CeTPD Journal Club

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

March 2024

Centre for Targeted Protein Degradation
University of Dundee

innovate
collaborate
inspire

Journal Club

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Meet this Month's Editors

This month's editors are (from left to right): Bharat Gadakh, Natalia Karolak and Roberta Ibba

"Though it is my first time, I enjoyed contributing to the Journal club. It is great resource to keep myself up to date with PROTAC and adjacent field. I also enjoy the process of reading the paper, critically analyzing the impact and scope of the paper and expressing my opinion."

Bharat completed his PhD in Medicinal Chemistry from the KU Leuven, Belgium where he worked on improving the uptake of antimicrobial compounds by Trojan-Horse Strategy (exploiting iron-channel or peptide transporter). He joined the AC-BI team as a Medicinal Chemist in October 2022.

"The Journal Club is a great opportunity to be up to date with relevant literature from TPD field. It was a pleasure to be a part of it as one of this month's editors."

Natalia completed her MSc in molecular biotechnology and PhD in chemistry at the University of Warsaw and joined AC-BI as a structural biologist and biophysicist in the PROTAC collaboration project with Boehringer Ingelheim in December 2023.

"*Contributing to the JC was a nice challenge this month, the JC is a great resource for scientists in the TPD to be up to date with the latest paper in the field."*

Roberta completed her undergraduate degree in Pharmaceutical Chemistry and Technology at the University of Sassari (Italy) followed by a PhD in Chemical Sciences focused on Medicinal Chemistry in the same University. She joined the Academic Group at the CeTPD in February 2024 as a Postdoctoral Medicinal Chemist.

Funding news: University of Dundee's CeTPD is part of the Cancer Grand Challenges KOODAC team

Contributor: Alessio Ciulli

I am delighted to share the news that we are part of the Cancer Grand Challenges KOODAC team to develop new drugs for tackling solid tumours in children. The team will be led by Yael Mossé, MD, Professor of Pediatrics in the Cancer Center at Children's Hospital of Philadelphia (CHOP), and Martin Eilers, Professor of Biochemistry from the Theodor-Boveri-Institute at the University of Würzburg in Germany, and it brings together clinicians, advocates and scientists with expertise in structural biology, chemical biology, paediatric oncology and medicinal chemistry, across ten institutions and five countries to develop transformative new therapies for previously undruggable forms of childhood cancer.

Funded by two of the largest funders of cancer research in the world – Cancer Research UK (CRUK) and the National Cancer Institute (NCI) in the US – Cancer Grand Challenges will fund overall five teams for five years with sums of up to $25 million each to address some of the most pressing challenges in cancer research and care. For this funding round, 176 global teams last year submitted bold ideas for tackling such challenges, and 12 teams were shortlisted. Our project *"Developing a suite of oncoprotein degraders for childhood solid cancers"* nicknamed Team KOODAC (acronym for "Knock out oncogenic drivers and cure kids), emerged as one of five teams to receive funding after a year-long rigorous selection process. To tackle this challenge, our KOODAC team brings together the expertise of researchers from the United States, Austria, France, Germany, and us from the United Kingdom.

KOODAC aims to develop new degrader drugs that breakdown five of the most compelling oncoproteins in children with high-risk oncogene-driven cancers, including neuroblastoma, medulloblastoma, fibrolamellar hepatocellular carcinoma, rhabdomyosarcoma, Ewing sarcoma and other tumours that are driven by these essential oncoproteins. The team is addressing this challenge working together with Nurix Therapeutics, a US-based biopharmaceutical company focused on developing degrader drugs, which will offer a path forward for clinical translation of discoveries made by the team. The team is also supported by a robust international patient advocacy committee that will amplify the voice of childhood cancer communities globally. "We were honoured to be invited and immediately felt a sense of purpose, a strong focus on team science, and realised the unique opportunity and call ahead of us", I said when interviewed. "Revolutionising paediatric solid tumour treatment demands global collaboration in the face of persisting outdated therapies. We have assembled a team with unrivalled technology and expertise to realise our ambitious goal of driving innovation in targeted paediatric cancer therapeutics. Our vision is to pioneer drugs that will become the new standards of care for children with oncoprotein-driven solid malignancies. We are currently recruiting a senior lead scientist, a structural biologist, a chemical biologist, and a medicinal chemist, who will be based within my research group at CeTPD to work on this project. We encourage spreading the word to anyone who might be interested."

The team was announced on Wednesday 8th March at the Cancer Grand Challenges Summit event in London, which I attended. KOODAC is one of two teams funded by the Cancer Grand Challenge to tackle paediatric cancer. KOODAC is funded by Cancer Research UK, Institut National Du Cancer and KiKa (Children Cancer Free Foundation). A joint team photo was taken with those attending the meeting from both teams and their patient advocates.

