MX from an Industrial Perspective

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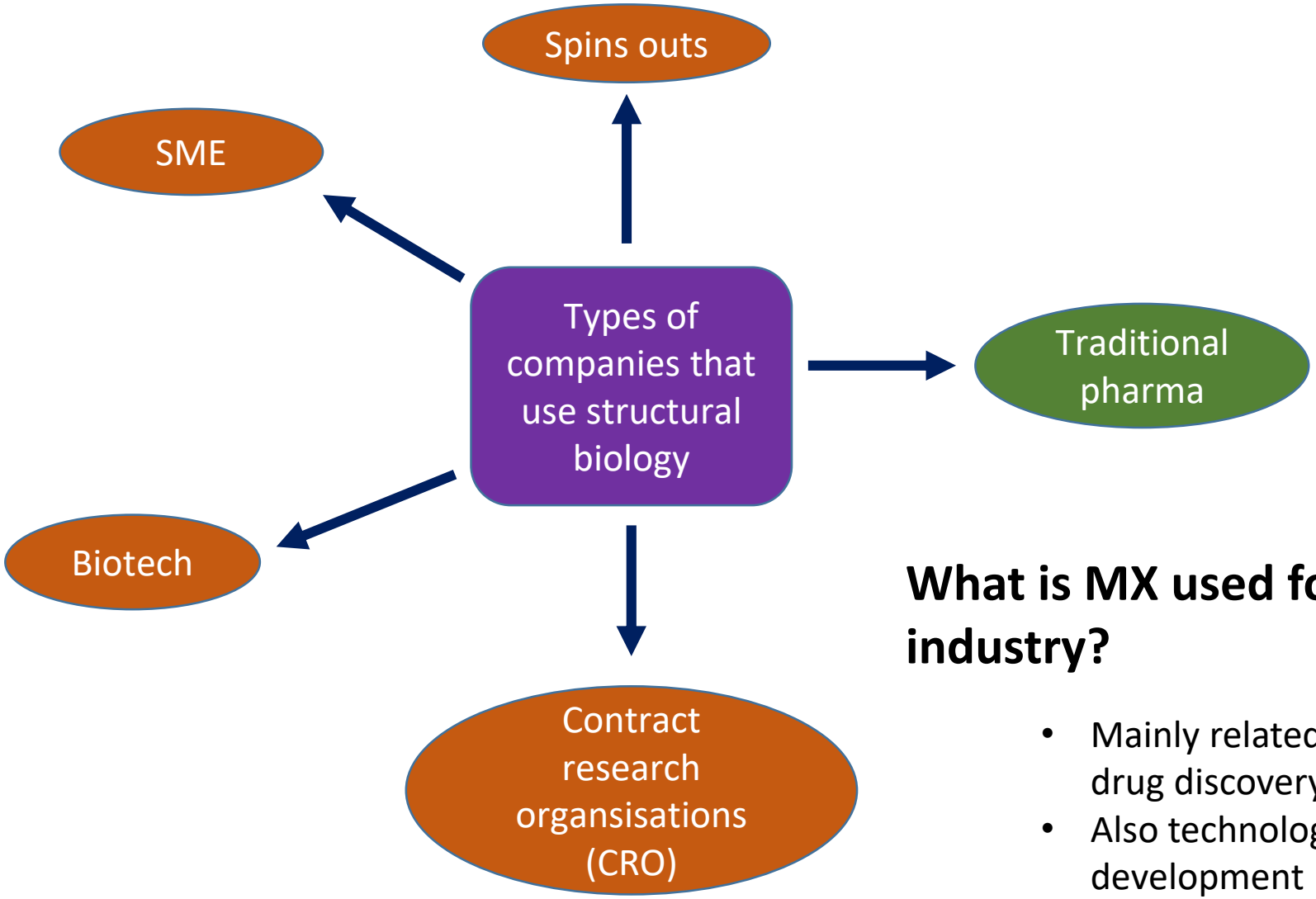
diamond

Types of industry and advantages of using MX

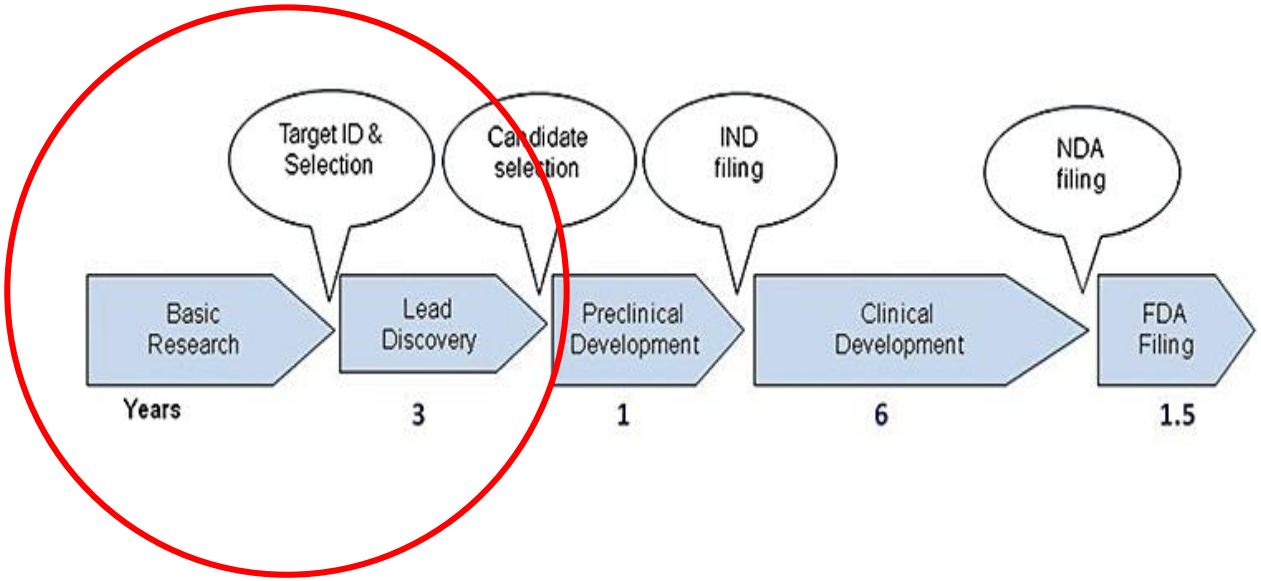


What is MX used for in industry?

