The Institute of Cancer Research **PHD STUDENTSHIP PROJECT PROPOSAL : iCASE SCHEME**

PROJECT DETAILS	
Project Title:	Building the monovalent degrader toolbox for cancer drug discovery

SUPERVISORY TEAM	
Primary Supervisor(s):	Olivia Rossanese, Target Evaluation and Molecular Therapeutics
Associate Supervisor(s):	Ben Bellenie, Medicinal Chemistry 4
Industry supervisor:	J. Willem M. Nissink, AstraZeneca
Secondary Supervisor:	Swen Hoelder
DIVISIONAL AFFILIATION	
Primary Division:	Cancer Therapeutics
Primary Team:	Target Evaluation & Molecular Therapeutics
Other Team:	Medicinal Chemistry 4

PROJECT PROPOSAL

SHORT ABSTRACT

Most drugs work by binding to a target to inhibit its function. Targeted protein degradation (TPD) offers an alternative – compound binding induces the destruction of a protein target, enabling longer lasting and sometimes different biological effects.[1–3] Current methods for inducing TPD include PROTAC (bivalent molecules that bind to both the target and components of the cell's degradation machinery called E3 ligases, bringing them together)[2,4] and IMiDs (monovalent molecules that bind to E3 ligases and alter which targets they bind to and degrade).[5,6] Monovalent degraders which bind to a target and enable its recognition for selective degradation are underexplored, and have great potential for therapeutic use.

In this project, we will combine computational methods and chemical synthesis to explore structural features that can trigger degradation, and design and prepare sets of compounds incorporating those features into known inhibitors. We will test our compounds for degradation activity in cells using chemoproteomics, and explore the mechanism of identified degraders using molecular and cell biology. Through this multidisciplinary approach we will seek to build a "monovalent degrader toolbox" – sets of molecular design principles that can be applied to the discovery of novel degraders for targets of therapeutic interest.

BACKGROUND TO THE PROJECT

Small-molecule mediated degradation of protein targets has developed into a new therapeutic modality and a valuable tool for target validation.[1–3,6,7] The design principles of novel bivalent (PROTAC-type, Fig 1) degraders are well established; a target-binding moiety is connected to an E3-ligase binding moiety via a linker, and optimisation of these components can enable ternary complex (target:PROTAC:ligase) formation. Because the PROTAC binds to target and E3 ligase independently, a decrease in degradation occurs at high compound concentrations (a "hook effect"). PROTAC have proven valuable as in vitro

tools; however, the relatively large size of these molecules presents a challenge in developing PROTAC for in vivo and clinical applications.

In contrast, monovalent degraders bind to only one component. IMiDs (including lenalidomide, iberdomide) bind to the E3 ligase Cereblon and modify its surface, causing it to bind to and trigger degradation of non-natural binding partners. These compounds are smaller than PROTAC and have physicochemical properties that facilitate their application *in vivo.* Although approaches to enable prediction of degradation targets are being explored,[2] currently it is not considered possible to design an IMiD to degrade a specific target of interest, limiting their broader applicability.

An alternative approach is to take a known ligand that binds to a target of interest and make chemical modifications which lead to target degradation. There are a number of isolated examples of this modified target-ligand (MoTaL, fig 1) approach.[8] Addition of "hydrophobic tags" has been shown to trigger degradation; for example the clinically-important selective estrogen degraders (SERDs),[9,10] and a degrader of EZH2.[11] Addition of a "BocArg" tag has been shown to trigger degradation by direct engagement of the proteasome.[12]

