

92(15):7021-7025; 1995) and strategies described in Lewis *et al.* (2014) "Generation of bispecific IgG antibodies by structure-based design of an orthogonal Fab interface." *Nat Biotechnol* 32, 191-8; Liu *et al.* (2015) "A Novel Antibody Engineering Strategy for Making Monovalent Bispecific Heterodimeric IgG Antibodies by Electrostatic Steering Mechanism." *J Biol Chem*. Published online January 12, 2015, doi:10.1074/jbc.M114.620260; Mazor *et al.* 2015. "Improving target cell specificity using a novel monovalent bispecific IgG design." *Mabs*. Published online January 26, 2015, doi: 10.1080/19420862.2015.1007816; WO 2014/081955, WO 2014/082179, and WO 2014/150973.

[0006] There nevertheless remains a need in the art for methods of reducing mispaired heavy chain/light chain by-products and increase yield of correctly assembled bispecific antibody.

SUMMARY

[0007] In one example, the present disclosure refers to a method of improving preferential pairing of a heavy chain and a light chain of an antibody, comprising the step of substituting at least one amino acid at position 94 of a light chain variable domain (V_L) or position 96 of the V_L , from a non-charged residue to a charged residue selected from the group consisting of aspartic acid (D), arginine (R), glutamic acid (E), and lysine (K), wherein the amino acid numbering is according to Kabat. In one aspect, the disclosure refers to a method of improving preferential pairing of a heavy chain and a light chain of an antibody, comprising the step of substituting at least one amino acid at position 94 of a light chain variable domain (V_L) or position 96 of the V_L , from a non-charged residue to a charged residue selected from the group consisting of aspartic acid (D), arginine (R), glutamic acid (E), and lysine (K), wherein the amino acid numbering is according to Kabat, wherein (i) the amino acid at position 94 is substituted with D; (ii) the amino acid at position 96 is substituted with R; or (iii) the amino acid at position 94 is substituted with D and the amino acid at position 96 is substituted with R. In some examples, the method comprises the step of substituting each of the amino acids at position 94 and position 96 from a non-charged residue to a charged residue. In some examples, the amino acid at position 94 is substituted with D. In some examples, the amino acid at position 96 is substituted with R. In some examples, the amino acid at position 94 is substituted with D and the amino acid at position 96 is substituted with R. In some examples, the amino acid at position 95 of a heavy chain variable domain (V_H) is substituted from a non-charged residue to a charged residue selected from the group consisting of aspartic acid (D), arginine (R), glutamic acid (E), and lysine (K), wherein the amino acid numbering is according to Kabat. In some examples, the amino acid at position 94 of the V_L is substituted with D, the amino acid at position 96 of the V_L is substituted with R, and the amino acid at position 95 of the V_H is substituted with D.

[0008] In some examples, a method provided herein further comprises subjecting the antibody (*e.g.*, the antibody that has been modified to improve preferential pairing of the heavy chain and the light chain) to at least one affinity maturation step, wherein the substituted amino acid at position 94 of the V_L is not randomized. Additionally or alternatively, in some examples, the substituted amino acid at position 96 of the V_L is not randomized. Additionally or alternatively, in some examples, the substituted amino acid at position 95 of the V_H is not randomized.

[0009] In some examples, the antibody is an antibody fragment selected from the group consisting of: a Fab, a Fab', an F(ab')₂, a one-armed antibody, and scFv, or an Fv. In some examples,

