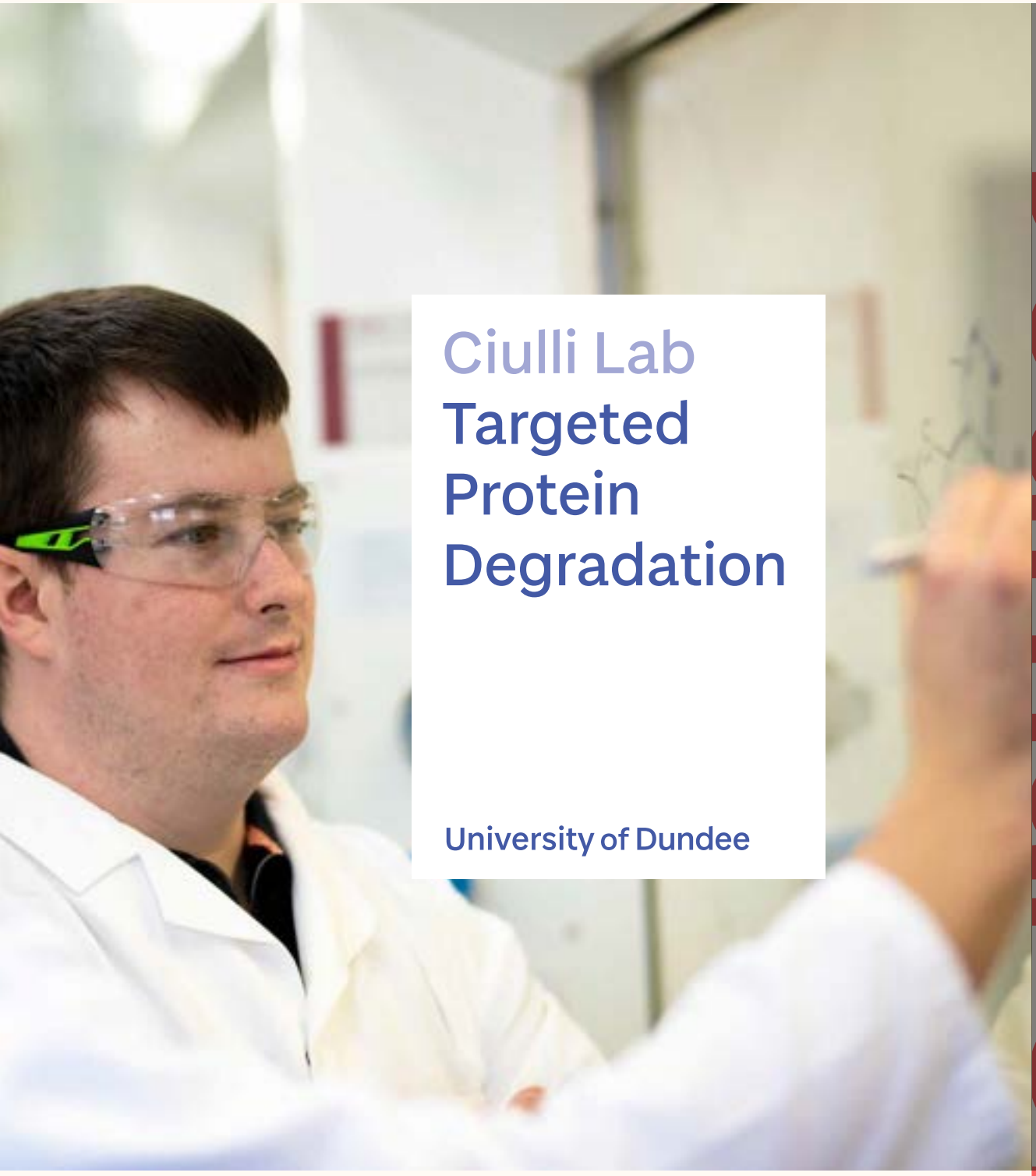
Targeted protein degradation, medicinal chemistry & chemical structural biology literature highlights



Ciulli Lab
Targeted Protein Degradation

University of Dundee

Journal Club

January 2022

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Meet this Month's Editors

This month's editors are (from left to right): Aina Urbina Teixidor, Kevin Haubrich and Sohini Chakraborti

"This Journal Club is an amazing resource to keep us up-to-date with the latest discoveries in the targeted protein degradation field."

Aina completed her undergraduate degree in Pharmacy before pursuing her Ph.D. in Organic Chemistry in the research group of Prof. Joan Bosch and Prof. Mercedes Amat at the University of Barcelona. In May 2021, she joined the Ciulli group in the AC-Almirall collaboration as a synthetic chemist to develop novel PROTACs, keen on expanding her knowledge of structure-based drug design and protein-ligand interactions.

"Most scientists share the feeling not to read enough. The journal club is a reminder that reading widely and beyond our own narrow areas of research is an essential part of science and encourages us to dedicate time to it."

Kevin obtained his BSc and MSc in Chemistry from the Heidelberg University and pursued a Ph.D. at EMBL, where he explored the interplay of RNA binding and catalytic activity in the E3 ligase TRIM25 using NMR, SAXS and other biophysical techniques. He joined the Ciulli group in November 2020 where he studies E3 ligases but with a new focus on the development of chemical probes.

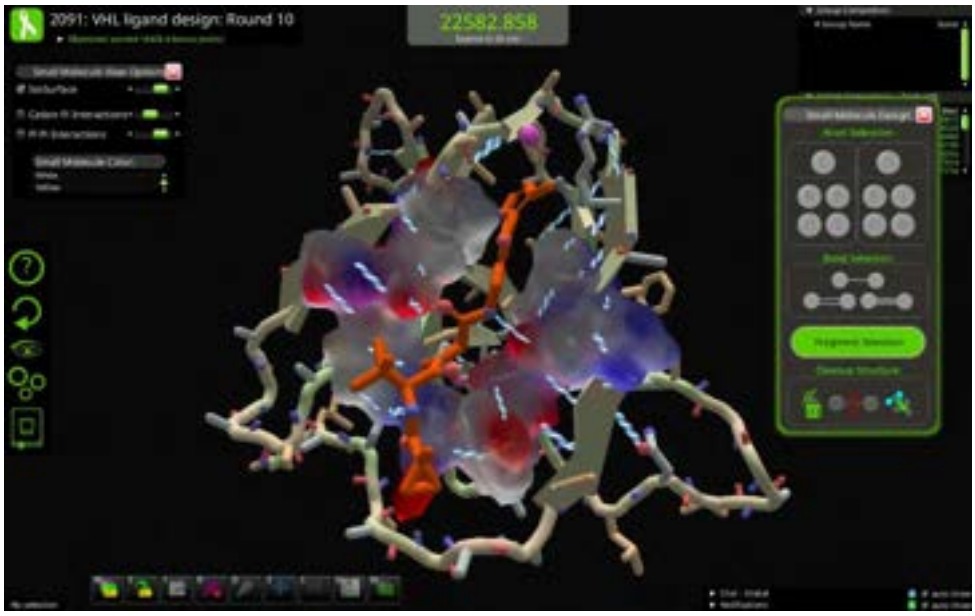
"*The Journal Club is a treasure that helps to navigate through exciting recent literature, and it was a joy to contribute to this resource."*

Sohini joined the Ciulli group in November 2021 as a computational drug design and molecular modelling scientist to work in the AC-Boehringer Ingelheim team. She received her undergraduate degree in Pharmacy from Jadavpur University and an M.S. (Pharm.) in Pharmacoinformatics at NIPER-Hajipur before pursuing a Ph.D. in Computational Biophysics in the research group of Late Prof. N. Srinivasan at the Indian Institute of Science, Bangalore.

Gamifying VHL binder design with DrugIt

Contributor: Valentina

Special thanks to Dr. Chris Smethurst.



DrugIt is a small molecule drug design game that harnesses the Foldit platform and is the product of a collaboration between Boehringer Ingelheim (BI) and Vanderbilt University and it confirms what we have always (secretly) suspected about medicinal chemistry - it's all a game! DrugIt launched its VHL binder design puzzles in October 2021 and presented players with ten rounds to flex their drug design muscles. The final round closed in January 2022. Each round gave the players different design objectives, exposing them to key drug design concepts, with the central theme being to optimise and create novel VHL binders. For example, the objective in the first round was to optimise a known VHL binder, which had several amide groups, to make it less peptide-like, with players scoring higher for having fewer amide bonds. However, chemistry even when gamified, isn't quite that simple and players had to simultaneously monitor changes to criteria such as the ligand's hydrogen bond donors, topological polar surface area and of course that all important synthetic feasibility. Balancing out these different considerations guides the player through the mind of a medicinal chemist and the decisions and compromises that are made when designing compounds.

What is truly unique about this game is that virtual success will become a reality as BI has committed to evaluate molecules that the players have designed and to synthesise the compounds that pass their internal criteria for small molecule development. These compounds will then be tested in binding studies and structural biologists will attempt to solve crystal structures for any successful protein-small molecule complexes.