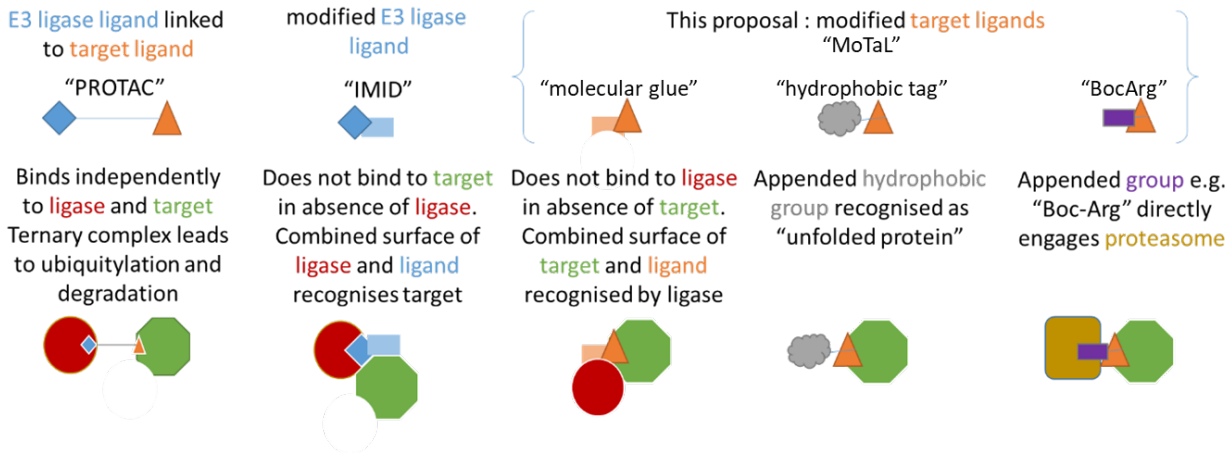

Fig 1: Small molecule degraders classified by their general chemical architecture

Recently, a novel class of degraders have been discovered which work on the "molecular glue" principle.[8,13,14] The compound binds strongly to the degradation target, and the resulting combined protein-ligand surface is recognised by an E3-ligase which sticks to it and triggers degradation. To date, these have been largely been discovered serendipitously, or through target-agnostic screening. Unlike the IMiDs, which bind to the E3 ligase, MoTaLs allow for addressing a specific disease-linked hypothesis directly, by targeting a specific protein. Building an understanding of the molecular features leading to degradation would enable the discovery of low molecular weight degraders for specific targets of therapeutic or biological interest.

PROJECT AIMS

- Use computational tools to analyse published and in house structural data on "molecular glue" and other modified target-ligand (MoTaL) degraders, looking at common features and properties of the combined protein-substrate surfaces. Generate structure-based design hypotheses for converting ligands into monovalent degraders.
- Design libraries of compounds which incorporate features and principles identified onto known ligands, in order to test structure-based design hypotheses. Incorporate medicinal chemistry design principles to enable cell permeation.
- Synthesise designed libraries using parallel solution-phase chemistry techniques, including solid-supported reagents and catch-and-release purification.
- Test synthesised compounds for ability to degrade target of interest in cells, using Western blotting and chemoproteomics.
- Explore mechanisms of degradation of identified degraders, including dependence on ubiquitin / proteasome system using pharmacologic and genetic tools. If indicated, use an siRNA library to determine the critical E3 ligase involved.

RESEARCH PROPOSAL

Recently, we and others have reported efficient monovalent degraders that bind to the degradation target.[13,15,16] We refer to these as "modified target-ligand" or MoTaL degraders, to differentiate from PROTAC or ligase-modifying degraders such as IMiDs. To date, only a small number of degraders of this type have been reported. In this project, we will analyse common features of in house and published degraders and use this information to design libraries of potential degraders for targets of interest. We will use parallel chemical synthesis to prepare sets of compounds, and test their ability to degrade their protein targets. We aim to identify new monovalent degraders, optimise them into useful chemical tools, and build an understanding of the mechanisms of degradation.

The functions of the cell's protein degradation machinery include the control of protein half-life (particularly for short lived proteins), and quality control - the destruction of misfolded or mutant proteins, or excess isolated subunits of larger protein complexes. Misfolded or isolated subunit proteins present unexpected structural features, for example aberrant hydrophobic patches on their surfaces, causing them to be recognised for degradation. The cell's degradation machinery must therefore be able to recognise a diverse range of "unexpected hydrophobicity" and a large range of adaptor proteins fulfils this function. Each adaptor recognises specific shapes or patterns of charge and hydrophobicity, allowing the cell to detect and destroy a broad range of undesired proteins.

Research question 1: How many MoTaL degraders are known in the literature, and what targets have been degraded?