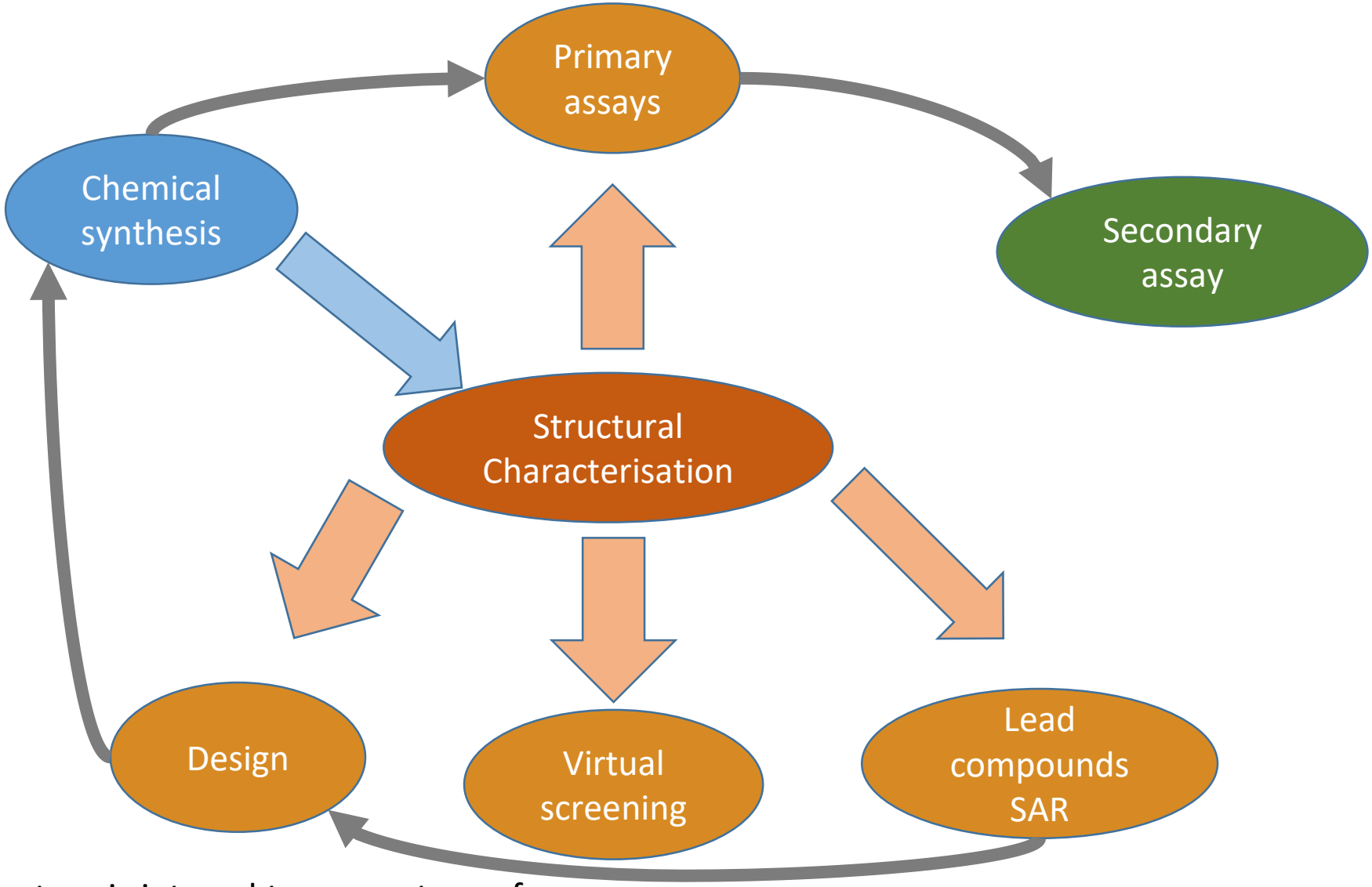
- Mainly related to drug discovery
- Also technology development

Why use structural biology

Invaluable insights at the drug discovery phase



- Protein structures can be used for the following:
  - Basic research – characterisation of the protein target (academic and industrial)
  - Lead discovery – identification of compounds (industrial and academic)



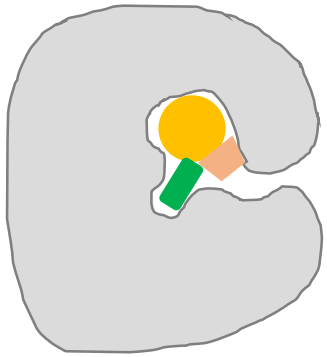
Structure is integral to many steps of the discovery process

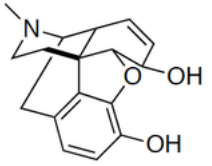
How structures contribute to screening

Structural aided drug design	• Use of crystal structures to help design molecules	• Have docked a compound into the crystal and use this to help predict where modifications could be added to provide increased potency or selectivity • Often used as an adjunct to other screening strategies within big pharma
Virtual screen	• Docking models: use of a virtual compound library with the X-ray structure of the protein • If have a known ligand, as a base to develop further compounds on	• Can provide the starting structures for a focused screen without the need to use expensive large library screens • Can also be used to look for novel patent space around existing compound structures
Fragment screen	• Soak small compounds into crystals with often low mM activity	• Join selected fragments together to fit into the chemical space to increase potency • Requires a crystal structure to be available

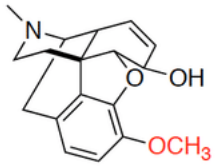
SAR – Structure activity relationships

- Link between the drug target and the lead compound.
- Structures of the target with and without the lead compound act as a guide that can allow computational chemists and molecular modellers to identify binding site interactions

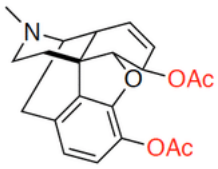




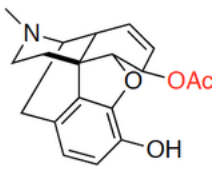
morphine
relative potency = 1
addictive



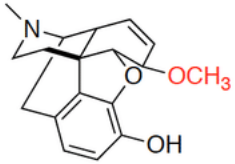
codeine
relative potency = 0.2
addictive



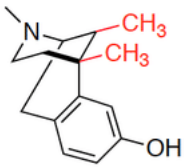
heroin
relative potency = 2
addictive



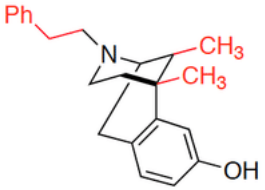
acetylmorphine
relative potency = 4
addictive



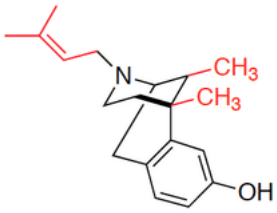
heterocodeine
relative potency = 5
addictive



metazocine
relative potency = 1
addictive



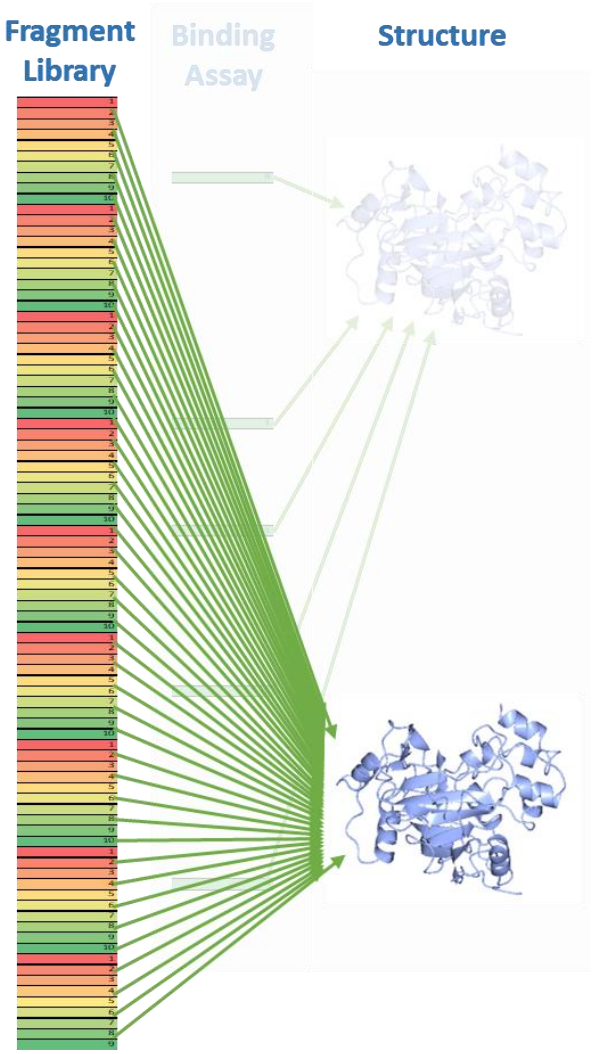
phenazocene
relative potency = 4
non-addictive



