Ciulli Group Journal Club

Targeted Protein Degradation and Other Literature Highlights

April 2021

To celebrate the 1st anniversary of the Journal Club (established April 2020), we commissioned two special illustrations by Jagoda Sadowska, graduate of the DJCAD, University of Dundee, and freelance illustrator and printmaker (jagodasadowska.com).

1) **Front cover**: This past year, we have seen a number of lockdowns, meaning that in-person social interaction has sadly been extremely limited. As a very social group, the Ciulli Lab has continued to meet and socialise through Zoom and Teams. We have also enjoyed online conferences, seminars, webinars and socials from across the scientific community!

2) **Inside cover**: Jagoda captures the key processes for producing the Journal Club, based on reading, writing, creating, collaborating, communicating, and delivering to our readers.

Contents

Feature of the Month: Happy First Birthday, Journal Club!

Contributor: Alessio

Our Journal Club marks its First Year Anniversary this month. What a year it has been. It seems like yesterday that Siying (now at Vernalis) came up with the idea of starting a new journal club, and she, Will and myself met to brainstorm how to move from idea to reality. Twelve months later, here we are. A lot has happened in the past year. In this piece, I want to celebrate our Journal Club, take stock of these last 12 months, and try to look into the future.

Whenever something not done before starts, whether it be a new project, a new team, or a new experience, there are the vibes of excitement and expectation, and the desire to learn how they develop. With the Journal Club, we wanted not only to meet its primary purpose of training scientists to review and communicate the literature as it emerges; but also, we aspired to create a platform for reaching out to the ever so rapidly growing field, and serve the community. Something that would not just be useful and interesting, but purposeful and fun, for everyone involved: the Executive at the helm (Charlotte today), the Editors, the contributors, everyone in our group – and beyond to all our readers and colleagues in the field. I hope this spirit and vision emerges in this month's Issue. Everyone in the group has worked hard to put together this Special Anniversary edition, and we also have special contributions from our own amazing illustrator Jagoda Sadowska to mark this event with original fun illustrations.

TPD is a field that grows exponentially and so rapidly, evident from the progress of many activities around the world – publications, conferences and events, investments, companies, number of PROTACs and other types of degraders becoming available and progressing through to the clinic, and so forth. It is almost impossible to capture all these developments even on a monthly basis with our Journal Club, but we hope to provide a platform for keeping the field as updated and aware as possibility, through the perspective of Dundee, Scotland, as we live, ride and see this new exciting wave of science and innovation. Indeed, so much has happened even in just the past month or so, and I want to highlight three landmarks achievements:

1) £38 million investment in University spinout Amphista therapeutics: Congratulations to the whole team at Amphista, led by CEO Nicki Thompson, for achieving an oversubscribed Series B – one of the largest fundraises of this kind to ever be made in Scotland – bringing on board investors Forbion, Gilde, Novartis Venture Funds, and Eli Lilly to join the existing investors Advent Life Sciences and BioMotiv. I am delighted to be the scientific founder of Amphista and to have contributed to its inception and growth. Amphista incubated between 2017-19 in our labs in Dundee, with former group members Andrea Testa and Scott Hughes leading chemistry and biology. I am proud of their achievements and look forward to watching them go from strength to strength.
2) Arvinas unveils PROTAC structures: As Ingo Hartung said, the whole TPD field was going to be watching. And we did. Congratulations are in order to Arvinas, who unveiled the chemical structures of their clinical PROTACs, ARV-110 for prostate cancer, and ARV-471 for breast cancer. We are so delighted to finally see structures that emblemize such wonderful pioneering work. The whole group was quietly hoping for them to based on the VHL ligand (our baby), and I will have to buy some beers at some point, but we actually knew already… indeed, many congratulations to Tasuku for correctly predicting the chemical structures, of both compounds as it turns out. Learn more about patent-magic skills from his own article directly next!
3) First-of-its-kind centre to lead protein degradation revolution: Hot off the press, the University announced the establishment of a new Centre, the next home for our TPD research. We are all really excited by this opportunity, a development that will allow Dundee to bolster and accelerate its efforts in this exciting new area of research. This is our future, so more to follow, for sure, in the coming Issues.

So Happy Birthday, Journal Club! Come join us in the celebrations, and I hope you will enjoy this Special Issue in full!

Feature of the Month: Arvinas PROTAC Structure Prediction

Contributor: Tasuku

The reasons that ARV-110 was estimated to be described in WO2018071606, not other patents, e.g. WO2016118666 (VHL type AR PROTACs)

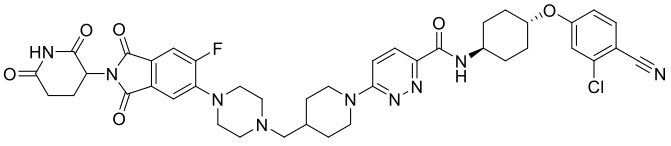
- ✓ Submission date (submitted on 11th October 2016 and 3rd July 2017) was about a year before Arvinas announced they were planning to introduce ARV-110 in clinic.
- ✓ In vivo data for Example 163 in WO2016118666 did not show a clear dose response even in p.o administration.
- ✓ WO2018071606 described more precise in vitro data.
- ✓ It seems the compounds in WO2018071606 were more drug-like than others.

Method

- ✓ Extracted examples of which the activities in the xenograft model (i.p. administration) were "A" (32 compounds in total).
- ✓ Generated structures of them from their IUPAC names.
- ✓ Calculated predicted physicochemical and DMPK properties.
- ✓ Linked each compound and corresponding data manually.

