CeTPD Journal Club

Targeted protein degradation, medicinal chemistry, chemical structural biology & cell biology

January 2024

Centre for Targeted Protein Degradation
University of Dundee

innovate
collaborate
inspire

Journal Club

Content

Meet this Month's Editors

This month's editors are (from left to right): Alex Hallatt, Aitana de la Cuadra Baste, Dylan M. Lynch

"Having worked in the TPD field during my PhD, the CeTPD Journal Club was great for navigating the ever-expanding sea of TPD literature and it was particularly useful when writing my PhD thesis introduction chapters too! It feels both strange and humbling to now be on the other side, helping to contribute to this invaluable resource."

Alex obtained his MChem degree from Newcastle University in 2019, during which he undertook an industrial placement year at GSK (Stevenage, UK) and gave Alex his first foray into the world of 'Med Chem'. Alex then returned to Newcastle to undertake a PhD in medicinal chemistry, developing bifunctional degraders against a novel protein target. In October 2023, Alex joined the Ciulli lab as a postdoc working with the EUbOPEN consortium.

"The JC is a great opportunity to keep up with the key publications in the field of Targeted Protein Degradation."

Aitana is originally from Valencia, Spain. In July 2022 she completed her bachelor's degree in biotechnology, at the Polytechnical University of Valencia. She moved to Dundee and joined the group in September 2022 for an Erasmus Internship. She loved the Scottish weather so much that she decided to return and she started her PhD the Ciulli lab in September 2023.

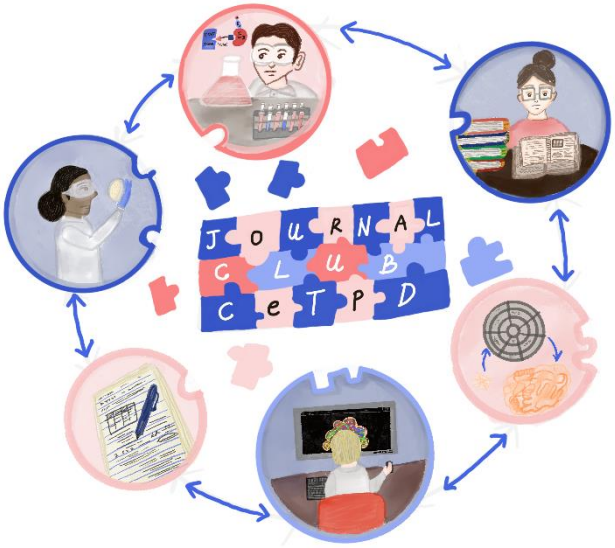
"*The CeTPD JC provides an avenue for scientists in the TPD space to pause and reflect on the advancements in the field, in a digestible format. This whistle-stop tour helps keep all of us involved in an ever-advancing field."*

Dylan completed his PhD in organic chemistry in his hometown of Dublin, Ireland. He worked in a number of Irish research labs, before starting his TPD research at the University of Washington, Seattle. He then moved to Dundee as a postdoc in the Ciulli lab, where he hunts down ligands for untapped E3 ligases to expand the TPD toolbox.

Editors-in-Chief for the CeTPD Journal Club

Contributor: Yuting, Illustration from Padma @padmartistry

The CeTPD Journal Club (previously Ciulli Group Journal Club) was spearheaded by Siying Zhong in 2020, mainly developed by Charlotte Crowe, and is now being led by PhD students. It is so popular in the TPD field that I knew of it already when I was in China long before I was joining the Ciulli group in 2021.



Andreas and I took over the Editors-in-Chief job in January 2023 when Charlotte left the JC. It has been a great opportunity and such a pleasure for me to join the team and work with so many people. In the past year, we highlighted 123 papers, 33 editors contributed for targeted protein degradation literature highlights and many people contributed other parts of the issue as well. I would like to say thank you to all of you, the journal club 2023 wouldn't be possible without all your contributions.

As a third year PhD student, I'll need to focus more on my own projects and have to quit the journal club. Starting this month another student will take over the baton. Aitana de la Cuadra Baste did her Erasmus internship in the Ciulli group and then she decided to stay here for her PhD. She will work together with Andreas Holmqvist to lead and coordinate the journal club from January 2024. I am sure they will do a great job and lead the journal club to a new chapter.

Please make sure to follow their "X" account @DeLaCuadraBaste @Holmqvist89, and our CeTPD official account @UoDCeTPD .

