CLAIMS

What is claimed is:

1. Peptide ligase having the activity of OaAEP1 Cys247Ala set forth in SEQ ID NO:1 comprising or consisting of:

(a) the amino acid sequence set forth in SEQ ID NO:1;

(b) an amino acid sequence that shares at least 85% sequence identity with the amino acid sequence as set forth in SEQ ID NO:1, provided that said peptide ligase comprises the amino acid sequence set forth in SEQ ID NO:2 at the positions corresponding to residues 247-264 of SEQ ID NO:1, and the amino acid at position 246 is alanine;

(c) a functional fragment of (a) or (b) provided that said peptide ligase comprises the amino acid sequence set forth in SEQ ID NO:2 at the positions corresponding to residues 247-264 of SEQ ID NO:1, and the amino acid at position 246 is alanine, wherein the functional fragment is at least 150 amino acids in length; or

(d) a polypeptide sequence containing the functional fragment of (c),

with the proviso that said peptide ligase is not the wild-type OaAEP1 having the amino acid sequence set forth in SEQ ID NO:3 or butelase 1 having the amino acid sequence set forth in SEQ ID NO:4.

2. The peptide ligase of claim 1, wherein the peptide ligase comprises or consists of an amino acid sequence that shares at least 95% sequence identity with the amino acid sequence as set forth in SEQ ID NO:1, provided that said peptide ligase comprises the amino acid sequence set forth in SEQ ID NO:2 at the positions corresponding to residues 247-264 of SEQ ID NO:1, the amino acid at position 247 is alanine.

3. The peptide ligase of claim 1, wherein the peptide ligase comprises or consists of the amino acid sequence set forth in SEQ ID NO:1.

4. Method of forming a peptide of Formula (I)

$$P^1\text{-Asx-Xaa}^1\text{-Xaa}^2\text{-}P^2 \quad \text{(I)},$$

by ligating a first peptide of Formula (II)

$$P^1\text{-Asx-Xaa}^3\text{-Leu-COOH/CONH}_2 \text{ (II)}$$

to a second peptide of Formula (III)

H_2N-Xaa^1-Xaa^2-P^2 (III),

wherein P^1 and P^2 are each independently any peptide, modified or unmodified, and optionally can combine such that the peptides of formula (II) and (III) are the termini of the same peptide; Asx is Asp or Asn; Xaa^1 is any naturally occurring amino acid; Xaa^2 is any naturally occurring amino acid with the exception of Pro, preferably Leu or Ile; and Xaa^3 is any naturally occurring amino acid, preferably selected from the group consisting of His, Ala, Ser, Cys, Asn, Gly, Arg, Met, Lys, Gln, Leu, and Glu,

by enzymatically cleaving the bond between "Asx" and "Xaa^3" in the first peptide of Formula (II) and ligating the fragment P^1-Asx of the first peptide via its C-terminus to the N-terminus of the second peptide of Formula (III) to form a ligated peptide of Formula (I), wherein the enzymatic cleavage and ligation reaction is catalyzed by a peptide ligase according to claim 1 under conditions suitable for said cleavage and ligation reaction.

5. Method of preparing a dimer, oligomer, or multimer of one or more peptides of interest, the method comprising the steps of:

(a) providing one or more peptides of interest having C-terminal Asx-Xaa^3-Leu-$COOH/CONH_2$ residues and a scaffold molecule having two or more copies of N-terminal H_2N-Xaa^1-Xaa^2 residues or, alternatively, providing one or more peptides of interest having N-terminal H_2N-Xaa^1-Xaa^2 residues and a scaffold molecule having two or more copies of C-terminal Asx-Xaa^3-Leu-$COOH/CONH_2$ residues, wherein Asx is Asp or Asn; Xaa^1 is any naturally occurring amino acid; Xaa^2 is any naturally occurring amino acid with the exception of Pro, preferably Leu or Ile; and Xaa^3 is any naturally occurring amino acid, preferably selected from the group consisting of His, Ala, Ser, Cys, Asn, Gly, Arg, Met, Lys, Gln, Leu, and Glu;

(b) providing a peptide ligase according to claim 1;

(c) preparing a mixture of the one or more peptides of interest, the scaffold molecule, and the peptide ligase;

(d) subjecting the mixture to conditions that allow the peptide ligase to catalyze the ligation of the one or more peptides of interest to the scaffold molecule.

