Claims

[Claim 1]

A library consisting essentially of a plurality of ~~antigen-binding molecules~~antibodies each comprising a light chain variable region differing in sequence from each other,

wherein ~~an antigen-binding domain~~ the light chain variable region in each of the ~~antigen-binding molecules~~antibodies comprises at least one amino acid residue that changes the antigen-binding activity of the ~~antigen-binding molecule~~ antibody depending on calcium ion concentration conditions between a low-calcium concentration selected from the range of 0.1 μM to 30 μM and a high-calcium concentration selected from the range of 100 μM to 10 mM,

wherein the at least one amino acid residue is an amino acid having a calcium-chelating effect selected from serine, threonine, asparagine, glutamine, aspartic acid, and glutamic acid, and is located at any one or more of positions 30, 31, and 32 defined by the Kabat numbering in the CDR1 of the light chain variable region, amino acid position 50 defined by the Kabat numbering in the CDR2 of the light chain variable region and the amino acid position 92 defined by the Kabat numbering in the CDR3 of the light chain variable region.

~~[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 2~~17~~]

The library according to a~~ny of~~ claim~~s~~ 1~~2 and 9 to 16~~, wherein a light chain framework region in the ~~antigen-binding molecule~~antibody comprises a germline framework sequence.

[Claim 3~~18~~]

The library according to ~~any of~~ claim~~s~~ 1 or 2~~ and 9 to 17~~, wherein a heavy chain variable region of the ~~antigen-binding molecule~~antibody 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 4~~23~~]

A library consisting essentially of a plurality of fusion polypeptides each comprising ~~antigen-binding molecules~~antibodies according to any of claims 1 to 3~~22~~, wherein each of the fusion polypeptides

is a fusion product of a heavy chain variable region of the ~~antigen binding molecule~~antibody and at least a portion of a viral coat protein.

[Claim 5~~24~~]

The library according to claim 4~~23~~, 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 6~~25~~]

A composition comprising a plurality of polynucleotide molecules each encoding ~~antigen-binding molecules~~antibodies differing in sequence from each other according to any of claims 1 to 3~~22~~ or fusion polypeptides differing in sequence from each other according to claim 4~~23~~ or 5~~24~~.

[Claim 7~~26~~]

A composition comprising a plurality of vectors each comprising a plurality of polynucleotide molecules according to claim 6~~25~~ in an operably linked state.

[Claim 8~~27~~]

The composition according to claim 7~~26~~, wherein the vectors are replicable expression vectors.

[Claim 9~~28~~]

The composition according to claim 8~~27~~, 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 10~~29~~]

The composition according to claim 8~~27~~ or 9~~28~~, 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 11~~30~~]

A composition comprising a plurality of viruses each comprising vectors according to any of claims 7~~26~~ to 10~~29~~.

[Claim 12~~31~~]

A composition comprising a plurality of viruses each displaying on their surface ~~antigen-binding molecules~~antibodies differing in sequence from each other according to any of claims 1 to 3~~22~~ or fusion polypeptides differing in sequence from each other according to claim 4~~23~~ or 5~~24~~.

[Claim 13~~32~~]

A library comprising ~~antigen-binding molecules~~antibodies differing in sequence from each other according to any of claims 1 to 3~~22~~ or fusion polypeptides differing in sequence from each other according to claim 4~~23~~ or 5~~24~~, wherein the library has 1 x 10^6 to 1 x 10^{14} distinct variable region sequences.

[Claim 14~~33~~]

The library according to claim 5~~3~~13, wherein the library has 1 x 10^8 or more distinct variable region sequences.

[Claim 15~~34~~]

A method for preparing a library consisting essentially of a plurality of ~~antigen-binding molecules~~antibodies each comprising an antibody light chain variable region differing in sequence from each other, the method comprising producing a plurality of ~~antigen-binding molecules~~antibodies designed so that ~~an antigen-binding domain~~the light chain variable region in each of the ~~antigen-binding molecules~~antibodies comprises at least one amino acid residue that changes the antigen-binding activity of the ~~antigen-binding molecule~~antibody depending on calcium ion concentration conditions between a low-calcium concentration selected from the range of 0.1 μM to 30 μM and a high-calcium concentration selected from the range of 100 μM to 10 mM,

wherein the at least one amino acid residue is an amino acid having a calcium-chelating effect selected from serine, threonine, asparagine, glutamine, aspartic acid, and glutamic acid, and is located at any one or more of positions 30, 31, and 32 defined by the Kabat numbering in the CDR1 of the light chain variable region, amino acid position 50 defined by the Kabat numbering in the CDR2 of the light chain variable region and the amino acid position 92 defined by the Kabat numbering in the CDR3 of the light chain variable region.

