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Summary ~~of Invention~~

Technical Problem

[0014]

The present invention has been made in light of such a situation, and an object of the present invention is to

concentration, particularly, calcium ion concentration, between plasma and early endosome or on the pHs of these environments and found that use of antigen-binding molecules having calcium-dependent or pH-dependent antigen-binding activity enables promotion of cellular uptake of antigens by the antigen-binding molecules and preparation of a library consisting essentially of antigen-binding molecules that reduce antigen concentration in plasma.

[0016]

Specifically, the present invention relates to 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 at least one amino acid residue that changes the antigen-binding activity of the antigen-binding molecule depending on ion concentration conditions, a composition comprising a plurality of polynucleotide molecules each encoding the antigen-binding molecules, a composition comprising a plurality of vectors each comprising the polynucleotide molecules, a method for selecting the antigen-binding molecules, a method for isolating the polynucleotide molecules, a method for producing the antigen-binding molecules, a pharmaceutical composition comprising any of the antigen-binding molecules, etc.. More specifically,

in a first aspect, the present invention refers to 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. In another aspect, the present invention refers to a library consisting essentially of a plurality of fusion polypeptides each comprising antigen-binding molecules according to the present invention, 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. In another aspect, the present invention refers to a composition comprising a plurality of polynucleotide molecules each encoding antigen-binding molecules differing in sequence from each other according to the present invention or fusion polypeptides differing in sequence from each other according to claim 8 or 9. In still another aspect, the present invention refers to a composition comprising a plurality of vectors each comprising a plurality of polynucleotide molecules

according to the present invention in an operably linked state. In another aspect, the present invention refers to a composition comprising a plurality of viruses each comprising vectors according to the present invention. In still another aspect, the present invention refers to a composition comprising a plurality of viruses each displaying on their surface antigen-binding molecules differing in sequence from each other according to the present invention or fusion polypeptides differing in sequence from each other according to claim 8 or 9. In another aspect, the present invention refers to a library comprising antigen-binding molecules differing in sequence from each other according to the present invention or fusion polypeptides differing in sequence from each other according to claim 8 or 9, wherein the library has 1×10^6 to 1×10^{14} distinct variable region sequences. In still another aspect, the present invention refers to a method for preparing a library consisting essentially of a plurality of antigen-binding molecules differing in sequence from each other, the method comprising producing a plurality of antigen-binding molecules designed so that 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. In another aspect, the present invention refers to 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 antigen-binding molecules differing in sequence from each other according to the present invention or fusion polypeptides differing in sequence from each other according to claim 8 or 9; 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). In still another aspect, the present invention refers to 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 according to the present invention or fusion polypeptides differing in sequence from each other according to claim 8 or 9; 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); and f) isolating the polynucleotides from the isolated virus. In another aspect, the present invention refers to 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 according

to the present invention or fusion polypeptides differing in sequence from each other according to claim 8 or 9; 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); 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). In another aspect, the present invention refers to 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 according to the present invention or fusion polypeptides differing in sequence from each other according to claim 8 or 9; 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); 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). ~~the~~ The present ~~invention~~ disclosure also ~~relates~~ describes~~to~~ the following:

Claims

[Claim 1]

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.

[Claim 2]

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

[Claim 3]

The library according to claim 1 or 2, wherein a heavy chain variable region of the antigen-binding molecule comprises the amino acid sequence of a naive sequence.

[Claim 4]

The library according to any of claims 1 to 3, wherein the amino acid residue forms a calcium-binding motif.

[Claim 5]

The library according to claim 4, 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 6]

The library according to any of claims 2 to 5, wherein the amino acid residue is an amino acid having a metal-chelating effect.

[Claim 7]

The library according to claim 6, 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 8]

A library consisting essentially of a plurality of fusion polypeptides each comprising antigen-binding

molecules according to any of claims 1 to 7, 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.

[Claim 9]

The library according to claim 8, 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]

A composition comprising a plurality of polynucleotide molecules each encoding antigen-binding molecules differing in sequence from each other according to any of claims 1 to 7 or fusion polypeptides differing in sequence from each other according to claim 8 or 9.

[Claim 11]

A composition comprising a plurality of vectors each comprising a plurality of polynucleotide molecules according to claim 10 in an operably linked state.

