Enhance PROTAC Drug Discovery with a Comprehensive No-Wash Technology Platform: A BTK Case Study

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1 Introduction

Targeted protein degradation has emerged as a new approach to selectively decrease protein expression levels referred to as PROteolysis TArgeting Chimeras (PROTACs). Unlike conventional small molecules that inhibit protein function, such as kinase inhibitors, PROTACs harness the cell's Ubiquitin Proteasome System (UPS) to destroy undesired proteins. Besides improving potency and efficacy, PROTAC strategy is expected to unlock parts of the proteome that have traditionally been considered "undruggable". PROTACs are heterobifunctional molecules comprising one ligand referred to as the Warhead that selectively binds to the targeted protein, and a second ligand that binds to an E3 ubiquitin ligase (E3), plus a linker that connects the two ligands. PROTACs act as a bridge that brings the target of interest into proximity with an E3 ubiquitin ligase, thereby promoting target ubiquitination and its subsequent proteasomal destruction. Finally, PROTAC molecules can be recycled back and destroy other newly synthesized proteins.

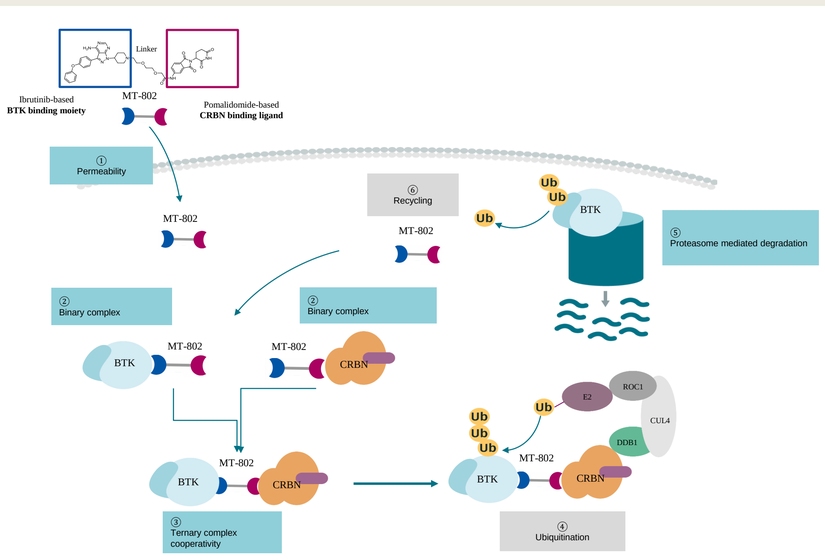
2 Materials

See below for PerkinElmer supplied materials. For additional material and method information please refer to the following Application Note available on our website - Enhance PROTAC Drug Discovery with a Comprehensive No-Wash Technological Platform: A BTK Case Study.

	Reagent	Supplier	Part Number
Microplates	ProxiPlate-384 Plus, White 384-shallow well	PerkinElmer	6008280/9
	AlphaPlate-384, Shallow well light gray	PerkinElmer	6008350/9
Buffer	HTRF PROTAC Binding Buffer 1	PerkinElmer	64BDE31RDF
	Diluent 9 (5X)	PerkinElmer	62DL9DDC
HTRF detection reagents	Dasatinib-Red	PerkinElmer	62KB02REDC
	Thalidomide-Red	PerkinElmer	64BDCRBNRED
	MAb Anti GST-Eu cryptate Kinase Binding	PerkinElmer	62KBGSTKAF
	Streptavidin-Eu cryptate Kinase Binding	PerkinElmer	62KBSAKAF
	MAb Anti GST-Eu Cryptate (PPI)	PerkinElmer	61GSTKLA
	Streptavidin-XL665 (PPI)	PerkinElmer	610SAXLF
Alpha Beads	AlphaLISA Glutathione Acceptor beads	PerkinElmer	#AL109C/M/R
	Alpha Streptavidin Donor beads	PerkinElmer	#6760002/2S/2B
HTRF kits	HTRF® Cereblon binding kit	PerkinElmer	64BDCRBNPEG/H
	HTRF® Total BTK kit	PerkinElmer	63ADK064PEG/H
AlphaLISA kits	AlphaLISA® Cereblon binding kit	PerkinElmer	AL3147C/F
	AlphaLISA® *SureFire Ultra* total BTK kit	PerkinElmer	ALSU-TBTK-A500

