

VMXm – getting the most from micron sized crystals

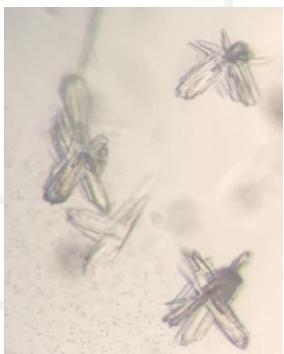
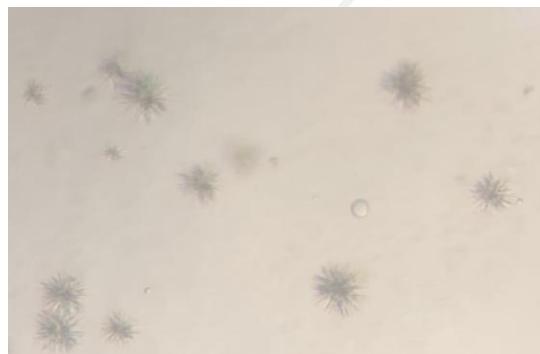
Anna Warren, Senior Beamline Scientist, VMXm

anna.warren@diamond.ac.uk



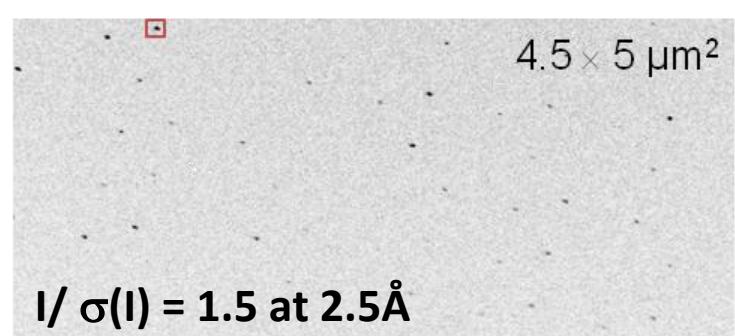
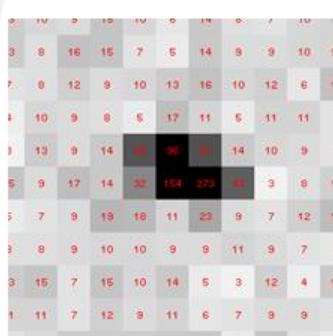
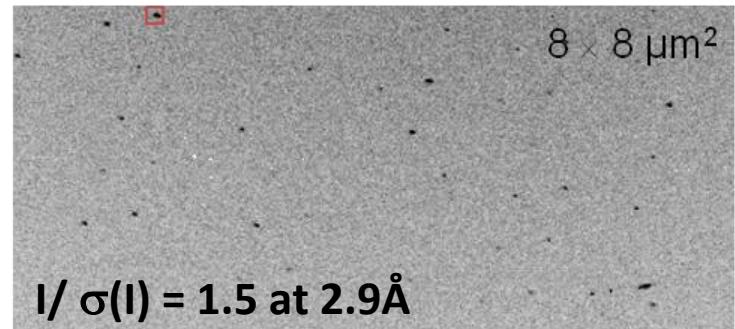
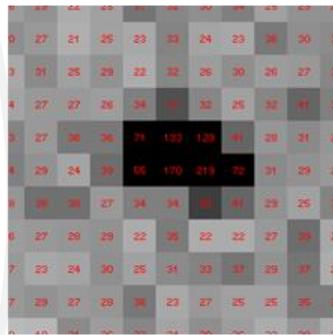
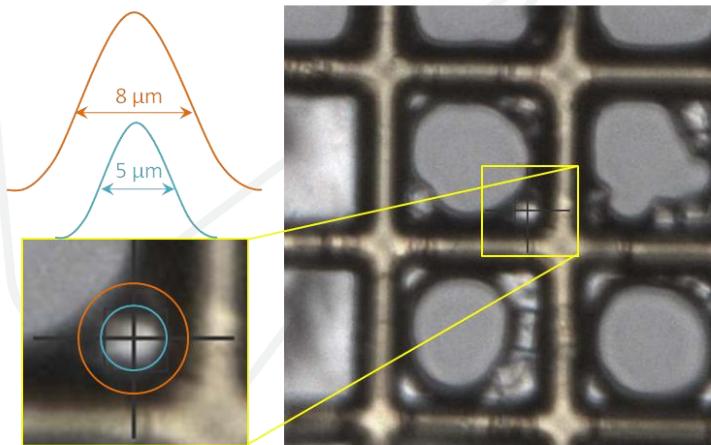
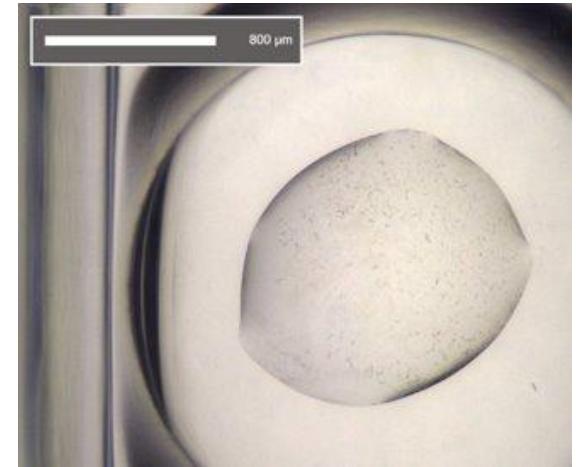
Introduction

- 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 with these challenging targets
- However, some complex proteins are difficult to crystallise and don't form large uniform crystals



Challenges of microcrystals ($<20 \mu\text{m}$)

