CeTPD Journal Club

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

Journal Club

April 2024

Centre for Targeted Protein Degradation
University of Dundee

innovate
collaborate
inspire

Contents

Meet this Month's Editors

This month's editors are (from left to right): Alexandra Harris, Tom Webb and Gemma Little

"The JC is a valuable tool for researchers for keeping up to date with the ever-expanding TPD field. It has been a great experience to have contributed to this edition."

Alexandra obtained her MSc by Research in November 2023 from the University of Dundee, where she undertook a year-long project within the Ciulli Group. As she greatly enjoyed this experience, she opted to continue her studies in the group as a PhD student. In her spare time, Alexandra enjoys travelling, socialising and learning Italian.

Tom joined the Ciulli group as an iCASE PhD student funded by the MRC-DTP and AstraZeneca in October 2020. Over the last four years, Tom's research has involved developing novel high-throughput approaches to mapping the ligandability of E3 ligases via fragment screening and high-throughput chemistry approaches to fragment expansion.

"*The JC allows researchers across all disciplines, including those not actively working within the area, to have a concise and summarised take on the new and upcoming technologies and findings within the advancing field of TPD. I think this is a great opportunity for researchers with varying expertise to freely express their opinions, and build on their skills to constructively analyse other people's work."*

Gemma joined the Ciulli group as a research scientist (medicinal/synthetic chemistry) as part of the Boehringer Ingelheim collaboration in late October 2023. She completed her MChem in Pure and Applied Chemistry at the University of Strathclyde in 2019. Remaining in Strathclyde, she joined the group of Professor Craig Jamieson to carry out her PhD studies focusing on the synthesis and design of BRD4 inhibitors using computer-aided drug design.

Behind the paper:

Targeted protein degradation via intramolecular bivalent glues

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Contributor: Valentina Spiteri

Behind the paper: Targeted protein degradation via intramolecular bivalent glues

Nature. ***2024*** *Mar; 627(8002):204-211.*

Oliver Hsia, Matthias Hinterndorfer, Angus D. Cowan*, Kentaro Iso, Tasuku Ishida, Ramasubramanian Sundaramoorthy, Mark A. Nakasone, Hana Imrichova, Caroline Schätz, Andrea Rukavina, Koraljka Husnjak, Martin Wegner, Alejandro Correa-Sáez, Conner Craigon, Ryan Casement, Chiara Maniaci, Andrea Testa, Manuel Kaulich, Ivan Dikic, Georg E. Winter & Alessio Ciulli*

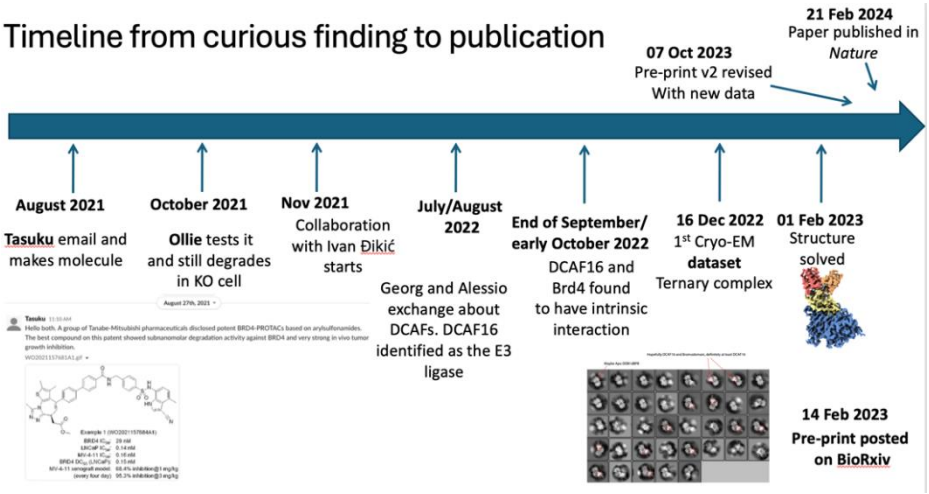
At the CeTPD, there are several projects brewing at any one time and generally, as in most labs, projects are either ticking along, hitting hurdles, or sometimes bringing joyous surprises. The great thing about CeTPD is that the culture is one of openness. We all get to partake in the highs and lows of each other's projects. A recent project that created a swirl of excitement was the intramolecular bivalent glue story, a collaborative project led by Ollie and Angus from the CeTPD here in Dundee and Matthias from the CeMM in Vienna. To my mind, this story echoes the saying, "fortune favours the trained eye". This team could not have been better placed in terms of experience and technical ability to deconvolve what they were seeing experimentally. To fully appreciate the story and how it came together so elegantly I wanted to delve deeper behind the paper and so had an interview with the team to get their perspectives.

