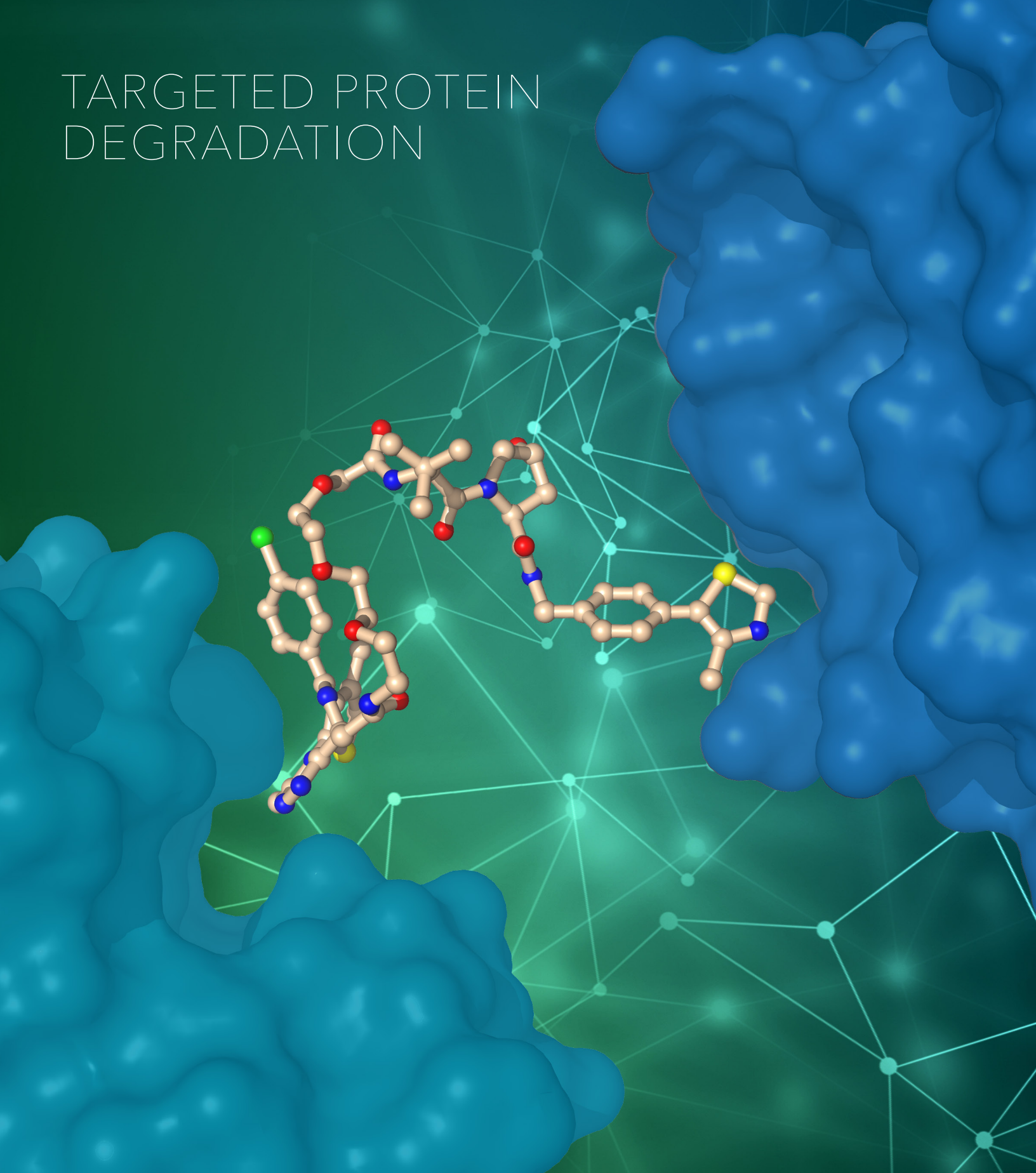


TARGETED PROTEIN DEGRADATION



biotechne®

Targeted Protein Degradation

The Bio-Techne family of brands offer a unique portfolio of high-quality reagents, instruments and services for researchers working in the rapidly growing field of Targeted Protein Degradation. Bio-Techne provides a bespoke range of tools and reagents, including Active Degraders, TAG Degradation Platform (aTAG, dTAG), Degradation Building Blocks, Assays for Protein Degradation, Ubiquitin Proteasome System Proteins and Assays, and Custom Degradation Services. Visit [tocris.com/tpd](https://www.tocris.com/tpd)

