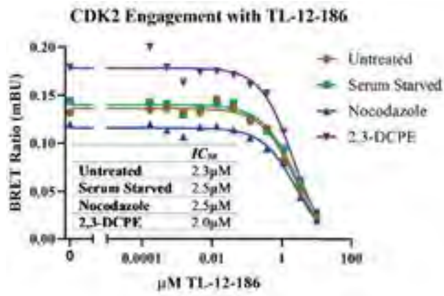
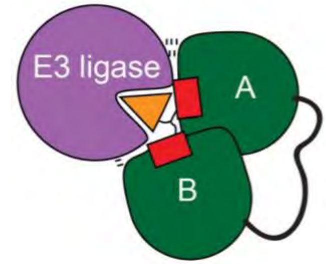
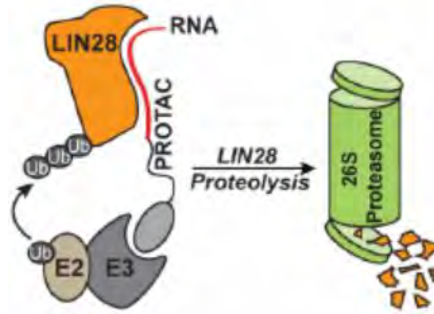
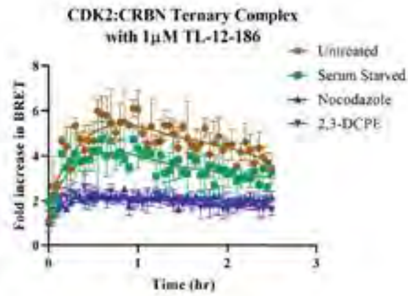
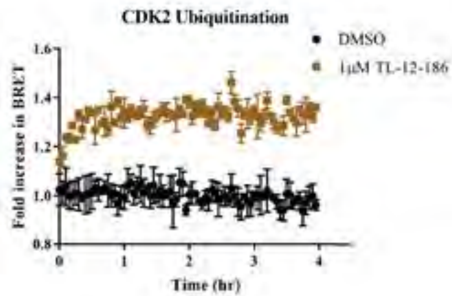
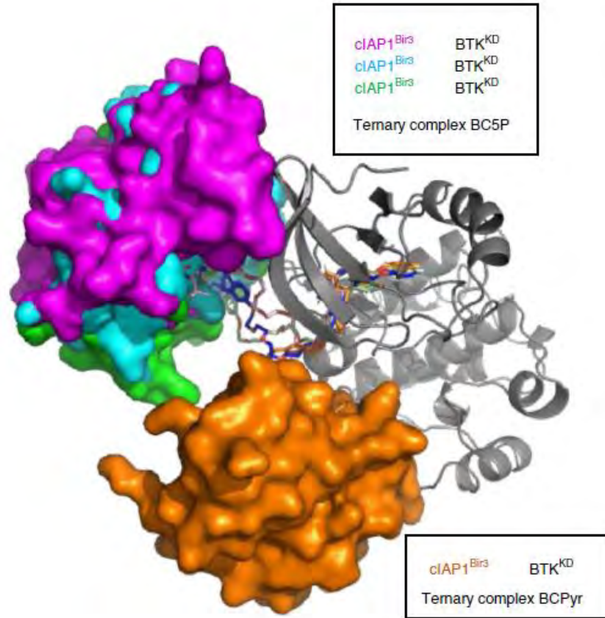


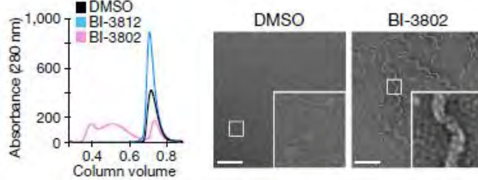


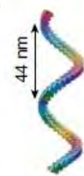












Ciulli Group Journal Club

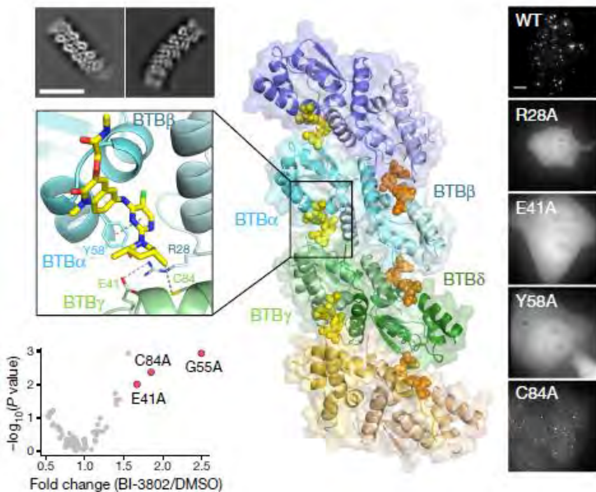
Targeted Protein Degradation,

Medicinal Chemistry and

Chemical Structural Biology

Literature Highlights

November Edition



Ciulli Group Journal Contents

Features of the month 1
Targeted Protein Degradation 5
Others 14

Features of the month

In Memory of Chris Abell

The sudden and unexpected passing of Chris Abell on 26th October 2020, at the age of 62, has been a tragic devastating loss for the many people whose lives, like mine, were touched by him, academically, scientifically, socially or personally.

Chris Abell. Credit: Royal Society

Trained as a chemist, Chris held an independent position in the Department of Chemistry at Cambridge since 1984. Over the past 36 years, Chris led pioneering interdisciplinary research in areas as diverse as mechanistic enzymology, drug discovery, and technology development. In all these areas Chris has made long-lasting impacts, innovating by crossing boundaries and straddling the interfaces of conventional fields of chemistry, biology and medicine. Extensive track record of impact is evidenced by many widely cited publications and the translation to real-world effect through entrepreneurship and founding of biotech companies. An example of this is Astex Pharmaceuticals, one of the world's pioneering companies in the area of fragment-based drug design, an approach now widely adopted in the pharmaceutical industry and academia. These achievements were recognized by many accolades, including election as Fellow of the Academy of Medical Sciences, Fellow of the Royal Society, and more recently, by the award of the Interdisciplinary Prize of the Royal Society of Chemistry. Importantly, over all these years, Chris trained, mentored and inspired many young scientists who have gone on to take important roles in academia, industry or beyond.

I feel very fortunate to have been one such young scientist. With this piece, I would like to take the opportunity to offer a personal remembrance and some reflections on the many aspects in which Chris touched my life.

