How to access and efficiently perform crystallographic fragment screening at XChem

XCHEM Diamond Fragment Screening

diamond

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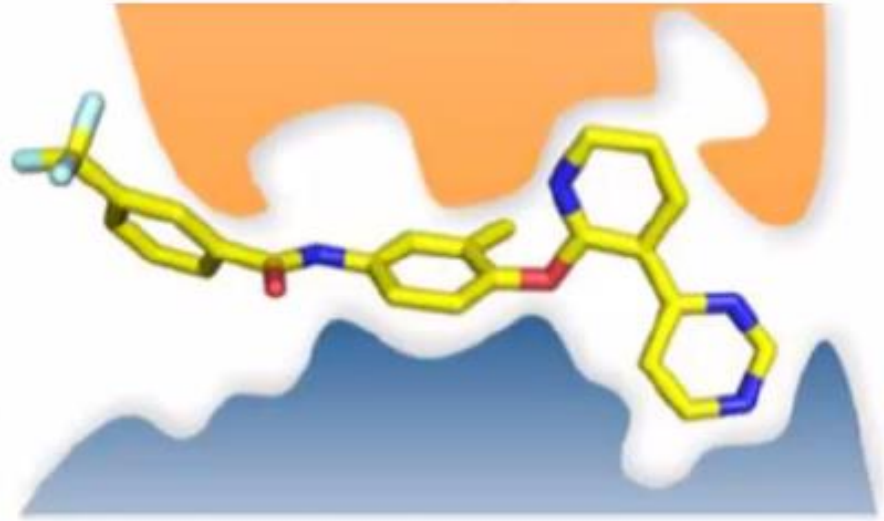
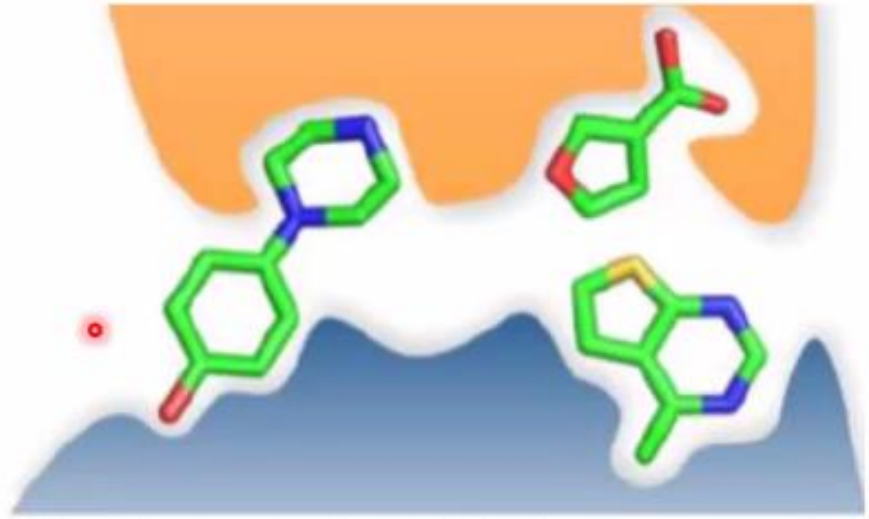
Notes 109%