Profile of Example 406 (ARV-110)

- ✓ MW: 812.3
- ✓ clogD: 2.85
- ✓ TPSA: 181.17
- ✓ DC_{50}: 0.24 nM, D_{max}: 82.4%



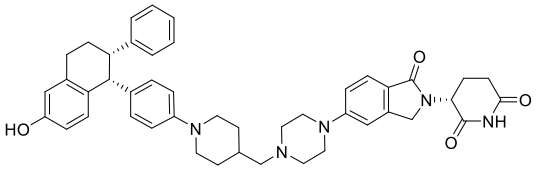
Example 406

The reasons why Example 406 was estimated as ARV-110

- ✓ Exact data for DC_{50} and D_{max} were described in the table.
- ✓ Only 4 out of 32 compounds had disclosed the exact values of both DC_{50} and D_{max}.
- ✓ The predicted physicochemical and DMPK data for Example 406 was better than the others.
- ✓ This type of linkage has appeared several times in this patent.
- ✓ The synthetic procedure of it has been described in detail.

Profile of Example 411 (ARV-471)

- ✓ MW: 723.92
- ✓ clogD: 5.02
- ✓ TPSA: 96.43
- ✓ ERα IC_{50}: 1.12 nM, DC_{50}: <5 nM, D_{max}: >75%

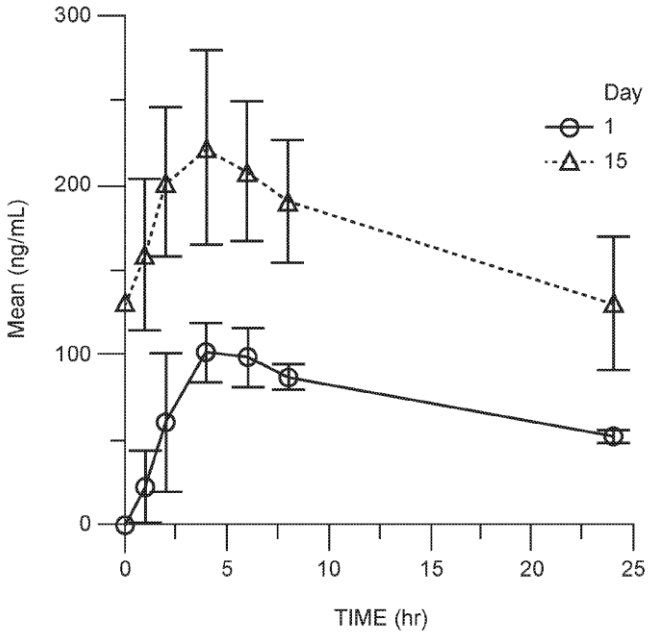


Example 411 (WO2018102725)

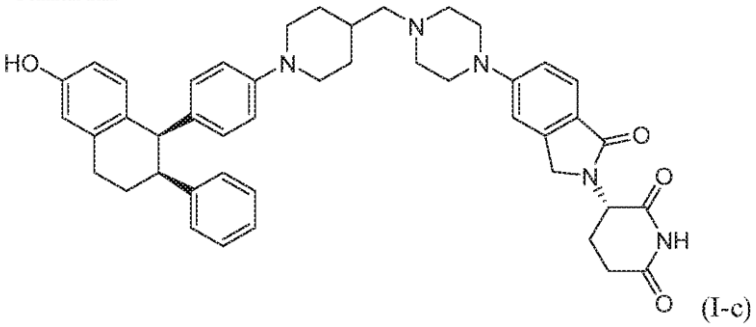
The reasons why Example 411 was estimated as ARV-471

- ✓ It has the same linker as ARV-110.
- ✓ The synthetic procedure of it has been described in detail.
- ✓ The reaction scale of the preparation of Example 411 was larger than other example compounds (964 mg as a final product).

In early March 2021, Arvinas divulged a new patent (WO2021041348) that included the data from phase 1 clinical data that is the exact same as the data they disclosed previously (see next page):

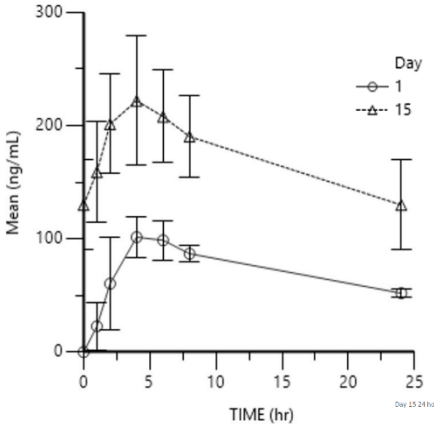


[0055] FIG. 3 is a pair of line graphs which show the mean concentration of the compound of Formula (I-c) (ng/mL) over the course of 24 hours post-dosing on both day 1 and day 15 in a Phase I clinical trial.



