New Avenues in Room Temperature Crystallography at VMXi

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APS training 2023

Research Complex at Harwell

diamond
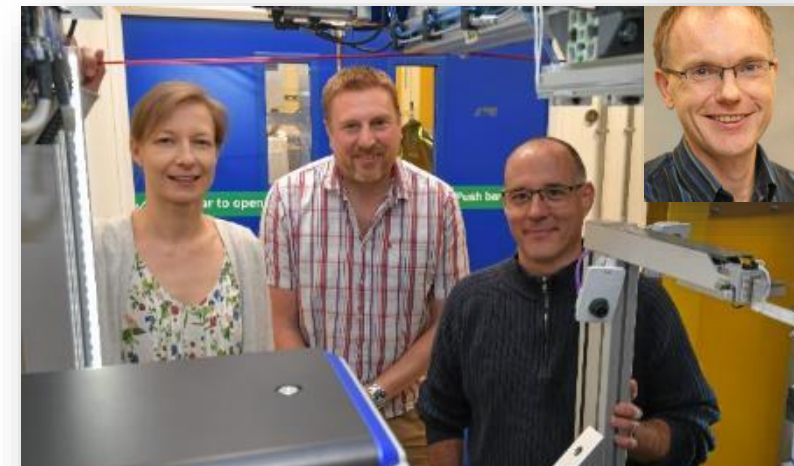
VMXi *in situ*

- VMXi is fully dedicated to room temperature experiments

- 10x10 µm^2 beam size, pink beam 5x10^{13} ph/sec, 16 keV (tunable)

- Intrinsic crystal quality without handling, disturbance. Rapid feedback for optimisation

- Routine, high quality room temperature structures from multiple crystals of hundreds to 10 µm dimensions

- Highly automated remote operation including machine learning crystal finding and data processing

- Expanding remit to time resolved serial crystallography and fragment screening
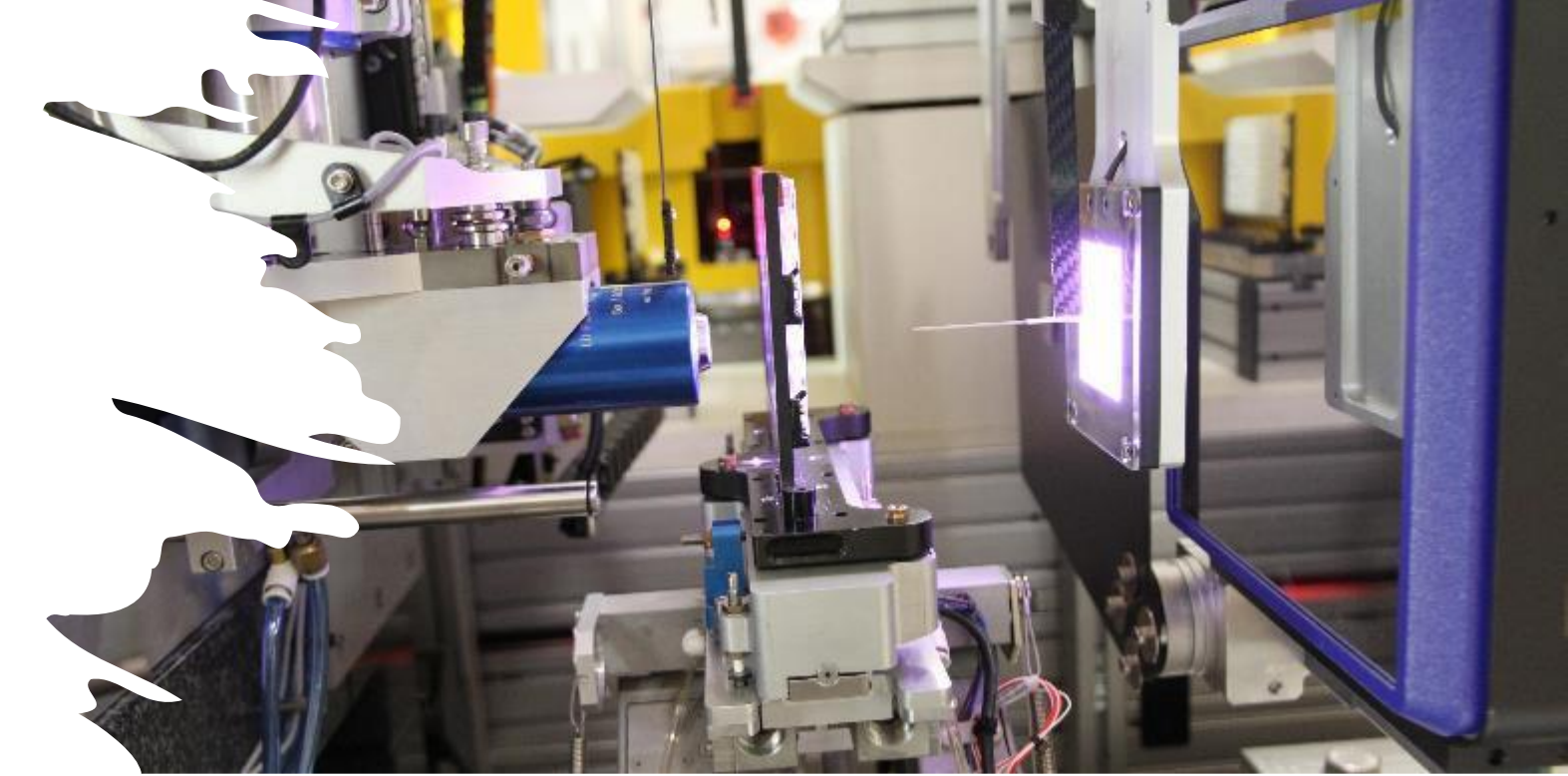
Workflow at VMXi

