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


# Ciulli Lab
# Targeted Protein Degradation

University of Dundee

Journal Club

July 2021

# Table of Contents

**Targeted Protein Degradation ..... 1**

Martin Reynders *et al*., *Cell Chem. Biol.* Optical control of targeted protein degradation ..... 1

Troy A. Bemis *et al*., *J. Med. Chem.* Unravelling the role of linker design in Proteolysis Targeting Chimeras ..... 2

Guilherme Vieira de Castro *et al*., *ChemRxiv* Estimating the cooperativity of PROTAC- induced ternary complexes using $^{19}$F NMR displacement assay ..... 3

Neil P. Grimster *et al*., *RSC Med. Chem*. Covalent PROTACs: the best of both worlds? ..... 4

Shipeng He *et al*., *Angew. Chem. Int. Ed.* Aptamer-PROTAC Conjugates (APCs) for Tumor-specific Targeting in Breast Cancer ..... 4

Peter Stacey *et al*., *SLAS Discov.* A Phenotypic Approach for the Identification of New Molecules for Targeted Protein Degradation Applications ..... 5

Mingxing Teng *et al*., *ACS Med. Chem. Lett.* Exploring Ligand-Directed *N*-Acyl-*N*-alkylsulfonamide-Based Acylation Chemistry for Potential Targeted Degrader Development ..... 6

Vittoria Massafra *et al*., *J. Immunol.* Proteolysis-Targeting Chimeras Enhance T Cell Bispecific Antibody-Driven T cell Activation and Effector Function through Increased MHC Class I Antigen Presentation in Cancer Cells ..... 7

Amy Ryan *et al*., *J. Am. Chem. Soc.* Targeted Protein Degradation through Fast Optogenetic Activation and Its Application to the Control of Cell Signalling ..... 8

Brandon Dale *et al*., *Nat. Rev. Cancer* Advancing targeted protein degradation for cancer therapy ..... 9

Ashwin Narain *et al*., *Mol. Cell* Targeted protein degradation reveals a direct role of SPT6 in RNAPII elongation and termination ..... 10

**Other Paper Highlights ..... 11**

Willem den Besten *et al*., *J. Am. Chem. Soc.* Primary Amine Tethered Small Molecules Promote the Degradation of X-Linked Inhibitor of Apoptosis Protein ..... 11

Laurent David *et al*., *ChemRxiv* Prediction of chameleonic efficiency ..... 12

David L. McKinzie *et al*., *J. Med. Chem.* Discovery and Early Clinical Development of LY3202626, a Low-Dose, CNS-Penetrant BACE Inhibitor ..... 13

John Jumper *et al*., *Nature* Highly accurate protein structure prediction with AlphaFold ..... 14

Cassandra R. Kennedy *et al*., *ACS Chem. Biol.* A Probe for NLRP3 Inflammasome Inhibitor MCC950 Identifies Carbonic Anhydrase 2 as a Novel Target ..... 15

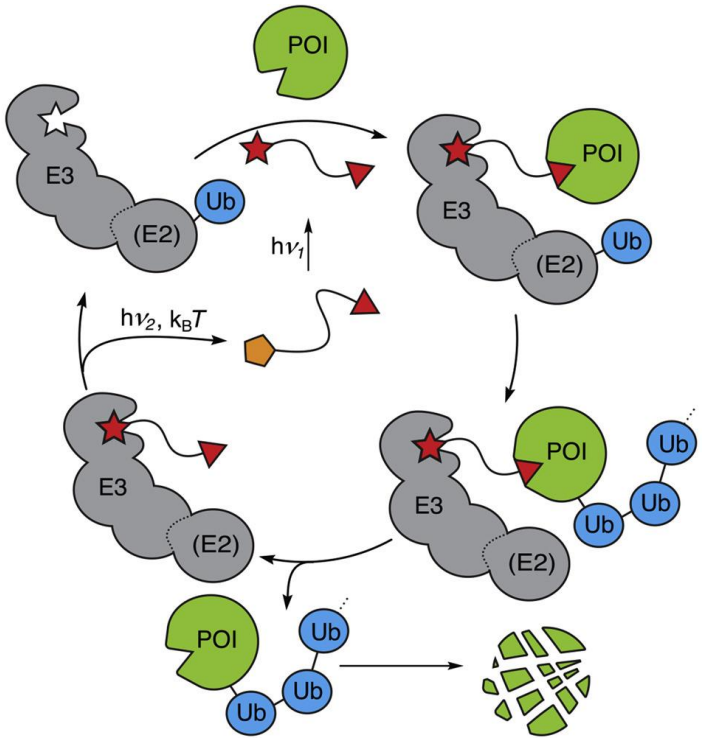
# Targeted Protein Degradation

Contributor: Suzanne

**Optical control of targeted protein degradation**

Martin Reynders[§], Dirk Trauner*

*Cell Chem. Biol.* **2021,** *28*, 969




This paper summarises recent approaches that employ light as an additional stimulus to control induced protein degradation. Light responsive degraders enable the precise temporal and spatial control of protein levels, making them useful research tools but also potential candidates for human precision medicine. The authors propose that providing an external stimulus that could activate and deactivate a PROTAC at a chosen time and location, could avoid potentially harmful systemic side effects. Optical control can also be used to induce oscillations of protein levels and study the functional consequences.

For the successful optical control of targeted protein degradation, the formation of a productive ternary complex must be controlled with light. Two main strategies have emerged to render small molecules responsive to light – either by masking of the pharmacophore with a photocleavable protecting group (PPG) or by incorporation of a reversible photoswitch into the molecule that renders the compound pharmacologically inactive in one form and active in another.

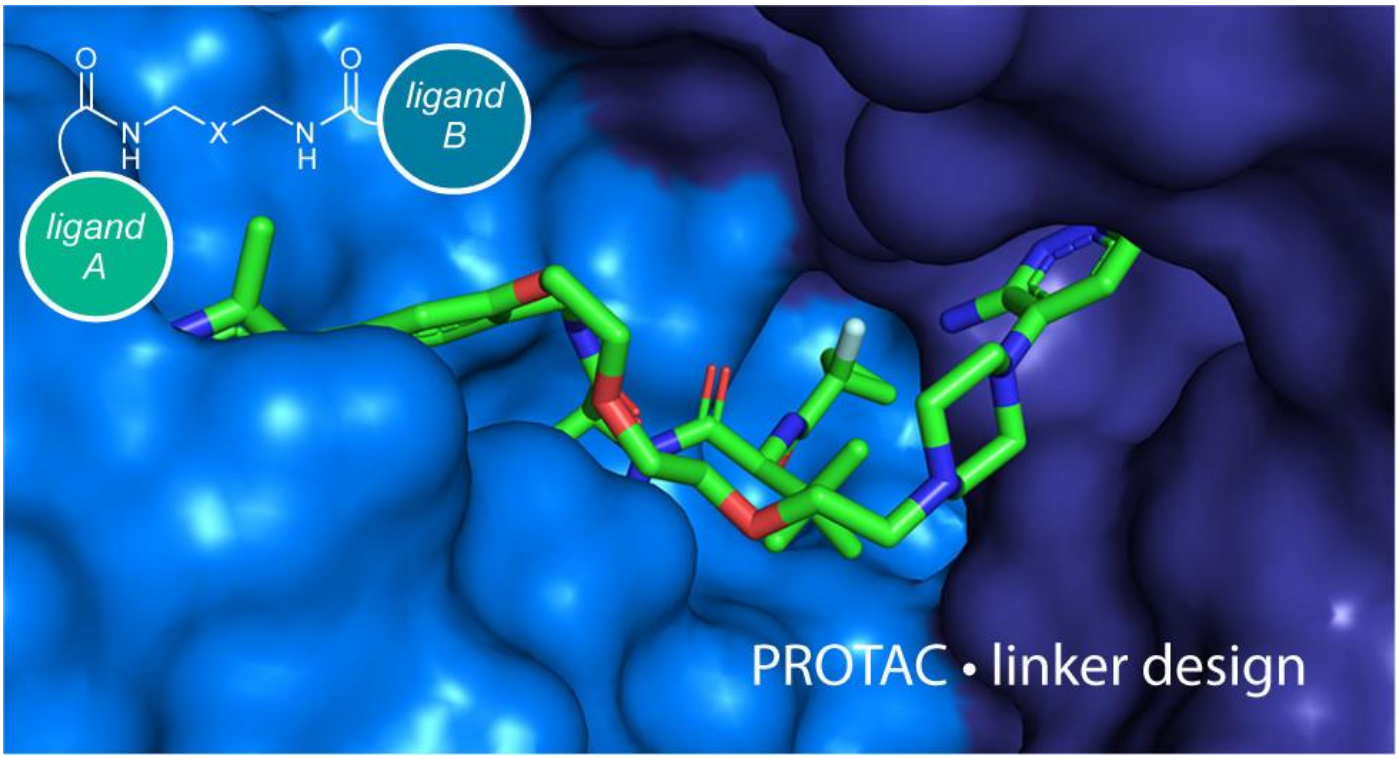
Photocaged and photoswitchable PROTACs represent a useful addition to the chemical toolbox for manipulating cellular function, with the ability to remove a target protein at a specific place and time. However, one potential drawback with the technology as it currently stands is the use of UV or violet light to activate degraders which limit clinical application to surface exposed diseases and is incompatible with applications that require deep tissue penetration. Important goals for the field are the development of PPG and photoswitches which can be activated by alternative means and the efficient and clean photochemical release of the active PROTAC is crucial.

