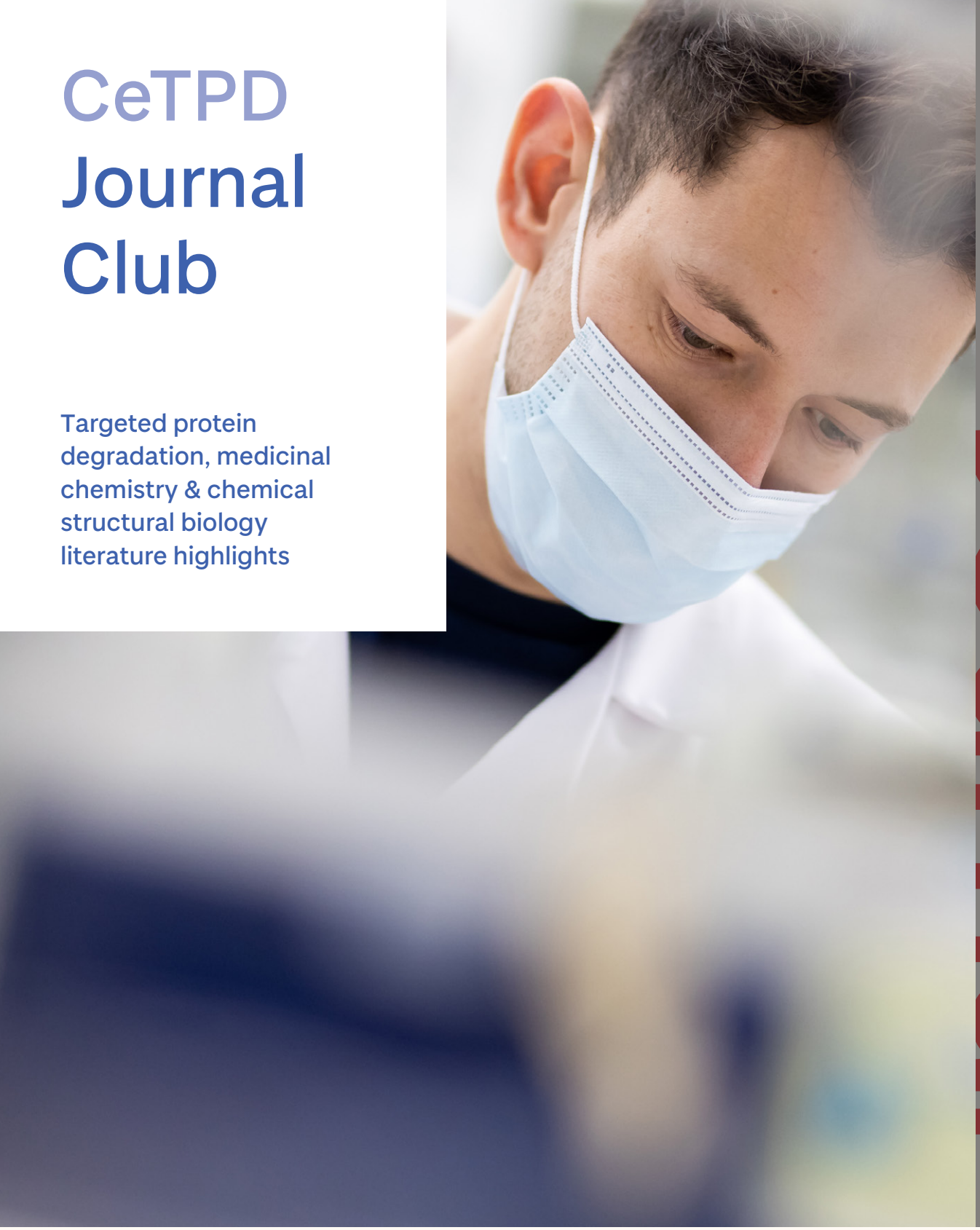
CeTPD Journal Club

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



Journal Club

September 2022

Centre for Targeted Protein Degradation
University of Dundee

innovate
collaborate
inspire

Content

Meet this Month's Editors


This month's editors are: Dylan Lynch, Manjula Nagala and Alessandra Salerno

"The JC is a great way to keep up with and reflect upon the latest TPD literature. It keeps our team focused on the latest developments in the TPD space, while also presenting breakthrough discoveries in a format accessible to a wide audience. "

Dylan completed his PhD at Trinity College Dublin with Prof. Eoin Scanlan, where he worked at the interface between organic synthesis and chemical biology. Originally training as a medicinal chemist, he spent his postgraduate years developing radical-based synthetic methodology for accessing biomolecules, often centered around a carbohydrate scaffold. He joined the Ciulli group in May 2022 as a postdoctoral scientist on the EUbOPEN project, working on small-molecule probes for E3 ligases.

