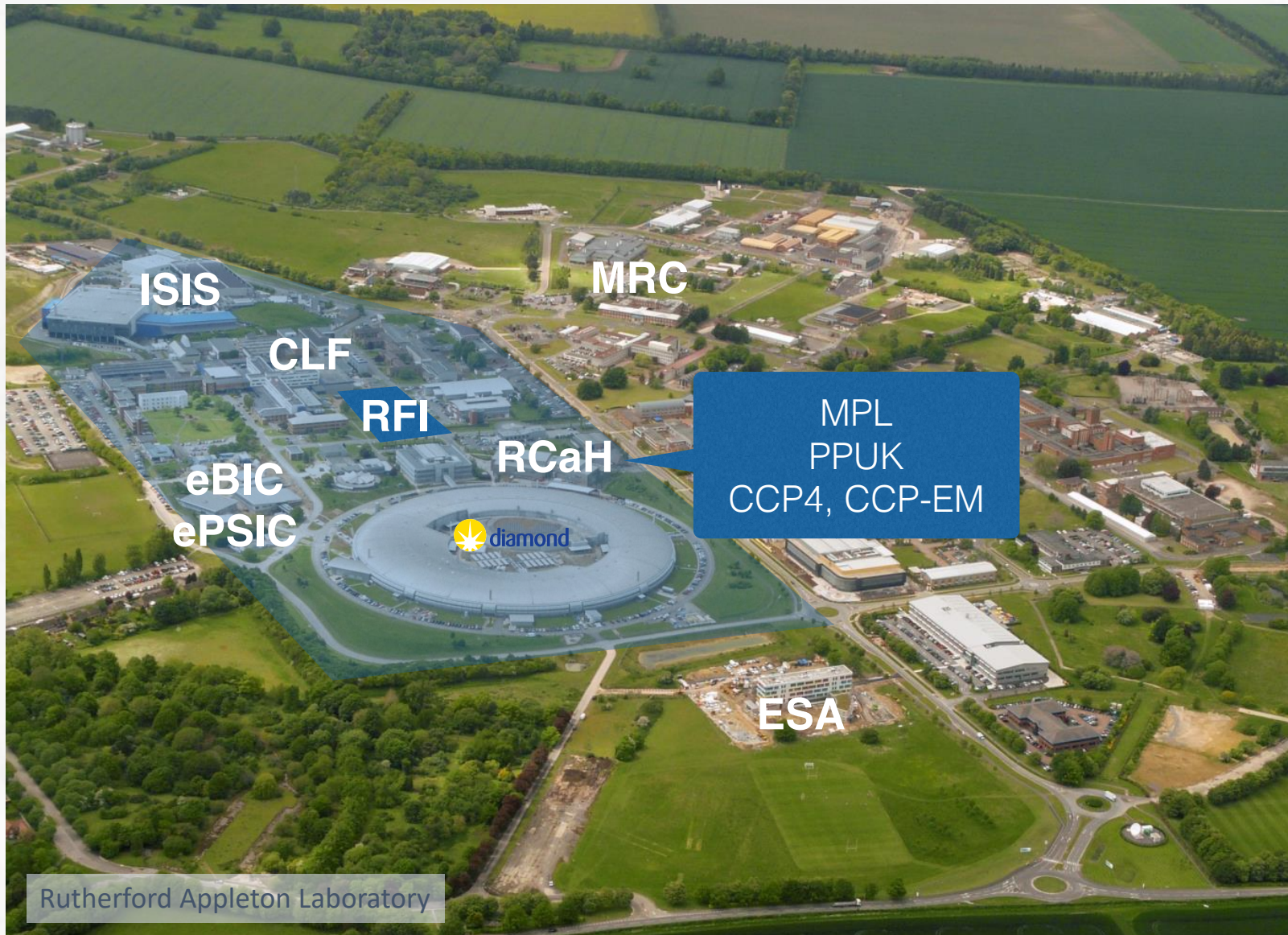


Macromolecular Crystallography

at  diamond

Marco Mazzorana
Diamond-CCP4 workshop 30/11/2020

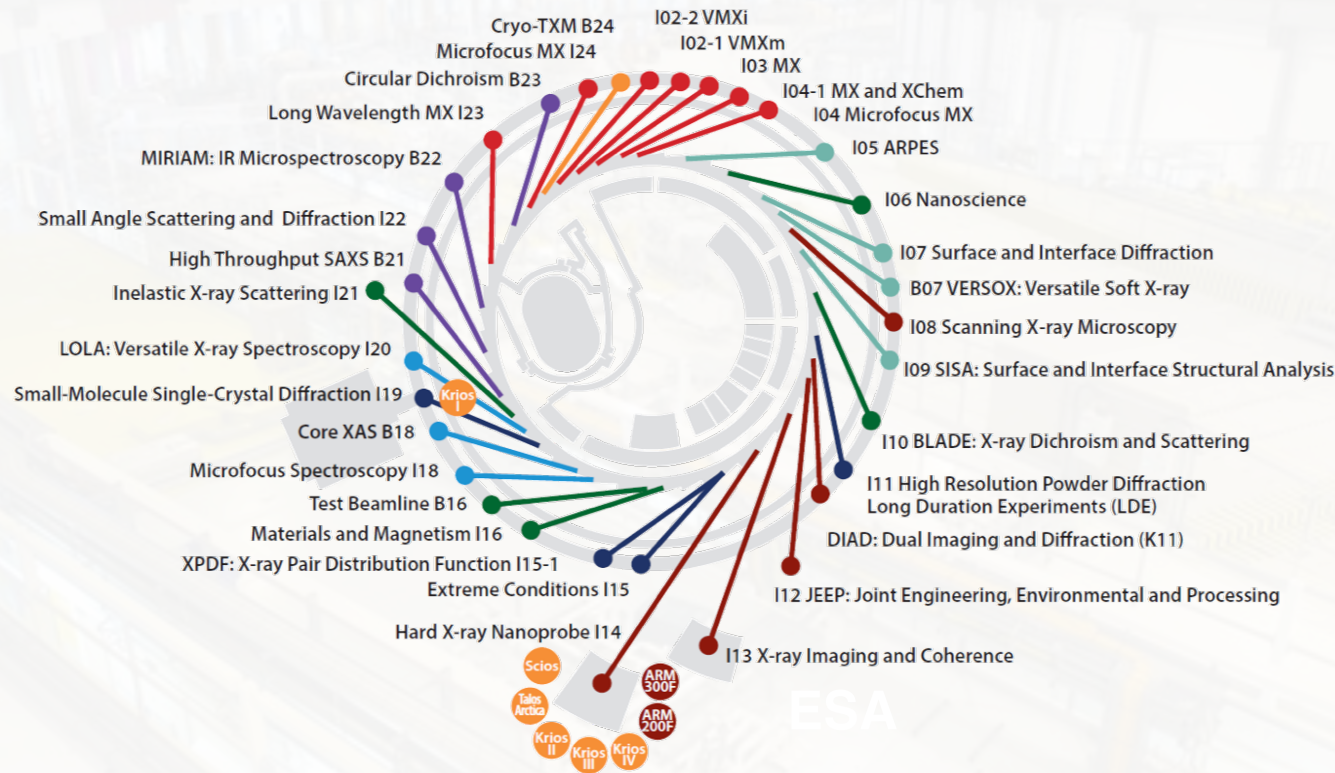
Diamond and the Harwell Campus



The UK national synchrotron

Funding: 86% UKRI, 14% Wellcome Trust

33 beamlines in 8 groups

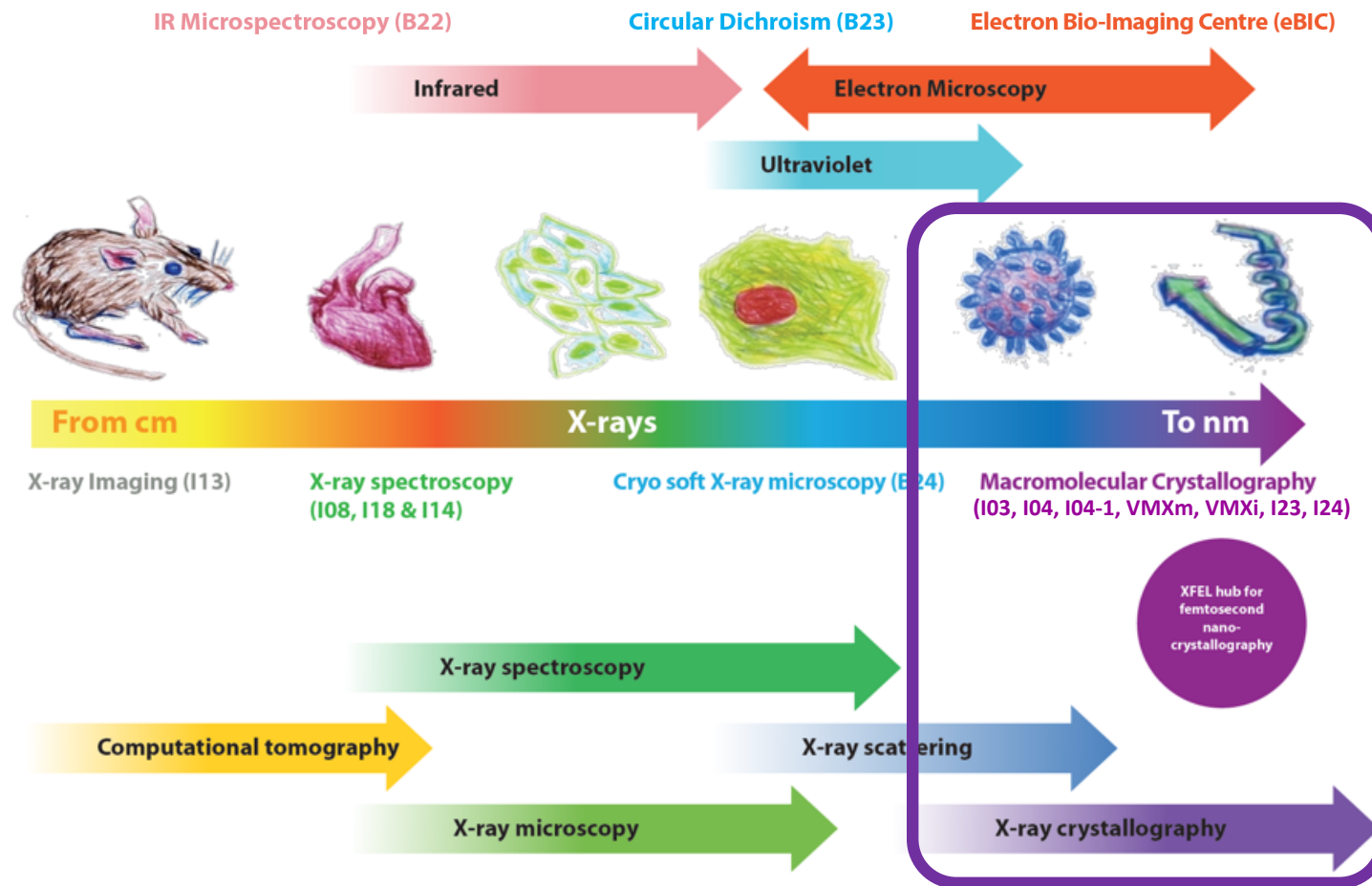


2 national cryo-EM facilities

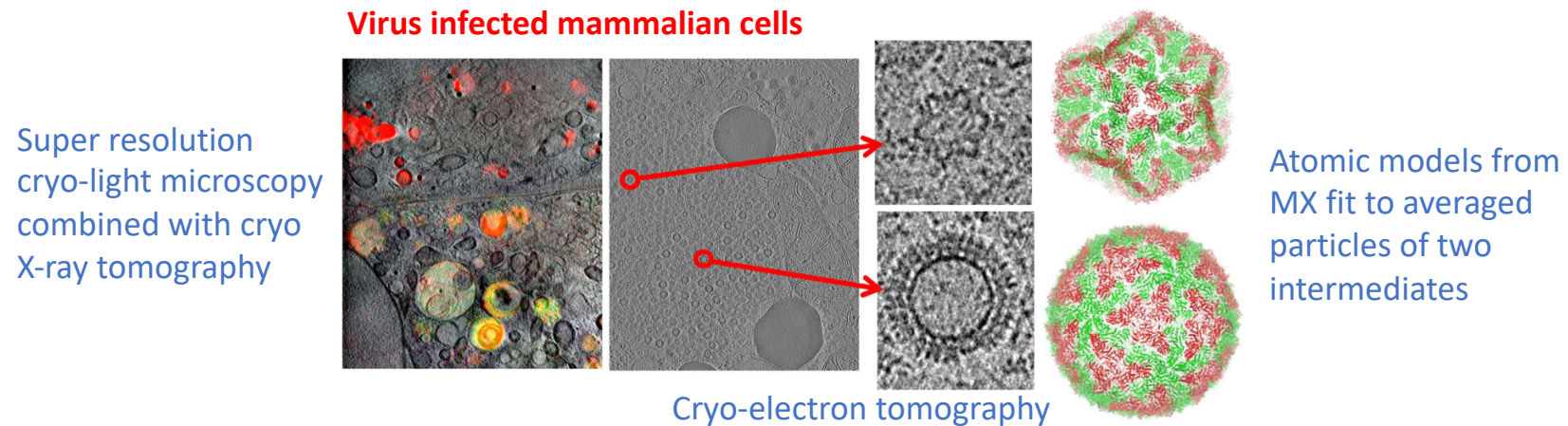
>12k Users

>100 Companies

Investigating the structure of biological systems



Combining techniques



Understand interactions and action across a large range of spatial and time domains (often weak and transient)

Reveal inner workings of the cell with a full dynamic picture of living organisms

Molecular description of cellular organization can transform fundamental science and understanding of disease processes

What can we learn from structures?

