CeTPD Journal Club

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

Journal Club

May 2024

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): Matylda Izert-Nowakowska, Jack Robertson, Alessandra Salerno

"The Journal Club is a valuable resource of knowledge about the recent developments in the TPD field. I am happy to contribute to this month's edition and share my excitement about the latest papers."

Matylda obtained her PhD at the University of Warsaw where she worked on developing methods for protein degradation in *E. coli* using plasmid-encoded peptides. In January 2024 she joined the Farnaby Group at the CeTPD as a Postdoctoral Scientist in cell biology and biophysics.

"The journal club is an excellent resource to help keep on top of the latest literature within the ever-expanding field of TPD "

Jack joined the Ciulli group in 2021 as a chemical biologist in the AC-Almirall collaboration after undertaking his PhD under Prof. Glenn Burley at the University of Strathclyde. Here he focussed on developing peptidomimetics for application in Cell-Penetrating Peptides combining both synthetic chemistry and cell biology techniques.

"*Serving as month's editor of the Journal Club allows me to stay engaged with the latest literature and expand my knowledge beyond my background in medicinal chemistry. I hope it encourages others to dive into their reading lists and broaden their horizons in the TPD field!"*

Alessandra completed her PhD at the University of Bologna and joined the CeTPD as a postdoctoral researcher in June 2023. Her scientific interests include developing novel linker moieties for PROTACs and advancing proximity labeling methodologies.

A Celebration of Science, Collaboration, and Scotland: The TPD Symposium in Dundee

Contributor: Alessandra

A Celebration of Science, Collaboration, and Scotland: The TPD Symposium in Dundee

After months of meticulous preparation, this month we hosted the "Protein Degradation in Focus: A Special Symposium to Celebrate the Opening of CeTPD" in Dundee. With a mix of science through the past, present, and future of TPD, the event was an amazing opportunity for inspiration, innovation, and collaboration.

Where It All Began. The idea for the Symposium was born as a grand opportunity to bring together the TPD field and celebrate the scientific opening of our new Centre for Targeted Protein Degradation in Dundee.

Whispers of the Symposium started swirling at the BioMed Induced Proximity conference in Barcelona a year ago, and finally took shape last October. Thanks to the enthusiastic reception, the invaluable contributions from all the participants, and the hard work of the Scientific Organizing Committee (Charlotte Crowe, Valentina Spiteri, Brenda Schulman, Craig Crews, and Alessio Ciulli), the dream became reality! The goal? To foster collaboration, spark innovation, and promote knowledge exchange among researchers from both academia and industry.

Symposium in Numbers. Over 3 absorbing days, the symposium explored the latest scientific advancements featuring 3 keynote lectures, 22 invited talks, 14 short presentations and 3 poster sessions showcasing an impressive 130 presentations.

But it wasn't all just about science! The Symposium buzzed with networking opportunities and cross-disciplinary interactions! Early-career researchers were provided with dedicated sessions for career development and mentorship opportunities, including the Careers in Academia & Industry Round Tables, Network Panel, and a Breakfast Social in partnership with the Women in TPD Network.

And the fun didn't stop there. We treated our participants to a trip to St. Andrews and whisky tasting on Sunday morning, unlimited pizza and beer on Monday night, and countless hours of traditional Scottish ceilidh dancing on Tuesday. Plus, we made sure our delegates felt the love with 300 packed goodie bags modelled after three iconic things that Dundee is famous for "Jute, Jam and Journalism".

The organizers are thrilled with the flood of positive feedback after the event: "*The wonderful messages we've received mean the world to us. It's fantastic to know that our delegates had an inspiring time in Dundee and are now motivated to push the frontiers of TPD research even further. Here's to more innovation, collaboration, and unforgettable moments!*"

For everyone at CeTPD, it was a pleasure to welcome everyone to Dundee and witness the extraordinary science, side by side with inspiring leaders in the field. It's thrilling to see this community growing closer and stronger together. It has been a massive success, in which we hope everyone enjoyed every minute of it!

Want to dive deeper into all the excitement about the Symposium? **Stay tuned!**

Targeted Protein Degradation

Cell Biology | Chemistry

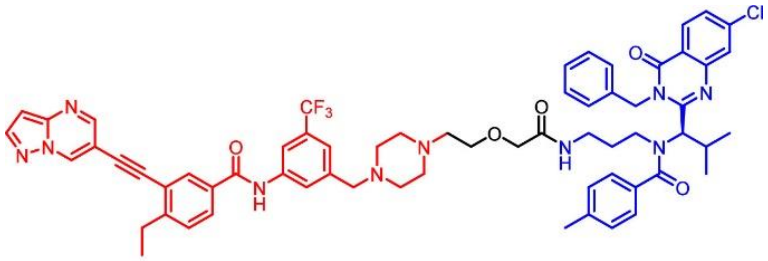
Contributor: Jack

Discovery of LLC355 as an Autophagy-Tethering Compound for the Degradation of Discoidin Domain Receptor 1

