CeTPD Journal Club

April – May 2025

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

Centre for Targeted Protein Degradation
University of Dundee

innovate
collaborate
inspire

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MEET THIS MONTH'S EDITORS

Click here for info on the editor

ALEJANDRO CORREA-SÁEZ

Alejandro completed his master's degree in Biomedicine and his PhD at the University of Córdoba (Spain), where he characterized the cell signaling events underlying members of the DYRK kinase family in cell cycle control. He obtained an FPU fellowship from the Spanish Ministry of Science for his PhD research. In 2023, he joined the Ciulli group as a postdoctoral MSCA-UKRI fellow where he focuses on innovative strategies for the rational design of molecular glues.

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Ryan Casement

Ryan, originally from Belfast, joined the Ciulli lab in 2018 after completing his undergraduate degree in medicinal chemistry at Trinity College Dublin. During this time he undertook a year long industrial placement at GSK working on targeted protein degradation. He completed his PhD in 2023 where he investigated ElonginB/C containing E3 ligase complexes using fragment-based ligand design and is now working on the structure guided design of molecular glue degraders in collaboration with Eisai.

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GIULIA BONASEGALE

Giulia obtained both her bachelor's and master's degree in chemistry at Università degli Studi di Pavia in Italy. Driven by her interest in medicinal chemistry, she then joined Rodriguez's group at Institut Curie in Paris to work on cancer research, as part of 6-months Erasmus Traineeship project. In October 2024, she started her PhD on molecular glues in Cossar's group at the Centre for Targeted Protein Degradation in Dundee.

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MHAIRI GIBSON

Mhairi completed her master's degree in chemistry with drug discovery and the University of Strathclyde in Glasgow. During this time she undertook a year long industrial placement at GSK working as part of the green chemistry team. In 2024 she completed her PhD at the University of Strathclyde working on novel bioorthogonal labelling techniques. She joined the Centre for Targeted Protein Degradation in 2024 as part of the ACBI collaboration with Boehringer Ingelheim.

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TARGETED PROTEIN DEGRADATION

CHEMISTRY | STRUCTURAL BIOLOGY & BIOPHYSICS | CELL BIOLOGY | MODELLING

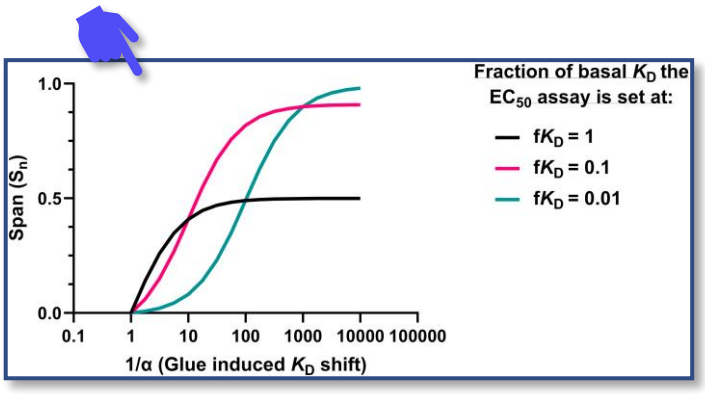
"Every two months, we spotlight the latest and most significant literature in the field of targeted protein degradation, spanning chemistry, biophysics, cell biology, and computational modeling"

Literature review from 21st March to 21st April 2025

| *Ryan*

A high throughput compatible workflow for the biochemical identification and characterisation of molecular glues

Ryan Guilbert§, Maxime Couturier§, ..., Argyrides Argyrou*
J. Biol. Chem. ***2025****, 30, 6, 108526*



