which a single antigen-binding molecule can bind before its degradation; use of the modified FcRn-binding domain for improving pharmacokinetics of an antigen-binding molecule; and methods for producing said antigen-binding molecules.

Solution to Problem

[0010] The present inventors conducted dedicated studies on modified FcRn-binding domains which have an enhanced affinity for FcRn at neutral pH and on antigen-binding molecules comprising said FcRn-binding domain which have low immunogenicity, high stability and form only few aggregates. As a result, the present inventors discovered that substitutions at specific positions of the FcRn-binding domain increases the affinity for the FcRn at neutral pH without substantially increasing the immunogenicity, without substantially decreasing the stability and/or without substantially increasing the ratio of high molecular weight species.
Furthermore, the present inventors conducted dedicated studies on modified FcRn-binding domains with an enhanced affinity for FcRn at neutral pH or acidic pH but without a significantly increased binding activity for a pre-existing anti-drug antibody and on antigen-binding molecules comprising such an FcRn-binding domain. As a result, the present inventors discovered that substitutions at specific positions of the FcRn-binding domain decrease the affinity for a pre-existing anti-drug antibody at neutral pH without substantially decreasing the FcRn-binding activity.

In a first aspect, there is provided an antigen-binding molecule comprising a modified FcRn-binding domain, wherein the modified FcRn-binding domain comprises an amino acid substitution at one or more positions selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU258, EU286, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436, wherein the numbers indicate the position of the substitution according to the EU numbering, wherein the antigen-binding molecule has a binding activity for a pre-existing anti-drug antibody that is not significantly increased as compared to the binding affinity of a control antibody comprising an intact FcRn-binding domain, wherein the FcRn binding domain further comprises an amino acid substitution at one or more positions selected from the group consisting of EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440.

In a second aspect, there is provided the use of the antigen-binding molecule as disclosed herein in the manufacture of a medicament for improving antigen-binding molecule-mediated antigen uptake into cells.

In a third aspect, there is provided the use of the antigen-binding molecule as disclosed herein in the manufacture of a medicament for reducing the plasma concentration of a specific antigen, wherein the antigen-binding molecule comprises an antigen-binding domain which can bind said antigen.

In a fourth aspect, there is provided a method for improving the pharmacokinetics of an antigen-binding molecule, comprising the step of introducing an amino acid substitution into an FcRn-binding domain of said antigen-binding molecules at one or more of the positions selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU258, EU286, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436.

In a fifth aspect, there is provided a method for delaying the elimination of an antigen-binding molecule in a subject, comprising the step of introducing an amino acid substitution

into an FcRn-binding domain of said antigen-binding molecule at one or more of the positions selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU258, EU286, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436.

In a sixth aspect, there is provided a method of prolonging the plasma retention time of an antigen-binding molecule, comprising the step of introducing an amino acid substitution into an FcRn-binding domain of said antigen-binding molecule at one or more of the positions selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU258, EU286, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436.

In a seventh aspect, there is provided a method for increasing the plasma antigen-elimination rate of an antigen-binding molecule, comprising the step of introducing an amino acid substitution into an FcRn-binding domain of said antigen-binding molecule at one or more of the positions selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU258, EU286, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436.

In an eighth aspect, there is provided a method for increasing the ability of an antigen-binding molecule to eliminate plasma antigen, comprising the step of introducing an amino acid substitution into an FcRn-binding domain of said antigen-binding molecule at one or more of the positions selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU258, EU286, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436.

In a ninth aspect, there is provided a method for producing antigen-binding molecules as disclosed herein, which comprises the steps of: (a) selecting a parent FcRn-binding domain and altering the parent FcRn by introducing an amino acid substitution at one or more positions selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU258, EU286, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436; (b) selecting an antigen-binding domain of an antigen-binding molecule and altering at least one amino acid in the antigen-binding domain in order to get a pH-dependent antigen-binding domain or a calcium-ion dependent antigen-binding domain; (c) obtaining a gene encoding an antigen-binding molecule in which the human FcRn-binding domain and the antigen-binding domain prepared in (a) and (b) are linked; and (d) producing an antigen-binding molecule using the gene prepared in (c).

