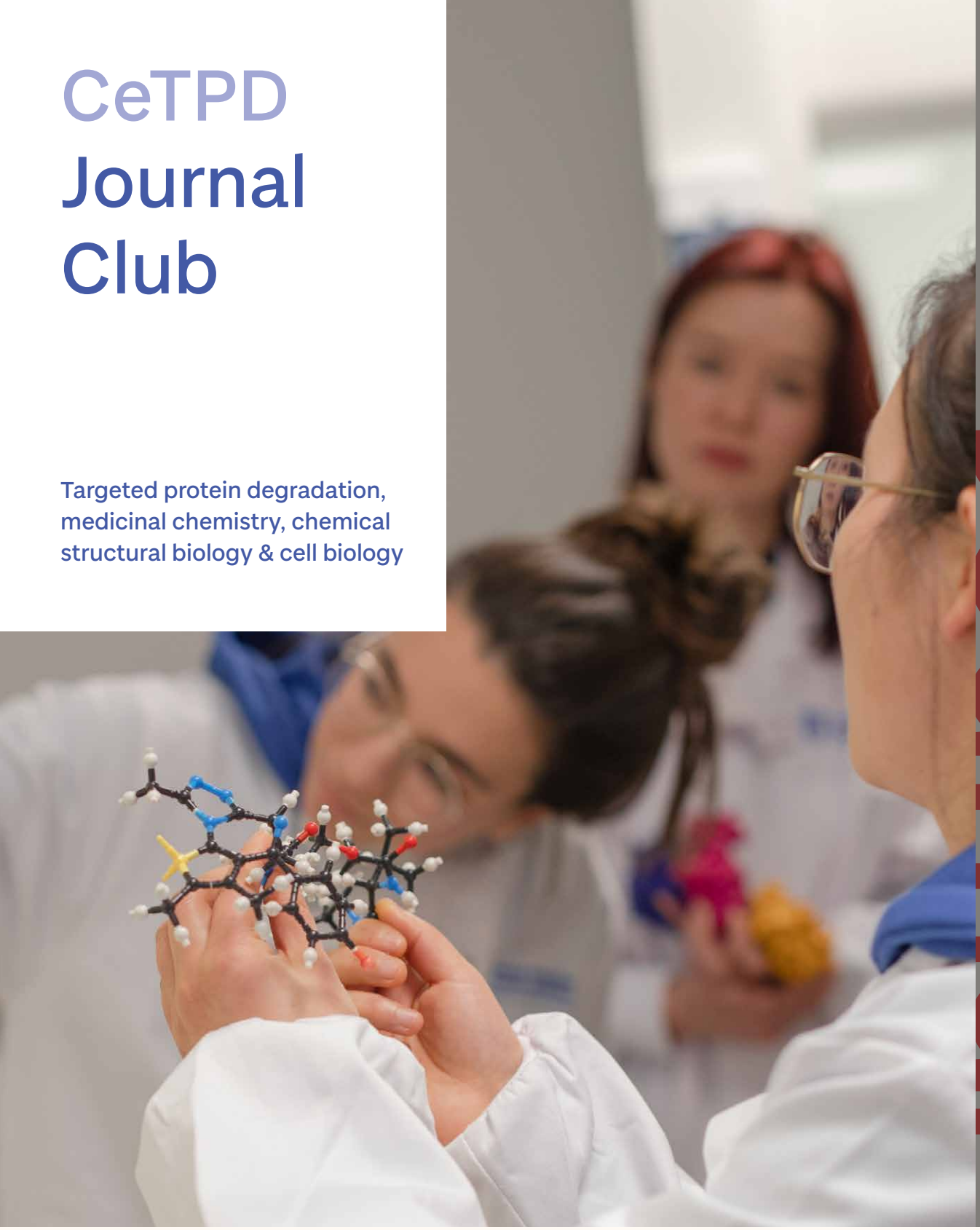
CeTPD Journal Club

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



Journal Club

January 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): Kevin Haubrich, Sohini Chakraborti, and Yuting Cao

"Most scientists share the feeling not to read enough. The journal club is a reminder that reading widely and beyond our own narrow areas of research is an essential part of science and encourages us to dedicate time to it."

Kevin obtained his BSc and MSc in Chemistry from the Heidelberg University and pursued a Ph.D. at EMBL, where he explored the interplay of RNA binding and catalytic activity in the E3 ligase TRIM25 using NMR, SAXS and other biophysical techniques. He joined the Ciulli group in November 2020 where he studies E3 ligases but with a new focus on the development of chemical probes.

"JC is a great resource to keep up with the exponentially growing literature in the TPD space."

Sohini completed her undergraduate degree in pharmacy at Jadavpur University and M.S. (Pharm) in Pharmacoinformatics at NIPER-Hajipur, India. She joined the ACBI team at CeTPD as a Computational Drug Design and Molecular Modelling scientist in November 2021 after completing her Ph.D. in Computational Biophysics from the Indian Institute of Science, Bengaluru.

"Journal Club provides us with an opportunity which enables us to follow the TPD field efficiently and quickly"

Yuting is a second year PhD student in AC acdemic group. She joined the goup in September 2021 through China Schorlarship Council Programme. Previous Dundee she was a master student in Nankai University, China. She is a chemist also studying biology!

New Editors-in-Chief for the CeTPD Journal Club

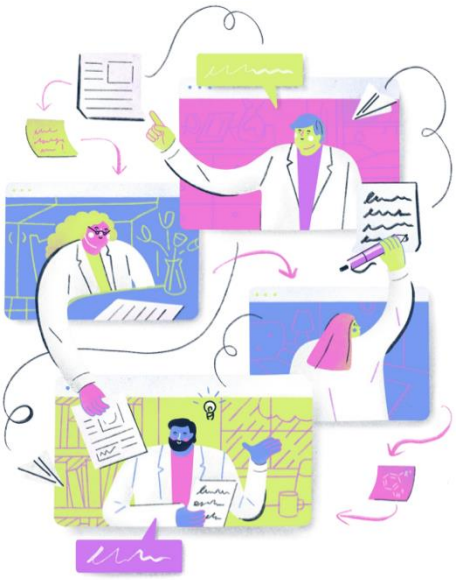
Contributor: Charlotte

The CeTPD Journal Club (formerly known as the Ciulli Lab Journal Club) was established in April 2020 by Siying Zhong, formerly a postdoctoral researcher in Alessio's lab, and now a scientist at Vernalis, Cambridge, UK.

