

Prasad Sulkshane<sup>1</sup>, Romit Bose<sup>1</sup>, Keith Woodley<sup>2</sup>, Alice Willer<sup>2</sup>, Shubham Gourana<sup>1</sup>, Anagha Dahake<sup>1</sup>, Animesh Pandit<sup>1</sup>, Jackline Agwenge<sup>2</sup>, Christian Mutti<sup>2</sup>, Simon Stockwell<sup>2</sup>, Rajesh Yesodharan<sup>1</sup>, Sunil Shah<sup>1,2</sup>, Prashant Shah<sup>1,2</sup> and Gayathri Sadasivam<sup>1,2\*</sup>

<sup>1</sup> *In vitro* Biology, o2h Discovery Pvt. Ltd., Changodar, Ahmedabad, India

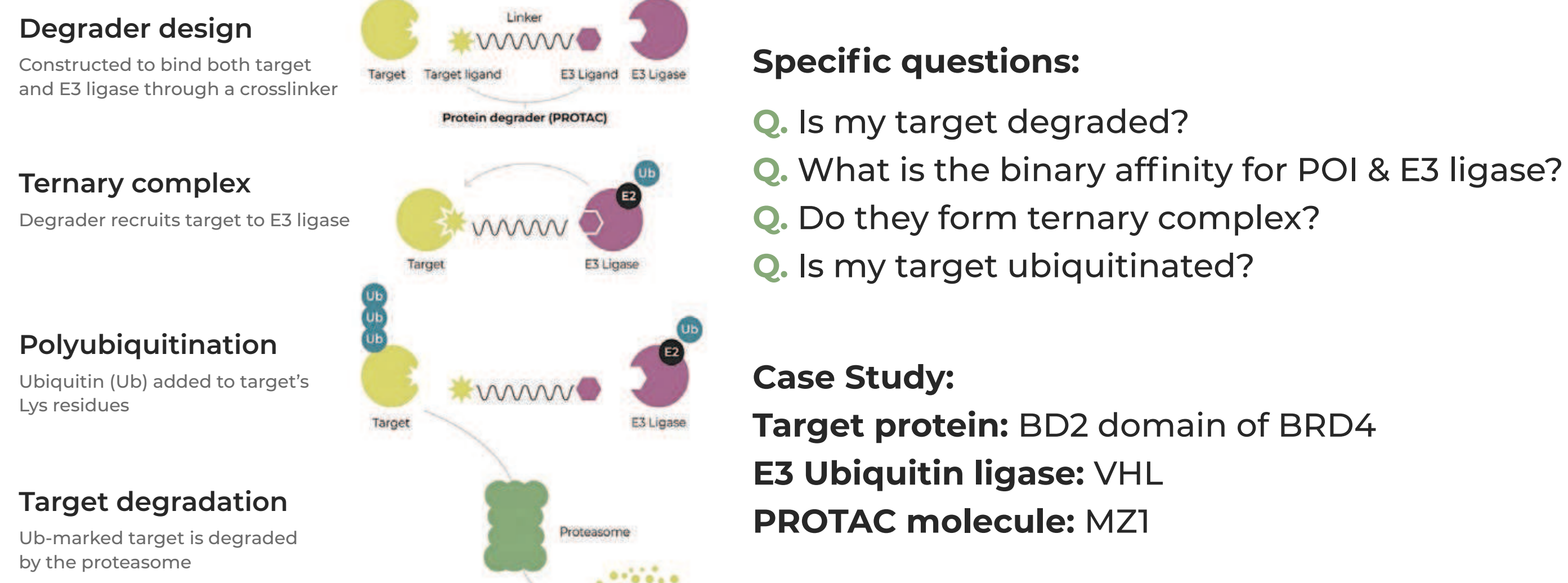
<sup>2</sup> *In vitro* Biology, o2h Discovery Pvt. Ltd., Mill SciTech Park, Hauxton, Cambridgeshire, UK