(I-c)

ARV-471 Phase 1 Dose Escalation— First Cohort Pharmacokinetics



Day 15 24 hour value is imputed from time zero

T_{max} = 4 hours

$t_{1/2}$ = estimated to be ~24 hours

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ARVINAS

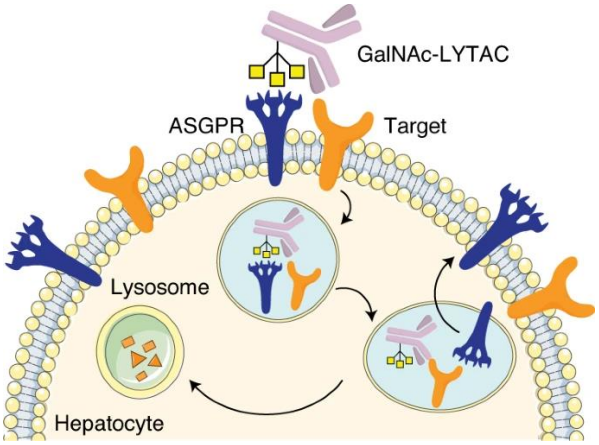
Targeted Protein Degradation

Contributor: Andre

LYTACs that engage the asialoglycoprotein receptor for targeted protein degradation

Green Ahn[§], …, Carolyn R. Bertozzi*

Nat. Chem. Biol. **2021**, DOI: 10.1038/s41589-021-00770-1



Lysosome-targeting chimeras (LYTACs) target extracellular and membrane proteins for degradation through the lysosomal degradation pathway. The first LYTACs utilize the cation-independent mannose-6-phosphate receptor (CI-M6PR) that is ubiquitously expressed. This paper describes the development of liver-specific LYTACs that engage the asialoglycoprotein receptor (ASGPR) using a triantenerrary *N*-acetylgalactosamine (tri-GalNAc) motif. Various GalNAc-LYTACs were designed by conjugation with antibodies or peptides. These are proven to be able to degrade various oncogenic membrane proteins such as the epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2) and tumor-associated integrins. These observed degradations are demonstrated to be ASGPR-mediated through the co-treatment with siRNA targeting ASGPR or autophagy inhibitors; bafilomycin A1 and chloroquine. Furthermore, GalNAc-LYTAC is shown to be cell-specific as it degrades targets in $ASGPR^+$ (HEP3B) but not in $ASGPR^-$ background (HeLa). The authors also explore site-specific conjugation that improves the pharmacokinetic profile of antibody-based GalNAc-LTYACs significantly.

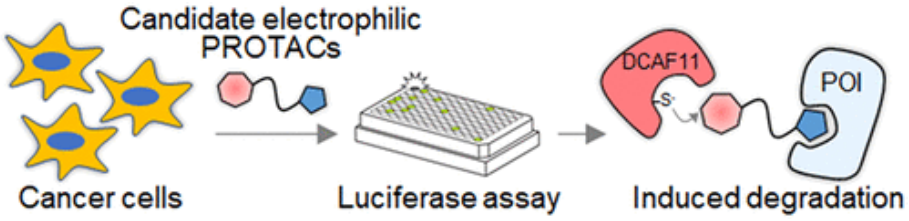
This paper has shown a substantial development in the LYTACs specificity, demonstrated their efficacy and offer potential application in the treatment of hepatocellular carcinoma. It will be interesting to see further structure-function studies that could determine the optimal architecture of LYTACs.

Contributor: Claudia

DCAF11 Supports Targeted Protein Degradation by Electrophilic Proteolysis-Targeting Chimeras

Xiaoyu Zhang[§], …, Benjamin F. Cravatt*

J. Am. Chem. Soc. **2021,** *143,* 5141



Successful targeted protein degradation depends on the recruitment of an E3 ligase by interaction with a small molecule, yet suitable small molecule ligands have been discovered only for a subset of E3 ligases. This work describes the combination of a cell-based screening approach with chemical proteomics to discover novel bifunctional degraders covalently engaging E3 ligases. A focused library of FKBP12-recruiting electrophilic PROTAC candidates was initially screened in four human cancer cell lines and analysed by a luciferase assay. The candidate library exhibited diverse activity, including compounds that reduced FKBP12 levels in a single or in multiple cell lines. The activity of one hit compound, 21-SLF, causing significant decrease of FKBP12 levels only in 22Rv1 cells, was studied by chemical proteomics and knock-out experiments identifying DCAF11 as degradation-inducing E3 ligase. Mutational analysis of DCAF11 indicates that 21-SLF can engage with three cysteines (C443, C460 and C485), although the presence of C460 alone already allows for efficient degradation. Furthermore, DCAF11-directed electrophilic PROTACs also induced androgen receptor degradation, qualifying DCAF11 as an E3 ligase supporting ligand-induced protein degradation.

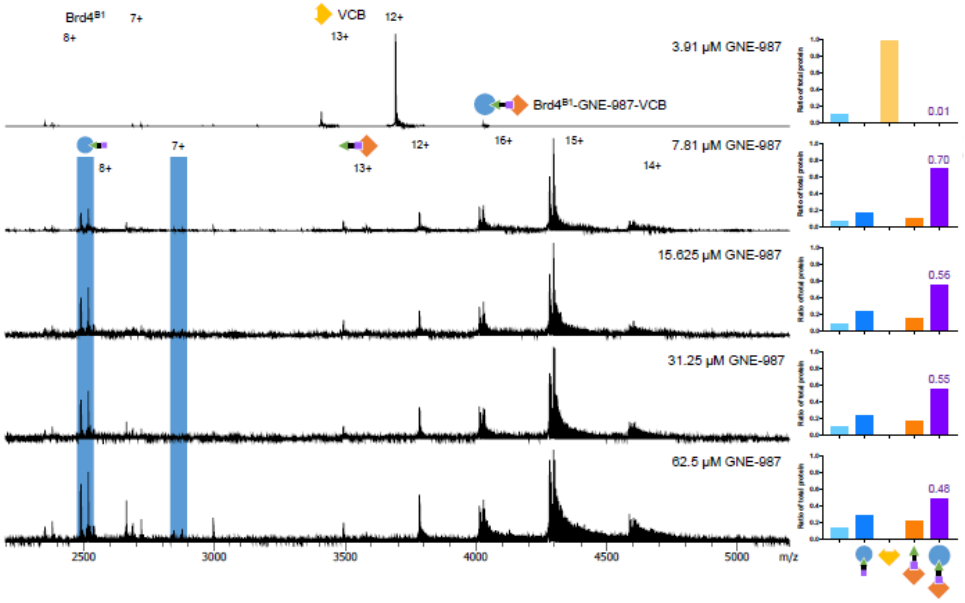
Although selectivity for FKBP12 degradation in 22Rv1 remains unexplained, 21-SLF provides a good starting point for further development of DCAF11 ligands. The applied screening approach presents a valuable tool facilitating discovery of additional E3 ligases engaging with covalent electrophilic PROTACs.

