CeTPD Journal Club

June – July 2024

Targeted protein degradation, medicinal chemistry, chemical structural biology & cell biology

Centre for Targeted Protein Degradation
University of Dundee

innovate
collaborate
inspire

TABLE OF CONTENTS

MEET THIS MONTH'S EDITORS

Click here for info on the editor

JEFF WONG

Jeff obtained his Mchem degree at the University of York, undertaking his final year project under the supervision of Prof. Peter O'Brien and Dr Thomas Farmer developing methodologies for obtaining lead-like nitrogen heterocycles from the renewable starting material 2-MeTHF. Jeff then joined the research group of Dr Graeme Barker in 2017 as the inaugural PhD student, where he developed novel C-H functionalisations of alkylazoles under batch and continuous flow conditions using organometallic chemistry. Jeff then joined the AC-BI team in 2021 as a medicinal chemist.

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JEYAN OSMAN

Jeyan completed his Medicinal Chemistry Degree with Honours at Monash Institute of Pharmaceutical Science (MIPS) at Monash Univeristy of Melbourne, Australia in 2019. He is currently in the final year of his PhD, as supervised by Prof. Martin Scanlon and A/Prof. Ben Capuano. Now, he joined the Ciulli Group as a visiting PhD student for 6-months, supported by the Australian Research Council's Centre of Fragment Based Design (ARC CFBD - https://cfbd.org.au/).

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MANON STURBAUT

Manon got her Master's degree in medicinal chemistry in 2016 from University of Lille (France) and remained in Lille to undertake her DPhil studies. Following her PhD, she joined NICR group in Newcastle in 2020 to begin a two-year postodoctoral position with Prof. Mike Waring. Since April 2022, she has joined AC-BI team in Dundee as a medicinal chemist.

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VESNA VETMA

Vesna got her MSc in Molecular Biology in 2014 from University of Zagreb, Croatia. She was awarded MSCA stipend in 2015 to pursue a PhD in Cell biology across international and interdisciplinary institutions, graduating with a PhD from University of Stuttgart, Germany in 2019. The same year she joined AC-BI team in Dundee as a cell biologist and from 2022 she is a senior scientist in cell biology at CeTPD.

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INTRODUCING THE NEW CETPD JOURNAL CLUB!

We are excited to introduce the new format of the CeTPD Journal Club with this issue!

Since its birth in 2020, the Journal Club has been led by different PhD students in the CeTPD, and we have had great feedback from the TPD community. Four years later, we are rebranding the Journal Club, and introducing some new and exciting changes.

Over the past few years, we have seen an increase in the amount of TPD-related published pre-prints, so we feel the need to cover these in a separate way. Starting from this issue you will find a section dedicated **to pre-prints of the month**, where we will highlight some pre-prints in a concise format. This way, when the paper is published in a journal, we will cover it thoroughly.

Additionally, we will increase the amount of "**editors of the month**" from three to four, and the issues will be published **bi-monthly**, resulting in six issues per year.

We would like to introduce our new "formatting editor"; Alessandra Salerno, who has designed the new layout of the CeTPD Journal Club.

We welcome any feedback on the new format. Please feel free to reach out to us via LinkedIn or Twitter/X.

We look forward to hearing from you!

All the best,

The "editors in chief":
Andreas Holmqvist 𝕏@***Holmqvist89***
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TARGETED PROTEIN DEGRADATION

CHEMISTRY | STRUCTURAL BIOLOGY & BIOPHYSICS | CELL BIOLOGY | MODELLING

"Every two months, we spotlight the latest and most significant literature in the field of targeted protein degradation, spanning chemistry, biophysics, cell biology, and computational modeling"

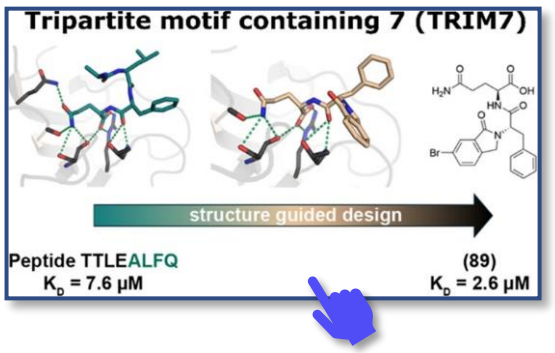
Literature review from 21st May to 20th July 2024

| *Manon*

A C-Degron Structure-Based Approach for the Development of Ligands Targeting the E3 Ligase TRIM7

