CeTPD Journal Club

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

Journal Club

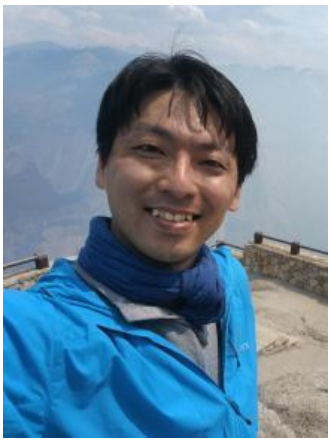
October 2022

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): Alena Kroupova, Kentaro Iso, Laura Casares Perez

"The Journal Club is a unique resource for me as a researcher and so it is a delight to contribute to it as one of the editors."

Alena completed her undergraduate studies at the University of Edinburgh after which she pursued a PhD in structural biology at the University of Zurich. She joined the exciting PROTAC field in September 2021 as a structural biologist/biophysicist within the CeTPD-Almirall collaboration.

"I realised how much attention is being paid and how fast the technology is advancing in the field of protein degraders by editing the Journal Club "

Kentaro joined the Ciulli group as a visiting postdoctoral scientist (medicinal chemistry/biochemistry) from Eisai Co., Ltd. as part of the collaboration project with Eisai in April 2022. He completed his undergraduate degree and his PhD in organic synthesis at Tohoku University. And he has experienced medicinal chemistry in the oncology field in industry.

"*Being an editor of the Journal Club is a great way to keep up with new literature in the field."*

Laura joined the Ciulli group as a cell biologist on the AC-Boehringer Ingelheim collaboration in February of 2022. Laura completed a 5-year degree in Pharmacy at the University of Santiago de Compostela (Spain) and specialized in cell biology and cancer research during her PhD at the University of Dundee.

Feature of the month

Contributor: Valentina

Cristina Mayor Ruiz visits Dundee!

This past month we had the pleasure of welcoming Cristina to the School of Life Sciences (SLS) and Centre for Targeted Protein Degradation (CeTPD) at the University of Dundee. Cristina hails from the Institute for Research in Biomedicine (IRB), in Barcelona, Spain, having recently been appointed as an independent researcher. This past year Cristina has been busy starting up her own research group following her ERC award, whose research will focus on the rational design of molecular glues.

With Cristina's blessing we were able to plan for her an absolutely jam-packed two-day visit to Dundee. The visit started off with a wonderful seminar from Cristina, that gave us all an insight into the latest goings on of her research group. The focus of Cristina's visit was two-fold, firstly, it was an opportunity to bring CeTPDers together and secondly it was a chance for Cristina to interact with as many people from across the CeTPD community as possible. One of our main ambitions with the CeTPD seminar series has been to make it distinct to other seminars, in that the speaker has a real opportunity to get stuck in and interact with as many researchers from across the community as possible. This with the belief that the best way to inspire our young scientists is for them to have a real opportunity to interact with visiting speakers.

Cristina's visit was particularly poignant for us as it has been a year in the making and came at a transitional time for the CeTPD. Over the last year we have been in the process of expanding the team and this visit was the first time many of the newer CeTPDers were all in the same room. We therefore also used the visit as an opportunity for us to plan some team building activities. The activity we chose for this event was a group walk around Tentsmuir National Park, a mere 30 minutes away from Dundee and offers both a coastal and forest route. We were fortunate that Cristina was able to bring the weather with her to Scotland and thus the group enjoyed a magnificent day of sunshine. We were also spoiled by the appearance of some Scottish seals, that were expertly photographed by resident photographer (and scientist) Kevin Haubrich.

We are very grateful that Cristina was able to make the journey to be with us, and we are very happy that she graciously took on everything we threw at her over the two days – pool, darts and Shuffl board included! We are particularly appreciative of her giving us the opportunity to get to know more about the exciting science brewing in her lab- and we look forward to seeing those projects unfold in the future!

Landmark Paper

Contributor: Sohini

Stereochemistry of polypeptide chain configurations

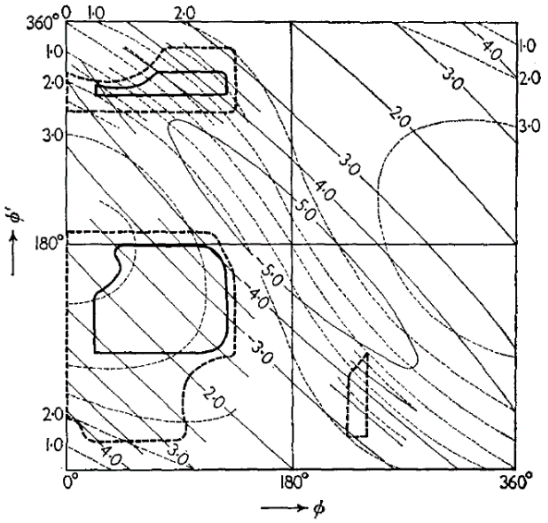
G.N.Ramachandran, C.Ramakrishnan, V.Sasisekharan

J Mol Biol **1963**, 7, 95-99

This month, the protein science community is celebrating the birth centenary of Professor G. N. Ramachandran (popularly known as GNR), a biophysicist who was ahead of his time. GNR was born on the 8th of October 1922 at Ernakulam, a town in the south-western part of India. As a tribute from the CeTPDers, we would like to feature in our current JC issue, the landmark paper on Ramachandran plot first published in JMB in the year 1963.

