CeTPD Journal Club

June – July 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

Aileen Frost

After completing her Dphil in Organic Chemistry at the University of Oxford, Aileen undertook post-doctoral research in the areas of Organocatalysis and Radiofluorination, at the University of St Andrews and the Max-Planck-Institut für Kohlenforschung, respectively.
Aileen has worked in PROTAC drug discovery since 2018, when she joined the collaboration between CeTPD and Boehringer Ingelheim. As a Senior Scientist in Medicinal Chemistry, Aileen has responsibility for leading drug discovery projects in the TPD space.

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Hirotake Furihata

Hirotake completed his PhD in biochemical and biophysical sciences in Japan, where he investigated the teratogenic mechanisms of thalidomide metabolites in collaboration with multiple research groups. Since joining the Ciulli Lab, his research has focused on evaluating molecular glues and PROTACs, particularly how these compounds promote protein-protein interactions to drive targeted biological outcomes.

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Ilaria Puoti

Ilaria is originally from Naples, Italy and in 2017 she moved to Glasgow to start her PhD at CRUK Beatson Institute in Sara Zanivan's lab. During her PhD, Ilaria focused on the application of an advanced proteomic technology called cell type-specific labelling using amino acid precursors (CTAP) to decipher the CAF-cancer crosstalk signalling in high-grade serous ovarian cancer. In 2022, Ilaria joined the Ciulli group as a proteomic and cell biology scientist as part of the PROTAC collaboration with Bohringer Ingelheim.

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Mokhitli Morake

Mokhitli completed his MSc in Pharmaceutical Chemistry (2017) at North-West University working on the synthesis of artemisinin-based compounds against malaria and tuberculosis. He joined H3D, the drug discovery and development centre at the University of Cape Town (UCT), as a scientific officer in medicinal chemistry. He then completed his PhD in Chemistry (2022) under the supervision of Prof Kelly Chibale and Dr Greg Basarab. He has been part of the ACBI Team in CeTPD since 2023.

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NEW EDITORS-IN-CHIEF FOR THE CETPD JOURNAL CLUB

| *Andreas*

The CeTPD Journal Club was founded back in April 2020 by Siying Zhong, a former postdoctoral researcher in Alessio's lab, and was later developed further by Charlotte Crowe, a former PhD student in the Ciulli group. Charlotte then handed it over to Yuting and me.

As I now enter the final year of my PhD, I feel I need to focus more on my research projects, so it's time for me to pass the torch.

I'm delighted to announce that **Giullia Bonasegale**, a first-year PhD student in the Cossar group, will be taking over as our ***new co-editor-in-chief***.

Giullia will be working alongside Aitana De La Cuadra Baste to lead and coordinate the journal club moving forward. I have every confidence they'll do an outstanding job!

I want to sincerely thank all of you who have supported and followed the Journal Club, it has been a real pleasure!!

All the best,
Andreas Holmqvist

TARGETED PROTEIN DEGRADATION

CHEMISTRY | STRUCTURAL BIOLOGY & BIOPHYSICS | CELL BIOLOGY | MODELLING

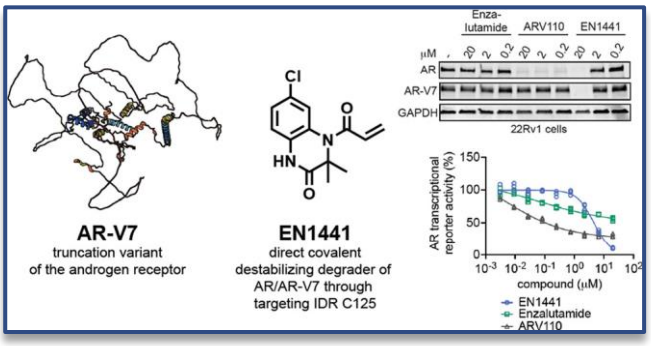
"Every two months, we spotlight the latest and most significant literature in the field of targeted protein degradation, spanning chemistry, biophysics, cell biology, and computational modeling"

Literature review from 22nd May to 21st July 2025

| *Aileen*

Covalent Destabilising Degrader of AR and AR-V7 in Androgen Independent Prostate Cancer Cells

Charlotte Zammit, Cory Nadel ….. Ryan Potts* and Daniel Nomura*
J. Am. Chem. Soc. **2025**, *147*, 20512-20524



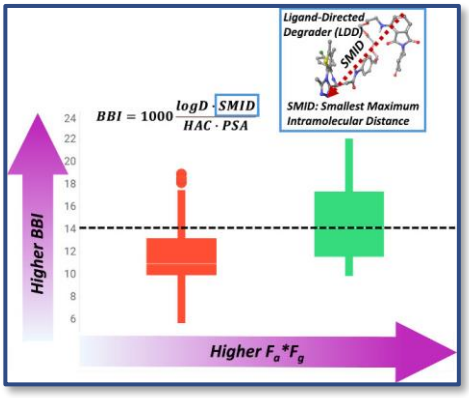
This report presents EN1441, a covalent molecule which is capable of effecting degradation of both AR and truncated variant AR-V7, which occurs due to drug resistance. AR-V7 is an intrinsically disordered protein, which lacks the AR ligand binding domain and thus cannot be targeted by current known therapies. The characterisation of EN1441 includes an investigation into selectivity via proteomics, identification of the covalent binding site utilising mass spectrometry, and shows that the compound is capable of inhibiting AR transcriptional activity more robustly than both inhibitor enzalutamide and degrader ARV110. The authors demonstrate that treatment with EN1441 causes destabilisation of the target protein, which leads to aggregation and therefore inhibition of AR activity. In this instance, proteasomal degradation appears to be a later consequence of protein aggregation, rather than the driver of activity.