Christian J. Muñoz Sosa§, Christopher Lenz§, …, Stefan Knapp*
ACS Chem. Biol. **2024**, 19, 7, 1638-1647

TRIM7 is an E3 ligase with regulatory functions such as autophagy and ferroptosis. It contains a PRYSPRY domain (TRIM7^{PRYSPRY}) that specifically recognise degron sequences containing C-term glutamine. The TRIM family has been poorly explored in terms of ligandability. The authors described here the development of a peptidomimetic ligands that mimic the glutamine degron and developed assay to study the binding *in vitro* and *in cellulo*.



Click on the picture for link to the journal

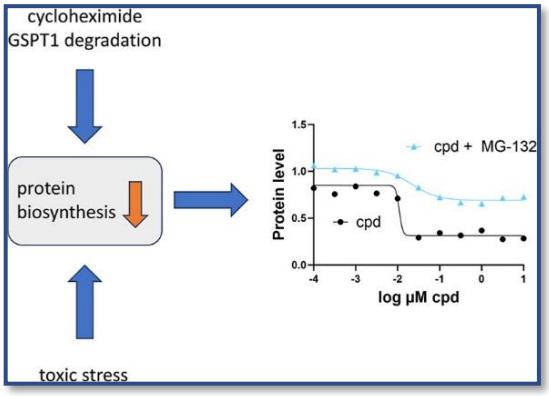
The compounds present C-terminal glutamine and a hydrophobic moiety linked by amide or sulfonamide (see picture). The library has been screened by DSF and FP with good correlation (R^2 = 0.88) leading to the discovery of new hits. However, peptidomimetic compounds are known to be poorly permeable render their cellular evaluation difficult, especially due to the presence of the carboxylic acid. In this case, the cellular target engagement assay allows the determination of binding constant in permeabilised cells only.

It is interesting to see the development of new ligand for poorly explored E3 ligase like TRIM7. It is a nice starting point for ligand development with low micromolar potency and good selectivity. However, for further use, it might be required to tackle the poor permeability of the compound. Moreover, the presence of Cys501 in the binding pocket opens new opportunities for the design and the development of new covalent TRIM7 binders.

| *Manon*

Cofounding factors in targeted degradation of short-lived proteins

Vesna Vetma§, …, Manfred Koegl*
ACS Chem. Biol. **2024**, 19, 1484-1494



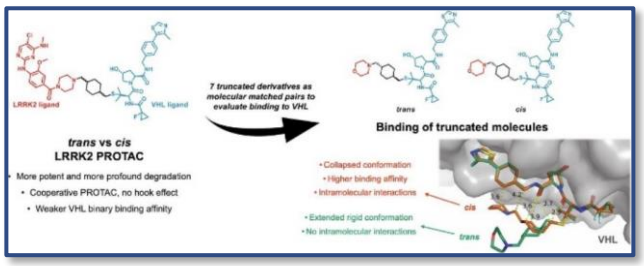
PROTACs can achieve potent degradation with long-life protein, nevertheless this is not true for short-lived protein. The lower half-life, the less effective degradation you get. This can be predicted by a mathematic model which take into consideration the half-life/synthesis rate of the protein, the concentration of the PROTAC, and the treatment time. As an example, ACBI2, a SMARCA2 degrader, is less efficient with HiBit tagged version of SMARCA2 ($t_{1/2}$ = 4h) than endogenous SMARCA2 ($t_{1/2}$ = 24h). Indeed, to have an effect, the PROTAC degradation rate should exceed the fast natural decay rate of the protein. A library of 6500 compounds has been screened to target CRAF, a short-lived protein. Different experiments show that the observed CRAF degradation is indirect and is caused by the degradation of GSPT1. Indeed, degraders of GSPT1, a known off-target for CRBN-based PROTACs, will artificially appear as degraders of short-lived proteins due to their effect on protein synthesis. This has been confirmed by looking at the protein level of other short-lived protein such as MYC or MCL1. Moreover, cytotoxic agents could also cause a reduction in short-lived protein level causing artifacts. To tackle this, the authors suggest (i) to judge the feasibility of a project with the help of a mathematical model and (ii) confirm the mode of action of PROTACs targeting short-lived protein with several controls.