With several lockdowns happening in the UK during 2020 and 2021, in-person social interactions were extremely limited. With the world "standing still", Siying had the opportunity to reflect on activities which would bring the lab together through Zoom and Teams, help to train our lab's scientists during the lockdown, and create an online resource to serve the community online.

COVID-19 caused devastation and damage. Despite this, the pandemic gave rise to a boom in online conferences, seminars, webinars and virtual social events across the scientific community. Amidst this, the Journal Club kept the Ciulli lab busy reading, writing, creating, collaborating, communicating, and delivering to our readers.

After Siying left Dundee to take up her new position at Vernalis, as a brand new first-year PhD student in Alessio's lab I was excited to volunteer, to contribute to the project and to drive development in the Journal Club. Now a third-year PhD student, it is time to pass the baton over to Yuting Cao, a second-year PhD student in Alessio's Ciulli's team, and Andreas Holmqvist, a first-year PhD student in Will Farnaby's team, both in the CeTPD.



The key processes for producing the monthly Journal Club, as illustrated for our April 2021 issue by Jagoda Sadowska.

It's been a huge pleasure to work with everyone over the past 2 years. The level of work being produced by the editors and the entire team with special features and other paper highlights each month is extremely high and made my role so much easier and enjoyable. It's been amazing to see how conscientious, attentive to detail and thorough the entire team is, to be able to meet and interact with everyone in the lab, and to share this project with our readers and colleagues in the field.

I'm certain that Yuting and Andreas will do a fantastic job continuing to coordinate the publishing of informative and inspiring issues as well as continuing to grow the JC as a platform for reaching out to the rapidly-growing TPD field. With the opening of the new Centre for Targeted Protein Degradation here at the University of Dundee this January 2023, our new and future home for TPD research, we are all excited to see the field accelerate in this wave of science and innovation.

Finally – Yuting and Andreas will also be posting the Journal Club on Twitter, so make sure to give them a follow! *@yutingcao1018* *and* *@Holmqvist89*

The 2nd Annual CeTPD Christmas Retreat

Photo credits: Kevin Haubrich. **Contributor: Andreas Holmqvist**

The group held their 2nd annual CeTPD Christmas Retreat on 25th of November at the Piperdam Leisure Resort, which gave us all a chance to look back and reflect over how far we have come, our proudest moments from previous year, and what we can be excited about for the upcoming year. For this year, we had so much to celebrate, with several new awards, 44 new lab members, 14 publications and a new academic group that joined the centre. Last year we were celebrating that we were about to open and move into the new Centre for Target Protein Degradation, which unfortunately had to be delayed. However, this year we could celebrate with good conscience that the centre was finished and we could start moving over in the beginning of 2023.

Over the course of the evening, we had Tom Webb as the MC who did a great job putting a big smile on all the attendees together with Alessio who gave a good speech and behind the scenes talk about the developing process of the new centre and highlighting all the key people who made this possible. We also had several presentations: Charlotte gave an overview of the Journal Club and highlighted some of the amazing feedback we received from it and introduced the two new Journal Club coordinators Andreas and Yuting. Valentina and Selma gave an CeTPD overview of whats been done in the CeTPD outreach. Conner gave the Academic Annual Review of the Ciulli group, highlighting some of the many publications done by the group and introducing some of the new members. William talked about his time as the collaboration leader in the Boehringer Ingelheim collaboration team and his new position as Principal Investigator for the Farnaby Group. He then introduced the new collaboration team leader Dr Kirsten McAuley who gave an overview of the progress made by the group. Ollie gave an update in the progess done by the collaboration between the Cuilli group and Eisai which was followed by David who finished off with updating us on the progess made by the Almirall collaboration team. We then ended the night with a 3-course dinner, quiz hosted by Angus and several hours of dancing.

CeTPD is showcased at Universities Scotland Parliamentary Event

Contributor: Alessio

I had a unique opportunity to visit the Scottish Parliament on January 30th, 2023, as part of a Universities Scotland reception showcasing the economic impact of universities.
A drinks reception was hosted by Universities Scotland to highlight universities' contribution to economic transformation as framed through the Scottish Government's National Strategy for Economic Transformation (NSET). The event took place in the evening of January 30th, 2023 in the Garden Lobby of the Scottish Parliament. The event will kick-start a bigger project, on the theme of economic transformation, planned to run throughout 2023.

The following speeches took place:

1) Willie Rennie MSP provided a welcome to the event, as event sponsor
2) Professor Dame Sally Mapstone, as the Convener of Universities Scotland, outlined the purpose of the event
3) Shirley-Anne Somerville delivered the keynote speech

The event aimed to tell a set of 19 (one per HEI) stories of economic transformation in our nation, which touch on one or more of the five over-arching "programmes of action" or themes in the NSET:

- Entrepreneurial people and culture
- New market opportunities
- Productive businesses and regions
- Skilled workforce
- A fairer and more equal society (which we think offers scope for stories of access)

The case study submitted to represent the University of Dundee was our Centre for Targeted Protein Degradation (CeTPD) – as a new translational research centre that will support the development of new treatments based on fundamental research. CeTPD's research pioneers a new drug type that has allowed previously believed to be untreatable conditions to be medicated, and has stimulated over $4 billion of investment in the global targeted protein degradation sector.

Representing CeTPD in Japan

Contributor: Alessio

In early December, I was very fortunate to take part on an intense and highly productive trip to Tokyo, Japan. I was invited to participate in two international symposia, organized by Yasushi Saeki, from the Tokyo Metropolitan Institute of Medical Science (TMIMS), together with co-organizers Keiji Tanaka (also from TMIMS), Shigeo Murata and Mikihiko Naito (from The University of Tokyo), and Kazuhiro Iwai (Kyoto University). The first symposium, titled *"Ubiquitin New Frontier: from Neo-Biology to Targeted Protein Degradation"*, was originally scheduled for October 2020 but was postponed twice due to COVID-19, and finally realized on 3-4 December 2022 *at Ito Hall, the University of Tokyo*. The second satellite meeting was the 23rd TMIMS International Symposium on *"New Frontiers in Ubiquitin Proteasome System"* which took place on 6 December at TMIMS.

The purpose of these symposia were to bring together an international community of researchers working on chemical biology and biochemistry of the ubiquitin-proteasome system, and included lot of work on TPD. There, I had the privilege to be part of a delegation of invited international speakers and all respected scientists and leaders in the UPS and wider fields, including Claudio Joazeiro (ZMBH, Heidelberg University, Germany), Dan Finley (Harvard Medical School, USA), David Komander (WEHI, Australia), Eri Sakata (Göttingen University, Germany), Hsueh-Chi Sherry Yen (Institute of Molecular Biology, Academia Sinica, Taiwan), Ivan Dikic (Goethe University Frankfurt, Germany), and Kylie Walters (National Cancer Institute, USA). Both symposia had >150 participants, including students and researchers from both academia and industry, and proved to be very enjoyable and fruitful meetings, generating lot of new ideas and opportunities for collaborations.

