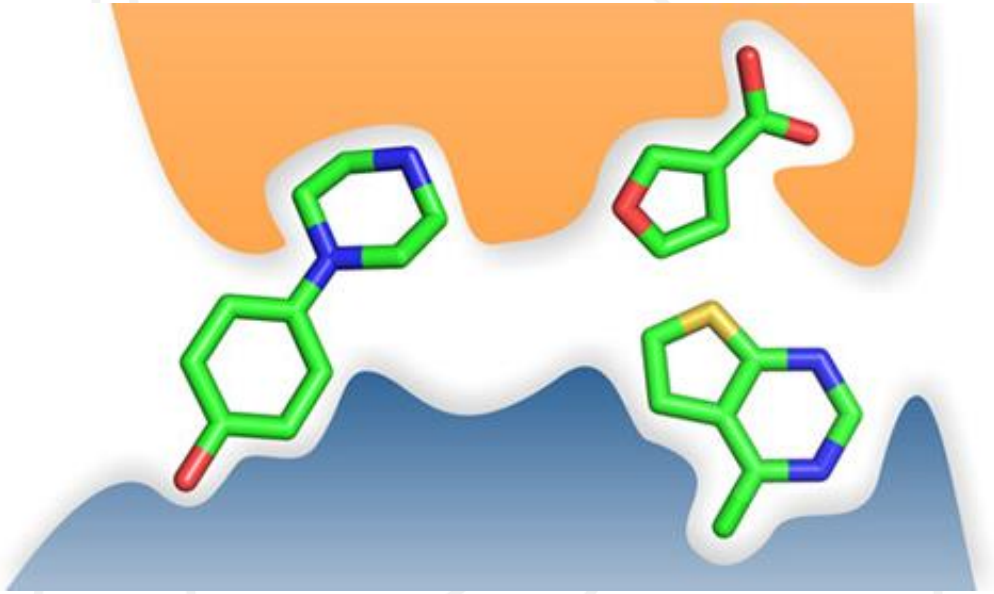
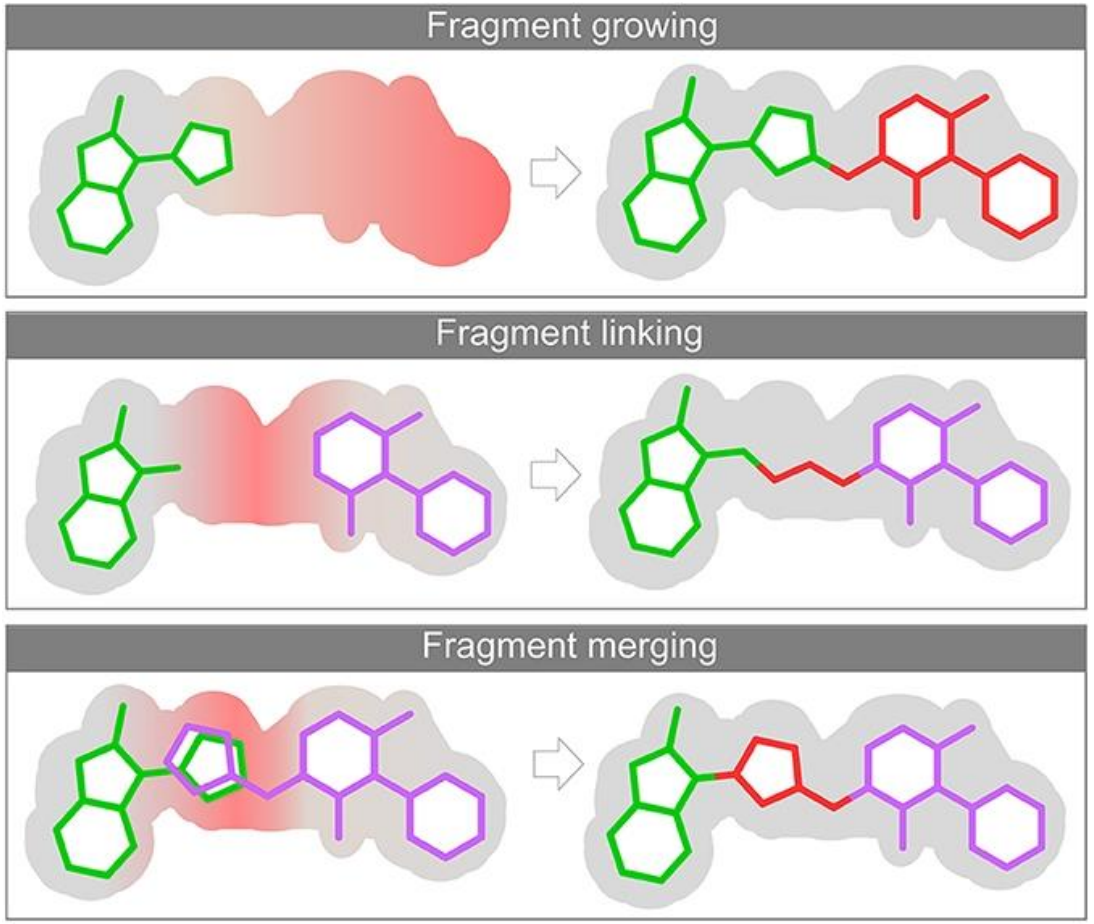
The XChem platform at Diamond Light Source

2024

Fragment Based Drug Discovery

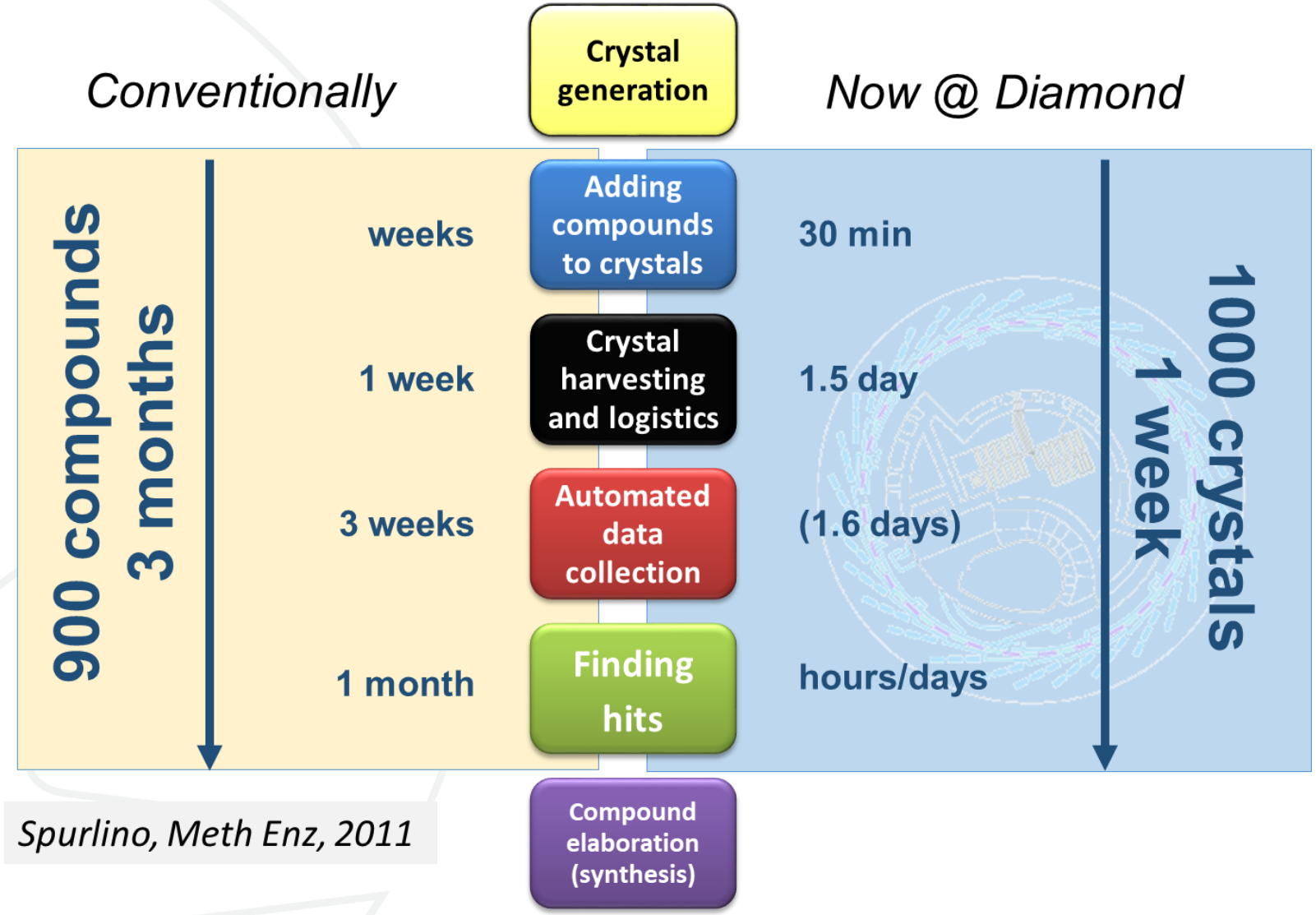


- Libraries of 500-1000 compounds
- Molecular weight < 250 Da
- mM-µM affinities **BUT** with high atom efficiency
- Often identified by biophysical methods
- Drives iterative optimisation



