CeTPD Journal Club

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

Journal Club

April 2023

Centre for Targeted Protein Degradation
University of Dundee

innovate
collaborate
inspire

Content

Meet this Month's Editors

This month's editors are (from left to right): Ilaria Puoti, Maria Rodriguez-Rios and Oliver Hsia

"The JC is an extremely valuable resource that allows to share the latest discoveries from the TPD field with opinions from scientists with different backgrounds promoting briskly discussion and connection among the TPD community"

Ilaria obtained her PhD in Cancer Science at the University of Glasgow-CRUK Beatson Institute under the supervision of Prof. Sara Zanivan working on the crosstalk signalling between ovarian cancer cells and fibroblasts using a proteomic methodology called CTAP. Keen to apply her proteomic expertise for profiling new degraders that could improve patients' life, Ilaria joined the Ciulli group in September 2022 as cellular and proteomic scientist to work in the AC-Boehringer Ingelheim team.

"*The JC is a fantastic resource that helps the community keep up to date with the latest advances in the fast-evolving TPD field. Its unique format helps the reader put the research in context from a critical point of view."*

Maria joined the Ciulli group in January 2023 as a postdoctoral scientist. She obtained her PhD in Chemistry at the University of Edinburgh under the supervision of Prof. Mark Bradley working in fluorogenic probes for detection of proteases in inflammation. Maria is now working on the development of tag-based dregon systems.

"The JC provides a unique platform for experienced researchers in the TPD space to give their opinion and analysis of papers in an easy-to-read format. I think this is of value to a wide audience as there is often nuance and context in research articles that can be hard to pick up on for those less familiar with the field."

Oliver joined the Ciulli group as a postdoctoral scientist (Cell Biology) as part of the Eisai project in May 2020. Ollie completed his undergraduate degree in biochemistry as well as his PhD in molecular biology at The University of Glasgow. Oliver has over 7 years in the protein homeostasis field, with 3 years working in targeted protein degradation.

Journal Club at 3 – Happy Birthday!

Contributor: Dr. William Farnaby and illustrator Padma Srinivasan

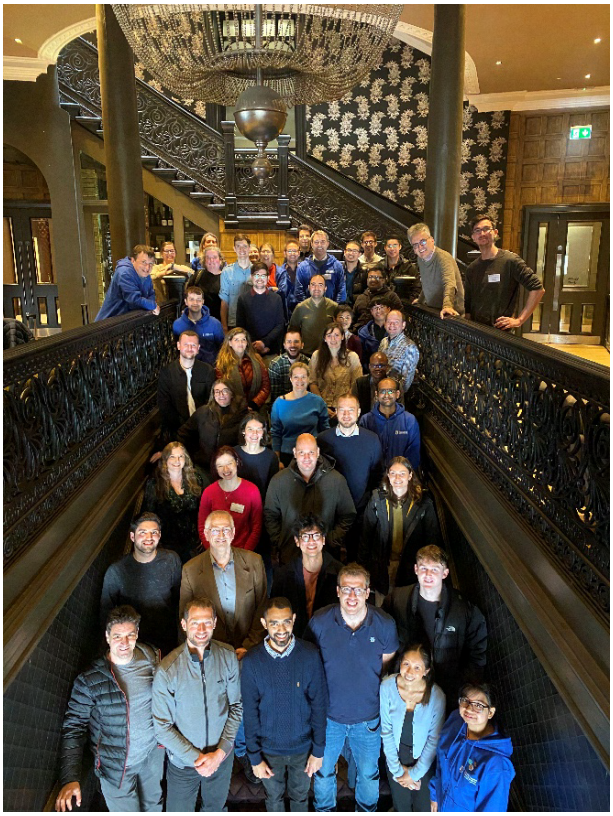
This month marks the 3rd anniversary of the CeTPD journal club, the first edition then known as the Ciulli Group Journal Club, being released in April 2020. The origins of the journal club came from an idea put forward by a former group member, Siying Zhong. During the global turbulence of February 2020 and whilst standing at a fumehood in our lab she made the observation that the sheer volume and diversity of TPD related literature emerging at the time, whilst exciting to see, created a challenge in trying to stay aware of everything that was being published. She was keen to find and drive towards a solution, proposing that we could combine the shared awareness of our scientists and capture that together. Days later, we found ourselves with labs shut and locked down at home, the perfect opportunity to put these ideas in to action. With Alessio and Siying, the three of us at this point discussed not only the format but also the idea that the problem we were trying to solve maybe faced by many in the field and even more acutely by those on the edges starting to get in. We decided that to work, this would be something that required input and participation by as wide a group of people as possible, clear guidance and leadership (provided by Siying during the first phase) and to make it as widely available as possible beyond our own group. Since then, we would like to believe, this has become not only a useful resource for those wishing to know what's new in the TPD world, but has also become a platform for scientists within CeTPD from many career levels, disciplines and career paths to provide their perspectives on key advances within TPD and beyond.

In 2021, the journal club was placed under new leadership, in the hands of PhD student Charlotte Crowe. Now being a student led initiative, Charlotte brilliantly and diligently developed the concept to another level, with extra features on visitors, global scientific events and CeTPD news as well as sharpening the format to improve readability. Having steered this key phase successfully, Charlotte then handed over to co-editors in chief Andreas Holmqvist (1st year PhD student in the Farnaby Group) and Yuting Cao (2nd Year PhD student in the Ciulli Group) in early 2023. In many ways the development of the journal club over the last two years has reflected the parallel development of Dundee CeTPD, encompassing an ever-growing body of teams, perspectives, skills and ambitions. Having had the privilege of seeing this initiative evolve from the beginning, it is exciting to think about how future members of CeTPD, collaborators and visitors can embellish the reach and relevance even further. For those of you reading now, you may see this as a useful source of information. As a PI in CeTPD, my perspective is that is gives all scientists in the CeTPD a framework and platform to contribute to their field whilst honing and expressing their scientific awareness, vital for all of us in every discipline and role.