This project is part of BI's continued efforts to support open science, an area where BI is a firm leader, mostly notably through their opnMe initiative, in which pre-clinical compounds are made available for free in an open manner for non-clinical research. DrugIt is the brainchild of Dr. Chris Smethurst, from BI, who told us that we "first had the idea in 2015. The idea from the outset was to help democratize drug discovery as far as possible. Rather than develop a tool on our own we decided to approach the Foldit community to see if they wanted to move in the direction of small molecule design". The scope of delivering a project like this, that needs to have wide appeal does not come without challenges. Dr. Smethurst told us that one of the key considerations was "trying to make the design games appeal to and be playable by many different people, ranging from experts through to complete novices". Indeed, every aspect of putting together DrugIt required careful planning including the "complex task" of aligning on target selection. So, bringing this novel idea to players is a testament to the team's determination especially considering they were "working with limited resources to create the tool and run the games".

We encourage our Journal Club readers to keep their eyes peeled for future DrugIt puzzles that will be available in the second quarter of this year. You can also get the latest updates on the project by following the DrugIt team on Twitter!

An Interview with: Hannah Kiely-Collins

Contributor: Charlotte

Alessio usually advertises the Ciulli lab Journal Club at the end of his webinars. On one occasion in March 2021 Hannah Kiely-Collins, a PhD student at the University of Cambridge, was in the audience. She suggested including interviews of TPD researchers – therefore unknowingly volunteering herself as our first candidate!

Hannah and I met up via Teams last Thursday morning. It was a slightly grey day in Dundee, I was reliably informed the weather was pretty similar in Cambridge. Hannah started by telling me a little about her background and how she got into TPD: She did her MSci in Chemistry with Medicinal Chemistry at Imperial College London. Her Master's thesis in the Barnard group was concerned with photocrosslinkable α-helix mimetics of P53, so here she gained interest in protein-protein interactions. She also first heard about TPD through Prof. Ed Tate, who had recently started working in the field.

Now, she is in the 3rd year of her PhD in the Bernardes group. Although Hannah is a medicinal chemist, she has also delved into various areas of biology, for example establishing and optimising cell-based assays to study the activity and toxicity of the compounds she has made. To begin with, Hannah's project was distinctive from the research being done in the rest of the lab – she was the first person in her lab to work on TPD and making PROTACs. However, these PROTACs were based on a ligand for a natural product, one of the Bernardes' group's main areas of research.

For Hannah, one of her favourite parts about her work has been using structural information – in this case from CryoEM with her initial set of PROTACs – to guide the rational design of her new PROTACs. In addition, after reading this paper (Zaidman *et al.*, 2020 also covered by Adam in our June 2020 Journal Club), Hannah was keen to collaborate with the authors to computationally design PROTACs for her project. After putting together PowerPoint slides and presenting her case to her supervisor in support of starting a collaboration, she found out that Daniel Zaidman (the paper's first author) would be joining the lab as a postdoc. Now, there is a small TPD team within the lab.

During the March 2020 lockdown, Hannah was also able to author a TPD paper on reversible and irreversible covalent PROTACs (also covered in April 2021 Journal Club). The paper was spurred by the fact there were not so many papers on covalent PROTACs at the time and there was controversy as to whether they worked – Hannah wanted to "address this controversy, showcasing PROTACs which do work and encourage more researchers to develop covalent PROTACs". She finds it particularly interesting that PROTACs can be covalent on E3 ligases, particularly as it is important for expanding scope, for tissue and degradation selectivity and matching a target to an E3 ligase.

When asked about her motivations for doing chemical biology research, Hannah had two very specific answers: 1. She was inspired after reading the Stuart Schreiber article Small molecules: the missing link in the central dogma and 2. Being involved in contributing to the target 2035 A probe for every protein. Also, being a PhD student means developing a valuable skillset and having the freedom to read papers and then incorporate the ideas or the strategy inter her own project. A cool example of this is after she read a paper about CANDDY degraders, she was able to synthesise the modified proteasome ligand and linker to enable her to explore this novel chemical knockdown strategy in future.

As a reasonably new PhD student myself, I was curious to know what Hannah's top suggestions for PhD students entering the field of TPD were. Her advice was:

- To "religiously" read the Ciulli lab Journal Club (she said it, not me!)
- To watch the Dana Farber Targeted Protein Degradation webinar series which are especially useful for relating to research groups and their work
- To join Twitter – for a little while Hannah was the only person in her group to work on PROTACs, so TPD Twitter helped her feel a part of the TPD community. Twitter is also helpful with mental health advice for researchers, for feeling part of the PhD community, and the science memes are good!
- To read some of the many really great TPD reviews

After our chat, I asked Hannah if she would be interested in guest editing one of our future Journal Club issues – she said yes, so stay tuned for her joining the editorial team in summer 2022!

I really enjoyed being able to spend 30 minutes of my day meeting someone new in the TPD field, being able to chat 1:1 and learn about what Hannah does in the context of TPD. Perhaps an interesting idea could be to have occasional virtual TPD "coffee mornings" pairing up students and early career researchers for half an hour?

With special thanks to Hannah! It was fantastic to be able to talk to you, look forward to interacting more in the future!

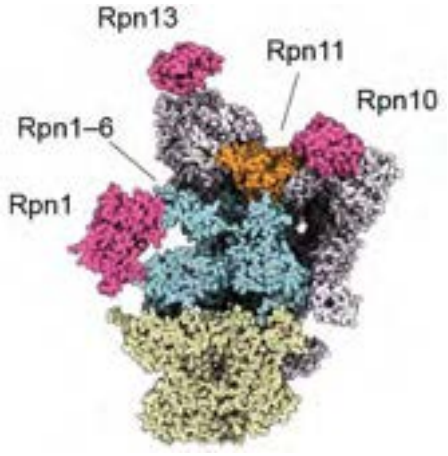
Structural Biology/Biophysics

Contributor: Kevin

Structural and biochemical elements of efficiently degradable proteasome substrates

Takuya Tomita§*

J. Biochem. **2021**, DOI: 10.1093/jb/mvab157



TPD by PROTACs is a complex multistep process of binary target recruitment, complex formation, neosubstrate ubiquitination and proteasomal degradation. While a lot of work has been done on the first two steps of this process, ubiquitination and proteasomal degradation are rarely studied separated from each other. This review however makes an excellent case for the importance of better understanding the mechanism and structural requirements for efficient degradation by the proteasome. It discusses the role of different ubiquitin chains in recognition by the proteasome, requirements for the initialisation sequence, the influence of mechanical stability against pulling forces and the role of substrate remodelling.

Of relevance to TPD is the discussion of the requirement for ubiquitin chains to position the initialisation sequence in such a way that it can be pulled into the gate, leading to potentially very different degradation efficiencies for different ubiquitination sites. Reading the review left me with the question how many PROTACs fail not because they don't induce neosubstrate ubiquitination, but rather because they engage targets that are particularly resistant to proteasomal degradation or cause ubiquitination on sites that are unsuitable to induce degradation.

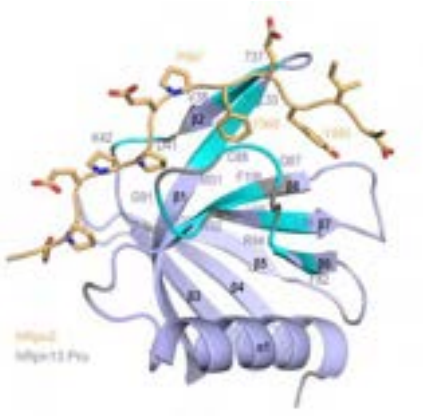
Cell Biology | Structural Biology/Biophysics

Contributor: Kevin

Structure-guided bifunctional molecules hit a DEUBAD-lacking hRpn13 species upregulated in multiple myeloma

Xiuxiu Lu §, …, Kylie J. Walters*

Nat. Commun. **2021**, *12*, 7318



The list of proteins that can be degraded by PROTACs is continuously expanding, but this paper adds a particularly odd candidate to it: hRpn13 is a subcomponent of the 26S proteasome and together with Rpn1 and Rpn10 recognizes ubiquitin chains. In this study, ligands that covalently target a cysteine in the Pleckstrin-like receptor for ubiquitin (Pru) domain were identified in a virtual screen and validated by DSF and NMR. A ligand with 1.5 µM K_D and reduced off-target binding compared to previous hRpn13 ligands was identified. Through detailed structural characterisation by NMR a suitable attachment point for PROTAC development was identified and a selection of PROTACs recruiting VHL, CRBN and IAP were designed and tested. A VHL-recruiting PROTAC preferentially degraded an isoform that carried the intact Pru domain, but lacked the DEUBAD domain, that interacts with the Pru in the absence of ubiquitinated substrate. While D_{max} and DC_{50} might not be impressive, it is still remarkable that a PROTAC can target a protein for degradation that acts as a gear in the machinery required for its own degradation.