This is a very useful paper with negative results that will help biologists to look at their results with a critical eye.

| *Jeff*

Stereochemical inversion at a 1,4-cyclohexyl PROTAC linker fine-tunes conformation and binding affinity

Martina Pierri§, Xingui Liu§, …, Alessio Ciulli*
Bioorg. Med. Chem. Lett. **2024**, 110, 129861



Pierri and Liu et al. has conducted a biophysical study of XL01126 and XL01134. A pair of LRRK2 PROTACs which differs in the stereochemistry in the cyclohexyl linkers. Pervious work found that although the trans isomer (XL01126) exhibits better degradation profile than the cis isomer (XL01134), XL01134 contradictory showed better binary affinity towards VHL than XL01126. To better understand of this phenomenon, 6 truncated VHL fragments of XL01126 and XL01134 were synthesised. Their VHL affinities were then tested by florescence polarisation (FP) and isothermal titration calorimetry (ITC) assays, it was found that

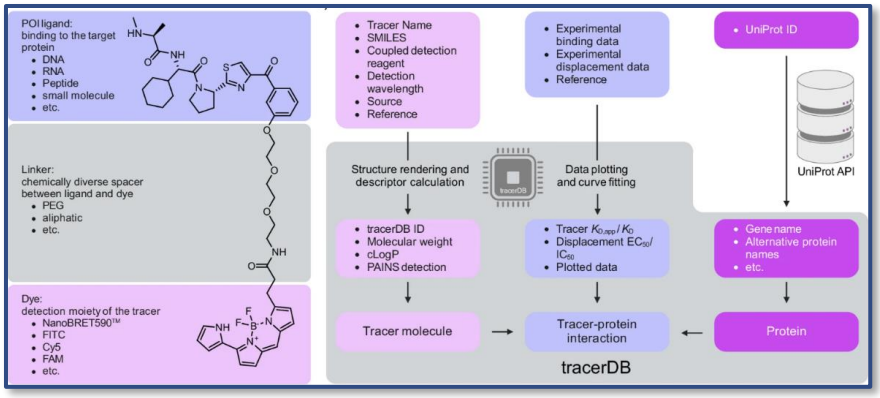
the cis fragments have consistently showed stronger VHL affinities than the trans isomers. Finally, co-crystal structures of the cis isomer with VCB showed the ligand adopted a collapsed conformation, allowing the formation of additional weak interaction with VHL to increase affinity. Whilst the trans isomer adopted a more rigid conformation away from the VHL ligand, promoting ternary complex formation by pre-organising the two proteins together. The paper nicely demonstrated how biophysics could allow us to gain better understanding on how chemical probe works in the biological settings.

The findings from this study along with computational docking could allow better design of VHL PROTACs with similar linkers.

| *Vesna*

tracerDB: a crowdsourced flourescent tracer database for target engagement analysis

Johannes Dopfer§, …, Martin P. Schwalm*
Nat. Commun. **2024**, 15, 5646



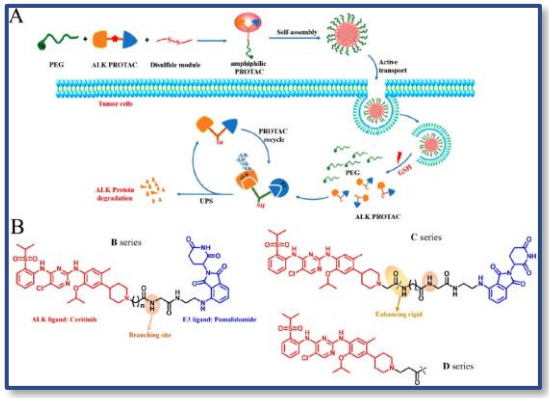
The authors have created tracerDB, a freely available, curated database of validated tracers used for cell biology or structural biology (https://www.tracerdb.org/). Tracers are compounds that can be used to measure ligand:protein interactions through displacement or binding. These molecules contain a ligand for binding the target, a linker and a detection moiety. The optimized tracers in the database are described for bioluminescence resonance transfer (BRET), time-resolved Forster resonance transfer (FRET) and fluorescent polarisation assays (FP). The authors encourage the users to add any tracers they might have optimised by submitting all the info required, such as tracer details, target details, assay details including thorough statistical and biochemical validation.

