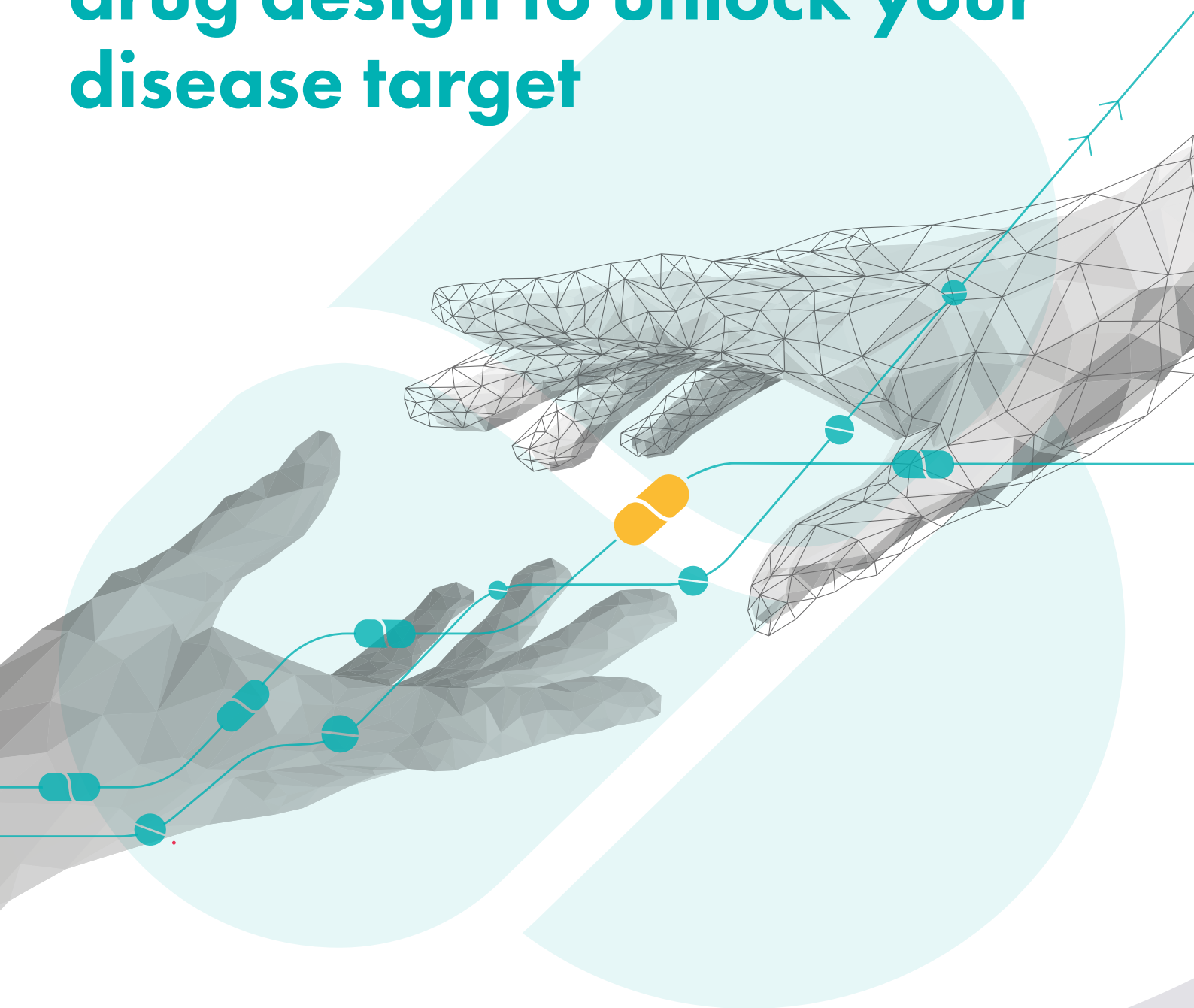
FragmentBuilder

Efficient Fragments to Quality Leads

Integrated fragment-based drug design to unlock your disease target



Domainex

domainex.co.uk

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Efficient Fragments to Quality Leads

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, sp^3-rich fragment library of ~1200 members
- Fragment screening, typically by Microscale Thermophoresis (MST)/Temperatute Related Intensity Change (TRIC) 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 mode

Our FBDD platform complements other approaches to hit discovery such as virtual screening and HTS that are also established at Domainex. It delivers early fragment binding and kinetic insight, more options for optimisation and potentially uncovers 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/TRIC 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
- Domainex generates high resolution crystal structures providing invaluable structural information
- 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 generate crystallography-grade proteins in multi-mg quantities using *E. coli*, baculoviral-infected insect and mammalian cell expression systems.