Finally, I was delighted to have an opportunity to give a keynote lecture at 26th Japanese Foundation for Cancer Research International Symposium on Cancer Chemotherapy (JFCR-ISCC) that was held on 7 December as a hybrid meeting, with a theme on *"New Antitumor Agents under Development in the US, Europe, and Japan"*. The purpose of the Symposium was to discuss recent progress in the development of new antitumor agents and future perspectives in cancer care.

One of the highlights of my trip was visiting our collaborators at Eisai and meeting again with Tasuku, of "patent-magic" fame. It felt good to reunite again in Japan, not too long after Tasuku left Dundee, and to meet with his fantastic colleagues and at their Tsukuba laboratory.

Being in Japan, a major highlight of the whole visit was the amazing food and the hospitality, courtesy and kindness of the people. Science offers such a unique opportunity to visit beautiful parts of the world, foster friendship, making connections and starting collaborations, all of which greatly enriches our experiences and lives as scientists and individuals. I love Japan. This was my fourth time in Japan, and certainly one of the most intense and special. This trip reminded me of the privileged and fortunate role that I have as scientist, group leader, and now as Director to represent CeTPD around the world, and to share the fantastic science that goes on in my group and in CeTPD. I am fully aware of how important it is to take full advantage of opportunities such as this one, as experiences like this not just enrich one's own life but also improve the lives of others. It is this sense of belonging, responsibility and purpose that makes what we do ever so more special and important.

Targeted Protein Degradation

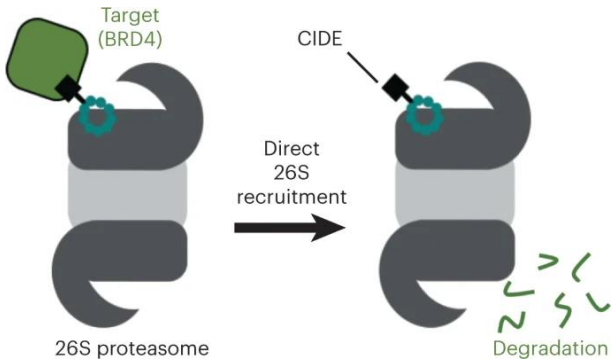
Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Kevin

Targeted degradation via direct 26S proteasome recruitment

Charlene Bashore[§], …, Ingrid E. Wertz*, Claudio Ciferri* & Erin C. Dueber*
Nat. Chem. Biol. **2023**, *19, 55*

Traditionally, most TPD approaches depend on recruiting E3 ligases. In the time frame covered by this JC edition several alternative approaches have been published that recruited other components of the UPS for TPD. The first of these is based on the direct recruitment of the 26S proteasome, eliminating the need for ubiquitination altogether. So far this has been prevented by the lack of good handles for the proteasome. In this paper, Bashore et al. present and characterize a peptide macrocycle targeting the 26S subunit PSMD2 obtained by phage display. They show target engagement, cell permeability and solved a 2.5 Å cryo EM structure of the ligand/PSMD2 complex. To demonstrate the use of this ligand in TPD, they synthesized several chimeric molecules of the macrocycle fused to a BRD4-targeting ligand via different exit vectors. One of these compounds mediated ternary complex formation in cell lysates, is readily cell permeable and mediates BRD4 degradation with sub-micromolar DC50.



Direct proteasome recruitment offers intriguing possibilities for TPD as PSMD2 and other 26S subunits are essential for the cell and universally expressed, reducing the risk of resistance formation. As this approach does not require ubiquitination also proteins without lysins accessible for ubiquitination can be degraded.

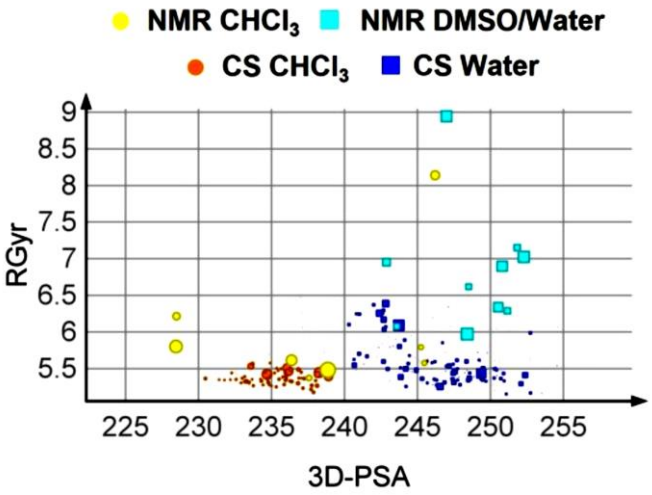
Computational Chemistry | Modelling/Simulation | Structural Biology/Biophysics | Chemistry

Contributor: Sohini

Conformational Sampling Deciphers the Chameleonic Properties of a VHL-Based Degrader

Giuseppe Ermondi[§],…, Giulia Caron*
Pharmaceutics **2023**, *15, 272*

The oral bioavailability of PROTACs and other therapeutics that are in the beyond-Rule-of-5 (bR5) chemical space are often found to be poor. Evidence suggests ability of these molecules to act as chameleons, i.e., exhibit dynamic physicochemical behaviour in polar vs. non-polar environments could aid in their cellular permeability. Experimental techniques to identify chameleons involve NMR spectroscopy and determining chromatographic indexes such as ChamelogD. Computational methods to predict the chameleonicity of bR5 molecules through conformational sampling in different implicit solvent environments are much faster. However, there are lack of benchmark studies to validate the computational results against experimental data. In this report, the authors used a model system PROTAC-1 to compare the molecular properties (3D PSA, Rgyr, IMHBs) that describe its chameleonic behaviour obtained from NMR- and computationally-generated conformers in water and chloroform.



The authors found that to derive an understanding of the 3D PSA variation in the polar vs non-polar solvents, default settings of Schrodinger suite's conformational search algorithm proved to be sufficient. However, a more accurate reproduction of NMR solution conformers could only be obtained with careful modulation of the algorithm that

required tweaking the number of conformers and energetic considerations. Conducting a similar study with an expanded dataset would be helpful to derive thorough insights and the learning could then hopefully be implemented in prospective drug design.

