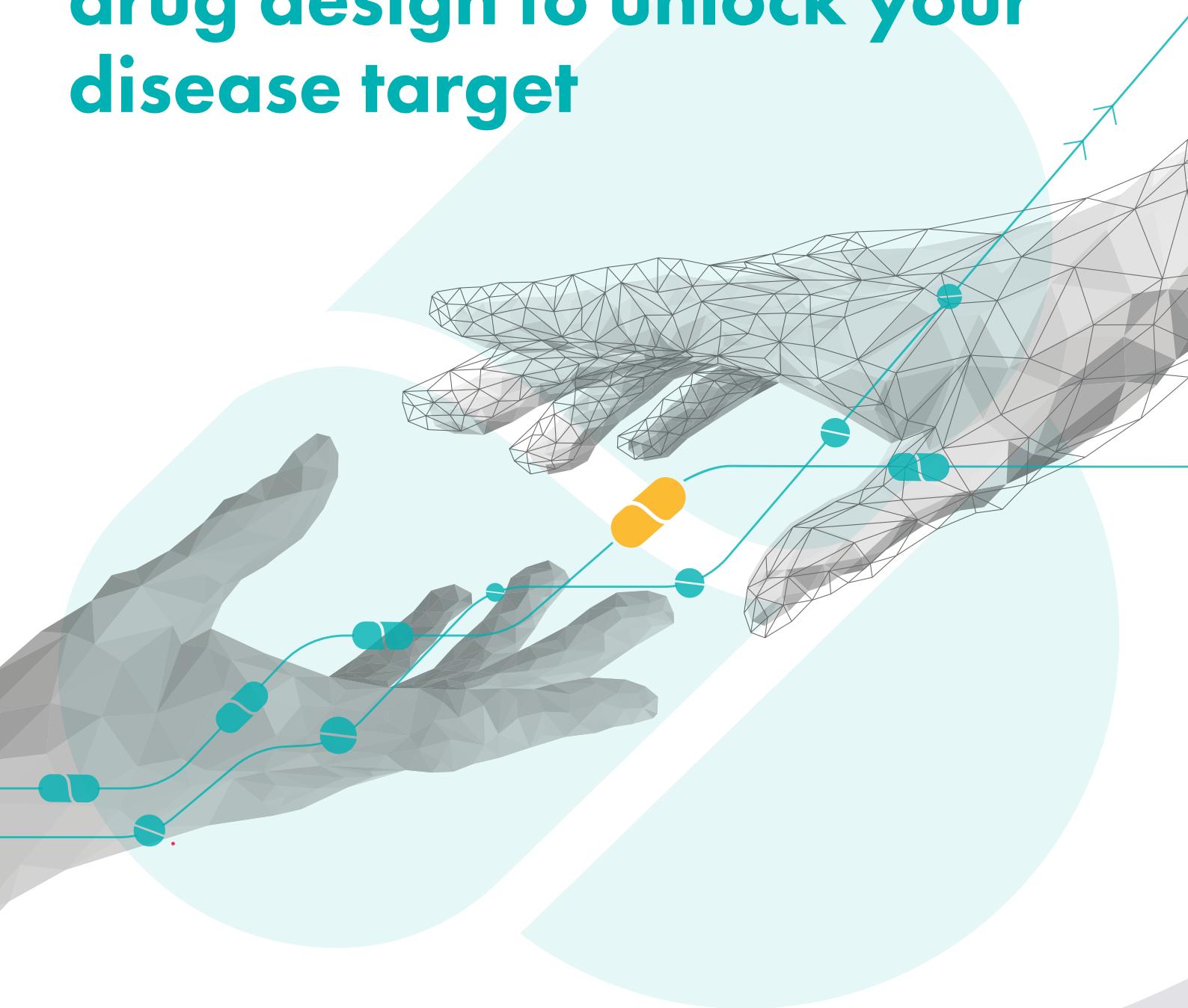




FragmentBuilder

Efficient Fragments to Quality Leads

Integrated fragment-based drug design to unlock your disease target



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 drug target, 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
- Fragment screening, typically by Microscale Thermophoresis (MST) or Grating Coupled Interferometry (GCI)
- 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 complements other approaches to hit discovery such as virtual screening and HTS that are also established at Domainex. FBDD will deliver earlier binding and kinetic insight, more options for optimisation and potentially uncover novel modes of binding too.-

Why Choose FragmentBuilder?

Domainex is one of the leading experts in the generation and interpretation of biophysical data. Both MST and GCI are excellent techniques for fragment screening.

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 will 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 will generate crystallography-grade proteins in multi-mg quantities using *E. coli*, baculoviral-infected insect cell and mammalian cell expression systems.

