De novo Semi-synthetic Platform for Monitoring Protein degradation in Live Cells

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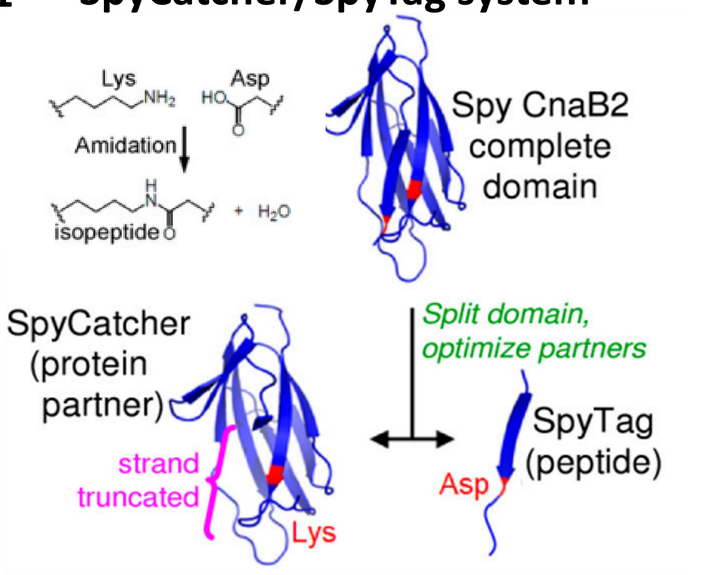
Introduction

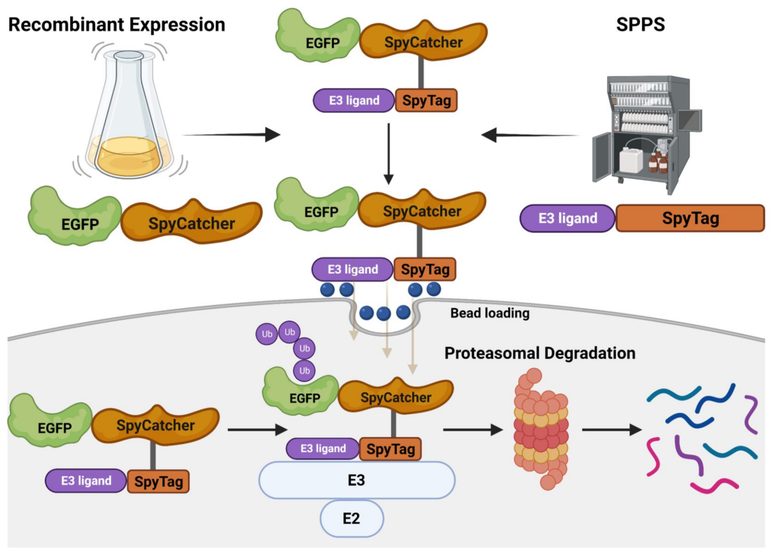
Depletion or inactivation of proteins is a powerful tool for evaluating their involvement in cellular pathways and in medicine. Proteolysis targeting chimera (PROTAC) is promising for both the development of therapeutics and studying protein turnover in biological pathways. The development of PROTAC depends on a known protein of interest (POI) binder and a suitable selection of an E3 recruiter. Although advanced progress has been made for the development of small molecule ligand for several proteins, developing a PROTAC requires significant efforts to optimize such a bifunctional molecule, containing a specific linker. Moreover, for many POIs there is a lack for a specific ligand to be used as part of the PROTAC, and the system requires extensive validation for the biological effect and its phenotype. Engineering degron technologies require a recombinant expression of a degron system, or genetically modifying the DNA to introduce the degrader to the POI. However, these methods are still restricted by the existence of a few ligands for E3 ligases. Inspired by these important approaches we thought to design a novel platform that is more flexible and systematically can be manipulated as well as can be activated on demand. Here we report our first steps towards building such a platform based on SpyTag/SpyCatcher system that allows tagging the POI with E3 ligand in test-tube, followed by cell delivery to monitor its degradation via the UPS.

