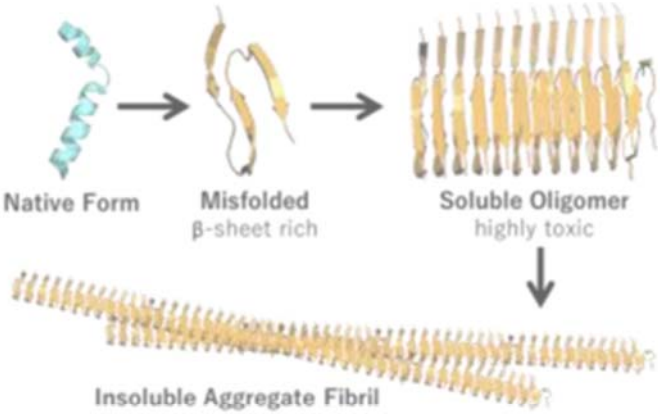
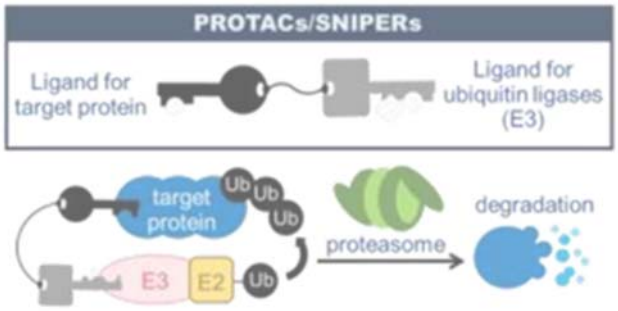
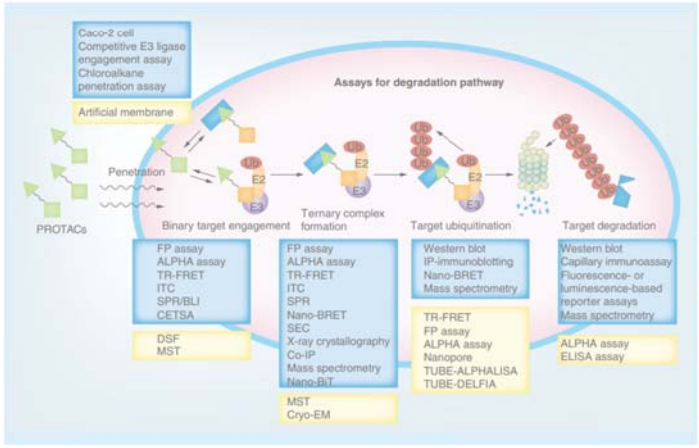
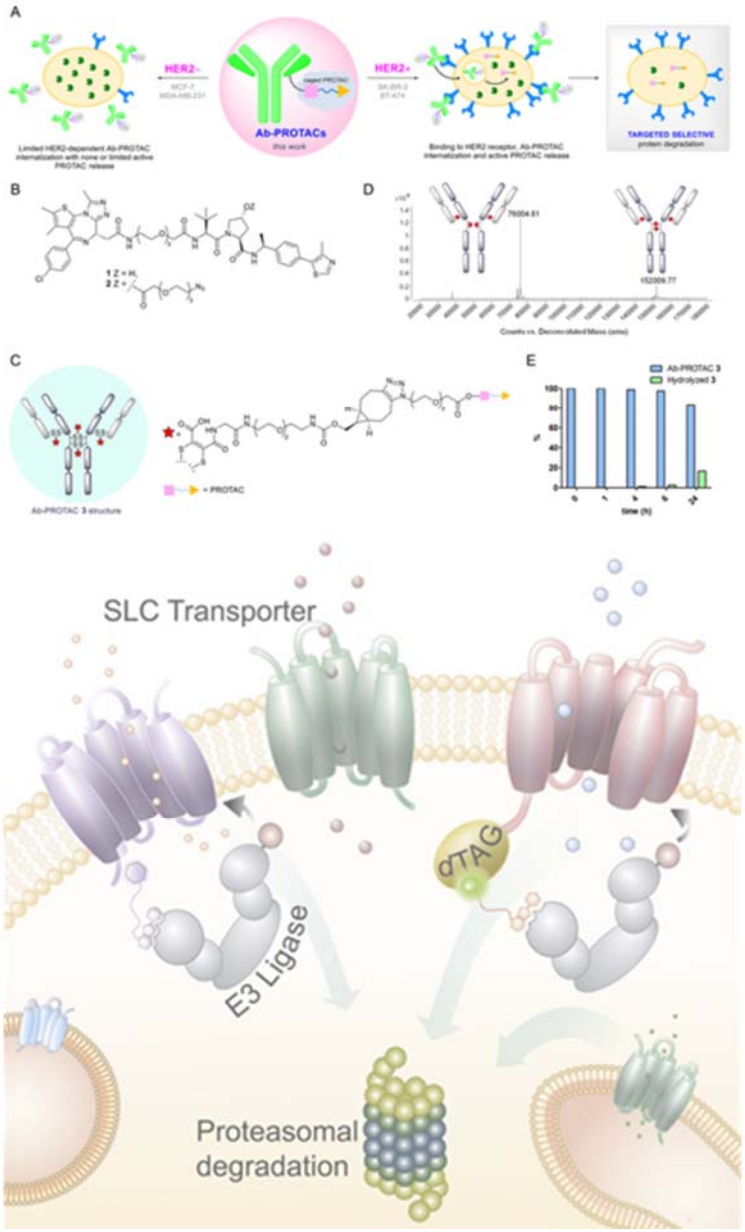
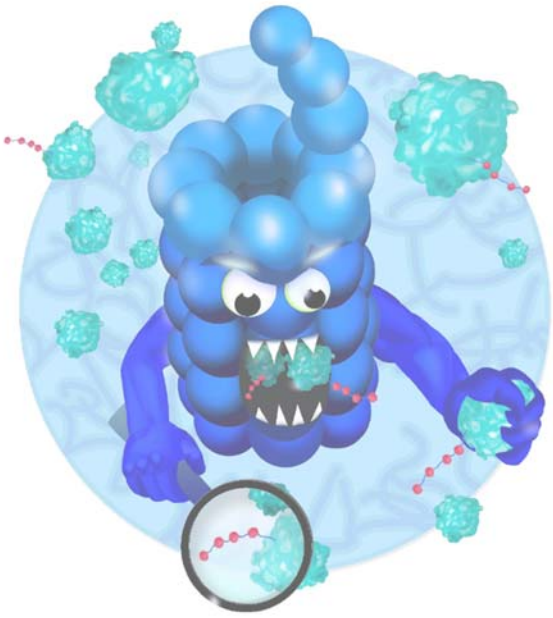










Ciulli Group Journal Club

Targeted Protein Degradation, Medicinal Chemistry and Chemical Structural Biology Literature Highlights

May 2020 Edition

Ciulli Group Journal Club Contents

Landmark Feature: In Memoriam Professor Huib Ovaa

Contributor: Alessio

On Wednesday May 20th, while browsing my twitter feeds, I picked by chance an odd one by chemical biologist Sander van Kasteren, quoting "I hate this year. I am going to deeply miss my old mentor and friend", and a link below titled "In Memoriam Professor Huib Ovaa". With a sense of incredulity, I opened the link. Gone to prostate cancer, at the age of 46. What a shock. I could not believe it. Huib and I were together at a Ubiquitin meeting in Bilbao at the end of February, just as the coronavirus was spreading worldwide and right before the subsequent lockdown. As ever, we talked science at that meeting, sat together at dinner enjoying good food and wine. We were so looking forward to collaborating as part of the soon-to-be launched EUbOpen consortium. That was one of a handful of conferences where our paths crossed over the past few years. I so vividly recall when he accepted my invitation to give a talk at a protein degradation session I organized for the 2017 Chinese Pharmaceutical Association (CPA) meeting, jointly with EFMC, which was held in Bejiing and hosted >1,700 attendees. We also lectured in the same session at the 2019 EFMC-ACSMEDI meeting in Krakow last summer. We talked PROTACs, DUBs, proteasome, chemical probes, and his strong passion and curiosity for science always struck me. I did not know he was unwell, and it certainly did not look like that. Gone way too soon. We all lost a creative scientist, a colleague and a friend, and our thoughts are with his beloved ones. RIP Huib Ovaa.

