

# Poster Abstracts

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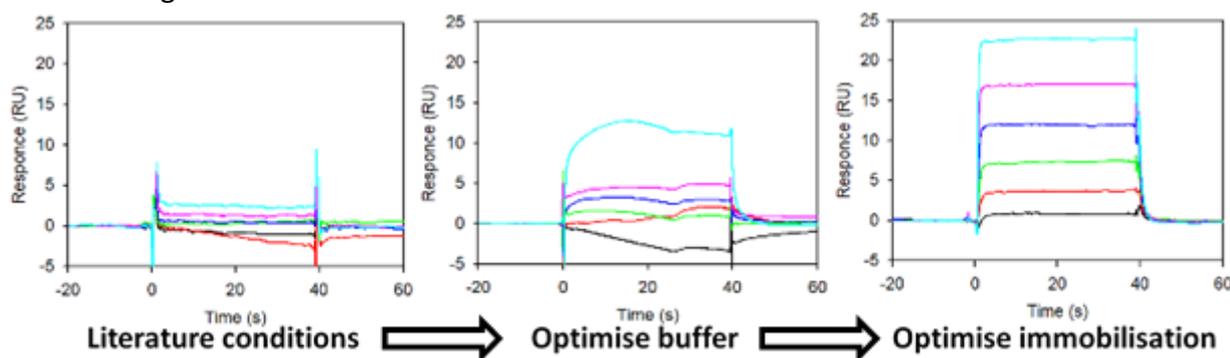
## Getting to Know Dengue Polymerase: The Development of Optimised Assay Conditions Using Stability Screening

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Dengue virus is a mosquito born *flavivirus* that causes 390 million infections each year. 40 % the world's population live in at-risk areas and there are no known small molecule treatments. Amongst the four serotypes of dengue, the RNA-dependent RNA polymerase (RdRP) is the most conserved of the non-structural viral proteins, making it an attractive target for the development of pan-serotype Dengue virus inhibitors. Small molecules that bound to a pocket on RdRP, known as the p-pocket, were discovered by the Novartis Institute of Tropical diseases and were found to inhibit viral replication.<sup>1</sup> The current p-pocket inhibitors have poor solubility and permeability,<sup>2</sup> and are therefore not suitable for development. Hence there is a need for novel inhibitor chemotypes for RdRP with improved physicochemical properties.

We have conducted a fragment screen against RdRP from Dengue serotype 3 using ligand detect NMR and identified several fragment hits which compete with a known p-pocket ligand. To aid rapid development of higher affinity analogues a robust, high throughput, quantitative assay is required. We therefore developed a Surface Plasmon Resonance (SPR) assay, to allow for determination of affinity as well as kinetics and enable rapid development of fragment series via Off-Rate Screening (ORS).<sup>3</sup> ORS uses the slow dissociation kinetics of high-affinity compounds to identify the best analogues which are generated in microscale parallel libraries and tested without purification.<sup>3</sup> Herein we describe the process of developing a robust SPR assay combining data from thermal shift analysis to investigate protein stability. We plan to use the developed SPR assay to conduct ORS and identify high affinity pan-serotype inhibitors of Dengue RdRP.



1. Noble, C. G.; Lim, S. P.; Arora, R.; Yokokawa, F.; Nilar, S.; Seh, C. C.; Wright, S. K.; Benson, T. E.; Smith, P. W.; Shi, P. Y. A Conserved Pocket in the Dengue Virus Polymerase Identified through Fragment-Based Screening. *J Biol Chem* **2016**, 291, 8541-8.
2. Yokokawa, F.; Nilar, S.; Noble, C. G.; Lim, S. P.; Rao, R.; Tania, S.; Wang, G.; Lee, G.; Hunziker, J.; Karuna, R.; Manjunatha, U.; Shi, P.-Y.; Smith, P. W. Discovery of Potent Non-Nucleoside Inhibitors of Dengue Viral Rna-Dependent Rna Polymerase from a Fragment Hit Using Structure-Based Drug Design. *J. Med. Chem* **2016**, 59, 3935-3952.
3. Murray, J. B.; Roughley, S. D.; Matassova, N.; Brough, P. A. Off-Rate Screening (Ors) by Surface Plasmon Resonance. An Efficient Method to Kinetically Sample Hit to Lead Chemical Space from Unpurified Reaction Products. *J. Med. Chem* **2014**, 57, 2845-2850.

## An automated and systematic approach to the prioritisation of fragments for chemical development through analogue screening

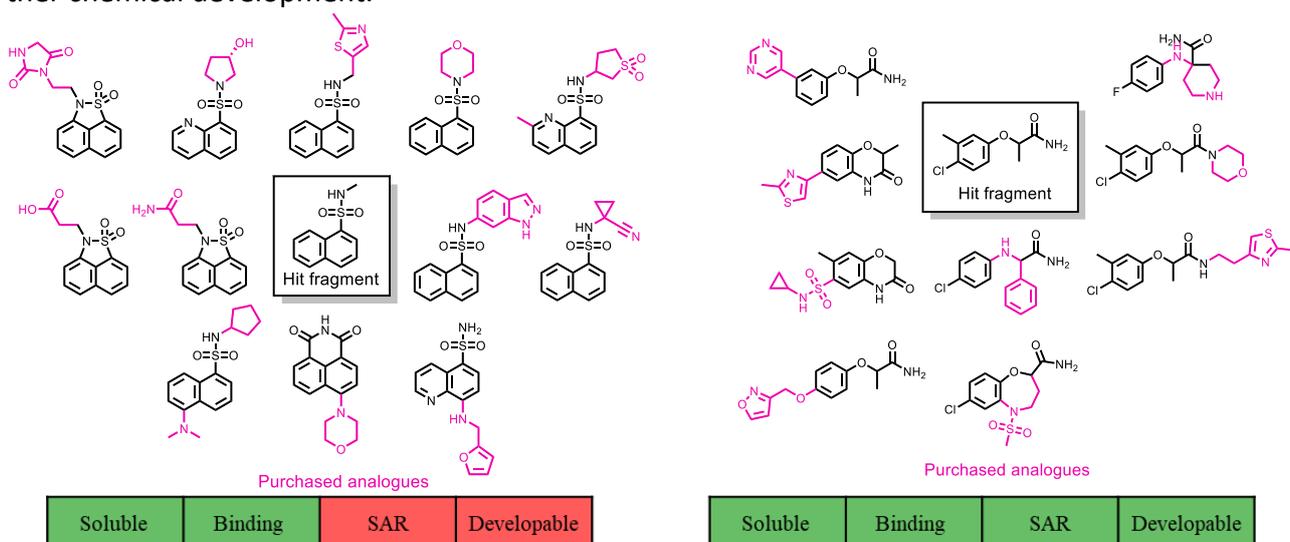
Ashley Taylor<sup>1</sup>, Bradley Doak<sup>1</sup>, Alicia Currie<sup>1</sup>, Olga Ilyichova<sup>1</sup>, Biswaranjan Mohanty<sup>1</sup>, Matrin Williams<sup>1</sup>, Sally Oxenford<sup>2</sup>, Alice Hooper<sup>2</sup>, Mary Wheldon<sup>2</sup>, Trevor Askwith<sup>2</sup>, Ben Allsop<sup>2</sup>, Richard Angell<sup>2</sup>, Martin Scanlon<sup>1</sup>

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Fragment-based drug design (FBDD) has become a well-established technique for drug discovery in both industry and academia.<sup>1</sup> A significant challenge that is faced in almost all FBDD campaigns is to elaborate the initial fragment hits, which typically bind weakly to the target protein into more potent lead molecules. An associated challenge is to select the most developable fragment hits to progress into development. Hit rates of 10% are not uncommon in fragment screening, which for typical libraries containing  $\approx 1,000$  fragments represent an unworkable number of possibilities.<sup>2,3</sup> It is therefore imperative to identify and prioritise the most developable fragments from the initial screen and identify the most promising positions/vectors for expansion to guide medicinal chemistry. These decisions must often be made in the absence of any structural data for the fragment bound to its target protein.

Our group has developed a workflow to select commercially available analogues of hit fragments that can be screened to assess fragment developability for a target. For each fragment hit the aim is to assess one vector at a time to determine its potential to improve affinity, whilst maintaining good physicochemical properties (solubility, stability etc.). To select suitable fragment analogues we have developed a semi-automated method for generic R group analysis and decomposition (GRADe). This method can identify, decompose and group analogues by the vector which they explore even when the core of the fragment has changed. Analogues can be scored and ranked to generate a suitable set to establish SAR for each fragment core ( $\sim 20$  analogues per fragment). Analysis of the resultant SAR can then be used to identify which fragments are amenable to elaboration as well as which vectors are most open to modification for further chemical development.



1. Erlanson, D. A. et al. *Nature Reviews Drug Discovery* **2016**, 15, 605–619.
2. Hann, M. M. et al. *Journal of Chemical Information and Computer Sciences* **2001**, 41, 856–864.
3. Kresu, G. M. et al. *Journal of Medicinal Chemistry* **2016**, 59, 8189–8206.

## **Discovery of a B-Cell Lymphoma 6 Protein–Protein Interaction Inhibitor by a Biophysics-Driven Fragment-Based Approach**

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<sup>2</sup>*Axcelead Drug Discovery Partners Inc.*

B-cell lymphoma 6 (BCL6) is a transcriptional factor that expresses in lymphocytes and regulates the differentiation and proliferation of lymphocytes. Therefore, BCL6 is a therapeutic target for autoimmune diseases and cancer treatment. This report presents the discovery of BCL6–corepressor interaction inhibitors by using a biophysics-driven fragment based approach. Using the surface plasmon resonance (SPR)-based fragment screening, we successfully identified fragment 1 (SPR  $K_D = 1200 \mu\text{M}$ , ligand efficiency (LE) = 0.28), a competitive binder to the natural ligand BCoR peptide. Moreover, we elaborated 1 into the more potent compound 7 (SPR  $K_D = 0.078 \mu\text{M}$ , LE = 0.37, cell-free protein–protein interaction (PPI)  $IC_{50} = 0.48 \mu\text{M}$  (ELISA), cellular PPI  $IC_{50} = 8.6 \mu\text{M}$  (M2H)) by a structure-based design and structural integration with a second high-throughput screening hit.