\*Correspondence to gayathri.sadasivam@o2h.com

## o2h Discovery

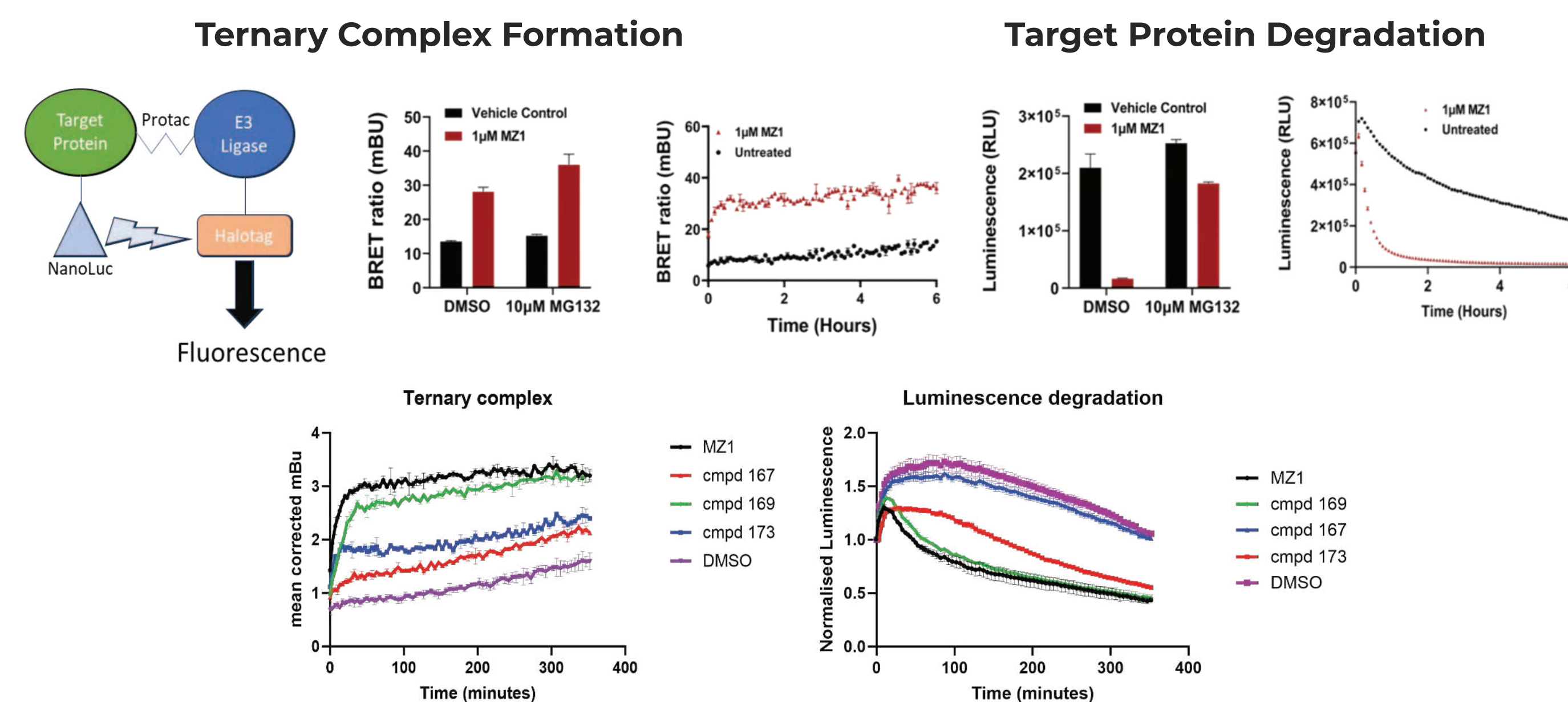
- o2h Discovery provides early-stage drug discovery services from its advanced facilities in Ahmedabad, India, and Cambridge, UK.
- The company has expanded its expertise in targeted protein degradation (TPD), offering a comprehensive suite of solutions for PROTAC design, synthesis, screening, and a customizable "off-the-shelf" PROTAC toolbox. Our experienced biology team provides assays for binary and ternary complex formation, protein degradation, and DC50 estimation, ensuring fully integrated capabilities.
- Leveraging expertise in small molecule discovery and bespoke assay development, o2h enables screening of small molecules that bind to unique RNA folds.
- Drawing on insights from Tong et al. (2023), o2h Discovery is applying these findings to design RNA-targeting molecules. By conjugating RNA-binding small molecules with RNase L-recruiting agents, or RIBOTACs, o2h aims to develop novel RNA degraders using a PROTAC-like approach, enhancing RNA-targeted drug discovery.
- Looking ahead, o2h is also exploring the use of DUBTACs for protein stabilization and other TAC-based mechanisms to clear protein aggregates, broadening its scope in targeted degradation strategies.