The discovery and characterisation of EN1441 provides an interesting case study for targeting disordered proteins. The authors are realistic about the limitations of this molecule, highlighting that an improvement in selectivity, potency and physicochemical properties would be necessary to progress the study further. The overall strategy in which disordered proteins are targeted for destabilisation is reminiscent of the "hydrophobic tagging" work published by Crews *et al*, and it will be interesting to see how this may be applied in a therapeutic context to allow the targeting of currently undruggable disordered protein targets.

| Aileen

New Multiparameter Index is a Strong Predictor of Oral Bioavailability for Heterobifunctional Degraders

Javier Baylon et al.
J. Med. Chem. Lett. ***2025****, 16, 1108-1113*



This team from Bristol-Myers Squibb share a new multiparameter index which promises to aid the design of orally bioavailable bifunctional degraders. Building on the previously reported "Balanced Permeability Index" (BPI), which combines size, polarity and lipophilicity, the "Bifunctional Bioavailability Index" (BBI) takes 3D shape into account by incorporating "smallest maximum intramolecular distance" or SMID. After showing the BBI was a better classifier of oral absorption than either BPI or EPSA, LogD, SMID or heavy atom count alone on their internal data set, the parameter was applied to a public data set of 55 degraders and utilising calculated LogD and TPSA values. Whilst BBI performed well on this public data set, the authors note that SMID/TPSA was also found to be a good predictor of high oral bioavailability, enabling application where experimental EPSA and LogD data is not available.

Developing orally bioavailable bifunctional degraders is a long-standing challenge in the field, as these molecules sit well outside classic drug-like chemical space. Our understanding has improved over recent years, as companies such as Abbvie, Arvinas and AstraZeneca have published guidelines for beyond rule of 5 compounds or degraders which have helped them in their optimisation efforts. BBI makes a nice addition to the PROTAC drug discoverers' toolkit. By demonstrating the success of the index when utilising solely calculated rather than experimental data, the authors give confidence that this parameter could be used to prioritise prospective designs in a campaign. The authors also highlight the challenges associated with measuring permeability of bifunctional degraders via a Caco-2 assay, presenting an opportunity for BBI to reduce the reliance on this often-inconsistent assay.

Hot topic: methodologies for evaluating potential ligandable sites on E3 ligases capable of effecting Targeted Protein Degradation

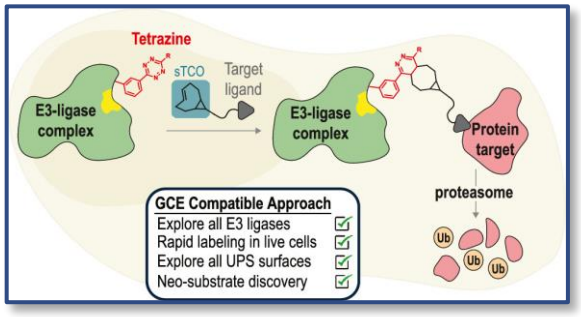
Whilst >600 E3 ligases are available for recruitment by degraders in principle, in practice, the field is still largely reliant on just two – VHL and CRBN. The bar to choosing to base a campaign on a new E3 ligase is understandably high, as developing a new ligand before degradation has been demonstrated would require significant investment, which may not pay off. This month, three different research groups have published their approaches to addressing this challenge.

Check the three following entries!

| *Aileen*

In-Cell Approach to Evaluate E3 Ligases for Use in Targeted Protein Degradation

Yunan Zheng§. . . Justin Reitsma* and Ryan Mehl*
J. Am. Chem. Soc ***2025****, 147, 21560-21574*

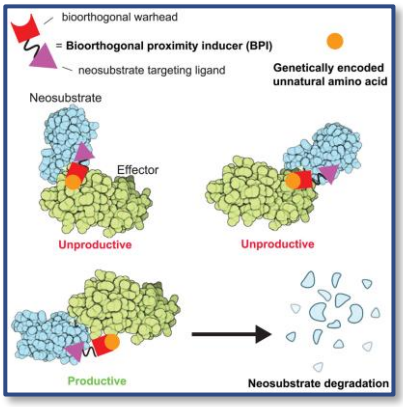


In this report, Zheng and co-workers outline their new methodology for evaluating the suitability of E3 ligase/POI pairs in a cellular context using an "E3-ligand-free degrader" (ELF degrader). This involves encoding a chosen E3 ligase with a tetrazine handle using genetic code expansion (GCE) and then using click chemistry to conjugate the E3 ligase to a POI binder. The authors used CRBN and BRD2/4 as a proof-of-principle pair to show that this methodology could indeed result in the CRBN-mediated degradation of BRD2/BRD4. Exploration of other sites on CRBN led to varying degrees of BRD2/4 degradation, showing that CRBN has a degree of structural plasticity as an E3 ligase. This also provided an example of surface mapping which would enable investigation into the most promising sites to affect degradation for new E3 ligases. This method was applied next to the E3 ligase SPOP for which BRD2 is a natural degron substrate, and which has no known ligand binding sites. Degradation of BRD2 was demonstrated via ELF-degrader, achieving protein levels lower than those achieved naturally, through recognition of the natural degron.

| *Aileen*

Site-resolved assessment of targeted protein degradation

Ricardo Moreno-Ballesteros... Satpal Virdee.*
Cell Chem. Bio. ***2025****, 32, 969-981*



