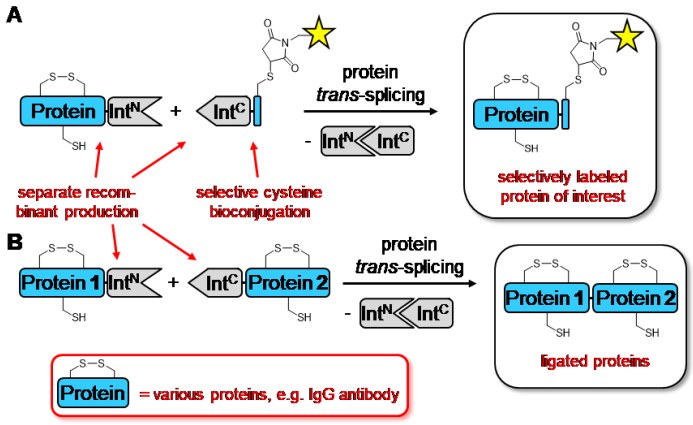
Split Inteins Without Active Site Cysteine

New route to protein-coupling via intein trans-splicing in the absence of reducing agents

Invention

Inteins are protein domains that auto-catalyze protein-splicing of their flanking regions with a peptide bond. In protein trans-splicing two parts of a split-intein can be used to ligate or cyclize polypeptides in a traceless manner. The reaction is robust, can be performed in-vivo or in-vitro, using biologically or chemically synthesized peptides or proteins. The sequence of only 2-3 flanking amino acids are constrained. As shown in the figure below, the N and C terminal intein fragments (IntN & IntC) associate and fold into the active domain thereby linking the flanking sequences with a peptide bond. All previously known split inteins only function in reducing conditions due to their use of one or two catalytic cysteines. In this invention novel cysteine-less split inteins are capable of robust trans-splicing at ambient temperatures and without the requirement of any chemical reducing or denaturation steps. This allows the preservation of disulfide bonds within the target protein. The use of this split intein was demonstrated for a full-length IgG, an Fc fragment of an IgG antibody and two nanobodies as representatives of therapeutically relevant proteins. The reactions are high yielding (> 90%) at low to medium micromolar concentrations.



Commercial Opportunities

The present invention allows for new strategies in the semisynthetic synthesis and site-selective bioconjugation of proteins and in biotechnology. On behalf of University of Muenster and Yeda, PROvendis offers a patent license as well as a research collaboration with licensing option to innovative companies.

Current Status

In case of interest we will be pleased to inform you about the patent status.

Relevant Publications

Bhagawati, M. et al. (2019): A mesophilic cysteine-less split intein for protein trans-splicing applications under oxidizing conditions. Proc. Natl. Acad. Sci. U S A, 116 (44): 22164-22172

An invention of the University of Münster and Weizmann Institute of Science, Israel.

Competitive Advantages

- Performed in absence of reducing agents
- Reactions at ambient temperature; high yield
- Novel access to protein semi synthesis and protein modification without denaturation
- Preservation of disulfide bonds and free cysteines

Technology Readiness Level

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

Technology validated in lab

Industries

- Biotechnology Industry
- Pharmaceutical Industry

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