Claims

[Claim 1]

A method for selecting an antigen-binding molecule whose antigen-binding activity is changed depending on calcium ion concentration conditions, the method comprising the steps of:

a) preparing a library consisting essentially of a plurality of

antigen-binding molecules differing in sequence from each other, wherein an antigen-binding domain in each of the antigen-binding molecules comprises a light chain variable region which comprises at least one amino acid residue that changes the antigen-binding activity of the antigen-binding molecule depending on calcium ion concentration conditions, wherein the amino acid residue is located at any one or more of positions 30, 31, 32, 50 and 92 defined by the Kabat numbering in the light chain variable region,or

fusion polypeptides differing in sequence from each other

and each comprising the antigen-binding molecules, wherein each of the fusion polypeptides is a fusion product of a heavy chain variable region of the antigen-binding molecule and at least a portion of a viral coat protein;

b) contacting the library with antigens under two or more different calcium ion concentration conditions;

c) sorting, from the library, a subpopulation of antigen-binding molecules whose antigen-binding activity is changed depending on the calcium ion concentration conditions; and

d) isolating each antigen-binding molecule whose antigen-binding activity is changed depending on the calcium ion concentration conditions from the subpopulation sorted in the step c), wherein an antigen-binding molecule having lower antigen-binding activity under a low-calcium concentration condition than that under a high-calcium concentration condition is selected,

wherein the low-calcium concentration condition is 0.1 μM to 30 μM and the high-calcium concentration condition is 100 μM to 10 mM.

[Claim 2]

A method for isolating a polynucleotide encoding an antigen-binding molecule whose antigen-binding activity is changed depending on calcium ion concentration conditions, the method comprising the steps of:

a) preparing a library comprising a plurality of replicable expression vectors each comprising, in an operably linked state, a plurality of polynucleotides each encoding antigen-binding molecules differing in sequence from each other , wherein an antigen-binding domain in each of the antigen-binding molecules comprises a light chain variable region which comprises at least one amino acid residue that changes the antigen-binding

activity of the antigen-binding molecule depending on calcium ion concentration conditions, wherein the amino acid residue is located at any one or more of positions 30, 31, 32, 50 and 92 defined by the Kabat numbering in the light chain variable region or

fusion polypeptides differing in sequence from each otherand each comprising the antigen-binding molecules, wherein each of the fusion polypeptides is a fusion product of a heavy chain variable region of the antigen-binding molecule and at least a portion of a viral coat protein;

b) allowing a plurality of viruses each transformed with the expression vectors contained in the library to express on their surface the antigen-binding molecules or the fusion polypeptides differing in sequence from each other encoded by the polynucleotides;

c) contacting the plurality of viruses with antigens under two or more different calcium ion concentration conditions;

d) sorting, from the library, a subpopulation of viruses whose antigen-binding activity is changed depending on the calcium ion concentration conditions;

e) isolating each virus whose antigen-binding activity is changed depending on the calcium ion concentration conditions from the virus subpopulation sorted in the step d) , wherein an antigen-binding molecule having lower antigen-binding activity under a low-calcium

concentration condition than that under a high-calcium concentration condition is selected,
wherein the low-calcium concentration condition is 0.1 μM to 30 μM and the high-calcium concentration condition is 100 μM to 10 mM; and
f) isolating the polynucleotides from the isolated virus.

[Claim 3]

The method according to claim 1 or 2, wherein a light chain framework region in the antigen-binding molecule comprises a germline framework sequence.

[Claim 4]

The method according to any of claims 1 to 3, wherein a heavy chain variable region of the antigen-binding molecule comprises the amino acid sequence of a naive sequence.

[Claim 5]

The method according to any of claims 1 to 4, wherein the amino acid residue forms a calcium-binding motif.

[Claim 6]

The method according to claim 5, wherein the calcium-binding motif is any calcium-binding motif selected from a cadherin domain, an EF hand, a C2 domain, a Gla domain, a C-type lectin, a domain, an annexin, a

thrombospondin type 3 domain, an EGF-like domain, a domain of Vk5, a domain represented by SEQ ID NO: 10, and a domain represented by SEQ ID NO: 11.

[Claim 7]

The method according to any of claims 3 to 6, wherein the amino acid residue is an amino acid having a metal-chelating effect.

[Claim 8]

The method according to claim 7, wherein the amino acid having a metal-chelating effect is any one or more amino acids selected from serine, threonine, asparagine, glutamine, aspartic acid, and glutamic acid.

[Claim 9]

The library method to claim 1 or 2, wherein the viral coat protein is selected from the group consisting of protein pIII, major coat protein pVIII, pVII, pIX, Soc, Hoc, gpD, pv1, and variants thereof.

[Claim 10]

The method according to claim 2, wherein the steps c) and d) are additionally repeated at least once.

[Claim 11]

The method according to claim 1 or 2, wherein the low-calcium concentration condition is 3 μM.

[Claim 12]

The method according to claim 1 or 2, wherein the high-calcium concentration condition is 2 mM.