In a tenth aspect, there is provided an antigen-binding molecule comprising a modified FcRn binding domain, wherein the modified FcRn binding domain comprises an amino acid substitution at one or more of the positions selected from the group consisting of EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440, wherein the binding affinity of said antigen-binding molecule for a pre-existing anti-drug antibody (ADA) at a neutral pH is not significantly increased as compared to the binding affinity of antigen-binding molecule comprising an intact FcRn binding domain, wherein the antigen-binding molecule further has an increased binding affinity for an FcRn in the neutral or acidic pH ranges.

In an eleventh aspect, there is provided a method for decreasing the binding activity for a pre-existing ADA of an antibody comprising an FcRn binding domain having an increased binding activity for an FcRn at neutral or acidic pH and an increased binding activity for a pre-existing ADA at a neutral pH, said method comprising the steps of: a) providing an antibody with an FcRn binding domain having an increased binding activity for FcRn at neutral or acidic pH and an increased binding activity for a pre-existing ADA at a neutral

pH; and b) substituting an amino acid in the FcRn binding domain at one or more of the positions selected from the group consisting of EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440 to yield an antibody with a modified FcRn binding domain, such that the binding activity for a pre-existing ADA of the antibody will be decreased.

In a twelfth aspect, there is provided method for increasing the total number of antigens to which a single antibody can bind without significantly increasing the binding activity for a pre-existing ADA at neutral pH as compared to a parent antibody, said method comprising the steps of: a) providing an antibody comprising a parent FcRn binding domain, b) altering the parent FcRn binding domain of step a) by substituting an amino acid in the amino acid sequence of the parent FcRn binding domain at one or more of the positions selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU256, EU258, EU286, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436, such that binding activity for an FcRn at neutral pH will be increased; and c) altering the modified FcRn-binding domain of step b) by substituting an amino acid in the amino acid sequence of the parent FcRn-binding domain at one or more positions selected from the group consisting of EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440, such that the binding activity for a pre-existing ADA of the antibody will be decreased.

In a thirteenth aspect, there is provided a method for facilitating the extracellular release of an antigen-free antibody taken up into cells in an antigen-bound form without significantly increasing the binding activity of said antibody for a pre-existing ADA at neutral pH as compared to a parent antibody, comprising the steps of: a) providing an antigen-binding molecule comprising a parent FcRn-binding domain, b) altering the parent FcRn binding domain by substituting an amino acid in the amino acid sequence of the parent FcRn-binding domain at one or more positions selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU256, EU258, EU286, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436, and EU428, such that binding ativity for an FcRn at neutral pH will be increased.; and c) altering the modified FcRn-binding domain of step b) by substituting an amino acid in the amino acid sequence of the parent FcRn-binding domain at one or more positions selected from the group consisting of EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440, such that the binding activity for a pre-existing ADA of the antibody will be decreased.

In a fourteenth aspect, there is provided a method for increasing the ability of an antibody to eliminate plasma antigen without significantly increasing the binding activity for pre-existing ADA at neutral pH compared to parent antibody, said method comprising the steps of: a) providing an antibody comprising a parent FcRn-binding domain, b) altering the parent FcRn binding domain by substituting an amino acid in the amino acid sequence of the parent FcRn-binding domain at one or more positions selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU256, EU258, EU286, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436, and EU428, such that binding activity for an FcRn at neutral pH will be increased; and c) altering the modified FcRn-binding domain of step b) by substituting an amino acid in the amino acid sequence of the parent FcRn-binding domain at one or more positions selected from the group consisting of EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440, such that the binding activity for a pre-existing ADA of the antibody will be decreased.

In a fifteenth aspect, there is provided a method for improving the pharmacokinetics of an antibody without significantly increasing the binding activity for a pre-existing ADA at

neutral pH as compared to a parent antibody, said method comprising the steps of: a) providing an antibody comprising a parent FcRn-binding domain, b) altering the parent FcRn-binding domain by substituting an amino acid in the amino acid sequence of the parent FcRn-binding domain at one or more positions selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU256, EU258, EU286, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436, such that binding activity for an FcRn at neutral pH will be increased; and c) altering the modified FcRn-binding domain of step b) by substituting an amino acid in the amino acid sequence of the parent FcRn-binding domain at one or more positions selected from the group consisting of EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440, such that the binding activity for a pre-existing ADA of the antibody will be decreased.

