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

Ciulli Lab
Targeted Protein Degradation

University of Dundee

Journal Club

October 2021

Content

Feature of the Month

Contributor: Giorgia

Targeted Protein Degradation makes IUPAC's list of Top Ten Emerging Technologies for 2021

Chemistry International, 43, 4, **2021**, 13

Since the IUPAC's 100th anniversary in 2019, a panel of judges have compiled a yearly list of technologies which have been deemed to be "developments on the verge of becoming game-changing commercial breakthroughs". On the inaugural list, asymmetric organocatalysis was featured, which I'm sure many can agree is a ground-breaking methodology – exemplified by being this year's Nobel prize in chemistry winning topic.

On the 18th of October, the list for 2021 was released and included many interesting and highly important fields of research, including targeted protein degradation.

The judges and nominees recognised TPD's enhanced therapeutic potential over traditional inhibitor drugs as a ground-breaking "technology". The article highlights that Arvinas and many others have entered clinical trials with PROTAC candidates as a treatment for many cancers, and that companies are also looking at the use of TPD in the treatment of Parkinson's and Alzheimer's, showing the hopefully wide target set for this emerging technology.

The other featured topics might be less familiar to journal club readers but are all extremely interesting and it doesn't take much imagination to see how they may lead to a brighter and more sustainable future.

The nominations are already open for 2022's top ten, so it will be interesting to see what technologies are in the running next year, and of course, how this year's top ten shape the world as they have been predicted to.

The list has been highlighted in C&EN and on the IUPAC website for further reading.

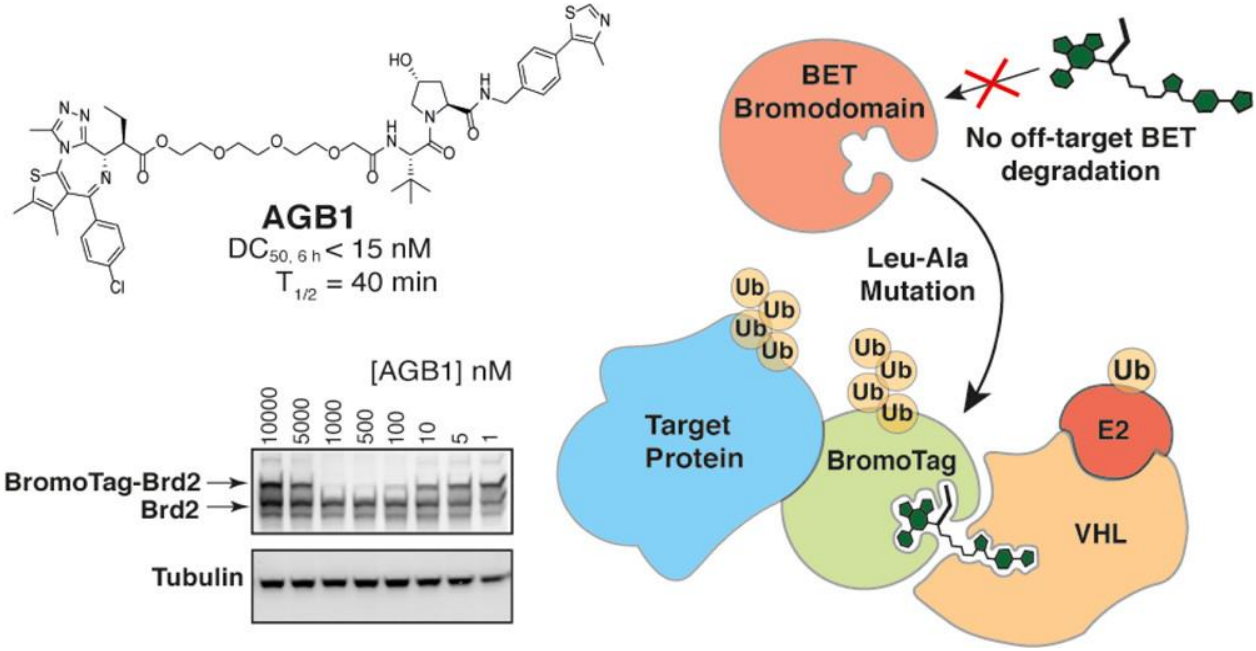
Targeted Protein Degradation

Contributor: Vesna

Development of BromoTag: A "Bump-and-Hole"–PROTAC System to Induce Potent, Rapid, and Selective Degradation of Tagged Target Protein

Adam G. Bond[§], Conner Craigon[§], …, Alessio Ciulli*

J. Med. Chem. **2021**, *64*, 15477



The authors present a novel way of inducing protein degradation in un-ligandable targets by using a degron tag that recruits the E3 ligase and leads to ubiquitination and subsequent degradation of the target. The authors point out the need for additional techniques due to disadvantages of the already existing AID, HaloTag and dTAG methods. The approach they developed is called Bump-and-Hole – where a "bump" is a ligand that carries a methyl or ethyl moiety that fits into a "hole", an engineered bromodomain that can fit methylated or ethylated compound. The bumped ligand causes the steric clash with the wild-type protein allowing for allele selectivity. The degron BromoTag is a BRD4 BD2 domain with leucine 387 mutated into alanine to accommodate the ethyl or methyl bump.

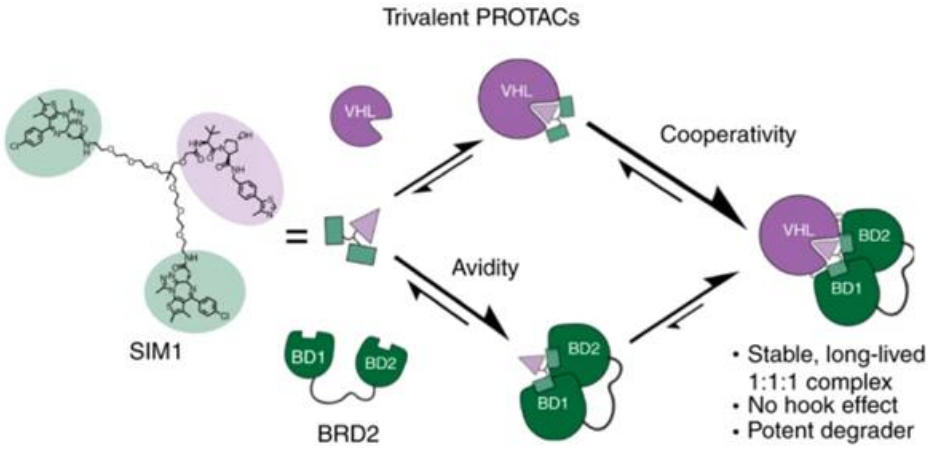
The authors have engineered a CRISPRed HEK293 cell line containing BromoTagged BRD2 to easily identify degraders with the best potency and selectivity on endogenously expressed protein. Degradation of BromoTag-BRD2 was considered on-target and degradation of any other BET protein off-target, enabling the easy screening. They synthesised two generations of compounds based on different BET binders – I-BET762 and JQ1. Extensive chemical optimisations and efforts in synthesising enantiomerically pure diastereomers have led to identification of compound AGB1, a selective and potent BromoTag-BRD2 degrader. This compound showed remarkable proteome-wide on-target effect, safe cytotoxicity profile, good PK profile and plasma stability making it a suitable for use in both in vitro and in vivo models.

Contributor: Giorgia

Trivalent PROTACs enhance protein degradation via combined avidity and cooperativity

Satomi Imaide[§], Kristin M. Riching[§], Nikolai Makukhin[§], …, Danette L. Daniels*, Alessio Ciulli*

Nat. Chem. Biol. **2021**, *17*, 1157



With the hypothesis that a trifunctional protein degrader could be more efficacious than the usual bifunctional PROTACs, the group assessed the X-ray structure of bivalent BET inhibitor MT1 and noted that the centre of the PEG linker was solvent exposed – and thus, an area that could possibly be further diversified and attached to an E3 ligase ligand to produce a trifunctional PROTAC. In order to produce an additional point of attachment on the inhibitor, a portion of the linker is exchanged for trimethylolethane which is structurally similar to the PEG linker previously used.