To me, Chris fulfilled many important roles over several formative years. First and foremost, he was my PhD supervisor. In September 2002, I left Italy to start my PhD at Cambridge under his guidance, thanks to the generous support of a Gates Cambridge Scholarship and a BBSRC industrial studentship (CASE) award with Astex (at the time Astex Technology), which Chris had co-founded a couple of years earlier. My second industry supervisor was Glyn Williams, who had just recently joined Astex to head a new Biophysics group.

Around that time, fragment-based screening approaches were not yet mainstream. The most popular methods being used by the founding groups were X-ray crystallography (a main focus at Astex) and NMR spectroscopy. The remit of my PhD project was to explore different biophysical methods to study weak protein-ligand interactions and applications for fragment-based drug design. These included techniques such as ITC, SPR and mass spectrometry, to complement NMR and X-ray. As a model system for my PhD research, I began to study an enzyme in the pantothenate pathway, so joined an ongoing collaboration Chris had with the groups of Alison Smith and Tom Blundell in studying enzymes of this pathways from bacteria and plants. Eventually, in February 2006, I defended my PhD thesis with a most-enjoyed viva discussion, together with Walter Ward and Carol Robinson, who duly grilled me as external and internal examiner, respectively.

During these PhD years, Chris provided me with the freedom and confidence to formulate and explore ideas and to take my research in different directions. He showed me how to take a calm, grounded, and rational approach, when questioning assumptions or posing hypotheses. Most importantly, he taught me to be thorough and un-approximate in my preparation and study, and so in my thinking. During our 1:1s, or during presentations at group meetings, he would

often ask: *"how does this method/technique work? why do you do the experiment this way? can you approach this experiment in another way? Or using a different method?".* This taught me to question my assumptions, and to refine my thinking, over and over, again and again.

There were many bumpy rides in my PhD project. Lot of things "did not work" as we would call it. For at least the first half of my PhD, lot of techniques I was trying did not work, or at least did not give the kind of data we were hoping to obtain. Yet, despite this, I never sensed a loss of his confidence in my abilities. This meant a lot to me. It helped me to keep going, to bring resilience. He used to tell me I had broad shoulders, so I believed him! In the end, the main first-author paper of my PhD published in J Med Chem shortly after my viva, and it is one that I remain most fond of.

Chris's supervisory style was what you might call "hands-off", because he did not work himself in the lab. I and the other fellow students, therefore, had to "learn the ropes" by ourselves. Yet he would always be there for us if we had any questions; he would always make time for a chat in his office or in the tea-room. Importantly, he created a safety space for us. On reflection, I feel he provided me with just the right balance of support and stir, carrot and stick, so to speak, to help me develop and find that "rope" within myself. He hardly ever explicitly told me what to do; hardly ever imposed the specifics of the experiments he expected me to do. He commanded respect not with status, entitlement or fear, but with clarity of thinking, and perspective.

As researchers, scientists and academics, we are the progeny of our advisors and mentors. Chris continued naturally in these roles, as I decided to stay on at Cambridge, not only for my postdoctoral research as a College Junior Research Fellow, but also later on when I started my own independent research group.

Around 2006, Chris and Tom began to bring back fragment-based approaches into their academic labs, and to apply those to a challenging disease area such as tuberculosis. I so had the opportunity to be involved in this new direction of research during my post-doctoral years, and to learn new techniques of structural biology. I joined the collaboration networks that they became part of, such as the European network led by Stuart Cole and the Gates Foundation network led by Clif Barry. I was fortunate to share with Chris so many collaboration meetings in various beautiful parts of the world, and to get to meet many scientists as a result. I have so vivid memories of personal experiences beyond science, too. Crucially, over these postdoctoral years, I learnt so much about how to collaborate, how to work with others, and how to lead others – which have greatly influenced how I later approached my own independent path. Chris was ever so supportive and encouraging of my desire to try new directions and carve out a space for me to try go my own way. Which in the end was made possible thanks to the securing of a BBSRC David Phillips Fellowship, which provided the funding that allowed me to start a group.

As Chris's and my own careers grew, so our own relationship evolved over those years, and we became closer and closer, like friends. He never quite managed to convince me to become a marathon runner (thank God!). Yet, when some hamstring problems forced him out of running for a few months, he decided to get seriously into road cycling. Aware that I had recently acquired a flashy new Bianchi road bike, one day he approached me: *"Hi Alessio, I want to get into cycling and get a road bike, and need your advice!"* He quickly got one, not quite as "Italian" as mine, but equally fast and light – so he could not blame me for any unfair advantage on the road! I really can't claim to have gotten him in to cycling though, as he had already decided that. That was classic Chris: you could not stop him, so you might as well follow him! I will always remember our many bike rides together around Cambridge, often early-morning rides, to my joy. A yearly routine was the often-frosty Sunday morning ride before the tennis Australian Open men's final, which was shown live on tv at 8:30am. We would watch this at his home, with Katherine ever so kindly providing warm coffee and cake for us to recover. Chris and I would make separate predictions and support the different players, just for the sake of fun competition and arguments.

Famously, Chris got me into playing bridge by calling my mobile while I was in the lab running experiments one afternoon. *"Alessio, we are short of a player for bridge tonight, would you like to join in?"*. Despite knowing many card

games, I had never played bridge before. Yet, I felt that would not be a good enough excuse for him to bear. In fact, even before I had a chance to say anything, he continued: *"We'll go through the rules altogether, it's very easy, you'll learn quickly!"*. In order not to arrive unprepared, I shamelessly thought I could learn how to play by joining an online bridge website, trying out a few hands for a couple of hours just before the real game. Not surprisingly, that did not help, as I got regularly kicked-out by the other online players for being slow and non-sensical. Chris and his bridge mates Finian and Alison could not help making fun of me for the entire evening. Somehow, I had made the test!

Chris was a person of significant achievements, who worked to make a difference. He strived to excel in everything he did, and always thrived with ideas. He inspired everything he touched with warmth and vision. Chris played pivotal roles in every organizations or group that he was part of and led or contributed to. Not just within the University and the Department, but also at Christ's College, where he was a Fellow for many years. It was thanks to him that I was provided with the opportunity to also become a Fellow at Christ's College in 2009. There, I was fortunate to hold the position of Director of Studies in Chemistry, which kept me much involved with undergraduate teaching.

Ever since I joined Dundee, I watched Chris taking on more important responsibilities at Cambridge. He was a founding Board Director of Cambridge Enterprise, advising companies and helping them with seed funds. He became the first Director of the University's Postdoctoral affairs, playing a key role to help postdoctoral fellows with their social as well of scientific experiences. And in 2016, Chris became Pro-Vice Chancellor for Research, being responsible for the overall research strategy of the University.