Read more about the international KOODAC team here:

https://cancergrandchallenges.org/teams/koodac and

https://news.cancerresearchuk.org/2024/03/06/changing-childhood-cancer-treatment-cancer-grand-challenges-koodac/

To apply to join our KOODAC team please follow the links to the positions from our website:

https://sites.dundee.ac.uk/alessio-ciulli/positions/

Industry-Academia Collaboration Award for our ACBI PROTAC Team

Contributors: Alessio Ciulli, Maria Lopalco and Kirsten McAulay

University of Dundee and Boehringer Ingelheim secured the prestigious Cancer Research Horizons (CRH) Innovation & Entrepreneurship Award 2024

The Centre for Targeted Protein Degradation, at the University of Dundee, and Boehringer Ingelheim research collaboration (ACBI PROTAC Team) have secured the prestigious Cancer Research Horizons (CRH) - Innovation and Entrepreneurship Awards 2024 in the category Further, Faster, Together (Industry-Academia Collaboration). The CRH Innovation & Entrepreneurship Awards aim to celebrate researchers and innovators actively bridging the gap between oncology discoveries and tangible patient outcomes.

This accolade is a recognition of the exceptional teamwork and dedication shown by the team over the years.

The journey began in July 2016 when the collaboration was formally established, bringing together the expertise of Prof. Alessio Ciulli and his team (at the time based within the Division of Biological Chemistry and Drug Discovery) with Boehringer Ingelheim's expertise in drug discovery and clinical development of new therapeutic agents. Since the incept, the team has focussed on translating fundamental academic discoveries into potential benefits for cancer patients through the development of proteolysis targeting chimeras (PROTACs); a revolutionary class of drugs that degrade proteins instead of only inhibiting them as conventional drugs do. The team's pioneering structure-guided approach to PROTAC design has allowed them to drug previously "undruggable" cancer targets.

The partnership was expanded in both 2018 and 2021 and it has continued to thrive since, with a large multi-disciplinary team across the two organisations. The joint team's research has led to the development of highly selective and potent first-in-class PROTACs against difficult-to-target proteins. Notable outcomes include the successful development of novel degraders with *in vivo* activity against two targets previously considered undruggable: the chromatin remodelling complex SMARCA2 (Farnaby et al. *Nat. Chem. Biol.* 2019; Kofink et al. *Nat. Commun.* 2022) and the cancer driver KRAS (Popow et al. *BioRxiv* 2023).

Moreover, the collaboration has embraced the ethos of open science. Several protein degraders developed by the joint team (including the SMARCA2 degraders ACBI1 and ACBI2) have been made freely available through OpnMe, an online portal established by Boehringer in 2017, thereby fostering a culture of knowledge exchange and advancing research initiatives for the benefit of patients with high unmet medical needs. The opnMe portal also provides access to two PROTAC degraders developed previously by the Ciulli academic group itself, namely the popular BET degrader MZ1 and the Brd7/Brd9 degrader VZ185.

The impact of this partnership extends beyond scientific breakthroughs. It serves as a model for effective academia-industry partnerships, as highlighted by Dr. Clive Wood, Corporate Senior VP, and Global Head at Boehringer, who stated, *"Our collaboration with Professor Ciulli and colleagues has brought significant success. This is an exceptional joint team positioned to discover and advance medicines that strike at the root cause of cancer."*

On the evening of Wednesday 20th March, the ACBI team attended the Cancer Research Horizons Innovations and Entrepreneurship Award Ceremony in central London. The team was represented at the ceremony by Alessio, Kirsten McAulay (ACBI team collaboration leader), Vesna Vetma (Senior Scientist, Cell Biology), Aileen Frost (Senior Scientist, Medicinal Chemistry), as well as Stephanie Glaser from Boehringer Ingelheim (Director for Alliance Management at BI's Global of Business Development and Licensing Boehringer Ingelheim). We were delighted and slightly surprised to be named as winner as we shared the prize with the Edinburgh University / Nuvectis, a collaboration deal which Maria Lopalco helped to secure during her previous tenure at Edinburgh (so a double-win for her!).

Following the announcement of the award, Alessio offered a brief speech where, on behalf of the whole team, he thanked the committee and extended our congratulations to all colleagues at Dundee involved in the establishment and support of our successful collaboration over so many years, with particular mention to Anne Muir at RIS who helped to establish and manage the collaboration, the ACBI team leaders, Will Farnaby since 2016 and Kirsten since 2022, who have impeccably led the team at Dundee, and all our colleagues at BI who have championed the collaboration over the years. This achievement will be duly celebrated also at the upcoming joint QR/JSC in-person meeting in Dundee in May!

In conclusion, this collaboration between the University of Dundee and Boehringer Ingelheim serves as a beacon of innovation in academia-industry partnership, exemplifying the true spirit of teamwork and offering a pioneering model for others to emulate.