Crystallisation plates arrive at the beamline

↓

Beamline images crystallisation plates

↓

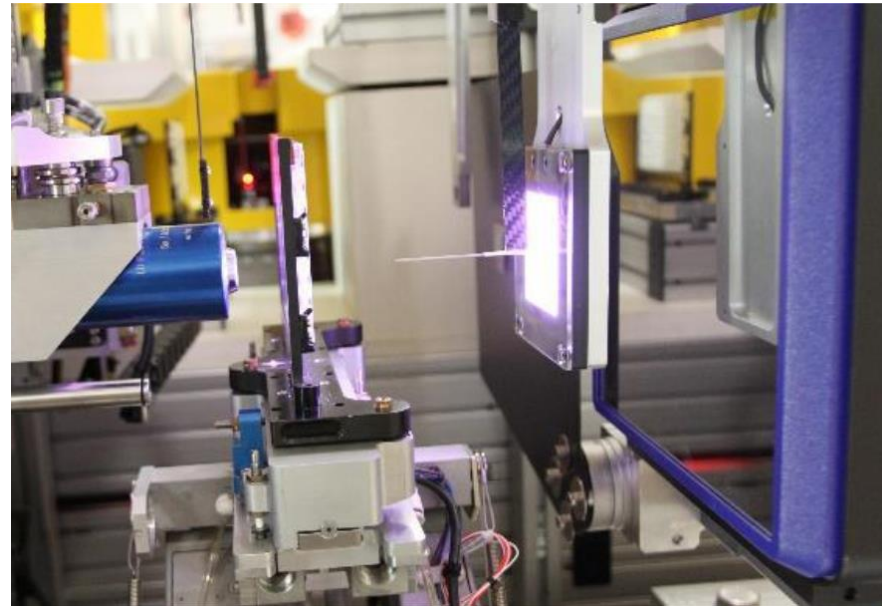User reviews results and selects point for DC

