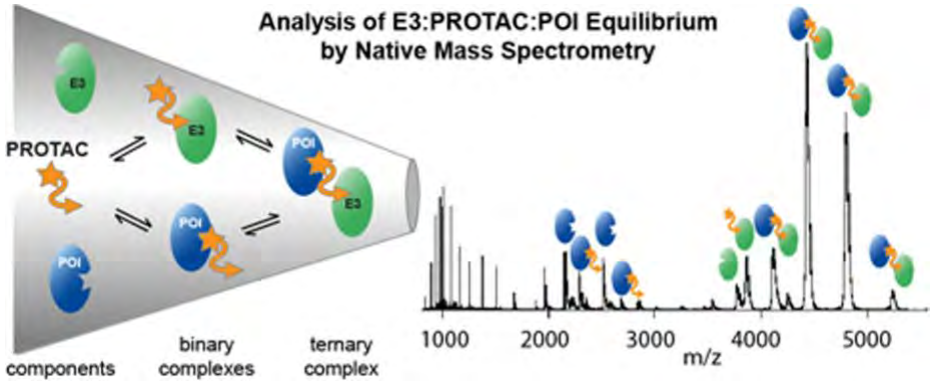
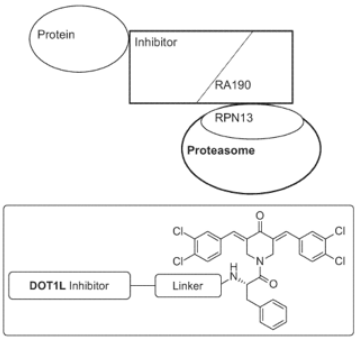
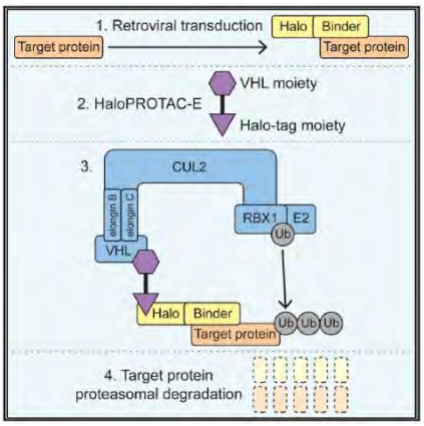
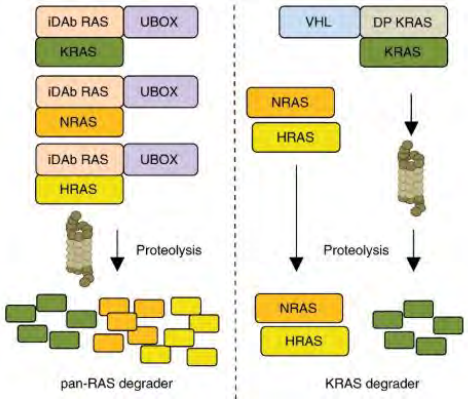
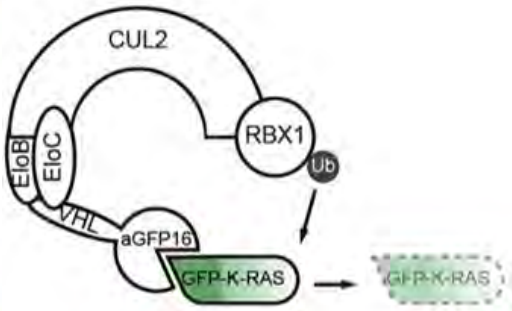
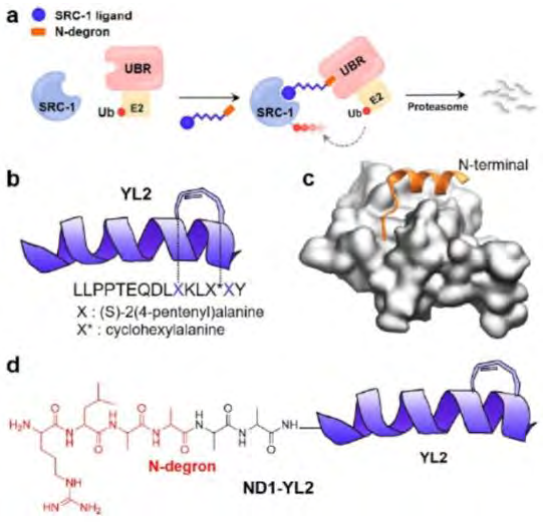


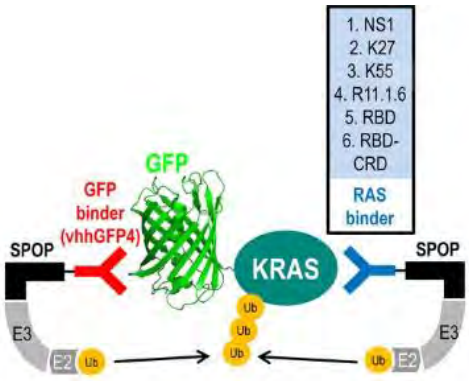


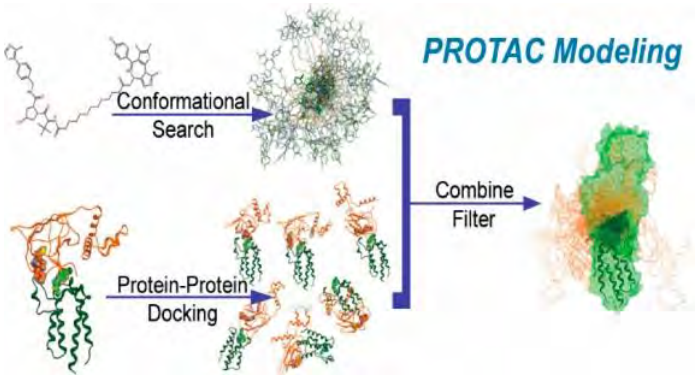












Ciulli Group Journal Club

Targeted Protein Degradation,

Medicinal Chemistry and

Chemical Structural Biology

Literature Highlights

July 2020 Edition

Ciulli Group Journal Contents

Landmark Paper

Contributor: Alessio

In vivo half-life of a protein is a function of its amino-terminal residue

Andreas Bachmair, Dan Finley and Alexander Varshavsky*

Science **1986**, *234*, 179-186.

