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Conditional protein depletion using small degradation tags and CRISPR/Cas9 systems

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In the last decade, metabolically engineered microorganisms have been developed for the bio-based production of chemicals, fuels, bioactive compounds, proteins or materials. For these purposes, metabolic engineers need a toolset to accurately control the native and heterologous reactions inside the cell, by manipulating the involved enzymes¹. However, selective post-translational inhibition of enzymatic activity is still challenging since proteins prevail in cells for much longer than their transcription or translation has been inhibited.

Here, we provide a conditional protein knockdown technology that allows the *in vivo* regulation of protein abundance inside the cells. This strategy, termed “Protein interference” (PROTi), is based on the use of small degradation tags (N-degrons)² that can be integrated by CRISPR/Cas9-based genome editing, targeting proteins for degradation. Further, combination of the PROTi technology with gene repression by CRISPRi system³, accelerated cellular depletion of the targeted proteins. This technology provides a valuable tool for balancing cellular pathways at the post-translational level for metabolic engineering purposes, for studying essential proteins or for discovering novel antibiotic targets.

1. Holtz, W.J. and Keasling, J.D. 2010. Engineering static and dynamic control of synthetic pathways. *Cell* 140: 19–23.

2. Erbse, A. *et al.*, 2006. ClpS is an essential component of the N-end rule pathway in *Escherichia coli*. *Nature* 439: 753–756.

3. Qi, L.S. *et al.*, 2013. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell* 152: 1173–1183.