DLS-CCP4 Data Collection and Structure Solution Workshop
6 November 2023

MX at Diamond

Marco Mazzorana

Diamond and the Harwell Campus

Research Complex at Harwell

The Rosalind Franklin Institute

CCP4

Medical Research Council

Science and Technology Facilities Council

diamond

What is Diamond

The UK national synchrotron
(86% STFC, 14% Wellcome Trust)

20 years from foundation

15 years of operations

What is Diamond

Phase 1

7 beamlines completed 2007

Phase 2

+15 beamlines completed 2012

Phase 3

+11 beamlines to be available by 2020

Many different instruments…

33 beamlines
8 Science Groups

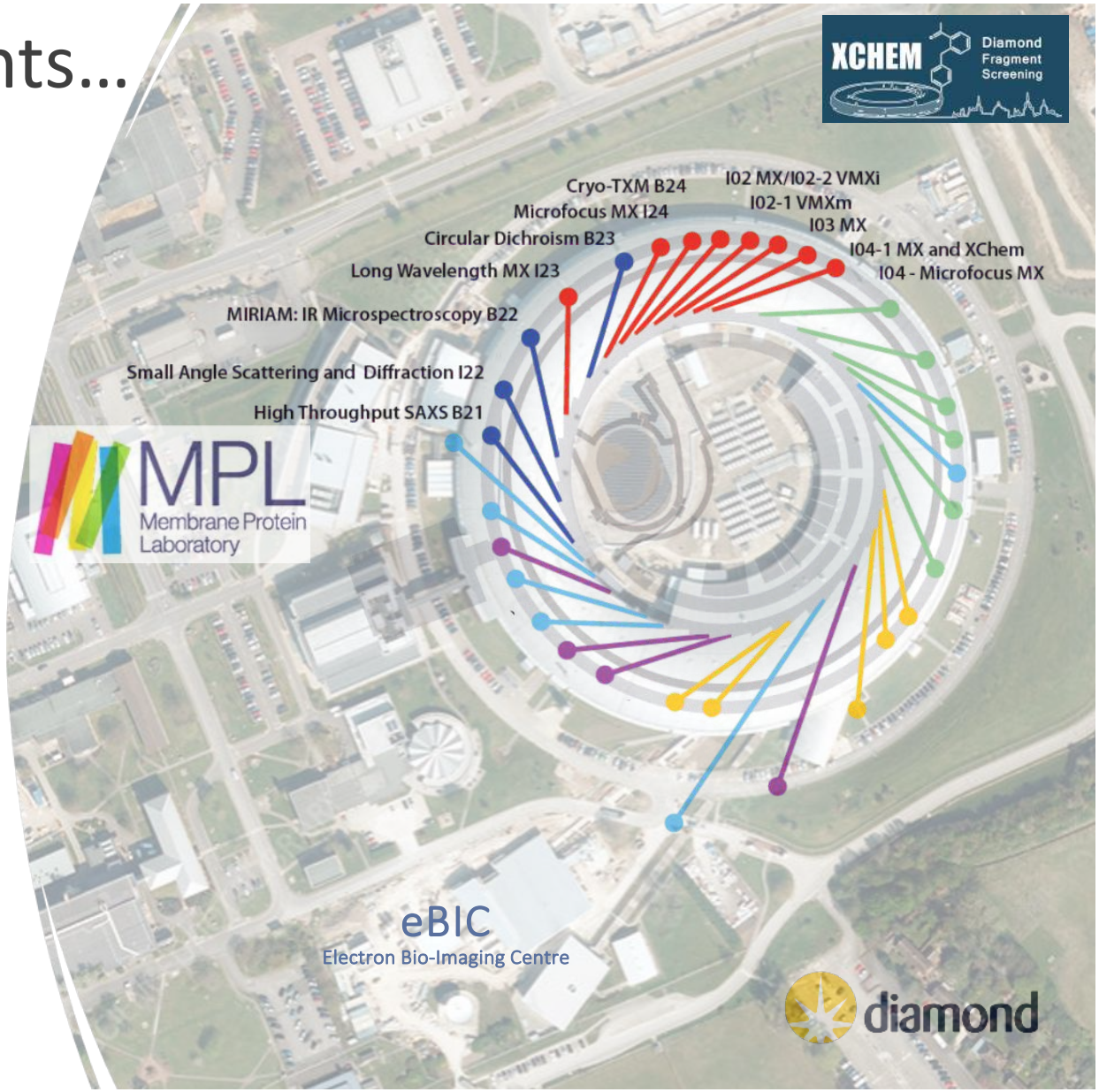
2 national cryo-EM facilities

12k+ users

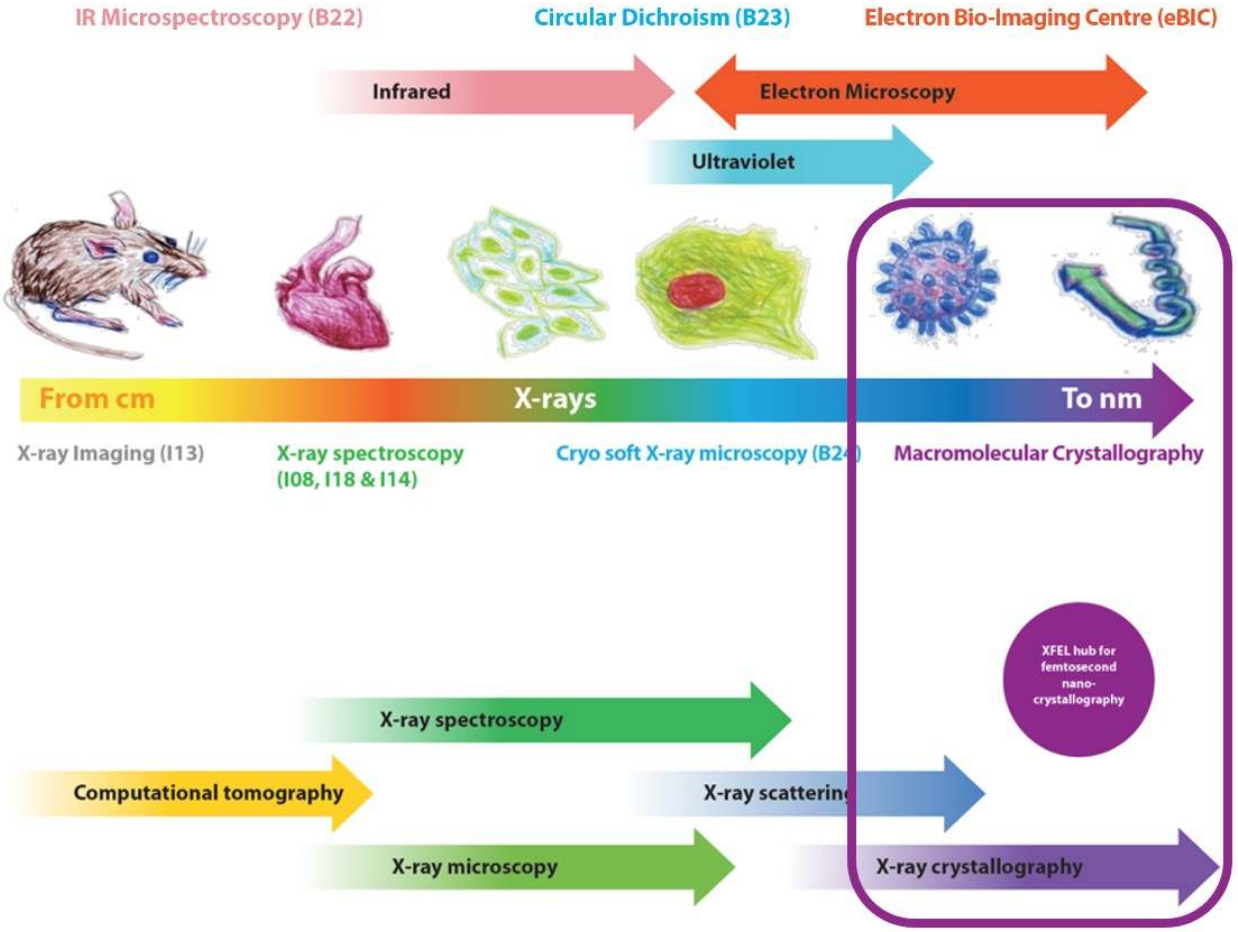
100+ companies

Structural biology labs
MPL, crystallization facility XChem

UK-XFEL Hub



…to investigate the structure of biological systems



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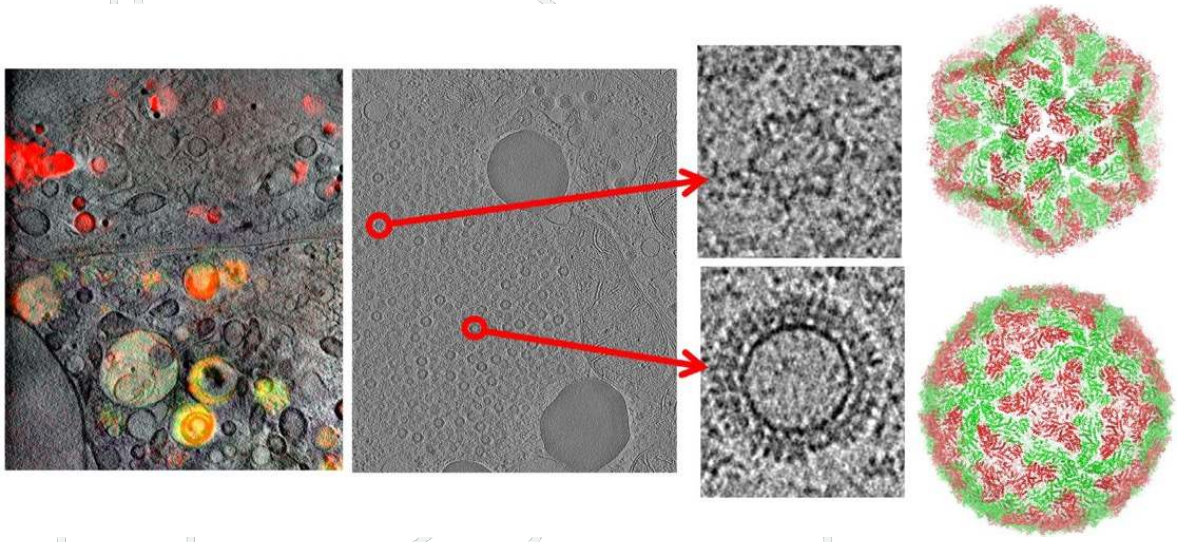
The power of combining techniques



