CeTPD Journal Club

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

Journal Club

July 2022

Centre for Targeted Protein Degradation
University of Dundee

innovate
collaborate
inspire

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Meet this Month's Editors

This month's editors are (from left to right): Alejandro Correa Sáez, Manon Sturbaut, and Mark A. Nakasone

"I´ve been reading this Journal Club before I joined the Ciulli lab as a vising PhD student. Now, having the opportunity to be an editor has enabled me to have a wider insight of this fascinating area by keeping up with the latest developments"

Alejandro completed the master of Translational Biomedical Research (2018) at the University of Córdoba (Spain). Currently he is a fourth-year PhD student, thanks to a FPU Fellowship (Spanish Science Ministry). During his doctoral studies, on the one hand, Alejandro has characterized novel substrates of a key kinase (DYRK2) in the DNA damage response and the cell cycle control. On the other hand, he has helped to elucidate the action mechanism of certain hypoxic-mimetic compounds. As a result, Alejandro developed his skills as a molecular and cell biologist focused on the study of cell signalling pathways. He joined the Ciulli lab in June 2022 as a visiting PhD student to satisfy his growing interest in the TPD field.

"The Journal Club helps us keep updated on the latest TPD publications. It is nice to have a quick overview of the literature each month"

Manon got her Master's degree in medicinal chemistry in 2016 from University of Lille (France) and remained in Lille to undertake her DPhil studies at the same university. Manon's PhD project was the development of inhibitors targeting the YAP-TEAD protein-protein interaction. Following her PhD, she joined NICR group in Newcastle in 2020 to begin a two year postodoctoral position with Mike Waring. Manon joined AC-BI team in Dundee as a medicinal chemist in April 2022.

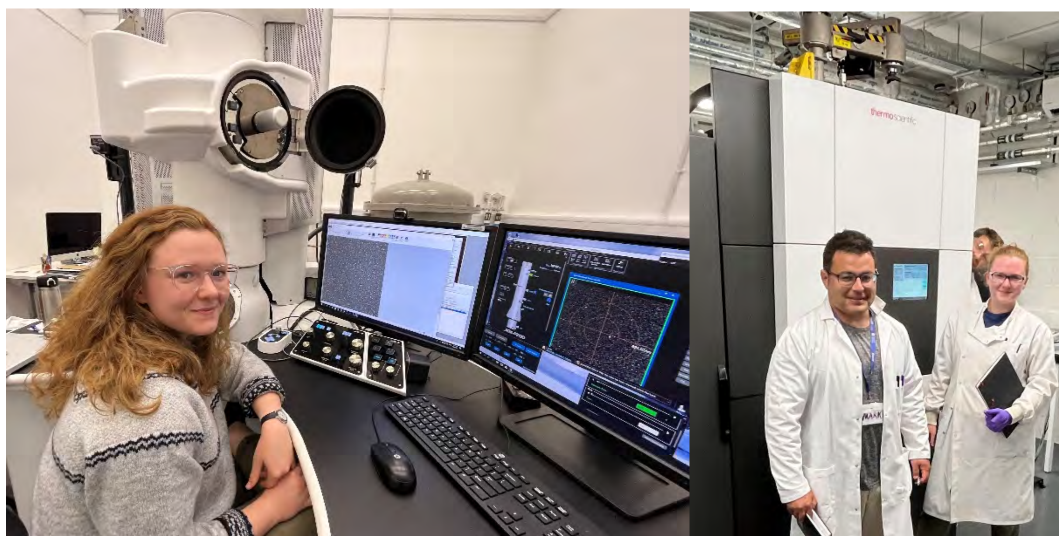
"Sometimes we all get busy, but the CeTPD journal club is an essential resource for updates in TPD."

Mark began his path in research as an undergraduate researcher at McDaniel College (USA), studying protein-folding under Melanie R. Nilsson. In 2008 Mark entered the ubiquitin field as a graduate student in David Fushman's NMR lab at the University of Maryland. For his doctoral work, Mark carried out the first structural study on "complex" polymeric ubiquitin. Upon earning his doctorate in 2013, the presidentially appointed Fulbright board awarded Mark a two year fellowship to carry out his ubiquitin research at the Technion in Haifa, Israel. While hosted in Michael H. Glickman's lab, Mark undertook many collaborative projects and discovered the substrate preferences of proteasome-associated deubiquitinases. In 2016 Mark joined Danny T. Huang's lab at the CRUK Beatson Institute in Glasgow. As an ERC research fellow, Mark characterised biologics that inhibit E3 ligases, novel ADP-ribosylated ubiquitin, and developed chemical methods to isolate E2/E3/substrate transition complexes for cryo-EM and X-ray crystallography. In April 2022, Mark joined CeTPD to further explore the potential of E3 ligases.

