Claims

1. A composition of the formula:
[FGF21-1st Linker]-[Ab]-[2nd-Linker-Ex4]; wherein
FGF21 is an FGF21 homologue; and
Ex4 is an Exendin4 homologue; and
Ab is an aldolase catalytic antibody or antigen binding portion thereof; and
the 1st linker is covalently attached to the side chain of a protein-linking residue in FGF21 and to a combining site of the antibody, and
the 2nd linker is covalently attached to the side chain of a peptide-linking residue in Ex4 and to a combining site of the antibody, and wherein the first and second linker are the same or different; or a salt, solvate, stereoisomer or tautomer thereof.

2. The composition as claimed in claim 1, wherein the 1st linker is covalently attached to the FGF21 homologue through the side chain of a protein-linking amino acid residue located at one of positions 129, 125 or 79 according to the numbering of SEQ ID NO:3.

3. The composition as claimed in any one of the preceding claims, wherein the FGF21 homologue comprises a sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, and SEQ ID NO:13.

4. The composition as claimed in any one of the preceding claims, wherein the FGF21 molecule comprises SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10.

5. The composition as claimed in any one of the preceding claims, wherein the 2nd linker is covalently attached to the Exendin4 homologue through the side chain of a peptide-linking amino acid residue located at one of positions 12, 14, 19, 20, 21 or 40, according to the numbering of SEQ ID NO:60.

6. The composition as claimed in any one of the preceding claims, wherein the Exendin4 homologue comprises a sequence selected from the group consisting of SEQ ID NO:38, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:53, SEQ ID NO:55, 60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, and SEQ ID NO:77.

7. The composition as claimed in any one of the preceding claims, wherein the Exendin4 homologue comprises an amino terminal capping group selected from the group consisting of CH_3, C(O)CH3, $C(O)CH_2CH_3$, $C(O)CH_2CH_2CH_3$, and $C(O)CH(CH_3)CH_3$, and preferably $C(O)CH_3$, and optionally, further comprises a carboxyl terminal capping group selected from the group consisting of OH, NH_2, $NH(CH_3)$, $NHCH_2CH_3$, $NHCH_2CH_2CH_3$, $NHCH(CH_3)CH_3$, $NHCH_2CH_2CH_2CH_3$, $NHCH(CH_3)CH_2CH_3$, NHC_6H_5, $NHCH_2CH_2OCH_3$, $NHOCH_3$, $NHOCH_2CH_3$, a carboxy protecting group, a lipid fatty acid group or a carbohydrate, and preferably NH_2.

8. The composition as claimed in any one of the preceding claims, wherein the Exendin4 homologue comprises the sequence SEQ ID NO:77.

9. The composition as claimed in any one of the preceding claims, wherein the first and/ or second linker comprises the formula X-Y-Z; wherein X is a biologically compatible connecting chain including any atom selected from the group consisting of C, H, N, O, P, S, F, Cl, Br, and I, and may comprise a polymer or block co-polymer, and is covalently linked to the peptide- or protein-linking residue where the linker is linear, Y is an optionally present recognition group comprising at least a ring structure; and Z is an attachment moiety comprising a covalent link to an amino acid side chain in a combining site of the antibody.

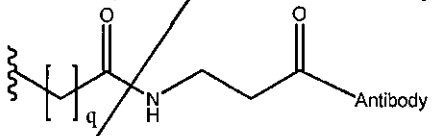
10. The composition as claimed in claim 9, wherein Y has the optionally substituted structure:



or

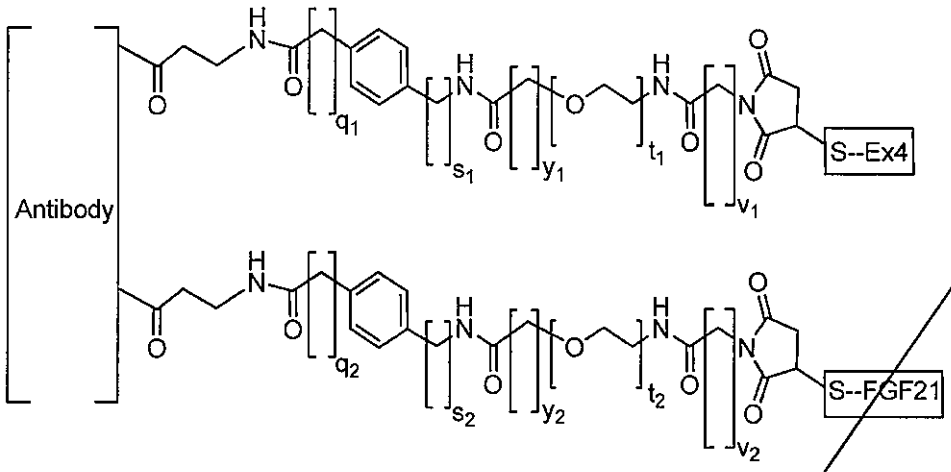

wherein a, b, c, d, and e are independently carbon or nitrogen; f is carbon, nitrogen, oxygen, or sulfur, and Y may be phenyl.

11. The composition as claimed in any one of claims 9-10, wherein Z has the formula



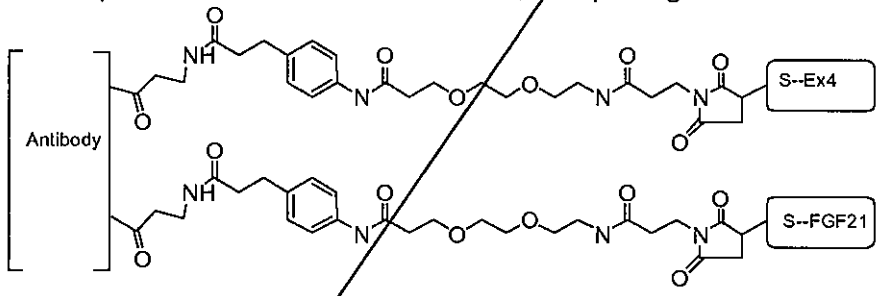
when covalently attached to the antibody, and q =1 or 2.

12. The composition as claimed in any one claims 9-11, comprising the formula:



wherein each independently of v_1 and v_2 is 1 or 2, each independently of t_1 and t_2 is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, each independently of y_1 and y_2 is 0, 1 or 2; each independently of $s_1$and s_2 is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, and each independently of q_1 and q_2 is 1, 2, 3, 4, or 5, S—Ex4 denotes a covalent link through a thiol group to the peptide linking residue of Ex4, and S—FGF21 denotes a covalent link through a thiol group to the protein linking residue of FGF21.

13. The composition as claimed in claim 12, comprising the formula:



14. The composition as claimed in any one of the preceding claims, wherein the antibody is a Fab, Fab', $F(ab')_2$, F_v, dsF_v, scF_v, V_H, V_L, diabody, or minibody, or is a full length antibody, and is preferably selected from the group consisting of IgG1, IgG2, $IgG_{2\Delta a}$, IgG3, IgG4, $IgG_{4\Delta b}$, $IgG_{4\Delta c}$, IgG_4 S228P, $IgG_{4\Delta b}$ S228P and $IgG_{4\Delta c}$ S228P.

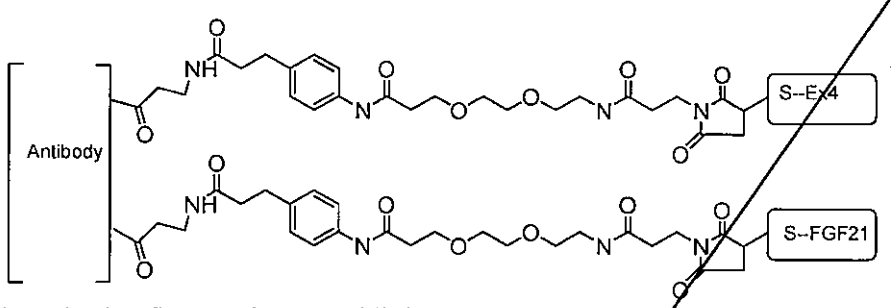
15. The composition as claimed in any one of the preceding claims, wherein the first and the second linker are each covalently conjugated to the ε-amino group of a lysine residue of one of the heavy chains of the antibody, the lysine being located at position 93 of the heavy chain according to Kabat numbering.