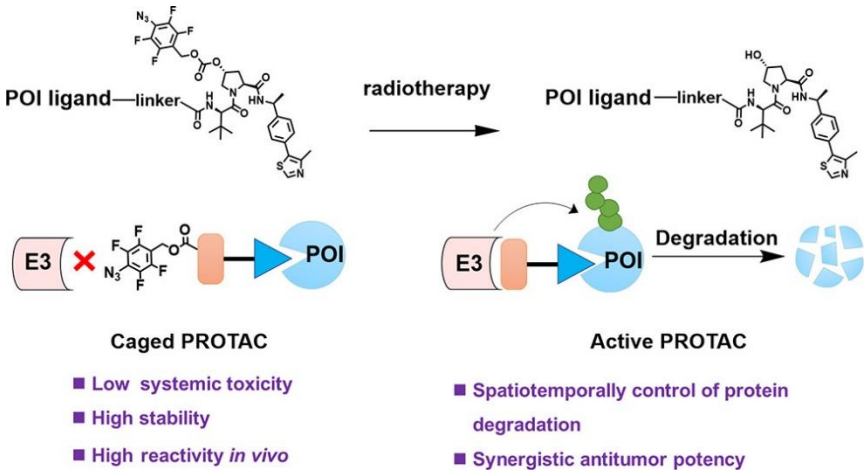
Cell Biology | Chemistry

Contributor: Yuting

Radiotherapy-Triggered Proteolysis Targeting Chimera Prodrug Activation in Tumors

Changrong Yang …, Jinghong Li*
J. Am. Chem. Soc. **2023**, *145*, 385

The undesired off-tissue protein degradation of PROTACs may cause potential systemic toxicity therefore limit their clinical application. Recently, several prodrug activation strategies have been reported which use clinically relevant doses of X-ray radiation to control the release of the chemically-modified cages on chemotherapeutic prodrugs. One example is that the phenyl azide cage (4-azido-2,3,5,6-tetrafluorophenyl)methanol can be reduced to 4-(hydroxymethyl)-2,3,5,6- tetrafluoro aniline under the effect of radicals generated by X-ray radiation.



In this paper, the authors using ARV-771, the well-studied VHL-based BRD protein degrader as reference compound, incorporated the phenyl azide-cage, and developed the first RT-PROTAC compound (RT-PRO). The cage on RT-PRO could also be reduced to aniline, which further undergoes a 1,6-elimination and decarboxylation reaction and releases RT-PRO. RT-PRO degrades BRD4 in a radiation-, proteasome, VHL-dependent manner. Further in vivo experiment shows that the protein degradation activity of RT-PRO can be precisely controlled by radiotherapy, and the activated RT-PRO together with radiotherapy effectively inhibits tumour growth in tumour-bearing mice without significant body weight loss.

Overall, this work provides new a strategy of precisely activating prodrugs into PROTACs thereof reducing the systemic toxicity. Moreover, the strategy shows great potential in various types of cancer which could be beneficial to clinical outputs.

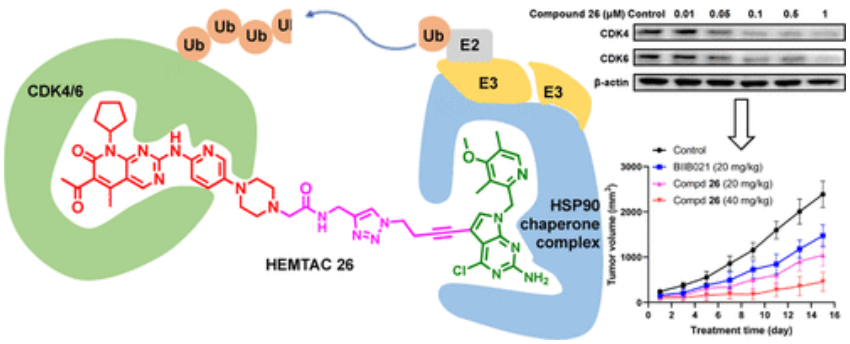
Cell Biology | Chemistry

Contributor: Kevin

Targeted Protein Degradation Induced by HEMTACs Based on HSP90

Zhenzhen Li[§], …, Minyong Li*
J. Med. Chem. **2023**, *6*, 733-751

This paper presents another novel strategy towards TPD that rather than recruiting a canonical component of the ubiquitin/proteasome system recruits the chaperone HSP90. HSP90 is best known for assisting the folding of proteins in an ATP dependent manner, thereby mediating the response of the cell towards stress, such as heat exposure, but it also associates with about 30 % of the human E3 ligases and can thereby accelerate the degradation of misfolded proteins. HSP90 is of therapeutical interest as it is strongly overexpressed in many tumors. Starting from the first orally



bioavailable HSP90 inhibitor BIIB02 and the CDK4/6 ligand Palbociclib the authors synthesized chimeric molecules designed to recruit HSP90 to CDK4/6, which they termed HEMTACs. The best HEMTAC showed low nanomolar DC50 and up to 92 % degradation. While their cytotoxicity for healthy cells remained much lower than the parent compound BIIB02, they had a stronger inhibitory effect on xenograft models in zebrafish and mice.

In summary, the paper proves that HSP90 recruitment is a promising method of TPD. The indirect recruitment of multiple E3 ligases and HSP90 being essential for many tumor cells likely lowers the risk of resistance formation against HEMTAC treatment. However, this indirect mode of action may also make the outcome of new HEMTACs more difficult to predict and it will be interesting to see how HEMTACs against other targets will perform.

