RSC INTEREST GROUP
CHEMICAL BIOLOGY AND BIOORGANIC

UNIVERSITY OF OXFORD

Royal Society of Chemistry

Chemical Biology and Bio-organic Group

Postgraduate Symposium

Monday 3rd April 2023
Department of Pharmacology, University of Oxford

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Contents

Venue

Department of Pharmacology

Mansfield Road,
Oxford, OX1 3QT,
United Kingdom

Access is from Mansfield Road:

Travel

By rail the department is ~20 min walk from Oxford train station, head straight along George St which leads into Broad St, and turn left along Mansfield Road.

By bus/coach the department is ~15 min walk from Oxford's Gloucester Green bus terminus, which is located on George St and follow the above directions.

By car the most easily accessibly parking is at Westgate shopping centre, Queen St, OX1 1PE (~20 min walk). Take Cornmarket St, then turn right on George St and follow as above.

Symposium Rooms

Oral presentations will be held in the **David Smith Lecture Theatre** (**Level 1**).

Poster sessions will be in both the **Seminar Room** (**Level 1**) and **Common Room** (**Level 4**).

Lunch & the wine reception will be served in the **Common Room** (**Level 4**).

Online Attendees

To watch oral presentations online, please use the following Microsoft Teams meeting:
Meeting ID: 391 611 694 183; Passcode: wNM66i

Programme

9:00	**Registration & Refreshments**	
10:00	**Welcome:** **CBBG Chair Dr Sarah Barry** (King's College London) **Housekeeping:** **Dr Thomas Lanyon-Hogg** (University of Oxford)	
	Session 1 Chemical Biology in Health and Disease (David Smith Lecture Theatre, Level 1)	
10:10	GPR84 structural–functional selectivity relationship (SFSR) reveals distinct responses induced by biased agonists	**Pinqi Wang** University of Oxford
10:30	Discovery of internal ligand inhibitors targeting SHANK1 PDZ domain guided by dynamic ligation screening strategy	**Yue Li** University of Leeds
10:50	A High Throughput Screen of Genetically Encoded Cyclic Peptides Identifies a Novel Antibiotic Precursor	**Leonie Windeln** University of Southampton
11:10	Target-directed synthesis of α-helix mimetics	**Sachi Sharma** Imperial College London
11:30	**Keynote**: Breaking resistance of pathogenic bacteria by chemical dysregulation	**Prof Stephan Sieber** Technical University of Munich
12:20	**Lunch** (Common Room, Level 4)	
13:00	**Poster session 1** (Seminar Room, Level 1, and Common Room, Level 4)	
13:00	**Poster session 1 (Even)**	13:30 **Poster session 1 (Odd)**
	Session 2 Chemical Tools to Shine Light on Biological Processes (David Smith Lecture Theatre, Level 1)	
14:00	Lights, Capture, Extraction! A Photoaffinity Probe for Zinc Metalloprotein Profiling in Live Cells	**Sean McKenna** Trinity College Dublin
14:20	One Trillion Photoswitchable Cyclic Peptides: Shining Light on Questions in Chemical Biology	**Thomas Jackson** Imperial College London
14:40	Screening and Optimisation of a Molecular Tool for the Investigation of O-GalNAc Glycans	**William Browne** Francis Crick Institute
15:00	Late-stage difluoromethylation of nucleosides	**Otto Linden** University of Strathclyde
15:20	**Poster session 2 and coffee break** (Seminar Room, Level 1, and Common Room, Level 4)	
15:20	**Poster session 2 (Even)**	15:40 **Poster session 2 (Odd)**
16:00	**Keynote**: Cyclic peptides as versatile tools for chemical biology	**Prof Akane Kawamura** Newcastle Univesity
16:50	**Prizes & Closing remarks: Dr Sarah Barry**	
17:00	**Wine reception**	
18:00	**Symposium End**	

ORAL ABSTRACTS

Keynote lecture

Prof. Stephan A. Sieber,
Technical University of Munich

Breaking resistance of pathogenic bacteria by chemical dysregulation

Multiresistant bacterial pathogens such as Methicillin-resistant *Staphylococcus aureus* (MRSA) are responsible for a variety of severe infections that pose a significant threat to global health. To approach this challenge new chemical entities with an unprecedented mode of action are desperately needed. This presentation will cover our latest efforts to identify new anti-bacterial targets and corresponding chemical inhibitors. A proteome mining approach will be presented to identify cofactor-dependent enzymes as novel antibiotic targets. Small molecule cofactor mimics infiltrate the bacterial metabolic machinery leading to their incorporation in cofactor-dependent enzymes. Their analysis via mass-spectrometry revealed the function of uncharacterized proteins in important bacterial pathways as well as the identification of novel antibiotic hits along with their mode of action.

In a separate approach we identified new synthetic or natural product derived compound classes that effectively kill pathogenic bacteria. Chemical synthesis of improved derivatives led to the identification of active molecules with nanomolar potency and suitable metabolic stability. The mode of action was investigated by diverse methodologies including affinity based protein profiling (AfBPP). For example, one compound stimulates a signal peptidase correlating with enhanced secretion of extracellular proteins. These included essential cell-wall remodeling enzymes whose dysregulation likely explains the associated antibiotic effects.

Keynote lecture

Prof. Akane Kawamura,
Chemistry – School of Natural and Environmental Sciences, Newcastle University

Cyclic peptides as versatile tools for chemical biology

Peptides, which can match the complexity and structural diversity of proteins, are attractive modalities for chemical biology probes and therapeutics development. Using mRNA-display, high affinity de novo peptides, in particular structurally constraint cyclic peptides, can rapidly be developed for many different protein targets including those considered 'challenging' by small molecules. This powerful display platform couples genetically encoded library with cell-free *in vitro* translational system to generate and screen peptide libraries with immense chemical diversity ($>10^{12}$). Enriched binding peptides against the protein targets can be rapidly identified through next generation sequencing, chemically synthesised and biochemically/biophysically validated. We have developed potent and selective cyclic peptides against a wide range of proteins using this method - including inhibitors, stabilisers, and functional / affinity probes for enzymes and PPI targets. The talk will provide an overview of our recent work in this area, with a particular focus on the development and application of cyclic peptides as chemical tools to study epigenetic proteins.

Talks

T01 GPR84 structural–functional selectivity relationship (SFSR) reveals distinct responses induced by biased agonists

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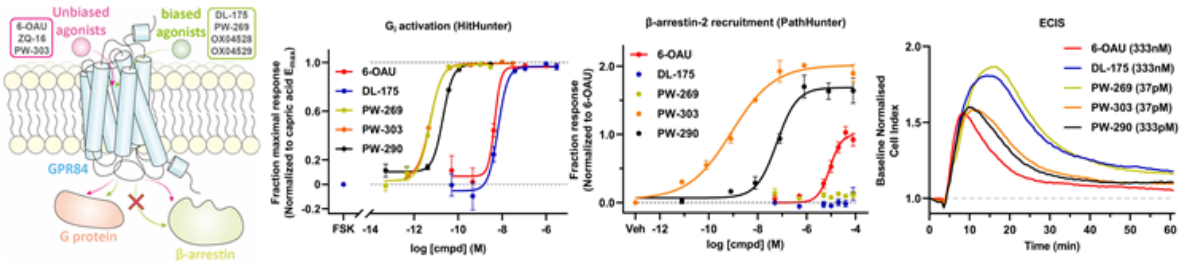

Orphan G-protein-coupled receptor 84 (GPR84) is a receptor that has been linked to cancer, inflammatory and fibrotic diseases. In our previous work we reported DL-175 as a biased agonist at GPR84 which showed differential signalling via Gαi/cAMP and ß-arrestin, but which is rapidly metabolised.[1] With extensive structure–activity relationship studies followed by structure–functional selectivity relationship investigations, highly potent ligands with high backbone similarities and low picomolar EC_{50} values (G-protein activation) are designed and synthesized with different level of bias, including highly biased agonists PW-269, OX04528, OX04529, medium biased agonist PW-290 and balanced agonist PW-303. We then conducted *in vitro* ADME, selectivity, and *in vivo* PK studies on four compounds, all of which showed appropriate metabolic stability, selectivity, and oral bioavailability that allow further *in vivo* investigations. In Electric Cell-substrate Impedance Sensing (ECIS) studies, highly biased PW-269 and DL-175 exhibited different impedance patterns than medium biased PW-290 and balanced PW303, 6-OAU at their EC_{80} concentration, with a higher maximum activation response and a slower decrease of response, indicating the differences in activating the receptor between biased and balanced ligands.

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T02 Discovery of internal ligand inhibitors targeting SHANK1 PDZ domain guided by dynamic ligation screening strategy

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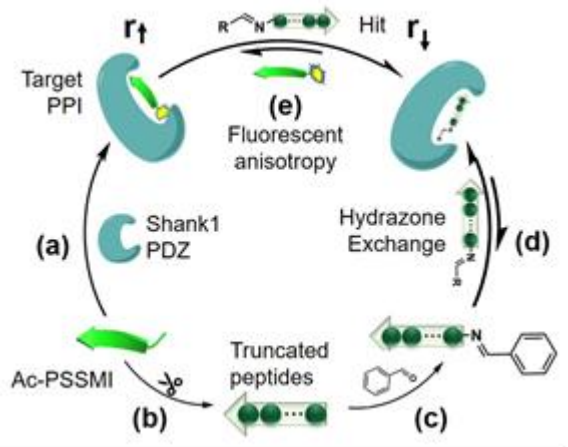
Protein-protein interactions (PPIs) have received extensive recent attention as targets for drug discovery given their key role in controlling many biological processes. In terms of studies on PPI inhibitors, rather than well-known α-helix mediated PPIs, we focus on less explored β-strand mediated PPIs in this presentation. The model PPI employed here is the SHANK1 PDZ domain[1] and a nine-mer sequence named Ac-PSSMI used as an internal template ligand for modification. Using a dynamic ligation screening strategy[2], we identified hybrid peptide-fragment hits from a library of 165 aldehyde fragments using peptide acyl hydrazones as anchors. Instead of time-consuming screening, we achieved rapid localization of peptide terminal building blocks leveraging a combination of hydrazone exchange reactions and fluorescent anisotropy assays. As a result, we obtained a series of promising peptide-small molecule hybrids with low-micromolar inhibitory activities towards interactions of SHANK1 PDZ protein with fluorophore labelled template sequence FA_PSSMI as tracer. Further study of these compounds through biophysical and structural methods will provide the basis for the design of therapeutically pertinent inhibitors of PPIs involving PDZ domains.



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T03 A High Throughput Screen of Genetically Encoded Cyclic Peptides Identifies a Novel Antibiotic Precursor

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The spread of antibiotic resistant pathogens is an increasing problem, giving rise to a need for new antibiotics.[1] To this end, we have developed an innovative high-throughput, drop-out screening strategy to produce cyclic peptides intracellularly with the goal of identifying antibiotic molecules. Our approach uses split-intein circular ligation of peptides and proteins (SICLOPPS) libraires, in combination with next generation sequencing.

As a proof of principle, we built an SXXXXX (where X is any of the 20 canonical amino acids) SICLOPPS library in *E. coli*. Up to 3.2 million different cyclic peptides were produced in these bacteria (one library member per bacterium) which only grow if the specific cyclic peptide doesn't inhibit cellular function. All DNA was then collected from the surviving cells, and deep sequenced along with the initial library to identify the missing sequences, which are potentially lethal to bacteria.

A set of python scripts were developed to deconvolute the cyclic peptide sequences and to find common motifs in those cyclic peptides. Screening of a focused library of active tetrapeptide motifs led to the identification of a series of potential hits. Expression of a cyclic peptide containing the active motif as a SICLOPPS construct was shown to inhibit bacterial growth. Furthermore, a hit peptide and a scrambled control were synthesized by solid phase peptide synthesis (SPPS) and tested in an agar diffusion assay. Here, only the hit showed growth inhibition (*via* halo formation) with 1 mg/disc loading.

This suggests that the identified sequence has an antimicrobial effect which is sequence specific, and it may be a potential lead for a novel antibiotic. We are currently working to decipher the mechanism of action of this hit and develop a better understanding of the structure-activity relationship (SAR). The technique developed is currently being expanded for the identification of antibiotics in other bacterial species.