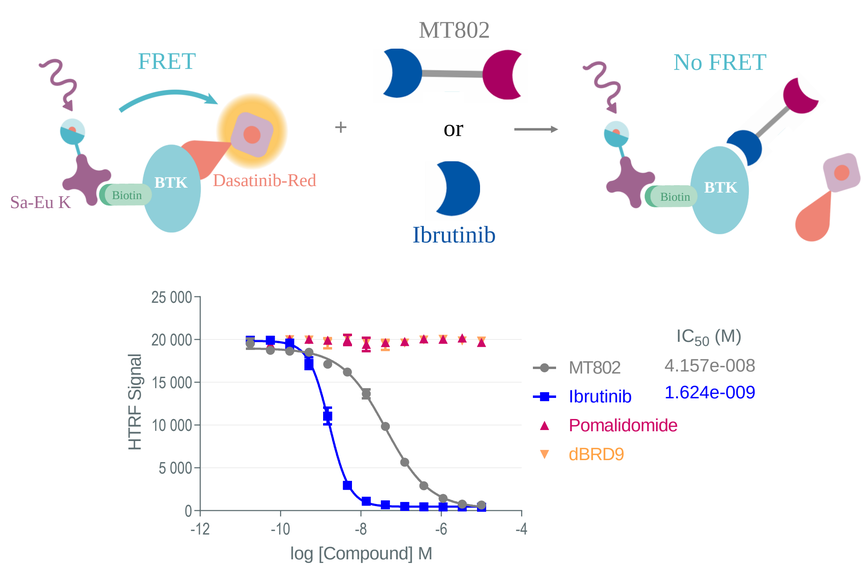
3 Mechanism of Action of PROTAC MT-802

AlphaLISA and HTRF biochemical and cell-based assays have been exemplified on Bruton Tyrosine Kinase (BTK) as the targeted protein of interest and MT-802 as the PROTAC compound. As depicted on the scheme, the formation of the binary (steps ②) and ternary (step ③) complexes were first investigated by biochemical approaches, then cell-based assays were implemented to assess the permeability (step ①) and the induction of BTK proteasomal degradation (step ⑤) of the MT-802 compound.

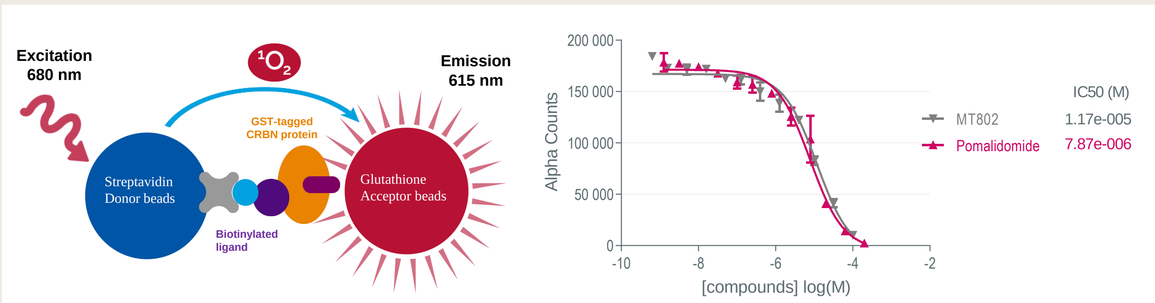


4 Assessment of the Binary Complexes Formation

BTK:MT-802 Complex. A competitive experiment was performed using increasing concentrations of either MT-802 or Ibrutinib, as well as two irrelevant compounds Pomalidomide and dBRD9 (negative controls). In the absence of competitors, a FRET signal occurs between the streptavidin-K and the Dasatinib-Red, while in the presence of competitor, the FRET signal decreases.

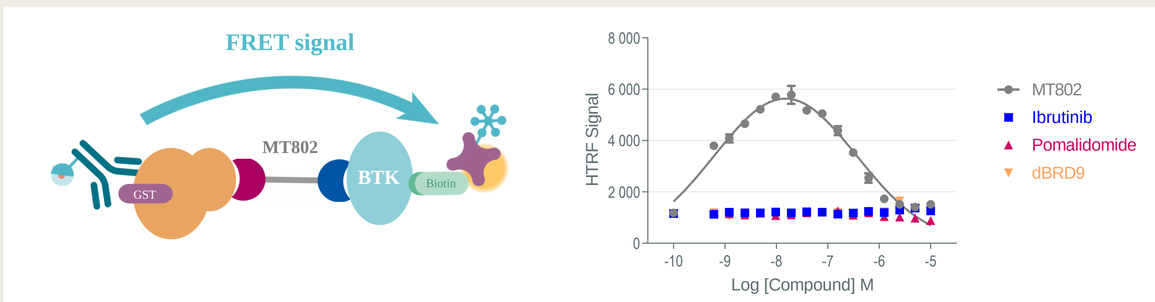


Cereblon:MT-802 Complex. A competitive experiment was performed according to the protocol described (PerkinElmer #AL3147C/F) in the presence of increasing concentrations of MT-802 or Pomalidomide. In the absence of competitors, Alpha signal occurs between the Streptavidin donor beads and Glutathione AlphaLISA acceptor beads, while in the presence of competitor, the signal decreases.



