New tricks for an old system – Bacterial system enables site-specific ubiquitylation of proteins in living cells

Reference No: B78098

CHALLENGE
The covalent attachment of ubiquitin (Ub) to target proteins represents one of the most versatile and common post-translational modifications in eukaryotic cells. Ubiquitylation plays a crucial role in many fundamental cellular processes ranging from protein degradation, DNA repair, nuclear transport, endocytosis to chromosomal organization. Importantly, many diseases including different types of cancer and neurodegenerative diseases such as Parkinson’s link to dysfunctions in ubiquitylation. However, how these essential modifications influence biological processes is not fully understood. The process of ubiquitylation is catalyzed by a complex and specialized cascade involving E1/E2/E3 ligases. Similarly, target proteins can also be covalently modified by ubiquitin-like-proteins (Ubls) such as SUMO. As a major obstacle for a detailed in-vitro analysis of ubiquitylation, chemical generation of Ub- and Ubl-conjugates has proven to be difficult and are often limited to easy and refoldable target proteins.

INNOVATION
We present a new method allowing for the site-specific ubiquitylation and SUMOylation of target proteins – both in vitro and in cellulo*. By genetic code expansion, a modified amino acid is incorporated into the target protein serving as the platform for a bacterial enzyme, named "sortase". In a transpeptidation step, the enzyme attaches an ubiquitin or ubiquitin-like molecule to the target protein thereby generating cleavage-resistant and ligase-independent Ubs and Ubls.

![SORTYLATION Diagram](image)

The process of "sortylation" provides a powerful tool to modify non-refoldable and multidomain proteins whilst retaining their physiological integrity and biological function. This allows for a detailed analysis of ubiquitin marks in cellular processes as well as potential involvement in different human diseases.

COMMERCIAL OPPORTUNITIES
• Inducible attachment of ubiquin-modifiers to complex proteins independent of ligase-system
• Potential to site-specifically conjugate other polypeptide to proteins of interest
• Synthesized proteins allow for quantitative characterization of cellular interactions

DEVELOPMENT STATUS
Proof of concept in vitro and in cellulo.

REFERENCES: