

WE CLAIM:

1. An IgA heterodimeric Fc (IgA HetFc) construct comprising a first Fc polypeptide and a second Fc polypeptide, the first Fc polypeptide comprising a first CH3 domain sequence and the second Fc polypeptide comprising a second CH3 domain sequence, the first and second CH3 domain sequences forming a modified CH3 domain,

wherein the first and second CH3 domain sequences comprise amino acid mutations that promote formation of a heterodimeric Fc over a homodimeric Fc,

wherein:

the amino acid mutations in the first CH3 domain sequence comprise an amino acid substitution at position A6085Y selected from A6085YF, A6085YY, A6085YM, A6085YW and A6085YH, and an amino acid substitution at position T6086 selected from T6086Y, T6086F, T6086M, T6086W and T6086H, and

the amino acid mutations in the second CH3 domain sequence comprise an amino acid substitution at position W6081 selected from W6081T, W6081L, W6081A, W6081V and W6081I,

wherein the heterodimeric Fc is formed with a purity of 70% or higher,

and wherein the numbering of amino acid positions is according to IMGT numbering.

2. The IgA HetFc construct according to claim 1, wherein the modified CH3 domain has a melting temperature (T_m) that is 60°C or higher.

3. The IgA HetFc construct according to claim 1, wherein the modified CH3 domain has a melting temperature (T_m) that is + 10°C of the T_m of a corresponding wild-type IgA CH3 domain.

4. The IgA HetFc construct according to any one of claims 1 to 3, wherein the amino acid substitution at position A6085Y is A6085YF, A6085YY or A6085YW.

5. The IgA HetFc construct according to any one of claims 1 to 3, wherein the amino acid substitution at position A6085Y is A6085YF.

6. The IgA HetFc construct according to any one of claims 1 to 5, wherein the amino acid substitution at position T6086 is T6086Y, T6086F or T6086W.

7. The IgA HetFc construct according to any one of claims 1 to 5, wherein the amino acid substitution at position T6086 is T6086Y.

8. The IgA HetFc construct according to any one of claims 1 to 7, wherein the amino acid substitution at position W6081 is W6081T or W6081L.

9. The IgA HetFc construct according to any one of claims 1 to 3, wherein the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions A6085YF, and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitution W6081T or W6081L.

10. The IgA HetFc construct according to claim 9, wherein the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitution W6081T.

11. The IgA HetFc construct according to any one of claims 1 to 10, wherein the amino acid mutations in the second CH3 domain sequence further comprise an amino acid substitution at position L6079 selected from L6079V, L6079T, L6079A and L6079I.

12. The IgA HetFc construct according to any one of claims 1 to 10, wherein the amino acid mutations in the second CH3 domain sequence further comprise the amino acid substitution L6079V.

13. The IgA HetFc construct according to any one of claims 1 to 12, wherein the amino acid mutations in the second CH3 domain sequence further comprise an amino acid substitution at position I6088 selected from I6088L, I6088A, L6088V and L6088T.

14. The IgA HetFc construct according to any one of claims 1 to 12, wherein the amino acid mutations in the second CH3 domain sequence further comprise the amino acid substitution I6088L.

15. The IgA HetFc construct according to any one of claims 1 to 14, wherein the amino acid mutations in the first CH3 domain sequence further comprise an amino acid substitution at position T6022 selected from T6022V, T6022I, T6022L and T6022A.

16. The IgA HetFc construct according to any one of claims 1 to 14, wherein the amino acid mutations in the first CH3 domain sequence further comprise an amino acid substitution at position T6022 selected from T6022V, T6022I and T6022L.

17. The IgA HetFc construct according to any one of claims 1 to 16, wherein the amino acid mutations in the second CH3 domain sequence further comprise an amino acid substitution at position L6007 selected from L6007F, L6007Y, L6007M, L6007W, L6007H and L6007I.

18. The IgA HetFc construct according to any one of claims 1 to 16, wherein the amino acid mutations in the second CH3 domain sequence further comprise the amino acid substitution L6007F.

19. The IgA HetFc construct according to any one of claims 1 to 18, wherein the amino acid mutations in the first CH3 domain sequence further comprise an amino acid substitution at position H6005 selected from H6005Y, H6005F, H6005M and H6005W.

20. The IgA HetFc construct according to any one of claims 1 to 18, wherein the amino acid mutations in the first CH3 domain sequence further comprise the amino acid substitution H6005Y.

21. The IgA HetFc construct according to any one of claims 1 to 20, wherein the amino acid mutations in the second CH3 domain sequence further comprise an amino acid substitution at position H6005 selected from H6005Y, H6005F, H6005M and H6005W.

22. The IgA HetFc construct according to any one of claims 1 to 20, wherein the amino acid mutations in the second CH3 domain sequence further comprise the amino acid substitution H6005Y.

23. The IgA HetFc construct according to any one of claims 1 to 10, wherein the modified CH3 domain further comprises amino acid substitutions to introduce cysteine residues capable of forming a disulfide bond.

24. The IgA HetFc construct according to claim 23, wherein the modified CH3 domain comprises two amino acid substitutions to introduce cysteine residues that form one disulfide bond in the modified CH3 domain, or four amino acid substitutions to introduce cysteine residues that form two disulfide bonds in the modified CH3 domain.

25. The IgA Het Fc construct according to claim 23, wherein the amino acid substitutions to introduce cysteine residues comprise the mutation H6005C in one CH3 domain sequence and the mutation P6010C in the other CH3 domain sequence.

26. The IgA HetFc construct according to claim 23, wherein the amino acid substitutions to introduce cysteine residues comprise the mutations H6005C and P6010C in one CH3 domain sequence and the mutations P6010C and H6005C in the other CH3 domain sequence.