References

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T04 Target-directed synthesis of α-helix mimetics

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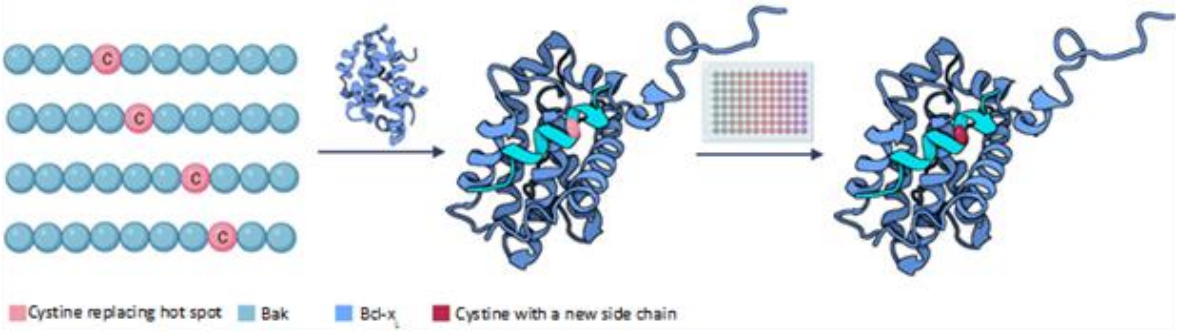
Protein-protein interactions (PPIs) play a significant role in almost all biological processes and, when misregulated, often result in disease.[1] A noteworthy example is the PPI between Bcl-xL and Bak, which has a prominent impact on apoptosis regulation and, thus, is a well validated oncological target.[2]

PPIs are commonly mediated by α-helices, with key hot spot residues contributing the majority of the binding energy.[3] As such, molecules which successfully mimic the hot spot residues found on the native helix, α-helix mimetics, have the potential to behave as a competitive inhibitors.[4] Therefore, α-helix mimicry has long been recognised as a promising strategy for the disruption or recapitulation of relevant PPIs.

This presentation will describe a novel approach to the synthesis and identification of α-helix mimetics targeting the Bcl-xL/Bak PPI via the development of an electrophilic covalent screen. This approach will allow the rapid identification of potent mimetics with high structural diversity. This project employs a target directed strategy via the synthesis and implementation of a thiol functionalised mimetic. The thiol handle is capable of reacting with electrophilic fragments in a 'click' reaction. This will enable screening for highly diverse side chains, in the presence of the target protein, expanding the chemical space beyond fragments found in nature and enabling the rapid identification of high affinity helix mimetic building blocks. By inserting the thiol functionality at different locations within the binding mimetic, the preferred binding motif at several positions can be identified to develop a ready optimised scaffold. The extent to which the electrophilic fragments react with the thiol handle is analysed using the quantitative irreversible tethering assay. This assay is founded on the principle of fluorogenic thiol quantification, whereby the progress of the reaction is monitored through the measurement of residual thiol concentration at various timepoints.



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T05 Lights, Capture, Extraction! A Photoaffinity Probe for Zinc Metalloprotein Profiling in Live Cells

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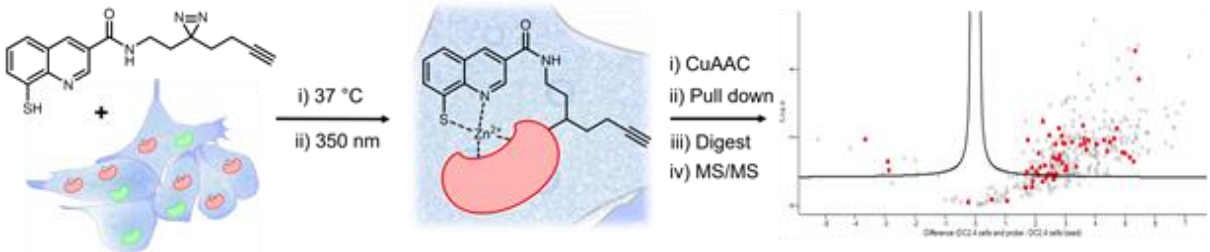
Zinc imparts vital structural characteristics and catalytic function to a wide variety of cellular proteins.[1,2] These zinc-dependent metalloproteins are exciting targets for investigation towards the development of novel therapeutics. However, their covalent capture for protein profiling studies can be highly challenging.[3] Here, we describe the design and preparation of a novel, photoactivatable affinity-based probe bearing an 8 mercaptoquinoline motif, a privileged ligand able to engage several zinc metalloproteins.[4,5] We report the synthesis of the probe and downstream proteomic analysis of metalloproteins labelled in a competitive and UV-dependent manner. Furthermore, we report the successful translation of this photoaffinity probe to labelling proteins in live DC2.4 cells. This work represents an important contribution to the library of cell-permeable probes with inducible reactivity for profiling a range of therapeutically significant biomolecules.[6]



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T06 One Trillion Photoswitchable Cyclic Peptides: Shining Light on Questions in Chemical Biology

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Cyclic peptides are an emerging class of chemical tools used to probe protein targets classically defined as undruggable. Novel cyclic peptides can be identified via RaPID mRNA display (*Random Non-standard Peptide Integrated Discovery*).[1] This platform enables the screening of >1 trillion-member cyclic peptide libraries incorporating non-proteogenic amino acids, against any protein of interest.

Despite the emerging use of cyclic peptides as novel Chemical Biology tools, the fine-tuned druggability of complex biological pathways remains elusive. Light is an incredibly powerful non-invasive stimulus to introduce temporal control within a system. Photoswitches are small molecules that undergo isomerisation via the stimulus of specific light wavelengths.[2] This project focuses upon the integration of light controllable 'photoswitches' within every macrocycle of the trillion-member library, via genetic code re-programming; inducing global conformational changes within the macrocyclic peptides structure to give switchable binding.

As a proof-of-concept, methodology for the identification of photoswitchable macrocyclic peptides has been developed against human Peptidyl Arginine Deiminase II (hPADI2). Identified peptides show up to 16-fold differential binding and inhibition of hPADI2 between each of the two light-dependent (E/Z) macrocyclic isomers. Further work to improve the binding differential is underway, honing the methodology to allow the robust identification of light-dependent photoswitchable macrocyclic peptides against any protein target of interest within complex biological systems.

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T07 Seening and Optimisation of a Molecular Tool for the Investigation of O-GalNAc Glycans

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O-GalNAc glycosylation is a ubiquitous post translational modification defined by the addition of a GalNAc sugar to a Ser or Thr. These glycans are involved in cell-cell interactions, protein folding and immune recognition. Mucins are a family of heavily O-GalNAc glycosylated glycoproteins that have been extensively linked to a range of cancer types and are believed to play a major role in enhancing the metastatic potential of primary tumours.[1,2] Previous studies have shown that the intracellular enzyme UDP-Galactose-4-Epimerase (GALE) is essential for mucin type O-GalNAc glycan biosynthesis.[3] Inhibiting the activity of GALE would inhibit O-GalNAc glycosylation and provide a valuable tool in studying the role these glycans in human biology. Additionally, GALE is potential novel therapeutic target for down-regulating mucin biosynthesis via inhibition of mucin type OGalNAc glycosylation.

Therefore, the project aim is to use a fragment-based approach to develop a selective small molecule and cell permeable inhibitor of GALE. Screening of both covalent and non-covalent fragments using intact mass spectrometry and x-ray crystallography respectively, yielded several promising hit compounds. Covalent hits show *in vitro* enzyme inhibition, whilst non-covalent hits have been elaborated based on virtual screening results to improve binding from high to low micromolar binding affinity.

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T08 Late-stage difluoromethylation of nucleosides

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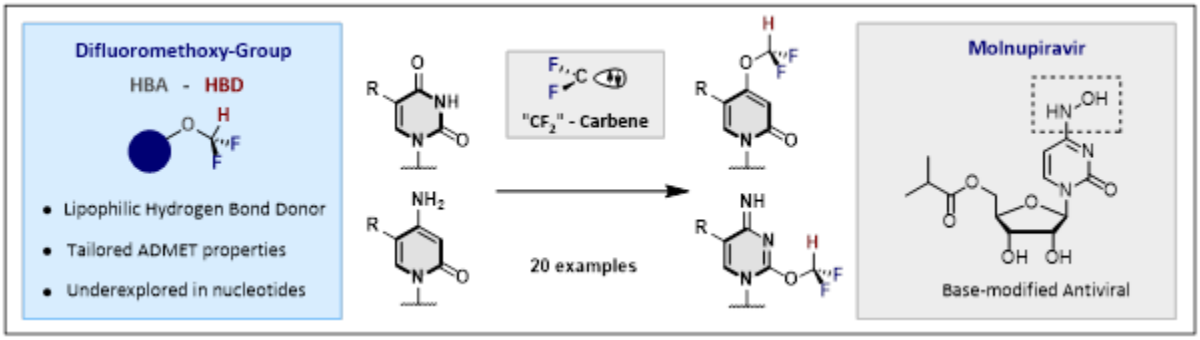
RNA based therapeutics represent the cutting edge of treatments for a range of applications, including genetic disorders, oncology, and infectious disease. To enhance the efficacy of these treatments, extensive modifications of the nucleoside scaffold are employed to enhance binding affinity and pharmacokinetic parameters. Difluoromethyl (CF_2H) modifications are used extensively throughout medicinal chemistry due to the associated high lipophilicity, polarity, and enzymatic stability, whilst the polarisation of the CH bond imparts hydrogen bond donating ability.[1] Thus far, utility of this modification in nucleic acid chemistry remains underexplored due to poor selectivity and the harsh reaction conditions required for installation.[1]

Through utilisation of state-of-the-art difluorocarbene chemistry, we have demonstrated that regioselective late-stage electrophilic difluoromethylation of nucleosides can be achieved using deceptively mild conditions.[2] Selective reaction at the 4-O position can be achieved on uridine nucleobases, whilst cytidine demonstrates high reactivity for the 2-O position. These findings have allowed for modification of a range of natural and un-natural nucleosides, which are under investigation for viral polymerase inhibition. Furthermore, reaction on the ribose sugar can be achieved through application of adequate protecting group systems.



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POSTER TITLES

Poster presentations

Posters sessions are held in the **Seminar Room** (**Level 1**) and **Common Room** (**Level 4**).

Even numbered posters will present in the first half of each session; odd numbered posters will present in the second half of the session.

01 Towards a Cure for Malaria: Development of a Covalent Inhibitor of PfCLK3 | Skye Brettell, University of Glasgow

02 A Multistep High-Throughput Chemistry Direct-to-Biology Platform to take PROTAC Synthesis and Testing to a New Level | Rebecca Stevens, GSK, Stevenage, UK/University of Strathclyde

03 Identification of GDNF peptide mimetics for Parkinson's Disease | Emily Atkinson, University College London

04 Photoaffinity Labelling using the Fungicide Mandipropamid as a Probe | Jacob Webb, University of Leeds

05 Affinity based protein profiling of MDM2 inhibitor, Navtemadlin | Amrita Date, Imperial College London

06 N-acyloxymethyl-phthalimides deliver genotoxic formaldehyde to human cells | Liam Lewis, University of Leicester

07 Global Target Profiling of Bromodomain Inhibitors | Ludwig Bauer, University of Oxford

08 Elucidating the activation cycle of the stress-response kinase GCN2 | Melissa Marx, University of Oxford

09 Developing chemical probes for investigation of human neuraminidase isoforms | Emma Wadforth, Newcastle University

10 High Throughput Production of Chemically Linked Bispecific Antibody Fragments | Caitlin Fawcett, GSK, Stevenage, UK/University of Strathclyde

11 VHL and UPS: delivering PROTACtion | Johanna Fish, University of Southampton/University of Sussex

12 Breaking the Barrier of Antimicrobial Resistance: Exploring the Mechanism of Action of Calcium-Dependent Antibiotics Cadasides and Malacidins | Katharina Webhofer, University College London

13 Chemical and enzymatic macrocyclisation of antibiotic peptides | Yaoyu Ding, King's College London

14 Development of Lanthanide Tags for Site Selective Labelling of Peptides and Proteins | Lydia Topping, Loughborough University

15 Peptide Technologies to Probe Chemokine Specificity | Tim Bell, Newcastle University

16 Developing inhibitors of Bacterial DNA-repair and SOS Response Pathways | Jacob Bradbury, University of Oxford

17 Cryopreservation of Liver-Cell Spheroids towards 'off the shelf' 3-D cellular models | Yanan Gao, University of Warwick/Southern University of Science and Technology, China

18 High throughput screening and identification of synthetically lethal cyclic peptides in MTAP deletion cancer cell lines | Monika Papayova, University of Southampton

19 Revealing the detail in i-Motif DNA Structures | Effrosyni Alexandrou, University College London

20 Design of lanthanide probes for time-resolved detection of peroxynitrite in specific cellular compartments | Jamie Webb, University of Loughborough

