Unlocking the potential of your drug discovery programme

Innovative screening
The leading fragment screening platform with MicroScale Thermophoresis at its core

Domainex expertise
High quality results from our world-class discovery team

Exclusive library
Expertly selected fragments for the best start to your drug discovery programme

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Introduction

All drug candidates have to start somewhere and at Domainex we believe that they start small. Our fragment-based drug discovery services are designed to provide high quality lead compounds at a speed and quality not matched by our competitors.

Our approach is simple:

• Exclusive fragment library – our expertly selected fragments will give you the best chemical starting point for your drug discovery programme
• Innovative screening – we’ll identify your hit compounds quickly and effectively, using leading assay techniques
• Attention to detail – high quality results with expert interpretation from our world-class discovery team

We are confident that our customised approach will provide you with high quality hit compounds. But that is just the beginning. From identifying soluble protein domains using our proprietary Combinatorial Domain Hunting platform, to transforming hit molecules into clinical candidates using the skills and experience of our protein scientists, assay biologists, medicinal and computational chemists, we’ll be with you every step of the way (and everywhere in between).

Why fragment screening?

The search for hit compounds often starts by screening large numbers (~10^5-10^6) of compounds against a biological target using High Throughput Screening (HTS) technology. But if you have a well characterised target protein, fragment screening offers a practical and efficient alternative. It will save you time and money, providing you with the best start to your drug discovery programme faster.

• Smaller than standard screening compounds, fragments are more likely to bind to the target, which leads to screening programmes with significantly higher hit rates (up to 5-7%) than HTS
• Fragment libraries offer more comprehensive coverage of the chemical diversity space, increasing the chances of finding your next hit molecule
• With the application of expert medicinal chemistry, fragments offer greater scope for enhancing potency within the desired physicochemical property range of drug-like candidates

Fragments are small, low MW molecules which generally adhere to the ‘rule of three’ criteria:

• molecular weight < 300
• clogP ≤ 3
• number of hydrogen bond donors ≤ 3
• number of hydrogen bond acceptors ≤ 3

Fragments bind to a target protein with a high degree of efficiency, to create an ‘anchor’ point to the target protein. This can be developed through application of expert medicinal chemistry (‘fragment growth’ or ‘fragment expansion’) to lead to selective and potent drug candidates.
The Domainex fragment library

Our fragment library contains approximately 1,000 fragments and has been carefully designed to maximise the chances of finding suitable chemical starting points for your drug discovery programme.

The library gives good coverage of ‘ideal fragment space’ by optimising a number of factors, including:

- number of pharmacophores represented
- diversity of chemical structures
- molecular properties - heavy atom count, ring count, polar surface area, number of hydrogen bond donors and acceptors, number of rotatable bonds
- physical properties such as aqueous solubility and lipophilicity

Additional filters were applied to remove:

- compounds containing atoms other than H, C, N, O, S, F, Cl, Br
- reactive functional groups

Our unique set of fragments covers a diverse range of pharmacophores, within a range of molecular and physicochemical properties, making it the ideal starting point for your next drug candidate.
MicroScale Thermophoresis (MST) is the leading technology used by our team to identify your hit compounds quickly and effectively. The high-throughput technique quantifies molecular interactions and structural dynamics, to present binding affinity results in just a few minutes. Based on the movement of molecules across temperature gradients, thermophoresis detects changes to molecular size, charge and hydration shell. When a binding event occurs, one or more of these properties is affected, enabling measurements to be recorded with unrivalled levels of sensitivity. The applications range from small-molecule binding events to protein-protein interactions, and interactions of multi-protein complexes.

There are many reasons we think MST offers advantages over alternative molecular interaction assay methods:

- Fast, flexible assay set up and a capability of making 400 measurements per hour
- Measurements are carried out in solution, meaning:
  - free choice of assay buffer systems
  - no surface immobilisation of target proteins
- Ternary and quaternary systems can be measured, allowing any fragment interactions with different binding sites (eg. substrate/co-factor) to be identified
- Live detection of absorption, aggregation and precipitation effects
- Broad concentration range (from pM to mM)
- Small sample consumption (fragment screening using as little as 400μg of protein)

**MST is quick, sensitive and efficient.**

How does it work?

