CeTPD Journal Club

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

November 2023

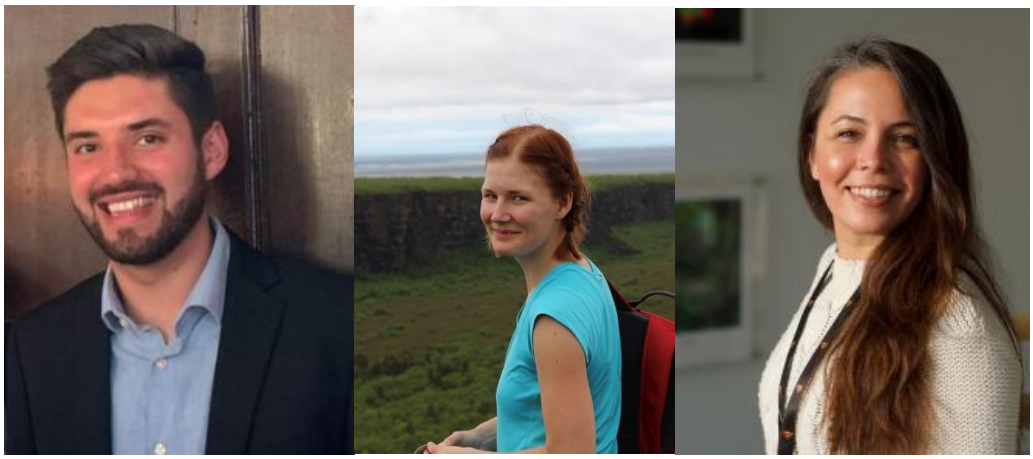
Centre for Targeted Protein Degradation
University of Dundee

innovate
collaborate
inspire

Journal Club

Contents

Meet this Month's Editors



This month's editors are (from left to right): Adam Pinto, Alena Kroupova and Nur Kocaturk

"The Journal Club provides an excellent basis for the exploration of TPD literature, giving concise snapshots of the most relevant papers."

Adam received his MChem in 2022 from the University of Oxford. He then joined the Ciulli group as an Associate Scientist in Medicinal and Organic Chemistry, within the Boehringer Ingelheim collaboration team. At present he is a first year PhD student in the Farnaby group, focussing on molecular glue development in the CNS area.

"The Journal Club is a unique resource for me as a researcher in the field providing a quick insight into relevant literature and so it is a delight to contribute to it as one of the editors."

Alena completed her undergraduate studies at the University of Edinburgh after which she pursued a PhD in structural biology at the University of Zurich. She joined the exciting PROTAC field in September 2021 as a structural biologist/biophysicist within the CeTPD-Almirall collaboration.

"The Journal Club is a great tool highlighting most recent research in TPD field in a practical way."

Nur joined Farnaby group as a postdoctoral scientist (Cell Biology and Chemoproteomics) in July 2023. Nur completed her PhD in Molecular Biology and Genetics focusing on cellular degradation systems and their crosslink during mitophagy in Sabanci University. She started working on TPD in Parkinson's Disease research at MRC PPU with Prof. Gopal Sapkota in 2021 and now working on molecular glue and PROTAC discovery in CNS disease models.

University of Dundee and Amphista Theraputics Win the PraxisAuril KE Awards 2023 for Commercialisation Achievement of the Year

Contributor: Alessio

On Thursday 23rd November I joined David McBeth, VP for Enterprise and Economic Transformation at Dundee, and colleagues from the University's RIS and Amphista therapeutics in Leeds city centre for the 2023 PraxisAuril KE awards ceremony.

The PraxisAuril KE Awards embody the high standards of UK Knowledge Exchange as a sector and recognise the people, partnerships, deals and initiatives that underpin this world-class activity. The Ceremony revealed the much-anticipated KE Awards winners for each category. The University of Dundee and Amphista were nominated as finalists in the category for "Commercialisation Achievement of the Year". Each nominee was required to give a 1-2 mins speech to introduce themselves and their nomination, David delivered the pitch for the University and Amphista (summary below). Shortly after, the usual drum-roll came: *"And the winner is…."*, to our surprise and delight it was Dundee and Amphista! Georgia Chapman, from Amphista, and I joined David on stage to receive the award. We each said brief thank-yous on stage, followed by the customary award-winning photographs. The usual social media and direct messaging frenzy then unfolded (see my tweet here). All in all, it was a jolly good evening, which witnessed discussions and open conversations about the challenges and opportunities in innovation and research commercialisation with colleagues. Following a brief rest, I managed to take the 5:41am train back to CeTPD and write this 😉! I feel humble and touched to have had the opportunity to join colleagues in Leeds on this occasion. This award really belongs to everyone at Dundee, the company, the investors and beyond, who have contributed to bring Amphista along its journey so far and helped achieving the commercialisation success that was recognised by the Award.

