Ciulli Lab

Targeted Protein Degradation

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

Journal Club

March 2022

Centre for Targeted Protein Degradation
University of Dundee

innovate
collaborate
inspire

Content

Meet this Month's Editors

This month's editors are (from left to right): Aileen Frost, Conner Craigon, and Andre Wijaya

Aileen completed her MChem and DPhil at the University of Oxford. In 2015, she moved to the University of St Andrews for a postdoctoral position in organocatalysis with Professor Andy Smith. She subsequently undertook a second post-doc with Dr Matthew Tredwell at the Max-Planck-Institut für Kohlenforschung in the field of radiochemistry. In November 2018 Aileen joined the AC-BI team as a Medicinal Chemist, and since January 2022 has been working as a Senior Drug Discovery Scientist within the collaboration.

Conner completed his MRes and recently his PhD at the Univeristy of Dundee. He is currently as of 2022 working in an academic postdoctoral position in the Ciulli group as a cell biologist.

Andre obtained his undergraduate degree in Applied Biology with Biotechnology in 2015 from the Hong Kong Polytechnic University. He then moved to UK in 2016 to pursue his PhD study in Biological Chemistry at University of Cambridge under the supervision of Dr Martin Welch. He joined the Ciulli group in October 2020 as a structural biologist/biophysicist on the PROTAC collaboration projects with Boehringer Ingelheim.

Feature of the Month

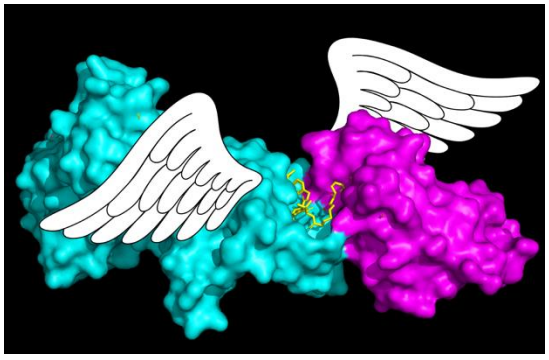
Contributor: Valentina

CeTPD welcomes Prof. Frank Sobott

On the 22nd and 23rd of March we had the pleasure of welcoming Prof Frank Sobott to Dundee for a visit that was co-hosted by the Biological Chemistry and Drug Discovery division, the Molecular Interactions team and the CeTPD. Frank is an expert in structural mass spectrometry techniques, including native mass spectrometry methods, ion mobility and hydrogen deuterium exchange mass spectrometry, chemical crosslinking, and covalent labelling techniques, predominantly Fast Photochemical Oxidation of Proteins (FPOP). His work at the University of Leeds has a strong focus of integrating MS with other structural biology techniques, in particular electron microscopy and developing computational modelling methods. Frank's group develops new tools and methods to address complex problems such as understanding membrane proteins-lipid interactions and amyloid formation by intrinsically disordered proteins.

As several key papers have been published in the TPD field using structural MS techniques (covered in a short review in this issue) curiosity has grown in understanding these techniques from across the community and so Frank's visit was eagerly awaited by our team. While Frank was here, he graciously gave a seminar outlining his techniques and current work as well as an in-depth workshop, giving us the opportunity to gain a deeper understanding of his approach and how we might be able to apply similar methodologies in our work.

Wings for ternary complexes



Several key articles have recently been published investigating ternary complexes via structural mass spectrometry. The first of these was published by Zorba *et al.* in 2018 and focused on understanding the role of cooperativity in cereblon-recruiting degraders targeting BTK.

The study utilised hydrogen-deuterium exchange (HDX)-MS, a technique based on the rapid exchange of deuterium with solvent exposed amide hydrogens in deuterated buffer. This is useful for the investigation of ternary complexes as one would expect to see shielding (little to no exchange) of residues at the protein-protein interface in a ternary complex relative to the binary complex and apo state of the proteins.

However, despite comprehensive experiments, Zorba et al were not able identify any interacting residues. This may have been due to the use of a truncated cereblon construct which included only the CULT (thalidomide-binding) domain. A more recent study by Eron *et al.* (2021), utilised a more complete cereblon construct (that includes the N-

terminal Lon domain in addition to the CULT domain) and demonstrated that the CFT-1297 BRD4 (BD1) degrader was able to induce shielding of several residues at the BD1-cereblon interface, in particular at the Lon domain. However, in a similar experiment with a dBET6 degrader they were not able to detect additional shielding at this interface suggesting that for the dBET6 degrader, the degrader-induced interface might not be resolved effectively on the HDX timescales. Both studies focussed on cereblon and suggest a more complicated conformational landscape for cereblon-recruiting degraders. This is especially in respect to another study by Dixon *et al.* (2021) (pre-print), that uses HDX-MS to study VHL-recruiting degraders and demonstrated clear and significant protection of residues at the protein-protein interface. For the 2021 studies HDX-MS data was fed into *in silico* docking studies, demonstrating the utility of this method in enriching computational techniques.

In a departure from HDX-MS, Beveridge et al. (2020) showed that native mass spectrometry can effectively predict PROTAC efficacy in a clear correlation between a higher fraction of ternary complex observed in the spectra being linked to other favourable biophysical parameters such as longer ternary half-lives and increased cooperativity. This study focussed predominantly on the BRD:MZ1/AT1:VCB system and it would be interesting to uncover if similar correlations could be made for other systems. Finally, a study by Song *et al.* (2021), used ion-mobility MS to show that BRD4:MZ1:VCB was able to occupy six distinct conformations. This technique that allows distinct conformations to be resolved as ions travel through the drift tube, with more compact conformations travelling faster than more elongated conformations. Moreover, the study employed the gas-phase fragmentation methods, collision induced dissociation (CID) and electron capture dissociation (ECD), to allow for the mapping of ligand interaction sites. Using CID, it was observed that the more compact ternary complexes have protein-protein interactions that allow a binary VCB:BRD4 complex to persist when MZ1 is eliminated. This is not observed in solution without MZ1, demonstrating the significance of those PPIs in stabilising the ternary complexes.

Together these articles demonstrate how structural MS techniques can augment our understanding of ternary complexes as they give us more information on the whole conformational landscape (via IM-MS). Where through HDX-MS we can gather information on the protein-protein interface which can be hugely transformative for understanding systems that suffer from an absence of X-ray/EM data, however when coupled with high resolution data it gives us an orthogonal read out in solution. As advancements have been made across the structural biology techniques from the resolution revolution with X-ray crystallography to being able to study multi-subunit complexes with cryo-EM and flying molecular elephants (proteins) with electrospray mass spectrometry, it's critical that we understand see how these techniques (and others) can enhance one another. This is especially true when it comes to *in silico* modelling, where additional restraints and data can only ever serve to generate more reliable outputs.

