

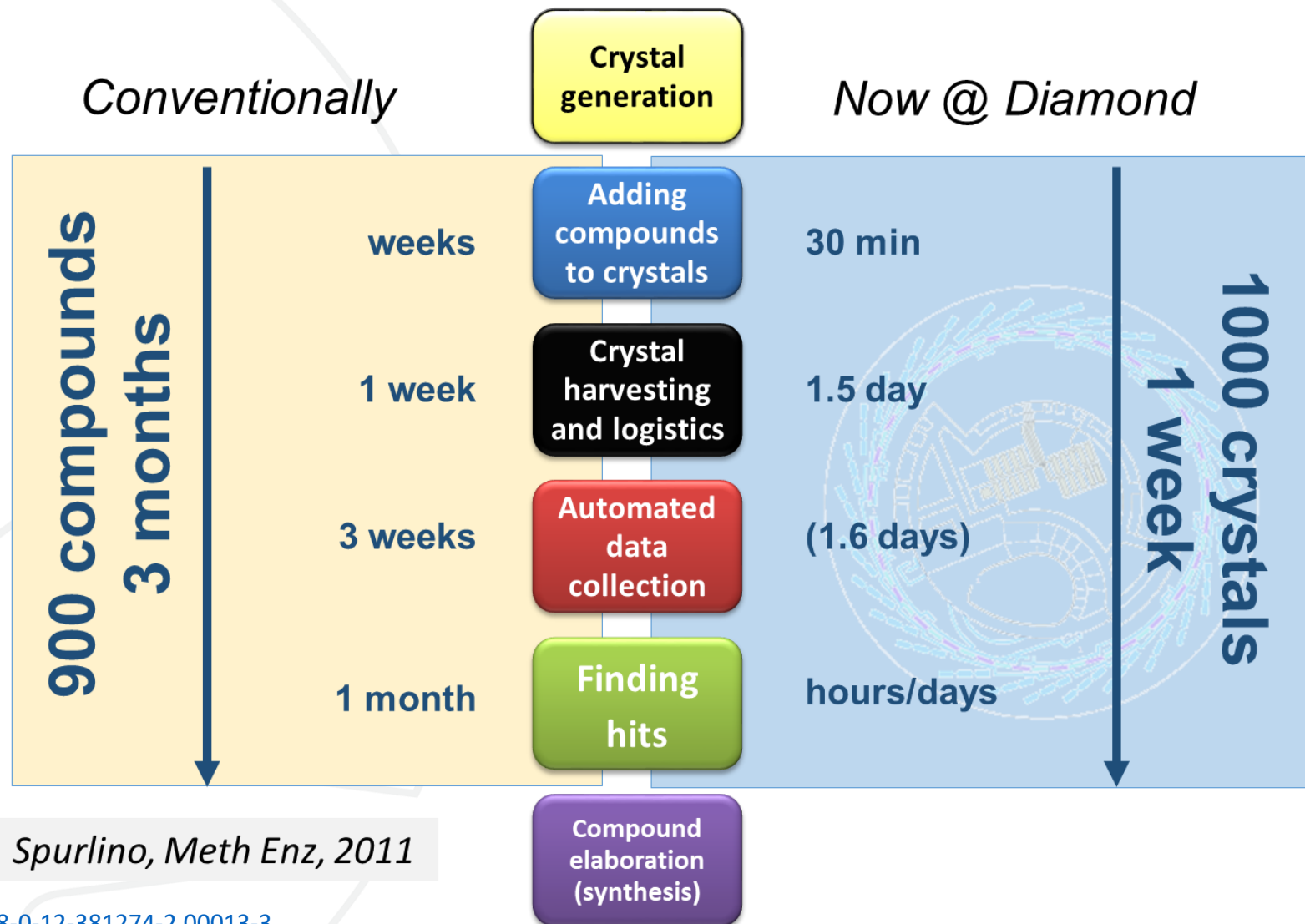
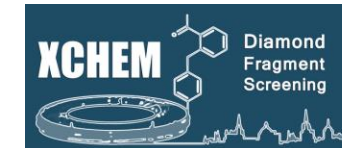


