CeTPD Journal Club

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

April 2022

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): Charlotte Crowe, Ollie Hsia and Zoe Rutter

"Not only does the JC help me keep up-to-date with latest TPD papers, I really enjoy reading everyone's opinions on the TPD entries. As a very multidisciplinary lab, the "other paper highlights" section captures everyone's individual research interests. The special feature sections are fun to read and write, and overall I think it's great to have a platform where everyone can express themselves"

Charlotte obtained her MChem in Chemistry at the University of St Andrews, where she undertook her Master project in an interdisciplinary lab developing mild-aqueous cross-coupling chemistries to enable late-stage C–H functionalisation in combination with halogenases. Interested in spending more time at the interface between chemistry and biology, she moved across the Tay to the University of Dundee and started her PhD in the Ciulli lab in October 2020.

"The JC provides a unique platform for experienced researchers in the TPD space to give their opinion and analysis of papers in an easy-to-read format. I think this is of value to a wide audience as there is often nuance and context in research articles that can be hard to pick up on for those less familiar with the field."

Oliver joined the Ciulli group as a postdoctoral scientist (Cell Biology) as part of the Eisai project in May 2020. Ollie completed his undergraduate degree in biochemistry as well as his PhD in molecular biology at The University of Glasgow, and has extensive experience in the NEDD8 and ubiquitin field.

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

Zoe completed her undergraduate degree in biochemistry at Newcastle University followed by a PhD in Structural Biology in the lab of Prof. Rick Lewis. In April 2021 she joined the ACBI team as a structural biologist/biophysicist.

Journal Club 2nd Anniversary

Contributor: Alessio Ciulli

Foreword

It is a special pleasure for me to watch our TPD Journal Club marking its second anniversary. This special issue celebrates this important achievement with a range of exciting articles and coverage of the most recent TPD literature, as the field continues to grow and grow. It illustrates the breadth of activities within Dundee and beyond, and some of the breath-taking advances seen across the globe.

I want to extend a huge thank you to everyone within the Group who have edited and written for the Journal Club to date, to our guests who have featured, and indeed, most importantly, to all of our world-wide readers and followers. Many of you have offered invaluable feedback, suggestions and constructive comments for improvement over these past two years, via social media, emails, DMs or verbal feedback – something we are most grateful for and continue to welcome. I salute all those involved and who have been part of this journey. We hope our readership feels a part of this exciting journey as much as we do.

This anniversary offers an opportunity to reflect on the seeds of the idea: how the JC was born, its vision and motivation, and how we and the field have so rapidly evolved over the past two years. It still seems like just yesterday when Siying, working back then as part of the AC-BI team, suggested to Will and I that we could try something new to keep track of what's going on in literature. Siying envisaged something in the form of an illustrated, written PDF document, that might best complement the conventional "literature review" in-person meetings we had in place at the time. Siying had been involved and so enjoyed such a written "Journal Club" in her previous research group, while a PhD student, and had found that experience not only enjoyable but also very useful to keep on top of literature. It was March 17, 2020, we had just shut-down our Labs and were preparing for the first lockdown. Following exchanges and discussions about how to best put the idea into practice, we decided to open it up to the public by posting it on the group webpage and on social media, and got on to organize the work for the first Issue. Just over a month later, in early May 2020 we debuted the first issue of our Journal Club, edited by Siying and Will as due credit for seeding and implementing this idea into practise. Read next the special feature "An interview with Siying Zhong" by Charlotte to gain further insights on this from Siying herself!

Since then, we have watched monthly Issues emerging, with now 10 issues per year, with due pauses for Christmas and mid-summer breaks. It has been so satisfying to have witnessed such a concerted group-wide and community-led project evolving. Editing responsibilities and coverage of TPD-field focused pieces are taken in turn by everyone in the group, while everyone is encouraged to volunteer non-TPD articles or other special articles. I have very much loved taking up a share of writing and editing, and contributing some special articles, including "Landmark papers", as the opportunity arises. As Siying left the group at the end of 2020, our first-year PhD student Charlotte took the baton and has been at the helm ever since. It has been wonderful to watch her leading the development of the Journal Club, and work so closely with everyone in the group and beyond, including guests and interviewees. Her leadership has helped to drive development of what I hope will be recognized today as a more elaborate and fit-for-purpose Journal Club, in terms of format, layout and content. I want to congratulate Charlotte for her hard work and efforts. Combining the JC work and activities with her main PhD Lab project is very complex and time consuming, and she deserves enormous credit for doing this so graciously and effortlessly!

A lot has happened in the past two years. The group has since witnessed 37 new people joining, while at the same time 10 have left, an overall net increase of +27. This means we have more than doubled to our current size of ~45! No doubt, recruitment has taken a huge amount of attention and efforts, and we seem to have done well at that, despite the pandemic and covid restrictions. The Journal Club hence witnessed the announcement of our new Centre for Targeted Protein Degradation (CeTPD), that I have the privilege to have founded and now to direct. The CeTPD, expected to open in the late Summer/early Fall, will be the exciting new home for our TPD research at Dundee. The Centre has been expanding in scale, including the appointment of senior roles, and new Fellows, exemplified by the

award of a Marie-Sklodloskwa Individual Fellowship to Xingui that was just recently announced . Indeed, the CeTPD is also expanding its own leadership, and we look forward to recruit, promote and nurture the next generation of leaders and innovators in the field, and support them to base their research efforts here with us. This is illustrated by the recent appointment of Will as CeTPD's very first Principal Investigator. Since 2016, Will has outstandingly led the AC-BI team, and will now build and base his own academic group in the new Centre. Will's own reflections on this fantastic achievement and exciting development appear in this month's issue too. In part to reflect our own rapidly evolving CeTPD, expanding beyond my own research group, our Journal Club is re-branding from now on as **"CeTPD Journal Club".**

The past two years have also witnessed a significant development and growth of the wider TPD field. The number of published papers (30 from our group), patents, new companies and deals inked in this space have just exploded. Of note for us are the PROTAC drug discovery deal with Almirall, the expansion of the collaboration with Boehringer Ingelheim, and, just a few days ago, the deals announced by Amphista Therapeutics, the TPD spin-out that had its foundations from science emerging from our own laboratory, with big pharma giants Merck and BMS. Beacon and Hanson Wade are amongst some of the many organizations doing a great job at helping researchers in their attempt to capture and navigate this rapidly evolving landscape. A piece from Vale in this issue captures the outcome of a very fruitful visit of CeTPD by Charlotte Price and Sofia Rodriguez, whom we were delighted to recently host.

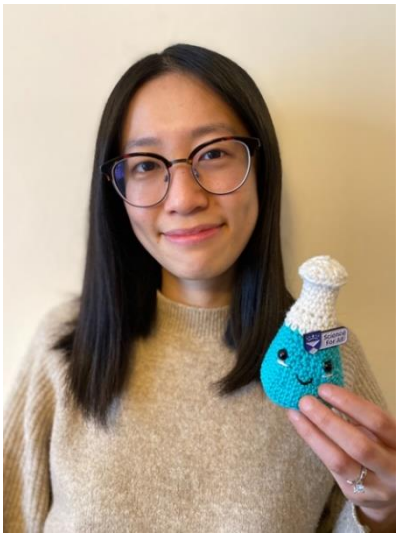
The future is bright for our new Centre in Dundee. We are excited to grow the TPD community and bring the world to Dundee. We hope to drive forward more and better innovation and collaboration, by inspiring each other and encouraging sharing of our findings and tools to best advance and improve people's quality and purpose of life as a result. I congratulate our Journal Club for its anniversary, all in the CeTPD group for a remarkable couple of years, and look forward to the exciting progress ahead of us.

