

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: 335-344, 818-824, 830-836, 1008, 1131, 1132, 1189, 1190, 1192-1194, 1196, 1198, 1201, 1202, 1206, 1209, 1210, 1212, 1215, 1216, 1218, 1219, 1221, 1222, 1226-1229, 10 1231-1233, 1242, 1253, 1254, and 1257, 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 wherein the ligand-binding molecule comprises an antibody VH and an antibody VL, wherein the antibody VL and the antibody VH in the ligand-binding molecule are associated 15 with each other, and the association is eliminated by cleavage of the protease cleavage sequence with the protease. 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 in which the ligand is fused with the ligand-binding molecule as disclosed herein. In another aspect, the present disclosure 20 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 comprising the step of culturing the host cell comprising a polynucleotide encoding the ligand-binding molecule of any one of claims 1 to 8 or a vector comprising the polynucleotide. In yet 25 another aspect, the present disclosure refers to a protease substrate comprising a sequence selected from the sequences shown in SEQ ID NOs: 335-344, 818-824, 830-836, 1008, 1131, 1132, 1189, 1190, 1192-1194, 1196, 1198, 1201, 1202, 1206, 1209, 1210, 1212, 1215, 1216, 1218, 1219, 1221, 1222, 1226-1229, 1231-1233, 1242, 1253, 1254, and 1257. In yet another aspect, the present disclosure refers to a polypeptide comprising one or more sequences selected 30 from the sequences shown in SEQ ID NOs: 335-344, 818-824, 830-836, 1008, 1131, 1132, 1189, 1190, 1192-1194, 1196, 1198, 1201, 1202, 1206, 1209, 1210, 1212, 1215, 1216, 1218, 1219, 1221, 1222, 1226-1229, 1231-1233, 1242, 1253, 1254, and 1257.

[0010]

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

- 5 (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 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.
- 10 (3) The ligand-binding molecule according to (1) or (2), wherein the cleavage site comprises a protease cleavage sequence.
- (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.
- 15 (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.
- (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
20 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 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.
- 25 (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.
- (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
30 flexible linker consisting of a glycine-serine polymer.
- (13) The ligand-binding molecule according to any of (1) to (12), wherein the ligand-binding molecule comprises antibody VH, antibody VL, and an 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 sequences selected from the sequences shown in SEQ ID NOs: 335-344, 818-824, 830-836, 1008, 1131, 1132, 1189, 1190, 1192-1194, 1196, 1198, 1201, 1202, 1206, 1209, 1210, 1212, 1215, 1216, 1218, 1219, 1221, 1222, 1226-1229, 1231-1233, 1242, 1253, 1254, and 1257, 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
wherein the ligand-binding molecule comprises an antibody VH and an antibody VL,
wherein the antibody VL and the antibody VH 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.
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 cleavage sequence is cleaved by a cancer tissue-specific protease or an inflammatory tissue-specific protease.
4. The ligand-binding molecule of any one of claims 1 to 3, which comprises an antibody constant region.
5. The ligand-binding molecule of claim 4, wherein the protease cleavage sequence is located between amino acid position 101 (Kabat numbering) of the antibody VH and amino acid position 140 (EU numbering) the antibody heavy chain constant region, or/and between amino acid position 96 (Kabat numbering) of the antibody VL and amino acid position 130 (EU numbering) of the antibody light chain constant region.
6. The ligand-binding molecule of any one of claims 1 to 5, wherein the ligand is a biologically active molecule, and wherein the ligand-binding molecule inhibits the biological activity of the ligand by binding to the ligand.

7. The ligand-binding molecule of any one of claims 1 to 6, wherein the binding of the ligand-binding molecule to the ligand in a state where the protease cleavage sequence is uncleaved inhibits the binding of the ligand to its binding partner.
- 5 8. The ligand-binding molecule of any one of claims 1 to 7, wherein the ligand is a cytokine or a chemokine.
9. The ligand-binding molecule of any one of claims 1 to 8, wherein the ligand-binding molecule is an IgG antibody.
- 10 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.
11. A fusion protein in which the ligand is fused with the ligand-binding molecule of any one of claims 1 to 8.
- 15 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.
- 20 13. A method of producing the ligand-binding molecule comprising the step of culturing the host cell comprising a polynucleotide encoding the ligand-binding molecule of any one of claims 1 to 8 or a vector comprising the polynucleotide.
- 25 14. A protease substrate comprising a sequence selected from the sequences shown in SEQ ID NOs: 335-344, 818-824, 830-836, 1008, 1131, 1132, 1189, 1190, 1192-1194, 1196, 1198, 1201, 1202, 1206, 1209, 1210, 1212, 1215, 1216, 1218, 1219, 1221, 1222, 1226-1229, 1231-1233, 1242, 1253, 1254, and 1257.
- 30 15. A polypeptide comprising one or more sequences selected from the sequences shown in SEQ ID NOs: 335-344, 818-824, 830-836, 1008, 1131, 1132, 1189, 1190, 1192-1194, 1196, 1198, 1201, 1202, 1206, 1209, 1210, 1212, 1215, 1216, 1218, 1219, 1221, 1222, 1226-1229, 1231-1233, 1242, 1253, 1254, and 1257.