21 The biosynthesis of *Daphniphyllum* alkaloids | Barbara Radzikowska, University of York

22 Elucidating the biosynthesis of *Securinega* alkaloids | Catharine Wood, University of York

23 Chemical Biology Approaches to Explore the Ligandability of TRIM E3 Ligases | Katherine McPhie, The Francis Crick Institute

24 New Methods for Scaled Synthesis of Nucleoside 5'-Phosphates | Alex Greer, Durham University

25 Assessment of the Bioorthogonality of the Nitrile Imine 1,3-Dipole | Mhairi Gibson, University of Strathclyde

26 Functional characterisation of yet-unexplored methylated lysine reader domains in epigenetic regulation | Filomena Saulino, Newcastle University

27 Development and Biological Evaluation of Novel α-Helix Mimetic Prodrugs as Leads for Prostate Cancer Treatment | Natalia Swiatek, Imperial College London

28 Chemical tools to understand fatty acid uptake, trafficking, metabolism and signalling in type 2 diabetes | Ríona Devereux, University of Oxford

29 Understanding the activity of the P450 RufM | Jessica Peate, King's College London

30 Exploiting Dual-Ligand Cooperativity for Enhancing Enzymatic Inhibition of Pin1 Prolyl Isomerase | Adem Ozleyen, University of Leicester

31 Small Molecules to Control IFN-I Induced Neuroinflammation | Kallie Friston, Newcastle University

32 Modulating protein-surfactant interactions for enhanced protein thermal stability and bioactivity| Jiaming Mu, Queen's University Belfast

33 Glycopeptide Probes in Pathogen Detection| Liam Tucker, University of East Anglia

34 The development of novel near-infrared squarylium dyes towards new antimicrobial agents | Sarah Brennan, University of Central Lancashire

35 Using Phage Display to Identify Novel Glycopeptide Ligands for Target Lectins | Hassan Boudjelal, University of East Anglia

36 Targeting i-Motif DNA of ALOX5 Promoter with Small Molecule Ligands | Robert Yuan, University College London

37 The development of novel thioxanthone photoantimicrobials | Natalie Valentine, University of Central Lancashire

38 Exploring the Potential Antimicrobial Activity of a Range of Heptamethine Cyanine Dyes | William Stockburn, University of Central Lancashire

39 Developing a biophysical toolbox to study protein molecular glue kinetics | Beth Thurairajah, University of Leicester

POSTER ABSTRACTS

P01 Towards a Cure for Malaria: Development of a Covalent Inhibitor of PfCLK3

Skye B. Brettell*, Andrew. B. Tobin¥, Andrew G. Jamieson*

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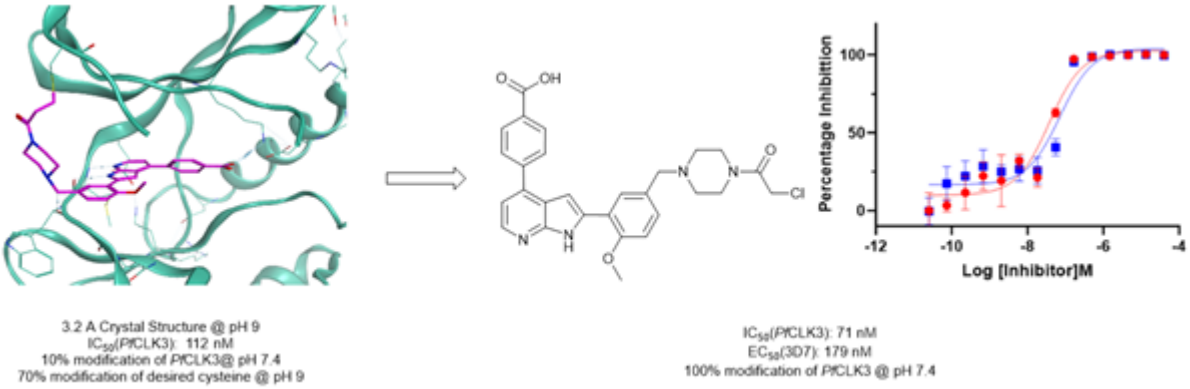
Despite global efforts, malaria continues to devastate the developing world.[1] *Plasmodium falciparum* (Pf), is the parasite responsible for most malaria mortality.[2] It has developed resistance to frontline drugs, which has now spread across multiple regions of the globe.[3] In 2020 malaria caused an estimated 627,000 deaths, a 12% increase from 2019.[1] There is therefore an urgent need for new antimalarials with novel mechanisms of action to combat this trend.

A promising class of drug not yet utilised in malarial therapeutics are kinase inhibitors, which already represent 20-33% of global drug discovery efforts.[4] An emerging trend within this field is the development of covalent kinase inhibitors, which benefit from increased potency and improved pharmacodynamic properties.[5]

In this work, a covalent inhibitor of the essential malarial kinase PfCLK3 has been rationally designed from the reversible inhibitor TCMDC-135051.[6] TCMDC-135051 offers prophylactic, transmission blocking, and curative potential.[7] Selection of a non-conserved nucleophilic cysteine residue for covalent attack is hoped to improve the selectivity of this hit molecule. Molecular modelling has allowed structure-guided drug design of several electrophilic ligands. Synthesis, whole protein mass spectrometry, and X-ray crystallography have yielded a compound which covalently binds the desired cysteine of PfCLK3 at pH 9. An optimised inhibitor demonstrates covalent inhibition at physiological pH and low nanomolar potency. This represents a promising lead compound in the fight against malaria.



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P02 A Multistep High-Throughput Chemistry Direct-to-Biology Platform to take PROTAC Synthesis and Testing to a New Level

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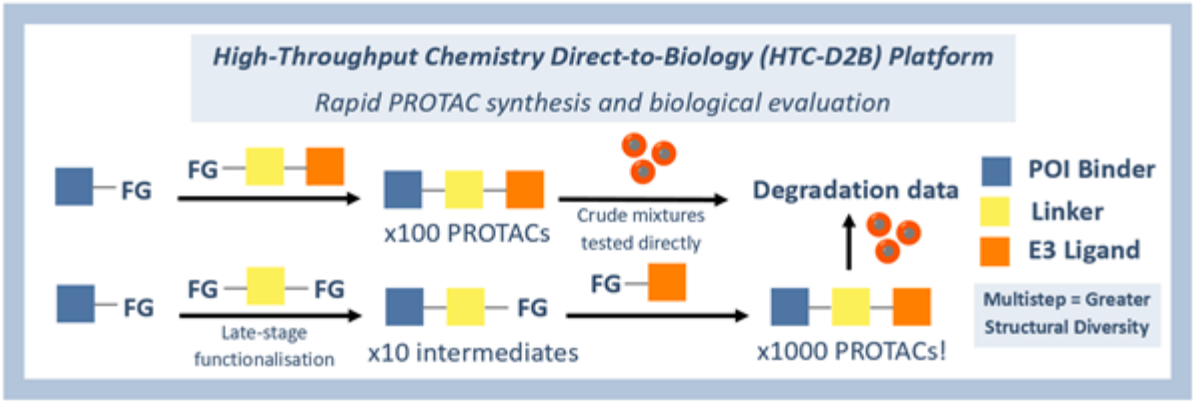
Proteolysis Targeting Chimeras (PROTACs) are a rapidly evolving new drug modality that is currently sparking great excitement in the pharmaceutical and biotechnology industries. Their unique mechanism of action offers opportunity to target the traditionally 'undruggable' parts of the proteome, alongside a series of other advantages for the development of new drug candidates.

Despite substantial investment into PROTACs over the last two decades, their synthesis and design remain relatively underexplored, resulting in limited chemical space exploration. This PhD project aims to develop a multistep platform for the synthesis and biological evaluation of PROTACs, incorporating the use of high-throughput chemistry (HTC) and modern chemical transformations into their synthetic routes. Employing these advanced chemical transformations at a late-stage in PROTAC synthesis will not only increase the rate of analogue synthesis, but also explore much more diverse chemical space in the PROTAC field, in an efficient and sustainable manner. Carrying out this chemistry in nanoscale plates followed by plate-based testing of the crude reaction mixtures eliminates the bottleneck of purification. Furthermore, a cellular degradation readout for hundreds of PROTACs at once allows for a rapid evaluation of chemical matter and significantly accelerates the hit identification and lead optimisation processes, as the results can inform future generations of PROTAC design.

Within this poster, we will outline how the use of a multistep high-throughput chemistry direct-to-biology platform was utilised to synthesise a large set of 900 novel BRD4 PROTACs in a single nanoscale reaction plate, identifying many highly potent hits.



P03 Identification of GDNF peptide mimetics for Parkinson's Disease

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Parkinson's disease is the second most prevalent neurodegenerative disorder, affecting over 6.1 million individuals worldwide and with no cure, there is an urgent need for advanced therapeutics.[1] Glial cell-derived neurotrophic factor (GDNF) has shown promise in clinical trials when locally delivered to the brain, however patients required repeated dosing due to the short half-life *in vivo*.[2] Peptide growth factor mimetics can be used to overcome this, with an increase in stability and a decrease in size. Therefore, the aim was to design a novel GDNF mimetic using rational design and phage display screening methods.

From inspection of the GDNF-GFRα1 crystal structure and the alanine mutagenesis studies, two GDNF hot spot sequences were identified. Hits from phage display screening have been sequenced and GDNF mimetics will be rationally designed. Mimetics are currently being synthesised using solid-phase peptide synthesis and tested in GFRα1 binding assays.

SpotOn and Peptiderive applications were utilised to identify key amino acids contributing most to GDNF-GFRα1 binding, known as hot spots, by performing theoretical alanine mutagenesis studies on GDNF to calculate the change in ΔG binding. Commercial phage-displayed 7-12-mer linear and cyclic peptide libraries were screened against GFRα1 to identify tightly binding sequences.

From inspection of the GDNF-GFRα1 crystal structure and the alanine mutagenesis studies, two GDNF hot spot sequences were identified. Hits from phage display screening have been sequenced and GDNF mimetics will be rationally designed. Mimetics are currently being synthesised using solid-phase peptide synthesis and tested in GFRα1 binding assays.

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P04 Photoaffinity Labelling using the Fungicide Mandipropamid as a Probe

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Oomycetes are phytopathogens responsible for some of the most significant plant diseases and their rapid development of resistance to fungicides is cause for concern. Uncovering the mode of action of fungicides is important for design of bioactive compounds. Phenotypic drug discovery (PDD) is a common method employed to identify bioactive compounds. Unfortunately, this approach leaves targets unclear. The aim of this project is to establish a chemical proteomics approach to define the targets of fungicides in oomycete pathogens.

Metalaxyl and mandipropamid are two oomyceticides used for control of the oomycete phytopathogens *Phytophthora infestans* and *Phytophthora capsici*. Our aim is to functionalise these compounds with photoaffinity labels – allowing the formation of a covalent bond between the fungicide and its target protein when exposed to UV radiation. With the aid of click chemistry, the fungicide – with the target protein appended – can be isolated from the cell lysate. The target protein can then be sequenced and identified.

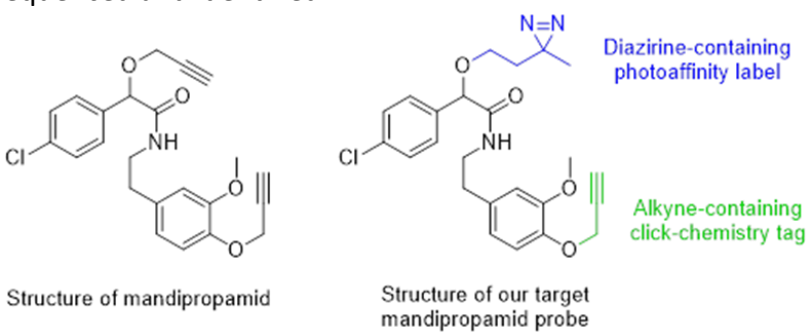

Figure 1: The structures of mandipropamid (a fungicide used for control of oomycete pathogens) and our target mandipropamid probe.

Work so far has focussed on mandipropamid. A variety of probes have been designed based on literature structure activity relationship and their chemical synthesis has been completed (the structure of one of these probes is shown above). Chemical assays have been carried out to assess the probes' potency towards *Phytophthora capsici*. Work is currently focussed on carrying out photoaffinity labelling workflows on each probe to identify their active binding partners. A control probe has also been synthesised and tested for activity.