The XChem platform at Diamond Light Source

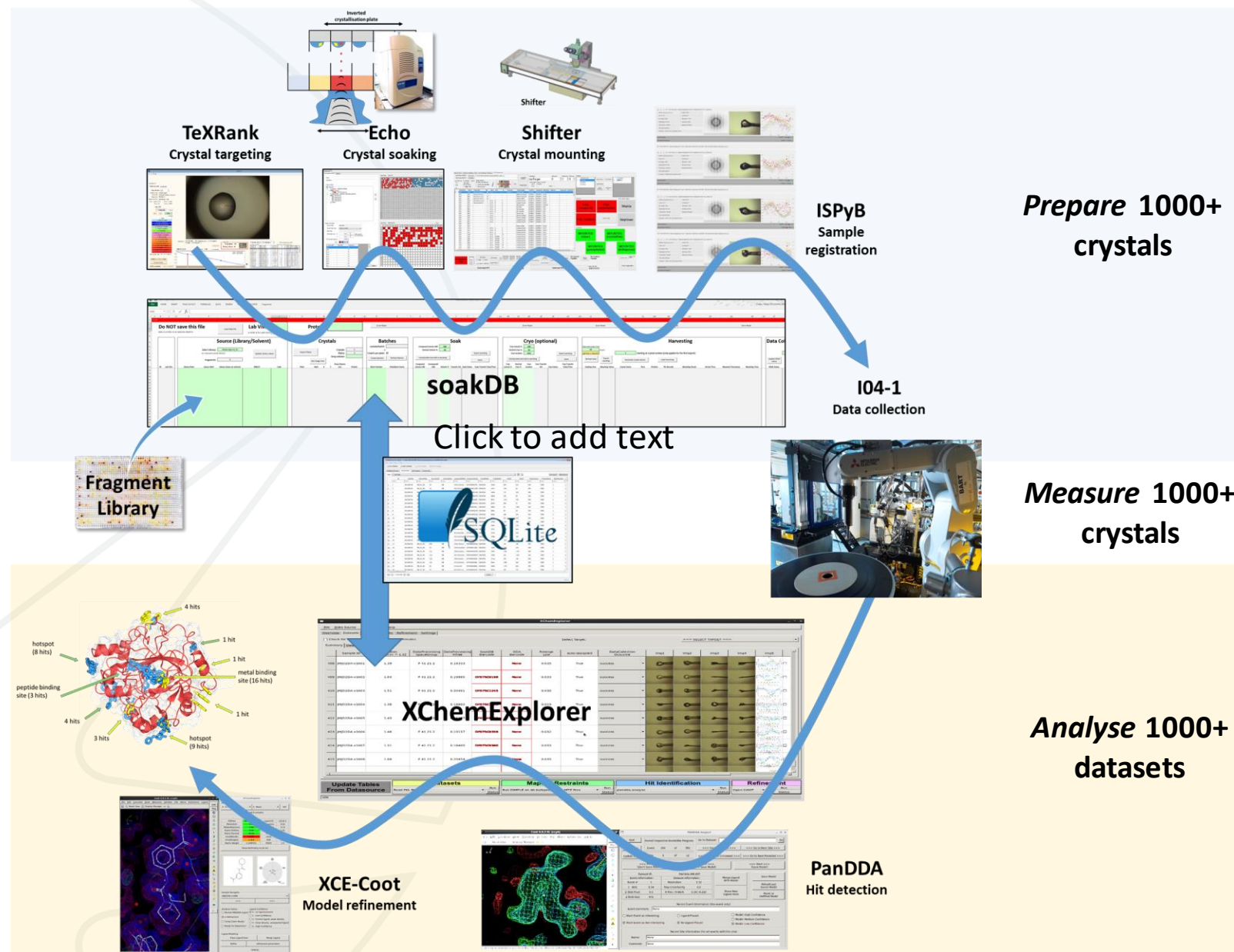
May 2022



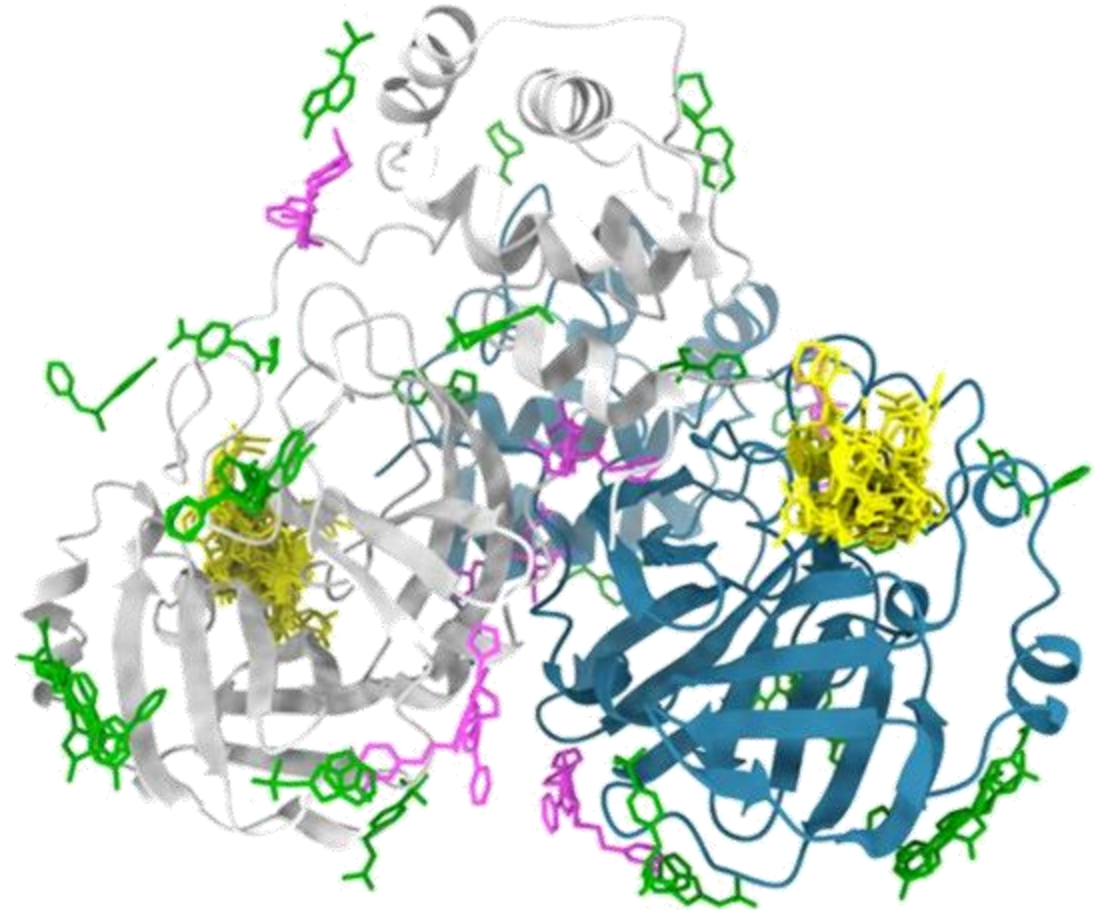