phenazocene
relative potency = 0.33
non-addictive

XChem Platform

Fragment Screening Platform - XChem



Standard practice

- Cascade of biophysical methods
  - SPR, NMR, MST…
- Require crystal structures

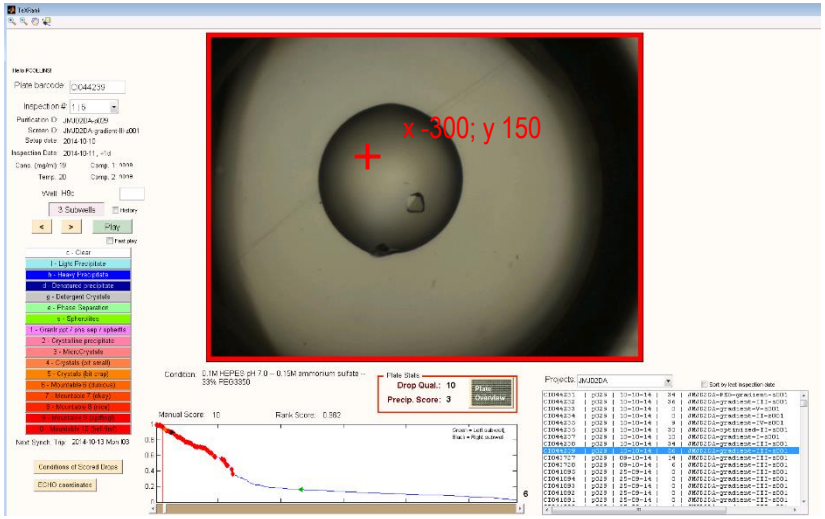
Via crystallography

- Very sensitive method
- High compound concentration
- Significant experimental overheads

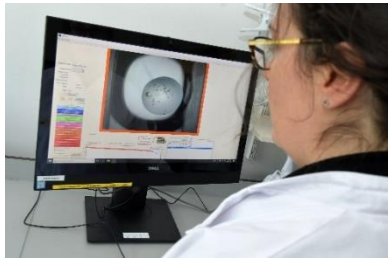
XChem platform

- >100 campaigns completed
  - 2-20% hit rate (project dependent)

Crystallisation and Plate Imaging



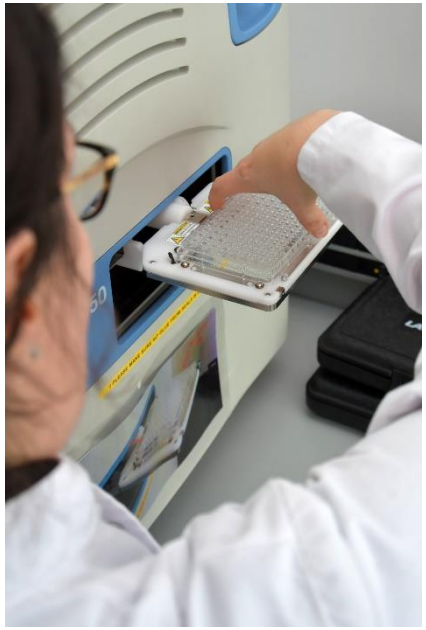
TexRank, Ng et al, 2014. Acta Cryst



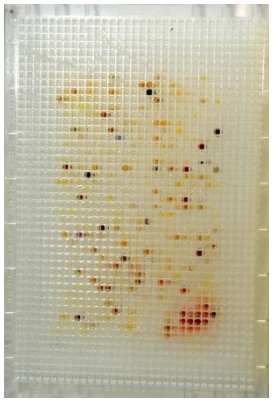
SwissCi 3 crystallisation plate

- Crystallisation facility
  - Mosquito
  - Hydra
  - Gryphon LCP
  - Rocker imagers 1000
- Compatible plate format
  - SwissCi 3 lens plate
- TexRank:
  - Image analysis per plate
  - User defined crystal ranking
  - User defined echo transfer coordinates

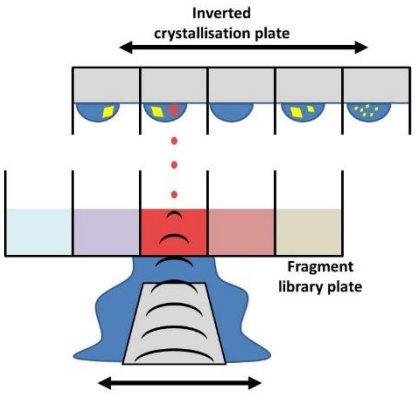
Libraries and Compound Transfer



Echo 550, commercialised by Labcyte

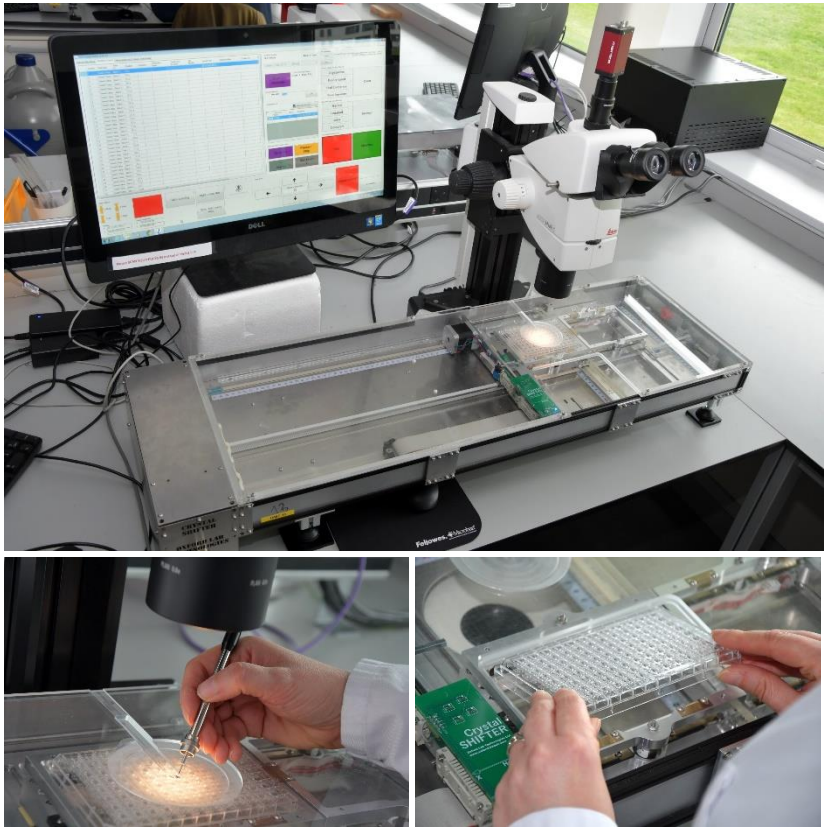


DSiP library in 1536 plate



- Libraries
  - DSiP
    - ~700 compounds
    - 500mM in DMSO
  - External libraries
    - Echo compatible plate format
- Echo 550
  - Acoustic liquid dispensing
    - Very accurate
    - transfer sequentially 2.5nl droplets
  - Cherry pick compounds
  - ~10min to transfer our DSIP library

