CeTPD Journal Club

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

Journal Club

November 2022

Centre for Targeted Protein Degradation
University of Dundee

innovate
collaborate
inspire

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Meet this Month's Editors

This month's editors are (from left to right): Sarah Chandler, Calum McLaughlin and Giacomo Padroni

"Reading and contributing to the journal club has provided me with a great overview of the rapidly expanding field of TPD "

Sarah is a 3rd year PhD student working between the Ciulli academic group and Ron Hay's group, focusing on ubiquitination in TPD. She previously studied biomedical sciences at the University of Manchester with a placement year working in the high-throughput biology group at Boehringer Ingelheim, Biberach.

"Being new to the TPD field, the JC helps me keep up-to-date with the latest literature and provides context in a rapidly-advancing research area. I enjoy reading the diverse content and everyone's analyses in a concise format."

Calum joined the Centre for Targeted Protein Degradation in September 2022 as a postdoctoral researcher in biological chemistry. Previously, he was an Alexander von Humboldt postdoctoral research fellow in photochemistry in the group of Prof. Ryan Gilmour at the Westfälische Wilhelms-Universität Münster (Germany) and carried out his PhD at the University of St Andrews in the lab of Prof. Andrew Smith researching enantioselective organocatalysis.

"*The JC is great resource for keeping up to date with the field of TPD."*

Giacomo completed his undergraduate degree in pharmaceutical science at the University of Ferrara followed by a PhD in Chemical Biology in the lab of Prof. Glenn Burley at Strathclyde University. After two postdoctoral positions (Duke and ETH Zurich), he joined the ACBI team as medicinal chemist in July 2022.

Launch event for the TPD Early Career Network (EC-TPD)

Contributor: Valentina

On the 14th of November we held the launch event for EC-TPD at the School of Life Sciences, University of Dundee. The event brought together 120 researchers from across Scotland, the United Kingdom and Europe. The purpose of the event was to bring together ECRs in TPD to discuss their work and encourage network building. The event was organised by CeTPDers Yuting Cao, Xingui Liu, Sarah Chandler, Diane Purves, Emelye Macqueen and me. The EC-TPD event was made possible by generous funding from a SULSA grant and additionally supported by Promega and TakaraBio.

Over the course of the day, we had some great talks. Kicking things off was Rebecca Beveridge (University of Strathclyde) who gave the audience an insight into how native mass spectrometry could be used to study ternary complexes. She was followed by Luke Simpson and Lorraine Glennie (both University of Dundee) who told us about their work on the correlation between protein localisation and degradability. Lauryn Benbow (University of Lancaster) discussed her work on enhancing DNA damaging therapies by abrogating the G2/M checkpoint using degraders. Craig Malcolm and Lucy Wheatley from Promega gave the audience an explanation on how their protein degradation tools, including how HiBiT and Lumit™ can be used to study degraders. Alice Wicks (University of Glasgow) told us about her work on using alternative E3 ligases in TPD. Finally, Charlotte Crowe closed the day by telling us about the importance of ternary complex formation in PROTAC-mediated ubiquitination and degradation.

In between these great talks we had a networking lunch with a poster session, where we had ten posters from early career researchers in TPD. We had three poster prize winners: Bill Carton, Daniel Webb and Amanda Thomaz. The day also included a career-development panel featuring Alessio Ciulli, Danette Daniels, Mike Hann, Andrea Testa and Chiara Maniaci.

Contributor: Mark & Charlotte

Morning seminars delivered by cryo-EM experts

Group photo of iSCEM-DD network launch attendees

Tour of the Dundee cryo-EM facility, led by Subbu Sundaramoorthy

Breakout session discussion (pictured: Ed Morris, Charlotte Crowe, Paula Da Fonseca and Olawale Raimi)

This November CeTPD launched the inclusive Scottish Cryo-Electron Microscopy in Drug Discovery (iSCEM-DD) network, with generous support from the Scottish University Life Science Alliance (SULSA) and Thermo Fisher Scientific.

The launch event attracted a mix of over 60 new and experienced cryo-EM users across Scotland. The event included talks from a range of drug discovery and cryo-EM experts: Alessio Ciulli (University of Dundee), Paula Da Fonseca (University of Glasgow), Tom Owen-Hughes (University of Dundee), Martin Rennie (University of Glasgow), Marcus Wilson (University of Edinburgh), James Streetley (University of Glasgow), Subbu Sundaramoorthy (University of Dundee) and Atieh Aminian (Thermo Fisher Scientific).