Features of the month

Contributors: Charlotte Crowe, Mark Nakasone, Alessio Ciulli

CeTPD wins award to create a cryo-EM in drug discovery training network



Funding from the Scottish Universities Life Sciences Alliance (SULSA) was awarded to Mark Nakasone and Charlotte Crowe to create the inclusive Scottish cryo-electron microscopy in drug discovery (iSCEM-DD) network. The primary objective of iSCEM-DD is to support new cryo-EM users undertaking drug discovery projects by encouraging early-career researchers (ECRs) to get trained and involved. The network also has the specific focus of supporting inclusivity, diversity, and to aid gender and demographic balance. At present, iSCEM-DD has a working framework of trainees at every level, along with experts from the University of Dundee and the University of Glasgow to support structural biology, biochemistry, and medicinal chemistry. Professor Alessio Ciulli (Dundee's CeTPD) will support chemical biology and Professor Ronald T. Hay (Dundee) will support biochemistry, alongside cryo-EM experts Professor Paula da Fonseca (Glasgow) and Professor Tom Owen-Hughes (Dundee). In addition, cryo-EM facility managers James Streetley (SCMI/Glasgow) and Ramasubramanian "Subbu" Sundaramoorthy (Dundee) have agreed to support iSCEM-DD.

The inaugural iSCEM-DD workshop will be held in November of 2022, following the commissioning of Dundee's Glacios 200 kV electron microscope equipped with Falcon 4i detector. Differing from similar events, iSCEM-DD will attract ECRs to discuss their nascent proposals, as well as ECRs with more developed projects. The entire focus of this event is to develop ECRs as electron microscopists and accelerate their projects by forming collaborations with experts.

With Dundee's cutting edge 200 kV and 16k direct electron detector, it will be possible to determine near-atomic resolution (~3 Å) structures of biological molecules, providing a new avenue for small molecule drug discovery. Within the CeTPD, we look forward to advancing TPD with single particle cryo-EM and supporting the iSCEM-DD network. In 2023, iSCEM-DD will be expanded to the whole of Scotland and support more ECRs as well as the recruitment of other experts. Therefore, iSCEM-DD will be a key mechanism to train new users and to accelerate collaborative drug discovery projects with single particle cryo-EM.

Contributor: Alessio Ciulli

Early Career Researcher Award from Arvinas

In keeping with the important theme of supporting Early Career Researchers in our field, here another great initiative this time by @ArvinasInc who have launched their Early Career Research Award. This award aims to recognize researchers bringing innovation, new approaches and creative thinking to advance the field of targeted protein degradation. For more information, please click the link here. Deadline is **August 1st** so hurry up!

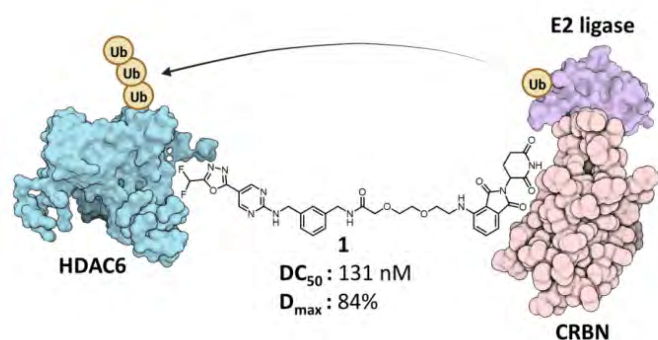
Cell Biology | Chemistry | Modelling/Simulation

Contributor: Manon

Development of the first non-hydroxamate selective HDAC6 degraders

Tim Keuler[§], Beate König[§], ..., Michael Gutschöw*, Finn K. Hansen*

ChemRxiv **2022**, DOI: 10.26434/chemrxiv-2022-3rhqf



HDAC6 is overexpressed in several cancers and the knock-out of the protein does not lead to significant defect. It is composed of two catalytic domains (CD1 and CD2) as well as a zinc-finger ubiquitin binding domain (ZnF-UBD). So far, all selective HDAC6-PROTACs contain a hydroxamate zinc-binding group (ZNB) which coordinates the zinc ion in the active site of HDAC6 CD2, but appears to have mutagenic and genotoxic potential. To avoid these effects, the authors report non-hydroxamate selective degraders containing difluoromethyl-1,3,4-oxadiazole. Using docking, they designed PROTACs able to recruit VHL as well as CRBN E3 ligases with different linkers and exit vectors. All PROTACs demonstrated HDAC6 inhibition with IC_{50} values between 0.6 to 1.9 µM *in vitro*- and present selectivity of HDAC6 over HDAC1-3. In cells, all compounds are able to degrade HDAC6 at 1 µM without showing any toxicity. Both VHL and CRBN recruiting series show ternary complex formation.

Despite the moderate potency, this paper presents a novel perspective and tools for the development of non-hydroxamate degraders. Only six compounds have been described so far with minimal changes in the structure. We look forward to new designs and more SAR optimization. The authors only investigated PEG or alkyl linkers, a diversification of the linkers to improve potency would be excellent future work.