The obvious first question was, how did it all start? Ollie explained that it all started at the end of August 2021. The Dundee team was working on developing DCAF15 based PROTACs in collaboration with Eisai, and Tasuku (visiting scientist from Eisai), working with Ollie at Dundee, had found the PROTAC-like compound that was to become IBG1 in a patent which had just published. Incidentally, Tasuku shared the information on the compound chemical structure with Ollie and Alessio on Alessio's wedding day. Alessio reassures me that "he definitely did not reply to that message (on that day)". The team at Dundee hoped that this compound (at the time dubbed as compound **1**) could finally act as a benchmark positive control compound for their project, as they had worked for several years by then to try to degrade proteins using the DCAF15 ligand indisulam, to little avail. However, the team very quickly found out that, while the compound was confirmed to be a potent degrader as claimed in the patent, it was not degrading via DCAF15 as expected. This was a surprising and fascinating result, that could have potentially been dismissed as a "negative result". Instead, it sparked the curiosity of Ollie, Tasuku and Alessio, who began to investigate how the compound might work instead. They first reached out to Ivan Đikić, with whom Alessio had initial discussions on this in Munich in October 2021 during his first face-to-face conference since the pandemic, and Ivan agreed for his team to help to develop cytotoxicity screening assays to address this question.

Georg and Matthias leading the CeMM team. Image taken by Anna Yuwen)

Meanwhile, Matthias was in Georg Winter's group in Vienna. He was setting up CRISPR screens and had a list of compounds that would be interesting to put into these screens. One of these compounds was GNE-0011, a monovalent molecular-glue-like compound, which he found was degrading via DCAF16. The team in Vienna were aware that there was interest by other groups in this compound and so wanted to move fast. As it turned out, Georg and Alessio were already collaborating on another project (later published in Hanzl et al. *Nat Chem Biol*, **2023**, *19*, 323–333) and so were in regular contact anyways. On 22nd July 2022 (Alessio's birthday), Georg sent Alessio a WhatsApp message: "DCAF16 is not by chance among the ligases you enabled biochemically or structurally, is it?". To which Alessio replied: "Yes it is! And curiously, we also have a compound we want to find what ligase it goes by!". That triggered an initial call where the teams shared their respective exciting data, and it was agreed that Dundee would help CeMM work on mechanism for GNE-0011 and that CeMM would help Dundee find the E3 ligase for IBG1, with the likely outcome being two separate stories. However, that was not to be the case as Matthias quickly found that IBG1 also degraded BRD4 via DCAF16, which offered further twists of excitement to the entire team.

Angus was the resident DCAF expert, who at the time was expressing and purifying as many DCAF proteins as he could and characterising them with the eventual aim of trying to find some ligands for them. He was aware of Ollie's and Tasuku's work on compound **1** and Ollie would quip to Angus "God wouldn't it be great if it was one of the ones you were working on?" to which Angus replied, "there's no way". He had initially started working with GNE-0011, which was luckily for Angus degrading via DCAF16, one of the DCAFs that he had been working on and in fact it was one of those which expressed most beautifully into good yields of purified soluble protein.



"Just to say, the structure is quite something.'' As the team were working all this out the DCAF office got a lot of visits from Alessio (see picture). Alessio loves sharing in the excitement of projects and couldn't resist real-time updates. But when he saw the structure, he was absolutely mesmerised, as was everyone else. In picture, Ollie, Angus, Kentaro and Alessio. (Image taken by Valentina on 3rd of February 2023).

I asked the team, when did they know this was something different? Ollie jumps in and says, "it was the structure". Up until that point, they had "a PROTAC that kind of behaved like a glue" and they couldn't really work it out exactly. Angus explained that up until the structure was determined, "everyone was working on the assumption that you had two compounds binding each bromodomain, basically creating these big hydrophobic surfaces that would make them stick." Being very honest he adds, "I can't pretend we had any inkling that we would be working in that bivalent manner". When they saw the structure, everything fell into place, and it made sense why you needed the tandem domains BD1-BD2 to get the degradation. At that point the team had all the data they needed and moved quickly. They solved the structure in January 2023 and were able to submit the preprint to bioRxiv on, Ollie jumped in with a chuckle, "Valentine's Day".

I had to ask, "how did it feel?"- Angus says, "the cliché, but a genuine eureka moment" and adds "it's the best thing about being a structural biologist, sometimes you get to unlock something that you couldn't do in any other way". Matthias mentioned there was a lot of shared excitement, with Alessio WhatsApp messaging Georg and Georg forwarding the messages to Matthias.

At the time of the preprint a motivating factor was that the team knew that another group was working on potentially a related story. What's amazing is that the two teams coordinated the release of the stories to bioRxiv and Ollie said it was a testament to the mutual respect of the groups involved that that level of coordination happened. In the end the stories presented different findings and complemented each other.

Angus recalls working hard to try to get the structure as quickly as possible while learning how to process electron microscopy data. In a sweet gesture of team spirit Ollie would bring Angus pastries in on the way to work to fuel him for the day. Angus makes the point that initially, the quality of the map was not good enough to build DCAF16 properly and building the chains *de novo* was not easy. It was after the initial preprint and further work on the structure, facilitated by improvements in the EM prediction tools, that he was able to build in a better model.

