CeTPD Journal Club

October – November 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

ADAM PINTO

Adam, originally from Hertfordshire, completed his MChem in 2022 at the University of Oxford. In August of that year Adam joined the Ciulli group at the Centre for Targeted Protein Degradation as an Associate Scientist in Medicinal and Organic Chemistry, within the Boehringer Ingelheim collaboration team. He remains at the CeTPD for his PhD, now in his third year, developing kinase-targeted molecular glues in the Farnaby group. Outside of the lab, Adam enjoys travelling, watching the F1 and live music.
LinkedIn: https://www.linkedin.com/in/adam-pinto

MATT PARKIN

Matt completed his MChem in medicinal and biological chemistry from the University of York in 2023, investigating the utility of sp^2-sp^3 Suzuki-Miyaura cross-couplings in fragment-based drug discovery in his final year project. He then joined flavour and fragrance company Natara Global as an R&D chemist investigating new synthetic routes to flavour and fragrance molecules. Since joining the Farnaby group in 2024, he has worked as knowledge transfer associate in collaboration with BioAscent Discovery investigating new methodologies in high throughput TPD drug discovery.
LinkedIn: https://www.linkedin.com/in/matt-parkin-646942278/

ELIZABETH HOGG

Lizzy joined CeTPD in 2025 as a Cell Biologist within the PROTAC Drug Discovery collaboration with Boehringer Ingelheim. Previously, she completed her PhD at the CRUK Manchester Institute studying fibroblasts in the pancreatic cancer microenvironment. In 2021 she moved to Dr Findlay's lab in the MRC PPU, Dundee, as a postdoc researching kinase and ligase signalling mechanisms in embryonic stem cells. Outside of the lab she is interested in baking and cricket.
LinkedIn: http://www.linkedin.com/in/elizabeth-hogg-phd-7b66a997

LIANNE WIESKE

Lianne is from the Netherlands, where she obtained a bachelor's degree in chemistry. In the summer of 2023, she finished her PhD studies at Uppsala University under supervision of Professor Máté Erdélyi where she employed liquid-state NMR spectroscopy to answer a diverse set of research questions. After receiving her PhD degree, she moved to Mülheim an der Ruhr in the summer of 2023 for a postdoctoral training with Dr. Christophe Farès at the Max-Planck-Institute. In October 2024 Lianne joined the Ciulli group as a postdoctoral scientist.

NEW EDITOR-IN-CHIEF FOR THE CETPD JOURNAL CLUB

| *Giulia*

The CeTPD Journal Club, founded in 2020 by Siying Zhong and later developed by Charlotte Crowe, is a great opportunity for our Centre —and the wider TPD community— to stay up to date with the TPD literature and the exciting science being published.

The current Journal team is composed of Aitana De La Cuadra Basté and myself as co-editors-in-chief, and Alessandra Salerno as our help in editing and formatting.

We are delighted to announce that **Yuting Zhi**, a first-year PhD student in the Farnaby group, will join the team as a ***new co-editor-in-chief***.

Yuting will help lead and coordinate the journal club moving forward. It is a pleasure to see the team growing!

All the best,
The JC Team

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 September to 20th November 2025

