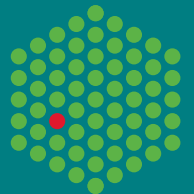


# News from ID30B

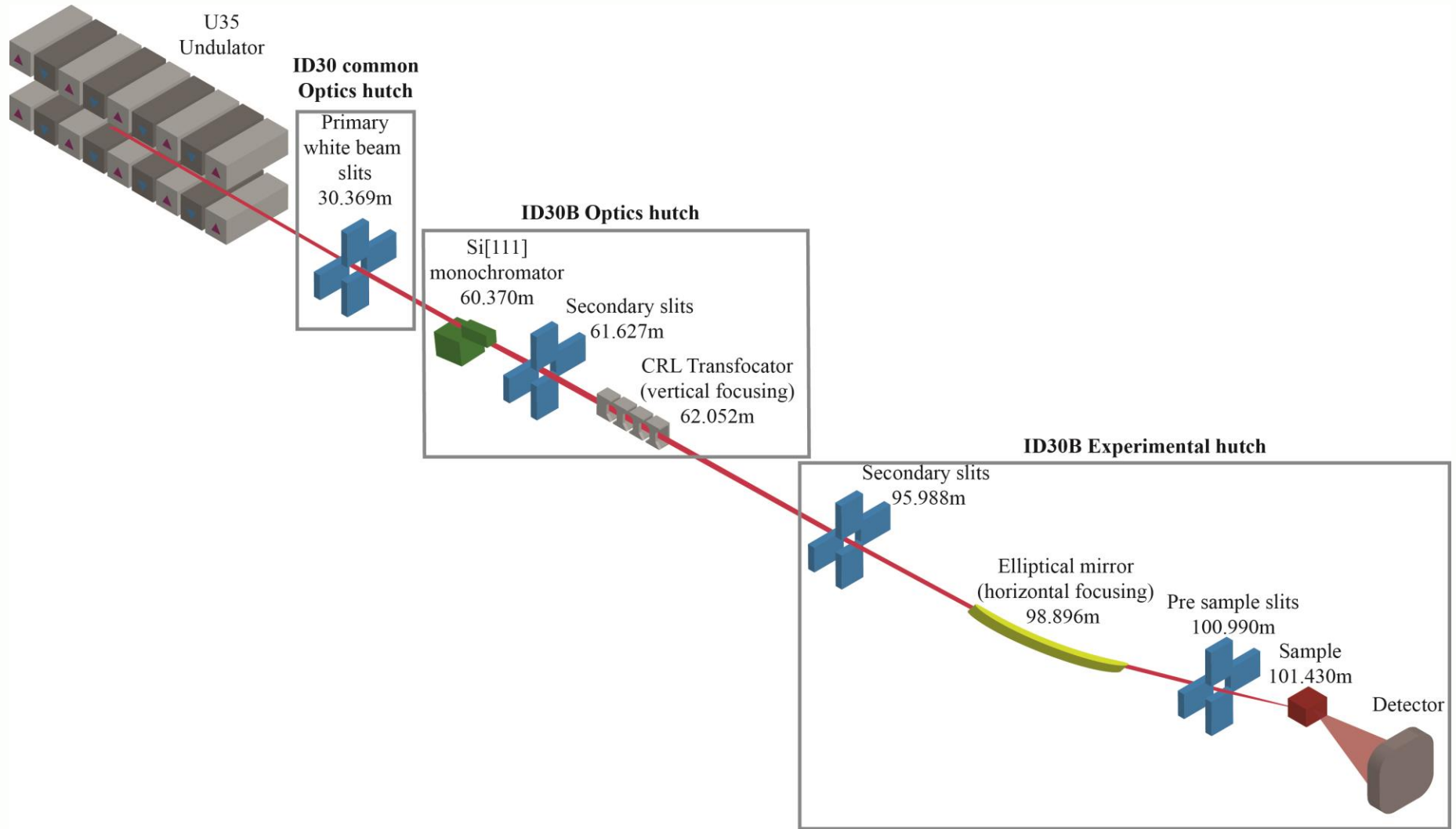
*Andrew McCarthy (EMBL)*

*Christoph Mueller-Dieckmann (ESRF)*

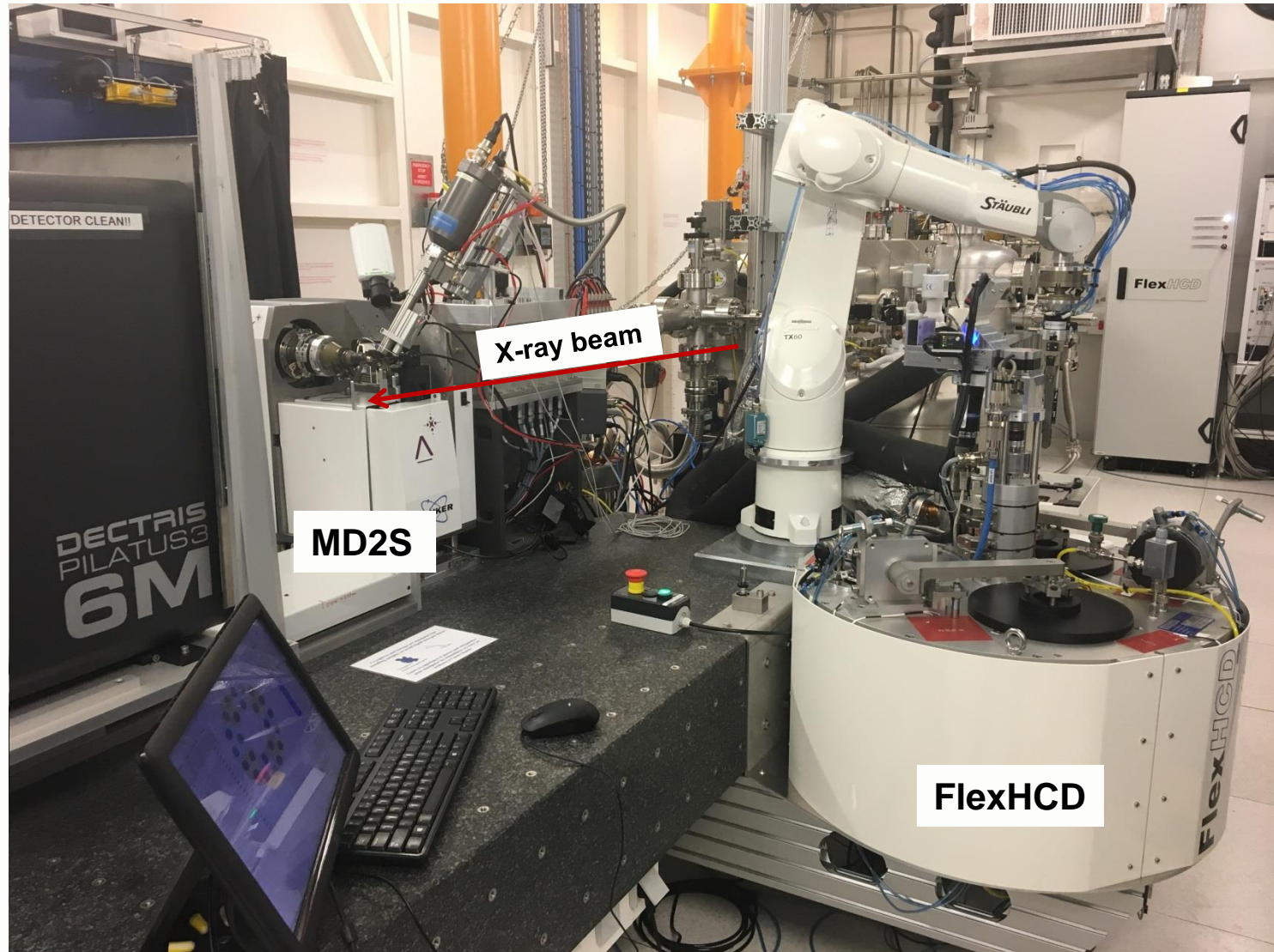
EMBL



# ID30B – Optical layout



# ID30B – Experimental hutch



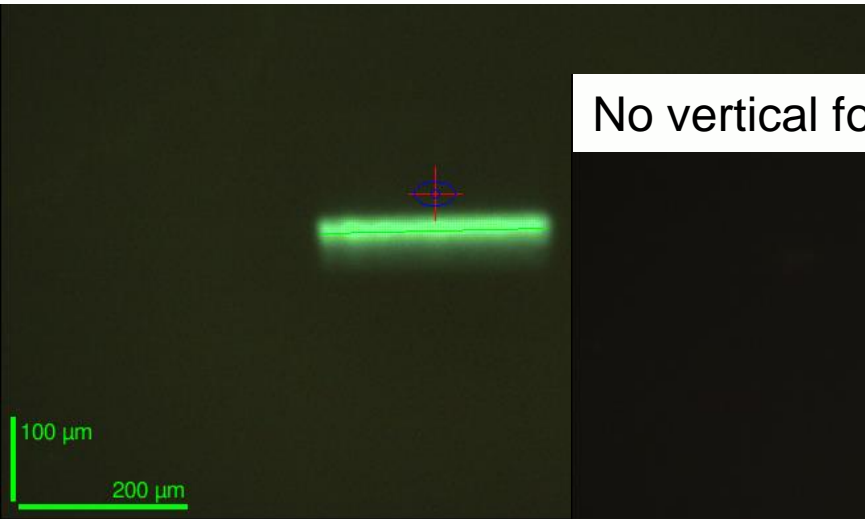
# ID30B – Variable focusing capabilities

