CeTPD Journal Club

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

Journal Club

February 2024

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): Elisha McCrory, Liam Martin and Simon Krols

" The journal club is a fantastic resource to explore exciting new research from the ever-expanding field of TPD."

Elisha is originally from Durham, UK and completed both her BSc and MSc in Pharmacology at the University of Liverpool in 2019. Elisha completed her PhD in the lab of Prof. Sir. Philip Cohen at the MRC-PPU, University of Dundee in 2023 where she worked on identifying sites of atypical ubiquitin attachment onto the hydroxyl groups of serine and threonine residues of proteins and onto hydroxyl groups of sugars. Elisha joined the AC-BI team in December 2023 as a Cell Biologist. In her spare time Elisha enjoys hiking, travelling, cooking and running.

"The CeTPD journal club is a great tool for keeping up with recent development in the field of TPD and beyond. Covering a range of disciplines, it can help you to form new perspectives and ideas that you can apply to your own research"

Liam received his BSc in Biomolecular Science from the University of St Andrews in 2015, before moving to London to complete an MRes in Drug Design at UCL. Liam remained at UCL to study for a PhD with Profs Helen Hailes and Sanjib Bhakta, focusing on the development of antitubercular compounds. He then moved to the University of Glasgow in 2021 to work as a postdoc with Dr David France on the discovery of antiviral PROTACs. In November 2022 Liam joined the AC-BI team in Dundee as a medicinal chemist. In his spare time, Liam enjoys playing the drums, relaxing with his cats, and travelling.

"This Journal Club is an invaluable resource for staying updated on the latest discoveries in targeted protein degradation. Covering diverse disciplines from chemistry to biology, it serves as a great tool for learning more about areas outside your expertise."

Simon graduated as a pharmacist with a Master of Sciences in Drug Development. In 2019, he embarked on a PhD program in Medicinal Chemistry at Ghent University, with a focus on developing PROTAC-based therapies for pediatric cancers. To further expand his knowledge in TPD, he joined the CeTPD as a visiting student in the beginning of October 2023.

Visit by Parkinson's Dundee Research Interest Group (DRIG)

Contributor: Nur

On Tuesday 6th February, we hosted Parkinson's patients and their families interested in understanding how research in CeTPD and the wider School of Life Sciences (SLS) could contribute to the understanding of disease mechanisms and the discovery of potential treatments.

Communicating what we do to patients and carers

To help scientists translate their research aims and ambitions into language that can be understood by a non-scientific audience, Dr. Andy Howden (UoD SLS) and co-chairs of the DRIG, Brendan and Joanne Goodburn, paired researchers with patients or their carers and tasked each pair with compiling a presentation on the researcher's work.

Five fantastic talks were delivered, sparking a huge variety of a questions and discussions around current and future challenges for patients and research into Parkinson's disease. Will was partnered with Joanne, a caregiver of a Parkinson's patient, and they delivered a presentation on the work carried out in the Farnaby Group on autophagy/mitophagy activator discovery for neurodegenerative diseases (**Photo 1**).

A presentation given by Jo on Farnaby Group research strategies was very well received and initiated great discussions among the visitors during and after the talk, with lots of super interesting questions. Over a lunch break, Nur and Will had a chance to dive into deeper conversations with visitors on Parkinson's disease, their research, and shared their perspectives on possible strategies for coping with their illness as well as life in general.

Visiting CeTPD labs

The visitors then came over to CeTPD where they met with a wider group of CeTPD researchers. Following brief introductions into the scientists' work in CeTPD, Giorgia Kidd led a presentation of our outreach activities (**Photo 2**) followed by a lab tour (**Photo3**). This was a unique experience for me to meet with people who live with PD. Some genuinely wanted to chat and to be heard because they think their families, consultants or even the PD nurses don't always understand them. Their questions also highlighted how urgently we need to strive to find better treatments to meet their needs. They all wanted to get together more often with researchers and enjoyed their time in CeTPD. We recieved great feedback from the guests, and the event was overall a great success. Huge thanks to everyone who made contributions to this success, including the admin and the operations teams.

