VMXm – getting the most from micron sized crystals

Anna Warren, Senior Beamline Scientist, VMXm

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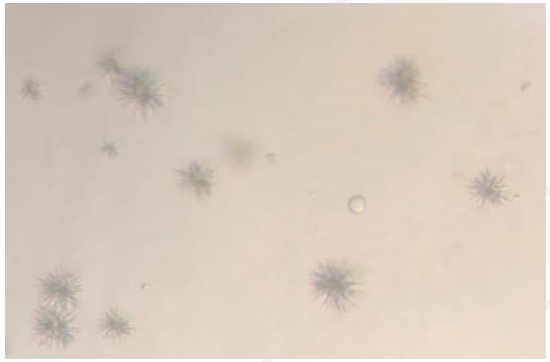
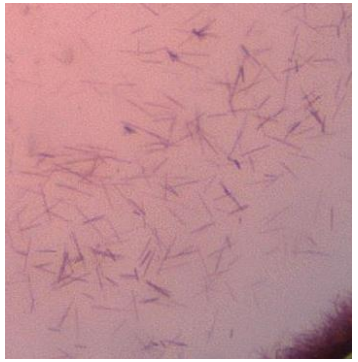
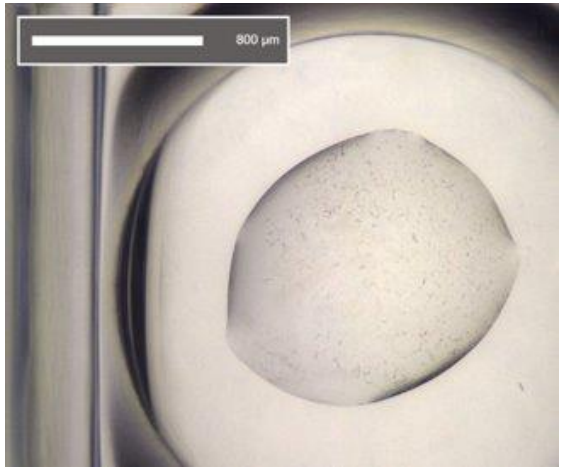
Introduction

- Crystallography remains one of the gold standards for the determination of macromolecular structures and ligand bound structures
- Production of suitable crystals remains one of the largest bottlenecks
- Many crystallisation strategies have been developed
- Synchrotron beamlines have also improved to help researchers with their challenging targets
- However, some complex proteins are difficult to crystallise and don’t form large uniform crystals

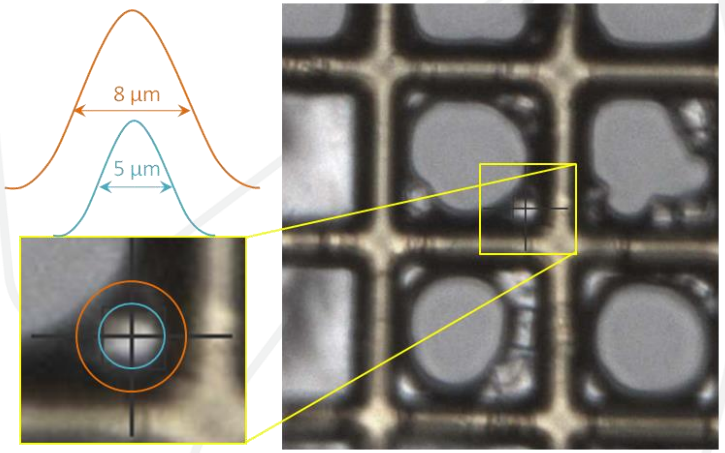


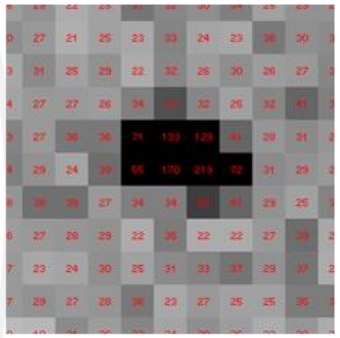


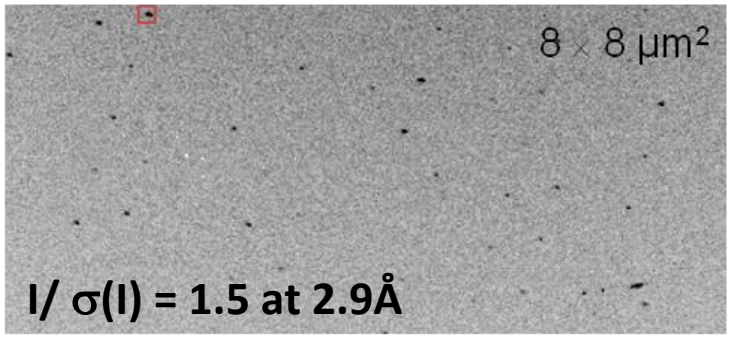
Challenges of microcrystals (<20 µm)

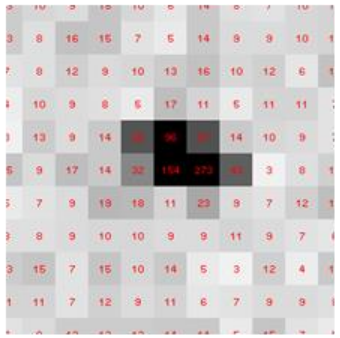


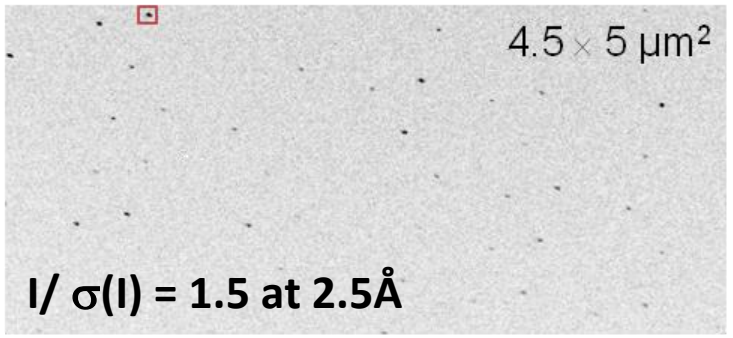
- Sometimes only small crystals are formed
- Can design ideal experiment to give optimal data quality
- As crystals get smaller, signal lowers, several problems emerge
  - Stability, alignment, visualization…
- Experimental setup becomes ever more critical











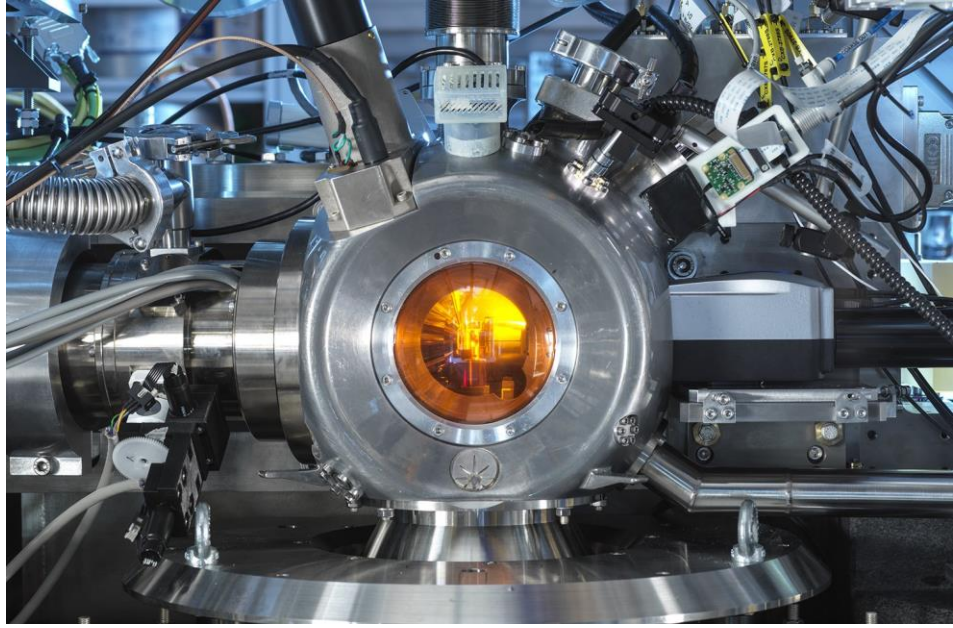
G. Evans, D. Axford and R. L. Owen (2011) *Acta Cryst.* **D67**, 261-270.