Energy range: 6-20 keV

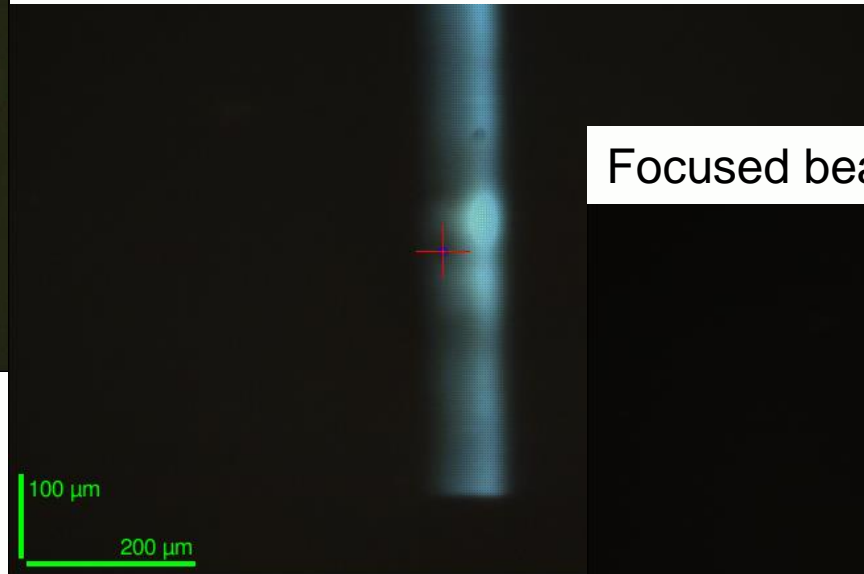
Flux  $\sim 5 \times 10^{12}$  phs/sec/mm<sup>2</sup> at 12.7 keV

Beam size:  $< 40 \mu\text{m}^2$  (apertures – 10, 20, 30, 50 and 75  $\mu\text{m}^2$ )

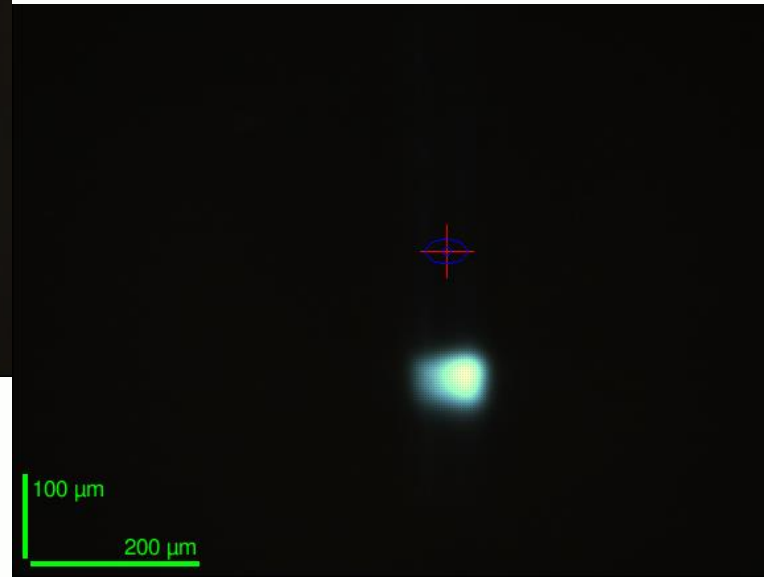
Vertical focusing, no horizontal focusing  
(mirror unbent)



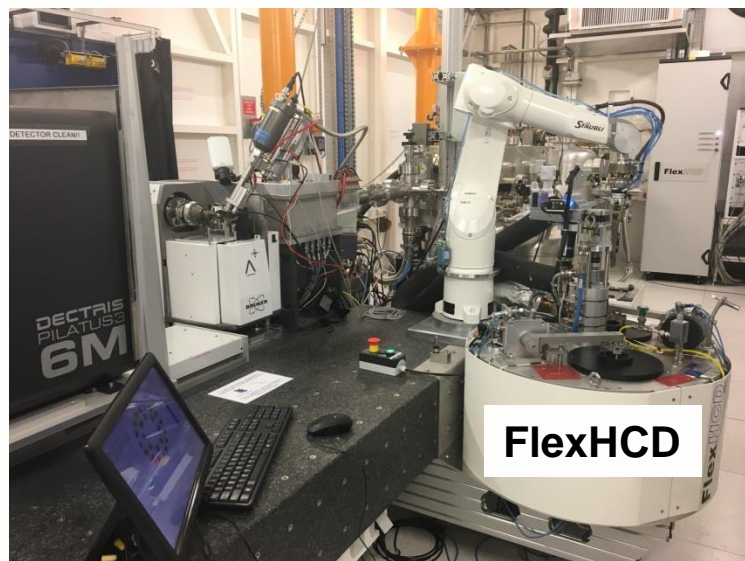
No vertical focusing – mirror bent



Focused beam



# FlexHCD – A versatile sample changer



FlexHCD

SPINE baskets (x12) = 120 samples

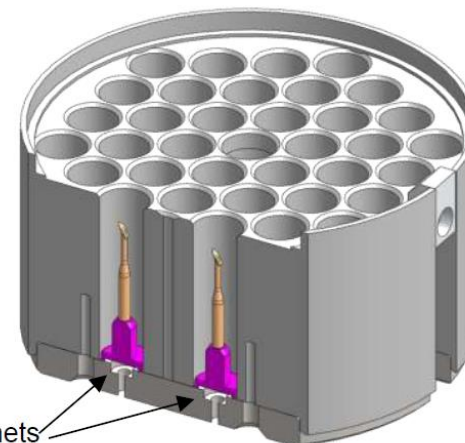
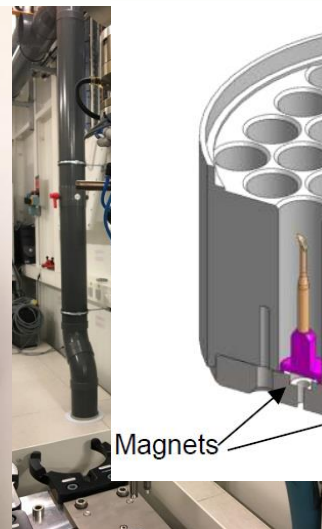


ESRF  
SLS  
BESSY  
PETRAIII  
ALBA

Unipucks (x11) = 176 samples

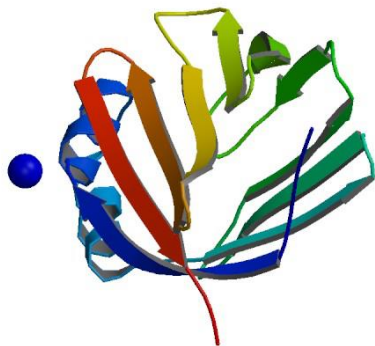


New sample holder types (36 samples)

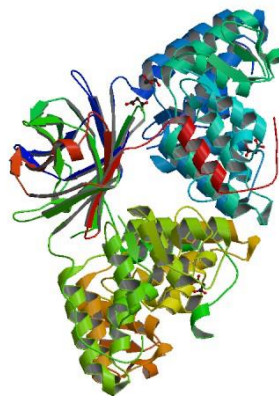


Magnets

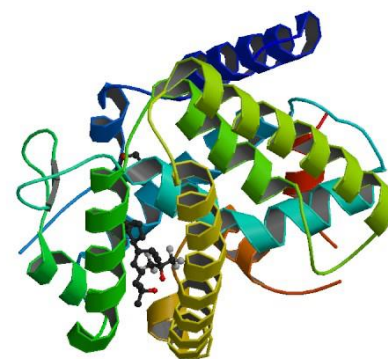
# 8 PDB depositions (6 publications)