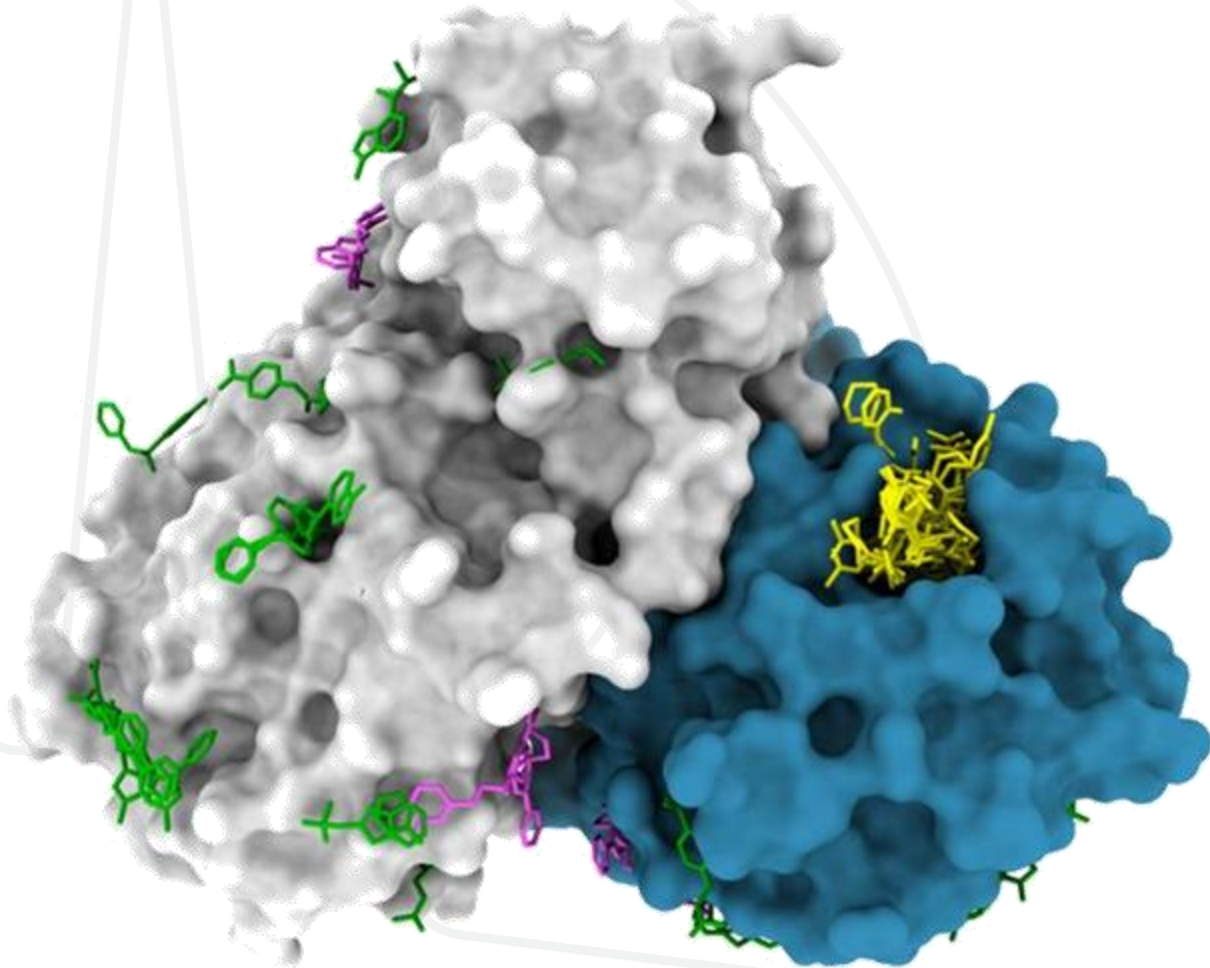
Fragment screening at Diamond: XChem



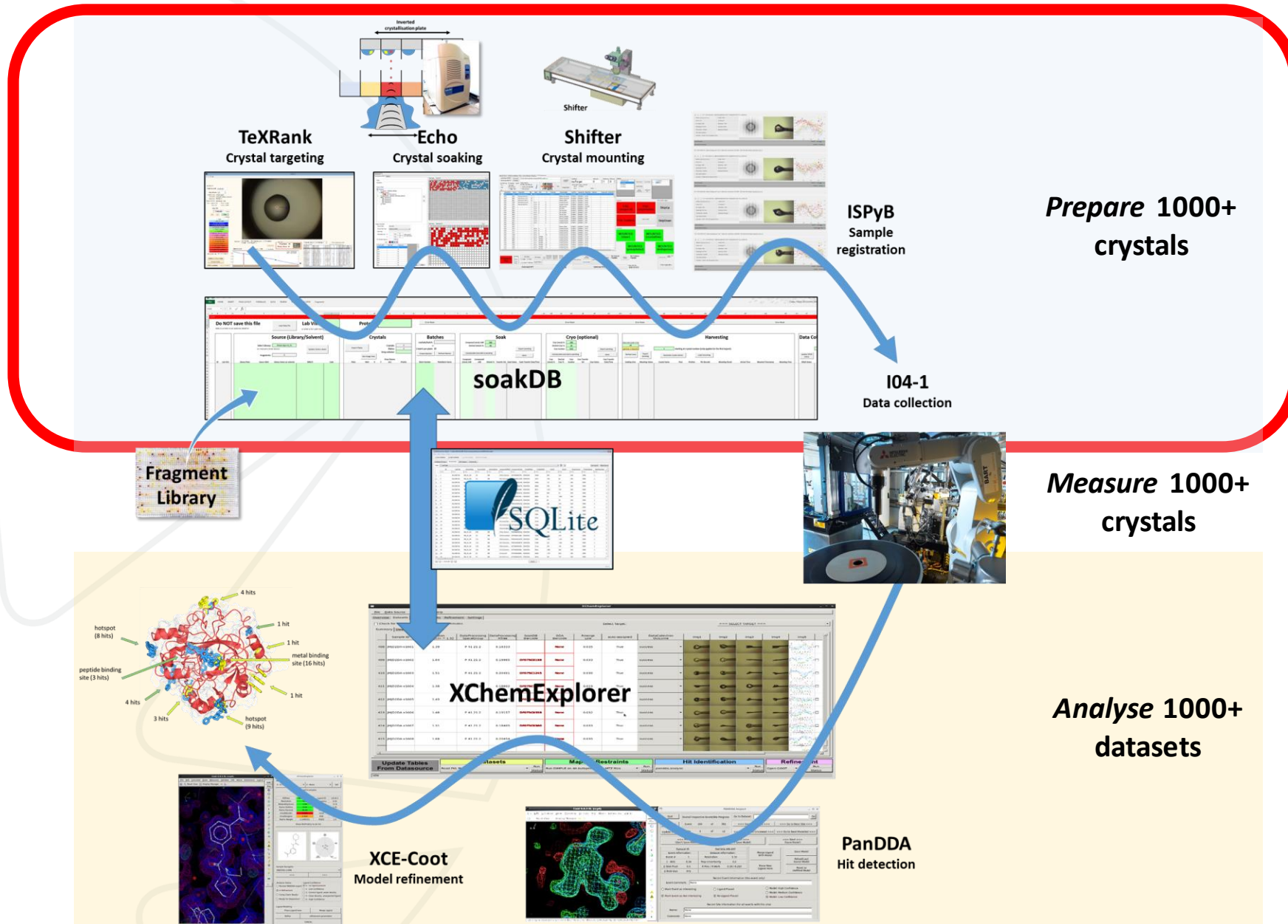
Complete screening experiment: crystal-to-model



