Routine room temperature structure determination from protein crystals *in situ* at the Diamond beamline VMXi using the Crystallisation Facility at Harwell

Dr Halina Mikolajek

CCP4 2023

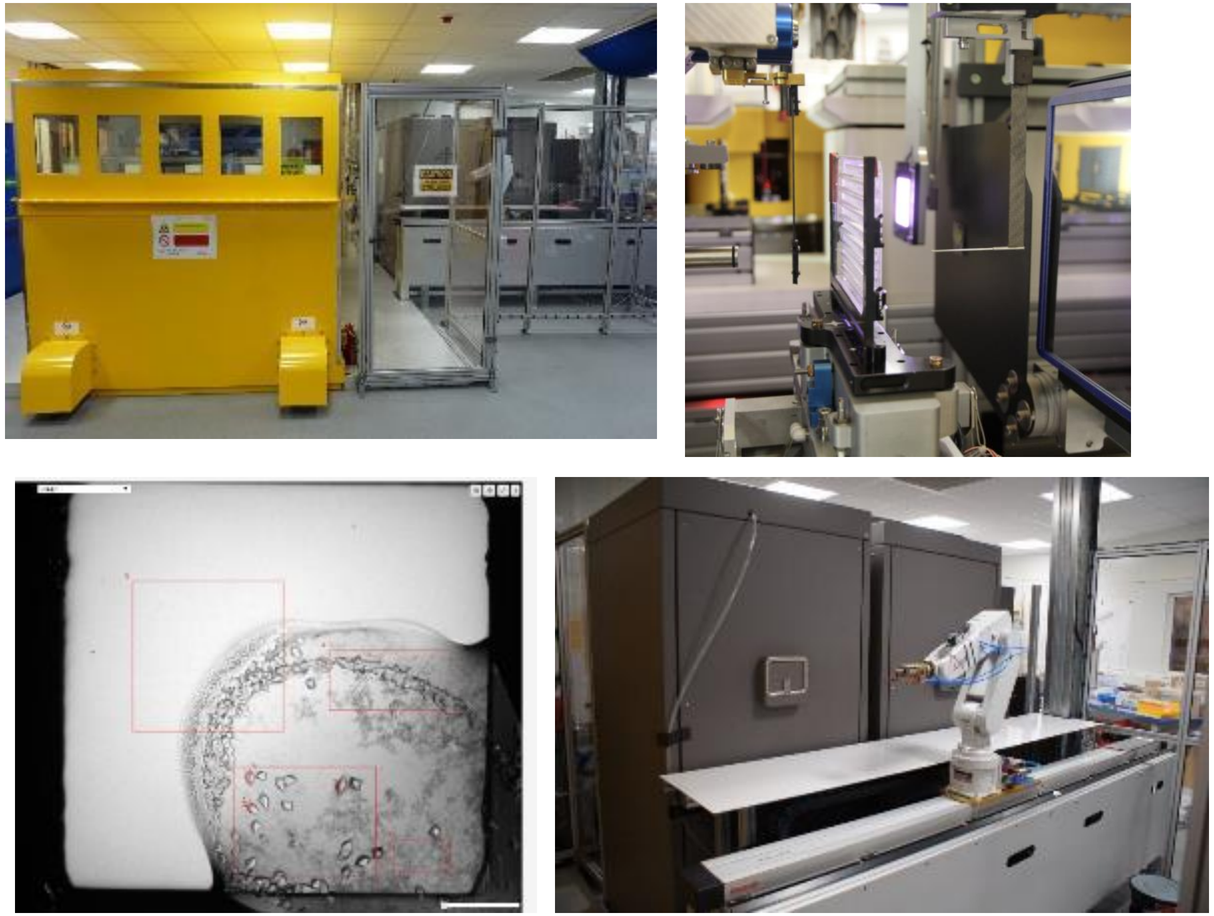
Research Complex at Harwell diamond

The VMXi team

Prof Mike Hough (PBS)
Dr Juan Sanchez-Weatherby (Senior Beamline Scientist)
Dr James Sandy (Senior Beamline Scientist)
Dr Halina Mikolajek (Research Scientist and Crystallisation Facility Manager)

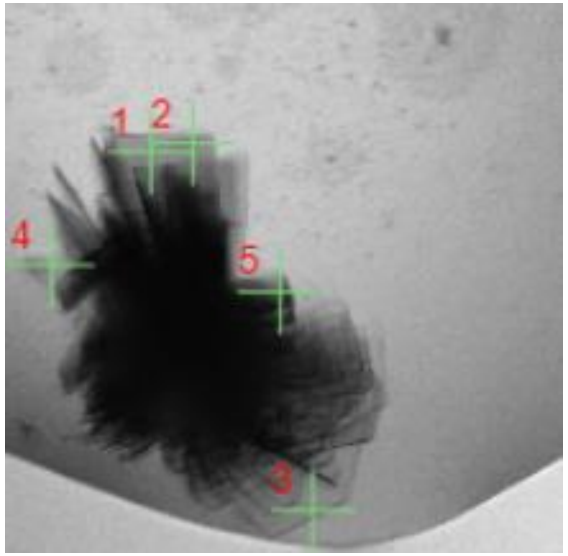
Beamline concept and vision

- VMXi is fully dedicated to room temperature experiments with samples in a number of different formats.
- 10x10 μm^2 beam size, pink beam $5x10^{13}$ ph/sec, 16 keV (tunable)
- Routine, high quality room temperature structures from multiple crystals of hundreds to 10 µm dimensions
- Highly automated operation including machine learning crystal finding and data processing
- Expanding remit to time resolved serial crystallography and fragment screening

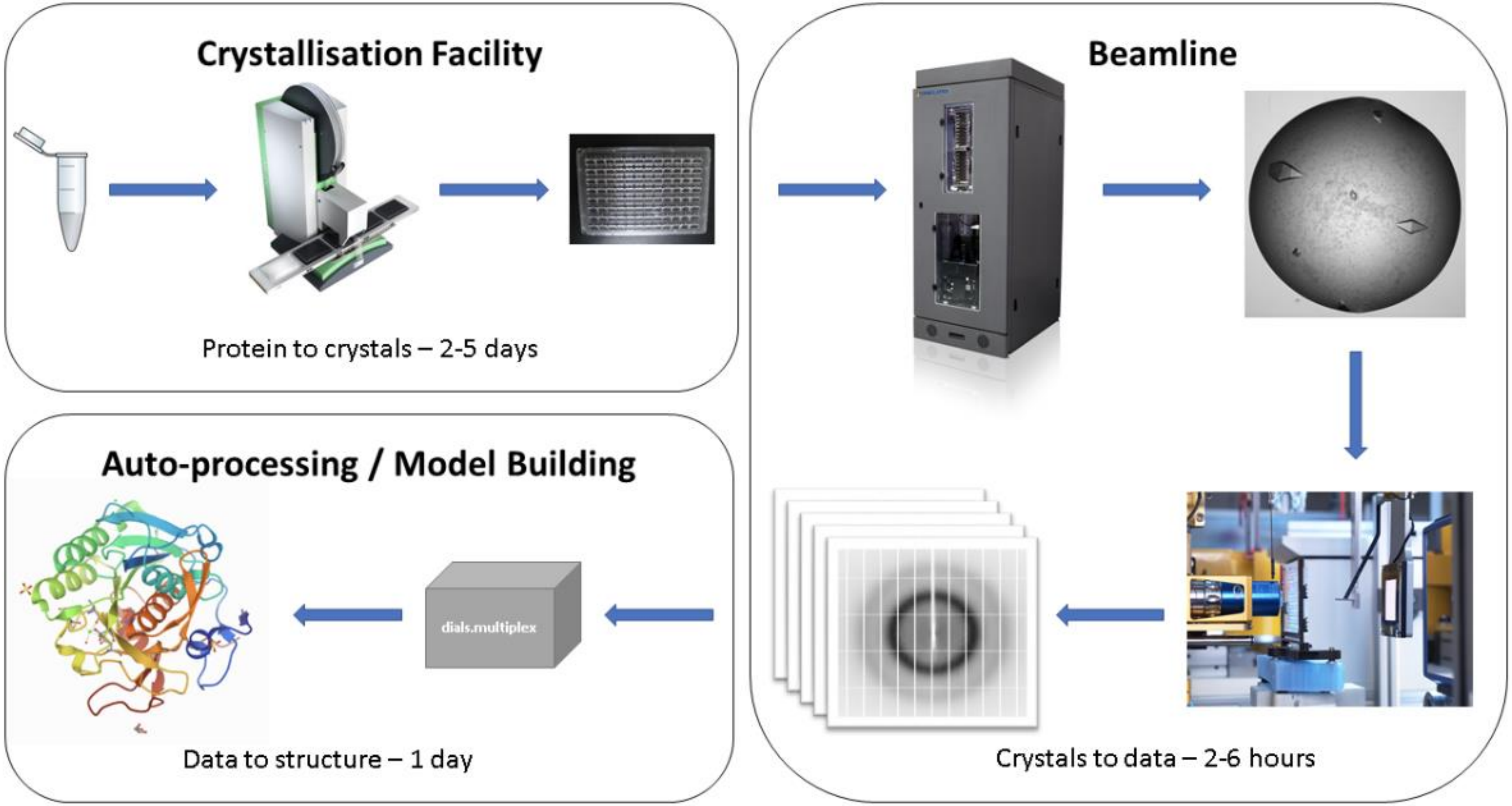


Why collect data at VMXi?
-> high quality RT datasets & structures

- Determining the intrinsic quality of crystal without introducing external variables
  - Dehydration
  - Manual handling
  - Cold shock
  - Cryoprotection
- Rapidly determining structures
  - Speeds up the early crystal optimisation cycles (hit finding and optimisation)
  - From click to structure in minutes
  - Not requiring fishing, cooling, cryo-optimising, transporting, loading, etc.
  - Rapidly solve and visualise multiple ligand structures
- Samples that do not cryo-protect well or are difficult to harvest
  - Complexes
  - Membrane proteins
  - Large crystals