We hypothesise that MoTaL degraders are less rare than the number of reports to date suggest – that certain compounds already prepared as target inhibitors may also act as degraders, and that many ligands occupying an appropriate solvent-facing site can be converted into monovalent degraders. We anticipate further publications in this area prior to project start, so the initial task will be to *review the literature and analyse data*. Computational tools including MOE will be used to ***review published and in-house structural data***.

Research question 2: What structural features can trigger degradation?

We will examine the properties of the residues surrounding the surface-exposed region of published and in-house ligands and ***identify patterns of lipophilic, electronic and steric features***. We will generate structure-based hypotheses based on these features. We have already developed simple early design hypotheses based on reported literature and will use this here to illustrate how we will apply these to library design of novel degraders.

Slabicki *et al* have reported "molecular glue" type degraders of cyclin K.[13] The ligand CR8 binds to the CDK12-cyclic K complex recruiting the CUL4 adaptor protein DDB1. A crystal structure of the resulting complex (fig. 2) shows a hydrophobic biaryl group clearly visible pointing out from a cleft in CDK12. DDB1 binds to the combined surface formed by CDK12 and the ligand.

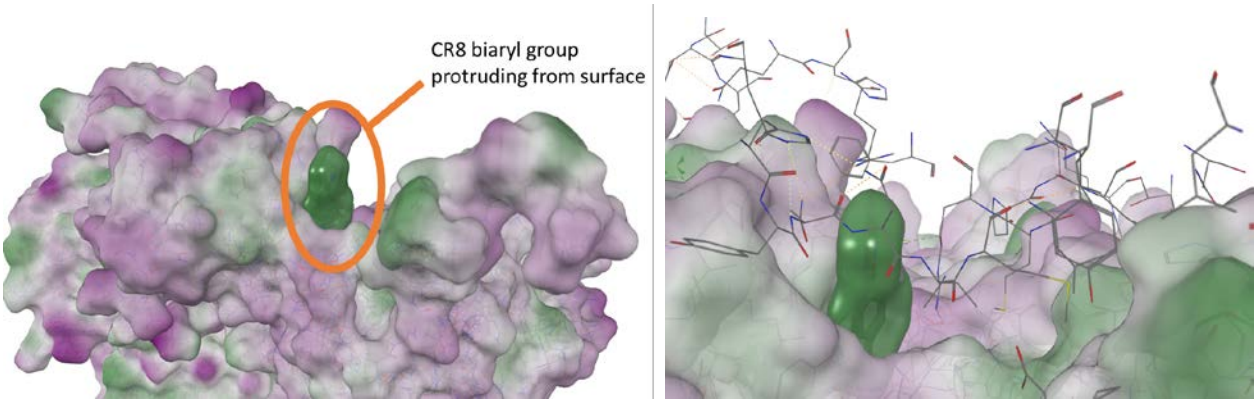

Figure 2: CDK12-cyclin K bound to ligand CR8 (PDB code 6TD3, DDB1 removed from left image). Green surface represents hydrophobic regions; purple hydrophilic.

From our own research, we show that a dimethyl piperidine group protruding from a binding cleft triggers BCL6 degradation (Fig. 3, left), whereas the analogous, less hydrophobic morpholine (Fig 3, right) binds in a similar fashion but does not cause degradation.

