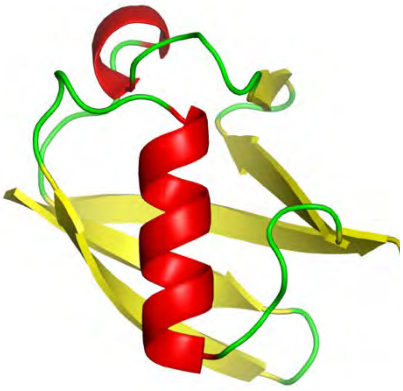
Landmark paper

Isolation of a polypeptide that has lymphocyte-differentiating properties and is probably represented universally in living cells

Gideon Goldstein,* …., Hugh D. Niall

Proc. Natl. Acad. Sci. USA **1975**, *72(1)*, 11



Goldstein et al. were the first to discover ubiquitin, as early as 1975. They isolated a small polypeptide of 8,500 Da following a series of painstaking extractions and purifications from bovine (calf) thymus (or 'sweetbread' in culinary term). Radioimmunoassay (^{125}I) allowed protein detection from extracts of various animal tissues. They also performed good old-fashioned N-terminal amino acid sequencing by Edman degradation. Because the protein was found to be widespread in tissues and was thought to participate in the maturation of white blood cells, it was provisionally named ***"ubiquitous immunopoietic polypeptide"*** *(**UBIP**).*

*The TPD/PROTAC field stands on the shoulder of many ground-breaking discoveries in the late 1970s and early 1980s, showing that protein degradation in cells takes place in a series of step-wise reactions that result in the proteins to be destroyed being "**labelled**" with a small polypeptide protein, called **ubiquitin**. We shall look at the key landmark papers that enabled this discovery, later recognized by the Nobel Prize in Chemistry in 2004.*

*To get the ball rolling, here I cover the first paper that reported biochemical isolation of the molecule that would later prove to be itself the "**label**" that marks out a protein for degradation. Interestingly, the researchers did not demonstrate a link between the newly identified protein and protein degradation, and the basic function remained unknown. They also did not give the isolated protein the name it is known today. Only later, when it was found in numerous organisms – but not in bacteria – it was given the name ubiquitin (from Latin ubique, "everywhere"). Sometime a small thing about how we give names to our discoveries can make a big difference!*

Targeted Protein Degradation

Medicinal Chemistry of Inhibiting RING-Type E3 Ubiquitin Ligases

Nicole Blaquiere, Elisia Villemure and Steven T. Staben*

J. Med. Chem. **2020**, DOI: 10.1021/acs.jmedchem.9b01451

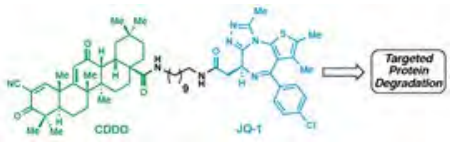
Scientists from Genentech published a review of medicinal chemistry optimisation of inhibiting five RING-type E3 ubiquitin ligases (IAPs, VHL, MDM2, KEAP1, and Skp2).

The small molecule inhibitors are developed by using either the E3 ligase substrate peptides (IAPs & VHL) or nonpeptidic competitors identified (MDM2 & KEAP1) as starting points. The authors highlighted the challenges associated with developing orally bioavailable E3 ligase inhibitors. They proposed that allosteric inhibitors of E3 ligases could possess more favourable physicochemical properties.

Targeted Protein Degradation via a Covalent Reversible Degrader Based on Bardoxolone

Bingqi Tong, [§] Mai Luo, [§] …., Thomas J. Maimone,* and Daniel K. Nomura*

ChemRxiv **2020**, DOI: 10.26434/chemrxiv.12055935.v1



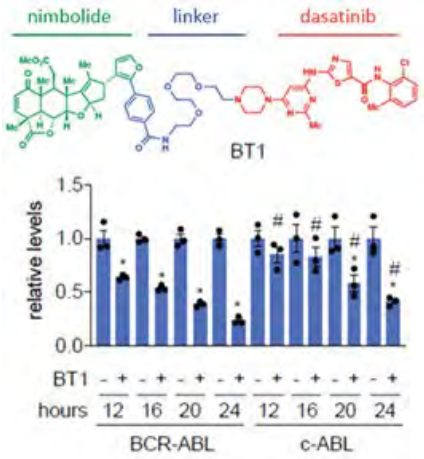
The authors synthesised a PROTAC recruiting KEAP1 using a covalent reversible binder based on bardoxolone (a.k.a. CDDO). In addition to BRD4 degradation (selectivity over related BET family members not measured), this PROTAC also degrades the E3 ligase KEAP1 at high concentration (> 1 μM).

It is unclear whether degradation of KEAP1 is a result of treating cells with electrophilic stressors, or neosubstrate degradation induced by CDDO-based PROTAC. In addition to activation of Nrf2 (through targeting cysteine residue of KEAP1), CDDO also has other pharmacologic targets so the authors proposed the degradation observed could be due to one or more cullin-family E3 ligases.

A Nimbolide-Based Kinase Degrader Preferentially Degrades Oncogenic BCR-ABL

Bingqi Tong, [§] Jessica N. Spradlin, [§] …., Thomas J. Maimone,* and Daniel K. Nomura*

BioRxiv **2020**, DOI: 10.1101/2020.04.02.022541



The terpene theme continues. Here is another PROTAC paper using covalent E3 ligase binder nimbolide to recruit RNF114 for protein degradation. Nimbolide-based PROTAC (BT1) shows preferential degradation of oncogenic human kinase BRC-ABL over c-ABL, whereas the corresponding VHL- and CRBN-based PROTACs preferentially degrade c-ABL over BRC-ABL. In addition to the preferential degradation of BRC-ABL, BT1 also induces stabilisation of tumour suppressor p21.