Brief synopsis of David McBeth's nomination speech, which included a mention to CeTPD:

- Amphista was formed in 2017 on the basis of Prof Alessio Ciulli's group research on targeted protein degradation (TPD).
- TPD is an entirely new approach to therapeutics – it works by reducing the levels of proteins that drive disease, opening new ways to target proteins previously thought as "undruggable" and so create medicines that matter to patients with genuine unmet needs.
- Amphista develops next-generation TPD platforms and medicines. Venture Capital firm Advent Life Sciences was in from the start working with the University RIS team and the founders to develop a highly differentiated, and crucially, innovative concept to arrive at degrader medicines.
- Rapid technological progress, first within the Ciulli Lab at Dundee, and later at BioCity, Newhouse (near Glasgow) saw series A ($7.5M) and B rounds in short order with the latter (in 2021) raising $53M and Winning a Scrip award in 2021.

- The commercialisation achievements for 2022 mentioned in the nomination were:
    - Licensing collaborations with big pharma Merck and Bristol Myers Squibb valued at more than US$2.125bn, representing the largest deal globally to date in the TPD space
    - Now >70 employees in their corporate HQ in Grant Park, Cambridge
    - Recent recognition in PWC Life Sciences Future 50 high growth UK biotechs
- Dundee's role as a recognised leader in TPD is now solidified with the University's creation of a new Centre for Targeted Protein Degradation (CeTPD) under the directorship of Ciulli. CeTPD is a flagship new Institute to the University and was praised in the recent Government's DSIT response to the Nurse review of the research landscape (which published on the same day). CeTPD is integral to the University's ambition to establish a Life Sciences Innovation District cluster to further foster innovation and translation at Dundee.

Targeted Protein Degradation

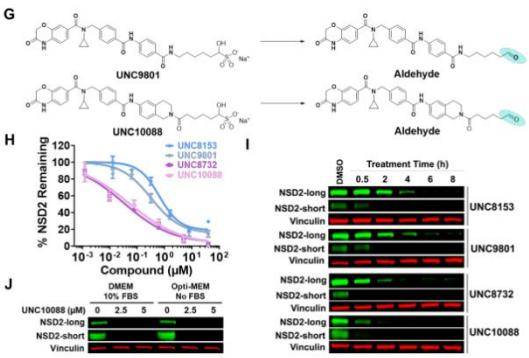
Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Ollie

Recruitment of FBXO22 for Targeted Degradation of NSD2

David Y. Nie[§], John R. Tabor[§], …, Cheryl H. Arrowsmith*

bioRxiv **2023**, DOI: 10.1101/2023.11.01.564830



This preprint is a fascinating mechanistic follow-up to a recently reported degrader of NSD2, an important gene regulator whose aberrant activity or somatic mutations are frequently present in MM and ALL patients. To date, traditional inhibition of NSD2 has not proved to be of therapeutic benefit. The present study unravels the mechanism by which the first-generation compound UNC8153, and the improved second-generation compound UNC8732, can degrade NSD2. Unexpectedly, despite the initial intended mechanism being to target 'N-recognin' E3 ligases including UBR1, UBR2, UBR4, and UBR5; the authors determine that the primary amine of the alkyl 'tail' is converted to an aldehyde species inside cells, which is then able to covalently engage C326 of the FBXO22 in a reversible manner. SCF^{FBXO22} is then able to promote the proteasomal degradation of NSD2.

Intriguingly, and speaking to some generality of this mechanism, the authors also demonstrate that an unrelated XIAP-targeting degrader, similarly harbouring a primary amine 'tail', is also able to promote SCF^{FBXO22}-dependent degradation. In both degrader cases, the activity is blocked by an amine oxidase inhibitor, which may allude to some sort of quality control mechanism by which SCF^{FBXO22} regulates oxidative stress-induced aldehyde formation. The authors note that FBX022 has elevated expression in certain tumour tissues, thus making it a potentially attractive substrate receptor for therapeutic interventions.

