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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 enzymes[1]. 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 system[3], 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.

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