Lianchao Liu[§], Lijie Zhao[§], Lujun Yang[§] …, Zhen Wang* and Ke Ding *

J. Med. Chem. **2024**, 67, 8043

This work by Liu *et al.* aims to address the current drawbacks with cancer target Discoidin domain receptor 1 (DDR1) by leveraging autophagy lysosomal degradation. DDR1 is a trans membrane receptor kinase in which several potent inhibitors have been developed. Due to various noncatalytic functions of DDR1 current inhibitors have failed to modulate tumour progression in DDR1 related cancers. The work aimed to target autophagy key protein LC3, which has been used for heterobifunctional autophagosome-tethering compounds (ATTECs), using previously reported ligands.



DDR1 degrader **LLC355**
DC_{50} = 150.8 nM

Two ligands, GW5074 and ipinesib, for LC3 were selected for ATTEC synthesis in combination with potent DDR1 inhinbitor **1**. Seven ATTEC compounds with varying alkyl and PEG linkers were made containing LC3 ligand GW5074. All GW5074 based ATTECs returned only modest degradation of DDR1 with **10g** performing the best with 60% degradation at 1 µM. Employing ipinesib as the LC3 binding moiety returned significantly better degradation with **LLC355** showing 92% degradation at 1 µM and 40% degradation at 0.3 µM. **LLC355** was able to degrade DDR1 in both a dose-dependent and time-dependant manner with a DC_{50} of 150.8 nM. The mechanism of **LLC355** was confirmed to be dependent on both DDR1 and LC3 binding. Degradation was not observed when removing the hinge binding moiety of **LLC355**, thus showing degradation was dependant on DDR1 binding. Further experiments inhibiting autophagy using chloroquine and bafilomycin A1 were able to rescue levels of DDR1 indicating degradation was dependent on autophagy. **LLC355** was also shown to selectively degrade DDR1 over DDR2 however, when conducting global proteomic profiling no significant reduction of DDR1 levels were observed.

Finally, the authors demonstrate that **LLC355** could inhibit migration and invasion of NCI-H23 cells in a trans well based assay. Overall, this paper demonstrated the identification of **LLC355** as a new ATTEC for the lysosomal degradation of DDR1. **LLC355** appears to be a good starting point for further development of ATTECs for DDR1 though further explanation of proteomic results would have clarified the compounds' selectivity. Nevertheless, this highlights the advantages of using a lysosomal approach to degrade trans-membrane proteins

Cell Biology | Chemistry

Contributor: Jack

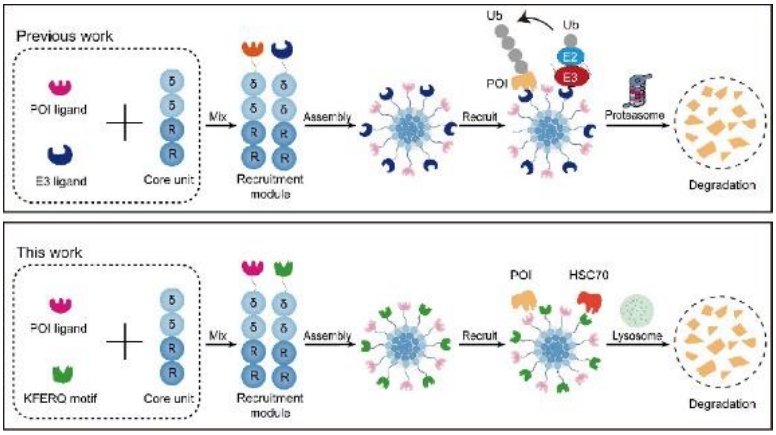
A Novel Lysosome Targeting Chimera for Targeted Protein Degradation via Split-and-Mix Strategy

Jinpeng Wang[§], …, Fei Lu*, Zigang Li*, Feng Lin*

ACS Chem. Biol. **2024**, 19, 1161

This work by Wang *et al.* seeks to build upon their previous work where the group developed a platform for assessing whether a protein could be an appropriate target for proteasome mediated degradation, termed ’split and mix PROTAC’. Here a similar ’split and mix’ approach is used but aimed at assessing protein degradation through a lysosome mediated pathway.

Specifically aiming to develop the platform for Chaperone-mediated autophagy chimera (CMAC), nanometric assemblies were formed which contained recruiting motifs for both HSC70 and a POI. Tamoxifen was selected as the first POI ligand for the assembly and nanoparticle formation was confirmed before degradation of ERα was assessed. The tamoxifen-based assembly was able to deplete ERα levels in both TF7D and MCF7 cell lines after 12 h incubation and depletion levels were sustained until 36 h. The mechanism of degradation was confirmed to be by a lysosomal pathway as inhibition experiments with hydroxychloroquine rescued the level of ERα whereas proteasomal inhibition, with MG-132, did not stop ERα depletion. The platform was then extended to three further protein targets: AR, MEK1/2 and BCR-ABL. Using established binders for each protein (Enzalutamide, Cobimetinib and Dasatinib) three separate assembles were characterised and confirmed to be able to degrade their target protein by a lysosomal mediated pathway in a concentration-dependant manner. Furthermore, both tamoxifen and enzalutamide based assembles demonstrated an ability to inhibit the proliferation of breast cancer and prostate cancer tumour cells respectively.



