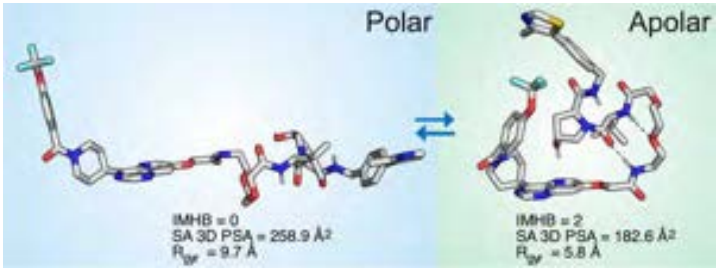
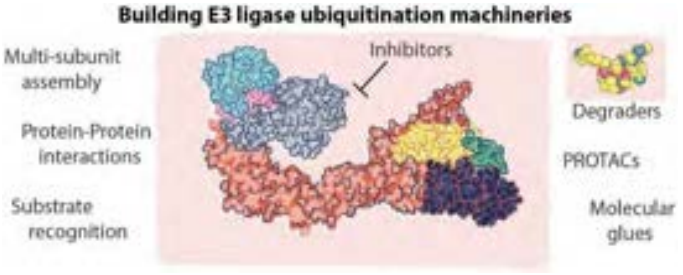
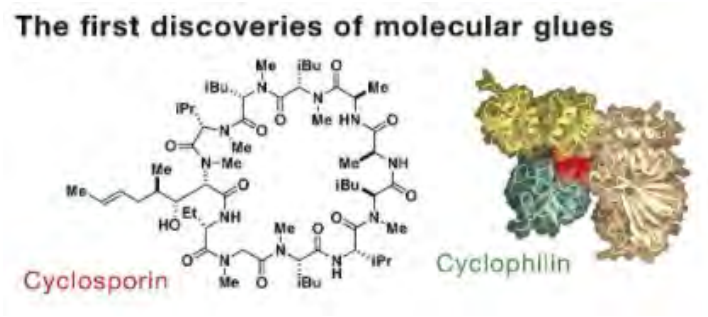
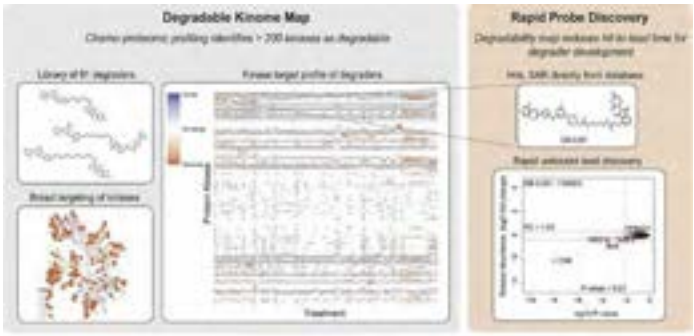
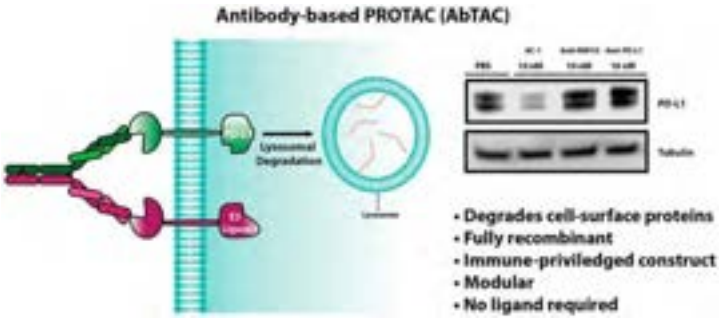
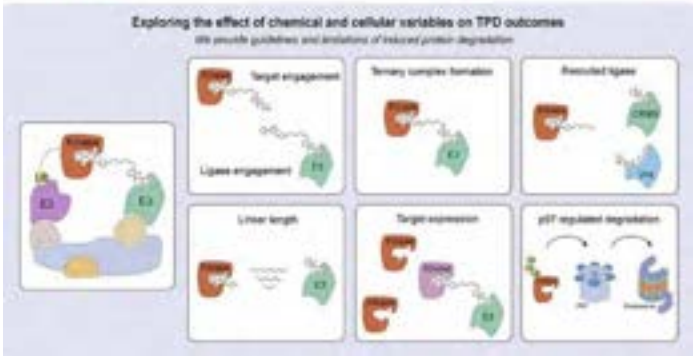


