



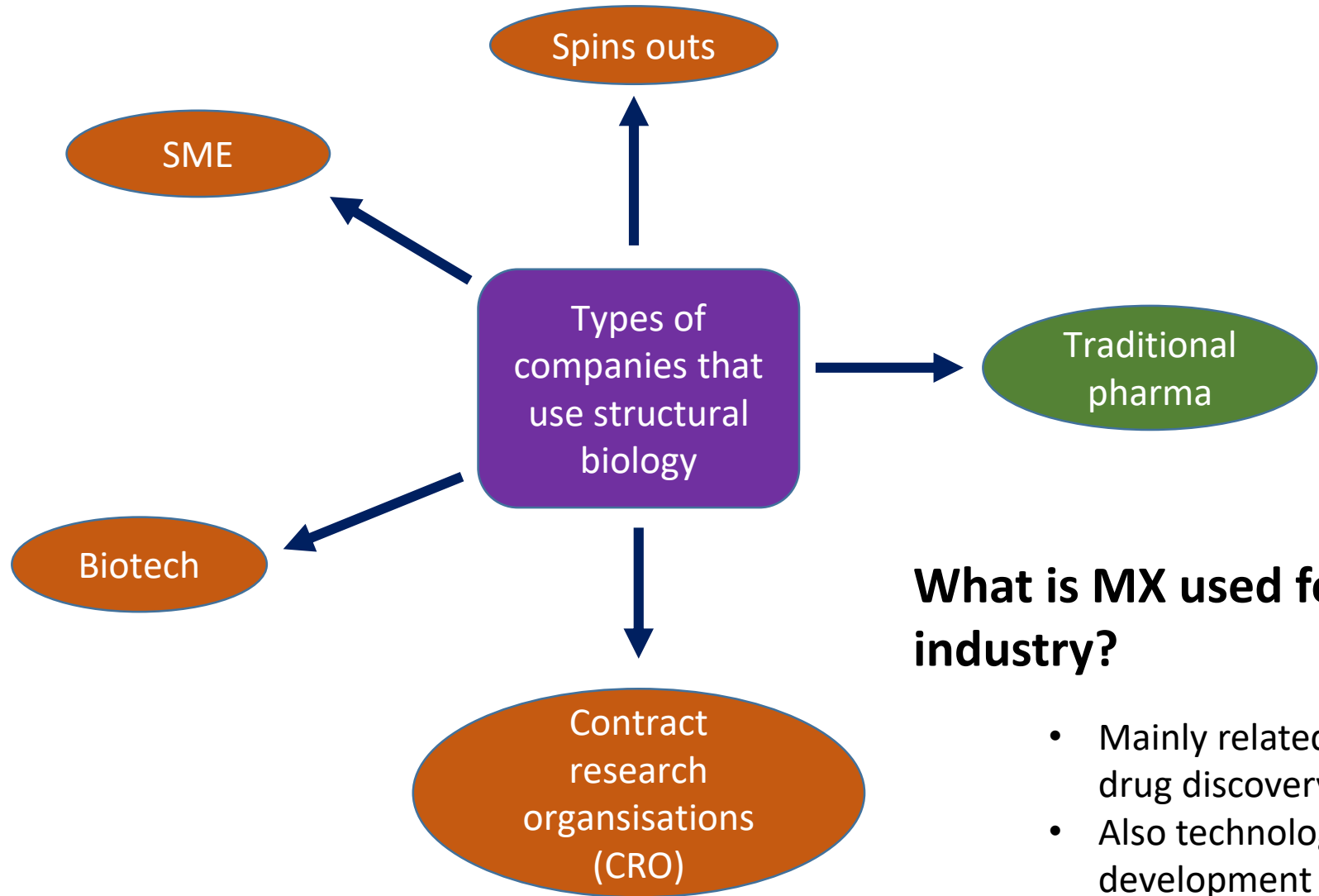
MX from an Industrial Perspective

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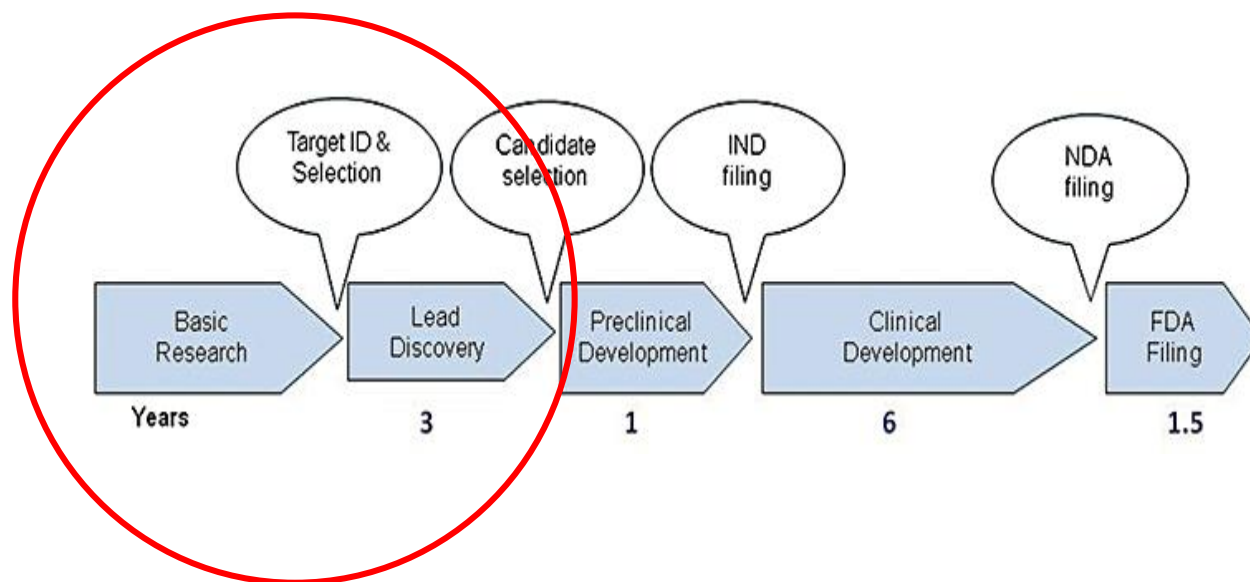