Our scientists will 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 our strategic partner SpiroChem, Domainex has curated a diverse collection of fragments offering novel potential starting points.

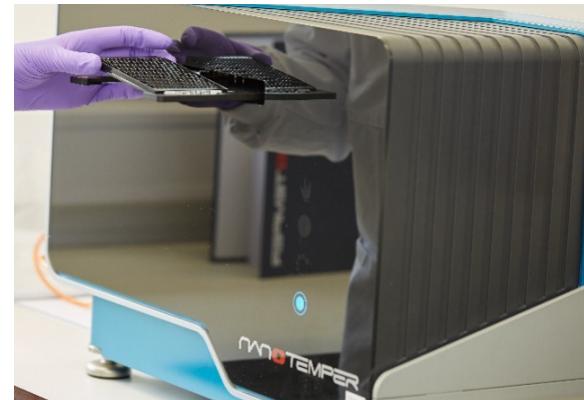
- Multi-parameter scoring function used to select compounds
- Molecular fingerprints used and compared with ChEMBL fragments to ensure good coverage of bioactive space
- All compounds soluble at 1 mM in 1% DMSO

Fragment Screening

At the heart of the platform is fragment screening using the biophysical techniques of MST or GCI. Domainex typically investigates both techniques in parallel so that we can select the right approach for your target.

MST

Domainex has invested in both the Dianthus NT.23PicoDuo and Monolith NT. Automated high-end instruments from NanoTemper Technologies GmbH. The Dianthus is a 384-well plate-based system that can screen several thousand samples in 24 hours, lending its use to medium-throughput screening. Whereas the Monolith is a 96-capillary-based system that is very sparing on protein use. A set of samples can be analysed in under 30 minutes, allowing for the efficient screening of fragment collections.



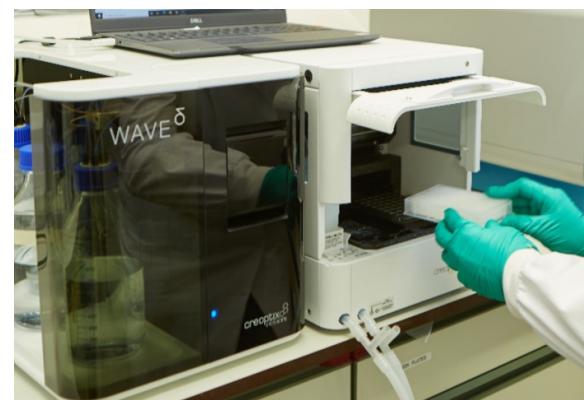
Key Advantages of MST

- Minimal assay development time
- 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 (therefore suitable for studying weak binders such as fragments)
- A high throughput technique
- Eliminates false positives early



GCI

Domainex has invested in the Creoptix WAVEdelta platform which uses the biophysical technique of Grating-Coupled Interferometry (GCI). GCI is analogous to Surface Plasmon Resonance (SPR). In both techniques, the target protein is immobilised onto specialised sensor chips and the passage of analytes over the chips' surface are monitored as time-dependent changes in refractive index, which can indicate bi-molecular interactions.



Key advantages of GCI

- More sensitive than SPR
- Varied pulse duration to minimise cycle time (waveRAPID® technology), increase throughput and also to allow compound titrations in one run
- Extra channel opens up the possibility of running a reference or selectivity protein in parallel to the test protein as part of the same run
- Fast and accurate measurements of kinetic rates
- Accurate determination of dissociation rates of up to 10 sec⁻¹ (therefore suitable for studying weak binders such as fragments)
- "Clog-free" and therefore suitable for analysing plasma, serum and crude cell lysates

Orthogonal & Functional Testing

We will establish competition assays to determine the mechanism of binding of identified screening hits and will use a range of orthogonal tests, including:

- MST (if not selected for the primary screen)
- GCI (if not selected for the primary screen)
- Saturation Transfer Difference (STD) – NMR
- Differential Scanning Fluorimetry (DSF) and nanoDSF
- Homogeneous Time Resolved Fluorescence (HTRF)

Once the project has progressed, the team will 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.



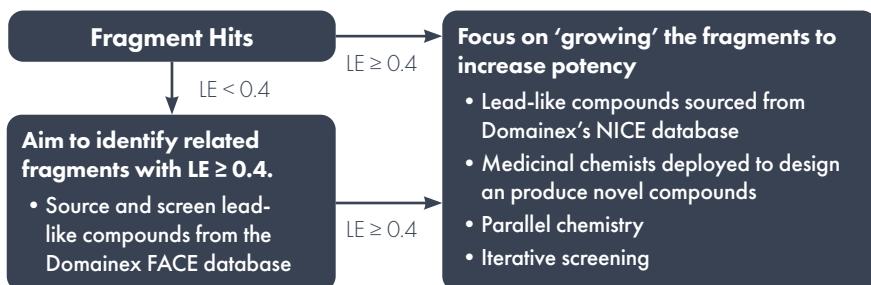
X-ray Crystallography & SBDD

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.



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:



FACE (Fragment Analogues for Chemical Evaluation):

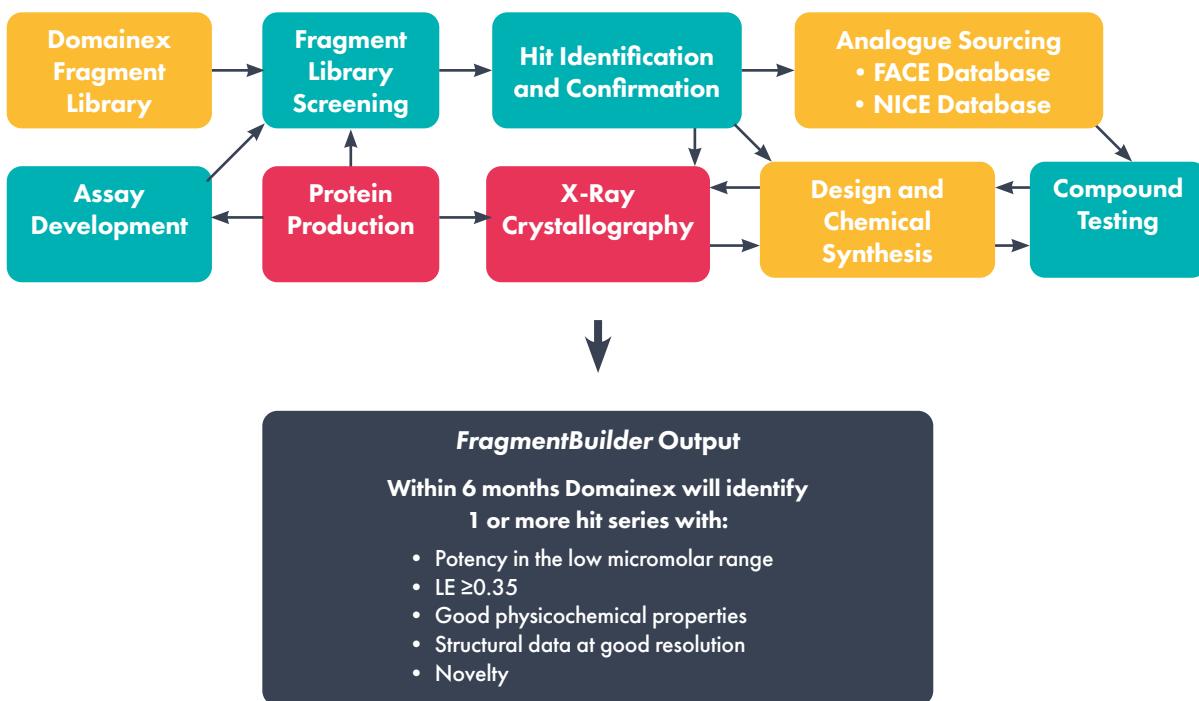
~300,000 commercially available compounds that meet our fragment criteria

NICE (Number of Interesting Chemical Entities):

~2 million lead-like commercially available compounds

LE = Ligand Efficiency

The Process



Case Studies

Case Study 1: E3 ligase

A primary screen of Domainex's Fragment Library (~1100 fragments) was conducted using GCI in RAPID mode against a full-length E3 ligase target protein (120 kDa) at 250 μM . The entire screen was completed in 4x 15-hour experimental runs. Recorded kinetic data was filtered by association error, dissociation error, R_{\max} and K_D to identify fragment hits. Figure 1 shows the distribution of fragment hits observed in an example k_a/k_d plot for one of the runs. The plot also demonstrates that very good reproducibility of the positive control was observed. In total 30 fragment hits were identified.

The fragment hits identified from this primary screening were then counter screened against the substrate binding domain of the E3 ligase to triage fragments binding to the substrate domain or ubiquitin binding domain. Comparable kinetic parameters were identified for six fragments against the full-length and substrate binding domain. Traditional kinetic measurements confirmed data obtained using RAPID mode (example data is shown in Figure 2), validating the new screening methodology. Furthermore, all six fragments also showed binding in a thermal shift assay using nanoDSF.

Here we have shown that using the new waveRAPID technology significantly shortens screening times compared to traditional SPR while maintaining excellent data resolution even at large ligand:analyte MW ratios.

