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

Ciulli Lab
Targeted Protein Degradation

University of Dundee

Journal Club

November 2021

Contents

Meet this Month's Editors

This month's editors are (from left to right): Valentina Spiteri, Shakil Khan and Alena Kroupova.

"Having been fond of this Journal Club before I ever joined the Ciulli group, so I was very excited to have the chance to contribute to this amazing resource."

Valentina obtained her BSc in Biochemistry at the University of Surrey in 2016 and her PhD in Structural and Molecular Biology at University College London under the supervision of Prof Stephen Perkins and Prof Paul Dalby in 2021. In August 2021 Valentina joined the PROTAC collaboration with Boehringer Ingelheim as a structural biologist and biophysicist.

"The Journal Club has kept me up-to-date with the latest discoveries in the developing PROTAC field, therefore participating in putting this resource together was a joy."

Shakil joined the Ciulli group as a Cell Biologist as part of the PROTAC collaboration with Boehringer Ingelheim. Shakil completed his master's degree in Drug Discovery at the University of Aberdeen before pursuing a PhD at Aston University.

"I enjoyed putting together the journal club and the purpose it brings to my perusing of the targeted protein degradation literature."

Alena completed her undergraduate studies in Medicinal and Biological Chemistry at the University of Edinburgh followed by a PhD in Structural Biology in the lab of Prof. Martin Jinek at the University of Zurich in Switzerland. In September 2021 she joined the exciting PROTAC field as a structural biologist/biophysicist within the Ciulli group – Almirall collaboration.

Feature of the Month

The Contergan scandal – a TPD perspective

Contributor: Kevin

This journal club usually aims to project an optimistic picture of the bright future that lies ahead of the TPD field. In this feature, however, we look back at the events of the last weeks of the year 1961, when a German newspaper brought to light the devastating effects of thalidomide, a molecule central to our field, and thereby one of the biggest scandals in modern medicine. We will give a brief account of the science and history of the Contergan scandal and discuss its implications for the modern TPD field.

Thalidomide was first synthesized in 1954 at the German pharma company Chemie Grünenthal. While early animal experiments in mice showed no adverse effects even at very high doses, they also failed to show any medically exploitable properties. Intrigued by the apparent lack of toxicity, Grünenthal nevertheless regarded thalidomide as a cure in need for a treatable condition and distributed samples among doctors in Switzerland and Germany with no indicated use. This was possible as approval of a drug at the time only required limited data showing safety in animal models, not efficacy. Thalidomide was first applied for the treatment of epilepsy, where it proved unsuccessful. Patients however reported its calming and sleep-inducing side effects. Although animal experiments showed no evidence for a sedative effect, Grünenthal recognized a potential market for a safe, non-addictive alternative to barbiturates and in October 1957 introduced it under the tradename Contergan as an over-the-counter drug to the German market. Contergan became a commercial success, being outsold only by aspirin and making up more than half of Grünenthal's revenue. This acted as a strong incentive to ignore early warning signs of neurotoxicity in patients and aggressively market Contergan even to pregnant women, where it was found effective against morning sickness.

Following the introduction of thalidomide, the number of children born with missing or malformed limbs and severe inner organ damage increased sharply. However, after the systematic murder of disabled people in the Third Reich, there was widespread concern about the central registration of birth defects in Germany and so this increase remained undetected until the end of 1960. It took almost another year until September 1961 for the physicians Widukind Lenz in Germany and William McBride in Australia to independently identify thalidomide as the cause of this epidemy in birth defects. Grünenthal was alerted by Lenz but denied any connection and refused to suspend the sale of Contergan. Only when several German newspapers picked up the story and made the allegations known to a wider audience, Contergan was withdrawn from the market on the 27th of November 1961. In the following years clear evidence for the teratogenic effect of thalidomide in rabbits and primates, but not mice, emerged, one of the first examples of inter-species differences in drug response.

By that time, thalidomide had been approved for use in at least 46 countries, including the UK, Canada and Australia. It is estimated that more than 10,000 children were born with thalidomide-induced birth defects. Only about 5,000 of them survived into adulthood. Additionally, around 40,000 adult patients suffered from neuropathies following thalidomide treatment. Thalidomide has also caused an unknown number of miscarriages.

The Contergan scandal prompted changes to the drug approval process, leading to the system we know today that requires proof of safety and efficacy from animal models and extensive clinical trials. The USA, where thalidomide had not previously been approved by the FDA on account of concerns over its neurotoxicity and lack of data on efficacy in animal models, took a lead here. The manufacturer Grünenthal and international distributors were mostly spared from legal consequences. Several Grünenthal officials were charged with negligent homicide and injury, but the case was settled out of court in 1970. As part of the settlement, Grünenthal contributed to a foundation for the support of Contergan victims, that however, received most of its funding from the German government. Similar settlements were reached in other countries, including the UK. It was only in 2012, that Grünenthal issued an apology to the victims of thalidomide. The company still denies that it could have anticipated the side-effects of thalidomide, a questionable claim given early evidence for thalidomide's neurotoxicity and the serious concerns brought forward by the FDA.

While the public still remembers thalidomide as the cause for one of the biggest disasters in modern medicine, its story took an unexpected turn only three years after its withdrawal from the market; in a French hospital, physician Jacob Sheskin attended a patient suffering from severe leprosy. Feeling he had nothing to lose he administered Contergan in a desperate attempt to mediate the pain of the dying patient and allow him to find some sleep. The

effect of thalidomide far exceeded Sheskin's expectations: not only did the patient sleep, but he made a significant recovery. Thalidomide's effectiveness in the treatment of leprosy was confirmed in clinical trials and it remained essential for the treatment of leprosy until the 2000s, when drugs with less severe side-effects became available. By this time thalidomide had been found effective in the treatment of other severe diseases including Morbus Crohn, the wasting syndrome in AIDS patients and multiple myeloma. As a result of this, in 1998 it finally gained FDA approval and in 2008 was once again approved for restricted use in the EU and UK. Its derivatives, lenalidomide and pomalidomide, became two of the most successful cancer drugs of the 2010s.

It was not until 2018, that the mechanism of thalidomide and its derivatives was resolved. They are what we now call "molecular glues", recruiting several transcription factors as neo-substrates to the E3 ligase Cereblon and mediating their degradation. Thalidomide was therefore the first inadvertent TPD drug. As the TPD field produces more and more molecules that employ a similar mechanism, it is critical that we are mindful of thalidomide's history and the memories it has left in the public consciousness. We must demonstrate to the public that we have learned our lesson, point out the factors that lead to the Contergan scandal and show the safety measures in place in modern drug development. While we must acknowledge the suffering of the victims of thalidomide treatment and support their ongoing fight for justice, we should also emphasize that thalidomide was one of the most important and versatile drugs of the 20th century, giving humanity an effective weapon against illnesses that have plagued it for centuries, extending and improving the lives of millions.

Ode to a science heroine

Contributor: Valentina

Dr Frances Kathleen Oldham Kelsey had her work cut out for her when she joined the United States Food and Drug Administration (FDA) in 1960 as part of a shoestring team of only seven full-time physicians. She was tasked with reviewing Richardson-Merrell's application for thalidomide, which was to be marketed as a treatment for morning sickness, amongst other indications. At this time the drug had already been approved across Europe and Canada, and she was facing immense pressure to approve the drug for use in the US. However, Dr Kelsey refused and asked for more tests, along with conclusive evidence that the drug did not cause harm to the unborn child. Her judgment was quickly proven sound after the story broke in Europe, and her stalling no doubt helped avert a similar crisis in the US. Moreover, her approach had a significant impact on the future direction of the FDA and helped to build its reputation as the foremost authority on drug safety. Dr Kelsey has since been hailed a heroine and received many accolades including the President's Award for Distinguished Federal Civilian Service from President John F. Kennedy. However, being as shrewd as she was, she understood that such praise is fickle; she remarked that had her assessment been proven incorrect, she would have been considered "unreasonable". Dr Kelsey fully understood the weight of her responsibility as the arbiter of what may ultimately enter people's bodies. As drug discovery scientists we are in the humbling position to be able to develop new therapeutics to help people and save lives; but we must never forget to be vigilant against the unforeseen risks these drugs may pose. This is especially true in a time when there is still so much ground to cover and so much trust to win. Frances lived a long life and passed away in 2015 at the age of 101.

Further reading: An article from the Washington Post published in 1962 describing the story.