Contributor: Suzanne

**Unravelling the role of linker design in Proteolysis Targeting Chimeras**

Troy A. Bemis[§], James J. La Clair[§], Michael D. Burkart*

*J. Med. Chem,* **2021**, *64,* 8042




One of the challenges with developing PROTACs is the empirical nature of linker structure activity relationships (SARs) which are often time and labour intensive. This paper details the synthetic approaches that have been developed to increase synthetic throughput and the advances in structural biology and computational chemistry that have led to rational PROTAC design.

The current approaches to streamline linker variant SAR studies are summarised including orthogonally protected bifunctional linkers, solid phase synthesis, copper catalysed click chemistry, activated esters and Staudinger ligation chemistry. X-ray crystal structures of the ternary complex, when available, enable more rational linker design and can identify crucial stabilising interactions of the linker. The paper also discusses, with examples, the computational methods that have been developed in efforts towards rationally designed *de novo* PROTAC development for example using Molecular Operating Environment (MOE) and the open source Rosetta software suites. In silico PROTAC development aims to minimise the number of synthesised and biologically evaluated molecules required for efficient target degradation. The ability of the protocols to rationalize biological trends in potency and selectivity is highly encouraging.

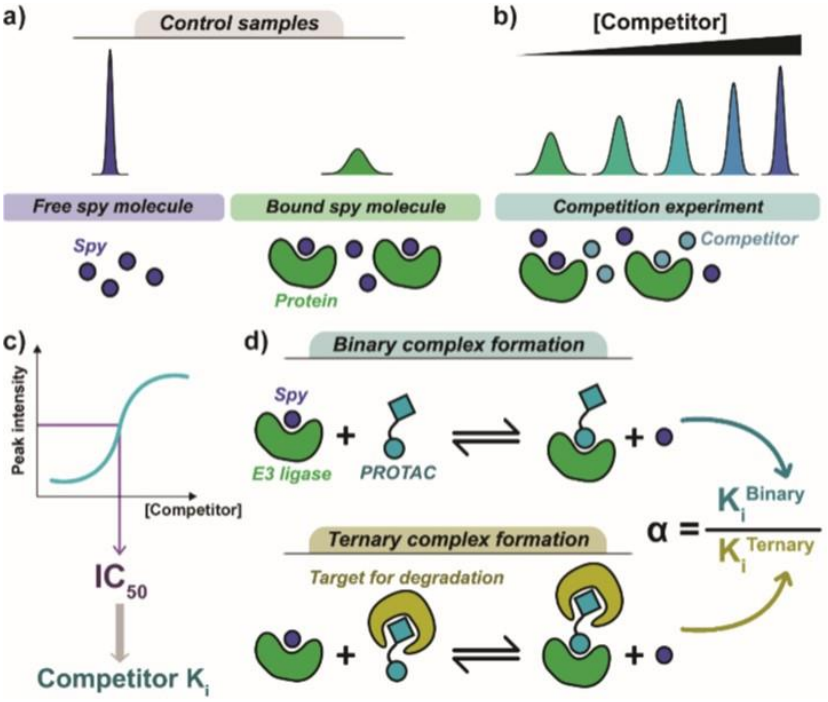
The empirical nature of PROTAC development is gradually being replaced by the development in synthetic methodologies, structural characterisation of ternary complexes and advances in computational protocols. This is certainly a positive step forward for the rational design of efficient targeted protein degraders.

Contributor: Suzanne

**Estimating the cooperativity of PROTAC- induced ternary complexes using $^{19}$F NMR displacement assay**

Guilherme Vieira de Castro[§], Alessio Ciulli*

*ChemRxiv* **2021**, DOI: 10.33774/chemrxiv-2021-zj19k




In this paper the authors describe the development of fluorine NMR competition binding experiments to determine cooperativity (α) of PROTACs. Cooperativity, which can be positive or negative, is an important parameter to understand the ternary complexes formed by protein degraders. The correlation between biophysical ternary complex formation and cellular activity motivates the development of methods for monitoring and measuring the cooperativity of ternary complexes to design effective degraders.

Previously reported methods to measure cooperativity include isothermal titration calorimetry, fluorescence polarisation, TR-FRET, surface plasmon resonance and native mass spectrometry. This is the first report of NMR based methods to monitor cooperativity of PROTAC ternary complexes. A highly sensitive competitive $^{19}$F NMR assay was developed to detect small molecules binding to the VHL E3 ligase. The $^{19}$F CPMG spectra of the fluorinated spy molecule free in solution and in the presence of protein are recorded as controls. The titration of increasing concentrations of a competitor causes the displacement of the spy molecule, which changes the shape and intensity of its $^{19}$F peak. The cooperativity of the ternary complex can be measured by comparing the titrations of a PROTAC with the E3 ligase in the absence and presence of the protein targeted for degradation.

$^{19}$F ligand observed NMR spectroscopy enabled the rapid monitoring of both positive and negative cooperativity in the examples studied. Negative cooperativities were accurately measured, however, large positive cooperativity could be underestimated due to tight binding under assay conditions. The described assay allowed at least semi-quantitative rapid estimates of PROTAC cooperativities and is a valuable additional method for the screening and characterisation of PROTACs.

Contributor: Jeff

**Covalent PROTACs: the best of both worlds?**

Neil P. Grimster[§]*

*RSC Med. Chem* **2021**, DOI: 10.1039/D1MD00191D

This opinion article describes the development of covalent PROTACs, a recently emerging area which combines PROTACs and targeted covalent inhibitors (TCI) for protein degradation. By introducing bond forming functional groups onto the POI binders, the potency, selectivity, and pharmacokinetics of the drug could be improved. The author outlined the potential advantages and challenges for covalent PROTAC designs when employing reversible and irreversible covalent warheads onto POI and E3 ligase binders, as well as notable examples such as EN219, in which combined with BCR-ACL inhibitors showed selectivity against c-ACL.

Covalent PROTACs are an exciting new area with a lot of potential, especially with a reversible covalent mode of action in place to allow enhanced selectivity of POI while maintaining catalytic mechanism. However, the additional complexities of covalent PROTACs pose extra challenges in terms of synthetic optimisation and suitable biological assays to understand their functions.