This publication and this database will be very useful as these types of assays are regularly used in PROTAC/glue drug discovery, either in cell biology or structural biology. From the perspective of a cell biologist who often uses NanoBRET target engagement assay, I think that it's very useful to have a concise database with validated tracers, however, I would recommend using the given concentrations as a starting point and titrating the tracers, especially if a different cell line than the one on the website is used. It would also be useful to know how the cell lines were transfected as the amount of donor protein (for example: Nanoluc VHL + carrier DNA, NanoBRET TE assay) can differ and that can also affect the concentration of the tracer used.

| *Jeff*

Novel Amphiphilic PROTAC with Enhanced Pharmacokinetic Properties for ALK Protein Degradation

Shirui Wang§, Zhan Zhan Feng§ …, Yan Zhang*, Rui Li*
J. Med. Chem. **2024**, 67, 9842



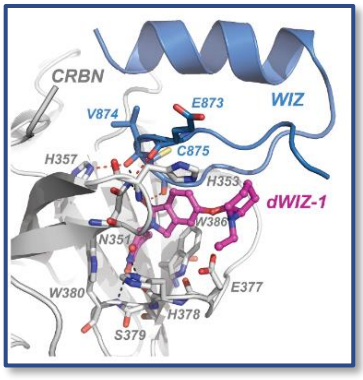
PROTACs featuring alkyl linkers often suffer from poor DMPK properties and low solubility. To address these issues, the authors have conducted a proof-of-concept study of amphiphilic EML4-ALK PROTACs. The development of amphiphilic PROTACs started from the linker exploration between Ceritinib, an ALK inhibitor and pomalidomide. It was found that either di- or triamido alkyl linkers gave optimal degradation profile by western blot. PROTAC B1 was then transformed into amphiphilic PROTAC B1-PEG by tethering a PEG-62 chain onto an amide of the linker, connected with a disulfide bond. B1-PEG shows good antitumor activity in a glutathione rich environment and good cellular uptake. These results proofs that B1-PEG could enter the cell by self-assembling into micelles and reach the tumour to selectively release the active B1 by disulfide bond cleavage. Finally, PK studies of B1 and B1-PEG show that B1-PEG has a 20% higher absolute bioavailability than B1.

Overall, this is very extensive study of first-in-class amphiphilic PROTACs. It would be interesting to see whether one can improve a PROTAC with low bioavailability using this approach.

| *Vesna*

A molecular glue degrader of the WIZ transcription factor for fetal hemoglobin induction

Pamela Y. Ting§, …, James E. Bradner*
Science ***2024***, 385, 91-99



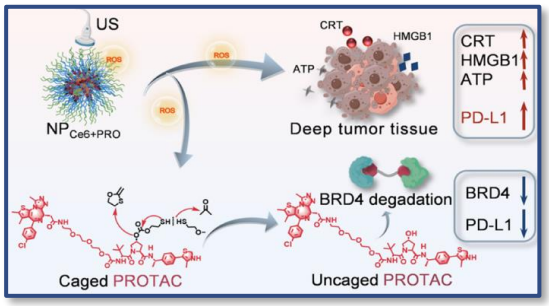
In this publication, the authors seek for a compound that can induce the expression of fetal hemoglobin as a viable treatment for sickle cell disease, without causing erythroblast differentiation or halt in proliferation. They identified dWIZ-1 (and later on, chemichally improved dWIZ-2), a compound degrading protein WIZ, through a phenotypic screen of large cereblon-based compound library. The authors performed extensive *in vitro* and *in vivo* profiling to validate WIZ and its degraders as a target in sickle cell disease. The authors also used biophysical methods and crystallography to characterize dWIZ-2 as a molecular glue and identify the zinc finger domain necessary for glueing of cereblon and WIZ and identified WIZ bining in the beta globin locus using CUT&RUN method for studying protein-DNA interactions.