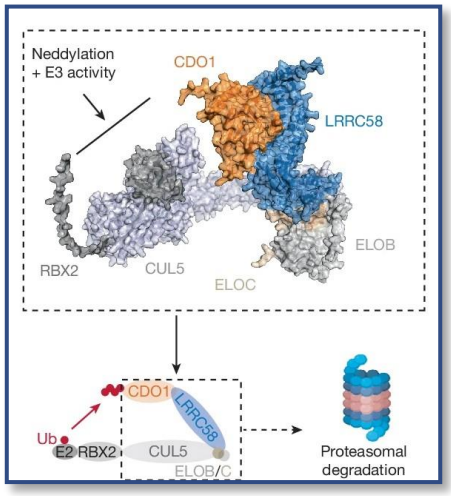
| *Lianne*

Covariation MS uncovers a protein that controls cysteine catabolism

Haopeng Xiao§, …, Edward T. Chouchani*
Nature **2025**, *647*, 268

Discussed internally

This paper reports CDO1 as the substrate for the cullin-RING E3-ligase substrate receptor LRRC58, playing a key-role in cysteine regulation. The authors investigated close to 12 000 proteins and 285 metabolites in brown adipose tissue and hepatocytes of mice by covariation MS. A positive correlation between hypotaurine and CDO1, and a negative correlation for both FMO1 and LRRC58 and hypotaurine were identified. CDO1 is a critical enzyme in the cysteine catabolism pathway where it converts cysteine into cysteine sulfinic acid as a first step on its way to taurine. FMO1 catalyses the last step of hypotaurine to taurine, explaining the observed correlations for CDO1 and FMO1 with hypotaurine. However, LRRC58 has no established metabolic role. Through LRRC58 knockdown (LRR58^{KD}) studies, an increase in taurine and CDO1 levels were observed in absence of LRRC58. The effect of taurine levels was shown to be a result of LRRC58 modulation of CDO1. Further investigation into the interaction partners allowed the authors to model a Rbx2/Cul5/EloC/EloB/LRRC58/CDO1 complex which has a CDO1 orientation that is compatible with ubiquitination. Flag immunoprecipitation followed by western blotting, as well as *in vitro* studies showing a 202.9 kDa complex being formed, supported the interaction between LRRC58 and CDO1, Cul5, EloB and EloC. TR-FRET studies on the complex indicated a K_D of 4.67 µM between eGFP-tagged LRRC58 and terbium-chelate-labelled CDO1. Furthermore, *in vivo* studies using proteasome inhibitors in hepatocytes resulted in accumulation of CDO1, indicating that CDO1 degradation is a proteasomal process.



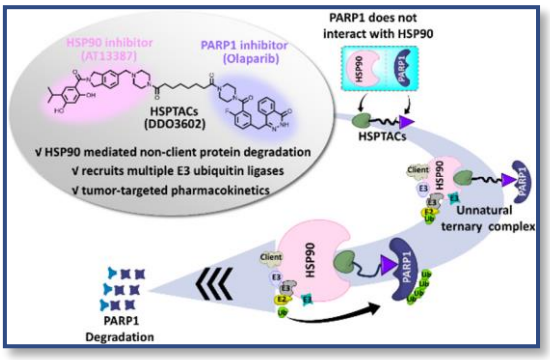
This paper sheds light on cysteine regulation, identifying the involvement of an E3-ligase that plays a key role in the process. It is always nice to see a less common substrate receptor entering the TPD scene.

| Matt

HSP90 Mediates Targeted Degradation of Nonclient Protein PARP1 for Breast Cancer Treatment

Wei Liu§, Jianfeng Liu§, Huangliang Shu§, ..., Xiaoli Xu*, Qidong You*, and Lei Wang*.
J. Med. Chem. ***2025***. *68, 19, 19933–1995.*