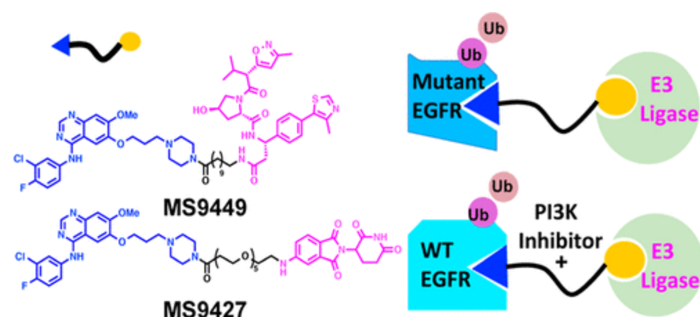
Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Manon

Exploring Degradation of Mutant and Wild-Type Epidermal Growth Factor Receptors Induced by Proteolysis-Targeting Chimeras

Xufen Yu[§], Meng Cheng[§] ..., Jing Liu*, Yue Xiong*, Jian Jin*

J Med Chem **2022**, *65*, 8416-8443, DOI: 10.1021/acs.jmedchem.2c00345



EGFR is a receptor tyrosine kinase involved in multiple intracellular pathways and its upregulation is associated with oncogenesis. Over the past decades, many kinase inhibitors have been developed and several PROTACS have recently been reported. Thus far most EGFR ligands are selective for EGFR mutants. The authors describe a SAR study investigating diverse lengths and motifs for the linker and E3 ligase ligands by immunoblot. The best compounds, for both VHL and CRBN recruiting series, were evaluated in cell growth inhibition assays leading to the discovery of two novel EGFR degraders with an antiproliferative potency of 1 µM and 0.87 µM on HCC-827 cells, for VHL and CRBN series respectively. Both compounds present nanomolar binding affinity for the wild-type and mutant EGFR, but are only able to degrade mEGFR. The mechanism of action study suggests that the degradation depends on the UPS, but also the autophagy/lysosome pathway. Interestingly, addition of PI3K inhibitors induces wtEGFR degradation, promotes the ternary complex formation, and inhibits the growth of cancer cells. These results provide a new therapeutic strategy for patients with wtEGFR overexpression.

We are really interested to discover how the autophagy/lysosome pathway is involved in EGFR degradation by PROTACs in future work.

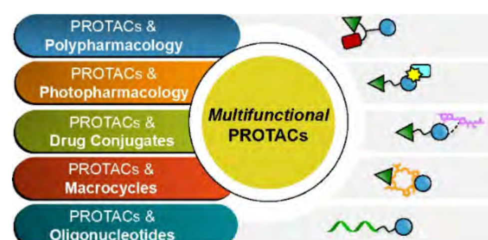
Chemistry

Contributor: Manon

Enriching proteolysis targeting Chimeras with a second modality : When two are better than one.

Alessandra Salerno§, …, Maria Laura Bolognesi *

J Med Chem **2022**, DOI: 10.1021/acs.jmedchem.2c00302



PROTACs/TPD has gained interest as a research field over the past decade, with the first PROTAC in clinical trial in 2019. TPD gives a new potential and advantages for drug design over inhibitors, including "undruggable" proteins, use of sub-stoichiometric quantity, or even minimizing resistance mechanisms. Nevertheless, PROTACs have some limitations, they don't respond to the established concept in drug discovery and still need more understanding and improvement, especially selectivity. Some limitations have already been overcome by new tools, highlighted by the authors. Multitarget PROTACs can be used for polypharmacology in the case of complex diseases to get better therapeutic windows. Photopharmacology uses light control to modify drug action by changing their PK or PD profiles. Use of photoactivable PROTACs offers spatiotemporal control of protein degradation and might result in a better selectivity. Another therapeutic approach consists of targeting a specific site or tissue by antibody/small molecule drug conjugates. Tissue-specific degradation can be achieved by high expression levels of a E3 ligase. Based on that, preliminary studies on PROTAC-conjugates has been published to enhance the selectivity and reduce side effects. Macrocycles have emerged as a new strategy to improve metabolic stability, cell permeability or oral bioavailability or PD profile. For these reasons, macrocycles are now combined with PROTACs, used as a POI ligand or a linker. The latest strategy consists of merging oligonucleotides and PROTACs. Oligonucleotides interact with specific sequence of its target *via* complementary base pairs, rendering them highly selective and able to modulate undruggable targets.

In this perspective, the authors give us an overview of PROTACs and new modalities described in the literature to develop multifunctional PROTACs. All of these new approaches and tools, successful or not, open many opportunities to explore, understand, and advance the TPD field.

