Protein-ligand X-ray Crystallography in support of the Development of a selective small molecule inhibitor

Katie Day, Philip Leonard, Hayley Jackson
Domainex Ltd, Chesterford Research Park, Little Chesterford, Saffron Walden, Essex, CB10 1XL, UK

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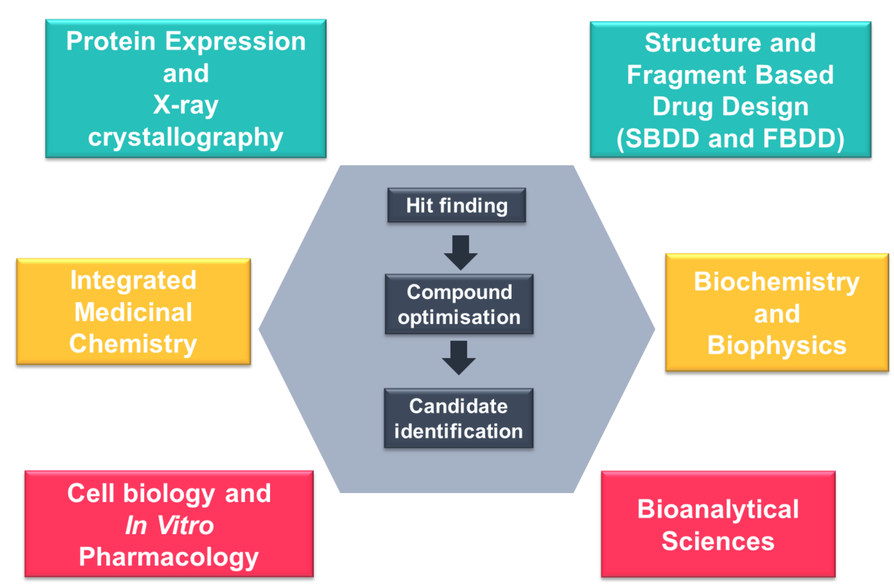
Structural Biology
Revealing New Dimensions

Introduction

Domainex offers a range of drug discovery services including **protein production, structural biology, assay development**, **medicinal chemistry, computational chemistry, analytical chemistry and bioanalysis.** Projects can be standalone or fully-integrated across the company.

The purpose of this project was the development of a selective small molecule inhibitor. We successfully developed a **co-crystallization** process & **soaking** system for both the target protein and the off-target. This led to the determination of several high-resolution crystal structures of many potential drug compounds bound to both proteins. **Lead optimization** was carried out by the **medicinal chemistry** team, using **structure-aided drug design**.

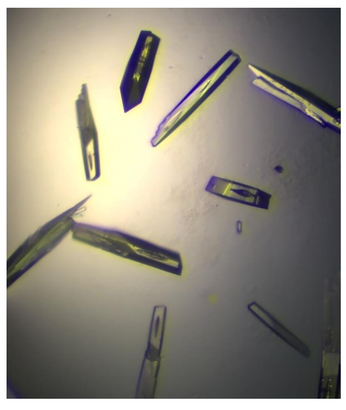
Domainex routinely generates **high-resolution crystal structures.** All crystallisation trials and data analysis are performed in-house, and X-ray diffraction data is collected at the Diamond Light Source facility in Oxford.



Co-crystallisation (Target protein)

Co-crystallisation of the target protein with compounds of interest was very successful. **More than 50 co-crystal structures** were generated, a large proportion have resolution better than **2 Å**.

Optimisation of the crystallisation precipitant solution was required. For example, co-crystals grown in HEPES buffer conditions led to better data than those grown in MES precipitant solutions. MES prevented protein cofactor binding and resulted in co-crystal structures with lower compound occupancy. For compounds which did not initially co-crystallise in HEPES conditions, cross-seeding using MES-grown co-crystals was used to grow co-crystals in HEPES buffer conditions.

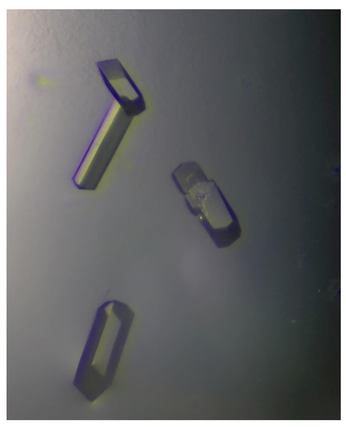


Soaking system (Off-target)

Apo off-target protein crystals were obtained through crystallisation in the presence of a compound, which was found to aid crystallisation in a form suitable for soaking but does not show up in the crystal structure.