Super resolution cryo-light microscopy

Cryo X-ray tomography

Cryo-electron tomography

Atomic models from MX fit to averaged particles of two intermediates

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

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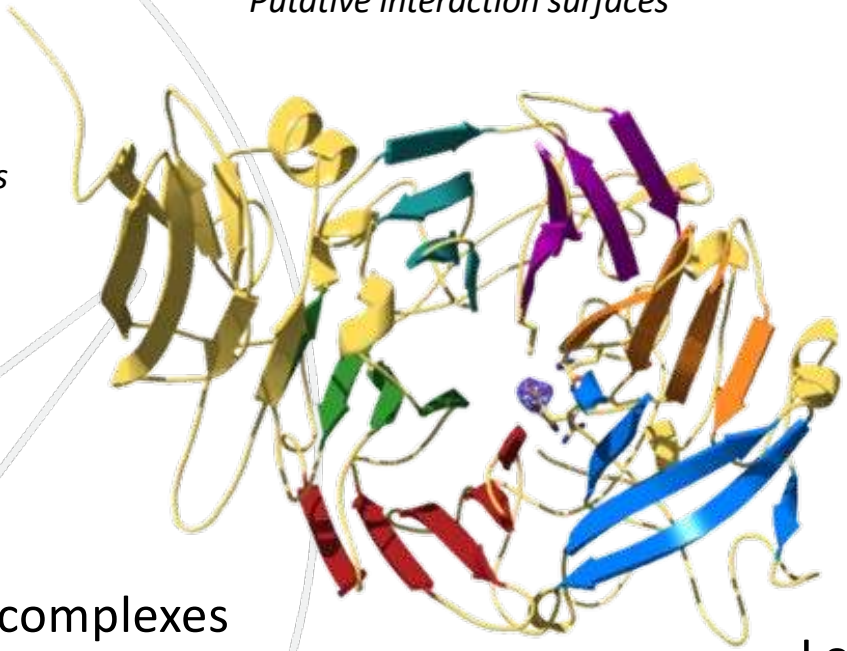
What crystallography tells us about macromolecules

Shape

Crystal packing
Putative interaction surfaces

Electrostatics

Interactions with other molecules
Docking sites



Fold

Putative function
Evolutionary relationships

Protein-ligand complexes

Functional sites
Druggability

Local structure

Catalytic mechanism
Conformation determinants

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Challenges and synergies

- 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

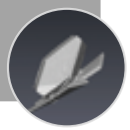
Experimental Phasing

- *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

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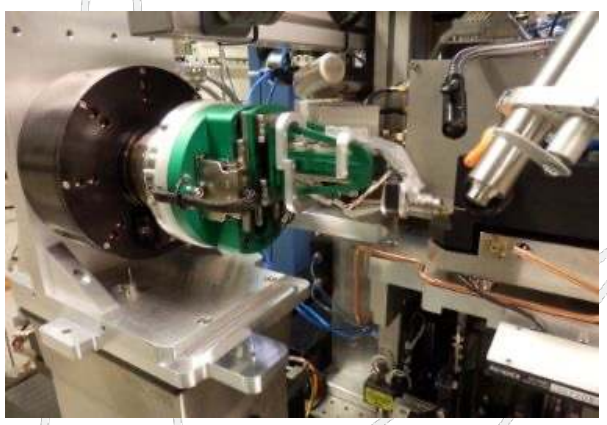
Dealing with the smallest crystals: microfocus

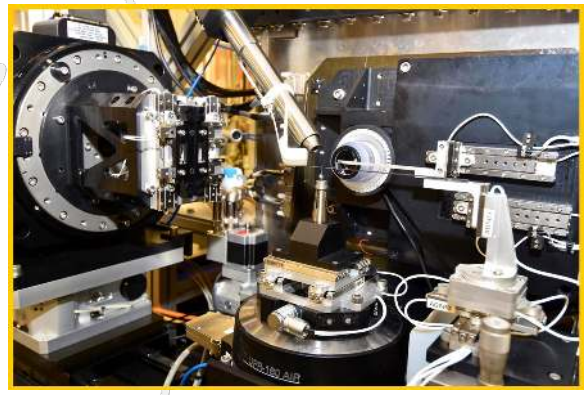
- Tuneable
- Rapid
- Variable focus
- High flux
- *In situ*

Microfocus

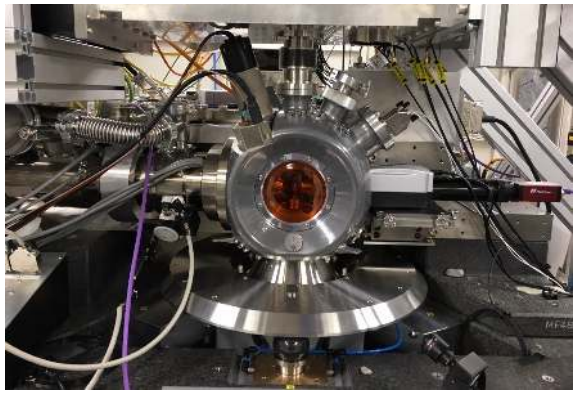
3 microfocus beamlines



I04



I24



VMXm

diamond

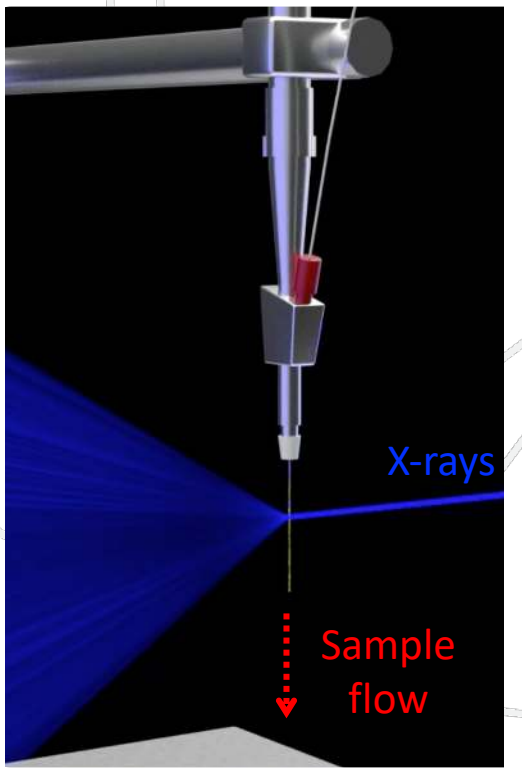
Microfocus for time-resolved studies

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

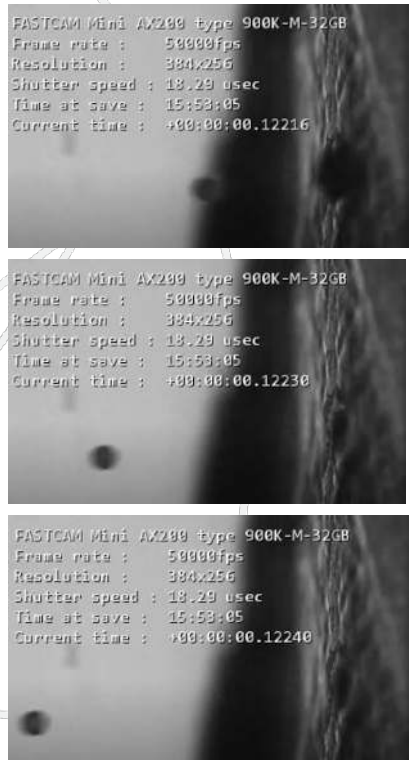
Microfocus

Sample delivery for serial crystallography (SSX and SFX)