- 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

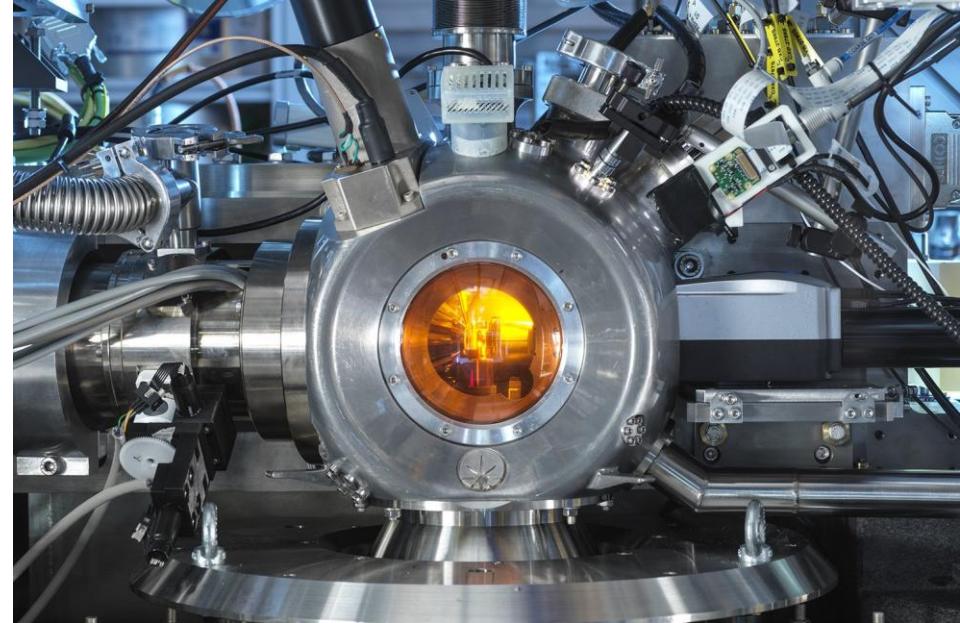


Current Limits

- 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

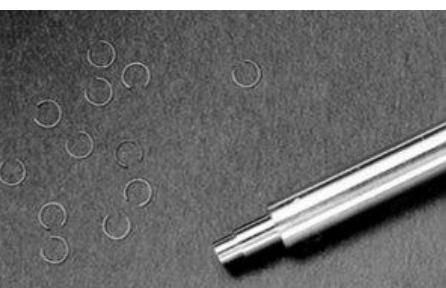
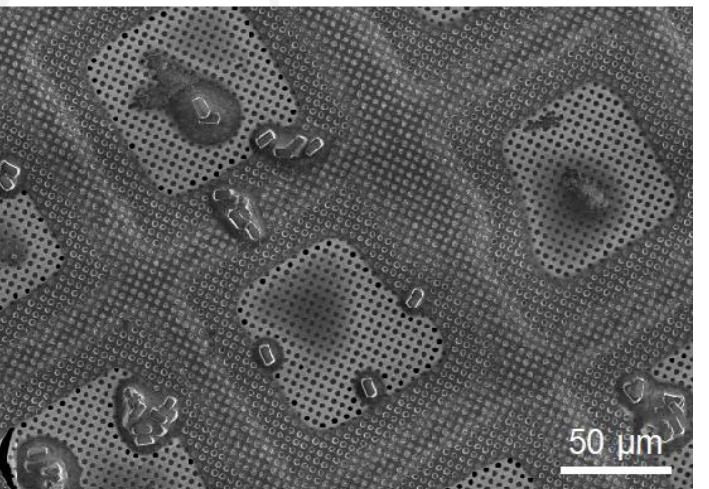
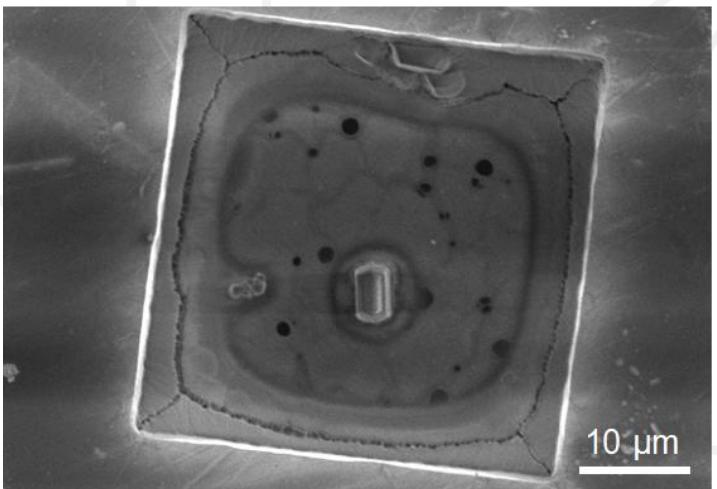
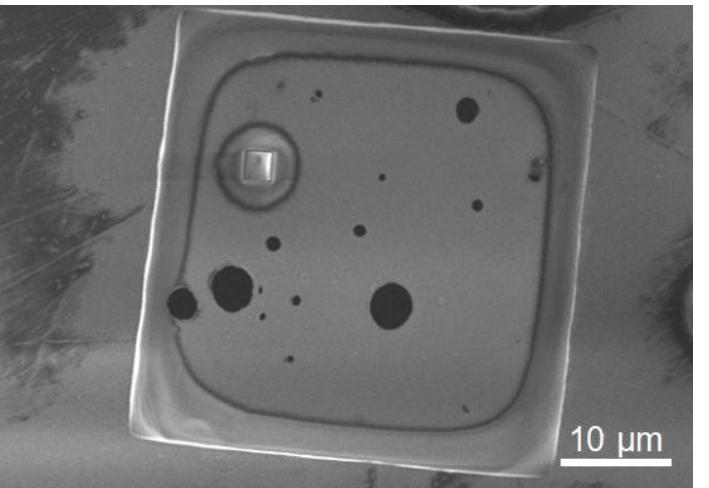
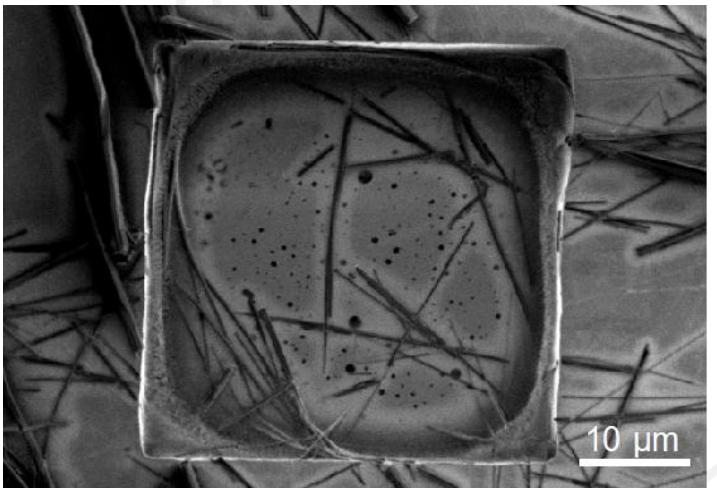
- Very low background X-ray diffraction data
 - Sample chamber under vacuum to give zero air scatter
 - Crystals mounted on cryoEM grids with minimal surrounding solvent
 - Reduce beamsize to match that of the crystal
- Rotation data from v. small crystals
 - Potential to collect rotation data on samples down to ~500 nm
 - High energy > 20 keV 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



