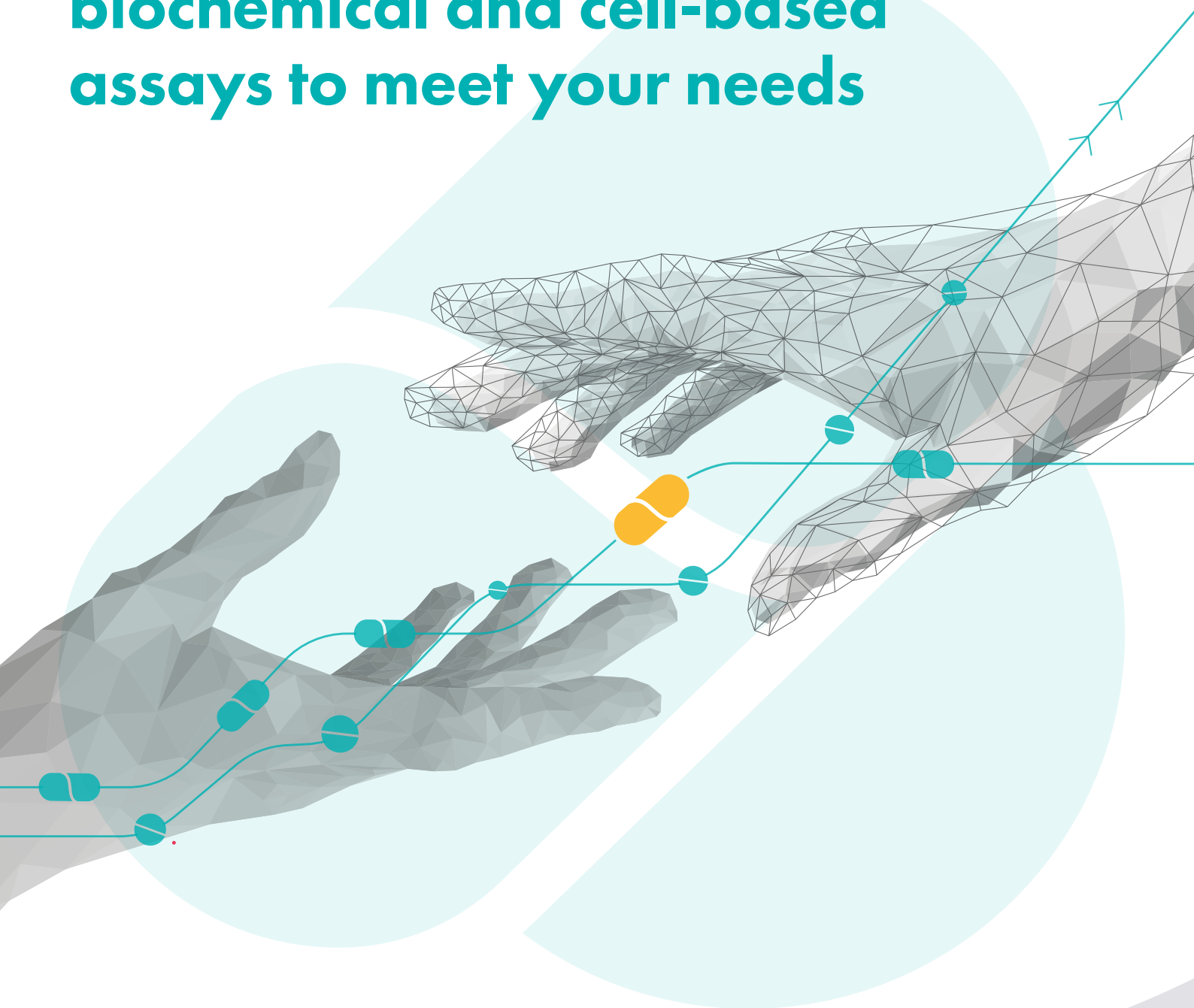
Assay Biology
Quality Data Throughout

Customised biophysical, biochemical and cell-based assays to meet your needs



Domainex

domainex.co.uk

Assay Biology
Quality Data Throughout

Customised assay development and screening

Domainex has wide-ranging expertise in developing biophysical, biochemical and cell-based assays that are optimised to give high-quality data. This enables the efficient progression of your drug discovery project at every step of the way, developing assay systems to identify hit compounds and subsequently running screening cascades as part of hit-to-lead and lead optimisation projects.