Timelines at VMXi



Crystallisation Facility

Protein to crystals – 2-5 days

Beamline

Crystals to data – 2-6 hours

Auto-processing / Model Building

Data to structure – 1 day

Workflow at VMXi

Crystallisation plates arrive at the beamline

Beamline images crystallisation plates

User reviews results and selects point for DC

Beamline loads samples

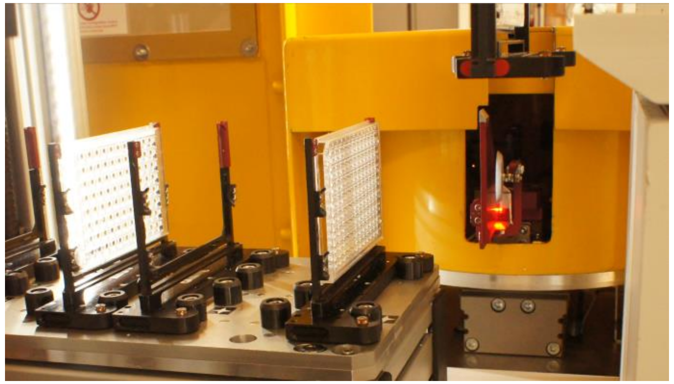
Beamline defines where users wants to collect data

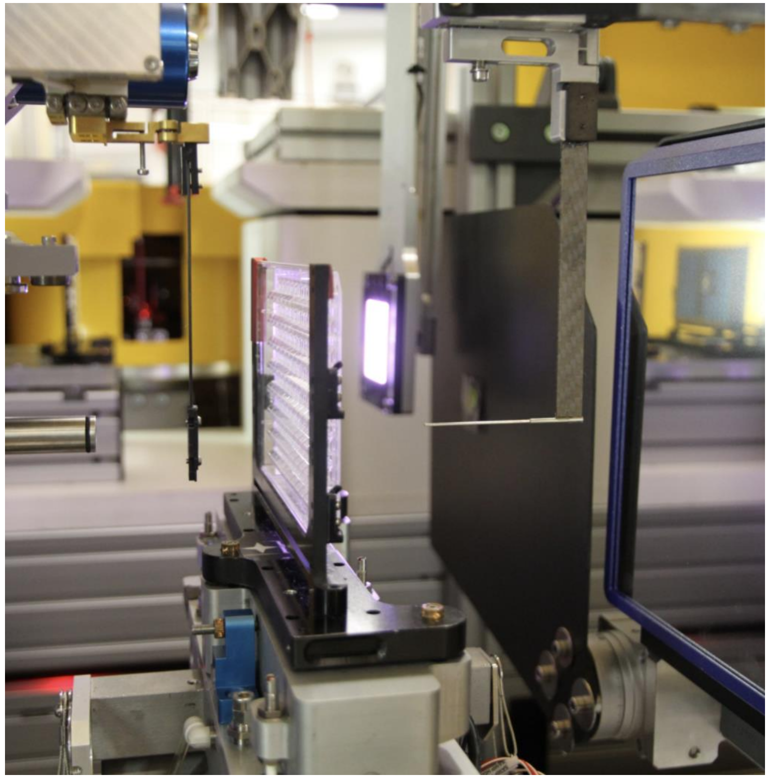
Beamline sets correct DC parameters

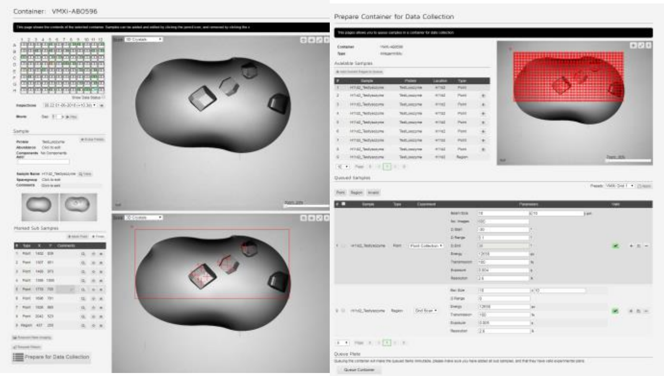
Beamline collects data

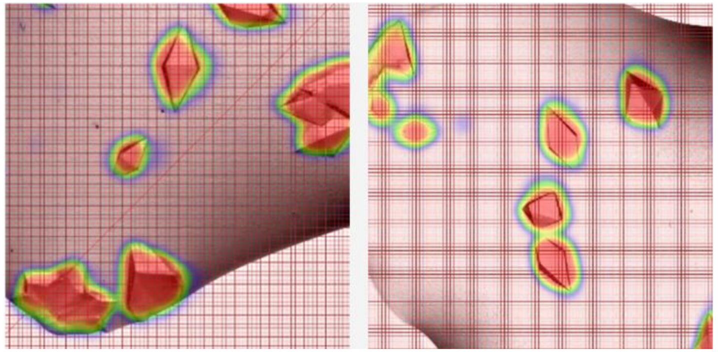
Beamline unloads samples

Beamline automatically processes data









Access

If you would like access to VMXi, please email:
VMXi@diamond.ac.uk or Dr Halina Mikolajek for soluble proteins and Dr Andrew Quigley for membrane proteins.
Complete a project form describing the proposed experiments, enter sample into ERA of active MX proposal.
Courier sample or visit site with sample or plates

Options include

- *Bringing plates to RCaH or VMXi directly*
- *Sending a researcher to setup plates in Research Complex*
- *Sending protein (by prior arrangement/discussion)*

Access to VMXi and Crystallisation laboratory within RCaH

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- Sending protein (by prior arrangement/discussion)

- Crystallisation robotics: Mosquito and Gryphon (4°C & 20°C, humidity & light control, LCP)
- Scorpion and Formulator for making screens
- Automated imaging systems with MFI technology (4°C & 20°C, LCP) with remote viewing

Crystallisation laboratory

Screens

- Molecular Dimensions
- Hampton Research

Plate types for VMXi

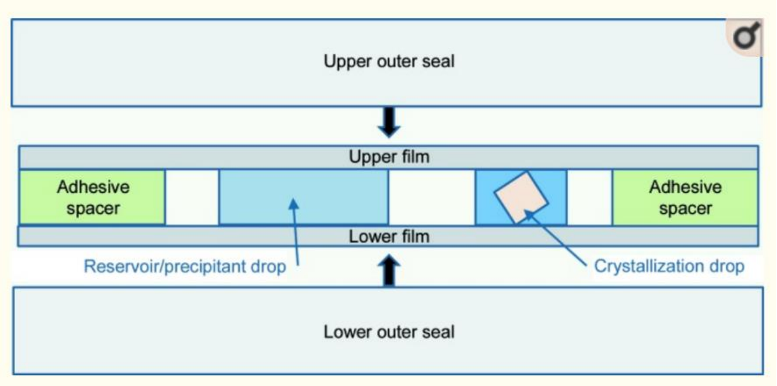
- Mitegen *in situ*
- Greiner Crystal Quick X

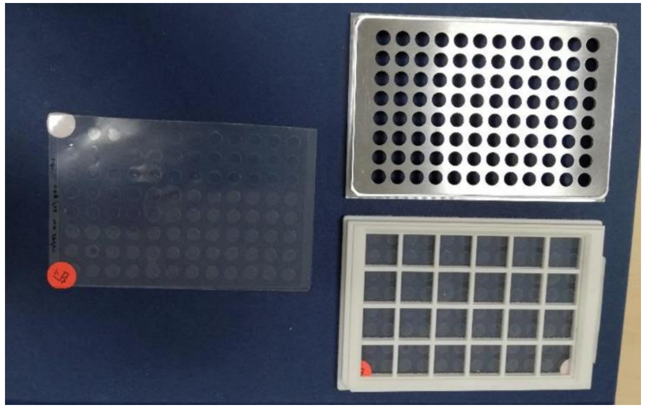
Films

- Very low background
- Ideal for small wedge data collection
- Ideal for microcrystals