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

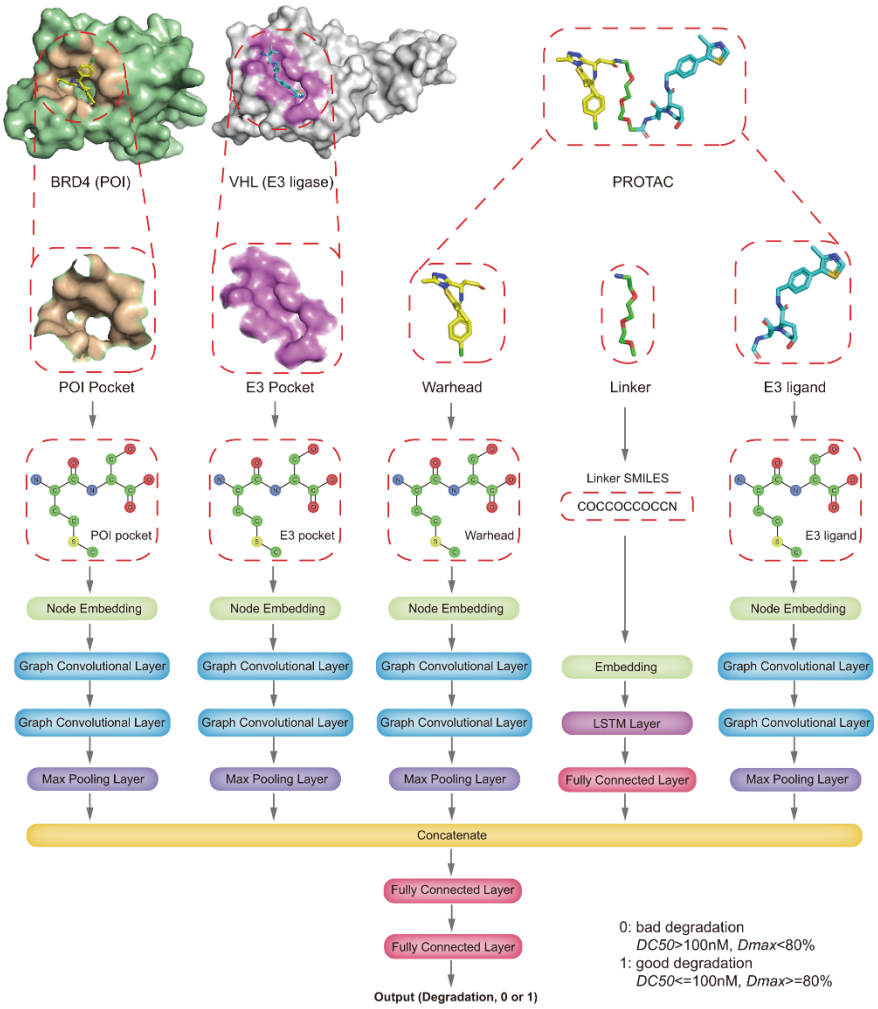
Contributor: Sohini

DeepPROTACs is a deep learning-based targeted degradation predictor for PROTACs

Fenglei Li[§], …, Xiaobao Yang*, Shenghua Gao*, Fang Bai*

Nat. Comm. **2022,** *13,* 7133

Predicting the degradation efficiency of PROTACs using AI/ML methods is not trivial due to the limited availability of SAR data. In this study, the authors proposed a deep neural network model, DeepPROTACs. This model can predict the degradation efficiency of proposed PROTAC molecules using structural data of the target protein and E3 ligase. The model has been trained using 2832 labelled data, where a PROTAC with DC_{50} of 100 nM and D_{max} of 80% was labelled as active (or 'good degrader') else inactive or 'bad degrader'. The input structures of the proteins and the PROTACs are represented as graphs and fed separately into Graph Convolutional Networks for feature extraction. In the test set, the DeepPROTACs model is ~78% accurate, while in experimental dataset and novel targets the accuracy rate has been found to range from 65% to 80%. Ternary complex modelling of a subset of test PROTACs with the target protein and E3 ligase was performed to get structural insights into the success and failure of this predictive DL model.



Although the DeepPROTAC model performed well, future improvements to incorporate the chirality information of the PROTAC into the model and expansion of the training dataset would be required to generate hopefully more accurate predictions and thus, to provide better guidance for PROTAC designing.

Cell Biology | Chemistry | Structural Biology/Biophysics

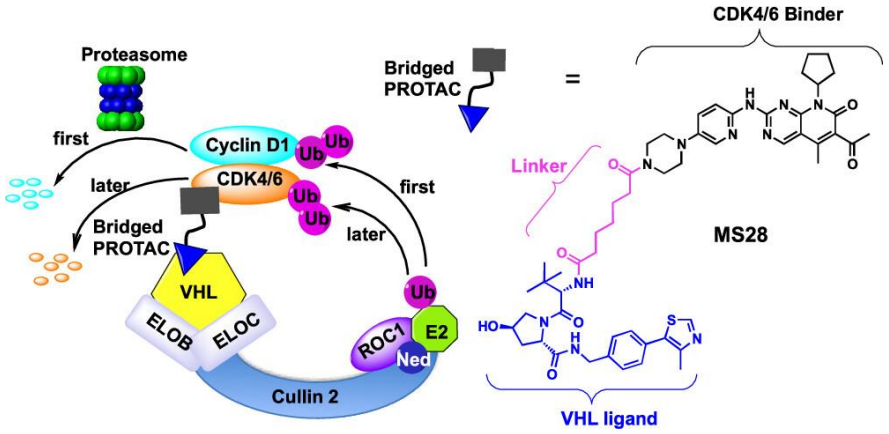
Contributor: Yuting

Bridged Proteolysis Targeting Chimera (PROTAC) Enables Degradation of Undruggable Targets

Yan Xiong §, Yue Zhong §, Hyerin Yim § …, Jian Jin*

J. Am. Chem. Soc. **2022**, *144*, 22622

Cyclin D1 is an important cell cycle regulator that activates cyclin-dependent kinase 4/6 (CDK4/ 6), which is also frequently amplified and overexpressed in many types of cancer and is a promising therapeutic target. However, cyclin D1 is undruggable, because there are no reported small molecules binding to cyclin D1 and therefore cannot be targeted by the PROTAC strategy. Several protein complex degraders have been reported in the field; however, degradation of these partner proteins is due to the initial degradation of the protein that directly binds the PROTAC and subsequently causing the destabilization of the protein complex.



In this paper, the authors using CDK4/6 inhibitor Palbociclib, linking the solvent-exposed moiety piperazine to E3 ligand VHL-1, developed different linker length of the bridged PROTACs. Among those compounds, MS28 could preferentially degrade the target protein CyclinD1 over the bridged protein CDK4/6. The degradation induced by MS28 is dependent on VHL, CDK6, and UPS. MS28 also inhibited the proliferation and tumorigenesis in NSCLC cells much more effectively than CDK4/6 inhibitors and degraders.

In summary, the bridged PROTAC strategy has the potential to target currently undruggable proteins which lack small-molecule binders but could form a protein complex with bridge proteins with well-characterized binders.