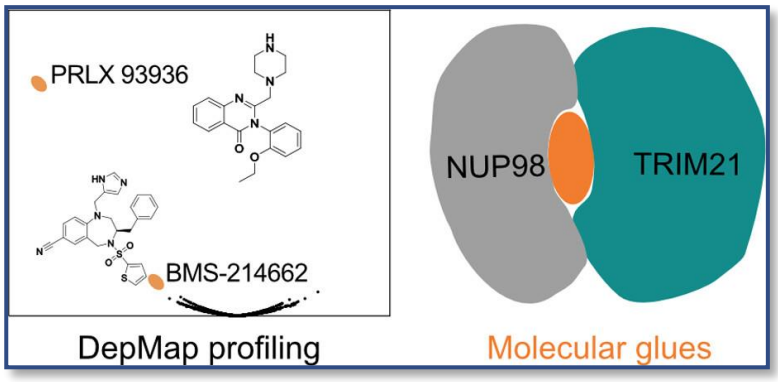
In this paper the authors annotate a workflow for the characterisation of molecular glues, with fluorescence-based assays such as TR-FRET. The system used was the β-TrCP1, β-catenin interaction for which molecular glues were reported by Simonetta et al. in 2019. Using the TR-FRET assay and compounds described in this work the relationship between K_D shift and EC_{50} span was investigated and validated, two metrics which are important for the optimisation of molecular glues.

Characterizing molecular glues in this context is inherently a multiparametric challenge, as it involves assessing both the K_D shift (reflecting cooperativity) and the EC_{50} (indicating potency). This work does an excellent job of utilising a high-quality literature example and in a step-by-step manner describing how to set-up the assay, the assumptions underlaying the results and a way to extract K_D shift without the need for costly matrix titrations.

| *Ryan*

TRIM21-NUP98 Interface Accommodates Structurally Diverse Molecular Glue Degraders

Yalong Cheng, …, Ting Han*
ACS Chem. Biol. **2025**, 20, 953−959



Building on a previous paper published in December 2024, which identified a TRIM21-based molecular glue, this study expands the findings by leveraging drug sensitivity data from DepMap to uncover two additional chemotypes with similar functionality. These two compounds were validated through TRIM21 knockout experiments, which resulted in reduced cytotoxicity, confirming their TRIM21-dependent mechanism. Both compounds were shown to induce degradation of nuclear pore proteins in a proteasome-dependent manner.
It was interesting that while BMS-214662 demonstrated approximately 50-fold greater binding affinity to TRIM21 (SPR) compared to PRLX-93936, both compounds exhibited similar activity in cell viability assays, albeit PRLX-93936 appeared cleaner in terms of dependency on TRIM21.

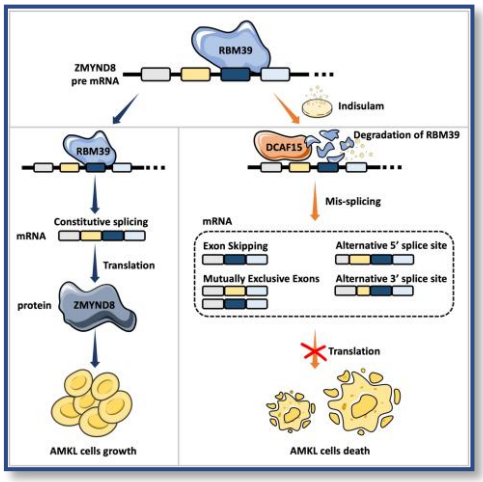
This paper exemplifies how publicly available databases can serve as valuable resources for uncovering new insights into the mechanisms of action of various compounds. It makes a meaningful contribution to the expanding body of research on TRIM21 as an E3 ligase in targeted protein degradation (TPD) in particular for the degradation of multimeric proteins.

| *Alejandro*

The RBM39 degrader indisulam inhibits acute megakaryoblastic leukemia by altering the alternative splicing of ZMYND8

Ying Yang§, Zhiheng Li§, Yang Yang§, Peifang Xiao§ … Jian Pan*, Shaoyan Hu*, Xiaoyan Yang*
Cell Biosci, ***2025,*** *46. https://doi.org/10.1186/s13578-025-01380-3*

Discussed internally



Acute MegaKaryoblastic Leukemia (AMKL) accounts for 4% to 15% of pediatric acute myeloid leukemia (AML) cases and has a significantly lower five-year survival rate compared to other leukemia subtypes. Alternative splicing (AS) plays a crucial role in the pathogenesis of AMKL, and targeting this process may represent a potential therapeutic strategy for its treatment. Indisulam is a well-known RBM39 degrader that alters AS events. Its efficacy has been demonstrated in various Phase I and Phase II clinical trials involving patients with advanced cancers.
However, the effectiveness of indisulam has not been tested specifically in AMKL.