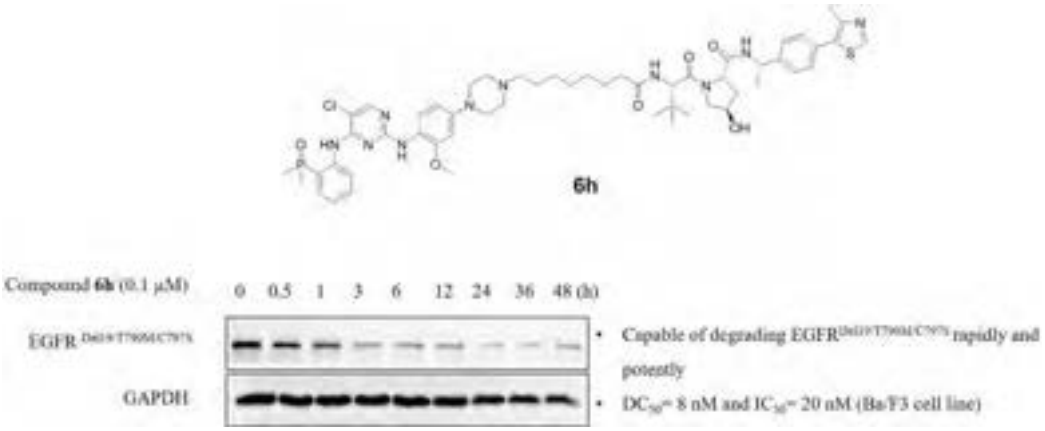
Cell Biology | Chemistry

Contributor: Aina

Design, Synthesis, and Biological Evaluation of Novel EGFR PROTACs Targeting Del19/T790M/C797S Mutation

Hualin Zhang[§], Ruliang Xie[§], …, Jian Li*, Fang Xu*, Tianfeng Xu*

ACS Med. Chem. **2022**, DOI: 10.1021/acsmedchemlett.1c00645



Lung cancer is one of the most commonly diagnosed cancer worldwide, the non-small cell lung cancer (NSCLC) being the most prevalent. To treat NSCLC, many efforts have been made to inhibit the epidermal growth factor receptor (EGFR) at its ATP-binding site. Unfortunately, acquired drug resistance to EGFR inhibitors emerged shortly after administering to the patients, due to several mutations (L858R or Del19, T790M, C4797S).

In this paper, the authors report a new series of EGFR brigatinib based PROTACs that were selectively targeting Del19/T709/C797S-mutated EGFR. All PROTACs were evaluated *via* antiproliferation and degradation assays in Ba/F3-EGFR cells, where compound **6h** was identified as the most active degrader (IC_{50} = 20 nM). Further studies demonstrated that the representative compound **6h** reduced $EGFR^{Del19/T709/C797S}$ protein levels in Ba/F3 cell in a dose- and time-dependent manner. The protein reduction was mediated specifically by the VHL-associated proteasome pathway. The degrader **6h** also achieved an excellent DC_{50} value of 8 nM and efficiently blocked the activation of EGFR downstreams signals ERK and AKT.

The potential ability of PROTACs to overcome clinical drug resistance could be applied in the case of **6h**. This could lead to a new class of treatment for resistant NSCLC.

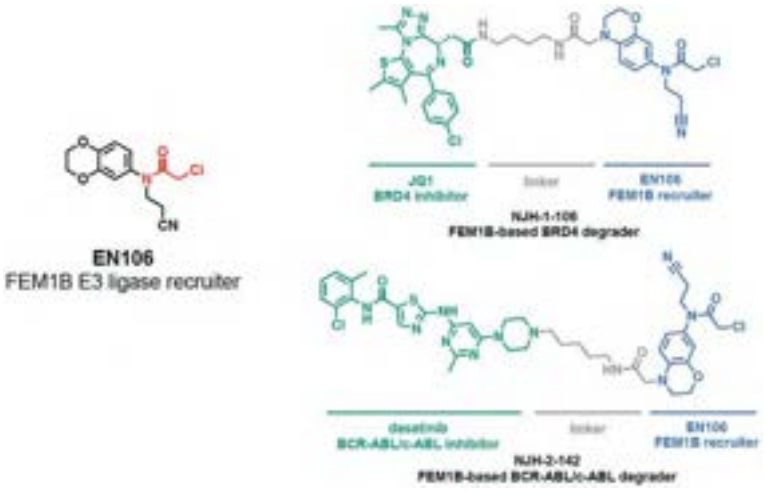
Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Aina

Discovery of a Covalent FEM1B Recruiter for Targeted Protein Degradation Applications

Nathaniel J. Henning[§], Andrew G. Manford[§], …, Michael Rape*, Daniel K. Nomura*

J. Am. Chem. Soc. **2022**, *144*, 701



Targeted protein degradation (TPD) is based on compounds which induce the proximity of E3 ubiquitin ligases to target proteins of interest and lead to their degradation. This field has become a sought-after therapeutic modality in drug discovery because of its potential ability to degrade any disease-causing protein in the cell, including classically undruggable targets. Currently, only a small number of E3 ligase recruiters are available for over 600 E3 ligases that exist in human cells.

The usefulness of chemoproteomics-enabled covalent ligand discovery platforms to disclose

novel covalent E3 ligase recruiters for targeted protein degradation was initially revealed by Backus *et al.*, where they demonstrate the ligandability of E3 ligases with cysteine-reactive covalent ligands. Since then, similar strategies have been used to discover and apply additional covalent ligands that recruit E3 ligases as protein degradation triggers.

Recently, the CUL2 E3 ligase FEM1B was discovered as a critical regulator of the cellular response to persistent depletion of reactive oxygen species. Reductive stress arises from prolonged antioxidant signaling or mitochondrial inactivity and can block stem cell differentiation or lead to diseases. In this study, the authors screened a library of cysteine-reactive covalent ligands in a competitive fluorescence polarisation assay to discover a covalent FEM1B recruiter, the chloroacetamide **EN106**. Further work demonstrated that **EN106** or its derivative **NJH-2-030** functionally engaged FEM1B in cells without detectable off-target effects and that it can target the endogenous E3 ligase. Furthermore, they synthesised a series of FEM1B-based BRD4 and BCR-ABL/c-ABL degraders, to demonstrate that **EN106** could be used as a covalent FEM1B recruiter in TPD applications.

Although more medicinal chemistry efforts are required to improve potency, selectivity and drug-like properties, **EN106** represents the first synthetic small-molecule ligand for FEM1B. **EN106** could be an attractive tool in the targeted protein degradation field.

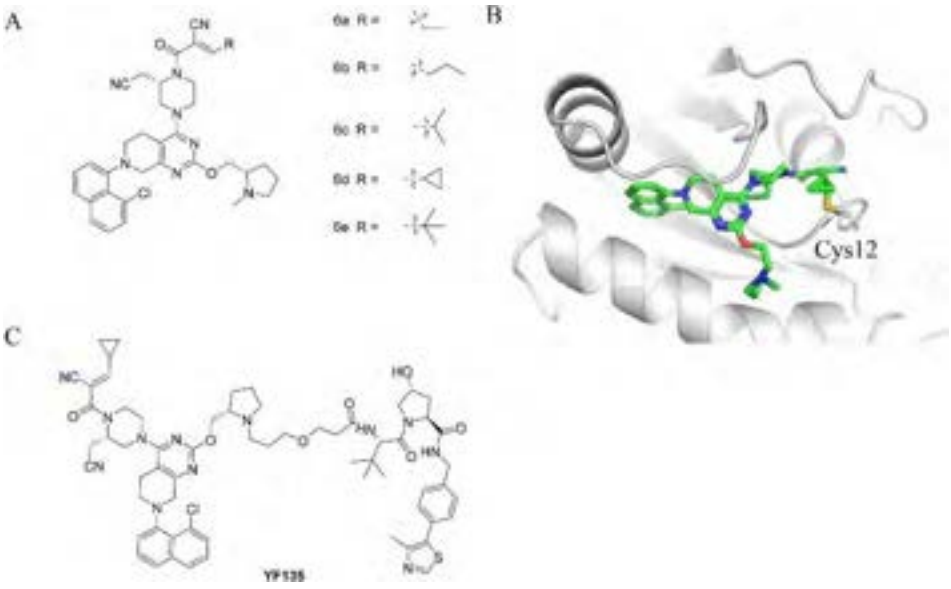
Cell Biology	Chemistry	Computational Chemistry	Modelling/Simulation

Contributor: Sohini

Efficient targeted oncogenic KRAS^{G12C} degradation via first reversible-covalent PROTAC

Fang Yang[§], Yalei Wen[§], …, Tongzheng Liu*, Xiaoyun Lu*

Eur. J. Med. Chem. **2022**, *230*, 114088



The most frequent mutation on KRAS in lung cancer is G12C. The Cys12 provides an opportunity to covalently target KRAS^{G12C}. Chemical degradation of KRAS^{G12C} could be a potential therapeutic strategy for lung cancer. However, the irreversible binding mode of PROTACs may compromise the substoichiometric activity of PROTACs, thus, decreasing the potency.