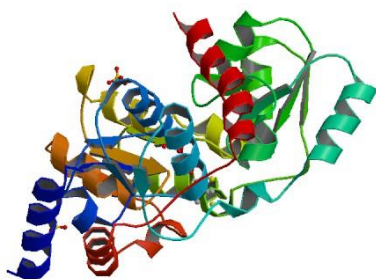
5LJK



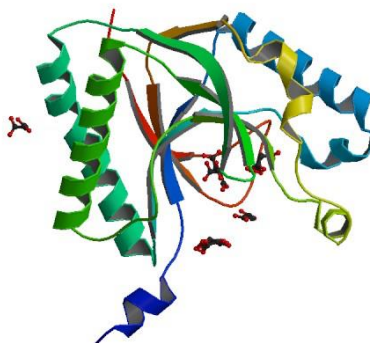
5L6M



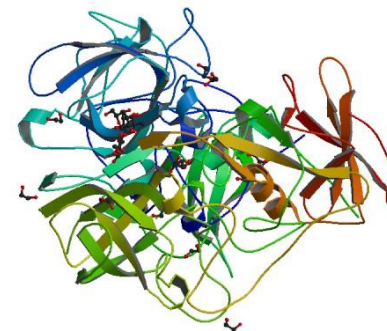
5LGA



5F33/5LQM



5JJ2



5HZA/5HZB

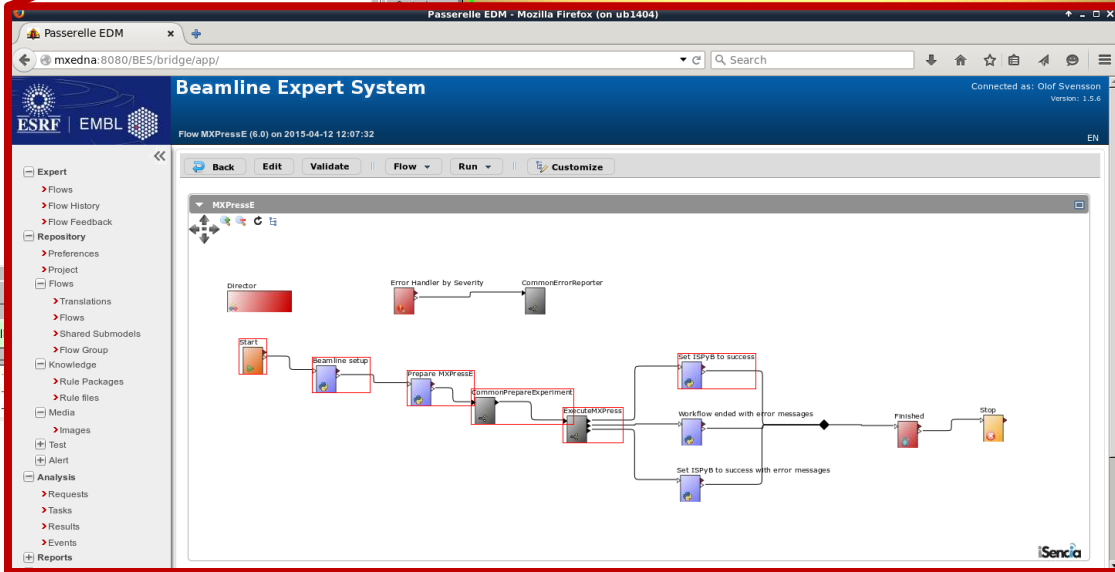
# Workflows implemented in MxCuBE

The screenshot displays the MxCuBE (MxCuBE) software interface. The top menu bar includes 'File', 'Instrumentation', and 'Help'. Below the menu, there are several control panels:

- User:** User: opid-30b, Group: [blank], Set, Logout
- Sample list:** Mode: Sample changer, Centring: Semi Automatic, Show SC-details, Synch ISPyB
- Sample centring:** Omega: 310.00, Kappa: 90.0, Phi: 0.00, Holder length: 23.067, Sample video: Back Light: 0.50, Focus: -0.200, Front Light: 0.0, Zoom: 5
- Collection method:** Standard Collection, Characterisation, Helical Collection, Energy Scan, XRF Spectrum
- Machine current:** 160.6 mA, 7/8 multibunch, 04:09, Flux: 1.94e+12 ph/s, Energy: 12.7000 keV, Current: 0.976 A, Resolution: 1.498 Å, Current: 267.84 mm, Transmission: 100.00%
- Safety shutter:** opened
- Fast shutter:** closed
- Beamstop:** unknown
- Capillary:** out
- Current users:** My name: bacon

A central video feed shows a sample being imaged. A red box highlights a list of implemented workflows:

- X-ray Centring
- Mesh Scan
- Kappa Re-orientation
- Visual Re-orientation
- Helical characterisation
- Mesh and collect
- Enhanced characterisation
- Burn strategy
- Dehydration
- Trouble shooting



# Fast mesh scans implemented in MxCuBE

The screenshot displays the MxCuBE software interface in 'Expert mode'. The main window is titled 'mxcube (opid-30b)'. The interface is divided into several sections:

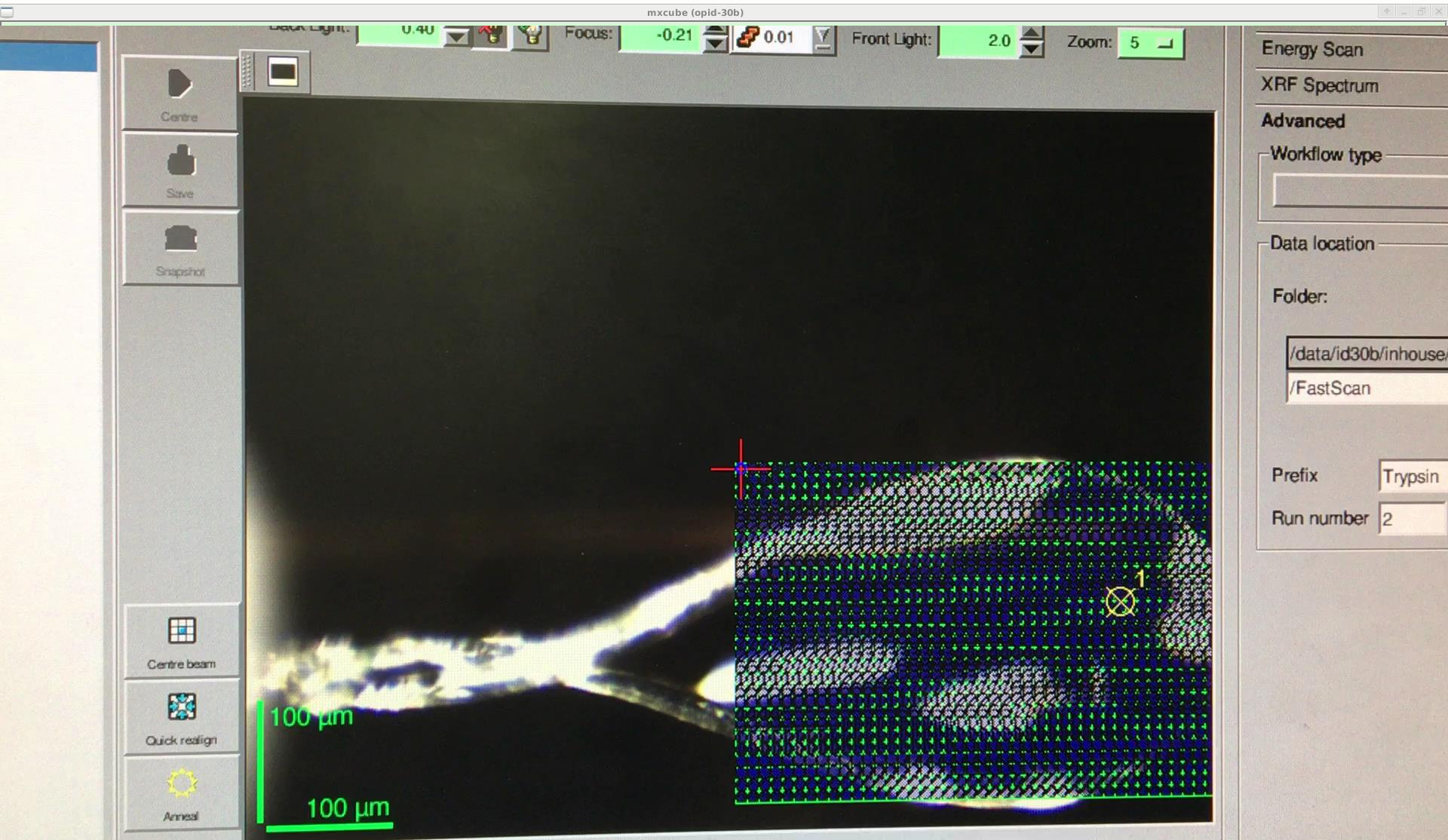
- Top Bar:** 'File Instrumentation Help' and 'Expert mode'.
- Left Panel:** 'Sample list' showing a tree view with 'manually-mounted' expanded to 'X-ray Centring - 1' and 'Workflow task - 0'. Below it are controls for 'Mode' (Manually mounted) and 'Centring' (Semi Automatic).
- Center Panel:** 'Sample centring' and 'Workflow' tabs. The 'Workflow' tab is active, showing a table of parameters for a 'micro-molecules' sample. The 'Use fast mesh (beta)' parameter is set to 'true' and is circled in red. Other parameters include 'Exposure' (0.02), 'Total oscillation range' (1.0), and 'Transmission' (100.0). Below the table are 'Stop', 'Pause', and 'Continue' buttons.
- Right Panel:** 'Machine current' section showing '155.5 mA' and '7% mxtbunch'. Other sections include 'Flux' (+0.00 ph/s), 'Energy' (13.5651 keV, 0.914 A), 'Resolution' (2.999 Å, 658.00 mm), 'Transmission' (100.00%), 'Beamstop distance' (38.0), 'Cryo' (99.99 K), 'Safety shutter' (closed), 'Fast shutter' (closed), 'Beamstop' (out), and 'Capillary' (out). A 'Current users' section at the bottom shows 'bacon' as the user.

Log messages at the bottom of the interface:

- [2017-02-05 08:43:39] Workflow: phiz: 0.258
- [2017-02-05 08:43:39] Workflow: phiy: -1.164
- [2017-02-05 08:43:40] Workflow waiting for input, verify parameters and press continue.



# Fast mesh scans implemented in MxCuBE



[2017-02-05 08:43:40] Workflow waiting for input, verify parameters and press continue.

