CeTPD Journal Club

November 2024 – January 2025

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

CHARLOTTE CROWE

Charlotte is originally from Strasbourg, France. She moved to Scotland in 2015 to study an MChem in Chemistry at the University of St Andrews, graduated with Honours in 2020. She then joined Prof. Alessio Ciulli's lab at the University of Dundee in 2020 for her PhD, focusing on using chemical, structural and biophysical approaches to understand the mechanism of action of small molecule degraders in Targeted Protein Degradation (TPD), with a particular focus on Cullin RING E3 ligases. In 2024, she joined the Ciulli lab as a postdoctoral researcher. In January 2025, she joined the CeTPD arm of the MJFF LITE project as a structural biologist.

LinkedIn: Charlotte Crowe
X: @CharlCrowe

ANDREAS HOLMQVIST

Andreas joined the Farnaby Group as a PhD student in medicinal chemistry in September 2022. His research focuses on developing blood-brain barrier-permeable degraders to restore neuronal function in neurodegenerative diseases. He earned his master's in organic and medicinal chemistry from the University of Gothenburg in 2022 and pursued a PhD at the Centre for Targeted Protein Degradation (CeTPD) to further explore his passion for targeted protein degradation.

LinkedIn: Andreas Holmqvist
X: @Holmqvist89

JON MACICIOR

After completing his BSc in Chemistry, Jon obtained his MSc in Organic Chemistry at the Universidad Complutense de Madrid, working on the stereocontrolled synthesis of bis(triflyl)enones. He then obtained an FPU scholarship to do his PhD in the Medicinal Chemistry Laboratory (Universidad Complutense de Madrid), working on the development of PROTACs for the treatment of progeria. In his spare time he is a keen padel player and songwriter.

LinkedIn: Jon Macicior

MARIA RODRIGUEZ-RIOS

Maria was born in Galicia, Spain. She obtained her PhD studies from the University of Edinburgh working on the synthesis of fluorogenic peptide-based probes for imaging inflammation-related proteases under the supervision of Prof. Mark Bradley. Maria joined Prof. Alessio Ciulli's lab in January 2023 as a postdoctoral scientist working on the development of the next generation tag-based system "BromoTag" with support from Tocris / Bio-Techne

Bluesky: @mariarrios.bsky.social
LinkedIn: Maria Rodriguez Rios

CeTPD END-OF-YEAR SYMPOSIUM

| *Jon*

The Centre for Targeted Protein Degradation **End of Year Symposium** is always a highlight in the calendar and 2024 was no exception! The event took place at the fabulous Invercarse Hotel and was the perfect way to round off the year. The Centre has had a busy year, with the publication of several papers in high-impact journals such as Nature and Science. *Congratulations to everyone involved!* The evening kicked off with a Secret Santa game that brought joy and laughter, even if some might have preferred a more daring version where you could steal gifts from others... While we missed the great Tom Webb as our Master of Ceremonies, Angus Cowan stepped up to the plate and led the event with style, introducing Alessio Ciulli, who gave an inspiring speech highlighting all the work the centre has done this year.

Alessio Ciulli's academic group, including Eisai, KOODAC and LITE teams

Next up was Charlotte Crowe, who perfectly summed up the 2024 academic group. Then came the talk by Jon Macicior, who gave us the rundown of the Eisai collaboration and their recent trip to Japan. Suzanne O'Connor followed with the summary of the brand-new AC-KOODAC team (congrats and best of luck to them!). As in the previous year, Kirsten McAulay gave a summary of the excellent year of the AC-BI collaboration.

Alessio Ciulli's Boehringer Ingelheim (AC-BI) collaboration team

From left to right: Peter Cossar's academic group, Will Farnaby's academic group, and Alessio Ciulli's Almirall (AC-Almirall) collaboration team

This was followed by the presentation of the new CeTPD group led by Peter Cossar. ***Welcome Peter and Giulia to CeTPD!*** This time, it was Will Farnaby himself who presented the update of the Farnaby Group, and finally we were able to witness the farewell of the successful AC-Almirall collaboration and its team leader David Zollman.
Congratulations to everyone for all their hard work and good luck in the future, David!

After some closing remarks by MC Angus Cowan, we enjoyed a delicious three-course meal, a quiz presented by Zoe Rutter and Manon Sturbaut, and finished the evening dancing to a traditional Ceilidh.

Last but not least, a big round of applause to Kevin Haubrich for taking wonderful photos of each team. On behalf of the organising committee, Happy New Year to all!

CeTPD SUSTAINABILITY AWARD

| *Manon*

Laboratories are using 3 to 10 times more energy than standard offices and are responsible for 2% of global plastic waste (LEAF, https://www.ucl.ac.uk/sustainable/take-action/staff-action/leaf-laboratory-efficiency-assessment-framework).

At CeTPD, we are committed to improve our impact on plastics, water and energy. But what can you do to make your lab greener and environmentally sustainable? The LEAF programme, initiated by UCL, proposes to carry out an analysis of your lab based on different criteria. It also provides tools and ideas to improve the sustainability of the lab and help reduce carbon emissions. Labs are encouraged to reduce their waste (especially single-use plastics) and recycle whenever possible. They can increase their ULT freezer from -80°C to -70°C or also share equipment and resources to reduce electricity consumption for example.

CATEGORY	Bronze	Silver	Gold
Waste	Provide recycling bins in the lab	Single-use plastic waste has been reduced (guidance provided)	Recycling rates have been increased, or overall waste produced has been decreased
People	Samples owned by departing staff are cleared or tracked	The lab has engaged other labs on LEAF and sustainability	One action to reduce travel has been implemented
Sample & Chemical Management	Labels are legible, and there's a common labeling system in place	Procedures are in place in case cold storage equipment breaks down	At least 80% of all samples and/or chemicals are clearly catalogued
Equipment	Equipment is turned off when not in use	There is a system in place for communal equipment booking	Excess equipment is repaired, sold, and/or donated
Ventilation	There is a clear reporting system for building issues	Fume cupboard sashes are kept closed when not in use	Solvent vapours are condensed and disposed and not released into the atmosphere

Last December, CeTPD was proud to receive the **Silver Award** in recognition of its efforts to improve sustainable practices.