Current Limits

- Dose tolerance of samples cannot be changed – Henderson/Garman limit fixed
- Reduce dose on sample to measure given data quality:
  - Reduce experimental background
  - Cleaner sample mounting
- Improve analysis for weak and multicrystal data
- Record rotation data to improve data quality
- Visualization of micron and sub-micron crystals
- Take advantage of photoelectron escape

VMXm

- Very low background X-ray diffraction data
  - Sample chamber under vacuum to give zero air scatter
  - Crystals mounted on cryoEM grids with minimal surrounding solvent
  - Reduce beamsize to match that of the crystal
- Rotation data from v. small crystals
  - Potential to collect rotation data on samples down to ~500 nm
  - High energy > 20keV data collection benefitting from photoelectron escape
- Benefits
  - Lower number of crystals for structure determination
  - Higher resolution data
- Can collect data from both protein and chemical crystallography samples



- 7 – 22 keV energy range
- 0.4 – 10 µm (v) & 1.2 – 5 µm (h)
- Full beam flux at 21.8 keV is 3.2×10^{11} ph/s
- Indirectly cryocooled samples
- Eiger2 X 9M CdTe sensor detector

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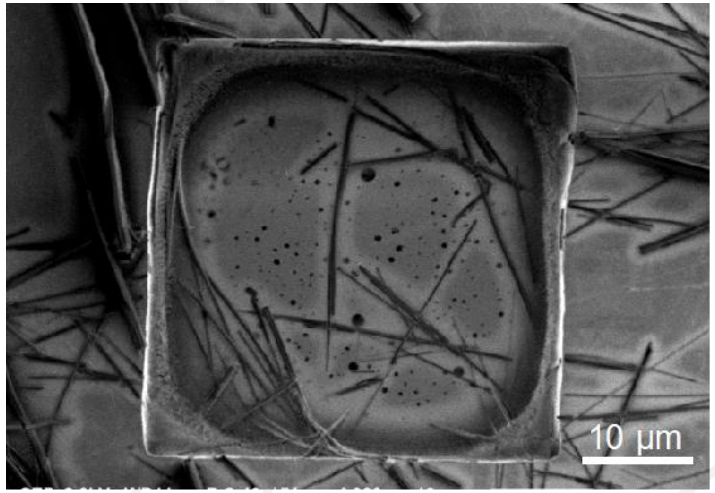
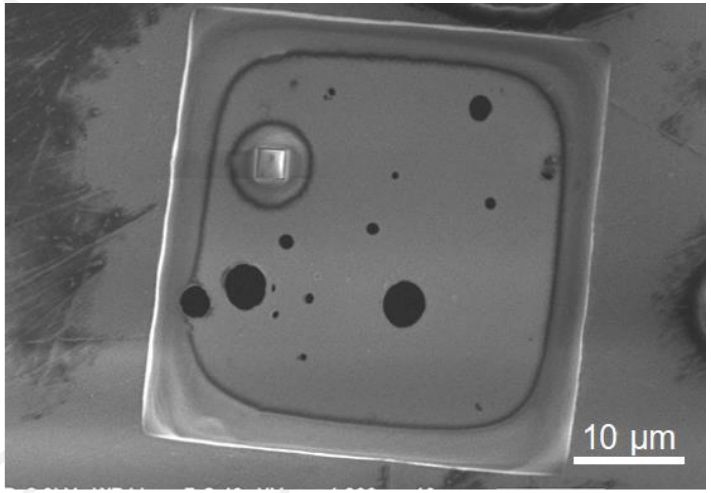
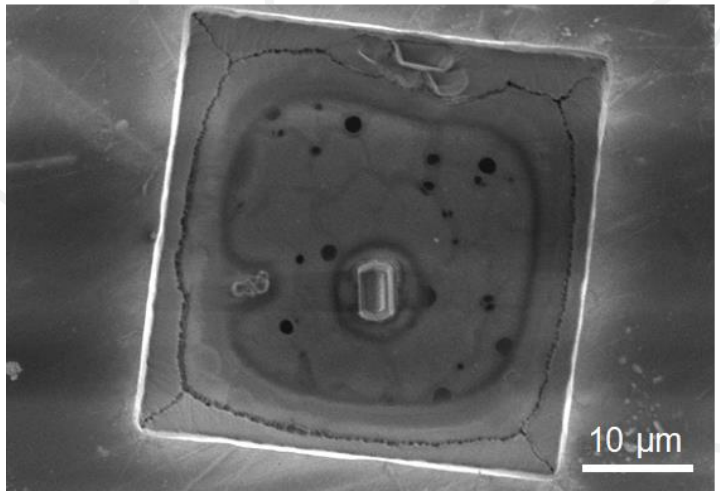
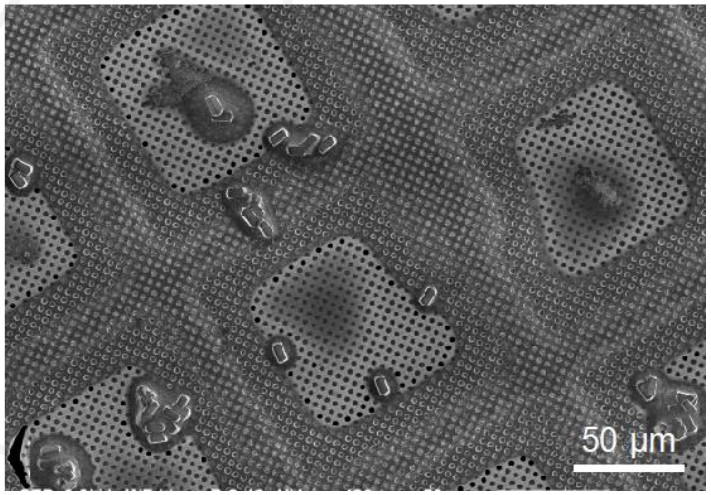
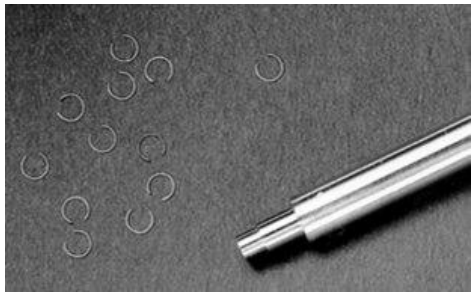
VMXm – sample preparation











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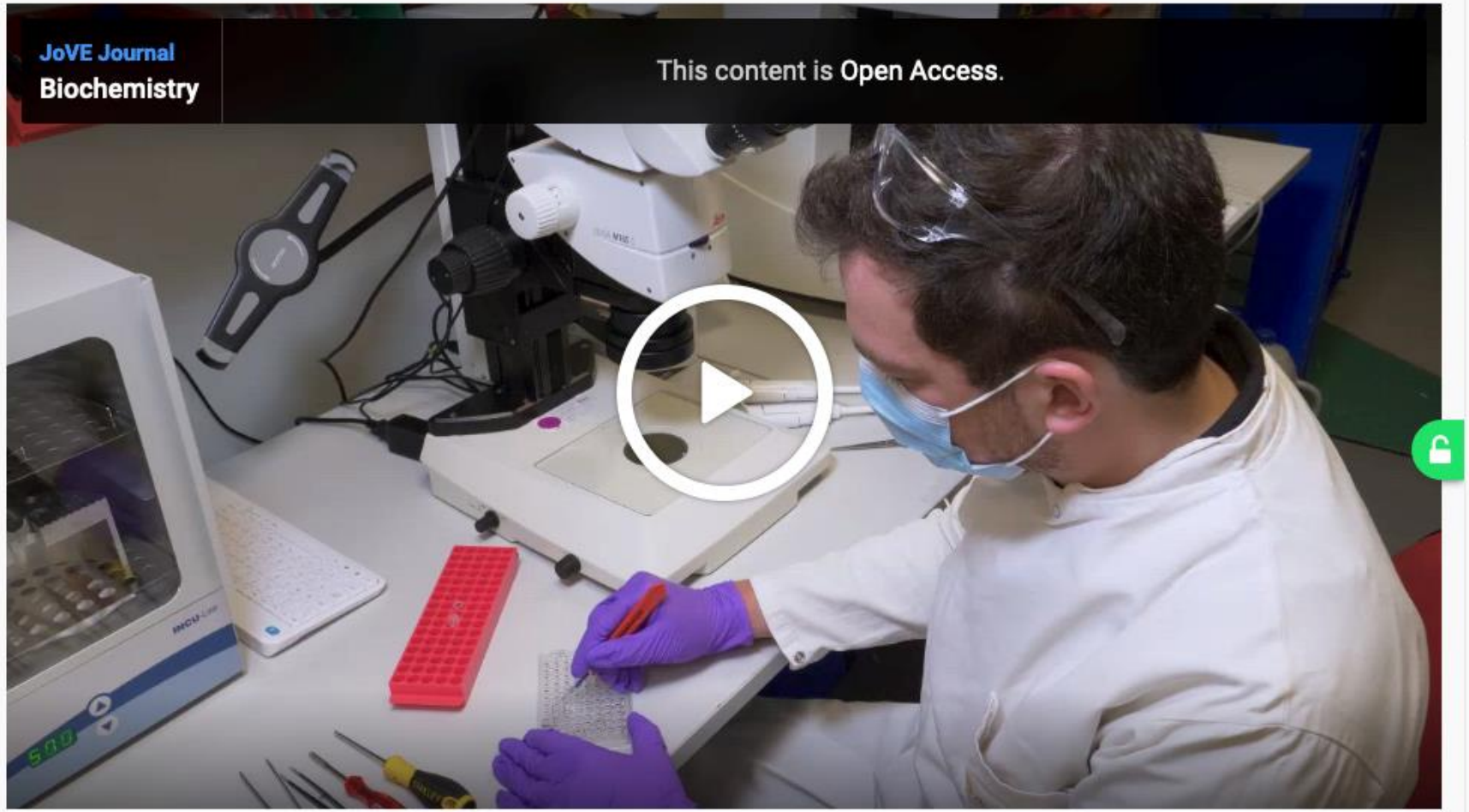