Contributor: Charlotte Crowe

An Interview with Siying Zhong

Over the past 12 months the Ciulli Lab has been rapidly expanding, welcoming more than 15 new scientists – and now the CeTPD is also welcoming our first new PI recruit, Will Farnaby and his group. The *Ciulli Lab Journal Club* is now re-branding as the *CeTPD Journal Club*, **a project which brings the CeTPD together as a community**.
In this 2nd Year Anniversary issue, I wanted to take the opportunity to interview Siying Zhong, the scientist who founded the Journal Club. Not only was I keen to catch up with her, I also wanted her to share the story about the context in which she set up the JC, to communicate this with our current readers and also our lab members who are newer to the CeTPD!

About Siying



Siying is from Guangzhou, China, and moved to the UK in 2009 for her integrated Master of Chemistry degree at the University of Sheffield. She moved to Bristol's Synthetic Chemistry Centre of Doctoral Training in 2014 and became the first joint PhD student between the labs of Varinder Aggarwal and Craig Butts. Her PhD work focused on studying molecular conformations of flexible small molecules with chiral centres. She was able to predict 3D shapes of small molecules through computational modelling, synthesise these in the lab, and use NMR spectroscopy to study their experimental 3D molecular conformations. Discussions about potential applications of her PhD work prompted Siying to learn more about PROTACs and begin to explore the idea of utilising conformationally constrained linkers for PROTACs to bias formation of productive ternary complexes.
Reading into the TPD field, Siying says that Alessio's name kept coming up. She came across the Ciulli group website and noticed that a postdoctoral position was open – and decided to apply! After her methodology-focused PhD, she was keen to broaden into medicinal chemistry. And as a friend has told her, "Med chem is like total synthesis, but with more fun"!
In October 2018, Siying joined the AC-BI team in Dundee as a synthetic chemist. In her final months here, she gravitated back toward computational chemistry. Siying described her time in the AC-BI team as exciting, multidisciplinary, and highly collaborative. In an "industrial postdoctoral" position, she said it felt very freeing to combine the curiosity-driven academic culture within a fast, result-driven industrial team.

Founding the Ciulli Lab Journal Club

Siying and I chatted about the context in which the TPD Journal Club idea hatched. In the midst of April 2020 lockdown, all lab members were working from home. As a predominantly lab-based chemist, Siying wanted to have a project to work on during the lockdown. With the world "standing still", she had the opportunity to reflect on the activities of her PhD: in the Aggarwal group, Dr Adam Noble ran a weekly JC where each lab member would submit an interesting paper, and Adam collated these into a document to share around the lab. With this idea of a TPD Journal Club in mind, Siying met (virtually) with Alessio and Will to brainstorm how to move this from idea to reality. The key outcomes of the discussion were:

- The primary purpose: to train our scientists to review and communicate literature as it emerges;
- The issues would appear monthly, to allow enough time to read, understand & distil the information;
- Each issue would comprise of at least 2 sections: TPD literature and other literature highlights;
- Each issue would be co-edited by members of the AC academic group, the AC-BI collaboration group (and now also the AC-Almirall collaboration group) to give everyone opportunity to work closely together;
- The final version would be shared online and made open access to serve the community!

Not only has this resource been very successful (Siying was even awarded the University of Dundee Science for All badge for her contributions to public engagement in research), this was also a valuable opportunity for Siying to develop mediation, leadership, communication and resource management skills, all of which she has found extremely valuable moving into her industry-based role.

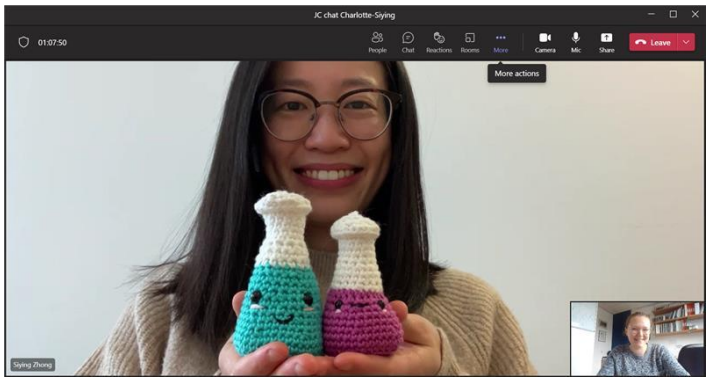
Leaving Dundee & next steps

Since January 2021, Siying has been at Vernalis, a Cambridge biotech company focusing on fragment & structure-based drug discovery. She initially joined as a lab-based synthetic chemist but she was still drawn to molecular shapes and design. Thanks to the company's flexibility, she took the opportunity to integrate into the computational chemistry team. She now works alongside a team of medicinal chemists, offering advice on compound design guided by computation modelling. Simultaneously she aids the computational chemists with her expertise in synthesis. After only a few months of working in her new role, she had her first success with one of her *in silico* designed compounds showing promising results *in vitro*.

When asked if she still keeps up to date with the Journal Club (I had to ask! 😉) Siying answered that yes, she does – not only is it still going on (keeping up the momentum thanks to CeTPDers' motivation & contributions), she has also been pleased to see the innovation over the past years. The JC is not just a TPD resource, but also a fun and purposeful platform to engage with a wider audience.

As a PhD student myself, I was curious if Siying had any advice for students or early career researchers which she could share with me. Her advice was:

- **Take opportunities** to attend workshops on "softer" skills. Recently, Siying attended a workshop on improving communication and influencing skills – this has helped with finding the right approach for communicating with different people in the company to achieve a common goal – she also mentioned that attending such a workshop would have made a big difference in her PhD years.
- **To "do good science"** as much as you can & as well as you can, and to be result-driven. To consciously develop a higher level of understanding, to leverage all data & information possible to inform experimental design, interpretation, and future work.
- **To not be scared of impacts and repercussions** if you want something – particularly in the context of being a female scientist – and even though career progression may possibly take longer, we can find our own moment of lift.



Screen capture of our conversation over Teams. I asked Siying if she has taken up any new hobbies recently, and she surprised me by showing these amazing and friendly-looking conical flasks she has crocheted!

With special thanks to Siying! I really enjoyed our chat and we were overdue a catch-up. I look forward to keeping in touch and hearing about the great science you will be doing!

Contributor: Will Farnaby

The Farnaby Group: A new adventure begins

On 1st March I had the honour and privilege of being the first new Principal Investigator appointed within the University of Dundee Centre for Targeted Protein Degradation (CeTPD). Of course, for me this represents an incredibly exciting step that will allow me to cultivate my independent research vision and help to develop the next generation of talent in fundamental and translational chemical biology.

What will the Farnaby group do?

We will be a multi-disciplinary group that explores new chemical approaches to discover and develop molecules that can illuminate disease biology. There will be a particular focus (though not exclusively) on Central Nervous System (CNS) diseases, because this is an area where there is particularly desperate need for new small molecule modalities that go beyond classical inhibitors and receptor antagonist/agonist modes of action. There are currently unmet CNS needs for conditions affecting hundreds of millions of people globally and we intend to discover a new generation of chemical tools to help address understanding how diseases of the CNS work and could be treated. This will involve reimagining how we find small molecules that can degrade target proteins *and* have the potential to do it in a way relevant for accessing and being active within the CNS. We will also seek to go beyond degradation and provide strategies for using induced proximity chemistry to impact protein function in a manner tailored to the endpoint desirable for a given therapeutic concept. This will of course require a lot of innovation in the chemistry we do as well as the assay platforms we use to interrogate our molecules.

How did I get here?