Residue X in ub-X-βgal	Radius of gyration of X (Å)	Deubiquitination of ub-X-βgal	$t_{1/2}$ of X-βgal
Met	1.80	+	
Ser	1.08	+	
Ala	0.77	+	
Thr	1.24	+	>20 hours
Val	1.29	+	
Gly	0	+	
Ile	1.56	+	~30 minutes
Glu	1.77	+	
Tyr	2.13	+	~10 minutes
Gln	1.75	+	
Phe	1.90	+	
Leu	1.54	+	
Asp	1.43	+	~3 minutes
Lys	2.08	+	
Arg	2.38	+	~2 minutes
Pro	1.25	−*	~7 minutes

*The rate of in vivo deubiquitination of ub-Pro-βgal is extremely low. The $t_{1/2}$ shown is that of the initial ub-Pro-βgal fusion protein (Fig. 4, lanes j to p).

It has been known for a long time that cellular proteins have different shelf life before they get degraded and recycled into the constitutive amino acids. For example, many structural proteins, or proteins that assemble into complex machines, are very long-lived, while many regulatory proteins e.g. transcription factors can be extremely short-lived. Most proteins are selectively targeted for degradation (and so turned over) via the ubiquitin-proteasome system (UPS), but what determines whether and when a protein should be degraded? One of the most significant advances of the last century was from research addressing such fundamental question, aiming at elucidating specific molecular features of proteins that would signal to make them susceptible to UPS mediated degradation. These features are commonly termed "degrons" and are defined as the minimal elements within a protein that are sufficient to make them recognized by the degradative UPS.

Degron can reside anywhere within a protein sequence, but the first degron was discovered to be strikingly simple: one that lays at the extreme N-terminus of proteins. Work by Andreas Bachmair, Dan Finley and Alexander Varshavsky in this landmark paper in 1986 showed that protein half-lives in vivo could be determined by the identity of their N-terminal residue – a feature they called "N-end rule". To do that, using molecular biology they elegantly designed chimeric ubiquitin-fusions of a protein called -galactosidase (gal). Once expressed in yeast, this fusion would undergo proteolytic cleavage at the ubiquitin- gal junction (of note: such cleavage did not occur when the fusion was expressed in E. coli). Cleavage occurred irrespective of the nature of the first amino acid residue of the gal sequence – thus allowing them to reveal otherwise identical gal proteins that differed in sequence only by the N-terminal residue. Using 35S methionine labelling followed by cycloheximide pulse-chase experiments, they made the unexpected observation that the different gal proteins had remarkably different half-lives in the yeast, from >20 h to < 3 min, depending on the nature of the revealed N-terminal residues. Destabilizing N-end residues included basic residues such as Arg and Lys, as well as bulky hydrophobic ones such as Leu, Phe, Tyr and Pro. Asp and Glu were also found to be somewhat destabilizing. Addition of a methionine residue n-terminal to those residues invariably led to stable constructs (half-life of ~20 h), comparable to a control gal protein whose gene had not been fused to ubiquitin – and so did construct bearing Met, Ser, Ala and Thr residues at the N-end.

This was a discovery of fundamental reach – revealing a simple yet powerful link between protein sequence, stability and regulation that has held up to date. Later studies revealed for example that the most destabilizing N-degrons

determine protein substrate specificity for binding to members of the UBR family of E3 ubiquitin ligases, which transfer ubiquitin on Lys residues present at some distance downstream in the sequence. Not surprisingly, it has been more recently found that an analogous set of rules apply to C-degrons – but that will be a subject for another day!

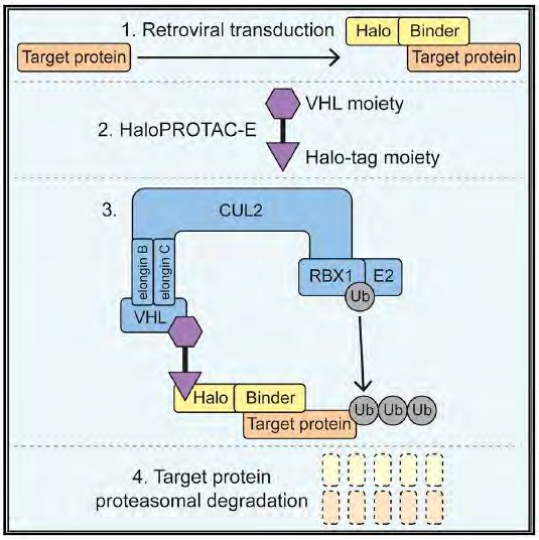
Targeted Protein Degradation

Contributor: Nicole

Inducible Degradation of Target Proteins through a Tractable Affinity-Directed Protein Missile System

Luke M. Simpson, … Gopal P. Sapkota*

Cell Chem. Biol., **2020**, *27*, 1-17



The past decade has seen the emergence of many new tools and technologies to promote the study of targeted protein degradation. To name but a few, there are compounds such as molecular glues and bifunctional degraders, as well as chemical biology approaches such as the auxin-inducible degron (AID), dTAG and HaloPROTAC platforms. With each approach there are advantages and limitations, therefore there is always room for one more. In this paper, the Sapkota group - based in the MRC Protein Phosphorylation and Ubiquitylation Unit at the University of Dundee - have expanded on their previously published Affinity-Directed Protein Missile System (AdPROM). The original AdPROM used genetic modification of the E3 ligase VHL to recruit GFP-tagged target proteins and induce their ubiquitylation and degradation. Here, recruitment of the target is facilitated by a POI binder conjugated to Halo-tag (termed Ligand-inducible AdPROM or L-AdPROM). The tag is then recruited to VHL via HaloPROTAC-E, which was designed by members of the Ciulli lab and reported in 2019. The benefit of this new technology is to gain control of POI degradation through the timing and concentration of HaloPROTAC treatment, as well as providing a generalised approach to target proteins, some of which have proven challenging for existing methods to date. This work was published alongside a second paper which is also highlighted in this month's edition of the Ciulli Group Journal Club.