The additional diversification handle was then used to append either CRBN or VHL ligands, thus, forming the trivalent PROTAC. While all the synthesised PROTACs showed some activity, the VHL based molecules showed superior activity compared to bifunctional PROTAC MZ1. Further investigation of the best degrader SIM1 showed that degradation is fast, and selective to the BET proteins, with a higher propensity for degradation of BRD2. Biophysical investigations showed that SIM1 forms a 1:1:1 complex with VHL and both BD1 and BD2. Pleasingly, the PK profile for SIM1 both *via* intravenous and subcutaneous dosing is promising, with low clearance and a long half-life, showing that while it is a large compound, the PK profile achieved can be comparable to the usual small molecules.

The concept of trifunctional PROTACs is very interesting and this study showed that the addition of a further binding unit for the same protein can lead to increased activity due to the avidity of the dual protein binding. However, it is also alluded to that trifunctional molecules could also be used to bind to two distinct proximal protein domains along with an E3 ligase, hopefully giving an improved therapeutic effect.

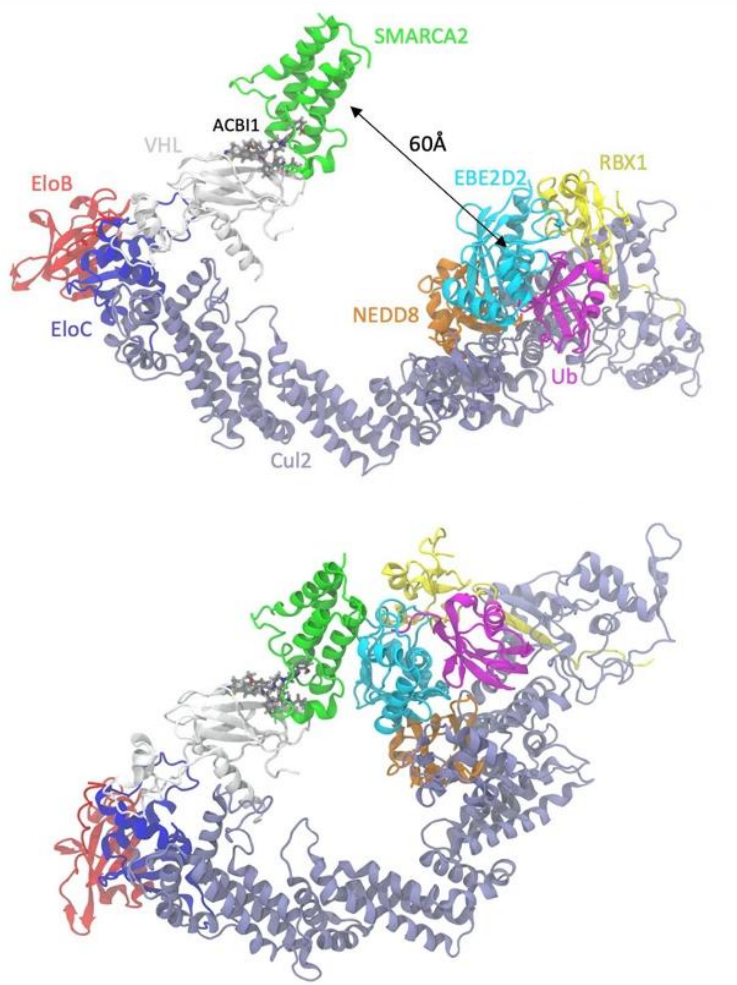
This paper is very in depth and explains well the thought process throughout, and pleasingly the hypothesis is met with the development of a trifunctional PROTAC that outcompetes both bifunctional predecessors. While the development and use of trifunctional molecules has been underexplored until this point, this paper shows that it is possible to have a larger trifunctional molecule with drug-like properties which- I'm sure will inspire further work in this area.

Contributor: Valentina

Atomic-Resolution Prediction of Degrader-mediated Ternary Complex Structures by Combining Molecular Simulations with Hydrogen Deuterium Exchange

Tom Dixon[§], Derek MacPherson[§], Barmak Mostofian[§], …, Alex Dickson*, Huafeng Xu*, Woody Sherman*, Jesus A. Izaguirre*

BioRxiv **2021**, DOI: 10.1101/2021.09.26.461830v1



This comprehensive article uses simulations to address three aspects of the targeted protein degradation process, 1) the formation of ternary complexes, 2) the conformational heterogeneity of the ternary complexes, and 3) the degrader efficiency *via* the full Cullin Ring Ligase. The authors augment their computational approaches by using experimental HDX-MS data to improve the efficiency and accuracy of the ternary structure predictions of the bromodomain of cancer target SMARCA2 with VHL mediated by three PROTACS. They also determine the structure of the ternary complex induced by the ACBI1 PROTAC (PDB ID: 7S4E). Their simulations were able to accurately predict model with RMSDs of 1.1 to 1.6 Å to experimentally solved structures.

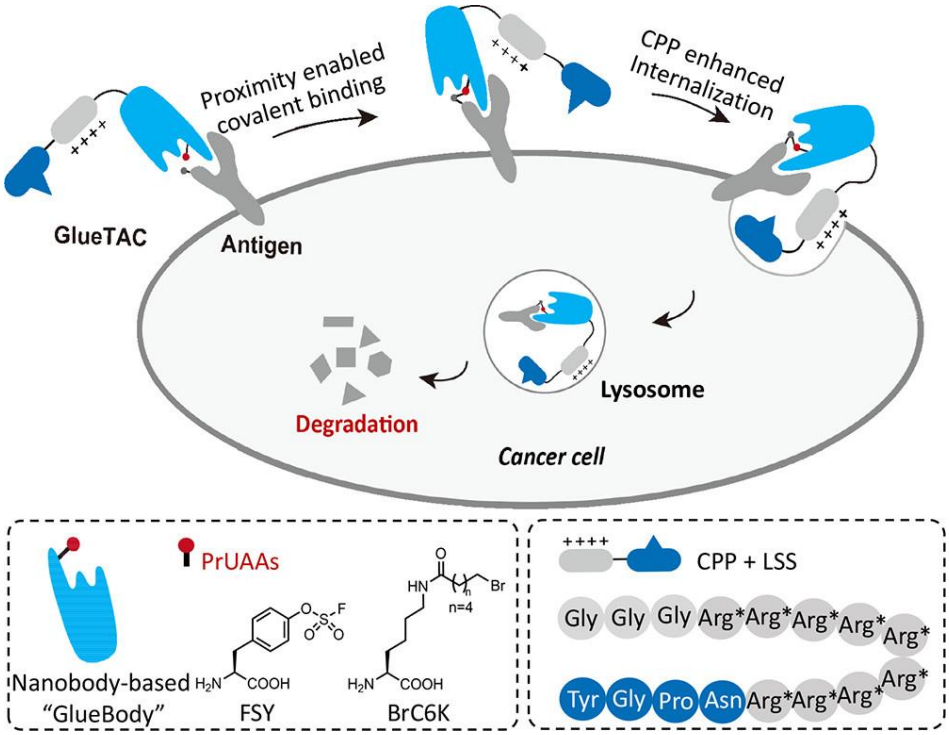
The investigation of the conformational heterogeneity is a wholly welcome aspect of this work, especially given the inclusion of in solution-based techniques, that allow us to break away from thinking about static structures. This is especially vital as the authors highlight that the crystal structures of the ternary complexes induced by the three PROTACs does not account for differences in cooperativity and degradation. The protocol that the team have developed based on weighted ensemble simulations (WES) approach seems very promising and will hopefully be adopted more widely.