The 1st Annual CeTPD Christmas Retreat

Photo credits: Tasuku and Kevin. Contributor: Valentina

The group held their 1st Annual CeTPD Christmas Retreat on the 25th of November at Piperdam Leisure Resort. The evening was not only a party, but also a chance to take stock of how far we've come this last year, and what we can be excited for in the next. We had much to celebrate; 19 new lab members, 14 publications and the new Centre for Targeted Protein Degradation, which will officially open in 2022. Alessio gave the party a status update for the Centre and gave special thanks for the work of the many people who are busy behind the scenes making all of this possible, including Anne Muir and Rachel Simpson, from the University's Research and Innovation Services; and Letty Gibson, the Research School Services Manager.

Over the course of the evening we had several presentations: Will updated us on the progress made by the Boehringer Ingelheim collaboration team, David on that of the Almirall collaboration team, and Ryan updated on the goings on of the academic group. Adam highlighted the key publications which have resulted from the research of these groups. Charlotte then gave us an overview of the achievements of this Journal Club and Valentina introduced the new CeTPD Seminar Series. Scott Hughes and Andrea Testa, from Amphista joined us for the evening and told us about life at a start-up. We all enjoyed a quiz, hosted by Angus and Tom, and topped off the evening with several hours of dancing!

Behind-the-scenes heroines: Anne and Rachel

Huge thanks to organisers: Diane, Vesna, Selma and Manjula

Amphista Wins Scrip Award!
Contributor: Alessio

Awards news this month, Amphista Therapeutics, the fast-growing TPD company that spun-out from our lab, was awarded the 'Financing Deal of the Year' award 2021 at the Scrip Awards, announced on Thursday 2nd December. I was fortunate to be a guest at the Award Ceremony on the evening, with Amphista's CEO Nicki Thompson and Director of Strategic Projects Diane Simmons amongst the company's guests (pictured below).

This recognition reflects the incredible work the Amphista team has put in and the amazing progress achieved over the past year, resulting in an oversubscribed $53M Series B funding round. The financing, which was announced in March this year, has helped Amphista advance their next-generation targeted protein degradation assets. This prestigious award marks an accolade not just for the company, but for us all in the University of Dundee where the company scientific foundations were built, as well as Scotland at large. It is an outstanding example of just how great an entrepreneurial and translational impact our research can have. Congratulations again to the Amphista team, and to all the other nominees in the same category for all their amazing achievements and raises this year!

(Pictured from left to right: Lucie Ellis-Taitt, Alessio Ciulli, Nicki Thompson, and Amol Rajan; Diane Simmons, Nicki and Alessio.)

Landmark Paper

Contributor: Valentina

Isolation of a Polypeptide That Has Lymphocyte-Differentiating Properties and Is Probably Represented Universally in Living Cells

Gideon Goldstein[§], …, Hugh D. Niall*

PNAS **1975**, *72*, 11

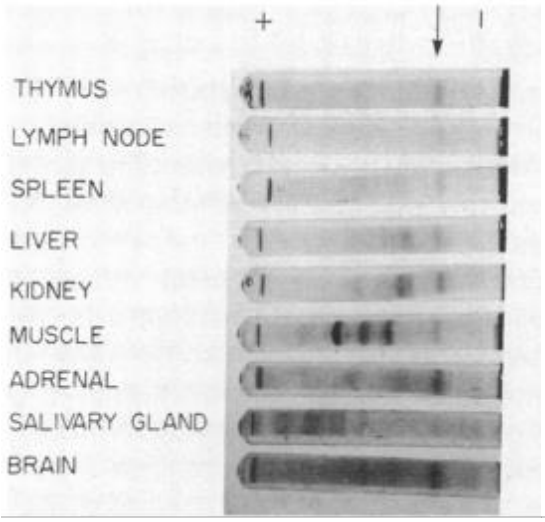


TABLE 3. *UBIP levels (μg/g) in various tissues and cells as measured by radioimmunoassay*

Guinea pig: lymph node 125, spleen 100, liver 100, kidney 65, muscle 35, salivary gland 25, thymus 5. *Mouse, outbred:* muscle 3, kidney 15, liver 4, ASCI (T cell leukemia) 4, RADI (T cell leukemia) 50, 3T3 fibroblasts (tissue culture) 2, SV101 fibroblasts (tissue culture, transformed) 32. *Mouse (nu/nu), athymic:* muscle 30, kidney 15, liver 5. *Chicken:* 10 day embryo 22, 13 day embryo 45, fibroblasts (tissue culture) 32. *Hamster,* BHK kidney (tissue culture) 10. *Fish (perch)* muscle 18. *Squid* muscle 100. *Celery* stalk 0.11. *Eggplant* fruit 0.05. *Carrot* root 0.14. *Pear* fruit 0.05. *Apple* fruit <0.05.
Yeast, brewers bottom 10. *Candida utilis (torula)* 10. *Escherichia coli* (ATCC 8739) 1.2, strain W (ATCC 9637) 1.8, strain B (ATCC 11303) 0.3, strain K12 0.3. *Bacillus subtilis* (ATCC 6633) 0.4. *Clostridium histolyticum* (ATCC 8034) <0.05. *Clostridium kluyveri* (grown on synthetic media) 0.2.

This Goldstein et al. 1975 paper is the first to report the discovery of ubiquitin. Gideon Goldstein was trying to isolate thymopoietin from bovine thymus and when he ran into an 8,500 Da polypeptide, he dubbed it ubiquitous immunoprotein polypeptide (UBIP), giving ubiquitin its first moniker. Following the isolation of the 76-residue polypeptide, Goldstein *et al.* were able to detect the same polypeptide in many other tissues and bacterial strains (see table and figure above). The authors conclude that UBIP can induce the differentiation of T and B cells, independently of thymopoietin. Although they do concede that this is likely to be limited to *in vitro* studies and anticipated that the same findings would not extend to *in vivo* assays, as athymic mice have many pro-thymocytes and lack T cells but had normal levels of UBIP, therefore UBIP could not be responsible for differentiation. The authors convey their confusion in the conclusion of the paper and ask the reader, in a scientific breaking of the 4th wall, "what, then, is the physiologic function of UBIP, since UBIP from such phylogenetically disparate sources as mammals and plants show such uniformity by structural and functional, and immunological criteria?"

Jumping into the future, we now know that ubiquitin plays no direct role in immunocyte differentiation, and this mystery was solved in later work by Allan Goldstein who was able to attribute this thymopoietic activity to an endotoxin contamination. We also know that prokaryotes do not express ubiquitin, and the presence in bacteria was a result of contamination of the bacteria by yeast extract in the media, and when grown in synthetic media ubiquitin was no longer present (explained in an interview of Prof Ciechanover, 2005). The name ubiquitin is therefore now, somewhat of a misnomer, that owes its persistence to the long-observed scientific tradition of awarding naming rights to the discoverer of a novel protein, often to the chagrin of students of the biological sciences.

Gideon Goldstein shared some of his ubiquitin samples with Keith D. Wilkinson and in 1980 Wilkinson et al., were able to show that ATP-dependent proteolysis factor 1 (APF-1), a key component of the ATP-dependent proteolytic system (Ciechanover, A., Hod, Y., and Hershko. A., 1978), and ubiquitin were one and the same (Wilkinson et al., 1980).

Goldstein et al., 1975 point out that their "recognition of UBIP was incidental to a study on the thymus which had quite a different objective", but they were piqued by UBIP's phylogenetic conservation. Despite its many twists and turns, ultimately this story lends itself to thinking about the human side of research, the person at the bench, the one that runs into problems and decides if they are worth pursuing. We often have discussions on projects in the group on

whether to risk falling down a rabbit hole, but you just never know what might be waiting for you when you dive in, a Cheshire cat with a toothy grin, mocking you for trying, or the discovery of ubiquitin?

Further reading: Ubiquitin: A Nobel Prize. Essay by Keith D. Wilkinson.
The discovery of ubiquitin-dependent proteolysis. Essay by Keith D. Wilkinson.