The depth of this study is quite astounding: not only have the authors successfully demonstrated the use of HaloPROTAC/L-AdPROM for three model systems (GFP-labelled proteins: UKL1, FAM83D and SGK3), but they further interrogated the downstream consequences of these degradation events in a high level of detail. The investigation of the mechanism of action for the protein missile system is rigorous and, where appropriate, is benchmarked by comparison with the activity of an existing inhibitor or PROTAC. If that isn't enough for you, there is an extension of the study towards targeting endogenous RAS proteins using L-AdPROMs with an anti-HRas monobody, proving that this approach is applicable to untagged targets. There is more to this story, which is revealed in the second paper.

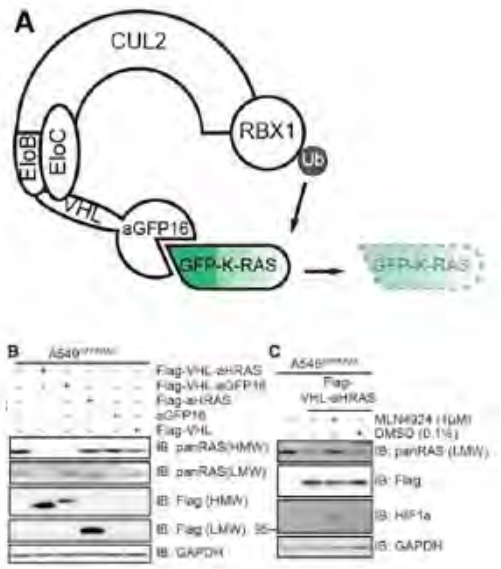
Contributor: Nicole

Targeting Endogenous K-RAS for Degradation through the Affinity-Directed Protein Missile System

Sascha Röth, … Gopal P. Sapkota*

Cell Chem. Biol., **2020**, *27*, 1-13

Here is the second highlight of work published by the Sapkota group this month. In this paper, the authors sought to assess the ability of their AdPROM system to degrade GFP-tagged and endogenous KRAS – a protein which has garnered significant attention in the drug discovery community (as well as in this month's edition of the Journal Club). The druggability of KRAS is often questioned and one proposed approach is targeted degradation. The hunt for a molecule capable of KRAS degradation requires a serious investment of time and resources; this search is also not rendered easy by the same features which give KRAS its reputation as "undruggable". The results of the VHL-aHRAS AdPROM experiments suggest that degradation of endogenous KRAS is viable and that in particular, VHL-recruiting degraders might be successful.



The challenge and impact of targeting KRAS can be described as "high-risk, high-reward", therefore the drug discovery community should applaud the efforts of those who seek to offset that risk. This work complements the research published by Nabet et al., (Nat. Chem. Biol., 2018) who used the dTAG approach to degrade an FKBP12-KRAS^{G12V} fusion protein, indicating that CRBN-recruiting degraders might prove successful at the task of degrading KRAS. If the activity of these model systems translates to endogenous processes, then having a second choice of E3 ligase to recruit is a bonus. The further added bonus here is that the AdPROM system can degrade untagged KRAS. In terms of using AdPROM to study the downstream effects of KRAS degradation, the authors acknowledge that one drawback is the selectivity of the monobody ligand, which also binds to HRAS. Little is known about the interaction of the monobody with various KRAS mutants and indeed, different phenotypic effects were observed across a panel of cell lines.

Contributor: Ryan

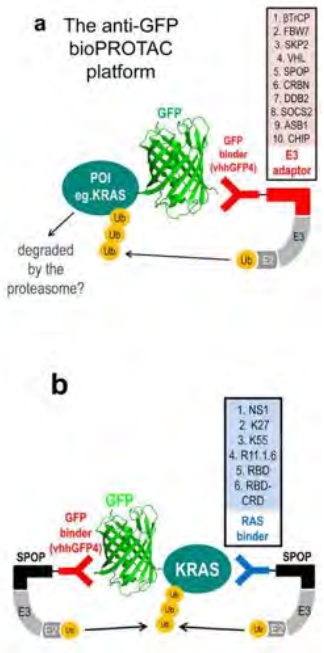
bioPROTACs establish RAS as a degradable target and provide novel RAS biology insights

Shuhui Lim, … ,Anthony W. Partridge*

bioRxiv, **2020**, DOI: 10.1101/2020.06.26.174565

In what seems to be a recurring theme this month this study describes the development of 'bioPROTACs' which operates in a similar way to the AdPROM and DARPin degrader systems also described in this month's issue. The authors first apply their technology to an impressive 10 CRL family members via fusion to a GFP-binding nanobody before individual transfection in HEK293 cell lines expressing GFP or GFP-KRAS. The GFP signal intensities were reduced in 8/10 bioPROTACs in the GFP-KRAS cell lines while GFP alone was poorly degraded. The focus then shifted to targeting endogenous RAS using a variety of RAS-binding macromolecules fused to the SPOP E3 ligase. One example tested (K-27 SPOP) used the DARPin K27 which is specific for GDP-loaded KRAS (but pan-RAS).

The expression of K27-SPOP led to complete disappearance of pan-RAS bands within 4 hours post-induction and the effect persisted for >24 hours along with inhibition of phosphor-ERK1/2. An mRNA transfection technique was then used to increase the efficiency compared to a typical DNA plasmid transfection, allowing the host cells to directly transcribe the bioPROTAC upon mRNA uptake. This had a similar outcome in all mRNA concentrations tested and caused growth inhibition of AsPC-1 cells ($KRAS^{G12D}$). When evaluated against a panel of cell lines of common KRAS mutations it was found that that rate of degradation correlated with the hydrolysis rate of GTP to GDP. The authors then incorporate the K19 KRAS specific DARPin (published during manuscript preparation) into a K19-SPOP bioPROTAC which selectively degrades KRAS independently of nucleotide state



This study is an extremely thorough characterisation of RAS targeting bioPROTACs using an impressive panel of E3 ligases and later RAS targeting macromolecules. While these technologies are largely utilised for understanding biology, the authors comment that the in-vivo delivery of therapeutic mRNA (to cause transcription of a protein of interest, an option for vaccines but also diseases such as cystic fibrosis) is becoming more common and indeed demonstrated this technique in-vitro for their bioPROTAC platform.

