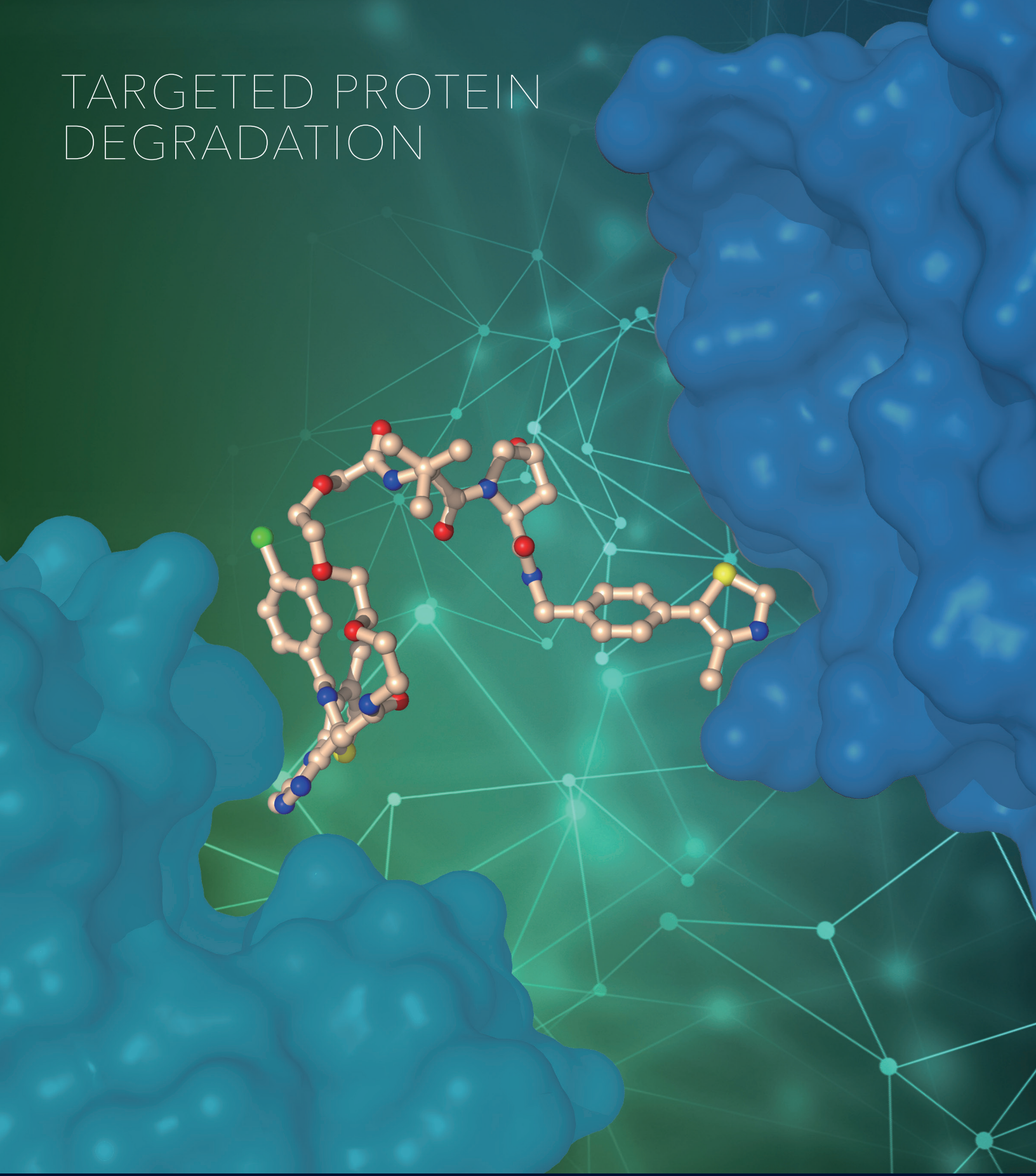
TARGETED PROTEIN DEGRADATION



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nacalai tesque
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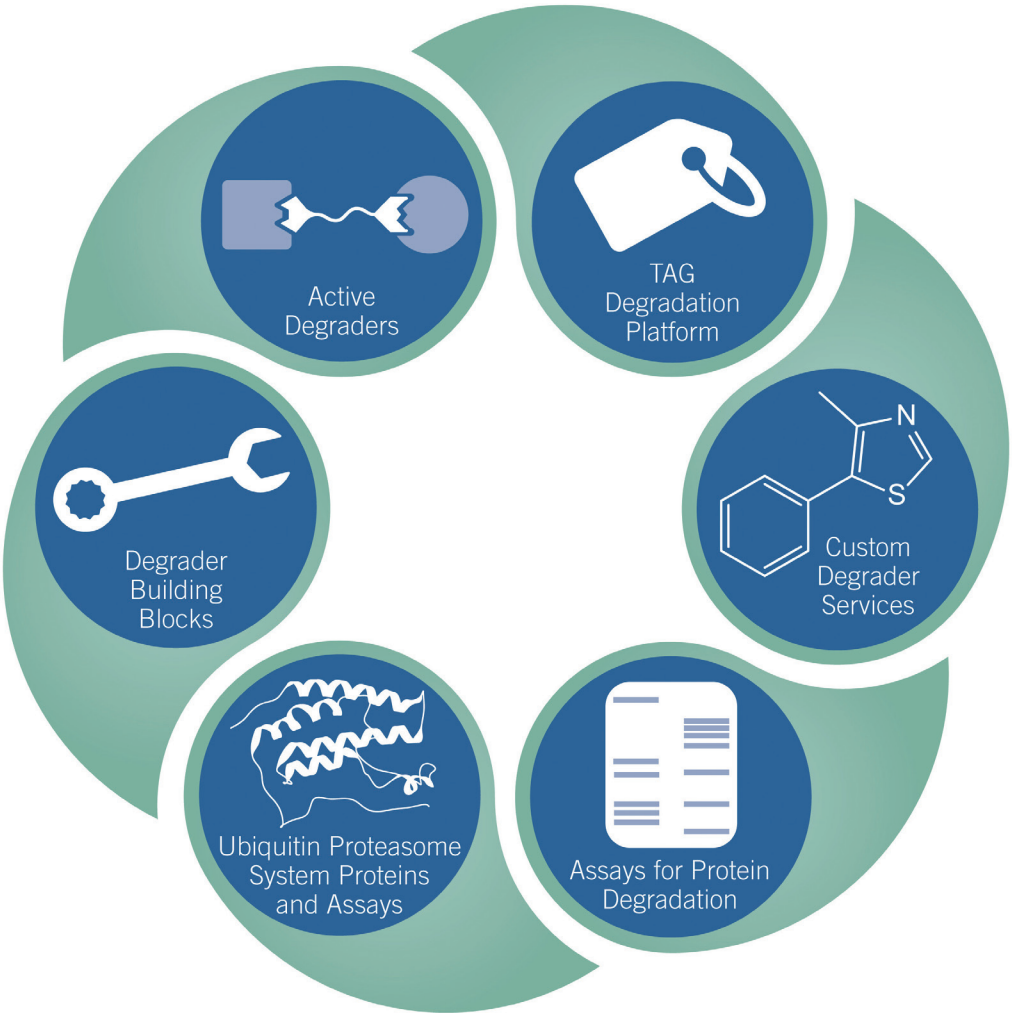
Targeted Protein Degradation

Bio-Techne グループは急成長している標的タンパク分解（Targeted Protein Degradation; TPD）研究に注目し、Active Degraders、TAG Degradation システム（aTAG, dTAG）、Degrader Building Blocks などのキメラ分子やそのパーツとなる分子を提供しています。



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Introduction to Targeted Protein Degradation

