and thereby producing a thioester.

One aspect as reported herein is a method for the enzymatic formation of a thioester bond comprising the following step

- incubating

i) a first polypeptide comprising the amino acid sequence LPXTG (SEQ ID NO: 01, wherein X can be any amino acid residue),

ii) a second polypeptide that has an amino acid sequence CGGG (SEQ ID NO: 02) or CAAA (SEQ ID NO: 03) at its N-terminus, and

iii) a third polypeptide that is a sortase A or a catalytically active fragment thereof,

and thereby forming a thioester bond.

In one embodiment the third polypeptide is derived from Staphylococcus aureus sortase A or Listeria monocytogenes Sortase A.

In one embodiment the method is for the enzymatic conjugation of two polypeptides.

In one embodiment the second polypeptide has at its N-terminus a cysteine amino acid residue followed by one to three glycine or alanine amino acid residues. In one preferred embodiment the second polypeptide has the amino acid sequence CGG, CGGG (SEQ ID NO: 02), CAA or CAAA (SEQ ID NO: 03) at its N-terminus.

In one embodiment the incubating is further in the presence of a thiol additive. In one embodiment the thiol additive is selected from the group consisting of thiophenol, 4-mercaptophenylacetic acid (MPAA), 2-mercaptoethanesulfonate (MESNA) and combinations thereof. In one preferred embodiment the incubating is in the presence of 2-mercaptoethanesulfonate.

In one embodiment the first polypeptide comprises at its C-terminus the amino acid sequence LPXTG (SEQ ID NO: 01, wherein X can be any amino acid residue). In one embodiment the first polypeptide comprises at its C-terminus the amino acid sequence LPETG (SEQ ID NO: 04).

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: 01, 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: 05 or SEQ ID NO: 06.

Detailed Description of the Invention

The current invention is based at least in part on the finding that a sortase A accepts as nucleophile a polypeptide that has a cysteine residue at its N-terminus.

Patent Claims

1. A method for the enzymatic formation of a thioester bond comprising the following step
   - incubating
     i) a first polypeptide comprising the amino acid sequence LPXTG (SEQ ID NO: 01, wherein X can be any amino acid residue),
     ii) a second polypeptide that has an amino acid sequence CGGG (SEQ ID NO: 02) or CAAA (SEQ ID NO: 03) at its N-terminus , and
     iii) a third polypeptide that is a sortase A or a catalytically active fragment thereof,

   and thereby forming a thioester bond.

2. The method according to claim 1, wherein the third polypeptide is Staphylococcus aureus sortase A or Listeria monocytogenes Sortase A or a catalytically active fragment thereof.

3. The method according to any one of claims 1 to 2, wherein the method is for the enzymatic conjugation of two polypeptides.

4. The method according to any one of claims 1 to 3, wherein the second polypeptide has at its N-terminus a cysteine amino acid residue followed by one to three glycine or alanine amino acid residues.

5. The method according to any one of claims 1 to 4, wherein the incubating is further in the presence of a thiol additive.

6. The method according to claim 5, wherein the thiol additive is selected from the group consisting of thiophenol, 4-mercaptophenylacetic acid (MPAA), 2-mercaptoethanesulfonate (MESNA) and combinations thereof.

7. The method according to any one of claims 1 to 6, wherein the first polypeptide comprises at its C-terminus the amino acid sequence LPXTG (SEQ ID NO: 01, wherein X can be any amino acid residue).

8. The method according to any one of claims 1 to 7, wherein the first polypeptide comprises at its C-terminus the amino acid sequence LPETG (SEQ ID NO: 04).

9. The method according to any one of claims 1 to 8, 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, and a peptide comprising the amino acid sequence LPXTG (SEQ ID NO: 01, wherein X can be any amino acid residue), a linker and a non-sortase motif moiety.

10. The method according to any one of claims 1 to 9, wherein the third polypeptide has the amino acid sequence of SEQ ID NO: 05 or SEQ ID NO: 06.