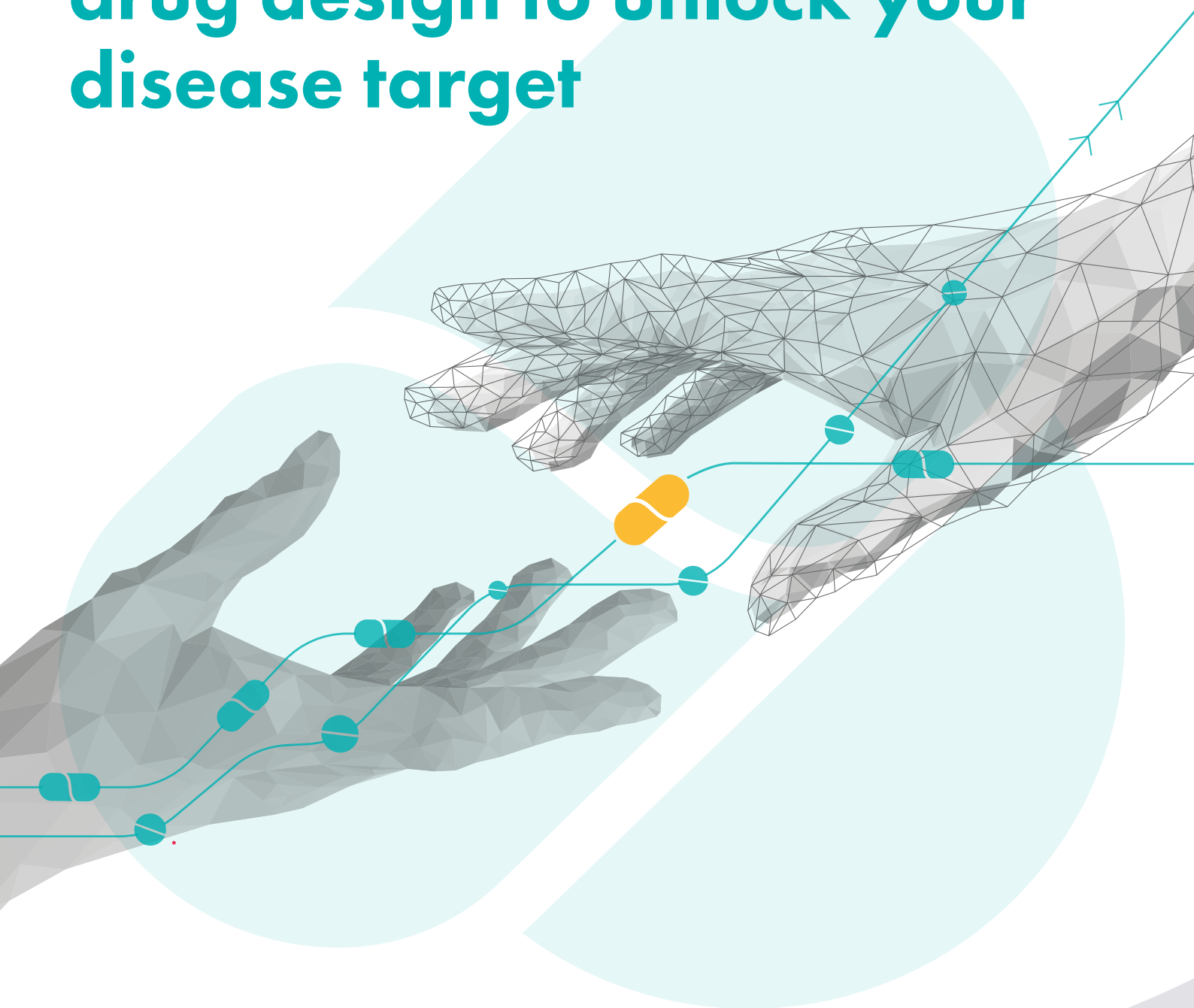
FragmentBuilder

Efficient Fragments to Quality Leads

Integrated fragment-based drug design to unlock your disease target



Domainex

domainex.co.uk

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What is *FragmentBuilder*?