Chris and I kept in touch after my Lab's move to Dundee. I always took an opportunity to congratulate him for his awards and achievements, and tried to keep him abreast of my own developments. Partly his full schedule as University Pro-VC, partly my own increasingly busy schedule, and the geographical distance, meant we did not get a chance to see each other as much as I would have liked in the past few years, unfortunately.

Chris was a unique and remarkable human being. A biological chemist of the finest, brightest intellect, and a brilliant teacher. Chris was clearly a great mentor to me. As a matter of fact, he was a role model to me. Pride meant I would not always admit that, to myself or to others. I have no regrets, but if I have to find one that would be that I wished I would have reminded him more often of how much he enabled me to grow, as a person and as a scientist. But I knew he knew that, and I sensed that if I did attempt to remind him, he would just stop me and say: *"Right, right, Alessio! Let's go for a bike ride next week-end, shall we?!"*.

It is this inspiring, galvanizing attitude, a so natural, unique mix of modesty, energy and kindness, that I will miss the most.

Rest in peace, Chris. And thank you, for all of this.

Alessio

Ciulli group members took part in Movember

This month, members of the Ciulli group – along with many of our friends, families and colleagues around the world – have been growing moustaches and getting active in aid of the Movember Foundation. The Movember Foundation is the leading men's health charity, operating in three key areas:

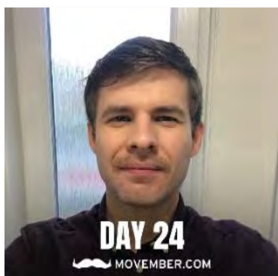
1. **Mental health and suicide prevention**
   Movember looks at mental health through a male lens, focusing on prevention, early intervention and health promotion. The rate of male suicide is alarmingly high: 3 out of 4 suicides in the UK are by men, and globally, on average, 1 man dies by suicide every minute of every day. With many people throughout the world currently living their lives under very difficult circumstances, the power of reaching out cannot be underestimated. We would really like to encourage you to take the time to check-in with your friends and family, and if you need to speak to someone you can contact NHS 111 on 111 or contact Samaritans on 116 123.

2. **Prostate cancer**
   1 in 8 men will be diagnosed with prostate cancer in their lifetime. Prostate cancer is the most commonly diagnosed cancer in men in the UK. Globally, more than 1.3 million men are diagnosed with prostate cancer each year. Across the country, there are more than 333,500 men living with and beyond the disease. Many are dealing with serious side effects from treatment. The Movember Foundation are trying to unite researchers and experts across the world to chase down breakthroughs in prostate cancer, from early detection through to diagnosis, treatment and support.

3. **Testicular cancer**
   Testicular cancer is the most common cancer in young men in the UK. At greater than 95%, the odds of survival for men with testicular cancer are better than good – but for some men, long-term treatment-related side effects, mean quality of life is severely compromised. The Movember Foundation focuses on getting these predominantly young men back to living full and healthy lives.

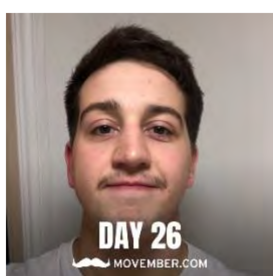
The above information was adapted from the 'About Us' section of the Movember Foundation website – follow this **link** to learn more about their work and strategies. To support our cause, donate to the Team here!

And because we could all use a good laugh right now, please take a look at some of our Ciulli group fundraisers progress:

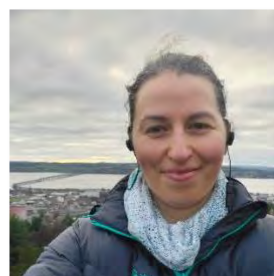


Angus

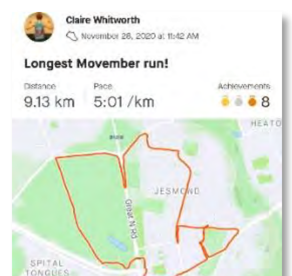
Tom



Adam



Emelyne



Claire

£958

Target: £800

Team Moves
257.59 km

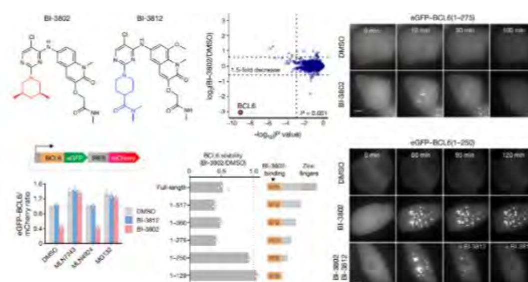
Targeted Protein Degradation

Contributor: Emelyne

Small-molecule-induced polymerization triggers degradation of BCL6

Mikolaj Slabicki §, Hojong Yoon §, Jonas Koeppel §, … Eric S. Fisher* and Benjamin L. Ebert*

Nature **2020**, *588*, 164



This paper describes the elucidation of the mode of action of a monovalent BCL6 degrader. Prior screening and chemical optimisation work by a team at Boehringer Ingelheim had led to the development of compounds BI-3802 and BI-3812, both targeting BCL6, a known driver of oncogenesis in lymphoid malignancies (*Cell Rep.* **2017**, *20*, 2860–2875). Both compounds are targeting BCL-6 with equal potency but through different mechanisms. BI-3812 acts as an inhibitor while BI-3802 shows higher efficacy as a degrader. The scientists aimed at determining the mechanism by which BI-3802 triggers degradation of BCL6.

Cellular experiments, including proteomics approaches, showed that BI-3812 selectively degrades BCL6, mediated by a non-Cullin E3 ubiquitin ligase. After observing the formation of BI-3802 induced foci by live-cell fluorescence microscopy, the authors studied the behaviour of BCL-6 on a molecular level. They observed that BCL6 was forming, in the presence of BI-3802, regular structures of higher molecular weight. Cryo-EM showed well-dispersed helical filaments. Further structural investigations and mutagenesis studies showed that BI-3802 favours assembly of BCL6 dimers, while BI-3812 induces steric clashes that impair polymerization. Blocking polymerization of BCL6 prevents BCL6 degradation and cellular toxicity in lymphoma cells.

To identify the cellular machinery involved in the BI-3802 induced degradation of BCL6, CRISPR-Cas9 genetic screens were performed. Only one gene stood out: *SIAH1*. Various *in vitro* and cellular assays were performed to confirm that BCL6 is degraded through SIAH1, when treated with BI-3802.