The 3rd Annual CeTPD Christmas End of Year Review

Contributor: Lexie

The Centre for Targeted Protein Degradation hosted their 3rd End of Year Review at the Apex Hotel on the 22nd of November. This event marks almost one year since the move to the new CeTPD building, and what a year it's been! The number of lab members within the centre has drastically grown, and we've seen the introduction of the CeTPD seminar series and international potluck events, along with the release of several impactful publications. The evening kicked off with a centre-wide *Secret Santa* exchange, generating lots of laughter and joy among participants. Following on from this, our fabulous MC Tom Webb settled everyone, before inviting Alessio to give an introductory speech which celebrated how far the centre had come over the past year. The presentations began with an operations team overview kindly presented by Louise McGreavey. Calum McLaughlin gave a cracking and unforgettably funny academic review which will be remembered for years to come. The AC-BI review was given by the collaborations team leader, Kirsten McAulay, marking her first successful year in this position. Andreas presented a thorough update on the Farnaby Group and their exciting research, before the Almirall review was given by David Zollman. The overview of the Eisai collaboration was up next, with Ryan Casement giving a professional but hilarious run down of their work. Last but certainly not least, was Giorgia Kidd, who presented an overview of all the outreach events and work experience that took place across the year. The night continued with a tasty 3-course meal, an amazing quiz hosted by Zoe Rutter, Aitana De La Cuadra Baste, Alexandra Harris and Angus Cowan and then a night filled with dancing.

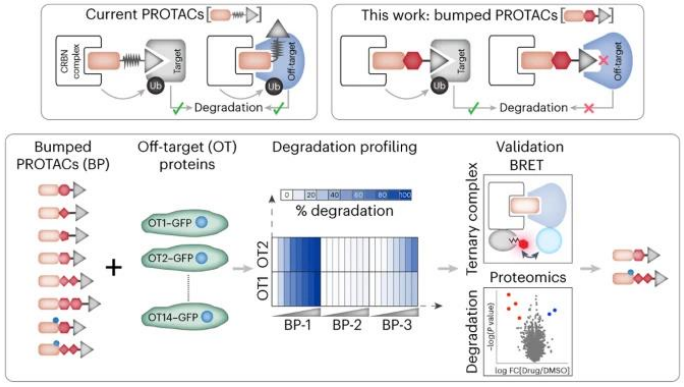
Targeted Protein Degradation

Cell Biology | Chemistry

Contributor: Alex

Proteolysis-targeting chimeras with reduced off-targets

Tuan M. Nguyen[§], Vedagopuram Sreekanth[§], Arghya Deb[§], Praveen Kokkonda[§], …, Amit Choudhary*
Nat. Chem. **2023**, DOI: https://doi.org/10.1038/s41557-023-01379-8



Nguyen *et al.* aimed to overcome the promiscuous nature of CRBN degraders towards proteins containing Zinc-Finger (ZF) domains by systematically exploring the chemical space around the pomalidomide chemotype in a high-throughput manner. The library of pomalidomide analogues was then profiled in various *in vitro* methods, including a novel automated imaging assay, to determine their propensity to degrade ZF domains. Through this study, the authors deduced that the greatest overall improvements in selectivity were as a result of changing the exit vector from the C4 to the C5 position of the phthalimide ring.

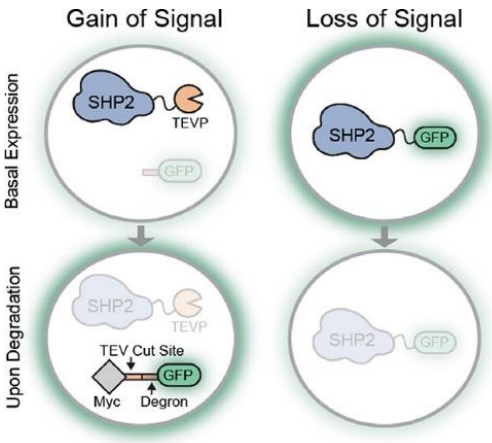
The promiscuity of CRBN degraders has become a notable challenge in their development in recent years, particularly with regards to molecular glues where the chemical space is more restricted. The systematic approach of this work aims to deconvolute the chemical space around the phthalimide ring, which will greatly benefit the development of CRBN PROTACs and glues alike. Perhaps the centrepiece of this work is not the outcomes, but rather the methods in which they were achieved. The automated imaging assay which uses a representative panel of tandem GFP-ZF constructs is an elegant and time-efficient method for gauging the promiscuity of CRBN based degraders. The limited panel (n = 14) is validated by global mass-spec proteomics, although it is yet to be seen whether the proposed time-saving benefits of this new method will be enough for this technique to be adopted by the TPD community.