Types of industry and advantages of using MX

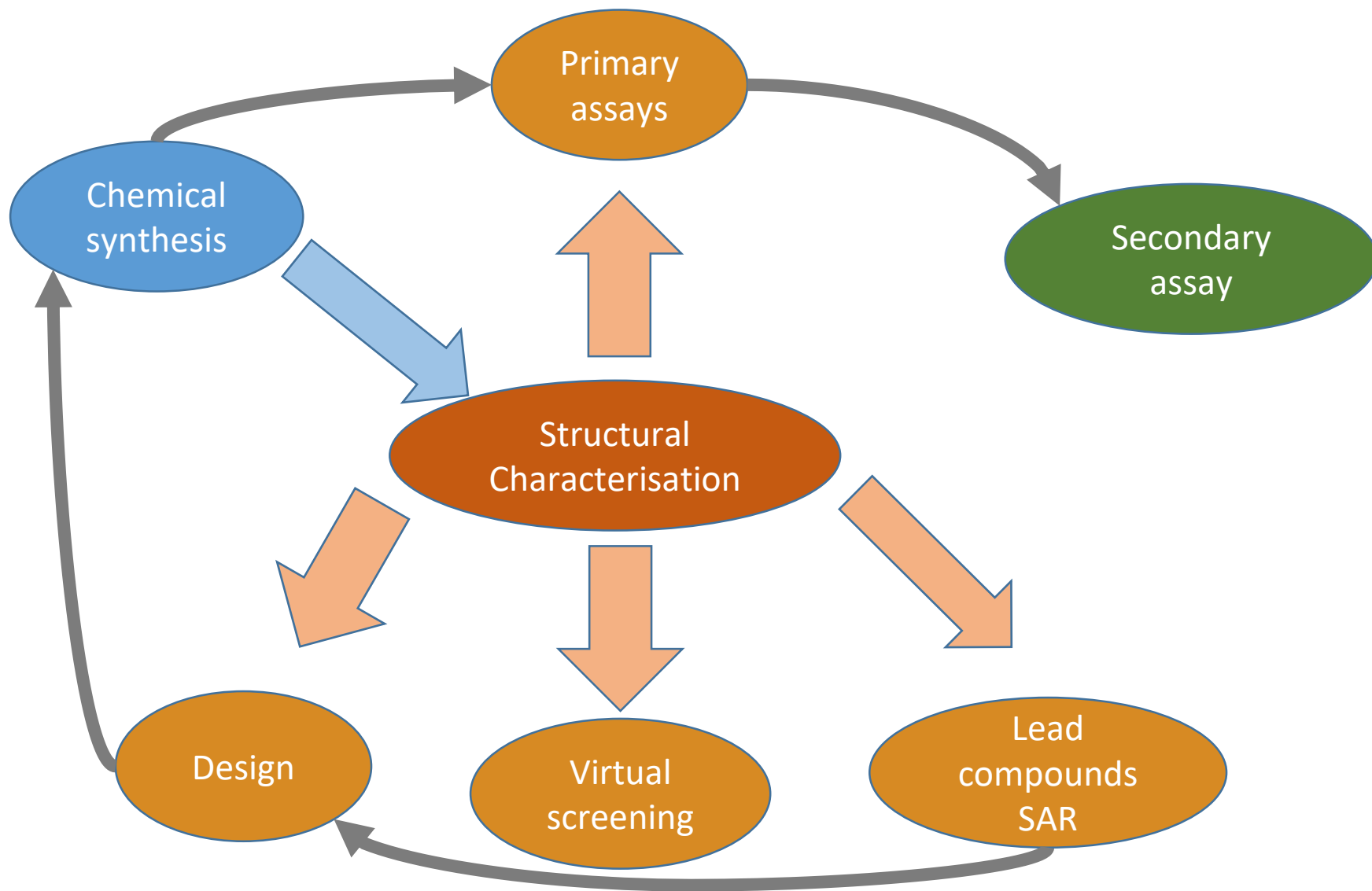


Why use structural biology

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

- Crystal structures help design molecules

- Bound ligand in structure used 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

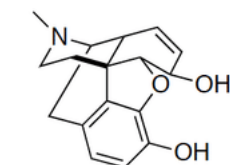
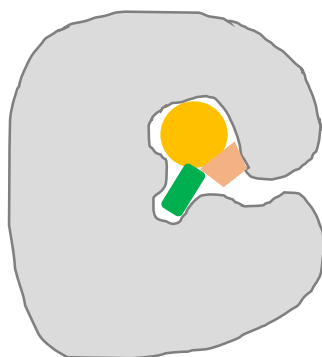
- Soak small compounds into crystals with often low mM activity

- Identify new binding sites
- Identify novel chemistry for known sites
- Join selected fragments together to fit into the chemical space to increase potency

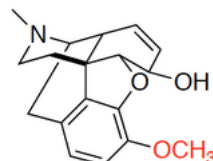
SAR – Structure activity relationships



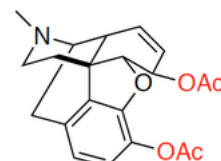
- Link between the drug target and the lead compound.
- Structures of the target with and without the lead compound act as a guide that can allow computational chemists and molecular modellers to identify binding site interactions



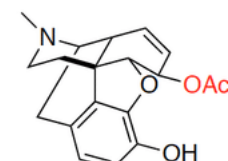
morphine
relative potency = 1
addictive



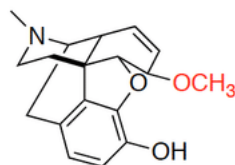
codeine
relative potency = 0.2
addictive



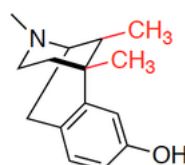
heroin
relative potency = 2
addictive



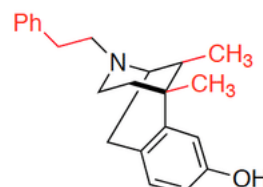
acetylmorphine
relative potency = 4
addictive



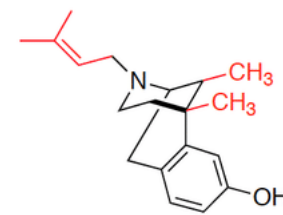
heterocodeine
relative potency = 5
addictive