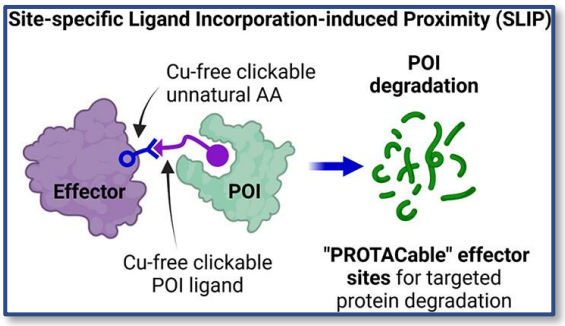
In this publication, the authors present methodology to explore the potential binding sites of TPD effector proteins. Using genetic code expansion technology, surface residues of VHL (His110, Thr105, Thr100, Asn90, Glu94, Asn67) and CRBN (Glu377, His353, Asn351) are conjugated to a JQ1-ligand, allowing impact on degradation of BRD2, BRD3 and BRD4 to be assessed. Interestingly, different conjugation sites showed differences in degradation profile, which in the case of VHL seemed to correlate with successful exit vector positions from known PROTACs. Investigation into degradation mediated by E2 ligase UBE2D1 is also undertaken, with modest BRD4 degradation observed when JQ1 is conjugated at positions Cys111 and Arg90. The opportunity to probe recruitment site geometry demonstrated here would have applications in helping to identify the most promising sites for effector protein ligand development for TPD.

| Aileen

Identification of Actionable Targeted Protein Degradation Effector Sites through Site-Specific Ligand Incorporation-Induced Proximity (SLIP)

Zhangping Xiao* ... Edward Tate*
J. Am. Chem. Soc. ***2025**, 147, 21549-21559*

Discussed internally



In Xiao *et al*'s approach, cysteine residues within E3 ligases are identified which are precedented for covalent binding and/or as handles for degraders. Using genetic code expansion (GCE), an unnatural amino acid containing a *trans*-cyclooctene is incorporated at the position of the identified cysteine, allowing for conjugation to a POI binder flanked by a tetrazine via *in cellulo* click chemistry. Degradation of FKBP12^{F36V} mediated by VHL is used as a model system, in which a FKBP12^{F36V}-mCherry fusion is utilised to provide a fluorescent read-out. Tagging VHL at different cysteine positions leads to differing levels of FKBP12^{F36V}-mCherry degradation, enabling a survey of possible "PROTACtable" sites to be undertaken. Expansion of this methodology was undertaken next to evaluate a rage of 22 potential sites across 17 effector proteins, with the focus on known covalently ligandable residues. Of these, SOCS2 Cys111 and RNF Cys185 were found to be the most efficient degraders of FKBP12^{F36V}-mCherry, with MARCHF5 Cys118 and RNF114 Cys65 also showing potential, along with E2 ligases UBE2B Ser25 and UBE2D4 His55. Investigation into the degradation of Aurora A kinase found that CRBN_H353, SOCS2_C111 and RNF_C185 were all capable of effecting endogenous protein degradation via the SLIP methodology.

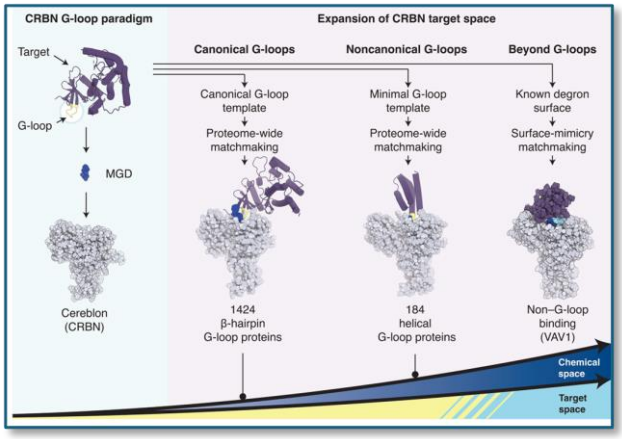
These methodologies provide an opportunity to study the feasibility of recruiting alternative E3 or E2 ligases for application in TPD, via site specific chemical modification of a chosen protein. A recent complementary report from the Dieters lab (*RSC Chem. Biol.*, 2025, 6, 240-248) showcases modification of a POI in order to conjugate an E3 ligand, and taken together these reports have an opportunity to make a huge impact within the field. The prospect to probe the most suitable sites to promote degradation afforded by these studies is particularly attractive to medicinal chemists and structural biologists, who could capitalise on such information to drive SBDD efforts towards new E3 ligase binders. Overall, it is clear to see how such studies could provide the foundation to enable us to expand therapeutic potential of TPD, for example by exploiting differing expression levels of E3 ligases in different disease-relevant tissue cells. It will be exciting to see how these methodologies are received and capitalised upon.

| Hirotake

Mining the CRBN target space redefines rules for molecular glue–induced neosubstrate recognition

Georg Perzold §, Pablo Gainza §, ..., Markus Warmuth * and John C. Castle *
Sci., **2025**, 389, 6736

Discussed internally



Petzold *et al*. combined computational and experimental approaches to identify over 20 new CRBN-recruitable proteins, revealing that both β-hairpin and helical G-loop motifs, as well as G-loop–independent surfaces like in VAV1, can mediate molecular glue–induced degradation. The CRBN binding interface shows high plasticity, allowing both structural and topological adaptability — opening doors for neosubstrate discovery through both β-hairpin motifs and alternative surfaces. CRBN's target landscape is far broader than previously known, encompassing over 1600 predicted G-loop–compatible human proteins, many beyond the C2H2 ZF class. Differentiated chemistries will be essential to access structurally diverse G-loop and non–G-loop targets, such as VAV1, which recruits CRBN via surface mimicry and not structural homology. Future computational designs will focus on matching dynamic surface features of CRBN–MGD complexes with the human proteome, enabling rational expansion of CRBN-targeting therapeutics.