Overall, this research builds on the group's previous work by developing a platform that can be tuned to degrade different proteins by a lysosomal pathway. The platform may offer opportunity to assess the suitability of lysosomal degradation of new protein targets, though established binders of any target would be essential.

Cell Biology **Chemistry**

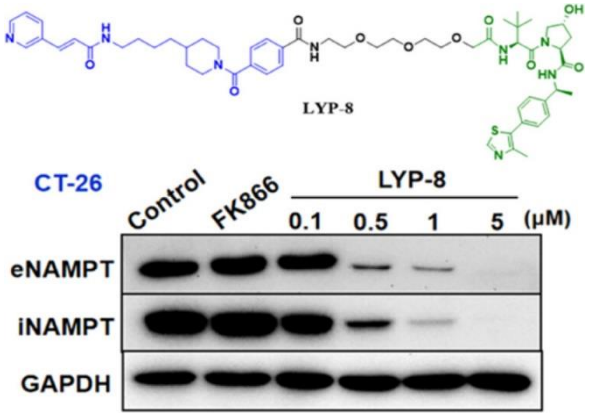
Contributor: Jack

Design of FK866-Based Degraders for Blocking the Nonenzymatic Functions of Nicotinamide Phosphoribosyltransferase

Tiangong Lu[§],Fangfang Chen[§], Jian Yao[§], Zixuan Bu[§] …, Hong Liang*, Nouri Neamati *, Yanghan Liu*

J. Med. Chem. **2024**, 67, 8099

This work by Liu *et al.* aims to address the issue with targeting the NAMPT protein for cancer therapy. NAMPT exists in two different forms, intracellular enzyme iNAMPT and extracellular cytokine eNAMPT. Dues to the two differing forms conventional inhibitors have proven challenging to develop.



Starting from a known potent NAMPT inhibitor FK866 (K_i = 0.3 nM), a series of PROTACs with differing linkers including alkyl, PEG aznd cyclic linkers were assessed and linked to either CRBN recruiting pomalidomide or VHL recruiting VH032. Assessing iNAMPT degradation in SKOV-3 cells, the first series of CRBN based PROTACs with alkyl linkers did not demonstrate any significant degradation after 24 h. Assessment of both PEG and cyclic linkers also returned poor levels of degradation when linked to pomolidamide with only a single compound showing degradation of 50%. VH032 linked PROTACs returned more fruitful results, with two PEG linker based PROTACs (**LYP-8** and **LYP-11**) returning near maximal degradation of iNAMPT at 0.5 µM after 24 h. Assessment of conformationally restricted linkers identified piperidine containing **LYP-35** as the most potent in the cyclic linker series, reducing iNAMPT levels by 74% at 0.5 µM.

Further evaluation of **LYP-8** and **LYP-11** showed that both compounds reduce iNAMPT levels in both a dose-dependent and time-dependant manner in three different cell lines. **LYP-8** was able to achieve DC_{50} values of 26, 6.8 and 520 nM in SKOV-3, MKN-45 and CT-26 cell lines, respectively. Furthermore, degradation was confirmed to be proteasomal

dependant as NAMPT levels were not reduced when using proteasome inhibitor MG 132. Importantly eNAMPT levels were also able to be reduced by **LYP-8** in a dose-dependent manner. *In vivo* studies were carried out using **LYP-8** in which both tumour growth and tumour size were suppressed in CT-26 bearing mice. **LYP-8** also demonstrated better *in vivo* safety when compared to its parent inhibitor FK866.

The research here again demonstrates the advantages of a PROTAC approach when conventional inhibitors are not sufficient. The research also highlights that assessing multiple E3 ligases can be crucial in PROTAC identification.