Cell Biology | Structural Biology/Biophysics

Contributor: Dylan

A Novel Gain-of-Signal Assay to Detect Targeted Protein Degradation

Megan Hoffman[§], Keith Ming Hong Cheah, and K. Dane Wittrup*
ACS Synth. Biol. **2024**, *Article ASAP*



Hoffman *et al.* described a novel gain-of-signal assay, based on a fluorescence signal correlating to protein depletion, with additional controls to obviate false positives. The authors used this platform to screen 192 macromolecules against the oncogenic phosphatase SHP2, wherein a reporter cell line increases expression of GFP in response to the degradation of the protein. Hoffman *et al.* designed this assay platform to avoid off-target ubiquitination, by removing solvent-accessible lysine residues other than those on the target protein.

One aspect of this work that I found noteworthy were the parallel screens for validating 'true' SHP2 degraders. The authors used their gain-of-signal assay and detected SHP2 and bioPROTAC expression levels (detected by Western blot) to validate their degradation results as true positives rather than false positives.

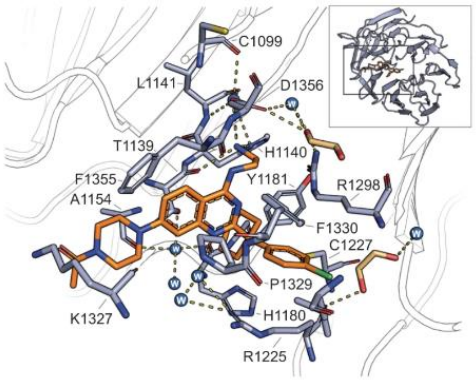
Chemistry | Structural Biology/Biophysics | Cell Biology

Contributor: Dylan

DCAF1-based PROTACs with activity against clinically validated targets overcoming intrinsic- and acquired-degrader resistance

Martin Schröder, Martin Renatus[§], …, Claudio R. Thoma*

Nat. Commun., **202 4**, 15, 275



Schröder and Renatus *et al.* describe a selective, non-covalent DCAF1 binder for targeted protein degradation. The authors validated $CRL4^{DCAF1}$-driven degradation through various rescue experiments, and functionalised their ligand into an effective BRD9-degrading PROTAC. Furthermore, the authors showed efficient degradation of Bruton's tyrosine kinase (BTK) in cells with resistance to CRBN-based PROTACs using a DCAF1-BTK PROTAC, and highlighted their DCAF1-BRD9 PROTAC as an alternative strategy to classical VHL-based degraders.

One experiment that stood out in the authors extensive chemical and genetic rescue experiments was their sgRNA rescue screen for the DCAF1-BRD9 PROTAC treatment. BRD9-HiBit cell lines underwent lentiviral sgRNA infection, followed by a selection step with puromycin to kill unifected cells. This genetic rescue screen provided a really useful HiBiT readout following treatment with 1 µM of the PROTAC.

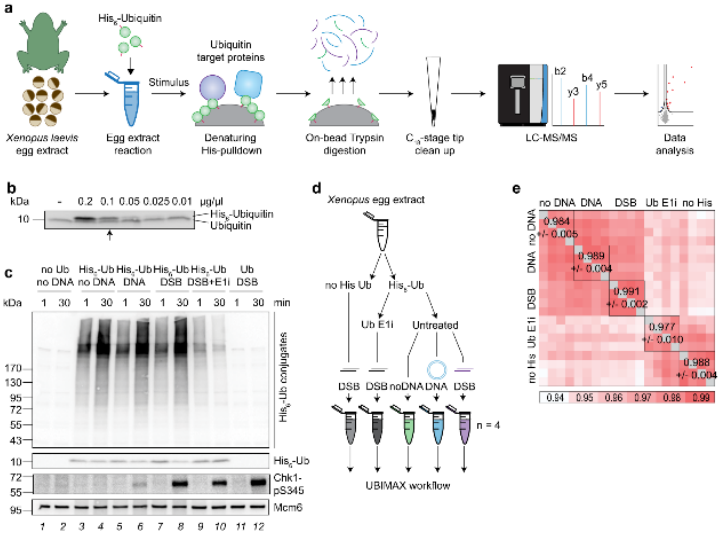
Cell Biology | Structural Biology/Biophysics

Contributor: Alex

Profiling ubiquitin signalling with UBIMAX reveals DNA damage- and SCFβ-Trcp1-dependent ubiquitylation of the actin-organizing protein Dbn1

Camilla S. Colding-Christensen[§], Ellen S. Kakulidis[§], …, Julien P. Duxin*, Michael L. Nielsen*

Nat. Commun. **2023**, *14*, 8293



This work describes the development of UBIMAX (UBiquitin target Identification by MAss spectrometry in Xenopus egg extracts) as a method to quantitatively study protein ubiquitylation under tuneable conditions. The combination of the Xenopus egg extract system with His-tagged ubiquitin enables the pulldown and enrichment of ubiquitylated proteins which can then be accurately quantified using high-resolution mass spectrometry. Additionally, this system is designed to analyse changes in ubiquitylation events resulting from specific stimuli. To this end, the UBIMAX system was used to investigate ubiquitylation events as a to double-strand DNA breaks and was able to identify Dbn1 as a substrate for the $SCF_{β\text{-}Trcp1}$ ligase.