However, the unique aspect of iSCEM-DD was the focus on accelerating collaboration between cryo-EM experts and new users to advance early-stage projects and ideas. In addition, other cryo-EM experts from Glasgow, Edinburgh and Dundee participated in discussions and breakout groups. Alessio Ciulli's talk on cryo-EM in Targeted Protein Degradation was inspiring and laid out the general challenges of structural biology for TPD. There was much excitement about the Glasgow (SCMI) and Dundee cryo-EM facilities, which are both capable of delivering maps approaching ~2Å resolution. Since the beginning of SCMI in 2018, the cryo-EM community in Scotland has been growing exponentially and the CeTPD is excited to be involved.

The event was initially hosted at the University of Dundee and led by Charlotte Crowe and Mark Nakasone, with support from Alessio Ciulli, Diane Purves and Emelye Macqueen. The success of the iSCEM-DD network will lead to a future 2023 meeting (to be announced). With special thanks to Kevin Haubrich (CeTPD postdoctoral researcher) for the photography!

Contributor: Valentina, Charlotte & Ollie

Alessio and Danette sporting their CeTPD hoodies

Ollie, Vesna, Danette and Alessio saying goodbye before Danette's taxi to Edinburgh airport

A great shot captured by Alessio in video here!

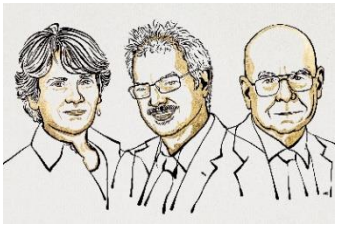
We were so happy to host Danette at the University of Dundee for a two-day visit between the 14-15th of November, a long overdue visit after her first visit was cancelled in 2020 due to the pandemic. Danette has collaborated with the Ciulli group over several projects including the trivalent PROTAC project and it was hugely exciting to her have finally visit us!

Danette Daniels is the VP of the Protein Degrader Platform at Foghorn Therapeutics. Prior to joining Foghorn, Danette was a R&D group pleader of functional proteomics at Promega Corporation. Danette is a veteran of TPD and has developed innovative approaches to monitor the kinetics of degradation in whole cells.

During her visit with us, Danette attended and contributed to the launch event for EC-TPD, including participating in a career panel to give her insight and advice to building a career to ECRs. The day after the EC-TPD launch event Danette gave an inspiring seminar on the work she & her team are doing at Foghorn Therapeutics bringing degraders to the clinic. Later in the day, Danette participated in a cell biology for degraders workshop with other cell biologists in the CeTPD, where she shared her invaluable knowledge and insight on developing cell-based assays for studying degraders. The day ended with dinner followed by a CeTPD game of pool – as Alessio commented, her shots are getting better and better, just like her degraders!

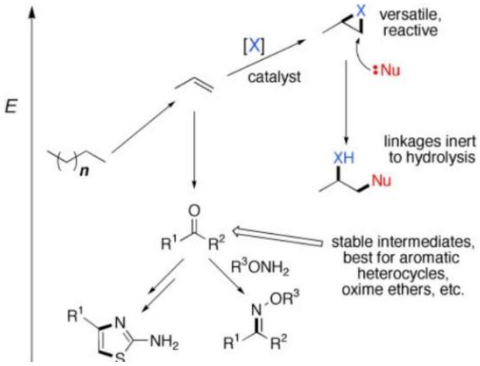
Contributor: Calum

Nobel Prize in Chemistry 2022



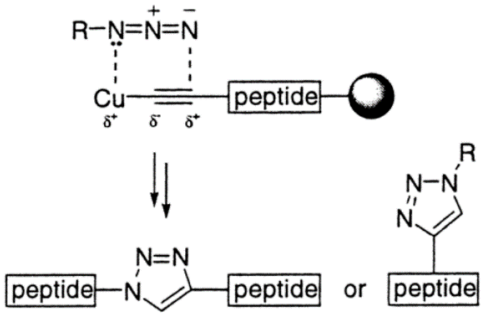
On 5th October 2022, the Nobel Prize in Chemistry was awarded to **Carolyn R. Bertozzi** (Stanford University, USA), **Morten Meldal** (University of Copenhagen, Denmark) and **K. Barry Sharpless** (Scripps Research, La Jolla, USA) for their outstanding contributions towards "the development of click chemistry and bioorthogonal chemistry". Below are landmark papers from each winner.

Click Chemistry: Diverse Chemical Function from a Few Good Reactions
Hartmuth C. Kolb[§], M. G. Finn, and K. Barry Sharpless*
Angew. Chem. Int. Ed. **2001**, *40*, 2004



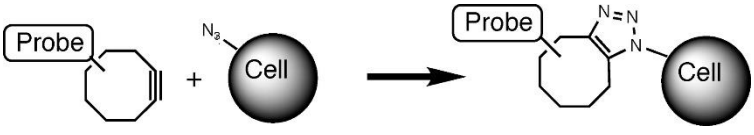
In this article, K. Barry Sharpless, who was previously awarded the Nobel Prize in Chemistry in 2001 "for his work on chirally catalysed oxidation reactions", and co-workers introduced the concept of click chemistry. They envisioned streamlined and robust organic synthesis with reactions that would possess ideal characteristics including (amongst others) modularity, wide scope, mild reaction conditions, use readily available building blocks and involve facile product isolation. Independently, Meldal and Sharpless would go onto discover what is considered as the gold-standard click reaction (*vide infra*) which is now in widespread use.