↓

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

10-60 degrees rotation

Typically 1-30 crystals

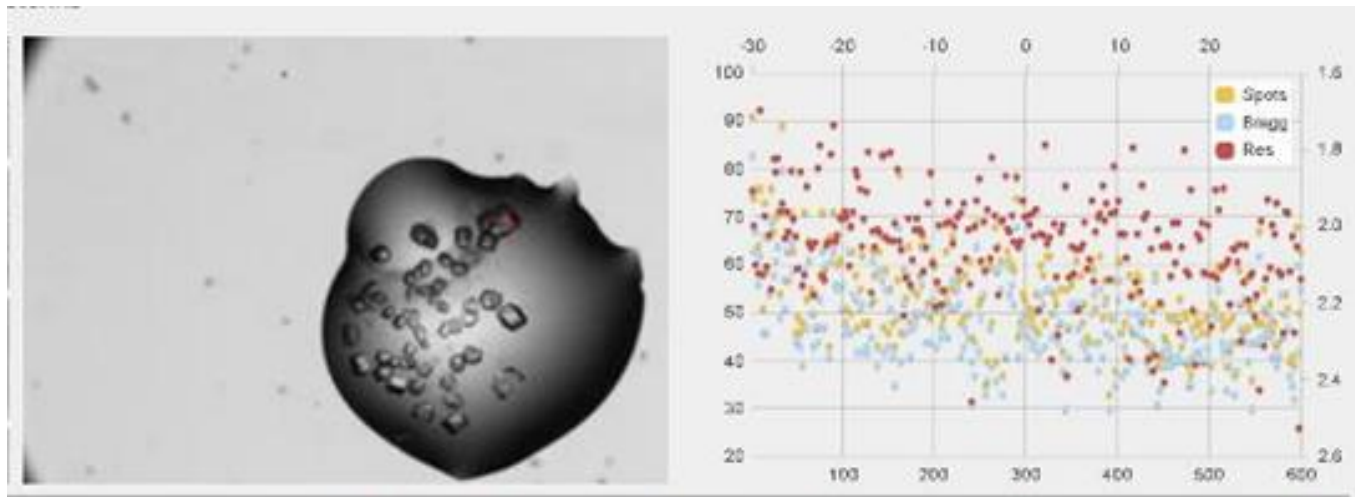*Crystals tested to 7 microns*

Plate arrival to processed data can be within 1 day

Access to Crystallisation laboratory within RCaH

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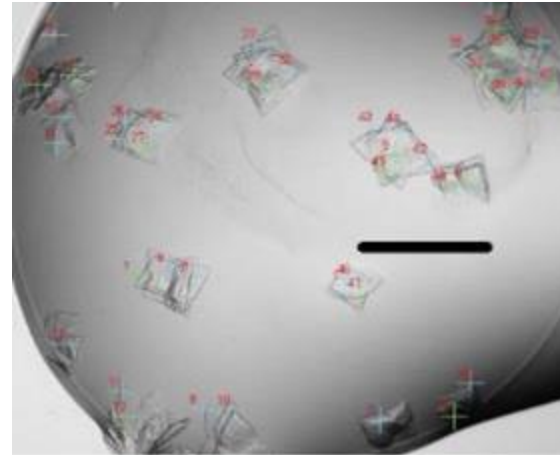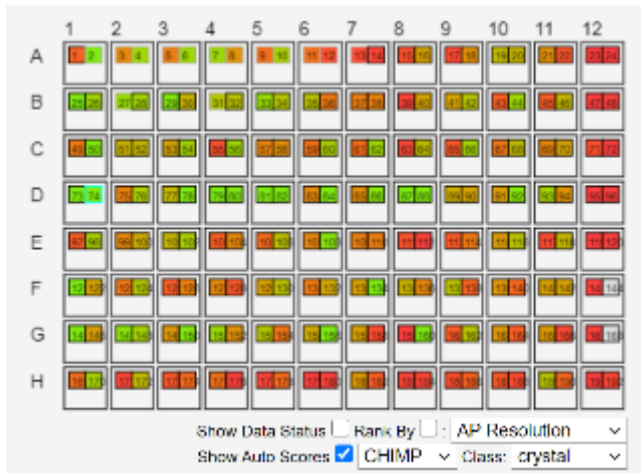- Sending protein (by prior arrangement/discussion)

- Crystallisation robotics: Mosquito and Gryphon (4^oC & 20^oC, humidity & light control, LCP)

  – Mitegen in situ plates or Greiner Crystal QuickX
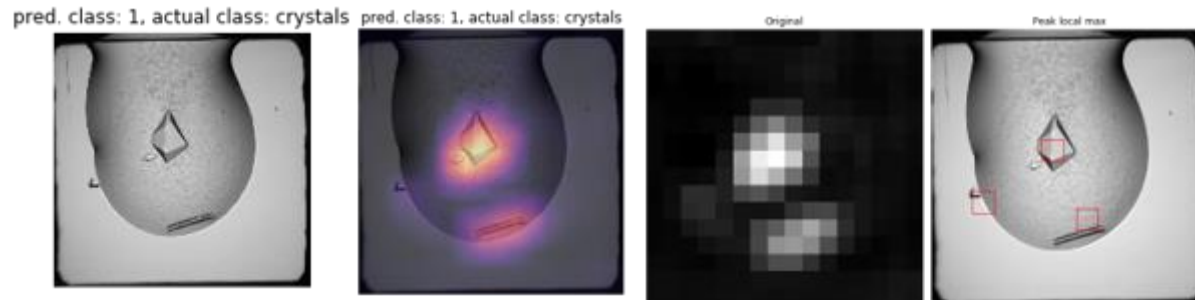
- Scorpion and Formulator for making screens

If you would like access to VMXi, please email: VMXi@diamond.ac.uk or for the facility Dr Halina Mikolajek for soluble proteins and Dr Andrew Quigley for membrane proteins.

Recent VMXi developments

- Automated crystal finding/marking

- **Crystal Hits in My Plate (CHIMP)** software (Olly King, DLS).

Automatically finds crystal centre of mass from images – eliminates tedious manual step

pred. class: 1, actual class: crystals   pred. class: 1, actual class: crystals   Original   Peak local max

- **xia2.multiplex** runs in the background and has transformed merging data at VMXi.

- Data collected in the same drop from several crystals gets automatically processed and output via iSpyB.

- We can collect smaller wedge data, which reduces radiation damage and get the best out of room temperature data collection.

- Critical for room temperature fragment screening experiments.
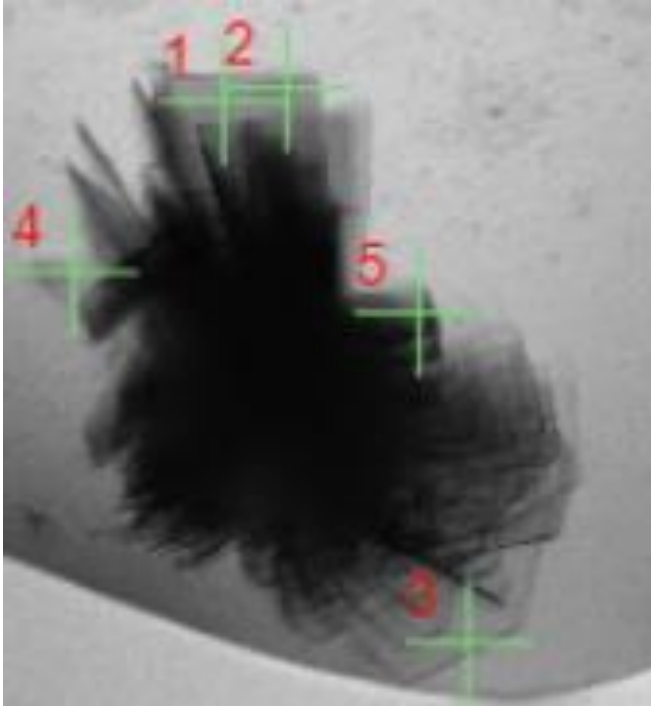
VMXi Crystal (Rogues?) Gallery