We will strive to establish high-quality assay biology systems using a wide range of assay read-outs that are validated pharmacologically. Our expert biologists create and deliver whole screening cascades or stand-alone assays – all tailored to your needs and budget.

Our range of services enable the comprehensive assessment of your compounds for potency, efficacy, kinetics, mechanism of action, selectivity and more – all under conditions that are as close to being physiologically relevant as possible. Our state-of-the-art automated liquid-handling systems (including the Integra VIAFLO384, STP Labtech mosquito® crystal, STP Labtech dragonfly® and Agilent Bravo) and Dotmatics® informatics platform, enable us to deliver rapidly assay data for each test compound. This, combined with our *FragmentBuilder* and *LeadBuilder* hit identification platforms and high throughput screening capabilities delivers high quality assay results. We will provide you our conclusions and recommendations along the way. As your project advances, our platforms provide rapid turnaround of data enabling Structure-Activity-Relationships (SARs) to be identified, quickly and efficiently, meaning that your project can be rapidly progressed to the next phase of research.

Additionally, our protein science team are able to clone and express your target protein to high purity, to support assay development and screening.

Assay design principles

- Intellectual input to design robust and high-quality assays
- Development of tailored assay systems
- Consultative approach throughout
- Quality control of reagents
- Understanding of assay limits
- Validation of *in vitro* pharmacology parameters with known ligands
- Assessment of assay robustness (Z'-factor, signal:background ratio, intra- and inter-plate variability, etc.)
- Decision-enabling data sets delivered in a time and cost-effective manner
- Ability to offer a complete screening solution
- Ensure that results are efficiently linked to medicinal chemistry

Biophysical methodologies available at Domainex

- MicroScale Thermophoresis (MST)/Temperature Related Intensity Change (TRIC)
- Grating-Coupled Interferometry (GCI)
- X-ray Crystallography
- Differential Scanning Fluorimetry (DSF) and nanoDSF
- NMR STD & WaterLOGSY
- Surface Plasmon Resonance (SPR)
- Isothermal Titration Calorimetry (ITC)

Biophysics

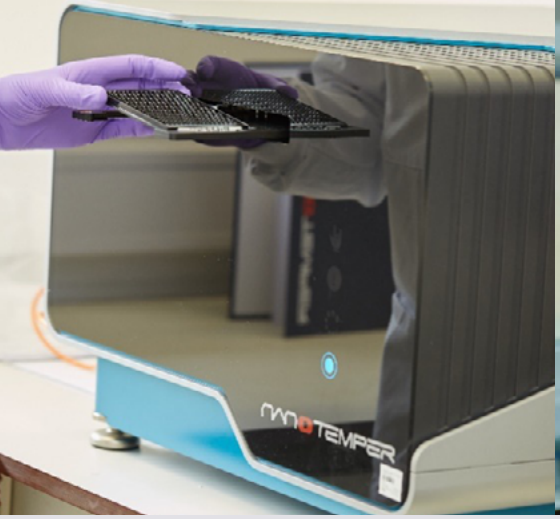
Domainex is one of the leading experts in the generation and interpretation of biophysical and structural data to gain crucial insights into ligand-target engagement, such as affinities (dissociation constants), kinetics (k_{on} and k_{off}) and energetics (enthalpic and entropic contributions to drug binding).