Contributor: Andre

Native Mass Spectrometry for the study of PROTAC GNE-987-containing ternary complexes

Louise M. Sternicki[§], Jim Nonomiya, Miaomiao Liu, Melinda M. Mulvihill, Ronald J. Quinn*

ChemMedChem. **2021**, DOI: 10.1002/cmdc.202100113



GNE-987 is a VHL-based bromodomain-containing protein 4 bromodomain 1 and 2 ($BRD4^{B1}$ and $BRD4^{B2}$) degrader that shows more potency *in vitro* in comparison to MZ1 and ARV-771. Previous SPR-based ternary complex half-life ($t_{1/2}$) measurements indicated that $BRD4^{B1}$ ternary complex ($BRD4^{B1}$:GNE-987:VCB) was more stable than $BRD4^{B2}$ ternary complex ($BRD4^{B2}$:GNE-987:VCB). This paper explores a native nano-electro spray ionization mass spectrometry (nESI-MS) method to 1) determine and quantify the ternary complex formation and its mass accurately, 2) determine the ternary complex stoichiometry and all species that are present in the equilibrium, and 3) quantify the stability of the PROTAC induced ternary complex.

The paper starts with measuring binary binding percentage of GNE-987 towards $BRD4^{B1}$, $BRD4^{B2}$ and VCB. GNE-987 binds to $BRD4^{B1}$ and $BRD4^{B2}$ comparably. This is expected as their binary affinity is comparable (4.7 nM and 4.4 nM, respectively). In an equimolar mixture of $BRD4^{B1}$ or $BRD4^{B2}$ and VCB, minor amount of protein-protein interaction between $BRD4^{B1}$ or $BRD4^{B2}$ and VCB were detected. However, with titrations of GNE-987, peaks corresponding to expected molecular weight of a 1:1:1 ternary complex of $BRD4^{B1}$ or $BRD4^{B2}$, VCB, and GNE-987 were observed. From this experiment, a ratio of the ternary complex formed relative to free proteins can be calculated. This gives a rough quantification of the protein species present in the equilibrium. However, this assumes that all species ionize and are transmitted within the MS with the same efficiency. Agreeing with the SPR data, this method revealed a greater ternary complex formation for $BRD4^{B1}$ over $BRD4^{B2}$; therefore, this method allows a semi-quantitative analysis of PROTAC ternary complexes. The authors also indicate the advantages of nESI-MS: label-free, fast, automated, and relatively low sample requirement, which are suitable for a high-throughput screening approach in detecting ternary complex formation.

It is interesting to see another method that could orthogonally support SPR in determining ternary complex stability.

Contributor: Nicole

Advances in Protein Degradation

M. Paola Castaldi*, Stewart L. Fisher*

SLAS Discov. **2021**, *26*, 471

This editorial introduces a special issue of SLAS Discovery titled “Targeted Protein Degradation”. The issue features a wide range of interesting papers, including recent reviews and perspectives on topics across the field: e.g. ligands for E3 ligases, PROTACs, BiDACs, AUTACs and LYTACs. There are also entries featuring technical guides on assay technologies to discover and characterise new degraders. Two research papers are highlighted in the issue: one reporting novel selective degraders of CDK proteins and the other detailing the development of a new SNAP-tag assay for the quantification of membrane protein levels.

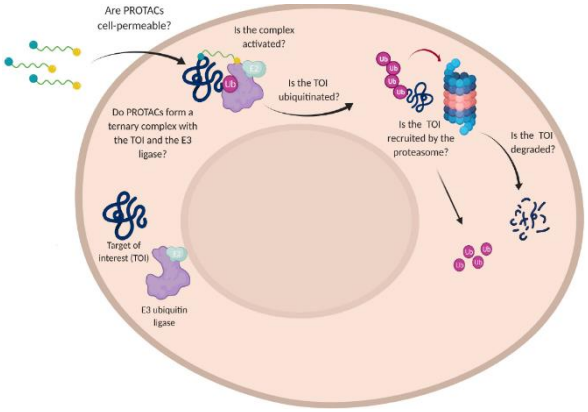
This is a recommended read for those wishing to keep up with the latest views and developments within the field of targeted protein degradation.