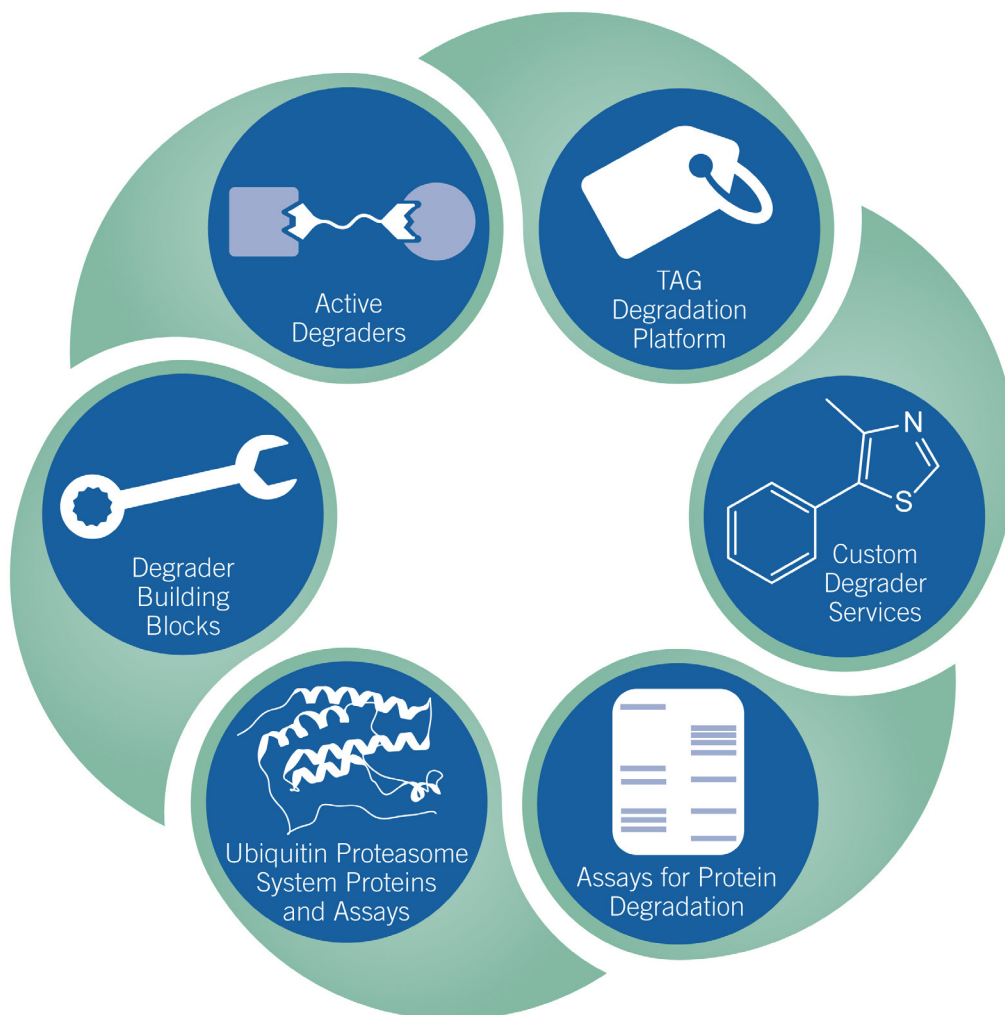


Table of Contents

Introduction to Targeted Protein Degradation	3
Active Degraders	4
Degradation Negative Controls	4
Degradation Building Blocks	5
TAG Degradation Platform	6-7
Custom Degradation Services	8
Assays for Targeted Protein Degradation	9-10
Ubiquitin Proteasome System Proteins	11

Introduction to Targeted Protein Degradation

The use of heterobifunctional small molecule Degraders (e.g. PROTAC® molecules, SNIPERs etc) to elicit targeted protein degradation (TPD) is an area of increasing research interest. The approach employs two small molecules joined by a linker. One binds to the target protein of interest, the other binds and recruits an E3 ligase to form a ternary complex. This initiates the ubiquitination of the target protein and its subsequent destruction by the proteasome. Using this technology, efficient and highly selective protein knock-down can be achieved both *in vitro* and *in vivo*. Degraders act catalytically by repeatedly engaging and directing the ubiquitination of target molecules and can therefore be used at very low doses to achieve sustained knock-down. Bio-Techne offers a range of products and services to support your research in this field.