HTS vs Fragment-Based Drug Discovery

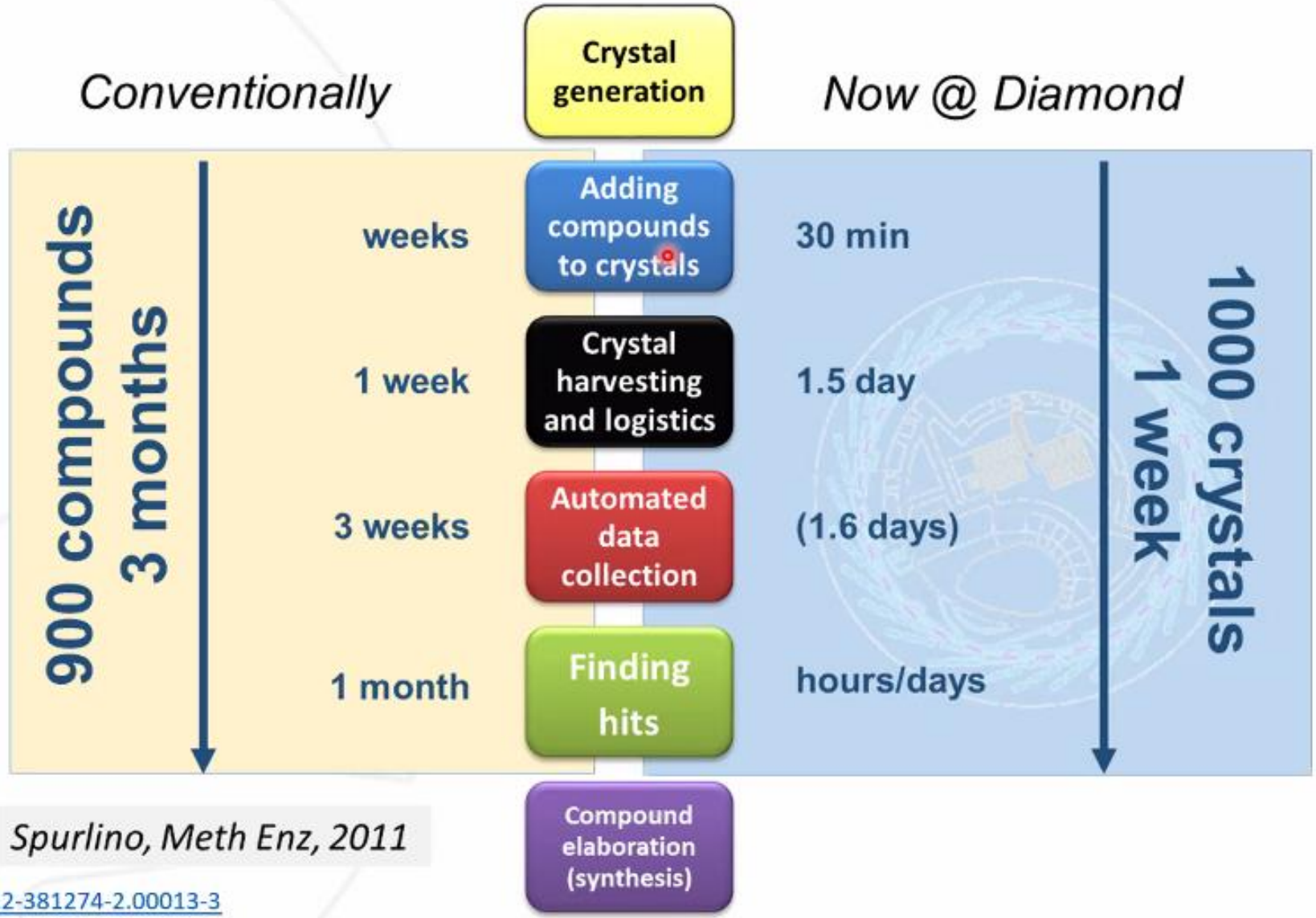


- Libraries typically >100,000 compounds
- Molecular weight >300 Da
- Affinities typically in µM-nM range
- Usually identified by biochemical or cellular assays
- Can be difficult to optimise



- Libraries typically 500-1000 compounds
- Molecular weight < 250 Da
- Affinities typically in mM-µM range **BUT** compounds are very efficient
- Usually identified by biophysical methods
- Iterative optimisation driven by structure-based methods