In a sixteenth aspect, there is provided a method for reducing total or free antigen plasma concentration without significantly increasing the binding activity for a pre-existing ADA at neutral pH as compared to a parent antibody, said method comprising the steps of: a) providing an antibody comprising a parent FcRn-binding domain, wherein the antigen-binding molecule comprises an antigen-binding domain which can bind said antigen, b) altering the parent FcRn-binding domain by substituting an amino acid in the amino acid sequence of the parent FcRn-binding domain at one or more positions selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU256, EU258 EU286, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436, such that binding activity for an FcRn at neutral pH will be increased; and c) altering the modified FcRn-binding domain of step b) by substituting an amino acid in the amino acid sequence of the parent FcRn-binding domain at one or more positions selected from the group consisting of EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440, such that the binding activity for a pre-existing ADA of the antibody will be decreased.

In a seventeenth aspect, there is provided a method for producing an antibody comprising an FcRn binding domain having an increased binding activity for an FcRn at neutral or acidic pH and a decreased binding activity for an pre-existing ADA at neutral pH, said method comprising the steps of: (a) providing an FcRn binding domain having an increased binding activity for an FcRn at neutral or acidic pH ranges and pre-existing ADA at neutral pH ranges, (b) substituting an amino acid at one or more of the positions selected from the group consisting of EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440, such that the binding activity for a pre-existing ADA of the antibody will be decreased,

(c) selecting an antigen-binding domain of an antibody and altering at least one amino acid in the antigen-binding domain in order to get a pH-dependent antigen-binding domain, or selecting an calcium-ion dependent antigen-binding domain;
(d) obtaining a gene encoding an antibody in which the human FcRn-binding domain and the antigen-binding domain prepared in (a) and (b) are linked and
(e) producing an antibody using the gene prepared in (c), wherein said antibody produced has an increased binding activity for an FcRn at neutral or acidic pH and a decreased binding activity for an endogenous ADA at neutral pH as compared to the antigen-binding molecule of a).

[0011] Specifically, the present ~~invention~~ disclosure relates to:
[1] An antigen-binding molecule comprising a modified FcRn-binding domain, wherein the modified FcRn-binding domain comprises an amino acid substitution at one or more positions selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU258, EU286, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436, wherein the numbers indicate the position of the substitution according to the EU numbering.
[2] The antigen-binding molecule according to [1], wherein the FcRn-binding domain has
a) an amino acid substitution of the amino acid at position EU252 and EU434; and
b) an amino acid substitution at one or more positions selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU256, EU258, EU286, EU387, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436.
[3] The antigen-binding molecule according to [1] or [2], wherein the modified FcRn-binding domain comprises
at position EU238 an aspartic acid,
at position EU250 a valine,
at position EU252 a tyrosine,

[CLAIMS]

[Claim 1] An antigen-binding molecule comprising a modified FcRn-binding domain, wherein the modified FcRn-binding domain comprises an amino acid substitution at one or more positions selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU258, EU286, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436, wherein the numbers indicate the position of the substitution according to the EU numbering~~.~~,

wherein the antigen-binding molecule has a binding activity for a pre-existing anti-drug antibody that is not significantly increased as compared to the binding affinity of a control antibody comprising an intact FcRn-binding domain,

wherein the FcRn binding domain further comprises an amino acid substitution at one or more positions selected from the group consisting of EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440.

[Claim 2] The antigen-binding molecule according to claim 1, wherein the FcRn-binding domain has
a) an amino acid substitution of the amino acid at position EU252 and EU434; and
b) an amino acid substitution at one or more positions selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU256, EU258, EU286, EU387, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436.