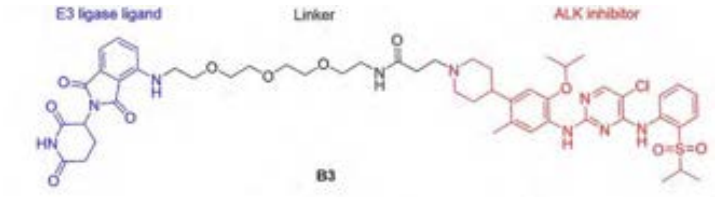



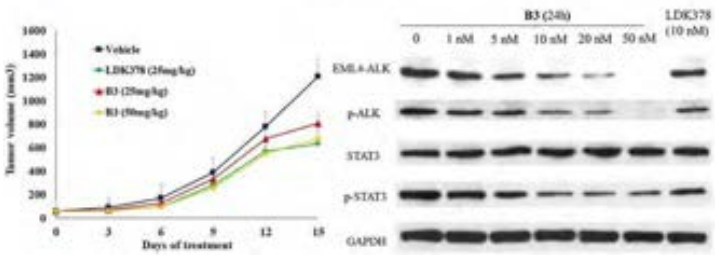




# Ciulli Group Journal Club

*Targeted Protein Degradation,*

*Medicinal Chemistry and*

*Chemical Structural Biology*

*Literature Highlights*

## January 2021 Edition

## Ciulli Group Journal Contents

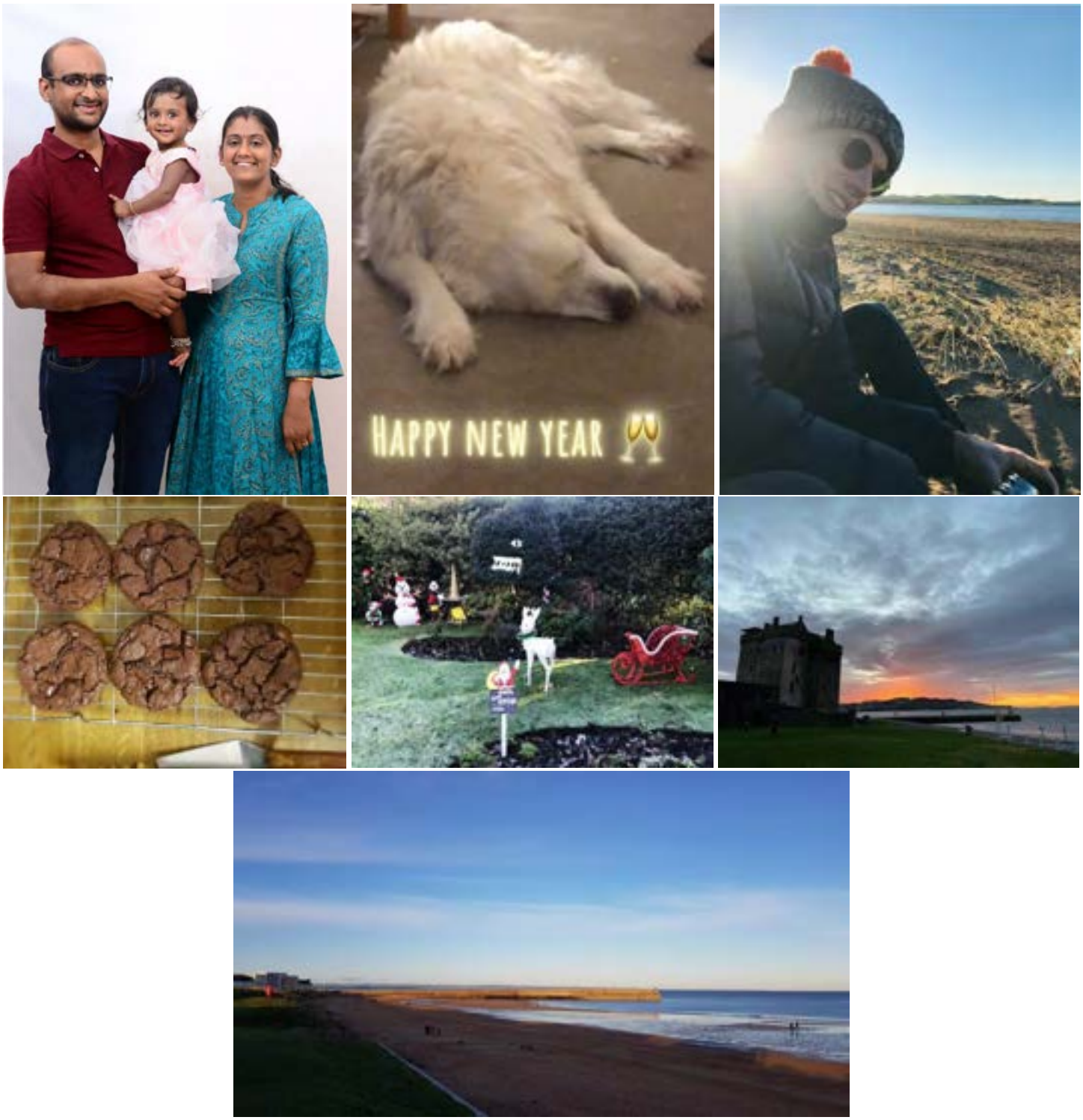
# Happy New Year from the Ciulli Lab!










The Journal Club has now been running since April 2020! We would like to take this opportunity to thank Siying Zhong, who started up the Journal Club and coordinated the activity until the end of 2020. Thank you to Siying! We welcome Charlotte Crowe who takes the baton from Siying and takes on the role of coordinating activities. We look forward to working with Charlotte and to a new exciting year for our JC!

To keep improving the Journal Club, we would like to hear your thoughts! Please follow the link below to fill out a brief survey. All responses are anonymous.
https://forms.office.com/Pages/ResponsePage.aspx?id=OTEyrjoJKk2Bpl0zS82QGe58NyjrjHVJszyhwkrfbqZUNlNMTkpJWUVNRkY5RlhZS05LWktBQlhPWi4u

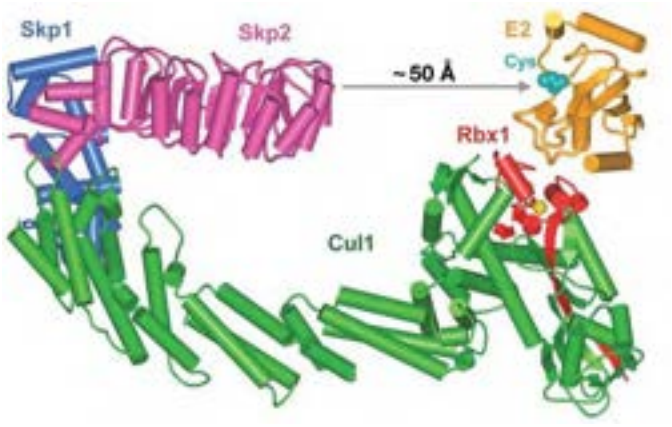
# Landmark Paper

Contributor: Alessio

**Structure of the Cul1–Rbx1–Skp1–Fbox$^{Skp2}$ SCF ubiquitin ligase complex**

Ning Zheng[§], Brenda A. Schulman[§], …, Nikola P. Pavletich*

*Nature* **2002**, *416 (6882)*, 703-709




We open 2021 with a Landmark feature that exemplifies the beauty and power of structural biology! Back in the early 2000, with the ubiquitin-proteasome system figured out in most of its components, it was clear that protein ubiquitination was mediated by enzymes (E3 ubiquitin ligases) that conferred substrate specificity for ubiquitination. A prominent class of multicomponent ligases contain a subunit called Really Interesting New Gene (RING). The atomic detail of the overall architecture and mechanism of RING-type ligases had remained poorly understood. In this paper the lab of Nikola Pavletich, a leading structural biologist at the Memorial Sloan Kettering Cancer Center renowned for tackling many high-profile disease-related proteins using X-ray crystallography, solved a first structure of the full complex for an archetypical member of a subfamily of ligases called Cullin-RING ligases (CRLs). The complex contains a central scaffold subunit (Cullin1) bridging a substrate binding unit composed of adaptor protein Skp1 and substrate recognition subunit Skp2 at one end, and the RING protein (Rbx1) intimately bound to Cul1 at the other end. Hence the nomenclature SCF (for Skp1–Cullin1-F-box protein) complex. Using elegant structural comparisons and evolutionary analyses, unprecedented features of the complex were highlighted to provide functional insights. Key feats of overall architecture and subunit-subunit interactions led the authors to infer roles of cullin neddylation in CRL regulation, and structural determinants of catalysis and specificity. The structure and devised model, a picture worth itself more than a 1,000 words, so strikingly highlighted how the whole CRL complex assembles to bridge and bring into correct proximity the substrate protein and the ubiquitin protein (activated as a thioester conjugate with an E2 conjugating enzyme), providing spatial and structural restraints to facilitate catalysis.