Fragment screening at Diamond: XChem



Spurlino, Meth Enz, 2011

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

XChem Workflow

Well curated libraries
Best practice protocols

Crystal imaging and ranking

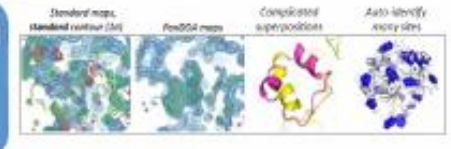
Compound dispensing

Sample harvesting

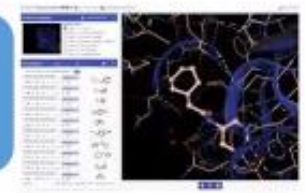
Unattended data collection

Automated data processing

Hit identification (PanDDA) and modelling

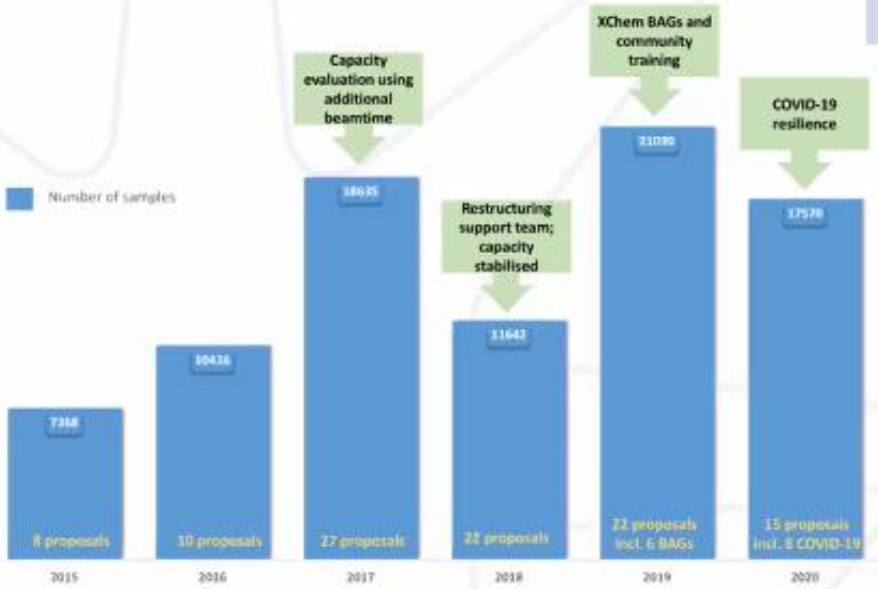


Model dissemination (Fragalysis)

