CeTPD

Journal Club

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

Journal Club

August 2022

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): Luke Simpson, Martina Pierri, Petr Zhmurov

"Throughout my PhD, the Ciulli Group monthly Journal Club was a fantastic way to keep up-to-date with relevant TPD-based literature. As an editor of this month's issue, I hope that others find this month's issue a helpful resource."

Luke completed his MSci in Biomedical Science at the University of Aberdeen and his PhD under the supervision of Professor Gopal Sapkota and Dr Ian Ganley in the MRC PPU at the University of Dundee. Here, Luke's doctoral research centred around exploring technologies for targeted protein modification and involved the combined use of nanobody- and PROTAC-based technologies for targeted protein degradation. Luke joined the Ciulli Group in March 2022 as a Cell Biologist as part of the PROTAC Drug Discovery collaboration with Boehringer Ingelheim.

"The Journal Club is a great way to pay attention to how the TPD field is evolving month by month. I am glad to have had the opportunity to contribute to this experience as one of the editors."

Martina completed her master's degree in Chemical and Pharmaceutical Technologies at the University of Salerno carrying out a thesis in the computational chemistry field. She is currently a PhD student with Prof. Stefania Terracciano at the University of Salerno where she is involved in the design and synthesis of new small molecules with promising anticancer activity. Martina joined Ciulli's lab in May 2022 as a visiting PhD student to pursue her research in the PROTAC field.

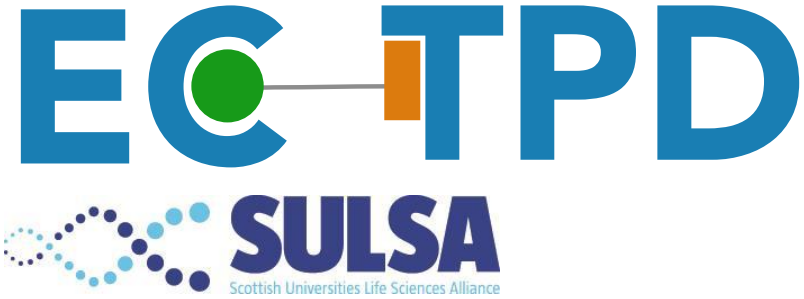
"*It is a Golden Age of medicinal chemistry. Nevertheless, publishing process can't get away from its outdated model making data search and extraction quite sophisticated. I hope this community-based platform can help scientists to navigate in our rapidly growing field of targeted protein degradation."*

Petr completed his PhD in Organic Chemistry under the supervision of Prof Alexey Sukhorukov in the Zelinsky Institute of Organic Chemistry. In 2019 he joined Prof Dmitry Perekalin group in the Nesmeyanov Institute of Organoelement Compounds. Then Petr moved to the Saint Petersburg State University to proceed postdoctoral studies in the group of Prof. Mikhail Krasavin and develop novel druglike PARP inhibitors. Since February 2022, he joined CeTPD as a Medicinal Chemist in the collaboration project with Boehringer Ingelheim.

Feature of the month

Contributor: Valentina

Network for Early Career Researchers in Targeted Protein Degradation



We are very excited to announce that we have been awarded a Scottish Universities Life Sciences Alliance (SULSA) grant to support the launch event of a new network aimed at bringing early career researchers (ECRs) together from across the TPD community. This grant was supported by researchers across universities in Scotland, including Prof Helen Walden and Prof Danny Huang at the University of Glasgow, Dr Rebecca Beveridge at the University of Strathclyde and Dr Will Farnaby and Prof Alessio Ciulli at the University of Dundee.

The idea to create this network was sparked after conversations with ECRs that highlighted the need for events that specifically bring together early-stage researchers to facilitate the sharing of knowledge and generation of new ideas. Moreover, given the staggering pace at which the TPD field is growing, we wanted this to be a network that could help minimise barriers for new ECRs.

The event with be held at the University of Dundee at the newly opened Centre for Targeted Protein Degradation (CeTPD) on **Monday the 14th of November** (with registration opening soon). We will invite all ECRs to bring a poster and submit a short abstract to potentially be selected to give a short talk. As part of the day, we will have a break-out session so people from similar disciplines can get together for in dept conversations. Finally, we are assembling a panel of TPD experts to give their perspective on building a career in TPD and equality and diversity in the field.

Targeted Protein Degradation

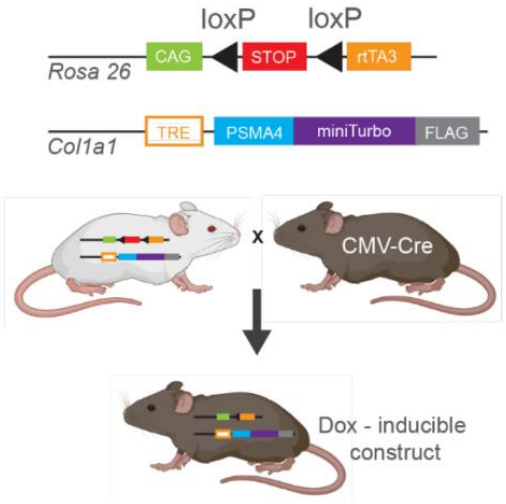
Cell Biology | Modelling/Simulation | Structural Biology/Biophysics

Contributor: Luke

ProteasomeID: quantitative mapping of proteasome interactomes and substrates for in vitro and in vivo studies

Aleksandar Bartolome[§], …, Alessandro Ori*

Biorxiv **2022**, DOI: 10.1101/2022.08.09.503299



Although abnormal proteasomal activity has been linked to various human disease, and that the proteasome has been successfully hijacked to degrade disease-causing proteins, robust methodologies to study proteasomal interactions *in vivo* have been lacking. Examples have been described where compound-mediated E3 recruitment and target protein ubiquitylation has not induced target protein degradation. Therefore, technologies to monitor whether compounds can promote the recruitment of the target protein directly to cellular proteasomes both *in cellulo* and *in vivo* could improve degrader development. In this paper, by tagging the proteasome subunit PSMA4 or PSMC2 with a promiscuous biotin ligase, BirA* or TurboID, Bartolome *et al*. developed ProteasomeID. The authors demonstrate that ProteasomeID does not impair proteasomal activity and can label proteins that come into proximity, within approximately 10 nm, of the proteasome with biotin. Biotinylated proteins can then be isolated from cell or tissue lysates and analysed by mass spectrometry. Bartolome *et al*. demonstrate that ProteasomeID can be employed to quantify endogenous proteasome interactors, both previously reported and novel, as well as those that are degrader-mediated in cultured human cells and mouse models. It will be exciting to see how applicable ProteasomeID will be for additional degraders in different cell lines and animal models to aid in degrader development.

