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Summary ~~of Invention~~

Technical Problem

[0009] The present ~~invention~~disclosure was conceived in view of the circumstances described above. An objective of the present ~~invention~~disclosure is to provide a modified FcRn-binding domain which has an enhanced affinity for the FcRn at neutral pH; an antigen-binding molecule comprising said FcRn-binding domain, wherein said antigen-binding molecule has low immunogenicity, high stability and forms only few aggregates; a modified antigen-binding molecule having an increased FcRn-binding activity at neutral or acidic pH without an increased binding activity at neutral pH for a pre-existing anti-drug antibody; use of the antigen-binding molecules for improving antigen-binding molecule-mediated antigen uptake into cells; use of the antigen-binding molecule for reducing the plasma concentration of a specific antigen; use of the modified FcRn-binding domain for increasing the total number of antigens to

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 improved pharmacokinetics compared to a control antibody comprising an intact FcRn binding domain, wherein a binding activity of the antigen-binding molecule ~~has a binding activity~~ for a pre-existing anti-drug antibody (ADA)~~that~~ is not significantly increased as compared to ~~the~~a binding affinity of ~~the~~a control antibody comprising an intact FcRn-binding domain for ADA, 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 for treating an autoimmune disease, a transplant rejection (graft-vs.-host disease), an inflammatory disease or an allergic disease; wherein the medicament improvesing 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 treating an autoimmune disease, a transplant rejection (graft-vs.-host disease), an inflammatory disease or an allergic disease; wherein the medicament reducesing 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 without significantly increasing the binding activity of said antigen-binding molecule for a pre-existing anti-drug antibody (ADA), comprising the steps of a) 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; and b) introducing further 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 EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440.

In a fifth aspect, there is provided a method for delaying the elimination of an antigen-binding molecule in a subject without significantly increasing the binding activity of said antigen-binding molecule for a pre-existing anti-drug antibody (ADA), comprising the steps of a) 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; and b) introducing further 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 EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440.

In a sixth aspect, there is provided a method of prolonging the plasma retention time of an antigen-binding molecule without significantly increasing the binding activity of said antigen-binding molecule for a pre-existing anti-drug antibody (ADA), comprising the steps of a) 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; and b) introducing further 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 EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440.

In a seventh aspect, there is provided a method for increasing the plasma antigen-elimination rate of an antigen-binding molecule without significantly increasing the binding activity of said antigen-binding molecule for a pre-existing anti-drug antibody (ADA), comprising the steps of a) 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; and b) introducing further 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 EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440.

In an eighth aspect, there is provided a method for increasing the ability of an antigen-binding molecule to eliminate plasma antigen without significantly increasing the binding activity of said antigen-binding molecule for a pre-existing anti-drug antibody (ADA), comprising the steps of a) 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; and b) introducing further 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 EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440.

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~~one embodiment, 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~~one embodiment, 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~~one embodiment, 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~~one embodiment, 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~~embodiment, 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~~one embodiment, 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~~embodiment, 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).

[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 improved pharmacokinetics compared to a control antibody comprising an intact FcRn binding domain,

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

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.

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

[Claim 24] Use of the antigen-binding molecule according to any one of claims 1 to 23 in the manufacture of a medicament for treating an autoimmune disease, a transplant rejection (graft-vs.-host disease), an inflammatory disease or an allergic disease; wherein the medicament improves~~ing~~ antigen-binding molecule-mediated antigen uptake into cells.

[Claim 25] Use of the antigen-binding molecule according to any one of claims 1 to 23 in the manufacture of a medicament for treating an autoimmune disease, a transplant rejection (graft-vs.-host disease), an inflammatory disease or an allergic disease; wherein the medicament reduces~~ing~~ the plasma concentration of a specific antigen, wherein the antigen-binding molecule comprises an antigen-binding domain which can bind said antigen.

[Claim 26] A method for improving the pharmacokinetics of an antigen-binding molecule without significantly increasing the binding activity of said antigen-binding molecule for a pre-existing anti-drug antibody (ADA), comprising the steps of a) 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; and b) introducing further 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 EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440.

[Claim 27] A method for delaying the elimination of an antigen-binding molecule in a subject without significantly increasing the binding activity of said antigen-binding molecule for a pre-existing anti-drug antibody (ADA), comprising the steps of a) 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; and b) introducing further 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 EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440.

[Claim 28] A method of prolonging the plasma retention time of an antigen-binding molecule without significantly increasing the binding activity of said antigen-binding molecule for a pre-existing anti-drug antibody (ADA), comprising the steps of a) 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; and b) introducing further 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 EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440.

[Claim 29] A method for increasing the plasma antigen-elimination rate of an antigen-binding molecule without significantly increasing the binding activity of said antigen-binding molecule for a pre-existing anti-drug antibody (ADA), comprising the steps of a) 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; and b) introducing further 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 EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440.

[Claim 30] A method for increasing the ability of an antigen-binding molecule to eliminate plasma antigen without significantly increasing the binding activity of said antigen-binding molecule for a pre-existing anti-drug antibody (ADA), comprising the steps of a) 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; and b) introducing further 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 EU387, EU422, EU424, EU426, EU433, EU436, EU438 and EU440.

[Claim 31] The method according to any one of claims 26 to 30, wherein further an amino acid substitution at position EU256 into the FcRn binding domain is introduced.

~~[Claim 32] The method according to any one of claims 26 to 31, 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 ~~33~~32] A method for producing antigen-binding molecules according to any one of claims 1 to 23, 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 ~~34~~33] The method according to claim ~~33~~32, wherein in step a) further an amino acid substitution at position EU256 into the FcRn binding domain is introduced.

[Claim ~~35~~34] The method according to any one of claims ~~33~~32 to ~~34~~33, 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 ~~36~~35] 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 ~~37~~36] The antigen-binding molecule according to claim ~~36~~35, 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 ~~38~~37] The antigen-binding molecule according to any one of claims ~~36~~35 to ~~37~~36, 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 ~~39~~38] The antigen-binding molecule according to any one of claims ~~36~~35 to ~~37~~36,

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

[Claim 4039] The antigen-binding molecule according to any one of claims 3635 to 3938, 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 4140] The antigen-binding molecule according to any one of claims 3635 to 4039, 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 4241] The antigen-binding molecule according to claim 4140, 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 4342] The antigen-binding molecule according to claim 4241, 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 4443] The antigen-binding molecule according to any one of claims 3635 to 3938 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 4544] The antigen-binding molecule according to claim 4443, 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 4645] The antigen-binding molecule of any one of claims 3635 to 4544 wherein

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

~~[Claim 47] 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.~~

~~[Claim 48] The method according to claim 47, 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 49] The method according to claim 47, 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 50] A 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.~~

~~[Claim 51] 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.

[Claim 52] 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.

[Claim 53] 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.

[Claim 54] 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.

[Claim 55] 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).

[Claim 56] The method according to claim 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 57] The method according to any one of claims 50 to 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 58] The method according to any one of claims 50 to 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.