Huib was an accomplished figure and a creative scientist who used chemical approaches to study the ubiquitin system. With his colleagues, he discovered the OTU family of de-ubiquitinating enzymes and pioneered cell permeable activity-based probes to study proteasomal and DUB activity and inhibition. Huib and his research group made many impactful discoveries in the fields of chemical biology and ubiquitin-proteasome system. Their powerful and widely used chemical probes of UPS components such as proteasomes and DUBs will be amongst many of his lasting legacies to the scientific community. He founded the biotech company UbiQ which commercializes his reagents amongst others. He served on the scientific advisory board of the journal Cell Chemical Biology (Cell Press) and was editor of Frontiers Chemical Biology. Huib (co-)authored over 200 scientific papers (189 on PubMed) and patent applications, with over 13,000 citations, and an h-index of 63 (Google Scholar). This month's landmark paper will be at the reader's choice amongst the many articles that Huib published in his distinguished career.

Special Feature: CHIP Collective – Living with cancer, our stories

Contributor: Vesna

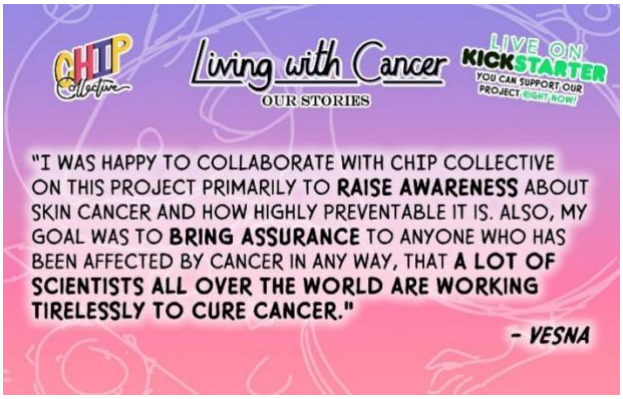
Kickstarter-funded charity comic anthology raising awareness about cancer

: @chip_collective

Last year I moved to Dundee and met my neighbour Ashling Larkin. At the time, she was working on creating an educational and helpful comic book about different aspects of living with cancer. It was a tribute to her mom Walkiria who passed away after a short battle with stage IV glioblastoma. Ashling wanted to create an educational, comforting, and simple comic book that could help anyone who is struggling or feeling lost in battling cancer.

The comic book comprises of stories of six different people and how cancer impacted their lives in different ways. The story of Ashling's mom is about the uncertainty and confusion her family felt after she was diagnosed. They didn't know what to expect or what services were available to them. Many people that spoke to them had misconceptions about cancer and didn't realise how different cancers require different treatments or how they can affect people in a variety of ways.

I was very happy to tell my part of the story as a freshly minted cell/cancer biologist. At the time I was so busy with finishing lab work, submitting the thesis, moving to another country, and starting a new job. Participating in creating this comic reminded me of the bigger picture and how my (our) work is important. It is easy to get lost in everyday tasks and issues and lose motivation and stories like these can inspire us to be better scientists. I hope my part of the comic also sent a message to the wider audience, one that maybe isn't very obvious to everyone outside of the scientific community: scientists all over the world are working very hard and are very dedicated to finding new treatments for cancer. And, we are in this together: collaboration between different disciplines of science, medicine, and pharma is crucial in tackling a heterogeneous disease that is cancer.



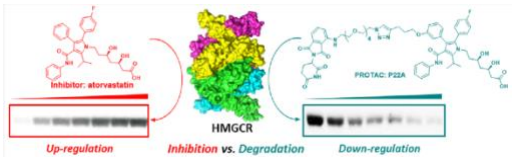
Targeted Protein Degradation

Contributor: Aileen

Degradation versus Inhibition: Development of Proteolysis-Targeting Chimeras for Overcoming Statin-Induced Compensatory Upregulation of 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase

Mei-Xin Li, . . . , Jie Luo*, Yu Rao*

J. Med. Chem. **2020**, *63*, 4908



Despite being effective drugs for treating cardiovascular disease, statins also cause upregulation of HMGCR protein. This can cause problems for patients, such as skeletal muscle damage. The authors envisage that degradation of HMGCR protein using PROTAC methodology could help overcome this issue. Reduction in HMGCR protein level was seen upon treatment with PROTAC P22A. Cholesterol biosynthesis was also blocked and less compensatory upregulation of HMGCR was observed. This study demonstrates proof of concept that elimination of HMGR via degradation may have therapeutic potential over inhibition by statin treatment alone.

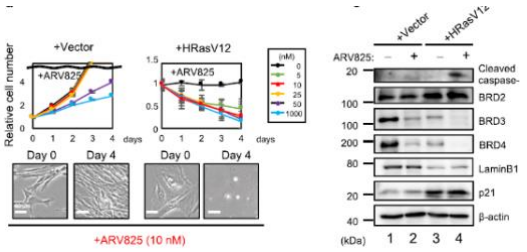
PROTAC design is based on the conjugation of the statin atorvastatin to the CRBN binder. The authors propose that the statin warhead will inhibit HMGCR activity, with the CRBN ligand recruiting CRBN to cause ubiquitination and subsequent degradation of upregulated HMGCR. The best compound, P22A (DC_{50} = 0.1 μM), is shown to inhibit cholesterol biosynthesis with similar potency to the parent drug, atorvastatin.

Contributor: Aileen

A BET family protein degrader provokes senolysis by targeting NHEJ and autophagy in senescent cells

Masahiro Wakita, . . ., Eiji Hara*

Nat. Commun. **2020**, *11*, 1935. DOI: 10.1038/s41467-020-15719-6



The authors use high-throughput screening in their aim to find new senolysis drugs. Senolysis describes the process of elimination of senescent cells, which accumulate with age and can cause chronic inflammation or tumorigenesis. The best senolytic agent arising from the screen of 47,000 compounds was the Arvinas BET degrader ARV825, which resulted in cell death at 5-10 nM concentration. A study into elucidating the mode of action of ARV825 follows, identifying BRD4 as the senolysis target. It is concluded that degradation of BRD4 exacerbates DNA double strand breaks (DSBs) causing inhibition of the non-homologous end joining (NHEJ) machinery, and activates the autophagy machinery, thus promoting senolysis.

In vivo studies show that ARV825 reduces liver cancer development in obese mouse livers and, in a xenograft tumour study, can increase the efficacy of a chemotherapeutic agent. The authors caution that healthy cells that rely on NHEJ, such as non-dividing quiescent cells, may be affected by this approach. However, at the concentrations of ARV825 used, cell death of quiescent cells was not observed.

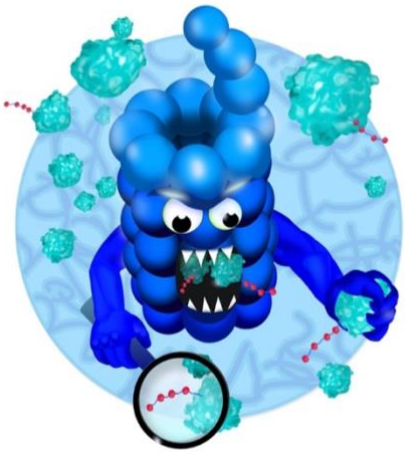
Contributor: Alessio

Prey for the Proteasome: Targeted Protein Degradation – A Medicinal Chemisť s Perspective

Laura M. Luh, ….. , Philipp M. Cromm*

Angew. Chem. Int. Ed. **2020**, DOI: 10.1002/anie.202004310

This review covers with adequate breadth and depth established and emerging TPD approaches, primarily focusing on those involving protein degradation induced by small molecules. It is well-written and well supported by nice graphics - including an icon-style Table 1 that provides a useful overview of pros and cons as well as scope of various TPD methods. It contains a good coverage of the prior literature while still allowing the authors to give their perspective and viewpoints, which add value to the piece. Figure 7 exemplifies this last point by providing the author's view on a representative PROTAC screening cascade – an area where many organizations differ in flavour and tactics. Cool frontispiece graphics – that's for sure!