Mechanism of Degradation Action

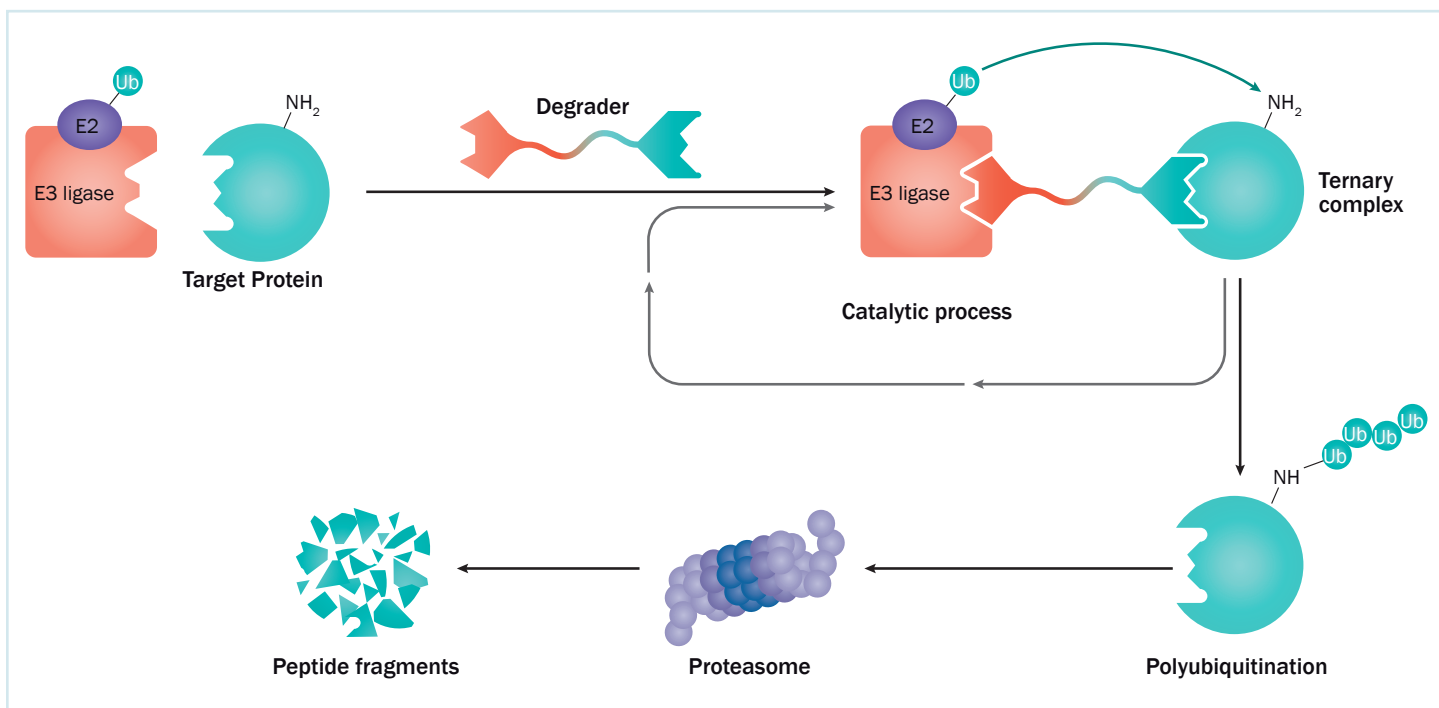


Figure 1: Schematic showing the catalytic mode of action of heterobifunctional degrader molecules. Degraders initiate the formation of a ternary complex between an E3 ubiquitin ligase and a target protein which results in polyubiquitination of the target protein, its recognition by the proteasome and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



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 Degradator 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 <i>in vitro</i> and downregulates MYC in mice bearing human AML xenografts**
dBRD9	6606	BRD9	Potent and selective BRD9 degrader**
dTRIM 24	6607	TRIM24	Degradator 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 <i>in vitro</i> **
TL 13-112	6745	ALK	Selective ALK Degradator; 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 Degradators, and a range of related reagents for the Ubiquitin Proteasome System, including Proteasome inhibitors. A selection of related products is listed below.