# Fast mesh scans implemented in MxCuBE

mxcube (opid-30b) \_ Expert mode

File Instrumentation Help

Collect System Feedback Chat

User: opid-30b Group:  Set Logout

Sample list

Mode: Manually mounted Show SC-details

Centring: Semi Automatic Synch ISPyB

manually-mounted

- X-ray Centring - 1
- Workflow task - 0
- X-rayCentring - 3
- mesh-mesh-mesh-Trypsin\_3 Collection done
- line-mesh-mesh-Trypsin\_4 Collection done
- Characterisation - 1
- sample-centring
- ref-Trypsin\_1 (Point - not defined) Done
- Diffraction plan - 1
- Trypsin\_w1\_1 (Point - not defined)

Sample centring Characterisation

## Characterisation Results

Indexing summary: Selected spacegroup: P222

a (Å)	b (Å)	c (Å)	alpha (°)	beta (°)	gamma (°)
60.687	63.963	70.141	90.000	90.000	90.000

Collection plan strategy: 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	57.00	0.05	2280	0.020	2.00	2.83	433.19

Minimal oscillation ranges for different completenesses

compl

Wilson plot B-factor = 21.8 Å<sup>2</sup>

B

Relative Intensity total vs. Dose, D1/2 = 7.63 MGy

I2D

Maximal oscillation width to avoid overlapped spots

phi\_overlap

### Diffraction Plan

Forced space group	Anomalous data	Aimed multiplicity	Aimed completeness	Aimed I/sigma at highest res.	Aimed resolution (Å)	Min osc. width
	False	Default-cbr>(optimized)	0.99	2.00	0.00	Default

### Image quality indicators

File	Dozor score (1)	Tot integr signal (2)	Spot total	In-Res Total	Good Bragg	Ice Rings	Meth 1 Res	Meth 2 Res	Max unit cell
ref-Trypsin_1_0001.cbf	10.1	1611	24	24	22	0	3.37	NA	92.4
ref-Trypsin_1_0002.cbf	126.5	236894	294	274	232	1	2.37	2.09	104.6
ref-Trypsin_1_0003.cbf	75.0	132342	355	322	312	1	2.08	2.07	104.2
ref-Trypsin_1_0004.cbf	141.3	329322	312	306	270	0	2.55	2.07	104.6

1. Dozor score: criteria of diffraction signal strength that uses intensities over background vs resolution. Popov 2014, to be published.

2. Total integrated signal, spot total etc.: results from cctbx Spotfinder

Collect Queue Pause View parameters

Machine current

**202.1 mA**

Flux: 6.36e+11 ph/s

Energy

Current: 13.5651 keV

0.914 Å

Move to:  keV

Resolution

Current: 2.002 Å

420.04 mm

Move to:  Å

Transmission

Current: 100.00%

Set to:  Filters

Beamstop distance

21.0

Cryo

99.99 K

Safety shutter

opened

Fast shutter

closed

Beamstop

in

Capillary

in

Current users

Selecting gives control

Allow timeout control

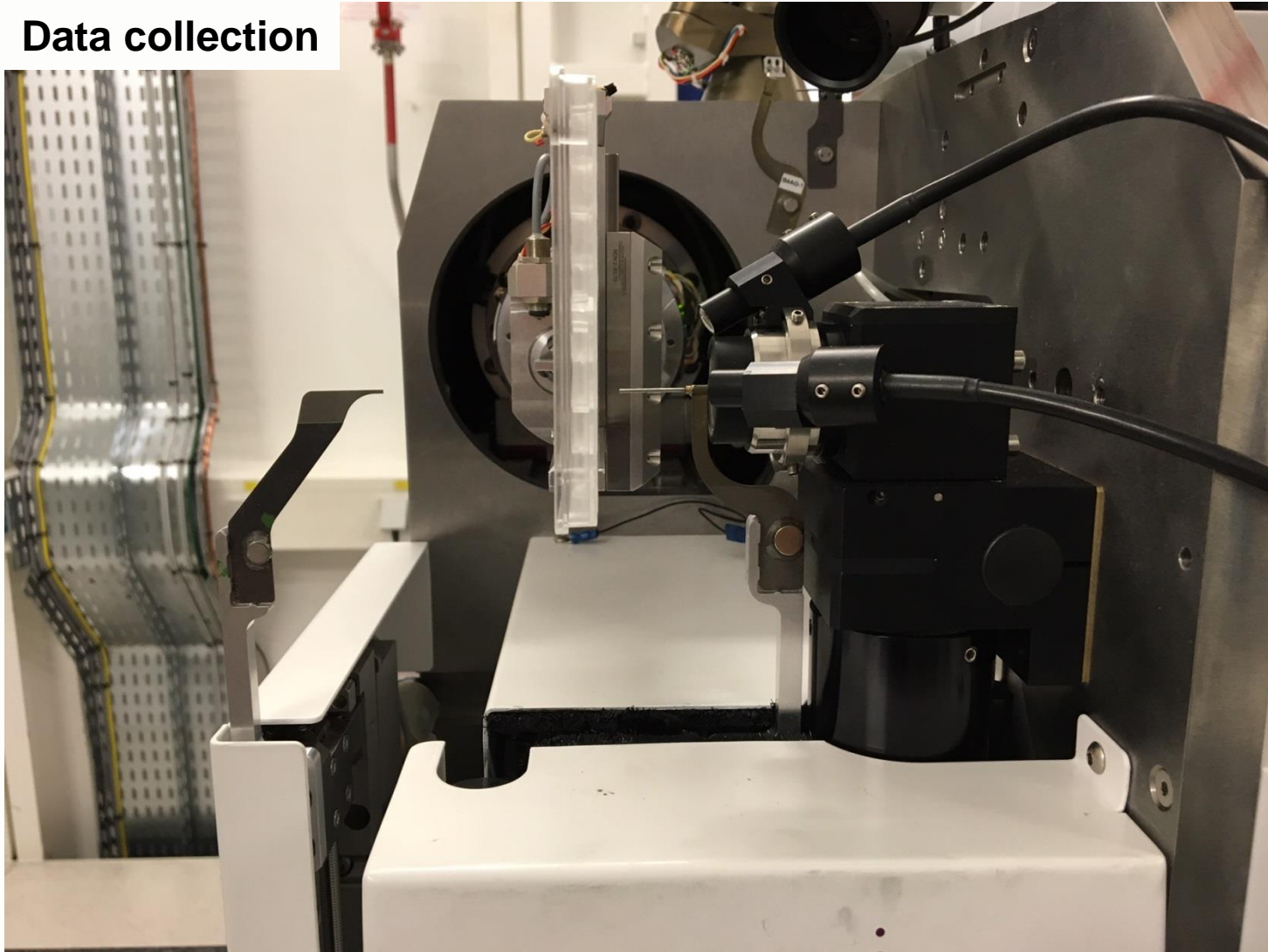
Take control

My name: bacon

[2017-02-05 09:03:24] Characterisation: Strategy: total no images 2280, total exposure time 45.6 [s]  
 [2017-02-05 09:03:24] Characterisation: Strategy calculation successful.  
 [2017-02-05 09:03:31] Characterisation completed.

# ID30B – MD2S allows plate screening capability

Data collection



# ID30B – SBS plate format supported

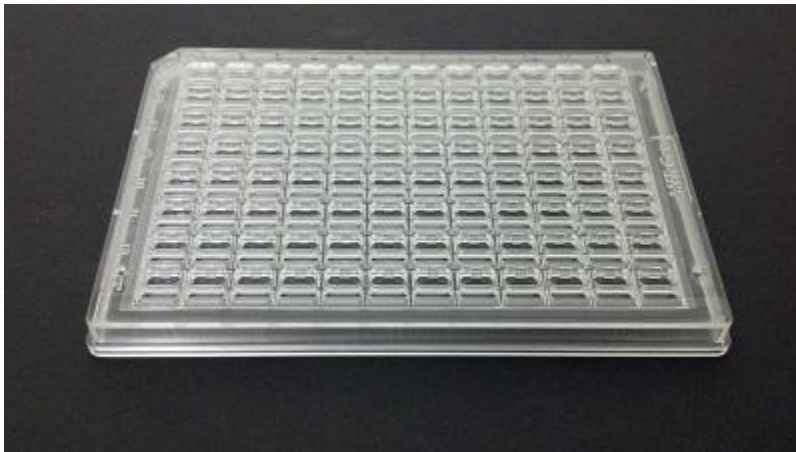
**Crystal Direct™**



**CrystalQuick™ X**



**In Situ-1™**



Other SBS plate formats can be accommodated but need templates from users

# In situ plate data collection in MxCuBE

File Instrumentation Help Expert mode

Collect System Feedback Chat

User: mx-1743 Group: [ ] Set Logout

Sample list  
Mode: Plate Show SC-details  
Centring: No Centring Synch ISPyB

Sample centring  
Sample position  
Omega: 316.36 90.0 Kappa: 0.00 1.0 Phi: 0.00 0.1  
Holder length: 32.500 0.1

Sample video  
Back Light: 0.60 Focus: -0.729 0.02 Front Light: 0.0 Zoom: 5  
Centre Save Snapshot  
Centre beam Quick realign  
Point no. 2 selected  
Aperture diameter: 50

Collection method  
Standard Collection  
Acquisition  
Oscillation range: 0.1 First image: 1  
Oscillation start: 316.36 Number of images: 10  
Kappa: 0.0 Phi: 0.0  
Detector mode: [ ]  
Exposure time (s): 0.037  
Energy (keV): 12.7 MAD ip: [ ]  
Resolution (Å): 1.997  
Transmission (%): 100.0  
Inverse beam Subwedge size: [ ]  
Shutterless

Data location  
Folder: /data/visitor/mx1743/id30b/20151104/RAW\_DATA /XlyA/A3-2  
File name: xyla\_23\_###.cbf Browse  
Prefix: xyla  
Run number: 23

Processing  
N.o. residues: 200 Space group: [ ]  
Unit cell:  
a: 0 b: 0 c: 0  
α: 0 β: 0 γ: 0