There are numerous review pieces that emerge on a regular basis in the burgeoning TPD field, so it is difficult to find ways to make one stand out. This is an excellent and timely perspective that achieves this task in many different ways so should attract attention amongst the many out there already.

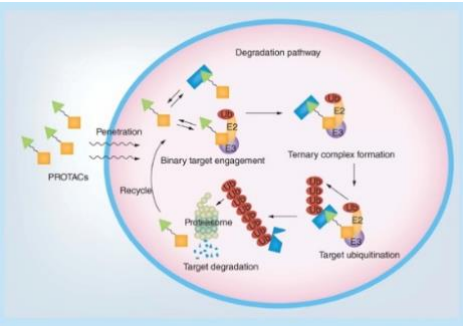
Contributor: Alessio

Assays and technologies for developing proteolysis targeting chimera degraders

Xingui Liu, ….. , Daohong Zhou*

Future Med. Chem. **2020**, DOI: 10.4155/fmc-2020-0073

Comprehensive review of the palette of assays and technologies that have been developed to date to characterise PROTACs' mechanism of action en-route from entering cells to protein degradation. Covers broadly a range of relevant assays – from cell permeability and cellular target engagement, to biophysical techniques to detect and measure binary and ternary complexes (FP, Alpha, TR-FRET, ITC, SPR, SEC etc.), ubiquitination and degradation assays.

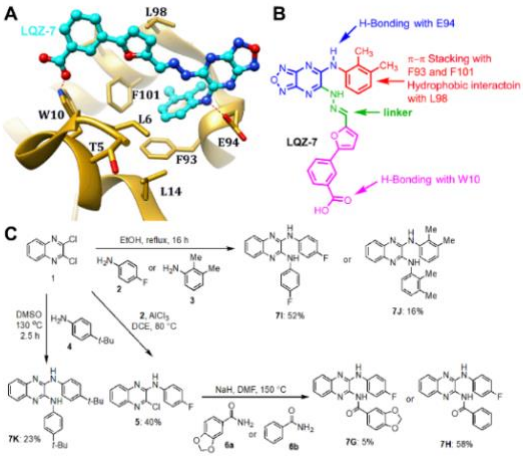


This review covers very familiar territory right up our street! I congratulate the authors on doing a fine job with it. I like the extensive referencing of key papers that developed or applied each assay, and the inclusion of the chemical structures of key PROTACs used in those individual studies. A good reference for the Introduction sections of students' PhD theses for sure.

Contributor: Aileen

Synthesis and identification of a novel lead targeting survivin dimerization for proteasome-dependent degradation

Robert C. Peery, . . ., Jian-Ting Zhang* *J. Med. Chem.* **2020** DOI: 10.1021/acs.jmedchem.0c00475



This study targets survivin, a protein which is commonly overexpressed in solid tumours and is necessary for cancer cell survival, but which has been considered "undruggable". The strategy involves inhibition of survivin in order to disrupt protein dimerisation, which leads to proteasomal degradation. The authors describe the optimisation of one of their previously developed inhibitors, LQZ-7: a number of simplified analoges of LQZ-7, are made and tested. The authors are successful in their primary goal of replacing the hydrazone and furazanopyrazine motifs in the parent compound, giving 7I which has increased activity (IC_{50} = 3.1 µM vs IC_{50} = 9.1 µM, C4-2 cells). This discovery will be used in further optimisation studies towards monomeric degraders of survivin.

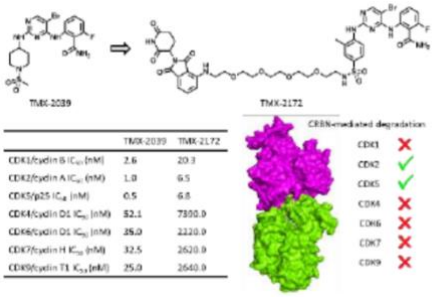
This reads as a clear and well thought out study. Improved inhibitory effect of 7I tracks to reduced levels of survivin protein. The authors verify that the compound can induce apoptosis, and supress tumour growth in xenograft studies in mice. It is noted that the compound was dissolved in corn oil for oral administration. Parent compound LQZ-7 has previously been shown to cause apoptosis and subjected to xenograft studies, see:Cancer Res. ***2016****, 76, 453.*

Contributor: Aileen

Development of CDK2 and CDK5 Dual Degrader TMX-2172

Mingxing Teng[§], Jie Jiang[§] , . . ., Tinghu Zhang*, Nathanael S. Gray*

Angew. Chem. Int. Ed. **2020** DOI: 10.1002/anie.202004087



Gray and co-workers demonstrate selective degradation of CDK2 and CDK5 by PROTAC TMX-2172. Some cancers, including high grade serious ovarian cancers (HGSOC), have shown vulnerability to inhibition of CDK2. However, inhibition of the closely related kinase CDK1 causes toxic effects, and thus development of selective CDK2 inhibitors has been problematic. It is demonstrated that treatment with TMX-2172 causes degradation of CDK2 and CDK5 in Jurkat cells, and inhibits growth rate in ovarian cancer cell line OVCAR8 (GR_{50} = 33 nM).

The development of a PROTAC that shows ~3 fold inhibitory selectivity for CDK2 over CDK1 leads to selective degradation of CDK2. The authors demonstrate that degradation of CDK2 is responsible for anti-proliferative effects in OVCAR8 cells. Proteomic analysis is undertaken for further proof of the degradation selectivity of TMX-2172. The discovery of a selective CDK2 degrader warrants further investigation to assess the therapeutic potential of this strategy.

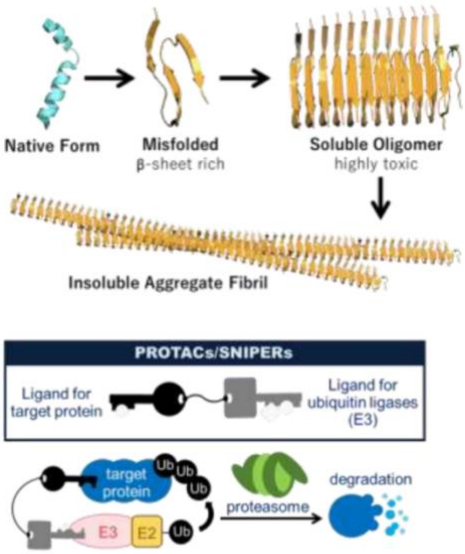
Contributor: Aileen

Recent Progress in PROTACs and Other Chemical Protein Degradation Technologies for the Treatment of Neurodegenerative Disorders

Shusuke Tomoshige, Minoru Ishikawa*

Angew. Chem. Int. Ed. **2020** DOI: 10.1002/anie.202004746

This mini-review discusses current progress in the application of protein degradation to the treatment of neurodegenerative disorders (NDs) and outlines the challenges and current limitations of the field. Protein degradation is promoted as a worthy approach, as the underlying cause of NDs is misfolded protein aggregates. These are considered undruggable via a traditional inhibition approach as protein aggregates are by definition not performing a function which can be inhibited. A number of strategies are covered, including peptidic PROTACs, small molecular PROTACs, targeted autophagy inducers and hydrophobic tagging.



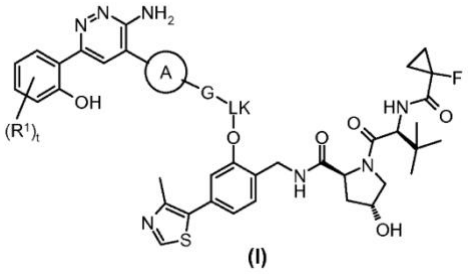
This review gives an interesting overview of the field and features a particularly nice conclusion, which outlines the key challenges along with a critical appraisal of the relative advantages and disadvantages of current aproaches.