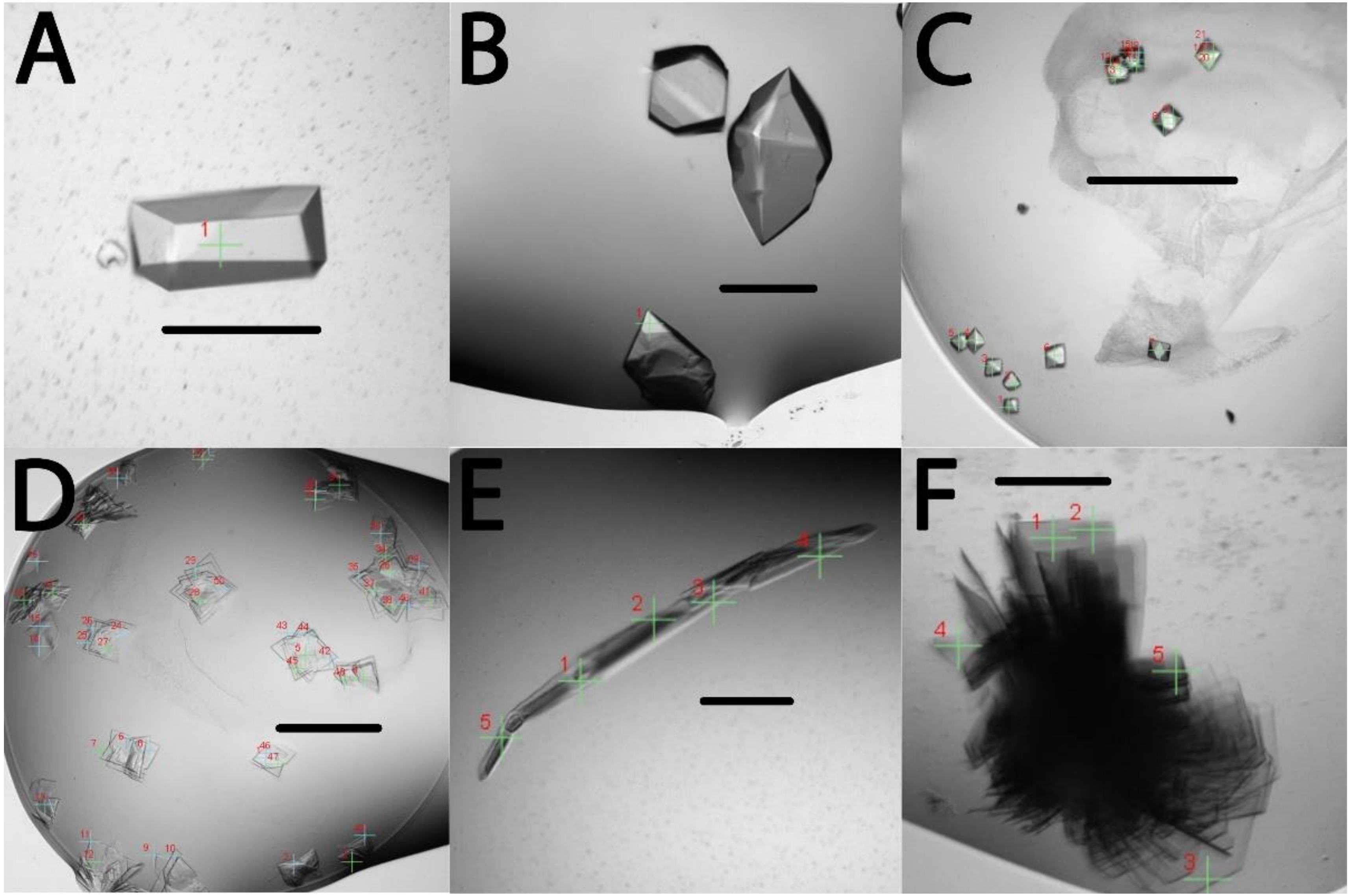
Molecular Dimensions

HAMPTON RESEARCH



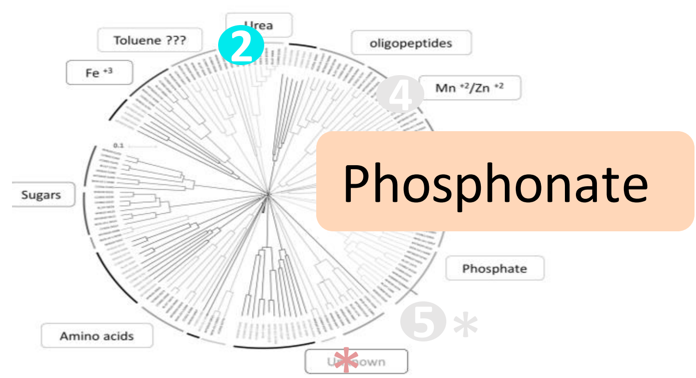


User cases

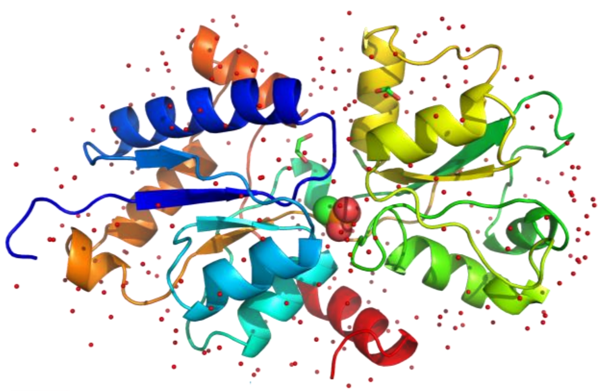


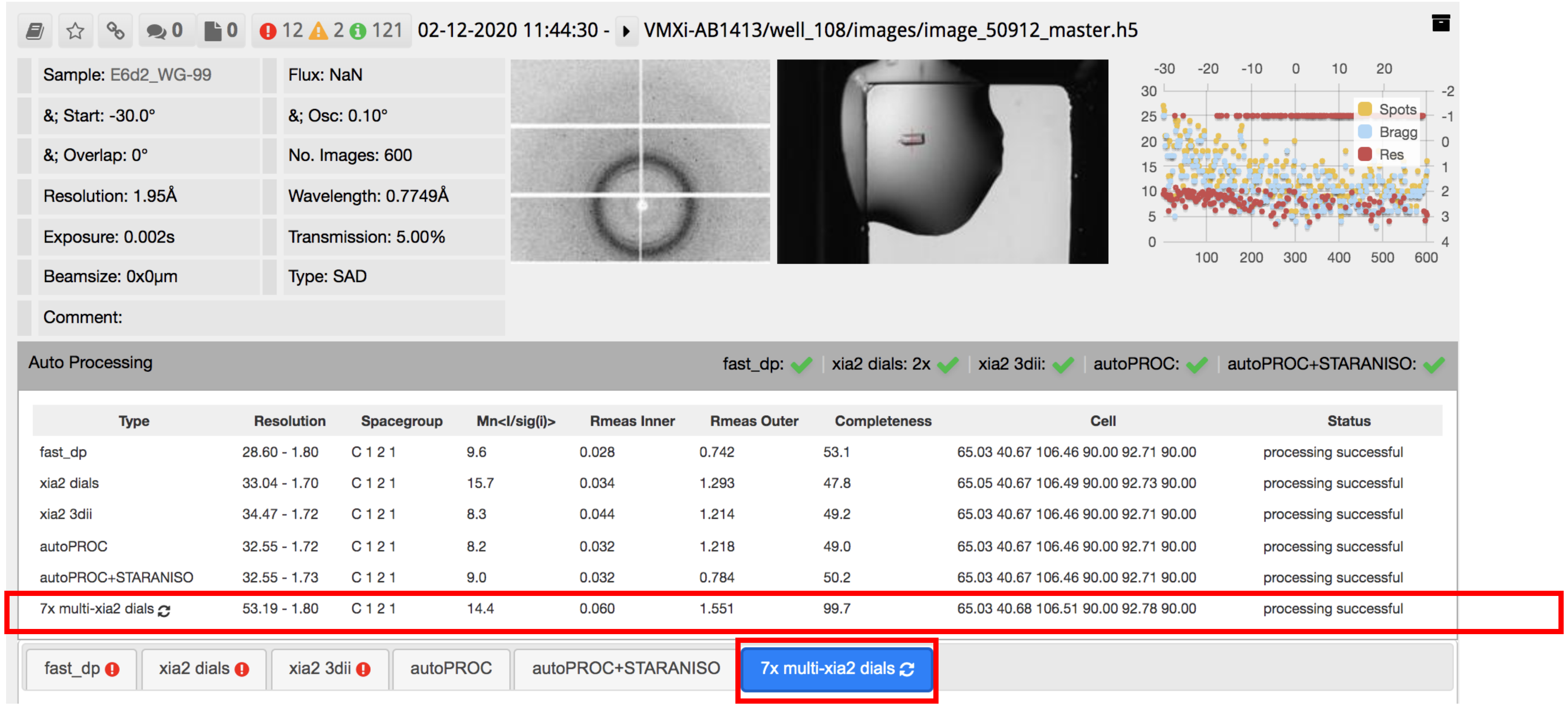
User 1: RT Crystal structures of Substrate Binding Proteins

This project required merging from 7 crystals using multi-xia2 dials to obtain a complete dataset.