The elegant work outlined in this paper provides compelling evidence for the utility of the UBIMAX system. New methods for examining ubiquitylation in response to specific stimuli are likely to be extremely beneficial to the wider TPD field. Whilst the traditional dogma is to degrade proteins that confer diseased states when overexpressed, this methodology may open the door to identify proteins which are degraded during diseased states, and thus may provide targets for deubiquitinase-targeting chimera (DUBTACs).

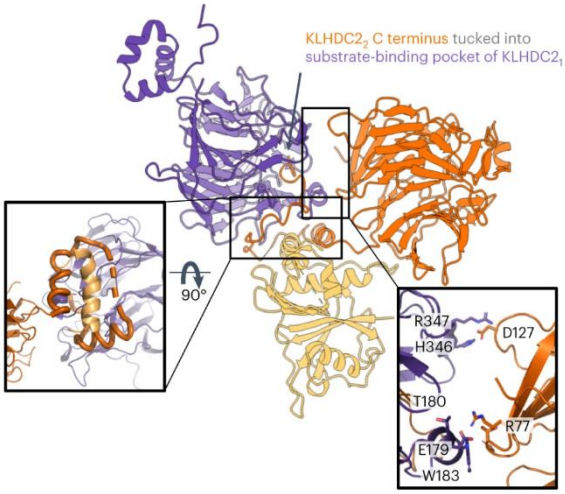
Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Dylan

Co-opting the E3 ligase KLHDC2 for targeted protein degradation by small molecules

Christopher M. Hickey, Katherine M. Digianantonio, …, Miklós Békés*
Nat. Struct. Mol. Bio, **2024**, *doi.org/10.1038/s41594-023-01146-w*

In a further expansion of the the E3 ligase toolbox Hickey and Digianatonio *et al*. performed biochemical characterisation of new KLHCD2 ligands, and functionalised these validated ligands into BET-family and androgen receptor degrading PROTACs. Throuhg a complement of X-ray crystallography, biochemical studies and cryo-EM, the authors characterised KLHDC2 E3 ligase assemblage and identified that it forms a dynamic tetramer, held together by its C-terminus.



The authors highlight that expression levels of other E3 ligases in varying tissue or cell lines can be guiding factors in considering untapped E3 ligases. The consistent research efforts to expand the E3 toolbox is exciting, especially as acquired resistance or incompatible cell lines can hamper the application of 'classical' E3s in TPD.

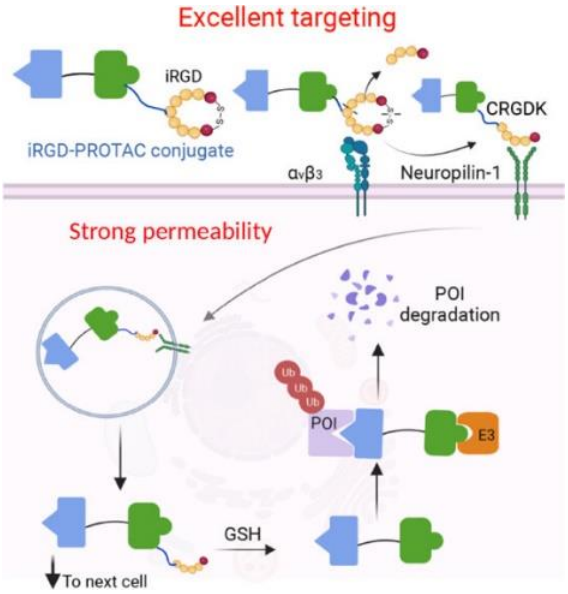
Cell Biology | Chemistry

Contributor: Alex

Enhanced Tumor Targeting and Penetration of Proteolysis-Targeting Chimeras through iRGD Peptide Conjugation: A Strategy for Precise Protein Degradation in Breast Cancer

