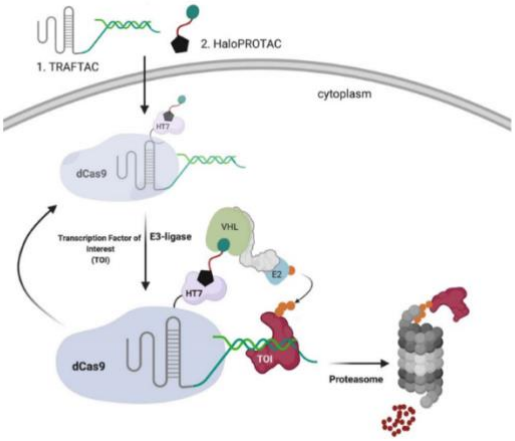
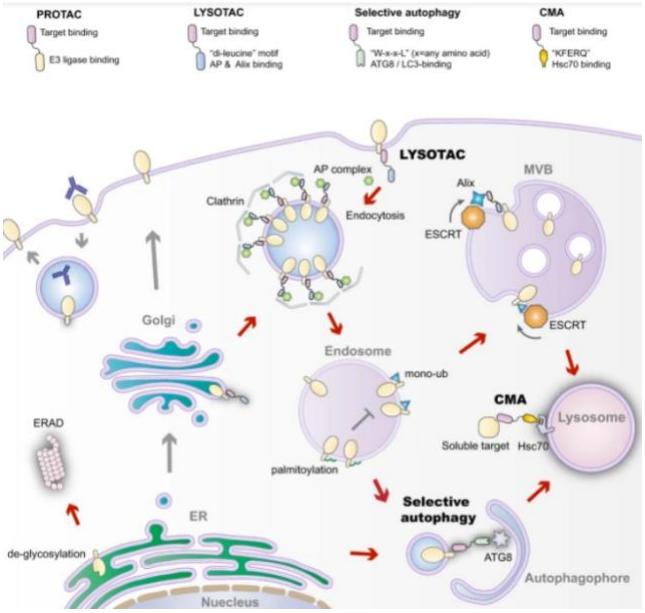
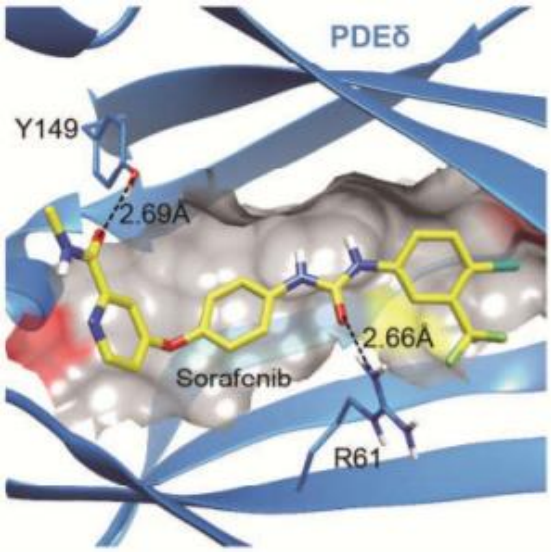






Ciulli Group Journal Club

Targeted Protein Degradation,

Medicinal Chemistry and

Chemical Structural Biology

Literature Highlights

October 2020 Edition

Ciulli Group Journal Contents

Landmark Paper

Contributor: Alessio

A heat-stable polypeptide component of an ATP-dependent proteolytic system from reticulocytes

Aharon Ciehanover [*alas Aaron Ciechanover*], Yaacov Hod, Avram Hershko

Biochem. Biophys. Res. Commun. **1978**, *81 (4),* 1100-1105

TABLE 1: Resolution of the ATP-Dependent Cell-Free Proteolytic System Into Complementing Activities

Enzyme fraction	Degradation of [^{3}H]globin percent/h	
	-ATP	+ATP
lysate	1.5	10.0
fraction I	0	0
fraction II	1.5	2.7
fraction I and fraction II	1.6	10.6

Enzyme fractions were separated by DEAE-cellulose as described under "Methods" and supplemented at the following amounts (mg of protein/ml reaction volume): lysate, 28; fraction I, 45; and fraction II, 3.5. Where indicated, ATP was added together with phosphocreatine and creatine phosphokinase.

The Landmark feature is back and seeing as October is the month of Nobel prize announcements, it seems fitting to look back at some of the early Nobel prize-winning work on the ubiquitin-proteasome system. My last entry covered the 1975 discovery and purification of the protein UBIP/APF-1 (later to be known as ubiquitin) - and it was only a few years later that its links to proteolysis began to emerge.