Peptidotriazoles on Solid Phase: [1,2,3]-Triazoles by Regiospecific Copper(I)-Catalyzed 1,3-Dipolar Cycloadditions of Terminal Alkynes to Azides
Christian W. Tornøe[§], Caspar Christensen, and Morten Meldal*
J. Org. Chem. **2002**, *67*, 3057



Prior work on Huisgen 1,3-dipolar cycloadditions required elevated temperatures and commonly resulted in mixtures of the 1,4 and 1,5 triazole regioisomeric products. In 2002, Morten Meldal and co-workers described the copper-catalysed azide-alkyne cycloaddition (CuAAC) to give 1,4-substituted triazoles using CuI. The reaction proceeded in high yields, at ambient temperatures, and was amenable to solid-supported peptide synthesis. Sharpless also developed a regioselective CuAAC using $CuSO_4$ to form 1,4-substituted triazoles and demonstrated the reliability and broad scope of the reaction, which could be carried out in water (*Angew. Chem. Int. Ed.* **2002**, *41*, 2596).

A Strain-Promoted [3 + 2] Azide-Alkyne Cycloaddition for Covalent Modification of Biomolecules in Living Systems
Nicholas J. Agard[§], Jennifer A. Prescher, and Carolyn R. Bertozzi*
J. Am. Chem. Soc. **2004**, *126*, 15046



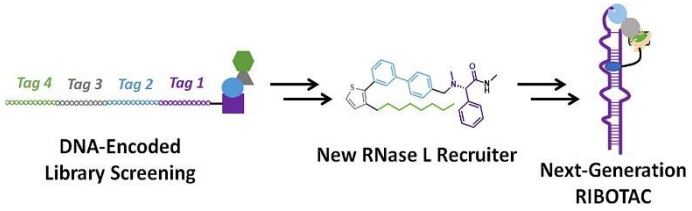
In 2004, Carolyn Bertozzi and co-workers reported a strain-promoted [3 + 2] azide-alkyne cycloaddition using cyclooctyne derivatives under physiological conditions. The reactions were used to selectively modify biomolecules (glycans) and living cells without any apparent toxicity. These biorthogonal reactions are now widely used to explore cells and track biological processes and have improved the targeting of pharmaceuticals.

Chemistry | Cell Biology

Contributor: Giacomo

DNA-Encoded Library Screening to Inform Design of a Ribonuclease Targeting Chimera (RiboTAC)

Samantha M. Meyer[§], …, Matthew D. Disney*
J. Am. Chem. Soc. **2022**, 144, 21096



As therapeutically relevant RNA targets and the principles for their recognition are rapidly emerging, this work expands the toolbox for proximity-induced degradation of RNA (RiboTAC). Disney and co-workers describe a DNA-encoded library strategy to identify novel recruiters of RNAse L. From an initial library of 2.8 billion compounds, a 2- benzylidine-3-thiophenone scaffold emerged as a RNAse L recruiter in its active dimeric form. An optimized recruiter was then conjugated to Dovitinib, a known kinase inhibitor that also targets pre-miR-21, which is highly expressed in many cancers. A series of *in vitro* assays evaluated target engagement, ternary complex formation and mechanism of action. Finally, the RiboTAC was shown to reduce miR-21 dependent cellular phenotypes in TNBC cell line MDA- MB-231 and its mode of action corroborated by a transcriptome and proteome-wide analysis. Overall, this provides proof-of principle that the new RNAse L recruiter is effective.

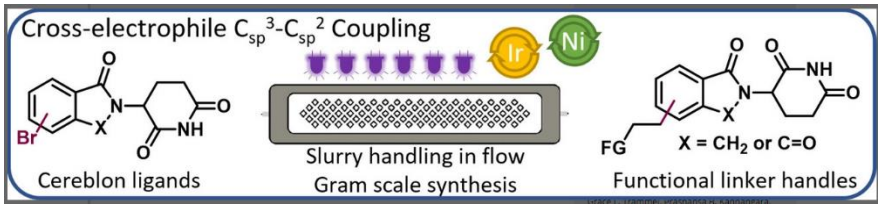
As the RNA world is gaining momentum, RiboTACs add an extra layer of complexity to the realm of induced-proximity targeted degradation. Arguably, very fast degraders are required for short-lived RNA targets. On the RiboTACs side is that the digesting enzyme is directly recruited, reducing the chances of ineffective steps in the degradation pathway.