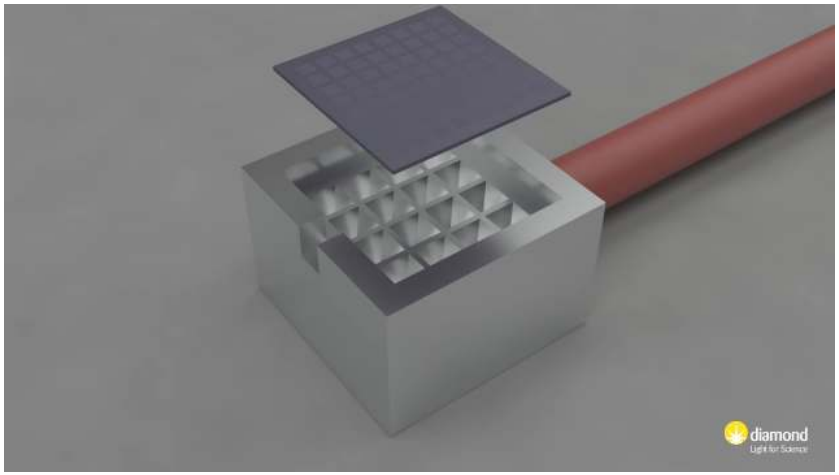
Liquid jets/ LCP extruders



Acoustic droplet ejection



Fixed target



Silicon nitride chips
25,600 positions/chip

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Microfocus for time-resolved studies

- Tuneable
- Rapid
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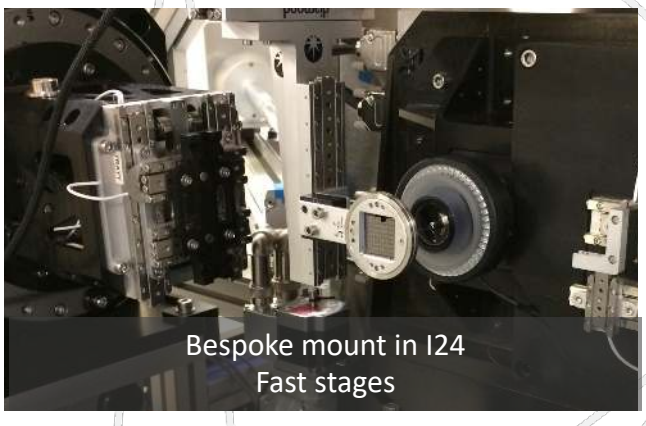
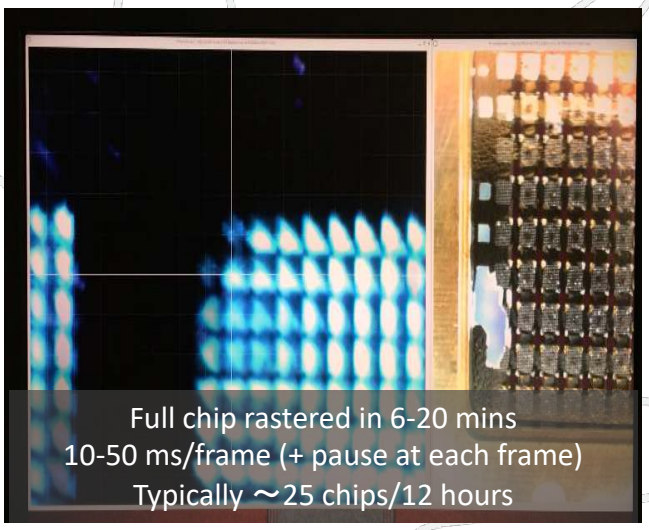
Microfocus

Fixed-target at I24

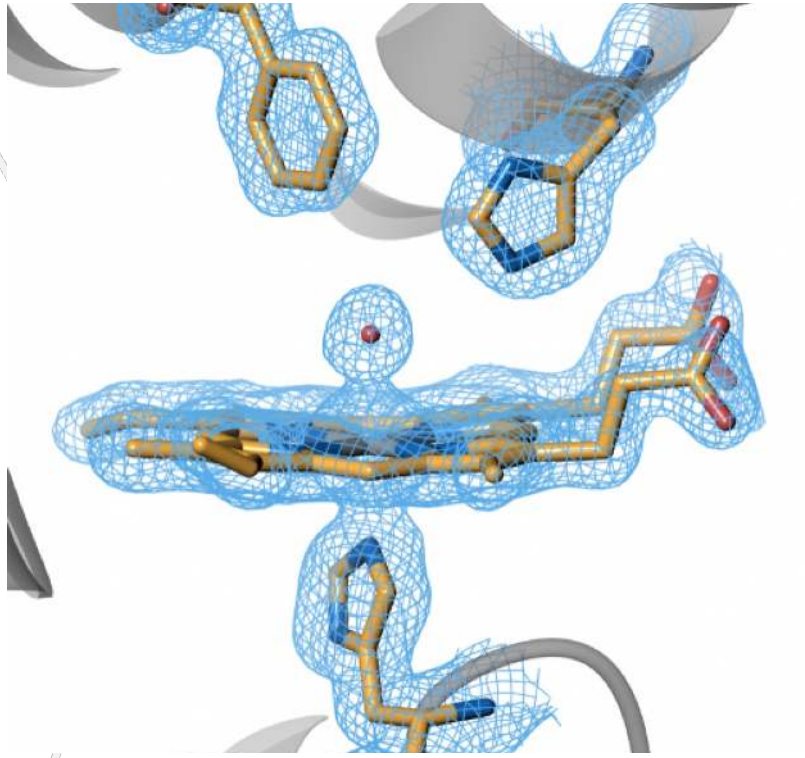


Bespoke mount in I24
Fast stages



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

Multi-crystal structures (from stills)



1.5 Å structure
from 9000 crystals

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The UK-XFEL Hub

European XFEL

LCLS

SACLA

PAL
POHANG ACCELERATOR LABORATORY

UK XFEL

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

Microfocus

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)

BBSRC bioscience for the future

W wellcome

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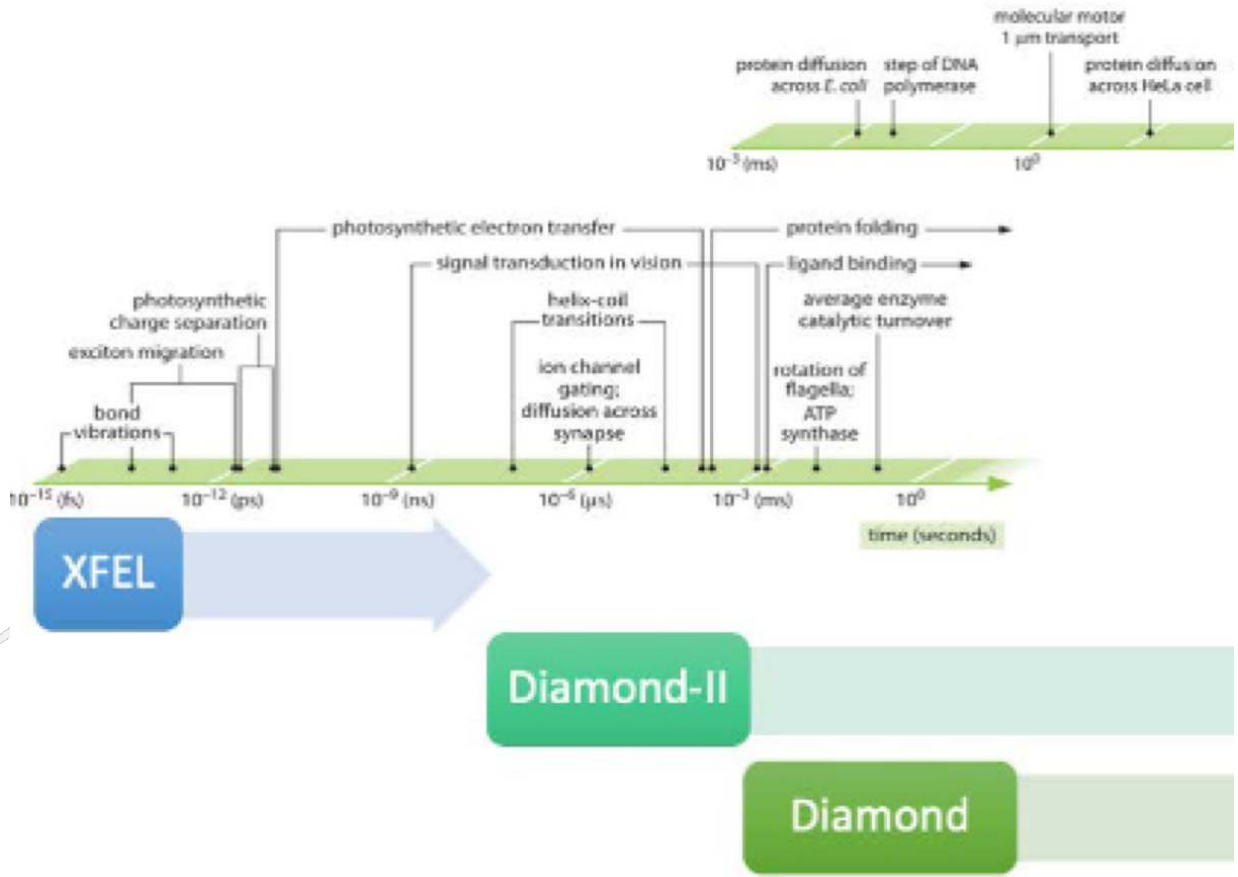
Extending the time range