Landmark Paper

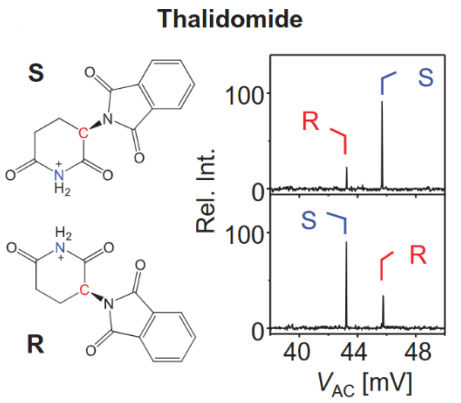
Chemistry

Contributor: Alex

Differentiating enantiomers by directional rotation of ions in a mass spectrometer

Xiaoyu Zhou[§], …, Zheng Ouyang*

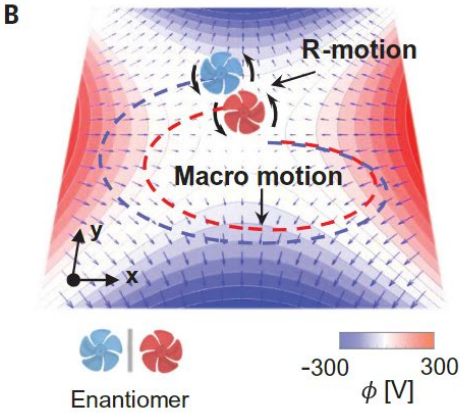
Science **2024**, *383*, 612-618, DOI: 10.1126/science.adj8342

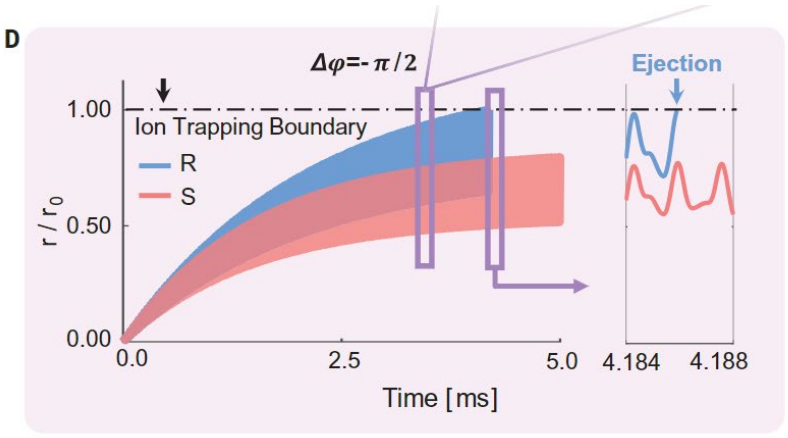


Zhou et al. report a novel mass spectrometry (MS) technique which enables the differentiation of enantiomers. By being able to distinguish between enantiomers, the stereochemical purity of compounds can also be determined. In its current form, this method can determine *ee* values up to 98%, although the authors suggest that this upper limit may be improved with further method development. As chiral molecules only interact differently when subjected to a chiral environment, this technique uses dual alternating current excitations to induce rotations around the centre of mass ("R-motion") as well as rotations around the centre of the trap ("macro motion"). This subtle combination of R-motion and macro motion enables the two enantiomers to be distinguished.

The authors validated this approach on various biologically relevant molecules such as, amino acids, sugars and drugs, including thalidomide. Additionally, the authors showcased this technique's potential application to asymmetric catalysis and the high-throughput screening of catalysts and methodologies.

Although this method is currently unable to determine the absolute stereochemistry of molecules without the use of a standard, there may be relationships between absolute chirality and the induced rotations in the ion trap. If this would be possible, it could greatly improve the speed and ease at which the absolute chirality of molecules could be determined. The ramifications of this work are not to be overlooked; being able to differentiate the chirality of molecules in the gas phase, under vacuum, whilst hurtling at immense speed through a charged tunnel is something of a marvel.





Targeted Protein Degradation

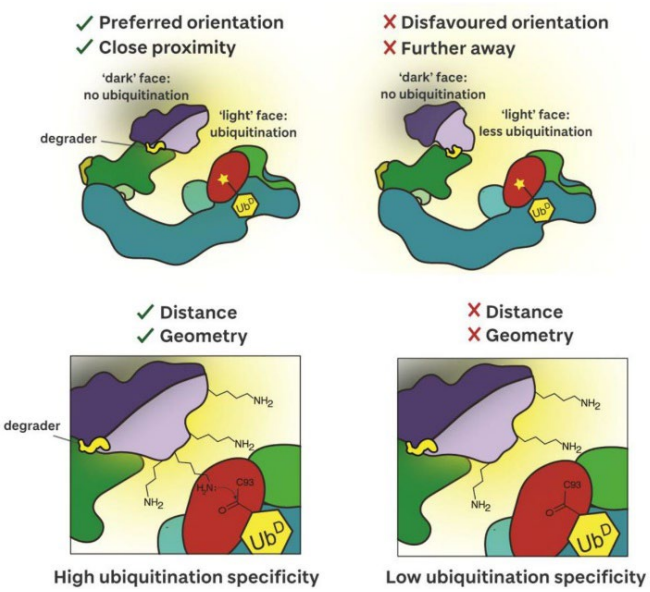
Cell Biology | Computational Chemistry | Modelling/Simulation | Structural Biology/Biophysics