Having invested in a suite of state-of-the-art biophysical instruments, Domainex will deploy this high-end technology platform on your project. This technology base includes the following NanoTemper instruments:

i. Monolith NT.Automated, a 96 capillary-based instrument for fragment screening which uses the techniques of MST/TRIC. The Monolith can assess the binding affinities of up to 50 compounds per day with full concentration response curves.

ii. Dianthus NT.Pico, a 384-well based instrument for high throughput screening which uses the techniques of MST/TRIC. The Dianthus can assess the binding affinities of up to 100 compounds per day with full concentration response curves.

iii. Prometheus NT.48, another capillary-based instrument that measures protein folding in a label-free manner using the technique of nanoDSF



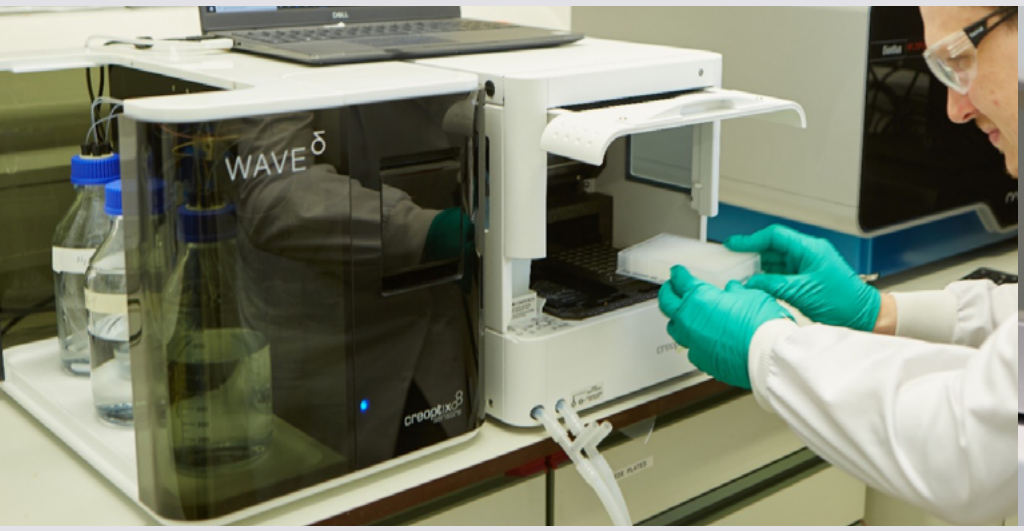
Advantages of MST/TRIC

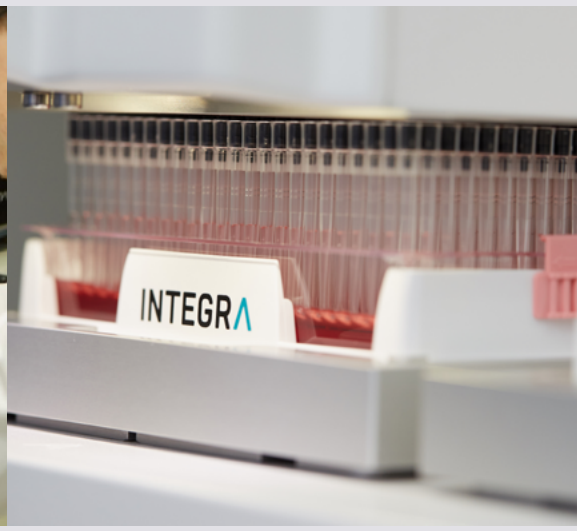
- Target remains in solution and therefore in its native folded state, without the risk of binding sites being occluded by immobilisation
- Very low protein consumption
- No molecular weight restrictions on the molecules that can be analysed
- Eliminates false positives early
- Minimal assay development time
- Wide range of buffers can be used
- Supports challenging biology (e.g. ternary or quaternary systems)
- Readily allows competitive binding studies to be performed, and orthosteric and allosteric binders to be discovered
- Binding affinity determinations performed easily, with a large dynamic range (pM – mM)
- Higher throughput than other biophysical techniques

Advantages of GCI

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

More recently, Domainex has invested in the Creoptix WAVEdelta instrument that utilizes Grating-Coupled Interferometry (GCI), a superior immobilisation-based technology that is analogous to SPR. These instruments are at the core of Domainex's fragment screening platform, *FragmentBuilder*, and we are widely recognised as a world-leader in their use. We also use these instruments to screen and characterise compounds arising from our *LeadBuilder* virtual screens and subsequently through lead optimisation campaigns.