From left to right: Aileen Frost, Stephanie Glaser, Alessio Ciulli, Kirsten McAulay, Vesna Vetma

Cell Biology

Contributor: Roberta

Proteome-scale discovery of protein degradation and stabilization effectors

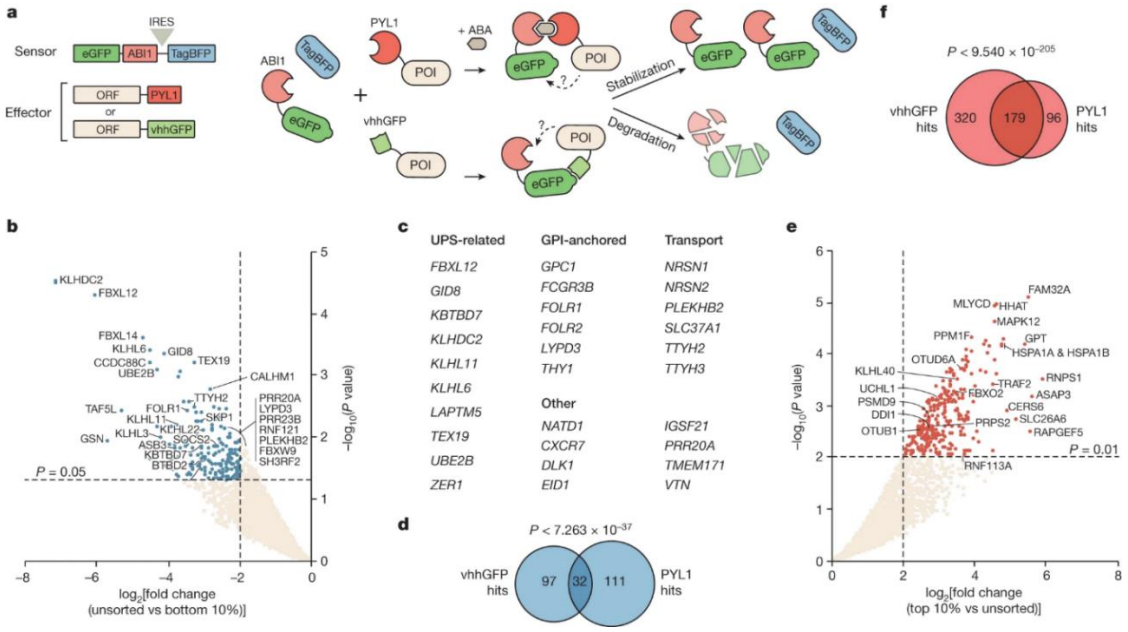
Juline Poirson, …, Mikko Taipale*

Nature **2024**, DOI:10.1038/s41586-024-07224-3

Poirson *et al.* reported the identification of human proteins that can promote the degradation or stabilisation of a targeted protein, triggered by proximity through the development of a synthetic proteome-scale protocol. The human genome expresses hundreds of E3 ubiquitin ligases and deubiquitinases, but just a few of them are, to date, being engaged by small molecules to induce degradation or stabilisation of the target. The identification of new effectors of degradation or stabilisation might open the possibility of recruiting them with small molecules, such as PROTACs, DUBTACs or molecular glues. To identify proximity-depending effectors at the proteome scale, they engineered 293T cells to stably express eGFP-ABI1 fusion protein and tagBFP. The cells were then used to express effectors fused to GFP nanobody (vhhGFP) or to PYL1, which binds ABI1 in the presence of abscisic acid (ABA). The degradation or stabilisation is then measured as a result of proximity-induced effect.

The method was validated by using two known E3 ligases and was then applied to a human ORFeome library of open reading frames (ORFs) tagged with either vhhGFP or PYL1 and hundreds of hits were identified. The same protocol was then applied to a selection of 300 E3 ligases and detached E2 ligases to verify the ubiquitination-dependent degradation of selected targets. The best degraders identified were proven also more potent than currently used E3 ligases, in the degradation of several disease-related proteins. The protocol was verified for biases and the results were confirmed through *in vitro* and *in vivo* models and resulted in a list of potential effectors that can be targeted in the near future for the development of small molecules as degraders or stabilisers.

Since targeted protein degradation (TPD) and targeted protein stabilisation (TPS) are among the most promising developments in drug discovery, the identification of new effectors of protein degradation or stability could drive the ligand discovery and the exploitation of these effectors for TPD and TPS. The synthetic proteome-scale protocol reported by Poirson and colleagues offered a new cell biology tool that coupled with proteomic mass analysis, represents a powerful screening approach that can support target identification for drug discovery in TPD and beyond.



Targeted Protein Degradation

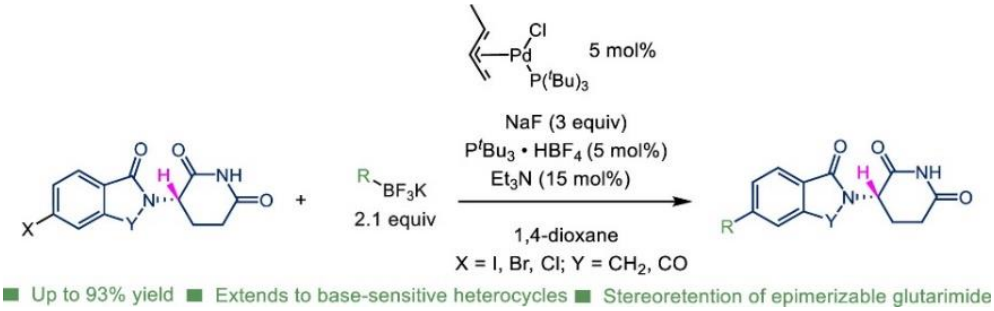
Chemistry

Contributor: Bharat

Anhydrous and Stereoretentive Fluoride-Enhanced Suzuki–Miyaura Coupling of Immunomodulatory Imide Drug Derivatives

