Claims

[Claim 1]

A molecule comprising a moiety [a], a moiety [b], and a moiety [c] indicated below and binding to a target antigen:

a moiety [a]: a moiety binding to the target antigen;

a moiety [b]: a first peptide recognizing a target antigen-binding site in the moiety [a]; and

a moiety [c]: a second peptide consisting of an amino acid sequence comprising amino acids that are converted from an uncharged state to a positively charged state in response to a pH change from neutral to acidic conditions at a pH 7.5 or lower.

[Claim 2]

The molecule according to claim 1, which exhibits higher binding affinity to the target antigen under acidic conditions than under neutral conditions.

[Claim 3]

The molecule according to claim 1 or 2, which comprises one or two or more of the moiety [a], the moiety [b], and the moiety [c] preferably wherein the number of the moiety [a], that of the moiety [b], and that of the moiety [c] are identical to each other.

[Claim 4]

The molecule according to claim 1 or 2, which comprises 2 moieties [a], 2 moieties [b], and 2 moieties [c].

[Claim 5]

The molecule according to any one of claims 1 to 4, wherein the second peptide consists of an amino acid sequence comprising 4 or more amino acids that are converted from an uncharged state to a positively charged state in response to a pH change from neutral to acidic conditions at a pH 7.5 or lower and has a pI value exceeding 6.4, preferably 6.8 or higher, more preferably 7.2 or higher, and even more preferably 7.6 or higher.

[Claim 6]

The molecule according to any one of claims 1 to 5, wherein the second peptide consists of an amino acid sequence comprising no negatively charged amino acids under acidic pH conditions.

[Claim 7]

The molecule according to any one of claims 1 to 6, wherein the second peptide consists of an amino acid sequence that does not comprise aspartic acid and/or glutamic acid.

[Claim 8]

The molecule according to any one of claims 1 to 7, wherein the second peptide consists of an amino acid sequence having the configurations (1) and (2) below:

(1) at least 1 of (a) an amino acid sequence consisting of 2 or more consecutive amino acids having basic functional groups with pKa of 7 or lower in side chains or (b) an amino acid sequence derived from the amino acid sequence (a) with insertion or addition of an arginine or lysine residue at any position (including an amino- or carboxyl terminus) (the amino acid sequence (a) and the amino acid sequence (b) are each referred to as a cluster of basic amino acids); and

(2) an amino acid sequence comprising 4 or more of the amino acids having basic functional groups with pKa of 7 or lower in side chains.

[Claim 9]

The molecule according to any one of claims 1 to 8, wherein the second peptide consists of an amino acid sequence having the configurations (1) and (2) below:

(1) comprising at least 1 amino acid sequence derived from the amino acid sequence consisting of 2 or more consecutive amino acids having basic functional groups with pKa of 7 or lower in side chains with insertion or addition of an arginine residue at any site (including an amino or carboxyl terminus); and

(2) comprising an amino acid sequence comprising 4 or more of the amino acids having basic functional groups with pKa of 7 or lower in side chains.

[Claim 10]

The molecule according to any one of claims 1 to 9, wherein the second peptide consists of an amino acid sequence having the configurations (1) and (2) below:

(1) comprising an amino acid sequence derived from the amino acid sequence consisting of 2 or more consecutive amino acids having basic functional groups with pKa of 7 or lower in side chains by insertion or addition of an arginine residue at any site (including an amino or carboxyl terminus) and/or comprising an amino acid sequence represented by RHHH; and

(2) comprising an amino acid sequence comprising 4 or more of the amino acids having basic functional groups with pKa of 7 or lower in side chains.

[Claim 11]

The molecule according to any one of claims 1 to 8, wherein the second peptide consists of an amino acid sequence comprising at least 1 of the 2 or more consecutive amino acids having basic functional groups with pKa of 7 or lower in side chains and 4 or more of the amino acids having basic functional groups with pKa of 7 or lower in side chains.

[Claim 12]

The molecule according to any one of claims 1 to 7, wherein the second peptide consists of an amino acid sequence comprising amino acids having basic functional groups with pKa of 7 or lower in side chains at every other position.

[Claim 13]

The molecule according to any one of claims 8 to 12, wherein the basic functional group has pKa of 5.0 to 7.0, and more preferably 5.5 to 6.5.

[Claim 14]

The molecule according to any one of claims 8 to 13, wherein the amino acids comprising basic functional groups with pKa of 7 or lower in side chains are natural amino acids.