[Claim 3] The antigen-binding molecule according to claim 1 or 2, wherein the modified FcRn-binding domain comprises
at position EU238 an aspartic acid,
at position EU250 a valine,
at position EU252 a tyrosine,
at position EU254 a threonine,
at position EU255 a leucine,
at position EU256 a glutamic acid,
at position EU258 an aspartic acid or an isoleucine,
at position EU286 a glutamic acid,
at position EU307 a glutamine,
at position EU308 a proline,
at position EU309 a glutamic acid,
at position EU311 an alanine or a histidine,
at position EU315 an aspartic acid,
at position EU428 an isoleucine,
at position EU433 an alanine, a lysine, a proline, an arginine, or a serine,
at position EU434 a tyrosine, or a tryptophan, and/or
at position EU436 an isoleucine, a leucine, a valine, a threonine, or a phenylalanine.

a) a lower binding activity for the antigen at pH 5.5-6.5 than at pH 7-8 or
b) a "calcium concentration-dependent binding" activity for the antigen.

[Claim 14] The antigen-binding molecule according to any one of claims 1 to 5, wherein the binding activity of said binding molecule for the FcRn at pH 7 is 50-150nM, Tm is higher than 63.0 degrees C, and Epibase score is less than 250.

[Claim 15] The antigen-binding molecule according to any one of claims 1 to 3 and claims 6 to 7, and wherein the binding activity of said binding molecule for FcRn at pH 7 is 15-50nM, Tm is higher than 60 degrees C, and Epibase score is less than 500.

[Claim 16] The antigen-binding molecule according to any one of claims 1 to 3 and claims 8 to 9, and wherein the binding activity of said the binding molecule for FcRn at pH 7 is stronger than 15nM, Tm is higher than 57.5 degrees C, and Epibase score is less than 500.

[Claim 17] The antigen-binding molecule according to any one of claims 1 to 3, wherein the FcRn-binding domain comprises an amino acid substitution
a) at the positions EU238, EU255 and/or EU258, and
b) at three or more positions, wherein the three or more positions are one of the combinations set forth in Tables 4 to 7.

[Claim 18] The antigen-binding molecule according to any one of claims 1 to 17, wherein
a) at the position EU257 of the FcRn-binding domain the amino acid is not an amino acid selected from the group consisting of alanine, valine, isoleucine, leucine, and threonine, and/or
b) at the position EU252 of the FcRn-binding domain the amino acid is not tryptophan.

~~[Claim 19] The antigen-binding molecule according to any one of claims 1 to 18, wherein the antigen-binding molecule has a binding activity for a pre-existing anti-drug antibody that is not significantly increased as compared to the binding affinity of a control antibody comprising an intact FcRn-binding domain.~~

~~[Claim 20] The antigen-binding molecule according to claim 19, wherein the FcRn binding domain further comprises an amino acid substitution at one or more positions selected from the group consisting of EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440.~~

[Claim ~~21~~19] The antigen-binding molecule according to claim ~~20~~1, wherein the FcRn

binding domain comprises one or more amino acid substitutions selected from the group consisting of
at position EU387 an arginine,
at position EU422 a glutamic acid, an arginine, or a serine, an aspartic acid, a lysine, a threonine or a glutamine;
at position EU424 a glutamic acid or an arginine, a lysine, or an asparagine;
at position EU426 an aspartic acid, a glutamine, an alanine, or a tyrosine;
at position EU433 an aspartic acid;
at position EU436 a threonine;
at position EU438 a glutamic acid, an arginine, a serine, or a lysine; and
at position EU440 a glutamic acid, aspartic acid or a glutamine.

[Claim ~~2220~~] The antigen-binding molecule according to any one of claim 1 to ~~2119~~, wherein the modified FcRn binding domain comprises three or more substitutions, wherein the three or more substitutions are one of the combinations set forth in Tables 12 to 13.

[Claim ~~2321~~] The antigen-binding molecule according to any one of claims 1 to ~~2220~~, wherein the modified FcRn-binding domain comprises three or more substitutions, wherein the three or more substitutions are one of the combinations set forth in Tables 14 to 15.