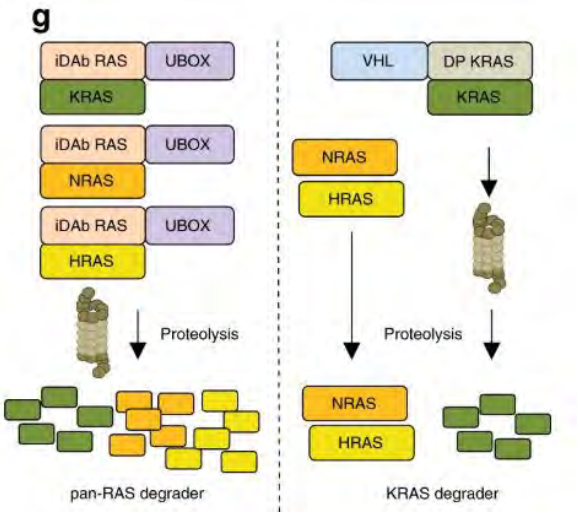
Contributor: Ryan

A potent KRAS macromolecule degrader specifically targeting tumours with mutant KRAS

Nicolas Bery, … ,Terry Rabbitts*

Nat Commun. **2020**, *11*, 3233.

The authors describe the development of both a pan-RAS and a KRAS specific macromolecular degrader. The degraders consist of either a DARPin (DP) which is KRAS specific or an intracellular single domain antibody (iDab) which is pan-RAS binding, these are then fused to an E3 ligase domain. They utilised both the VHL E3 ligase and the UBOX domain of the CHIP ligase and found that both the ligase choice and linkage vector (C or N terminus) was important to optimise for each system. They found that the iDAB-UBOX and DP-VHL systems were effective in pan-RAS and KRAS specific degradation respectively in a panel of cancer cell lines. Impressively the system was also applied in subcutaneous xenograft mouse models using cell lines which are either $KRAS^{WT}$ or $KRAS^{MUT}$. While the pan-RAS degrader impeded tumour burden in both models the KRAS degrader induced tumour regression in the KRAS mutated model only.



Despite the KRAS degrader being effective in degrading all KRAS proteins (mutant and wildtype) in a panel of cancer cell lines and xenograft mouse models it only inhibits cancer cell lines expressing mutant KRAS. The finding that a KRAS

specific degrader could spare non-mutant RAS cells is an extremely important one and supports KRAS degradation as a powerful strategy.

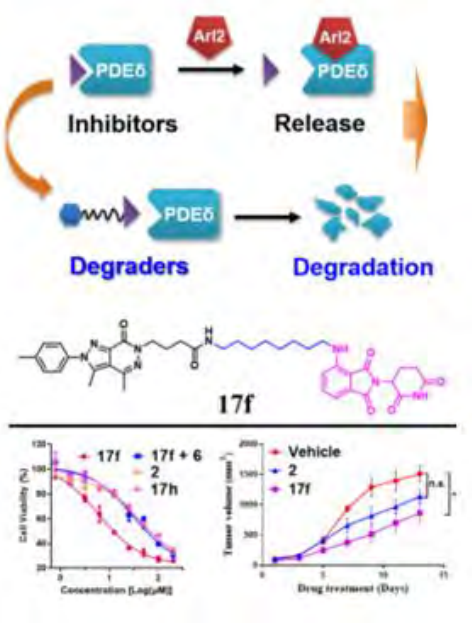
Contributor: Nicole

Discovery of Novel PDEδ Degraders for the Treatment of KRAS Mutant Colorectal Cancer

Junfei Cheng, Yu Li, Xu Wang, Guoqiang Dong* and Chunquan Sheng*

J. Med. Chem. **2020**, DOI: 10.1021/acs.jmedchem.0c00929

KRAS is an attractive target for cancer therapy, however, it has been a notoriously elusive protein, thwarting many drug discovery efforts. Attention has therefore turned towards proteins which support KRAS function. PDEδ is one such example: this shuttling factor protein binds to farnesylated KRAS and prevents its binding to endomembranes. The release factor, Arl2, frees KRAS from PDEδ and delivers it to the plasma membrane, where KRAS can carry out signalling functions. Therefore, targeting PDEδ could result in reduced levels of KRAS at the plasma membrane and attenuate signalling in KRAS mutant cancer cells. Potent PDEδ inhibitors (in vitro K_D < 10 nM) are known, however they display only moderate cellular potency. The authors of this paper attribute this potency shift to the release of the inhibitors by Arl2 and further propose that degradation of PDEδ could overcome this effect. In this paper they report and characterise the first PDEδ PROTACs.



*The authors made a good case for using a degradation approach to target PDEδ and their theory appears to hold true: the PROTAC **17f** displayed improved anti-proliferative activity against KRAS mutant colorectal cancer cells vs. the parent inhibitor **2**. The search for degraders began with an empirical approach to PROTAC design using a crystal structure of the inhibitor bound to PDEδ to guide positioning of the linker. Attachment of the CRBN binder, pomalidomide, using various linkers afforded the first set of molecules. These were characterised in terms of binding affinity for PDEδ by FP and DC_{50} of the target in CRC cells; PROTAC **17f** was identified as the best in both assays. The rank order for binding affinity appears to correlate with degradation potency, however two questions remain here: 1) what are the affinities of the ternary complexes? 2) why are the DC_{50} values an order of magnitude greater than the binding affinities? The authors proposed that poor permeability and high efflux ratio, as evidenced by Caco-2 assays, were plausible reasons for the discrepancy. I tend to agree with this assessment but would also propose that study of the ternary complex kinetics/stability may shed additional light on the potency shift and offer insight to aid optimisation of the next round of compounds.*

*One further question from me would be: what is the degradation selectivity of the lead PROTAC? The authors monitored degradation of IKZF1 and IKFZ3 as typical off-targets of CRBN binders, however assessment of wider proteomic analysis would have satisfied my curiosity about the broader selectivity profile of **17f**.*

*There are some nice results from experiments interrogating the effects of PROTAC-induced degradation of PDEδ. Firstly, the low micromolar DC_{50} value of the lead compound correlates with an anti-proliferative IC_{50} of similar potency. Downstream effects of the lead compound were observed for RAS signalling proteins, indicating the important role of PDEδ in the maintenance of KRAS activity. Immunofluorescence staining provided further evidence of target degradation and RAS localisation to endomembranes. In vivo experiments suggested that **17f** was tolerated and induced significant tumour growth inhibition.*