***FragmentBuilder* is a fully integrated fragment-based drug design (FBDD) platform that delivers well qualified starting points for lead optimisation. Starting from your gene, we provide the following suite of services to advance your FBDD project seamlessly:**

- Protein expression and characterisation
- Assay development
- An established diverse, sp3-rich fragment library of ~1200 members
- Biophysical screening, including Microscale Thermophoresis (MST)
- Orthogonal techniques for hit confirmation
- Analogue-by-catalogue screening to establish initial SAR
- Parallel synthetic chemistry to drive potency and selectivity
- X-ray crystallography to determine binding site

Our FBDD platform is the route of choice over undertaking a wasteful, time-consuming and expensive HTS! It will deliver greater insight, more options for optimisation and potentially uncover novel modes of binding too.

Why Choose *FragmentBuilder*?

Domainex has established a world-leading position in the use of MST in fragment screening. Requiring little assay development time or quantity of target protein, MST allows proteins to stay in solution. Each fragment can interact with 'fresh' protein rather than sequential exposure of immobilised protein as with SPR (and hence it being prone to interference by irreversible binders).

Features and advantages

- Domainex has a proven track record in FBDD
- Access to Domainex's carefully constructed library of ~1200 diverse fragments
- Choice of primary screening methods available
- Domainex can generate high resolution crystal structures of bound fragments
- Orthosteric and/or allosteric configurations possible
- Speed: fragment hits in <1 month
- Orthogonal techniques for rapid fragment hit confirmation
- Rapid access to a database of >300K fragments for near neighbour analysis
- Immediate route into parallel chemistry

High Quality Protein Supply

Domainex's highly experienced Protein Sciences team can generate crystallography-grade proteins in multi-mg quantities using *E. coli* and baculoviral-infected insect cell expression systems.

Our scientists can utilise standard bioinformatics/literature-informed approaches or Domainex's proprietary technology, Combinatorial Domain Hunting (CDH).

- CDH can quickly identify soluble, highly expressible protein constructs of drug target proteins
- To find out more about CDH please request a copy of our separate CDH brochure or visit www.domainex.co.uk

Diverse Fragment Collection

Along with its strategic partner SpiroChem, Domainex has curated a diverse collection of fragments.

- Multi-parameter scoring function used to select compounds
- Molecular fingerprints used and compared with ChEMBL fragments to ensure good coverage of bioactive space
- Access to Spirochem fragments provides novel starting points
- All compounds soluble at 1 mM in 1% DMSO

Fragment Screening

The heart of the platform is screening of fragments by MicroScale Thermophoresis (MST, Nanotemper Technologies GmbH). This capillary-based, homogenous technology is able to quantify even weak binding events in a solution-based manner. The Domainex fragment collection can be screened in a matter of a few days to determine initial Kd values of fragment hits. Domainex has alternative screening methods available if required, such as HTRF, nanoDSF, SPR and direct-binding mass spectrometry.

MST Advantages Over Alternative Methods

- Minimal assay development
- Little protein required
- Solution-based, so no immobilisation
- Measure up to quaternary biological systems
- Capture orthosteric and allosteric binders
- Sensitive across the nM—mM range
- A high throughput technique
- Eliminates false positives early

Orthogonal & Functional Testing

The Domainex Assay Biology team can establish competition assays to determine the mechanism of binding of identified screening hits and can use a range of orthogonal tests, including:

- Saturation Transfer Difference (STD) – NMR
- Surface Plasmon Resonance (SPR)
- Differential Scanning Fluorimetry (DSF)
- HTRF

Once the project has progressed, the team can run cellular assays to further profile novel compounds received from the medicinal chemistry team. In parallel, *in vitro* ADME assays are run on the most promising compounds to help identify tractable leads.

X-ray Crystallography & SBDD

Domainex has the expertise to undertake crystal screens and, through its access to the synchrotron at Diamond Light Source (Oxfordshire, UK), can obtain high-resolution structures of fragment hits bound to target proteins. These can then be used by computational and medicinal chemists at Domainex to guide an efficient fragment elaboration process.

