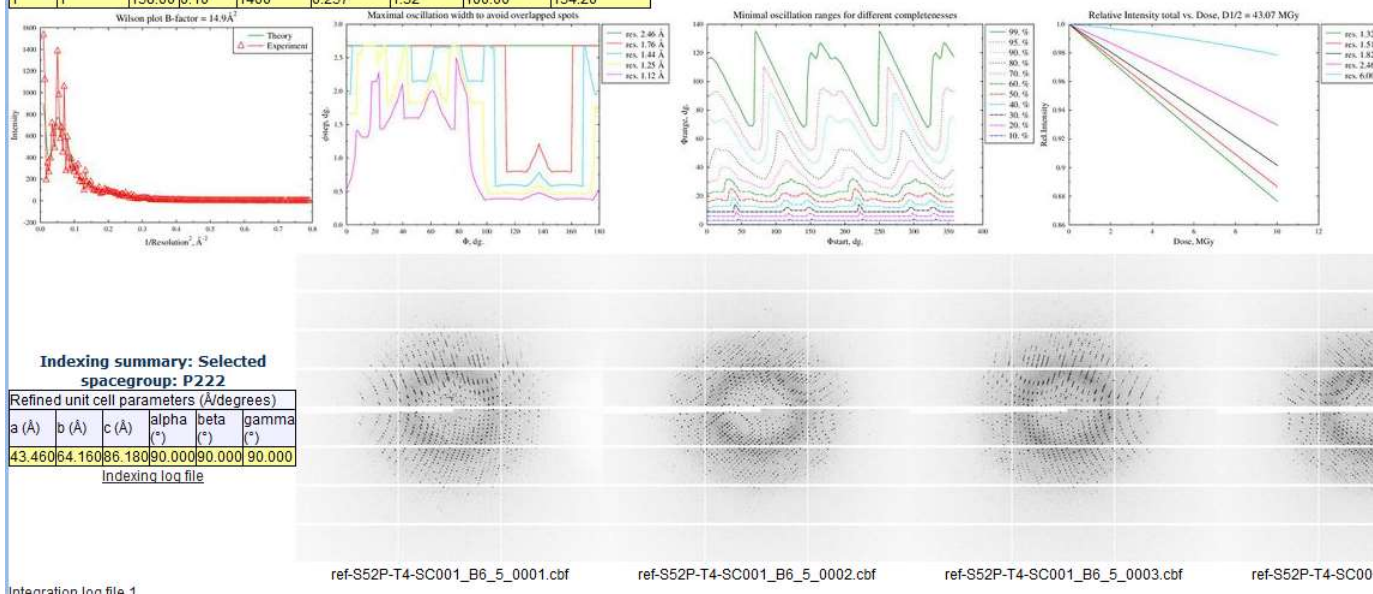


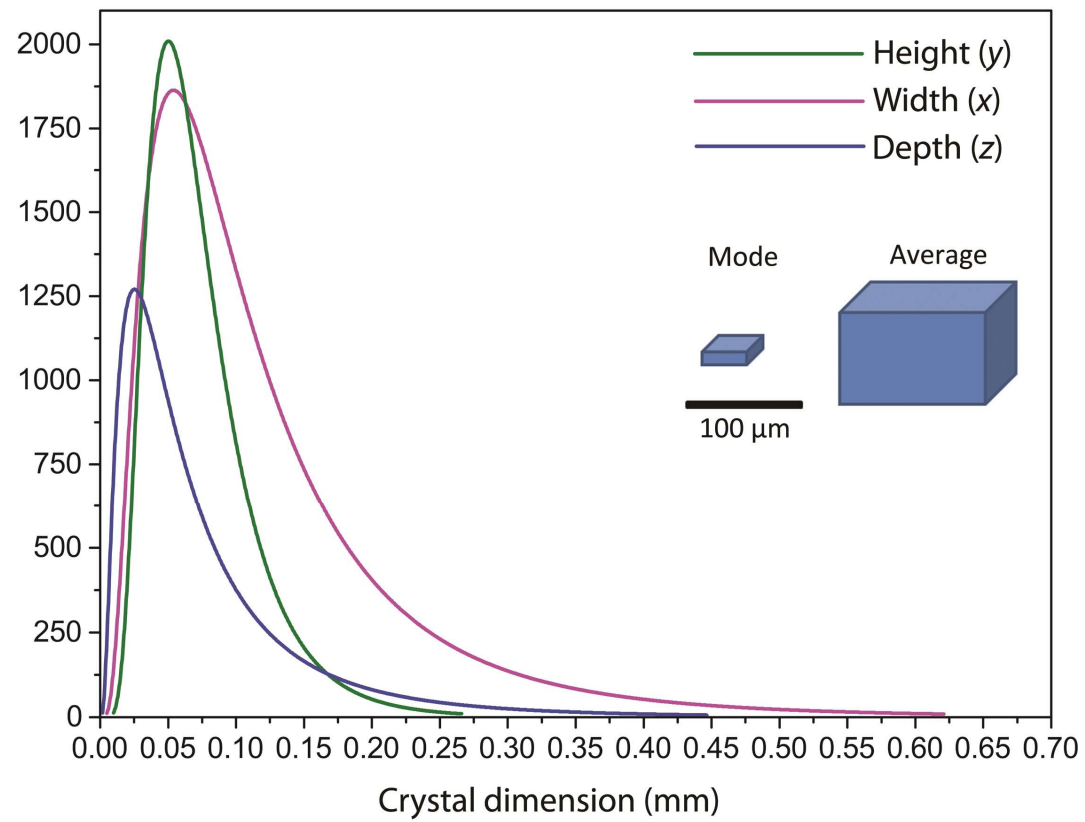
space group	data	multiplicity	completeness	at highest res.	resolution (Å)
None	False	Default (optimized)	Default (≥ 0.99)	BEST Default	Default (highest possible)

Collection plan strategy (**RADDOS** log file , **BEST** log file)

Resolution limit is set by the radiation damage

Wedge	Subwedge	Start (°)	Width (°)	No images	Exp time (s)	Max res (Å)	Rel trans (%)	Distance (mm)
1	1	158.00	0.10	1400	0.257	1.32	100.00	134.20





Bowler M.W., Svensson, O and Nurizzo, D. (2016) *Cryst. Rev.*, **22**, 229-245

The difference measurements make.....