In this work, the authors report the development of the first reversible-covalent PROTAC (**YF135**) that induces VHL mediated proteasomal degradation of KRAS^{G12C}. They introduced a series of cyanoacrylamide-based warheads to the piperazine part of MRTX849 (a known KRAS^{G12C} inhibitor) employing structure-based design approaches. The inhibitory activity of the synthesized compounds against KRAS^{G12C} were evaluated by HTRF assay. Molecular docking studies with the most potent compound (**6d**) suggested that it occupies the allosteric S-IIP of KRAS^{G12C} with a similar binding mode to that of MRTX849 – a trans configuration of the covalently bonded cyanoacrylamide with Cys12 was noted. The docked pose of **6d** further indicated that the methylpyrrole group was exposed to solvent which served as an exit vector to link an E3 ligase ligand for the PROTAC design. Reversible covalent binding mode of **6d** was confirmed by MALDI-TOF-MS and dialysis assay. Subsequently, the PROTAC **YF135** was synthesized by tethering **6d** with a VHL ligase ligand and ethylenedioxy as a linker. **YF135** inhibited the proliferation of H358 and H23 cells with IC50 values of 153.9 nM and 243.9 nM, respectively. **YF135** decreased the level of KRAS^{G12C} and phospho-ERK in a dose-dependent manner with DC_{50} values of 3.61 mM and 1.68 mM in H358 cells, respectively. In H23 cells, it also dose-dependently decreased the level of KRAS^{G12C} and phospho-ERK with DC_{50} values of 4.53 mM

and 1.44 mM, respectively. However, no effects on inhibition and levels of either KRAS protein or phospho-ERK were observed in A549 cells harboring $KRAS^{G12S}$ mutation. The ternary complex of **YF135** with $KRAS^{G12C}$ and VHL was modelled to gain insights into the molecular mechanism of degradation.

Overall, the results from this study indicate that **YF135** is an effective and selective $KRAS^{G12C}$ reversible-covalent degradation agent and could be pursued as a promising new lead for the development of $KRAS^{G12C}$ degraders. The combination of rational structure-based design, molecular modelling, medicinal chemistry, and cell biology approaches used in the study could undoubtedly guide the development of future reversible covalent PROTACs.

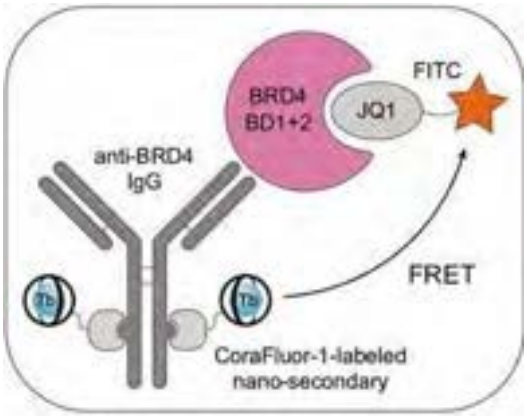
Cell Biology | Structural Biology/Biophysics

Contributor: Will

A direct high-throughput protein quantification strategy facilitates discovery and characterization of a celastrol-derived BRD4 degrader

N. Connor Payne[§], Semer Maksoud, Bakhous A. Tannous, Ralph Mazitschek*

BioRxiv **2021**, DOI: 10.1101/2021.12.08.471806



Within the targeted protein degradation field and others there is a growing need to generate assay methodologies that are both high-throughput and accurate for the detection of target protein levels. Whilst significant progress has been made such as via use of ELISA, capillary electrophoresis or the Promega split nanoluciferase system, they all have caveats. In particularly few methods currently present an option for accurate detection of endogenously expressed proteins in 96-well plate format without the need for time consuming cellular engineering. Here, the authors present an approach that can be utilised in two different modes, either towards measurement of target engagement or for measurement of target protein levels. To do this, they employ a Förster resonance energy (TR-FRET) based platform. This simple approach uses an antibody for the protein of interest in combination with a nano-secondary antibody labelled with CoraFluor-1. They then utilise a protein of interest tracer consisting of binder (in this BRD4 ligand JQ1) labelled with FITC. The assay set-ups can then be formatted according to the desired read-out and were miniaturised into 96-well format. The authors use this assay to direct them towards the discovery of celastrol-derived BRD4 degraders which may function via the recruitment of several E3 ligases including KEAP-1.

Whilst the application detailed here, with respect to recruitment of E3 ligases with a celastrol derived ligand, leaves a number of unanswered questions regarding mechanism of action, the assay platform presented will find significant utility. This study proposes a much-desired solution towards the measurement of endogenous level proteins of interest in unmodified cell lines in a robust and high-throughput manner. The requirements for the assay also play to the strengths of degrader approaches, or example tracers of this kind (or similar) are often already generated for other degrader related assay platforms. A technical caveat may be the 1-hour wash-out step utilised prior to lysis of cells, potentially limiting the assay to use in longer time-point, end-point assay set-ups and applicability to more than just a single protein of interest is needed.

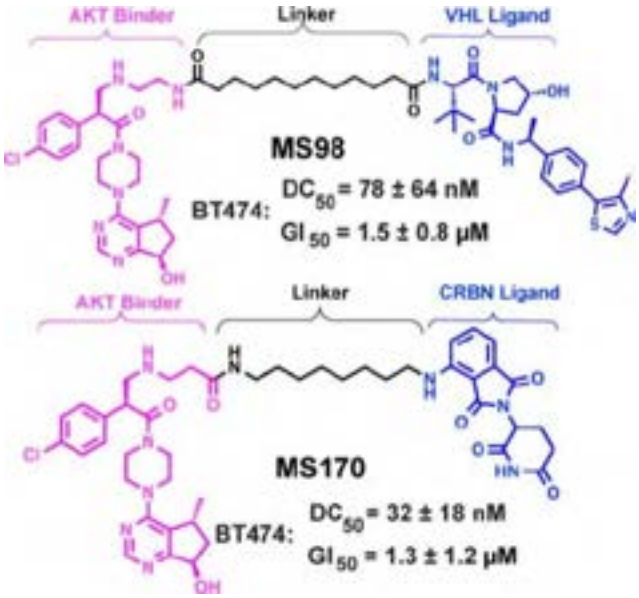
Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Aina

Design, Synthesis, and Evaluation of Potent, Selective, and Bioavailable AKT Kinase Degraders

Xufen Yu[§], Jia Xu[§], …, Jing Liu*, Ramon E. Parsons*, Jian Jin*

J. Med. Chem. **2021**, *64*, 18054



AKT has been recognized as an oncogenic therapeutic target for several decades, as it is an important component of the PI3K/AKT/m-TOR signalling pathway and regulates fundamental cellular and physiological processes. This signalling pathway is one of the most frequently dysregulated pathways in the initiation and propagation of human cancers. Several AKT inhibitors have been reported which are currently being investigated in clinics for oncology applications. Despite some success, there are some limitations to AKT inhibitors such as lack of selectivity or low antitumor activity. In the TPD field, only two AKT degraders have been published but lacking any structure-activity relationship (SAR).

This publication explores the use of GDC-0068, an ATP-competitive inhibitor, to construct PROTACs with the aim of studying their SAR and degrading AKT. The cocrystal structure of AKT1 with GDC-0068 was used to identify a solvent-exposed site, suitable for linker attachment. Several VHL- and CRBN-based PROTACs were synthesised. After a preliminary degradation assay in BT474 cells, compounds **MS98** and **MS170** were selected for further biological characterizations and pharmacokinetic studies in mice. These two compounds selectively induced robust AKT protein degradation, inhibited downstream signalling, suppressed cancer cell proliferation and exhibited good plasma exposure levels in mice.

Comprehensive SAR studies resulted in the discovery of two novel, potent and selective AKT PROTAC degraders. These compounds could be potentially useful chemical tools to investigate biological and pathogenic functions of AKT *in vitro* and *in vivo*. It would be valuable to compare this AKT degradation approach with AKT inhibitors.

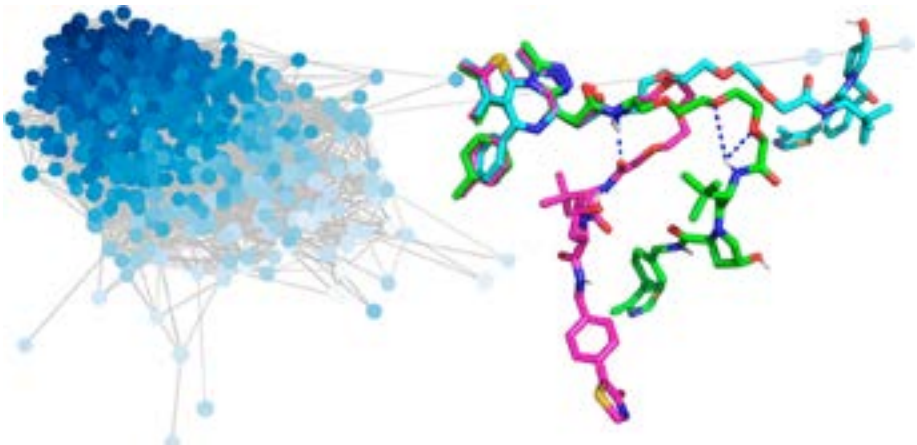
Computational Chemistry | Structural Biology/Biophysics

Contributor: Kevin

Impact of PROTAC Linker Plasticity on the Solution Conformations and Dissociation of the Ternary Complex

Dhanushka Weerakoon[§], Rodrigo J. Carbajo[§], Leonardo De Maria, Christian Tyrchan, Hongtao Zhao*

J. Chem. Inf. Model. **2022**, *62*, 340



The linker connecting the warhead and the neosubstrate binding moiety of a PROTAC plays a crucial role in complex stability and specificity, as it determines the entropic penalty involved in binding.