Discussed internally



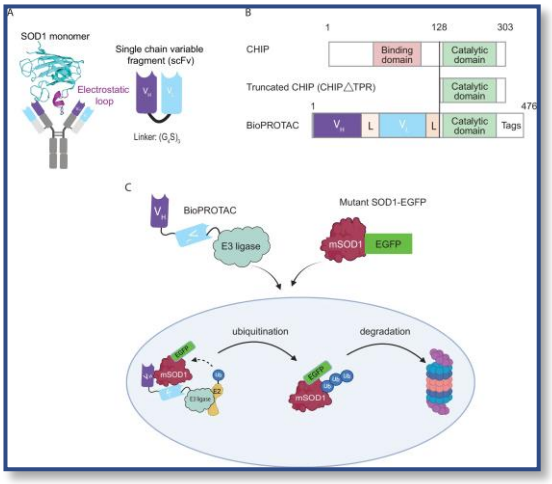
This paper shows the development of a heterobifunctional degrader for poly(ADP-ribose) polymerase 1 (PARP1) using Heat Shock Protein 90 (HSP90) as the E3 ligase recruiter. HSPTACs as they are known, were initially only thought to be useful to degrade cooperative proteins of HSP90. However, the authors demonstrate in this paper that this is not the case. Through a BLI assay, HSP90 showed no binding affinity towards PARP1, showing PARP1 to be a non-client protein of HSP90. The authors identified two small molecule inhibitors for both PARP1 and HSP90 and through an iterative linker optimisation, DDO3602 was identified to be a potent heterobifunctional degrader of PARP1 with DC_{50} of 490 nM and a D_{max} of 69.7%. It was also discovered that DDO3602 exhibits *in-vivo* anti-tumour properties with significant tumour regression noted at 20 mg/kg dosing in xenograft mice studies. Due to the non-traditional nature of the E3 recruiter, the authors decided to investigate the mechanism of degradation for DDO3602. Through Co-IP assays and quantitative proteomics, it was discovered that DDO3602 induces a ternary complex between HSP90 and PARP1 followed by polyubiquitination by a range of E3 ligases including TRIM25, MYCBP2 and TRIM50 amongst others. Degradation pathways using HSP90 then follow the traditional proteosome degradation pathway used with traditional PROTACs.

It is interesting to see the authors using new and underutilised E3 ligase recruiters in TPD strategies helping to overcome resistance mechanisms when using only one E3 ligase recruiter. The authors provide important evidence that the degradation of PARP1 is due to the degrader and not favourable protein-protein interactions between HSP90 and PARP1 showcasing the utility of using this method when designing heterobifunctional degrader compounds. It will be interesting to see how useful this HSPTAC strategy can be when degrading other HSP90 non-client proteins along with using other heat shock proteins such as HSP70 in the same vein.

| Matt

Development of a targeted BioPROTAC degrader selective for misfolded SOD1

Rachael Bartlett,* ..., Christen G. Chisholm,* Jeremy S. Lum.
Nat Commun., **2025**, ***16,*** *9713*



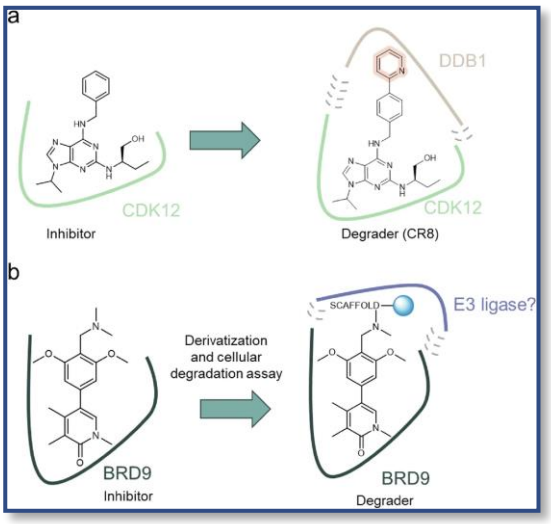
In this paper, the authors set out to design and validate a biological PROTAC (BioPROTAC) targeting mutant forms of superoxide dismutase (SOD1), found in patients with ALS. Using single chain variable fragments (scFvs) targeting an electrostatic loop exposed in mutant variants of SOD1 allows the BioPROTAC to selectively target different mutant variants of the protein. The authors investigated a range of BioPROTACs using a range of linkers and E3 ligases. Their final candidate to push into in-vivo studies was using the catalytic domain of CHIP and a $(G_4S)_3$ linker. By designing mice with expression for a SOD1 mutant and cross breeding these with BioPROTAC expressing mice, they produced 4 pools. The SOD1 mutant mice saw significant deterioration in their weight but also neural capability. In the SOD1 mutant/BioPROTAC mice, they saw a similar deterioration in body weight however, without the decline in neural capability. The expression of the BioPROTAC did not increase the lifespan of the mice, however the SOD1/BioPROTAC group tended to be euthanised due to the drop in body weight as opposed to their neural capabilities. Finally, the authors wanted to determine the mechanism for degradation. They expressed both an unmodified BioPROTAC and a BioPROTAC with the catalytic domain of CHIP removed with either a proteasome or lysosomal inhibitor. This confirmed that the truncated BioPROTAC utilises an exclusively lysosomal degradation mechanism. Whereas the unmodified BioPROTAC utilises both proteasome and lysosomal mechanisms of degradation allowing for more efficient degradation.