Degradator 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

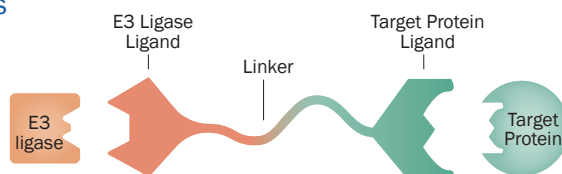
Proteasome Inhibitors		
Product Name	Catalog #	Action
MG 132	1748	Proteasome and calpain inhibitor. Inhibits NF-κB activation
Lactacystin	2267	Cell-permeable, potent and selective proteasome inhibitor

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



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 —NH₂ Amine Carboxylic Acid —N₃ 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

For advice, support and custom projects for developing novel Degraders, see page 8



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?

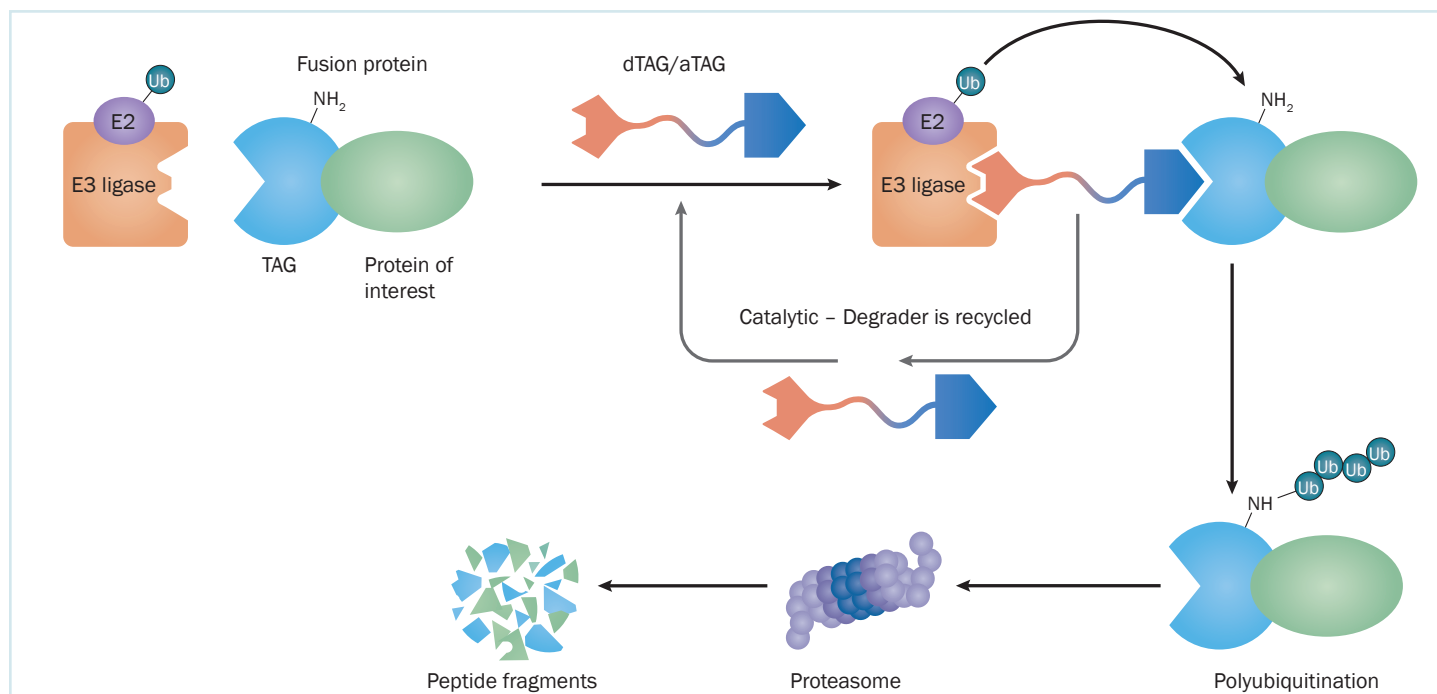


Figure 2: Schematic showing the mode of action of dTAG/aTAG Degraders. A protein of interest is expressed as a fusion with a “TAG” protein. For the dTAG system the protein of interest is tagged with single-point mutant FKBP12 (F36V); the aTAG system uses MTH1 as the TAG. The dTAG/aTAG Degrader, which 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	*	***	*	*	**



