CeTPD Journal Club

August – September 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

AITANA DE LA CUADRA BASTE

Aitana is originally from Valencia, Spain. In July 2022 she completed her bachelor's degree in biotechnology, at the Polytechnical University of Valencia. As part of her thesis placement under Dr. Alejandro Ferrando, she studied the interaction between plant translation factors and autophagy-related proteins. She moved to Dundee and joined the Ciulli group in September 2022 for an Erasmus Internship for 9 months, and in September 2023 she started her PhD in the Ciulli group.

𝕏: @DeLaCuadraBaste
LinkedIn: www.linkedin.com/in/aitana-de-la-cuadra-baste

NIKKI MURDOCH

Nikki completed her undergraduate degree in Chemistry with Drug Discovery at the University of Strathclyde in 2020. She then moved to the University of St Andrews to undertake a PhD as part of the EaSI-CAT programme working with Dr Craig Johnston. Following her PhD, she joined the CeTPD in September 2024 working as a medicinal chemist on the AC-BI team

𝕏: @_nicolamurdoch
LinkedIn: https://www.linkedin.com/in/nicolamurdoch1/

MICHAEL KRUMMHAAR

Michael studied biomedical sciences and biochemistry in the University of Reutlingen and Tuebingen, before joining the group of Christian Roth at the Max-Planck Institute of colloids and Interfaces for his PhD. Here he focused on the functional and structural characterisation of glycosyltransferases involved in the synthesis of antimicrobial peptides using automated peptide synthesis, x-ray crystallography and biophysical methods. In 2024, he was awarded his Doctorate in Biochemistry from the Free University of Berlin for this work. He joined the CeTPD in the same year.

QINGZHI ZHANG

Qingzhi received her BSc and MSc degree from China. She had been teaching Organic Chemistry at Henan Normal University before pursing PhD under the supervision of Prof Derek Woollins at St Andrews. She stayed in St Andrews working with the late Dr. Nigel Botting as postdoctoral research associate on food chemistry and 13C-labelling. She then sprinted to industry at EPP for one year, and returned to St Andrews working with Prof David O'Hagan on fluorine chemistry. She joined the AC-BI team in Nov 2022.

FEATURE OF THE MONTH

Happening at CeTPD.. Dundee Science Centre Explorathon Event

A small representation of the CeTPD (Roberta, Ruben, Aitana, Maria R-R, Jon, Alessandra, and Giorgia) helped at the Explorathon outreach event at the Dundee Science Centre for the **European Researchers' Night 2025**. It was a very busy day, with around 400 attendees at the event. Lots of keen people visited our stand to learn about our work at CeTPD, either by chatting to one of us, or by playing the hook the bad protein, or ubiquitinate the target game!

Here are some quotes from the audience:

"The kids have had an amazing time. This is the first time we visited the Science Centre, and it was good that it was free."

"I liked chatting to all the scientists. I didn't know that so many people from all over the world do science in Dundee."

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"

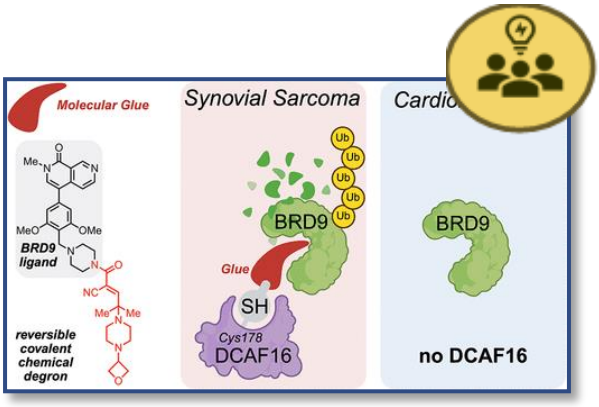
Literature review from 21st July to 20th September 2025