"*The JC is a great resource for keeping up to date with the literature and for helping the less familiar to approach to the field. I have been following the Ciulli JC since it was launched during the pandemic, and I am glad to have been part of it for this month. It feels to be part of a community"*

Alessandra started her PhD under the supervision of Prof M. L. Bolognesi (University of Bologna, Italy), focused on the design and synthesis of PROTACs for neglected and neurodegenerative diseases. She joined the Ciulli Group in June 2022 for six months as a visiting PhD student, keen on expanding her knowledge of the PROTAC field.

Landmark Paper

Contributor: Dylan

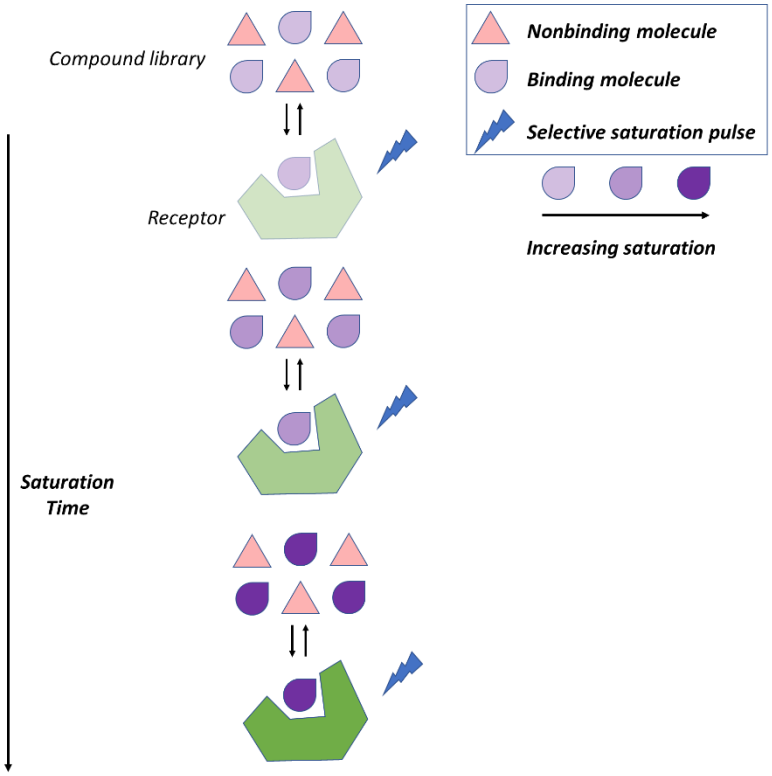
Characterization of Ligand Binding by Saturation Transfer Difference NMR Spectroscopy

Moriz Mayer and Bernd Meyer

Angew. Che. Int. Ed. **1999**, 38, 1784 DOI: 10.1002/(SICI)1521-3773(19990614)38:12<1784::AID-ANIE1784>3.0.CO;2-Q

This month, the CeTPD had the pleasure of being joined by Moriz Mayer, who is visiting from Boehringer Ingelheim until the end of November. With this in mind, the CeTPD journal club would like to feature Moriz' 1999 paper; a pioneering account discussing saturation transfer difference NMR (STD-NMR).

STD-NMR is an invaluable addition to the toolbox of techniques for studying protein-ligand binding. This technique relies on the saturation transfer to molecules which are in direct contact with the protein, facilitating fast identification of binding activity from diverse mixtures of potential ligands. This technique generates both 1D and 2D spectra which display only signals from compounds with binding affinity, even with as little as 1 nmol of protein. In addition, the exact binding epitope of a given ligand can be elucidated, as the functional groups in direct contact with the protein will yield stronger STD signals. In a more technical sense, the intermolecular transfer of magnetisation from protein-to-ligand results in a progressive ligand saturation. The authors highlighted that a particular limitation is that the mixture of molecules should not be affected by the initial selective saturation pulse.



Mayer and Meyer demonstrated the use of STD-NMR for studying the binding of *N*-acetylglucosamine (GlcNAc) to wheat germ agglutinin (WGA). The technique was further validated by the inclusion of several non-binding saccharides for comparative purposes. Furthermore, the screening of the library of WGA and ligand (in 20-fold excess) *via* a standard 1D STD NMR experiment could be accomplished in two minutes.

This account forged a breakthrough in bioaffinity NMR methods and laid the groundwork for what is a widely employed and indispensable technique, crucial for investigating the binding of molecules directly from mixtures. This technique works to obviate the risk of false positives for binding affinity and there are few limitations on the size of the molecule being screened, if the protein can still be selectively excited.

Since this publication in 1999, there have been over 1,000 research papers based on STD-NMR deposited in the Scopus literature database. The Ciulli group has relied on this technique as part of our toolbox for many years, and the contribution of this landmark paper to the field of targeted protein degradation and beyond cannot be understated.