[Claim 13]

A method for producing an antigen-binding molecule whose antigen-binding activity is changed depending on calcium ion concentration conditions, the method comprising the steps of:
a) preparing a library comprising a plurality of replicable expression vectors each comprising, in an operably linked state, a plurality of polynucleotides each encoding antigen-binding molecules differing in sequence from each other , wherein an antigen-binding domain in each of the antigen-binding molecules comprises a light chain variable region which comprises at least one amino acid residue that changes the antigen-binding activity of the antigen-binding molecule depending on calcium ion concentration conditions, wherein the amino acid residue is located at any one or more of positions 30, 31, 32, 50 and 92 defined by the Kabat numbering in the light chain variable region or
fusion polypeptides differing in sequence from each otherand each comprising the antigen-binding molecules,

wherein each of the fusion polypeptides is a fusion product of a heavy chain variable region of the antigen-binding molecule and at least a portion of a viral coat protein;

b) allowing a plurality of viruses each transformed with the expression vectors contained in the library to express on their surface the antigen-binding molecules or the fusion polypeptides differing in sequence from each other encoded by the polynucleotides;

c) contacting the plurality of viruses with antigens under two or more different calcium ion concentration conditions;

d) sorting, from the library, a subpopulation of viruses whose antigen-binding activity is changed depending on the calcium ion concentration conditions;

e) isolating each virus whose antigen-binding activity is changed depending on the calcium ion concentration conditions from the virus subpopulation sorted in the step d) , wherein an antigen-binding molecule having lower antigen-binding activity under a low-calcium concentration condition than that under a high-calcium concentration condition is selected,

wherein the low-calcium concentration condition is 0.1 μM to 30 μM and the high-calcium concentration condition is 100 μM to 10 mM;

f) isolating the polynucleotides from the isolated virus;

g) culturing a host cell transfected with a vector having an operably linked insert of the isolated polynucleotides; and
h) collecting the antigen-binding molecules from the cultures of the cell cultured in the step g).

[Claim 14]

A method for producing an antigen-binding molecule whose antigen-binding activity is changed depending on calcium ion concentration conditions, the method comprising the steps of:
a) preparing a library comprising a plurality of replicable expression vectors each comprising, in an operably linked state, a plurality of polynucleotides each encoding antigen-binding molecules differing in sequence from each other , wherein an antigen-binding domain in each of the antigen-binding molecules comprises a light chain variable region which comprises at least one amino acid residue that changes the antigen-binding activity of the antigen-binding molecule depending on calcium ion concentration conditions, wherein the amino acid residue is located at any one or more of positions 30, 31, 32, 50 and 92 defined by the Kabat numbering in the light chain variable region or
fusion polypeptides differing in sequence from each otherand each comprising the antigen-binding molecules, wherein each of the fusion polypeptides is a fusion product of a heavy chain variable region of the antigen-

binding molecule and at least a portion of a viral coat protein;

b) allowing a plurality of viruses each transformed with the expression vectors contained in the library to express on their surface the antigen-binding molecules or the fusion polypeptides differing in sequence from each other encoded by the polynucleotides;

c) contacting the plurality of viruses with antigens under two or more different calcium ion concentration conditions;

d) sorting, from the library, a subpopulation of viruses whose antigen-binding activity is changed depending on the calcium ion concentration conditions;

e) isolating each virus whose antigen-binding activity is changed depending on the calcium ion concentration conditions from the virus subpopulation sorted in the step d) , wherein an antigen-binding molecule having lower antigen-binding activity under a low-calcium concentration condition than that under a high-calcium concentration condition is selected,

wherein the low-calcium concentration condition is 0.1 μM to 30 μM and the high-calcium concentration condition is 100 μM to 10 mM;

f) isolating the polynucleotides from the isolated virus;

g) linking the isolated polynucleotides in frame with a polynucleotide encoding an antibody constant region;

h) culturing a host cell transfected with a vector having an operably linked insert of the polynucleotides linked in the step g); and
i) collecting the antigen-binding molecules from the cultures of the cell cultured in the step h).

[Claim 15]

The method according to claim 13 or 14, wherein a light chain framework region in the antigen-binding molecule comprises a germline framework sequence.

[Claim 16]

The method according to any of claims 13 to 15, wherein a heavy chain variable region of the antigen-binding molecule comprises the amino acid sequence of a naive sequence.

[Claim 17]

The method according to any of claims 13 to 16, wherein the amino acid residue forms a calcium-binding motif.

[Claim 18]

The method according to claim 17, wherein the calcium-binding motif is any calcium-binding motif selected from a cadherin domain, an EF hand, a C2 domain, a Gla domain, a C-type lectin, a domain, an annexin, a thrombospondin type 3 domain, an EGF-like domain, a

domain of Vk5, a domain represented by SEQ ID NO: 10, and a domain represented by SEQ ID NO: 11.

[Claim 19]

The method according to any of claims 15 to 18, wherein the amino acid residue is an amino acid having a metal-chelating effect.

[Claim 20]

The method according to claim 19, wherein the amino acid having a metal-chelating effect is any one or more amino acids selected from serine, threonine, asparagine, glutamine, aspartic acid, and glutamic acid.

[Claim 21]

The library method to claim 13 or 14, wherein the viral coat protein is selected from the group consisting of protein pIII, major coat protein pVIII, pVII, pIX, Soc, Hoc, gpD, pv1, and variants thereof.

[Claim 22]

The production method according to claim 13 or 14, wherein the steps c) and d) are additionally repeated at least once.

[Claim 23]

The production method according to claim 13 or 14, wherein the low-calcium concentration condition is 3 μM.

[Claim 24]

The production method according to claim 13 or 14, wherein the high-calcium concentration condition is 2 mM.