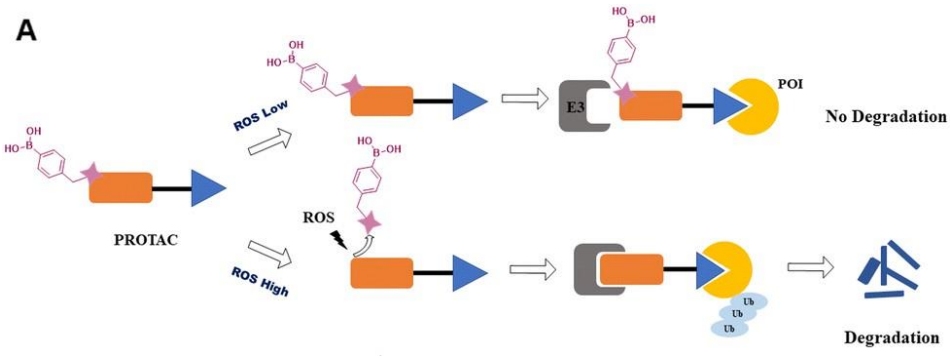
Cell Biology | Chemistry

Contributor: Luke

Reactive oxygen species-responsive Pre-PROTAC for tumor-specific protein degradation

Haixia Liu[§], Chaowei Ren[§], …, Xiaobao Yang*, Biao Jiang*, Hongli Chen*

Chem. Commun. **2022**, DOI: 10.1039/D2CC03367D



To improve therapeutic efficacy and minimise systemic toxicity, efforts have been made to modify degraders so that their pharmacological activity is shielded prior to being activated by a specific trigger in a particular cellular environment. For example, light-regulated PROTACs have been previously described, however light may be a limited stimulus due to inadequate tissue penetration. Reactive oxygen species (ROS) have been reported to be significantly upregulated in the tumour microenvironment in comparison to normal cells, therefore may be a suitable stimulus to activate PROTACs exclusively in cancer cells. Therefore, Liu *et al.* set out to develop ROS-responsive Pre-PROTACs for tumour-specific target protein degradation. To do so, Liu *et al*. introduced arylboronic acid, a ROS-triggered leaving group, onto the CRBN-binding moiety of a JQ1-based BRD degrader, to prevent CRBN binding, and therefore prevent BRD degradation, in the absence of ROS. In the presence of ROS, the arylboronic acid would be removed, facilitating CRBN binding and BRD degradation. In this study, the authors demonstrate that Pre-PROTAC-

mediated BRD3 degradation occurs in high ROS-containing T47D cells, but not in low ROS-containing HEK293T cells, and is inhibited in the presence of the ROS scavenger NAC. It will be most interesting to see how this approach and ROS-responsive Pre-PROTACs perform in additional cell lines with high or low ROS levels, when targeting alternative proteins, and how efficacious and tolerable they are in *in vivo* tumour models.

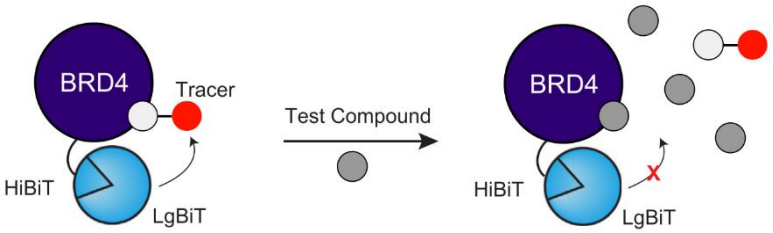
Cell Biology

Contributor: Luke

BRET measurement on CCD camera-based microtiter plate readers

Kelvin F. Cho[§], Noelle Javier[§], Kaylee Choi*

SLAS Discov. **2022**, DOI: 10.1016/j.slasd.2022.08.002



Cellular NanoBRET assays have been developed for analysing protein-protein interactions and for measuring target protein engagement with compounds in live cells. NanoBRET-based assays employ NanoLuc luciferase as a donor for proximity-dependent bioluminescence energy resonance transfer (BRET) to a small-molecule BRET acceptor. With regards to degrader profiling, NanoBRET technologies can be used to quantify target protein or E3 engagement, ternary complex formation and target protein ubiquitylation *in cellulo*. Typically, plate-based NanoBRET assays are measured using a plate reader, for example the EnVision, the PHERAstar or the GloMax. However, these plate readers measure single wells individually, which can take several minutes to measure a complete 96- or 384-well plate, potentially hindering their application when performing kinetic assays, specifically when analysing quicker kinetic processes. To overcome this limitation, Cho *et al*. employ charge-coupled device (CCD) camera-based plate readers to image the entire plate simultaneously. By adapting a NanoBRET BRD4-based PROTAC target engagement assay in HEK293 cells, the authors demonstrate that CCD-based plate readers can be used to perform NanoBRET measurements with high signal-to-background ratios, generating comparable IC_{50} values to those calculated using the EnVision, but taking less time to perform reads. It will be most interesting to test the capabilities of CCD-based readers using various cell lines and with additional NanoBRET applications, such as ternary complex formation and target protein ubiquitylation assays, specifically in a kinetic format.