16. The composition as claimed in any one of the preceding claims, wherein the antibody comprises a light chain variable region (V_L) comprising a V_L CDR1, V_L CDR2, and V_L CDR3 of the V_L sequence shown in SEQ ID NO:27; and a heavy

chain variable region (V_H) comprising a V_H CDR1, V_H CDR2, and V_H CDR3 of the V_H sequence shown in SEQ ID NO:28.

17. The composition as claimed in any one of the preceding claims, wherein the antibody comprises a light chain comprising SEQ ID NO:25 and a heavy chain comprising SEQ ID NO:26.

18. The composition as claimed in any one of the preceding claims, comprising the formula



wherein the first and second linker are each covalently connected to one of the two antibody combining sites, and the antibody comprises SEQ ID NO:25 and SEQ ID NO:26, and S—Ex4 denotes a covalent link through a thiol group to K(SH) of SEQ ID NO:64, and S—FGF21 denotes a covalent link through a thiol group to C^{129} of SEQ ID NO:10.

19. A process for preparing the composition as claimed in any one of the preceding claims, comprising

(i) mixing the FGF21 homologue and the 1st linker together at a ratio of between about 1:4 and about 1:1 to form the complex [FGF21-1st linker];

(ii) mixing [FGF21-1st linker] and Ab together at a ratio of between about 1.1:1 and about 1:5 so as to form a mixture containing [Ab], [Ab]- [FGF21-1st linker]$_1$ and [Ab]- [FGF21-1st linker]$_2$;

(iii) extracting the [Ab]- [FGF21-1st linker]$_1$ molecules from the mixture formed in (ii);

(iv) mixing Ex4 and 2nd linker together at a ratio of between about 2:1 and about 1:2, to form the complex [2nd linker-Ex4];

(v) mixing [Ab]-[FGF21-1st linker]$_1$ with [2nd linker-Ex4] at a ratio of between about 2:1 and about 1:2 so as to form a mixture containing [FGF21-1st linker]$_1$-[Ab]-[2nd linker-Ex4]$_1$.

20. The process as claimed in claim 20, wherein the [Ab]- [FGF21-1st linker]$_1$ molecules are extracted from the mixture formed in (ii) by reverse phase chromatography.

21. The process as claimed in claim 20, wherein the reverse phase chromatography is over a butyl column.

22. The process as claimed in claim 21, wherein butyl column comprises butly conjugated resin beads of between about 30 µM and about 40 µM diameter, the beads comprising pores of between about 950Å and 1050 Å.

23. The process as claimed in any one of claims 22, wherein the [FGF21-1st linker]$_1$ species is extracted using a buffer comprising a linear gradient of 1,6 hexanediol, wherein the linear gradient begins at a 1,6 hexanedilol concentration of between about 2% and about 3%, and ends at a 1,6, hexanediol concentration of between about 6% and about 5%

24. A process for preparing the composition as claimed in any one of claims 1-18, comprising

(i) mixing Ex4 and 2nd linker together at a ratio of between about 2:1 and about 1:2, to form the complex [2nd linker-Ex4];

(ii) mixing [2nd linker-Ex4] and Ab together at a ratio of between about 1:1 and about 1:3 so as to form a mixture containing [Ab], [Ab]-[2nd linker-Ex4]$_1$ and [Ab]-[2nd linker-Ex4]$_2$;

(iii) extracting the [Ab]-[2nd linker-Ex4]$_1$ molecules from the mixture formed in (ii);

(iv) mixing FGF21 and the 1st linker together at a ratio of between about 1:4 and about 1:1 to form the complex [FGF21-1st linker];

(v) mixing [FGF21-1st linker] with [Ab]-[2nd linker-Ex4]$_1$ at a ratio of between about 2:1 and about 1:2 so as to form a mixture containing [FGF21-1st linker]$_1$-[Ab]-[2nd linker-Ex4]$_1$.