## PROTAC (PROteolysis Targeting Chimera)



### Demonstration of ternary complex formation & target protein degradation by Nanoluciferase assay:

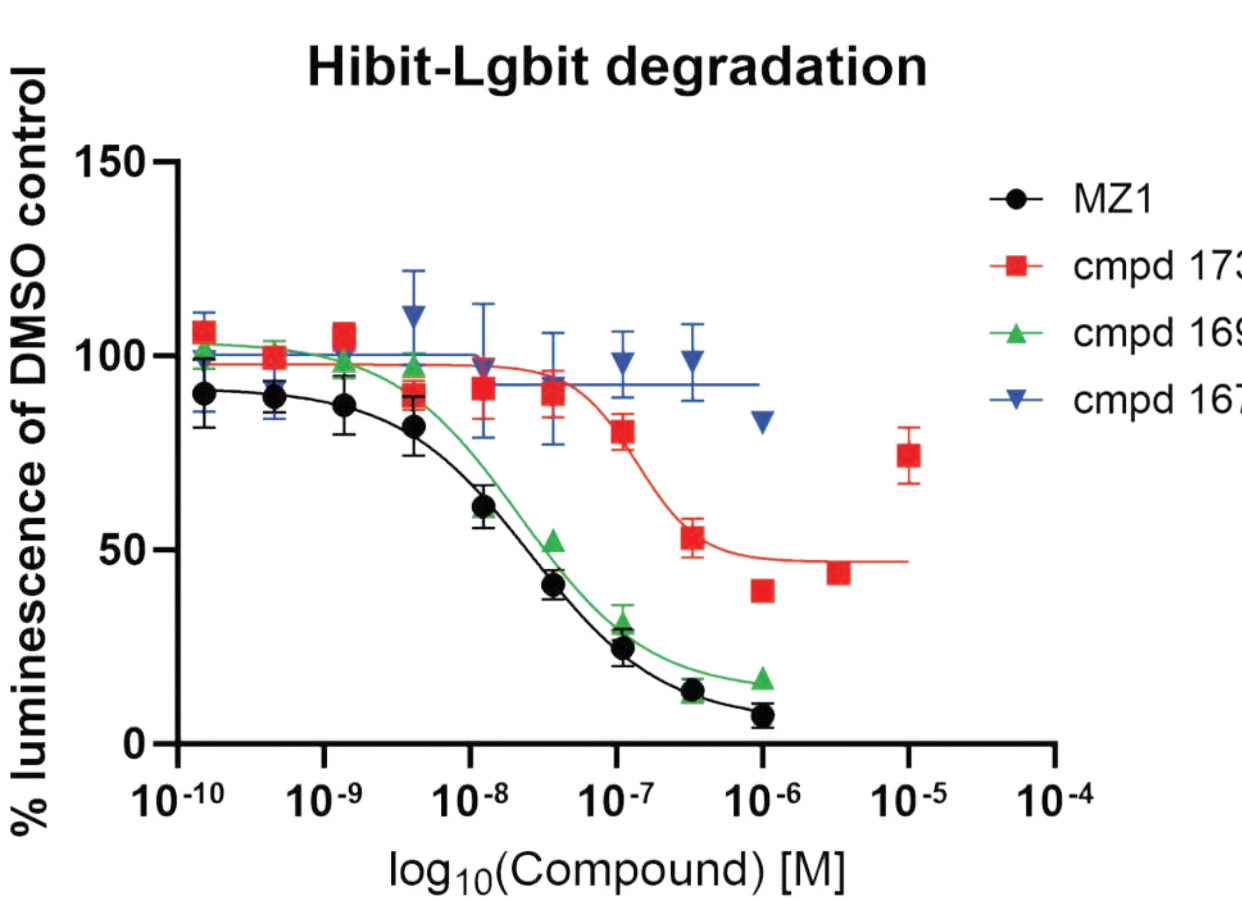
HEK293 cells expressing NanoLuc-BRD4 (Donor) and Halo-Tag-VHL (acceptor) at 1:100 ratio.



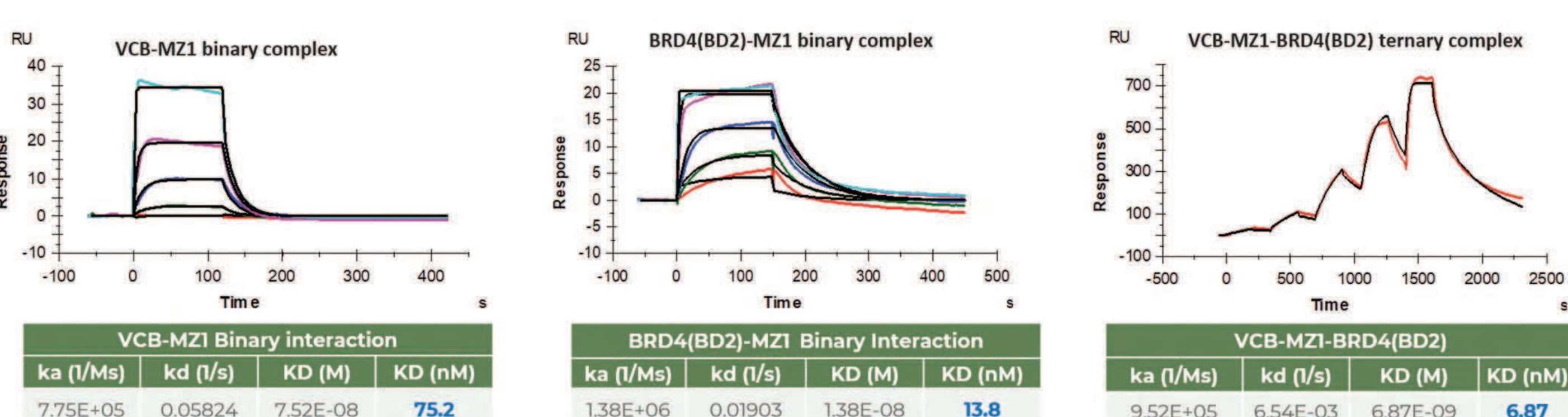
### Demonstration of target protein (BRD4) degradation by split Nanoluciferase assay:

- In HEK293 cells stably expressing LgBit nanoluciferase fragment, we knocked in the corresponding HiBit fragment at the endogenous locus of BRD4.
- The LgBit fragment has a high affinity for the HiBit fragment, thereby reconstituting the active NanoBIT® (Nanoluciferase) enzyme.
- Expression of BRD4 therefore, creates an active nanoluciferase.
- Degradation of BRD4 thus causes a decrease in the Nanoluciferase signal.

