

CLAIMS

1. A multispecific binding protein that binds a first antigen and a second antigen, the multispecific binding protein comprising:
 - a first antigen binding domain comprising a first light chain Fab region and a first
 - 5 heavy chain Fab region, wherein the first light chain Fab region is a kappa light chain and comprises:
 - a lysine at amino acid residue 143 (EU numbering) and a lysine at amino acid residue 199 (EU numbering);
 - a lysine at amino acid residue 143 (EU numbering), a lysine at amino acid
 - 10 residue 199 (EU numbering), and an alanine at amino acid residue 109 (EU numbering);
 - a lysine at amino acid residue 143 (EU numbering), a lysine at amino acid residue 199 (EU numbering), and an aspartic acid at amino acid residue 110 (EU numbering);
 - 15 a lysine at amino acid residue 143 (EU numbering), a lysine at amino acid residue 199 (EU numbering), an alanine at amino acid residue 109 (EU numbering), and an aspartic acid at amino acid residue 110 (EU numbering);
 - an aspartic acid at amino acid residue 110 (EU numbering) and a lysine at amino acid residue 143 (EU numbering);
 - 20 an aspartic acid at amino acid residue 110 (EU numbering), a lysine at amino acid residue 143 (EU numbering) and an alanine at amino acid residue 109 (EU numbering);
 - an aspartic acid at amino acid residue 110 (EU numbering) and a lysine at amino acid residue 199 (EU numbering);
 - 25 an aspartic acid at amino acid residue 110 (EU numbering), a lysine at amino acid residue 199 (EU numbering) and an alanine at amino acid residue 109 (EU numbering);
 - an alanine at amino acid residue 109 (EU numbering) and a lysine at amino acid residue 143 (EU numbering);
 - 30 an alanine at amino acid residue 109 (EU numbering) and a lysine at amino acid residue 199 (EU numbering); or

an alanine at amino acid residue 109 (EU numbering) and an aspartic acid at amino acid residue 110 (EU numbering); and

a second antigen binding domain comprising a second light chain Fab region and a second heavy chain Fab region,

5 wherein the first antigen binding domain binds the first antigen and the second antigen binding domain binds the second antigen.

2. The multispecific binding protein of claim 1, wherein:

10 if the first light chain Fab region comprises a lysine at amino acid residue 143 (EU numbering) and a lysine at amino acid residue 199 (EU numbering), then the second light chain Fab region does not comprise a lysine at amino acid residue 143 (EU numbering) and a lysine at amino acid residue 199 (EU numbering);

15 if the first light chain Fab region comprises a lysine at amino acid residue 143 (EU numbering), a lysine at amino acid residue 199 (EU numbering), and an alanine at amino acid residue 109 (EU numbering), then the second light chain Fab region does not comprise a lysine at amino acid residue 143 (EU numbering), a lysine at amino acid residue 199 (EU numbering), and an alanine at amino acid residue 109 (EU numbering);

20 if the first light chain Fab region comprises a lysine at amino acid residue 143 (EU numbering), a lysine at amino acid residue 199 (EU numbering), and an aspartic acid at amino acid residue 110 (EU numbering), then the second light chain Fab region does not comprise a lysine at amino acid residue 143 (EU numbering), a lysine at amino acid residue 199 (EU numbering), and an aspartic acid at amino acid residue 110 (EU numbering);

25 if the first light chain Fab region comprises a lysine at amino acid residue 143 (EU numbering), a lysine at amino acid residue 199 (EU numbering), an alanine at amino acid residue 109 (EU numbering), and an aspartic acid at amino acid residue 110 (EU numbering), then the second light chain Fab region does not comprise a lysine at amino acid residue 143 (EU numbering), a lysine at amino acid residue 199 (EU numbering), an alanine at amino acid residue 109 (EU numbering);