J. Org. Chem. **2024**, DOI:https://doi.org/10.1021/acs.joc.3c02873

William F. Tracy[§], … Jesus Moreno*, Emily C. Cherney*, Huw M. L. Davies*



Ever since the thalidomide tragedy, our understanding of these glutarimide-containing drugs has evolved. Now, these toxic compounds, often referred to as immunomodulatory imide drugs (IMiDs) or Cereblon E3 Ligase Modulators (CELMoDs), have been repurposed for anticancer therapy utilizing Targeted Protein Degradation (TPD) strategy. The key pharmacophore of these drugs is the glutarimide ring which often poses synthetic challenges due to its poor solubility, propensity to hydrolysis and epimerization. As a result, late-stage functionalization of these drug molecules is often challenging. Tracy *et al*, reported the use of inorganic fluoride (NaF) base under anhydrous conditions to perform Suzuki Miyaura Couplings (SMCs) with good to excellent yield. It was observed that inorganic fluoride source enhances the reactivity irrespective of metal cation, though NaF performed slightly better than KF. This method is particularly useful for alkene-type potassium trifluoroborates and base-sensitive heteroarenes. SMC with phenyl trifluoroborate or electron-deficient heteroarene gave poor yield. Computational studies on phenyl trifluoroborate concluded that π complexation of trifluorobarate with Pd is an endothermic process in contrast to exothermic for vinyl trifluoroborate. As a result, phenyl transfer becomes a less efficient process leading to a poor yield.

This paper provides an excellent method which is mild enough to prevent hydrolysis and racemization of the glutarimide ring. Overall, this study represents an excellent addition to a chemist's toolbox who would like to perform late-stage modifications on glutarimide-containing drugs. Further optimization (different bases, temperature, catalyst etc) is warranted for electron-poor arene to improve the yield and widen the scope of the methodology.

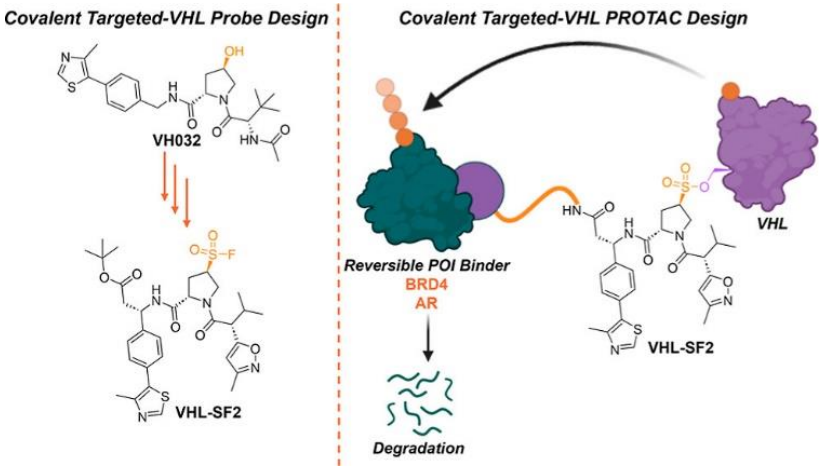
Cell Biology | Chemistry | Computational Chemistry | Structural Biology/Biophysics

Contributor: Bharat

Structure-Guided Design and Optimization of Covalent VHL-Targeted Sulfonyl Fluoride PROTACs

Rishi R. Shah*,…., Edward W. Tate*

J. Med. Chem. **2024**, 67, 6, 4641–4654



Von Hippel-Linadu (VHL) is one of the widely used E3 ligase in TPD field. The VHL binder consists of (R)-hydroxyproline which interacts with Ser110 in the HIF1α binding site of VHL. These interactions are very essential for VHL recognition. Recently several covalent PROTACs bearing an electrophilic warhead (acrylamide or chloroacetamide) targeting cysteine have been reported. In addition, a covalent CRBN binder bearing a sulfonyl fluoride has been reported as a molecular glue. In the same line, the authors sought to convert a hydroxyl group into a sulfonyl fluoride as an electrophilic warhead to

generate first generation VHL covalent ligand (**VHL-SF1**) which failed to completely displace a fluorophore-labelled HIF1α peptide up to 100 µM. Swapping tert-leucine moiety for a methyl isoxazole yielded a second-generation covalent ligand (**VHL-SF2**). VHL-SF2 showed improved efficacy with IC_{50} of 35µM and showed 65% conversion in 24 hours by intact LCMS. VHL-SF2 was further incorporated into PROTACs and checked for degradation of BRD4 and AR. Though the degradation efficiency does not match with non-covalent PROTACs, the sulfonyl fluoride-based PROTACs were capable of inducing UPS-dependent protein degradation.