Contributor: Nicole

From Conception to Development: Investigating PROTACs Features for Improved Cell Permeability and Successful Protein Degradation

Carlotta Cecchini[§], Sara Pannilunghi[§], Sébastien Tardy, Leonardo Scapozza*

Front. Chem. **2021**, DOI: 10.3389/fchem.2021.672267



This review provides perspectives on the features which contribute to the design of successful PROTACs. The authors first discuss factors which impact PROTAC activity inside cells, such as affinity for the target of interest (TOI) and the stability of the ternary complex between E3, PROTAC and TOI. The linker region of a PROTAC is suggested to be an important area to optimise, not only towards enabling productive ternary complexes, but to tune physicochemical properties, which are the focus of a significant proportion of the review. The cell permeabilities of PROTACs are often limited, which motivated the authors to review strategies to overcome this issue. Examples include the design of intramolecular hydrogen bonding (IMHB) to shield polar atoms as well as tuning the polarity and lipophilicity of PROTACs within a narrow range to promote optimal permeability and solubility. The authors also endeavour to provide insight into the various assays to assess PROTAC cellular uptake such as PAMPA, Caco-2, LC-MS, in cell NanoBRET and the chloroalkane penetration assay (CAPA).

A nicely accessible and detailed review, which includes useful insights into the design of balanced physiochemical properties while also providing advice on the range of available methods to characterise these properties.

Contributor: Andre

Transforming targeted cancer therapy with PROTACS: A forward-looking perspective

William Farnaby*, Manfred Koegl*, Darryl B. McConnell, Alessio Ciulli

Curr. Opin. Pharmacol. **2021**, *57*, 175

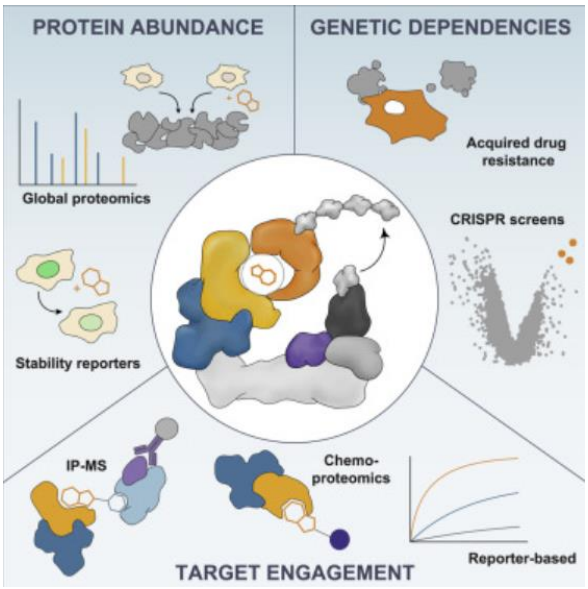
This perspective gives an interesting and concise recap on recent advances and limitations of Proteolysis-Targeting Chimeras (PROTACs), as well as future challenges that lie ahead in the context of cancer drug research. Several unique features of PROTACs that have been demonstrated to tackle challenges in cancer drug research i.e. target scope, resistance, and selectivity, are presented in an unbiased manner. PROTACs increase the druggable target scope by eliminating the necessity to bind to a functional binding site. This is exemplified in the development of ACBI1, a SMARCA ATPase degrader that binds into the non-essential bromodomain of SMARCA2 and SMARCA4. Additionally, as resistance is inevitable for most drugs, PROTACs might offer a solution to this issue. A covalent inhibitor of Bruton's tyrosine kinase (BTK), ibrutinib, loses its activity when the essential cysteine residue is mutated leading to a loss in affinity. An ibrutinib-based PROTAC, however, still retains its anti-proliferative effects despite this loss of affinity mutation. This can be explained by the fact that the requirement for high-affinity binders is relaxed for PROTACs. Although ultimately, resistance towards VHL and cereblon-based PROTACs will eventually emerge and have been observed. This is often mediated by E3 ligase mutational loss or reduced expression. Fortunately, this could be circumvented by employing different E3 ligases. In addition, since there are more than 600 E3 ligases available, more ways to circumvent resistance are explorable. Lastly, PROTACs exhibit exceptional selectivity for their targets. This is illustrated by a pan-inhibitor based PROTAC, MZ1, that shows a significant preference in degrading BRD4 in comparison to its paralog, BRD2 and BRD3, a characteristic that was not displayed by the inhibitor on which the PROTAC is based. This astonishing specificity can be attributed to the specific recognition within the PROTAC-induced ternary complex.

Contributor: Nicole

Identification and selectivity profiling of small-molecule degraders via multi-omics approaches

Natalie S. Scholes[§], Cristina Mayor-Ruiz, Georg E. Winter*

Cell Chem. Biol. **2021**, DOI: 10.1016/j.chembiol.2021.03.007



Thorough investigation of the mechanism of action and selectivity of degraders is essential to assess the quality of these molecules as potential drugs or chemical probes. Here the authors explain various proteomics and functional genomics techniques to characterise degrader activities. Case studies are used to exemplify the use of these assays: for example, one section highlights the various proteomics assays which revealed the degrader activity of immunomodulatory drugs such as thalidomide. The authors explain how the combination of proteomics and functional genomics approaches led to identification of the target protein and E3 ligase recruited by selected aryl sulfonamide molecular glues. The combination of these strategies also allows the characterisation of some of the common cellular determinants of TPD. In addition to revealing information about the on or off-target mechanism of degraders, the integrated multi-omics approaches described here are recommended to enable characterisation of resistance mechanisms.