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P05 Affinity based protein profiling of MDM2 inhibitor, Navtemadlin

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MDM2 is an E3 ubiquitin-protein ligase which acts as a key negative regulator of the tumour suppressor protein p53.[1] The p53/MDM2 protein-protein interaction has been identified as a promising, yet highly challenging target in cancer therapeutics. Several compounds have been reported to act as competitive inhibitors of this interaction, however, none have yet been approved for clinical use.[2] Navtemadlin (formerly AMG-232) is the "best-in-class" small molecule inhibitor of MDM2,[3] and is currently being tested in Phase II/III clinical trials for the treatment of various cancers.[4]

While the binding affinity, phenotypic activity, and pharmacokinetic parameters of this molecule have been extensively studied, little is known about its selectivity for MDM2. This project aims to utilize photoaffinity labelling and affinity-based protein profiling to carry out the proteome-wide target profiling of Navtemadlin. To achieve this, photoaffinity probes of the molecule have been synthesised and their activity and binding affinity have been benchmarked against that of the parent molecule (Figure 1). These are now being used in mass spectrometry-based experiments, to enable the identification of off-targets of Navtemadlin.

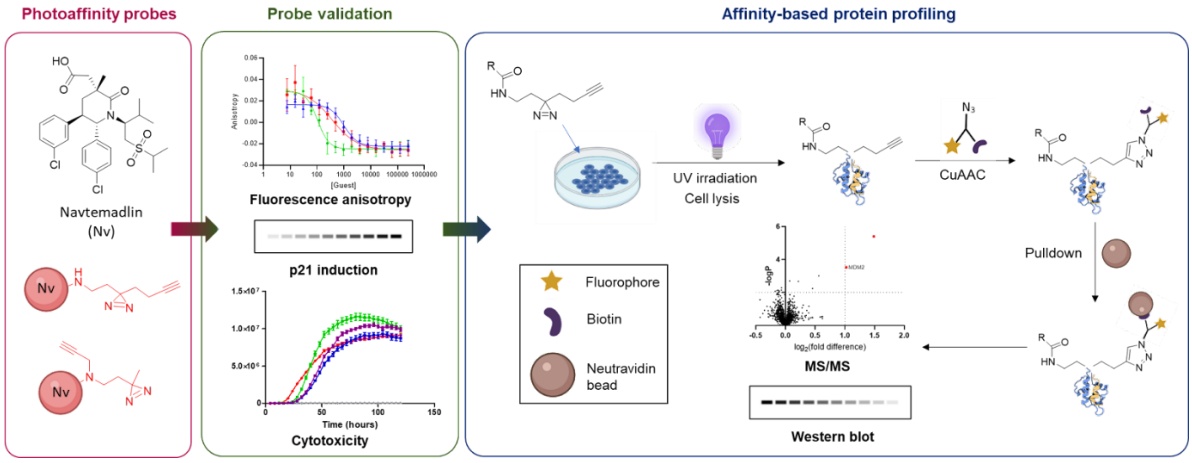

Figure 1: Overview of experiments conducted for probe validation and affinity-based protein profiling

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P06 N-acyloxymethyl-phthalimides deliver genotoxic formaldehyde to human cells

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Formaldehyde (HCHO) is a small and highly reactive electrophile which plays wide-ranging roles in human biology. Above baseline levels, HCHO exhibits toxic effects in humans; however, it is also a key metabolite in essential thymidine biosynthesis and is endogenously produced from a range of enzymatic processes such as histone demethylation. Due to its reactivity and volatility, studying HCHO in a cellular environment is difficult.

This work outlines the development of small molecule HCHO releasers designed for use in a cellular environment. These toolkit compounds are composed of N-acyloxymethyl-phthalimide moieties which are cleaved *in-vitro* by the action of esterases to yield transient hydroxymethyl-phthalimide. This intermediate spontaneously decomposes to yield formaldehyde, phthalimide and a carboxylate product. The biochemistry and stability of these compounds in an aqueous environment is characterised by NMR spectroscopy and degradation products are characterised.

Treatment of human cell lines with the tool compounds led to comparable or less-toxic effects than HCHO treatment but increased intracellular formaldehyde levels. Furthermore, the lead compound PFAc induced cell death in a gemcitabine-resistant pancreatic cancer cell line. Overall, these bench-stable, solid tool compounds enable controlled and quantitative HCHO release in cells, and will therefore stimulate ongoing functional and biomedical studies.

P07 Global Target Profiling of Bromodomain Inhibitors

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Chemical probes that target bromodomain-containing proteins (BCPs) have enabled researchers to dissect their role in epigenetic gene regulation. Drug candidates have been advanced to clinical trials to leverage their therapeutic potential.[1] However, the proteome-wide target space of many of these inhibitors has not been evaluated even though the knowledge of overall selectivity is crucial to deconvolute the exerted phenotype. Here, we designed a set of affinity probes based on bromodomain inhibitors targeting different branches of the bromodomain phylogenetic tree and evaluated their binding profiles within complex lysates by chemical affinity purification coupled to mass spectrometry.

This chemoproteomic profiling approach confirmed cognate targets but also revealed unexpected off-targets. We found that the pan-BRPF chemical probe NI-57 binds the oxidoreductase NQO2, a frequent kinase inhibitor off-target.[2] Our results demonstrate the power of proteome-wide approaches to evaluate global selectivity and target engagement.

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P08 Elucidating the activation cycle of the stress-response kinase GCN2

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GCN2 is a human protein kinase activated during pro-survival responses to cell stress, termed the integrated stress-response (ISR), and elevated GCN2 activity is associated with cancer, pulmonary hypertension and intestinal inflammation. [1-3] Despite the role of GCN2 in human disease, most structural and biochemical studies are conducted in yeast, and the sequence of events and cellular components involved is still under investigation. [1,4-7] We aim to understand human GCN2 activation and function under normal and stress conditions, using chemical and structural biology methods. We have successfully undertaken protein expression, purification, differential scanning fluorometry of the GCN2 kinase domain with known kinase inhibitors and successfully co-crystallised the kinase domain with Dovitinib. These results demonstrate that the GCN2 kinase domain can be expressed and co-crystallised for XChem screening, which will allow us to use available compounds to interrogate GCN2 structure, activation and role within the cell and how this relates to disease onset.

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P09 Developing chemical probes for investigation of human neuraminidase isoforms

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Neuraminidases (NEU), otherwise known as sialidases, are responsible for cleaving terminal sialic acid residues from glycoproteins. Hence, these enzymes have significant roles in regulatory pathways, with expression levels linked to various disease pathologies, including neuroblastoma and lysosomal storage disorders (LSDs). In humans, these exist as four isoenzymes (NEU1, NEU2, NEU3 and NEU4), with differing subcellular localisation and substrate specificity.[1] Despite having overall sequence similarity of <40%, the highly conserved active sites make finding specific binders particularly difficult.[2] While several viral NEU inhibitors (Zanamivir, DANA (Neu5Ac2en), and Siastatin B) used as influenza treatments exhibit weak inhibition against some human isoenzymes,3 selective chemical probes for human NEU are needed to study their cellular function and as starting points for drug discovery.

Initially, recombinant human NEU2 was expressed in *E. coli* in high yield and purity (3.1 mg, >95% purity) and used for sialidase assays. Differential Scanning Fluorometry (DSF) was developed to screen small molecule ligands that thermally stabilised NEU2, and fluorescence-based assay was used to check NEU2 enzyme activity. Viral NEU small molecule inhibitors were used to validate these assays, which successfully corroborated literature IC50 inhibition against human NEU2 (Zanamivir 5.3 µM (literature), 3.2 µM (experimental)). Detailed kinetic studies were performed to investigate the mode of inhibition of Zanamivir, which confirmed competitive binding with the substrate 4-(methylumbelliferyl)-N-acetylneuraminic acid (MUNANA), a structural mimetic of sialic acid. Next, we tested Zanamivir against NEU activity in mouse brain homogenates, which showed inhibition in this model. We will use these validated assays and biophysical techniques to screen novel compounds for binding and activity, to help develop chemical tools against human NEUs to increase our understanding of NEU in different disease pathways.

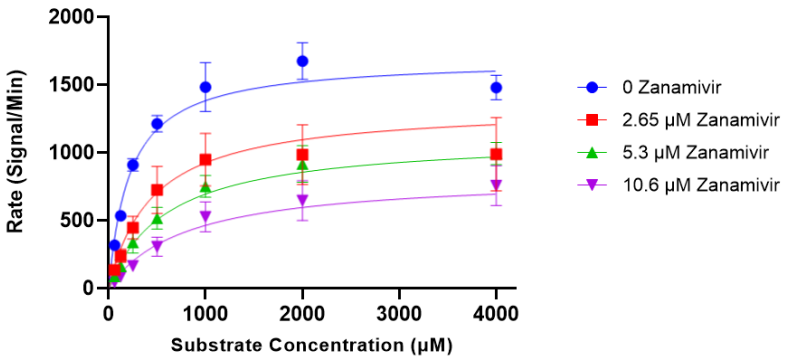

Figure 1: Kinetic time-course data for hNEU2 (15 nM) activity against MUNANA substrate, with varying concentrations of Zanamivir over one hour, fluorescence reading at 460 nm. Analysis performed in GraphPad Prism (Ki 1.69 µM).

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P10 High Throughput Production of Chemically Linked Bispecific Antibody Fragments

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Bispecific antibodies can provide a differentiated mechanism of action compared with monoclonal antibodies, via the binding and modulation of two targets simultaneously. This can, for example in the case of IgG-format bispecific antibodies, provide enhanced efficacy compared with a mixture of monoclonal antibodies, whilst maintaining desirable properties such as an *in vivo* half-life typical of antibody therapies. Methods to generate bispecific antibodies with sufficient purity for direct biological analysis in an expedient and low-cost manner are limited, resulting in a lack of standardised method for the high-throughput production of bispecific antibodies, for use within early stage functional screening assays of a discovery campaign.

Chemically linked bispecific antibodies have previously been produced by the replacement of the disulfide linkage between two halves of an antibody or antibody fragments with a chemical linker, bearing a click handle.[1] Two different Fab-conjugates can be subsequently connected using biorthogonal click reactions, producing bispecific antibodies in a specific and modular manner. This allows bispecific antibodies to be produced in high yields and purities, with simple purification steps, needed only within building block production (both chemical linker synthesis and antibody fragment expression).

Herein is described a strategy for the high-throughput production of chemically linked bispecific antibody fragment panels, to rapidly produce large numbers of bispecific Fabs with high yields and purities, which to our knowledge has not been previously described on a large scale, using chemical approaches. Click chemistry and bioconjugation techniques are explored to find the optimal pairing for this purpose, alongside the synthesis of chemical linkers, and the design, expression and purification of bespoke antibody fragments. By generation of an automated, high-throughput method to produce large panels of bispecific antibodies, toolboxes can be created for specific early-stage screening assays, such as target validation and identification, and epitope mapping activities, to aid in the design and development of novel bispecific antibodies.

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P11 VHL and UPS: delivering PROTACtion

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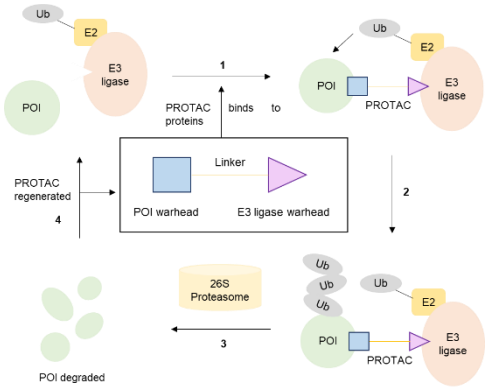

Figure 1. PROTAC-assisted proteasomal degradation[1].

Dysregulation of the ubiquitin proteasomal system (UPS) can cause accumulation of damaged or misfolded proteins, and disruption of other signalling pathways, leading to a wide array of maladies[2]. The abundance of E3 ligase signalling enzymes in this system provides a new modality to assist protein degradation through catalytic UPS 'hijackers', namely, PROteolysis-TArgeting Chimeras (PROTACs). These molecules comprise three parts: a protein of interest (POI) warhead and an E3 ligase targeting warhead connected by a linker to facilitate degradation by the UPS (Figure 1)[1]. Despite the benefits of protein degradation over reversible inhibition, they suffer from poor pharmacological properties. Thus, modifications to improve cell permeability and bioavailability is pertinent for their therapeutic viability.