	Crystal larger than beam		Crystal smaller than beam	
	Default Crystal Size	Measured Crystal Size	Default Crystal Size	Measured Crystal Size
Crystal dimensions (μm)	100 x 100 x 100	603 x 238 x 397	100 x 100 x 100	74 x 37 x 13
Beam diameter (μm)	50	50	50	50
Space group	C2 ₁	C2 ₁	P222	P222
Unit cell dimensions a, b, c (Å) α, β, γ (°)	129.00, 208.38, 117.50, 90, 109.3, 90	129.00, 208.38, 117.50, 90, 109.3, 90	86.37, 90.66, 113.25, 90, 90, 90	86.37, 90.66, 113.25, 90, 90, 90
Flux (ph/s)	1.2 x 10 ¹²	1.2 x 10 ¹²	1.2 x 10 ¹²	1.2 x 10 ¹²
Transmission (%)	100	100	100	54.6
Dose (MGy)	13.23	36.38	13.54	4.93
Total exposure time (s)	178.9	507.1	91.5	60.4
Oscillation Range (°)	108 - 252	108 - 252	170 - 275	9 - 160
Detector resolution (Å)	2.16	2.05	2.86	3.06

β_1 adrenergic GPCR



Thanks to Tony Warne

β_1 adrenergic GPCR

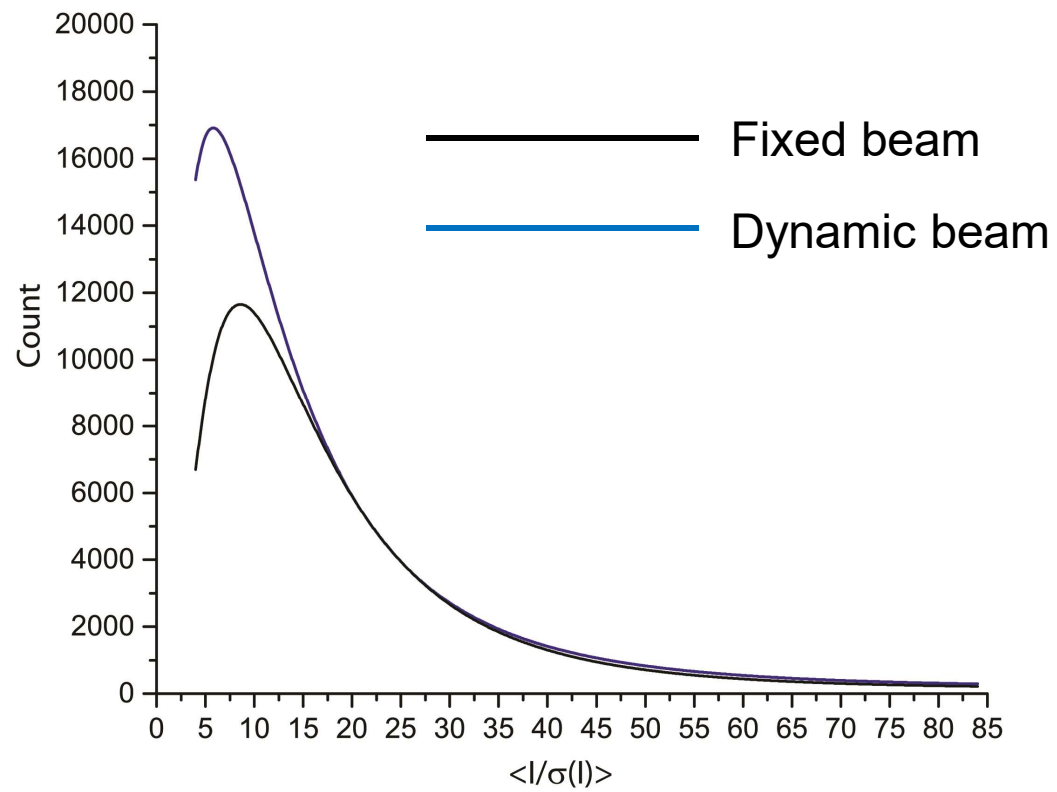
Crystal	Crystal dimensions (x, y, z, mm)	Fixed beam diameter		Adaptable beam diameter	
		Resolution limit (Å)	$\langle I/\sigma(I) \rangle$	Resolution limit (Å)	$\langle I/\sigma(I) \rangle$
adrcpt-For41	0.109 x 0.053 x 0.025	3.77	6.7	4.13	4.4
adrcpt-For42	0.084 x 0.025 x 0.025	4.22	4.3	3.53	10.6
adrcpt-For45	0.035 x 0.045 x 0.051	3.95	6.2	-	-
adrcpt-For47	0.105 x 0.061 x 0.051	3.74	4.7	3.72	5.4
adrcpt-For48	0.105 x 0.039 x 0.064	-	-	3.8	5.7
adrcpt-For58	0.169 x 0.050 x 0.061	3.88	6.6	4.11	4.5
adrcpt-For59	0.042 x 0.024 x 0.025	3.25	9.2	3.16	8.3
adrcpt-For67	0.064 x 0.026 x 0.031	-	-	3.8	5.6