| *Aitana*

Discovery of BRD9 Molecular Glue Degraders That Spare Cardiomyocytes

Woong Sub Byun§, … Nathanael S.Gray*

J. Am. Chem. Soc., Article ASAP DOI: 10.1021/jacs.5c09857



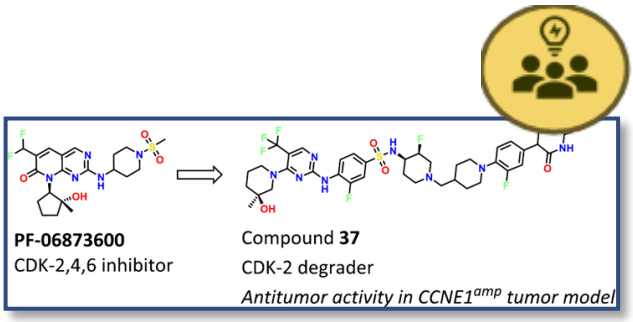
This paper reports the development of a new molecular glue degrader (MGD), **ZZ7**, that selectively degrades **BRD9**, a component of the SWI/SNF chromatin-remodelling complex implicated in malignancies such as synovial sarcoma. The authors applied a previously developed "chemocentric" approach, functionalising a potent BRD7/9 ligand with a library of covalent chemical degrons. An initial hit, ZZ6, bearing a cyanoacrylamide warhead, showed weak BRD9 degradation. Further optimization led to ZZ7, which incorporates a piperazinyl-oxetane moiety and showed improved potency. Competition experiments with excess warhead showed no rescue of BRD9, supporting that ZZ7 acts as a molecular glue degrader. **DCAF16** was identified as the recruited E3 ligase, and compound-dependent ternary complex formation was validated using FLAG immunoprecipitation, a TR-FRET assay, and complementary reporter assays. ZZ7 covalently engages Cys178 on DCAF16, in contrast to previously reported DCAF16 BRD4 MGDs, which target Cys58. Importantly, ZZ7 selectively degrades in disease-relevant cells, with minimal effect on human iPSC-derived cardiomyocytes, likely due to a lower expression of DCAF16 in these cells. This tissue-specific BRD9 degradation overcomes the cardiac toxicity commonly observed with conventional BRD9 PROTACs.

This study describes the discovery of a BRD9-selective MGD, that achieves potent efficacy while minimizing cardiotoxicity, overcoming an issue in the current treatment options. It is worth mentioning that another MGD of BRD9 via DCAF16 was described previously in a preprint, AMPTX-1. I found it particularly interesting that ZZ7 engages Cys178 on DCAF16, whereas AMPTX-1 and BRD4 MGDs engage different cysteine residues (mainly Cys58). This emphasises how the same ligase can mediate different glue interactions depending not only on the target protein but also on the degrader scaffold.

| Qingzhi

Discovery of Selective and Orally Bioavailable Heterobifunctional Degraders of Cyclin-Dependent Kinase 2

Philip N. Collier§, … Nicholas P. Kwiatkowski*
J. Med. Chem. ***2025***, *68, 17, 18407−18422*



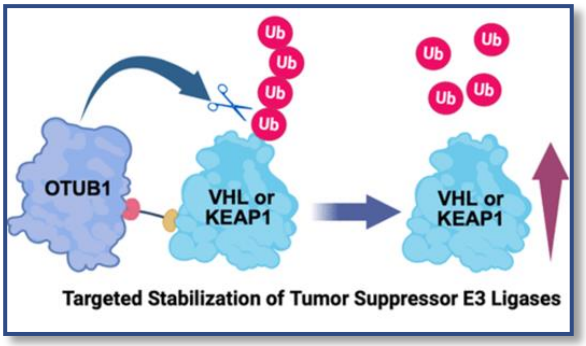
The authors reported a CDK2 selective and oral bioavailable degrader. They started from modifying the known sulfonamide CDK1,2,5 and CKD2,4,6 inhibitors to get highly CDK2-selective binders as a PROTAC building block. Several series of PROTACs were obtained by variation of the linkage, modification of the CRBN and the CDK2 binder. The optimised CDK2-selective and oral bioavailable degrader **37** exclusively down regulates the CDK2 in proteomic analysis. It demonstrated improved phenotypic selectivity compared to a clinical CDK2 inhibitor, with greater specificity for disease-relevant cyclin E1 (CCNE1)-amplified cancer cells vs. nonamplified cohort. The antitumor activity of **37** in mice bearing CCNE1-amplified HCC1569 tumors correlated with sustained >90% degradation of CDK2 and sustained 90% inhibition of Rb phosphorylation.

