CeTPD Journal Club

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

March 2023

Centre for Targeted Protein Degradation
University of Dundee

innovate
collaborate
inspire

Journal Club

Content

Meet this Month's Editors

This month's editors are (from left to right): Andre Wijaya, Guillem Loren and Sarath Ramachandran.

"The journal club provides a nice overview of the recent development in the TPD field. Personally, I found it very useful and glad to be able to contribute to it"

Andre obtained his undergraduate degree in Applied Biology with Biotechnology in 2015 from the Hong Kong Polytechnic University. He then moved to Cambridge in 2016 to pursue his PhD study in Biological Chemistry at University of Cambridge under the supervision of Dr Martin Welch. He joined the Ciulli group in October 2020 as a drug discovery scientist on the PROTAC collaboration projects with Boehringer Ingelheim.

"Keeping up with the TPD literature is a hard task. As an editor, I appreciate the opportunity to make it a bit easier for the community"

Sarath did his Ph.D. in structural from National University of Singapore and moved to Prof. Ciulli's lab in February 2017 as Post-doctoral Research Scientist. He joined the AC-Boehringer Ingelheim collaboration as a Senior Drug Discovery Scientist in March 2022

"*It's a good way to keep up with the literature in the field*."

Guillem completed his bachelor's degree and master in organic chemistry at the University of Barcelona. After being employed by GATT Technologies B.V. in the Netherlands as a Junior Scientist, he moved back to Barcelona to undertake his PhD at the IRB Barcelona under supervision of Prof. Antoni Riera. There he works on the design and synthesis of bifunctional molecules targeting degradation (PROTACs) and phosphorylation of various proteins of interest.

The launch of CeTPD Outreach

Contributor: Dr Selma Gulyurtlu

Special Acknowledgement: University's Public Engagement Network, Dr Amy Cameron, Shabnam Wasim and Erin Hardee for their help and guidance

The Centre for Targeted Protein Degradation (CeTPD) is committed to achieving all angles of our slogan "Innovate, Collaborate, Inspire". Correspondingly, this is not limited to stakeholders of the scientific community, but the commitment for us goes wider into the general public. As part of many thriving new technologies, comes the desire to reach out to raise awareness of progress in the scientific, medical and pharmaceutical discovery fields, as well as create aspirations for the youth or anyone looking for a new adventure. As such, at the start of 2022 the prospect of CeTPD Outreach started to become a reality.

An idea first thought of by one of our structural biology postdoctoral scientists, Dr Valentina Spiteri, and henceforth developed and coordinated by one of our cell biology postdoctoral scientists, Dr Selma Gulyurtlu, CeTPD Outreach is our public engagement sub-team that works closely with the University's public engagement team to deliver enjoyable, innovative and educational activities to different target audiences of the general public.

To start us off, with an involvement from over 20 members of the centre (not limited to scientists, but also our support staff), we identified the first target audiences we were interested in and created focus groups for specialised activity development. This includes families with small children, adolescence and young adults, seniors (retirement age+) and board game development. Over 2022 the sub-team have worked hard to think of events and tabletop activities that best showcase the research we produce in the centre. An exciting prospect that has joined both focus groups families and adolescence & young adults, is the creation of "The Degrader Quest".

The Degrader Quest (See Fig.1) is being developed as a series of challenges that are involved in making a degrader (To us, a bivalent degrader or PROTAC). These challenges will take form as tabletop activities that would be done in a sequential manner that teach the participant about the intricacies and involvement of different scientific fields to make a degrader. To make this prospect a reality, CeTPD Outreach have been working on prototype tabletop activities to trial in different public engagement festivals and events throughout 2023.