1 SpyCatcher/SpyTag system



Zakeri, *et al*. 2012, *PNAS.*

Our strategy



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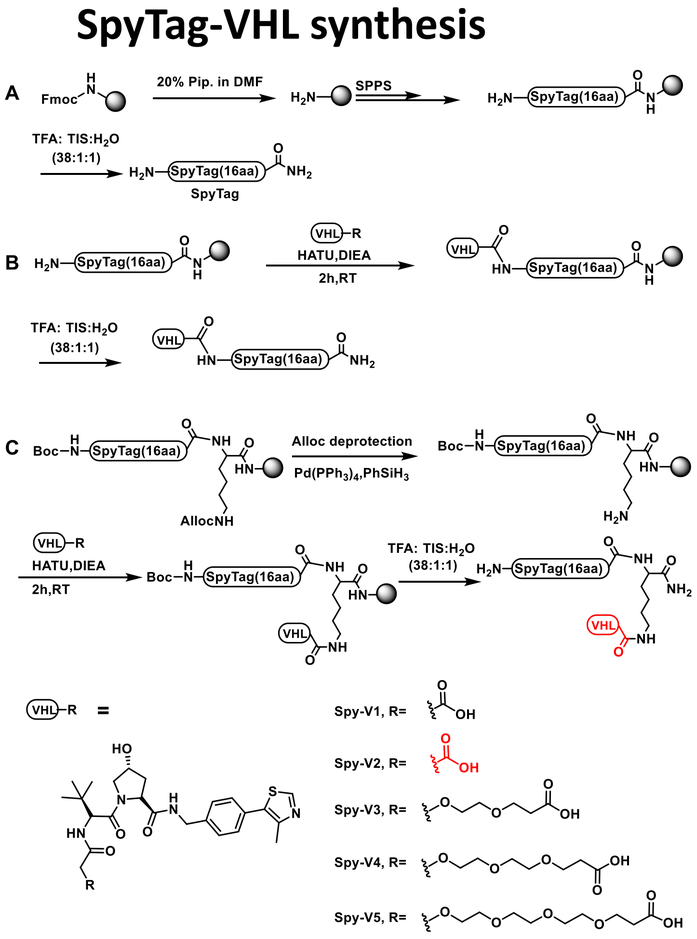
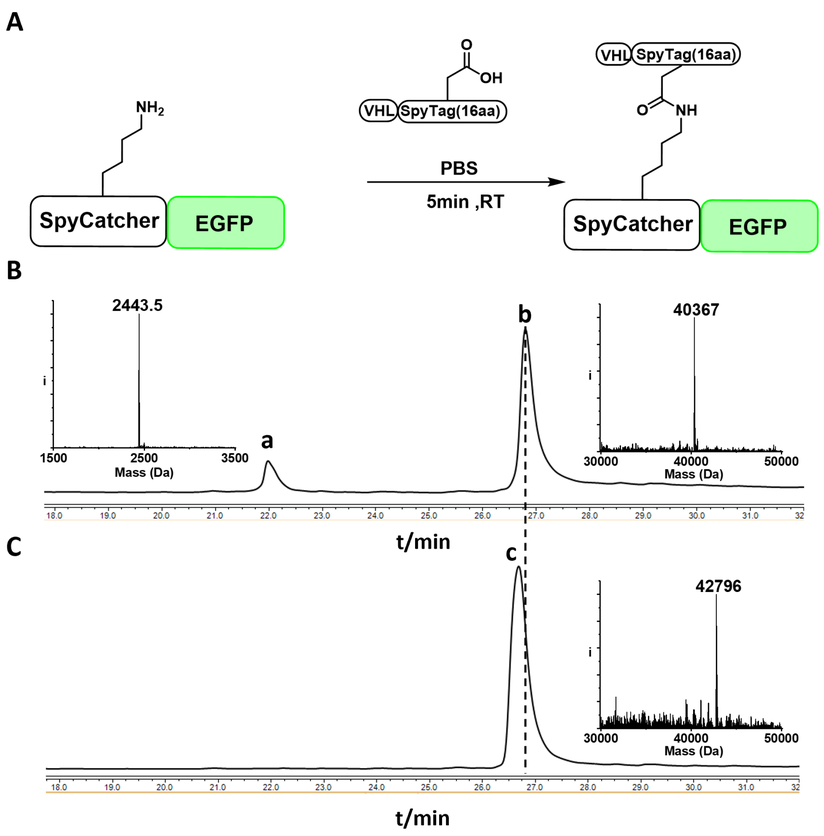
2 SpyTag-VHL synthesis