My journey has taken me from industry to industry-academia collaborations and now to a full academic position. This has not been a deliberate plan in any sense, but rather defined by taking the next challenge that most interested me each time a fork in the career path arrived. Before leaving University with an MChem degree I had done two years in industry in process chemistry and then in medicinal chemistry. At this point I had eyes only for a career as a medicinal chemist and spent nearly 9 years at Takada Pharmaceutical company doing just that. Most of the projects I worked on were CNS focussed and I had the experience of a lifetime when working for 6 months as a secondee at Takeda's research site in Osaka. Of course, we had quite a few failures, but some important successes too with a couple of molecules now in the clinic which remain some of my proudest scientific and personal moments. In 2016 my TPD journey began when hired by Alessio Ciulli to lead a new PROTAC collaboration that had just been established between his research group and Boehringer Ingelheim, what has become known as the 'ACBI Team'. Impossible to do justice to the influence on me as a scientist and lessons learned without writing a whole other feature article, but of most relevance here is that it is really quite difficult to be within such a dynamic team of people, within such a dynamic field as TPD between 2016 and 2021 and not be inspired to try new things and to drive innovation. I would see my current step as the ultimate development of a desire to push my own ideas and visions on where we can take the fundamental science in this area, something that has been undoubtedly nurtured by the freedom and support from within both the University of Dundee and BI over the years. Few things of value are gained without a lot of hard work, but the importance of the mentors I've had and the encouragement of my peers cannot be overstated and is something that I am very lucky to have received. Many are not so fortunate and this is something I hope I can play my part in addressing in my new role as a PI.

Why University of Dundee School of Life Sciences and CeTPD?

A desire to provide a hub for the TPD community, that acts as a place for the best and most creative scientists to interact with as collaborators, students or staff members, is really in the Farnaby group DNA, so the chance to be a future part of CeTPD was never a difficult choice. Through my role leading the ACBI team, I have been a part of

establishing CeTPD from its roots and could not be more excited to not only observe its emergence but be an active and passionate contributor to that vision.

Just as important, as a new PI pitching myself as a chemical tool builder, there cannot be many finer places to be than the University of Dundee School of Life Sciences. The greatest breakthroughs occur at the interface of disciplines and organisations. I am fortunate to be surrounded by several Divisions full of world class biology groups interested in using the latest chemical probes to test their ideas. This fertile ground for collaboration was a big pull for wanting to stay in Dundee when building my own research group.

Getting started…

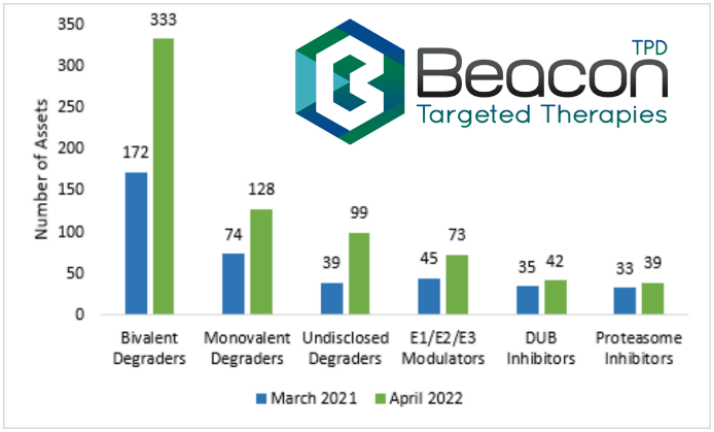
There will be plenty of opportunities to join the adventure! Right now we are advertising for an iCASE studentship focussed on novel bifunctional chemistries for unbiased exploration of TPD mechanisms, part funded by leading life sciences company Tocris. We are also establishing our first masters projects and expect to be advertising post-doctoral positions in chemical biology in early 2023. Those potentially interested in our projects should feel free to reach out to me : w.farnaby@dundee.ac.uk.

Contributor: Valentina Spiteri

CeTPD Welcomes Beacon Targeted Therapies

We recently welcomed Sofia Rodriguez, a TPD analyst, and Charlotte Price, a Research Commercial Liaison Manager, both at Beacon Targeted Therapies, to the University of Dundee. Beacon Targeted Therapies is a software-as-service platform that consolidates clinical trial and drug data with analysts acting as your eyes and ears. They survey TPD (and many other therapeutic areas) to leave you with more time for science. Sofia and Charlotte showcased their software to the Centre where they demonstrated how it worked and what sort of information one could parse. What was clear to see was just how much innovation the field was generating so quickly!

We asked Sofia, given that she is a keen observer of the field, what she finds so captivating about TPD. She said that she finds "the dynamism of TPD incredibly exciting" with more "innovation in the space every day." The team had launched TPD on Beacon in March 2021 and Sofia said that "the number of bivalent degraders has grown by 93% which reflects the investment and interest from developers in the field." Additionally, the rate at which bivalent degraders are entering the clinic is nothing short of staggering, having gone from "two in 2019 to 17 currently, with promising data already being announced by companies like Kymera Therapeutics". Sofia also highlighted that she finds "the variety in TPD interesting, there are a variety of modalities being used to tackle undruggable targets which is encouraging developers to bring their technologies to the table, thus increasing the possibilities of meeting unmet clinical needs." These modalities such as molecular glues "have seen increased interest from developers including Amgen, Plexium and Salarius Pharmaceutical which will hopefully increase the arsenal of tools TPD has." Sofia was kind enough to provide us with a graphic (below) generated using the Beacon TPD platform showing the number of viable assets (compounds) added to the public domain across the several key TPD modalities in March 2021 versus April 2022. This graphic is a clear snapshot of the breakneck speed at which the field is currently moving.



Beacon Targeted Therapies is part of Hanson Wade, which apart from offering data products is a conference specialist. Hanson Wade conferences are particularly significant for the TPD field as they were the first to deliver a TPD-centred conference in 2018, having identified TPD as a growing field that necessitated its own conference. As part of their Undruggable Series, Hanson Wade now hosts two TPD-focussed events annually. This January they held the 2nd TPD Europe Summit in London and will host the 5th Annual TPD Summit in Boston in October. At these conferences attendees include key pharmaceutical and biotechnology researchers who will present clinical and preclinical progress.

While Sofia and Charlotte were at Dundee, they had the opportunity to record a podcast interview with Alessio. This episode is the first in a new podcast series, called Beacon Brainstorms. In the interview Sofia and Alessio discuss the new Centre for Targeted Protein Degradation, the growth of the TPD field, opportunities for novel degradation mechanisms and new modalities of proximity-based pharmacology beyond PROTACs. We encourage our readers to stay tuned for future episodes of this podcast which will be available across most podcast streaming services and YouTube.