Targeted Protein Degradation

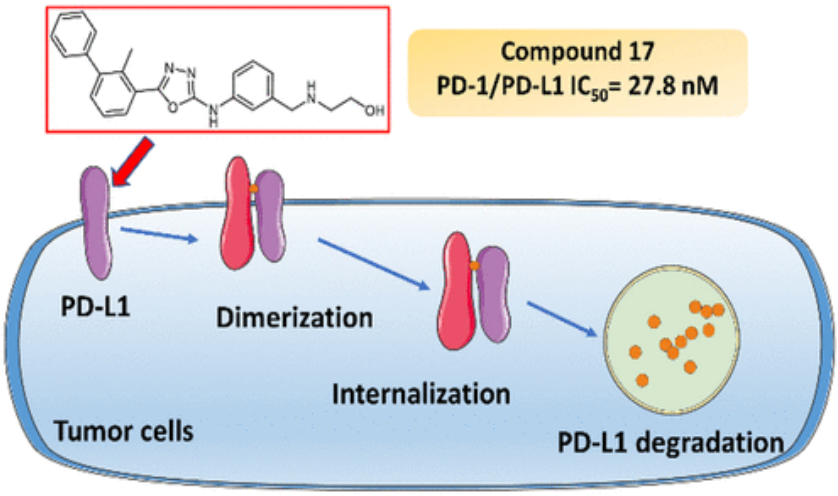
Cell Biology | Chemistry

Contributor: Conner Craigon

Discovery of Small-Molecule Inhibitors of the PD-1/PD-L1 Axis That Promote PD-L1 Internalization and Degradation

Tianyu Wang[§], Shi Cai[§], Yao Cheng[§], …, Yibei Xiao,* Sheng Jiang*

J. Med. Chem. **2022**, *65*, 3879; DOI: doi.org/10.1021/acs.jmedchem.1c01682



Under normal physiological conditions engagement of the programmed cell death-1 (PD-1) with programmed cell death-ligand, 1 (PD-L1) leads to a negative feedback loop against immune cell activation. Separation of PD-L1 from PD-1 has been successfully exploited in the clinic against non-small-cell lung cancer (NSCLC), urothelial carcinoma (UC), and Merkel cell carcinoma using monoclonal antibodies (mAbs) directed against the PD-1/PD-L1 interaction which leads to the reactivation of the body's antitumor immunity which mediates the killing of tumour cells expressing PD-L1. Antibody therapies have severe limitations including limited oral bioavailability, expensive production, unfavourable immune activation, and poor uptake into tumour tissues, so alternative strategies are in urgent demand, one such approach is small-molecule inhibitors. In this paper, Wang, Cai, and Cheng describe the discovery of a bifunctional inhibitor of the PD-1/PD-L1 interaction termed compound 17. Compound 17 was established from an initial pharmacophore database virtual screening search followed by docking analysis and subsequent validation by TR-FRET. Through crystallisation of PD-L1 and Compound 17, it was shown that compound 17 inhibits the interaction of PD-1/PD-L1 and promotes subsequent PD-L1 dimerization. Through immunostaining of a GFP tagged PD-L1, the authors were able to demonstrate time dependant PD-L1 internalization upon treatment with compound 17. It was also established through western blot that compound 17 induces degradation of PD-L1 in a lysosomal dependant mechanism. Furthermore, the authors go on to show *in vivo* data that demonstrated compound 17 suppresses the growth of CT26 mouse model tumour in BALB/c mice. The work in BALB/c mice also demonstrated no obvious bodyweight loss or mortality during the treatment indicating the suitability of compound 17's for in vivo applicability.

This is a fascinating paper that demonstrates a unique form of induced protein-protein interaction that results in the degradation of a well-studied pathway known to have clinical efficacy against common cancer types but without the negative effects of using antibody therapies. The authors use a diverse range of approaches from computational chemistry to biophysics and cellular biology to establish the efficacy of their compound 17 against PD-L1.

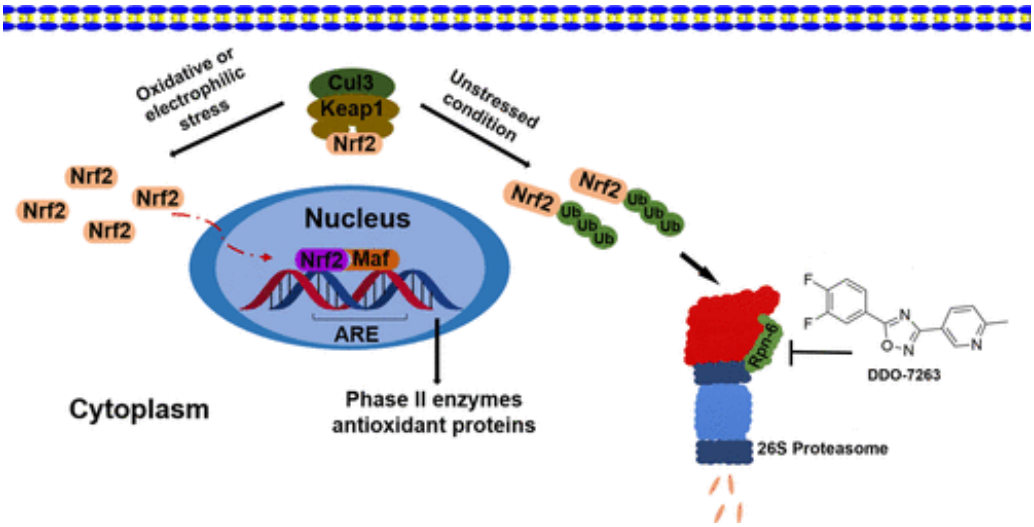
Cell Biology | Chemistry

Contributor: Conner Craigon

Target Fishing Reveals a Novel Mechanism of 1,2,4-Oxadiazole Derivatives Targeting Rpn6, a Subunit of 26S Proteasome

Zhen Dai[§], Lu-yan An, …, Qi-dong You*, Bin Di*, Li-li Xu*

J. Med. Chem. **2022**, *65,* 5029; DOI: 10.1021/acs.jmedchem.1c02210



The Keap1−Nrf2−ARE pathway regulates intracellular oxidation and therefore cellular homeostasis in humans. Nrf2, as the main regulator of intracellular redox, is a primary drug target for antioxidant stress and cancer chemoprevention. Due to this, a growing number of Nrf2-ARE activating compounds, such as the 1,2,4-oxadiazole heterocycles in inflammation-related diseases. However, it is unclear how these heterocycles induce Nrf2 activation, which hinders their drug development. This work by Dai et al. was initiated with the development of fluorescent and biotinylated derivatives probes of DDO-7263, an Nrf2 activating compound, which they establish as similarly Nrf2−ARE activating through luciferase ARE gene activation and Nrf2 localisation and demonstrate antioxidant and anti-inflammatory activity through detection of H2O2 mediated cell cytotoxicity and IL-1B suppression. Following this, the probes were used to perform an affinity pulldown of PC12 cell lysates followed by MS/MS identification of DDO-7263 engaging targets. This analysis established that DDO-7263 pull-down enriches for Rpn6 – a component of the 26s proteasome - which was subsequently validated by in vitro binding and in-cellulio competition assays as a target of DDO-7263. In this paper Dia et al. established a mechanism of 1,2,4-oxadiazole mediated Nrf2 activation and a novel target for subsequent drug discovery of the Nrf2 pathway.