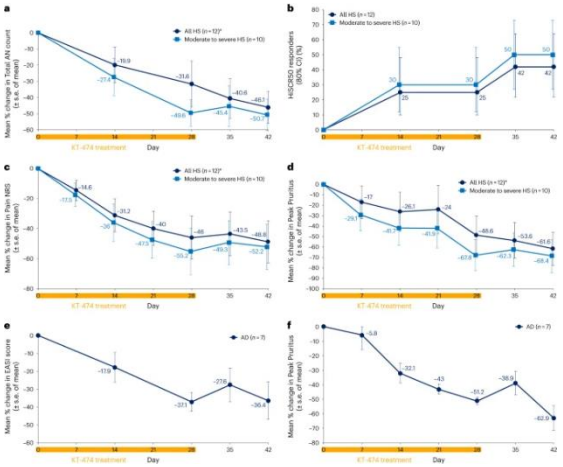
Cell Biology

Contributor: Ollie

IRAK4 degrader in hidradenitis suppurativa and atopic dermatitis: a phase 1 trial

Lindsay Ackerman[§], …, Jared A. Gollob*

Nature Medicine, **2023**, DOI: 10.1038/s41591-023-02635-7



In this landmark, first publication of clinical trial results for a heterobifunctional degrader, the team at Kymera Therapeutics present encouraging phase 1 data for their CRBN-recruiting IRAK4 PROTAC for the treatment of chronic inflammatory skin diseases. This also represents a key milestone in the treatment of non-lethal and non-oncological diseases, which remain under-investigated areas using targeted protein degradation.

The risk of any off-target effects for IRAK4 degraders is even less tolerable than for lethal diseases, and as such Ackerman and colleagues made significant alterations to the IMiD scaffold employed to recruit CRBN, so as to avoid any undesirable degradation of canonical IMiD substrates. Orally administered KT-474 promoted profound and sustained degradation of IRAK4, but the authors observed a distinct PK-PD relationship in skin compared to blood highlighting the challenges with appropriate dosing regimen for the target tissue. Despite some mild side effects, KT-474 demonstrated both objective and subjective improvement in clinical measures for patients with hidradenitis suppurativa and atopic dermatitis as well as a systemic reduction in inflammation. This study was limited to 28 days of dosing, but the data are consistent with IRAK4 degradation being superior to inhibition, and hopefully further placebo-controlled and longer-duration studies will further corroborate and advance PROTACs for the treatment of non-oncology diseases.

Cell Biology | Chemistry

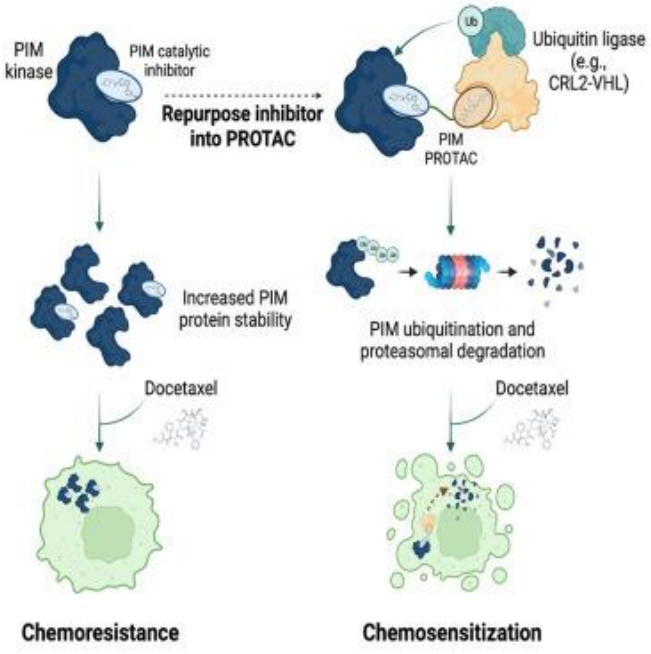
Contributor: Nur

PIM1 targeted degradation prevents the emergence of chemoresistance in prostate cancer

Pedro Torres-Ayuso[§], …, Noel A. Warfel*, John Brognard*

Cell Chem Bio **2023**, DOI: 10.1016/j.chembiol.2023.10.023

PIM kinases are involved in oncogenic roles, and therefore have been a long-term focus for drug discovery. Consequently, multiple PIM kinase inhibitors have entered clinical trials. In this paper the authors showed that current inhibitors stabilise all three isoforms of PIM (1/2/3) which leads resistance to PIM inhibitors. They designed PROTACs by coupling various E3-ubiquitin ligase warheads to PIM kinase inhibitors *via* a linker and found that VHL-recruiting PROTACs displayed preference and more potency for PIM1. PIMs were targeted for degradation effectively *via* the proteasome, with greater efficacy at inducing cell death than inhibition.



This study provides extensive evidence of kinase activity-independent tumorigenic roles of the PIM proteins in various pancreatic cell lines. Despite off-target effects, re-purposing inhibitors and targeting PIMs for degradation to overcome chemoresistance is an attractive strategy when combinatorial approaches are utilised, and further *in vivo* validations have been done.