Contributor: Elisha

Mechanism of degrader-targeted protein ubiquitinability

Charlotte Crowe (s)[§], …, Alessio Ciulli*

BioRxiv **2024**, https://doi.org/10.1101/2024.02.05.578957



In this study, the authors solve a cryo-EM structure of the Cullin 2 RING ligase complexed with BRD-targeting PROTAC, MZ1, alongside its BRD4 substrate and associated E2-ubiquitin bound. Using *in-vitro* ubiquitylation assays coupled with mass spectrometry the team identify eight lysine residues on $BRD4^{BD2}$ that become ubiquitylated during this process. Of these residues, several are conserved between the BET proteins, but two residues are strictly conserved in BRD4 and BRD2 and thus may confer some specificity of MZ1 activity toward these substrates compared to BRD3.

The structure highlights the ability of MZ1, once bound to VHL, to adopt a favourable orientation in which one face of the BRD4 substrate is faced toward the E2 and its lysines within range of reacting with the E2~thioester. The authors discuss that having favourable ubiquitin residues facing toward the E2, termed the 'light face' may become a key factor in designing more fast and potent degraders and conversely, may be useful in engineering specificity between alternative protein paralogs or isoforms.

These findings demonstrate the importance of understanding ternary complex formation for ubiquitinability of substrates and will be interesting to see how this can aid further degrader design and optimisation.

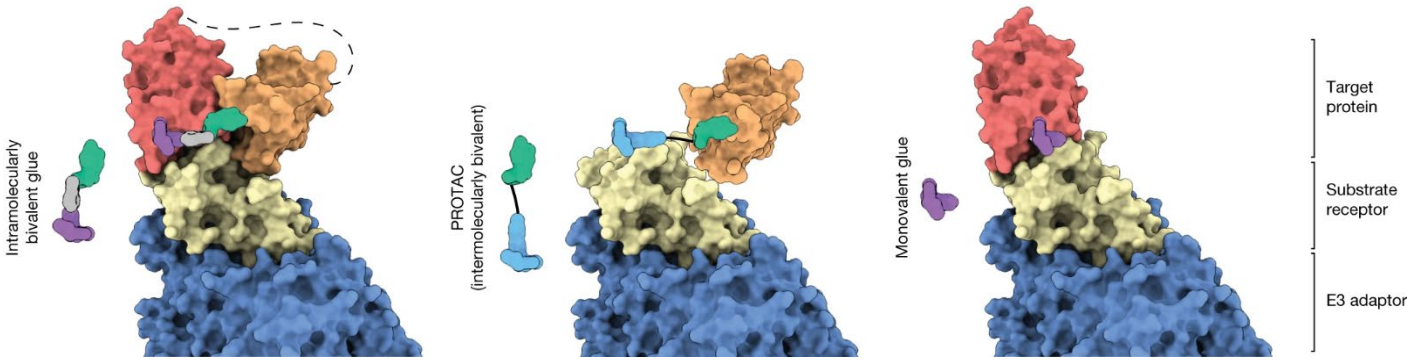
Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Elisha

Targeted protein degradation via intramolecular bivalent glues

Oliver Hsia[§], Matthias Hinterndorfer[§], Angus D. Cowan[§], …, Georg E. Winter *, Alessio Ciulli*

Nature **2024**, https://doi.org/10.1038/s41586-024-07089-6



We previously discussed this article in our journal club when it was initially published on bioRxiv. Given the significant changes made in the final version, we believe it is worthwhile to revisit and cover it again here:

This exciting study introduces the novel concept of 'intramolecular bivalent glues', degraders that are capable of simultaneously engaging two adjacent domains of the target protein. The authors utilise the compound IBG1, comprising of BET family inhibitor JQ1 tethered to E8720, a sulphonamide used for the degradation of RBM39 via the DCAF15 CRL complex. IBG1 was shown to efficient activity against BET family proteins BRD2 and BRD4. Interestingly,

despite being based on E8720, a previously characterised DCAF15-targeting molecular glue, the authors identified members of the CRL4-DCAF16 complex as key for IBG1-mediated degradation of BRD2 and BRD4.