This is a well thought out study that logically establishes a link between the Nrf2 ARE activating properties of the 1,2,4-oxadiazole compounds and the proteasomal component Rpn6. Key to convincing the reader of the veracity of this research is their validation of the phenotypic similarities of their probe compounds to the DDO-7623 original compound, in so doing, they demonstrate that they haven't lost DDO-7623 on-target activity. Upon identifying Rpn6 they further convince the reader by demonstrating the engagement between Rpn6–DD07623 both in vitro and in-cellulio. This paper, in my estimation, establishes a clear and rational workflow for the derivation of unidentified drug targets, provides the larger drug discovery community with novel probes of the 26s proteasome beneficial to the larger drug discovery community and most importantly has established a novel connection between Rpn6 activity and Nrf2 ARE activation that can be exploited in future drug discovery endeavours.

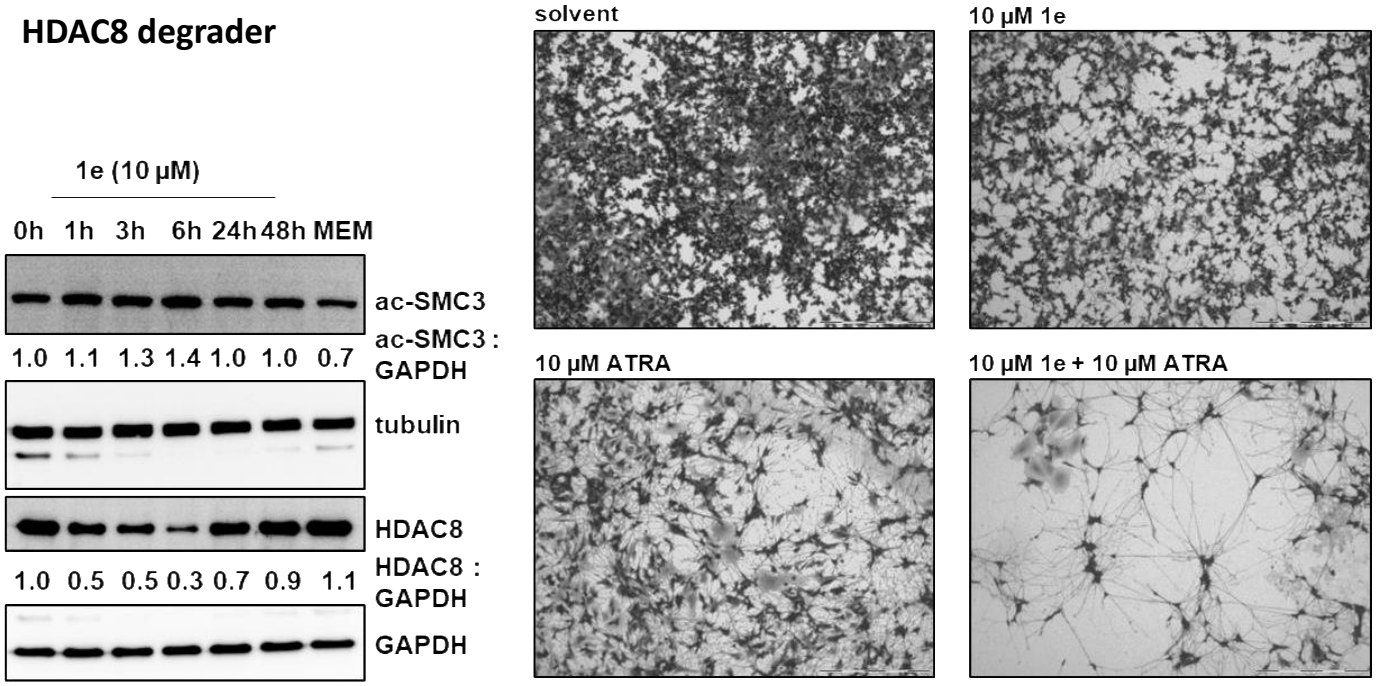
Cell Biology | Chemistry

Contributor: Conner Craigon

Design, Synthesis and Biological Characterization of Histone Deacetylase 8 (HDAC8) Proteolysis Targeting Chimeras (PROTACs) with Anti-Neuroblastoma Activity

Salma Darwish[§], Ehab Ghazy, Tino Heimburg, Daniel Herp, …, Wolfgang Sippl*

ChemRix. **2022**, DOI: 10.26434/chemrxiv-2022-blb5v



Abnormal acetylation of histones and non-histone proteins have been found to contribute to the development of various diseases including cancers of different organs such as lung, breast, and liver along with lymphoma and neuroblastoma. Histone deacetylases (HDACs) regulate the removal of acetylation markers on histones and other proteins and are therefore often dysregulated in cancers. The HDAC, HDAC8, plays a critical role in inhibiting apoptosis and enabling the invasion and metastasis of cancer. As a result, HDAC8 is considered a potential target in the treatment of cancer forms such as T-cell lymphoma, gastric adenocarcinoma, hepatocellular carcinoma, and childhood neuroblastoma. In this paper, Darwish et al. developed the first in class PROTACs against HDAC8 derived from benzhydroxamates – a selective HDAC8 inhibitor. Through *in vitro* analysis for HDAC inhibition, cytotoxicity/colony formation assays, western blot analysis of PROTAC treated SK-N-BE (2)-C neuroblastoma cells and observation of neuronal differentiation upon PROTAC treatment, Darwish et al. was able to identify two HDAC8 selective degraders that exhibit HDAC8 mediated anti-neuroblastoma activity.

It is great to see the application of PROTACs to new biological pathways and Darwish et al have done demonstrated this well in this paper. Darwish et al. has demonstrated clearly in this research novel PROTACs against HDAC8, and while they aren't particularly effective degraders in their current form, they have opened the door for future research into better second-generation degraders as well as validated HDAC8 as being phenotypically receptive to degradation in Neuroblastoma cell model.

