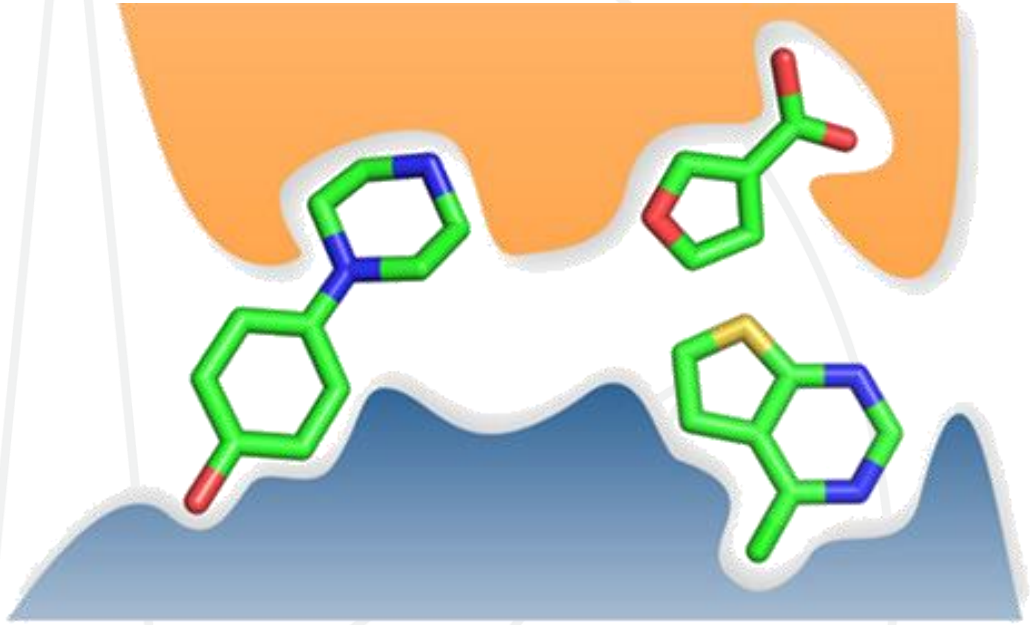
Achieving efficient crystallographic fragment screening using the XChem facility at Diamond Light Source

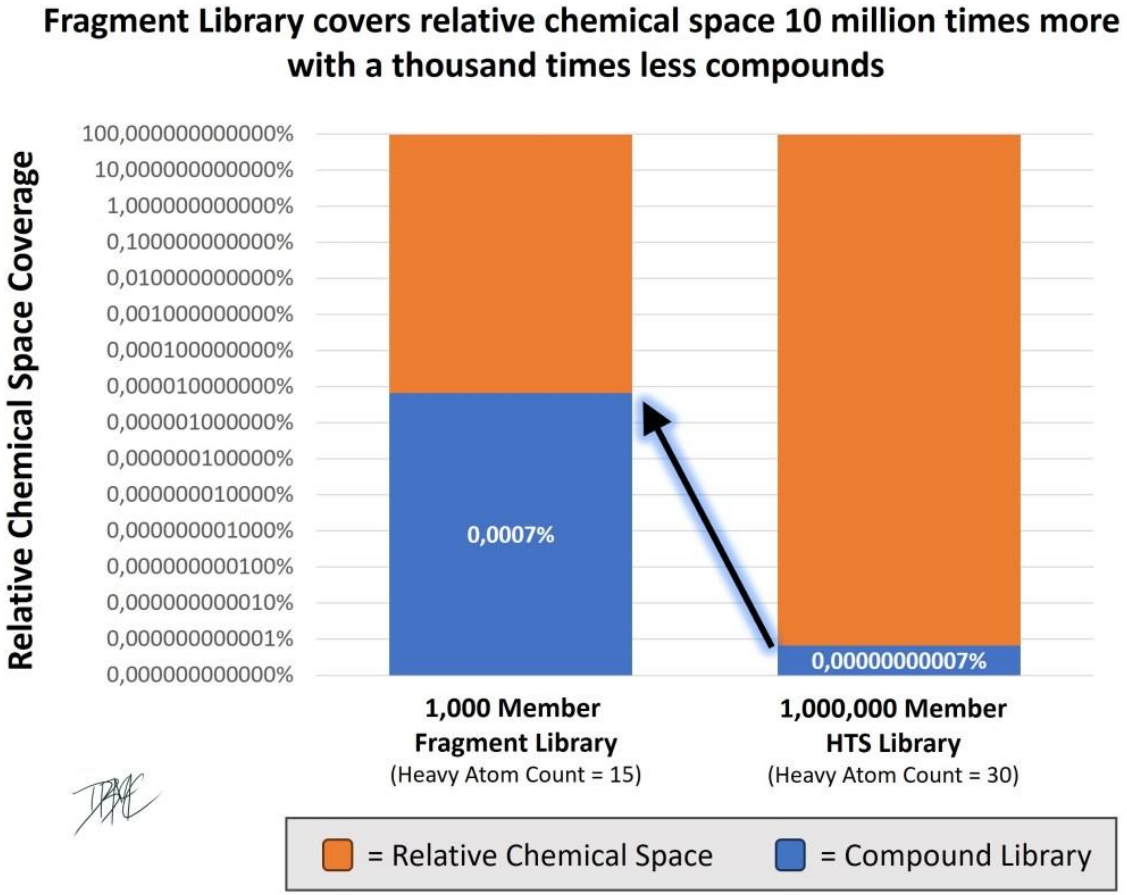
Daren Fearon
Senior Beamline Scientist (XChem)
daren.fearon@diamond.ac.uk

Blake Balcomb
Beamline Scientist (XChem)
blake.h.balcomb@diamond.ac.uk

Fragment-Based Drug Discovery



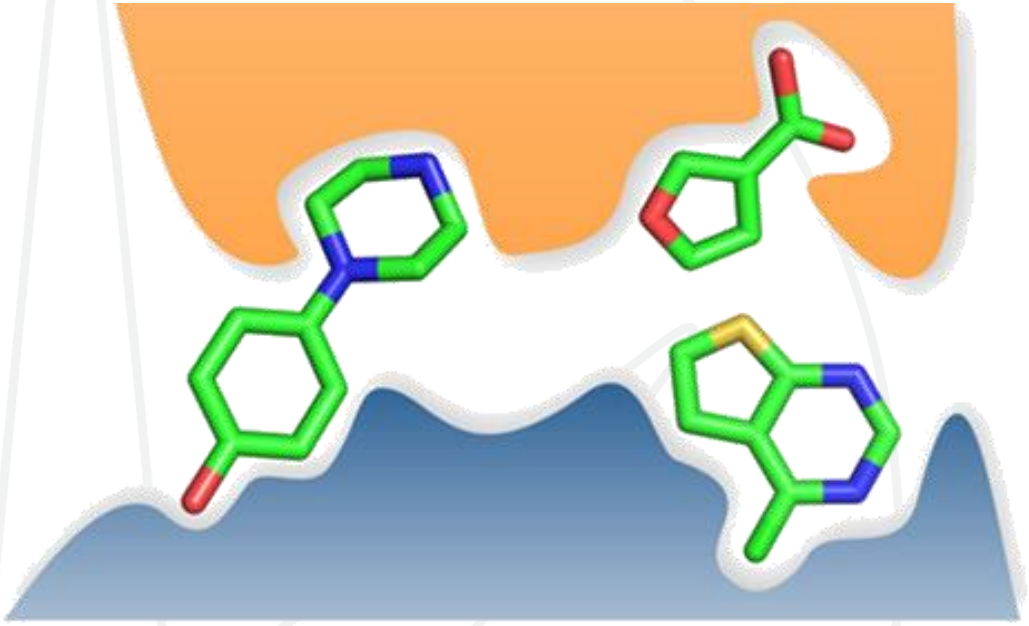
- Molecular weight < 300 Da
- mM-µM affinities **BUT** highly efficient
- Often identified by biophysical methods
- Libraries typically 500-1000 compounds



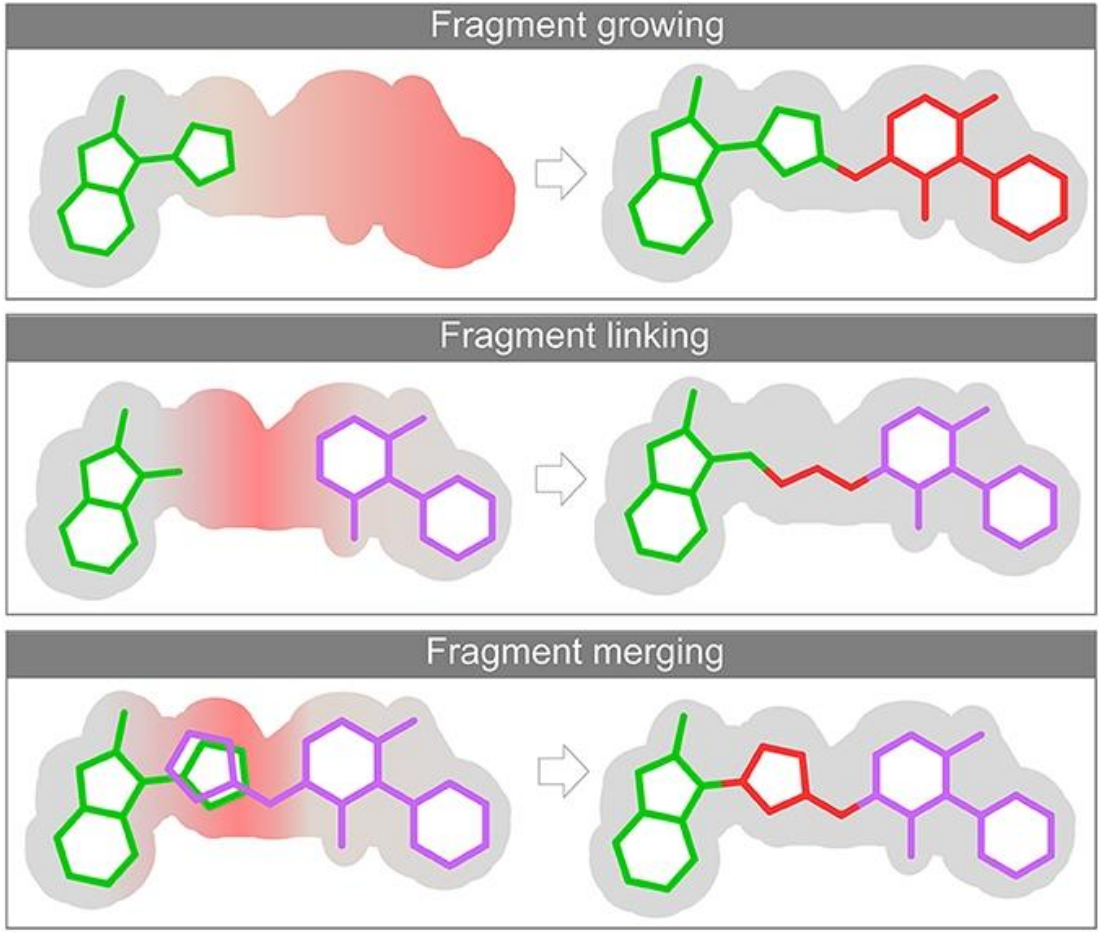
doi.org/10.1021/bi3005126
doi.org/10.3389/fchem.2020.00093
https://mcconnellsmedchem.com/2024/01/09/chemical-space-is-big/