Pure nimbolide is ridiculously expensive (2020 Sigma price: $77,000 USD/gram according to the authors). In the first nimbolide PROTAC paper published by the Maimone and Normura groups, pure nimbolide was purchased and 100 mg (~$7,000, give or take a few hundred $) was used for synthesis (ouch). This time, they extracted ~ 1 g analytically pure nimbolide from 450 g neem leaf extract (which costs $14 according to the authors).

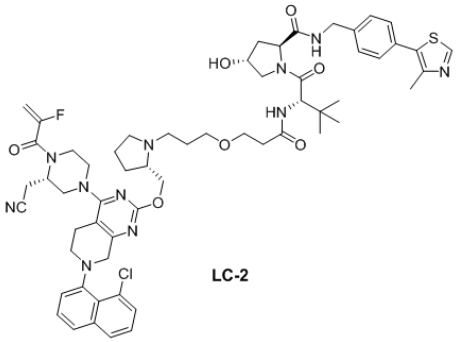
I am sure with the increasing interest/demand of nimbolide in the community, the price will drop. Until then, the incredibly detailed and beautiful extraction procedure in the supporting information should allow more groups to access reasonable-priced nimbolide for other biological/TPD applications.

Degradation of Oncogenic KRAS^{G12C} by VHL-recruiting PROTACs

Michael J. Bond,[§] Ling Chu,[§] Dhanusha A. Nalawansha, Ke Li, Craig M. Crews*

ChemRxiv **2020**, DOI: 10.26434/chemrxiv.12091176.v1

Crews and co-workers demonstrate that degradation of endogenous KRAS^{G12C} can be achieved using a VHL-based PROTAC with a covalent warhead (developed by Mirati Therapeutics). LC-2 induced degradation of KRAS in 5 different cell lines with DC_{50} values ranging from 0.25 to 0.76 µM and D_{max} of >40-85%. Attenuation of downstream pErk signalling for up to 72 hours was also observed in several cancer cell lines.



LC-2

The authors demonstrated that degradation occurs via PROTAC mechanism by rescuing LC-2 induced KRAS^{G12C} degradation via the treatment of cells using the hydroxyproline epimer of LC-2 (instead of LC-2), excess of the VHL ligand, proteasome inhibitor and neddylation inhibitor. The kinetics of KRAS^{G12C} engagement and degradation induced by LC-2 was monitored in two cell lines by western blotting and the rates for each step were found to be cell line dependent. Rapid engagement of KRAS^{G12C} (~ 1 hour of treatment) was observed in both cell lines with D_{max} observed at 8 hours the earliest and the degradation of KRAS^{G12C} persist for 24 hours.

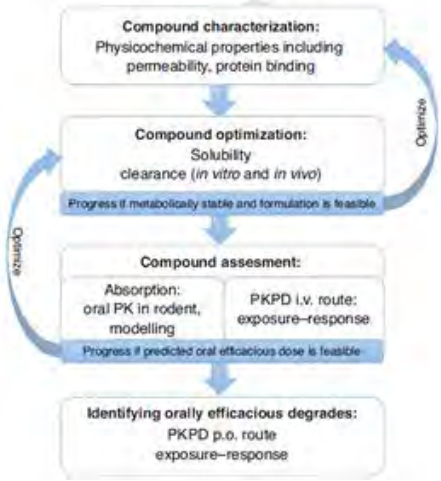
It is unclear how the authors select/prioritise the 'starting point' for the MedChem optimisation campaign. It seems like they may have taken PROTACs that have good affinity to KRAS^{G12C} and synthesised analogues with different linker lengths. Worth noting that a patent application *(ACS Med. Chem. Lett. 2020, 11, 1, 5-6) that published late in 2019 also showed degradation of endogenous KRAS^{G12C}, but in that case using a different KRAS^{G12C} inhibitor.*

Fundamental aspects of DMPK optimization of targeted protein degraders

Carina Cantrill,[§] …., Caroline Rynn,[§] Jeannine Petrig Schaffland,[§] Isabelle Walter,[§] and Matthias B. Wittwer[§]*

Drug Discovery Today **2020**, DOI: 10.1016/j.drudis.2020.03.012

Scientists from Roche and C4 therapeutics shared their analysis and learnings of DMPK optimisation towards oral PROTACs. Their analysis shows that metabolic stability and solubility are key parameters to optimise for oral degraders (as long as permeability is not a rate-limiting factor). A DMPK optimisation cascade (on the right) was proposed based on their learnings.

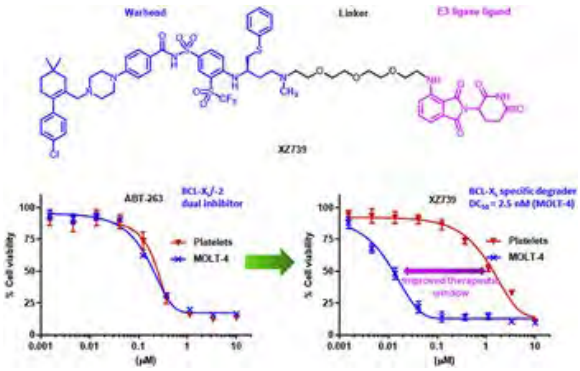


This is a very enjoyable read. It contains a very good overview of common methods used for permeability, solubility, and plasma protein binding measurements in drug discovery programmes. It is also interesting to see the use of computational tool (Gastroplus) to access which properties (solubility vs permeability) are more sensitive to oral absorption.