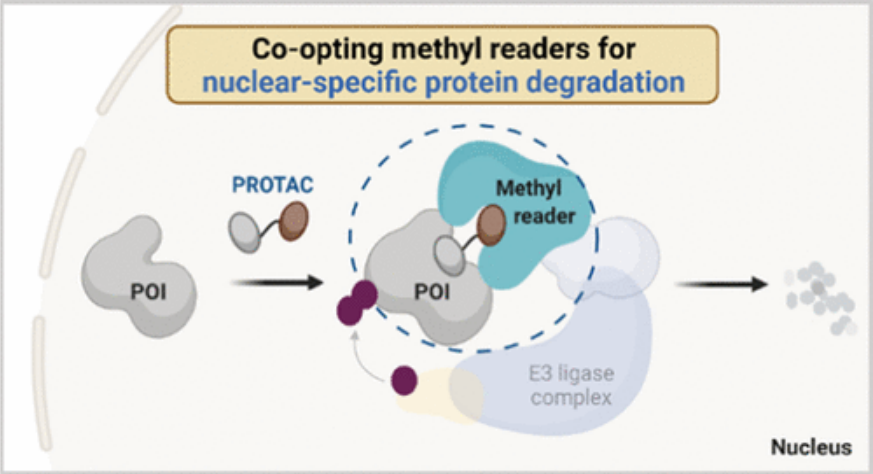
Cell Biology | Chemistry

Contributor: Conner Craigon

Hijacking Methyl Reader Proteins for Nuclear-Specific Protein Degradation

Dhanusha A. Nalawansha [§], Ke Li[§], John Hines, Craig M. Crews*

J. Am. Chem. Soc. **2022**, *144*, 5594



Most PROTACs to date degrade target proteins by utilizing either VHL or cereblon as the recruited E3 ligase. This focus on a limited number of E3 ligases is due to the paucity of liganded E3 ligases in the proteome to recruit via a small-molecule-based PROTAC. Therefore, expanding the E3 ligase toolbox is an active area of research in TPD. Research in 2018 identified that the methyl-lysine reader protein, L3MBTL3 (Lethal (3) malignant brain tumorlike protein 3), is in complex with the $Cul4^{DCAF5}$ E3 ligase, targets methylated proteins such as DNMT1, SOX2, and E2F1 for proteasomal degradation. Nalawansha et al. in this study, as a proof-of-concept, utilised the L3MBTL3 antagonist, UNC1215, as a handle to recruit the L3MBTL3-bound E3 ligase complex to the vicinity of the selected target proteins: $FKBP12^{F36V}$ and BRD2. Both the $FKBP12^{F36V}$ targeting, L3MBTL3-recruiting, KL-4 and their BRD-targeting, L3MBTL3-recruiting, KL-7, were shown to be time-, concentration-, and proteasome-dependent as identified through western blotting analysis in titration and competition assays. Interestingly, KL-7 displays selective degradation of BRD2 over BRD4 among multiple cell lines. The L3MBTL3-recruiting PROTACs demonstrate nuclear-specific protein degradation owing to the nuclear localization of L3MBTL3.

This paper demonstrates the first in class L3MBTL3 recruiting PROTACs, thereby opening the door for other researchers to develop PROTACs against their protein targets. While these PROTACs themselves are slow acting, they act as a platform for future PROTAC development. Interestingly, as L3MBTL3 is nuclear specific protein, this research implies that L3MBTL3 PROTACs could be useful in localisation specific degradation research projects, further expanding the scope of TPD.

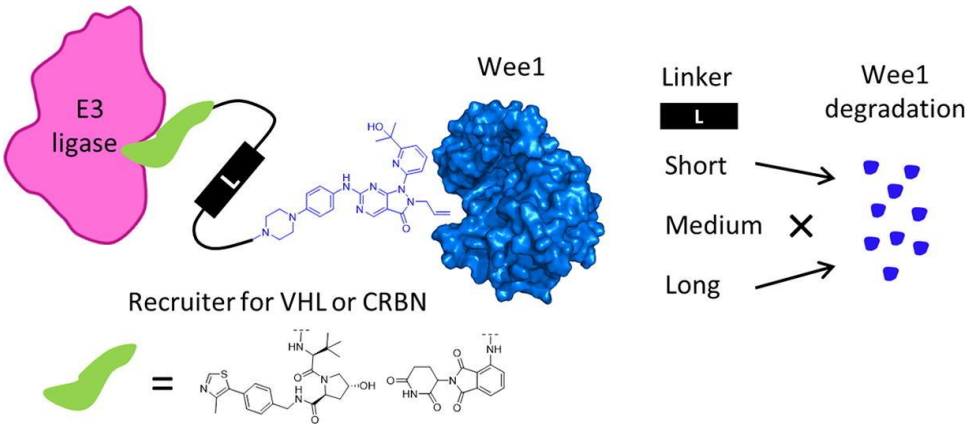
Cell Biology | Chemistry

Contributor: Aileen Frost

Selective Wee1 degradation by PROTAC degraders recruiting VHL and CRBN E3 ubiquitin ligases

Marine C. Aublette[§], …, Morgan S. Gadd*

Bioorg. Med. Chem. Lett. **2022**, *64*, 128636; DOI: 10.1016/j.bmcl.2022.128636



Wee1 regulates the G2/M checkpoint and is overexpressed in many p53 mutated cancers. Inhibition can cause cancer cells to be sensitive to DNA-damaging therapies, as this abrogates the G2/M DNA repair checkpoint. Clinical candidate AZD1775 inhibits Wee1 and has been shown to cause apoptosis of p53-inactive cells, in combination therapy with DNA-damaging agents. However, other kinases are also known to be inhibited by AZD1775, including PLK1, which negatively regulates Wee1 activity. Application of PROTAC technology to this protein target could result in enhanced selectivity, afforded via selective ternary complex formation.

The authors have generated both CRBN and VHL PROTACs, by using AZD1775 as a Wee1 ligand and have explored linker SAR. Degradation is relatively fast (~ 4 h), and control experiments involving E3 ligase negative binding controls and proteasome inhibitors support a PROTAC mode of action. Enhanced degradation selectivity is demonstrated over other inhibition targets of AZD1775, namely PLK1, PLK2, PLK3, MAP3K4 and JAK2, via Western Blot analysis. However, antiproliferation experiments show that degradation of Wee1 with these PROTACs is not as effective as by treatment with the parent inhibitor, and indeed the synthesised negative controls exhibit similar anti-proliferative IC_{50}s. The authors postulate that optimisation of their PROTACs could perhaps deliver better efficacy and could help to distinguish the effects of degradation vs inhibition.

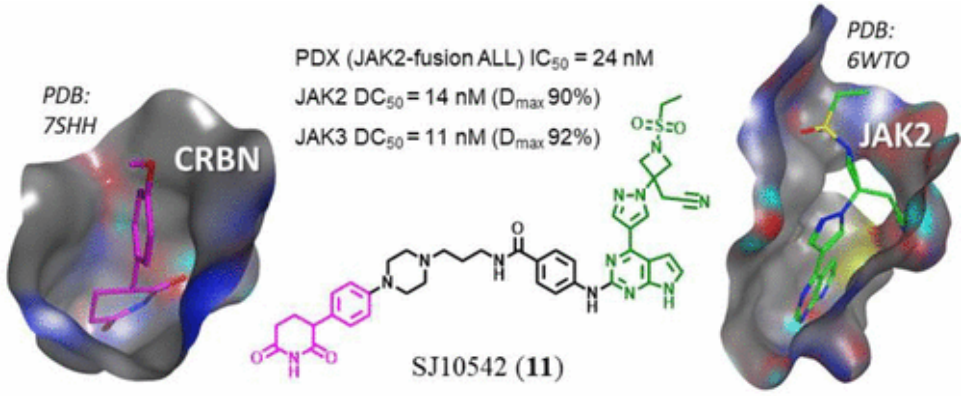
Cell Biology	Chemistry	Structural Biology/Biophysics

Contributor: Aileen Frost

Development of Potent and Selective Janus Kinase 2/3 Directing PG–PROTACs

Lisa J. Alcock[§], …, Charles G. Mullighan,* Zoran Rankovic*

ACS Med. Chem. Lett. **2022**, *13*, 475; DOI: 10.1021/acsmedchemlett.1c00650



Activation of the JAK-STAT signalling pathway is implicated in several subtypes of acute lymphoblastic leukemia (ALL), with JAK2 mutations augmenting signalling. Unfortunately, JAK2 inhibitors have been found to have limited efficacy, as reactivation of JAK-STAT signalling can occur upon trans-phosphorylation of inhibited JAK2, mediated by heterodimerisation with JAK1/TYK2. An earlier study by the same authors details the identification of dual JAK2/3 and GSPT1 CRBN-recruiting degraders. GSPT1 is a translation termination factor, and a known CRBN neo-substrate. Degradation of this off-target shuts down protein resynthesis so can confound results, and in this case was shown to mediate potent anti-tumour activity.