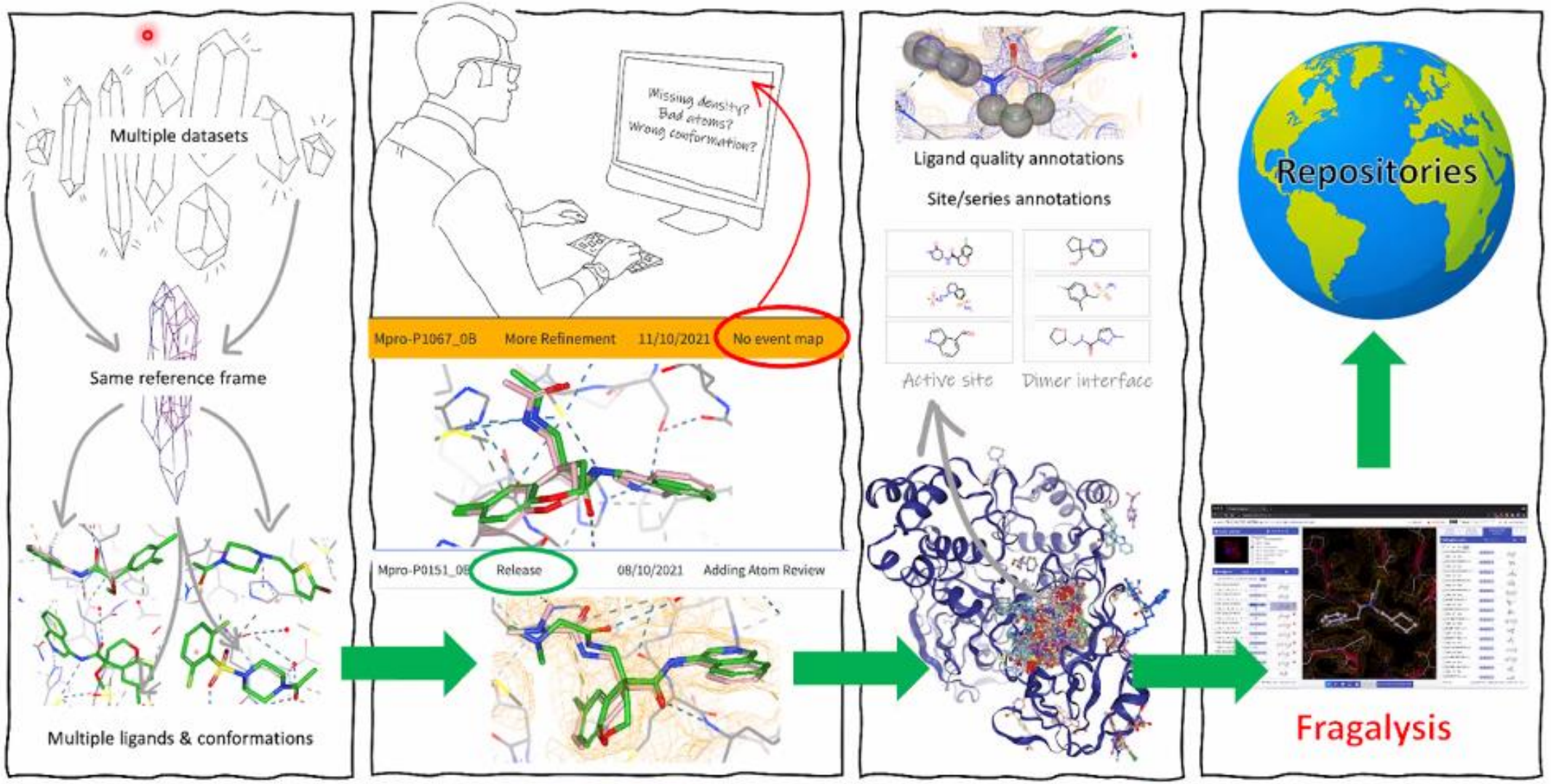


Streamlining in MX village
Technology for user labs

New algorithms
New platforms



Data Review & Dissemination