This study discussed the potential advantages of transforming the ternary complex into simple binary interactions between a POI and covalently modified E3 ligase (VHL). At the VHL binder level, there is no direct evidence of covalent modifications at the desired site (Ser110). Based on the absence of evidence for modifications of the protein at other sites and the absence of Ser110-containing peptides in samples treated with VHL-SF2, it was concluded that VHL-SF2 is bound or attached to the predicted site. Direct evidence is needed as significant loss of potency was observed for VHL-SF2 compared to VH032. Moreover, the reasons for the loss of potency of VHL-SF2 are not clear. It could be due to modification at the critical hydroxyproline site or hydrolytic stability of sulfonyl fluoride at physiological pH and temperature. Further investigation is required to answer this question. This study further paves the way for medicinal chemistry efforts to optimize the pharmacokinetic and pharmacodynamic properties of these covalent sulfonyl fluoride PROTACs. Other sites on VH032 could be considered for modification as the H-bond interaction of hydroxyproline is crucial for VHL recognition.

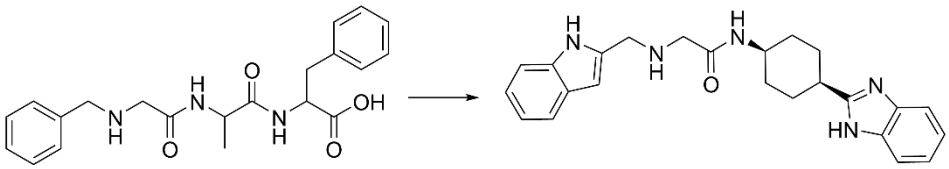
Cell Biology | Chemistry | Computational Chemistry | Structural Biology/Biophysics

Contributor: Bharat

Chemical tools for the Gid4 subunit of the human E3 ligase C-terminal to LisH (CTLH) degradation complex

Aliakbar Khalili Yazdi[§], …, Dafydd R. Owen*

RSC Med. Chem., **2024**,*15*, 1066.



The ortholog C-terminal to LisH (CTLH) degradation complex in humans has the same function of glucose-induced degradation (GID)-like ubiquitin ligase activity as in the yeast. In addition to the formation of multi-protein supramolecular assemblies, the CTLH complex also retains the structurally homologous Gid4 substrate receptors. In this paper, Yazdi *et al.* have developed a novel tool to interrogate and understand primary and secondary functions of CTLH complex. They embarked on unbiased Affinity Selection Mass Spectrometry (ASMS) screening on 500000 compounds and found tripeptide **2** (Bn-Gly-Ala-Phe-OH) as a hit. The hit was further validated by orthogonal methods like Fluorescence Polarization (FP) and Surface Plasmon Resonance (SPR). Now, rather than establishing stereochemical makeup of **2**, they perform a substructure search for *N*-benzylated glycinamide on a multimillion Pfizer compound library to obtain benzylated glycinamide **3-5**. It was observed that the *N*-benzylated glycine mimics the N-terminal proline found in synthetic tetrapeptide degron (compound **1**). Further screening to diversify the scaffold beyond benzylamide yielded Compound **7** (also called PFI-E3H1) which had a K_d of 0.5 µM in SPR and excellent cell penetration. Furthermore, 91 compounds were synthesized and tested to explore the exit vector and optimize the physicochemical properties. Compound **9** (also called PFI-7) has been developed as a chemical probe with Kd of 0.08µM in SPR and has excellent cell penetration. Acetylation of glycine moiety yielded negative control (K_d >50 µM).

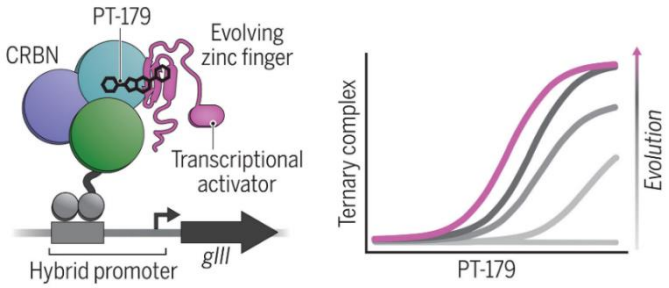
This paper narrates the story of the discovery and optimization of a chemical probe for Gid4 and paves the way to understand the biology of Gid4 and the human CTLH degradation complex. CTLH could be explored as an alternate E3 ligase if it has a degradative function. PFI-7 is an excellent addition to the non-covalent E3 ligase ligands though the use case of the probe is not disclosed.

Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Natalia

Continuous evolution of compact protein degradation tags regulated by selective molecular glues

Jaron A. M. Mercer[§], Stephan J. DeCarlo[§], Shourya S. Roy Burman[§], …, Amit Choudhary*, Eric S. Fischer*, David R. Liu*
Science 2024, 383, eadk4422,