It was initially thought that regulation of cellular processes occurred mostly at transcriptional (DNA => RNA) and translational (RNA => protein) levels, and as a result work on protein recycling had remained a neglected area of research. The discovery of the lysosome and elucidation of lysosomal degradation pathways made scientists initially assume that cellular proteins were degraded primarily within this organelle. However, accumulating evidence since had suggested that another mechanism of intracellular proteolysis must be occurring, that required energy and ATP hydrolysis. To explore such new mechanism, in a quest to identify the components involved, the authors employ a classic biochemical method of stepwise separation on diethylaminoethanol (DEAE) cellulose, a positively charged resin for ion-exchange chromatography widely used at the time to purify proteins and nucleic acids from cell extracts. They chose to use cell extracts from reticulocytes, which are terminally differentiated red blood cells, because those cells do not contain lysosomes and because prior work had shown that rabbit reticulocytes could degrade artificial haemoglobin and could provide a suitable model system for studying cell-free non-lysosomal proteolysis. Initial fractionation of crude reticulocyte

cell extracts yielded two fractions which were both required to reconstitute energy-dependent proteolytic activity: a flow-through Fraction I, containing unbound proteins, primarily haemoglobin and a few other basic proteins, and Fraction II, containing bound proteins, eluted in high salt. Further characterization allowed them to purify the active component from Fraction I, which was found to be a small, ~9 kDa heat-stable protein, that was required for ATP-stimulated protein degradation. Because Fraction II contained proteolytic activity (while Fraction I did not), the authors hypothesized that the protein purified in Fraction I might serve as an "activating factor" of some sort for an unknown protease. These early observations began to point to the presence of a two-component system for intracellular non-lysosomal proteolysis. Later work would, indeed, identify such system as the ubiquitin-proteasome system. The authors conclude the article with a forward-looking statement, anticipating what will later turn out to be correct: "*It might well be that the heat-stable polypeptide participates in such an early event that precedes the actual proteolytic reactions*".

After-dinner drinks / pub stories on this article: the first author's first and surname were both misspelled (should be Aaron Ciechanover) – which means this landmark article won't have featured in PubMed or other citation searches for a while... At least they gave the Prize to the right individual! Ah, and of note: this crucial data was published in a journal that does not get mentioned remotely as often as other high-impact ones. When we think the journal matters, let's think twice!

Feature of the month

October is breast cancer awareness month!

We therefore dedicate this issue of our monthly Journal club to brave women and men who are fighting breast cancer and to the ones who have lost the fight. We also urge all of you (both women and men of any age) to do regular check-ups and seek medical help if you notice anything out of ordinary. Infographics from Know your lemons® campaign can help you understand and recognise 12 signs of breast cancer.

Did you know?

- The original ribbon for Breast Cancer Awareness was not pink, but peach coloured. In 1991, Charlotte Haley, a breast cancer survivor, handmade the ribbons and attached a packet of 5 ribbons to a postcard to be distributed. The postcard read: “The National Cancer Institute’s annual budget is $1.8 billion, only 5 percent goes for cancer prevention. Help us wake up legislators and America by wearing this ribbon.” When she refused to work with them, bigger corporations were advised by legal counsel that a change in colour wouldn’t require Charlotte’s permission to use her idea. That’s how the pink ribbon for Breast

Cancer Awareness was born. (learn more at https://www.bcaction.org/2014/06/24/in-memoriam-charlotte-haley-creator-of-the-first-peach-breast-cancer-ribbon/)

- One of the earliest reports of breast cancer dates to Ancient Egypt, 3000-2500 BCE. The Edwin Smith Surgical Papyrus describes eight cases of tumours or ulcers of the breast. The first reported mastectomy was performed on Theodora, empress of Byzantine in 548 BCE
- French surgeon Jean Louis Petit and later Scottish surgeon Benjamin Bell were the first to surgically remove lymph nodes, breast tissue, and chest muscle as a treatment for the disease. This method was the first successful treatment of breast cancer
- Tattoo artists are helping breast cancer survivors who had to undergo surgeries on their breasts, by covering the scars from the surgery with beautiful designs or giving them areola tattoo (learn more at https://www.theverge.com/2017/2/21/14669588/david-allen-tattoos-breast-cancer-survivors-healing)
- Mammography/mammogram can detect breast cancer cells before they can be physically felt
- Breast cancer is the most common type of cancer in the UK*
- From 2015-2017 there were 150 cases of breast cancer diagnosed daily in the UK*
- Breast cancer survival is improving and has doubled in the last 40 years in the UK*
- When diagnosed at its earliest stage, all (100%) people with breast cancer will survive their disease for one year or more, compared with 2 in 3 (66%) people when the disease is diagnosed at the latest stage*

*Taken from cancerresearchuk.org

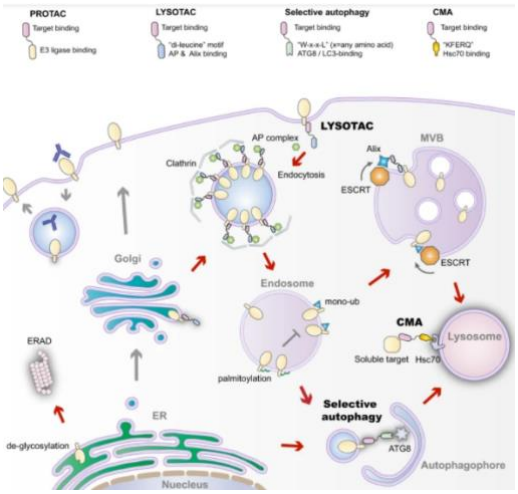
Targeted Protein Degradation

Contributor: Vesna

Targeted degradation of immune checkpoint proteins: emerging strategies for cancer immunotherapy