Our scientists utilise literature-informed or our proprietary technologies, such as Combinatorial Domain Hunting (CDH), to express your target(s) of interest.

- CDH can quickly identify soluble, highly expressible protein constructs of drug target proteins
- To find out more about CDH please see our separate brochure at www.domainex.co.uk/services/cdh-target-gene-fragmentation or contact us.

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
- Our fragments have suitable properties for progression (low molecular weight, a limited number of hydrogen bonding groups, a balance of polarity and lipophilicity, and a small number of rotatable bonds)

Fragment Screening

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

MST/TRIC

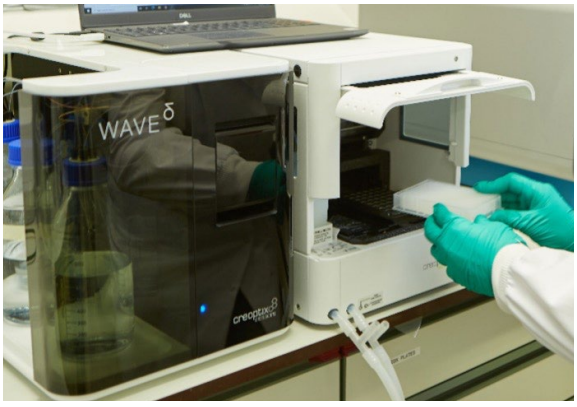
Domainex has invested in both the Dianthus NT.23PicoDuo and Monolith NT.Automated instruments from NanoTemper Technologies GmbH. The Dianthus is a 384-well plate-based system that can screen several thousand samples in 24 hours, lending its use to medium-throughput screening. Whereas the Monolith is 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/TRIC

- 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

Covalent Fragment Screening

Domainex also perform covalent fragment screening. Targeted covalent inhibitors have the potential advantages of prolonged duration of action, improved potency and high levels of selectivity for the target of interest. Covalent fragments can be detected by mass spectrometry which can be beneficial if a sensitive biophysical detection method, essential for classical fragment screening, cannot be established for the protein target of interest.

Orthogonal & Functional Testing

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

- MST/TRIC (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.

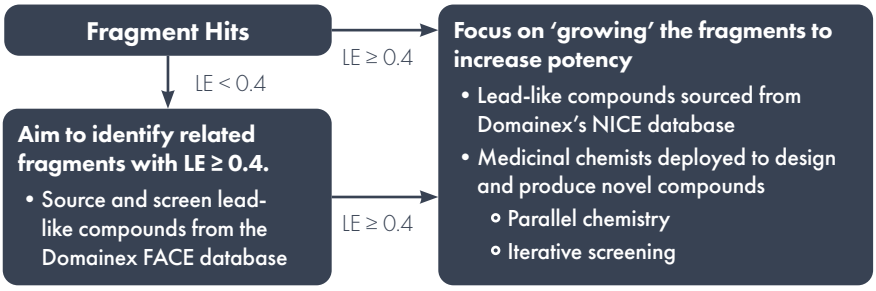
X-ray Crystallography & SBDD

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:

Medicinal Chemistry
Delivering Drug Candidates

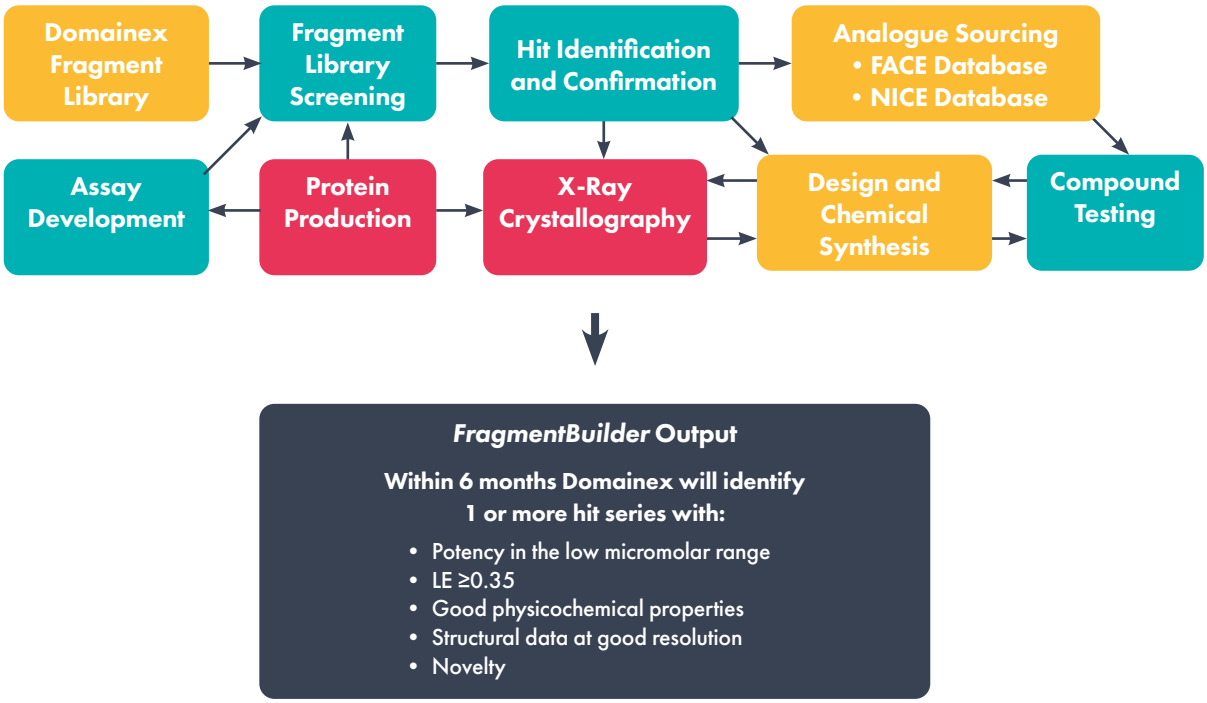


FACE (Fragment Analogues for Chemical Evaluation):
~300,000 commercially available compounds that meet our fragment criteria

NICE (Number of Interesting Chemical Entities):
~4.9 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.

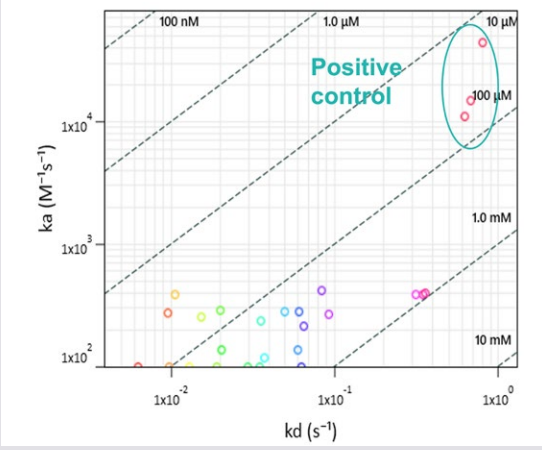
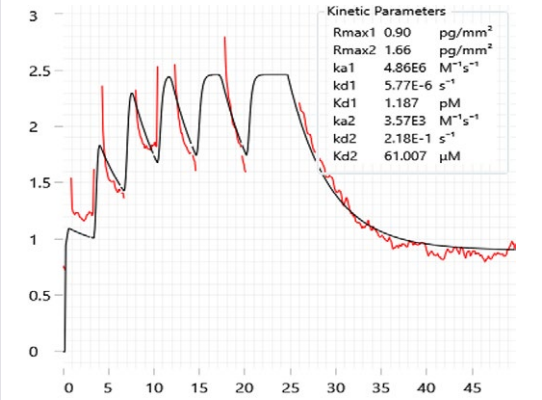


Figure 1: Figure 1: k_a/k_d plot for one of the runs which shows very good reproducibility of the positive control and the distribution of fragment hits.