We are developing the E3 ligase targeting moiety of a novel PROTAC for drug optimisation, which recruits the von Hippel-Lindau (VHL) tumour suppressor protein, a component of an E3 ubiquitin ligase complex[3]. Through structure-based design and in silico studies, we aim to identify new synthetic ligands of VHL to improve the physicochemical properties of their resulting PROTACs. We have developed scalable syntheses for these scaffolds, incorporating our first generation of modifications. These will undergo evaluation using a range of biophysical methods and biological assays. The results will direct our next round of modifications and later we will evaluate the properties in the overall PROTAC construct. These molecules, applied across a wider range of diseases, could be significant for drug discovery and improving patient outcomes.

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P12 Breaking the Barrier of Antimicrobial Resistance: Exploring the Mechanism of Action of Calcium-Dependent Antibiotics Cadasides and Malacidins

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With antimicrobial resistance constantly rising, and estimations reaching 10 million annual deaths by 2050,[1] finding new antibacterial agents that are effective against such microorganisms is becoming more and more pressing. Calcium-dependent antibiotics (CDAs), an emerging class of cyclic lipid peptide antibiotics, offer a possible solution to this problem.[2] Besides their potency against resistant bacteria, little is known about their mechanism of action, and few efficient chemical synthesis methods have been established. Two subgroups of CDAs, cadasides and malacidins, are of particular interest, due to their deviation from the highly conserved structure. Despite this difference, they are potent against resistant bacteria, making them an interesting target to study the impact of structural differences in terms of efficacy and mechanism of action. Our goal is to identify the mechanism of action, define residues relevant for binding, and thereby potentially improve the structure of these molecules to improve their pharmacokinetics or activity.

To synthesize the peptides, a novel synthesis method using a sophisticated protecting group strategy has been established. The peptides and their analogues (i.e. alanine-substituted, varying stereochemistry and fatty acids) are being assembled via solid phase peptide synthesis. Synthesised peptides will be tested *in vitro* for antimicrobial activity on Gram-positive bacteria, as well as in binding assays with bactoprenol and geranyl phosphate to further elucidate the mechanism of action of these molecules.

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P13 Chemical and enzymatic macrocyclisation of antibiotic peptides

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Antimicrobial resistance is a rising global concern, it is important to speed up the discovery of novel antibiotics. Compared to other natural products inhibitors, the non-ribosomal peptide rufomycins have outstanding anti-mycobacterial activity by inhibiting bacteria enzyme ClpC1 proteolysis in *M. tuberculosis* and *M. abscessus*.[1] The rufomycin biosynthetic gene cluster has been identified from two strains.[2,3] The cyclic precursor is released through macrocyclisation by RufT thioesterase (TE) domain. The molecule is further functionalised by two cytochrome P450s, which were proved active *in vitro* bioassay.[4] Our goal is to combine peptide synthesis with biocatalysis and also chemical cyclisation to generate derivatives of rufomycin for future structure - activity studies.

Herein, we used Solid Phase Peptide Synthesis (SPPS) to assemble linear peptide precursor and developed a chemoenzymatic synthesis employing the RufT-TE domain to cyclise and afford rufomycin analogues. Alternatively, we also developed an efficient and green cyclisation method as an *in-situ* reaction to give cyclic peptide on a preparative scale. Notably, it can also cyclise peptides that cannot be achieved through enzymatic reaction. We diversify peptide building blocks through SPPS, then either chemically or enzymatically macro cyclise peptide (Fig.1). Furthermore, one-pot style enzyme cascade reaction with other tailoring enzymes to modify this scaffold will be investigated in future.

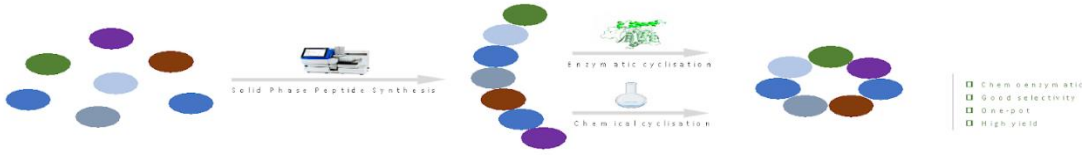

Figure 1. General strategy to synthesise rufomycin analogues.

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P14 Development of Lanthanide Tags for Site Selective Labelling of Peptides and Proteins

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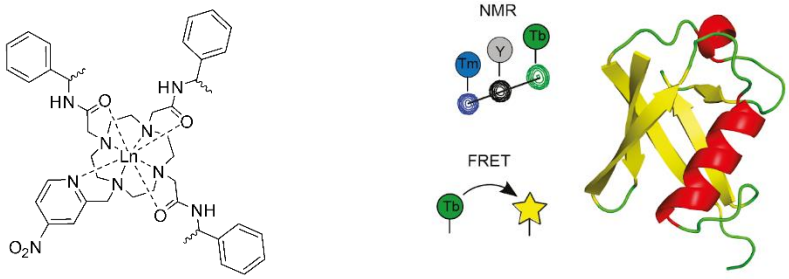
Site-specific labelling of cysteine residues of proteins with lanthanide complexes offers a powerful tool for studying protein structure and their interactions using a range of spectroscopic techniques, including nuclear magnetic resonance (NMR), electron paramagnetic resonance (EPR) and luminescence spectroscopy.[1] Useful tags require the tether connecting the lanthanide ion with cysteine residue to be rigid, with the smallest possible number of rotatable bonds, as precise structural information can be obtained only if the lanthanide ion does not move relative to the protein. At the same time, the tag must not affect the protein structure.[2] Additionally, the lanthanide complex of the tag needs to be kinetically and thermodynamically stable in order not to dissociate in aqueous or biological media. Fulfilling all these criteria presents a challenging synthetic task.

We have developed a small library of macrocyclic lanthanide tags that fulfil the above criteria and are capable of quantitative reactions with cysteine residues forming a stable thioether bond.[3,-5] We have investigated the effect of varying the Ln(III) tag structure on the rate of reaction and emission spectral changes that occur. Specifically, we have compared 1) chiral and achiral macrocyclic complexes, 2) nitropyridine versus N-oxide coordination to the lanthanide ion, 3) pyridine versus quinoline antenna and 4) 1-point versus 2-point attachment to the protein.



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P15 Peptide Technologies to Probe Chemokine Specificity

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Chemokines are small proteins responsible for the recruitment of leukocytes in and out of the vasculature in response to damage/infection or homeostatic signals. Chemokines are implicated in many disease presentations, including inflammatory diseases, cancer, and atherosclerosis, but their disease physiology is not always well characterised. Chemokines form a complex, overlapping network, with each chemokine engaging multiple different receptor, glycocalyx and homo/heterodimer interactions while different monocytic leukocytes express various combinations of receptors. This complex inflammation physiology makes specific chemokine/receptor inhibition somewhat ineffective and broad-spectrum chemokine inhibition chemically challenging. Chemical probes that can inhibit chemokine interactions with a variety of modalities are thus needed to study the effects of chemokine network modulation.

As an extra-cellular target, peptides are a promising class of molecules for this task, providing significant chemical space to adapt their chemokine specificity, while retaining deliverability and low toxicity. Here we describe the derivation of peptides from natural multi-chemokine-binding proteins called evasins and present a pipeline for the development of peptides with tuneable chemokine binding properties. Evasin (P672) was studied in complex with chemokine CCL8 by Hydrogen-Deuterium Exchange Mass Spectrometry (HDX-MS) and the chemokine-binding sequence elucidated[1]. Peptide BK1.3 derived from this sequence maintains parent evasin properties with improved tractability. CelluSpots™[2] technology has now been employed to replicate HDX-MS interaction mapping and can elucidate binding patterns across the chemokine network. CelluSpots™ are also enabling the design of modified peptides to be selectively employed to study chemokine function.

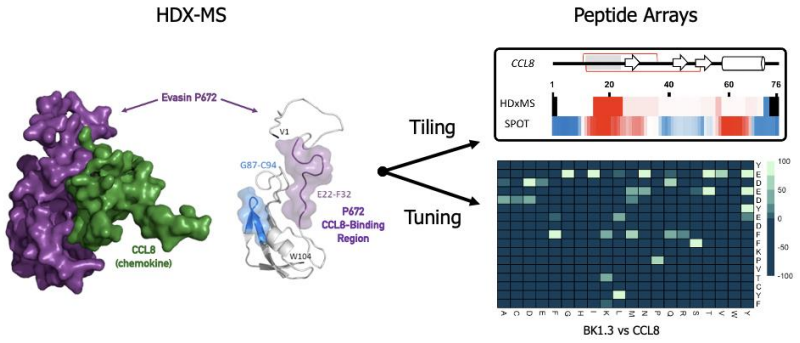

Figure 1: HDX-MS data elucidate the primary binding region between P672 and CCL8 – this can now be replicated by peptide arrays and these same arrays used to find sequence changes that improve the binding of derived peptides.

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P16 Developing inhibitors of Bacterial DNA-repair and SOS Response Pathways

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Antimicrobial resistance (AMR) has the potential to make many life-saving medical advances redundant, causing 10 million deaths per year by 2050.[1] New compounds with novel targets are required to address the challenge of AMR.[2] One such target is the DNA damage repair process which allows bacterial survival under stress from antibiotics or immune attack. Loading of the DNA repair enzyme AddAB onto damaged DNA facilitates double stranded break repair and upregulates the SOS response, which activates virulence, persistence, and resistance mechanisms.[4] IMP1700 inhibits AddAB, potentiating the DNA damaging antibiotic Ciprofloxacin (CFX) and inhibiting the SOS response in methicillin-resistant *Staphylococcus aureus* (MRSA).3 IMP1700 contains a fluoroquinolone (FQ) motif associated with 'black box' toxicity and understanding of the structure-activity relationship (SAR) of this series is limited, both of which hinder future translation. To investigate the SAR of IMP1700 and develop improved lead compounds, over 90 analogues were synthesised (Fig. 1A), and two high-throughput assays were developed to assess compound activity. Firstly, compound growth inhibition was determined with and without half-minimum inhibitory concentration (MIC) of CFX to probe DNA-repair inhibition (Fig. 1B) and fold increase in potency with CFX (ΔCFX) calculated. Secondly, SOS response inhibition after activation by CFX was measured using a reporter system expressing GFP under control of an SOS-responsive promoter (Fig. 1C). OXF077 showed greater synergy with CFX-induced DNA damage than IMP1700 (ΔCFX = 260 and 106-fold, respectively) and stronger SOS inhibition (IC_{50} = 75 and 132 nM, respectively). Interestingly, a selection of compounds, such as OXF030 and OXF031 showed only SOS response inhibition (IC_{50} = 455 and 607 nM, respectively) without strong synergy with CFX-induced DNA damage, suggesting the potential existence of a second target or mechanism of action (Fig. 1D). OXF030 also does not contain the CFX core associated with off-target 'black-box' toxicity. Overall, this work has uncovered a divergent SAR in DNA-repair and SOS response inhibitors, suggesting a new mechanism of action for this series which could provide a useful tool to combat the threat of AMR.

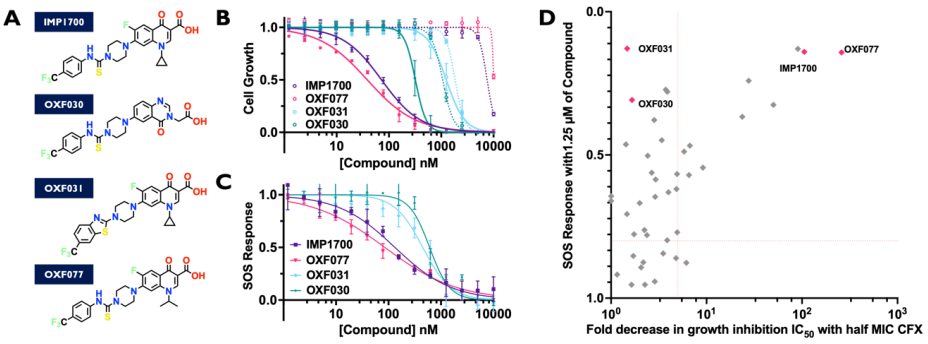

Figure 1: A) DNA repair and SOS response inhibitors of interest. **B)** Growth inhibition with half MIC CFX (solid line) and without (dashed line). **C)** SOS response inhibition measured in GFP reporter assay. **D)** Comparison of ΔCFX and SOS response inhibition with 1.25 μM of compound.