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 FKBP12^{F36V} fusions proteins *in vitro* and *in vivo*
- Corresponding plasmids are available through Addgene

Sold under license from the Dana-Farber Cancer Institute, USA

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

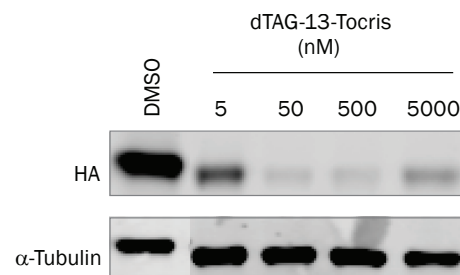


Figure 3: Western blot data showing potent knockdown of a HA-tagged FKBP12^{F36V}-fusion protein after application of dTAG-13

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

Sold under exclusive license from C4 Therapeutics, USA

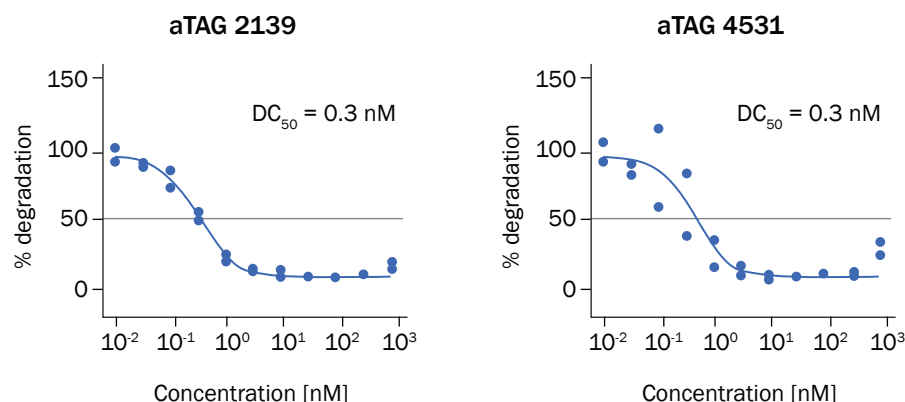
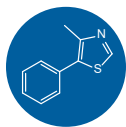


Figure 4: Dose response curves showing degradation of exogenously expressed CAR fused to MTH1 in human Jurkat cells at 4 hours.

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



Bio-Techne, through the Tocris brand, has pioneered the commercialization of Degraders (PROTACs) as research tools, and as a result we have a high level of expertise in the chemical synthesis and purification of this new class of molecule. To support the growing demand for design and synthesis of Degraders for the pharmaceutical industry, we offer a specific custom service in this area.

Our chemistry centre of excellence is based in Bristol, UK, with new state of the art, dedicated synthetic chemistry and analytical chemistry laboratories. We have generated a set of guidelines, based on published Degradar structures, relating to the physicochemical properties necessary to generate cell-permeable Degradar molecules (see Maple *et al.* (2019) MedChemComm. Developing degraders: principles and perspectives on design and chemical space). This information is used to guide customers in the choice of linker length and type, E3 ligase ligand and exit vectors that are likely to be appropriate for their target ligand.

We offer the following services

1. **Custom Chemistry** for the design and chemical synthesis of Degraders against a target selected by the customer using our expertise in PROTAC design and synthesis to inform selection of candidate Degraders
2. **Custom Degradar Building Blocks:** We build panels of Degradar Building Blocks for customers to support specific development projects, or for customers' internal libraries. From mg to g scale, we offer unparalleled quality and customer service.
3. **Biological Testing** of Degraders via automated western blot (Simple Western™) provides the percentage knockdown of the target protein in a chosen cell line, relative to a DMSO-only control. The results can be used to inform further chemical optimization work on the Degradar molecules, either by Bio-Techne or by the customer.
4. **Custom E3 Ligases:** Bio-Techne brand, Boston Biochem offers industry leading experience in the custom production, purification and characterization of UPS proteins, particularly E3 ligase enzymes. For further information on this service, visit: www.rndsystems.com/services/ubiquitin-proteasome-custom-services

What Our Customers Say

"The Tocris team demonstrated an exceptional ability to solve challenging synthetic problems while preparing an array of complex PROTACs for us. Their commitment to quality was commendable, and their efforts led to the delivery of substantial compound quantities with excellent purities in a timely fashion."