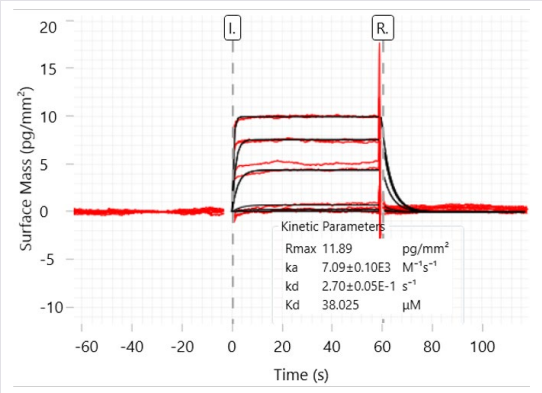


Figure 2: Top panel shows example data obtained in RAPID mode. Bottom panel shows traditional kinetic measurements obtained for the same fragment.

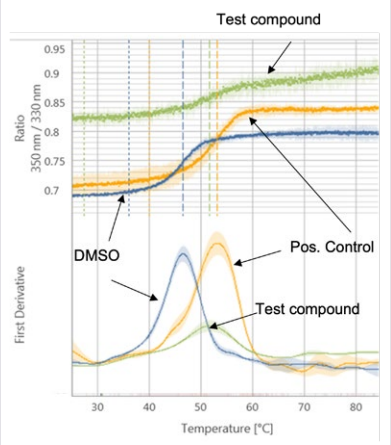


Figure 3: Orthogonal confirmation of fragment binding by nanoDSF. Data demonstrating thermal stabilisation due to fragment binding. The top panel shows the raw data and the bottom panel shows the first derivative plot highlighting the stabilisation due to compound binding

Case Studies

Case Study 2: G9a

G9a is a lysine methytransferase which mono- or di-methylates substrates at histone 3/ lysine 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

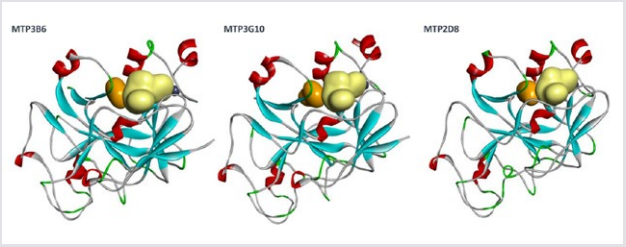


Figure 1: In-house G9a-fragment structures (orange: fragment, yellow: SAM

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 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.

For further information please see the following publication:

Quevedo CE *et al* (2018) Small molecule inhibitors of RAS-effector protein interactions derived using an intracellular antibody fragment. *Nature Comms* **9**, 3169.

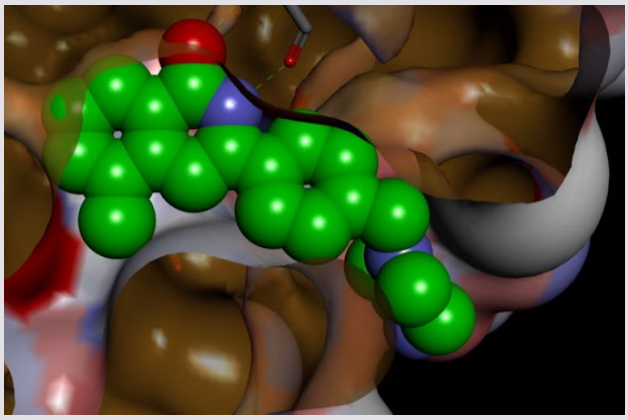


Figure 1: Lead molecule bound to RAS

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. We have ambitious growth plans and are expecting to reach 110 biologists and chemists in the near future. We provide integrated services, from disease target selection to candidate drug nomination. We have a very strong reputation for contributing innovative ideas, undertaking high-quality experiments and for generating intellectual property on behalf of our clients. We strive to build strong, dynamic relationships. In 2021 we served over 60 clients from the UK, Europe, the United States, Japan and Australia and had a project renewal rate of over 80%.

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 bio/analytical chemists, *in vitro* pharmacologists and ADME scientists – 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 across a wide range of drug targets and therapeutic areas. From our sites within Europe's leading bioscience hub at Cambridge, UK and 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

Social

Domainex

@Domainex_UK

Chemistry & Bioanalytical Centre of Excellence
Churchill Building
Chesterford Research Park
Little Chesterford
Saffron Walden
CB10 1XL
UK

Biology Centre of Excellence
Iconix 2, Unity Campus
London Road
Pampisford
Cambridge
CB22 3EG
UK

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