Characterisation  
Helical Collection  
Energy Scan  
XRF Spectrum  
Advanced Add to queue

Machine current: 185.4 mA uniform multibunch 08:57  
Flux: +0.00 ph/s  
Energy: 12.7000 keV Current: 0.976 A  
Move to: [ ] keV  
Resolution: 1.997 Å Current: 391.52 mm  
Move to: [ ] Å  
Transmission: 100.00%  
Set to: [ ] Filters

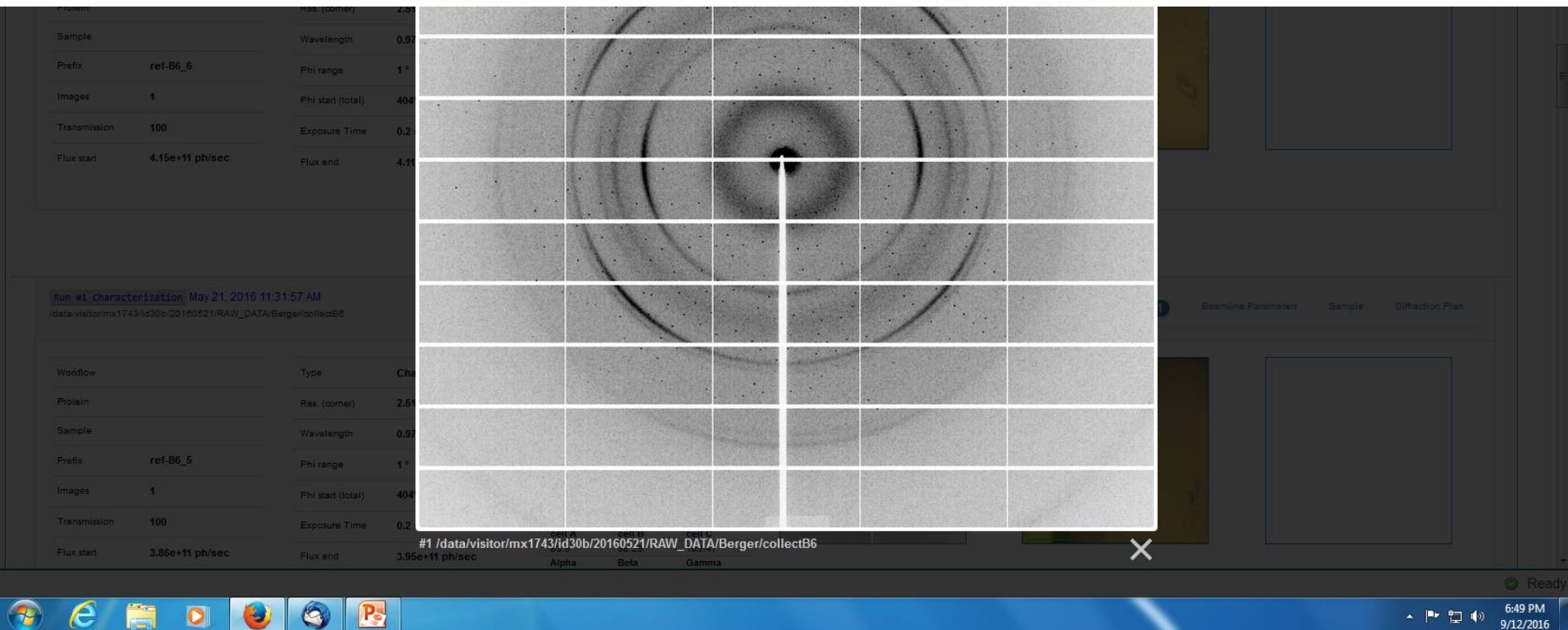
Safety shutter: closed  
Fast shutter: closed  
Beamstop: in  
Capillary: unknown  
Current users: [ ]  
Selecting gives control Allow timeout control  
Ask for control  
My name: bacon

Collect Queue Pause

[2015-11-04 11:49:00] Asking for input files writing  
[2015-11-04 11:49:00] Preparing acquisition, start=314.730000, wedge size=10  
[2015-11-04 11:49:04] Collection completed

# MX-1743 (EMBL)

- CrystalDirect plates from the HTX lab
  - Test of diffraction quality at RT
  - Highest resolution is 2.5 Å (cryo)
  - Best we saw was ~3 Å (maybe 2.8 Å)



# Thaumatococcus crystals (12.7 keV)

Workflow	Type	OSC	P 42 21 2	Completeness	Res.	Rmerge			
Protein	Res. (corner)	1.5 Å (1.18 Å)	Inner	91%	4.1	0.1			
Sample	Wavelength	0.977 Å	Outer	100%	1.5	0.7			
Prefix	Phi range	0.1 °	Overall	98%	1.5	0.1			
Images	Phi start (total)	340° (50°)	cell A	cell B	cell C				
Transmission	Exposure Time	0.02 s	58.5689	58.5689	151.592				
Flux start	Flux end	3.39e+10 ph/sec	Alpha	Beta	Gamma				
			90	90	90				

Workflow	Type	OSC	P 4 21 2	Completeness	Res.	Rmerge			
Protein	Res. (corner)	1.5 Å (1.18 Å)	Inner	93%	4.2	0.1			
Sample	Wavelength	0.977 Å	Outer	99%	1.6	0.8			
Prefix	Phi range	0.1 °	Overall	99%	1.6	0.1			
Images	Phi start (total)	340° (50°)	cell A	cell B	cell C				
Transmission	Exposure Time	0.02 s	58.5727	58.5727	151.579				
Flux start	Flux end	3.51e+10 ph/sec	Alpha	Beta	Gamma				
			90	90	90				

# Thaumatin crystals

## Data collection statistics

Wavelength (Å)	0.9763	0.7085
Phs/sec (Single bunch 4x10 mA and 20 μm aperture)	$3.5 \times 10^{10}$	$9.3 \times 10^{10}$
Exposure time (s)	0.02	0.02
Oscillation range (degrees)	50	50
Total dose (MGy) – <i>flux2dose (Sasha)</i>	0.42	0.53
Space group	P4 <sub>1</sub> 2 <sub>1</sub> 2	P4 <sub>1</sub> 2 <sub>1</sub> 2
Cell Dimensions a, b, c (Å)	58.6, 58.6, 151.6, 90, 90, 90	58.6, 58.6, 151.5, 90, 90, 90
Resolution (Å) (final shell)	50-1.5 (1.53-1.5)	50-1.5 (1.53-1.5)
Observed Reflections	149,639 (7,107)	153,095 (7,675)
Unique Reflections	42,124 (2,031)	40,896 (2,027)
Completeness (%) (final shell)	98.2 (98.4)	95.1 (98.2)
R <sub>meas</sub> (%) (final shell)	14.2 (137)	12.6 (111)
<I/σ(I)> (final shell)	5.7 (1.0)	7.1 (1.3)

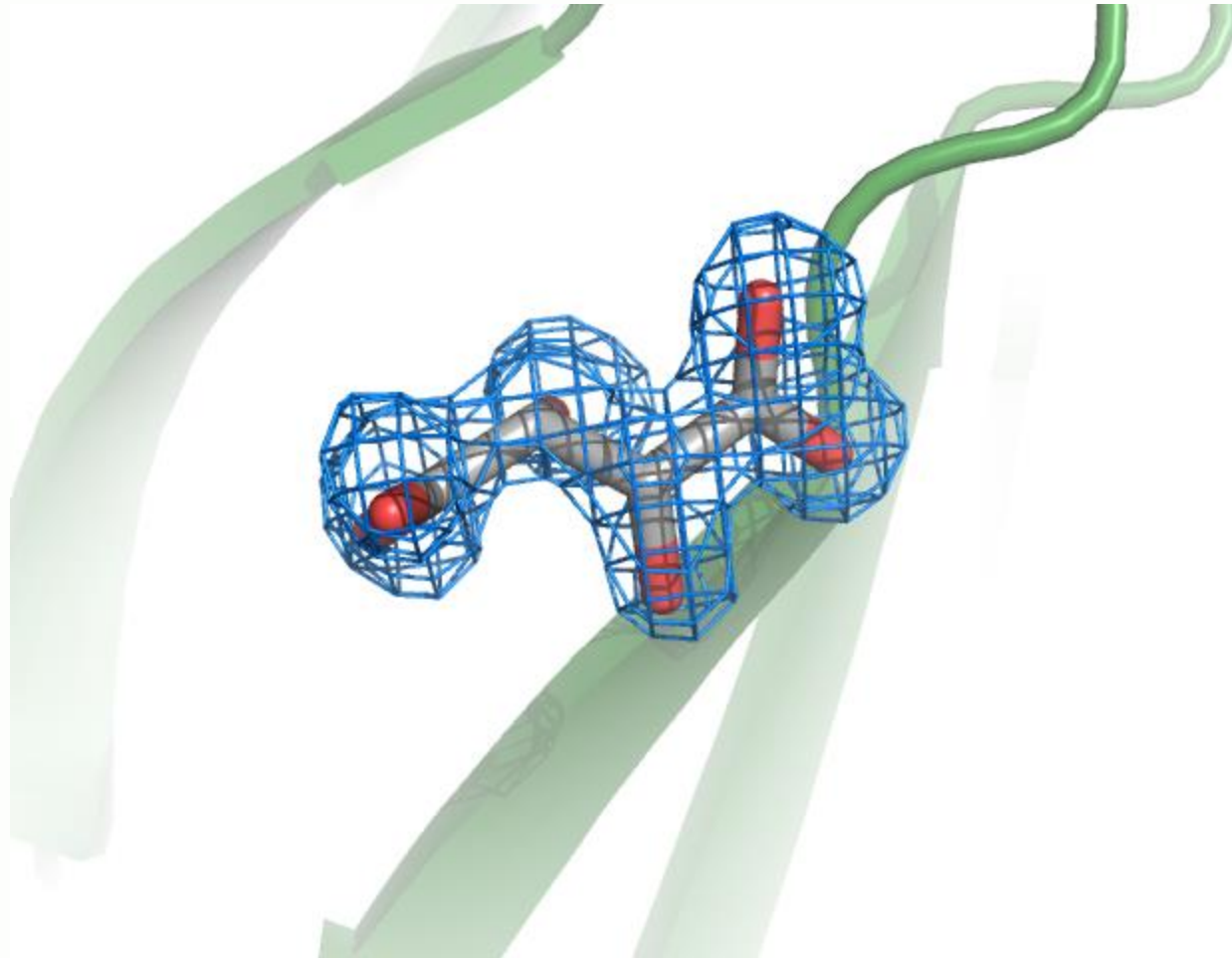
## Model quality indicators

R <sub>cryst</sub> (%) / R <sub>free</sub> (%)	16.5/18.6	16.8/18.8
rms deviations, bonds (Å)/angles (°)	0.008/0.97	0.008/0.97