A Sample Preparation Pipeline for Microcrystals at the VMXm Beamline

DOI: 10.3791/62306-v

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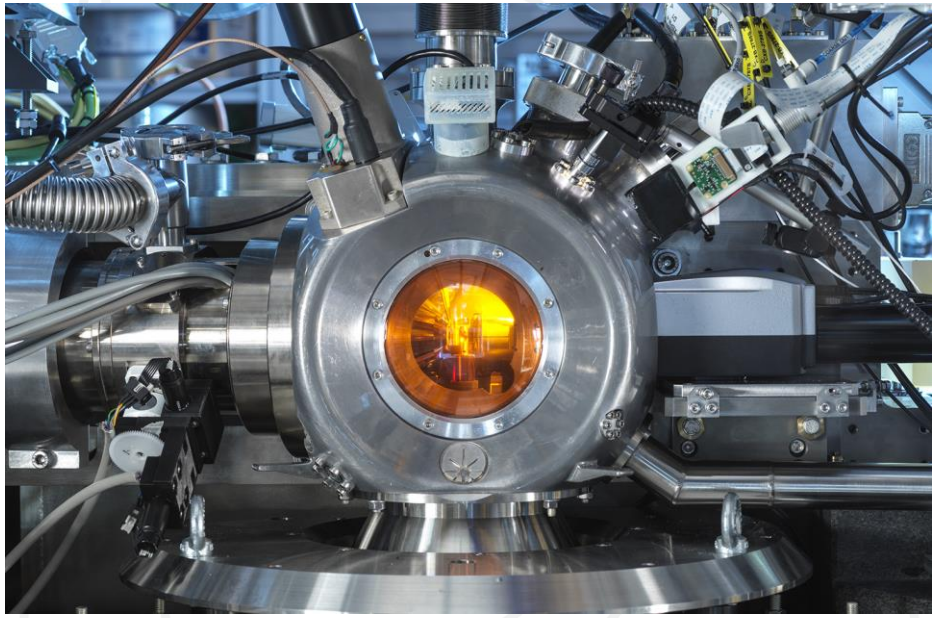
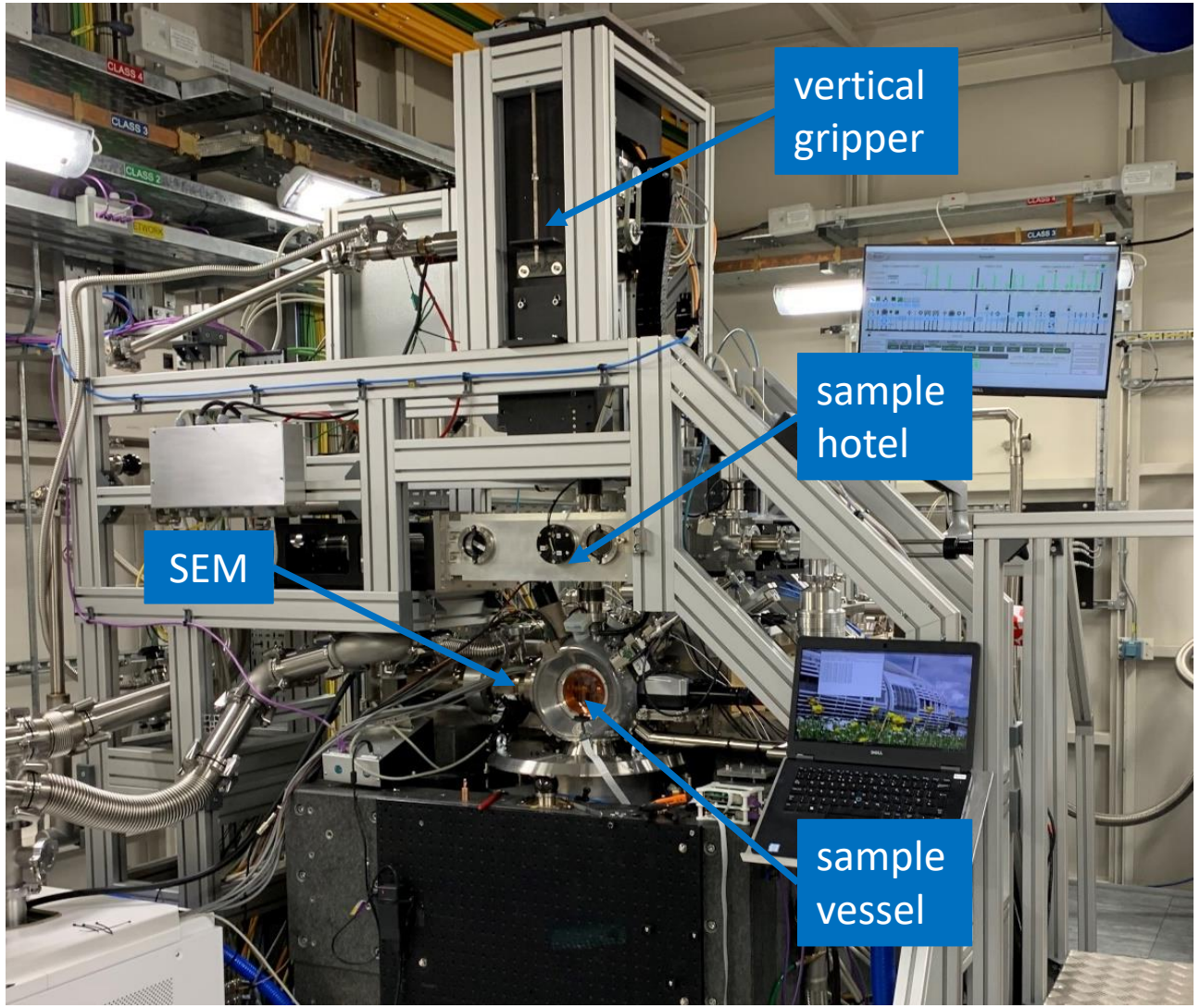
[1]Diamond Light Source Ltd, Harwell Science and Innovation Campus, [2]Paul Scherrer Institut, [3]Central Laser Facility, Science and Technologies