Computational Chemistry | Structural Biology/Biophysics

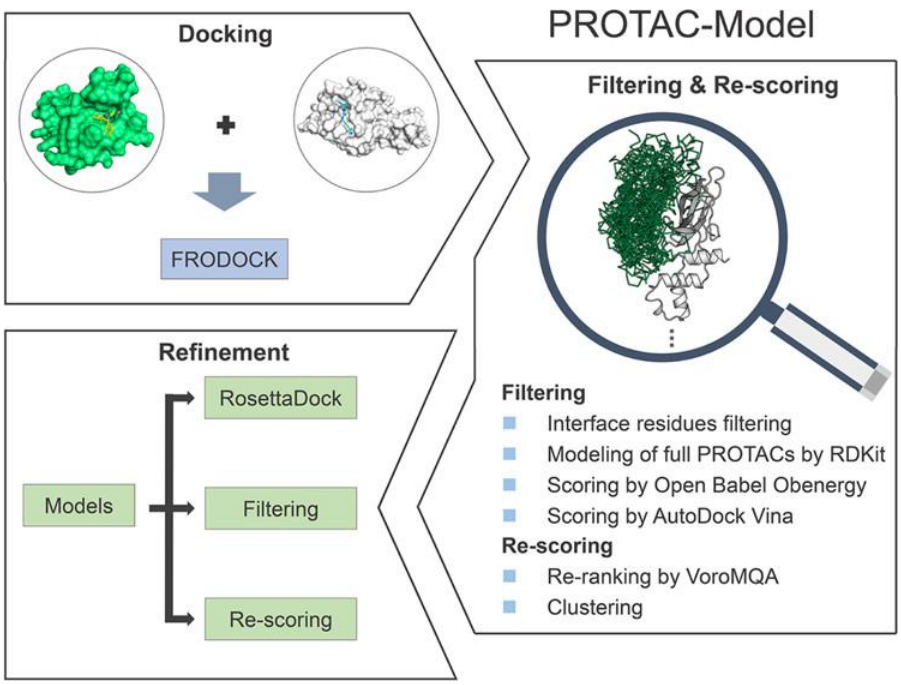
Contributor: Valentina

Integrative Modeling of PROTAC-Mediated Ternary Complexes

Gaoqi Weng§, …, Yu Kang*, Tingjun Hou*

J. Med. Chem. **2021**, *64*, 16271-16281

In this work the authors present PROTAC-Model, a computational method that combines the FRODOCK-based docking protocol, RosettaDock for structural refinement, and RDkit for modelling PROTAC conformations, with the aim of predicting ternary complexes. To gauge the quality of the predicted models a critical assessment of predicted interactions (CAPRI) method was employed to categorise models into acceptable, medium, and high quality. The method was tested on 14 PROTAC-mediated ternary structures found on the PDB and found that medium- or high-quality predictions was achieved in 12 cases.



The authors demonstrate that their methodology performs better than PRosettaC and the method developed by Drummond *et al*. However, despite the ability of the method to robustly produce high quality predictions it struggled in cases where ternary complexes had a high degree of flexibility with a limited number of contacts. The method was also weaker in predicting cereblon based ternary structures, which the authors judge to be due to larger discrepancies between bound and unbound structures. At present, there is a paucity of structural data available for ternary complexes so predictive methods can only be evaluated against a limited dataset. Perhaps, a future itineration of this protocol can include structural restraints derived from techniques such as HDX-MS to help further narrow down the search.

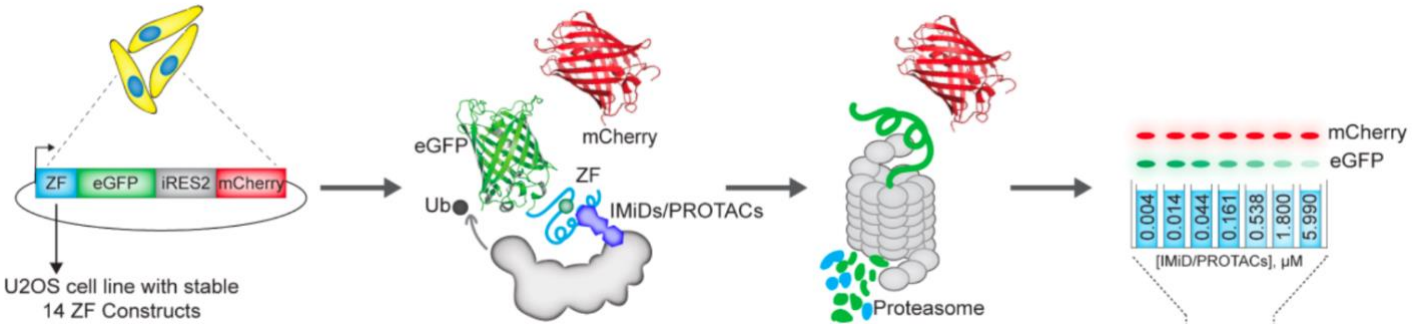
Chemistry | Cell Biology

Contributor: Valentina

Proteolysis Targeting Chimeras with Reduced Off-targets

Tuan M. Nguyen[§], Arghya Deb[§], Praveen Kokkonda[§], Vedagopuram Sreekanth[§], …, Amit Choudhary*

BioRxiv **2021**, DOI: 10.1101/2021.11.18.468552



Pomalidomide is a widely exploited E3 ligase binder that is associated with neo-substrate activity, in particular several zinc-finger containing proteins. Nguyen *et al.* developed a high-throughput automated imaging assay that interrogates the off-target degradation of zinc finger domains. The assay included the zinc finger degrons of 11 zinc finger proteins that were reported to be degraded by pomalidomide, and three zinc finger protein degrons that were not degraded by pomalidomide which were included as controls. The zinc finger degrons were incorporated into a lentivirus degradation reporter vector to compare the fluorescence of zinc finger-tagged eGFP to untagged mCherry. Compounds were tested against 14 stable U20S cell lines and found that the automated imaging assay agreed well with corresponding proteomics data.

From their data, the authors were also able to derive an understanding of which PROTAC structural features are most amenable to engage in off-target activity. They identified that PROTACs with common exit vectors including arylamine, -ether, -carbon and -amide were more susceptible to degrading zinc finger domains. Additionally, PROTACs with linkers on the 4th position of the aryl ring induces significant off-target degradation of zinc finger domains.

Armed with this insight the authors were able to reengineer the potent ALK PROTAC MS4078 by introducing alkyne exit vectors to reduce off-target degradation and achieve a 1.8-2-fold higher target potency after systematic linker length optimisation.

A limitation of this assay is that it is designed to be sensitive to pomalidomide based PROTACs and not the other IMiD analogs. Despite this, the assay has several advantages to conventional mass spectrometry-based methods, namely that it is not limited by cell type specific expression levels and does not depend on the zinc finger being disengaged from its native protein complex. Moreover, given the expense of proteomics experiments this high-throughput assay to identify key off target activity is no doubt a welcome addition to the field.

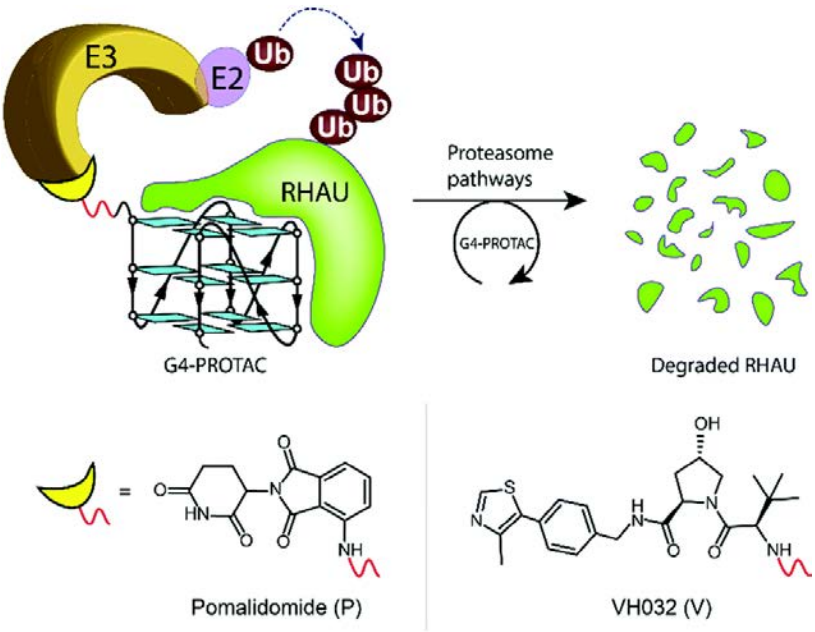
Chemistry | Cell Biology

Contributor: Alena

G4-PROTAC: Targeted Degradation of a G-quadruplex Binding Protein

Kiran M. Patil[§], …, Anh Tuân Phan*

ChemComm **2021**, DOI: https://doi.org/10.1039/D1CC05025G



G-quadruplexes (G4s) are nucleic acid secondary structures composed of multiple planar guanine tetrads stacked on top of each other forming a four-stranded helix. Their importance has been established in various cellular processes including maintaining the length of telomeric ends or gene regulation. Consequently, G4s and G4-binding proteins have been implicated in several disorders.

Pomalidomide (P)

VH032 (V)

In this study, Patil *et al.* developed PROTACs targeting the G4-binding protein RHAU, a therapeutically relevant DEAH-box helicase. Their G4-PROTACs consist of a standard E3 ligase-binding moiety linked to a G-quadruplex-forming DNA sequence recognized by the protein of interest. They show that both cereblon and VHL-based G4-PROTACs successfully degrade RHAU in a dose-, time- and proteasome-dependent manner.