Chemistry

Contributor: Giacomo

Photoredox C_{sp}^3–C_{sp}^2 Reductive Cross-Couplings of Cereblon Ligands for PROTAC Linker Exploration in Batch and Flow

Alexander Steiner[§], …, Hans-Michael Eggenweiler*, Jason D. Williams*, C. Oliver Kappe*
ChemCatChem **2022,** DOI: 10.1002/cctc.202201184



In this manuscript, Steiner and co-workers showcase a photoredox mediated C_{sp}^3- C_{sp}^2 coupling for a variety of Cereblon-binding chemotypes that is proven to be efficient both under batch and flow conditions. The main advantage of this approach is the possibility to directly functionalize the aromatic core of these chemotypes with aliphatic moieties (both linear and cyclic) expediting medicinal chemistry efforts in the generation of new PROTAC molecules. The conditions are compatible with the relatively-labile imide core and several functionalizations of the aliphatic moieties such as halogens, alkynes, esters and dioxolane-protected aldehydes. Some chemical groups (such as azides) and substrates like α-halo esters and benzylic halogens are instead not compatible due to interference with the catalyst or fast proto-dehalogenation. This work also provides an exhaustive characterization of the reaction in an oscillatory flow system (to handle slurry generated by the reaction) together with the detailed reasoning behind the optimization of parameters required for achieving fast and efficient transformations in larger scale.

The systematic study of this type of chemical transformations in the context of a PROTAC-focused chemical space is warmly welcomed by the numerous medicinal chemists invested in targeted protein degradation.

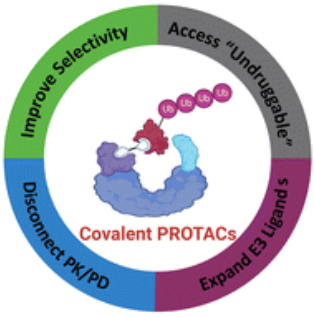
Chemistry | Structural Biology/Biophysics | Cell Biology

Contributor: Giacomo

Applications of covalent chemistry in targeted protein degradation

Dong Lu[§], …, Jin Wang*

Chem. Soc. Rev. **2022**, *51*, 9243



This review provides a comprehensive description of the state of the art of covalent interactions in PROTACs. Advantages of covalent binders such as enhanced target selectivity and engagement as well as the possibility to address poorly targetable proteins are analyzed in the context of ternary systems. As per the inherent increased complexity of PROTACs compared to inhibitors, careful choices need to be made before investing in drug programs which involve covalent binders of the POI. Whilst this approach might be required for addressing poor engagement or achieve higher selectivity, it results in non-catalytic degradation and potentially discrepant results. Reversible covalent bonds (such as the one resulting from α-cyano acrylamide warheads) could address this. However, determining the underpinning mechanism of degradation of these compounds might not be straightforward. On the other side, the advantages resulting from covalent E3 ligase binders are clearer as the catalytic degradation is retained and further simplified into a pseudo-binary system.

Undoubtedly, exploiting covalent interactions is and will be a primary avenue to expand the toolbox of hijackable E3 ligases.

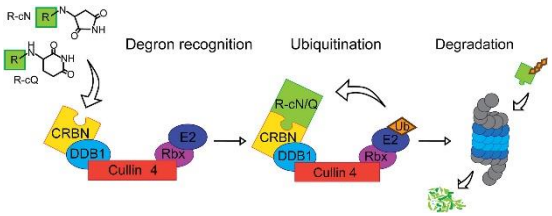
Structural Biology/Biophysics | Chemistry

Contributor: Giacomo

Identification and structural basis of C-terminal cyclic imides as natural degrons for cereblon

Christopher Heim[§], …, Marcus D. Hartmann*

Biochem. Biophys Res Commun. **2022**, *637*, 66



This work provides the structural basis of cereblon (CRBN) recognition of C-terminal peptidic imides further corroborating the evidence of the regulatory role of CRBN for naturally occurring degrons. This systematic top-down study identified preferred degron motifs by exploiting truncated versions of the peptide APP, whose C-terminal portion is known to interact with CRBN. By combining microscale electrophoresis and x-ray crystallography, the authors demonstrated how a key C-terminal asparagine residue binds to the aromatic cage of MsCl4 (a CRBN isoform of *Magnetospirillum gryphiswaldense*). Serendipitously, it was found that only the cyclized-imide asparagine by-product formed during the solid phase synthesis of the peptide is recognized by MsCl4. This imide is indeed reminiscent of naturally occurring chemical motifs found in ageing proteins. The authors further showed how 5 and 6-membered imide rings that are specifically located at the C-terminus of peptides share similar binding modes to MsCl4 and have analogous affinity for the human isoform of CRBN.