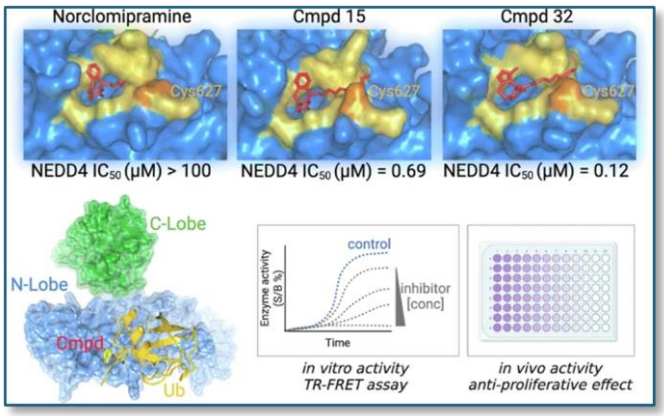
Alternative approaches may exist for identifying CRBN neosubstrates induced by molecular glues, and surface mimicry here presented could offer a distinct methodology to uncover substrates that engage in protein-protein interactions facilitated by these glues. Even though non-G-loop targets can be mediated for degradation, other structural frames – still underexplored – could still be effective neosubstrate candidates.

| Hirotake

Structure-based design of potent and selective inhibitors of the HECT ligase NEDD4

Elena Maspero §, Anna Cappa §, ..., Ciro Mercurio* and Simona Polo*
Commun. Chem., **2025**, 8:164

A covalent selective inhibitor for NEDD4 (Neural precursor cell expressed developmentally down-regulated 4) has been developed to block NEDD4-mediated ubiquitin chain elongation while preserving monoubiquitinated interaction unaffected. Norclomipramine, a broad-spectrum inhibitor of HECT-type E3 ligases, allows the formation of ubiquitin dimer formation but specifically prevents further ubiquitin chain elongation. The development of a covalent inhibitor specifically targeting the



non-catalytic cysteine C627, based on crystallographic approach, inhibits NEDD4-mediated polyubiquitination while retaining the characteristics of Norclomipramine. Further modification of aromatic ring resulted in excellent potency and demonstrated a well-suited pharmacokinetics profile, representing step towards targeting the oncogenic functions of NEDD4. This compound, which also selectively targets NEDD4-like members of the NEDD4 family, is expected to be useful for controlling NEDD4-mediated ubiquitination due to its high selectivity conferred by covalent binding.

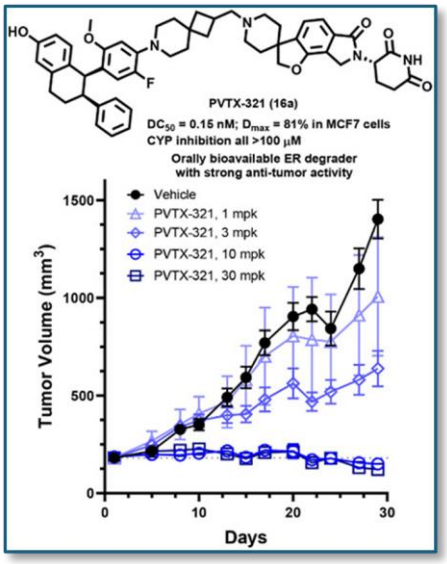
The shallow region above the NEDD4 catalytic site, which contains the active cysteine responsible for thioester formation and ubiquitin transfer, poses a challenge for stable compound binding, making it difficult to directly inhibit NEDD4 activity. However, the compound described in the paper covalently binds to a region distinct from the catalytic site, yet still effectively and selectively inhibits ubiquitination activity. This suggests an alternative and promising strategy for targeting NEDD4

| *Hirotake*

Discovery and Characterization of PVTX-321 as a Potent and Orally Bioavailable Estrogen Receptor Degrader for ER+/HER2– Breast Cancer

Guozhang Xu §*, …, Xuqing Zhang*
J. Med. Chem. ***2025***, *68, 11299-11321*

PVTX-321 a novel, orally bioavailable heterobifunctional degrader targeting estrogen receptor a (ERa). It developed from the early ER degrader ERD-12310A, effectively inducing degradation of both wild-type ERa and clinically relevant ESR1 mutants in ER-positive breast cancer cell lines. Futhremore, PVTX-321 demonstrates exceptional efficacy by inhibiting tumor growth and inducing substantial tumor regression, while also exhibiting favourable oral bioavailability in murine models. The spirocyclic CRBN ligand reduced the degradation of CRBN substrate CK1a, IKZF1, and SALL4, while ARV-471 still has phthalimide ring concerns binging proteins that contribute side-effect. Optimization and fine-tunning lead to high efficacy and efficient degradation can be applied to other ERa degrader developments. Altogether, PVTX-321 holds strong promise for clinical development as a treatment for ER+/HER2− breast cancer.



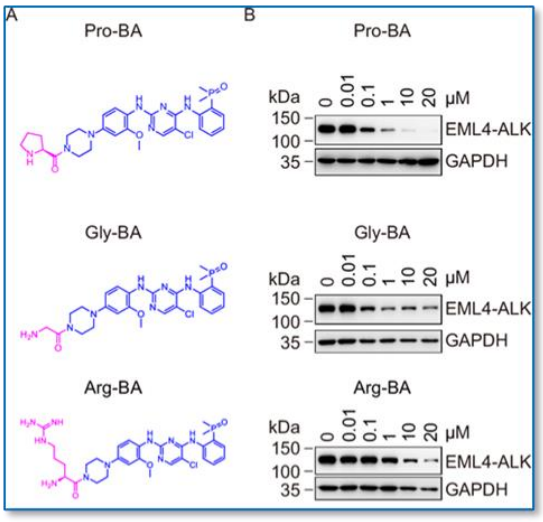
Given the recent development of multiple ERa degraders, there is a growing need for studies that dissect how individual components—such as the ligand and linker—contribute to the degradation mechanism. Such mechanistic insights will be essential for guiding the next generation of ERa-targeting degraders. In this work, the spirocyclic group on CRBN biner, which extends slightly beyond the core structure, may introduce steric hindrance that reduces off-target interactions compared to the phthalimide backbone, which merely fits within the thalidomide-binding pocket. This spirocyclic ligand, combined with optimised linkers and an improved ERa binder, contributes to a highly effective ERa degrader that surpasses ARV-471, pointing to the potential for a parallel and potent ERa degrader development pathway.