metazocine
relative potency = 1
addictive



phenazocene
relative potency = 4
non-addictive



phenazocene
relative potency = 0.33
non-addictive

Crysalin Ltd

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

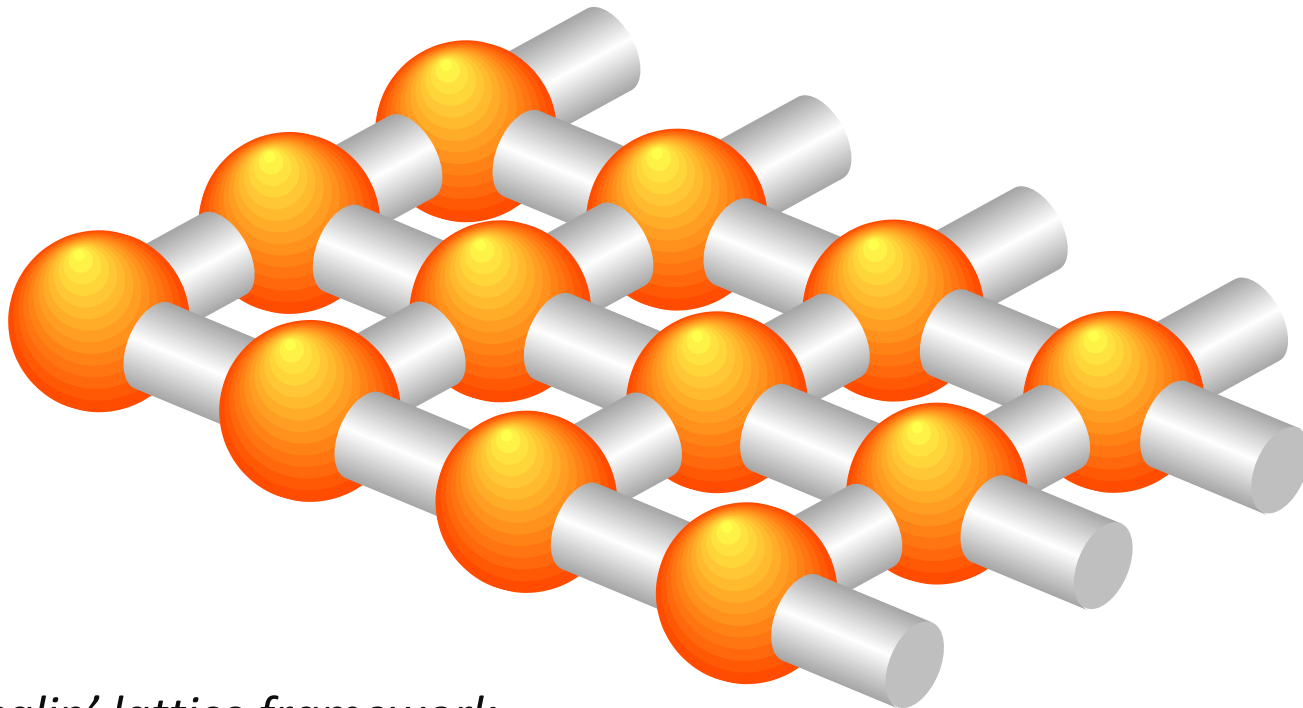
How to achieve this?

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

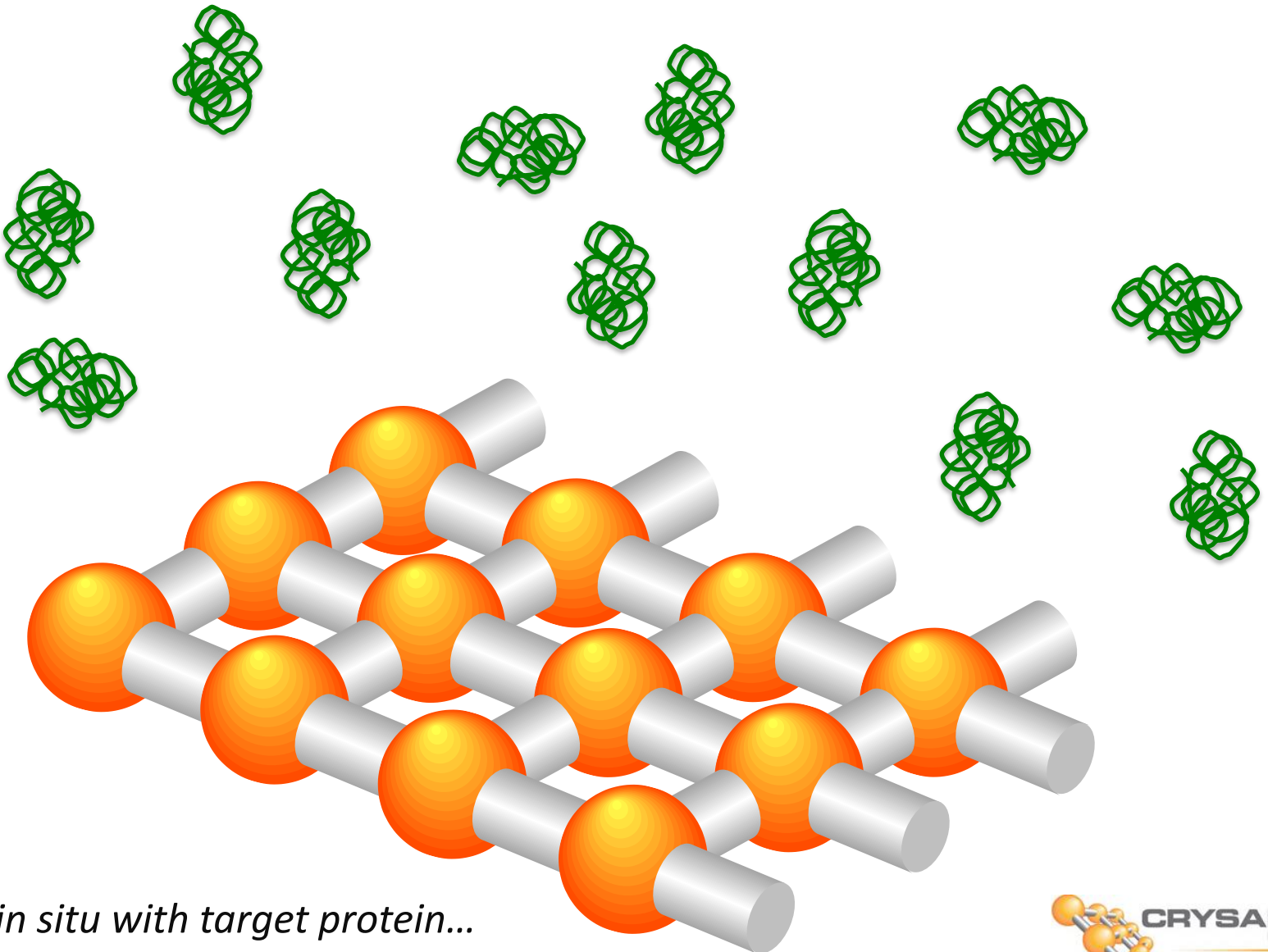
An industrially robust and consistent process with predictable results