Here, the authors demonstrate the development of non-GSPT1 degrading JAK2/3 PROTACs, via use of phenyl glutarimide (PG) CRBN ligands. Swapping the thalidomide based CRBN binder for a range of PGs enabled the identification of a JAK2/3 degrader, without GSPT1 off-target effects. This provided a useful tool for independently assessing the effect of JAK degradation. The cytotoxicity of lead JAK2 degrader **11** in MHH-CALL-4 cells was assessed alongside inhibitors ruxolitinib and baricitinib. Although more cytotoxic than the inhibitors, PROTAC **11** did not achieve full efficacy, which the authors attribute to JAK2 redundancy in the pathway activation of JAK-STAT signalling. They further postulate that combination therapies could be explored, to assess the potential of JAK2 PROTACs as a therapy for ALL.

The publication presents a valuable example of the removal of off-target GSPT1 activity from a CRBN-recruiting PROTAC and provides a tool compound for deconvoluting this from desired on-target JAK2 degradation. Worthy of note is the co-crystal structure of a phenyl glutarimide ligand with the CRBN thalidomide binding domain, which will certainly be of interest to structural biologists and medicinal chemists alike!

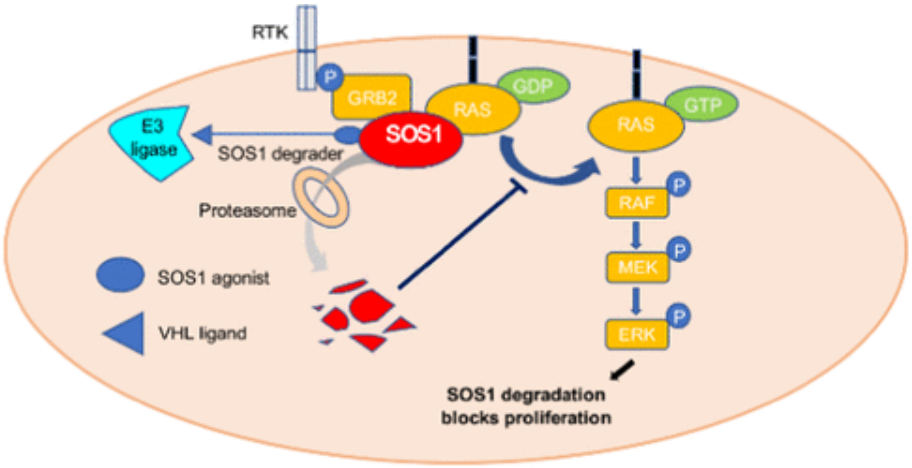
Cell Biology | Chemistry | Structural Biology/Biophysics

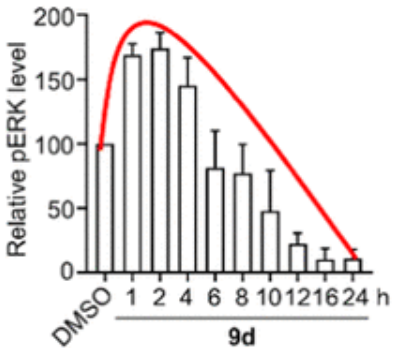
Contributor: Aileen Frost

Discovery of the First-in-Class Agonist-Based SOS1 PROTACs Effective in Human Cancer Cells Harboring Various KRAS Mutations

Chaun Zhou[§], …, Mingyue Zheng,* Sulin Zhang,* Tianfeng Xu*

J. Med. Chem. **2022**, *65*, 3923; DOI: 10.1021/acs.jmedchem.1c01774





Cell line	KRAS mutation	IC_{50} (μM)	DC_{50} (μM)
NCI-H358	G12C	0.525	0.098
MIA PaCa-2	G12C	0.218	0.255
AsPC-1	G12D	0.307	0.119
SK-LU-1	G12D	0.115	0.104
SW620	G12V	0.199	0.125
A549	G12S	0.232	0.022

SOS1 is a GEF that regulates the GDP-GTP cycle of KRAS, catalysing this exchange and thereby activating the MAPK pathway. The authors propose that targeted degradation of SOS1 may provide a strategy for treating KRAS-driven cancers, as SOS1 deletion has been shown to inhibit KRAS mutant tumour growth. This comprehensive study details the design and characterisation of VHL-recruiting SOS1 PROTACs based on clinical candidate BI 1701963.

Once best degrader **9d** is identified, its characterisation is thorough. A PROTAC mode of action is supported by synthesis of the non-VHL binding control, and via experiments including the proteasome inhibitor MG132. Interrogation of the ternary complex is achieved via use of the HTRF assay and ternary SPR measurements, in which cooperativity is also calculated (α = 15.1). By blotting also for SOS2 and KRAS it is demonstrated that **9d** does not degrade these proteins, and the PROTAC is shown to successfully inhibit cell growth over a number of KRAS mutant cell lines. Furthermore, in vivo PK experiments show that degradation of SOS1 indeed leads to inhibition of tumour growth in KRAS mutant xenograft models in mouse, via intraperitoneal dosing.

Overall, this is a well thought out and detailed study, and an enjoyable read. The investigation of SOS1 as a potential target for the treatment of KRAS-mutant cancers provides an interesting case study for the community.

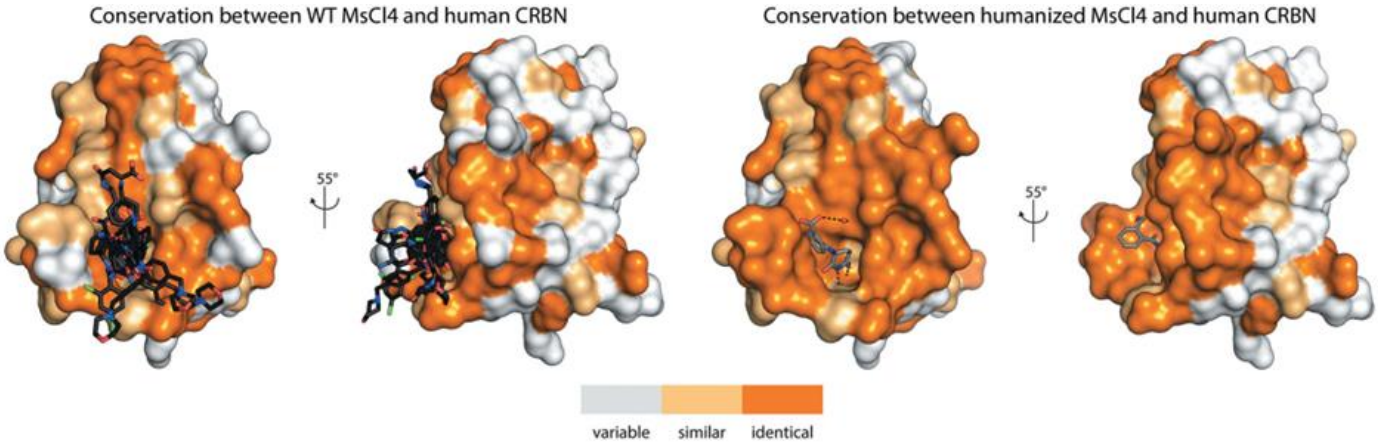
Structural Biology/Biophysics

Contributor: Andre Wijaya

High-resolution structures of the bound effectors avadomide (CC-122) and iberdomide (CC-220) highlight advantages and limitations of the MsCl4 soaking system