# Thaumatococcus (12.7 keV) – tartaric acid



Fo-Fc omit map contoured at  $5\sigma$

# Thaumatococcus crystals – S-SAD

Extended ISPyB

exi.esrf.fr/mx/#/mx/datacollection/session/54178/main

Library: Main - Intranet

ExiMX Extended ISPyB for MX<sub>BETA</sub>

Version: 0.9.6  
Released: 2016/11/15

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

search by protein acronym

Log out OPID30b

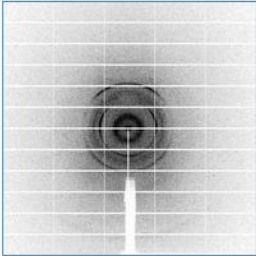
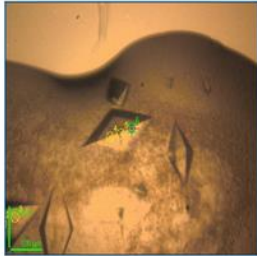
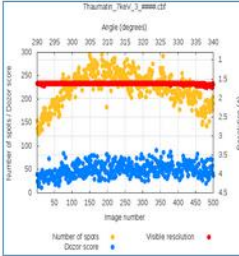
New Tab

Run #3 OSC Sep 12, 2016 10:12:13 AM

/data/id30b/inhouse/opid30b/20160912/RAW\_DATA/Thaumatococcus7keV/D6\_2x2

Summary Beamline Parameters Data Collections 1 Sample Results 14

Workflow	Type	OSC	P 41 21 2	Completeness	Res.	Rmerge
Protein	Res. (corner)	2 Å (1.71 Å)	Inner	79%	7.7	6.9
Sample	Wavelength	1.772 Å	Outer	15	1.7	42.5
Prefix	Phi range	0.1 °	Overall	77%	1.7	7.2
Images	Phi start (total)	340° (50°)	cell A	cell B	cell C	
Transmission	Exposure Time	0.02 s	58.5	58.5	151.6	
Flux start	Flux end	1.1e+11 ph/sec	Alpha	Beta	Gamma	
			90	90	90	

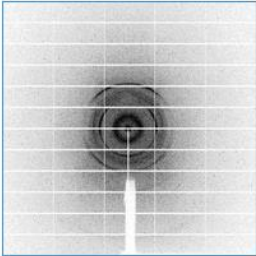
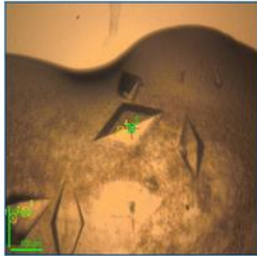
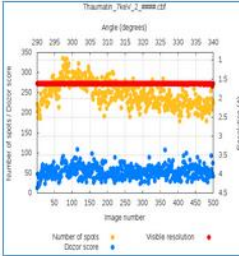




Run #2 OSC Sep 12, 2016 10:11:09 AM

/data/id30b/inhouse/opid30b/20160912/RAW\_DATA/Thaumatococcus7keV/D6\_2x2

Summary Beamline Parameters Data Collections 1 Sample Results 12

Workflow	Type	OSC	P 41 21 2	Completeness	Res.	Rmerge
Protein	Res. (corner)	2 Å (1.71 Å)	Inner	80%	8.1	5.7
Sample	Wavelength	1.772 Å	Outer	52%	1.8	28.4
Prefix	Phi range	0.1 °	Overall	86%	1.8	6.4
Images	Phi start (total)	340° (50°)	cell A	cell B	cell C	
Transmission	Exposure Time	0.02 s	58.5	58.5	151.6	
Flux start	Flux end	1.12e+11 ph/sec	Alpha	Beta	Gamma	
			90	90	90	

# Thaumatin crystals – S-SAD

## XSCALE merge of 4 data sets

Resolution (Å)	Completeness (%)	I/SigI	R-meas (%)	CC(1/2)	Anomal Corr	SigAno	Nano
15	97.50	25.71	9.90	98.7*	32	1.454	16
8	100.00	25.82	9.40	99.2*	37*	1.581	158
6	99.50	23.24	9.50	99.3*	45*	1.633	285
4	100.00	25.18	7.90	99.3*	32*	1.177	1271
3.5	99.80	23.38	7.20	99.4*	19*	0.974	919
3	100.00	21.58	7.20	99.6*	21*	0.965	1675
2.5	100.00	16.58	8.90	99.4*	15*	0.899	3341
2.2	100.00	12.52	12.00	99.1*	11*	0.896	3754
2	100.00	8.91	17.10	98.3*	5	0.875	3939
1.9	87.60	5.85	24.10	95.5*	6	0.908	2111
total	98.20	14.46	8.90	99.6*	14*	0.943	17469

# Thaumatin crystals – S-SAD

## Data collection statistics (merge 4 partial datasets)

Wavelength (Å)	1.7712
Phs/sec (Single bunch 4x10 mA and 20 μm aperture)	1.1 x 10 <sup>11</sup>
Exposure time (s)	0.02
Oscillation range (degrees)	50
Total dose (MGy) – <i>flux2dose (Sasha)</i>	0.42
Space group	P4 <sub>1</sub> 2 <sub>1</sub> 2
Cell Dimensions a, b, c (Å)	58.6, 58.6, 151.6, 90, 90, 90
Resolution (Å) (final shell)	50-1.9 (1.95-1.9)
Observed Reflections	220,045 (8,883)
Unique Reflections	21,397 (1,349)
Anomalous Completeness (%) (final shell)	99.2 (88.5)
R <sub>meas</sub> (%) (final shell)	9.3 (27.9)
<I/σ(I)> (final shell)	18.1 (6.3)

## Model quality indicators

R <sub>cryst</sub> (%) / R <sub>free</sub> (%)	16.5/18.6
rms deviations, bonds (Å)/angles (°)	0.008/0.97



# Thaumatin (7 keV) – Anomalous map



S anomalous difference peaks using experimental anomalous differences and model phases contoured at  $5\sigma$

# Lysozyme-Benzamide crystals

Save Comments View All Summary info View All DataCollection Expand All Collapse All Clear

Exp. Type	Image Prefix	Run#	Parameters	Results	Image Thumbnail	Crystal snapshot	Graph	Comments
-----------	--------------	------	------------	---------	-----------------	------------------	-------	----------

Start  
time: 15:22:56  
11-07-2016 (1 Item)

OSC	lysozyme_benz	2	<p>Nb images: 250 Exp. time: 0.037 s Phi range: 0.10 ° Flux: 2.17E10 ph/sec Detector resolution: 1.50 Å Transmission: 1.00 Wavelength: 0.912 Å Total expo time: 9.25 s</p>	<p>EDNA_proc <span style="color:red">●</span> grenades_fastproc <span style="color:green">●</span> grenades_parallelproc <span style="color:green">●</span></p> <p>Space Group: P 4 2 2</p> <p>Completeness:</p>				
-----	---------------	---	--	--	--	--	--	--

Start  
time: 15:21:34  
11-07-2016 (1 Item)

OSC	lysozyme_benz	1	<p>Nb images: 250 Exp. time: 0.037 s Phi range: 0.10 ° Flux: 2.17E10 ph/sec Detector resolution: 1.50 Å Transmission: 1.00 Wavelength: 0.912 Å Total expo time: 9.25 s</p>	<p>EDNA_proc <span style="color:red">●</span> grenades_fastproc <span style="color:green">●</span> grenades_parallelproc <span style="color:green">●</span></p> <p>Space Group: P 4 2 2</p> <p>Completeness:</p>				
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Start  
time: 15:19:24  
11-07-2016 (1 Item)