Molecules that are optically visible can be measured using MST, so a fluorescent tag is added to the sample. The concentration of the fluorescent molecule is kept constant and the concentration of a binding partner is increased. An infra-red laser creates a localised temperature gradient, which allows any changes to fluorescence intensity, caused by the movement of the molecules, to be observed. Initially the molecules are distributed evenly and diffuse freely in solution. By switching on the IR-laser, the molecules typically move out of the heated spot. In the steady state, this molecule flow is counterbalanced by ordinary mass diffusion. After turning off the laser, molecules re-establish a homogeneous distribution. The change in thermophoresis from the unbound to the fully bound state, enables binding affinities to be determined.

![The Monolith NT, developed by NanoTemper Technologies (Munich, Germany), can house 4x24 capillary cartridges simultaneously and take up to 96 measurements in 16 minutes.](image)

![MST-on time versus Fnorm](image)
Case study - G9a

G9a is a lysine methyltransferase which has an important role in the progression of solid tumours, promoting cell proliferation and survival under hypoxic conditions, as well as metastasis, thus making it an important cancer target.[1]

Domainex used MST to successfully screen 320 compounds from our fragment library against the lysine methyltransferase G9a with a hit rate of 5.3%. Screening the same fragments using Differential Scanning Fluorimetry (DSF) or the activity-based AlphaScreen yielded only one hit per method (0.3% hit rate), due to the small shift (0.5-1°C) observed in DSF using this ternary system and the high false positive (TrueHits) rate using AlphaScreen. Eleven fragment hits identified by MST were taken into secondary screening to determine their binding affinity (Kd) to the G9a-SAM complex (Figure D). MST was able to identify high and low affinity binders and provide an affinity-based ranking. As a follow-up, the Kd values for nine of the fragment hits were determined in the absence of the co-factor SAM. This revealed different mechanisms of action and highlighted the importance of being able to study a ternary system.

Figure 1 A) MicroScale Thermophoresis single shot fragment screen data overview. Temperature jump plotted vs compound fragment was measured. B) Thermophoresis traces of selected fragments, positive control and DMSO reference. C) Thermophoresis trace of fragment induced protein aggregation – false positive identification. D) Summary table of fragments taken into secondary and tertiary screening

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<tr>
<th>Ligand Name</th>
<th>Kd + SAM [µM]</th>
<th>Kd - SAM [µM]</th>
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About Domainex

Domainex is a fully integrated drug discovery service company based near Cambridge, UK serving pharmaceutical, biotechnology, academic and patient foundations globally. Domainex’s drug discovery service business was established in 2001 and since that time has continued to expand to serve a wider range of clients across the world including UCB, FORMA Therapeutics, St George’s University, The Institute of Cancer Research and Auspherix. Our expertise and commitment to providing high quality services has resulted in a strong success record in drug discovery, delivering an average of one candidate drug every year for the past six years.

How Can Domainex Help Your Drug Discovery Project?

Domainex’s highly experienced molecular biologists, assay biologists, medicinal, computational and analytical chemists can be leveraged through our CRO services. Domainex provides highly efficient and well considered scientific solutions to enable successful drug discovery programmes against a wide range of drug targets. Whether your project is at an early stage of drug discovery or has already identified chemical matter, our processes have been shown to result in a 30% time-saving compared to industry standards and use less resource, allowing prudent management of your own budget.

Contacts

If you would like to know more about Domainex’s discovery services, or speak to us regarding your own drug discovery needs, please contact us at: enquiries@domainex.co.uk

Alternatively we can be contacted directly as follows:

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References

[1] Casciello et al., Front Immunol., 2015, (6), 1-12

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