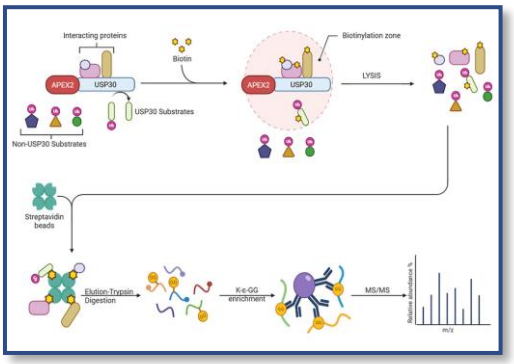
In this study, the authors first revealed that the AMKL subtype is particularly sensitive to indisulam treatment. They then characterized the downstream signaling events responsible for this sensitivity through a multi-omics approach combining proteomics and transcriptomics. Their findings showed that DCAF15-dependent degradation of RBM39 by indisulam led to alterations in transcription profiles and protein downregulation. They narrowed their focus to a list of 22 candidate proteins, ultimately validating indisulam impact on ZMYND8 AS and protein levels.

This research exemplifies the importance of understanding cancer-specific cell signaling events to enhance the clinical translation of degraders into treatment options.

| *Alejandro*

Integrative proximal-ubiquitomics profiling for deubiquitinase substrate discovery applied to USP30

Andreas Damianou§, Hannah B.L. Jones§…. Benedikt M.Kessler*
Cell Chem Biol. ***2025****; 32, 5, 736-751*



The identification of E3 ligase and deubiquitinating enzyme (DUB) substrates is a promising field that could lead to the discovery of novel therapeutics. While there are several emerging and exciting methods available for studying direct E3 ligase substrates, such as UbPOD and BioE3, we currently lack a reliable methodology for identifying direct substrates of DUBs. In this study, the authors developed a technique called proximal-ubiquitomics, using USP30 as a proof of concept for DUB analysis. This method involves a proximity labeling enzyme, such as APEX2, to induce biotinylation of nearby proteins. Following biotin enrichment by using streptavidin beads, a second purification step is performed through K-ε-GG immunoprecipitation to focus on changes in the ubiquitination profiles of close interactors. Using this method, the authors optimized the methodology for the DUB USP30. Then, by using a USP30 inhibitor (Compound 39), the authors were able to validate known substrates of USP30, such as TOMM20 and FKBP8, as well as identify a novel substrate, LETM1.

The development of technologies that combine proximity labelling with a second readout, such as ubiquitinomics, is of great interest. This approach allows researchers to study changes in ubiquitination following the expression of a protein of interest, without the confounding effects of downstream events. This capability is particularly valuable for identifying substrates of E3 ligases and DUBs.

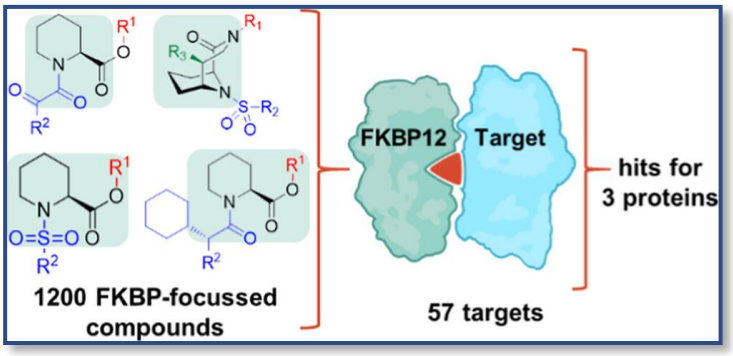
| *Ryan*

Broad Target Screening Reveals Abundance of FKBP12-Based Molecular Glues in Focused Libraries

Johannes K. Dreizler§, Christian Meyners§, …, Felix Hausch*
J. Med. Chem., **2025**, 68, 9525−9536

Discussed internally

This work describes the screening of a FKBP focused library for the identification of non-degradative molecular glues across a broad range of targets using recombinant proteins. The authors developed a TR-FRET assay to screen 57 different targets, carefully tuning protein concentration in order to be able to detect weak molecular glues. Of particular importance was the robust orthogonal validation of hits using FP, native MS and photo crosslinking. Hits for 3 proteins were obtained, two of which are considered 'undruggable' which highlights one of the key advantages of this mechanism of action. A follow-up chemistry effort was initiated for BRD4 BD2, and the ternary complex formation was improved by >10fold with a small library of analogues. Of particular note was the selectivity over BRD4 BD1, highlighting another of the key advantages of harnessing surface PPIs where there is less conservation between domains.