Shipeng He[§], Yuxin Fang[§], Minghao Wu[§], …, Honggang Hu*, Chunquan Sheng*, Guoqiang Dong*
J. Med. Chem. **2023**, *66*, 16828-16842

In this work, He *et al.* have conjugated the tumour-targeting cyclic peptide iRGD with the BRD4 PROTAC MZ1, in order to direct the degrader towards tumour sites and improve tissue penetration. The iRGD peptide is recognised and selectively cleaved by recognised by $\alpha_v\beta_3$ integrin, which is overexpressed in tumour cells, and the resulting peptide fragment contains a NRP1 recognition sequence which enables the PROTAC to be to be preferentially uptaken into cancerous tissues. The two-pronged approach of tumour-targeting and tumour uptake widens the therapeutic window of the parent degrader compound. The authors were able to demonstrate this improved anti-tumour activity in murine models and patient-derived organoids.



While iRGD conjugation has already been successfully applied to small-molecule inhibitors and biologics, this is the first time that the technology has been applied in a TPD setting. It is encouraging to see that the iRGD motif led to improvements in the anti-tumour activity whilst also improving the aqueous solubility of the compound, further improving the profile of the degrader. Whilst this paper provides great proof-of-concept for the synergy between iRGD and TPD, it would have been beneficial to see this applied on systems other than BRD4 and VHL.

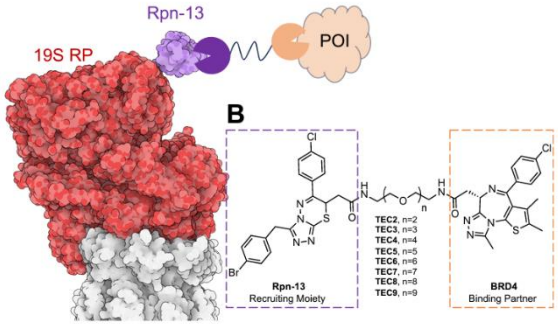
Cell Biology | Chemistry

Contributor: Dylan

ByeTAC: Bypassing an E3 Ligase for Targeted Protein Degradation

Eslam M. H. Ali, Cody A. Loy, Darci J. Trader*
BioRxiv **2024**, *doi.org/10.1101/2024.01.20.576376*

In this account, authors Ali and Loy *et al*. describe a TPD methodology which bypasses E3 ligase binding, and instead binds a 26S proteasome subunit. These so-called Bypassing E3 Targeting Chimeras (ByeTACs) function through binding Rpn-13, a non-essential ubiquitin receptor for the 26S proteasome. The authors generated a number of Rpn-13-BRD4 bifunctional degraders, with varying linker lengths. Furthermore, the authors demonstrated that the induced degradation upon compound treatment is dependant upon both proteasome activity and the expression levels of Rpn-13.



There has been a recent expansion of TPD methodologies that don't rely on the traditional POI-E3-PROTAC ternary complex, for accomplishing a variety of phenotypic outcomes. While the authors compare their compounds ability to other published degraders, it would be beneficial to see degradation beyond the classical BRD4 target to truly cement this technology in the current TPD state-of-the-art.

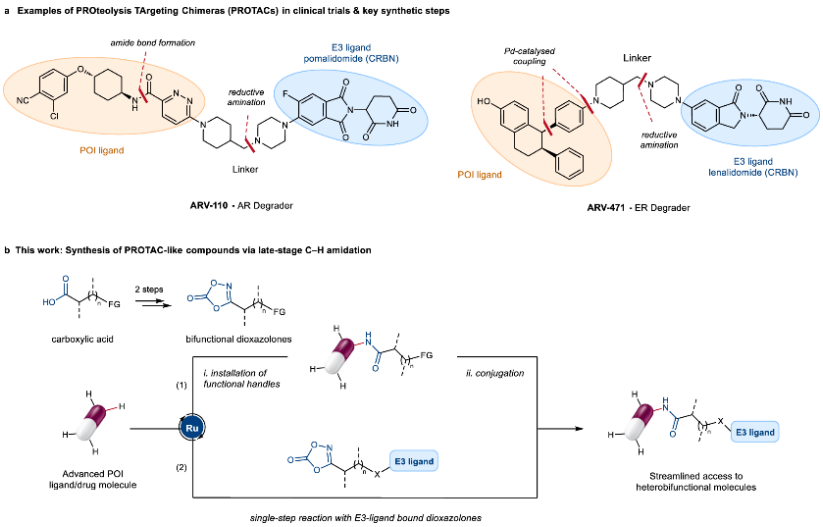
Chemistry

Contributor: Alex

Late-stage synthesis of heterobifunctional molecules for PROTAC applications via ruthenium-catalysed C–H amidation

Daniele Antermite[§], …, Lutz Ackermann*, Magnus J. Johansson*
Nat. Commun. **2023**, *14*, 8222

Antermite *et al.* report a novel method for the late-stage amidation of drug-like scaffolds, with a particular emphasis on the applicability towards assembling heterobifunctional modalities such as PROTACs. In this approach, a ruthenium catalyst facilitates the amidation between a dioxazolone (as a nitrene source) and a (hetero)aryl coupling partner bearing an activated C-H bond. The utility of this new method was demonstrated by the scope of the tolerated coupling partners at either end, where at least one partner was already a large, drug-like scaffold.