Targeted Protein Degradation

Cell Biology | Chemistry

Contributor: Dylan

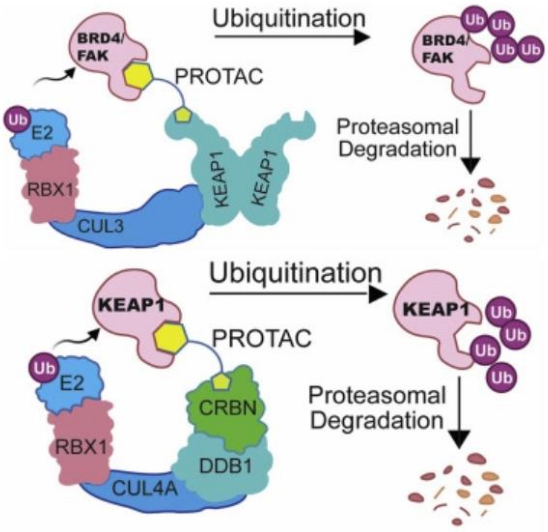
Exploring the target scope of KEAP1 E3 ligase-based PROTACs

Guangyan Du[§], Jie Jang[§], …, Eric S. Fischer*, Nathanael S. Gray*

Cell Chem. Biol. **2022**, DOI: 10.1016/j.chembiol.2022.08.003

In this endeavour by Du *et al.*, the authors sought to use KI696, an inhibitor of Kelch-like ECH-associated protein 1 (KEAP1), as a recruitment handle for E3 ligase-mediated degradation. They were able to generate a number of KEAP1-recruiting degraders of the BET family proteins, as well as certain kinases. Furthermore, having identified the limited scope of KEAP1, they synthesised a conjugate of a KEAP1-binding ligand and a CRBN-binding ligand, which induced KEAP1 degradation without deterioration of CRBN.

One particular outcome is the evidence that KEAP1-based PROTACs are less versatile than CRBN or VHL-based systems. Another interesting point is the 'linkerology' involved in their design of the KEAP1-BRD4 compounds, which shows a clear trend on the level of BRD4 degradation based on linker length and the number of oxygen atoms in the linker chain. Finally, one interesting point regarding their results is their discussion of the studies limitations – particularly the variance between cell lines, and that degradation may only work in a small number of them.



Cell Biology | Chemistry

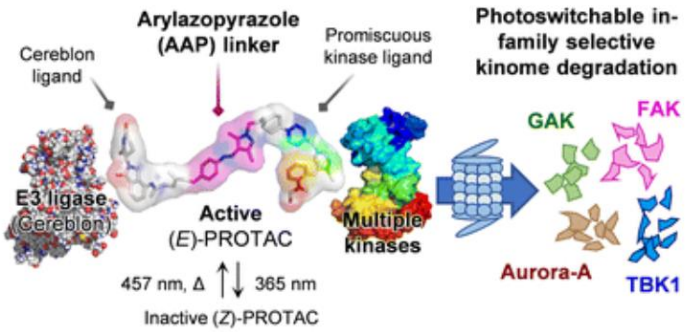
Contributor: Dylan

Light-mediated multi-target protein degradation using arylazopyrazole photoswitchable PROTACs (AP-PROTACs)

Qisi Zhang,[§] Cyrille S. Kounde, …, Edward W. Tate*

Chem. Comm. **2022**, DOI: 10.1039/d2cc03092f

In this account, light-activated control of PROTAC-induced degradation was accomplished with an arylazopyrazole linker. By varying the wavelength of light, Zhang *et al.* have achieved selective on/off optical control over the degradation of a number of valuable kinase targets. The inactive (*Z*)-isomer is transformed to the active (*E*)-PROTAC which induces ternary complex formation and ultimately, protein degradation. The authors propose that this "selective switchable degradation" is due to augmented affinity for kinase binding, and the ability of the active isomer to form a catalytically competent kinase/PROTAC/CRBN ternary complex.



One interesting application of this work is in the applicability of the photoswitchable linker to wide range of other PROTACs. Those working in TPD know the dramatic effects that linker modifications can furnish, and with the potential use of this arylazopyrazole scaffold in a 'plug-and-play' setup for bifunctional compounds, there is a wide scope of future applications of this research.

Cell Biology | Chemistry

Contributor: Dylan

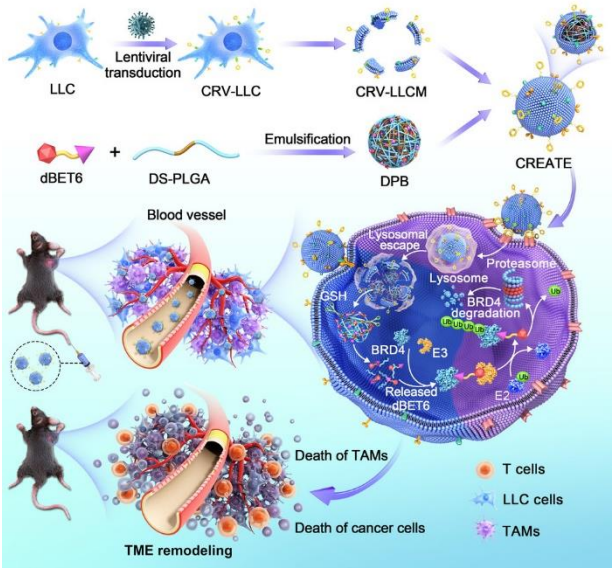
Versatile Nano-PROTAC-Induced Epigenetic Reader Degradation for Efficient Lung Cancer Therapy

