CLAIMS

1. An *in vitro* method for production of a ~~heterodimeric protein~~full-length bispecific antibody comprising the following steps:

a) incubating a first ~~homodimeric protein~~full-length antibody with a second ~~homodimeric protein~~full-length antibody under reducing conditions sufficient to allow reduction of the inter-chain disulfide bonds in the hinge region, and

~~wherein said first homodimeric protein comprises an Fc region of an immunoglobulin wherein said immunoglobulin is either an IgG1 or an IgG4 isotype, said Fc region comprising a first CH3 region and said second homodimeric protein comprises an Fc region of an immunoglobulin wherein said immunoglobulin is either an IgG1 or an IgG4 isotype, said Fc region comprising a second CH3 region, wherein said first homodimeric protein has an amino acid substitution in the CH3 region at a position selected from the group consisting of: 366, 368, 370, 399, 405, 407 and 409, and said second homodimeric protein has an amino acid substitution in the CH3 region at a position selected from the group consisting of: 366, 368, 370, 399, 405, 407 and 409, and wherein said first homodimeric protein and said second homodimeric protein is not substituted in the same positions so~~wherein the CH3 region of said first full-length antibody is a first CH3 region and has an Arg at position 409, and the CH3 region of said second full-length antibody is a second CH3 region and has:

(i) an Asp, Glu, Gly, Asn, Arg, Ser, Thr, Val, or Trp at position 368;

(ii) a Phe, His, Lys, Arg or Tyr at position 399;

(iii) a Gly, Leu, Met or Trp at position 407 and a Lys at position 409, and wherein the first CH3 further has a Tyr at position 407; or

(iv) an amino acid other than Phe, Arg or Gly at position 405 and has a Lys at position 409, and wherein the first CH3 further has a Phe at position 405,

such that the sequences of said first and second CH3 regions are different and are such that the heterodimeric interaction between said first and second CH3 regions is stronger than each of the homodimeric interactions of said first and second CH3 regions, wherein the amino acid positions are numbered according to the EU-index~~,~~ as described in Kabat, wherein said first and second full-length antibodies bind different epitopes, wherein said first and second full-length antibodies:

1) each have an Fc region with an amino acid sequence that is of an IgG1 isotype;

2) each have an Fc region with an amino acid sequence that is of an IgG2 isotype;

3) each have an Fc region with an amino acid sequence that is of an IgG3 isotype; or

4) each have an Fc region with an amino acid sequence that is of an IgG4 isotype; and

wherein said first and second antibodies are humanized antibodies or wherein said first and second antibodies each comprise a full-length heavy chain of a human antibody; and further wherein step a) comprises incubation for at least 90 minutes at a temperature of at least 20°C in the presence 2-mercaptoethylamine at a concentration of at least 25 mM;

b) subjecting at least 10 mL of the composition obtained from step a) to oxidizing conditions sufficient to allow oxidation of cysteines in the ~~heterodimeric protein~~full-length bispecific antibody to inter-chain disulfide bonds, and

~~c) obtaining the heterodimeric protein, wherein the method yields a heterodimeric protein wherein more than 80% of the total product is the desired heterodimeric protein.~~

~~2. An *in vitro* method according to any of the preceding claims, wherein the reducing conditions in step a) comprise adding a reducing agent.~~

c) obtaining the full-length bispecific antibody.

~~3.~~2. An *in vitro* method according to claim ~~2, wherein the reducing agent is selected from the group consisting of: 2-mercaptoethylamine, a chemical derivative of 2-mercaptoethylamine, L-cysteine,~~1, wherein both first and ~~D-cysteine~~second full-length antibodies comprise a Cys-Pro-Pro-Cys sequence in the hinge region.

~~4.~~ An *in vitro* method according to any of the preceding claims, wherein step a) comprises adding a metal chelating agent~~.~~

~~5.~~3. ~~An *in vitro* method according to claim 4, wherein the metal chelating agent is,~~ such as EDTA, EGTA or citric acid.

~~6.~~4. An *in vitro* method according to any one of the preceding claims, wherein step a) is performed under reducing conditions with a redox potential between -150 and -600 mV, such as between -350 and -450 mV.