G.N. Ramachandran (1922-2001)

The triple helical model of collagen structure as proposed by GNR and his group invoked huge controversy. The main objection was raised by Rich and Crick suggesting that in GNR's collagen model, atoms were too close that would lead to steric hindrance based on van der Waal's radii of atoms. This instigated GNR to set the most unique example of responding to criticism ever known in the history of science. GNR who always wanted to tackle problems at the basic levels decided to examine crystal structures of various small molecules known at the time. The idea was to see the limiting distance to which two atoms can be brought together before the conformation become impossible due to steric hindrances. It was clear from the analysis of structural data on small molecules, compensating features in crystal structures, such as hydrogen bonds or other attractive forces in the neighbourhood could lead to bringing two atoms closer than their sum of van der Waals radii. Thus the "extreme limits" beyond which two atoms cannot approach closer to each other is less than the "normally allowed limits". Based on this understanding, GNR and his student C. Ramakrishnan (CR) worked out the values of the dihedral angles (ϕ, ψ) in trans-peptide units linked at an α-carbon atom that keep the two non-bonded atoms at such a distance where the conformation is "fully allowed", "partially allowed", and "disallowed". These calculations gave birth to what is today widely known as the "*Ramachandran Map/Plot*" (Figure alongside).



Contours of constant *n* (_____) and constant *h* (- - - - - -) corresponding to the angle N-αC-C' = 111°. The boundaries of the fully allowed and outer limit regions are also shown.

At a time when computers were unknown in India, CR had to undertake a painstaking marathon exercise of calculating the possible combinations of ϕ, ψ using electronic desk calculators that can only perform four basic operations: addition, subtraction, multiplication, and division and had no memory allocation. What resulted out of these calculations is an important validation test that every protein structure must pass through and today no biochemistry is complete without a φ,ψ-plot of the alanine dipeptide. In the words of Professor George Rose, "*the ϕ,ψ-plot is a model of physical reality, and its validity needed to be tested by experiment. That test was passed with flying colours as an increasing number of experimentally determined protein structures was solved. Now, of course, it is theory that is used to validate experiments, not the reverse.*"

GNR's life and his work is a massive source of inspiration to scientists of all generations, reassuring that science speaks for itself and simple, pure, honest approaches towards science can help in addressing some difficult problems.

Targeted Protein Degradation

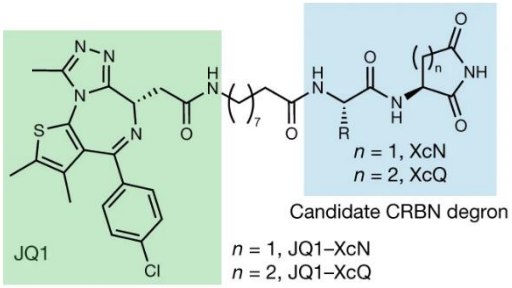
Cell Biology

Contributor: Alena

The E3 ligase adapter cereblon targets the C-terminal cyclic imide degron

Saki Ichikawa[§], …, Christina M. Woo*

Nature **2022**, *610*, 775



Ever since the discovery of thalidomide's mode of action through CRBN, the search for endogenous substrates of CRBN has been at the forefront of the targeted protein degradation field. In this study, Ichikawa *et al*. use chemical biology and proteomics approaches to uncover C-terminal cyclic imides as the physiological degron of CRBN. In particular, C-terminal cyclic glutarimide (cQ) and aspartimide (cN) were identified from a set of thalidomide analogues to act as functional degraders of BRD4 when associated with the JQ1 ligand. Further analysis shows that C-terminal cyclic imide post-translational modifications (PTMs) are ubiquitous and temporary, nevertheless have a half-life sufficient for CRBN-induced degradation. Attaching a cQ/cN degron to C-terminus of GFP showed CRBN-dependent ubiquitination and degradation whilst cQ- and cN- containing peptides increase upon the inhibition or knockout of CRBN.

This robust study is a true game changer in the hunt for endogenous CRBN substrates that has been the focus point of TPD research for years. It further highlights the importance of improving our understanding of the formation and role of C-terminal cyclic imides to understand the impact of CRBN on the proteome. Finally, a perplexing question remains: are there other degrons than cyclic imides recognized by CRBN?

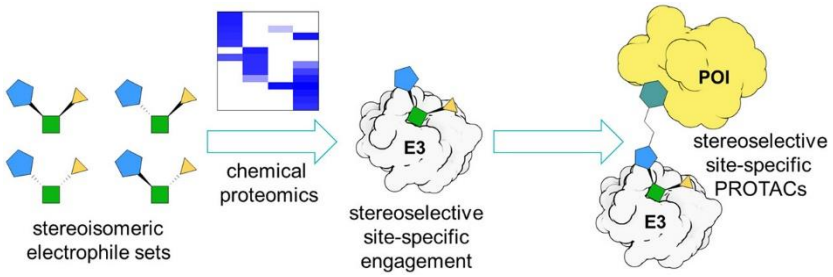
Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Kentaro

Targeted Protein Degradation by Electrophilic PROTACs that Stereoselectively and Site-Specifically Engage DCAF1

Yongfeng Tao*, …, Xiaoyu Zhang*, Benjamin F. Cravatt*

J. Am. Chem. Soc. **2022**, *144*, 18688



The authors discovered azetidine acrylamides that stereoselectively react with a cysteine in DCAF1 by their activity-based protein profiling (ABPP) strategy. A set of azetidine acrylamide stereoprobes were screened, and it was found that one of these compounds strongly bound to DCAF1 compared to other probes. By mass spectrometry they revealed the compound was covalently binding to C1113 in DCAF1. Then they developed DCAF1-directed electrophilic PROTACs by connecting their ligand to SLF (ligand for FKBP12) and JQ1 (ligand for BRD4), and confirmed degradation of the POIs in DCAF1 dependent manner.

To date, only a limited number of E3 ligases are utilized by PROTACs. Expanding the available E3 ligases is one of the challenges the TPD field faces. In addition to this work, the authors discovered new ligands for E3 ligases such as DCAF11 using their ABPP technology. Their strategy would provide one of solutions to the challenge.

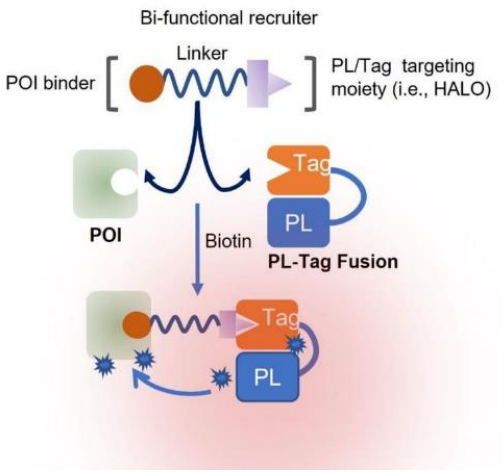
Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Kentaro

Drug interaction mapping with proximity dependent enzyme recruiting chimeras

John D Venable, …, Ansgar Brock*
bioRxiv **2022** DOI: 10.1101/2022.09.26.509259

Identification of the molecular target of compounds is still challenging. The authors expanded proximity-dependent labelling such as BioID and TurboID to low molecular weight compound guided labelling. Their ligand mediated proximity labelling is conceptually similar to the PROTAC approach. The POI binder is connected to proximity labelling enzyme via a linker. The feasibility study using GNF2133 (kinase inhibitor), SLF (ligand for FKBP12), and JQ1 (ligand for BRD4) demonstrated that their strategy worked well to detect the validated target engagement. With JQ1 mediated proximity labelling, not only BRD2, 3, and 4, but also several bromodomain interacting proteins are picked up as the hit. This ligand mediated proximity labelling therefore would provide an additional tool to clarify the molecular mechanism of protein degradation and other investigation of protein complexes.



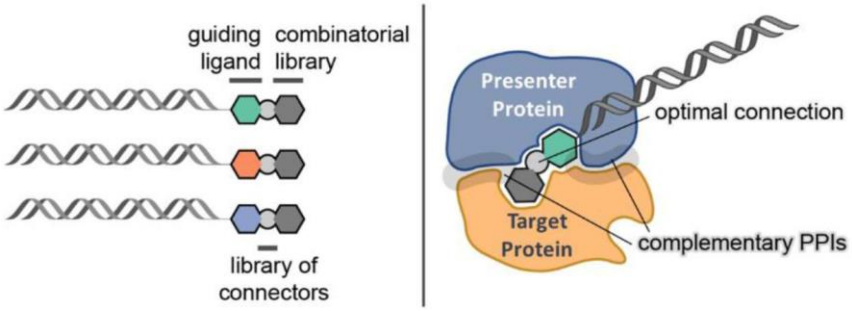
Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Kentaro

DNA-encoded library (DEL)-enabled discovery of proximity-inducing small molecules

Jeremy W. Mason, …, Frédéric J. Zécri*, Karin Briner*, Stuart L. Schreiber*
bioRxiv **2022** DOI: 10.1101/2022.10.13.512184

The authors developed a platform for the discovery of chemical inducers of proximity (CIP) using DEL. They demonstrated the efficiency of their approach with BRD4 and VHL-elongin C-elongin B (VCB) complex. CIP-DEL library was composed of VHL ligand, 15 linkers and various POI targeting moiety, and the library contains 1,083,150 potential CIPs. For 21 hit library members, off-DNA compounds were synthesized and evaluated. As a result it was found the enrichment of CIP-DEL was correlated to the binary and ternary Kd in SPR and HiBiT DC50. In addition the crystal structure of the ternary complex of BRD4(BD1), VBC complex, and CIP1 (one of the hit compounds) was solved. Interestingly their CIP-DEL is designed based on MZ1, the orientation of the proteins are different from that of MZ1.



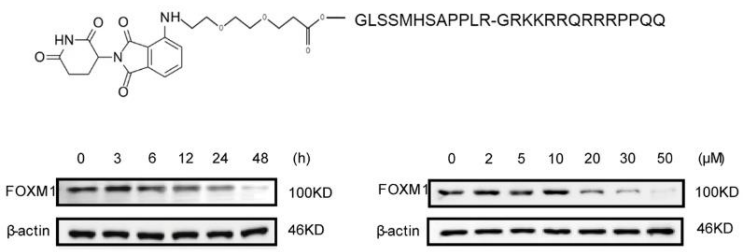
The approach reported here can directly identify the inducers of proximity, and the binding site is not restricted. Although there can be still some difficulty in quality control of DEL, it is a powerful tool to find inducers of proximity and binders to the protein of interest.