[Claim 15]

The molecule according to any one of claims 8 to 13, wherein the amino acids comprising basic functional groups with pKa of 7 or lower in side chains are unnatural amino acids.

[Claim 16]

The molecule according to any one of claims 8 to 14, wherein the amino acids comprising basic functional groups with pKa of 7 or lower in side chains are histidine residues.

[Claim 17]

The molecule according to any one of claims 1 to 16, wherein the second peptide consists of an amino acid sequence comprising 1 to 4 clusters of basic amino acids.

[Claim 18]

The molecule according to any one of claims 1 to 17, wherein the second peptide consists of 10 to 60 amino acids, preferably 12 to 50 amino acids, more preferably 15 to 40 amino acids, and even more preferably, 20 to 35 amino acids.

[Claim 19]

The molecule according to any one of claims 1 to 11 and 13 to 18, wherein the second peptide comprises an amino acid sequence represented by any one of SEQ ID Nos: 49 to 53, 55, 59 to 61, 65, 66, and 75 to 81 (Figure 54 and Figure 64), or

the molecule according to any one of claims 12 to 18, wherein the second peptide comprises an amino acid sequence represented by SEQ ID NO: 54 (Figure 54).

[Claim 20]

The molecule according to any one of claims 1 to 19, wherein the EC_{50} ratio is 3 or higher, preferably wherein the EC_{50} ratio is 10 or higher, and more preferably wherein the EC_{50} ratio is 30 or higher.

[Claim 21]

The molecule according to any one of claims 1 to 20, wherein the second peptide consists of an amino acid sequence comprising 10 or fewer amino acids having basic functional groups with pKa of 7 or lower in side chains.

[Claim 22]

The molecule according to any one of claims 1 to 21, wherein the amino acid sequence of the second peptide comprises 2 or more clusters of basic amino acids, a cluster of basic amino acids is positioned at the amino or carboxyl terminus of the amino acid sequence, and other clusters of basic amino acids are not positioned at the amino or carboxyl terminus of the amino acid sequence.

[Claim 23]

The molecule according to any one of claims 1 to 21, wherein the amino acid sequence of the second peptide comprises 2 or more clusters of basic amino acids, a cluster of

basic amino acids is positioned at the amino terminus of the amino acid sequence, and another cluster of basic amino acids is positioned at the carboxyl terminus.

[Claim 24]

The molecule according to any one of claims 1 to 21, wherein none of the clusters of basic amino acids comprised in the amino acid sequence of the second peptide are positioned at the amino or carboxyl terminus of the amino acid sequence.

[Claim 25]

The molecule according to any one of claims 1 to 22 and 24, wherein the amino acid sequence of the second peptide comprises an amino acid sequence cleaved by an intracellular and/or extracellular protease and an amino acid sequence comprising a cluster of basic amino acids, wherein the amino acid sequence of the second peptide comprises:

(1) an amino acid sequence comprising a cluster of basic amino acids and an amino acid sequence cleaved by an intracellular and/or extracellular protease connected in that order from the amino terminus toward the carboxyl terminus; or

(2) an amino acid sequence cleaved by an intracellular and/or extracellular protease and an amino acid sequence comprising a cluster of basic amino acids connected in that order from the carboxyl terminus toward the amino terminus.

[Claim 26]

The molecule according to any one of claims 8 to 25, wherein the number of amino acids having basic functional groups with pKa of 7 or lower in side chains in the amino acid sequence of the second peptide is 5% to 30% of the total number of amino acids comprised in the amino acid sequence.

[Claim 27]

The molecule according to any one of claims 1 to 26, which comprises the first peptide, the second peptide, and the moiety binding to a target antigen connected in that order.

[Claim 28]

The molecule according to any one of claims 1 to 27, wherein the moiety binding to a target antigen is a polypeptide consisting of an amino acid sequence that is not comprised in the first peptide or the second peptide.

[Claim 29]

The molecule according to any one of claims 1 to 28, which consists of a polypeptide.

[Claim 30]

The molecule according to claim 29, which comprises the first peptide, the second peptide, and the moiety binding to a target antigen connected from the amino terminus toward the carboxyl terminus.

[Claim 31]

The molecule according to any one of claims 1 to 30, wherein any one of a moiety selected from the group consisting of the first peptide, the second peptide, and the moiety binding to a target antigen is connected to either or both of the other two moieties by a linker or spacer (or linkers or spacers).

[Claim 32]

The molecule according to any one of claims 1 to 31, wherein a pH of acidic conditions is 6.5 or lower, preferably 6.0 or lower and more preferably 5.5 or lower.

