01, wherein X can be any amino acid residue) or LPXTA (SEQ ID NO: 41, wherein X can be any amino acid residue),

ii) a second polypeptide comprising i) a glycinyl, an alaninyl, or a cysteinyl compound at its N-terminus (i.e. a compound that comprises a cysteine amino acid residue with free alpha amino group, e.g. as NH_2 or NH_3^+, and a carboxy group, which is part of a peptide bond, at position 1, or 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, which is part of a peptide bond, at position 1, or a compound that comprises a glycine amino acid residue with free alpha amino group, e.g. as NH_2 or NH_3^+, and a carboxy group, which is part of a peptide bond), at position 1, or ii) an oligoglycine, or oligoalanine, or a cysteine amino acid residue followed by one to three glycine or alanine amino acid residues at its N-terminus, or iii) a lysine amino acid residue within its 5 N-terminal amino acid residues, and

iii) a third polypeptide with sortase A activity (i.e. a third polypeptide that is a sortase A or a catalytically active fragment thereof, i.e. that has sortase A activity),

in a deep eutectic solvent

and thereby producing a polypeptide.

One aspect as reported herein is a method for the enzymatic production of a polypeptide 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) or LPXTA (SEQ ID NO: 41, wherein X can be any amino acid residue),

ii) a second polypeptide that has an oligoglycine, or oligoalanine, or a cysteine amino acid residue followed by one to three glycine or alanine amino acid residues at its N-terminus, and

iii) a third polypeptide with sortase A activity wherein the third polypeptide

a) has the amino acid sequence of Staphylococcus aureus sortase A or Listeria monocytogenes Sortase A with 0 to 10 amino acid sequence changes, or

b) is a shortened variant of the amino acid sequence of Staphylococcus aureus sortase A or Listeria monocytogenes Sortase A,

in a deep eutectic solvent which is a mixture of choline chloride with glycerol at a molar ratio of 1:2 comprising up to 5 % (v/v) aqueous co-solvent

and thereby producing a polypeptide.

In one embodiment the third polypeptide with sortase A activity is derived from Staphylococcus aureus sortase A, or from Streptococcus pyogenes Sortase A, or from Listeria monocytogenes Sortase A.

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

In one embodiment the third polypeptide with sortase A activity is derived from Staphylococcus aureus sortase A, or from Streptococcus pyogenes Sortase A, from Listeria monocytogenes Sortase A, or a catalytically active fragment thereof.

In one embodiment the third polypeptide comprises the amino acid sequence of SEQ ID NO: 05, SEQ ID NO: 06, or SEQ ID NO: 38. In one preferred embodiment the third polypeptide comprises the amino acid sequence of SEQ ID NO: 38.

Patent Claims

1. A method for the enzymatic production of a polypeptide 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) or LPXTA (SEQ ID NO: 41, wherein X can be any amino acid residue),

      ii) a second polypeptide that has an oligoglycine, or oligoalanine, or a cysteine amino acid residue followed by one to three glycine or alanine amino acid residues at its N-terminus, and

      iii) a third polypeptide with sortase A activity wherein the third polypeptide

         a) has the amino acid sequence of Staphylococcus aureus sortase A or Listeria monocytogenes Sortase A with 0 to 10 amino acid sequence changes, or

         b) is a shortened variant of the amino acid sequence of Staphylococcus aureus sortase A or Listeria monocytogenes Sortase A,

   in a deep eutectic solvent which is a mixture of choline chloride with glycerol at a molar ratio of 1:2 comprising up to 5 % (v/v) aqueous co-solvent

   and thereby producing a polypeptide.

2. The method according to claim 1, wherein the method is for the enzymatic conjugation of two polypeptides.

3. The method according to any one of claims 1 to 2, wherein the second polypeptide has at its N-terminus the amino acid sequence GGG, AAA, CGG, or CAA.

4. The method according to any one of claims 1 to 3, 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) or LPXTA (SEQ ID NO: 41, wherein X can be any amino acid residue).

5. The method according to any one of claims 1 to 4, wherein the first polypeptide comprises at its C-terminus the amino acid sequence LPETG (SEQ ID NO: 04) or LPETA (SEQ ID NO: 42).

6. The method according to any one of claims 1 to 5, 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) or LPXTA (SEQ ID NO: 41, wherein X can be any amino acid residue), a linker and a non-sortase motif moiety.