[Claim 12]

The composition according to claim 11, wherein the vectors are replicable expression vectors.

[Claim 13]

The composition according to claim 12, wherein each of the replicable expression vectors is an expression vector in which the polynucleotide is operably linked to a promoter region selected from the group consisting of a lacZ promoter system, an alkaline phosphatase phoA promoter (Ap), a bacteriophage λPL promoter (temperature-sensitive promoter), a tac promoter, a tryptophan promoter, a pBAD promoter, and a bacteriophage T7 promoter.

[Claim 14]

The composition according to claim 12 or 13, wherein each of the replicable expression vectors is an M13, f1, fd, or Pf3 phage or a derivative thereof, or a lambdoid phage or a derivative thereof.

[Claim 15]

A composition comprising a plurality of viruses each comprising vectors according to any of claims 11 to 14.

[Claim 16]

A composition comprising a plurality of viruses each displaying on their surface antigen-binding molecules differing in sequence from each other according to any of claims 1 to 7 or fusion polypeptides differing in sequence from each other according to claim 8 or 9.

[Claim 17]

A library comprising antigen-binding molecules differing in sequence from each other according to any of claims 1 to 7 or fusion polypeptides differing in sequence from each other according to claim 8 or 9, wherein the library has 1×10^6 to 1×10^{14} distinct variable region sequences.

[Claim 18]

The library according to claim 17, wherein the library has 1×10^8 or more distinct variable region sequences.

[Claim 19]

A method for preparing a library consisting essentially of a plurality of antigen-binding molecules differing in sequence from each other, the method comprising producing a plurality of antigen-binding molecules designed so that 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.

[Claim 20]

The preparation method according to claim 19, wherein the antigen-binding molecules are antigen-binding molecules according to any of claims 1 to 7.

[Claim 21]

The preparation method according to claim 19 or 20, wherein a heavy chain variable region of each of the antigen-binding molecules is fused with at least a portion of a viral coat protein.

[Claim 22]

The preparation method according to any of claims 19 to 21, 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 23]

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 antigen-binding molecules differing in sequence from each other according to any of claims 1 to 7 or fusion polypeptides differing in sequence from each other according to claim 8 or 9;

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).

[Claim 24]

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 according to any of claims 1 to 7 or fusion polypeptides differing in sequence from each other according to claim 8 or 9;
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); and
f) isolating the polynucleotides from the isolated virus.

[Claim 25]

The method according to claim 60, wherein the steps c) and d) are additionally repeated at least once.

[Claim 26]

The method according to any one of claims 23 to 25, 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.

[Claim 27]

The method according to claim 26, wherein the low-calcium concentration condition is 0.1 μM to 30 μM.

[Claim 28]

The method according to claim 26 or 27, wherein the high-calcium concentration condition is 100 μM to 10 mM.

[Claim 29]

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 according to any of claims 1 to 7 or fusion polypeptides differing in sequence from each other according to claim 8 or 9;

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);
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 30]

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 according to any of claims 1 to 7 or fusion polypeptides differing in sequence from each other according to claim 8 or 9;
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);
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 31]

The production method according to claim 29 or 30, wherein the steps c) and d) are additionally repeated at least once.

[Claim 32]

The production method according to any one of claims 29 to 31, 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.

[Claim 33]

The production method according to claim 32, wherein the low-calcium concentration condition is 0.1 μM to 30 μM.

[Claim 34]

The production method according to claim 32 or 33, wherein the high-calcium concentration condition is 100 μM to 10 mM.

Claims

[Claim 1]

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 at least one amino acid residue that changes the antigen-binding activity of the antigen-binding molecule depending on ion concentration conditions.

[Claim 2]

The library according to claim 1, wherein the ion concentration is a calcium ion concentration.

[Claim 3]

The library according to claim 2, wherein the amino acid residue is contained in the antigen-binding domain in a heavy chain of the antigen-binding molecule.

[Claim 4]

The library according to claim 3, wherein the antigen-binding domain in a heavy chain is a heavy chain variable region.

[Claim 5]

The library according to claim 4, wherein the amino acid residue is contained in CDR3 of the heavy chain variable region.