Crystallography can tell us how a protein works

Shape

Crystal Packing
Putative interaction surfaces

Fold

Putative function
Evolutionary relationships

Electrostatics

Interactions with other molecules
Docking sites

Protein-ligand complexes

Functional sites
Druggability

Local structure

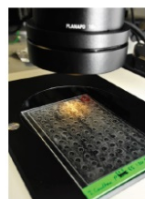
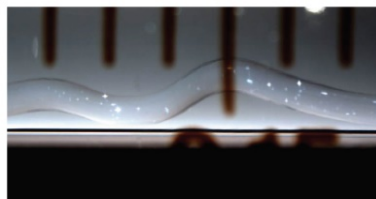
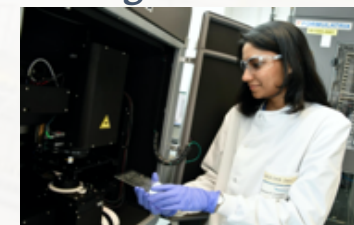
Catalytic mechanism
Conformation determinants



From gene to protein structures

Crystallization Facility @ Harwell

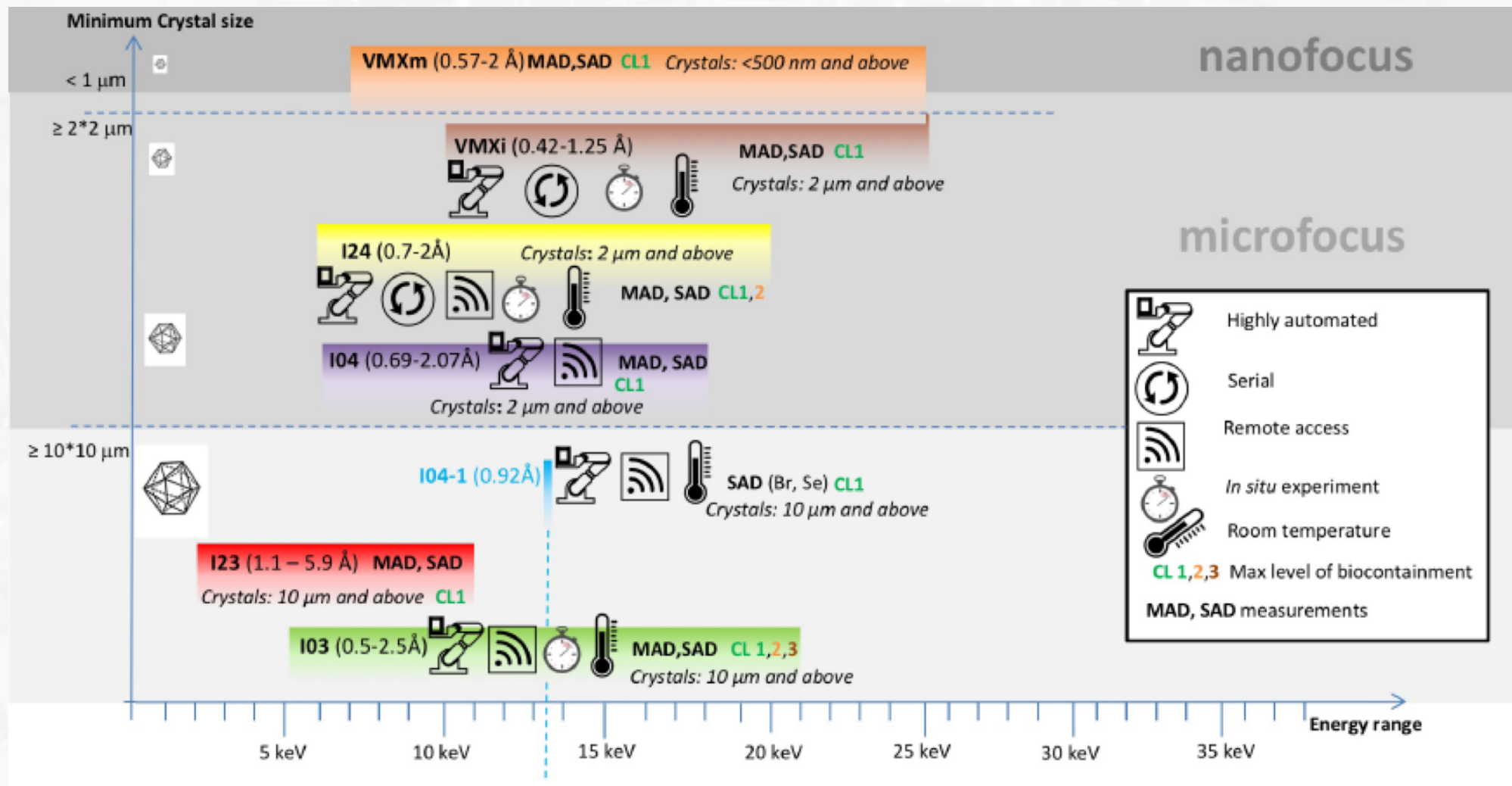
A high throughput platform open to academic groups and industry that provides users with an easy route into the VMXi beamline, the fully automated in situ and serial crystallography beamline at Diamond Light Source.



MPL (membrane protein laboratory)

A research and training facility for scientists interested in solving the 3D structures of these challenging proteins

MX - Wide portfolio of instruments



Beamline capabilities

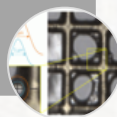
- PADs
- Automation
- Pipelines
- Serial Crystallography
- Access (rapid/freq)

High Throughput



- Tuneable
- Rapid
- Variable focus
- High flux
- *In situ*

Microfocus



- Tuneable beamlines
- I23 dedicated
- Smart data collection strategies
- Expert systems

Long Wavelength



- VMXi
- I24
- I03
- Screening
- Data collection
- CLIII

In situ



- Long wavelength
- Multi-axis strategies
- Inverse beam
- Wedged MAD
- Pipelines

Experimental Phasing



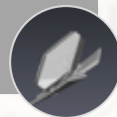
- *In situ*
- Containment Level II & III
- Schedule 5

Biological Containment



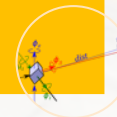
- Humidity control
- Spectroscopy
- Tomography
- Dynamics
- Fluorescence

Complementary information



- Diffraction image processing for SR Electron and XFEL data
- Collaborative effort
- Led and managed by DLS

Dials Project



- Experiment database
- Synchweb
- Data archive
- Data access
- Data reprocess

Data Management



the MX group team



Software



Controls



Technicians



Engineers



Challenges and synergies

I23
I24
VMXi
VMXm
I03
I04-1
I04

Microcrystals

Challenging phasing

Throughput

Alternative approaches

Doing smaller things better: microfocus

Google the best beamline in the world

All Images Shopping Videos News More Settings Tools

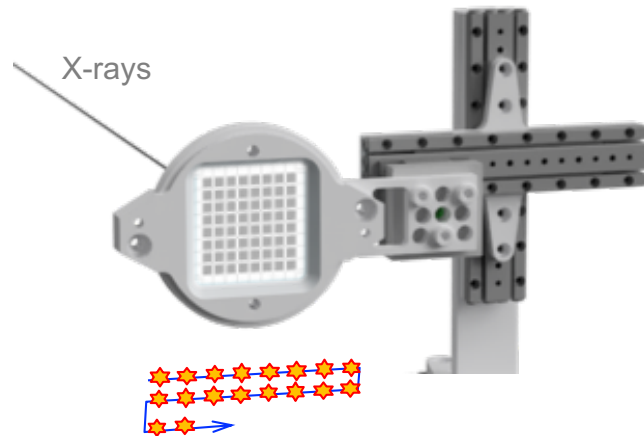
About 1,410,000 results (0.49 seconds)

Welcome to I24 - MX - Diamond Light Source

<https://www.diamond.ac.uk> Instruments

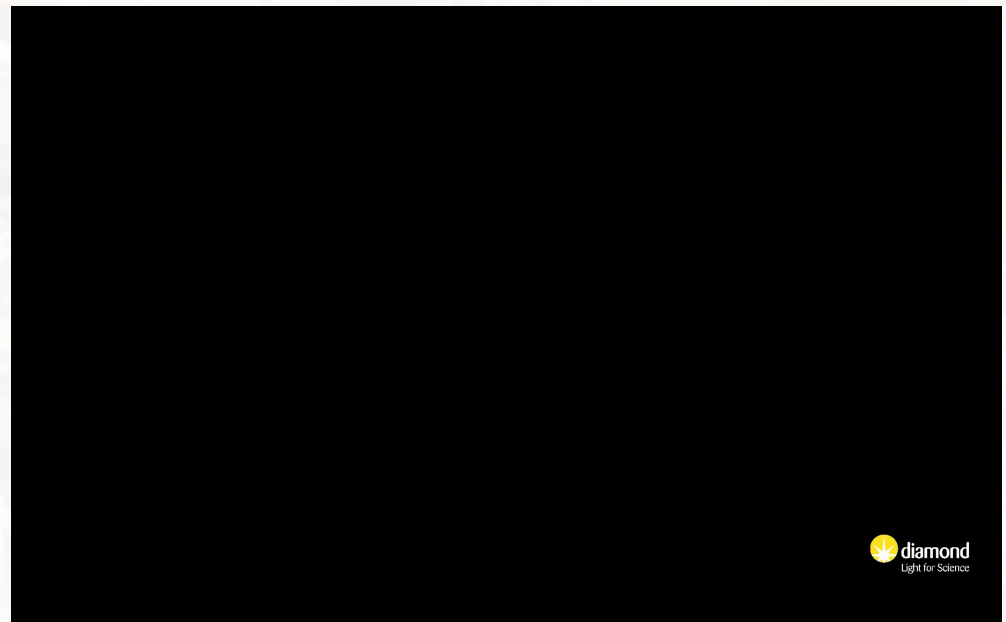
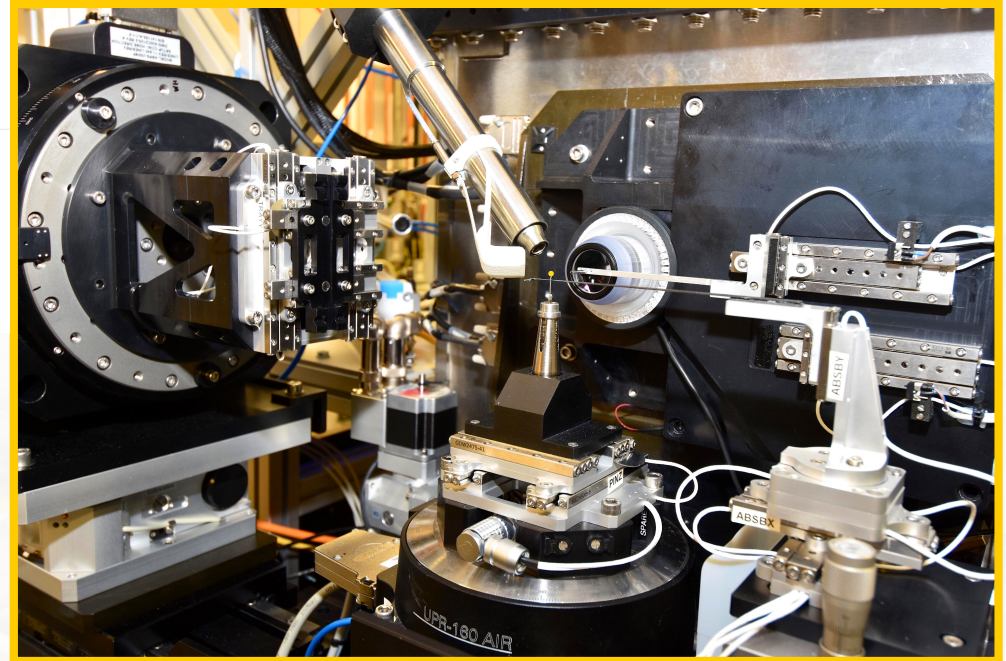
I24 is a tuneable microfocus beamline for MX. It was the first beamline of its kind to be built in Europe and has been operating since 2008. In 2015/2016 the ...