This paper describes work carried out on compounds shared on the BI openMe platform, and as such is an excellent example of how science advances by sharing results and compounds with the scientific community. The original story was already by itself very interesting from a structural and chemistry point of view, but this paper adds another layer of excitement, by going deeper into the molecular mechanisms on why BI-3802 is an inhibitor while BI-3812 is a degrader, and how the different mode of action underpins their different effects on cancerous cells.

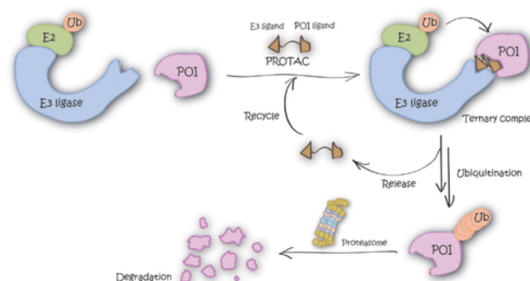
Contributor: *Emelyne*

E3 Ligase Ligands for PROTACs: How They Were Found and How to Discover New Ones

Tasuku Ishida[§] and Alessio Ciulli*

SLAS Discov. **2020**, DOI: 10.1177/2472555220965528

The authors offer an overview on E3 ligases ligands, a high-interest research topic within targeted protein degradation. Each commonly used ligand family is described, from their discovery to application. Other E3 ligases ligands and methods for discovery of novel moieties are highlighted.



There are 600 E3 ligases in the human body, and this is motivating an exponential interest in finding novel ligands to expand the PROTAC drug discovery toolbox. Most reviews focus on degraders alone, but it is also important to remember that each part of the molecule play an important role to boost degrader discovery, including the E3 ligand.

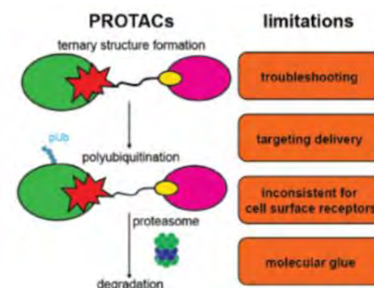
Contributor: *Emelyne*

PROTAC Compatibilities, Degrading Cell-Surface Receptors, and the Sticky Problem of Finding a Molecular Glue

Conghe Tian[§] and Kevin Burgess*

ChemMedChem **2020**, DOI: 10.1002/cmdc.202000683

The authors aim at describing some of the recent progress made to overcome the challenges met when developing PROTACs, such as E3 ligase selection. They also describe alternative methods to selectively degrade a Protein of Interest (Ab-PROTAC, LYTACs, Identification of Molecular Glues).



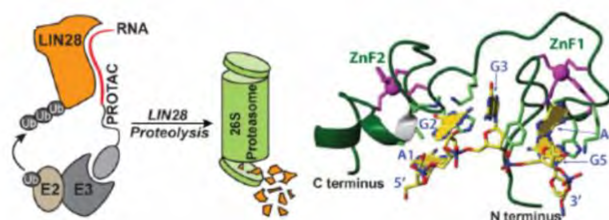
Contributor: *Emelyne*

RNA-PROTACs – degraders of RNA-binding proteins

Alice Ghidini,[§] Antoine Cléry, François Halloy, Frédéric H. T. Allain and Jonathan Hall*

Angew. Chem. Int. Ed. **2020**, DOI: 10.1002/anie.202012330

This work highlights the efforts made towards RNA-PROTACs to overcome the challenges presented by targeting RNA binding proteins (RBPs) with conventional drugs. Two RBPs were targeted for selective protein degradation – stem cell factor LIN28 and splicing factor RBFOX1.



Small RNA mimics were attached to a HIF-1α derived peptide. Various assays were used to establish whether the RNA-PROTACs would be able to bind and compete with the native RNAs. Ubiquitination assays were performed and showed poly ubiquitination of LIN28 when treated with RNA-PROTACs, while their negative controls showed no effect. Pull-down RNA ELISA confirmed that the RNA-PROTACs were entering the cells and engaging with endogenous LIN28. Degradation assays showed ~50% degradation in cells treated with 2 µM of the key compounds.

Negative controls showed no degradation effects and treatment with a proteasome inhibitor rescued LIN28 levels, confirming UPS mediated degradation.
To solidify their findings, the authors applied this strategy to another RBP: RBFOX1 and observed the same results as previously described.

This paper shows how promising targeted protein degradation is, in order to overcome challenges met by conventional ways of drugging proteins. Although the degradation was not complete (~50% at 2 µM), the RNA-PROTACs haven't been optimised and I hope this proof of concept motivates researchers to explore further this modality and bring it down the drug discovery pipeline.

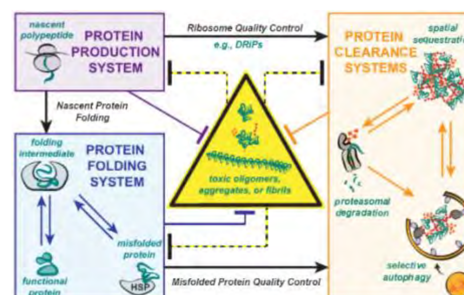
Contributor: Emelyne

Alternative systems for misfolded protein clearance: life beyond the proteasome

Harvey E. Johnston§ and Rahul S. Samant*

FEBS J. **2020**, DOI: 10.1111/febs.15617

This review describes the different protein quality control systems in the body. Under normal physiological conditions, the main protein clearance happens through ubiquitination and degradation by the proteasome. But when under acute stress and/or ageing related decline, this route can be overwhelmed. Consequently, other protein clearance systems such as autophagy and other mechanisms, are activated, which is the focus of this review. There is an increased need to understand those mechanisms as numerous components of the protein clearance system are involved in neurodegenerative diseases and other ageing related diseases, diabetes, cancers etc.



Most of targeted protein degradation relies on the ubiquitin proteasome system. But recent reports have also emerged where other protein clearance systems have been exploited for degrading proteins of interest. This review highlights the alternatives to the UPS, with clear explanations on the mechanisms and their potential for therapeutic advances for healthy ageing and other diseases. It is well written and a clear addition to the literature library on protein degradation and mechanisms.