Phosphonate





02-12-2020 11:44:30 - VMXi-AB1413/well_108/images/image_50912_master.h5

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&; Osc: 0.10°
&; Overlap: 0°
No. Images: 600
Resolution: 1.95Å
Wavelength: 0.7749Å
Exposure: 0.002s
Transmission: 5.00%
Beamsize: 0x0μm
Type: SAD
Comment:

Auto Processing

fast_dp: ✓ | xia2 dials: 2x ✓ | xia2 3dii: ✓ | autoPROC: ✓ | autoPROC+STARANISO: ✓

Type	Resolution	Spacegroup	Mn<I/sig(i)>	Rmeas Inner	Rmeas Outer	Completeness	Cell	Status
fast_dp	28.60 - 1.80	C 1 2 1	9.6	0.028	0.742	53.1	65.03 40.67 106.46 90.00 92.71 90.00	processing successful
xia2 dials	33.04 - 1.70	C 1 2 1	15.7	0.034	1.293	47.8	65.05 40.67 106.49 90.00 92.73 90.00	processing successful
xia2 3dii	34.47 - 1.72	C 1 2 1	8.3	0.044	1.214	49.2	65.03 40.67 106.46 90.00 92.71 90.00	processing successful
autoPROC	32.55 - 1.72	C 1 2 1	8.2	0.032	1.218	49.0	65.03 40.67 106.46 90.00 92.71 90.00	processing successful
autoPROC+STARANISO	32.55 - 1.73	C 1 2 1	9.0	0.032	0.784	50.2	65.03 40.67 106.46 90.00 92.71 90.00	processing successful
7x multi-xia2 dials	53.19 - 1.80	C 1 2 1	14.4	0.060	1.551	99.7	65.03 40.68 106.51 90.00 92.78 90.00	processing successful

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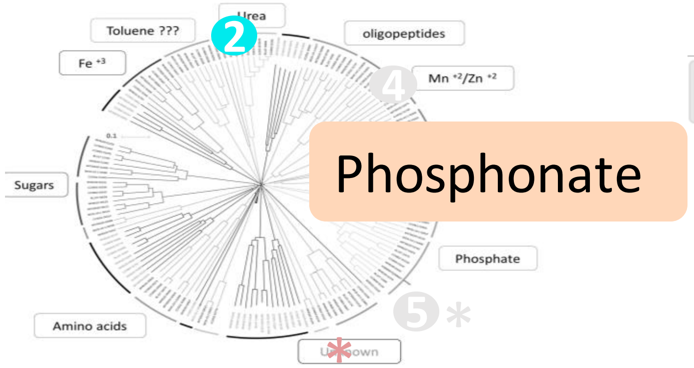
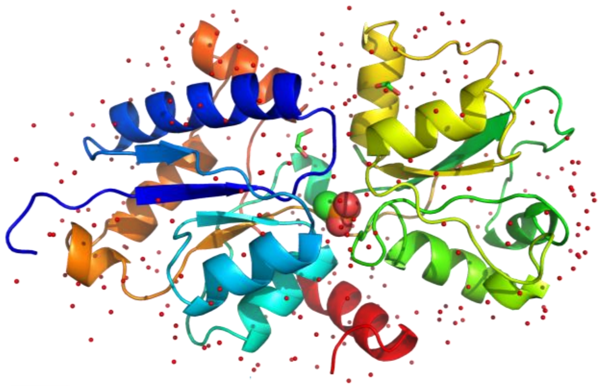
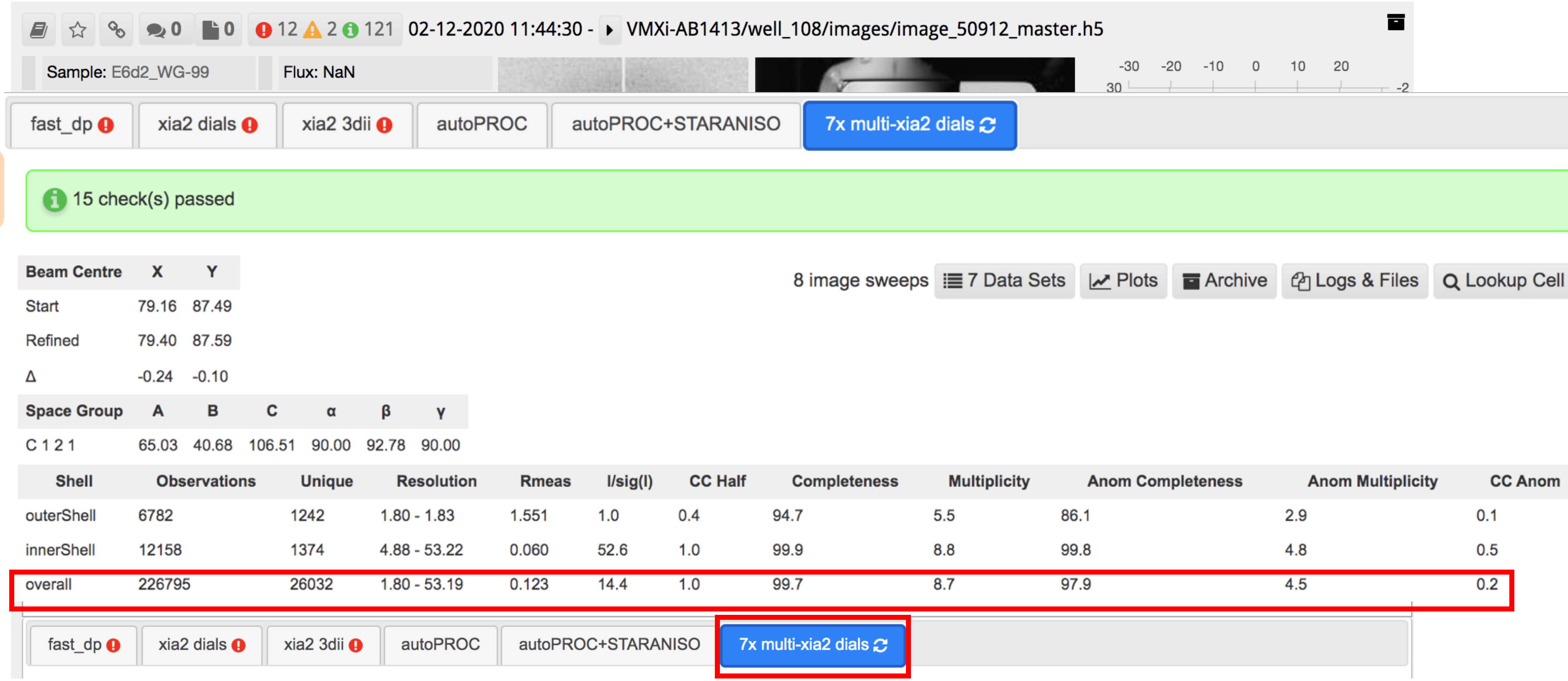






02-12-2020 11:44:30 - VMXi-AB1413/well_108/images/image_50912_master.h5

Sample: E6d2_WG-99

Flux: NaN

15 check(s) passed

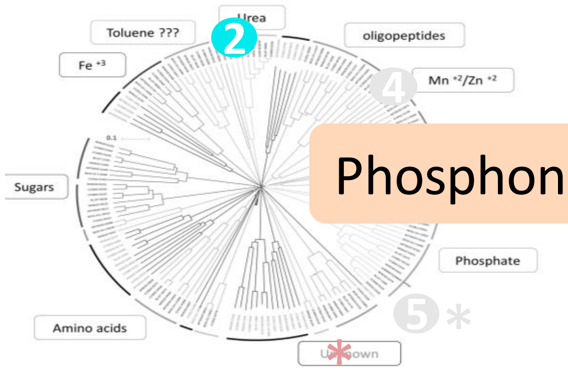
Beam Centre	X	Y
Start	79.16	87.49
Refined	79.40	87.59
Δ	-0.24	-0.10

8 image sweeps

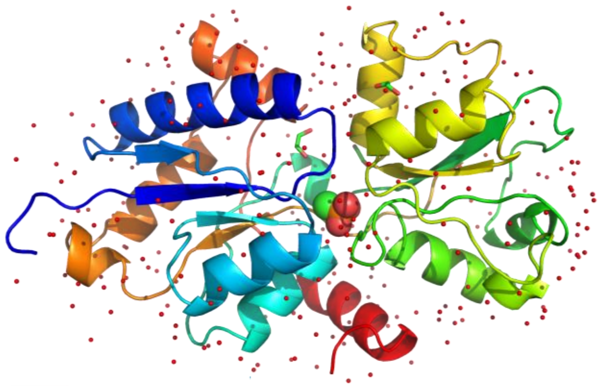
Space Group	A	B	C	α	β	γ
C 1 2 1	65.03	40.68	106.51	90.00	92.78	90.00