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P17 Cryopreservation of Liver-Cell Spheroids towards 'off the shelf' 3-D cellular models

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The cryopreservation of tissues and organs is challenging, compared to cells in suspension. 3-D cell models, such as spheroids, are widely used as a tool to avoid or reduce the use of animals in drug discovery and basic science, but there is a time and skill barrier to preparing these and ready-made spheroids are hard to store frozen. Herein, human Caucasian lung carcinoma cell (A549) and hepatocellular carcinoma (HepG2) spheroids are cryopreserved by a unique combination of pre-conditioning and controlled ice nucleation. L-proline (L-pro) is a protective osmolyte amine could penetrate cells and keep balance of turgor pressure and water stress, preparing cells for freezing. Secondly, ice nucleating (IN) polysaccharides from pollen washings allow precise nucleation of extracellular ice, which reduces intercellular ice. We show increased recovery and intact morphology of spheroids suggesting the significant benefit from L-pro preincubation and DMSO addition with ice nucleation for cryopreservation when compared to DMSO alone group. This combination protocol of L-pro & ice nucleation is a promising method to improve the efficacy of cryopreservation of large-size tissues.

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P18 High throughput screening and identification of synthetically lethal cyclic peptides in MTAP deletion cancer cell lines

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MTAP is a metabolic enzyme and deletions occur in 15% of all cancer types, rising to >50% of glioblastoma multiforme. MTAP catalyses the conversion of MTA (a polyamine) into both adenine and methylribose-1-phosphate. MTAP deleted cells contain metabolic vulnerabilities, via the MAT2A/PRMT5/RIOK1 axis[1] and several clinical studies have also shown that MTAP deleted cancers have a worse prognosis[2]. MTAP is expressed ubiquitously in healthy cells, thus compounds targeting only cells with MTAP deletions may present a novel, specific cancer therapeutic strategy.

SICLOPPS is a genetically encoded method for the generation of intracellular libraries of 3.2 million cyclic peptides. We use a SICLOPPS library that is functional in human cells, combined with isogenic HCT116 colon cancer cell lines, with one containing a MTAP deletion (MTAP-/-). Our screening strategy involved a high throughput drop out screen to identify peptides that are synthetically lethal to the MTAP-/- cells, but not the isogenic MTAP +/+ cells. We used next generation sequencing in combination with high throughput data analysis to identify and characterise such peptides. Given that most activity from the cyclic peptide is derived from di- and tri-peptide motifs, our in-house data analysis tools identified “dropped out” peptides and identify recurring motifs that may indicate a di- or tripeptide pharmacaphore.[3] Focused libraries were generated based on the identified “dropped out” motifs and were screened in the target cells using a series of bio-panning experiments to amplify the differences between toxicities of the motifs. 10 motif “hits” were identified which displayed a progressive decrease in the copy number ratio between MTAP and HCT116 cells, across pans. Individual hexapeptide constructs will be tested in cell viability experiments to further validate their toxicity. Following the viability screening, candidate cyclic peptides will be synthesized and tested in the cells to verify their efficacy and potency, and the target protein will be identified using standard chemical biology techniques.

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P19 Revealing the detail in i-Motif DNA Structures

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The cellular functions of nucleic acids rely not only on their sequence but also on their structure. Beyond the most widely known double helix,[1] DNA can adopt other classes of secondary structural motifs, including the cytosine-rich four-stranded helical i-motifs.[2] Such non-canonical DNA conformations are formed *in vivo*,[3] but to date, structural information on i-motifs is very limited. Our aim is to reveal more structural detail about i-motif DNA structures using a range of biophysical methods including X-ray crystallography. Here we describe our initial crystallography studies with sequences from the human insulin gene-linked polymorphic region and the regulatory region of nitric oxide synthase from *Paracoccus denitrificans*.

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P20 Design of lanthanide probes for time-resolved detection of peroxynitrite in specific cellular compartments

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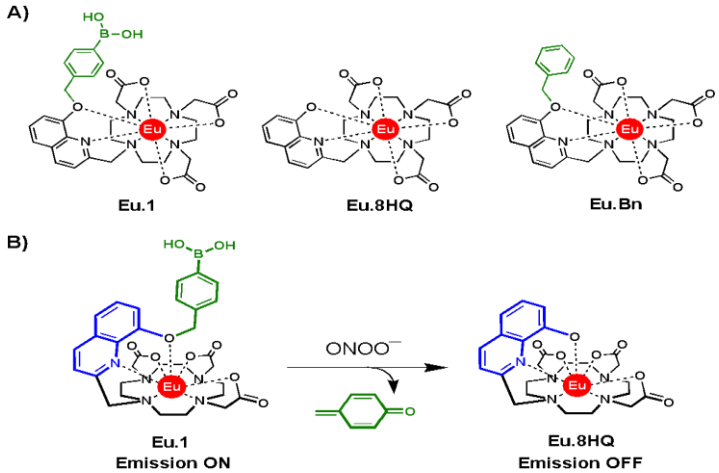
Peroxynitrite (ONOO–) is a short-lived reactive oxygen and nitrogen species that has been implicated in several diseases and is an important therapeutic component of cold atmospheric plasma commonly used for skin wound treatment.[1] Methods to detect ONOO– in biological media could help elucidate the role of this reactive species in biology, disease and therapeutics. Previously, reactivity based probes have been developed bearing a boronic acid moiety capable of detecting ONOO– (and hydrogen peroxide) using fluorescence spectroscopy.[2,3] However, there are drawbacks inherent to organic fluorescent probes, including small Stokes' shift, short lifetimes and limited solubility in aqueous solutions.[4] Lanthanide based probes have the potential to overcome these drawbacks.

We present a long-lived luminescent probe Eu1 for sensing ONOO– in aqueous media (Figure 1). This probe contains an 8-alkoxyquinoline group functionalised with a phenylboronic acid. Upon rapid and selective oxidation of the phenylboronic acid residue by peroxynitrite, the Eu(III) emission is switched off (Figure 1). Our probe has the advantage of a long luminescence lifetime, allowing ONOO– to be detected in complex biological fluids (e.g. blood serum) and for changes in ONOO– levels in living cells to be monitored using time-gated imaging. We will present the next generation of Eu(III) probes bearing a pendant arm that can be readily functionalised to promote its localisation to specific cellular compartments, including the cell membrane. Our ultimate goal is to detect ONOO– transport across lipid bilayers to better understand how this species is delivered to cells upon external treatment with cold atmospheric plasma.



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P21 The biosynthesis of Daphniphyllum alkaloids

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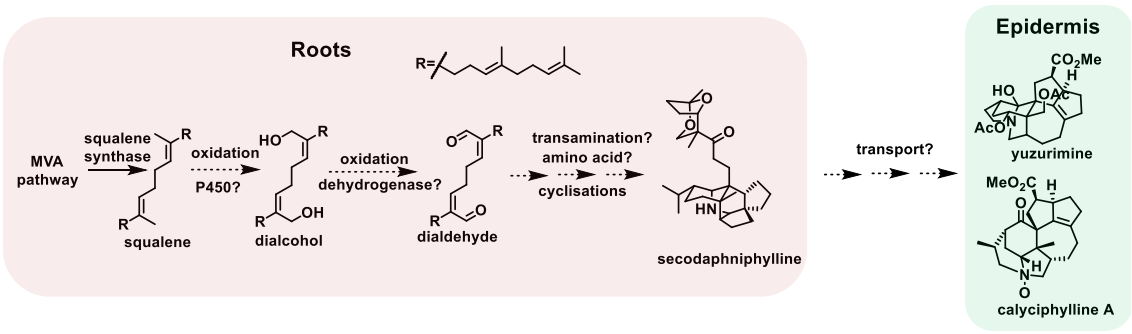
Plants from the genus *Daphniphyllum* have been used in Traditional Chinese Medicines to treat a range of ailments. The medicinal properties of this plant are largely due to its variety of complex polycyclic alkaloids that exhibit a range of bioactivities[1], however their biosynthesis in plants is still unknown. Prior studies have shown that *Daphniphyllum* alkaloids are likely derived from the isoprenoid squalene[2] that undergoes series of oxidations, introduction of a nitrogen and cyclisation to form secodaphniphylline that is then diversified into other *Daphniphyllum* alkaloids. The current progress towards the understanding of the biosynthetic pathway will be discussed, using synthetic chemistry, metabolomics, LC/GCMS and alkaloid isolation. These results have indicated that the early steps of the biosynthetic pathway occur in the roots and compounds are later transported and diversified in other tissues.



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P22 Elucidating the biosynthesis of Securinega alkaloids

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Alkaloids are a large and diverse group of naturally occurring, nitrogen-containing organic compounds. By understanding how plants make these valuable compounds, we can gain direct access to the alkaloids themselves, and also the enzymes involved in their biosynthesis, which can be used as biocatalysts. One understudied alkaloid is securinine, the bioactive alkaloid from the plant *Flueggea suffruticosa*[1]. Securinine has been investigated for a broad spectrum of bioactivities including neuroprotection and anticancer[2]. In this study, we built a de novo transcriptome of *F. suffruticosa* to identify candidate genes involved in securinine biosynthesis. We identified candidates using transcriptome annotations and expressed these genes in planta and used mass spectrometry to characterise their activity. Substrates not commercially available were chemically synthesised to incubate in feeding assays *to F. suffruticosa* protein extract. We continue to study enzymes *in vitro* to better understand their mechanism.

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P23 Chemical Biology Approaches to Explore the Ligandability of TRIM E3 Ligases

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Ubiquitination is a key protein post-translational modification which regulates many cellular processes, from protein degradation to immune signalling. There is significant interest in developing small molecules that target the ubiquitin system for therapeutic purposes. Protein ubiquitination is catalysed by a three-enzyme-cascade: E1-activating, E2-conjugating, and E3 ligase enzymes. The E3 ligases are the largest class, and mediate the final step of ubiquitin transfer, from E2 to the substrate. As such, E3s are important in conferring substrate specificity so offer an attractive point at which to chemically target the ubiquitin system, both for therapeutic purposes and chemical probe development. Moreover, within the targeted protein degradation field, ligands for novel E3 ligases are required to further develop PROTACs and molecular glues.[1] In this work, fragment-based drug discovery has been used to ligand the TRIM family of RING-type E3 ligases. TRIM E3s have a conserved domain architecture, characterized by an N-terminal tripartite motif and a C-terminal substrate-binding domain. Over half of TRIMs contain the PRYSPRY substrate-binding domain, which comprises a conserved β-sandwich fold linked by structurally diverse variable loops. A range of orthogonal fragment screening techniques have been employed to find chemical binders of the PRYSPRY domain of two TRIM E3 ligases, TRIM21 and TRIM25. Non-covalent fragment screening by differential scanning fluorimetry (DSF) has been complemented by covalent fragment screening by mass spectrometry. Additionally, crystallographic fragment screening has been carried out at the XChem platform at Diamond Light Source.[2]

A number of hits have arisen from these screens, and these fragments are being validated by biophysical methods such as SPR, and biochemical activity assays. Guided by the crystallographic information obtained on ligand-bound proteins, validated fragment binders will undergo iterative optimisation to develop more potent and selective ligands. The goal is to use these compounds as valuable tools to explore the biology of the ubiquitin system and identify novel points for therapeutic intervention.

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P24 New Methods for Scaled Synthesis of Nucleoside 5'-Phosphates

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Nucleoside phosphates are involved in many biological processes such as DNA replication and signalling pathways. They are also key intermediates for sequencing technologies and the growing field of synthetic biology. Despite their increasing usefulness, there is still no standardised approach to synthesise these compounds, primarily because of practical issues, including sensitivity to moisture and complex chromatographic purification.

We aim to deliver efficient synthetic strategies to access nucleoside triphosphates through flow technologies and improved purification methods. Our work builds upon procedures reported by Jessen and co-workers, using a phosphitylating reagent developed by Bialy and Waldmann, and improved upon by Desmaële and co-workers.[1,2,3] Jessen and co-workers have focused on batch approaches towards an iterative addition of phosphates to nucleoside 5'- phosphate substrates. Owing to instability in both acidic and basic environments, purification of the phosphitylating reagent proved challenging. Thus, we optimised the synthesis of the phosphitylating reagent through solvent testing, and other improvements to workflow to increase mass recovery (85%) and levels of purity (93% via 31P NMR). We delivered time-efficient, gram-scale strategies that provided material, which proved effective for phosphate addition. With access to large quantities of phosphitylating reagent, we translated phosphate addition to a flow setup. We are therefore able to report reliable, consistent multigram procedures for phosphate addition to several nucleotides.

