CeTPD Journal Club

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

Journal Club

August 2023

Centre for Targeted Protein Degradation
University of Dundee

innovate
collaborate
inspire

Content

Meet this Month's Editors

This month's editors are (from left to right): Manjula Nagala, Marco Serafini and Ryan Casement

Manjula moved to Prof. Ciulli's lab in July 2021. Manjula comes from St Andrews where she formerly was Research Fellow at the BSRC Mass Spec services. Prior to that she worked in Dundee's School of Life Sciences, in both CSI (Prof.Paul R Crocker) and GRE (Dr.Joost Zomerdijk) on cell signalling, gene regulation and mass spec proteomics. Manjula obtained her BSc in chemistry, MSc in Biochemistry from Madras University and PhD from Centre for Biotechnology, Anna University, India. In her spare time Manjula enjoys travel, playing badminton and chess.

"The JC is a valuable tool not only for keeping pace with the growing TPD literature but also to experience being editor"

Marco obtained his master's degree in Pharmaceutical Chemistry and Technology in 2020 from the University of Pisa, Italy. During his master thesis, he was involved in a project focused on drug discovery of new selective thyromimetics with potential therapeutic use, under the supervision of Prof. Simona Rapposelli and in collaboration with Dr. Sheraz Gul from the Fraunhofer Institute (ITMP, Hamburg, Germany). In 2021, he started his PhD at the Research Institute for Medicines (iMed), University of Lisbon, Portugal under the supervision of Prof. Rui Moreira and co-supervision of Prof. Alessio Ciulli. His doctoral studies are directed towards the design and development of novel PROTACs capable to induce specific intracellular degradation of Akt protein kinase for the treatment of cancer. He joined the Ciulli group as a PhD Visiting Student to carry out a six months research internship aimed at gaining further experience and skills in targeted protein degradation field.

"The journal club provides an easily digestible summary of key publications In the field"

Ryan, originally from Belfast, joined the Ciulli lab in 2018 after completing his undergraduate degree in medicinal chemistry at Trinity College Dublin. During this time, he undertook a year long industrial placement at GSK working on targeted protein degradation. He completed his PhD in 2023 where he investigated ElonginB/C containing E3 ligase complexes using fragment-based ligand design. In his spare time Ryan enjoys playing basketball, going skiing and going to the pub.

Launch of the CeTPD work experience programme in collaboration with the "Auricher Wissenschaftstage"

Contributor: Claudia

Special Acknowledgement: University's Public Engagement Network, Erin Hardee and Dr Amy Cameron for their help and guidance in establishing the CeTPD work experience programme

Being committed to engaging with the general public to raise awareness to scientific progress and discoveries in life sciences, particularly in the innovative field of targeted protein degradation, outreach activities targeting different focus group have been developed within the CeTPD, as highlighted in our March 2023 Journal Club issue. As part of this ongoing outreach initiative, we were keen to implement a work experience programme at the CeTPD allowing aspiring next-generation scientists a peek behind our lab doors.
We envisioned the CeTPD work experience programme as a chance for high school students with an interest in sciences to experience the day-to-day work of the CeTPD's scientists, to learn about the field of targeted protein degradation and to gain some hands-on experience in various lab techniques. Hosting only one or two students at a time for a one- to two-week period, we could offer a research experience beyond lab tours and shadowing, as the students would be able to work side-by-side with scientists from all disciplines represented within the CeTPD, i.e., chemistry, cell biology, structural biology, biophysics, protein production, proteomics and computational chemistry. This programme was planned to be complemented by some workshops, e.g., an introduction into drug discovery, or a workshop on how to prepare presentations & write reports, as well as social mixers with and with undergraduate students of the CeTPD and the wider CeTPD community.

Initiated and organised by Claudia Diehl, postdoctoral researcher in organic chemistry and chemical biology who benefitted from work experience stays at research institutions herself as high school student, a collaboration with the scholarship programme "Auricher Wissenschaftstage" was set in place. The "Auricher Wissenschaftstage" is an established scientific initiative in Aurich, Germany, encompassing an annual scientific lecture series for the public and a scholarship program enabling final-year high school students to visit collaborating scientific institutions for work experience stays. As a host institution of the "Auricher Wissenschaftstage", the CeTPD joins a network of over 30 research centres and political institutions providing insights into their activities to the participating students.

Kicking off the CeTPD's work experience programme, we hosted our first work experience visitors, Hanna Windau and Johanna Dahl, from 24th July until 4th August at the CeTPD.