The authors then went on to solve the cryo-EM structure of the ternary complex formed between BRD4, IBG1 and DCAF16-DDB1. In this cryo-EM structure, a single molecule of IBG1 simultaneously engages both bromodomains of BRD4. Here, IBG1 demonstrates both a canonical binding of JQ1 to the acetyllysine pocket of BD2 and an unexpected binding of E8720 to the equivalent pocket of BD1. On these structural insights, a further series of degraders were synthesised showing improvements in BR2 and BRD4 degradation to picomolar potencies.

Intramolecular glueing is an exciting novel concept and it will be interesting to see this whether this mechanism can be exploited to target a wider range of disease-causing proteins for degradation.

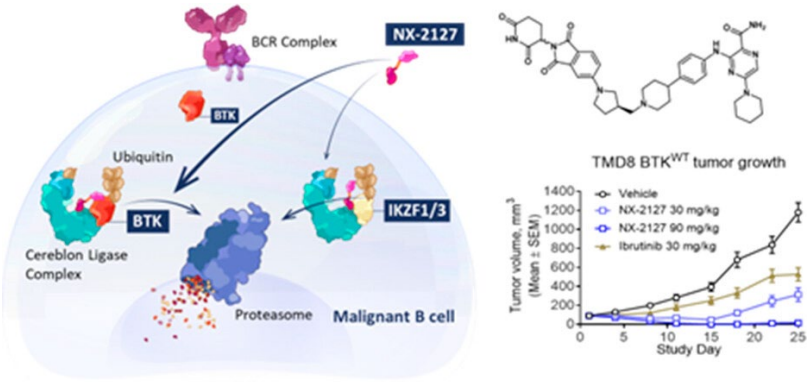
Cell Biology **Chemistry**

Contributor: Simon

Discovery and Preclinical Pharmacology of NX-2127, an Orally Bioavailable Degrader of Bruton's Tyrosine Kinase with Immunomodulatory Activity for the Treatment of Patients with B Cell Malignancies

Daniel W. Robbins[§], …, Frederick Cohen(s)*

J Med Chem **2024**, *67*, 2321



Bruton's tyrosine kinase (BTK) is pivotal in B-cell receptor signalling, making it an appealing target in B-cell malignancies. This article outlines the preclinical discovery process of NX-2127, an advanced BTK degrader. The authors utilized a combinatorial synthesis approach, assessing various BTK ligands and flexible linkers connected to thalidomide- based CRBN binders to identify BTK degraders. Further optimization involved changing the exit vector position of thalidomide, linker rigidification, and reducing the MW by optimizing the BTK ligand. In addition to BTK degradation, the authors retained the thalidomide moiety's molecular glue activity, enabling concurrent degradation of IKZF1/3 thereby providing additional immunomodulatory effects. Their efforts culminated in NX-2127, a potent triple BTK/IKZF1/IKZF3 degrader effective *in vitro* and *in vivo*, demonstrating antitumor effects in both wild-type and inhibitor-resistant BTK^{C481S} mutant tumor mouse models. Comprehensive preclinical evaluation, including pharmacokinetics, pharmacodynamics and toxicology studies supported the progression of NX-2172 into clinical trials (NCT04830137).

This paper provides an excellent overview of a state-of-the-art medicinal chemistry campaign aimed at developing an orally bioavailable clinical candidate PROTAC. In addition to the structure-activity relationship studies discussed, it is noteworthy for the extensive pharmacokinetic and corresponding *in vivo* pharmacodynamic studies nicely discussed here.

Cell Biology | Structural Biology/Biophysics

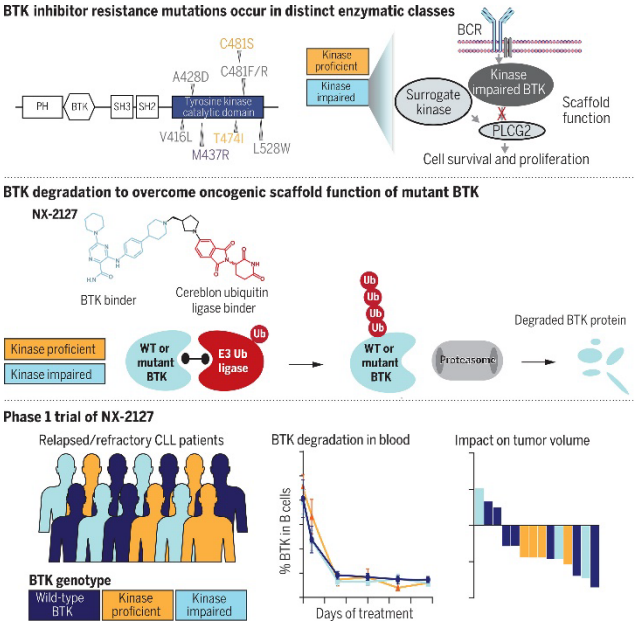
Contributor: Simon

Kinase-impaired BTK mutations are susceptible to clinical-stage BTK and IKZF1/3 degrader NX-2127