This work is well designed based on structure. They provided a clear rationale for each round of optimisation, addressing specific challenges along the way- such as improving selectivity, reducing microsomal intrinsic clearance, mitigating CYP3A4 inhibition liability, and enhancing oral bioavailability. There are no toxicity data available, but they mentioned that the preclinical safety study will be reported later.

| Qingzhi

Deubiquitinase-Targeting Chimeras Mediated Stabilization of Tumor Suppressive E3 Ligase Proteins as a Strategy for Cancer Therapy

Li Chen§,…, Jian Jin*, Wenyi Wei*
J. Am. Chem. Soc. ***2025***, *147, 29875−29883*



Targeted protein stabilization has emerged as a promising therapeutic strategy to combat various diseases related to aberrant protein degradation. In this paper, the authors employed deubiquitinase targeting chimeras (DUBTAC) to stabilize tumor-suppressive E3 ligase VHL (naturally degrading HIF) and KEAP1 (negatively regulating NR) by avoiding them from ubiquitination and degradation *via* UPS pathway. They developed two series of PRO-DUBTACs, VHL-DUBTAC and KEAP1-DUBTAC, through conjugation corresponding E3 ligase binders with a small-molecule ligand of the deubiquitinase OTUB1 *via* a linker. The DUBTACs could effectively stabilize the tumor-suppressive E3 ligases VHL and KEAP1 in cells, respectively, in an OTUB1-dependent manner to retard tumor cell growth.

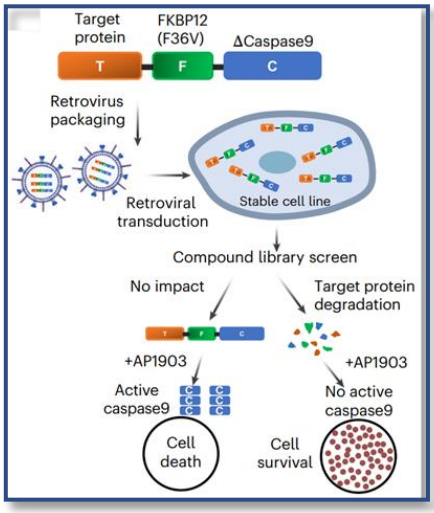
Ubiquitination and deubiquitination work together in a dynamic balance to maintain protein homeostasis. Degradation of disease-causing proteins through ubiquitination mediated by PROTACs has strived into clinic. This work illustrated an alternative approach: deubiquitinating E3 ligases to maintain their appropriate levels, thereby downregulating their client tumor-related proteins through UPS. This work demonstrated the concept using DUBTACs built from an OTUB1 ligand, two E3 ligase binders and simple linkers. It would be interesting to see more work in this area involving other POIs and a greater variety of linkages.

| *Qingzhi*

A rapid imaging-based screen for induced-proximity degraders identifies a potent degrader of oncoprotein SKP2.

Yankai Chu§,...Hai Jiang*
Nat. Biotechnol. https://doi.org/10.1038/s41587-025-02793-8

In this work, the authors developed a high throughput screening system, DEath FUSion Escaper (DEFUSE), to identify small-molecule protein degraders. They fused the POI with death protein FKBP12-F36V-ΔCaspase9 (F-C) and expressed POI-F-C conjugate in HEK293 cells by retroviral transduction. When the resultant cells are treated with a library of small molecules, the active degrader will eliminate the POI along with the F-C fusion. The cells deprived of suicidal POI-F-C protein will survive while those with abundant POI-F-C will die rapidly upon treatment with AP1903. The survival versus death imaging provides clear-cut readouts. Through this method, they swiftly discovered a degrader SKPer1 for oncoprotein SKP2. Based on the same imaging screen, they pinpointed SKP2's degradation partner STUB1 by RNAi knock-down of ~600 genes involved in ubiquitination and degradation. SKPer1 acts as an induced-proximity degrader by inducing interaction between SKY2 and STUB1.