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



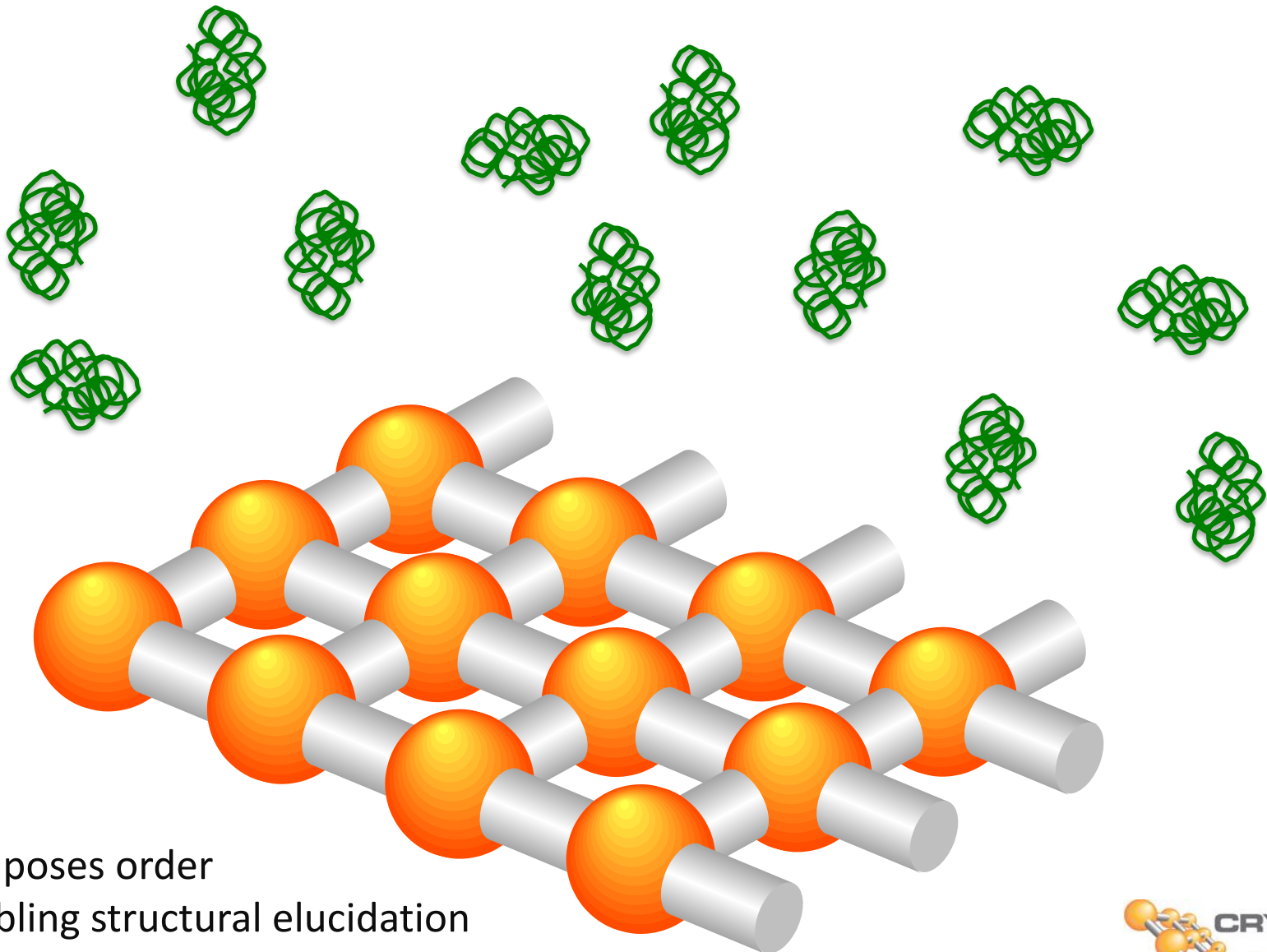
The 'Crysalin' lattice framework...

Technical concept 2D

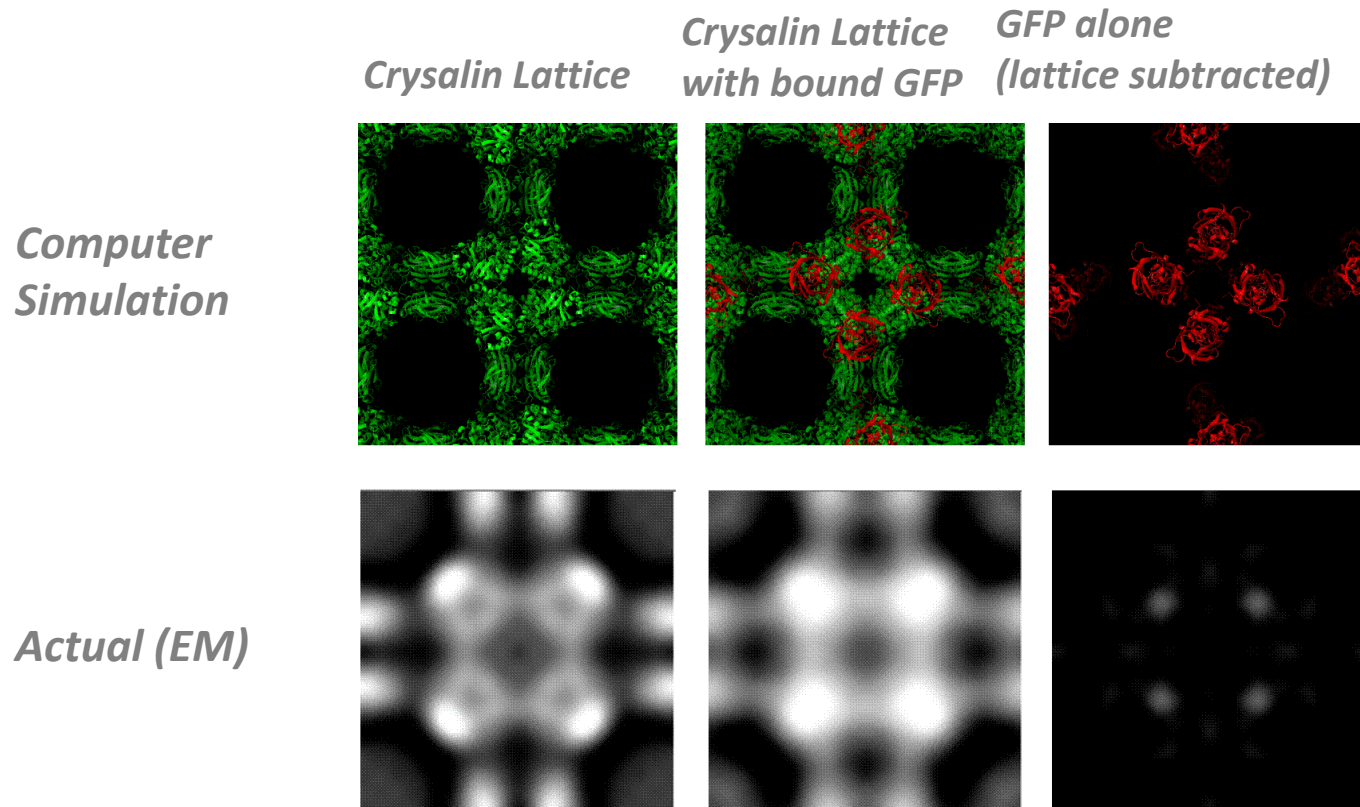


...placed in situ with target protein...