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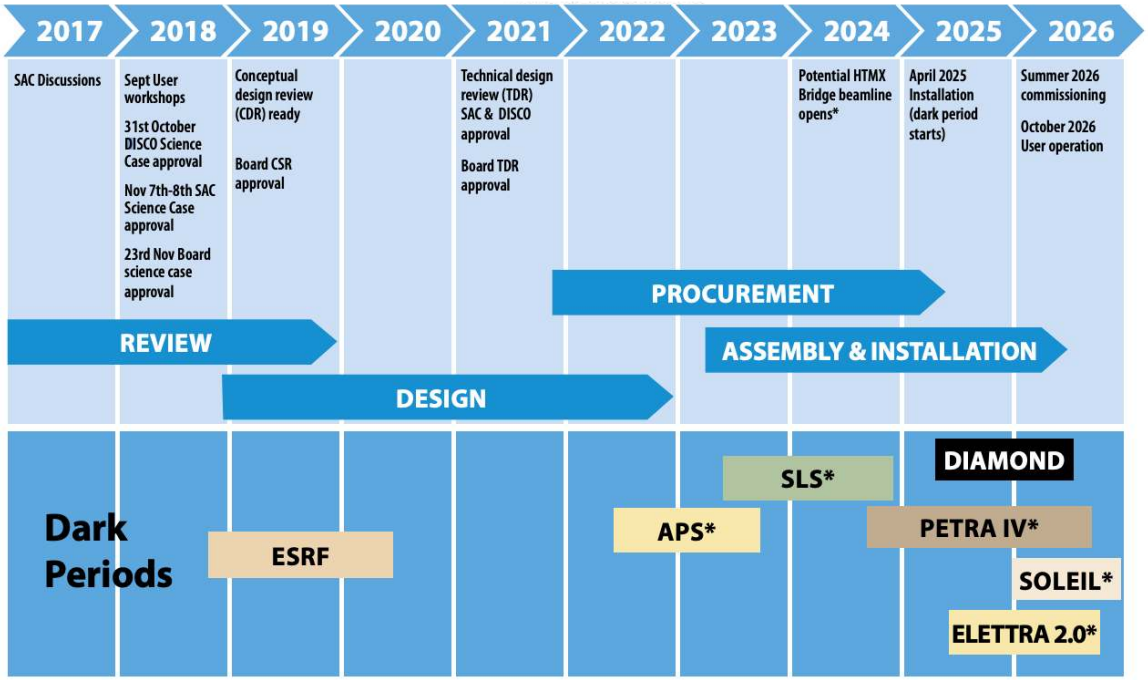
Microfocus

diamond

The Diamond-II project

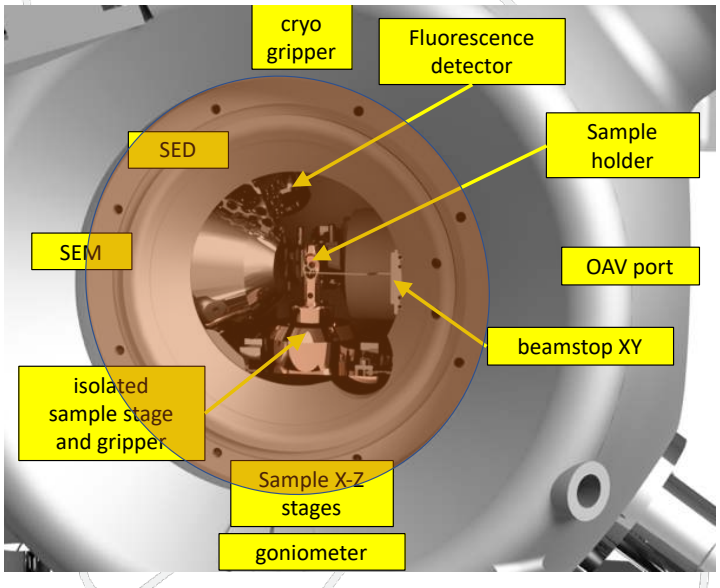
4th generation synchrotrons and XFELs have hotter beams



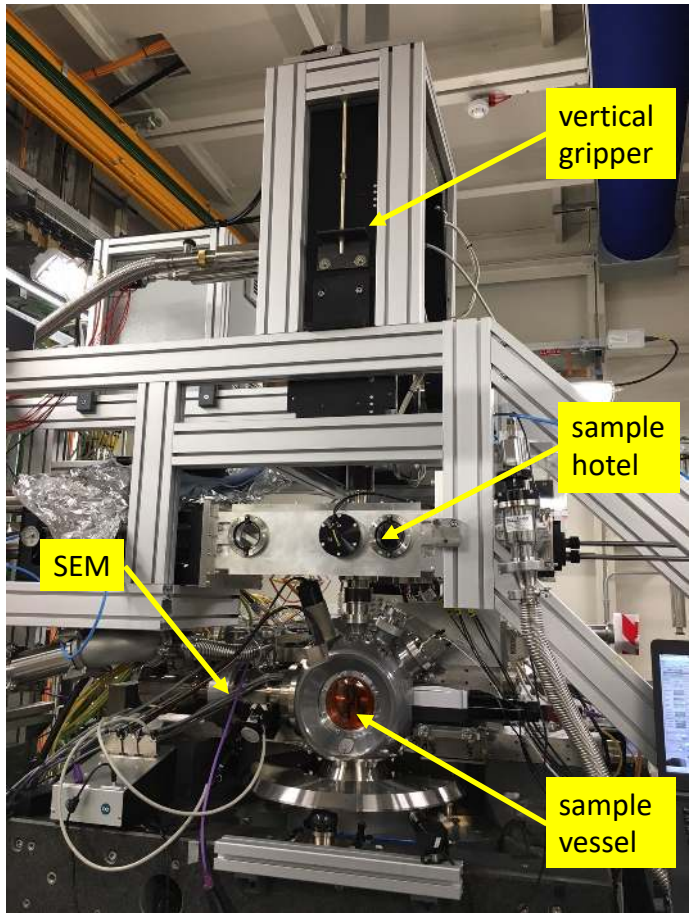
diamond

Smaller than visible

VMXm: the first nano-focus beamline



< 500 nm beamsize
Integrated SEM
In vacuum
Tunable 7-25 keV (2.0-0.57Å)



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Microfocus

diamond

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VMXm: the first nano-focus beamline

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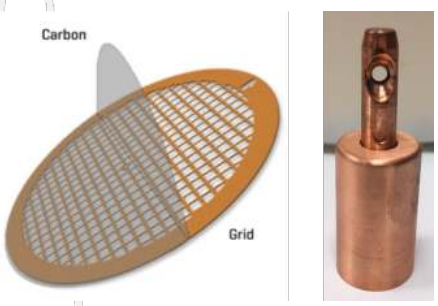
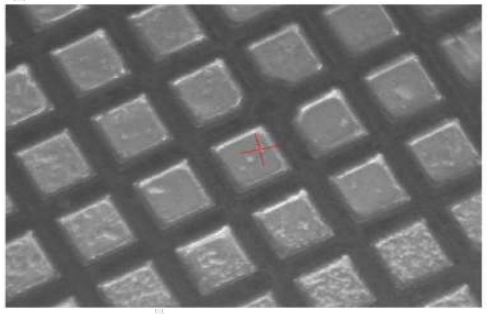
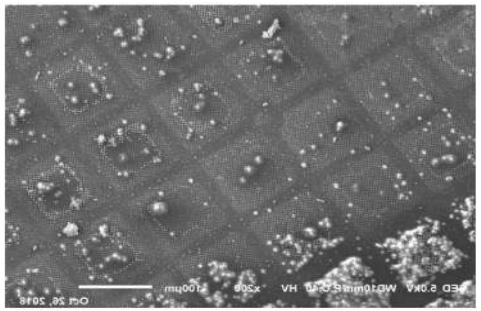
Microfocus