Christopher Heim[§], Marcus D. Hartmann*

Acta Cryst. D. **2022**, *78*, 290



The absence of a suitable crystal-soaking system for human cereblon protein has led the authors to develop a robust, single-domain crystal system based on a bacterial homologue; cereblon isoform 4 from *Magnetospirillum gryphiswaldense* (MgCl4). Using this system, the authors have characterized the binding of various thalidomide analogs, metabolites, and recently the next generation thalidomide-derived immunomodulatory drugs (IMiDs), including avadomide and iberdomide at atomic resolution. This clearly shows the advantage of this system as it is a very reproducible system that also generate high-resolution structures. However, in the latter structures, the binding mode of these next generation IMiDs which is larger in size in comparison to thalidomide analogs, revealed the limitation of the current system as the ligands are starting to reach the non-conserved residues of the MgCl4. The authors then attempted to 'humanized' MgCl4 and managed to obtain a more representative structure of human cereblon.

This study is highly relevant to the cereblon structure-based drug design work and can certainly and immensely help the wider community in exploring the chemical space of cereblon binders.

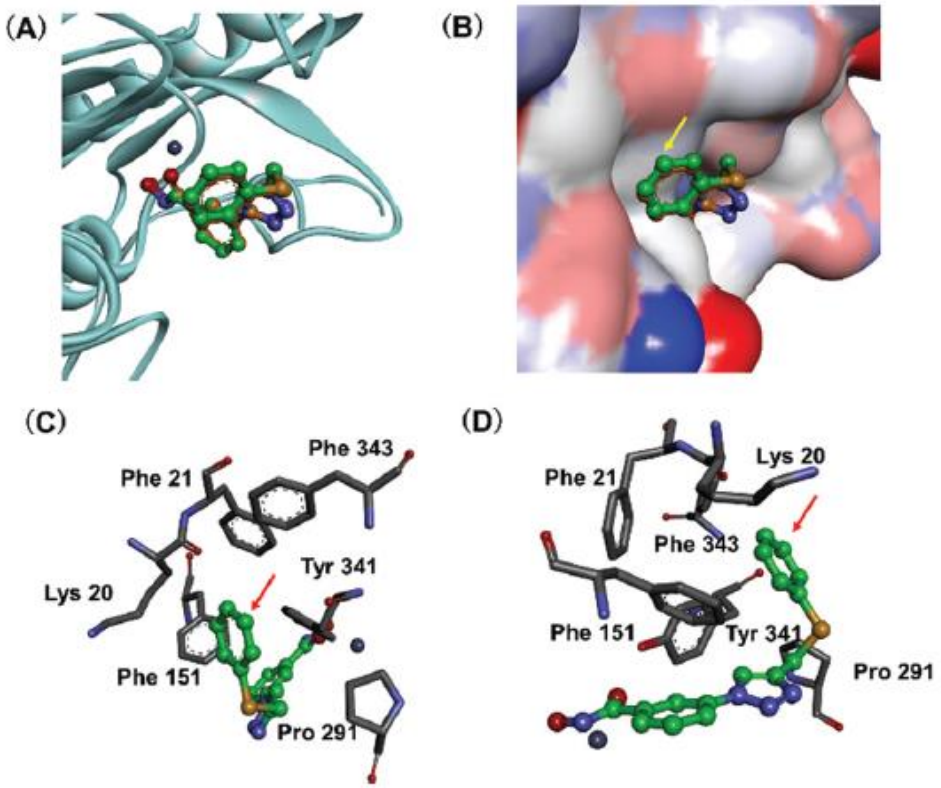
Chemistry | Structural Biology/Biophysics

Contributor: Andre Wijaya

Selective degradation of histone deacetylase 8 mediated by a proteolysis targeting chimera (PROTAC)

Jiranan Chotitumnavee[§], …, Yukihiro Itoh*, Takayoshi Suzuki*

Chem. Commun. **2022**, DOI: 10.1039/d2cc00272h



Histone deacetylase 8 (HDAC8) is a zinc-dependent deacetylase that has been shown to be involved in cancer development. Despite this, there is still uncertainty in the field on whether its catalytic or scaffolding function, or even both are essential for its role in cancer development. Therefore, the addition of PROTAC technology would be advantageous to elucidate this as successful target degradation eliminates the scaffolding function of the protein of interest (POI).

In this work, the authors used the classical structure-guided approach in designing their PROTACs, starting with a docking study on the crystal structure of humanized *Schistosoma mansoni* HDAC8 to determine the exit vector which they conclude with *para*-position to be the most desirable. They then chose pomalidomide as the E3 ligand due to its popularity and began making PROTACs with various linkers length. HDAC8 cellular degradation assay revealed Compound **4c** (with C11 at *meta*-position) to be the most potent degrader. An analogous compound (C11 at para-position), **4d**, showed no degradation as expected from the docking study, highlighting the importance of exit vector design and structure-guided approach. Degradation selectivity assay consisting of HDAC1, HDAC2, and HDAC6 showed that **4c** only degrade HDAC8, retaining the selectivity of its parent compound. Co-treatment with the parent compound, pomalidomide, MLN7243 and bortezomib reduced and/or abolished the **4c** induced HDAC8 degradation which suggest that the degradation is facilitated by the ubiquitin proteasome system. Another success story of PROTAC degrading potentially high-profile target.

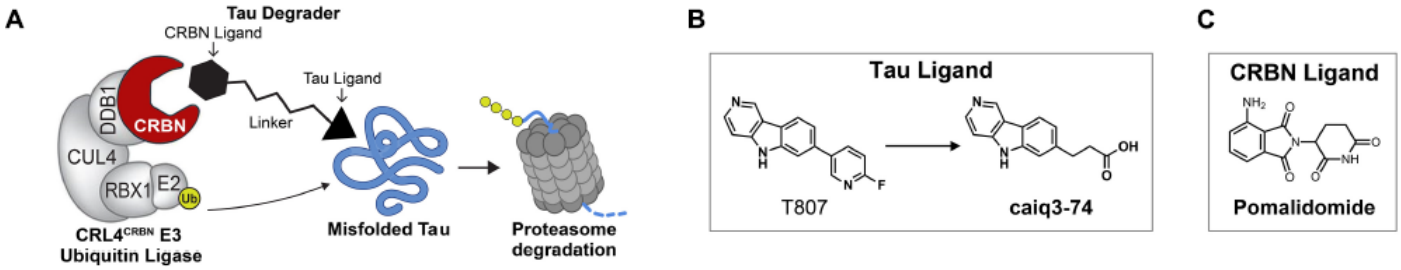
Cell Biology | Chemistry

Contributor: Andre Wijaya

Discovery and Optimization of Tau Targeted Protein Degraders Enabled by Patient Induced Pluripotent Stem Cells-Derived Neuronal Models of Tauopathy