Cell Biology | Chemistry

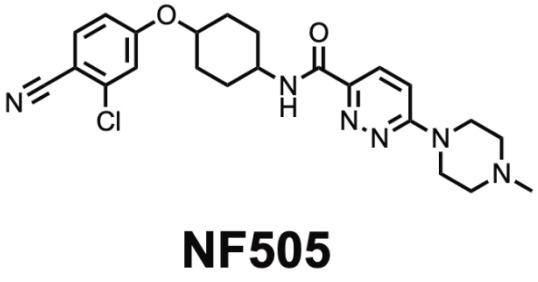
Contributor: Kevin

Targeted Protein Degradation through E2 Recruitment

Nafsika Forte§, …, Daniel K. Nomura*

bioRxiv **2023** , *DOI: 10.1101/2022.12.19.520812*

This preprint from Forte et al. is the third work describing recruitment of non-E3 components of the UPS for TPD. Here the UBE2D family of E2 conjugating enzymes is recruited. This is achieved by a fragment specifically modifying a conserved allosteric cysteine C111 identified by a gel-based ABPP screen. When the authors synthesized PROTACs using this fragment as a warhead and the BRD4 ligand JQ1 as the POI binder, they observe selective degradation of only the short isoform of BRD4. A similar PROTAC featuring the androgen receptor binder used by the Arvinas PROTAC ARV-110 induced significant degradation of the receptor. Interestingly, for both targets pretreatment with the NEDDylation inhibitor MLN4924 attenuated degradation, suggesting that although the E2 is directly recruited, this process is still Cullin dependent.



The unusual selectivity in the case of BRD4-degrading PROTACs and the likely independence of specific substrate receptors, although not CRLs, make E2-recruitment a promising addition to the TPD toolkit. It may prove advantageous that rather than a single member these compounds target all four members of the UBE2D family, decreasing the likelihood of resistance emergence.

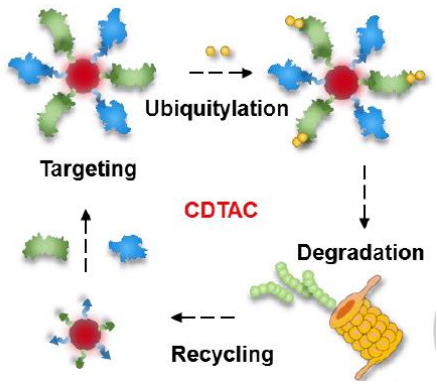
Cell Biology | Chemistry

Contributor: Yuting

Targeted Degradation of PD-L1 and Activation of the STING Pathway by Carbon-Dot-Based PROTACs for Cancer Immunotherapy

Wen Su[§], Mixiao Tan[§], …, Guangjun Nie*, Hai Wang*
Angew. Chem. Int. **Ed.** 2023, DOI: 10.1002/anie.202218128

Membrane proteins are not considered as ideal targets for PROTACs because the distribution of ubiquitin proteasome system is in the cytoplasm. In this paper, the authors reported a carbon-dots (CDs)-based PROTAC (CDTACs) which uses the ubiquitin proteasome system to efficiently degrade membrane protein PD-L1 protein in tumour cells. The mechanism should be that "CDTACs recognize and bind PD-L1 distributed in tumour cells and transport to lysosomes through endocytosis. Ubiquitinated PD-L1 is degraded by the proteasome, and CDTACs are recycled to bind another copy of PD-L1, showing a catalytic-like reaction.''



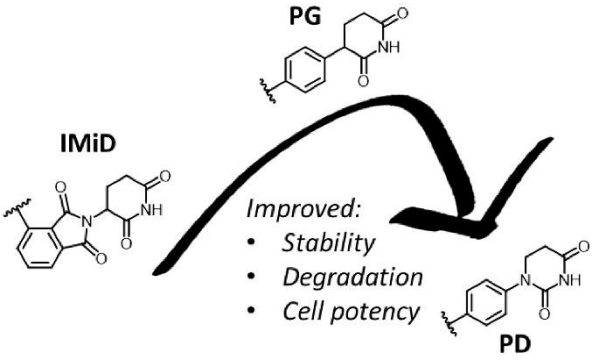
CDTACs could effectively and selectively degrade PD-L1 over SPT1, IKZF1 and CK1α in A549 tumour cells. CDs or CDTACs activated the STING pathway in cells, thereby promoting DC maturation and T cell priming CDTACs induced immune responses. It is also worth noting that FMD treatment augmented the degradation efficacy of PD-L1. Consequently, CDTACs with FMD treatment efficiently inhibits the growths of CT26 and B16-F10 tumours without obvious systemic toxicity. CDTACs have multiple advantages, such as membrane protein degradation, tumour accumulation, immune system activation, and fluorescent in vivo tracking, which may provide a new strategy for advancing PROTAC technology in cancer therapy.

Chemistry | Structural Biology/Biophysics | Cell Biology

Contributor: Sohini

Phenyl Dihydrouracil: An Alternative Cereblon Binder for PROTAC Design

Jamie A. Jarusiewicz[§], Satoshi Yoshimura[§],…, Jun J. Yang*, Zoran Rankovic*
ACS Med. Chem. Lett. **2023**, DOI: 10.1021/acsmedchemlett.2c00436



IMiD binders are frequently used in cereblon (CRBN)-based PROTACs. However, IMiDs are inherently unstable and readily undergoes hydrolysis. The authors of this article had previously reported phenyl glutarimide (PG)-based PROTACs that retain affinity and have improved clinical stability compared to IMiDs-based PROTACs but they still hydrolyze and racemize. In the current study, the authors replaced the glutarimide C-3 carbon in the PG moiety with a nitrogen atom resulting in phenyl dihydrouracil (PD) PROTACs that are not susceptible to racemization and have improved chemical stability. PG-, PD-, and IMiD-based LCK targeting PROTACs were synthesized for comparing their physicochemical and pharmacological properties. Despite lower CRBN affinity of PD-PROTAC 2, it still displayed a higher extent of ternary complex stabilization and similar LCK degradation profile as PG PROTACs. Further, similar modification in the linker chemistry had significantly improved the degradation profile of the PD-PROTACs compared to PG-PROTACs, resulting in PD-PROTAC 5 to be the most potent among all reported in this paper.

The data collectively suggests PDs could be an alternative to the the IMiDs in PROTAC design to tackle stability and racemization issues. However, like other PROTACs , these are also found to have lower cell permeability. Thus, future designs focussing on achieving better permeability with the PD-PROTACs would be helpful.