XChem Screen Readout



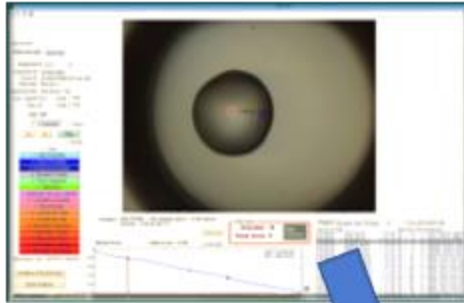
Complete screening experiment: crystal-to-model



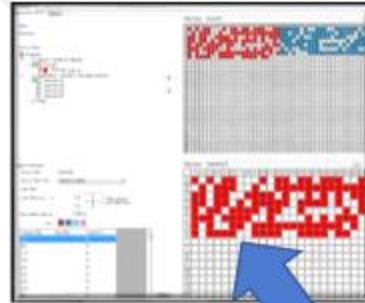
SoakDB



TeXRank



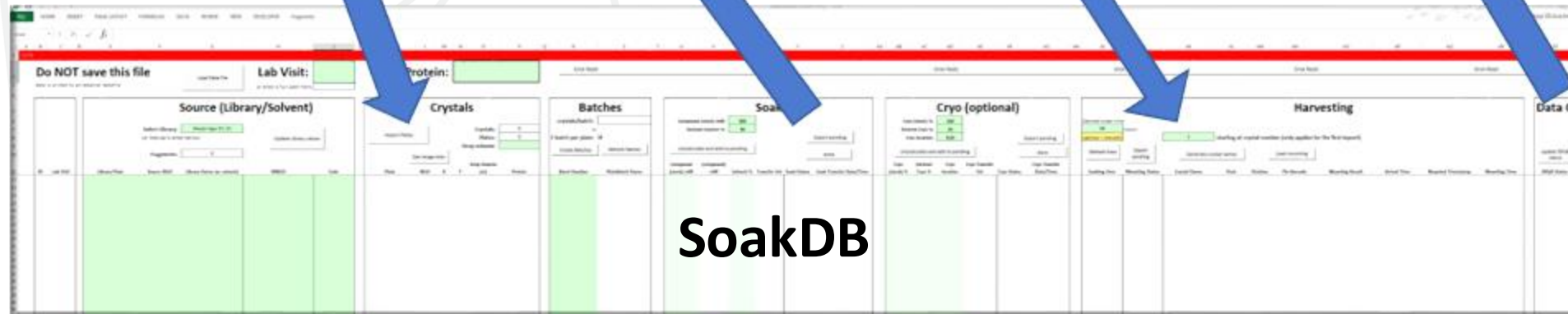
Echo



Shifter



ISPyB



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

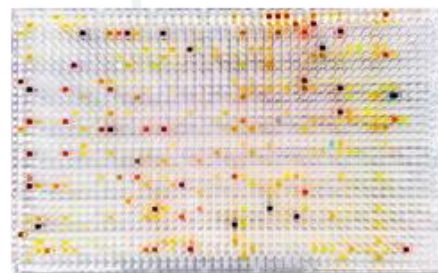
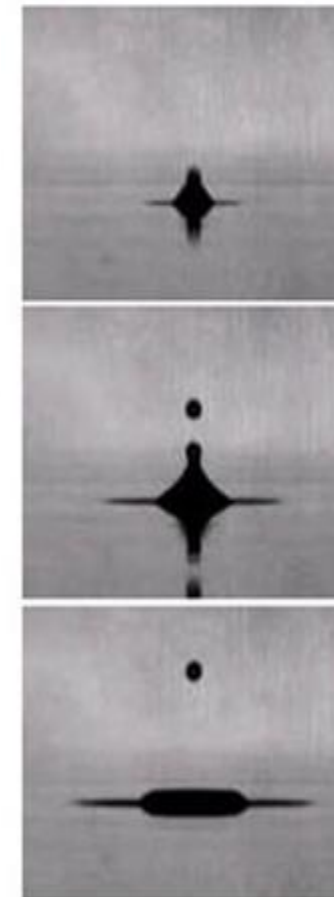
Library	Description	# of cpds	conc. in DMSO (mM)	conc. in EG (mM)	Available to Industry?	Contact person for follow-ups
DSiP	Poised reaction for quick follow-ups	768	500	100	yes	XChem
Collaborative libraries						
EUbOPEN-DSiP extended	Poised saturated heterocycles	108	500	100	TBC	Adam Nelson
EU-OPENSREEN	Possibility of follow-ups through EU-OPENSREEN	968	100	-	TBC	EU-OPENSREEN 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 electrophiles (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>