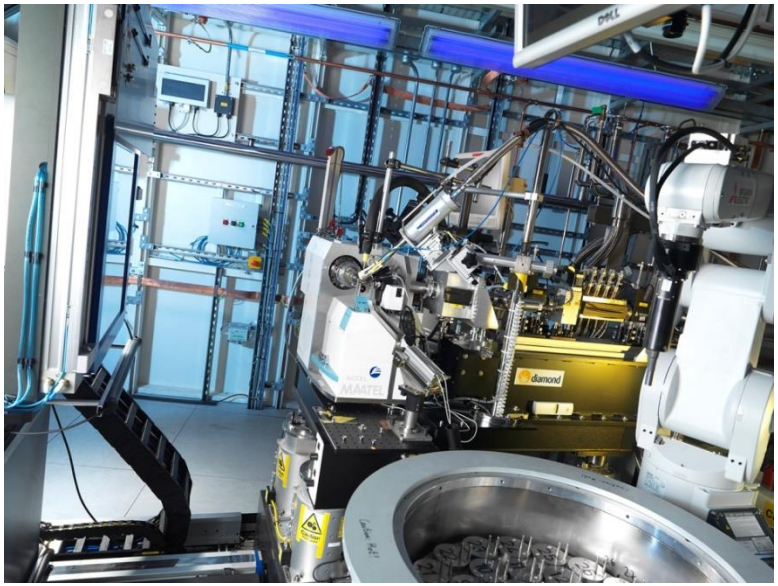
Crystal Harvesting



Crystal SHIFTER, commercialised by OLT

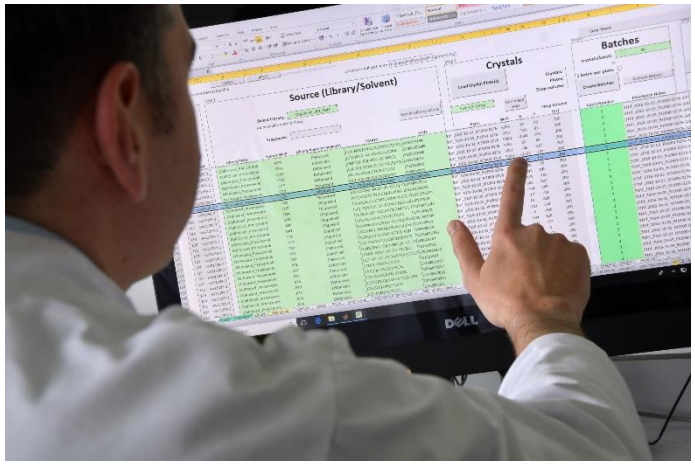
- Crystal SHIFTER
  - SwissCi 3 lens plate
  - Motorised X&Y stage under a microscope
  - Touch screen graphical interface
    - Record events (time stamps)
      - Crystal mounted
      - Crystal melted
      - Precipitate
      - etc.
  - Linked to SoakDB
    - XChem data management system

Fully Automated X-ray Data Collection

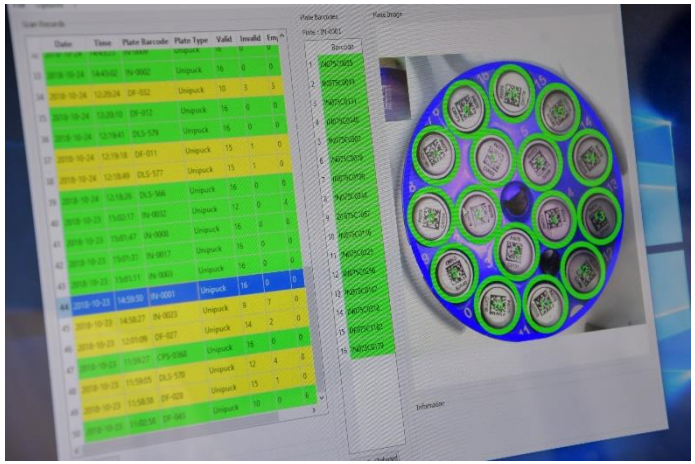


- I04-1 MX beamline
  - Automated loop centring
    - Optical or X-ray
  - Bart robot
    - Dewar capacity ~600 samples
  - Process up to 32 samples per hour
  - Large pixel area detector
    - 60s data collection
  - Auto-processing pipelines
    - Xia2, Dials and AutoProc

Data Management System: SoakDB



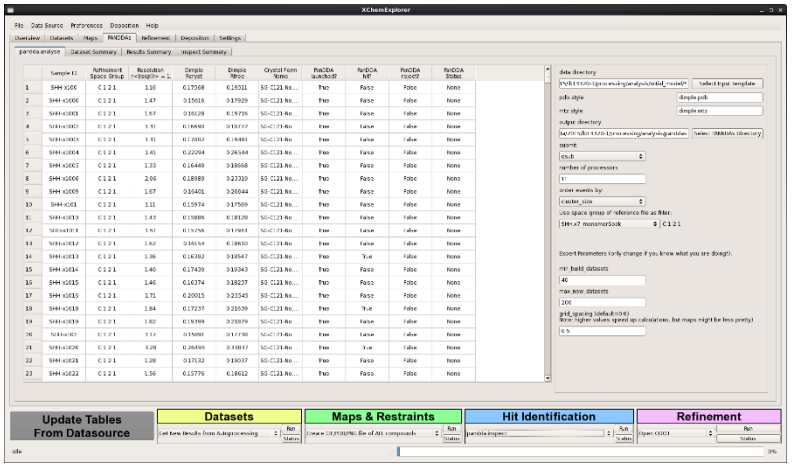
SoakDB interface



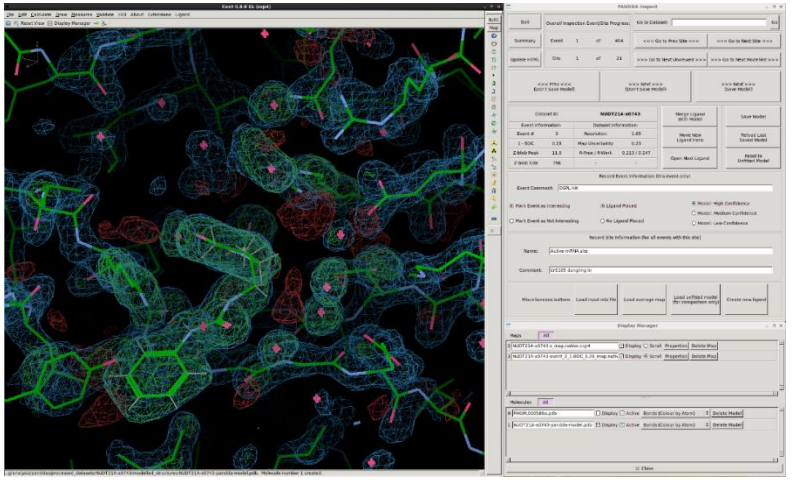
Puck & pins barcode reader

- Experiment tracking interface
- Linked to all components
  - Libraries, TexRank, Echo, Shifter and ISPyB
  - Generate and import CSV files
- Record all information
  - Synced to our SQLite database

