

Claims

- 1) An isolated multispecific antibody comprising a first CH2-CH3 region comprising a mutation Q311R, Q311K, T307P/L309Q, T307P/V309Q, T307P/L309Q/Q311R or T307P/V309Q/Q311R and a second CH2-CH3 region comprising a wild-type amino acid residue at positions 307, 309 and 311, wherein residue numbering is according to the EU Index.
- 2) The isolated multispecific antibody of claim 1, wherein the antibody is an IgG1, an IgG2 or an IgG4 isotype.
- 3) The isolated multispecific antibody of claim 1 or 2, wherein the first CH2-CH3 region has reduced binding to protein A ligand when compared to the second CH2-CH3 region.
- 4) The isolated multispecific antibody of claim 3, wherein the protein A ligand comprises Staphylococcal Protein A, Z-domain or Y-domain.
- 5) The isolated multispecific antibody of claim 4, wherein Z-domain comprises an amino acid sequence of SEQ ID NO: 1.
- 6) An isolated polynucleotide
 - a) comprising the polynucleotide encoding the first CH2-CH3 region comprising a mutation Q311R, Q311K, T307P/L309Q, T307P/V309Q, T307P/L309Q/Q311R or T307P/V309Q/Q311R;
 - b) comprising the polynucleotide encoding the first CH2-CH3 region comprising a mutation Q311R, Q311K, T307P/L309Q, T307P/V309Q, T307P/L309Q/Q311R or T307P/V309Q/Q311R and the second CH2-CH3 region comprising a wild-type amino acid residue at positions 307, 309 and 311;
 - c) comprising a polynucleotide sequence of SEQ ID NOs: 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 87, 88 or 91.
- 7) A vector comprising
 - a) the isolated polynucleotide encoding the first CH2-CH3 region comprising a mutation Q311R, Q311K, T307P/L309Q, T307P/V309Q, T307P/L309Q/Q311R or T307P/V309Q/Q311R;
 - b) the isolated polynucleotide comprising a polynucleotide sequence of SEQ ID NOs: 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 87, 88 or 91;
 - c) the isolated polynucleotide comprising the polynucleotide encoding the first CH2-CH3 region comprising a mutation Q311R, Q311K, T307P/L309Q, T307P/V309Q, T307P/L309Q/Q311R or T307P/V309Q/Q311R and the second CH2-CH3 region comprising a wild-type amino acid residue at positions 307, 309 and 311;
 - d) the isolated polynucleotide comprising

- i) SEQ ID NOs: 27, and 47, respectively;
 - ii) SEQ ID NOs: 28 and 47, respectively;
 - iii) SEQ ID NOs: 29 and 47, respectively;
 - iv) SEQ ID NOs: 30 and 47, respectively;
 - v) SEQ ID NOs: 31 and 48, respectively;
 - vi) SEQ ID NOs: 32 and 48, respectively;
 - vii) SEQ ID NOs: 33 and 48, respectively;
 - viii) SEQ ID NOs: 34 and 48, respectively;
 - ix) SEQ ID NOs: 35 and 49, respectively;
 - x) SEQ ID NOs: 36 and 49, respectively;
 - xi) SEQ ID NOs: 37 and 49, respectively;
 - xii) SEQ ID NOs: 38 and 49, respectively;
 - xiii) SEQ ID NOs: 39 and 50, respectively;
 - xiv) SEQ ID NOs: 40 and 50, respectively;
 - xv) SEQ ID NOs: 41 and 50, respectively;
 - xvi) SEQ ID NOs: 42 and 50, respectively;
 - xvii) SEQ ID NOs: 43 and 51, respectively;
 - xviii) SEQ ID NOs: 44 and 51, respectively;
 - xix) SEQ ID NOs: 45 and 51, respectively;
 - xx) SEQ ID NOs: 46 and 51, respectively;
 - xxi) SEQ ID NOs: 87 and 89, respectively;
 - xxii) SQ ID Nos: 87 and 90, respectively;
 - xxiii) SEQ ID NOs: 88 and 89, respectively;
 - xxiv) SQ ID Nos: 88 and 90, respectively;
 - xxv) SEQ ID NOs: 92 and 89, respectively; or
 - xxvi) SQ ID Nos: 92 and 90, respectively.
- 8) A host cell comprising the vector of claim 7.
- 9) The host cell of claim 8, wherein the host cell is a hybridoma, a myeloma, SP2/0, NS0, U266, CHO, CHO-K1SV, CHO-K1, DG44 or Hek293.
- 10) A method of making an isolated multispecific antibody comprising a first heavy chain or fragment thereof comprising a mutation Q311R, Q311K, T307P/L309Q, T307P/V309Q, T307P/L309Q/Q311R or T307P/V309Q/Q311R and a second heavy chain or fragment thereof comprising wild-type amino acid residue at positions 307, 309 and 311, comprising
- a) providing a first parental antibody comprising the first heavy chain or fragment thereof comprising the mutation Q311R, Q311K, T307P/L309Q, T307P/V309Q, T307P/L309Q/Q311R or T307P/V309Q/Q311R and a first light chain;