What's more impressive than the record speed of the DCAF16 story was how quickly the DCAF11 element came into play after the initial preprint that greatly added to the story and showed that the intramolecular bivalent glue idea was more generalisable. Angus mentioned that having "chemists on the team is key", and Kentaro and Ryan agree!

When discussing how you know if this is a rabbit hole worth pursuing, Ollie says it was a combination of "the right environment, right people and enough curiosity" that allowed them to chase this. Angus credits the robust system that Matthias had that allowed them to find the tandem dependency. Matthias said it's a "testament to how fortunate everything played out" because he would not have thought of testing the tandem, and only tested it to help guide structural efforts which initially were directed to the question of whether the compound was recruiting BD1 or BD2.

I asked the guys what would they personally be taking away from this experience? Ollie said that he's learned that it's important to "play to the strengths of the team members and collaborators". Matthias said that this experience further confirmed what he knew before "functional genetics and structural biology go really well together" and that he'd like his research to pursue working on the interface of the two fields. Angus said that "following the data even when it doesn't make sense and having regular meetings to bounce off ideas" is what he'll take with him from working on this project.

Targeted Protein Degradation

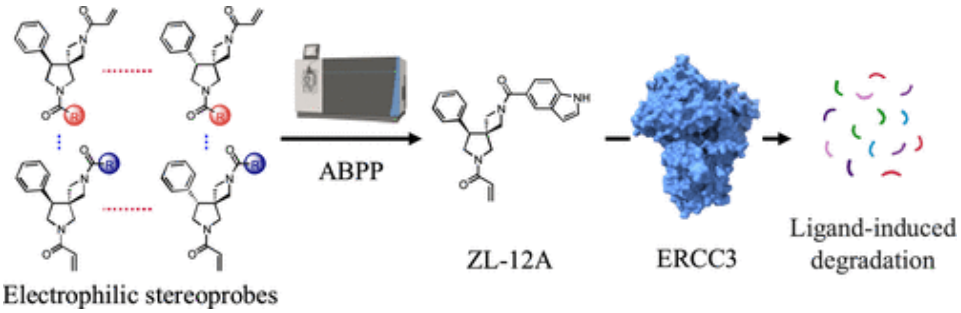
Cell Biology | Chemistry

Contributor: Tom

Proteomic Ligandability Maps of Spirocycle Acrylamide Stereoprobes Identify Covalent ERCC3 Degraders

Zhonglin Liu[§], …, Bruno Melillo*, Benjamin F. Cravatt*

J. Am. Chem. Soc., 2024, 146, 10393



The authors present a covalent stereoprobe strategy to activity-based protein profiling (ABPP). The covalent stereoprobes were found to frequently engage cysteine residues with site specificity, and in a stereoselective manner, versus non-covalent fragment hits which are often promiscuous (in part due to their weak affinity) and are therefore challenging to use in cell biology studies. The authors demonstrate that this screening technique can be applied to detect specific engagement of a range of protein classes (monitored by chemo-proteomics) and are able to demonstrate compound induced proteasomal degradation of transcription factor IIH complex component helicase ERCC3.

The authors' use of spirocyclic acrylamides to generate their stereoprobes gives, in most cases, direct access to negative controls for hit compounds, and they note that 'the enantiomer of the stereoprobe hit does not react' with the cysteine engaged by the hit compound of interest. This is a powerful advantage over other covalent and non-covalent fragment screening techniques, where deconvoluting the mechanistic drivers of a given phenotypic effect is a major challenge. This study also highlights the growing utility of bespoke library design applied to diversity-orientated synthesis, where the authors find that 'the limited overlap in proteins liganded by azetidine and tryptoline stereoprobes' provides evidence that 'their distinct stereochemically defined, entropically constrained cores bestow each compound family with a selected number of productive protein interactions'. This is perhaps best highlighted by the fact that they are able to demonstrate distinct functional outcomes in covalent compounds engaging the same cysteine residue in ERCC3; spirocyclic acrylamide ligands engaging cysteine-342 (C342) were found to induce its proteosomal degradation, while covalent engagement by the natural product triptolide instead promoted collateral degradation of RNA polymerases.

The study is thorough, and we highly recommend a detailed read for further information on the experimental details.

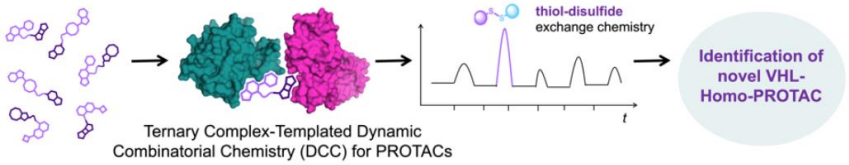
Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Gemma

Ternary Complex-Templated Dynamic Combinatorial Chemistry for the Selection and Identification of Homo-PROTACs

Claudia J. Diehl[§], Alessandra Salerno[§], and Alessio Ciulli*

Angew. Chem. Int. Ed., **2024**, e202319456



Dynamic combinatorial chemistry (DCC) provides an effective hit-identification strategy in drug discovery to establish optimal ligands for a target protein of interest (POI). Application of this methodology towards multiple protein template systems has been rarely observed. This proof-of-concept study aims to discover chimeric molecules inducing proximity between two proteins, in this case VHL homo-PROTACs, using a DCC approach.