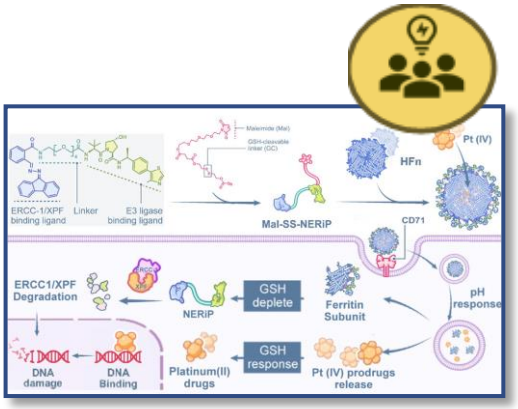


High-throughput screening of degrader compounds remains challenging due to the low visibility of efficacy at the endpoint. The authors creatively employed the apoptosis mechanism to devise a simple and effective black-and-white type protein degradation screening system. I am really impressed by the work and find the name of DEFUSE and SKPer very imaginative and vivid.

| Nikki

Ferritin-Conjugated PROTAC Strategy for ERCC1/XPF Degradation and Platinum Sensitization in Resistant Tumors

Shenghui Wang§…Xiyun Yan*, Bing Jiang*
J. Med. Chem. **2025**, 68, 18, 19002–19021



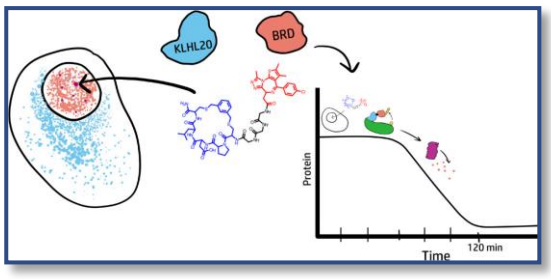
This study presents a dual-delivery nanosystem, HFn-NERiP-Pt(IV), designed to overcome cisplatin resistance, which is frequently driven by enhanced DNA repair. The system combines a glutathione-responsive PROTAC (NERiP) with a ferritin nanocarrier to enable targeted degradation of ERCC1/XPF and improved delivery of a platinum(IV) prodrug. NERi, previously reported as a nucleotide excision repair (NER) inhibitor, was computationally predicted to interact with ERCC1 and XPF, leading to its conversion into a PROTAC. Among several linker variants, P4-NERiP emerged as the most effective degrader of ERCC1/XPF. Proteasome inhibition studies confirmed degradation via the ubiquitin–proteasome system. Incorporating P4-NERiP into the ferritin nanocarrier alongside Pt(IV) resulted in enhanced DNA damage and tumour growth inhibition in cisplatin-resistant esophageal squamous cell carcinoma models.

This paper offers a promising approach for treating drug-resistant cancers. The authors provide robust data showing that this strategy effectively sensitises resistant cells to cisplatin while maintaining a favourable safety profile. It was particularly interesting to see PROTACs used in a synergistic system to overcome therapeutic resistance and enhance the efficacy of an already widely used chemotherapeutic.

| Nikki

Temporal and Spatial Characterization of CUL3^{KLH20}-Driven Targeted Protein Degradation of BET Family BRD Proteins by the Macrocycle-Based Degrader BTR2004

Phoebe H. Fechtmeer§, Cameron Martinez, Johannes T.-H. Yeh*
ACS Chem. Biol. ***2025****, 20, 9, 2056–2062*



It is of wide interest to expand the targeted protein degradation (TPD) landscape beyond the well-characterized CRBN and VHL E3 ligases. Previous studies validated CUL3^{KLHL20}, a BTB-Kelch family adaptor protein broadly expressed in cancer, as a viable E3 ligase for TPD via the development of BTR2000, a selective macrocyclic peptide ligand targeting the KLHL20 Kelch domain. Attachment of BTR2000 to JQ1 via a peptide linker identified BTR2004, a potent and selective degrader of BET family proteins, particularly BRD2. This represents the first KLHL20-engaging PROTAC. This study outlines the temporal and spatial profiling of BTR2004 and reveals this PROTAC demonstrates rapid cellular entry and efficient BRD2 degradation. These findings underscore the importance of subcellular localisation in PROTAC

