

the ligand-binding molecule is useful for producing a medicament for the treatment of a disease. The present inventors also developed a method for producing the ligand-binding molecule and thus completed the present disclosure.

[0009a]

5 In one aspect, the present disclosure refers to a ligand-binding molecule which is capable of binding to a ligand, wherein the molecule is a polypeptide comprising at least one protease cleavage sequence comprising one or more sequences selected from the sequences shown in SEQ ID NOs: 1003, 701, 1008, 338, 1023, 719, 1131, 1143, 822, 1384, 1132, 1144, 823, 1161-1180 and 1392-1411 or the sequences set forth in Table 1, wherein the binding of the ligand-
10 binding molecule to the ligand in a state where the protease cleavage sequence is cleaved is weaker than the binding of the ligand-binding molecule to the ligand in a state where the protease cleavage sequence is uncleaved. In another aspect, the present disclosure refers to a complex which is formed of the ligand and the ligand-binding molecule as disclosed herein which is bound with the ligand. In another aspect, the present disclosure refers to a fusion protein
15 in which the ligand is fused with the ligand-binding molecule as disclosed herein. In another aspect, the present disclosure refers to a pharmaceutical composition comprising the ligand-binding molecule as disclosed herein, the complex as disclosed herein, or the fusion protein as disclosed herein. In yet another aspect, the present disclosure refers to a method of producing the ligand-binding molecule as disclosed herein, the complex as disclosed herein, or the fusion
20 protein as disclosed herein. In yet another aspect, the present disclosure refers to a protease substrate comprising a sequence selected from the sequences shown in SEQ ID NOs: 1161-1180 and 1392-1411 and the sequences set forth in Table 1. In yet another aspect, the present disclosure refers to a polypeptide comprising one or more sequences selected from the sequences shown in SEQ ID NOs: 1161-1180 and 1392-1411 and the sequences set forth in Table 1.

25 [0010]

The present disclosure is based on the above findings and specifically encompasses exemplary examples described below.

- (1) A ligand-binding molecule, which is a molecule capable of binding to a ligand, wherein the molecule is a polypeptide having at least one cleavage site and the binding of which to the ligand
30 is attenuated in a state where the molecule is cleaved at at least one cleavage site.
- (2) The ligand-binding molecule according to (1), wherein the ligand is released from the ligand-binding molecule in a state where the cleavage site is cleaved.
- (3) The ligand-binding molecule according to (1) or (2), wherein the cleavage site comprises a protease cleavage sequence.
- 35 (4) The ligand-binding molecule according to (3), wherein the protease is a target tissue specific protease.

- (5) The ligand-binding molecule according to (4), wherein the target tissue is a cancer tissue, and the target tissue specific protease is a cancer tissue specific protease.
- (6) The ligand-binding molecule according to (4), wherein the target tissue is an inflammatory tissue, and the target tissue specific protease is an inflammatory tissue specific protease.
- 5 (7) The ligand-binding molecule according to any of (3) to (6), wherein the protease is at least one protease selected from matriptase, urokinase (uPA), and metalloprotease.
- (8) The ligand-binding molecule according to (3), wherein the protease cleavage sequence is a sequence comprising a sequence selected from the sequences represented by SEQ ID NOs: 3, 34, 66, 70, 71, 72, 73, 35, 75, 76, 335 to 345, 1161 to 1180, 1392 to 1411, and the sequences set
10 forth in Table 1.
- (9) The ligand-binding molecule according to any of (3) to (8), wherein a first flexible linker is further attached to one end of the protease cleavage sequence.
- (10) The ligand-binding molecule according to (9), wherein a second flexible linker is further attached to the other end of the protease cleavage sequence.
- 15 (11) The ligand-binding molecule according to (9), wherein the first flexible linker is a flexible linker consisting of a glycine-serine polymer.
- (12) The ligand-binding molecule according to (10), wherein the second flexible linker is a flexible linker consisting of a glycine-serine polymer.
- (13) The ligand-binding molecule according to any of (1) to (12), wherein the ligand-binding
20 molecule comprises antibody VH, antibody VL, and an antibody constant region.

(100) A polypeptide comprising one or more sequences selected from the sequences shown in SEQ ID NOs: 1161-1180, 1392-1411, and the sequences listed in Table 1.

[0011]

The present disclosure can also specifically include examples exemplarily described below.

(B1) A ligand-binding molecule which is capable of binding to a ligand, wherein the molecule is a polypeptide comprising at least one protease cleavage sequence comprising one or more sequences selected from the sequences shown in SEQ ID NOs: 1161-1180 and 1392-1411 and the sequences set forth in Table 1, wherein the binding of the ligand-binding molecule to the ligand in a state where the protease cleavage sequence is cleaved is weaker than the binding of the ligand-binding molecule to the ligand in a state where the protease cleavage sequence is uncleaved.