Structural Biology/Biophysics

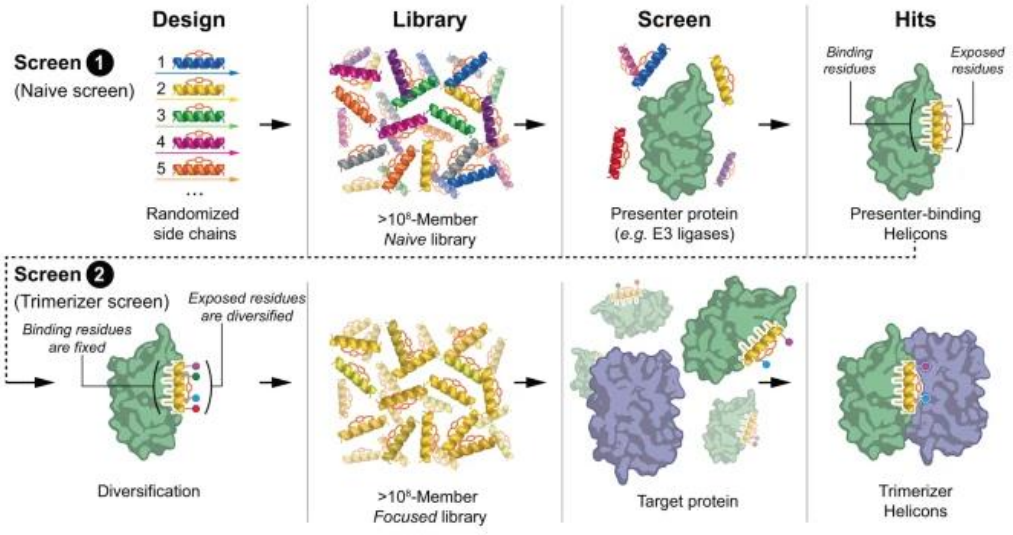
Contributor: Alena

Recognition and reprogramming of E3 ubiquitin ligase surfaces by α-helical peptides

Olena S. Tokareva[§], …, Gregory L. Verdine*, John H. McGee*

Nat. Commun. **2023**, *14*, 6992

The lack of known binders for most E3 ubiquitin ligases presents one of the main reasons why it is challenging to harness them for targeted protein degradation. In this paper, Tokareva and colleagues describe a new method to discover molecular glue-like α-helically constrained polypeptides, termed trimeriser Helicons, which bind to E3 ubiquitin ligases and induce new protein-protein interactions.



They used a two-step screening approach whereby the first naive screen allows the identification of E3-binding Helicons followed by a focused screen to identify trimerisers for a specific protein of interest. Specifically, they identified trimerisers that induce the formation of cooperative ternary complexes between CHIP E3 ligase and TEAD4 or PPIA, and the MDM2 E3 ligase and ß-catenin, demonstrating utility of their method for variety of E3 ligases and substrates.

This intriguing study expands the traditional small-molecule molecular glue space with providing elegant methodology for efficient design of new peptide degraders. Whilst the authors provide a thorough biophysical and structural analysis of the trimeriser-induced ternary complexes, it remains to be determined whether these binding modes are productive for degradation of the target proteins in vivo.

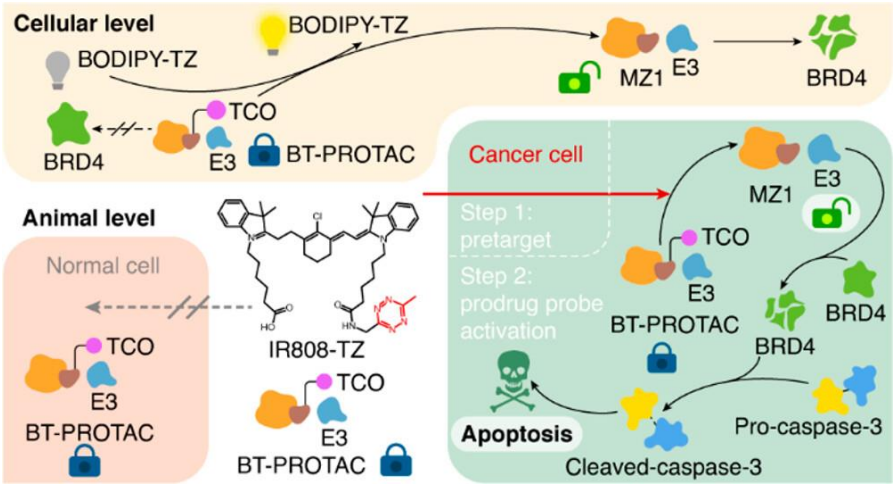
Cell Biology | Chemistry

Contributor: Alena

Rational design of bioorthogonally activatable PROTAC for tumor-targeted protein degradation

Tao Bi[§], …, Sijin Yang*, Wei Ren*, Zengjin Liu*

J. Med. Chem. **2023**, *66*, 14843



Systemic cytotoxicity of PROTACs presents a challenge for their clinical use and different approaches are being explored to overcome this issue. In this study, Bi and colleagues design a pre-target strategy that allows the biorthogonal activation of BT-PROTAC, an MZ1-derived prodrug, specifically in cancer cell. The activated BT-PROTAC shows comparable proteasome-dependent degradation of BRD4 to MZ1 in vitro. Using the tumour targeting dye IR808 to deliver the activator tetrazine BODIPY-TZ, they also achieve excellent tumour growth inhibition in vivo.