Huan-Tian Zhang,[§] Rui Peng, …, Zheng-Gang Zha*, Mengmeng Yi*, Lingmin Zhang*

Adv. Sci. **2022**, DOI: 10.1002/advs.202202039

This account details a novel nano-PROTAC based on a disulfide bond-linked polymer scaffold, which responds to pH/glutathione, and is used to load a BRD4-targeted PROTAC into cells. This DS-PLGA scaffold emulsifies the PROTAC dBET6 to facilitate loading into cells. The authors report that their 'CREATE' methodology furnishes simultaneous targeting of both tumour associated macrophages (TAMs) and lung cancer cells. Additionally, this paper reports a remodelling of the tumour cell microenvironment via direct elimination of lung cancer cells and TAMs.

This work provides an interesting insight into stimuli-responsive nanoparticles and their potential in novel treatments. The authors highlight that among the stimuli that can trigger drug release from these nanoparticles, redox potential is currently a front runner in the field, due to the redox potential gradient at the tumour site.



Cell Biology | Structural Biology/Biophysics

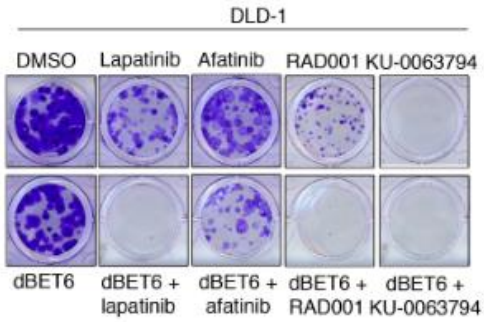
Contributor: Dylan

The drug efflux pump MDR1 promotes intrinsic and acquired resistance to PROTACs in cancer cells

Alison M. Kurimchak[§], Carlos Herrera-Montávez[§], … James S. Duncan*

Sci. Signal. **2022**, DOI: 10.1126/scisignal.abn2707

Drug resistance is a huge obstacle in the field of oncology, and in the TPD field, resistance to PROTACs has been reported in several cancer cell lines to date. In this work, the authors identified both acquired and intrinsic resistance mechanisms to PROTACs in several cancer cell models, *via* proteomic analysis. They further elucidated that this resistance was mediated by increased production of the drug efflux pump MDR1. This account concludes that in the clinical use of PROTACs for protein degradation, if a therapeutic response in cancer is desired, a concurrent MDR1 blockade will likely be required.



An interesting note from this paper is that the gene which encodes for the drug efflux pump, ABCB1, appears to have limited expression in some cancer cell models, particularly those concerned with lymphoma. The knock-on effect of this is that there may be a possible patient population for whom the desirable therapeutic outcomes of PROTAC treatment would be more likely.

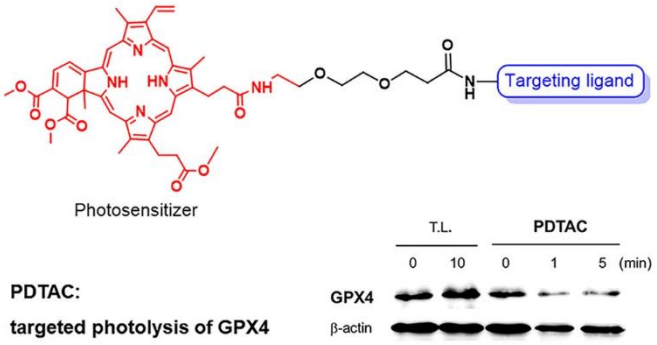
Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Alessandra

PDTAC: Targeted Photodegradation of GPX4 Triggers Ferroptosis and Potent Antitumor Immunity

Sijin Liu,[§] Xi Zhao,[§] … Guoquan Liu*

J. Med. Chem. **2022**, 65, *18*, 12176



In this work, the authors propose an unprecedented protein degradation strategy based on a targeted photolysis approach. The photodegradation-targeting chimeras (PDTACs) have been designed by conjugating a clinically approved photosensitizer (verteporfin, VPF) to noninhibitory Glutathione Peroxidase 4 (GPX4)-targeting peptides with the purpose to direct photoinduced ROS to degrade GPX4. After confirming the unchanged binding affinity and *in vivo* target engagement, the degradation capacity of those chimeras was assessed in both cell lysates and living cells upon red-light irradiation. Eventually, the targeted photolysis of GPX4 resulted in dominant ferroptotic cell death in cancer cells. Although in living cells, VPF alone also promiscuously degraded several proteins, the authors made the effort to prove PDATCs-mediated selective degradation. Of note, given the amphiphilic structures, the chimeras were identified by transmission electron microscopy (TEM) to be self-assembled into nanoparticles, which were highly taken by cancer cells.