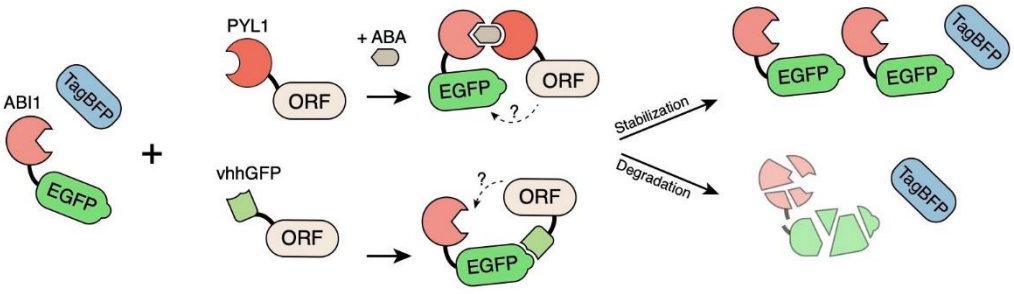
Cell Biology **Modelling/Simulation** **Structural Biology/Biophysics**

Contributor: Luke

Proteome-scale induced proximity screens reveal highly potent protein degraders and stabilizers

Juline Poirson[§], …, Mikko Taipale*

Biorxiv **2022**, DOI: 10.1101/2022.08.15.503206



To date, only a handful of E3 ligases and deubiquitylases (DUBs) have been harnessed to induce target protein degradation or stabilisation, respectively. In this paper, Poirson *et al*. establish a proteome-scale induced proximity platform to identify additional proteins that can potentially be hijacked to promote target protein degradation or stabilisation. The platform involves the co-expression of an EGFP-ABI1-IRES-TagBFP reporter along with an effector protein conjugated to a tag that can bring two proteins together, either constitutively using an anti-GFP nanobody (vhhGFP and GFP), or chemically mediated in

the presence of ABA (PYL1 and ABI1). Following the expression of a pooled human ORFeome library tagged with either vhhGFP or PYL1, EGFP-ABI1 protein levels were quantified and sorted by flow cytometry, normalised to TagBFP, and sequenced. This elegant approach identified hundreds of proteins that mediated EGFP-ABI1 degradation or stabilisation, including multiple E3s and DUBs, but also various unexpected hits. Interestingly, the authors identify and validate UBE2B, an E2 conjugating enzyme, as a potent degrader, which requires UBE2B catalytic activity, but is E3-independent. The authors also investigate the applicability of 50 effector hits against 10 different protein targets located in various cellular compartments, including disease-relevant, challenging-to-target example proteins. From this, some effectors, including VHL, displayed varying effects across the targets tested, whereas others displayed potent effects across all targets, with some being more efficient degraders than both VHL and CRBN. Following on from this study, it will be interesting to explore these effectors in additional cell lines and will be most exciting to see their translatability to small-molecule development for targeting endogenous proteins.

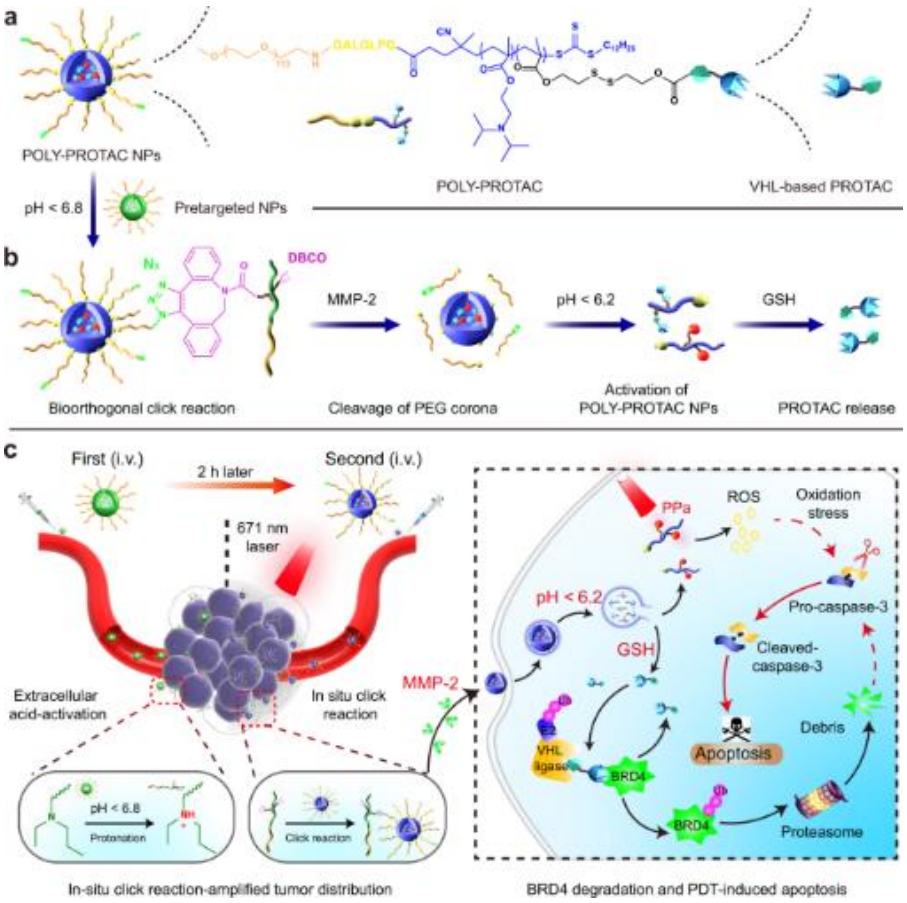
Cell Biology | **Chemistry** | **Structural Biology/Biophysics**

Contributor: Martina

Engineered bioorthogonal POLY-PROTAC nanoparticles for tumour-specific protein degradation and precise cancer therapy