NEW LRRK2 INVESTIGATIVE THERAPEUTICS EXCHANGE (LITE) TEAM

| *Aina, Jack, Alex & Charlotte*

Parkinson's Disease (PD) is a progressive neurodegenerative disease that damages both motor and non-motor functions. Although often associated with tremors and slowness of movement (bradykinesia), PD can manifest as a wide-range of symptoms which can often make it difficult to diagnose and treat. Current estimates suggest that more than 6 million people suffer from PD worldwide, and it is the fastest-growing neurodegenerative disease in total number of cases. **Despite this, there is currently no cure for PD.**

LRRK2 (Leucine-rich repeat kinase 2) Investigative Therapeutics Exchange (LITE) is a new academic-industrial global network supported by the Michael J Fox Foundation for Parkinson's Research (MJFF). CeTPD are collaborating with our University of Dundee partners in the Drug Discovery Unit and MRC-PPU to accelerate the development of Parkinson's Disease therapeutics.

The mission of this collaboration team is centred on the design, synthesis, evaluation and optimisation of induced-proximity small molecules targeting different regions of LRRK2.

Aina Urbina Teixidor, Jack Robertson, Alex Hallatt, and Charlotte Crowe, members of the new LITE team. They will be joined in the coming months by Tan Zher Yin and Claudine Greenwood

The team focuses on three synergistic projects:

1) Discovery and optimization of PROTAC degraders of LRRK2, building on the learnings from chemical probe XL-01126 (Liu *et al*., *J. Am. Chem. Soc*. 2022);
2) Discovery of LRRK2-kinase targeting glue degraders;
3) Reactivating 14-3-3/LRRK2 binding as a strategy towards small molecule therapeutics.

For more information on MJFF, please visit https://www.michaeljfox.org/

THE MICHAEL J. FOX FOUNDATION
FOR PARKINSON'S RESEARCH

NOTE FROM THE EDITORS

| *Alessandra*

With the relaunch of our CeTPD Journal Club last June we introduced some new features to make our contributions clearer and more engaging.

At the same time, we made some internal changes, including a **dedicated one-hour discussion** on selected papers chosen by the Editors of the Month and kindly organised by Angus C. Our impression is that in this way the Journal Club is not just beneficial for the wider community (as we hope!) but it is also helping us develop our critical thinking and allowing to bring you the best insights possible on this fast moving field.

For this reason, from this issue onwards, you will notice **special labels** on papers we have **discussed internally**:

On another note, we realised that deciding whether or not to include papers from CeTPD is always a tricky decision for the Editors of the Month. Since we have also reduced the number of papers we cover, we do not want any paper that deserves it to lose visibility. We have decided to introduce a dedicated section: "**Papers from CeTPD**." Here, you will find papers from the centre with added commentary and/or insights from the authors themselves.

We really hope you enjoy this new format and that it makes your reading experience even more enjoyable!

Alessandra,
on behalf of the JC Editors

TARGETED PROTEIN DEGRADATION

CHEMISTRY | STRUCTURAL BIOLOGY & BIOPHYSICS | CELL BIOLOGY | MODELLING

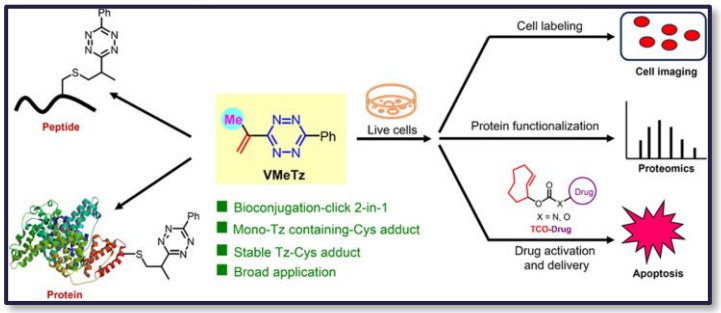
"Every two months, we spotlight the latest and most significant literature in the field of targeted protein degradation, spanning chemistry, biophysics, cell biology, and computational modelling"

Literature review from 21st November to 20th January 2024

| *Jon*

Dual-Performing vinyltetrazine for rapid, selective bioconjugation and functionalization of cysteine Proteins

Mengyang Chang, …, Wei Wang*
ACS Chem. Biol. **2025**, *20*, 153-161



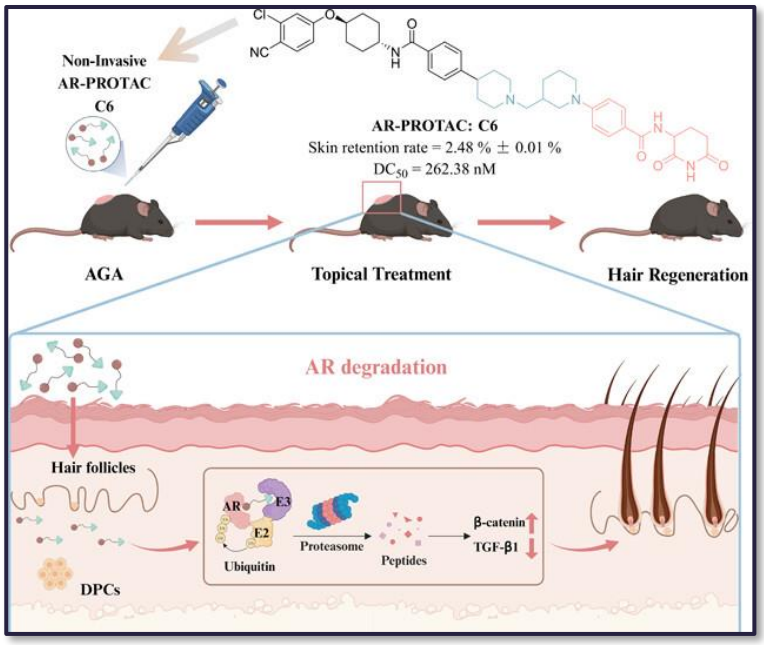
Although methods for **Cys-specific bioconjugation** and functionalization of proteins have been developed and widely used, reagents for this purpose are generally designed to accomplish bioconjugation only. In this work, authors describe a new, simple, **dual-performing bioconjugation-functionalization reagent, VMeTz**, which possesses both a Michael receptor for selective conjugation with Cys, a site for click reactions introduce functionality and a methyl group that prevents the formation of multiple VMeTz-containing Cys-adducts. Furthermore, VMeTz selectively activates *trans*-cyclooctene-caged (TCO-caged) compounds (such as PROTAC ARV-771) in HeLa cells following a "**click-release**" mechanism, achieving therapeutic effects comparable to the parent drugs. VMeTz demonstrated high potential in selectively modifying Cys-containing peptides and proteins, being highly permeable and showing low toxicity. Current studies are exploring the potential application of this reagent in other therapeutic protein bioconjugation and functionalization processes, including antibody-drug conjugates (ADCs).