#### Assessing BRD4 degradation by HiBit-LgBit assay

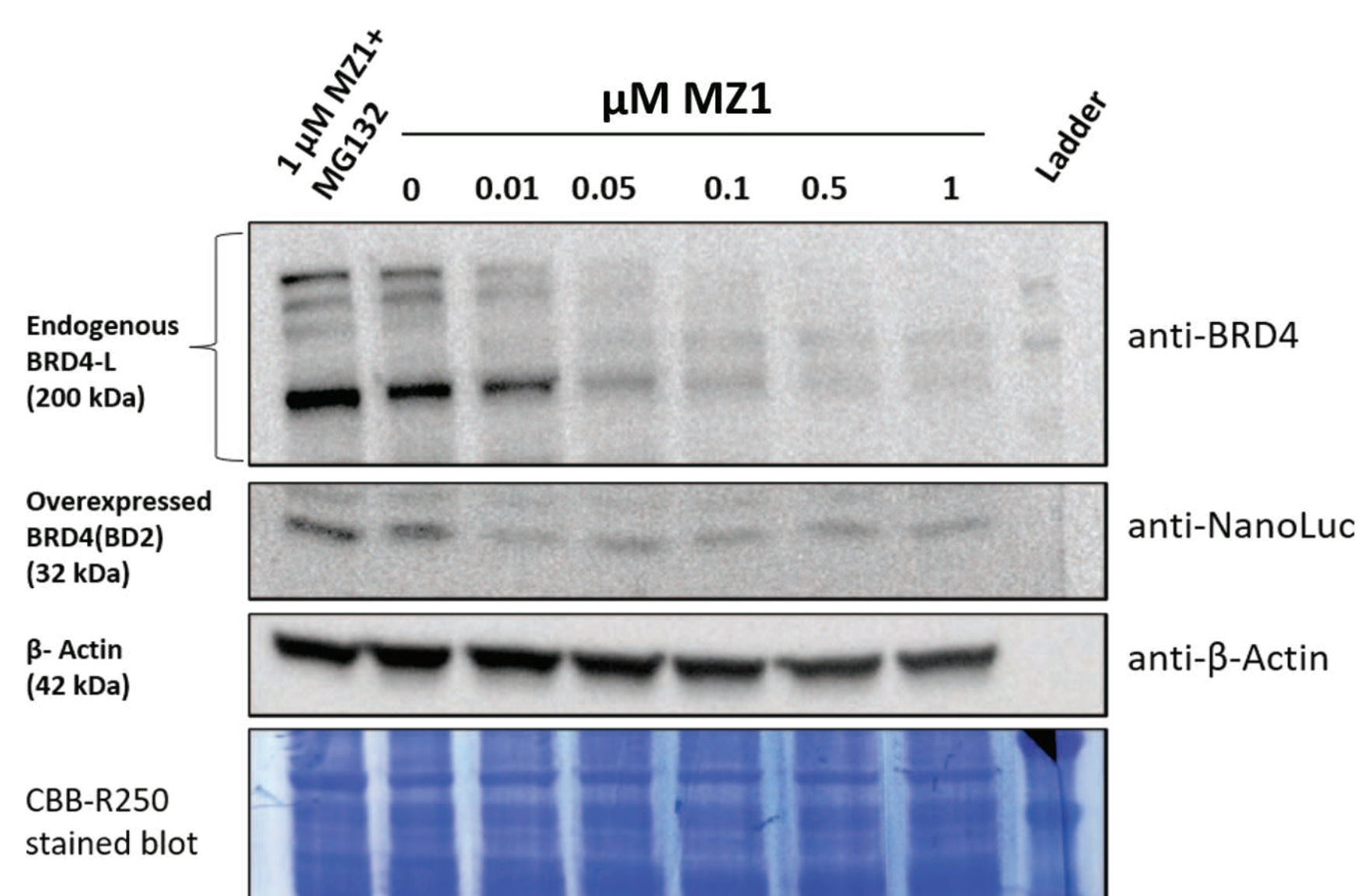


### Demonstration of binary and ternary complex formation by SPR:



### Demonstration of target protein degradation by western blotting:

HeLa cells transfected with the NanoLuc-BRD4(BD2) plasmid and 24 hours later treated with indicated concentrations of MZ1 for 6 hours. The cell lysates were analyzed by western blotting for the indicated proteins.