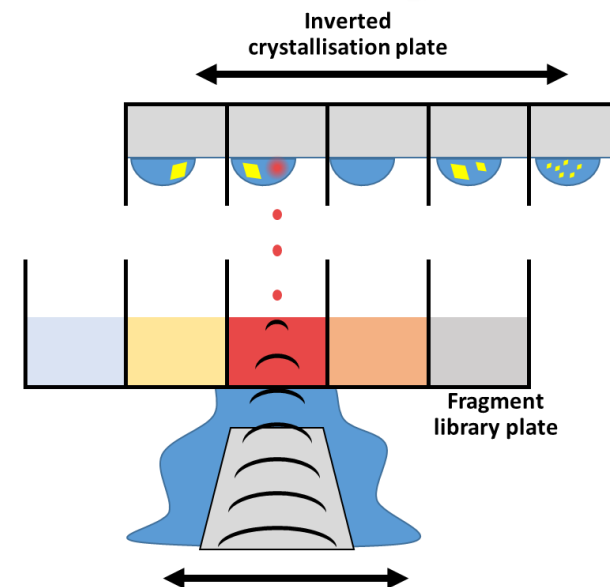


Crystal soaking – Acoustic dispensing

- 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

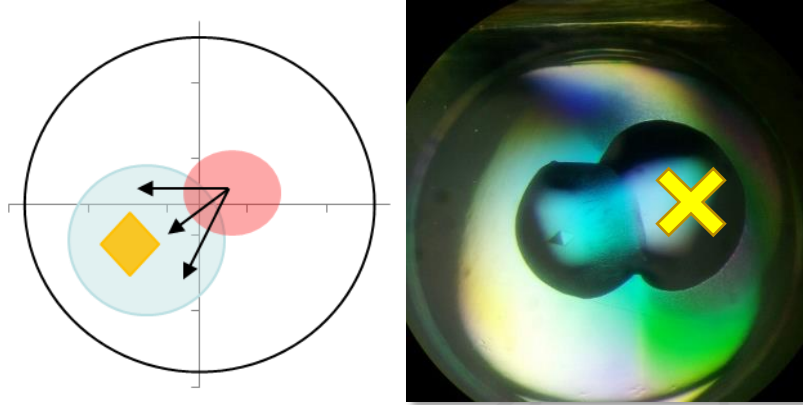


**Fragment
library**



Crystal soaking – Acoustic dispensing

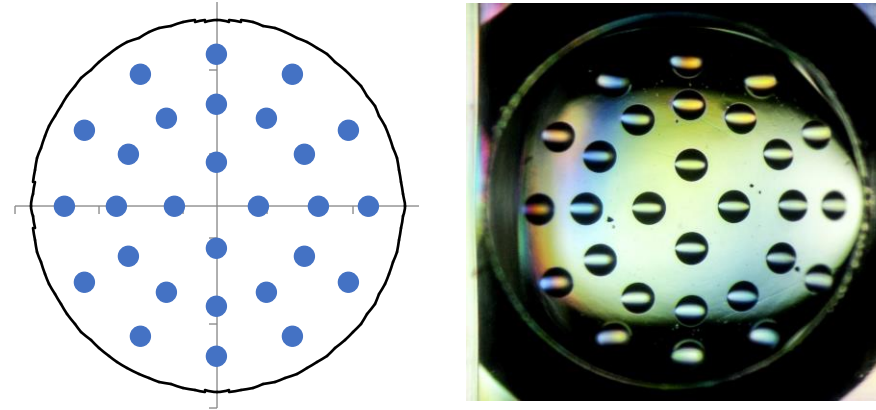
Compound/solvent diffusion across drop



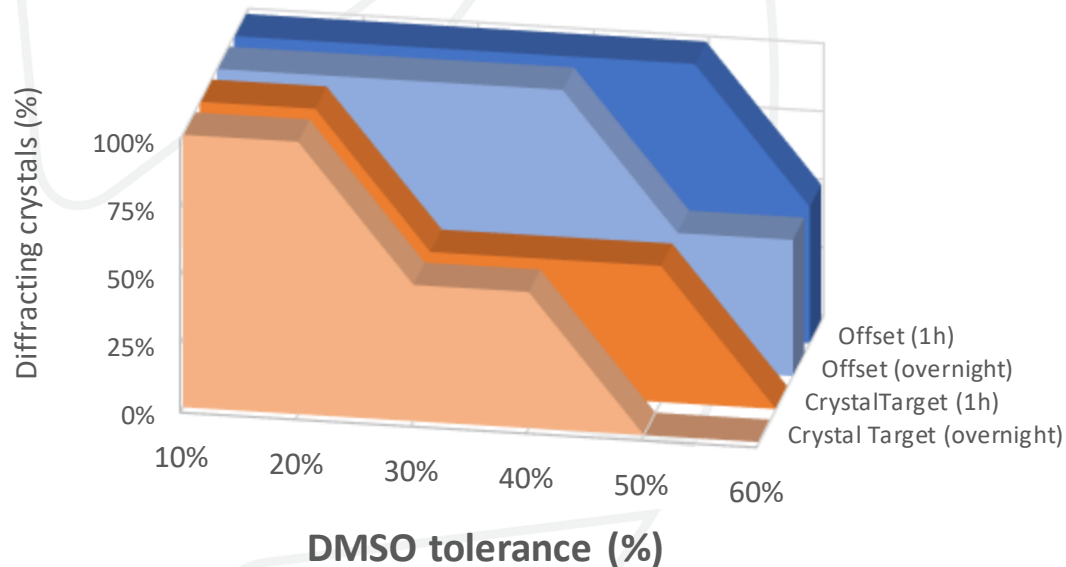
Crystallisation plate 2.5 nL Echo drops

Requested

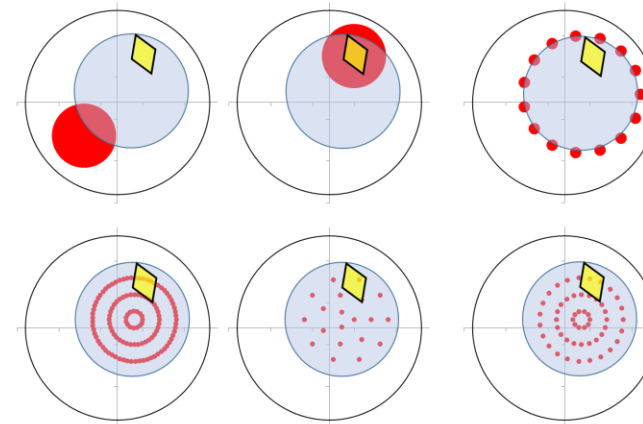
Delivered



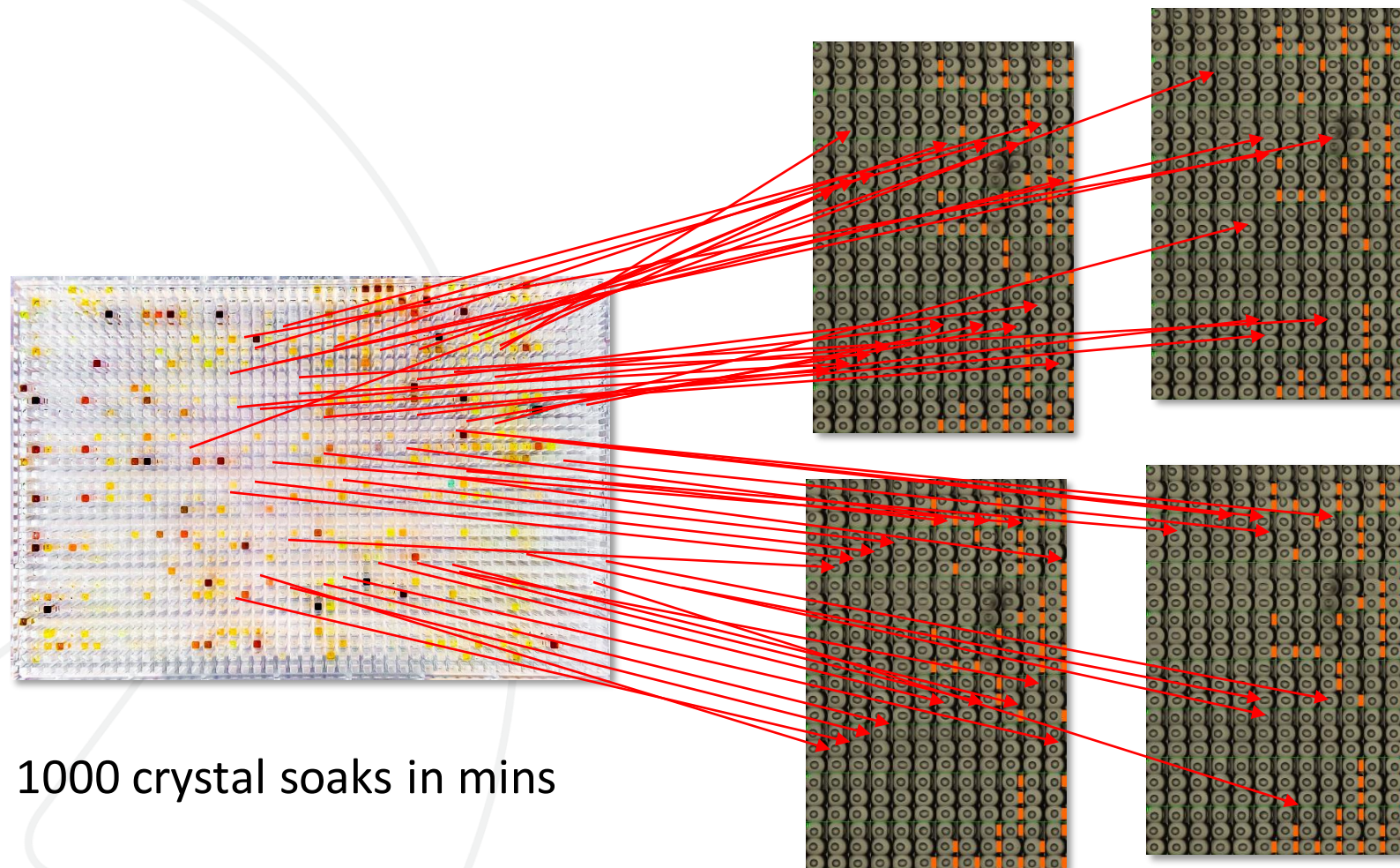
Crystal Survival Rate



Compound dispensing patterns



Rapid crystal soaking

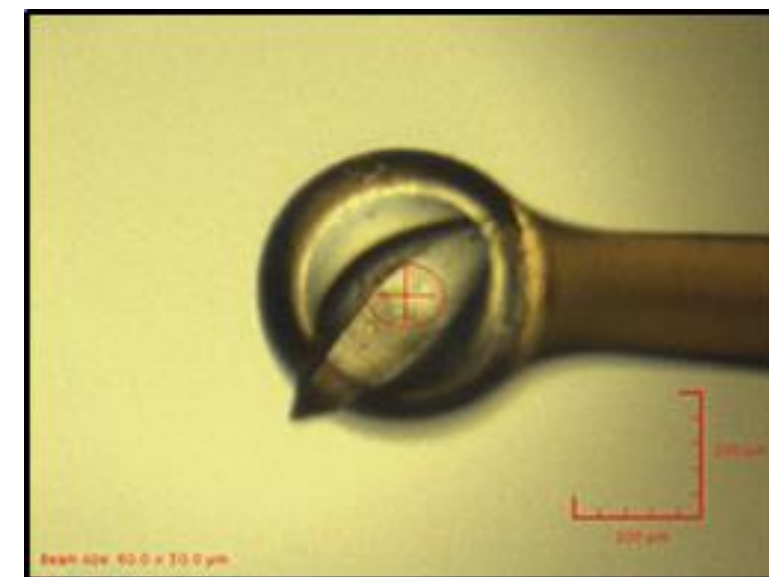
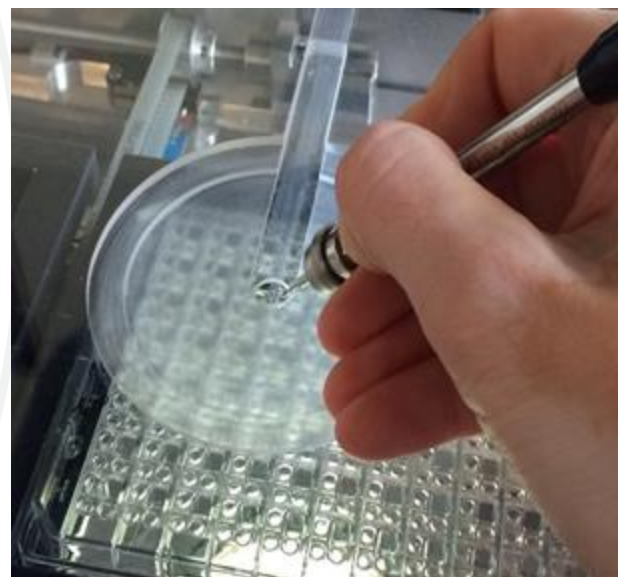