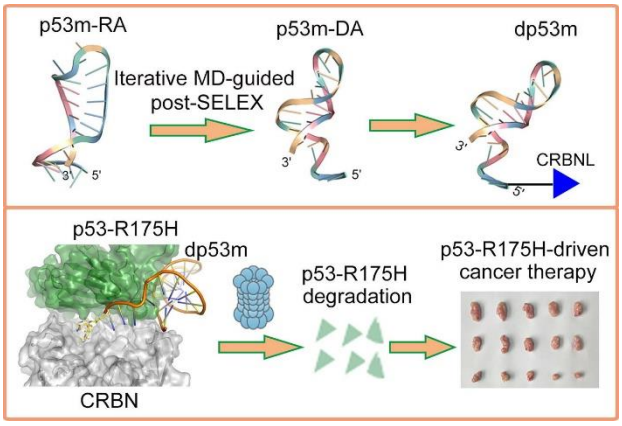
Cell Biology | Modelling/Simulation | Structural Biology/Biophysics

Contributor: Matylda

An engineered DNA aptamer-based PROTAC for precise therapy of p53-R175H hotspot mutant-driven cancer

Lingping Kong[§], Fanlu Meng[§], Ping Zhou[§] …, Sijin Wu*, Diansheng Zhong*, Songbo Xie*

Sci. Bull. **2024**, *https://doi.org/10.1016/j.scib.2024.05.017*



In this paper, the authors present a selective DNA aptamer PROTAC against p53-R175H. The R175H mutation is associated with loss of p53 tumour suppressor function, higher risk of metastasis, and poor prognosis. The research presented in the paper is a follow-up of an article describing the development of an RNA aptamer PROTAC targeting p53-R175 published by the authors in 2023. The authors used molecular dynamics simulations to design a new, improved DNA aptamer which had only slightly improved affinity towards p53-R175H, but notably higher serum stability in comparison to the RNA aptamer, overcoming the main limitation of the RNA-based PROTAC. The PROTAC was obtained by click conjugating of thalidomide to the DNA aptamer selectively targeted the p53-R175H mutant without affecting wild type p53. The PROTAC inhibited the tumour cell proliferation in both cellular and BALB/c nude mouse models. Additionally, the PROTAC increased the sensitivity of cells with p53-R175H to cisplatin suggesting that the PROTAC could be used in combination with other therapies which were previously ineffective against tumours with p53 mutations.

Although the paper shows promising data on the effectiveness of the developed PROTAC, it is worth noting that nucleic acids can be recognised as pathogen-associated molecular patterns (PAMPs) and trigger the immune response. Therefore, cautious evaluation of the immune effects of such aptamer-based PROTACs should be performed before considering them as potential therapeutics.

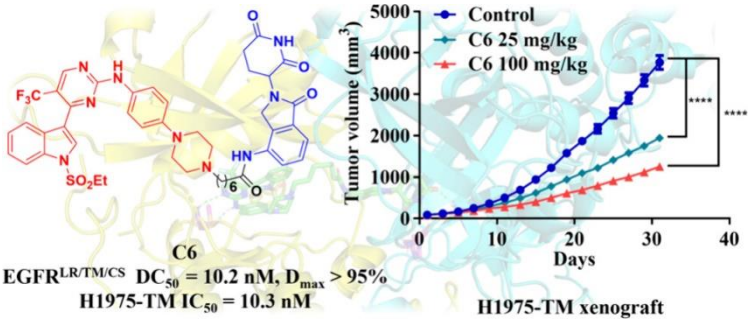
Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Matylda

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

Yasheng Zhu[§], Xiuquan Ye[§], Yuxing Wu[§], Hao Shen[§], …, Xiao Wang*, Yibei Xiao*, Peng Yang*

J. Med. Chem. **2024**, *67*, 7283



The paper presents the synthesis and testing of a new PROTAC against the EGFR-C797S mutant. This mutation was found to arise in nonsmall cell lung cancer and contribute to resistance of the third-generation EGFR inhibitor Osimertinib. Several PROTACs targeting this specific mutation have been developed in the past, however, none of them have been approved for clinical trials. The authors have synthesised a new PROTAC based on the selective

EGFR-C797S inhibitor, which they developed in 2023. Using the docking model, they identified a suitable exit vector for linker attachment and synthesised a series of PROTACs with lenalidomide as an E3 recruiter. They tested a range of linkers and identified the most potent compound C6 with DC_{50} = 10.2 nM. The authors evaluated the dose-response and mutant EGFR degradation kinetics in response to C6. Although C6 had reduced affinity towards mutant EGFR in comparison to the parental ligand, it showed a promising antiproliferative effect in cellular studies and mouse xenograft models. Finally, the authors tested the pharmacokinetic properties of the C6 compound. They suggest that testing different rigid linkers could improve the PK properties of the presented PROTAC.

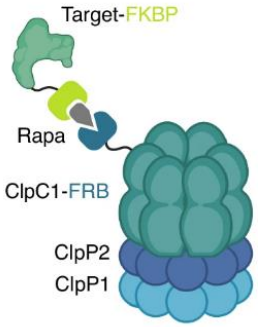
This paper is an example of a comprehensive study on the development of a new PROTAC based on an existing mutant-selective inhibitor and highlights the importance of testing multiple linker lengths and compositions.