This paper was highly significant at the time for several reasons: not only because of its impact to structural biology, which in the pre-cryoEM era was largely reliant on the success of crystallographers tackling difficult-to-work-with proteins to illuminate new biology; but also to the ubiquitin field because of the important roles CRL and SCF complexes play to regulate fundamental biological processes e.g. the cell cycle, and their emerging roles in disease such as cancer. It was early days for PROTACs, yet interestingly the landmark 2001 PROTAC paper by Sakamoto *et al.* targeted indeed an SCF complex – in that case SCF$^{\beta\text{-TrCP}}$. The emergence of a first SCF structure thus provided fresh insights to the possibility that, with the right chemistry, one might productively hijack these molecular machineries to artificially induce target ubiquitination and degradation. Fast forward 20 years, we now know well what chemistry allows us to hijack many such CRL complexes, such as CRL2$^{VHL}$ and CRL4$^{CRBN}$ with PROTACs, or SCF$^{\beta\text{-TrCP}}$, CRL4$^{DCAF15}$ and CRL4$^{CRBN}$ with smaller degraders. The authors' list of this landmark paper includes, in addition to Pavletich, so many pioneers who have contributed major discoveries to the ubiquitin system, including Elledge, Pagano, the Conaways, Harper, and the two co-first authors Ning Zheng and Brenda Schulman, who later went on to establish their own successful prolific laboratories, and have made so many other significant contributions. Too many landmark papers out there from all these researchers! I hope this particular one will inspire you to look out for and learn more about them.

# Targeted Protein Degradation

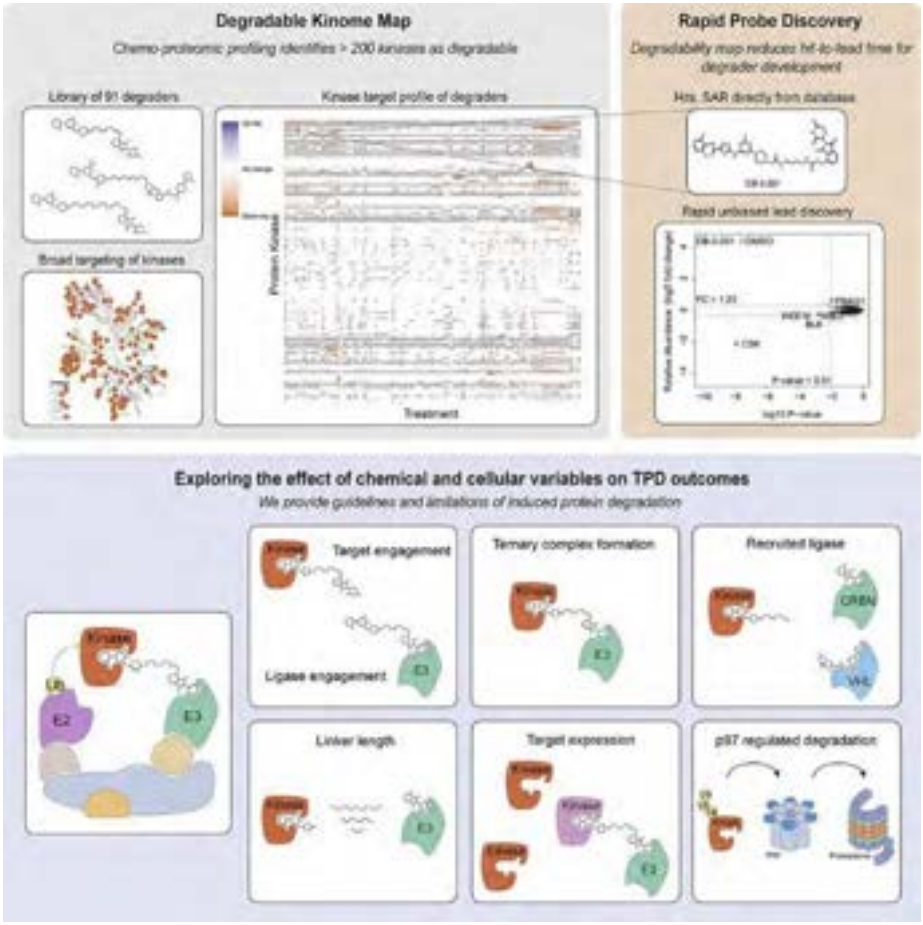
Contributor: Angus

**Mapping the Degradable Kinome Provides a Resource for Expedited Degrader Development**

Katherine A. Donovan[§], Fleur M. Ferguson[§], …, Taebo Sim*, Nathanael S. Gray*, Eric S. Fischer*

*Cell* **2020**, *183*, 1714

In this ambitious collaborative effort, the authors use chemo-proteomics to build an experimental map of the degradable kinome. In Part 1 of the manuscript, they design a library of 91 degraders using both specific and multitargeted kinase inhibitors to theoretically cover recruitment of roughly 70% of the human kinome. Both CRBN- and VHL-recruiting ligands are employed, and linker lengths, compositions, and attachment chemistries are varied to increase the chances of a kinase forming a productive ternary complex which will result in ubiquitination and degradation. Global proteomics of 7 cell lines treated with the degraders revealed degradation of 212 protein kinase/kinase-related targets, the majority of which had not been identified as degradable in the literature. Importantly, kinases refractory to degradation are also identified and characterised. On top of this, the authors calculate a degradability score for the identified targets and provide examples of how this degradable kinome database can be utilised to accelerate lead discovery.




In Part 2, they delve into the effects of chemical and cellular variables on TPD outcomes, using the power of the large dataset to examine the role of cellular target engagement, stable ternary complex formation, target abundance on degradative efficacy. Surprisingly and despite evidence to the contrary in smaller studies, they find these variables do not predict degrader efficacy. An interesting finding that does agrees with the literature is that choice of the recruited ligase (CRBN or VHL) influences target degradation, both in terms of which targets will be degraded and the degradative efficacy of the degrader molecules. Tolerance for changes in linker length, chemistries and attachment points is also investigated and found to be variable depending on the target. Finally, the authors use degrader molecules in combination with an inhibitor of the $AAA^+$ ATPase p97 to show proteasomal degradation of most kinases in the study is dependent on p97's unfoldase activity.