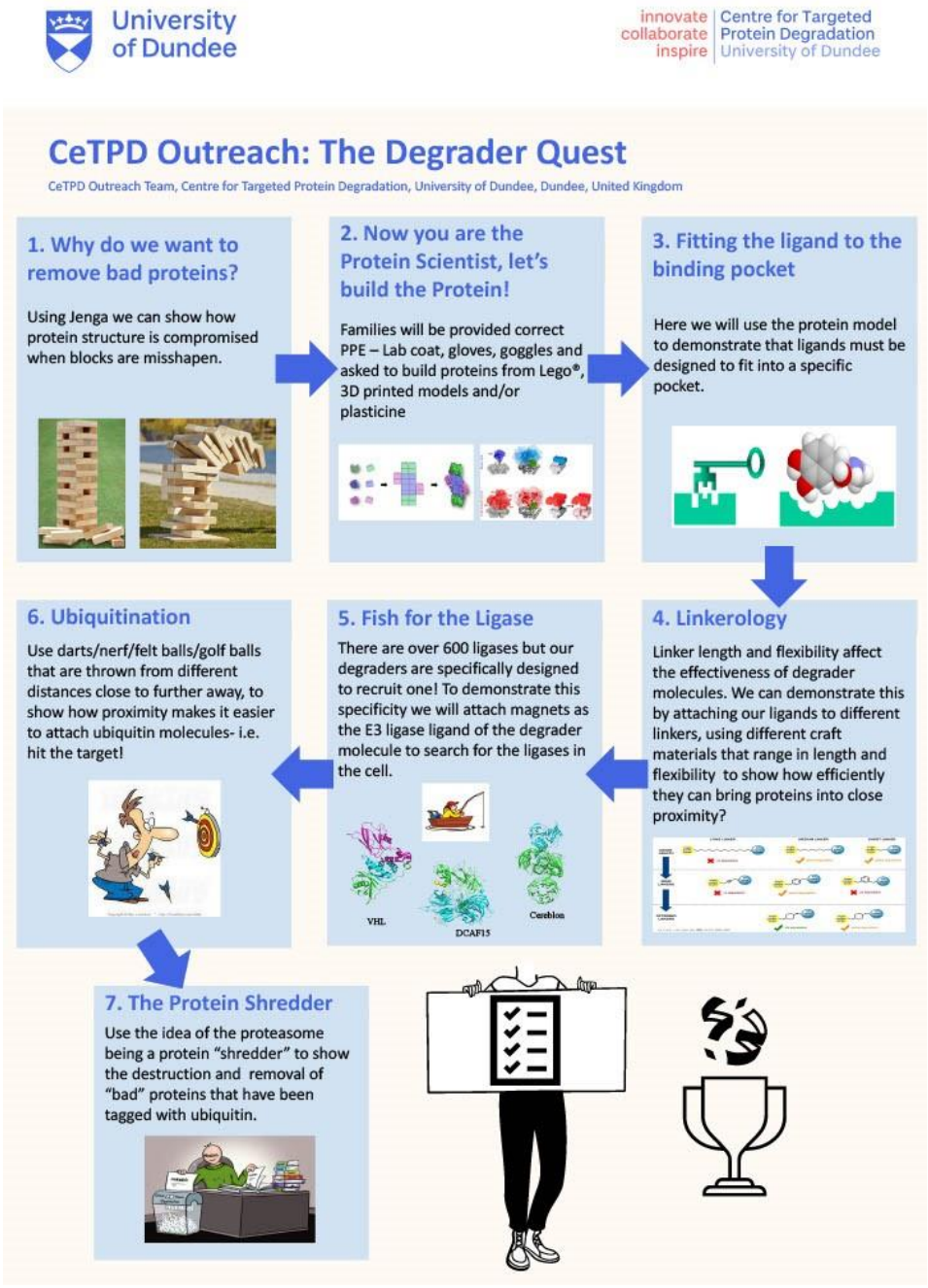


Figure 1: The Degrader Quest Poster. *Image of the CeTPD Outreach poster outlining The Degrader Quest challenge. This challenge is set as 7 different tabletop activities that participants need to complete to make their own degrader. The activities start by looking into the importance of removing bad proteins, then how you can design a ligand that binds selectively to your protein of interest, attaching the right linker to your ligand, recruiting a ligase, targeting via ubiquitination and the importance of close proximity, and finally the turnover of the protein.*

March 2023 was our big debut where we brought two tabletop activities to our first festival event: Family Fun with Women in STEM, hosted by ScrapAntics in the Wellgate Shopping Centre as part of the Dundee Women's Festival on the same week as International Women's Day (See Fig.2). This target audience were mainly families with small children. For this event we had the following volunteers on site: Dr Sohini Chakraborti, Claudia Diehl, Darren Darren, Dr Suzanne O'Conner, Dr Valentina Spiteri, Dr Ilaria Puoti and Dr Selma Gulyurtlu.

Figure 2: Photos of the CeTPD Outreach Team at the Family Fun with Women in STEM event as part of Dundee Women's Festival. *(A) Image of the stall used with the posters including a lay introduction of what a degrader is and celebrating the women in CeTPD. (B) The volunteers from left to right: Darren Darren, Dr Ilaria Puoti, Dr Selma Gulyurtlu, Dr Sohini Chakraborti, Dr Valentina Spiteri, Claudia Diehl and Dr Suzanne O'Conner. (C) and (D) showcase the centre's director, Prof. Alessio Ciulli trying the tabletop activities "Fish for the Bad Protein" and "Ubiquitination" respectively.*

Our first activity, "Fish for the bad protein", consisted of a tray of hand-made clay protein structures, where white one were healthy and blue ones needed removal into the proteosome. We wanted to teach our participants that our bodies have their own quality control mechanism that removes bad proteins, but our degraders exploit this pathway. As such, to make them differentially targeted, our blue ones had little hoops as well as being magnetised. With this, a small fishing rod was made, where the hook was compatible with the little hoops to remove the bad proteins from the tray. Then, by attaching magnet to the hook, we simulate what it's like with a degrader by making the process of removal much more efficient. This led to all participants managing to remove significantly more bad proteins with the magnet within 30 seconds.

Our second activity was teaching the participant about ubiquitination, how it can target a protein for destruction, and how this is eased by close proximity created by the presence of a degrader. Here, we set up a Velcro dart board, known as the bad protein in this context, and taped 2 distances away from it: a "Degrader Distance", which was further away, and a "No Degrader Distance", which was closer to the board. There were a series of soft balls, known here as the ubiquitin, which we asked the participant to throw at the bad protein from the two distances. We evaluated and discussed together not just the number of ubiquitin balls they were able to attach to the bad proteins, but also how it was much easier from a closer distance.

This event was very successful as our first with personalised activities, and the feedback was extremely positive, where all of the ratings collected were marked as "Excellent". One of our participants also stated that the activities were very well described and very visual. A special visitor we had that day was our own director, Prof. Alessio Ciulli, who had this to say about the event:

"I was thrilled to join the team showcasing for the first time our new degrader games and activities at a public event. The family fun event was a fantastic opportunity to engage with the local community and it was wonderful to watch children learning more about science, while playing and having fun!"

We also had one of our own Principal Investigators and former AC-BI Collaboration team leader Dr William Farnaby, who brought his own family to the event, where his son described the event as fun and elaborated with:

"I liked that I could do lots of different things and meet lots of different people"

Henceforth, we were able to bring our experience to another event also this March, which was a closed event welcoming local Girl Guides and Brownies, hosted by the Dundee Science Centre (See Fig.3). This event gave the opportunity to girls aged from 7 to 15 to learn about different careers in STEM. Here, we took the same activities (Fishing for bad proteins and Ubiquitination) and our volunteers were Dr Kirsten McAuley, Dr Sohini Chakraborti, Dr Alena Kroupova, Dr Giorgia Kidd and Dr Selma Gulyurtlu. Much of the success for the first event was translated into this event, where the participants were greatly engaged, asked lots of innovative questions and also rated our stall as "Excellent". In addition, one of the participants also stated that they "Liked the games and I got the concept". This left our sub-team energised and ready to take on more challenges.

Figure 3: Photo of the CeTPD Outreach Team at the Girl Guides and Brownies event in the Dundee Science Centre. *Image of our volunteers and the stall used with the two tabletop activities "Fish for the Bad Protein" and "Ubiquitination", as well as the posters that included a lay introduction of what a degrader is and celebrating the women in CeTPD. Our volunteers from left to right: Dr Sohini Chakraborti, Dr Giorgia Kidd, Dr Kirsten McAuley, Dr Alena Kroupova and Dr Selma Gulyurtlu*

Some of these challenges now branch into new adventures, including some of our members now getting ready to take on Café Science in the senior community in May. This event will also be paired with our board game development group as those members have created different card games to accompany the session. We look forward to showcasing ourselves in this new environment, seeing what we can learn from this new target audience, and sharing some science with them!

Promoting Entrepreneurship in Science: Welcoming Converge to CeTPD

Contributor: Dr Selma Gulyurtlu

The Centre for Targeted Protein Degradation (CeTPD) and its members share a commitment to drive innovation in the TPD field. Every day it does this by the excellent and novel research ideas and techniques it strives towards, but there are also other ways to drive innovation, one of which is through entrepreneurship. Whether it be to start a business, collaborate with industry or looking into any other commercialisation options of one's research outputs, it is important that the scientists that aim to innovate and inspire others with an entrepreneurial mindset understand their options of expanding the reach of their outputs. As such, in March, CeTPD welcomed two special guests for a visit: **Executive Director of Converge, Dr Claudia Cavalluzzo** and **Converge's Enterprise Executive for Northeast Scotland, Richard Cormack Corrigan**.