During their two-week stay, Hanna and Johanna learned about targeted protein degradation and drug discovery while getting introduced into the different disciplines of research performed at CeTPD shadowing scientists and performing experiments themselves. Starting off, they were introduced to the CeTPD with a tour of the Centre guided by Suzanne O'Connor, senior scientist in medicinal/organic chemistry, and Diane Cassidy, senior biology technician, and learned about targeted protein degradation and drug discovery from Suzanne O'Connor and about our outreach activities from Giorgia Kidd, scientist in in medicinal/organic chemistry and CeTPD's outreach coordinator.
Spending two days with Aina Urbina Teixidor, scientist in in medicinal/organic chemistry, and Maria Rodriguez-Rios, postdoctoral scientist in medicinal chemistry, Hanna and Johanna were introduced to various techniques of synthetic organic chemistry and performed their first own chemical experiment setting up an S_N2 reaction.
They took a deep dive into structural biology and biophysics, with a master class in protein expression and purification producing YFP and Tomato red with Valentina Spiteri and Kevin Haubrich, both postdoctoral researchers in structural biology and biophysics, an introduction to crystallography with Zoe Rutter, scientist in structural biology/biophysics,

including crystalising lysozyme and performing a fluorescence polarisation assay with Darren Darren, associate scientist in structural biology/biophysics.
Moving into our molecular biology/cell biology facilities, they performed an *in vitro* deubiquitination assay with Hirotake Furihata, postdoctoral researcher in chemical biology, were introduced into tissue culture work by Diane Cassidy and Alessandra Salerno, postdoctoral researcher in organic chemistry and chemical biology, to prepare for a cellular degradation assay performed with Shakil Khan, scientist in cellular biology, and Ilaria Puoti, scientist in cellular biology and proteomics, followed by Western blotting with Jakub Krstev, associate scientist in cellular biology, and Claudia Diehl.
Hanna and Johanna developed some of their personal and transferable skills through a presentation/writing workshop with Maria Rodriguez-Rio and were introduced to further research areas represented in CeTPD with workshops in proteomics with Ilaria Puoti, and in computational chemistry with Sohini Chakraborti, scientist in computational drug design and molecular modelling.

Outside the labs, Hanna and Johanna joined lots of social activities, such sport activities in the evenings and joined in for the first CeTPD sports day, which coincided with their last day at CeTPD.

It has been a tremendous pleasure to host both Hanna and Johanna at the CeTPD and to show them what our work is all about! Inspired by the success of this first work experience visit, we are already looking forward to and started organising the next visit of work experience students.

Our visiting work experience students, Hanna and Johanna, together with their lab supervisors.

Hanna and Johanna have kindly agreed to share their thought on their work experience at CeTPD, which you will find on the next page:

“The collaboration between the "Auricher Wissenschaftstage" and the CeTPD was a great experience for me. Having never been in a laboratory before, it was very interesting to learn a bit about the work of professional scientists. A lot of the practices we were able to try were talked about in class already. Being at the CeTPD was a way of actually leaving the theoretical level of school classes and becoming active. Things we learned in school were actually given a purpose in research. That was really nice to see. I had not heard of Targeted Protein Degradation before I came here, but it was very well explained to us. In general, we were welcomed really nicely, and everybody was motivated to show us what they do in their field of work. Being able to get a peek into professional chemistry and biology enriched my experience. That is why I liked this work experience very much and want to thank all the people who made it possible!”

Hanna Windau

“Being among the first students to participate in the program at the CeTPD was a truly remarkable journey. From the beginning, I found myself enveloped in a realm of pioneering scientific investigation and collaborative endeavors at the forefront of research. The CeTPD's unique approach to bridging the gap between academia and practical application not only expanded my understanding of targeted protein degradation but also provided me with an invaluable opportunity to work alongside leading experts in the field.
Through hands-on experiments, stimulating conversations, and exposure to state-of-the-art laboratory techniques, I gained insights that transcended traditional classroom learning. The CeTPD's commitment to fostering a dynamic and inclusive learning environment empowered me to ask questions, challenge assumptions, and contribute meaningfully to the ongoing research. This experience has definitely left an indelible mark on my academic and professional journey. Moreover, the warmth and camaraderie of the center's staff added a delightful layer to the encounter. From shared dinners to sports activities, these interactions really showed me the importance and special feature of forging personal connections and friendships at a professional setting and that the bonds we form and the memories we create are just as significant as the discoveries we make. As I reflect on my time at the Centre, I am filled with gratitude for the opportunity to be a part of this exceptional journey and the memories I was able to make.”