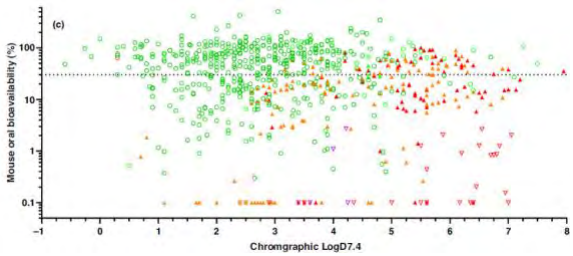
Contributor: Siying

Optimising proteolysis-targeting chimeras (PROTACs) for oral drug delivery: a drug metabolism and pharmacokinetics perspective

Andy Pike,* Beth Williamson, Stephanie Harlfinger, Scott Martin and Dermot F. McGinnity

Drug Discov. Today **2020**, DOI: 10.1016/j.drudis.2020.07.013

Scientists from AstraZeneca published a feature article detailing challenges with achieving oral PROTACs and the established in vitro ADME screening cascade for PROTACs in AZ. This is a well-written article with lots of interesting insights. The authors based their opinions on the PK studies of a set of cereblon- and VHL-based PROTACs (which may or may not show cellular activities). The data disclosed shows that cereblon-based PROTACs are more likely to achieve mouse oral bioavailability > 30%, and the optimal cLogP and ChromLogD values for achieving oral bioavailability are around 5-7 (although more data points required to study oral bioavailability of PROTACs with LogD around 2).



(Dotted line marked 30% mouse oral bioavailability. Open circle: small molecules. Solid triangle: cereblon PROTACs. Inverted open triangle: VHL PROTACs.)

Challenges of measuring PROTAC ADME properties due to their poor solubility and high nonspecific binding are highlighted and experimental approaches to yield less misleading results are discussed. What I found particularly though-provoking is the PK studies of PROTAC metabolites generated from linker cleavage. The metabolites could have a longer $t_{1/2}$ and a significantly larger free fraction than the parent PROTAC. The implication is that the metabolites could compete with PROTAC to block its mode of action, therefore highlighting the importance of optimising metabolic stability of PROTACs.

We are at the beginning of understanding the ADME properties of PROTACs as more data emerge. Very exciting to learn more about the properties of this class of beyond the rule of five compounds.

Contributor: Nicole

DOT1L degraders and uses thereof

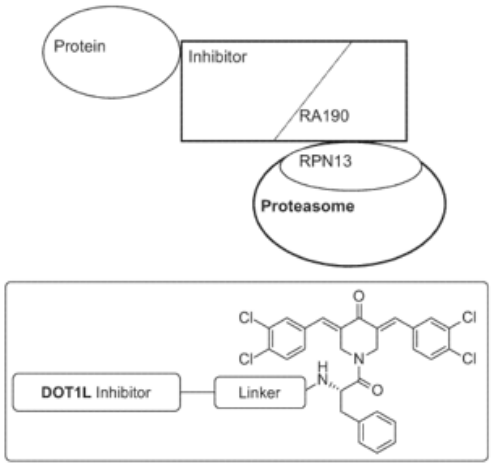
Scott Armstrong and Jun Qi

WO 2020/146561A1

Scientists from the Dana-Farber Cancer Institute disclose the invention of novel DOT1L degraders. DOT1L is a histone lysine methyltransferase, which writes mono-, di- and trimethyl modifications to lysine 79 of histone 3. In certain

diseases, such as leukaemia or lymphoma, hypermethylation occurs via mis-regulated DOT1L activity, which leads to aberrant transcription.

The bifunctional degrader molecules of this invention are comprised of a DOT1L binder and RA190, a binder of the ubiquitin receptor RPN13. The significance of this is that rather than hijacking an E3 ligase to ubiquitylate and mark the target for degradation - as with the majority of known degrader molecules - RA190 covalently binds to RPN13 and directly delivers DOT1L to the proteasome to facilitate its degradation. The patent includes figures depicting RA190 binding to RPN13 and a crystal structure of a DOT1L degrader binding to DOT1L. Exemplary molecules degrade DOT1L in a concentration and time-dependent manner and inhibit cell growth in three leukaemia cell lines.



I had the opportunity to study the chemical biology of DOT1L during my PhD and have followed the progress of DOT1L inhibitor pinometostat in Phase I clinical trials. Since joining research efforts in the field of TPD, I became curious to see whether a degradation approach to target DOT1L would be explored. This patent gives me reason to believe that DOT1L degraders could not only act as tools to study the protein but could also boost efforts to tackle aggressive diseases such as MLL. Looking forward to seeing more from this research journey.

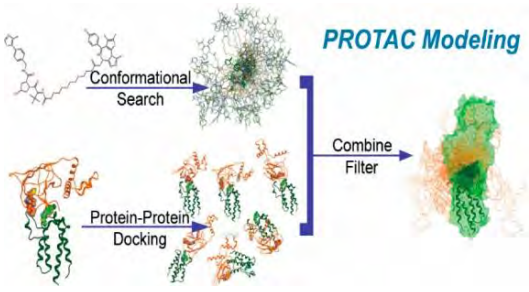
Contributor: Siying

Improved Accuracy for Modeling PROTAC-Mediated Ternary Complex Formation and Targeted Protein Degradation via New *In Silico* Methodologies

Michael L. Drummond,* Andrew Henry, Huifang Li and Christopher I. Williams

bioRxiv **2020**, DOI: 10.1101/2020.07.10.197186

Scientists from the Chemical Computing Group reported an improved version ('method 4b') of a previously reported method for modelling ternary complexes in silico, as well as a new method ('method 5') as a faster (but less accurate) alternative. In the previously reported method 4, protein-protein docking is first performed (in the absence of PROTAC linker) using target protein + binder and E3 ligase + binder binary co-crystal structures. PROTAC conformations are generated separately with no constraint and combined with the protein-protein docking poses (if compatible) and scored. In this preprint, the authors show significant improvements in the 'hit rate' of generating crystal-like modelled ternary complexes by increasing the diversity of protein-protein docked poses, applying constraints in PROTAC conformer generation and clustering the predicted ternary complexes. Method 4b shows poor to good hit rate (0.4 – 99%) in validation studies using six VHL- and cereblon-based PROTACs targeting bromodomain-containing proteins, and it seems to work better for VHL-based PROTACs than cereblon-based PROTACs.



