DLS-CCP4 Data Collection and Structure Solution Workshop
29 October 2024

MX at Diamond

Marco Mazzorana

The UK national synchrotron

2003 Established

2007 First User

(7 beamlines of which 3 MX)

Medical Research Council

Science and Technology Facilities Council

central laser facility

Research Complex at Harwell

The Rosalind Franklin Institute

CCP4

3 rounds of funding

Many different instruments…

33 beamlines
8 Science Groups

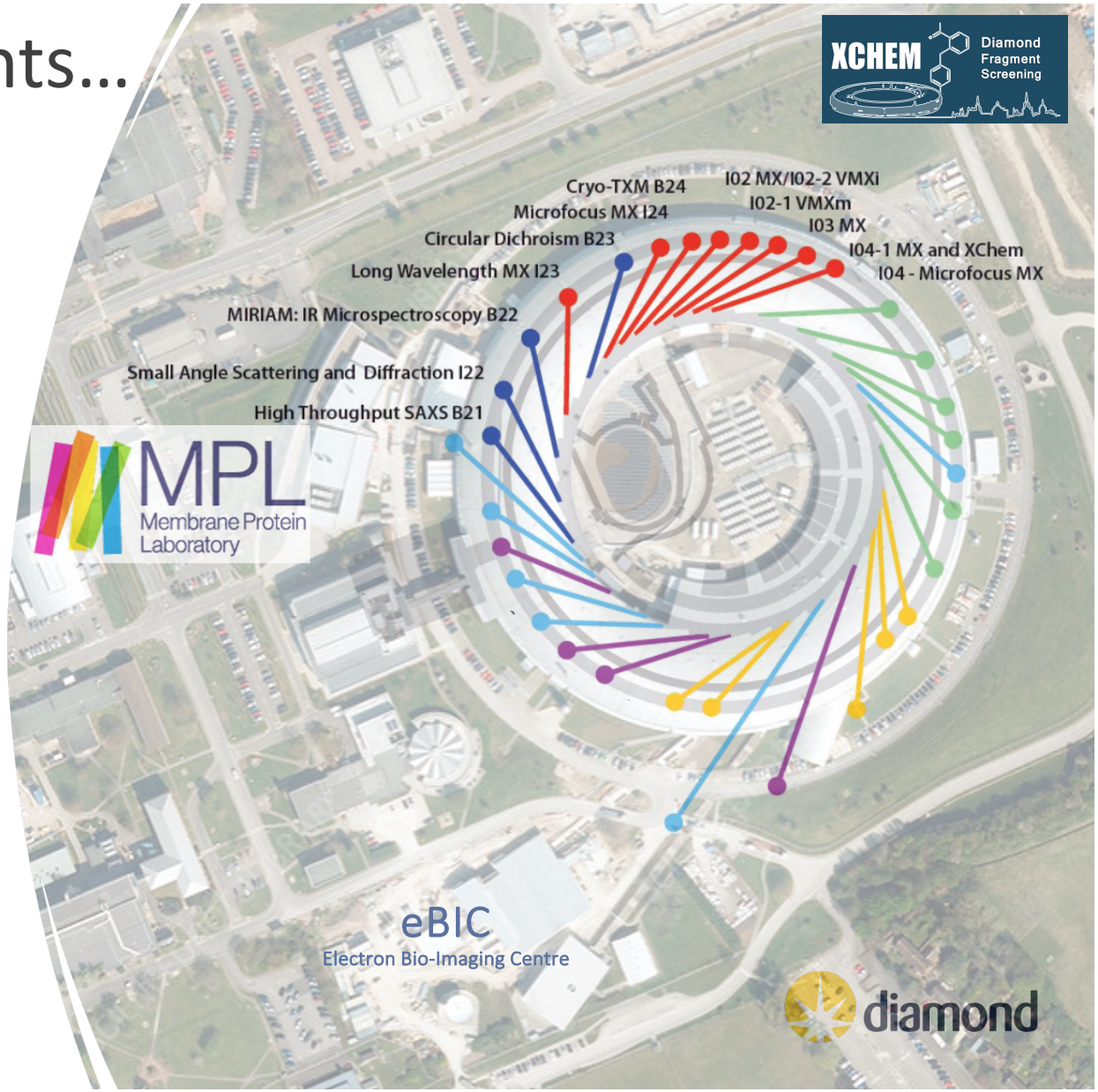
2 national cryo-EM facilities

12k+ users

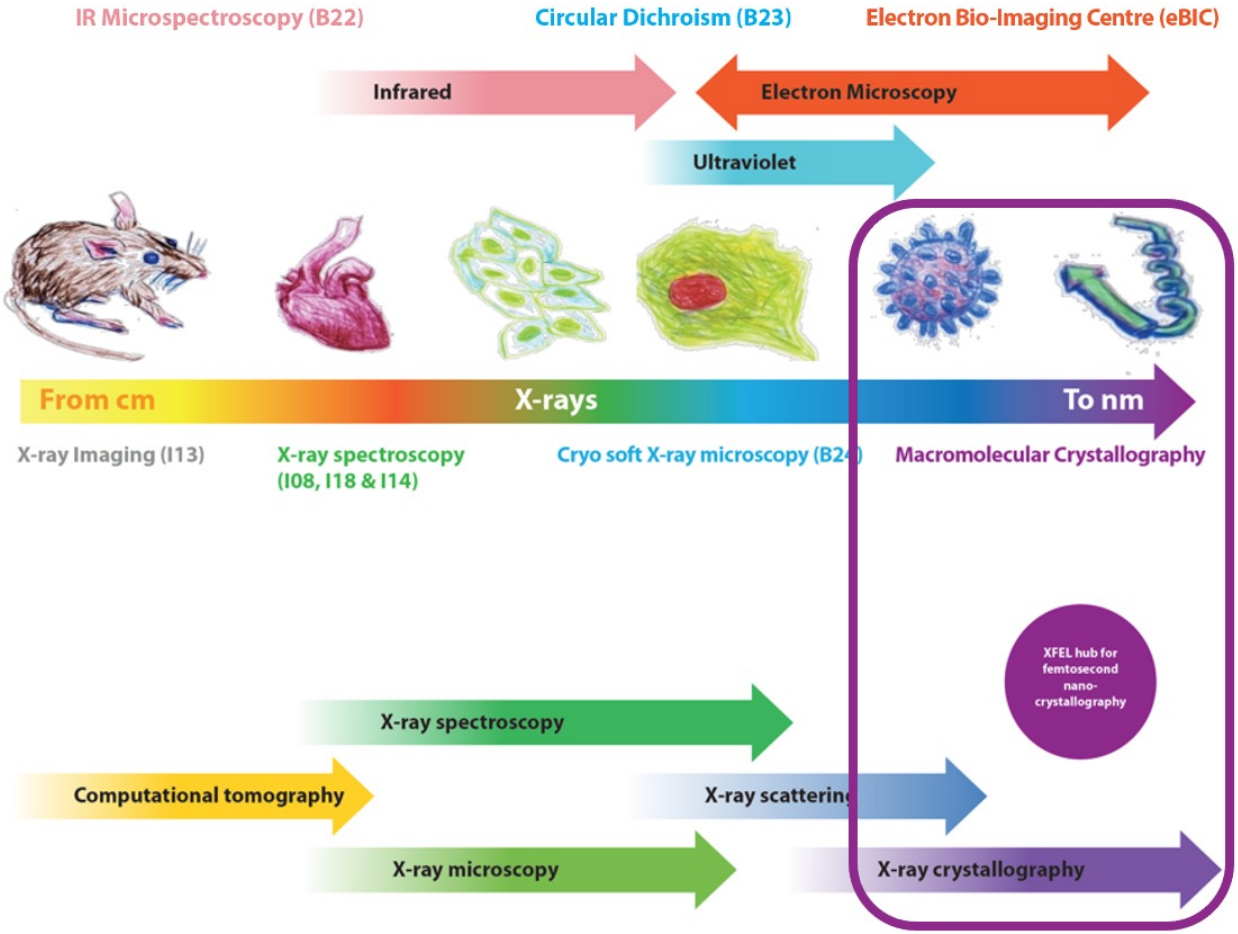
100+ companies

Structural biology labs
MPL, crystallization facility XChem

UK-XFEL Hub

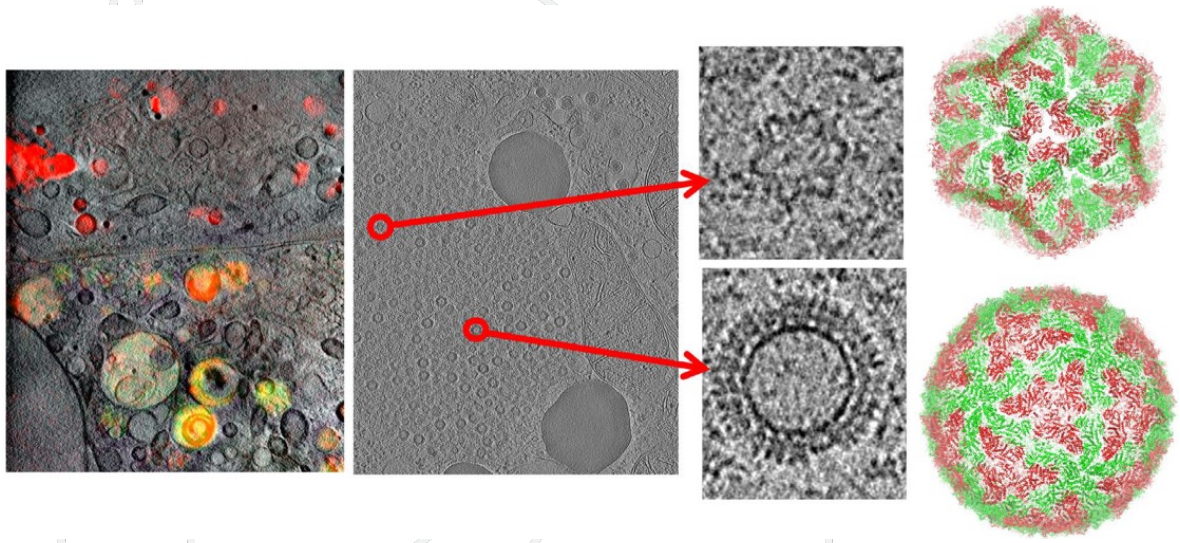


…to investigate the structure of biological systems



diamond

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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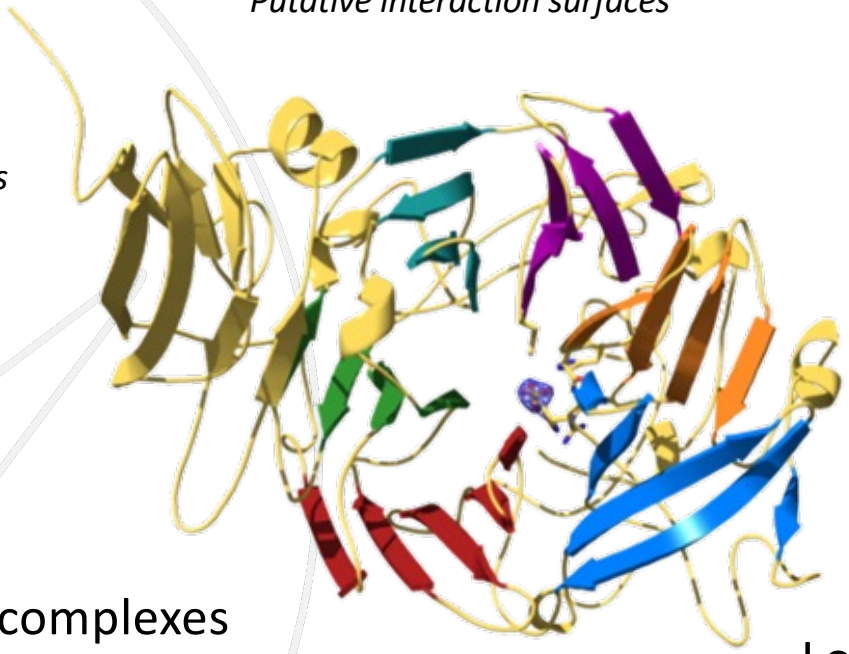
What crystallography tells us about macromolecules

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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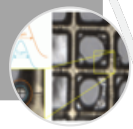
Challenges and synergies

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

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



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

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

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

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

Complementary information
- Humidity control
- Spectroscopy
- Tomography
- Dynamics
- Fluorescence

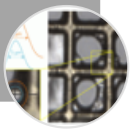
Dials Project
- Diffraction image processing for SR Electron and XFEL data
- Collaborative effort
- Led and managed by DLS

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

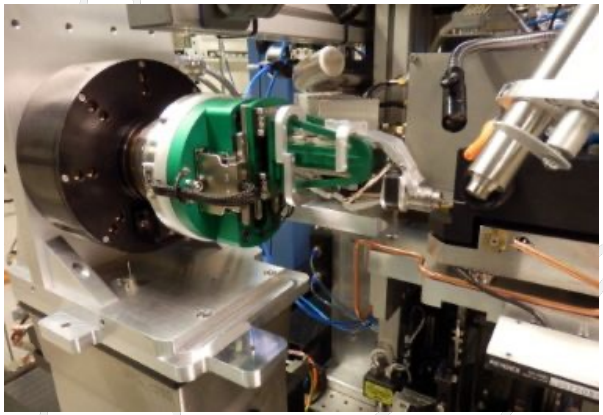
Dealing with the smallest crystals: microfocus