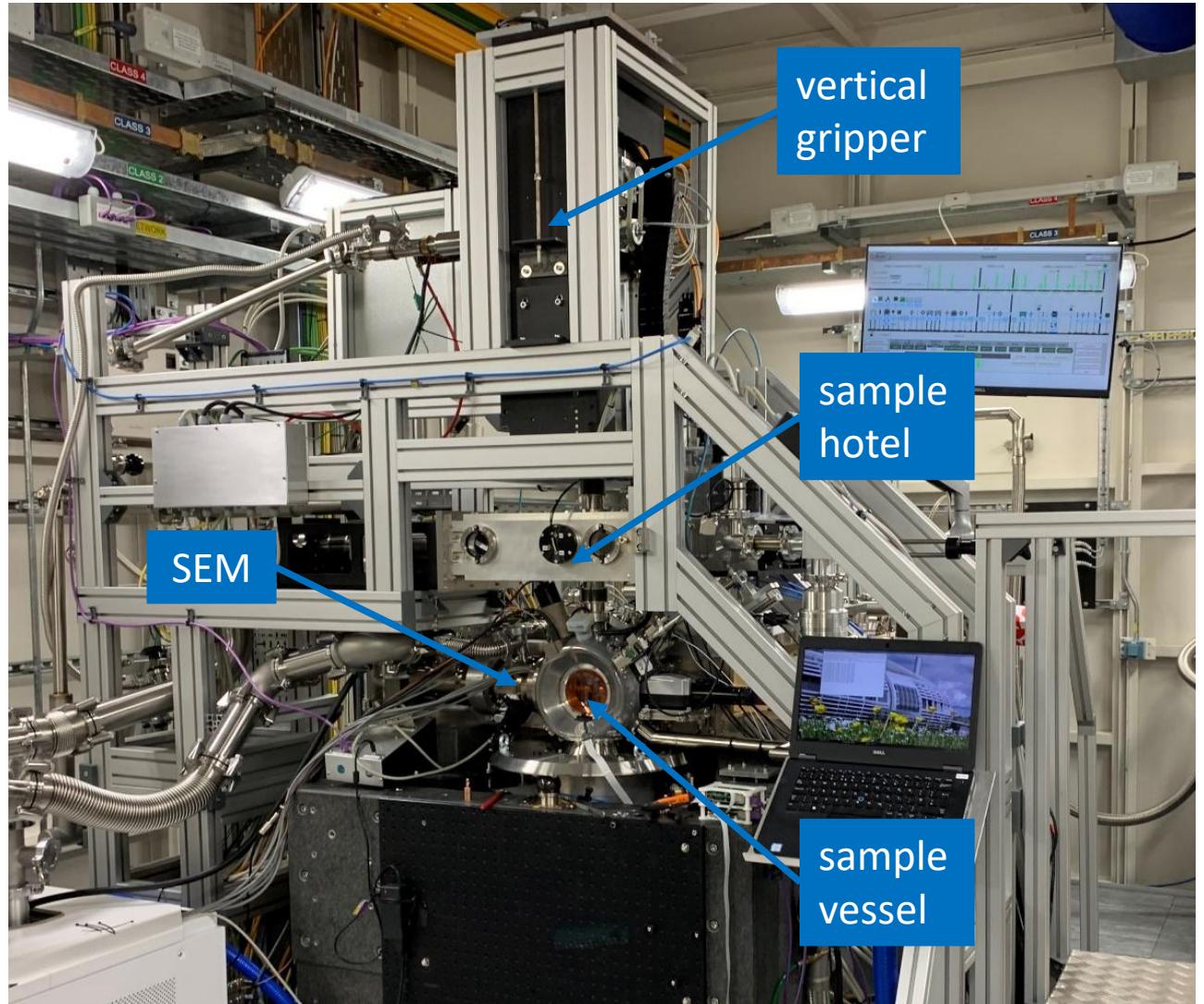
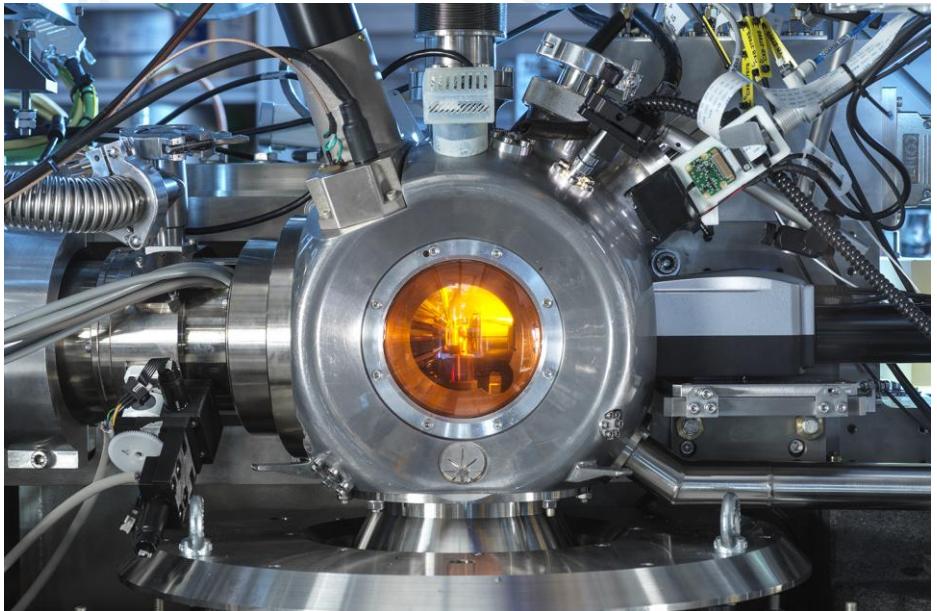
- 7 – 22 keV energy range
- 0.4 – 10 μm (v) & 1.2 – 5 μm (h)
- Full beam flux at 20.4 keV:
 - 6.4×10^{11} ph/s
- Indirectly cryocooled samples
- Eiger2 X 9M CdTe sensor detector