Mercer *et al.* decided to address the problem of off-target degradation which arises when using existing degron systems based on zinc finger (ZF) degron tags and immunomodulatory drugs (e.g. thalidomide, lenalidomide and pomalidomide). They took advantage of the short life cycle of bacteriophage to evolve new, more specific degron tags. They generated a platform for molecular glue-phage-assisted continuous evolution (MG-PACE). In the system, a phage propagation is dependent on the interaction between CRBN, IMiD derivative and protein of interest (here a ZF degron tag sequence). As the IMiD derivative, a PT-179 compound was used. It is a pomalidomide analogue with a morpholine ring that doesn't degrade endogenous neosubstrates. Although it has similar affinity to DDB1-CRBN as pomalidomide, in a BRET assay, it is 7-fold less potent. To evolve a ZF tag that can efficiently engage PT-179 bound CRBN, the authors constructed MG-PACE. As a starting point, they used a 60 amino acid 'super-degron' tag. Although first trials with both full-length and CTD CRBN were unsuccessful, Mercer *et al.* used structure-based mutagenesis followed by less stringent phage-assisted noncontinuous evolution (PANCE) with 16 different derivatives to obtain better ternary complex formation with PT-179. To further increase selectivity, rounds of PANCE/PACE experiments were conducted followed by mutagenesis and degron tag truncation, resulting in 36 amino acid 'SD40' sequences for which PT-179 had a DC_{50} of 4.5 nM. The SD40 tag is small enough for efficient in-frame insertions both at the N- or C-terminus using prime editing tools, which yielded a lower by-product knock-in rate than CRISPR. The cryoEM structure gave an insight into a mechanism of CRBN – PT-179- SD40 binding, showing that SD40 engage both N- and C-terminal domains of CRBN keeping it in the closed state. Finally, SD40 was used to successfully evolve a degron tag engaging mouse CRBN.

To sum up, the authors narrate an exciting story of generating a platform for remodelling molecular glue interactions and applying it to develop a selective molecular glue-small degron tag pair that expands the suite of tools available to TPD researchers.

Cell Biology | Chemistry | Modelling/Simulation | Structural Biology/Biophysics

Contributor: Natalia

Orthogonal IMiD-Degron Pairs Induce Selective Protein Degradation in Cells

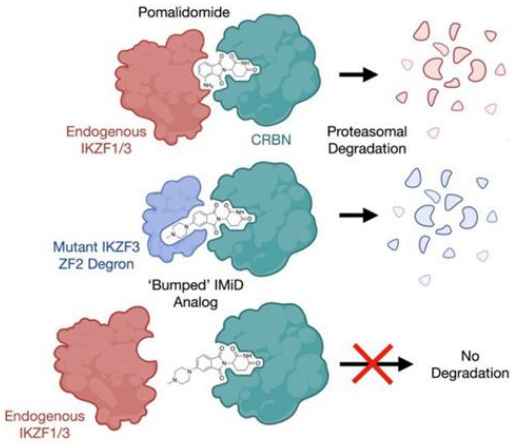
Patrick J. Brennan[§], …, Lewis L. Brayshaw*, Stuart J. Conway*
bioRxiv ***2024****, DOI: 10.1101/2024.03.15.585309*

The authors of this preprint have taken a 'bump and hole' approach to tackle the lack of selectivity in a zinc finger-immunomodulatory drug degron system. As a degron tag, they used IKZF3 ZF2 sequence (aa 130-189). Based on the CRBN-pomalidomide-IKZF1 crystal structure, a Q147A mutation in the degron sequence was introduced to disrupt the interaction with CRBN E378. Then, Brennan *et al.* generated 36 'bumped' analogues of thalidomide with different modifications at 4- and 5- position and analysed the degradation of wild type or mutated ZF-eGFP in Jurkat cells. The derivatives with modifications at 5- position (with 5-hydroxy, 5-*tert*-butyl, and 5-N-methylpiperazine groups) induced more potent degradation of Q147A tag (and less potent degradation of the unmodified tag compared to thalidomide). To increase the selectivity of IMID degron pair, further mutations were introduced. As investigating all possible mutations in the 9 amino acids at the interface of the proteins interactions gave too high number of possible degron sequences, the *in-silico* screening with Rosetta and FoldX was performed resulting in 8380 different ZF mutants. The library of the ZF-eGFP mutants was screened with 6 different compounds (including previously selected tert-butyl and N-methyl piperazine derivatives), sorted by FACS based on eGFP level and sequenced by NGS. The most selective (with a 35-fold increase in selectivity) pair turned out to be N-methyl piperazine derivative with Q147W, N149E, Q150I

mutations and unmodified S154 – 'WEIS' sequence. With the tert-butyl analogue, a 25- and 28-fold increase in selectivity was obtained with 'HEIP' and 'AEVK' variants, respectively. To assess possible interactions in ternary complexes with these modified ZFs, docking analysis was performed.

New interactions were observed between CRBN and the three variants that were not seen in a crystal structure of the WT sequence, including a hydrogen bond between E149 (N149 in WT) of ZF and Y355 of CRBN for all three variants. In the final part of the paper, authors performed the immunoblotting analysis of ZF-eGFP and endogenous IKZF3 proteins levels, followed by global proteomics after WEIS-TRIM28 protein degradation, which confirmed increased degradation selectivity.

In summary, the authors describe a successful story of the structure-based design of new combination of IMiD small molecule and ZF-based degron.