Linker-free PROTACs efficiently induce the degradation of oncoproteins

Jianchao Zhang, Congli Chen, …, Hai Rao*

Nat Commun, ***2025***,*16, 4794*

Discussed internally



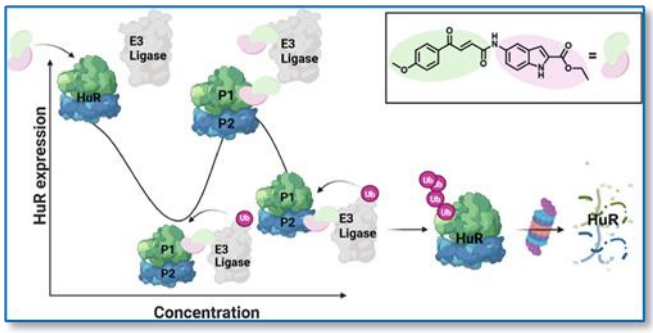
The design of the optimal linkers is essential in heterobifunctional molecules such as PROTACs, as this impact physicochemical properties and size of the molecules. There are currently no guidelines for the choice of linkers. This paper explores the use of linker-free PROTACs by addition of the amino acids as N-degrons that are recognized in the N-end rule pathway for degradation. The authors describe a potent Brigatinib analog (BA) appended with proline, dubbed Pro-BA, which degrades the EML4-ALK more efficiently than its PEG linked matched pairs. Pro-BA demonstrated antiproliferative effects in the H3122 cells and the mechanism of degradation was confirmed to be via proteolysis. Ternary complex formation is achieved, and Pro-BA is described to be essential for the interaction of EML4-ALK and GID4 (the CTLH E3 ligase substrate receptor) which leads to EML4-ALK ubiquitylation. This linker-free strategy could also be extended to other targets (BCR-ABL) and compounds (Dasatinib analogs) to achieve similar degradation levels. Pro-BA was orally bioavailable and demonstrated *in vivo* efficacy in xenograft models.

In the absence of universal linker design principles, the linker-less strategy offers an alternative and streamlined approach for the design of degraders. This strategy also uses the simple degradation signals such as single amino acids, which directly impacts the size of the PROTACs, leading to drug-like molecules (<500 Da). This has favourable advantages in that it reduces the size to levels of molecular glues, maintains good physicochemical properties requirements, and uses simple amino acids as the potent degrons that can bind E3 ligases for N-end rule pathway degradation.

| Mokhitli

Druglike Molecular Degraders of the Oncogenic RNA-Binding Protein HuR

Liann Kassabri and Raphael I. Benhamou
J. Am. Chem. Soc. Au, ***2025***, https://doi.org/10.1021/jacsau.5c00551



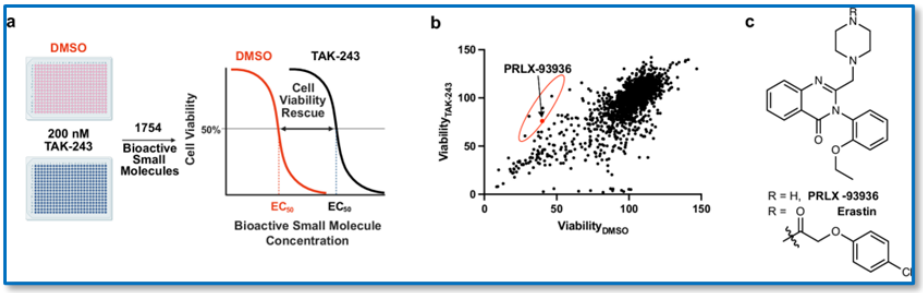
This paper reports on the novel PROTACs and molecular glue (MG) compounds which were explored for the degradation of Hu antigen R (HuR), which acts as an RNA binding protein promoting stabilization of oncogenes such as Bcl2 and FOXQ1. MG appears to be the best approach with superior effects than the PROTAC strategy in inhibiting tumour growth. The authors demonstrate that the mechanism of action of this compound is through proteasomal degradation. Interestingly, despite reaching hook effect at intermediate concentrations, a second degradation phase ensues at high doses, resulting in a biphasic degradation profile. This is suggested to lead to enhanced degradation of the target protein. The most active compound in the study, MG-HuR2, is a MG and has druglike properties, which are essential for further optimization.

These authors managed to identify drug-like degraders that target challenging RBPs like HuR, which tend to have structural plasticity that makes their inhibition challenging with small molecule inhibitors. The superior activity of the molecular glue with drug-like properties addresses the challenges in the field of heterobifunctional molecules with high molecular weight and physicochemical properties that are unfavourable. The biphasic degradation profile of the lead molecule suggested that it can bind to two pockets of the same protein, and this has the consequence of a compound that can lead to enhanced ubiquitination and degradation of the target proteins. It is therefore imperative that the crystal structure be obtained to understand the binding mode of the degraders identified herein and thus inform future designs for further optimization. The authors have demonstrated that the RBPs can be targeted, and this results in downstream activities that lead to tumour growth inhibition.

| Mokhitli

Elaboration of molecular glues that target TRIM21 into TRIMTACs that degrade protein aggregates

Marc A. Scemama de Gialluly, …, & Drew J. Adams
Nat Commun, ***2025***,*16, 6548*



This work describes the phenotypic screening of compounds that were aimed at independently targeting E3 ligases. From this screening, PRLX-93936 and BMS-21466233 are identified as molecular glue degraders