Data Analysis: XChemExplorer & PanDDA



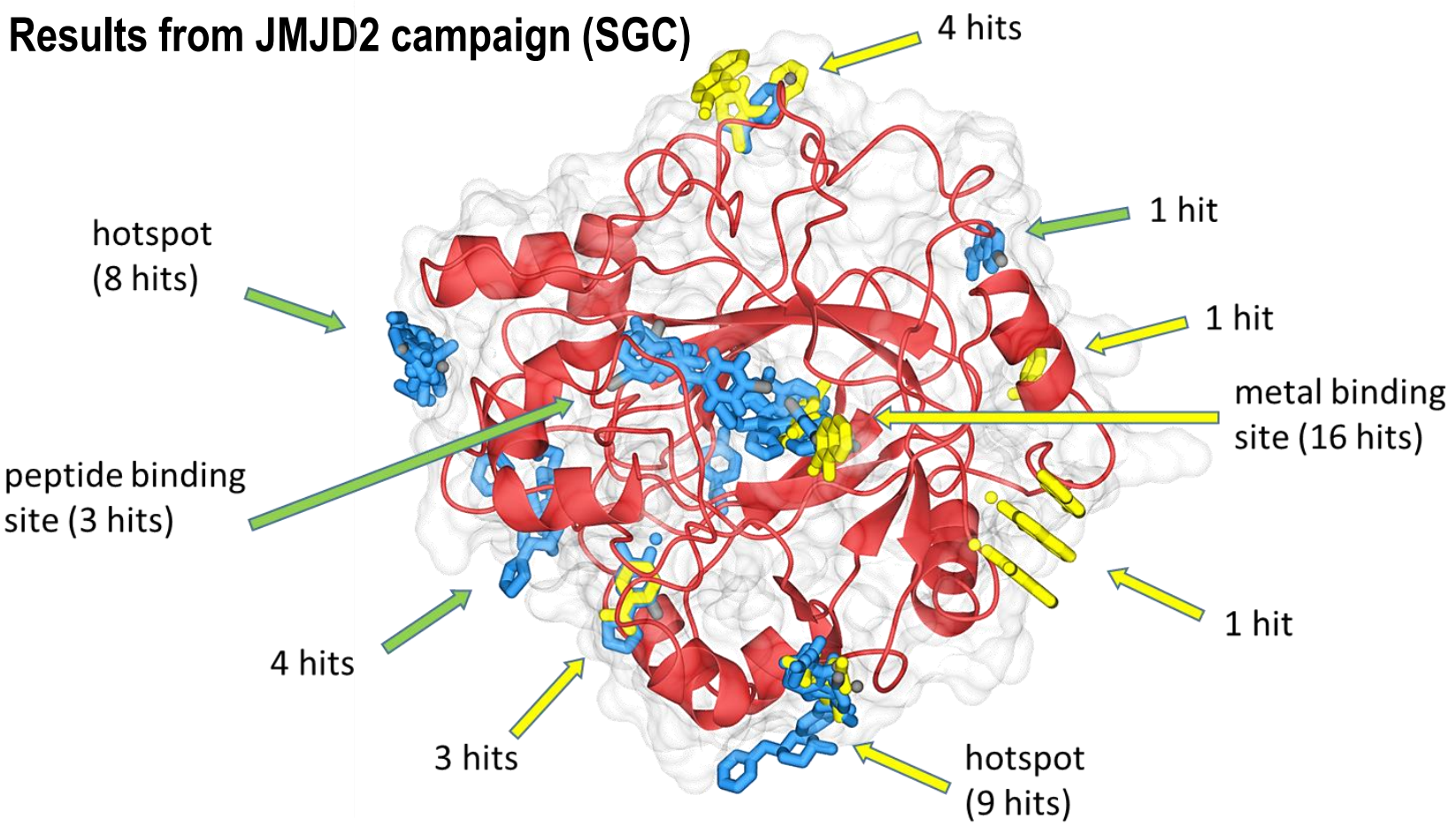
Krojer *et al*, 2017, ActaD



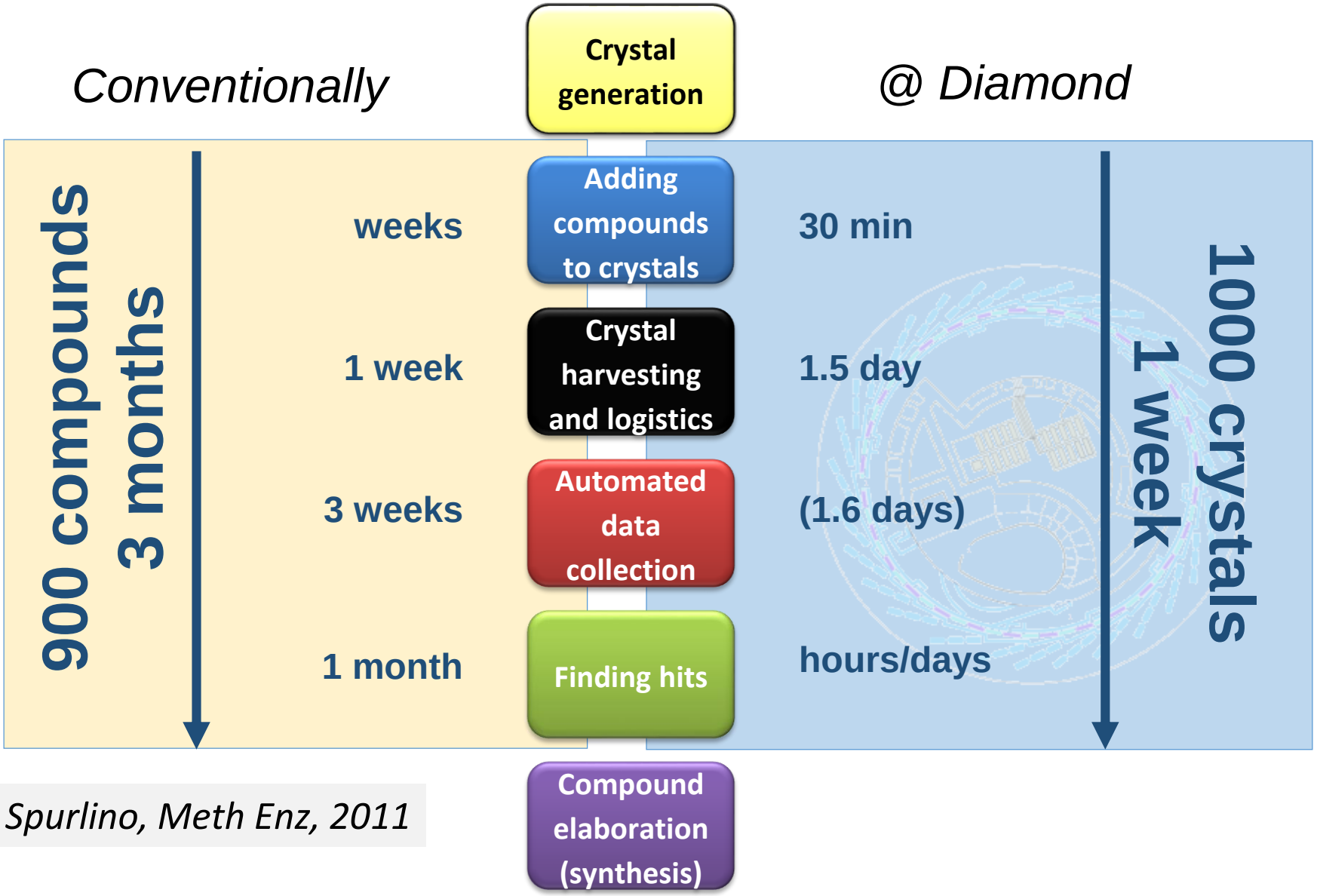
PanDDA. Pearce et al, 2017, Nat. Comm.

- XChemExplorer
  - Record all information
    - Same SQLite database as SoakDB
  - Rapid review of data collection
    - Selection of processed files
      - Reprocess datasets, if required
  - Generation of maps
    - Run Dimple on user's PDB model
  - PanDDA user interface
    - Pre-PanDDA
      - Generate accurate PDB model of "background state"
    - Coot Plugin
      - Facilitate ligand fitting
      - Quickly browse through events and record comments

Fragment Screening Platform - XChem



Order of magnitude speedup



Spurlino, Meth Enz, 2011

Crysalin Ltd

R&D based spin out

Venture capital funded spin out from University of Oxford

Founded by John Sinclair and Martin Noble

- Aim: to provide rapid determination of protein structures, irrespective of target class, at sub 3Å resolution

How to achieve this?