This paper gives an interesting alternative to traditional heterobifunctional degraders using intrabodies. By using these, it allows the BioPROTAC to overcome the selectivity issues that traditional PROTACs struggle with. However, it will be interesting to see how this methodology could be developed into be used in a therapeutic setting to treat patients suffering with these SOD1 mutations without the use of gene editing.

| *Matt*

Mode of action of a DCAF16-recruiting targeted glue that can selectively degrade BRD9

Scott J. Hughes§, Wojciech J. Stec§, Colin T. R. Davies§, …, Louise K. Modis* & Andrea Testa*.
Nat Commun., **2025**, 16, 8516



In this paper, the authors discover a targeted molecular glue (AMPTX-1) against BRD9. This is achieved through attaching an electrophilic cyanoacrylamide to the solvent exposed region of a BRD9 inhibitor. By doing so, this alters the protein-ligand interface and can encourages a ternary complex to form. One enantiomer of AMPTX-1 was found to be more active with a DC_{50} of 0.2 nM and a D_{max} of 94%. The authors discovered that AMPTX-1 recruits DCAF16 as the E3 ligase through a ternary complex pulldown experiment. This glue shows a more blended MoA compared to traditional molecular glues as a hook effect was seen, usually observed with heterobifunctionals. This suggests that AMPTX-1 maintains binding to the E3 ligase after ubiquitination of BRD9 through the covalent link with a cysteine at residue 58. Proteomic experiments show only BRD9 is degraded with BRD7 showing no detectable degradation, even though the inhibitor used in this study is shown to recruit BRD7. The selectivity comes from favourable PPIs forming upon ternary complexation favouring ubiquitination on BRD9 and not BRD7. AMPTX-1 was also found to follow a proteasomal degradation pathway. The authors also ran mouse xenograft models to test in-vivo efficacy. A 50 mg/kg oral dose was administered at t = 0 h and t = 8 h. At 2 h post last dose, a statistically significant 82% degradation of BRD9 was observed in tumour samples.

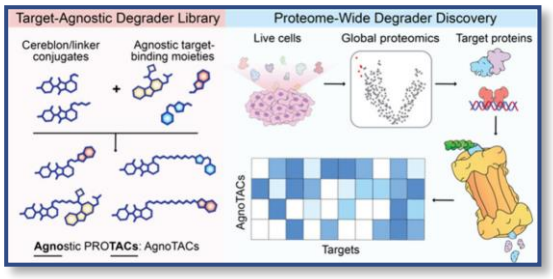
This paper shows a nice approach to discovering and designing molecular glues by using existing inhibitors. It also shows a nice way to overcome issues surrounding selectivity of small molecule inhibitor through using favourable protein-protein interactions rather than altering the ligand itself. One downside to introducing this methodology into a drug discovery program would be the lack of rational design with targeted glues, unlike heterobifunctionals. However, glues have huge potential in the area of orally bioavailable degraders moving into the future.

| *Adam*

Proteome-Wide Discovery of Degradable Proteins Using Bifunctional Molecules

*Ines Forrest§, …, Christopher G. Parker**
ACS Cent. Sci. ***2025****, 11, 11, 2240–2256*