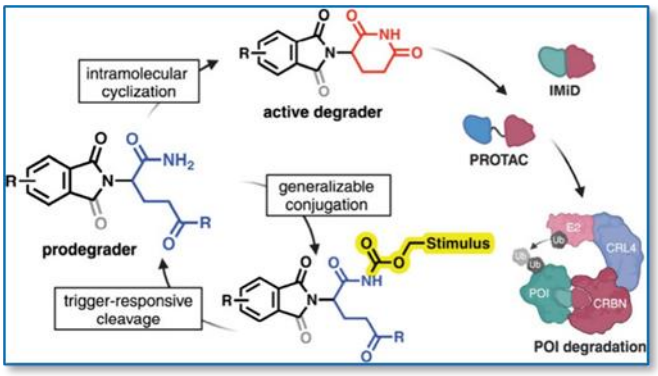
that target TRIM21. This is confirmed by a suite of experiments including the genetic knockouts of TRIM21 and unbiased proteomics which show that TRIM21 is essential for the activity of these degraders. Additionally, proteomics data identified that these compounds induce the degradation of nucleoproteins, a feature related to TRIM21, which targets ubiquitination of multimeric protein complexes such as nucleoproteins. The authors were able to also convert these glues into TRIMTACs, whose activity was largely influenced by the stereochemistry, and one atropisomer showed superior degradation. TRIMTACs were also found to degrade protein aggregates, as was observed for molecular glues.

The results of this study expand on the knowledge of the E3 ligase repertoire that can be targeted for the treatment of various cancers. TRIM21 adds to the options of E3 ligases that can be explored in the development of degraders to target a variety of cancers, in addition to the commonly reported CRBN and VHL ligases. The ability of this ligase to ubiquitinate multimeric protein aggregates is also a unique feature that can be extended to tumours with high expression of TRIM21. This work further sheds light on the mechanism of action of PRLX-93936 and BMS-21466233, which is driven by TRIM21-mediated ubiquitination. In general, the paper provides evidence for the relevance of TRIM21 as a key E3 ligase in targeted protein degradation for molecular glues and PROTACs/TRIMTACs discovery campaigns.

| *Mokhitli*

Design and Application of Cereblon-Recruiting Prodegraders

Davis H. Chase, § Alicia Stein,§ …, Craig M. Crews*
J. Am. Chem. Soc. ***2025****, 147, 24527−24537*



Immunomodulatory Imide Drugs (IMiDs) are frequently employed as key binding partners of cereblon (CRBN) E3 ligase for ubiquitination of a variety of proteins of interest (POIs) by PROTACs, and some of these are already in the clinical trials. As the main feature of IMiDs, the glutarimide ring is essential for CRBN binding. The paper describes a novel approach through a design of prodegraders bearing the glutamine and asparagine that undergo intramolecular cyclization to form glutarimide and aspartimide, respectively, *in vitro* and *in cellulo*. These prodegraders demonstrate the degradation of the POIs *in vitro* and *in cellulo*, albeit longer time point since the cyclization process progresses with time. This strategy was applicable to the PROTACs, which could be synthesized with uncyclized amino acid (glutamine) and cyclize to the desired degraders that are equipotent to the parent IMiD-based degrader. Furthermore, the authors apply this strategy to Degrader Antibody Conjugates (DACs), and in this case the advantage of the prodegraders is that they provide handles for conjugation that enable enzymatic release of the prodegrader payload. In this way, CRBN-based prodegraders can be successfully employed in the development of DACs.

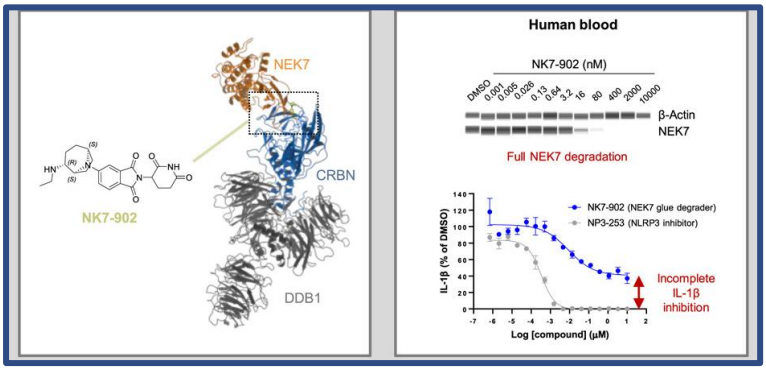
The prodegrader concept described by the authors can help in overcoming stability challenges that comes with IMiDs ring opening during PROTACs development. This phenomenon has been previously observed in our lab. However, the work presented

herein is encouraging in that this moiety can be designed in an open ring form and still achieve degradation of targeted proteins *in cellulo*. The limitation is that the use of esters may present metabolic stability issues *in vivo*, leading to the hydrolysis and removal. It will be essential to assess, at least in the first instance, whether these prodegraders are stable in microsomes. Moreover, the slow cyclization required for conversion of the prodegrader to the degrader will likely be limiting as well. However, the application of this strategy to DACs provides a better access for conjugation with options for functionalization directly on the linkers. The paper has demonstrated that the IMiD ring opening is not a limiting factor in CRBN PROTACs design, as this can cyclize *in vitro* or *in cellulo* to the desired degrader.

| *Ilaria*

A cereblon-based glue degrader of NEK7 regulates NLRP3 inflammasome in a context-dependent manner

Aude Sylvain, ...Dennis L. Buckley and Zuni I. Bassi
Cell Chem. Bio., ***2025,*** *32, 955–968*