Jie Xu,* Jean Philippe Brosseau & Hubing Shi

Oncogene **2020***, DOI:* 10.1038/s41388-020-01491-w



Immune checkpoint inhibitors have revolutionized cancer therapy. PD-1 ligand (PD-L1) is often overexpressed by cancer cells and thus "hides" it from the surveillance of immune T-cells. Monoclonal antibodies like Pembrolizumab and Nivolumab (and others) can block PD1/PD-L1 interaction and make cancer cells visible to the immune system. However, the drawbacks of the immune therapy include adverse side effects (overactivated immune system), resistance and low responsiveness. This review suggests considering degrading such targets by using different approaches of targeted protein degradation – PROTACs, LYTACs, selective autophagy or chaperone-mediated autophagy. Immune checkpoint inhibitors and ligands have a relatively long half-life and they are regulated by endocytosis-exocytosis cycle rather than translation and degradation. Besides their primary role on the surface of the cell, these molecules participate in downstream signalling driving survival of cancer cell and chemoresistance. The authors suggest that degradation of PD-1/PD-L1 could possibly produce a more durable inhibitory effect compared to solely steric inhibition by monoclonal antibodies.

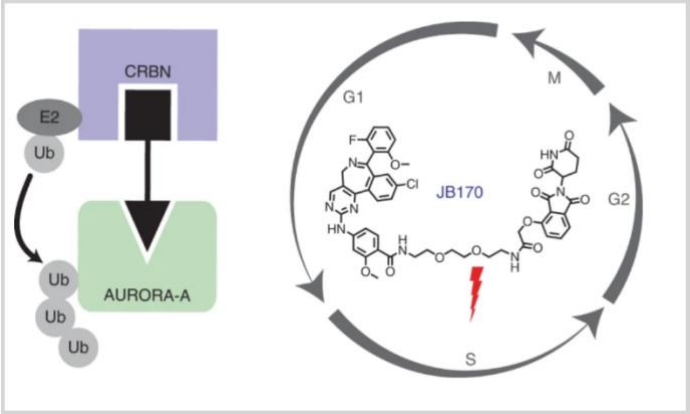
Immune checkpoints are interesting targets, and I found this review very concise and nicely explaining the benefits of different approaches in degradation of PD-1/PD-L1. In my opinion, the biggest issue remains the overactivated immune system. The authors have briefly mentioned methods of precise drug delivery to minimise the effect on healthy cells, but there is still a lot of work to be done to overcome this issue!

Contributor: Vesna

PROTAC-mediated degradation reveals a non-catalytic function of AURORA-A kinase

Bikash Adhikari, § Jelena Bozilovic, § … Stefan Knapp,* Elmar Wolf*

Nature Chemical Biology **2020**, *16, 1179-1188*



Deregulated cell cycle is a hallmark of cancer. Cell cycle is regulated by cyclin-dependent kinases (CDK) and mitotic kinases. One of the prominent oncogenic mitotic kinases is AURORA-A. Alisertib, a potent AUROR-A inhibitor, is currently in phase III trial, but the evidence for its efficacy are mixed. Besides catalytic function, AURORA-A also exhibits non-catalytic functions that might contribute to low efficacy of the inhibitor in some studies. There is evidence that as part of its non-catalytic functions, AURORA-A binds to Myc proteins and protects them from proteasomal degradation or facilitates stem-cell like properties in breast cancer. Degrading AURORA-A instead of inhibiting it seems like an elegant solution to abrogate both catalytic and non-catalytic functions of this enzyme. The authors have synthesised a potent cereblon-based PROTAC JB170 and demonstrated its specificity for AURORA-A over other isoforms, but also analysed the off-target degradation by MS that confirmed its high specificity. They also analysed the ternary complex formation by utilising computational approaches followed by verification in vitro and proved that degradation of AURORA-A causes S-phase arrest and apoptosis in cancer cells.

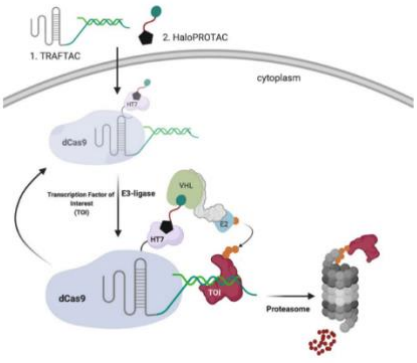
This paper again proves the advantages of PROTACs over classical inhibitors.

Contributor: Vesna

Targeted Degradation of Transcription Factors by TRAFTACs: Transcription Factor Targeting Chimeras

Kusal T. G. Samarasinghe, § Saul Jaime-Figueroa, Katherine Dai, Zhenyi Hu and Craig M. Crews*

BioRxiv **2020**, *DOI:* 10.1101/2020.10.12.336529



Transcription factors (TFs) bind DNA and regulate gene expression. They are often deregulated and involved in oncogenic processes. Development of inhibitors of TFs is challenging due to lack of enzymatic activity and ligandable pockets, except for oestrogen and androgen receptors that have been drugged by small molecules and, most recently, PROTACs. In this paper the authors present a novel way of degrading TFs with TRAFTACs – TRAnscription Factor TArgeting Chimeras, a customizable system that doesn't require finding a ligand for TFs. TRAFTACs consist of TF-binding double-stranded DNA covalently linked to CAS9 CRISPR-binding RNA that binds to dCas9-HT7 (ectopically expressed as Halo Tag fusion protein without enzymatic CAS9 activity). Addition of Halo-PROTAC (that binds but does not degrade HT7) recruits VHL E3 ligase and

brings it to the proximity of the transcription factor that subsequently gets ubiquitinated and degraded. The authors show development of this system and its efficacy on two transcription factors – NF-kB and brachyury. They also announce further work on degrading DNA-bound promotors that would introduce another level of specificity by repressing single gene rather than plethora of genes regulated by a single transcription factor.