This compelling study extends the repertoire of prodrug strategies to enable tissue specificity and tackle systemic toxicity of PROTACs. A compelling proof-of-concept, the applicability of this system will likely require extensive optimization for each particular clinical target.

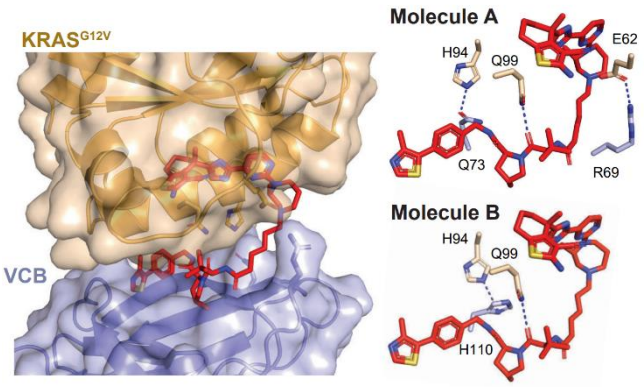
Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Alena

Targeting cancer with small molecule pan-KRAS degraders

Johannes Popow[§], William Farnaby[§], Andreas Gollner[§], Christiane Kofink[§] …, Peter Ettmayer*, Alessio Ciulli*

bioRxiv **2023**, DOI: 10.1101/2023.10.24.563163



The KRAS oncogene is a prototype of a high-profile cancer target previously considered undruggable with a limited set of mutation-specific inhibitors available for the multitude of KRAS variants. In this preprint, Popow and colleagues present a pan-KRAS VHL-based PROTAC, ACBI3, which effectively degrades 13 out of the 17 most prevalent oncogenic KRAS mutants in vitro and in vivo.

This study showcases the power of structure-based PROTAC design where the optimization of the stability and durability of the ternary complex leads to rapid improvement in target degradation and ultimately tumour regression. The comprehensive suite of structural, biophysical and cell biology techniques all the way to *in vivo* proof of principle illustrates an elegant journey through a PROTAC drug discovery project.

Chemistry | Structural Biology/Biophysics

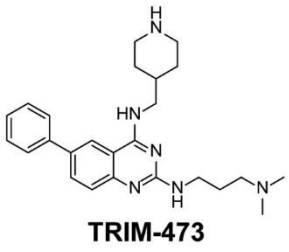
Contributor: Adam

Discovery of Ligands for TRIM58, a Novel Tissue-Selective E3 Ligase

Klemens Hoehenauer[§], …, Martin Renatus[§]*, John S. Reece-Hoyes*

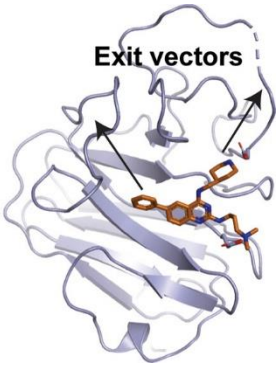
ACS Med. Chem. Lett. **2023**, DOI: 10.1021/acsmedchemlett.3c00259

The authors report the discovery of novel ligands for the PRY-SPRY domain of TRIM58, a RING ligase. TRIM58 is expressed specifically in erythroid precursor cells, which could allow PROTACs that recruit the E3 ligase to degrade neo-substrates in a tissue specific manner. In the study Hoehenauer *et. al.* used a differential scanning fluorimetry screen to identify primary hits, one of which was validated by protein-observed NMR, and SPR. A basic SAR of the chemotype was established *via* the use of an FP assay.



TRIM-473

DSF hit
confirmed by SPR and NMR
FP EC_{50} = 5.3 μM



At present the vast majority of PROTACs recruit either cereblon or VHL as the E3 ligase. A lack of known ligands for other, tissue specific, E3 ligases presents an untapped opportunity for the development of degraders with improved safety profiles. Although this work suggests that developing a significantly more potent binder of the PRY-SPRY domain of TRIM58 may be difficult due to the shallow binding surface, this ligand may still allow the development of effective PROTACs, as it has been shown that weak ligase binders can still lead to potent degraders. This work highlights the necessity for the development of binders for currently non-liganded E3s.