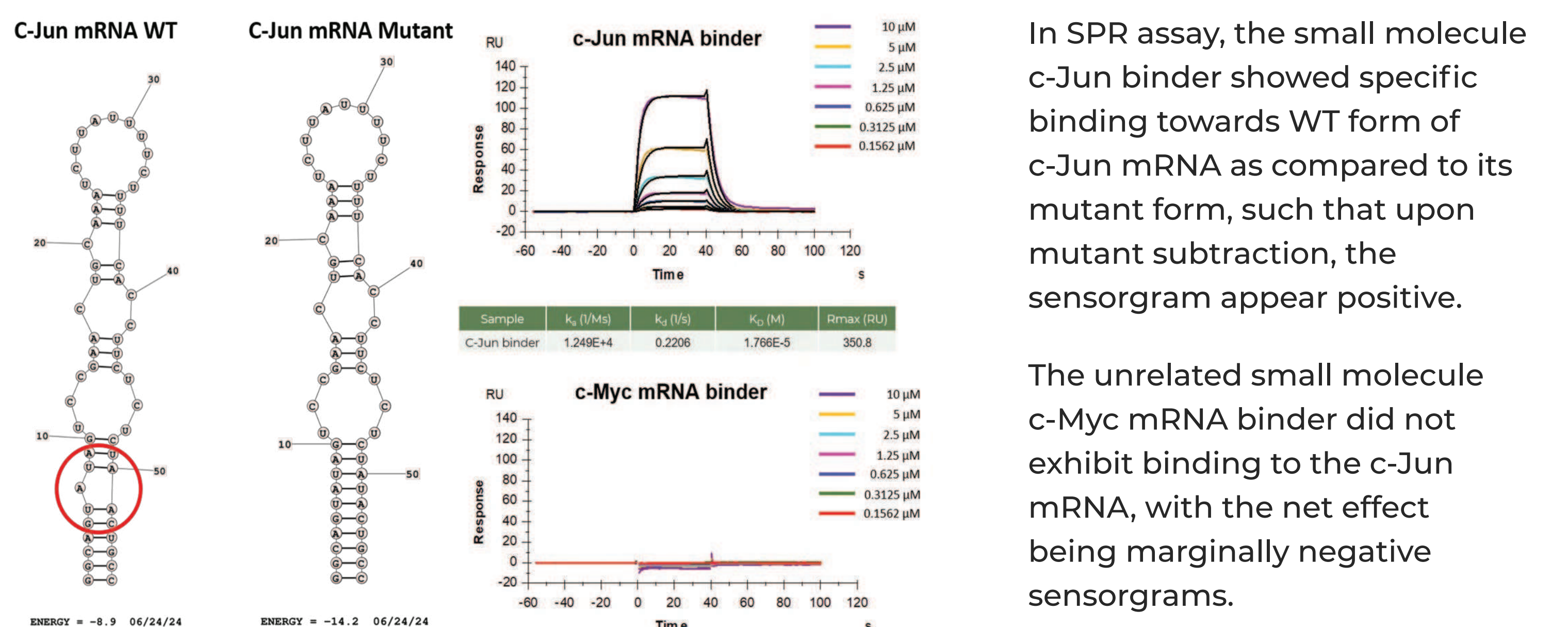
## RiboTAC (RNA-TARGETING Chimera)

- Current RNA-targeting modalities have been limited to antisense oligonucleotides, which mediate Ribonuclease-dependent RNA cleavage.
- However, RNA is remarkably structured and intricately folded as hairpin loops through its intramolecular base pairing, forming a series of unique secondary structures.
- These RNA folds, similar to motifs or domains in proteins, may offer a unique opportunity for targeting by small molecules through specific binding.
- The biological outcome of such RNA binding small molecules can be further enhanced by appending a functional group to it, which can recruit a Ribonuclease, activate it and cleave the target RNA.
- We have developed SPR & Fluorescence Dye Displacement assays as robust and highly sensitive methods to study these specific RNA-small molecule interactions.

### Case Study

- Studying the specific interactions between c-Jun mRNA and a small molecule [Ref. Tong et al. (2023)].
- A specific part of c-Jun mRNA bearing a unique loop/kink – Wild Type (WT), where a small molecule – c-Jun mRNA binder binds.
- Negative control: Mutant c-Jun mRNA, which lacks the unique kink (the binding site).
- Unrelated small molecule: c-Myc mRNA binder.

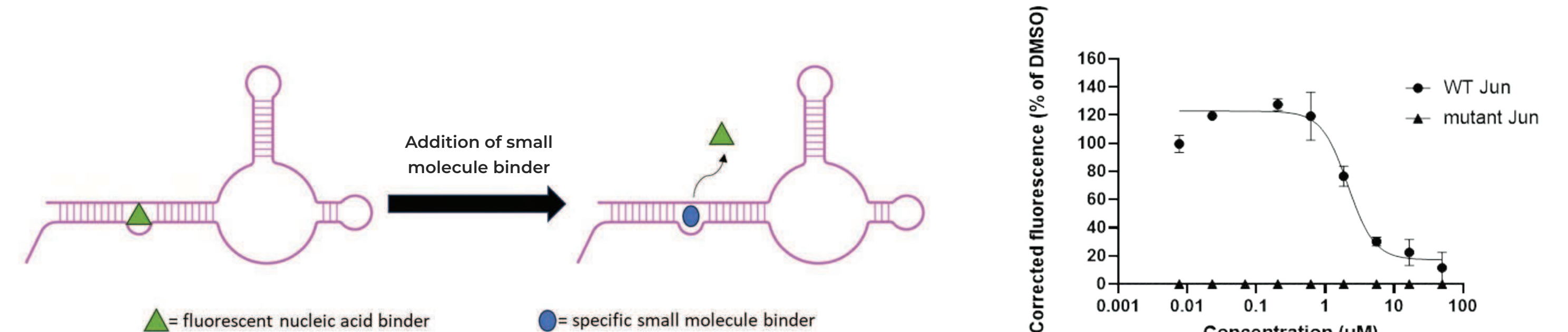
### Demonstration of RNA-small molecule interactions by SPR:



In SPR assay, the small molecule c-Jun binder showed specific binding towards WT form of c-Jun mRNA as compared to its mutant form, such that upon mutant subtraction, the sensorgram appear positive.