Very clever and interesting approach for degradation of transcription factors. I'm looking forward to seeing how this method further develops.

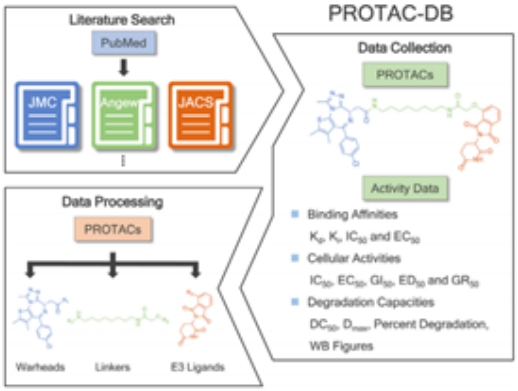
Contributor: Nikolai

PROTAC-DB: an online database of PROTACs

Gaoqi Weng, ….., Jian Wu*, Tingjun Hou*

Nucleic Acids Research **2020***, DOI:* 10.1093/nar/gkaa807

A web-based open-access database is a remarkable addition to the exponentially growing targeted protein degradation field. The database provides structural information and experimental data of PROTACs. Current catalogue consists of 1662 PROTACs, 202 warheads, 65 E3 ligands, 806 linkers, as well as their chemical structures, biological activities, and physicochemical properties. User-friendly PROTAC-DB enables researchers to easily query, browse and analyse the structures, degradation capacities, binding affinities and cellular activities of PROTACs in the database. The database is freely accessible at http://cadd.zju.edu.cn/protacdb/



Another online PROTAC database is created by London Lab http://protacdb.weizmann.ac.il/ptcb/main

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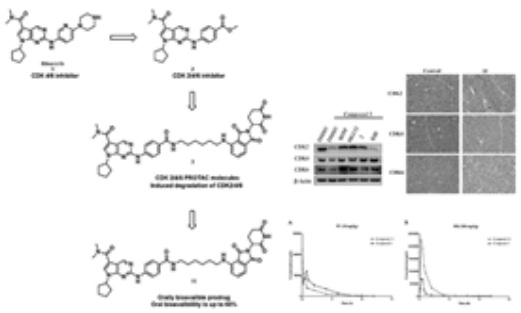
Contributor: Nikolai

First Orally Bioavailable Prodrug of Proteolysis Targeting Chimera (PROTAC) Degrades Cyclin-Dependent Kinases 2/4/6 *in vivo*

Mingming Wei,[§] Rui Zhao,[§] Yuting Cao,[§] Yujiao Wei,[§] Ming Li,[§] Zhiqiang Dong,[§] Yulin Liu,[§] ….., Guang Yang*, Cheng Yang*

Eur. J. Med. Chem. **2020***, DOI:* 10.1016/j.ejmech.2020.112903

Cyclin-dependent kinases (CDKs) are important cellular enzymes and play a vital role in regulating eukaryotic cell division and proliferation. Enhanced activity or temporally abnormal activation of CDKs leads to the development of many types of tumours. Therefore, inhibition or degradation of CDKs represent attractive chemotherapy strategies. Previously, CDK6 and CDK9 degraders have been already described. This paper illustrates the development of CDK2/4/6-CRBN PROTACs based on the CDK4/6 inhibitor Ribociclib. Author showed that a structural modification of Ribociclib (replacement of piperazine fragment by methoxycarbonyl) improved its affinity to CDK2. The created binder was used for PROTAC design. PROTAC



3 demonstrated efficient CDK2/4/6 degradation in vivo, inducing cell cycle reset and apoptosis in malignant melanoma cells. Moreover, an orally bioavailable prodrug was developed with 68% bioavailability (in rats) using pivaloyloxymethyl masking group.

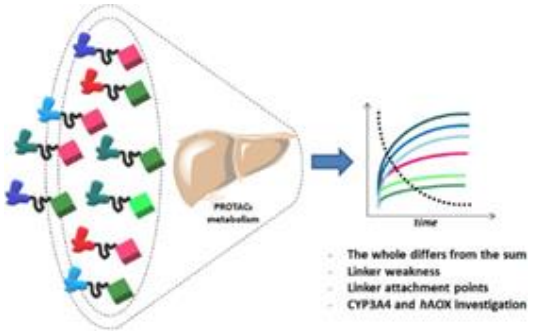
CRBN-based PROTACs are known to have poor oral bioavailability (<1%). Applying a prodrug strategy, the first orally bioavailable PROTAC prodrug was developed. This approach might provide a general solution for oral administration of PROTAC molecules with CRBN and other E3 ligase ligands.

Contributor: Nikolai

Understanding the Metabolism of Proteolysis Targeting Chimeras (PROTACs): The Next Step toward Pharmaceutical Applications

Laura Goracci, ….., Gabriele Cruciani*

J. Med. Chem. **2020**, 63, 20, 11615–11638



This important study represents the first analysis of the metabolic stability of PROTACs applied to a wide collection of compounds. The metabolism of 40 PROTACs were assessed in cryopreserved human hepatocytes at multiple time points. To cover a large chemical diversity, various combinations of ligands for four target proteins (BET, CK2, PARP and AR), ligands for two E3 ligases (VHL and CRBN), and nineteen linkers were selected. Both metabolic rate (half-life value) and soft spot identification were investigated. In addition, a subset of compounds was also tested for metabolism by human cytochrome CYP3A4 and human aldehyde oxidase (*h*AOX) for deeper data interpretation.