SpyCatcher/SpyTag reaction



3 Results

SpyCatcher/SpyTag system promote ubiquitination and degradation of EGFP

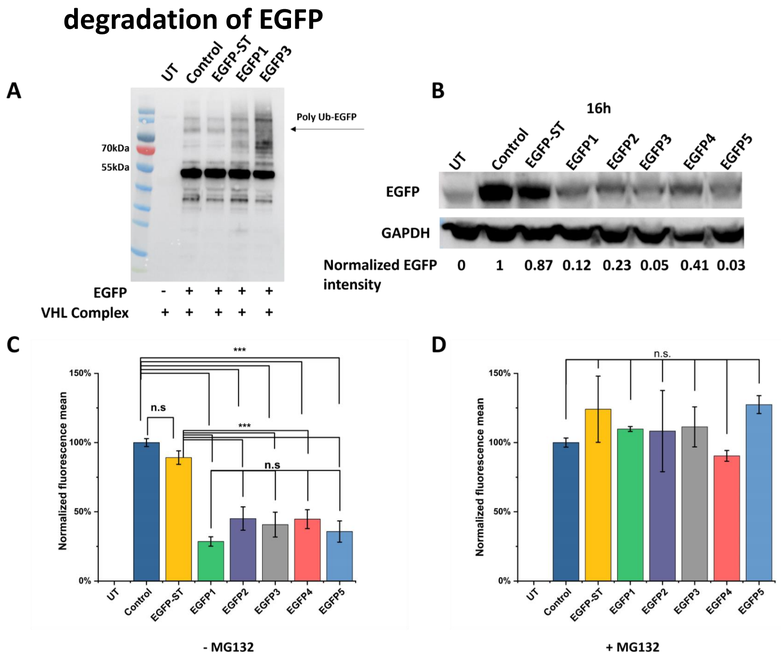


Figure 1.(A) In vitro ubiquitination assay using VHL complex. (B) WB analyses of the degradation of EGFP constructs in U2OS cells. (C) Flow cytometry analyses of the degradation of EGFP constructs, after 16h incubation in U2OS cells. (D) degradation rate of EGFP constructs in U2OS cells in the presence of proteasome inhibitor, MG132

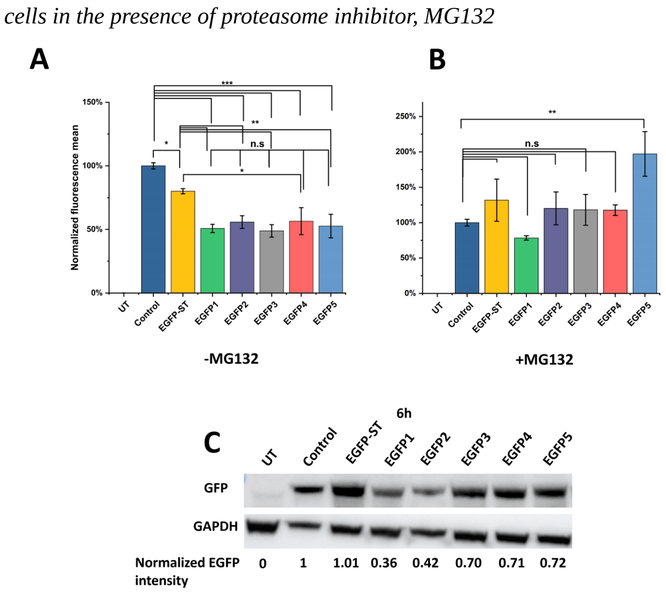
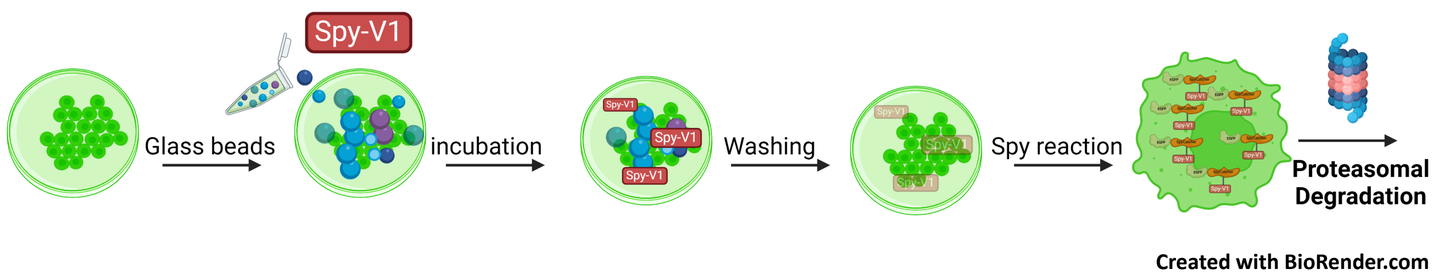