[Claim ~~2422~~] The antigen-binding molecule according to any one of claims ~~20-1~~ to ~~2321~~, wherein the FcRn-binding domain comprises:
a) at position EU252 a tyrosine, at position EU387 an arginine, at position EU434 a tyrosine, and at position EU436 a valine; or
b) at position EU252 a tyrosine, at position EU422 a glutamic acid, at position EU434 a tyrosine, and at position EU436 a valine; or
c) at position EU252 a tyrosine, at position EU422 an arginine, at position EU434 a tyrosine, and at position EU436 a valine; or
d) at position EU252 a tyrosine, at position EU422 a serine, at position EU434 a tyrosine, and at position EU436 a valine; or
e) at position EU252 a tyrosine, at position EU424 a glutamic acid, at position EU434 a tyrosine, and at position EU436 a valine; or
f) at position EU252 a tyrosine, at position EU424 an arginine, at position EU434 a tyrosine, and at position EU436 a valine; or
g) at position EU252 a tyrosine, at position EU434 a tyrosine , at position EU436 a valine, and at position EU438 a glutamic acid; or

h) at position EU252 a tyrosine, at position EU434 a tyrosine , at position EU436 a valine, and at position EU438 an arginine; or
i) at position EU252 a tyrosine, at position EU434 a tyrosine, at position EU436 a valine, and at position EU438 a serine; or
j) at position EU252 a tyrosine, at position EU434 a tyrosine, at position EU436 a valine, and at position EU440 a glutamic acid.

[Claim ~~25~~23] The antigen-binding molecule according to any one of claims 1 to ~~24~~22, wherein the antigen-binding molecule is an antibody.

[Claim ~~26~~24] Use of the antigen-binding molecule according to any one of claims 1 to ~~25~~ 23 in the manufacture of a medicament for improving antigen-binding molecule-mediated antigen uptake into cells.

[Claim ~~27~~25] Use of the antigen-binding molecule according to any one of claims 1 to ~~25~~ 23 in the manufacture of a medicament for reducing the plasma concentration of a specific antigen, wherein the antigen-binding molecule comprises an antigen-binding domain which can bind said antigen.

[Claim ~~28~~26] A method for improving the pharmacokinetics of an antigen-binding molecule, comprising the step of introducing an amino acid substitution into an FcRn-binding domain of said antigen-binding molecules at one or more of the positions selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU258, EU286, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436.

[Claim ~~29~~27] A method for delaying the elimination of an antigen-binding molecule in a subject, comprising the step of introducing an amino acid substitution into an FcRn-binding domain of said antigen-binding molecule at one or more of the positions selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU258, EU286, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436.

[Claim ~~30~~28] A method of prolonging the plasma retention time of an antigen-binding molecule, comprising the step of introducing an amino acid substitution into an FcRn-binding domain of said antigen-binding molecule at one or more of the positions selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU258, EU286, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436.

[Claim ~~31~~29] A method for increasing the plasma antigen-elimination rate of an antigen-binding molecule, comprising the step of introducing an amino acid substitution into an FcRn-binding domain of said antigen-binding molecule at one or more of the positions selected from the group

consisting of EU238, EU250, EU252, EU254, EU255, EU258, EU286, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436.

[Claim 3230] A method for increasing the ability of an antigen-binding molecule to eliminate plasma antigen, comprising the step of introducing an amino acid substitution into an FcRn-binding domain of said antigen-binding molecule at one or more of the positions selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU258, EU286, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436.

[Claim 3331] The method according to any one of claims 28-26 to 3230, wherein further an amino acid substitution at position EU256 into the FcRn binding domain is introduced.

[Claim 3432] The method according to any one of claims 28-26 to 3331, wherein the method further comprises a step of introducing into the FcRn-binding domain an amino acid substitution at one or more positions selected from the group consisting of EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440.

[Claim 3533] A method for producing antigen-binding molecules according to any one of claims 1 to 2523, which comprises the steps of
(a) selecting a parent FcRn-binding domain and altering the parent FcRn by introducing an amino acid substitution at one or more positions selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU258, EU286, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436;
(b) selecting an antigen-binding domain of an antigen-binding molecule and altering at least one amino acid in the antigen-binding domain in order to get a pH-dependent antigen-binding domain or a calcium-ion dependent antigen-binding domain;
(c) obtaining a gene encoding an antigen-binding molecule in which the human FcRn-binding domain and the antigen-binding domain prepared in (a) and (b) are linked and
(d) producing an antigen-binding molecule using the gene prepared in (c).