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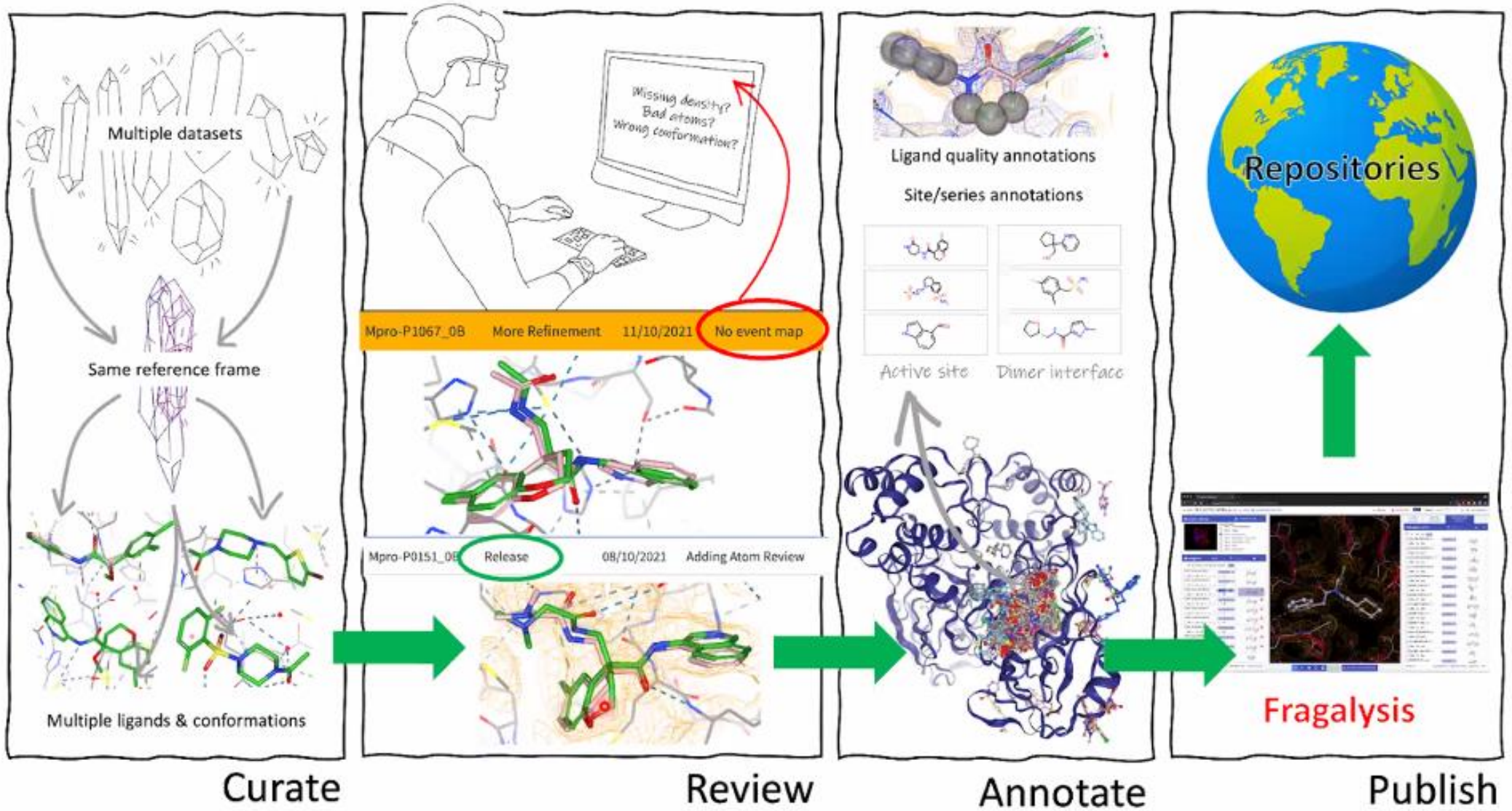
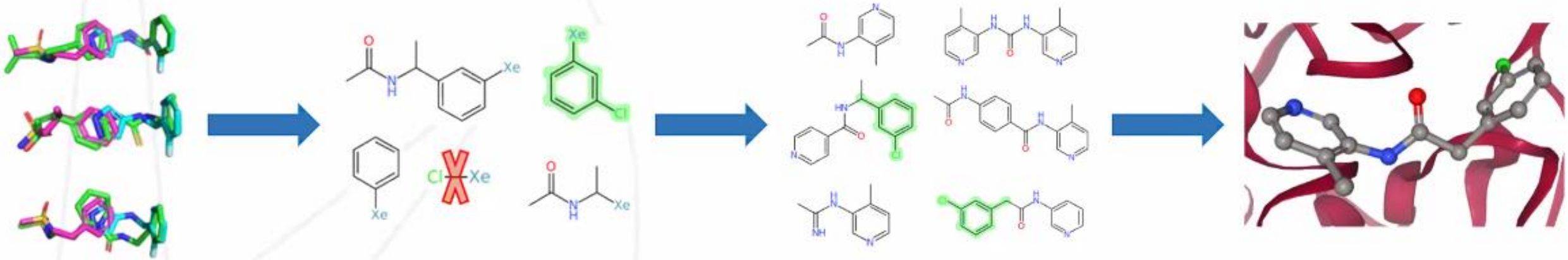