Jing Gao[§], …, Zhiai Xu*, Huixiong Xu*, Haijun Yu*

Nat. Commun. **2022**, *13*, 1



This paper presents an excellent design of tumour microenvironment-activatable polymeric PROTACs (POLY-PROTACs) nanoplatform, which were engineered by covalently linking PROTACs onto the backbone of an amphiphilic diblock copolymer. This work aims to achieve tumour-specific delivery and protein degradation, in order to potentiate the antitumor potency of conventional PROTACs. Indeed, despite PROTACs holding promising potential for cancer therapy, generally, they lack tumour specificity, which might cause systemic toxicity due to the distribution in normal tissue. For proof-of-concept, the authors rationally engineered a POLY-PROTAC nanoplatform for precise targeted degradation of BRD4. Starting with the synthesis of a small set of VHL-based PROTACs, they designed reduction-activatable POLY-PROTACs able to self-assemble into micellar nanoparticles (NPs) for systemic delivery. Subsequently, they engineered dibenzocyclooctyne-loaded pretargeted NPs to enhance intratumoural accumulation and retention. Upon internalisation into the tumour cells, the POLY-PROTAC nanoparticles release the PROTAC via glutathione-mediated reduction. Furthermore, the authors are currently investigating the combination of POLY-PROTAC nanoplatform with radiotherapy and chemotherapy.

This study provides a valuable strategy for potentiating tumour-specificity of PROTAC-based therapy. It will be interesting to see if its application will finally boost the clinical translation of PROTAC, which is still a tough challenge.

Cell Biology | Chemistry | Structural Biology/Biophysics

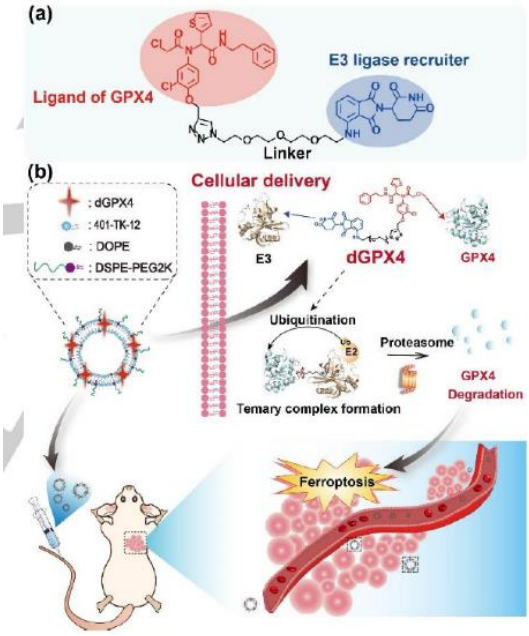
Contributor: Martina

Intracellular Delivery of Glutathione Peroxidase Degrader Induces Ferroptosis In Vivo

Tianli Luo[§], …, Ming Wang*

Angew. Chem. Int. Ed. **2022**, DOI: 10.1002/anie.202206277

The importance of a targeted protein degradation strategy using PROTACs encapsulated in biodegradable lipid nanoparticles for selective intracellular cancer delivery is confirming as a highly effective therapeutic modality able to reduce side effects and improve pharmacokinetic parameters. In this study is reported the first proof-of-concept of designing PROTAC-based protein degraders able to deplete endogenous glutathione peroxidase 4 (GPX4) and to induce cancer cell ferroptosis, namely a new form of regulated, non-apoptotic cell death. The authors rationally designed a GPX4 degrader starting from an already known inhibitor whose therapeutic potential was greatly limited by the low efficiency of regulating cell ferroptosis *in vivo*. Notably, the potency of the designed PROTAC was five-fold higher for inducing cancer cell ferroptosis compared to the known inhibitor. Furthermore, the most interesting thing about the paper is the development of a lipid nanoparticle formulation that not only encapsulates the PROTAC for *in vivo* administration but also, triggered by the tumour microenvironment, controls selectively the release of the degrader in cancer cells.



This article is a good read and an important reference in furthering the development of cell-selective protein degradation.

Cell Biology | Chemistry

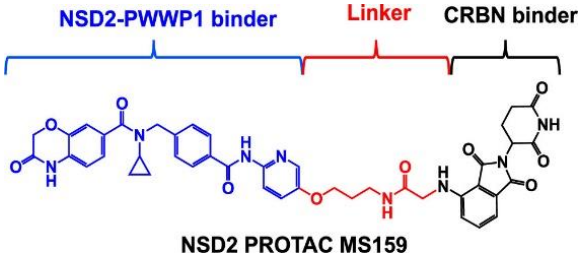
Contributor: Martina

Discovery of a First-in-Class Degrader for Nuclear Receptor Binding SET Domain Protein 2 (NSD2) and Ikaros/Aiolos

Fanye Meng[§], …, H. Ümit Kaniskan*, Gang Greg Wang*, Jian Jin*

J. Med. Chem. **2022**, *65*, 10611

The nuclear receptor binding SET domain protein 2 (NSD2) is a lysine methyltransferase associated with diverse human diseases and it is considered an attractive therapeutic target for multiple myeloma (MM). Nevertheless, only limited progress has been made in developing selective inhibitors and yet none are effective in suppressing MM cell proliferation. In this scenario, Meng *et al.* break the silence by presenting the first-in-class NSD2 PROTAC degrader, which effectively reduced NSD2 protein level in cells in a concentration-, time-, and proteasome-dependent manner. Moreover, they also evaluated and reported the degradation of CRBN:IMiD neosubstrates IKZF1 and IKZF3, which are validated oncotargets of multiple myeloma. Notably, the PROTAC degrader was much more effective than the parent compound in inhibiting the proliferation in two multiple myeloma cells, suggesting that the pharmacological degradation of NSD2 and IKZF1/3 is a superior therapeutic strategy. Furthermore, *in vivo* pharmacokinetic studies revealed that the PROTAC degrader is bioavailable and well tolerated in mice.