Contributor: Manjula

Covalently Engineered Nanobody Chimeras for Targeted Membrane Protein Degradation

Heng Zhang[§], Yu Han[§], …, Jian Lin*, Peng R. Chen*

J. Am. Chem. Soc. **2021**, *143*, 16377



Monoclonal antibodies have revolutionized cancer therapy, antibodies can be used to interfere with the function of their target membrane proteins, for example blocking signalling and thereby inhibiting growth or inducing apoptosis of cancer cells. However, Ab delivery to tumour cells in vivo is hampered by the large size (150 kDa) of conventional antibodies. This article presents a covalent nanobody (Nb)-based (and non-UPS based) proteolysis-targeting chimeras (PROTAC) strategy, termed GlueTAC, as an alternative approach to eliminate cell-surface target proteins and thereby restrict the function of target membrane proteins/receptors. The recently reported LYTACs (lysosome-targeting chimeras), AbTACs (antibody-targeting chimeras), and bispecific aptamer chimeras offers other attractive approaches for membrane protein elimination by recruiting lysosome targeting receptors (LTRs) or RNF43 with glycopolypeptides, a single-chain variable fragment (scFv), or aptamers. However, the expression levels of specific LTRs or the membrane E3 ligase in some tumour cells are relatively low. In addition, there are stability issues with aptamers that remains to be solved and further endocytosis of large complexes is difficult due to the IgG-based antibody scaffolds. Therefore, this approach has several advantages: 1. It takes the advantage of small sized nanobodies (heavy-chain-derived antibodies) as they possess remarkable stability and high permeability. 2. Given that nanobodies have relatively low binding affinity, they created covalently engineered nanobody chimeras that irreversibly react with membrane proteins. This improved Nb- on-target retention and stabilised (Nb−Ag) complex as well as minimising off-target effects during endocytosis. 3. They conjugated this engineered nanobody with CPP-LSS to allow rapid protein degradation in lysosome and to induce cell-type-independent degradation. In brief, the GlueTAC containing a proximal reactive uncanonical amino acid covalently binds to the membrane antigen on tumour cells via proximity-enabled crosslinking. The resulting Nb−Ag complex is internalized and degraded in lysosomes by conjugated cell penetrating peptide consisting of nine D-arginines and a lysosomal sorting sequence consisting of NPGY. Here, using a PD-L1 specific covalent nanobody-GlueBody, they demonstrate that this strategy is particularly attractive when encountering tumour cells which are usually highly heterogeneous, where a cell-type-independent degradation strategy would allow the elimination of tumour markers (such as PD-L1) regardless of the cell differentiation within solid tumours.

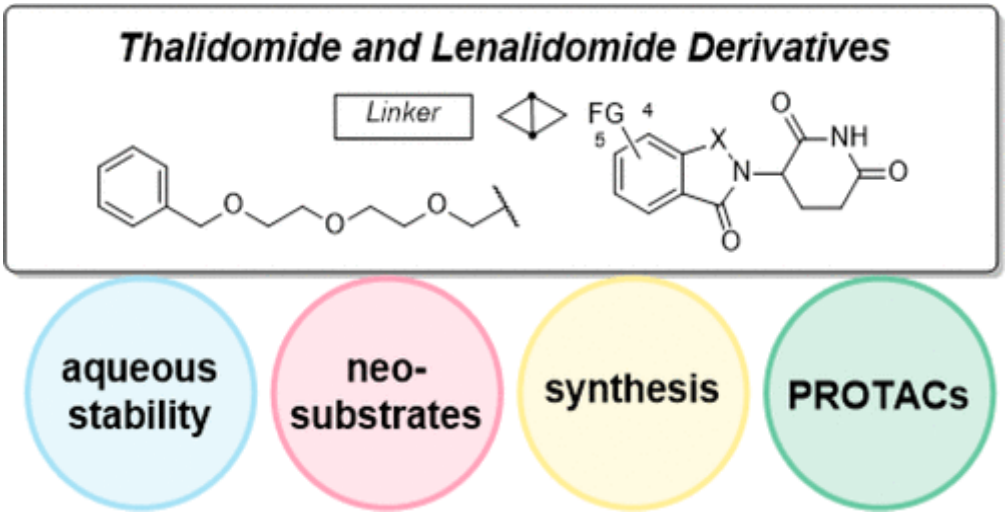
This is an interesting strategy with potential therapeutic applications in several diseases, where targeting cell surface proteins provides protective measures. However, cell type specific degradation cannot be achieved by this strategy, unless the target protein expression is cell type or disease specific.

Contributor: Giorgia

Influence of Linker Attachment Points on the Stability and Neosubstrate Degradation of Cereblon Ligands

Aleša Bricelj[§], …, Christian Steinebach*

ACS Med. Chem. Lett. **2021**, ASAP DOI: 10.1021/acsmedchemlett.1c00368



Whilst CRBN ligands are widely studied in PROTAC design, IMiD based structures can be prone to instability *in vivo* through hydrolytic or metabolic cleavage. CRBN ligands are also know to interact with many neosubstrates, and in this paper, the authors investigate whether the linker type, and attachment point have any correlation to the stability and promiscuity of the ligands.

16 Thalidomide derivatives, and 6 lenalidomide based binders were synthesised and tested for hydrolytic stability in a variety of conditions, showing that while there was minimal degradation of thalidomide derivatives in acidic solutions, at a physiological pH of 7.4 degradation was more pronounced. Amino-based linkers resulted in the most stable compounds, while alkynyl- and carboxamide-linkers were degraded fully after 24 h at pH 7.4. In general, linker attachement at C4 resulted in the most stable compounds. Lenalidomide-based ligands have before been seen to be more stable than their thalidomide counterparts, and this was also the case here, as the six compounds tested showed good stability at pH 7.4.

The degradation of known CRBN neosubstrate IKZF1 was also probed, and in general was lower with the lenolidamide derivatives. In plasma stability tests, amide based linkers were found to be less stabe than others tested.

The ^{13}C NMR shifts of the carbon at the linker position were found to correlate well to stability, and thus, this could be an easy way to preempt the stability of any ligands not covered in this literature.