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

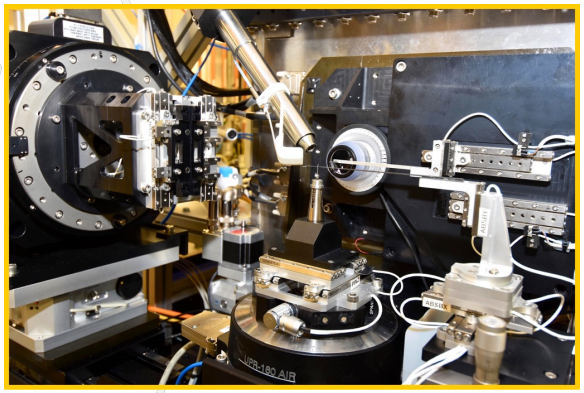
Microfocus



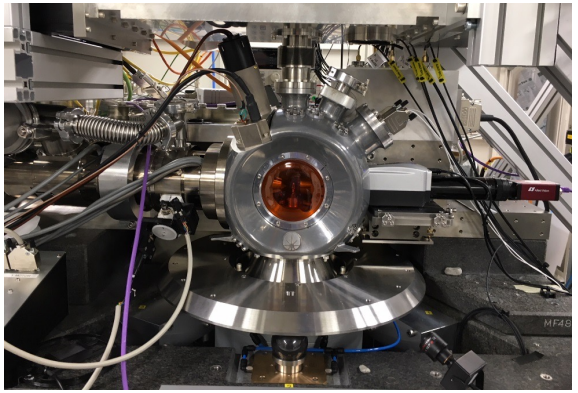
3 microfocus beamlines



I04



I24



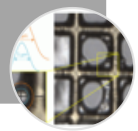
VMXm

diamond

Microfocus for time-resolved studies

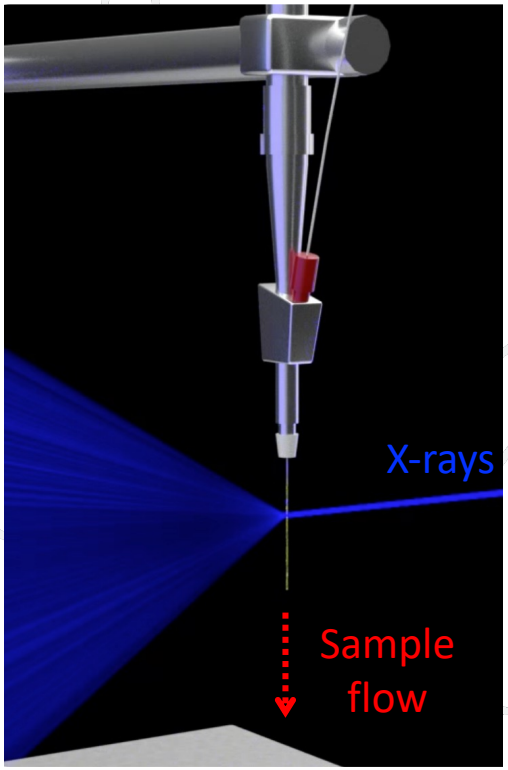
- Tuneable
- Rapid
- Variable focus
- High flux
- *In situ*

Microfocus

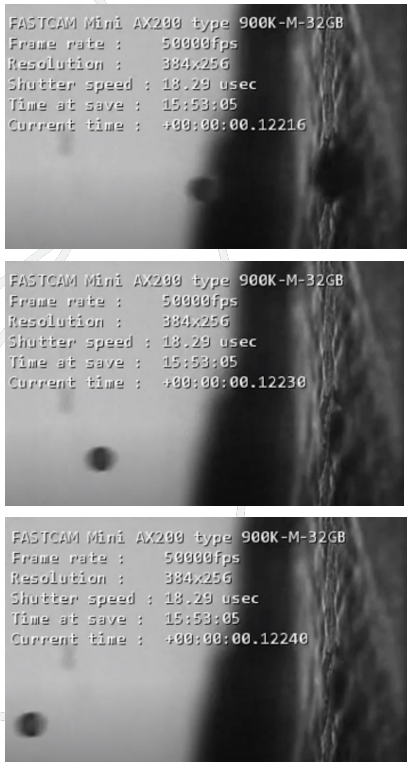


Sample delivery for serial crystallography (SSX and SFX)

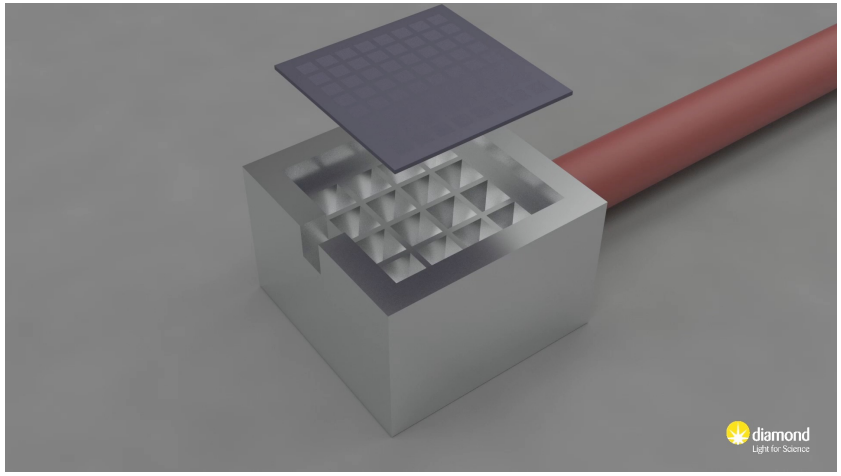
Liquid jets/ LCP extruders



Acoustic droplet ejection



Fixed target



Silicon nitride chips
25,600 positions/chip

diamond

Microfocus for time-resolved studies

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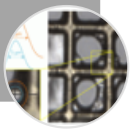
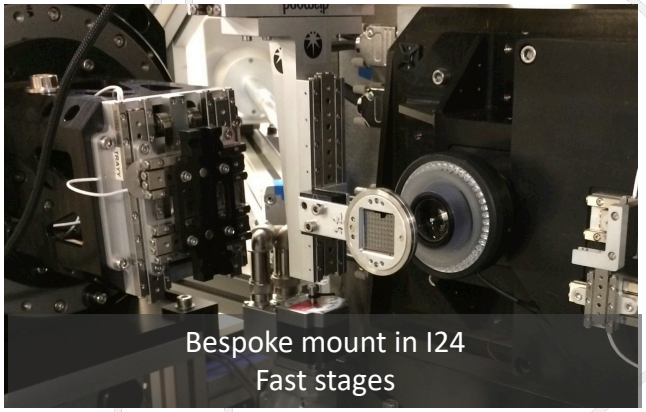
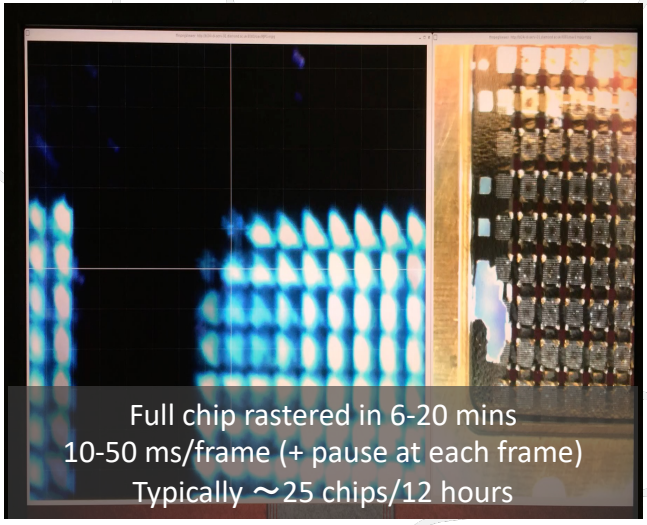
Microfocus



Fixed-target at I24

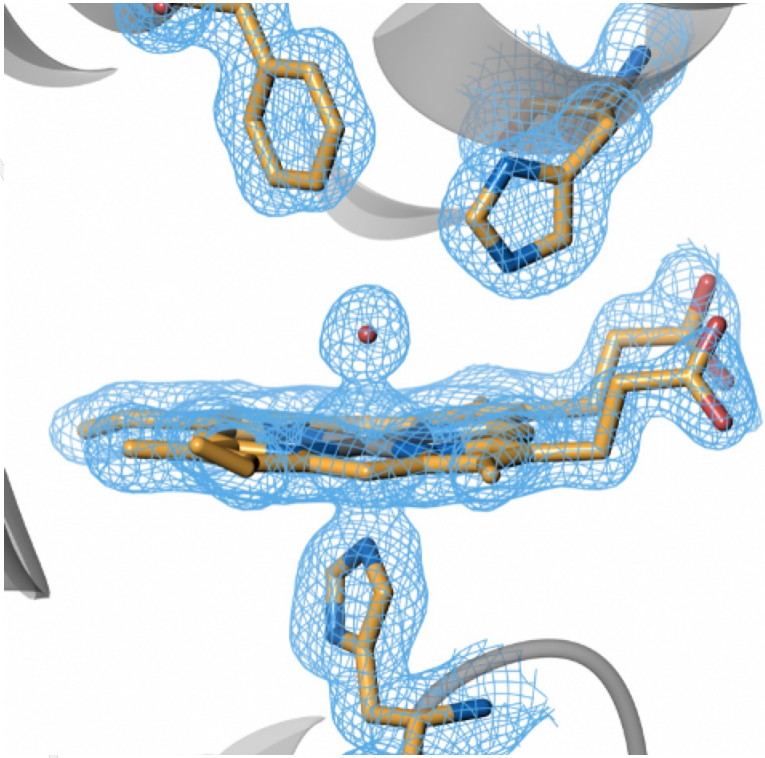


Bespoke mount in I24
Fast stages



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

diamond

The UK-XFEL Hub

European XFEL

LCLS

SACLA

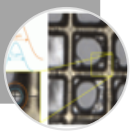
PAL

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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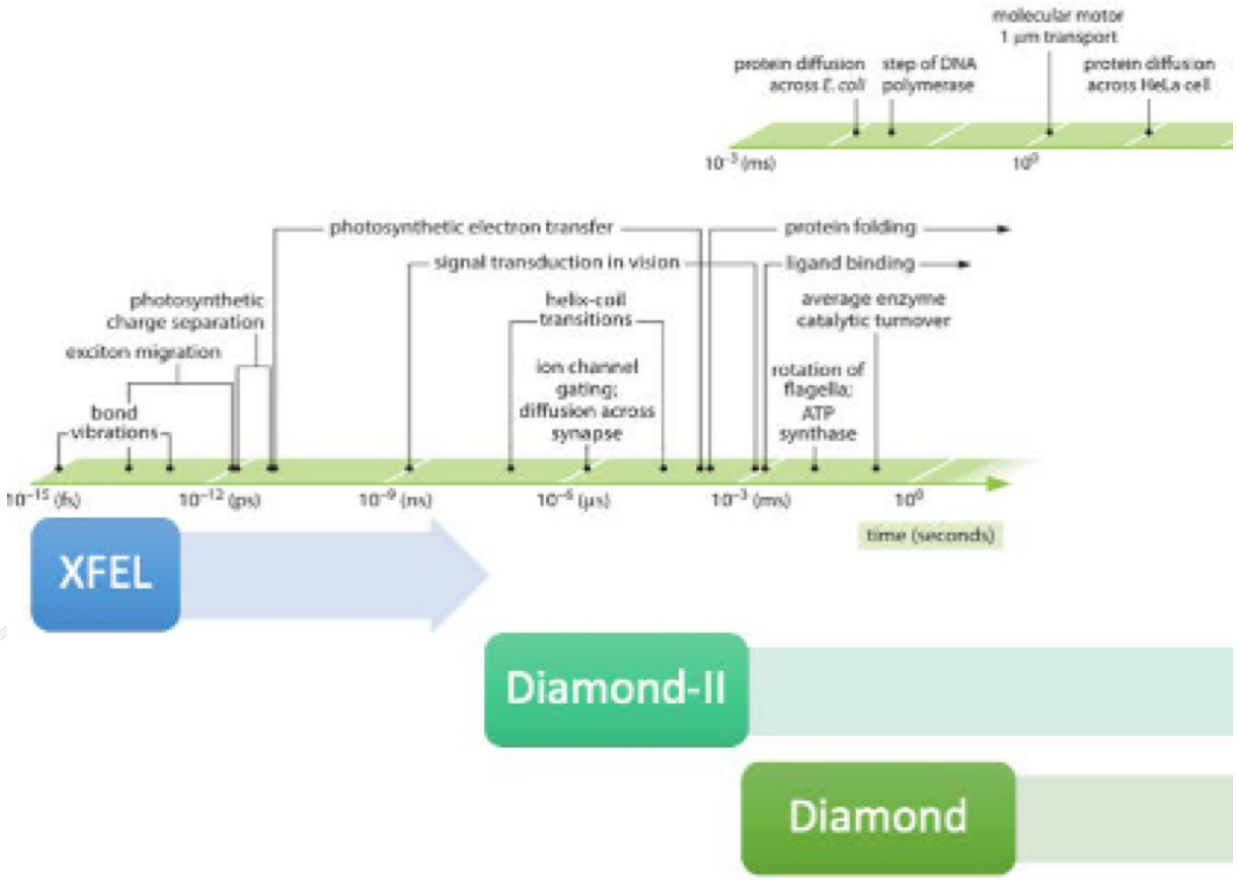
Extending the time range