Overall, the PDATACs technology may be a promising application since (i) light activation endows high spatiotemporal control and (ii) the independence from E3 ligase system broad the application to those contexts where the ubiquitin–proteasome system is unknown or not present

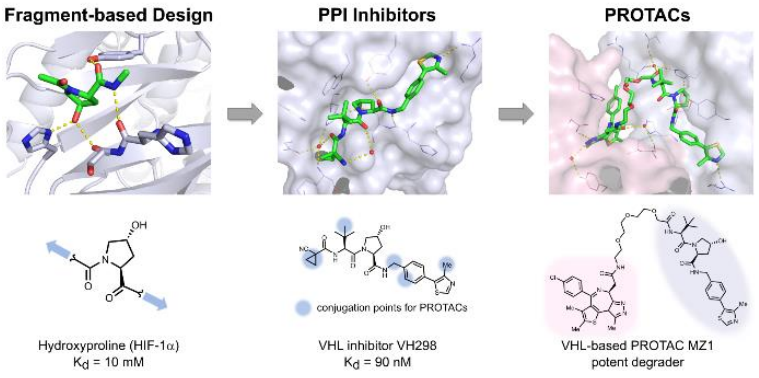
Chemistry | Computational Chemistry | Structural Biology/Biophysics

Contributor: Alessandra

Discovery of small molecule ligands for the von Hippel-Lindau (VHL) E3 ligase and their use as inhibitors and PROTAC degraders

Claudia J. Diehl[§] and Alessio Ciulli*

Chem. Soc. Rev. **2022**, DOI: 10.1039/D2CS00387B



This article is a journey towards the discovery of VHL inhibitors, from the early fragment-based design to the subsequent rational structure-guided optimization. In the reading, extensive and critical "med chem" discussions support each stage of the development campaigns alongside insights into the structural counterpart.

Besides the obvious application as chemical probes for disrupting the VHL/HIF-1a interaction, VHL inhibitors have served as a platform also for further chemical developments, for example, VHL recruiting fluorescence or NMR probes for biophysical assays. The limitation of such biophysical techniques and the unsuccessful optimization stories have been fairly reported throughout. Eventually, the embodiment of those high specificity small-molecule ligands for VHL into PROTACs has boosted their use and popularity among the TPD community. The second half of the article reports selected examples of VHL-recruiting PROTACs, with a purposed focus on ligand's scaffold and linker tethering vectors and also on chemical and spatiotemporal control innovation.

The lack of good E3 ligase ligands was (and partially still is) a damper on the PROTACs development. The article sheds light on the past and present of VHL inhibitors, from their early development to the latest pieces of research and for this reason, it appears to be hugely significant not only for the ones involved in VHL-based research but for the entire TPD community.

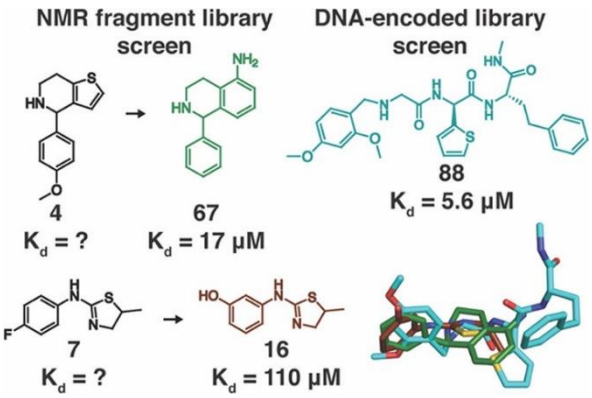
Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Alessandra

Discovery and Structural Characterization of Small Molecule Binders of the Human CTLH E3 Ligase Subunit GID4

Chetan K. Chana,[§] … Anne-Claude Gingras,* and Frank Sicheri*

J. Med. Chem. **2022**, DOI: 10.1021/acs.jmedchem.2c00509



Structural information and small-molecule binders for the expansion of the E3 ligase toolbox are nowadays a major challenge in TPD field. The authors herein reported the identification of new small-molecule binders for the human CTLH GID4 E3 Ligase. Firstly, the GID4 subunit of the CTLH E3 ligase has been genetically proven to induce protein degradation in living cells by direct recruitment of the model substrate EGFP. After the proof-of-concept, small molecule binders of CLTH GID4 were identified by two different and parallel approaches. NMR-based fragment screening and DNA encoding library (DEL) screening campaigns led to the identification of three and five hit compounds, respectively. The X-ray crystal structures of the identified hits were harnessed to pursue extensive structure-guided SAR exploration which led to more affine binders. Binding affinity was indeed assessed by differential scanning fluorimetry (DSF) for the initial hits and by fluorescence polarization (FP) competition assays and isothermal titration calorimetry (ITC) for the optimized compounds. The prioritized compounds (**16**: Kd = 110 µM, **67**: Kd = 17 µM, **88**: Kd = 5.6 µM) displayed cellular engagement by cellular thermal shift assay (CETSA) experiments, which positively confirmed permeability and binding to a 3xFLAG epitope-tagged full-length GID4. Eventually, thermal proteome profiling of HEK293 lysates treated with one of the compounds showed selective engagement and low off-targets binding.