VMXm – beamline





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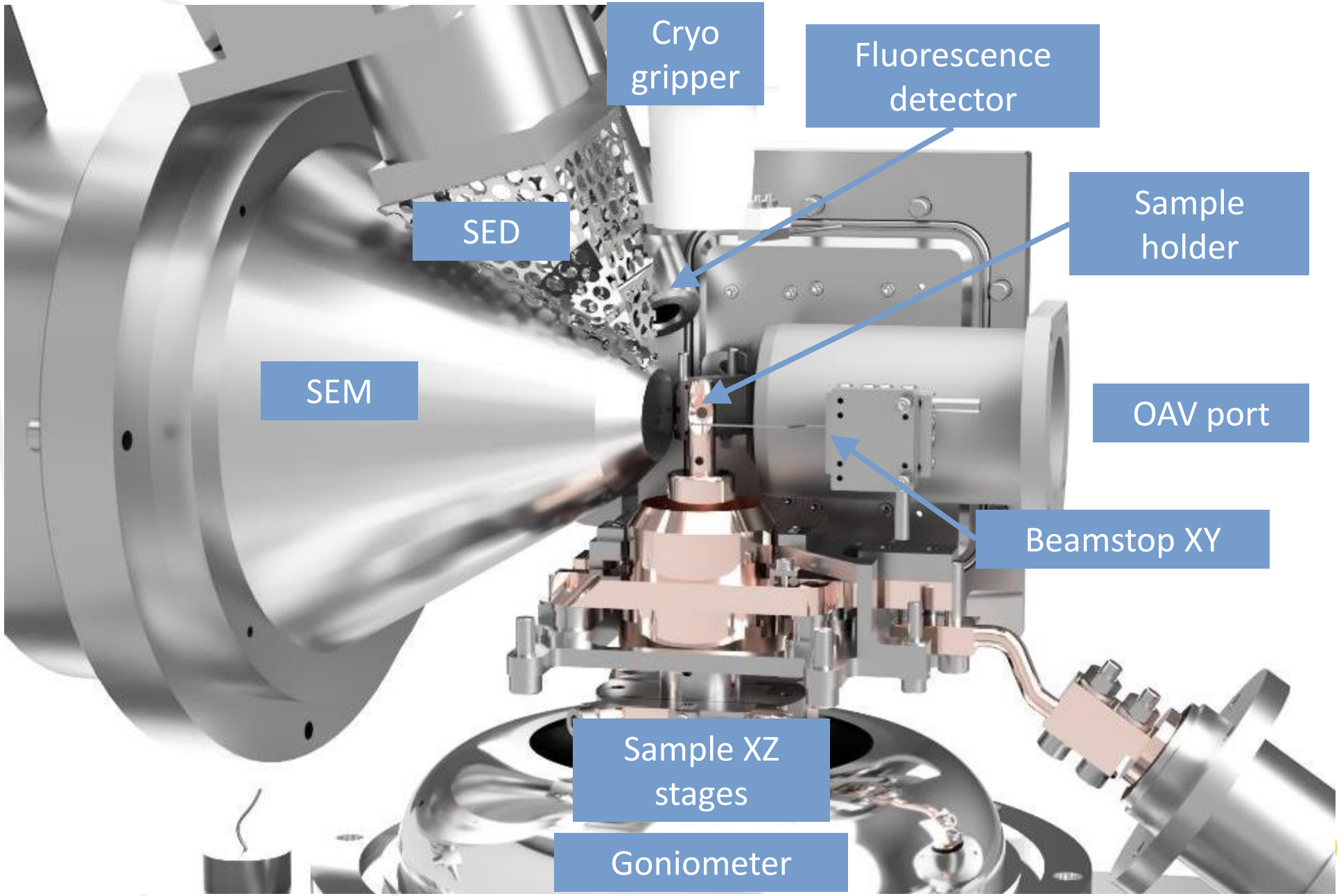
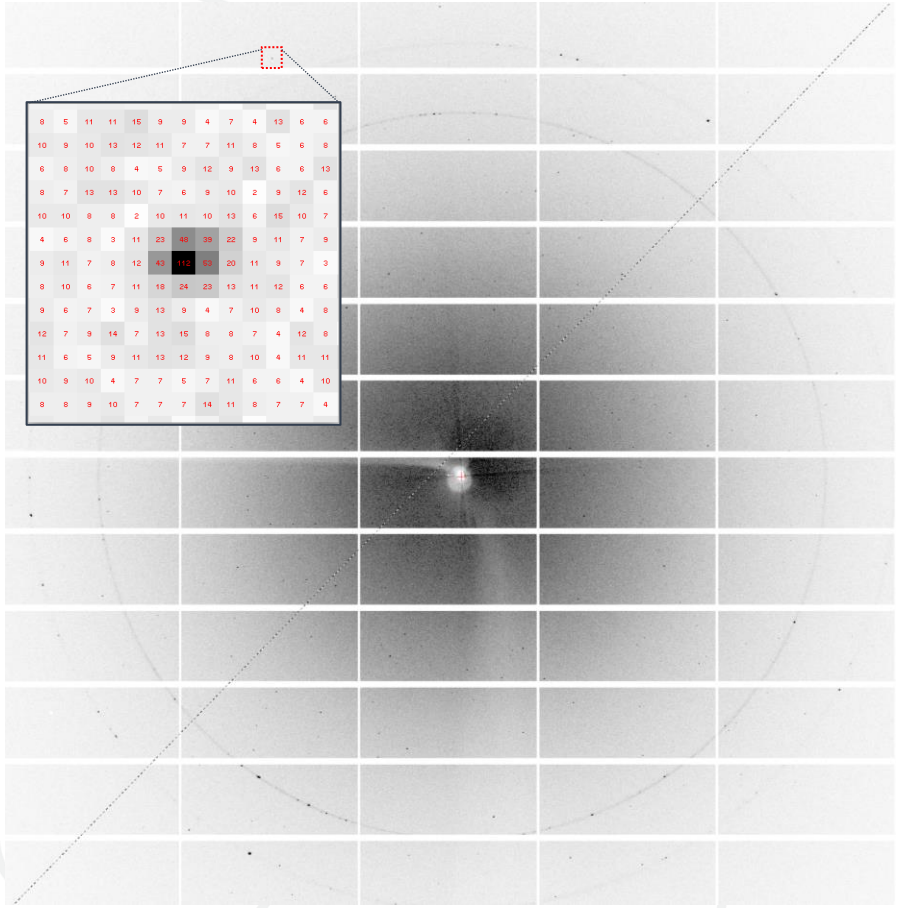
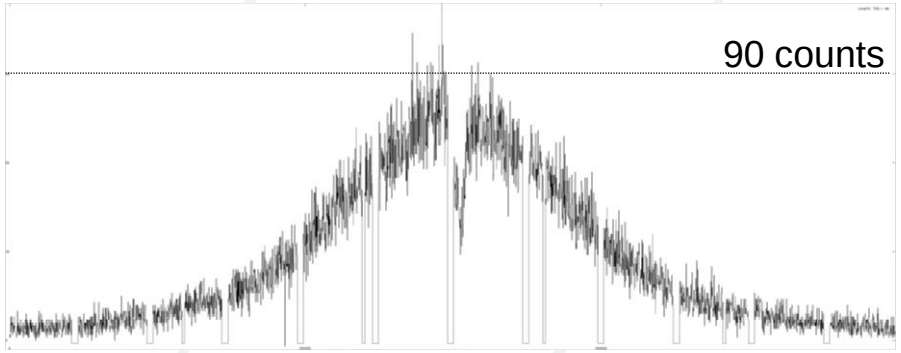
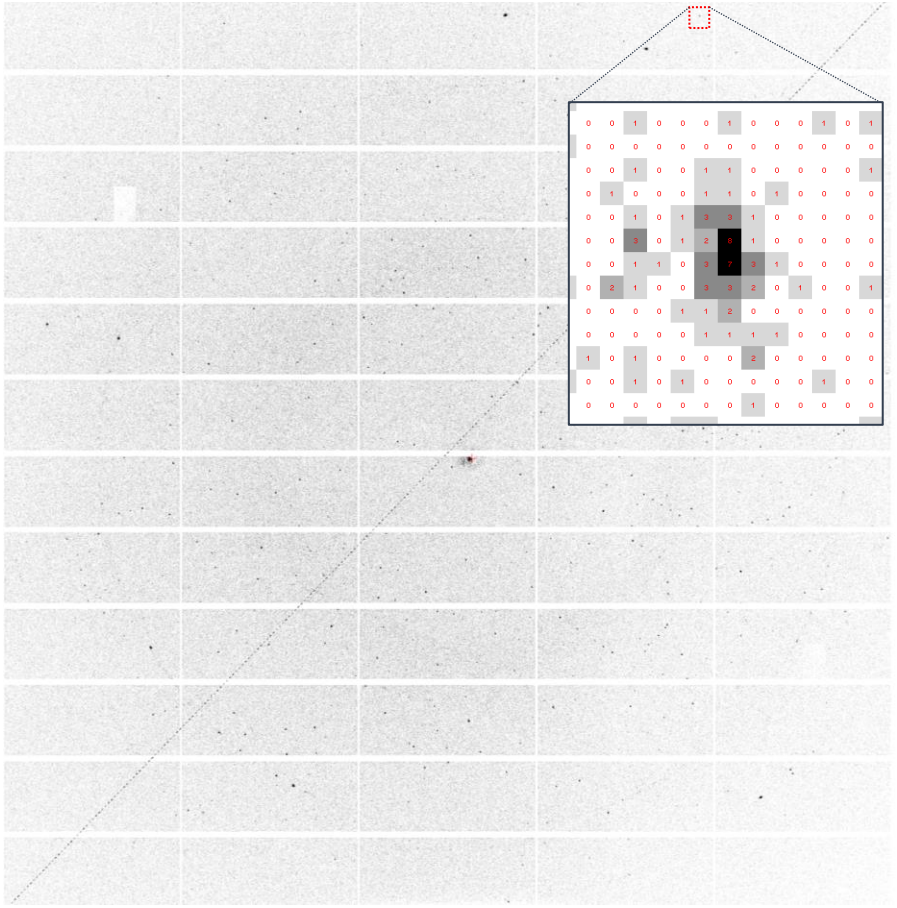