Cell Biology | Chemistry

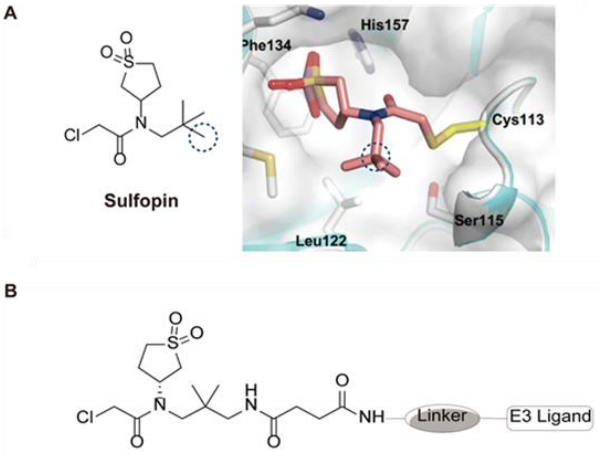
Contributor: Roberta

Discovery of potent PROTAC degraders of Pin1 for the treatment of acute myeloid leukemia

Yunkai Shi[§], Minmin Liu[§], Mengna Li[§], …, Wenlong Wang*, Yubo Zhou*, Jia Li* and Bing Zhou*

Chem. Sci., **2024**, *15*, 5027

In this paper, Shi *at al*. reported the development of a potent, first-in-class, covalent Pin1 PROTAC. Pin1 is a peptidyl-prolyl isomerase also known by the full name Peptidyl-prolyl cis/trans isomerase NIMA-interacting 1; Pin1 has been recognised to play a key role in oncogenesis and is overexpressed or overactivated in several human cancers.



The authors aimed at the development of a covalent PROTAC, using a covalent ligand for the targeted protein. Sulfopin was selected as a selective Pin1 inhibitor and through a structure-based approach they identified the appropriate exit vector to which install the linker to design the PROTACs. P1D-34 was identified as the most potent in inducing Pin1 degradation and antiproliferative activity on a series of acute myeloid leukaemia cell lines.

The antiproliferative mechanism of action was proved to be wide, from the downregulation of the Pin1 clients in a dose-dependent manner, to the down-regulation of the UPR pathway inducing DNA damage, to the further increase of ROS products that all together lead to apoptosis. With the publication of this highly promising Pin1 chemical probe by Shi and colleagues, a novel treatment approach for acute myeloid leukemia and other Pin1-related diseases may now be explored.

Structural Biology/Biophysics

Contributor: Roberta

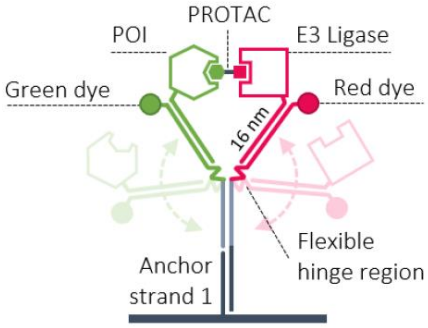
Proximity Biosensor Assay for PROTAC Ternary Complex Analysis

Irene Ponzo[§], Alice Soldà[§], …, Stefan Geschwindner*, Alessio Ciulli* and Ulrich Rant*

ChemRxiv **2024**, DOI: 10.26434/chemrxiv-2024-8w4zb

PROTACs are bifunctional small molecules consisting of two protein-binding moieties joined by a linker, one that targets a protein of interest (POI) while the other binds an E3 ubiquitin ligase. The simultaneous engagement of the two proteins originates a ternary complex which enables ubiquitination of the POI which is then degraded via proteasome. The formation of the ternary complex is the result of molecular interactions that rely on cooperativity

and avidity effect. It is essential to understand the ternary complex formation and how to induce it through in vitro assays to foster the design and development of efficacious PROTACs.



In this pre-print, Ponzo and colleagues reported a new Proximity Biosensor Assay for the analysis of PROTAC ternary complexes formation. They developed an assay that used the fluorescence energy transfer (FRET) technique to measure the formation of the complex and used a Y-shaped DNA scaffold to support the POI and the E3 ligase. The two proteins are anchored to mobile swivel arms of a DNA scaffold each, the arms are connected to the rigid body by flexible linkers that allow the arms to swing. Green and red fluorophores are attached to the distal ends of the swivel arms to detect binary binding via fluorescence quenching (FPS mode) and ternary binding via FRET signal, which is generated when the arms are closed. The assay was developed and optimised to have the best detection sensibility, and the length of the arms was optimised. The assay was validated by using it on two different E3 ligases (CRBN and VHL), four known PROTACs (AT1, MZ1, dBETs and ARV-825) and the two bromodomains of Brd2, Brd3, Brd4 and BrdT were used as POIs. In this preprint, the authors describe a very practical tool that allows the measurement of proximity-mediated binding enhancements as well as ternary and binary binding kinetics, simplifying and speeding up the development of PROTACs and molecular glues.