6. Method of modifying or tagging the surface of a target cell by one or more peptides of interest, the method comprising the steps of:

(a) providing the one or more peptides of interest having C-terminal Asx-Xaa^3-Leu-$COOH/CONH_2$ residues and/or having N-terminal H_2N-Xaa^1-Xaa^2 residues, wherein Asx is Asp or Asn; Xaa^1 is any naturally occurring amino acid; Xaa^2 is any naturally occurring amino acid with the exception of Pro, preferably Leu or Ile; and Xaa^3 is any naturally occurring amino acid,

preferably selected from the group consisting of His, Ala, Ser, Cys, Asn, Gly, Arg, Met, Lys, Gln, Leu, and Glu;

(b) providing a peptide ligase according to claim 1;

(c) contacting the target cell with the one or more peptides of interest and the peptide ligase;

(d) subjecting the target cell to conditions that allow the peptide ligase to catalyze the ligation of the one or more peptides of interest to a cellular surface protein of the target cell.

7. The method of claim of claim 4, the method of claim 5, or the method of claim 6, wherein:

(a) Asx is Asn or Asp; and/or

(b) Xaa^1 is any naturally occurring amino acids; and/or

(c) Xaa^2 is Leu or Ile; and/or

(d) Xaa^3 is selected from the group consisting of His, Ala, Ser, Cys, Asn, Gly, Arg, Met, Lys, Gln, Leu, and Glu.

8. The method of forming a peptide according to claim 7, the method of preparing a dimer, oligomer, or multimer of one or more peptides of interest according to claim 7, or the method of modifying or tagging the surface of a target cell by one or more peptides of interest according to claim 7, wherein two or more of features (a)-(d) are met, i.e. (a) and (b), (a) and (c), (a) and (d), (b) and (c), (b) and (d), or three or more features are met, i.e. (a), (b) and (c), (a), (b) and (d), (a), (c) and (d), (b), (c) and (d) or, most preferably all four are met.

9. The method of of forming a peptide according to any one of claims 4 and 7-8 , the method of preparing a dimer oligomer, or multimer of one or more peptides of interest according to any one of claims 5 and 7-8, or the method of modifying or tagging the surface of a target cell by one or more peptides of interest according to any one of claims 6 and 7-8, wherein

- Asx is Asn, Xaa^1 is any naturally occurring amino acid, Xaa^2 is Leu, and Xaa^3 is His, Ser, Cys, Gly, or Ala; and/or

- Asx is Asn, Xaa^1 is Arg or Gly, Xaa^2 is Leu, and Xaa^3 is His, Ala, or Gly.

10. The method of forming a peptide according to any one of claims 4 and 7-9, wherein the first and second peptides are termini of the same peptide (i.e. P^1 and P^2 combine to form a single core peptide sequence) such that the method cyclizes said peptide.

11. The method of claim 10, wherein the first and/or second peptide further comprises a labeling moiety, optionally the labeling moiety is an affinity tag, therapeutic agent, detectable label, or scaffold molecule.

12. The method of forming a peptide according to any one of claims 4 and 7-11, wherein,

- the first and/or second peptide is coupled to a solid support material; and/or

- the first and/or second peptides is a cellular surface protein such that the method results in the modification or tagging of the cellular surface protein and in result the cellular surface as such.

13. The method of modifying or tagging the surface of a target cell by one or more peptides of interest according any one of claims 6 and 7-9, wherein

- the method further comprises removing the unligated one or more peptides of interest from the taget cell after step (d); and/or

- the one or more peptides of interest comprise a labeling moiety, wherein optionally the labeling moiety is an affinity tag, therapeutic agent, detectable label, or scaffold molecule; and/or

- the one or more peptides of interest are coupled to a solid support material; and/or

- the target cell recombinantly expresses a surface polypeptide having N-terminal H_2N-Xaa^1-Xaa^2 residues or C-terminal Asx-Xaa^3-Leu-COOH residues for ease of tagging by the one or more peptides of interest.

14. Kit for use in any of the methods of claims 4-13, wherein the kit comprises a peptide ligase according to claim 1.

15. Use of a peptide ligase according to claim 1 for the ligation of two peptides.