Converge is Scotland's most progressive and pioneering company creation programme. It helps students, graduates and staff across the Scottish Universities and research institutes to achieve their entrepreneurial goals. They do this by providing the right tools to turn creative and innovative ideas into commercial reality. Thus, we were delighted to welcome Claudia and Richard for a tour of our centre and listen to Claudia's valuable insight in her seminar "The Science of Entrepreneurship" (See Fig.1).

Originally a chemist with a PhD in Organic Chemistry, focussing on peptides and peptidomimetics, Claudia changed her career direction from research into driving business creation after becoming a Saltire Fellow. This flourished into her former roles as Head of the Saltire Fellowship at Entrepreneurial Scotland, and then Deputy Director at Converge Challenge before being appointed as Executive Director. She is passionate about supporting innovators, creators and problem solvers achieve their impact through business creation.

In her seminar, to the intrigue of many members of the Centre, Claudia shared her journey from the laboratory bench to the boardroom. It was discussed the different skills, techniques and knowledge that distinguishes scientists and entrepreneurs, but also how they are related and transferable. It was also outlined how Converge can be a platform for anyone wishing to make their creative ideas into a commercial reality.

Correspondingly, Richard has experienced a varied career journey involving travel, conferences & events, hospitality, tourism, corporate responsibility, business acceleration and enterprise in education. Since his appointment at Converge, he has been working to help support new businesses and spinouts within the Scottish University sector, responsible for those in the Northeast of Scotland (which includes the University of Dundee). We look forward to further developing these working relationships and seeing how Converge can help our members achieve their entrepreneurial goals in the future.

Figure 1. Photographs of the Converge Visit in CeTPD. *(A) Richard and Claudia at our CeTPD Outreach Display receiving an activity demonstration from postdoctoral scientists Dr Giorgia Kidd and Dr Sohini Chakraborti. (B) Claudia's seminar on "The Science of Entrepreneurship". (C) Start of the CeTPD tour with Operations Manager Dr Louise McGreavey and Postdoctoral Scientist Dr Selma Gulyurtlu. (D) Discussion with PhD candidate, Yuting Cao, in one of the chemistry lab tours. (E) Group photo of the visit*

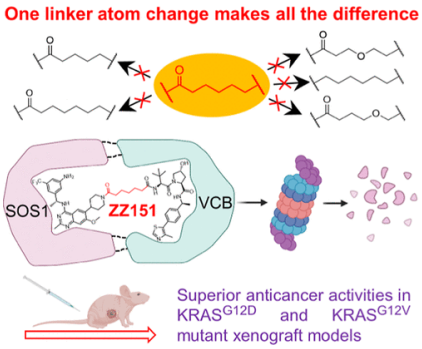
Targeted Protein Degradation

Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Guillem

Discovery of a Potent, Cooperative, and Selective SOS1 PROTAC ZZ151 with In Vivo Antitumor Efficacy in KRAS-Mutant Cancers

Zehui Zhou[§], Guizhen Zhou[§], Chuan Zhou[§], …, Mingyue Zheng*, Tianfeng Xu*, Sulin Zhang*
J. Med. Chem. **2023**, *66*, 4197



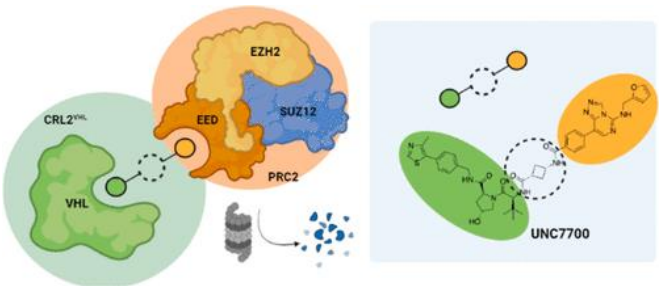
Zhang and co-workers report the discovery of ZZ151, a selective SOS1 PROTAC. They describe the design and characterization of the compound and illustrate its antitumoral efficacy against a panel of KRAS mutant cancer cell lines and xenografts in mice. Son of Sevenless Homologue 1 (SOS1) is a guanine nucleotide exchange factor which interacts with KRAS and catalytically promotes its activation; it has been proposed as an indirect way of tackling this elusive enzyme. The authors link a SOS1 inhibitor through a solvent-exposed piperidine to the VHL linker through a length-diverse array of linkers. The compound is able to degrade SOS1 in a panel of KRAS mutated cell lines with DC50 values in the low nanomolar range and shows moderate in vivo efficacy in KRAS mutated xenograft mouse models.
This paper shows the PROTAC's journey from the design starting at a POI inhibitor to in vivo experiments proving its efficacy. The authors raise interesting questions about the importance of the linker composition in the conformation adopted by the PROTAC and its role in producing a cooperative ternary complex that favours degradation potency; however, the second generation of linkers incorporating oxygen atoms fail to deliver better potency and cooperativity, giving the impression that rational design is still falling short compared to serendipity or brute force SAR. Indirect targeting of KRAS should prove a good marketing strategy, since mutations of this protein are critical in many lung, pancreatic and colorectal cancers, and so far only one inhibitor has been approved by the FDA for the treatment of lung cancer. Although PROTACs still must prove their value in the clinic, works like this show a promising proof of concept.

Cell Biology | Chemistry

Contributor: Guillem

PROTAC Linkerology Leads to an Optimized Bivalent Chemical Degrader of Polycomb Repressive Complex 2 (PRC2) Components

Frances M. Bashore[§], Caroline A. Foley[§], …, Lindsey I. James*
ACS Chem. Biol. **2023**, *18*, 494



Previous work by James et al reported the discovery of UNC6852, a PROTAC targeting EED, a member of the PRC2 complex. Also constituted by EZH2 and SUZ12, this complex is responsible for propagation of histone 3 lysine 27 trimethylation, a common hallmark in a variety of cancers. In this work, the authors introduce saturated cyclic linker modifications to UNC6852, obtaining compounds able to affect PRC2 degradation; degrading EED and EZH2 at the nanomolar range and SUZ12 to a lesser extent.

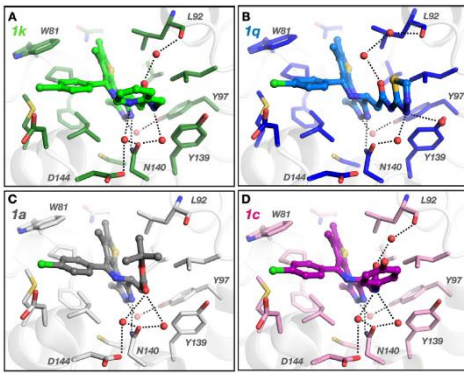
Although awkward, the term linkerology emphasises the fact that chemical modifications of a PROTAC aiming at improving potency or other PK properties generally must be carried out in the linker. The paper focuses on two stereoisomers of the same cyclobutane linker analogue. It attempts to elucidate why the *cis* compound shows higher potency while the *trans* displays selective degradation, although a more modest D_{max}, towards EED. CAPA analysis and MD conformational analysis point to a better permeability of the *cis* analogue, and computational analysis also shows a more stable tertiary complex, although FRET experiments are not conclusive. To round it up, they show antiproliferative effect of the PROTACs in DB cells.