[Claim 6]

The library according to any of claims 2 to 5, wherein the amino acid residue is located at any one or more of positions 95, 96, 100a, and 101 defined by the Kabat numbering in the heavy chain CDR3.

[Claim 7]

The library according to any of claims 2 to 6, wherein an amino acid sequence except for the amino acid residue comprises the amino acid sequence of a naive sequence.

[Claim 8]

The library according to any of claims 3 to 7, wherein a light chain variable region of the antigen-binding molecule comprises the amino acid sequence of a naive sequence.

[Claim 9]

The library according to claim 2, wherein the amino acid residue is contained in the antigen-binding domain in a light chain of the antigen-binding molecule.

[Claim 10]

The library according to claim 9, wherein the antigen-binding domain in a light chain is a light chain variable region.

[Claim 11]

The library according to claim 10, wherein the amino acid residue is contained in CDR1 of the light chain variable region.

[Claim 12]

The library according to claim 11, wherein the amino acid residue is located at any one or more of positions 30, 31, and 32 defined by the Kabat numbering in the CDR1.

[Claim 13]

The library according to any of claims 10 to 12, wherein the amino acid residue is contained in CDR2 of the light chain variable region.

[Claim 14]

The library according to claim 13, wherein the amino acid residue is located at position 50 defined by the Kabat numbering in the light chain CDR2.

[Claim 15]

The library according to any of claims 10 to 14, wherein the amino acid residue is contained in light chain CDR3.

[Claim 16]

The library according to claim 15, wherein the amino acid residue is located at position 92 defined by the Kabat numbering in the light chain CDR3.

[Claim 17]

The library according to any of claims 2 and 9 to 16, wherein a light chain framework region in the antigen-binding molecule comprises a germline framework sequence.

[Claim 18]

The library according to any of claims 2 and 9 to 17, wherein a heavy chain variable region of the antigen-

binding molecule comprises the amino acid sequence of a naive sequence.

[Claim 19]

The library according to any of claims 1 to 18, wherein the amino acid residue forms a calcium-binding motif.

[Claim 20]

The library according to claim 19, 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 21]

The library according to any of claims 2 to 20, wherein the amino acid residue is an amino acid having a metal-chelating effect.

[Claim 22]

The library according to claim 21, 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 23]

The library according to claim 1, wherein the ion concentration conditions are pH conditions.

[Claim 24]

The library according to claim 23, wherein the amino acid residue is contained in the antigen-binding domain in a heavy chain of the antigen-binding molecule.

[Claim 25]

The library according to claim 24, wherein the antigen-binding domain in a heavy chain is a heavy chain variable region.

[Claim 26]

The library according to claim 25, wherein the amino acid residue is located at any one or more of positions 27, 31, 32, 33, 35, 50, 52, 53, 55, 57, 58, 59, 61, 62, 95, 96, 97, 98, 99, 100a, 100b, 100d, 100f, 100h, 102, and 107 defined by the Kabat numbering in the heavy chain variable region.

[Claim 27]

The library according to claim 26, wherein an amino acid sequence except for the amino acid residue at any one or more of positions 27, 31, 32, 33, 35, 50, 52, 53, 55, 57, 58, 59, 61, 62, 95, 96, 97, 98, 99, 100a, 100b, 100d, 100f, 100h, 102, and 107 defined by the Kabat numbering in the heavy chain variable region comprises the amino acid sequence of a naive sequence.

[Claim 28]

The library according to any of claims 23 to 27, wherein a light chain variable region of the antigen-binding molecule comprises a germline sequence.

[Claim 29]

The library according to claim 23, wherein the amino acid residue is contained in the antigen-binding domain in a light chain of the antigen-binding molecule.

[Claim 30]

The library according to claim 29, wherein the antigen-binding domain in a light chain is a light chain variable region.

[Claim 31]

The library according to claim 30, wherein the amino acid residue is located at any one or more of positions 24, 27, 28, 30, 31, 32, 34, 50, 51, 52, 53, 54, 55, 56, 89, 90, 91, 92, 93, 94, and 95a defined by the Kabat numbering in the light chain variable region.

[Claim 32]

The library according to claim 30 or 31, wherein the amino acid residue is contained in CDR1 of the light chain variable region.

