Obtaining protein-fragment structures in a Fragment Based Drug Discovery campaign

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Fragment Based Drug Discovery

Fragment-based approaches are becoming increasingly significant in the lead discovery process in the pharmaceutical industry (1). The availability of structural information of how small fragments are bound is crucial to success. However, that put a very high demand on the crystallisation process since many structures of weak binding fragments is required in a short time span.

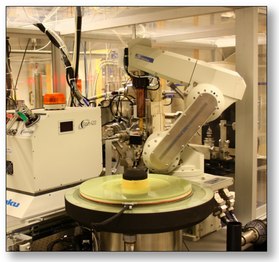
The crystals we aim for:

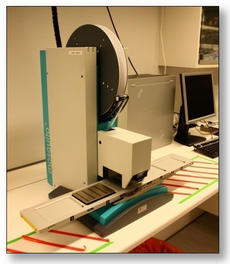
- Easy to produce
- Robust
- Ligand-free
- Tolerable of high soaking concentrations
- Diffract in-house below 2.5 Å

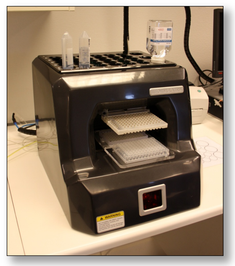
How to achieve this:

- Well-equipped high-throughput laboratory
- Well-organised lab and data system for working with many crystals, compounds, datasets and structures
- Frontloading of crystallisation early in campaigns with clear stop/go decision points
- Close collaboration in campaign team with protein chemist, biophysicist and chemist to make sure we have the most suitable protein and compounds available

Some of the high-throughput equipment in our laboratory



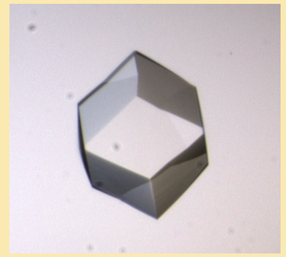




How to proceed when weak fragments fail to deliver structures:

- Careful analysis of the ligand-free structure to rule out the presence of dmso, cryo, precipitant molecules etc in the active site that potentially could compete with fragment binding
- Comparison of crystallisation/soaking condition to assay condition
- Verify compound binding in the crystallisation/soaking condition
- Investigate different compound concentrations, temperatures and times in the soaking setup
- Compare the result of co-crystallisation and soaking
- Explore different crystal forms
- Test the effect of different co-solvents or a powder soak
- Cross-link with glutaraldehyde (2) to allow soaking in very high compound concentrations
- Include compound in the cryo-protective solution
- Investigate stability, purity and identity of compound

Example 1: Serine protease



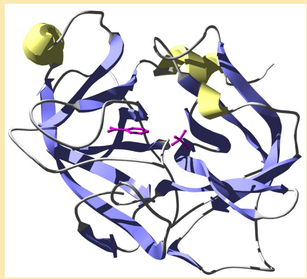
Problems:

1. Crystals dissolve in dmso
2. Not possible to obtain ligand-free crystals: benzamidine (or other active site binder) necessary for crystal formation

Solution:

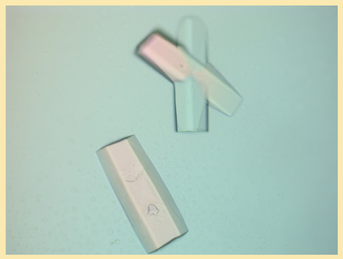
Three different methods in place for obtaining structures of weak binding fragments:

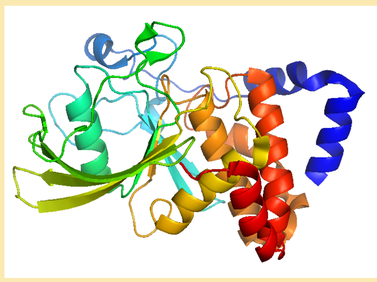
1. Cross-linking with glutaraldehyde (2) to allow soaking in high concentration of compound dissolved in dmso.
   Drawback: loss of diffraction, necessary to collect data at synchrotron
2. Solubilise compounds with cyclopentanol and soak in high concentration.
   Drawback: cyclopentanol is not as efficient as dmso to solubilise compounds
3. Co-crystallisation
   Drawback: time- and protein consuming



The combination of these three methods allowed many structures of weak binding fragments in complex with the protease to be determined. This facilitated structure-guided design resulting in potent lead compounds.

Example 2: Phosphatase





Problem:

Could not obtain structure of highly soluble fragment (K_d=7mM) despite soaking in very high compound concentration (200mM).

Solution:

Compound binding in the soaking solution was measured with NMR but could not be detected. Further analysis revealed that $MgCl_2$ (necessary for crystal formation) prevented compound binding. Soaking crystals without $MgCl_2$ gave the structure of the phosphatase in complex with the weak fragment.

References:

1. Murray C.W., Rees DC (2009) The rise of fragment-based drug discovery *Nat Chem* **3**,187-92
2. Lusty CJ (1999) A gentle vapor-diffusion technique for cross-linking of protein crystals for cryocrystallograpy *J. Appl. Cryst* **32** 106-12

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