27. An IgA heterodimeric Fc (IgA HetFc) construct comprising a first Fc polypeptide and a second Fc polypeptide, the first Fc polypeptide comprising a first CH3 domain sequence and the second Fc polypeptide comprising a second CH3 domain sequence, the first and second CH3 domain sequences forming a modified CH3 domain,

wherein the first and second CH3 domain sequences comprise amino acid mutations that promote formation of a heterodimeric Fc over a homodimeric Fc,

wherein:

(a) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: A6085YY and T6086L, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: L6079T, W6081L and I6088L; or

(b) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: A6085YY and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: L6079T, W6081L and I6088L; or

(c) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: L6079V, W6081L and I6088L; or

(d) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: L6079V, W6081T and I6088L; or

(e) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: T6022V, A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: L6079V, W6081T and I6088L; or

(f) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: T6022L, A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: L6079V, W6081T and I6088L; or

(g) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: T6022I, A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: L6079V, W6081T and I6088L; or

(h) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: L6007F, L6079V, W6081T and I6088L; or

(i) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: H6005Y, A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: H6005Y, L6079V, W6081T and I6088L; or

(j) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: H6005C, A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: P6010C, L6079V, W6081T and I6088L; or

(k) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: P6010C, A6085YF and T6086Y, and the amino acid mutations in the second CH3

domain sequence comprise the amino acid substitutions: H6005C, L6079V, W6081T and I6088L;
or

(l) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: H6005C, P6010C, A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: H6005C, P6010C, L6079V, W6081T and I6088L,

and wherein the numbering of amino acid positions is according to IMGT numbering.

28. An IgA heterodimeric Fc (IgA HetFc) construct comprising a first Fc polypeptide and a second Fc polypeptide, the first Fc polypeptide comprising a first CH3 domain sequence and the second Fc polypeptide comprising a second CH3 domain sequence, the first and second CH3 domain sequences forming a modified CH3 domain,

wherein the first and second CH3 domain sequences comprise amino acid mutations that promote formation of a heterodimeric Fc over a homodimeric Fc,

wherein:

(a) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: L6079V, W6081T and I6088L; or

(b) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: L6007F, L6079V, W6081T and I6088L; or

(c) the amino acid mutations in the first CH3 domain sequence comprise the amino acid substitutions: H6005Y, A6085YF and T6086Y, and the amino acid mutations in the second CH3 domain sequence comprise the amino acid substitutions: H6005Y, L6079V, W6081T and I6088L,

wherein the heterodimeric Fc is formed with a purity of 70% or higher,

wherein the modified CH3 domain has a melting temperature (T_m) that is 60°C or higher,

and wherein the numbering of amino acid positions is according to IMGT numbering.

29. The IgA HetFc construct according to claim 28, wherein the modified CH3 domain has a melting temperature (T_m) that is + 5°C of the T_m of a corresponding wild-type IgA CH3 domain.

30. The IgA HetFc construct according to any one of claims 1 to 29 further comprising one or more target binding domains.

31. The IgA HetFc construct according to claim 30, wherein the one or more target binding domains are antigen-binding antibody fragments.

32. The IgA HetFc construct according to claim 31, wherein each of the one or more antigen-binding antibody fragments are independently selected from a Fab and an scFv.

33. The IgA HetFc construct according to any one of claims 30 to 32, wherein the IgA HetFc construct comprises two target binding domains and is bispecific.

34. The IgA HetFc construct according to any one of claims 1 to 33, wherein the modified IgA CH3 domain comprises an α -tailpiece.

35. The IgA HetFc construct according to any one of claims 1 to 33, wherein the modified IgA CH3 domain lacks an α -tailpiece.

36. A conjugate comprising the IgA HetFc construct according to any one of claims 1 to 35 and one or more therapeutic, diagnostic or labeling agents.

37. An IgA HetFc multimer comprising two or more IgA HetFc constructs according to any one of claims 1 to 34 and a J chain, wherein two of the IgA HetFc constructs are joined by the J chain.

38. A pharmaceutical composition comprising the IgA HetFc construct according to any one of claims 1 to 35 and a pharmaceutically acceptable carrier or diluent.

39. A pharmaceutical composition comprising the conjugate according to claim 36 and a pharmaceutically acceptable carrier or diluent.

40. A pharmaceutical composition comprising the IgA HetFc multimer according to claim 37 and a pharmaceutically acceptable carrier or diluent.

41. An isolated polynucleotide or set of polynucleotides encoding the IgA HetFc construct according to any one of claims 1 to 35.

42. A vector set or set of vectors comprising one or more polynucleotides encoding the IgA HetFc according to any one of claims 1 to 35.

43. A host cell comprising one or more polynucleotides encoding the IgA HetFc according to any one of claims 1 to 35.

44. A method of preparing the IgA HetFc construct according to any one of claims 1 to 35 comprising transfecting a host cell with one or more polynucleotides encoding the IgA HetFc construct, and culturing the host cell under conditions suitable for expression of the IgA HetFc construct.

45. A method of preparing the IgA HetFc multimer according to claim 37 comprising transfecting a host cell with one or more polynucleotides encoding the IgA HetFc construct according to claim 34 and a polynucleotide encoding a J chain, and culturing the host cell under conditions suitable for expression of the IgA HetFc construct and the J chain.

46. An IgA HetFc construct of any one of claims 1 to 35, wherein the IgA Het Fc includes one or more mutations to eliminate binding to a binding target.

47. An IgA HetFc construct of any one of claims 1 to 35, wherein the IgA HetFc includes one or more mutations to introduce binding to Neonatal Fc Receptor (FcRn).