[Claim 16~~35~~]

The preparation method according to claim 1534, wherein the ~~antigen-binding molecules~~antibodies are ~~antigen-binding molecules~~antibodies according to any of claims 12 to 322.

[Claim 1736]

The preparation method according to claim 1534 or 1635, wherein a heavy chain variable region of each of the ~~antigen-binding molecules~~antibodies is fused with at least a portion of a viral coat protein.

[Claim 1837]

The preparation method according to any of claims 1534 to 1736, 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 1938]

A method for selecting an ~~antigen-binding molecule~~antibody 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~~antibodies differing in sequence from each other according to any of claims 1 to 322 or fusion polypeptides differing in sequence from each other according to claim 423 or 524;

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~~antibodies whose antigen-binding activity is changed depending on the calcium ion concentration conditions; and

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

[Claim 2039]

A method for isolating a polynucleotide encoding an ~~antigen-binding molecule~~antibody 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~~antibodies differing in sequence from each other according to any of claims 1 to 3~~22~~ or fusion polypeptides differing in sequence from each other according to claim 4~~23~~ or 5~~24~~;

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~~antibodies 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 21~~40~~]

The method according to claim 20~~39~~, wherein the steps c) and d) are additionally repeated at least once.

~~[Claim 41]~~

~~The method according to any of claims 38 to 40, wherein the ion concentration is a calcium ion concentration.~~

[Claim 22~~42~~]

The method according to any one of claims ~~41~~19 to 21, wherein an ~~antigen-binding molecule~~antibody having lower antigen-binding activity under a low-calcium concentration condition than that under a high-calcium concentration condition is selected.

[Claim 23~~43~~]

The method according to claim 22~~42~~, wherein the low-calcium concentration condition is 0.1 μM to 30 μM.

[Claim 24~~44~~]

The method according to claim 22~~42~~ or 23~~43~~, wherein the high-calcium concentration condition is 100 μM to 10 mM.

[Claim 25~~45~~]

A method for producing an ~~antigen-binding molecule~~antibody 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~~antibodies differing in sequence from each other according to any of claims 1 to 3~~22~~ or fusion polypeptides differing in sequence from each other according to claim 4~~23~~ or 5~~24~~;

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~~antibodies 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~~antibodies from the cultures of the cell cultured in the step g).

[Claim 26~~46~~]

A method for producing an ~~antigen-binding molecule~~antibody 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~~antibodies differing in sequence from each other according to any of claims 1 to 3~~22~~ or fusion polypeptides differing in sequence from each other according to claim 4~~23~~ or 5~~24~~;

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~~antibodies 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~~antibodies from the cultures of the cell cultured in the step h).

[Claim 27~~47~~]

The production method according to claim 25~~45~~ or 26~~46~~, wherein the steps c) and d) are additionally repeated at least once.

~~[Claim 48]~~

~~The production method according to any of claims 45 to 47, wherein the ion concentration is a calcium ion concentration.~~

[Claim 28~~49~~]

The production method according to any one of claims ~~48~~25 to 27, wherein an ~~antigen-binding molecule~~antibody having lower antigen-binding

activity under a low-calcium concentration condition than that under a high-calcium concentration condition is selected.

[Claim 29~~50~~]

The production method according to claim 28~~49~~, wherein the low-calcium concentration condition is 0.1 μM to 30 μM.

[Claim 30~~51~~]

The production method according to claim 28~~49~~ or 29~~50~~, wherein the high-calcium concentration condition is 100 μM to 10 mM.

~~[Claim 51A]~~

~~An antigen-binding molecule produced by a production method according to any of claims 45 to 51.~~

~~[Claim 52]~~

~~A pharmaceutical composition comprising an antigen-binding molecule according to claim 51 or a modified form thereof.~~