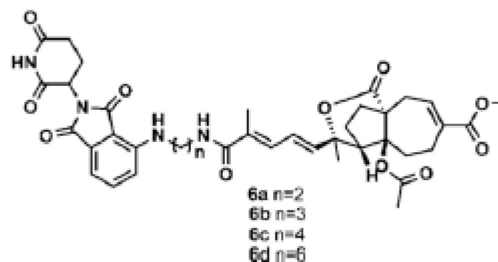
Contributor: Will

Targeted Degradation of CD147 proteins in melanoma

Zhe Zhou§, Jing Long§,....., QianBin Li*, Cong Peng*, and Xiang Chen*

Bioorg. Chem. **2020**, *105*, 104453

This study focuses on the discovery of CD147 PROTACs for treating cutaneous melanoma. CD147 is a transmembrane glycoprotein believed to play a broad role in tumour progression. No small molecule therapeutics currently exist for CD147 targeting, though a CD147 monoclonal antibody, Licartin, has been used for hepatocellular carcinoma to some effect. Studies by others have previously shown that natural product pseudolaric acid B (PAB) is an antagonist of CD147. This previous study had also demonstrated that modification of the carboxylate group of (PAB) was not detrimental to CD147 binding, suggesting to the authors of this manuscript a possible exit vector for PROTAC conjugation. CRBN based PROTACs were made and profiled for degradation and antiproliferative activity, with the best compound showing >80% degradation at 4 µM.



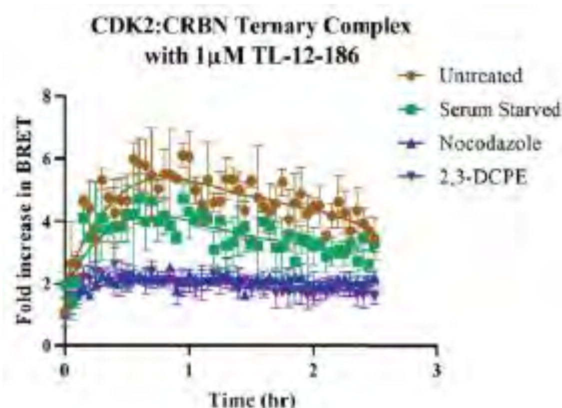
Contributor: Will

CDK Family PROTAC Profiling Reveals Distinct Kinetic Responses and Cell Cycle-Dependent Degradation of CDK2

Kristin M. Riching§,....., Danette L. Daniels*

SLAS Discov. **2020**, *DOI*: 10.1177/2472555220973602

Riching *et al.* herein investigate a pan-kinase PROTAC TL121-86 with respect to its propensity for degradation of cyclin-dependent kinase family members. They evaluate in detail the kinetics and maximal degradation of CDK proteins, focussing on CDK2 degradation which is shown to be reliant on cell-cycle phase. Whilst PROTAC-induced degradation of CDK2 is observed in unsynchronised or G1-arrested cells, minimal effect is observed when cells are locked in a S or G2/M state. These studies are enabled by endogenous tagging of CDK proteins with the nanoluciferase fragment, Hibit, in HEK293 cells. Using these cellular models the authors are also able to show accompanying Ligase:PROTAC:CDK2 ternary complex data and CDK target engagement data further supporting the cell-cycle dependency of PROTAC function.



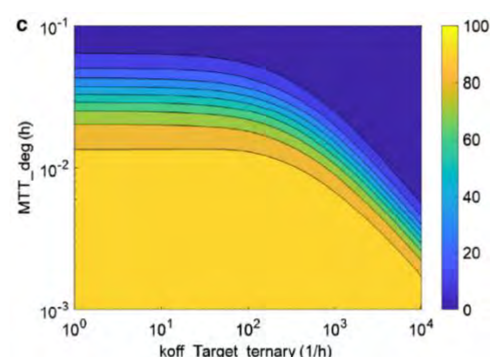
Whilst the CDK data put forward here is interesting in its own right, this study also more broadly illuminates how PROTAC-induced ternary complex formation and subsequent degradation may be cell-state and/or protein sub-population dependent. This has broader implications beyond the CDK family and shows how detailed cellular interrogation of PROTAC function is vital for pushing back the boundaries of understanding in the field.

Contributor: Will

A kinetic proofreading model for bispecific protein degraders

Derek W. Bartlett[§*] and Adam M. Gilbert

J. Pharmacokinet. Pharmacodyn. **2020**, DOI: 10.1007/s10928-020-09722-z



Targeted protein degradation induced via bifunctional/bispecific small molecules constitutes a complex multi-step process from dose to target engagement through to protein degradation. The authors of this manuscript detail a mathematical framework towards enabling more sophisticated pharmacokinetic/pharmacodynamic (PK/PD) modelling in this field. They do so by use of a kinetic proofreading concept which accounts for not only all of the requirements from dose (cellular or *in vivo*) up to and including ternary complex formation and stability, but also accounts for the time delay (mean transit time (MTT_deg), between this step and subsequent protein degradation, accounting for competing processes downstream of ternary complex engagement. The authors are thus able to show not only how binary and ternary kinetic rate constants effect ternary complex stability and concentration, but how Mtt_deg reflects what these values must be to result in protein degradation. The article then moves to demonstrating simulating how repeated *in vivo* dosing can drive down protein levels for target proteins with slow resynthesis rates, which has been experimentally supported by others.

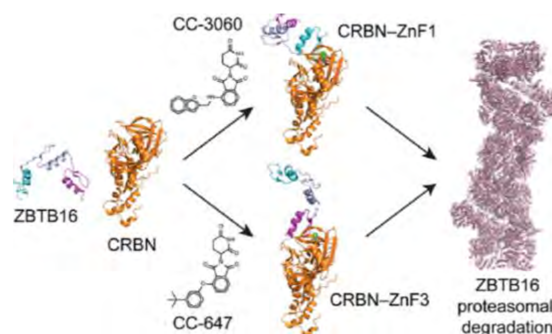
The authors rightly point to a dearth of detailed PK/PD modelling in the field of bispecific protein degradation and in that regard this work is illuminating, partly because it highlights the experimental parameters drug discovery practitioners in this area will be required to have a strong grasp of to enable efficient *in vivo* translation. Mtt_deg could be viewed as a mathematical descriptor of so-called 'ternary complex productivity' and understanding the relationship between this and ternary complex kinetics is shown to be vital for degrader design/prioritisation. The importance of understanding endogenous protein turnover rates is also highlighted with an interesting observation that the advantages of slow protein resynthesis for targeted protein degradation approaches contrast with genetic modalities such as siRNA which tend to be enabled by the opposite.