[Claim 33]

The library according to claim 32, wherein the amino acid residue is located at any one or more of positions 24, 27, 28, 30, 31, 32, and 34 defined by the Kabat numbering in the light chain CDR1.

[Claim 34]

The library according to any of claims 30 to 33, wherein the amino acid residue is contained in light chain CDR2.

[Claim 35]

The library according to claim 34, wherein the amino acid residue is located at any one or more of positions 50, 51, 52, 53, 54, 55, and 56 defined by the Kabat numbering in the light chain CDR2.

[Claim 36]

The library according to any of claims 30 to 35, wherein the amino acid residue is contained in light chain CDR3.

[Claim 37]

The library according to claim 36, wherein the amino acid residue is located at any one or more of positions 89, 90, 91, 92, 93, 94, and 95a defined by the Kabat numbering in the light chain CDR3.

[Claim 38]

The library according to any of claims 29 to 37, wherein a light chain framework region comprises a germline framework sequence.

[Claim 39]

The library according to any of claims 29 to 38, wherein a heavy chain variable region has a naive sequence.

[Claim 40]

The library according to any of claims 23 to 39, wherein the amino acid residue is an amino acid having a side chain pKa of 4.0 to 8.0.

[Claim 41]

The library according to any of claims 23 to 40, wherein the amino acid residue is glutamic acid.

[Claim 42]

The library according to any of claims 23 to 39, wherein the amino acid residue is an amino acid having a side chain pKa of 5.5 to 7.0.

[Claim 43]

The library according to any of claims 23 to 40 and 42, wherein the amino acid residue is histidine.

[Claim 44]

A library consisting essentially of a plurality of fusion polypeptides each comprising antigen-binding molecules according to any of claims 1 to 43, 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.

[Claim 45]

The library according to claim 44, wherein the viral coat protein is selected from the group consisting of protein pIII, major coat protein pVIII, pVII, pIX, Soc, Hoc, gpD, pvi, and variants thereof.

[Claim 46]

A composition comprising a plurality of polynucleotide molecules each encoding antigen-binding molecules differing in sequence from each other according to any of claims 1 to 43 or fusion polypeptides differing in sequence from each other according to claim 44 or 45.

[Claim 47]

A composition comprising a plurality of vectors each comprising a plurality of polynucleotide molecules according to claim 46 in an operably linked state.

[Claim 48]

The composition according to claim 47, wherein the vectors are replicable expression vectors.

[Claim 49]

The composition according to claim 48, wherein each of the replicable expression vectors is an expression vector in which the polynucleotide is operably linked to a promoter region selected from the group consisting of a lacZ promoter system, an alkaline phosphatase phoA promoter (Ap), a bacteriophage λPL promoter (temperature-sensitive promoter), a tac promoter, a tryptophan promoter, a pBAD promoter, and a bacteriophage T7 promoter.

[Claim 50]

The composition according to claim 48 or 49, wherein each of the replicable expression vectors is an M13, f1, fd, or Pf3 phage or a derivative thereof, or a lambdoid phage or a derivative thereof.

[Claim 51]

A composition comprising a plurality of viruses each comprising vectors according to any of claims 47 to 50.

[Claim 52]

A composition comprising a plurality of viruses each displaying on their surface antigen-binding molecules differing in sequence from each other according to any of claims 1 to 43 or fusion polypeptides differing in sequence from each other according to claim 44 or 45.

[Claim 53]

A library comprising antigen-binding molecules differing in sequence from each other according to any of claims 1 to 43 or fusion polypeptides differing in sequence from each other according to claim 44 or 45, wherein the library has 1×10^6 to 1×10^{14} distinct variable region sequences.

[Claim 54]

The library according to claim 53, wherein the library has 1×10^8 or more distinct variable region sequences.

[Claim 55]

A method for preparing a library consisting essentially of a plurality of antigen-binding molecules differing in sequence from each other, the method comprising producing a plurality of antigen-binding molecules designed so that an antigen-binding domain in each of the antigen-binding molecules comprises at least one amino acid residue that changes the antigen-binding activity of the antigen-binding molecule depending on ion concentration conditions.

[Claim 56]

The preparation method according to claim 55, wherein the antigen-binding molecules are antigen-binding molecules according to any of claims 2 to 43.

