WHAT IS CLAIMED IS:

1. A composition, comprising a physiologically active polypeptide-immunoglobulin Fc fragment conjugate which maintains the intrinsic binding affinity of the immunoglobulin Fc fragment for FcRn,

the conjugate comprising only one molecule of a physiologically active polypeptide is linked via a non-peptidyl linker having two ends to an immunoglobulin Fc fragment comprising an FcRn-binding region,

wherein the immunoglobulin Fc fragment is not further modified with a non-peptidyl polymer and the immunoglobulin Fc fragment is an IgG4 Fc fragment,

and a binding ratio of the amount of the conjugate bound to FcRn at pH 6.0 and the amount of the conjugate bound to FcRn at pH 7.4 is within the range of ± 6% of a binding ratio of the immunoglobulin Fc fragment determined under the same conditions as those used for the conjugate, wherein the binding ratio of the conjugate and the binding ratio of the immunoglobulin Fc fragment are determined using the following equation:

Equation 1

Binding ratio (%) = (amount bound to FcRn at pH 7.4/ amount bound to FcRn at pH 6.0) X 100,

wherein the composition comprises only monomeric forms of said conjugate; and

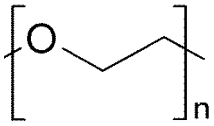
wherein the physiologically active polypeptide is selected from the group consisting of glucagon-like peptide-1 (GLP-1), erythropoietin (EPO), glucagon, oxyntomodulin, insulin, growth hormone releasing hormone, growth hormone releasing peptide, interferon receptors, G-protein-coupled receptor, interleukins, interleukin receptors, interleukin binding proteins, cytokine binding proteins, macrophage activating factor, macrophage peptide, B cell factor, T cell factor, protein A, allergy inhibitor, cell necrosis glycoproteins, immunotoxin, lymphotoxin, tumor necrosis factor, tumor suppressors, metastasis growth factor, alpha-1 antitrypsin, albumin, α-lactalbumin, apolipoprotein-E, highly glycosylated erythropoietin, angiopoietins, hemoglobin, thrombin, thrombin receptor activating peptide, thrombomodulin, blood factors VII, VIIa, VIII, IX and XIII, plasminogen activating factor, fibrin-binding peptide, urokinase, streptokinase, hirudin, protein C, C-reactive protein, renin inhibitor, collagenase inhibitor, superoxide dismutase, leptin, platelet-derived growth factor, epithelial growth factor, epidermal growth factor, angiostatin, angiotensin, bone growth factor, bone stimulating protein, calcitonin, atriopeptin, cartilage inducing factor, elcatonin, connective tissue activating factor, tissue factor pathway inhibitor, follicle stimulating hormone, luteinizing hormone, luteinizing hormone releasing hormone, nerve growth factors, parathyroid hormone, relaxin, secretin, somatomedin, insulin-like growth factor, adrenocortical hormone, cholecystokinin, pancreatic polypeptide, gastrin releasing peptide, corticotropin releasing factor, thyroid stimulating hormone, autotaxin, lactoferrin, myostatin, cell surface antigens, virus derived vaccine antigens, hormone, cytokine, and incretin.

2. The composition of claim 1, wherein the conjugate is obtained by reacting the physiologically active polypeptide having the non-peptidyl linker linked thereto with the immunoglobulin Fc fragment at a pH of 4.0-9.0, thereby linking the physiologically active polypeptide via the non-peptidyl linker to a portion excluding the FcRn-binding region of the immunoglobulin Fc fragment.

3. The composition of claim 1, wherein the non-peptidyl linker is selected from the group consisting of polyethylene glycol, polypropylene glycol, an ethylene glycol-propylene glycol copolymer, polyoxyethylated polyol, polyvinyl alcohol, polysaccharide, dextran, polyvinyl ethyl ether, a biodegradable polymer, a lipid polymer, chitin, hyaluronic acid, and combinations thereof.

4. The composition of claim 1, wherein the non-peptidyl linker is a polyethylene glycol polymer represented by the following formula 1:

Formula 1



wherein n ranges from 10 to 2400.

5. The composition of claim 1, wherein the non-peptidyl linker has a reactive group selected from the group consisting of an aldehyde group, a propionaldehyde group, a butyraldehyde group, a maleimide group, and succinimide derivatives.

6. The composition of claim 1, wherein the immunoglobulin Fc fragment comprising the FcRn-binding region comprises a CH2 domain, a CH3 domain, or both.

7. The composition of claim 1, wherein the immunoglobulin Fc fragment is in a non-glycosylated form.

8. The composition of claim 1, wherein the immunoglobulin Fc fragment further comprises a hinge region.

9. The composition of claim 1, wherein the physiologically active polypeptide is hormone, cytokine, or incretin.

10. The composition of claim 1, wherein the physiologically active polypeptide is insulin, or glucagon-like peptide-1 receptor agonist.