Cell Biology | Chemistry

Contributor: Kentaro

Peptide-based PROTAC degrader of FOXM1 suppresses cancer and decreases GLUT1 and PD-L1 expression

Kun Wang, …, Laiqiang Huang*
J Exp Clin Cancer Res **2022**, *41*, DOI: 10.1186/s13046-022-02483-2



The transcription factor FOXM1 is considered as an attractive oncotarget. The authors group developed a peptide based PROTAC targeting FOXM1. FOXM1 targeting peptide was screened by phage display, and one of the peptides named F-1 was identified as the strongest inhibitor in a cell viability assay. To make the peptide even more membrane permeable, cell membrane penetrating peptide TAT was attached to the N-terminal of the F-1. To develop the FOXM1 degrader, the TAT-F-1 peptide was chemically modified with Pomalidomide-PEG2-COOH. The resulting PROTAC showed higher inhibitory activity of cell viability compared to the original peptide F-1. And the degradation of FOXM1 was observed with the PROTAC while the parent peptide did not degrade. It was noteworthy that the peptide based PROTAC suppressed tumour growth *in vivo* without side effects including body weight loss.

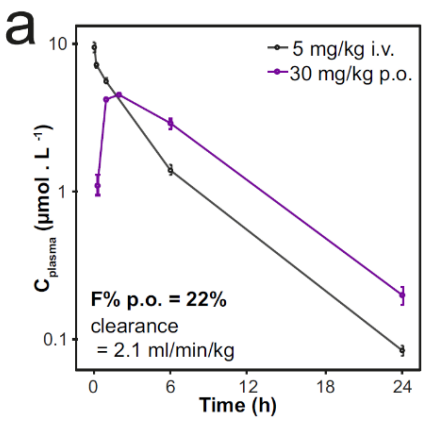
Peptides have some advantages over small molecules in terms of affinity and target specificity, but they often have disadvantages like poor membrane permeability and metabolic instability. Once these issues are resolved, the peptide-based PROTACs would be more widely applied in the TPD field.

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

Contributor: Laura

A selective and orally bioavailable VHL-recruiting PROTAC achieves SMARCA2 degradation in vivo

Christiane Kofink[§], Nicole Trainor[§], Barbara Mair[§], …, Harald Weinstabl*, William Farnaby*
Nat Commun **2022**, *13*, 5969



Oral dosing of degraders in the clinic so far has been confined to CRBN-based molecules, greatly limiting the therapeutic scope of PROTACs. This paper presents ACBI2, an orally bioavailable VHL-recruiting PROTAC that preferentially degrades SMARCA2 over other closely related isoforms. Authors started by designing a novel SMARCA2 binder with improved physicochemical properties. Then, crystallographic knowledge of ternary complex modes guided exit vector optimisation to obtain rapid and potent degraders that form high affinity complexes. Lastly, the linker was elongated and modified to achieve compact structures with improved oral bioavailability along with selectivity for SMARCA2. ACBI2 is a highly potent SMARCA2 degrader with a >30-fold window over SMARCA4 and an oral bioavailability of 22% in mouse. Authors also tested this PROTAC in a lung cancer mouse xenograft model, leading to near-complete degradation of SMARCA2 and tumour growth inhibition.

This paper highlights the feasibility of designing VHL-based degraders in the orally efficacious space, broadening the potential scope of degraders in the clinic. Furthermore, both tool compounds ACBI2 and ACBI1 (SMARCA2/4 degrader) are available to researchers so it will be interesting to read further studies into the differential effect of these PROTACs in other cancer models.

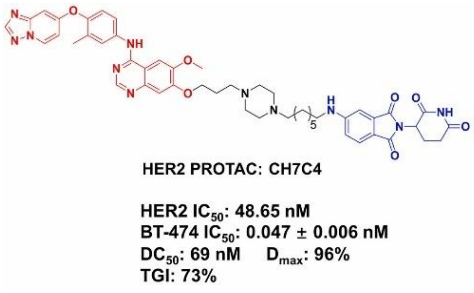
Cell Biology | Chemistry

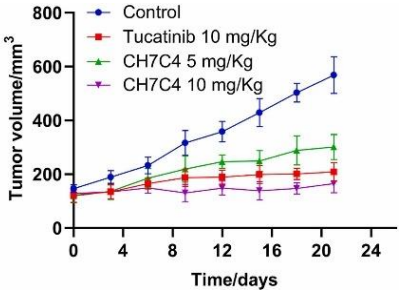
Contributor: Laura

Discovery of potent and selective HER2 PROTAC degrader based Tucatinib with improved efficacy against HER2 positive cancers

Mingxing Hu[§], …, Binwu Ying*, Yongmei Xie*

Eur J Med Chem **2022**, *244*, 114775





Inhibitors of receptor HER2 are first-line therapies for HER2 breast and gastric positive cancers. However, these compounds can cause a lot of side effects due to their pan-HER inhibition. This paper describes a potent and selective CRBN-based HER2 PROTAC that is efficient against tumours *in vivo*. Authors designed a panel of compounds using Tucatinib as a HER binder and both CRBN and VHL ligands. From these compounds, CH7C4 was selected because of its low IC50 in antiproliferation assays as well as high degradation percentage. CH7C4 is shown to be more potent than Tucatinib at inducing apoptosis and supressing the downstream pathway in HER2-dependent cell lines. Results of both competition and inhibition of UPS experiments suggest PROTAC mode of action for this compound. Furthermore, results in a HER2-dependent xenograft mouse show that CH7C4 was well tolerated and slightly more efficient at reducing tumour volume than Tucatinib.