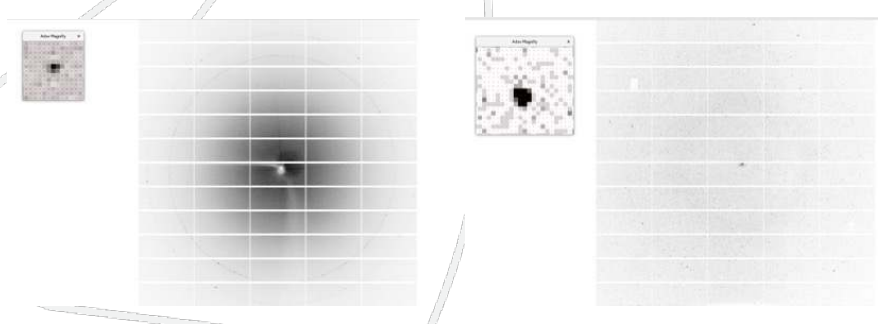
EM-grids as sample holders



OAV



SEM

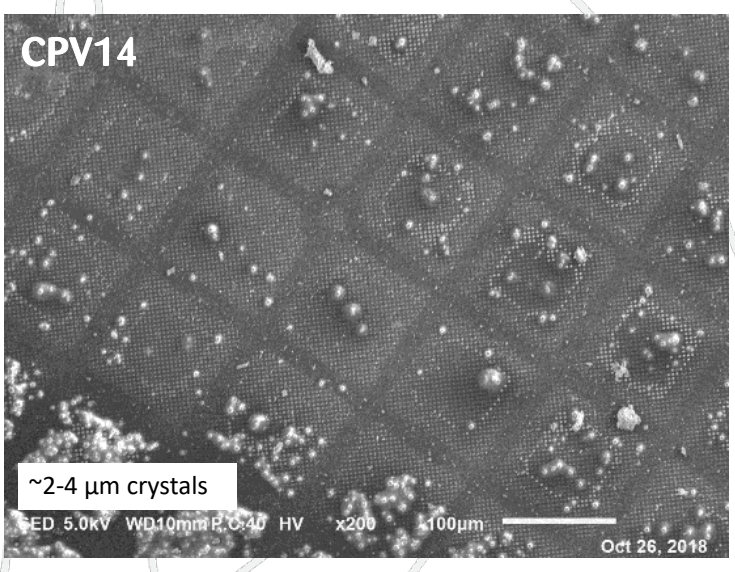


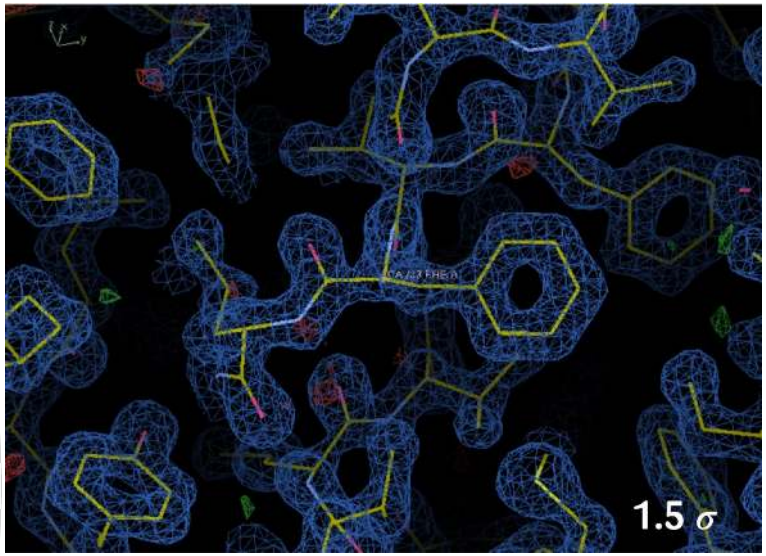
extremely low background

diamond

Smaller than visible

VMXm: the first nano-focus beamline





27 crystals
1.5-2.0 deg/data per crystal

Rigid body+restrained refinement
Dials, CCP4, Refmac, Coot

Summary of merging statistics

	Overall	Low resolution	High resolution
Resolution (Å)	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

- Tuneable
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Microfocus

diamond

The need for longer wavelenght

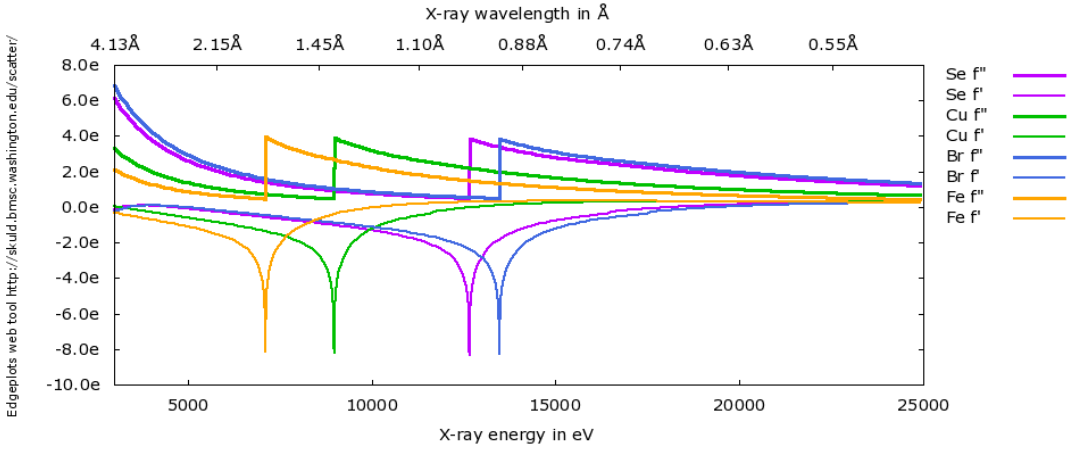
- Tuneable beamlines
- I23 dedicated
- Smart data collection strategies
- Expert systems

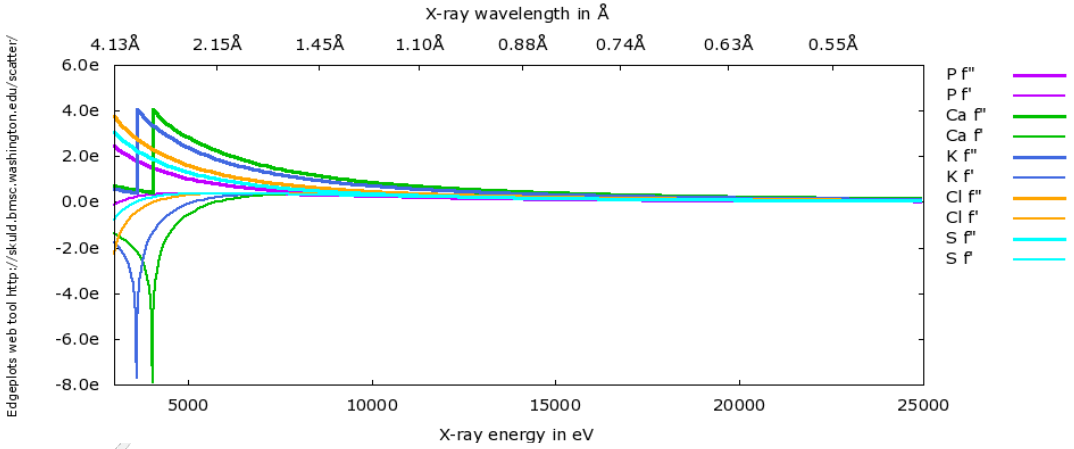
Long Wavelength

Biologically relevant elements

	e (keV)	λ(Å)
P	2.146	5.779
S	2.472	5.016
Cl	2.822	4.393
K	3.607	3.437
Ca	4.038	3.070

Xray absorption edges outside the capabilities of standard beamlines





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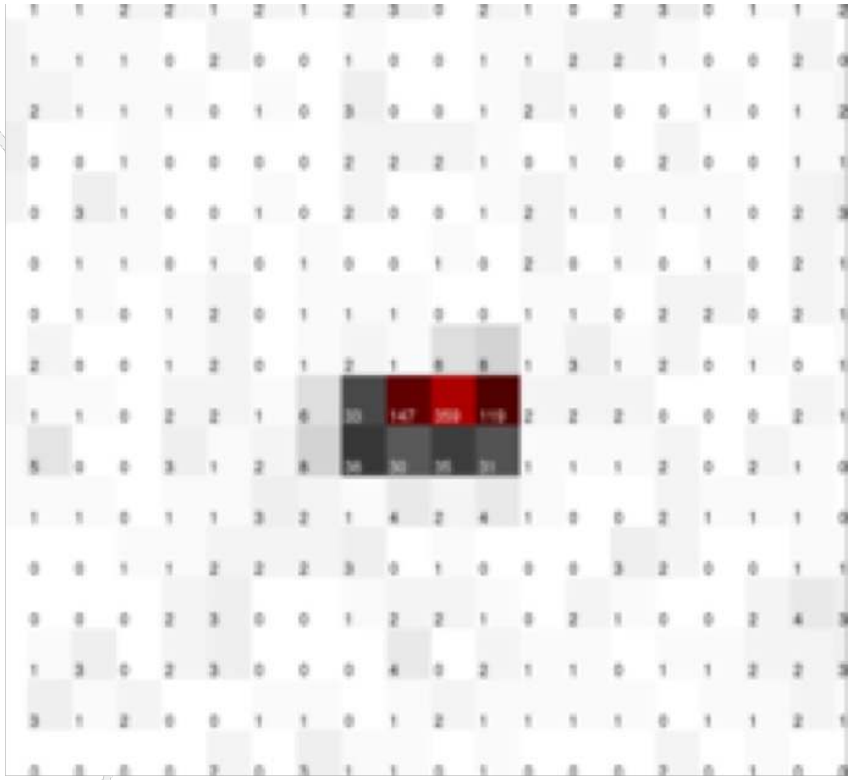
Challenges and solutions



- Tuneable beamlines
- I23 dedicated
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Long Wavelength

Long wavelength increases the angle of diffraction
Need a curved detector to measure high resolution spots