(B2) The ligand-binding molecule of (B1), wherein the ligand is released from the ligand-binding molecule in a state where the protease cleavage sequence is cleaved.

(B3) The ligand-binding molecule of (B1) or (B2), wherein the protease is a target tissue-specific protease.

(B4) The ligand-binding molecule of (B3), wherein the target tissue is a cancer tissue, and the target tissue-specific protease is a cancer tissue-specific protease.

(B5) The ligand-binding molecule of (B3), wherein the target tissue is an inflammatory tissue, and the target tissue-specific protease is an inflammatory tissue-specific protease.

(B6) The ligand-binding molecule of any one of (B1) to (B5), wherein the protease is at least one protease selected from matriptase, urokinase (uPA), and metalloprotease.

(B7) The ligand-binding molecule of any one of (B1) to (B6), wherein a first flexible linker is further added to one end of the protease cleavage sequence.

(B8) The ligand-binding molecule of (B7), wherein the first flexible linker consists of a glycine-serine polymer.

(B9) The ligand-binding molecule of (B7) or (B8), wherein a second flexible linker is further added to the other end of the protease cleavage sequence.

(B10) The ligand-binding molecule of (B9), wherein the second flexible linker consists of a glycine-serine polymer.

(B11) The ligand-binding molecule of any one of (B1) to (B10), which comprises an antibody VH, an antibody VL, and an antibody constant region.

(B12) The ligand-binding molecule of (B11), wherein the protease cleavage sequence, the protease cleavage sequence and the first flexible linker, or the protease cleavage sequence, the first flexible linker, and the second flexible linker, are located within the antibody constant region.

CLAIMS

1. A ligand-binding molecule which is capable of binding to a ligand, wherein the molecule is a polypeptide comprising at least one protease cleavage sequence comprising one or more
5 sequences selected from the sequences shown in SEQ ID NOs: 1003, 701, 1008, 338, 1023, 719, 1131, 1143, 822, 1384, 1132, 1144, 823, 1161-1180 and 1392-1411 or the sequences set forth in Table 1, wherein the binding of the ligand-binding molecule to the ligand in a state where the protease cleavage sequence is cleaved is weaker than the binding of the ligand-binding molecule to the ligand in a state where the protease cleavage sequence is uncleaved.
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2. The ligand-binding molecule of claim 1, wherein the ligand is released from the ligand-binding molecule in a state where the protease cleavage sequence is cleaved.
3. The ligand-binding molecule of claim 1 or 2, wherein the protease is a cancer tissue-specific
15 protease or an inflammatory tissue-specific protease.
4. The ligand-binding molecule of any one of claims 1 to 3, which comprises an antibody VH, an antibody VL, and an antibody constant region.
- 20 5. The ligand-binding molecule of claim 4, wherein the protease cleavage sequence is located near the boundary between the antibody constant region and the antibody VH, or/and near the boundary between the antibody constant region and the antibody VL.
6. The ligand-binding molecule of claim 4 or 5, wherein the antibody VL and the antibody VH
25 in the ligand-binding molecule are associated with each other, and the association is eliminated by cleavage of the protease cleavage sequence with the protease.
7. The ligand-binding molecule of any one of claims 1 to 6, wherein the ligand is a biologically active molecule, and wherein the ligand-binding molecule inhibits the biological activity of the
30 ligand by binding to the ligand.
8. The ligand-binding molecule of any one of claims 1 to 7, wherein the ligand is a cytokine or a chemokine.
- 35 9. The ligand-binding molecule of any one of claims 1 to 8, wherein the ligand-binding molecule is an IgG antibody.

10. A complex which is formed of the ligand and the ligand-binding molecule of any one of claims 1 to 8 which is bound with the ligand.
- 5 11. A fusion protein in which the ligand is fused with the ligand-binding molecule of any one of claims 1 to 8.
12. A pharmaceutical composition comprising the ligand-binding molecule of any one of claims 1 to 8, the complex of claim 10, or the fusion protein of claim 11.
- 10 13. A method of producing the ligand-binding molecule of any one of claims 1 to 8, the complex of claim 10, or the fusion protein of claim 11.
14. A protease substrate comprising a sequence selected from the sequences shown in SEQ ID
15 NOs: 1161-1180 and 1392-1411 and the sequences set forth in Table 1.
15. A polypeptide comprising one or more sequences selected from the sequences shown in SEQ ID NOs: 1161-1180 and 1392-1411 and the sequences set forth in Table 1.