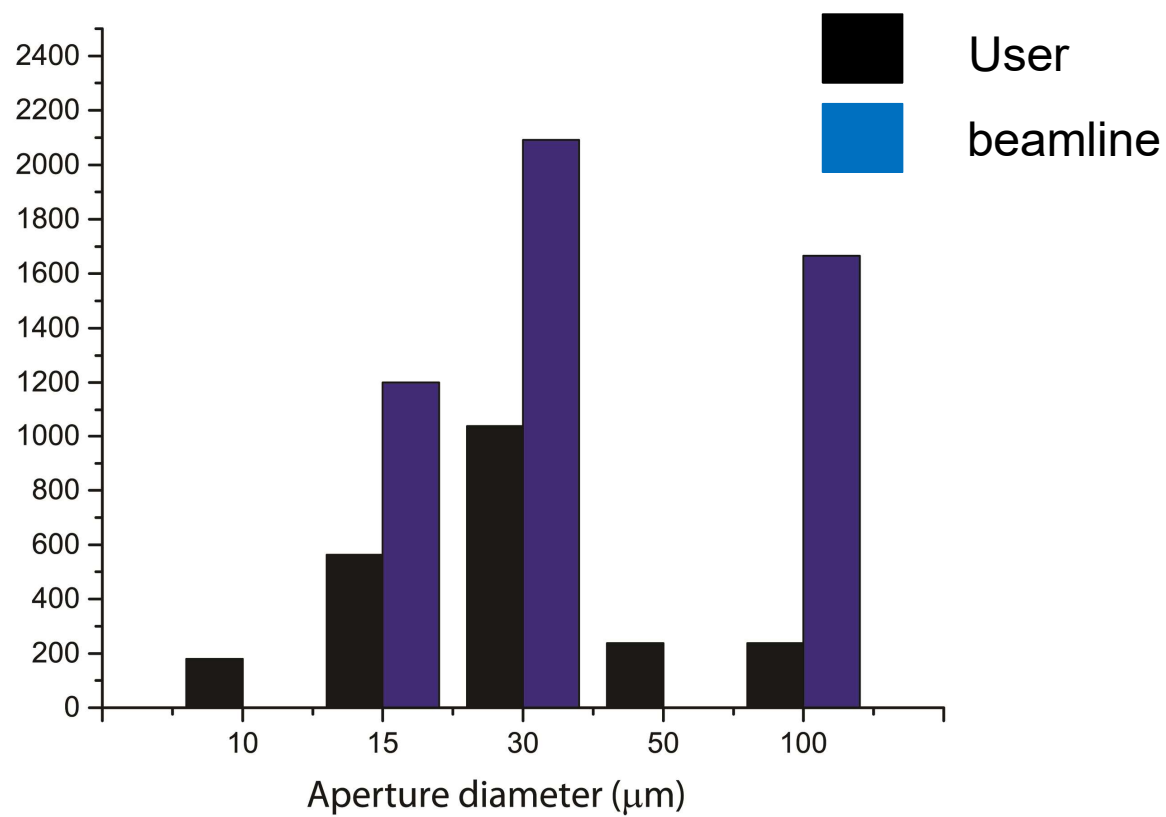
β_1 adrenergic GPCR

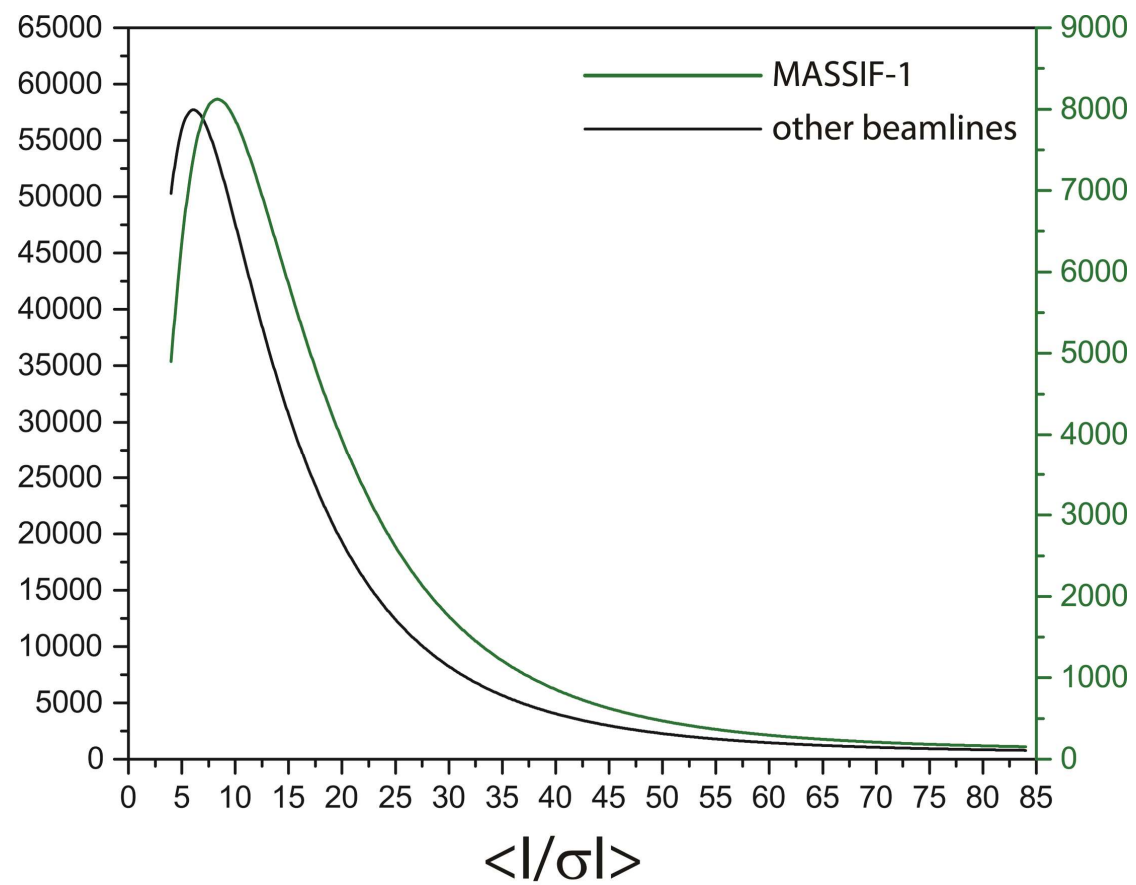
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B₁ adrenergic GPCR

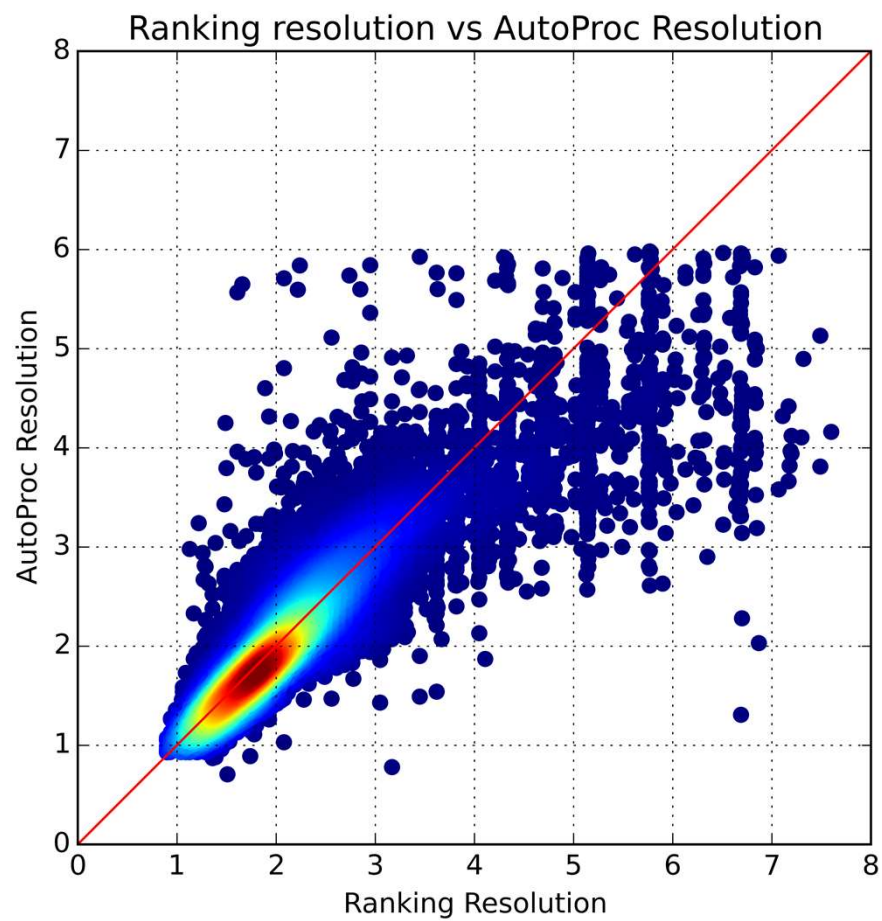
Crystal	adrcpt-For42	adrcpt-For42
Beam diameter (μm)	50	30
Space group (no.)	P2 ₁ 2 ₁ 2 ₁ (19)	P2 ₁ 2 ₁ 2 ₁ (19)
Cell parameters (Å, °)	116.8, 121.1, 129.5 90, 90, 90	116.5, 120.78, 128.7 90, 90, 90
Flux (ph/s)	5.7 x 10 ¹¹	2.2 x 10 ¹¹
Rotation width (°)	0.15	0.05
Total oscillation Range (°)	149.1	124.0
Total dose (MGy)	5.25	5.92
Detector resolution	4.05	3.9
Wilson B-factor (Å ²)	94.0	97.0
Resolution (Å)	48.2 – 3.95 (4.09 – 3.95)	48.7 – 3.53 (3.66–3.53)
Completeness (%)	98.2 (90.9)	81.5 (33.0)
Observed reflections	15,940 (1413)	18,785 (721)
Average redundancy	4.1 (2.7)	4.4 (1.6)
< I/σ(I) >	4.3 (0.8)	10.6 (0.6)
R _{meas}	0.23 (1.45)	0.1 (1.64)
R _{merge}	0.18 (1.05)	0.077 (1.17)
CC _{1/2}	0.99 (0.43)	1 (0.33)