## Fragment screening for a WDR5 inhibitor

Matthew Dennis<sup>1</sup>

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The WD40-repeat protein WDR5 scaffolds various epigenetic writers, and is a critical component of the mammalian SET/MLL histone methyltransferase (HMT) complex. Dysregulation of MLL1 catalytic function is associated with mixed-lineage leukemia, and antagonism of the WDR5-MLL1 interaction by small molecules has been proposed as a therapeutic strategy for MLL-rearranged cancers. Small molecule binders of the “WIN” site of WDR5 that cause displacement from chromatin have been additionally implicated to be of broader use in cancer treatment. In this study, a fragment screen with Surface Plasmon Resonance (SPR) was used to identify a highly ligand-efficient imidazole-containing compound that bound in the WIN site. The subsequent medicinal chemical campaign—guided by a suite of high-resolution co-crystal structures with WDR5—progressed the initial hit to a low micromolar binder. One outcome from this study is a moiety that substitutes well for the sidechain of arginine; a tripeptide containing one such substitution was resolved in a high-resolution structure (1.5 Å) with a binding mode analogous to the native tripeptide. This novel scaffold therefore represents a possible means to overcome the potential permeability issues of WDR5 ligands that possess highly basic groups like guanidine. The series reported here furthers the understanding of the WDR5 WIN site, and functions as a starting point for the development of more potent WDR5 inhibitors that may serve as cancer therapeutics.

## Developing molecules that target an essential bacterial protein for broad-spectrum antibiotics

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Given the increasing incidence of antibiotic-resistant infections, antibiotics that employ new strategies are urgently needed. Bacterial survival is dependent on proper function of the signal recognition particle (SRP; a protein:RNA complex) and its receptor (FtsY). Deletions of SRP/FtsY components or mutations that inhibit SRP/FtsY interactions have been shown to result in severe bacterial growth defects or cell death (1). A unique set of interactions within the FtsY:SRP-RNA complex represent a novel target that has never been previously pursued for antibiotic development. Furthermore, the nature of the interactions is unique to prokaryotes, minimising any potential off-target effects in humans.

We have previously used a Fragment Based Drug Design approach to identify fragment hits that bind FtsY (2). We are currently using a combined approach involving medicinal chemistry, structure-guided design, molecular docking, biophysical binding experiments and whole-cell activity assays to develop structure-activity relationships (SAR) for the fragment hits and their derivatives. Here, we will report some of our recent work towards developing a set of tool compounds with increasing binding affinity, specificity and biological activity; with the ultimate goal of developing them into antibiotic leads.

1. Siu, F. Y., Spanggord, R. J., and Doudna, J. A. (2007) SRP RNA provides the physiologically essential GTPase activation function in cotranslational protein targeting. *Rna* **13**, 240-250
2. Faoro, C., Wilkinson-White, L., Kwan, A. H., and Ataide, S. F. (2018) Discovery of fragments that target key interactions in the signal recognition particle (SRP) as potential leads for a new class of antibiotics. *PLoS one* **13**, e0200387

## Development of Zika protease inhibitors using fragment-based drug design

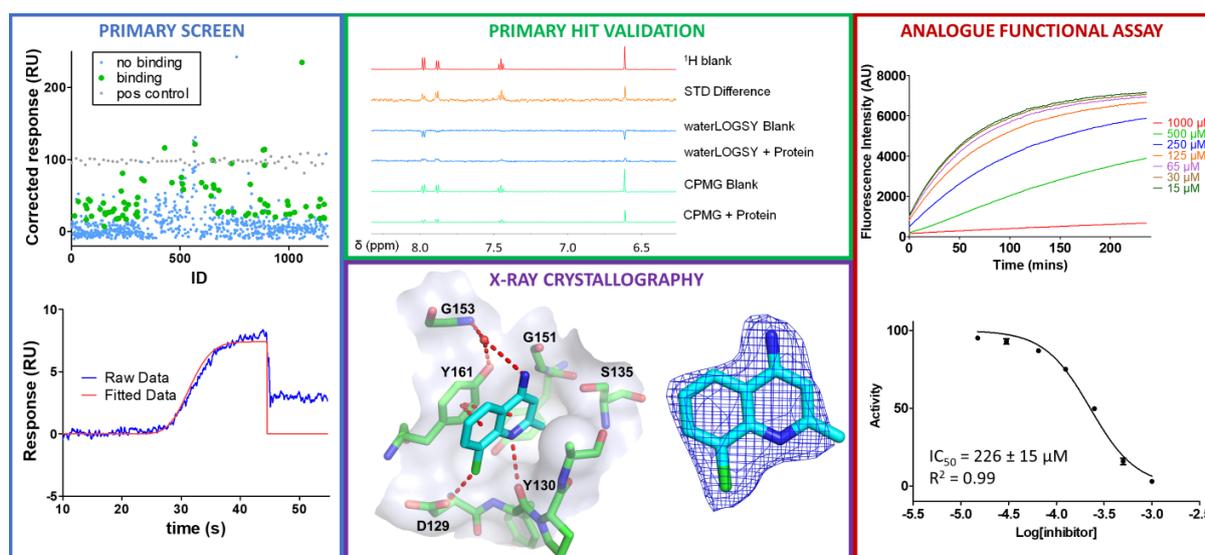
Grace M. Curtis<sup>1</sup>, Menachem Gunzburg<sup>1</sup>, Hong Yang<sup>1</sup>, Martin L. Williams<sup>1</sup>, Hang Nguyen<sup>1</sup>, Biswaranjan Mohanty<sup>1</sup>, Indu Chandrashekar<sup>1</sup>, Dahai Luo<sup>2</sup>, Bradley C. Doak<sup>1</sup>, Martin J. Scanlon<sup>1</sup>

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Zika virus is a mosquito-borne *flavivirus* which is linked to microcephaly in fetuses and Guillain-Barré syndrome in adults.<sup>1,2</sup> Currently there are no treatments or vaccines available for Zika virus. Zika NS2B:NS3 protease (ZiPro) is an attractive drug target due to its key role in viral replication and availability of 3D protein model determined by X-ray crystallography.<sup>3</sup> However there are no reported high affinity, small molecule inhibitors of Zika protease.