Discovery of PROTAC BCL-X_L degraders as potent anticancer agents with low on-target platelet toxicity

Xuan Zhang,[§] Dinesh Thummuri,[§] …., Daohong Zhou,* Guangrong Zheng*

Eur. J. Med. Chem. **2020**, *192*, 112186



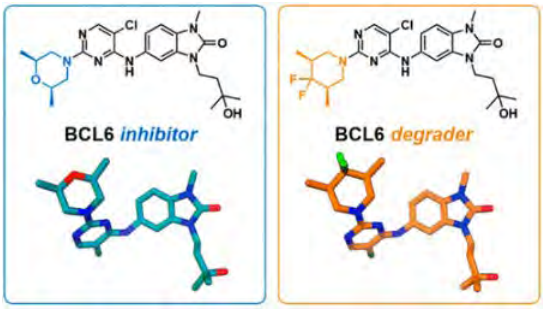
In this paper, the authors achieved selective degradation of BCL-X_L, in terms of specificity against BCL-2 and site-selectivity, using VHL and CRBN based PROTACs with a dual inhibitor of antiapoptotic proteins BCL-X_L and BCL-2. By exploiting the difference in VHL/CRBN expression between tumour cells and human platelets, the authors developed a potent degrader (XZ739) which shows >100-fold selectivity for the tumour cell line over human platelets. The authors reason that these degraders can reduce the on-target toxicity in platelets, which are dependent on BCL-X_L for survival and have a low expression of VHL/CRBN.

From Angus: I don't know the authors of this paper, but I worked on the BCL-2 protein family during my PhD and the ABT-737/263/199 series of inhibitors were developed by my PhD supervisor and others at WEHI. The original problem with these compounds was the on-target toxicity on platelets due to BCL-X_L inhibition. Platelets are dependent on BCL-X_L for survival, so a BCL-2-specific compound ABT-199 was developed which is now in the clinic as Venclexta for the treatment of a molecular subtype of chronic lymphocytic leukaemia. There are some cancers that are BCL-X_L-dependent, hence the interest in mitigating the on-target platelet toxicity. These are some of the largest PROTACs developed (according to the authors) due to the already high-MW BCL-2/BCL-X_L compound that is a PPI inhibitor.

Achieving *In Vivo* Target Depletion through the Discovery and Optimization of Benzimidazolone BCL6 Degraders

Benjamin R. Bellenie, …., Swen Hoelder*

J. Med. Chem. **2020**, *63*, 4047



BCL6, via interaction with its co-repressor proteins, down regulates expression of genes associated with cell cycle control, cell differentiation and cell death. Targeting dysregulated BCL6 which drives B-cell lymphoma proliferation is thus viewed as a therapeutic concept of value. Through linking/merging of low affinity hits the authors then identify sub-micromolar BCL6 inhibitors, from which they serendipitously identify BCL6 degraders. A subsequent optimisation (mainly focussed on ADME) leads to a chemical probe of sufficient quality to demonstrate in vivo BCL6 degradation in lymphoma xenograft mice following oral dosing.

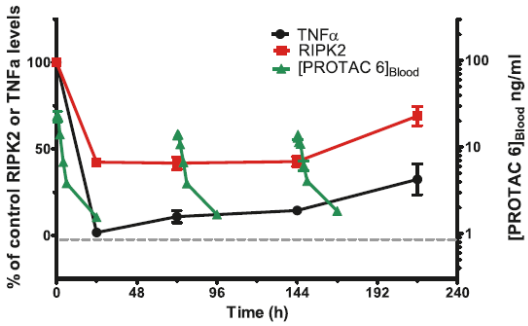
This manuscript describes a huge amount of work and is full of quality and valuable learnings. The journey from hit to sub-micromolar affinity inhibitor alone would have made a nice story even without the intriguing discovery of

degraders. There is a logical and well thought out medicinal chemistry approach to the ADME optimisation as well as smart use of various assay platforms at various points in the story. Whilst there is a good discussion and some opinions offered regarding the mechanism of action of the degrader, I very much hope the authors (or others) will attempt to go further and unravel how exactly these compounds function (see also Kerres et al. Cell Reports, 2017, 20, 2860). Well worth a read for all experience levels and disciplines.

Extended pharmacodynamic responses observed upon PROTAC-mediated degradation of RIPK2

Alina Mares,[§] …., John Harling*

Commun. Biol. **2020**, *3*, 140



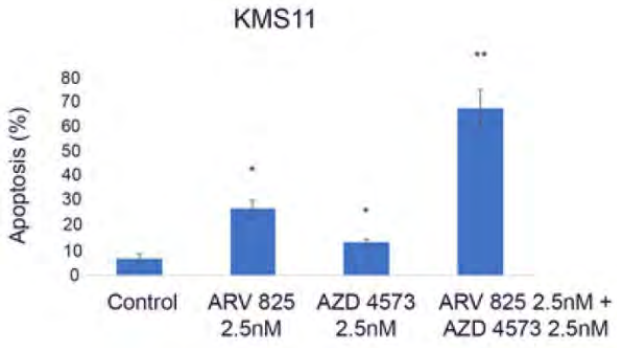
Coming from scientists formerly part of the Degradation DPU at GSK, this manuscript is the first to demonstrate sustained in vivo pharmacodynamic effect beyond the period of detectable PROTAC plasma exposure. The optimised compound, PROTAC 6, is an IAP based Receptor-Interacting Serine/Threonine Protein Kinase 2 (RIPK2) degrader. Dysregulation of RIPK2 is associated with inflammation-based diseases such as IBD. PROTAC 6 demonstrates extremely potent and selective RIPK2 degradation in cellular experiments. Combined with a suitable ADME profile this enables in vivo suppression of TNFα (disease biomarker) over a 9-day period using just four administrations of a 0.15 mg/kg s.c. dose.

