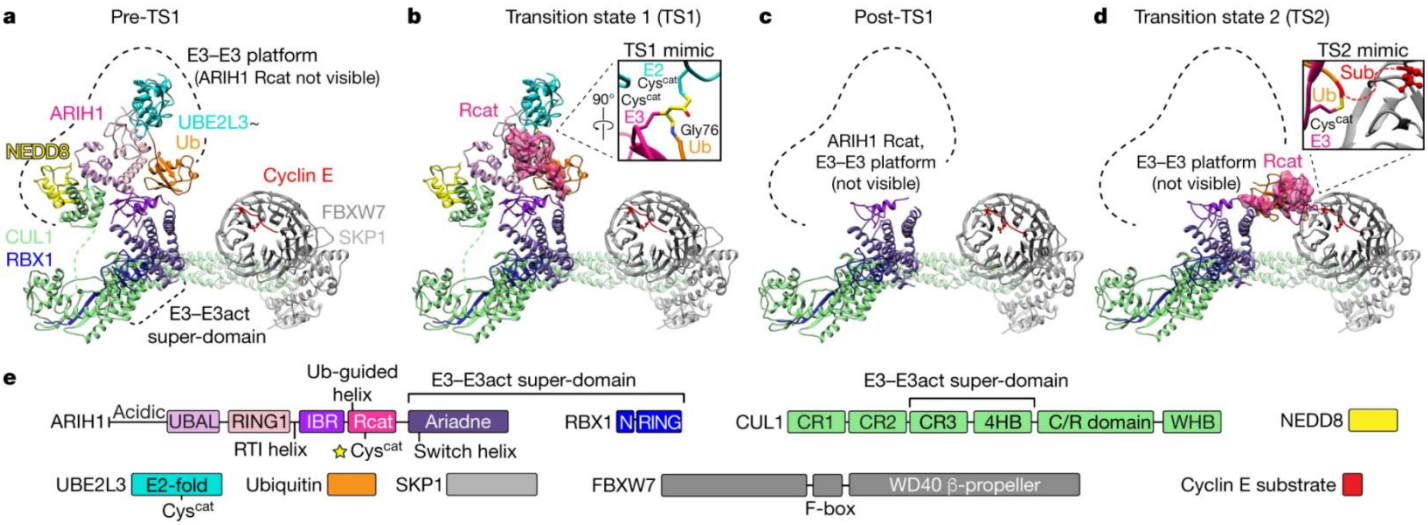

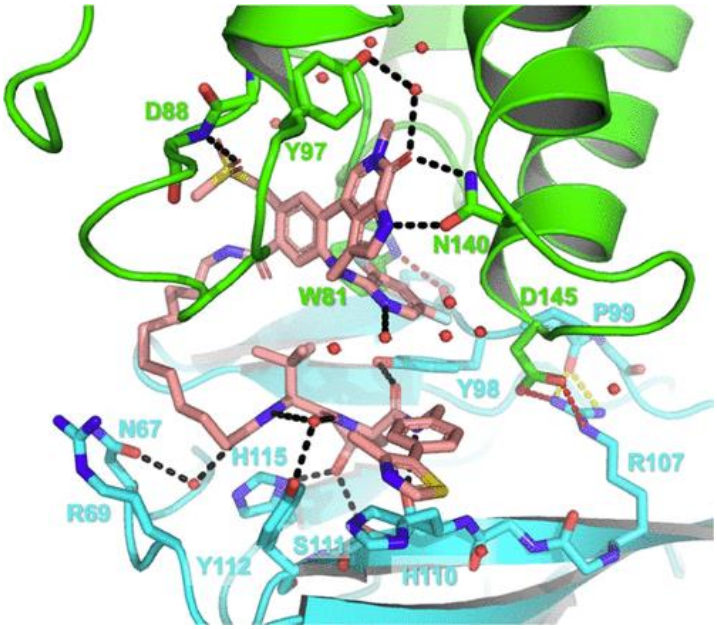

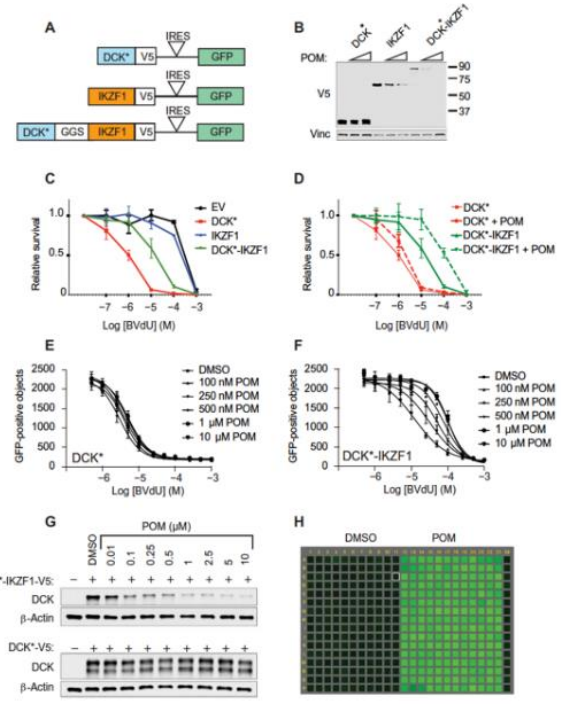

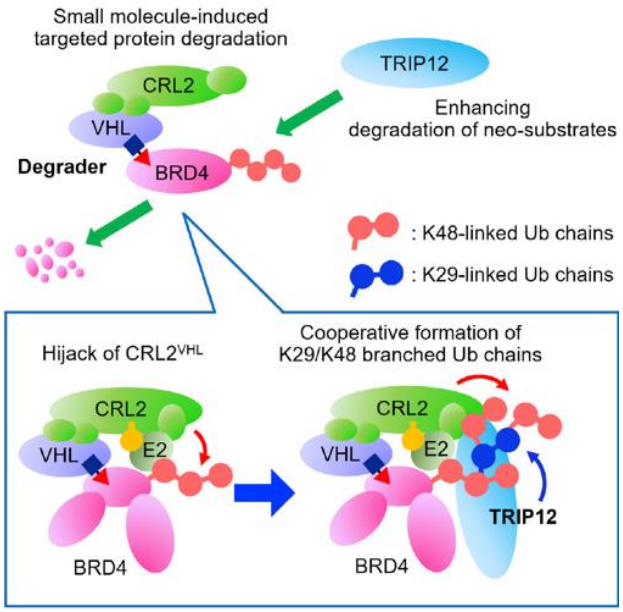

Ciulli Group Journal Club

Targeted Protein Degradation,
Medicinal Chemistry and
Chemical Structural Biology
Literature Highlights

February 2021 Edition

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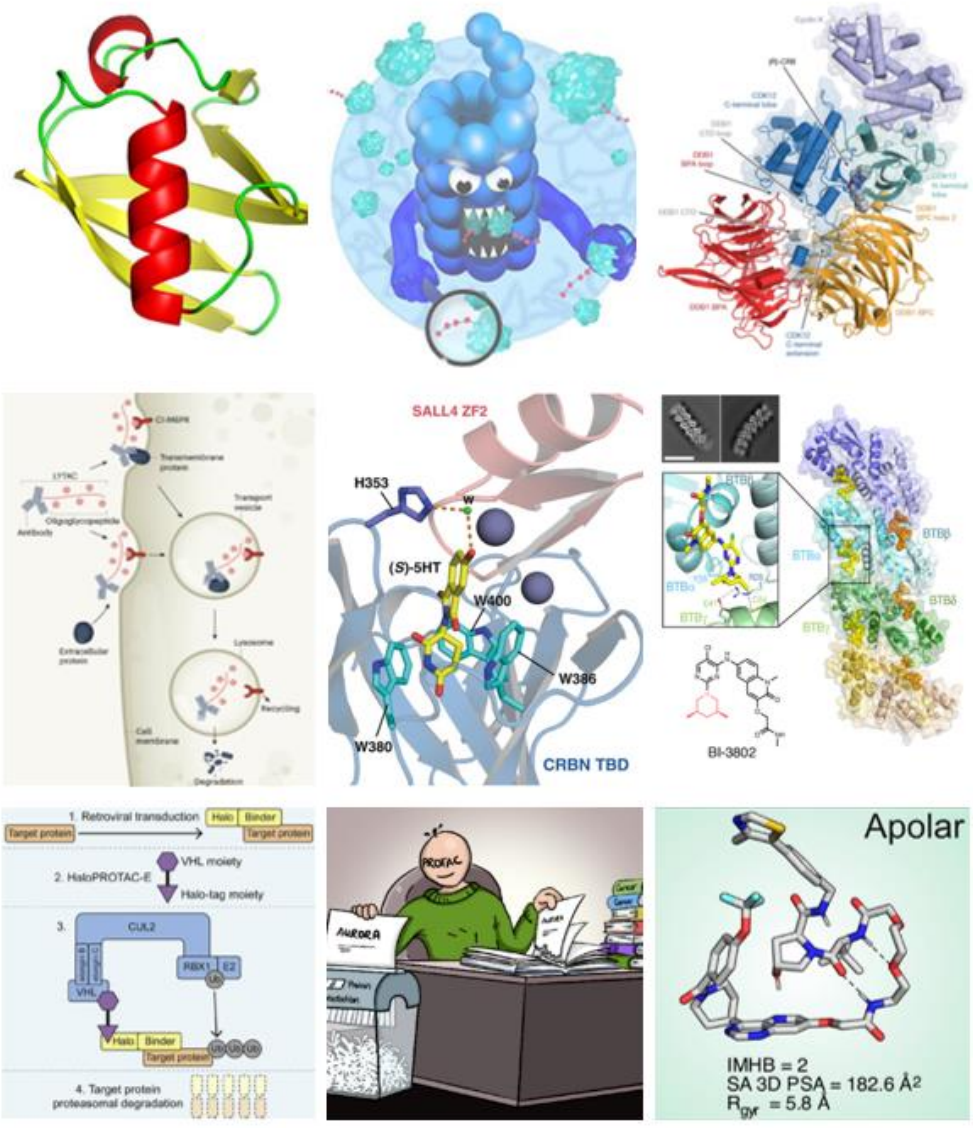
Feature of the Month

Our Journal Club survey is still open!

https://forms.office.com/Pages/ResponsePage.aspx?id=OTEyrjoJKk2Bpl0zS82QGe58NyjrjHVJszyhwkrfbqZUNlNMTkpJWUVNRkY5RlhZS05LWktBQlhPWi4u



We wanted to thank all those who have filled out the Journal Club survey. We have been enjoying reading through your fantastic feedback and suggestions which will help shape the future of the Journal Club.