We conducted a fragment screen against Zika protease using ligand-detect NMR, protein-detect NMR and surface plasmon resonance (SPR) to identify and validate hits. Analogues of the validated hits were then selected and screened, which generated preliminary SAR for multiple series that also showed inhibitory activity in a functional enzyme assay. In addition, crystal structures of the ligand bound were obtained for two of the fragment hits to aid medicinal chemistry elaboration. These represent promising starting points for development of high affinity compounds as potential Zika virus inhibitors.



1. Cao-Lormeau, *et al. Lancet*. **2016**, 387, 1531-1539.
2. Rasmussen, *et al. N. Engl. J. Med.* **2016**. 374, 1981-1987.
3. Kang, *et al. Trends Microbiol.* **2017**, 25, 797-808.

## Efficient development of fragments via biophysical screening of crude parallel libraries

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The efficient development of fragment hits into higher affinity tool compounds and leads is challenging. It can be especially difficult when structural data on the binding mode of fragments to the target is lacking. Here we present our workflow for the Rapid Elaboration of Fragments into Leads (REFiL). This approach employs microscale parallel synthesis to generate libraries of compounds, which are screened without extensive purification and characterisation using biophysical methods. We have chemoinformatically designed reagent libraries (~ 96 reagents) to support the most commonly used chemical transformations. The reagents are designed to maximise “3D 1-point pharmacophore” diversity in the resulting library and can efficiently explore the binding site from multiple vectors of a fragment hit. With difficult targets, we typically identify fragment hits which bind with low affinity ( $K_D > 500$   $\mu$ M). In this situation, we have employed X-ray crystallography to screen a parallel library and identified multiple higher affinity analogues that make unexpected but favourable interactions with the target. For higher affinity fragments ( $K_D < 200$   $\mu$ M) we have used the approach of off-rate screening by SPR<sup>1</sup> to develop multiple higher affinity ( $K_D < 10$   $\mu$ M) ligands. This approach does not require structural information. These techniques are exemplified against two targets and demonstrate the power and utility of biophysical screening coupled to chemoinformatically designed parallel libraries for efficient development of fragments.

1. Murray, J. B. *et al. J. Med. Chem.* **2014**, 57, 2845–2850.

## Similarity implicated exploration of the fragment galaxy

Bilal Nizami<sup>1</sup>, Gábor Imre<sup>1</sup>, Ákos Tarcsay<sup>1</sup>

<sup>1</sup>*ChemAxon*

Fragment space is a marginal subgroup of the druggable universe of chemical entities considering its population. However, it spans astonishingly high number of structures. GDB-13, the largest virtually enumerated collection counts nearly 1 billion structures of fragment size compounds. Chemically intelligent navigation in this vast dataset demands special purpose solutions. Our study focuses on making the very large chemical datasets live by ultra fast similarity search method. As a use-case, we search GDB-13 to finding similar structures to that of the FDA approved drugs not exemplified in the space of patented structures available within SureChEMBL. This framework represents a scaffold hopping approach exploiting the GDB-13 under the hood and demonstrates the benefit of using MadFast Similarity Search technique to support early phase drug discovery.

## Towards the development of herbicide cocktails with a novel mode of action

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Weeds pose a threat to ecosystems and agricultural industries. Herbicides are currently the most effective and economical weed management strategy however weeds are rapidly evolving resistance. Many successful herbicides inhibit the biosynthesis of amino acids in plants, however lysine biosynthesis is yet to be exploited as a herbicide target. Our goal is therefore to identify inhibitors of lysine biosynthesis enzymes and validate them as novel herbicide leads. Additionally, we aim to circumvent the emergence of resistance by combining inhibitors into a 'herbicide cocktail.' Such a cocktail should prevent the emergence of resistant weeds by ensuring that a single resistance-conferring mutation does not result in plant survival. The present research is investigating the structure-activity relationship of small molecule inhibitors of lysine biosynthesis. Screening is being conducted *in vitro* against recombinant proteins, as well as *in planta* against pre and post emergent *Arabidopsis thaliana* plants. The relationship between lead inhibitor mechanisms and physiological outcomes is being probed using x-ray crystallography, toxicity assays, and systems biology. Lastly, we will ascertain if any synergistic effects may be afforded by combining these inhibitors and investigate potential resistance mechanisms.

## Inhibitors of Lysine Biosynthesis as Novel Herbicides

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Invasive weeds are a significant contributor to decreased yield and quality of crops globally, as well as posing a threat to infrastructure, livestock and native flora, ultimately culminating in an enormous economic burden.<sup>1-3</sup> Traditionally, herbicides have been used the most efficient and economic option for the control of noxious weeds.<sup>4</sup> However, weeds have now developed resistance to almost all known herbicide modes of action, yet there has been a void in herbicide discovery for upwards of 30 years.<sup>5-7</sup> This study investigates the efficacy of inhibiting lysine biosynthesis in plants as a novel herbicide approach.<sup>8</sup> Specifically, we focus on the enzyme dihydrodipicolinate synthase (DHDPS), which catalyses the first committed and rate limiting step of the lysine biosynthetic pathway.<sup>8</sup> We have employed a combination of X-ray crystallography and rational inhibitor design, to generate a novel class of inhibitors that target a previously unknown binding site within plant DHDPS. Furthermore, we have shown that these inhibitors exhibit low micromolar efficacy both *in vitro* and *in planta* using enzyme kinetics and pre- and post-emergence assays, respectively. Thus, this study provides proof-of-concept that inhibiting lysine biosynthesis represents an attractive target for the development of herbicides with a novel mode of action to combat noxious weeds.