Much more has been postulated than actually demonstrated regarding the potential in vivo advantages of degrading vs inhibiting a target protein. Via a concise round of PK/PD studies, this study is able to provide strong data that highlights for the right target, PD effect beyond PK exposure can be achieved and is a potentially powerful therapeutic approach. Furthermore, there is a paucity of in vivo data for PROTACs beyond oncology targets and a need for more PK/PD based degrader studies to be published. This manuscript addresses both of those gaps. It would have been nice to see in vivo data for the cis controls and/or direct comparison to the inhibitor alone.

Multiple Myeloma: Combination Therapy of BET Proteolysis Targeting Chimeric Molecule with CDK9 Inhibitor

Su-Lin Lim,*[§] …., Liang Xu[§]

BioRxiv. **2020** DOI: 10.1101/2020.04.08.031583



CDK9 and BET proteins both contribute to transcriptional activation by RNA Polymerase II. The authors of this disclosure investigate the synergistic effect of combining CRBN based BET degrader ARV-825 and selective CDK9 inhibitor AZD4573 in multiple myeloma (MM) cell lines, via both cellular and in vivo studies. They analyse data from anti-proliferation studies in three cell lines and demonstrate a reduction in combination index (signalling synergy) in two of them with low doses (2.5-

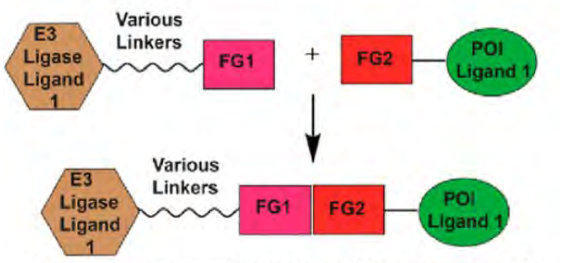
20 nM) of each compound. They also demonstrate enhanced effects on apoptosis and tumour burden in vivo. To rationalise these effects a reduction in the phosphorylation of RNA polymerase II following treatment of AZD4573 alone and reduction in BET protein levels following treatment with ARV-825 (but not vice versa) is also shown in MM cell lines.

Whilst it is great to see such positive responses in vivo when these two compounds are combined, a measurement of synergy is only applied to cellular anti-proliferation data. It will be interesting to see how this study looks post-peer review.

Two-stage Strategy for Development of Proteolysis Targeting Chimeras and its Application for Estrogen Receptor

Brett L. Roberts,[§] …., Weiping Tang*

ACS Chem Biol. **2020** DOI: 10.1021/acschembio.0c00140



Stage 1: Simultaneously examine multiple parameters of a library of unstable PROTACs by in-cell ELISA
Stage 2: Form stable PROTACs by bioisosteric replacement

The authors present an approach for generating PROTAC libraries via combination of hydrazides on ER receptor binders with 'ligase+linker' building blocks containing an aldehyde handle. The combined building blocks yield acyl hydrazides that are screened directly in cellular degradation assays without purification required. Approximately 100 PROTACs were made in a first round encompassing CRBN and VHL binders with multiple ER binder exit vectors. From this first stage ~10 nM degrader is identified. The acyl hydrazide based PROTACs do show signs of instability over 24 and 48 hour assay time points. This is addressed by making secondary amide molecular matched pairs of the best compounds leading to a 1.1 nM amide linked ER degrader.

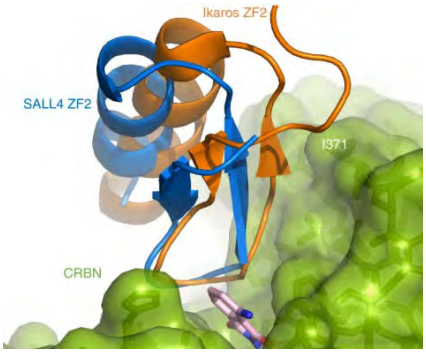
This a creative and pragmatic approach that could easily be transferred to other targets. The authors have found a rapid way to navigate an initial, empirical phase of PROTAC hit ID. A key limitation may be that whilst the final step does not require purification, expanding to larger libraries with more diverse aldehyde containing building blocks may still require synthetic effort close to what would be required to make an equivalent library of secondary amide based PROTACs in the first place. Nevertheless the challenges they discuss and are trying to solve are relevant and it works well in this case.

Contributor: Alessio

Crystal structure of the SALL4–pomalidomide–cereblon–DDB1 complex

Mary E. Matyskiela,* …., Philip P. Chamberlain*

Nat. Struct. Mol. Biol. **2020**, *27*, 319



SALL4 is one of the many "neo-morphic" cereblon substrates recruited by immunomodulatory drugs (ImIDs). Here the Cellgene team solved a new crystal structure of CRBN bound to pomalidomide and SALL4. The structure features both similar (same conserved glycine-loop degron) and unique (shifted binding mode of the zinc-finger and steric clash with $I371^{CRBN}$) features compared to previously observed CRBN:ImIDs ternary complexes.