Matthew Fyfe
Head of Chemistry and Intellectual Property
Sitryx Therapeutics.

Assays for Targeted Protein Degradation



Simple Western™

Gel-free, blot-free, hands-free Western blots

Simple Westerns™ from ProteinSimple let you separate and analyze proteins by size or charge from 2-440 kDa either by immunoassay or total protein analysis, in just 3 hours.

You'll get quantitative results, reproducibility that's spot on, and use less sample in the process.

Simple Western automates the entire protein separation and detection process so you'll have more time to get down to real science.



Jess

Size assays on up to 25 samples
Chemiluminescence and Fluorescence



Wes

Size assays on up to 25 samples
Chemiluminescence



Sally Sue

Size assays on up to 96 samples
Chemiluminescence



Peggy Sue

Size and charge assays on up to 25 samples
Chemiluminescence

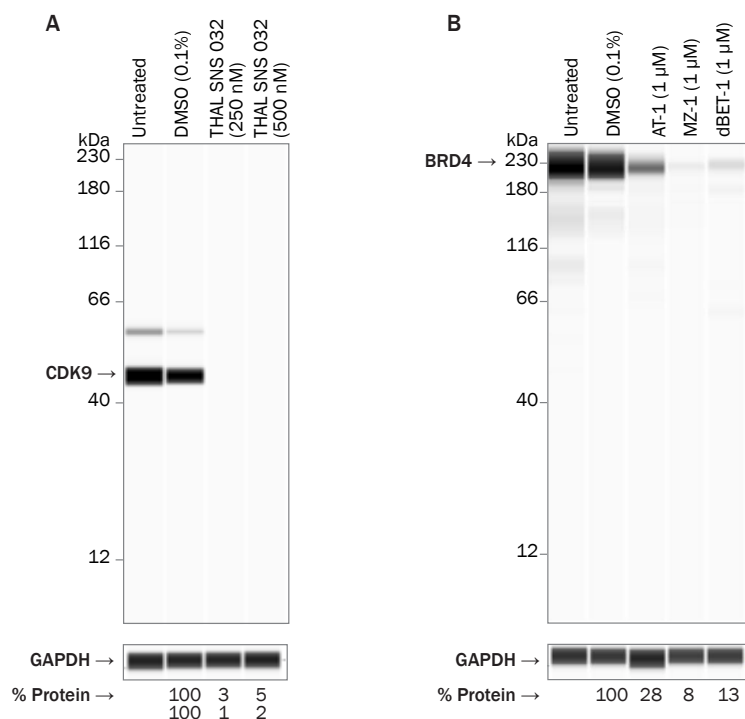


Figure 5: (A) Wes data showing knockdown of both CDK9 isoforms after THAL SNS 032 (Tocris Cat. No. 6532) treatment of MOLT-4 cells (4 h incubation). **(B)** Wes data showing knockdown of BRD4 long isoform after AT-1 (Cat. No. 6356, 1 µM), MZ-1 (Cat. No. 6154, 1 µM) or dBET-1 (Cat. No. 6327, 1 µM) treatment of HeLa cells. Protein quantification (relative to DMSO-only control) is shown beneath the corresponding lane.



In vitro Ubiquitination Assay

Boston Biochem is the leading global provider of Ubiquitin Proteasome System (UPS)-related research products. Superior quality proteins from Boston Biochem enable construction of assays to investigate *in vitro* ubiquitination of a substrate protein. This is a powerful approach to evaluate whether a target protein is ubiquitinated in the presence of a Degradar molecule, in a cell-free system. These assays provide a useful metric for Degradar discovery programs, without complicating factors such as Degradar cellular permeability and efflux.