About Domainex

Domainex is a fully integrated drug discovery service company based in Cambridge, UK. We serve a wide range of pharmaceutical, biotechnology, academic organisations and patient foundations globally. We have ambitious growth plans and currently have over 100 scientists. 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 and work with our clients to provide customised services.

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 the pre-clinical drug discovery process. 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.

Contact

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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Biochemistry

At Domainex we understand fully that providing reliable and reproducible data on key parameters such as potency, mode of inhibition, selectivity and kinetics, is essential for any drug discovery project – all the way from hit identification, through the subsequent medicinal chemistry optimisation process, to the characterisation of candidate drugs.

Our skilled assay biologists routinely develop and validate biochemical assays for enzymes, receptors and protein-protein interactions (PPI) using cutting-edge detection and liquid-handling instruments for quantifying a variety of read-outs, including:

- Light absorbance
- Fluorescence intensity and polarisation
- HTRF®, TR-FRET
- Luminescence, BRET
- AlphaLISA®, AlphaScreen®
- FLIPR
- LC-MS

Case Study 1

sosei HEPTARES

Establishment of a biochemical assay for the SARS-Cov-2 main protease

Domainex formed a collaboration with Sosei Heptares to identify inhibitors of the SARS Cov 2 main protease (M^{pro}). As part of this programme, the enzyme kinetics were thoroughly characterised (Figure 1) and a screening assay was developed with excellent reproducibility and robustness (Figure 2).

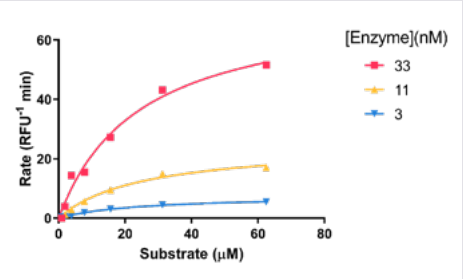


Figure 1: Michaelis-Menten Kinetics of SARS-CoV2 M^{pro}

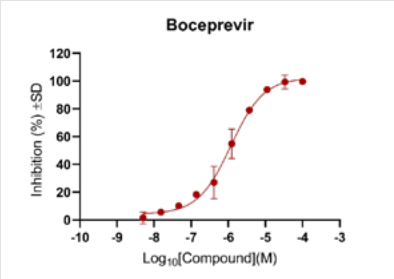
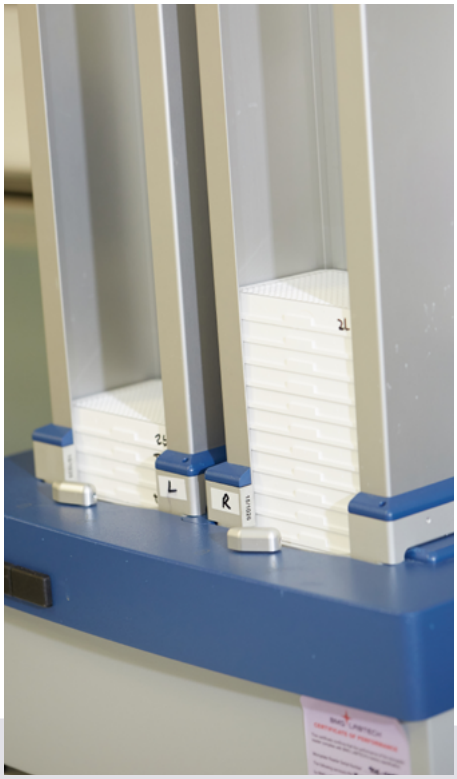