Cell Biology | Chemistry | Structural Biology/Biophysics

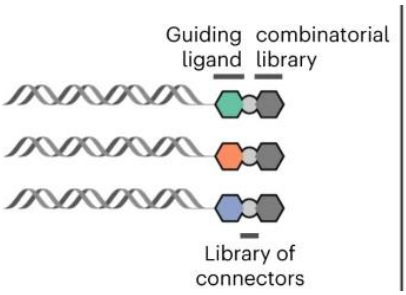
Contributor: Adam

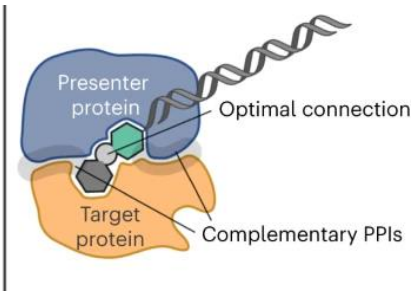
DNA-encoded library-enabled discovery of proximity-inducing small molecules

Jeremy W. Mason[§], …, Shuang Liu*, Frédéric J. Zécri*, Stuart L. Schreiber*

Nat. Chem. Biol. **2023**, DOI: 10.1038/s41589-023-01458-4

A platform for the discovery of compounds that can induce PPIs between two proteins using DELs has been developed. Approximately 1 million DNA-encoded compounds possessing a VHL targeting moiety were generated by a split-and-pool combinatorial approach. The DEL was screened against bromodomains, in the presence and absence of VHL, allowing identification of compounds that could form a ternary complex with $BRD4^{BD1}$ and VHL. MZ1, a known BRD4 degrader, was used as a template for initial library design, it was tagged with an alkyne handle allowing click ligation of a DNA headpiece. The DEL was then generated by identifying a series of linker-ligand combinations that could displace a HIF-1α derived peptide from the VCB complex. Each linker-ligand combination was then DNA-tagged. The linkers contained a terminal amine which could be functionalised with a triazine core. A diverse set of low-MW amine fragments could then complete the functionalisation of the triazine by S_NAr. Affinity based screening of the DEL, followed by off-DNA SPR, TR-FRET and HiBiT degradation assays identified potent degraders of $BRD4^{BD1}$ with a novel BRD4 ligand.





The true power behind DNA-encoded library screening lies with one's ability to test a vast number of compounds against target protein(s) in a single shot. The authors state that a second benefit is that a DEL approach is not restricted to active-site ligands, as all accessible pockets are interrogated. It would be an exciting follow-up to this work to see the same approach used to develop proximity inducing probes for novel, currently intractable target proteins.

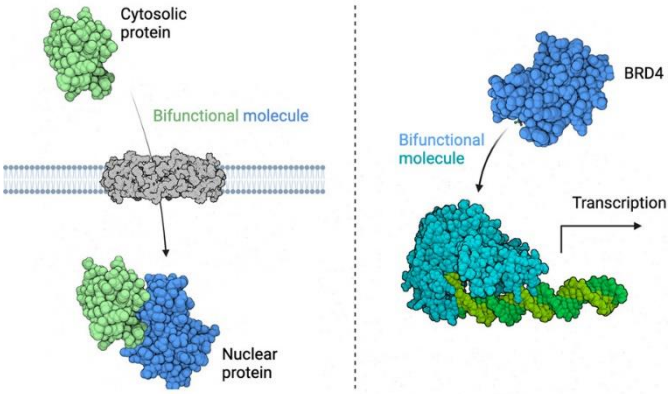
Cell Biology | Chemistry

Contributor: Adam

Bifunctional Small Molecules That Induce Nuclear Localization and Targeted Transcriptional Regulation

William J. Gibson[§*], Ananthan Sadagopan[§], …, Matthew Mayerson*, Stuart L. Schreiber*

J. Am. Chem. Soc. **2023**, *145*, *48*, 26028



Atypical localisation of proteins plays an important role in the development of a variety of cancers and neurodegenerative diseases. In this paper the authors demonstrate that heterobifunctional compounds can induce nuclear import of cytosolic proteins using BRD4 as a carrier for co-import and nuclear trapping. NICE-01, a compound consisting of a BRD4 ligand linked by a PEG2 diamine to an $FKBP^{F36V}$ ligand, is shown to be able translocate a $FKBP^{F36V}$-mEGFP fusion protein rapidly to the nucleus. Gibson *et. al.* went on the elucidate the mechanism of translocation to be predominantly passive diffusion into the nucleus followed by sequestration of the nuclear fraction by BRD4, thus preventing export. It was also shown that cytosol-localised transcription factor IRF1 could be imported into the nucleus and specifically switch on expression of target genes.