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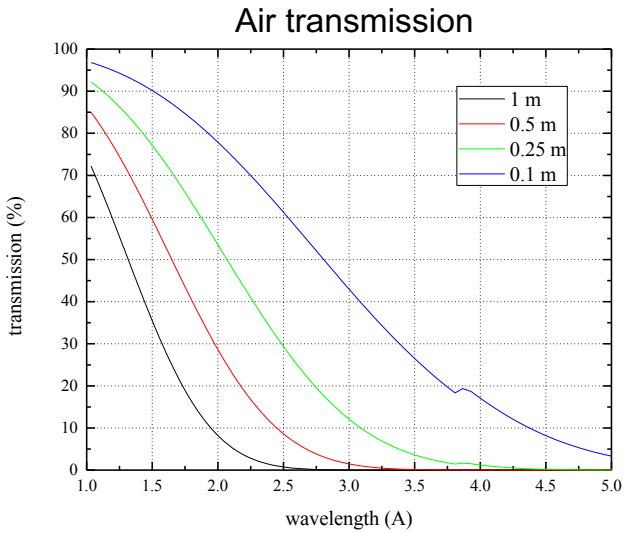
Challenges and solutions

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

Long Wavelength

Absorption by air of long wavelength X-rays



In vacuum beamline

Bespoke sample holders

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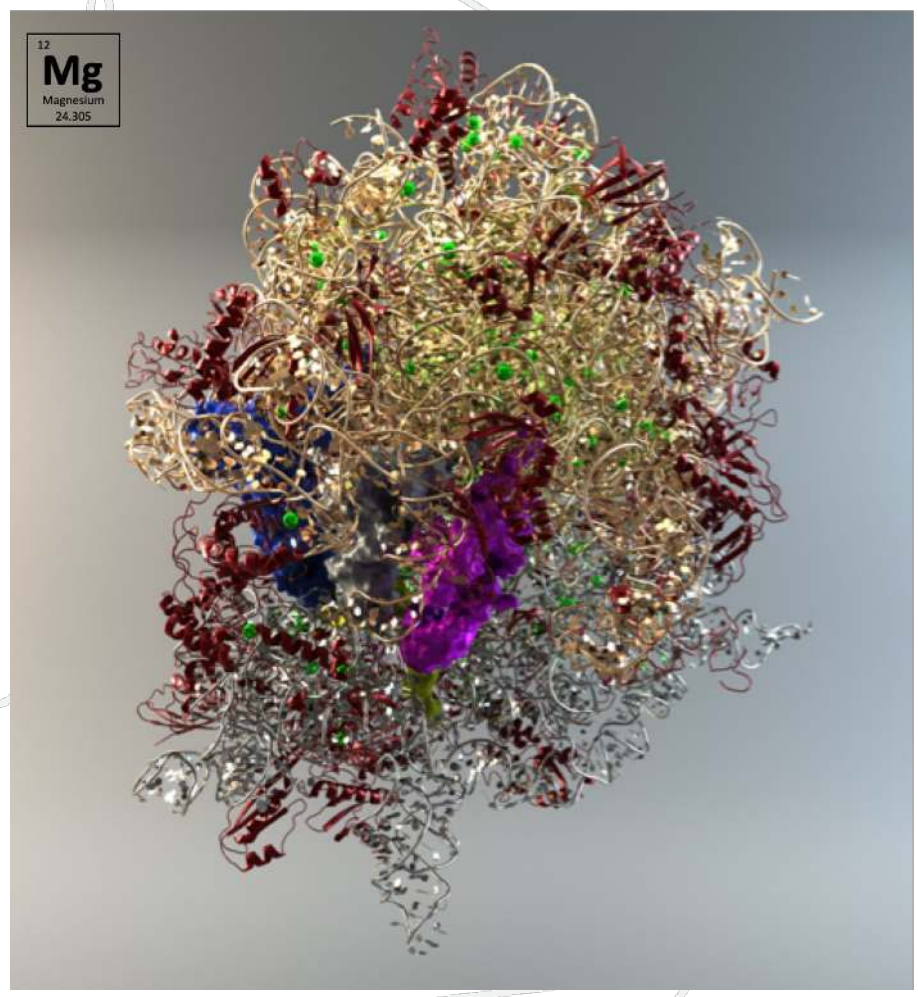
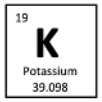
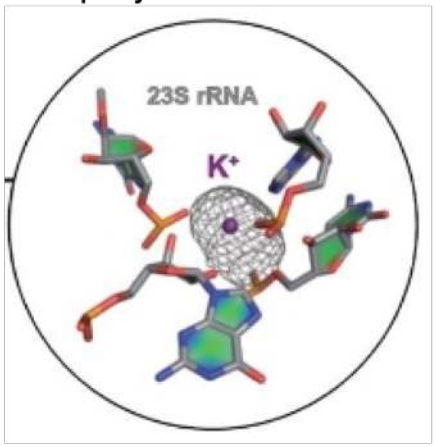
Results: metal identification

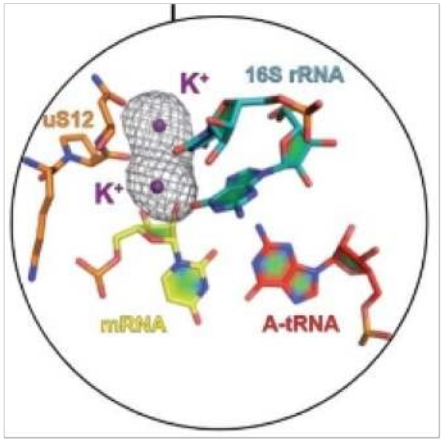




Peptidyl transferase center



Decoding center



- Tuneable beamlines
- I23 dedicated
- Smart data collection strategies
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Long Wavelength

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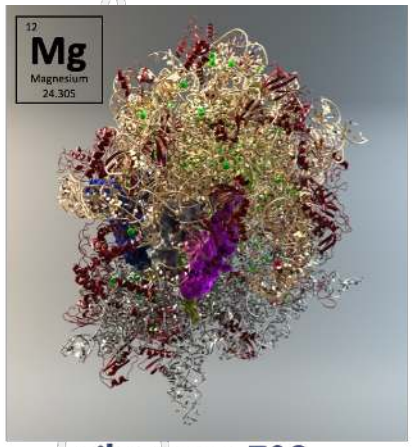
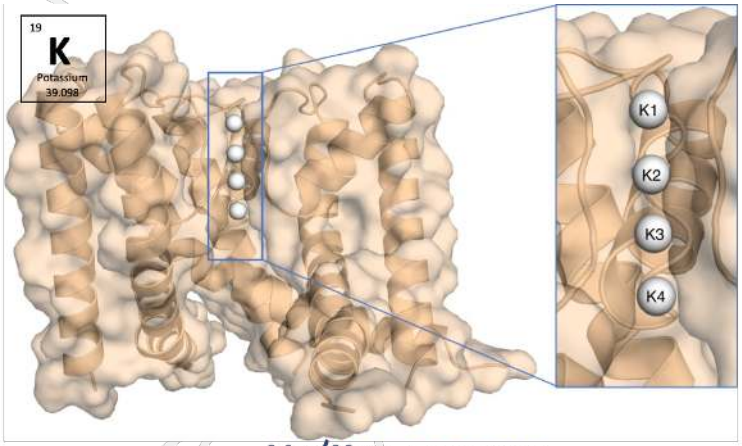
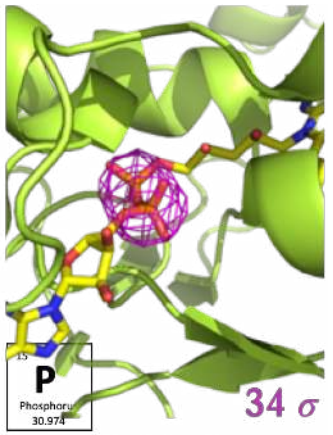
Extending the element 'palette'



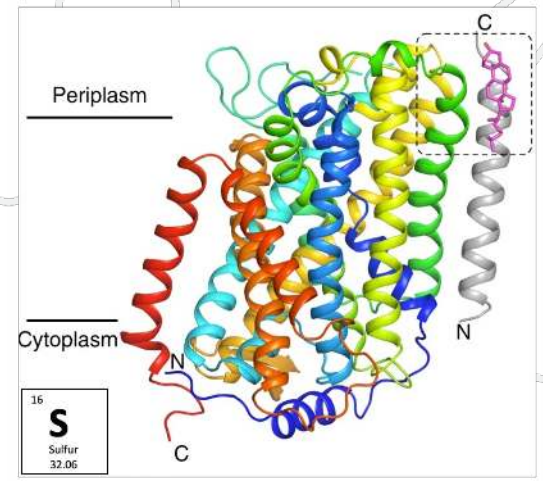
ribosome 70S



Na/K transporter

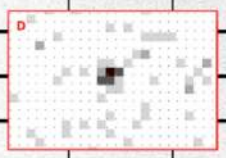


FAD-binding

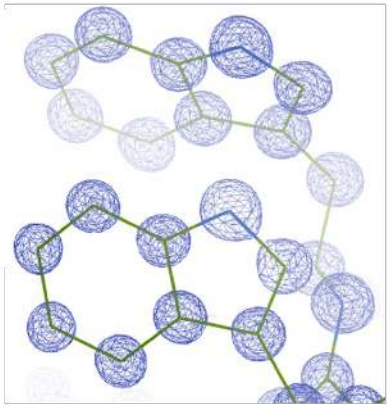


SLC7 transporter

- Data to 0.92 Å resolution
- No signs of specific radiation damage
- Very low background (1.6 counts in water ring, 0.1 counts at 1.0 Å resolution) from curved detector in vacuum