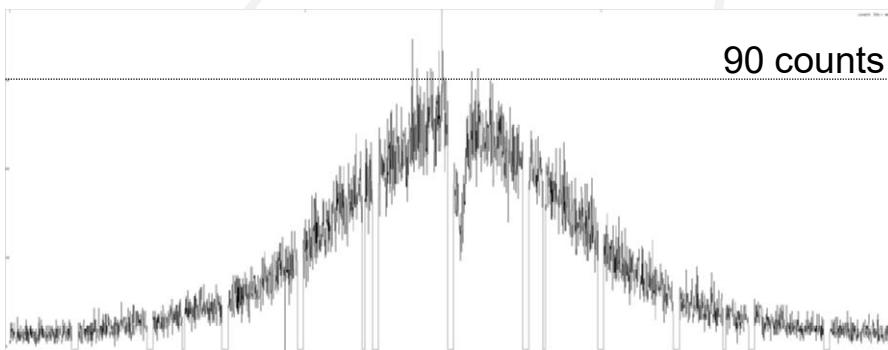
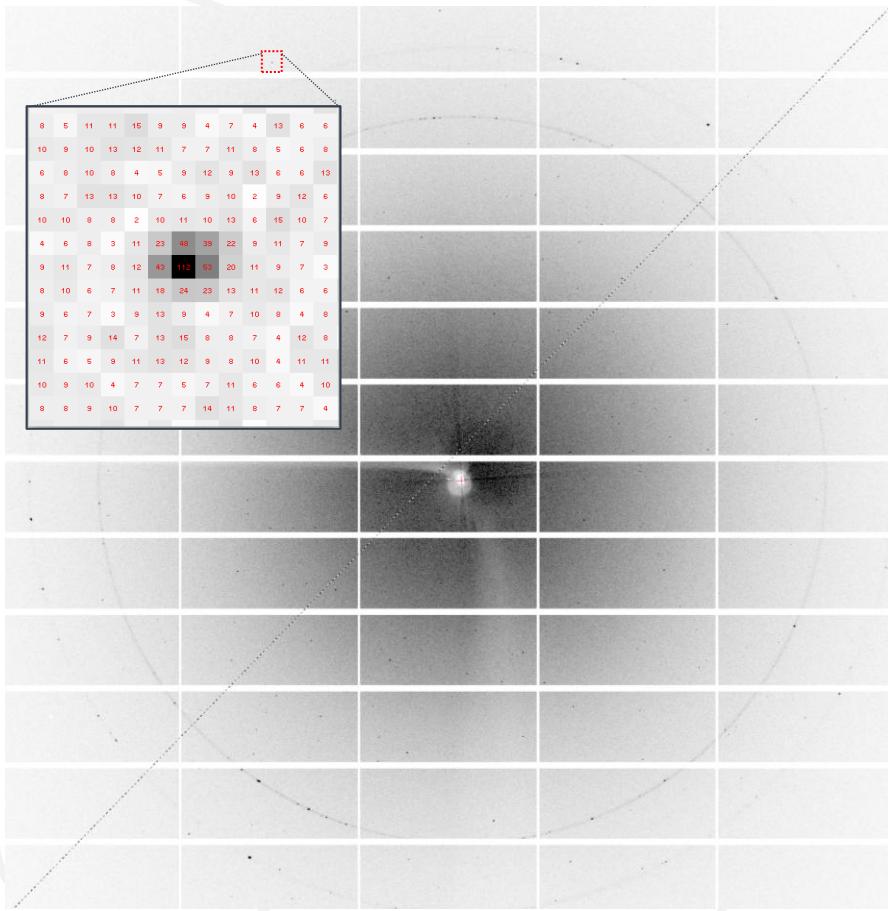
VMXm – sample preparation



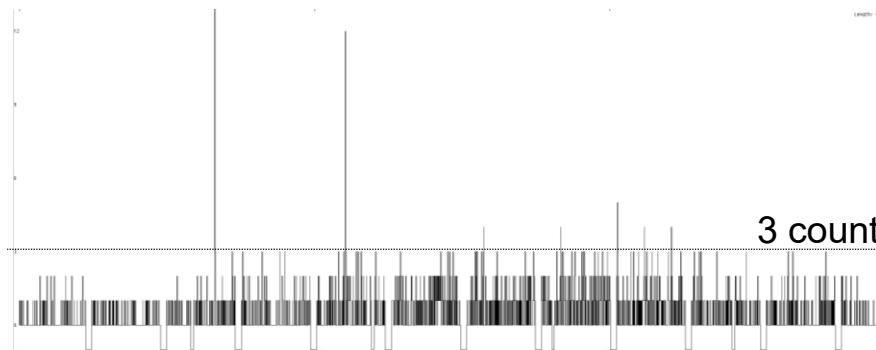
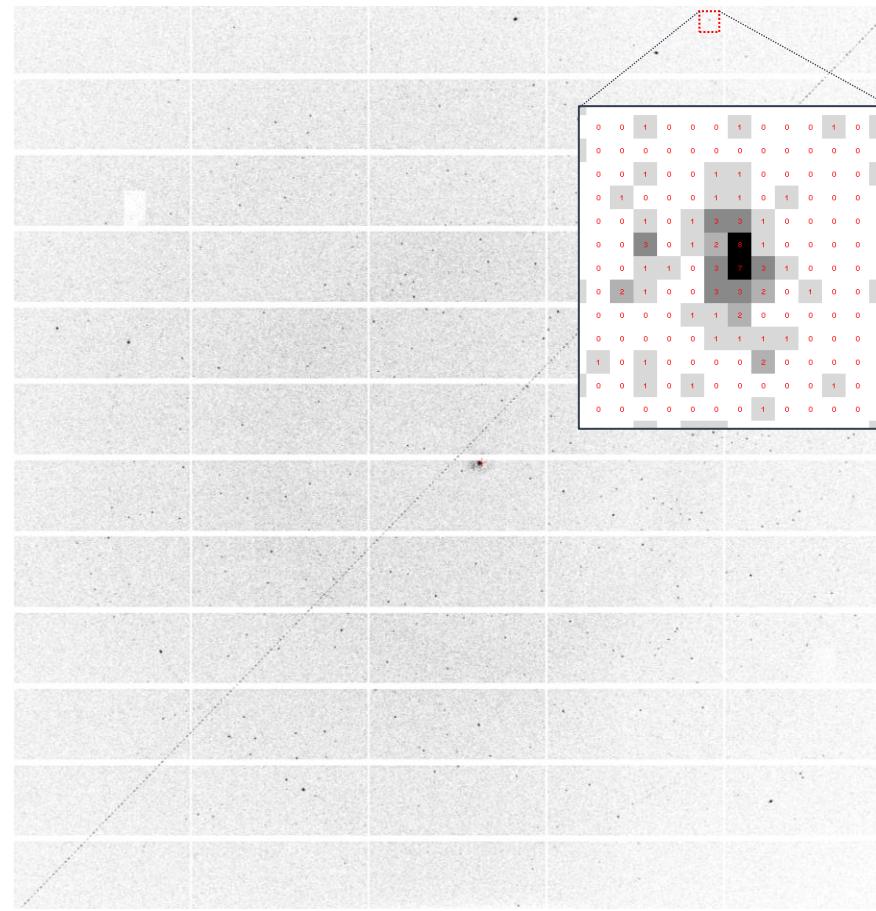
VMXm – beamline