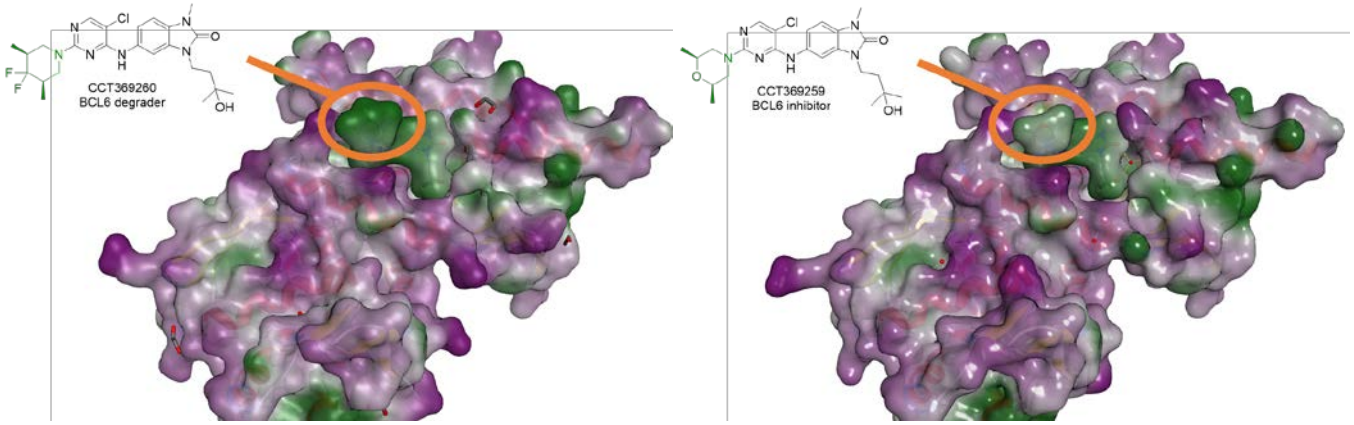

Figure 3: BCL6 BTB domain bound to degrader CCT369260 (left) or inhibitor CCT369259 (right). PDB codes 6TOM and 6TOL

In both cases highlighted, the ligand presents a ***conformationally inflexible, hydrophobic surface*** sticking out from the surface of the protein, with potential ***space adjacent to the ligand*** for an adaptor protein or similar to bind. Additional hypotheses will be generated and existing ones refined as more data on monovalent degraders is published or generated in house.

Research questions 3: How specific are the structural requirements for degradation? and
4: Can different degradation mechanisms be invoked for the same target?

In house experience both at AZ[17] and ICR[15] and has shown that small changes to chemical structures can abrogate degradation. Initial work will seek to ***systematically explore small structural changes around a known degrader*** and understand the tolerance of structural change. We also hypothesise that it will be possible to find alternative structures that trigger degradation, by modifying the combined surface to attract a different component of the cell's protein degradation machinery. We will therefore also seek to explore more diverse structures to test the structural hypotheses generated in section 2.

One possible example is described to illustrate this. Based on the literature starting point CR8, we will make small modifications to explore what structural changes are tolerated. We will then target additional compounds where DDB1 binding is prevented or disfavoured, and explore whether degradation can be induced by a different binding partner or mechanism, guided by the information and hypotheses generated in part 2. Examples of target compound can be found in Figure 5.

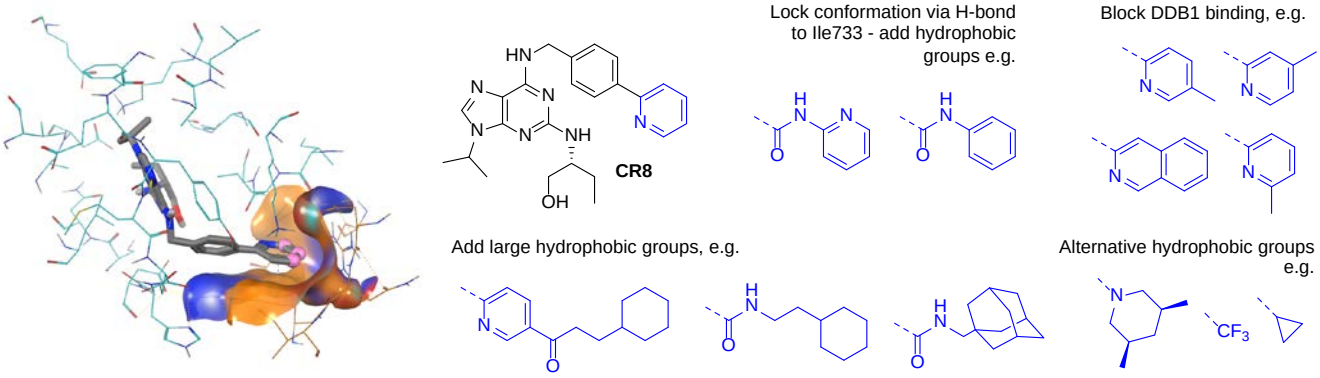

Figure 5: CDK12-cyclin K bound to ligand CR8 (left), and potential library target molecules (right). Substitution from the pyridine at positions highlighted (pink spheres) will induce a clash with DDB1 (orange surface).