1. Sinden, J.; CRC for Australian Weed Management *The economic impact of weeds in Australia: report*; The CRC: Glen Osmond, South Aust., 2004; ISBN 978-1-920932-47-3.
2. Vats, S. Herbicides: History, Classification and Genetic Manipulation of Plants for Herbicide Resistance. In *Sustainable Agriculture Reviews*; Lichtfouse, E., Ed.; Springer International Publishing: Cham, 2015; Vol. 15, pp. 153–192 ISBN 978-3-319-09131-0.
3. Oerke, E.-C. Crop losses to pests. *The Journal of Agricultural Science* 2006, 144, 31–43.
4. Hay, J.R. Gains to the Grower from Weed Science. *Weed Science* 1974, 22, 439–442.
5. Duke, S.O. Why have no new herbicide modes of action appeared in recent years? *Pest Management Science* 2012, 68, 505–512.
6. Heap, I. Global perspective of herbicide-resistant weeds. *Pest Management Science* 2014, 70, 1306–1315.
7. Heap, I. The International Survey of Herbicide Resistant Weeds. <http://www.weedscience.com> 2019.
8. Griffin, M.D.W.; Billakanti, J.M.; Wason, A.; Keller, S.; Mertens, H.D.T.; Atkinson, S.C.; Dobson, R.C.J.; Perugini, M.A.; Gerrard, J.A.; Pearce, F.G. Characterisation of the First Enzymes Committed to Lysine Biosynthesis in *Arabidopsis thaliana*. *PLoS ONE* 2012, 7, e40318.

## Rapid Elaboration of Fragments into Leads (REFiL) as applied to BRD3-ET

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The bromodomain and extra-terminal domain (BET) family of proteins, bromodomain-containing (BRD)2, 3, 4 and T, are a class of epigenetic regulators that have been implicated in a number of diseases but most significantly cancer. Despite concentrated efforts in the development of BET inhibitors with a significant number currently in clinical trials,<sup>1</sup> the understanding of how the BET-family of proteins regulate gene expression is not well established. In order to delineate some of the molecular mechanisms by which these proteins function we have been studying the ET domain of BRD3.<sup>2</sup>

Recently we have applied a fragment-based approach to develop inhibitors of BRD3-ET, utilising our in house REFIL (Rapid Elaboration of Fragments into Leads) technique. We utilised Off-Rate Screening by SPR to rapidly evaluate small molecule binding.<sup>3</sup> Examination of the SPR off rates enabled the identification of potential leads, from an initial hit, from crude reaction products. Solution phase parallel synthesis in 96 well plates was developed using a set of reagents that enabled the most efficient coverage of chemical space. This combined with a computational analysis of suitable vectors for expansion enabled us to rapidly improve binding affinity upwards of 30-fold in 2 rounds of screening off a single vector.

The work presented will demonstrate REFIL as applied to BRD3-ET and how this strategy could be applied to a number of proteins for an efficient method of fragment evolution to drug leads.

1. M. Perez-Salvia, M. Esteller *Epigenetics* **2017**, *12*, 323.
2. D. C. Wai, T. N. Szyszka, A. E. Campbell, C. Kwong, L. E. Wilkinson-White, A. P. G. Silva, J. K. K. Low, A. H. Kwan, R. Gamsjaeger, J. N. Chalmers, W. M. Patrick, B. Lu, C. R. Vakoc, G. A. Blobel, J. P. Mackay *J. Biol. Chem.* **2018**, *293*, 7160-7175.
3. J. B. Murray, S. D. Roughley, N. Matassova, P. A. Brough *J. Med. Chem.* **2014**, *57*, 2845-2850.

## REFiL – an efficient workflow for early stage hit-to-lead optimisation in FBDD

Beatrice Chiew<sup>1,2</sup>, Menachem Gunzburg<sup>1,2</sup>, Stephen Headey<sup>4</sup>, Linsey Ingerman<sup>3</sup>, Caroline Foley<sup>3</sup>, Stephen Frye<sup>3</sup>, Bradley Doak<sup>1,2</sup> and Martin Scanlon<sup>1,2</sup>

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Fragment based drug design (FBDD) is an established means of drug discovery. A key difficulty in working with fragments is that initial fragment hits generally display weak binding. As such, there is a need to generate a large number of analogues to improve potency. Regarding this, parallel synthesis is an efficient means of generating large numbers of compounds.

Slow dissociation from a given target drives improvements in potency. As such, Off-rate Screening (ORS) via Surface Plasmon Resonance (SPR) provides a means to evaluate products of parallel synthesis.<sup>1</sup> Furthermore, we may take advantage of the sensitivity of this assay such that parallel synthesis may be conducted on microscale, and there is little need for purification as we are able to distinguish the slowest dissociating component from the reaction. This further increases the efficacy of early stage optimisation as purification of compounds is often lengthy, and we need not waste this time pursuing undesirable analogues. This approach relies on structural information and unfortunately during early stage development, it is often not feasible to obtain structural information of the initial fragments bound to the target.