Cell Biology | Chemistry

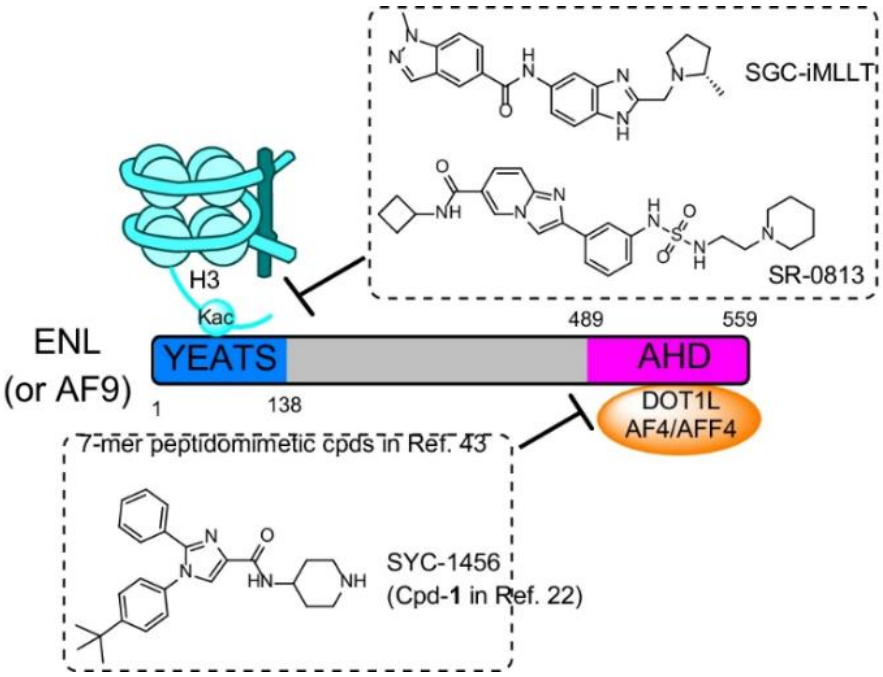
Contributor: Zoe

A Proteolysis-targeting chimera molecule selectively degrades ENL and inhibits malignant gene expression and tumor growth

Xin Li[§], Yuan Ya, Fangrui Wu, Yongcheng Song*

J. Hematol. Oncol. **2022**, DOI: 10.1186/s13045-022-01258-8

The transcription cofactor ENL is a constituent of the super elongation complex that promotes aberrant gene transcription, oncogenesis and maintenance of MLL1-rearranged (MLL1-r) leukemia. With inhibitor drugs in clinical trials, only non-specific treatments such as chemotherapy are currently used to treat MLL1-r leukemia with a high demand for targeted therapies. ENL and its paralog AF9, contain an N-terminal YEATS and C-terminal AHD domain linked by an intrinsically disordered linker and ENL specifically is essential for MLL1-r leukemia making it a potential drug target.



In this study, a novel PROTAC was developed that specifically degraded ENL causing inhibition of gene expression and tumour growth. Three PROTAC molecules were designed that consisted of a YEATS inhibitor SGC-iMLLT covalently linked to thalidomide to target the protein for degradation by Cereblon. Compound **1** showed to be the most efficient ENL-specific degrader with a DC_{50} of 37 nM and depletion at 500 nM (D_{max} ~ 95 %). The authors demonstrated that malignant gene signatures were significantly suppressed as a result of compound **1** medicated ENL reduction. Furthermore, significant antitumour activity in a mouse model of MLL1-r leukemia was observed.

The authors suggested that the increased number and accessibility of lysines in ENL could be the reason why compounds 1-3 could bind to both ENL and AF9 but only degrade ENL and it would be interesting to have this confirmed experimentally. The study could have benefited from some structural biophysics data to gain an understanding of the ternary complex formation but overall, a lead compound for further anticancer drug development has been established.

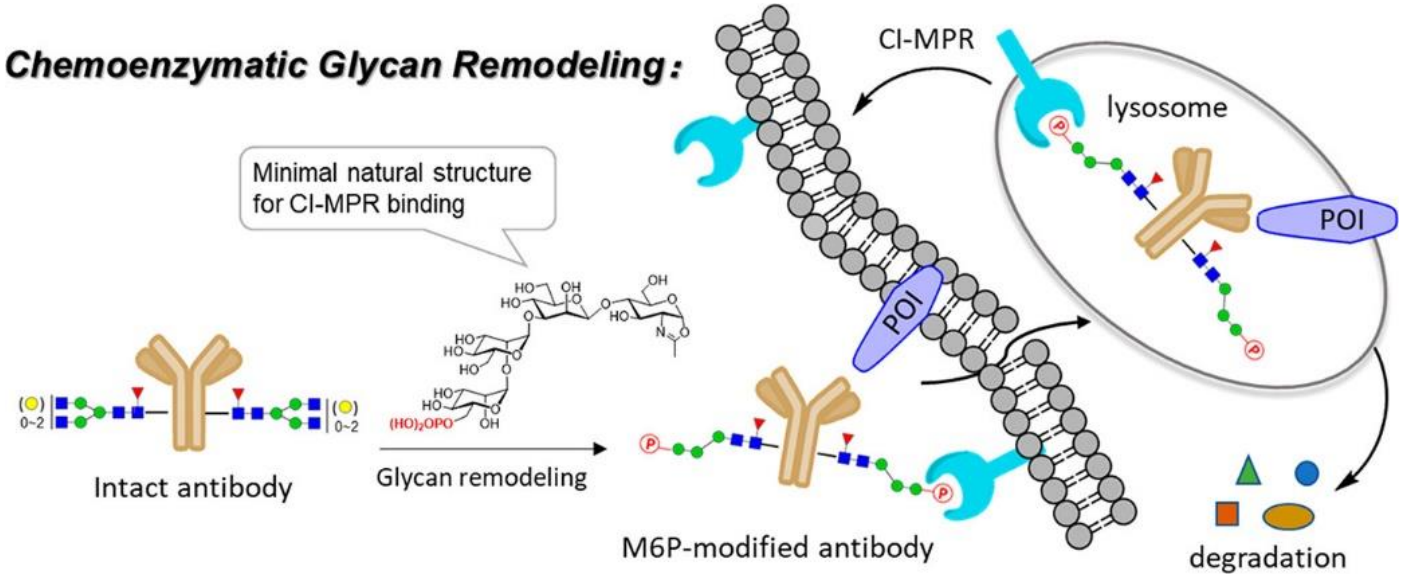
Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Zoe

Site-Specific Chemoenzymatic Conjugation of High-Affinity M6P Glycan Ligands to Antibodies for Targeted Protein Degradation

Xiao Zang[§], Huiying Liu, Jia He, Chong Ou, Thomas C. Donahue, Musleh M. Muthana, Lishan Su, Lai-Xi Wang*

ACS Chem. Biol. **2022**, DOI: 10.1021/acschembio.1c00751



Lysosome-targeting chimeras (LYTACs) offer an opportunity for the targeted degradation of membrane associated and extracellular proteins that cannot be targeted to the proteasome. This work builds on the LYTAC proof of concept study by Bertozzi *et al*., 2020 and successfully demonstrates that LYTACs can be generated by site-specific conjugation of a M6P glycan ligand which leads to CI-MPR (cation-independent mannose-6-phosphate receptor) mediated degradation of membrane associated cancer targets.

The authors tested a number of endoglycosidases and found that Endo- S and Endo- S2 enzymes can be used for the site-specific transfer of M6P glycan ligands to the Fc domains of the therapeutic antibodies trastuzumab and cetuximab. Cell-based assays showed that selective degradation of HER2 and EGFR was achieved using M6P-trastuzumab and M6P-cetuximab, respectively. M6P glycan-antibody conjugates had a high affinity for CI-MPR, binding affinities in the nanomolar range were identified by SPR. These binding modes could be further characterised by an orthologous binding assay such as ITC.

The method developed in this study enables the generation of homogeneous M6P glycan-antibody conjugates without the requirement of protein engineering or click chemistry, an important step in the development of LYTACs.