Johanna Sophie Dahl

Targeted Protein Degradation

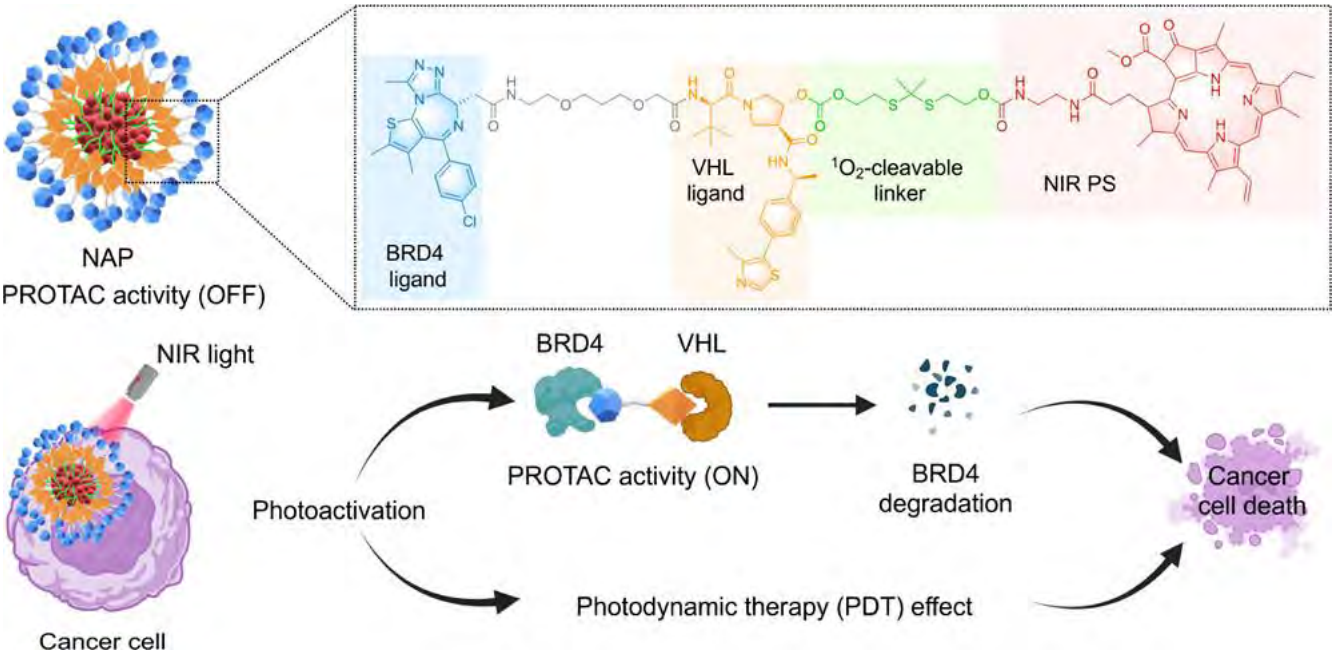
Cell Biology | Chemistry

Contributor: Manjula

Self-Assembled Nano-PROTAC Enables Near-Infrared Photodynamic Proteolysis for Cancer Therapy

Weishan Wang [§], …, Jinbo Li*

J. Am. Chem. Soc. ***2023****, 145, 30, 16642–16649*



The author describes a near-infrared (NIR) photoactivatable nano-PROTAC (NAP) for remote-controllable proteolysis of target protein in tumor-bearing mice in spatiotemporal control manner. They prepare the NAP by molecular self-assembly from an amphiphilic conjugate of PROTAC linked with an NIR photosensitizer through a singlet oxygen (1O2)-cleavable linker. The nanoformulation of NAP promoted tumor accumulation of PROTAC. The PROTAC is inactive initially but can be remotely switched to active PROTAC upon NIR photoirradiation to generate $^1O2$ by the photosensitizer. In this paper they demonstrated that NAP enabled tumor-specific degradation of bromodomain-containing protein 4 (BRD4) in an NIR light-instructed manner and show effective suppression of tumor growth in vivo. This work thus presents a novel approach for spatiotemporal controlled degradation of target protein by PROTAC molecules.