Crystallographic Fragment Screening (CFS)

CFS is a powerful technique for hit identification

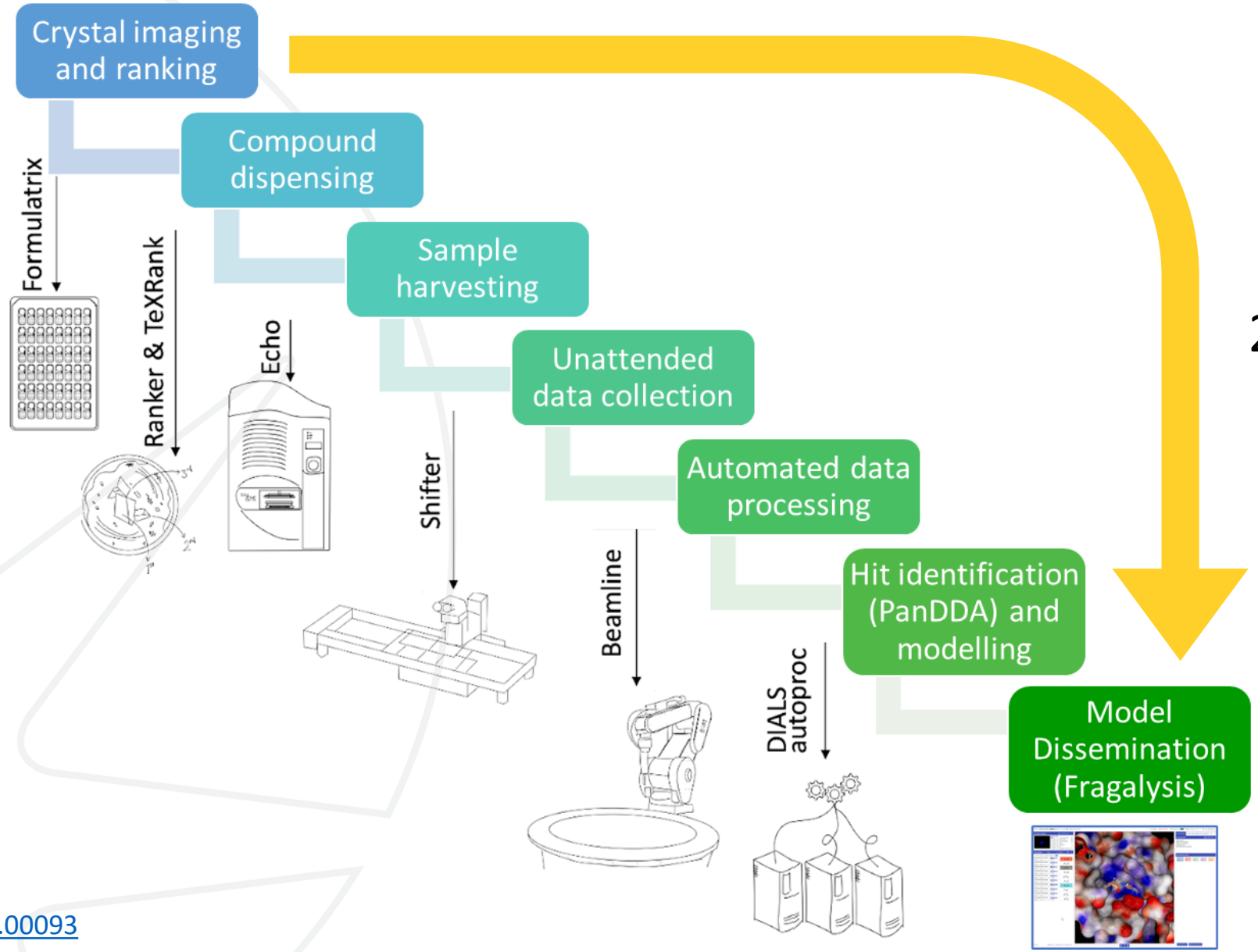


- Highly sensitive
- Directly yields structural information that can drive hit-to-lead and lead optimisation



doi.org/10.1021/bi3005126
doi.org/10.3389/fchem.2020.00093
https://mcconnellsmedchem.com/2024/01/09/chemical-space-is-big/

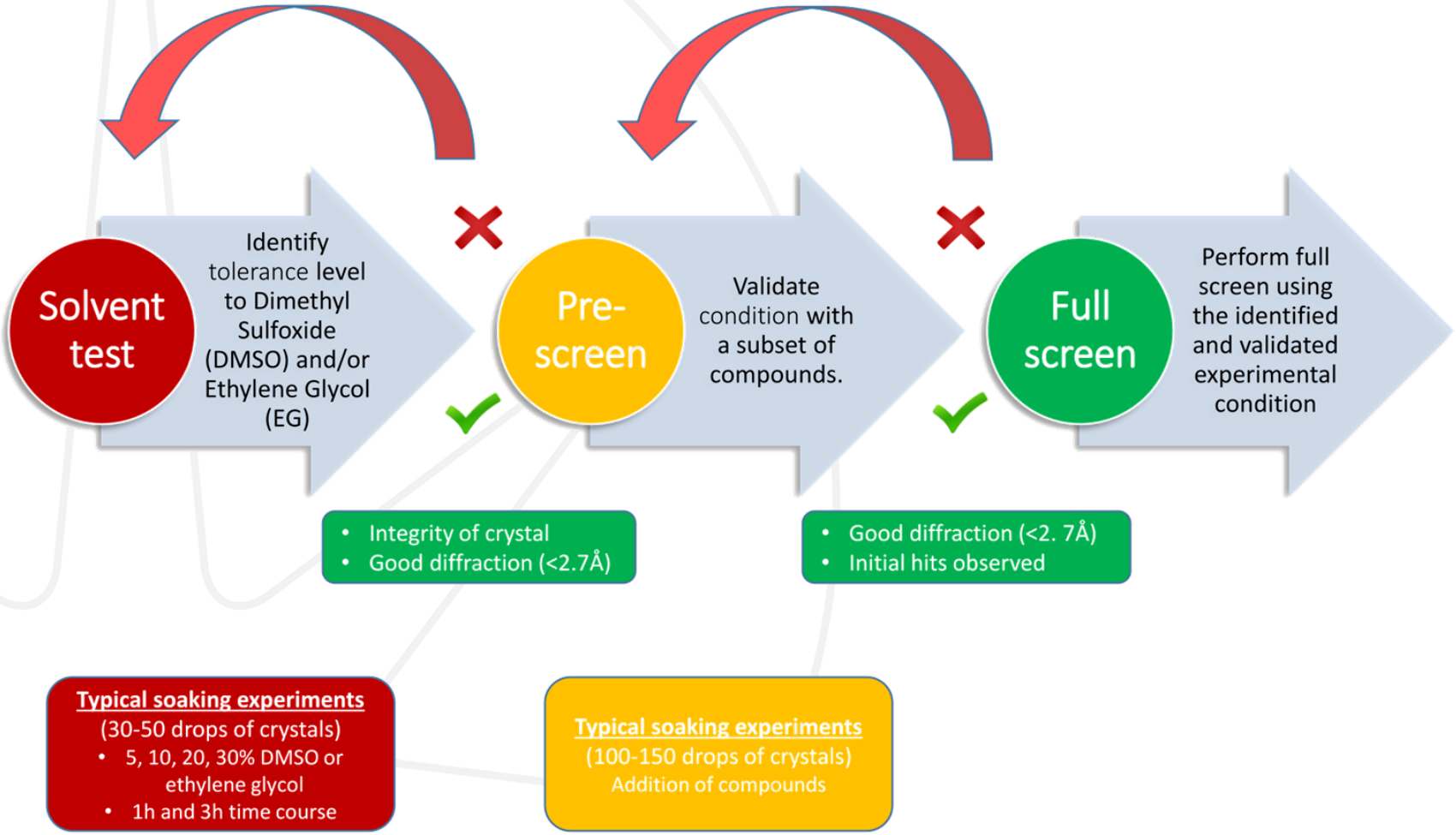
CFS at Diamond: XChem

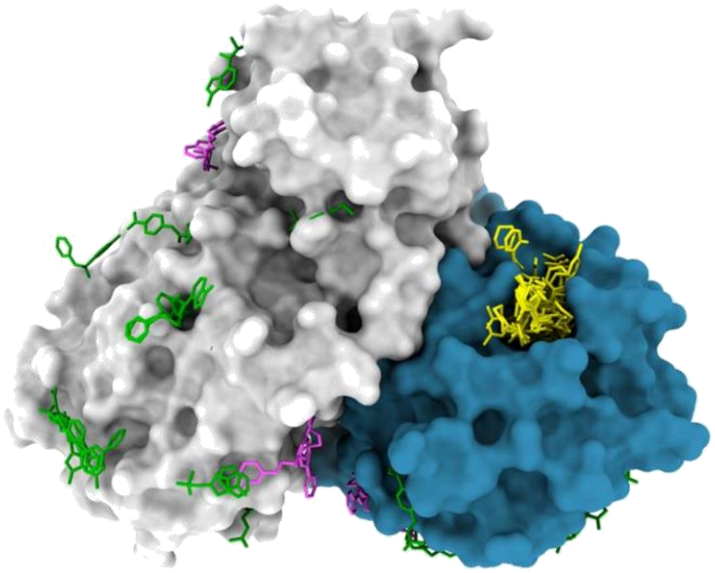


2-3 weeks

doi.org/10.3389/fchem.2020.00093

XChem workflow



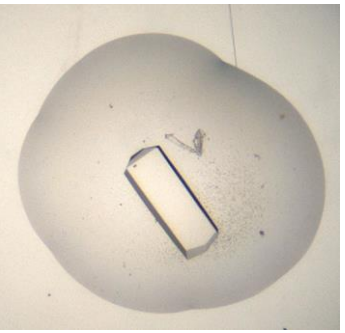


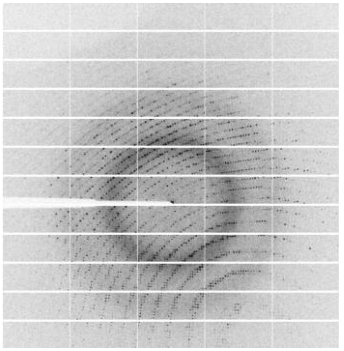
Screening outcome:
- 5-10% hit rate typical
- Fragments bound at range of sites

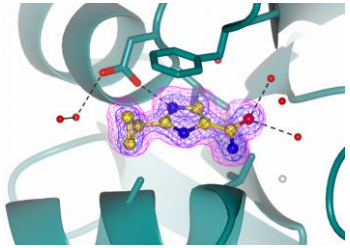
doi.org/10.3791/62414

Ideal “XChem ready” crystal systems

- Grow reproducibly (>50% drops) in SWISSCI 3-drop plates
- Consistently diffract to high resolution (<2.5 Å)
- Tolerate high solvent concentrations
- Don't require complicated cryoprotection
- Crystals are chunky, rather than needles
- Don't stick to the plate
- Don't grow skin on the drop