Discussed internally



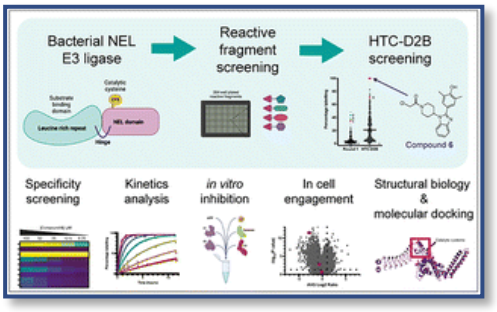
The authors argue that overreliance on pre-existing, well-characterised ligands inherently restricts PROTAC scope. They aimed to address this by synthesising 72 "AgnoTACs" consisting of thalidomide attached, using one of four simple linkers, to a set of 18 structurally diverse, druglike small molecules. These target-agnostic bifunctionals were then screened by total proteomics, enabling identification of downregulated proteins. The team identified more than 50, functionally diverse, proteins downregulated by their compounds. Investigations into the mechanism of action of some of these hits showed that, despite many conventionally acting degraders, they also identified degradation events that occurred non-canonically. For example, degradation of ETS-1 and TMEM205, whilst proteasomally mediated, are seemingly independent of neddylation activity, suggestive of a MoA separate from CRBN activity. The authors go on to examine the effect of linker composition on the degradation activity of their AgnoTACs. They show that linker composition was of high importance, as most targets were uniquely degraded by a single linker type. CRBN dependence was established for BRD2 and PLOD2 degradation by generation of negative control compounds, unable to bind CRBN, these compounds were also unable to degrade their targets. Overall, the authors have used the power of proteomic profiling to identify starting points for degraders of underexplored target proteins via their AgnoTAC platform.

The authors present an unorthodox approach towards mapping the chemically degradable proteome. Although the hits are of low potency and degradation efficiency, they still provide a valuable starting point for PROTAC development against novel targets. Whether these hits can be developed into chemical probe quality tools remains to be seen, however, this would serve as an exciting follow-up story. This paper also highlights the importance of carrying out a full set of suitable control experiments, to ascertain whether our compounds act in the way we expect them to. The robustness of the control experiments (negative probes, target engagement assays and effector controls) give confidence many of the hits in this paper represent genuine degradation events.

| *Adam*

Covalent fragment screening to inhibit the E3 ligase activity of bacterial NEL enzymes SspH1 and SspH2

Cassandra R. Kennedy§, …, David House* and Katrin Rittinger*
RSC Chem. Biol., ***2025***, *Advance Article*



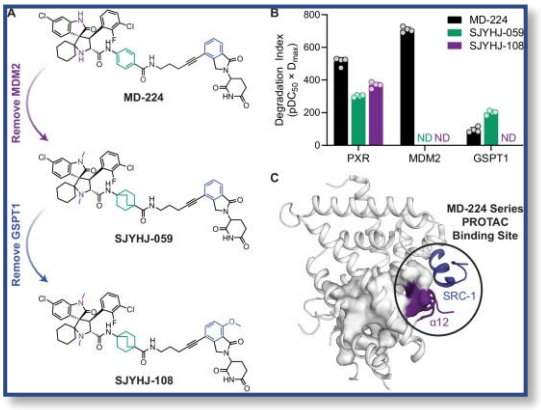
This study reports the first successful chemical-inhibition of bacterial NEL-family E3 ubiquitin ligases, using a covalent fragment-based screening strategy. The authors screened a chloroacetamide fragment library for compounds able to covalently label the catalytic cysteine of NEL E3s. Fragments were screened against recombinant SspH1, from Salmonella, and IpaH9.8, from Shigella, using intact MS. Hits identified for SspH1 were then optimized via a high-throughput direct-to-biology platform, which generated a set of related chloroacetamide analogues able to fully label SspH1 under the MS assay conditions. The two most potent binders were investigated in ubiquitin discharge and substrate ubiquitination assays. It was shown that compounds 6 and 7 were able to functionally inhibit SspH1, blocking ubiquitin discharge and substrate ubiquitination. IA-DTB chemoproteomics showed target engagement of compound 6 in both cell lysate spiked with SspH1 and SspH2, as well as in live cells transiently expressing the targets. Compound 7 also showed target engagement in lysate, however, was cytotoxic in live cells. Compound 6 now represents a useful starting point for the further development of chemical probes able to inhibit SspH.