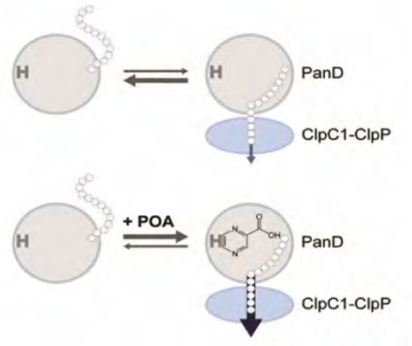
The new structure is significant because SALL4 was recently implicated in the teratogenic activity of thalidomide – although others identified a different target (p63), so this remains unsettled. For this reason, SALL4 is also an unwanted degradation off-target of CRBN-recruiting PROTACs. To fully rationalize the structural basis of thalidomide teratogenicity, the structure should have been solved ideally with thalidomide bound, rather than pomalidomide. Nevertheless, this new structure could enable more rational PROTAC design strategy to "dial-out" this fastidious off-target. I found the characterization of various mutants in ternary complex pull-down assays quite elegant and high-throughput. To aid more quantitative data, future biophysical work are warranted to further characterize the thermodynamics and kinetics of these ternary complexes.

Contributor: Alessio

Pyrazinamide triggers degradation of its target aspartate decarboxylase

Pooja Gopal,[§] Jickky Palmae Sarathy,[§] Michelle Yee,[§] …., Thomas Dick*

Nat. Commun. **2020**, *11*, 1661



Pyrazinamide (PZA) has been used as a front-line drugs against tuberculosis since 1952. Drug resistance mutations in Mycobacterium tuberculosis were recently identified on the gene PanD (encoding the enzyme aspartate decarboxylase), and genes encoding the degrading protease complex ClpC1-ClpP. Yet the mode of action of PZA remained long elusive. Here, the authors provide convincing evidence to support that PZA works as a degrader, by inducing ClpC1-ClpP-dependent degradation of PanD in M. tuberculosis.

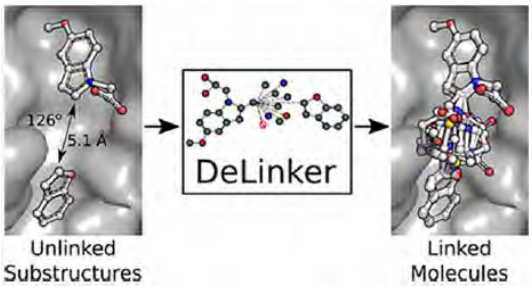
This paper is significant as it identifies perhaps for the first time an antibacterial drug that works as a targeted protein degrader. Personally, this strikes some good-old chords as it brings together research topics of my past and current career. You might know that as a PhD student I studied enzymes in the pantothenate biosynthesis (PanE, which works two steps downstream of PanD), and as a postdoc worked on targeting M. tuberculosis PanC (just one step downstream of PanD). It's a small world! Future work elucidating the mechanism down to atomic details are warranted!

Deep Generative Models for 3D Linker Design

Fergus Imrie, Anthony R. Bradley, Mihaela van der Schaar, Charlotte M. Deane*

J. Chem. Inf. Model. **2020**, DOI: 10.1021/acs.jcim.9b01120

This paper describes the development of a graph-based machine learning model, 'DeLinker', for linker design between two fragments using protein-context-dependent 3D structural information (relative distance and orientation between the fragments) in the design process. The application of DeLinker is demonstrated by fragment linking, scaffold hopping and PROTAC linker design (using the SMARCA PROTAC developed by the AC-BI team). In all cases, DeLinker designed linker molecules that are highly similar to (in some cases the same as) the 'experimental' endpoints.



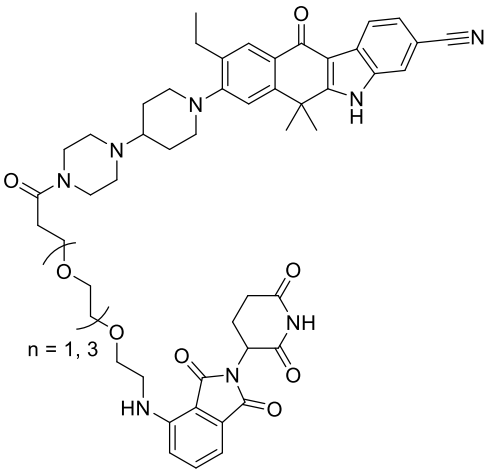
DeLinker is trained to reproduce the linked molecule from a combination of the encodings of the fragments and linked molecule using 417,997 selected fragment-molecule pairs from the ZINC database. In a large scale evaluation, the performance of DeLinker is compared to a traditional approach where the linker molecule is selected from a pre-defined database. It was found that DeLinker designed 60% more molecules with high 3D similarity to the original molecule and massively outperformed (200%!) in the design of longer linkers with at least five atoms. In addition, DeLinker is able to design novel linkers that are outside of the machine learning training set, which is super cool. The source code of DeLinker is available at https://github.com/oxpig/DeLinker

Degraders That Target ALK And Therapeutic Uses Thereof

Nathanael Gray, John M. Hatcher, Chelsea E. Powell, Pasi A. Janne

WO2020069106A1

Scientists from Dana-Farber Cancer Institute published a patent on degradation of Anaplastic lymphoma kinase (ALK) using pomalidomide-based degraders. PROTACs using alectinib to recruite ALK with PEG linkers show partial degradation of ALK (D_{max} < 60%) across a range of concentration (0.01 – 1 µM) and comparable anti-proliferative activity to the parental inhibitors across both ALK-positive and ALK-negative Ba/F3 cancer cell lines. These PROTACs also induced degradation of other kinases such as Aurora A and PTK2 at higher concentrations (> 50 µM) and longer time points (16 hour).



Compared to previous generation of cereblon-based ALK PROTACs using ceritinib to recruit ALK, the novel PROTACs disclosed in this patent claims reduced off-target degradation of PTK2.