Although the best compounds of this work resulted less active than the reported GID4 binder PFI-7, the outlined pipeline, which spans from the discovery to the biological characterization of new binders, could be of help and inspiration to expand and accelerate new E3 ligase ligands discovery.

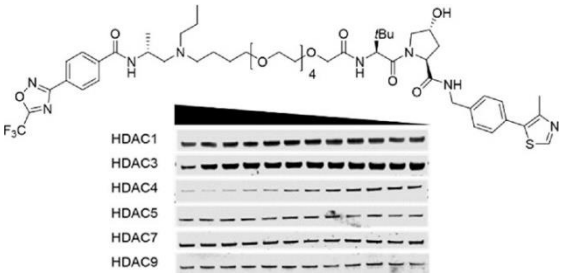
Cell Biology | Chemistry

Contributor: Alessandra

Developing HDAC4-Selective Protein Degraders To Investigate the Role of HDAC4 in Huntington's Disease Pathology

Natsuko Macabuag,[§] …, Elizabeth M. Doherty,* Celia Dominguez

J. Med. Chem. **2022**, 65, *18*, 12445–12459



HDAC4 has been shown to associate and colocalize with cytoplasmic aggregates of mutant huntingtin protein and for this reason HDAC4 inhibition has been already investigated in preclinical Huntington's Disease (HD) models. In this work, panHDAC inhibitors belonging to two chemical classes have been turned into PROTACs as a companion strategy to further elucidate the role of HDAC4 in HD. The HDAC4 degraders were profiled in *in vitro* and cellular enzymatic inhibition in comparison with the unmodified HDAC inhibitors, showing no pronounced selectivity towards class IIa isoforms. Interestingly, as for other cases described in literature, although starting from HDAC pan-inhibitors, the developed PROTACs showed selective HDAC4 degradation in Jurkat cell line and HD mouse model-derived cortical neurons. However, the degradation profile was not evident in other neuronal cell lines, likely due to P-gp efflux mechanisms. This study encompasses the challenges of designing CNS-directed PROTACs and highlights how poor permeability across the BBB due efflux mechanisms and/or variability towards cell models should always take into consideration in PROTAC early development.

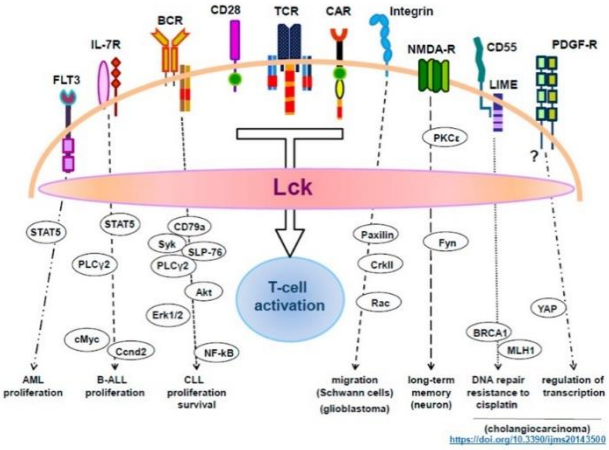
Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Manjula

Preclinical evaluation of proteolytic targeting of LCK as a therapeutic approach in T cell acute lymphoblastic leukemia

Jianzhong Hu[§], Jamie Jarusiewicz[§], …, Zoran Rankovic*, Jun J Yang*

Sci. Transl. Med. **2022**, DOI: 10.1126/scitranslmed.abo5228.



In recent years T-cell kinase, the lymphocyte-specific protein tyrosine kinase (Lck) a key molecule regulating T-cell functions is widely implicated in blood malignancies. Previous targeting of Lck with dasatinib has proven to inhibit/delay leukemia growth in preclinical studies and in patients with T acute lymphoblastic leukemia (T-ALL). However, this is transient in most cases. To irreversibly suppress and degrade LCK, Hu *et al*. created a PROTAC using dasatinib as an LCK ligand and phenyl-glutarimide as a cereblon-directing moiety. In this study they identified a lead compound, SJ11646 which performed better than dasatinib *in vitro* (three orders higher cytotoxicity in LCK-activated T-ALL cell lines and primary leukemia samples) and *in vivo* (through pharmacokinetic and pharmacodynamic profiling which indicated a 630% increase in the duration of LCK suppression by SJ11646 over dasatinib). SJ11646 extended leukemia-free survival over dasatinib in mice (as analysed using patient-derived xenograft models of T-ALL) and represents a promising therapy for patients that require further clinical evaluation. Further, the study shows SJ11646 has high binding affinity to 51 human kinases, particularly ABL1, KIT, and DDR1, all of which are known drug targets in other cancers, hence can be a valuable tool in other cancer models which needs further evaluation.