- b) providing a second parental antibody comprising the second heavy chain or fragment thereof comprising wild-type amino acid residue at positions 307, 309 and 311 and a second light chain;
 - c) contacting the first parental antibody and the second parental antibody in a sample;
 - d) incubating the sample; and
 - e) purifying the multispecific antibody using protein A ligand affinity chromatography.
- 11) The method of claim 10, wherein the isolated multispecific antibody further comprises asymmetric stabilizing mutations in the first heavy chain or fragment thereof and in the second heavy chain or fragment thereof.
- 12) The method of claim 11, wherein the asymmetric stabilizing mutations in the first heavy chain or fragment thereof and in the second heavy chain or fragment thereof or in the second heavy chain or fragment thereof and in the first heavy chain or fragment thereof are
- a) F405L and K409R, respectively;
 - b) wild-type and F405L/R409K, respectively;
 - c) T366W and T366S/L368A/Y407V, respectively;
 - d) T366Y/F405A and T394W/Y407T, respectively;
 - e) T366W/F405W and T394S/Y407A, respectively;
 - f) F405W/Y407A and T366W/T394S, respectively;
 - g) L351Y/F405A/Y407V and T394W, respectively;
 - h) T366I/K392M/T394W and F405A/Y407V, respectively;
 - i) T366L/K392M/T394W and F405A/Y407V, respectively;
 - j) L351Y/Y407A and T366A/K409F, respectively;
 - k) L351Y/Y407A and T366V/K409F, respectively;
 - l) Y407A and T366A/K409F, respectively;
 - m) D399K/E356K and K409D/K392D, respectively; or
 - n) D399K/E356K/E357K and K409D/K392D/K370, respectively.
- 13) The method of any one of claims 10-12, wherein the isolated multispecific antibody is an IgG1, an IgG2 or an IgG4 isotype.
- 14) The method of claim 10, wherein a reducing agent is added during step d).
- 15) The method of claim 14, wherein the reducing agent is 2- mercaptoethylamine (2-MEA), dithiothreitol (DTT), dithioerythritol (DTE), glutathione, tris(2-carboxyethyl) phosphine (TCEP), L-cysteine or beta-mercaptoethanol.
- 16) The method of claim 15, wherein 2-MEA is present at a concentration of about 10 mM to about 100 mM.
- 17) The method of claim 16, wherein 2-MEA is present at a concentration of about 25 mM to about 75 mM.