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

Contributor: Guillem

From PROTAC to inhibitor: Structure-guided discovery of potent and orally bioavailable BET inhibitors
Paper Title

Mladen Koravovic[§], Anand Mayasundari[§], …, Vladimir Savic*
Eur. J. Med. Chem., **2023**, 5, 251



Previous work by the authors identified a JQ1-based BET PROTAC that recruits CRBN and is assembled via copper catalysed alkyne azide coupling (CuCAAC), thus bearing a triazole at the linker. Structural studies show novel interactions with the protein absent in the JQ1 binding mode, notably hydrogen bonding between the amide triazole and His433. In order to incorporate such interactions into a potential improved BET inhibitor, they synthesized JQ1 amide derivatives to probe the subpocket occupied by the 1,2,3-triazole. Incorporation of diverse heterocyclic and polar groups led to the discovery of SJ1461, which was further characterized and tested against a panel of cancer cell lines. The SAR study conducted is very focused, in fact the compounds provided could be fitted into the same chemical series as birabresib, a JQ1 amide derivative currently in clinical trials. The authors benchmark their lead compound with birabresib, which also exploits the same strategy of adding interactions to JQ1 via amide derivatization. SJ1461 has comparable PK parameters to the clinical candidate but overall higher IC50 values in the cell lines tested. Although SJ1461 stands as a promising BET inhibitor, the authors will need to put in some work before they can challenge Merk's candidate.

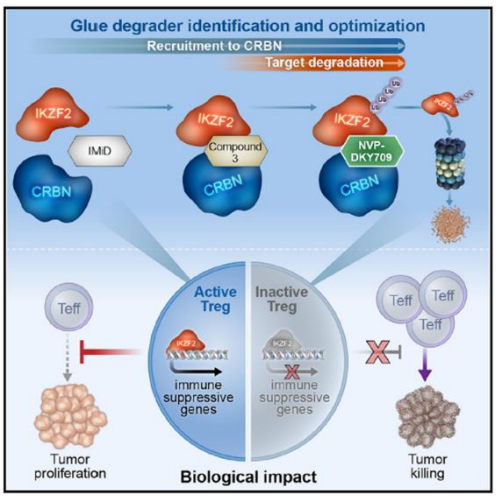
Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Sarath

Discovery and characterization of a selective IKZF2 glue degrader for cancer immunotherapy

Simone Bonazzi[§]*, Eva d'Hennezel[§]*, Rohan E.J. Beckwith[§], …, Jonathan M. Solomon*
Cell Chem. Biol. **2023**, *30*, 235–247

This manuscript describes the development of a selective molecular glue degrader NVP-DKY709 for IKZF2 (Helios), which spares other closely related members of the Ikaros transcription factor family, IKZF1 and IKZF3. NVP-DKY709 reduces Treg suppression *in vitro* and has entered the clinic in a phase 1 trial for solid tumour indications (ClinicalTrials.gov NCT03891953). Furthermore, a recent publication from Verano et al (DOI: 10.1021/acschembio.2c00439) utilised DKY709 as a PROTAC handle capable of co-degrading Helios and CDK4/6 to result in synergistic effects. Here, Bonazzi et al. demonstrate a novel approach in design of IKZF2 selective degraders, by screening for glues that enhance IKZF2 recruitment to CRBN. DDB1:CRBN:DKY709:IKZF2(ZF2) X-ray structure was used to rationalise the IKZF family selectivity to the residue Histidine 141, present only in IKZF2 and IKZF4, forming CH-π interaction with bulky piperidine in DKY709. Intriguingly, CRBN adopts an 'open' conformation when in complex with IKZF2(ZF2) but adopts a 'closed' conformation with IKZF2(ZF2-3). This inconsistence hypothesis from Watson et al. (DOI: 10.1126/science.add7574), that suggests that ligand binding to CRBN thalidomide binding pocket, orders its sensor loop to create the closed conformation.



Cell Biology | Chemistry

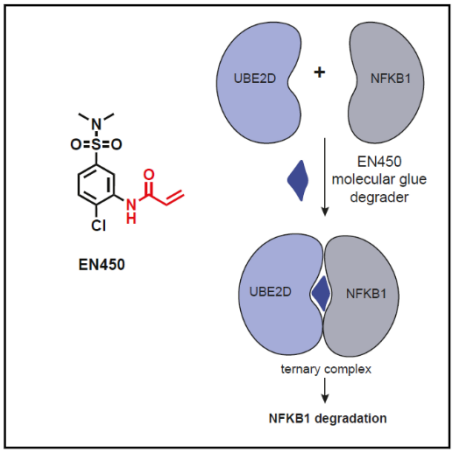
Contributor: Sarath

Chemoproteomics-enabled discovery of a covalent molecular glue degrader targeting NF-kB

Elizabeth A. King[§], …, Daniel K. Nomura*

Cell Chem. Biol. **2023**, *30*, 1-9

In this study, King et al. coupled phenotypic and chemoproteomic approaches to discover a cysteine reactive covalent molecule EN450, which mediates degradation of NFKB1 by gluing it directly to the E2 ubiquitin-conjugating enzyme UBE2D. The manuscript provides a template for a platform that enables rapid discovery of hits that confer antiproliferative effect by engaging ubiquitin-proteasome system (UPS). The glue EN450, like the CR8 glue reported in Slabicki et al. (DOI:10.1038/s41586-020-2374-x), engages UPS components other than the substrate receptors for its activity. The authors show that the degradation of NFKB1 is proteasome dependent. However, the neddylation-dependency suggests that the glue requires the CRL machinery for its activity. The mode of action of these glues requires greater exploration as it is also not clear if the glue can bypass the need for an E3 ligase/ substrate receptor while it engages and ubiquitinates NFKB1. It would be interesting to see if future studies on this glue include structural characterisation of the ternary complex to help design non-covalent glues that retain the potency but enhances selectivity. Like many glues in the past, Forte et al. (DOI: 10.1101/2022.12.19.520812) have already extended the application of this UBE2D binder as a PROTAC handle.