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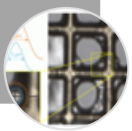
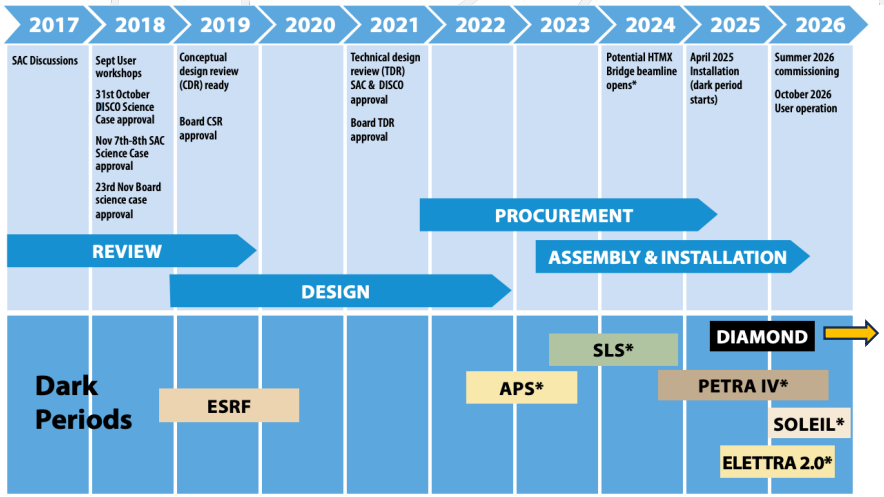
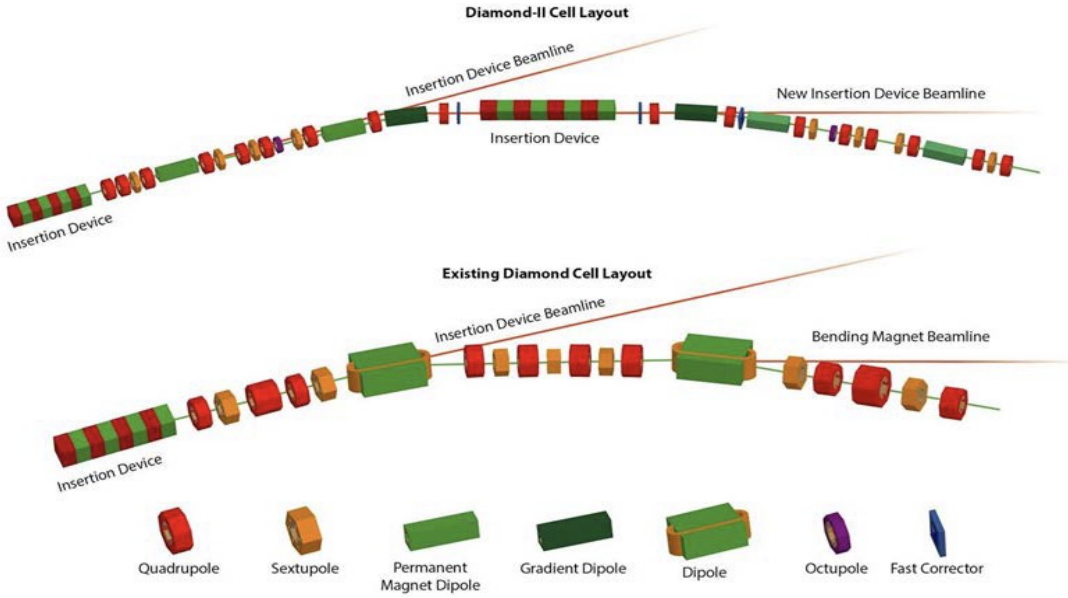
Microfocus



The Diamond-II project

4th generation synchrotrons and XFELs have hotter beams

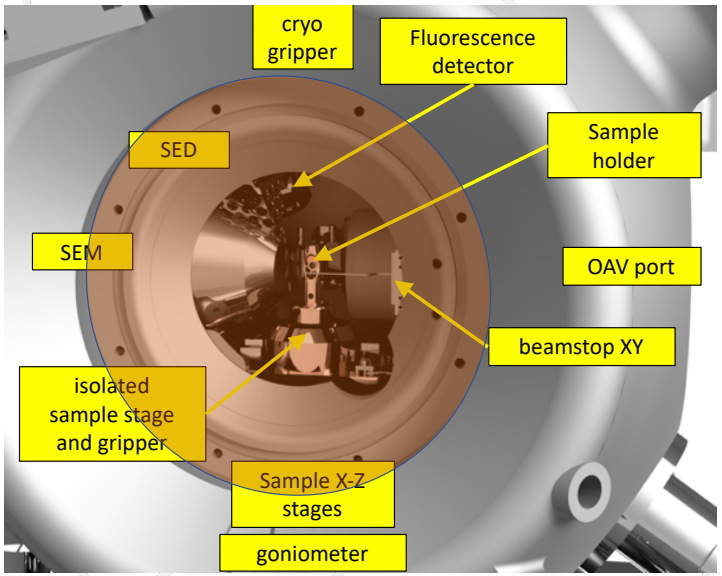




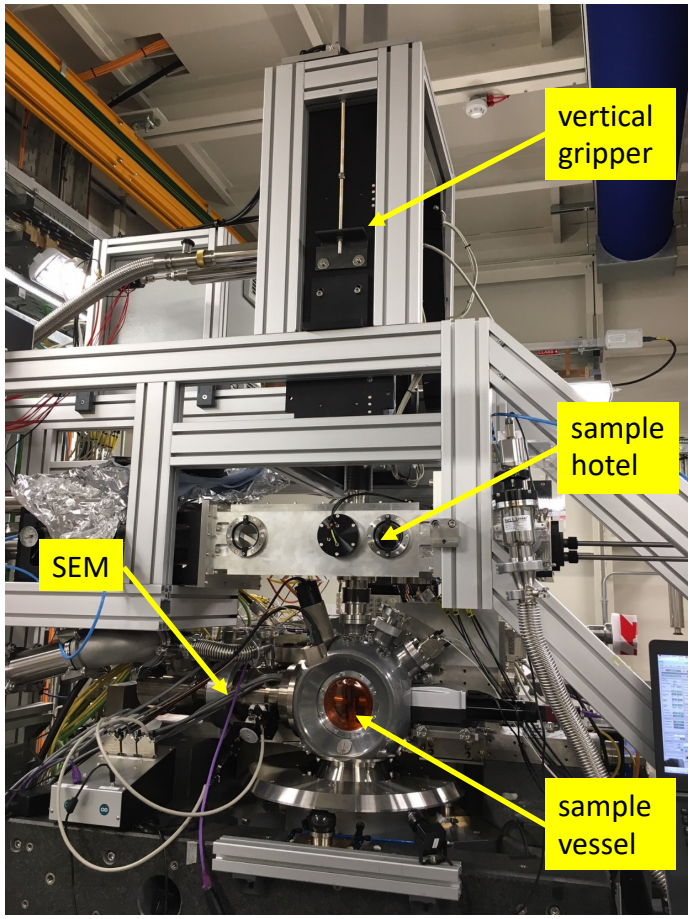
diamond

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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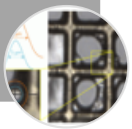
Microfocus



diamond

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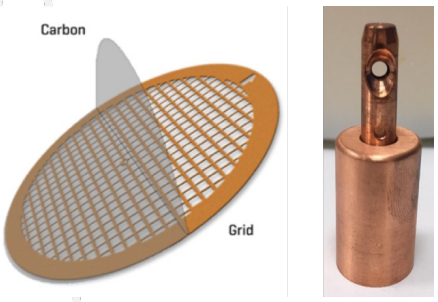
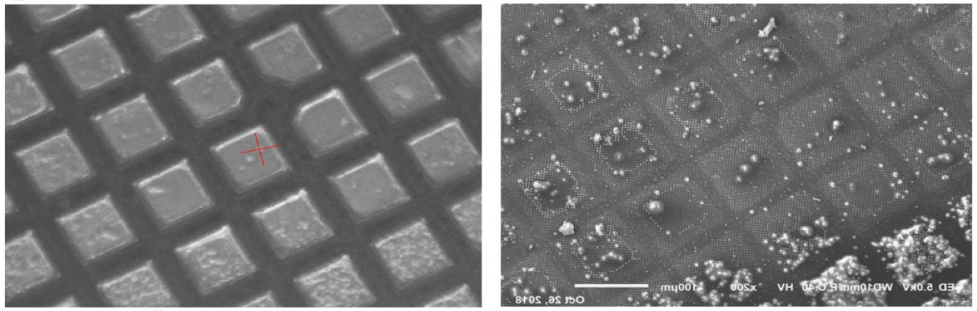
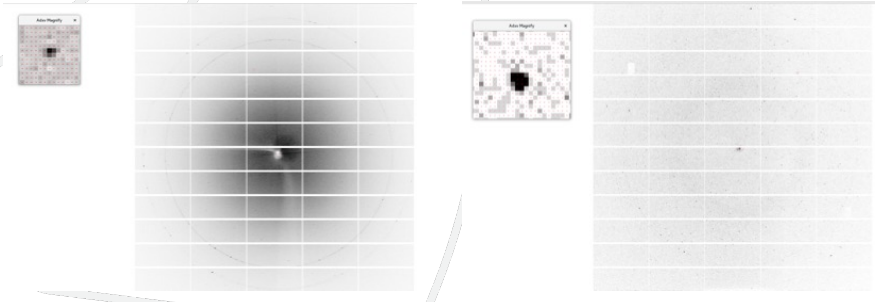


EM-grids as sample holders



OAV

SEM

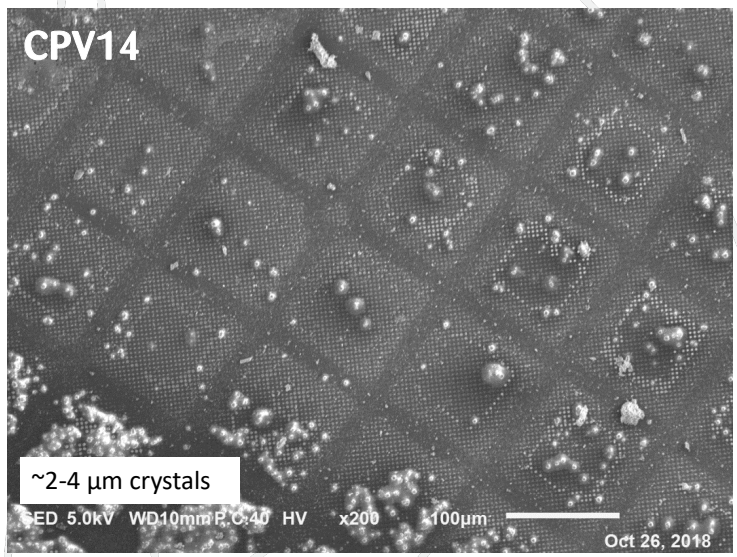


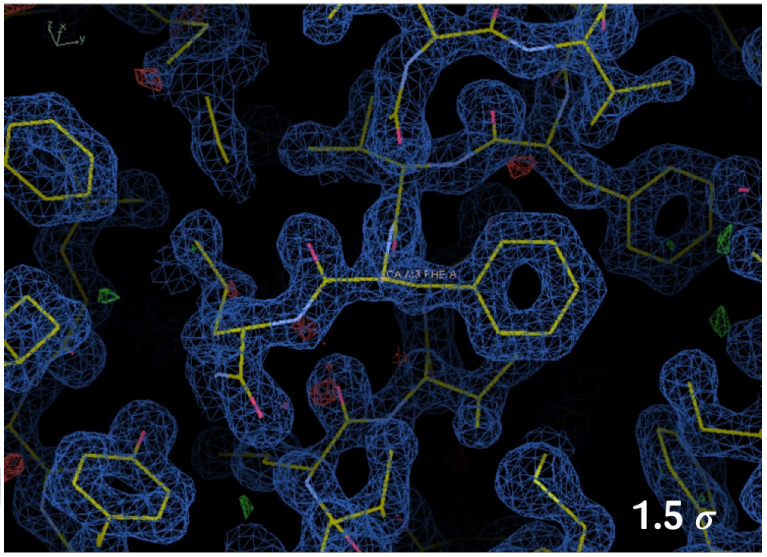
extremely low background

diamond

Smaller than visible

VMXm: the first nano-focus beamline





27 crystals
1.5-2.0 deg/data per crystal

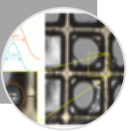
Rigid body+restrained refinement
Dials, CCP4, Refmac, Coot

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

Microfocus



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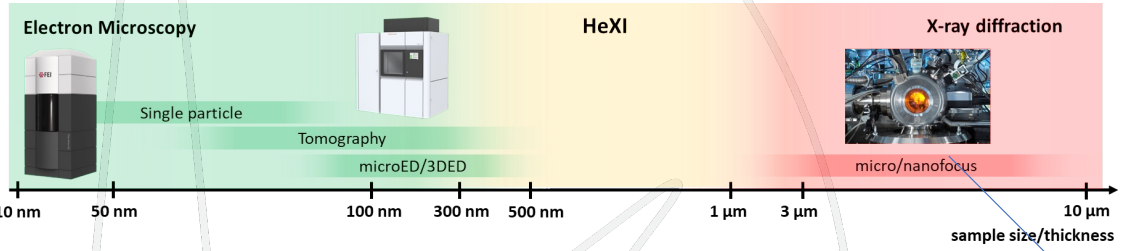
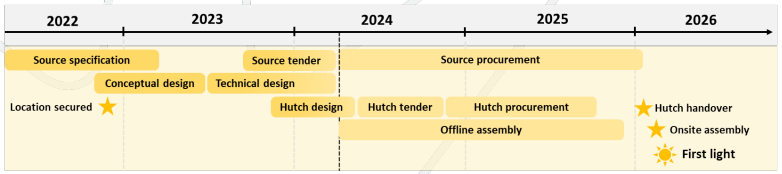
Not just photons – The HeXI beamline

High energy electrons for structure determination

- HeXI will target 300 nm to 3 µm crystals with high energy electrons
- Bridge the gap between Commercial electron Microscopes and MX beamlines



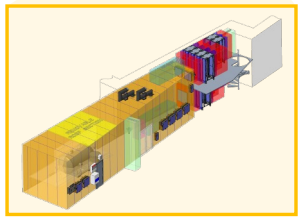
Project Milestones



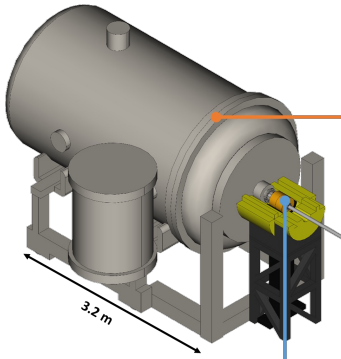
- Funded by the Wellcome ELS grant
- Conceptual design approved
- Technical design submitted
- Electron source tender submitted
- Commencing construction

First Light by 2026

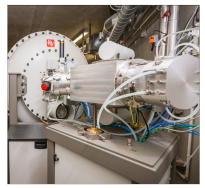
Operation through the *"Dark Period"*

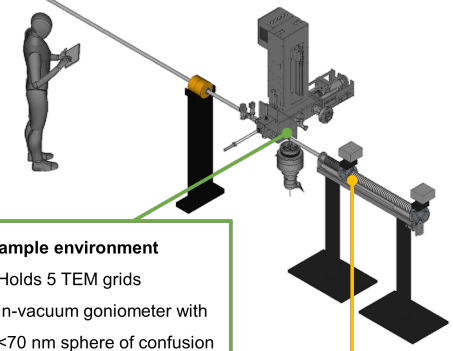


Dedicated MeV electron diffractometer

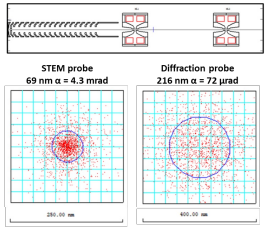


1 MeV electron source
- Cockcroft-Walton High-Voltage power supply
- LaB_6 emitter
- 10 ppm stability

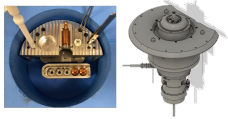




Electron optics
- Bespoke 0.4 T solenoid lenses
- 300 nm parallel beam
- Low-resolution (~100 nm) STEM imaging capabilities