The results obtained demonstrate general trends in PROTAC metabolism: 1) metabolism cannot be predicted from the one of the ligands used for their design and synthesis; 2) linkers' chemical nature and length play a major role in the PROTACs' liability; 3) instability is mainly localized at the attachment points to ligands; 4) VHL-based PROTACs could undergo *h*AOX metabolism at the 5-phenyl-thiazole moiety.

Of note, to overcome nonenzymatic degradation of thalidomide-based PROTACs in aqueous solutions, the authors developed a protocol which reduced this degradation during storage in the autosampler for LC–MS analysis and improved reproducibility of the results.

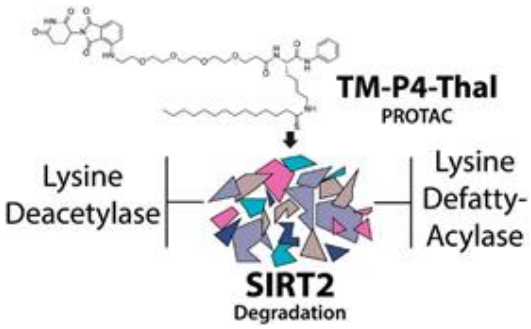
This insight into PROTAC metabolism represents a solid base for considering metabolism in the rational PROTAC design to develop degraders with good *in vivo* performance.

Contributor: Nikolai

Simultaneous Inhibition of SIRT2 Deacetylase and Defatty-Acylase Activities via a PROTAC Strategy

Jun Young, ….., Hening Lin*

ACS Med. Chem. Lett. **2020**, *DOI:* 10.1021/acsmedchemlett.0c00423



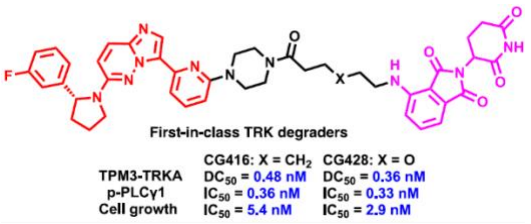
SIRT2 is an attractive target for cancer treatment due to promoting tumorigenesis through deacetylation and defatty-acylation of various substrates. Inhibition of SIRT2 activity has been shown to impede cancer cell growth. However, SIRT2-selective small molecule inhibitors could not abrogate both SIRT2 activities in cells. In this paper, the authors employed a PROTAC approach for inhibiting both SIRT2 deacetylation and defatty-acylation. The developed CRBN-based PROTACs (TM-P4-Thal), comprised thiomyristoyl lysine ligand and PEG-linker, promoted efficiently and selectively SIRT2 degradation in living cells, which allowed complete eradication of both enzyme activities. Moreover, the degradation of SIRT2 led to stronger cytotoxicity, suggesting the importance of defatty-acylation activity of SIRT2 in cancer cells. The authors propose that inhibiting the defatty-acylation activity of SIRT2 could enhance the anticancer activity of SIRT2 inhibitors.

Contributor: Ross

Discovery of First-In-Class Potent and Selective Tropomyosin Receptor Kinase Degraders

Liqun Chen, …, Xiao-Ran Han*, Jing Liu*

J. Med. Chem. **2020** DOI: 10.1021/acs.jmedchem.0c01342



The tropomyosin receptor kinase (TRK) receptor family affect neuronal tissue survival through a variety of signalling pathways. There has been a recent renewed interest in the TRK family as it relates to its role in human cancers following the identification of NTRK1 (TRKA), NTRK2 (TRKB) and NTRK3 (TRKC) gene fusions and other oncogenic alterations in various tumour types. The authors utilise, and slightly modify, two FDA approved TRK inhibitors as the POI binder. They employ a general PROTAC strategy to create a small library of potent CRBN based degraders.

The authors showed that two PROTACs reduced levels of TRKA fusion protein in KM12 cells at sub-nanomolar concentrations, and inhibition of downstream PLCγ1. As expected, both compounds were shown to exhibit better antiproliferative activity than the parental inhibitor. PK studies also found high levels of plasma concentration by IP injection, which should allow the use of these compounds as useful *in vivo* tool compounds.

While CRBN was chosen as the E3 ligand for "potentially favourable PK properties", it would be interesting to have shown the applicability of various E3 ligands, as well as more exotic linker configurations.