The reported conditions are mild, robust and display great chemoselectivity over aryl halides, alkenes, alcohols and other reactive groups. The regioselectivity was less clear to predict *ab initio*, however, this could be overcome by the careful use of directing groups such as amides or azoles. This wide tolerability enables this methodology to be suited towards late-stage functionalisation, but the variability in conversion may limit its use in direct-to-biology settings.

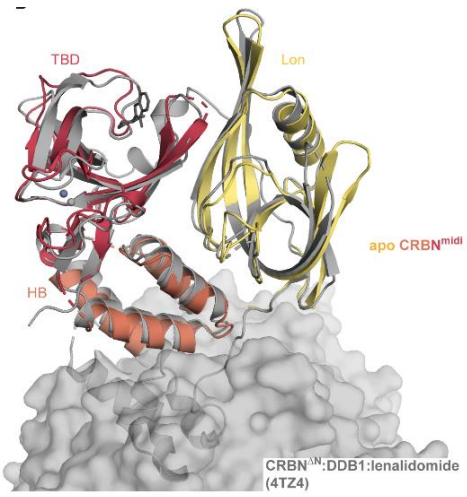
Structural Biology/Biophysics

Contributor: Aitana

Design of a Cereblon construct for crystallographic and biophysical studies of protein degraders

Alena Kroupova[§], …, David Zollman*, Alessio Ciulli*

BioRxiv **2024**, *doi.org/10.1101/2024.01.17.575503*



The structure-based design of Cereblon (CRBN) recruiting degraders has been considerably limited due to the challenges of producing a suitable recombinant CRBN protein, which requires co-expression with the adaptor protein DDB1, or truncation to the thalidomide-binding domain only which does not fully represent the full-length protein.

In this preprint, the authors develop a soluble and stable recombinant CRBN protein, which expresses in *E.coli* as a monomer, without the need of co-expression with DDB1. They design 15 new constructs, from which the most promising (referred to as CRBN^{midi}), lacks the DDB1-interacting region and incorporates 12 stabilising mutations. This construct adopts a relevant biological fold, shown by the crystal structure that the authors solved to a resolution of 3.11 Å. They also show its ability to crystallize in ligand bound form which validates its use for structure-based design. Through a range of biophysical assays, they benchmark the functionality of CRBN^{midi} to the wild type, which demonstrates that it is suitable for ligand binding studies in solution.

This will be a very valuable tool for the field of Targeted Protein Degradation, CRBN^{midi} solves many limitations of the previously described constructs. This work will accelerate the design and optimisation of cereblon recruiting degraders.

Chemistry | Modelling/Simulation | Structural Biology/Biophysics

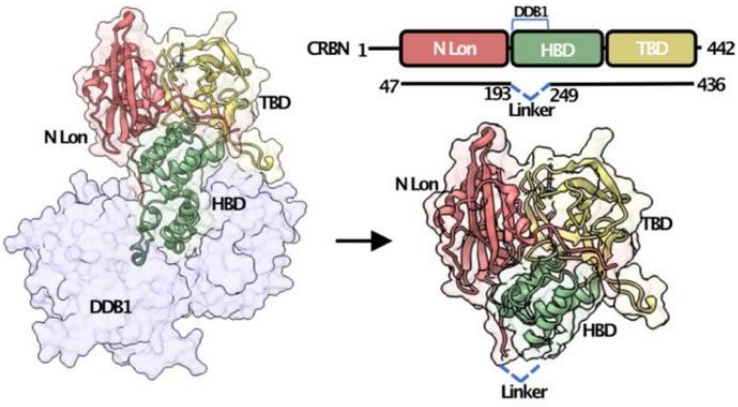
Contributor: Aitana

Engineering CRBN for rapid identification of next generation binders

Henry J. Bailey[§], Jonathan Eisert[§], …, Fiona J. Sorrell*, Ivan Dikic*.