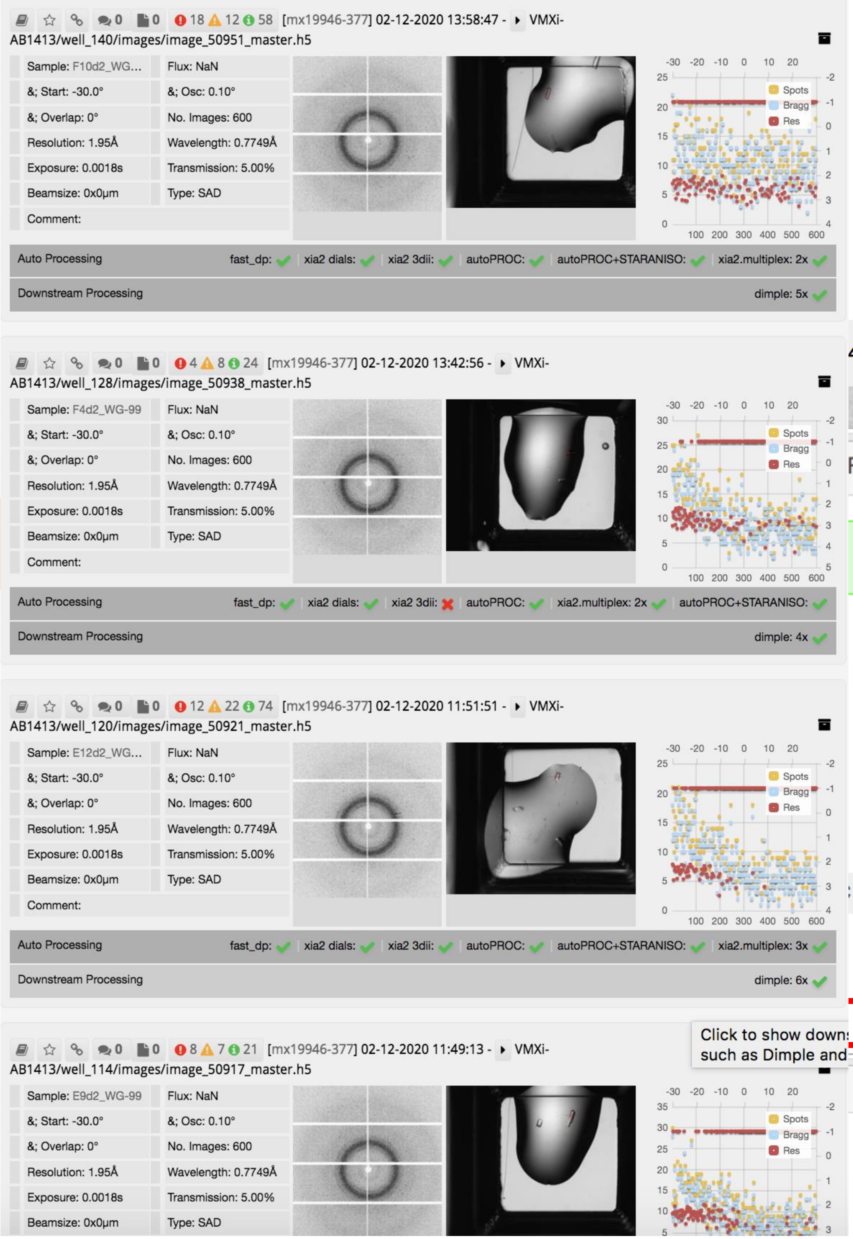
Shell	Observations	Unique	Resolution	Rmeas	I/sig(I)	CC Half	Completeness	Multiplicity	Anom Completeness	Anom Multiplicity	CC Anom
outerShell	6782	1242	1.80 - 1.83	1.551	1.0	0.4	94.7	5.5	86.1	2.9	0.1
innerShell	12158	1374	4.88 - 53.22	0.060	52.6	1.0	99.9	8.8	99.8	4.8	0.5
overall	226795	26032	1.80 - 53.19	0.123	14.4	1.0	99.7	8.7	97.9	4.5	0.2

User 1: RT Crystal structures of Substrate Binding



Phosphonate





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No. Images: 600
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Wavelength: 0.7749Å
Exposure: 0.0018s
Transmission: 5.00%
Beamsize: 0x0μm
Type: SAD
Comment:

Auto Processing fast_dp: ✓ | xia2 dials: ✓ | xia2 3dii: ✓ | autoPROC: ✓ | autoPROC+STARANISO: ✓ | xia2.multiplex: 2x ✓

Downstream Processing dimple: 5x ✓

[mx19946-377] 02-12-2020 13:42:56 - VMXi-AB1413/well_128/images/image_50938_master.h5

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Wavelength: 0.7749Å
Exposure: 0.0018s
Transmission: 5.00%
Beamsize: 0x0μm
Type: SAD
Comment:

Auto Processing fast_dp: ✓ | xia2 dials: ✓ | xia2 3dii: ✗ | autoPROC: ✓ | xia2.multiplex: 2x ✓ | autoPROC+STARANISO: ✓

Downstream Processing dimple: 4x ✓

[mx19946-377] 02-12-2020 11:51:51 - VMXi-AB1413/well_120/images/image_50921_master.h5

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No. Images: 600
Resolution: 1.95Å
Wavelength: 0.7749Å
Exposure: 0.0018s
Transmission: 5.00%
Beamsize: 0x0μm
Type: SAD
Comment:

Auto Processing fast_dp: ✓ | xia2 dials: ✓ | xia2 3dii: ✓ | autoPROC: ✓ | autoPROC+STARANISO: ✓ | xia2.multiplex: 3x ✓

Downstream Processing dimple: 6x ✓

[mx19946-377] 02-12-2020 11:49:13 - VMXi-AB1413/well_114/images/image_50917_master.h5

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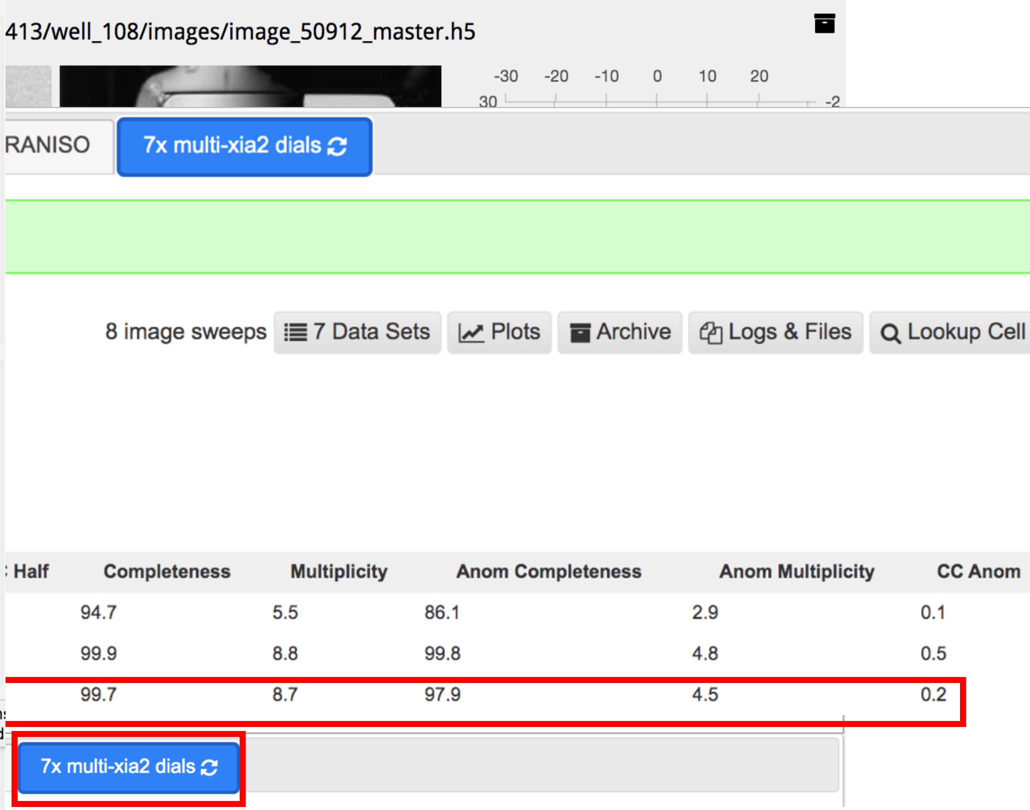
&; Overlap: 0°
No. Images: 600
Resolution: 1.95Å
Wavelength: 0.7749Å
Exposure: 0.0018s
Transmission: 5.00%
Beamsize: 0x0μm
Type: SAD



413/well_108/images/image_50912_master.h5

Half	Completeness	Multiplicity	Anom Completeness	Anom Multiplicity	CC Anom
	94.7	5.5	86.1	2.9	0.1
	99.9	8.8	99.8	4.8	0.5
	99.7	8.7	97.9	4.5	0.2

Click to show down such as Dimple and

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User 2: Quick feedback on difficult to harvest crystals -> Biofuel Project