[Claim 57]

The preparation method according to claim 55 or 56, wherein a heavy chain variable region of each of the antigen-binding molecules is fused with at least a portion of a viral coat protein.

[Claim 58]

The preparation method according to any of claims 55 to 57, 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 59]

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

a) preparing a library consisting essentially of antigen-binding molecules differing in sequence from each other according to any of claims 1 to 43 or fusion polypeptides differing in sequence from each other according to claim 44 or 45;

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

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

d) isolating each antigen-binding molecule whose antigen-binding activity is changed depending on the ion concentration conditions from the subpopulation sorted in the step c).

[Claim 60]

A method for isolating a polynucleotide encoding an antigen-binding molecule whose antigen-binding activity is changed depending on 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 according to any of claims 1 to 43 or fusion polypeptides differing in sequence from each other according to claim 44 or 45;

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 ion concentration conditions;

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

e) isolating each virus whose antigen-binding activity is changed depending on the ion concentration conditions from the virus subpopulation sorted in the step d); and

f) isolating the polynucleotides from the isolated virus.

[Claim 61]

The method according to claim 60, wherein the steps c) and d) are additionally repeated at least once.

[Claim 62]

The method according to any of claims 59 to 61, wherein the ion concentration is a calcium ion concentration.

[Claim 63]

The method according to claim 62, 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.

[Claim 64]

The method according to claim 63, wherein the low-calcium concentration condition is 0.1 μM to 30 μM.

[Claim 65]

The method according to claim 63 or 64, wherein the high-calcium concentration condition is 100 μM to 10 mM.

[Claim 66]

The method according to any of claims 59 to 61, wherein the ion concentration conditions are pH conditions.

[Claim 67]

The method according to claim 66, wherein an antigen-binding molecule having lower antigen-binding activity in an acidic pH condition than that in a neutral pH condition is selected.

[Claim 68]

The method according to claim 66, wherein the acidic pH condition is pH 4.0 to 6.5.

[Claim 69]

The method according to claim 67 or 68, wherein the neutral pH condition is pH 6.7 to 10.0.

[Claim 70]

A method for producing an antigen-binding molecule whose antigen-binding activity is changed depending on 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 according to any of claims 1 to 43 or fusion polypeptides differing in sequence from each other according to claim 44 or 45;

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 ion concentration conditions;
d) sorting, from the library, a subpopulation of viruses whose antigen-binding activity is changed depending on the ion concentration conditions;
e) isolating each virus whose antigen-binding activity is changed depending on the ion concentration conditions from the virus subpopulation sorted in the step d);
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 71]

A method for producing an antigen-binding molecule whose antigen-binding activity is changed depending on 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 according to any of claims 1 to 43 or fusion polypeptides differing in sequence from each other according to claim 44 or 45;

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 ion concentration conditions;

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

e) isolating each virus whose antigen-binding activity is changed depending on the ion concentration conditions from the virus subpopulation sorted in the step d);

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 72]

The production method according to claim 70 or 71, wherein the steps c) and d) are additionally repeated at least once.

[Claim 73]

The production method according to any of claims 70 to 72, wherein the ion concentration is a calcium ion concentration.

[Claim 74]

The production method according to claim 73, 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.

[Claim 75]

The production method according to claim 74, wherein the low-calcium concentration condition is 0.1 μM to 30 μM.

[Claim 76]

The production method according to claim 74 or 75, wherein the high-calcium concentration condition is 100 μM to 10 mM.

[Claim 77]

The production method according to any of claims 70 to 72, wherein the ion concentration conditions are pH conditions.

[Claim 78]

The production method according to claim 77, wherein an antigen-binding molecule having lower antigen-binding activity in an acidic pH condition than that in a neutral pH condition is selected.

[Claim 79]

The production method according to claim 78, wherein the acidic pH condition is pH 4.0 to 6.5.

[Claim 80]

The production method according to claim 78 or 79, wherein the neutral pH condition is pH 6.7 to 10.0.

[Claim 81]

An antigen-binding molecule produced by a production method according to any of claims 70 to 80.

[Claim 82]

A pharmaceutical composition comprising an antigen-binding molecule according to claim 81 or a modified form thereof.