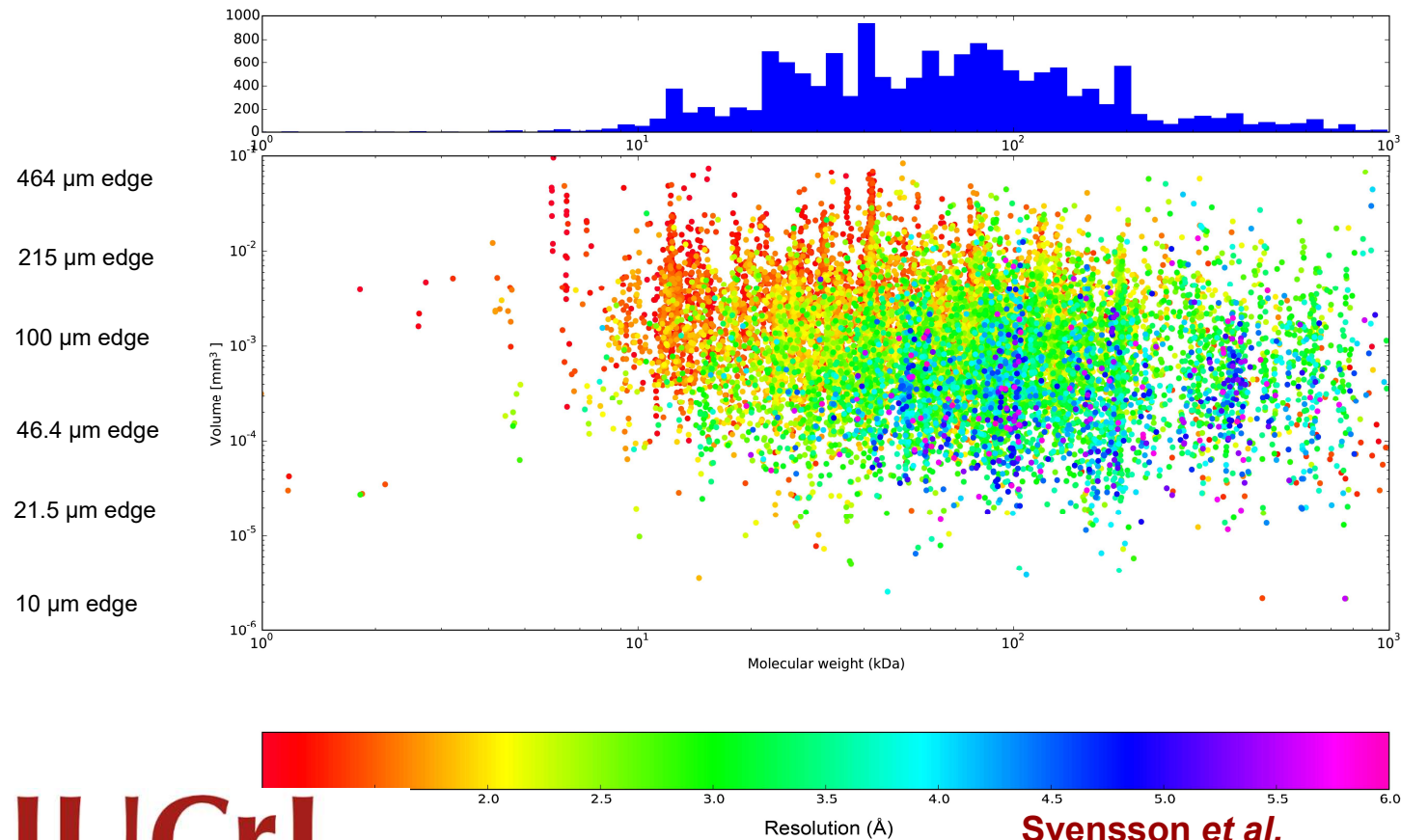




Bowler M.W., Svensson, O and Nurizzo, D. (2016) *Cryst. Rev.*, **22**, 229-245



Molecular-weight dependence of the minimum required crystal size



