

CLAIMS

1. A conjugated immunoglobulin comprising an immunoglobulin, or antigen-binding portion thereof, and a functional agent, wherein

a) the immunoglobulin, or antigen-binding portion thereof, comprises an engineered lysine residue, wherein the engineered lysine residue is a natural amino acid residue which has been mutated to a lysine residue,

wherein the natural amino acid residue which has been mutated to a lysine residue is selected from the group consisting of:

Threonine 135 (T135K), Serine 136 (S136K), Leucine 193 (L193K), Aspartic acid 221 (D221K), Threonine 223 (T223K), Histidine 224 (H224K), Threonine 225 (T225K), Methionine 252 (M252K), Asparagine 297 (N297K), and Proline 445 (P445K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof,

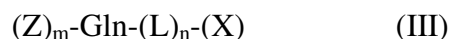
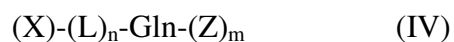
Leucine 201 (L201K) and Serine 202 (S202K) on a kappa light chain of the immunoglobulin, or antigen-binding portion thereof, and

Glutamic acid 213 (E213K) on a lambda light chain of the immunoglobulin, or antigen-binding portion thereof;

wherein the amino acid residues are numbered according to the EU numbering system;

b) the engineered lysine residue is conjugated to a glutamine residue on an acyl donor substrate, wherein the acyl donor substrate further comprises a reactive group,

wherein the acyl donor substrate is according to one Formulae (III) or (IV):



wherein

Z is a carboxylbenzyloxy (CBZ) group or an amino acid residue;

Gln is a glutamine amino acid residue;

each L is independently a straight or branched linker from 1 to 20 carbon atoms, wherein one or more of the carbon atoms may be optionally and independently replaced with a nitrogen, oxygen or sulfur atom, and wherein each carbon and nitrogen atom may be optionally substituted; or each L is optionally and independently an amino acid residue;

m is an integer from 0 to 5;

n is an integer from 0 to 5; and

X is a reactive group;

c) the reactive group is conjugated to a functional agent, wherein the functional agent is a therapeutic agent or a diagnostic agent.

2. A pharmaceutical composition comprising the conjugated immunoglobulin of claim 1, and a pharmaceutically acceptable carrier.

3. The conjugated immunoglobulin of claim 1, wherein the heavy chain further comprises an amino acid residue which has been added to its C-terminus at position 448, and wherein said amino acid residue is not proline or an acidic amino acid residue.

4. The conjugated immunoglobulin of claim 3, wherein the at least one amino acid residue which has been added to the C-terminus at position 448 is leucine.

5. The conjugated immunoglobulin of claim 1, wherein the immunoglobulin, or antigen-binding portion thereof, further comprises a second engineered lysine residue, wherein the second engineered lysine residue is a second lysine residue insertion or a second natural amino acid residue which has been mutated to a lysine residue, and wherein the second engineered lysine residue is conjugated to the glutamine residue of the acyl donor substrate.

6. The conjugated immunoglobulin of claim 5,

wherein the natural amino acid residue which has been mutated to the engineered lysine residue is Threonine 135 (T135K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof, and the second natural amino acid residue which has been mutated to the second engineered lysine residue is Leusine 201 (L201K) on a kappa light chain of the immunoglobulin, or antigen-binding portion thereof; or

wherein the natural amino acid residue which has been mutated to the engineered lysine residue is Threonine 135 (T135K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof, and the second natural amino acid residue which has been mutated to the

second engineered lysine residue is Serine 202 (S202K) on a kappa light chain of the immunoglobulin, or antigen-binding portion thereof;

wherein the amino acid residues are numbered according to the EU numbering system.

7. The conjugated immunoglobulin of claim 6, wherein the heavy chain further comprises at least one amino acid residue which has been added to its C-terminus at position 448, and wherein the at least one amino acid residue is not proline or an acidic amino acid residue.

8. The conjugated immunoglobulin of claim 7, wherein the at least one amino acid residue which has been added to the C-terminus at position 448 is leucine.

