However, all these approaches have the drawback, that they produce or employ an artificial motive or structure, which may result later on in problems in vivo, like immunogenicity.

Summary

It has been found that the combination of an oligo-alanine as nucleophile with the LPXTG Sortase motif results in suppressed or even completely eliminated back-reaction or hydrolysis of the reaction product as LPXTA is not accepted as substrate.

Thus, as reported herein is a method for producing an enzymatic conjugation product of two polypeptides comprising the following steps

- incubating
  - i) a first polypeptide comprising (within the 20 C-terminal amino acid residues) the amino acid sequence LPXTG (SEQ ID NO: 20, wherein X can be any amino acid residue),
  - ii) a second polypeptide that has an oligo-alanine A_m (m = 2 (SEQ ID NO: 26), or 3 (SEQ ID NO: 27), or 4 (SEQ ID NO: 28), or 5 (SEQ ID NO: 29)) amino acid sequence at its N-terminus,
  - iii) a third polypeptide with sortase activity which is derived from *Staphylococcus aureus* Sortase A, and
- recovering the conjugate from the reaction mixture and thereby producing the enzymatic conjugation product of two polypeptides.

Thus, as reported herein is a method for producing an enzymatic conjugation product of two polypeptides comprising the following steps

- incubating
  - i) a first polypeptide comprising (within the 20 C-terminal amino acid residues) the amino acid sequence LPXTG (SEQ ID NO: 20, wherein X can be any amino acid residue),
  - ii) a second polypeptide that comprises i) an alaninyl compound at its N-terminus, or ii) an oligo-alanine A_m (m = 2 (SEQ ID NO: 26), or 3 (SEQ ID NO: 27), or 4 (SEQ ID NO: 28), or 5 (SEQ ID NO: 29)), or iii) a cysteine amino acid residue followed by one to three alanine amino acid residues at its N-terminus,

iii) a third polypeptide with sortase activity which is derived from *Staphylococcus aureus* Sortase A, and

- recovering the conjugate from the reaction mixture and thereby producing the enzymatic conjugation product of two polypeptides.

In one aspect as reported herein is a method for producing an enzymatic conjugation product of two polypeptides comprising the following steps

- incubating

i) a first polypeptide comprising the amino acid sequence LPXTG (SEQ ID NO: 20, wherein X can be any amino acid residue),

ii) a second polypeptide polypeptide that comprises i) an alaninyl compound at its N-terminus, or ii) an oligo-alanine A_m (m = 2 (SEQ ID NO: 26), or 3 (SEQ ID NO: 27), or 4 (SEQ ID NO: 28), or 5 (SEQ ID NO: 29)), or iii) a cysteine amino acid residue followed by one to three alanine amino acid residues at its N-terminus,

iii) a third polypeptide with sortase activity which is derived from *Staphylococcus aureus* Sortase A, and

- recovering the conjugate from the reaction mixture and thereby producing the enzymatic conjugation product of two polypeptides.

In one embodiment the first polypeptide comprises at its C-terminus the amino acid sequence LPXTG (SEQ ID NO: 20, wherein X can be any amino acid residue).

In one preferred embodiment the first polypeptide comprises at its C-terminus the amino acid sequence LPETG (SEQ ID NO: 30).

In one embodiment the second polypeptide has an oligo-alanine of SEQ ID NO: 26 or SEQ ID NO: 27 at its N-terminus.

In one embodiment the first polypeptide and the second polypeptide are independently of each other selected from an antibody variable domain, an antibody heavy chain Fab-fragment, an antibody Fc-region, a tag, and a peptide comprising the amino acid sequence LPXTG (SEQ ID NO: 20, wherein X can be any amino acid residue), a linker and a non-sortase motif moiety.

In one embodiment the third polypeptide has the amino acid sequence of SEQ ID NO: 21.

As reported herein is the use of the sortase motif amino acid sequence LPXTG (SEQ ID NO: 20, wherein X can be any amino acid residue) in combination with a peptide comprising an oligo-alanine A_m (m = 2 (SEQ ID NO: 26), or 3 (SEQ ID NO: 27), or 4 (SEQ ID NO: 28), or 5 (SEQ ID NO: 29)) amino acid sequence at its N-terminus for increasing the yield of a Sortase catalyzed conjugation reaction between the sortase amino acid sequence and the oligo-alanine comprising peptide.

As reported herein is the use of the Sortase motif amino acid sequence LPXTG (SEQ ID NO: 20, wherein X can be any amino acid residue) in combination with a peptide comprising an oligo-alanine A_m (m = 2 (SEQ ID NO: 26), or 3 (SEQ ID NO: 27), or 4 (SEQ ID NO: 28), or 5 (SEQ ID NO: 29)) amino acid sequence at its N-terminus for reducing by-product formation in a Sortase catalyzed conjugation reaction between the sortase amino acid sequence and the oligo-alanine comprising peptide.

As reported herein is the use of the sortase motif amino acid sequence LPXTG (SEQ ID NO: 20, wherein X can be any amino acid residue) in combination with a peptide comprising an oligo-alanine A_m (m = 2 (SEQ ID NO: 26), or 3 (SEQ ID NO: 27), or 4 (SEQ ID NO: 28), or 5 (SEQ ID NO: 29)) amino acid sequence at its N-terminus for shifting a sortase catalyzed conjugation reaction between the sortase amino acid sequence and the oligo-alanine comprising peptide to the product side.

Detailed Description of the Invention

I. DEFINITIONS

In the present specification and claims the numbering of the amino acid residues in an immunoglobulin heavy chain Fc-region is that of the EU index of Kabat (Kabat, E.A., et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991), NIH Publication 91-3242, expressly incorporated herein by reference).

The term "an alaninyl compound" denotes a compound that comprises an alanine amino acid residue with free alpha amino group, e.g. as NH_2 or NH_3^+, and a carboxy group at position 1 that is in a peptide bond with another moiety, whereby the moiety can be any amino group containing moiety, such as an isolated amino acid residue, a peptide, a polypeptide, a protein, a small molecule, a dye, or a (chemical or peptidic) linker.

The term "comprising" when used herein expressly includes the term "consisting of".

The term "alteration" denotes the mutation, addition, or deletion of one or more amino acid residues in a parent amino acid sequence, e.g. of an antibody or fusion polypeptide comprising at least an FcRn binding portion of an Fc-region, to obtain a variant antibody or fusion polypeptide.

The term "amino acid mutation" denotes a modification in the amino acid sequence of a parent amino acid sequence. Exemplary modifications include amino acid substitutions, insertions, and/or deletions. In one embodiment the amino acid mutation is a substitution. The term "amino acid mutations at the position" denotes the substitution or deletion of the specified residue, or the insertion of at least one amino acid residue adjacent the specified residue. The term "insertion adjacent to a