If you read the Journal Club and have not yet given feedback, please consider doing so! The survey only takes a couple of minutes (at most) to complete. All responses are anonymous. We would love to hear what you think!

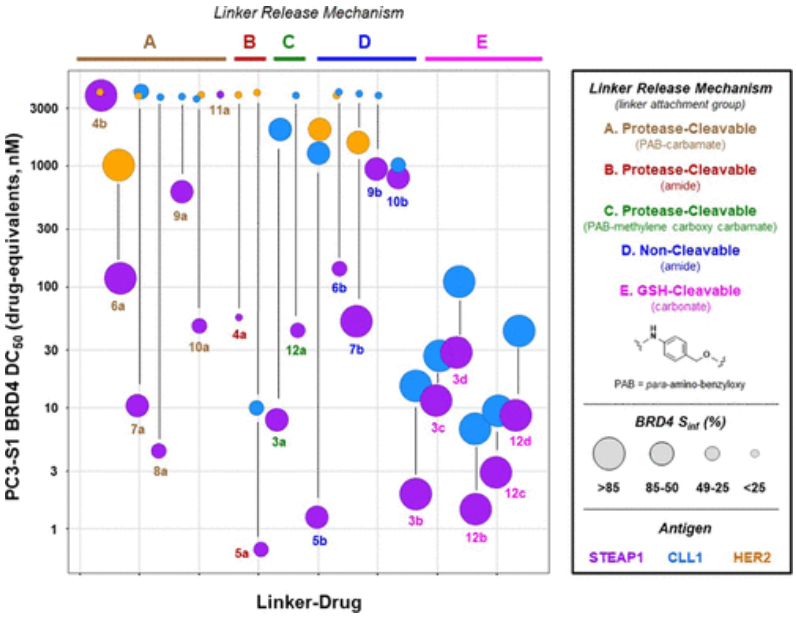
Targeted Protein Degradation

Contributor: Ryan

Antibody-Mediated Delivery of Chimeric BRD4 degraders. Part 1: Exploration of Antibody Linker, Payload Loading, and Payload Molecular Properties

Peter S. Dragovich[§]*, …, Xiaoyu Zhu

J. Med. Chem. **2021**, DOI: 10.1021/acs.jmedchem.0c01845



This paper describes the first of a two-part story detailing the development of antibody-drug conjugates (ADCs) carrying a BRD4 degrader payload. Starting with the known degrader MZ1 the authors describe a systematic exploration of antibody linkage (cleavable/non-cleavable), type of cleavage, vector and PROTAC vector/linker composition. In addition to conjugation to a STEAP1 antibody (STEAP1 is a cell surface antigen overexpressed in many cancers, especially prostate), HER2 and CLL-1 antibodies were also conjugated as a control as these proteins are not expected to be present at high levels in the selected PC3-S1 prostate cancer cell line. It was found that the PROTAC 'scaffolds' (without ADC linker) had a range of DC_{50}s which correlated well with their ternary complex $t_{1/2}$s with (BRD4 BD1 + BD2) as determined by SPR, a correlation which was improved when accounting for predicted cell permeability. The authors tested their hypothesis that poor degrader permeability could be overcome by antibody conjugation by including a sulfonic acid group on one of their degrader scaffolds. Even when antibody-conjugated, this was found to be completely inert – raising an important question about the requirements for payload permeability in ADCs.

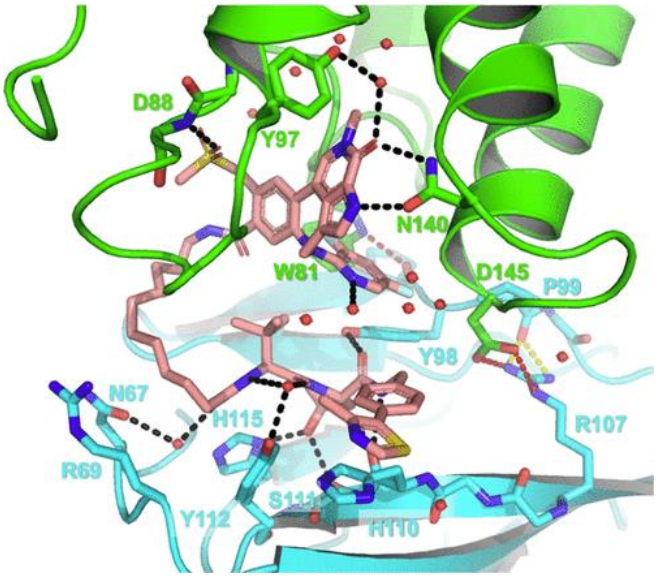
Ultimately it was found that although many of the conjugates could degrade BRD4 (albeit with suboptimal D_{max}) they were inactive in antiproliferation assays against the PC3-S1 cell line. While one of the conjugates STEAP1-8a-D6 offered antiproliferative effects in LNCaP cells, it was the only ADC to show this effect. The authors reflect that although the 'parent' PROTACs are cytotoxic, they are significantly weaker than typical ADC payloads which are generally in the low nanomolar to picomolar range. They thus identify increasing the degrader potency as well as the payload:antibody ratio as a key focus of the next phase of their research which is described in part 2. This paper is a great read and is packed with high quality data which can be visualised easily thanks to the well thought out figure design such as the one included here.