Contributor: Ross

A tale of two tails - efficient profiling of protein degraders by specific functional and target engagement readouts

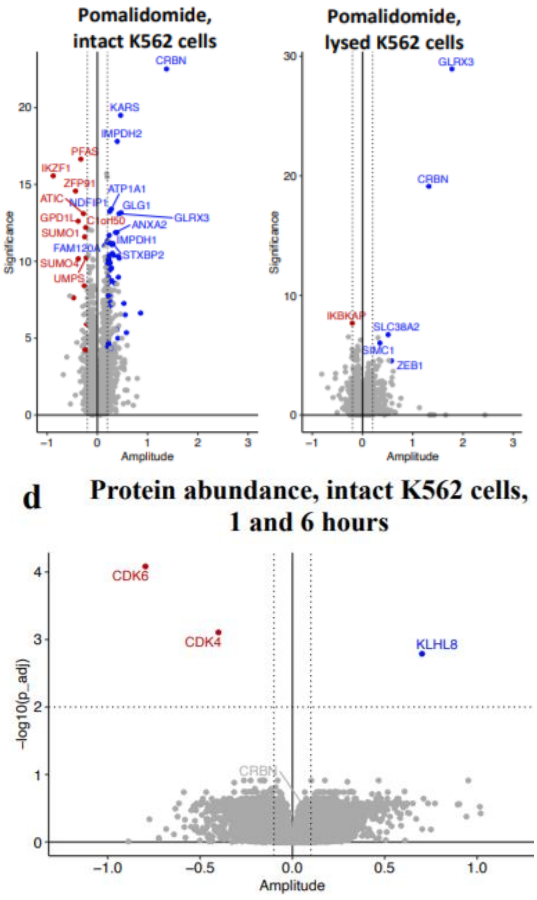
Alexey L. Chernobrovkin, …, Daniel Martinez Molina*

BioRxiv **2020**, *DOI:* 10.1101/2020.09.22.307926

Immunomodulatory drugs (IMiDs) are well known for their teratogenicity that is driven by targeting cereblon (CRBN). These molecules have been repurposed for anticancer agents and utilised widely in the PROTAC field as E3 ligase recruiters.

The authors describe the use of a CETSA MS experiment to robustly profile a molecule's interactions and downstream effects on a global proteomic scale. 2D CETSA MS profiling of pomalidomide, lenalidomide and thalidomide were conducted in intact (live, biologically active) cells (K562, iPSC and EB) and in lysed (biologically inactive) cells. Thermal stability effects, resulting from direct interaction and downstream activities, indicated the same primary target described in the previous two decades. Through the CETSA MS experiment they postulate a mode of action with respect to IMiD activity in the inosine pathway.

The method was used in relation to BSJ-03-204 (IMiD-based PROTAC), and in doing so determined target engagement and degradation profile in K562 cells, highlighting CDK4/6 as degraded proteins and increased levels of KLHL8.



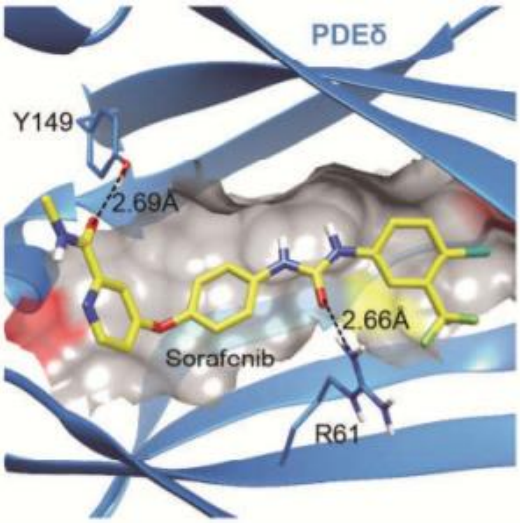
The CESTA MS approach appears to track both target engagement and downstream efficacy, in a single experiment, affording the same results as known in the literature. Suggesting it could be of great benefit as a tool for the profiling of PROTAC molecules in the future.

Contributor: Ross

Identification of PDE6D as a potential target of sorafenib via PROTAC technology

Yang Li, § Qingshi Meng, § …, Niu Huang*

BioRxiv **2020***, DOI:* 10.1101/2020.05.06.079947



Sorafenib is a multi-kinase inhibitor and is used to treat a multitude of cancers and has also been shown to target non-kinase receptors, including 5-hydroxytryptamine receptors. This group decided to use PROTACs to investigate the promiscuity of sorafenib and found that PDE6D, a novel non-kinase target, could be degraded.

The authors designed a sorafenib-3PEG-CRBN PROTAC (PROTAC T-S). PROTAC T-S significantly downregulated 6 proteins (3 kinases, 3 non-kinases) by TMT-based proteomics, with PDE6D showing the most significant depletion. Degradation of PDE6D could be depleted, but not abrogated with the use of sorafenib, thalidomide or a combination even at high concentrations indicating the necessity of ternary complexation to induce degradation. CETSA and SPR experiments also proved that PDE6D is a novel non-kinase target of sorafenib. They conclude by constructing biotinylated-sorafenib to purify sorafenib targets, however while PDE6D was not observed in this set of proteins, another non-kinase (ATXN10) was observed.

This communication elegantly describes the potential for PROTACs as a complementary method for drug target identification, with the major advantage being a superior signal-to-noise ratio to other approaches. Though also mention the reliance on ternary complexation that could miss some targets.

Others

Contributor: Nicole

A Turing Test for Molecular Generators

Jacob T. Bush, … Darren V. S. Green*

J. Med. Chem. **2020**, 63, 20, 11964–11971

One of the key steps to success in any medicinal chemistry project is molecule generation. The field of drug discovery now has at its disposal several machine learning tools to aid and expedite the design of new ideas: but how can we assess the performance of these tools? The authors of this paper report the results of three Turing-inspired tests to rate the performance of three molecular generators. The tests involve: 1) whether the machines can imitate human molecular generators (i.e. medicinal chemists); 2) whether the chemists would consider the machine-made ideas for synthesis; 3) whether the machines could iteratively generate ideas based on legacy projects from patents. One of the generators showed superior performance in all three tests, indicating its utility for machine-assisted molecular design.