At this milestone I'd like to thank all of our contributors, the many who are still in CeTPD and those who have now moved on to new adventures and roles elsewhere. We are also indebted and gratefult to Siying, Charlotte, Andreas and Yuting for their guidance and leadership. I would also like to thank so many of you who read this and have shared it with your colleagues or provided us with feedback to help us improve it or simply to encourage us to keep going. This will be our 32nd journal club edition and the collection as a whole, when looking back through them (I would really recommend doing that!), provides an intriguing storyline of its own for TPD enthusiasts, mapping the milestones and progress that has erupted in that time. My hope is that this rich collection of fascinating science inspires us all to be future contributors through the research we are all doing right now…I look forward to seeing your paper summarised in the CeTPD journal club during year 4!

Boehringer Ingelheim visit

Contributor: Kirsten



This April, our collaborators from Boehringer Ingelheim (BI) were welcomed to Dundee for a scientific exchange meeting and the unveiling of the newly named 'Boehringer Ingelheim seminar room' within the Centre for Targeted Protein Degradation (CeTPD). The event kicked off with tours and a reception, with speeches from the Centre director, Alessio Ciulli, and Senior Vice President at BI, Darryl McConnell. A dinner celebrating the collaboration was subsequently held in the School of Life Sciences to mark the occasion. Over 60 people from across the two organisations were involved, highlighting the strength of the partnership which is now in its seventh year.

Following the launch event, a two and a half-day meeting was held at the Malmaison in Dundee. An array of talks and posters were given, bolstering discussion, and making way for further innovation, with Denzel Gonzales (CeTPD) and Alex Bentley (CeTPD) receiving the coveted poster prizes. Non-scientific highlights included a personality workshop from Zoe Rutter (CeTPD) and a talk on psychological safety from Manfred Koegl (BI), as well as birthday celebrations for Adam Pinto (CeTPD) and Stefan Walke (BI). The team were also delighted to welcome back Will Farnaby (CeTPD) to give a talk on his current research as a PI.

The event was also a great opportunity for many in the team to meet face to face for the first time. For several, the pinnacle of the week was the social event which saw the team embarking on a (rather muddy) walk to Maspie Den in Falkland estate, followed by a tour of Lindores Abbey distillery and whiskey tasting. Thankfully, there weren't too many sore heads the next day.

Contributor: Valentina Spiteri

Systemwide disassembly and assembly of SCF ubiquitin ligase complexes

Kheewoong Baek§, Daniel C. Scott, Lukas T. Henneberg, Moeko T. King, Matthias Mann, Brenda A. Schulman*

Baek *et al*'s working on understanding the disassembly and assembly of the SCF ubiquitin ligases complexes is a vertible tour de force, that one could only expect from the Schulman lab. The work includes a prolific 47 cryo-EM structures that help to delineate in dazzling detail the mechanism for recycling CUL1 to form new SCF complexes. To read this paper is to grow to know a cast of characters, and how they come together to fall apart. One could indulge some creative liberty and summarise this work as though it were a Shakespearean play, let's call it:

The Taming of the SCF ubiquitin ligase complexes

In fair Cell the authors lay their scene.

Characters in the play:

SCF- complex composed of SKP1-CUL1-F box protein E3 ubiquitin ligases – founding members of cullin-RING ligase superfamily.

- *CUL1*- elongated protein binds RBX1 on end and SKP1-Fbp on the other.
- *RBX1*- Ring box 1 binds to CUL1 mediates neddylation and ubiquitylation by interacting with E2 enzymes
- *SKP1*- S-phase kinase associated protein 1- binds to F-box of Fbps and CUL1
- Fbp- F box protein, ~70 different Fbps identified- recognise unique substrate degron motifs

CUL1-RBX1 subcomplex promotes ubiquitylation of substrates that are recognised by SKP1-Fbp subcomplexes.

CAND1- Cullin-associated NEDD8-dissociated protein 1- fundamental to E3 ligase-dependent regulation. Has an elongated sinusoidal structure, with an N-terminal arch termed the anti-neddylation domain that binds CUL1's empty neddylation site, prevent neddylation and a C-terminal arch termed anti-SKP1 domain, with β-hairpin that interacts with a CUL1 groove that otherwise accommodates SKP1 binding.

CAND1 mutants: β-hairpin and β-hairpin ++

UBE2M- E2 enzyme that transfers NEDD8 to CUL1.

CSN- COP9 signalosome deneddylase- deconjugates NEDD8 from CUL1, causing CUL1 deactivation.

NEDD8- Ubiquitin-like protein- conjugated to CUL1's C-terminal WHB domain.

The Prologue

Activity of the E3 ligase is dependent on CUL1 being conjugated to NEDD8. SCFs become deactivated when NEDD8 is removed by CSN. However, this deconjugation only happens when the SCF is not bound to a protein substrate. Additionally, unneddylated CUL1-RBX1 binds CAND1 which inhibits neddylation of CUL1.

Despite both CSN and CAND1 playing a role in respectively, removing or prevent neddylation, they are both required for degradation of many SCF substrates. How does this work? CSN and CAND1 are thought to be supply chain managers; CUL1-RBX1 are in lower abundance relative to the populous SKP1-Fbps, therefore CSN and CAND1 prevent simultaneous unproductive production of all possible, long-lived, SCFs.

Interlude: A brief history of CAND1

CAND1 seems to also play a role in providing Fbps access to CUL1-RBX1 via the following:

From biochemical observations, unneddylated SCFs partially release bound SKP1-Fbp subcomplex in the presence of CAND1 and CAND1 dissociates from the CAND1-CUL1-RBX1 complex when SKP1-Fbp is added.

From ubiquitylation assay observations, CAND1 was also found to allow an isolated SKP1-Fbp subcomplex to form on SCF with CUL1-RBX1, which could only happen following disassembly of another SCF.

From proteomic observations, cellular cohorts of Fbps bound to CUL1 depend on CAND1 and the neddylating and deneddylation machineries.

From structural data, so far X-rays of CAND1-CUL1-RBX1 or CUL1-RBX1-SKP1-Fbp- but never all together, didn't help piece together the mechanism of how this could happen.

Act one: Complex Rock'n'roll

[Enter neddylation assays and cryo-EM]

As the curtain lifts, the authors were able to show biochemically that CAND1 was able to facilitate SKP1-Fbp swapping that in the presence of a substrate resulted in a slow-down of deneddylation of CUL1 by CSN.