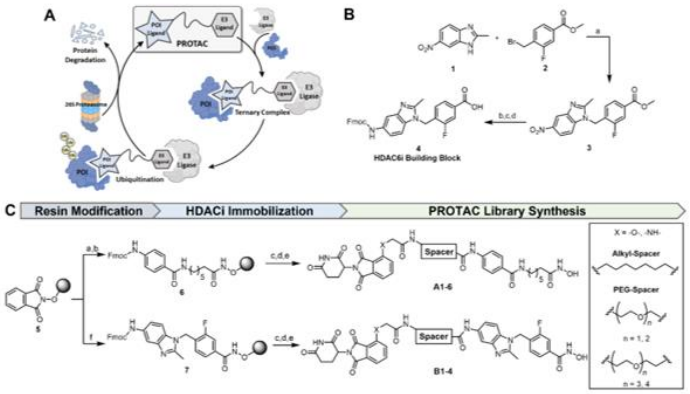
Cell Biology | Chemistry

Contributor: Zoe

Solid-phase synthesis of cereblon-recruiting selective histone deacetylase 6 degraders (HDAC6 PROTACS) with anti-leukemic activity

Laura Sinatra[§], Jing Yang[§], …, , Sanil Bhatia* and Finn K. Hansen*

Chemrxiv, **2022**, DOI: 10.26434/chemrxiv-2022-dntx4



In this study, the authors developed two series of cereblon-recruiting PROTACs that selectively degraded HDAC6 in leukemia cell lines. Series A was based on an unselective vorinostat-like HDAD ligand and series B was based on a selective benzimidazole HDAC6 ligand. The authors claimed that rapid preparation of this PROTAC library was performed using a solid-phase parallel synthesis approach, achieving 95 % purity with total yields between 27-71 %.

The most potent degraders from each series were A6 and B4 with a D_{max} around 78 % and DC_{50} values of 3.7 nM and 13.3 nM, respectively, quantified by automated capillary western blot. A6 and B4 both contained an 8-aminooctanoic acid linker moiety, highlighting a potential importance in ternary complex formation. It was demonstrated by pre-incubation of cells separately with both HDAC6 inhibitors and proteasome inhibitors that the PROTACs degrade by ternary complex formation and via the ubiquitin-proteasome pathway.

A further understanding of ternary complex formation could aid in advancing this PROTAC series, including the comparison of the ternary complex biophysical parameters with the different linkers, specifically the 8-aminooctanoic acid linker compared to the PEG-based linker. In this field, PROTAC technology offers an opportunity to control the cellular processes that are influenced by the first catalytic domain of HDAC6 (CD1) that are not inhibited by the well-established second catalytic domain (CD2) inhibitors.

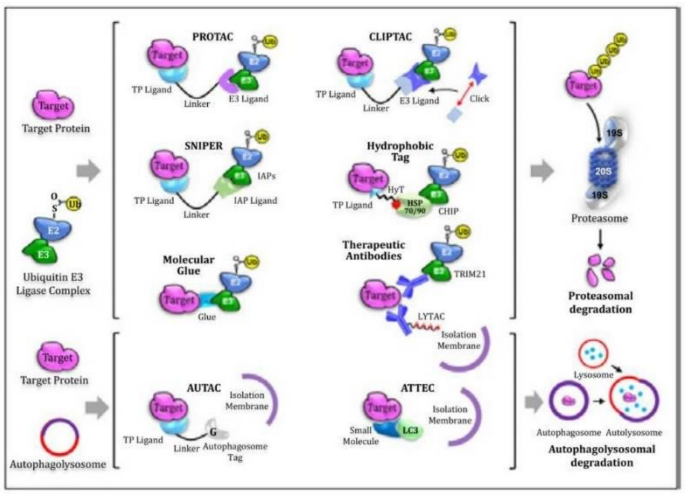
Cell Biology | Chemistry

Contributor: Zoe

Targeted protein degraders march towards the clinic for neurodegenerative diseases

Dhiraj Kumar and Md. Imtaiyaz Hassan*

Ageing Res. Rev. **2022**, DOI: https://doi.org/10.1016/j.arr.2022.101616



In this review, the authors explore and examine what is currently know about targeted protein degraders in the neurodegenerative disease (NDD) field. In section 2, the degrader technologies are summarised and the current applications with regards to NDD are discussed, these include: PROTACs, CLIPTACs, SNIPERs, hydrophobic tagging, molecular glues, AUTACs and ATTECs. A nice figure aids in the comparison of these degraders and in section 3 of the paper the advantages and disadvantages of each technique are highlighted with respect to clinical candidates. In sections 4 and 5 the pharmacological considerations and bottlenecks hampering degrader development are discussed. Overall, this is a nice overview that gets the reader up to speed with the current developments in protein degradation of NDD targets.

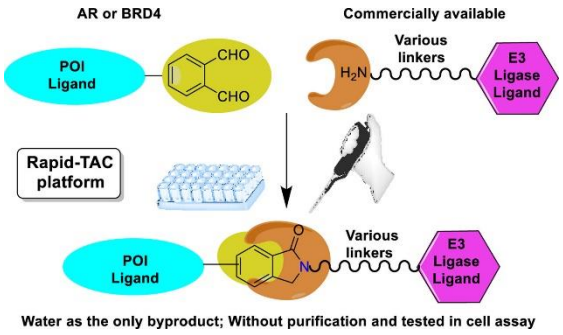
Cell Biology | Chemistry

Contributor: Charlotte

A platform for the rapid synthesis of proteolysis targeting chimeras (Rapid-TAC) under miniaturized conditions

Le Guo[§], Yaxian Zhou[§], Xueqing Nie[§], …, Weiping Tang*

Eur. J. Med. Chem. **2022**, DOI: 10.1016/j.ejmech.2022.114317



The authors propose a method named "Rapid-TAC" for the rapid synthesis of PROTACs, based on **ortho-phthalaldehyde (OPA)-amine** coupling chemistry. They performed the reactions in DMSO at 80 °C for 48 h and carried out biological screening in cells using the crude compounds without any further purification. With water as the only side-product, the authors state that up to 99% purity can be achieved with this reaction in the presence of 10 equivalents of HOAc. To test this system, the authors synthesised 42 PROTACs using:

- **POI ligands** for AR or BRD4 with an **OPA motif** (*synthesised in-house*);
- **E3 ligase ligands** with **varying linker lengths and types (alkyl or PEG)**: 9 compounds with pomalidomide (CRBN ligand) and 11 compounds with VH032 (VHL ligand) (*commercially available*)

Their biological activity at 1 µM and 10 µM was evaluated by Western Blot in LNCap cells for AR degraders and in MV-4-11 cells for BRD4 degraders. For both AR and BRD4, no degradation was observed with CRBN PROTACs, whereas degradation, trends based on varying linker lengths/types and "hook" effect were all observed for VHL PROTACs. The best degrader for each POI were taken forward for a dose response study: a DC50 of 41.9 nM was observed for the AR and 8.9 nM for BRD4.

To conclude, the authors present an interesting method for rapid PROTAC synthesis. It is curious that none of the CRBN PROTACs mediated degradation, so it remains to be shown whether this system can be applied or optimised for CRBN PROTACs.

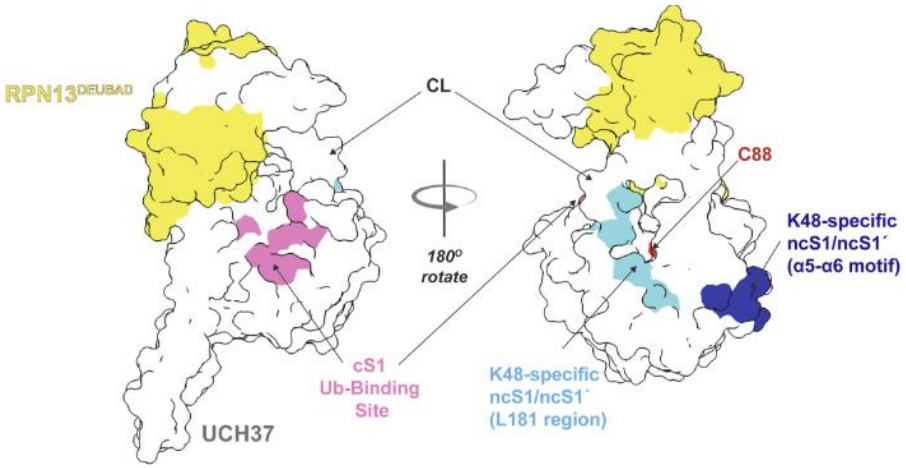
Cell Biology | Computational Modelling | Structural Biology/Biophysics