30 if the first light chain Fab region comprises an aspartic acid at amino acid residue 110 (EU numbering) and a lysine at amino acid residue 143 (EU numbering), then

the second light chain Fab region does not comprise an aspartic acid at amino acid residue 110 (EU numbering) and a lysine at amino acid residue 143 (EU numbering);

if the first light chain Fab region comprises an aspartic acid at amino acid residue 110 (EU numbering), a lysine at amino acid residue 143 (EU numbering) and an alanine at amino acid residue 109 (EU numbering), then the second light chain Fab region does not comprise an aspartic acid at amino acid residue 110 (EU numbering), a lysine at amino acid residue 143 (EU numbering) and an alanine at amino acid residue 109 (EU numbering);

if the first light chain Fab region comprises an aspartic acid at amino acid residue 110 (EU numbering) and a lysine at amino acid residue 199 (EU numbering), then the second light chain Fab region does not comprise an aspartic acid at amino acid residue 110 (EU numbering) and a lysine at amino acid residue 199 (EU numbering);

if the first light chain Fab region comprises an aspartic acid at amino acid residue 110 (EU numbering), a lysine at amino acid residue 199 (EU numbering) and an alanine at amino acid residue 109 (EU numbering), then the second light chain Fab region does not comprise an aspartic acid at amino acid residue 110 (EU numbering), a lysine at amino acid residue 199 (EU numbering) and an alanine at amino acid residue 109 (EU numbering);

if the first light chain Fab region comprises an alanine at amino acid residue 109 (EU numbering) and a lysine at amino acid residue 143 (EU numbering), then the second light chain Fab region does not comprise an alanine at amino acid residue 109 (EU numbering) and a lysine at amino acid residue 143 (EU numbering);

if the first light chain Fab region comprises an alanine at amino acid residue 109 (EU numbering) and a lysine at amino acid residue 199 (EU numbering), then the second light chain Fab region does not comprise an alanine at amino acid residue 109 (EU numbering) and a lysine at amino acid residue 199 (EU numbering); and

if the first light chain Fab region comprises an alanine at amino acid residue 109 (EU numbering) and an aspartic acid at amino acid residue 110 (EU numbering), then the second light chain Fab region does not comprise an alanine at amino acid residue 109 (EU numbering) and an aspartic acid at amino acid residue 110 (EU numbering).

3. A multispecific binding protein that binds a first antigen and a second antigen, the multispecific binding protein comprising:
 - a first antigen binding domain comprising a first light chain Fab region and a first heavy chain Fab region, wherein the first light chain Fab region is a kappa light chain and comprises a lysine at amino acid residue 143 (EU numbering) and a lysine at amino acid residue 199 (EU numbering); and
 - a second antigen binding domain comprising a second light chain Fab region and a second heavy chain Fab region,
wherein the first antigen binding domain binds the first antigen and the second antigen binding domain binds the second antigen.
4. The multispecific binding protein of Claim 3, wherein the first light chain Fab region further comprises an aspartic acid at amino acid residue 110 (EU numbering).
5. The multispecific binding protein of any of Claims 3 or 4, wherein the first light chain Fab region further comprises an alanine at amino acid residue 109 (EU numbering).
6. A multispecific binding protein that binds a first antigen and a second antigen, the multispecific binding protein comprising:
 - a first antigen binding domain comprising a first light chain Fab region and a first heavy chain Fab region, wherein the first light chain Fab region is a kappa light chain and comprises an aspartic acid at amino acid residue 110 (EU numbering) and a lysine at amino acid residue 143 (EU numbering); and
 - a second antigen binding domain comprising a second light chain Fab region and a second heavy chain Fab region,
wherein the first antigen binding domain binds the first antigen and the second antigen binding domain binds the second antigen.
7. The multispecific binding protein of Claim 6, wherein the first light chain Fab region further comprises an alanine at amino acid residue 109 (EU numbering).