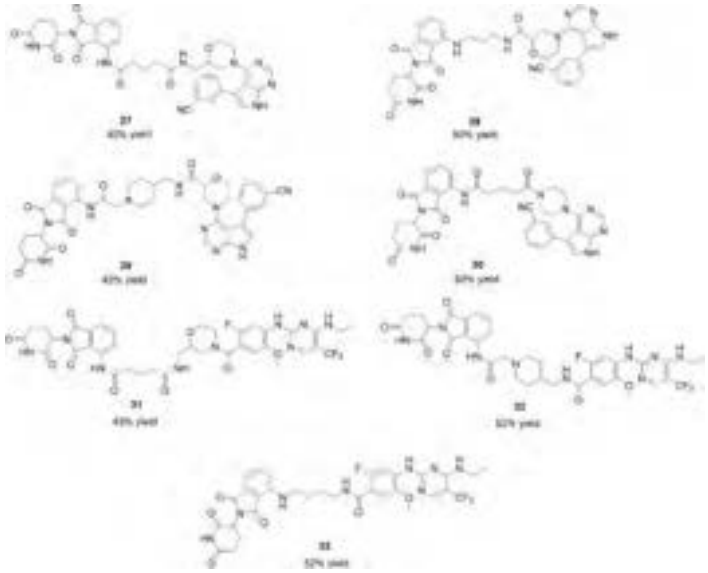
A truly impressive collaborative body of work with myriad interesting findings that will have a big influence in the TPD field. I look forward to future studies on the degradability of other protein families and also to the kinase TPD drug development programs this work will inspire and inform.

Contributor: Angus

**The tale of proteolysis targeting chimeras (PROTACs) for Leucine-Rich Repeat Kinase 2 (LRRK2)**

Markella Konstantinidou[§], …, Alexander Dömling*

*ChemMedChem* **2021**, DOI: 10.1002/cmdc.202000872



Leucine-rich repeat kinase 2 (LRRK2) is implicated in Parkinson's disease (PD) and is a target of interest for treatment of the disease. In this study, the authors explore targeted degradation of LRRK2, designing several PROTACs based on two ATP-competitive inhibitor scaffolds: PF-06447475 from Pfizer and GNE-7915 from Genentech. CRBN is expressed in the brain where PD pathogenesis occurs and was therefore chosen as the E3 ligase for recruitment. The authors synthesised four PF-06447475- and three GNE-7915-based PROTACs for evaluation. Despite demonstrable target engagement in cells, the PROTACs failed to induce degradation of LRRK2.

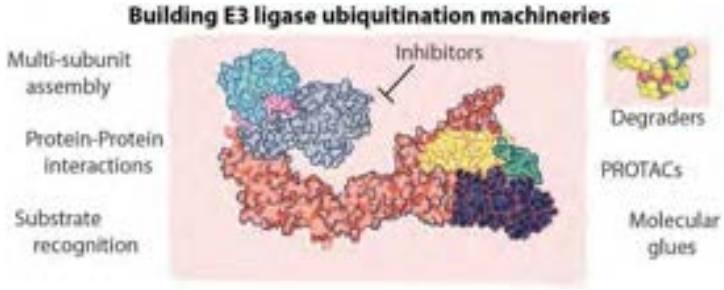
Publication of negative results is an important and underreported aspect of scientific progress and the authors should be commended for taking the time to prepare and publish this manuscript on a challenging and relevant disease target. As they mention in their discussion, a patent for degradation of LRRK2 was recently published indicating it is amenable to the TPD approach (patent number: WO 2020/081682 A1) and interestingly, LRRK2 also appeared in the degradable kinome map in the publication covered above.

Contributor: Angus

**Building ubiquitination machineries: E3 ligase multi-subunit assembly and substrate targeting by PROTACs and molecular glues**

Sarath Ramachandran[§], Alessio Ciulli*

*Curr. Opin. Struct. Biol.* **2021**, *67*, 110




An excellent review of recent literature on multi-subunit E3 ligases and PROTACs/molecular glues from Sarath and Alessio with a structural biology focus. Topics covered include regulation and assembly of multi-subunit E3 ligases, substrate recognition by CRL receptor subunits, and ternary complex formation between CRLs and neosubstrates induced by molecular glues or PROTACs.

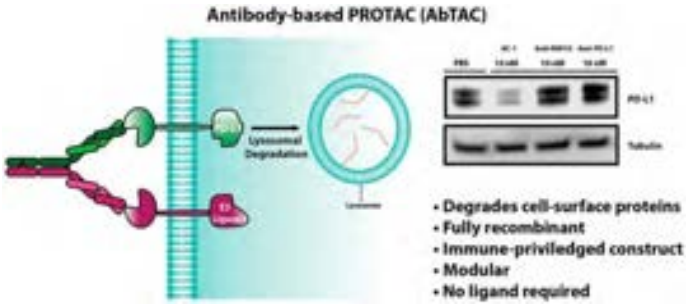
The review makes a clear case for the importance of structural information in informing the rational design of potent degrader molecules, including not only structures of small molecule-induced ternary complexes, but also structures that give a broader mechanistic understanding of ubiquitin transfer mediated by CRLs. A good read with some great structural figures

Contributor: Angus

**Development of Antibody-Based PROTACs for the Degradation of the Cell-Surface Immune Checkpoint Protein PD-L1**

Adam D. Cotton[§], Duy P. Nguyen, Josef A. Gramespacher, Ian B. Seiple, James A. Wells*

*J. Am. Chem. Soc.* **2021**, *143*, 593




Extracellular proteins of interest (POI) and plasma membrane POI that lack ligandable cytoplasmic domains have been out of reach for targeted protein degradation until recently with the development of lysosome-targeting chimaeras (LYTACs) (which were covered in the August 2020 JC issue and a news and views piece from Claire and Alessio (*Nature*, **2020**, *584*, 193-194). Here, Cotton *et al.* outline a novel method for degradation of plasma membrane proteins using recombinant bispecific antibody-based PROTACs (AbTACs). AbTACs are recombinant IgG antibodies produced in two halves with different specificities in the Fv regions, but complementary Fc regions which ensures correct pairing of heavy chains. Following some initial characterisation, the authors develop a bispecific antibody, AC-1, which hijacks the plasma membrane-resident single-pass E3 ligase RNF43 to degrade an important cell surface cancer target PD-L1 with a $DC_{50}$ of 3.4 nM and $D_{max}$ of 63% at 24 hours. Degradation is shown to be lysosome-dependent and proteasome-independent using inhibitors bafilomycin and MG132, respectively.

Another nice addition to the growing targeted protein degradation toolbox. A strength of the method is that a small molecule ligand is not required for the target protein nor the E3 ligase. Further mechanistic studies are warranted, for example are RNF43 and AC-1 also degraded in the lysosome as part of the RNF43:AC-1:POI complex? The authors perform whole cell proteomics to look for general changes in protein levels following treatment with AC-1 and see minimal changes but note that low abundance of cell-surface proteins such as PD-L1 (and presumably RNF43) makes detection difficult. Unlike small molecule PROTACs, AbTACs may be non-catalytic if the E3 ligase and AbTAC used to degrade the target are also degraded. If this is the case, the choice of E3 ligase will be important to ensure a high $D_{max}$ (i.e. higher concentrations of the ligase than the POI) and to minimise the unwanted effects caused by a decrease in levels of the ligase at the cell surface.