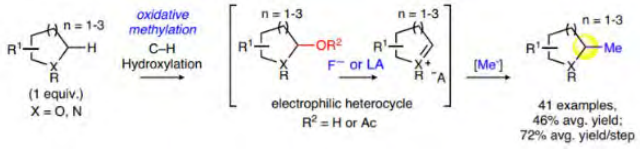
Others

Contributor: Aileen

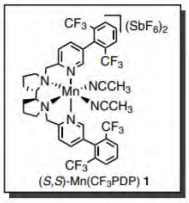
Late-stage oxidative C(sp^3)-H methylation

Kaibo Feng, …., M. Christina White*

Nature **2020**, DOI: 10.1038/s41586-020-2137-8



The installation of methyl groups, especially adjacent (α) to heteroatoms, can drastically increase the potency of bioactive molecules. Current methylation methods display limited scope and have not been demonstrated in complex settings. This paper describes a regio- and chemoselective oxidative C(sp3)–H methylation method compatible with late-stage functionalization of drug scaffolds and natural products. This combines a highly site- and chemoselective C–H hydroxylation with a mild, functional-group-tolerant methylation.



The methodology is demonstrated on 41 substrates, including medicinally important cores and pharmacologically relevant molecules (drugs and natural products), which contain motifs including electron-rich aryls, heterocycles, carbonyls and amines. Methylation occurs site-selectively at the most electron rich, least sterically hindered position. This publication builds on the authors' previous work regarding site-selective C-H oxidation, see: J. Am. Chem. Soc. ***2015****, 137, 14590; Nature Chemistry* ***2019****, 11, 213.*

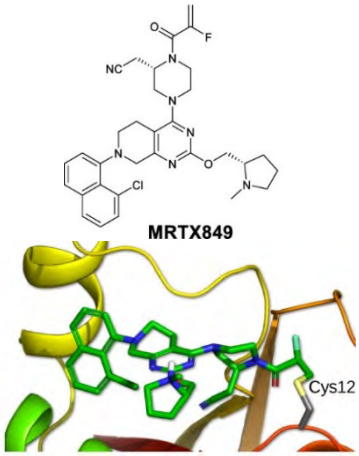
Contributor: Ross

Identification of the Clinical Development Candidate MRTX849, a Covalent KRAS^{G12C} Inhibitor for the Treatment of Cancer

Jay B. Fell,* …., Matthew A. Marx*

J. Med. Chem. **2020**, 10.1021/acs.jmedchem.9b02052

Capping off an era marred by drug development failures and punctuated by waning interest and presumed intractability toward direct targeting of KRAS, new technologies and strategies are aiding in the target's resurgence. As previously reported, the tetrahydropyridopyrimidines were identified as irreversible covalent inhibitors of KRAS^{G12C} that bind in the switch-II pocket of KRAS and make a covalent bond to cysteine 12. Using structure-based drug design in conjunction with a focused in vitro absorption, distribution, metabolism and excretion screening approach, analogues were synthesized to increase the potency and reduce metabolic liabilities of this series. The discovery of the clinical development candidate MRTX849 as a potent, selective covalent inhibitor of KRASG12C is described.



This paper describes a structure-based optimisation of a previously described inhibitor of KRAS (ACS Med. Chem. Lett. ***2018****, 9, 1230) which has led to the discovery of MRTX849, an orally bioavailable clinical development candidate. The introduction of a 2-fluoroacrylamide proved essential in imparting stability while retaining cellular potency. Proteomic*

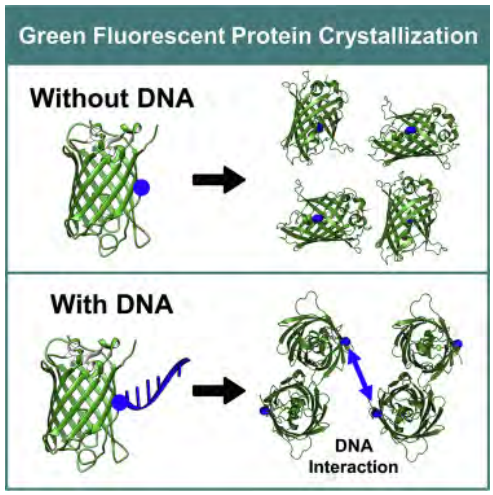
analysis also showed "exquisite selectivity" towards KRAS^{G12C}, and tumor regression was observed until the 70-day monitoring period had concluded at the highest doses.

Contributor: Sarath

DNA-Directed Protein Packing within Single Crystals

Peter H. Winegar, …., Chad A. Mirkin*

Chem **2020**, *6*, 1007



Designed DNA-DNA interactions are investigated for their ability to modulate protein packing within single crystals of mutant green fluorescent proteins (mGFPs) functionalized with a single DNA strand (mGFP-DNA). The authors probe the effects of DNA sequence, length, and protein-attachment position on the formation and protein packing of mGFP-DNA crystals. Notably, when complementary mGFP-DNA conjugates are introduced to one another, crystals form with nearly identical packing parameters, regardless of sequence if the number of bases is equivalent. DNA complementarity is essential, because experiments with non-complementary sequences produce crystals with different protein arrangements. Importantly, the DNA length and its position of attachment on the protein markedly influence the formation of and protein packing within single crystals. This work shows how designed DNA interactions can be used to influence the growth and packing in X-ray diffraction quality protein single crystals and is thus an important step forward in protein crystal engineering.