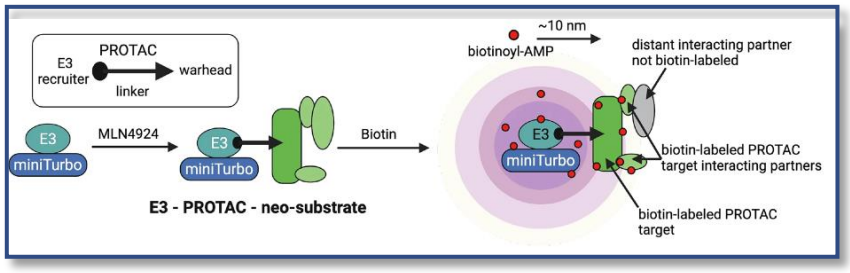
function and highlight the potential of KLHL20-recruiting PROTACs as a new modality for proximity-induced protein degradation.

This is an interesting study that demonstrates how BTR2004, a macrocyclic peptide–based PROTAC with a molecular weight exceeding 1200 Da, can achieve efficient cellular permeability, challenging classical Lipinski constraints. The work highlights the emerging therapeutic potential of bRo5 (beyond Rule-of-Five) degraders and supports the viability of macrocyclic scaffolds in PROTAC design. It will be particularly intriguing to see whether future studies can optimise BTR2004 or related compounds to improve pharmacokinetic properties, especially with the aim of achieving oral bioavailability.

| *Aitana*

Characterization of PROTAC specificity and endogenous protein interactomes using ProtacID

Suman Shrestha§..., Cheryl H. Arrowsmith*, Brian Raught*
J. Med. Chem. **2025**, 68, 18, 19002–19021



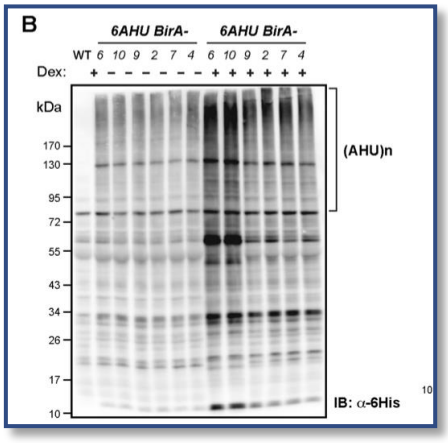
Studying the specificity of degraders has been difficult. The most common method is global proteome analysis, which identifies changes in protein abundance upon treatment. However, observed decreases in protein levels can occur indirectly due to the loss of interacting partners, while some recruited proteins may not be degraded but may still exhibit changes in activity, localization, or interactions. This study introduces ProtacID, a method that directly maps PROTAC-induced proximity interactions in living cells. The authors fused a miniTurbo biotin ligase to an E3 ligase (VHL or CRBN) and treated the cells with a neddylation inhibitor, DMSO, or the PROTAC, along with biotin. The biotinylated proteins were pulled down and analysed by mass spectrometry. This system allows the identification of PROTAC-mediated interactions, both productive (degraded) and non-productive. Using ACBI1 as a model, the authors confirmed known substrates including SMARCA2, SMARCA4, and PBRM. They also showed that ProtacID can distinguish between different BAF complex assemblies recruited by distinct PROTACs. They also identified a non-productive interactor, KIF20B. The method was further validated using $CRBN^{midi}$-based degraders and tested across additional target systems and cell lines.