M. Catarina Silva[§], …, Fleur M. Ferguson*, Stephen J. Haggarty*

Front. Cell. Neurosci. **2022**, DOI: 10.3389/fncel.2022.801179/full



Accumulation of misfolded, insoluble, and hyper-phosphorylated Tau protein inside neurons is the hallmark of Tauopathies, which includes frontotemporal dementia (FTD) and Alzheimer's disease (AD). Previous works from this group indicate that degradation of the misbehaving Tau proteins could be a promising therapeutic intervention for these neurodegenerative disorders, and discovered a Tau-specific degrader, **QC-01-175**. The discovery was made possible by an *ex vivo* disease model which is FTD-patient specific, as it is derived from the FTD-neurons induced pluripotent stem cells (iPSC). Through linker and E3 ligands exploration, the authors managed to discover nanomolar degraders and found that their cereblon-based degraders outperform the VHL-based counterpart. They reasoned that this could be due to the VHL E3 ligase system being disrupted in this particular disease model. They then carried out another optimization round on the cereblon-ligand end which resulted in a molecule with 10-fold increase in potency, **FMF-06-049**. Remarkably, **FMF-06-049** preferentially degrades the insoluble form of Tau protein.

The usage of a highly relevant disease model for screening, FTD-neurons iPSC derived from patients in this case, in my opinion, is the key to the success in this study. Additionally, by measuring the Tau protein degradation level in both soluble and insoluble forms rather than just as a whole, they discovered a different degradation preference profile among the degraders, adding an extra layer of complexity to the kinetic of the degraders.

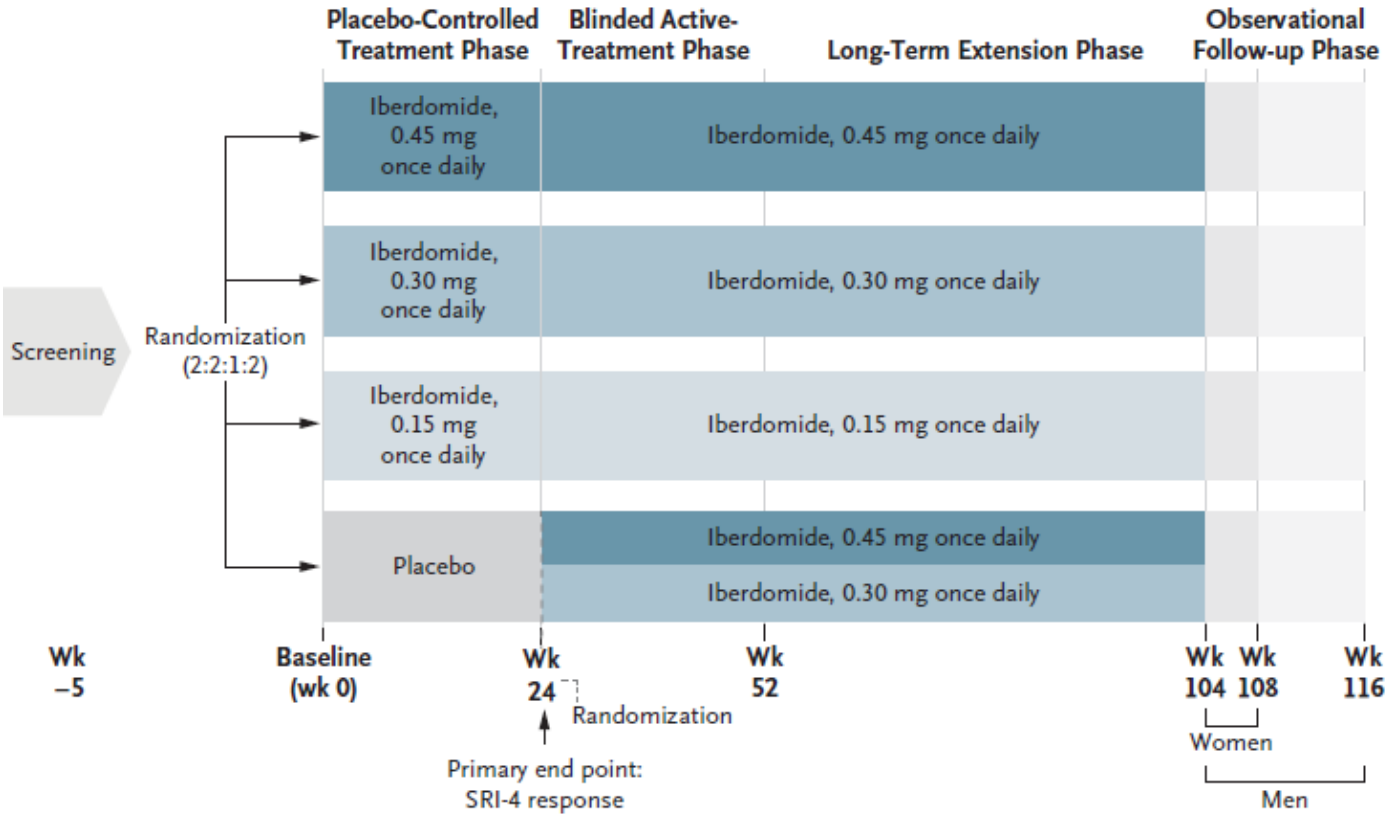
Cell Biology

Contributor: Andre Wijaya

Phase 2 Trial of Iberdomide in Systemic Lupus Erythematosus

Joan T. Merrill[§], ..., Nikolay Delev*

N. Engl. J. Med. **2022**, *386*, 1034



Iberdomide is a next generation IMiD that recently has gained popularity due to its antitumor and immunostimulatory properties. The effect of iberdomide on treating systemic lupus erythematosus (SLE) is evaluated in this phase 2 trial study. 288 patients randomly assigned into 3 treated and 1 placebo groups were subjected to 24-week long iberdomide treatment. 54% of patients from the highest dosage group (0.45 mg daily, n=81) showed better prognosis. Interestingly, 35% of the placebo group (n=83) also deemed the same. Mild and moderate adverse effects were found to be more frequent among patients who received treatment. Some of the most frequent adverse events in treated groups are neutropenia and infections including urinary tract, upper respiratory tract, and influenza. Furthermore, the rate of serious adverse effects in all treated group are comparable to those of the placebo group, highlighting the tolerability of iberdomide at dosage up to 0.45 mg/day. Lastly, the authors conclude that, at the highest dosage tested, iberdomide is superior to placebo with respect to their definition of efficacious treatment.

It is very interesting and encouraging to see a targeted protein degrader going further in clinical trial study and shows promising results. This will inspire other protein degraders across the globe (including us, CeTPDers) to keep degrading other disease-relevant proteins!