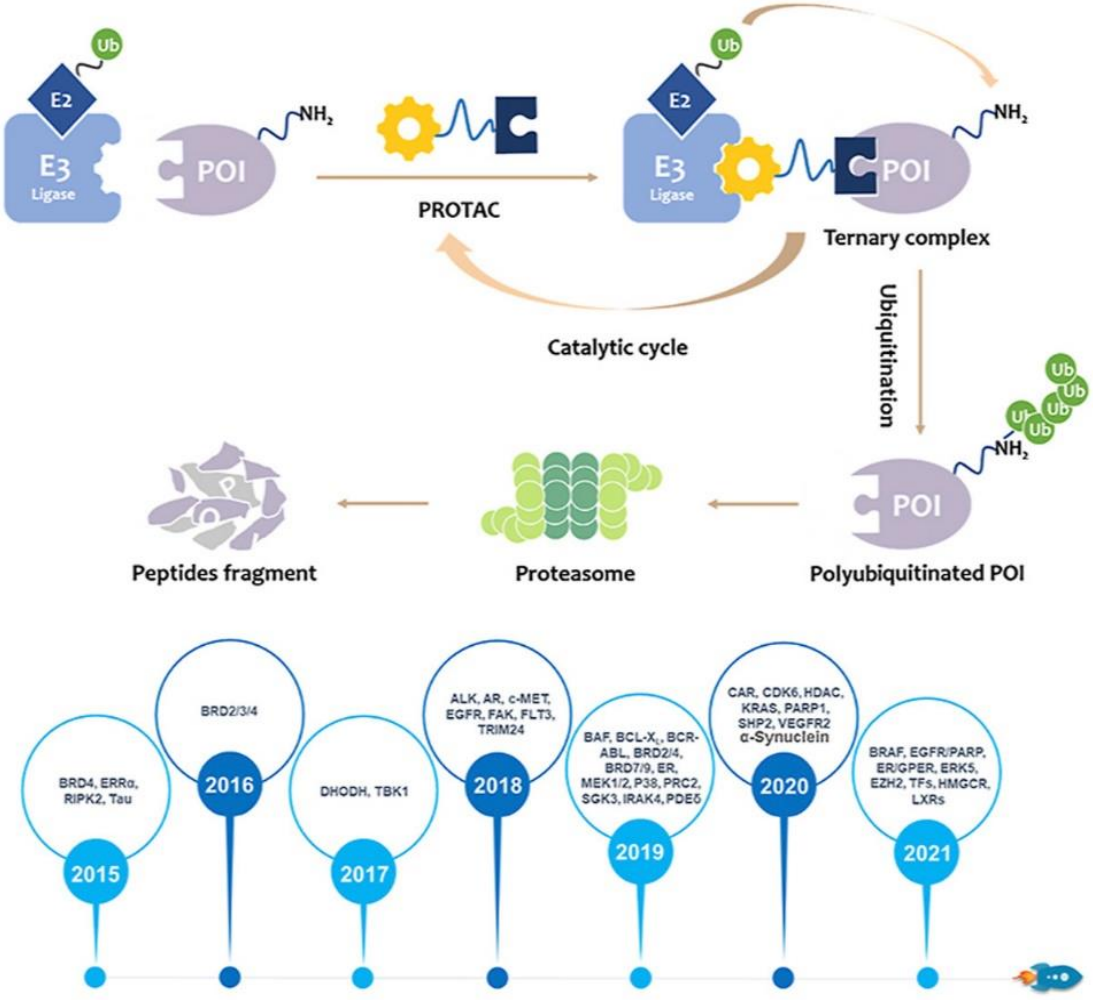
Overall, this paper makes a good piece of reference literature for CRBN based PROTAC design, in order to asses what features might lead to a more stable, and less promiscuous compound. The range of molecular structures tested was moderate, but consists of a representative range of linkers, and the techniques used could be applied to further compounds in the readers labs.

Contributor: Vesna

VHL-based PROTACs as potential therapeutic agents: Recent progress and perspectives

Chao Wang[§], Yujing Zhang, Jie Wang, Dongming Xing*

Eur. J. Med. Chem. **2021**, *227*, 113906



VHL-based PROTACs dominate the field of targeted protein degradation. VHL binders are specific, well characterised and have acceptable physicochemical properties which led to development of a number of PROTACs targeting different proteins in various diseases.

This review summarises major events and milestones of VHL PROTACs developed for targets in cancer, cardiovascular, immune and neurodegenerative diseases. The first chapter covers 32 different targets, including EGFR, MEK, KRAS, PARP1 and many other "hot" targets in cancer biology.

Since I'm a cancer biologist I found other targets that I don't encounter daily more interesting to read about. These targets include: HMGCR (3-hydroxy-3-methylglutaryl coenzyme A reductase) which plays a role in regulating cholesterol levels in blood, IRAK4 and RIP2 with important roles in inflammation and immunity and PROTACs that target α-synuclein and tau protein, two major targets in Parkinson's and Alzheimer's disease. Authors also point out PROTAC targeting PDEδ as crucial chemical tool in elucidating the full biological role of this protein.

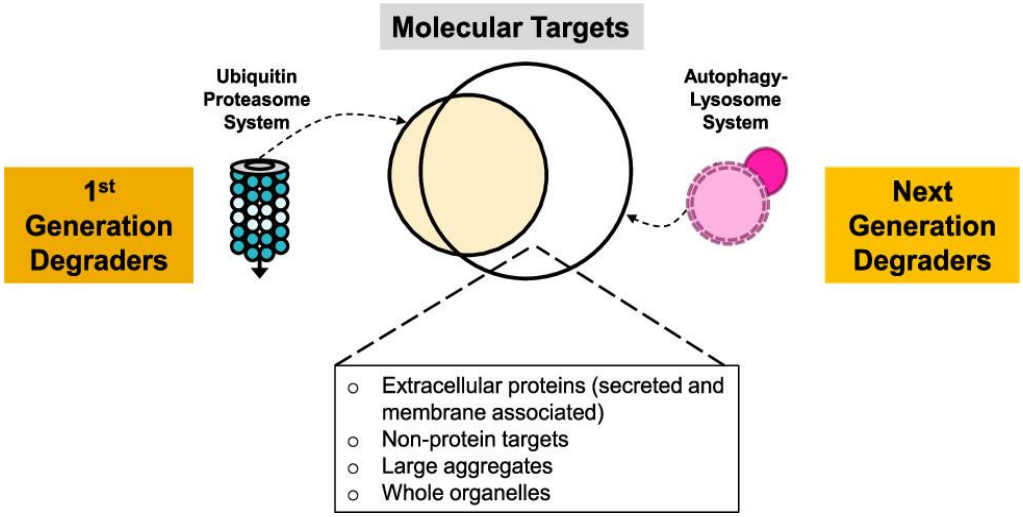
Authors note that there are about 600 E3 ligases in human proteome and not even 1% have been successfully utilised due to lack of ligands. Therefore, exploring new chemical space will be crucial in advancement of protein degradation field.

Contributor: Manjula

Redefining the Scope of Targeted Protein Degradation: Translational Opportunities in Hijacking the Autophagy−Lysosome Pathway Katelyn

Katelyn Cassidy*, Heng Zhao*

Biochemistry **2021**, DOI: 10.1021/acs.biochem.1c00330



This review covers an array of current TPD modalities. In this review, in addition to briefly specifying the clear advantages of the widely adopted UPS based TPD modalities (molecular glues and PROTACs) in the pharmaceutical/ biotechnology industries, the reviewer focuses on their blind spots and discusses their potential challenges, some examples are:

1. UPS-mediated degradation is limited to intracellular protein targets and nearly 40% of the human proteome resides in the extracellular space (e.g. secreted proteins, membrane proteins, insoluble proteins, and even whole cellular organelles) and therefore are difficult/unsuitable targets for UPS-mediated degradation.
2. The absence of universal guidelines for degrader design or platforms to streamline the process of designing and optimizing degraders and finding new ligands for novel E3 ligase partners.
3. Until mid-2021, there has been only one example of a PROTAC reported for an “undruggable” target.
4. The need to build PK and PD evaluation tools for PROTACs and other protein degraders.

The authors give their perspective and viewpoints and provide a good coverage of the prior literature.

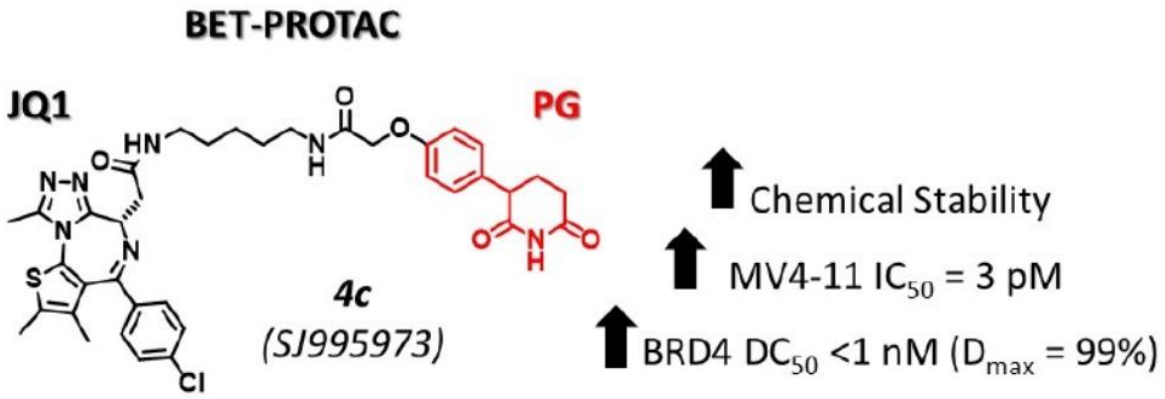
The authors further emphasise the importance of moving beyond UPS-based TPD modalities and discuss possibilities for other non-UPS based TPD modalities (LYTAC, AUTAC, and ATTEC) such as hijacking the autophagy and lysosomal degradation pathway, to expand the landscape of what is possible to target. There are numerous interesting, valuable review sections, worth reading for those in the TPD field.