In conclusion, this paper is an enjoyable read presenting a well-thought-out and detailed study strengthened by the consistent use of negative controls.

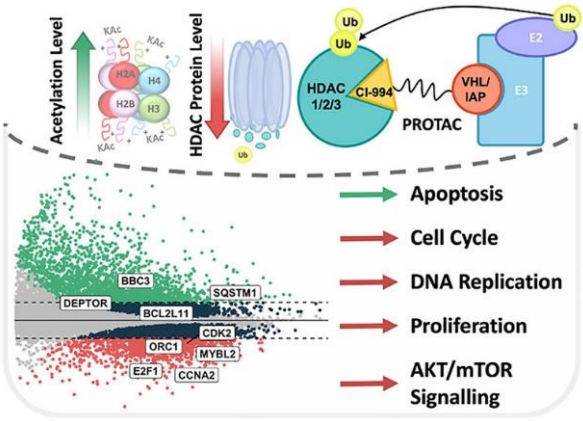
Cell Biology | Chemistry

Contributor: Martina

Comprehensive Transcriptomic Analysis of Novel Class I HDAC Proteolysis Targeting Chimeras (PROTACs)

India M. Baker[§], Joshua P. Smalley, Khadija A. Sabat, James T. Hodgkinson*, Shaun M. Cowley*

Biochemistry **2022**, DOI: 10.1021/acs.biochem.2c00288



The class I histone deacetylase (HDAC) enzymes are well-known proteins involved in the regulation of chromatin accessibility and gene activity. Over the last few years, several research groups had been focused on the development of small molecule inhibitors, which have shown beneficial effects in cancer treatments. Recently, the idea to develop PROTACs for class I HDACs that both bind and degrade HDAC1-3 is spreading around the scientific community. Interestingly, in this article, the authors report a library of PROTACs incorporating the benzamide-based HDAC inhibitor CI-994, which was connected through an alkyl linker to two different E3 ligase ligands, including the inhibitor of apoptosis protein (IAP) ligand. Surprisingly, the human colorectal carcinoma cells HCT116 treated with IAP-based PROTAC were significantly more sensitized to apoptosis at much lower concentrations than either the parental molecule (CI-994) or VHL-derived PROTACs, while the IAP ligand alone had no effect. Furthermore, thanks to RNA sequencing analysis, the authors provided a powerful overview of the transcriptional events that occur upon PROTAC treatment of colorectal cancer cells, identifying a distinct gene expression signature in which cell cycle and DNA replication machinery are repressed.

This article provides the first PROTAC directed against HDAC1/2 that uses a ligand belonging to the IAP family of E3 ligases. The use of the novel described degraders offers an exciting potential avenue for the clinical treatment of colon cancer.

Cell Biology | Chemistry

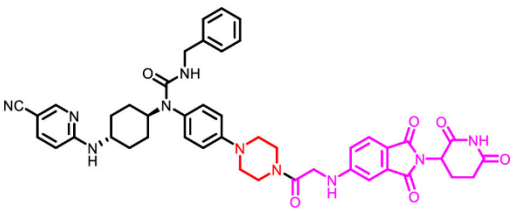
Contributor: Petr

Discovery of a Highly Potent and Selective Dual PROTAC Degrader of CDK12 and CDK13

Jianzhang Yang[§], …, George Xiaoju Wang*, Arul M. Chinnaiyan*, Ke Ding*

J. Med. Chem. **2022**, *65*, 11066

CDK12 and its homologue CDK13 are transcription-associated cyclin-dependent kinases. Their complexes with cyclin K (CycK) regulate the transcription of DNA damage response genes. It was previously reported that inhibition of CDK12/13 is an effective strategy to inhibit tumour growth and promote synergy with DNA-damaging chemotherapy and PARP inhibitors.



dual CDK12/13 degrader 7f

CDK12 DC_{50} = 2.2 nM in MDA-MB-231
CDK13 DC_{50} = 2.1 nM in MDA-MB-231

In this work, the authors report the development of a highly potent and selective dual CDK12/13 degrader based on a previously reported CDK12/13 dual inhibitor and cereblon-binding ligand. Compound **7f** effectively degraded CDK12 and CDK13 in MD-MBA-231 breast cancer cells with DC50 values of 2.2 and 2.1 nM, respectively. Moreover, **7f** displayed outstanding selectivity and suppressed cancer cell proliferation in the combo-treatment with cisplatin and PARP inhibitor Olaparib.

Unfortunately, **7f** demonstrated poor solubility and unsuitable pharmacokinetic properties. That is why its close analogue **7b** was used for a preliminary pharmacodynamic study and displayed strong and fast degradation efficiency *in vivo*.

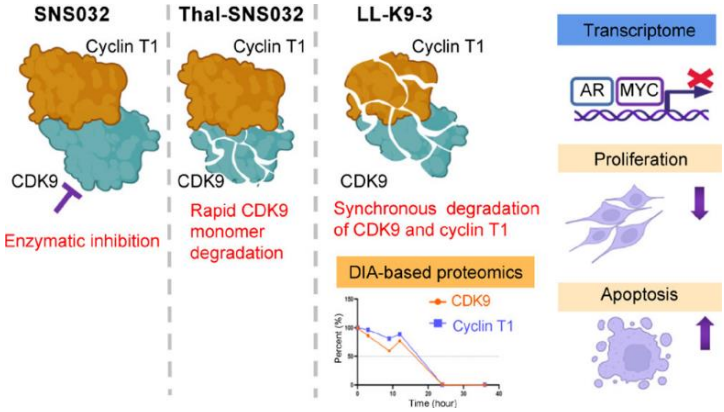
Cell Biology | Chemistry | Modelling/Simulation

Contributor: Petr

Discovery of Small-Molecule Degraders of the CDK9-Cyclin T1 Complex for Targeting Transcriptional Addiction in Prostate Cancer

Jiacheng Li[§], …, Hu Zhou*, Hua Lin*, Cheng Luo*

J. Med. Chem. **2022**, *65*, 11034



Hydrophobic tagging (HyT) has become a powerful strategy to induce POI degradation. However, this approach is still not widely adopted in PROTAC-based drug discovery. In this research, Li *at al.* developed a highly selective and potent small-molecule HyT degrader **LL-K9-3** which contains a CDK9 inhibitor **SNS032** core, novel (-)-menthoxyacetyl hydrophobic tag and sulfonyl linker. The degrader demonstrated rapid synchronous degradation of the prominent transcription-associated CDK9-cyclin T1 complex.