This proof-of-concept study extends the PROTAC technology from the traditional small-molecule binders to target proteins based on their nucleic acid substrate, and in particular its secondary structure rather than sequence specificity. Although further studies will need to establish the specificity of G-quadruplex binders especially for therapeutic use, G4-PROTACs represent a novel tool to study G4-interacting partners in cells. This is an innovative study that broadens the scope of the PROTAC field.

Chemistry | Cell Biology

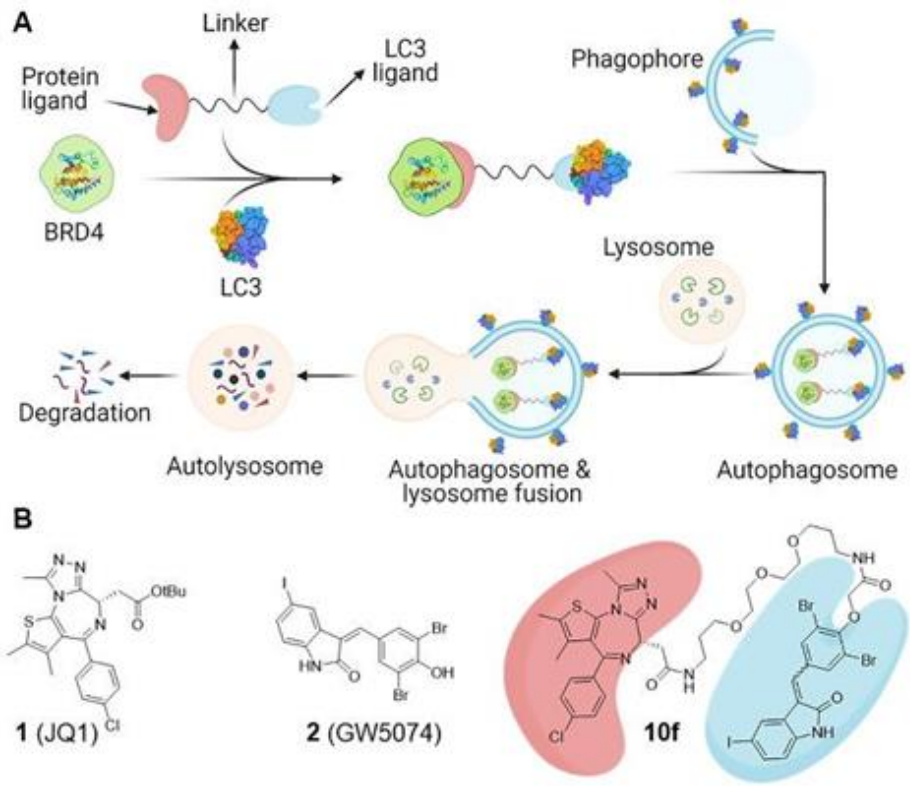
Contributor: Valentina

Developing potent LC3-targeting AUTACs tools for protein degradation with selective autophagy

Fir Junping Pei [§], Xiaoli Pan [§], Aoxue Wang [§], …, Guan Wang*, Liang Ouyang*

Chem.Comm. **2021**, DOI: https://doi.org/10.1039/D1CC04661F

Pei et al., have developed a novel autophagy-targeting chimera (AUTAC), 10f, that degrades BRD4 by autophagy key protein, LC3. 10f had over 80% and 99% degradation efficiency for BRD4 in several TNBC and MDA-MB-231 cell lines, respectively. BRD4 degradation was observed from two hours, and complete degradation was evident after four hours. 10f promoted dose-dependent apoptosis in MDA-MS-231 cells. The authors were able to confirm that 10f induces cell apoptosis via autophagy through a series of control experiments. Moreover, they demonstrated that 10f can degrade BRD4 without influencing overall autophagic flux. Typically, autophagy is viewed as a non-selective process, however the authors were able to demonstrate target specificity for BRD4.



This work adds to the ever-growing targeted protein degradation arsenal and demonstrates that there are feasible opportunities to target clinically relevant targets with autophagy-based degradation.

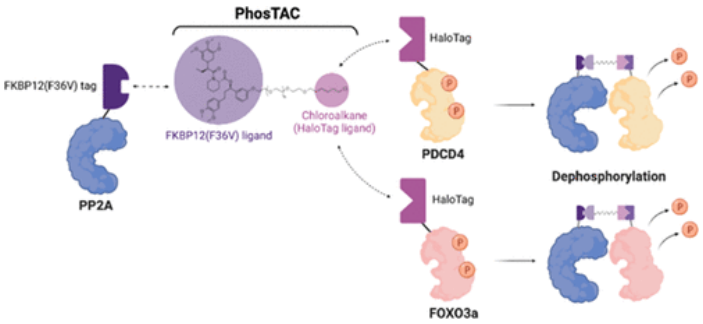
Chemistry | Cell Biology

Contributor: Valentina

Modulation of Phosphoprotein Activity by Phosphorylation Targeting Chimeras (PhosTACs).

Po-Han Chen[§], Zhenyi Hu[§], ..., Craig M. Crews*

ACS Chemical Biology **2021**, DOI: https://doi.org/10.1021/acschembio.1c00693



This proof-of-concept study describes phosphorylation targeting chimeras (PhosTACs), a novel class of targeting chimeras that aim to manipulate protein phosphorylation. PhosTACs induce ternary complexes of a Ser/Thr phosphatase to a phosphosubstrate, which is dephosphorylated. Protein phosphorylation is a dynamic process that is critical in regulating cell function, mediated by kinases (phosphorylate proteins) and phosphatases (dephosphorylate proteins). Dysregulation in phosphorylation has been implicated in many diseases and inhibitors targeting kinases have long been an area of intense focus in drug discovery. However, the efficacy of traditional inhibitors is often encumbered by acquired resistance.

PhosTACs were designed with a FKBP12(F36V) ligand, that binds to FKBP12(F3V) tag fused to a phosphatase in this case PP2A, a PEG linker with various lengths (2-7) and a chloroalkane to bind to the HaloTag fused to the target protein. PDCD4 and FOXO3a PP2A A/ PDCD4 stably expressing cells were treated with PhosTACs with different linker lengths, and this was followed by a halotrap pulldown assay to confirm ternary complex formation, and determined that PhosTACs with longer linkers, PhosTACs6 and 7 more favourably induced ternary complexes. PhosTAC-7 was able to induce a maximum of 90% dephosphorylation of PDCD4 at 16 h of incubation. For FOXOa, 30% dephosphorylation (when normalised to its total protein level) was reported and this led to transcriptional activation of a FOXO3a-responsive reporter gene.

This is an excellent study that goes beyond the protein degradation paradigm and opens the door for more research to focus on more precisely tuning protein activity. There will no doubt be tremendous interest from the community to follow the progress of this work as it moves into the next stages of development.

Chemistry

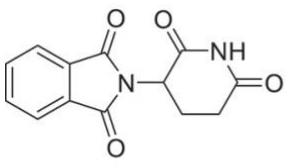
Contributor: Alena

Ligands for Cereblon: 2017-2021 Patent Overview

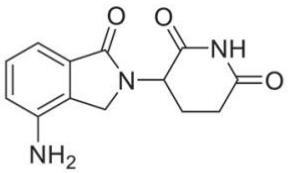
Alexander Kazantsev[§], Mikhail Krasavin*

Expert Opin. Ther. Pat. **2021**, DOI: 10.1080/13543776.2022.1999415

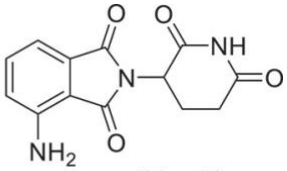
Cereblon is the most promising E3 ubiquitin ligase currently explored in the PROTAC field. Thalidomide was the first cereblon ligand identified and its derivatives, including pomalidomide and lenalidomide, are still some of the most explored as cereblon-recruiters in PROTACs. Their significance is not limited to PROTACs as several thalidomide derivatives are approved for clinical use in diseases such as multiple myeloma.



thalidomide



lenalidomide



pomalidomide

The authors introduce all cereblon binders patented in the last 5 years and group them into two categories: thalidomide-like and those distinct to thalidomide. This comprehensive list is supplemented with examples of the efforts in academic literature to identify novel cereblon ligands. The authors argue that the advancement of the field lies in the development of new non-thalidomide-like ligands which will enable new players to claim intellectual property for new PROTAC drug candidates.