Contributor: Giorgia

Phenyl-Glutarimides: Alternative Cereblon Binders for the Design of PROTACs

Jaeki Min[§], Anand Mayasundari[§], Fatemeh Keramatnia[§], Barbara Jonchere[§], …, Zoran Rankovic*

Angew. Chem. Int. Ed. **2021**, DOI: 10.1002/anie.202108848



While IMiDs are commonly used CRBN ligands in PROTACs, as previously mentioned their hydrolytic stability can lead to low *in vivo* efficacy, and difficulties in preparation. In order to combat this instability, additional CRBN ligands can be utilised to hopefully retain the ability to recruit cereblon, with increased stability over IMiDs. Noting that while a range of alternative CRBN ligands have been disclosed in patents more recently, their physicochemical properties are understudied and would provide more rational for their use over more classical ligands, and so the authors design and thoroughly assess a range of phenyl glutarimides (PGs), looking at the stability and activity of these new ligands.

Based on the facts that the phthalimide ring in IMiDs is not within the binding pocket and can be attributed to increased instability of the glutarimide, the authors choose to append a simple phenyl moiety to the glutarimide. This hypothesis was looked into by docking the new structure into a previous X-ray structure with thalidomide bound and predicted the phenyl group to be deeply bound in the pocket, hopefully suggesting tighter binding of the glutarimide, meaning CRBN binding should be retained.

Pleasingly, when assessing the binding of the ligands, as well as PROTACs synthesised with the ligand through a fluorescence polarisation assay, the phenyl glutarimides showed similar binding affinities to IMiDs, and when taking into account their smaller size, in fact have higher ligand efficiencies.

The stability of the PGs was improved in comparison to the IMiDs, but also pleasingly the target degradation was improved when using PG based PROTACs, with both lower DC_{50}'s as well as a much longer T_{max} seen.

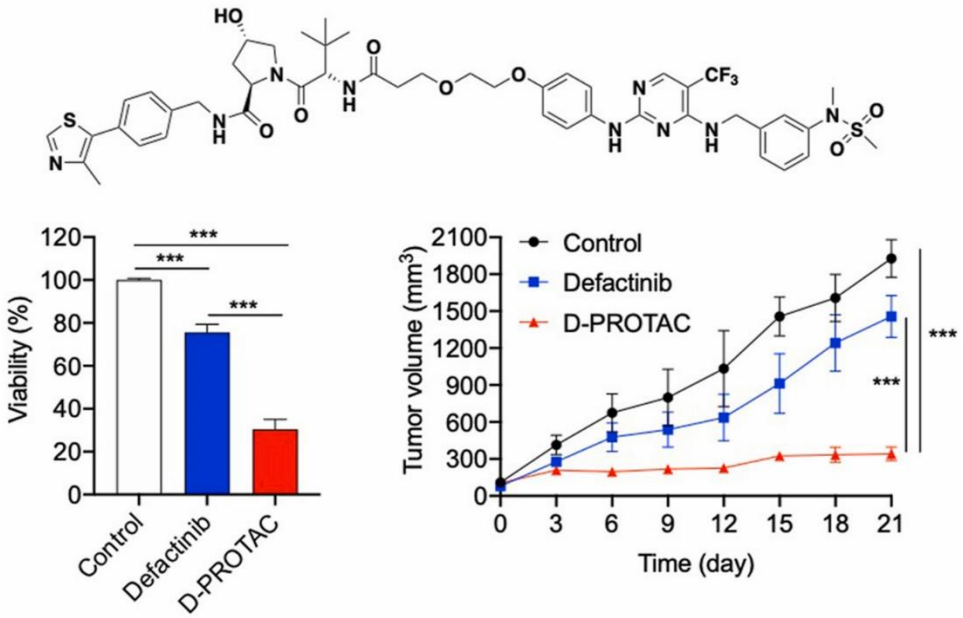
PGs seem like a worthy competitor for IMiD ligands, hopefully being more easily handled in synthesis, as well as more effective. I would expect these to be widely utilised in future PROTACs and other TPD.

Contributor: Vesna

FAK-targeting PROTAC demonstrates enhanced antitumor activity against *KRAS* mutant non-small cell lung cancer

Jinyuan Liu[§], Lei Xue, Xiang Xu, Jinhua Luo, Shijiang Zhang*

Exp. Cell Res. **2021**, *408*, 112868



KRAS mutation occurs in approximately 30% cases of non-small cell lung cancer (NSCLC). FAK (focal adhesion kinase) protein is a cytoplasmic tyrosine kinase that plays a role in cell growth, invasion and metastasis and is frequently found overexpressed in cancer, particularly in NSCLC. Studies revealed that FAK acts downstream of KRAS and inhibiting FAK suppresses the growth of KRAS mutated NSCLC. Defactinib is a kinase inhibitor of FAK and it showed a tolerable safety profile in clinical trials, but also modest antitumour activity.

The authors of this study hypothesised that elimination of FAK protein could prove to be more useful than inhibition due to scaffolding properties for signal transduction in addition to kinase role. Therefore, they've set out to compare Defactinib inhibitor and Defactinib-based PROTAC called D-PROTAC *in vitro* and *in vivo*. They have demonstrated superiority of D-PROTAC in inducing anti-proliferative effects and reducing invasion potential in cancer cells *in vitro* and later in a xenograft model of KRAS-mutated cell line A427 compared to Defactinib.

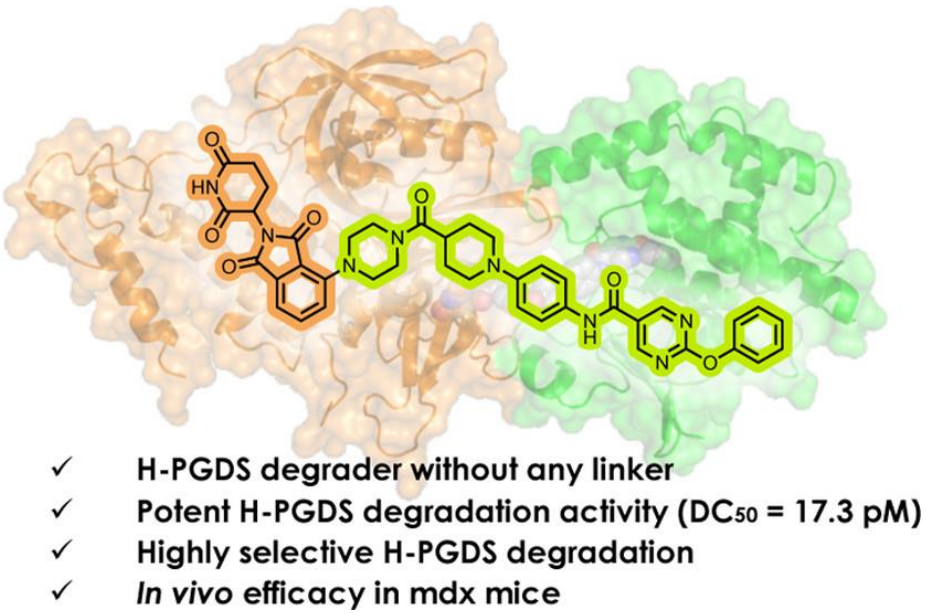
This paper is a nice proof of concept for benefits of degrading FAK versus just inhibiting its kinase function. To further verify these claims, the cell line panel should be extended to include various KRAS mutated cell lines.