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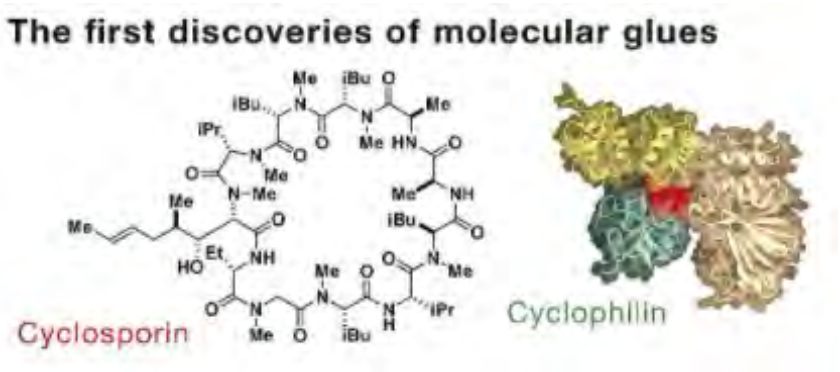
Contributor: Adam

**The Rise of Molecular Glues**

Stuart L. Schreiber[§]*

*Cell* **2021**, *184*, 3




This concise overview marks the 30th anniversary of the revelation that natural products cyclosporin A and FK506 act as molecular glues, kickstarting the field of heterodimerization by small molecules and eventually targeted protein degradation with PROTACs. Schreiber highlights the order of events which took place for the development of molecular glues, starting with the early natural products cyclosporin A and FK506, to the first chemical inducer of proximity (CIP), FK-CsA, and eventually to the well know iMID glues such as thalidomide and lenalidomide.

A nice overview of molecular glues summed up in just a few pages. I like how Schreiber has written this as a story with some great figures showing the molecular glue timeline and modes of action.

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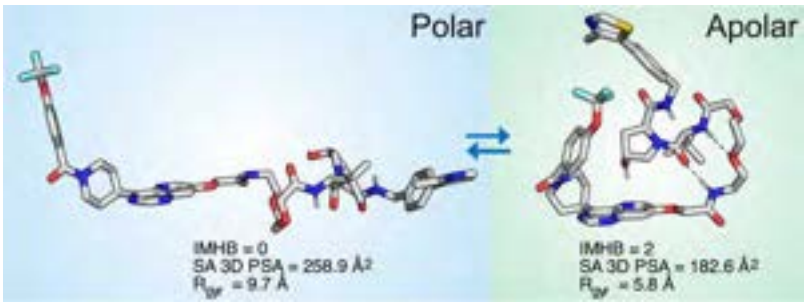
Contributor: Adam

**Solution Conformations Shed Light on PROTAC Cell Permeability**

Yoseph Atilaw[§], Vasanthanathan Poongavanam[§], Caroline Svensson Nilsson[§], …, Jan Kihlberg*

*ACS Med. Chem. Lett.* **2021**, *12*, 107




PROTAC cellular permeability has always puzzled medicinal chemists, due to their high molecular weights and disregard for drug-like physicochemical properties such as Lipinski's rule of five. In this paper, the authors have used NMR methods to reveal structural insights into how certain VHL based PROTACs behave in solution. They determined conformational ensembles for an ERK5 targeting PROTAC using NMR analysis of molecular flexibility in solution (NAMEFIS) algorithm and proton-proton distances from the nuclear Overhauser effect (NOE). This was conducted in both polar (DMSO-$d_6$, DMSO-$d_6$ - $D_2O$) and apolar ($CDCl_3$) solvents to mimic the aqueous extra- and intracellular environments and the interior of the cell membrane, respectively. They discovered that in the three most populated conformations, which are likely to be the permeating species, intramolecular hydrogen bonds (IMHBs) and $\pi$-$\pi$ stacking cause the PROTAC to fold in on itself and make the amide NH groups point inwards thus shielding them from the aprotic surrounding environment. The authors finish by suggesting that the ability to adopt folded conformations, stabilised by intramolecular interactions that minimise the radius of gyration ($R_{gyr}$) and solvent accessible 3D polar surface area (SA 3D PSA) is likely to be required for other VHL-based PROTACs to effectively enter cells and induce target protein degradation.

A nice paper highlighting potential mechanisms for PROTAC cellular permeability. Worth a read!

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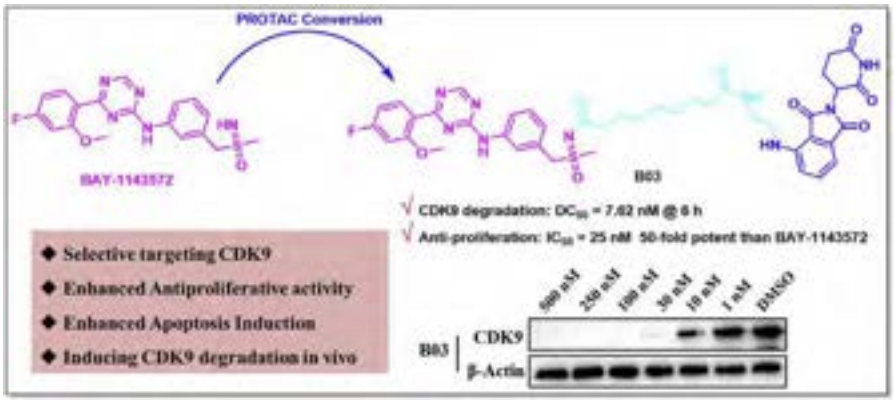
Contributor: Adam

**Discovery of selective CDK9 degraders with enhancing antiproliferative activity through PROTAC conversion**

Xiaqiu Qiu[§], Yuanqing Li[§], …, Jubo Wang*, Jinlei Bian*

*Eur. J. Med. Chem.* **2021**, *211*, 113091




Cyclin-dependant kinase 9 (CDK9) plays a key role in transcriptional regulation of cancer suppressors and oncogenes. Overexpression of CDK9 has been linked with cancers such as leukaemia and malignant melanoma. CDK9 degraders have been reported before which use non-selective CDK9 inhibitor warheads but *in vivo* activity was not investigated. The authors of this paper use a CDK9 selective inhibitor, BAY-1143572, coupled to CRBN targeting pomalidomide via a variety of linkers differing in composition, rigidity and length. They found suitable linkage vectors on BAY-1143572 by molecular docking. The sulfonylimide was found to be the most suitable by measuring $IC_{50}$ affinities of their PROTACs to CDK9. Their best compound "**B03**" was found to have the best anti-proliferation activity in a variety of cancer cell lines, showing a 50-fold increase in anti-proliferation compared to the parent inhibitor in MV4-11 cell line. **B03** was found to have the highest degradation efficiency with a $DC_{50}$ of 8 nM in MV4-11 cells compared to the other degraders.