Cell Biology **Chemistry**

Contributor: Roberta

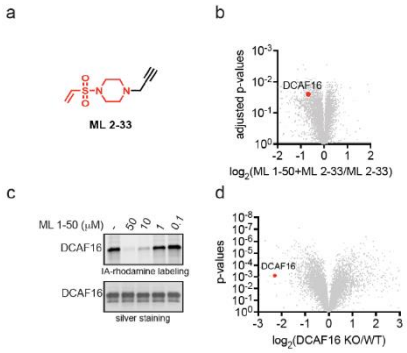
DCAF16-Based Covalent Handle for the Rational Design of Monovalent Degraders

Melissa Lim[§], Thang Do Cong[§], Daniel K. Nomura*

BioRxiv **2024**, DOI: 10.1101/2024.02.20.580683

Lim *et al.* present the identification of a transplantable covalent handle that can be applied to the exit vector of several proteins of interest (POI) ligands to recruit DCAF16 as E3 ligase to induce the degradation of their respective targets. The handle was identified among 18 warheads, linked to the well-known BET family inhibitor JQ1, the obtained 18 compounds were tested for BRD4 both short and long isoforms degradation, in different cell lines.

ML-150, the compound responsible for the highest degradation rate of both isoforms, was used to map the E3 ligase which was recruited. A simplified probe was synthesised and a chemoproteomic experiment was carried out, from which DCAF16 turned out as one of the handle-containing compound's targets. As might be expected, the small covalent compound bonded with a number of proteins, but DCAF16 was the only recruited E3 ligase. Through competitive activity-based protein profiling (ABPP) they observed that C119 was the targeted cysteine. Controls on DCAF16 knockout cells proved the degradation of BRD4 was obtained through DCAF16 engagement. Thereafter in this study, they appended the DCAF-16-binding covalent handle to a series of POI ligands, achieving the degradation of CDK4, the androgen receptor, BTK, SMARCA2/4, and BCR-ABL/c-ABL. This study also proved that different chemical handles might recruit different E3 ligases opening to a new approach to the design of covalent molecular glues. The authors also demonstrated a practical use of the covalent chemoproteomic approach to identify new ligases or their clients for TPD applications.



Other Paper Highlights

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

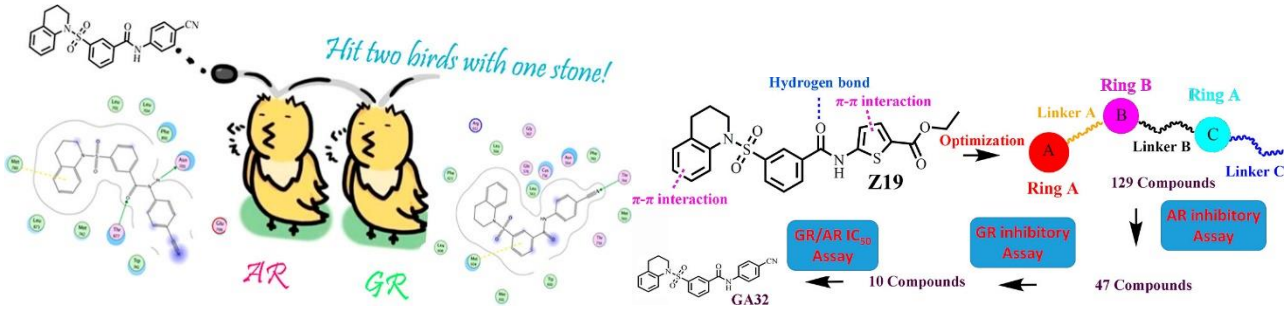
Contributor: Bharat

Design and Synthesis of Dual-Target Inhibitors Targeting Androgen Receptors and Glucocorticoid Receptors to Overcome Antiandrogen Resistance in Castration-Resistant Prostate Cancer

Chenfan Li[§], …. Jianlong Wang*, Meng Wu*, and Jinming Zhou*

J. Med. Chem. **2024**, *67*, 5, 3419–3436.

Simultaneously blocking of the Androgen receptor (AR) and glucocorticoid receptor (GR) signalling has become an efficient strategy for the treatment of castration-resistant prostate cancer (CRPC). In 2019, the Zhou group rationally reported dual AR/GR antagonist Z19. The predicted binding mode of Z19 in the AR/GR pocket indicated that rings A and C formed a π-π interaction, and the amide carbonyl formed an H-bond with the targets. Based on this observation, they have performed a structure-based, stepwise optimization of each ring and the linkers connecting these rings to yield GA32 as a potent dual antagonist. It was observed that a modification of ring C and linker C greatly improves the dual antagonistic activity. Particularly, the cyano group on the phenyl ring of GA32 formed a hydrogen bond interaction with Thr744. Modification of ring C remained an effective way to improve the binding affinity through additional hydrophobic interactions. Though GA32 is a potent dual antagonist, it has poor pharmacokinetic properties ($T_{1/2}$ = 1.16 h). Further optimization of GA32 is required to improve metabolic stability.



This is an excellent study on the optimization of Z19 as dual AR/GR inhibitors. However, attempts to improve pharmacokinetic properties were not disclosed (probably ongoing!). GA32 could serve as a starting point for developing dual PROTACs targeting AR/GR as POI. Authors also discussed mutations in the AR as a resistance mechanism and believed that a combination of dual AR/GR inhibitors with a selective AR degrader could expand the application in prostate cancer treatment.

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