A faster alternative to method 4b (named 'method 5') was also developed. In method 5, a single protein-protein docking is performed (using the same input as method 4b) and the binders are connected with a linker via the shortest path mapped out by computed water molecules between the binders. The entire PROTAC is then subjected to restrained minimisation and the results are scored by four filters based on how likely the PROTAC adopts the conformation found by the solvation-based pathfinding approach, PROTAC conformer energies, and total direct protein-protein interfacial surface area.

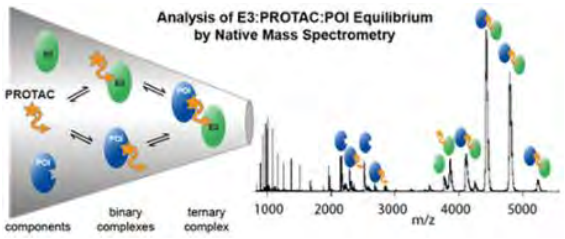
These methods are then applied to rank PROTACs in seven case studies, covering a wide range of scenarios (VHL-based macrocyclic PROTAC, VHL-based PROTACs for the degradation of wild type vs mutant bromodomains, cereblon-based PROTACs (which are known to induce weak direct protein-protein interactions), one PROTAC vs multiple targets, multiple PROTACs vs multiple targets, cIAP1-based PROTACs). It is interesting to see a correlation between the population of the largest cluster of predicted ternary complexes and potency, as well as the use of this population to rank PROTACs in different case studies. It is also interesting to see the use of docked protein-ligand poses (in scenarios when co-crystal structures are not available) as input files for the reported methods. Although the methods described perform less well for cIAP1-based PROTACs, which is not surprising given the methods are developed using VHL- and cereblon-based PROTACs, some of the experimental trends are still recapitulated. The methods described perform well at predicting the best performing PROTACs and the general trend of their potency. Further refinement of the methods should enable it to be a very valuable tool to prioritise PROTACs for synthesis and further development.

Contributor: Siying

Native Mass Spectrometry Can Effectively Predict PROTAC Efficacy

Rebecca Beveridge,* Dirk Kessler, Klaus Rumpel, Peter Ettmayer, Anton Meinhart, and Tim Clausen*

ACS Cent. Sci. **2020**, DOI: 10.1021/acscentsci.0c00049



Formation of a ternary complex (which consists of protein of interest – PROTAC – E3 ligase) is crucial for PROTAC-mediated ubiquitination (and subsequently degradation) of the protein of interest. Therefore, studying ternary complex formation could offer opportunities for PROTAC development and optimisation. Scientists from Research Institute of Molecular Pathology and Boehringer Ingelheim report a native mass spectrometry (nMS) approach using nanoelectrospray ionisation (nESI) and label-free proteins to monitor the formation of binary and ternary complexes.

Two well-characterised VHL-based PROTACs targeting bromodomain proteins (MZ1 and AT1) were used as model compounds in the nMS studies. For both PROTACs, the formation of ternary complexes with VCB and $BRD4^{BD2}$ were detected and MZ1 shows a higher proportion of ternary complex formation (at PROTAC concentration > 5 M), indicative of the higher stability of ternary complex formed by MZ1 as expected. The possibility to study the specificity of a PROTAC vs different protein targets ($BRD4^{BD2}$, $BRD3^{BD2}$, and $BRD4^{BD1}$) is also established. For both PROTACs, the most 'preferred' protein target identified by nMS experiments agrees with that identified by our group using other

biophysical methods (Gadd et al. Nat Chem Biol. 2017, Roy et al. ACS Chem. Biol. 2019), and the more selective PROTAC (AT1) shows a larger window of the proportion of ternary complexes formed with different proteins.

The most exciting feature (in my opinion) of the reported nMS methodology is the possibility to perform competition experiment 'in one shot' with unlabelled proteins. Mixtures of PROTAC (MZ1 or AT1), VCB, and up to 5 label-free bromodomain proteins were analysed, and the most preferred protein target, as well as the relative % of ternary complexes formed, agree well with our group's SPR data. Although ternary complexes formed with $BRD2^{BD2}$ and $BRD3^{BD2}$ could not be deconvoluted (due to a very small difference in mass), the results are still highly informative in understanding what proteins are (preferentially) recruited by PROTACs.

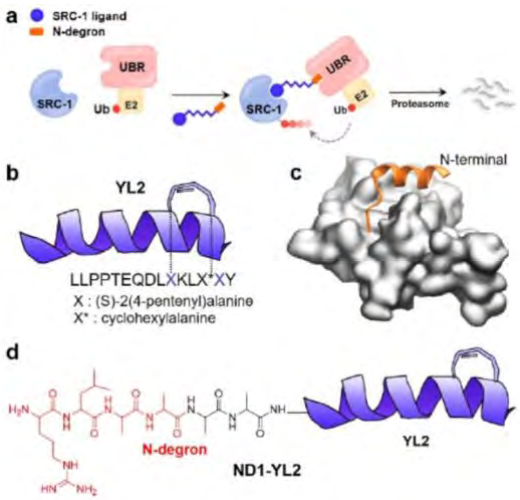
The current nMS methodology is not fully quantitative and does not provide K_D values. Also, one should bear in mind that ionisation efficiency of different species (apo protein and binary complexes) could affect the observed relative population of ternary complex. Indeed, this was observed by the authors in the analysis of a mixture containing $BRD4^{BD2}$, VCB, and a high concentration (20 M) of PROTACs. Due to the poor ionisation efficiency of the $BRD4^{BD2}$-PROTAC binary complex, the signal of the binary complex is low, which then artificially increases the relative percentage of $BRD4^{BD2}$-PROTAC-VCB ternary complex observed. I wonder how well this method will work with other E3 ligases, and whether this could be used as a tool to predict the efficiency of PROTACs with different E3 ligase recruiters.