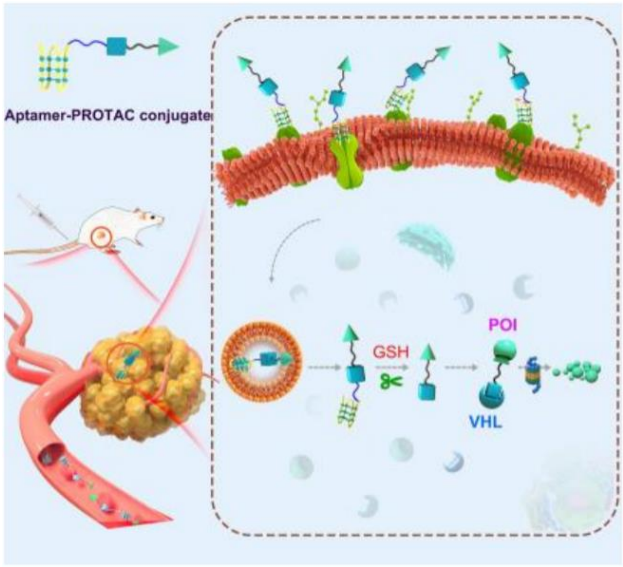
---

Contributor: Jeff

**Aptamer-PROTAC Conjugates (APCs) for Tumor-specific Targeting in Breast Cancer**

Shipeng He[§], Fei Gao[§], Junhui Ma[§], …, Guoqiang Dong*, Chunquan Sheng*

*Angew. Chem. Int. Ed.* **2021**, DOI: 10.1002/anie.202107347




Conventional PROTACs often suffer from poor cell membrane permeability, unfavourable pharmacokinetic profiles, and lack of target specificity due to their high molecular weight and hydrophobicity. This paper disclosed the development of aptamer conjugated BET PROTACs, which show increased selectivity against MCF-7 breast cancer cells. The use of single-stranded nucleic acid aptamers, which are connected by a disulfide-ester linker onto the hydroxy group of VHL ligand allowed selective release of active PROTACs in cancer cells. The stability, cellular uptake, efficacy, toxicity and *in vivo* distribution of APCs are also studied.

The selective tumour uptake of APCs, as well as their reduced *in vivo* toxicity are certainly attractive features to develop potent PROTACs for therapeutic use. It would be interesting to see whether this approach could be extended to CRBN based or other cancer targeting PROTACs as well.

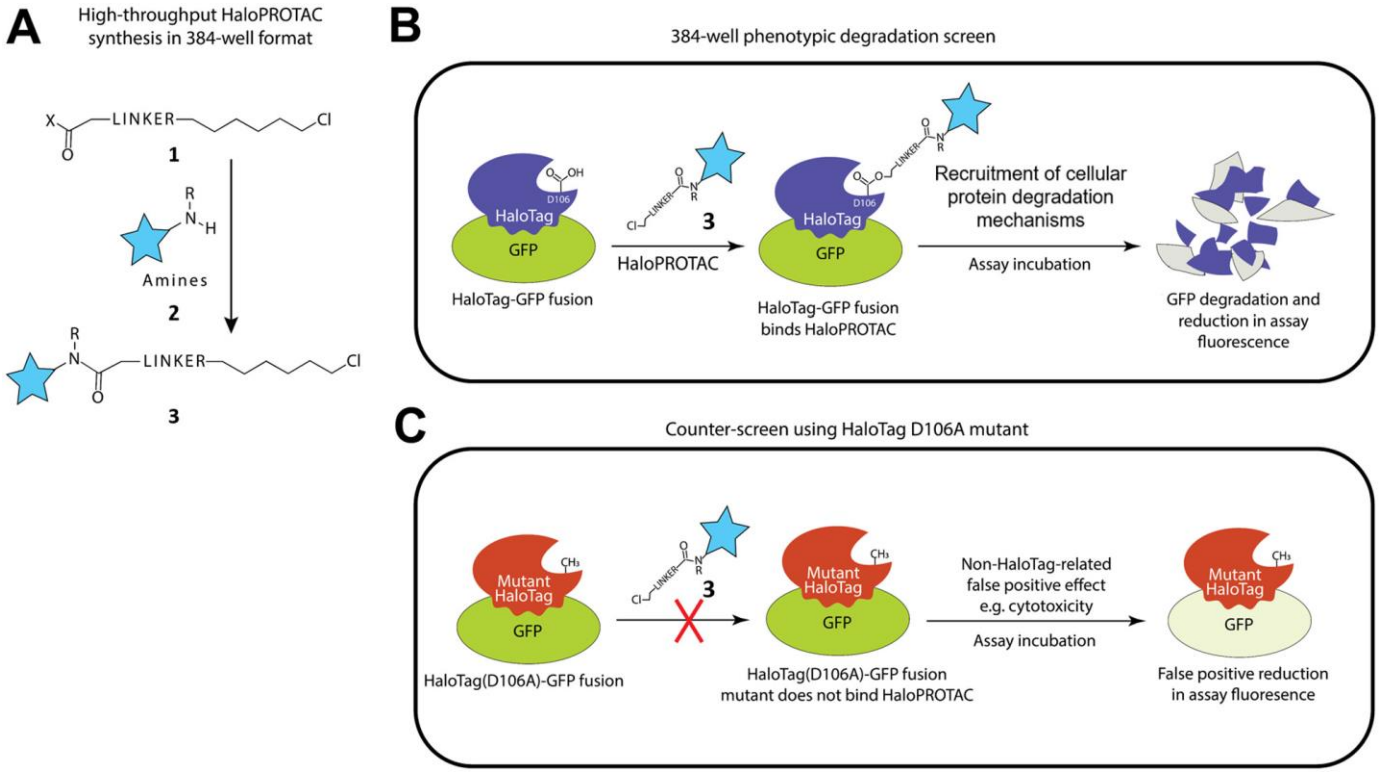
---

Contributor: Jeff

**A Phenotypic Approach for the Identification of New Molecules for Targeted Protein Degradation Applications**

Peter Stacey[§], Hannah Lithgow[§], …, Markus A. Queisser*

*SLAS Discov.* **2021**, *26*, 885




Although there have been numerous successful PROTACs in the field for the degradation of different POIs, the currently known PROTACs only recruit a small number of E3 ligases in the human genome. VHL and CRBN are the dominant choices of current PROTAC design. The authors attempted to identify novel E3 ligase binders by employing high throughput synthesis of a HaloPROTAC library. This was done by reacting 2934 amines with a chloroalkane linker, where 1956 amines showed good (>50%) conversion to the corresponding HaloPROTACs. These HaloPROTACs were then used for degradation assays against HaloTag (D106A)-GFP cells. Nine compounds were identified to be potential degraders and two were able to give reproducible results. Unfortunately, the two hits were not found to be true protein degraders after further investigation.