Case Studies

Case Study 1: G9a

G9a is a lysine methytransferase which mono- or di-methylates Histone 3 at Lysine residue 9 (H3K9), repressing gene expression. G9a is involved in mechanisms of carcinogenesis, making it an attractive oncology target. As a proof-of-concept study, a randomly selected 320-compound subset of our fragment library was screened at 1 mM against a G9a-SAM complex using MST. By using a saturating concentration of SAM, we ensured that the co-factor binding pocket was not available for fragment binding, as we specifically wanted to identify substrate-competitive hits. Several fragment hits with high ligand efficiencies were identified (5.3% hit rate) from the screen. Three fragment hits were successfully crystallised bound to G9a, confirming that they were substrate rather than co-factor competitive inhibitors as desired, and this structural data enabled a SBDD programme for this target. In just one round of fragment elaboration a 10-fold increase in affinity was achieved.

Frag ID	K_d [µM]	LE	STD-NMR Positive binding	X-ray Structure Resolution
MTP3B6	19	0.66	✔	1.5 Å
MTP2C3	56	0.41	X	
MTP4E1	115	0.41	✔	X
MTP2D8	327	0.54	✔	2.0 Å
MTP3G10	411	0.53	✔	1.8 Å
MTP2H9	564	0.44	✔	X
MTP3G1	718	0.36	X	

Table 1: Screening summary

Case Study 2: Ras

Activating RAS mutations are associated with approximately 30% of cancers; therefore, blocking RAS-effector interactions with biological reagents has beneficial effects in cancer models. Our collaborator, Prof Terry Rabbitts of the University of Oxford, commissioned Domainex to help with the identification of small molecules which bind potently to activated RAS and inhibit binding of effectors such as PI3K and Raf. A fragment hit (Abd-1) that demonstrated selective binding to GTP-bound (i.e. the active form) of RAS was identified from a SPR screen of a fragment library of 656 compounds. Our medicinal chemists explored the SAR around this initial hit to generate Abd-2, which had increased RAS-binding affinity and improved aqueous solubility. These superior properties facilitated structural biology studies and enabled the binding mode to be confirmed by X-ray crystallography. Domainex utilised its extensive experience of fragment- and structure-based drug design to further develop the series and identified a lead molecule with efficient binding to RAS. This and related compounds were shown to inhibit the RAS-effector interaction and tumour viability in cellular assays.