Figure 2: Inhibition of SARS-CoV2 M^{pro} by Boceprevir

Biochemical assay portfolio

Enzyme assays	• Measurements of enzyme kinetic parameters such as k_{cat} and K_M for your target • Mechanism of action studies to determine competitive, non-competitive, uncompetitive binding • In-depth assays to determine inhibitor on/off rates, K_i values and k_{inact}/K_I for slow off-rate or covalent binders
Receptor ligand-binding assays	• Determination of receptor B_{max} and K_d values of known ligands • Optimisation of assay systems ahead of screening for levels of agonism and/or antagonism with known ligands and test compounds • Use of our proprietary *PoLiPa* technology for detergent-free solubilisation of membrane targets
Protein Protein Interaction assays	• AlphaLISA®, FRET and fluorescence polarisation technologies established • Promega Nanoluc®-derived BRET or HiBiT/LgBiT systems delivered



Cell and molecular biology

Our experienced team of cell biologists can test a large variety of different cell types and offers a wide range of cellular assay services to enable screening and MOA studies on your compounds.

We have also cultured successfully many different cell types and are familiar with a number of cell differentiation protocols that allow us to make the required cell types from progenitor cells.

We can also design and build both transient and stable cell lines expressing your target of interest.

Our experience extends to working routinely with human cell assay systems including use of iPSC, whole blood, PBMC, T and B cell assay systems.

Cell biology assay portfolio

Phenotypic assays	• IncuCyte® imaging • Atmosphere-controlled long-term kinetic cellular readouts on a Clariostar® plate-reader • Cell viability, cell health, cell cycle and cell death • Immunology endpoints (e.g. phagocytosis and inflammasome readouts, T and B-cell proliferation) • Metabolic function • Mitochondrial function • 3D spheroid cultures • Phenotypic fibrosis model (scar-in-a-jar) • Cell-based drug combination/synergy studies
Receptor ligand-binding assays	• NanoBRET • IncuCyte® imaging • Fluorescent Activated Cell Sorting (FACS) • Western blotting
Biomarker profiling	• AlphasLISA® technology, IncuCyte® imaging and FACS • Able to analyse up to 20 cytokines in multiplex to study immune responses • Other biomarkers available such as secreted proteins, phosphorylation and kinase levels and histone methylation
Reporter assays for analysis of biochemical pathways	• FLIPR ion flux • Receptor second messengers • Protein-protein interactions

Case Study 2

A Fragment-based drug discovery (FBDD) programme to identify inhibitors of G9a using MicroScale Thermophoresis (MST)

Lysine methyltransferases (KMTs) are involved in epigenetic gene regulation by catalysing the transfer of methyl groups from *S*-adenosyl methionine (SAM) to lysine residues on histone proteins. G9a is a KMT that mono- or di-methylates Histone 3 at Lysine residue 9 (H3K9), repressing gene expression, and has been validated as an attractive oncology target.

As a proof-of-concept study, a randomly selected 320 compound sub-set of Domainex's fragment library (containing >1000 fragments) 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, and so we specifically targeted substrate-competitive hits. Fragments were declared as hits if a significant shift in the response compared to the reference was observed (Figure 1). A 5.3% hit rate was obtained, which compared favourably to the results of screening the same fragments using Differential Scanning Fluorimetry (DSF) or by an activity-based AlphaScreen assay – both of which showed only a 0.3% hit rate.