Contributor: Will

Cereblon Modulators Target ZBTB16 and Its Oncogenic Fusion Partners for Degradation via Distinct Structural Degrons

Mary E. Matyskiela[§],...., Joel W. Thompson*

ACS Chem. Biol. **2020**, DOI: 10.1021/acschembio.0c00674



In this study Matyskiela *et al*. at Bristol Myers Squibb (BMS) discover Pomalidomide-derived molecules capable of inducing CRBN-dependent degradation of the protein ZBTB16, a transcription factor. ZBTB16-Retinoic acid receptor alpha (RAR) chimeras are known to mediate rare forms of acute promyelocytic leukemia (APL) that are refractory to standard care and are shown here to be viable drug targets. Previous work by this group and others have detailed a range of C2H2 Zinc finger (ZnF)-containing proteins as CRBN neosubstrates whereby such neosubstrates are typically

characterised by a C2H2 ZnF with a glycine-containing ß-hairpin loop. ZBTB16 is the latest to be added to that list, both here by screening compounds in HT-1080 cells ectopically expressing ProLabel-tagged ZBTB16 as well as in another recent study by Yamanaka *et al.* (BioRXIV, 2020, DOI: 10.1101/2020.02.28.969071). In addition to showing partial degradation of ZBTB16 by Pomalidomide the authors also discover two derivatives, CC-3060 and CC-647 which show fuller and more potent effects. By cellular expression of point mutated full-length ZBZT16 constructs this work demonstrates the third ZnF domain (ZnF3) of the nine ZnFs present in ZBTB16 is a CRBN modulator structural degron for CC-647, whereas CC-3060-mediated degradation is driven predominantly through its first ZnF (ZnF1). Further, CC-647 is superior with respect to dose-dependent degradation of a RAR -ZBTB16 fusion which contains only the ZnF3-9 region, whereas CC-3060 is superior when it comes to degradation of a reciprocal ZBTB16-RAR fusion which contains only ZnF1 and ZnF2.

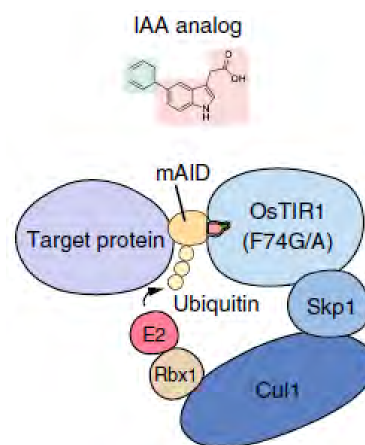
This is a compelling example of how CRBN modulators may expand the druggable proteome particularly with respect to transcription factors. This is a thorough study with regards to characterising the degradation behaviour of a novel CRBN neo-substrate with a clear therapeutic angle. It is worth noting the disconnect between *in vitro* and cellular preferences of the different ZBTB16 ZnFs to engage with CC-3060:CRBN and CC-647:CRBN complexes and the value shown here in following up with detailed cellular studies.

Contributor: Will

The Auxin-inducible degron 2 technology provides sharp degradation control in yeast, mammalian cells and mice

Aisha Yesbolatova[§],...., Masato T. Kanemaki*

Nat. Commun. **2020**, *11*, 5701



The well-established Auxin-inducible degron (AID) system is optimised in this disclosure of 'AID-2'. The AID system functions by expressing a protein of interest (POI) as a fusion with so-called mini-AID (mAID), a 7 kDa degron derived from *Arabidopsis* IAA17. When dosed with small molecule indole acetic acid (IAA) which is the most abundant natural auxin, in the presence of *Oryza Sativa* TIR1 (OsTIR1) which is capable of forming Skp1-Cul1-F-box E3 ligase complexes, proteasomal degradation of the POI is observed. Despite its utility, the AID system has some limitations including basal degradation of the POI-mAID fusions in the absence of IAA, as well as IAA itself which is often required to be dosed at concentrations in the 100-500 µM range. In this study the authors use a bump-and-hole approach, introducing an F47G mutation into the Auxin binding site of OsTIR1. Using an EGFP-tagged mAID reporter system, bumped IAA analogues were then screened revealing 5-Ph-IAA to be capable of inducing OsTIR1(F47G)-mediated degradation of the mAID reporter at 1 nM. This system is then applied to range of cellular and *in vivo* contexts demonstrating negligible basal degradation of POI-mAID constructs using the OsTIR1(F47G) system. Furthermore, this study shows that with a more potent and likely more permeable analogue in 5-Ph-IAA, robust *in vivo* efficacy can be achieved. This is demonstrated *via* the use of TOP2A and BRD4-mAID expressing HCT-116 cells grafted into nude mice. Tumour regressions/stasis are demonstrated with daily i.p. doses of <10 mg/kg.

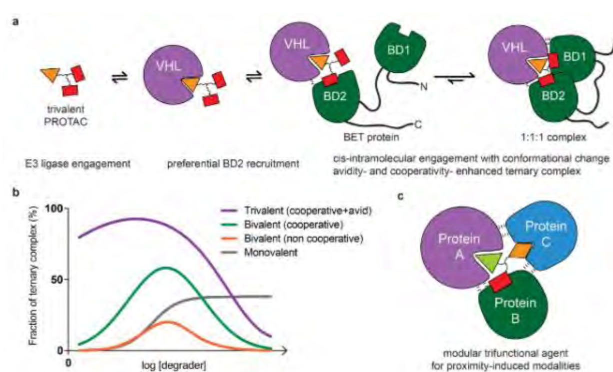
This study reveals an even more attractive and flexible tool for investigating the consequences of post-translational protein knockdown compared with AID. The progress shown with respect to speed of degradation, breadth of cell/species types and in particular the *in vivo* data for AID-2 show a significant enhancement of the approach. A caveat highlighted by the authors is that 5-Ph-IAA is unlikely to access the brain *in vivo* and so does limit *in vivo* studies to peripheral tissues.