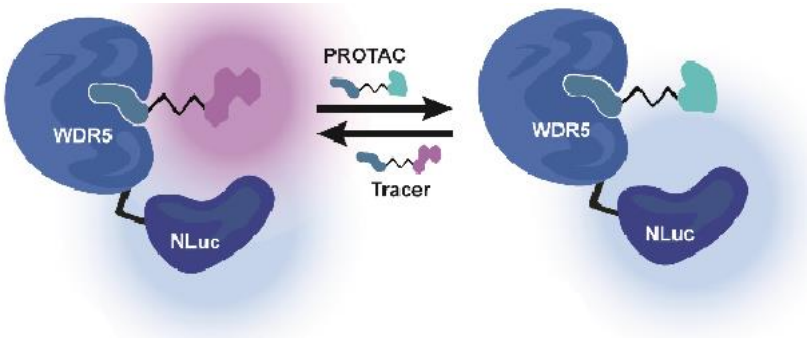
Cell Biology | Structural Biology/Biophysics

Contributor: Kevin

Tracking the PROTAC degradation pathway in living cells highlights the importance of ternary complex measurement for PROTAC optimization

Martin P. Schwalm[§], …, Stefan Knapp*
bioRxiv **2023**, DOI: *10.1101/2023.01.11.523589*

Targeted protein degradation by PROTACs is a multistage process, involving among others cell penetration, binary binding to the E3 ligase and POI, ternary complex formation, target ubiquitination and proteasomal degradation. Yet, when evaluating PROTACs often only degradation efficiency as the endpoint of this process is measured, leaving the cause of differences in degradation efficiency unclear. This preprint aims to address this by individually measuring binary and ternary affinity, kinetics of complex formation and degradation for a series of VHL-recruiting PROTACs degrading WDR5. Binary affinities of the PROTACs towards WDR5 were then measured by NanoBRET with NLuc tagged WDR5 and a competing tracer, or CETSAs using HiBIT tagged WDR5. A large difference in binary engagement between intact cells and cell lysates was found, indicating that cell permeability is limiting these PROTACs.



Ternary affinities were measured similarly using BRET between HaloTagged WDR5 and NLuc-tagged VHL. This allowed for time-resolved measurements, yielding not only affinities, but also on- and off-rates of complex formation. Lastly, D_{max} and DC_{50}s were measured by monitoring POI concentrations using HiBIT-WDR5. A strong correlation between ternary affinity and degradation efficiency was found. The authors proceeded to solve crystal structures of several ternary complexes, demonstrating a correlation between contact area of PPIs and ternary affinities.

This study is a valuable step towards identifying the bottleneck of TPD by PROTACs and focusing efforts where they make the biggest impact. For future work, it might be very interesting to further include ubiquitination rates and try to further deconvolute cell permeability from binding kinetics.

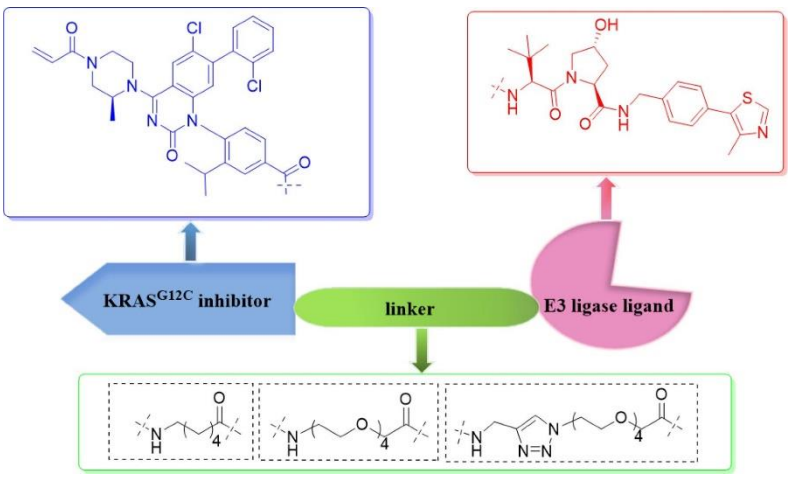
Cell Biology | Chemistry | Computational Chemistry | Modelling/Simulation

Contributor: Sohini

Design, synthesis and biological evaluation of KRAS^{G12C}-PROTACs

Xiaoyi Zhang[§], Tong Zhao[§],…, Huibin Zhang *
Bioorg. Med Chem. **2023**, 78, 117153

KRAS mutations account for over 83% of all RAS mutations in human malignancies. In May 2021, FDA approved the first KRAS^{G12C} inhibitor, AMG-510, for the treatment of NSCLC. However, small-molecule inhibitors of KRAS^{G12C} may induce adaptive resistance and reactivation of MAPK signalling. Targeting KRAS with PROTACs can be a complementary strategy to improve anti-tumour effects. In this study, the authors designed and synthesized a series of novel KRAS^{G12C}-PROTACs based on AMG-510. These include CRBN- as well as VHL-based PROTACs. S1, an analogue of AMG-510, that is structurally similar to AMG-510 and equally effective was chosen as the starting point to achieve improved synthetic feasibility that led to introducing linkers to a site favourable for ligase recruitment.



Molecular docking simulations of the modified handles in $KRAS^{G12C}$ predicted a similar binding pose as that of AMG-510 in $KRAS^{G12C}$. Cellular assays indicate these PROTACs induce degradation of the $KRAS^{G12C}$ via the ubiquitin-proteasome pathway. SAR shows PROTACs with flexible linkers are more effective degraders than their rigid linker counterparts. Also, VHL-based PROTACs gave higher degradation than CRBN-based PROTACs with same linkers.

The SAR study in this work highlights the advantage of VHL ligands in developing $KRAS^{G12C}$ PROTACs. The ADME studies on these VHL-PROTACs would be helpful to understand how far the well known challenges related to oral bioavailability of VHL-PROTACs could be overcome.

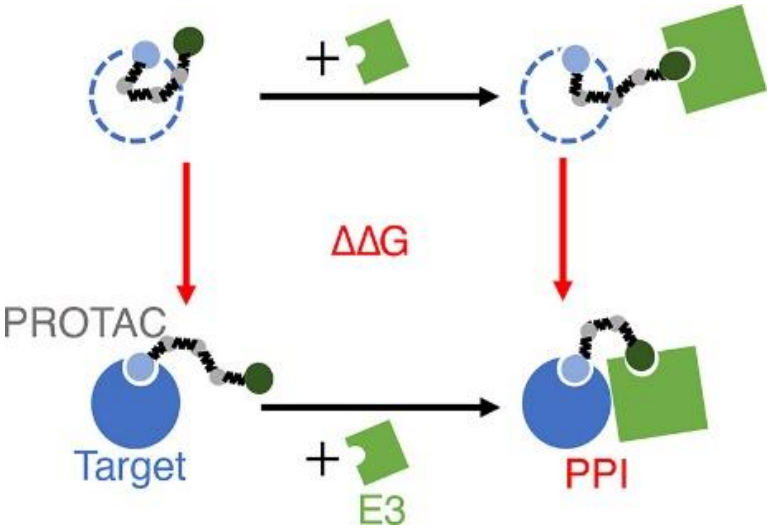
Computational Chemistry | **Modelling/Simulation**