Sample environment
- Holds 5 TEM grids
- In-vacuum goniometer with <70 nm sphere of confusion
- Cryogenic sample hotel



CMOS detector
- Translatable stage

New Grant will Electrify Life Sciences with Breakthrough Technologies

diamond

The need for longer wavelenght

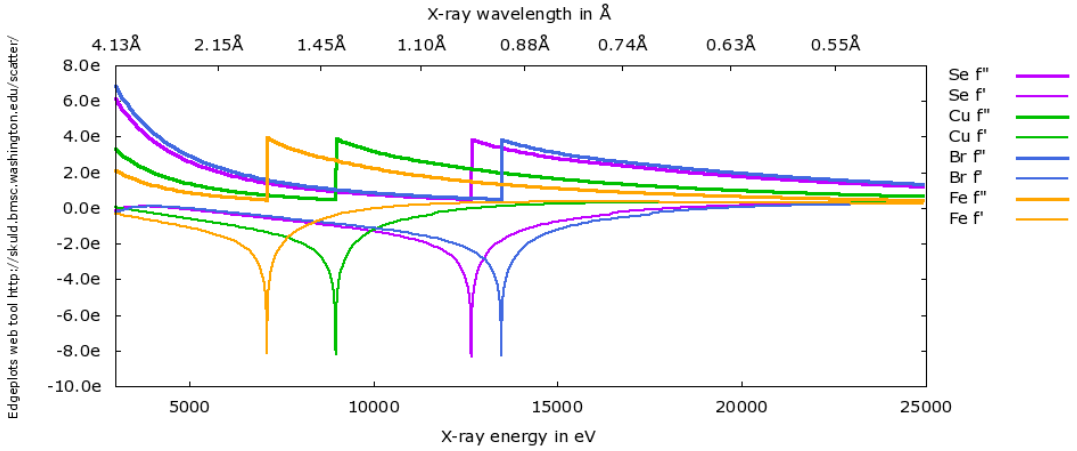
- Tuneable beamlines
- I23 dedicated
- Smart data collection strategies
- Expert systems

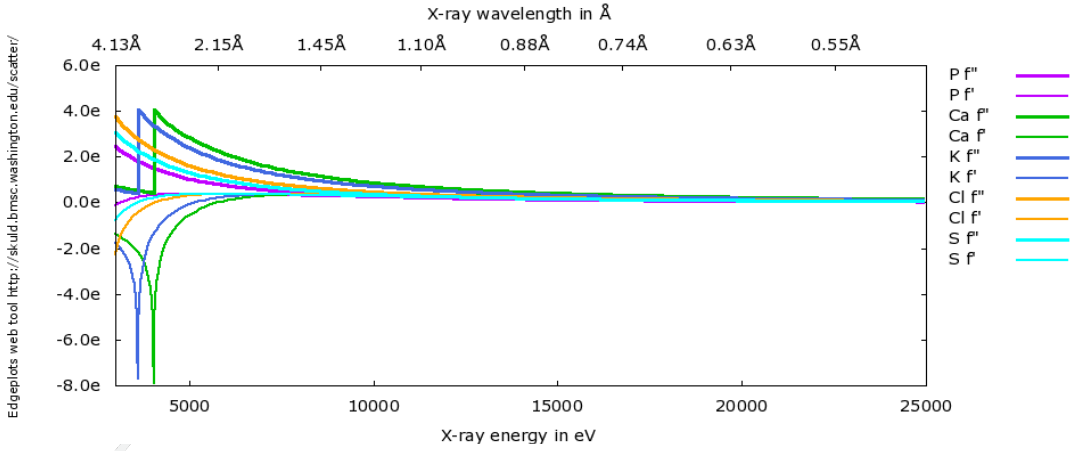
Long Wavelength

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

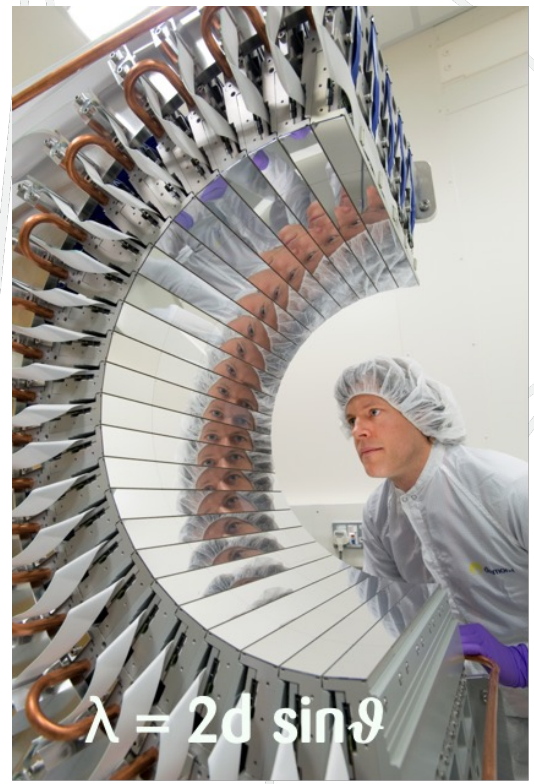


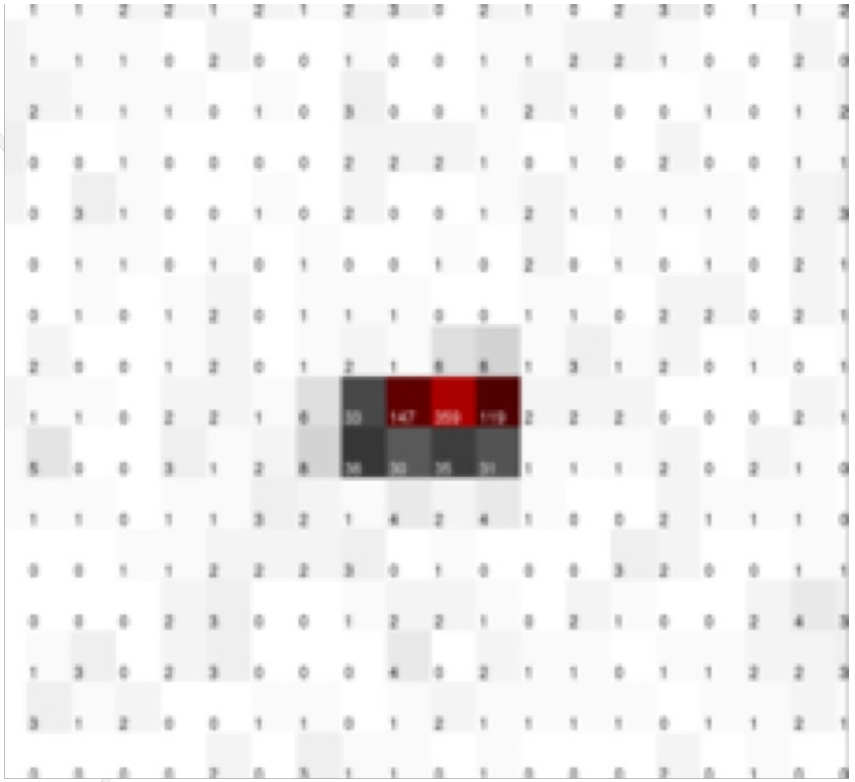


Challenges and solutions

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

Long Wavelength





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

diamond

Challenges and solutions

- Tuneable beamlines
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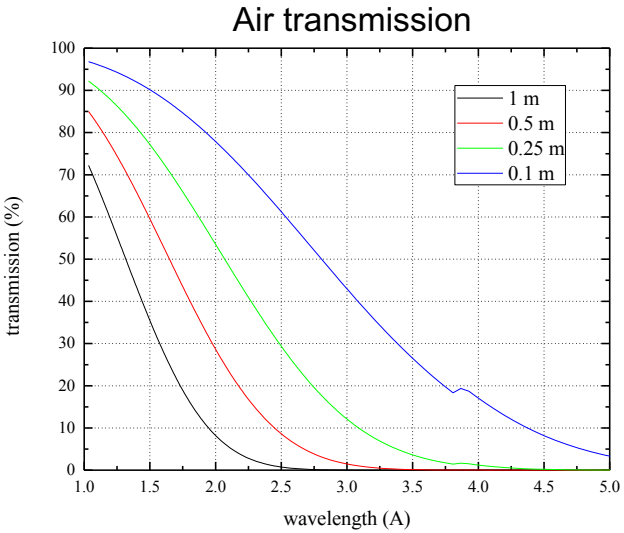
Long Wavelength

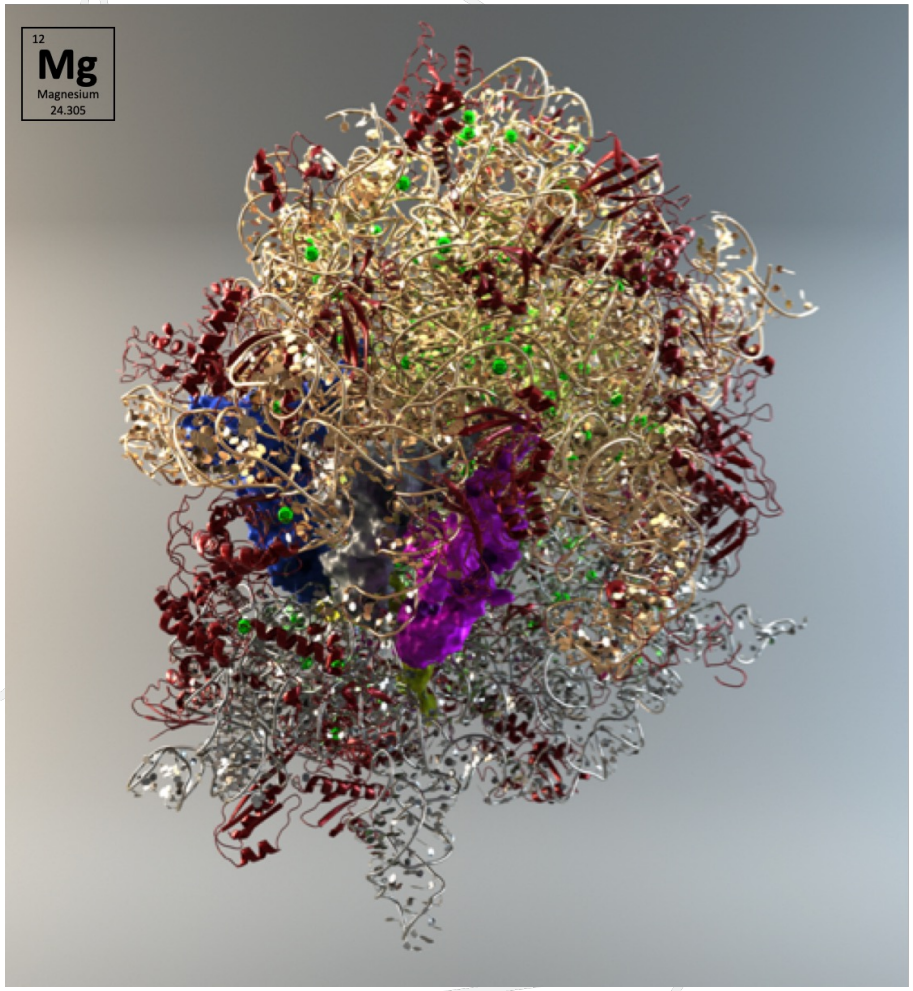
Absorption by air of long wavelength X-rays

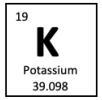


In vacuum beamline

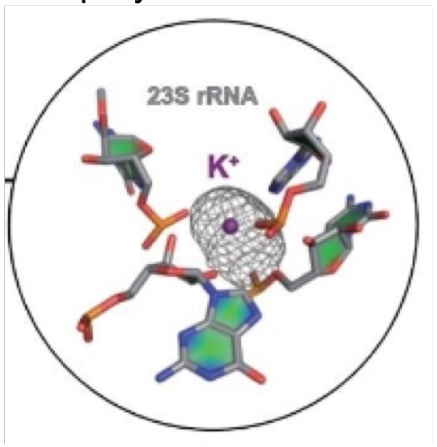
Bespoke sample holders

Results: metal identification

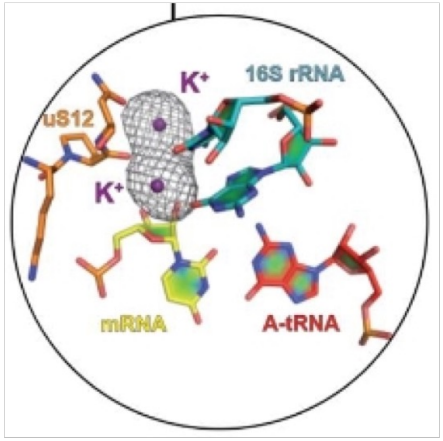




Peptidyl transferase center



Decoding center

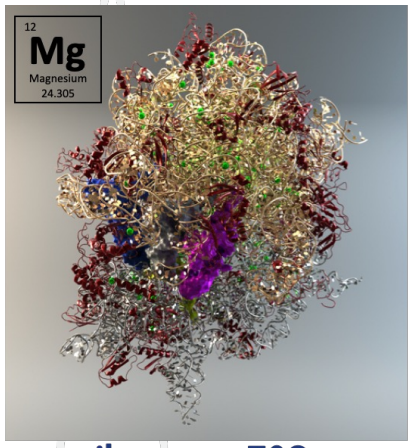


- Tuneable beamlines
- I23 dedicated
- Smart data collection strategies
- Expert systems