3 microfocus beamlines: I24, I04 & VMXm



fixed target silicon nitride chip
25,600 crystals/chip

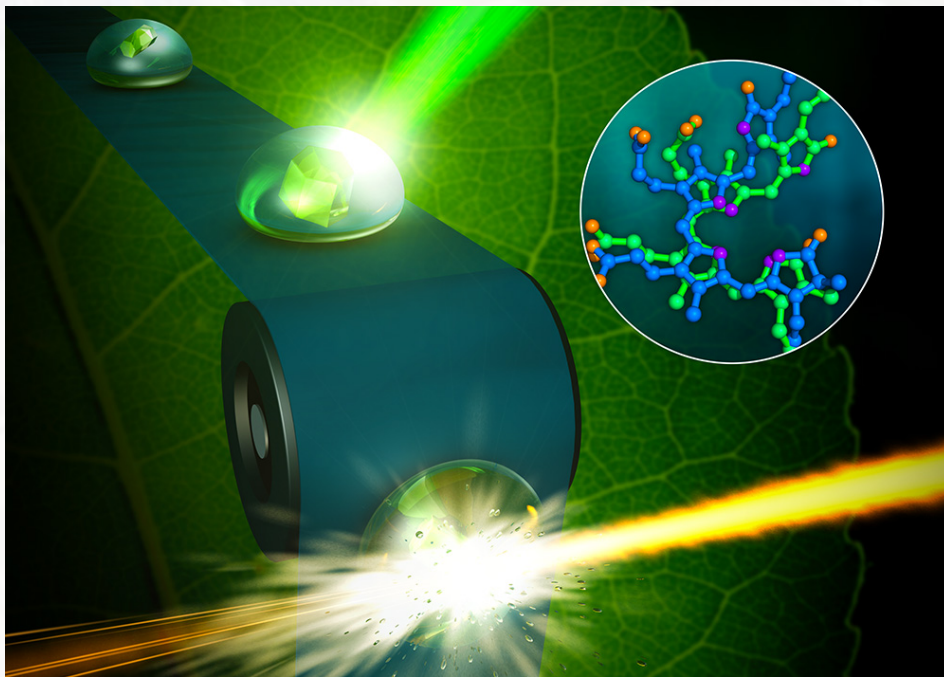
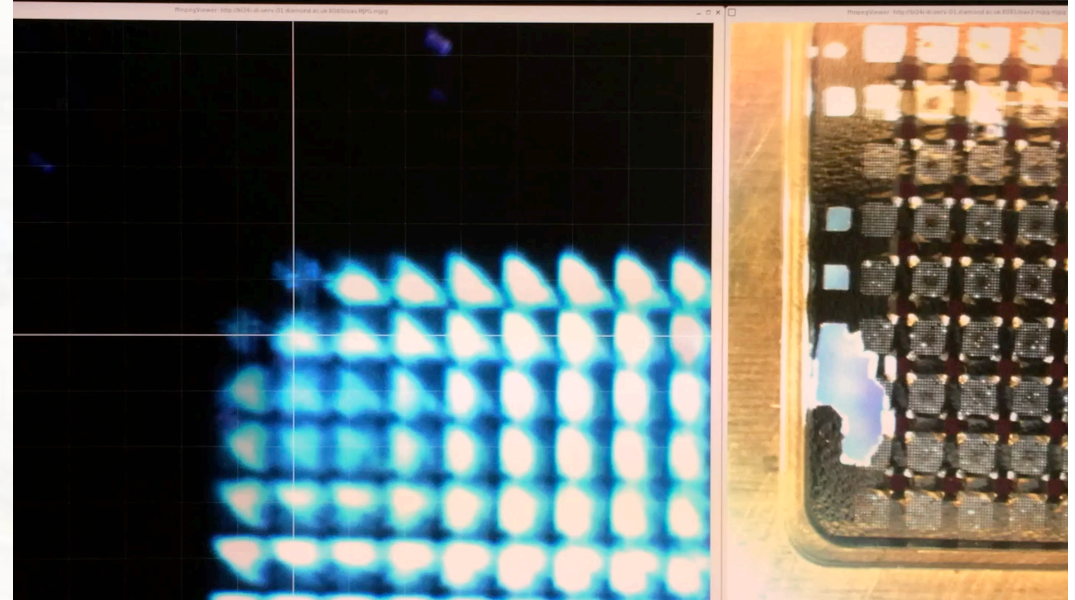
Full chip rastered in 6-20 mins
10-50 ms/frame (+ pause at each frame)
Typically ~25 chips/12 hours



diamond
Light for Science

XFEL-Hub + I24 @  diamond

Serial approaches



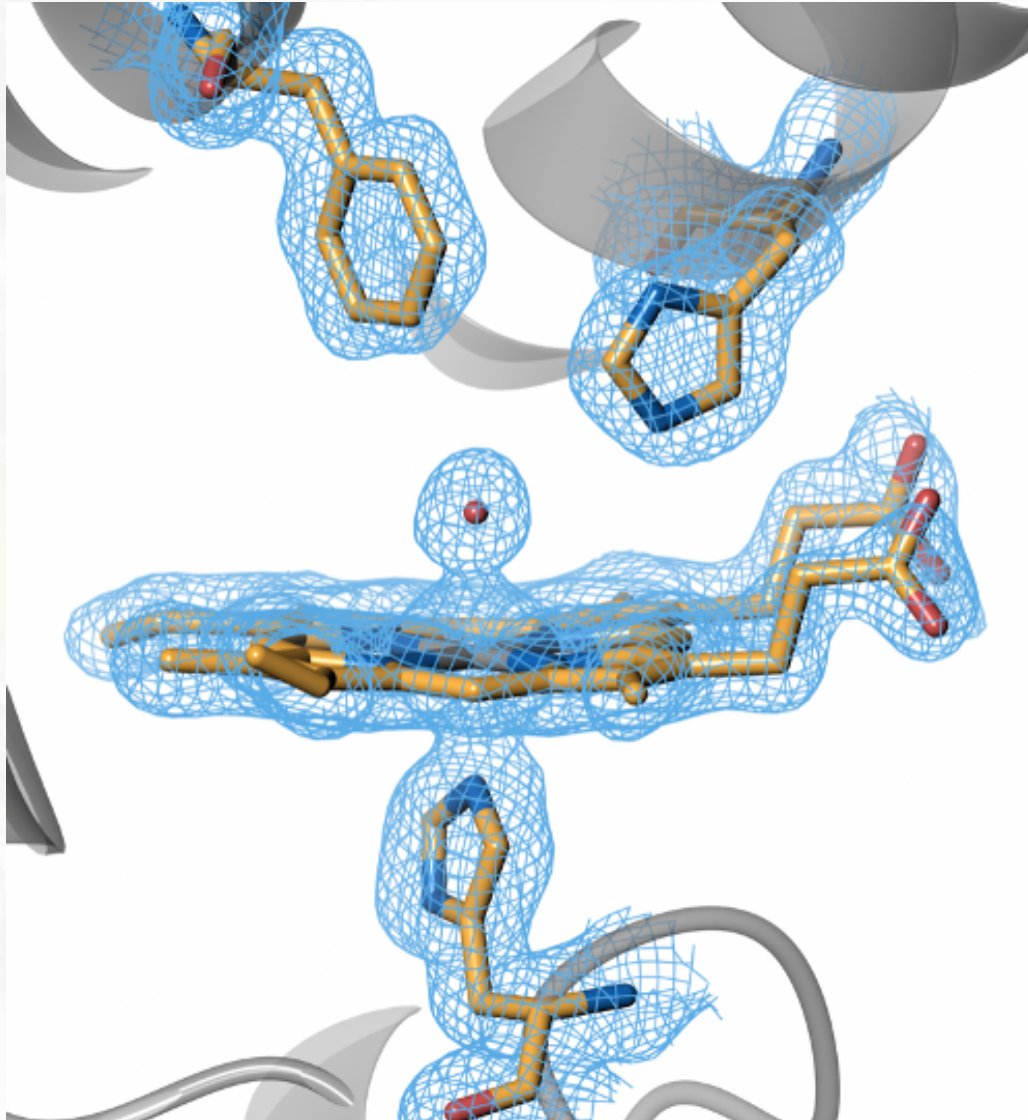
for SSX/SFX (UK XFEL-Hub)

- acoustic droplet ejection
- liquid jets / LCP extruders
- crystal levitation
- Pump/probe dynamical studies