[Claim 3634] The method according to claim 3533, wherein in step a) further an amino acid substitution at position EU256 into the FcRn binding domain is introduced.

[Claim 3735] The method according to any one of claims 35-33 to 3634, wherein the

method further comprises a step of introducing in the FcRn-binding domain an amino acid substitution at one or more positions selected from the group consisting of EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440.

[Claim 3836] An antigen-binding molecule comprising a modified FcRn binding domain, wherein the modified FcRn binding domain comprises an amino acid substitution at one or more of the positions selected from the group consisting of EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440, wherein the binding affinity of said antigen-binding molecule for a pre-existing anti-drug antibody (ADA) at a neutral pH is not significantly increased as compared to the binding affinity of antigen-binding molecule comprising an intact FcRn binding domain.,

wherein the antigen-binding molecule further has an increased binding affinity for an FcRn in the neutral or acidic pH ranges.

~~[Claim 39] The antigen-binding molecule according to claim 38 wherein the antigen-binding molecule further has an increased binding affinity for an FcRn in the neutral or acidic pH ranges.~~

[Claim 4037] The antigen-binding molecule according to claim 3638 ~~or 39~~, wherein the amino acid substituting one or more of the positions selected from the group consisting of EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440 is selected from the group consisting of
a) at position EU387 an arginine;
b) at position EU422 a glutamic acid, an arginine, a serine, aspartic acid, lysine, threonine, or glutamine;
c) at position EU424 a glutamic acid, an arginine, a lysine, or asparagines;
d) at position EU426 an aspartic acid, a glutamine, an alanine, or a tyrosine;
e) at position EU433 an aspartic acid
f) at position EU436 a threonine
g) at position EU438 a glutamic acid, an arginine, a serine, or a lysine; and
h) at position EU440 a glutamic acid, an aspartic acid, or a glutamine.

[Claim 4138] The antigen-binding molecule according to any one of claims ~~38~~ 36 to 4037, wherein the modified FcRn binding domain comprises an amino acid substitution at one or more positions or one of the combinations set forth in Table 10.

[Claim 4239] The antigen-binding molecule according to any one of claims ~~38~~ 36 to 4037,

wherein the modified FcRn binding domain comprises any one of the amino acid substitution or substitution combinations set forth in Table 11.

[Claim 4340] The antigen-binding molecule according to any one of claims ~~39~~ 36 to ~~42~~39, wherein the modified FcRn binding domain further comprises an amino acid substitution at one or more positions of the FcRn binding domain selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU256, EU258, EU286, EU307, EU308, EU309, EU311, EU315, EU428, EU434, and EU436, wherein said substitutions confer an increase in FcRn binding activity in the neutral pH or acidic pH range.

[Claim 4441] The antigen-binding molecule according to any one of claims ~~39~~ 36 to ~~43~~40, wherein the modified FcRn binding domain comprises amino acid substitutions at the FcRn binding domain positions
i) a) EU438 / EU440 or b) EU424; and
ii) a) EU434, b) EU252/EU254/EU256; c) EU428/EU434; or d) EU250/EU428.

[Claim ~~45~~42] The antigen-binding molecule according to claim 4441, wherein the modified FcRn binding domain comprises amino acid substitutions
i) a) EU438R/EU440E or b) EU424N; and
ii) a) M434H; b) M252Y/S254T/T256E; c) M428L/N434S; or d) T250Q and M428L (EU numbering).

[Claim ~~46~~43] The antigen-binding molecule according to claim ~~45~~42, wherein the modified FcRn binding domain comprises three or more amino acid substitutions wherein the three or more substitutions are one of the combinations set forth in Tables 13 and 15.

[Claim ~~47~~44] The antigen-binding molecule according to any one of claims ~~39~~ 36 to ~~42~~ 39 wherein the modified FcRn binding domain comprises substitutions
a) at one or more of the positions selected from the group consisting of EU387, EU422, EU424, EU438, EU440, EU433, or at two or more positions wherein the two positions are one of the combinations of the group consisting of EU422/EU424, and EU438/EU440; and
b) two or more positions wherein the two positions are one of the combinations set forth in Table 9.