Technical concept 2D

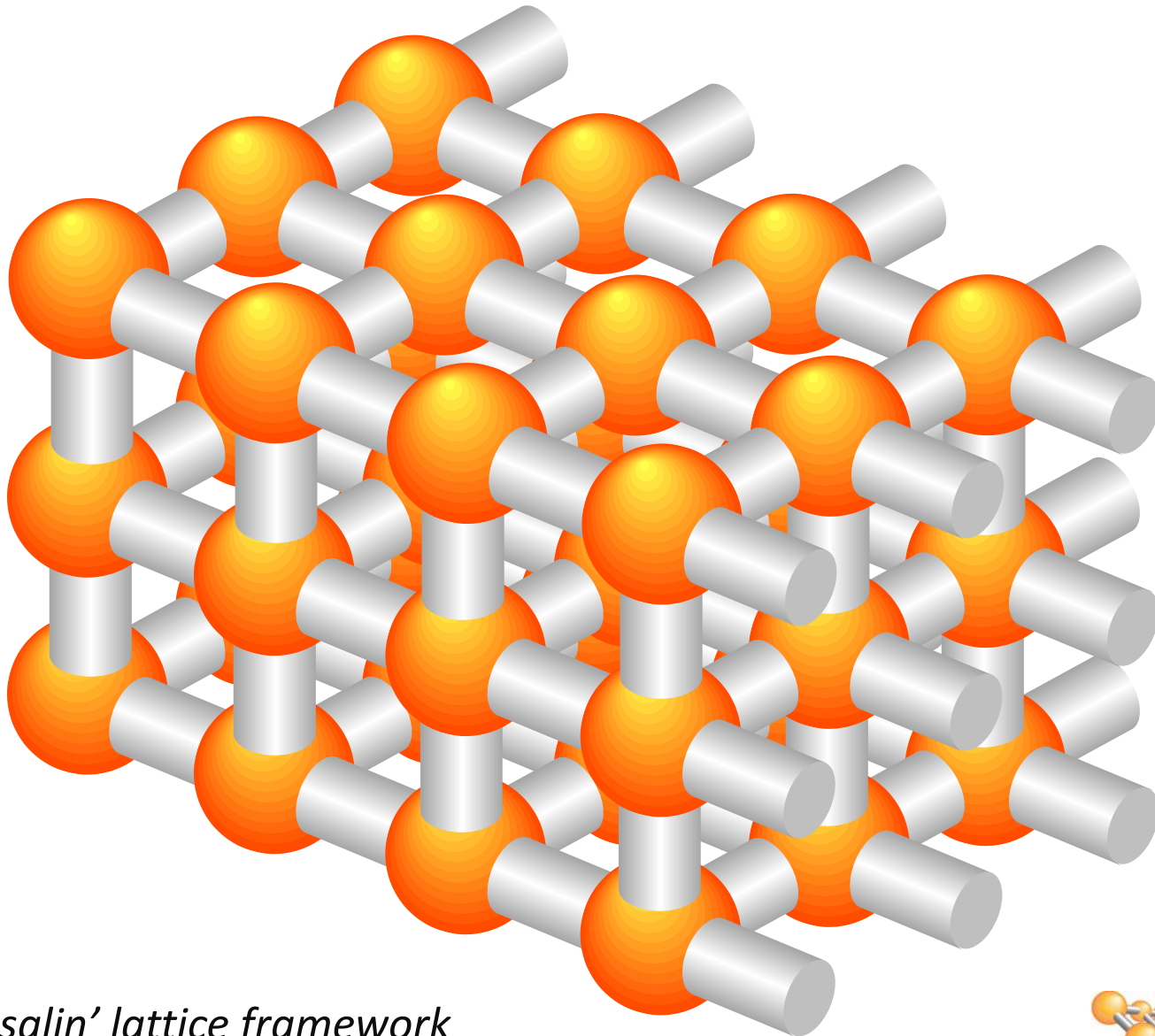


2D Crysalin lattice



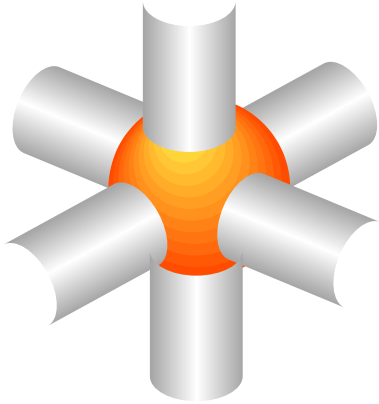
Crysalin lattice manufacture sufficient to generate medium resolution structures (circa 12Å)*

* *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)

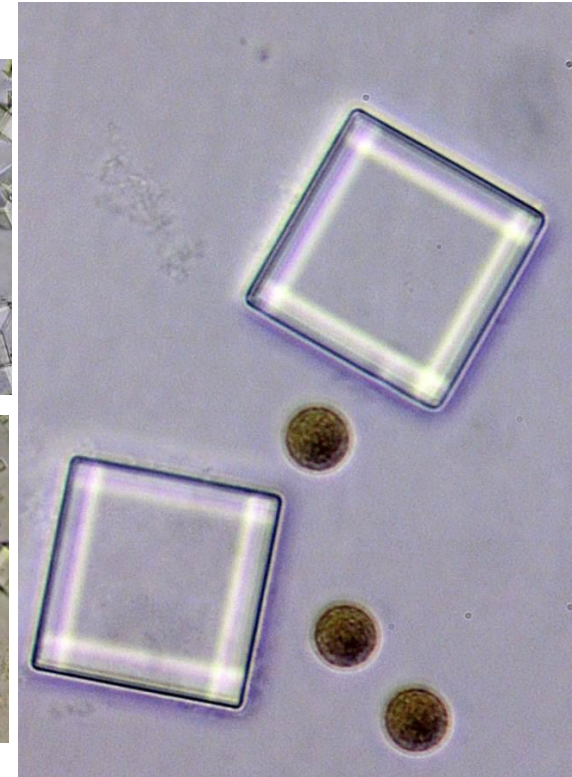
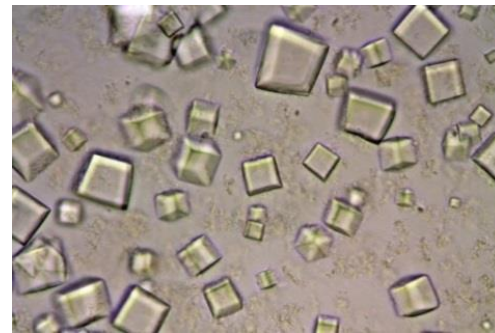
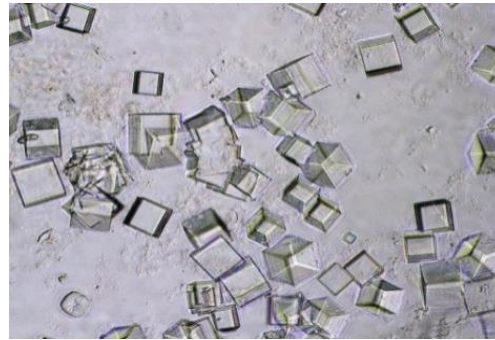