Contributor: Ryan

Antibody-Mediated Delivery of Chimeric BRD4 Degraders. Part 2: Improvement of In Vitro Antiproliferation Activity and In Vivo Antitumor Efficacy

Peter S. Dragovich[§]*, …, Xiaoyu Zhu

J. Med. Chem **2021**, DOI: 10.1021/acs.jmedchem.0c01846



As the second in the series describing BRD4 PROTAC ADCs this paper describes the attempt to improve the anti-proliferative effects of the conjugates. As described previously the focus was on first improving the potency of the PROTAC payload to be more in line with typical ADC cytotoxic payloads. The initial work reveals a move away from JQ1 to a more potent BRD4 targeting scaffold and the discovery of GNE-987 (compound **9**) an extremely potent BRD4 degrader. While the chemical difference between compounds **8** and **9** is essentially switching from a PEG to a carbon linker this provides a start potency difference resulting in a ~100x improvement in DC_{50}. The ternary crystal structure partially rationalises this, showing a hydrophobic collapse of the compound. Indeed, it appears that the formation of this ternary complex is so favourable the 'normal' VHL ligand binding mode is scrambled at one end. It is notable that in this study a good correlation between DC50 and ternary complex stability can only be achieved once an 'outlier' (compound **12**) is removed – even when normalised for permeability.

Ultimately when conjugated the properties of some of the most potent degraders caused aggregation issues when part of an ADC, an issue which required careful tuning of the antibody linker region. The authors present a large amount of data showing the high activity of the new and improved degrader ADCs in cells and *in vivo*, importantly with large separations between the active and control conjugates (evidence of successful targeting).

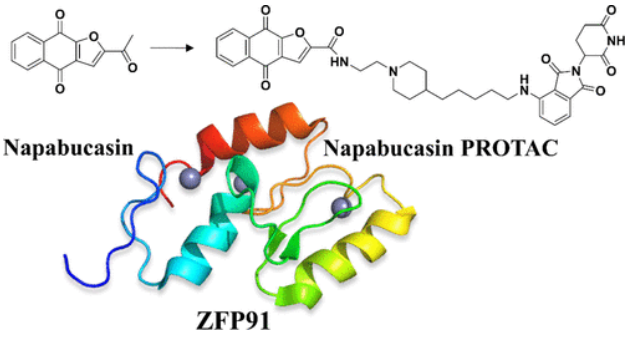
In these two papers the authors clearly establish antibody-mediated delivery as a viable option for PROTACs and provide a high level of detail and discussion on the optimization of the many variables present in these complicated modalities. The sheer volume of data in these papers is impossible to summarise adequately here and so careful reading is recommended!

Contributor: Ryan

Discovery of a Napabucasin PROTAC as an Effective Degrader of the E3 Ligase ZFP91

Maha Hanafi[§], Xinde Chen[§], Nouri Neamati*

J. Med. Chem. **2021**, *64*, 1626



This paper describes the synthesis and biological evaluation of PROTACs utilising napabucasin as a warhead, a compound which has orphan drug status for cancer indications and is thought to act through inhibition of the STAT3 signalling pathway. The authors describe their motivation to develop these compounds which was to understand the mechanism of action of napabucasin.

The paper then describes PROTAC optimisation based on cytotoxicity in two pancreatic cancer cell lines. The most cytotoxic compound XD2-149 (twofold increase in cytotoxicity vs. napabucasin) was taken for further mechanistic studies. Two VHL recruiting PROTACs are also evaluated which were not cytotoxic in the pancreatic cancer cell lines. On this evidence the authors suggest that VHL is therefore not optimal for the design of napabucasin-based PROTACs. XD2-149 reduced STAT3 expression in a proteasome-independent manner and inhibited cell growth in an MTT assay (3x more potent than negative control PROTAC). Following proteomics to aid target identification, 136 proteins were found to be dysregulated. Among the downregulated proteins was the E3 ligase ZFP91, a known IMiD dependent neo-substrate of CRBN, but when tested side by side for degradation of ZFP91, XD2-149 was found to be 5-fold more potent than pomalidomide. While the authors report that the cytotoxicity of XD2-149 is partially dependent on ZFP91, the cell death mechanism was found to be similar to napabucasin, via reactive oxygen species mediated by NQO1.

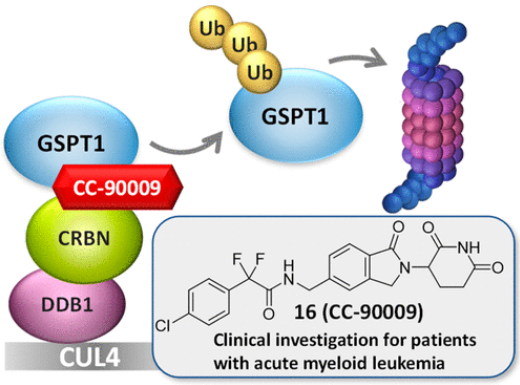
This work represents an extensive investigation into the mechanism of a tool PROTAC compound based on napabucasin. While the compound is effective in degrading ZFP91, it appears to also have cytotoxicity due to the usage of a napabucasin warhead. In further work it would be interesting to evaluate the minimum structural requirements for this enhancement in ZFP91 degradation vs. pomalidomide.