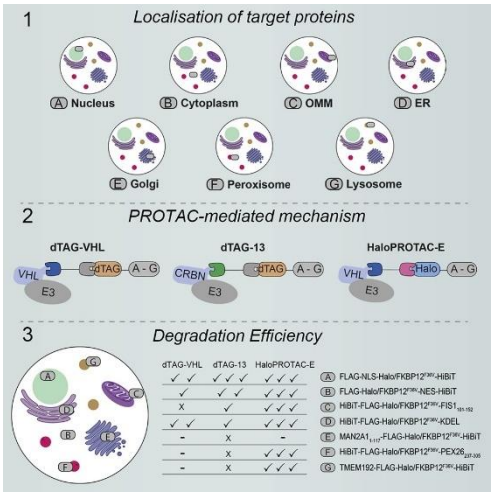
Cell Biology | Chemistry | Computational Chemistry | Structural Biology/Biophysics

Contributor: Manjula

Target protein localization and its impact on PROTAC-mediated degradation

Luke M. Simpson[§], Lorraine Glennie[§], …, Gopal P. Sapkota*

Cell Chem. Biol. **2022**, DOI: 10.1016/j.chembiol.2022.08.004



TPD uses degraders such as PROTACs with the idea of bringing two proteins together that aren't normally in proximity, to promote degradation of target protein. This method has opened a whole new avenue of drug discovery and PROTACs have several advantages over small-molecule traditional inhibitors and gene silencing approaches. Given that PROTACs rely on bringing endogenous cellular degradation machinery to mediate target protein degradation, Simpson *et al*. interrogated whether the subcellular context of the target protein and its access to the E3 ligase being recruited potentially impacts PROTAC efficacy. To probe this, they expressed either Halo or FKBP12F36V (dTAG) constructs consisting of varying localization signals and thereby the target protein was localized to the nucleus, cytoplasm, outer mitochondrial membrane, endoplasmic reticulum, Golgi, peroxisome or lysosome. Following expression of target proteins to various cellular compartment, they tested the efficacy of their degradation by von Hippel-Lindau (VHL)- or cereblon (CRBN)-recruiting PROTACs targeting either Halo or dTAG and noticed differentially localized Halo or FKBP12F36V proteins displayed varying levels of degradation using the same respective PROTACs. This suggests that it is important to take into consideration the subcellular context of the target protein and the E3 degradation machinery while choosing the PROTAC, for better efficiency in PROTAC-mediated target protein degradation.

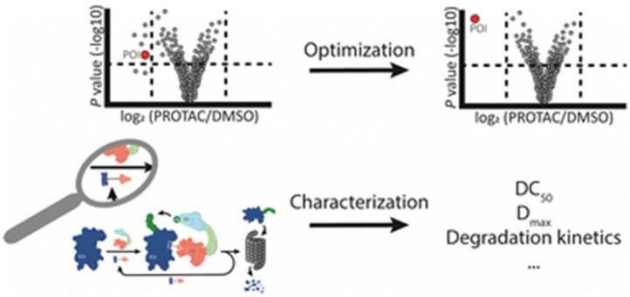
Cell Biology | Chemistry | Computational Chemistry

Contributor: Manjula

PROTAC degraders as chemical probes for studying target biology and target validation

Václav Němec[§], Martin P. Schwalm[§], Susanne Müller*, Stefan Knapp*

Chem. Soc. Rev. ***2022***, *51*, 7971-7993



PROTACs have several advantages over small-molecule traditional inhibitors due to its catalytic mode of action and its ability to target undruggable proteins, and thus represent an attractive new strategy for drug development. In this review the author discusses about the benefits of PROTACs strategy for drug development, the recent progress in the development of assay systems for PROTAC and further discusses the need for comprehensive characterization of PROTACs, PROTAC-specific quality criteria that need to be defined by the chemical biology community. The authors discuss the development and characterization of PROTACs that requires an array of additional assay systems that track the degradation pathway leading ultimately to degradation of the POI, identifying critical steps for PROTAC optimization. In addition to their exciting translational potential, PROTACs represent versatile chemical tools that considerably expanded our chemical biology toolbox and significantly enlarged the proteome that can be modulated by small molecules. Similar to conventional chemical probes, PROTACs used as chemical probes in target validation require comprehensive characterization. As a consequence, PROTAC-specific quality criteria should be defined by the chemical biology community. These criteria need to comprise additional or alternative parameters compared to those for conventional occupancy-driven chemical probes, such as the maximum level of target degradation (Dmax), confirmation of a proteasome dependent degradation mechanism and, importantly, also kinetic parameters of POI degradation. The kinetic aspects are particularly relevant for PROTACs that harbor covalent binding moieties. The author review recent progress in the development of assay systems for PROTAC characterization and suggests a set of criteria for PROTACs as high quality chemical probes.