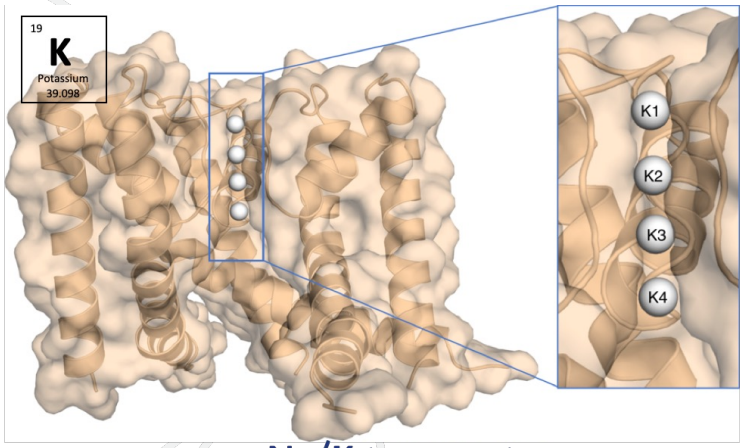
Long Wavelength

diamond

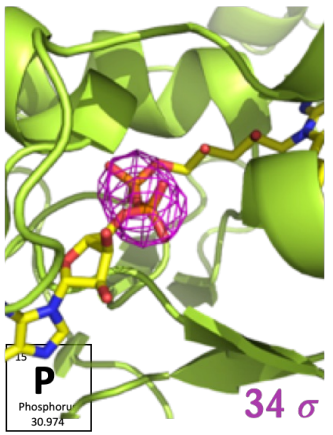
Extending the element 'palette'



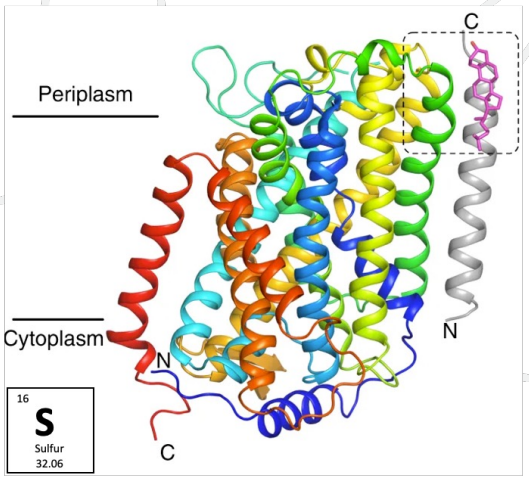
ribosome 70S



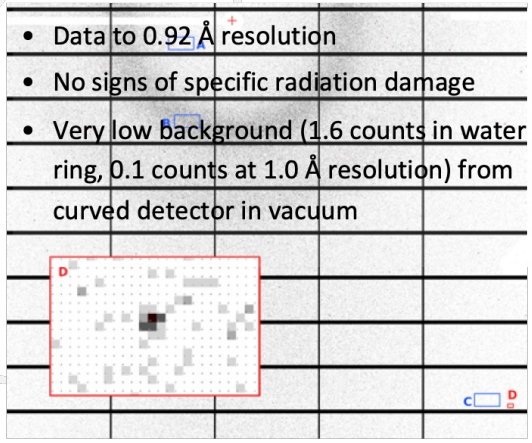
Na/K transporter



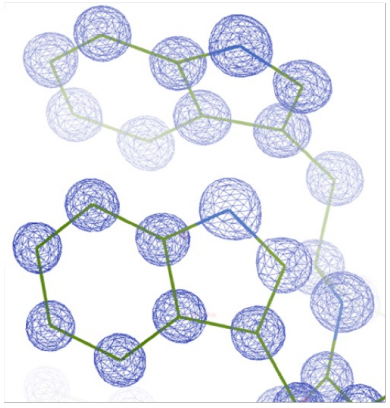
FAD-binding



SLC7 transporter



PETase

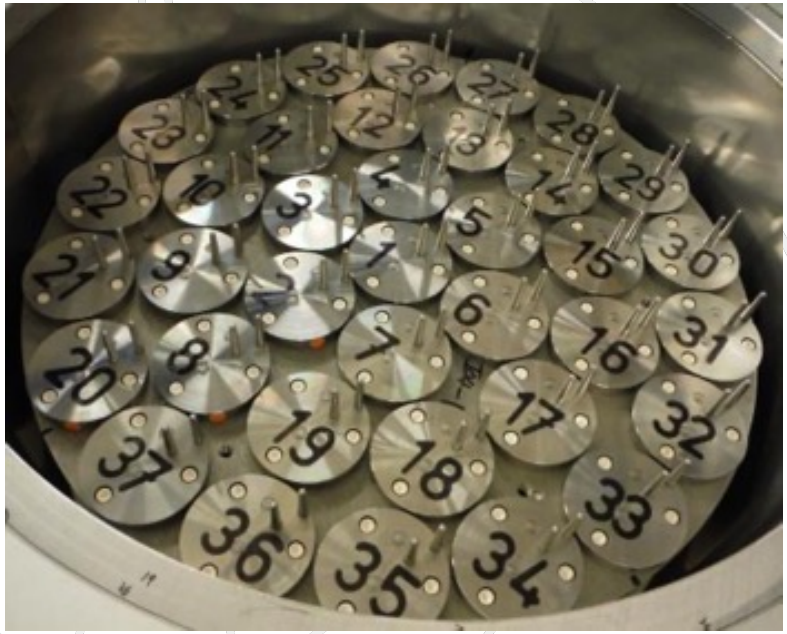


- Tuneable beamlines
- I23 dedicated
- Smart data collection strategies
- Expert systems

Long Wavelength

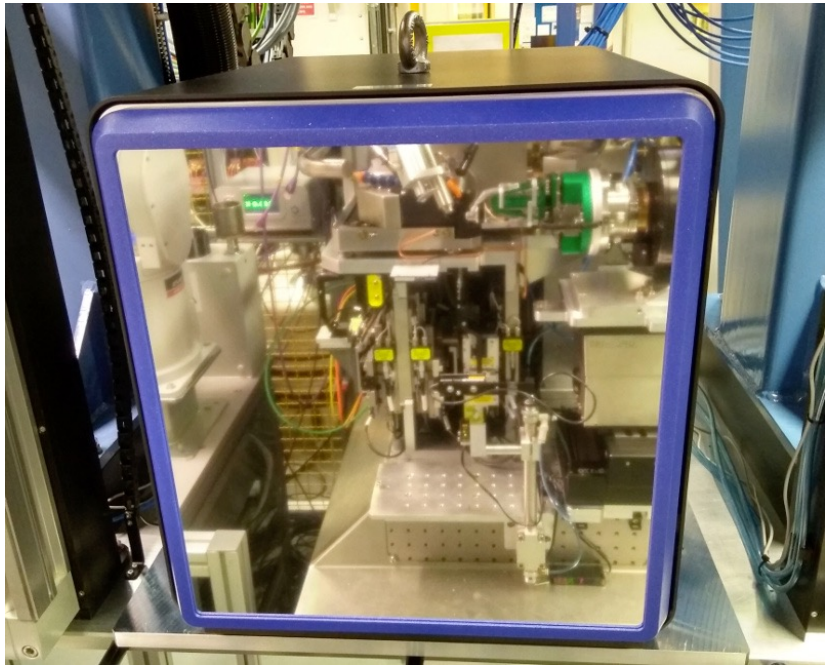
diamond

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)

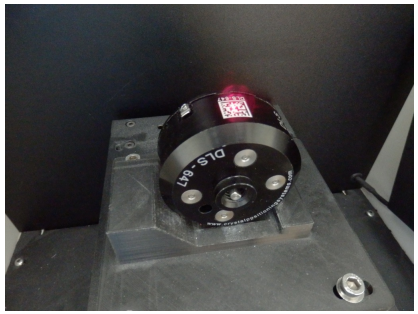
High Throughput

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

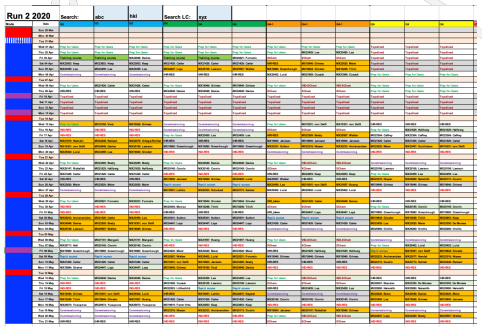
diamond

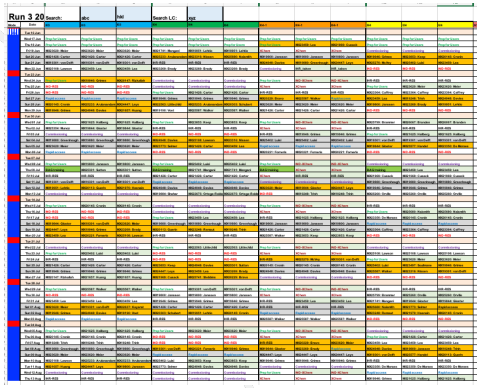
Responsive/dynamic scheduling

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

High Throughput

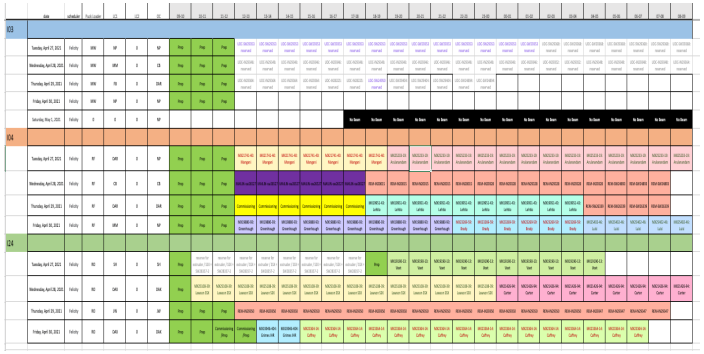
2019





2020

2022



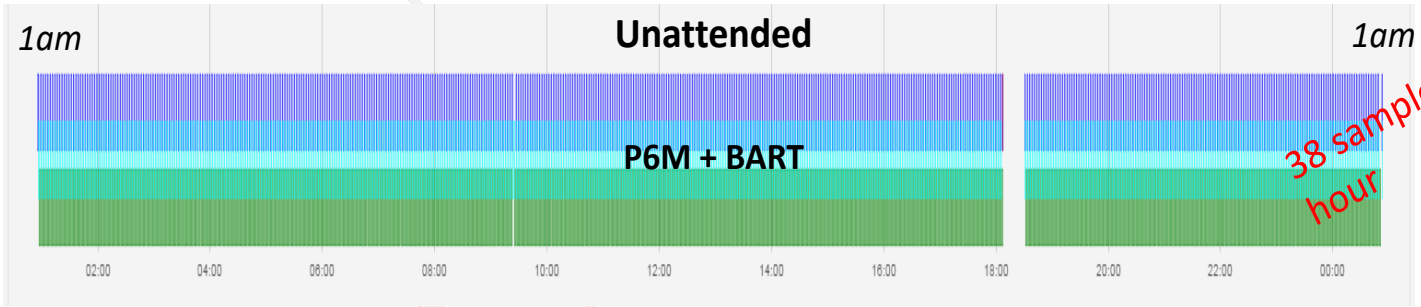
diamond

Unattended data collection

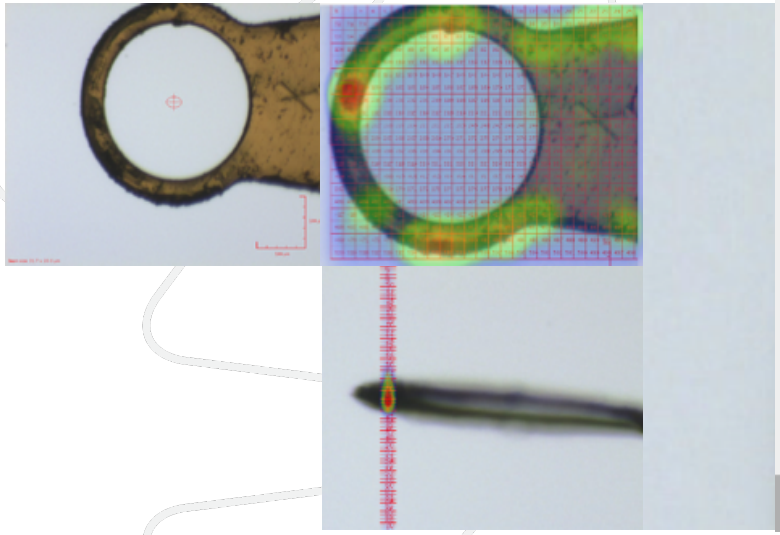
- PADs
- Automation
- Pipelines
- Serial Crystallography
- Access (rapid/freq)

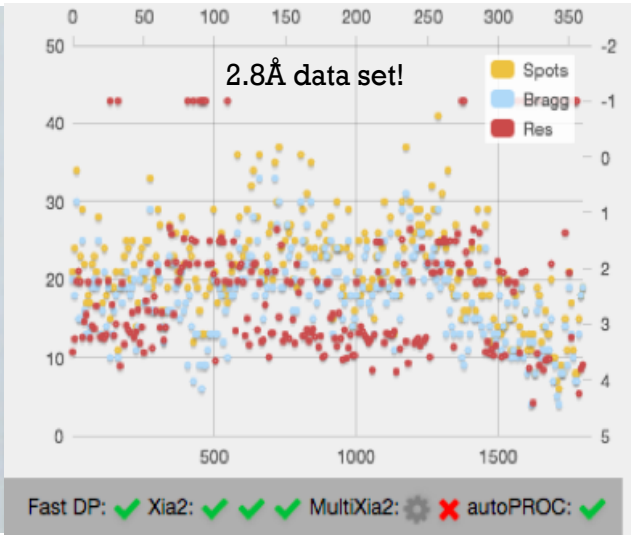
High Throughput

Optimized for speed



Xray-centering and strategies





diamond

Automated processing pipelines

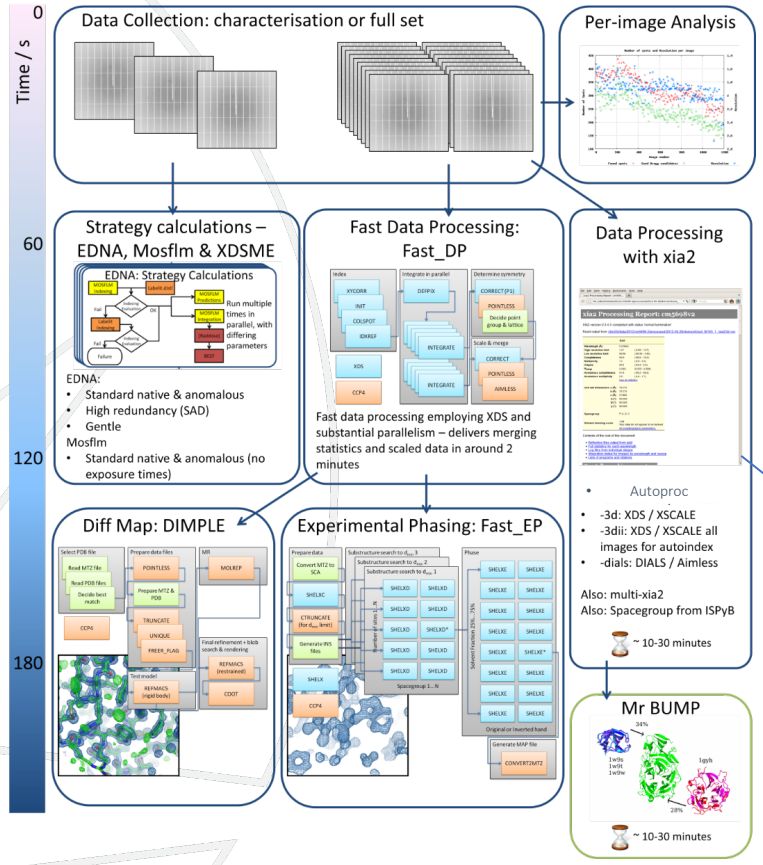
- PADs
- Automation
- Pipelines
- Serial Crystallography
- Access (rapid/freq)