Contributor: Manjula

Discovery of a Highly Potent and Selective Degrader Targeting Hematopoietic Prostaglandin D Synthase via In Silico Design

Hidetomo Yokoo[§], …, Norihito Shibata*, Mikihiko Naito*, Kosuke Aritake*, Yosuke Demizu*

J. Med. Chem. **2021**, DOI: 10.1021/acs.jmedchem.1c01206



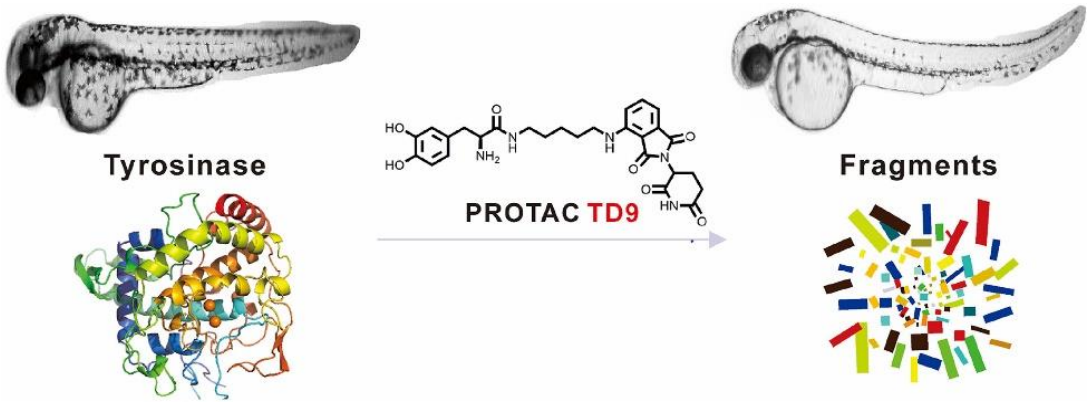
This study demonstrates the effectiveness of *in silico* simulation for the rational development of PROTACs. PROTAC development requires selection of an appropriate linker, an E3 ligase ligand, and a target protein ligand. Given there is no streamlined process for designing and optimizing degraders, it is necessary to synthesize a large number of PROTACs through trial and error. In this study the authors have successfully established a docking simulation of the ternary complex of a hematopoietic prostaglandin D synthase (H-PGDS) degrader and cereblon to develop a PROTAC (H-PGDS)-7 (compound 6). This compound showed potent and selective degradation activity (DC50 = 17.3 pM) and potent suppression of prostaglandin D2 production in KU812 cells. Thus, the study put forward the potential implications of *in silico* docking simulation as an effective strategy for the rational design and development of PROTAC compounds with potent activity.

Contributor: Giorgia

Design, synthesis and biological evaluation of tyrosinase-targeting PROTACs

Dingqiang Fu[§], …, Guangxun Li*, Yun Deng*, Zhuo Tang*

Eur. J. Med. Chem. **2021**, *226*, 113850



In order to treat skin diseases which lead to over pigmentation, the authors wanted to design a new tyrosinase degrading PROTAC. Many of the existing tyrosinase inhibitors are based on natural products, which do lead to effective inhibition, however, with many downsides such as toxicity or instability. Designing a PROTAC to degrade human tyrosinase would hopefully lead to an improved therapy without the caveats that many of the inhibitors have.

The authors synthesised a range of PROTACs using known tyrosinase binders kojic acid or L-DOPA, and with pomalidomide and various linker structures, and assessed their degradation affinity for tyrosinase. Pleasingly, whilst the kojic acid based PROTACs showed no significant activity, many of the L-DOPA based PROTACs showed moderate levels of degradation, as well as less cell toxicity than L-DOPA as an inhibitor. The best degrader in the series **TD9** had a DC_{50} of ~50 µM and D_{max} of 61% at 100 µM. While the degradation was moderate, **TD9** was also able to act as an inhibitor so may still have benefits over only inhibitor-based therapies. Along with the promising cellular activity, **TD9** was seen to reduce the melanin production *in vivo* with zebrafish.

Molecular docking of **TD9** into human tyrosinase showed the key interactions between the phenolic moieties with both Cu^{2+} in the active site, and residue Asn260, along with interactions between the 1^{γ} amine of L-DOPA with Gly281. Presumably these interactions are conserved when using L-DOPA on its own as an inhibitor.

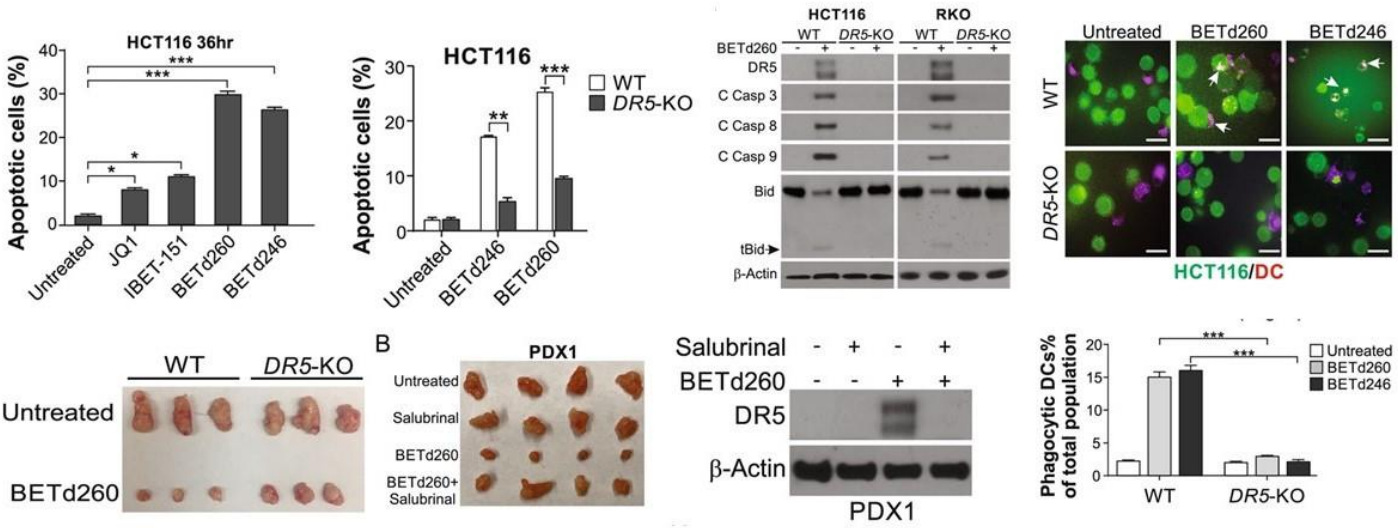
The study seemed thorough, with synthesis of a selection of PROTACs based on the two inhibitors chosen with simple chemistry. Since many simple inhibitors are known for the target, it could have been nice to see an additional few used in the PROTAC design, to hopefully improve the moderate DC_{50} and D_{max} of **TD9**.