https://doi.org/10.1021/bi3005126

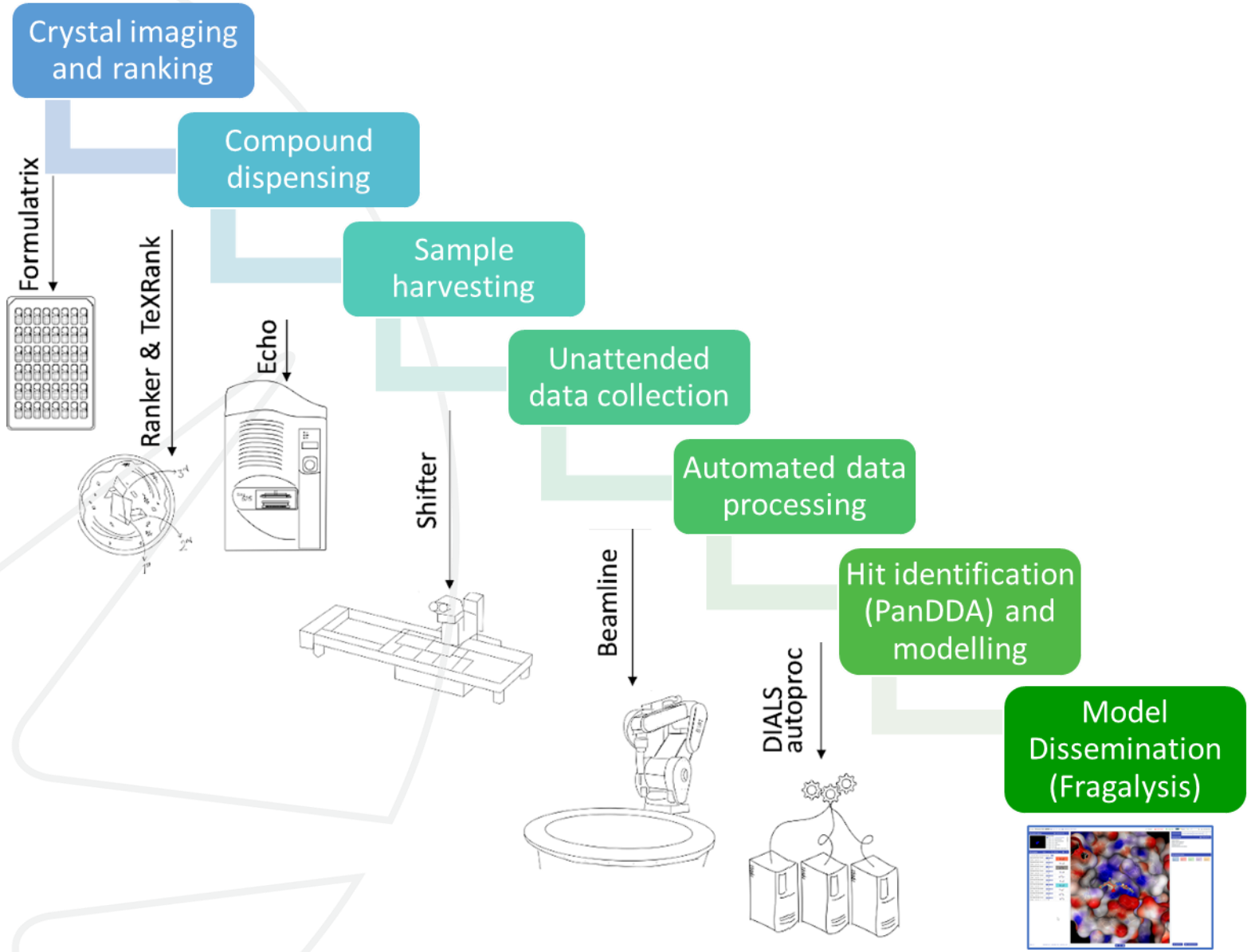
Fragment screening at Diamond: XChem



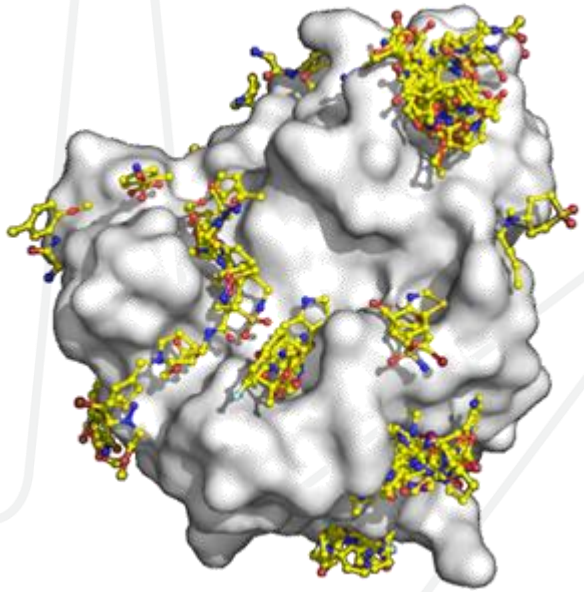
Spurlino, Meth Enz, 2011

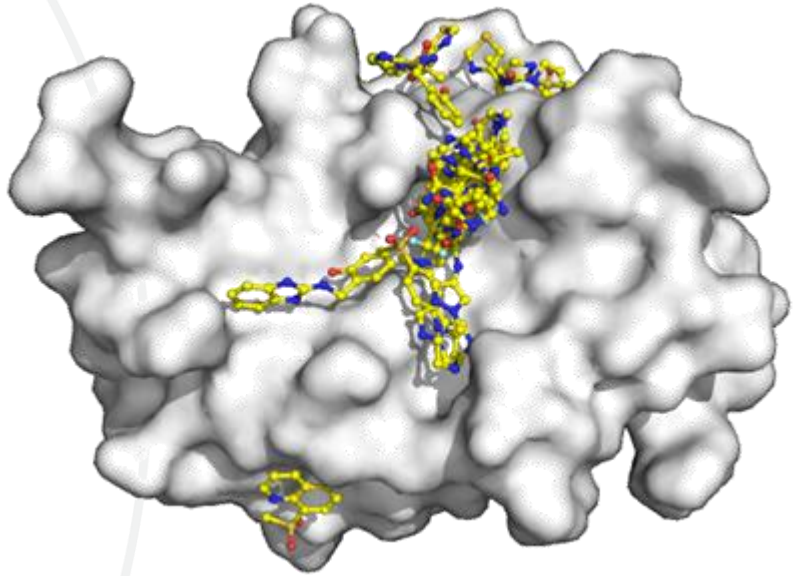
https://doi.org/10.1016/b978-0-12-381274-2.00013-3

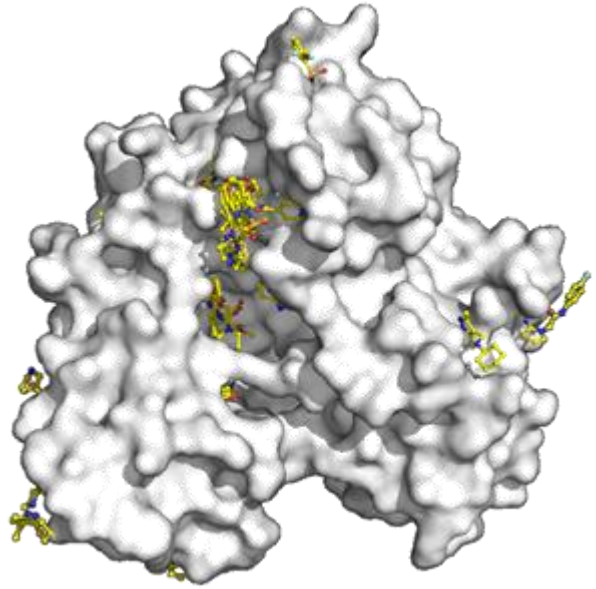
Complete screening experiment: crystal-to-model