In the example below, a functional VHL E3 ligase complex (Cat. No. E3-655) was used to investigate poly-ubiquitination of the native substrate, HIF1α. The biotinylated HIF1α peptide substrate was evaluated for polyubiquitination using an anti-Biotin antibody and analyzed by western blot (**Figure 6 below**).

In vitro Ubiquitination Assay for the VHL E3 Ligase

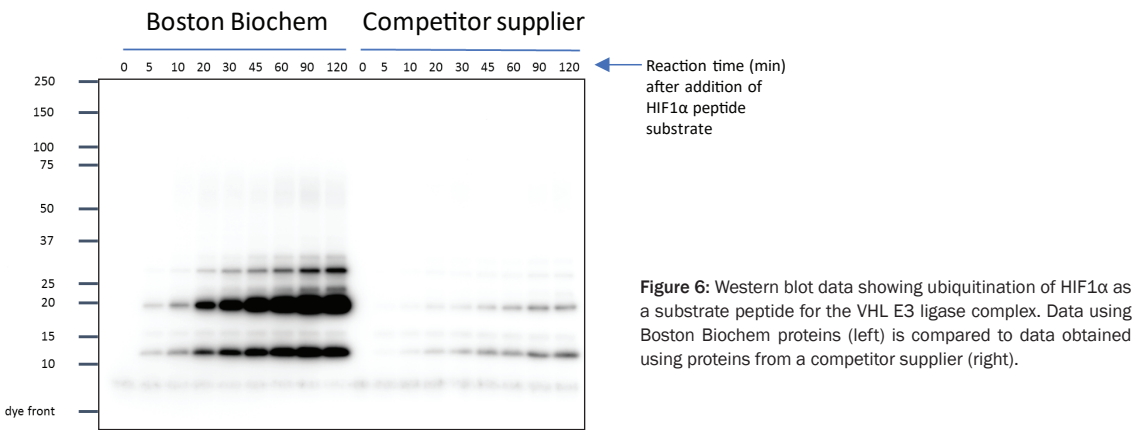
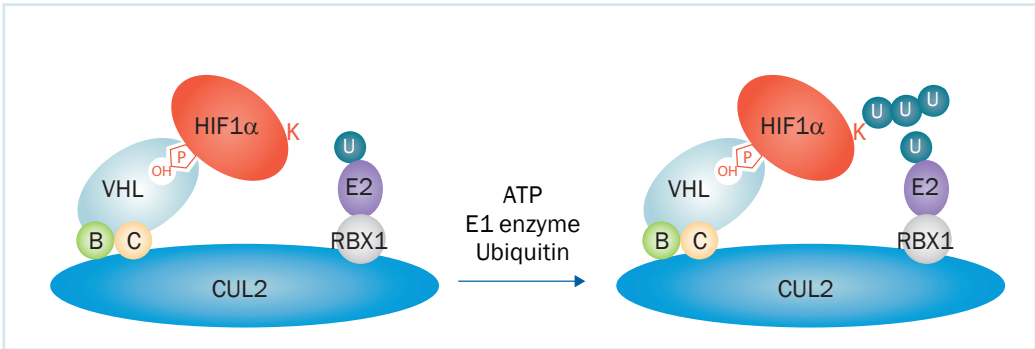


Figure 6: Western blot data showing ubiquitination of HIF1α as a substrate peptide for the VHL E3 ligase complex. Data using Boston Biochem proteins (left) is compared to data obtained using proteins from a competitor supplier (right).



HIF1α: Master transcriptional regulator of hypoxia response. During normoxia, two prolines (P402, P546) in HIF are hydroxylated, leading to its VHL-dependent ubiquitination and proteasome-dependent degradation.

VHL: von Hippel-Lindau disease tumor suppressor. Recognition subunit for Cullin-2 based ligase. Binds a range of substrates with hydroxylated proline residues.

B/C: Elongin B & Elongin C are adapter proteins between VHL and Cullin-2.

CUL2: Cullin-2

RBX1: Adapter protein between Cullin and Ubiquitin-charged E2.