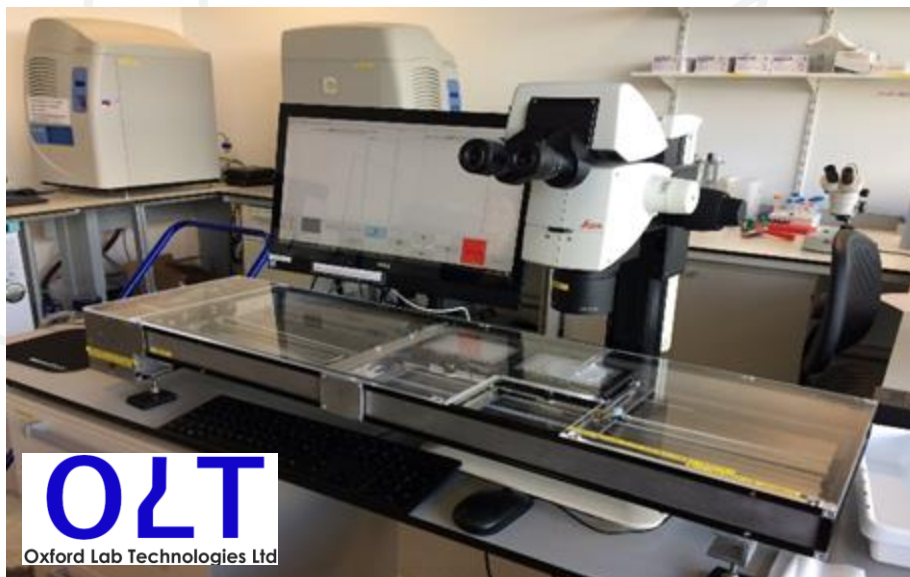


1000 crystal soaks in mins

Crystal targets

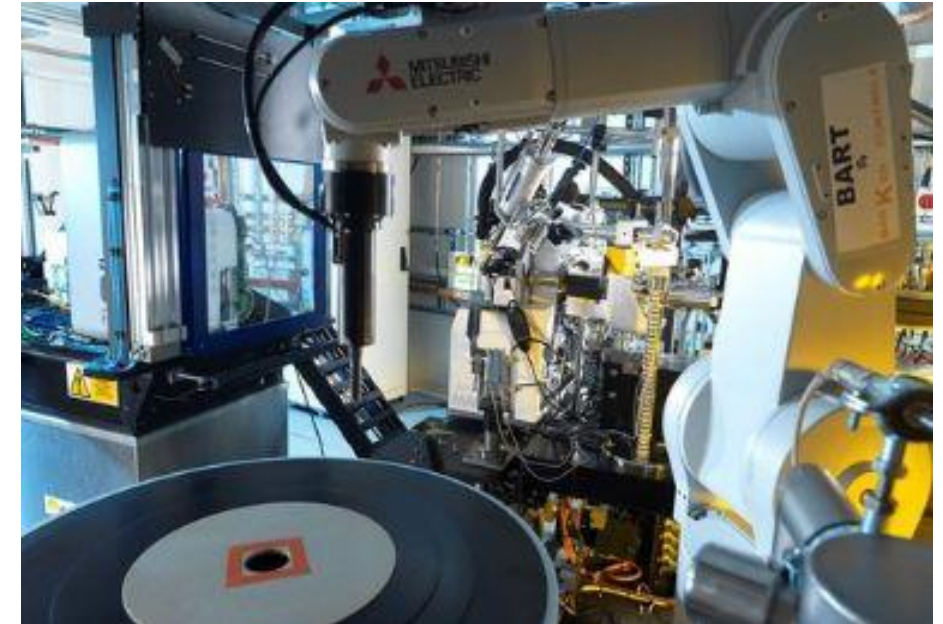
Crystal Harvesting - Crystal Shifter

- 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



Automated/Unattended Data Collection on i04-1

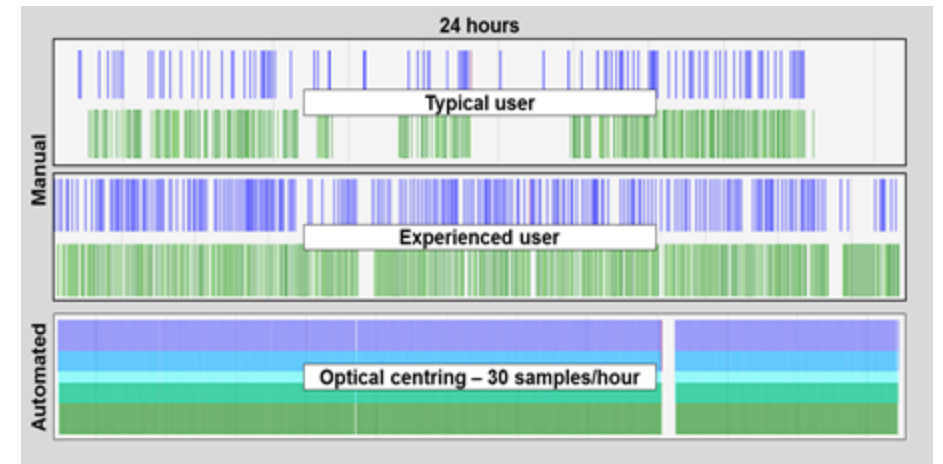
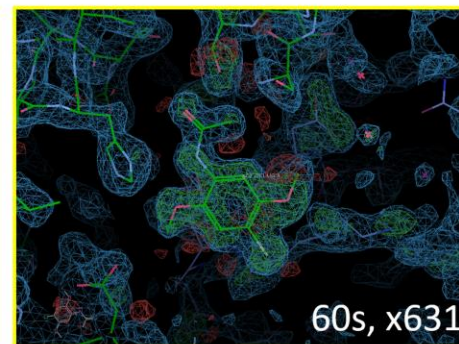
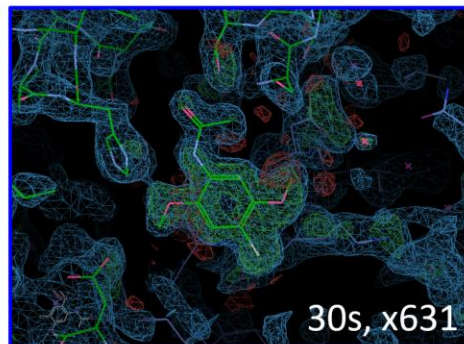
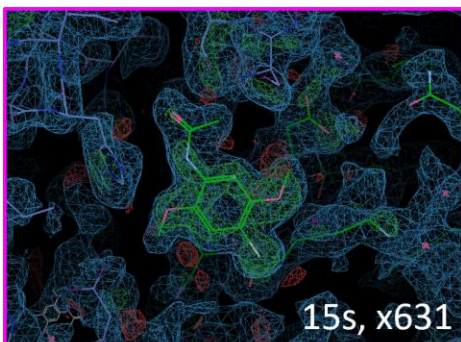
- Optical-based or X-ray based centring
- Crystal mounting (~32 samples/hour)
- Rapid sample exchange (20 s/sample)
- High-flux (0.92 Å fixed wavelength)
- BART high capacity dewar (592 samples)
- New Dectris Eiger 9MXE