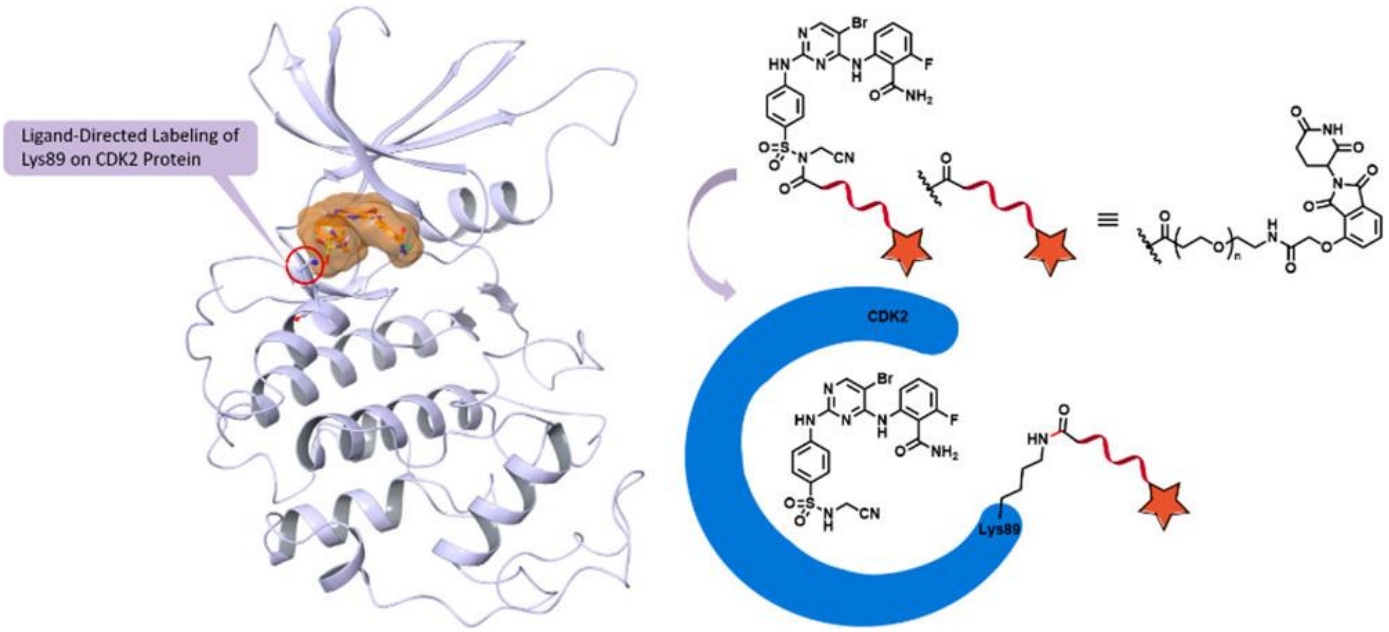
Even though the authors were unable to identify any novel E3 ligase binders, this proof-of-concept study has shown to have potential to discover new E3 ligase binders which are unknown. Since only amide bond formation and one type of linker were used in this study, the chance of success would certainly increase by expanding the library synthesis to different chemical motifs, different linkers, as well as different cell lines.

Contributor: Jeff

**Exploring Ligand-Directed *N*-Acyl-*N*-alkylsulfonamide-Based Acylation Chemistry for Potential Targeted Degrader Development**

Mingxing Teng[§], Jie Jiang[§], Scott B. Ficarro[§], …, Jarrod A. Marto*, Nathanael S. Gray*

*ACS Med. Chem. Lett.* **2021**, DOI: 10.1021/acsmedchemlett.1c00285




Strong ligand-protein binding interactions are often required for effective PROTAC degradation and other small molecule inhibitors, which makes the degradation of 'unligandable' POIs using PROTAC technology difficult. As a biorthogonal strategy, the authors of this paper developed a series of CRBN binders containing *N*-acyl-*N*-alkylsufonamide (NASA) based covalent probes, which enabled the transfer of the E3 ligase binder part onto the lysine residue of CDK2 for degradation. The formation of the CDK2:CRBN complex using TMX-3054 was successful and verified by MS, X-ray crystallography and HiBit assay. However, no degradation of CDK2 was observed.

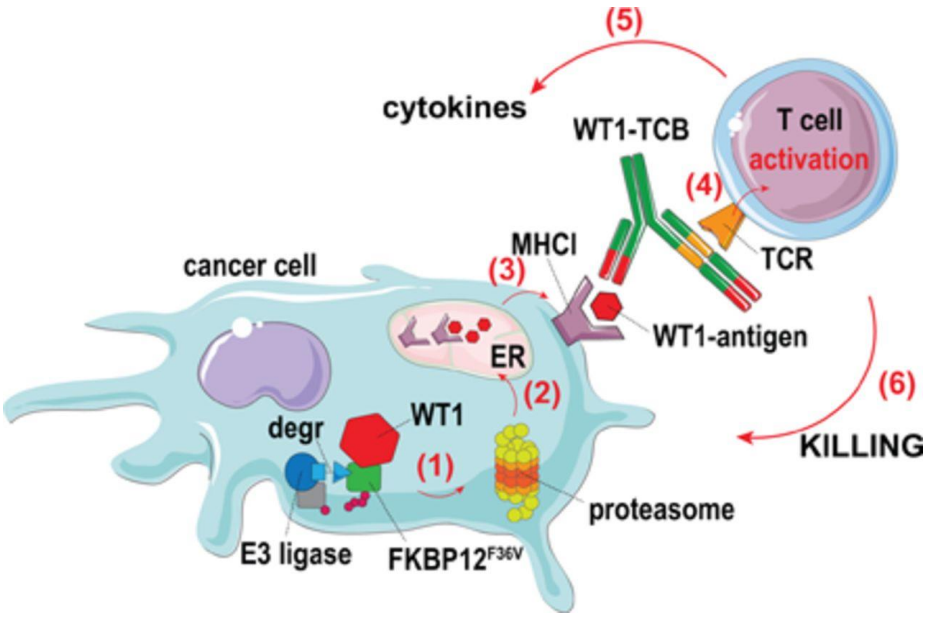
The use of a biorthogonal covalent tether to facilitate protein degradation would provide a complementary tool to help future PROTAC design towards difficult therapeutic targets. Although the authors were unable to demonstrate successful degradation of CDK2, further optimalisation of the linker to enable interaction of the binary complexes, as well as employing different E3 ligase binders such as VHL would be interesting for further development of this strategy.

Contributor: Selma

**Proteolysis-Targeting Chimeras Enhance T Cell Bispecific Antibody-Driven T cell Activation and Effector Function through Increased MHC Class I Antigen Presentation in Cancer Cells**

Vittoria Massafra[§], …, Yvonne A. Nagel*

*J. Immunol.* **2021**, *207*, 493




This paper follows on from some of the work by Moser et al (2018), who originally showed that the activity of a degrader increased Antigen (Ag) presentation on MHC Class I (MHC-I) in murine cell lines. Its aim was to demonstrate the immune response to cancer cells following enhanced protein degradation. The study starts by using dBET1 and MZ1 in the SKM-1 leukemic cell line, and identifying peptides bound to MHC-I that uniquely resemble BRD4 using liquid chromatography-tandem mass spectrometry. Peptide elutions from cellular MHC-I complexes were mixed with isotopically labelled peptides to quantify relative abundances. It was shown that both compounds presented significant increases in abundance of BRD4-bound peptides to MHC-I complexes. To show how this is relevant to the immune response, BRD4 was fused to a peptide derived from Wilms tumour 1 (WT1), a tumour-associated antigen which has previously been shown to engage T cells. Here they used MDA-MB-231 cells (breast cancer origin) because this engagement results in killing of the cells by T cells as a response, and therefore they monitored cancer cell viability. Using dBET6, they were able to show a decrease in viability. Furthermore, using a fusion of WT1 to a mutant protein of FKBP12 ($FKBP12^{F36V}$) and an $FKBP12^{F36V}$ degrader, they were able to show an increased percentage of $CD8^+$ and $CD4^+$ T cells expressing the activation marker CD69, and subsequently an increased presence of $CD25^+$ and dividing $CD8^+$ cells. This demonstrated evidence of enhanced early and late activation of T cells, respectively. Lastly, this was coupled with enhancement of T cytotoxic cytokine secretion, particularly GZMB, TNF-α and IFN-γ. Overall this study has shown that with increased proteolytic degradation, Ag presentation on MHC-I was enhanced and led to activation of $CD8^+$ T cell and effector function.