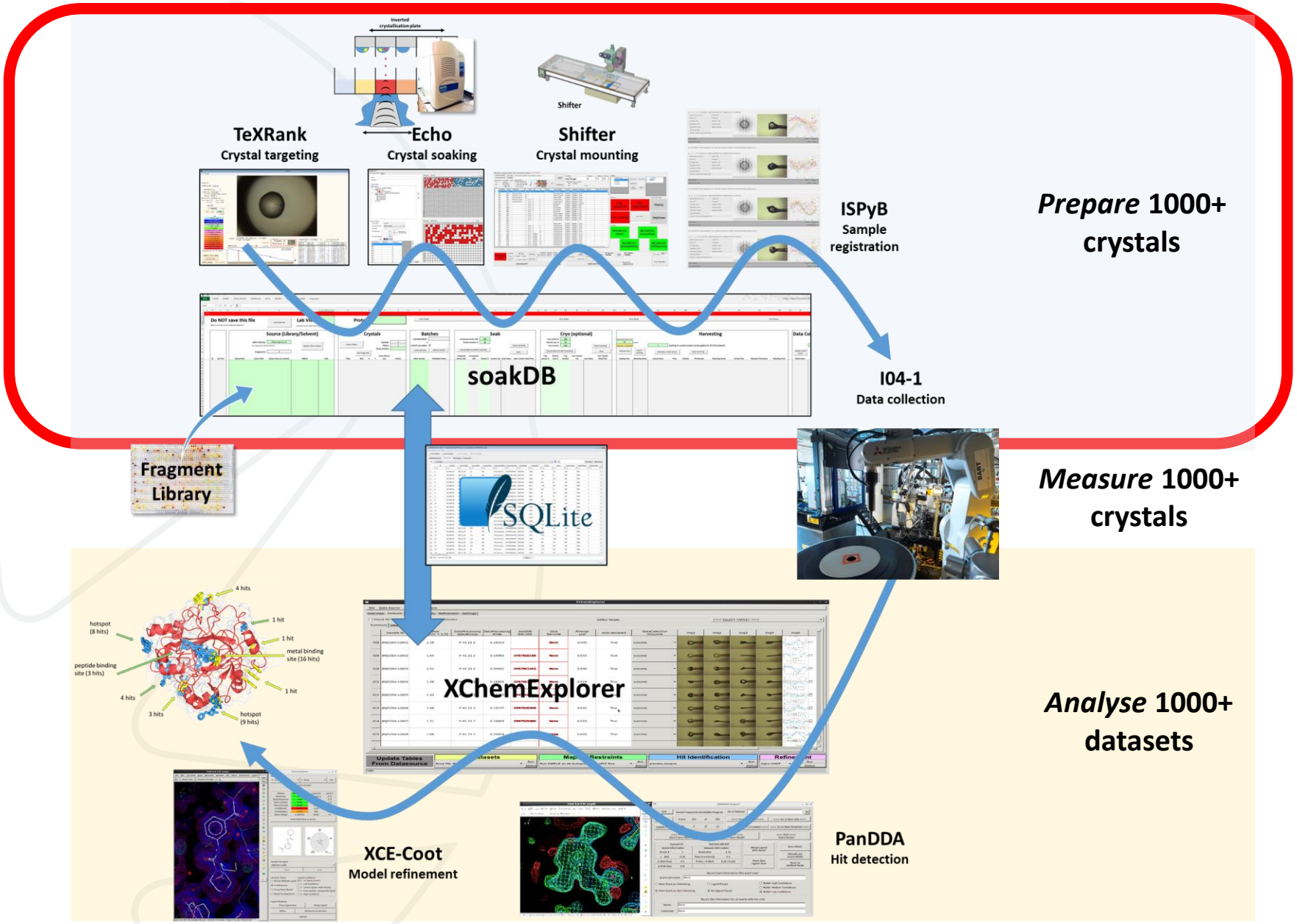
XChem Screen Readout



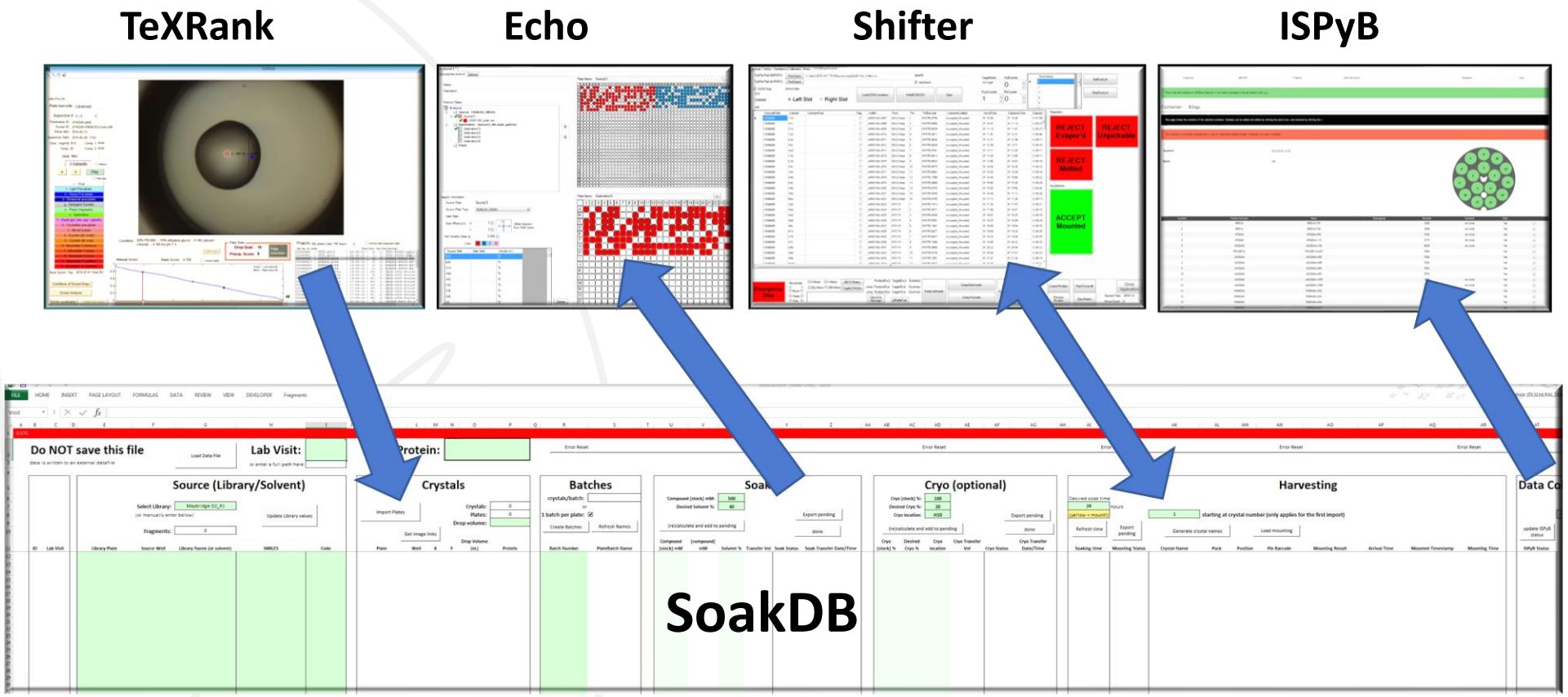




Complete screening experiment: crystal-to-model



SoakDB

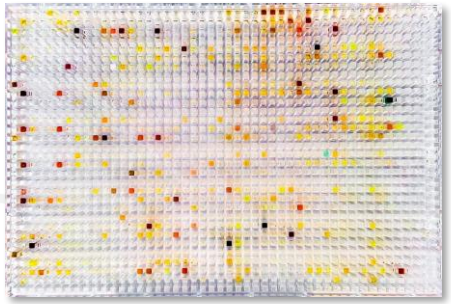


Launched from a .bat file in the lab36 directory, eg here:
Y:\data\lb18145\lb18145-216\processing\lab36\soakDB.bat

Libraries

Several diverse fragment libraries available

- Over 3,000 compounds in total
- All available in d6-DMSO at 100-500 mM
  - Some also offered in ethylene glycol (EG)
    - Typically at 100 mM
- Not bound by any IP
- All commercially available or published
- Custom libraries can also be used



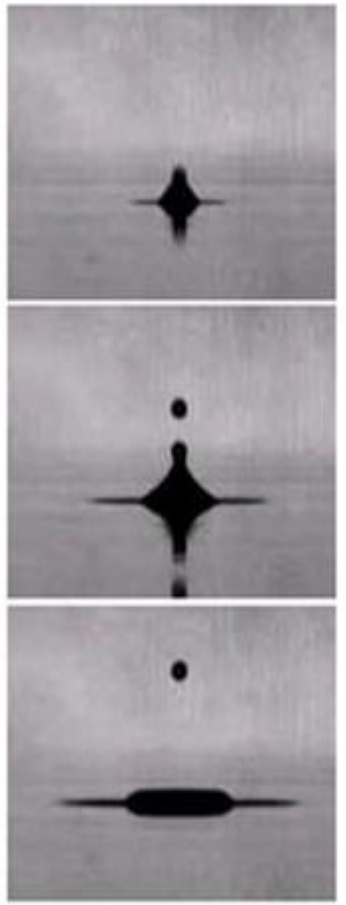
List of live fragment libraries

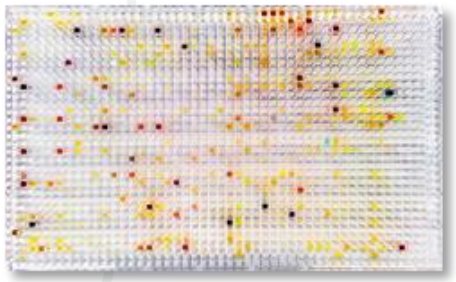
Library	Description	# of cpds	conc. in DMSO (mM)	conc. in EG (mM)	Available to Industry?	Contact person for follow-ups
DSiP	Poised reaction for quick follow-ups	768	500	100	yes	XChem
Collaborative libraries						
EUbOPEN-DSIP extended	Poised saturated heterocycles	108	500	100	TBC	Adam Nelson
EU-OPENSCREEN	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