Contributor: Sohini

Exploring PROTAC Cooperativity with Coarse-Grained Alchemical Methods

Huanghao Mai*

J. Phys. Chem. B **2023**, 127, 446



Systematic experimental characterization of PROTAC-mediated binding cooperativity remain scarce. Available computational modelling methods suffer from biasness of scoring functions towards natural PPIs or would require sufficiently large time scale of simulations to capture non-native PPIs as seen in PROTAC-mediated PPIs between the target protein and ligase. In this work, the authors report a computational framework that explores a coarse-grained (CG) approach to model interactions in the ternary complex, which enables converged thermodynamic estimations using alchemical free energy calculation methods despite an unconventional scale of perturbations in a model system studied in this work (BTK-PROTAC-CRBN). Even with the minimal parameterization, the study is able to capture the thermodynamic principles of cooperativity that favour intermediate PROTAC linker length, striking the right balance of compensation between penalty and reward terms. These simulations are less time-consuming, being highly dependent on the size of the system and the level of details included in the parameterization. However, quantitative modelling of cooperativity remains difficult and minimal parametrization is found to be insufficient for systems that lead to cooperativity due to involvement of specific interactions (such as JQ1-based PROTACs in $BRD4^{BD2}$-VHL).

Albeit CG enables efficient computation, parameterization for force field development will remain a major hurdle to overcome for achieving accurate predictions of PROTAC cooperativity.

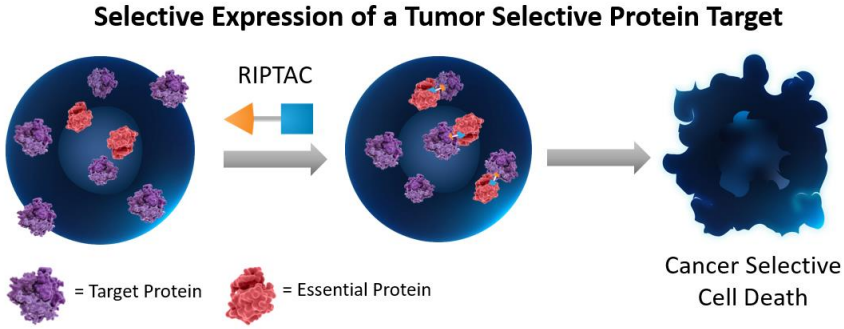
Cell Biology | **Chemistry**

Contributor: Yuting

Regulated Induced Proximity Targeting Chimeras (RIPTACs): a Novel Heterobifunctional Small Molecule Therapeutic Strategy for Killing Cancer Cells Selectively

Kanak Raina[§*], …, Craig M. Crews*

bioRxiv **2013**, DOI: 10.1101/2023.01.01.522436



Some subsets of high-affinity and selective ligands have the potential to accumulate in cells which express their protein target. Therefore, the authors hypothesis that relying on the expression of a target protein they could block the function of a pan essential effector protein in a target-expressing, cell-selective manner. They engineered a HEK293-derived cell line (293_HFL) with lentiviral overexpression of Flag-tagged HaloTag7-FKBPF36V (hereafter HaloTag-FKBP) with a C-terminal P2A-EGFP sequence as target protein. Then conjugating to either covalent or non-covalent ligands such as JQ1 (BET inhibitor), BI2536 (PLK1 inhibitor), TMX3013 (multi-CDK inhibitor), and dinaciclib (multi-CDK inhibitor).

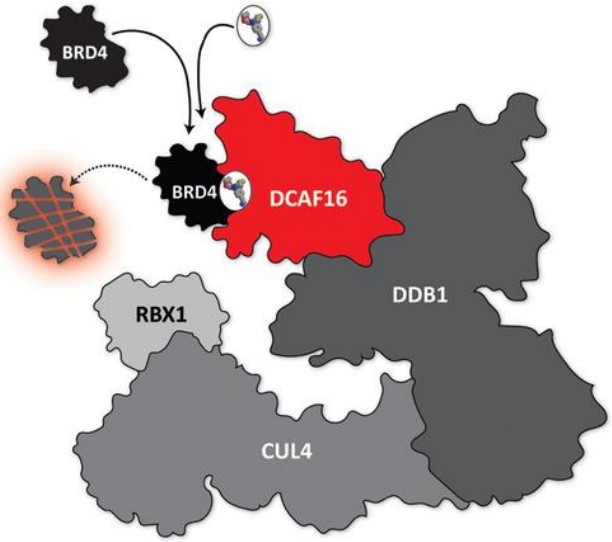
The RIPTAC activity is related to the abundance of the target protein and the pan-essential effector protein and localization between these two proteins. In viability assay, an interesting feature is that the enhancement of their selectivity for target-expressing cells under washout conditions relative to continuous treatment. Most importantly, RIPTAC modality is that it is agnostic to the identity of the oncogenic driver of the disease. In all, RIPTACs is a new heterobifunctional modality which selectively kills cells by differentially accumulating an antiproliferative agent to induce Protein-Protein interaction (PPI) , this approach opens new target space by leveraging differentially expressed intracellular proteins and has the advantage of not requiring the target to be a driver of disease.

Cell Biology **Chemistry**

Contributor: Kevin

CRISPR Screen Reveals BRD2/4 Molecular Glue-like Degrader via Recruitment of DCAF16

Andrea G. Shergalis[§], …, Justin M. Reitsma*
ACS Chem.Biol. **2023**, DOI: *10.1021/acschembio.2c00747*



The discovery of a novel molecular glue is always exciting due to the lack of rational design strategies for glues. This paper describes a monovalent degrader of BRD2/4 derived from JQ1. A CRISPR/Cas9 screen identified this degradation to be dependent on DCAF16, a substrate receptor of CRL4. Pulldown experiments showed increased association of DCAF16 with BRD4 in the presence of the compound. As expected for a molecular glue it does not show a hook effect.

Unfortunately, unlike earlier molecular glues like thalidomide or indisulam this glue is expected to have binary affinity for the BRDs, but not the E3 ligase, meaning it will not be a suitable starting point for PROTAC design. Nevertheless, as so few gluable E3 ligase/neosubstrate pairs are known further structural and mechanistic characterisation of this system is warranted.

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