The NLRP3 inflammasome is a key immune signaling pathway and its aberrant activation is linked to various diseases. While NLRP3 inhibitors are in clinical trials, NEK7 has emerged as an alternative target to block NLRP3 activity. Given that NEK7 function in the NLRP3 inflammasome is independent of its kinase activity, the authors proposed the development of a molecular glue degrader as unique opportunity to block this pathway. They identified NK-902, a cereblon-based molecular glue that potently and selectively degrades NEK7 in human monocytes, whole blood and iPS cells. Structural analysis revealed that NK-902 engages NEK7 via a conserved β-hairpin degron. Functionally, NK-902 inhibits NLRP3-dependent IL-1β release in donor- and stimulus-dependent manner, highlighting partial NEK7 independence in NLRP3 activation. NEK-902 had acceptable PK properties in mice with good oral exposure, enabling assessment of its activity in vivo. Unlike most CRBN-based molecular glue, NEK-902 was effectively degrading NEK7 in murine relevant disease models such as in acute peritonitis and CAPS. However, in cynomolgus monkeys NEK7 degradation mediated by NEK-902 did not consistently suppress IL-1β, indicating species specific disconnects between NEK7 depletion and inflammasome inhibition. While the findings do not support the applicability of molecular glue degrader as strategy to block NLRP3 pathway, the authors provide a chemical tool with in vivo activity that will contribute to further elucidate the role of NEK7 into NLRP3 signaling pathway.

The authors are realistic about the limitations of the study and temper enthusiasm about the degrader's clinical utility for NLRP3 driven diseases. However, the discovery of NEK7-902 reported by Sylvain *et al.* represents an interesting case study that highlights how targeted protein degradation can also advance the understanding of the biology of disease-causing proteins such as NEK7. The authors provide important evidence that NLRP3 activation can occur without NEK7 challenging prevailing assumptions and reinforcing emerging models of context-dependent inflammasome activation. The reason for residual inflammasome activity despite NEK7 degradation

requires to be fully elucidated, alternative pathways are discussed but not experimentally dissected. It would be interesting to see if NK-902 as valuable tool compound will contribute to reveal insights into inflammasome regulation

| *Ilaria*

Covalent Recruitment of NEDD4 for targeted protein degradation: Rational design of small molecular degraders

Xiaoqiang He, … Ke Ding, Tangzheng Liu, Yi Tan and Zhengqiu Li
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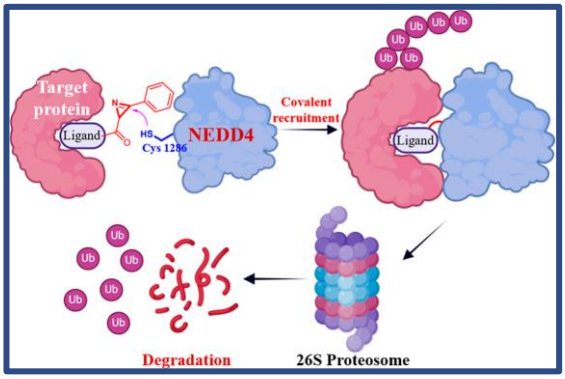
Discussed internally



To address drug resistance in NSCLC, the authors aimed to develop novel degraders targeting EGFR-C797S mutation, which many patients acquire after treatment with third-generation EGFR TKIs. They selected LS-106 a derivative of brigatinib as basal moiety for $EGFR^{L858R/T709M/C797S}$ recognition and for modification with various electrophilic warheads toward new covalent degrader. Among the several electrophilic moieties screened, 2H-azirine emerged as the most effective chemical handle for $EGFR^{L858R/T709M/C797S}$ degradation. Firstly, the authors identified compound ZSH-1136 that degraded $EGFR^{L858R/T709M/C797S}$ by > 70% and showed inhibitory activity, outperforming brigatinib. Then, they found that varying the linker length critically affected degradation efficiency leading to the identification of ZSH-2117 that selectively degraded resistant $EGFR^{L858R/T709M/C797S}$ mutant while sparing wild type and other variants, achieving nanomolar potency with up to 96% degradation. Using chemoproteomics approach such as ABPP and Co-IP/MS, the authors identified NEDD4 as E3 ligase mediating $EGFR^{L858R/T709M/C797S}$ degradation and pinpointed Cys1286 on NEDD4 as the covalent binding site for ZSH-2117. Knockdown of NEDD4 abolished both EGFR degradation and ubiquitination, confirming its central role. ZSH-2117 showed superior inhibition of cancer cell growth compared to its parent inhibitor and significantly reduced tumor burden in vivo without affecting body weight. Beyond $EGFR^{L858R/T709M/C797S}$, the authors attached the 2H-azirine handle to ligands for PDE5, CDK4, BTK, BRD4, SMARCA2/4 and Bcr/Abl^{T315I}. This resulted in efficient degradation of these targets, highlighting the versatility of this chemical handle and its relevance for expanding E3 ligase toolkit in small molecular degrader design.

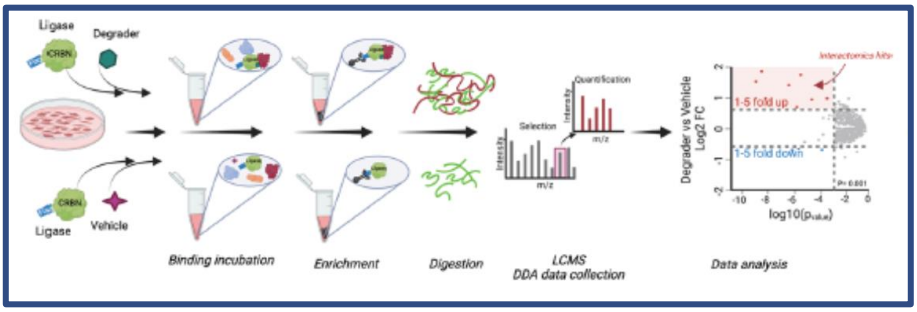
The study presents NEED4 as an alternative E3 ligase for targeted protein degradation, recruited via a 2H-azirine chemical handle that covalently binds to the target. While previous work by Nomura and Gray demonstrated the potential of covalent chemistry to identify E3-recruiting handles, this study offers the first proof-of-concept for using NEDD4 as an E3 ligase in degrader design, expanding the toolbox beyond CRBN and VHL. Importantly, the study provides a new strategy for degrading proteins resistant to CRBN/VHL-mediated degradation. Given NEDD4's role in neuronal signaling, this approach could be explored for targeting proteins involved in neurological diseases. Despite its innovation, the study has limitations, including a lack of structural data and in vivo evaluation limited to xenograft models without pharmacokinetic or toxicity assessment—critical for clinical translation. The use of a brigatinib-derived scaffold raises concerns about selectivity and off-target effects, which were not addressed, such as through global proteomics. Additionally, NEDD4's promiscuity could risk degradation of unintended substrates. Nonetheless, this work marks a significant step forward in