As antimicrobial resistance is one of the greatest challenges facing global health, new approaches to the development of antibiotics are essential. This work represents a significant first step towards the development of inhibitors of NEL E3s. The data the authors generate suggests that further optimisation of the reversible binding of compounds 6 and 7 may prove difficult due to low ligand efficiency, but similar screens of different fragment libraries could lead to improved hits with potential for further development.

| Lizzy

PROTAC repurposing uncovers a noncanonical binding surface that mediates chemical degradation of nuclear receptors

Andrew D. Huber§, …, Taosheng Chen*
Nat. Commun., ***2025***, *16, 9805.*



Nuclear receptors are transcription factors that sense cellular stimuli via ligand binding and regulate gene expression. Nuclear receptors are a highly targeted area of research, however, there are challenges in obtaining selectivity of small molecules or PROTACs between the receptor family due to the largely non-selective ligand binding domain. The authors describe here the discovery of degraders targeting a subfamily of nuclear receptors, including PXR, which upregulate enzymes involved in drug metabolism and transport. Aiming to repurpose existing PROTACs, a 45-compound PROTAC library screen was performed, and the MDM2 degrader MD-224 was identified as a potent PXR PROTAC. Performing CRISPR/Cas9, mutagenesis and chemical competition experiments with known PXR chemical enhancers or blockers, MD-224 was shown to engage the AF-2 region at helix a12 adjacent to the ligand binding pocket and induce degradation of PXR in a CRBN-dependent manner. The authors present this new degron surface as noncanonical for CRBN neosubstrates and show that whilst MD-224 induced degradation of four nuclear receptors containing this degron, two nuclear receptors also containing the degron were unaffected, indicating further structural differences within this subfamily. Through structure-guided design of MD-224 analogue PROTACs, and screening using TR-FRET for CRBN-complexes, potent PXR degraders were identified that lacked off-target MDM2 and GSPT1 activity.

Perhaps serendipitously the authors identify a 'noncanonical' binding mode for promiscuous PROTAC MD-224 and take advantage of this to improve SAR analyses and gain selectivity for a subfamily of nuclear receptors. This publication is a useful exercise in interrogative mechanistic and chemical biology techniques to find out the why and how a degrader displays unexpected functionality. However, the difficulties of reliably detecting endogenous PXR by proteomics limit conclusions on whole-proteome off-target effects for their compound, and without the crystal structure of MD-224 in complex with PXR, the exact binding mode remains to be determined.

PRE-PRINTS

| *Lianne*

bioRχiv

Cysteine availability tunes ubiquitin signaling via inverse stability of LRRC58 E3 ligase and its substrate CDO1

*Gisele A. Andree§, Luca Stier§, …, Brenda A. Schulman**

In this study active cullin-RING ligase (CRL) profiling and quantitative proteomics revealed the inverse relationship between LRRC58 and CDO1, adding to the number of studies supporting that CDO1 degradation is a proteasomal process. The substrate receptor LRRC58 can form an active CRL complex with Cul2 as well as Cul5, both of which ubiquitinate CDO1 at Lys8. The authors went on to solve the CryoEM structure of two CDO1/LRRC58/CRL complexes, making this the first study to report the structure of these complexes.

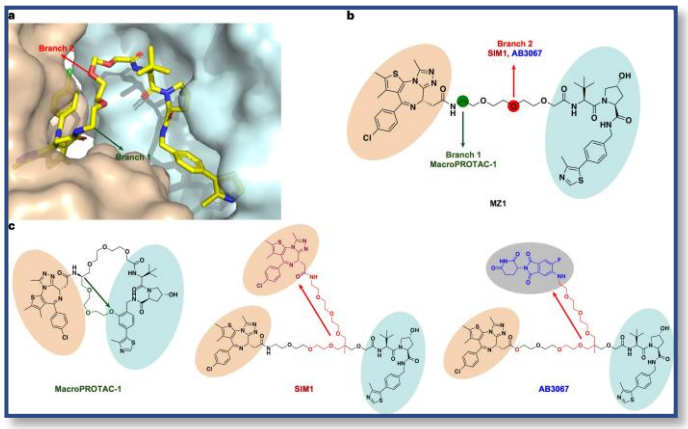
PAPERS AND PRE-PRINTS FROM CeTPD

| *Yuting & Alexandra*

Branching beyond bifunctional linkers: synthesis of macrocyclic and trivalent PROTACs