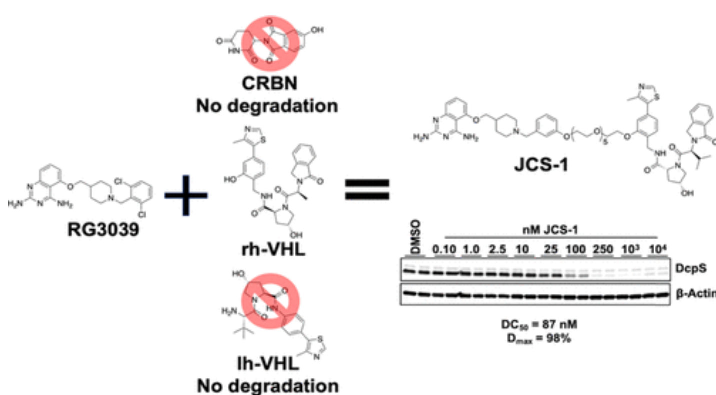
Cell Biology | Chemistry

Contributor: Manon

Targeted degradation of mRNA decapping enzyme DcpS by a VHL-recruiting PROTAC

Jack C. Swartzel§, Michael J. Bond§ , …, Craig M. Crews*

ACS Chem. Biol. **2022**, *17*, 1789-1798, DOI: 10.1021/acschembio.2c00145



The RNA decapping scavenger protein, DcpS, is implicated in several diseases and is a driver of acute myeloid leukemia (AML). Both inhibition or shRNA knockdown of DcpS are antiproliferative and the protein is not essential in normal cells. Based on that, the authors have developed a degrader using PROTAC mechanism starting from the inhibitor RG3039, a phase I clinical candidate. They synthesized 20 PROTACs recruiting CRBN or VHL as the E3 ligase. Only one compound coupled to the VHL ligand with the phenol linkage on the right-hand side shows degradation of DcpS with D_{max} ~ 98% at nanomolar concentration. Their PROTAC induces rapid and highly sustained degradation of DcpS across different AML cell lines. Unfortunately, JCS-1 is not as antiproliferative as expected. The mRNA decapping activity does not have a direct effect on mRNA expression or stability. As the role of DcpS in mRNA is not totally understood, the PROTAC could be used as a useful chemical biology tool for understanding the function of DcpS in disease. As a probe, it would be interesting to assess the degradation of DcpS in the context of enzymatic or scaffolding roles.

Here again, looking at the chemistry investigation, the authors only observed degradation with 17-atom linker while moderate or even inactive PROTACs with one or two extra atoms, showing the real importance of ternary complex formation for degradation.

Cell Biology | Structural Biology/Biophysics

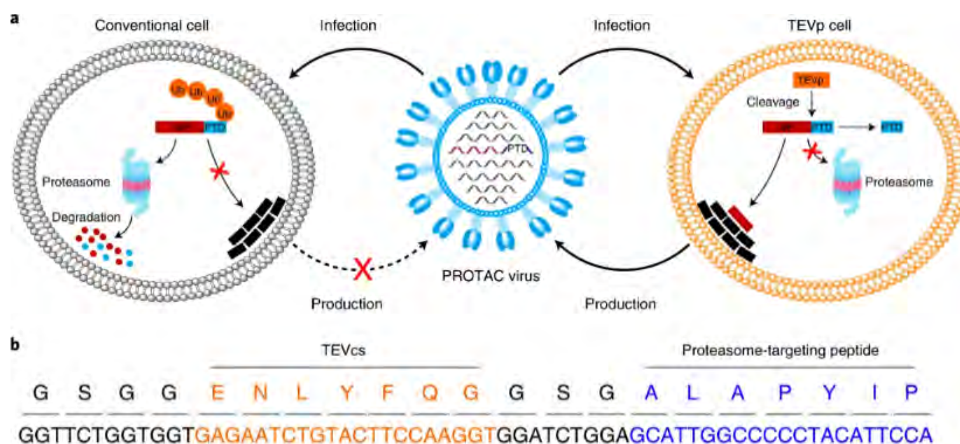
Contributor: Alejandro

Generation of a live attenuated influenza A vaccine by proteolysis targeting

Longlong Si[§]*, Quan Shen[§], Jing Li[§]…

Nat Biotechnol **2022**, DOI: 10.1038/s41587-022-01381-4

Attenuated vaccines have been traditionally considered of great relevance among the strategies to prevent infectious diseases as it boosts the immune response. However, there are great concerns in terms of safety, immune escape, and, inefficient manufacturing processes. In this article, Longlong Si and colleagues engineered influenza A viruses inserting a VHL recognition sequence which codifies a proteasome-targeting peptide (PTD). As a result, the proteasome complex could selectively degrade the modified viral proteins, allowing the construction of an attenuated virus vaccine termed "PROTAC virus". Another interesting feature is that protease TEVp enables the PTD cleavage from the viral proteins sparing the virus from degradation. Therefore, TEVp stable cell lines were generated to achieve a large-scale production of this PROTAC virus. Following these principles, the authors conducted multiple approaches to determine the safety of the virus vaccine (named M1-PTD), as well as the robustness of the immune responses in mice and ferrets, as well as protection against homologous and heterologous viruses.



This study presents a new technology which could overcome the current challenges in attenuated vaccines studies. Limiting the virus' ability to replicate leads to the absence of potential risk in live-attenuated vaccines of returning to a more virulent strain due to spontaneous mutations. Finally, the simplicity of this method would allow its use in other viruses and its adaptation to many other labs.