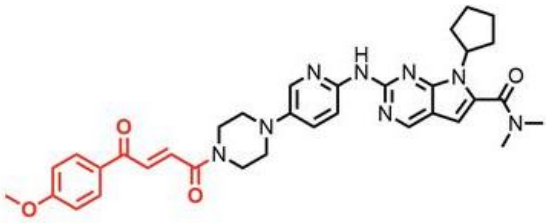
The discovery of CRBN and its function is indeed a peculiar one. A rare event when an unfortunate drug design led the way to a new revolutionary generation of therapeutics, and eventually facilitate the identification of the natural substrates of a protein.

Cell Biology | Chemistry

Contributor: Calum

Rational Chemical Design of Molecular Glue Degraders

Ethan S. Toriki[§], James W. Papatzimas[§],…, Daniel K. Nomura*
bioRxiv **2022**, DOI: 10.1101/2019.12.11.123456



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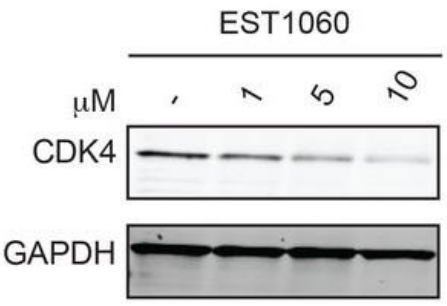


Compared to the modularity of PROTACs, there are limited rational chemical design principles for the discovery of novel molecular glues, with previous findings relying on fortuitous breakthroughs or using cell-based phenotypic screening. In this study Nomura and co-workers sought to identify a transposable chemical handle which could be appended to existing ligands of various targets thereby converting these to molecular glue degraders with minimal structural modification.

Using Ribociclib (CDK4/6 inhibitor) as a model substrate, the authors initially identified a trifluoromethylphenyl cinnamamide moiety to induce CDK4 degradation. Structure-activity relationship investigations led to identification of the optimal chemical handle (fumarate-piperazine derivative) necessary for RNF126 recognition. The authors showed the generality of the covalent tag by appending it onto ligands against several targets (CDK4, BCR-ABL and c-ABL, PDE5, BRD4, SMARCA2, and LRRK2) to induce degradation.

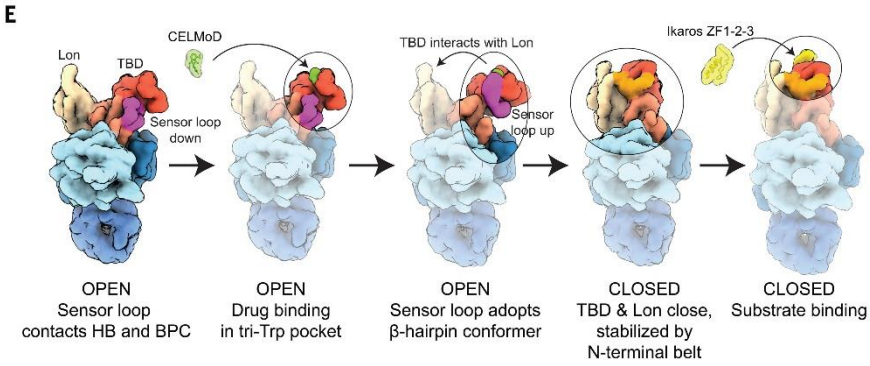
This proof-of-concept study demonstrates a systematic approach to the design of new molecular glues. The wide utility demonstrated by attaching a relatively simple handle is impressive and the potential physicochemical benefits are noticeable (lower molecular weight compounds with more "drug-like" features). It should be noted that it appears RNF126 is also degraded, which could limit the catalytic ability of the method.

Structural Biology/Biophysics

Contributor: Calum

Molecular glue CELMoD compounds are regulators of cereblon conformation

Edmond R. Watson[§], …, Gabriel C. Lander*
Science. **2022**, *378*, 549



In this article, Lander and co-workers leverage cryogenic electron microscopy (CryoEM) to resolve the structure of DDB1-CRBN complexes and examine the effect of CELMoD molecular glue compounds on the mechanism of allosteric neosubstrate binding. They found that the DDB1-CRBN complexes exist in a default "open" conformation, which then transitions from the "open" to "closed" form in the presence of various CELMoDs (pomalidomide, iberdomide, mezigdomide) via triggered rearrangement of the sensor loop.