CLAIMS

1. A method of improving preferential pairing of a heavy chain and a light chain of an antibody, comprising the step of substituting at least one amino acid at position 94 of a light chain variable domain (V_L) or position 96 of the V_L , from a non-charged residue to a charged residue selected from the group consisting of aspartic acid (D), arginine (R), glutamic acid (E), and lysine (K), wherein the amino acid numbering is according to Kabat,
wherein
 - (i) the amino acid at position 94 is substituted with D;
 - (ii) the amino acid at position 96 is substituted with R; or
 - (iii) the amino acid at position 94 is substituted with D and the amino acid at position 96 is substituted with R.
2. The method of claim 1, comprising the step of substituting each of the amino acids at position 94 and position 96 from a non-charged residue to a charged residue.
3. The method of claim 1 or 2, wherein the amino acid at position 94 is substituted with D.
4. The method of any one of claims 1-3, wherein the amino acid at position 96 is substituted with R.
5. The method of any one of claims 1-4, wherein the amino acid at position 94 is substituted with D and the amino acid at position 96 is substituted with R.
6. The method of any one of claims 1-5, wherein the amino acid at position 95 of a heavy chain variable domain (V_H) is substituted from a non-charged residue to a charged residue selected from the group consisting of aspartic acid (D), arginine (R), glutamic acid (E), and lysine (K), wherein the amino acid numbering is according to Kabat.
7. The method of any one of claims 1-6, wherein the amino acid at position 94 of the V_L is substituted with D, the amino acid at position 96 of the V_L is substituted with R, and the amino acid at position 95 of the V_H is substituted with D.
8. The method of any one of claims 1-7, further comprising subjecting the antibody to at least one affinity maturation step, wherein the substituted amino acid at position 94 of the V_L is not randomized.

9. The method of claim 8, wherein the substituted amino acid at position 96 of the V_L is not randomized.
10. The method of claim 8 or 9, wherein the substituted amino acid at position 95 of the V_H is not randomized.
11. The method of any one of claims 1-10, wherein the antibody is an antibody fragment selected from the group consisting of: a Fab, a Fab', an $F(ab')_2$, a one-armed antibody, and scFv, or an Fv.
12. The method of any one of claims 1-11, wherein the antibody is a human, humanized, or chimeric antibody.
13. The method of any one of claims 1-12, wherein the antibody comprises a human IgG Fc region.
14. The method of claim 13, wherein the human IgG Fc region is a human IgG1, human IgG2, human IgG3, or human IgG4 Fc region.
15. The method of any one of claims 1-14, wherein the antibody is a monospecific antibody.
16. The method of any one of claims 1-14, wherein the antibody is a multispecific antibody.
17. The method of claim 16, wherein the multispecific antibody is a bispecific antibody.
18. The method of claim 17, wherein the bispecific antibody comprises a first C_H2 domain (C_{H2_1}), a first C_H3 domain (C_{H3_1}), a second C_H2 domain (C_{H2_2}), and a second C_H3 domain;

wherein C_{H3_2} is altered so that within the C_{H3_1}/C_{H3_2} interface, one or more amino acid residues are replaced with one or more amino acid residues having a larger side chain volume, thereby generating a protuberance on the surface of C_{H3_2} that interacts with C_{H3_1} ; and

wherein C_{H3_1} is altered so that within the C_{H3_1}/C_{H3_2} interface, one or more amino acid residues are replaced with one or more amino acid residues having a smaller side chain volume, thereby generating a cavity on the surface of C_{H3_1} that interacts with C_{H3_2} .

19. The method of claim 17, wherein the bispecific antibody comprises a first C_H2 domain (C_H2₁), a first C_H3 domain (C_H3₁), a second C_H2 domain (C_H2₂), and a second C_H3 domain;

wherein C_H3₁ is altered so that within the C_H3₁/ C_H3₂ interface, one or more amino acid residues are replaced with one or more amino acid residues having a larger side chain volume, thereby generating a protuberance on the surface of C_H3₁ that interacts with C_H3₂; and

wherein C_H3₂ is altered so that within the C_H3₁/ C_H3₂ interface, one or more amino acid residues are replaced with one or more amino acid residues having a smaller side chain volume, thereby generating a cavity on the surface of C_H3₂ that interacts with C_H3₁.

20. The method of claim 18 or 19, wherein the protuberance is a knob mutation.

21. The method of claim 20, wherein the knob mutation comprises T366W, wherein amino acid numbering is according to the EU index.

22. The method of claim 18 or 19, wherein the cavity is a hole mutation.

23. The method of claim 22, wherein the hole mutation comprises at least one, at least two, or all three of T366S, L368A, and Y407V, wherein amino acid numbering is according to the EU index.