Cryo gripper
Fluorescence detector
SED
Sample holder
SEM
OAV port
Beamstop XY
Sample XZ stages
Goniometer

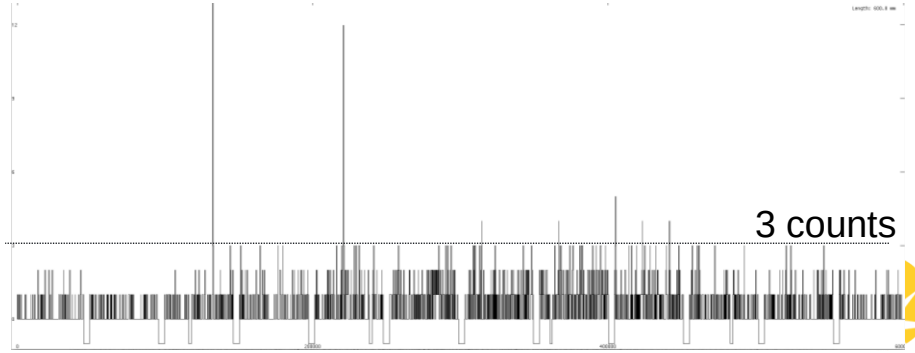
Standard in air MX





VMXm *in vacuo*





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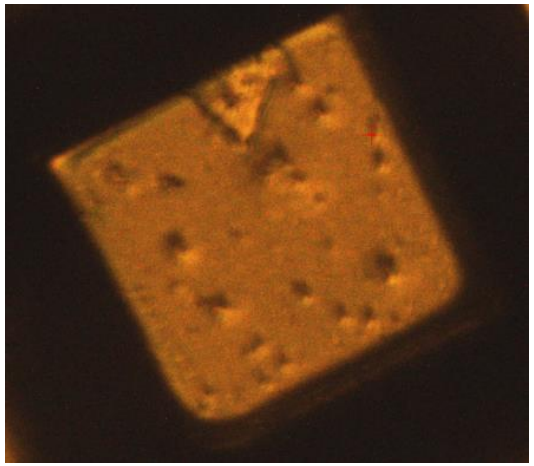
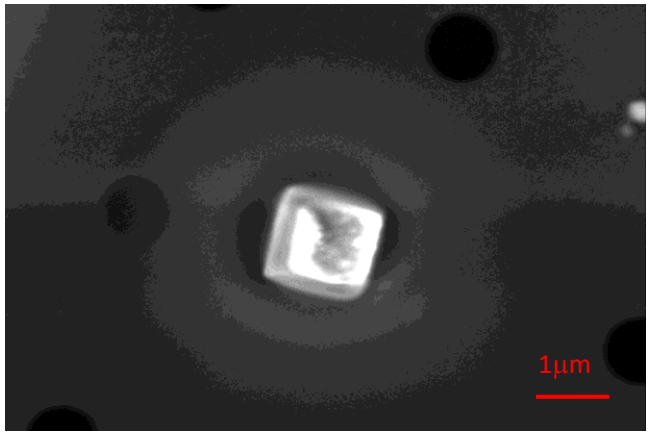
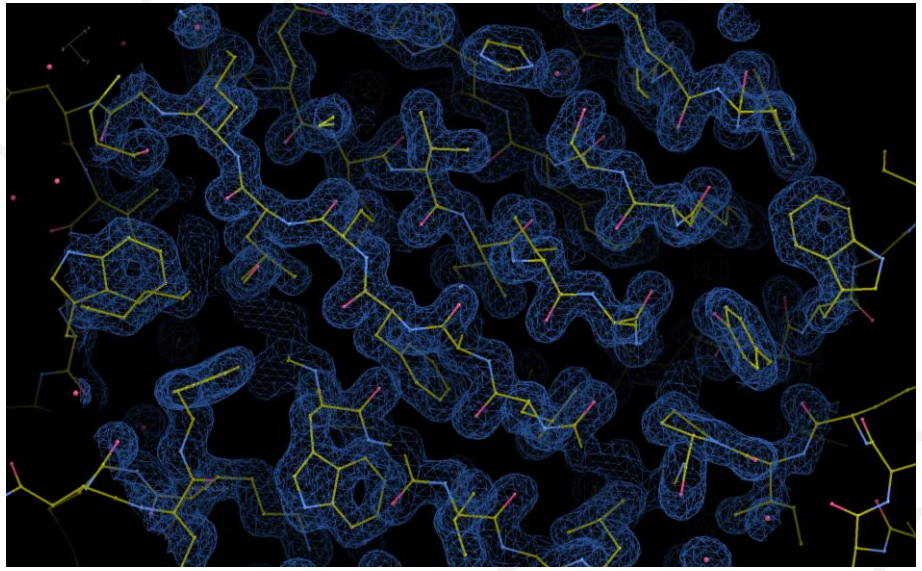
CPV Us17 – Cytoplasmic polyhedrosis virus

Previously measured on I24 and at CXI instrument @LCLS. Initially solved my MR using LCLS data to 1.75 Å and then extended with better XFEL data analysis to 1.46 Å.

- Cytoplasmic polyhedrosis virus
- Spacegroup I23
- Unit cell a=b=c=105 Å
- Average crystal size 1.2 µm
- Data collected at 21.3keV
- Eiger2 X CdTe 9M





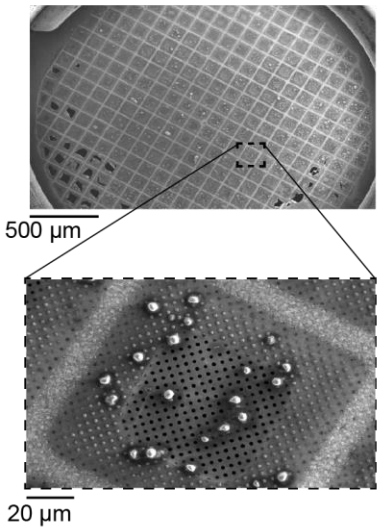


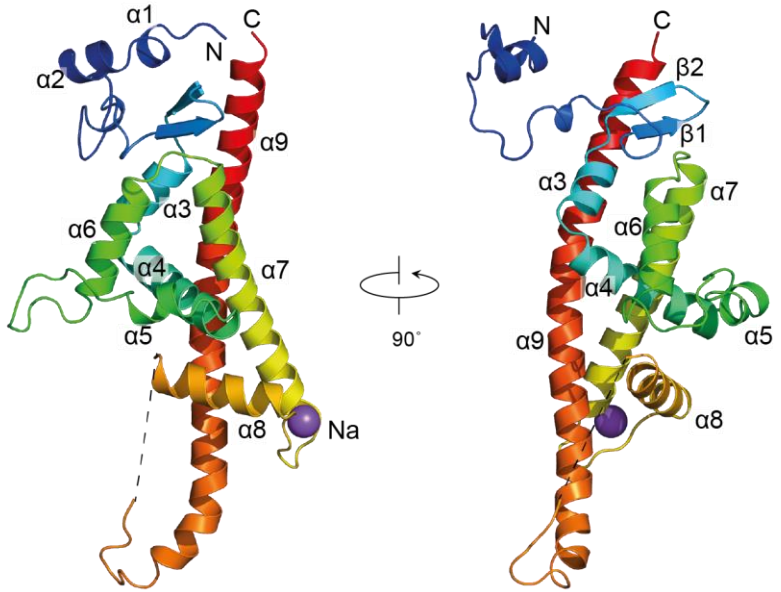
	VMXm	XFEL	I24
Number of crystals	14	6537	768
Resolution	74.2–1.45 (1.48-1.45)	25.0-1.46 (1.485-1.46)	74.16-2.20 (2.26-2.20)
Unique Reflection	33145(1699)	34369 (-)	9376(931)
Completeness (%)	97.2(100)	99.5(92.8)	99.9(100)
R_{meas} (%)	89.1(668.1)	19.0(71.4)	0.665(0.000)*
I/σI	9.2(1.1)	3.2 (-)	6.4(1.4)
CC1/2	0.986(0.302)	0.999(0.331)	--
R_{work}/R_{free} (%)	15.2/16.6	11.1/15.8	14.7/19.9

X. Ji et al. (2015). *Journal of Structural Biology*, **192**, 88–99.
H. M. Ginn et al. (2015). *Acta Cryst. D*, ***71***, 1400-1410.