This patent review collates the vast number of CRBN binders explored in drug discovery and will undoubtedly become a useful reference for medicinal chemists in their quest for developing cereblon-based PROTACs.

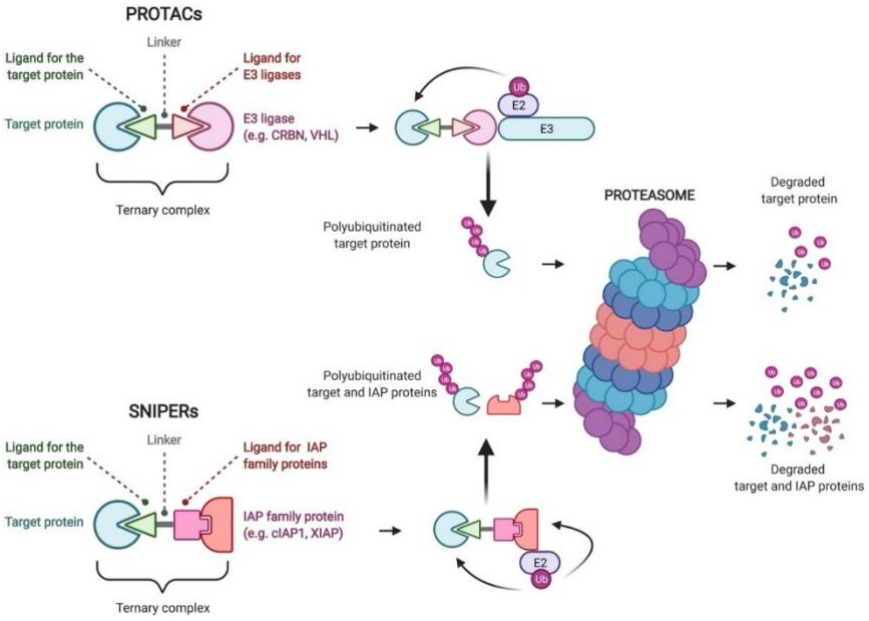
Cell Biology | Chemistry

Contributor: Alena

Targeted Protein Degraders from an Oncologist Point of View: the Holy Grail of Cancer Therapy?

Margherita Ambrosini[§]*, Giovanni Fucà*

Crit. Rev. Oncol. Hematol. **2021**, DOI: 10.1016/j.critrevonc.2021.103532



Cancer therapy constitutes a prime field for applying PROTAC technology due to the challenges many current or potential treatments face, including drug resistance and target selectivity.

This review provides a detailed summary of the cancer targets that have been under investigation so far with a focus on thorough evaluation of each drug candidate and their progress into clinical trials. The PROTAC strategy is set into context by providing a comparison with existing small-molecule inhibitor and monoclonal antibody approaches. In addition to traditional PROTACs, it also mentions the utility of the SNIPER (Specific and Non-genetic Inhibitor of apoptosis protein-dependent Protein Erasers) technology in cancer which degrades the inhibitor of apoptosis proteins (IAP) that are often overexpressed in cancer cells. Importantly, this paper discusses the unknowns that are yet to be understood as PROTAC drug candidates proceed through early-stage clinical trials, including their safety profile and potential for on-target toxicity when wild-type target proteins are completely degraded.

This is a great resource on how far the cancer-targeting PROTAC field has come and the promise it holds for the future.

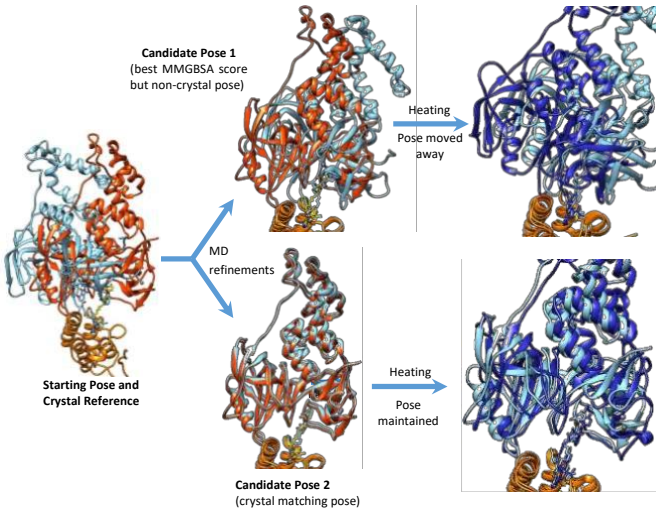
Computational Chemistry | Structural Biology/Biophysics

Contributor: Alena

In Silico Modeling and Scoring of PROTAC-Mediated Ternary Complex Poses

Junzhuo Liao[§], Xueqing Nie, Ilona Unarta, Spencer Ericksen*, Weiping Tang*

ChemRxiv **2021**, DOI: 10.26434/chemrxiv-2021-ldzzz-v2



Structure-based design can play a critical role in the optimization of PROTACs. However, with the resource-intensive and unpredictable nature of generating experimental crystal structures of ternary complexes, there is also an evident need for the development of in silico modeling workflows to predict a PROTAC's mode of action.

Several methods have been developed previously for the docking of the crystal structures of an E3 ligase and protein of interest to predict the ternary complex they would form in the presence of a PROTAC. However, previous docking workflows, such as PRosettaC, lacked a scoring system that would enable the identification of the native pose in the absence of experimental evidence. Liao *et al*. present a method to score MD-generated poses of ternary complexes by using an MM/GBSA enthalpy calculation in the first step followed by heating-accelerated pose change trials (HAPOC) that involve the determination of total pose-residence time over a series of elevated temperatures. The authors propose cut-off values of 17.5 time score and 380 temperature score over 10 or more runs above which a pose can be considered native using this approach, providing confidence in the docking prediction in the absence of experimentally-determined structures. The authors tested four systems for which they generated the MD-based poses themselves as well as re-scoring PRosettaC-generated poses for two systems. The HAPOC scoring successfully distinguished the native pose from non-native ones. The inclusion of entropy component, which plays a significant role in protein-protein interactions compared to classical protein-ligand binding appeared to be crucial in the success of this scoring method.

The study addresses an important issue of ternary complex predictions where in the absence of experimental evidence, it is difficult to determine the accuracy of a given docking pose.

Cell Biology	Chemistry	Structural Biology/Biophysics

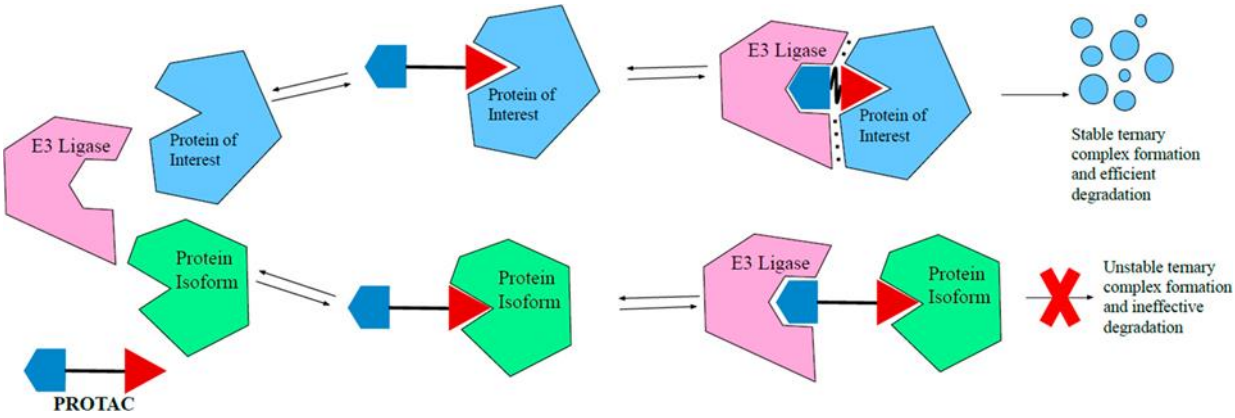
Contributor: Shakil

Degradation of Protein Kinases: Ternary Complex, Cooperativity, and Selectivity

Claire E. Grigglestone§, Kap-Sun Yeung*

ACS Med. Chem. Lett. **2021**, *12*, 1629

Protein kinases are important in phosphorylation of proteins, dysregulation of phosphorylation leads to excess cellular



proliferation or continued survival of cancerous motifs. Intensive research has been carried out to discover kinase inhibitors, however discovering a selective kinase inhibitor is a key issue. Acute myeloid leukaemia cell lines have shown a specific dependency on CDK6. However, there are currently only dual CDK4/6 inhibitor drugs, which have been approved for the treatment of breast cancer. It was hypothesized that a PROTAC could induce selective degradation of CDK6 over CDK4 by inducing PPIs that would result in differential ternary complex formation. In agreement with this hypothesis, it was shown that PROTAC BSJ-03-123 only formed stable complexes with CDK6. BSJ-03-123 was able to selectively disrupt the proliferation of CDK6-dependent AML cells while sparing CDK4-dependent cancer cell lines. Work conducted by our lab also supported the idea that selectivity arises from differential ternary complex formation specifically the development of an SGK3 selective degrader, SGK3-PROTAC1. This viewpoint highlights how understanding the structural differences in ternary complex formation could drive the development of selective PROTACs.