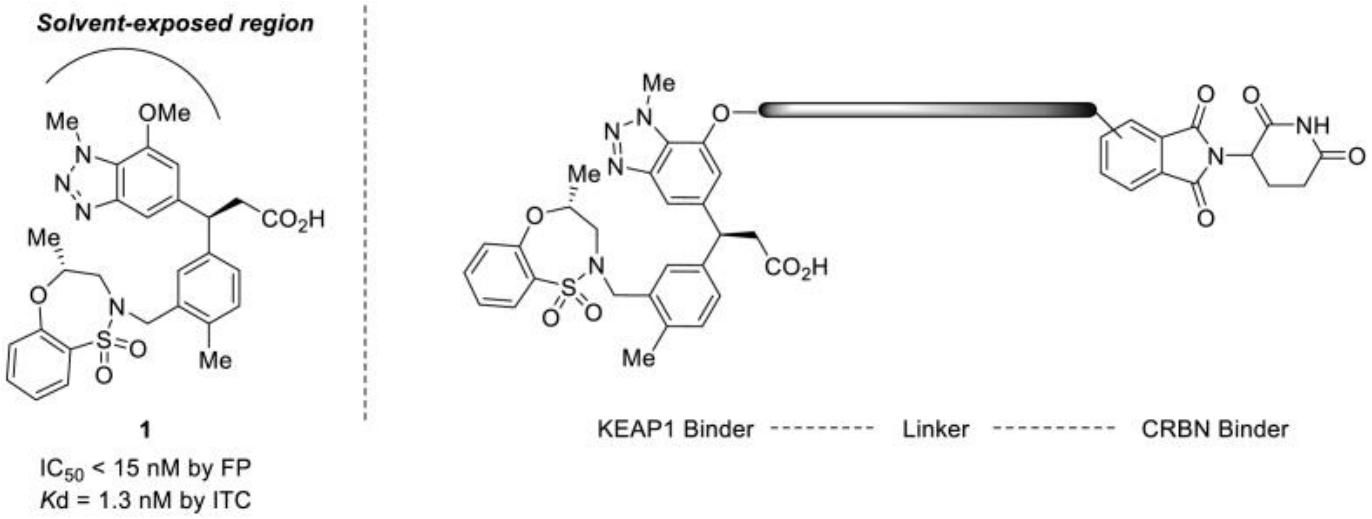
Cell Biology | Chemistry

Contributor: Manjula

Development of KEAP1-targeting PROTAC and its antioxidant properties: In vitro and in vivo

Se Yong Park §, …, Seung Hyun Oh*

Redox Biol. **2023,** *DOI: 10.1016/j.redox.2023.102783*



The KEAP1-NRF2 pathway is the principal protective response to oxidative and electrophilic stresses. Under homeostatic conditions, KEAP1 forms part of an E3 ubiquitin ligase, which tightly regulates the activity of the transcription factor NRF2 by targeting it for ubiquitination and proteasome-dependent degradation. In this article, the authors generated a cereblon (CRBN)-based PROTAC (SD2267) that induces the proteasomal degradation of KEAP. Furthermore, they provide evidence that KEAP1 degradation by SD2267 was proteasomal degradation-dependent and autophagy-independent by studying KEAP1 degradation efficiency using by MG132 (a proteasomal degradation inhibitor) and chloroquine (an autophagy inhibitor). Further in hepatocytes they showed that KEAP1 degradation by SD2267 leads to nuclear translocation of NRF2, expression of NRF2 target genes, attenuation of ROS accumulation induced by acetaminophen (APAP) and attenuated APAP-induced liver damage. Furthermore SD2267 could be used to treat hepatic diseases related to oxidative stress. SD2267 was not fully profiled from a drug candidate perspective, SD2267 oral pharmacokinetic parameters were much lower than those of IP, suggesting that future studies to optimize pharmacokinetic properties including solubility, metabolic stability and permeability are still required, however they show for first time, KEAP1-targeting PROTACs have potential use as therapeutic agents for oxidative stress-related liver diseases.

Cell Biology | Chemistry

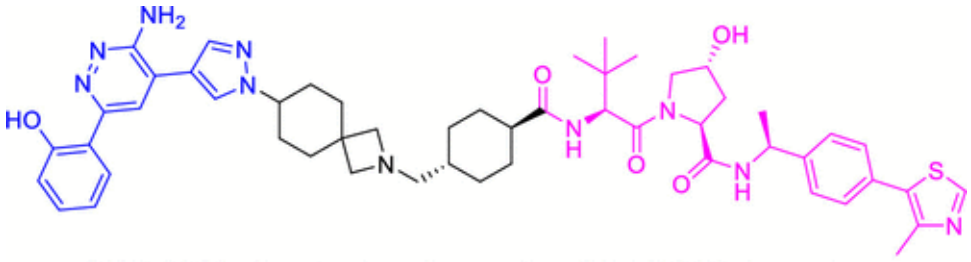
Contributor: Manjula

Discovery of SMD-3040 as a Potent and Selective SMARCA2 PROTAC Degrader with Strong in vivo Antitumor Activity

Lin Yang [§], …, Shaomeng Wang *

J. Med. Chem. ***2023***, *66, 15, 10761–10781*

This study presented the design, synthesis, and biological evaluation of PROTAC degraders of SMARCA proteins using a potent and dual SMARCA2/4 bromodomain ligand and ligands for VHL/Cullin 2. In this study they investigated the linker length, composition and rigidity, and the linker attachment position in the VHL ligand, and identified a set of potent and selective degraders of SMARCA2 protein over SMARCA4, with SMD-3040. They show that SMD-3040 degrades SMARCA2 protein with a low nanomolar DC50 and Dmax > 90% and demonstrated degradation selectivity for SMARCA2 protein over SMARCA4 protein. SMD-3040 displayed potent cell growth inhibitory activity in a panel of SMARCA4-deficient cancer cell lines.