We present an efficient means to overcome scarce structural information using a Rapid Expansion of Fragments into Leads (REFiL) workflow. This highlights targeted analogue sets to establish SAR and parallel small scale synthesis using typical medicinal chemistry reactions and chemoinformatically designed diverse reagents sets to efficiently explore chemical space around a vector. With high-throughput means to assess synthesis conversion (LCMS) and evaluate compound binding (ORS by SPR); kinetic, affinity and selectivity information can be rapidly acquired.

We demonstrate the practical application of REFiL to overcome key difficulties in fragment development.

1. Murray, J. B. et al (2014), "Off-Rate Screening (ORS) By Surface Plasmon Resonance. An Efficient Method to 2. Kinetically Sample Hit to Lead Chemical Space from Unpurified Reaction Products", *J. Med Chem*, 57, 2845.

## Chemical deuteration at the Australian National Deuteration Facility

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<sup>1</sup>National Deuteration Facility, Australian Nuclear Science and Technology Organisation, Lucas Heights, Sydney, Australia. [www.ansto.gov.au/ndf](http://www.ansto.gov.au/ndf)

The National Deuteration Facility (NDF) at the Australian Nuclear Science and Technology Organisation (ANSTO) provides deuteration for a diversity of molecules and applications. Deuterium (<sup>2</sup>H or D) is a naturally occurring stable isotope of hydrogen (<sup>1</sup>H or H). Deuteration can provide contrast and improved resolution to assist investigations into the relationship between molecular structure and function of molecules of both biological and synthetic origin. Molecular deuteration of organic compounds significantly increases the options in complex structure function investigations using neutron scattering and reflectometry, nuclear magnetic resonance (NMR), mass spectrometry (MS) and other techniques but there had been limited global initiatives in the production and thus availability of complex deuterated small molecules such as lipids and fatty acids for such experiments.

The NDF has developed a suite of chemical deuteration capabilities that utilise catalysed <sup>1</sup>H/<sup>2</sup>H exchange followed by custom chemical synthesis for deuteration of small organic molecules, providing access to a large range of deuterated molecules for research and industry. The development of protocols at the NDF for a broader range of molecular classes unavailable commercially and a tailoring of deuteration approach has increased the range of systems that can be investigated using deuterated molecules. Lipids, phospholipids (including head or tail or head/tail deuterated mono-unsaturated lipids such as POPC and DOPC), heterocyclics, aromatics, surfactants, ionic liquids, branched chain alcohols (e.g. phytantriol), mineral oils, polyalkene, sugars (e.g. sucrose and trehalose), ligands, saturated fatty acids (C4 – C20), unsaturated fatty acids (e.g. oleic acid), substituted aromatics, radiotracers/radiopharmaceuticals, cholesterol and detergents have been deuterated. Some of the diverse applications that can benefit from availability of custom deuterated molecules include structural biology, thin film devices and molecular electronics, energy and gas adsorption materials, drug delivery and drug metabolism. Selected examples of chemically deuterated molecules and collaborator studies will be shown and described.

For further detail and information refer to <http://www.ansto.gov.au/ndf>

The National Deuteration Facility is partly supported by the National Collaborative Research Infrastructure Strategy – an initiative of the Australian Government.

## Deuteration of biomolecules at the Australian National Deuteration Facility.

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The National Deuteration Facility (NDF) at the Australian Nuclear Science and Technology Organisation (ANSTO) is the only facility of its type in the Southern Hemisphere, having the specialised expertise and infrastructure for both biological and chemical molecular deuteration. Deuterium (<sup>2</sup>H or D) is a naturally occurring stable isotope of hydrogen (<sup>1</sup>H or H) which contains a neutron in addition to the proton and electron found in the naturally abundant protium <sup>1</sup>H. Molecular deuteration of molecules significantly increases the options in complex structure function investigations using NMR, neutron scattering, MS and other techniques. Deuteration can provide greater contrast and improved resolution to assist investigations into the relationship between molecular structure and function of molecules of both biological and synthetic origin.

The Biodeuteration group of the NDF has developed reliable and robust methods for the deuteration and multiple isotope labelling of a broad range of proteins by recombinant expression in *Escherichia coli* BL21.<sup>1</sup> Partially deuterated and perdeuterated protein has been produced for small angle neutron scattering (SANS) and neutron crystallography investigations, with triple- (<sup>2</sup>H/<sup>15</sup>N/<sup>13</sup>C), double-labelled (<sup>2</sup>H/<sup>15</sup>N or <sup>13</sup>C/<sup>15</sup>N) and selectively-labelled protein produced for NMR studies. Further capability development is continually undertaken to expand the range of NDF methods in the deuteration of biomolecules, increasing the options available to research and industry groups. Recently microbial systems have been utilised to biosynthetically deuterate cellulose and cholesterol.

An overview of the NDF Biodeuteration facility and capabilities will be presented along with some selected examples of research utilising isotopically labelled biomolecules produced by the NDF.<sup>2,3</sup> Various modes of access are available to facility users. Since the open access user program began in 2010 we have delivered over 280 different labelled molecules to collaborators.

For further detail and information refer to <http://www.ansto.gov.au/ndf>.

The National Deuteration Facility is partially funded by the National Collaborative Research Infrastructure Strategy (NCRIS) – an initiative of the Australian Government.