# User 1: Gas binding c-type cytochromes: Project 1
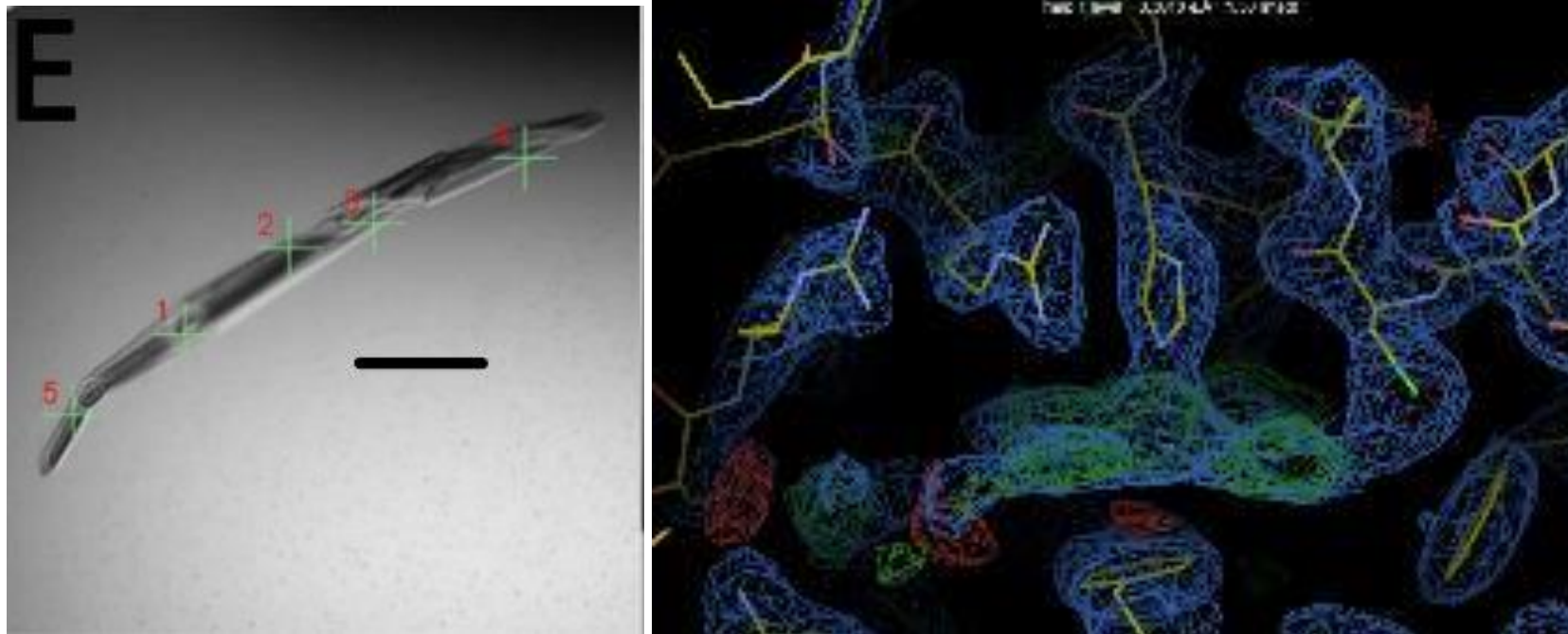
Simple case: High symmetry but few crystals
- Multiple wedges from long crystal

Analysis	No. crystals	Resolution (Å)	Space group	Mn (I/sigI)	Rmeas (inner)	Completeness (%)
Xia2 dials	1	56-2.08	P6222	7.1	0.094	99.4
Xia2 multiplex	4	56-1.88	P6222	7.9	0.113	100.0