Skye Montoya[§], Jessie Bourcier[§], Mark Noviski[§], Hao Lu[§], …, Gwenn M. Hansen*, Omar Abdel-Wahab*, Justin Taylor*

Science, **2024**, *383*, 496

This study elucidates the mechanism behind acquired BTK mutations leading to current BTK inhibitor resistance. Through BTK enzymatic activity assays, two distinct groups of BTK inhibitor resistance mutations are identified: those retaining kinase activity and those impairing kinase function. Despite reduced catalytic activity of the latter, they sustain downstream BCR signalling in malignant B cells. Utilizing IP mass spectrometry, kinase impaired BTK mutants are shown to sustain downstream signalling through protein interactions with surrogate kinases. This scaffolding function can be efficiently targeted by the BTK degrader NX-2127, inhibiting BCR signalling more efficiently. Furthermore, NX-2127 can degrade all recurrent drug-resistant forms of mutant BTK *in vitro* and demonstrated significant degradation in a phase I clinical trial involving patients with diverse BTK mutations.



These findings once again highlight the effectiveness of a degrader strategy in overcoming mutations that confer resistance to multiple classes of enzymatic inhibitors by targeting scaffolding functions. Additionally, NX-2127 demonstrated clinical benefits in patients with tumors resistant to current BTK inhibitors, thereby filling the clinical pipeline with the next generation of BTK-targeting drugs. An elegant example showcasing that PROTACs continue to deliver on their potential!

Cell Biology | Chemistry | Structural Biology/Biophysics

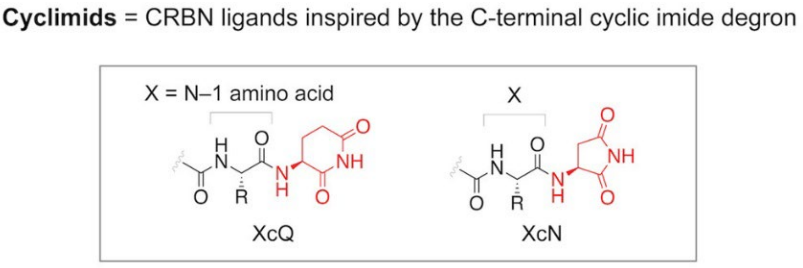
Contributor: Liam

The cyclimids: Degron-inspired cereblon binders for targeted protein degradation

Saki Ichikawa [§], Connor Payne[§], …, Ralph Mazitschek*, Christina M. Woo*

Cell Chem. Biol. **2024**, 31, e1

Ichikawa *et al.* report the synthesis and evaluation of a library of dipeptidic CRBN binders, termed cyclimids, for use in bifunctional degraders. The cyclimids investigated in this study each featured either a cyclised asparagine or glutamine at the C-terminus and one of the 20 natural amino acids at the N-1 position. Interestingly, they found that cyclimids with a 5-membered cyclic asparagine as the tryptophan cage-engaging moiety were more effective at engaging CRBN than their 6-membered glutarimide counterparts.



They also observed that judicious choice of N-1 amino acid could be exploited to confer selectivity for degradation of BRD4-BD1 over BRD4-BD2 and CDK6 over CDK4. Of particular interest, they observed that their cyclimid-based degraders did not induce off-target degradation of known IMiD neosubstrates, including a range of zinc finger proteins, SALL4 and GSPT1. Taken together, these findings provide very useful insights into the design of highly selective CRBN-based bifunctional degraders.