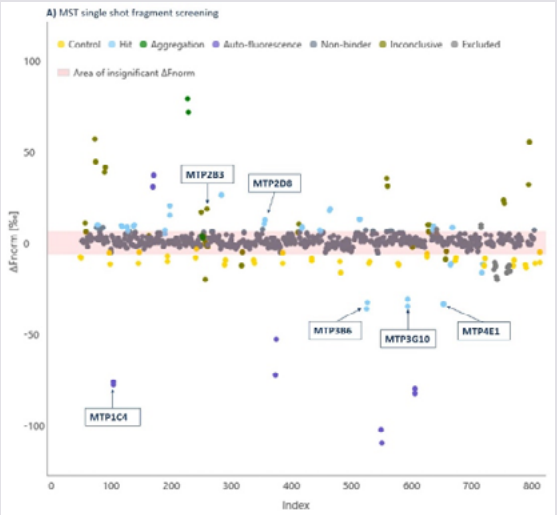


Figure 1: Hits identified from the MST screen

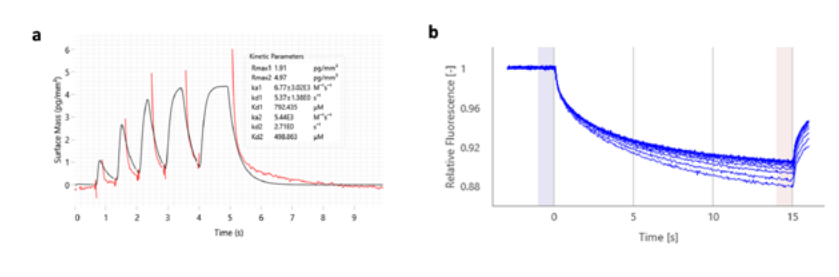


Figure 2: a) waveRAPID kinetic data obtained using the Creoptix WAVEdelta system for G9a fragment hit, Frg331. A K_d value of 499 µM was obtained. B) Microscale Thermophoresis (MST) data for the same fragment hit. A K_d value of 460 µM was obtained using this technique.

Hits were taken into secondary screening to determine their binding affinities (K_d) to the G9a-SAM complex using MST. Fragment hits were identified with good affinities, and hence excellent ligand efficiency. Orthogonal confirmation of hit binding to G9a was demonstrated by STD NMR spectroscopy and GCI (Example data is shown in Figure 2).

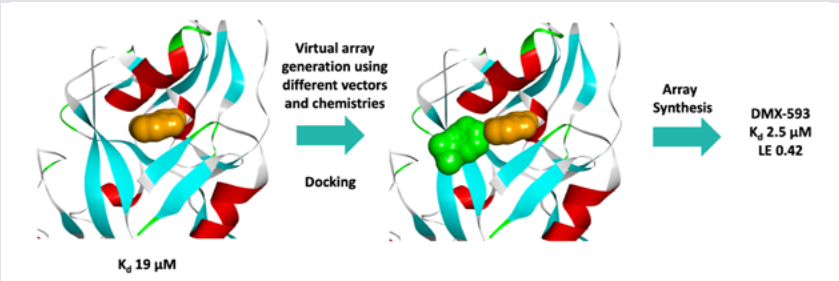


Figure 3: Rapid elaboration of the fragment hits

Three G9a-fragment crystal structures were solved in-house in the presence of the co-factor, SAM, with a resolution of 1.5–2.0 Å, which revealed different fragment binding modes. These three series were progressed and in just one round of fragment elaboration (~50 compounds) a 10-fold increase in affinity was achieved. Figure 3 shows the efficient process used by our medicinal and computational chemists.

Case Study 3

Profiling phagocytosis inhibitors in differentiated THP-1 macrophages

THP-1 cells were differentiated to M0 phenotype by exposure to phorbol 12-myristate 13-acetate (PMA) followed by polarisation to M1 and M2, as per Figure 1 below. Phagocytosis was measured by exposure of the cells to pH sensitive fluorescent zymosan bioparticles; there is an increase in fluorescence in low pH intracellular compartments upon phagocytosis of the particles. Fluorescence imaging (Figure 2) and quantification (Figure 3) were performed kinetically using the IncuCyte system. As predicted from well-defined phenotypic profiling of THP-1 cells, phagocytosis was promoted in the M2 phenotype and inhibited in the M1 state compared to M0 cells (Figure 3).