While molecular glue degraders have seen widespread interest in academia and industry, this work highlights the power of non-degradative molecular glues and outlines a robust screening cascade to identify them.

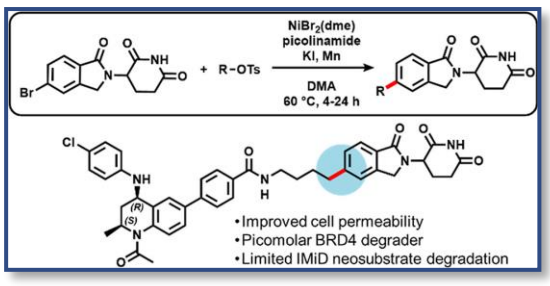
| *Giulia*

Reductive C(sp²)-(sp³) Coupling Protocol to Enable Linker Exploration of Cereblon E3-Ligase BRRD4 Proteolysis-Targeting Chimeras

Kaytlin Lovato, … Jean-François Brazeau*
J. Med. Chem. ***2025***, *68, 10, 10061−10074*

Discussed internally

In the context of PROTAC design, the linker plays a crucial role in defining the physicochemical properties of the compound. PROTACs often rely on amide bond formation to connect the two warheads. This paper presents an optimized procedure to achieve a C(sp²)–C(sp³) bond, overcoming challenges found in previously published methodologies, such as scalability issues, a limited pool of starting materials, and incompatibility with the glutarimide scaffold. The authors performed reaction condition optimization and a substrate scope study to evaluate the method's applicability. The procedure was then applied to the synthesis of BRD4-PROTACs to assess how the presence of a carbon–carbon linkage near the CRBN-binding moiety influences the properties of the PROTAC.

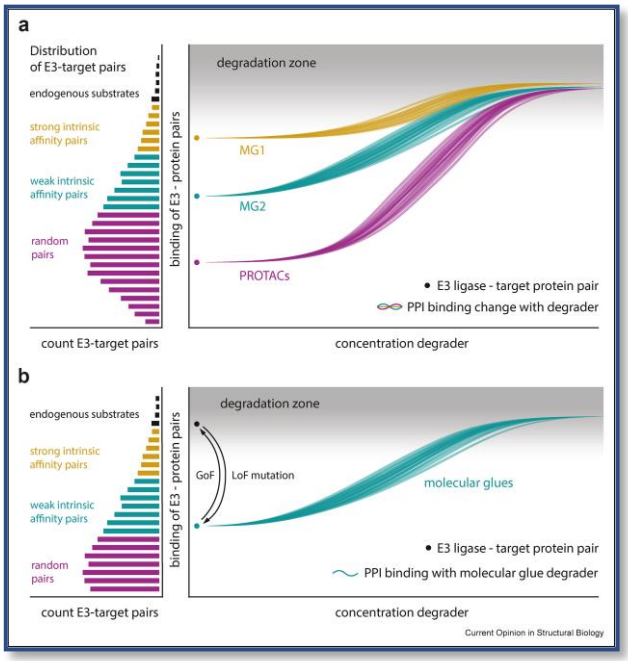


The optimized protocol has synthetic interest and could be exploited to achieve the formation of an alkyl linker in PROTACs. The collected data suggest that a C(sp^2)–C(sp^3) bond close to the CRBN-binding scaffold can have a positive influence on the properties of the PROTAC. Nevertheless, the paper presents some underexplored aspects, such as the observed toxicity of the compounds, which has not been further investigated, and the selectivity, which has been assessed only on very specific targets, leaving the full proteome unexamined.

| *Alejandro*

Primed for degradation: How weak protein interactions enable molecular glue degraders

Alexander Hanzl, Clara Inghelram§, Stefan Schmitt§, Nicolas H Thomä*
Curr Opin Struct Biol. **2025**. 6:92:103052.