The unrelated small molecule c-Myc mRNA binder did not exhibit binding to the c-Jun mRNA, with the net effect being marginally negative sensorgrams.

### Demonstration of RNA-small molecule interactions by Fluorescent dye displacement assay



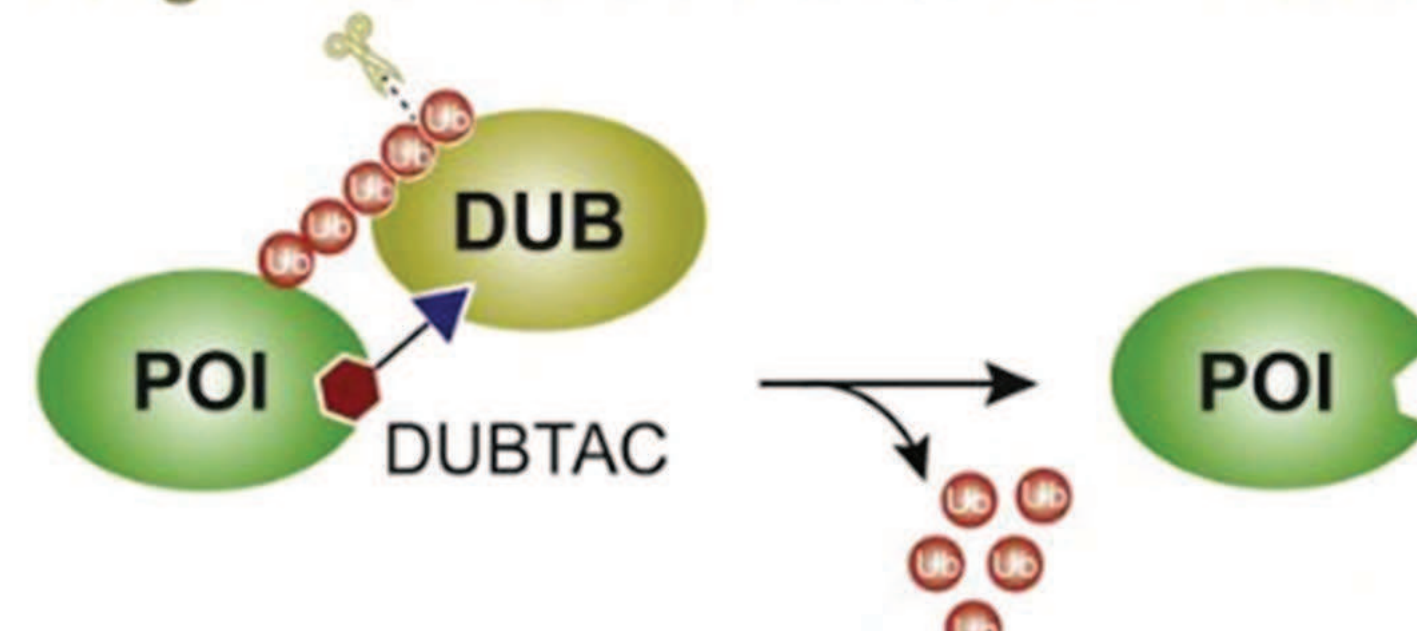
Fluorescence readout showing displacement of the ToPro RNA binder by the c-Jun binder. As the concentration of the c-Jun binder increases, fluorescence decreases. No displacement is observed with the mutant c-Jun mRNA.

Next, we plan to demonstrate the ternary complex of RNA-RiboTAC-RNaseL by SPR and evaluate the RiboTAC efficacy in cellular studies.

## Future Plans

## DUBTAC (Deubiquitinase-Targeting Chimera)

Targeted Protein Stabilization: DUBTAC



## Autophagy-Targeting Chimera