二つのリガンドをリンカーで接続した低分子化合物 "Degraders"（PROTAC®、SNIPER）で標的タンパク質分解（TPD）を行う研究は、急速に注目を集めています。二つのリガンドのうち、一つは標的タンパク質に結合し、もう一つは E3 リガーゼに結合します。E3 リガーゼを足場として、E2 リガーゼから標的タンパク質がユビキチン化され、続いてプロテアソームによるユビキチン化タンパク質の分解が行われます。この技術を利用することにより、効率的に高い選択性を持ったプロテインノックダウンを *in vivo* でも *in vitro* でも行うことが可能になります。Degarder は触媒反応のように、繰り返し作用するため、少量でも十分なノックダウン効果を得ることが可能です。Bio-Techne は標的タンパク質分解に関連する研究をサポートする、さまざまな製品やサービスを提供しています。

Mechanism of Degrader Action

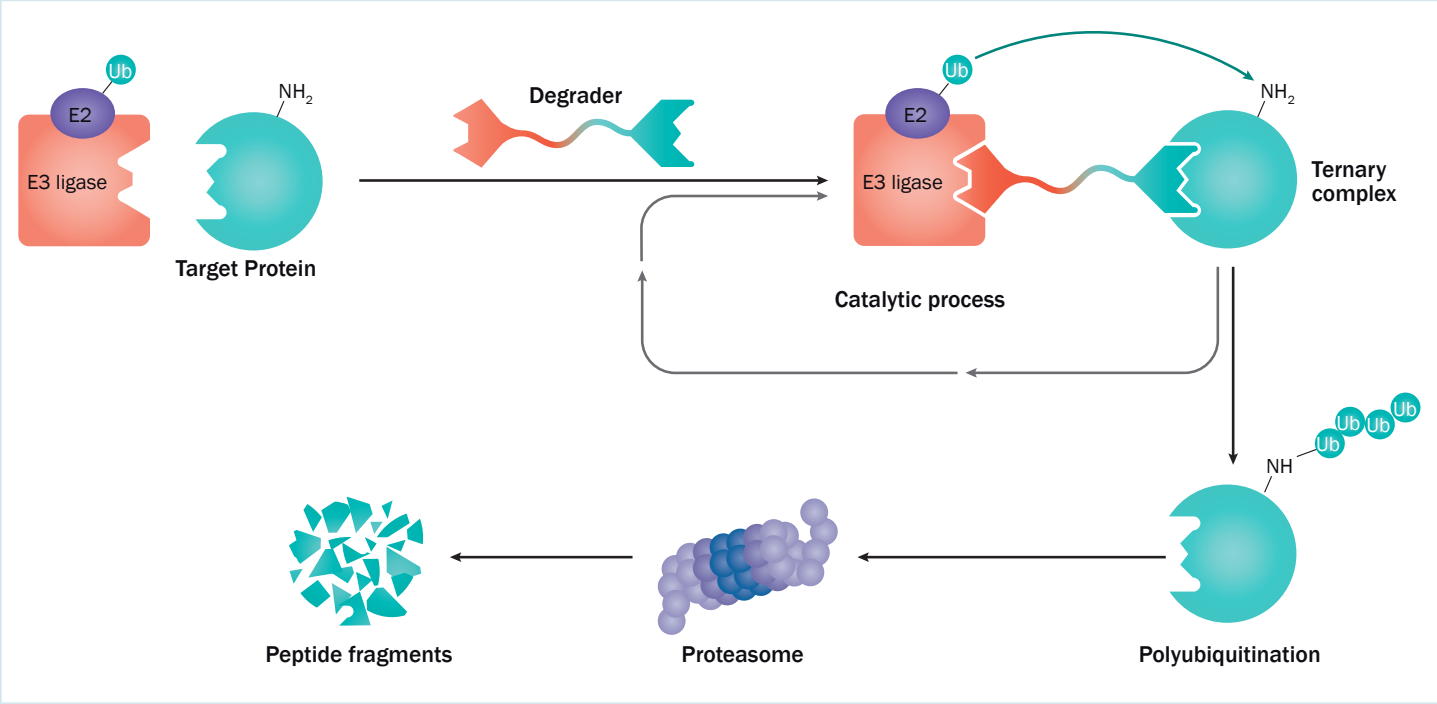

Figure 1: Schematic showing the catalytic mode of action of heterobifunctional degrader molecules. Degraders initiate the formation of a ternary complex between an E3 ubiquitin ligase and a target protein which results in polyubiquitination of the target protein, its recognition by the proteasome and subsequent degradation.