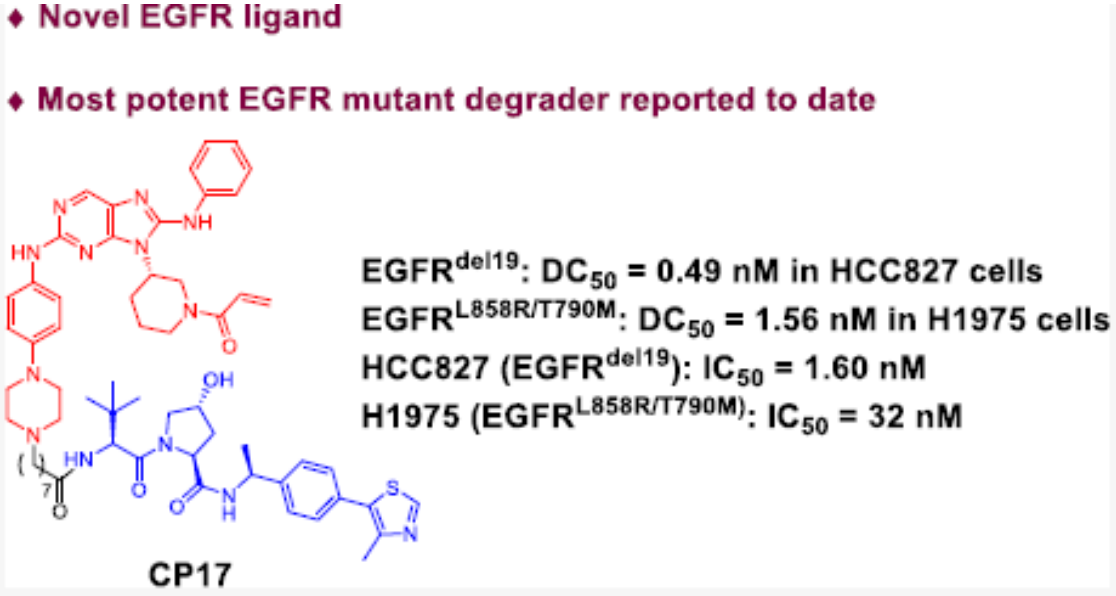
Cell Biology | Chemistry

Contributor: Aileen Frost

Discovery of Potent PROTACs Targeting EGFR Mutants through the Optimization of Covalent EGFR Ligands

Hong-Yi Zhao[§], …, San-Qi Zhang*

J. Med. Chem. **2022**, *65*, 4709; DOI: 10.1021/acs.jmedchem.1c01827



Inhibition of EGFR tyrosine kinase at the ATP binding site has been previously shown to block EGFR signalling and result in antiproliferation of tumour cells, with therapeutic potential in NSCLC. However, EGFR mutations have resulted in drug resistance, limiting the utility of inhibition as a treatment strategy. It is postulated therefore that targeted protein degradation could be an effective alternative to target these mutant forms. The authors present an optimisation of covalent PROTACs to simultaneously degrade both the EGFR$^{L858R/T790M}$ and EGFR^{del19} mutants, building on a previous non-covalent study.

Chemical optimisation involving amendments to the core and a reversible covalent strategy are detailed, with antiproliferation in EGFR mutant cell lines used as they key readout. Ultimately, covalent purine based **CP17** is identified as the lead compound and further characterisation, involving degradation assays in a number of cell lines, impact on downstream signalling, and mechanistic considerations, is undertaken. Experiments are described to validate the PROTAC mode of action, including the synthesis of non-binding negative controls. Key findings include that whilst abrogating VHL binding removes EGFR mutant degrading ability, the negative control compound still maintains a similar level of antiproliferation activity, implying that this **CP17** also functions as an inhibitor. Interestingly, inclusion of the proteasome inhibitor MG132 did not change the extent of degradation, whereas the lysosome inhibitor chloroquinine was found to partly inhibit EGFR$^{L858R/T790M}$ degradation, implying that the degradation was related to lysosomes.

Whilst the authors clearly consider the role of ternary complex formation in their discussion, this is mostly probed indirectly in this article, for example through the use of competition experiments. It would be interesting to see some biophysical or biochemical data to both interrogate the ternary complexes formed by **CP17**, and to support the compound optimisation process.

Other Paper Highlights

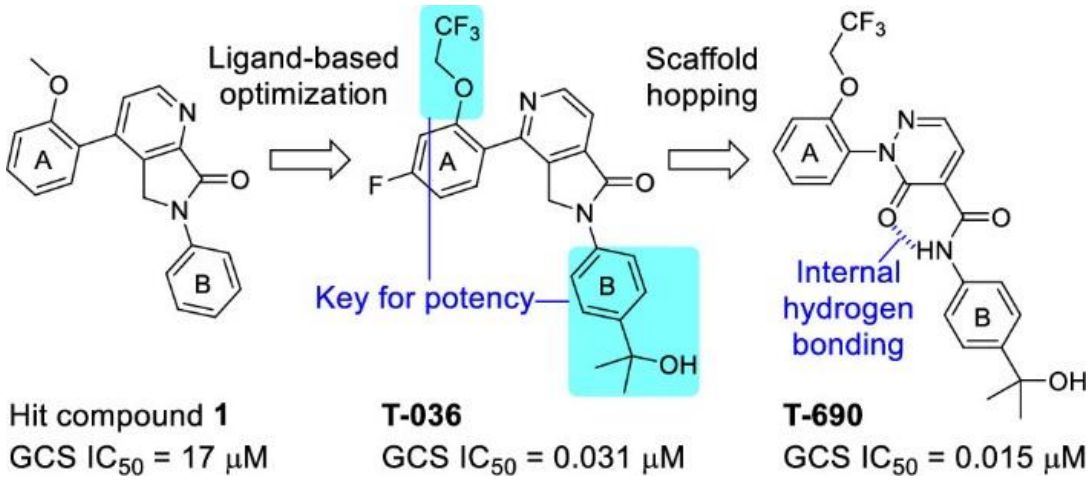
Chemistry

Contributor: Tasuku

Discovery of Brain-Penetrant Glucosylceramide Synthase Inhibitors with a Novel Pharmacophore

Yuta Tanaka*, …, Yuta Tanaka*

J. Med. Chem. **2022**, *65*, 4270



It is known that the dysfunctional mutation in the beta-glucocerebrosidase (GCase) gene is the key factor that causes Gaucher's disease (GD). Inhibition of glucosylceramide synthase (GCS), which is a main enzyme in glycolipid synthesis, potentially reduces glycosphingolipids and is the one of the approaches used to treat patients with GD. Some GSC inhibitors are in clinic to manage peripheral symptoms of GD, but efficacy of those compounds in central nerve system is still not sufficient. In this paper, the authors investigated developing novel GSC inhibitors which have a good brain permeability. Their high throughput screening campaign and following medicinal chemistry effort identified T-036, but it has a potential toxicological risk that caused by some off-target inhibitions. They speculated that the planarity of the central bicyclic ring is important for GSC inhibition, and they took a scaffold-hopping approach to replace it with a monocyclic ring. It was found that formation of the intramolecular hydrogen bond retained strong GSC inhibitory activity and T-690 showed strong activity with moderate brain permeability. T-690 was tested in *in vivo* mouse models and showed meaningful reduction of glucoceramid in plasma and brain tissues. I guess that it would be a great clinical candidate for treatment of GD.

My image is that the formation of bicyclic rings is one of the standard approaches to improving brain penetration and physicochemical profile, so I'm interested in their opposite approach. And I was a bit surprised that the names of the first author and the last author are the same.

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