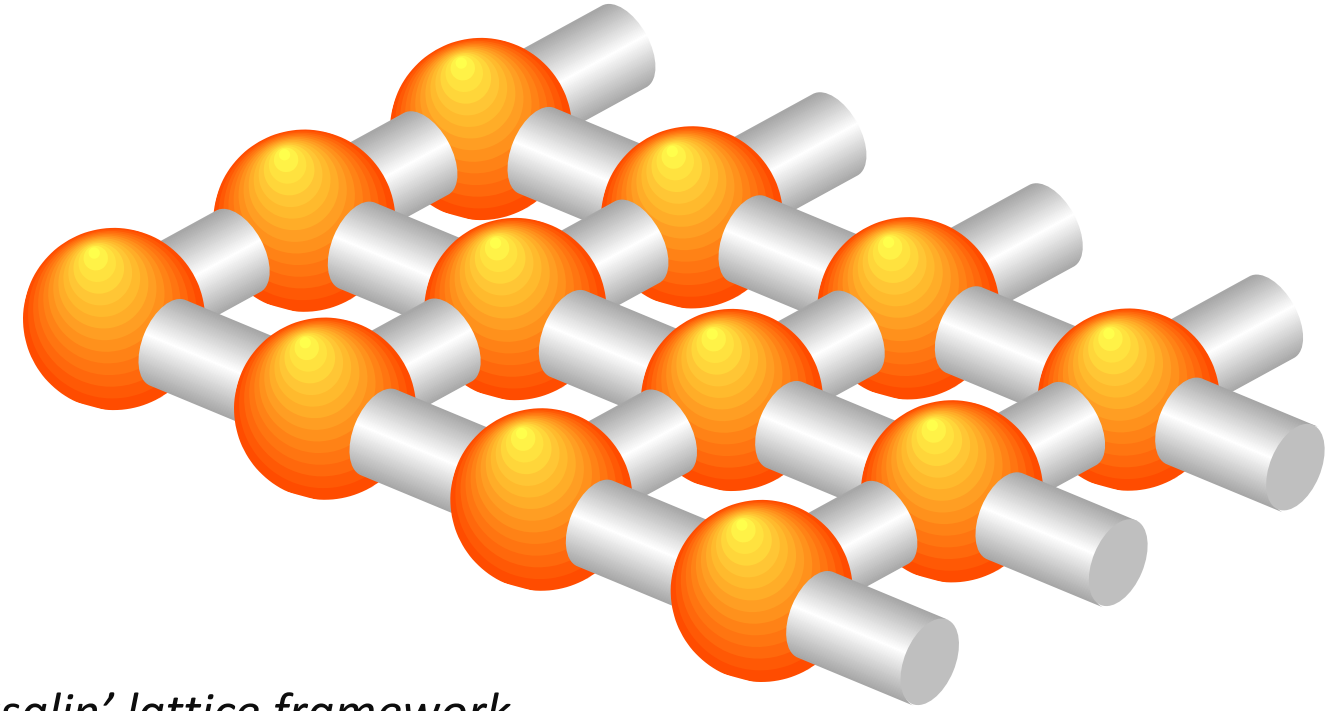
Crysalins are a biological nanomaterial – a self assembling protein lattice

- Remove need for protein crystallisation
- No reproducibility issues (poor/variable resolution, etc)
- No target class restriction (ion channels/GPCRs)
- Remove need for large quantities of highly purified protein
- Increase speed of process
- Increase certainty of result

An industrially robust and consistent process with predictable results

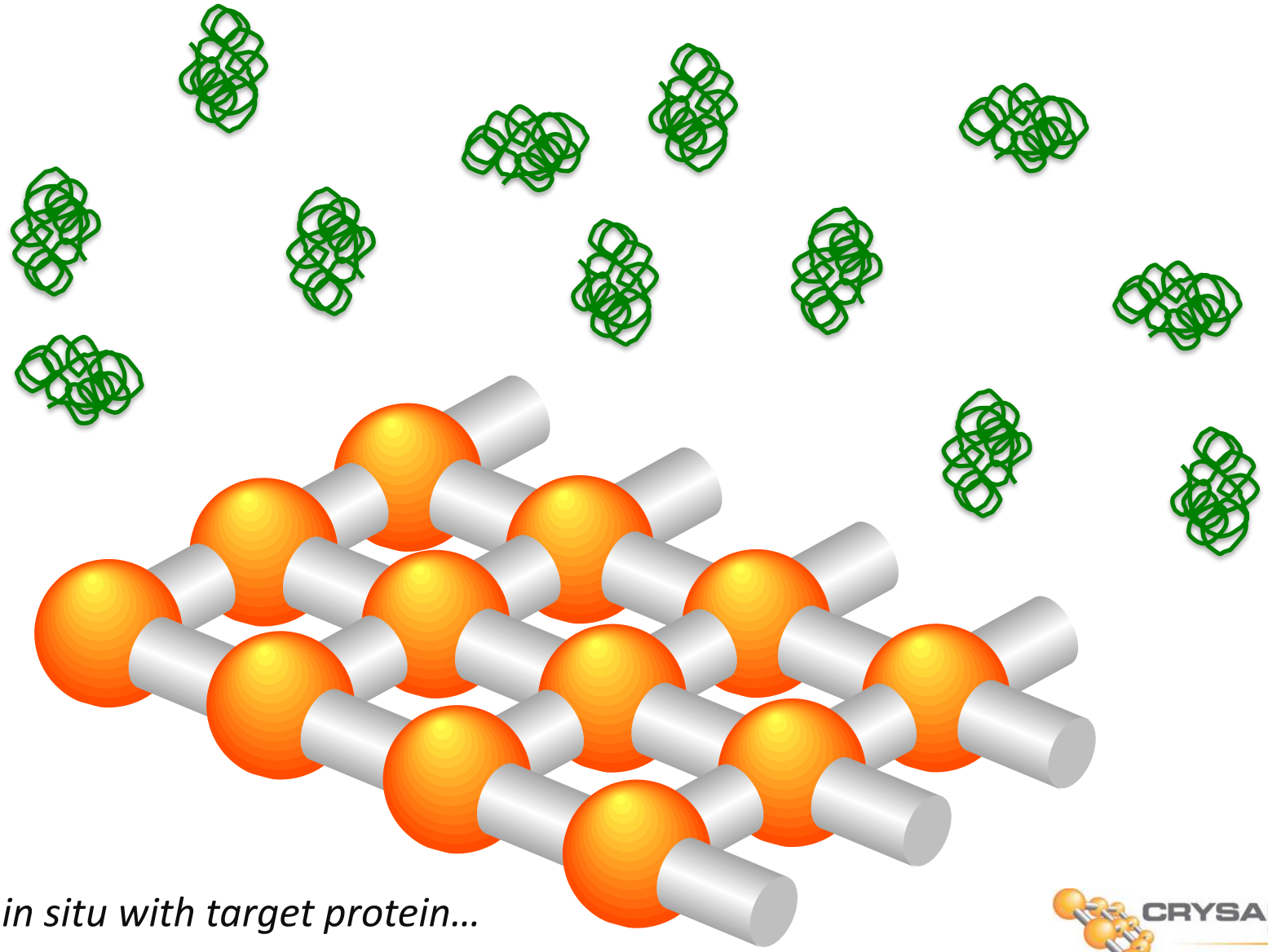
The freedom to choose which target to work on due to scientific validity of the target not the technical limitations of the methodology