Adapted from Tinworth et al. (2016) MedChemComm 7 2206.

Why Use Degraders?

As an approach for target protein knockdown within cells, Degraders offer several advantages over genetic manipulation:

- **Ease of use:** Tocris Degraders are cell-permeable small molecules that can be applied directly to cells, with no need for transfection or expression vectors
- **Applicable to multiple cells lines**, with no requirement that cells are easily transfectable
- **Duration of effect is adjustable** and reversible on compound washout
- **Catalytic mode of action**, allowing use at sub-stoichiometric concentrations

Active Degraders

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The Tocris brand has pioneered commercialization of tool Degraders to make them available to the research community. They provide an easy-to-use alternative to genetic manipulation for investigating phenotypic consequences of target protein knockdown. A selection of our growing range is provided in the table below, and the full range is available through our website: www.tocris.com/product-type/active-degraders

Product Name	Catalog #	Target Protein	Action
AT 1	6356	BRD4	Selectively degrades BRD4, with negligible loss of BRD2 and BRD3; most selective BRD4 Degrader available*
BSJ-03-123	6921	Cdk6	Selective Cdk6 degrader**
BSJ-03-204	6938	Cdk4/6	Selective Cdk4/6 degrader; induces G_1 cell-cycle arrest and inhibits proliferation of a mantle cell lymphoma cell line**
CM 11	6416	pVHL30	Homo-PROTAC for self-degradation of the long form of VHL, pVHL30*
CRBN-6-5-5-VHL	6948	CRBN	Potent and selective cereblon degrader; induces complete degradation of cereblon in MM1S cells; cell-permeable
dBET1	6327	BET bromodomains	Depletes BET bromodomains in cancer cell lines *in vitro* and downregulates MYC in mice bearing human AML xenografts**
dBRD9	6606	BRD9	Potent and selective BRD9 degrader**
dTRIM 24	6607	TRIM24	Degrader targeting TRIM24; demonstrates antiproliferative effects in MOLM-13 cells**
MZ 1	6154	BRD4	Selectively degrades BRD4 over BRD2 and BRD3; exhibits potent antiproliferative and cytotoxic effects in AML cell lines*
THAL SNS 032	6532	Cdk9	Potently and selectively degrades Cdk9**
TL 12-186	6524	Multikinase	Multikinase degrading PROTAC; degrades a range of kinases *in vitro***
TL 13-112	6745	ALK	Selective ALK Degrader; inhibits proliferation of ALK+ cancer cell lines**
TL 13-12	6744	ALK	Exhibits higher selectivity for ALK over Aurora A kinase compared with TL 13-112 (Cat.No. 6745)
ZXH 3-26	6713	BRD4	Potent and selective BRD4 degrader**
VZ 185	6936	BRD7/9	Coming soon!*

*Sold under license from the University of Dundee, UK ** Sold under license from the Dana-Farber Cancer Institute, USA

Controls and Related Small Molecules

Tocris also offers negative controls for some of the active Degraders, and a range of related reagents for the Ubiquitin Proteasome System, including Proteasome inhibitors. A selection of related products is listed below.

Degrader Negative Controls		
Product Name	**Catalog #**	**Action**
BSJ-Bump	6922	Negative control for BSJ-03-123
cis MZ 1	6155	Negative control for MZ 1
CMP 98	6417	Negative control for CM11
TL 13-110	6746	Negative control for TL 13-112
TL 13-22	6747	Negative control for TL 13-12
TL 13-27	6525	Negative control for TL 12-186

Proteasome Inhibitors		
Product Name	**Catalog #**	**Action**
MG 132	1748	Proteasome and calpain inhibitor. Inhibits NF-κB activation
Lactacystin	2267	Cell-permeable, potent and selective proteasome inhibitor

To discuss potential licensing opportunities for Degraders and related products, please contact our licensing team at: **licensing@bio-techne.com**