Cell Biology

Contributor: Matylda

Targeted protein degradation in mycobacteria uncovers antibacterial effects and potentiates antibiotic efficacy

Harim I. Won[§], Samuel Zinga[§], …, Eric J. Rubin*, Junhao Zhu*
Nat. Commun. **2024**, *15*, 4065



Targeted protein degradation in bacteria is a developing field giving an exciting perspective for exploring new antimicrobial approaches. In this work, the authors created a new method for interrogating antimicrobial targets in mycobacteria and asses their potential as putative TPD candidates. They developed a system based on chemically induced dimerization between ClpC1-FRB and POI-FKBP-eGFP fusions in *Mycobacterium smegmatis*. By adding rapamycin to the bacterial cultures, they induced the proximity between the fusion POI and mycobacterial ClpC1P1P2 protease complex, which was previously used in the development of BacPROTACs by Morreale *et al*. in 2022. The authors have selected 54 essential proteins which are highly conserved between *M. smegmatis* and pathogenic *Mycobacterium tuberculosis* and tested their degradation by the created system. They observed different degradation potency of the tested fusion proteins which they linked to the accessibility of the unstructured N-terminus. They found that the efficient degradation of the essential targets can significantly reduce mycobacterial growth which identifies the proteins as putative drug targets. Additionally, the degradation of several proteins was found to sensitise bacteria against known antibiotics.

Although the presented approach is limited by the experimental setup since the C-terminal fusion of eGFP might perturb the degradation of the proteins with unstructured C-termini, it presents an interesting tool for the identification of putative drug targets. The research described in the paper can help to accelerate the development of successful TPD strategies in antimicrobial research to develop new antimicrobials, or to sensitise drug-resistant bacteria to the known forms of treatment.

Cell Biology **Modelling/Simulation**

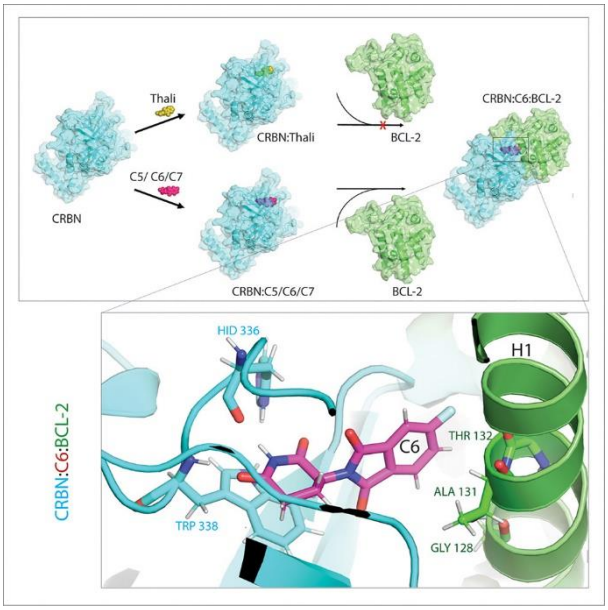
Contributor: Matylda

Thalidomide derivatives degrade BCL-2 by reprogramming the binding surface of CRBN

Jianhui Wang[§], …, Xinlai Cheng*
Cell. Rep. Phys. Sci. **2024**, *5*, 101960

The anti-apoptotic protein BCL-2 is a promising target in anti-cancer treatment. However, in some chronic lymphocytic leukemia patients, BCL-2 mutations contribute to treatment resistance. In this paper, the authors studied thalidomide derivatives in search of new BCL-2-specific molecular glues. The proteomic screen revealed that several thalidomide derivatives (C5, C6, and C7) with modification on the phthaloyl-ring cause decreased BCL-2 levels without affecting other proteins from the BCL-2 family. The BRET assay and *in vitro* alpha assay confirmed that the compounds induce the formation of a ternary complex with CRBN and BCL-2. Further analysis of the C5 compound effect showed that the degradation of BCL-2 is dependent on CRBN, neddylation, and ubiquitination of K17 and K22 BCL-2 residues.

Compound C5 was also active against common BCL-2 variants which are resistant to treatment by commonly used inhibitor venetoclax. Molecular modeling and MD simulations of the ternary complexes helped to identify three BCL-2 residues essential for interactions with the molecular glues. It also suggested a new motive in the BCL-2 sequence which could be a putative degron recognised by CRBN. The essential residues identified by MD modelling were later confirmed by the abolishment of the degradation and ternary complex formation by BCL-2 triple mutant. Finally, the C5 glue was tested in *Drosophila* tumor model. C5 significantly reduced tumor growth and mortality outperforming thalidomide and venetoclax.



The paper provides an interesting insight into altering CRBN binding surface and thus substrate specificity by different thalidomide derivatives and present a putative alternative treatment against venetoclax-resistant leukemia. It also includes an interesting finding of a new putative degron mediating interactions with thalidomide derivatives which might have implications for finding new substrates for thalidomide-based molecular glues.

Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Alessandra

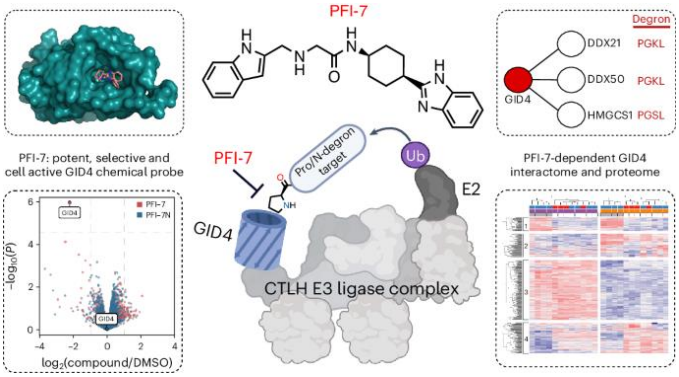
A chemical probe to modulate human GID4 Pro/N-degron interactions

Dominic D. G. Owens[§], Matthew E. R. Maitland[§]… Cheryl H. Arrowsmith*

Nat. Chem. Biol. ***2024***, https://doi.org/10.1038/s41589-024-01618-0

Identifying new ubiquitin ligases and their interactors and substrates has been a major challenge in cellular biology and the TPD field. In this work the C-terminal to LisH (CTLH) complex has been presented as a ubiquitin ligase that targets substrates with Pro/N-degrons through its receptor, Glucose-Induced Degradation 4 (GID4). However, its precise roles and substrates in humans remain unclear.

The authors proved a significant advancement with **PFI-7**, a potent, selective, and cell-active chemical probe that inhibits Pro/N-degron binding to human GID4. Using proximity-dependent biotinylation and quantitative proteomics with **PFI-7**, this innovative tool has enabled the identification of numerous GID4 interactors, shedding light on how GID4 recruits substrates to the CTLH complex. They discovered that GID4 interactors are rich in nucleolar proteins, including RNA helicases DDX21 and DDX50, but also identified proteins regulated by GID4, such as the metabolic enzyme HMGCS1. Surprisingly, blocking GID4 did not always reduce protein degradation, indicating GID4's dual roles in both degradative and non-degradative pathways.



Despite the presence of over 600 E3 ligases in the human proteome, an accurate map of their substrates and interactors remains challenging. In this work, the developed probe and study pipeline offer valuable insights into GID4 ubiquitin ligases and their role, while also laying the groundwork for the discovery of new E3 ligases. Moreover, **PFI-7** will be an invaluable tool for future research on the CTLH complex and for developing strategies that harness CTLH E3 ligase activity for targeted protein degradation.

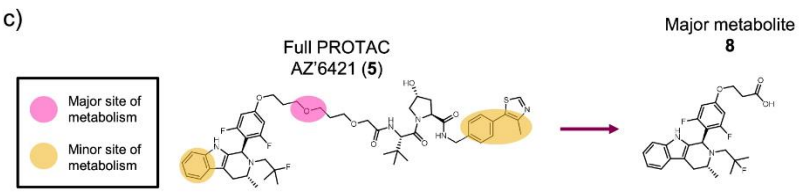
Cell Biology | Chemistry

Contributor: Alessandra

Metabolism-driven in vitro/in vivo disconnect of an oral ERα VHL-PROTAC

Thomas G. Hayhow*, Beth Williamson, Mandy Lawson … Claire Crafter

Commun. Biol. **2024**, 7, 563.



Targeting the estrogen receptor alpha (ERα) pathway is a proven clinical approach for treating ER+ breast cancers. In this work, orally bioavailable PROTACs of ERα that recruit VHL were developed based on the reported core of the SERD AZD9496, by inserting an ether-linked PEG chain connected to the VHL ligand. PROTAC **AZ'6421** emerged as a promising candidate due to its excellent potency, degradation efficiency, and suitable hepatic clearance for pharmacokinetic (PK) and pharmacodynamic (PD) studies in mice. Notably, solution NMR spectroscopy was employed to study the 3D conformational behavior and intramolecular hydrogen bond properties of **AZ'6421**, aimed at understanding its potential to form a ternary complex with ERα and VHL. Furthermore, preliminary in vitro studies demonstrated that **AZ'6421** effectively degrades ERα and acts as an antagonist in ER+ breast cancer cell lines, retaining a SERD-like activity partially dependent on VHL.

However, in vivo testing showed less ERα degradation than expected from the in vitro results. Several biological factors were systematically ruled out (e.g., VHL levels, tumor heterogeneity, hypoxic vs. normoxic conditions) to understand this discrepancy. Further investigation revealed that the metabolic instability of the PROTAC linker led to the production of metabolites—resulting from cleavage and oxidation—that competed with the full PROTAC for ERα binding, thereby reducing degradation.

Overall, this work highlights the importance of designing metabolically stable PROTACs to ensure maximum efficacy. PROTAC metabolites might have their own pharmacological effects, which is particularly relevant in contexts where minor molecular changes can lead to significant activity shifts (e.g., antagonism to agonism as in this case). Therefore, optimizing the linker is essential in PROTAC design to achieve the desired therapeutic outcomes and should be considered early in the development process.

Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Alessandra

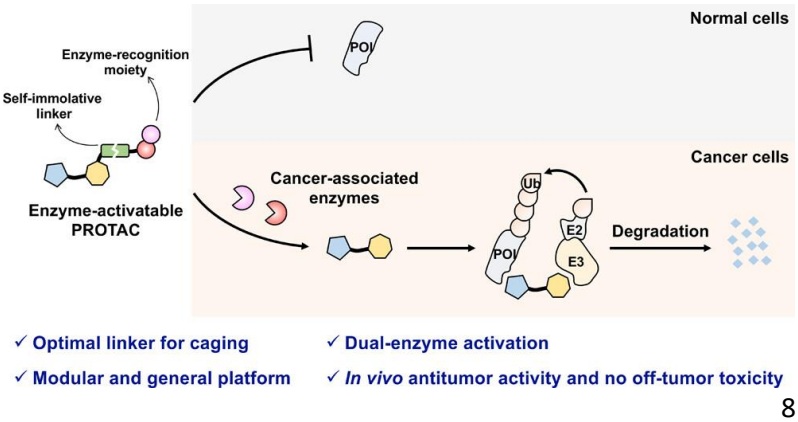
Modular Development of Enzyme-Activatable Proteolysis TargetingChimeras for Selective Protein Degradation and Cancer Targeting

Yanchi Chen[§], Lina Zhang[§], Lincheng Fang[§], …Tao Peng*

JACS Au **2024**, https://doi.org/10.1021/jacsau.4c00298

Cellular selectivity is still a crucial aspect to be addressed in TPD. The authors herein developed enzyme-activatable PROTACs, which use enzyme-recognition moieties to prevent protein degradation until activated by high enzyme levels in cancer cells, enabling cell-specific degradation and targeted therapy.

Previous prodrug approaches used self-immolative carbonates to cage the VHL ligand but were unstable under physiological conditions. The authors identified the methylene alkoxy carbamate (MAC) unit as an optimal self-immolative linker, offering high stability and efficient release. The MAC linker outperformed carbonate units in stability, release efficiency, and cell-selective protein degradation. Using the MAC linker, they developed several enzyme-



activatable MZ1-based PROTACs targeting specific expressions in cancer cells and tested them in relevant cancer systems.

To enhance specificity, dual-enzyme-activatable PROTACs were also designed, requiring processing by two cancer-associated enzymes to reduce off-tumor activation. By incorporating aminoacid-based substrate motifs for HDACs and CTSB into MZ1 *via* the MAC linker, they achieved selective protein degradation in cancer cells with potent *in vivo* antitumor efficacy and no off-tumor toxicity.

This study demonstrates the versatility of enzyme-activatable PROTACs using the MAC linker for various targets and E3 ligases (CRBN and VHL) and highlights their potential to minimize toxicity in normal tissues. Additionally, the study explores an array of cell lines as proper controls based on POI or E3 ligase protein levels

Enzyme-activatable PROTACs offer significant advantages in minimizing toxicity to normal tissues, a crucial goal in targeted protein degradation drug discovery. Although this strategy might be less likely to translate to clinical applications due to the higher structural complexity of the compounds, the interest in finding clinical solutions to reduce off-target effects remains strong in the field of drug discovery for TPD.

Cell Biology | **Chemistry** | **Structural Biology/Biophysics**

Contributor: Alessandra

Alkenyl oxindole is a novel PROTAC moiety that recruits the CRL4^{DCAF11} E3 ubiquitin ligase complex for targeted protein degradation

Ying Wang[§] ,Tianzi Wei[§] ,Man Zhao[§], …, Liang Hong* ,Rui Wang* ,Ruilin Tian* ,Guofeng Li*

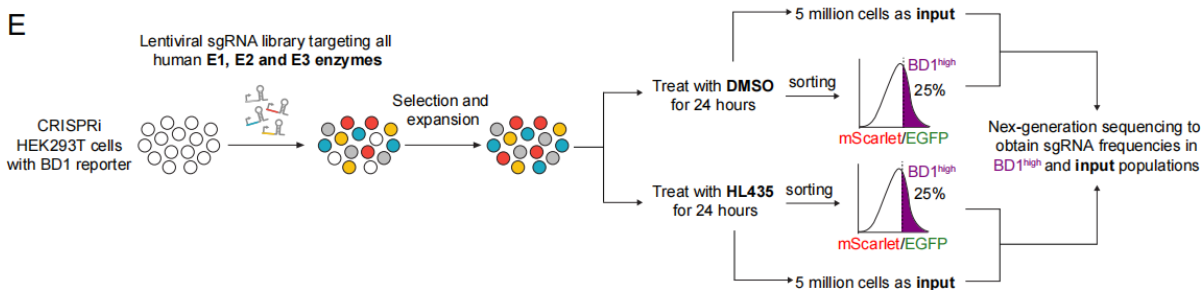
PLoS Biology ***2024***, https://doi.org/10.1371/journal.pbio.3002550

Alkenyl oxindoles have been identified as autophagosome-tethering compounds (ATTECs) targeting mutant huntingtin protein (mHTT) for lysosomal degradation. To expand their use in targeted protein degradation, a series of heterobifunctional compounds combining various alkenyl oxindoles with the BRD4 inhibitor JQ1 were designed and synthesized.