Routine at I24 on standard MX beamtime

XFEL-Hub + I24 @  diamond

Serial approaches



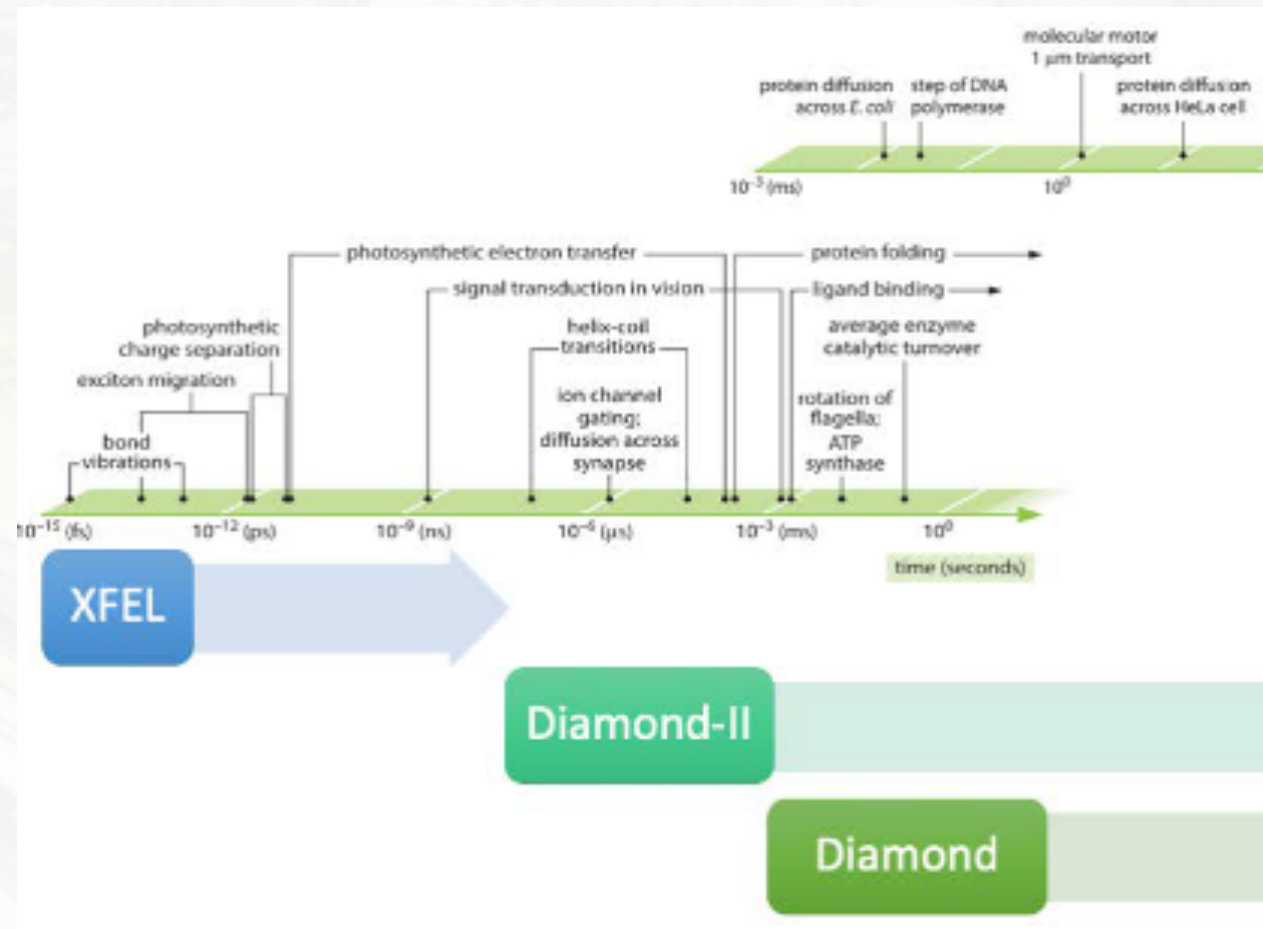
1.5 Å structure from 9000 crystals

XFEL-Hub + I24 @  diamond

Integrated structural biology offer

XFEL Hub: tools and strategies to enable dynamic structural biology facilitating

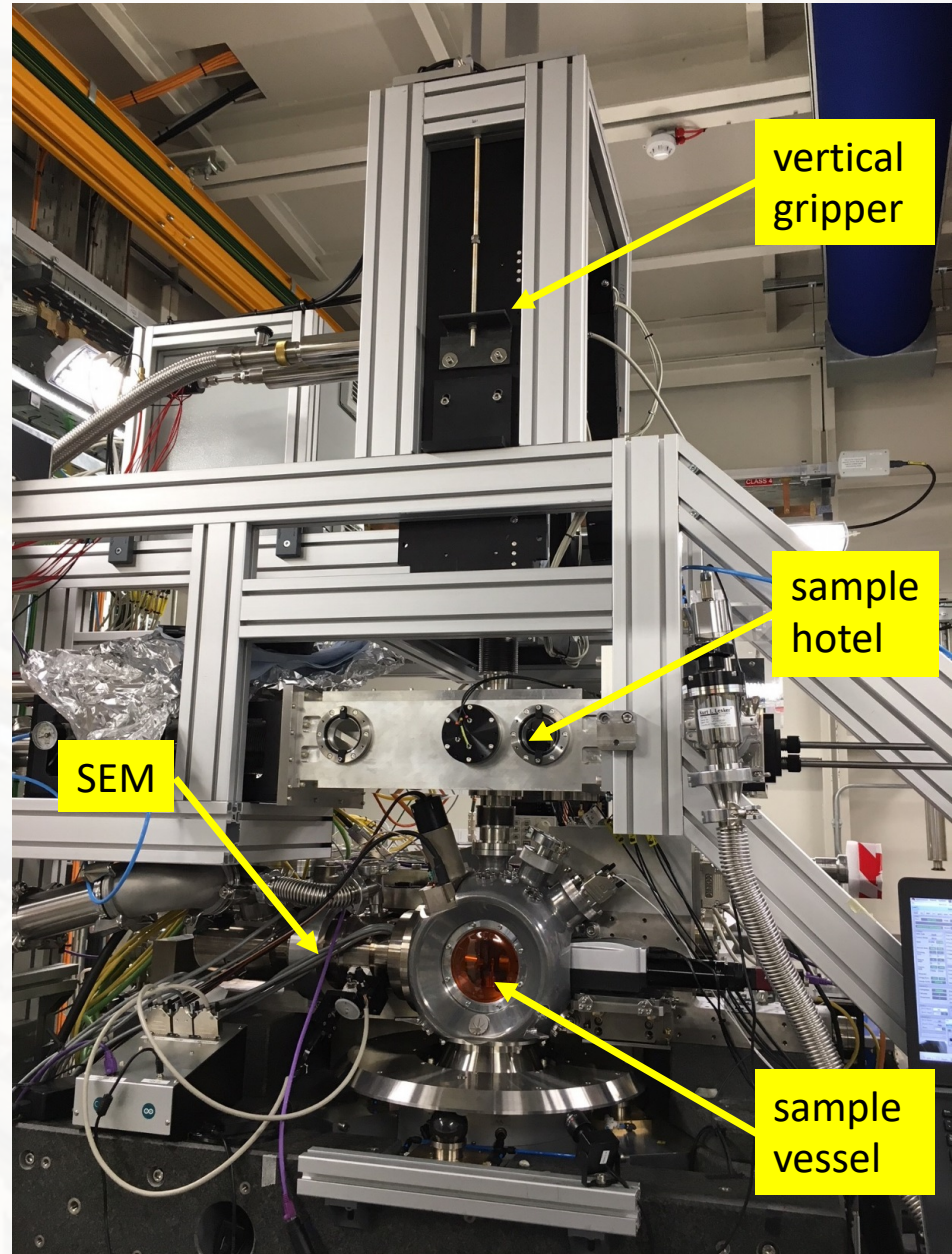
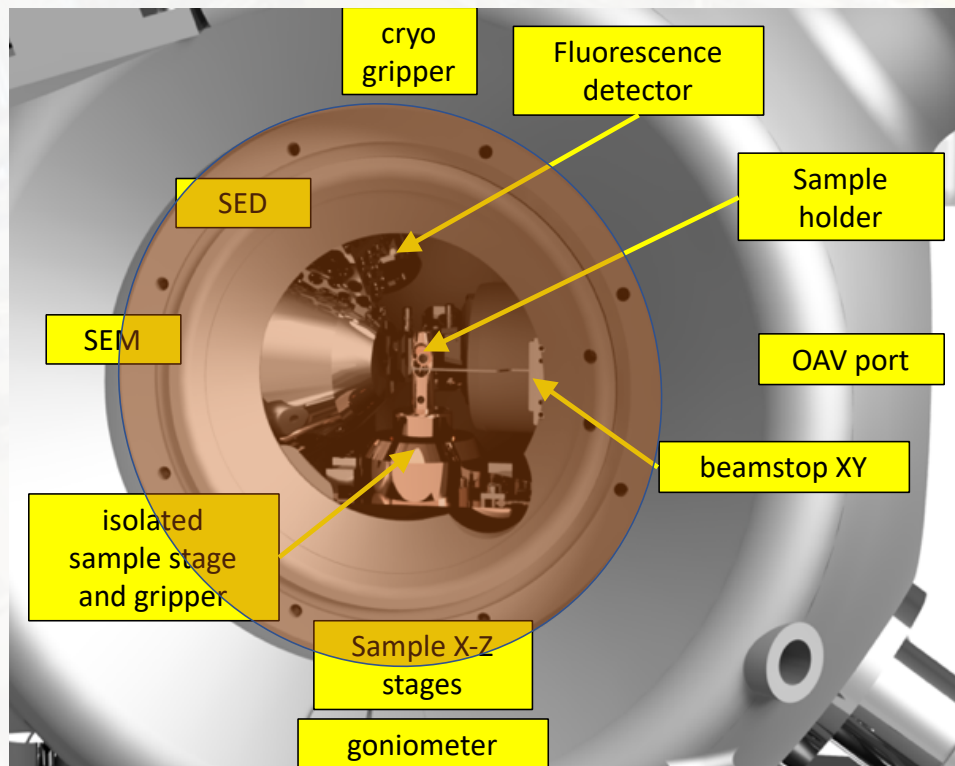
- (time-resolved) serial structural biology experiments via sample preparation, delivery, data collection, and processing
- the transfer of methods between XFEL, synchrotron, and/or cryo-EM sources
- access to, and data collection from complementary facilities (DLS, CLF, eBIC)



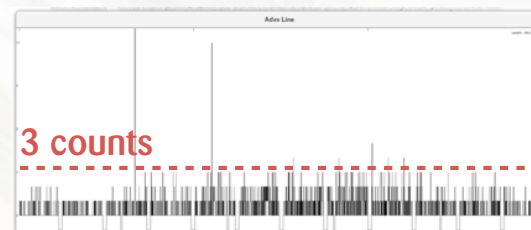
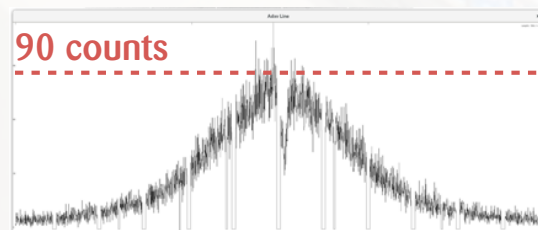
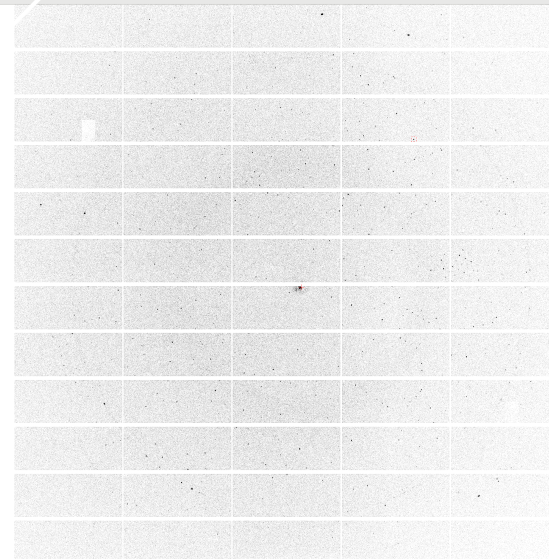
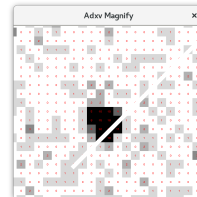
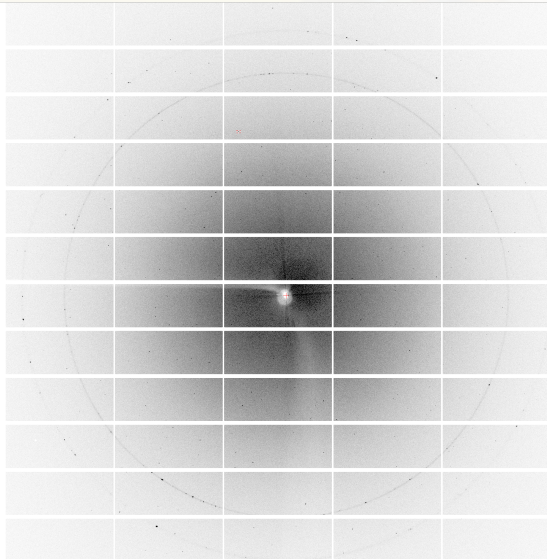
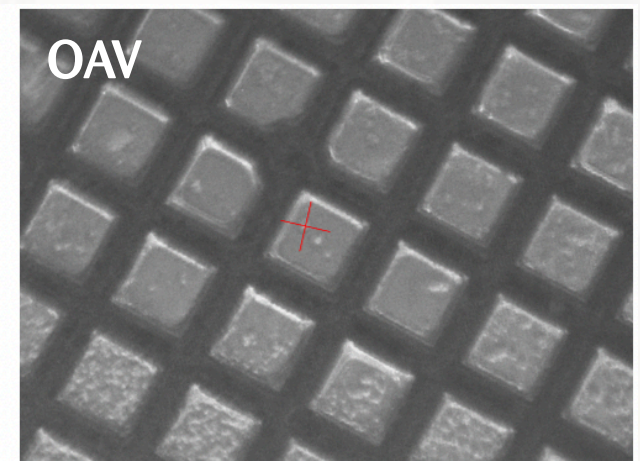
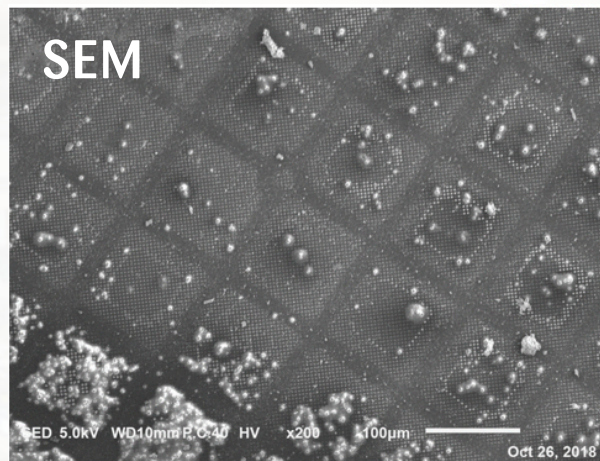
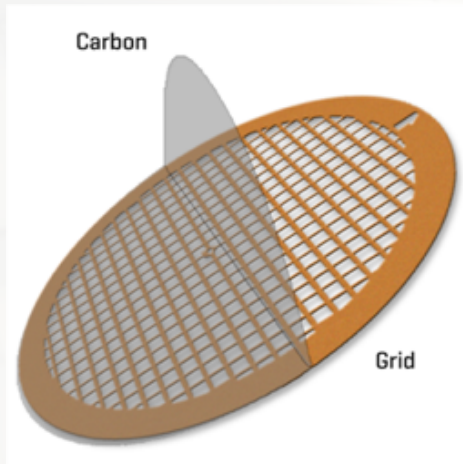
Going even smaller: VMXm