ProtacID is a powerful tool to dissect degrader specificity and to explore how protein complexes are "re-wired" by degraders. I think this could be particularly interesting when applying degraders to new protein complexes or when testing alternative E3 ligases, since the method could reveal unexpected recruitment patterns or non-productive interactions that standard proteomics might miss. This represents an emerging concept, as a comparable approach has been recently employed using AirID in place of MiniTurboID to investigate PROTAC-induced interactions.

| *Michael*

Biotinylation Interferes with Protein Ubiquitylation and Turnover in Arabidopsis—A Cautionary Insight for Proximity Labelling in Ubiquitylation Proteome Studies

Yang Li§, Peifeng Yu, Zhihua Hua*
Int. J. Mol. Sci. **2025**, 26, 8248



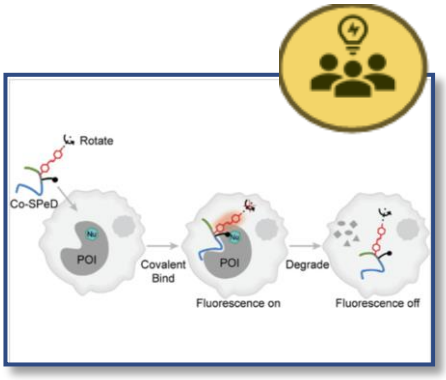
TurboID and other methods relying on biotinylation are a promising approach to discover natural targets of E3 ligases. Here, the authors set out to use this powerful method on the Skp1-cullin1-F-box ligase of *Arabidopsis Thaliana*. However, the authors report that such approaches aren't always straightforward in probing E3 ligases as the act of biotinylating the target protein interferes with the homeostasis of the modified plant cells. The authors demonstrated that this occurs as a combination of two factors: first, biotinylated proteins appeared to be more stable. The precise mechanism of this stability is unknown, though the authors suggest that this may be related to a disruption of the proteasomal degradation. Secondly, high levels of biotinylated ubiquitin were found to be developmentally lethal. Combined, these problems made it impossible to accurately probe this Scf complex in plant cells with TurboID. As an alternative approach, the authors suggest to use a TurboID based tag on a known substrate of the ligase, instead of the ligase itself.

This paper urges caution when attempting to probe the interactome of E3 ligases by proximity-based biotinylation methods. While the study focuses exclusively on plants, a similar effect may also occur in mammalian cells as well. In this manner, this paper supplements the ProtacID publication featured in this issue of the journal club

| *Michael*

A Covalent Self-Reporting Peptide Degrader Enables Real-Time Monitoring of Targeted Protein Degradation In Vivo

Wei Zhang§, …, Xiaoding Lou*
Journal of the American Chemical Society **2025** 147 (31), 27862-27875



During the degradation of a target protein, monitoring the remaining concentration of said target can be informative for ongoing treatments or for the development of better molecules. While the use of protein tags enables detection of the POI in real time, fusing such a reporter tag to the protein can alter its function, localization, and potentially its turnover. Here, the authors set out to develop a self-reporting PROTAC by utilising a rotor-fluorophore, whereby binding of the fluorophore to a protein decreases its rotational freedom and leads to an increase in its fluorescence. Based on molecular docking models, the authors developed a peptide-based covalent degrader

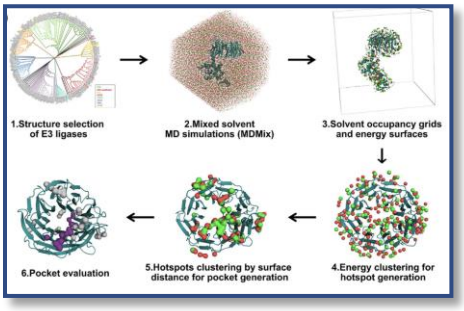
targeting MDM2, BCL-xL, GRP78 and KRAS(G12D), enabling the observation of protein degradation in real time, both *in vitro* and, notably, in living mice.