In this paper, M. Chang *et al.* describe a fast, effective and clean way to conjugate a 'click chemistry-ready' moiety to peptides and proteins containing reactive cysteines. The reagent described, VMeTz, is a major advance over the gold standard *N*-phenylmaleimide, improving key aspects such as conjugation efficiency, selectivity and by-product generation. While the concept itself is interesting, it remains to be seen whether VMeTz can be functionalised or incorporated into an existing ligand to try to achieve selectivity between cysteines of different proteins, which I think would be much more interesting.

| Jon

Discovery of a novel non-invasive AR PROTAC degrader for the topical treatment of androgenetic alopecia

Xinfei Mao, Weitong Hu, …, Xiaowu Dong*
J. Med. Chem. **2024**, *67*, 22218-22244



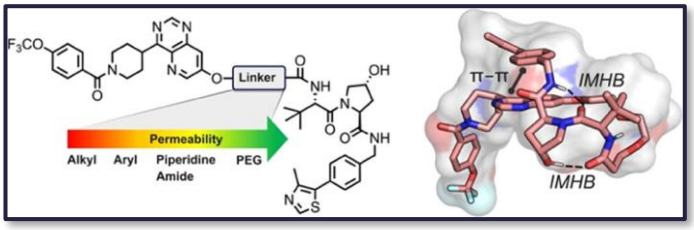
The pathogenesis of androgenetic alopecia (AGA) is not fully understood, but the androgen receptor (AR) is thought to play an important role. Despite efforts to improve the druggability of AR PROTACs, many **orally bioavailable compounds lack sufficient skin retention**, making them unsuitable for topical AGA treatment. Therefore, this research focuses on identifying key structural features to develop non-invasive AR PROTACs with good skin retention and effective AR. Among other findings, parameters such as higher logKp and clogP, and lower tPSA, MW, HBA and HBD were found to be favourable for improving skin retention. Structural optimisation of compounds led to the development of **C6**, which **effectively degraded AR** in HDPC cells and showed **good skin retention** properties. *In vivo* pharmacokinetics also showed favourable skin accumulation, reducing potential systemic side effects, and topical application of C6 in mice resulted in effective hair regeneration comparable to a currently used anti hair-loss drug.

X. Mao *et al.* describe the development of an effective AR PROTAC, with the particularity that it has an acceptable skin retention and is therefore not significantly distributed to other tissues and organs, distinguishing it from traditional AR degraders targeting prostate cancer. From a chemical point of view, we observe the search for the improvement of a relatively little studied property in the field, such as skin retention, and the ability to rationalise the incorporation of different chemical structures to improve this. On the other hand, we are starting to see the arrival of PROTACs in diseases besides the oncology field and using non-invasive administration routes. However, I think the article could be improved by including experiments to prove the formation of the ternary complex, as simulations alone are not considered irrefutable proof.

| *Jon*

Impact of linker composition on VHL PROTAC cell permeability

Yordanos Esubalew Abeje, Lianne H. E. Wieske, …, Daniel Meibom,* and Jan Kihlberg*
J. Med. Chem. **2025**, *68*, 638−657



The discovery of orally bioavailable von Hippel-Lindau **(VHL) PROTACs** is challenging, as their large structures with multiple polar moieties usually place them **beyond the outer limits of the oral druggable space**. The length and chemical composition of the linker are critical for the formation of a ternary complex between the POI and the E3 ligase, which influences the physicochemical and DMPK properties of PROTACs. Since VHL ligands are larger, more polar and more flexible than those for CRBN, the choice and optimisation of the linker is even more crucial. The authors used a series of nine VHL PROTACs and found that **high permeability in solution** correlated with their **ability to adopt folded conformations** with a low solvent-accessible polar surface area. NMR spectroscopy studies in chloroform provided a rationalisation for the observed differences in membrane permeability. The authors conclude that rational design of oral VHL PROTACs could focus on **linkers that facilitate the dynamic hiding of large polar surface areas** in the VHL ligand in an apolar but not in a polar environment.

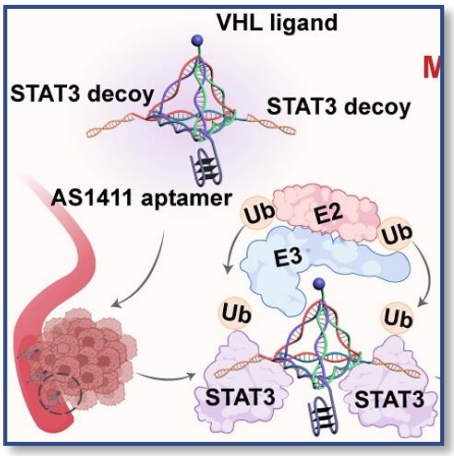
In this paper, the authors highlight the importance of the composition and length of the linker in VHL-based PROTACs on cell permeability. "Linkerology" is a crucial aspect of PROTACs, so although much research has been done, I believe it is still extremely important to describe methods or parameters to rationalise linker design and implementation.

| *Charlotte*

DNA-tetrahedron-driven multivalent proteolysis-targeting chimeras: enhancing protein degradation efficiency and tumor targeting

Shiqing Li, Tao Zheng, …, Chunhua Lu*, Huanghao Yang*
J. Am. Chem. Soc. **2025**, *147*, 2168-2181

The authors assemble a new 'Multivalent PROTAC' composed of a VH298-based chemical VHL ligand, two STAT3 decoy oligonucleotides, and a 'guidance module' targeting nucleolin which is overexpressed on tumour cell membranes. These four components are assembled around a tetrahedral DNA nanostructure. The authors clearly show proteasome-, time- and concentration-dependent degradation of STAT3 by western blot. They also show their PROTAC can induce downstream effects of STAT3 downregulation, and can target tumours in vitro and *in vivo* with mouse studies.