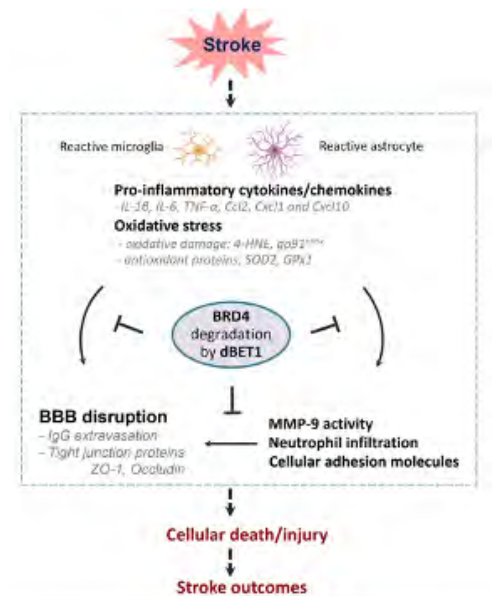
Cell Biology

Contributor: Alejandro

Targeted BRD4 protein degradation by dBET1 ameliorates acute ischemic brain injury and improves functional outcomes associated with reduced neuroinflammation and oxidative stress and preservation of blood–brain barrier integrity

Lei Liu [§], …, Eduardo Candelario-Jalil*

J Neuroinflammation **2022**, DOI: 10.1186/s12974-022-02533-8



Ischemic stroke triggers blood-brain barrier (BBB) disruption mediated by an excessive inflammatory and oxidative response. Intriguingly, Bromodomain-containing protein 4 (BRD4) has recently achieve significant attention as a key activator of this response in ischemia and its blockade is of great interest in the clinical field. In this study, the dBET1 PROTAC was employed to determine whether BRD4 degradation could elicit neuroprotective effects against transient focal ischemic stroke. The authors first showed attenuation of several inflammatory and oxidative mediators followed dBET1 treatment in the ischemic cortex. More importantly, dBET1 prevents BBB disruption as a result of Matrix Metallopeptidase 9 activity attenuation, neutrophil infiltration reduction, and a decrease of cellular adhesion molecules expression (such as ICAM-1). Finally, dBET1 ameliorates the neurological deficits induced by ischemia while, at the same time, it reduces the brain lesion volume.

These findings are of great relevance as they showed dBET1 to be a promising therapeutic strategy to reduce ischemic brain injury. Intriguingly, the use of this PROTAC could also be of interest in other pathologies where BBB is disrupted such as Traumatic Brain Injury. However, it would be also interesting to know the neuroprotective effects of dBET1 compared to other BET degraders. Moreover, as dBET1 targets other BET family members it will be necessary to further verify that its neuroprotective effect is exclusively BRD4 dependent.

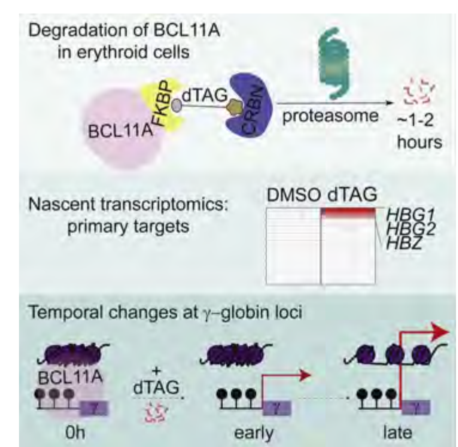
Cell Biology | Chemistry

Contributor: Alejandro

Temporal resolution of gene derepression and proteome changes upon PROTAC mediated degradation of BCL11A protein in erythroid cells

Stuti Mehta[§], Altantsetseg Buyanbat[§], Yan Kai[§],…, Stuart H. Orkin*

Cell Chem Biol **2022**, DOI: 10.1016/j.chembiol.2022.06.007



Reactivation of fetal (HbF) from adult (HbA) hemoglobin has been considered a promising strategy to treat β-hemoglobinopathies. BCL11A has attracted significant interest due to its role in γ-globin gene repression, thus contributing to the switch from HbF to HbA. For that reason, its clinical modulation is at the forefront of efforts to treat β-hemoglobinopathies. Here, the authors employed the dTAG PROTAC system to degrade BCL11A, providing more insight into the kinetics of BCL11A's action compared to previous gene editing strategies (shRNA or CRISPR-Cas9). First, nascent transcription identifies only 31 genes directly repressed by BCL11A degradation, with the globin coding genes (HBG and HBZ) being the most altered. Then, quantitative proteomics revealed that the effects of BCL11A depletion are highly specific. Finally, combination of BCL11A depletion and the use of a DNMT1 inhibitor enabled the authors to differentiate between BCL11A repression and the impact of methylation in the γ-globin promoter at the locus.

Overall, thanks to a powerful combination of high throughput techniques, the authors were able to temporally dissociate the impact of BCL11A blockage in transcriptional induction, chromatin accessibility, and DNA methylation at the the γ-globin locus. This article elegantly reinforces the advantages of small molecules over gene therapy in the study of β-hemoglobinopathies. It also raises the question of how many other processes would be better studied using TPD instead of gene editing.