The soaking solution was also developed and **optimised** – starting from the crystallisation precipitant solution. Ammonium sulphate concentration was reduced to avoid phase separation & PEG concentration was increased. **CryoSol** (Molecular Dimensions) was also used to identify a solubilisation solution which aided compound solubility in the precipitant solution.

More than 30 crystal structures containing compounds of interest have been solved using soaking, most of these have resolution better than **2 Å**.



Protein production
Design and express protein construct(s) suitable for crystallography
Protein purification and QC
Binding confirmation of identified hits by most suitable assay

Crystallisation
Enter protein into crystallisation trials as apo and/or in the presence of identified hits followed by optimisation to obtain suitable crystals for X-ray analysis

Co-crystal structures
If soaking is suitable crystals will be soaked with compounds otherwise co-crystals will be optimised and used for structure determination

Data collection and structural solution
Co-crystals will be analysed and if resolution is better than 2.8 Å resolution, a data set will be collected for subsequent structure solution

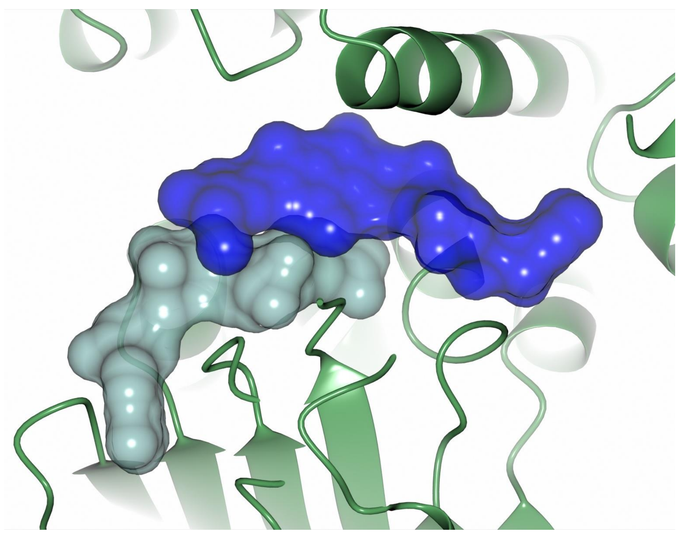
Structure-aided drug design

The **structural biology** team at Domainex worked closely with the **medicinal chemistry** and **assay biology** teams on this integrated drug discovery project.

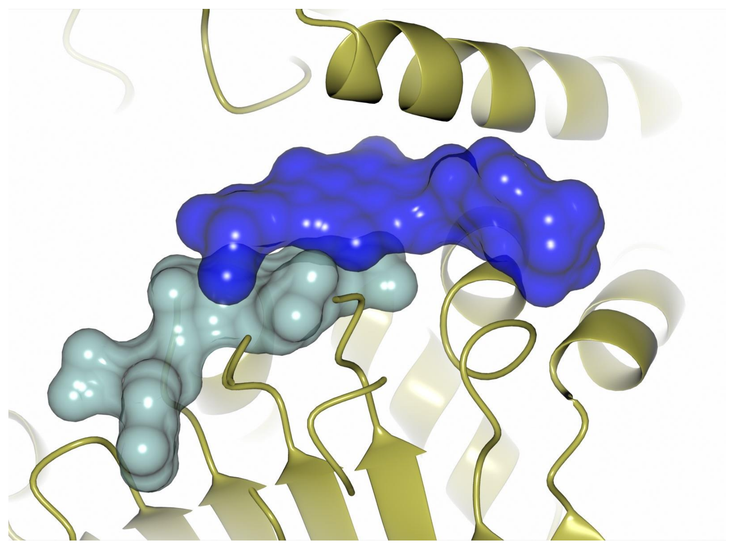
The X-ray crystal structures have been invaluable for determining compound binding modes in the target & off-target proteins and have allowed many iterations of **targeted improvements** to the compounds to aid **selectivity**, **binding** and other properties.

Project Outcomes
Vast improvements have been made in the lead compound candidates since the beginning of Domainex's involvement with the project. The **selective lead candidate** is more than **80-fold selective** for the protein target, whilst also **retaining potency** & having **significantly improved solubility** and other **ADME properties.** In addition, an unselective lead candidate has also been identified with similarly desirable properties.

Target protein + Compound A



Off-target protein + Compound A



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enquiries@domainex.co.uk