As a chemist, I was impressed with how the authors determined the efficacy of both CDK9 and Cyclin T1 degradation, the degradation selectivity of **LL-K9-3** and its anti-proliferative effects. A special place in this research is given to the comparison between **LL-K9-3** and the previously reported thalidomide-based CDK9 degrader **Thal-SNS032**, which showed only slow, delayed and partial reduction of cyclin T1.

LL-K9-3 had increased anti-proliferative and pro-apoptotic effects than its parental molecule **SNS032** in prostate cancer cells, however, there was no significant contrast between these two degraders. However, CDK-cyclin heterodimer degradation seems to be a highly powerful tool in the field of anti-cancer drug design and this paper provides a good starting point for its further development.

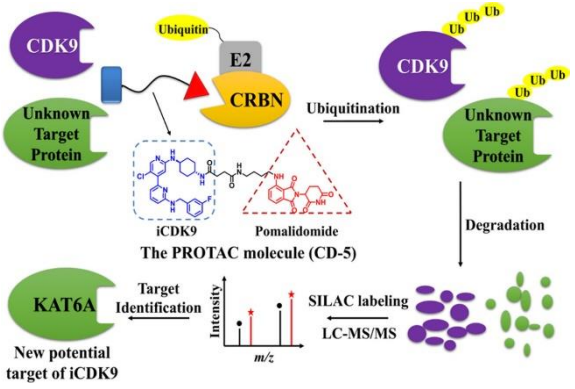
Cell Biology | Chemistry

Contributor: Petr

The synthesis of PROTAC molecule and new target KAT6A identification of CDK9 inhibitor iCDK9

Mingtao Ao[§], Jun Wu[§], …, Yuhua Xue*, Meijuan Fang*, Zhen Wu*

Chin. Chem. Lett. **2022**, 107741



PROTAC technology proved to be a useful tool for protein level regulation and has broad applicability in studying the roles of different proteins in biological systems. It has been recently shown that stable-isotope labelling with amino acids in cell culture (SILAC) analysis can be used for understanding the specificity of PROTAC-mediated POI degradation.

A new study has demonstrated the utility of PROTAC treatment-SILAC quantitative proteomics experiments as a novel method to identify unknown off-target proteins. Ao *et al*. synthesised five small molecule degraders based on previously reported highly selective CDK9 inhibitor iCDK9 and identified protein abundance based on mass spectrometric analysis after PROTAC probe treatment. For example, non-kinase protein KAT6A was most efficiently degraded with a more than 85% decrease in protein expression. Formation of the KAT6A:PROTAC:CRBN ternary complex was proven by adding pomalidomide as a CRBN competitive agent. These results suggest that KAT6A might be a non-kinase target of previously reported iCDK9.

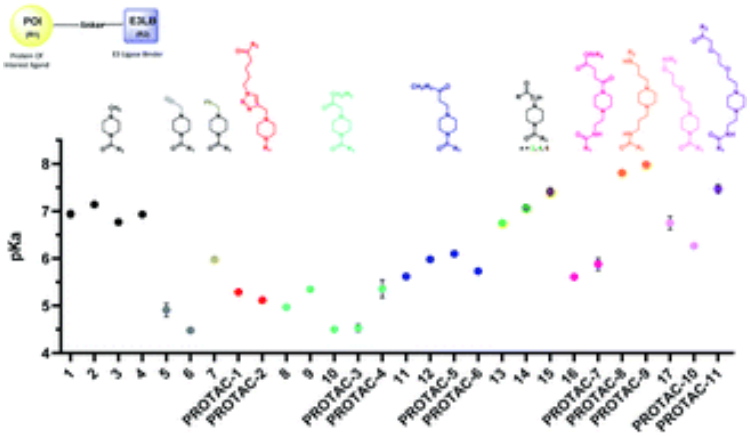
Chemistry | Computational Chemistry

Contributor: Petr

PROTACs bearing piperazine-containing linkers: what effect on their protonation state?

Jenny Desantis[§], Andrea Mammoli[§], …, Laura Goracci*

RSC Adv. **2022**, *12*, 21968



PROTAC's large and flexible structures are beyond the rule of five chemical space and usually have permeability and solubility issues. One of the most common routes to optimise these parameters is the incorporation of rigid and more polar linkers. The piperazine motif has attracted a lot of attention due to its rigidity and ability to increase the solubility upon protonation. However, the insertion of an additional protonable nitrogen atom adds a new element to the optimization of physicochemical properties.

In this paper, the authors described how neighbouring groups affect the protonation state of piperazine. They synthesised a small model set of piperazine-bearing degraders and experimentally measured their pKa. It was shown that the basicity of piperazine can significantly vary depending on the linker design, with pKa values ranging from 4.5 to 8. Moreover, they demonstrated the applicability of *in silico* prediction of pKa values for the basic centres even in such large molecules as model PROTACs.

It will be interesting to see an analysis of not only different piperazine linkers but structurally close motifs such as 4-aminopiperidine, spiroazetidine or 4,4-difluoropiperidine. Despite these limitations, this paper will be very helpful during hit-to-lead PROTAC optimisation indeed.