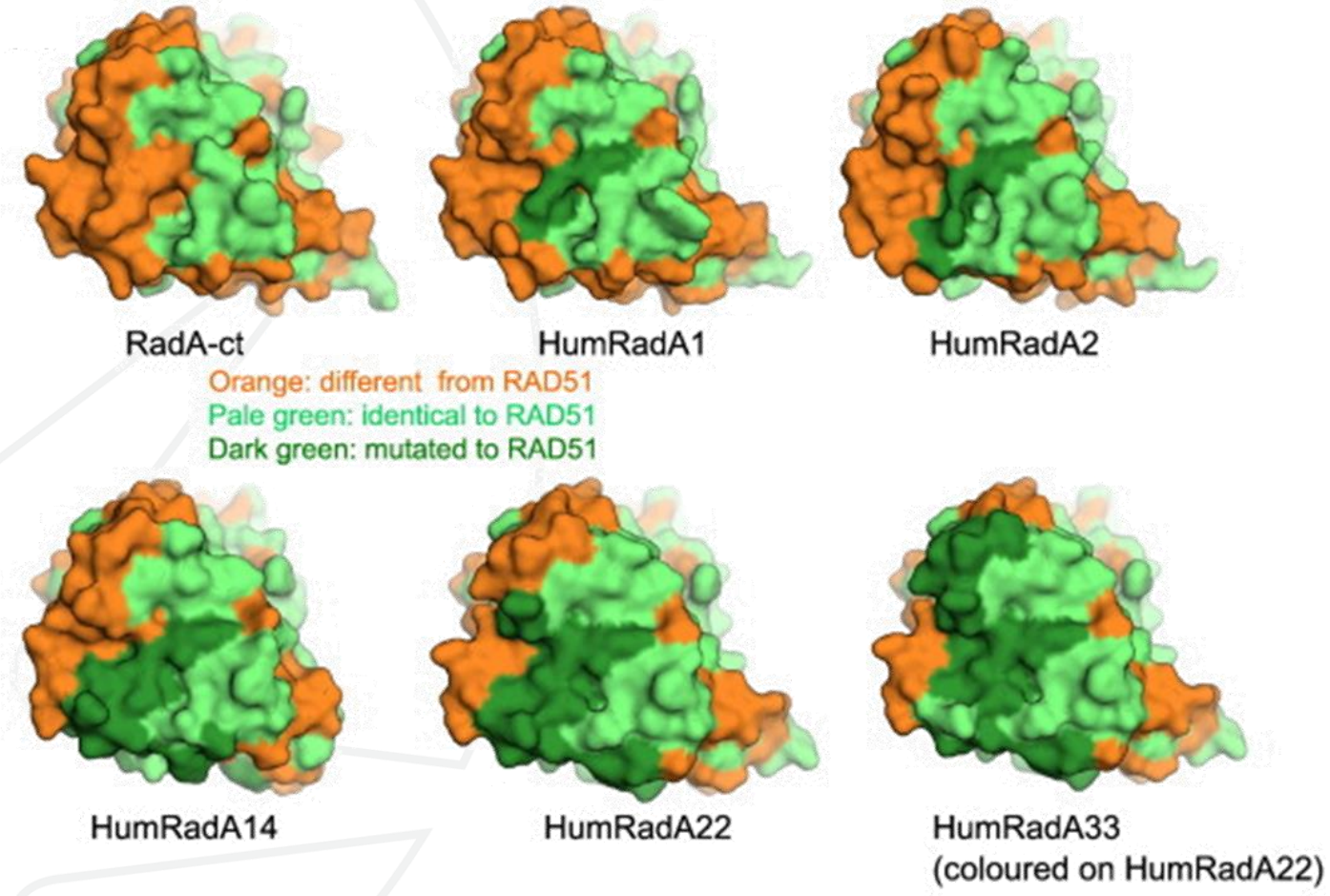
But non-ideal crystals are feasible!

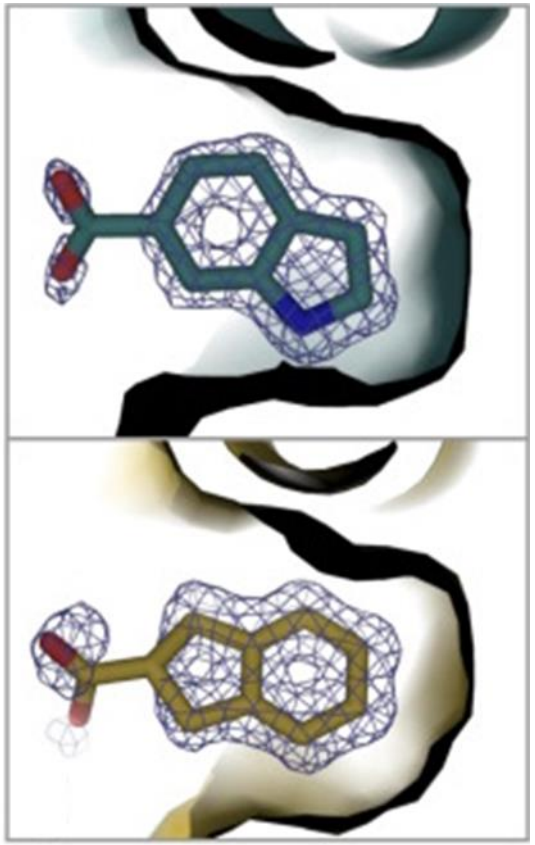
https://www.diamond.ac.uk/Instruments/Mx/Fragment-Screening/Troubleshooting-Tips-Tricks/Crystallisation-And-SamplePrep.html

doi.org/10.20944/preprints201809.0383.v1

Protein engineering/surrogate systems

Can be useful if not possible to produce target in a form that is compatible with the requirements of FBDD

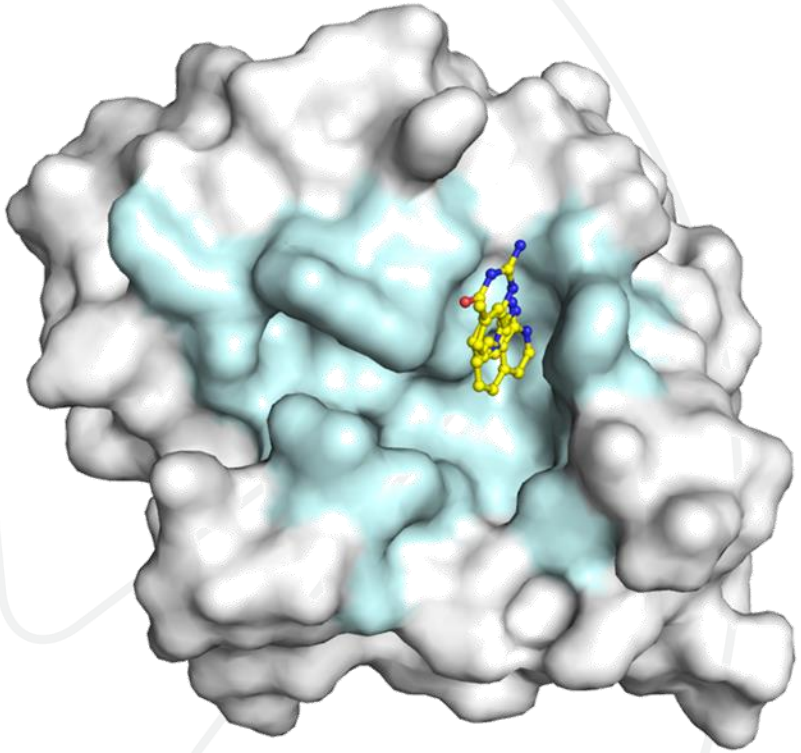




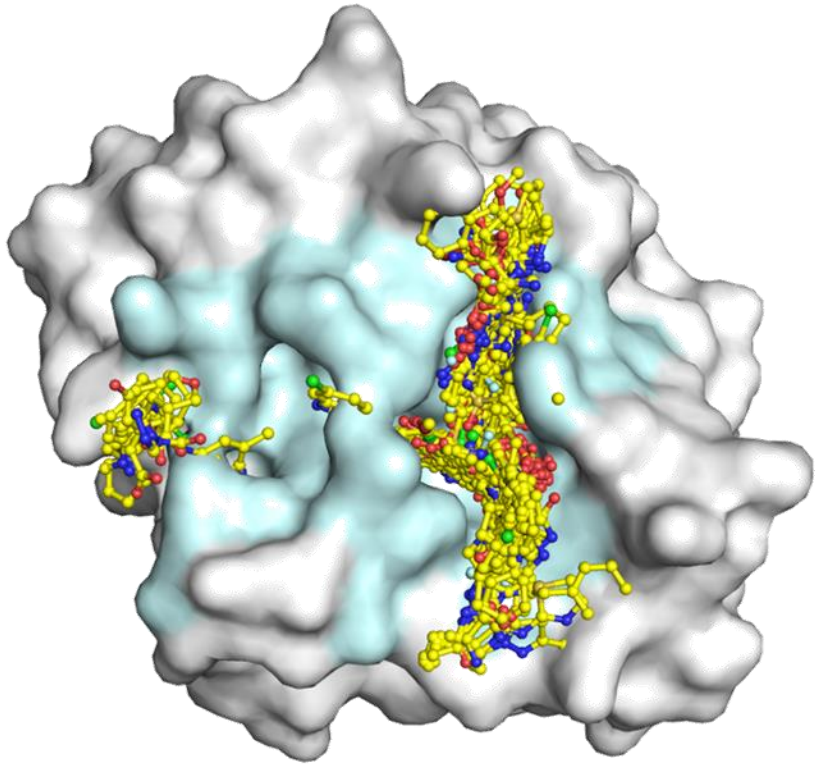
doi.org/10.1016/j.jmb.2016.10.009

Finding the “right” crystal form

SARS-CoV-2 Nsp3 Macrodomain



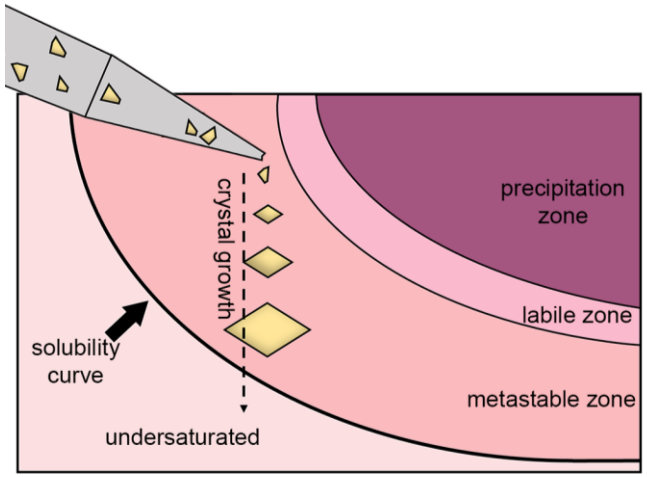
C2 crystal form
234 datasets
3 fragments in ADPr site (1.3%)