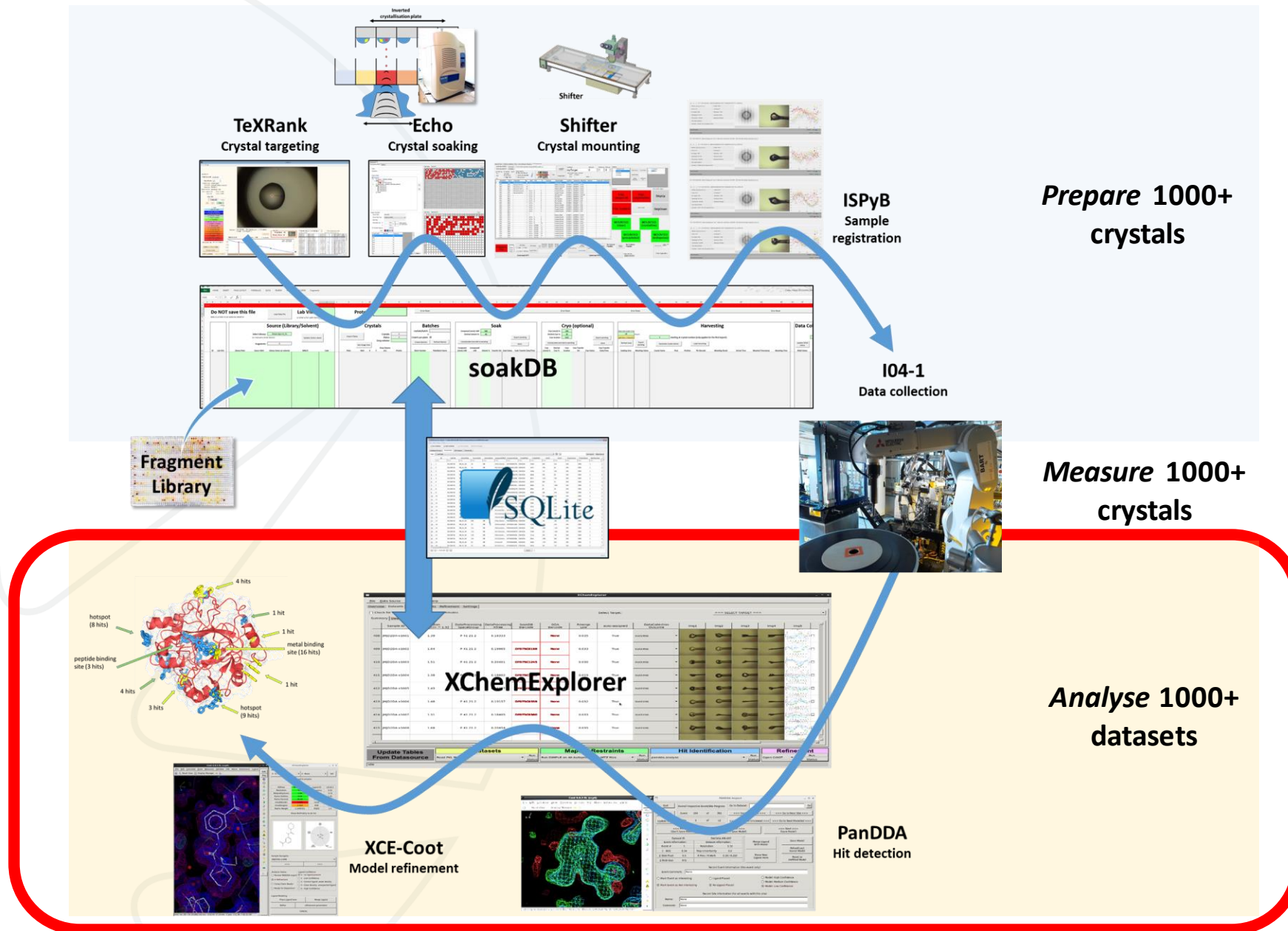
15sec dose

30sec dose

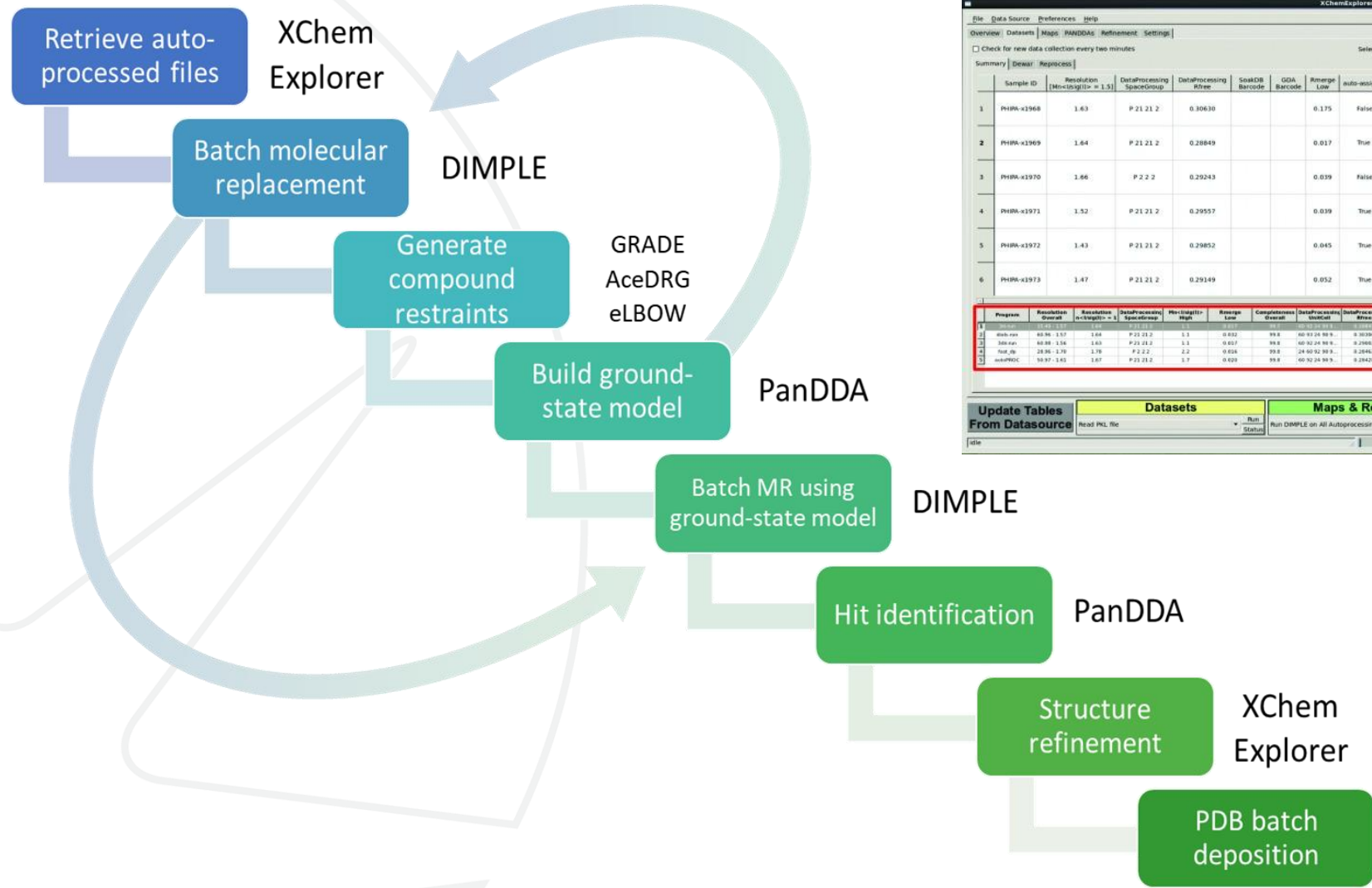
60sec dose



Complete screening experiment: crystal-to-model



Data Analysis Workflow



XChem Explorer

Overview | Datasets | Maps | PANDDAs | Refinement | Settings

Check for new data collection every two minutes

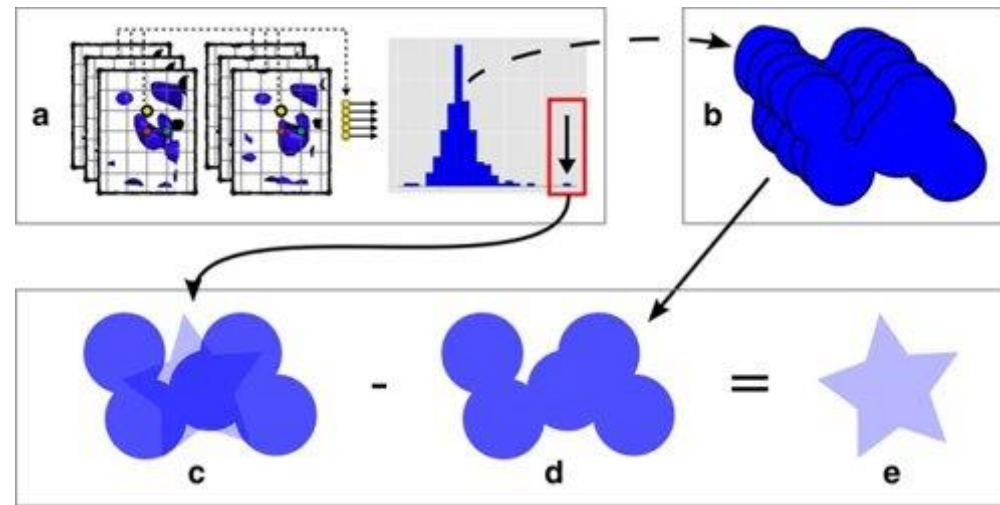
Select Target: == SELECT TARGET ==

Sample ID	Resolution [MaxCsg] = 1.51	DataProcessing SpaceGroup	DataProcessing Attrs	ScaleDB Barcode	QDA Barcode	Average Low	auto-assigned	DataCollection Outcome	img1	img2	img3	img4	img5	Show Details
1	PH00-43968	1.63	P 21 21 2	0.30630		0.175	False	success						
2	PH00-43969	1.64	P 21 21 2	0.28849		0.017	True	failure						
3	PH00-43970	1.66	P 2 2 2	0.29243		0.039	False	failure						
4	PH00-43971	1.52	P 21 21 2	0.29557		0.039	True	success						
5	PH00-43972	1.43	P 21 21 2	0.29852		0.045	True	success						
6	PH00-43973	1.47	P 21 21 2	0.29149		0.052	True	success						

Program	Resolution Overall	Resolution (MaxCsg) = 1	DataProcessing SpaceGroup	MaxCsg[1]	Average Low	Completeness Overall	DataProcessing Overall	DataProcessing Attrs	DataProcessing Score
1	1.43	1.51	P 21 21 2	1.1	0.032	99.8	60 92 24 99.9	0.30386	0
2	1.64	1.56	P 21 21 2	1.1	0.017	99.8	60 92 24 99.9	0.29852	0
3	1.70	1.70	P 2 2 2	2.2	0.024	99.8	24 60 92 99.9	0.26462	0
4	1.62	1.67	P 21 21 2	1.7	0.031	99.8	60 92 24 99.9	0.29429	0

Update Tables From Datasource | Datasets | Maps & Restraints | Hit Identification | Refinement

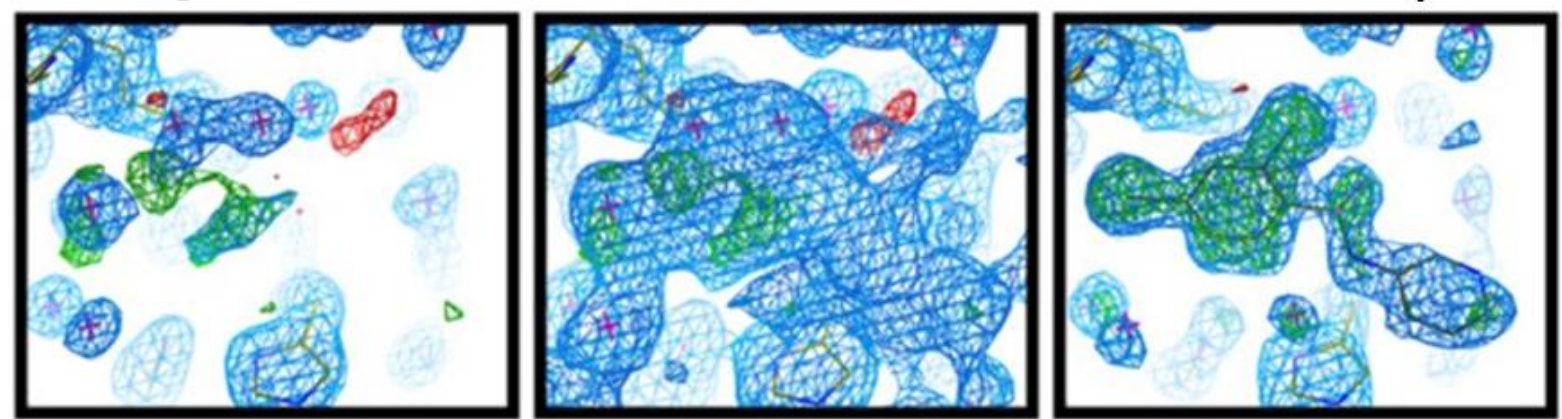
Pan Density Dataset Analysis (PanDDA)