Standard in air MX



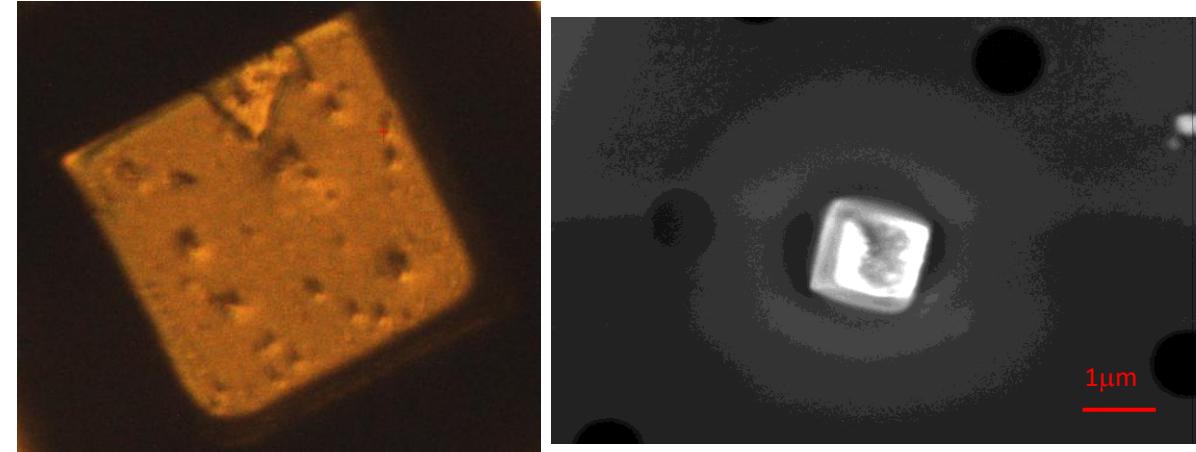
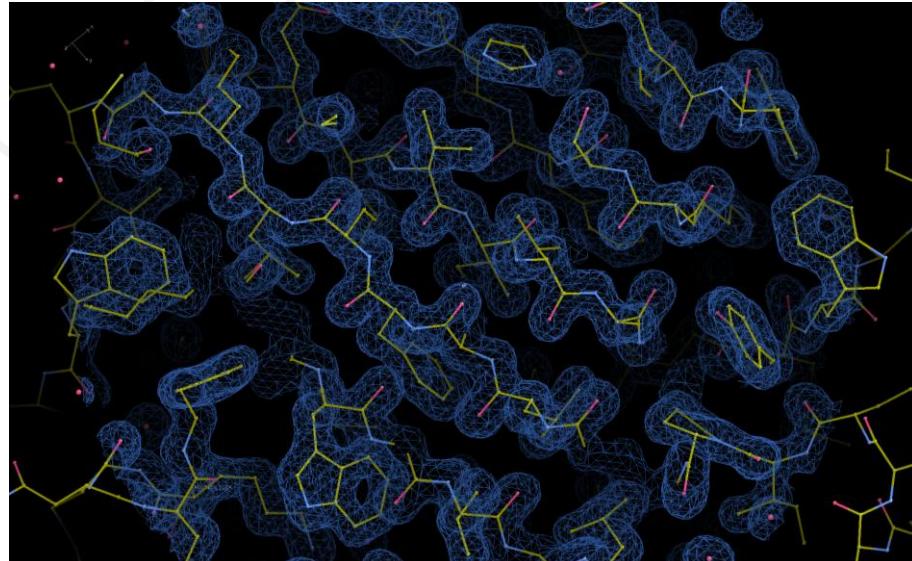
VMXm *in vacuo*



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 $a=b=c=105\text{ \AA}$
- Average crystal size $1.2\text{ }\mu\text{m}$
- Data collected at 21.3keV
- Eiger2 X CdTe 9M



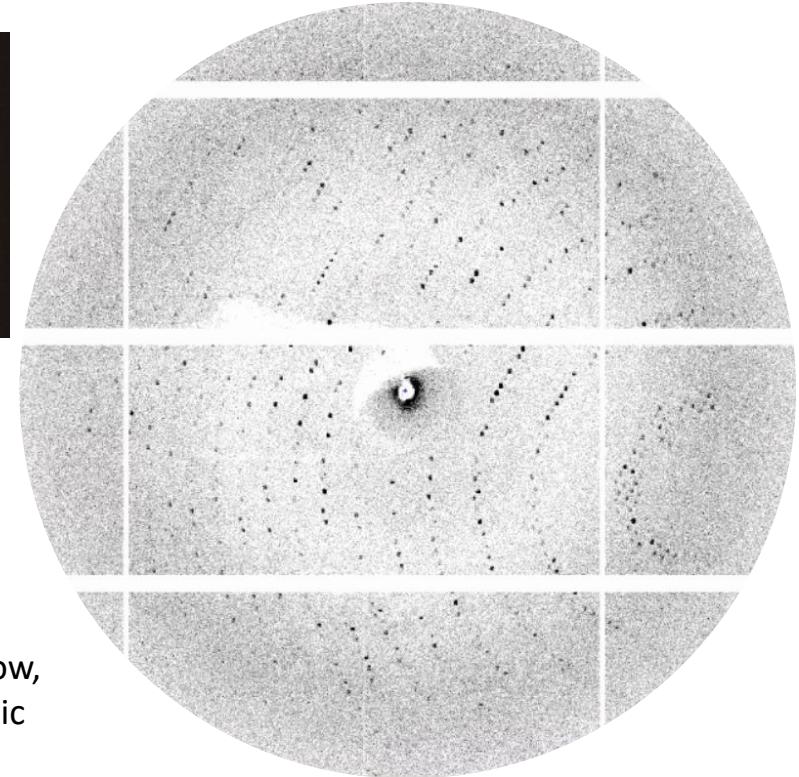
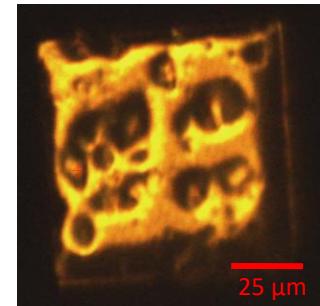
	VMXm	XFEL	I24
Number of crystals	14	6537	768
Resolution	74.2–1.45 (1.48–1.45)	25.0–1.46 (1.485–1.46)	74.16–2.20 (2.26–2.20)
Unique Reflection	33145(1699)	34369 (-)	9376(931)
Completeness (%)	97.2(100)	99.5(92.8)	99.9(100)
R_{meas} (%)	89.1(668.1)	19.0(71.4)	0.665(0.000)*
$I/\sigma I$	9.2(1.1)	3.2 (-)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



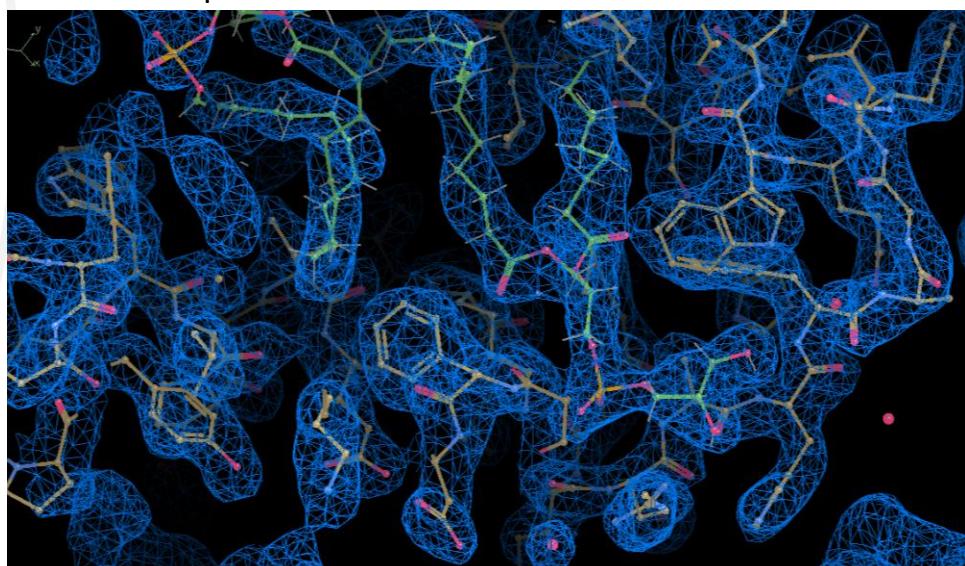
Bdellovibrio lipid transporter

- 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)
CC%	0.995 (0.294)