Features:

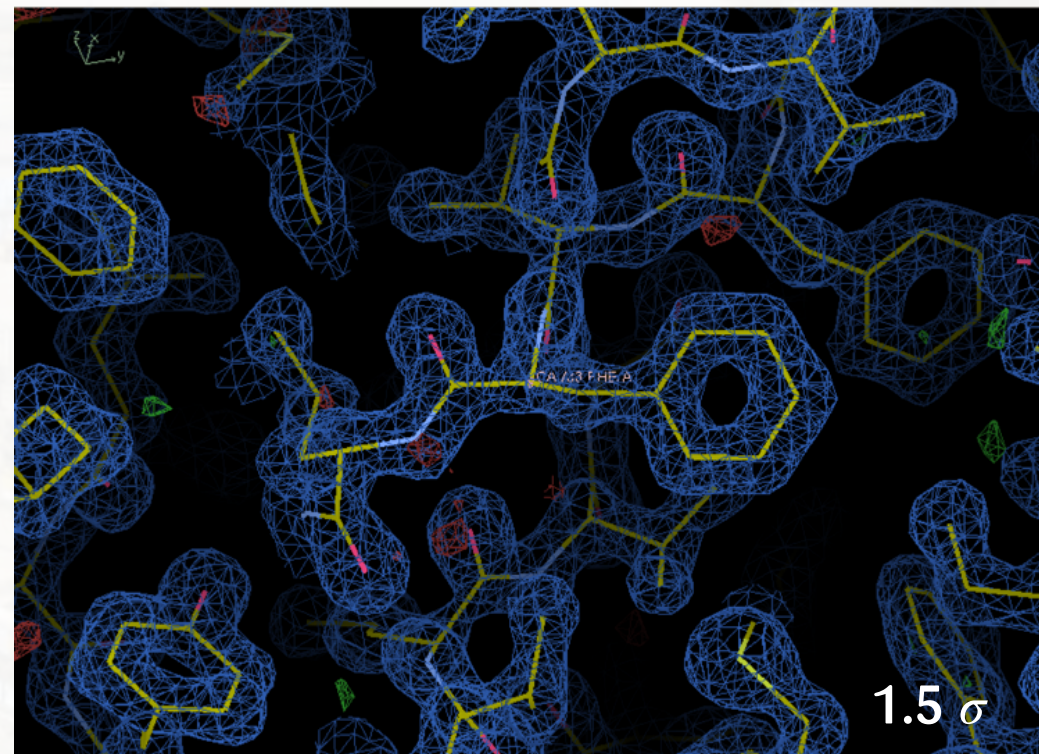
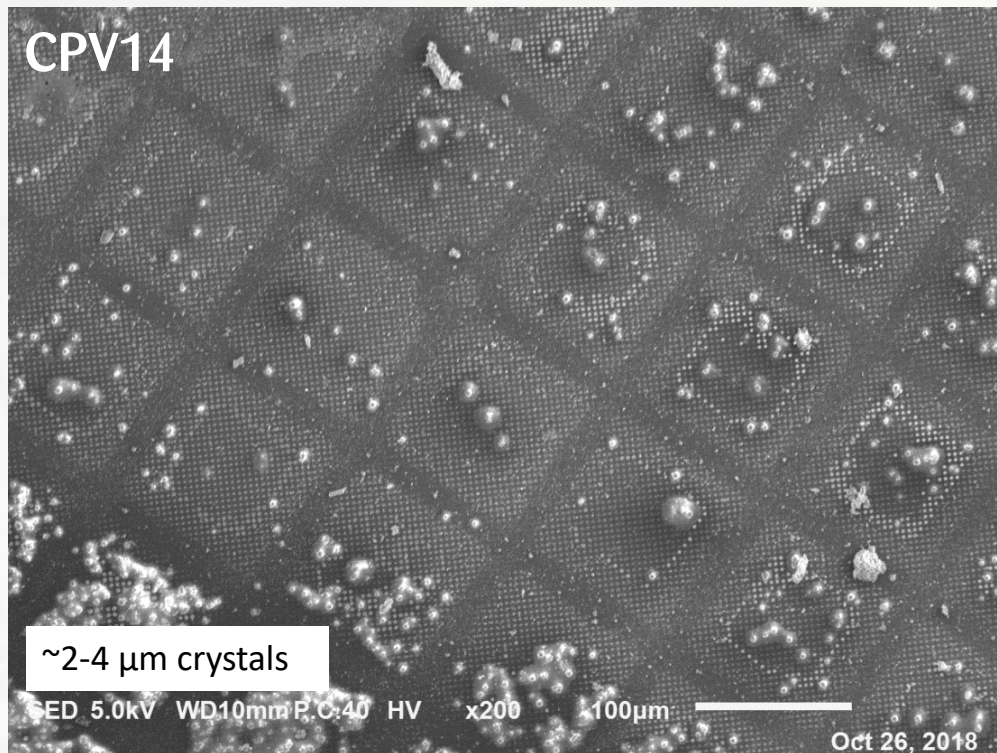
< 500 nm beamsizes
in vacuum
tunable 7-25 keV (2.0-0.57 Å)



Going even smaller: VMXm



VMXm: the first structures



Summary of merging statistics

	Overall	Low resolution	High resolution
Resolution ($\text{\AA}$)	36.57 - 1.50	36.58 - 4.07	1.53 - 1.50
Observations	116543	7779	1406
Unique reflections	25790	1405	833
Multiplicity	4.5	5.5	1.7
Completeness	87.23%	90.30%	57.21%
Mean I/sigma(I)	5.1	13.4	0.7
Rmerge	0.195	0.128	0.775
Rmeas	0.217	0.140	1.017
Rpim	0.088	0.053	0.646
CC1/2	0.969	0.970	0.319

27 crystals
1.5 – 2.0° data per crystal

Rigid body + restrained refinement
DIALS, CCP4, REFMAC, COOT

R/Rfree: 17.7/20.8

New phasing capabilities

Periodic Table of the Elements

1 1A 1A H Hydrogen 1.008	2 IIA 2A He Helium 4.003																
3 Li Lithium 6.941	4 Be Beryllium 9.012																
11 Na Sodium 22.990	12 Mg Magnesium 24.305																
19 K Potassium 39.098	20 Ca Calcium 40.078	21 Sc Scandium 44.956	22 Ti Titanium 47.88	23 V Vanadium 50.942	24 Cr Chromium 51.996	25 Mn Manganese 54.938	26 Fe Iron 55.933	27 Co Cobalt 58.933	28 Ni Nickel 58.693	29 Cu Copper 63.546	30 Zn Zinc 65.39	31 Ga Gallium 69.732	32 Ge Germanium 72.61	33 As Arsenic 74.922	34 Se Selenium 78.09	35 Br Bromine 79.904	36 Kr Krypton 84.80
37 Rb Rubidium 84.468	38 Sr Strontium 87.62	39 Y Yttrium 88.906	40 Zr Zirconium 91.224	41 Nb Niobium 92.906	42 Mo Molybdenum 95.94	43 Tc Technetium 98.907	44 Ru Ruthenium 101.07	45 Rh Rhodium 102.906	46 Pd Palladium 106.42	47 Ag Silver 107.868	48 Cd Cadmium 112.411	49 In Indium 114.818	50 Sn Tin 118.71	51 Sb Antimony 121.760	52 Te Tellurium 127.6	53 I Iodine 126.904	54 Xe Xenon 131.29
55 Cs Cesium 132.905	56 Ba Barium 137.327	57-71 Lanthanide Series	72 Hf Hafnium 178.49	73 Ta Tantalum 180.948	74 W Tungsten 183.84	75 Re Rhenium 186.207	76 Os Osmium 190.23	77 Ir Iridium 192.22	78 Pt Platinum 195.08	79 Au Gold 196.967	80 Hg Mercury 200.59	81 Tl Thallium 204.383	82 Pb Lead 207.2	83 Bi Bismuth 208.980	84 Po Polonium [209]	85 At Astatine [210]	86 Rn Radon 222.018
87 Fr Francium 223.020	88 Ra Radium 226.025	89-103 Actinide Series	104 Rf Rutherfordium [261]	105 Db Dubnium [262]	106 Sg Seaborgium [266]	107 Bh Bohrium [264]	108 Hs Hassium [269]	109 Mt Meitnerium [268]	110 Ds Darmstadtium [271]	111 Rg Roentgenium [272]	112 Cn Copernicium [285]	113 Uut Ununtrium [288]	114 Fl Flerovium [289]	115 Uup Ununpentium [294]	116 Lv Livermorium [293]	117 Uus Ununseptium [294]	118 Uuo Ununoctium [294]
			70 La Lanthanum 138.905	71 Ce Cerium 140.116	72 Pr Praseodymium 140.908	73 Nd Neodymium 144.24	74 Pm Promethium [145]	75 Sm Samarium 150.36	76 Eu Europium 151.964	77 Gd Gadolinium 157.25	78 Tb Terbium 158.925	79 Dy Dysprosium 162.50	80 Ho Holmium 164.930	81 Er Erbium 167.26	82 Tm Thulium 168.934	83 Yb Ytterbium 173.04	84 Lu Lutetium 174.967
			89 Ac Actinium 227.028	90 Th Thorium 232.038	91 Pa Protactinium 231.036	92 U Uranium 238.029	93 Np Neptunium 237.048	94 Pu Plutonium 244.064	95 Am Americium [243]	96 Cm Curium 247.070	97 Bk Berkelium 247.070	98 Cf Californium 251.080	99 Es Einsteinium [254]	100 Fm Fermium 257.095	101 Md Mendelevium 258.1	102 No Nobelium 259.101	103 Lr Lawrencium [262]
			K		3.607		3.437										
			Ca		4.038		3.070										

e (keV) λ (Å)