Cell Biology | Chemistry | Structural Biology/Biophysics

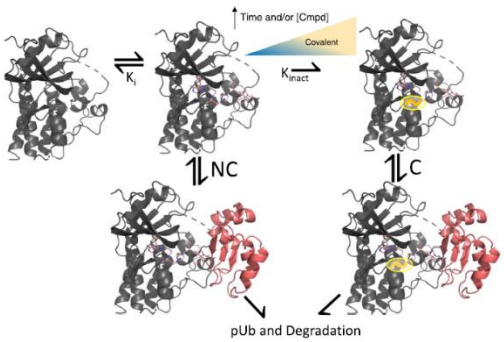
Contributor: Sarath

A covalent BTK ternary complex compatible with targeted protein degradation

James Schiemer[§], …, Matthew F. Calabrese*

Nat. Commun. **2023**, *14(1)*, 1189

Schiemer et al., in this study demonstrate that PROTACs that employ warheads to covalently engaging target proteins are still compatible with the protein degrader mechanism of action. The authors make the point with a PROTAC that utilises an Ibrutinib-like BTK warhead and co-opts cIAP to mediate proteasomal degradation of BTK. The crystal structure of the complex coupled with NMR show a stable yet dynamic ternary complex.



Unlike earlier efforts that used chimeric degraders to covalently engage KRAS and ERK1/2 to mediate their degradation, the authors here use some clever cellular tools to demonstrate that degradation is not just because of the fraction of PROTAC that engages the target reversibly. The use of inducible cellular system to pre-form a stable population of covalently modified target prior to the expression of E3 ligase is an interesting approach that can be adopted to understand the contribution of covalency in the mechanism of action of PROTACs that recruit E3 ligases covalently.

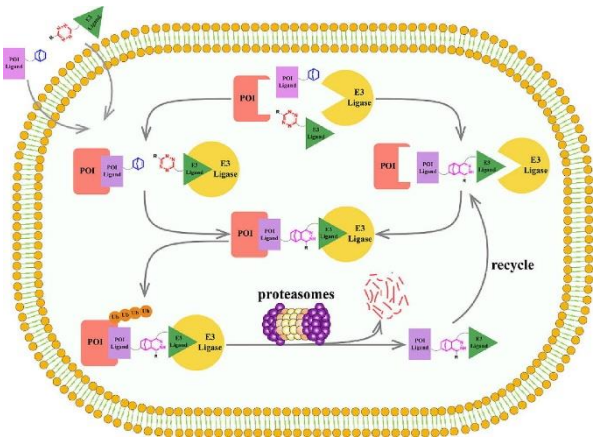
Covalent modification on the target protein is usually not a lucrative option as the PROTACs lose the catalytic activity, requiring higher doses to achieve therapeutic levels of degradation. However, this approach could be a starting point to engage target proteins that are non-ligandable and have no known reversible binder. The ternary structures can then be a springboard for optimisation of reversible and potent degraders.

Cell Biology **Chemistry**

Contributor: Guillem

Design, synthesis and bioactivity evaluation of self-assembled PROTACsbased on multi-target kinase inhibitors

Ru Si[§], Huanjie Zhu[§], ..., Jie Zhang*
Bioorganic Chemistry, **2023**, *134*, 106439.



The authors aim to address the problem of the typically low cell permeability exhibited by PROTACs by engineering a self-assembly system using the inverse electron demand Diels-Alder (IEDDA), a type of cycloaddition between a conjugated diene and a dienophile that has been widely used as a ligation tool in chemical biology due to its bio-orthogonality and fast reaction rate. The authors draw on previous work reporting the discovery of PROTACs targeting the VEGF and PDFE growth factors, the low activity of which they attribute to its large molecular weight (>1000 Da), leading to poor cell permeability. To overcome this problem they synthesize two fragments, one bearing the warhead and half of the linker terminated in a norbornene unit (a strained alkyne which is very reactive towards IEDDA), and the other constituted of lenalidomide or VH032 linked to a tetrazine unit. After demonstrating that the assembly of the fragments proceeds in a PBS/acetonitrile mixture, they perform the assembly in cells by sequential treatment of the two PROTAC fragments. After 24 hours of treatment, they analysed cell lysates by HPLC-MS, showing assembly of the PROTAC and moderate degradation of the target proteins. Although this approach is innovative, it is not the first time that in cell assembly of a PROTAC is investigated [1]: in-cell click-formed PROTACs have been dubbed CLIPTACs. The synthesis of CLIPTACs requires the introduction of very specific chemical moieties, unusual in conventional linker design. Moreover, the biological activity of the compounds

is not better than that of a well optimized lead compound, and the requirement of sequential administration of the fragments makes an in-vivo administration more challenging.

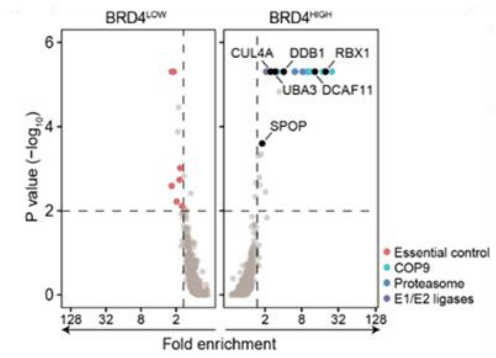
1: ACS Cent. Sci. **2016**, 2, *12*, 927–934

Cell Biology | Chemistry

Contributor: Sarath

Discovery of a Drug-like, Natural Product-Inspired DCAF11 Ligand Chemotype

Gang Xue[§], Jianing Xie[§], …, Georg Winter*, Herbert Waldmann*
bioRxiv **2023**, DOI: 10.26434/chemrxiv-2023-zmh4f



Xue et al. in this study, design chimeric degraders that would recruit autophagy-related LC3B-protein to mediate autophagy-dependent degradation of target proteins like PDEδ lipoprotein chaperone, the BRD2/3/4-bromodomain containing proteins and the BTK- and BLK kinases. However, while investigating the mechanism of action, the authors realized that the degraders function via UPS and employ a CRL. With the help of the target protein stability CRISPR screening platform, they demonstrate that the degraders utilize CRL^{DCAF11} complex for their activity. Conjugating the E3 binder with a fluorophore dye, enable them to show that arylidene-indolinones covalently bind DCAF11. The tempered electrophilic reactivity to DCAF11 in contrast to the chloroacetamide group reported in Zhang et al. (DOI: 10.1021/jacs.1c00990), has the potential to reduce off-target effects.