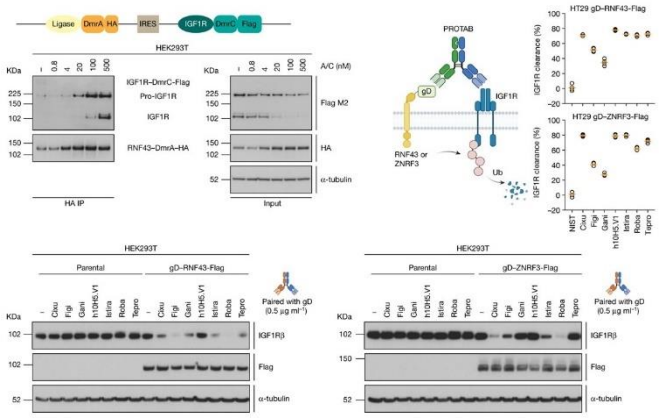
Cell Biology | Computational Chemistry | Modelling/Simulation | Structural Biology/Biophysics

Contributor: Manjula

Antibody targeting of E3 ubiquitin ligases for receptor degradation

Hadir Marei,[§] Wen-Ting K Tsai, … Felipe de Sousa E Melo*

Nature, **2022**. DOI: 10.1038/s41586-022-05235-6



Cell membrane proteins/receptors are considered as gatekeepers of the cell and play a major role in regulating various processes. A plethora of human pathologies are associated with the altered expression or dysfunction of cell membrane proteins, making them interesting therapeutic drug targets. However, typical mammalian membrane proteins comprise multiple domains that execute discrete but coordinated activities. Thus, inhibition of one domain often incompletely suppresses the function of a protein. In this study they developed new strategy proteolysis-targeting antibodies (PROTABs) that tether cell-surface E3 ubiquitin ligases to transmembrane proteins, resulting in target degradation both in vitro and in vivo. They utilised cell surface zinc- and ring finger 3 (ZNRF3), a Wnt-responsive E3 ligase as a degradation machinery and show PROTAB can degrade target protein and enable colorectal cancer-specific degradation. Notably, by examining a matrix of additional cell-surface E3 ubiquitin ligases and transmembrane receptors, they demonstrate that this technology is amendable for 'on-demand' degradation. Furthermore, they offer insights on the ground rules governing target degradation by engineering optimized antibody formats. In summary, this work describes a strategy for the rapid development of potent, bioavailable and tissue-selective degraders of cell-surface proteins.

Other Paper Highlights

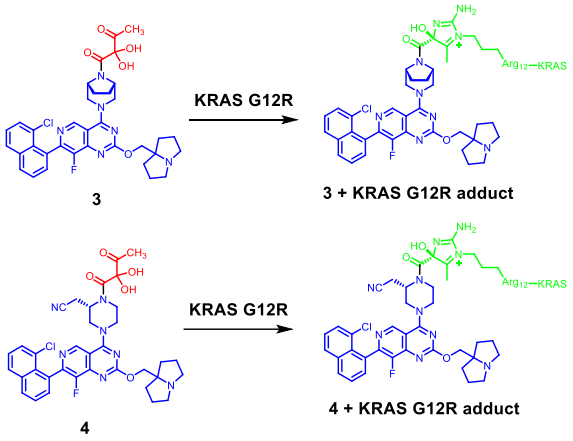
Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Xingui

Chemoselective Covalent Modification of K-Ras(G12R) with a Small Molecule Electrophile

Ziyang Zhang*, …, Kevan M. Shokat*

J. Am. Chem. Soc. **2022**, *144*, 15916



A new chemical strategy to selectively target KARS G12R mutation is reported. Inspired by the fact that vicinal dicarbonyl compounds can selectively modify arginine residues on proteins, the authors attached an α,β-diketoamide moiety to the K-Ras Switch-II ligand, resulting compounds (*e.g.* **3** and **4,** existing as hydrates) that can react covalently and selectively with KRAS G12R. Compound **3** engages with arginine 12 of KRAS G12R in a pH dependent manner because pH can influence the nucleophilicity of arginine residue. Compound **3** also depends on GDP to engage with KRAS G12R because the KRAS switch-II ligand preferentially binds to the GDP nucleotide state of KRAS. Nevertheless, endogenous KRAS G12R exists predominantly in the GTP bound state, which may explain the lack of cellular activity in KRAS G12R cell line and limit the therapeutic application of these compounds. This study not only provided a starting point to conquer cancers driven by KRAS G12R mutation, but also offered a new drug design strategy that can be used to target arginine residues in the protein. As to date, strategies for targeting G12C (Sotorasib), G12D (MRTX1133), G12R and G12S (Zhang *et al.*, *Nat. Chem. Biol.*, 2022) mutations of KRAS are all available, the "to-do list is getting shorter".

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