~~7.~~ An *in vitro* method according to any of the preceding claims, wherein the first and second ~~homodimeric proteins~~full-length antibodies are in a buffer~~.~~

~~8.~~5. ~~An *in vitro* method according to claim 7, wherein the buffer~~, which comprises in the range of 1-100 mM phosphate, such as 1-50 mM phoshate or in the range of 1-25 mM, or in the range of 5-20 mM.

6. An *in vitro* method according to claim 5, wherein the buffer has pH in the range of 4.5-8.5, such as in the range of 6.5 to 7.5.

7. An *in vitro* method according to claim 5, wherein the buffer is selected from the group consisting of a) 8.1 mM sodium phosphate (Na_2HPO_4-$7H_2O$), 1.5 mM potassium phosphate (KH_2PO_4), 138 mM sodium chloride (NaCl), 2.7 mM potassium chloride (KCl) pH 5.0; b) 8.1 mM sodium phosphate (Na_2HPO_4-$7H_2O$), 1.5 mM potassium phosphate (KH_2PO_4), 138 mM sodium chloride (NaCl), 2.7 mM potassium chloride (KCl) pH 7.0; 3) 20 mM Tris-HCl, pH 7.8

8. An *in vitro* method according to any of the preceding claims, wherein step b) comprises a pH in the range of 6-8.5.

9. An *in vitro* method according to any of the preceding claims, wherein step b) comprises a redox potential of at least -300 mV.

~~9.~~10.An *in vitro* method according to any of the preceding claims, wherein the oxidizing conditions in step b) comprise the presence of at least 0.05 mM oxygen.

~~10.~~11. An *in vitro* method according to any of the preceding claims, wherein the oxidizing conditions in step b) comprise adding oxygen, wherein the adding of oxygen is performed by the methods comprising: mechanically, e.g. by agitating, stirring, increasing pressure or creating flow, or by sparging with oxygen or air.

~~11. An *in vitro* method according to claim 10, wherein adding oxygen is performed mechanically, e.g. by agitating, stirring, increasing pressure or creating flow.~~

~~12. An *in vitro* method according to any of claims 10-11, wherein adding oxygen is performed by sparging with oxygen or air or increasing pressure.~~

~~13.~~12. An *in vitro* method according to any of the preceding steps, wherein the oxidizing conditions in step b) comprise an oxidizing agent~~.~~, such as e.g. dehydroascorbic acid (dhAA).

~~14. An *in vitro* method according to claim 13, wherein the oxidizing agent is dehydroascorbic acid (dhAA).~~

~~15. An *in vitro* method according to any of the preceding claims, wherein step b) comprises separating the heterodimeric protein and the reducing agent.~~

~~16.~~ An *in vitro* method according to any of the preceeding claims , wherein step b) comprises subjecting the composition obtained from step a) to chromatography ~~or filtration.~~

~~17. An *in vitro* method according to claim 16, wherein the chromatography is,~~ such as e.g. column chromatography~~.~~

~~18. An *in vitro* method according~~ or to ~~claim 16, wherein the~~ filtration ~~is,~~ preferably diafiltration~~.~~

~~19.~~13. ~~A method according to claim 18, wherein the diafiltration is~~ such as tangential flow filtration (TFF) or normal flow filtration (NFF).

~~20.~~ An *in vitro* method according to claim ~~19, wherein the diafiltration is TFF.~~

~~21.~~14. ~~An *in vitro* method according to claim 18~~13, wherein the diafiltration is performed by circulating the composition through a hollow fiber cartridge comprising a cut-off value in the range of 10-50 kDa, and with a surface area in the range of 0.05-1 m^2, and with a cartridge inlet pressure in the range of 70-280 kPa, until one to seven volumes of buffer exchange have taken place.