Other Paper Highlights

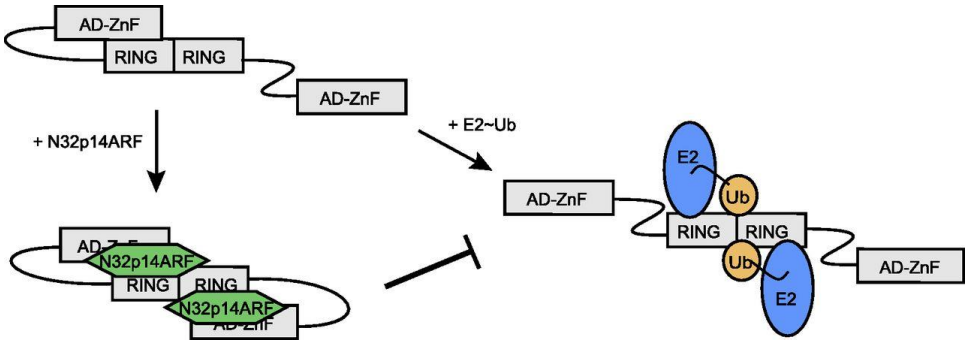
Cell Biology | Computational Chemistry | Modelling/Simulation | Structural Biology/Biophysics

Contributor: Mark

Bivalent binding of p14ARF to MDM2 RING and acidic domains inhibits E3 ligase function

Dominika Kowalczyk[§], Mark A. Nakasone, Brian O. Smith, Danny T. Huang*

Life Sci. Alliance. **2022**, DOI: 10.26508/lsa.202201472



Kowalczyk and co-workers applied a spectrum of *in vitro* methods to uncover new regulatory modalities of the RING E3 ligase, Mdm2. Their solution NMR validated a new self-regulatory mechanism for Mdm2, in which the RING domain binds the acidic and zinc finger (AD-ZnF) region. Subsequent activity and binding assays confirmed that the presence of AD-ZnF decreased the E3 ligase activity of Mdm2 and reduced binding of E2~Ub. Considering that the tumor suppressor p14ARF is reported to bind the AD-ZnF region in Mdm2, Kowalczyk *et al*. addressed how the RING domain responds to p14ARF. With a solution NMR approach, they discovered p14ARF inhibits Mdm2 through a "bivalent" mode by simultaneously binding AD-ZnF and RING. Application of protein engineering showed that the RING domain alone is still recruited to p14ARF/AD-ZnF, suggesting a robust complex. Functional assays and NMR revealed the p14ARF/AD-ZnF/RING complex sequesters the E2~Ub binding site, resulting in significantly reduced E3 ligase activity, in respect to AD-ZnF alone. Importantly, this study further advances our understanding on how Mdm2 is inhibited by p14ARF.

With an interest in exploiting Mdm2 as an E3 ligase for TPD, this study provides insight on several regulatory layers of Mdm2. In addition, Mdm2 and p14ARF both have great interest in cancer research but are challenging proteins to work with due to their tendency to aggregate and highly charged regions. This study and others like it will undoubtedly advance our understanding of new E3 ligases for TPD.

Computational Chemistry | Modelling/Simulation | Structural Biology/Biophysics

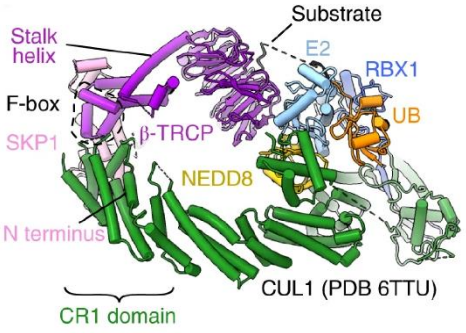
Contributor: Mark

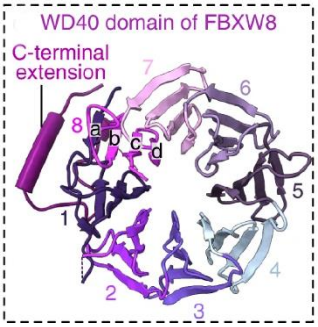
Structure of CRL7^{FBXW8} reveals coupling with CUL1–RBX1/ROC1 for multi-cullin-RING E3-catalyzed ubiquitin ligation

Linus V. M. Hopf[§], …, Brenda A. Schulman*

Nat. Struct. Mol. Biol. **2022**, DOI: 10.1038/s41594-022-00815-6

Hopf and co-workers use single particle cryo-electron microscopy (cryo-EM) to uncover the specificity of CUL7 for its F-box partner, FBXW8. The cullin RING ligase E3 complex, CUL7-RBX1-SKP1-FBXW8 (CRL7^{FBXW8}), is essential for development in mammals, yet mechanistically elusive. With a size of ~190 kDa, CUL7 is abnormally large for a cullin and known to interact with CUL9, both OBSL1 and CCDC8 in the 3M complex, and CUL1-RBX1 through





FBXW8. Hopf and co-workers determined a 2.8 Å resolution structure of $CRL7^{FBXW8}$ with a "T-shape" architecture unique among known CRLs. This Hopf *et al*. study shows $CRL7^{FBXW8}$ is resistant to NEDD8 modification, has low autoubiquitination with known human E2s, and RBX1 presents in a non-catalytic position. Therefore, $CRL7^{FBXW8}$ is not such an active E3 ligase, however $CRL7^{FBXW8}$ recruits neddylated CRL1 and they determined a 4.6 Å resolution structure of the complex using cryo-EM. Biochemical assay confirmed this multi-CRL complex: $CRL7^{FBXW8}$ and neddylated CRL1, was a potent E3 ligase for TP53, a known CUL7 substrate.

Overall, this study advances our understanding of CRLs and applies the cutting edge of structural biology to explore the understudied roles of $CRL7^{FBXW8}$. Clearly, there is a pattern of two E3 ligases working together, and the structural work in this study suggests that such multi-E3 complexes have potential for novel TPD approaches.