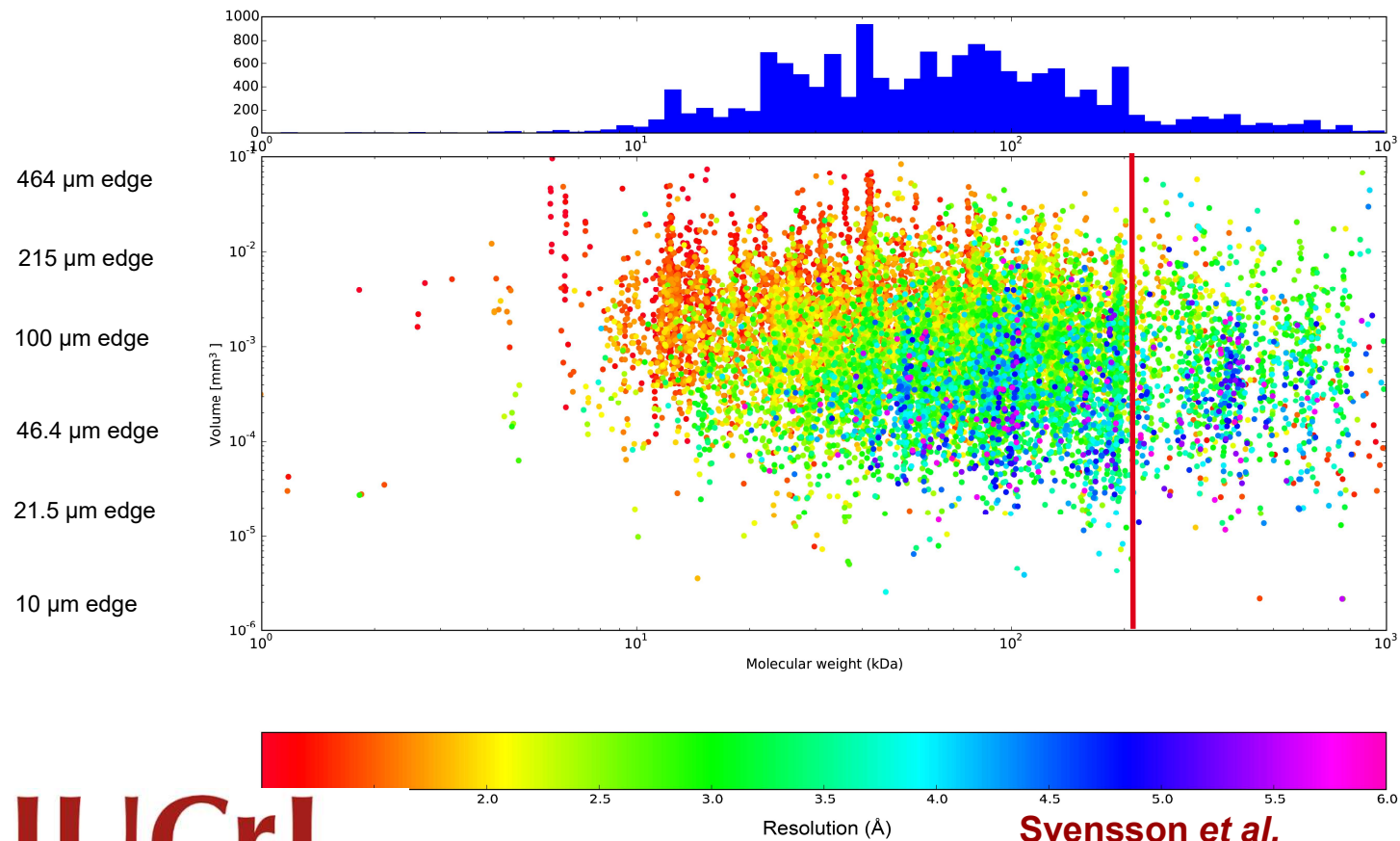
IUCrJ

Svensson *et al.*

Volume 6 | Part 5 | September 2019 | Pages 822–831 |



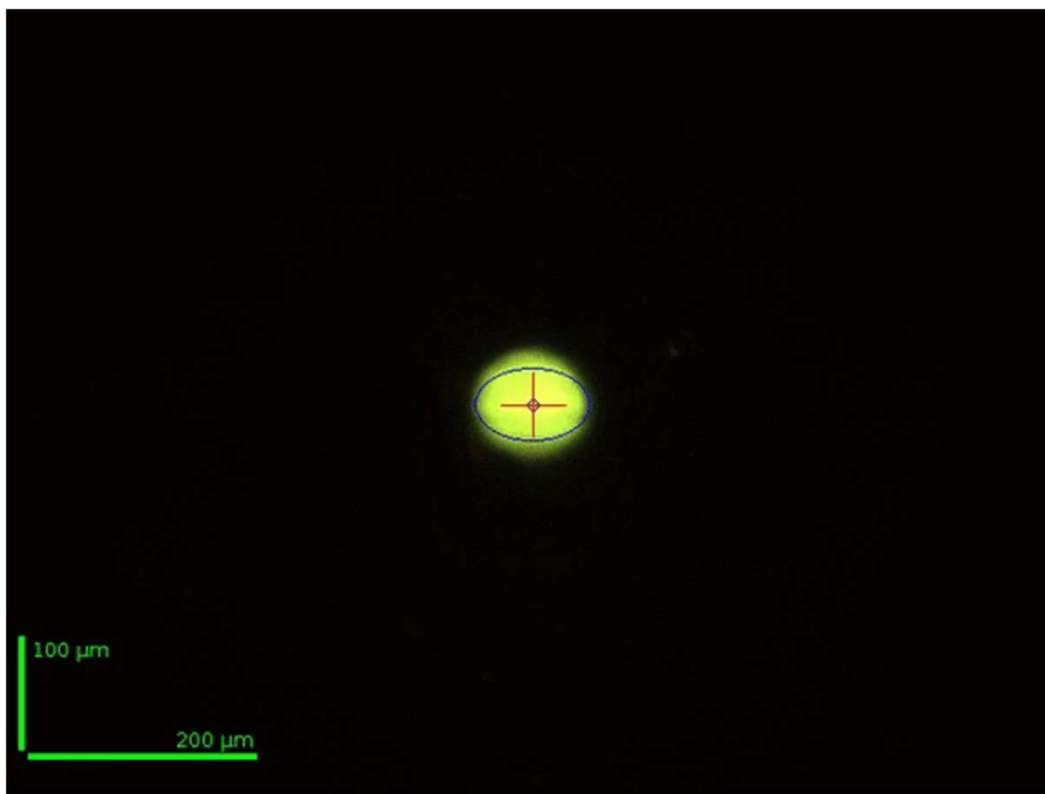
Molecular-weight dependence of the minimum required crystal size