- User obtained crystals after 75 days. Screened at VMXi and determined crystal to be protein. Tricky MR and low completeness meant harvesting of remaining crystal and collecting at I03. Structure solved.
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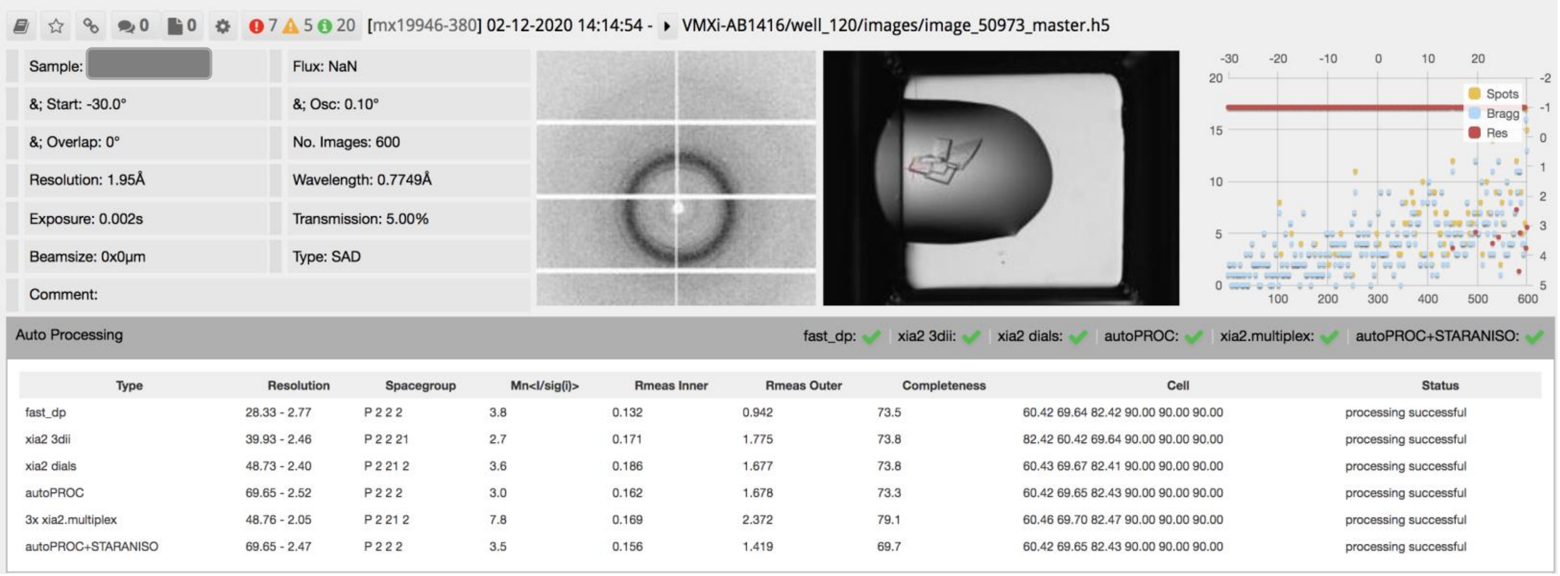

0 0 7 5 20 [mx19946-380] 02-12-2020 14:14:54 - VMXi-AB1416/well_120/images/image_50973_master.h5

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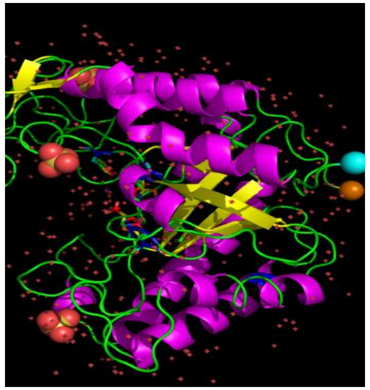
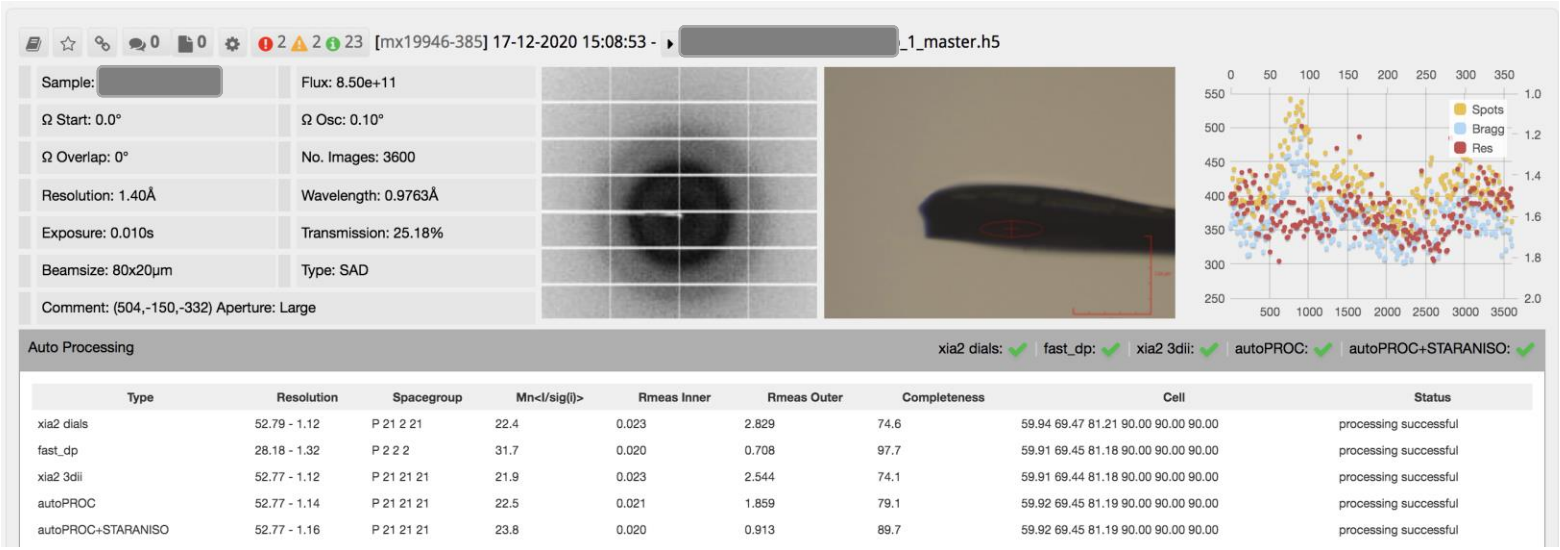
&; Overlap: 0°
No. Images: 600
Resolution: 1.95Å
Wavelength: 0.7749Å
Exposure: 0.002s
Transmission: 5.00%
Beamsize: 0x0µm
Type: SAD
Comment:

Auto Processing

fast_dp: ✓ xia2 3dii: ✓ xia2 dials: ✓ autoPROC: ✓ xia2.multiplex: ✓ autoPROC+STARANISO: ✓

Type	Resolution	Spacegroup	Mn<I/sig(i)>	Rmeas Inner	Rmeas Outer	Completeness	Cell	Status
fast_dp	28.33 - 2.77	P 2 2 2	3.8	0.132	0.942	73.5	60.42 69.64 82.42 90.00 90.00 90.00	processing successful
xia2 3dii	39.93 - 2.46	P 2 2 21	2.7	0.171	1.775	73.8	82.42 60.42 69.64 90.00 90.00 90.00	processing successful
xia2 dials	48.73 - 2.40	P 2 21 2	3.6	0.186	1.677	73.8	60.43 69.67 82.41 90.00 90.00 90.00	processing successful
autoPROC	69.65 - 2.52	P 2 2 2	3.0	0.162	1.678	73.3	60.42 69.65 82.43 90.00 90.00 90.00	processing successful
3x xia2.multiplex	48.76 - 2.05	P 2 21 2	7.8	0.169	2.372	79.1	60.46 69.70 82.47 90.00 90.00 90.00	processing successful
autoPROC+STARANISO	69.65 - 2.47	P 2 2 2	3.5	0.156	1.419	69.7	60.42 69.65 82.43 90.00 90.00 90.00	processing successful





0 0 2 2 23 [mx19946-385] 17-12-2020 15:08:53 - [redacted]_1_master.h5

Sample: [redacted]
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Ω Start: 0.0°
Ω Osc: 0.10°
Ω Overlap: 0°
No. Images: 3600
Resolution: 1.40Å
Wavelength: 0.9763Å
Exposure: 0.010s
Transmission: 25.18%
Beamsize: 80x20µm
Type: SAD
Comment: (504,-150,-332) Aperture: Large