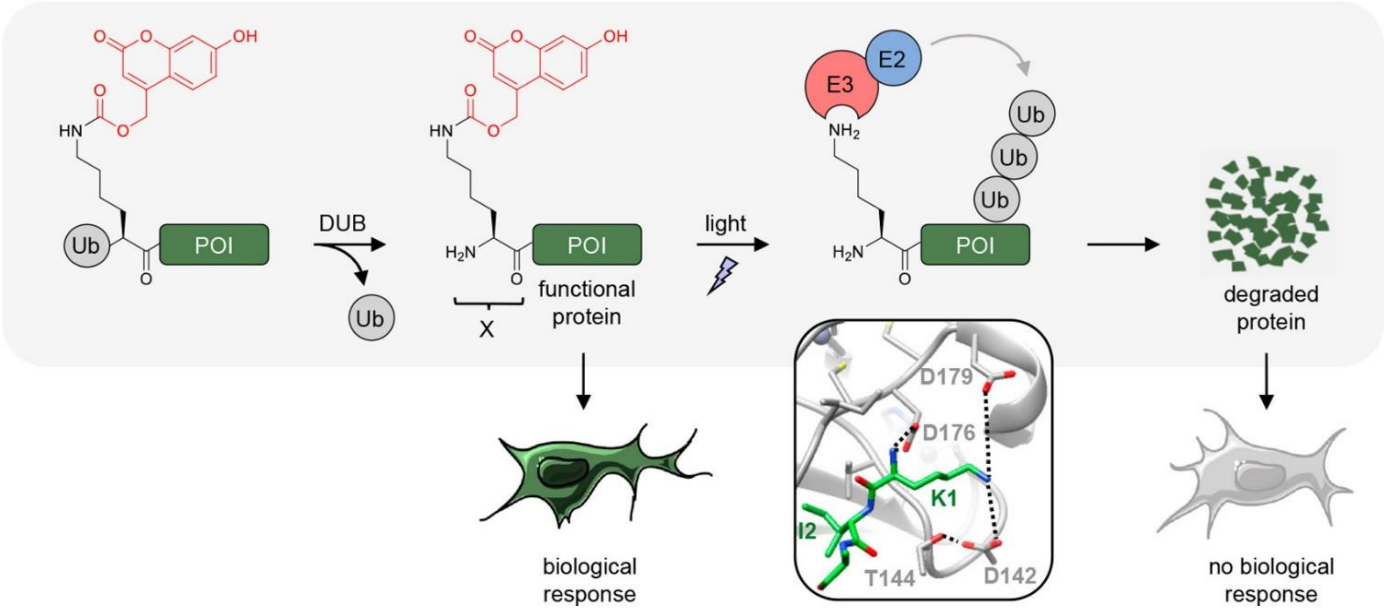
It is discussed that low levels of Ag presentation have presented a challenge in efficacy of Ag-targeting cancer immunotherapeutics. Proteolytic degradation is the main mechanism contributing to MHC-I display of peptides that leads to activation of the immune response. Therefore, this paper demonstrates another benefit to degraders apart from removal of a protein and its role, it demonstrates how degradation can also lead to increased effector functions downstream.

Contributor: Selma

**Targeted Protein Degradation through Fast Optogenetic Activation and Its Application to the Control of Cell Signalling**

Amy Ryan[§], Jihe Liu, Alexander Deiters*

*J. Am. Chem. Soc.* **2021**, *143*, 9222




This study designed a light-controlled peptide degron to control proteolytic activity. It comprised of a 14-amino acid peptide, made up of a photocaged N-terminal amino acid and a lysine-rich 13-residue linker, which allowed optical control of protein degradation through the N-end proteolytic pathway. By designing a primary destabilising residue (or an N-degron) that is exposed following light activation, this allowed recruitment of proteasomal machinery. Using this, the investigators had temporal control of rapid protein degradation. Specifically, the crystal structure of an N-terminal lysine tetrapeptide bound to Ubiquitin Protein Ligase E3 Component N-Recognin 1(UBR1) showed electrostatic interactions between positively charged amino groups and the negatively charged binding pocket of the E3 ligase, and thus the investigators introduced a photocaged hydroxycoumarin lysine at the N-terminal position of different targets, that would block the recognition by E3 ligases such as UBR1. This system was primarily validated by using EGFP fusions to analyse the kinetics using fluorescence microscopy, and then moved on to show temporally controlled degradation of proteins in the ERK/MAPK cell signalling pathway.

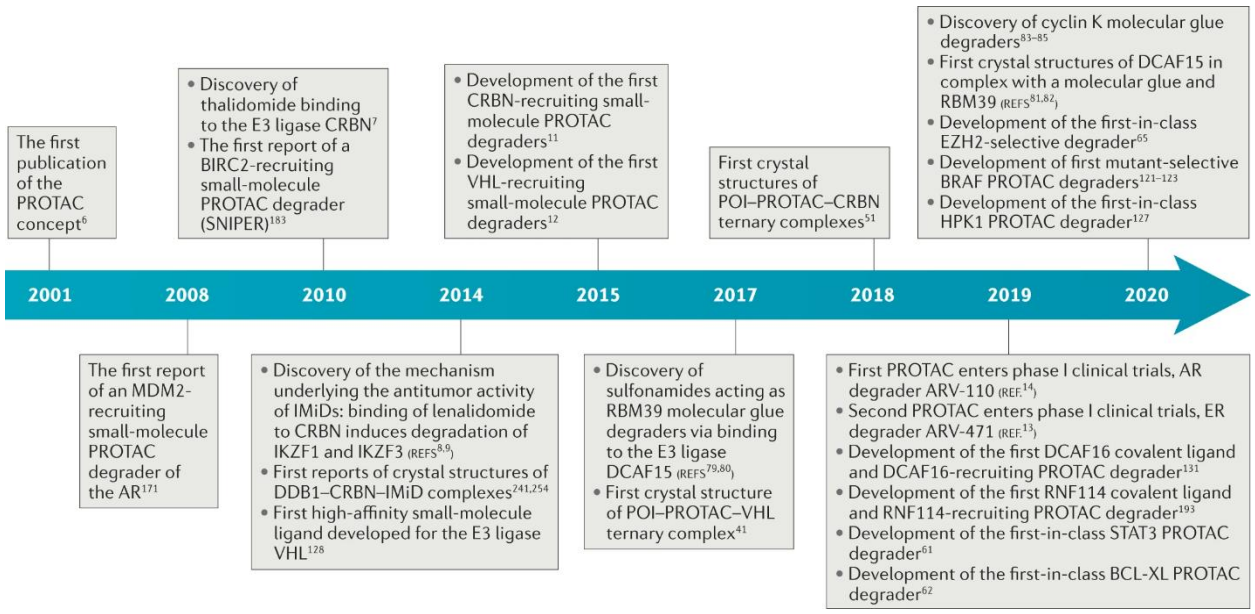
This study used fluorescence microscopy for their quantitation and focussed very heavily on EGFP degradation. This presents a few limitations in relying on a live imaging time course with likely photobleaching events, without replicating the time series using Western Blot or other protein quantifying methods. Whilst the results look promising to investigate the kinetics of degradation, there would need to be a lot more controls to make the “3-minute rapid degradation” reliable, for example.

Contributor: Selma

**Advancing targeted protein degradation for cancer therapy**

Brandon Dale[§], Meng Cheng[§], …, Yue Xiong*, Jian Jin*

*Nat. Rev. Cancer* **2021**, DOI: 10.1038/s41568-021-00365-x




This review gives a rounded overview of targeted protein degradation - what it is and how the field of TPD was developed before homing in on some key examples within oncology. It presents a timeline of the key discoveries and developments in the field, from 2001 where the first PROTAC publication was made, to now where there are several degraders in development, as well as those that have now entered Phase I of clinical trials. It leads into describing the Ubiquitin-proteasome system, and how it can be hijacked with heterobifunctional small molecule degraders. It describes mainly the Molecular Glues and PROTACs on the ten E3 ligase ligands that have mainly been used to date. The focus then becomes primarily on PROTACs in cancer therapy, describing how the degraders for AR, ER, STAT3 BCL-XL, EZH2, BRAF and HPK1 were designed, developed and why they are important and relevant. Henceforth, the review explores some design/development considerations, particularly in novel E3 ligand design, pharmacokinetics and oral bioavailability before brushing onto some of the key limitations of TPD technology. These limitations focussed on cell toxicity and acquired resistance by impairing specific E3 ligase function following prolonged exposure.

This was a well-rounded review into TPD and good material for anyone starting off in this field to see what has already been done. It would be good to see a similar article for non-cancerous applications.