25. A composition produced according to the process as claimed in any one of claims 19-24.

26. A pharmaceutical composition comprising a therapeutically effective amount of the composition as claimed in any one of claims 1-18 or claim 25, in combination with a pharmaceutically acceptable carrier.

27. A formulation comprising between about 0.1 and about 200mg/ml of the composition as claimed in any one of claims 1-18 or 25, and further comprising between about 1 and 150mM glutamic acid, pH between about 4.0 and about 5.0; and at least one of the following:

(i) between about 10 to about 150mg/ml cryoprotectant;

(ii) between about 0.001 and about 1.0mg/ml chelator;

(iii) between about 0.02-2.0mg/mL surfactant.

28. A formulation suitable for lyophlization comprising about 30mg/mL of the composition as claimed in any one of claims 1-18 and 25, and further comprising:

(i) about 20mM glutamic acid, pH 4.5 ± 0.5;

(ii) about 8.5% trehalose dehydrate;

(iii) about 0.005% disodium EDTA dihydrate; and

(iv) about 0.02% polysorbate 80.

29. A method of treating diabetes, diabetes related conditions, obesity, dislipidemia, hypertension, hepatosteaotosis, or cardiovascular disease; or controlling or reducing weight levels; or controlling glycemic control; or increasing insulin secretion, or levels of non-esterified free fatty acids or adipsin; or reducing levels of blood glucose, glucagon, triglyceride, fructosamine, low density cholesterol, or C-reactive protein; comprising administering a therapeutically effective amount of the composition as claimed in any one of claims 1-18, 25, the pharmaceutical composition as claimed in claim 26, or the formulation as claimed in any one of claims 27-28 to a subject.

30. Use of the composition as claimed in any one of claims 1-18, or 25, the pharmaceutical composition as claimed in claim 26, or the formulation as claimed in any one of claims 27-28, in the preparation of a medicament for treating diabetes related conditions, obesity, dislipidemia, hypertension, hepatosteaotosis, or cardiovascular disease; or controlling or reducing weight levels; or controlling glycemic control; or increasing insulin secretion, or non-esterified free fatty acid levels; or reducing levels of blood glucose, glucagon, triglyceride, fructosamine, low density cholesterol, or C-reactive protein.

31. A method of assessing patient's suitability for a treatment regime for a condition, comprising

(i) measuring the gene expression levels of one or more genes selected from the group consisting of Abcd2, Acot3, Cidea, Cyp2b9, Cyp4a14, Fmo2, Gstm5, Hmgcrk, Klb, Lepr, Saa1/2, Scd1, and Srebf2 prior to treatment;

(ii) measuring the expression levels of the genes measured in step (i) after the patient has been treated with the composition as claimed in any one of claims 1-18 or 25, the pharmaceutical composition as claimed in claim 26, or the formulation as claimed in any one of claims 27-28;

(iii) comparing the gene expression levels from steps (i) and (ii) and identifying increases or decreases in expression;

(iv) continuing treatment of the patient with the composition as claimed in any one of claims 1-19 or 26, the pharmaceutical composition as claimed in claim 27, or the formulation as claimed in any one of claims 28-29; based on an change in gene expression of one or more genes selected from the group consisting of Abcd2, Acot3, Cidea, Cyp2b9, Cyp4a14, Fmo2, Gstm5, Hmgcrk, Klb, Lepr, Saa1/2, Scd1, and Srebf2.

32. The method as claimed in claim 31, wherein the continuation of treatment is based on one or more of an increase in gene expression of Acot3 and a decrease in gene expression of Saa1/2.