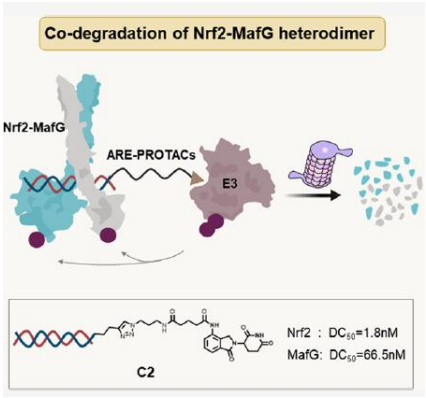
This paper is a perfect example of the importance of systematic investigation of mechanism of action for PROTACs. The CRISPR screening method has become an elegant setup to identify novel E3 ligases that can be recruited for TPD (DOI: 10.1101/2023.02.14.528511) .

Cell Biology | Chemistry

Contributor: Sarath

ARE-PROTACs Enable Co-degradation of an Nrf2–MafG Heterodimer

Jianai Ji[§], …, Qidong You*, Zhengyu Jiang*
J. Med. Chem. **2023**, DOI: *10.1021/acs.jmedchem.2c01909*



This study reports a first in class degrader targeting Nrf2 to proteasomal degradation and downregulation of Nrf2-ARE transcriptional activity. The antioxidant response elements (ARE) -based proteolysis targeting chimeras (ARE-PROTAC), C2 utilises a DNA element binding to the transcription factor Nrf2, and CRBN handle lenalidomide to co-degrade the heterodimer transcription complex Nrf2-MafG. The authors demonstrate that the C2 increased oxidative stress by decreasing total GSH levels (and the ratios of GSH/GSSG) and increasing ROS levels. Furthermore, the ARE-PROTACs enhanced the sensitivity of NSCLC cells to therapeutic drugs, including doxorubicin, ErbB family inhibitor Afatinib, ALK inhibitor Crizotinib, and MEK1 inhibitor Trametinib.

Interestingly, C2 can bind all the three heterodimers of Nrf2 and MafF/MafK/MafG, but mediates selective degradation of only Nrf2-MafG complex. This highlights the added benefit of E3 layered selectivity offered by the PROTAC modality.

Chemistry

Contributor: Andre

Design, synthesis and biological evaluation of the tumour hypoxia-activated PROTACs bearing caged CRBN E3 ligase ligands

Weiyan Cheng§*, Shasha Li§, …, Xiaojian Zhang*

Bioorg Med Chem. **2023**, *82*, 117237

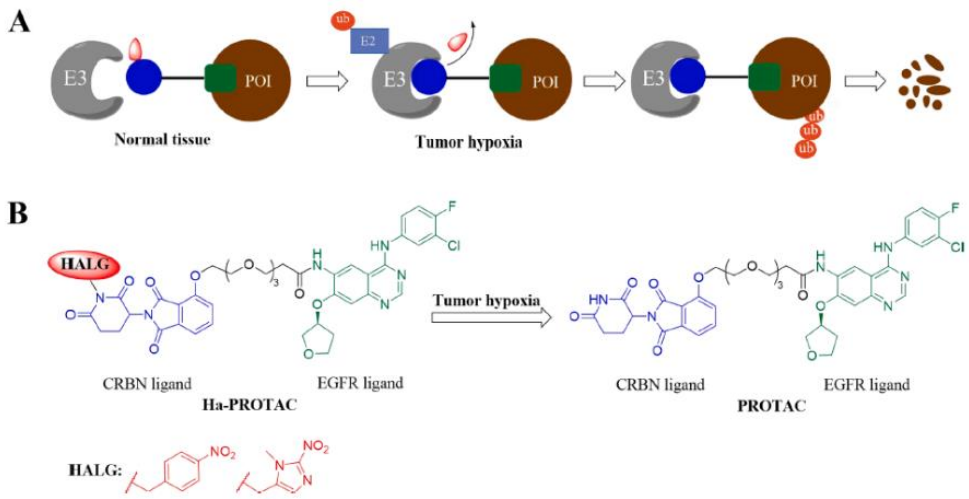


Fig. 1. (A) Schematic diagram of the mechanism of action of ha-PROTACs; (B) Structures of designed ha-PROTACs.

The epidermal growth factor receptor exon 19 mutation ($EGFR^{Del19}$) is an in-frame deletion located at the exon 19 of the EGFR gene that is observed in 15-20% of lung cancer patients. Here, the authors exploit the hypoxia environment of solid tumours to activate their hypoxia-activated $EGFR^{Del19}$ targeting PROTACs (HA-PROTACs). This strategy is argued to be able to alleviate the off-target effects of PROTACs. Previous work from the group has explored similar idea by attaching the hypoxia-activated leaving groups (HALGs, (1-methyl-2-nitro-1H-imidazol-5-yl) methyl or 4-nitrobenzyl group) at the POI ligands. Current work describes the introduction of HALGs to the CRBN E3 handle moiety.

The synthesized HA-PROTAC **9** & **10** showed a more potent degradation profile in the hypoxia condition compared to normoxia. Cell viability assays also showed two-fold hypoxia selective activity. Cell migration inhibition and apoptosis assay further confirm the hypoxia mode of action of these HA-PROTACs. Significant inhibition of cell migration and doubling of the number of apoptotic cells were observed in hypoxia condition.

Although the authors did not perform the usual PROTACs mode of action control experiments, I think this could be an interesting approach to activate PROTAC in a specific tumour environment and hopefully reducing the toxicity.

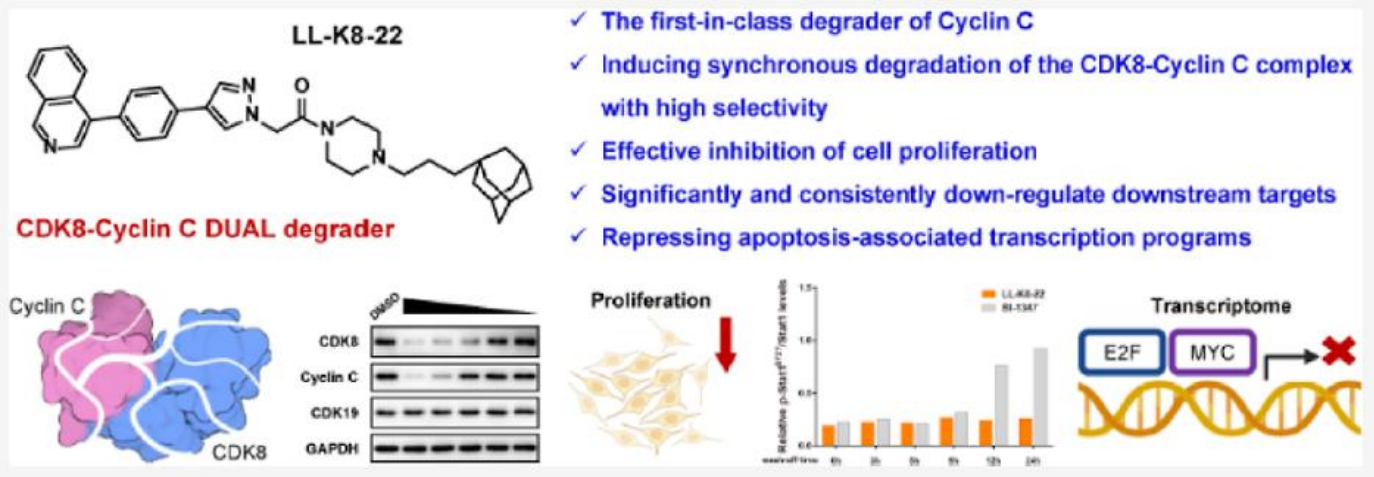
Chemistry

Contributor: Andre

Discovery of LL-K8-22: A Selective, Durable, and Small-Molecule Degrader of the CDK8-Cyclin C Complex