~~22. An *in vitro* method according to any of claims 18-21, wherein separating the heterodimeric protein and the reducing agent comprises exchanging the buffer or solution of the composition obtained from step a) with a buffer or solution without said reducing agent.~~

~~23. An *in vitro* method according to claim 22, comprising in the range of 3-12 volumes of buffer or solution exchanges, such as in the range of 4-10 volumes.~~

~~24. An *in vitro* method according to any of claims 16-22, wherein separating the heterodimeric protein and the reducing agent is a continous process.~~

~~25. An *in vitro* method according to any of claims 16-22, wherein separating the heterodimeric protein and the reducing agent is a batch process.~~

~~26. An *in vitro* method according to any of the preceding claims, wherein the oxidizing conditions in step b) comprise the steps of:~~

~~I) diafiltration of the composition obtained from step a)~~

~~II) incubation of the retentate obtained from step I)~~
~~III)diafiltration of the composition obtained from step II).~~

~~27. An *in vitro* method according to claim 26, wherein the diafiltration in steps I) and/or III) comprises in the range of 3-12 volumes of buffer exchange.~~

~~28. An *in vitro* method according to any of claims 26-27, wherein step II) comprises incubation at a temperature in the range of 15-35°C for a period of 12-48 hours.~~

~~29.~~15. An *in vitro* method according to any of the preceding claims, wherein the concentration of ~~heterodimeric protein~~full-length bispecific antibody in the composition obtained from step a) is in the range of 1-100 g/L.

~~30.~~16. An *in vitro* method according to any of the preceding claims, wherein the oxidizing conditions in step b) comprise ~~a metal ion~~adding a metal ion, preferably a metal ion selected from the group consisting of: Copper, Manganese, Magnesium, Iron, Nickel and Cobalt, wherein the metal ion concentration is preferably in the range of from 0.1 to 100 µM.

~~31.~~ An *in vitro* method according to ~~claim 30~~any of the preceding claims, wherein the ~~oxidizing conditions~~ratio of first to second full-length antibody in step ~~b) comprise adding a metal ion.~~

~~32.~~17. ~~An *in vitro* method according to any of claims 30-31, wherein the concentration of the metal ion~~) is in the range of ~~0.~~1:1.01 to ~~100 µM.~~1:2.

~~33.~~18. An *in vitro* method according to any of the preceding claims, wherein either the first full-length antibody or the second ~~homodimeric protein~~full-length antibody is not able to bind to Protein A and/or Protein G.

~~34.~~19. An *in vitro* method according to any of the preceding claims, wherein the first and second ~~homodimeric protein~~full-length antibodies comprise different light chains.

~~35. An *in vitro* method according to any of the preceding claims, wherein step c) comprises subjecting the composition obtained from step b) to a purification method.~~

~~36. An *in vitro* method according to any of the preceding claims, wherein step b) comprises subjecting at least 30 mL of the composition obtained from step a) to oxidizing conditions sufficient to allow oxidation of cysteines in the heterodimeric protein to inter-chain disulfide bonds.~~

~~37.~~20. An *in vitro* method according to any of the preceding claims, wherein the total concentration of first ~~homodimeric~~full-length antibody and second ~~homodimeric protein~~full-length antibody in step a) is at least 0.25 mg/mL.

~~38.~~ An *in vitro* method according to any of the preceding claims, wherein said first ~~homodimeric protein has no more than one amino acid substitution in the CH3 region, and the second homodimeric protein has no more than one amino acid substitution in the CH3 region relative to the wild-type CH3 regions.~~

~~39. An *in vitro* method according to any of the preceding claims, wherein said first homodimeric protein has~~ full-length antibody comprises a Phe at position 405 and an ~~amino acid other than Lys, Leu or Met~~ Arg at position 409 and said second ~~homodimeric protein has an amino acid substitution at a position selected from the group consisting of: 366, 368, 370, 399, 405~~full-length antibody comprises a Leu at position 405 and ~~407.~~

~~40.~~21. ~~An *in vitro* method according to any of the preceding claims, wherein said first homodimeric protein has an amino acid other than Lys, Leu or Met~~a Lys at position 409 ~~and said second homodimeric protein has an amino acid other than Phe at position 405~~.

~~41. An *in vitro* method according to any of the preceding claims, wherein said first homodimeric protein has an amino acid other than Lys, Leu or Met at position 409~~

~~and said second homodimeric protein has an amino acid other than Phe, Arg or Gly at position 405.~~

~~42. An *in vitro* method according to any of the preceding claims, wherein the first and/or second homodimeric protein do not contain the c-terminal lysine.~~

~~43. An *in vitro* method according to claim 42, wherein the first and/or second homodimeric proteins are genetically modified to lack the c-terminal lysine in the heavy chain.~~

~~44. An *in vitro* method according to claim 42, wherein said method comprises a step of removing the c-terminal lysine from the heavy chain.~~