Contributor: Ryan

Targeted Degradation of Transcription Co-activator SRC-1 through the N-Degron Pathway

Yeongju Lee, … ,Hyun-Suk Lim*

Angew. Chem. Int. Ed. **2020**, DOI: 10.1002/anie.202005004



In this study the authors describe the design of degraders targeting steroid receptor co-activator-1 (SRC-1), a transcription coactivator which when abnormally activated has been linked to cancer progression and metastasis. To recruit SRC-1 the group uses a previously developed cell-permeable stapled peptide (K_i = 140nM) while the UBR E3 ligase family was recruited through an 'N-degron'. In this case the N-degron was a tetrapeptide (RLAA) which is known to bind to the UBR box (yeast Ubr1) with low micromolar affinity. This resulted in a final peptidic PROTAC ND1-YL2 which was able to catalyse degradation of SRC-1 with a DC_{50} of 10 µM. PROTACs were then synthesised which utilised Pomalidomide rather than UBR as a comparison and it was found that these were also effective with a similar activity to ND1-YL2. The tool peptide was then used to study the downstream effects of SRC-1 degradation which included the downregulation of genes such as CSF-1 and upregulation of E-cadherin consistent with previous siRNA experiments in the literature. The compound was also taken into a study in mice where it was found that SRC-1 degradation by ND1-YL2 suppresses the metastasis of breast cancer cells.

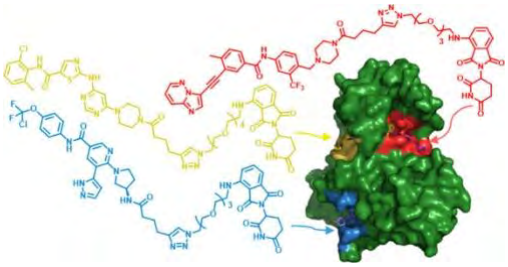
As the authors point out, despite there being an estimated >600 E3 ubiquitin ligases, only a handful have so far been hijacked for use in degrader molecules and so the use of an N-degron to target UBR is a welcome addition. Although the DC_{50} of the lead molecule may seem modest by some standards, it is impressive that this large, peptidic and charged compound is permeable enough to result in degradation with a comparable activity to a more typical cereblon hijacking PROTAC.

Contributor: Siying

Global PROTAC Toolbox for Degrading BCR–ABL Overcomes Drug-Resistant Mutants and Adverse Effects

Yiqing Yang, Hongying Gao, Xiuyun Sun, Yonghui Sun, Yueping Qiu, Qinjie Weng,* and Yu Rao*

J. Med. Chem. **2020**, DOI: 10.1021/acs.jmedchem.0c00967



The genetic abnormality in chromosome 9 and 22 generates BCR-ABL fusion oncoprotein, which leads to constitutive activation of ABL tyrosine kinase and causes chronic myeloid leukemia (CML). Although several inhibitors have been developed as a targeted treatment for CML, intolerance and drug resistance limit their application to a wider range (>60%) of the patient population. The authors aim to address these limitations by developing PROTACs to degrade wild type BCR-ABL, as well as the T315I mutant which contributes to drug resistance.

*A series of PROTACs were synthesised by linking pomalidomide to BCR-ABL inhibitors (imatinib, dasatinib, asciminib, or ponatinib) which are known to bind at 3 different sites of BCR-ABL. It was found that PROTACs with inhibitors from either of the three binding sites can induce wild-type degradation. Imatinib-based PROTACs do not degrade wild-type BCR-ABL and asciminib-based PROTACs (such as compound **14**) show moderate degradation. Both dasatinib- and ponatinib-based PROTACs (compounds **10** and **19**) induce potent degradation of wild-type BCR-ABL with DC_{50} values of 10 and 20 nM at 32-hour time point. For mutant degradation, either asciminib- and ponatinib-based PROTACs (compounds **14** and **19**) are effective, although less well compared to wild-type degradation. The degradability of dasatinib-based PROTAC is rescued by* structure-based modification of dasatinib to regain the binary binding of *PROTAC to the mutant. Compound **19** shows good antiproliferative activities in a mutant dependent cell line and does not exhibit cytotoxic effect like its parent inhibitor (ponatinib) and bind weaker (by 10 to 250-fold) to ponatinib off-target kinases. A synergistic combination of compound **19** with hedgehog pathway inhibitor GDC-0449 is proposed to further reduce adverse effects.*

The authors made an interesting observation that good affinity of the inhibitor to BCR-ABL (determined by antiproliferative IC_{50} values of the inhibitors) is important for PROTAC-induced formation of ternary complex and therefore degradation of wild-type BCR-ABL. It will be good to see additional biophysical data (BCR-ABL binary affinity and ternary affinity of PROTACs) to rule out potential effects of linkers on the BCR-ABL binary affinity.

*Another area biophysics could provide more insight is to rationalise the different degradation efficiency observed for compounds **10**, **14** and **19** by studying the kinetics and stability of ternary complexes. The authors modelled ternary*

*complex formed by compounds **10**, **14** and **19** in silico to rationalise the observed degradation potency based on the area of direct protein-protein interaction interface (as a proxy for the stability of ternary complexes). It will be interesting to see whether the biophysical data agrees with the computational analysis. Since modelling PROTAC-induced ternary complex is a rapidly evolving topic (so there is no 'standard' workflow), it will be good if the authors could either provide a reference to their computational workflow, or a validation study using the workflow. In addition, it would be interesting to apply the 'method 4b/method5' developed by CCG for modelling ternary complex in silico (which is featured in this month's journal club) to rank these three PROTACs.*

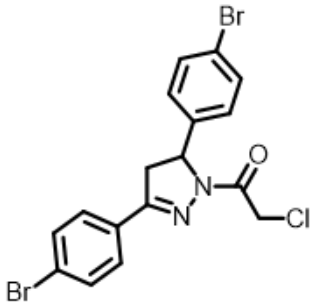
*Furthermore, it would be good to see attempts to demonstrate the degradation of BCR-ABL by compound **19** is via PROTAC mechanism (ie. E3 ligase-dependent and proteasome-dependent degradation). In the study of lead PROTAC (compound **19**) selectivity, only the inhibition and degradation of known off-targets of parent binder ponatinib is considered, but not the typical off-targets of cereblon binders (eg. IKZF1 and KIFZ3). Perhaps a proteomic analysis would provide more insights into the selectivity of compound **19**.*