Assay Component	Product Name	Catalog #
E3 ligase complex	Cul2/Rbx1/ELOB/ELOC/VHL	E3-655
E2 enzyme	E2: UBE2D1	E2-616
E1 enzyme	E1: UBE1	E3-306
Ubiquitin	Ubiquitin mutant No K	UM-NOK-01M
ATP	ATP disodium salt	Tocris #3245

For custom E3 ligase enzymes, visit:
www.rndsystems.com/services/ubiquitin-proteasome-custom-services

Ubiquitin Proteasome System Proteins

BostonBiochem
a biotechne brand



The Boston Biochem catalog includes superior quality UPS proteins such as E3 ligase enzymes, as well as offering custom manufacture of proteins not currently available in the range. Boston Biochem products and services are now exclusively available through R&D Systems.

E3 ligase enzymes assemble into multi-subunit complexes using (in the case of the Cullin-RING type of E3 ligase) a repertoire of substrate receptors (e.g. cereblon (CRBN)), adapters (e.g. DDB1), Cullin scaffolds (e.g. CUL4A) and RING-box proteins (e.g. Rbx1).

Product Name	Catalog #
DDB1/DCAF16	E3-502
DDB/DCAF16/CUL4A/RBX1	E3-664
CUL1/RBX1	E3-410
CUL2/RBX1	E3-420
CUL2/RBX1/ELOB/ELOC/VHL	E3-655
CUL4A/RBX1/DDB1/CRBN	E3-650
ELOB/ELOC/VHL	E3-600
RNF4	E3-210
RNF114	E3-304
Skp1/Skp2	E3-521

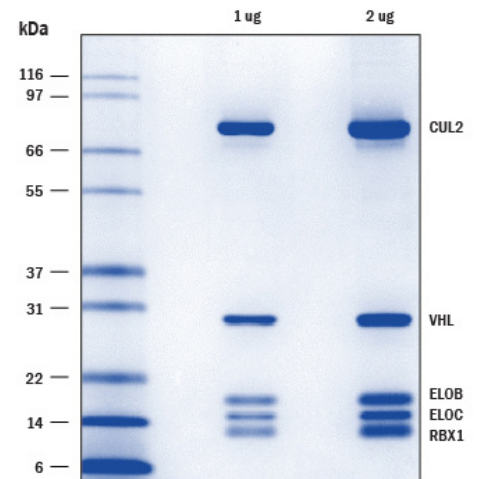


Figure 7: SDS-PAGE gel stained with colloidal Coomassie blue with 1 µg and 2 µg loading of CUL2/RBX1/ELOB/ELOC/VHL (Cat. No. E3-655)

Tandem Ubiquitin Binding Entities (TUBEs)

- Tandem Ubiquitin Binding Entities (TUBEs) have been developed for the isolation and identification of ubiquitinated proteins.
- TUBEs display increased affinity for poly-ubiquitin moieties over the single Ubiquitin Binding Associated domain (UBA).
- TUBEs also display a protective effect on poly-ubiquitinated proteins, allowing for detection at relatively low levels.

Product Name	Catalog #
Recombinant Human Ubiquilin 1 Tandem UBA (TUBE2) Agarose	AM-130-250
Recombinant Human HR23A Tandem UBA (TUBE1) Agarose	AM-125-250
Recombinant Human His6-Ubiquilin 1 Tandem UBA (TUBE2)	UBE-110-250
Recombinant Human Ubiquilin 1 Tandem UBA (TUBE2) Biotin	UBE-115-250
Recombinant Human His6-HR23A/Rad23A Tandem UBA (TUBE1)	UBE-210-250
Recombinant Human HR23A/Rad23A Tandem UBA (TUBE1) Biotin	UBE-215-25
Recombinant Human His6-Fluorescein-Tandem UBA (TUBE1)	UBE-212-100

A selection of the growing range is provided in the table below, check out the website for the full range:
<https://www.rndsystems.com/products/boston-biochem-products-and-services>

WHERE SCIENCE
INTERSECTS INNOVATION™

bio-technne®

bio-technne.com

R&DSYSTEMS

NOVUS
BIOLOGICALS

TOCRIS

proteinsimple

A&D

exosomed_x

Global info@bio-technne.com bio-technne.com/find-us/distributors TEL +1 612 379 2956 North America TEL 800 343 7475
Europe | Middle East | Africa TEL +44 (0)1235 529449 China info.cn@bio-technne.com TEL +86 (21) 52380373

For research use or manufacturing purposes only. Trademarks and registered trademarks are the property of their respective owners.

BR_TPD_MKT20-11320