In this study, the conformation of PROTACs MZ1 and dBET6 were investigated using ROESY-based NMR experiments and Molecular Dynamics simulations. Both PROTACs sample a

large number of unfolded conformations in DMSO with no contacts between the protein binding moieties. Upon titration with water a collapsed conformation with hydrophobic contacts between the aromatic moieties becomes dominant. The authors also simulate the MZ1-induced ternary complex and its dissociation. They find that the model from crystallography is stable over a 200 ns simulation and MZ1 shows a very similar binding mode as in the crystal structure. Interestingly, even though MZ1 has higher binary affinity to BRD4 than VHL, in steered MD simulations BRD4 dissociates sooner than VHL. It's exciting to see more and more work that aims to characterize the structure and dynamics of PROTACs and their ternary complexes in solution even though experimental limitations, such as poor solubility so far restrict this to computational modelling approaches in many cases.

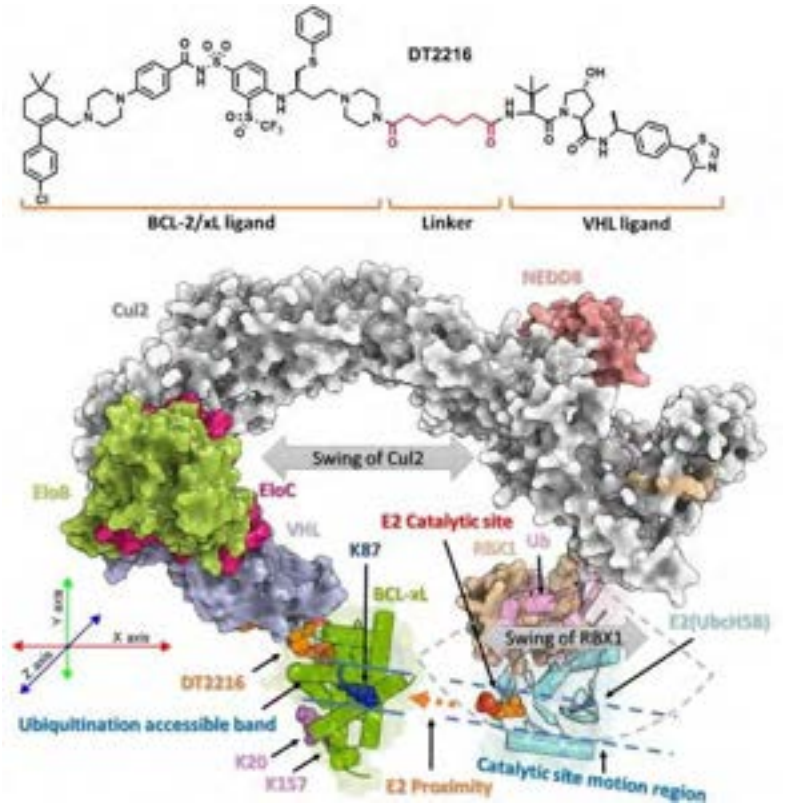
Cell Biology	Computational Chemistry	Structural Biology/Biophysics

Contributor: Aina

Development of a BCL-xL and BCL-2 dual degrader with improved anti-leukemic activity

Dongwen Lv[§], Pratik Pal[§], …, Yaxia Yuan*, Guangrong Zheng*, Daohong Zhou*

Nat. Commun. **2021**, *12, 6896*



BCL-xL and BCL-2 belong to the anti-apoptotic BCL-2 protein family and play an important role in promoting tumor initiation, progression and development of drug resistance by protecting tumor cells from apoptosis. Inhibition and degradation of these BCL-2 family proteins have been extensively investigated. BT2216 is a VHL-based PROTAC that potently degrades BCL-xL but not BCL-2 despite binding to both proteins with high affinities. Although BCL-xL and BCL-2 are homologous proteins sharing about 45% sequence identity, they have distinct surface lysine distributions with only one conserved surface lysine. It is known that one of the possible mechanisms for PROTACs target selectivity is the differential distribution and orientation of lysines on the surface of different targets. The differential lysine distribution makes BCL-xL/2 an ideal model system to study lysines accessibility to the E2 component of the ubiquitination complex.

Based on the computational modelling of the entire NEDD8-VHL Cullin RING E3 ubiquitin ligase complex structure, the authors found that this complex can only ubiquitinate the lysines in a defined band region on BCL-xL. Using this approach, they developed a potent BCL-xL/2 dual degrader with significantly improved antitumour activity against BCL-xL/2-dependent leukemia cells. A different linker site on the BCL-xL/2 binder may have changed the geometry of BCL-xL/2-E3 interaction with the exposure of different lysines toward the E2 for more efficient BCL-xL/2 degradation.

This extensive study demonstrates that computational models have the potential to aid the development of PROTACs to improve their potency as well as selectivity.

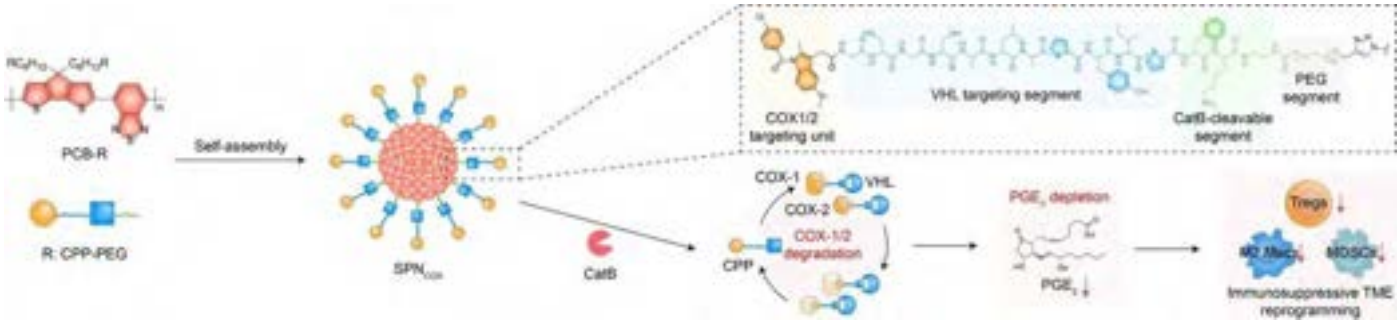
Cell Biology | Chemistry

Contributor: Kevin

Smart Nano-PROTACs Reprogram Tumor Microenvironment for Activatable Photo-metabolic Cancer Immunotherapy

Chi Zhang[§], Shasha He, Ziling Zeng, Penghui Cheng, Kanyi Pu*

Angew. Chem. Int. Ed. **2021**, DOI: 10.1002/anie.202114957



Due to their size and chemical properties PROTACs, especially when peptide derived, can pose challenges for drug delivery. This paper proposes nanoparticle-based drug delivery vehicles, that carry peptide-based PROTAC recruiting VHL for the degradation of COX-1/2 conjugated to a semiconducting core by a cathepsin B (CatB)-cleavable segment. This leads to specific release of the PROTAC in CatB-expressing tumor cells. Crucially, these nanoparticles allow for combination therapy with phototherapy, as the semiconducting core will generate singlet oxygen when irradiated with near-infrared light. The paper shows both increased singlet oxygen levels and efficient, proteasome-dependent degradation of COX1/2 in mouse models, leading to reduced tumor volume and increased tumor immunicity. The article thereby highlights the potential of PROTACs when used as one element of a combinatorial therapy and offers a possibility to not only overcome the challenges of peptide-based therapeutics but give them added value through the possibility of tumor-specific activation.

Cell Biology | Chemistry | Computational Chemistry | Modelling/Simulation

Contributor: Sohini

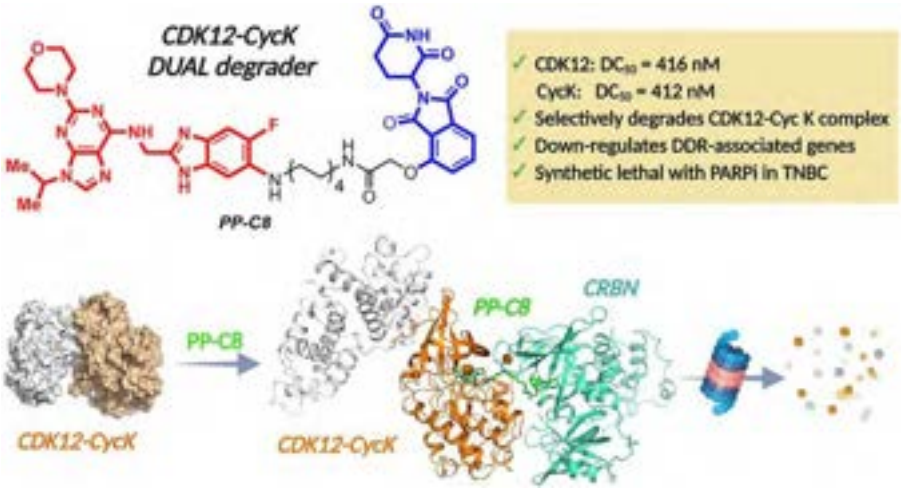
Noncovalent CDK12/13 dual inhibitors-based PROTACs degrade CDK12-Cyclin K complex and induce synthetic lethality with PARP inhibitor

Tian Niu[§], Kailin Li[§], Li Jiang[§], …, Bo Yang*, Cheng-Liang Zhu*

Eur. J. Med. Chem. **2022**, *228*, 114012

Cyclin-dependent kinase 12 (CDK12) is a validated anti-cancer target that plays a crucial role in the transcription of DNA-damage response (DDR) genes. Development of CDK12 specific inhibitors that do not target CDK13 (an isoform of CDK12) remains a challenge.