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P25 Assessment of the Bioorthogonality of the Nitrile Imine 1,3-Dipole

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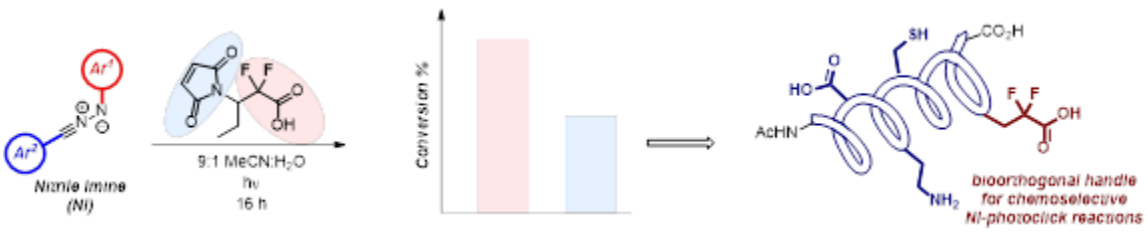
The nitrile imine (NI) 1,3-dipole is a highly reactive and readily accessible synthetic intermediate generated via the photolysis of 2,5-disubstituted tetrazoles.[1] The NI species exhibits broad reactivity with dipolarophiles,[2] as well as a range of nucleophilic functionalities.[3-5] This reactivity has enabled its utility in a variety of synthetic applications,[6,7] including bio-orthogonal chemistry.[8]

The reaction of the NI dipole with a range of carboxylic acid moieties was quantified and revealed an enhancement in reactivity with decreasing pKa. This work has been expanded to assess the biorthogonality of the dipole through the competitive reaction of an activated dipolarophile versus a highly acidic fluorinated carboxylic acid competitor. A range of NI species were generated through photolysis of a 2,5-disubstituted tetrazole and their reactivity with a model substrate was quantified. A suitable NI precursor for bio-orthogonal photoclick reactions has been identified which enhances chemoselectivity for the fluorinated carboxylic acid handle, suppressing the reactivity of other endogenous nucleophiles. Work is ongoing to incorporate a fluorinated bio-orthogonal handle into a range of peptide sequences to explore NI-mediated bio-orthogonal labelling.



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P26 Functional characterisation of yet-unexplored methylated lysine reader domains in epigenetic regulation

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Histone proteins play a crucial role in chromatin organisation. They are chemically modified by writers or erasers that, respectively, install or remove histone post-translational modifications (PTMs). Histone PTMs have activating or repressive effects on gene transcription. There are different classes of reader domains that recognise specific types of PTMs (bromodomains, chromodomains, PHD fingers). Plant homeodomain (PHD) fingers are Zn-coordinating domains that recognise unmodified or methylated lysine. They are present in more than 100 proteins across the human proteome, often associated with other domains involved in transcriptional regulation, cell cycle control, chromatin remodelling and nuclear signalling.[1] PHD fingers are potential targets in oncology; for instance, PHD domain in NSD2 are reported to be important for cellular activity and transcriptional activation of oncogenes in multiple myeloma.[2] Despite their involvement in cancer development, many PHD fingers, such as NSD2 PHD fingers, remain uncharacterised and as yet, no chemical probes are available for this domain. This project aims to clone, express, and purify PHD fingers of NSD2 for further characterisation of their potential binding partners through biophysical assays and development of potential chemical probes. Production of those domains was not straightforward due to solubility issues. Therefore, the expression in E.Coli and purification of NSD2PHD5 with different tags have been carried out to improve protein solubility. An AlphaScreen binding assay on PHD fingers and biotinylated histone peptides has been established.

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P27 Development and Biological Evaluation of Novel α-Helix Mimetic Prodrugs as Leads for Prostate Cancer Treatment

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The androgen receptor (AR) transcription pathway has been identified to play a critical role in the proliferation of prostate cancer (PCa) cells.[1] Consequently, traditional PCa treatment strategies focus on androgen suppression and deprivation using AR antagonists and castration.2 These reduce AR activity however the onset of resistance is inevitable.3 Our research has focused on the design and development of a novel small molecule coactivator binding inhibitor targeting the activating function 2 domain in the AR ligand-binding domain (LBD) to hinder androgen-dependant transcription in tumour cells.4 Through computational modelling studies, this novel CBI-1 was designed to contain an azabicyclo[2.2.2] octane amide-based α-helix mimetic scaffold. Through a plug-and-play model this scaffold can be used to project functionalities present at protein-protein interfaces, which are involved in binding, in 3D space similar to that in a protein α-helix. In particular, we are aiming to imitate the unique FXXLF epitope of AF2-binding coactivators (Fig. 1). Following the development and optimisation of a 12-step synthetic route, an *in-vitro* directing binding fluorescence polarisation assay showed promising binding activity between CBI-1 and the AR LBD, with an IC50 value of 29.4 μM, suggesting that CBI-1 is able to disrupt AR-coactivator binding.[5] However, the compound was inactive in cell-based AR activity assays. We hypothesise this may be due to poor cell permeability of CBI-1 as a result of its two free carboxylic acid groups. Thus, the current research is focused on the synthesis and screening of a library of ester prodrug derivatives of CBI-1 which are anticipated to be cleaved to the active CBI-1 drug by action of endogenously expressed esterases or proteases inside PCa tumour cells.

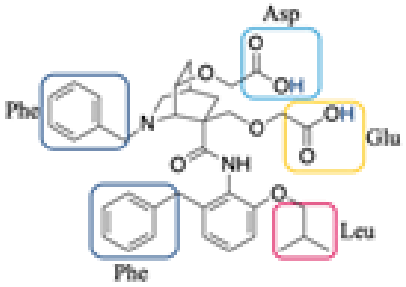

Fig. 1a: **CBI-1**, an Asp-Phe-Glu-X-Leu-Phe (FXXLF) Spivey α-helix mimetic scaffold structure.

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P28 Chemical tools to understand fatty acid uptake, trafficking, metabolism and signalling in type 2 diabetes

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Fatty acids are the predominant fuel in the body, with many functions including; cell membrane composition, metabolism and immune cell differentiation. Too much fatty acid metabolism can contribute to many diseases including the cardiac complications that occur in type 2 diabetes.1 Despite the prevalence of clinical data demonstrating this role of dysregulated fatty acid metabolism in type 2 diabetes, we do not currently understand with which biomolecules the fatty acids or their metabolites interact with in cells and its impact on the disease. Therefore, this project aims to develop a library of chemical probes and various methodologies to begin to address the following research questions: which long chain fatty acids or metabolites are most prevalent in type 2 diabetes, with which biomolecules do these long chain fatty acids/metabolites interact with and what are the outcomes of these lipid-biomolecule interactions with regards to type 2 diabetes? The synthesised chemical probes contain both photoaffinity fatty acid probes (containing both a diazirine photocrosslinking group and a pulldown “Click” alkyne handle) and photocatalytic fatty acid probes (μMap).2,3,4 Using both probe types in parallel will allow for elucidation of both the covalent interactions and non-covalent associations between specific fatty acids and proteins of interest. Photoaffinity labelling (PAL) incorporates a stoichiometric photoactivatable group, however using the photocatalytic chemical probes, where the reactive warhead (diazirine) is decoupled from the fatty acid, will allow for catalytic signal amplification and result in multiple labelling events per probe, ultimately leading to higher levels of target enrichment. These probes will be used as tools for elucidating lipid function – through exploring molecular interactions and live cell imaging. Lipidomic and proteomic technologies will also be used to analyse interaction partners and metabolites of the fatty acids within the cells. Recent results and next steps for this work will be presented.

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P29 Understanding the activity of the P450 RufM

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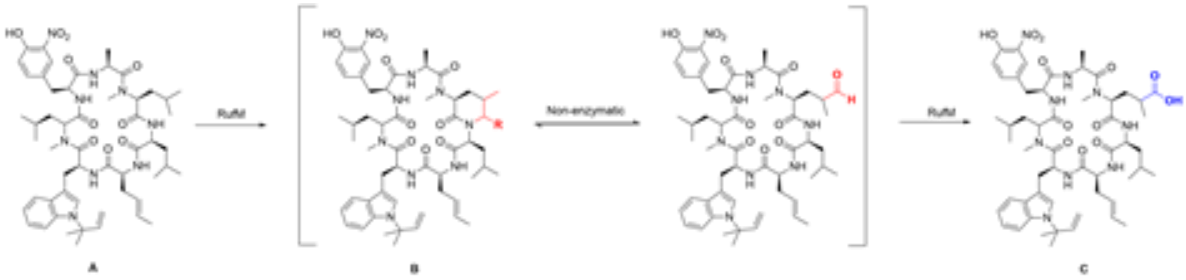
Tuberculosis (TB) is one of the most lethal infections of the modern world. With many strains gaining resistance to traditionally used antibiotics, it is imperative that new compounds be developed. The cyclic non-ribosomal peptide known as rufomycin, is a natural product that has known activity against TB.[1]

In the biosynthesis of rufomycin, the cytochrome p450 RufM performs a unusual consecutive oxidation reaction on the cyclised form.[2] Thereby producing compounds with improved biological activity. Compound B has the highest activity amongst the known derivatives but is a minor product of the reaction. RufM's mechanism and what factors favour the formation of carboxylic acid product over the aldehyde product is not fully understood.

This study explores how changing highly conserved and predicted catalytically important residues affect product output. Additional screens on several rufomycin derivatives have helped determine what peptide residues may affect substrate binding.



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P30 Exploiting Dual-Ligand Cooperativity for Enhancing Enzymatic Inhibition of Pin1 Prolyl Isomerase

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Pin1 is a peptidyl-prolyl cis-trans isomerase enzyme that exhibits high specificity for substrates containing phosphorylated serine-proline and/or threonine-proline[1]. Pin1 activity plays an important role in regulating the function of over 200 substrate proteins that are involved in the regulation of cell cycle, cell motility, apoptosis, and neuronal differentiation. Dysregulation of Pin1 has been linked to the development of various severe diseases, including cancer, obesity, diabetes, and inflammation. Several Pin1 inhibitors, including juglone, EGCG, KPT-6566, and sulfopin, have been identified, but they often possess undesirable pharmacological properties such as poor substrate specificity, low cell-permeability, solubility, and off-target effects[2,3]. To address these issues, we have employed a novel dual-ligand cooperativity strategy to achieve more potent Pin1 inhibition[4].

In this poster, the cooperation between Pin1 inhibitors has been demonstrated as observed through a spectrophotometric prolyl isomerase activity assay. We have also identified that specific ligands preferentially inhibit substrate binding to distinct Pin1 domains using fluorescence polarisation substrate binding assays. Ongoing work involves utilizing native mass spectrometry to gain insights into the structural mechanisms of our findings, and demonstrating their effects in mammalian cancer cell models by performing the CellTiter-Glo® luminescent cell viability assay. Our ultimate goal is to develop even more potent bitopic inhibitors that covalently link two cooperative inhibitors, which could have a significant impact on Pin1's development as a drug target.

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P31 Small Molecules to Control IFN-I Induced Neuroinflammation

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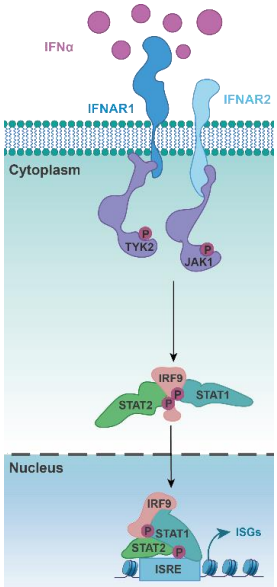

Though type-I interferons (IFN-I) help regulate the innate immune response, they can exacerbate neuroinflammation.[1] The role of neuroinflammation in neurodegenerative disease is becoming increasingly clear,[2] with studies showing that aberrant IFN-I signalling contributes to the progression of diseases such as Alzheimer's Disease.3

As such, the IFN-I signalling pathway (Figure 1) is an attractive therapeutic target. Current drugs that modulate IFN-I signalling inhibit "druggable" JAK proteins. Since most JAK inhibitors interact with multiple JAKs, this leads to broad and unselective effects such as susceptibility to infection. Selective modulation of IFN-I by targeting another point in the cascade has therefore become an area of interest for drug discovery.

This poster will detail a phenotypic approach to the discovery of new IFN-I signalling inhibitors. We hope to uncover molecules that modulate less commonly manipulated targets in the pathway and validate where in the cascade provides an optimal balance between anti-inflammatory activity and undesirable side effects.

Following hit identification and validation, the structure-activity relationship was explored through the design and synthesis of new compounds. These were profiled via western blotting and fluorescent imaging, with percentage inhibition of the IFN-I response measured in cells. Increased potency was observed, with apparent close to full knockdown in some cases. A lack of biochemical JAK inhibition supports that modulation of a target in the IFN-I signalling pathway (other than JAK1/TYK2) is possible and can lead to inhibition of the IFN-I response.