Yuting Cao§, Alexandra L. Harris§ and Alessio Ciulli*
Nat Protoc. **2025**

CeTPD authors: Yuting Cao, Alexandra L. Harris and Alessio Ciulli



In this protocol, we detail the synthesis of macrocyclic and trifunctional linkers. We report our pioneering rationally-designed macrocyclic PROTAC macroPROTAC-1, trivalent PROTAC SIM1, and more recent heterotrivalent PROTAC AB3067. These explore designs beyond the conventional bifunctional compounds, with the potential to expand the scope of targeted protein degradation and other proximity-based pharmacology. In this work, we describe the step-by-step synthesis of macroPROTAC-1 and SIM1, detailing the generation of the macrocyclic and trivalent cores and their subsequent conjugation to the respective ligands. These designs aimed to enhance protein degradation by constraining the PROTAC in its bioactive conformation or increasing avidity and cooperativity within the PROTAC ternary complex by augmenting the binding valency to the target protein, respectively. This two-part procedure is expected to take ~14 d for the synthesis of macroPROTAC-1 and 10 d for the synthesis of SIM1. In this protocol, we also provide a brief introduction into the biophysical and cellular evaluation of these unusual molecules, representative structures of key negative control compounds and their utility, and highlight recent developments and expansion beyond pioneering exemplars.

| *Charlotte and Lianne*

bioRχiv

LRRC58 defines an E3 ubiquitin ligase complex sensitive to cysteine abundance

Dylan Ramage§, …, *Richard Timms**

CeTPD authors (past and present): Lianne H. E. Wieske, Charlotte Crowe, Mark A. Nakasone, Kevin Haubrich, Mark Dorward and Alessio Ciulli

This paper brings us one step closer to understanding the cysteine regulation through saturation mutagenesis stability profiling. The authors show that CDO1-levels inside the cell are regulated in response to extracellular cystine concentration. One of the key findings is that the substrate receptor LRRC58 (which can form a cullin-RING E3-ligase complex with both Cul2 as well as Cul5) is both responsive to extracellular cystine levels and able to degrade CDO1.

OTHER PAPER HIGHLIGHTS

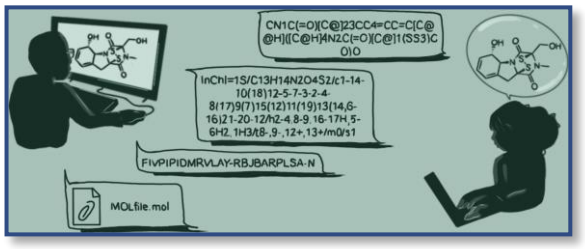
CHEMISTRY | STRUCTURAL BIOLOGY & BIOPHYSICS | CELL BIOLOGY | MODELLING

| *Lianne*

Machine-Readable Structural Information Is Essential for Natural Products Research

Karin Steffen, Nicholas H. Oberlies*, Antonis Rokar**

J. Nat. Prod. ***2025****, 88, 2815−2821*



How and where chemical structures of secondary metabolites are deposited and reported varies wildly. Despite the vast amount of structural data being avaible, the inconsistent (and sometimes ambiguous) ways in which these structures are reported complicates data-mining. The authors of this paper encourage researchers to publish, and journals to require, the reporting of an InchKey with novel secondary metabolites, which will fasciliate "big data" in the future.

This paper showcases the importance of transparent and consistent data reporting and how thinking beyond our own field will enable our work to make the greatest impact. Depositing raw data and using standardised data formats will allow science to progress more quickly.

TPD WORDSEARCH

(FOR YOUR COFFEE BREAK)

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