In this work, the authors developed a potent CRBN-recruiting PROTAC degrader, **PP-C8**, based on the two noncovalent dual CDK12/13 purine inhibitors and achieved specificity for CDK12 over CDK13. In contrast to earlier reported degrader **BSJ-4-116** which only leads to CDK12 degradation, **PP-C8** degrades CycK simultaneously. Further, **PP-C8** demonstrated profound synthetic lethal effect with PARP inhibition in triple negative breast cancer cell (TNBC) growth. Also, no



degradation was observed for CK1α and GSPT1 that are known to bind with imide-based compounds, suggesting the exceptional selectivity of **PP-C8**. Computational analysis indicated that differential stability of ternary complex might contribute towards the isoform selectivity of PP-C8 against CDK12 versus CDK13. **PP-C8** and other PROTAC degraders developed in this study could serve as valuable tools in uncovering the potential kinase-dependent and independent functions of CDK12-CycK complex.

More detailed investigation to better understand the molecular mechanism of action and selectivity of the reported PROTACs would help in the design of better degraders as is already mentioned and undertaken by the researchers of this article. Nonetheless, this study highlights the benefits of PROTACs over conventional inhibitors in achieving target selectivity and would boost the enthusiasm of the TPD community to explore isoform selectivity against other challenging drug targets using degrader molecules.

Cell Biology | Chemistry | Structural Biology/Biophysics

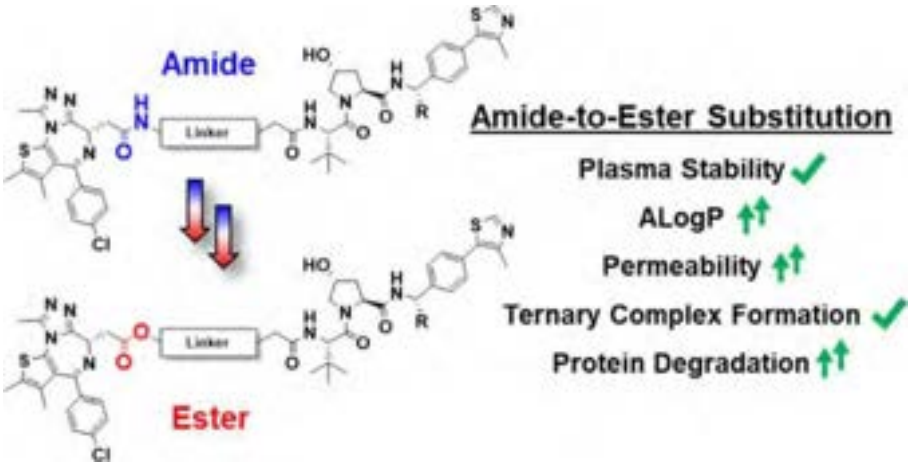
Contributor: Aina

Amide-to-Ester Substitution as a Strategy for Optimizing PROTAC Permeability and Cellular Activity

Victoria G. Klein[§], Adam G. Bond[§], Conner Craigon, R. Scott Lokey*, Alessio Ciulli*

J. Med. Chem. **2021**, *64*, 18082

The bifunctional nature and chemical composition of PROTACs make them go beyond the "Rule of 5" and can hinder their pharmaceutical development. Permeability is an important pharmacokinetic barrier for these compounds. In this paper, the authors demonstrate how a bioisosteric amide-to-ester substitution can enhance physicochemical properties and, thus, bioactivity of PROTACs.



After an extensive and well conducted study using model compounds, bearing either amides or esters, the parameters for optimal lipophilicity and permeability were identified. The strategy was then applied to a set of novel amide-to-ester substituted, VHL-based BET degraders with the goal to improve the degrader activity through increased permeability. In fact, ester PROTACs in the optimal range of lipophilicity showed an improved permeability as well as an increase in degradation potency against BET proteins in HEK293 cells, compared to their amide counterparts. Further biophysical characterisation showed that ester PROTACs were slightly less cooperative than their amide counterparts, demonstrating that increased protein degradation for these compounds must be driven by increased cell permeability rather than ternary complex formation.

It is known that amides can become a hurdle for pharmacokinetics properties. Amide-to-ester substitution can be a simple and convenient bioisosteric replacement to consider when optimising PROTAC's permeability.

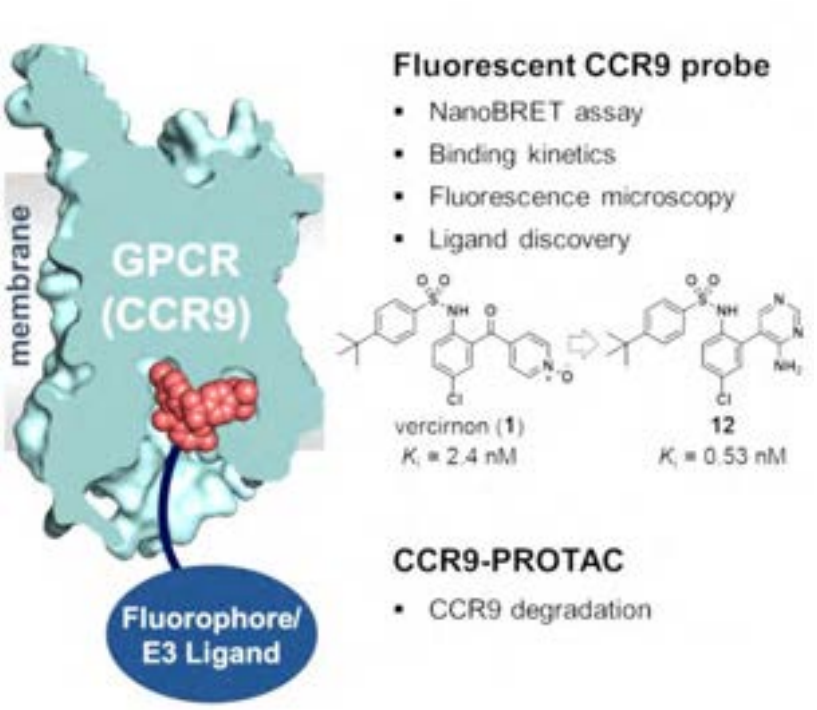
Cell Biology | Chemistry | Computational Chemistry

Contributor: Sohini

A Chemical Biology Toolbox Targeting the Intracellular Binding Site of CCR9: Fluorescent Ligands, New Drug Leads and PROTACs

Max E. Huber[§], …, Matthias Schiedel*

Angew. Chem. Int. Ed. **2021**, DOI: 10.1002/anie.202116782



A highly conserved intracellular allosteric binding site (IABS) that enables the binding of small molecule antagonists has recently been identified in several G-protein coupled receptors (GPCRs), such as chemokine receptors (CCR2, CCR7, CCR9) and the beta-2 adrenergic receptor (β2AR). Starting from vercirnon – an intracellular CCR9-selective antagonist and a previous phase III clinical candidate for the treatment of Crohn's disease, the authors of this study developed the first small molecule-based fluorescent ligand targeting the IABS of CCR9.

Molecular docking studies enabled identification of a suitable position for installation of the linker that allowed the conjugation of vercirnon with a functional label such as a cell-permeable tetramethylrhodamine (TAMRA) fluorophore. This fluorescent probe enabled binding studies via NanoBRET, fluorescence microscopy, and the discovery of a 4-aminopyrimidine analogue as a new intracellular CCR9 antagonist with improved affinity. An azido-functionalized CCR9 ligand was conjugated with an alkynylated analogue of (S,R,S)-AHPC (VHL ligand), to obtain a potential CCR9-PROTAC. This CCR9-PROTAC demonstrated substantial reduction in CCR9 level in a concentration-dependent manner. It binds to the IABS of CCR9 with Ki values of 78.0 nM (pKi = 7.13 ± 0.06) and 151 nM (pKi = 6.86 ± 0.08) under membrane-based and cell-based conditions, respectively. Further, no cytotoxicity was observed in HEK293T cells upon treatment with this PROTAC and it selectively induced a reduction in CCR9 levels, without affecting the levels of the related chemokine receptors CCR2 and CCR7.

This is the first report of a PROTAC targeting IABS of GPCRs and is certainly an important contribution to broaden the target space for PROTACs. Future studies focussed on the functional consequences of CCR9 degradation and improvement of degradation efficacy of CCR9-PROTACs will help to gain better insights into the therapeutic potential of the current findings.