2.146 5.779

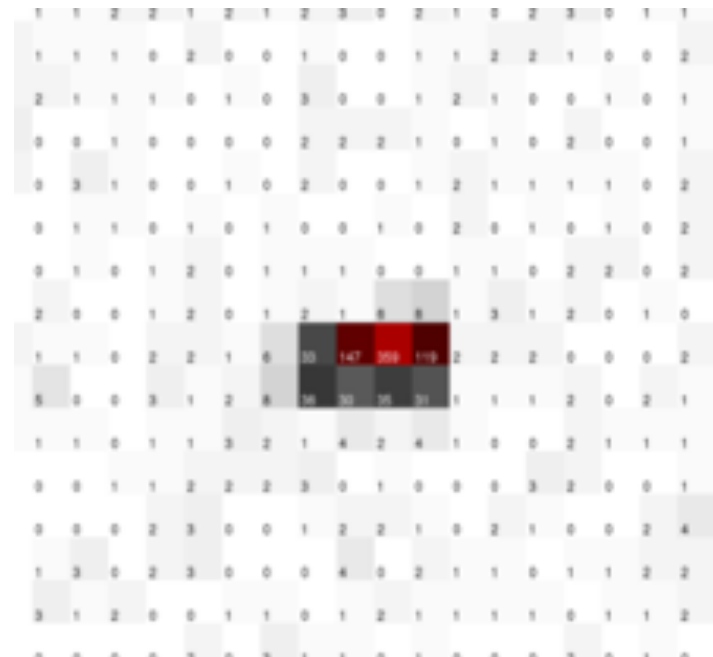
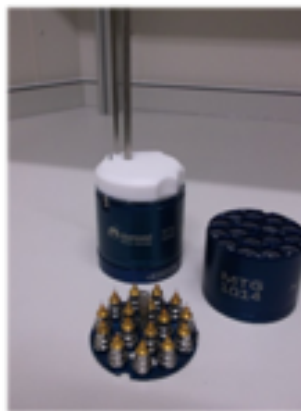
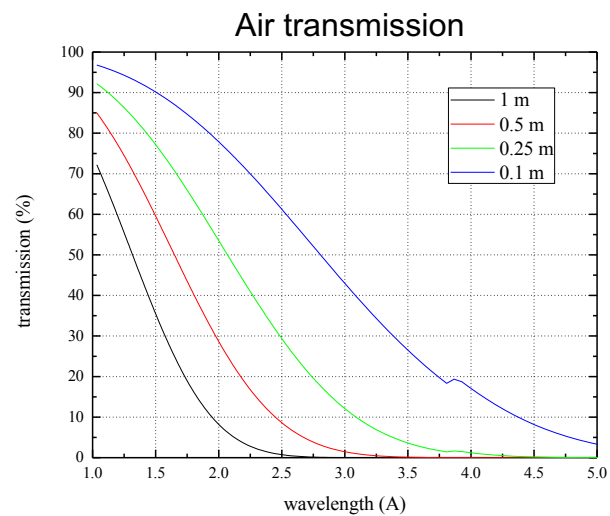
2.472 5.016

2.822 4.393

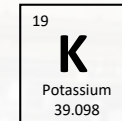
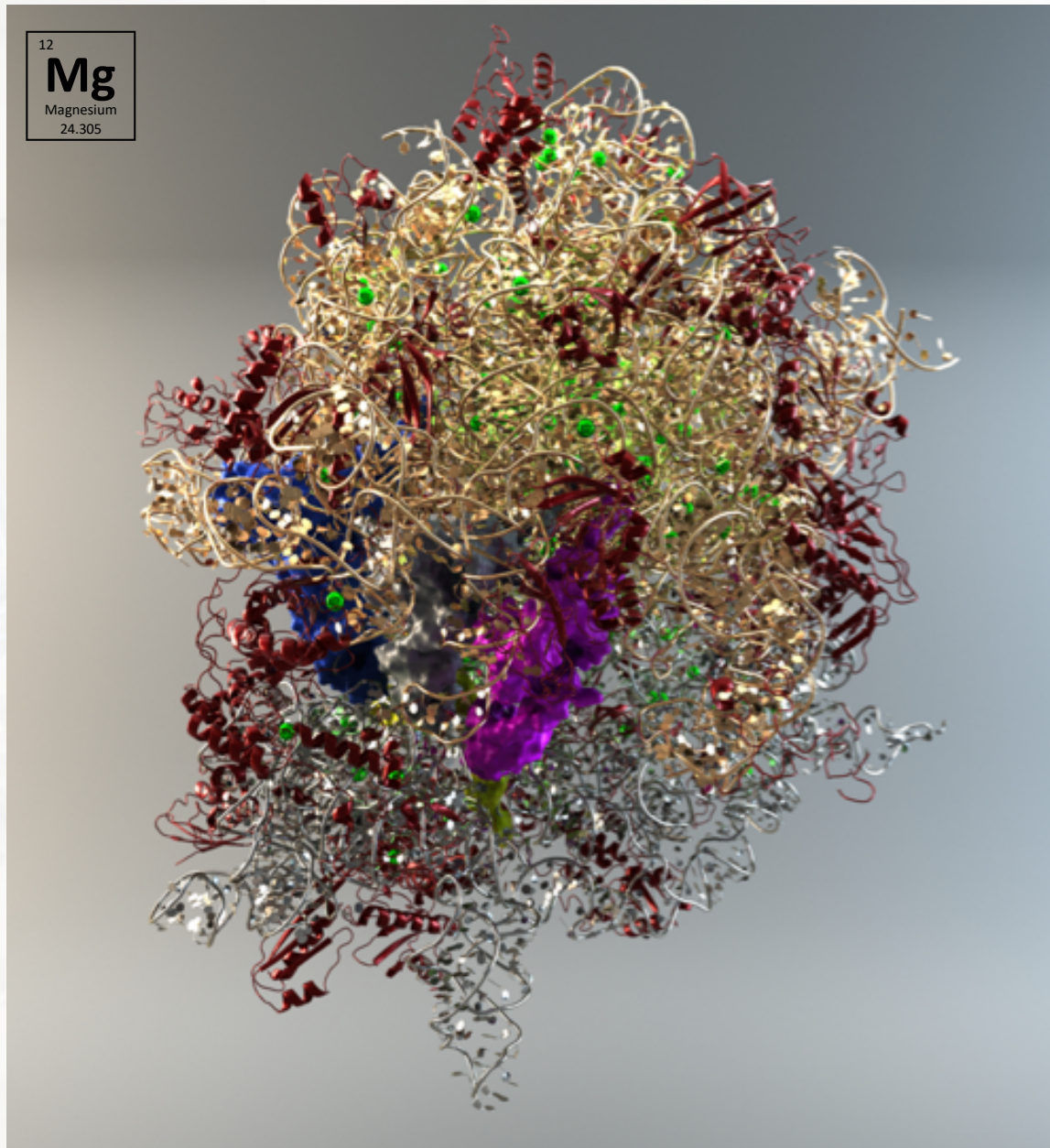
© 2013 Todd Helmenstine
sciencenotes.org