Mingyu Wang§, Rongkun Lin§, Jiacheng Li§, Yuying Suo§, …, Cheng Luo*, Hua Lin*

J. Med. Chem. **2023**, *66*, 4932



This study reports the first in class dual degrader of CDK8-Cyclin C, an important anti-tumour target which was previously deemed undruggable. Utilizing an inhibitor that binds to CDK8-Cyclin C complex, **BI-1347**, Wang et al. designed hydrophobic tag-based (HyT-based) degraders by attaching a linker and a large hydrophobic moiety (adamantine or menthoxy group). The attached hydrophobic moiety mimics the denatured state of the bound-target protein and triggers the proteasomal mediated degradation. Iterative linker optimization resulted in **LL-K8-22**. **LL-K8-22** has a remarkable selectivity profile, barely degrading closely related CDK and Cyclin family members (CDK2, 4, 5, 6, 7, 9, 12 & 19, and Cyclin A2, B1, E1, H, K, T1), while potently degrades CDK8 & Cyclin C (D_{max} > 90%, DC_{50} value of 2.5 µM). Degradation mechanism is confirmed to be UPS mediated. **LL-K8-22** also has a more potent and prolonged antiproliferation activity compared to the parent **BI-1347**.

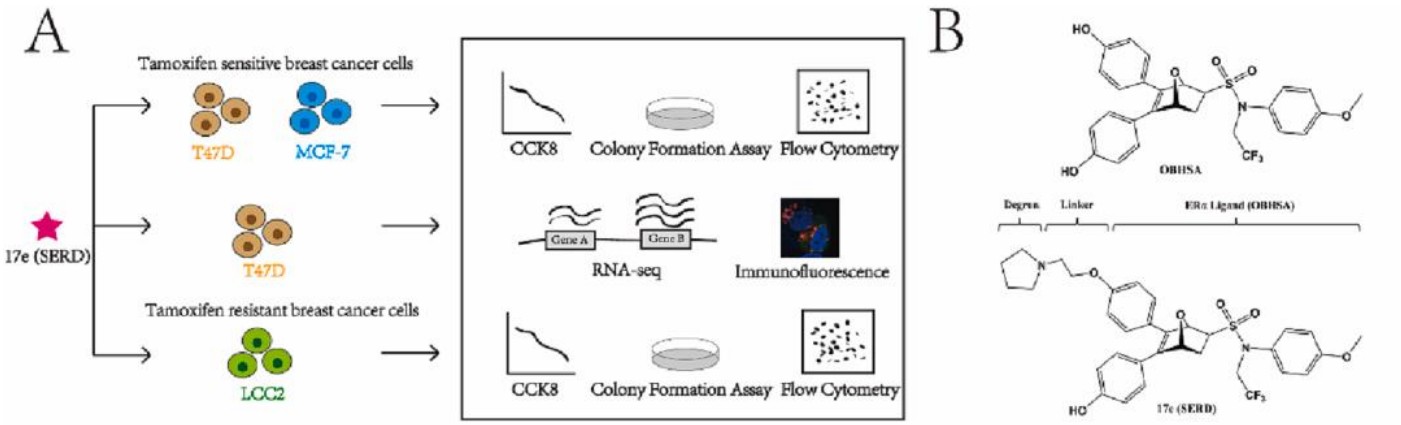
This is a very interesting approach in targeting an undruggable target; by degrading the whole complex of CDK8-Cyclin C. The small size of hydrophobic tags in comparison to most E3 ligase handles could be a significant plus for HyT-based degraders. Although the authors barely mention this, I would like to see the basis of the remarkable selectivity demonstrated by **LL-K8-22**.

Cell Biology | Chemistry

Contributor: Andre

A novel selective estrogen receptor degrader induces cell cycle arrest in breast cancer via ERα degradation and the autophagy-lysosome pathway

Jiawei Zhou[§], Rong Shen[§], …, Hai-Bing Zhou*, Jian Huang*
Bioorg Med Chem. **2023**, *82*, 117235



Estrogen receptor α (ERα) is one of the most important therapeutic targets for estrogen-dependent breast cancer. This study reports a detailed mode of action of a novel PROTAC-like selective estrogen receptor degrader (SERD), compound **17e**. Compound **17e** was designed by attaching a linker and degron into a full ER antagonist, 7-oxabicyclo [2.2.1] heptene sulfonamide (OBHSA). Encouragingly compound **17e** inhibits breast cancer growth in both *in vitro* and *in vivo* settings, as well as in a tamoxifen resistant breast cancer cell line, LCC2. Mechanistic study revealed that compound **17e** induces ERα degradation via the proteasome pathway and promotes the autophagy-lysosome pathway. This is not the case for other ER antagonists.

It is striking to see that just by attaching a small degron moiety, one could significantly trigger different pharmacological affects; from an antagonist into a target protein degrader and simultaneously activates the autophagy-lysosome pathway.

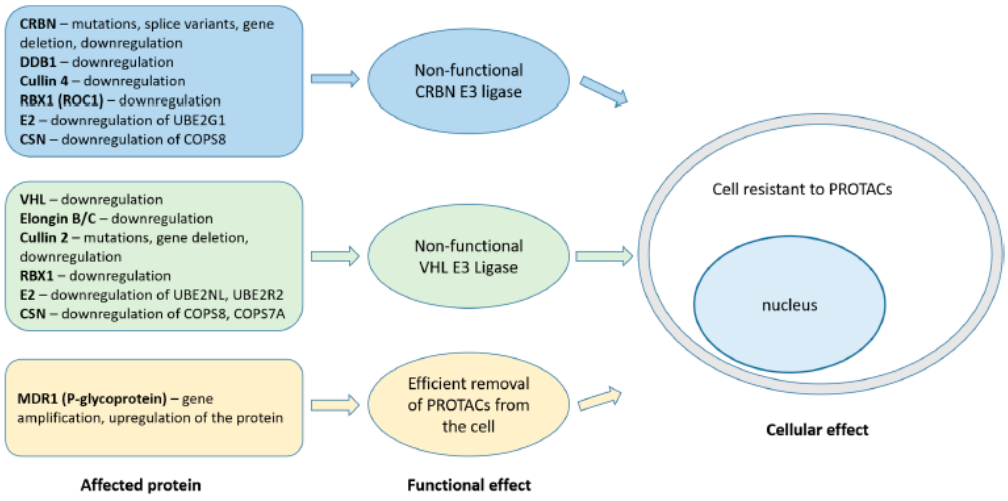
Cell Biology **Chemistry**

Contributor: Andre

Cereblon-Recruiting PROTACs: Will New Drugs Have to Face Old Challenges?