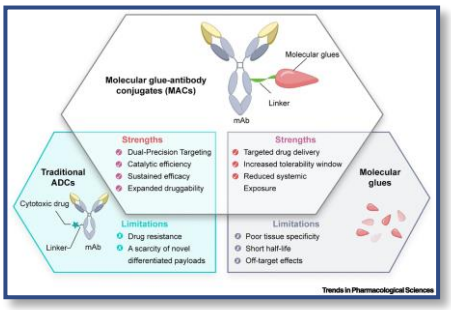
This comprehensive review from Nicolas Thomä's lab provides an in-depth look at Molecular Glue Degraders (MGD), exploring their mechanisms and novel strategies for rational design. It is widely known that MGDs are reported to primarily enhance the intrinsic basal affinities between two proteins. Therefore, the authors highlited in this work the importance of protein complementarity and binding affinities between these interacting pairs, raising the question of the necessary affinity level for effective molecular glue action. The review begins builing this argument by highlighting the great success of CRBN-based MGDs, such as IMiDS, in targeting Zinc Finger (ZF) proteins. These molecules create a dynamic interface for CRBN that facilitates the recruitement of a substantial amount of intrinsic interactions, particularly those that had a weak/non-productive interaction. Additionally, the authors examine examples that demonstrate how gain-of-function mutations can also mimic the surface complementation achieved by MGDs, leading to target degradation. The review concludes by discussing various receptors of other E3 ligases, including DCAF16, DCAF11, and GID/CTLH.

A better understanding of the requirements for minimal surface complementarity is essential for effectively driving the rational design of molecular glues. To achieve this, using exploratory proteomics methods alongside biophysical characterization of PPIs could significantly enhance our understanding in this field.

| Giulia

Molecular glue meets antibody: next-generation antibody–drug conjugates

Yiran Tao§, Ying Lu§, Bin Yu* and Yuxi Wang*
Trends Pharmacol Sci, **2025**, *46*, (6), *520 – 534*



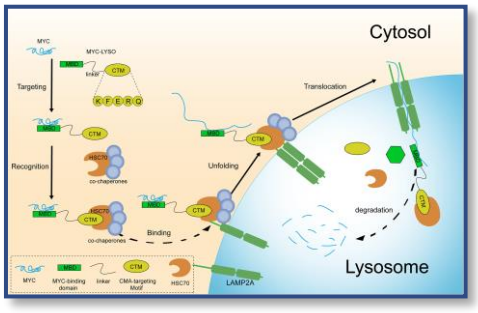
Antibody-drug conjugates (ACDs) are a class of targeted anticancer therapeutics composed of a monoclonal-antibody, a potent cytotoxic payload and a chemical linker. Although a significant number of ADC products have already been approved for several clinical indications, the selectivity and potency of these compounds could be further improved. The constrain to this development is payload diversity, as current payloads are often derived from natural sources and cover only a limited number of cytotoxic mechanisms. Molecular glue-antibody conjugates (MACs) combine the approach of ACDs with the positive features of molecular glues, such as the catalytic, even-driven mechanism of action that leads to protein degradation, and improved selectivity. This review analyses this innovative class of compounds, highlighting their characteristics, molecular mechanism and advances, as well as describing challenges and future directions in the area of MACs therapeutics.

This review explores a different application of molecular glues, showing the potency of combining different approaches. MACs are an interesting class of compounds, and an exhaustive list of examples is here provided, along with evidence of their anticancer activity, both in vitro and *in vivo*.

| Giulia

Structure-based artificial intelligence-aided design of MYC-targeting degradation drugs for cancer therapy

Donghua Liu§, Yize Jiang, Bohan Ma,* Lei Li*
Biochem Biophys Res Commun., ***2025***, *766, https://doi.org/10.1016/j.bbrc.2025.151870*



This work focuses on the development of a novel MYC peptide inhibitor, Se-MYC-LYSO, in the context of cancer treatment. To achieve this goal, the authors exploit a combination of innovative approaches: an AI-based method, the Rosetta platform, to identify a high affinity peptide for the target, and a nano-selenium delivery system to address the challenge of poor membrane permeability. The newly developed peptide has been characterized at a molecular level, at a cellular level and from the point of view of its mechanisms of action. Se-MYC-LYSO relies on the autophagy pathway to achieve the degradation of the target IDP and the consequential inhibition of cell proliferation and apoptosis.

