The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL : MRC iCASE SCHEME

PROJECT DETAILS	
Project Title:	Investigating the druggability of the motif-mediated interactome with peptide PROTACs
Short Project Title:	Investigating the druggability of the motif-mediated interactome with peptide PROTACs
SUPERVISORY TEAM	
Primary Supervisor(s):	Norman Davey
Associate Supervisor(s):	Sebastian Guettler
Industry supervisor:	Stefan Jaekel
Backup Supervisor:	Jon Pines
Lead contact person for the project:	Norman Davey
DIVISIONAL AFFILIATION	
Primary Division:	Cancer Biology
Primary Team:	Short Linear Motifs
PROJECT PROPOSAL	

SHORT ABSTRACT

Short, Linear Motif (SLiMs) are ubiquitous compact interaction interfaces that direct key cellular processes. PROTACs targeting SLiM-binding pockets can specifically promote ubiquitin ligase-dependent degradation of regulatory proteins in critical cellular pathways. We will apply a scalable method using genetically encoded peptide PROTACs to provide proof of principle for the therapeutic relevance of targeted degradation of essential proteins. Peptide PROTACs provide a fast and cost-effective approach to mirror the mode of action of a small molecule PROTAC, providing evidence for the spatiotemporal compatibility of the E3 and target, and the phenotype of target protein degradation.

BACKGROUND TO THE PROJECT

Proteolysis targeting chimeras (PROTACs) are a promising strategy to target previously "undruggable" cellular proteins for therapy. PROTACs emerged from the study of a class of SLiMs known as degrons that act as docking motifs for E3 ubiquitin

ligases. PROTACs function by promoting the poly-ubiquitination and consequent degradation of the target protein by encoding an E3-binding and a target-binding region separated by a short linker.

The development of small molecule PROTACs is expensive and time-consuming; consequently, careful target selection is vitally important. We will use genetically encoded peptide PROTACs targeting SLiM-binding pocket in key regulatory proteins to provide substantive evidence for prioritisation of targets for further development or triage. The peptide PROTACs approach allows medium-throughput testing of the spatiotemporal compatibility of the E3 and target, and the phenotype of dose-dependent target degradation, without the development steps required for cell-penetrating peptides or small molecule discovery and optimisation.

The project will focus on SLiM-binding pockets in the cell cycle pathway. SLiM-mediated interactions build dynamic complexes required by many core cell cycle pathways. As a result of the central role of SLiM-mediated interactions in cell cycle regulation, the deregulation of SLiM-containing or -binding proteins can lead to catastrophic defects, making them a potent target for therapies. Importantly, SLiM-binding pockets are optimised to specifically bind short peptides (usually between 3 and 8 amino acids in length); in most cases, the peptide binding determinants are well understood as the peptides that bind the pocket have been characterised.

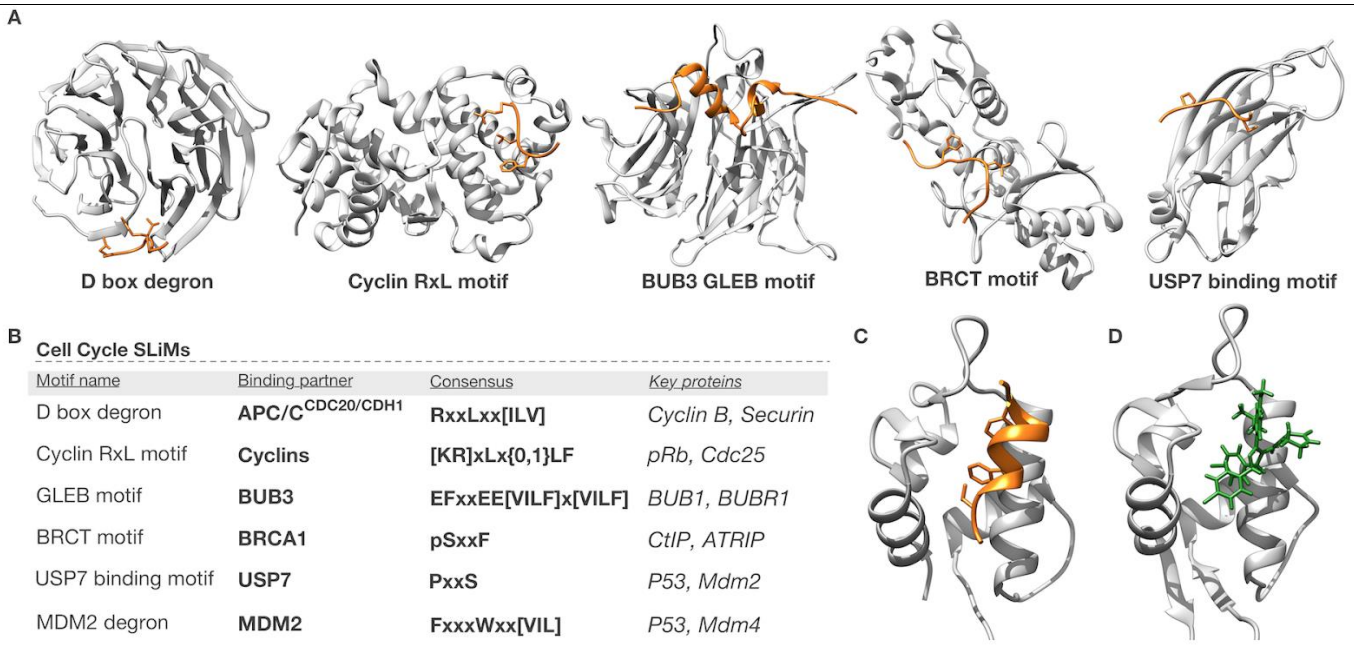
Approximately 250 classes of SLiM-binding pocket have been experimentally characterised. Yet, only a handful of the proteins containing these motif-binding pockets have been explored for their potential as PROTAC targets. The goal of the project is to determine the therapeutic relevance of targeted degradation of the SLiM-binding proteins of the cell cycle, and create the experimental framework required to scale-up the screening approach to the whole proteome level.