[Claim ~~48~~45] The antigen-binding molecule according to claim ~~47~~44, wherein the modified FcRn binding domain comprises three or more the amino acid substitutions wherein the three or more the amino acid substitutions are one of the combinations set forth in Tables 12 or 14.

[Claim ~~49~~46] The antigen-binding molecule of any one of claims ~~39~~ 36 to ~~48~~ 45 wherein

said antigen-binding molecule comprises a pH-dependent antigen-binding domain or a calcium ion-dependent antigen-binding domain.

[Claim ~~5047~~] A method for decreasing the binding activity for a pre-existing ADA of an ~~antigen-binding molecule~~ antibody comprising an FcRn binding domain having an increased binding activity for an FcRn at neutral or acidic pH and an increased binding activity for a pre-existing ADA at a neutral pH, said method comprising the steps of
a) providing an ~~antigen-binding molecule~~ antibody with an FcRn binding domain having an increased binding activity for FcRn at neutral or acidic pH and an increased binding activity for a pre-existing ADA at a neutral pH; and
b) substituting an amino acid in the FcRn binding domain at one or more of the positions selected from the group consisting of EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440 to yield an antibody ~~antigen-binding molecule~~ with a modified FcRn binding domain~~.~~, such that the binding activity for a pre-existing ADA of the antibody will be decreased.

[Claim ~~5148~~] The method according to claim ~~5047~~, wherein step b) comprises substituting an amino acid at three or more positions wherein the three or more positions are one of the combinations set forth in Table 10.

[Claim ~~5249~~] The method according to claim ~~5047~~, wherein step b) comprises introducing three or more the amino acid substitutions into the FcRn-binding domain wherein the three or more the amino acid substitutions are one of the combinations set forth in Table 11.

[Claim ~~5350~~] A method for increasing the total number of antigens to which a single antibody ~~antigen-binding molecule~~ can bind without significantly increasing the binding activity for a pre-existing ADA at neutral pH as compared to a parent antibody, said method comprising the steps of
a) providing an antibody ~~antigen-binding molecule~~ comprising a parent FcRn binding domain,
b) altering the parent FcRn binding domain of step a) by substituting an amino acid in the amino acid sequence of the parent FcRn binding domain at one or more of the positions selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU256, EU258, EU286, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436, such that binding activity for an FcRn at neutral pH will be increased; and
c) altering the modified FcRn-binding domain of step b) by substituting an amino acid in the amino acid sequence of the parent FcRn-binding domain at one or more positions selected from the group consisting of EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440, such that the binding activity for a pre-existing ADA of the antibody will be decreased.

[Claim ~~54~~51] A method for facilitating the extracellular release of an antigen-free antibody ~~antigen-binding molecule~~ taken up into cells in an antigen-bound form without significantly increasing the binding activity of said antibody ~~antigen-binding molecule~~ for a pre-existing ADA at neutral pH as compared to a parent antibody, comprising the steps of
a) providing an antigen-binding molecule comprising a parent FcRn-binding domain,
b) altering the parent FcRn binding domain by substituting an amino acid in the amino acid sequence of the parent FcRn-binding domain at one or more positions selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU256, EU258, EU286, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436, and EU428, such that binding ativity for an FcRn at neutral pH will be increased.; and
c) altering the modified FcRn-binding domain of step b) by substituting an amino acid in the amino acid sequence of the parent FcRn-binding domain at one or more positions selected from the group consisting of EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440, such that the binding activity for a pre-existing ADA of the antibody will be decreased.

[Claim ~~55~~52] A method for increasing the ability of an antibody ~~antigen-binding molecule~~ to eliminate plasma antigen without significantly increasing the binding activity for pre-existing ADA at neutral pH compared to parent antibody, said method comprising the steps of
a) providing an antibody ~~antigen-binding molecule~~ comprising a parent FcRn-binding domain,
b) altering the parent FcRn binding domain by substituting an amino acid in the amino acid sequence of the parent FcRn-binding domain at one or more positions selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU256, EU258, EU286, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436, and EU428, such that binding activity for an FcRn at neutral pH will be increased; and
c) altering the modified FcRn-binding domain of step b) by substituting an amino acid in the amino acid sequence of the parent FcRn-binding domain at one or more positions selected from the group consisting of EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440, such that the binding activity for a pre-existing ADA of the antibody will be decreased.