Mixed CAND1, CUL1-RBX1 and SKP1-Fbp, using different Fbps, where subject to cryo-EM and resulted in a mixture of CAND1-CUL1-RBX1 and SCFs alone. Multiple CAND1 conformations were found. First, "rolling-1"- where only the anti-neddylation domain is visibly bound to SCFs. Second, "rolling-2"- CAND1's central arch spirals away from SCF, directing CAND1's anti-SKP1 domain away from SKP1-FbP. Third, CAND1 fully visible similar structure of CAND1-CUL1-RBX1 alone. This is permissible by "rocking" of the CUL1-SKP1-Fbox interface. Authors suggest that varying conformations lower barrier for SKP1-Fbp dissociation from CUL1-RBX1 through allosteric remodelling.

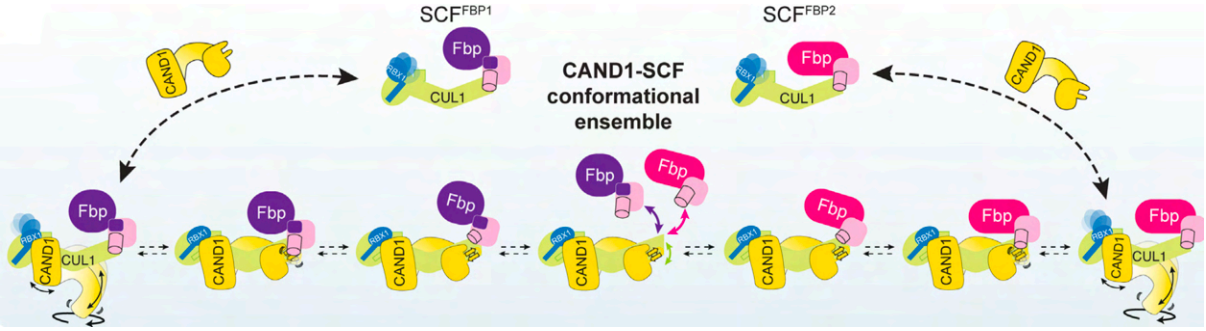
Act two: Rolling away

Looking at CAND1-SCF^{FBXW7} structures showed that CAND1's anti-SKP1 domain was disengaged from CUL1. Going through the EM maps of the CAND1-SCF complex revealed that an assortment of conformations are accessible. Generally, when SKP1 is engaged and blocking the CUL1 WHB domain, CAND1 rolls around CUL1 and has a reduced number of contacts compared to prior CAND1-CUL1-RBX1 structure, which explains CAND1 dissociation when SKP1-Fbp is bound to CUL1.

Act three: β-hairpin

CAND1 curling around SCF partially rocks the CUL1, SKP1 and F box region. Rocking of the SKP1-F box unit around CUL1 creates a crack that allows CAND1's β -hairpin to be inserted, consequently freeing CUL1 from one edge of SKP1. On the other edge interactions are maintained at a pivot point. A β-hairpin mutant was created to allow for better delineation of the role of this motif.

Act four: Putting the mechanism together



Engagement of CAND1'a β-hairpin is linked to SCF rocking and destabilisation of the complex.

Disengagement of CAND1's β-hairpin is linked to CAND1 rolling and its dissociation resulting in SCF E3 activation.

On the anti-neddylation end of the interaction, in "rolling-2" conformation CAND1 does not interact with RBX1's RING domain, which becomes poorly resolved hinting that this region is now more dynamic. CAND1's loss of interaction with RBX1 allows for a shifting in CUL1's WHB, α/β and 4HB domains (that also interact with CAND1 at the anti-neddylation domain). It's possible to imagine further rolling states that result in more loss of contacts between CAND1 and CUL1 and RBX1 that in turn allows for neddylation.

Act five: *In cellulo*

[enter cells and mass spectrometry]

The structural observations were tested in cells by observing $SCF^{\beta\text{-}TRCP}$ dependent degradation of phosphorylated IkBα, in a CAND1 and CAND2 deleted cell line which had resulted in impaired reduction of phosphorylated IkBα. In these cells degradation could be rescued by expressing CAND1 WT, however the authors found that CAND1 β-hairpin and β-hairpin ++ mutants failed to rescue.

Finally, to access the effect of WT and mutant CAND1 on CUL1 occupancy across 44 Fbps and SKP1 in CAND1 and CAND2 knockout cell line. The expression of WT CAND1 restored SCF repertoire to that of the parental cells, whereas β-hairpin mutants resulted in no restoration of SCF repertoire.

Epilogue

The ancient rivalry between CAND1 and SKP1-Fbp for CUL1's affections has thus been the two hours' traffic of our stage.

This impeccable work demonstrates the power of structural biology and the wealth of information that can be gleamed not just from high-resolution structures but also from lower resolution maps that tell a compelling story of how these characters interact with one another.

As with Shakespeare, whose plays were intended to be watched, I highly suggest viewing the stellar supplementary videos that accompany this work.

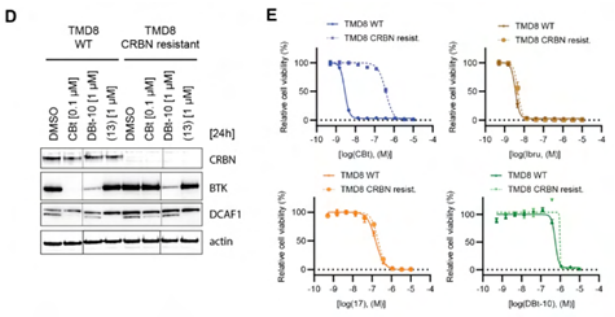
Targeted Protein Degradation

Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Ollie

Reinstating targeted protein degradation with DCAF1 PROTACs in CRBN PROTAC resistant settings

Martin Schröder[§], Martin Renatus[§] , …, Claudio R. Thoma*
bioRxiv **2023**, doi: 10.1101/2023.04.09.536153



With CBRN and VHL dominating as the E3 ligases of choice for PROTACs in the clinic, and with the emergence of resistance mechanisms in pre-clinical settings, there is an ever-increasing need to expand the repertoire of ligases in degrader design. This study from Schröder and colleagues leverages the cullin 4 substrate receptor DCAF1 for the design, development and in-depth characterization of PROTACs targeting BRD9 and BTK.