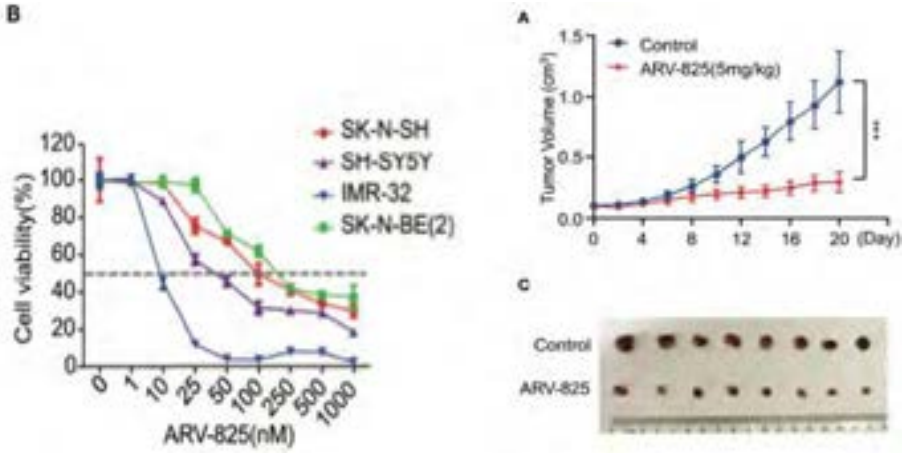
A well written paper which highlights a variety of effective CDK9 degraders. Yet another example of how degrading a protein and removing it entirely can have a much bigger impact than standard inhibition.

Contributor: Conner

**PROTAC Bromodomain Inhibitor ARV-825 displays Anti-Tumour Activity in Neuroblastoma by Repressing Expression of *MYCN* or *c-MYC***

Zhiheng Li[§], Su Lin Lim[§], Yanfang Tao[§], …, Sheng Xiao*, Shaoyan Hu*, Jian Pan*

*Front. Oncol.* **2020**, DOI: 10.3389/fonc.2020.574525




Neuroblastoma (NB) is a common paediatric malignancy with demonstrated sensitivity to the pan-selective BET inhibitor JQ1. Treatment of MYCN-amplified NB cells with JQ1 leads to cell cycle arrest and promotes apoptosis. Although previous work has shown promising results of BET inhibition in interfering with BRD4 function using JQ1 and OTX015, this process is reversible, causing the re-accumulation of BRD4 and incomplete suppression of MYC. ARV-825 is a previously published PROTAC degrader that demonstrates rapid, efficient and sustained degradation of BRD4. ARV-825 is a conjugate of the BET inhibitor OTX015 and a CRBN binding moiety. This publication is a continuation of the research into ARV-825 properties in both *in cellulo* and *in vivo* using NB cell lines. Li and colleagues demonstrate that NB cell lines IMR-32, SK-N-BE2, SK-N-SH and SH-SY5Y treated with ARV-825 display a dose-dependent reduction in cell viability. They go on to show that ARV-825 treatment induced antiproliferative effect demonstrated by an observable increase in the number of G1 phase cells accompanied by a decrease in S and $G_2$ phase cell proportions across all NB cell lines used. An increase in apoptotic cells after treatment with ARV-825 was also observed. RT-PCR analysis of SK-N-BE(2) cells demonstrated MYCN-associated super enhancer genes ISL1, PHOX2B, HAND2, TBX2, & GATA3 were dramatically repressed following treatment with ARV-825. To further investigate the *in vivo* activity of ARV-825, they developed a pre-clinical model of NB using the MYCN-amplified SK-N-BE(2) cells in nude mice, in the presence of ARV-825. Xenografted tumours of SK-N-BE(2) cells treated with ARV-825 had reduced weight, lower levels of Ki67, and downregulated expression of BRD4 and MYCN. Finally, they observed similar dose-dependent reductions in cell viability using all four NB cell lines treated with different BRD4 degraders; dBET1, MZ1 and GNE987.

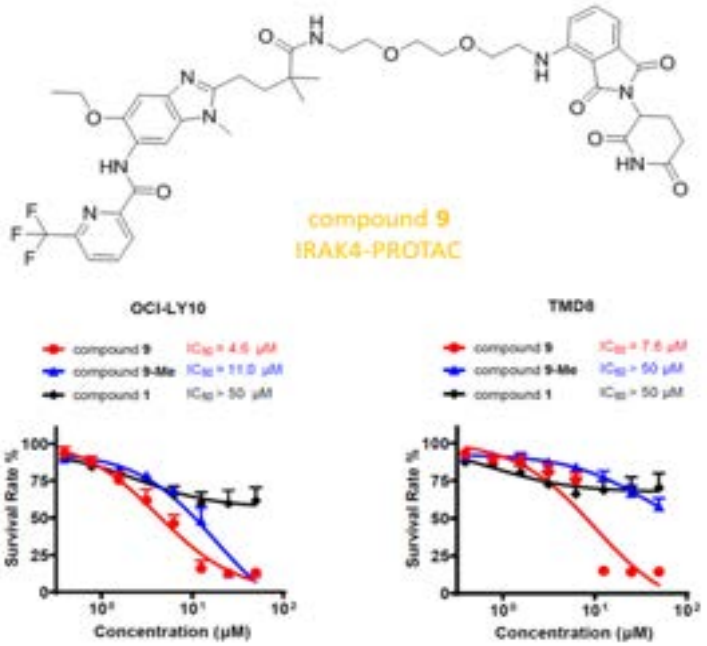
This paper not only very clearly underlines the reliance of neuroblastomas on BET expression for survival, but also demonstrates the potential that BET degraders have for the treatment of neuroblastoma in the future. This paper more broadly should inspire others to look further into the development of degraders for the treatment of disease currently refractory to treatment and further emphasises the need to have a better understanding of BET protein biology.

Contributor: Conner

**Design, Synthesis, and Biological Evaluation of IRAK4-Targeting PROTACs**

Yun Chen[§], Yi Ning[§], …, Huibin Zhang*, Hua Xie*, Jian Ding*, Wenhu Duan*

*ACS Med. Chem. Lett.* **2021**, *12*, 82




Interleukin-1 receptor associated kinase 4 (IRAK4) is a promising therapeutic target in diffuse large B-cell lymphoma, which is driven by mutations in MYD88. Both MYD88 and IRAK4 are part of the myddosome complex that is responsible for phosphorylation of I B kinase (IKK) and constitutive activation of NF- B driving proliferation in B-cells. The poor activity of previously established inhibitors is thought to be due to the now established non-kinase functions of IRAK4. In this paper they disclose a series of novel PROTAC degraders that are derived from the conjugation of the previously established IRAK4 kinase domain inhibitor **1** and the CRBN ligand pomalidomide. The degraders **2**-**9** showed linker lengths of increasing size. Through the use of activated B-cell-like diffuse large B-cell lymphoma (ABC-DLBCL) cell lines, Chen and colleagues demonstrate firstly that linker length is of importance in targeting IRAK4. Shorter length degraders **2**-**8** were unable to degrade IRAK4, but the longer PEG2 linking degrader **9** was able to show a statistically significant reduction in IRAK4 protein levels in OCI-LY10 and TMD8 cell lines. Following this, they go on to show that degradation of IRAK4 in these cell lines using degrader **9** leads to the complete abrogation of NF- B signalling and a 10-fold improvement in the antiproliferative effect of degrader **9** when compared to the original inhibitor