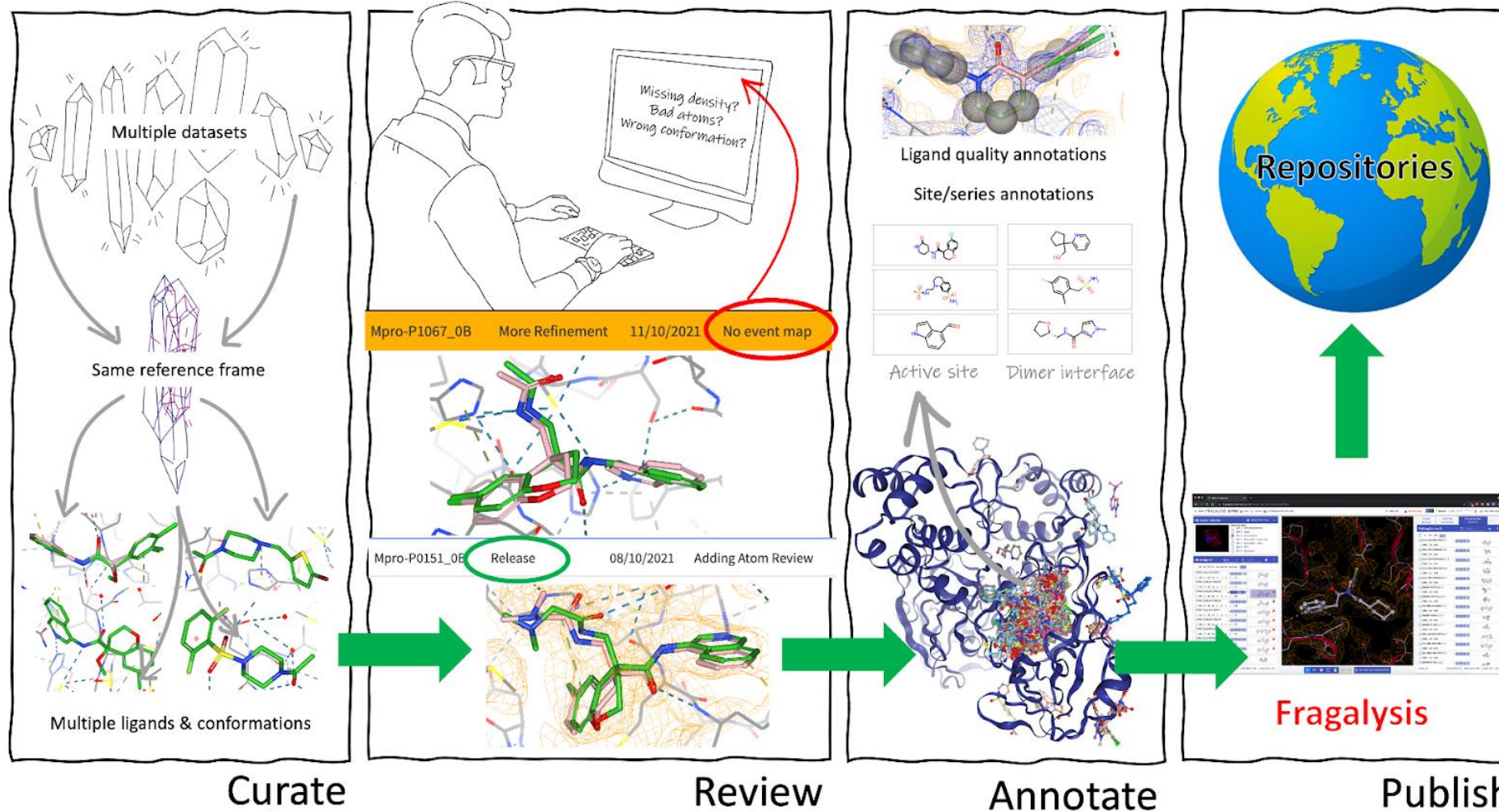
High Contour

Low Contour

PanDDA Map



Data Review & Dissemination



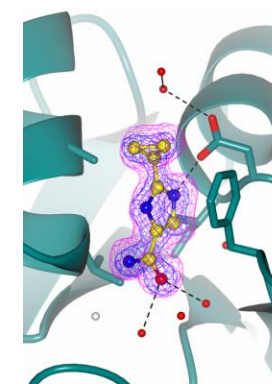
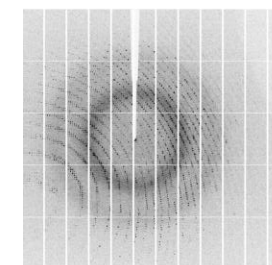
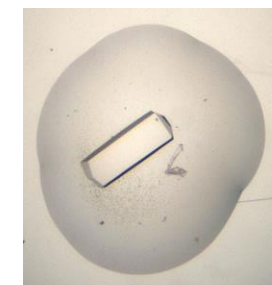
How to access - Standard Academic Access



- 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 (**new**): Follow-up support (200-300 compounds)
 - Feasibility previously established and design rationale articulated for peer review

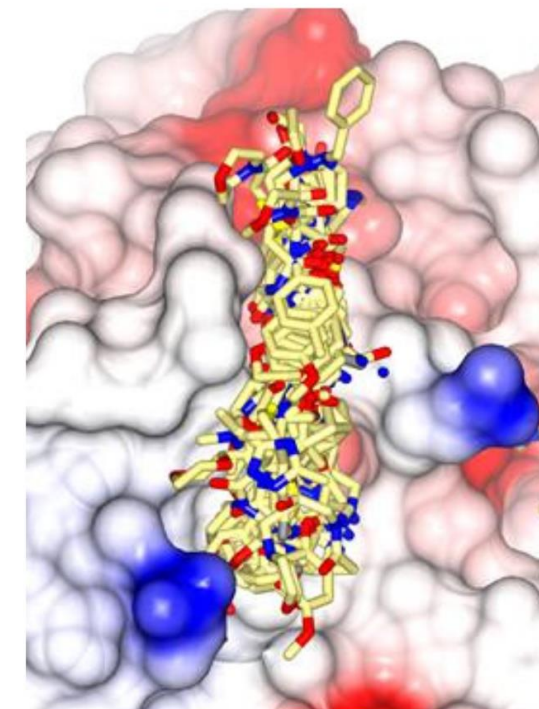
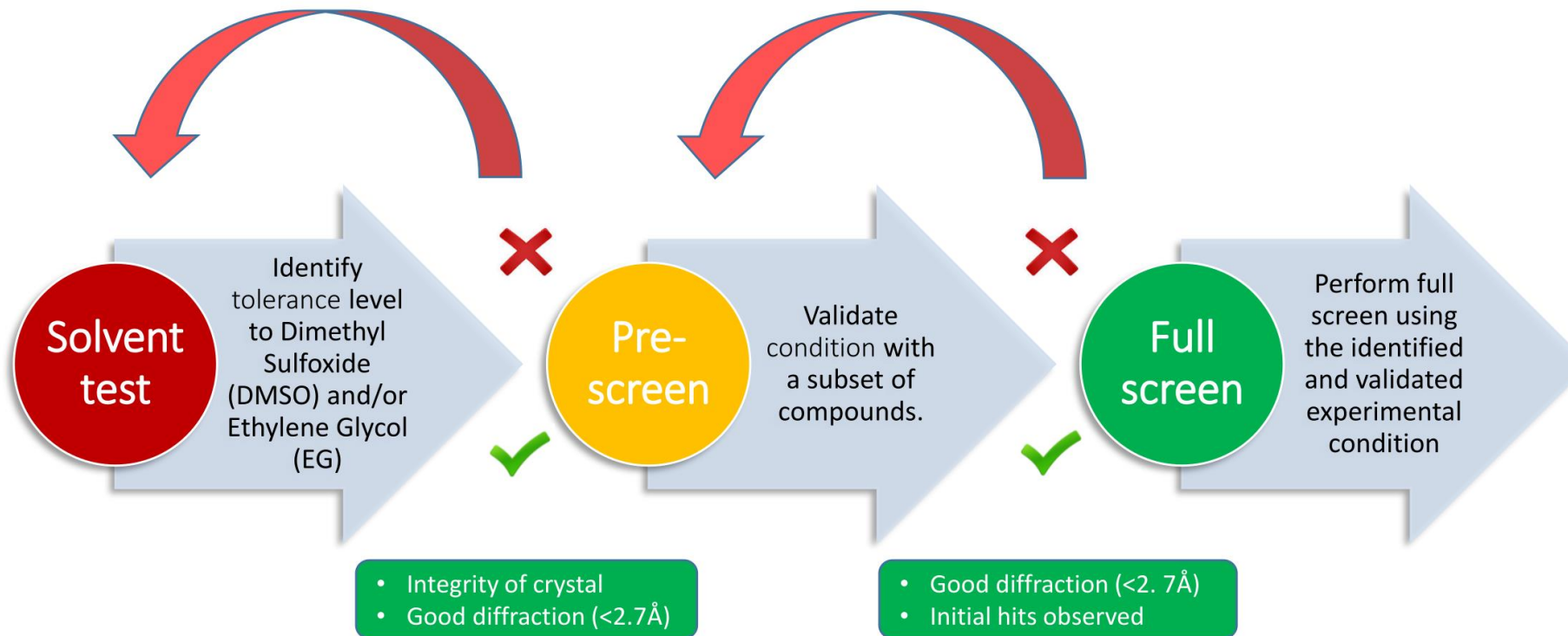
Ideal XChem ready crystal systems

- 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!

Establishing “XChem ready” systems



Screening outcome

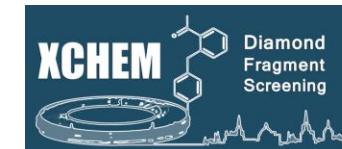
Typical soaking experiments

- (30-50 drops of crystals)
- 5, 10, 20, 30% DMSO or ethylene glycol
 - 1h and 3h time course

Typical soaking experiments

- (100-150 drops of crystals)
- Addition of compounds

Useful Contacts and Links



- **Frank von Delft (PBS):** 8997, frank.von-delft@diamond.ac.uk
- **Daren Fearon (ACD):** 8936, daren.fearon@diamond.ac.uk
- **Charlie Tomlinson (ACD):** charlie.tomlinson@diamond.ac.uk
- **Jose Brandao-Neto (I04-1):** 8506, jose.brandao-neto@diamond.ac.uk
- **Louise Dunnett (I04-1):** 8263, louise.dunnett@diamond.ac.uk
- **Alex Dias (Industry):** 8200, alexandre.dias@diamond.ac.uk
- **Ailsa Powell (Industry):** 7524, ailsa.powell@diamond.ac.uk
- **Warren Thompson (Chemistry):** 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:

- Krojer, T., *et al.* **The XChem Explorer graphical workflow tool for routine or large-scale protein-ligand structure determination.** *Acta Cryst D*, **73**, 267-278 (2017). <https://doi.org/10.1107/S2059798316020234>

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>