The 3D 'Crysalin' lattice framework

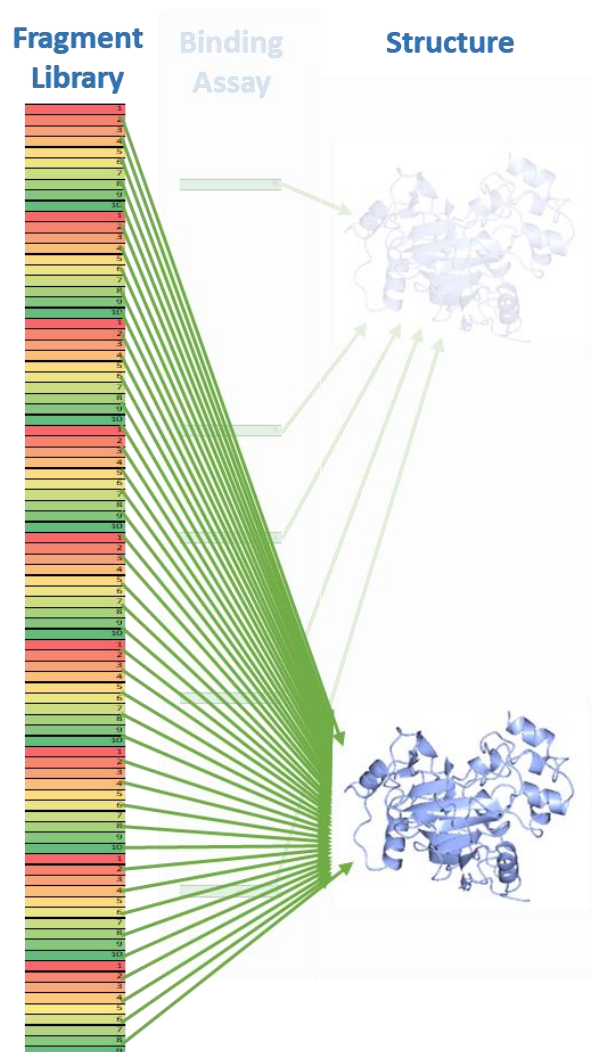
Crysalin formation



- Make the lattice components
- Crysalin lattice self assembles under the correct conditions
- Crysalins form as crystalline cubes varying in size from $20\mu\text{m}$ to $300\mu\text{m}$
- Typical diffraction ranges of base lattice from $3.5\text{-}2.7\text{\AA}$ (best diffraction to $2.5\text{\AA}$)



XChem Platform



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

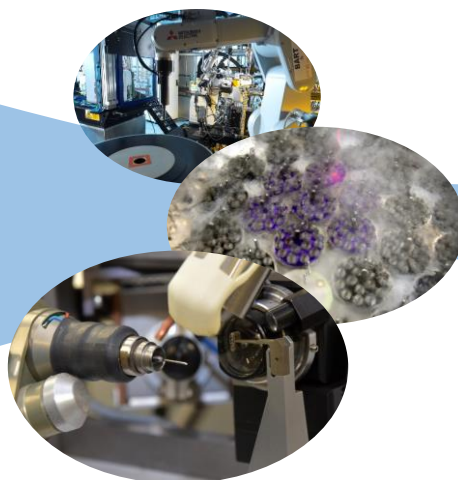
- >100 campaigns completed
 - 2-20% hit rate (project dependent)

Overview of process

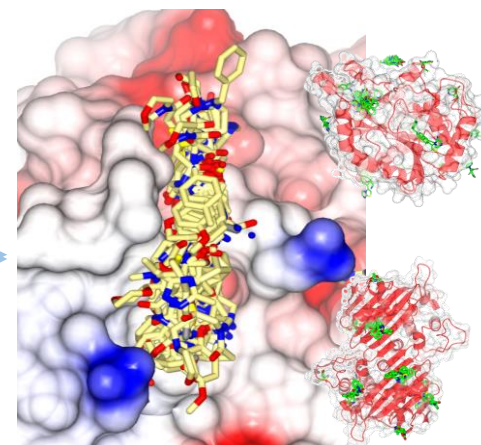
XChem Lab



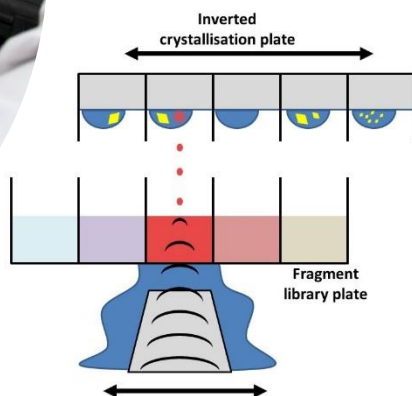
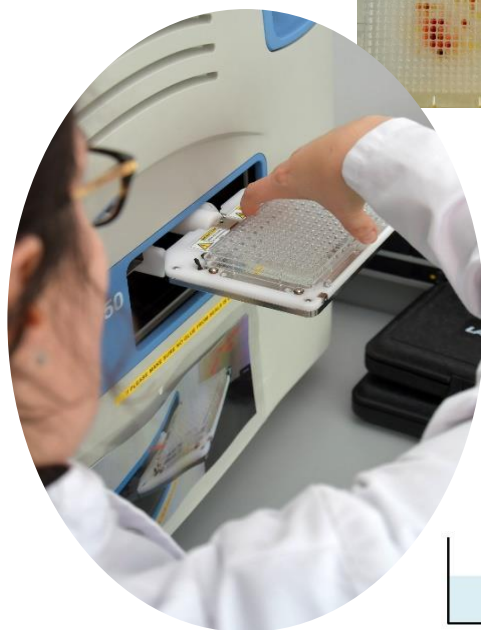
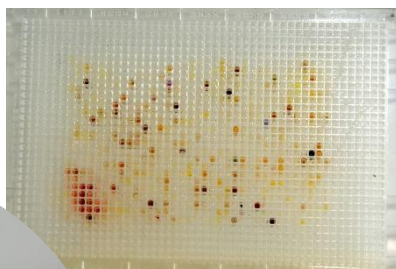
Beamline I04-1



Example readout



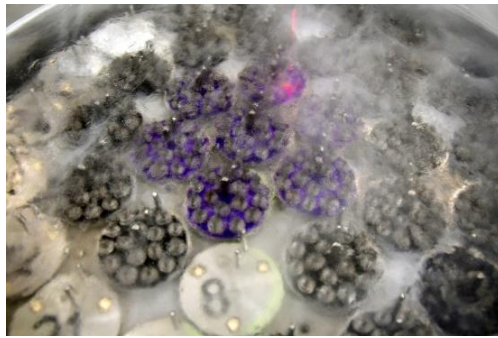
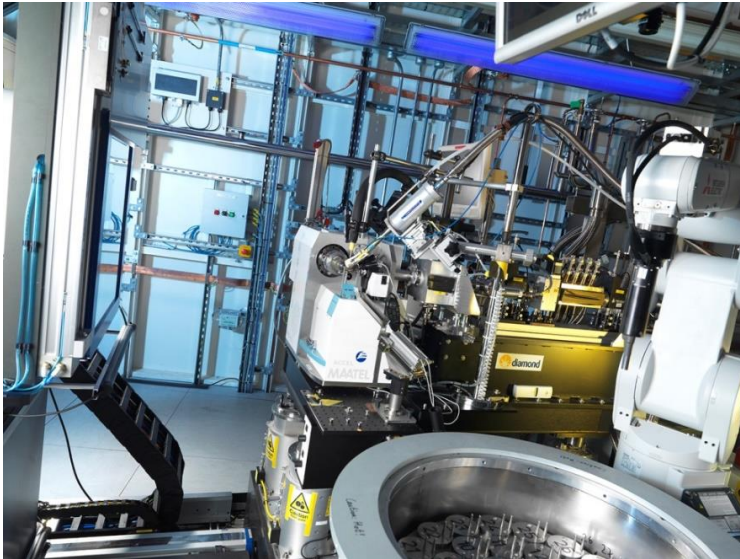
DSiP library in 1536 plate