8. A multispecific binding protein that binds a first antigen and a second antigen, the multispecific binding protein comprising:
- 5 a first antigen binding domain comprising a first light chain Fab region and a first heavy chain Fab region, wherein the first light chain Fab region is a kappa light chain and comprises an aspartic acid at amino acid residue 110 (EU numbering) and a lysine at amino acid residue 199 (EU numbering); and
- 10 a second antigen binding domain comprising a second light chain Fab region and a second heavy chain Fab region, wherein the first antigen binding domain binds the first antigen and the second antigen binding domain binds the second antigen.
9. The multispecific binding protein of Claim 8, wherein the first light chain Fab region further comprises an alanine at amino acid residue 109 (EU numbering).
- 15 10. A multispecific binding protein that binds a first antigen and a second antigen, the multispecific binding protein comprising:
- 20 a first antigen binding domain comprising a first light chain Fab region and a first heavy chain Fab region, wherein the first light chain Fab region is a kappa light chain and comprises an alanine at amino acid residue 109 (EU numbering) and a lysine at amino acid residue 143 (EU numbering); and
- 25 a second antigen binding domain comprising a second light chain Fab region and a second heavy chain Fab region, wherein the first antigen binding domain binds the first antigen and the second antigen binding domain binds the second antigen.
11. A multispecific binding protein that binds a first antigen and a second antigen, the multispecific binding protein comprising:
- 30 a first antigen binding domain comprising a first light chain Fab region and a first heavy chain Fab region, wherein the first light chain Fab region is a kappa light chain and comprises an alanine at amino acid residue 109 (EU numbering) and a lysine at amino acid residue 199 (EU numbering); and

a second antigen binding domain comprising a second light chain Fab region and a second heavy chain Fab region,
wherein the first antigen binding domain binds the first antigen and the second antigen binding domain binds the second antigen.

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12. A multispecific binding protein that binds a first antigen and a second antigen, the multispecific binding protein comprising:

a first antigen binding domain comprising a first light chain Fab region and a first heavy chain Fab region, wherein the first light chain Fab region is a kappa light chain and comprises an alanine at amino acid residue 109 (EU numbering) and an aspartic acid at amino acid residue 110 (EU numbering); and

a second antigen binding domain comprising a second light chain Fab region and a second heavy chain Fab region,
wherein the first antigen binding domain binds the first antigen and the second antigen binding domain binds the second antigen.

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13. The multispecific binding protein of any one of Claims 1 to 12, wherein the first antigen binding domain further comprises a first heavy chain Fc region.

14. The multispecific binding protein of Claim 13, wherein the first heavy chain Fc region comprises a human IgG1, a human IgG2 or a human IgG4 constant region.

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15. The multispecific binding protein of any one of Claims 1 to 14, wherein the second antigen binding domain further comprises a second heavy chain Fc region.

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16. The multispecific binding protein of Claim 15, wherein the second heavy chain Fc region comprises a human IgG1, a human IgG2 or a human IgG4 constant region.

17. The multispecific binding protein of any one of Claims 13 to 16, wherein the first heavy chain Fc region comprises an arginine at amino acid residue 311 (EU

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numbering) and a glutamic acid at amino acid residue 317 (EU numbering).

18. The multispecific binding protein of any one of Claims 13 to 17, wherein the
second heavy chain Fc region comprises an arginine at amino acid residue 311
5 (EU numbering) and a glutamic acid at amino acid residue 317 (EU numbering).

19. The multispecific binding protein of any one of Claims 13 to 18, wherein both the
first and second heavy chain Fc regions comprise a human IgG1 constant region;
both comprise a human IgG2 constant region; or both comprise a human IgG4
10 constant region.

20. The multispecific binding protein of any one of Claims 13 to 19, wherein both the
first and second heavy chain Fc regions comprise an arginine at amino acid
residue 311 (EU numbering) and a glutamic acid at amino acid residue 317 (EU
15 numbering).

21. The multispecific binding protein of any one of Claims 1 to 20, wherein the
second light chain Fab region does not comprise an alanine at amino acid residue
109; does not comprise an aspartic acid at amino acid residue 110; does not
20 comprise a lysine at amino acid residue 143; or does not comprise a lysine at
amino acid residue 199.

22. The multispecific binding protein of any one of Claims 1 to 21, wherein the
25 second light chain Fab region is a Kappa light chain.

23. The multispecific binding protein of any one of Claims 1 to 22, wherein the
second light chain Fab region is a Lambda light chain.