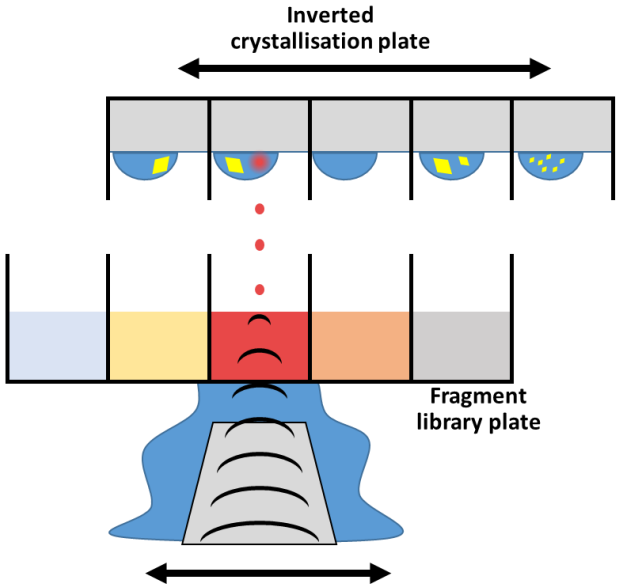
Crystal soaking – Acoustic dispensing

- Contactless, acoustic liquid dispensing technology
- High precision/accuracy dispensing
- Optimised for DMSO liquid handling
- Can soak hundreds of crystals in minutes
- Preferred tray format SWISSCI 3 drop