Exploiting their previously reported SAR of VHL homo-PROTACs, the authors were able to incorporate a reversible disulfide linker to allow the enrichment of the most stable homo-PROTACs within the DCC library, based on their ability to induce ternary complex formation. To first confirm their hypothesis, a diverse set of disulfide-containing homo-PROTACs were synthesised and tested in both cellular degradation and biophysical ternary complex formation assays. Incorporation of the disulfide moiety was well-tolerated as exemplified by **SH2**, for which compound induced ternary complex formation and VHL degradation was demonstrated. Next, the authors were able to successfully develop a ternary complex directed DCC assay to identify a novel unsymmetric homo-PROTAC, **H2-H4**. Crucially, good correlation was observed between the enrichment of the dynamic combinatorial library (DCL) members and their respective biophysical and cellular potency. This highlights the importance of the thermodynamic driving force towards the formation of a stable ternary complex.

This compelling study provides proof of concept for the utility of ternary complex directed DCC assays to identify potent degraders. The question regarding the applicability of this strategy being directly employed towards other E3 ligase templates using homo-PROTACs should be readily transferable. However, the design of hetero-PROTACs will likely be more challenging due to the fact that homo-species may still form in this context, and careful experimental design will be required to overcome this. Overall, this DCC approach extends the repertoire of hit identification strategies in early-stage discovery programs for PROTAC design.

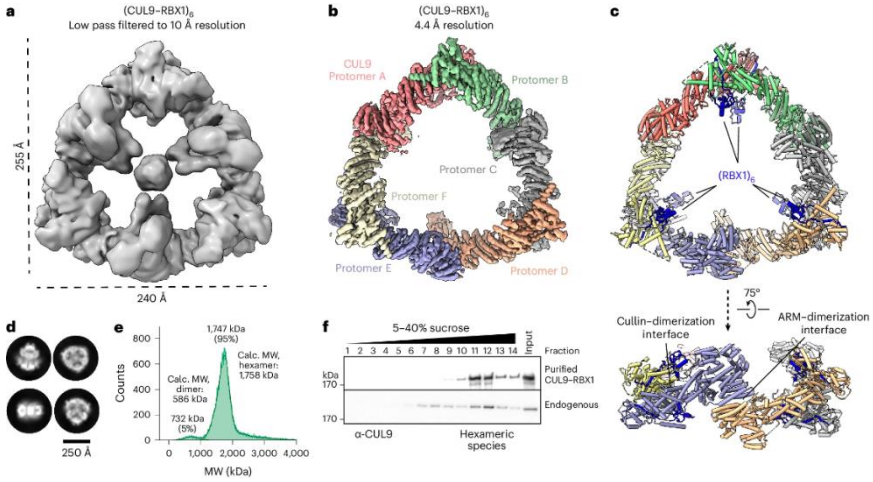
Cell Biology | **Modelling/Simulation** | **Structural Biology/Biophysics**

Contributor: Alexandra

Noncanonical assembly, neddylation and chimeric cullin–RING/RBR ubiquitylation by the 1.8 MDa CUL9 E3 ligase complex

Daniel Horn-Ghetko [§], Linus V. M. Hopf[§], …, Brenda A. Schulman*

Nat. Struct. Mol. Biol., ***2024*** *DOI: https://doi.org/10.1038/s41594-024-01257-y*



This work describes the non-canonical hexameric triangular assembly of the vertebrate specific 1.8 MDa CUL9 E3 ligase complex, which interestingly encompasses both cullin-RING and RBR E3 catalytic domains. Through the proficient use of Cryo-EM, biochemical studies and cellular assays, a detailed depiction of this mega-complex comes to life: notably, the three dimeric sub-complexes (each composed of two CUL9-RBX1 protomers), their dimerisation interfaces and their neddylation-state driven conformations. Interestingly, the authors determine the neddylation pathway of CUL9. Firstly, they confirm that the neddylation of CUL9 was dependent on the activity of NEDD8 E1 (NAE). Independent knockdown of both NEDD8 E2s, UBE2M or UBE2F, in U2OS cells revealed that surprisingly, the poorly characterised metazoan specific E2 UBE2F is crucial for neddylation of CUL9. This was further confirmed by the incubation of CUL9$^{\Delta ARIH\text{-}RBR}$–RBX1 with NAE, MgATP, fluorescent NEDD8 and either UBE2F or UBE2M. This demonstrated the neddylation of K1881 when in the presence of UBE2F and not UBE2M. Their data demonstrates the complexes' ability to ubiquitinate substrates *via* chimeric CRL – RBR E3 ubiquitin ligase activity.