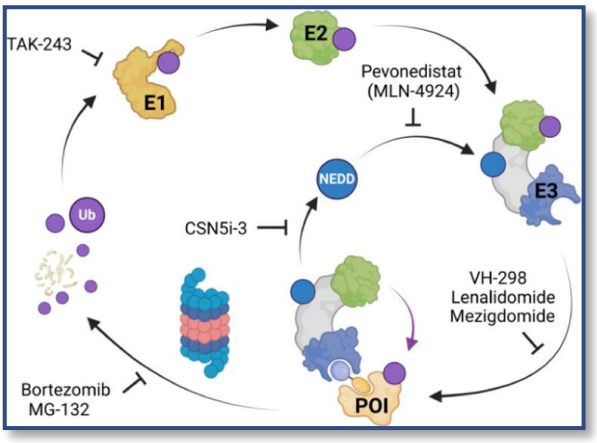


This is yet another interesting derivative of the 'traditional' heterobifunctional small molecule PROTAC. It will be interesting to see if this DNA scaffold can be extended in terms of substrate scope and optimised in terms of potency. I would like to see testing with well-known UPS and Cullin RING inhibitors, for example inhibiting the E1, and inhibiting CRL2^{VHL}-neddylation.

| *Charlotte*

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

Martin P. Schwalm*, …, Stefan Knapp*
ACS Chem. Biol. **2025**, *20*, 94-104



The authors evaluate 12 inhibitors acting in the mechanistic steps in the TPD pathway, using MZ1 and dBET6 PROTACs. As a readout, they monitor HiBit-tagged Brd4 levels in live and lysed HEK293 cells. The key findings are:

- **E1** inhibitor TAK-243 rescues Brd4. In contrast, the **E1** inhibitor PYR-41 is only partially effective.
- **Neddylation** inhibitor MLN-4942 rescues Brd4.
- The **COP9-signallosome** inhibitor CSN5i-3 should block deneddylation and thus maintain the cullin RING E3 ligase in a constantly activated neddylated state, capable of degrading Brd4 but also potentially degrading the substrate receptor. CSN5i-3 has previously been shown to destabilise both VHL and CRBN. As expected, CSN5i-3 rescues Brd4 in the presence of dBET6, likely due to CRBN destabilisation. But interestingly, treatment of CSN5i-3 in the presence of MZ1 yielded complete degradation of Brd4 and no rescue.
- **Substrate engagement** to the E3 ligase was blocked at high concentrations of lenalidomide and VH298.
- VCP/p97 inhibitor NMS-873 rescues Brd4.
- Rpn13 inhibitor RA190 had off-target activity due to its highly reactive covalent warhead.
- MG-132 was a more potent inhibitor of the **proteasome** than oprozomib, however bortezomib was shown to be the most suitable due to its increased potency and lack of nonspecific Brd4 downregulation in the absence of PROTAC.

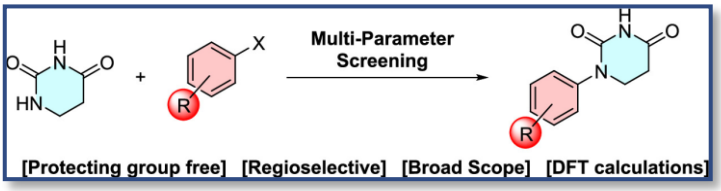
'Tool' molecules are crucial for informing on pathways and validating mechanisms of action. However, these tool compounds are often used at toxic concentrations which may lead to assay artifacts. By titrating each of these inhibitors, the authors help inform on which inhibitors are suitable and at which concentrations they can be used for assays.

There have been reports of E2 inhibitors, which are not tested in this study. However, due to the higher number of E2 ubiquitin-conjugating enzymes and their ability to buffer against the loss of one member or family in knock-out cell lines, inhibition of all E2s could be challenging.

| *Andreas*

One-step regioselective synthesis of *N*-1-SubstitutedDihydrouracils: A motif of growing popularity in the targeted protein degradation Field

Ian D. G. Nixon, Joseph M. Bateman, …, Peter J. Lindsay-Scott*
J. Org. Chem. **2024**, *89*, 18301-18312



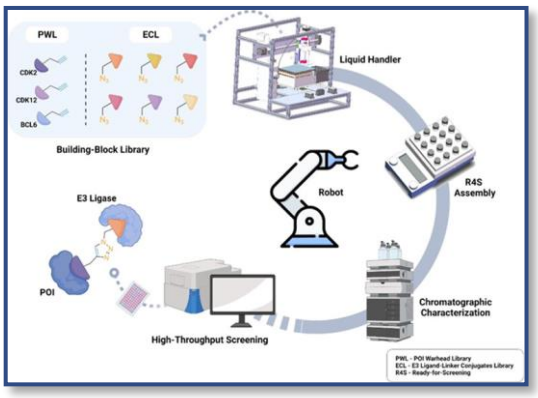
In this paper, the team from AstraZeneca introduces a highly efficient, one-step method for synthesizing *N*-1-substituted dihydrouracils, a motif of increasing importance in Cereblon (CRBN)-based PROTAC and molecular glue research. The approach utilizes Pd-catalysed cross-coupling with aryl halides, offering a cost-effective, regioselective, and scalable alternative to previous multi-step strategies. Importantly, the method is compatible with diverse coupling partners, including aryl bromides (Condition A), aryl chlorides (Condition B), and aryl iodides (Condition C). Functional group tolerance is excellent, accommodating both electron-donating and electron-withdrawing groups. The significance of this work lies in its versatility for advancing CRBN-related discovery. Rapid access to dihydrouracil scaffolds facilitates both molecular glue discovery and PROTAC development. The scalability and avoidance of protecting groups further enhance its utility for medicinal chemistry workflows. Overall, this study provides a powerful tool to expand the development of CRBN ligands and improve targeted protein degradation efforts.