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P32 Modulating protein-surfactant interactions for enhanced protein thermal stability and bioactivity

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Surfactant-mediated stabilisation of proteins is a commonly used strategy in the food, chemical and pharmaceutical industries.[1] Through non-covalent association, surfactants can enhance protein stability and bioactivity in non-native environments.[2] These favourable local conformational changes have enabled valuable biocatalytic transformations in organic solvents, helped with the formulation of therapeutic monoclonal antibodies and facilitated protein encapsulation in polymeric and lipid-based drug delivery systems. Protein-surfactant binding is however complex and dynamic, with concurrent interactions leading to stabilisation and destabilisation processes. **Deconstructing this assortment of interactions is key to rationalising surfactant-mediated stabilisation and advancing protein applications**. In this study, we report the impact that sodium dodecyl sulfate (SDS) has on the thermal stability and biocatalytic activity of the antimicrobial enzyme lysozyme. Lyzoyme-SDS interactions were assessed at increasing SDS concentrations. Differential scanning calorimetry measurements showed that the melting temperature of lysozyme increased with surfactant association, highlighting the role of SDS in thermal stabilisation. Maximum binding efficiency at pH 4 in the presence of 5 μM SDS led to lysozyme precipitation and loss of aqueous solubility. Increasing ionic strength led to surfactant dissociation and protein re-solubilisation, emphasising the role that SDS has on modulating protein aqueous solubility. Protein structural and bioactivity assays are currently on-going to deconvolute the impact of surfactant association.

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P33 Glycopeptide Probes in Pathogen Detection

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Glycan-binding proteins, known as lectins, are extensively used by pathogens such as Pseudomonas aeruginosa and uropathogenic Escherichia coli for host cell-binding and biofilm formation. Traditional methods of targeting the carbohydrate recognition domain are often hampered by poor binding affinity of the pathogenic lectins. Recently, phage display has been utilised to find candidate binders that could be used in developing new diagnostics or pharmaceutical products. Peptide phage display libraries can be modified by N-terminus modification to include glycans for increased target specificity1 and constraining via cysteine residues to increase proteolytic stability and increase binding2.

Using both already available MatLab and custom Python scripts, constrained glycopeptide candidates that bind to the lectin targets LecA and LecB are found through Illumina sequencing in single round unamplified panning. Candidates were found through filtering for statistical significance and abundance compared against controls to remove non-specific binders and in favour of sequences that bind with both the respective glycan and the peptide. In the future, we aim to attach candidates to gold nanoparticles to demonstrate pathogen detection, along with potential disruption of pathogenic functions such as binding and biofilm formation.

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P34 The development of novel near-infrared squarylium dyes towards new antimicrobial agents

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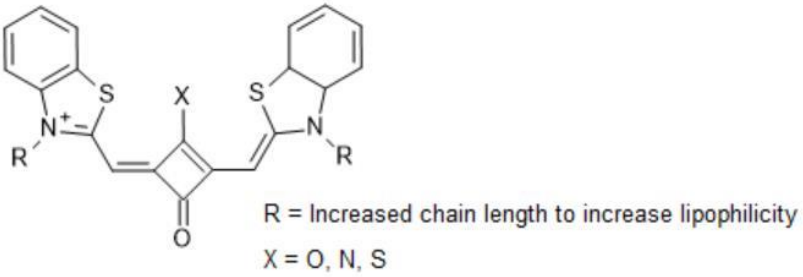
Although conventional drugs such as the penicillin's led the golden age of antimicrobial chemotherapy, the alarming rise in antimicrobial drug resistance due to its single mode of action, means alternative approaches to infection control and disinfection needs to be rapidly considered [1]. In contrast, photoantimicrobials produce highly reactive oxygen species and thus offers both multiple and variable sites of action at the pathogenic target. This project seeks to develop a new range of near-infrared squarylium dyes capable of producing a large amount of reactive oxygen species in order to cause a localised photobiocidal response in pathogenic targets [2].



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P35 Using Phage Display to Identify Novel Glycopeptide Ligands for Target Lectins

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Phage display is a recombinant screening technique that allows for the selection of high-affinity peptides for a given target molecule. Peptides are specifically selected from libraries containing 106-109 randomized sequences by iterative screening. Lectins are carbohydrate binding proteins that mediate a wide range of biological processes, therefore it would be therapeutically valuable to develop inhibitors/agonists for many of these interactions. This is challenging due to the weak binding between lectins and their natural carbohydrate ligands1. We aim to combine the selectivity of glycan binding to the lectin carbohydrate recognition domain (CRD) with the enhanced affinity of peptide binding. Phage libraries have been engineered to contain an N-terminal serine, used to covalently attach a glycan monomer, creating glycopeptide display libraries that can be screened against glycan-binding lectins2.

In this project, three glycan-conjugated cyclized peptide libraries were cloned and screened against six lectins including LecB (P. aeruginosa) and RCA120 (R. communis). One round of panning was performed, and the output sequenced by Illumina sequencing. Nine candidate peptides were identified from NGS by utilizing MATLAB and Python scripts, filtering for peptides that were significantly ($p < 0.05$) enriched by a ratio of ≥5 in panning against a specific lectin compared to controls. The candidate peptides were synthesized by solid phase peptide synthesis, cyclised and conjugated to the relevant glycan monomer. The affinity of these glycopeptides for their target lectin will be determined by surface plasmon resonance and compared to the native galactose monomer ligand. Glycopeptide ligand identification can easily be adapted to investigate other lectins by replacing the N' terminal galactose with other sugar monomers.

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P36 Targeting i-Motif DNA of ALOX5 Promoter with Small Molecule Ligands

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i-Motif is a form of alternative DNA secondary structure that is formed from cytosine rich sequences. Instead of the canonical B-form double helix [1], the i-motif consists of four strands and is stabilized by hemi protonated C-C base pairing [2]. There is less known about the i-motif DNA structure compared to other forms of DNA secondary structures such as the G-quadruplex. Both i-motif and G-quadruplex DNA structures have been shown to exist *in vivo* and that ligands targeting these structures have been shown to affect gene expression and the activity of telomerase. The working hypothesis indicates i-motifs are involved in the regulation of gene expression and targeting these structures with binding ligands can affect gene expression [3].

There are many GC-rich regions across the genome that may fold into i-motif structures. Here we focus on one sequence that shares sequence with GC-boxes, which are known transcription factor binding sites. In this poster we will describe the characterisation of a repetitive GC-rich sequence found across the human genome, including the promoter region of the gene ALOX5 [4], compounds that target the i-motif structures derived from this sequence and their effects in ALOX5 gene expression using a reporter gene assay.

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P37 The development of novel thioxanthone photoantimicrobials

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The increasing and alarming rise in antimicrobial resistance is forcing health care professionals to seek alternative approaches to infection control and disinfection. One such approach is the development of photoantimicrobials which produce highly reactive oxygen species offering multiple and variable sites of action at the pathogenic target in comparison to single mode of action antimicrobials such a penicillin.

The project looks at developing a range of thioxanthone photoantimicrobials with charged N-alkylated motifs, with the increasing linear chain lengths as shown in figure 1. We postulate that these compounds could be attracted to the negatively charged yeast membrane, subsequently distorting the lipid bilayer and then causing a phototoxic response using light of an appropriate wavelength[1,2].

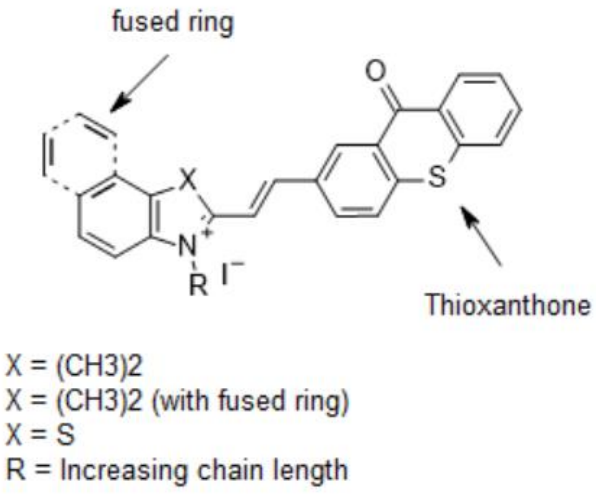

Figure 1: *N*-alkylated thioxanthone photoantimicrobials

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P38 Exploring the Potential Antimicrobial Activity of a Range of Heptamethine Cyanine Dyes

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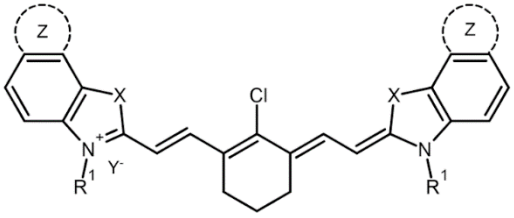
The increasing presence of antimicrobial resistance organisms has made the pursuit of new, effective medicinal compounds ever more urgent [1]. The research presented aims to explore the potential antimicrobial activity of a range of already medicinally established compounds known as Heptamethine Cyanine Dyes (figure 1). Whilst their capacity as fluorescent imaging agents has been extensively characterised, exemplified in the FDA approved use of Indocyanine Green [2], their capacity for antimicrobial activity has remained largely unexplored. Herein we have focussed on establishing the minimum inhibitory concentration of a range of heptamethine cyanine dyes against E. coli, S. aureus and C. albicans.



X = S		X = $(CH_3)_2$		X = $(CH_3)_2$ Z = Benzyl	
R	Y^-	R	Y^-	R	Y^-
C_1-C_{10}	I^-	C_1-C_{10}	I^-	C_1-C_{10}	I^-
-CH_2-C_6H_5	Br^-	-CH_2-C_6H_5	Br^-	-CH_2-C_6H_5	Br^-
$(CH_2)_3SO_3^-$	N/A	$(CH_2)_3SO_3^-$	N/A	$(CH_2)_3SO_3^-$	N/A

Figure 1: The range of heptamethine cyanine dyes

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P39 Developing a biophysical toolbox to study protein molecular glue kinetics

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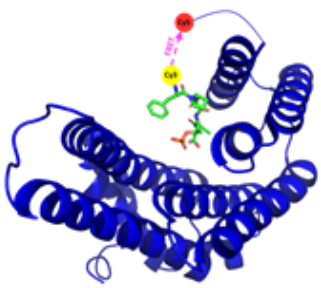
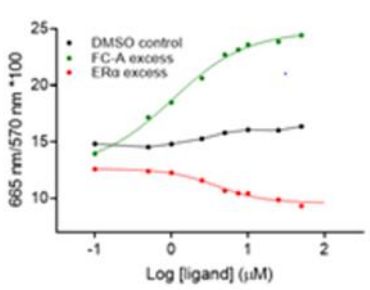
Protein-protein interaction (PPI) interfaces provide an abundance of novel opportunities for therapeutic intervention. Well-defined 'hot spots' within binding interfaces have proven to be targets for small molecule modulation. PPI inhibition has been greatly explored, however, the design and development of molecular glues stabilising PPIs at these interfaces is yet to be exploited. This is partly because the cooperative and dynamic nature of ternary complex formation between a glue and two interacting proteins is not well understood. This poster presents our progress toward building a biophysical toolbox to study the temporal aspects of ternary complex formation.

Our research is focused on the well-characterised PPI between 14-3-3σ and Estrogen Receptor α (ERα). ERα is key in the cell proliferation of breast cancer cells and self-dimerisation is crucial to its activity. 14-3-3σ interacts strongly with a phosphorylated threonine at the C-terminus of ERα preventing this self-dimerisation. Thus, stabilisation of this PPI by molecular glues such as Fusicoccin A (FC-A) provides an entry point for further drug development.

Commonly used biophysical techniques give important insight into thermodynamics of protein-protein binding but not binding kinetics. We have therefore been developing complimentary experimental approaches to study molecular glue kinetics: biolayer interferometry (BLI) and an in-solution FRET-based assay. BLI experiments have shown an interesting development in the kinetic profile of this PPI. Immobilisation of biotinylated ERα on a streptavidin-coated biosensor allowed the observation of 14-3-3σ binding through changes in the optical thickness. Data collected suggests much higher affinities than previously thought with very slow off rates, creating new kinetic avenues to explore.

Non-radiative FRET also presented a useful in-solution method to study this PPI and this research concluded that direct labelling of the protein partners is most efficient and an ideal FRET pair is Cy3/Cy5. These conditions led to efficient small-scale measurements showing concentration dependent FRET. This preliminary data has been proven accurate through stabilisation by FC-A and a competition assay with unlabelled ERα. In the future, this system will be transferred into liposomes allowing observation of on and off rates on a single molecule level.





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