Judicious DNA design and attachment onto proteins may direct protein crystallization and dictate how proteins are arranged in crystals, ultimately facilitating the discovery and application of protein structure and function.

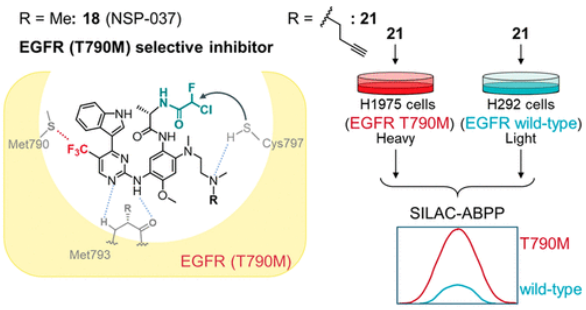
Contributor: Tasuku

Selective Covalent Targeting of Mutated EGFR(T790M) with Chlorofluoroacetamide-Pyrimidines

Mami Sato, …., Akio Ojida*

ACS Med. Chem. Lett. **2020**, DOI: 10.1021/acsmedchemlett.9b00574

They reported the development of a targeted covalent inhibitor for mutated EGFR (L858R/T790M) using α-chlorofluoroacetamide (CFA) as the reactive group. The chemically tuned weak reactivity of CFA was suitable for the design of third-generation EGFR inhibitors that possess the pyrimidine scaffold.



*They developed novel mutated EGFR targeted covalent inhibitor which has a unique warhead they developed previously. See: Nat Chem Biol **2019**, 15, 250.*

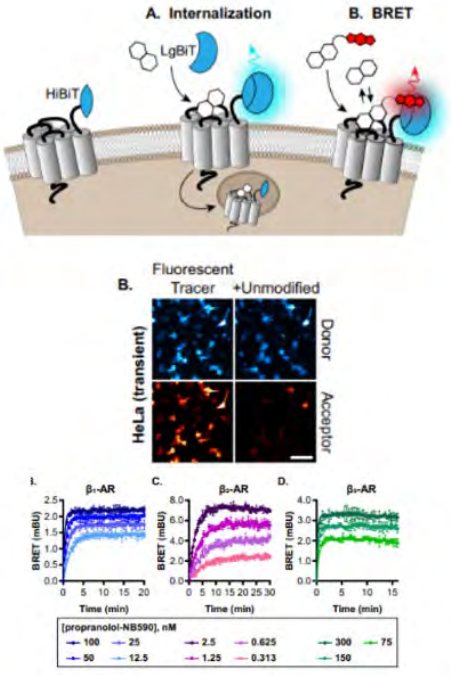
Contributor: Claire

The luminescent HiBiT peptide enables selective quantitation of GPCR ligand engagement and internalization in living cells

Michelle E. Boursier, …., Rachel Friedman Ohana*

Journal of Biological Chemistry **2020**, *15*, 5124

There is increasing interest in evaluation of binding dynamics under nonequilibrium conditions, which are postulated to be more relevant for predicting in vivo compound efficacy. Additionally, because ligand-binding assays cannot differentiate agonists from antagonists, binding evaluations typically require complementary cell-based functional assays that monitor the biological consequences of receptor engagement. Promega developed complementary cellular assays that enable equilibrium and real-time analyses of GPCR ligand engagement and consequent activation, measured as receptor internalization. These assays utilize GPCRs genetically fused to an N-terminal HiBiT. Using the – adrenergic receptor family as a model, they demonstrate the versatility of these assays by utilizing the same HiBiT construct in analyses of multiple aspects of GPCR pharmacology.



Promega's NanoLuc/NanoBRET platforms are gaining momentum in drug discovery research and are super useful (and relatively straight forward) to apply to assess many aspects of intracellular compound characteristics. This paper is a nice example of how their technology can be adapted in a project-specific manner using the HiBiT/NanoLuc tag for detection across various assay set ups and assay modes.

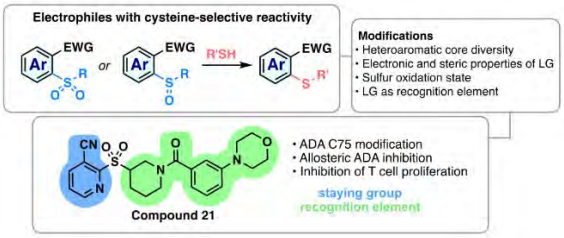
Contributor: Tasuku

2-Sulfonyl pyridines as tunable, cysteine-reactive electrophiles

Claudio Zambaldo,[§]* Ekaterina V. Vinogradova,[§]* …., and Michael J. Bollong*

J. Am. Chem. Soc. **2020**, DOI: 10.1021/jacs.0c02721

They used the endogenous electrophile sensor of mammalian cells — the KEAP1-NRF2 pathway — to discover cysteine-reactive electrophilic fragments from a reporter-based screen for NRF2 activation. This strategy identified a series of 2-sulfonyl pyridines that selectively react with biological thiols via nucleophilic aromatic substitution (SNAr).