Contributor: Aileen

Proteolysis Targeting Chimera (PROTACs) as Degraders of SMARCA2 and/or SMARCA4

Alessio Ciulli, Christian Dank, . . ., Steffen Steurer, Nicole Trainor WO 2020/078933

A patent from the Dundee-Boehringer Ingelheim collaboration detailing the degradation of SMARCA2 and/or SMARCA4, ATPases found in the BAF complex whose mutations are implicated in lung, liver and colon tumours. Conjugation of a SMARCA2/A4 bromodomain ligand to a VHL ligand results in PROTACs which are shown to degrade SMARCA2/A4 protein with reported DC_{50}s in the range 10-1000 nM with Dmax >80% in most cases, in A549 cells.

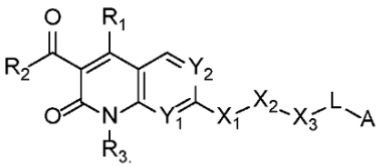


Degradation of SMARCA2 and SMARCA4 has been demonstrated. Congratulations to everyone involved!

Contributor: Aileen

Proteolysis-Targeting Chimeras

Joseph Savino, Bruno Calabretta, Marco De Dominici, You Cai Xiao WO 2020/092662



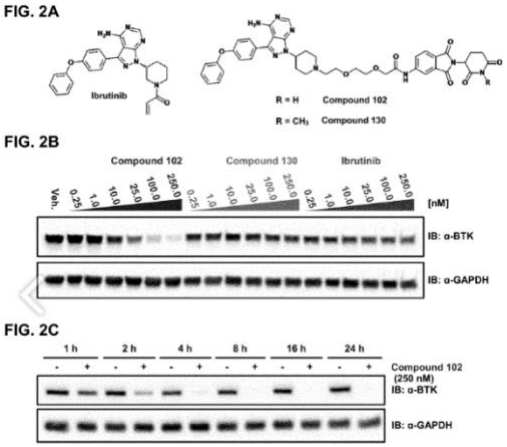
Scientists from The Wistar Institute of Anatomy and Biology and Thomas Jefferson University have published a patent which details the targeted degradation of CDK4 or CDK6, with a focus on delivering PROTACs which have improved efficacy, selectivity, and bioavailability for the treatment of cancers, including leukemia. Conjugation of CDK6 inhibitor palbociclib to a CRBN E3 ligase binder gave a PROTAC which selectively degraded CDK6, whereas MDM2 based PROTACs were equally efficient degraders of CDK4 and CDK6.

In vivo experiments showed slower disease progression in mice treated with the PROTAC verses the inhibitor. CDK6 selective PROTACs are expected to overcome toxicity associated with dual CDK4/6 inhibitors, which have caused neutropenia in breast cancer patients.

Contributor: Aileen

Modulators of BTK Proteolysis and Methods of Use

Craig M. Crews, Saul Jaime-Figueroa, Momar Toure US 20200121684



Discoveries from the Crews laboratory detailing the degradation of BTK, a target for the treatment of chronic lymphocytic leukemia, are covered in this patent. Covalent inhibitor ibrutinib has shown efficacy as a treatment, however patients can experience relapse. This has been attributed to C481S mutated BTK, which no longer allows for the irreversible inhibition by ibrutinib required for effective treatment. The authors have shown that CRBN-recruiting PROTACs based on the ibrutinib scaffold successfully degrade both wild type and C481S mutant BTK. Lead compound ***102*** *has a* DC_{50} *of 9.1 nM, and exhibits no hook effect up to 2.5 µM. A* D_{max} *of >99% is reported at 250 nM for this compound.*

The acrylamide motif within Ibrutinib is essential for its irreversible binding to the target, but is also responsible for off-target inhibiton of other kinases, including ITK. PROTAC 102 exhibited better target specificity, with no degradation of ITK observed upon treatment of Jurkat cells.

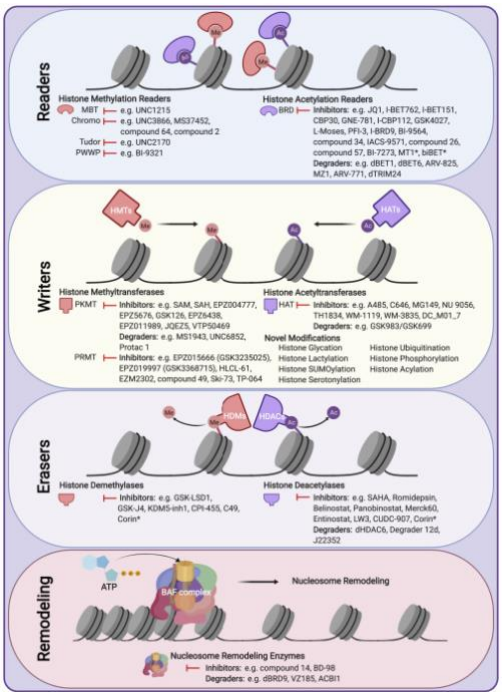
Contributor: Alessio

Using Chemical Epigenetics to Target Cancer

Virangika K. Wimalasena,[§] Tingjian Wang,[§] Logan H. Sigua,[§] Adam D. Durbin,* Jun Qi*

Mol. Cell **2020**, DOI: 10.1016/j.molcel.2020.04.023

This is an excellent review of the state of the art in the growing field of cancer chemical epigenetics. It covers with clarity and breadth the compelling case for targeting transcriptional processes in cancer by means of complementary chemical biology approaches. A nice focus of the review is on differential pharmacology that can be illuminated via comparing and contrasting domain inhibitors versus degraders of the full-length target protein. Particularly useful to the community will be the detailed and well-organized Supporting Table listing the available arsenal of inhibitors and degraders, catalogued by target type: writers, readers and erasers of chromatin post-translational modifications, as well as transcription factors and chromatin remodelling complexes. The narrative is readable and nicely illustrated and the targets and chemistry covered strike a good balance between comprehensiveness and selectivity.



Although not strictly a TPD article, epigenetic proteins account for some of the most successful PROTAC targets right since the 2015 papers on BET PROTACs and this is evidently accounted in this nice piece.

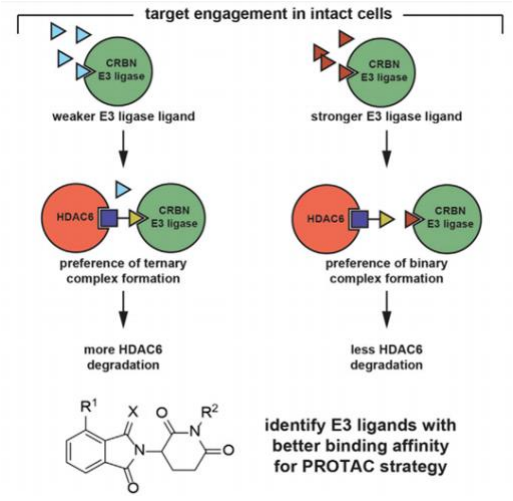
Contributor: Conner

A Cell-Based Target Engagement Assay for the Identification of Cereblon E3 Ubiquitin Ligase Ligands and Their Application in HDAC6 Degraders

Ka Yang[§], Yu Zhao[§],…, Haibo Xie*, and Weiping Tang*

Cell Chem. Biol. **2020**, DOI: 10.1016/j.chembiol.2020.04.008

A large proportion of published PROTACs have relied upon thalidomide and its derivative ligands to recruit CRBN E3 Ligase to a target protein. However, PROTACs based on thalidomide and its derivatives, such as pomalidomide and lenalidomide, can still degrade some neo-substrates, including IKZFs and GSPT1. For this reason, it is vital to expand upon the current toolbox of ligands of E3 ligases for use in targeted protein degradation. This is often achieved using in-vitro cellular techniques, but these assays involve the expression and purification of a large amount of proteins that often yield ligands that are inactive in cell-based assays due to poor cell permeability and stability among other reasons. In this research, they demonstrate the development of a competitive cell-based target engagement assay that they used to evaluate CRBN ligands. This approach functions via an HDAC6 degradation readout that can be analysed using western blot and mass



spectrometry. The ligands identified using this approach are then subsequently used in the development of successful HDAC6 degraders.