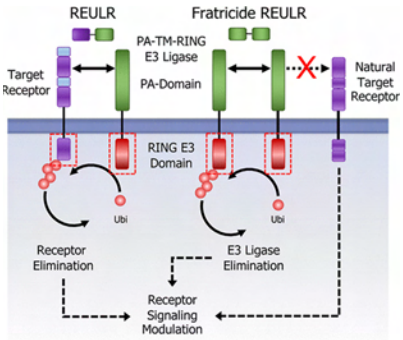
Not only is it refreshing to see novel ligases being recruited by PROTACs, but this study also nicely demonstrates the utility of employing such a strategy. Namely, the rescue of CBRN-based degrader-resistant cells by now using a DCAF1-based PROTAC for the same target. The implications extend beyond academic curiosity and may add to the strategies available to overcome any anticipated PROTAC-induced resistance in clinical settings.

Cell Biology

Contributor: Ollie

Receptor Elimination by E3 Ubiquitin Ligase Recruitment (REULR): A Targeted Protein Degradation Toolbox

Dirk H. Siepe[§], Lora K. Picton and K. Christopher Garcia*
ACS Synthetic Biology **2023**, *12*, 1081



Proteasomal TPD is no longer limited to the targeting of intracellular proteins via the recruitment of intracellular E3 ligases, with the recent availability of cell-surface strategies including AbTACs and PROTABs which are both antibody-based systems. Siepe and colleagues present here a nanobody-based modality, termed Receptor Elimination by E3 Ubiquitin Ligase Recruitment (REULR). In brief, this involves the design of heterobifunctional nanobodies (VHH) raised against one of 5 transmembrane E3 ligases coupled to nanobodies raised against receptor proteins of interest. Treatment with the POI-REULRs was shown to result in POI degradation.

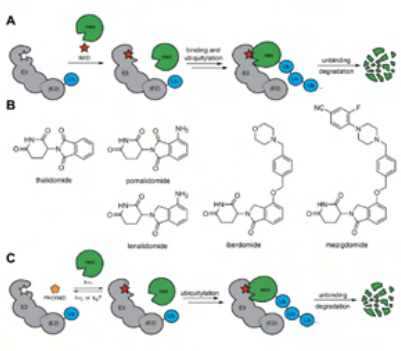
This strategy is similar to existing antibody-based strategies, but avoids some of the challenges. Namely, nanobodies are much smaller and exhibit better PK properties. There are however certain drawbacks for the immediate clinical application of nanobodies, including their rapid renal clearance. The modular nature of REULRs was also used in this study to homodimerize or heterodimerize the E3 ligases themselves resulting in loss of cell surface ligase levels and this latter finding may facilitate the study of the biological role of these ligases in different disease models and drug discovery programmes, which is made easier by the human and mouse cross-reactivity of of the E3 ligase nanobodies.

Cell Biology | Chemistry

Contributor: Ollie

Photoswitchable Molecular Glues Enable Optical Control of Transcription Factor Degradation

Christopher J. Arp[§], …, Dirk Trauner*
bioRxiv **2023**, doi: 10.1101/2023.04.09.536172



Temporal control in the TPD is an area of interest, with photo-activatable PROTACs (PHOTACs) having been recently described by various groups. In addition to adding temporal control, other use cases for light-controlled PROTACs include localization-specific activation such as in a specific disease tissue (e.g. skin) which can help to mitigate systemic toxicity issues. Arp et al demonstrate here the design of photoswtichable molecular glues, which are a traditionally monovalent TPD modality. PHOIMiDs are based on the IMiD family of molecular glue degraders, modified with an azobenzene photoswitch at the C4 or C5 positions of the pthalimide ring.

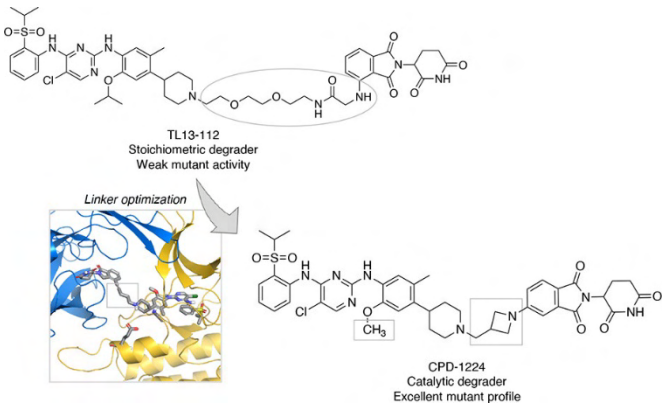
These compounds are shown here to be UV-activated and able to degrade Ikaros and Aiolos selectively over other transcription factors, opening the door to the spatiotemporal study of the degradation of these targets. The authors also suggest that this system may also be exploted in existing IMiD-inducble degron systems, adding additional granularity and breadth to TPD in fundamental biological research.

Cell Biology | Chemistry | Computational Chemistry

Contributor: Maria

Catalytic Degraders Effectively Address Kinase Site Mutations in EML4-ALK Oncogenic Fusions

Yang Gao[§], Baishan Jian[§], Hellen Kim[§], …, Nathanael S. Gray*, and Lyn H. Jones*
J. Med. Chem. **2023**, *66, 8, 5524*



Catalytic degraders present a promising solution to overcome the challenge of binding site mutants in kinase protein targeting, which has been a longstanding issue in the development of kinase inhibitors. Unlike competitive small molecule inhibitors, catalytic degraders achieve protein downregulation with exceptionally low occupancy levels, significantly lower than the stoichiometric amount required by traditional competitive inhibitors. The authors use a model of anaplastic lymphoma kinase (ALK) and echinoderm microtubule-associated protein 4 (EML4), that drive non small-cell lung cancer (NSCLC) through consistent activation of ALK by EML4. Several ALK-ATP competitive inhibitors have been developed to treat NSCLC, but they are sensible to resistance by mutation in the ATP binding site following repeated treatment, even in inhibitors of later generations that underwent optimisation for mutant profiles here, PROTACs are proposed as an alternative when binding affinity to mutants is reduced.

