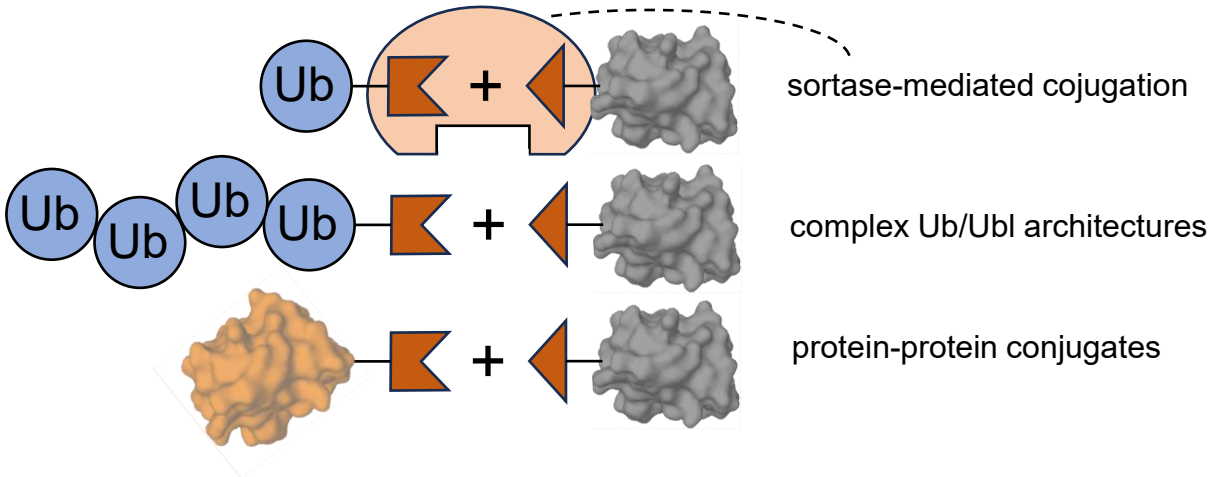
ETH zürich

Licensing Opportunity

Modular toolbox for the study of ubiquitylation pathways *in cellulo*



Application

This platform produces ubiquitin–protein conjugates as tools for biochemical assays and structural studies of the ubiquitin system. The platform also generates site-specific protein–protein conjugates, which are powerful modalities for next-generation biologics and therapeutic candidates.

Features & Benefits

- Site-specific protein-protein conjugation based on genetic code expansion technology
- Enzyme based methodology that works under physiological conditions *in vitro* and *in cellulo*

Publication

- ”Site-specific ubiquitylation and SUMOylation using genetic code expansion and sortase”, Nat. Chem. Biol. 2019, 15, 276-284, https://doi.org/10.1038/s41589-019-0227-4
- ”A modular toolbox to generate complex polymeric ubiquitin architectures using orthogonal sortase enzymes”, Nature Communications (2021) 12:6515, https://doi.org/10.1038/s41467-021-26812-9
- Patent pending, WO/2020/007899

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Technology Readiness Level

1 2 3 4 **5** 6 7 8 9

Background

Many different human diseases, including different types of cancer and neurodegenerative diseases, are linked to the dysfunction of ubiquitylation pathways. The ability to study the effects of ubiquitylation is limited by the difficulty to prepare homogenously modified proteins in vitro and by the impossibility to selectively trigger specific ubiquitylation events in living cells.

Invention

The invention provides a chemoenzymatic method for the site-specific and programmable attachment of Ubiquitin (Ub) and ubiquitin-like (Ubls) to proteins using genetic code expansion and sortase-mediated transpeptidation. A noncanonical lysine derivative is incorporated at a defined position in a target protein and serves as a selective acceptor for sortase-catalyzed transpeptidation with engineered Ub/Ubl variants, resulting in Ub/Ubl-protein conjugates bearing a native isopeptide bond.

The method enables inducible modification under native conditions and in living cells, is applicable to complex and non-refoldable proteins, and can be extended through orthogonal sortase enzymes to generate defined and diverse polyUb chains, including branched and hybrid Ub/Ubl architectures.

The technology is currently in the experimental validation stage. The core methodology has been established and successfully demonstrated for programmable site-specific Ub/Ubl protein conjugation under native conditions, both in vitro and in living cells. Current work in the lab focuses on testing the platform with diverse protein targets, optimizing conjugation chemistries and orthogonal sortase systems, and validating applications including the generation of defined polyubiquitin chains and activity-based probes for studying ubiquitin signalling.