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

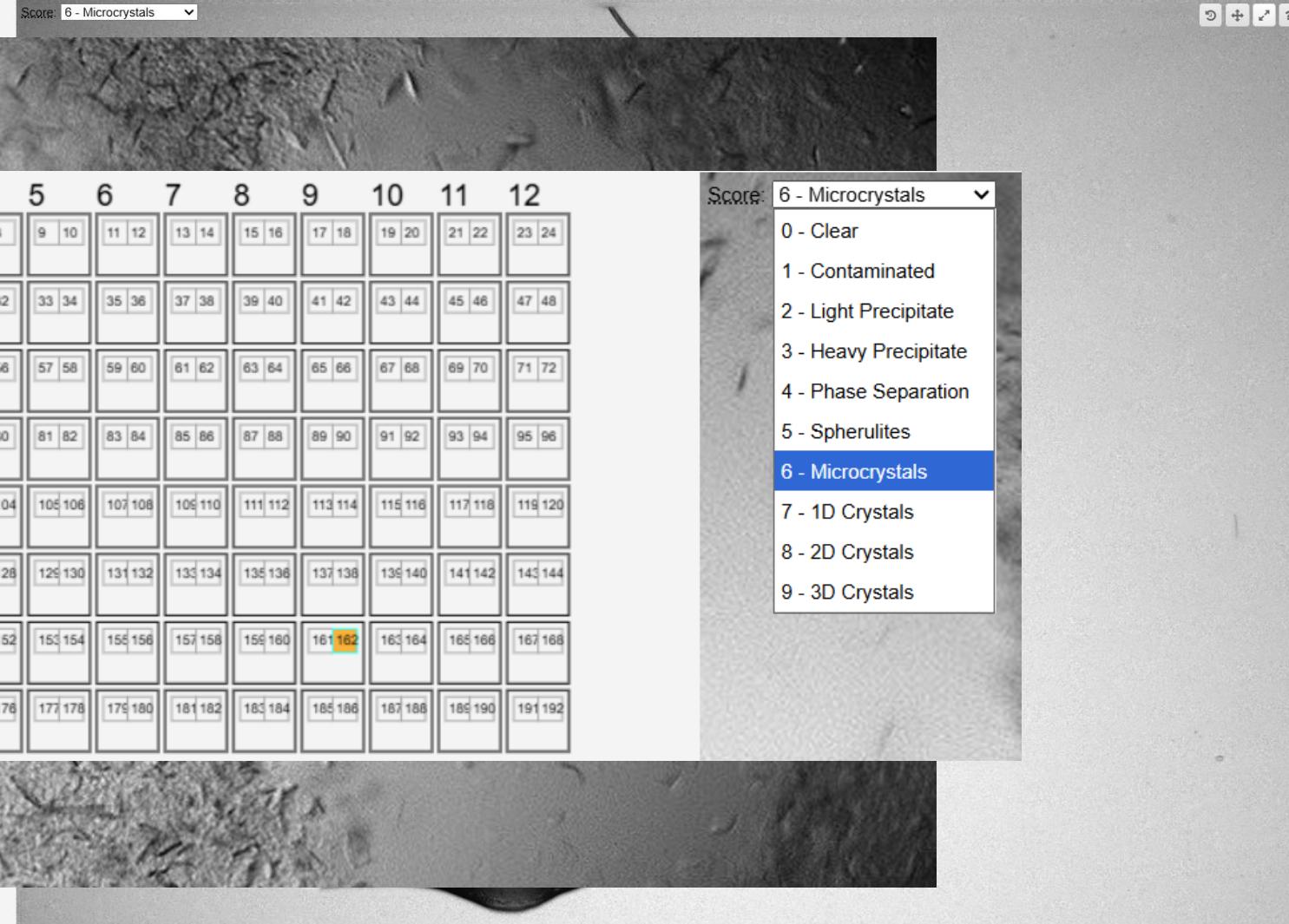


Rwork/Rfree
0.21/0.26



VMXi to VMXm pipeline

Score: 6 - Microcrystals



Score: 6 - Microcrystals

0 - Clear
1 - Contaminated
2 - Light Precipitate
3 - Heavy Precipitate
4 - Phase Separation
5 - Spherulites
6 - Microcrystals
7 - 1D Crystals
8 - 2D Crystals
9 - 3D Crystals

	1	2	3	4	5	6	7	8	9	10	11	12
A	1 2	3 4	5 6	7 8	9 10	11 12	13 14	15 16	17 18	19 20	21 22	23 24
B	25 26	27 28	29 30	31 32	33 34	35 36	37 38	39 40				
C	49 50	51 52	53 54	55 56	57 58	59 60	61 62	63 64				
D	73 74	75 76	77 78	79 80	81 82	83 84	85 86	87 88				
E	97 98	99 100	101 102	103 104	105 106	107 108						
F	121 122	123 124	125 126	127 128	129 130	131 132						
G	145 146	147 148	149 150	151 152	153 154	155 156						
H	165 170	171 172	173 174	175 176	177 178	179 180						

Display

Current Score
 Max Scores
 Data Status - Rank By
 CHIMP Auto Scores C

Inspections

17:58 13-11-2025 (+0)