To validate their theory, the authors developed a series of PROTACs targeting oncogenic EML4-ALK fusions hijacking the CRBN ligase using PROTAC TL13-112 as a prototype. They performed several optimisation rounds, starting by adding flexibility to the ceritinib ALK ligand by replacing the isopropoxy group by a smaller methoxy group. Linker optimisation, supported by computational docking studies, reduced the molecular weight and H donor capacity by shortening the linker and by using a more aliphatic chain. Rigidity was also introduced at the linker to improve oral bioavalability and reduce hemolysis, keeping the linker short, they added an azetidine ring while maintaining the piperidine motif in ceritinib. On the E3 ligase ligand side, thalidome derivates containing phthalimide instead of isoindoline showed superior efficacy across the clinically relevant mutations.

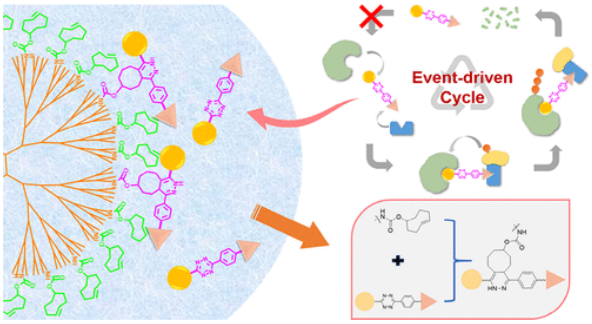
This exhaustive study provides valuable insights for the development of more effective mutant resistant kinase targeted degraders in the future.

Cell Biology | Chemistry

Contributor: Maria

Ligation to Scavenging Strategy Enables On-Demand Termination of Targeted Protein Degradation

F Yuhui Jin, …, Ning Li*, Qidong You*, Zhengyu Jiang*
J. Am. Chem. Soc. ***2023****, 145, 13, 7218*



Jin et al. present a "ligation to scavenging" platform to control the degradation event of PROTACs by scavenging intracellular free chimeras and terminating their catalytic activity. The platform is based on trans-cyclooctene functionalised PAMAM dendrimer that can scavenge tetrazine linked PROTACs via bioorthogonal reactions. The trans-cyclooctene in the PAMAM dendrimers reacts with the tetrazine linker via inverse electron demand Diels–Alder (IEDDA). The "ligation to scavenging" dendrimers successfully scavenged the Tz-PROTAC, that beared a JQ1 as the POI ligand and thalidomide the E3 ligase recruiter for Brd4 degradation. The authors demonstrate that they can terminate degradation of Brd4 in cells and prevent over-degradation of the target.

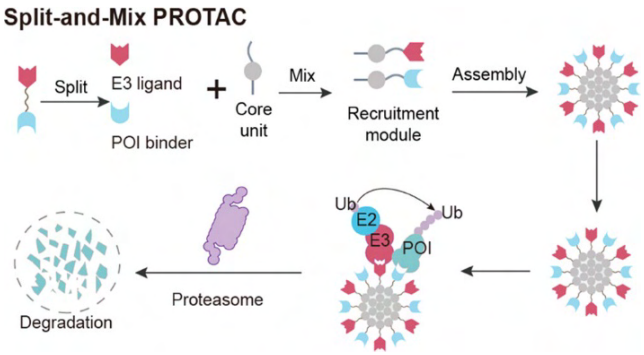
This platform is an alternative to other controlled PROTAC activity strategies that might be leaky or unable to fully terminate degradation such as photoswitchable PHOTACs or caged/activatable PROTACs and may be useful for phenotypic characterisation with live cell imaging upon treatment with the degrader. However, the system is irreversible (as opposed to PHOTACs for instance), upon exposure to the scavenging dendrimer, the PROTAC activity cannot be recovered and due to the "excess" cyclooctene composition of the dendrimer, further PROTAC treatment will be trapped immediately. Another limitation is the linker structure, which is restricted by the requirement of a tetrazine, limiting the linkerology in the PROTAC design. Finally, the scavenged PROTAC remains attached to the dendrimer via the linker, so the system might still engage the promiscuous POI/ligase targets while bound to the dendrimer.

Cell Biology | Chemistry

Contributor: Maria

Targeted Biomolecule Regulation Platform: A Split-and-Mix PROTAC Approach

Fenfang Yan[§], Qinhong Lu[§], …, Feng Yin,* and Zigang Li *
J. Am. Chem. Soc. ***2023****, 145, 7879−7887*



In this article, the authors describe a combinatorial split and mix PROTAC nanoplatform. The system consists of programmable self-assembling PROTAC "nanoballs". The so-called SM-PROTACs present POI ligands and E3 ligase recruiters at the surface allowing for binding to the protein target and recruitment of the E3 ligase of choice. The self-assembly is driven by a hydrophobic core unit consisting of a diphenyl glycine motif that is fused to the E3/POI ligand, which drives assembly by pi-pi stacking.

An interesting advantage of the platform is its programmability, allowing easy tuning of the ratio of the E3 and POI ligands to achieve optimal degradation of the desired target. Besides, the system can be extended to other combinatorial options where three or more modules can be used to include tissue specific motifs, dual POI targeting or dual ligase recruitment. This system can be a useful tool for screening of different ligases for a given target on early stage PROTAC development without the need of extensive synthesis and linkerology. The platform can also be used as an alternative to trivalent or multivalent "small molecule" PROTACs, that often require extensive optimisation on the multivalent linkers. The size of these nanospheres may affect the interaction between the POI and ligase, potentially limiting their effectiveness for complex protein targets.

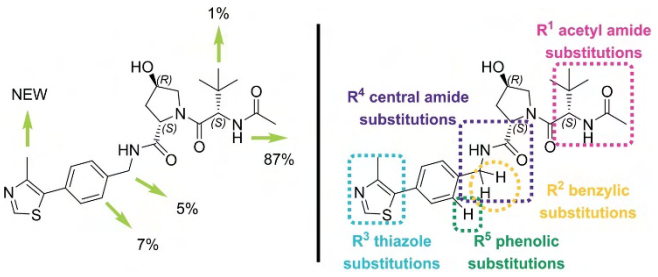
While the system has been shown to degrade various targets, including BRD2/4, MEK1/2, BCR-ABL, EGFR, and AR, its utility may be limited for more complex protein targets.