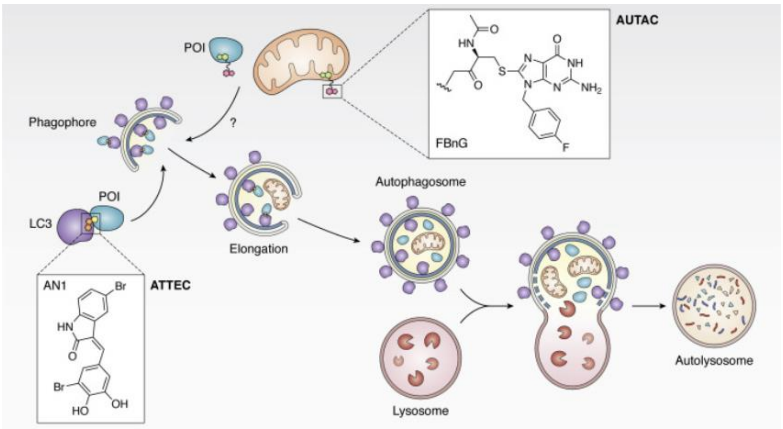
The article provides useful summary tables to illustrate the pros and cons of the featured techniques to guide readers on when to apply a particular assay and how these approaches can complement each other to elucidate the mechanism of action of a degrader.

Contributor: Nicole

Major Advances in Targeted Protein Degradation: PROTACs, LYTACs, and MADTACs

Shanique Alabi[§], Craig Crews*

J. Biol. Chem. **2021**, DOI: 10.1016/j.jbc.2021.100647



From the first PROTAC in 2001 to the recent progression of the first degrader molecules to clinical trials (two milestones which were achieved by Craig Crews and colleagues), the field of TPD has received an exponential increase in interest from the scientific community. This has led to innovative co-opting of alternative cellular mechanisms to the ubiquitin-proteasome system (UPS) to effect protein degradation. In this review, the authors discuss what is currently known about various types of degraders: PROTACs and molecular glues which hijack the UPS, LYTACs which target the endo-lysosomal pathway and MADTACs, (AUTACs and ATTECs) which utilise the macroautophagic pathway. The review also provides a perspective on the future of targeted protein degradation.

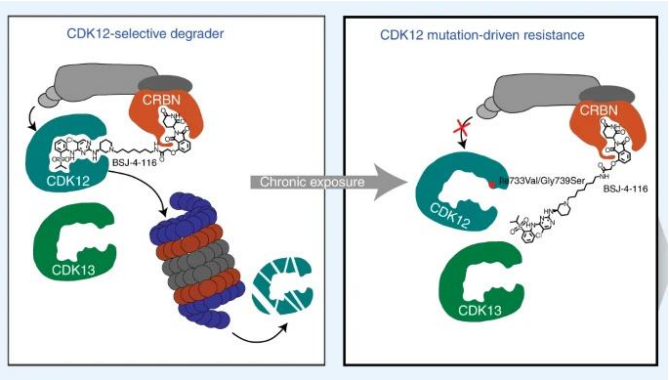
This article includes a comprehensive and historical review of the various types of degrader and key components in the cellular machineries they hijack, therefore it may be of particular interest to those new to working in TPD.

Contributor: Claudia

Discovery and resistance mechanism of a selective CDK12 degrader

Baishan Jiang[§], Yang Gao[§], Jianwei Che[§], …, Tinghu Zhang*, Nathanael S. Gray*

Nat. Chem. Biol. **2021**, DOI: 10.1038/s41589-021-00765-y



Cyclin-dependent kinase 12 (CDK12) regulates transcription of DNA-damage response (DDR) genes, attracting attention as a target for cancer therapy. Due to large homology with other CDKs, especially CDK13, CDK12-selective inhibitors remain elusive to date. In this study, a CDK12-selective degrader, BSJ-4-116, was rationally designed using ligand efficiency calculations and computational modelling of potential ternary complexes with CDK12 and CRBN. Using a range of cell-based experiments, multiplex MS proteomic analysis, and NanoBRET live-cell ternary complex assays, BSJ-4-116 was identified as potent CDK12-selective degrader. Employing BSJ-4-116, CDK12 was validated to induce increasing poly(adenylation) resulting in DDR gene downregulation and early transcriptional termination. Regarding potential therapeutic application, strong growth inhibition effects both of BSJ-4-116 as degraders alone and in combination with a PARP inhibitor were found in T-ALL cell lines and drug-resistance of the CDK12^{C1039F} mutation was overcome. Chronic high-dose exposure with BSJ-4-116 however induced drug-resistance through point-mutations in the target protein CDK12.

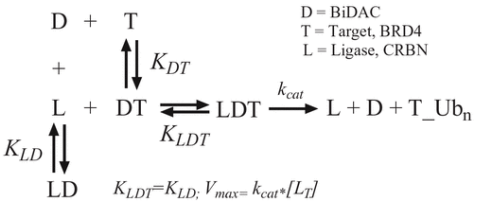
Following recent successes in designing related CDK degraders, BSJ-4-116 now allows selective targeting of CDK12 enabling elucidation of its biological function in cell-cycle regulation as well as therapeutic application. Notably, CDK12 represents the first example of resistance to a bifunctional degrader based on point mutations in the target protein, highlighting the importance of resistance studies in degrader evaluation.

