Claims

[Claim 1]

A method for selecting an antigen-binding molecule or a fusion polypeptide 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 or fusion polypeptides whose antigen-binding activity is changed depending on the calcium ion concentration conditions,

wherein the antigen-binding activity is measured by surface plasmon resonance (SPR) method; and

d) isolating each antigen-binding molecule or fusion polypeptides 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 1 μM to 5 μM and the high-calcium concentration condition is 500 μM to 2.5 mM,

wherein KD (3 μM Ca)/KD (2 mM Ca) is between 2 and 10,000.

[Claim 2]

A method for isolating a polynucleotide encoding an antigen-binding molecule or a fusion polypeptide 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 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) 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 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,

wherein the antigen-binding activity is measured by surface plasmon resonance (SPR) method;

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 1 μM to 5 μM and the high-calcium concentration condition is 500 μM to 2.5 mM,

wherein KD (3 μM Ca)/KD (2 mM Ca) is between 2 and 10,000; 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 one of claims 1 to 4, wherein the amino acid is any one or more amino acids selected from serine, threonine, asparagine, glutamine, aspartic acid, and glutamic acid.

[Claim 6]

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 7]

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

[Claim 8]

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

[Claim 9]

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

[Claim 10]

A method for producing an antigen-binding molecule or a fusion polypeptide 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 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) 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 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,

wherein the antigen-binding activity is measured by surface plasmon resonance (SPR) method;
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 1 μM to 5 μM and the high-calcium concentration condition is 500 μM to 2.5 mM,

wherein KD (3 μM Ca)/KD (2 mM Ca) is between 2 and 10,000;
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 olynucleotides; and

h) collecting the antigen-binding molecules or the fusion polypeptides from the cultures of the cell cultured in the step g).

[Claim 11]

A method for producing an antigen-binding molecule or a fusion polypeptide 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 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) 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 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,

wherein the antigen-binding activity is measured by surface plasmon resonance (SPR) method;

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 1 μM to 5 μM and the high-calcium concentration condition is 500 μM to 2.5 mM,

wherein KD (3 μM Ca)/KD (2 mM Ca) is between 2 and 10,000;

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 or the fusion polypeptides from the cultures of the cell cultured in the step h).

[Claim 12]

The method according to claim 10 or 11, wherein a light chain framework region in the antigen-binding molecule comprises a germline framework sequence.

[Claim 13]

The method according to any of claims 10 to 12, wherein a heavy chain variable region of the antigen-binding molecule comprises the amino acid sequence of a naive sequence.

[Claim 14]

The method according to any one of claim 10 to 13, 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 15]

The library method to claim 10 or 11, 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 16]

The production method according to claim 10 or 11, wherein the steps c) and d) are additionally repeated at least once.

[Claim 17]

The production method according to claim 10 or 11, wherein the low-calcium concentration condition is 3 μM.

[Claim 18]

The production method according to claim 10 or 11, wherein the high-calcium concentration condition is 2 mM.