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SUMOvera is a truly orthogonal cleavage module – Enabling versatile purification strategies for broad application in eukaryotic hosts

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Background

Classical protein purification strategies rely on recombinant expression of an affinity-tagged protein. After chromatography, the affinity tag is removed by a specific protease to yield the untagged protein. Affinity tags may be small peptides or proteins e.g. small ubiquitin-like modifiers (Ubl). The use of Ubl-fusions has the ultimate advantage, that corresponding proteases exist, which recognize the folded Ubl and cleave directly at the protein-Ubl junction but never within the protein of interest. The cleavage is highly selective and can be performed on column, which renders this process very fast and efficient.
However, because endogenous Ubl-proteases are widely expressed in eukaryotes, the Ubl purification strategy is mostly limited to prokaryotic expression hosts. To date, the only system applicable to eukaryotic hosts is the SUMOstar system from LifeSensors. This system relies on an engineered yeast SUMO protein (designated SUMOstar, SUMO = small Ubl) with its cognate engineered protease (designated SUMOstar protease). Importantly, the SUMOstar protease cleaves endogenous yeast SUMO and SUMOstar fusions with comparable efficiency, thus does not constitute a proper orthogonal system.

Technology

Scientists from the Max-Planck Institute of Biophysical Chemistry in Göttingen set out to evolve proteases and protease-cleavage modules to novel specificities. They developed the SUMOvera cleavage module, which is highly resistant to cleavage by previously described SUMO proteases, including SUMOstar protease, and thus allows the stable expression of SUMOvera-fusion proteins in any eukaryotic host (1). Furthermore, a corresponding SUMOvera protease was engineered, which leaves SUMOstar and wild type SUMO fusion proteins intact, but cleaves SUMOvera fusion proteins efficiently. Extensive testing confirmed that the SUMOvera system is orthogonal to other SUMO/SUMO protease systems.
The SUMOvera system can be used for protein expression, purification and efficient tag-removal as well as for purification of individual proteins and protein complexes by the “affinity capture and proteolytic release” strategy. In combination with the SUMOstar system, the purification of untagged and stoichiometric hetero-dimeric protein complexes is possible. Finally, in contrast to endogenous Ubl-proteases, SUMOvera protease causes no toxicity when over-expressed in a prokaryotic or eukaryotic host, enabling *in-vivo* site-specific proteolysis.

Patent Information: A European priority application has been filed in June 2018.

We are currently seeking licensing partners for this technology.

Reference:

(1) Vera Rodriguez et al., J Cell Biol. 2019, doi: 10.1083/jcb.201812091
(The SUMOvera system is herein referred to as $SUMO^{Eu}$/$SENP1^{Eu}$ pair or $SUMO^{Eu}$ system.)