Degrader Building Blocks

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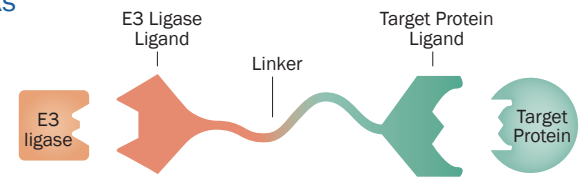
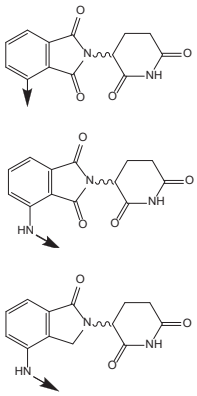
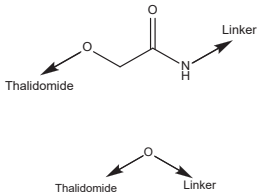
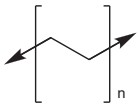
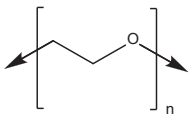
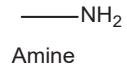
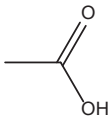
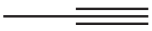
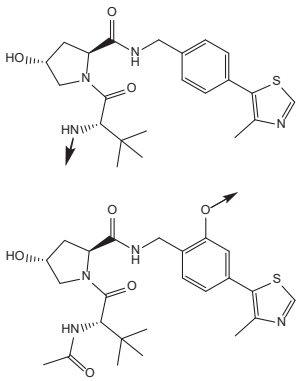
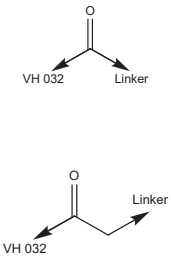
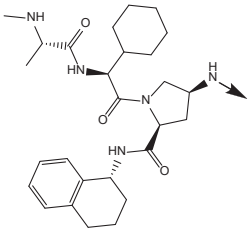
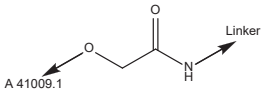
Develop your Degraders with our toolbox of functionalized building blocks

Tocris now supplies chemical building blocks (functionalized E3 ligase ligands plus linkers) to enable researchers to develop their own Degraders. Degraders are modular in design, consisting of binding moieties for an E3 ubiquitin ligase and a target protein joined by a linker. Our Degrader components have functional handles for easy conjugation to ligands/linkers of interest. The range includes the most effective and commonly used E3 ubiquitin ligase ligands, functionalized at positions known not to interfere with binding affinity. E3 ligase ligands conjugated to common linker groups are also supplied. For more information on our range visit: www.tocris.com/product-type/degrader-building-blocks



	E3 Ligase Ligand	Example Exit Vectors	Standard Linkers	Conjugation Functionality
CRBN		The exit vector bridges the E3 ligand to the linker group 	The choice and length of linker is critical for achieving optimal formation of the ternary complex. It is also a key determinant of the physicochemical properties of the final Degrader molecule. The majority of Degraders for proof-of-concept studies use either a PEG or alkyl linker  	Linkers are functionalized with a reactive chemical 'handle' to enable coupling to your target ligand of interest  Amine  Carboxylic Acid  Azide  Alkyne
VHL				
IAP				

Bulk quantities available. To find out more about our offering visit: **www.tocris.com/support/bulk-quantities-form**
For custom building blocks get in touch with our team: **www.tocris.com/services/custom-degrader-services**
Target Protein Ligandのカスタム合成も行っていますので、お気軽にご相談ください。

TAG Degradation Platform

Tag, Degrade, Discover

The TAG Degradation Platforms (dTAG and aTAG) are a TPD based approach to target validation that use a heterobifunctional Degrader targeting a TAG domain that is expressed as a fusion with a protein of interest. This technology allows rapid and highly selective degradation of a protein of interest, without the requirement of developing a specific Degrader for each target protein, and is generalizable to a range of fusion proteins.

How Does TAG Degradation Work?