Mechanisms of degradation can be studied as described below.

Research question 5: Can we discover degraders for different targets based on the same ligand?

It has previously been demonstrated that non-selective ligands can be converted into selective PROTACs – although the resulting PROTACs bind to multiple targets, productive ternary degradation complexes do not form equally readily for all targets. We will investigate whether the same is true for monovalent degraders. We will use the information generated from previous sections to ***design focussed libraries based around multi-target inhibitors*** which target 5-10 kinases. By focussing on multi-target ligands from a single target class, we can:

1. Take advantage of the broad structural similarity between kinases in our design – molecules are likely to show similar binding modes between targets, and the broad similarities will aid analysis and design
2. Efficiently screen for degradation using proteomics as described below.
3. Increase our chances of identifying degraders – each molecule combines with multiple targets generating multiple distinct protein-ligand binding surfaces which may be recognised for degradation

Novel degraders can be further optimised to useful chemical tools, profiled for broader selectivity, and mechanism of degradation studied.

Research question 6: Can we discover degraders for a specific target of interest?

The ultimate goal of this project is to develop a "degrader toolbox" – degrader groups and structural knowledge which can be applied to convert inhibitors to degraders. Using knowledge and experience from previous sections, we will design and synthesise potential degraders for a target of interest in cancer research.

Research question 7: What mechanisms of degradation are observed?

For identified degraders, we will confirm monovalent degradation by checking for lack of a hook effect. Dependence on the proteasome and on ubiquitination will be probed using proteasome and neddylation inhibitors, as well as the E1 inhibitor TAK-243. If results suggest a ubiquitin-dependent mechanism, we will screen an E3 ligase siRNA library to determine which E3 ligase is involved.

Scientific techniques

This project will apply techniques from multiple scientific disciplines to answer the key scientific questions posed, and discover novel degraders. All necessary training will be provided, and the supervisory team spans the disciplines covered.

The student will apply parallel chemical synthesis techniques including solid-supported reagents, solid phase extraction and catch-release purification, to prepare multiple libraries each of 30-50 compounds. Chemical synthetic routes will be designed to facilitate this, and ICR and AZ labs have automated purification and analysis equipment available to support this work.

Using computational chemistry tools including MOE, the student will study published and in house X-ray crystal structures, build models and design compounds to test generated hypotheses. Medicinal chemistry principles will be used to ensure compounds are sufficiently soluble and able to permeate cells.

Compounds designed to degrade a specific target will be tested in an appropriate cell-line expressing that target, and degradation assessed by Western blotting using a selective antibody. Chemical inhibitors of components of the cell's protein degradation machinery will be used to investigate the mechanism of degradation. Biochemical binding assays will be used to confirm that substituent groups do not abrogate binding. For kinase-focussed degraders, we will use proteomics to broadly assess kinase degradation, and competition experiments with parent kinase inhibitors will be used to check whether degradation can be abrogated. Kinobeads will be used to capture endogenous kinases, simplifying the analysis.

Supervisory team

Dr. Olivia Rossanese (Target Evaluation and Molecular Therapeutics team) has extensive experience in cell and molecular biology with particular expertise in unravelling the molecular mechanism of action of small molecules and more recently in characterising monovalent degraders.

Dr Ben Bellenie (part of Prof. Swen Hoelder's team, Medicinal Chemistry 4) brings experience in medicinal and synthetic chemistry, including library design and high-throughput parallel synthesis. Both teams collaborated on the discovery of monovalent BCL6 degraders.[15]

Dr. Willem Nissink (AstraZeneca) will fill the role of industrial supervisor. He is a computational chemist with extensive experience in drug design, including development of monovalent small-molecule degraders and PROTACs.

