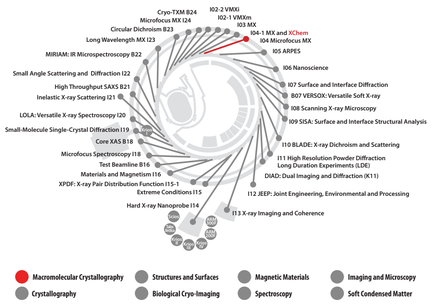


Crystallographic fragment screening

Fragment-based screening is now well-established as a powerful approach to early drug discovery. Synchrotron based MX has played a major role in this area in the past 20 years but often there are limitations in how widely it can be used for activities such as primary screening of drug targets.

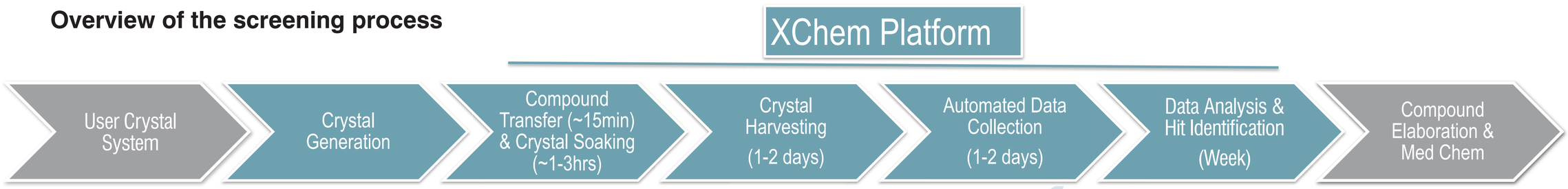
At the fixed wavelength monochromatic MX beamline I04-1, with the XChem facility, the full X-ray screening experiment has now been implemented as a highly streamlined process, allowing up to 1000 compounds to be screened individually in less than a week, using less than 40 hours of automated beamtime.

I04-1 and XChem: a High throughput *In-Crystallo* Fragment Screening Platform

The platform for X-ray accelerated Chemistry, or XChem, has been running at Diamond since April 2016, and grew out of a partnership started in 2012, between Diamond and the Structural Genomics Consortium (SGC) in Oxford. It leverages on SGC sample production developments and their experience as a user of synchrotron facilities coupled with the optimisation of beamline I04-1 at Diamond as a high processing capacity hub.

The process covers soaking, harvesting, automatic data collection, and data analysis; drug fragment libraries are available, although some users can bring their own.

Overview of the screening process



XChem process

Current requirements

- Crystallise in SwissCi 3 lens wells
  - Robust and reproducible crystals
  - Diffract to <2Å (up to 2.5Å)
  - Yield >50%
  - Small drop volume 200-400nl

Available Libraries:

Fragment library in 1536 wells format

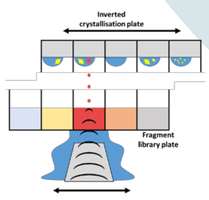
- >2000 compounds
- DSI-poised
- Maybridge
- Edelris
- Your own!

Acoustic droplet ejection

- Fast and accurate
- Small volume (2.5nl per droplet)
- ~10-15min to transfer compounds from a library

Transfer of compounds into SwissCi 3 lens plates

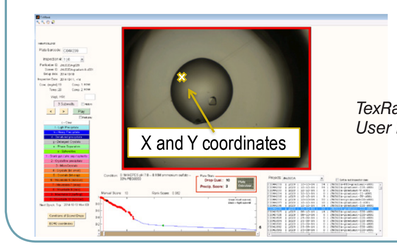
Echo dispenser (Labcyte)



Schematic view of transfer

Crystal location and transfer coordinates

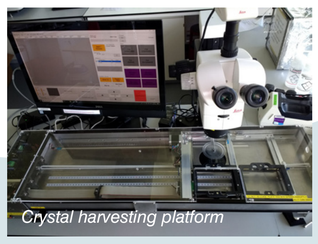
- Plates are imaged via a Rockimager
- TexRank locates the crystals
- The user defines the transfer coordinates
- Target away from crystals
  - Slower diffusion
  - Direct hit damages crystal