the field of targeted protein degradation and lays a strong foundation for further exploration of NEED4-based strategies for therapeutic applications.

| *Ilaria*

Unveiling the hidden interactome of CRBN molecular glues

Kheewoong Baek, … Katherine A. Donovan & Eric S. Fischer
Nat Commun, ***2025****, 16, 6831*



In this study, the authors introduced a high-throughput lysate-based affinity proteomic workflow to comprehensively map the interactome of CRBN molecular glues. Using CRBN-DDB1ΔB spiked into lysates, the authors identified 298 enriched proteins recruited to CRBN by IMID-like degraders across multiple cell lines. These include known zinc finger (ZF) transcription factors and newly uncovered non ZF proteins, such as kinases and RNA-recognition motif domain proteins. Remarkably 270 targets were newly reported and validated with a computational screening pipeline that aligned G-loop degrons from AlphaFold2 predicted structures and scored their compatibility for recruitment of CRBN via Rosetta-based clash modelling. This affinity proteomic workflow complements traditional unbiased proteomic approaches, enabling the detection of interactors missed by global proteomic profiling. Validation using TR-FRET, ubiquitination assays and cryo-EM structures confirmed direct binding and degrader-induced ternary complexes for non-ZF proteins like PPIL4 and PDE6D. Moreover, the authors not only identified and validated a novel non-ZF protein PPIL4 enriched in their affinity proteomic screening but also discovered a selective molecular glue for this target. Importantly, many proteins were found to bind CRBN without being degraded, highlighting a previously underappreciated layer of molecular glue biology and challenging the assumption that ligase recruitment must lead to degradation. In addition, the authors updated the data onto their open-access proteomics portal envisioning the impact of their study for the TPD community.

The study expands our understanding of CRBN interactomes and introduces a robust and scalable high throughput affinity proteomics workflow for the identification of drug-induced protein interactions in cell lysate. The findings reported are confined to CRBN-IMiD chemistry, but the authors provide a valuable open-access resource to the entire TPD community. It would be exciting to see further applications of the affinity proteomics workflow and how can be adapted other E3 ligases for molecular glues discovery. Nevertheless, it will be interesting to see in the future how the field will elucidate biology behind the interaction of CRBN glues that do not trigger degradation. Perhaps new methodologies will contribute to differentiating molecular glues functions between modulators or productive recruiters.

PRE-PRINTS

| *Hirotake*

bioRχiv

Data-independent acquisition (DIA) approach for comprehensive ubiquitinome profiling in targeted protein degradation

*Gajanan Sathe... Gopal P. Sapkota**

This study established an optimized ubiquitinome workflow combining urea lysis, magnetic bead-based diGly enrichment, and DIA-NN analysis, enabling highly sensitive and reproducible identification of ubiquitination sites. Compared to previous methods, it offers deeper coverage without requiring overexpression or proteasome inhibition in all cases. This makes it especially valuable for TPD research, as it allows precise mapping of degrader-induced ubiquitination on endogenous targets, aiding mechanistic understanding and degrader design.

| *Hirotake*

ChemRxiv™

Understanding the role of H-bonds in the stability of molecular glue-induced ternary complexes

*Patricia Blanco-Gabella * ... Jordi Juárez-Jiménez **

They developed canonical molecular glue (MG) inducing ternary complex system by Steered Molecular Dynamics, which is tailored for precise quantification of individual H-bond strengths at interfaces. This establishes a framework for evaluating whether the molecular glue (MG) enhances pre-existing hydrogen bonds or facilitates the formation of new molecular interactions.

PAPERS AND PREPRINTS FROM CeTPD

| *Ilaria*

bioRχiv

Identification of a Highly Cooperative PROTAC Degrader Targeting GTP-loaded KRAS(on) Alleles

*Vesna Vetma§, Ilaria Puoti§, Natalia K. Karolak§,... Alessio Ciulli, Peter Ettmayer, Kirsten McAulay**

CeTPD authors (past and present): *Vesna Vetma, Ilaria Puoti, Natalia Karolak, Sohini Chakraborti, Emelyne Diers, Shakil Khan, Giorgia Kidd, Ross McLennan, Suzanne O'Connor, Nicole Trainor, Claire Whitworth, Andre Wijaya, Jeff Wong, David Zollman, William Farnaby, Alessio Ciulli, Kirsten McAulay*

In this study, the authors reported ACBI4 a potent and selective heterobifunctional VHL-based KRAS(on) PROTAC. ACBI4 forms a highly stable and cooperative ternary complex with VHL and GTP-bound KRAS, effectively degrading all KRAS variants and inducing a strong antiproliferative response in vitro. For years, the search for single agent capable of specifically and simultaneously targeting all oncogenic KRAS alleles represents a major challenge in drug discovery. The identification of ACBI4 marks unprecedented progress toward this goal. This new molecule stands out as valuable chemical probe and lays the groundwork for the development of future therapies for KRAS-driven cancers.

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