This is a very comprehensive publication, showcasing a very elegant and complex methodology in discovering and validating a target and a degrader at the same time.

| Jeyan

Ultrasound-Activated PROTAC Prodrugs Overcome Immunosuppression to Actuate Efficient Deep-Tissue Sono-Immunotherapy in Orthotopic Pancreatic Tumor Mouse Models

Ye Liu§, Haiyang Wang§ …, Youyong Yuan*
Nano Lett. **2024**, 8741



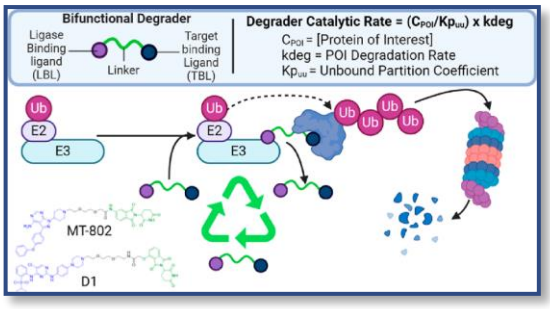
In efforts to address membrane permeability and selectivity limitations of PROTACs that target cancer, the authors have designed, synthesised and validated an ultrasound (US) responsive nanoparticle (NP) system for on-demand PROTAC delivery to tumors. Here, PROTACs are leveraged to overcome the PD-L1 mediated evasion of tumors from immunosurveillance, that's typically seen with sonodynamic treatment alone. The NP system is comprised of a reactive oxygen species (ROS) generating sonosensitiser, as well as a 'caged' MZ1 PROTAC (a potent BRD4 Degrader) that is released upon ROS generation, via US pulse. The biodistribution of PROTAC is monitored for all vital organs of mice, proving that the PROTAC is indeed selectively delivered to the tumor site. Overall, the sonodynamic NP technology results in the efficacious treatment of deep-seated tumors, when compared to PROTAC cleaved via light irradiation, as observed through a broad range of *in vitro* and *in vivo* experiments.

Getting PROTACs to the clinic, even outside of cancer, can be challenging due to cell permeability issues. I think this is an innovative way to potentially overcome that hurdle, so it would be interesting to see if this approach translates to the clinic. I also think the use of US to cleave the PROTAC is particularly useful, and begs the question, of whether there are any other ways 'caged' PROTAC NP systems can be employed. Perhaps specific microenvironmental differences between target tissue and non-target tissues, such as pH or enzyme presence/concentration, can be taken advantage of to treat non-superficial cancers as well.

| Jeyan

Quantitative Measurement of Rate of Targeted Protein Degradation

Thomas L. Lynch§, Violeta L. Marin§, …, Justin M. Reistma*
ACS Chem. Biol. **2024**, 19, 1604



Despite the generally accepted mechanism that protein degraders act substoichiometrically by nature, there are few examples that prove this. Herein, the authors establish a general method to calculate a degrader catalytic rate (DCR) based on the concentration of protein of interest per cell, number of unbound degrader molecules per cell and the degradation rate constant. They use BTK as proof of concept, along with a promiscuous kinase degrader MT-802, and D1, featuring a multikinase tageting warhead. They began by finding the degradation rate constant from HiBit assay. Then,

the intracellular unbound partition coefficient was calculated with measured unbound PROTAC concentration from cell media and intracellular fractions, as well as kinetic cellular accumulation PROTAC concentration. DCR of BTKs was calculated for both degraders as BTK molecules/h/free degrader. Cooperativity was also calculated and was strongly correlated with DCR. Additionally, a kinome wide DCR characterisation of D1 was carried out, which showed that DCR can inform complementarity between an E3 ligase and a POI.

Overall, I think this study has established a great tool for TPDers to gain understanding of how their compounds work. Moreover, I think there is a potential for proteome wide DCR characterisation to be used as a screening method for E3 ligase compatibility.

PRE-PRINTS

| *Manon*

bioRχiv

A potent agonist-based PROTAC targeting Pregnane X Receptor that delays colon cancer relapse

*Lucile Bansard§ , …, Jean-Marc Pascussi **

In this study, the design and synthesis of a novel PXR agonist-CRBN based PROTAC (JMV7048) is reported. This molecule decreased PXR protein expression in colon cancer stem cells and sensitized them to chemotherapy.

| *Aitana*

bioRχiv

Functional characterization of pathway inhibitors for the ubiquitin-proteasome system (UPS) as tool compounds for CRBN and VHL-mediated targeted protein degradation

*Martin P.Schwalm, …, Stephan Knapp **

Here, the authors use MZ1 and dBET6 to characterize established UPS pathway inhibitors commonly used in TPD. They provide a range of recommendations including the best concentrations to use these inhibitors (cell line and target dependent) and how to calculate these values. It is very interesting to see the differences between the VHL and CRBN- based degraders. This will help the TPD community with the characterization of the molecular mechanism of degraders!

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