The elegant work outlined in this paper provides an example of the use of Cryo-EM for the elucidation and further structural dissection of gigantic multi-subunit complexes and their mechanisms within the field of ubiquitination and targeted protein degradation.

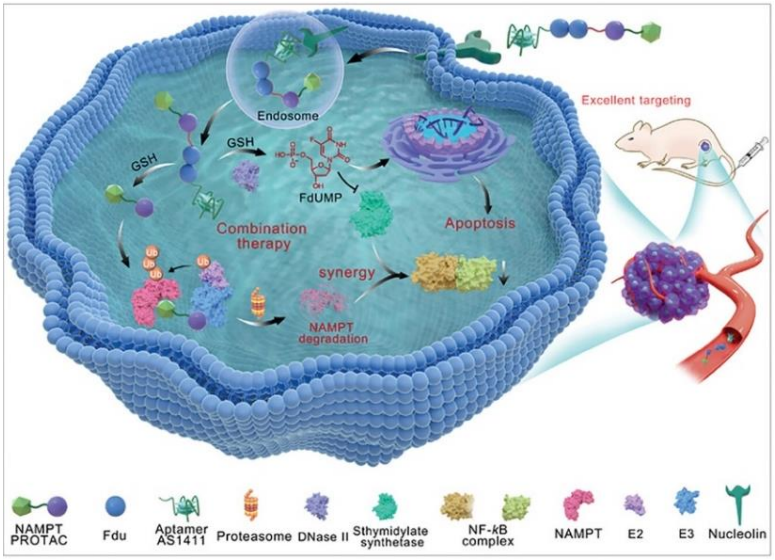
Cell Biology | Chemistry

Contributor: Gemma

Drugtamer-PROTAC Conjugation Strategy for Targeted PROTAC Delivery and Synergistic Antitumor Therapy

Shipeng He[§]*, Yuxin Fang[§], …, Guoqiang Dong*, Chunquan Sheng*

Adv. Sci., **2024**, 2401623



In this work, He and Fang *et al*. develop a novel multifunctional smart prodrug strategy to address some of the therapeutic limitations of PROTACs entering the clinic, such as systemic cytotoxicity.

The authors demonstrate an approach for improving the therapeutic efficacy of PROTACs leveraging "drugtamer-PROTAC" conjugates. This involves linking a cytotoxic agent (**FU**, fluorouridine nucleotides), an aptamer (**AS**) and a synergistic PROTAC (nicotinamide phosphoribosyltransferase (NAMPT)) to enhance tumour targeting and antitumour potency. The aptamer moiety selectively identifies the cell membrane receptor, which is overexpressed in tumour cells, and is then specifically internalized into the cell. Therefore, the aptamer fragment enables the PROTAC to be preferentially taken up into cancerous tissues. The cellular uptake of PROTAC conjugate, **AS-2F-NP**, by MDA-MB-231 cells was confirmed using flow cytometry and confocal laser scanning. *In vitro* dose-dependent degradation of NAMPT was observed for **AS-2F-NP** (DC_{50} = 18 nM, D_{max} >90%) which proved to be more efficient than the PROTAC (**NP**) alone, and the non-targeted **CRO-2F-NP** PROTAC conjugate, highlighting the significance of cellular uptake. To further validate **AS-2F-NP**, whole-cell proteomic experiments were performed, which confirmed NAMPT was significantly downregulated. *In vivo* assessment showed that conjugate **AS-2F-NP** displayed excellent tumour targeting capabilities in MDA-MB-231 xenograft mice models, following IV administration. Furthermore, *in vivo* efficacy was validated in a mouse MDA-MB-231 xenograft model whereby **AS-2F-NP** showed the best tumour growth inhibition (TGI > 92%), even when compared to combination therapy i.e., **FU** and **NP** (TGI = 79%).

This work is focused on breast cancer models only. Despite this, it proves to be a promising method which nicely compliments the existing prodrug strategies for PROTAC design to enable their precise delivery for efficient cellular uptake and *in vivo* distribution for reduced toxic potential.

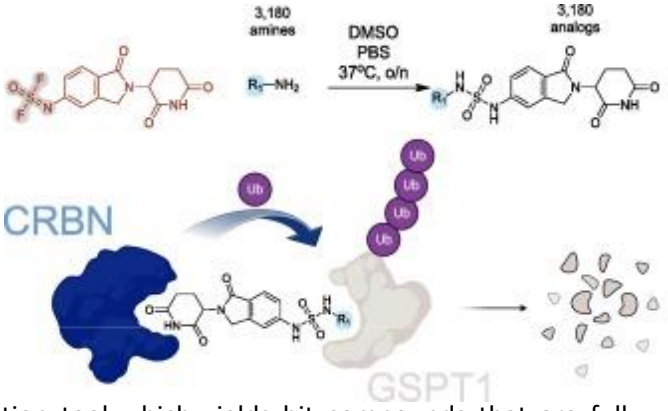
Cell Biology | Chemistry

Contributor: Tom

SuFEx-based chemical diversification for the systematic discovery of CRBN molecular glues