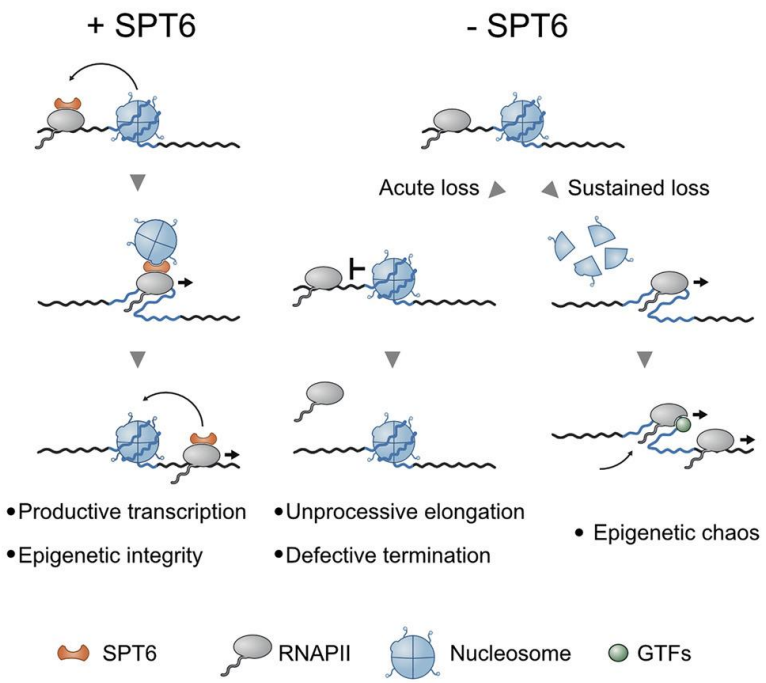
Contributor: Selma

**Targeted protein degradation reveals a direct role of SPT6 in RNAPII elongation and termination**

Ashwin Narain[§], Pranjali Bhandare[§], …, Florain Erhard*, Apoorva Baluapuri*, Elmar Wolf*

*Mol. Cell* **2021**, *81*, 1

Histone chaperones are crucial in maintaining epigenetic information during transcription to ensure correct re-assembly of nucleosomes with DNA during transcription. SPT6 is a histone chaperone that tightly binds with RNA polymerase II (RNAPII) during transcription elongation. Its depletion results in intragenic transcription initiation, aberrant levels of antisense transcription, pre-mature termination and changes in elongation rates. Yet, its primary role in transcription is still uncertain. This study used TPD to deplete SPT6 acutely using the auxin-inducible degron (AID) system within human U2OS cells, and analysed its effects on RNAPII and transcription. Here they used 4-thiouridine (4sU) labelling of transcripts to show that the distribution of reads over the gene body was highly altered with SPT6 depletion, with a correlation to gene length, demonstrating its key role in elongation. Furthermore, a failure to complete transcription was confirmed using qPCR, further suggesting a key role in termination. Lastly, they combined their genomic approaches (4sU-seq, DRB-4sU-seq RNAPII ChIP-Rx and SLAM-seq) with mathematical modelling to demonstrate that SPT6 leads to lowered processivity of RNAPII and slower elongation rates, a trait that was amplified with increased gene length.




Whilst this study emphasizes the importance of this histone chaperone in transcription, it doesn't achieve its primary aim of providing insight into its direct role. It uses an alternative form of TPD (auxin-inducible degron) to emphasize its importance in elongation and demonstrate a role in termination. However, only the discussion suggests that perhaps its direct role with RNAPII is to aid its passage through chromatinized DNA, via its ability to bind to histones. It would be a good next step in this investigation to look at the direct interaction between SPT6 and RNAPII, and try to hinder it without affecting their functionality (such as by using mutagenesis for their interaction site), and seeing if the same altered transcription effects are there.

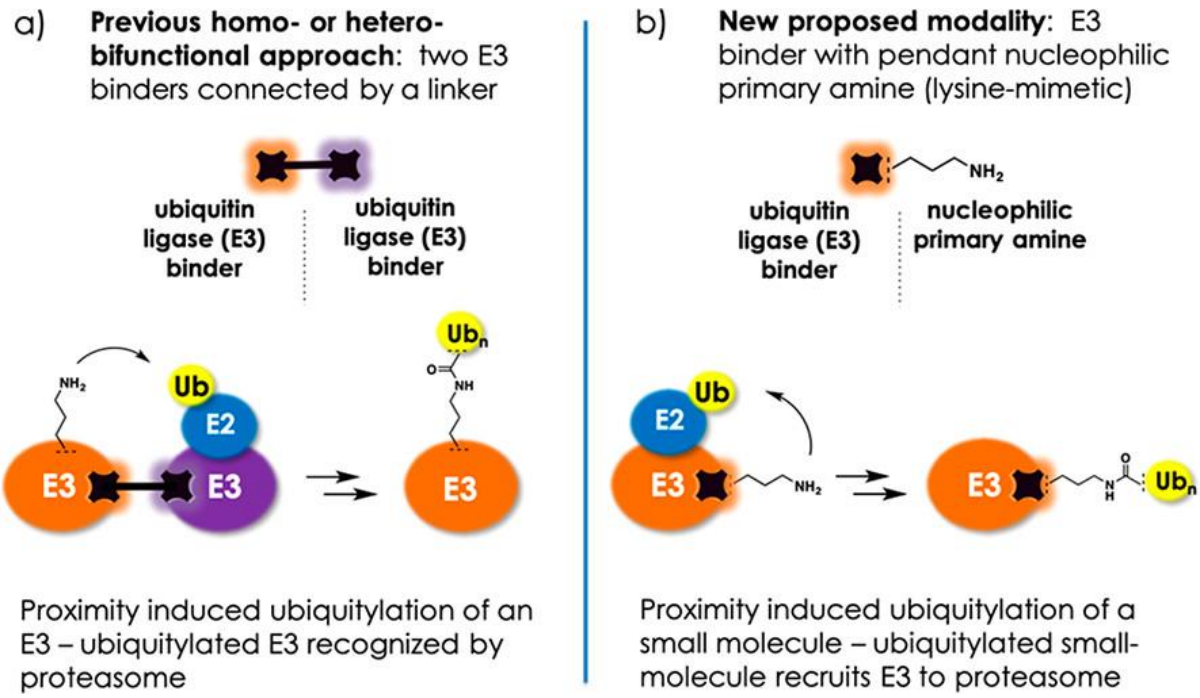
# Other Paper Highlights

Contributor: Alessio

**Primary Amine Tethered Small Molecules Promote the Degradation of X-Linked Inhibitor of Apoptosis Protein**

Willem den Besten[§], Kshitij Verma[§],…, Steven T. Staben*

*J. Am. Chem. Soc.* **2021**, *143*, 10571




The idea behind the paper is simple: the authors hypothesised that appending a terminal amine on a small molecule binding to an E3 ligase would project a new ubiquitination site, which might then lead to the E3 ligase ubiquitinating its small molecule binder and as a result itself be degraded. To test this hypothesis, they choose XIAP as model system, and carefully and duly control for the unwanted self-degradation mechanisms observed with cIAP binders. A series of amine-bearing analogues of a high-affinity and specific XIAP binder is designed to identify the best degrader (compound 10). They then go on to perform a series of well-designed and executed experiments that convincingly evidence ubiquitination, and XIAP degradation, consistent with the expected mechanism via dependency of compound 10 binding, and ligase and proteasome activity. Evidence that the small molecule itself gets poly-ubiquitinated is offered using mass spectrometry, BLI with anti-Ub antibody, and a clever TAMRA-ubiquitination assay. All in all, the paper provides convincing evidence to support the proposed idea and main claim. Future work expanding to other E3 ligases, such as members of the Cullin RING ligase family, will be necessary to establish broad applicability beyond XIAP.