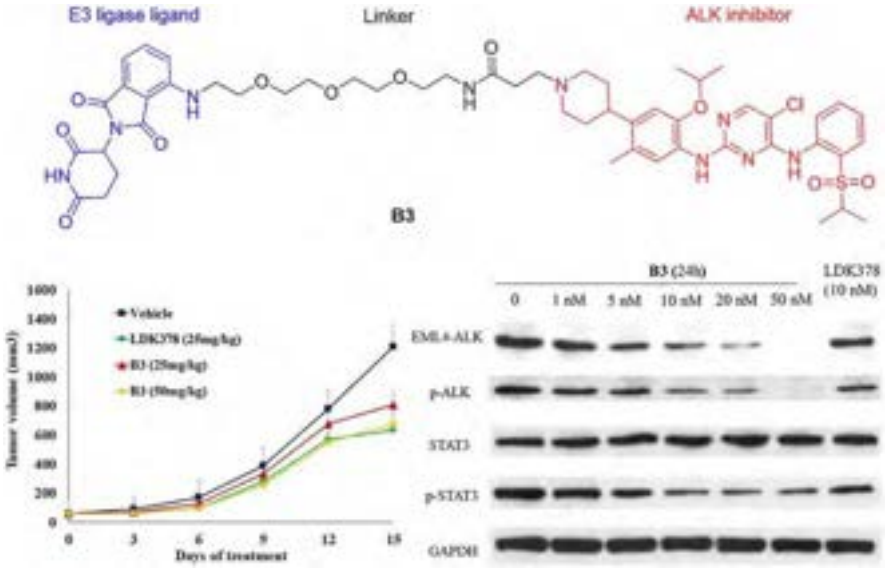
This paper is of great interest not only because it further demonstrates the value of applying PROTAC modality to improve upon inhibitor efficacy but also demonstrates the importance of considering linker length in PROTAC design. The experiments performed in this paper are clear, concise and lack ambiguity with regards to their results. Interestingly, compound **9** is a relatively weak degrader compound and therefore this paper should induce others to strive towards the development of significantly more potent compounds against this important target.

Contributor: Conner

**Discovery of a PROTAC targeting ALK with *in vivo* activity**

Guoyi Yan[§], Xinxin Zhong[§], Lin Yue[§], …, Rui Li*

*Eur. J. Med. Chem.* **2021**, DOI: 10.1016/j.ejmech.2020.113150




Anaplastic lymphoma kinase (ALK) is involved in the development of various cancer types including Anaplastic large-cell lymphoma and lung adenocarcinoma. Ceritinib (LDK378) is a potent ALK inhibitor as demonstrated in clinical trials, however, drug resistance of Ceritinib has been observed. In response to the discovery of ALK drug resistance, many groups have attempted to develop PROTAC degrader compounds against ALK, however, many of the previously published degrader compounds are not orally bioavailable. In this paper, Yan and colleagues develop PROTACs against ALK using the inhibitor LDK378 and various CRBN binding moieties. In total they produced 25 PROTACs, several of which showed promising inhibitory and antiproliferative effects in various cancer cell lines including H3122, H2228, H1299, A549 and HeLa. They demonstrate the improved antiproliferative effect of degrader **B3** against LDK378 in H3122 cell. Combining this and the discovery that compound **B3** is not any more toxic than LDK378, Yan *et al*. focus on investigating **B3** efficacy both *in cellulo* and *in vivo.* They confirm that degradation of ALK and inhibition of its downstream targets is possible with degrader **B3** and that it has better efficacy in BaF3-ALK-L1196M ALK mutant cells compared to LDK378, suggesting a possible use in the treatment of ALK drug resistant cancer. Finally, they go on to show favourable pharmacokinetics for degrader **B3** in a rat model and demonstrate that **B3** has antiproliferative effects in mouse H3122 xenograft tumours.

This paper highlights a clear advancement that has been made by Yan *et al*. towards the development of bioavailable degraders for ALK, this research should hopefully pave a path towards viable therapeutic ALK degrader candidates.

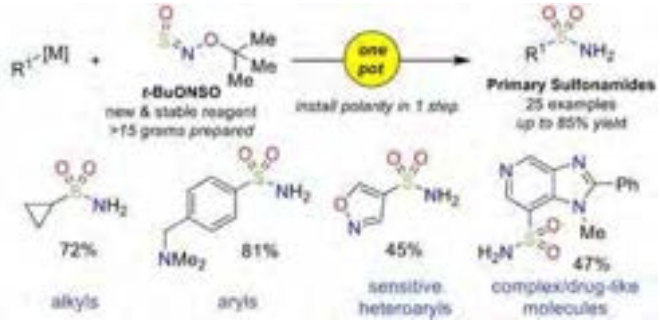
# Other Paper Highlights

Contributor: Tom

**Primary Sulfonamide Synthesis Using the Sulfinylamine Reagent *N*-Sulfinyl-*O*-(*tert*-butyl)hydroxylamine, *t*-BuONSO**

Thomas Q. Davies[§], Michael J. Tilby, David Skolc, Adrian Hall, Michael C. Willis*

*Org. Lett.* **2020**, *22*, 9495




Sulfonamides, in particular primary sulfonamides, are present in many marketed drug compounds. They are of particular interest to the targeted protein degradation community due to their presence in molecular glue degraders, such as indisulam. Therefore, new and simple synthetic routes to access sulfonamides could greatly accelerate developments in this field.

Willis *et al*. have developed a synthetic route to a novel sulfonating reagent, *N*-sulfinyl-*O*-(*tert*-butyl)-hydroxylamine (*t*-BuONSO), which was synthesised in one step from commercially available *O*-*tert*-butylhydroxylamine hydrochloride, thionyl chloride and triethylamine. Distillation under reduced pressure yields pure *t*-BuONSO (57% yield on decagram scale). This new reagent allows for a one-pot installation of primary sulfonamides via reaction with common alkyl and aryl halides such as Grignard and organolithium reagents. Optimal conditions for this reaction (-78°C, 1 eq. of organometallic reagent) also delivered good yields (>62%) at preparative scales.