- 18) The method of claim 10, wherein step d) is performed at a temperature of about 20°C to about 37°C about ninety minutes to about six hours.
- 19) An isolated antibody comprising two heavy chains or fragments thereof having identical amino acid sequences and two light chains or fragments thereof, wherein the two heavy chains comprise a mutation Q311R, Q311K, T307P/L309Q, T307P/V309Q, T307P/L309Q/Q311R or T307P/V309Q/Q311R, wherein residue numbering is according to the EU Index.
- 20) The isolated antibody of claim 19, wherein the two heavy chains or fragments thereof further comprise a mutation F405L, K409R, F405L/R409K, T366W or T366S/L368A/Y407V.
- 21) The isolated antibody of claim 19 or 20, wherein the antibody is an IgG1, an IgG2 or an IgG4 isotype.
- 22) A polynucleotide
 - a) encoding the antibody heavy chain comprising the CH2-CH3 region of SEQ ID NOs: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 52, 53 or 56; or
 - b) comprising the polynucleotide sequence of SEQ ID NOs: 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 87, 88 or 91.
- 23) A vector comprising the polynucleotide of claim 22.
- 24) A host cell comprising the vector of claim 23.
- 25) A multimeric protein comprising a first polypeptide and a second polypeptide, wherein the first polypeptide comprises a first CH2-CH3 region comprising a mutation Q311R, Q311K, T307P/L309Q, T307P/V309Q, T307P/L309Q/Q311R or T307P/V309Q/Q311R and the second polypeptide comprises a second CH2-CH3 region comprising a wild-type amino acid residue at positions 307, 309 and 311, wherein residue numbering is according to the EU Index.
- 26) The multimeric protein of claim 25, wherein the first CH2-CH3 region and the second CH2-CH3 region are an IgG1, an IgG2 or an IgG4 isotype.
- 27) The multimeric protein of claim 25 or 26, wherein the first CH2-CH3 region has reduced binding to protein A ligand when compared to the second CH2-CH3 region.
- 28) The multimeric protein of claim 27, wherein the protein A ligand comprises Staphylococcal Protein A, Z-domain or Y-domain.
- 29) The multimeric protein of claim 28, wherein Z-domain comprises an amino acid sequence of SEQ ID NO: 1.
- 30) The multimeric protein of claim 25, wherein the multimeric protein is an antibody.
- 31) The multimeric protein of claim 30, wherein the antibody is multispecific, bispecific or monospecific.

- 32) A method of making an isolated multimeric protein comprising a first CH2-CH3 region comprising a mutation Q311R, Q311K, T307P/L309Q, T307P/V309Q, T307P/L309Q/Q311R or T307P/V309Q/Q311R and a second CH2-CH3 region comprising a wild-type amino acid residue at positions 307, 309 and 311, comprising
- providing a first parental protein comprising the first CH2-CH3 region comprising the mutation Q311R, Q311K, T307P/L309Q, T307P/V309Q, T307P/L309Q/Q311R or T307P/V309Q/Q311R;
 - providing a second parental protein comprising the second CH2-CH3 region comprising the wild-type amino acid residue at positions 307, 309 and 311;
 - contacting the first parental protein and the second parental protein in a sample;
 - incubating the sample; and
 - purifying the multispecific protein using protein A ligand affinity chromatography.
- 33) The method of claim 32, wherein the isolated multimeric protein further comprises asymmetric stabilizing mutations in the first CH2-CH3 region and in the second CH2-CH3 region.
- 34) The method of claim 33, wherein the asymmetric stabilizing mutations in the first CH2-CH3 region and in the second CH2-CH3 region or in the second CH2-CH3 region and in the first CH2-CH3 region are
- F405L and K409R, respectively;
 - wild-type and F405L/R409K, respectively;
 - T366W and T366S/L368A/Y407V, respectively;
 - T366Y/F405A and T394W/Y407T, respectively;
 - T366W/F405W and T394S/Y407A, respectively;
 - F405W/Y407A and T366W/T394S, respectively;
 - L351Y/F405A/Y407V and T394W, respectively;
 - T366I/K392M/T394W and F405A/Y407V, respectively;
 - T366L/K392M/T394W and F405A/Y407V, respectively;
 - L351Y/Y407A and T366A/K409F, respectively;
 - L351Y/Y407A and T366V/K409F, respectively;
 - Y407A and T366A/K409F, respectively;
 - D399K/E356K and K409D/K392D, respectively; or
 - D399K/E356K/E357K and K409D/K392D/K370, respectively.
- 35) The method of any one of claims 32-34, wherein the first CH2-CH3 region and the second CH2-CH3 region are an IgG1, an IgG2 or an IgG4 isotype.
- 36) The method of claim 32, wherein a reducing agent is added during step d).

- 37) The method of claim 36, wherein the reducing agent is 2- Mercaptoethylamine (2-MEA), dithiothreitol (DTT), dithioerythritol (DTE), glutathione, tris(2-carboxyethyl) phosphine (TCEP), L-cysteine or beta-mercaptoethanol.
- 38) The method of claim 37, wherein 2-MEA is present at a concentration of about 10 mM to about 100 mM.
- 39) The method of claim 37, wherein 2-MEA is present at a concentration of about 25 mM to about 75 mM.
- 40) The method of claim 32, wherein step d) is performed at a temperature of about 25°C to about 37°C for about ninety minutes to about six hours.