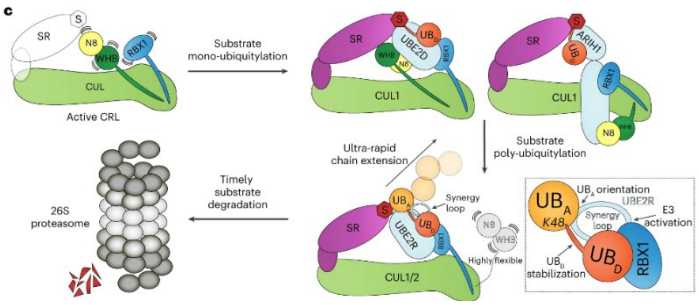
Cell Biology | Structural Biology/Biophysics

Contributor: Elisha

Mechanism of millisecond Lys48-linked poly-ubiquitin chain formation by cullin-RING ligases

Joanna Liwocha [§], Jerry Li[§], …, Brenda A. Schulman*, Gary Kleiger*

Nat Struct Mol Biol, **2024**, 31, 378



Here the Liwocha and Li *et al.* solve the cryo-EM structure of an active CRL complex containing the NEDDylated CUL2-RBX1-ELONGIN-B/C-FEM1C complex bound to a ubiquitin bound E2, UBE2R2, and linked to a primed ubiquitylated peptide substrate. Using this structure, the authors uncover the mechanism of CRL-mediated K48-ubiquitin chain elongation. Using various mutants, the authors uncover that UBE2R2 contains a unique loop near its C-terminal tail, termed the synergy loop, allowing simultaneous engagement of Rbx1, the donor ubiquitin and the acceptor ubiquitin. This engagement allows for optimal arrangement for efficient ubiquitin transfer to the substrate.

The authors are also able to demonstrate that whilst NEDDylation of CUL2 is required for catalysis, NEDD8 does not mediate proximity between UBE2R2 and the ubiquitin primed substrate as would be expected. Instead, NEDDylation of CUL2 releases the Rbx1 RING domain allowing optimal interactions with UBE2R2 and facilitating rapid millisecond catalysis. This work gives great insights into the mechanism of ubiquitin chain extension, and I am excited to see whether this mechanism is conserved for other CRL complexes.

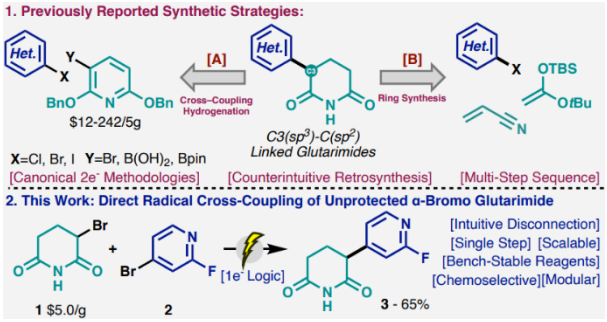
Chemistry

Contributor: Simon

Simplifying Access to Targeted Protein Degraders via Nickel Electrocatalytic Cross-Coupling

Philipp Neigenfind[§], Luca Massaro[§], …, Phil S. Baran*

Angew. Chem. In. ed. **2024**, *e202319856*



This manuscript discusses a novel method for synthesizing C(sp^2)-C(sp^3) linked (hetero)aryl-glutaramides, serving as alternative CRBN binders in PROTAC designs. The approach involves a direct, electrochemically enabled nickel-catalyzed cross-coupling of unprotected α-bromoglutarimide with various (hetero)aryl halides. A key discovery was the requirement of a Brønsted acid, such as AcOH, to facilitate the transformation. The utility of the method was further demonstrated by exploring its substrate scope with various (hetero)aryl moieties and the ease of up-scaling was showcased.

Thalidomide-based PROTACs have inherent issues like plasma instability and off-target degradation, prompting exploration alternative CRBN binders such as (hetero)aryl glutarimides, and dihydrouracils. However current synthesis methods for these glutarimides are multistep and low yielding. The strategy reported here, characterized by operational simplicity and broad functional group tolerability, clearly overcomes limitations of existing synthesis methods. The tested substrate scope contains useful scaffolds allowing further linker attachment, and I look forward to seeing this methodology in future PROTAC programs.