Trever R. Carter[§], …, Seiya Kitamura*, Michael A. Erb*

Bioorg. Med. Chem., **2024**, *104*, 117699



The authors herein present application of their previously disclosed sulfur(VI) fluoride exchange (SuFEx) 'click' chemistry (doi: 10.1002/anie.201902489) to library synthesis for the diversification of parent scaffold 5'-amino lenalidomide. Using this approach, a library of over 3000 analogues was synthesised and crude products taken forward for phenotypic screening (cell viability). Four of the most promising hit compounds were characterized for their ability to degrade G-to-S phase transition 1 (GSPT1) protein. This study adds to the ever-increasing number of success stories with respect to screening crude reaction products. This approach serves as a powerful SAR generation tool which yields hit compounds that are fully characterizable (and optimizable) with respect to desired phenotypic outcomes – in this case degradation of a protein of interest leading to cell death.

SuFEx chemistry relies on 'click' type reactions between iminosulfur oxydifluorides and aliphatic amines. These reactions have been shown to be high yielding and proceed to completion under mild aqueous conditions making them highly amenable to the 'direct-to-biology' approach applied herein. The parent lenalidomide imino-sulfur oxyfluoride derivative was reacted in parallel with 3180 primary and secondary amines and the crude products were screened for anti-proliferative effects against MOLT4, MV4;11 and BE(2)-C (3 different cancer cell lines representing both haematological and solid malignancies). Hits were tested in triplicate and re-tested in CRBN-deficient matched cell lines to monitor CRBN-dependency of antiproliferative activity. Validated hits were next screened for degradation of GSPT1. All four hit compounds are derived from primary amine parents and contain an *ortho*-substituted alkyl phenol ether group, demonstrating the importance of this SAR for cellular activity. The authors fully characterize the hit compounds as GSPT1 degraders *via* thorough immunoblot and HiBiT analysis (it is recommended to visit the full article for further details of these experiments).

Overall, this study provides a neat example of how powerful crude reaction screening can be as an SAR generation tool if the range of chemistries which can be leveraged are expanded. Furthermore, this study points to the fact that the SAR generated is tangible; it can be used to generate hit compounds which are fully characterizable and optimizable for degradation of a given protein of interest.

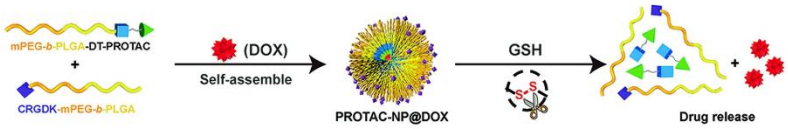
Cell Biology

Contributor: Gemma

Tumor-Targeted PROTAC Prodrug Nanoplatform Enables Precise Protein Degradation and Combination Cancer Therapy

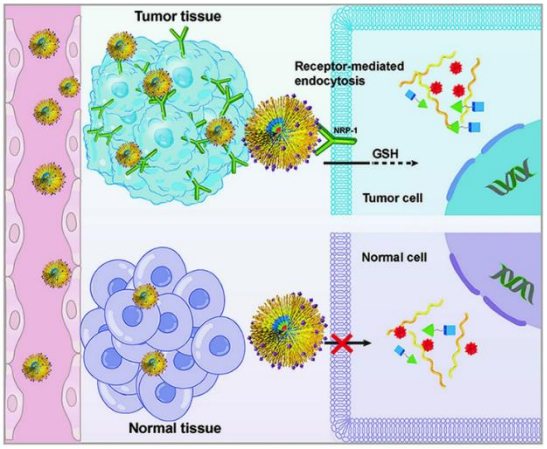
Zhi-feng Zou[§], Lei Yang[§], Hui-jun Nie[§], …, Jing Gao*, Hai-jun Yu*, Xiao-hua Chen*, and Zhi-ai Xu*

Acta Pharmacologica Sinica, **2024**, *0,* 1–12



Poor pharmacokinetic properties are often a concern for PROTAC development, and many strategies have been implemented in attempts to overcome this. Zou *et al*. describe self-assembling PROTAC prodrug nanoparticles (NP) which are internalised into tumour cells to selectively release the PROTAC (and chemotherapeutic agents) *via* reduction of a disulfide cleavable linker.