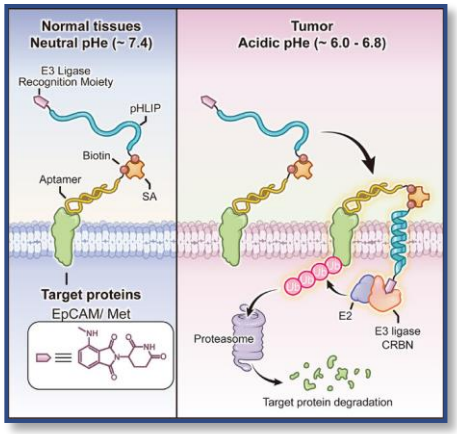
The paper shows the development of the desired compound and provides the confirmation its predicted properties and mode of action. The authors effectively address the challenges associated with this approach by employing innovative techniques and procedures. It would be valuable to next evaluate the system's efficacy *in vivo*.

| *Mhairi*

Membrane-Bounded Intracellular E3 Ubiquitin Ligase-Targeting Chimeras (MembTACs) for Targeted Membrane Protein Degradation

Mengwu Mo,..., Jinbiao Shang*, and Zhi Zhu*
Angew. Chem. Int. Ed. ***2025****, 64, e202501857*

Discussed internally



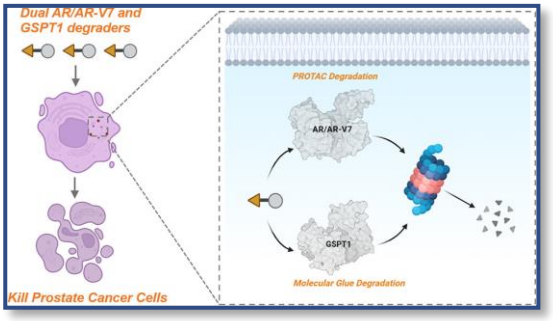
The development of PROTACs has enabled the degradation of many challenging substrates, however, current PROTAC technology have yet to achieve effective degradation of membrane proteins. Although alternative methods have been developed to degrade membrane proteins, these are limited by their poor cell permeability and off-target effects. In this exciting proof-of-concept study, the authors have demonstrated a novel PROTAC modality for the degradation of membrane bound proteins (MembTACs). The paper showcases the design of effective degraders which simultaneously recruit intracellular E3 ubiquitin ligases and enable extracellular binding of the protein of interest to induce selective degradation of therapeutically relevant membrane proteins. This new platform overcomes issues with cell permeability by utilising a low pH insertion peptide to link both ligands and enable communication between outer and inner cell membranes. Degradation is achieved under acidic conditions, found in tumour microenvironments, highlighting an additional level of selectivity over existing techniques. While this approach enabled effective degradation of the target protein, degradation was relatively slow and required multiple E3 ligase targeting motifs to facilitate degradation. However, the authors demonstrated low nM potency for the degradation of EpCAM using an antibody, suggesting efficiency can be improved with further development of this methodology.

Overall, this paper presents a promising strategy to enable efficient and selective degradation of membrane bound proteins, overcoming issues associated with poor cell permeability. With further optimisation, this strategy could significantly improve upon current methods for membrane protein degradation.

Rational Design of Dual Degraders by Incorporating Molecular Glue Structural Features into PROTAC Degraders

Bowen Zhang§, Shan Gao§..., Chong Qin*

J. Med. Chem. ***2025***, *68, 10268-10298*



This paper presents the development of novel dual-function degraders which possess both PROTAC and molecular glue characteristics to simultaneously target AR/AR-V7 and GSPT1 for the treatment of prostate cancer. This intuitive design merged the AR-NTD binding moiety of a previously reported PROTAC, BWA-522, with an aliphatic linker and CRBN molecular glue thalidomide to target GSPT1. Exploration of SAR enabled the discover of lead compound BWA-6047 which exhibits rapid degradation of both targets with a low DC_{50} value. Extensive mechanistic studies were used to validate the dual mechanism of the lead compound, which was further confirmed by molecular modelling to profile the binding mode of BWA-6047. Notably, BWA-6047 exhibited excellent antiproliferative effects in resistant cell lines demonstrating its potential in overcoming enzalutamide resistance. While the lead compound was able to achieve tumour suppression in vivo with no apparent toxicity, its antitumour activity was weaker than that of enzalutamide. Suboptimal bioavailability and membrane permeability may hinder the clinical development of the dual degrader; however, further SAR optimisation may overcome these issues in future studies.