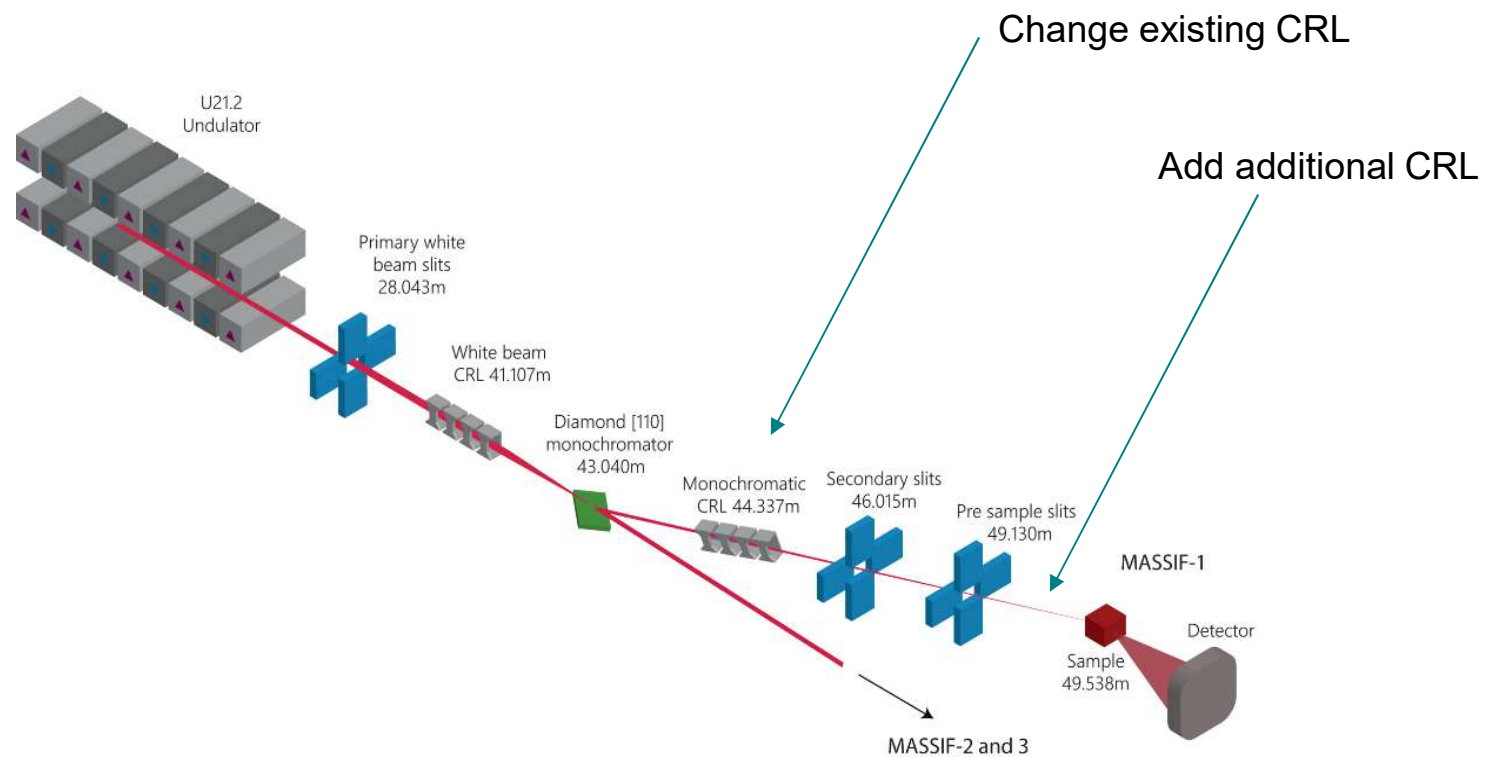


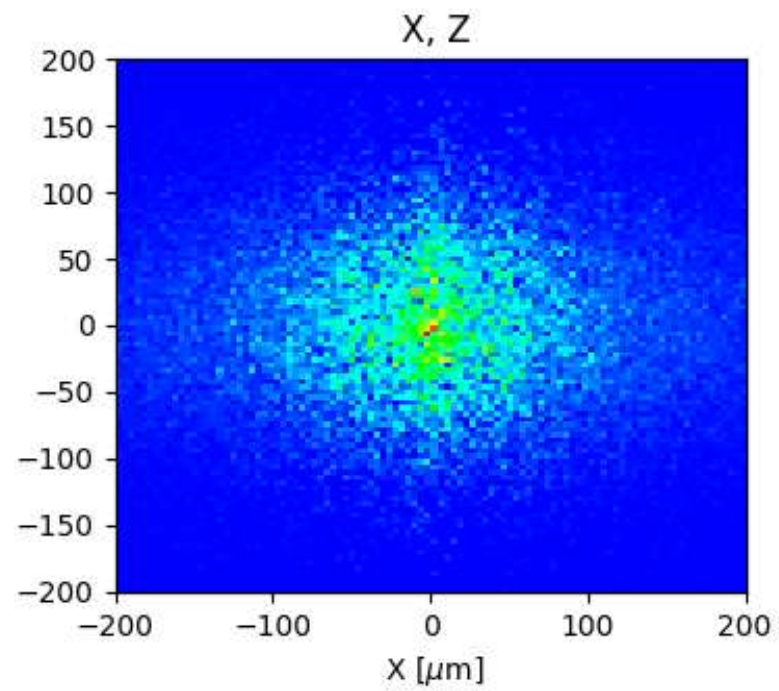
IUCrJ

Svensson et al.
Volume 6 | Part 5 | September 2019 | Pages 822–831 |



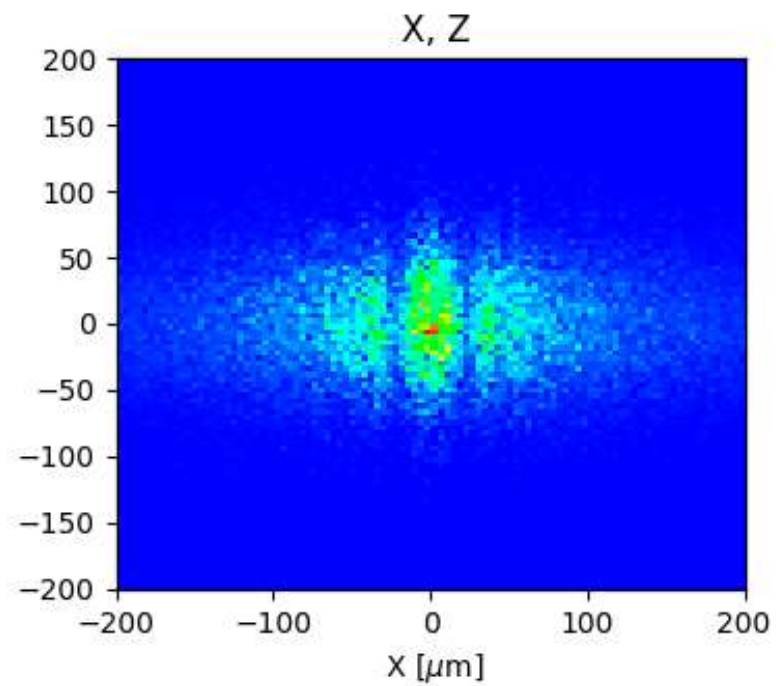






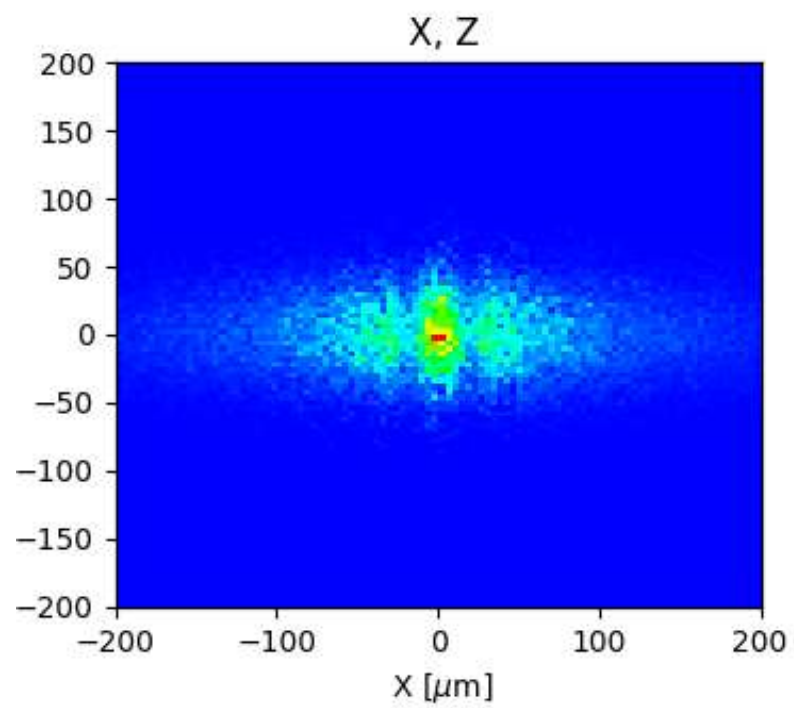
100 μm diameter beam

Flux: 5×10^{12} ph/sec



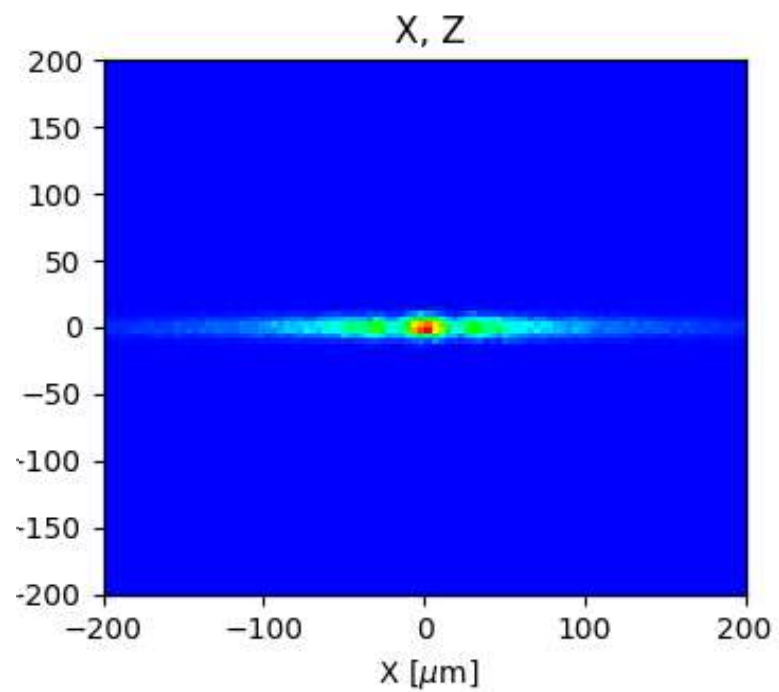
75 μm diameter beam

Flux: 5×10^{12} ph/sec