This paper opens a door for the development of new therapeutic strategies for the treatment of HER2-positive cancers. However, further validation of CH7C4 as selective degrader of HER2 is needed, as the authors did show selectivity for HER2 over EGFR (HER1) but did not check other HER receptors.

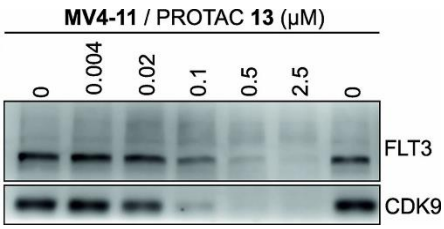
Cell Biology | Chemistry

Contributor: Laura

Modulation of FLT3-ITD and CDK9 in acute myeloid leukaemia cells by novel proteolysis targeting chimera (PROTAC)

Eva Řezníčková[§], Soňa Krajčovičová[§], …, Miroslav Souralb*, Vladimír Kryštofac*

Eur J Med Chem **2022**, *243*, 114792



Activating mutations of kinase FLT3 are one of the major drivers of disease in acute myeloid leukaemia (AML). Inhibitors of FLT3 have already been approved for AML treatment, however patients often develop resistance and relapse so degradation of this kinase via PROTACs might present a novel therapeutic approach. Furthermore, some studies have described a positive effect of simultaneous inhibition of FLT3 and CDK9 so in this paper authors aim to design a compound that degrades both targets. The authors arrived at CRBN-based "PROTAC **13**" through rational design using molecular models and information from previously reported FLT3 degraders. Treatment of FLT3 mutated cell lines with 100 nM of PROTAC **13** resulted in decreased levels of both FLT3 and CDK9 - which was not observed in the corresponding CRBN-deficient cell line. Competition experiments suggest that this compound degrades both targets in a proteasome-dependent manner. The authors also show this degrader was more efficient at decreasing expression of downstream genes involved in haematopoiesis, apoptosis and AML initiation compared to FLT3 inhibitors alone.

In summary, this paper provides an interesting tool to study the therapeutic potential of simultaneous degradation of FLT3 and CDK9 in AML. However, I think it would be beneficial to perform a global proteomics analysis to ensure selectivity towards these two targets before using it in further studies.

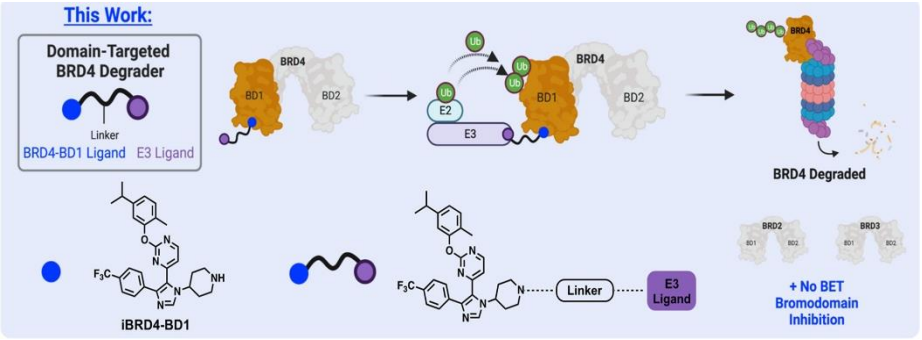
Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Laura

Development of an N-Terminal BRD4 Bromodomain-Targeted Degrader

Anand Divakaran[§], …, Daniel Harki*, William Pomerantz*

ACS Med Chem Lett **2022**, *13*, 1621



BET family proteins, BRD2, -3 and -4, are regulators of gene expression involved in inflammation and oncology. Considering that BRD4 is accepted as the most disease relevant BET protein and that targeting BRD2 and BRD3 can lead to dose-limiting toxicity, there is a need to design BRD4 selective degraders. This paper highlights dBRD4-BD1, a CRBN PROTAC based on novel selective iBRD4-BD1 inhibitor that binds to its bromodomain 1 (BD1). Authors hypothesise that by taking advantage of the structural information on their BD1-selective inhibitors, they could degrade BRD4 through an individual domain. Ternary complex TR-FRET assays were used to confirm dBRD4-BD1 selectively produced a signal with BRD4-BD1 and not BD2. This compound was also shown to degrade BRD4 in cells while upregulating BRD2/3, a surprising effect not seen with other BRD4-selective degraders. Competition experiments with domain-selective BET and proteasome inhibitors confirmed PROTAC mode of action.

Previous work has achieved BRD4 selective degradation by optimization of pan-BET ligands for interaction kinetics or linker geometries for ternary complex formation. However, they still result in BRD2/3 inhibition, which can obscure biological effects of BRD4 degradation and may result in BRD2/3-related toxicity. dBRD4-BD1 can be a great tool compound to further study the effects of BRD4 degradation.