Auto Processing

xia2 dials: ✓ fast_dp: ✓ xia2 3dii: ✓ autoPROC: ✓ autoPROC+STARANISO: ✓

Type	Resolution	Spacegroup	Mn<I/sig(i)>	Rmeas Inner	Rmeas Outer	Completeness	Cell	Status
xia2 dials	52.79 - 1.12	P 21 2 21	22.4	0.023	2.829	74.6	59.94 69.47 81.21 90.00 90.00 90.00	processing successful
fast_dp	28.18 - 1.32	P 2 2 2	31.7	0.020	0.708	97.7	59.91 69.45 81.18 90.00 90.00 90.00	processing successful
xia2 3dii	52.77 - 1.12	P 21 21 21	21.9	0.023	2.544	74.1	59.91 69.44 81.18 90.00 90.00 90.00	processing successful
autoPROC	52.77 - 1.14	P 21 21 21	22.5	0.021	1.859	79.1	59.92 69.45 81.19 90.00 90.00 90.00	processing successful
autoPROC+STARANISO	52.77 - 1.16	P 21 21 21	23.8	0.020	0.913	89.7	59.92 69.45 81.19 90.00 90.00 90.00	processing successful

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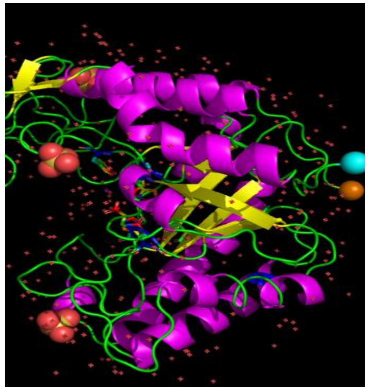

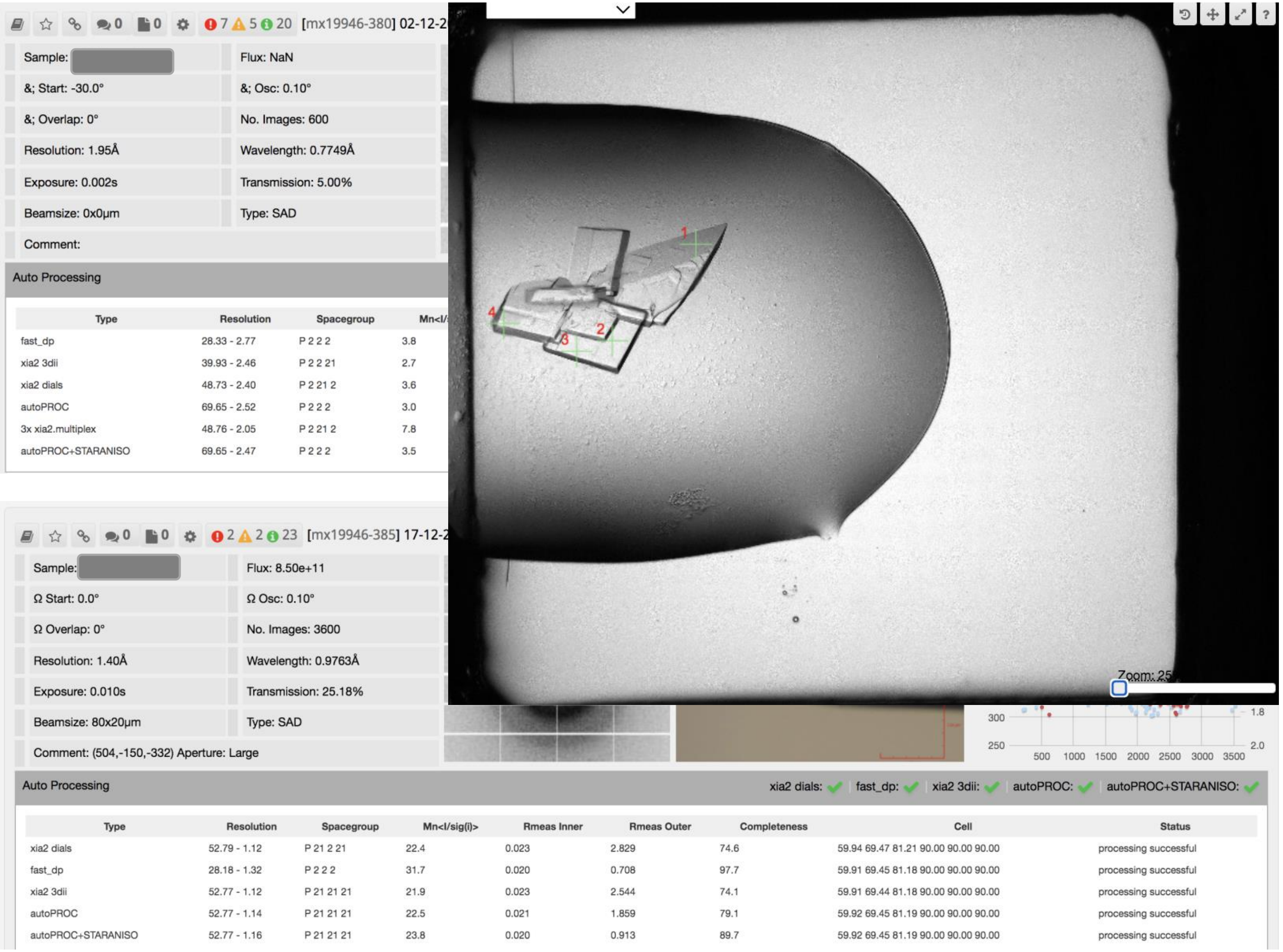

0 0 7 5 20 [mx19946-380] 02-12-2

Sample: [redacted]	Flux: NaN
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&; Overlap: 0°	No. Images: 600
Resolution: 1.95Å	Wavelength: 0.7749Å
Exposure: 0.002s	Transmission: 5.00%
Beamsize: 0x0µm	Type: SAD
Comment:	

Auto Processing

Type	Resolution	Spacegroup	Mn<I/
fast_dp	28.33 - 2.77	P 2 2 2	3.8
xia2 3dii	39.93 - 2.46	P 2 2 21	2.7
xia2 dials	48.73 - 2.40	P 2 21 2	3.6
autoPROC	69.65 - 2.52	P 2 2 2	3.0
3x xia2.multiplex	48.76 - 2.05	P 2 21 2	7.8
autoPROC+STARANISO	69.65 - 2.47	P 2 2 2	3.5

0 0 2 2 23 [mx19946-385] 17-12-2

Sample: [redacted]	Flux: 8.50e+11
Ω Start: 0.0°	Ω Osc: 0.10°
Ω Overlap: 0°	No. Images: 3600
Resolution: 1.40Å	Wavelength: 0.9763Å
Exposure: 0.010s	Transmission: 25.18%
Beamsize: 80x20µm	Type: SAD
Comment: (504,-150,-332) Aperture: Large	

Auto Processing xia2 dials: ✓ fast_dp: ✓ xia2 3dii: ✓ autoPROC: ✓ autoPROC+STARANISO: ✓