PETase



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

Long Wavelength

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High throughput

BART sample changer:
37 unipucks
597 samples
Exchange rate approx. 20 sec/sample

EIGER2 XE 16M
Pixel size: 75x75 µm^2
18,093,576 pixel
560 frames/sec (3600 frames in <7 sec)

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

High Throughput

diamond

Logistics

Weland/Randex automated store

high capacity (up to 272 dewars)
barcode tracking (dewars and pucks)
logistics integrated in Synchweb/ISPyB

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

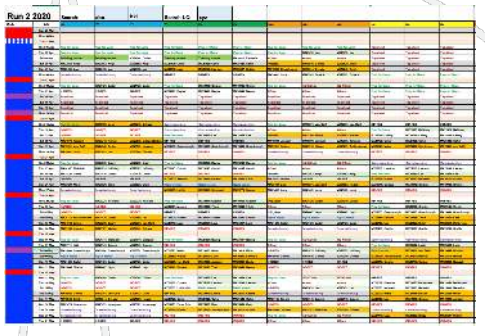
High Throughput

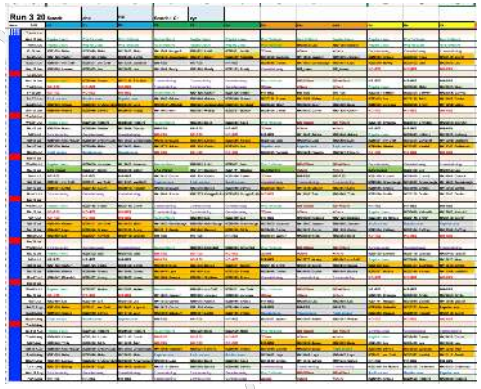
Responsive/dynamic scheduling

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

High Throughput

2019





2020

2022

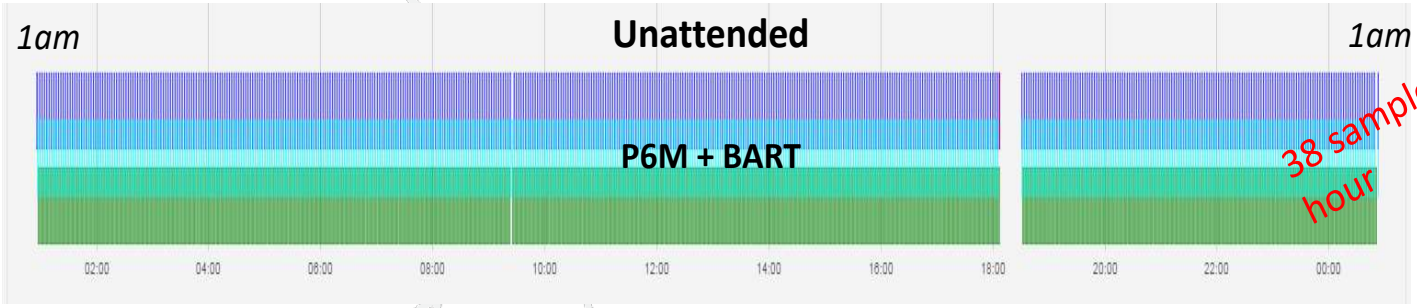
diamond

Unattended data collection

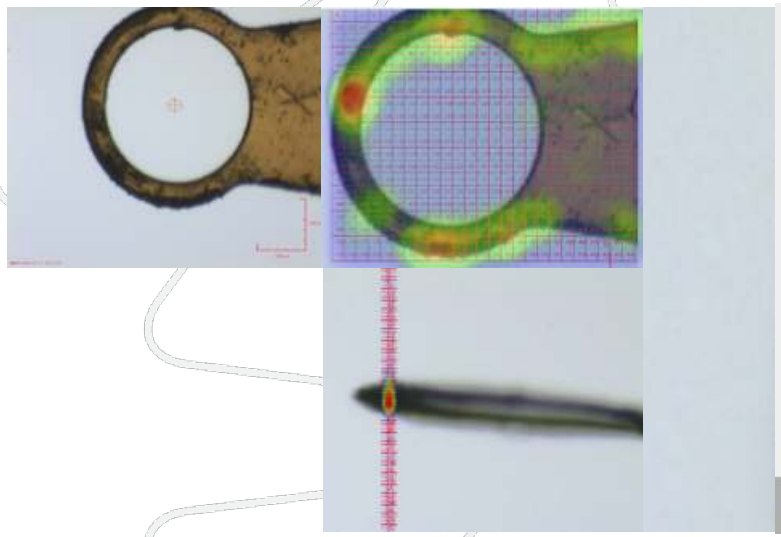
- PADs
- Automation
- Pipelines
- Serial Crystallography
- Access (rapid/freq)

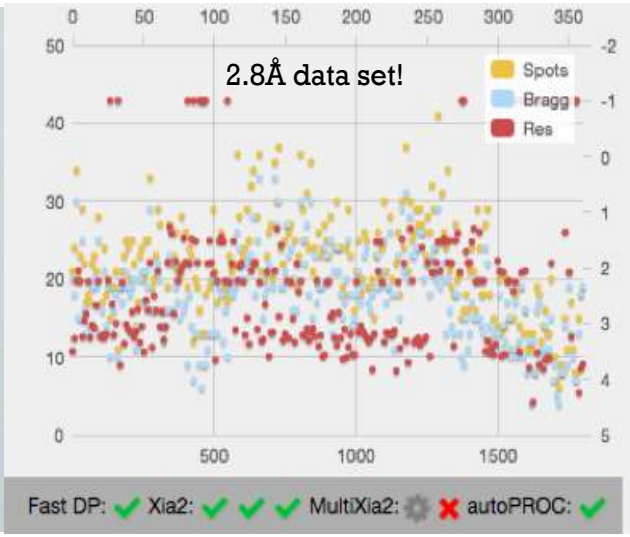
High Throughput

Optimized for speed



Xray-centering and strategies





diamond

Automated processing pipelines

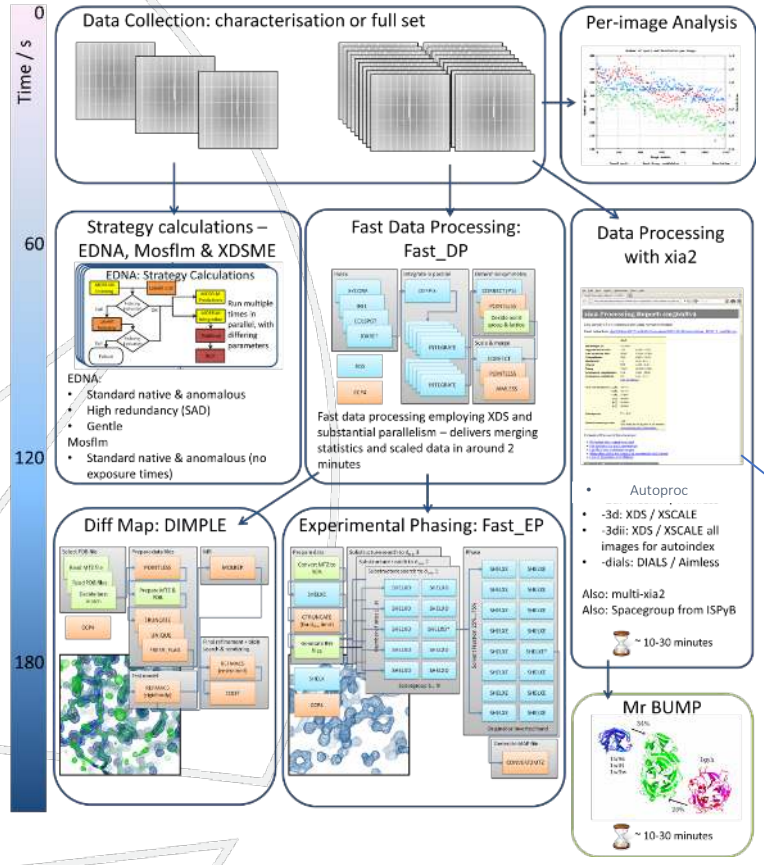
- PADs
- Automation
- Pipelines
- Serial Crystallography
- Access (rapid/freq)

High Throughput

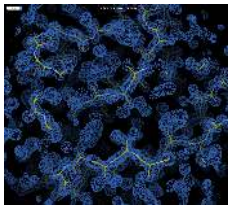
Data Collection

Data Reduction

Structure Solution



BigEp



Thorough Phasing Pipeline Up to 24 hours

diamond

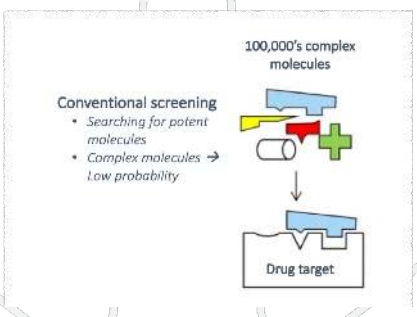
HT fragment screening

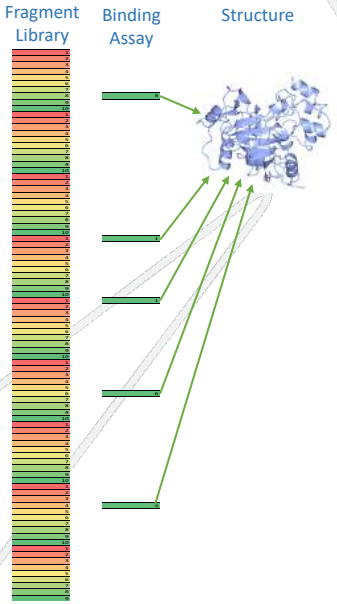
- PADs
- Automation
- Pipelines
- Serial Crystallography
- Access (rapid/freq)