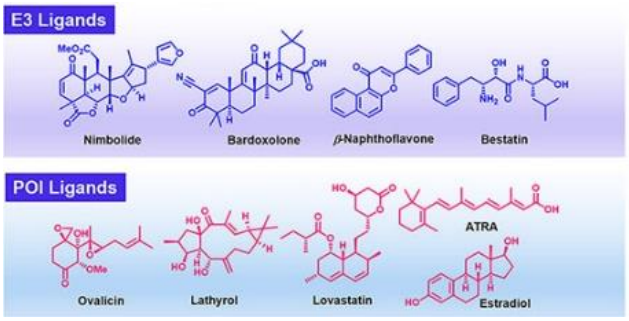
Chemistry

Contributor: Alena

Natural product-inspired targeted protein degraders: advances and perspectives

Jiao Li[§], Zhenyu Cai, Xu-Wen Li*, Chunlin Zhuang*

J Med Chem **2022**, *65*, 13533



Natural products played a major role in drug discovery and still are an important source of ligand templates. In this review, Li *et al*. discuss their role in targeted protein degradation based on their exhaustive overview of natural product-derived E3 ligase and POI ligands. The authors see a great opportunity in exploring natural products to expand the E3 ligase toolbox, an ever-present challenge in the TPD field, where the improvement of powerful multi-omic technologies is instrumental in enabling new discoveries. Furthermore, detailed insights into the mechanism of action are essential especially in the case of natural products that act as molecular glues, but also to guide optimization preventing off-target activity. Natural products will also open many TPD doors as chemical probes, or in new TPD modalities, such as LYTAC.

This is a very comprehensive perspective on a key part of the TPD space and undoubtedly a thought-provoking read.

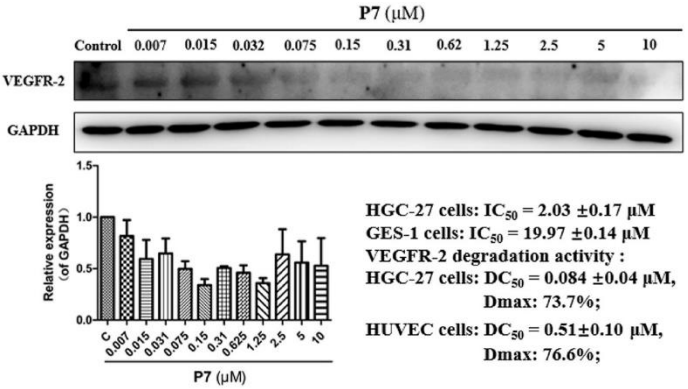
Cell Biology | Chemistry | Computational Chemistry

Contributor: Alena

Discovery of novel VEGFR-2-PROTAC degraders based on the localization of lysine residues via recruiting VHL for the treatment of gastric cancer

Xing-Rong Wang[§], …, Shi-Wu Chen*
Eur J Med Chem **2022**, *244*, 114821

Vascular endothelial growth factor-2 (VEGFR-2) plays a pivotal role in promoting tumour angiogenesis. Whilst its inhibition prolongs tumour patient survival, the accumulation of VEGFR-2 inhibitors leads to toxicity and emergence of drug resistance. To overcome the challenges of traditional inhibitors, in this study Wang *et al*. use rational design to identify a potent VEGFR-2-targeting VHL-based PROTAC. Compound **P7** exhibits high cytotoxicity is gastric carcinoma HGC-27 cells and low toxicity to human normal HEK293T, HUVEC and GES-1 cells with a VHL- and proteasome-dependent mechanism.



This paper sets out to use a structure-based approach to guide their linker design with an aim to access specific lysine regions for ubiquitination. The molecular docking simulations of the VEGFR-2 warhead binding mode enables rational linker design that leads to the promising candidate. It would be extremely informative for future studies to validate the original aim of using lysine residue distribution to guide PROTAC design by experimental approaches to identify whether the predicted lysine region is indeed accessible for ubiquitination in the ternary complex formed by compound **P7**.

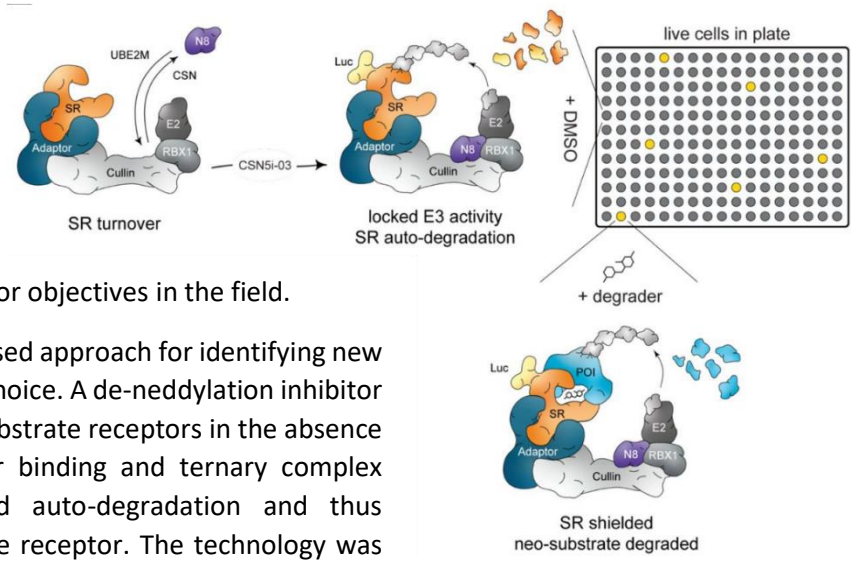
Cell Biology | Chemistry

Contributor: Alena

E3-specific degrader discovery by dynamic tracing of substrate receptor abundance

Alexander Hanzl[§], …, Georg E. Winter*
BioRxiv **2022**, *DOI: 10.1101/2022.10.10.511612*

Despite around 600 E3 ligases in the human genome, the TPD field has been largely focused on CRL4^{CRBN} and CRL2^{VHL}, especially in the context of current pre-clinical drug candidates. Harnessing the power of the full range of E3 ligases to expand the druggable proteome as well as exploit tissue- and cell-selective degradation remains one of the major objectives in the field.