long wavelength beamline

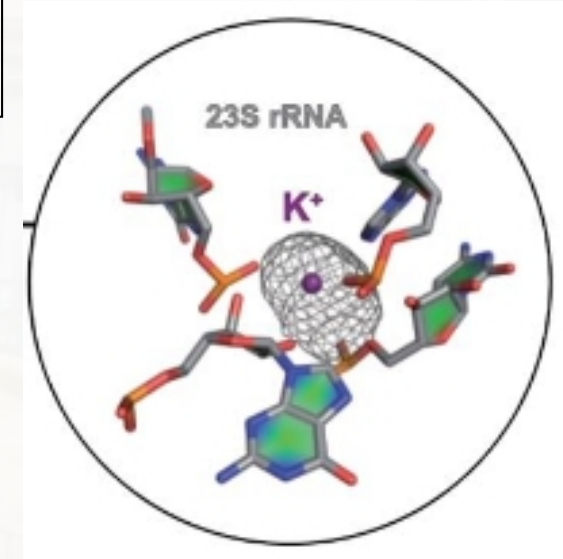
Challenges



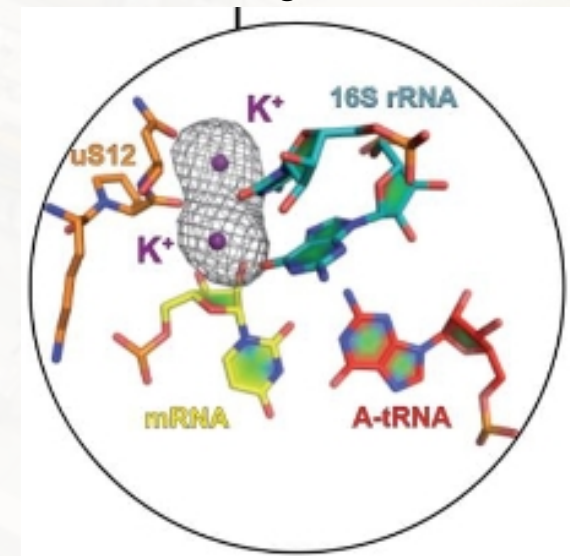
Results - ion identification



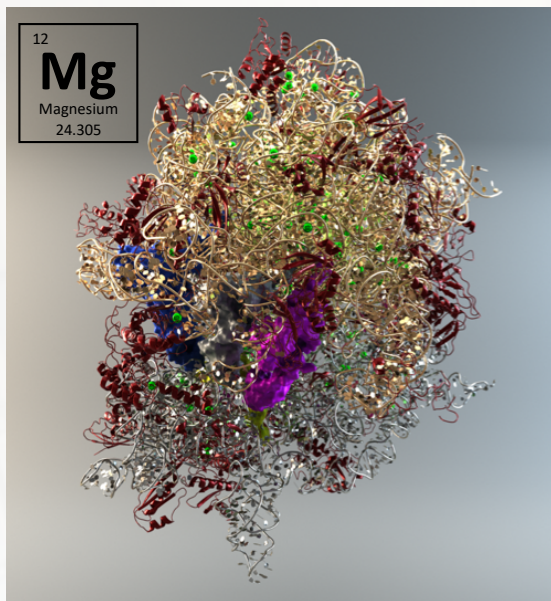
Peptidyl transferase center



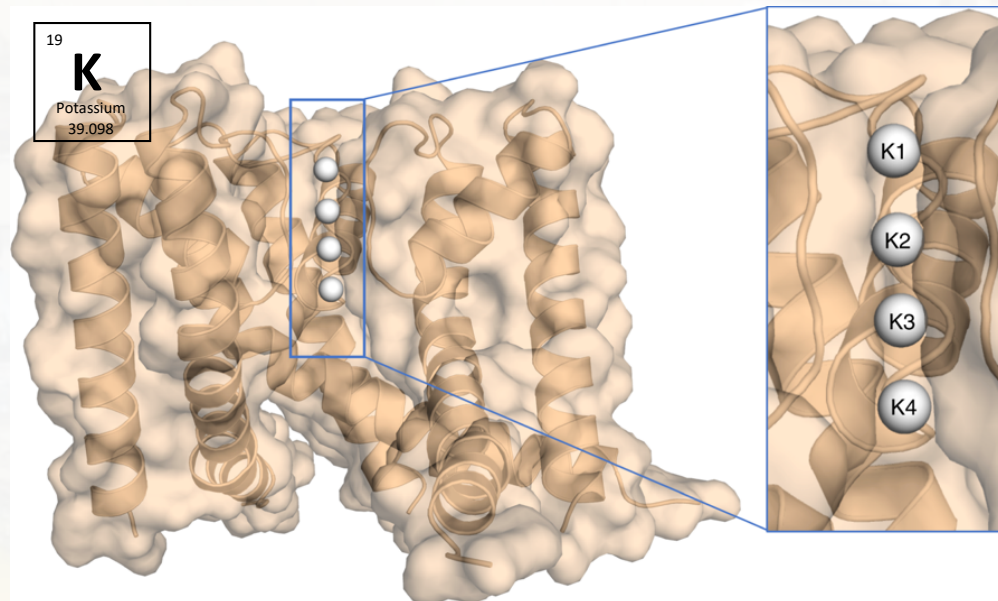
Decoding center



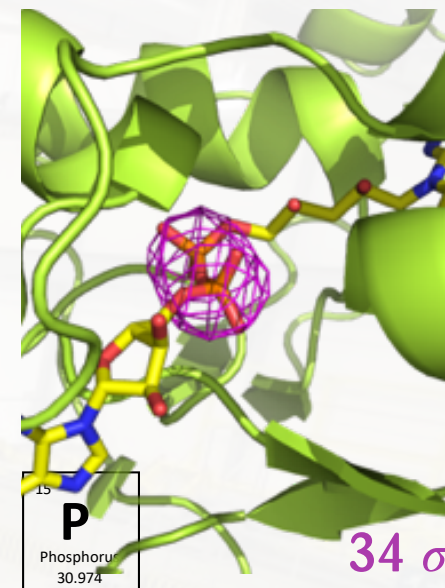
Results - ion identification



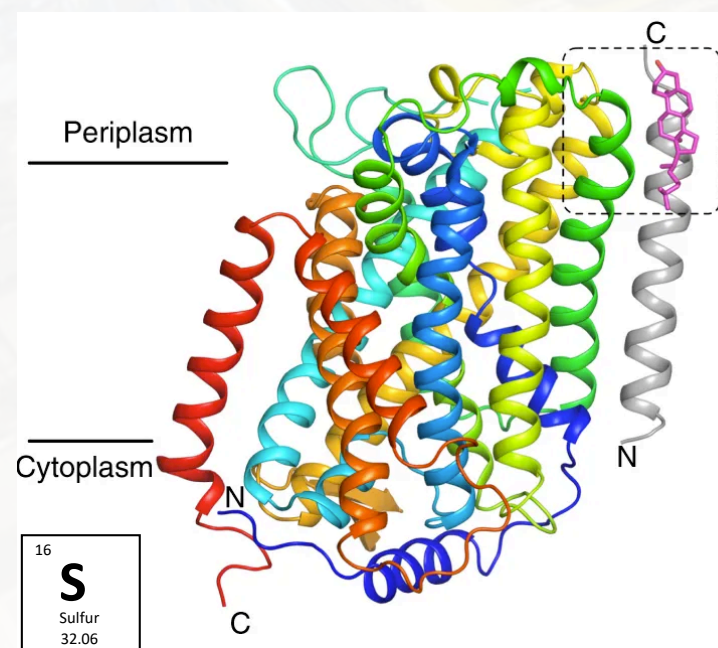
ribosome 70S



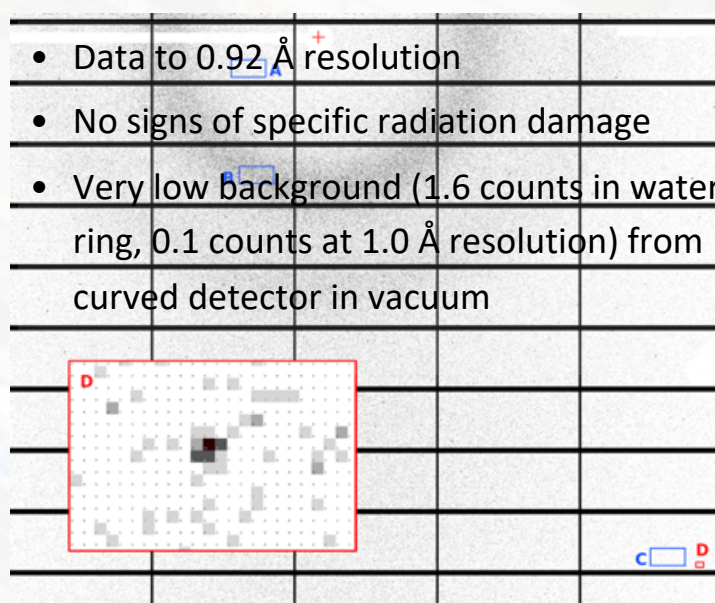
Na/K transporter



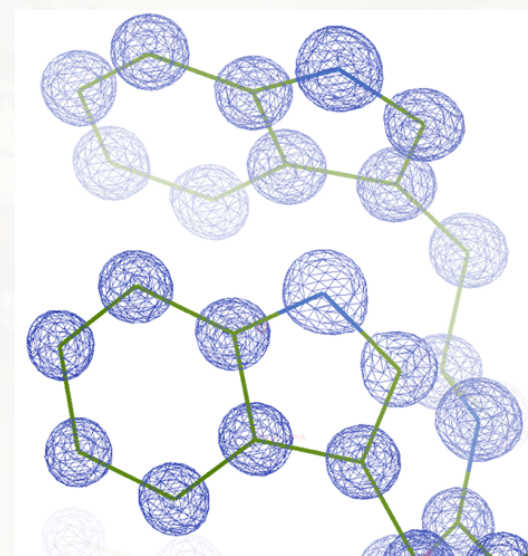
FAD-binding



SLC7 transporter



PETase



Increasing throughput



BART sample changer

37 Unipucks

597 samples

exchange rate ~20 sec/sample



2x EIGER2 XE 16M

Pixel size: 75x75 μm^2

Pixels number: 18,093,576

Frame rate: 560 Hz (EIGER 133 Hz)

- time resolved studies

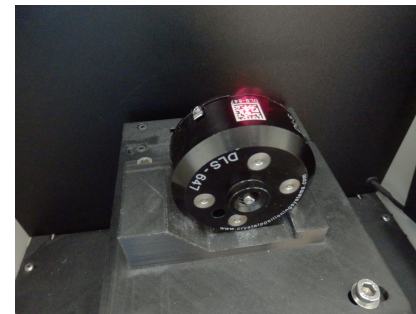
Virtually no readout time

Logistics and dynamic scheduling



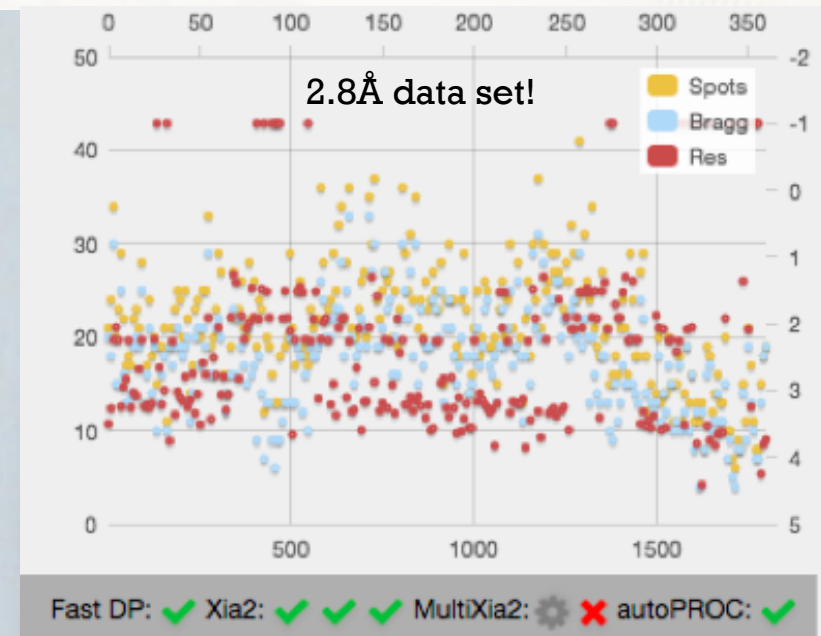
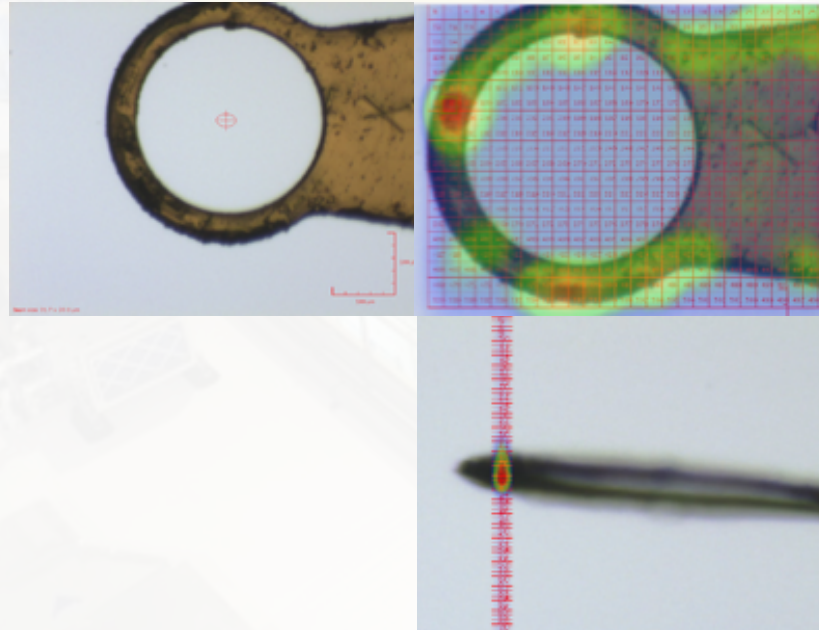
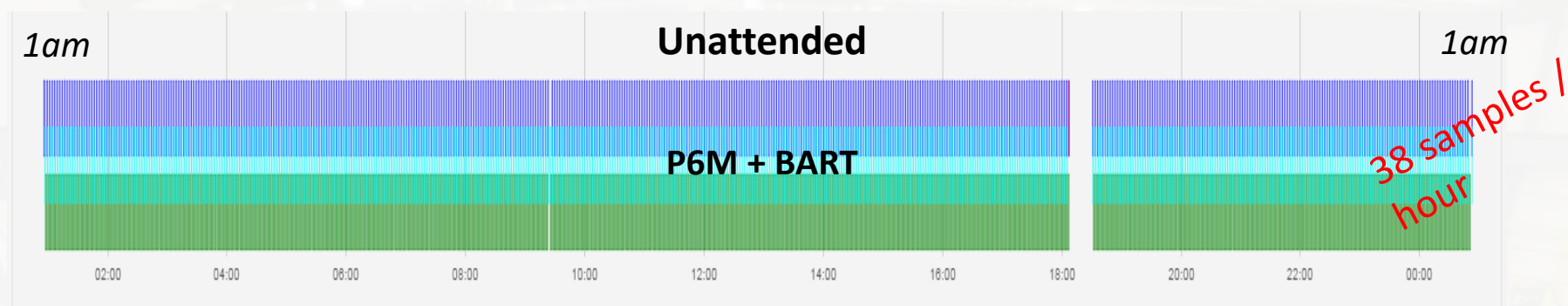
High capacity, automated store