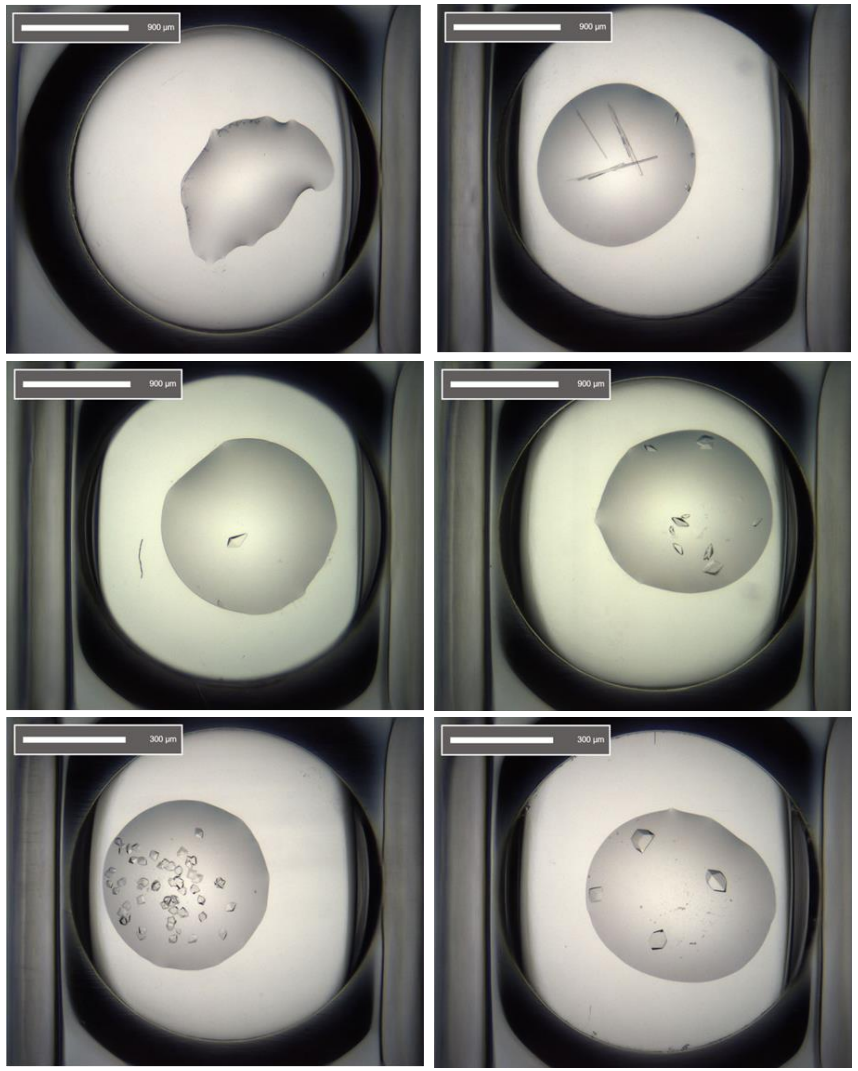


$P4_3$ crystal form
2,509 datasets
181 fragments in ADPr site (7.2%)

doi.org/10.1126/sciadv.abf8711

Crystal seeding can enable CFS





- Decouples nucleation from crystal growth
- Can help to:
  - Improve/standardise crystal quality
  - Increase reproducibility
  - Speed up crystallisation
  - Remove problematic buffer components
  - Identify (more) useful crystal forms
  - Aid co-crystallisation

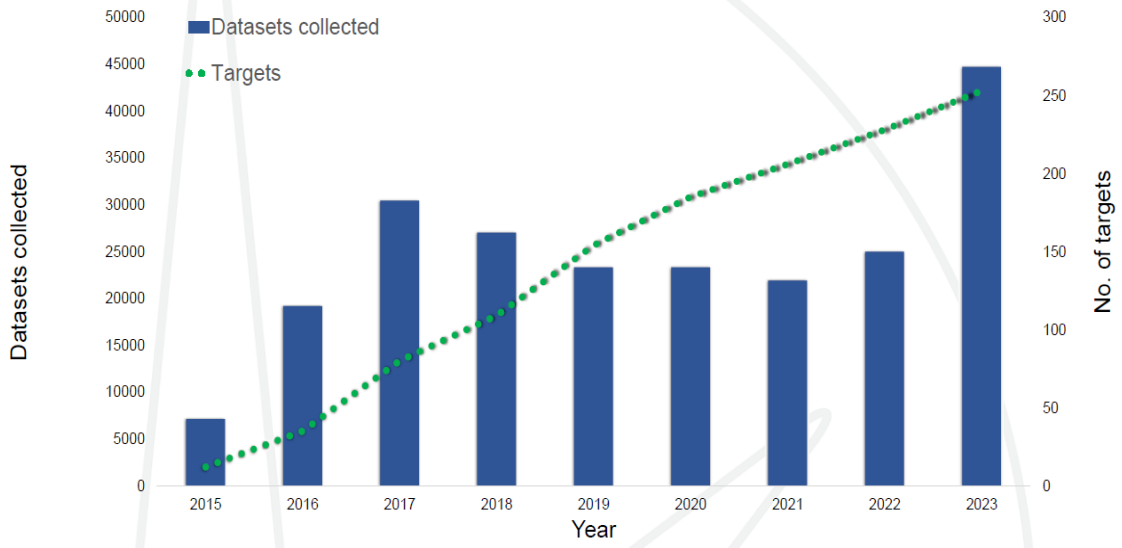
https://www.diamond.ac.uk/Instruments/Mx/Fragment-Screening/Troubleshooting-Tips-Tricks/Crystal-seeding.html
doi.org/10.1016/bs.mie.2023.08.005

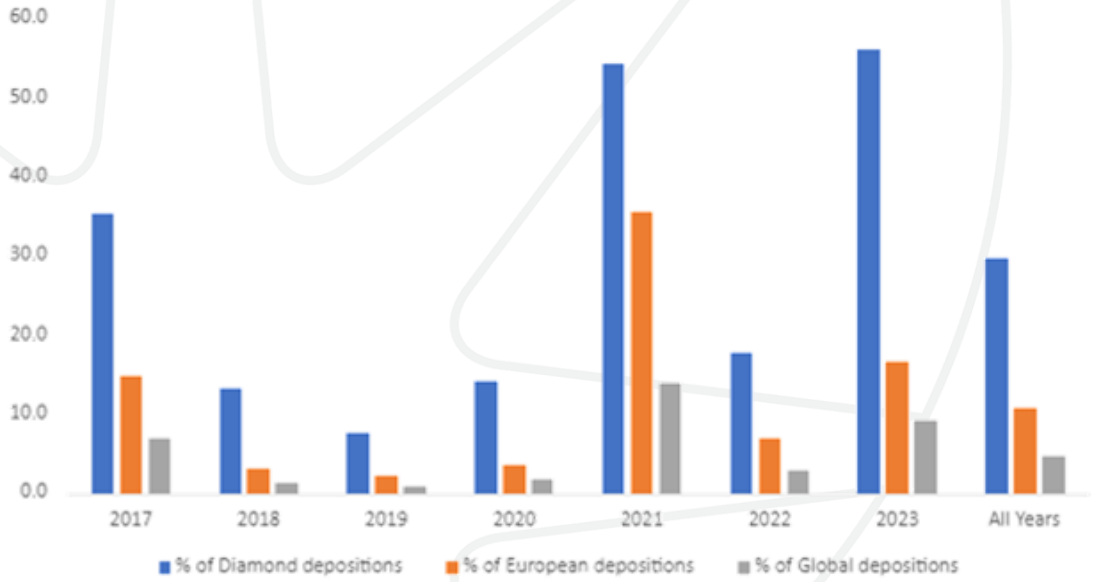
Establishing “XChem ready” systems

- Get the right protein for crystallisation
- Identify multiple crystallisation conditions/crystal forms
    - PEG preferable over high salt conditions
    - Be aware of pH and volatile solvents
- Establish robust seeding protocol
- Run QC for your protein batches and crystal trays
- Determine transferability of crystallisation/trays between locations and life span of crystals
- When robustness established, keep things consistent

https://www.diamond.ac.uk/Instruments/Mx/Fragment-Screening/Troubleshooting-Tips-Tricks/Crystallisation-And-SamplePrep.html
doi.org/10.20944/preprints201809.0383.v1

10 years of XChem



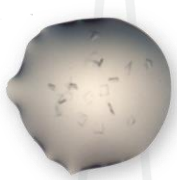


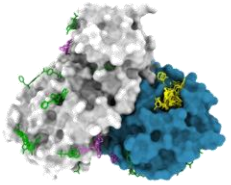
- Routine users since 2015
- **> 300 academic projects**
  - >240,000 datasets (45k in 2023)
  - **30-50k liganded structures**
  - 3,750 models deposited in PDB
    - > 600 depositions in 2023
- **Industry Team** – completed campaigns against **150 targets**

Impact of CFS – the Moonshot

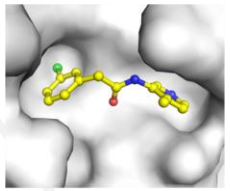
COVID Moonshot

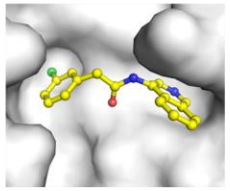
XCHEM Diamond Fragment Screening

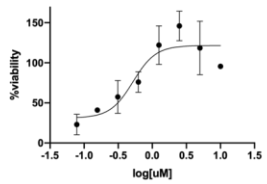


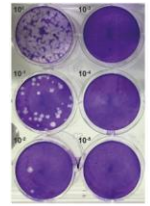


WORLDWIDE PDB PROTEIN DATA BANK

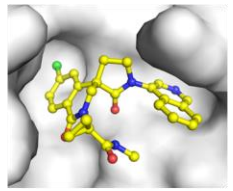






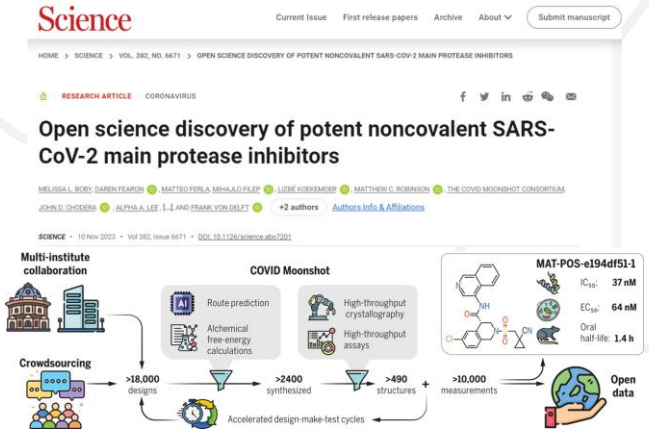


wellcome



Feb 2020	March 2020	April 2020	April 2020	July 2020	Aug 2020	Feb 2021	July 2021	Feb 2022
M^{pro} cloned, produced and crystallised at Diamond	Data collection complete for crystallographic fragment screen	78 fragment structures released	First µM fragment merge identified	First nM inhibitor identified	Measurable cellular potency achieved	First compound with sub µM antiviral activity	Received $11M funding from Wellcome Trust for IND enabling studies	Nominated pre-clinical candidates