High Throughput

Conventional screening

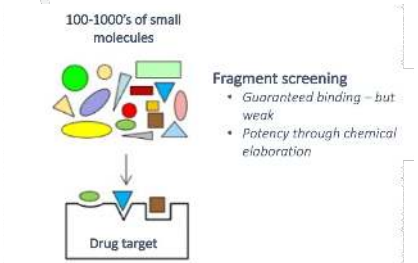
Preselection of best compounds

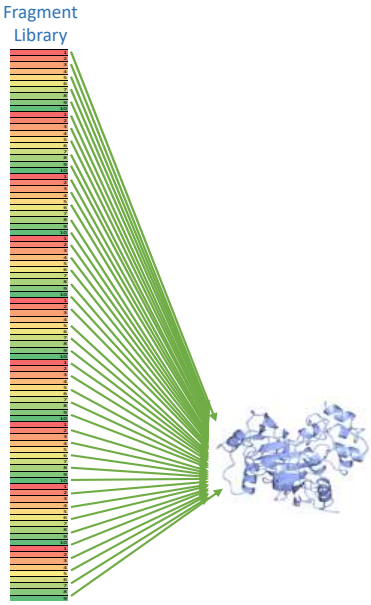




Fragment-based approach

Screen by crystal

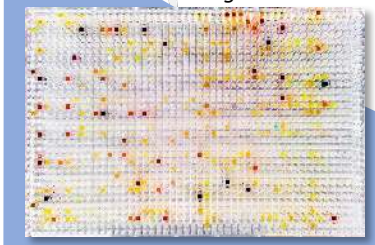




HT fragment screening

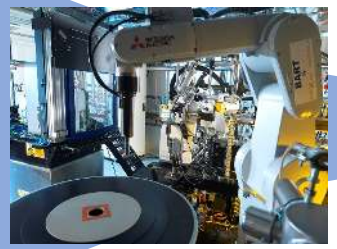
XChem Lab at Diamond

Fragment libraries

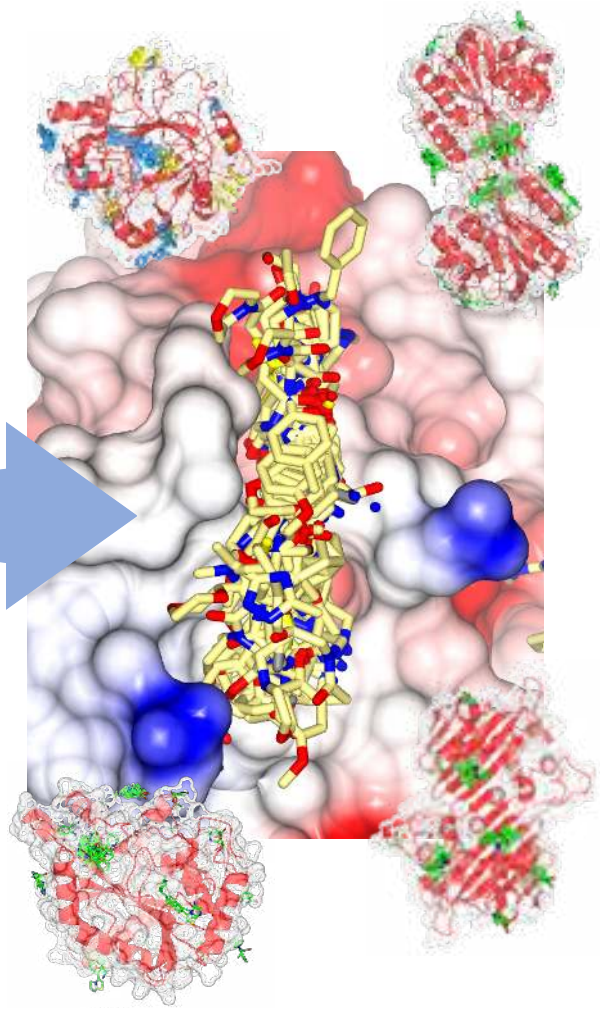


Protein crystals in plates

Beamline I04-1



~700 datasets/day, unattended



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

High Throughput

1 crystal – 1 fragment

Soak/harvest/collect data
(up to 1000 crystals/week)

Routine since 2016

March 2020 COVID-19 targets

diamond

Access to Diamond

Non-proprietary (academic)

Peer-review access based on quality of proposal
- Free access
- Publishable experiments

Access modes:
- **BAG** (2 year programme)
- **Single application** (6 months)
- **Rapid access** (apply anytime for one-off experiments)

MX beamlines, XChem

iNEXT

instruct ERIC

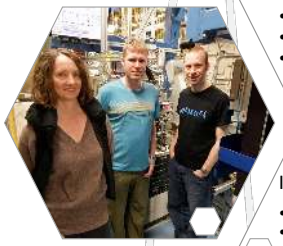
Proprietary (industry and academia)

Organized via Industrial Liaison Office
- No need to publish
- Allocated slots throughout the schedule
- 10% of allocated beamtime

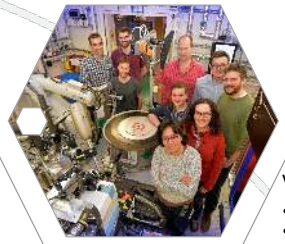
Services provided:
- Direct and remote access
- Unattended data collection
- Mail-in data collection and analysis service
- XChem fragment screening

diamond

The team

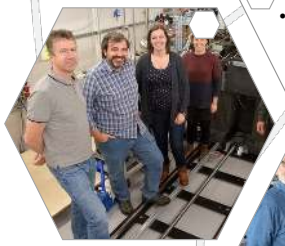


I04-1 / XChem
- **Frank von Delft**
- Jose Brandao-Neto
- Louise Dunnett
- Kutumbarao Nidamarthi
- Daren Fearon
- Charlie Thomlinson

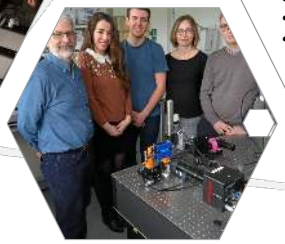


I04
- **Ralf Flaig**
- David Aragão
- Marco Mazzorana
- Kutumbarao Nidamarthi
- Pierpaolo Romano

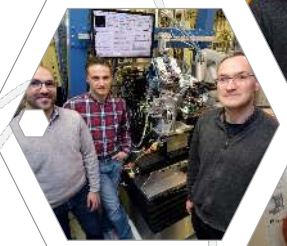
I03
- **Dave Hall**
- Neil Paterson
- Mark Williams
- Felicity Betram



VMXm
- **Gwyndaf Evans**
- Jose Trincao
- Anna Warren
- Adam Crawshaw

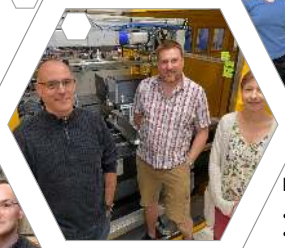


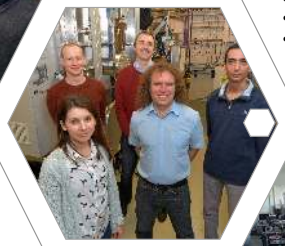
VMXi
- **Mike Hough**
- Juan Sanchez-Weatherby
- James Sandy
- Halina Mikolajek

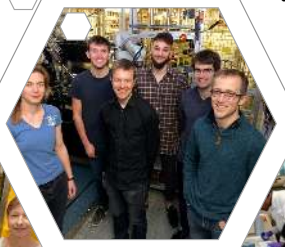


XFEL-Hub
- **Allen Orville**
- Pierre Aller
- Anastasiia Shilova
- Tiankun Zhou
- Jos Kamps

I24
- **Robin Owen**
- Danny Axford
- Sofia Jaho
- Do-Heon Gu
- Pierre Aller
- Anastasiia Shilova



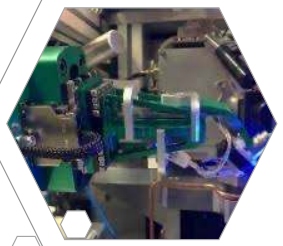




I23
- **Armin Wagner**
- Kamel el Omari
- Vitaliy Mykhaylyk
- Ramona Duman
- Christian Orr

MPL
- **Andrew Quigley**
- James Birch
- Harish Cheruvara

MX User Support
- Marco Mazzorana
- Kutumbarao Nidamarthi
- Felicity Bertram



Data Analysis & Management
- Graeme Winter
- Irakli Sikharulidze
- Neil Smith
- Karl Levik
- Stu Fisher

DIALS
- Nicholas Devenish
- James Beilsten-Edmands
- Luis Fuentes-Montero
- Graeme Winter
- David Waterman

diamond