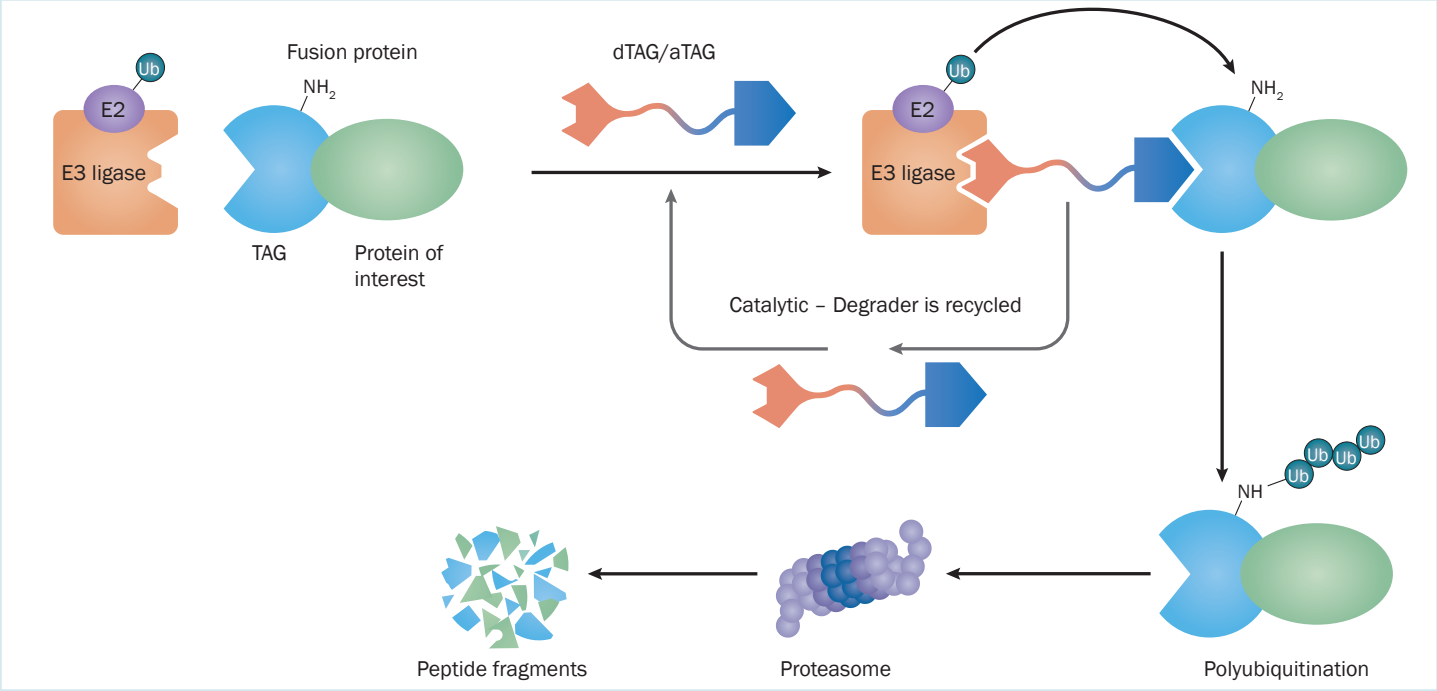


Figure 2: Schematic showing the mode of action of dTAG/aTAG Degraders. A protein of interest is expressed as a fusion with a "TAG" protein. For the dTAG system the protein of interest is tagged with single-point mutant FKBP12 (F36V); the aTAG system uses MTH1 as the TAG. The dTAG/aTAG Degrader, which comprises a ligand that selectively binds the TAG protein linked to an E3 ligase ligand, initiates the formation of a ternary complex between an E3 ubiquitin ligase and the fusion protein which results in polyubiquitination of the target protein, its recognition by the proteasome and subsequent degradation of the entire fusion protein. dTAG/aTAG molecules act catalytically, repeatedly engaging and directing the ubiquitination of target molecules.

TAG Degradation is a promising alternative to genetic methods for target validation and can be used in cell culture or *in vivo*. It offers a valuable approach to validating targets for which there are no known ligands. The table below provides a comparison of TAG Degradation with commonly used genetic knockout/knockdown approaches.