User 1: Gas binding c-type cytochromes: Project 2

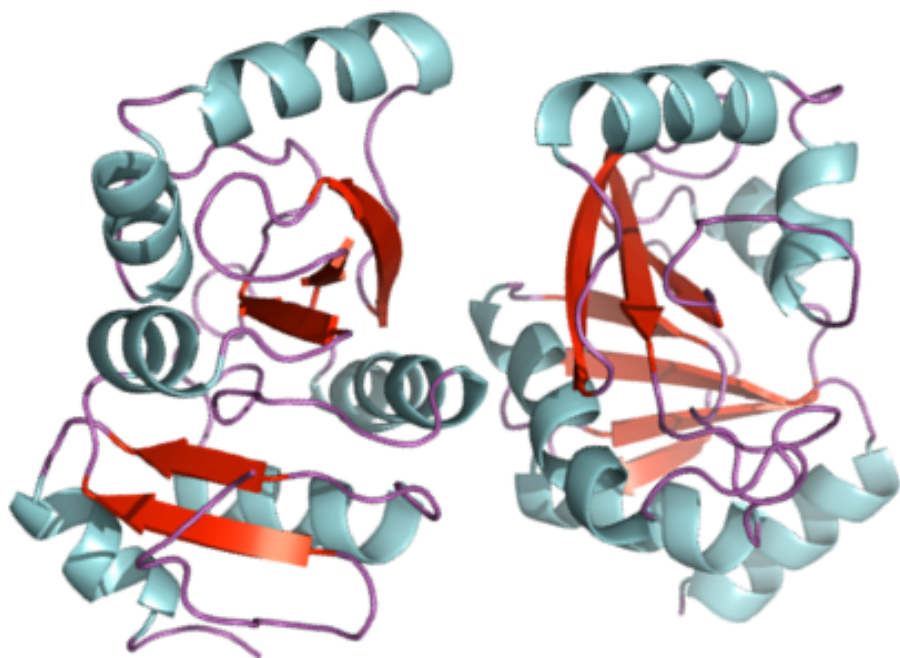C2 symmetry and challenging stacked plates crystallisation
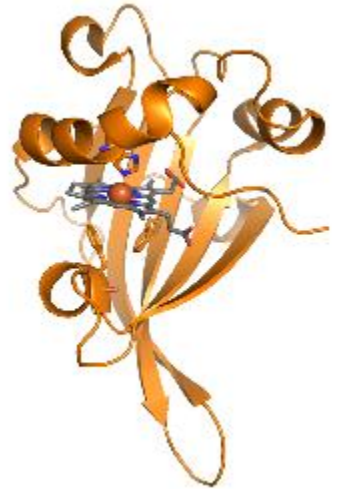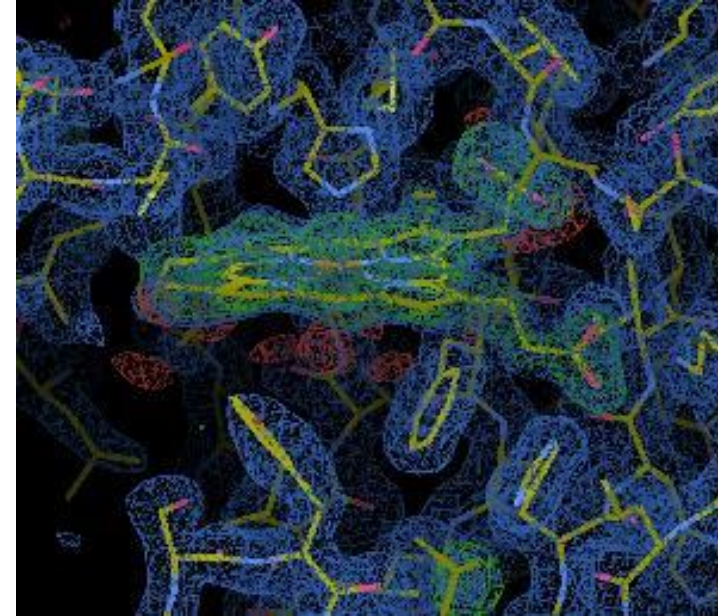
Analysis	No. crystals	Resolution (Å)	Space group	Mn (I/sigI)	Rmeas (inner)	Completeness (%)
Xia2 dials	1	27-1.7	C2	4.5	0.041	55.1
Xia2 multiplex	4	35-1.75	C2	9.9	0.061	93.9

# XChem On VMXi

**SARS-CoV-2 Macro domain**

- EU Open DMSO/cocktailing screens used
- Crystal conditions modified for more, smaller crystals

- 4 crystallisation plates in total/ 334 compounds
    - 3000 datasets collected in 14.5 hours

- Data all collected automatically at 293K *in situ* in VMXi

- Datasets automatically merged using processing pipelines. Typically 10-30 crystals yielded 1.4A resolution


Alice Poole, Oxford with XCHEM & VMXi teams
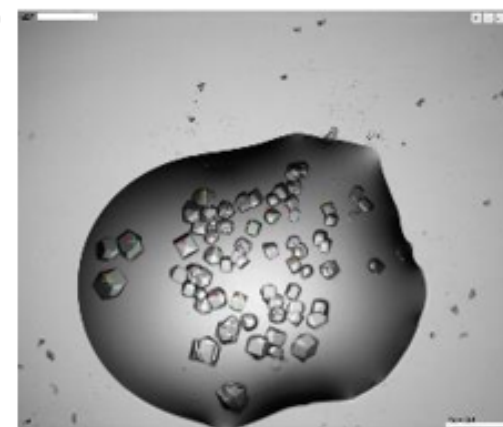
VMXi RT Fragment Screen
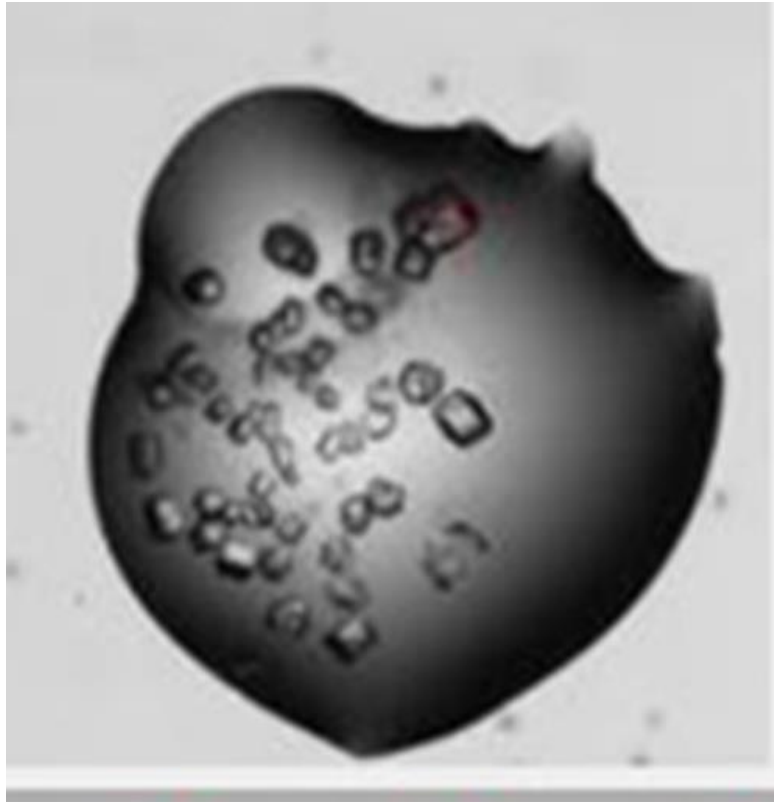
Reoptimised XCHEM condition to yield many smaller crystals