A significant aspect of this research was that the conformation of the complex could be linked to substrate binding: association of the neosubstrate protein (Ikaros ZF) was only observed to the "closed" conformer, demonstrating that the conformation is critical for recruitment of target proteins. This report provides great insights and highlights additional factors into the allosteric mechanism of molecular glues and CRBN. One problem endured in the TPD field has been the inability to achieve sufficient target protein degradation despite having compounds which bind tightly to

cereblon. The report herein gives greater understanding why this could be the case and what drugs must be able to do to achieve efficacy. In addition, an optimised procedure for the sample preparation of DDB1-CRBN complexes was developed, which will lower the barrier to researchers studying DDB1-CRBN using CryoEM and hopefully pave the way for significant advances in the TPD field.

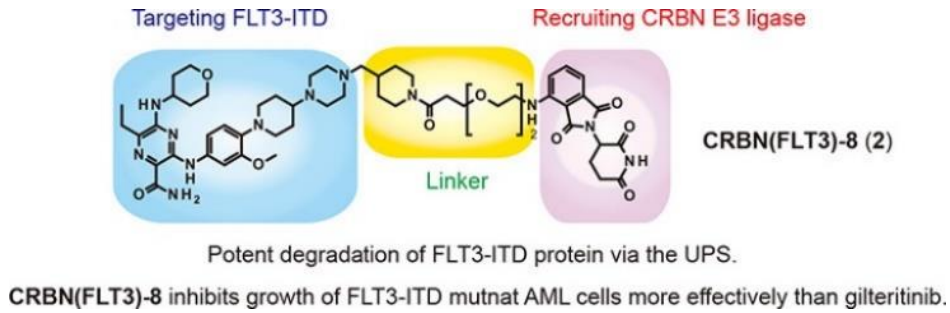
Cell Biology | Chemistry

Contributor: Calum

Development of Gilteritinib-Based Chimeric Small Molecules that Potently Induce Degradation of FLT3-ITD Protein

Nobumichi Ohoka*, …, Mikihiko Naito*

ACS Med. Chem. Lett. **2022**, DOI : 10.1021/acsmedchemlett.2c00402



Internal tandem duplication (ITD) in the gene encoding FMS-like tyrosine kinase 3 (FLT3) (FLT3-ITD) is the most common mutation (25%) observed in acute myeloid leukemia (AML). As an alternative to FLT3 kinase inhibitors which have been shown to be susceptible to drug resistance via reactivation of FLT3, Ohoka, Naito and co-workers designed and synthesised a series of PROTACs based on various FLT3 inhibitors (gilteritinib and crenolanib) and E3 ligase (IAP, VHL, CRBN) recruiting ligands. The compounds composed of gilteritinib and pomalidomide most effectively reduced protein levels in acute myeloma cell lines, and further linkerology revealed that a PEG2 was the optimal linker length (compound CRBN(FLT3)-8).

The authors carried out a very thorough investigation which included extensive studies into the protein degradation. Amongst others, they found that the degradation was UPS dependant and confirmed that $CRL4^{CRBN}$ was required for degradation activity. Intriguingly, they also showed that CRBN(FLT3)-8 inhibited cell proliferation of FLT3-ITD mutant AML cells more potently than gilteritinib, an FDA-approved inhibitor. Overall, it was enjoyable to read such a thorough and well-structured article.

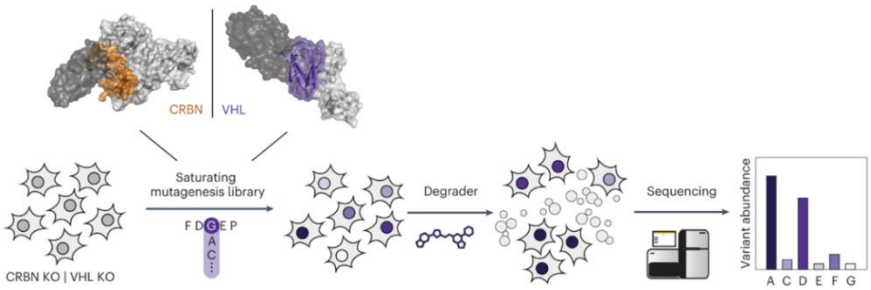
Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Sarah

Functional E3 ligase hotspots and resistance mechanisms to small-molecule degraders

Alexander Hanzl[§] ,.., Georg E. Winter*

Nat. Chem. Biol. **2022,** DOI: 10.1038/s41589-022-01177-2



Acquired resistance is a major challenge for TPD. Clinical data from patients treated with the IMiD, pomalidomide, show that up to one-third of refractory patients present with various types of alterations in the substrate receptor, CRBN. To support the prediction of resistance mechanisms this study uses a genetic approach to identify 'functional hotspots', which are defined as regions within the E3 interfaces involved in ternary complex formation. They use deep mutational sequencing (DMS) using cellular fitness as downstream readout, to identify hotspots in the CRBN and VHL substrate receptors. This is then complemented with biophysical and structural data. Comparing the SMARCA2/4 PROTAC, ACBI1 with a range of BET protein degraders they demonstrate that the hotspots identified in VHL are neo-substrate specific. To identify degrader specific hotspots in VHL they compared BET protein degraders and