Contributor: David

Trivalent PROTACs Enhance Protein Degradation Through Cooperativity and Avidity

Satomi Imaide,[§] … Danette L. Daniels*, Alessio Ciulli*

ChemRxiv **2020**, DOI: 10.26434/chemrxiv.13218695.v1



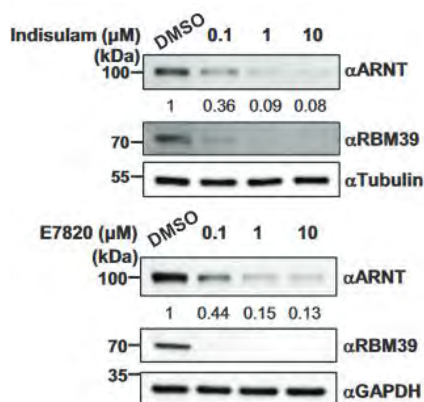
In a brand-new pre-print paper from our own group in collaboration with the group of Danette Daniels at Promega, Imaide *et al*. present a trivalent PROTAC as a promising new way to enhance PROTAC ternary complex structural recognition features through increased valency. The trivalent PROTAC SIM1 comprises a VHL binder and two BET bromodomain inhibitors connected via a branched linker. SIM1 was shown to engage both bromodomains of BET family proteins simultaneously forming a 1:1:1 complex with VHL. The ternary complex was stable and long lasting, resulting in fast degradation of the target BET proteins. Interestingly, SIM1 displayed faster degradation, and the most robust ubiquitination of BRD2 relative to the other BET proteins, a selectivity which is unprecedented for this class of BET PROTAC degraders. Despite the increased size of the trivalent PROTAC SIM1 relative to the constituent and analogous bivalent PROTAC MZ1 and bivalent inhibitor MT1, SIM1 was shown to be readily cell permeable, allowing it to enter cells and outperform parent bivalent BET inhibitors and PROTACs in relevant cellular disease assays. Imaide *et al*. also outline a new modular linker design strategy to help facilitate the synthesis of future trivalent PROTACs. While this paper focussed on degrading a target with two repeated binding domains, Imaide *et al*. highlight the possibility of using this mode of action to target proteins with distinct domains, or even two distinct proteins. Together, the results presented in this paper convincingly provide proof of concept that the PROTAC field should not consider themselves limited to bivalent PROTACs alone.

Contributor: David

Aryl Sulfonamides Induce Degradation of Aryl Hydrocarbon Receptor Nuclear Translocator through CRL4^{DCAF15} E3 Ligase

Sung Ah Kim,[§] … Boyung Chul Park,* Sunhong Kim,* and Jeong-Hoon Kim*

Mol. Cells **2020**, *43*, 935



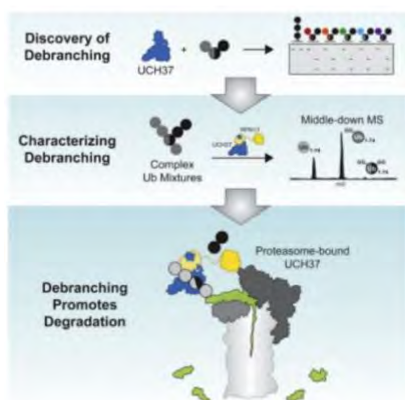
Aryl hydrocarbon receptor nuclear translocator (ARNT, also known as HIF1β) is critical to the cell's ability to respond to environmental stress such as hypoxia or xenobiotic exposure. Due to its vital role in responding to hypoxic conditions, the HIF pathway is an attractive target for anti-cancer therapeutics, although small molecules that can effectively target the HIF pathway have yet to be identified. Aryl sulfonamides have recently garnered significant interest due to their anti-cancer activity through induction of G1 cell cycle arrest leading to cell death. This anti-cancer ability has previously been attributed to aryl sulfonamides acting as a molecular glue, facilitating ternary complex formation between the E3 ligase substrate receptor DCAF15 and RNA-binding proteins RBM39 and RBM23. Kim *et al.* identified ARNT as an additional interacting protein of DCAF15 and showed that aryl sulfonamides promote proteosomal degradation of ARNT through CRL4^{DCAF15}. ARNT was identified as an interacting partner of DCAF15 by co-immunoprecipitating endogenous ARNT with overexpressed DCAF15. Treatment with aryl sulfonamides indisulam and E7820 induced degradation of ARNT in a proteosome-dependent manner. By indicating a new potential HIF-pathway target Kim *et al.* extend the potential therapeutic application of aryl sulfonamides.

Contributor: David

Proteasome-Bound UCH37/UCHL5 Debranches Ubiquitin Chains to Promote Degradation

Kirandeep Deol,[§] … Eric Strieter*

Mol. Cell **2020**, *80*, 796



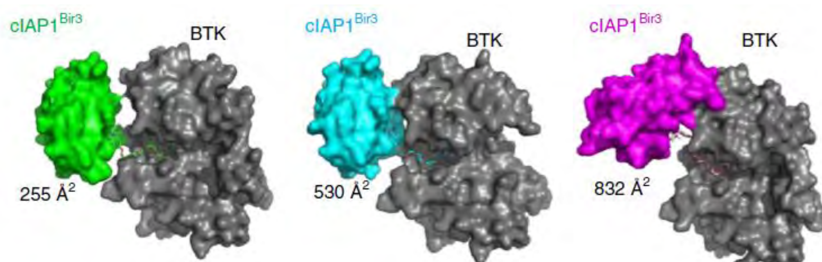
Strieter *et al.* present a study that expands on our understanding of the wider ubiquitination system. The architecture, linkage and length of ubiquitin chains are all important variables in the cells ability to maintain tight control over protein levels. The proteins that selectively recognise and process branched ubiquitin chains were hereto unknown, however, Deol *et al*. identify Ubiquitin C-terminal hydrolase 37 (UCH37) as a deubiquitinase able to selectively cleave K48 branched ubiquitin chains. Deol *et al.* go on to show that debranching of ubiquitin chains promotes degradation of the ubiquitinated substrates. Proteome-wide pulse-chase experiments show that loss of UCH37 activity negatively impacts on global protein turnover. The activity of UCH37 is shown to be greatly enhanced by binding in the context of the proteasome, or when binding to the proteasomal ubiquitin receptor RPN13. Together, this study presents the importance of UCH37 as a debranching deubiquitinase in the promotion of proteasomal degradation.

Contributor: David

Snapshots and ensembles of BTK and cIAP1 protein degrader ternary complexes

James Schiemer, [§] … Matthew Calabrese*

Nat. Chem. Biol. **2020**, DOI: 10.1038/s41589-020-00686-2



Currently VHL, cereblon and cIAP are the three E3 ligases most commonly utilised in PROTAC studies. Prior to this publication, PROTAC ternary crystal structures were only publicly available for complexes containing VHL or cereblon as the E3 ligase. Due to the immense value that structure-based design provides towards optimising small molecules including PROTACs, it is particularly exciting that Schiemer *et al*. present in this paper the first reported crystal structures of a cIAP-PROTAC ternary complex, in this case with the kinase BTK and the PROTAC BC5P. Schiemer *et al.* reveal three distinct binding modes observed in different asymmetric units within their cIAP-BC5P-BTK ternary crystal structure indicating high flexibility, which is in agreement with solution state NMR experiments as well as the fact that BC5P is a non-cooperative PROTAC ($\alpha \approx 1$) as measured by BLI experiments. A second homologous PROTAC with a shorter linker (BCPyr) was also produced, revealing increased cooperativity (α from 2.6 to 30). A second crystal structure comprising cIAP, BCPyr and BTK is also presented, showing only a single binding mode throughout the crystal asymmetric units. Interestingly, despite BCPyr showing modestly improved ternary affinity and greater cooperativity relative to BC5P, BCPyr was found to be a worse degrader of BTK than BC5P (BTK DC50 = 800 ± 94 nM and 182 ± 57 nM, respectively). The authors acknowledge that this disconnect between apparent ternary complex stability and degradation activity is counter to what might be expected and hypothesise that holding a complex tightly in an unproductive confirmation could be less effective at facilitating ubiquitination than tethering a complex together loosely. Schiemer *et al*. also recognise that biophysical experiments using minimal systems such as ligand binding domains only may not be able to fully reproduce the complexities of the ternary complex in a cellular environment.