50 μm diameter beam

Flux: 5×10^{12} ph/sec



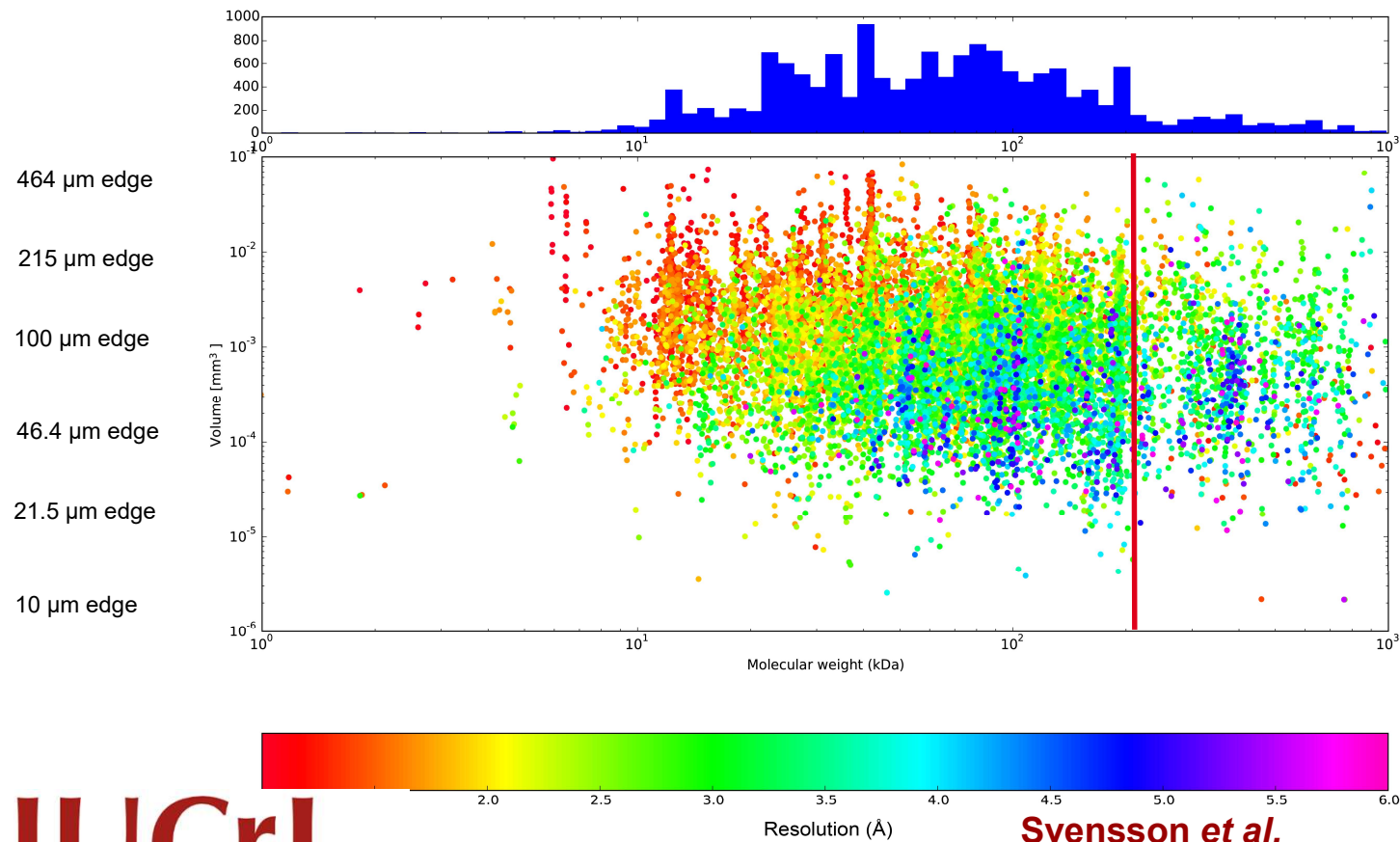
10 μm diameter beam

Flux: 5×10^{12} ph/sec



Flux: 5×10^{11} ph/sec

Molecular-weight dependence of the minimum required crystal size



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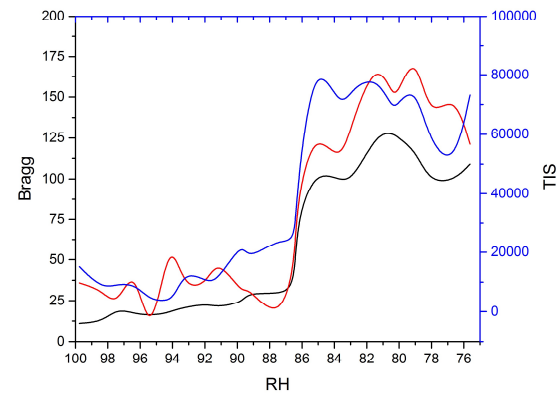
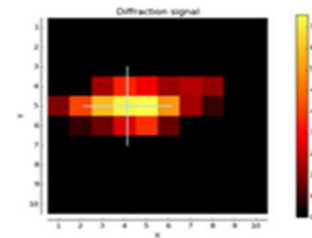
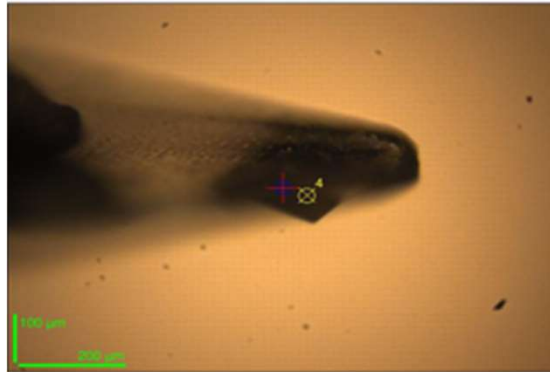