Fragment screen

Soaked and merged data from each drop/soak
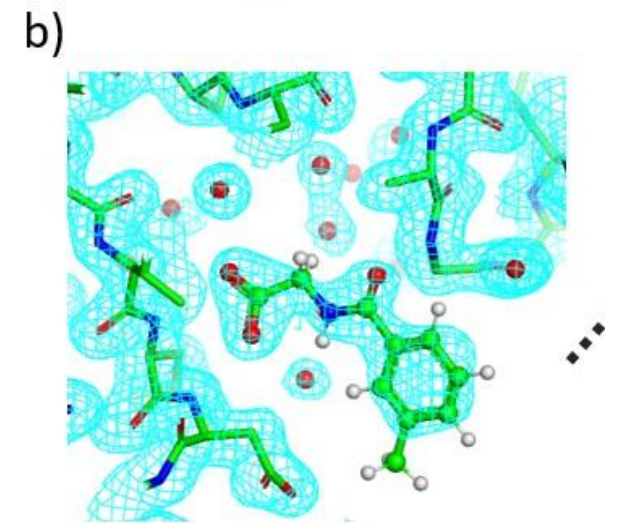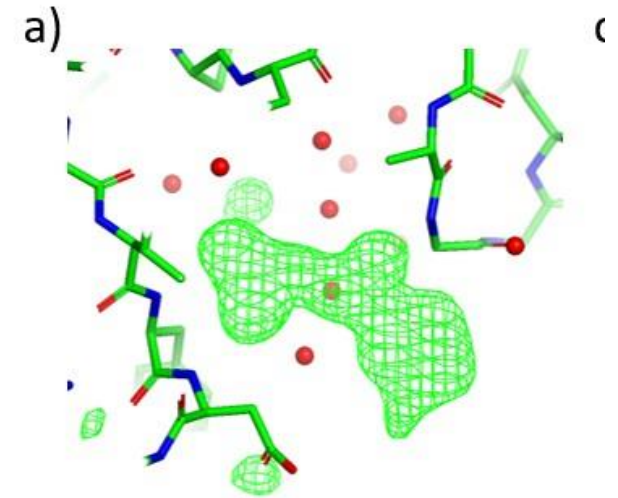
a)

b)

Alice Poole, Oxford & XCHEM

Analysis	No. crystals	Resolution (Å)	Space group	Mn (I/sigI)	Rmeas (inner)	Completeness (%)
Xia2 dials	1	44-1.4	P41	20.1	0.025	53.1
Xia2 multiplex	24	88-1.5	P41	31.2	0.048	100.0