Using a clinically approved biodegradable diblock copolymer (PEG-*b*-PLGA), a polymer-conjugated PROTAC prodrug platform was developed. The "reduction-activatable PROTAC prodrug NPs" were constructed from the self-assembly of the PEG-*b*-PLGA diblock copolymer conjugated PROTAC, with PEG-*b*-PLGA diblock copolymers functionalized with a CRGDK ligand. The CRGDK ligand on the peripheral of the PROTAC prodrug NPs is recognised by NRP1, which is overexpressed on the surface of tumour cells, and selectively internalised. BRD4 and CDK9 were shown to be efficiently degraded, and the PROTAC NPs were more effective at inhibiting tumour growth in an MDA-MB-231 triple-negative breast cancer (TNBC) model than their small-molecule PROTAC counterparts. To assess this methodology for a combinatorial therapy setting, incorporation of the anticancer agent DOX into the CRGDK ligand modified BRD4 PROTAC prodrug NP (**RPG7**) was engineered, to give **RPG7@DOX**. These NPs inhibited the proliferation of MDA-MB-231 tumour cells *in vitro* (IC_{50} = 36 nM) which confirmed the enhanced therapeutic efficacy of **RPG7@DOX** in relation to using PROTAC ARV-771 or DOX agent separately (IC_{50} = 510 and 81 nM, respectively). Furthermore, **RPG7@DOX** induced apoptosis by synergistically activating the caspase-3 pathway in TNBC models *in vivo*, with negligible histological damage to normal tissues being observed. The NPs that were engineered to contain both PROTAC and chemotherapeutic agents (**RPG7@DOX**) were far more effective at eradicating tumour growth in TNBC models *in vivo*, in comparison to the free DOX chemotherapeutic drug or the PROTAC prodrug NP **RPG7** dosed separately.



This paper showcases an alternative PROTAC delivery platform utilising target specific prodrug nanotechnology to simultaneously address precise tumour targeting and efficient delivery to the site of interest. This technology was able to overcome issues with the limited bioavailability of PROTACs and minimise off-target side effects and systemic toxicity. Using a clinically approved polymer, alongside well established E3 ubiquitin ligase ligands targeting CRBN and VHL provides a novel translational strategy for PROTAC-based combination anticancer therapies.

Cell Biology **Chemistry**

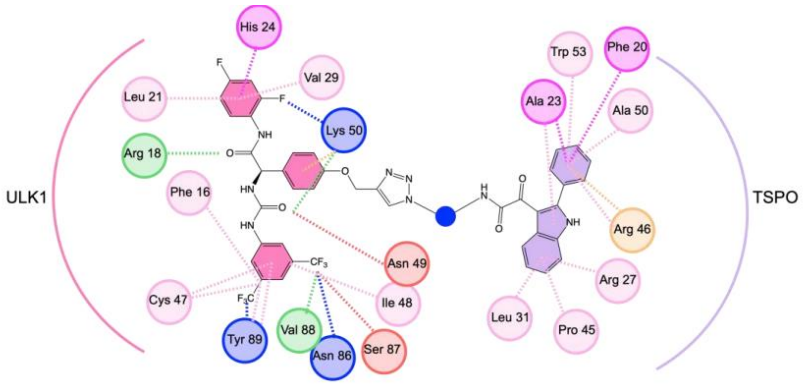
Contributor: Alexandra

Development of the ULK1-Recruiting Chimeras (ULKRECs) to enable proximity-induced and 3 ULK1-dependent degradation of mitochondria

Niyaz Zaman[§], …, Robin Ketteler*

bioRXiv, **2024**, DOI: 10.1101/2024.04.15.589474

In this pre-print, Zaman and Aley *et al.* detail the development of ULKRECs as an alternative strategy to AUTACs. Compared to AUTACs, which direct the target of interest for autophagosome-mediated degradation via linkage to an autophagy cargo protein, ULKRECs recruit ULK1 to initiate autophagosome biogenesis directly at the target site. They report the synthesis of 2 structurally similar chimeric compounds (NZ-65 and NZ-66), composed of a three-part scaffold: an ULK1 agonist (BL-918), a linker region, and a mitochondrial targeting ligand (2-phenylindole derivative TSPO ligand which is known to bind the peripheral benzodiazepine receptor on the outer mitochondrial membrane). Through the use of immunofluorescence, they firstly established the ULKREC-induced co-localisation of ULK1 and the mitochondria, before going on to visualise the induction of mitophagy by both western blot and the use of the mito-mKeima fluorescent reported assay. Importantly, the authors utilise Parkinson's disease patient-derived fibroblasts to explore the potential therapeutic effect of ULKRECs – critically highlighting that these novel



chimeric entities are able to induce mitophagy independent of the PRKN/PINK axis, a mitophagy pathway found to be mutated in many neurological diseases.

This work effectively provides a disease-relevant insight into the work of small molecule degraders beyond PROTACs, with ULKRECs providing a proof of concept for the local initiation of mitophagy at a target site.

Computational Chemistry | Modelling/Simulation | Structural Biology/Biophysics

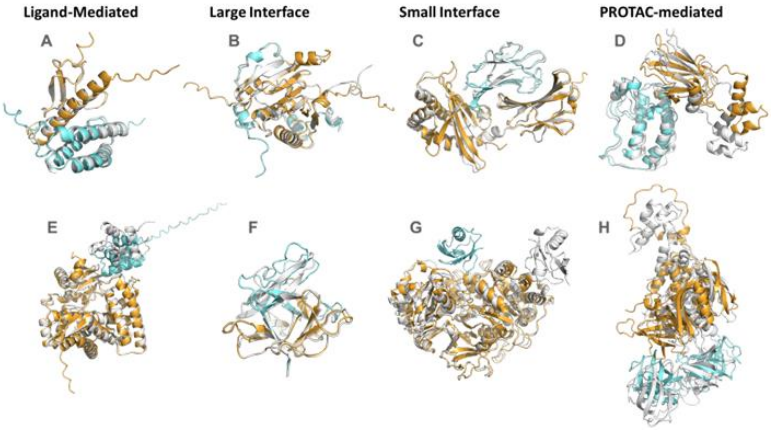
Contributor: Tom

AlphaFold-Multimer struggles in predicting PROTAC-mediated protein-protein interfaces

