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 *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.

Patent Claims

1. 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 *Staphylococcus aureus* Sortase A, and
   - recovering the conjugate from the reaction mixture and thereby producing the enzymatic conjugation product of two polypeptides.

2. The method according to claim 1, wherein 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).

3. The method according to claim 2, wherein the first polypeptide comprises at its C-terminus the amino acid sequence LPETG (SEQ ID NO: 30).

4. The method according to any one of claims 1 to 3, wherein the second polypeptide has an oligo-alanine of SEQ ID NO: 26 or SEQ ID NO: 27 at its N-terminus.

5. The method according to any one of claims 1 to 4, wherein 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, a linker and a non-Sortase motif moiety.

6. The method according to any one of claims 1 to 5, wherein the third polypeptide has the amino acid sequence of SEQ ID NO: 21.