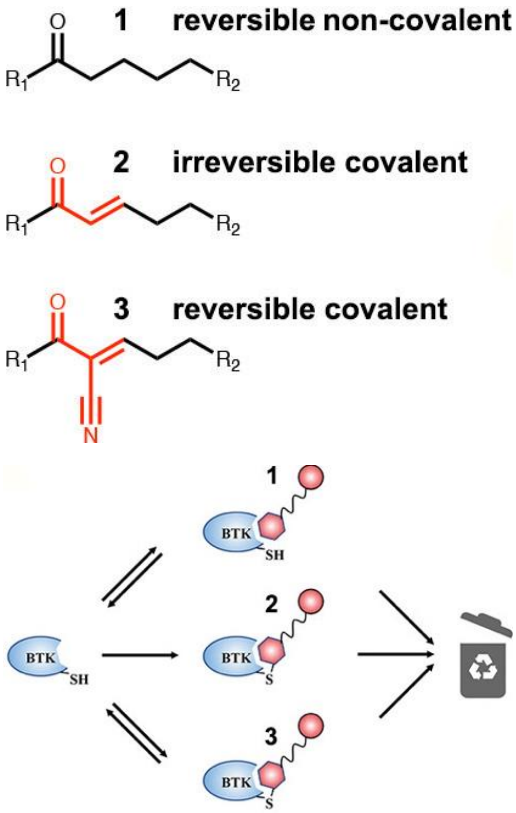
This is an interesting paper that demonstrates an approach for obtaining cell-based ligand-E3 ligase target and off-target binding data using a competitive PROTAC-mediated approach. A clear benefit of this approach is that unlike some other cell-based approaches, such as fluorescent tagging, the protein target doesn't require gene modification. One limitation of this approach, however, is that validated PROTACs for a target E3 ligase binding pocket must be available before it can be implemented towards identifying novel ligands for that binding pocket.

Contributor: Alessio

Efficient targeted degradation via reversible and irreversible covalent PROTACs

Ronen Gabizon, ….. , Nir London*

J. Am. Chem. Soc. **2020**, DOI: 10.1021/jacs.9b13907



London and colleagues compare non-covalent, covalent and reversibly-covalent PROTAC degraders of BTK, using Ibrutinib as the target ligand. The authors design a small series of reversible-covalent CRBN and VHL based PROTACs, and following selection of one of these as starting hit, they go on to design analogues where they vary the warhead group to explore the three regimes at targeting BTK: reversible covalent (RC: cyano-acrylamide); irreversible covalent (IC: acrylamide); and a non-covalent (NC) saturated alkyl analogues. To qualify the degraders, they perform degradation assays, kinase inhibition and covalent labelling assays, and cellular assays to assess proteome-wide selectivity and potency in primary B-cells. The key findings are: 1) IC PROTACs can be potent degraders due to their reversible binding component driving faster target ubiquitination/degradation than covalent bond formation; 2) RC PROTACs can be much less potent than the corresponding IC ones. However, they include one RC example that appears to be a potent degrader due to rapid covalent binding. While the NC PROTAC in this particular case was found to be the most potent compound class, the authors argue that there could be room for applications of IC and RC PROTACs on challenging targets.

The insights from this study are interesting, timely and significant because there exists some controversy in the field regarding how being covalent at the target of interest impacts target degradation, due to the non-catalytic nature of the interaction. The findings in this paper somewhat challenge previous observations that covalency on BTK hampered PROTAC-induced degradation (see ACS Chem. Biol. 2019, 14, 3, 342–347) – with the caveat that the compound structures were different between the studies. It is also relevant that two other manuscripts appeared online around the same time the London study was under review: Guo et al. BioRxiv, posted 30 Dec 2019, which describes a reversible-covalent PROTAC degrader of BTK with single-digit nanomolar DC50; and Xue et al. Chem Commun 2020, 56, 1521, which also showed potent covalent PROTAC degraders (for a recent review on BTK

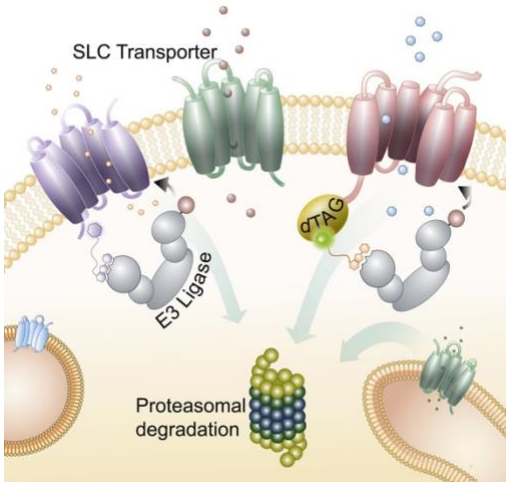
PROTACs see Arthur R et al. Explor Target Antitumor Ther. 2020 doi: 10.37349/etat.2020.00009). Such competitive situations occur not infrequently, particularly in the PROTAC field these days, and it is great to see a journal such as JACS giving authors a fair chance to report their work when submitting around the same time as the others.

Contributor: Alessio

Targeted Degradation of SLC Transporters Reveals Amenability of Multi-Pass Transmembrane Proteins to Ligand-Induced Proteolysis

Ariel Bensimon, ….. , Giulio Superti-Furga *

Cell Chem. Biol. **2020**, DOI: 10.1016/j.chembiol.2020.04.003



The solute carrier proteins (SLCs) regulate transport of key metabolites and are emerging as important targets because they sustain growth and function of highly-metabolically active cancer cells and T-cells. With over 450-members, SLCs are the second largest family of multi-pass transmembrane proteins (after GPCRs). Because of their extensive embedding within biological membranes, it had been questioned whether such class of proteins could be amenable to targeted protein degradation. Bensimon et al. set out to address this problem. They use the dTAG technology (HA-dTAG SLCs stably expressed in HAP1 cells) to show SLCs undergo TPD via the expected mechanism of action. They observe some dependency on subcellular localization e.g. SLCs located at the plasma membrane are most amenable, while those located at the inner mitochondria or Golgi compartments were less likely to undergo complete degradation. To demonstrate TPD of an endogenous SLC target, they first establish N-terminal dTAG knock-in of one SLC (SLC38A2) and show rapid PROTAC-induced degradation. They then leverage a previously reported ligand for SLC9A1 (also known as NHE1) and design a small series of five PROTACs of varying linkers length / composition – the most potent being PEG4. PROTAC d9A-2 potently induced rapid degradation of SLC9A1 and of several other members of the SLC9 family. Finally, they show that d9A-2 induced target degradation has functional consequence by potently and CRBN-dependently impairing the ability of cells to recover from acid load, and tested the degrader's cytotoxicity in a panel of cancer cell lines.

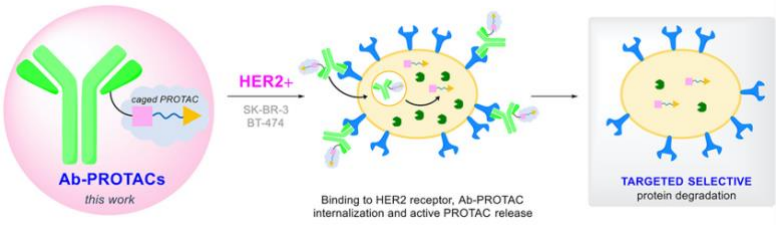
This paper is significant in that it establishes perhaps for the first time that multi-pass transmembrane proteins from different cellular localization are suitable to TPD. This was a non-trivial task considering the historic lack of good ligands for the intracellular portions of membrane proteins. Interestingly the warhead ligand they used was described over a decade ago (Atwal et al., BMCL, 2006). In that study, it was reported as pretty selective for NHE-1 over NHE-2 (SLC9A2) – so it is interesting that the resulting PROTAC appears to induce degradation of several members of the SLC9 family. Development of paralogue-selective degrader tools will be an important avenue in future, likely to be significantly fostered by development of selective tools for individual SLC members, which is one of the main thrusts of the large IMI-funded consortium ReSOLUTE, headed by Superti-Furga. From a technical point of view, I found it interesting how they report the area over dose response as a proxy for potency in cell viability assay, see last Figure in the Supp. Info.