9. The conjugated immunoglobulin of claim 5, wherein the immunoglobulin, or antigen-binding portion thereof, further comprises a third engineered lysine residue, wherein the third engineered lysine residue is a third lysine residue insertion or a third natural amino acid residue which has been mutated to a lysine residue, and wherein the third engineered lysine residue is conjugated to the glutamine residue of the acyl donor substrate.

10. The conjugated immunoglobulin of claim 9, wherein the immunoglobulin, or antigen-binding portion thereof, further comprises a fourth engineered lysine residue, wherein the fourth engineered lysine residue is a fourth lysine residue insertion or a fourth natural amino acid residue which has been mutated to a lysine residue, and wherein the fourth engineered lysine residue is conjugated to the glutamine residue of the acyl donor substrate.

11. The conjugated immunoglobulin of claim 1, wherein the immunoglobulin, or antigen-binding portion thereof, comprises a heavy chain which further comprises at least one amino acid residue which has been added to its C-terminus at position 448, and wherein the at least one amino acid residue is not proline or an acidic amino acid residue.

12. The conjugated immunoglobulin of claim 11, wherein the amino acid residue which has been added to the C-terminus at position 448 is leucine.

13. The conjugated immunoglobulin of claim 1, wherein the immunoglobulin, or antigen-binding portion thereof, comprises a light chain which comprises an insertion of one to four additional amino acids after cysteine 214, wherein the lysine residue insertion is a lysine residue which has been inserted after the one to four additional amino acids, and wherein a leucine residue has been inserted after the lysine residue.

14. The conjugated immunoglobulin of claim 13, wherein the insertion of one to four additional amino acids after cysteine 214, the lysine residue which has been inserted after the one to four additional amino acids, and the leucine residue which has been inserted after the lysine residue comprise a sequence selected from the group consisting of: GKL, GGKL, GGSKL, and GGSGKL.

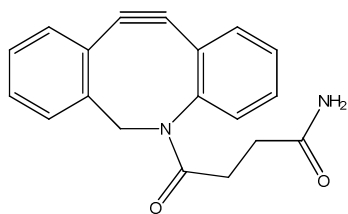
15. The conjugated immunoglobulin of claim 1, wherein the acyl donor substrate is according to formula (III), and wherein Z is a CBZ group; wherein each L is independently a polyethylene glycol moiety (PEG) (-O((CH₂)₂)-), ethyl amine (-NH((CH₂)₂)-) or propyl amine (-NH((CH₂)₃)-); and wherein n is 0, 1, 2, 3, 4, or 5; or

wherein the acyl donor substrate is according to formula (III), wherein Z is a CBZ group, and wherein one or more L is an amino acid; or

wherein the acyl donor substrate is according to formula (IV), wherein Z is a CBZ group; m is 1; n is 1, 2 or 3; and at least one L is Gly.

16. The conjugated immunoglobulin of claim 15,
 wherein L is Gly; m is 1; and n is 1; or
 wherein m is 1; and n is 0.

17. The conjugated immunoglobulin of claim 1, wherein X is a reactive group selected from the group consisting of (1R,8S,9s)-bicyclo[6.1.0]non-4-yn-9-ylmethanol (BCN),



(dibenzocyclooctyne; DBCO), trans-cyclooctene (TCO), azido (N_3),

alkyne, tetrazine methylcyclopropene, norbornene, hydrazide/hydrazine, and aldehyde.