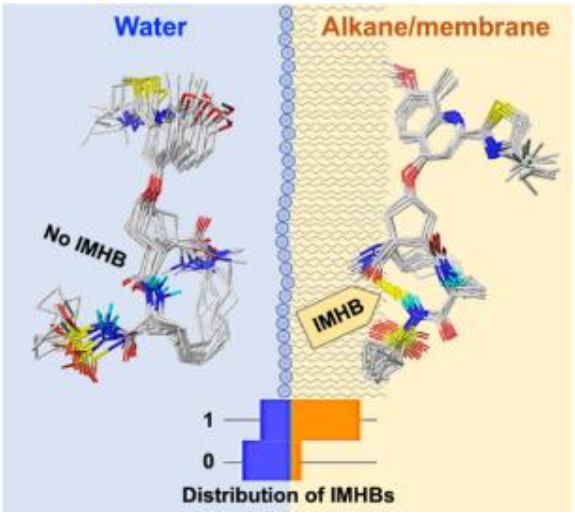
This is an excellent article and a mechanistically sound piece of science by Staben and colleagues at Genentech, and a new twist in the theme of E3 self-ubiquitination and degradation previously established with homo-PROTACs and related ligase-ligase small-molecule dimerizers (see Graphical Abstract). A minor limitation of the paper is that no evidence is provided that definitively links the degradation of the XIAP protein to its small-molecule ubiquitination, however this would be no small feat. It will be interesting to watch how this approach develops and expands in future.

Contributor: Will

**Prediction of chameleonic efficiency**

Laurent David[§], Mark Wenlock, Patrick Barton and Andreas Ritzen*

*ChemRxiv* **2021**, DOI: 10.26434/chemrxiv.14748879.v1




The ability for a molecule to hide polar functionality in non-polar environments and expose it in water has been described as 'chameleonic' behaviour. Whilst there have been studies reporting this phenomenon and its impact on drug solubility and permeability, there is a lack of methods that medicinal and computational chemists can use to prospectively design the degree to which a molecule is chameleonic. In this work the authors first validate models to predict chromatographic apparent polarity and Δlog *P*oct–tol, a measure of hydrogen bond donor potential. Key to accuracy of these models is the scaling of the surface areas of hydrogen bond donors and acceptors with their respective hydrogen bond strengths. The study then introduces two new indices to predict 'chameleonicity'. The chameleonic intramolecular hydrogen bonding efficiency (CHE) aims to predict the difference in mean numbers of intramolecular hydrogen bonds (IMHB) for a molecular dynamics generated conformational ensemble in non-polar (octanol) and water, where a value of zero signifies non-chameleonic behaviour. Secondly, the chameleonic hydrogen bond donor efficiency (CDE) aims to predict mean hydrogen bond donor potential between non-polar (octanol) and water environments, with zero representing no differences between the two environments and 1 representing no hydrogen bond exposure in octanol. The indices are applied to a diverse set of 25 molecules, with a macrocyclic HCV inhibitor demonstrating the highest CHE and a VHL based PROTAC demonstrating the highest CDE. This highlights the point that the two indices are fundamentally assessing different aspects of chameleonicity, CHE indicating if IMHBs are formed in membranes and CDE indicating the effect IMHB formation on hydrogen bond potential.

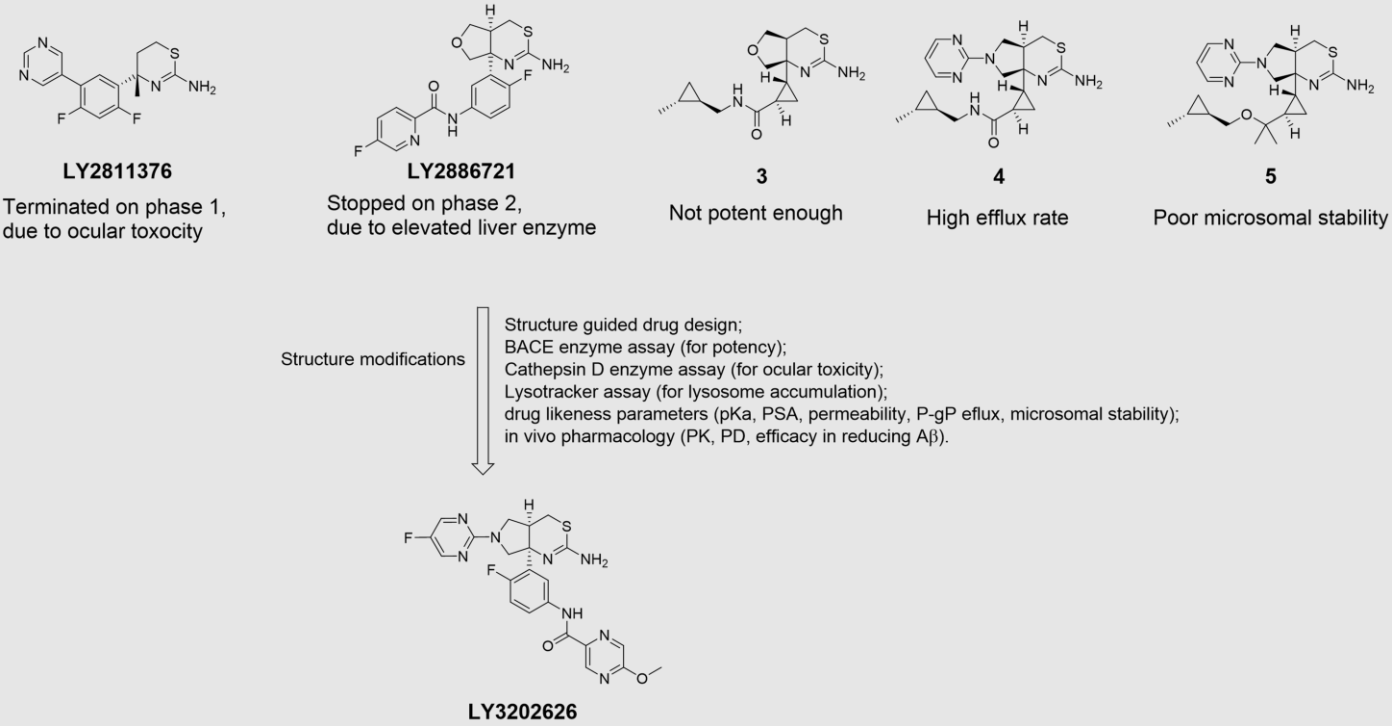
Balancing permeability and solubility is a fundamental medicinal chemistry challenge, one heightened when working with 'beyond-rule-of-5' molecules. Whilst it is now widely appreciated that classical 2D descriptors such as TPSA may be limited in aiding molecule design in this space, there is a paucity of validated tools that go beyond these that can be simply applied in a medicinal chemistry program. The tools presented here may provide medicinal chemists with the means to prospectively design ways to purposefully harness chameleonicity to tune permeability and solubility. It will be important to review to what degree indices such as those laid out in this study are taken up by the growing numbers of medicinal chemists working on larger, potentially 'chameleonic' molecules as well as future case studies outlining how they impacted optimisation. The methods outlined will be accessible to a wide audience which are strongly in their favour.

Contributor: Xingui

**Discovery and Early Clinical Development of LY3202626, a Low-Dose, CNS-Penetrant BACE Inhibitor**

David L. McKinzie[§], …, Dustin J. Mergott*

*J. Med. Chem.* **2021**, *64*, 8076




Beta-site APP cleaving enzyme 1 (BACE1) is involved in the production of amyloid-beta (Aβ), and deposits of Aβ plaques in the brain are a pathological hallmark of Alzheimer's disease (AD). Targeting BACE1 with small molecular inhibitors has therefore long been pursued as a potential AD therapy. Scientists from Eli Lilly have invested dramatic efforts to discover a highly potent, CNS-penetrant, and safe BACE1 inhibitor. The medicinal chemistry here, guided by crystal structures and a variety of biological assays, has successfully improved the potency, safety and drug likeness of their previous generations of amino thiazine BACE1 inhibitors, and ultimately led to the discovery of LY3202626. However, despite the good potency, low toxicity and optimal PK/PD profiles of LY3202626, it failed to benefit AD patients in phase 2 clinical trials. The failure of LY3202626, together with many other clinical trials of BACE1 inhibitors (e.g. atabecestat, verubecestat), suggests the use of BACE1 inhibitors as AD therapy should be reconsidered.