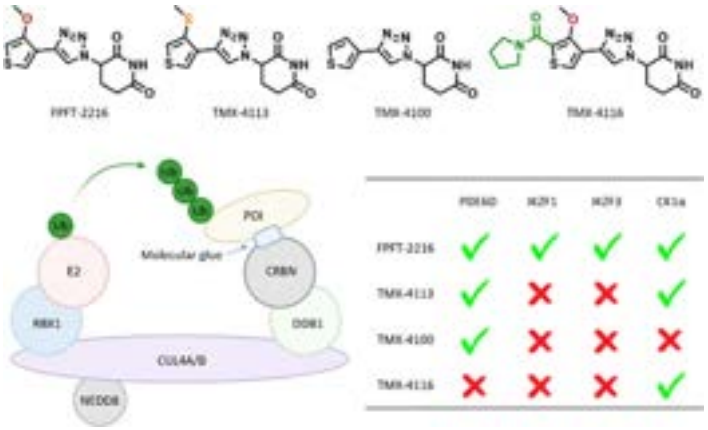
Chemistry | Structural Biology/Biophysics

Contributor: Tasuku

Development of PDE6D and CK1α Degraders through Chemical Derivatization of FPFT-2216

Mingxing Teng[§], Wenchao Lu[§], …, Eric S. Fischer*, and Nathanael S. Gray*

J. Med. Chem. **2022**, *65*, 747



Immunomodulatory drugs (IMiDs), like thalidomide, are one of the most well-investigated synthetic molecular glues. It is known that IMiDs induce proteosomal degradation for a variety of proteins, such as IKZFs, SLL4, and GSPT1, and research of their degradation activities is ongoing not only at academia but also pharmaceutical companies. In this paper, the authors found that FPFT-2216 degrades several proteins including PDE6. PDE6 is known to modulate the localization of KRAS, so they decided to optimize it to obtain PDE6 selective molecular glues.

A variety of derivatives were synthesised and they found that only minor changes would result in drastic changes in degradation profiles. After the SAR study, two compounds, TMX-4100 and TMX-4116, were identified as a PDE6 and CKα degrader, respectively. Each compound degrades the target protein at low concentration (DC_{50} < 200 nM), and is highly selective against other proteins. TMX-4100 was then assessed for cell growth inhibitory activity against KRAS-dependent cell lines and found that no clear effect was observed even though PDE6 was degraded, so they speculated that PDE6 is not essential for cell growth in KRAS-dependant cancers. Their results indicated that IMiDs still have the potential to degrade other proteins.

It is not clear to me why the degradation of PDE6 did not show any effect against KRAS-dependent cell lines though PDE6 knockdown by siRNA showed some growth inhibition activity.

Modelling/Simulation

Contributor: Sohini

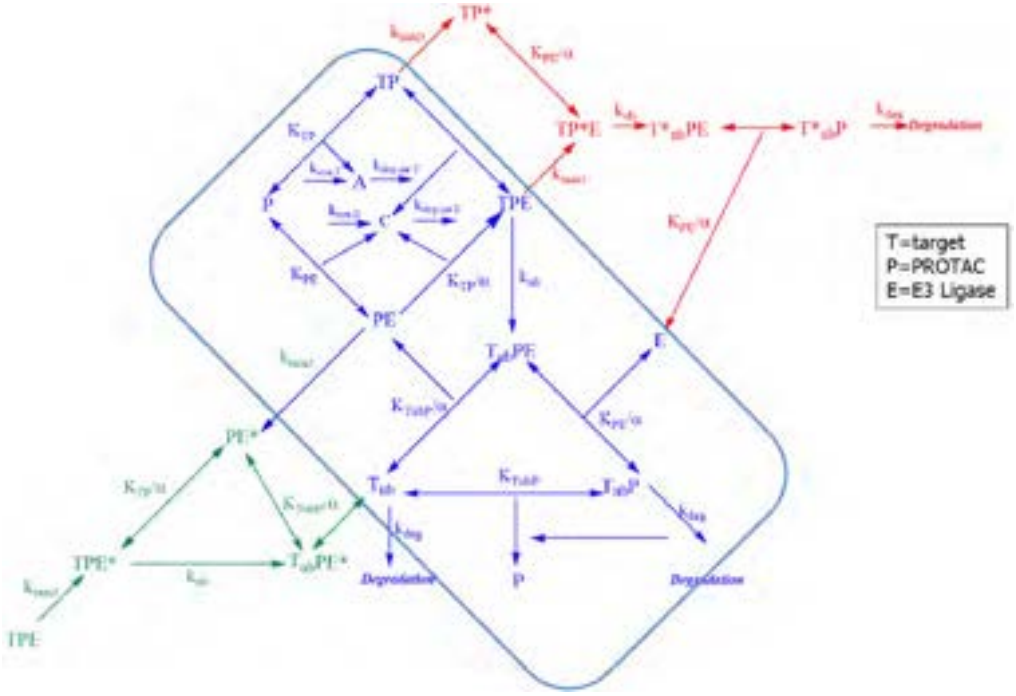
A Mathematical Model for Covalent Proteolysis Targeting Chimeras: Thermodynamics and Kinetics underlying Catalytic Efficiency

Charu Chaudhry[§]*

ChemRxiv **2021**, DOI: 10.26434/chemrxiv-2021-vw4qb

Theoretically, PROTACs that covalently bind to the POI or E3 ligase could help to address the challenges associated with weak ligand affinity and inefficient catalytic turnover. In this study, the author has provided a comprehensive theoretical framework to model the effects of covalently targeting E3 ligase or the POI on PROTAC efficacy. The model was implemented in MATLAB R2020a and all simulations were performed using the ODE15s integrator.

The model predicts that a covalent E3 ligase PROTAC can overcome weak E3 ligase binding affinity and stabilize ternary complex, resulting in an increase in degradation efficacy. Despite equivalent ternary complex stabilization, covalency to E3 ligase can enhance catalytic cycling of the E3 while covalency to the POI abolishes the catalytic benefits. Nonetheless, a covalent POI PROTAC could be advantageous in overcoming weak target binding that boosts ternary complex concentrations and promote degradation. The kinetic simulations indicate that high levels of target degradation can be achieved by engaging fractional E3 ligase. Collectively, the simulation results highlight that the most efficient degrader may not be the optimal degrader. Albeit the model is based on several assumptions, the available experimental data qualitatively correlates with the predicted results. In the absence of any published kinetic data to date, the model could not be quantitatively validated.



Testing the relevancy of this mathematical model upon availability of experimental kinetic data in future would enhance the confidence on the predictions. Nonetheless, the model has the potential to explore many parameters and generate testable predictions that can drive experimental design of covalent PROTACs with better degradation efficacy.

Other Paper Highlights

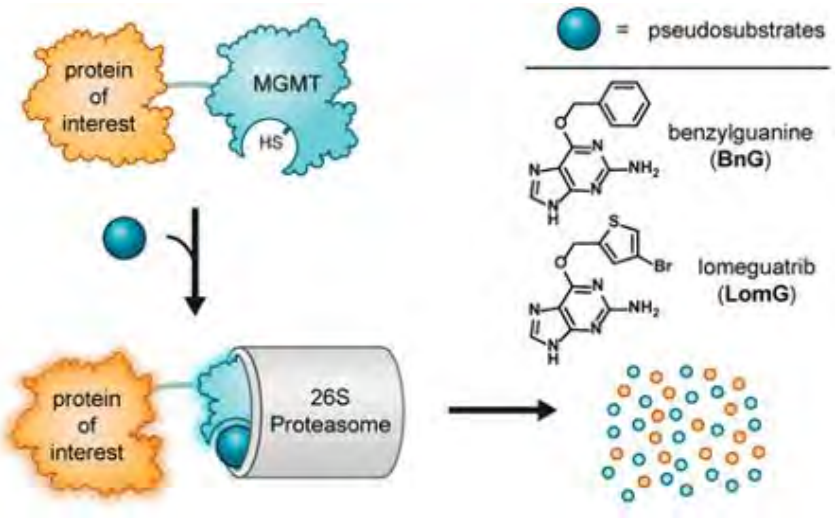
Cell Biology

Contributor: Marek

Repurposing the Damage Repair Protein Methyl Guanine Methyl Transferase as a Ligand Inducible Fusion Degron

Gosia M. Murawska[§], Caspar Vogel[§], …, Dennis Gillingham*

ACS Chem. Biol. **2022**, *17*, 24



In this publication, Murawska and colleagues expand on the repertoire of ligand inducible degron tags available for post-translational knockdown of proteins, by employing methyl guanine methyl transferase (MGMT). MGMT is a ubiquitously expressed 23kDa enzyme, the function of which is to protect cells against mutagenic effects of alkylation at the O^6 position of DNA base guanine.

The alkylation can be a result of exposure to environmental alkylating agents including those present in tobacco smoke and has also been explored in the context of induced cytotoxicity in oncology, using alkylating chemotherapeutics. In the latter, high MGMT expression is associated with resistance to alkylating drugs.

MGMT acts by transferring the alkyl group from the O^6 guanine to a cysteine residue present in its active site. Following the transfer, the enzyme is inactivated and degraded via the ubiquitin-proteasome pathway (UPP). MGMT pseudo-substrates (such as benzylguanine and lomeguatrib) have been previously used in the clinic to inhibit MGMT activity by binding to the cysteine in the active site, in an attempt to sensitise cancer cells to alkylating chemotherapeutics. The binding of the pseudosubstrates has also been associated with MGMT degradation via the UPP.

Murawska and colleagues decided to explore the feasibility of using MGMT as a ligand inducible degron tag. They verify MGMT degradation induced by lomeguatrib and benzylguanine across a panel of cell lines (Huh6, HeLa, HEK293T, MiaPaca2, Calu1, HCT116). They also show the inducible degradation of MGMT-$KRAS^{G12C}$ and CAR-MGMT fusion constructs. The authors suggest that their ligand inducible system benefits from using cheap small molecules that are also functional in mice and humans and present a good safety profile. In the future, it may be interesting to see how the system compares to more established dTAG or the recently disclosed “Bump-and-Hole” bromodomain systems, particularly in the *in vivo* context.