Marcin Cie´slak§ and Marta Słowianek*

Pharmaceutics **2023**, *15*, 812



The review focuses on CRBN based PROTACs that degrades various therapeutic oncology targets including kinases, transcription factors, enzymes, receptors, etc. The first section summarises the overview of the development and biological activities of numerous PROTACs. Then factors that could affect the clinical efficacy of PROTACs and potential resistance mechanism are discussed.

Expression of CRBN E3 ligase complex is proposed to be an important factor that could be leveraged in certain disease conditions; haematological cancer may respond better to IMiDs & CRBN based PROTACs due to the higher expression of CRBN mRNA in cell lines originating from blood compared to solid tissues. On the other hand, patients with head and neck, and pancreatic cancer are shown to have reduced expression of DDB1 and CUL4. Potentially, they could respond poorly to IMiDs. Genetic alteration of CRBN could also reduce the efficacy of CRBN based PROTACs. Nonsense and missense mutations in CRBN which are observed in patients that are refractory to IMiDs can impair the ability of PROTACs to bind CRBN.

This is a good read for those who wish to get an overview of the recent development in the CRBN PROTACs field and understand the potential challenges ahead.

Other Paper Highlights

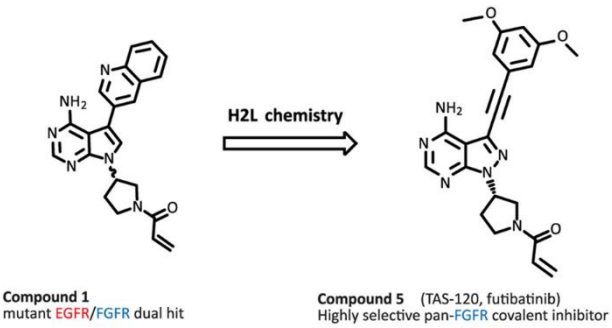
Chemistry

Contributor: Petr

Discovery of Futibatinib: The First Covalent FGFR Kinase Inhibitor in Clinical Use

Satoru Ito§*

ACS Med. Chem. Lett. **2023**, DOI:10.1021/acsmedchemlett.3c00006



Futibatinib* is the covalent fibroblast growth factor receptor (FGFR) inhibitor which got FDA accelerated approval for patients with previously treated, unresectable intrahepatic cholangiocarcinoma in 2022. In this paper, a group of authors from Taiho Pharmaceutical described an identification of a unique hit compound with inhibitory activities of both FGFR2 and EGFR del19/T790M mutations followed by SAR study. Based on 2D structure overlapping with well-known FGFR, BtK and EGFR inhibitors compound optimization led to the replacement of pyrrolopyrimidine core with pyrazolopyrimidine and changing quinoline fragment to 3,5-dimethoxybenzene ring for better binding with hydrophobic pocket close to the gatekeeper residue in the ATP-binding site. These modifications led to high selectivity against EGFR.

Interestingly, azetidine analogues showed poor performance in *in vivo* mouse exposure study despite good microsomal stability. Authors explained it by intestinal absorption and decreased plasma stability. Reducing ring size to azetidine at the nitrogen of acrylamide can increase intrinsic reactivity to the thiols in the body. The same observation has been reported, but still an important result for the development of acrylamide-based covalent inhibitors.

*(Previously reported in 2020 by Hiroshi Sootome et al., *Cancer Res* 2020;80:4986–97)

Cell Biology

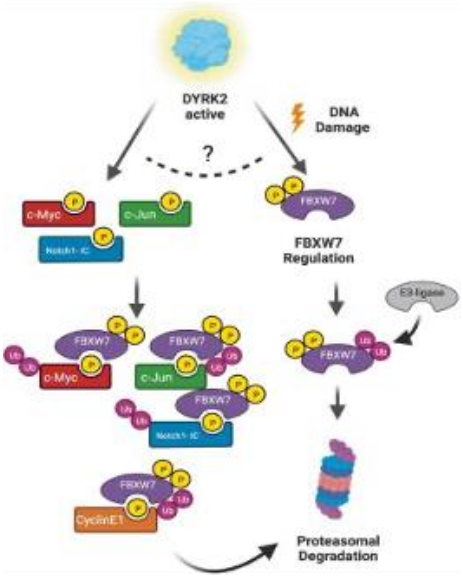
Contributor: Alejandro

FBXW7 tumor suppressor regulation by dualspecificity tyrosine-regulated kinase 2

Rafael Jiménez-Izquierdo§, Rosario Morrugares§, …, Marco A. Calzado*

Cell Death Dis. **2023** DOI: 10.1038/s41419-023-05724-0.

FBXW7 is key a substrate recognition component of the SKP1-Cullin (CUL1)-F-box (SCF) complex. This protein has critical roles in regulating the stability of several oncogenic proteins, such as Cyclin E1, c-Myc, and MCL1. Notably, alterations in FBXW7 expression and functionality have been shown to increase tumorigenesis, making it the most altered ubiquitin-proteasome-related protein. To fully comprehend the role of FBXW7 in cancer and to devise pharmacological interventions, a proper understanding of the post-translational mechanisms in charge of its



functionality is essential. In this publication the authors describe the protein kinase DYRK2 as a novel FBXW7 negative modulator in response to DNA damage with relevant roles on cell survival and sensitivity to Paclitaxel and BET inhibitors. Finally, this new mechanism might have important implications for tumour development control, and points at DYRK2 as a potential target to explain the increased tumorigenesis and chemotherapy resistance caused by alterations in FBXW7.

This work emphasizes the importance of gaining comprehensive understanding of the mechanisms involved in controlling ligase functionality and stability in different cellular contexts. Such understanding will pave the way for a broader range of ligases to be implemented in TPD, as it will enable researchers to determine the best scenarios and subcellular contexts where each ligase can be more successful hijacked.

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