[Claim 33]

The molecule according to any one of claims 1 to 32, wherein the target antigen is an antigen that is present in a tumor tissue, an endosome, or a lysosome.

[Claim 34]

The molecule according to any one of claims 1 to 33, wherein another moiety [d] binds to a moiety binding to the target antigen and the moiety [d] does not comprise the first peptide and the second peptide.

[Claim 35]

The molecule according to claim 34, wherein the moiety [d] is at least 1 substance selected from the group consisting of an antibody or an antigen-binding fragment thereof that is not a moiety binding to the target antigen, a peptide comprising an amino acid sequence that is not comprised in the first peptide or the second peptide, a cytokine, a toxin, a radioactive isotope, a label molecule, a photosensitizer, an immunostimulant, an antitumor compound, a drug, a payload, and a polymer.

[Claim 36]

The molecule according to claim 35, wherein the antitumor compound is a camptothecin derivative or a pyrrolobenzodiazepine derivative and the camptothecin derivative

is preferably N-[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]-2-hydroxyacetamide, and/or

wherein the immunostimulant is a cyclic dinucleotide derivative.

[Claim 37]

The molecule according to any one of claims 34 to 36, which consists of a polypeptide or the moiety [d] and a polypeptide.

[Claim 38]

The molecule according to any one of claims 1 to 37, wherein the first peptide is obtained by a method comprising steps (i) to (iii):

(i) a step of bringing a peptide library comprising a repeat sequence of aromatic amino acid and Pro into contact with the moiety binding to a target antigen as defined in [1];

(ii) a step of collecting a peptide binding to the moiety binding to a target antigen; and

(iii) a step of preparing the peptide by recombination, chemical synthesis, or peptide synthesis.

[Claim 39]

A method for producing the molecule according to any one of claims 1 to 37, which comprises a step of preparing a peptide comprising an amino acid sequence comprised in the first peptide and/or an amino acid sequence comprised in the second peptide by recombination, *in vitro* translation, chemical synthesis, or peptide synthesis.

[Claim 40]

A molecule binding to a target antigen, which is obtained by the method according to claim 39.

[Claim 41]

A method for producing the molecule according to any one of claims 29, 30, and 37 comprising a step (iv) below:

(iv) a step of introducing a polynucleotide comprising a nucleotide sequence encoding an amino acid sequence comprised in a moiety binding to a target antigen and, optionally, an amino acid sequence comprised in the first peptide, and/or an amino acid sequence comprised in the

second peptide into a cell, culturing the cell, and collecting a polypeptide binding to the target antigen from the culture product.

[Claim 42]

A molecule binding to a target antigen, which is obtained by the method according to claim 41.

[Claim 43]

A pharmaceutical composition comprising the molecule according to any one of claims 1 to 38, 40, and 42, a salt thereof, or a hydrate of the molecule or salt.

[Claim 44]

The pharmaceutical composition according to claim 43, which is an anti-cancer agent.

[Claim 45]

The pharmaceutical composition according to claim 43 or 44, wherein an antigen-binding intensity of a molecule binding to a target antigen under neutral pH conditions is adjusted to be lower than the antigen-binding intensity under acidic pH conditions, so as to reduce the toxicity of the molecule binding to a target antigen imposed on a subject compared with the toxicity of a molecule comprising no amino acids converted from an uncharged state to a positively charged state in response to a pH change of the second peptide from neutral to acidic conditions at a pH 7.5 or lower.

[Claim 46]

The pharmaceutical composition according to claim 43 or 44, wherein an antigen-binding intensity of a molecule binding to a target antigen under neutral pH conditions is adjusted to be lower than the antigen-binding intensity under acidic pH conditions, so as to increase the blood concentration of the molecule which binds to the target antigen in a subject, compared with that of a molecule comprising no amino acids that are converted from an uncharged state to a positively charged state in response to a pH change of the second peptide from neutral to acidic conditions at a pH 7.5 or lower.

[Claim 47]

A test drug, diagnostic drug, or reagent comprising the molecule according to any one of claims 1 to 38, 40, and 42 or the pharmaceutical composition according to claim 58.

[Claim 48]

A polynucleotide comprising a nucleotide sequence encoding the amino acid sequence of the molecule according to claim 29 or 37.

[Claim 49]

A vector comprising the polynucleotide according to claim 48.

[Claim 50]

A cell comprising the polynucleotide according to claim 48 or the vector according to claim 49 or producing the molecule according to claim 29 or 37.