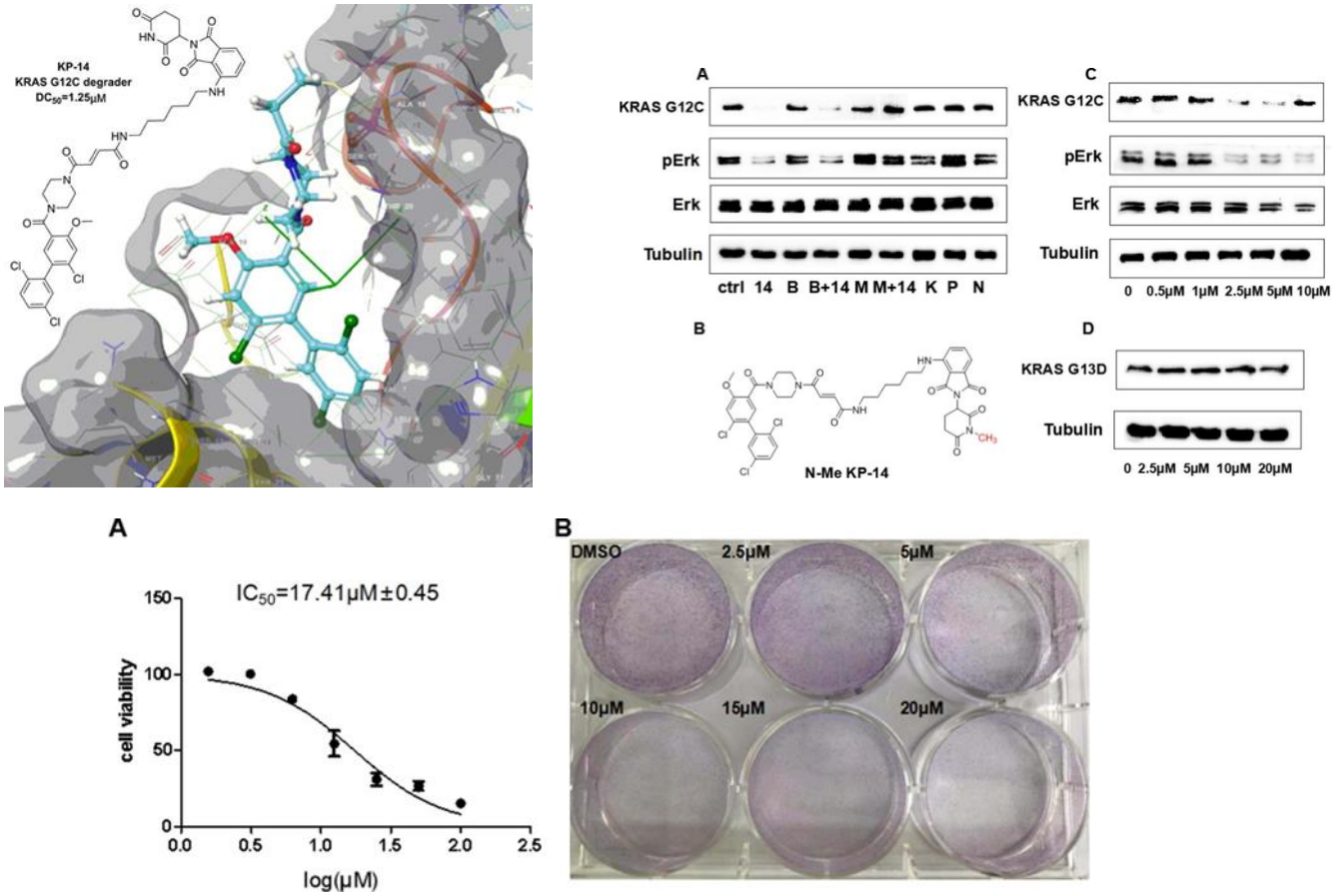
Chemistry	Cell Biology	Computational Chemistry

Contributor: Shakil

Discovery of KRas G12C-IN-3 and Pomalidomide-based PROTACs as degraders of endogenous KRAS G12C with potent anticancer activity

Ling Li[§], Yinrong Wu[§], Zichao Yang[§], Chenglong Xu[§], Huiting Zhao[§], Jin Liu[§], Jingxuan Chen*

Bioorganic Chemistry **2021**, *117*, 105447



Mutated KRAS causes constitutive activation of downstream signalling pathways such as RAS-RAF-MEK-ERK, leading to the development of cancer. Approximately 80% of KRAS mutations occur within codon 12, where the glycine can be mutated to a cysteine residue. Many KRAS G12C inhibitors have been developed, however drug resistance and MAPK signalling reactivation after KRAS G12C inhibitors have been reported. Zeng *et al*. and Bond *et al.* produced a series of PROTACs to address the issue of drug resistance and reactivation of MAPK signalling. However, these PROTACs produced either degraded exogenous KRAS G12C or had a molecular weight larger than 1100 Da. To overcome these issues, this paper designed a series of KRAS G12C-IN-3 and pomalidomide-based PROTACs. Among a series of compounds, KP14 could potently degrade KRAS G12C and suppress activation of the MAPK pathway in NCI-H358. Furthermore, KP-14 exhibited potent anti-proliferative activity against NCI-H358 cancer cells and was able to suppress the formation of NCI-H358 tumour colonies. KP14 may serve as an important tool to further design PROTACs for KRAS G12C degradation and potential investigation as an anticancer agent.

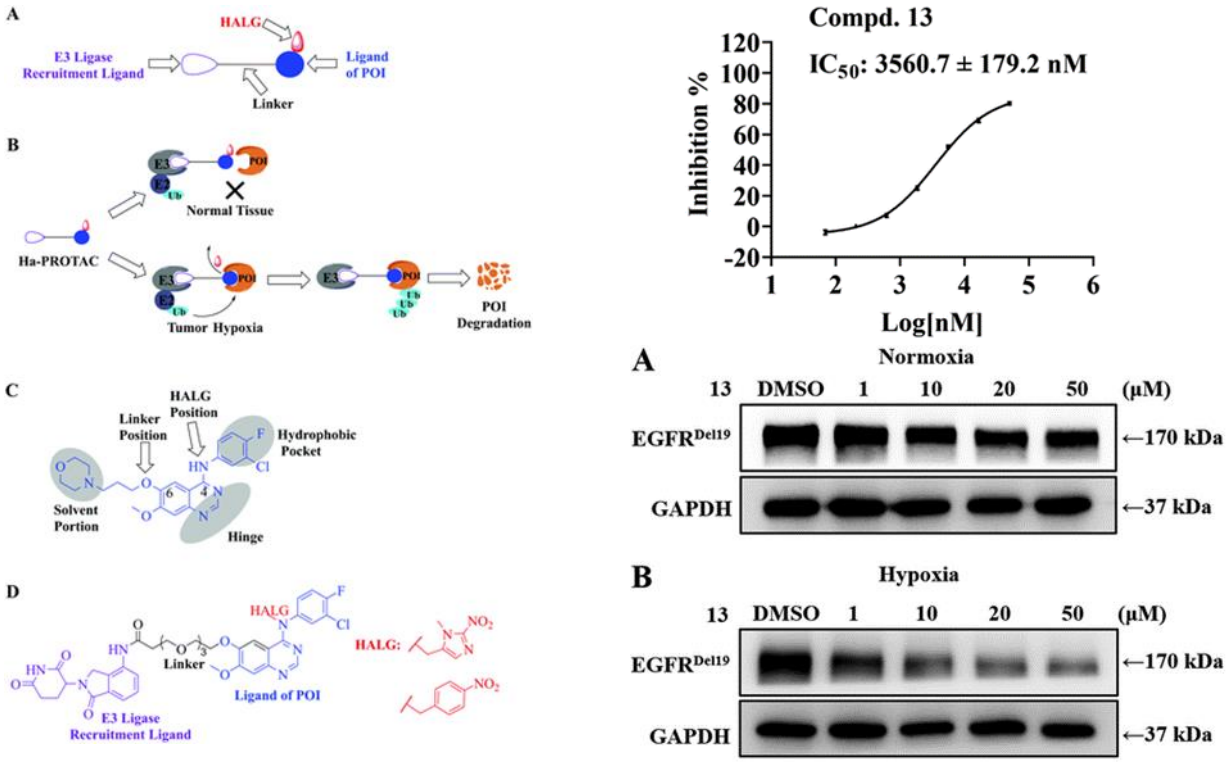
Chemistry | Cell Biology

Contributor: Shakil

Development of Hypoxia-activated PROTAC Exerting More Potent Effect in Tumour Hypoxia than in Normoxia