demonstrate using a fluorescence polarisation assay that VHL^{P71} is a functional hotspot specific to MZ1 and macroPROTAC-1 but not ARV-771. Finally, the authors integrate the DMS data with available clinical data and find CRBN hotspots that are mutated in multiple myeloma patients relapsing from treatment with lenalidomide and pomalidomide. It will be interesting to see whether the CRBN and VHL functional hotspots identified in this study will converge with data from ongoing clinical trials as this could prove to be a powerful and widely adopted approach to study degrader resistance mechanisms.

Cell Biology **Chemistry**

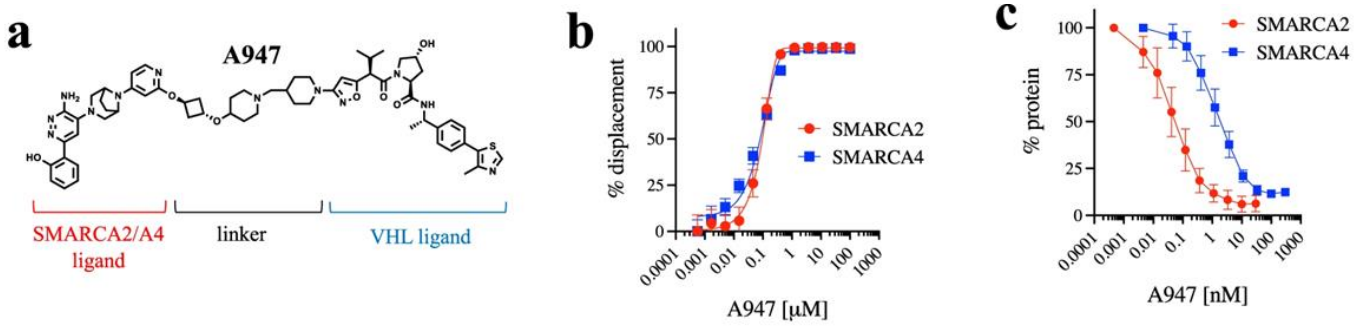
Contributor: Sarah

Selective PROTAC-mediated degradation of SMARCA2 is efficacious in SMARCA4 mutant cancers

Jennifer Cantley[§],…, Robert L. Yauch*

Nat. Comms. **2022**, *13*, 6814

The switch/sucrose non-fermentable (SWI/SNF or BAF) complex facilitates chromatin remodelling and has gained traction in cancer biology as ~20% of human cancers harbour mutations in specific core or accessory components of the complex. The ATP-dependent helicases, SMARCA2 and SMARCA4 provide catalytic activity to the complex in a mutually exclusive manner. SMARCA2 is an attractive drug target because the loss of SMARCA2 in cancers with inactivated SMARCA4 is synthetically lethal. However, selectivity for SMARCA2 over SMARCA4 is hampered by the close homology between the paralogs. Here, the authors linked a small-molecule ligand capable of binding the bromodomains of SMARCA2/4 to a VHL ligand yielding, PROTAC A947. Despite there being only minor differences in binding affinity to the SMARCA2 and SMARCA4 bromodomains (SMARCA2 Kd = 93 nM, SMARCA4 Kd = 65 nM) A947 degrades SMARCA2 with a DC_{50} of 39 pM but requires ~28-fold higher concentration (1.1 nM) to degrade SMARCA4. Global ubiquitination following A947 treatment (500 nM) was analysed, and no off-target degradation was identified. A947 inhibited growth of SMARCA4-mutant NSCLC cells and demonstrated activity *in vivo* in two different $SMARCA4_{mut}$ lung cancer xenograft models, HCC515 and HCC2302 inducing near complete growth inhibition and 60% tumour growth inhibition, respectively. Next, the authors performed a screen for combination therapies and identify a synergistic relationship between A947-dependent SMARCA2/4 degradation and MCL1 inhibition. The discovery of A947 as a potentially selective SMARCA2 degrader has positive implications for the treatment of SMARCA4-defective cancers. It would be beneficial for future studies to uncover the mechanism for SMARCA2 selectivity over SMARCA4.