I found this paper incredibly useful, as coupling DHU with aryl halides has traditionally been a challenging step, requiring either protected DHU or starting with an aniline and subsequently generating the ring. This process eliminates these complexities, saving significant time and effort. I believe this paper is a must-read for any chemist working on CRBN-based degraders, as it simplifies a critical step in their workflows and accelerates research progress.

| *Andreas*

Auto-RapTAC: A versatile and sustainable platform for the automated rapid synthesis and evaluation of PROTAC

Jiexuan Chen, Mingfei Wu, Jun Mo, …, Xiaowu Dong*, Jinxin Che*
J. Med. Chem., **2025**



The synthesis and optimization of bifunctional molecules like PROTACs remain empirical, requiring iterative cycles of chemical synthesis and biological testing. Current solutions, including high-throughput screening and direct-to-biology (D2B) approaches, are limited by complexity and resource demands. In this paper, Chen *et al*. present Auto-RapTAC, an automated platform integrating a modular building-block library with CuAAC click chemistry for rapid assembly. Its ready-for-screening (R4S) workflow enables direct biological evaluation

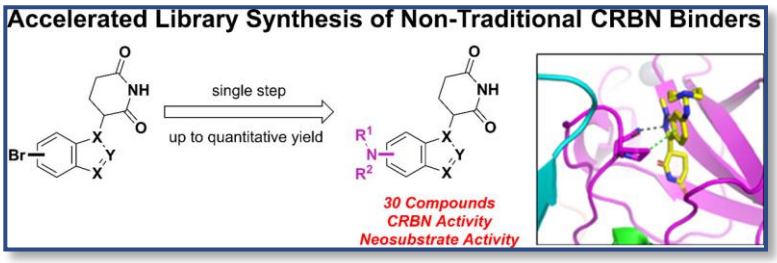
without extensive purification. The authors synthesized and tested 160 PROTACs targeting CDK2, CDK12, and BCL6 within just 8 days per target, addressing the bottlenecks of slow synthesis and limited assay throughput. Auto-RapTAC accelerates lead identification while enhancing reproducibility and scalability. Despite limited linker diversity and next-generation E3 ligase binders in its current design, its modular structure provides a solid foundation for future expansion and broader applications in chemical biology and drug discovery.

The authors successfully demonstrate the efficacy of their fully automated Auto-RapTAC platform, showcasing its ability to generate multiple PROTACs and identify potent BCL6 degraders. The inclusion of a video in the supporting information provides a clear view of the platform's operation. However, the library design misses an opportunity for greater structural diversity, notably rigid, cyclic, and aromatic linkers, as well as next-generation CRBN binders. While the authors suggest future updates, their current absence raises questions about the platform's robustness across diverse substrates. Furthermore, the lack of copper scavenger usage and negative control assays to account for potential copper residue interference in biological assays could affect reliability and interpretation of degradation results. Despite these limitations, the paper effectively highlights the platform's promising efficiency, although certain improvements would enhance its comprehensiveness and impact.

| *Andreas*

Development of a Buchwald–Hartwig amination for an accelerated library synthesis of cereblon binders

Anastasia Lejava, Giulianna A. Miseo, …, Natalie Holmberg-Douglas*
ACS Med. Chem. Lett. **2025**, *16*, 89–95



In this study, the research team from Bristol Myers Squibb developed an optimized Buchwald-Hartwig amination method for synthesizing *N*-linked glutarimide-based cereblon (CRBN) binders. Their initial reaction using RuPhos G2 and LHMDS at 90 °C for 16 hours yielded the product in only 5%–10%. Subsequent optimization through high-throughput catalyst screening revealed that $Pd_2(dba)_3$ with RuPhos or BrettPhos significantly improved yields and minimized glutarimide hydrolysis. Remarkably, LHMDS proved superior to traditional bases such as Cs_2CO_3 and KHMDS, likely due to a protective aggregation effect with deprotonated glutarimides. The refined conditions were tested with diverse amines and heteroaryl bromides, achieving yields between 45% and 95%. Additional optimization for some *N*-linked glutarimides at 45 °C with prolonged reaction times and citric acid quenching further improved conversions, yielding between 41%–66%.

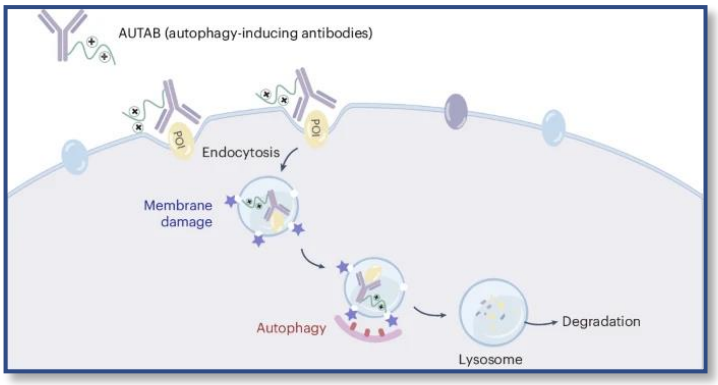
This paper effectively tackles a persistent challenge in synthesizing glutarimide-based CRBN binders: the hydrolysis of the glutarimide ring under basic conditions. The Bristol Myers Squibb team's optimization of Buchwald-Hartwig amination, using LHMDS as a base and Pd-catalyst systems, provides a robust solution that significantly mitigates this issue. Although the scope is comprehensive, including diverse coupling partners, it would have been insightful to see examples of dihydrouracil-based CRBN binders. Including even unsuccessful

cases could highlight the method's limitations and guide future improvements. Overall, this work presents invaluable strategies for reducing the frustrations of glutarimide hydrolysis and inconsistent reactivity, making it a must-read for chemists developing CRBN-based PROTACs and other degradation platforms. This paper stands as a key resource for avoiding the synthesis-related headaches that can plague this chemistry.

| *Maria*

Chemically engineered antibodies for autophagy-based receptor degradation

Binghua Cheng, Meiqing Li, …, Hongchang Li*
Nat. Chem. Bio., **2025**



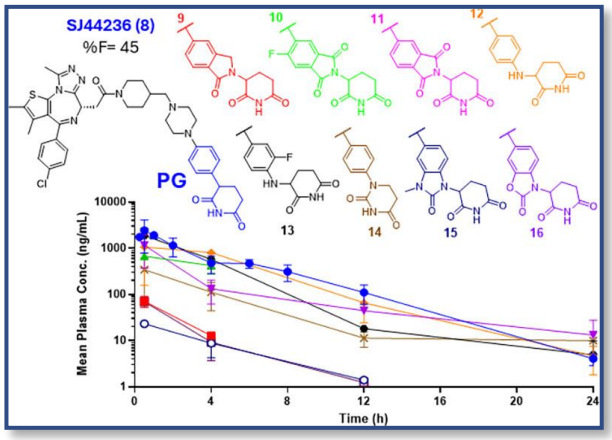
Autophagy-based degradation has emerged as an alternative to ubiquitin-proteasome-based (PROTACs) and lysosome-based (AbTACs, LYTACs) degraders. Autophagy targeting chimeras (AUTACS) have proven particularly useful for degrading large proteins, protein aggregates, and organelles. However, AUTACs are currently limited to trapping and degradation of cytosolic protein targets and organelles. In this work, the authors develop a platform for autophagy-based membrane-bound protein degradation, so-called **AUTABs** (autophagy-inducing antibodies). This strategy directs plasma membrane proteins for autophagic degradation simply by conjugating a target specific nanobody to the **autophagy inducer compound polyethylenimine (PEI),** that produces endosomal damage resulting in **increased LC3C levels,** which is responsible for triggering autophagy. The authors successfully degrade multiple membrane receptors in different cell lines demonstrating the broad compatibility and utility of the platform.