[Claim ~~56~~53] A method for improving the pharmacokinetics of an antibody ~~antigen-binding molecule~~ without significantly increasing the binding activity for a pre-existing ADA at neutral pH as compared to a parent antibody, said method comprising the steps of
a) providing an antibody ~~antigen-binding molecule~~ comprising a parent

FcRn-binding domain,
b) altering the parent FcRn-binding domain by substituting an amino acid in the amino acid sequence of the parent FcRn-binding domain at one or more positions selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU256, EU258, EU286, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436, such that binding activity for an FcRn at neutral pH will be increased; and
c) altering the modified FcRn-binding domain of step b) by substituting an amino acid in the amino acid sequence of the parent FcRn-binding domain at one or more positions selected from the group consisting of EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440, such that the binding activity for a pre-existing ADA of the antibody will be decreased.

[Claim ~~5754~~] A method for reducing total or free antigen plasma concentration without significantly increasing the binding activity for a pre-existing ADA at neutral pH as compared to a parent antibody, said method comprising the steps of
a) providing an antibody ~~antigen-binding molecule~~ comprising a parent FcRn-binding domain, wherein the antigen-binding molecule comprises an antigen-binding domain which can bind said antigen,
b) altering the parent FcRn-binding domain by substituting an amino acid in the amino acid sequence of the parent FcRn-binding domain at one or more positions selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU256, EU258 EU286, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436, such that binding activity for an FcRn at neutral pH will be increased; and
c) altering the modified FcRn-binding domain of step b) by substituting an amino acid in the amino acid sequence of the parent FcRn-binding domain at one or more positions selected from the group consisting of EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440, such that the binding activity for a pre-existing ADA of the antibody will be decreased.

[Claim ~~5855~~] A method for producing an antibody ~~antigen-binding molecule~~ comprising an FcRn binding domain having an increased binding activity for an FcRn at neutral or acidic pH and a decreased binding activity for an pre-existing ADA at neutral pH, said method comprising the steps of
(a) providing an FcRn binding domain having an increased binding activity for an FcRn at neutral or acidic pH ranges and pre-existing ADA at neutral pH ranges,
(b) substituting an amino acid at one or more of the positions selected from the group consisting of EU387, EU422, EU424, EU426, EU433,

EU436, EU438 and EU440, such that the binding activity for a pre-existing ADA of the antibody will be decreased,

(c) selecting an antigen-binding domain of an ~~antigen-binding molecule~~antibody and altering at least one amino acid in the antigen-binding domain in order to get a pH-dependent antigen-binding domain, or selecting an calcium-ion dependent antigen-binding domain;
(d) obtaining a gene encoding an ~~antigen-binding molecule~~antibody in which the human FcRn-binding domain and the antigen-binding domain prepared in (a) and (b) are linked and
(e) producing an ~~antigen-binding molecule~~antibody using the gene prepared in (c), wherein said ~~antigen-binding molecule~~antibody produced has an increased binding activity for an FcRn at neutral or acidic pH and a decreased binding activity for an endogenous ADA at neutral pH as compared to the antigen-binding molecule of a).

[Claim ~~59~~56] The method according to claim ~~58~~55, wherein the FcRn binding domain having an increased binding activity for FcRn and pre-existing ADA at neutral or acidic pH ranges and for pre-existing ADA in the neutral pH ranges comprises an amino acid substitution at one or more positions selected from the group consisting of EU238, EU250, EU252, EU254, EU255, EU256, EU258, EU286, EU307, EU308, EU309, EU311, EU315, EU428, EU433, EU434, and EU436.

[Claim ~~60~~57] The method according to any one of claims ~~53-~~50 to ~~57~~54, wherein the amino acid substitution introduced in step a) are at three or more positions wherein said three or more positions are one of the combinations set forth in Tables 4 to 7.

[Claim ~~61~~58] The method according to any one of claims ~~53-~~50 to ~~60~~57, wherein the amino acid substitutions introduced in step b) are at three or more positions wherein said three or more positions are one of the combinations set forth in Table 10.