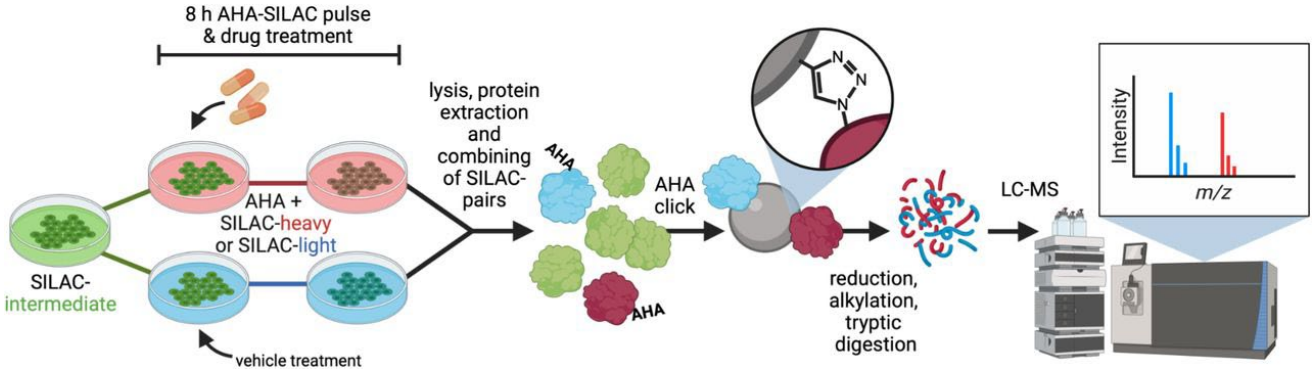
Cell Biology

Contributor: Liam

Degradome analysis to identify direct protein substrates of small-molecule degraders

Marco Jochem[§], …, Jeroen Krijgsveld*

BioRxiv, **2024**, doi: 10.1101/2024.01.28.577572



Jochem *et al.* present an elegant SILAC- and click-chemistry-based whole-cell proteomics workflow to establish the degradome of small molecule degraders. This approach allows for the specific isotopic labelling and enrichment of proteins which were synthesised prior to treatment with a degrader compound. This makes it possible to distinguish these proteins from those which were synthesised during treatment and consequently allows for differentiation between those proteins for which changes in abundance are a direct result of degradation and those for which it is a result of a downstream effect on a biological pathway, such as transcription or translation.

To demonstrate the utility of this approach, they profiled the molecular glues dCeMM2 (a Cyclin K, CDK12 and CDK13 degrader), and dCeMM4 (a Cyclin K degrader) and CC-885 (a GSPT1, IKZF1 and IKZF2 degrader) and found that their SILAC-based approach gave a more precise picture of the degradome of these compounds than was achieved with a TMT-labelling approach (for dCeMM2 and dCeMM4), or by label-free data independent acquisition MS (for CC-885). The also used their workflow to identify the targets of 'degrader compound 1' for which the targets were previously unknown.

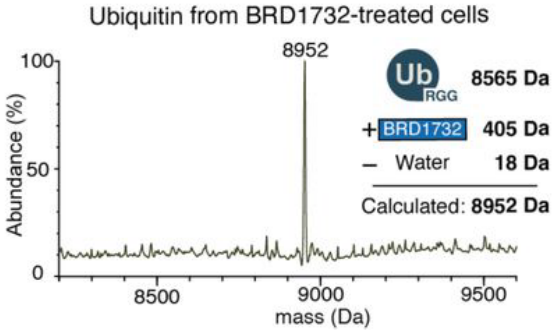
Cell Biology

Contributor: Liam

Highly specific intracellular ubiquitination of a small molecule

Weicheng Li[§], Enrique M. Garcia-Rivera[§], …, Jonathan M.L. Ostrem*

BioRxiv **2024**, doi: 10.1101/2024.01.26.577493



Li *et al.* present the characterisation of the small molecule BRD1732, a cytotoxic compound which is ubiquitinated in cells, resulting in a broad disruption of the UPS. This cytotoxicity of BRD1732 was found to be dependent on the E3 ligases RNF19A and RNF19B in a CRISPR-Cas9 resistance screen. A PRISM screen confirmed these findings and also implicated the E2 ligase UBE2L3.

The team observed a curious gel shift in monoubiquitin in cells that were treated with BRD1732 and decided to investigate further. MS analysis revealed that this was due to an apparent adduct between BRD1732 and ubiquitin, with fragmentation analysis suggesting that this adduct formed between the C-terminal glycine of ubiquitin and BRD1732. Quantitative proteomics revealed that treatment with BRD1732 significantly alters the abundance of over 300 different proteins, with a profile that resembled that of various proteasome inhibitors and the neddylation inhibitor MLN4924. It is clear from this study that BRD1732 interferes significantly with the UPS, however the precise MOA remains unclear.

It was highlighted by the authors that ubiquitination of a small molecule by the UPS opens the exciting possibility of using a small molecule as a conduit to deliver a posttranslational modification to a target protein.