Modification of the alkenyl oxindole core, particularly adding a trifluoromethyl group (R = 6-CF_3), enhanced degradation activity. This led to the creation of HL435, a highly effective BRD4 degrader with over 99% degradation efficiency at 1.0 µM. However, mechanistic studies indicated that BRD4 degradation by HL435 occurs through the ubiquitin-proteasome system, not the autophagy-lysosomal pathway as previously reported. Using a dual-fluorescence reporter system, CRISPR interference (CRISPRi) screening revealed that JQ1-alkenyl oxindole conjugates recruit the E3 ubiquitin ligase complex CRL4^{DCAF11} for substrate degradation. Additionally, HL435 demonstrated significant antitumor activity both in vitro and in a mouse xenograft model.

These findings are consistent with the research by Waldmann and Winter (*Nat Commun*, 2023, 14, 7908) confirming the recruitment of CRL4 ^{DCAF11} by alkenyl oxindoles. However, previous SAR studies on PROTACs have primarily examined the effects of different linkers on degradation activity, with limited focus on the structure-activity relationship of the E3 ligase ligand, as explored in this work.

Overall, this study emphasizes the urgent need to carefully evaluate the underlying mechanisms in TPD and highlights DCAF11's role as an E3 ligase in nuclear protein degradation by electrophilic PROTACs, underscoring the potential and interest in the DCAF family for next-generation targeted protein degradation.



Other Paper Highlights

Chemistry

Contributor: Jack

Azole reagents enabled ligation of peptide acyl pyrazoles for chemical protein synthesis

Peisi Liao[§] and Chunmao He*

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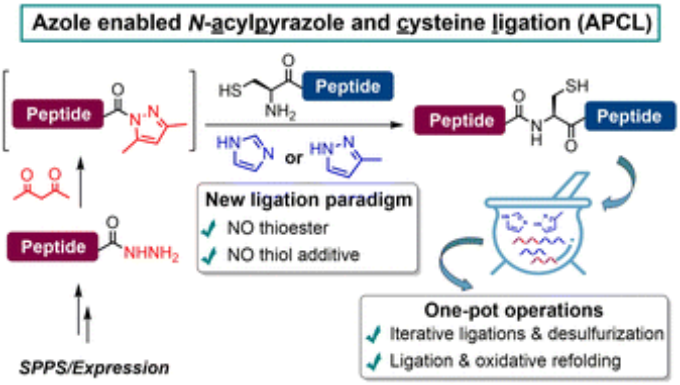
This work by Liao *et al*. seeks to expand the toolkit of native chemical ligation and chemical protein synthesis using azole-based reagents without the need for thioester or thiol additives. Chemical protein synthesis typically employs reactive peptidyl thioesters to enable chemical ligation between two smaller proteins, but also thiol-based catalysts to generate more reactive donors to facilitate the ligation. Metal-free desulphurisation chemistry has further allowed ligation beyond just two Cys sites, though is often not compatible with thiol-based catalysts.



Seeking sulphur-free additives, imidazole and 3-methylpyrazole were employed to achieve chemical ligation. The ligation process requires two steps. Firstly, a peptide hydrazide (synthesised by SPPS or recombinant expression) on the *C*-terminus of peptide **1** is converted to a *N*-acyl pyrazole through a reaction with acetylacetone. Secondly, an *N*-terminal Cys containing peptide and azole reagent are added to react with pyrazole peptide **1** and complete the ligation. Conditions were optimised using representative peptide fragments and it was found that optimal ligation was completed at pH 6.5 with imidazole or 5.0 when using 3-methylpyrazole. The complete synthesis of Ubiquitin was completed in 'one-pot' with imidazole-based ligation then metal-free desulphurisation. The full-length Ubiquitin was isolated in an overall yield of 34% without the need for elevated temperatures or prolonged reactions times. Larger and more complex proteins were isolated in good yields with tertiary structures confirmed by CD. Cu-containing azurin was isolated in a yield 31% and sulphated hirudin in a yield of 39%.

Overall, this work has demonstrated a relatively robust and facile 'one-pot' approach to chemical protein synthesis with various proteins without the need for thiol-based catalysts. The ligation is still limited to Cys residues at the *N*-terminal point of ligation, but these residues can be altered to Ala as conditions allow for metal-free desulphurisation.

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