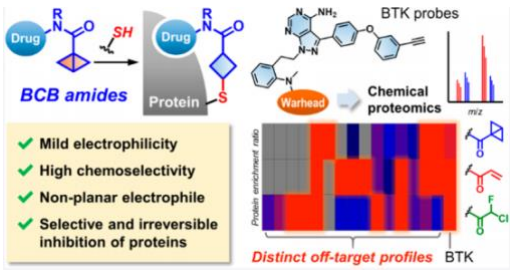
This paper is an enjoyable read and highly accessible for those who are curious about the developments in machine learning tools for drug discovery, without the need for expert knowledge of the algorithms behind them. It is interesting to see how and why one molecular generator comes out on top across the three tests. It is also encouraging to see some redundancy as well as complementarity between the human-generated and machine generated ideas. Overall this demonstrates an example of the utility of molecular generators in assisting the drug discovery process.

Contributor: Tasuku

Bicyclobutane Carboxylic Amide as a Cysteine-Directed Strained Electrophile for Selective Targeting of Proteins

Keisuke Tokunaga, … Naoya Shindo*, and Akio Ojida*

J. Am. Chem. Soc. **2020** 142, 43, 18522–18531



Introducing an appropriate warhead for the formation of covalent bonds to reactive amino acid residues is one of the attractive ways to develop target-selective chemical probes, inhibitors and degraders. In this paper, the authors developed bicyclo[1,1,0]butane (BCB) carboxylic amide as a reactive electrophile to cysteine residues and demonstrated its unique biological profile. First, they synthesized BCB amide derivatives and checked their reactivity with nucleophilic amino acids. It was found that BCB amides only reacted to cysteine and the reaction rate was tuneable by modifying the amine part. In addition, they observed a different protein binding profile of BCB amides from that of acrylamides. In the proteomics analysis of derivatives of ibrutinib (a selective BTK inhibitor), BCB amide, acrylamide, and chlorofluoroacetamide derivatives showed different off-target binding. It was found that only the BCB-

ibrutinib derivatives bound to MPV17 mitochondrial inner membrane protein like 2 (MPV17L2). These findings suggest that the BCB amide can be one of the best chemical motifs for the development of selective covalent binders.

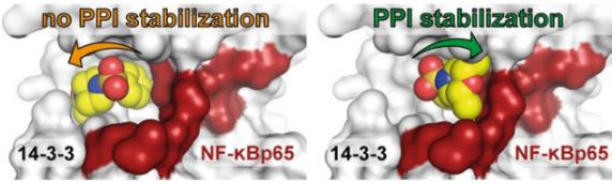
They also established convergent synthetic routes to attach this caged moiety to a wide range of small molecules. Their routes can also be useful for installing this type of unique spiro moieties.

Contributor: Emelyne

Fragment-Based Stabilizers of Protein-Protein Interactions through Imine-Based Tethering

Madita Wolter, Dario Valenti, ...Dimitrios Tzalis,*and Christian Ottmann*

Angew. Chem. Int. Ed. **2020***,* DOI: 10.1002/anie.202008585



Targeting Protein-Protein Interactions has emerged as one of the most exciting strategy in drug discovery. Inhibiting PPIs has seen successful attempts. On the other hand, while very promising, discoveries of PPI stabilization happened serendipitously (Lenalidomide, Rapamycin). The biggest challenge remains in the identification of chemical matter. In addition to FBDD as a "bottom-up" strategy for drug discovery, site-directed fragment "tethering" enables localization of the fragment to a specific site at the PPI interface, covalently.

The authors applied this strategy in a previously published work, to describe a first fragment-based stabilization of the 14-3-3 and ERα, through the insertion of the common covalent handle, cysteine, at the PPI interface.

In this work, a lysine (Lys122) is targeted at the PPI between 14-3-3/NF-κB, with the formation of an imine as a covalent anchor. Lys122 is of particular interest as a handle for tethered fragment screening as it sits in a preferred pocket for 14-3-3 PPI stabilization, and is predicted to be most amenable to imine bond formation (pKa~10).

A first screening of aromatic aldehydes through soaking into crystals of the NF-κBp65/14-3-3 complex yielded a selective fragment that was then grown into an extended library, of which two derivatives showed interesting properties. Crystal soaking and mass spectroscopy experiments confirmed the formation of the covalent imine bond. Both extended fragments show different behaviours but are interacting with both proteins and stabilizing the PPI as confirmed by biochemical studies. Both fragments do not stabilize other PPIs where 14-3-3 (Lys122) is involved.

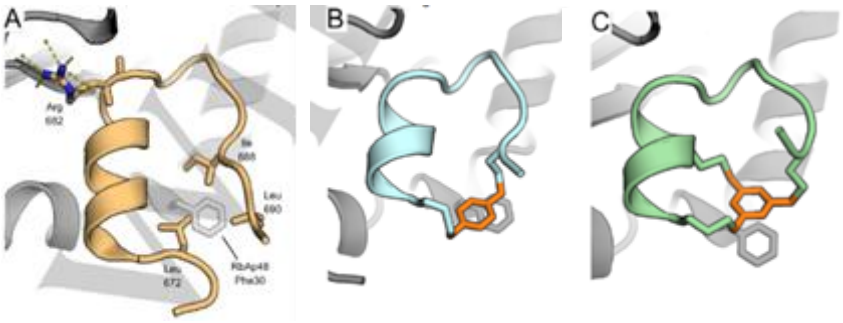
This paper shows a very nice example of tethered fragment-based drug design, and a first report of a small molecule that stabilize the 14-3-3/NF-κB complex. It works well here as the interface between the two proteins is quite unique, with a lysine in an optimal setting. Hopefully, there will be further reports of this kind with great potential for future drug discovery campaigns, for PPI stabilizers, molecular glues.