The authors also investigated substrate scope. Varying the aryl organometallic nucleophile showed that *para*-, *meta*- and *ortho*-methyl substituents were all well tolerated. Good yields (55-81%) were also obtained using aryl nucleophiles with electron-donating and electron-withdrawing groups. Further substrate scope studies showed that basic nitrogen heterocycles, including a fused imidazopyrimidine, and five-membered heterocycles could all be obtained in reasonable to good yields (43-74%), as could alkyl organomagnesiums. It was also possible to install primary sulfonamides on medicinally relevant compounds – a dopamine receptor motif and celecoxib – in 59% and 55% yield respectively. A reasonable mechanism is also provided from the results of preliminary mechanistic investigations.

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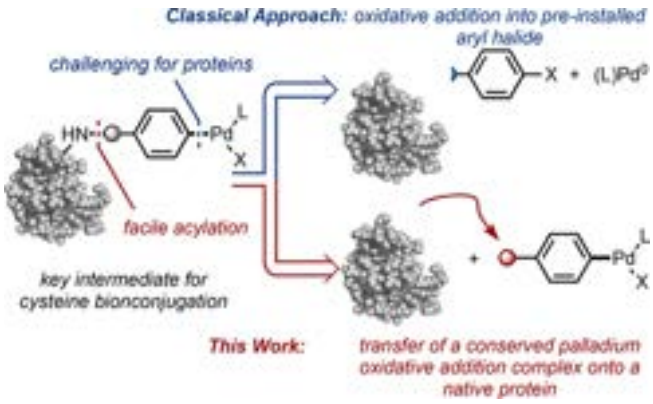
Contributor: Tasuku

**Palladium–Protein Oxidative Addition Complexes by Amine-Selective Acylation**

Heemal H. Dhanjee[§], Ivan Buslov[§], …, Bradley L. Pentelute*, Stephen L. Buchwald*

*J. Am. Chem. Soc.* **2020**, *142*, 21237




Palladium oxidative addition complexes (OACs) are useful precursors for coupling two components. OACs are especially reactive to thiols and form stable C-S bonds quickly in aqueous media and this type of reaction is useful to tether two proteins with covalent bonds. However, inert atmospheres and organic solvents are needed to prepare them so it's challenging to conjugate them onto proteins. In this paper, the authors prepared a stable lysine-selective organopalladium-containing reagent and successfully established a way to introduce that palladium complex onto proteins. They then applied those protein-organopalladium complexes to form a variety of protein-protein conjugates, for instance, antibody-protein conjugates and antibody-peptide conjugates. Their conjugation method has some limitations (e.g. one of the proteins needs to have one or more cysteines on the surface and another needs to have no cysteine on the surface), but this pre-isolated palladium complex approach not only establishes a method for functionalizing proteins but also impacts other fields in which preparation of OACs has previously been difficult due to the harsh conditions required.

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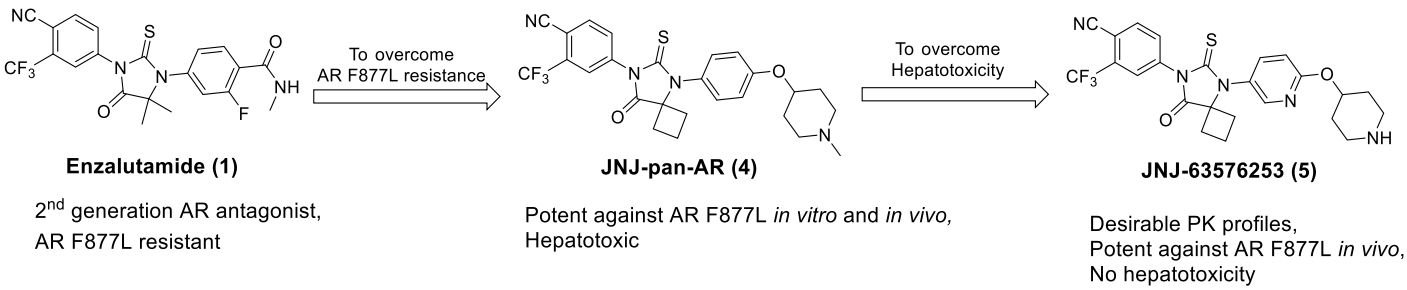
Contributor: Xingui

**Discovery of JNJ-63576253: A Clinical Stage Androgen Receptor Antagonist for F877L Mutant and Wild-Type Castration-Resistant Prostate Cancer (mCRPC)**

Zhuming Zhang*, …, Gilles Bignan*

*J. Med. Chem.* **2021**, *64*, 909




Prostate cancer is the second most common cancer worldwide. Since androgen receptor (AR) signalling is the major driver of prostate cancer cell growth, therapeutics targeting AR signalling, such as androgen deprivation therapy (ADT) and AR antagonists, have provided impressive therapeutic benefits in the clinic. However, acquired resistance develops after treatment, resulting in disease progression to metastatic castration-resistant prostate cancer (mCRPC). Various resistance mechanisms have been established, including AR amplification, overexpression, and mutation. The AR F877L point mutation was reported to confer resistance to the second generation of AR antagonists (e.g., enzalutamide (**1**)), leading to antagonist-to-agonist switch in response to preclinical treatment *in vitro*. Generation of novel antagonists is therefore warranted for targeting F877L mutant AR.

Through a library compound screening campaign, the authors identified JNJ-pan-AR (**4**). Side-by-side comparison of compounds **4** and **1** showed that compound **4** acted as antagonist to both AR WT and AR F877L mutant, while compound **1** was antagonist to AR WT, but agonist to AR F877L mutant. As such, compound **4** outperformed **1** in various *in vitro* and *in vivo* assays, including more potently inhibiting androgen-mediated transcriptional signalling and AR nuclear translocation, and completely inhibiting an AR F877L-driven xenograft tumor growth that is resistant to **1**. However, compound **4** displayed unacceptable hepatotoxicity due to the production of reactive phenolic metabolites *in vivo*. To evade this bioactivation pathway and block the formation of a phenolic metabolite, the authors adopted several structural modification strategies such as electronic alteration, carbon-linker in place of oxygen, and introduction of steric hindrance and extra metabolic soft spots, and synthesized more than 200 analogues of **4**. After systematic SAR exploration, combined with PD and PK studies as well as metabolism analysis, the authors arrived at JNJ-63576253 (**5**). Compound **5** exhibited desirable PK profiles, retained (slightly less potent than **4**) the anti-cancer activity of **4** in a prostate LNCaP SRα F877L tumor xenograft model, and more importantly, showed no hepatotoxicity. Compound **5,** now known as TRC253, is currently in phase 1/2A clinical study. It has the potential to be a first-in-class small molecule AR antagonist that brings hope to patients that develop resistant AR F877L tumors and fail the first and second-generation anti-androgen therapy.

Improving metabolic profiles and decreasing toxicity is a common practice during the lead to candidate modification, this paper provides an example of structural modifications that dramatically improved safety window with a subtle and simple N/C exchange of phenyl with bioisosteric pyridinyl.