BioRxiv **2024**, *doi.org/10.1101/2024.01.18.576231*

In this preprint the authors design an intermediate human CRBN (CRBN_ Δ HBD), which replaces the DDB1 interacting residues with a soluble linker and can be purified from an *E.coli* expression system. CRBN_ Δ HBD adopts the functional closed conformation according to Alphafold and has shown equivalent activity to CRBN:DDB1 in several assays, demonstrating its suitability to study binary and ternary complexes. The authors use this construct to screen a CRBN-focused library of possible scaffolds that may overcome the limitations of current binders, which have significant issues with off target toxicity and stability. Several hits are identified with higher binding potency than current scaffolds. However, these need to be characterized to and whether they have a reduced off target toxicity needs to be explored.



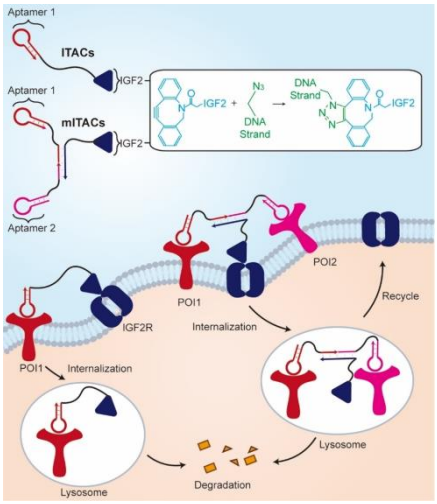
This is complementary to the work done by Kroupova *et al.*, where they have both created very powerful tools to solve the same problem. It is very interesting to see how they have taken different approaches, whilst one is structural biology centered, the other focuses on the finding of next-generation binders.

Cell Biology | Chemistry

Contributor: Aitana

Insulin-like Growth Factor 2-Tagged Aptamer Chimeras (ITACs) Modular Assembly for Targeted and Efficient Degradation of Two Membrane Proteins

Yuan Tian[§], …, Junyan Wang*,Da Han*
Angew. Chem. Int. Ed. ***2023****, e202316089*



To solve the current limitations with degradation of cell surface proteins, in this paper they develop Insulin-like growth factor 2 (IGF2)-tagged aptamer chimeras (ITACs). These are composed of two modules: IGF2, which binds to IGF2 receptor (IGF2R), and aptamers that recognize the protein of interest, bringing them together and inducing lysosomal degradation. ITACs have been shown to successfully degrade several target proteins, and the degradation is dependent on moderate IGF2R levels on the cell surface. The authors go a step further and develop a strategy to target two different membrane proteins (mITACs), which show a higher degradation efficiency than two separate ITACs combined.

This work gives some more insight into targeting cell membrane proteins as current methods still have some challenges. With the mITACs the authors show for the first time the ability to target more than one membrane protein. This provides a platform for the development of treatment for diseases related to overexpression of membrane proteins.

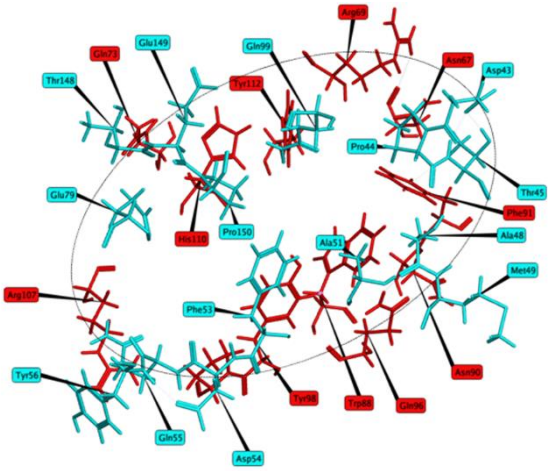
Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Dylan

A small molecule VHL molecular glue degrader for cysteine dioxygenase 1

Antonin Tutter[§],…, Gregory A. Michaud*
BioRxiv **2024**, doi.org/10.1101/2024.01.25.576086

A recent pre-print from Novartis discloses a VHL molecular glue degrader for cysteine dioxygenase 1 (CDO1). Using protein arrays, the authors bound the HIF1α binding pocket with a small molecule that functions as a MGD of the neosubstrate CDO1, achieving selective degradation. This account combines a full complement of characterisation techniques for the PPIs, including mutagenesis studies and X-ray structures of the VHL-CDO1-degrader ternary complex. After optimisation, Tutter *et al*. arrived at a potent degrader of CDO1, boasting absDC$_{50}$ of 8 nM/98% degradation.