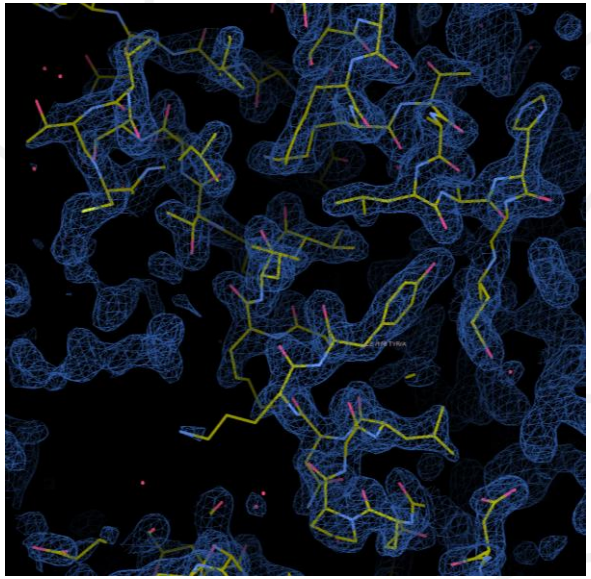
ToNV

- Polyhedra protein from a nudivirus
- Self-assembles into a dense lattice around new viral particles
- 3 x Met engineered into WT clone to allow SeMet protein
- $P3_121$ a=53.5 Å, c=105.2 Å, ɣ=120°
- Solvent content 21%
- Crystals ~ 5 – 7 µm (SeMet); 3 – 5 µm (WT)

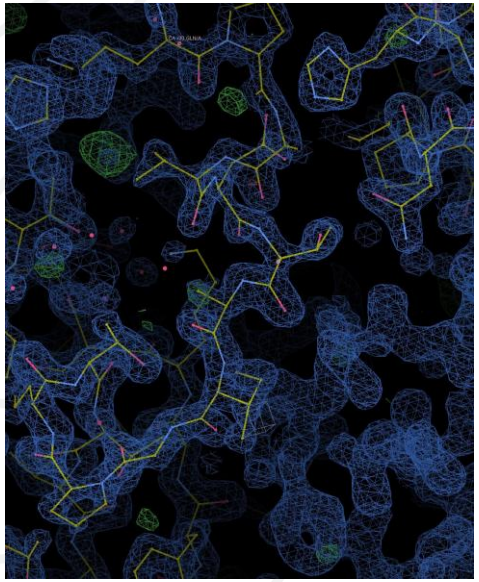




Se-Met to 1.9 Å



WT to 1.7 Å

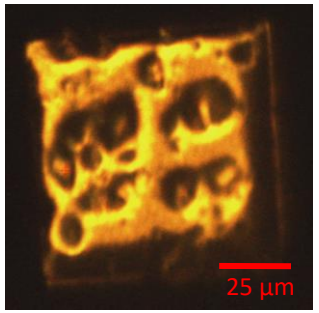


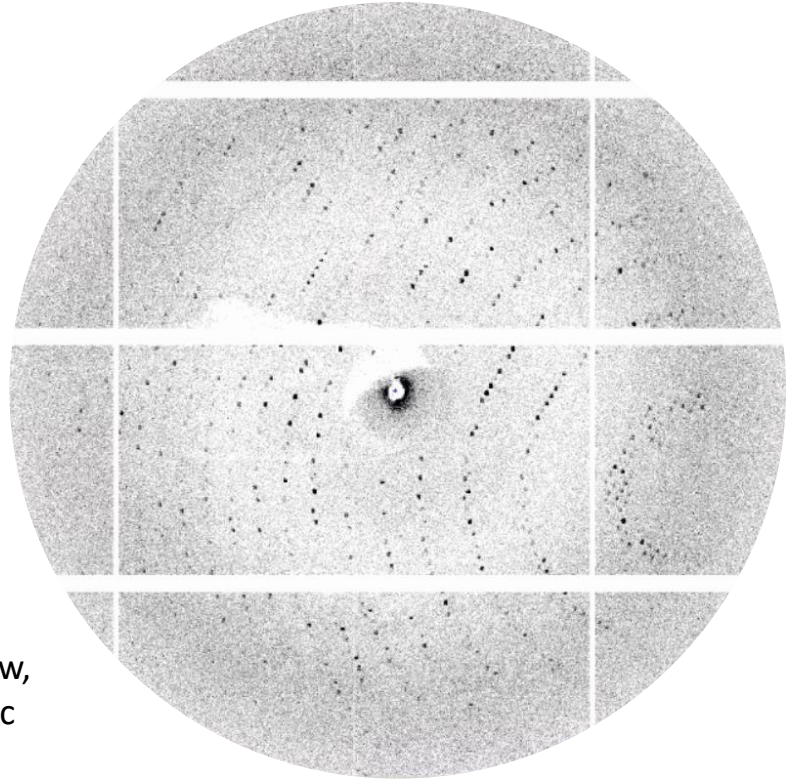
	WT	Se-Met
Detector	Eiger2 X CdTe 9M	Eiger2 X CdTe 9M
Number of crystals	16	55
Energy (keV)	19.6	12.67
Resolution (Å)	28.0-1.69 (1.75-1.69)	34.77-1.91 (1.98-1.91)
Unique Reflections	19056(1962)	13277(1129)
Completeness (%)	94.0(99.6)	93.9(79.7)
R_{pim}	0.18(0.38)	0.04(0.74)
I/σI	9.9(1.9)	17.7(0.7)
CC1/2	0.97(0.32)	0.95(0.28)
Beamsize	3.6 x 3.6 µm	3.6 x 3.6 µm

J. R. Keown *et al.* *Nat. Commun.* (2023), **14**, 4160.

Bdellovibrio lipid transporter

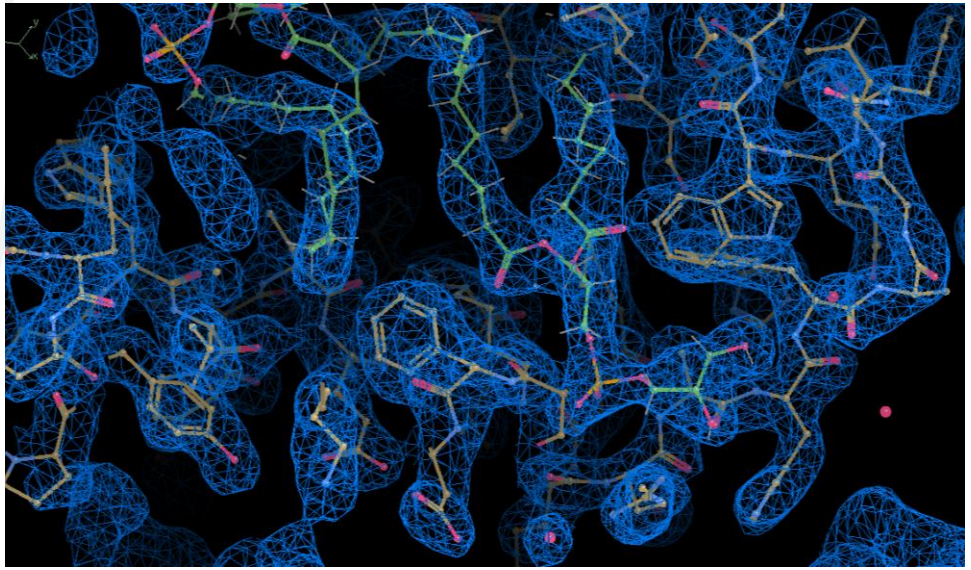
- Potential use as living antibiotic
- Limited sequence homology to other organisms
- Previous attempts to get structure from ~20 µm crystals gave ~7.5 Å resolution
- Crystallisation trials yielded crystals ~5-7 µm, suitable for VMXm
- Structure solved to 2.29 Å using AlphaFold model