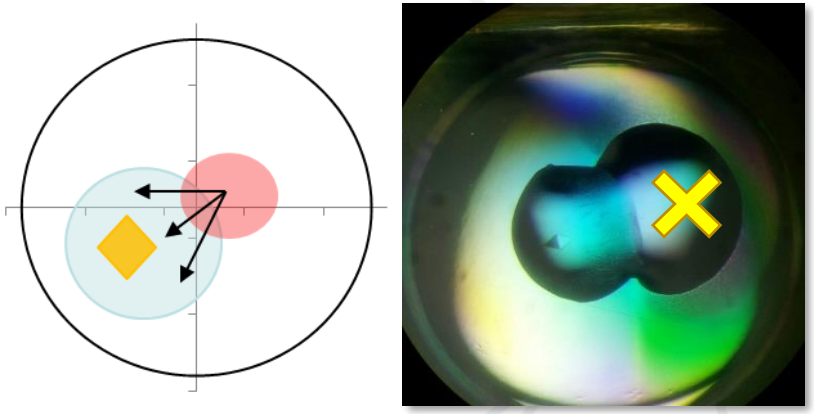
Fragment library



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

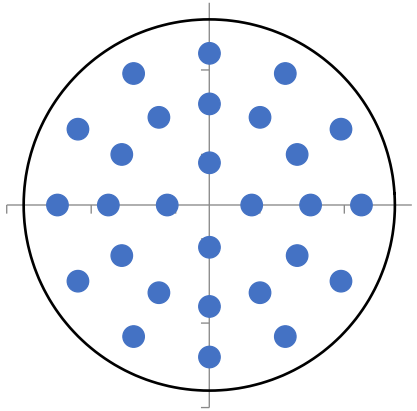
Crystal soaking – Acoustic dispensing

Compound/solvent diffusion across drop

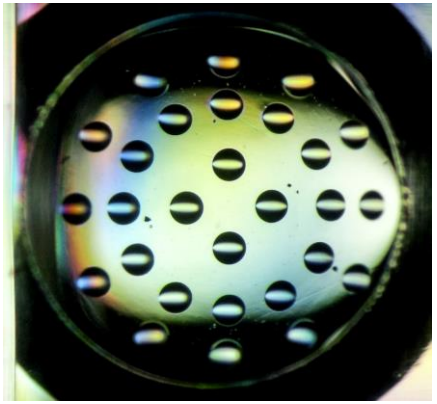


Crystallisation plate 2.5 nL Echo drops

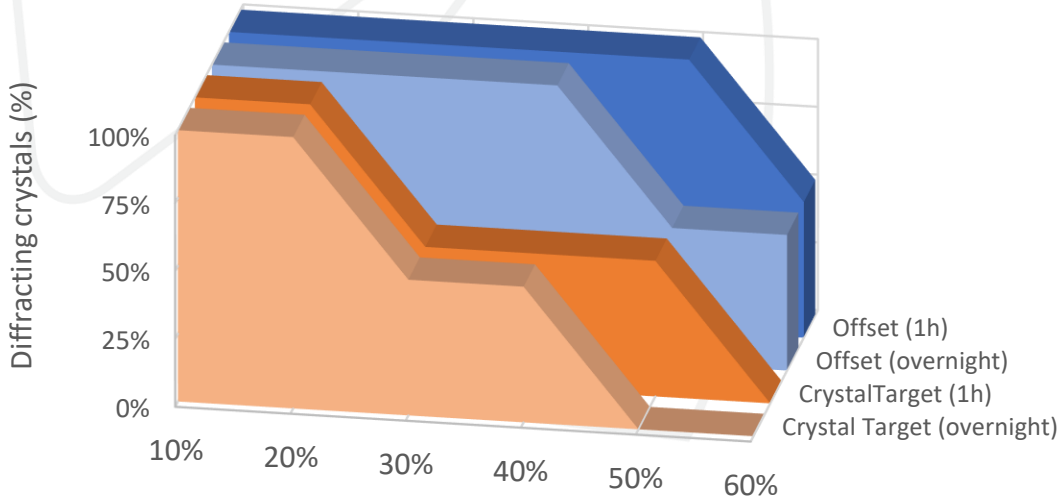
Requested



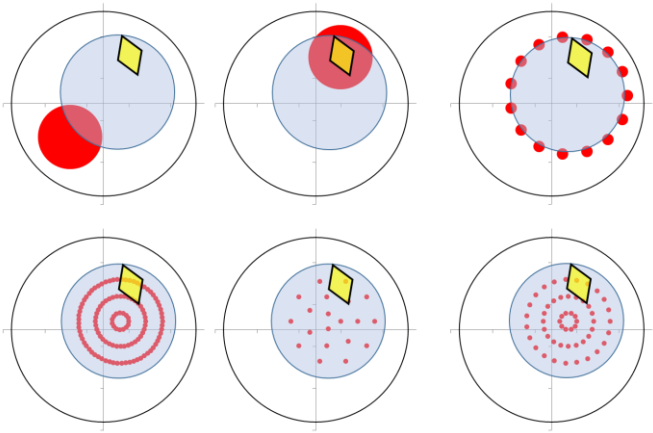
Delivered



Crystal Survival Rate

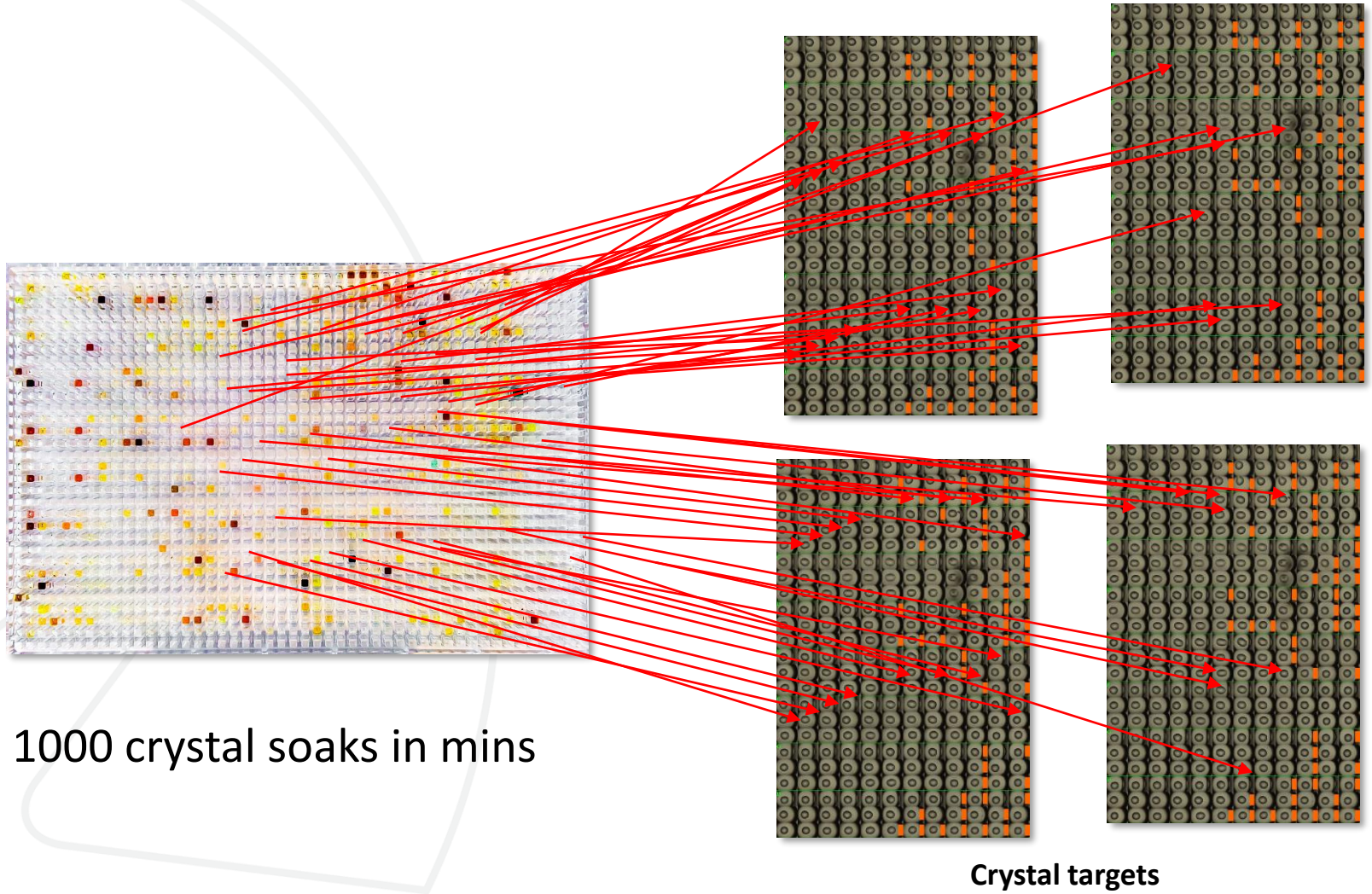


Compound dispensing patterns



https://doi.org/10.1107/S205979831700331X

Rapid crystal soaking



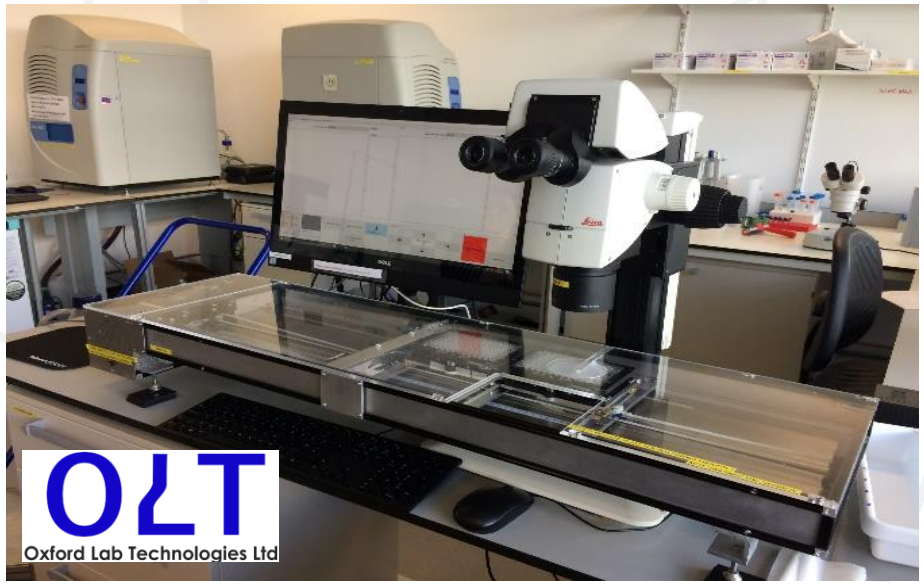
1000 crystal soaks in mins

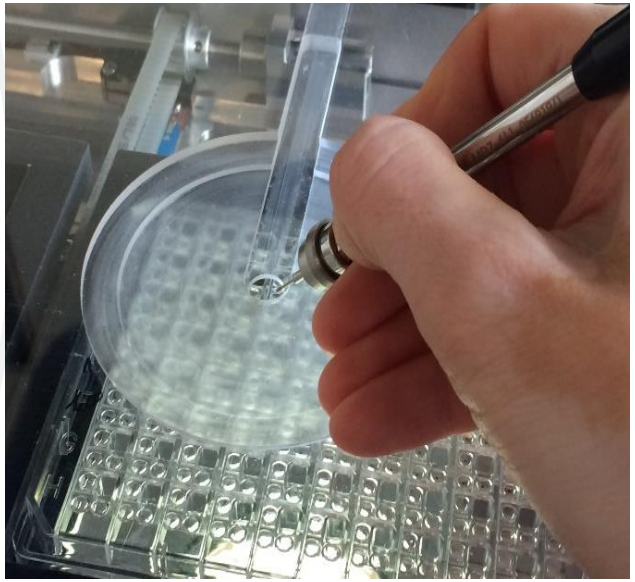
Crystal targets

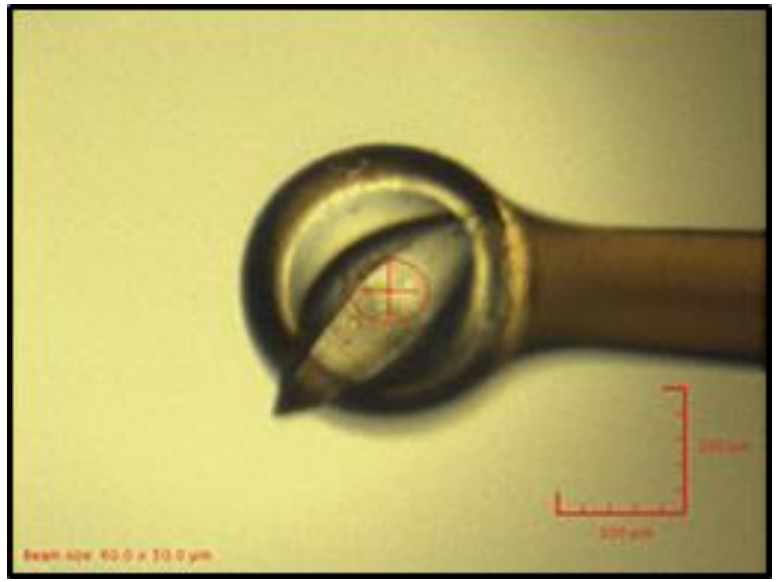
11

Crystal Harvesting - Crystal Shifter

- X/Y stage for microscope
- Allows rapid crystal harvesting (up to 200 samples/hour)
- Dedicated software with sample information recorded in database
- 3 units available in XChem lab