In under 2 years progressed from weak fragments to pre-clinical candidates


Science

Open science discovery of potent noncovalent SARS-CoV-2 main protease inhibitors

Multi-institute collaboration

COVID Moonshot

Crowdsourcing

Route prediction

Alchemical free-energy calculations

High-throughput crystallography

High-throughput assays

MAT-POS-e194df51-1

37 nM

64 nM

Oral half-life: 1.4 h

>18,000 designs

>2400 synthesized

>490 structures

>10,000 measurements

Open data

Accelerated design-make-test cycles

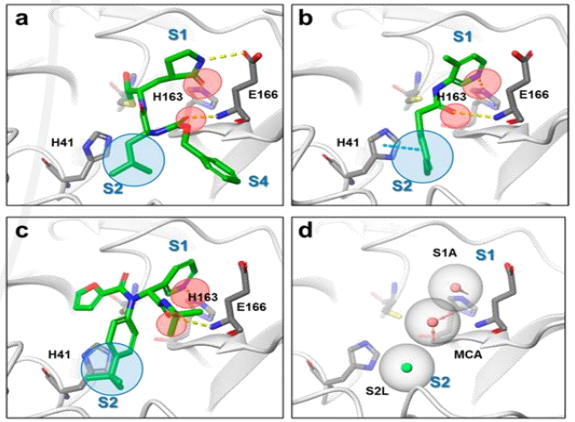


Figure 2. Binding modes of 3CL^{pro} inhibitors, their interactions, and defined pharmacophore filters for virtual screening. (a) Crystal structures of GC376 (PDB code: 6WTT), (b) 3-aminopyridine-like compound of the Postera COVID moonshot project (PDB code: 5RH2), and (c) ML188 (PDB code: 7L0D). The common H-bond acceptors are circled in red; the common hydrophobic features are circled in blue. (d) Common pharmacophore shared with inhibitors A–C. Red and green spheres represent H-bond acceptors and lipophilic features, respectively.

Moonshot molecules (and structures) used by Shionogi to identify starting point for clinically approved SARS-CoV-2 treatments in Japan (Ensetrilvir)

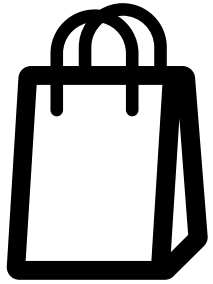
doi.org/10.1038/s41467-020-18709-w
doi.org/10.1126/science.abo7201
doi.org/10.1021/jacs.1c08402

diamond

XChem access modes

- Standard academic access - covers single target with three levels:
  - Tier 1: Exploratory projects (1 shift; 200-300 datasets)
  - Tier 2: Full screen (4 shifts; up to 1000 fragments)
  - Tier 3: Follow-up support (batches of 200-300 compounds)

- XChem BAG access - for groups, institutes or collaborations
  - Routinely have crystal systems for evaluation and screening
  - Hit-to-lead infrastructure in place
  - Stringent internal prioritisation process
  - Experienced crystallographers to organise and provide logistical support

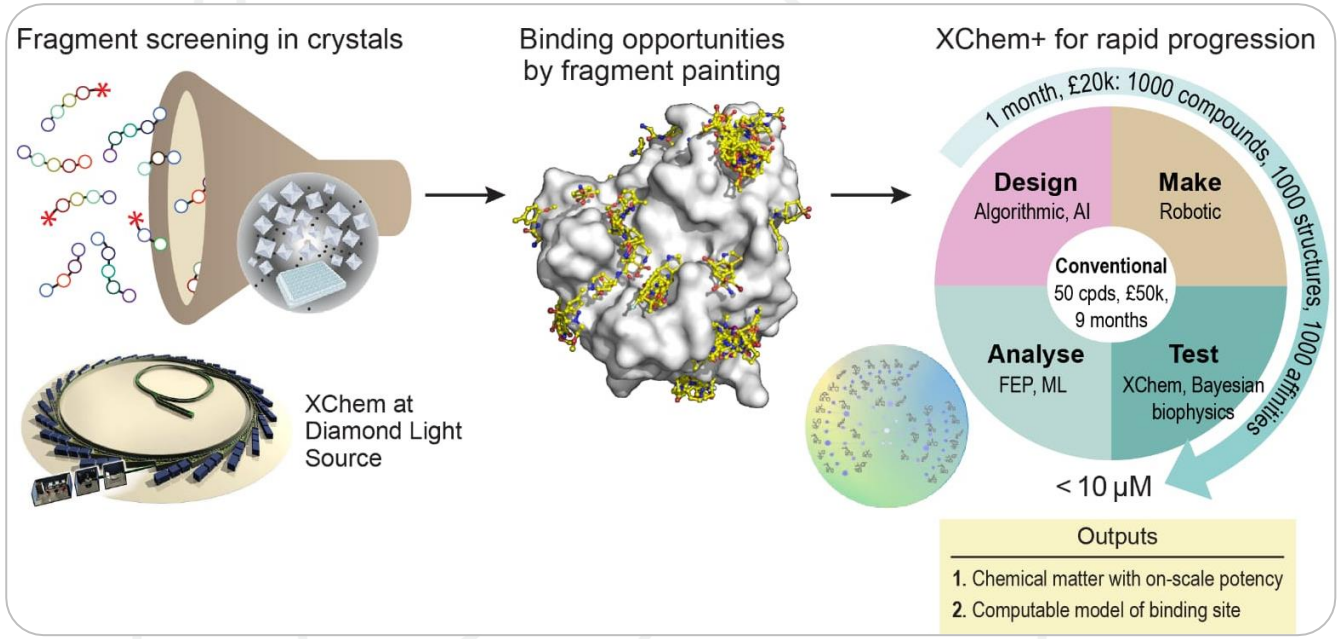


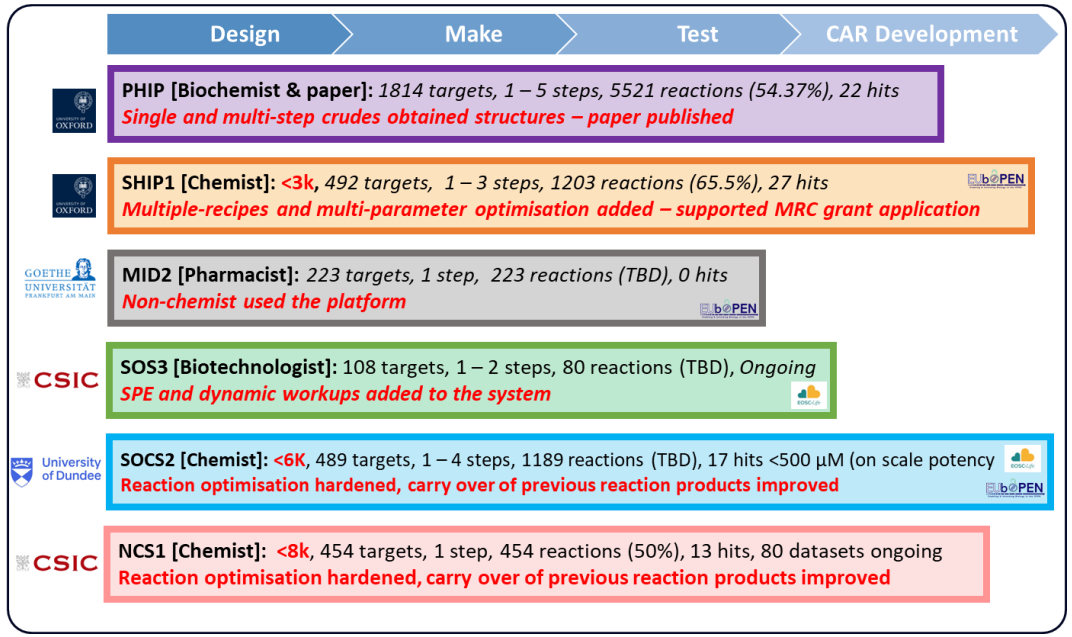
- Industrial/proprietary access:
  - Contact ailsa.powell@diamond.ac.uk

https://www.diamond.ac.uk/Instruments/Mx/Fragment-Screening/XChem-Applications.html
https://www.diamond.ac.uk/industry/Techniques-Available/Integrated-Structural-Biology/Fragment-Screening---XChem.html

Fast Forward Fragments (Tier 3)

- Goal: Augment fragment screen with one high-value, low-cost DMTA iteration





- Currently under development with internal projects and collaborators
  - 6 projects performed + 4 projects ongoing & scheduled (till Q2-2025)
- Flagship outcomes to date:
  - SOCS2 (Dundee): On-scale potency for mol-glue in 2 months *(vs 3 yrs)*
  - NCS1 (Madrid): Sampling of multiple PPI subsites + on-scale affinity
- Roll out to users late 2025

XChem/I04-1 Team

Professor Frank von Delft
Principal Beamline Scientist

Dr Daren Fearon
Senior Beamline Scientist (XChem)

Dr Warren Thompson
Senior Software Support Scientist

Dr Jasmin Aschenbrenner
PDRA - Antiviral research

Jose Brandao-Neto
Senior Beamline Scientist (I04)

Dr Blake Balcomb
Beamline Scientist (XChem)

Dr Conor Wild
PDRA – XChem Algorithms

Dr Ryan Lithgo
PDRA - Antiviral research

Dr Louise Dunnett
Beamline Scientist (I04-1)

Dr Ailsa Powell
Principal Industrial Scientist

Dr Max Winokan
PDRA – XChem Algorithms

Dr Eda Capkin
PDRA - Antiviral research

Dr Kutu Nidamarthi
Beamline Scientist (MX)

Grant Watt
Beamline Scientist (I04-1)

Isabel Barker
Industrial Liaison

Peter Marples
XChem Research Technician

Mathew Golding
Junior Research Scientist (Chemistry)