Chemistry

Contributor: Maria

Systematic Potency and Property Assessment of VHL Ligands and Implications on PROTAC Design

Johannes Krieger[§],..., Sarah Schlesiger*
ChemMedChem, **2023**, *18, e202200615*



This SAR and SPR study provides a thorough review of the latest trends in VHL ligand optimization, systematically assessing their impact on properties such as binding affinity, potency, and lipophilicity. The study covers trends found in patent literature, which often lack peer review and may not be easily accessible to the wider scientific community.

The study compares common modifications in the VHL ligand, including substitution at the acetyl amide, thiazole or the central amide motifs and explores the effect of methylation at the benzylic amide position. Findings show that substitutions at the acetyl amide position by an oxazole provide strong increase in binding affinity. In addition, replacement of the thiazole group by a phenyl or an imidazole group helps to increase lipophilicity while maintaining similar binding affinities. The authors evaluate the exit vectors at the phenolic and benzylic amide positions in VHL, that tolerate a wide variety of linkers without affecting the binding conformation, these vectors are useful alternatives to the standard acetyl amine vector, used in 87 % of the VHL based PROTACs (Diehl's insightful review offers a valuable and comprehensive analysis on the topic). Significantly, a new exit vector at the methyl-thiazole position was discovered that can provide new opportunities for PROTAC design. Using their optimised ligands, the authors built a small Brd4-PROTAC library demonstrating that the optimised PROTACs show improved degradation potency when compared to MZ1.

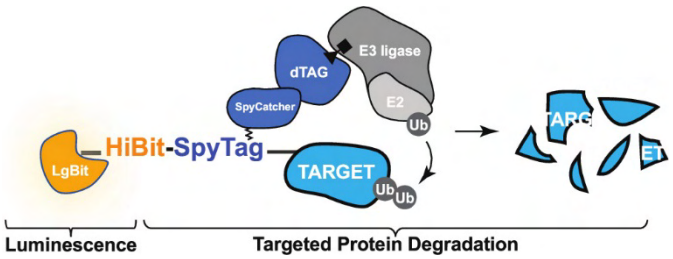
Importantly, this study compiles for the first time trends in VHL ligand modifications under the same umbrella, subjecting them to rigorous scrutiny using standardized biophysical assays, as well as consistent characterization techniques for evaluating physicochemical and ADME properties modifications. This work addresses the persistent challenge of assay variability and data accessibility when comparing results from diverse sources to inform decision-making in the development of optimal VHL-based PROTACs.

Cell Biology **Chemistry**

Contributor: Ilaria

HiBiT-SpyTag: A Minimal Tag for Covalent Protein Capture and Degrader Development

Tiffany Tsang[§], Fidel Huerta[§],..., Lyn H. Jones*, Breanna L. Zerfas*, Radoslaw P. Nowak*
ACS Chem.Biol. **2023**, *18, 993*



It is essential to validate target degradability via ubiquitin proteasome system at early stages of degrader development. In that regard, degron tags such as dTAG represent powerful tools to functionally characterize protein depletion. However, the degron tags currently available are large (>12kDa) and this limits the efficiency of their genetic knock in.

In this study the authors combined the HiBiT tag with 13 amino acid SpyTag or its other version with 14 amino acid SpyTag 002 to generate a single multifunctional tag of 24-25 amino acid long HiBiT-SpyTag/SpyTag002. The HiBiT-SpyTag fusion enables to get a readout of protein quantification via HiBiT and to covalenty capture protein fusions via d-TAG Spy Catcher with degradation induced upon dTAG degraders treatment.

Through the application of the d-TAG-SpyCatcher-HiBiT-SpyTag system the authors demonstrated for the first time the degradability of IRE1α, an attractive target for several cancers. Then, they generated a CRBN-based PROTAC CPD-2828 that selectively degrades IRE1α with $D_{max,\ 24h}$ of 65±2% and $DC_{50,24h}$ of 1.3 μM.

Whilst more studies would help to understand the factors that impact the efficiency of the labelling of the cells with the HiBiT-SpyTag and to expand the approach to different cell types, the d-TAG-SpyCatcher-HiBiT-SpyTag system proposed in this study represents a valuable tool, which undoubtedly could facilitate the assessment of target degradability and therefore guide the generation of new degraders.

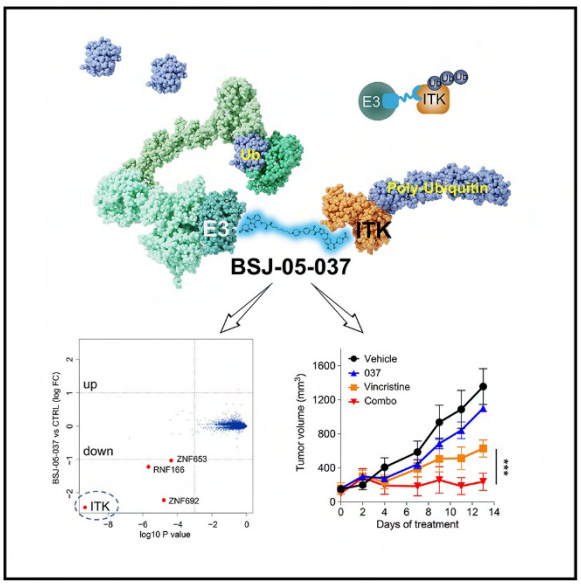
Cell Biology	Chemistry	Computational Chemistry

Contributor: Ilaria

ITK degradation to block T cell receptor signaling and overcome therapeutic resistance in T cell lymphomas

Baishan Jiang[§], …, Nathanael S. Gray*, Wenchao Wu*
Cell Chem. Biol **2023**, *30, 1*

T cell lymphomas (TCLs) account for the 10% non-Hodgkin lymphomas. Interleukin-2-inducible T cell kinase (ITK) belongs to the Tec family of kinases, and it has a crucial role in mediating TCR signalling to control T cell proliferation and differentiation. ITK is considered an attractive therapeutic target for TCLs but currently no inhibitors for ITK are available into the clinic.