SMD-3040: A potent and selective SMARCA2 degrader

DC_{50} = 12 nM and D_{max} = 91% against SMARCA2;

DC_{50} > 1000 nM and D_{max} = 44% against SMARCA4;

SMARCA2/4 selectivity: >80X;

Strong in vivo anti-tumor activity in H838 and SK-Mel-5 models

Chemistry | Structural Biology/Biophysics

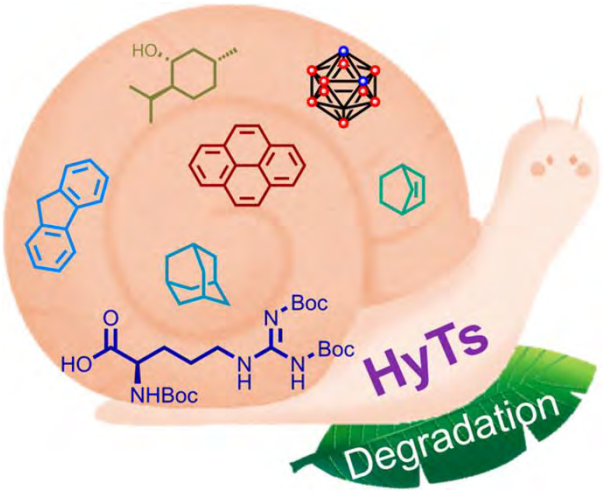
Contributor: Marco

Small-Molecule Hydrophobic Tagging: A Promising Strategy of Druglike Technology for Targeted Protein Degradation

Shaowen Xie[§], Jingjie Zhu[§], Hong Yao*, Jinyi Xu*, Shengtao Xu*

J. Med. Chem. 2023, ***66,*** *16, 10917–10933*

Targeted Protein Degradation (TPD), spearheaded by PROTACs, has emerged as a promising and innovative approach shifting the drug discovery landscape from an occupancy-driven pharmacology to an event-driven strategy. Although TPD, and in particular PROTACs, provided multiple advantages highlighting their potential in delivering more selective and effective therapies, the clinical development of these heterobifunctional degraders has been hampered by their poor drug-like properties. In this perspective, Hydrophobic tags (HyTs), defined as bifunctional molecules consisting of a target-binding moiety and a lipophilic small-molecule tag connected through a linker, and capable to induce the degradation of the protein of interest (POI) by provoking a destabilization or mimicking a partially unfolded protein state, are reported to be a valuable alternative due to their enhanced drug-like properties. As a matter of fact, hydrophobic fragments are usually endowed with lower molecular weight and a reduced number of hydrogen bond donors/acceptors related to E3 ligands.



The authors provide a thorough overview of the HyTs platform by listing various hydrophobic tags and highlighting the advantages and current limitations of the approach. HyTs approach bears the potential to further broaden and sustain the TPD field by merging the “designability” of PROTACs and the “druggability” of molecular glues.

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

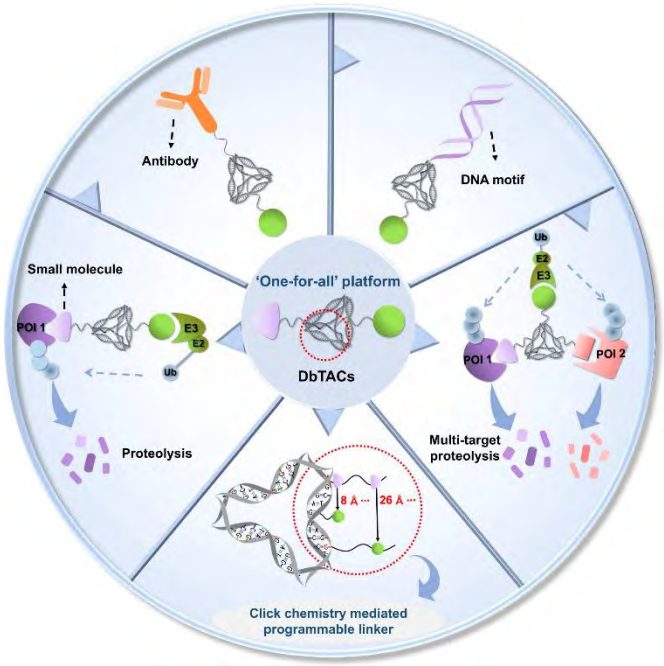
Contributor: Marco

DNA framework-engineered chimeras platform enables selectively targeted protein degradation

Li Zhou[§], Bin Yu[§], Mengqiu Gao[§], Yueqing Gu*, Jinlei Bian*, Yi Ma*

Nat. Commun. **2023**, July 23, 14:4510.