The straightforward design of this platform makes it widely versatile and scalable, and it could be a powerful platform for targeting membrane bound proteins even where recruitment of specific membrane or lysosome-bound ligases/receptors in AbTACs or LYTACs might be challenging. Importantly, authors perform a series of experiments to validate the positive-charge and endosomal-membrane-damage dependent mechanism (which differs from the LC3 ligand-based mechanism of ATTECs, whose mechanism has recently been challenged). Interestingly, they reported no reduction in cell viability, despite these types of highly positively charged compounds generally being linked to cell membrane disruption and high cell toxicity, often limiting their clinical progress.

| *Maria*

Evaluation of cereblon-directing warheads for the development of orally bioavailable PROTACs

Marisa Actis, Joel Cresser-Brown, …, Zoran Rankovic*
J. Med. Chem. **2025**, *ASAP*



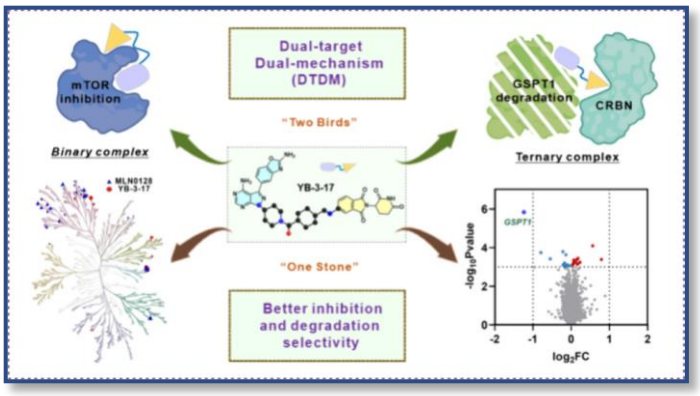
In this study, the authors optimized the **phenyl glutarimide-based BET PROTAC** SJ995973, that, despite its optimal *in vitro* potency, presents high clearance, and a short half-life *in vivo*. Authors evaluated multiple modifications in the linker and CRBN warheads to develop an orally bioavailable BET PROTAC. This **two-part study** first evaluated the effect that changes in the linker in SJ995973 (reducing rotatable bonds, hydrogen bond donors and topological polar surface area) had on ternary complex formation (AlphaScreen), degradation efficiency (HiBiT), specificity (proteomics), and ADME properties *in vivo*. The authors found an optimal linker, 1-(4-piperidinylmethyl)piperazine (**SJ44236**), that **retained the high degradation potency, while improving metabolic stability**. They then moved on to exploring variations in the CRBN targeted warhead in SJ44236. Interestingly, the warhead changes showed high variability in degradation efficacy and *in vivo* properties, with the phenyl glutarimide based PROTAC **SJ44236** demonstrating the best degradation, oral bioavailability and PK profile.

This work highlights the importance of the warhead selection when designing PROTACs and the significant impact that subtle changes can have in their behaviour *in vivo*. It was very interesting to see how the combined effect of changes on CRBN warheads and linker can lead to degradation of neo substrates by promoting new CRBN molecular glue interactions. When tested *in vivo*, only five out of the nine best tested showed measurable plasma levels at 24 h, showcasing the challenging route to progress potent PROTACs from *in vitro* to *in vivo*.

| Maria

A dual-target and dual-mechanism design strategy by combining inhibition and degradation together

Yongbo Liu, Xiuyun Sun, Qianlong Liu, Chi Han and Yu Rao*
J. Am. Chem. Soc., **2025**, *147*, 3110-3118



Glioblastoma is a highly aggressive brain tumour associated with high mortality rates. Clinical candidates for the treatment of glioblastoma include kinase inhibitors of mTOR (rapamycin) and molecular glue degraders for GSPT1 (G1 to S phase transition gene), both of which are overexpressed in glioblastoma and promote cancer growth. However, single therapies utilizing mTOR kinase inhibitors failed clinical trials due to high undesired toxicity. Similarly, GSPT1 degraders failed to balance degradation activity and selectivity, with only a limited number of GSPT1 degraders entering clinical trials. In this PoC study, the authors propose a **dual-target, dual-mechanism** small molecule design **that combines a kinase mTOR inhibitor and a GSPT1 molecular glue degrader** into a single molecule. This approach aims to leverage synergistic advantages, improving selectivity and reducing toxicity.

With this dual approach, the authors demonstrate an enhancement in glioblastoma tumour growth inhibition while significantly reducing the undesired toxicity associated with mTOR inhibitors. Interestingly, they were able to independently inhibit mTOR while synergistically degrading GSPT1 without triggering mTOR degradation. Authors demonstrated no degradation of mTOR by Western blots (WBs) and proteomics, ruling out a PROTAC-like mechanism. However, they did not fully explain the rationale behind this, which would be useful for expanding this strategy to other target designs. The selectivity results for GSPT1 degradation are not fully convincing, and the authors face the limitation of poor blood-brain barrier (BBB) permeability, with only 1% of YB-3-17 delivered to the brain. Moreover, factors such as dosing adjustments and the risk of PROTAC-like degradation add complexity to its implementation.