Algorithmic Fragment Progression

- Computational tools being developed to accelerate routine hit-to-lead



Fragment network for pure merges

Fragmenstein

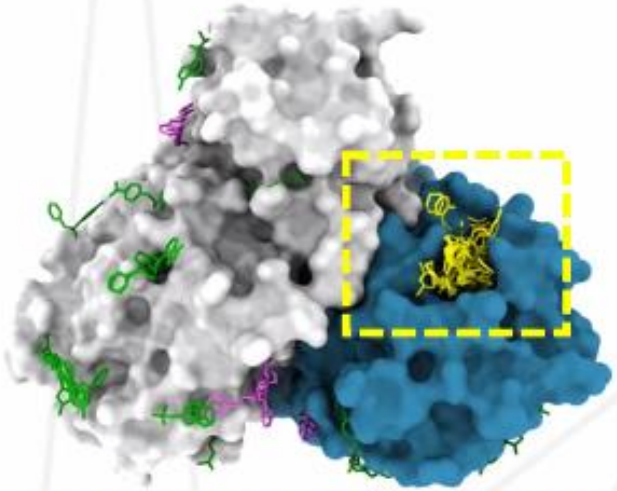
DEVELOP

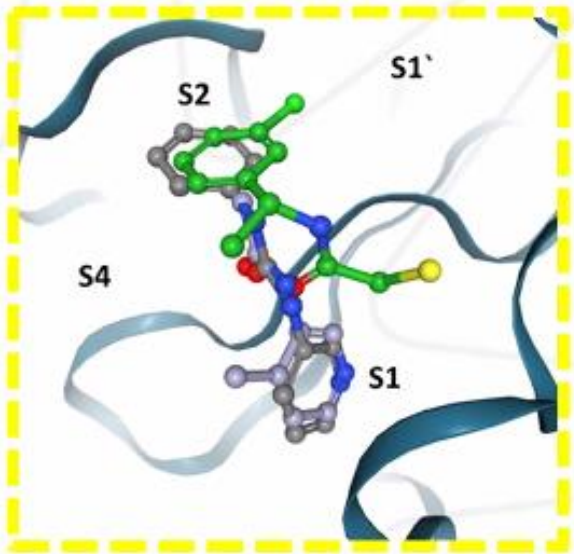
STRIFE

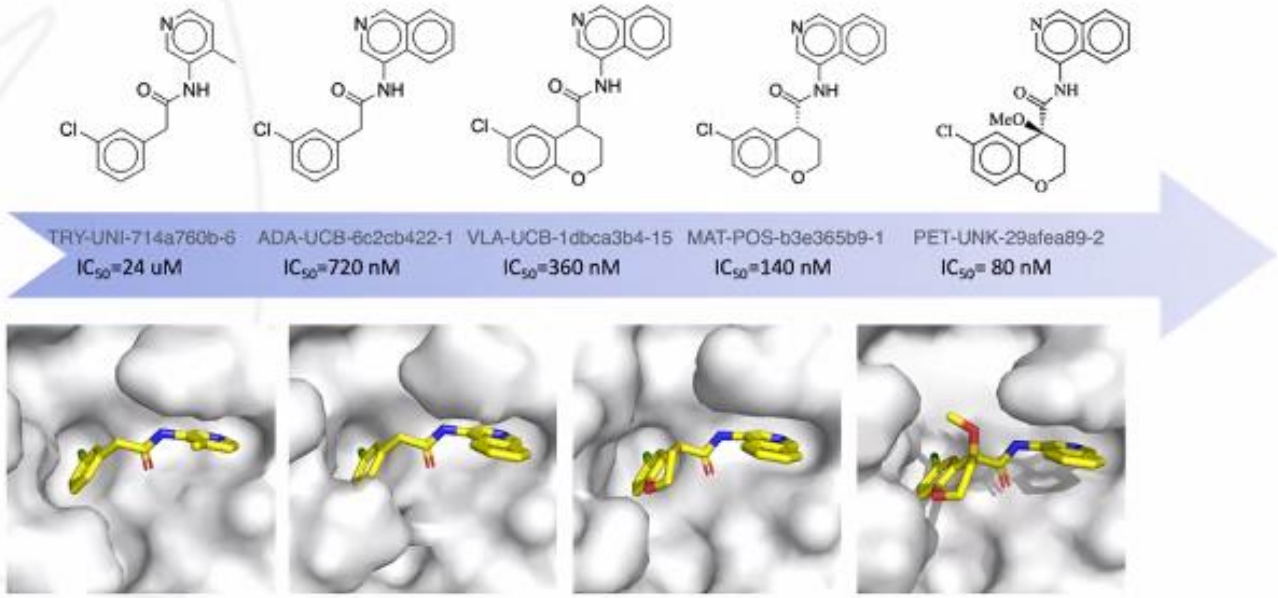
DeLinker

What results can I expect?

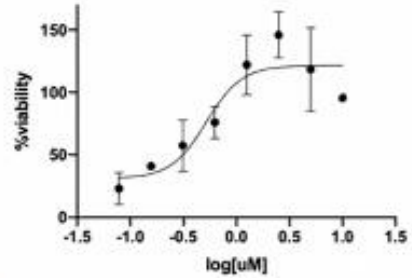
- Merging fragments can rapidly lead to potency

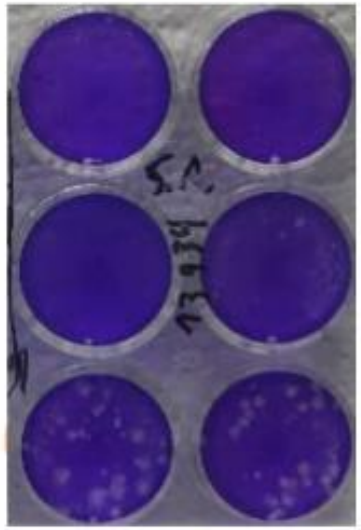






Lead compound active against live SARS-CoV-2





https://doi.org/10.1101/2020.10.29.339317

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

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

Academic BAG Access

For groups, institutes or collaborations that:

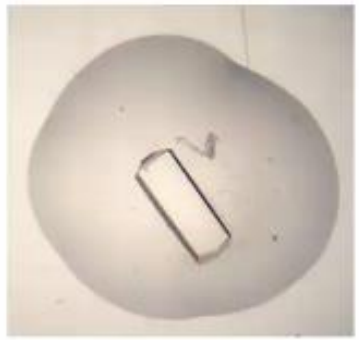
- Have a pipeline of projects all aimed specifically at compound development
- Have hit-to-lead infrastructure in place
- Will therefore routinely and predictably have crystal systems for evaluation and screening
- Have a stringent internal prioritisation process
- Have experienced XChem crystallographer (super-user) to organise and provide logistical support to BAG members

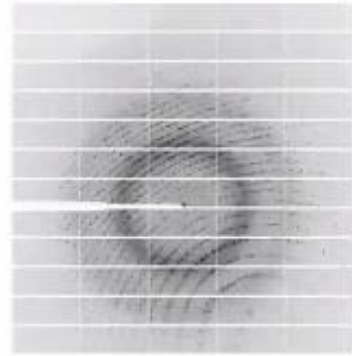
New BAG applications welcome!

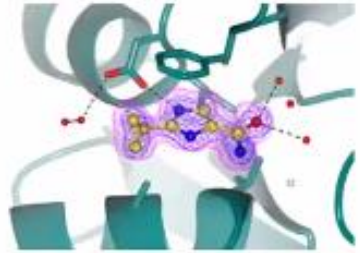
https://www.diamond.ac.uk/Instruments/Mx/Fragment-Screening/XChem-Applications/BAG-Access.html

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!

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

oi: 10.20944/preprints201809.0383.v1

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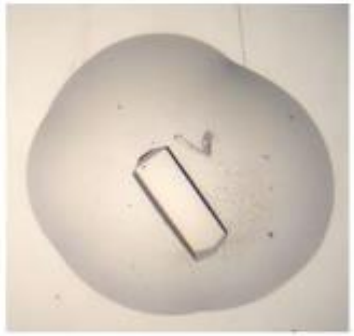

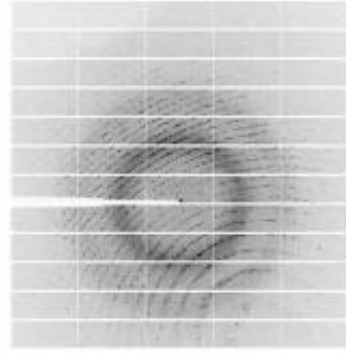

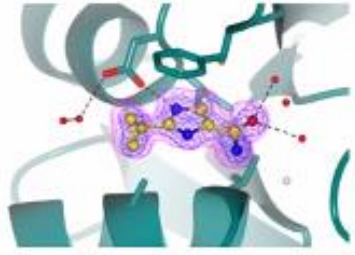

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Establishing “XChem ready” systems

- Explore multiple protein constructs and protein engineering
- Identify multiple crystallisation conditions/crystal forms
  - PEG preferable over high salt conditions
  - Be aware of pH and volatile solvents
- Run QC for your protein batches and crystal trays
- Use seeding!
- Determine crystal solvent tolerance and life span of crystals
- Test transferability of crystallisation/trays between locations
- When robustness established, keep things consistent