Crysalin concept 2D



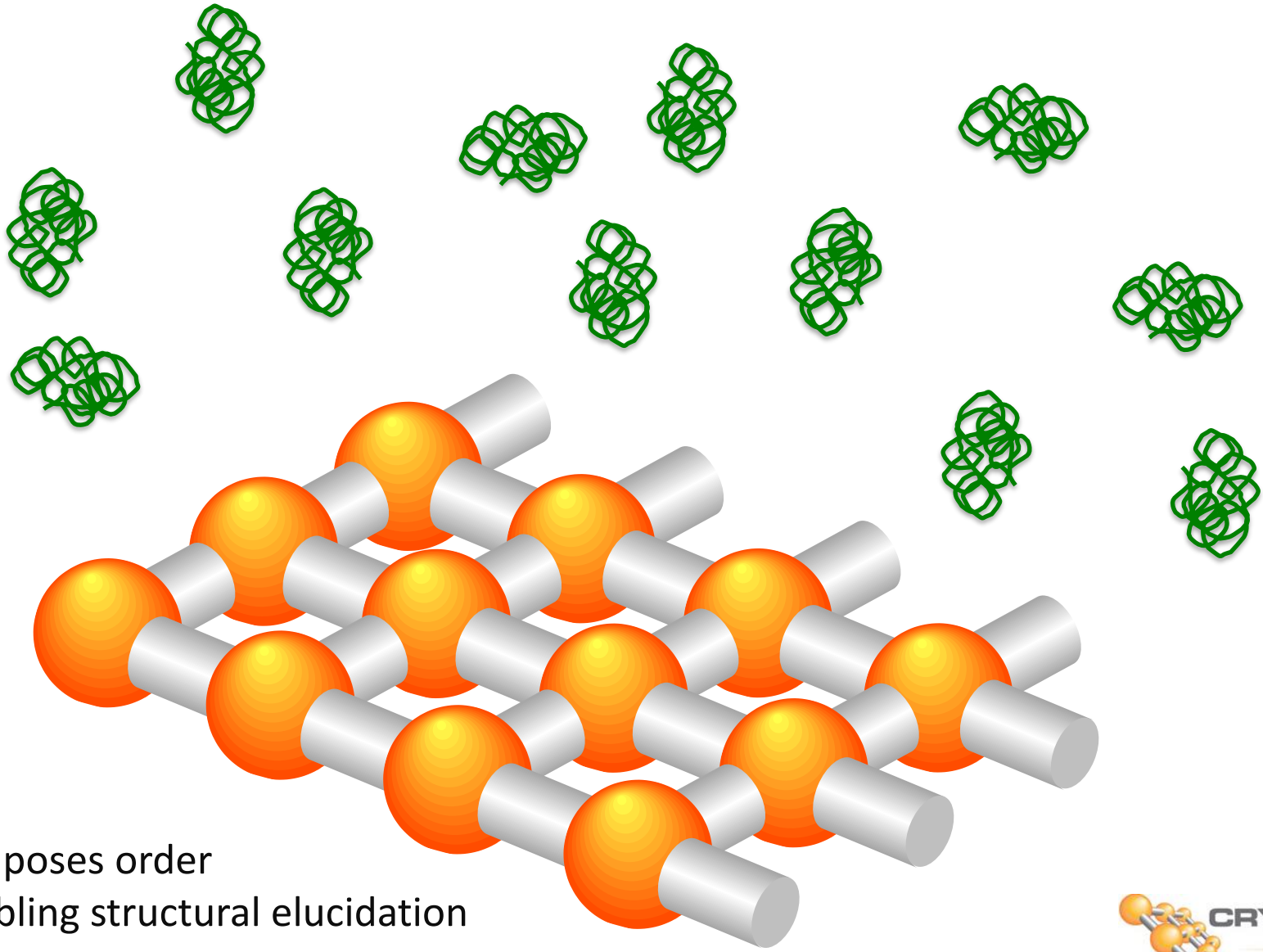
The 'Crysalin' lattice framework…

Technical concept 2D



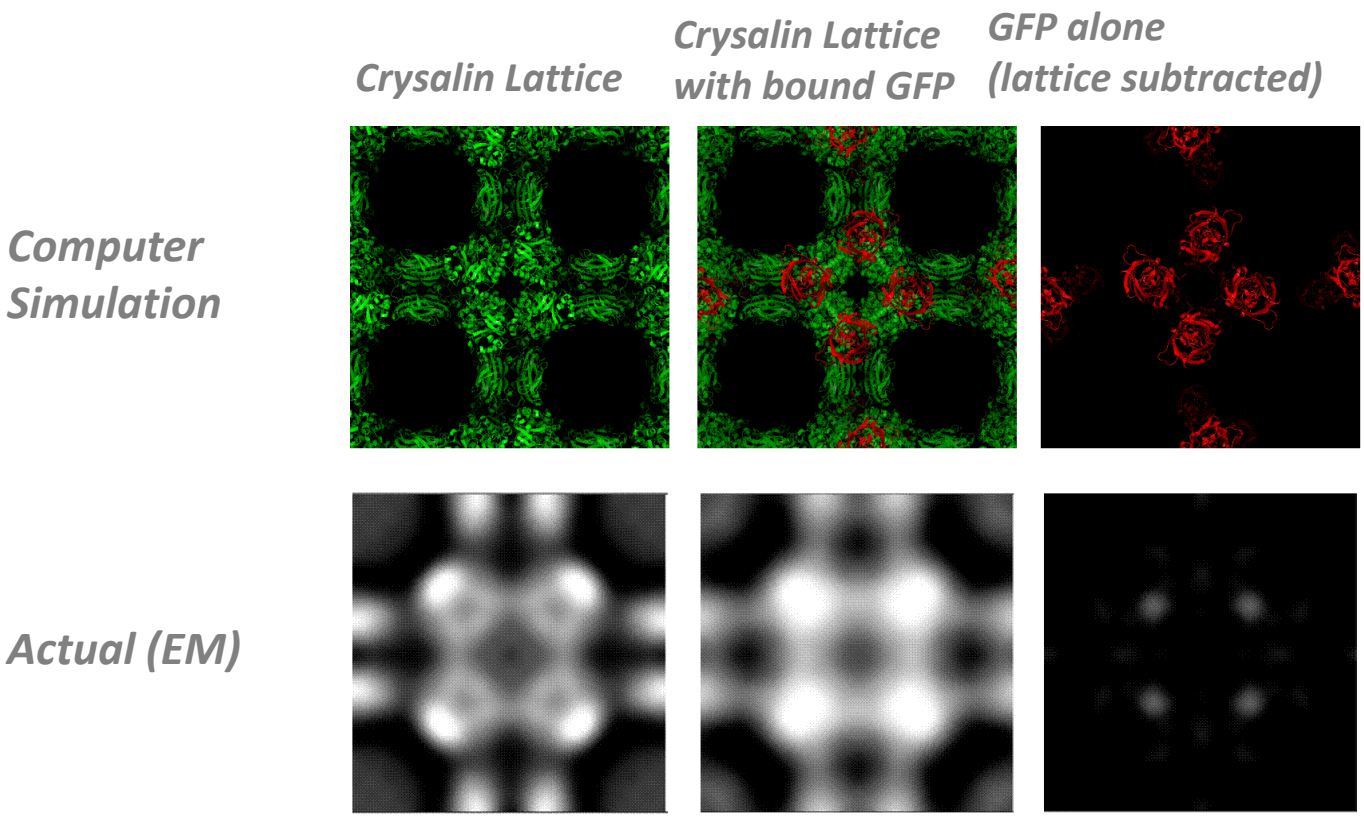
…placed in situ with target protein…

Technical concept 2D



…imposes order
enabling structural elucidation

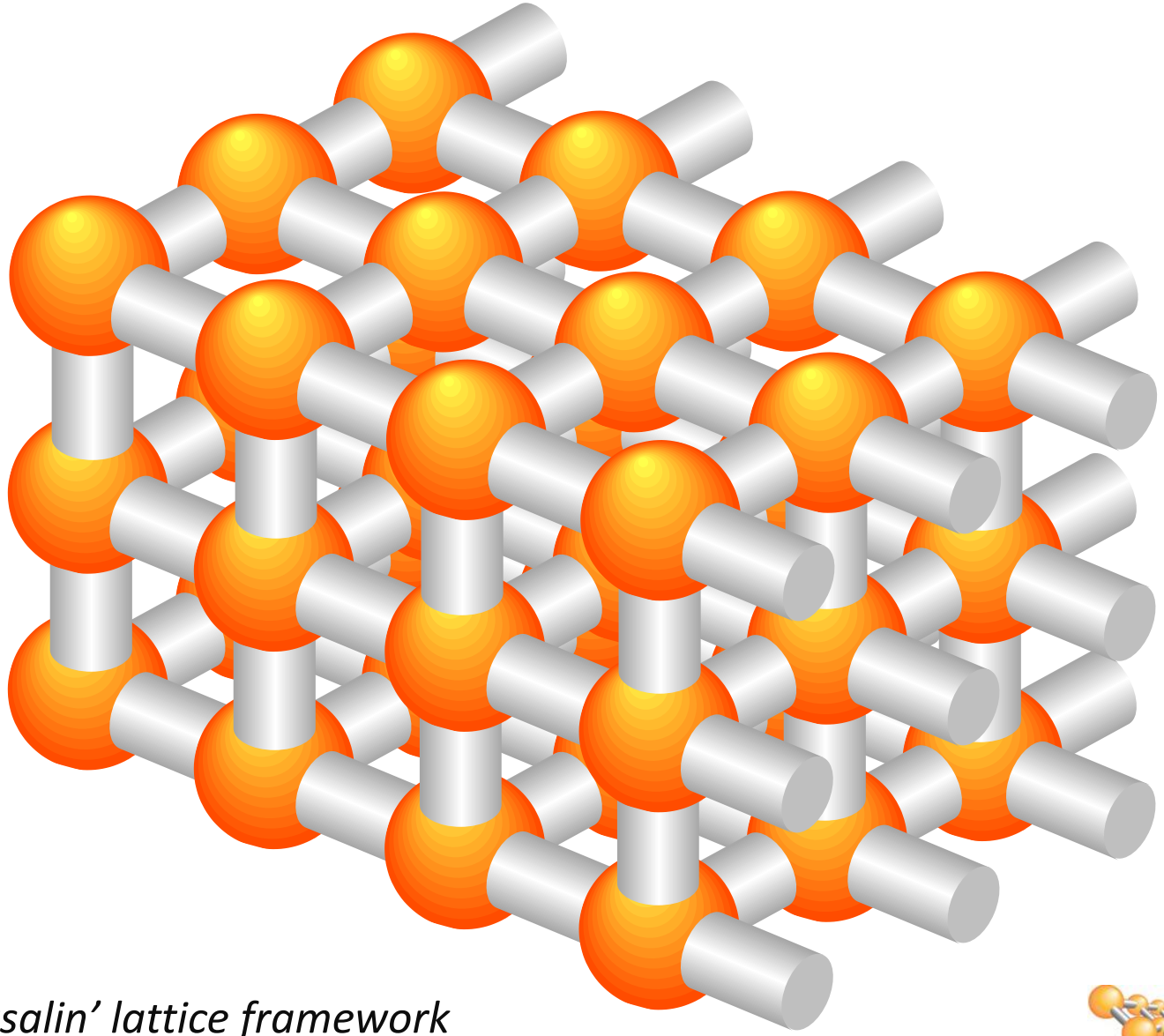
2D Crysalin lattice



Crysalin lattice manufacture sufficient to generate medium resolution structures (circa 12Å)*

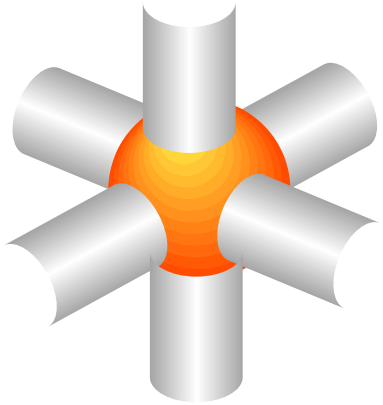
* *Generation of protein lattices by fusing proteins with matching rotational symmetry*, John C. Sinclair, Karen M. Davies, Catherine Vénien-Bryan & Martin E. M. Noble, Nature Nanotechnology 6, 558–562 (2011)

Technical concept 3D

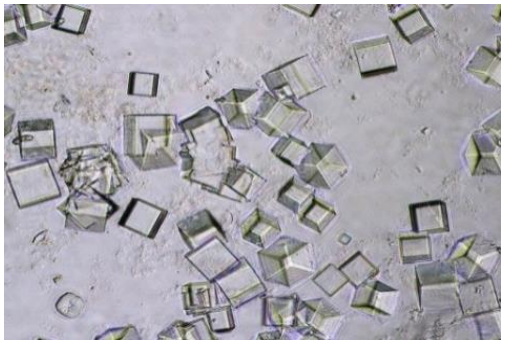


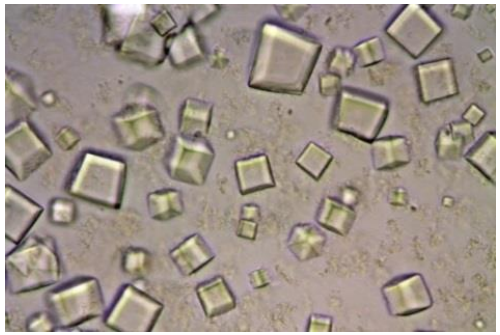
The 3D 'Crysalin' lattice framework

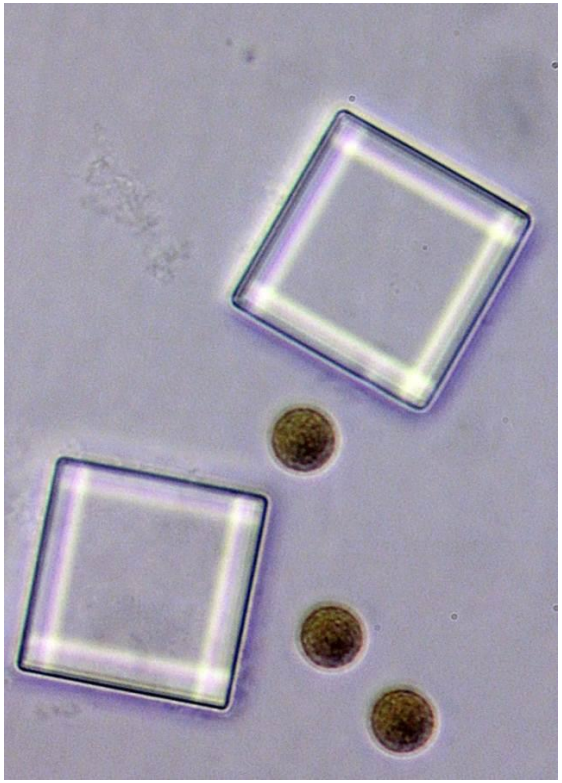
Crysalin formation



- Make the lattice components
- Crysalin lattice self assembles under the correct conditions
- Crysalins form as crystalline cubes varying in size from 20µm to 300µm
- Typical diffraction ranges of base lattice from 3.5-2.7Å (best diffraction to 2.5Å)







Working in MX for industry

- Rapid turnover of data
- Different approach to data collection
- Quick analysis of data to report to client/manager/board
- Steer the scientific direction
- Can be working on a well defined system, but also developing that system
- Expectation of success
- If a project isn't working, will be stopped
- Interactions across teams
- Rapid problem solving
- Working on multiple projects
- Development of transferable skills, eg.
  - Management
  - Business development

"I don't want to be a robot"

Definitely not a robot!