Contributor: Charlotte

CC-90009: A Cereblon E3 Ligase Modulating Drug That Promotes Selective Degradation of GSPT1 for the Treatment of Acute Myeloid Leukemia

Joshua D. Hansen[§]*, …, Antonia Lopez-Girona

J. Med. Chem. **2021**, DOI: 10.1021/acs.jmedchem.0c01489



CC-90009 is a molecular glue which recruits the CRBN E3 ligase and targets GSPT1 for the treatment of Acute Myeloid Leukemia (AML).

CC-885 had previously been reported as a potent and broad degrader against GSPT1 and IKZF3. However, this compound was considered unsuitable due to its lack of selectivity and cytotoxic activity, in particular against the non-tumorigenic THLE-2 cell-line. The authors build on their previous work by developing analogues of CC-885. Empirical SAR efforts showed that replacement of urea with a 2,2-difluoroacetamide component could drastically increase selectivity for GSPT1, leading to the creation of CC-90009.

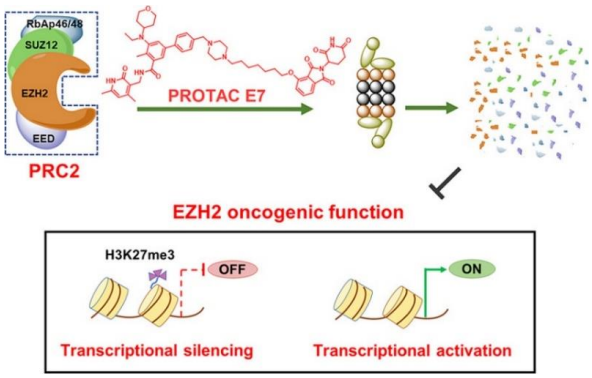
The drug has been in phase 1 clinical trials since 2020, and the manuscript includes a summary of pharmacokinetic and clinical efficacy data. Not only are the preliminary results promising, but the strategies leading to CC-90009 provide a pathway for development of other molecular glues targeting “undruggable” proteins.

Contributor: Ryan

Design and Synthesis of EZH2-Based PROTACs to degrade the PRC2 Complex for Targeting the Noncatalytic Activity of EZH2

Zhihao Liu[§], Xi Hu[§], …, Ningyu Wang*, Luoting Yu*

J. Med. Chem. **2021**, DOI: 10.1021/acs.jmedchem.0c02234



Despite the fact EZH2 is frequently overexpressed in many cancers the efficacy of inhibitors alone is limited. This paper describes the synthesis of EZH2-targeting PROTACs to abolish the noncatalytic activity of EZH2. The initial tool molecule (G12) described in this work decreased not only EZH2 levels but also EED and SUZ12, other members of the PCR2 complex. Following this early success, a library of CRBN-targeting PROTACs was generated exploring two different vectors from the EZH2 ligand. The ability to monitor the degradation of each subunit revealed interesting trends which were notably different depending on the compound series. Compound E7 resulted in reduction of H3K27me3 levels in a similar way to the parent inhibitor. However unlike the parent inhibitors, E7 was found to downregulate genes linked to cancer which are activated by EZH2 independent of its catalytic function, demonstrating their hypothesis that PROTACs could have an advantage over inhibitors in this way. The authors also highlight that previous publications have shown chemical knockdown of EED can lead to downregulation of EZH2 and SUZ12 due to the decreased stability of the PRC2 complex. To test this, EZH2 was knocked down by shRNA and from the results the authors hypothesise the decreased levels of SUZ12 may be due to the instability of the PRC2 complex, however they note that further evidence is required to ascertain the mechanism of the degradation of SUZ12, EED and RbAp48.

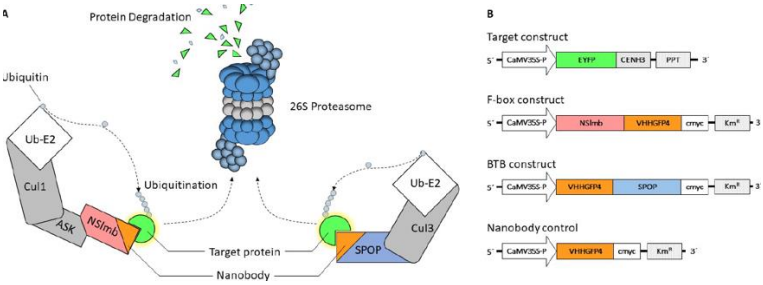
This paper has disclosed a valuable chemical tool E7 to investigate further the implications and potential benefits of abolishing the catalytic and noncatalytic activity of the PRC2 complex.