Weiyan Cheng[§], Shasha Li[§], …, Xin Tian*, Xiaojian Zhang*

ChemComm **2021**, *57*, 12852



Tumour hypoxia develops due to uncontrollable cell proliferation, altered metabolism, and abnormal tumour blood vessels resulting in a reduced transport of oxygen and nutrients. This results into overexpression of many carcinogenic proteins (such as HIF, VEGFR, EGFR, *etc.*) and enhances tumour resistance and survival rates. Anti-tumour prodrugs have been developed to contain a hypoxia-activated leaving group (HALGs). These prodrugs are activated by tumour hypoxia causing separation of the HALGs from the parent structure resulting in an active drug release *via* electron transfer and rearrangement, which selectively exert antitumor activity. Cheng *et al.* designed the first hypoxia-activated PROTACs (ha-PROTACs) that target $EGFR^{Del19}$ by incorporating HALGs into the structure of PROTACs. In normoxia, ha-PROTAC 13 degraded a small amount of $EGFR^{Del19}$ in HCC4006 cells. Interestingly, degradation of $EGFR^{Del19}$ by ha-PROTAC 13 was increased in hypoxia. This paper suggests that there is a therapeutic potential of PROTACs as a prodrug, however it is yet to be seen if ha-PROTAC 13 can have an anti-proliferative effect.

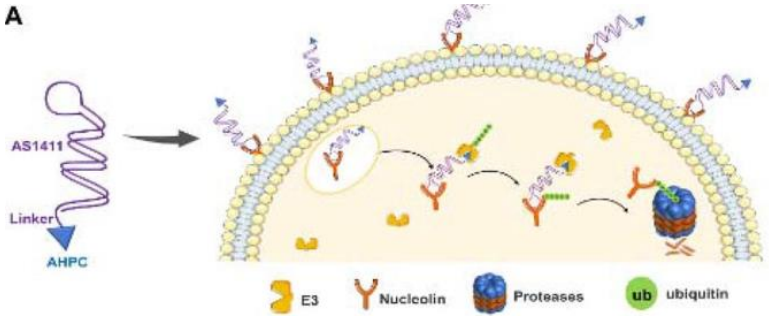
Cell Biology | Chemistry

Contributor: Shakil

Development of a novel PROTAC using the nucleic acid aptamer as a targeting ligand for tumour-selective degradation of nucleolin

Lin Zhang[§], Ling Li[§], …, Mao Ye*, Weihong Tan*

BioRxiv **2021**, DOI: https://doi.org/10.1101/2021.08.05.455211



PROTACs are highly efficient for protein degradation and therefore potential therapeutic strategy for cancer. However, most PROTACs lack tumour selectivity, this is due to the E3 ligases utilized by PROTACs, which are widely expressed in both normal and tumour tissues. This may result in on-target toxicity if POIs are not tumour specific. Aptamers are single-stranded oligonucleotides that fold into defined architectures and bind to targets such as proteins. Like antibodies, aptamers bind to their targets with high specificity and affinity. Aptamers hold characteristics derived from their oligonucleotide properties, such as low immunogenicity and toxicity, easy chemical synthesis and modification, rapid tissue penetration and excellent stability. In this first proof-of-concept study, the authors demonstrate the use of an aptamer to construct a PROTAC. Aptamer AS1411 was used as a targeting ligand for nucleolin and conjugated to a small molecule ligand of E3 ligase VHL via a DBCO-azide click reaction, which generated a nucleolin-targeting PROTAC ZL216. ZL216 promotes ternary complex formation of nucleolin-ZL216-VHL and potently degrades nucleolin in breast cancer cells *in vitro* and *in vivo*. This paper shows a novel way for aptamers to be used to develop PROTACs and provides a promising strategy for the development of tumour selective PROTACs. However, aptamers can range from 20 to 80 nucleotides, further studies are required to understand what effect aptamer length has on formation and stability of ternary complexes and the ability of a PROTAC to degrade its POI.

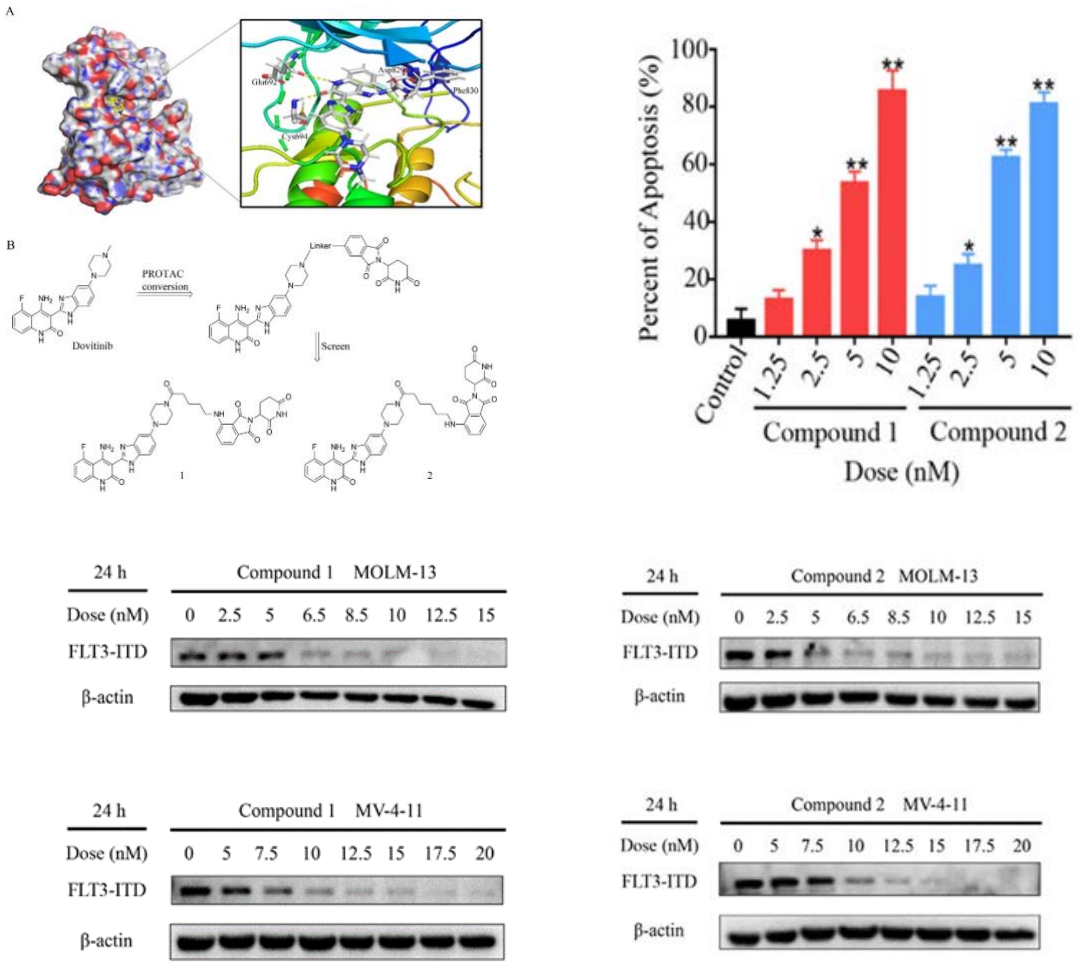
Cell Biology | Chemistry | Computational Chemistry

Contributor: Shakil

Proteolysis-Targeting Chimera (PROTAC) Modification of Dovitinib Enhances the Antiproliferative Effect against FLT3-ITD-Positive Acute Myeloid Leukemia Cell

Sheng Cao§, Lan Ma§, Yulin Liu§, Mingming Wei§, …, Xiaoji Wang*, Cheng Yang*, Guang Yang*

J. Med. Chem. **2021**, *64*, 16497



Acute myeloid leukemia (AML) is one of the most lethal blood malignancies worldwide. Mutation in the tyrosine receptor kinase 3 (FLT3) is the most frequent genetic alteration in AML, with the most dominant FLT3 mutation being the internal tandem duplication alteration (FLT3-ITD). There are a wide variety of FDA approved FLT3 inhibitors (e.g., sunitinib, pexidartinib, KW-2449, dovitinib, ponatinib, sorafenib, tandutinib, lestaurtinib, and midostaurin). However, there are undesirable off-target effects, differentiated metabolic issues, and clinical drug resistance problems. This study demonstrated that the chemical conversion of dovitinib into a CRBN-recruiting PROTACs could provide a more active anti-proliferative compound to terminate FLT3-ITD$^+$ AML cells than dovitinib alone, both in vitro and in vivo. This paper shows how drugs that are FDA approved can be chemically converted to a PRTOAC to overcome off-target effects and drug resistance.