HC1 and CrystalDirect allow automated RT

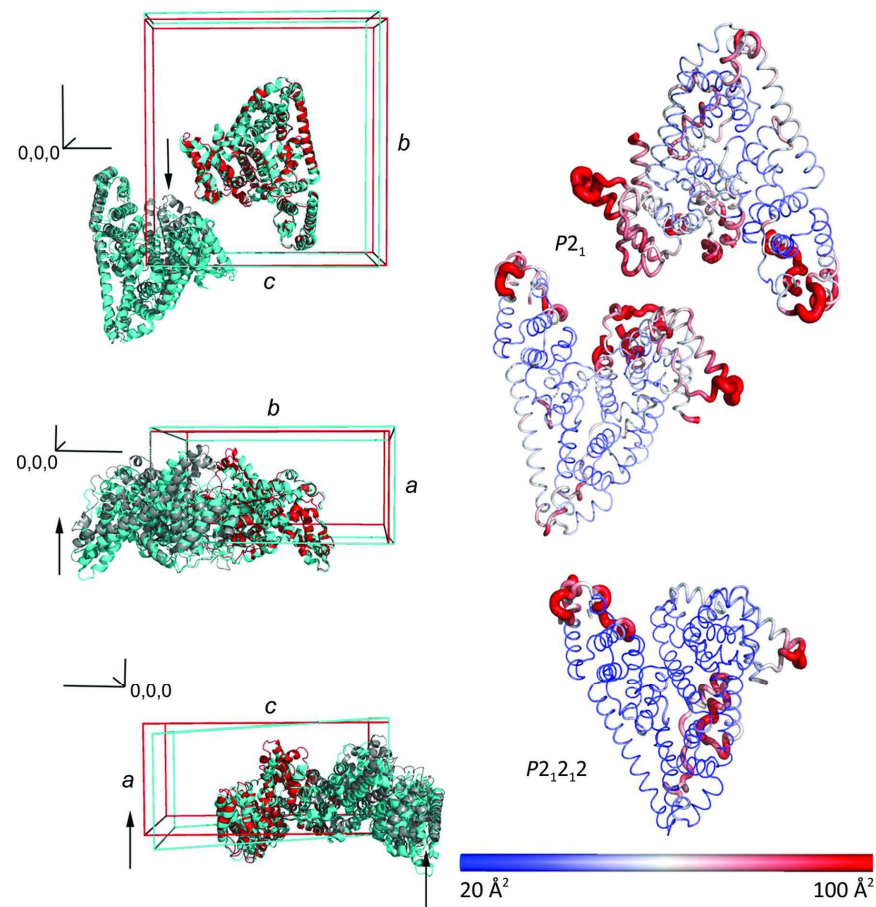


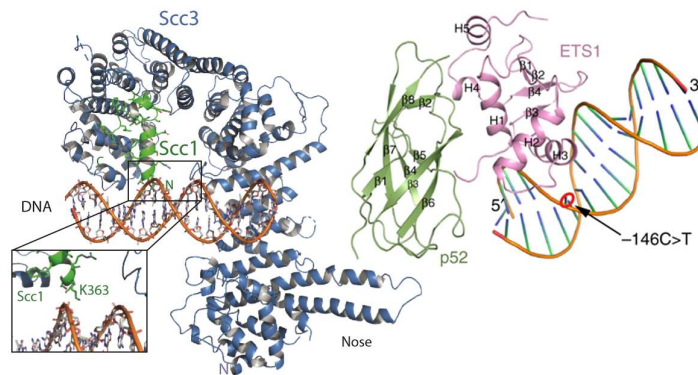
CrystalDirect-To-Beam: Opening the shortest path from crystal to data
AIP Conference Proceedings **2054**, 050009 (2019); <https://doi.org/10.1063/1.5084627>

Automated humidity control experiments



Automated humidity control experiments



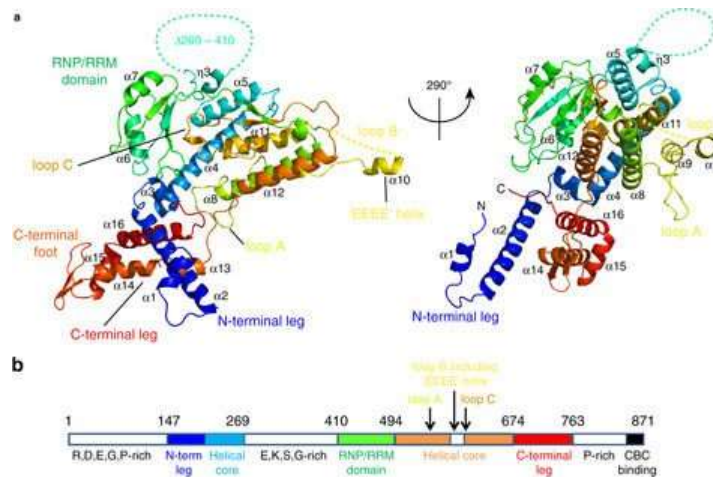


Not just for lysozyme!

Scc3/Scc1 complex
with DNA: – 600
crystals final 3.8 Å
data set

p52 and ETS1: 300
crystals final 3.0 Å
data set

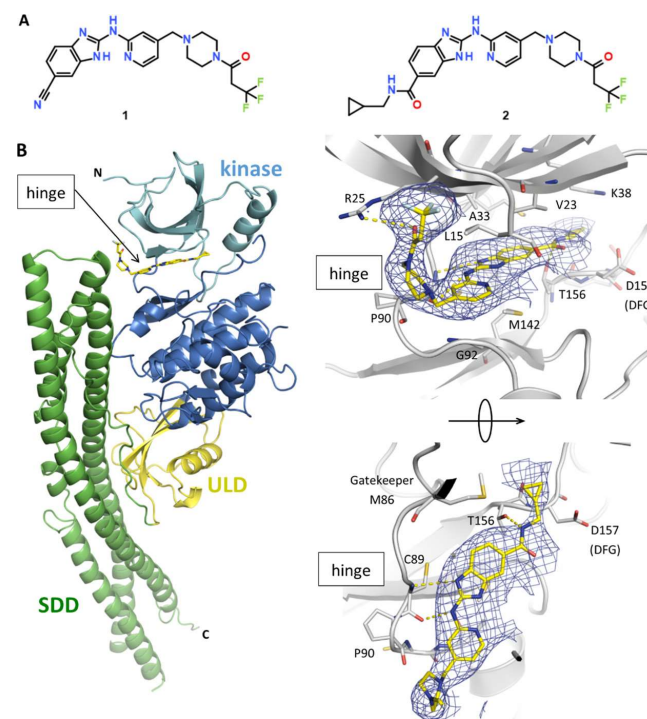
ARS2: 300 crystals
Se-Met phasing at
4.0 Å



MASSIF-1

Example – Bayer discover a new anti-inflammatory targeting Tank Binding Kinase (TBK)

- Problem: Crystals diffract poorly (1 in 20 good) but many fragments need to be screened
- Automation allowed large number of molecules to be screened (20 per molecule, over 1000 total) without human presence
- Optimised data collection allowed high enough resolution to be obtained to develop the molecule



Acknowledgements:

Didier Nurizzo
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Matias Guijarro
Olof Svensson
Debby Davison

Marcus Oscarsson
Marjolaine Bodin
Stephanie Monaco
Gordon Leonard
Christoph Mueller-Dieckmann
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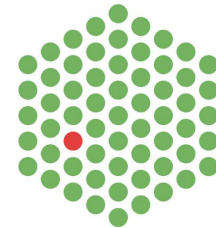


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