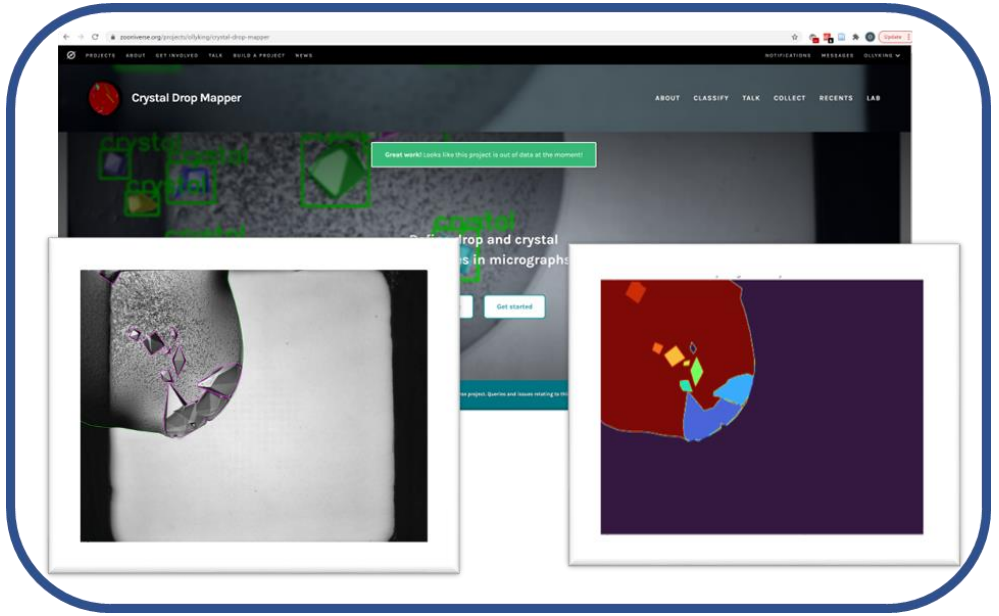
Felicity Bertram
Support scientist (MX)

http://www.diamond.ac.uk/Beamlines/Mx/Fragment-Screening.html

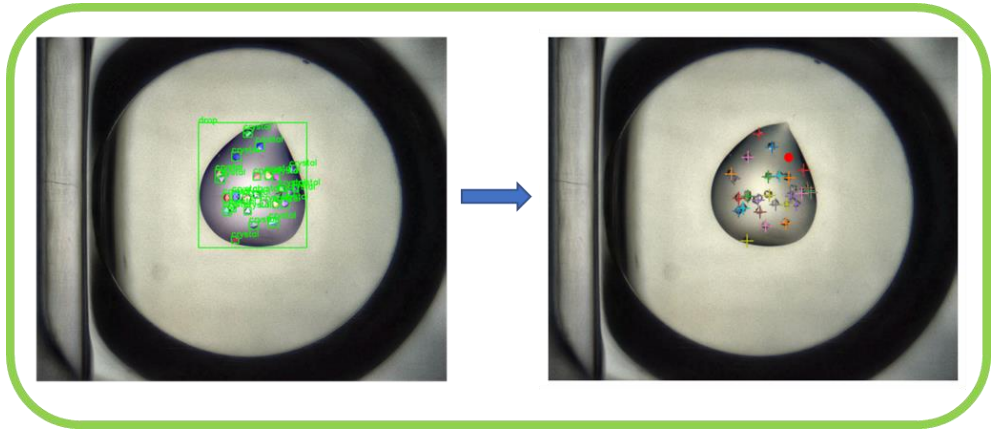
Supplementary slides

Crystal Hits in My Plate (CHiMP)

- Replacement for TeXRank with deep learning model (CHiMP)
- Training data generated with help of beamline staff and users
- Prediction produces detections and masks for drops and crystals
- Converted to position for dispensing compound into drop using Echo
- Information sent to web app (EchoLocator) for display to users

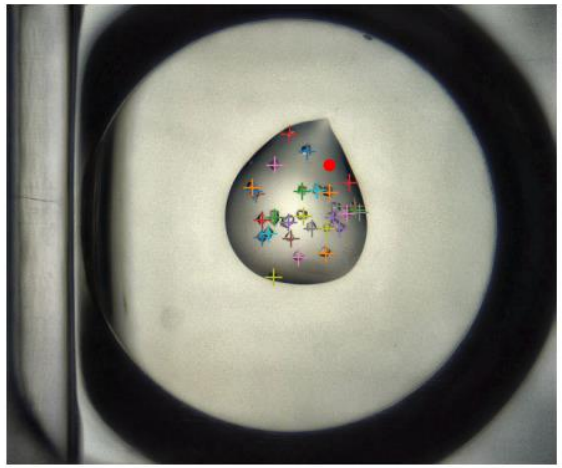


Olly King



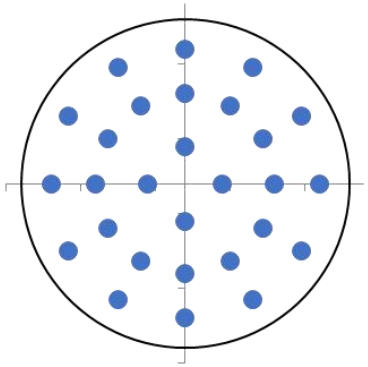
Crystal Soaking – Acoustic dispensing

- Contactless, acoustic liquid dispensing technology
- High precision/accuracy dispensing
- Optimised for DMSO liquid handling
- Can soak hundreds of crystals in minutes

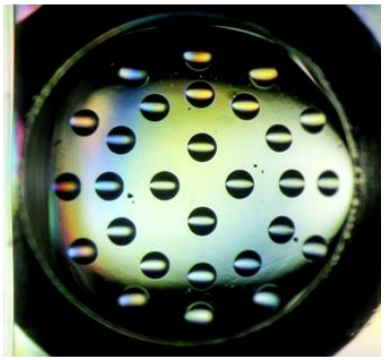


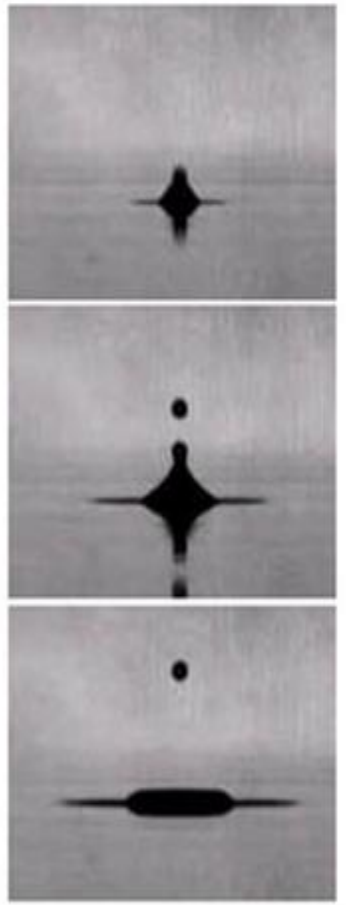
Crystallisation plate 2.5 nL Echo drops

Requested



Delivered





http://www.labcyte.com/
https://doi.org/10.1107/S1399004714017581
https://doi.org/10.1107/S205979831700331X

Fragment Libraries

Several diverse fragment libraries available:

- Over 3000 compounds in total
- All available in d6-DMSO at 100-500 mM
  - Some also offered in ethylene glycol (EG)
- All commercially available or published
- Stored at controlled humidity and oxygen levels (5%)