High Throughput

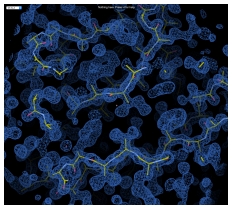
Data Collection

Data Reduction

Structure Solution



BigEp



Thorough Phasing Pipeline Up to 24 hours

diamond

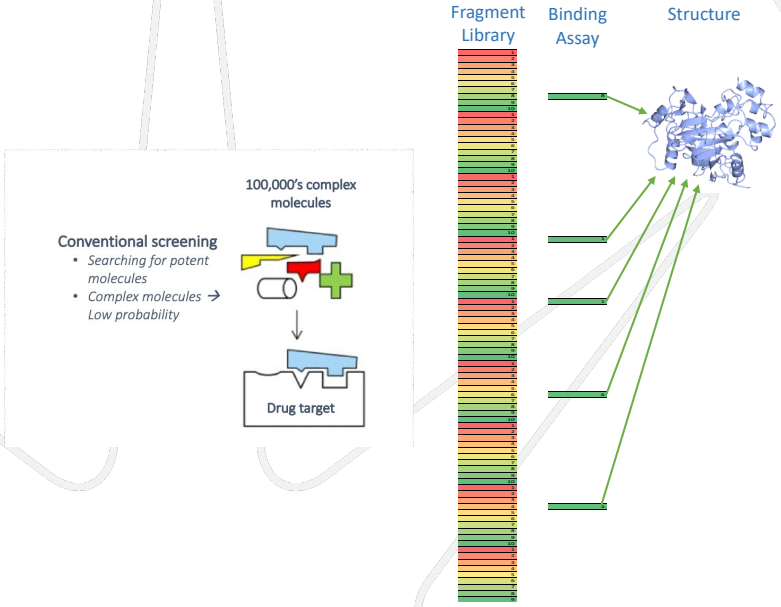
HT fragment screening

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

High Throughput

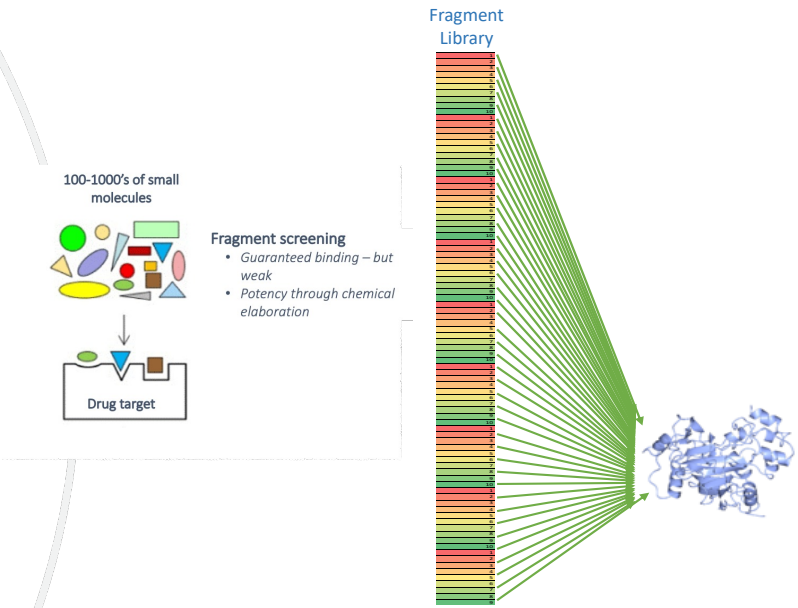
Conventional screening

Preselection of best compounds



Fragment-based approach

Screen by crystal

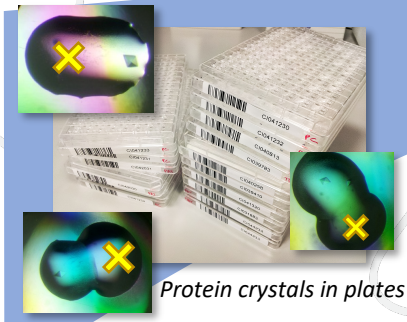


diamond

HT fragment screening

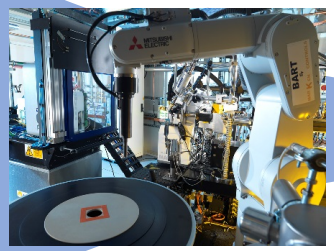
XChem Lab at Diamond

Fragment libraries

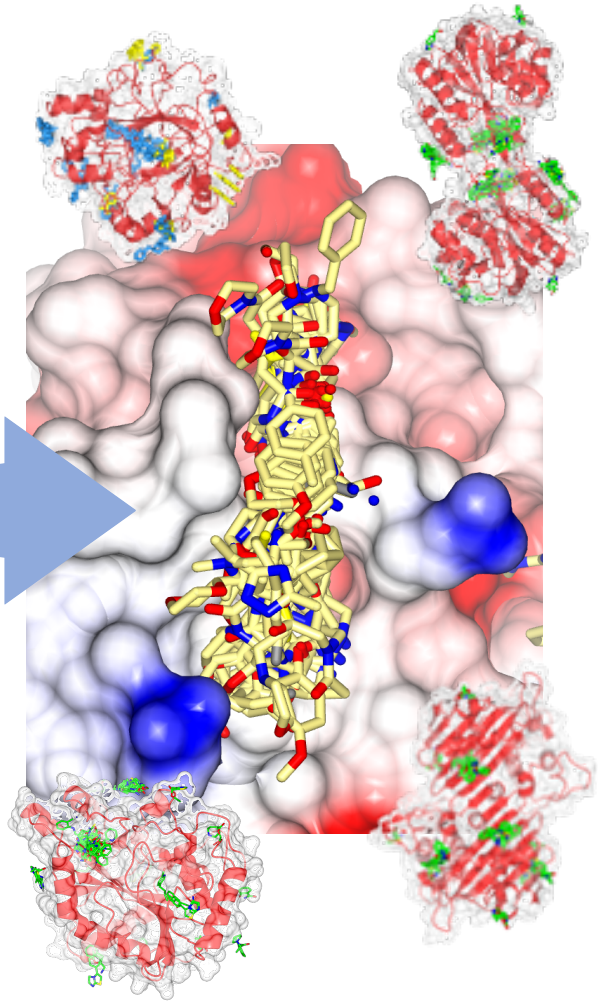


Protein crystals in plates

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

Multiple access ways

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

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

instruct-eric.org admin@instruct-eric.org @instructhub

Instruct–UK- Harwell Campus

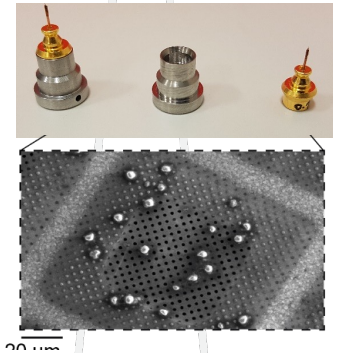
diamond UKRI Research Complex at Harwell instruct ERIC

diamond

Access to MX

Cryo

standard SPINE pins
I23 specific holders
VMXm – EM grids

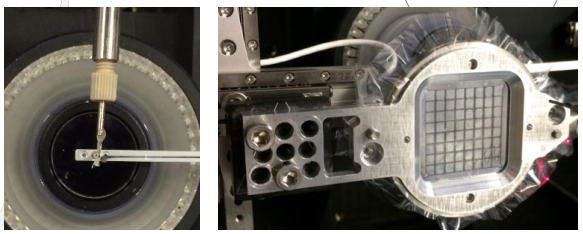


Room Temperature

VMXi dedicated beamline
I03 - biological containment

SSX (I24, VMXi and XFEL Hub)

Fixed targets
Thin films
Extruders
Acoustic drop ejection
Tape drives (in dev)

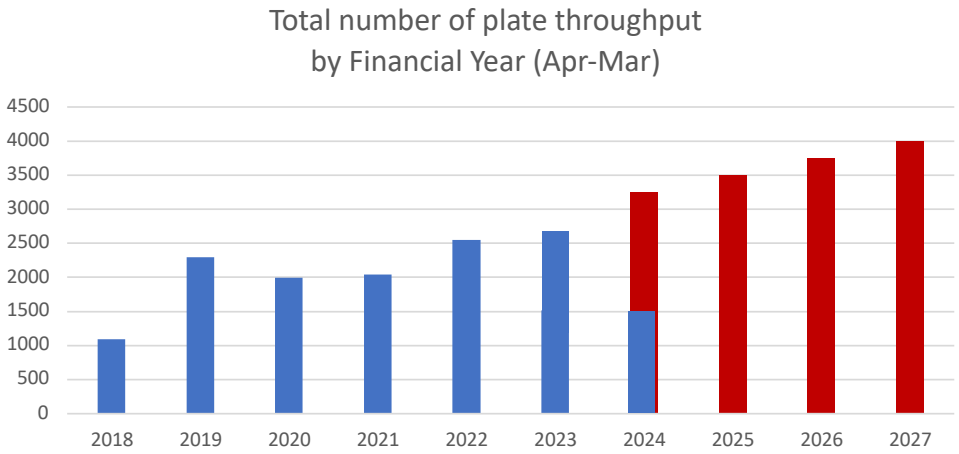


Crystallisation facility

>£1.4M invested in last 5 years (DLS/ RCaH)
Discussion over future partnership with ISIS
Rockmaker and Rockmaker web (Formulatrix)

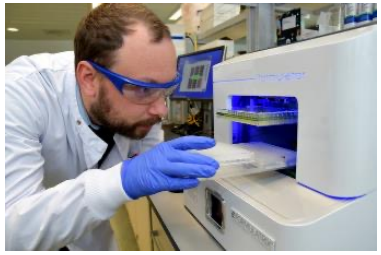
Future Plans

- Replacement of 4 °C imager
- Imager for nano crystals- VMXm/ microED
- Upgrade chemical storage including Echo dispensing
- Long-term technical support (1.0 FTE) for facility









2018
Formulatrix R1000
LCP plate Capacity

2018
Scorpion

2019
VMXi
Workflow

2022
Formulatrix R1000
LCP + MFI optics

2022
Formulator
+ LCP Mosquito

2023
Mass
Spectrometer

2024/25
Imaging for nano xtals
tracking system

In situ data collection

Fully automated handling of *in-situ* plates at 20 °C for data collection (100s of datasets/hr)

~10x10 µm^2 pink beam 3x10^{13} ph/s, 16 keV

Machine learning crystal location and selection

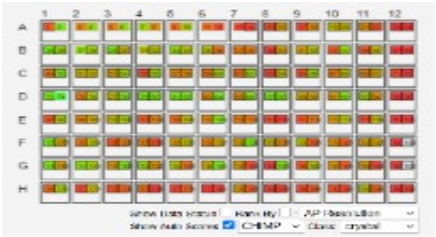
Users can remotely queue plates for unattended data collection

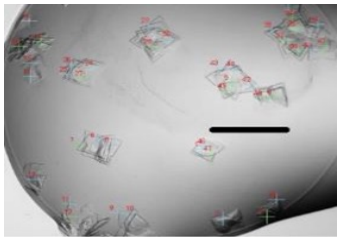
Optimisation of crystallisation conditions as well as high quality room temperature structures

Multi-crystal data merged using dials-multiplex and presented in ISPyB with downstream analysis

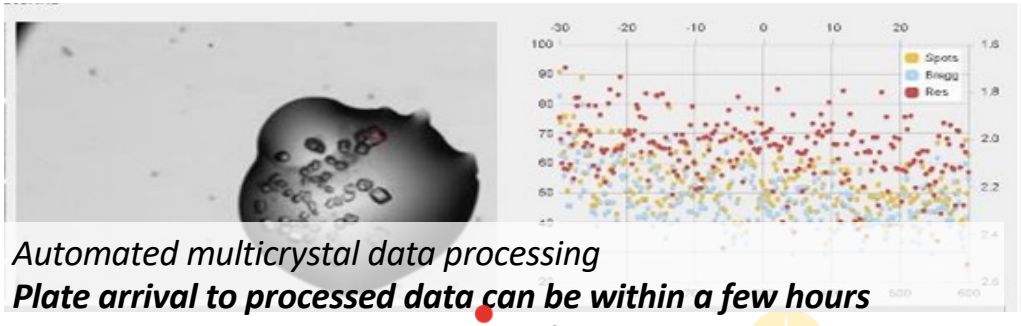
Crystal hotel to the beamline using robotic arm