No. crystals	27
Resolution (Å)	128.59 – 2.29 (2.33 – 2.29)
Observations	1389654 (70686)
Unique reflections	44985 (2236)
Multiplicity	30.9 (31.6)
Completeness (%)	100 (100)
Mean I/σ(I)	7.2 (0.5)
R_{merge}	0.413 (6.894)
R_{meas}	0.420 (7.007)
R_{pim}	0.075 (1.241)
CC½	0.995 (0.294)

Electron density map showing protein in yellow, and lipid in green interacting with hydrophobic residues on protein



Rwork/Rfree
0.21/0.26

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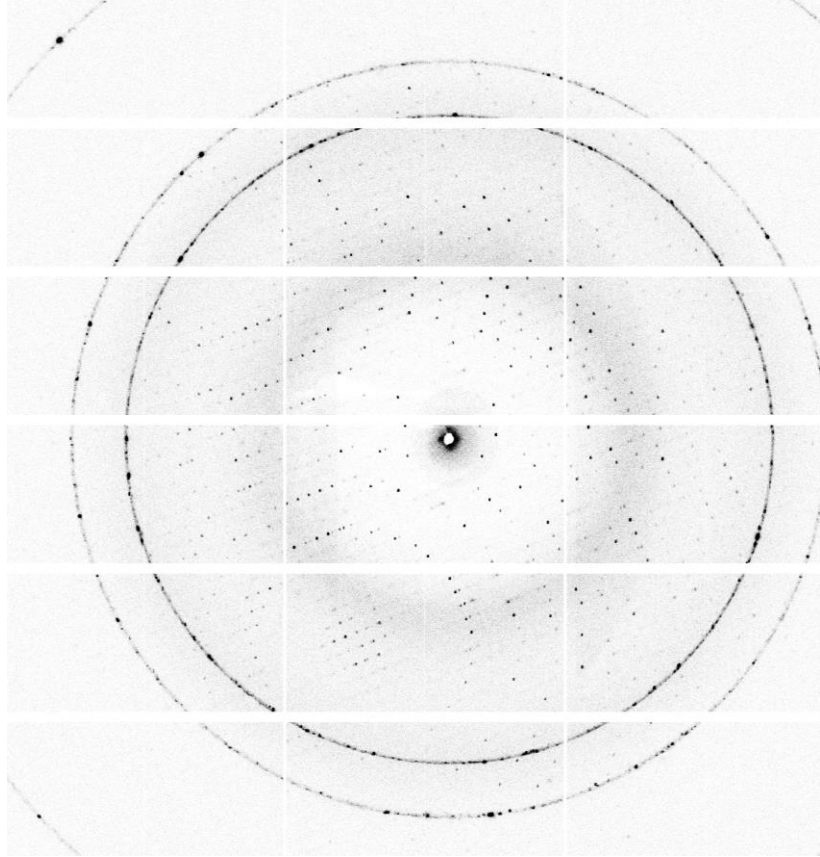
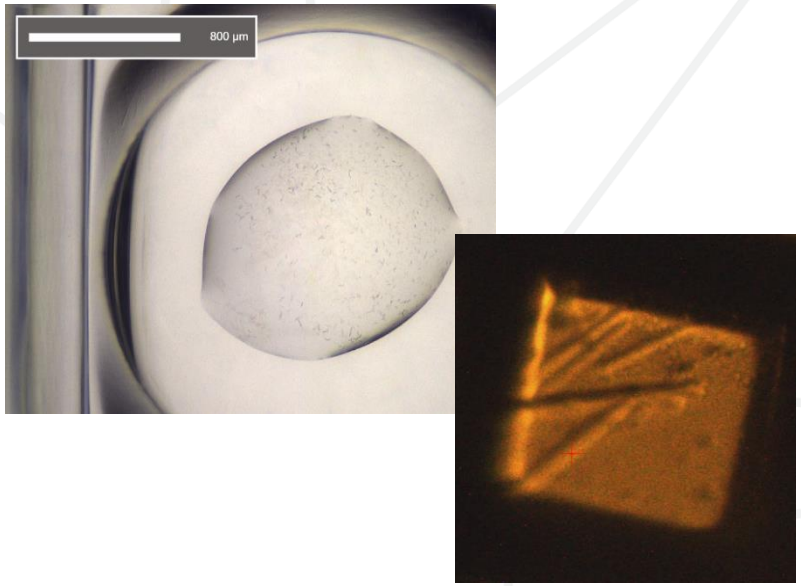
Stella Sultan (Birmingham), Andrew Lovering (Birmingham), Anna Warren (VMXm)

Mpro ligand co-crystals

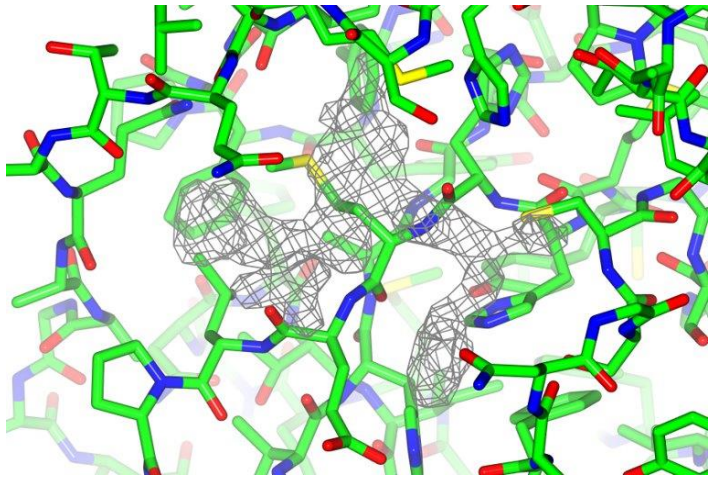
- Ligand absent from large crystal form
- Co-crystals with ligand of needle form grown
- Needle crystals (3-4 µm wide)
- 40° wedges of diffraction collected from 12 crystals
  - 3 x 3 µm beam at 21.3 keV
- dials.multiplex used to combine the 12 datasets

Resolution (Å)	22.53 – 1.81
Wavelength (Å)	0.5821
Observations	371253 (6992)
Unique Observations	23222 (1081)
Multiplicity	16.0 (6.5)
Completeness	81.34% (76.83%)
Mean I/σI	6.2 (0.6)
Rmeas	0.511(4.039)
Rpim	0.110 (1.386)
CC1/2	0.98 (0.031)





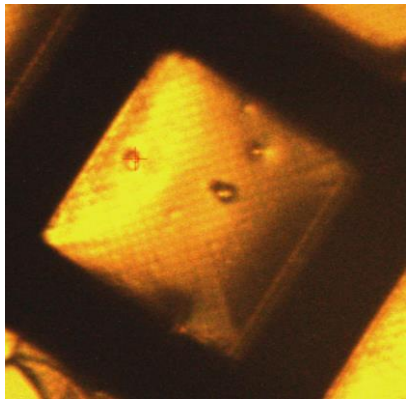
Stat	Value
Resolution	22.53 – 1.81
N. Reflections all/free	21520/1140
R/Rfree	0.19/0.24
RMS dev	
Bonds	0.01
Angles	1.459