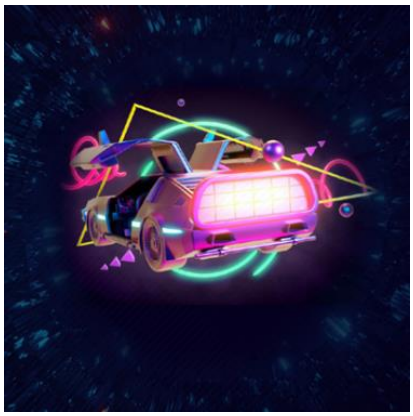
Chemistry

Contributor: Ross

Back to the Medicinal Chemistry Future

Mariateresa Giustiniano*, Christian W. Gruber*, Caitlin N. Kent*, and Paul C. Trippier*

J. Med. Chem. **2021**, *64*, 15515



This paper highlights essential literature in the medicinal chemistry field, from the first publication of *J. Med. Chem.* in 1959. The paper also exists as a virtual issue of the references within, covering the classical rules (ADMET and Lipinski's 'rule of 5') and beyond. Various other topics are covered including, but not limited to, BBB and CNS penetration, bioisosterism and computational discoveries. The final papers of note are on specific diseases and biological target, including COVID-19.

This selection of papers should be a go-to for all medicinal chemists, no matter the field you work in. The final four publications are those of 'recent strategic advances in medicinal chemistry', 'a medicinal chemist's guide to molecular interactions', 'essential medicinal chemistry of essential medicines' and 'what makes a good medicinal chemist?' Personal perspectives which provide nice overviews of the specified topics.

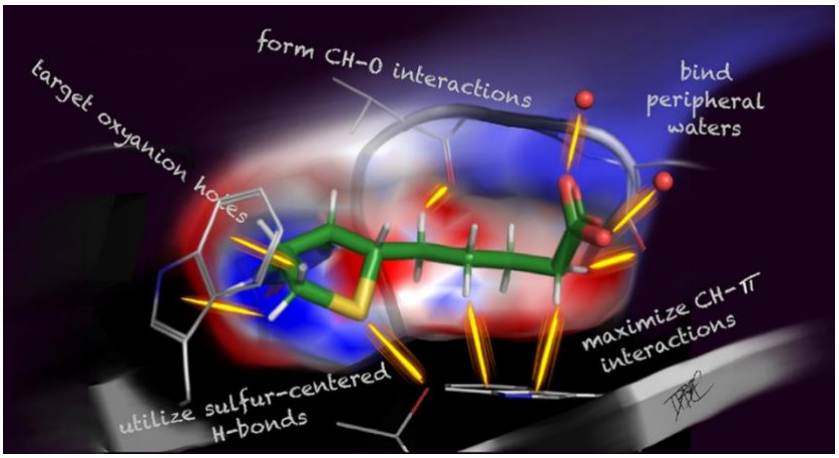
Chemistry | Structural Biology/Biophysics

Contributor: Alena

Biotin's Lessons in Drug Design

Darryl B. McConnell*

J. Med. Chem. **2021**, *64*, 16319



The interaction of biotin to avidin and streptavidin are the strongest non-covalent molecule-protein interactions known to date. With biotin achieving this with its mere 240 Da size. Such a strong interaction is what many drug discovery pipelines can only dream about. Yet, this comprehensive summary suggests that modern medicinal chemistry is not utilizing the full repertoire of tools at its disposal.

The author draws our attention to hydrogens which through non-standard protein-ligand interactions such as the CH-π hydrogen bonds provide the majority of the binding affinity. Similarly, biotin utilizes an oxyanion hole in its binding mode which is probably underutilized in drug design due to the inability of crystallography to unambiguously determine the position of hydrogens in binding pockets of proteins leading to many unidentified oxyanion holes that could be prime sites for binding optimization.

This fascinatingly detailed analysis, where no hydrogen is left unturned, challenges the fundamental rules of small molecule drug discovery, and allows a fresh perspective into the strategies medicinal chemists employ in their day-to-day decision making.

Chemistry

Contributor: Giorgia

The Open Reaction Database

Steven M. Kearnes§*, …, and Connor W. Coley*

J. Am. Chem. Soc. **2021**, *143*, 18820

This paper details a new interface for structuring and sharing reaction protocols and data, all on a relatively easy-to-use web app. The relevant data is uploaded to the "Open Reaction Database" (ORD) and allows a wide range of reactions to be formatted in a clear, consistent manner. Crucially, this consistency means that the database can be used as a tool for machine learning. While reaction protocols can be extracted from primary methodology papers, the information on ORD seems vast, detailing the vessel used, stirring rate, and reaction atmosphere, parameters which can often be up to the reader's interpretation when following literature preps. The unambiguity of the reaction setup as taken from ORD would hopefully help when implementing literature preps within your own lab, which can usually lead to mixed results.

Additional promise comes from the ORD in the ability to format reaction sets in a way which allows machine learning to analyse vast amounts of data and give additional information about reaction conditions, allowing predictions of yield, product, or conditions, based on the given data and extrapolation from known reaction outcomes.

The ORD is available online and the paper has links to some example reactions to look around, with the authors committed to keeping this an open access application. While it is in its early stages I can see this becoming a great platform for not only lab chemists looking for detailed literature preparations, but also for future development of prediction programs, which would help save a lot of time spent optimising reactions in the lab.

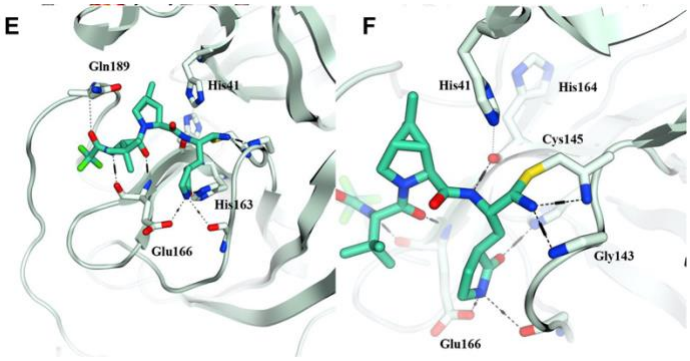
Chemistry	Cell Biology	Structural Biology/Biophysics

Contributor: Tasuku

An oral SARS-CoV-2 Mpro inhibitor clinical candidate for the treatment of COVID-19

Dafydd R. Owen* et al.

Science **2021**, DOI: 10.1126/science.abl4784



The global epidemic of SARS-CoV-2, which has resulted in over 235 million confirmed cases causing over 4.8 million deaths, is gradually settling down with the application of anti-COVID-19 vaccines. However, therapeutic agents for SARS-CoV-2 are still under development and their efficacy is limited. In this paper, the authors aimed to develop orally available compounds targeted for the coronavirus-specific protease Mpro based on PF-00835231, which was obtained at the time of SARS-CoV-1 and MARS. After optimization, the authors obtained PF-07321332 with a high biological activity against Mpro and improved oral bioavailability. One of the characteristic features of this compound is the formation of a reversible covalent bond between the nitrile group in the compound and the cysteine residue of Cys145 in the catalytic center of Mpro, which results in high activity and selectivity. Since PF-07321332 showed good in vitro and in vivo activities with excellent therapeutic index, the authors proceeded to take this compound to clinical trials to obtain pharmacokinetics and safety profiles. It was found that the blood concentrations required for showing the anti-virus activity was achieved and the concentration were maintained for 12 hours in clinical trials of 250 mg PF-07321332 and 100 mg ritonavir twice a day.

It was the first time I had seen a nitrile group act as a reversible covalent warhead. I also wondered why PF-07321332 was used with ritonavir in clinical trials, and in this paper, they mentioned that ritonavir was used as a CYP3A4 inhibitor to improve the pharmacokinetic profiles because PF-07321332 is mainly metabolized by CYP3A4.

This compound showed excellent antiviral activity against COVID-19 infection in phase2/3 trials.

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