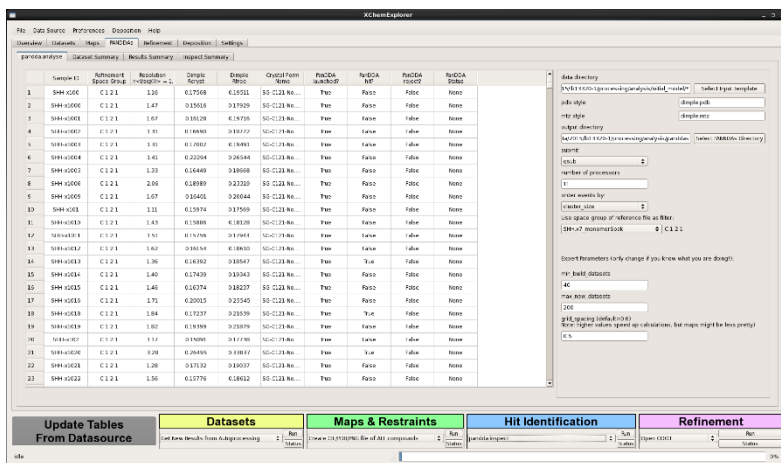
- Libraries
 - DSiP
 - ~700 compounds
 - 500mM in DMSO
 - External libraries
 - Echo compatible plate format
 - Cherry pick compounds
- Echo 550 (Labcyte)
 - ~10min to transfer our DSiP library



- Crystal SHIFTER
 - Motorised X&Y stage under a microscope
 - Allows for rapid crystal harvesting
 - Touch screen graphical interface
 - Record events (time stamps)
 - Crystal mounted
 - Crystal melted
 - Compound status
- Linked to SoakDB
 - XChem data management system

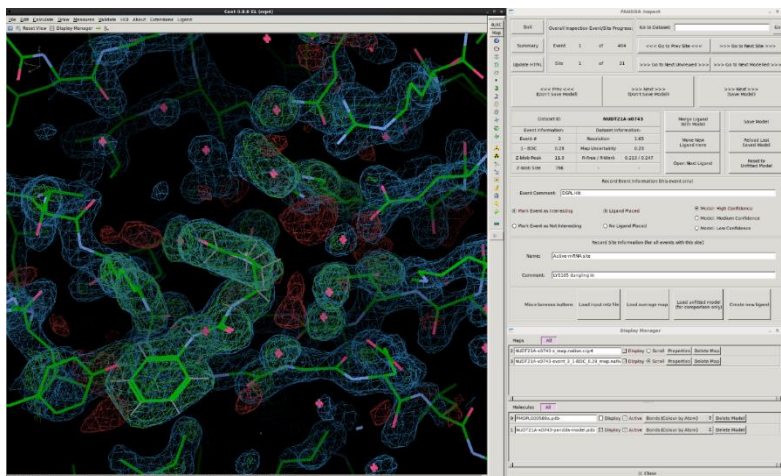


- I04-1 MX beamline
 - Automated loop centring
 - Optical or X-ray
 - Bart robot
 - Dewar capacity ~600 samples
 - Unattended data collection
 - Process up to 32 samples per hour
 - Large pixel area detector
 - 15s or 60s data collection
 - Auto-processing pipelines
 - Xia2, Dials and AutoProc



Sample ID	Substrate	Resolution (Å)	Crystals	Crystals (µm)	Crystals (mm)	PanDDA	PanDDA	PanDDA	PanDDA
						Hit	Hit	Hit	Hit
1	SHH-0001	1.15	1.15	1.15	1.15	True	False	False	False
2	SHH-0002	1.15	1.15	1.15	1.15	True	False	False	False
3	SHH-0003	1.15	1.15	1.15	1.15	True	False	False	False
4	SHH-0004	1.15	1.15	1.15	1.15	True	False	False	False
5	SHH-0005	1.15	1.15	1.15	1.15	True	False	False	False
6	SHH-0006	1.15	1.15	1.15	1.15	True	False	False	False
7	SHH-0007	1.15	1.15	1.15	1.15	True	False	False	False
8	SHH-0008	1.15	1.15	1.15	1.15	True	False	False	False
9	SHH-0009	1.15	1.15	1.15	1.15	True	False	False	False
10	SHH-0010	1.15	1.15	1.15	1.15	True	False	False	False
11	SHH-0011	1.15	1.15	1.15	1.15	True	False	False	False
12	SHH-0012	1.15	1.15	1.15	1.15	True	False	False	False
13	SHH-0013	1.15	1.15	1.15	1.15	True	False	False	False
14	SHH-0014	1.15	1.15	1.15	1.15	True	False	False	False
15	SHH-0015	1.15	1.15	1.15	1.15	True	False	False	False
16	SHH-0016	1.15	1.15	1.15	1.15	True	False	False	False
17	SHH-0017	1.15	1.15	1.15	1.15	True	False	False	False
18	SHH-0018	1.15	1.15	1.15	1.15	True	False	False	False
19	SHH-0019	1.15	1.15	1.15	1.15	True	False	False	False
20	SHH-0020	1.15	1.15	1.15	1.15	True	False	False	False
21	SHH-0021	1.15	1.15	1.15	1.15	True	False	False	False
22	SHH-0022	1.15	1.15	1.15	1.15	True	False	False	False