	Dose tuneability	Efficacy	Reversibility	Kinetics	Selectivity
TAG Degradation Platform (dTAG/aTAG)	***	****	****	***	****
Gene knockout e.g. CRISPR/Cas9	*	****	*	*	****
Gene knockdown e.g. RNAi	*	***	*	*	**

TAG Degradation Platform

Tocris now offers two options for TAG Degradation: dTAG and aTAG. The difference between them is the TAG protein used: dTAG uses mutant FKBP12, and aTAG uses MTH1. Both can be used *in vitro* and *in vivo*.

dTAG

- dTAG is a powerful new tag-based degradation platform for the specific knockdown of mutant FKBP12 fusion proteins
- FKBP12^{F36V} can be expressed as a fusion with a target protein of interest via transgene expression or CRISPR-mediated specific knock-in
- dTAG-13 exhibits rapid, reversible and tuneable knockdown of FKBP^{F36V} fusions proteins *in vitro* and *in vivo*
- Corresponding plasmids are available through Addgene

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Product Name	Catalog #
dTAG-13	6605
dTAG-7	6912

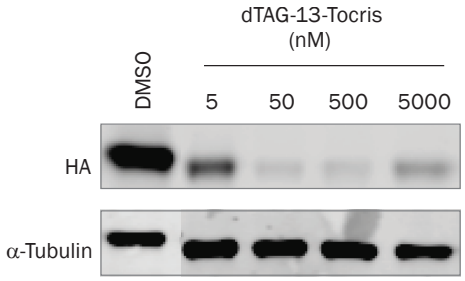

Figure 3: Western blot data showing potent knockdown of a HA-tagged FKBP12^{F36V}-fusion protein after application of dTAG-13

aTAG

- The aTAG degradation domain, MTH1, can be expressed as a fusion with a target protein of interest via CRISPR-mediated specific knock-in (protocol available: www.tocris.com/resources/protocols/crispr-cas9-based-genome-editing)
- MTH1 (NUDT1) is a small protein (17kDa) whose degradation or acute inhibition has no effect on cell viability: MTH1 knockout mice have no phenotypic differences when compared to wild-type mice. It can therefore be used as a TAG domain
- The aTAG Degraders, aTAG 2139 (Cat. No. 6970) and aTAG 4531 (Cat. No. 6971) can be applied both *in vitro* and *in vivo* to selectively and potently degrade MTH1 fusion proteins
- aTAG Degraders are cell-permeable and suitable for *in vivo* and *in vitro* use

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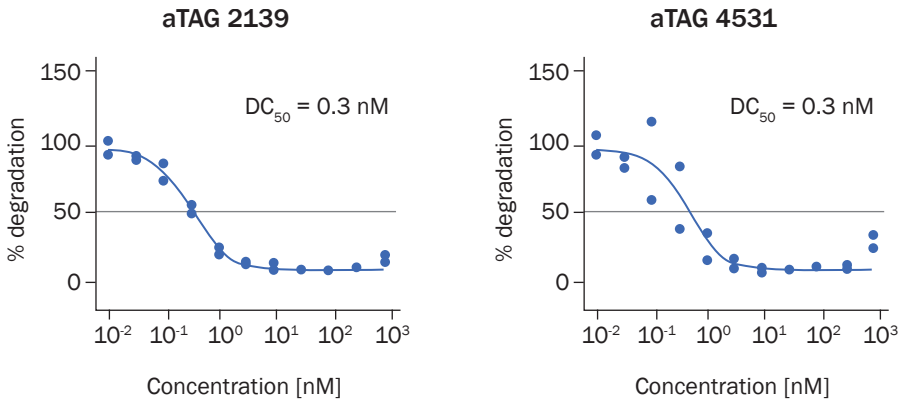

Figure 4: Dose response curves showing degradation of exogenously expressed CAR fused to MTH1 in human Jurkat cells at 4 hours.

Mouse DMPK Properties for aTAG Degraders		
	aTAG 2139 (Cat. # 6970)	aTAG 4531 (Cat. # 6971)
DC_{50}	0.27 nM	0.34 nM
D_{max}	92.1%	93.1%
CL	21.5 mL/min/kg	61.34 mL/min/kg
Half life	5.43 hours	2.83 hours

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