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

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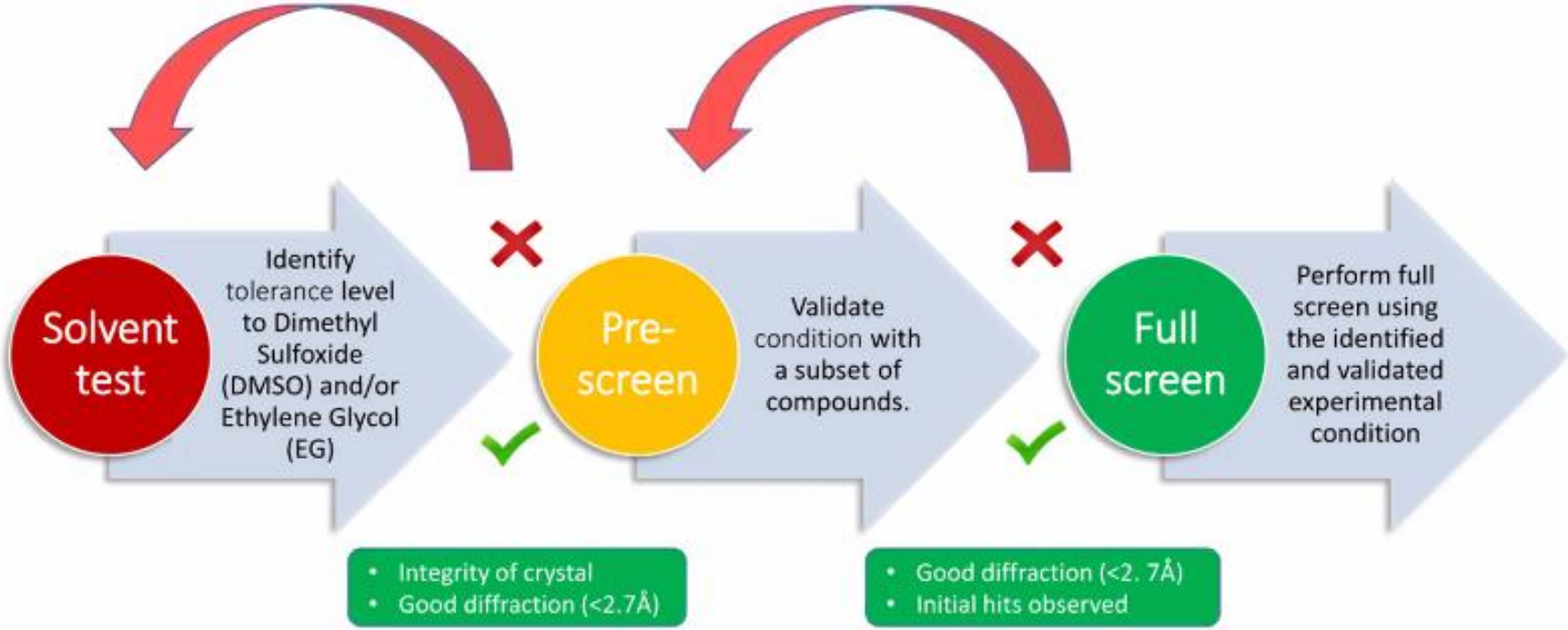
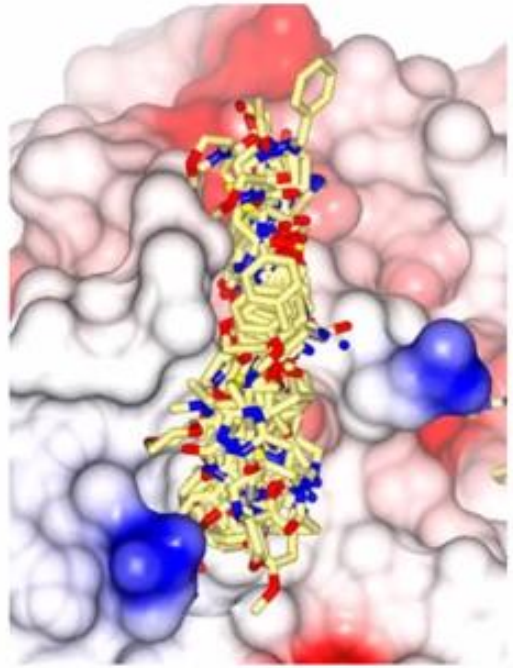
Flushing out the problems





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

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

Partnerships (hit to lead follow-ups):

- EUbOpen
  - Collaboration with members of the consortium (including 5 pharma partners) to help progress hits to chemical probes
  - Can include screening assays, crystallisation, HTS, chemistry etc
  - The target remains confidential between the applicant and collaborator(s)

- EU-OPENSCREEN
  - Offers open access to its shared resources including 140,000 compounds linked to EU-OS Fragment library and med. chem. support

https://www.diamond.ac.uk/Instruments/Mx/Fragment-Screening/Partnerships--access-and-hit-to-lead-follow-ups-.html
https://www.eubopen.org/
https://www.eu-openscreen.eu/

Useful Contacts and Links

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- 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