As disruption of protein localisation is seen in so many diseases, this work provides a highly significant proof of principle that bifunctional molecules are able to cause proteins to occupy different cellular compartments. If practical uses can be found for specific proteins, this work could be important for the development of localisation probes and potentially therapeutics.

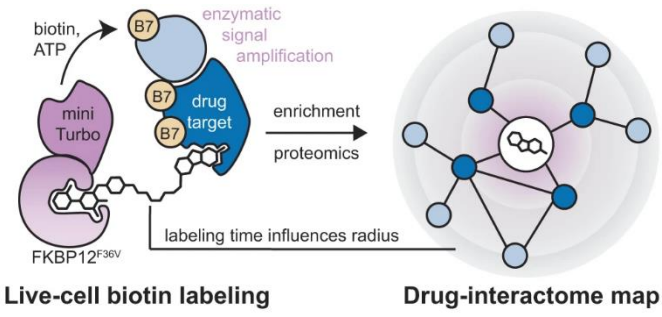
Cell Biology | Chemistry

Contributor: Adam

A biotin targeting chimera (BioTAC) system to map small molecule interactomes in situ

Andrew J. Tao[§], …, Justin G. English*, Fleur M. Ferguson*

Nat. Commun. **2023**, *14*, 8016



A chemoproteomic approach to the identification of small molecule interactomes using a biotinylating probe has been described. The BioTAC system comprises of a bifunctional molecule containing a $FKBP12^{F36V}$ ligand and a ligand for the protein of interest. The $FKBP12^{F36V}$ binding end recruits a miniTurbo tagged protein capable of biotinylating proximal compound-bound complexes, allowing subsequent affinity-based enrichment and purification. The authors showed that the technique successfully identified known proteins in the interactome of BRD2/3/4 using a (+)-JQ1 BioTAC. They also showed the value of the BioTAC platform for target ID, using an Alisertib derivative to identify its target, Aurora kinase A. Tao *et. al.* went on to identify interactors of MEK in the presence of a non-degrader molecular glue.

The BioTAC system provides an alternative approach to target identification and interactome mapping than state of the art photoaffinity labelling, as well as more recent advances such as photocatalytic µMap. When identifying the interactome of a small molecule's target labelling radius is a key consideration. µMap has a very tight labelling radius of ~4 nm and uses short lived carbenes to label making it effective at direct target identification. BioTACs provide a wider labelling radius of around 35 nm better suited to interactome mapping. The technique, especially when used in conjunction with photoaffinity methods will provide a new way to differentiate between off-targets and interactors, as well as enable discovery of how small molecules affect protein complexation.

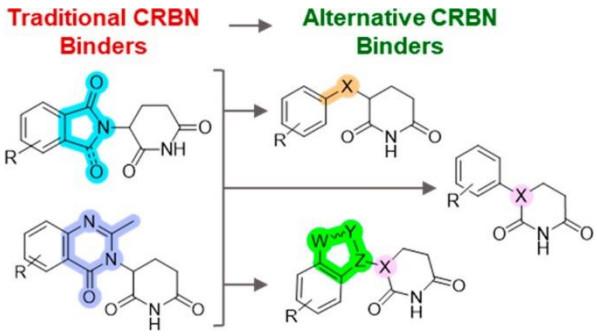
Chemistry

Contributor: Andreas

Design and Synthesis of Novel Cereblon Binders for Use in Targeted Protein Degradation

Stephen Norris§*, Xiaochu Ba§*, …, Deborah S. Mortensen*

J. Med. Chem. **2023**, *66*, *23*, 16388



Within the field of Targeted Protein Degradation (TPD), most bifunctional degraders are based on utilising the E3 ligase complex cereblon (CRBN) with binders such as Lendalidomide and Pomalidomide. In this article, the authors explore the optimisation of protein degraders by modifying CRBN binders to identify alternative CRBN binders that may have potential to serve as both molecular glues and to provide alternative vectors for bifunctional degraders. Challenges in achieving effective protein degradation, such as on-targeted effect and neosubstrate degradation selectivity, are addressed through the design and synthesis of novel CRBN binding moieties. In this study, they identify several alternative CRBN binders with a diverse set of chemical structures, emphasising the impact of subtle changes on compound profiles and degradation.

To overcome these challenges, the researchers explore a matrix of heterobifunctional molecules targeting CRBN-mediated degradation of the androgen receptor. Here, they demonstrate the potential of nontraditional CRBN binders in developing bifunctional degraders, showcasing their application in targeting specific proteins, as well as outlaying the synthetic route to obtain these novel binders. In conclusion, the article underscores the importance of understanding and modifying CRBN ligands for successful CRBN-mediated protein degradation, presenting alternative binders as promising candidates for the development of protein degraders with enhanced therapeutic potential.