18. The conjugated immunoglobulin of claim 1,
 wherein the therapeutic agent is an antibody or antigen-binding portion thereof, a chemotherapeutic agent, a drug agent, a radioactive agent, a cytotoxic agent, an antibiotic, a small molecule, a nucleic acid, or a polypeptide; or
 wherein the diagnostic agent is a fluorophore, a fluorescent dye, a radionuclide, or an enzyme.
19. The conjugated immunoglobulin of claim 1, wherein the immunoglobulin, or antigen-binding portion thereof
 - (i) is an IgG₁ immunoglobulin, or antigen-binding portion thereof; or
 - (ii) is an IgG₂, IgG₃, or IgG₄ immunoglobulin, or antigen-binding portion thereof; or
 - (iii) is an IgA₁, an IgA₂, or an IgM immunoglobulin, or antigen-binding portion thereof; or
 - (iv) is an IgD or IgE, immunoglobulin, or antigen-binding portion thereof; or
 - (v) is a fragment-antigen binding (Fab); or
 - (vi) is a human immunoglobulin, or antigen-binding portion thereof, or a humanized immunoglobulin, or antigen-binding portion thereof; or
 - (vii) is a chimeric immunoglobulin, or antigen-binding portion thereof, or a non-human immunoglobulin, or antigen-binding portion thereof; or
 - (viii) comprises two heavy chains and two light chains.
20. The conjugated immunoglobulin of claim 1, wherein the ratio of the functional agent to immunoglobulin, or antigen-binding portion thereof, is 1:1 to 100:1 or 1:1 to 200:1.
21. A method for generating a conjugated immunoglobulin, the method comprising:

i) contacting an immunoglobulin, or antigen-binding portion thereof, with a microbial transglutaminase and an acyl donor substrate,

a) wherein the immunoglobulin, or antigen-binding portion thereof, comprises an engineered lysine residue, wherein the engineered lysine residue is a natural amino acid residue which has been mutated to a lysine residue,

wherein the natural amino acid residue which has been mutated to a lysine residue is selected from the group consisting of:

Threonine 135 (T135K), Serine 136 (S136K), Leucine 193 (L193K), Aspartic acid 221 (D221K), Threonine 223 (T223K), Histidine 224 (H224K), Threonine 225 (T225K), Methionine 252 (M252K), Asparagine 297 (N297K), and Proline 445 (P445K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof,

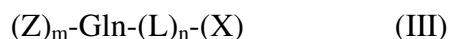
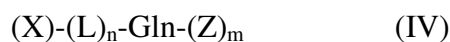
Leucine 201 (L201K) and Serine 202 (S202K) on a kappa light chain of the immunoglobulin, or antigen-binding portion thereof, and

Glutamic acid 213 (E213K) on a lambda light chain of the immunoglobulin, or antigen-binding portion thereof;

wherein the amino acid residues are numbered according to the EU numbering system; and

b) wherein the acyl donor substrate comprises a glutamine residue and a reactive group,

wherein the acyl donor substrate is according to one Formulae (III) or (IV):



wherein

Z is a carboxylbenzyloxy (CBZ) group or an amino acid residue;

Gln is a glutamine amino acid residue;

each L is independently a straight or branched linker from 1 to 20 carbon atoms, wherein one or more of the carbon atoms may be optionally and independently replaced with a nitrogen, oxygen or sulfur atom, and wherein each carbon and nitrogen atom may be optionally substituted; or each L is optionally and independently an amino acid residue;

m is an integer from 0 to 5;

n is an integer from 0 to 5; and

X is a reactive group;

wherein the microbial transglutaminase conjugates the engineered lysine residue of the immunoglobulin, or antigen-binding portion thereof, to the glutamine residue of the acyl donor substrate, and

ii) conjugating a functional agent to the reactive group of the acyl donor substrate, wherein the functional agent is a therapeutic agent or a diagnostic agent, thereby generating the conjugated immunoglobulin.

22. The method of claim 21, wherein the reactive group of the acyl donor substrate is conjugated to the functional agent by click chemistry.

23. The conjugated immunoglobulin of claim 1, wherein the natural amino acid residue which has been mutated to a lysine residue is Threonine 135 (T135K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof.

24. The conjugated immunoglobulin of claim 1, wherein the natural amino acid residue which has been mutated to a lysine residue is Leucine 193 (L193K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof.

25. The conjugated immunoglobulin of claim 1, wherein the natural amino acid residue which has been mutated to a lysine residue is Proline 445 (P445K) on a heavy chain of the immunoglobulin, or antigen-binding portion thereof.