Contributor: Aileen

Structure based design of bicyclic peptide inhibitors of RbAp48

Peter't Hart ,. . . and Herbert Waldmann*

Angew. Chem. Int. Ed. **2020**, DOI: 10.1002/anie.202009749



RbAp48 is a histone binding protein, which is found to be overexpressed in cancer types including breast cancer, thyroid carcinomas, hepatocellular carcinoma and colon cancer. The authors present a structure-based approach for the development of a bicyclic peptide which inhibits the RbAp48-MTA1 PPI, which is intended as a tool compound to study RbAp48 function.

The design strategy identifies a peptide sequence from MTA1 as a starting point and looks to the co-crystal structure with RbAp48 for inspiration. An initial hit is found via cyclisation, which reduces entropy loss upon binding and maintains hydrophobic interactions with RbAp48 via a benzylic linkage moiety. Co-crystallisation of RbAp48 with hit compound peptide **8** (IC_{50}=15.1 nM) informs further optimisation, enabling the identification of a suitable linkage point for the synthesis of a bicyclic analogue. Bicyclic peptide **33** was shown to be more active than **8** via ITC and showed greater stability in MDA-MB-231 cell lysate. Modification of **33** with an N-terminal TAT sequence (peptide **49**) was necessary to investigate the mode of action via a morphological cell painting assay. This indicated that the compound may cause an increase in the levels of tumour suppressor protein p53. This was validated by Western blot analysis of U2OS cells treated with **49** (treatment with TAT peptide alone elicited no response).

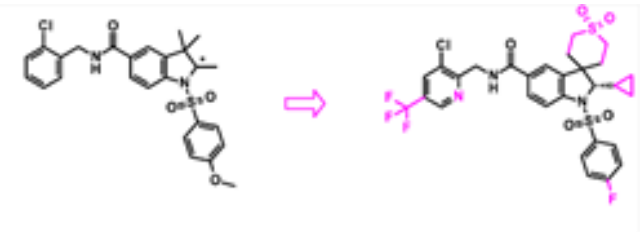
This paper is a nice example of rational inhibitor design which makes full use of structural information for both the inspiration and optimisation phases. The authors are able to show proof of concept that developed bicyclic peptides cause an increase in tumour suppressor protein, however the poor cell permeability of lead compound **33** limits further applications. As the authors suggest, TAT-modified peptide **49** may find use as a tool compound for further biological studies, and the bicyclisation approach could be applied to design further tool compounds for other PPIs.

Contributor: Will

Discovery and Characterization of BAY 1214784, an Orally Available Spiroindoline Derivative Acting as a Potent and Selective Antagonist of the Human Gonadotropin-Releasing Hormone Receptor as Proven in a First-In-Human Study in Postmenopausal Women

Olaf Panknin, … Gernot Langer*

J. Med. Chem. **2020**, *63, 11854*



Uterine fibroids (also known as uterine leiomyomas) are a common benign tumour type whose growth is sex-hormone dependent. Despite 20-50% of women of reproductive age presenting with leiomyoma related disease treatment options are limited. Gonadotropin-releasing hormone receptor (GnRH-R) has long been considered a viable drug target in this area due to its role in the production of gonadotropins Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH), which in turn also regulate estradiol and estrogen production. Currently approved and/or clinically evaluated GnRH antagonists, whilst efficacious, have shown adverse effects such as hot flushes, headache and loss of bone mineral density consistent with full GnRH antagonization and a drastic blockade of estrogen production. In this study scientists from Bayer aimed to identify a small molecule drug with superior PK/PD characteristics capable of yielding partial lowering of estradiols only. Starting with a cellular screen of a 2.5 million compound library they identified an indoline hit with weak activity against both human and rat GnRH-R. The ability to optimise equipotency across species was seen as a key facet to enable *in vivo* optimisation. The authors first incorporated a spiropiperidine to give a spiroindoline based molecule, a fragment considered to be a GPCR privileged structure. Such molecular cores are also well known to often induce human ether-a-go-go (hERG) potassium ion channel liabilities. In line with this whilst observing >10-fold improvements in GnRH potency the spiropiperidine based compounds also demonstrated significant hERG activity. A subsequent stepwise optimisation of all substituents of the indoline core, including switching the spiropiperidine for a cyclic sulfone, addressed a number of challenges including improvements in solubility, CYP liability, metabolic stability and hERG activity. Ultimately this optimisation approach yielded a clinical candidate that has demonstrated suppression of LH levels in post-menopausal women with good tolerability up to 450 mg po once daily.

This is a textbook med chem optimisation rich in examples of how series-specific liabilities can be addressed. This is a target that has long been pursued by a number of large (and small) organisations. Currently approved GnRH-R antagonists from Takeda and AbbVie have finally managed to start paving the way for effective small molecule therapies but there is much room for improvement that the authors seek to achieve here.