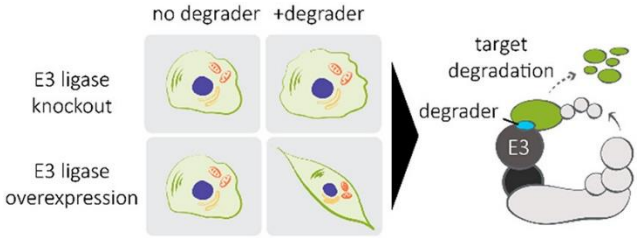
Cell Biology | Chemistry

Contributor: Andreas

Discovery of Molecular Glue Degraders via Isogenic Morphological Profiling

Amanda Ng§, Fabian Offensperger§, Jose A. Cisneros…, Georg E. Winter*

ACS Chem. Biol. **2023**, *18*, *12*, 2464



In this article the authors introduce a fascinating strategy in the field of targeted protein degradation where they utilise high-throughput chemistry in combination with Isogenic Cell Painting Assays (CPA) for the discovery of molecular glues. Molecular Glue Degraders (MGDs) is a novel class of drugs capable of targeting a broader spectrum of proteins, diverging from the traditional approaches. The CPA emerges as a pivotal screening tool, leveraging morphological changes in isogenic cell lines expressing varying levels of the E3 ligase CRBN.

The investigation centres on the practical implementation of this approach, where 132 CRBN binders are generated through a high-throughput chemistry approach based on SuFEx chemistry, underscored for its efficiency, streamlining the drug discovery pipeline. Within this library, FL2-14 stands out as a potent degrader of GSPT2, showcasing a unique preference for GSPT2 over GSPT1 in a CRBN-dependant manner.

A noteworthy aspect of the findings emphasises the significance of the morphological profiling approach in MGD discovery. The isogenic CPA process contribution in unravelling the complexity of nonessential therapeutic targets, offering a transformative perspective in drug development. The research not only contribute crucial insight into advanced drug development strategies but also highlights the success of the CPA in identifying a distinctive GSPT2 degrader, emphasising the role of morphological profiling in MGD discovery.

Other Paper Highlights

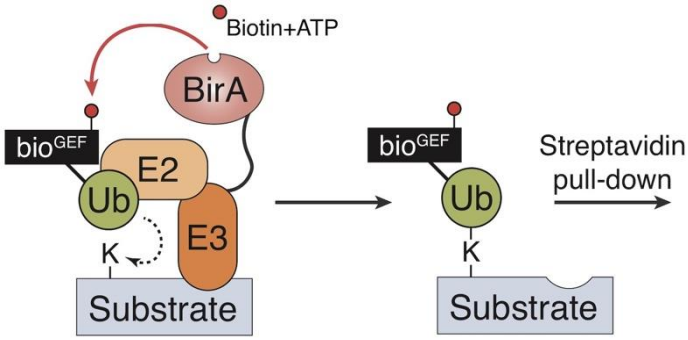
Cell Biology

Contributor: Alejandro

BioE3 identifies specific substrates of ubiquitin E3 ligases

Orhi Barroso-Gomila [§], Laura Merino-Cacho [§], Rosa Barrio*, James D. Sutherland*

Nat. Commun. **2023**, *23, 14(1),* 7656



Proximity labelling techniques, such as BioID, have been proven to be highly valuable in studying and identifying potential protein-protein interactions (PPIs). However, when specifically used for characterising E3 substrates, BioID has several limitations. For instance, it also recognises components of protein complexes and non-covalent interactors, which can lead to false positives. Therefore, there is a growing need to develop more reliable tools for identifying E3 ligase substrates. In this paper, the authors present BioE3, a method for identifying specific substrates of ubiquitin E3 ligases taking advantage of the biotin-avidin technology. First, the authors used a version of AviTag (called bioGEF) fused to a Ubiquitin gene and developed doxycycline-inducible stable cell lines. Subsequently, an E3 Ligase-BirA fusion construct was expressed into the bioGEF cell lines and treated with DOX and Biotin leading to the concomitant increase of BirA-E3 expression with the production and incorporation of bioGEF-Ub into its cellular substrates. The AviTag-BirA pairing enables specific biotinylation of ubiquitin bound to E3 substrates, allowing for subsequent streptavidin capture and identification by LC-MS.

This methodology holds significance not only in the broad field of Ub research but also in TPD. It can help speed up the characterisation of new E3 ligases to be used by degraders, as noted by the authors. The results are well-supported by a variety of assays, including confocal microscopy, proteomics, and bioinformatics. The candidate substrates identified were also validated using other methods such as gene knockout or RNAi. This paper is definitively a must-read for anyone interested in the characterisation of E3 Ligase PPIs.

Centre for Targeted
Protein Degradation
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