Overall, this study demonstrates a compelling strategy for dual-target degradation which enhances efficacy and broadens the therapeutic scope for the treatment of prostate cancer. The integration of two degradation strategies in a single molecule may inform the next generation of degraders targeting resistant cancers.

PREPRINTS

| *Mhairi*

Molecular surface mimicry enables CRBN to target G3BP2 for degradation

Stefano Annunziato§, Chao Quan§, Etienne J. Donckele§.....Pablo Gainza, and Georg Petzold**

In this work, the authors identified a G-loop independent CRBN neosubstrate, G3BP2, recruited by molecular glue MRT-5702, that forms a novel PPI interface by engaging in an unconventional binding site on the CRBN LON domain. By elucidating the binding mode, the authors discovered that CRBN mimics the endogenous binding partner of G3BP2 to engage a preexisting PPI hotspot. This work uses cellular, computation and structural approaches to demonstrate that neosubstrate recruitment does not require a G-loop recognition motif and ternary complex formation is not dependent on the primary G-loop binding site on CRBN. This work builds on the existing knowledge of molecular glue degraders to provide a generalisable approach towards the discovery of novel neosubstrate binding modes on CRBN and other E3 ligases.

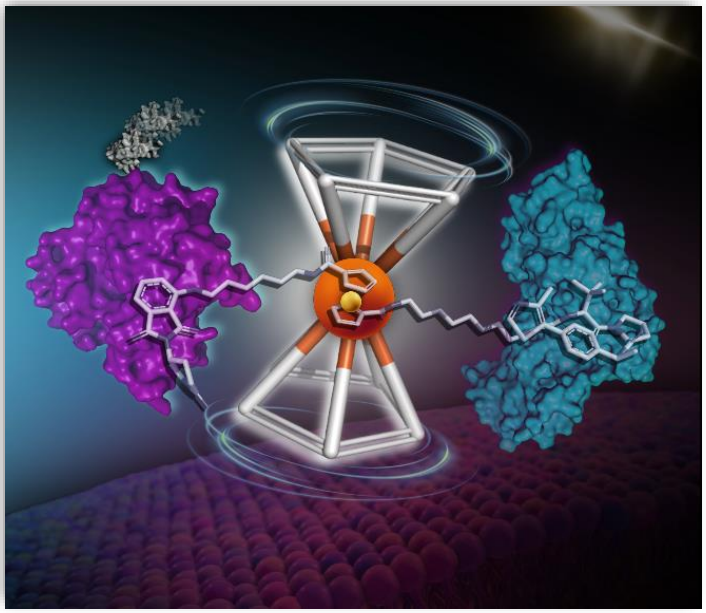
PAPERS AND PREPRINTS FROM CeTPD

| *Alessandra*

Rational Design of PROTAC Linkers Featuring Ferrocene as a Molecular Hinge to Enable Dynamic Conformational Changes

*Alessandra Salerno, Lianne H. E. Wieske, Claudia J. Diehl, Alessio Ciulli**
J. Am. Chem. Soc. ***2025****, 147, 16, 13328–13344*

CeTPD authors: Alessandra Salerno, Lianne H. E. Wieske, Claudia J. Diehl, Alessio Ciulli



Unpublished graphic of the FerroTAC design

In this study, we have explored an exciting new strategy with the development of **FerroTACs**, a rationally designed class of PROTACs utilising **ferrocene** as a molecular hinge. Ferrocene's organometallic structure, characterised by two cyclopentadienyl rings freely rotating around an Fe(II) centre offers a unique combination of rigidity and flexibility. This ferrocene`s property facilitates structural adaptability — also known as chameleonicity — which can significantly impact PROTACs` behaviour in cellular environments. We applied this design across three PROTAC architectures: VHL-VHL (homo-PROTACs), VHL-CRBN, and VHL-BETs. NMR-based conformational analyses revealed that ferrocene promotes intramolecular folding in apolar environments, leading to enhanced permeability and reduced cellular efflux—two key hurdles in PROTAC development. Cellular assays showed promising results as FerroTACs demonstrated efficient target degradation with permeability metrics that matched or exceeded those of established benchmark degraders such as CM11, 14a, and MZ1. In addition, we evaluated lipophilicity, solubility, and metabolic stability, all of which further support the potential of FerroTACs for further development.