In this study, the authors developed a highly selective heterobifunctional CRBN based degrader **BSJ-05-037** that induced potent ITK degradation dependent on the ubiquitin proteasome system in several TCL cells. Additionally, they showed that **BSJ-05-037** had more potent anti-proliferative effect than its parental inhibitor BMS-509744. TCR-ITK signalling through the ITK/NF-kB/GATA-3 axis facilitates the resistance to conventional chemotherapy such as vincristine in TCLs. Interestingly, they demonstrated that **BSJ-05-037** represents the first generation of CRBN based ITK degrader with in vitro and in vivo efficacy for reducing GATA-3 levels with subsequently increased sensitivity to vincristine.

Although **BSJ-05-037** is not orally bioavailable due to its limited drug properties as already stated by the authors, the contribution of this study to the field is considerable since this work provides a proof of principle of how PROTAC degraders can be combined with conventional chemotherapeutic agents and potentiate their effect overcoming chemoresistance.

Nevertheless, this study could suggest to exploit PROTAC degraders as a novel immunotherapeutic agent for cancer treatment.

Cell Biology | Chemistry

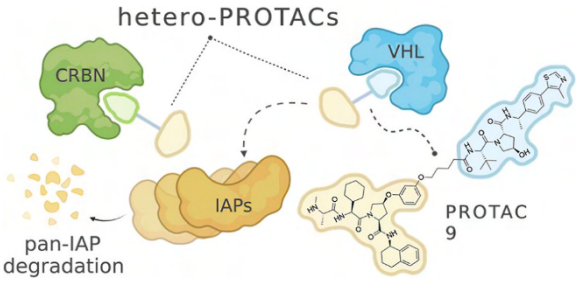
Contributor: Ilaria

Heterobifunctional Ligase Recruiters Enable pan-Degradation of Inhibitor of Apoptosis Proteins

Yuen Lam Dora Ng[§], Alesa Bricelj[§],…, Christian Steinebach*, Izidor Sosic*

J. Med. Chem. **2023**, *66, 4703*

Beyond driving ubiquitination events for TPD, several E3 ligases are involved in the control of biological processes that support tumorigenesis and therefore are considered of therapeutic relevance. Particularly, inhibitors of apoptosis proteins such as cellular IAP1, IAP2 and X-chromosome- linked IAP have been in the spotlight with several mimetics of the IAP-binding motif of SMAC (second mitochondria-derived activator of caspases) that function as endogenous IAP antagonist entering clinical trials.



The authors in this study proposed a different approach to tackle this challenging class of drug targets. They generated a heterobifunctional PROTAC based on an IAP antagonist linked to either VHL or CRBN recruiting ligand. Directing the E3 ligases against each other's lead to a potent and selective degradation of cellular IAPs including X-IAP. This study highlights the benefits of the hetero-PROTACs over monomeric or homobivalent SMAC mimetics in reaching pan-IAP selectivity thus boosting the interest in the TPD field for further development of heterobifunctional degraders targeting relevant ligases.

Cell Biology | Chemistry

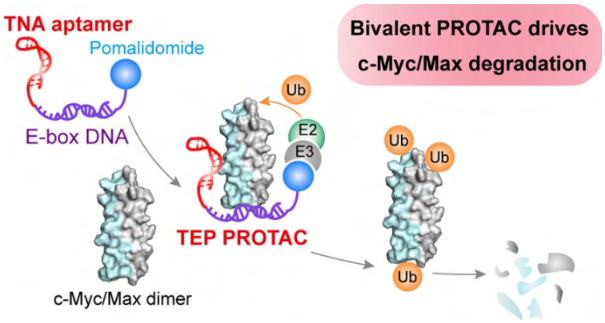
Contributor: Ilaria

c-Myc-Targeting PROTAC Based on a TNA-DNA Bivalent Binder for Combination Therapy of Triple-Negative Breast Cancer

Xintong Li[§] ,…, Hanyang Yu*, Xiaoxiang Guan*

J. Am. Chem. Soc, **2023**, *145, 16, 9334-9342*

c-Myc is a pleiotropic transcription factor, and it forms an obligate heterodimer with its partner Max for the binding of the DNA enhancement box E through which regulates the hallmark features of cancer. In multiple malignancies, including triple negative breast cancer (TNBC) c-Myc represents a tumour driver. Therefore, c-Myc is among the most enticing targets for drug discovery. In the TPD field, the development of potent PROTAC degraders for targeting transcriptional factors such as c-Myc is still a challenge due to the lack of defined ligand binding pockets in TFs. To circumvent this limitation, the authors in this study proposed an alternative approach with the design of a nucleic acid based PROTAC for targeting c-Myc. Indeed, they generated a bivalent binder for c-Myc/Max heterodimer consisting of TNA aptamer and the cognate DNA E-box that was further conjugated with a pomalidomide ligand. They demonstrated that the TNA-E box pomalidomide (TEP) mediates proteasomal degradation of endogenous c-Myc/Max protein showing also the most potent effect of TEP over small molecule inhibitor MYCi975 in inducing c-Myc degradation. Additionally, TEP inhibited TNBC cell proliferation, and it showed a synergistic effect with Palbociclib, an FDA approved cyclin-dependent kinase inhibitor for metastatic TNBC, in exerting a potent anti-tumour effect both in vitro and in vivo.



It will be interesting to further explore the efficacy of TEP PROTAC also in other tumours where c-Myc is a relevant clinical target, as this would be of significant utility to broaden the space of this promising nucleic acid based PROTAC for cancer treatment.