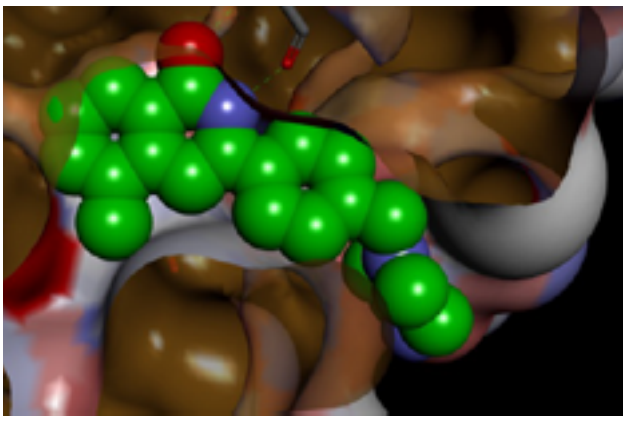

Figure 1: Lead molecule bound to RAS

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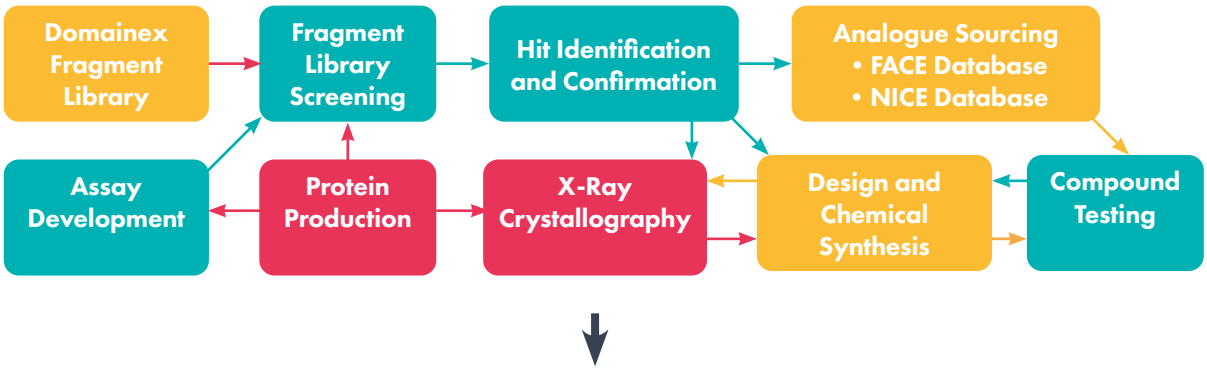
The Process



FragmentBuilder Output

Within 6 months Domainex will identify 1 or more hit series with:

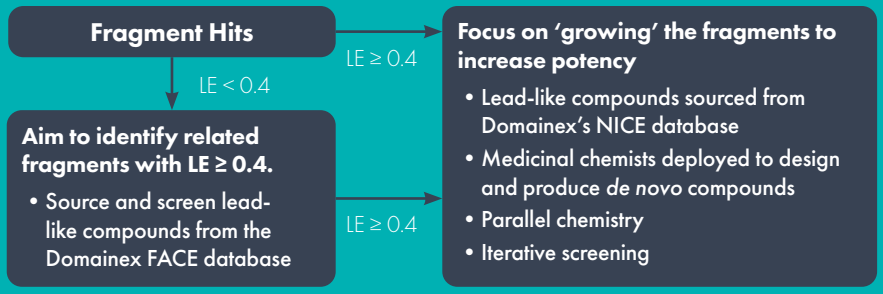
- Potency in the low micromolar range
- LE ≥0.35
- Good physicochemical properties
- Structural data at good resolution
- Novelty

Medicinal and Computational Chemistry

Domainex has a team of highly skilled chemists experienced in FBDD who will optimise your fragment hits. We understand the chemistry around our fragments and can quickly synthesise analogues/elaborated compounds. We have developed an efficient process for fragment hit expansion:

Medicinal Chemistry

Delivering Drug Candidates



FACE (Fragment Analogues for Chemical Evaluation):
~200,000 commercially available compounds that meet our fragment criteria

NICE (Number of Interesting Chemical Entities):
~2 million lead-like commercially available compounds

LE = Ligand Efficiency

About Domainex

Domainex is a fully integrated drug discovery service company based at Cambridge, UK. We serve pharmaceutical, biotechnology, academic organisations and patient foundations globally. With over 60 highly experienced biologists and chemists, we work in partnership with our clients from disease target through to candidate drug nomination. We have built a strong reputation for providing our clients with innovative ideas and undertaking high-quality, breakthrough experimental studies. We strive to build strong, dynamic relationships with our clients. In 2019 we served over 40 clients drawn from the UK, Europe, the United States and Australia and had a project renewal rate of over 70%.

How Can Domainex Help Your Drug Discovery Project?

Our highly experienced multi-disciplined scientists – molecular biologists, protein biochemists, assay biologists, structural biologists, medicinal, computational and analytical chemists – will support you to advance your drug discovery projects towards drug development effectively and efficiently. We provide customised programmes to address your specific needs at each stage of drug discovery. We draw from a wealth of expertise built up over the last 20 years against a wide range of drug targets and therapeutic areas. Being based at a single location with access to the very latest cutting-edge technologies, we are able to help you realise your goals and enrich your discovery pipeline.

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:

Dr. Thomas Mander MBA
Chief Executive Officer
tom.mander@domainex.co.uk
Tel: +44 (0) 1223 743174
Mob: +44 (0)7584 578024

Domainex
Chesterford Research Park
Little Chesterford
Cambridge
CB10 1XL, UK

Social
Domainex
@Domainex_UK