Contributor: Sarath

Engineered degradation of EYFP-tagged CENH3 via the 26S proteasome pathway in plants

Eberhard Sorge*, Dmitri Demidov, Inna Lermontova, Andreas Houben, Udo Conrad*

PLoS ONE **2021**, DOI: 10.1371/journal.pone.0247015



This work expands on the deGradFP work of Shin, Y.J. *et al.* (2015) and Caussinus, E. *et al.* (2012) on plants. This study reports 26S proteasomal degradation of target protein CENH3 of *A. thaliana* fused to EYFP via replacement of the interaction domain of the E3 ligase adaptor protein SPOP (Speckle-type POZ adapter protein) with a specific anti-GFP nanobody (VHHGFP4). The authors also created E3 constructs constituting nanobody swaps of WD40 substrate-binding domain of the F-box protein and evaluated their efficacy in degrading EYFP fused CENH3 protein. The modified SPOP BCR (BTB, Cullin, Rbx1) E3 ligase outperformed modified SCF (Skp1, Cullin3, F-box) E3 ligase in degrading nuclear localised CENH3. Modified SCF E3 ligase instead resulted in mislocalisation of CENH3.

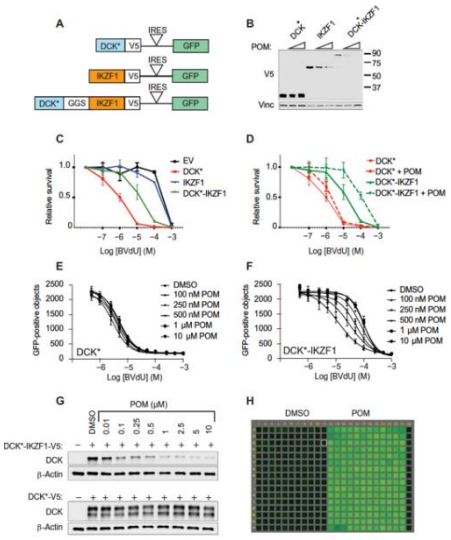
The deGradFP approach involving a genetically encoded method for direct and fast depletion of target green fluorescent protein (GFP) fusions overcome some limitations of classical RNAi-mediated knockdown of target proteins. RNAi-mediated downregulation is limited by their inefficiency at depleting long-lived proteins, their lack of immediate reversibility and their potential for off-target effects. But unlike the degron tags, deGradFP do not offer the option of time or dose dependent degradation of target proteins.

Contributor: Sarath

Targeting oncoproteins with a positive selection assay for protein degraders

Vidyasagar Koduri[§], Leslie Duplaquet[§] …, Matthew G. Oser*, William G. Kaelin Jr.*

Sci. Adv. **2021**, DOI: 10.1126/sciadv.abd6263



Traditional loss of signal ("down") assays for identifying degraders often exhibit poor signal-to-noise ratios, narrow dynamic ranges, and false positives from compounds that non-specifically suppress transcription or translation. This article establishes a new gain of signal ("up') positive selection screening method by fusing protein of interest (POI) to a modified version of deoxycytidine kinase (hereafter called DCK*) that converts the non-natural nucleoside 2-bromovinyldeoxyuridine (BVdU) into a poison and green fluorescent protein (GFP). Degraders of POI are expected to degrade fused DCK* promoting survival of DCK*-POI-GFP cells treated with BVdU. GFP is used to mark reporter-positive cells. As a proof-of-concept, the authors were able to develop a positive selection assay for IKZF1 degraders. With the help of high throughout screens, they were able to identify Spautin-1 as a novel degrader in addition to known IMiDs like LEN, POM, and avadomide. Interestingly, Spautin-1 mediated proteasomal downregulation of IKZF1 does not depend on cereblon or any other cullin based E3 ligase. Discovery of CDK2 as a regulator of ASCL1 highlights the ability of the platform to enable mechanism-agnostic searches for compounds and targets that regulate the abundance of previously undruggable proteins.

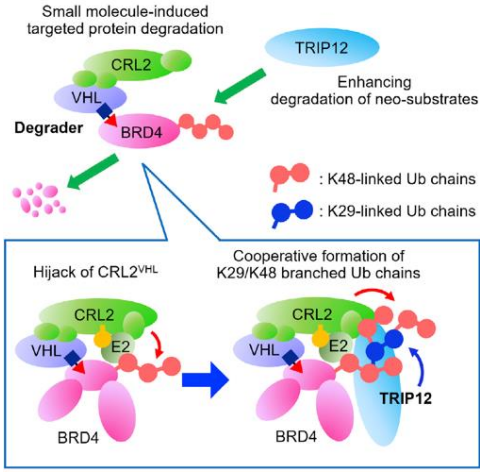
The positive selection assay developed in this article has the potential to accelerate the search of novel molecular glues which have largely been discovered serendipitously so far. The work has also been covered in the Dana-Farber TPD Webinar series.