This study presents a high-throughput cell-based approach for identifying new molecular glue degraders for an E3 ligase of choice. A de-neddylation inhibitor is used to amplify auto-degradation of CRL substrate receptors in the absence of their substrates. Consequently, degrader binding and ternary complex formation can be monitored by reduced auto-degradation and thus stabilization of the NanoLuc-tagged substrate receptor. The technology was validated on CRL4^{CRBN} and CRL2^{VHL}, its feasibility demonstrated for twelve other E3 ligases and a proof-of-concept study led to the discovery of dRRM-1, a new CRL^{DCAF15} molecular glue degrader of RBM39 and RM23.

This live-cell ligase tracing strategy has a major advantage of not being restricted to POIs that are essential for cellular fitness and will undoubtedly prove a fundamental technology to accelerate identification of new degraders.

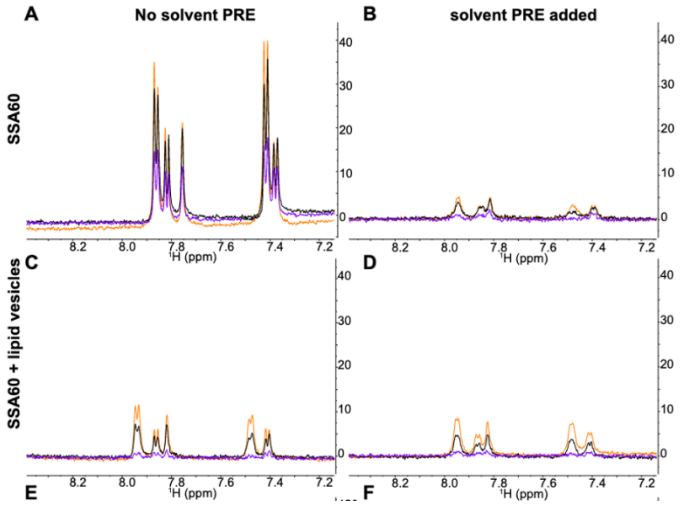
Other Paper Highlights

Cell Biology | Chemistry | Computational Chemistry

Contributor: Kevin

Elucidation of small molecule passive permeation across lipid membranes using conventional solution state NMR methods

Angela Serrano-Sanchez[§], …, Jose L. Ortega-Roldan*
BioRxiv. **2022**, DOI: doi.org/10.1101/2022.10.01.510446



Membrane permeability is a crucial property of any successful drug or chemical probe, but measuring it is far from trivial. It is commonly measured in artificial systems, such as PAMPA assays, that require specialised equipment and cannot easily be tuned to reflect the membrane composition of different organisms. This paper presents a quick and easy way to quantify passive membrane permeability and membrane interactions using NMR. The method employs the enhanced relaxation rates and resulting line broadening in the presence of paramagnetic substances in the sample (paramagnetic relaxation enhancement, PRE). CPMG spectra are collected on samples of the compound alone, with the paramagnetic agent Mn2+, reconstituted membrane vesicles and both Mn2+ and membrane vesicles. The intensity ratio between the paramagnetic samples with and without membrane vesicles is used to calculate the permeability factor. Similarly, the intensity ratios in the diamagnetic sample informs about possible interactions of the compound with the membrane.

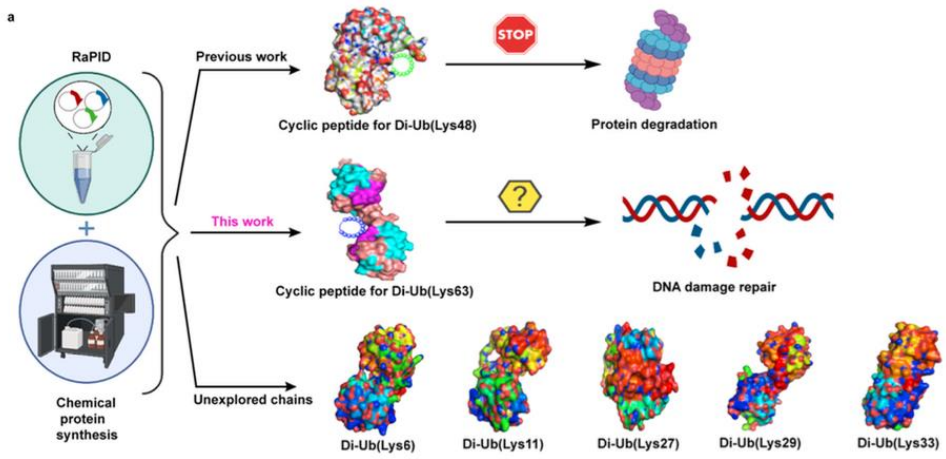
The reliance only on standard NMR equipment means that this experiment could be performed in nearly any chemical biology/drug discovery lab and even the most biology-averse chemist should be comfortable running and analysing these experiments. It is quick (20 min per compound) and could allow for higher throughput measurements of membrane permeability.

Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Mark

Selective macrocyclic peptide modulators of Lys63-linked ubiquitin chains disrupt DNA damage repair

Ganga B. Vamisetti[§], Abhishek Saha[§], …, Hiroaki Suga*, Ashraf Brik*
Nat Commun ***2022****, 13(1): 6174*



The polymeric nature of ubiquitin (Ub) enables a wide range of signalling outcomes and methodology to detect specific Ub-Ub linkages is vital to our understanding. Natural Ub-binding domains, antibodies, nanobodies, peptides, and small molecules were previously used for selection of the eight Ub-Ub linkages. Vamisetti and co-workers expand the toolbox of these

reagents in this study with the discovery of a cell permeable macrocyclic peptide for K63-linked polymeric-Ub. In this process, Vamisetti *et al*. screened a library of ~1 trillion cyclic peptides using their Random Non-standard Peptides Integrated Discovery (RaPID) method. Their lead, cyclic peptide 2, exhibited high selectivity for only K63-linked polymeric Ub, binding below 100 nanomolar, and permeable to human cells. Once in the cell, the Vamisetti cyclic peptides inhibited DNA damage repair and deubiquitination associated with K63-linked polymeric Ub. In addition, their cyclic peptides could be modified with fluorescent dyes, adding another dimension of functionality for these cyclic peptides. This expands on the K48 selective macrocyclic peptides from the Brik group (Nawatha, 2019) with implications as both research reagents and cytotoxic chemical modalities.

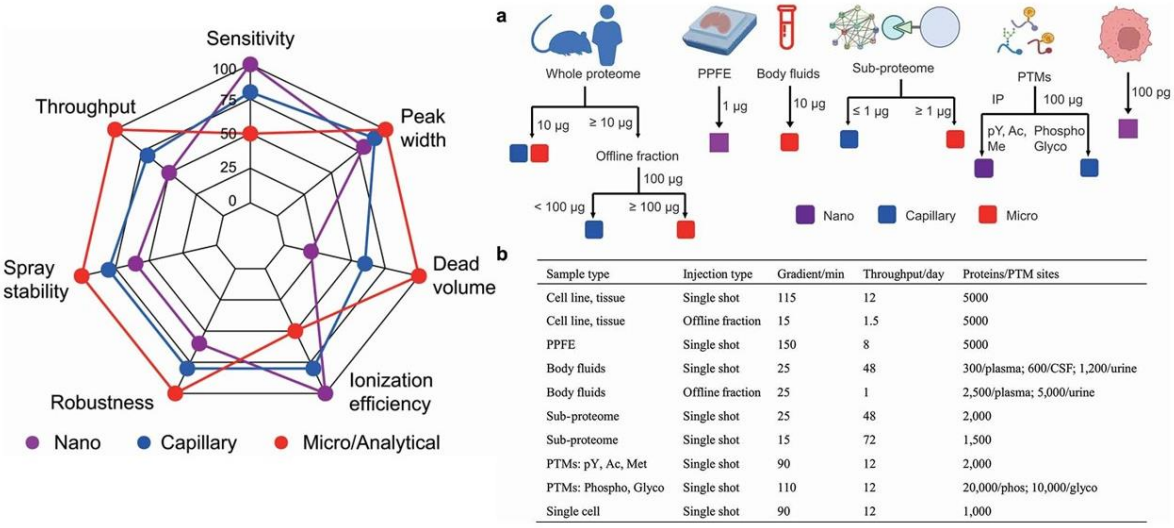
Cell Biology | **Chemistry** | **Structural Biology/Biophysics**

Contributor: Manjula

On the potential of micro-flow LC-MS/MS in proteomics

Yangyang Bian[§], Chunli Gaoa, Bernhard Kuster*

Expert Rev Proteomics **2022** *Mar;19(3):153-164.*



Sample type	Injection type	Gradient/min	Throughput/day	Proteins/PTM sites
Cell line, tissue	Single shot	115	12	5000
Cell line, tissue	Offline fraction	15	1.5	5000
PPFE	Single shot	150	8	5000
Body fluids	Single shot	25	48	300/plasma; 600/CSF; 1,200/urine
Body fluids	Offline fraction	25	1	2,500/plasma; 5,000/urine
Sub-proteome	Single shot	25	48	2,000
Sub-proteome	Single shot	15	72	1,500
PTMs: pY, Ac, Met	Single shot	90	12	2,000
PTMs: Phospho, Glyco	Single shot	110	12	20,000/phos; 10,000/glyco
Single cell	Single shot	90	12	1,000

Proteomics has developed into a diverse scientific discipline. In terms of sensitivity, sample throughput, quantitative precision, etc., applications that span single-cell analyses to population proteomics (the proteomic study of large numbers of subjects) have very different analytical requirements. In this article Yangyang *et al*., reviews the history of different chromatographic flow regimens coupled directly to mass spectrometry for discovery proteomics. They discuss the recent advances, advantages and disadvantages of nano-, capillary, micro-LC and analytical-flow chromatography in proteomic research. In particular, the author provides recent advances in application of micro-flow LC-MS/MS in discovery proteomics. The author discussed the advantages of micro-flow over the traditional nano-LC-MS/MS for large scale projects and in studies where decent sample material is available. They provide several valuable references including the study on PROTACs where a 'single-shot' approach was used to identify the protein targets of PROTACs directed against the kinase AURORA-A and WD-repeat-containing protein 5 (WDR5) using micro-flow (50 µL/min, 120 min gradient) LC-MS/MS. Further they discuss how micro-flow LC-MS/MS has the potential to bridge the fields of proteomics and metabolomics. There are numerous interesting and valuable review sections, worth reading. The authors provided their perspective, viewpoints and recommendations on which flow rate is most suitable for a given application and provided a good coverage of the prior literature to support their viewpoints.

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