It is widely reported that linker nature plays a pivotal role in the effectiveness and degradation activity of PROTAC molecules. However, despite numerous efforts have been directed towards a rational design of the linker, generating effective linkers remains empirical and challenging. In this paper, the authors implemented the use of engineered DNA frameworks as linkers endowed with high programmability and addressability to assembly a new generation of heterobifunctional degraders called DNA framework-based PROTACs (DbTACs). The feasibility and consistency of the approach has been demonstrated by developing a series of DbTACs bearing different linker lengths and evaluating them for degradation, ternary complex formation and stability, and selectivity, using ligands of cyclin-dependent kinase family protein and CRBN recruiters as template. Additionally, the innovative platform has been adopted for the generation of modular trivalent agents, bis-DbTACs, enabling multi-target depletion and degraders employing multiple types of warheads including antibodies and DNA motifs, thus proving broad applicability and generalizability.



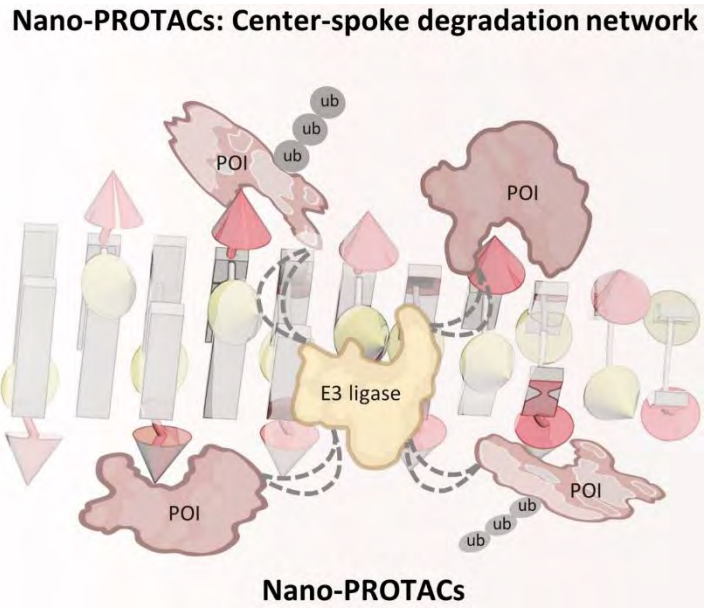
The strategy holds great potential, not only by allowing the generation of degraders provided with linkers of precisely controlled length, but also providing a universal platform liable to different applications and scenarios.

Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Marco

Nano Proteolysis Targeting Chimeras (PROTACs) with Anti-Hook Effect for Tumor Therapy

Ni-Yuan Zhang[§], Da-Yong Hou[§], Hong-Wei An*, Wanhai Xu*, Hao Wang*
Angew. Chem. **2023**, July 24, e202308049.



In this paper, an intracellular self-assembled NanoPROTAC system capable to accumulate selectively in tumor cells and induce potent and long-lasting protein degradation *in vitro* and *in vivo*, is reported. The system entails two independent hydrophilic peptide-based precursors bearing the ligand for the protein of interest (POI) or the E3 ligase recruiter respectively, which, upon in situ activation triggered by high GSH concentrations within cancer cells, are conjugated into assembly-driving monomers capable to self-assemble and generate stable and highly organized β-sheet nanostructures. Such structures establish a multi-binding surface where multiple POIs and E3 ligases can bind, thus creating polynary nanocomposites (E3)m: Nano-PROTACs: (POI)n in comply with an E3 ligase center-spoke POI degradation network.

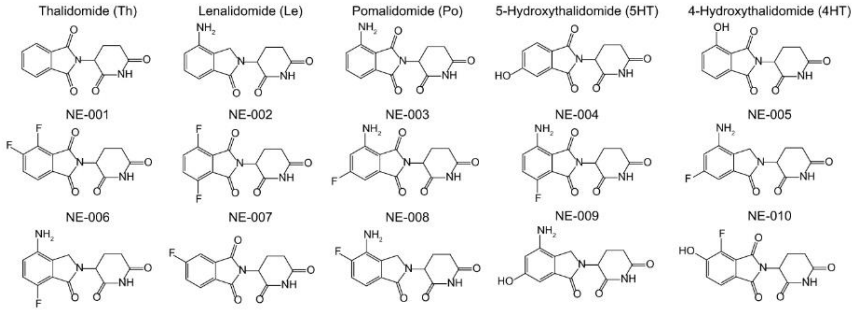
NanoPROTAC system holds promising value and allows to further broaden the TPD field by providing a tumor-selective platform capable to deliver sustained and robust protein degradation with “anti-hook effect”.

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

Contributor: Marco

Lenalidomide derivatives and proteolysis-targeting chimeras for controlling neosubstrate degradation

Satoshi Yamanaka[§], Tatsuya Sawasaki*
Nat. Commun. **2023**, August 18, 14:4683.