Gilberto P. Pereira[§], Corentin Gouzien[§], …, Juliette Martin*

bioRXiv **2024**, DOI: 10.1101/2024.03.19.585735

This work provides a performance analysis of the ability of AlphaFold (AF)-Multimer to predict a range of protein-protein interactions (PPIs). These are divided into four classes; (i) large PPIs, (ii) small PPIs, (iii) PPIs mediated by ligands and (iv) PROTAC-mediated PPIs. The authors highlight that AF-multimer can accurately predict the structure of PPIs which fall within the first three datasets but fail to do so for the PROTAC-mediated set. This may be accounted for by the fact that AF2 does not consider ligands and therefore, these 'PROTAC-induced' interfaces are hard to predict using AF-Multimer.



It is highlighted that AF performance analysis typically is conducted using a standard benchmark dataset, primarily comprising structures that closely resemble those encountered in the training dataset. However, this gives rise to challenges in predicting less represented PPIs/PPI structures, such as those mediated by PROTACs. In their analysis, the authors selected structures with a resolution better than 3Å, and the interface size was calculated as the sum of surface areas of the monomers minus the surface area of the complex (with and without heteroatoms to assess involvement of ligands at the interface). DockQ was used to analyse the similarity between the experimental structures employed and the predicted structures (software which provides a similarity score from 0 to 1, with 1 being perfect similarity). The authors found that large PPIs are consistently well predicted, and that accuracy deteriorates as the interface gets smaller in both the training and test datasets. Ligand-mediated interfaces are either well predicted or entirely incorrect. For the PROTAC-mediated set, the DockQ score is < 0.1 in all but three cases. The authors postulate a few reasons for this; (1) there are relatively few PROTAC mediated ternary complex structures versus the other PPI classes investigated, leading to a low population of this structure class available to AF-multimer; (2) PROTACs typically induce a *de-novo* PPI between two proteins which would not normally interact, and the ligand itself can contribute significantly to the entropic/enthalpic favourability of ternary complex formation (a contribution which is ignored by AF2) and; (3) PROTAC mediated crystal structures may contribute only one structure within a larger conformational ensemble.

Whilst bespoke examples exist in the literature for prediction of PROTAC-mediated ternary complexes, most fail to yield high-quality solutions and are not general. In most cases, the development of the tool is a project in itself. It is refreshing to see a study of this type highlighting the challenges in performing this type of prediction – this can often be more insightful in helping move the field forwards than examples successfully applied to very specific problems, as it better highlights the key problems which need to be addressed to enable general applicability. It will be fascinating to follow to what extent these problems can now be addressed with the release of AlphaFold 3.

Cell Biology

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Ubiquitin-Specific Proximity Labeling for the Identification of E3 Ligase Substrates

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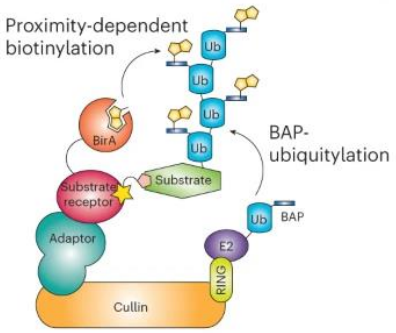

Huang *et al.* discuss the development of E-STUB, a ubiquitin-specific proximity labelling method that enables the identification of E3 ubiquitin ligase substrates. Initially, as proof of concept, they co-expressed a biotin acceptor peptide (BAP - AviTag) tagged ubiquitin with an E3 ligase conjugated to the Escherichia coli biotin ligase BirA in this hopes that it would biotinylate the ubiquitinated substrates of an E3 ligase. As a model system, they showed the pomalidomide-induced biotinylation of IKAROS family zinc finger 1 (IKZF1), a well-known ubiquitinated substrate of the E3 ligase Cereblon. However, during this, they observed background biotinylation across the ubiquitome, likely due to the high affinity between AviTagged-ubiquitin and BirA itself. In an attempt to optimise this system, they opted to investigate alternative BAP variant, aiming to tune down the affinity for BirA. By immunoblotting, they established the optimal BAP variant A3-ubiquitin that induced proximity-dependent biotinylation of IKZF1, while reducing the background biotinylation noise. They then effectively demonstrated that E-STUB is capable of detecting endogenous E3 ligase substrates.

This work is an exciting representative of the use of proximity-induced labelling within the field on targeted protein degradation. The potential future applications of E-STUB appear extremely promising, an example being the mapping of E3 ligase substrates based on the specific ligase family.

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