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

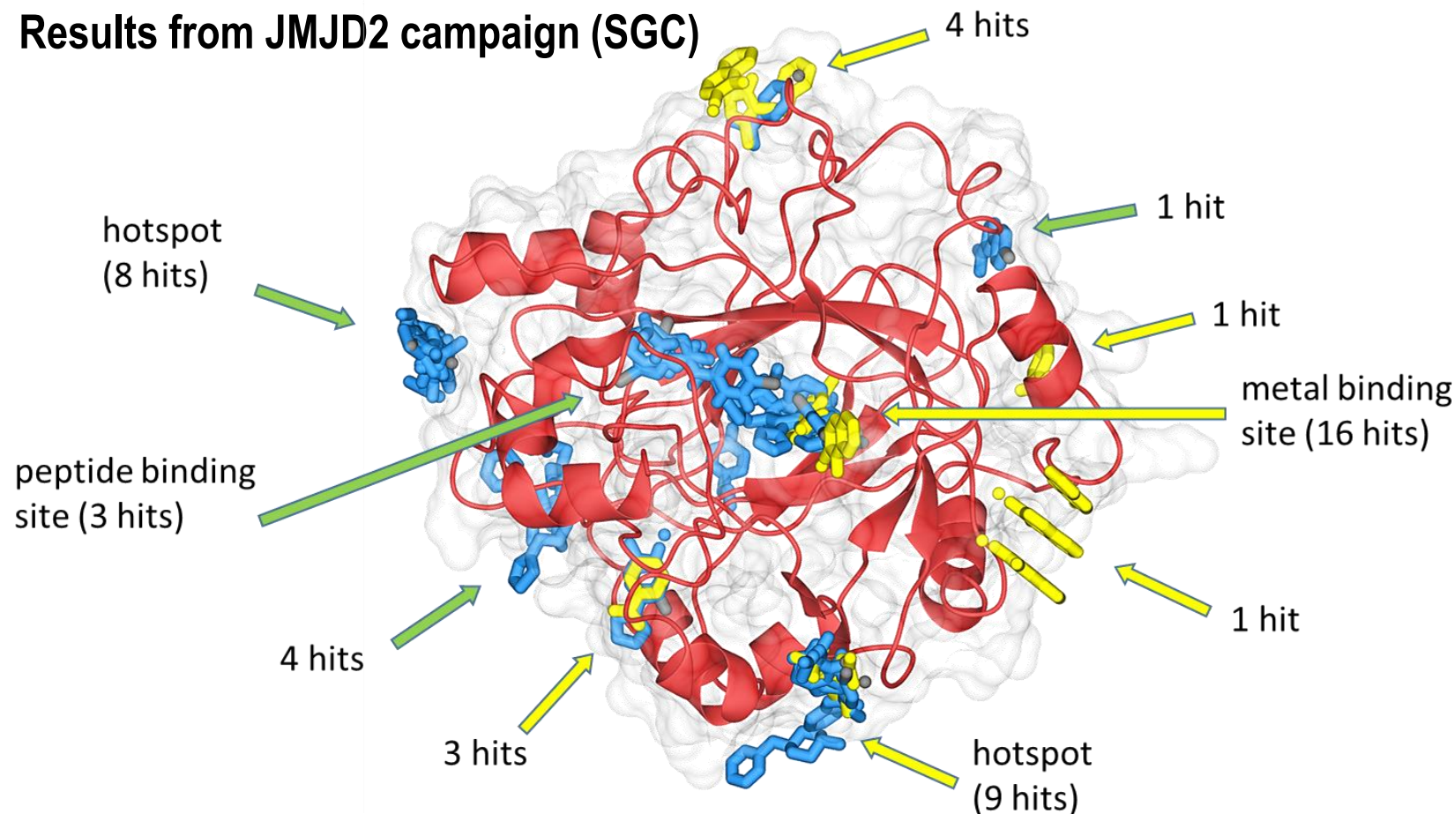
- Pre-PanDDA

- Generate accurate PDB model of "background state"

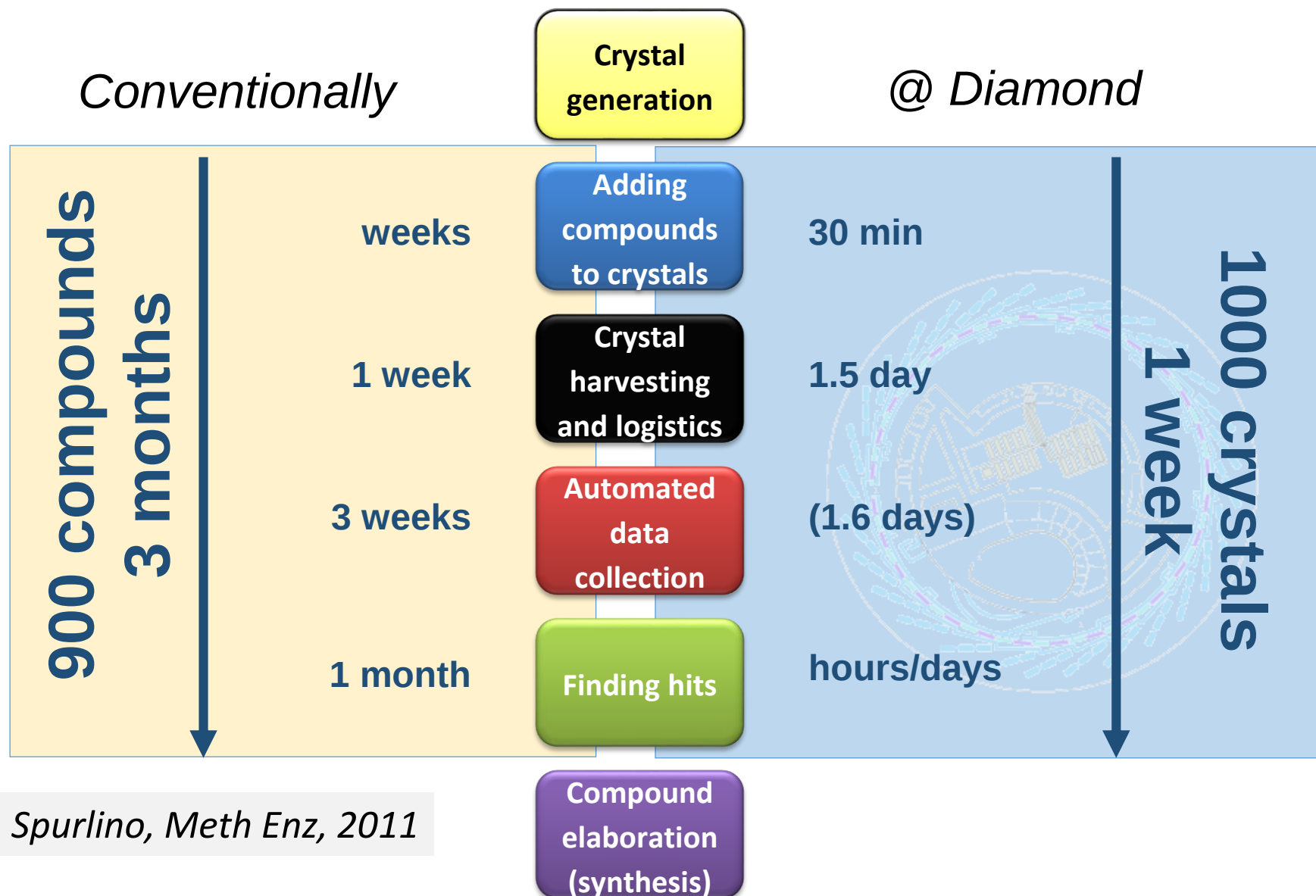
• Coot Plugin

- Facilitate ligand fitting
- Quickly browse through events and record comments

Results from JMJD2 campaign (SGC)



Order of magnitude speedup



Spurlino, Meth Enz, 2011

How is the platform used by industry?



- Trained on the platform and use it with support in place
- Send projects for “full service” access
 - Project run by members of Diamond’s ILO team
 - This can also include data analysis
- Run 4 campaigns per year for a number of companies
- Frequently have follow up compounds sent for testing
- Operate with a 6 month waiting list
- Increasing enquires from virtual and AI companies

- 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