Lenalidomide, a drug used as a first-line treatment of several haematological cancers, is a thalidomide derivative belonging to the immunomodulatory drugs (IMiDs) and it acts as a molecular glue. It exerts its functions by recruiting CRBN E3 ligase and degrading a series of neosubstrates, such as IKZF1, IKZF3, and CK1α, proteins involved in anti-haematological cancer activity. Given its lower molecular weight and the ubiquitous expression of CRBN E3 ligase in several tissues, lenalidomide has been widely used in the assembly of many PROTACs. However, its applicability has been limited by its poor selectivity among different neosubstrates and capability to degrade SALL4 and PLZF, proteins responsible for the teratogenicity of thalidomide. Accordingly, in this study, modifications on the phthalimide ring of lenalidomide aimed at improving selectivity and reducing side effects, have been implemented. In particular, the

authors showed that small substitutions on the 6-position of lenalidomide led to a valuable increase in selectivity towards IKZF1, IKZF3, and CK1α against SALL4 and PLZF. Additionally, lenalidomide derivatives showed stronger antiproliferative effects on haematological cancer cell lines such as multiple myeloma (MM) and 5q myelodysplastic syndromes (5q MDS), than the parent ligand. To further confirm the potential of these derivatives, a series of BET-targeting PROTACs, which proved to retain the same level of selectivity, have been generated.

Based on these considerations, lenalidomide derivatives and, modifications on the 6-positions, could represent a valuable tool for controlling neosubstrates selectivity and to generate degraders capable to establish a more selective and effective protein degradation.

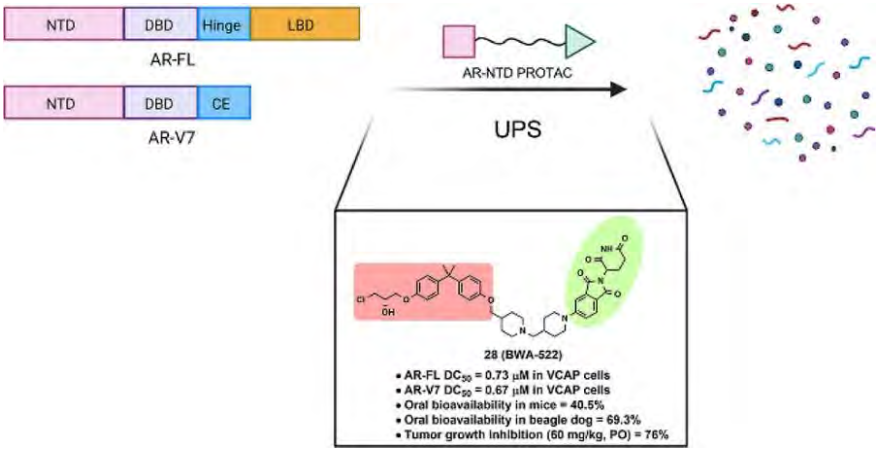
Cell Biology | Chemistry

Contributor: Ryan

Discovery of BWA-522, a First-in-Class and Orally Bioavailable PROTAC Degrader of the Androgen Receptor Targeting N-Terminal Domain for the Treatment of Prostate Cancer

Bowen Zhang[§], …, Chong Qin*

J. Med. Chem. ***2023****, 66, 16, 11158–11186*



This work describes the discovery of orally bioavailable PROTACs targeting the N-terminal domain (NTD) of the androgen receptor (AR), in contrast to previously reported PROTACs which generally target the ligand-binding domain (LBD). The significance of this is highlighted by the statement that AR mutations generally consist of point mutations on the LBD and splice variants such as AR-V7 which lack the LBD and hinge region. EPI-002, a putative covalent ligand for AR-NTD was conjugated to typical VHL and CRBN ligands *via* a variety of linkers. Compounds were evaluated at two concentrations with the most potent (BWA-522) reducing protein levels by 70-80% at 5 μM. Following PK studies BWA-522 was found to have 40.5% oral bioavailability in mice and 69% in beagle dogs. The compound was shown to inhibit the growth of AR-dependent prostate cancer cells and enzalutamide reistant cells with IC50s in the single-digit micromolar range. In a xenograft model BWA-522 inhibited tumour growth at 20 and 60mg/kg and does not appear to display the toxicity of the parent antagonist.

The compound developed and thoroughly evaluated in this work will be a valuable tool molecule in assessing the utility of targeting the NTD of the androgen receptor to degrade AR-full length and AR-V7 alongside the previously reported MTX-23 which targets the DNA binding domain.