33. The method as claimed in any one of claims 31-32, wherein the condition is selected from the group consisting of obesity, dislipidemia, hypertension, hepatosteaotosis, or cardiovascular disease; or controlling or reducing weight levels; or controlling glycemic control; or increasing insulin secretion, or non-esterified free fatty acid levels; or reducing levels of blood glucose, glucagon, triglyceride, fructosamine, low density cholesterol, or C-reactive protein.

34. A process for purifying a sample of h38C2 antibody or variant thereof, wherein both antigen binding sites are fully available for antigen binding in at least about 85% of the antibodies in the sample, comprising

(i) Equilibrating a HIC column with a pre-loading equilibration wash comprising a base buffer that comprises between about 15mM and about 100mM sodium phosphate, potassium phosphate or ammonium phosphate HEPES, Tris and bis-Tris, at between about pH6.5 to about 7.5, and further comprises a salt selected from the group consisting of NaCl, KCl, and monosodium citrate, at a first concentration of between about 0.5M and 1.5M; wherein the HIC column comprises phenyl conjugated resin beads below about 50μm in diameter and comprising pores of at least about 500Å;

(ii) Loading the column with a sample of h38C2 at between about 4 and about 80g/L in loading buffer comprising the base buffer and further comprising the salt at the first concentration;

(iii) Washing the column with post-loading equilibration wash comprising the base buffer and the salt at the first concentration;

(iv) Washing the column with a salt gradient, comprising the base buffer and further comprising a linear concentration gradient from about 1.5M to about 0.25M of the salt, characterised in that the salt concentration decreases by between about 90mM and 100mM per 1CV;

(v) Washing the column with a salt plateau wash, comprising between about 4CV and about 8CV of the salt at between about 0.25M and about 0.4M in the base buffer;

(vi) Washing the column with a buffer wash comprising the base buffer;

(vii) Eluting the h38C2 with an elution buffer, comprising the base buffer and a linear concentration gradient of 1,6 hexanediol beginning at a concentration of between about 0 to about 1% of 1,6 hexanediol and ending at an upper limit selected from the group consisting of about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, and 22% about 0% to about 22% of 1,6 hexanediol for between about 0.5CV to about 3CV or until the elution pool is collected.

(viii) Optionally running a further elution step comprising the base buffer and 1,6 hexanediol at a concentration selected from the group consisting of about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, and 22% about 0% to about 22%, for up to 5CV or until the elution pool is collected;

wherein the h38C2 antibody or variant thereof is an IgG1 antibody comprising a light chain variable region (V_L) as set forth in SEQ ID NO:27; and a heavy chain variable region (V_H) as set forth in SEQ ID NO:28.

35. The process as claimed in claim 34, wherein the antibody further comprises a light chain constant region at least 95% identical to one or more of SEQ ID NOs:78, 79, 80 and 81, and a heavy chain constant region at least 95% identical to SEQ ID NO:82.

Claims

1. A process for purifying a sample of h38C2 antibody or variant thereof, wherein both antigen binding sites are fully available for antigen binding in at least about 85% of the antibodies in the sample, comprising

   (i) Equilibrating a HIC column with a pre-loading equilibration wash comprising a base buffer that comprises between about 15mM and about 100mM sodium phosphate, potassium phosphate or ammonium phosphate HEPES, Tris and bis-Tris, at between about pH6.5 to about 7.5, and further comprises a salt selected from the group consisting of NaCl, KCl, and monosodium citrate, at a first concentration of between about 0.5M and 1.5M; wherein the HIC column comprises phenyl conjugated resin beads below about 50µm in diameter and comprising pores of at least about 500Å;

   (ii) Loading the column with a sample of h38C2 at between about 4 and about 80g/L in loading buffer comprising the base buffer and further comprising the salt at the first concentration;

   (iii) Washing the column with post-loading equilibration wash comprising the base buffer and the salt at the first concentration;

   (iv) Washing the column with a salt gradient, comprising the base buffer and further comprising a linear concentration gradient from about 1.5M to about 0.25M of the salt, characterised in that the salt concentration decreases by between about 90mM and 100mM per column volume (1CV);