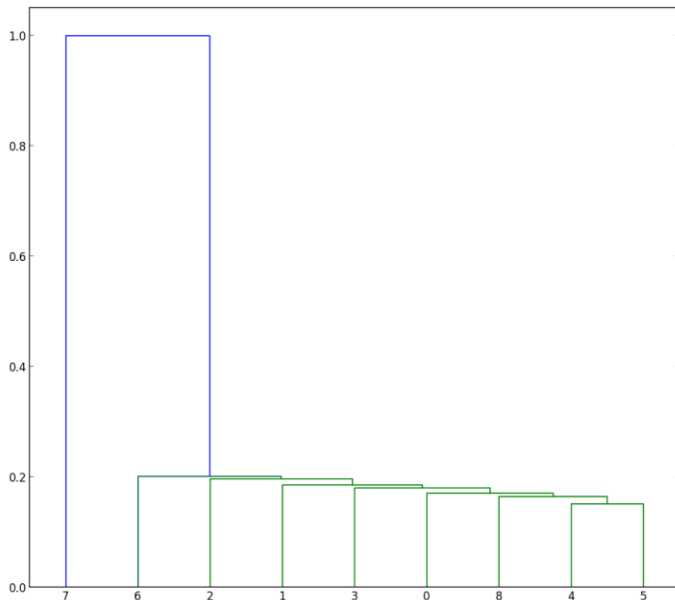
OSC	lysozyme_benz	5	<p>Nb images: 250 Exp. time: 0.037 s Phi range: 0.10 ° Flux: 2.17E10 ph/sec Detector resolution: 1.50 Å Transmission: 1.00 Wavelength: 0.912 Å Total expo time: 9.25 s</p>	<p>EDNA_proc <span style="color:red">●</span> grenades_fastproc <span style="color:green">●</span> grenades_parallelproc <span style="color:green">●</span></p> <p>Space Group: P 41 21 2</p> <p>Completeness:</p>				
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Start  
time: 15:18:31  
11-07-2016 (1 Item)

OSC	lysozyme_benz	3	<p>Nb images: 250 Exp. time: 0.037 s Phi range: 0.10 ° Flux: 2.2E10 ph/sec Detector resolution: 1.50 Å Transmission: 1.00 Wavelength: 0.912 Å Total expo time: 9.25 s</p>	<p>EDNA_proc <span style="color:red">●</span> grenades_fastproc <span style="color:green">●</span> grenades_parallelproc <span style="color:green">●</span></p> <p>Space Group: P 41 21 2</p> <p>Completeness:</p>				
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# Lysozyme-Benzamidine crystals

- 9 datasets collected: (24° or 36°; 1% transmission; 0.037s; 0.1° per image)
- 8 merged using (HCA)



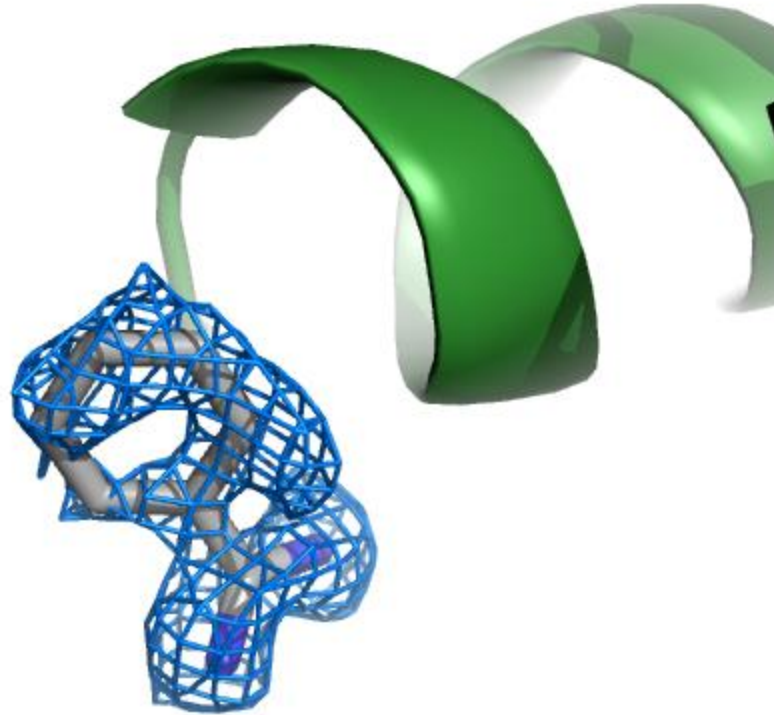
## Data collection statistics

Wavelength (Å)	0.91165
Space group	P4 <sub>3</sub> 2 <sub>1</sub> 2
Cell Dimensions a, b, c (Å)	79.4, 79.4, 38.0, 90, 90, 90
Molecules per asymmetric unit	1
Resolution (Å) (final shell)	50-1.5 (1.53-1.5)
Observed Reflections	324,244 (5,095)
Unique Reflections	18,246 (847)
Completeness (%) (final shell)	91.7 (87.5)
R <sub>meas</sub> (%) (final shell)	8.6 (48.6)
<I/σ(I)> (final shell)	21 (3.4)

## Model quality indicators

R <sub>cryst</sub> (%) / R <sub>free</sub> (%)	16.5/17.8
rms deviations, bonds (Å)/angles (°)	0.01/1.1

# Lysozyme-Benzamidine crystals

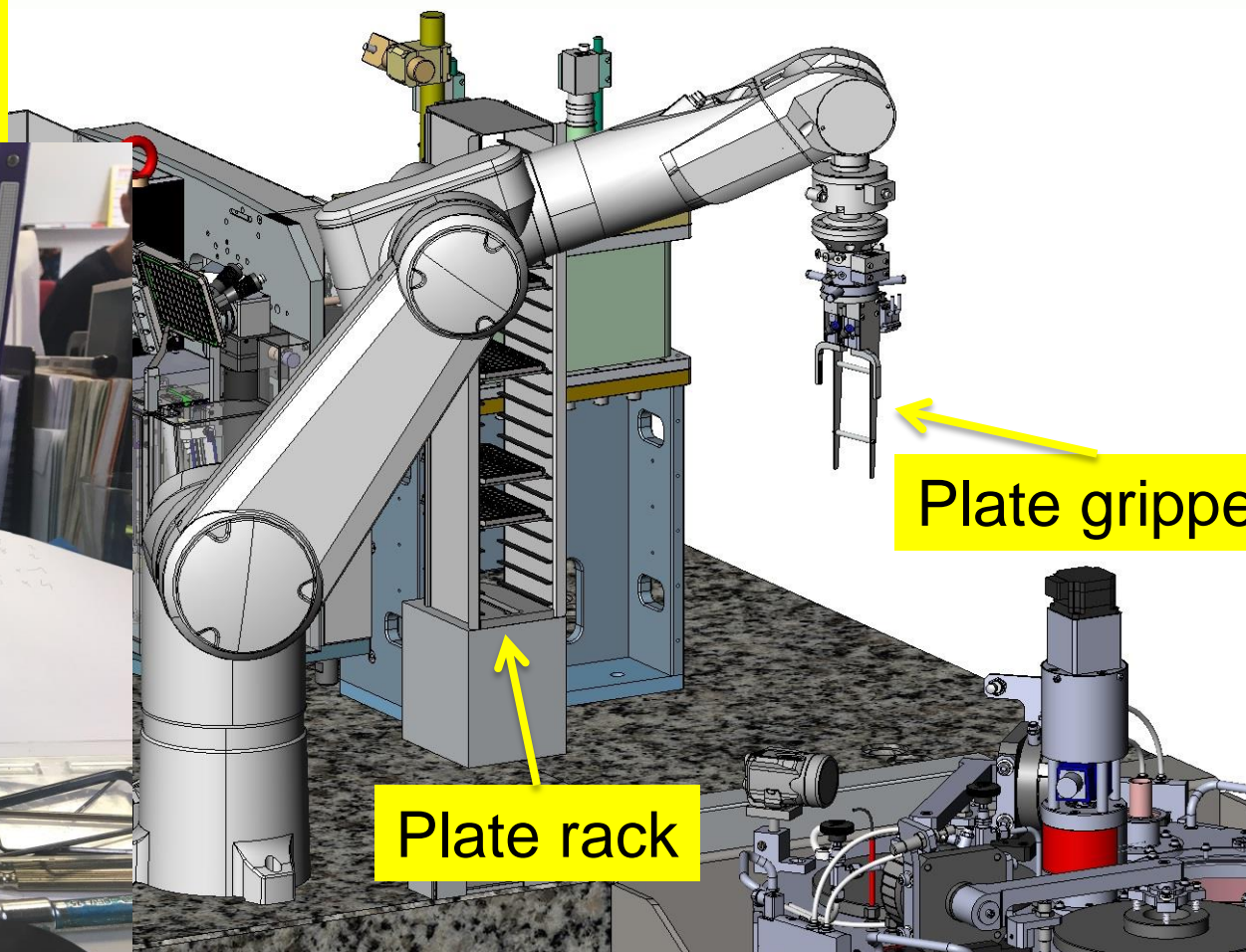
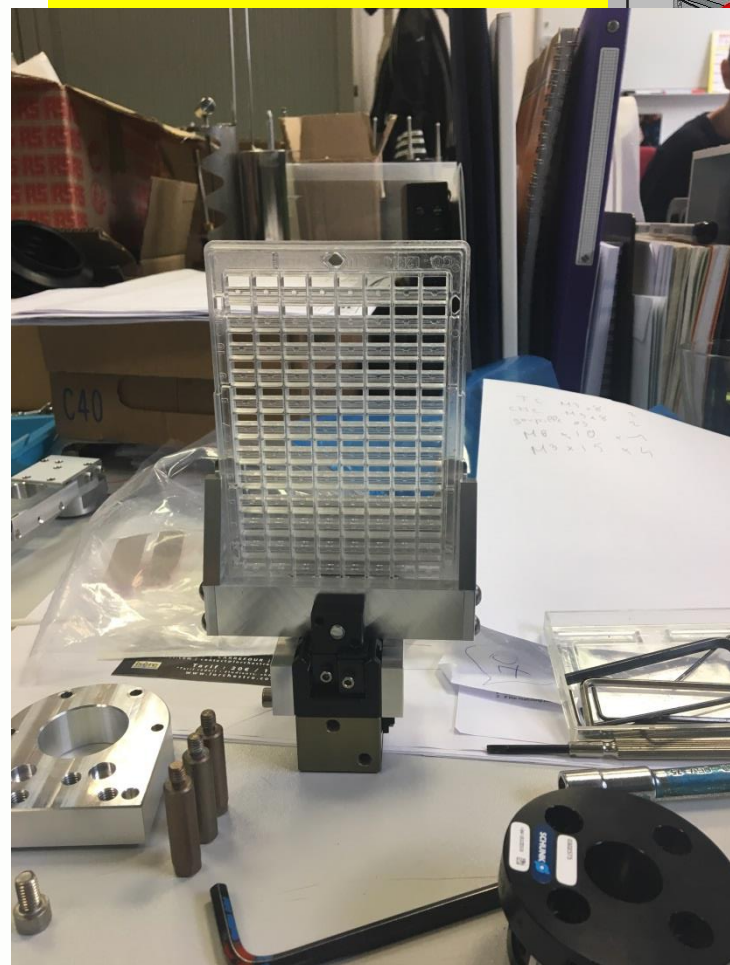