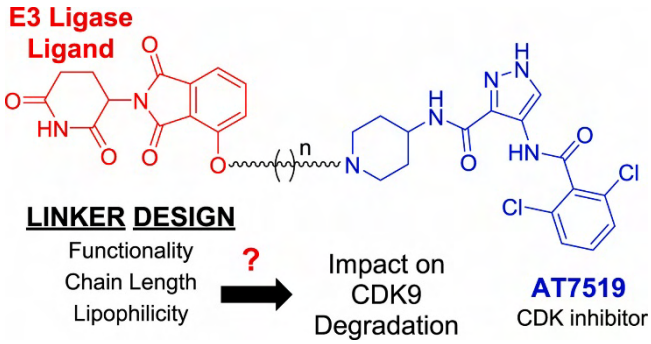
Cell Biology | Chemistry

Contributor: Maria

Bifunctional degraders of cyclin dependent kinase 9 (CDK9): Probing the relationship between linker length, properties, and selective protein degradation

Robert J. Tokarski[§], Chia M Sharpe[§], …, James R. Fuchs*

Eur. J. *Med*. Chem. ***2023***, 254, 115342



DK9 emerges as a promising therapeutic target due to its involvement in transcriptional regulation and its association with various cancers. While several PROTACs have been developed to target CDK9 through CRBN recruitment, the structural variability of CDK9 inhibitors and linker compositions has resulted in significantly different activity profiles. In this study, the authors aim to systematically evaluate the influence of linker structure on CDK9-targeting degraders and identify patterns for optimal design. They synthesize a library of PROTACs based on AT7519, a CDK9 ligand with promising results in Phase 2 clinical trials for cancer treatment, and a 4-hydroxythalidomide as the CRBN recruiter. The library includes the previously reported AT7519/CRBN PROTAC degrader by Zhou *et al.*, as well as degraders with variable linker lengths, compositions, and connectivity to the ligands. The authors assess the impact of linker modifications on the physicochemical properties of the degraders, their target degradation efficacy, and specificity in cells. Interestingly, despite demonstrating high potency for CDK9 degradation in western blot analysis, some of the most promising PROTACs do not exhibit the same level of potency in cell-based assays, with reductions observed in some cases of up to five-fold. This observation underscores the challenges associated with translating physicochemical properties to cellular assays and potentially in vivo scenarios.

This article shreds light on the importance of linker structure and its impact on the efficacy and specificity for CDK9 degradation. The findings enhance our understanding of PROTAC design strategies and offer guidance for the development of more effective CDK9-targeting therapeutics with potential clinical relevance.

Other Paper Highlights

Chemistry

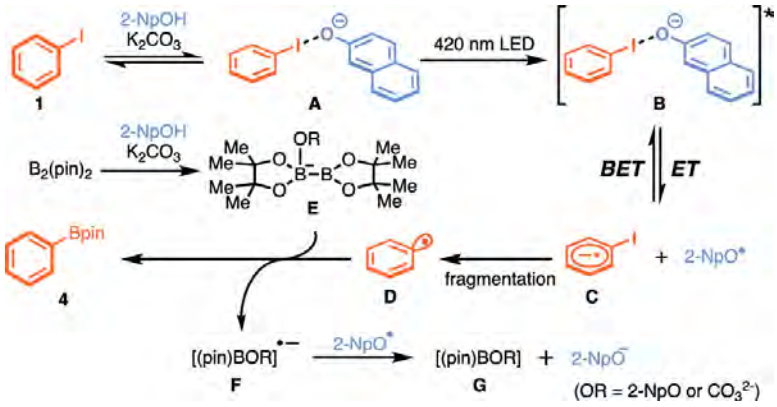
Contributor: Petr Zhmurov

Halogen-Bonding-Promoted Photoinduced C–X Borylation of Aryl Halide Using Phenol Derivatives

Kazuki Matsuo, Eiji Yamaguchi* and Akichika Itoh*

J. Org. Chem. **2023**, *https://doi.org/10.1021/acs.joc.3c00201*

An intriguing technique for arylboronates synthesizing was published by a group of scientists from Gifu Pharmaceutical University. Their approach involves a photoinduced C-H borylation of aryl halides, promoted by halogen bonding without using a complex Ir-based catalyst. The reaction mechanism was thoroughly analyzed and a varied series of borylated substrates was synthesised in moderate to high yields and exhibited excellent regioselectivities.



There was no information about arene homocoupling, however reaction required 3 eq. of bis(pinacolato)diboron, which can be a problem during purification. Moreover, basic conditions were used so the hydrolysis of the remaining diboron reagent is expected. On the other hand, use of mild reaction conditions also helps to prevent *in situ* decomposition of unstable boronates.

While this technique cannot replace Miyaura borylation and lithiation-borylation Methodology, it could prove to be a valuable tool for more complex late-stage functionalization reactions.

Cell Biology **Structural Biology/Biophysics**

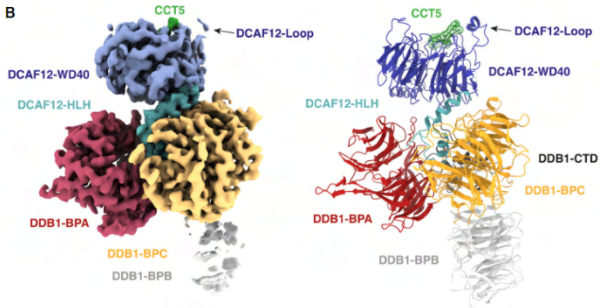
Contributor: Ollie

Recognition of the CCT5 di-Glu degron by CRL4^{DCAF12} is dependent on TRiC assembly

Carlos Pla-Prats[§], …, Nicholas H. Thomä*

The EMBO J. **2023**, *42*, e112253

DDB1 and CUL4-associated factors (DCAFs) are a large family of substrate receptors, with one of the most well-known members being CBRN – famed for its involvement in IMiD mode-of-action and concomitant tractability for PROTAC design. Other family members include DCAF15 and DDB2. There is a field-wide push to determine the molecular mechanism for substrate recognition for CRL substrate receptors, and the present study comprehensively characterizes CRL4^{DCAF12} recognition of the di-glutamate-containing TRiC subunit, CCT5.



Pla-Prats and colleague solve here the Cryo-EM structure of DDB1-DCAF12-CCT5 following the validation of CCT5 as a substrate for DCAF12. The structure reveals that DCAF12 binding to CCT5 via its C-terminal di-Glu degron is incompatible with the TRiC chaperonin complex assembly, and the authors suggest that the ligase thus acts as a sensor and an assembly quality control (AQC) regulator for TRiC assembly and disassembly. CRL4^{DCAF12} may play a role in the assembly/dissasembly of other protein complexes with subunits harbouring di-Glu motifs which are only solvent exposed when monomeric and not assembled in the complex, in addition to ubiquitin-independent functions.

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