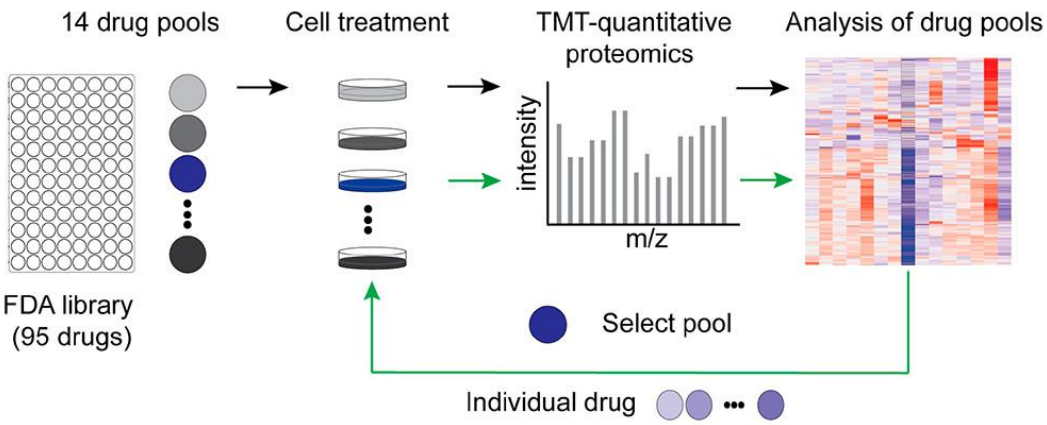
Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Manjula

Evaluation of a Pooling Chemoproteomics Strategy with an FDA-Approved Drug Library

Huan Sun[§], …, Junmin Peng*

Biochemistry **2022**, DOI: 10.1021/acs.biochem.2c00256



Huan Sun and co-workers developed TMT based low cost high-throughput pooled-chemoproteomics platform for profiling the responses of proteins to drug treatment. Proteomics profiling is not widely used for high-throughput screening (HTS) despite its deep proteome coverage due to its limited throughput and high cost. To improve the throughput and reduce the cost of proteomics-based screening, they introduced a drug pooling strategy and combined it with the multiplexed TMT methods. 14 drug pools were built from FDA-approved drug library (95 best-in-class drugs), each containing six or seven compounds of dissimilar chemical structures, thereby minimizing drug interaction by distributing similar drugs into different pools. After treatment with the pooled drugs, HEK293 cells were harvested and digested into peptides, followed by TMTpro labelling and LC-MS/MS analysis. Based on preliminary analysis of the proteome in drug pools, the selected pool was progressed to the next individual stage. Analysis of the proteomic data coming from an individual drug was then integrated with that from drug pools. The data clearly indicate that the drug pools have significant impacts on the cellular proteome with different patterns, consistent with their functional differences.

Overall, this TMT pooled-chemoproteomics platform provides high-throughput screening and more compounds might be mixed as long as individual compounds can reach their effective concentrations, and there is minimal target competition among mixed compounds. This feasible pooling strategy can be extended to other large compound library and cell types for probing novel protein targets, however additional studies are required to understand the molecular mechanisms, as protein changes may stem from one or a combination of the following possibilities e.g. change in transcription and/or translation, perturbation of protein degradation mediated by the UPS, deubiquitinase, interference of protein interaction with the lysosome/autophagy system.

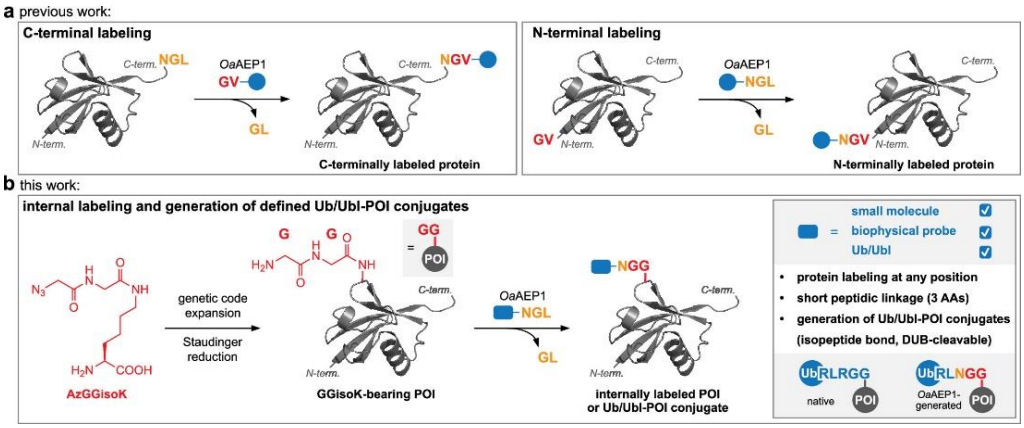
Cell Biology | Structural Biology/Biophysics

Contributor: Charlotte Crowe

Site-Specific Protein Labelling and Generation of Defined Ubiquitin-Protein Conjugates Using an Asparaginyl Endopeptidase

Maximilian Fottner[§], …, Kathrin Lang*

J. Am. Chem. Soc. **2022**, *144*, 13118



A good toolbox of methods for protein labelling is indispensable for monitoring, detecting and quantifying specific biological processes. Asparaginyl endopeptidases (AEPs) can be used for labelling protein N-termini or C-termini. Here, Fottner and colleagues use AEPs to label recombinant proteins at internal sites in a specific manner. Notably, the authors generate a number of ubiquitin or SUMO-linked POI conjugates, as well as di-ubiquitin, with DUB-cleavable isopeptide bonds.

Ubiquitinated proteins can also be built through sortylation (using the transpeptidase sortase). However, AEPs appear to have significant advantages using low concentrations (0.01 - 0.04 mol equivalents), and only a single point mutation necessary between the Ub/Ubl and POI. Furthermore, di-ubiquitin generated by sortase is resistant to DUBs, whereas the authors show that AEP-generated di-ubiquitin is cleaved by DUBs and therefore resembles the endogenous conjugate. All labelling methods will have their benefits and shortcomings, but this seems to be a very useful method for our toolboxes.

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

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