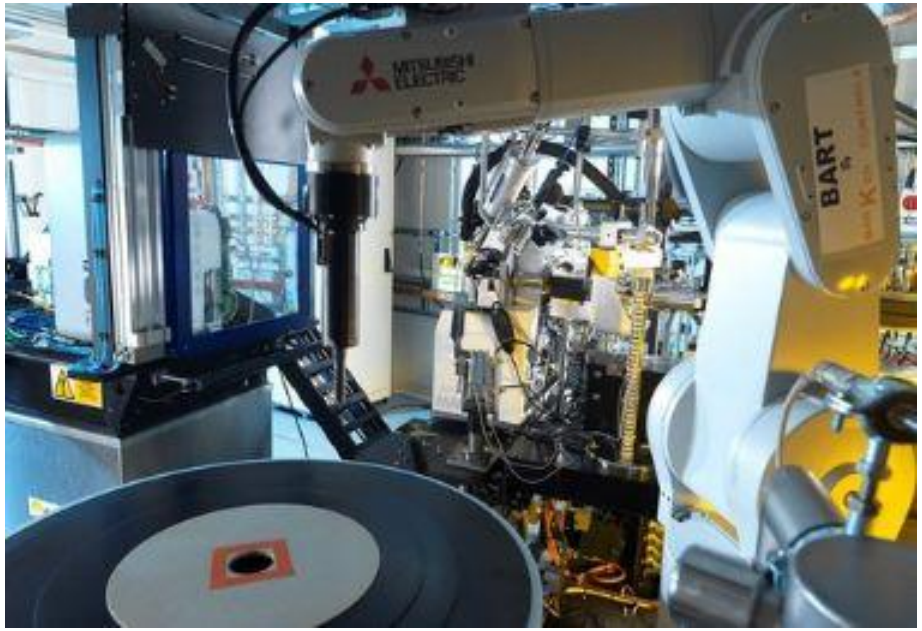


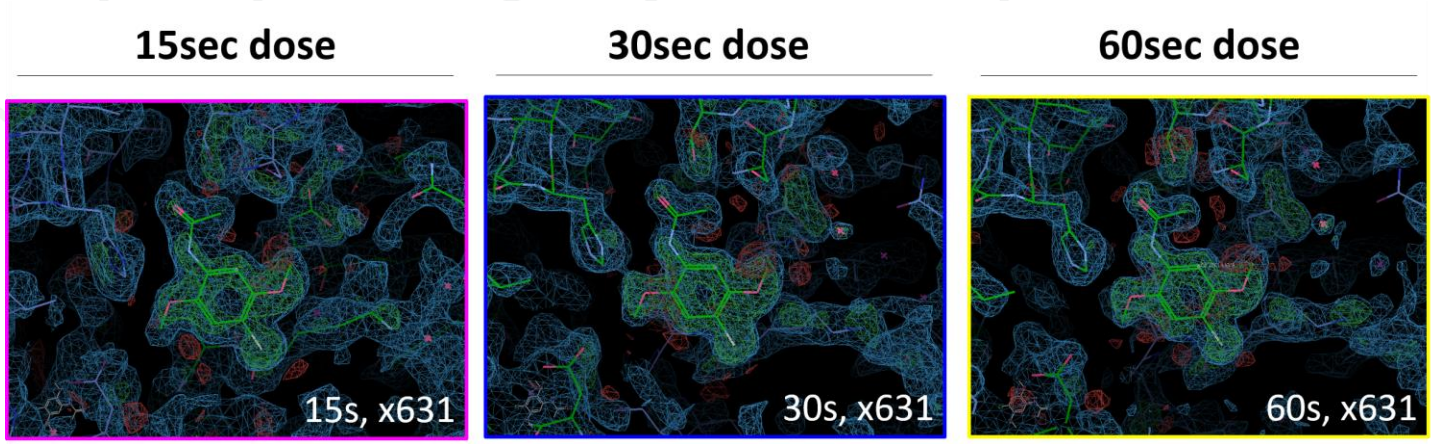


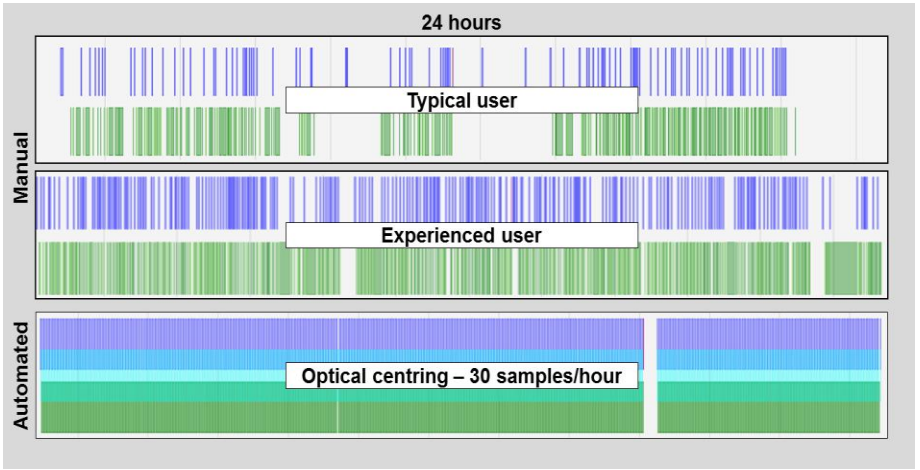
http://www.oxfordlabtech.com/
https://doi.org/10.1107/S2059798320014114