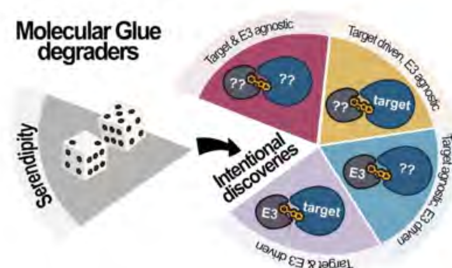
Cell Biology | Chemistry

Contributor: Alejandro

Chasing molecular glue degraders : screening approaches

Ana Domostegui[§], Luis Nieto-Barrado[§], Carles Perez-Lopez, Cristina Mayor-Ruiz*

Chem Soc Rev. **2022**, *51*, 5498, DOI : 10.1039/D2CS00197G



This review explores the current strategies for the rational discovery of molecular glues (MGs) for TPD. The authors first contextualized the place of MG degraders within TPD highlighting the main differences with PROTACs (more favorable drug-like properties, ability to target unligandable proteins, absence of hook effect, etc). A bibliographic classification of MGs is provided, with compounds categorized based on a number of physiochemical properties and further sub-categorised into natural and synthetic MGs as well as differentiating degraders from non-degraders. Specifically, as fortuity has driven synthetic MG degrader discovery, an extensive insight into the serendipitously discovered degraders was provided. These strategies were further categorised depending on whether the scope was to target a specific protein (target driven) or not (target-agnostic), employing a specific E3 ligase (E3-driven) or not (E3-agnostic). Finally, the authors also propose future perspectives and strategies to degrade undruggable targets (employing a target-agnostic approach) and accelerating drug discovery in this area (improving technological progress in high-throughput proteomics).

This work highlights the advances in MG screening strategies moving from serendipitous to intentional discovery, which has enabled the expansion of E3s and chemical scaffolds employed.

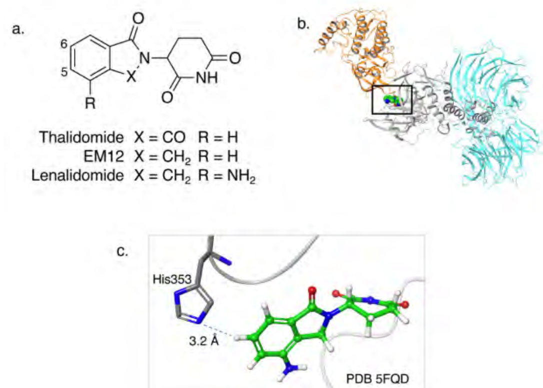
Cell Biology | Chemistry | Modelling/Simulation | Structural Biology/Biophysics

Contributor: Mark

Cereblon covalent modulation through structure-based design of histidine targeting chemical probes

Justin T. Cruite[§], Geoffrey P. Dann[§], Jianwei Che,…, Breanna L. Zerfas, Lyn H. Jones*

RSC Chem. Biol. **2022**, DOI: 10.1039/D2CB00078D



Cruite *et al*. expand on the available methods to covalently modify amino acid side chains, now adding histidine to the list including tyrosine, lysine, and cysteine. Using previous work on sulfonyl fluorides, this study combines a fluorosulfate functional group with the thalidomide analogue EM12. After optimizing the position of this functional group on EM12 in a NanoBRET assay, intact MS of CRBN confirmed the adduct of EM12-SO2F (307 Da). Next, degradation of IKZF1 by lenalidomide was inhibited by EM12-SO2F in MOLT4 cells, confirming the potency. Interestingly, closer inspection with proteomics revealed that fluorosulfate EM12 downregulated N-terminal glutamine amidohydrolase (NTAQ1). TR-FRET of CRBN/NTAQ1 confirmed their association in the presence of fluorosulfate EM12. While treatment with the NEDD8-activating enzyme (NAE) inhibitor MLN4924 partially rescued levels of NTAQ1 following addition of fluorosulfate EM12 to cells. Together this established NTAQ1 as an unexpected neo-substrate of CRBN covalently modified with fluorosulfate EM12.

Undoubtedly having established chemical methods to selectively modify amino acid side chains will greatly improve existing inhibitors and warheads for TPD. CRBN is one of the most studied E3s in TPD and existing structures support that covalent attachment to His-353 would aid in recruitment. In addition, the conservation of CRBN His-353 could expand TPD across species.

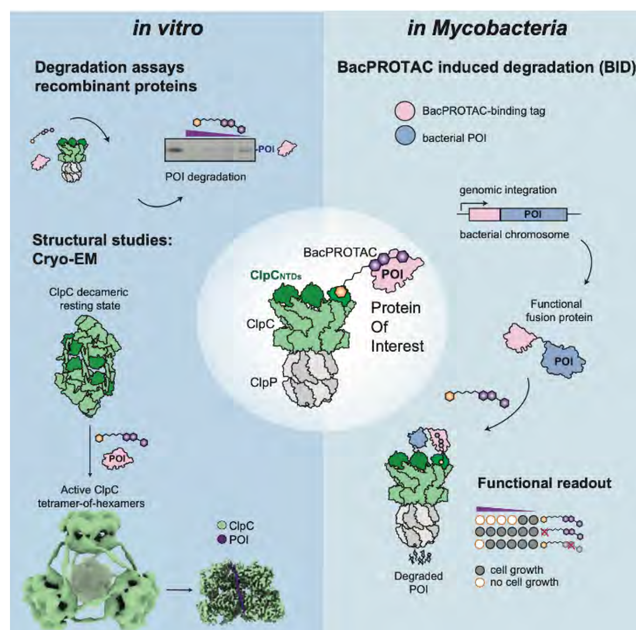
Cell Biology | Chemistry | Computational Chemistry | Modelling/Simulation | Structural Biology/Biophysics

Contributor: Mark

BacPROTACs mediate targeted protein degradation in bacteria

Francesca E. Morreale§, Stefan Kleine, Julia Leodolter, …, Markus Kaiser*, Tim Clausen*.