Up to 60° rotation of plates





Machine learning crystal identification & marking for data collection



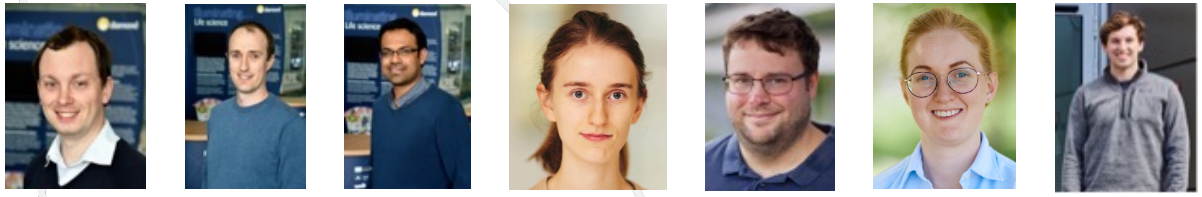
Automated multicrystal data processing

Plate arrival to processed data can be within a few hours

Membrane protein laboratory (MPL)

Contact: mpllab@diamond.ac.uk

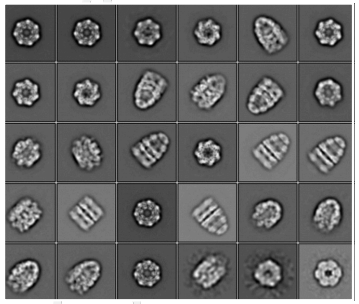
Andrew James Harish Claudia Peter Rebecca Charlie



- Funded by Wellcome - free at point of access
- Access: **Rapid proposal** & **Instruct-ERIC**
- **Platforms:**
  - HTP cloning and expression screening
  - Purification Optimisation
  - Sample preparation for cryo-EM, crystallisation and SAXS
  - Biophysical Characterisation
  - Data collection & structure solution

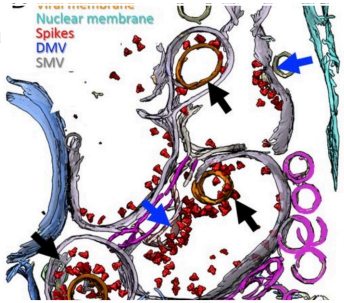
MPL Membrane Protein Laboratory diamond UKRI Research Complex at Harwell instruct ERIC wellcome

eBIC access through INSTRUCT



CryoEM SPA

Established Since start

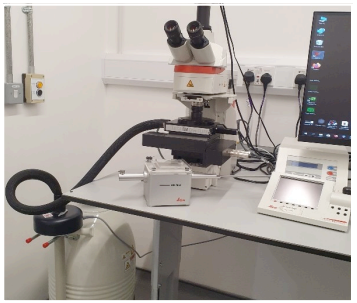


Cryo-Electron Tomography

Established & Developing

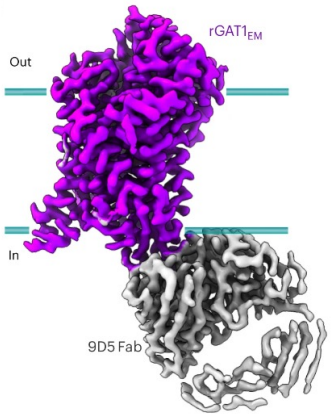
Chameleon & Electron Diffraction

Developing

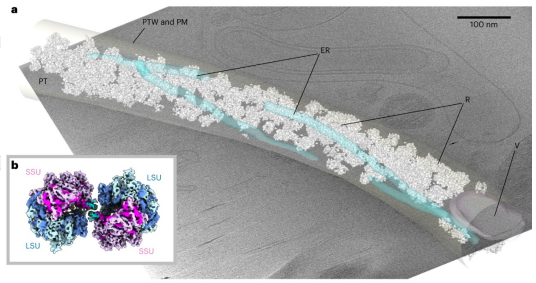


Leica CryoCLEM

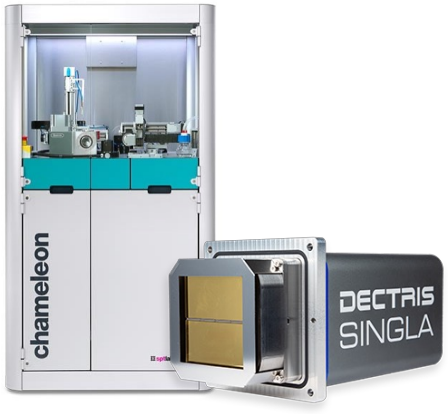
Developing



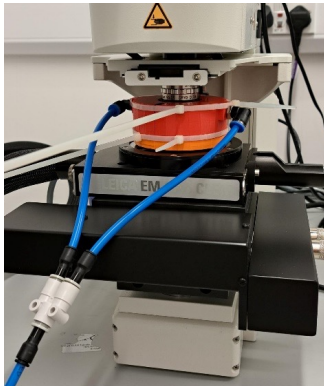
Nayak *et al.*, 2023 NSMB



McLaren *et al.*, 2023 Nat.Microbiology



Radiation hard 4KHz readout



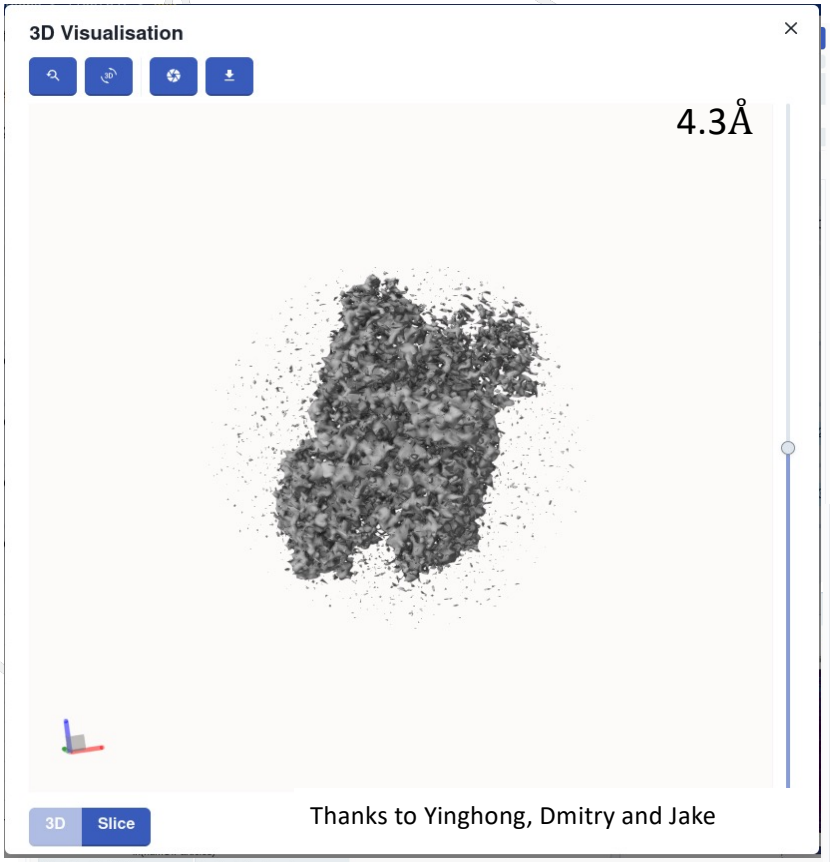
Humidity control chamber

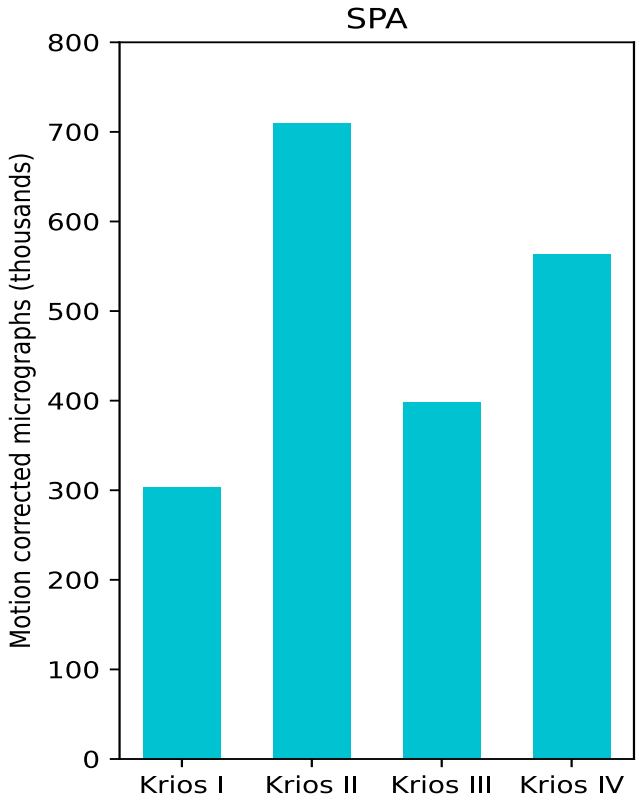
instruct ERIC diamond

RELION 4 Pipeline

with new web interface developed outside of SynchWeb Introducing PATo

(Dan Hatton, Anna Horstmann, Stephen Rigg, Guilherme De Freitas)



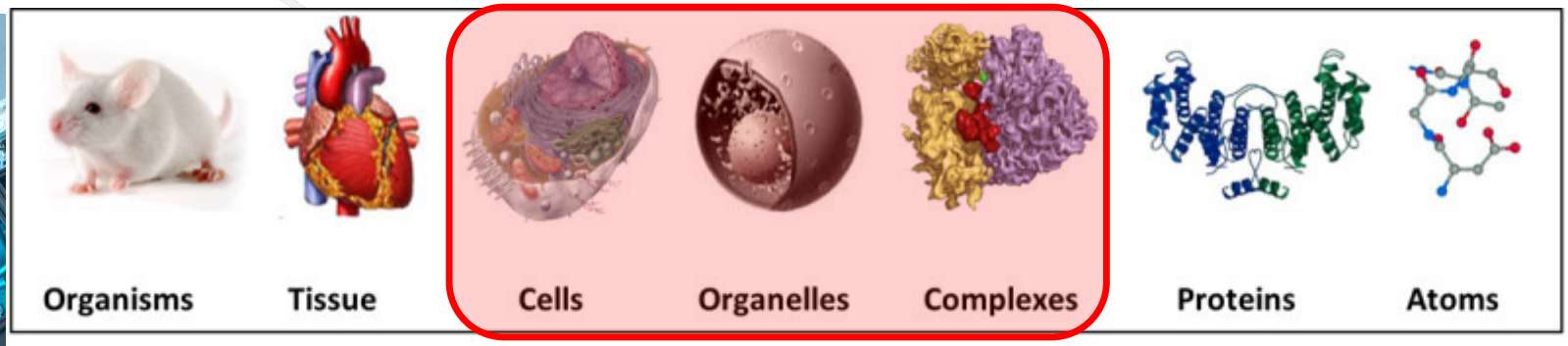


Data storage

18 TB/SPA visit

Integrated data transfer and triggering the pipeline - the local contact needs to input the dose per frame and everything else is automated (relion, cryolo, ice breaker) And it is Live!

B24 correlative imaging



nation

Computerised Tomography
1-2 mm

Fluorescence Microscopy
250 nm

Cryo-Soft X-ray Tomography
25 nm

Cell Electron Tomography
5 nm

Single Particle Electron Microscopy
0.2 nm

X-ray Crystallography
< 0.1 nm

mm ---------- Resolution ---------- nm

- Cryo soft X
(cryoSXT)
- 3D X-ray a
imaging

instruct ERIC diamond

Molecular Biophysics

2x ITC
2x AUC
3x RT-PCR
Biacore T200
SEC-MALS

~£1M invested in last 5 years (RCaH/ DLS)

Future Plans
Echo dispensing

2018
Stopped Flow

2019
NanoDSF

2020
Clariostar

2022
Fida1

2023
Mass Spectrometer

2025
Echo dispensing

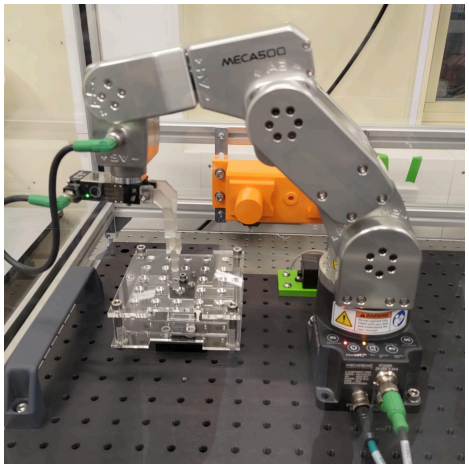
B21 – bioSAXS/WAXS

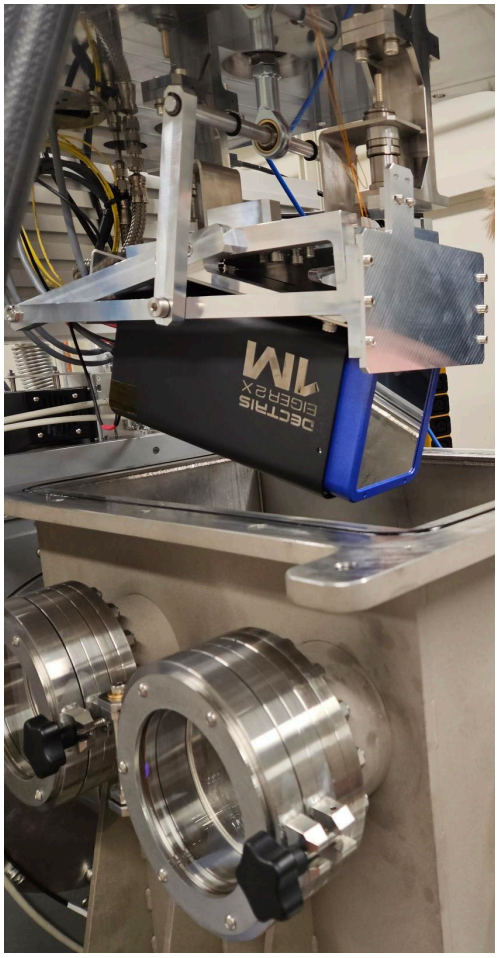
Source: bending magnet
Flux: $4x10^{12}$ ph/s
Wavelength: 0.9464 Å (13.1 keV)

Sample to SAXS detector distance: 3684.6 mm
SAXS Detector: EigerX 4M (Dectris)
WAXS Detector: EigerX 1M (Dectris)