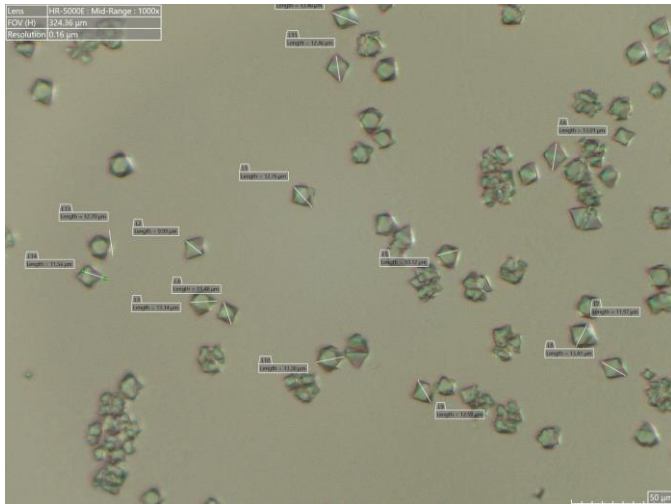# Serial crystallography project at VMXi – tape drive
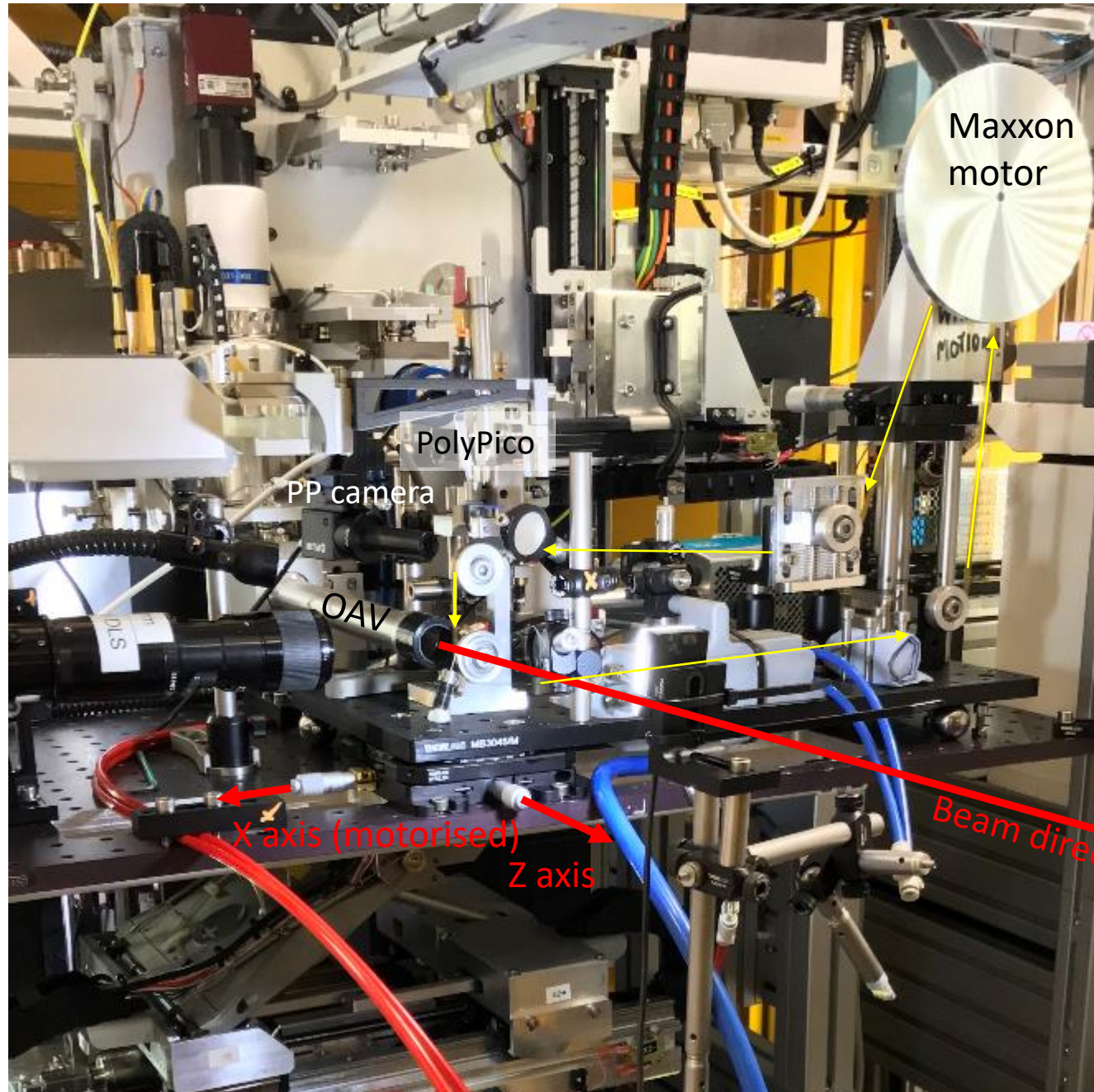
- Droplets on demand onto tape drive.
- PolyPico addition of ligands for mixing
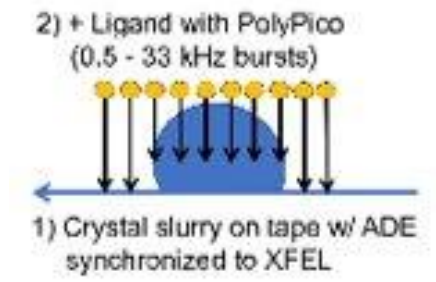
Reaction initiation by mixing or light. Microsecond to second time regimes Links to XES
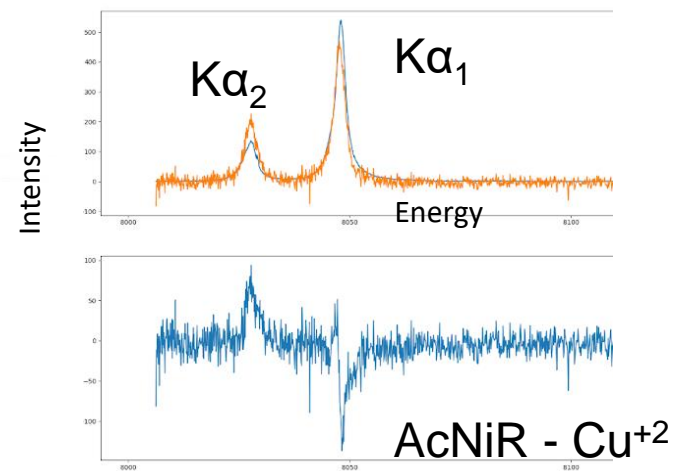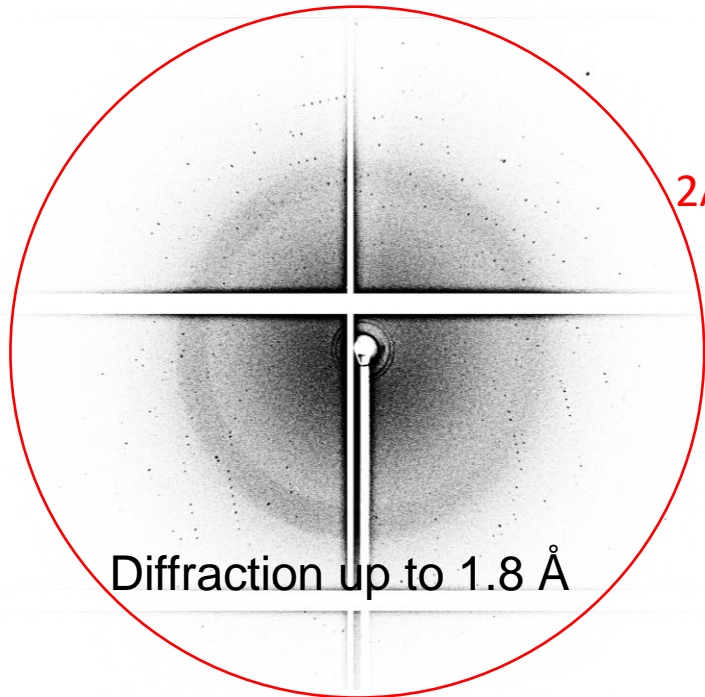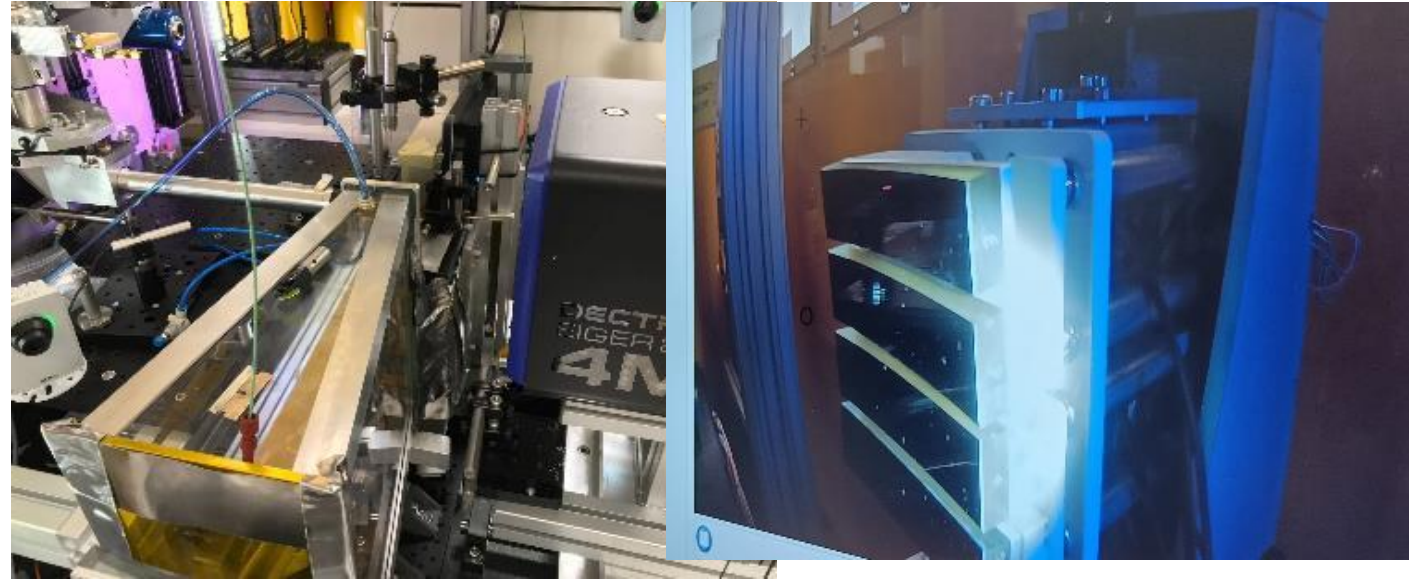
XES + XRD data collected from copper enzyme microcrystals at VMXi

average crystal size 12 µm

flowrate = 0.058 ml/min

2Å

Diffraction up to 1.8 Å

$K\alpha_2$ $K\alpha_1$

Intensity

Energy

AcNiR - Cu^{+2}

XES data from von Hamos spectrometer allows Cu(II) and Cu(I) states of the enzyme to be distinguished within crystals

Acknowledgements

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Olly King
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Graeme Winter

XFEL Hub team (Allen Orville and team)

Users contributing data