   (v) Washing the column with a salt plateau wash, comprising between about 4CV and about 8CV of the salt at between about 0.25M and about 0.4M in the base buffer;

   (vi) Washing the column with a buffer wash comprising the base buffer;

   (vii) Eluting the h38C2 with an elution buffer, comprising the base buffer and a linear concentration gradient of 1,6 hexanediol beginning at a concentration of between about 0 to about 1% of 1,6 hexanediol and ending at an upper limit selected from the group consisting of about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, and 22% about 0% to about 22%

of 1,6 hexanediol for between about 0.5CV to about 3CV or until the elution pool is collected.

(viii) Optionally running a further elution step comprising the base buffer and 1,6 hexanediol at a concentration selected from the group consisting of about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, and 22% about 0% to about 22%, for up to 5CV or until the elution pool is collected;

wherein the h38C2 antibody or variant thereof comprises a light chain variable region (V_L) comprising a V_L CDR1, V_L CDR2, and V_L CDR3 of the V_L sequence shown in SEQ ID NO:27; and preferably as set forth in SEQ ID NO:27, and a heavy chain variable region (V_H) comprising a V_H CDR1, V_H CDR2, and V_H CDR3 of the V_H sequence shown in SEQ ID NO:28, and preferably as set forth in SEQ ID NO:28.

2. The process as claimed in claim 1, wherein the antibody further comprises a light chain constant region at least 95% identical to one or more of SEQ ID NOs:78, 79, 80 and 81, and a heavy chain constant region at least 95% identical to SEQ ID NO:82.
3. The process as claimed in any one of the preceding claims, wherein the antibody is an IgG1, and may comprise SEQ ID NO:25 and 26.
4. The process as claimed in any one of the preceding claims, wherein HIC column comprises phenyl conjugated resin beads of about 35µM comprising pores of about 750Å.
5. The process as claimed in any one of the preceding claims, wherein the pre-loading equilibration wash of step (i) comprises about 20mM sodium phosphate, about 1M NaCl, about pH7.
6. The process as claimed in any one of the preceding claims, wherein at step (ii), the column is loaded with a sample of h38C2 at between about 15 and about 18g/L in about 20mM sodium phosphate at about pH7.
7. The process as claimed in any one of the preceding claims, wherein at step (iii), the post-loading equilibration wash comprises about 1 CV of 1M NaCl in about 20mM sodium phosphate at about pH7.

8. The process as claimed in any one of the preceding claims, wherein at step (iv), the NaCl gradient comprises 20mM sodium phosphate pH7, and wherein and the linear concentration gradient is from about 1M to about 0.33M of NaCl, and the salt concentration decreases by between about 90mM and 100mM per 1CV.
9. The process as claimed in any one of the preceding claims, wherein at step (iv), the linear salt gradient comprises at least about 4 CV.
10. The process as claimed in any one of the preceding claims, wherein the salt plateau wash of step (v) comprises about 5 CV about 0.33M of NaCl in about 20mM sodium phosphate at about pH 7.
11. The process as claimed in any one of the preceding claims, wherein the base buffer at step (vi) comprises about 2CV of 20mM sodium phosphate at about pH7.
12. The process as claimed in any one of the preceding claims, wherein the elution buffer of step (vii) comprises 20mM sodium phosphate pH7.
13. The process as claimed in any one of the preceding claims, wherein the linear concentration gradient of step (vii) comprises a linear concentration gradient of 1,6 hexanediol beginning at a concentration of between about 0 to about 1% of 1,6 hexanediol and ending at an upper limit selected from the group consisting of about 14%, about 15%, or about 16%, of 1,6 hexanediol for about 1CV.
14. The process as claimed in any one of the preceding claims, wherein the further elution step comprises 20mM sodium phosphate pH7 and 1,6 hexanediol at a concentration selected from the group consisting of about 14%, about 15%, and about 16%, for about 2 to about 5CV or until the elution pool is collected.
15. A composition comprising h38C2 prepared according to any one of the preceding claims.