This work highlights ferrocene as a versatile and tuneable linker motif, offering a novel platform for modulating molecular properties in next-generation PROTACs but also a dynamic space for medicinal chemistry innovation. It was both a challenging and rewarding experience to work on this project, which sits at the intersection of organometallic chemistry, TPD, and medicinal chemistry.

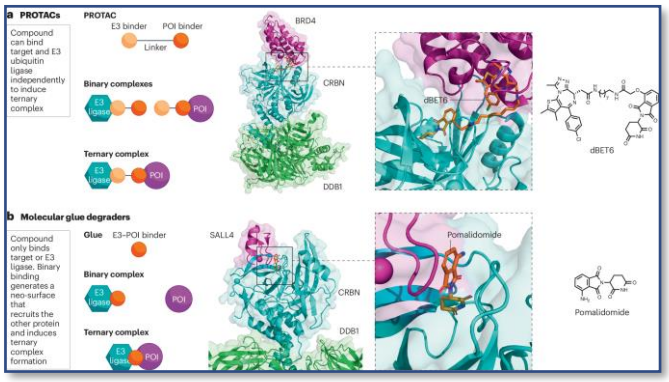
If you're curious to hear more, I presented these findings in a ***recent Dana-Farber TPD webinar series****— recommend checking it out for a deeper dive into the science behind FerroTACs!*

Abstract

Targeted protein degradation for cancer therapy

Matthias Hinterndorfer§, Valentina A. Spiteri§, Alessio Ciulli & Georg E. Winter**
Nat Rev Cancer, ***2025****. https://doi.org/10.1038/s41568-025-00817-8*

CeTPD authors: Valentina A. Spiteri, Alessio Ciulli



Targeted protein degradation (TPD) aims at reprogramming the target specificity of the ubiquitin–proteasome system, the major cellular protein disposal machinery, to induce selective ubiquitination and degradation of therapeutically relevant proteins. Since its conception over 20 years ago, TPD has gained a lot of attention mainly due to improvements in the design of bifunctional proteolysis targeting chimeras (PROTACs) and understanding the mechanisms underlying molecular glue degraders. Today, PROTACs are on the verge of a first clinical approval and recent structural and mechanistic insights combined with technological leaps promise to unlock the rational design of protein degraders, following the lead of lenalidomide and related clinically approved analogues. At the same time, the TPD universe is expanding at a record speed with the discovery of novel modalities beyond molecular glue degraders and PROTACs. Here we review the recent progress in the field, focusing on newly discovered degrader modalities, the current state of clinical degrader candidates for cancer therapy and upcoming design approaches.

| *Maria and Conner*

bioRχiv

BromoCatch: a self-labelling tag platform for protein analysis and live cell imaging

*Maria Rodriguez-Rios§, Conner Craigon§, ... Alessio Ciulli**

CeTPD authors: Maria Rodriguez-Rios, Conner Craigon, Mark A. Nakasone, Adam G. Bond, Mark Dorward, Alessio Ciulli

In this preprint from the Ciulli Lab, in collaboration with Tocris BioTechne, the team developed a novel self-labeling tag (SLP) platform called BromoCatch for protein analysis and live imaging. BromoCatch utilizes a small (~13 kDa) engineered bromodomain containing a nucleophilic cysteine, which serves as a protein fusion tag. This tag specifically binds to a para-acrylamide-based ligand designed for BromoCatch, enabling selective and efficient labeling of BromoCatch fused proteins. The ligand also features an orthogonal functionality, allowing its use in diverse applications such as live-cell imaging, biotin assays or bio-conjugation. This platform offers a versatile and precise tool for studying proteins in complex biological system

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