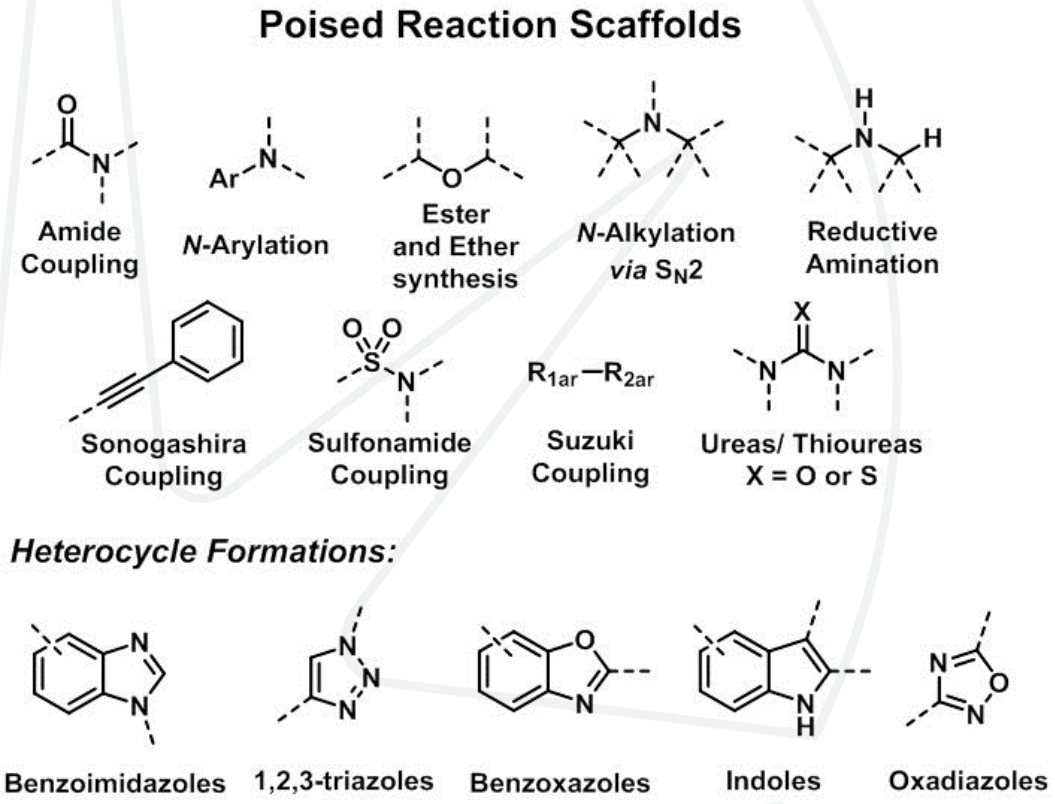
List of live fragment libraries

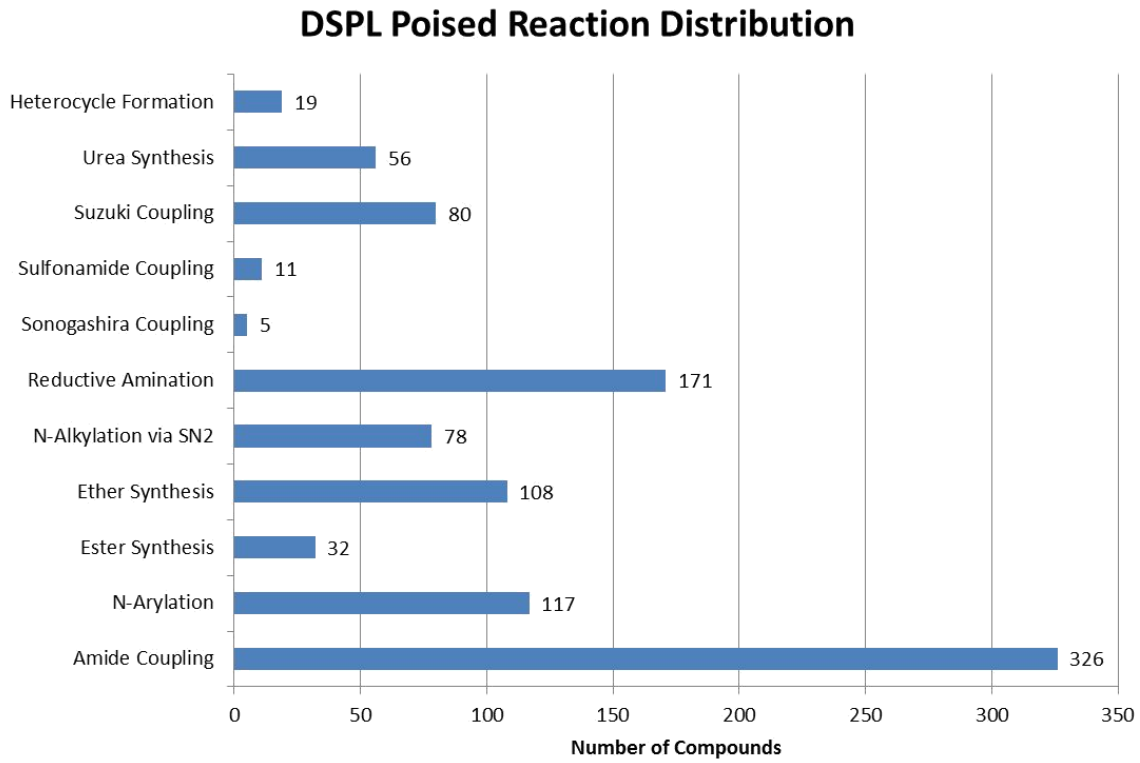
Library	Description	# of cpds	conc. in DMSO (mM)	conc. in EG (mM)	Available to Industry?	Contact person for follow-ups
DSiP	Poised reaction for quick follow-ups	768	500	100	yes	XChem
Collaborative libraries						
EUbOPEN-DSIP extended	Poised saturated heterocycles	108	500	100	TBC	Adam Nelson
EU-OPENSCREEN	Possibility of follow-ups through EU-OPENSCREEN	968	100	-	TBC	EU-OPENSCREEN consortium
SpotXplorer	Pharmacophore and binding hot spots	96	varied	-	TBC	György M Keserű
FragLites	Halogenated fragments	31	500	100	yes	Mike Waring and Martin Noble
PepLites	Halogenated peptidomimetics	25	500	100	TBC	Mike Waring and Martin Noble
Cambridge 3D	3D and poised	137	250-500	-	TBC	David Spring
York 3D	3D, substituted aliphatic heterocycle	106	500	100	yes	Peter O'Brien
Leeds 3D	3D, natural product-like scaffolds, high sp3	125	Varied	-	TBC	N/A
MiniFrags	Astex's pharmacophore	80	1M	in water available	yes	N/A
CovHetFrags	Small heterocyclic electrophyles (Covalent MiniFrags)	141	varied	-	TBC	György M Keserű
Cys Electrophyle	Cysteine covalent library	993	20	-	TBC	Nir London

https://www.diamond.ac.uk/Instruments/Mx/Fragment-Screening/Fragment-Libraries.html

DSi-Poised Library

- Design principle to allow rapid, cheap follow-up synthesis to establish SAR
- Generated by analysing commercial fragment space for “poised-ness” and selecting diverse subset available from Enamine REAL Database

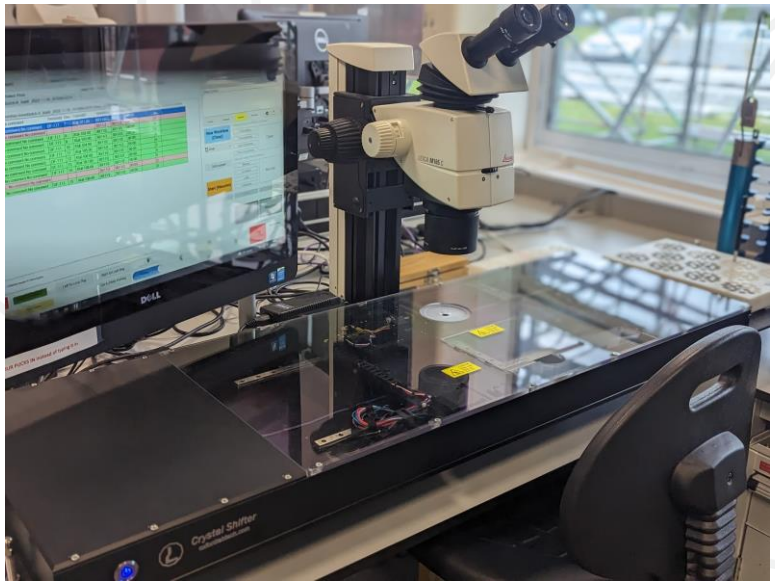


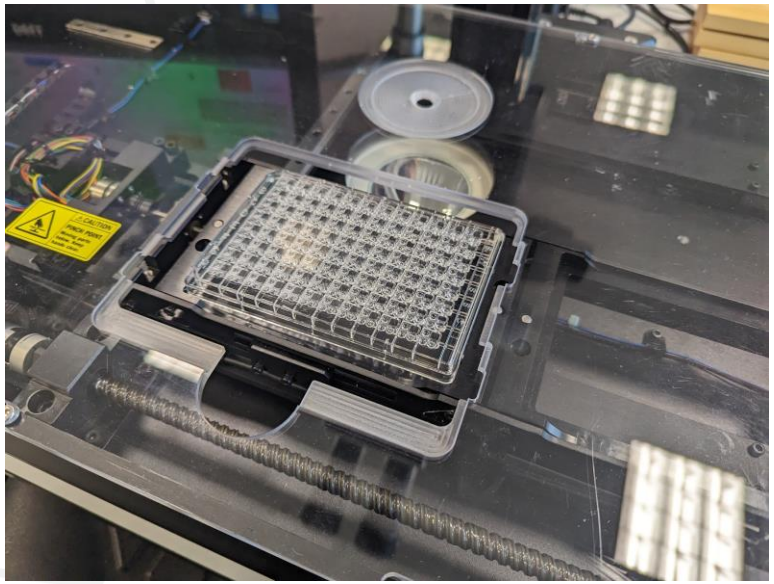


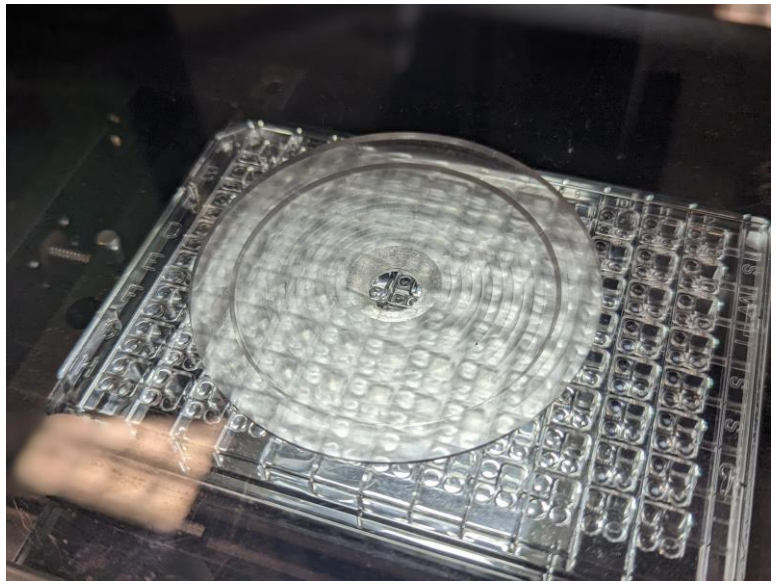
10.1039/c5sc03115j
https://enamine.net/compound-libraries/fragment-libraries/dsi-poised-library

Crystal Harvesting - Crystal Shifter

- X/Y stage for crystal harvesting
  - 100 samples/user is common but 200 samples/hour is possible
- 4 units available as part of XChem facility
- Dedicated software with sample information recorded in database