Automated/Unattended Data Collection on i04-1

- 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)
- Dectris Eiger 9MXE

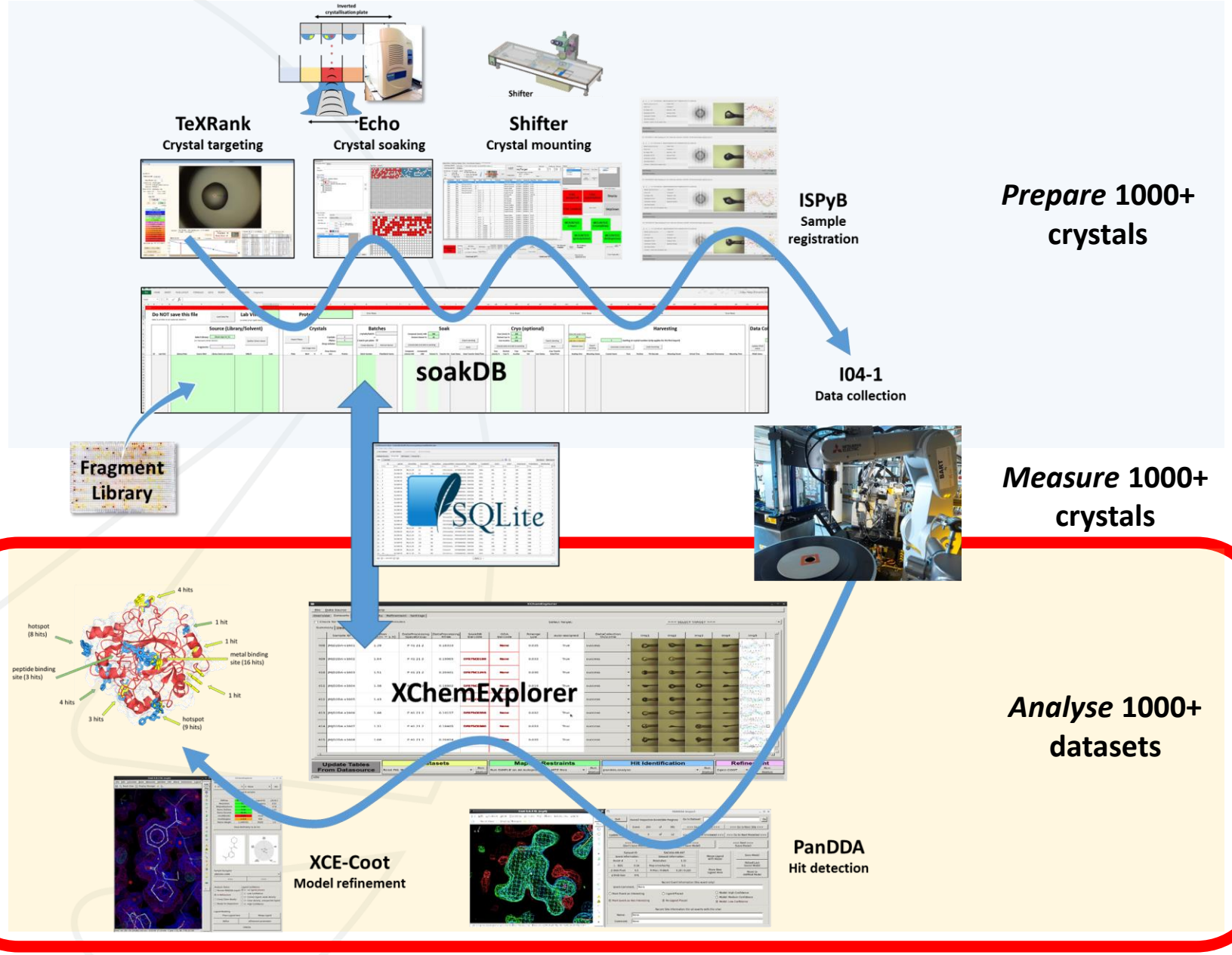




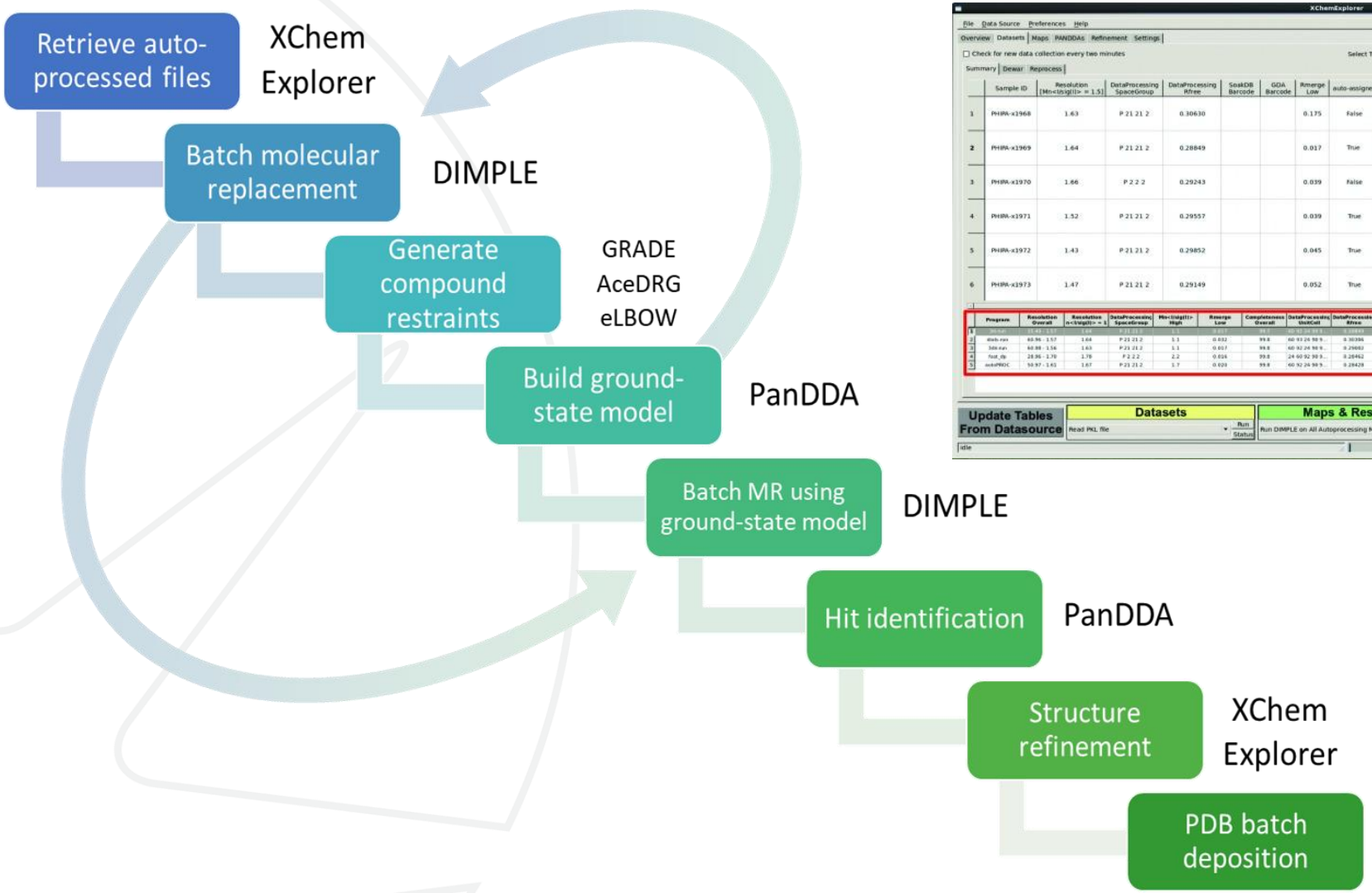


https://www.diamond.ac.uk/Instruments/Mx/I04-1.html

Complete screening experiment: crystal-to-model

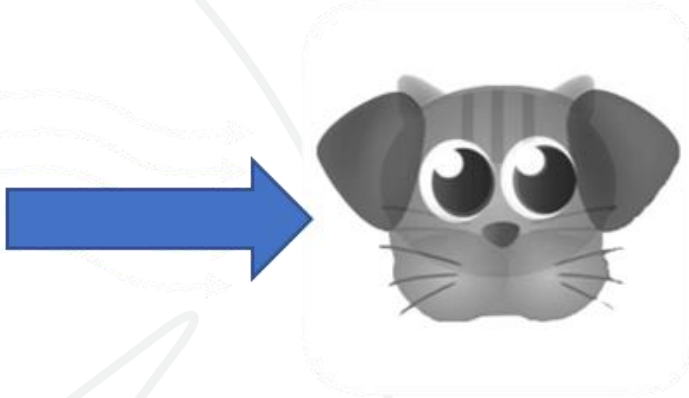


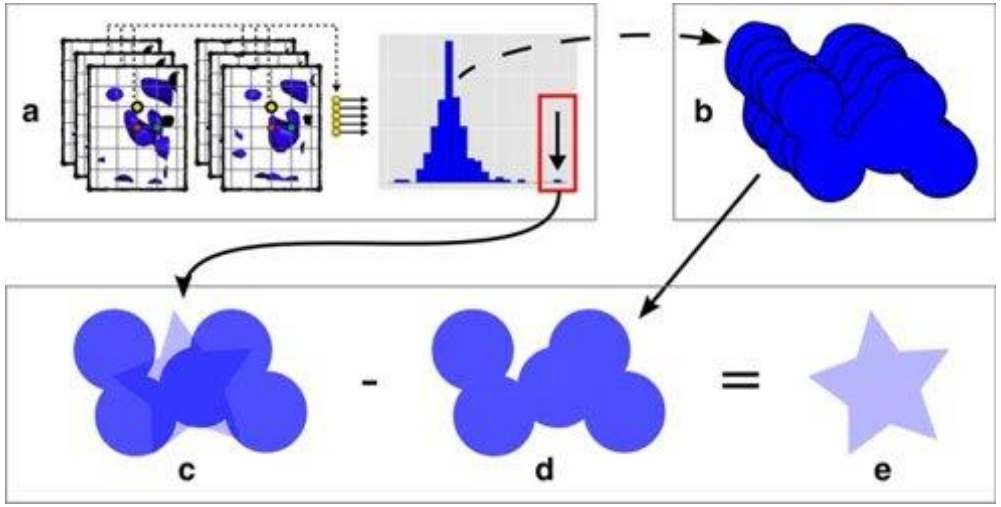
Data Analysis Workflow

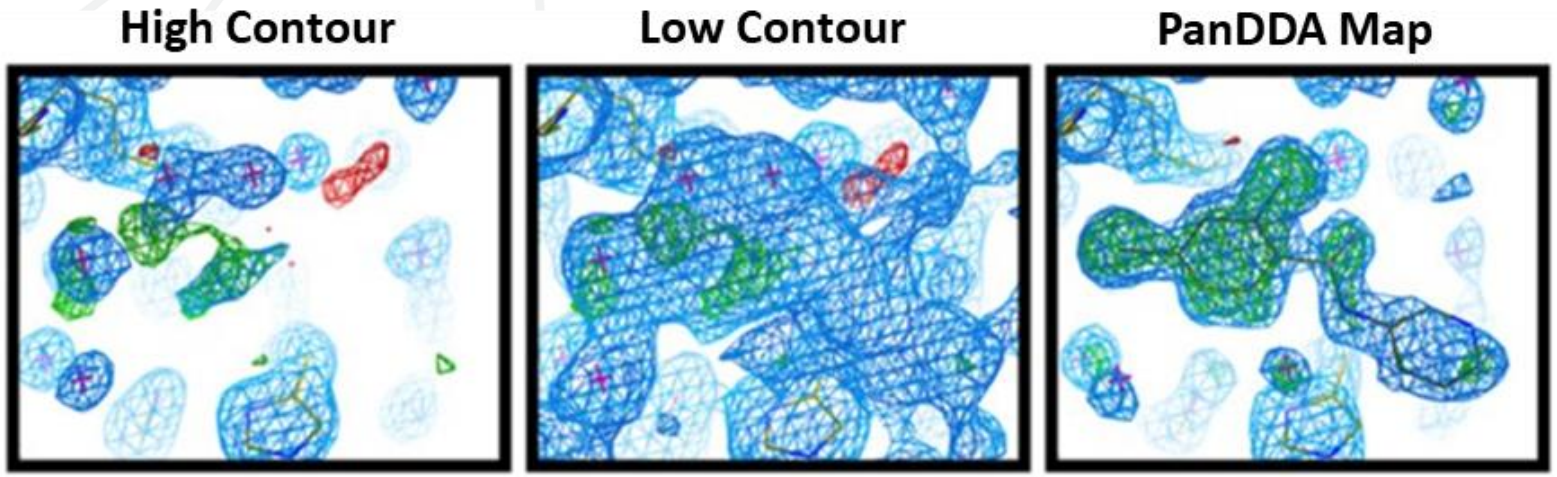


10.1107/S2059798316020234

Pan Density Dataset Analysis (PanDDA)

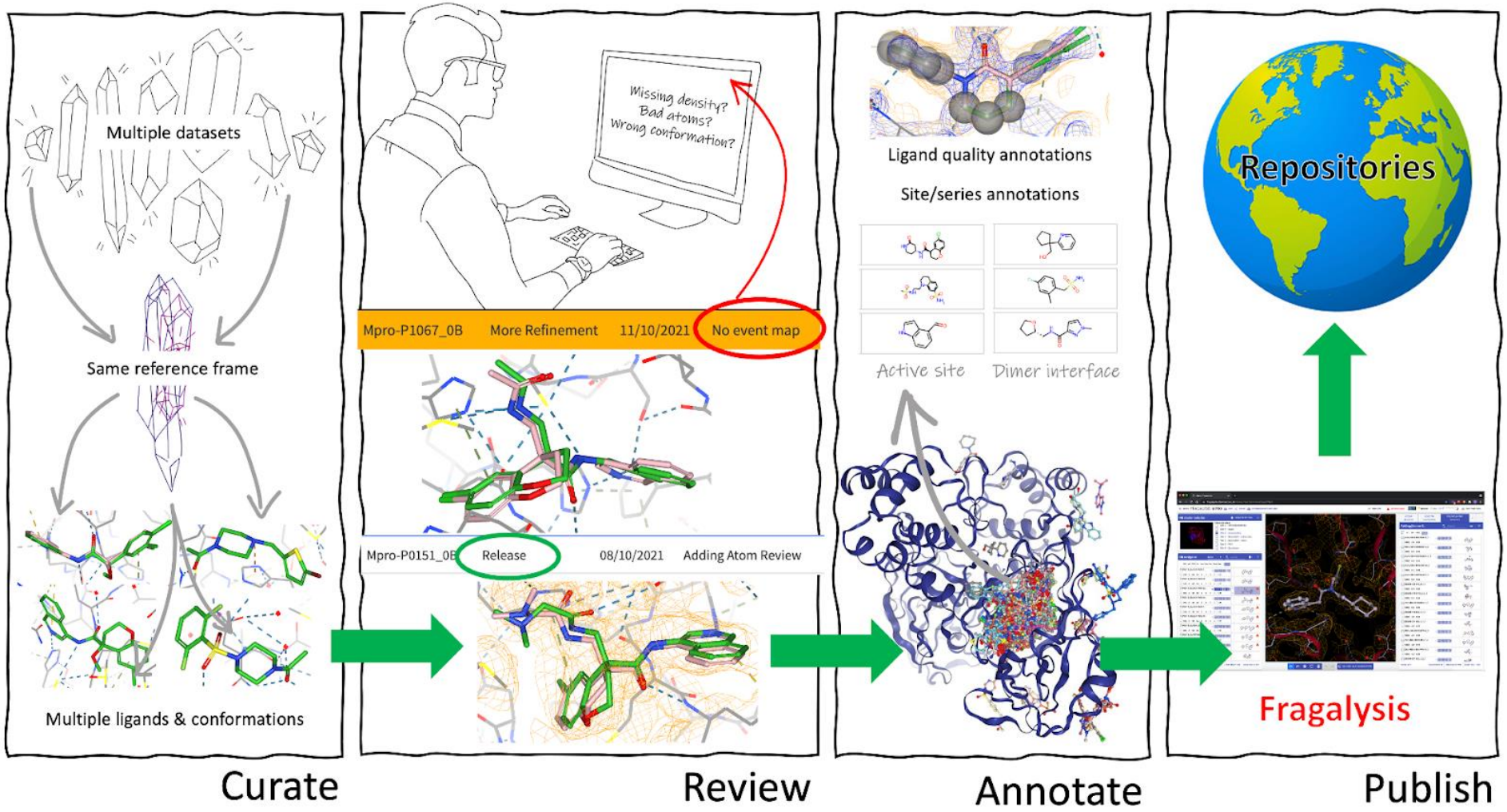






https://doi.org/10.1038/ncomms15123
https://doi.org/10.1063/1.4974176

Data Review & Dissemination



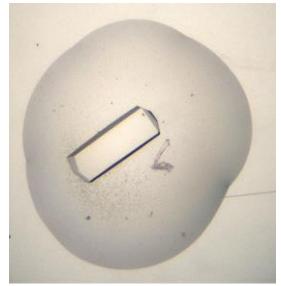
https://fragalysis.diamond.ac.uk/viewer

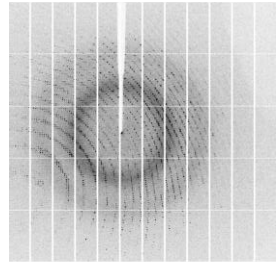
How to access - Standard Academic Access