Pharmacologic modulation of phagocytosis was investigated using a panel of PI3K inhibitors. Cells (M0) were incubated with compound for 1 hour prior to exposure with zymosan bioparticles. The higher potency of the PI3K-γ selective compound, IPI-549, compared with the PI3K-γ selective inhibitor, idelalisib, suggests that the effect is more PI3K-γ driven (Figure 4), in agreement with the literature.[1]

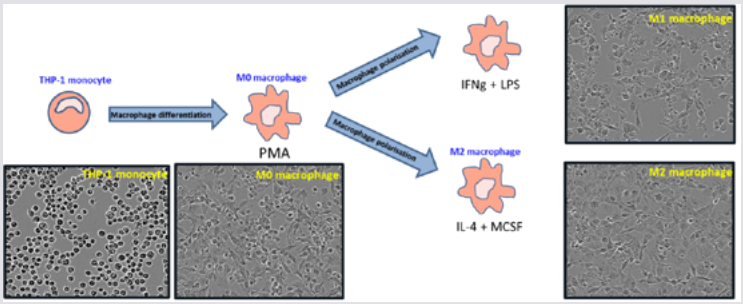


Figure 1: Schematic of THP-1 differentiation and polarisation with IncuCyte images of the cells at each phase.

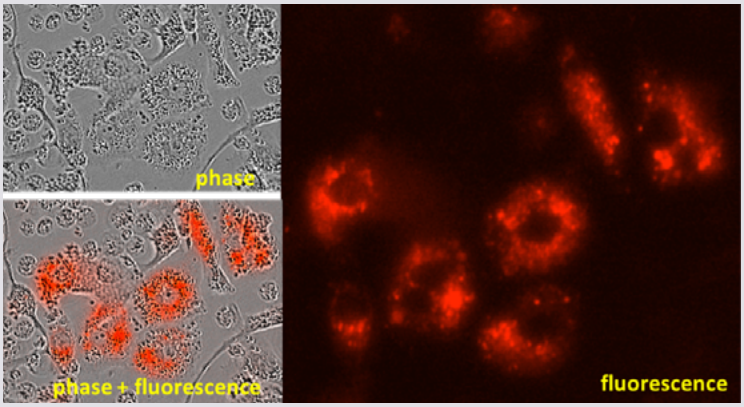


Figure 2: IncuCyte images of M1 THP-1 cells after phagocytosis of zymosan bioparticles (red fluorescent objects)

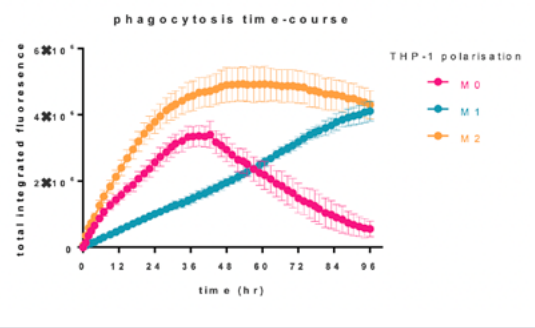


Figure 3: Time-course of fluorescence increase upon phagocytosis in THP-1 cells.

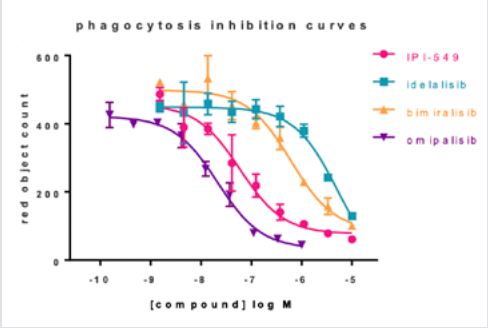


Figure 4: Pharmacologic modulation of phagocytosis in M0 THP-1 cells by PI3K inhibitors (24 hr timepoint).

Reference

1. Loss of phosphoinositide 3-kinase gamma decreases migration and activation of phagocytes but not T cell activation in antigen-induced arthritis. Michael Gruen, Christina Rose, Christian König, Mieczyslaw Gajda, Reinhard Wetzker and Rolf Bräuer. *BMC Musculoskeletal Disorders*, **2010**, 11:63.