Contributor: Manjula

BET protein degradation triggers DR5-mediated immunogenic cell death to suppress colorectal cancer and potentiate immune checkpoint blockade

Jingshan Tong[§], …, Lin Zhang*

Oncogene. **2021**, DOI: 10.1021/acs.jmedchem.1c01206



Bromodomain and extraterminal (BET) protein family (BRD2, BRD3, BRD4, and BRDT) are epigenetic readers that recognize acetylated lysine residues in histones or in other proteins such as transcription factors. BET proteins play a critical role in oncogenesis by controlling the expression of oncogenes such as c-Myc. Targeting BET family proteins has recently emerged as a promising anticancer strategy. In this paper the authors investigate the molecular mechanisms by which cancer cells respond to BET targeting in colorectal cancer and provide evidence for ER stress and DR5- dependent cancer cell killing following BET targeting. They observed that BET degraders (BETd; PROTACs BETd, BETd260 and BETd246) are more potent than BET inhibitor (BETi) in suppressing growth of CRC cell line HCT116. Furthermore, the authors discovered upregulation of DR5 expression via CHOP-mediated transcriptional activation after analysing WT and KO HCT116 cells treated with BETd260. Next, they provided evidence for the functional role of DR5 in response to BETd in CRC cells and showed that knock-out of DR5 in HCT116 cells abrogated growth suppression.

Further, treatment of BETd at low, non-apoptotic doses markedly enhanced DR5 induction, caspase activation, and apoptosis in WT HCT116 cells, but not in DR5-KO cells. Altogether, they demonstrated that the apoptotic and chemosensitization effects of BETd260 and BETd246 in CRC cells are dependent on DR5 via crosstalk between the two apoptotic pathways. Furthermore, they confirmed *in vivo* antitumor effects of BETd is mediated by DR5 by using xenograft tumours established from WT and DR5-KO HCT116 cells and using patient-derived xenograft (PDX) models in presence and absence of ER stress inducer salubrinal. They also showed that BETd induced DR5-dependent immunogenic cell death (ICD) in CRC cells, not by affecting the expression of PD-L1, but instead by inducing maturation of immune cells via uptake of ER chaperone calreticulin from dead cancer cells. Further, by using the immuno-competent CT26-BALB/cJ syngeneic tumour model, they show that BETd260 potentiates anti-PD-1 immunotherapy in a DR5-dependent manner, whereas either agent alone showed little or no efficacy in this model at these doses. Thus, this study demonstrated the therapeutic efficacy of BETd260 and BETd246, highlighting that BET degraders can be used in combination with 5-FU, oxaliplatin, TRAIL, or anti-PD-1 as they exhibit highly synergistic functions and can markedly improve therapeutic efficacy in colorectal cancer. It will be interesting to assess if the mechanisms are similar in other cancer types.

Contributor: Giorgia

Design, Synthesis, and Evaluation of Trivalent PROTACs Having a Functionalization Site with Controlled Orientation

Yifan Huang[§], ..., Naoki Kanoh*

ChemRxiv **2021**, DOI: 10.33774/chemrxiv-2021-8cp0q-v2

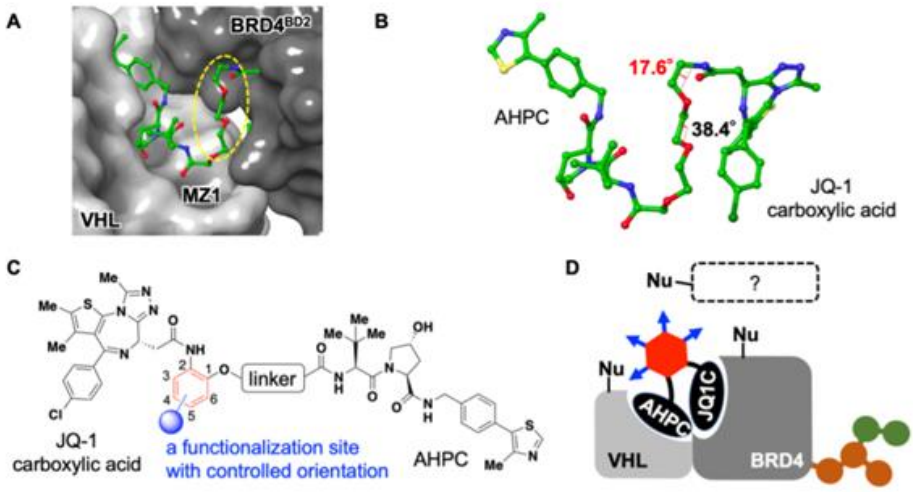


Figure 2. Design of trivalent PROTACs having a functionalization site with controlled orientation.

Inspired by the work of Kim *et al.* and Ciulli *et al.* on trifunctional PROTACs, this group set out to design and synthesise trivalent molecules where one of the functional groups is a point of further diversification. The point of functionalisation would allow synthesis of a range of trivalent PROTACs, or functional molecules for screening such as fluorescent probes or affinity labelling reagents.

Within this paper, the synthesis of PROTACs with the additional functionalisation handle at sites around a phenyl ring in the linker are carried out and assessing the dose dependant BRD4 degradation with these PROTACs shows variable results depending on substitution. Derivatisation at C3 showed lower activity than the patent PROTAC, suggesting that appending the functionalisation handle to this position is detrimental. Substitution at C4 and C6 showed a hook effect in the dose dependent degradation studies, again showing that functionalisation at these sites may not be beneficial. When C5 was functionalised, the activity seen was comparable to the initial PROTAC, and no hook effect was observed, possibly showing that the further functionalisation of this position could be useful for synthesis of probes. However, since no additional functionalisation was carried out in this paper, addition of any larger groups could lead to the effects seen with derivatisation of the other positions.

The concept laid out in the introduction of this paper is very interesting, and synthesis of trivalent probe molecule will surely be more prevalent in the future, however this paper did not delve into this and instead focussed on an intermediate molecule. It would have been nice to see further diversification with relevant structures such as fluorescent probes, to see the effect of this larger functionality and test the hypothesis further.

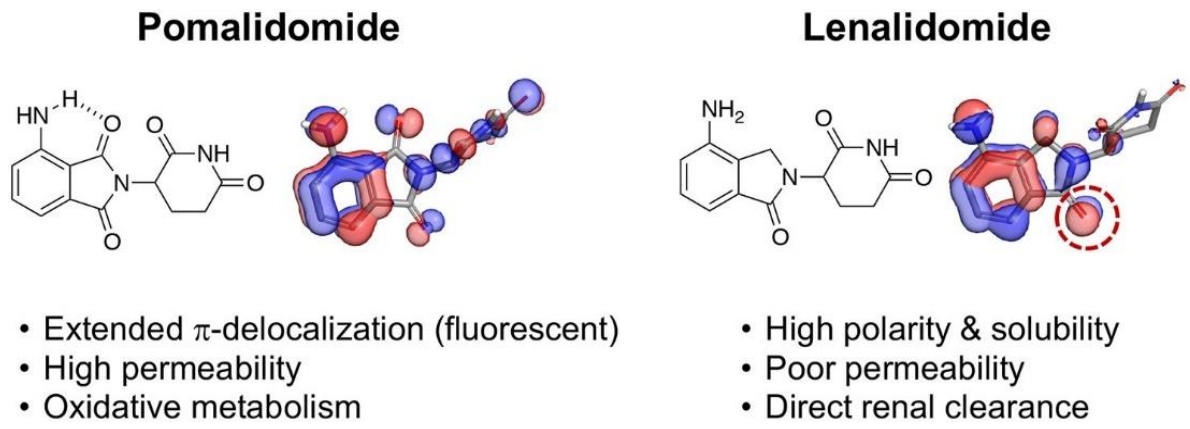
Other Paper Highlights

Contributor: Suzanne

Physicochemistry of cereblon modulating drugs determines pharmacokinetics and disposition

Nikki Kong[§], Hu Liu, Jianwei Che, Lyn Jones*

ACS Med. Chem. Lett. **2021**, ASAP, DOI: 10.1021/acsmedchemlett.1c00475



This paper examines the physicochemical properties of the IMiDs pomalidomide, thalidomide and lenalidomide as well as the phthalimide EM-12, and the candidate drug iberdomide. The mode of action of IMiDs is being intensely studied but here the focus is on gaining a better understanding of their pharmacokinetics by examining lipophilicity, solubility, metabolism, permeability, intracellular bioavailability, and cell-based potency.