Contributor: Andre

A Method for Determining the Kinetics of Small-Molecule-Induced Ubiquitination

Ellen F. Vieux[§], …, Stewart L. Fisher*

SLAS Discov. **2021**, DOI: 10.1177/24725552211000673



In addition to the binary affinities toward its target and E3 ligase, the rate of PROTAC-induced protein degradation is also determined by the ubiquitination rate. To determine the ubiquitination rate, this paper describes a cell-free lysate ubiquitination quantification protocol which uses a capillary-based immunodetection method. The benefit of using the cell-free lysates system is to provide a functional ubiquitination system that simultaneously bypasses cell membrane permeability issues. The authors applied the method by detecting the BRD4^{BD1} ubiquitination profile induced by CFT-0251, a JQ1 & phthalimide based PROTAC. The on-mechanism ubiquitination of BRD4^{BD1} was then validated by treatments with inhibitors of the different components of the system; ivebresib, CC-220 and MLN-4924. Treatment with inhibitors blocked CFT-0251 induced ubiquitination.

The authors then established a quantitative model for BRD4^{BD1} ubiquitination. Ubiquitination rate is dependent on two factors; 1) the ternary complex formation, which is driven by the binary affinities of the PROTAC to both the target and ligase, and 2) the kinetic rate of ubiquitination by ligases. Binary affinities (K_d) to both proteins were measured using FP while the ternary complex formation and inhibition constants (K_{TF} and K_{TI}) were quantified using an AlphaLISA-based assay. Although ternary complex formation is a prerequisite for ubiquitination, it does not necessarily determine the rate of ubiquitination; therefore, the authors measure the kinetics of the ubiquitination (V_{max}) by applying an essential enzyme activator model. The model is based on the rapid equilibrium assumption and non-cooperativity between the ligase and the PROTAC based on their binary affinity measurements. Interestingly, the amount of ternary

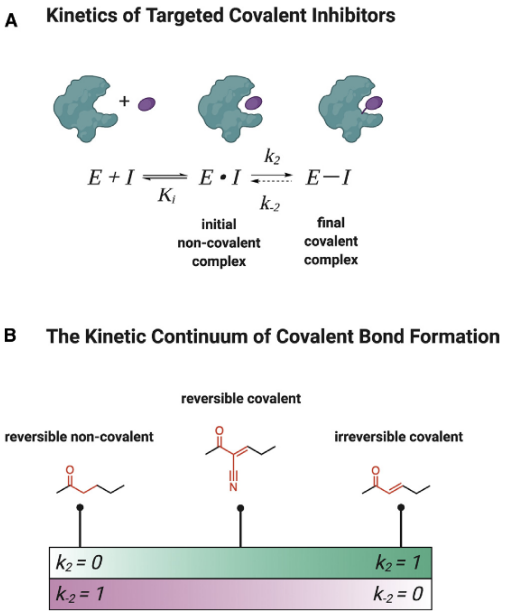
complex formation does not correlate with V_{max}. This highlights the key role of the kinetic component in the ubiquitination process.
This is an interesting read to give insight into why even PROTACs within a very closely related series might have very different ubiquitination and protein degradation profiles.

Contributor: Nicole

The role of reversible and irreversible covalent chemistry in targeted protein degradation

Hannah Kiely-Collins[§], Georg E. Winter, Gonçalo J. L. Bernardes*

Cell Chem. Biol. **2021**, DOI: 10.1016/j.chembiol.2021.03.005



Most PROTACs engage their target E3 ligase and protein of interest via reversible non-covalent binding interactions. This review discusses the potential advantages of harnessing targeted covalent inhibition (either reversible or irreversible) to facilitate protein degradation. Such benefits include improving selectivity, engaging poorly druggable targets/binding pockets, the discovery and recruitment of new E3 ligases, increasing ligand efficiency or achieving sustained target engagement. Case studies from the recent literature are highlighted, with one notable example being BTK, for which several groups have attempted to develop covalent PROTACs and have reported interesting observations and a variety of success. The case studies introduce important lessons in how covalent degraders are identified and validated, as well as the need for characterisation of their target engagement and cellular uptake, which can differ from their non-covalent analogues.

It is likely we will see a greater number of covalent degraders in the near future: as this article demonstrates, covalent targeting is enabling the community to access challenging or previously unknown POIs and E3 ligases, thus expanding the scope of targeted protein degradation. This article raises awareness of what is currently known about covalent PROTACs so that readers can be better equipped to develop and characterise these interesting molecules.

