Claims

1. A symmetric bispecific antibody of the class IgG4 comprising two heavy chains which each comprise a variable domain, C_H1 domain and a hinge region, wherein in each heavy chain:
the cysteine at position 127, numbered according to the Kabat numbering system, in the C_H1 domain which forms an inter-chain disulphide bond with a cysteine in a light chain is substituted with another amino acid; and
~~optionally~~ one or more of the amino acids positioned in the upper hinge region is substituted with cysteine, wherein the constant region sequence of each heavy chain is similar or identical and the variable region in each heavy chain is different.

2. A symmetric bispecific antibody according to claim 1, wherein the hinge region is an IgG1 type hinge.

3. A symmetric bispecific antibody according to claim 1 or 2 wherein the antibody is isolated.

~~4. A symmetric bispecific antibody according to any one of claims 1 to 3 where an inter-chain cysteine at position 127, numbered according to the Kabat numbering system, in the C_H1 domain in one or both heavy chains is substituted with another amino acid.~~

~~5~~4. A symmetric bispecific antibody according to any one of claims 1 to ~~4~~3, wherein the one or more amino acids positioned in the upper hinge region of the first heavy chain is substituted with cysteine is selected from 227, 228, 229 and 230, numbered according to the Kabat numbering system.

~~6~~5. A symmetric bispecific antibody according to claim ~~5~~4, wherein the glycine at position 230, numbered according to the Kabat numbering system, is substituted with cysteine.

~~7~~.6. A symmetric bispecific antibody according to claim ~~5~~4, wherein the tyrosine at position 229, numbered according to the Kabat numbering system, is substituted with cysteine.

~~8~~.7. A symmetric bispecific antibody according to claim ~~5~~4, wherein the lysine at position 228, numbered according to the Kabat numbering system, is substituted with cysteine.

~~9~~.8. A symmetric mixed antibody according to claim ~~5~~4, wherein the serine at position 227, numbered according to the Kabat numbering system, is substituted with cysteine.

~~10~~.9. A symmetric bispecific antibody according to any of claims 1 to ~~9~~8, wherein the cysteine at position 239 and the cysteine at position 242, numbered according to the Kabat numbering system, in one or both heavy chains is/are substituted with another amino acid.

~~11~~.10. A symmetric bispecific antibody according to any of claim 1 to ~~10~~9, wherein the cysteine at position 239, numbered according to the Kabat numbering system, in the first heavy chain is substituted with another amino acid.

~~12.~~11. A symmetric bispecific antibody according to any of claims 1 to ~~11~~10, wherein the cysteine at position 242, numbered according to the Kabat numbering system, in the first heavy chain is substituted with another amino acid.

~~13.~~12. A symmetric bispecific antibody of the class IgG4 comprising two heavy chains which each comprise a variable domain, C_H1 domain and hinge region, wherein in each heavy chain the inter-chain cysteine at position 127, numbered according to the Kabat numbering system, is substituted with another amino acid; and the cysteine at position 239 and/or the cysteine at position 242, numbered according to the Kabat numbering system, is substituted with another amino acid, wherein the constant region sequence of each heavy chain is similar or identical and the variable region in each heavy chain is different.

~~14.~~13. A symmetric bispecific antibody according to any of claims ~~10~~ 9 to ~~13~~12, wherein the cysteine at position 239 and/or the cysteine at position 242 of a first heavy chain is substituted by a non-thiol containing amino acid.

~~15.~~14. A symmetric bispecific antibody according to claim ~~14~~13, wherein the non-thiol containing amino acid is serine.

~~16.~~15. A symmetric bispecific antibody according to any preceding claim, wherein the cysteine at position 127 is substituted by a non-thiol containing amino acid.

~~17.~~16. A symmetric bispecific antibody according to claim ~~16~~15, wherein the non-thiol containing amino acid is serine.

~~18.~~17. A symmetric bispecific antibody according to any preceding claim, wherein one or both heavy chains is/are mutated to insert three amino acids between amino acids 226-243, numbered according to the Kabat numbering system.

~~19.~~18. A symmetric bispecific antibody according to claim ~~18~~17, wherein one or both heavy chains is/are mutated to insert three amino acids between positions 238 and 239, numbered according to the Kabat numbering system.

~~20.~~19. A symmetric bispecific antibody according to claim ~~19~~18, wherein three alanines are inserted between positions 238 and 239, of the first heavy chain numbered according to the Kabat numbering system.

~~21.~~20. A symmetric bispecific antibody according to claim ~~19~~18, wherein a threonine, a histidine and a further theronine are inserted between positions 238 and 239, of one or both of the heavy chains numbered according to the Kabat numbering system.

~~22.~~21. A symmetric bispecific antibody according to any preceding claim, wherein the serine at position 241, of one or both of the heavy chains numbered according to the Kabat numbering system, is/are substituted with proline.

~~23.~~22. A symmetric bispecific antibody according to any preceding claim, wherein in one or both of the heavy chains the glycine at position 230 is substituted with cysteine, the serine at position 227 is/are substituted with proline, the tyrosine at position 229 is substituted with serine, the proline at position 237 is substituted with aspartic acid, the proline at position 238 is substituted with lysine, the amino acid sequence threonine-histidine-threonine is inserted between positions 238 and 239 and the serine at position 241 is substituted with proline.

~~24.~~23. A symmetric bispecific antibody according to any preceding claim, wherein both the heavy chains comprises a C_H2 domain and/or a C_H3 domain.

~~25.~~24. A symmetric bispecific antibody according to any preceding claim, wherein hinge region in each heavy chain is identical.

~~26.~~25. A symmetric bispecific antibody according to any one of claims 1 to ~~25~~24, wherein each heavy chain comprises an upper hinge region and core region of 12 to 17 amino acids in length, for example 15 amino acids in length.

~~27.~~26. A symmetric bispecific antibody according to any preceding claim, which further comprises one or two light chains.

~~28.~~27. An expression vector comprising a sequence which encodes an antibody as defined in any one of claims 1 to ~~27~~26.

~~29.~~28. A host cell comprising a vector as defined in claim ~~28~~27.

~~30.~~29. An antibody as defined in any one of claims 1 to ~~27~~ 26 for use in the treatment of a disease or disorder.

~~31~~30. A method of generating a symmetric bispecific antibody comprising the step of mixing a first IgG4 antibody with a second IgG4 antibody *ex vivo*, under conditions conducive to heavy chain exchange, wherein the antigen specificity of variable regions in the first ~~the~~ antibody are different to antigen specificity of the variable regions in the second antibody and the first IgG4 antibody and the second IgG4 antibody each comprise a C_H1 domain and a hinge region as defined in any of claims 1 to 26.

~~32~~31. A method according to claim ~~31~~30, wherein the conditions conducive to heavy chain exchange are reducing conditions, for example in the presence of a suitable buffer.

~~33. A method according to claim 31 or claim 32, wherein the first IgG4 antibody and the second IgG4 antibody each comprise a C_H1 domain and a hinge region as defined in any of claims 1 to 26.~~