11. The composition of claim 10, wherein the glucagon-like peptide-1 receptor agonist is desamino-histidyl-exendin-4, beta-hydroxy imidazopropionyl-

exendin-4, beta-carboxy imidazopropionyl-exendin-4, dimethyl-histidyl-exendin-4, or imidazo-acetyl-exendin-4.

12. A method for preparing composition of claim 1, the method comprising:

(a) linking a physiologically active polypeptide via a non-peptidyl linker to an immunoglobulin Fc fragment comprising an FcRn-binding region to prepare a mixture of physiologically active polypeptide-immunoglobulin Fc fragment conjugates; and

(b) separating from the mixture physiologically active polypeptide-immunoglobulin Fc fragment conjugates that comprise only one molecule of a physiologically active polypeptide linked via a non-peptidyl linker to an immunoglobulin Fc fragment comprising an FcRn-binding region and which show a binding ratio within the range of ± 6% of the binding ratio of the immunoglobulin Fc fragment, as determined by substituting the amount of binding to FcRn at pH 6.0 and the amount of binding to FcRn at pH 7.4 into the following equation 1:

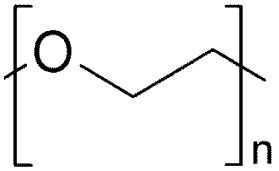
Equation 1

Binding ratio (%) = (amount bound to FcRn at pH 7.4/ amount bound to FcRn at pH 6.0) X 100,

wherein the physiologically active polypeptide is selected from the group consisting of glucagon-like peptide-1 (GLP-1), erythropoietin (EPO), glucagon, oxyntomodulin, insulin, growth hormone releasing hormone, growth hormone releasing peptide, interferon receptors, G-protein-coupled receptor, interleukins, interleukin receptors, interleukin binding proteins, cytokine binding proteins, macrophage activating factor, macrophage peptide, B cell factor, T cell factor, protein A, allergy inhibitor, cell necrosis glycoproteins, immunotoxin, lymphotoxin, tumor necrosis factor, tumor suppressors, metastasis growth factor, alpha-1 antitrypsin, albumin, α-lactalbumin, apolipoprotein-E, highly glycosylated erythropoietin, angiopoietins, hemoglobin, thrombin, thrombin receptor activating peptide, thrombomodulin, blood factors VII, VIIa, VIII, IX and XIII, plasminogen activating factor, fibrin-binding peptide, urokinase, streptokinase, hirudin, protein C, C-reactive protein, renin inhibitor, collagenase inhibitor, superoxide dismutase, leptin, platelet-derived growth factor, epithelial growth factor, epidermal growth factor, angiostatin, angiotensin, bone growth factor, bone stimulating protein, calcitonin, atriopeptin, cartilage inducing factor, elcatonin, connective tissue activating factor, tissue factor pathway inhibitor, follicle stimulating hormone, luteinizing hormone, luteinizing hormone releasing hormone, nerve growth factors, parathyroid hormone, relaxin, secretin, somatomedin, insulin-like growth factor, adrenocortical hormone, cholecystokinin, pancreatic polypeptide, gastrin releasing peptide, corticotropin releasing factor, thyroid stimulating hormone, autotaxin, lactoferrin, myostatin, cell surface antigens, virus derived vaccine antigens, hormone, cytokine, and incretin.

13. The method of claim 12, wherein the non-peptidyl linker is a polyethylene glycol polymer represented by the following formula 1:

Formula 1



wherein n ranges from 10 to 2400.

14. The method of claim 12, wherein the conjugate separated in step (b) has a structure in which the non-peptidyl linker is bound to the N-terminus of the immunoglobulin Fc fragment.

15. The method of claim 12, step (b) comprises determining a binding ratio of the conjugate and a binding ratio of the immunoglobulin Fc fragment using the equation 1 before separating the physiologically active polypeptide-immunoglobulin Fc fragment conjugate,

16. The method of claim 12, wherein the physiologically active polypeptide is hormone, cytokine, or incretin.

17. The method of claim 12, wherein the physiologically active polypeptide is insulin, or glucagon-like peptide-1 receptor agonist.

18. The method of claim 17, wherein the glucagon-like peptide-1 receptor agonist is desamino-histidyl-exendin-4, beta-hydroxy imidazopropionyl-exendin-4, beta-carboxy imidazopropionyl-exendin-4, dimethyl-histidyl-exendin-4, or imidazo-acetyl-exendin-4.

19. The method of claim 12, wherein

(a) the immunoglobulin Fc fragment comprising the FcRn-binding region comprises a CH2 domain, a CH3 domain, or both;

(b) the immunoglobulin Fc fragment is in a non-glycosylated form;

(c) the immunoglobulin Fc fragment further comprises a hinge region;

(d) the immunoglobulin Fc fragment is a Fc fragment derived from the group consisting of IgG, IgA, IgD, IgE, IgM, combinations thereof, and hybrids thereof; or

(e) the immunoglobulin Fc fragment is an IgG4 Fc fragment.