Contributor: Siying

Chemoproteomics-Enabled Ligand Screening Yields Covalent RNF114-Based Degraders that Mimic Natural Product Function

Mai Luo, § ….. , Daniel K. Nomura*

bioRxiv **2020**, DOI: 10.1101/2020.07.12.198150



EN219

The Nomura group reported the discovery of EN219 as a synthetically more accessible (and more drug-like) RNF114 recruiter that has the same binding mode as nimbolide. The disordered nature of the nimbolide binding region of RNF114 precludes structure-based ligand discovery. Therefore, an affinity-based protein profiling (ABPP) competition assay was employed to screen a library of 318 commercially available chloroacetamide- and acrylamide-based covalent ligand. The screening campaign identified EN219 as the most potent ligand (IC_{50} = 470 nM) and the site of EN219 covalent modification on RNF114 was confirmed by LC-MS/MS experiment. To further validate EN219 engagement of RNF114 in cells, competitive isotopic tandem orthogonal proteolysis-ABPP (isoTOPABPP) and Tandem mass tagging (TMT)-based quantitative proteomic experiments were performed. While RNF114 engagement was only observed in the isoTOPABPP experiment, other off target proteins (10 in total) were also identified and none of which are E3 ubiquitin ligases.

The application of EN219 in TPD was showcased in the degradation of BRD4 and BCR-ABL. EN219 was linked to a pan-selective BET bromodomain inhibitor JQ1 to degrade BRD4, and the degradation was shown to be proteasome- and RNF114-dependent manner. A TMT-based proteomic experiment shows that treatment of the EN219-based PROTAC (with JQ1 as warhead) upregulates tumour suppressor p21 and CTGF, a behaviour that is similar to the nimbolide-based PROTACs. To further illustrate the utility of EN219 in TPD, it was also connected to an ABL inhibitor dasatinib to degrade BCR-ABL. Similar to the degradation profile of nimbolide-based BCR-ABL PROTACs, preferential degradation of BCR-ABL was also observed for the EN219-based PROTACs.

It is interesting to see how one can identify novel covalent linkers with chemoproteomics when structural information is not available, as well as the generation of a double-digit nM degrader (in the case of BRD4 degradation) using an E3 ligase ligand identified directly from the screening campaign. I look forward to the further optimisation of EN219 and more studies on covalent E3-targeting PROTACs.

Others

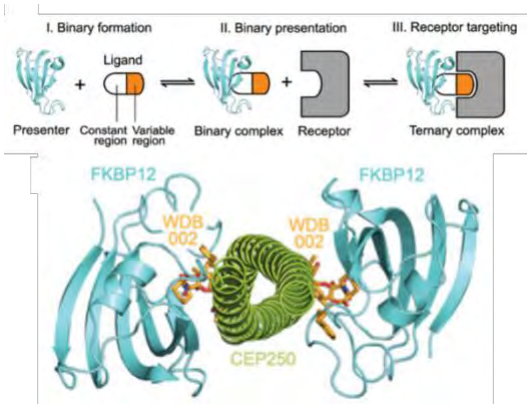
Contributor: Will

Genomic discovery of an evolutionarily programmed modality for small-molecule targeting of an intractable protein surface

Uddhav K. Shigdel[§]…Gregory L. Verdine*

PNAS **2020**, *29,* 17195 DOI: 10.1073/pnas.2006560117

Shigdel and co-workers herein mine the genomes of actinomycete species to identify biosynthetic gene clusters that could in turn allow access to natural products in the FK506- and Rapamycin-like structural class. The aim of this was to identify compounds capable of recruiting FKBP12 to enable the formation of FKBP12:small-molecule:protein-of-interest (POI) ternary complexes at POIs that do not contain binding sites classically deemed as druggable. The authors identify compound WDB002 and via pull-down of FKBP12 in cell lysates and mass spec identify CEP250 as a protein target for FKBP12:WDB002 complexes. A ternary crystal structure shows how WDB002 is able to induce binding of FKBP12:WDB002 to the featureless coiled-coil topology of CEP250.



The work laid out in this publicaiton demonstrates the potential of an exciting and novel modality for perturbing/binding otherwise intractable proteins and demonstrates the versitility of FKBP12 to use its different 'faces' to bind different protein partners when induced to do so by a small molecule.

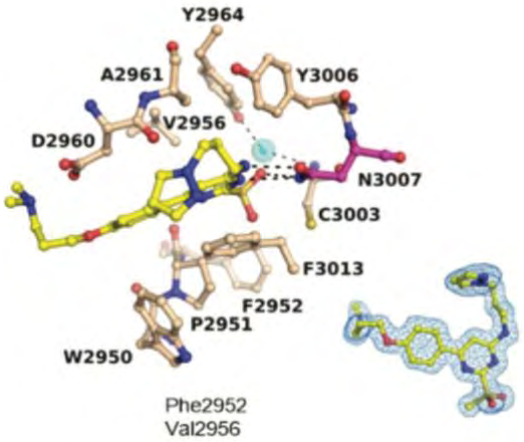
Contributor: Alessio

New inhibitors for the BPTF bromodomain enabled by structural biology and biophysical assay development

Peter D. Ycas,[§] Huda Zahid,[§] …, William C. K. Pomerantz*

Org. Biomol. Chem. **2020**, *18,* 5174 DOI: 10.1039/D0OB00506A

Ycas et al. describe a biophysical and crystallographic study of small molecules binding to the BPTF bromodomain. The BPTF bromodomain has been challenging to target with small molecules, and unlike e.g. the BET bromodomains, only very limited binding chemotypes were known, beyond compound AU1 that had been reported previously by the same group. They studied the binding of a small set of fragment-like molecules to BPTF using orthogonal biophysical methods: SPR, Alpha Screen, their previously-reported 19F-Trp observed protein (PrOF) NMR, and X-ray crystallography. The cross-validation of the different biophysical techniques, and the novel co-crystal structures



with ligands bound, are the highlight of the paper. Together they qualify new bona-fide binders and provide a solid platform for future ligand design.

It is commendable that the authors cross-validate ligand binding using multiple biophysical techniques – and certainly something we advocate as best practise in the field – so kudos to the group for doing that! Their best binders described in the paper still have a binding affinity comparable to their own lead inhibitor AU1 which was reported in 2015, so there is still plenty of room for optimization too.
