

What is claimed is:

1. A method of purifying a heterodimeric protein, comprising:
 - (a) introducing a mixture of a heterodimeric protein and impurities to an affinity matrix containing a protein-binding ligand, wherein the heterodimeric protein comprises first and second polypeptides with differing affinity for the protein-binding ligand, and wherein at least one impurity binds the protein-binding ligand and at least one impurity does not bind the protein-binding ligand, or has a reduced affinity for binding to the protein-binding ligand relative to the impurity that binds the protein-binding ligand;
 - (b) washing the affinity matrix with a first wash buffer comprising a salt at a concentration greater than 200 mM and a first pH of from 5 to 9 to remove the at least one impurity that does not bind the protein-binding ligand or has a reduced affinity for binding the protein-binding ligand;
 - (c) eluting and collecting the heterodimeric protein from the affinity matrix in a first elution buffer comprising a salt at a concentration greater than 200 mM and a second pH of from 4 to 5 to obtain a purified heterodimeric protein in a first eluate;
 - (d) washing the affinity matrix with a second wash buffer comprising a third pH of less than 4 to remove the at least one impurity that binds the protein-binding ligand;
 - (e) equilibrating the affinity matrix to a fourth pH of from 5 to 9;
 - (f) neutralizing the first eluate to a pH of 5 to 9, then reapplying the first eluate to the affinity matrix;
 - (g) washing the affinity matrix with a third wash buffer comprising less than 100 mM salt; and
 - (h) eluting and collecting the purified heterodimeric protein in a second eluate, wherein the second eluate comprises less than 100 mM of the salt.
2. The method of claim 1, wherein the third wash buffer comprises less than 50 mM salt.
3. The method of claim 1 or 2, wherein the impurities comprise homodimeric species of the first and second polypeptides.
4. The method of any one of claims 1-3, wherein the protein-binding ligand is Protein A, and the affinity matrix comprises the Protein A ligand affixed to a substrate.

5. The method of claim 4, wherein the Protein A ligand is an engineered Protein A comprising a Z-domain tetramer, an engineered Protein A comprising a Y-domain tetramer, or an engineered Protein A that lacks D and E domains.

6. The method of any one of claims 1-3, wherein the protein-binding ligand is Protein G, and the affinity matrix comprises the Protein G ligand affixed to a substrate.

7. The method of any one of claims 4-6, wherein the substrate is a particle and the affinity matrix comprises a multiplicity of the particles comprising a mean diameter of from 25 μm to 100 μm .

8. The method of claim 7, wherein the particles comprise a mean diameter of from 40 μm to 60 μm .

9. The method of claim 8, wherein the particles comprise a mean diameter of from 45 μm to 55 μm .

10. The method of claim 9, wherein the particles comprise a mean diameter of about 50 μm .

11. The method of any one of claims 4-10, wherein the substrate comprises any one or more of agarose, poly(styrene divinylbenzene), polymethacrylate, cellulose, controlled pore glass, and spherical silica.

12. The method of any one of claims 4-11, wherein the particles comprise pores having a mean diameter of about 1100 $\text{\AA}$.

13. The method of any one of claims 1-12, wherein the first elution buffer comprises a salt at a concentration of greater than 250 mM.

14. The method of claim 13, wherein the first elution buffer comprises a salt at a concentration greater than 300 mM or greater than 400 mM.

15. The method of claim 14, wherein the first elution buffer comprises a salt at a concentration of about 500 mM.

16. The method of any one of claims 1-15, wherein the salt is selected from a salt containing (i) Cl^- , Br^- , I^- , NO_3^- , $\text{N}(\text{CH}_3)_4^+$, NH_4^+ , Cs^+ , Rb^+ , K^+ , Na^+ , Ca^{2+} , Mg^{2+} , or Al^{3+} ; (ii)

combinations of Na^+ , Ca^{2+} , Mg^{2+} or Al^{3+} with Cl^- , Br^- , I^- , NO_3^- , or ClO_4^- , or (iii) CaCl_2 , MgCl_2 or NaCl .

17. The method of any one of claims 1-16, wherein the second eluate comprises less than 50 mM of the salt.

18. The method of claim 17, wherein the second eluate comprises less than 10 mM of the salt.

19. The method of any one of claims 1-18, wherein the purified heterodimeric protein is eluted and collected in the second eluate via the third wash buffer.

20. The method of any one of claims 1-19, wherein the first polypeptide comprises a CH3 domain that is capable of binding to the protein-binding ligand and the second polypeptide comprises a CH3 domain with a reduced affinity for binding to the protein-binding ligand relative to the CH3 domain of the first polypeptide.

21. The method of any one of claims 1-19, wherein the first polypeptide comprises a CH3 domain that is capable of binding to the protein-binding ligand and the second polypeptide comprises a CH3 domain that is not capable of binding to the protein-binding ligand.

22. The method of claim 4 or 5, wherein the first polypeptide comprises a CH3 domain that is capable of binding to Protein A and the second polypeptide comprises a CH3 domain with a reduced affinity for binding to Protein A relative to the CH3 domain of the first polypeptide.

23. The method of claim 22, wherein the first polypeptide comprises a CH3 domain that is capable of binding to Protein A and the second polypeptide comprises a CH3 domain that is not capable of binding to Protein A.

24. The method of claim 6, wherein the first polypeptide comprises a CH3 domain that is capable of binding to Protein G and the second polypeptide comprises a CH3 domain with a reduced affinity for binding to Protein G relative to the CH3 domain of the first polypeptide.

25. The method of claim 24, wherein the first polypeptide comprises a CH3 domain that is capable of binding to Protein G and the second polypeptide comprises a CH3 domain that is not capable of binding to Protein G.

26. The method of claim 22 or 23, wherein the second polypeptide comprises a HY to RF substitution in its CH3 domain.

27. The method of any one of claims 1-26, wherein the first pH is from 6 to 8.

28. The method of any one of claims 1-27, wherein the second pH is from 4.0 to 4.25.

29. The method of claim 28, wherein the second pH is 4.10 ± 0.05 .

30. The method of any one of claims 1-29, wherein the third pH is from 2.8 to 3.5.

31. The method of any one of claims 1-30, wherein the fourth pH is from 6 to 8.

32. The method of any one of claims 1-31, further comprising an additional chromatography step or viral inactivation step following step (h).

33. The method of claim 32, wherein the additional chromatography step or viral inactivation step are performed under conditions with less than 100 mM salt.

34. The method of claim 33, wherein the additional chromatography step includes ion exchange chromatography.

35. The method of claim 34, wherein the ion exchange chromatography is anion exchange chromatography and is performed under conditions with less than 50 mM salt.

36. The method of any one of claims 1-35, wherein the heterodimeric protein is a bispecific antigen-binding protein.

37. The method of claim 36, wherein the bispecific antigen-binding protein is a bispecific antibody.

38. The method of any one of claims 1-37, wherein the first and second polypeptides comprise a human heavy chain constant domain of isotype IgG1.

39. The method of any one of claims 1-37, wherein the first and second polypeptides comprise a human heavy chain constant domain of isotype IgG4.