PRE-PRINTS

| *Andreas*

bioRχiv

Small molecule-mediated *targeted protein degradation of voltage-gated sodium channels involved in pain*

*Alexander Chamessian, ..., Robert Gereau**

A very good pre-print provides a proof-of-concept study demonstrating the feasibility of inducing proximity-driven degradation of voltage-gated sodium channels (NaVs), specifically NaV1.7 and NaV1.8, involved in pain signaling. Using degron-tagged NaVs and PROTAC-mediated recruitment of cereblon or Von Hippel Lindau E3 ligases, the authors successfully induced targeted degradation, opening new avenues for pain therapeutics.

| *Aitana*

bioRχiv

Cullin-RING ligase BioE3 reveals molecular-glue-induced neosubstrates and rewiring of the endogenous Cereblon ubiquitome

Laura Merino-Cacho, ..., Rosa Barrio, James D. Sutherland **

In this preprint the authors apply their previously developed BioE3 to study the CRBN ubiquitome. They demonstrate that this technology is suitable to identify both native substrates and neosubstrates upon IMID treatment. It will be really interesting to see this applied to study novel E3 ligases with unknown native substrates.

| *Jon*

bioRχiv

Selective degradation of BRD9 by a DCAF16-recruiting targeted glue: mode of action elucidation and in vivo proof of concept

Scott J. Hughes, …, Louise K. Modis, Andrea Testa**

A very interesting and long-awaited work disclosing AMPTX-1, a potent and selective BRD9 degrader that acts via a "targeted glue" mechanism inducing selective recruitment of BRD9 to the E3 ligase DCAF16. The authors show that the covalent modification of DCAF16 is facilitated by the BRD9 protein, positioning the covalent warhead near DCAF16-Cys58. AMPTX-1 also shows high bioavailability *in vivo*, demonstrating a novel and promising approach to engage new protein surfaces.

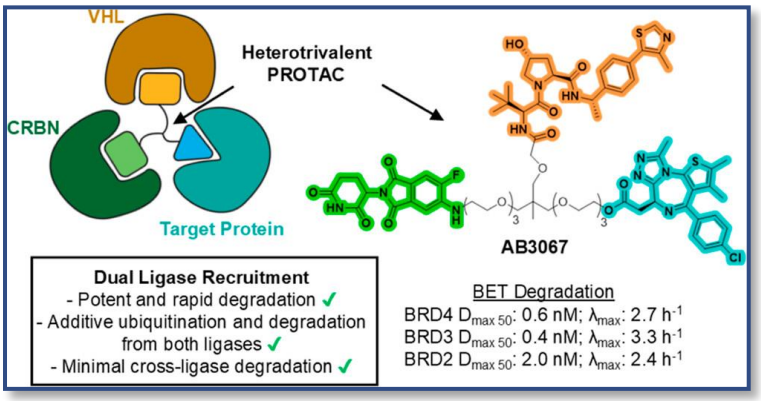
Centre for Targeted Protein Degradation
University of Dundee

PAPERS FROM CeTPD

| *Maria*

Leveraging dual-ligase recruitment to enhance protein degradation via a heterotrivalent proteolysis targeting chimera

Adam G. Bond, Miquel Muñoz i Ordoño, …, Georg E. Winter*, Kristin M Riching*, **Alessio Ciulli***
J. Am. Chem. Soc., **2024**, *146*, 33675-33711
Authors from the CeTPD: ***Conner Craigon, Manjula Nagala***



This study introduces a novel strategy of **dual-ligase recruitment to enhance protein degradation.** Inspired by the successful design of the SIM1 trivalent PROTAC (that used a single VHL ligand linked to two JQ1 ligands for BET protein degradation), researchers designed a **heterotrivalent PROTACs** that can simultaneously recruit two different E3 ligases (CRBN and VHL) to degrade BET proteins. In this work, they connect a CRBN and a VHL recruiting ligands with the JQ1 BET inhibitor via a branched trifunctional linker. Among the synthesized compounds, **AB3067** emerged as the most potent and fastest degrader of BET proteins, with **minimal cross-degradation of E3 ligases**. The comparative kinetic analyses in wild-type and ligase knockout cell lines robustly validated the dual-ligase recruitment (CRBN and VHL) and showed that BET protein ubiquitination and degradation induced by **AB3067** were additive. This dual-ligase approach was further expanded by developing: **(1)** a **heterotrivalent CRBN/VHL PROTAC** for the inducible BromoTag degron system **(2)** a **heterotetravalent PROTAC** with two BET ligand moieties, aiming to retain the avidity and BET bivalency that the previous SIM1 displayed, but now adding the ability to recruit two E3 ligases.

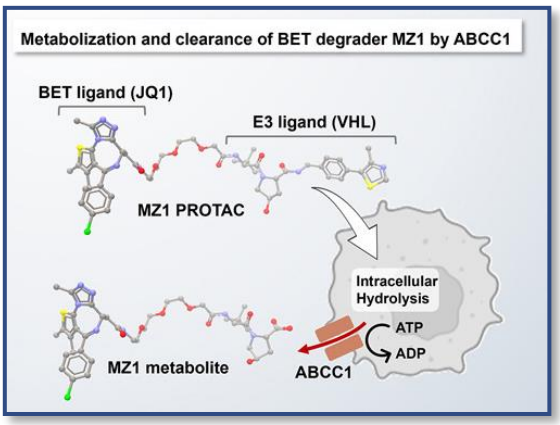
This research highlights the potential of dual-ligase recruitment to boost the efficiency of PROTAC-degradation. It is crucial to emphasize the necessity for minimal cross-degradation of E3 ligases and optimal cell permeability, both of which have been successfully achieved in this design. One thing to keep in mind is that these factors could be significantly impacted when adapting the platform to other ligase or protein target ligands or linkers.

| *Charlotte*

The efflux pump ABCC1-MRP1 constitutively restricts PROTAC sensitivity in cancer cells

Gernot Wolf, … , Giulio Superti-Furga*
Cell Chem. Biol. **2025**, *in press*
CeTPD authors: ***Conner Craigon, Diane Cassidy, Yuting Cao, Alessio Ciulli***

For a PROTAC library composed of different architectures (the bivalent MZ1, the trivalent SIM1 and the macrocyclic macroPROTAC-1), recruiting different E3 ligases (VHL and CRBN) and targeting different BET substrates, the authors evaluate ABCC1 transport affinity and cellular efflux efficacy. They explore ABCC1 chemical inhibition with reversan and tariquidar in several cancer cell lines, and find that inhibitors may increase PROTAC efficacy 'with tolerable side-effects'. They also reveal that MZ1 can be metabolised within the cell, suggesting that the amide bonds of the VHL ligand moiety are cleaved by intracellular hydrolases.