The extended π-electron system of thalidomide and pomalidomide affords higher lipophilicity and lower solubility compared to the more polar isoindolinone analogues EM-12 and lenalidomide. The intramolecular hydrogen bonding of the amino group with the phthalimide ring of pomalidomide further increases relative lipophilicity. Lenalidomide has the highest hydrophilicity of the IMiDs that imparts high aqueous solubility and remarkably low permeability.

The paper includes a table which can be used to help modify a structure to improve a property of interest. For example, the metabolic stability of a lipophilic phthalimide/pomalidomide-based degrader could be improved by switching to the more polar isoindolinone/lenalidomide scaffold. Conversely, the low permeability of a polar lenalidomide analogue could be addressed through conversion to the phthalimide. If stability in human plasma was proving problematic, a thalidomide motif could be changed to a lenalidomide one. Overall, this short paper has many useful comparisons of PK properties of IMiDs, helpful for the optimisation of new compounds for targeted protein degradation.

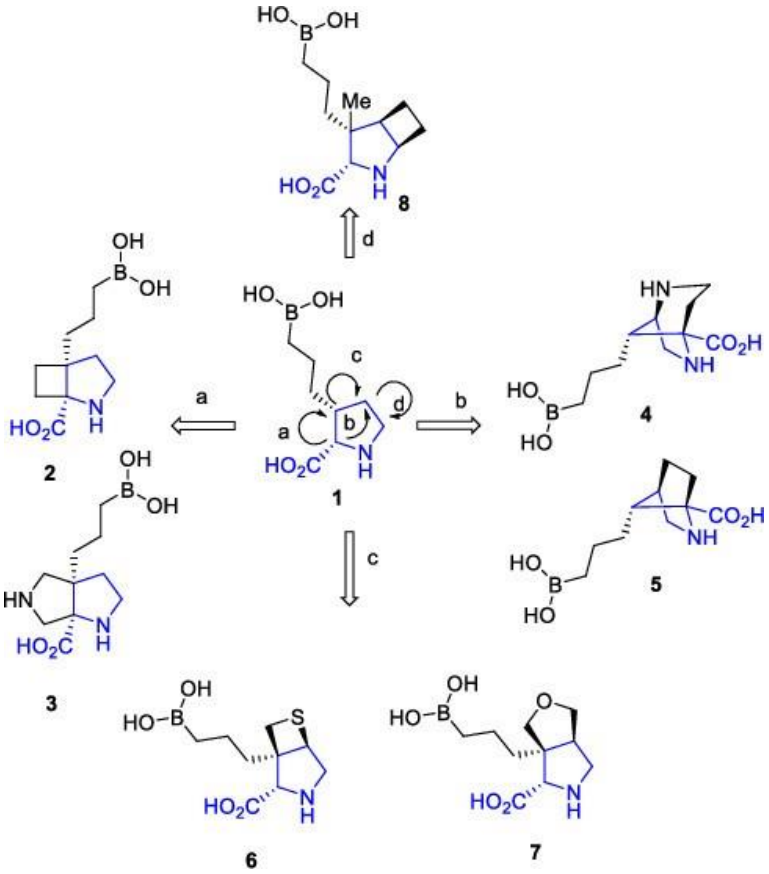
Contributor: Tasuku

Comprehensive Strategies to Bicyclic Prolines: Applications in the Synthesis of Potent Arginase Inhibitors

Derun Li[§]* *et al.*

ACS Med. Chem. Lett. **2021**, ASAP, DOI: 10.1021/acsmedchemlett.1c00258

L-Proline is one of the essential amino acids and this moiety is used for a variety of biologically active compounds and chemical organocatalysts. It is known that L-proline forms the Cγ-*exo* and Cγ-*endo* conformations and there is a quick equilibrium between the two states at room temperature. The authors previously found that Compound **1** showed significant inhibitory activity against human arginase 1 (hArg1) and that the cocrystal structural analysis of **1** in hArg1 revealed that the compound binds to hArg1 in Cγ-exo form. Therefore, they hypothesized that the restriction of the proline ring to the Cγ-exo form would improve the binding affinity to hArg1. Their strategy is to form of bicyclic prolines to fix the desired Cγ-exo form. They utilized elegant and stunning chemical strategies to develop a variety of bicyclic prolines successfully. The cell-free binding assay for hArg1 revealed that some of the bicyclic prolines inhibited hArg1 more strongly than Compound **1**. In addition, cocrystral structure analyses showed that the compounds showing great inhibitory activity bind to hArg1 with Cγ-exo conformation. Their findings not only help to design novel hArg1 inhibitors but also synthesize a broad range of conformationally restricted bicyclic proline derivatives.



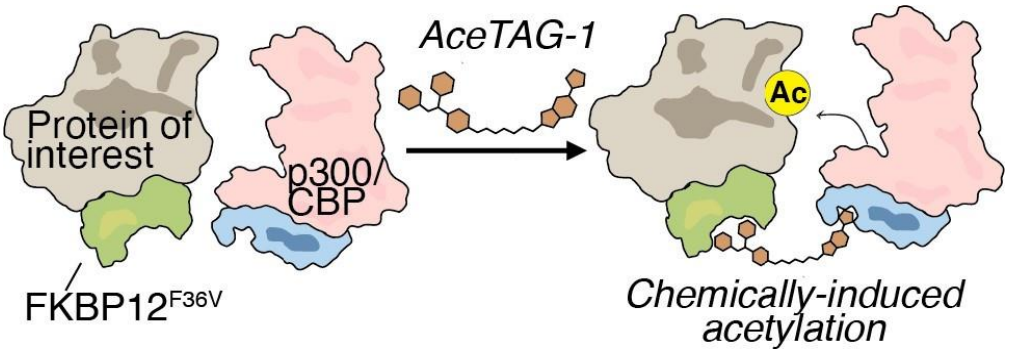
One of the must-read papers for medicinal chemists. I'm really impressed by their deep knowledge of organic chemistry. Perhaps it might be useful for new VHL ligands?

Contributor: Alessio

Targeted Protein Acetylation in Cells Using Heterobifunctional Molecules

Wesley W. Wang[§], …, Christopher G. Parker*

J. Am. Chem. Soc. **2021**, *143*, 16700



TAC-mania continues and expands to targeted protein acetylation (AceTAGs). Chris Parker and co-workers developed bifunctional small molecules that induce proximity between an acetyltransferase and an FKBP12-tagged protein leading to acetylation of the tagged protein. Their design leveraged the well-established bumped Ariad ligand for the FKBP12-mutant tag and a ligand for the bromodomains of the acetyltransferase proteins CREBBP/EP300. The researchers made three AceTAGs bearing different PEG/alkylic linkers. The best molecule, AceTAG-1, formed more ternary complex in AlphaScreen proximity and target engagement assays. They show compound-induced acetylation of three different tagged-proteins (interestingly all reported endogenous substrates of the hijacked acetyltransferase), identify Lys sites of acetylation, and characterise proteome-wide selectivity.

This paper is significant because it's a first on acetylation-induced molecules. It adds to proximity-based compounds beyond PROTACs that modulate post-translational modifications, such as phosphorylation (here and here). It is envisaged that targeted acetylation could tackle drug targets in new ways. For example, acetylated proteins could be stabilized, or their function altered by preventing Lys ubiquitination; transcription factors or RNA-binding proteins could be blocked by neutralizing the positively-charged Lys side chains on surfaces interacting with the negatively-charged DNA or RNA. The next step will be to develop AceTAGs targeting endogenous untagged proteins.

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