1. Duff AP, Wilde KL, Rekas A, Lake V, Holden PJ. *Robust high-yield methodologies for <sup>2</sup>H and <sup>2</sup>H/<sup>15</sup>N/<sup>13</sup>C labelling of proteins for structural investigations using neutron scattering and NMR* Methods in Enzymology, **565**, 3-25 (2015).
2. Morris VK, Linser R, Wilde KL, Duff AP, Sunde M, Kwan AH. *Solid-state NMR spectroscopy of functional amyloid from a fungal hydrophobin: a well-ordered –sheet core amidst structural heterogeneity* Angewandte Chemie Int Ed, **51**, 12621-12625 (2012).
3. Emily J. Furlong, Hassanul G. Choudhury, Fabian Kurth, Anthony P. Duff, Andrew E. Whitten, and Jennifer L. Martin. *Disulfide isomerase activity of the dynamic, trimeric *Proteus mirabilis* ScsC protein is primed by the tandem immunoglobulin-fold domain of ScsB*. J. Biol. Chem. **293**, 5793–5805 (2018)

## Structure determination and elaboration of a fragment inhibitor of BpsDsbA by solution NMR spectroscopy

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Fragment-based Drug Design (FBDD) is an established and successful method in the drug discovery and development process. Here we present the results of a fragment screening campaign against a 22 kDa bacterial thiol oxidoreductase enzyme from the gram-negative pathogen *B. pseudomallei* (BpsDsbA). Although fragments were identified that bound weakly to BpsDsbA, all attempts to generate a crystal structure were unsuccessful. Therefore, solution-state NMR was used to determine the structure of BpsDsbA bound to a fragment. Elaboration through analogue search led to the identification of a higher affinity inhibitor of BpsDsbA, and a structural model was again solved by NMR. The parent fragment and analogue were both observed to bind in the same location on the surface of BpsDsbA, and both inhibit the enzyme activity of BpsDsbA measured in a peptide oxidation assay.

Subsequent <sup>1</sup>H<sup>N</sup> and <sup>15</sup>N CPMG relaxation dispersion NMR experiments revealed protein backbone dynamics in oxidised BpsDsbA on the  $\mu$ s-ms timescale. The dynamic residues are in the same region where ligand binding was observed.

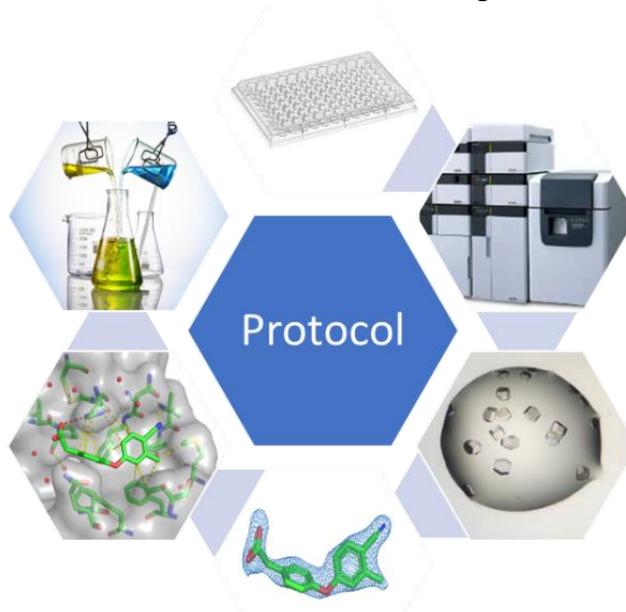
This information will be used to guide further medicinal chemistry, with the potential to develop a narrow-spectrum anti-virulence agent against *B. pseudomallei*.

## Rapid elaboration of fragments into leads by X-ray crystallography (REFIL<sub>x</sub>)

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Fragment based screening has become widely used as a method for finding starting points for the development of high affinity lead compounds. A review of the 2016 literature identified 28 examples where a fragment was developed into a lead compound.<sup>1</sup> In most cases, the initial fragment hits bound weakly to their target, and a significant medicinal chemistry effort in addition to high quality structural data was required to develop a lead compound. Advances in data collection and processing have increased the throughput of X-ray crystallography, which has made it more accessible for fragment screening and fragment elaboration. Dynamic combinatorial X-ray crystallography has also been shown to have the capability to extract and purify a known high affinity compound from a mixture of 30 possible analogues.<sup>2</sup> Herein we describe a general approach to support fragment-to-lead projects that employs X-ray crystallography as a means to analyse a library of compounds in order to identify those that bind with higher affinity. To do so we used parallel synthesis to prepare a library of amide analogues of a compound that had previously been shown to bind to the bacterial oxidoreductase enzyme DsbA. A diverse library of amines was designed to maximize chemical diversity of the resulting amide library and cover a wide pharmacophoric space. The amides were prepared using microscale parallel synthesis in a 96-well plate, verified using LCMS, and soaked into crystals of DsbA without purification. In total 4 hits were identified which gave clear density in the X-ray data and these were resynthesized on batch scale, and purified for full characterization,  $K_D$  determination and their mode of binding confirmed via X-ray crystallography.



1. Keserú, G. M.; Erlanson, D. A.; Ferenczy, G. G.; Hann, M. M.; Murray, C. W.; Pickett, S. D. Design Principles for Fragment Libraries: Maximizing the Value of Learnings from Pharma Fragment-Based Drug Discovery (FBDD) Programs for Use in Academia. *Journal of Medicinal Chemistry* **2016**, *59*, 8189-8206.
2. Congreve, M. S.; Davis, D. J.; Devine, L.; Granata, C.; O'Reilly, M.; Wyatt, P. G.; Jhoti, H. Detection of Ligands from a Dynamic Combinatorial Library by X-ray Crystallography. *Angewandte Chemie* **2003**, *115*, 4617-4620.