Contributor: Sarath

TRIP12 promotes small-molecule-induced degradation through K29/K48-branched ubiquitin chains

Ai Kaiho-Soma[§], …, Fumiaki Ohtake*

Mol. Cell **2021**, *81*, 1



Small molecule degraders like PROTACs induce degradation of neo-substrates by hijacking recruited E3 ubiquitin ligases. In this article, a HECT-type E3 ligase, TRIP12 is reported to function as a coregulator of PROTAC-driven targeted protein degradation. TRIP12 associates with BRD4 in a PROTAC- and $CRL2^{VHL}$-dependent manner to facilitate the formation of K29/K48-branched ubiquitin chains. TRIP12 is also shown to promote $CRL2^{VHL}$ mediated assembly of K48 linkage. In order to explain the dispensable nature of TRIP12 in degradation of the $CRL2^{VHL}$ natural intrinsic substrate HIF-1α, the authors propose a distinction between optimal rapid polyubiquitination and degradation of natural substrates v/s sub-optimal slower polyubiquitination of neo-substrates. TRIP12 is proposed to amplify the ubiquitination rate and accelerate degradation of these neo-substrates. This study also expands the coregulatory role of TRIP12 to other degraders that target CRABP2 or TRIM24, or recruit CRBN.

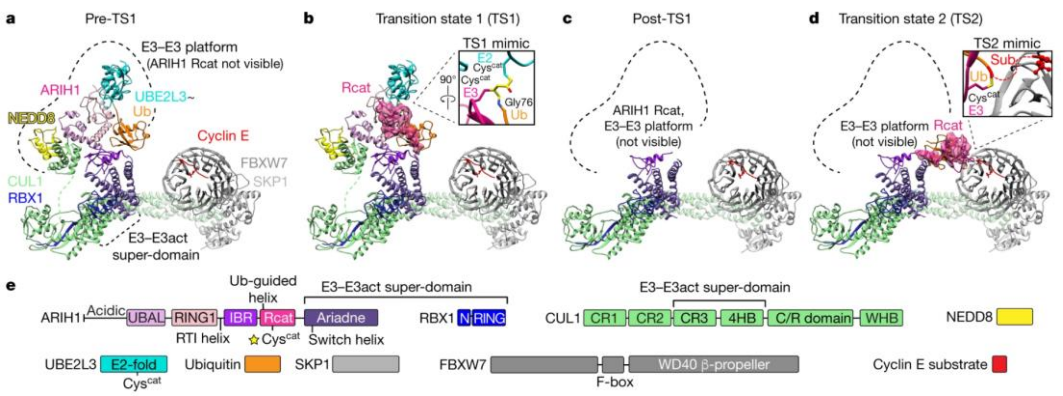
The observations that TRIP12 is partially recruited to BRD4 through association with CUL2 warrants a detailed analysis of all cullin binding partners to identify other novel coregulators. The discovery of coregulators in PROTAC mediated targeted degradation opens up an opportunity to exploit their variable levels of expression in different tissues or disease conditions to modulate PROTAC specificity.

Contributor: Charlotte

Ubiquitin ligation to F-box protein targets by SCF–RBR E3–E3 super-assembly

Daniel Horn-Ghetko, …, Brenda A. Schulman*

Nature **2021**, DOI: 10.1038/s41586-021-03197-9



Early studies of the UPS have taught us that substrate ubiquitination is achieved through an E1-E2-E3 enzyme cascade. Valuable details have progressively been revealed, for example certain E3s depend on NEDD8 to mediate substrate ubiquitination. In this study, Schulman and colleagues pinpoint key intermediates and interactions underpinning substrate ubiquitination by using chemical probes which enable visualisation through cryogenic electron microscopy. They show that an RBR E3, ARIH1, specifically binds Rbx1 and Cul1 from the SCF E3 via NEDD8. ARIH1 possesses a ubiquitin-guided helix which allows capture of ubiquitin from the E2 UBE2L3. Subsequently, ARIH1 is positioned in a way to directly guide ubiquitin toward the SCF-bound target substrate.

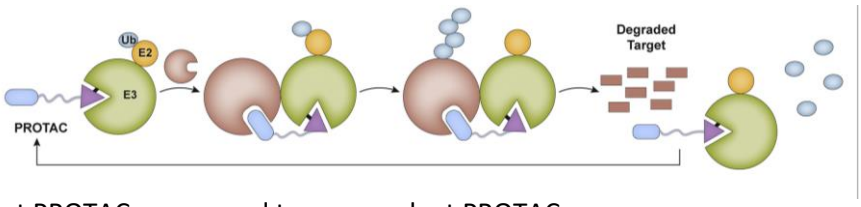
A "tag team" mechanism for ubiquitination with ARIH1 was suggested in 2016, however the mechanism through which ARIH1 is involved was unclear. The fact that an additional E3 is responsible for the final positioning of ubiquitin, enabling substrate ubiquitination and subsequent degradation, is remarkable. It will be fascinating to discover more about the intricacies of ubiquitination mediated by collaborative E3s and apply these findings to targeted protein degradation. The paper was enjoyable to read and describes the above system in a very visual way. I also highly recommend watching the webinar given by Prof. Brenda Schulman in the Dana-Farber TPD Webinar series.

Contributor: Charlotte

The rise of covalent proteolysis targeting chimeras

Ronen Gabizon[§]*, Nir London[§]*

Curr. Opin. Chem. Biol. **2021**, *62*, 24



This review explores the current status of covalent PROTACs. The authors first acknowledge the shortcomings of covalent PROTACs which bind the target protein of interest (POI) irreversibly. They then assess the efficacy of 'reversible' POI-binding covalent PROTACs compared to noncovalent PROTACs. The advantages of PROTACs covalently binding the E3 ligase are also discussed and strategies for their discovery are highlighted. To emphasise the unfulfilled potential of E3-binding covalent PROTACs, the authors compile a list of ligandable cysteines in E3 ligases. This provides a motivating and thought-provoking end to the review.