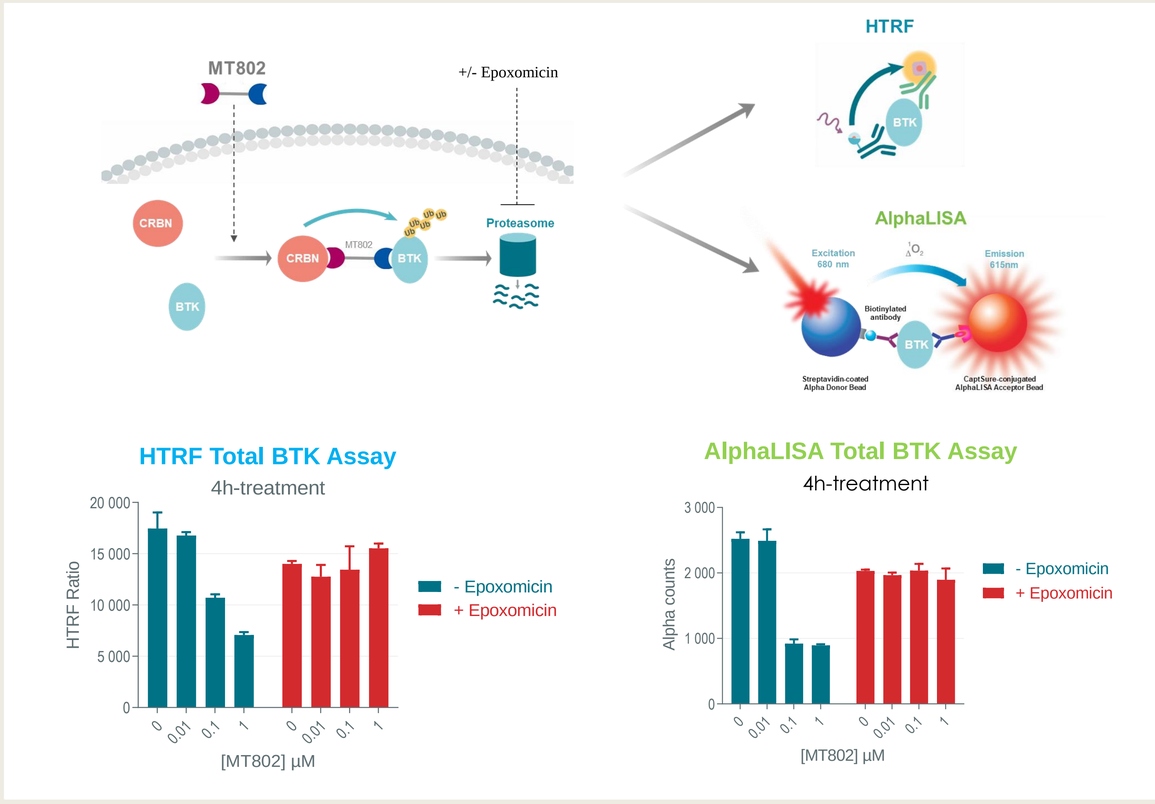
5 Assessment of the Ternary Complex Formation

BTK:MT-802:Cereblon Complex. The experiment was carried out using GST tagged Cereblon protein, Biotinylated BTK protein and increasing concentrations of compounds, followed by a mix of anti GST-Eu cryptate plus Streptavidin-XL665. Increasing concentrations of MT-802 result in an expected bell-shape curve where a hook effect is obtained at higher concentrations. Thus, this result demonstrates the ability of MT-802 alone to induce the formation of the ternary complex selectively, bridging the targeted BTK protein and the E3 ligase Cereblon.



6 Investigations with No-Wash HTRF and AlphaLISA *SureFire Ultra* Cell-based Assays

HTRF and AlphaLISA Biochemical assays demonstrated the efficacy of MT-802 PROTAC compound to 1) bind to BTK, 2) bind to Cereblon, and 3) induce the ternary complex formation. Further investigations were performed to address MT-802 efficacy to induce BTK degradation in a cellular environment. Experiments were carried out using Ramos cell line expressing endogenous BTK and Cereblon proteins. The expression level of BTK was determined either with HTRF total BTK assay or AlphaLISA *SureFire Ultra* total BTK assay after treatment with MT-802 in the presence or absence of the proteasome inhibitor epoxomicin.



7 Summary

- Consistent results are provided with both HTRF and AlphaLISA technologies in biochemical and cell-based context.
- MT-802 PROTAC can be easily monitored in the binary and ternary complex formed with BTK and Cereblon.
- HTRF and AlphaLISA *SureFire Ultra* total kits enable the level of endogenous protein expressed in cells to be monitored as seen in Ramos cell line experiments.
- Epoxomicin (proteasome inhibitor) blocks MT-802 induced degradation of BTK as shown in Ramos cells.
- HTRF and AlphaLISA technologies offer a versatile and straightforward platform ideally suited for PROTAC drug discovery.

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