2Fo-Fc Map contoured at 0.6  $\sigma$



# ID30B – Develop a flex plate gripper

Plate manipulator  
nozzle

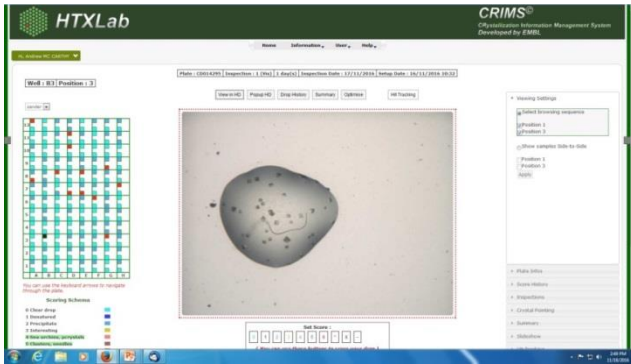


**EMBL instrumentation team**

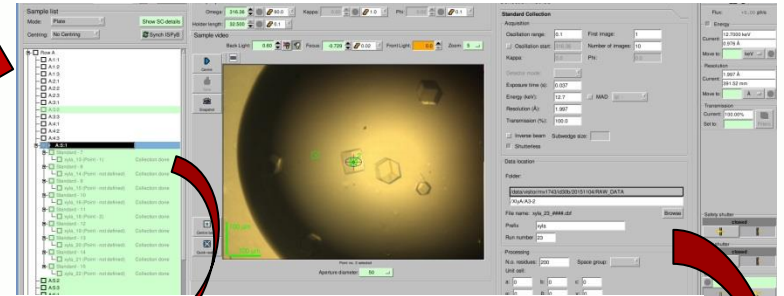


# Advanced instrumentation and software

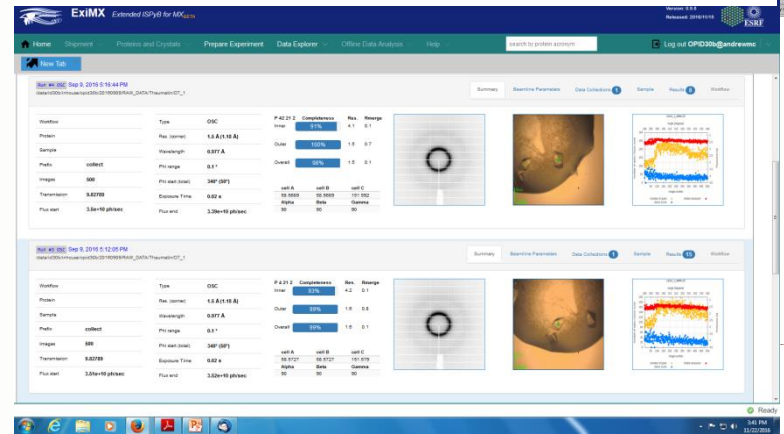
## CRIMS



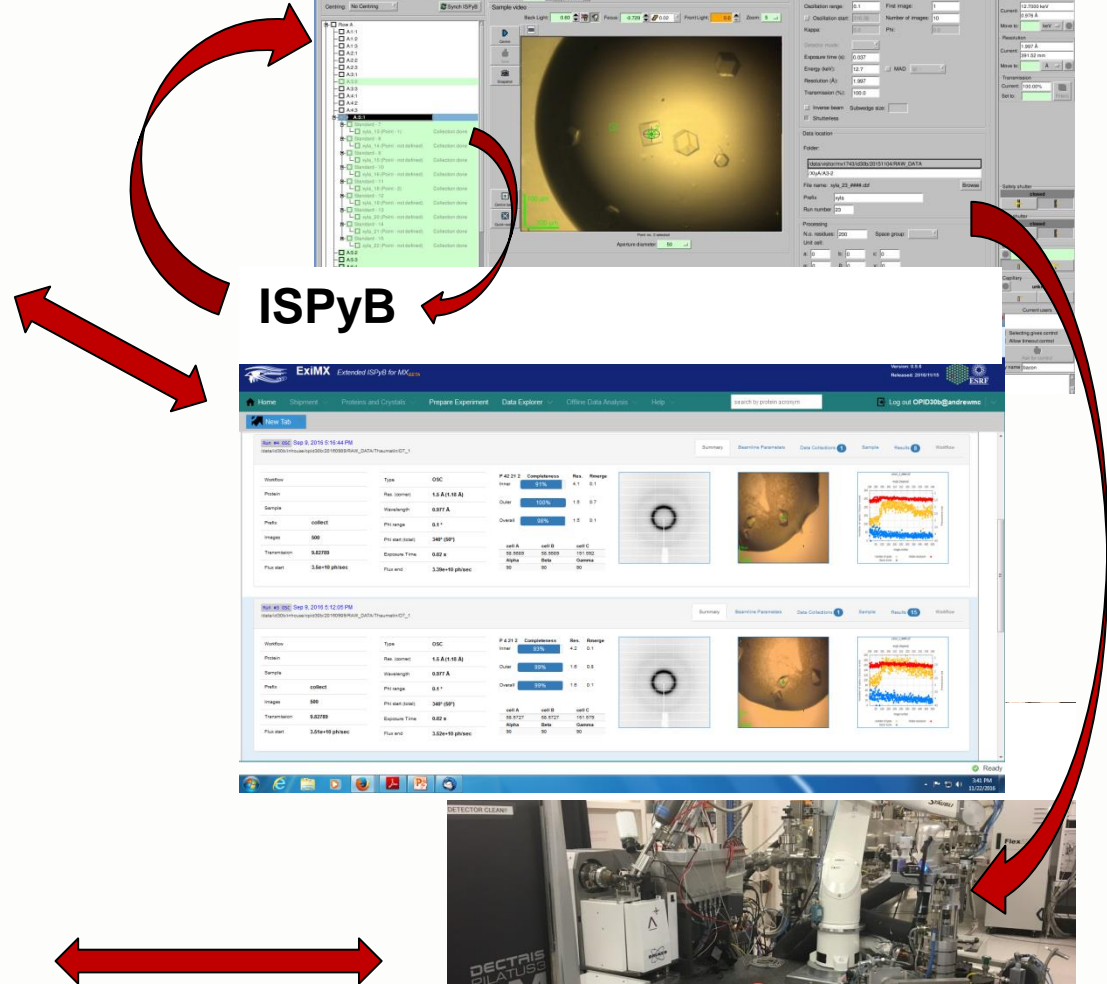
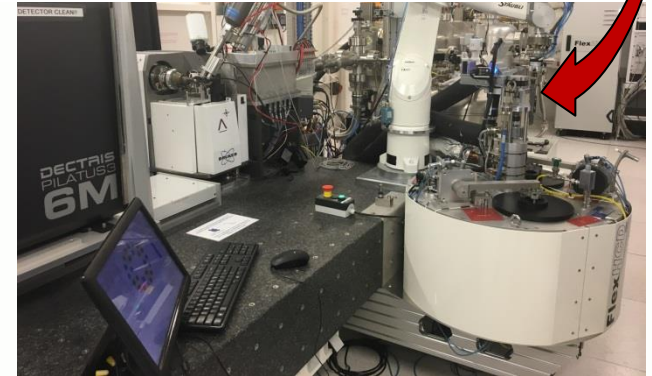
## MxCuBE



## ISPyB



## Crystal Direct™



# ID30B – To do

## Immediate future

- Finalise Unipuck ‘double’ gripper integration in MxCuBE
- Finalise *in situ* plate screening
- Finalise MXPress automatic data collection WFs (as on MASSIF-1)

## Near future

- Implement Flex plate gripper (prototype under construction)
- Develop plate functionalities in ISPyB/Exi ISPyB)
- Develop CRIMS-ISPyB connection for crystal ‘marking’
- Develop and implement dynamic beam size routines
- Add move to Si strip for low energy ranges

## Future

- Improve long term beam stability
- Automate data collection from plates (MASSIF style)
- Expand lower energy ranges (<6 keV)
- Phase plate commissioning (D. de Sanctis)

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