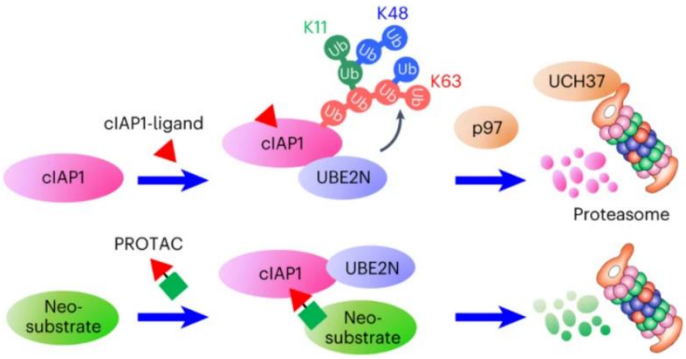
Cell Biology | Chemistry

Contributor: Sarah

cIAP1-based degraders induce degradation via branched ubiquitin architectures

Yoshino Akizuki[§] ,…, Fumiaki Ohtake*

Nat. Chem. Biol. **2022**, DOI: 10.1038/s41589-022-01178-1



The general dogma is that K48-linked ubiquitin chains target proteins for proteasomal degradation, and this is frequently observed for the $CRL2^{VHL}$ and $CRL4^{CRBN}$-targetting degraders. Here, the authors use the RING-type E3 ligase cellular inhibitor of apoptosis protein 1 (cIAP1) as a model to further understand the diversity within the degrader-generated ubiquitin code. Mimetics of the endogenous cIAP1 substrate, SMAC, induce the self-ubiquitination and subsequent proteasomal degradation of the RING E3, which induces cancer cell death. However, these mimetics are also used as the E3-binding moieties for the PROTACs known as specific and non-genetic IAP-dependent protein erasers (SNIPERs). Therefore, degradation of both cIAP1 and neo-substrates cooperatively induces cancer cell death. They use the SMAC mimetic, LCL-161 to precipitate endogenous cIAP from HCT-116 cells and identify a mixture of K11, K48 and K63-linked ubiquitin chains. They demonstrate that the chain initiating E2s UBE2D (1-4) and the K63-linkage specific chain extending E2, UBE2N are required for the cellular degradation of cIAP1. Reconstituting this system *in vitro,* they show that these E2s work in concert to build a branched ubiquitin chain with K63 linkages proximal to cIAP1 and distally located K11 and K48-linkages. Interestingly, they find that p97 and the K48-linkage specific proteasomal DUB, UCH37 interact with modified cIAP and are required for the degradation of cIAP1. Indicating that the distal K48-linked portion of the branched chain acts as the signal that targets the substrate to the proteasome. This study reveals a novel role for the E2, UBE2N, providing additional diversity to the mechanisms used for chemically induced targeted protein degradation.

Chemistry | Cell Biology | Structural Biology/Biophysics

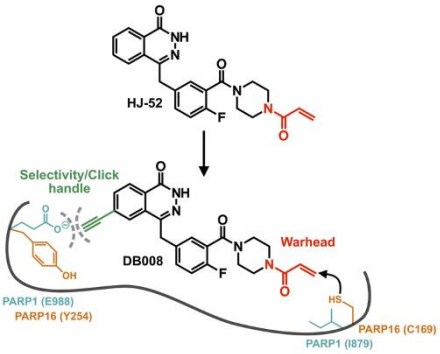
Contributor: Petr

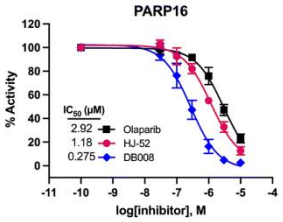
Structure-guided design and characterization of a clickable, covalent PARP16 inhibitor

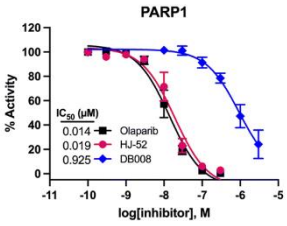
Daniel S. Bejan[§], ..., Michael S. Cohen*

Chem. Sci., **2022**, *13*, 13898

Poly(ADP-ribose) polymerase-16 (PARP-16) is a new highly promising anticancer target. This protein is a common non-canonical target of many PARP-1-4 inhibitors such as Talazoparib or Olaparib. However, relatively little is known about catalytic activity of PARP16. Selective membrane-permeable PARP16 inhibitor is a key point to a better understanding of physiological and pathophysiological function of this enzyme.







After a close look at the crystal structures of PARP1-bound Olaparib and overlaying it with PARP16 researchers found out that cyclopropyl moiety can be in a close proximity to PARP-16 Cys169. Simple replacement of cyclopropyl amide with an acrylamide designed to react with Cys169 of the D-loop in PARP16 led to a new covalent PARP-16 inhibitor HJ-52.

The next step of the modification was based on using Tyr254 as a “gatekeeper” to control access to a hydrophobic cavity adjacent to the nicotinamide sub-pocket of the NAD+ binding site. New analogue DB008 contains an ethynyl group at the C-6 position of the phthalazinone scaffold. This modification led to enhanced PARP family-wide selectively. DB008 irreversibly inhibits PARP16, but not other PARP family members.

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