Contributor: Alessio

Antibody-PROTAC conjugates enable HER2–dependent targeted protein degradation of BRD4

Maria Maniero, ….. , Edward W. Tate*

ACS Chem. Biol. **2020**, DOI: 10.1021/acschembio.0c00285



The recent PROTAC-ADC theme continues with this paper showing use of a PROTAC as cytotoxic payload for cell-specific TPD. Maneiro et al. derivatized a VHL-based BRD4 PROTAC (MZ1 with the extra methyl group on the VHL-ligand's benzylic position) at its Hyp-OH position with an anti-human epidermal growth factor receptor 2 (HER2) monoclonal antibody (trastuzumab). To do so, they leverage an elegant, previously reported maleimide-based antibody conjugation strategy. After evidencing the chemical identity and stability of their PROTAC-ADC at physiological pH, they show that it induces dose-dependent degradation of BRD4 after 4 h incubation in HER2-positive SK-BR-3 and BT-474, but not HER2-negative MCF-7 and MDA-MB-231 breast cancer cells. They then generate a fluorescently-labeled ADC-PROTAC version to track its receptor mediated internalization. They show using confocal microscopy that their AlexaFluor-ADC-PROTAC conjugate accumulates on the cell surface of HER2+ (but not HER2–) cells within 1 hour, and internalizes and colocalizes with lysosomes already at 4 h, and then more extensively at later time-points. They therefore postulate that hydrolysis in the more acidic lysosomal environment must release the active PROTAC to induce the observed BRD4 degradation.

This is a very nice study. It follows suit from earlier studies published last year by Genentech that demonstrated first proof-of-concept of the ADC-PROTAC idea with BET degraders conjugated to anti-CLL1 (Pillow et al. ChemMedChem 2019) and estrogen receptor alpha degraders conjugated to anti-HER2 (Dragovich et al., BMCL, 2020). Together these studies support the potential of combining PROTACs as potent, highly cytotoxic payload with the cancer cell-specific uptake of antibodies, as a strategy to enhance cell-type specificity and widen drug therapeutic index. It is interesting that in the Maneiro et al. study, the corresponding free MZ1-analogue PROTAC also appeared to exhibit more potent BRD4 degradation in HER2+ cells compared to HER2– cells, even without antibody conjugation. For future mechanistic studies it will be important to compare ideally between isogenic cell lines e.g. wild-type and receptor KOs, as different cell lines can respond very differently to PROTAC-induced degradation.

Others

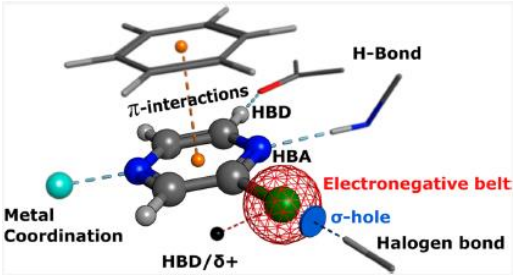
Contributor: Emelyne

Molecular interactions of Pyrazine-Based Compounds to Proteins

Martin Juhás and Jan Zitko*

J. Med. Chem. **2020**, DOI: 10.1021/acs.jmedchem.9b02021

Pyrazine-based compounds are of great importance in medicinal chemistry. Due to their heteroaromatic nature, they uniquely combine properties of heteroatoms (polar interactions) with the properties of aromatic moieties (nonpolar interactions). This review summarizes results of a systematic analysis of RCSB PDB database focused on important binding interactions of pyrazine-based ligands cocrystallized in protein targets



Well written review based on ~200 PDB entries that highlights the interactions of a common core in medicinal chemistry. It shows that pyrazine shouldn't be considered as an isosteric replacement for benzene but rather as a significant H-bonding partner. It also features links to reviews of interest to all wishing to expand their knowledge on interactions observed in crystal structures.

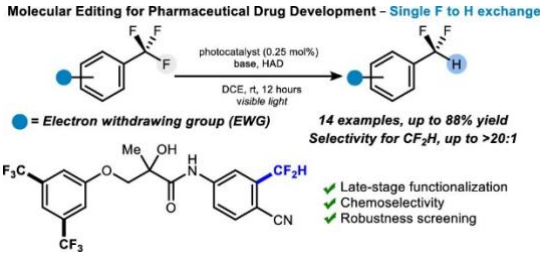
Contributor: Siying

Organophotoredox Hydrodefluorination of Trifluoromethylarenes with Translational Applicability to Drug Discovery

Jeroen B. I. Sap, …., Véronique Gouverneur*

J. Am. Chem. Soc. **2020**, DOI: 10.1021/jacs.0c03881

The difluoromethyl group (-CF_2H) acts as a lipophilic H-bond donor and has been used as a bioisoster for thiol and hydroxyl groups. The Gouverneur group reported the development of a reductive defluoronation procedure for the synthesis of electron-poor difluoromethylarenes and the application of this methodology for selective late stage functionalisation on several drug molecules.



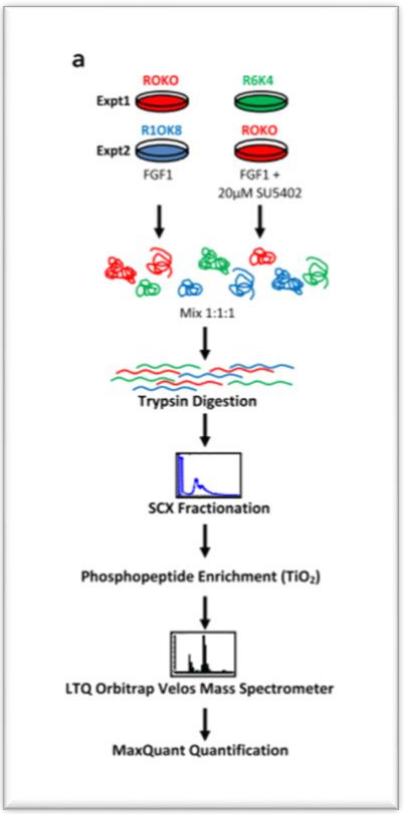
Starting from known photoredox defluorination conditions, the authors identified the use of 4-hydroxythiophenol as a hydrogen atom donor to achieve good selectivity towards CF_2H. The reaction tolerates a wide range of functional groups (nitrile, fluorine, methoxy, acetamide and sulfonamide) to generate desired molecules with moderate to good yield (34 – 93%) and CF_2H selectivity (from 5:1 to > 20:1 CF_2H/CH_2F). Poor selectivity (~3:1) is observed in substrates containing an ester moiety para to the CF_3 group. The tolerability of the reaction conditions is further demonstrated by the additive-based screening using heterocycles frequently seen in medicinal chemistry. Complete chemoselectivity (alkyl vs arene, arene vs arene) are demonstrated by late stage functionalisation of several drug molecules. The utility of this methodology is further illustrated with continuous flow conditions and the authors proposed a reductive quenching cycle as a plausible pathway based on their mechanistic studies.