Cell. **2022**, *185*, 2338-2353, DOI: 10.1016/j.cell.2022.05.009



Morreale *et al*. have opened a new frontier in TPD by reporting small molecule degraders "bacPROTACs" which function in bacteria. Their approach exploits substrate binding preferences of the proteasome equivalent: ClpC:ClpP in gram-positive bacteria and ClpC1P1P2 in mycobacteria. As their first bacPROTAC design, a phospho-arginine (pArg) moiety is used to anchor to ClpC and biotin is attached through a linker. Target degradation was successful for several monomeric streptavidin (mSA) fusions using *B. subtilis* targets. Negative stain and cryo-EM analysis of a ClpC:ClpP/bacPROTAC-1/mSA-Kre (neo-substrate) ternary complex did not show the expected hexamer, but rather a tetramer of hexamers, providing insight on how bacPROTACs activate ClpC. Switching to mycobacteria, Morreale *et al*. modified the natural ClpC1 binding cyclic peptide, CymA, to generate bacPROTAC-2 with biotin and the BET bromodomain inhibitor JQ1 based bacPROTAC-3,4, and 5. The co-crystal structure of their modified cyclicpeptide with ClpC1 and successful degradation of bromodomain-1 (BD1) of BRDT in *M. smegmatis* established another class of bacPROTACs. Using well designed *in vivo* assays, Morreale *et al*. then demonstrate the effectiveness of their bacPROTACs on several other proteins of interest (POIs).

It was notable that the length of the linker for bacPROTAC-1 was not as important compared to eukaryotic PROTACs. Transporting such large molecules into cells is always a challenge and likely even greater when it comes to bacteria. The concept of anchoring neo-substrates to the equivalent of the proteasome is efficient. This study proves that TPD is possible in bacteria and therefore promising for meeting our emerging public health needs.

Other Paper Highlights

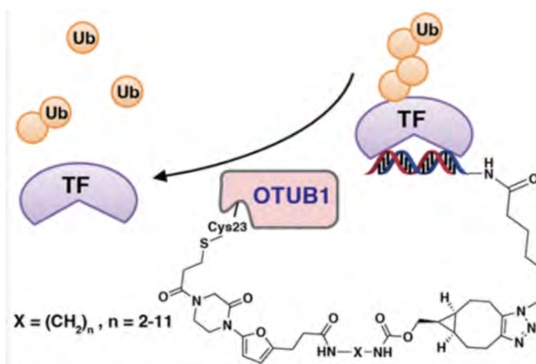
Cell Biology | Chemistry

Contributor: Mark

TF-DUBTACs Stabilize Tumor Suppressor Transcription Factors

Jing Liu [§], Xufen Yu [§], He Chen, …, Jian Jin *, Wendi Wei*

J. Am. Chem. Soc. **2022**, *144*, 12934-12941, DOI: 10.1021/jacs.2c04824



Liu and co-workers expand chemical biology to stabilize tumor suppressing transcription factors (TFs): p53, IRF3, and FOXO3A by recruiting the deubiquitinase (DUB), OTUB1. By combining the existing EN523 covalent OTUB1 modifier, with azide modified DNA, Liu *et al*. generated complete TF-DUBTACs with alkylene linkers ranging from n=2-11. The ~29 bp sense and anti-sense DNA easily anneals, resulting in a high affinity ligands for TFs. Their approach was ideal for linkers of six−eight methylenes, less efficient with longer linkers, and shorter linkers tended to interfere with productive target engagement. TF-DUBTACs were transfected into HEK293T or HeLa cells and Western blot analysis confirmed stabilization of the respective TF. Knock out of OTUB1 confirmed the DUB was required for stabilization and proteomics along with RT-qPCR demonstrated the impact of TF-DUBTACs on downstream targets.

This Liu *et al*. study is among several recent works demonstrating novel methods for manipulating TFs. We now have the technology to recruit either DUBs to rescue TFs from proteasome targeting or E3 ligases to rapidly degrade TFs. In the larger picture the emerging targeted protein stabilization (TPS) strategy to recruit of DUBs seems promising.