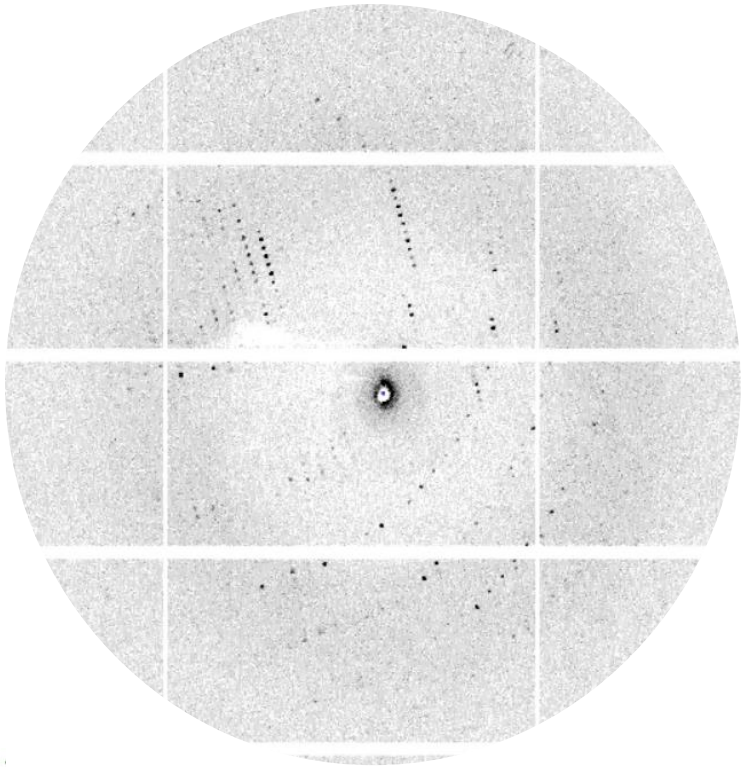


L. Brewitz *et al.* (2023) *J. Med. Chem.*, **66**, 2663-2680.

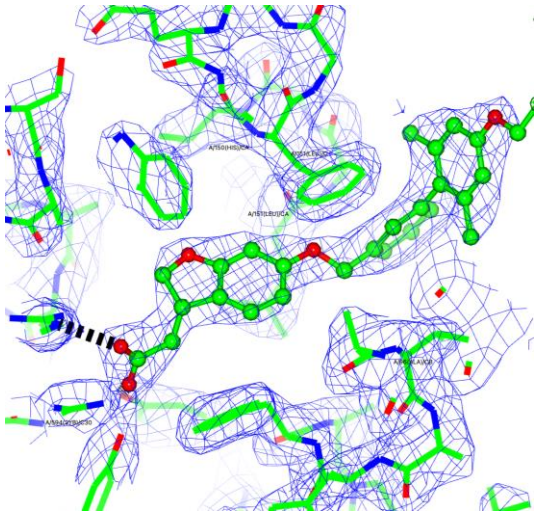
Membrane protein - FFAR1

- GPCR membrane protein – crystals ~5 µm
- Grown in classic glass plate sandwich in LCP – highly viscous, adds significant background scatter
- Crystallisation solution including 12% MPD used to wash the bolus out from under the glass
- Applied directly to grid mounted in plunge freezer as per soluble sample





	Overall	High resolution
Resolution (Å)	48.94 - 2.27	2.351 - 2.27
Observations	297894	30317
Unique reflections	30698	3052
Multiplicity	9.7	9.9
Completeness	98.17%	88.27%
Mean I/σ(I)	6.3	0.7
Rmerge	0.285	3.815
Rmeas	0.301	4.029
Rpim	0.095	1.255
CC½	0.997	0.292



R_{work}/R_{free} = 0.20/0.24

MPL Membrane Protein Laboratory

diamond

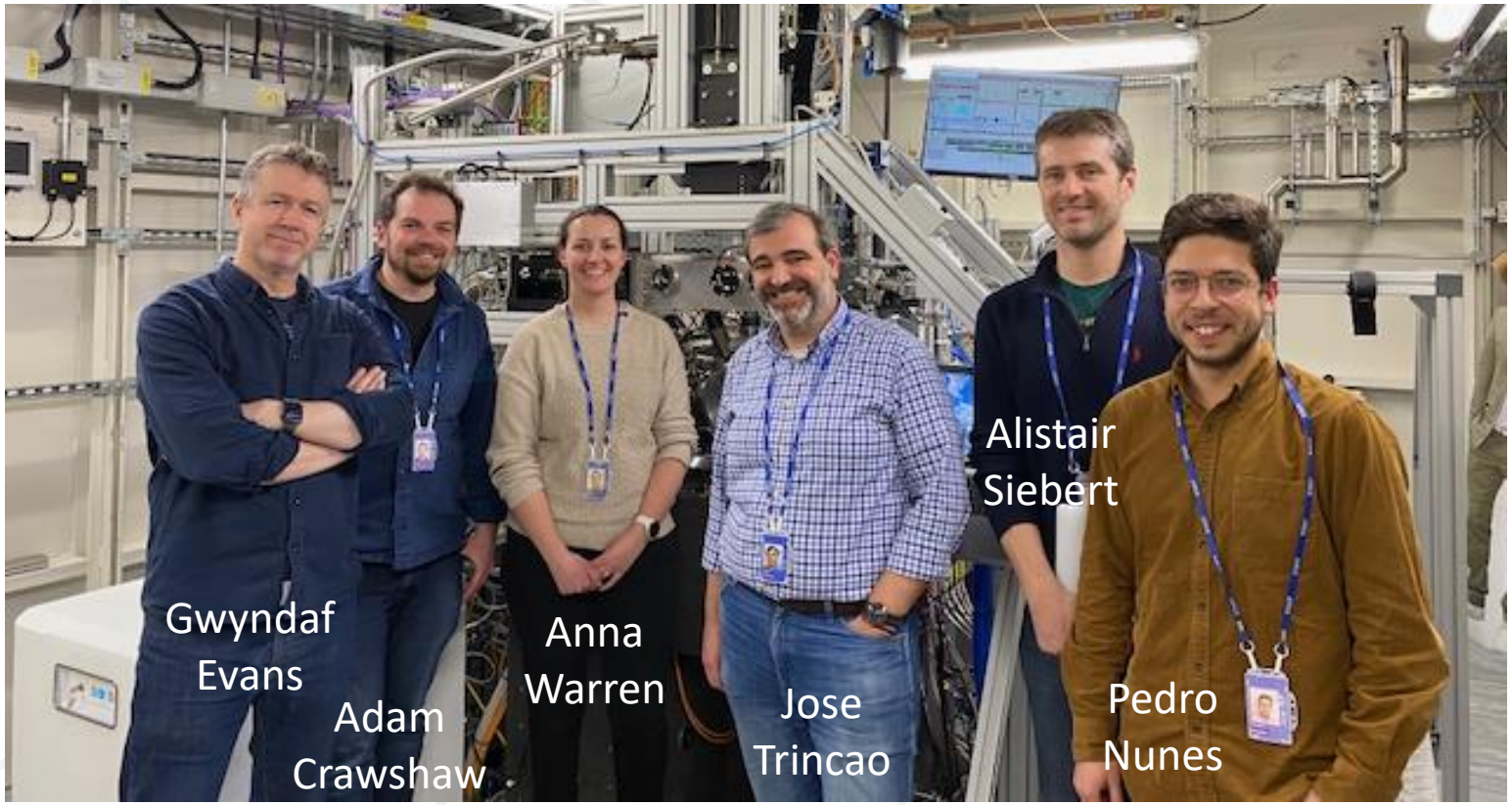
Andrew Quigley, Rebecca Parr (MPL) and Adam Crawshaw (VMXm)

Current Status

User programme:

- Currently commissioning with users – similar to rapid access route
  - Requires a brief scientific justification
- Sample size envelope is broad – not just micron sized
  - We can prepare grids for the beamline with crystals up to ~20 µm
- Send VMXm staff pictures of sample! VMXm@diamond.ac.uk
  - This will help us advise on suitability and approach
- Drops with low numbers of crystals are OK! Do not need high concentration of crystals!
- We have observed useful interactions between microED/VMXm/small molecule teams at Diamond in deciding most appropriate instrument for the problem

VMXm Team



Support team:

Graham Duller

Richard Littlewood

Andy Foster

Mark Lunnon

Contact a member of the VMXm team if interested to know more or use the beamline

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beamlines

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VMXm – A sub-micron focus macromolecular crystallography beamline at Diamond Light Source

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VMXm joins the suite of operational macromolecular crystallography beamlines at Diamond Light Source. It has been designed to optimize rotation data collections from protein crystals less than 10 µm and down to below 1 µm in size. The beamline has a fully focused beam of 0.3 × 2.3 µm (vertical × horizontal) with a tuneable energy range (6–28 keV) and high flux (1.6×10^{12} photons s^{-1} at 12.5 keV). The crystals are housed within a vacuum chamber to minimize background scatter from air. Crystals are plunge-cooled on cryo-electron microscopy grids, allowing much of the liquid surrounding the crystals to be removed. These factors improve the signal-to-noise during data collection and the lifetime of the microcrystals can be prolonged by exploiting photoelectron escape. A novel *in vacuo* sample environment has been designed which also houses a scanning electron microscope to aid with sample visualization. This combination of features at VMXm allows measurements at the physical limits of X-ray crystallography on biomacromolecules to be explored and exploited.