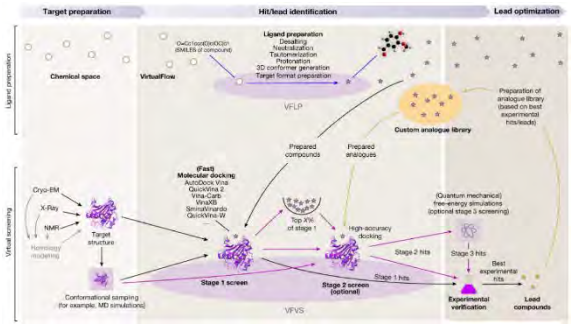
*It is a unique warhead for forming covalent bond to cysteine residue. One of great advantage of this warhead is you can control reactivity of it by changing substituent at 3 position. Recently another group reported similar type of warhead, see: J. Am. Chem. Soc. **2020**, 142, 1801.*

Contributor: Tasuku

An open-source drug discovery platform enables ultra-large virtual screens

Christoph Gorgulla,* ….. , Haribabu Arthanari*

Nature **2020**, DOI: 10.1038/s41586-020-2117-z



Here the authors designed VirtualFlow, a highly automated and versatile open-source platform with perfect scaling behaviour that is able to prepare and efficiently screen ultra-large ligand libraries of compounds. Using VirtualFlow, they have prepared the largest and freely available ready-to-dock ligand library available, with over 1.4 billion commercially available molecules.

Website: https://virtual-flow.org/

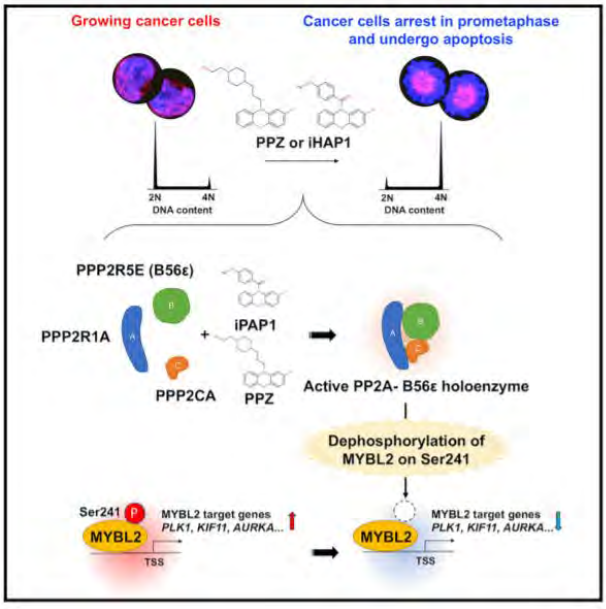
They developed open source ultra-large scale virtual screening software customized for cluster CPU machines for free of charge. In addition, you can select and download ready-to-use virtual compound library from over a billion compounds Enamine library.

Contributor: Vesna

Allosteric Activators of Protein Phosphatase 2A Display Broad Antitumor Activity Mediated by Dephosphorylation of MYBL2

Ken Morita, …., A. Thomas Look*

Cell **2020**, 181, 1



PP2A protein phosphatases are hetero-trimeric enzymes consisting of three subunits (A, B and C) that control catalytic core, substrate specificity and cellular localisation. Each subunit has different isoforms and is located on different chromosome. Due to this complexity PP2A enzymes are considered an enzyme family and researching them was complicated. They are tumour suppressors and often inactivated in cancer. The inactivation is connected to upregulation of other inhibitory proteins rather than usual inactivation by mutation. Therefore, the strategy to overcome this inhibition is to increase concentration of PP2A proteins by introducing compounds that bring different (but specific) subunits together. In this article, a class of small-molecules iHAPs (improved heterocyclic activators of PP2A) was identified as an allosteric activator of PP2A by assembling a specific PP2A holoenzyme. One compound, iHAP2 was found to be a potent allosteric activator that does not inhibit dopamine receptor D2, a mediator of neurologic toxicity caused by perphenazine, a related heterocyclic compound that activates PP2A. The PP2A complex activated by iHAP1 dephosphorylates the MYBL2 transcription factor on Ser241, causing irreversible arrest of leukemia and other cancer cells in prometaphase. In contrast, SMAPs, a separate class of

compounds, activate PP2A holoenzymes containing a different regulatory subunit, do not dephosphorylate MYBL2, and arrest tumor cells in G1 phase.

This is a very interesting paper, utilizing many different (and expensive) methods for research of this complicated protein phosphatase family. Most of the experiments are done in animal models (mice and zebra fish) as well as in human primary cell lines and "regular" cancer cell lines. They utilise CRISPR CAS, phospohoproteomics, epigenetics, behavioural techniques etc. This and a related back-to-back paper (DOI: 10.1016/j.cell.2020.03.038) are well worth a read!

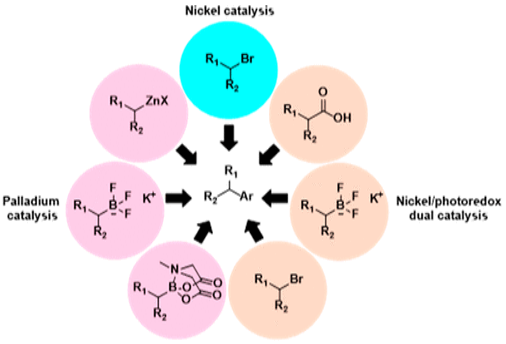
Contributor: Nikolai

Expanding the Medicinal Chemist Toolbox: Comparing Seven C(sp2)–C(sp3) Cross-Coupling Methods by Library Synthesis

Amanda W. Dombrowski, …., Ying Wang*

ACS Med. Chem. Lett. **2020**, *11*, 4, 597

A study was done comparing the ability of seven methods to directly install a diverse set of alkyl groups on "drug-like" aryl structures via parallel library synthesis. Each method showed substrates that it excelled at coupling compared with the other methods. The results reported herein should be used to inform future syntheses, assess reaction scope, and encourage medicinal chemists to expand their synthetic toolbox.



This study provides a guide to enable the installation of a variety of alkyl groups on heteroaromatic rings. General guidelines, recommending methods for each alkyl group type, are outlined.