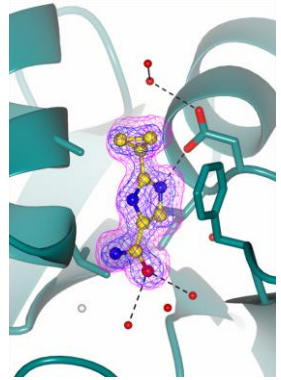
- Covers single target with calls issued twice a year (usually Apr and Oct)
  - Proposals evaluated by an independent peer-review panel
  - Includes iNEXT-Discovery applications
- Tier 1: Exploratory projects (100-200 fragments)
  - Feasibility is high (robust crystal system established) but credible strategy for follow-up of hits is not in place
- Tier 2: Full screen (700-1000 fragments)
  - Feasibility has been demonstrated and a robust strategy for progressing hits has been provided
- Tier 3: Follow-up support (batches of 200-300 compounds)
  - Feasibility previously established and design rationale articulated for peer review

Ideal XChem ready crystal systems

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





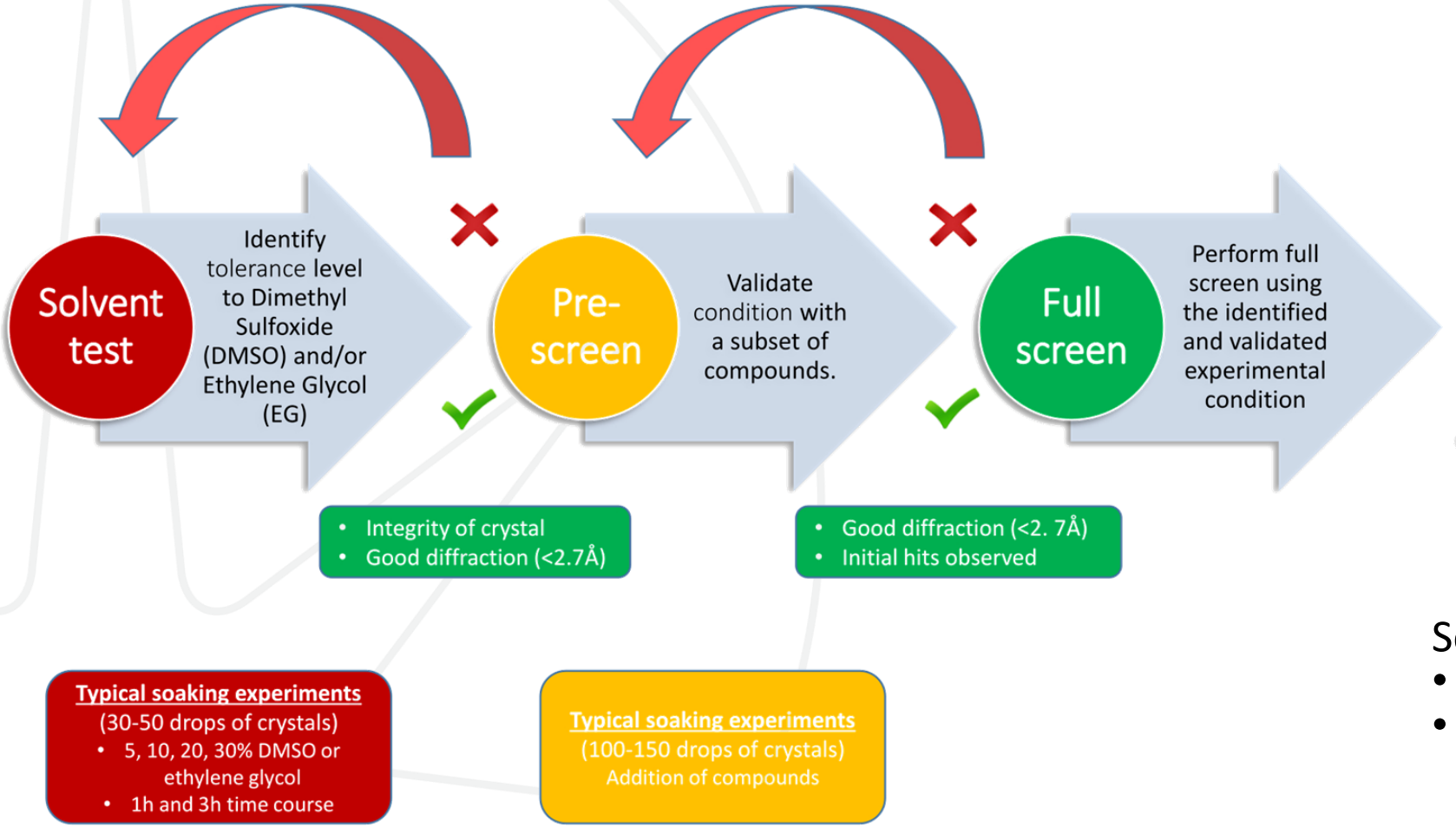


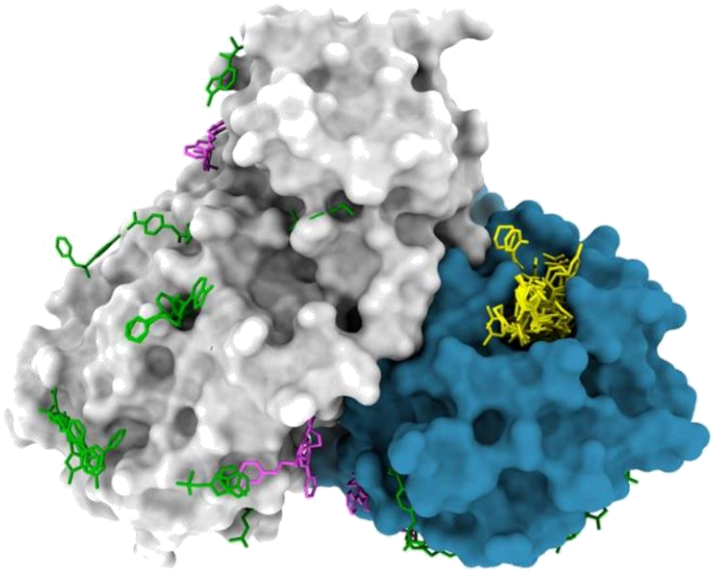
But non-ideal crystals are feasible!

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

doi: 10.20944/preprints201809.0383.v1

Establishing “XChem ready” systems





Screening outcome:

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

https://dx.doi.org/10.3791/62414

Useful Contacts and Links

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- **Alex Dias (Industry):** 8200, alexandre.dias@diamond.ac.uk
- **Ailsa Powell (Industry):** 7524, ailsa.powell@diamond.ac.uk
- **Warren Thompson:** warren.Thompson@diamond.ac.uk

- http://www.diamond.ac.uk/Beamlines/Mx/Fragment-Screening.html
- Achieving-efficient-fragment-screening-at-XChem-facility-at-Diamond Light Source: https://dx.doi.org/10.3791/62414

References on XChem

TexRank Crystal Targeting:

- Ng, J. T., *et al.* **Using textons to rank crystallisation droplets by the likely presence of crystals.** *Acta Cryst D,* **70**, 2701-2718 (2014). https://doi.org/10.1107/S1399004714017581

ECHO Fragment Dispensing:

- Collins, P. M., *et al.* **Gentle, fast and effective crystal soaking by acoustic dispensing.** *Acta Cryst D,* **73**, 246-255 (2017). https://doi.org/10.1107/S205979831700331X

Shifter Crystal Harvesting:

- Wright, N. D., *et al.* **The Low-Cost, Semi-automated Shifter Microscope Stage Transforms Speed and Robustness of Manual Protein Crystal Harvesting.** *Acta Cryst D*, **77**, 62-74 (2021). https://doi.org/10.1107/S2059798320014114

XChem Explorer:

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PanDDA:

- Pearce, N., *et al.* **Partial-occupancy binders identified by the Pan-Dataset Density Analysis method offer new chemical opportunities and reveal cryptic binding sites.** *Structural Dynamics,* **4**, 03210 (2017). https://doi.org/10.1063/1.4974176
- Pearce, N., *et al.* **A multi-crystal method for extracting obscured crystallographic states from conventionally uninterpretable electron density**. *Nat. Commun.,* **8**, 15123 (2017). https://doi.org/10.1038/ncomms15123

The XChem pipeline overview

- Douangamath, A., *et al*. **Achieving Efficient Fragment Screening at XChem Facility at Diamond Light Source.** *JoVE journal (2021). https://www.jove.com/t/62414/achieving-efficient-fragment-screening-at-xchem-facility-at-diamond*