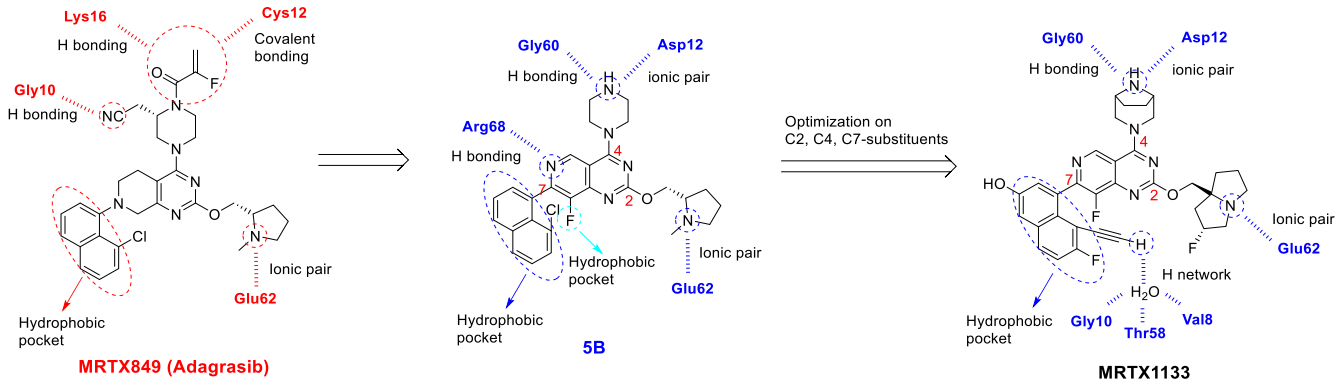
Chemistry | Computational Chemistry | Structural Biology/Biophysics

Contributor: Xingui

Identification of MRTX1133, a Noncovalent, Potent, and Selective KRAS^{G12D} Inhibitor

Xiaolun Wang[§]*, …, Matthew A. Marx*

J. Med. Chem. **2021**, DOI: 10.1021/acs.jmedchem.1c01688



KRAS is reputed to be the “holy grail” of cancer drug discovery because mutated KRAS, particularly the G12D, G12V, and G12C mutations, are the major drivers of many cancers, including colorectal cancer, lung cancer and the most-deadly pancreatic cancer. With AMG510 (Sotorasib) been approved by the FDA and multiple covalent KRAS^{G12C} inhibitors (e.g., MRTX849, JNJ-74699157, LY3537982) in clinical trials, the grail of KRAS cancer drugs is within our reach. However, G12C is only a fraction of KRAS mutations, G12D and G12V are the number one and number two mutations in terms of frequency and prevalence. In this paper, scientists from Mirati Therapeutics report the structure-guided design of the first non-covalent, potent and selective KRAS^{G12D} inhibitor.

They started the campaign by borrowing a few structural moieties from MRTX849, a promising covalent KRAS^{G12C} inhibitor they developed previously, so as to occupy the same switch Ⅱ pocket in KRAS. The α-fluoro-acrylamide was removed, allowing ionic interaction between protonated piperazine and mutant Asp12, which is the most important interaction responsible for potency and selectivity. The tetrahydropyridopyrimidine core is replaced with Pyrido[4,3-d] pyrimidine to have extra interactions in the pocket. Subsequent optimizations on the C2, C4, and C7-substituents of Pyrido[4,3-d] pyrimidine culminated MRTX1133. With sub picomolar binding affinity to KRASG12D and robust anti-cancer activities in vitro and in vivo, further advancement of MRTX1133 is guaranteed.

The discovery of MRTX1133 (currently in IND-enabling stage) represents another breakthrough in the KRAS field. While AMG510 was approved only for KRAS^{G12C} mutated non-small cell lung cancer (NSCLC), the success of MRTX1133 will likely bring hope to patients with pancreatic, colorectal or NSCLC cancer. Now that such excellent KRAS^{G12D} inhibitor is ready, it would be interesting to see if PROTACs based on MRTX1133 will be even superior and it is very likely that the race on KRAS^{G12D} PROTAC is already on.

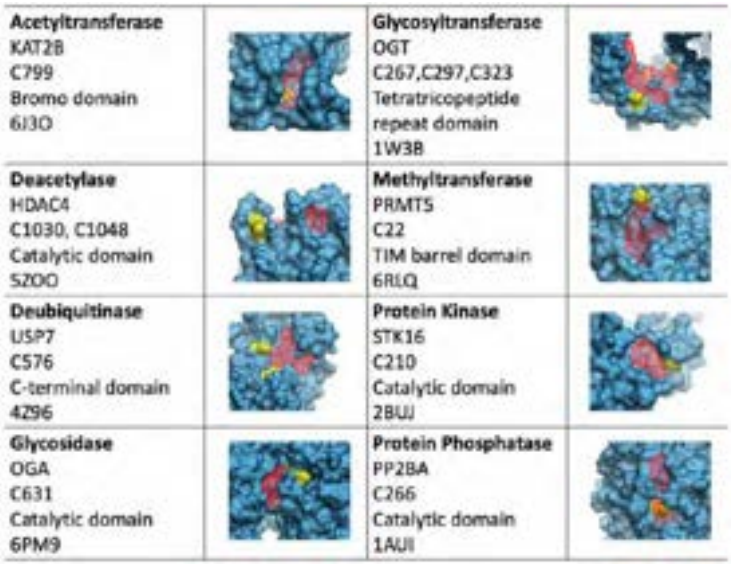
Computational Chemistry | Structural Biology/Biophysics

Contributor: Kevin

Structure-Based Survey of the Human Proteome for Opportunities in Proximity Pharmacology

Evianne Rovers[§], Matthieu Schapira*

BioRxiv **2022**, DOI: 10.1101/2022.01.13.475779



Proximity pharmacology (ProxPharm) is the attempt to generalize the concept of inducing protein/protein interactions to achieve modification of proteins pioneered by PROTACs. This study systematically screens the high-resolution structures of all protein-modifying enzymes for pockets that are suitable to develop ligands for it without affecting catalytic activity directly or by allostery. They found suitable pockets in 287 human proteins, including kinases, phosphatases, deubiquitinases, and writers and erasers of methyl, acetyl and glycosyl groups. Finally, they also screen for reactive cysteines that could be targeted as a nucleophile by covalent handles similar to electrophilic PROTACs for DCAF16. The article thereby highlights the promises of ProxPharm and identifies promising starting points for the development of modulators of protein modification beyond Targeted Protein Degradation!

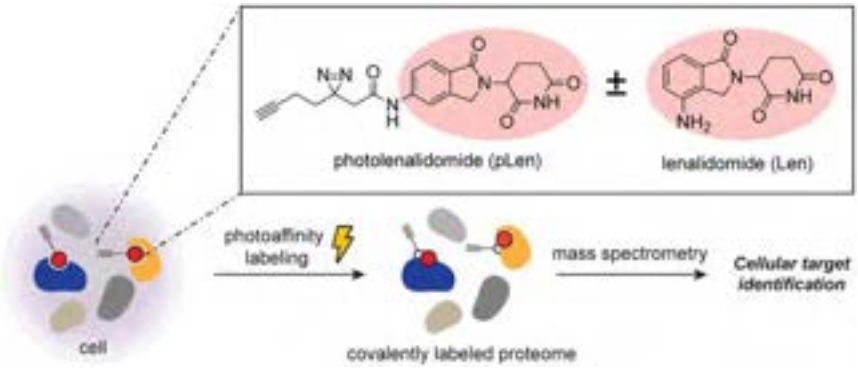
Cell Biology | Chemistry | Computational Chemistry | Structural Biology/Biophysics

Contributor: Sarah

Development of Photolenalidomide for Cellular Target Identification

Zhi Lin[§], …, Christina M. Woo*

J. Am. Chem. Soc. **2022**, 144, 1, 606



Only a handful of cereblon (CRBN)-dependent substrates targeted by Lenalidomide have been identified despite it having a wide range of therapeutic and teratogenic effects. This paper uses photoaffinity labelling to identify a novel Lenalidomide target in HEK293T cells. The authors develop photolenalidomide (pLen), a Len probe bearing an alkyl diazirine alkyne. The diazirine group is required for photocrosslinking and the alkyne handle is for enrichment of crosslinked products. Following irradiation, they use copper catalysed azide alkyne cycloaddition to conjugate azide linked affinity labels to the probe in both recombinant protein complexes and cell lysates. To verify that pLen labels CRBN and IKZF1 in the ternary complex in live cells, they perform co-immunoprecipitation and use an Alexa 488 azide to visualise photolabeling by in-gel fluorescent imaging. They use a cleavable biotin azide to enrich pLen targets from HEK293T cells and find eukaryotic translation initiation factor 3 subunit i (eIF3i) to be one of the most significantly enriched proteins. Interestingly, they establish that eIF3i is recruited to CRBN in a pLen dependent manner in HEK293T cells, but this doesn't result in the ubiquitination or degradation of eIF3i.

The authors nicely demonstrate the versatility of this probe and its ability to identify Lenalidomide targets independent of substrate degradation.

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