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**Martinez, Virginia; Lauritsen, Ida; Hobel, Tonja; Nørholm, Morten**

*Published in:*

Book of Abstracts. DTU's Sustain Conference 2015

*Publication date:*

2015

*Document Version*

Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

*Citation (APA):*

Martinez, V., Lauritsen, I., Wolff, T., & Nørholm, M. H. H. (2015). Conditional protein depletion using small degradation tags and CRISPR/Cas9 systems. In Book of Abstracts. DTU's Sustain Conference 2015 [B-4] Lyngby: Technical University of Denmark (DTU).

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

Virginia Martínez<sup>1</sup>, Ida Lauritsen<sup>1</sup>, Tonja Wolff<sup>1</sup> and Morten H. H. Nørholm\*<sup>1</sup>

1: DTU Biosustain

\*Corresponding author email: [morno@biosustain.dtu.dk](mailto:morno@biosustain.dtu.dk)

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<sup>1</sup>. 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)<sup>2</sup> 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<sup>3</sup>, 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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