Type	Resolution	Spacegroup	Mn<I/sig(I)>	Rmeas Inner	Rmeas Outer	Completeness	Cell	Status
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xia2 3dii	52.77 - 1.12	P 21 21 21	21.9	0.023	2.544	74.1	59.91 69.44 81.18 90.00 90.00 90.00	processing successful
autoPROC	52.77 - 1.14	P 21 21 21	22.5	0.021	1.859	79.1	59.92 69.45 81.19 90.00 90.00 90.00	processing successful
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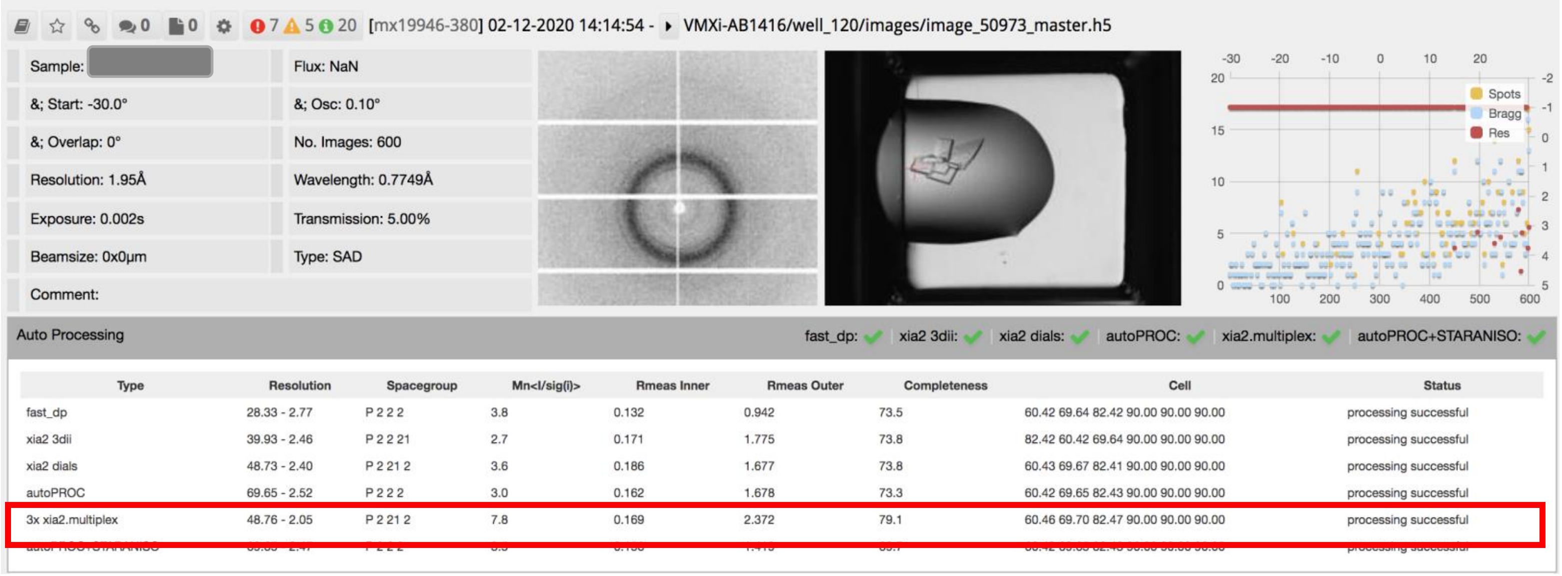
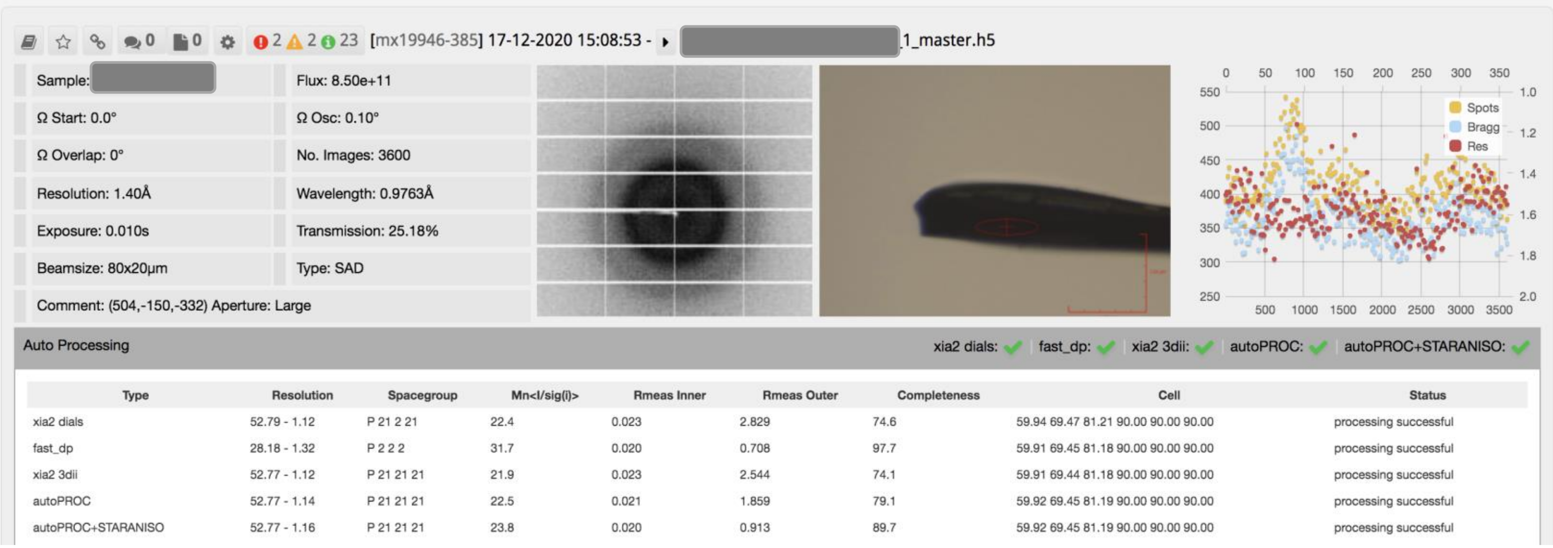


0 0 ❗7 ⚠5 ℹ20 [mx19946-380] 02-12-2020 14:14:54 - ▸ VMXi-AB1416/well_120/images/image_50973_master.h5

Sample: [redacted]	Flux: NaN
&; Start: -30.0°	&; Osc: 0.10°
&; Overlap: 0°	No. Images: 600
Resolution: 1.95Å	Wavelength: 0.7749Å
Exposure: 0.002s	Transmission: 5.00%
Beamsize: 0x0µm	Type: SAD
Comment:	

Auto Processing — fast_dp: ✓ xia2 3dii: ✓ xia2 dials: ✓ autoPROC: ✓ xia2.multiplex: ✓ autoPROC+STARANISO: ✓

Type	Resolution	Spacegroup	Mn<I/sig(I)>	Rmeas Inner	Rmeas Outer	Completeness	Cell	Status
fast_dp	28.33 - 2.77	P 2 2 2	3.8	0.132	0.942	73.5	60.42 69.64 82.42 90.00 90.00 90.00	processing successful
xia2 3dii	39.93 - 2.46	P 2 2 21	2.7	0.171	1.775	73.8	82.42 60.42 69.64 90.00 90.00 90.00	processing successful
xia2 dials	48.73 - 2.40	P 2 21 2	3.6	0.186	1.677	73.8	60.43 69.67 82.41 90.00 90.00 90.00	processing successful
autoPROC	69.65 - 2.52	P 2 2 2	3.0	0.162	1.678	73.3	60.42 69.65 82.43 90.00 90.00 90.00	processing successful
3x xia2.multiplex	48.76 - 2.05	P 2 21 2	7.8	0.169	2.372	79.1	60.46 69.70 82.47 90.00 90.00 90.00	processing successful

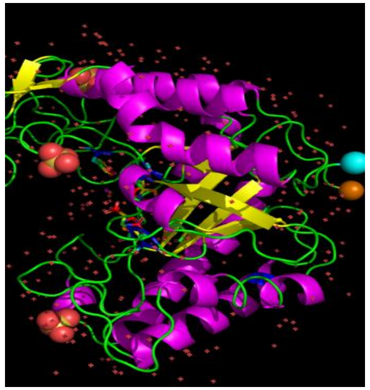


0 0 ❗2 ⚠2 ℹ23 [mx19946-385] 17-12-2020 15:08:53 - ▸ [redacted]1_master.h5

Sample: [redacted]	Flux: 8.50e+11
Ω Start: 0.0°	Ω Osc: 0.10°
Ω Overlap: 0°	No. Images: 3600
Resolution: 1.40Å	Wavelength: 0.9763Å
Exposure: 0.010s	Transmission: 25.18%
Beamsize: 80x20µm	Type: SAD
Comment: (504,-150,-332) Aperture: Large	

Auto Processing — xia2 dials: ✓ fast_dp: ✓ xia2 3dii: ✓ autoPROC: ✓ autoPROC+STARANISO: ✓

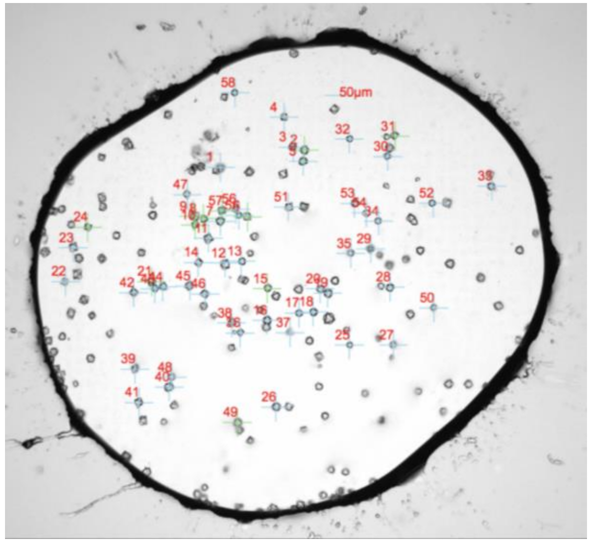
Type	Resolution	Spacegroup	Mn<I/sig(I)>	Rmeas Inner	Rmeas Outer	Completeness	Cell	Status
xia2 dials	52.79 - 1.12	P 21 2 21	22.4	0.023	2.829	74.6	59.94 69.47 81.21 90.00 90.00 90.00	processing successful
fast_dp	28.18 - 1.32	P 2 2 2	31.7	0.020	0.708	97.7	59.91 69.45 81.18 90.00 90.00 90.00	processing successful
xia2 3dii	52.77 - 1.12	P 21 21 21	21.9	0.023	2.544	74.1	59.91 69.44 81.18 90.00 90.00 90.00	processing successful
autoPROC	52.77 - 1.14	P 21 21 21	22.5	0.021	1.859	79.1	59.92 69.45 81.19 90.00 90.00 90.00	processing successful
autoPROC+STARANISO	52.77 - 1.16	P 21 21 21	23.8	0.020	0.913	89.7	59.92 69.45 81.19 90.00 90.00 90.00	processing successful



Future directions for VMXi

- Thin film for serial static and serial-oscillation options (small wedge data collection) for batch grown microcrystals





- Time resolved serial crystallography

- Integration with XCHEM for fragments and ligands at room temperature