## Discovery and design of novel *EcDsbA* inhibitors using fragment-based design strategies.

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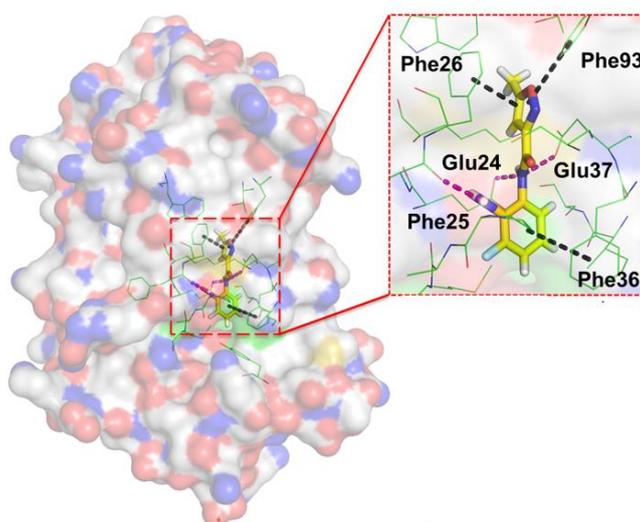
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Bacterial resistance to antibiotics has become a major global concern with numerous bacteria now resistant to almost all current antibiotics.<sup>1</sup> This highlights the urgent need for new antibacterials with novel mechanisms of action. Targeting bacterial virulence rather than bacterial growth is one approach to tackling the problem of bacterial resistance.<sup>2</sup> DsbA is a bacterial dithiol-disulfide oxidoreductase enzyme which is responsible for disulfide bond formation in Gram-negative bacteria such as *Escherichia coli*, *Burkholderia pseudomallei* and *Vibrio cholera*. DsbA plays an essential role in the folding of numerous virulence factors and bacteria lacking a functional DsbA display attenuated virulence. Therefore, DsbA is a promising and novel anti-virulence target.<sup>3</sup>

The development of DsbA inhibitors is challenging due to the large and relatively shallow groove that is the substrate binding site. In the current work we have analysed the dynamics of DsbA in conjunction with fragment screening to identify compounds that bind to a previously unreported cryptic pocket in *E. coli* DsbA. These compounds bind with moderate affinity and uncommonly slow kinetics. The discovery and development of this cryptic pocket-binding class of inhibitors is presented along with biophysical characterization, functional assays and collection of structural data on the *E. coli* DsbA-fragment complexes.



1. World Health Organisation, Antimicrobial resistance: global report on surveillance, **2014**.
2. Heras B., Shouldice S.R., Totsika M., Scanlon M.J., Schembri M.A., Martin J.L. *Nat Rev Microbiol.* **2009**, 7, 215.
3. Ireland, P. M., McMahon, R. M., Marshall, L. E., Halili, M., Furlong, E., Tay, S., Sarkar-Tyson, M. *Antioxid Redox Signal.* **2014**, 20, 606.

## In pursuit of isoform-selective inhibitors of fatty acid-binding proteins for the treatment of metabolic disorders and beyond

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Fatty-acid binding proteins (FABPs) are a family of small cytosolic intracellular lipid binding proteins that bind with high affinity to lipophilic ligands such as fatty acids and eicosanoids and mediate their intracellular transport.<sup>1</sup> FABPs are important mediators of metabolic processes and are potential targets for the development of therapeutics for metabolic diseases.<sup>2</sup> Previous studies have demonstrated that FABPs modulate the activity of nuclear hormone receptors (NHRs) in a ligand-selective manner.<sup>3</sup> We have shown that the mechanism that underpins the ability of FABPs to potentiate NHR activation by specific ligands involves the stabilization of a conformation of FABP that promotes interaction with the receptor.<sup>4</sup> Several known drugs bind to FABPs but most of these have similar binding specificities for NHRs thereby limiting their utility in selectively targeting FABPs. Development of inhibitors that display significant selectivity for FABP isoforms over nuclear hormone receptors will be valuable tools to dissect the cellular functions of FABPs.

We have conducted fragment screens against various isoforms of FABP using STD NMR. Primary STD NMR screen identified 392 hits, of which several fragments showed selectivity for the various FABP isoforms. The hits from the primary screen will be validated using protein-detected HSQC NMR and surface plasmon resonance assays against the various isoforms of FABP. Counter-screens will also be performed against nuclear hormone receptors to identify compounds that bind selectively to FABPs. Analogues of the validated hits will be screened against the FABP isoforms to determine the structure-activity relationship.

### References:

1. Kaikaus, R.M., Bass, N.M., and Ockner, R.K. (1990) *Experientia* 46, 617- 630
2. Hotamisligil, G.S. and Bernlohr, D.A. (2015) *Nat Rev Endocrinol* 11, 592-605.
3. Hughes, M. L., Liu, B., Halls, M. L., Wagstaff, K. M., Patil, R., Velkov, T., Jans, D. A., Bunnett, N. W., Scanlon, M. J., and Porter, C. J. (2015) *J Biol Chem* 290, 13895-13906
4. Patil, R., Mohanty, B., Liu, B., Chandrashekar, I. R., Headey, S. J., Williams, M. L., Clements, C. S., Ilyichova, O., Doak, B. C., Genissel, P., Weaver, R. J., Vuillard, L., Halls, M. L., Porter, C. J. H., and Scanlon, M. J. (2019) *J Biol Chem* 294, 3720 - 3734