30 24. A method of purifying a multispecific binding protein comprising a first antigen
binding domain that binds a first antigen and a second antigen binding domain that
binds a second antigen, the method comprising:
introducing into the first antigen binding domain a first light chain Fab region

comprising a lysine at amino acid residue 143 (EU numbering) and a lysine at amino acid residue 199 (EU numbering), wherein the first light chain Fab region is a kappa light chain;

expressing the multispecific binding protein, wherein the first antigen binding domain assembles with the second antigen binding domain; and

subjecting the multispecific binding protein to an affinity chromatography column; and

recovering purified multispecific binding protein.

25. The method of Claim 24, wherein the step of introducing further comprises introducing into the first antigen binding domain an alanine at amino acid residue 109 (EU numbering).

26. The method of any of Claims 24 or 25, wherein the step of introducing further comprises introducing into the first antigen binding domain an aspartic acid at amino acid residue 110 (EU numbering).

27. A method of purifying a multispecific binding protein comprising a first antigen binding domain that binds a first antigen and a second antigen binding domain that binds a second antigen, the method comprising:

introducing into the first antigen binding domain a first light chain Fab region comprising an aspartic acid at amino acid residue 110 (EU numbering) and a lysine at amino acid residue 143 (EU numbering), wherein the first light chain Fab region is a kappa light chain;

expressing the multispecific binding protein, wherein the first antigen binding domain assembles with the second antigen binding domain; and

subjecting the multispecific binding protein to an affinity chromatography column; and

recovering purified multispecific binding protein.

28. The method of Claim 27, wherein the step of introducing further comprises introducing into the first antigen binding domain an alanine at amino acid residue 109 (EU numbering).
- 5 29. A method of purifying a multispecific binding protein comprising a first antigen binding domain that binds a first antigen and a second antigen binding domain that binds a second antigen, the method comprising:
- introducing into the first antigen binding domain a first light chain Fab region comprising an aspartic acid at amino acid residue 110 (EU numbering) and a lysine at amino acid residue 199 (EU numbering), wherein the first light chain Fab region is a kappa light chain;
- 10 expressing the multispecific binding protein, wherein the first antigen binding domain assembles with the second antigen binding domain; and
- subjecting the multispecific binding protein to an affinity chromatography column; and
- 15 recovering purified multispecific binding protein.
30. The method of Claim 29, wherein the step of introducing further comprises introducing into the first antigen binding domain an alanine at amino acid residue 109 (EU numbering).
- 20 31. A method of purifying a multispecific binding protein comprising a first antigen binding domain that binds a first antigen and a second antigen binding domain that binds a second antigen, the method comprising:
- 25 introducing into the first antigen binding domain a first light chain Fab region comprising an alanine at amino acid residue 109 (EU numbering) and a lysine at amino acid residue 143 (EU numbering), wherein the first light chain Fab region is a kappa light chain;
- expressing the multispecific binding protein, wherein the first antigen binding domain assembles with the second antigen binding domain; and
- 30 subjecting the multispecific binding protein to an affinity chromatography

column; and

recovering purified multispecific binding protein.

32. A method of purifying a multispecific binding protein comprising a first antigen
5 binding domain that binds a first antigen and a second antigen binding domain that
binds a second antigen, the method comprising:
introducing into the first antigen binding domain a first light chain Fab region
comprising an alanine at amino acid residue 109 (EU numbering) and a lysine at
amino acid residue 199 (EU numbering), wherein the first light chain Fab region is
10 a kappa light chain;
expressing the multispecific binding protein, wherein the first antigen binding
domain assembles with the second antigen binding domain; and
subjecting the multispecific binding protein to an affinity chromatography
column; and
15 recovering purified multispecific binding protein.
33. A method of purifying a multispecific binding protein comprising a first antigen
binding domain that binds a first antigen and a second antigen binding domain that
binds a second antigen, the method comprising:
20 introducing into the first antigen binding domain a first light chain Fab region
comprising an alanine at amino acid residue 109 (EU numbering) and an aspartic
acid at amino acid residue 110 (EU numbering), wherein the first light chain Fab
region is a kappa light chain;
expressing the multispecific binding protein, wherein the first antigen binding
25 domain assembles with the second antigen binding domain; and
subjecting the multispecific binding protein to an affinity chromatography
column; and
recovering purified multispecific binding protein.