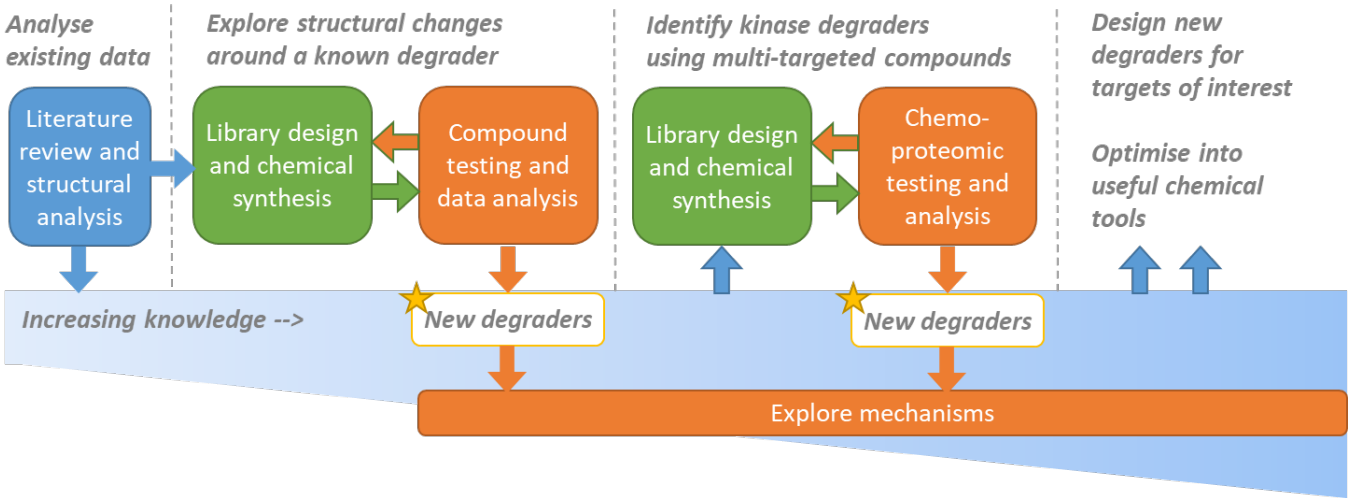

Figure 6: Schematic showing project plan.

Training and next steps

The student will gain valuable expertise in interdisciplinary research, including compound design and parallel chemical synthesis, cell biology and proteomics, and computational analysis, with one-to-one training from experienced postdoctoral scientists, and will benefit further from exposure to industrial research through a placement at AstraZeneca. Supporting this, our in house training programmes, along with seminars from AZ speakers, provide a broad understanding of drug discovery. As a result, past students in our groups have readily found employment as scientists in academia or the pharmaceutical industry.

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CANDIDATE PROFILE	
Note: the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1)	
Pre-requisite qualifications of applicants:	• At least a 2:1 degree in a relevant scientific subject • Some experience of synthetic chemistry is required

	• Enthusiasm to learn and work across disciplines including synthetic, medicinal and computational chemistry, and molecular biology
Intended learning outcomes (including those arising from the industry collaboration):	• Hands-on experience of compound synthesis, analysis of data, and use of data in compound design • Hands-on experience of computational chemistry including structure visualisation and analysis • Use of parallel chemical synthesis and purification techniques • Experimental cell biology techniques including tissue culture, Western blotting, compound testing, siRNA, protein overexpression and pulldown techniques • Application of chemoproteomics techniques • Broad experience of medicinal chemistry and drug discovery, from exposure to other projects within the unit and attendance of training seminars, problem sessions and workshops • Experience of working in an industrial environment through placement • Ability to present work verbally and in writing to a multidisciplinary audience, including writing and submitting of papers to high impact peer-reviewed journals
Potential publications arising from project:	• A general paper or review describing molecular rules governing the design and discovery of monovalent degraders and their application to multiple targets • Specific case study from a single target to include mechanism of degradation and the molecular consequences of target loss
Estimated amount and distribution of time spent with industrial partner:	• Placement of 3 – 6 months • Regular communication with industrial supervisor, including direct supervision of computational aspects
ADVERTISING DETAILS	
Project suitable for a student with a background in:	☒ Biological Sciences ☐ Physics or Engineering ☒ Chemistry ☐ Maths, Statistics or Epidemiology ☐ Computer Science ☐ Other
Keywords:	**1. Cancer drug discovery**
	2. Small molecule degraders
	3. PhD with industrial collaboration
	4. Medicinal and synthetic chemistry
	5. Multidisciplinary cancer research

	6. Structure-based design