Movie Gap: 0.5 s

Sample

Protein HOM18

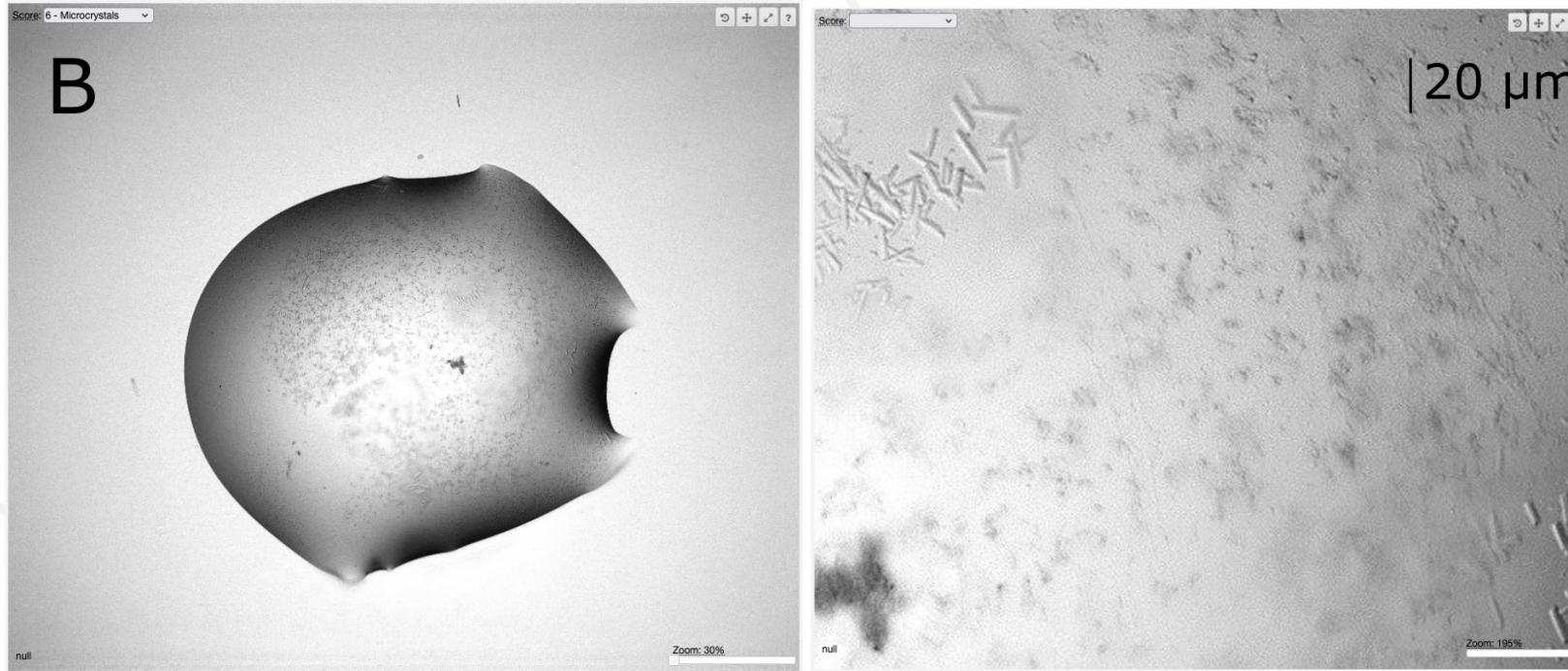
Abundance Click to edit

Components Add: No Components

Ligands Add: No Ligands

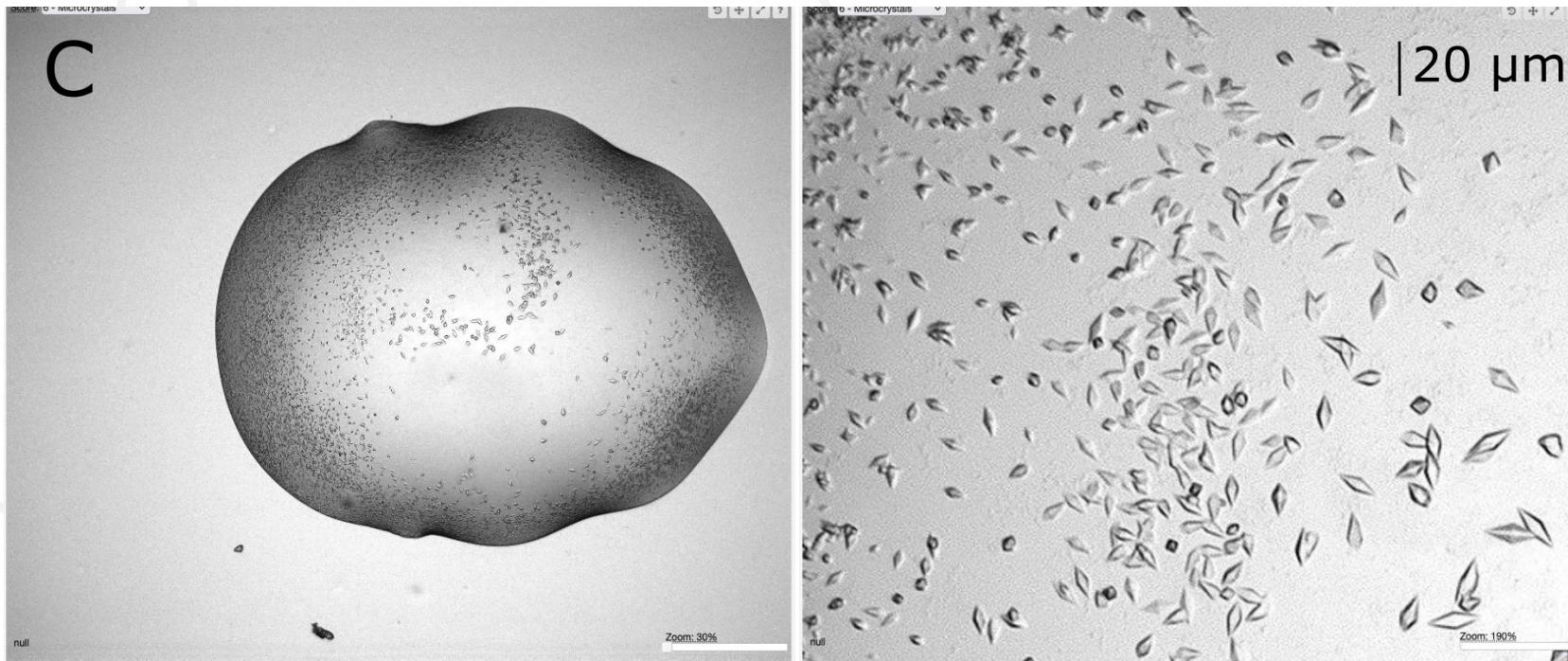
SMILES Code Click to edit

VMXi to VMXm pipeline



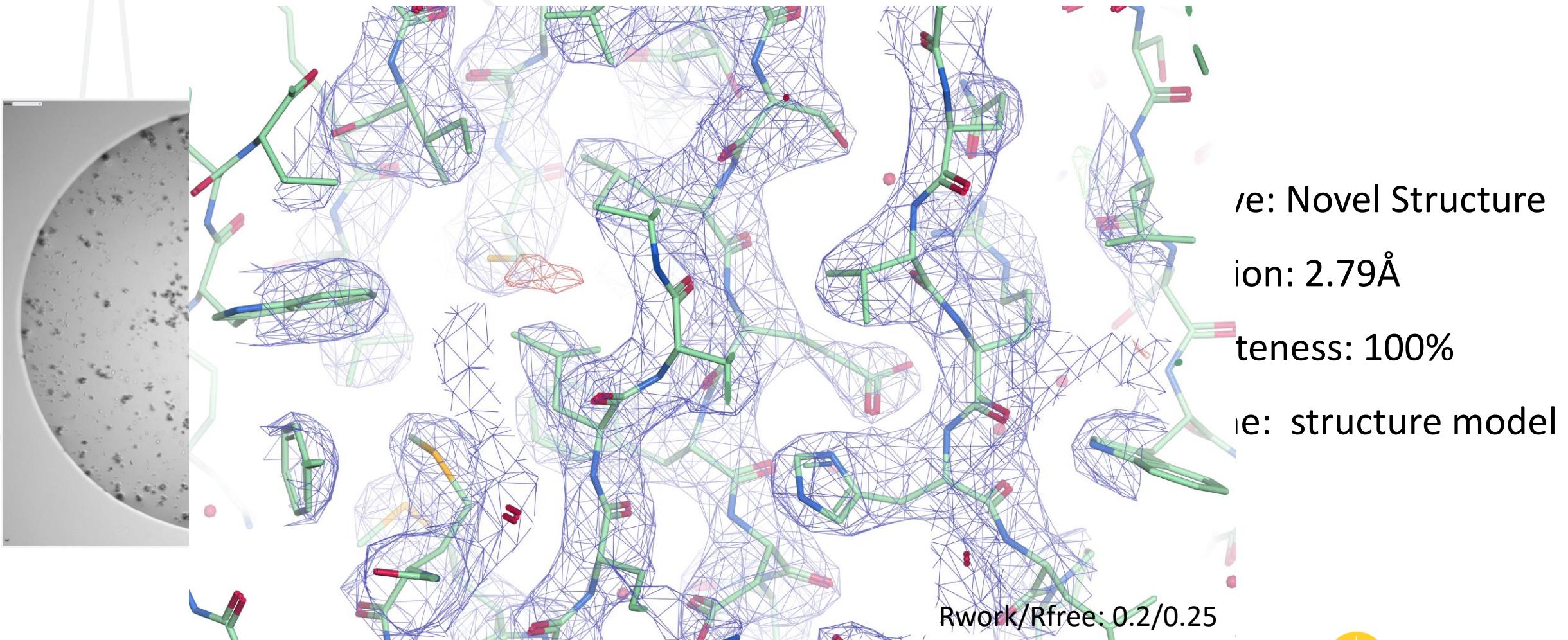
- Objective: new Structure
- Resolution: 2.1 Å
- Completeness: 61% (P1)
- Outcome: further data collection planned to complete data

VMXi to VMXm pipeline



- Objective: new structure
- Resolution: $\sim 8 \text{ \AA}$
- Completeness: --
- Outcome: optimising

VMXi to VMXm pipeline



Current Status

- Sample size envelope is broad – not just micron sized
- Send VMXm staff pictures of sample! VMXm@diamond.ac.uk
- Drops with low numbers of crystals are OK!