One aspect of this work that I appreciated was the optimisation of the structure-activity relationship for their degrader – the authors analysed the effects of small changes on the recruitment of CDO1, *via* TR-FRET, sPR, and NanoBiT. Furthermore, the authors sought to avoid phenotypic screening and instead identify neosubstrates for VHL using handles previously employed in bifunctional degraders, but without any explicit molecular glue activity, using protein arrays which allowed them to easily control the assay parameters.

Other Paper Highlights

Chemistry

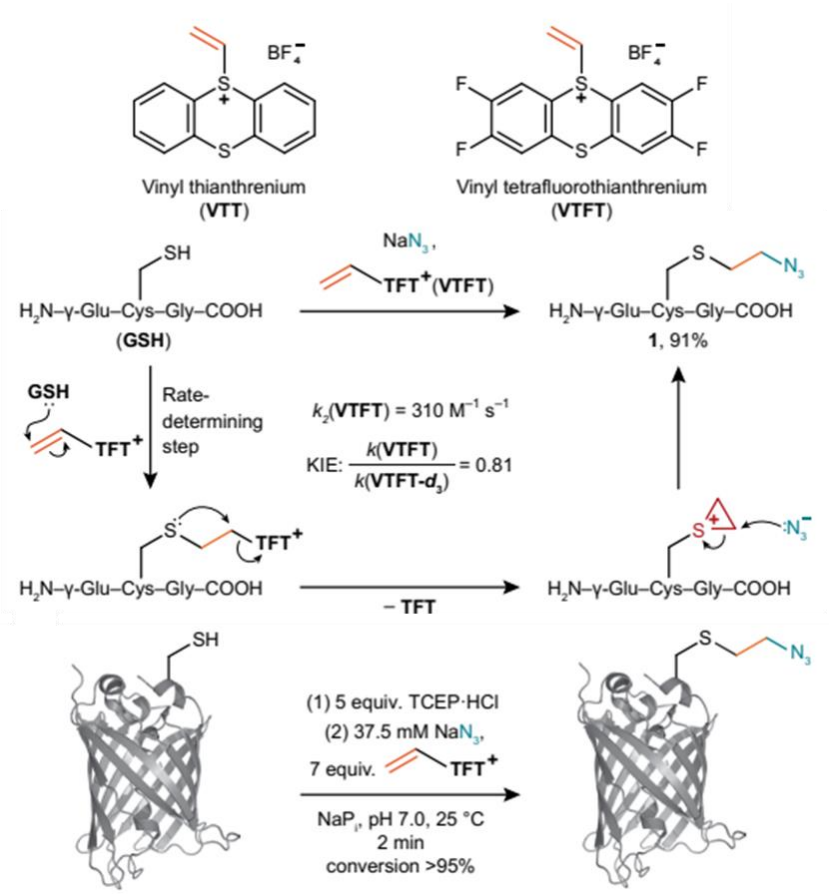
Contributor: Calum

Chemoselective umpolung of thiols to episulfoniums for cysteine bioconjugation

Philipp Hartmann[§], …,Tobias Ritter*

Nat. Chem. **2023**, DOI:10.1038/s41557-023-01388-7

Methods for the chemoselective bioconjugation of cysteine amino acid residues typically involve the use of electrophilic reagents (Michael acceptors, α-halocarbonyls, etc) or, alternatively, conversion of Cys to an electrophile for functionalisation in a two-step sequence.



In this work, Ritter and co-workers have developed a bifunctional vinyl thianthrenium (VTT) reagent, which is bench-stable and water soluble, for Cys-targeting covalent modification of biomolecules. Key to the success of this reagent is the cationic thianthrenium group which acts as both an electron-withdrawing group and as a latent leaving group. Upon initial Michael addition to engage the nucleophilic thiol, the formed alkylsulfonium intermediate can undergo displacement of thianthrene to generate the key episulfonium ion in situ. This highly reactive species can engage a diverse set of nucleophiles (azides, anilines, thiocyanates, thiophosphates, iodide) through ring-opening, enabling rapid access to a range of Cys modifications through a single common reagent in one step. In the absence of an exogenous nucleophile, stapling and macrocyclization of peptides could also be performed. The authors applied the process to the modification of peptides and native proteins, demonstrated chemoselectivity and site selectivity, and utilised isotopologues in quantitative proteomics.

Overall, the utility and wide applicability of the vinyl-thianthrenium-based reagent for bioconjugation has been demonstrated. The ability to introduce an azide chemoselectively into proteins which can then be utilised in copper-catalysed click reactions with alkynes is powerful. This protocol compliments the existing electrophilic and multi-step Cys-targeting technologies, and it is expected that this will find broad use in chemical biology.

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