TexRank Graphical User Interface

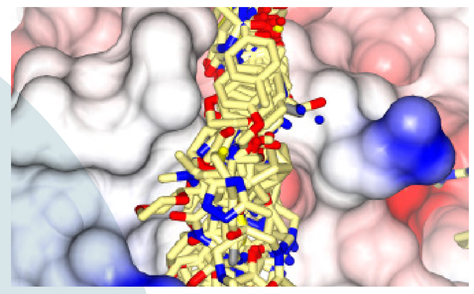
Crystal harvesting and plate shifter

- Easily mount >100 crystals per hour
- X,Y and Z motorised stage
- Touch screen user interface
- Easy to use
- Keep track record of experiments
- Mitegen loops curated per size (unitrays)
  - 35, 75 and 150μm loops



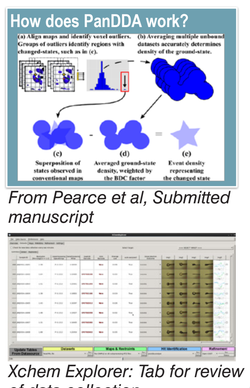
Crystal harvesting platform

Typical readout of a screen: a superposition of all hits



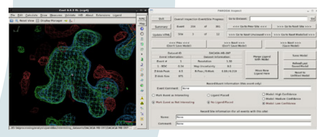
XChemExplorer & PanDDA

- Rapid review of the experiment
  - Assess processed data
  - Re-run Xia2 and Dimple
  - Launch pandda.analyse and pandda inspect



From Pearce et al, Submitted manuscript

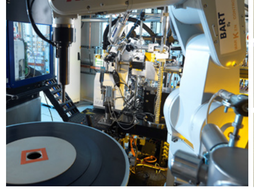
Xchem Explorer: Tab for review of data collection



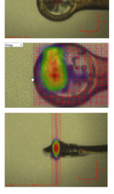
Pandda.inspect: coot plugin for rapid review

Unattended data collection on I04-1

- Fully automated
  - >30 samples per hour
  - Precise loop centring routine
- Fast pixel Area detector
  - Data collection 60s
  - New sample changer BART
- Sample exchange <15s
  - Capacity~600 samples



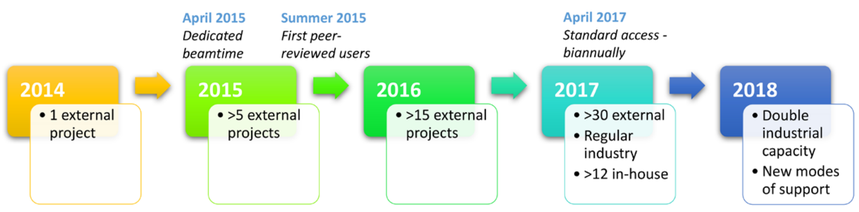
I04-1 end-station and loop centring



Routine X-ray Centring

References: Ng et al, 2017, ActaD. Pearce et al, 2015. Cox et al, 2016, Chemical Science. Krojer et al, 2017, ActaD. Collins et al, 2017, ActaD.

XChem progress



>> 80,000 crystals in < 2.5 years
>> 1,500 hits for >60 targets
(Papers: 2-3yr delay)

XChem is a world-first facility, offering crystal-based fragment screening as a globally accessible user programme. Up to 1000 compounds are screened every week by academic and industrial users. Annually it hosts well over 30 screening experiments from both usages. In 2017, the programme contributed 35,000 of the more than 50,000 crystals shot at the beamline, but using less than one third of the total beamtime, illustrating the efficiency of the automated queue mode. With the programme consistently oversubscribed, and demand from industry growing, the facility is expanding its capacity: a dedicated user support team has been assembled, with extra capacity and an increase in dedicated lab space. Additional beamtime has been assigned for the allocation period for 2018.