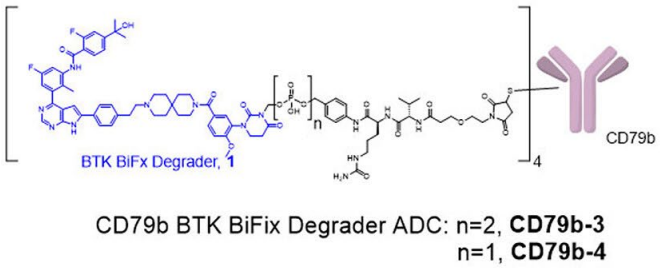
Cell Biology | Chemistry

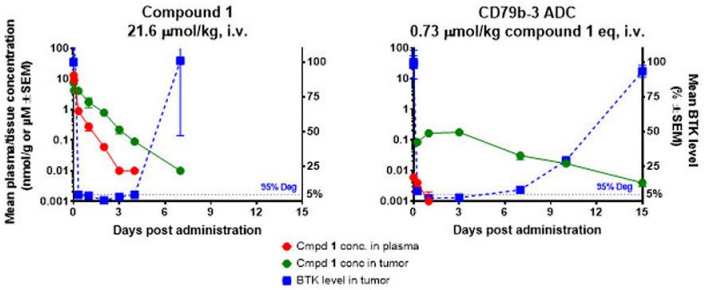
Contributor: Simon

Design, Synthesis, and In Vitro and In Vivo Evaluation of Cereblon Binding Bruton's Tyrosine Kinase (BTK) Degrader CD79b Targeted Antibody–Drug Conjugates

Alan Zhang[§], …, Matthew T. Burger*

Bioconjugate Chem. **2024**, *35*, 2, 140





This study details the development of a CD79b antibody drug conjugate (ADC) incorporating a bifunctional Bruton's tyrosine kinase (BTK) degrader. The ADC was constructed by linking the dihydrouracil nitrogen of the CRBN binder portion of the BTK degrader (compound 1) to a CD79b antibody through a cleavable linker. Results demonstrated that the CD79b-ADC exhibited comparable in vitro BTK degradation and antiproliferative effects when compared to the parent BTK degrader. In vivo pharmacokinetic/pharmacodynamic (PK/PD) studies indicated an extended exposure over time of the released payload in tumor cells for the CD79b-ADC, in contrast to intravenous administration of the free PROTAC. This exposure was associated with prolonged in-tumor BTK degradation for the CD79b-ADC compared to the free payload. Additionally, systemic exposure of the released PROTAC from CD79b-ADC was significantly reduced.

ADCs have proven to be a successful modality in anti-cancer research. This study clearly provides proof-of-concept for a BTK-degrader ADC to degrade BTK in a tumor mouse model more prolonged than the free payload, supporting further exploration of the technology. However, despite lower systemic concentrations being noticed, it is yet to be seen if the ADC show advantages in terms of on- (and off)-target toxicity.

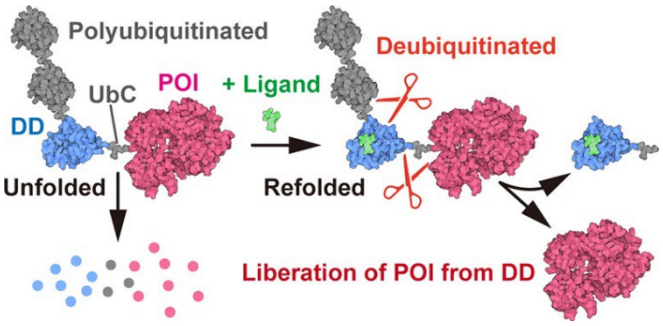
Cell Biology

Contributor: Liam

Ubiquitin-Derived Fragment as a Peptide Linker for the Efficient Cleavage of a Target Protein from a Degron

Yuki Utsugi[§], …, Yusaku Miyamae*

ACS Chem. Biol. **2024**, *19* (2), 497



Utsugi *et al*. present a creative method for the modulation of cellular protein levels. Their approach involves the expression of a POI as a fusion protein with a short DUB-cleavable linker and a destabilising domain (DD). When expressed in the absence of a stabilising ligand, the DD will be rapidly ubiquitinated and the fusion protein degraded. When the ligand is present, the DD adopts a stable fold, and the DUB-cleavable linker is cleaved to release the POI. The authors highlight that the advantages of this method over other traceless tagging methods are that it requires the expression of only one component, the linker is cleaved by endogenous protease machinery and the cleavage is initiated using ligands which do not have other activity in eukaryotic cells.

They use the proto-oncogene tyrosine-protein kinase (Src) to exemplify the utility of this method, as a free N-terminus is required for proper localisation, while the C-terminus is required for protein activation. Consequently, any tagging method which blocks these termini will prevent the protein from functioning correctly. They showed that upon treatment with stabilising ligand, Src was successfully cleaved from the fusion protein and was observed to localise correctly to the plasma membrane.

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