PROJECT AIMS

- Development of an optimised scaffold construct for an inducible genetically-encoded heterobifunctional peptide PROTAC
- Construct a set of cell lines expressing peptide PROTACs targeting motif-binding pockets of cell cycle proteins
- Assay the ability of peptide PROTACs to promote the degradation of the targeted proteins and to modulate cell cycle progression and cell viability
- Screen peptide PROTACs with relevant phenotypes in cancer cell lines and prioritise targets for further development

RESEARCH PROPOSAL

SLiMs are central to many key pathways of strong interest to the drug discovery community, including cell signalling, cell cycle regulation, DNA damage repair and transcriptional control (Van Roey et al. 2014). Given the expertise and current research in the participating labs, in this project, we will target the SLiM-mediated interactome of the cell cycle (Figure 1A-B). Cell cycle control is heavily dependent on ubiquitin-dependent degradation, and defects in the timing of degradation of cell cycle protein can result in cell cycle arrest, checkpoint deregulation or mitotic catastrophes (Davey & Morgan 2016). Targeting the machinery required by dividing cells has an obvious application as a cancer therapy.



Motif name	Binding partner	Consensus	Key proteins
D box degron	APC/C$^{CDC20/CDH1}$	RxxLxx[ILV]	*Cyclin B, Securin*
Cyclin RxL motif	Cyclins	[KR]xLx{0,1}LF	*pRb, Cdc25*
GLEB motif	BUB3	EFxxEE[VILF]x[VILF]	*BUB1, BUBR1*
BRCT motif	BRCA1	pSxxF	*CtIP, ATRIP*
USP7 binding motif	USP7	PxxS	*P53, Mdm2*
MDM2 degron	MDM2	FxxxWxx[VIL]	*P53, Mdm4*

Figure 1: Representative examples of the SLiMs bound to SLiM binding pocket in cell cycle proteins **(A)** Structures of cell cycle motifs (orange; *D box degron* PDB:4BH6, *Cyclin RxL motif* PDB:1H27, *BUB3 GLEB motif* PDB:2I3T, *BRCT motif* PDB:1T29, *USP7 binding motif* PDB:2FOJ) bound to their motif-binding domains (grey). **(B)** Table of examples from panel A. **(C)** Structure of the MDM2 degron of P53 bound to the SWIB domain of the E3 Ub ligase MDM2 (PDB:1YCR). **(D)** Bound structure of the MDM2 degron motif small molecule mimetic drug Nutlin (PDB:4HG7).

The primary output of the project will be a set of validated targets for further development of small molecule PROTACs. Successful peptide PROTACs will provide the phenotype of target degradation, information on the spatiotemporal compatibility of the E3 and target, molecular details of the target binding site, and a set of peptides as peptidomimetic scaffolds. This will allow evidence-based prioritisation of targets for the development of small molecule PROTAC targeting motif binding pockets (Figure 1C-D). A second important output of the project will be a scalable framework for the high-throughput characterisation of target pockets for PROTAC-based drugs. Both these outputs align with the pharmaceutical industries interest in discovering and developing novel PROTAC drugs targeting previously "undruggable" proteins.

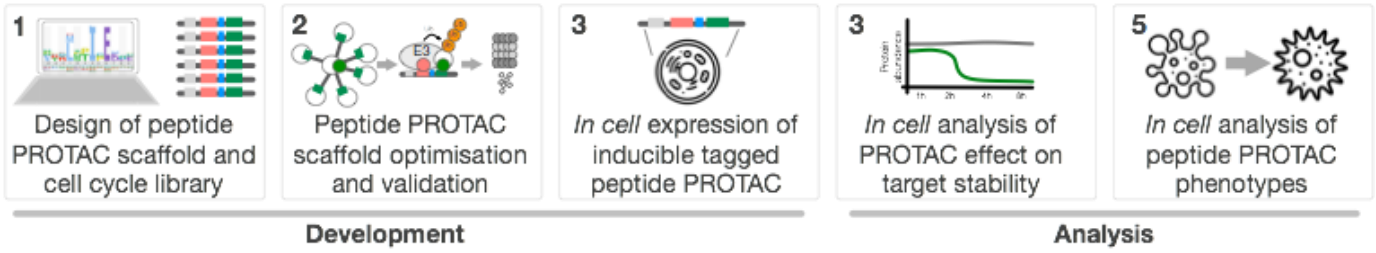


Figure 2: Overview of the project.