Others

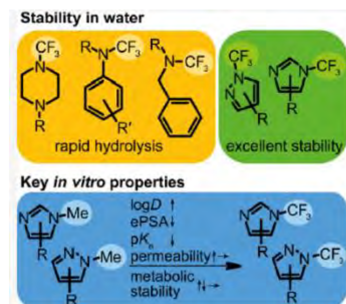
Contributor: Emelyne

N-Trifluoromethyl Amines and Azoles: An Underexplored Functional Group in the Medicinal Chemist's Toolbox

Stefan Schiesser,[§] …, Rhona J. Cox*

J. Med. Chem. **2020**, *63*, 13076

The introduction of a trifluoromethyl is a common strategy to modulate the properties of active entities. A team of scientists at AstraZeneca has synthesized *N*-trifluoromethyl amines and azoles. Those motifs are rarely encountered in molecules from the drug discovery pipeline, while trifluoromethyl attached to a carbon or an oxygen is quite often represented. This can be explained by the synthetic challenges to obtain this motif. But recent progress has been made. The remaining question was to investigate their *in vitro* properties (stability in aqueous solution, pK_a, permeability).



The group synthesized representative molecules. This study found out that *N*-trifluoromethyl amines might only be suitable in specific drugs, as they show very low stability in aqueous media with the by-product of hydrolysis, *N*-carbamoyl fluoride, being able to react with physiological nucleophiles, therefore raising concerns as to drug safety and specificity. On the other hand, *N*-trifluoromethyl azoles, showed excellent stability in aqueous solution. Changes in log D and pK_a were observed. Changes in permeabilities and metabolic stabilities were dependant on the compounds. The investigations also yielded that an *N*-trifluoromethyl azole can serve as a bioisostere to *N*-*iso*-propyl and *N*-*tert*-butyl azoles.

This paper is a nice addition to the publications on underused moieties that deserve to be added to the medicinal chemist's toolbox (*Angew. Chem. Int. Ed.* **2013**, *52*, 9399–9408, *Eur. J. Med. Chem.* **2017**, *126*, 225–245, *Org. Chem. Front.* **2019**, *6*, 1319–1324, *J. Med. Chem.* **2020**, *63*, 7081–7107.)

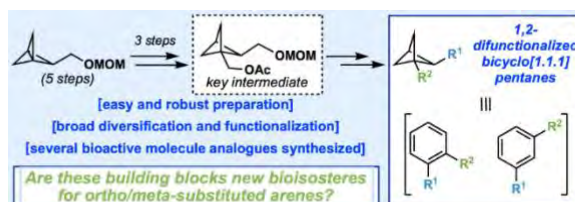
Contributor: Siying

1,2-Difunctionalized Bicyclo[1.1.1]pentanes: Long Sought After Bioisosteres for ortho/meta-Substituted Arenes

Jin-Xin Zhao,[§] …, Phil S. Baran*

ChemRxiv **2020***,* DOI: chemrxiv.13120283.v1

Scientists from Phil Baran's laboratory and Pfizer reported the preparation of a 1,2-disubstituted bicyclopentanes (BCP) building block ('key intermediate' in the diagram) and the application of this building block in the synthesis of several drug bioisosteres.



Building upon the literature-reported synthesis of the propellane with a methoxy methyl (MOM), the authors generated the key 1,2-disubstituted BCP in three steps. The key intermediate offers great synthetic versatility: in addition to the standard reaction conditions routinely used in medicinal chemistry (e.g. oxidation to

aldehyde/carboxylic acid, amide coupling, reductive amination), the application of decarboxylative functionalisation methodologies emerging from recent literature enables the introduction of aryl moieties, as well as pinacol boronic esters which are super-versatile reaction handles.

The authors demonstrated the synthetic utility of the key 1,2-disubstituted BCP intermediate by synthesising several drug bioisosteres and simple molecular matched pairs. Studies to validate the use of 1,2-disubstituted BCP as bioisosteres for ortho/meta-substituted arenes are currently ongoing.

Although the synthesis of the intermediate looks straightforward, further optimisation of the synthetic procedures to reduce step counts and improve yields would encourage the use of this intermediate in the community. The BCP building block reported in this paper could be useful in the synthesis of conformationally constrained PROTAC linkers.

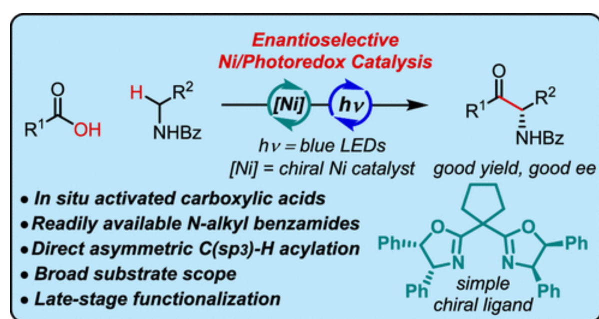
Contributor: Tasuku

Direct Enantioselective C(sp^3)–H Acylation for the Synthesis of α-Amino Ketones

Xiaomin Shu,[§] Leitao Huan,[§] Qian Huang, Haohua Huo*

J. Am. Chem. Soc. **2020**, *142*, 19058

α-aminoketones are one of the most common chemical motifs found in bioactive compounds, but direct and robust methods to afford α-aminoketones are limited. In this paper, the authors developed a direct and enantioselective C(sp^3)–H acylation reaction to afford α-aminoketones by *in situ* activation of carboxylic acids and following nickel/photoredox-catalyzed coupling with α-amino radical species. This reaction proceeds in relatively mild conditions and can be applied to a broad spectrum of substrates with moderate to high yield and high enantioselectivity. In addition, the authors demonstrated the application of this reaction for late-stage functionalization of natural products and bioactive compounds. Varieties of carboxylic acids and benzoyl protected primary amines can be used for this reaction, electing it as one of the best reactions to establish chiral α-aminoketones.



The reaction condition they developed is somewhat unique (e.g. 1 equiv. of ammonium chloride and sodium hydrogen phosphate, isopropyl acetate as a solvent, 15 °C), so it seems the authors optimized the reaction conditions well.