While the authors identify ABCC1 as an exporter for these VHL- and CRBN-recruiting BET degraders, other exporters may be involved for other E3 ligases and targeted substrates. Further studies will also be required to identify the mechanism of PROTAC cellular influx.

| *Maria*

ChemRxiv™

Rational design of PROTAC linkers featuring ferrocene as a molecular hinge to enable dynamic conformational changes

Alessandra Salerno, Lianne Wieske, Alessio Ciulli*

The chemical complexity of PROTACs often can lead to poor solubility and cell permeability, which are critical for PROTAC's bioavailability. This preprint introduces a new PROTAC design approach, where FerroTACs ferrocene-based linkers are used to achieve chamaleonicity. The authors demonstrate that ferrocene's unique organometallic structure enables dynamic conformational changes and improving the cellular permeability across three different PROTAC systems.

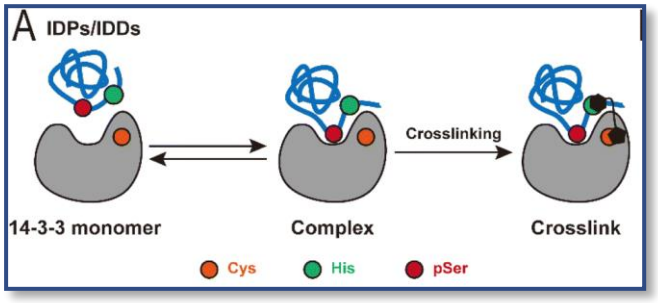
OTHER PAPER HIGHLIGHTS

CHEMISTRY | STRUCTURAL BIOLOGY & BIOPHYSICS | CELL BIOLOGY | MODELLING

| *Charlotte*

Proximity-enhanced cysteine-histidine crosslinking for elucidating intrinsically disordered and other protein complexes

Qi Wu, …, Luc Brunsveld*, Peter J. Cossar*
Chem. Sci. **2025**, *advance article*



The authors designed and synthesised a bifunctional tool molecule to serve as a dual-protein crosslinker between 14-3-3 and IDPs (intrinsically disordered proteins). **Maleimide (pink)** covalently modified the 14-3-3 surface-exposed cysteine located at the 14-3-3/phospho-partner PPI interface. At the end of the **short linker (green)**, a **2-cyclohexenone moiety (orange)** was installed, as it is reported to react with histidine residues. This is particularly relevant, as histidine is highly represented in IDPs. The authors confirm a high level of crosslinking between 14-3-3 and B-Raf, using time-dependent intact MS and FP. Further linker modelling and chemical optimisation is performed. The authors additionally observe crosslinking with other small peptides representative of 14-3-3 substrates, such as C-Raf, KSR1, CDK11B, and Tau. Finally, they show that crosslinking is possible between 14-3-3 and full-length Tau and identify a previously overlooked pSer356 14-3-3 binding site on Tau.

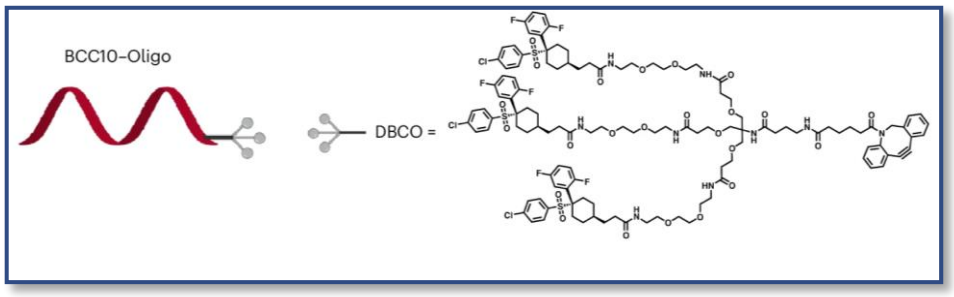
Intrinsically disordered protein domains are difficult to study owing to the lack of structural information. It would be interesting if purification of the crosslinked species could be used for further structural study, for example by cryo-EM, to further understand the binding mode of 14-3-3 to Tau.

All experiments in this study were performed *in vitro*, meaning that purified protein is required. By further functionalising this small molecule, maybe it would be possible to apply this dual-crosslinking in cells to identify new IDP binding partners.

| *Andreas*

Intravenous administration of blood–brain barrier-crossing conjugates facilitate biomacromolecule transport into central nervous system

Chang Wang, Siyu Wang, ..., Eric J. Nestler, Yizhou Dong*
Nat. Biotechnol. **2024**



This paper presents a novel approach to overcoming the blood-brain barrier (BBB) permeability, a key challenge in treating central nervous system (CNS) disorders. The authors developed a library of BBB-crossing conjugates (BCCs) and identified BCC10, derived from a γ-secretase inhibitor, as a lead candidate. BCC10 utilizes γ-secretase-mediated transcytosis to effectively deliver biomacromolecules, including oligonucleotides (oligos), into the brain. When combined with antisense oligonucleotides (ASOs), BCC10 demonstrated enhanced gene silencing in mouse models, human brain tissue *ex vivo*, and an ALS mouse model, outperforming previous delivery systems like cholesterol-conjugated oligos by up to 66-fold in brain penetration. Beyond novel therapeutics, BCC10 offers potential for reviving compounds designed to target CNS proteins but are limited by BBB permeability. It addresses unmet needs in diseases such as Alzheimer's and ALS. With high biocompatibility and no significant toxicity in preclinical models, BCC10 is a promising platform for advancing CNS drug delivery and improving understanding of neurological disease mechanisms.

This paper was incredibly interesting, particularly for someone working on larger molecules targeting CNS disease-causing proteins. The potential of BCC10 to overcome the challenges of BBB permeability is highly exciting. I would love to see this system tested with a PROTAC to determine whether it could not only deliver PROTACs into the brain but also induce effective degradation of target proteins. This could be a game-changer for orally challenging VHL-based degraders, which struggle with BBB penetration. For instance, coupling BCC10 with XL01126—a VHL-based LRRK2 degrader that has shown promise in degrading LRRK2 but suffers from low BBB permeability ($K_{p,brain}$: <0.0035)—could significantly enhance its activity in the brain. Such a combination might provide a transformative strategy for addressing neurological diseases where protein degradation is a therapeutic goal. Overall, this paper opens exciting possibilities for repurposing and optimizing previously limited CNS-targeting therapeutics.

Centre for Targeted Protein Degradation
University of Dundee

innovate
collaborate
inspire

Centre for Targeted Protein Degradation
School of Life Sciences
1 James Lindsay Place,
Dundee,
DD1 5JJ

Journal club – website

@alessiociulli @Farnaby84 @FarnabyLab @UoDCeTPD @DeLaCuadraBaste @Holmqvist89