- Sample triaging – movement of samples to most appropriate instrument – VMXi/microfocus Xray/microED

User programme:

- Currently under general user call (AP38 - October 2025 – March 2026)
 - If you're already in a BAG we can schedule through here
 - Still open for Rapid Access enquires through this time

Acknowledgements

VMXm

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Jose Trincao

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Software

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Support team

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Richard Littlewood

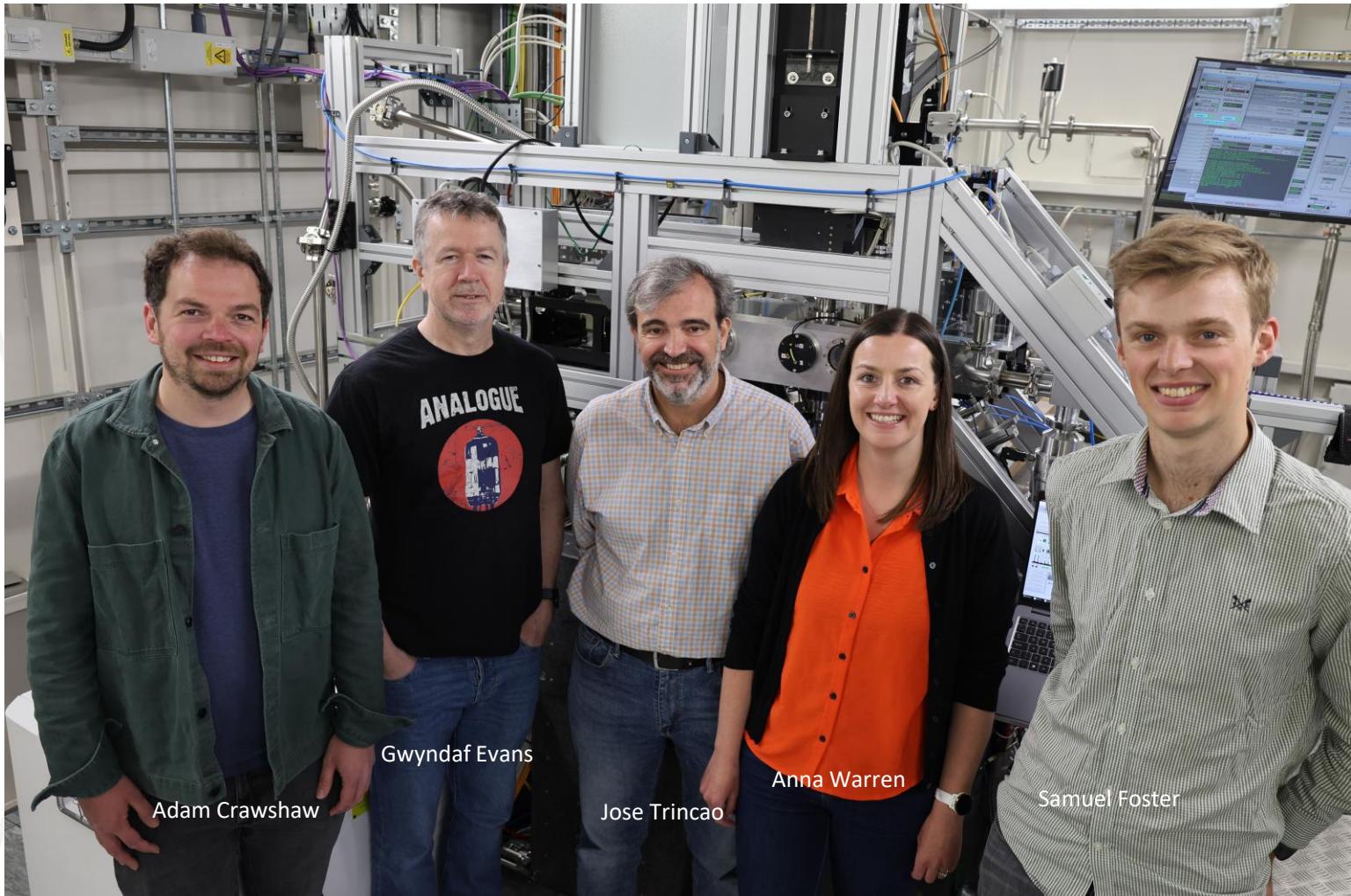
Andy Foster

Mark Lunnon

Adam Prescott

Adam Taylor

Dave Bowler



VMXm@diamond.ac.uk

 diamond