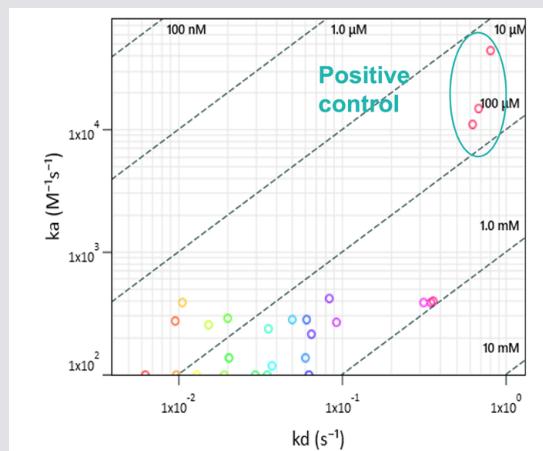


Figure 1: Figure 1: ka/kd plot for one of the runs which shows very good reproducibility of the positive control and the distribution of fragment hits.

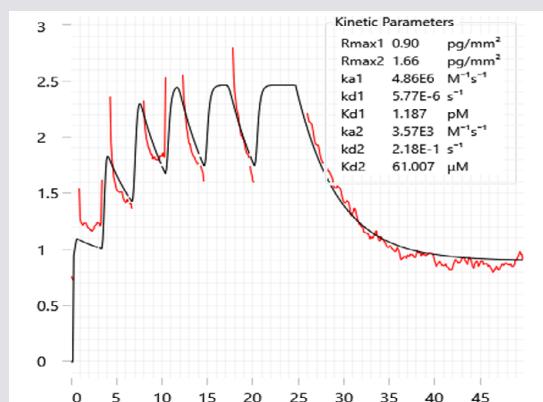


Figure 3: Orthogonal confirmation of fragment binding by nanoDSF. Data demonstrating thermal stabilisation due to fragment binding. The top panel shows the raw data and the bottom panel shows the first derivative plot highlighting the stabilisation due to compound binding

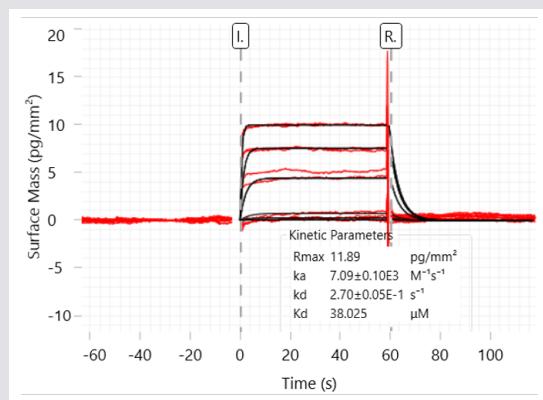


Figure 2: Top panel shows example data obtained in RAPID mode. Bottom panel shows traditional kinetic measurements obtained for the same fragment.

Case Studies

Case Study 2: G9a

G9a is a lysine methyltransferase 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	✗	
MTP4E1	115	0.41	✓	✗
MTP2D8	327	0.54	✓	2.0 Å
MTP3G10	411	0.53	✓	1.8 Å
MTP2H9	564	0.44	✓	✗
MTP3G1	718	0.36	✗	

Table 1: Screening summary

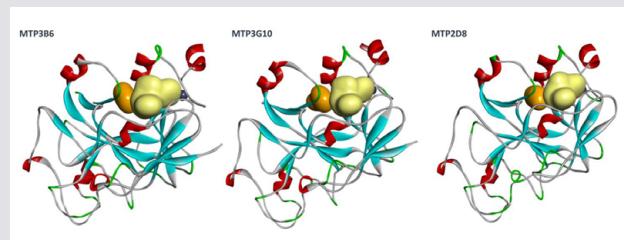


Figure 1: In-house G9a-fragment structures (orange: fragment, yellow: SAM)

Case Study 3: 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 Robbins 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.

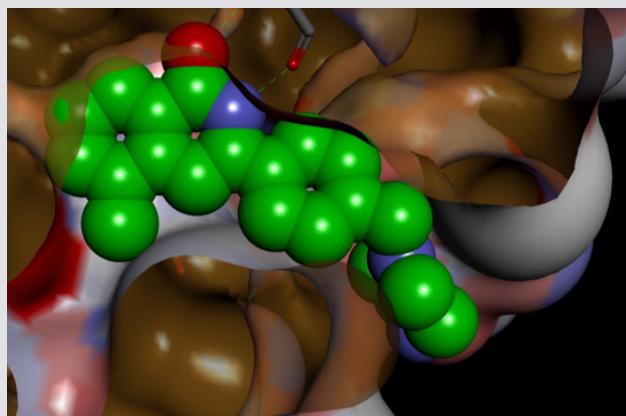


Figure 1: Lead molecule bound to RAS

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 70 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 2020 we served over 50 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

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