Contributor: Charlotte

A cryptic K48 ubiquitin chain binding site on UCH37 is required for its role in proteasomal degradation

Jiale Du[§], …, Eric Strieter*

eLife **2022**, DOI: 10.7554/eLife.76100



UCH37 is a deubiquitinase (DUB) which is activated by Rpn13, a receptor for ubiquitinated substrates in the 26S proteasome. It has previously been shown that UCH37 cleaves branched K48 polyubiquitin chains, which promotes degradation of the polyubiquitinated substrate. Using a suite of techniques (hydrogen-deuterium mass spectrometry, chemical crosslinking, small-angle X-ray scattering, NMR, molecular docking, targeted mutagenesis) the authors identify and characterise a previously unknown K48 ubiquitin chain specific site responsible for K48 chain debranching. Furthermore, (through quantitative proteomics, translation shutoff experiments, linkage-specific affinity tools) the authors investigate the effects of this binding site on 26S proteasomal degradation *in vitro* and in cells. Not only is this paper an enjoyable read from the variety of techniques used, but its' findings also constitute an interesting advance in our understanding of the UPS system and by extension the TPD mechanism.

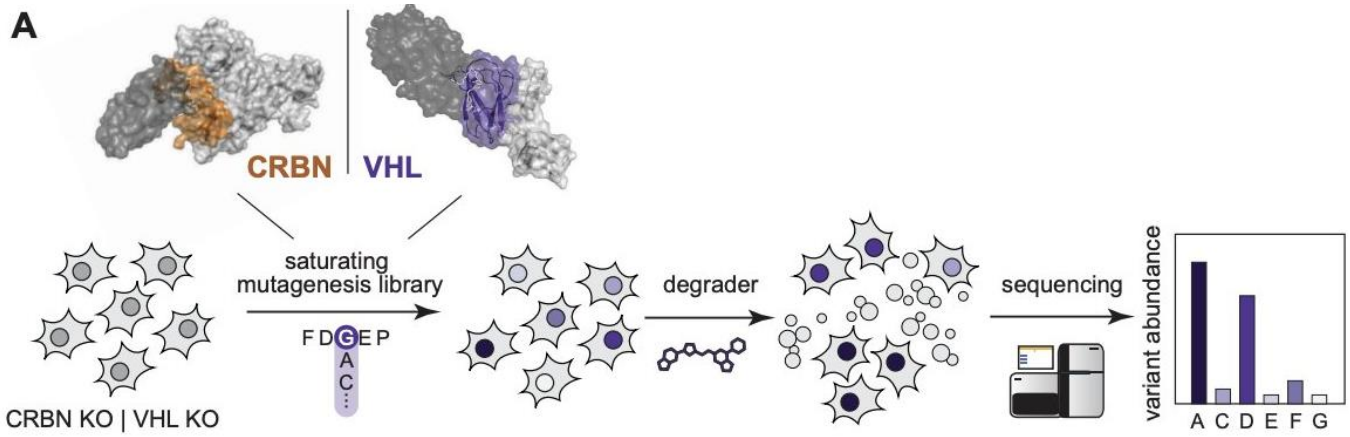
Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Charlotte

Charting functional E3 ligase hotspots and resistance mechanisms to small-molecule degraders

Alexander Hanzl[§], …, Alessio Ciulli*, Georg E. Winter*

BioRxiv **2022**, DOI: 10.1101/2022.04.14.488316v2



"Functional hotspots" refer to the interfaces between E3 ligase-small molecule-neosubstrate. The authors use haploid genetics in combination with structural biology to identify these functional hotspots, characterise the effects of mutations and understand mechanisms of resistance.

The authors show that VHL is an essential gene, whereas CRBN has previously been shown to be a non-essential gene. They also observe that in dBET6 resistant cells, the majority of disruptive mutations are on CRBN compared to the rest of the $CRL4^{CRBN}$ complex. In contrast, in ARV-771 resistant cells, there is a lower proportion of mutations in VHL compared to the rest of the $CRL2^{VHL}$ complex, and the mutations in VHL are less disruptive than in CRBN. Since most mutations conferring resistance were found proximal to the degrader binding pocket and at the neosubstrate interface, the authors examined the amino acids within 10 Å of the binding sites by DMS (Deep Mutational Scanning). With VHL, they show that specific hotspots could be identified for specific neosubstrates (SMARCA2/4 vs Brd4) and also for specific BET degrader with similar chemical structures and potencies (for example MZ1 vs ARV-771). They even show that certain mutations can even enhance degradation. Furthermore, the study identifies and validates CRBN hotspots involved in resistance mechanisms which align with clinically identified mutations.

Of particular note is the use of DMS as a central technique throughout the study – this displays a number of important advantages especially when used in combination with more routinely used crystallographic and biophysical techniques. The authors plan to make the DSM libraries for CRBN and VHL used in this study available for other to use through Addgene.

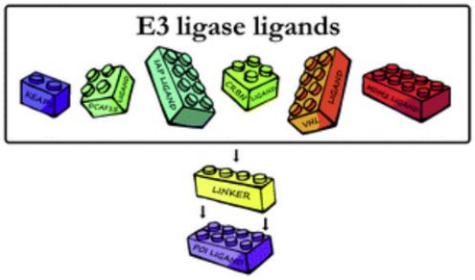
Chemistry

Contributor: Charlotte

E3 ligase ligand chemistries: from building blocks to protein degraders

Izidor Sosič[§], Aleša Bricelj, Christian Steinebach*

Chem. Soc. Rev. **2022**, DOI: 10.1039/d2cs00148a



This review provides an overview of the chemistries surrounding E3 ligase ligands and their derivatisation. The E3 ligases CRBN, VHL, MDM2, IAP, KEAP1 as well as "other ligases" (such as DCAF16, DCAF15, DCAF11, RNF114, RNF4, FEM1B, AhR, L3MBTL3) are discussed. It is a comprehensive summary, also reporting on compounds from patent literature, strategies such as E3 ligase ligand-derivatised chemical probes as well as solid-phase synthesis strategies. It is a well-summarised go-to guide for chemists which will be useful to keep on hand!

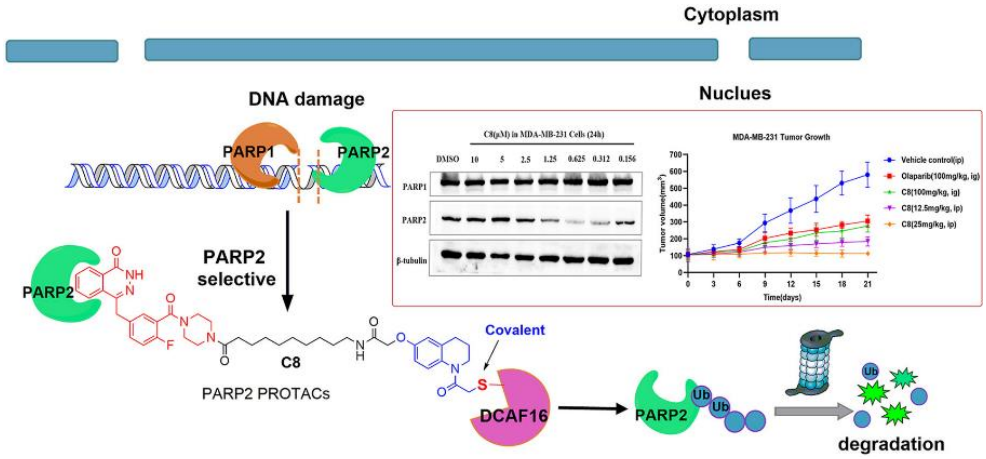
Cell Biology | Chemistry

Contributor: Oliver

Selective degradation of PARP2 by PROTACs via recruiting DCAF16 for triple-negative breast cancer