Chemistry

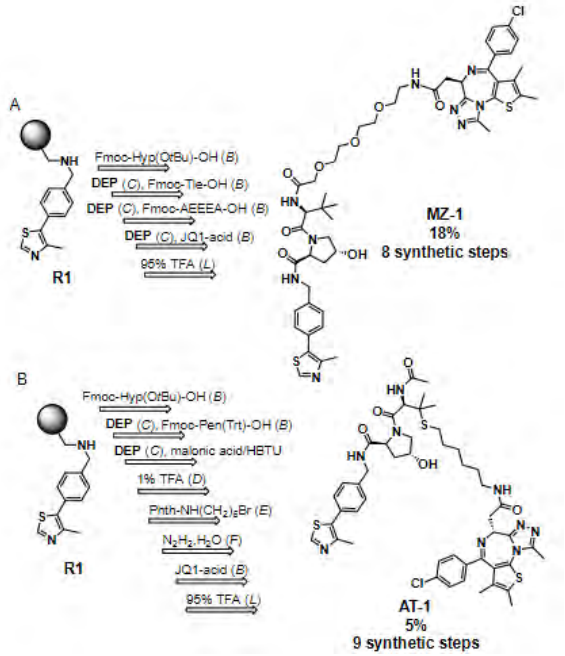
Contributor: Ryan

Solid-phase Synthesis of PROTACs and SNIPERs on Backbone Amide Linked (BAL) resin

Liam Hales[§], Philip Thomson*

Chem. Eur. J., ***2023***, *DOI: 10.1002/chem.202301975*

In this paper the authors describe the synthesis of a number of VHL and IAP recruiting PROTACs using solid phase synthesis and a backbone amide linked (BAL) resin. VHL The synthesis of VHL recruiting PROTACs utilising 4 different vectors and IAP PROTACs from one vector is achieved using an impressive range of on-bead chemical reactions. These compounds could be synthesised in yields of 2-18% with only one purification step required following resin cleaveage.



While this is by no means the first time solid phase organic synthesis has been leveraged for the synthesis of PROTAC libraries, th3 strategy implemented here allows exploration of structural diversity within peptidomemtic E3 ligase ligands. This is an attractive approach as it has been shown that there is no one size fits all for E3 ligase ligase ligand structure/linkage point across different protein targets due to differences in ternary complex assembly. This work also nicely highlights the various optimization that had to be carried out for certain reactions, which can be challenging due to difficulties in reaction monitoring.

Cell Biology | Chemistry | Computational Chemistry

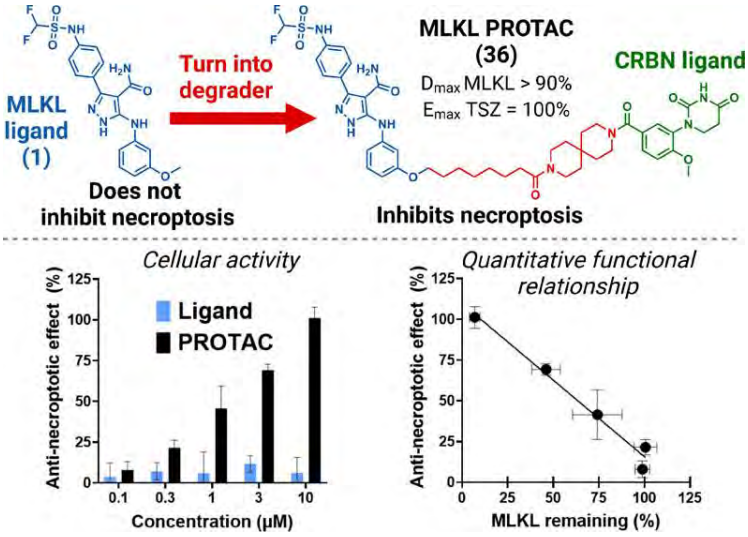
Contributor: Ryan

PROTACs Targeting MLKL Protect Cells from Necroptosis

Oliver H. Rathje[§] Lara Perryman, Richard J. Payne*, and Dieter W. Hamprecht*

J. Med. Chem. ***2023***, *66, 16, 11216–11236*

Mixed Lineage Kinase domain-Like pseudokinase (MLKL) is thought to be an ultimate effector of necroptosis and thus represents a promising therapeutic target for a number of pathologies. Ligands have previously been developed for various domains of MLKL however as this protein is thought to drive necroptosis through structural and scaffoldoing activity these efforts have been challenging. A pyrazole carboxamide-based ligand with sub-nanomolar MLKL binding affinity (but does not inhibit necroptosis) was selected and docking studies identified a solvent exposed vector for conjugation to lenalidomide based CRBN binders. In a subsequent round of synthesis phenyl dihydrouracil CRBN ligands were leveraged to generate the tool molecule ‘compound 36’ which demonstrated a single-digit micromolar DC50 and was found to protect against necroptosis which the parent ligand is not capable of. These effects could also be rescued in competition experiments by using the parent MLKL ligand or lenalidomide.



Although these first-generation MLKL PROTACs display modest potency when compared to the affinity of the parent ligand they provide extremely valuable tool molecules for investigating MLKL biology and it's importance in the necroptopic pathway.

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