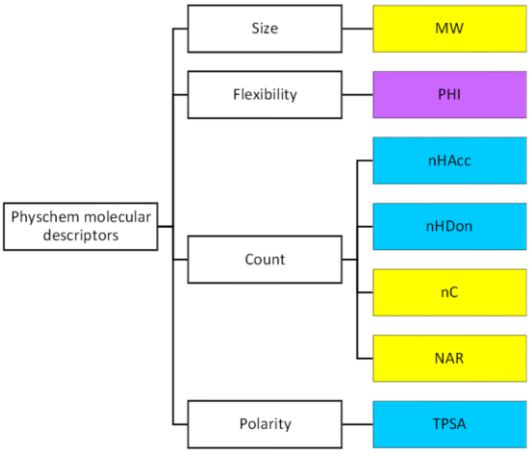
Overall, a clear and well-written overview of strengths and weaknesses of covalent PROTACs. I enjoyed that the review not only celebrates the successes of covalent PROTACs, but that their shortcomings are also discussed as well as potential pathways for progress in the area.

Contributor: Charlotte

PROTACs and Building Blocks: The 2D Chemical Space in Very Early Drug Discovery

Giuseppe Ermondi[§], Diego Garcia-Jimenez[§], Giulia Caron[§]*

Molecules **2021**, *26*, 672



Over 20 years ago, Lipinski's 'Rule of 5' was introduced as a method for describing 'drug-likeness' and serves as a guide for the early stages of small molecule drug discovery. The properties of PROTACs lie beyond the Rule of 5. To evaluate the 2D physicochemical molecular space of PROTACs, the authors devised a set of descriptors: molecular weight, Kier's flexibility index, number of carbon atoms, number of aromatic rings, number of H-bond acceptor atoms, number of H-bond donor atoms and topological surface area. The PROTACs, warheads, E3 ligases and ligands from databases PROTAC-DB and PROTACpedia were downloaded and analysed using a range of statistical and graphical tools. A number of trends were identified, providing a preliminary framework for PROTAC discovery. As expected by the authors, the 2D descriptors did not reveal any distinctions between active and inactive compounds, highlighting the additional need for pharmacodynamic descriptors.

PROTACs are an attractive and promising strategy for tackling challenging protein targets. As PROTACs are involved in a highly dynamic mechanism, it is clear that systematic approaches and models will be needed to evaluate potential PROTAC drugs, and early studies of PROTAC chemical space can help guide us in the right direction.

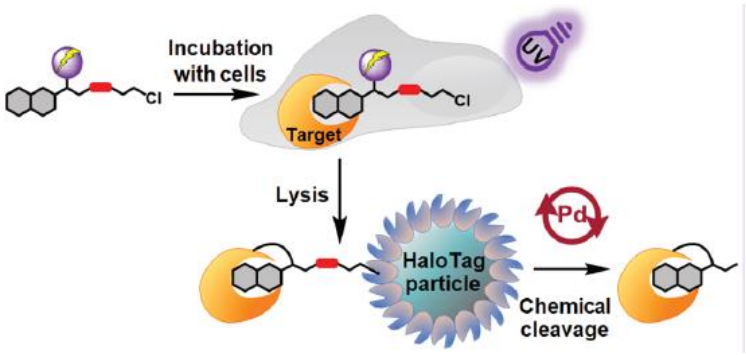
Other Paper Highlights

Contributor: Will

Streamlined Target Deconvolution Approach Utilizing a Single Photoreactive Chloroalkane Capture Tag

Rachel Friedman Ohana[§]*, Sergiy Levin[§], Robin Hurst[§], …, Thomas A. Kirkland

ACS Chem. Biol. **2021**, *16*, 404



In this manuscript Ohana and colleagues describe a novel approach for the capture and ID of physiologically relevant small molecule protein targets. Their method entails attachment of both a photoaffinity tag (for target protein capture even when interactions are low affinity or fleeting) in addition to a cleavable chloro alkane capture tag. This single, modular and (synthetically) easily applied system allows for treatment of live cells with tagged small molecules followed by UV-mediated crosslinking, lysis and capture/release using solid-supported HaloTag. A BRET assay is developed to aid identification of suitable photoaffinity labels, with the authors highlighting phenylazides as potentially underutilised and advantageous groups due to their lower inherent reactivity and thus decreased non-specificity, balanced by an extended lifetime of their reactive intermediates allowing for efficient cross-linking even if the azide itself was not optimally positioned relative to the protein target. They are also tunable by altering the phenyl ring electronics and sterics. The approach is validated using three infamous drug molecules, SAHA (pan-HDAC inhibitor), Dasatinib (BCR-ABL inhibitor) and Propranolol (betablocker that binds to the membrane bound beta-adrenergic receptor) with all on-targets and a number of known off-targets successfully identified.

A neat system and there is nice validation to show no obvious disadvantage with respect to cellular uptake or potency when using this compared with most commonly used alkyne tags for azide-biotin capture with some potential advantages in workflow. Some off-targets do seem to have been missed and the investigation of the different photoaffinity tags using the BRET assay is the highlight of this paper.