34. The method of any one of Claims 24 to 33, wherein the step of introducing further comprises introducing into the first antigen binding domain a first heavy chain Fc region.
- 5 35. The method of Claim 34, wherein the first heavy chain Fc region comprises a human IgG1, a human IgG2 or a human IgG4 constant region.
36. The method of any one of Claims 24 to 35, wherein the step of introducing further comprises introducing into the second antigen binding domain a second heavy
10 chain Fc region.
37. The method of Claim 36, wherein the second heavy chain Fc region comprises a human IgG1, a human IgG2 or a human IgG4 constant region.
- 15 38. The method of any one of Claims 34 to 37, wherein the step of introducing further comprises introducing into the first heavy chain Fc region an arginine at amino acid residue 311 (EU numbering) and a glutamic acid at amino acid residue 317 (EU numbering).
- 20 39. The method of any one of Claims 34 to 38, wherein the step of introducing further comprises introducing into the second heavy chain Fc region an arginine at amino acid residue 311 (EU numbering) and a glutamic acid at amino acid residue 317 (EU numbering).
- 25 40. The method of any one of Claims 24 to 39, wherein both the first heavy chain Fc region and the second heavy chain Fc region comprise a human IgG1 constant region; both comprise a human IgG2 constant region; or both comprise a human IgG4 constant region.
- 30 41. The method of any one of Claims 24 to 40, wherein the step of introducing further comprises introducing, into both the first heavy chain Fc region and the second

heavy chain Fc region, arginine at amino acid residues 311 (EU numbering) and glutamic acid at amino acid residues 317 (EU numbering).

42. The method of any one of Claims 24 to 41, wherein the second light chain Fab
5 region does not comprise an alanine at amino acid residue 109; does not
comprise an aspartic acid at amino acid residue 110; does not comprise a lysine
at amino acid residue 143; or does not comprise a lysine at amino acid residue
199.
- 10 43. The method of any one of Claims 24 to 41, wherein the second light chain Fab
region does not comprise an alanine at amino acid residue 109; does not
comprise an aspartic acid at amino acid residue 110; does not comprise a lysine
at amino acid residue 143; and does not comprise a lysine at amino acid residue
199.
- 15 44. The method of any one of Claims 24 to 43, wherein the second light chain Fab
region is a Kappa light chain.
45. The method of any one of Claims 24 to 43, wherein the second light chain Fab
20 region is a Lambda light chain
46. The method of any one of Claims 24 to 45, wherein the affinity chromatography
column comprises a kappa affinity ligand.
- 25 47. The method of any one of Claims 24 to 46, wherein the affinity chromatography
column comprises a lambda affinity ligand.
48. The method of any one of Claims 24 to 47, wherein the affinity chromatography
column comprises Protein A.
- 30 49. The method of any one of Claims 24 to 48, wherein the second light chain Fab

region binds to the affinity chromatography column with greater affinity than the first light chain Fab region.

50. The method of any one of Claims 24 to 49, wherein the first light chain Fab
5 region does not bind to the affinity chromatography column.

51. The method of any one of Claims 24 to 50, further comprising the steps of:
subjecting the purified multispecific binding protein to a second affinity
chromatography column after the step of recovering purified multispecific
10 binding protein; and
recovering purified multispecific binding protein after the step of subjecting
the purified multispecific binding protein to a second affinity chromatography
column.

15 52. The method of 51, wherein the second affinity chromatography column
comprises a kappa affinity ligand.

53. The method of any one of Claims 51 or 52, wherein the second affinity
chromatography column comprises a lambda affinity ligand.
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54. The method of any one of Claims 51 to 53, wherein the second affinity
chromatography column comprises Protein A.

55. The method of any one of Claims 51 to 54, wherein the second light chain Fab
25 region binds to the second affinity chromatography column with greater affinity
than the first light chain Fab region.

56. The method of any one of Claims 51 to 55, wherein the first light chain Fab
region does not bind to the second affinity chromatography column.
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