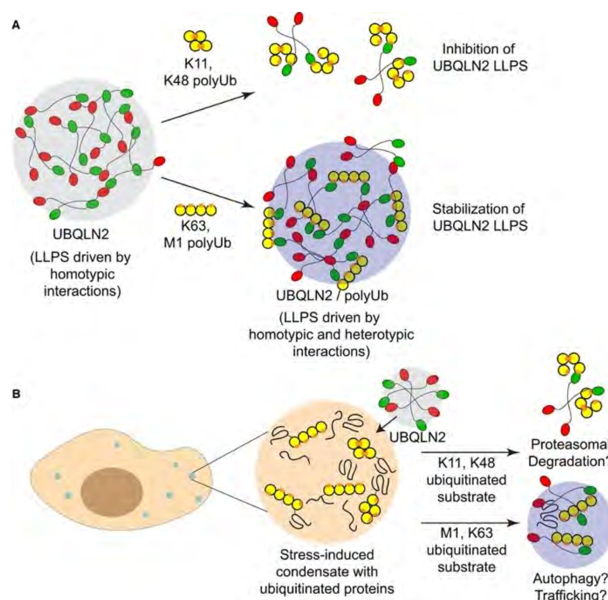
Cell Biology | Computational Chemistry | Modelling/Simulation | Structural Biology/Biophysics

Contributor: Mark

Mechanistic insights into enhancement or inhibition of phase separation by different polyubiquitin chains

Thuy P Dao [§], Yiran Yang [§], Weikang Ma,…, Stewart N Loh, and Carlos Castañeda*

EMBO Rep. **2022**, e55056, DOI: 10.15252/embr.202255056



Dao *et al*. continue expanding on the role of polymeric ubiquitin (polyUb) in driving UBQLN2 mediated liquid-liquid phase separation (LLPS). Although all polyUb chains link repeating units of Ub, this Dao study shows that the internal Ub-Ub linkage, as well as chain length are major factors controlling LLPS in UBQLN2/polyUb complexes. They demonstrate that K63-Ub_4 but not K63-Ub_2 or K48-Ub_4 readily undergoes LLPS with UBQLN2. By applying solution NMR they show that K48- and K63-polyUb are recognized by UBQLN2 differently, supporting their LLPS data. Small-angle X-ray scattering (SAXS) reveals that K48-polyUb binds UBQLN2 in an open conformation, distinct from K63-Ub_4 or M1-Ub_4. In the Dao study they go on to explore LLPS with other forms of polyUb including “HOTag6-G10-Ub" that is a tetramer with a Ub on each unit. The main finding is that LLPS is favoured with longer polyUb and Ub-Ub linkages that present extended conformations, with minimal interdomain contacts. Surprisingly, autoubiquitinated E2 enzymes also ended up in condensates with UBQLN2 during *in vitro* enzymatic synthesis of K63 linkages.

This study continues to add important insights in the growing field of LLPS and ubiquitin signalling pathways. The linkage selectivity coupled with LLPS in the case of UBQLN2 highlights the importance of internal Ub-Ub linkages. Furthermore, LLPS has been reported for other UBL/UBA shuttle proteins and Ub binding domains. This study represents one of the most robust *in vitro* LLPS/Ub studies.

Chemistry

Contributor: Yuting

Feasible Column Chromatography-Free, Multi-Gram Scale Synthetic Process of VH032 Amine, Which Could Enable Rapid PROTAC Library Construction

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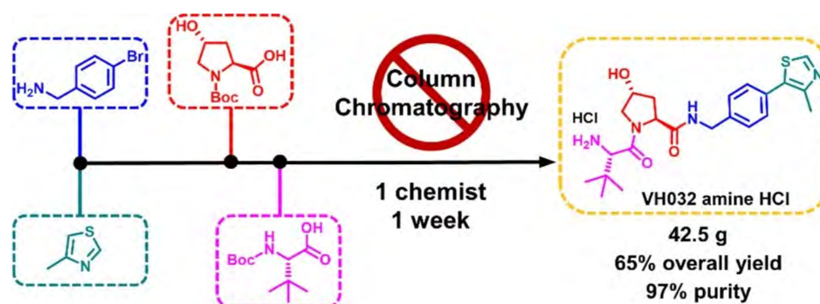

VH032 amine is a key building block for synthesis of VHL E3 ligase based PROTACs. Previous published methods toward the synthesis of this compound are either low yield or only work well at small scale. (4-(4-methylthiazol-5-yl) phenyl)methanamine is the key intermediate in synthesis of VH032 amine. In this report, the authors first optimised the reaction by investigating the Heck coupling for large scale synthesis. Upon increasing the reaction to 130 °C and they observed a complete transformation of materials within 4 hrs. They then used HCl instead of TFA to deprotect the Boc group, in which the HCl salt can form a fine solid and precipitate out in >95% purity without the necessity of further purification. The next steps were adding proline and leucine. They compared linear with convergent synthesis. However, the convergent synthetic route was less controllable because the materials were not UV active, making it challenging to monitor the reaction progress and product purity. Through the last step deprotection, 90% purity HCl salt compound was observed. The salt was then washed, basified, saturated, extracted and saltified with HCl again. In the final stage, a fine beige solid with 65% net overall yield and 97% purity was obtained.

In summary, a feasible column chromatography-free, multi- gram scale, high overall yield synthetic process of VH032 amine was developed. This could provide an useful approach towards rapid PROTAC library construction.

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