This paper shows an intriguing approach to observe TPD in real time with low background. However, further work will be needed to make this approach broadly applicable, as the current use of a fully peptide-based covalent degrader may limit its generalisability.

| *Michael*

Discovering Uncharted Binding Pockets on E3 Ligases Leads to the Identification of FBW7 Allosteric Modulators

Míriam Martínez-Cartró§, …, Carles Galdeano*
Adv. Sci. **2025**, e06068



While PROTACS have shown great promise, most of the degraders designed so far have been developed based on only two E3 ligases: VHL and CRBN. While these ligases have demonstrated broad promiscuity with regards to the degradable targets, expanding the arsenal of ligandable E3 ligases could be beneficial to help reduce the constraints in chemical space and enable built-in tissue specificity. At the same time, current research into finding new ligandable E3 ligases focuses mainly on the natural degron binding site. Here, the authors demonstrate a computational method to discover non-orthosteric binding pockets in E3 ligases. They use FBW7 as a proof-of-concept ligase and show that the molecule they designed based on their predictions can bind to this protein within the predicted pocket. Intriguingly, the designed binder appeared to act as an allosteric modulator of FBW7, increasing the degradation of c-MYC and c-Jun.

This paper shows an interesting computational approach to discover new binding sites in E3 ligases, though there is no reason to suspect it won't work for other proteins as well. While the methods section provides a general overview, additional detail would have been helpful for fully assessing and reproducing the experiments. Nevertheless, this approach may make the development of non-orthosteric PROTACS more straightforward. The FBW7 allosteric modulator itself requires rather high concentrations and has relatively low affinity, though its function of increasing the degradation of a challenging target, such as c-MYC, does show promise.

PRE-PRINTS

| *Nikki*

Targeted degradation of pathologic tau aggregates via AUTOTAC ameliorates tauopathy

Jihon Lee§ ,..., Sung Tae Kim, Chang Hoon*, Maja Zakošek Pipan*, and Yong Tae Kwon**

Several neurodegenerative diseases are associated with the misfolding and aggregation of tau, presenting it as an interesting target for drug discovery. This paper outlines the development of ATB2005A, a BBB-penetrating oral drug currently in Phase I clinical trials in Korea, as an AUTOTAC that simultaneously binds tau and the autophagic receptor p62/SQSTM1, leading to degradation of tau. Overall, this is an interesting paper that highlights the potential of AUTOTAC's.

| *Michael*

Predicting PROTAC-Mediated Ternary Complexes with AlphaFold3 and Boltz-1

*Nils Dunlop§, Francisco Erazo, Farzaneh Jalalypour, Rocío Mercado**

Predicting the structure of a PROTAC-mediated ternary complex remains a challenge. Two AI-based prediction software have been released recently that have the technical capability to predict these complexes: Alphafold 3 and Boltz. This paper compares these two algorithms with the "ground truth" of solved structures. While the use of Boltz-1 is now outdated following the release of Boltz-2 earlier this year, this paper nonetheless demonstrates that, despite considerable deviations of the PROTAC poses from the solved structures, the overall positioning of the POI relative to the E3 substrate-binding domain can still be accurate with an RMSD <4.

PAPERS AND PREPRINTS FROM CeTPD

| *Alejandro and Valentina*

bioRχiv

Dual E3 ligase recruitment by monovalent degraders enables redundant and tuneable degradation of SMARCA2/4

Valentina A. Spiteri§, Dmitri Segal§, Alejandro Correa-Sáez§,..., Alessio Ciulli*, Georg E. Winter*

CeTPD authors (past and present): *Vaentina A. Spiteri, Dmitri Segal, Alejandro Correa-Sáez, Kentaro Iso, Ryan Casement, Mark A. Nakasone, Gajanan Sathe, Hannah E. Peters, Mark Doward, Angus D. Cowan, Alessio Ciulli*

In this preprint, in collaboration with Georg Winter´s lab, we characterised monovalent SMARCA2/4 degraders that recruit two E3 ligases (DCAF16/FBXO22) in a redundant fashion. To decipher this intriguing mechanism, we combined a suite of in-cell and in vitro techniques, including CRISPR screens, DMS, in-cell proximity labelling, TR-FRET, intact MS, and Cryo-EM. Notably, this multi-disciplinary setup allowed us to show that E3 ligase preference can be chemically and genetically tuned, either by altering the "degradation tail" of the compound, or by introducing mutations in the E3 ligase.

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School of Life Sciences
1 James Lindsay Place,
Dundee,
DD1 5JJ

Journal club – website

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