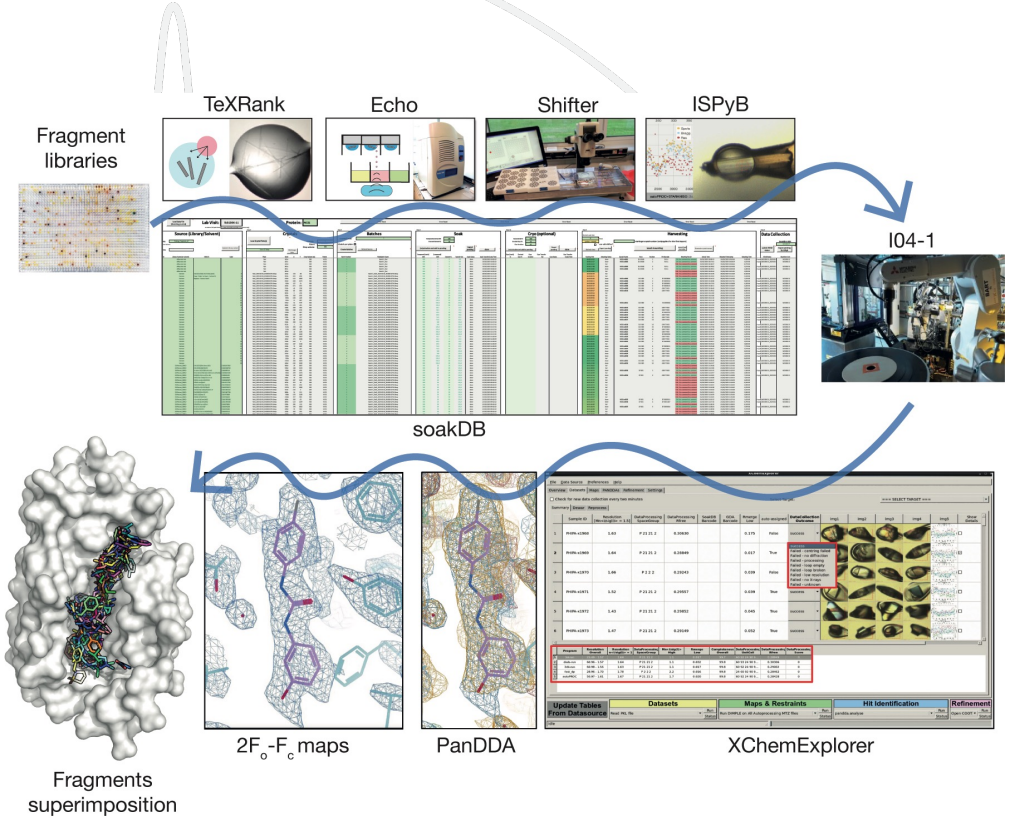
SAXS Q range:	0.0045-0.34 Å-1
NEW WAXS Q range:	0.8 -3.4 Å-1

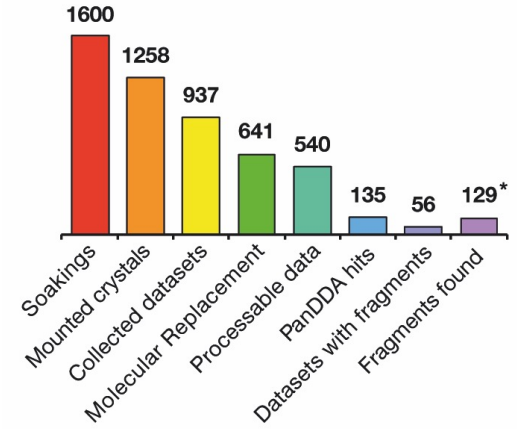
Upgrade plans:
New Agilent HPLC online by December 2024 with
Integrated GDA/HPLC controls in 2025
Robot Arm for solid and viscous samples installed by late 2025

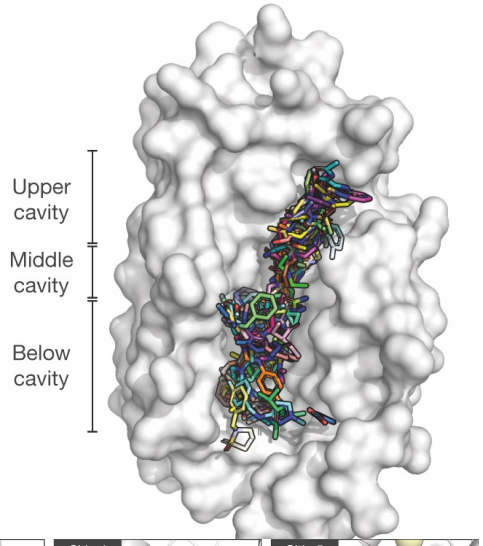


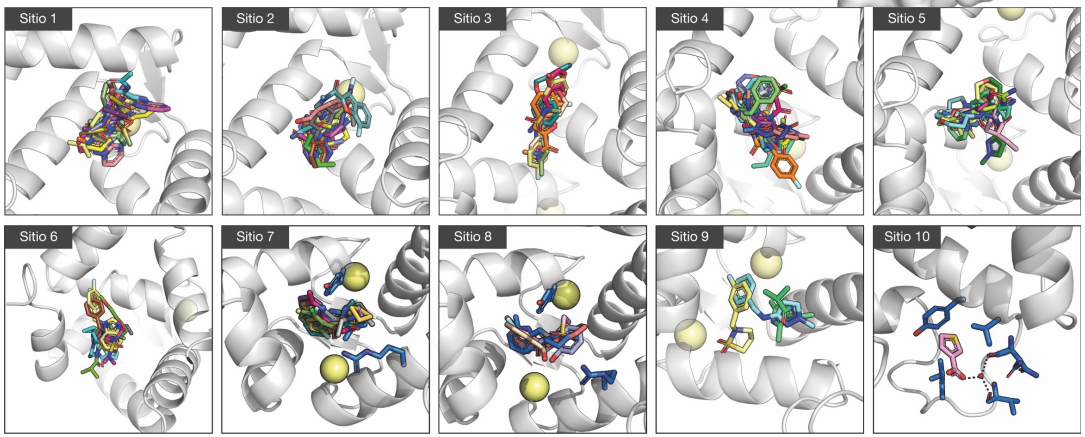


INSTRUCT internships – a success case









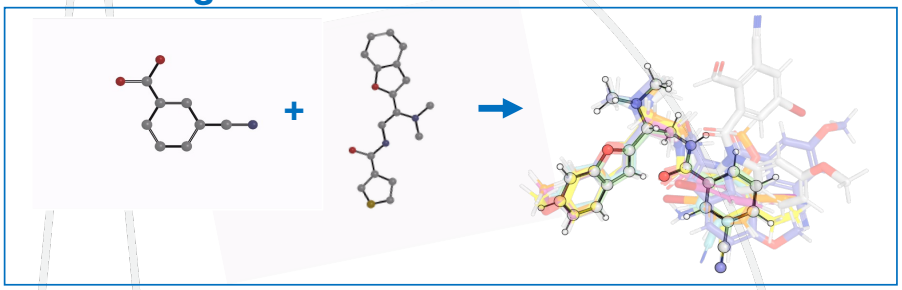
129 fragments bound to the protein

INSTRUCT internships – a success case

AI-based automated design

Syndirella
HIPPO

590 hypothetical elaborations (8000€)

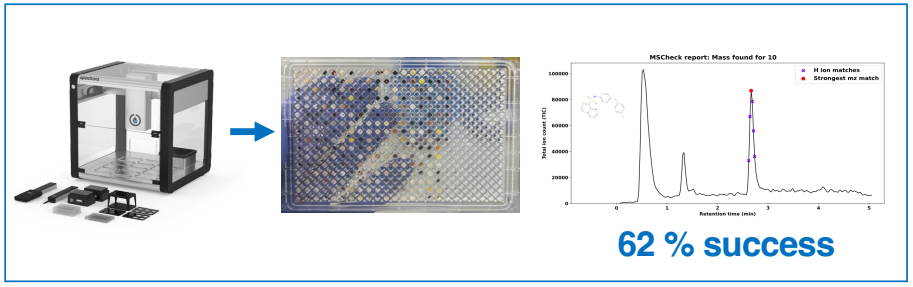


Robotic synthesis

CAR

454 elaborations

252 products

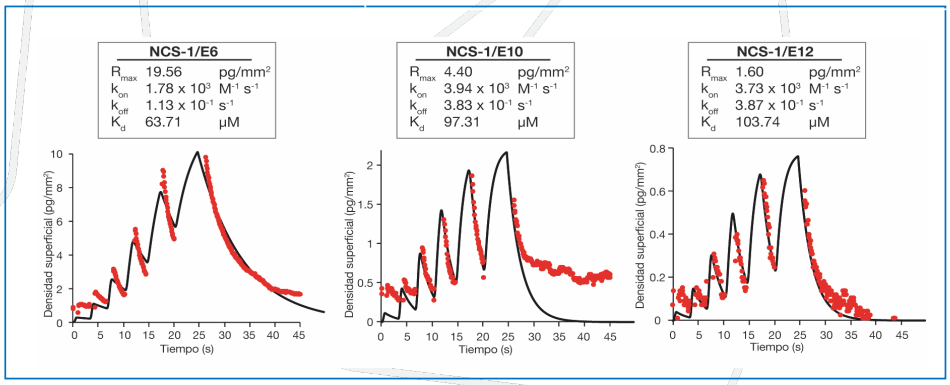


62 % success

Biophysical assays

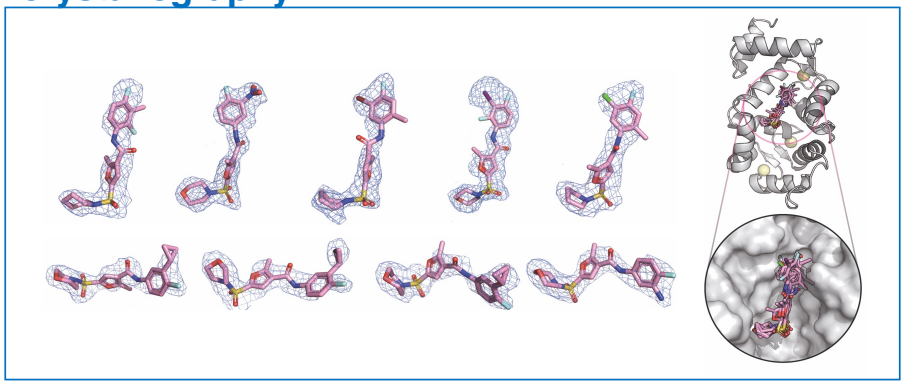
Creoptix

6 pure compounds with µM affinity for the protein



High-throughput crystallography

16 crystal structures from 3 scaffolds (crude reaction mixtures)



Instruct-UK at Harwell Campus

Diamond Light Source

- **Fragment Screening**
- Electron microscopy
- X-ray diff. & Bio-SAXS

Research Complex at Harwell

- Molecular biophysics

Admin Contact:
Jodie Lavender

Sci/Tech Contact:
Martin Walsh

Scientific Contact:
Gwyndaf Evans

Technical Contact:
Gemma Harris

Scientific Contact:
Andrew Quigley

Scientific Contact:
Frank von Delft

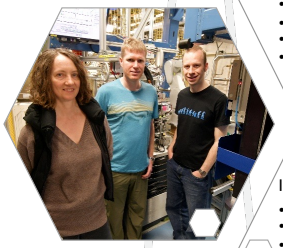
Technical Contact:
Halina Mikolajek

Scientific Contact:
Karen Davies

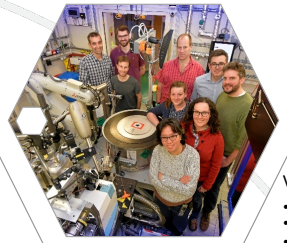
Technical Contact:
Daren Fearon

- *Membrane protein expression*
- Membrane protein crystallisation
- Crystallisation

The team



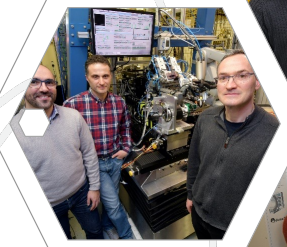
I04-1 / XChem
- **Frank von Delft**
- Jose Brandao-Neto
- Louise Dunnett
- Grant Watt
- Kutumbarao Nidamarthi
- Daren Fearon
- Blake Balcomb



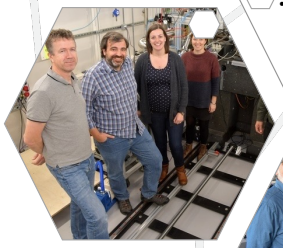
I04
- **Ralf Flaig**
- David Aragão
- Marco Mazzorana
- Pierpaolo Romano

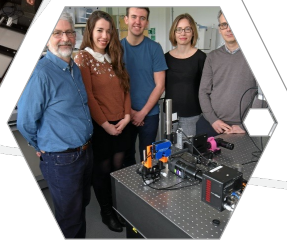
I03
- **Dave Hall**
- Neil Paterson
- Mark Williams
- Felicity Betram

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



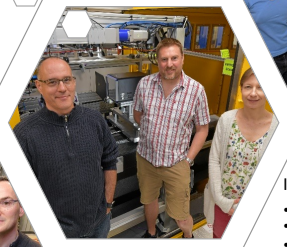
VMXm
- **Gwyndaf Evans**
- Jose Trincao
- Anna Warren
- Adam Crawshaw

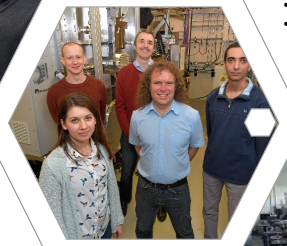




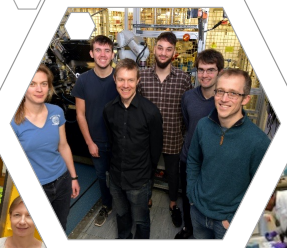
XFEL-Hub
- **Allen Orville**
- Pierre Aller
- Tiankun Zhou

I24
- **Robin Owen**
- Danny Axford
- Sofia Jaho
- Do-Heon Gu





DIALS
Diffraction Integration for Advanced Light Sources

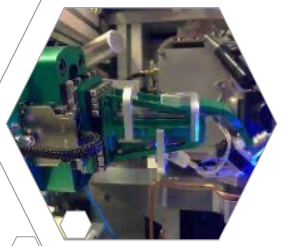


DIALS
- Nicholas Devenish
- James Beilsten-Edmands
- Luis Fuentes-Montero
- Graeme Winter
- David Waterman

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

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

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



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

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diamond