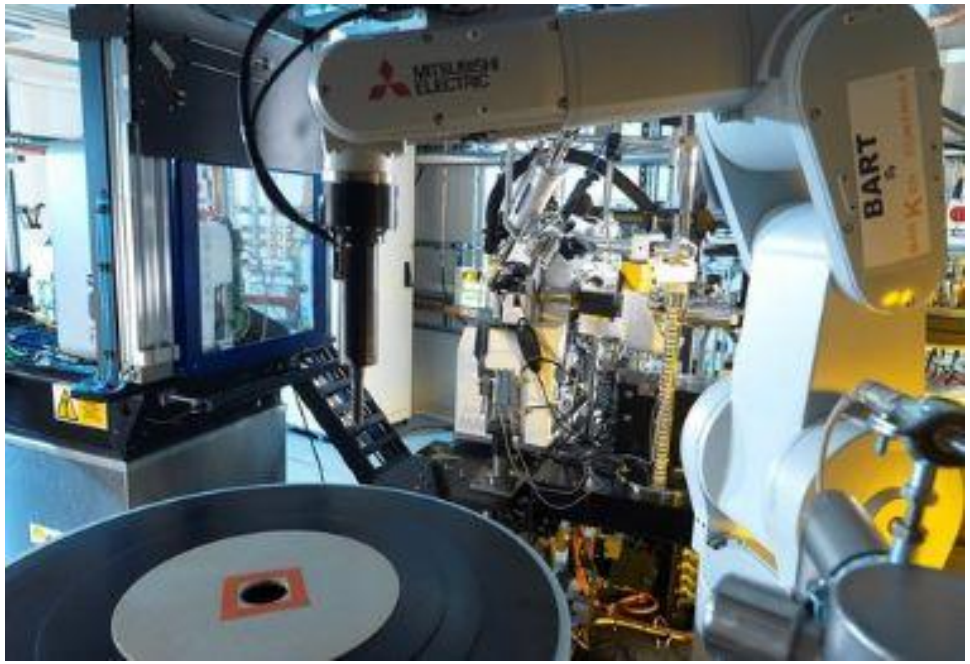


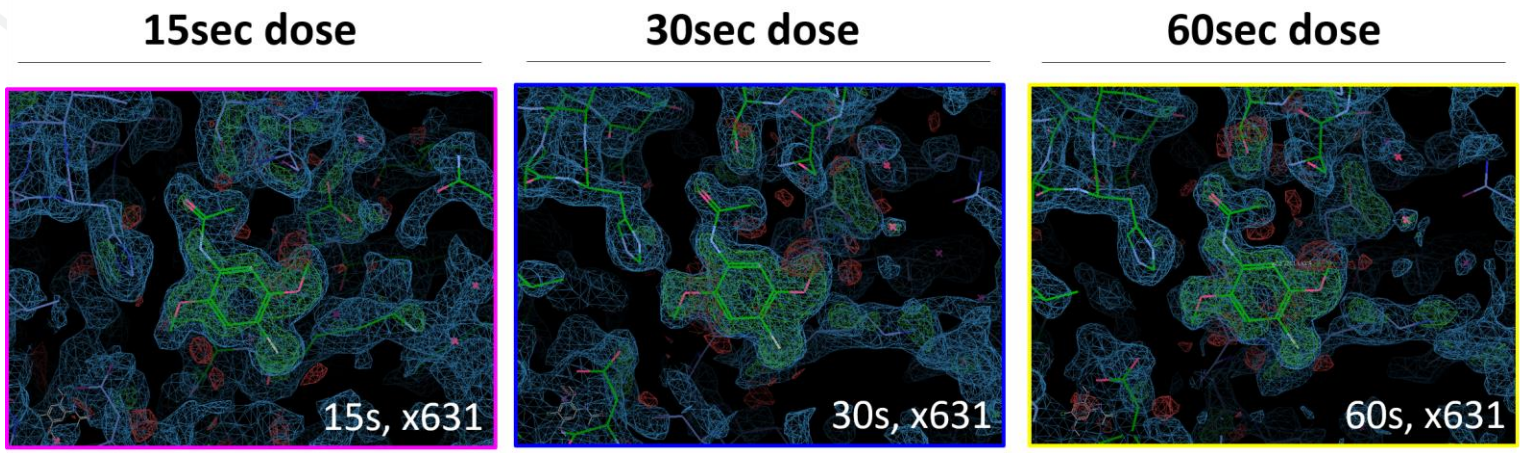


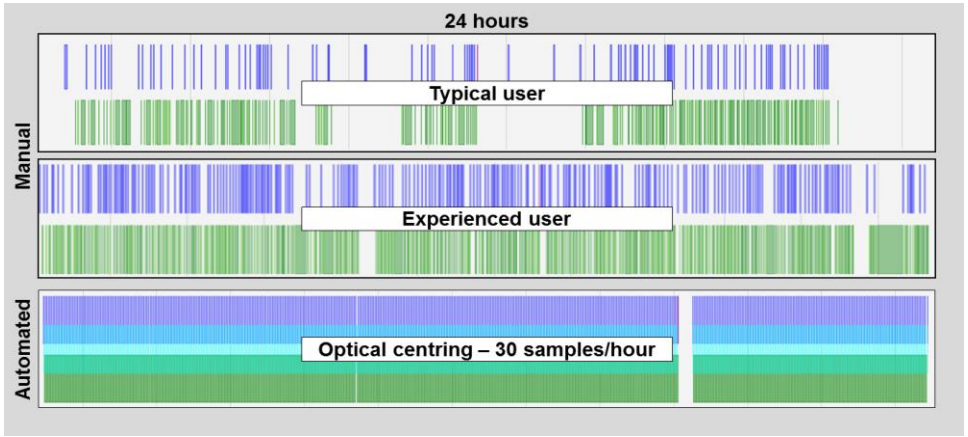
http://www.oxfordlabtech.com/
https://doi.org/10.1107/S2059798320014114

Data Collection – I04-1

- High-level automation
- Optical-based or X-ray based centring
- Crystal mounting (~32 samples/hour)
- Rapid sample exchange (20 s/sample)
- High-flux (0.92 Å fixed wavelength)
- BART high capacity dewar (592 samples)
- Eiger 2 XE 9M detector (500 Hz)

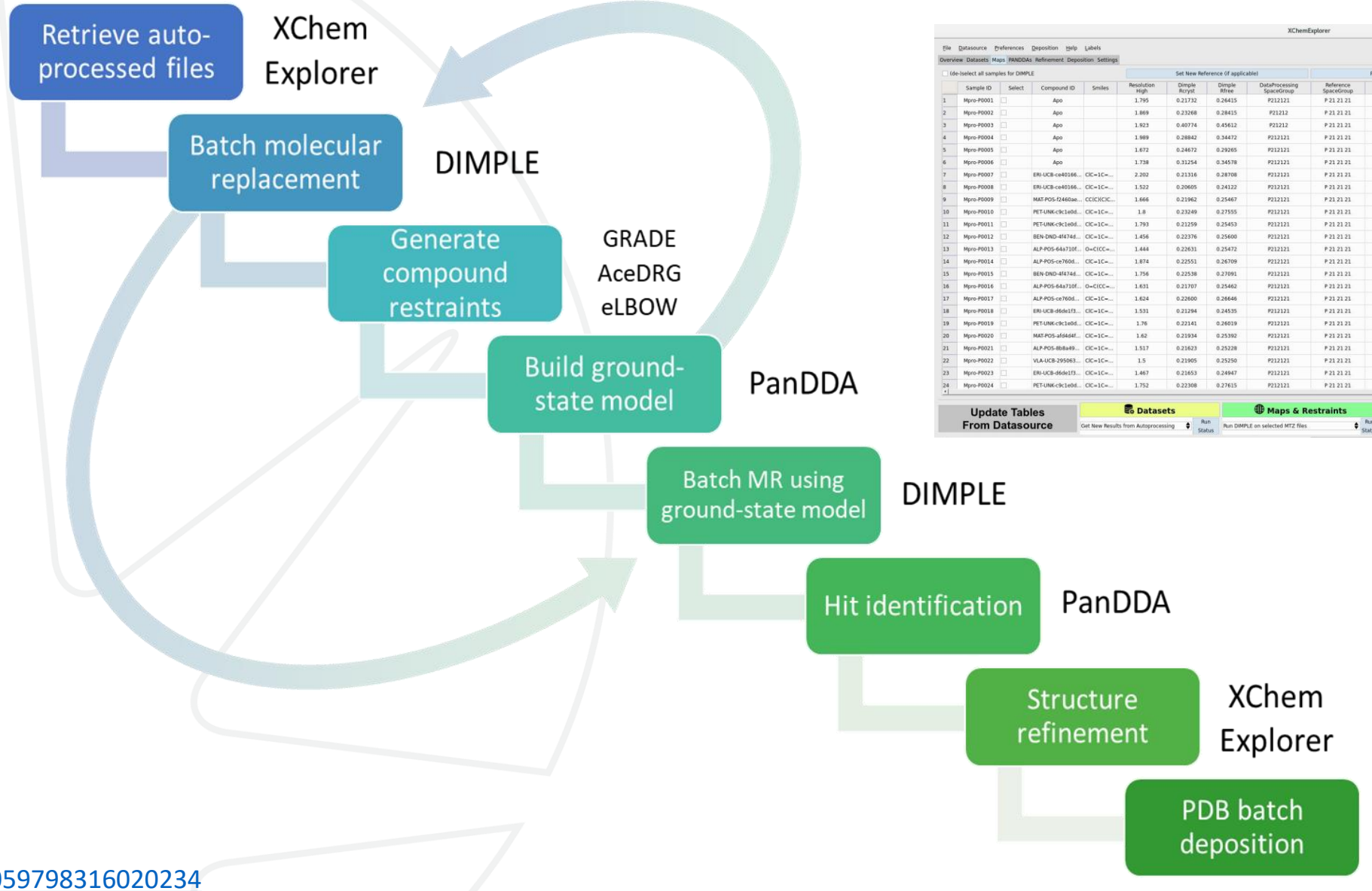






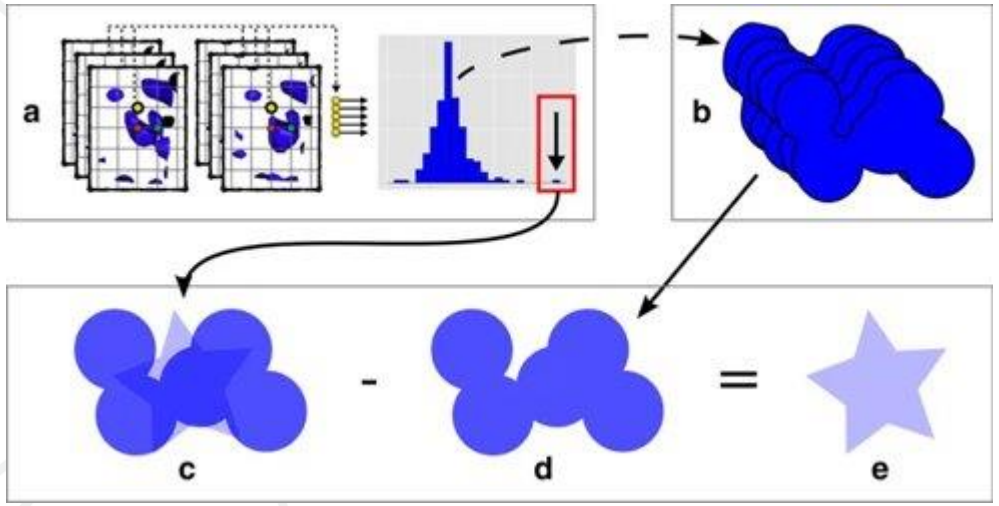
https://www.diamond.ac.uk/Instruments/Mx/I04-1.html

Data analysis workflow

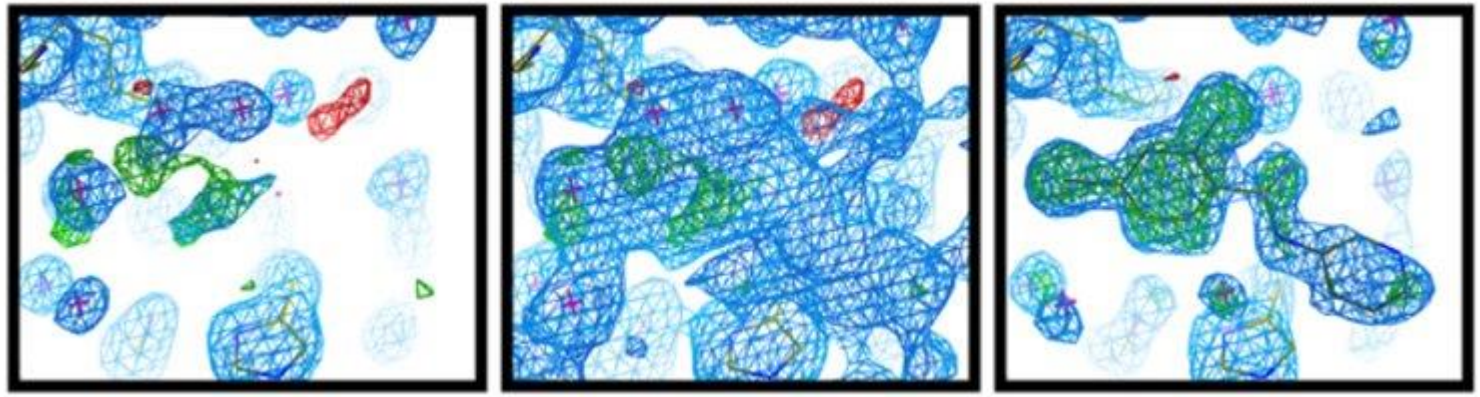


10.1107/S2059798316020234

Pan Density Dataset Analysis (PanDDA)



High Contour **Low Contour** **PanDDA Map**



https://doi.org/10.1038/ncomms15123
https://doi.org/10.1063/1.4974176

Tools for discovery: Fragalysis Cloud

- *Live on STFC Cloud: https://fragalysis.diamond.ac.uk*
- Addresses gaps:
  - *(1) Focus on hardening & deployment (not novelty-and-publish)*
  - *(2) Develop formulaic fragment-to-hit*
  - *(3) Non-proprietary*
  - *(4) Platform to test & compare premises*
- Supports:
  - Data Dissemination *(open)*
  - Design Collaboration *(closed)*
  - Execution of HPC algorithms
- Availability:
  - Fully open-source
  - Professionally developed web-stack
  - Launchable by anybody *(with expertise & cloud)*
  - *(https://github.com/xchem/fragalysis-stack)*