Chunlan Pu[§], Yu Tong[§], ..., Rui Li*

European Journal of Medicinal Chemistry **2022**, DOI: 10.1016/j.ejmech.2022.114321



Triple-negative breast cancer (TNBC) is characterised by the lack of expression of oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2), and represents roughly 10-15% of all breast cancers. Poly (ADP-ribose) polymerase (PARP) inhibitors have shown some promise in TNBC therapy but are often required to be combined with chemotherapy or radiotherapy and are thus fairly toxic with substantial side effects. PROTACs may overcome some of the resistance and toxicity issues that have been observed with PARP inhibitors, while also increasing selectivity for PARP2 over PARP1 which would enhance antitumour effects. Pu and colleagues here leverage the existing DCAF16 covalent ligand KB02 and couple this to the PARP inhibitor Olparib. The resulting PROTAC termed **C8**, exhibits PARP2-specific degradation and therapeutic effects *in vitro* and *in vivo*.

This work is interesting because it provides further evidence for the advantage PROTACs can have over other therapies, namely providing selective degradation (PARP2 over PARP1) based on an inhibitor which targets multiple proteins (in this case both PARP proteins). In addition, the *in vivo* data using a mouse xenograft model provides compelling preliminary evidence for the potential of this PROTAC to act as a single therapeutic agent in TNBC therapy. The covalent warhead for DCAF16 recruitment still enables the sub-stoichiometric mode-of-action of the PROTAC and has the potential added benefit of low fractional engagement of DCAF16, thus avoiding the antagonism of endogenous ligase function. The covalent DCAF16 ligand is also hypothesized by the authors to confer the specificity towards PARP2 due to a self-covalent binding event that occurs only with a PARP1-specific cysteine, resulting in loss of DCAF16 binding and thus preventing PARP1 degradation.

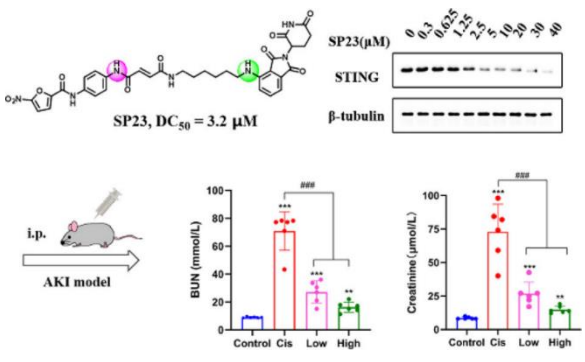
Cell Biology | Chemistry

Contributor: Oliver

Novel CRBN-Recruiting Proteolysis-Targeting Chimeras as Degraders of Stimulator of Interferon Genes with In Vivo Anti-Inflammatory Efficacy

Jin Liu[§],... , Jianjun Chen*

J. Med. Chem. **2022**, DOI: 10.1021/acs.jmedchem.1c01948



STING is an adaptor protein involved in the innate immune response via type I interferon stimulation and has been implicated in many autoimmune disorders due to its overactivation. As such, STING represents an important therapeutic target, however existing inhibitors are occupancy-driven and concomitantly subject to resistance mechanisms and reactivation of STING signalling pathways. Liu and colleagues present here data for the optimisation of a series of STING-

targeting PROTACs based on the STING inhibitor C-170 and the CRBN ligand pomalidomide. Their most promising compound, **SP23**, has a DC_{50} of 3.2µM and more interestingly exhibited high *in vivo* anti-inflammatory effects in a cisplatin-induced acute kidney injury mouse model. The downstream signals of the STING pathway were shown to be inhibited *in vivo* and in cells, while also demonstrating minimal toxicity in normal cells and mouse kidney samples.

This appears to be the first reported PROTAC targeting STING, and the *in vivo* data presented is particularly encouraging for the therapeutic potential for PROTACs in treating autoimmune and inflammatory diseases. It is interesting to note that despite only modest degradation activity in cells, SP23 shows potent inhibition of STING signalling *in vivo*, highlighting the importance of profiling PROTAC molecules in physiological and disease-relevant systems, and not simply in standard immortalised cancer cell lines.

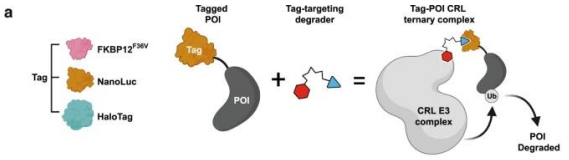
Cell Biology | **Chemistry**

Contributor: Oliver

Development of NanoLuc-targeting protein degraders and a universal reporter system to benchmark tag-targeted degradation platforms

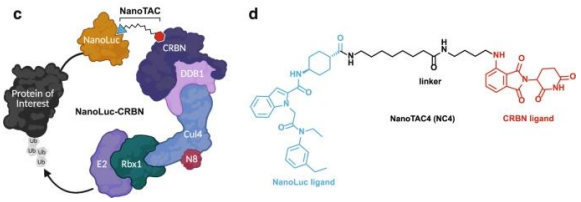
Christoph Grohmann[§], Charlene M. Magtoto[§], ..., Rebecca Feltham*
Nature Communications **2022**, DOI: 10.1038/s41467-022-29670-1

There are an increasing number of tag-targeted protein degrader (tTPD) systems such as HaloTag, BromoTag and dTAG, which couple an E3 ligase (typically CBRN or VHL) to the tag-targeting ligand. Grohmann and colleagues present here an interesting variation of these chemical biology tools by using in NanoLuc, a luciferase enzyme that can produce bioluminescence in the presence of the substate furimazine. This NanoLuc reporter system provides a luminescent readout for target degradation, which is a potential advantage compared with the other tTag systems. The NanoTACs, as they are termed in this work, in particular the CRBN-recruiting one NC4, exhibit comparable target degradation to the other more established systems, while also showing physiological effects in limiting MLKL-driven necroptosis via MLKL degradation in HT29 cells.



b

FKBP12^{F36V}, HaloTag and NanoLuc Tag Comparison								
Tag	MW (kDa)	CRBN degrader	VHL degrader	IAP degrader	Catalytic degraders	Antibodies	Luminescence properties	*in vivo* activity
FKBP12^{F36V}	12	Yes	Yes	X	Yes	Yes	X	Yes
NanoLuc	19	Yes	X	X	Yes	Yes	Yes	NT
HaloTag	33	X	Yes	Yes	X	Yes	X	Yes*



This work highlights the diverse tag-based degrader platforms available and provides a good reporter system for comparing the different technologies. Overall, the dTAG system offered the most superior levels of degradation amongst the tags tested, but NanoTACs offer a potential advantage in terms of degradation monitoring directly via the luminescence, although the caveat highlighted by the researchers here is that the NanoLuc inhibitor warhead can interfere with the nanoluciferase activity at concentrations close to the IC_{50} of the warhead, and thus this must be accounted for during degradation assays at higher concentrations.