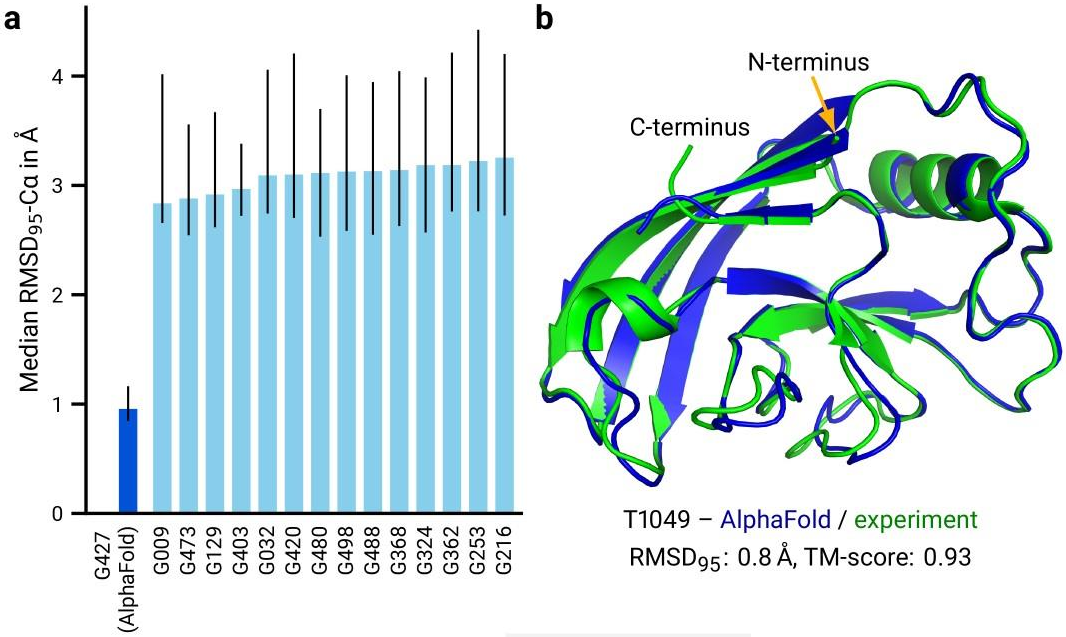
While BACE1 may not be a viable target for AD, this paper presented a beautiful medicinal chemistry study, very complex synthesis, and an elegant drug discovery story. It also demonstrates the challenges of modifying multiple parameters at the same time during lead optimisation.

Contributor: Tasuku

**Highly accurate protein structure prediction with AlphaFold**

John Jumper*, …, Demis Hassabis*

*Nature* **2021**, DOI: 10.1038/s41586-021-03819-2




The structural information of proteins is important for understanding their biological mechanism of action, but experimental approaches to obtaining 3D structures of proteins are time-consuming and labour-intensive. To overcome this so-called 'protein folding problem', computational prediction approaches are required to address this issue. The authors at DeepMind, the world-leading company for artificial intelligence, and Seoul National University, developed a landmark protein structure prediction model, AlphaFold2, and described this architecture and the theory of how to create the accurate structural model in detail not only in the main text but also the 62 pages of supporting information. Their model uses both approaches, historical structural knowledge and information about proteins, e.g. multiple sequence alignments (MSA) and PDB, and cutting-edge deep learning methodologies. As a result, AlphaFold2 won an overwhelming victory at the 14th Critical Assessment of Protein Structure Prediction (CASP14). In addition, they demonstrated that the accuracy of AlphaFold2 has reached nearly 90 GDT (Global Distance Score) for the first time which means the accuracy is almost the same as the experimental results. AlphaFold2 is now available as CC BY-NC 4.0 license and it's free to download from GitHub (https://github.com/deepmind/alphafold/) and install to your machine, or you can simply input protein sequences to the AlphaFold2 GoogleColab page: (https://colab.research.google.com/drive/1LVPSOf4L502F21RWBmYJJYYLDlOU2NTL).

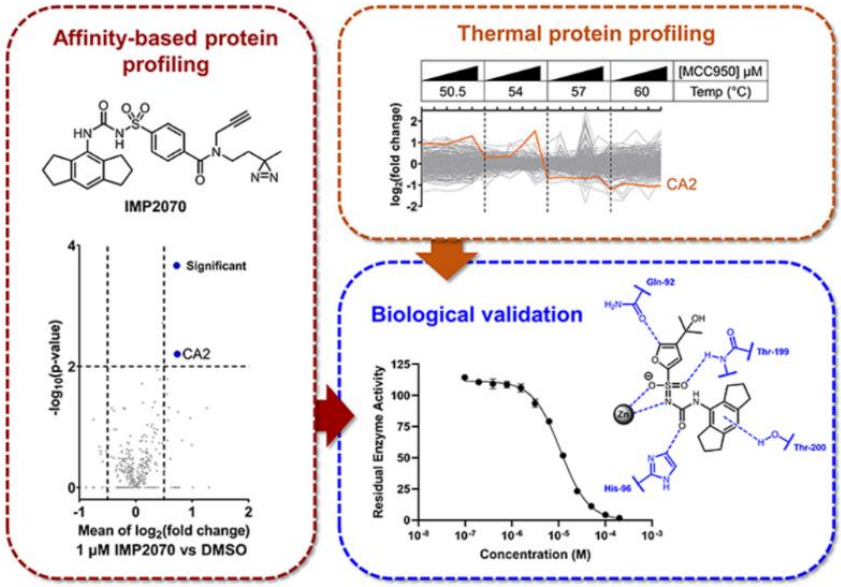
The impact of AlphaFold2 is spreading throughout the community of structural biology. For instance, a lot of structural biologists said they had solved X-ray diffraction data which was not possible to solve because of phase determination issues by using predicted structures. AlphaFold2 can also be used for predicting multiple complexes by simply connecting each sequence with a linker (not an official use). In addition, DeepMind released a database of more than 350,000 predicted structures of proteins and they're planning to expand it to cover 100,000,000 proteins. You can access this exciting database from Uniprot and EMBL-EBI web pages (https://alphafold.ebi.ac.uk/). From now, one of the objectives of structural biologists might be changed from solving the structures of proteins to validating the predicted structures experimentally.

Contributor: Sarah

**A Probe for NLRP3 Inflammasome Inhibitor MCC950 Identifies Carbonic Anhydrase 2 as a Novel Target**

Cassandra R. Kennedy[§], …, Avinash R. Shenoy*, Edward W. Tate*

*ACS Chem. Biol*. **2021**, *16*, 982




Photoaffinity labelling (PAL) is an increasingly important approach to map ligand/ biomacromolecule interactions. Here, the authors use PAL to uncover potential off-target mechanisms of MCC950, an NLR-family inflammasome pyrin domain-containing 3 (NLRP3) inhibitor and clinical candidate for the treatment of inflammatory disorders. A photoaffinity probe, IMP2070, was designed and synthesised to mimic MCC950. IMP2070 retains the essential features required to directly interact with NLRP3 with additional diazirine and alkyne functional groups for photo-crosslinking and bioorthogonal ligation, respectively. Affinity based chemical proteomics using IMP2070 in live macrophages identified carbonic anhydrase 2 (CA2) as a specific target. The authors utilised cellular thermal proteomic profiling as a complementary approach and demonstrated the stabilisation of CA2 by MCC950. Further CA2 esterase activity assays revealed MCC950 as a non-competitive inhibitor of CA2.

Despite identification using western blotting, no NLRP3 peptides were detected in any of the proteomic data sets. Regardless of this limitation, the paper nicely demonstrates the use of orthogonal target engagement approaches and highlights the value of PAL for target identification.

University of Dundee

**Ciulli Laboratory**
School of Life Sciences
Dow Street, Dundee,
DD1 5EH
United Kingdom

**lifesci.dundee.ac.uk/groups/alessio-ciulli/publications/journal-club**

@alessiociulli @CharlCrowe



23198