The project will have 2 major sections - Development and Analysis (Figure 2):

Section 1: Development - Create a library of cell lines expressing peptide PROTACs targeting the cell cycle machinery.
(1.1) *Development of an optimised scaffold for a heterobifunctional peptide PROTACs*
(1.2) *Design a peptide PROTAC library*
(1.3) *Construct a collection of cell lines expressing inducible peptide PROTACs*

Section 2: Analysis - Test the function and therapeutic potential of peptide PROTACs targeting the cell cycle machinery.
(2.1) *Test the functionality of the PROTAC in non-transformed cells.*
(2.2) *Test the functionality of the PROTAC in therapeutically relevant cell lines.*

The project aligns with our recent grant awards focussing on the Cell Cycle (a CRUK-funded Senior Cancer Research Fellowship entitled *"Discovery, characterisation and therapeutic targeting of motif-mediated interactions regulating cell cycle progression"*) and on Ubiquitin Ligases (the Horizon 2020-funded European Training Network (ETN) entitled *"UBIMOTIF: Short linear interaction motifs as specificity determinants in the ubiquitin system"*). These complementary projects will allow the recruited student to learn from the shared interests and expertise of the other group members.

PLEASE INDICATE WHETHER THIS PROJECT ALIGNS WITH ANY OF THE MRC STRATEGIC SKILLS PRIORITY AREAS?

☒ Quantitative skills ☒ Interdisciplinary skills ☐ Whole organism physiology

See the link below for further details: https://mrc.ukri.org/documents/pdf/mrc-strategic-skill-priorities/

LITERATURE REFERENCES

Collin, P. et al., 2013. The spindle assembly checkpoint works like a rheostat rather than a toggle switch. Nature Cell Biology, 15(11), pp.1378–1385.

Davey, N.E. & Morgan, D.O., 2016. Building a Regulatory Network with Short Linear Sequence Motifs: Lessons from the Degrons of the Anaphase-Promoting Complex. Molecular Cell, 64(1), pp.12–23.

Dinkel, H. et al., 2016. ELM 2016--data update and new functionality of the eukaryotic linear motif resource. Nucleic Acids Research, 44(D1), pp.D294-300.

Kruse, T. et al., 2018. The Ebola Virus Nucleoprotein Recruits the Host PP2A-B56 Phosphatase to Activate Transcriptional Support Activity of VP30. Molecular Cell, 69(1), pp.136-145.e6.

Ottis, P. & Crews, C.M., 2017. Proteolysis-Targeting Chimeras: Induced Protein Degradation as a Therapeutic Strategy. ACS Chemical Biology, 12(4), pp.892–898.

Sakamoto, K.M. et al., 2001. Protacs: chimeric molecules that target proteins to the Skp1-Cullin-F box complex for ubiquitination and degradation. Proceedings of the National Academy of Sciences of the United States of America, 98(15), pp.8554–8559.

Timms, R.T. et al., 2019. A glycine-specific N-degron pathway mediates the quality control of protein N -myristoylation. Science, 365(6448), p.eaaw4912.

Van Roey, K. et al., 2014. Short linear motifs: ubiquitous and functionally diverse protein interaction modules directing cell regulation. Chemical Reviews, 114(13), pp.6733–6778.

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: e.g. BSc or equivalent in specific subject area(s)	B.Sc or M.Sc in a biological science
Intended learning outcomes (including those arising from the industry collaboration): Please provide a bullet point list (maximum of seven) of the knowledge and skills you expect the	• Cell line development • Approaches to protein engineering for synthetic biology • Fluorescence microscopy and image analysis • Degradation assays

student to have attained on completion of the project.	• Protein-protein and protein-peptide interaction assays • Cancer cell phenotype screening
Potential publications arising from project:	The project will result in two papers: (1) A methods paper describing the peptide PROTAC scaffold design, peptide scaffold optimisation and screen setup. (2) An analysis paper describing the cell cycle peptide PROTACs, their ability to specifically degrade their targets, their phenotype, and the effect of the selected peptide PROTACs in select cancer cell lines.
Estimated amount and distribution of time spent with industrial partner: *MRC requires a cumulative period of no less than three months spent working in the facilities of the industrial collaborator.*	3-6 months
ADVERTISING DETAILS	
Project suitable for a student with a background in: (Please tick all categories that apply – your project will be advertised under all selected categories)	☒ Biological Sciences ☐ Physics or Engineering ☐ Chemistry ☐ Maths, Statistics or Epidemiology ☐ Computer Science ☐ Other (provide details)
Keywords: Please provide 4-6 words/short phrases that potential students may type into search engines (e.g. Google) to search for PhDs similar to yours – e.g. 'cancer predisposition genes', 'physics PhD London' etc.	**1.** PROTACs
	2. Cell Cycle
	3. Cancer
	4. Short linear motifs
	5. Synthetic biology
	6. Biology PhD London