Contributor: Vesna

Differential responses to kinase inhibition in FGFR2-addicted triple negative breast cancer cells: a quantitative phosphoproteomics study

Debbie L. Cunningham* ..., John K. Heath*

Scientific reports **2020**, *10*, 7950. DOI: 10.1038/s41598-020-64534-y



Many forms of cancer involve manifestations of aberrant fibroblast growth factor receptor 2 (FGFR2) signalling. Given the prevalence of FGFR genetic lesions in human tumours, the pathway has become a target for drug development. Despite exhibiting potent inhibition of FGFR kinase activity in preclinical models, clinical trials with these agents have displayed weak efficacy. Also, clinical responses are heterogeneous and vary according to the type of genetic lesion. There is, for example, increasing evidence that small molecule inhibition is most effective in the cases of tumours harbouring FGFR amplifications and fusions but less in the case of point mutations. A central regulatory mechanism in these networks is post translational modification by protein phosphorylation of crucial regulatory sites that control protein activity, associations, localisation and abundance. This is the core mechanism that controls the biological behaviour of the tumour. Therefore, analysing patterns of protein abundance and phosphorylation gives direct access to the activity status of the pathway in the tumour, allows stratification based on pathway activity, and identifies key nodes for therapeutic targeting and novel nodes for drug development.

In this study the authors compare the proteome and phospho-proteome of two FGFR-signalling addicted breast cancer cell lines before and after the treatment with FGFR kinase inhibitor SU540232. By utilising bioinformatic analysis the authors identified different groups of kinases active in two breast cancer cell lines after the treatment with SU540232. By comparing 1385 phosphosites found in both cell lines, the authors have identified different responses to SU540232 inhibition. This study reveals that even though two cell lines share common histopathological classification and FGFR-addiction they differ significantly in their response to FGFR kinase inhibition

This is an interesting and elegantly done study proving even more how heterogenous cancer is and how personalised medicine is the future. It also demonstrates how high-throughput techniques and computational methods can guide us in improving clinical efficacy of the drugs by carefully selecting patient population that can benefit from the treatment.

Contributor: Will

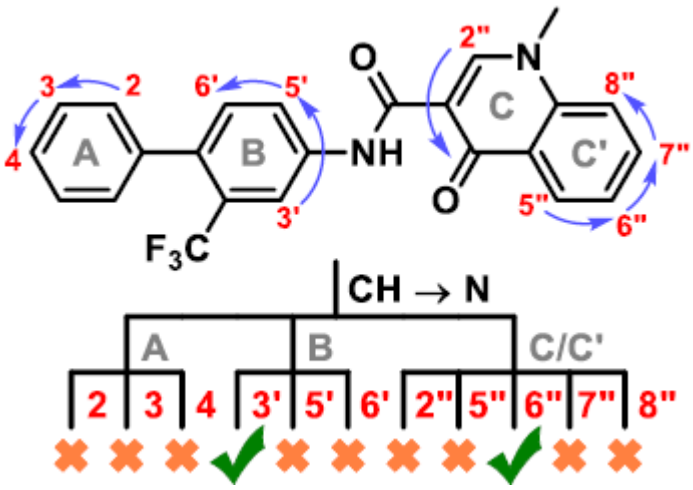
Positional Analogue Scanning: An effective strategy for multiparameter optimization in drug design

Lewis Pennington, …., Ingo Muegge*

J. Med. Chem. **2020**, DOI: 10.1021/acs.jmedchem.9b02092

Use of a 'nitrogen scan' or 'methyl walk' in medicinal chemistry is a common tactic employed for efficiently exploring chemical and biological space around a chemotype. This perspective uses an analysis of the ChEMBL database,

supported by specific case studies, to assess the value of this approach with respect to aromatic ring systems. It specifically looks at how molecular matched pairs, where a change of CH → N, CF, CMe or COH is implemented, impacts 13 common experimental parameters. They also dissect this data set to reveal the difference between positional analogues. E.g. highlighting that a CH → N switch in the 2-position has a greater chance of reducing CYP inhibition than increasing it. The authors conclude that the synthetic investment required for positional analogue scanning is, on balance, a high value exercise given the potential for rapid and simultaneous multi-parameter optimisation. They also state that this justifies a need for continued efforts towards synthetic methodologies for late stage functionalisation to further enable such approaches.



Whilst already a commonly used approach this perspective provides evidence for not only that it is indeed a worthy exercise, but more specifically where and when it can add particular value. Whilst analysis of such a general approach in the context of such a large data set inevitably brings a raft of caveats, the authors are transparent about these and endeavour to explain why they took the approach they did. The take home here is that if you have a series with multiple problems to solve (which most of us do!) you should do it, apart from where and when your data set already tells you shouldn't.

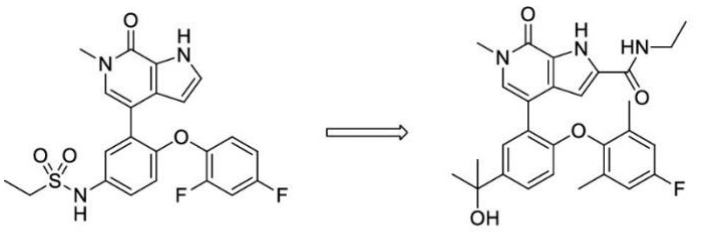
Contributor: Adam

Discovery of N-Ethyl-4-[2-(4-fluoro-2,6-dimethyl-phenoxy)-5-(1-hydroxy-1-methyl-ethyl)phenyl]-6-methyl-7-oxo-1H-pyrrolo[2,3-c]pyridine-2-carboxamide (ABBV-744), a BET Bromodomain Inhibitor with Selectivity for the Second Bromodomain

George S. Sheppard§, Le Wang§,….. Keith F. McDaniel*

J. Med. Chem **2020**, DOI: 10.1021/acs.jmedchem.0c00628

Structure-based modification of a dual BET bromodomain inhibitor, ABBV-075, to achieve selectivity for the second bromodomain (BD2). They targeted BD2-specific residues His and Pro with a combination of a secondary amide incorporated into the pyrrolopyridone core and a 2,6-disubsituted aryl ether containing a p-fluorine to prevent metabolism. To improve the ADME profile, they switched from the ethyl sulfonamide to a dimethyl carbinol which provided a hydrogen bonding interaction to an Asp residue in the channel adjacent to the ZA loop. These modifications ultimately led to ABBV-744, a BD2-selective inhibitor which showed high affinity for Brd4 BD2 (<2 nM) with a 330-fold selectivity over Brd4 BD1. ABBV-744 is currently under examination in Phase I clinical trials (ClinicalTrials.gov identifier NCT03360006).



A nice medicinal chemistry story where they have generated BD2 selectivity by introducing three modifications around the same core as the parent molecule. They tested a huge number of different functional groups for each modification

---

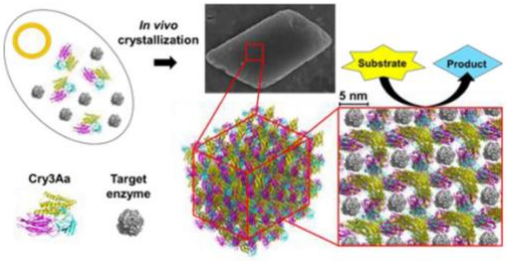
in a periodical and well-thought out manner. The chemistry used was fairly similar to the discovery of ABBV-075 with the exception of the pyrrole amide.

Contributor: Angus

In Vivo Enzyme Entrapment in a Protein Crystal

Bradley S. Heater, …. , Michael K. Chan*

J. Am. Chem. Soc. **2020**, DOI: https://doi.org/10.1021/jacs.9b13462



Nanoporous crystalline materials have many applications, such as in gas storage, molecular separations, and catalysis. Materials with pores large enough to accommodate macromolecules such as proteins are difficult to create synthetically, and naturally forming protein crystals provide a potential alternative that is easily modifiable by genetic manipulation. Cry3Aa naturally forms protein crystals in the bacterium Bacillus thuringiensis and had previously been used to create catalytic crystals using fusion proteins of Cry3Aa and enzymes. In this paper, Cry3Aa and the enzyme are co-expressed, rather than fused, and the growing Cry3Aa crystals incorporate the ideally sized Proteus mirabilus lipase (PML) enzyme cargo. Compared to a Cry3Aa--PML fusion protein crystal, the untethered Cry3Aa PML crystal showed a 10-fold higher catalytic turnover (k_{cat}), presumably because the untethered PML has higher conformational flexibility within the crystal. The superior characteristics of these catalytic crystals compared to the free enzyme (including improved thermostability and increased proteolytic resistance) increases their real-world applicability. In this case, the lipase crystal catalysts were used in the recycling of used cooking oil for biodiesel.