Structural Biology/Biophysics

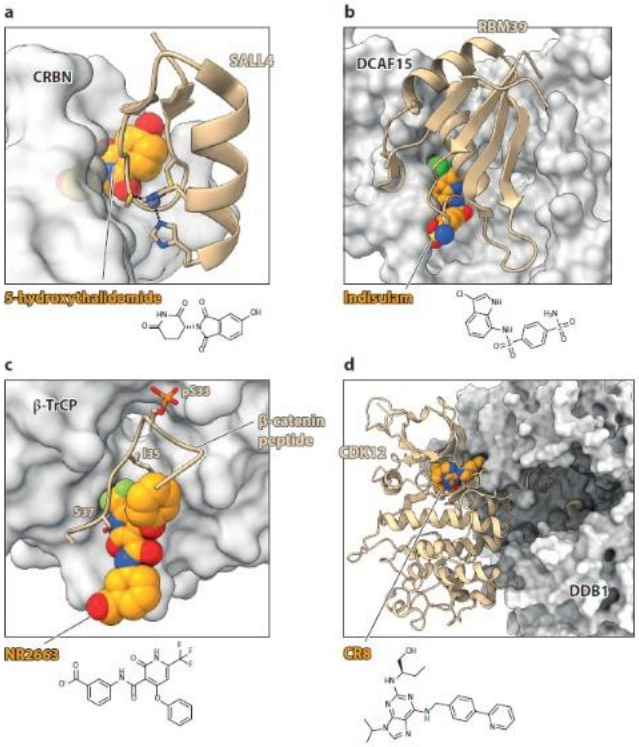
Contributor: Oliver

Driving E3 Ligase Substrate Specificity for Targeted Protein Degradation: Lessons from Nature and the Laboratory

Angus Cowan[§] and Alessio Ciulli*

Annual Review of Biochemistry. **2022***,* DOI: 10.1146/annurev-biochem-032620-104421

This comprehensive review, written by Angus and Alessio here in the Ciulli group, summarises the structural basis for substrate recruitment to E3 ligases for both naturally occurring ligands and 'man-made' ligands including monovalent molecular glues and bivalent PROTAC molecules. The complex and intricate structural dynamics involved in the ubiquitination and degradation of a target protein can be a daunting concept even for seasoned TPD researchers, and this review uses clear illustrations to display molecular glues and PROTACs with their binding partners accompanied by detailed accounts of the molecular basis of these interactions.



This review is a great resource for researchers in the TPD space, with up-to-date accounts of interactions including the novel molecular glue **BI-3802** which induces BCL6 polymerization by the non-cullin E3 ligase SIAH1 and another interaction induced by the CDK inhibitor CR8, which proceeds via the substrate adaptor DDB1 directly binding CDK12 to mediate the degradation of cyclin K. Unusually, this occurs independently of the cognate CRL4-DDB1 substrate receptors (DCAF proteins), and may be the first example of a wider mechanism yet to be fully explored in the TPD space.

Other Paper Highlights

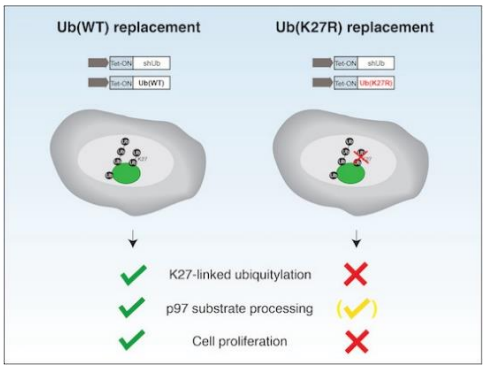
Cell Biology | Structural Biology/Biophysics

Contributor: Mark Nakasone

K27-linked ubiquitylation promotes p97 substrate processing and is essential for cell proliferation

Robert F Shearer[§], Dimitris Typas[§], …, Niels Mailand*

EMBO J. **2022**, DOI: 10.15252/embj.2021110145



Shearer *et al*. developed a cell-based assay to uncover a major role for ubiquitin (Ub) internally linked through lysine-27 (K27), in cell proliferation. Using their "ubiquitin replacement" method, a doxycycline inducible shRNA (shUb) for all four Ub encoding genes depleted endogenous Ub and replacement with Ub(K27R) or other Ub mutants exhibited varying degrees of cell viability. Proteomics confirmed that replacement with Ub(K27R) did not alter levels of other Ub linkages and replacement of Ub(wt) fully rescued the cells. Notably cells accumulated at the G2 phase, arresting mitosis and DNA replication. Immunofluorescence confirmed accumulation of K27 linkages in the nucleus. While whole proteome analysis comparing rescue with Ub(wt) and Ub(K27R) identified many proteins associated with the nuclear functions of p97. Next, using Ub(G76V)-GFP as a model substrate, Shearer *et al*. establish a p97 dependent proteasome degradation pathway initiated by p97's recognition of K27 linkages.

Of the eight Ub-Ub linkages (M1, K6, K11, K27, K29, K33, K48, and K63), K27 is the least understood and technically challenging due its rarity among linkage types. K48 linkages were the first linkage type reported to signal proteasome degradation with subsequent studies identifying K11 and K29 linkages. Shearer *et al*. describe a novel p97-dependent proteasome degradation pathway by K27 linkages.

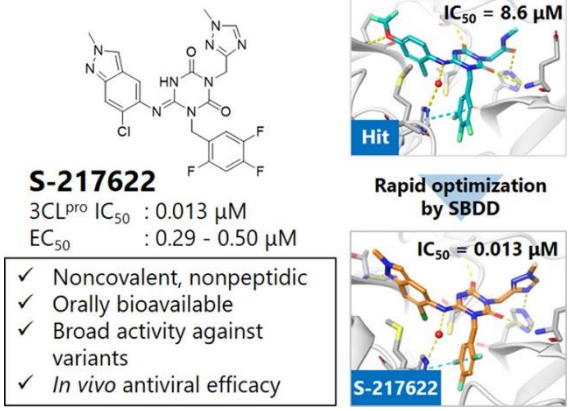
Chemistry | Computational Chemistry | Structural Biology/Biophysics

Contributor: Tasuku Ishida

Discovery of S-217622, a Noncovalent Oral SARS-CoV-2 3CL Protease Inhibitor Clinical Candidate for Treating COVID-19

Yuto Unoh[§], Shota Uehara[§], Kenji Nakahara[§], …, Yuki Tachibana*

J. Med. Chem. **2022**, DOI: 10.1021/acs.jmedchem.2c00117



One approach to target SARS-CoV-2 is selective inhibition of 3C-like protease (3CL^{pro}), which is essential for viral replication, but *Homo sapiens* don't have similar types of proteases. Recently, Pfizer launched nirmatrelvir, a selective 3CL^{Pro} inhibitor, in clinic for treatment of COVID-19, but it needs to be administered with ritonavir because of instability against CYP3A4. In this paper, the authors describe the development of **S-217622**, a novel non-peptidic and non-covalent-type 3CL^{pro} inhibitor. First, they conducted docking simulations with their over 100,000 compound libraries by using the cocrystal structure data of 3CL^{pro} with a non-covalent-type inhibitor. After identifying compound **1**, which has 8.6 μM IC_{50} with good biological stability, they then obtained the cocrystal structure of 3CL^{pro} with **1**. The compound binds to the protein as expected, except there were π-π interaction between the trifluorobenzene ring and the imidazole ring on His41 in the S2 pocket. They utilized this information to optimize **1** and then finally identified **S-217622**, which has a more than 600-fold strong IC_{50} while maintaining great bioavailability. **S-217622** showed great oral activity *in vivo* and potentially can be applied with QD dosing, which might be a great advantage against nirmatrelvir. This paper showed the power of structural information to shorten the development time. Unfortunately, their compound did not show the significant effects in phase 2 clinical trials with a small number of patients.

Centre for Targeted
Protein Degradation
University of Dundee

Centre for Targeted Protein Degradation
School of Life Sciences
Dow Street, Dundee,
DD1 5EH
United Kingdom

lifesci.dundee.ac.uk/groups/alessio-ciulli/publications/journal-club

@alessiociulli @CharlCrowe

23435