Figure 2.(A) Flow cytometry analysis of the degradation of EGFP constructs, after 6h incubation in U2OS cells. (B) Degradation rate of EGFP constructs in U2OS cells in the presence of proteasome inhibitor, MG132. (C) WB analyses of the degradation of EGFP constructs in U2OS cells

4 SpyTag VHL promote degradation of overexpressed EGFP-SpyCatcher U2OS cells after 4h



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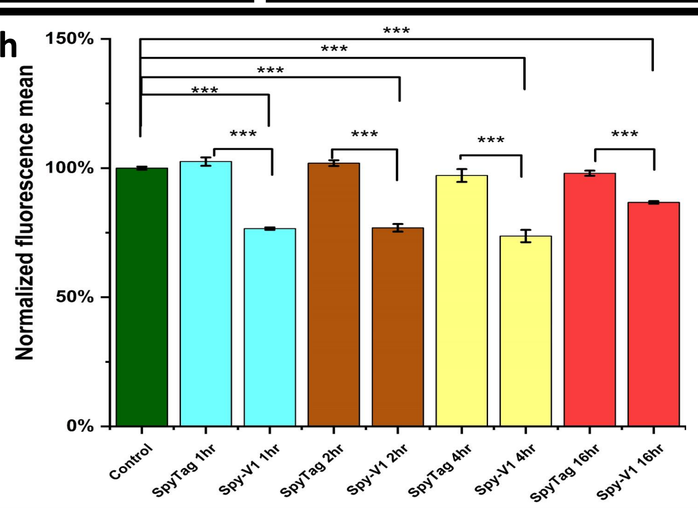

*Figure 3. Flow cytometry analysis of the degradation of overexpressed EGFP-SpyCatcher U2OS cells after 1,2,4 or 16 hours. SpyTag or Spy-V1 were delivered to U2OS via bead loading, the fluorescent intensity was normalized to DMSO treated cells (Control). Data are presented as normalized fluorescent mean ± SEM of two independent biological repeats with ≥10000 cells. The level of significance between the indicated treatments determined as: *p<=0.05, **p<0.01, ***p<0.001.*

Conclusion

- We have developed first generation of novel platform for monitoring the degradation of exogenous semi-synthetic proteins in live cells.
- We employed SpyCatcher/SpyTag system to modify EGFP with VHL ligand and used the bead loading approach to deliver it to live cells and monitor its proteasomal degradation.
- Our platform should allow rapid examination of different E3 ligands, as well as to introduce caged E3 such as the photocleavable VHL and bioorthogonal PROTAC that were recently developed.

Further reading

1- **Mahdi Hasan**, Deepanjan Panda, Guy Mann and Ashraf Brik. "De novo Semi-Synthetic Platform for Monitoring Protein degradation in Live Cells." *ChemBioChem* 25.3 (2024): e202300731.

2- Guy Mann, Pradeep Sadhu, and Ashraf Brik. "Multiplexed Delivery of Synthetic (Un) Conjugatable Ubiquitin and SUMO2 Enables Simultaneous Monitoring of Their Localization and Function in Live Cells." *ChemBioChem* 23.11 (2022): e202200122.

3- Bijan Zakeri, Jacob O Fierer, Emrah Celik, Emily C Chittock, Ulrich Schwarz-Linek, Vincent T Moy, Mark Howarth. "Peptide tag forming a rapid covalent bond to a protein, through engineering a bacterial adhesin." *PNAS* 109(12) (2012), E690-E697.