Barcodes tracking

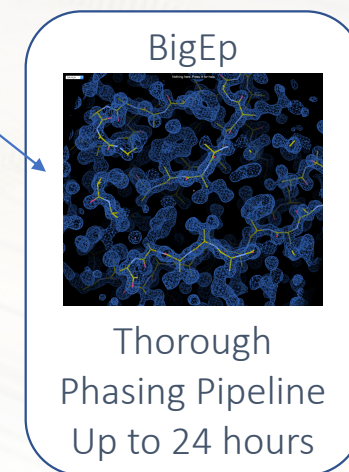
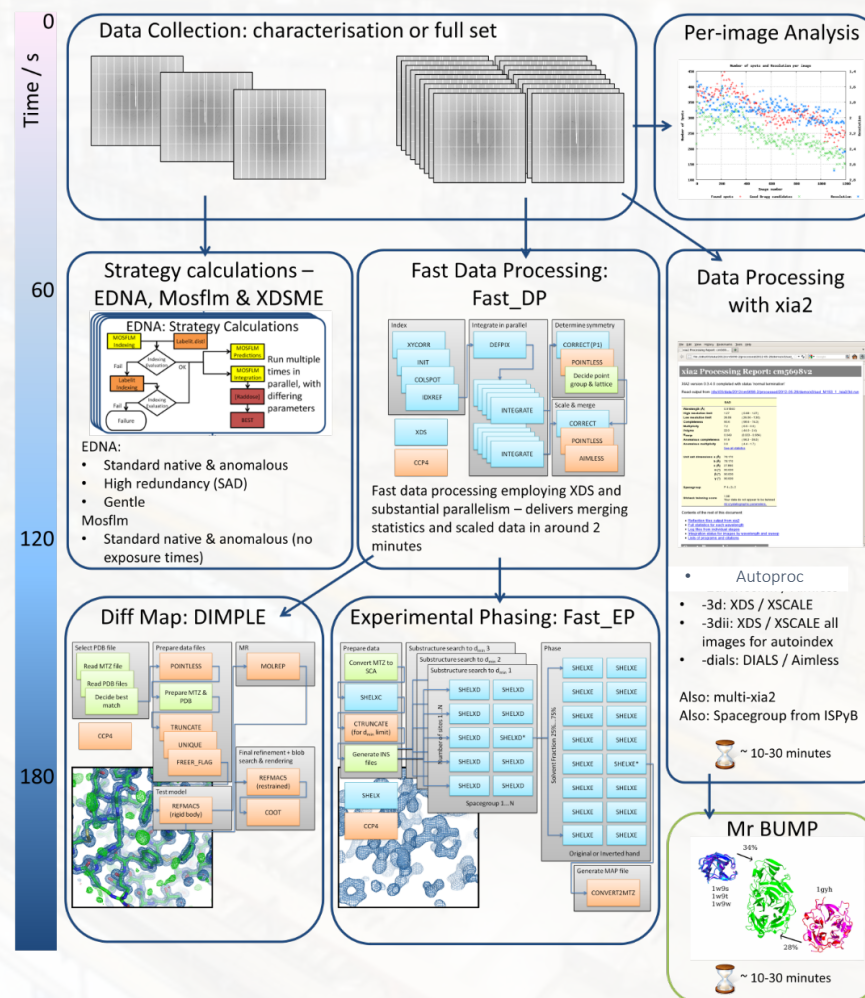
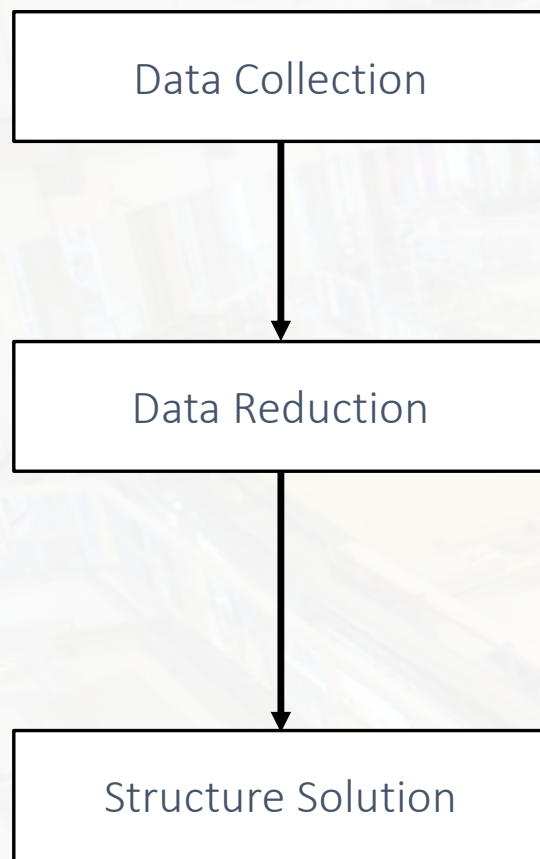


Dynamic scheduling

Automation - unattended data collection



Automated processing pipelines



VMXi - fully automated, user-free, in situ

Container: Z12368764

This page shows the contents of the selected container. Samples can be added and edited by clicking the pencil icon, and removed by clicking the x

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Inspections 08:47 09-10-2015 (+d) [Adhoc] [+](#)

Movie Gap: 0.5 s [Play](#)

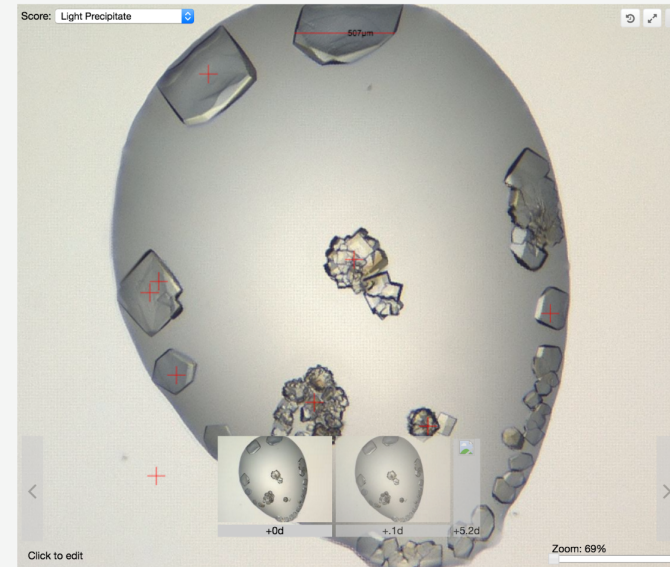
Sample

Protein pL_prot1 [+ Extra Fields](#)
Sample Name A1d1_x1 [View](#)
Spacegroup Click to edit
Comments a1d1



Screen Components

Component	Concentration	pH
No components for this group		



Manage Beamline Presets

Proposals cm14559 v Projects Unit Cell Search Feedback Help

Shutdown activities are complete, please report any Synchrotron issues to your local contact

Dashboard Imaging Schedules Crystallisation Screens Global Presets Beamline Parameters

Global Presets

Global presets are currently being saved into "cm14559" [Add Preset](#)

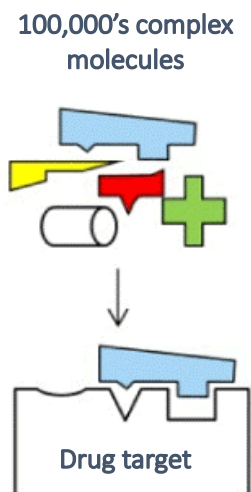
Beamline	Name	Exp. Type	Parameters
02-2	VMXi Grid 1	Grid Scan	Box Size 10 x 10 Q Range 0 Energy 12658 eV Transmission 100 % Exposure 0.00134 s Resolution 2.6 Å
02-2	VMXi Point 1	Point Collection	Beam Size 10 x 10 μm No. Images 600 Q Start -30 ° Q Range 0.1 ° Q End 30 ° Energy 12658 eV Transmission 25 % Exposure 0.0017 s Resolution 2.6 Å

15 Page: 1

High throughput fragment screening

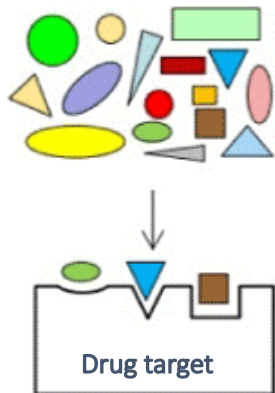
Conventional screening

- Searching for potent molecules
- Complex molecules → Low probability



VS

100-1000's of small molecules



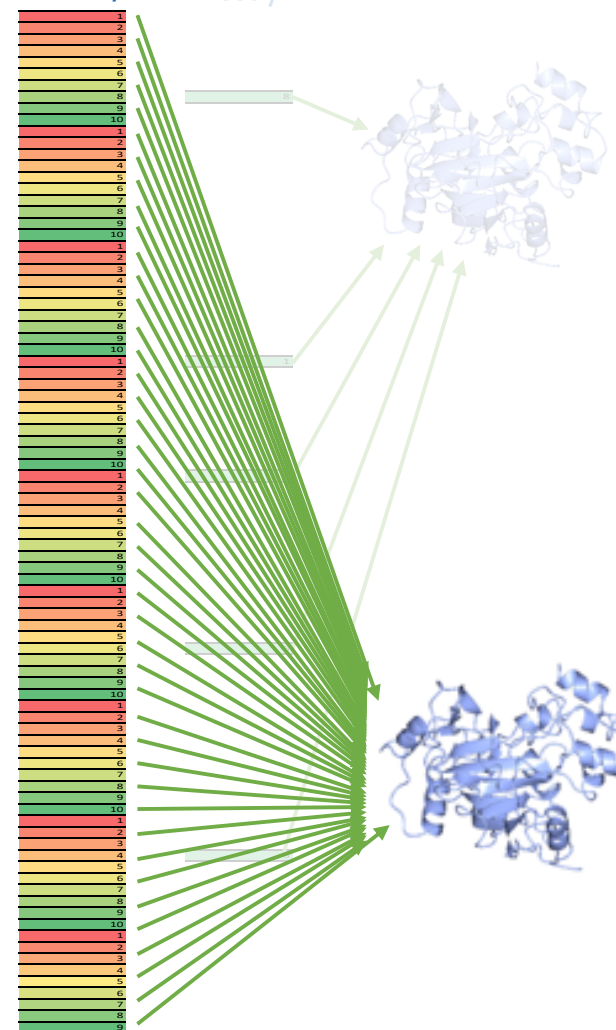
Fragment screening

- Guaranteed binding – but weak
- Potency through chemical elaboration

Fragment Library

Binding Assay

Structure



By selection of best compounds

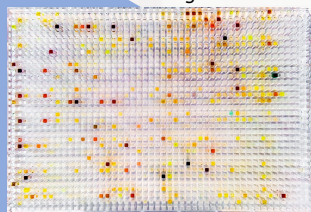


diamond

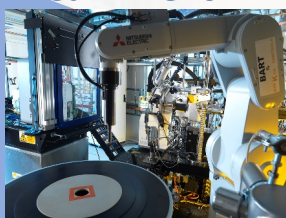
A screening pipeline

XChem Lab at Diamond

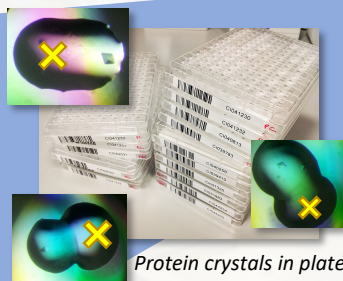
Fragment libraries



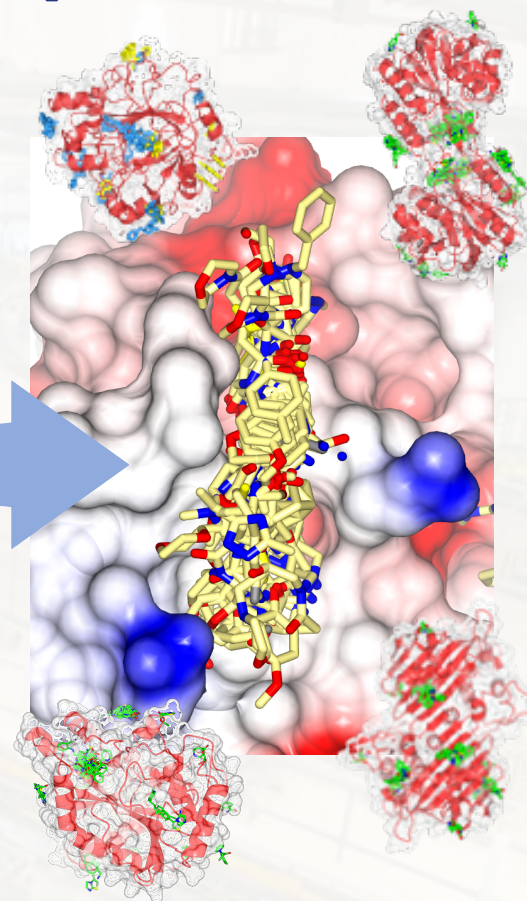
Beamline I04-1



~700 datasets/day, unattended



Protein crystals in plates



Fragment screening directly in crystals (one compound per crystal)

Crystal soaking/harvesting/data collection/processing (up to 1k crystal/week)

Routine since 2016

Since March 2020 COVID-19 targets/moonshot



Access to Diamond

Non proprietary (academic)

Peer reviewed access based on quality

- Free access
- Publishable experiments

Access modes

- **Single** application - 6 months proposal
- **BAG** – 2 year programme
- **Rapid access** – apply anytime
- XChem

<http://www.diamond.ac.uk/Home/ForUsers/academics.html>

Proprietary (industry and academia)

Organized via Industrial Liaison Office

- No need to publish (industry and academia)
- Allocated slots throughout the schedule
- 10% of allocated beamtime
- Direct access
- Remote access
- Mail-in data collection service
- XChem fragment screening

<http://www.diamond.ac.uk/Home/industry.html>





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bright ideas start here