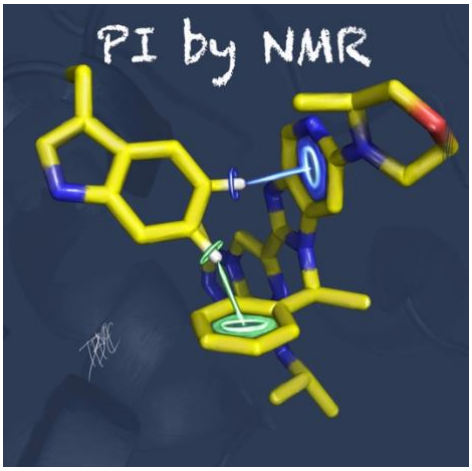
This is a fascinating use of naturally occurring protein crystals to create an entirely biological crystal catalyst. The incredible catalytic power of enzymes could be more widely applied in industry and other areas with these crystal catalysts, it will be interesting to see what other applications and modifications are thought up in the future.

Contributor: Ryan

PI by NMR: Probing CH-π Interactions in Protein-Ligand Complexes by NMR

Gerald Platzer[§], Moriz Mayer[§], …, Darryl B. McConnell*, Robert Konrat*

Angew. Chem. Int. Ed. **2020**, *DOI:* https://doi.org/10.1002/anie.202003732



Despite the fact most drugs contain at least one aromatic ring-system, no method exists to evaluate individual CH-π interactions. In this study the authors describe the development of PI by NMR which can evaluate the strength of protein-ligand CH-π interactions in solution. Leveraging specific amino acid labelling strategies (Trp in this case) the authors use ^{1}H-^{13}C protein NMR spectroscopy to monitor the CSPs induced by the CH-π interaction of a selection of ligands to BRD4-BD1. The authors can correlate the observed CSPs with interaction geometry and thus identify favourable CH-π interactions which is further demonstrated via a matched pair analysis comparing ligands with and without this pharmacophore.

The ability to deconstruct overall ligand affinity into individual contributions is a powerful way to guide lead optimisation. This technique allows for the quick identification of productive CH-π interactions and gives the ability to

---

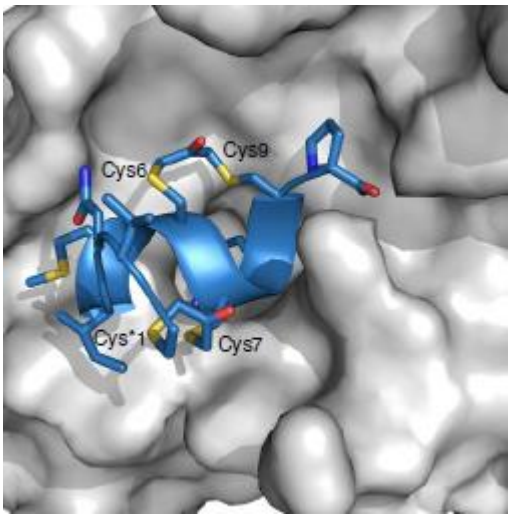
monitor how this this interaction changes e.g. when evaluating a congeneric ligand series, rationalise SAR and ultimately create a more informed ligand design.

Contributor: Nicole

De novo development of proteolytically resistant therapeutic peptides for oral administration

Xu-Dong Kong, …., Christian Heinis*

Nature Biomedical Engineering **2020**, *4,* 560. DOI: https://doi.org/10.1038/s41551-020-0556-3



Peptides are an important class of therapeutics, however, most clinically approved peptide drugs are unsuitable for oral administration. Poor physicochemical properties and vulnerability to proteases in the G.I. tract are cited as barriers towards oral efficacy. Natural peptides such as cyclosporine demonstrate that oral bioavailability can be achieved for this modality. Classical engineering approaches including cyclising, stapling, grafting, and formulation can transform early lead peptide hits into stable drug candidates. This may require 1000s of molecules to be synthesised and tested. The authors of this paper demonstrate an alternative approach applying "proteolytic phage display" which expedites hit discovery by simultaneously screening combinatorial peptide libraries for target affinity and stability. The authors exemplify the method using Factor XIa as the target and identify peptides which demonstrate high in vivo stability in various parts of the G.I. tract of a mouse. They further investigate use of the approach to find new proteolytically stable hits for IL-23R: a validated target for Crohn's disease and ulcerative colitis, for which no orally bioavailable drugs currently exist.

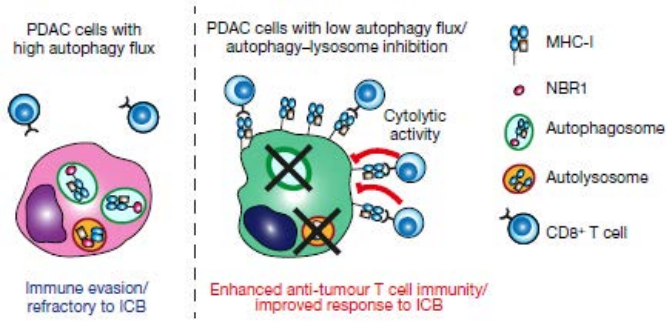
This is the first reported example of a de novo generated peptide which binds a target with high affinity while also achieving high stability towards proteases. The approach demonstrates broad scope for efficiently designing peptide drugs towards better oral bioavailability from the birth of a project towards clinical approval, where patients will ultimately benefit by accessing these drugs through oral administration rather than more invasive routes.

Contributor: Tasuku

Autophagy promotes immune evasion of pancreatic cancer by degrading MHC-I

Keisuke Yamamoto[§], Anthony Venida[§], …, Rushika M. Perera*, Alec C. Kimmelman*

Nature **2020**, *581,* 100-105. DOI: 10.1038/s41586-020-2229-5



Pancreatic ductal adenocarcinoma (PDAC) is one of the most 'notorious' cancers which do not respond to approved cancer therapies, so five-year survival rate of PDAC is still under 10%. It is known that immune checkpoint inhibitors (ICIs) do not show great effect to most of PDAC, but the reason of insensitivity to PDAC was unclear. In this paper, the authors found that lysosomal degradation of major histocompatibility complex class 1 (MHC-1) via autophagy-dependant mechanism prevents immune response of anti-tumour T cells. Moreover, inhibition of autophagy helps to change immune response of PDAC from 'cold' to 'hot'. These findings might open new opportunities to develop drugs targeted to PDAC cotreat with ICIs.

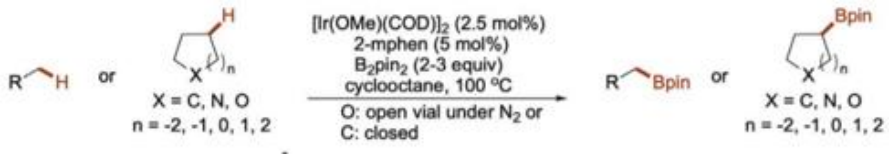
The authors used chloroquine, a well-known anti-malarial drug, as an autophagy inhibitor. If chloroquine could show same activity to PDAC in clinic, it might be helpful for patients who suffer PDAC even if the combination with PD-1 and CTLA-4 antibodies might have higher adverse event ratio.

Contributor: Nikolai

Diverse functionalization of strong alkyl C–H bonds by undirected borylation

Raphael Oeschger[§], Bo Su[§], …, John Hartwig*

Science **2020**, 368, 736–741. DOI: 10.1126/science.aba6146



Iridium catalysed borylation of primary C–H bonds is reported. When primary C–H bonds are absent or blocked, borylation of strong secondary C–H bonds is observed. Reactions at the resulting carbon-boron bond show how these borylations can lead to the installation of a wide range of carbon-carbon and carbon-heteroatom bonds at previously inaccessible positions of organic molecules.

One more landmark paper from the Hartwig group. The system reported enables the introduction of a range of functional groups at the strongest alkyl C–H bonds in organic molecules in an undirected fashion.