Contributor: Claudia

VHL inhibitor binding increases intracellular level of VHL

Julianty Frost[§], Sonia Rocha*, Alessio Ciulli*

bioRxiv **2021**, DOI: 10.1101/2021.04.12.439487

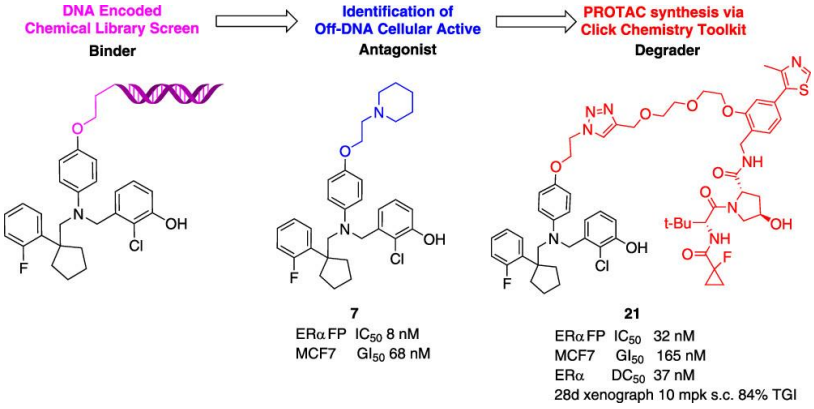
The tumour suppressor protein VHL, substrate recognition subunit of the multi-subunit Cullin RING E3 ligase ($CRL2^{VHL}$), specifically targets Hypoxia Inducible Factor-alpha subunits (HIF-α) for ubiquitination and degradation. As VHL inhibitors are widely used as chemical tools, e.g. to enable hijacking of VHL by bifunctional degraders or as therapeutics, understanding the effects of VHL inhibition to the intracellular proteome and identification of potential off-target effects is essential. This work studied the proteomic changes by VHL inhibitors in comparison to hypoxia and the prolyl-hydroxylase domain (PHD) enzyme inhibitor IOX2 using unbiased quantitative TMT-labelling based mass spectrometry. All examined HIF stabilizing agents lead to activation of HIF response and show a vast overlap in upregulated proteins. Interestingly, VHL inhibitors VH032 and VH298 were found to selectively upregulate VHL levels by VHL stabilization through ligand binding, resulting in reduced levels of HIF-1α after prolonged exposure to the inhibitor.
These results imply that changes to VHL levels on their own can regulate HIF-1α levels in cells, offering an opportunity for VHL inhibitor-based therapeutics. Beneficially, the high specificity of VH298 suggests low off-target effects in its response upon prolonged exposure, although further studies *in vivo* will be necessary to confirm this assumption. Upregulation of VHL levels through VHL stabilization upon PROTAC treatments has so far been neglected in VHL-based PROTAC evaluation but might be a relevant factor to monitor.

Contributor: Claudia

Bispecific Estrogen Receptor α Degraders Incorporating Novel Binders Identified Using DNA-Encoded Chemical Library Screening

Jeremy S. Disch[§]*, …, Shilpi Arora*

J. Med. Chem. **2021**, *64*, 5049



The estrogen receptor (ERα) gets overexpressed in 70% of all breast cancers, thus representing an attractive therapeutic target. Although some ER degraders are already in clinical trials and approved inhibitors exist, those are limited by acquired resistance through gain-of-function (GOF) mutations creating the need for novel therapeutics for both WT and GOF mutants of ERα. In this study, novel ERα binders as well as binding vectors for PROTAC design were identified by DNA-encoded chemical libraries (DECL) screening of ~120 billion encoded molecules against WT and three GOF mutants of ERα. "Hit" binder **7** was evaluated after off-DNA synthesis and featured nanomolar ERα binding, antagonism and degradation. Based on the results from the DECL and molecular docking, an initial PROTAC library was generated via click chemistry using different E3 ligases, leading to PROTACs **18** and **21** which proved to be nanomolar degraders of ERα efficiently inhibiting ER+ MCF7 tumour growth in mouse model when dosed subcutaneously.

The presented DECL platform offers an efficient approach for identification of novel potent inhibitors and bifunctional degraders in a timely manner. Advantageously for PROTAC development, the DECL approach not only identifies hit binders, but also suitable vectors for linker attachment as small molecules are screened in their linked presentation.

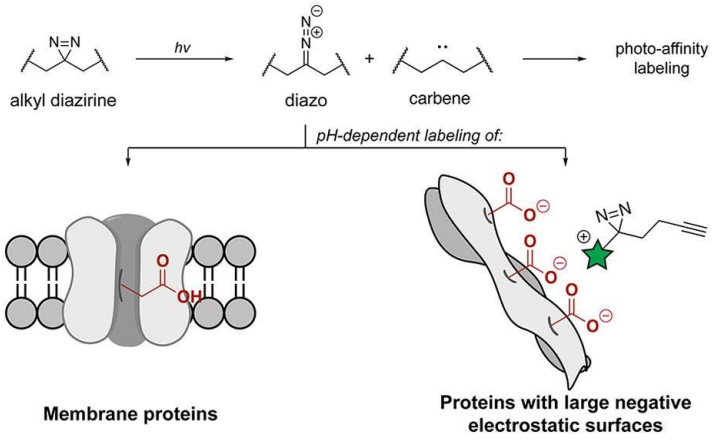
Other Paper Highlights

Contributor: Tasuku

Labeling Preferences of Diazirines with Protein Biomolecules

Alexander V. West[§], …, Christina M. Woo*

J. Am. Chem. Soc. **2021**, DOI: 10.1021/jacs.1c02509



Diazirines are one of the most common warheads for photoaffinity labelling, and probes that have diazirines are widely used for proteomics analyses and identification of binding sites of small molecules. However, the reactivity profile of diazirines has not been understood sufficiently in interpreting the results of those studies, which might be a limitation in designing new diazirine photoprobes.

In this paper, the authors investigated the reactive preference of amino acids and found that alkyl diazirines selectively react with acidic amino acids (e.g. glutamic acid and aspartic acid), while aryl diazirines react with a wide range of amino acids. The reactivity difference among 32 alkyl diazirine probes against BSA showed that probes that have positive charge have broader reactivity against proteins than other types of probes, and that glutamic acid is the most frequently reacted amino residue in positively charged probes despite no meaningful difference in neutral and negatively charged probes. In addition, they observed that alkyl diazirine probes have an enrichment bias for membrane proteins. These findings help us to interpret experimental results of photoaffinity labelling studies and guide the design and optimization of new diazirine photoprobes that have a preferred profile for each target.