

What is claimed is:

1. A method of removing a component from a chromatographic eluate, comprising:
 - (a) performing a first chromatography step in which the component is present in a first buffer applied to a chromatographic column;
 - (b) collecting an intermediate eluate from the first chromatography step, wherein the intermediate eluate contains a protein product and the component;
 - (c) reapplying the intermediate eluate to the chromatographic column, and eluting the protein product with a second buffer containing the component at a concentration lower than that of the intermediate eluate;
 - (d) collecting the chromatographic eluate from step (c), wherein the component is present in the chromatographic eluate at a concentration lower than that of the intermediate eluate; and
 - (e) applying the chromatographic eluate to a subsequent process step.
2. The method of claim 1, wherein the component is absent from the second buffer.
3. The method of claim 1 or 2, wherein the component is a salt.
4. The method of claim 3, wherein the concentration of the salt in the intermediate eluate is greater than 50 mM.
5. The method of claim 4, wherein the concentration of the salt in the intermediate eluate is ≥ 100 mM, ≥ 250 mM, or ≥ 500 mM.
6. The method of any one of claims 1-5, wherein the first chromatography step is selected from affinity chromatography or ion exchange chromatography.
7. The method of any one of claims 1-6, wherein the subsequent process step is a second chromatography step.
8. The method of any one of claims 1-7, wherein the subsequent process step is selected from affinity chromatography, ion exchange chromatography, mixed-mode chromatography, hydrophobic interaction chromatography, or viral inactivation.

9. The method of any one of claims 1-8, wherein the protein product is an antibody.
10. 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;
 - (b) washing the affinity matrix with a first wash buffer comprising a salt concentration greater than 200 mM and a first pH of from 5 to 9 to remove impurities;
 - (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 impurities;
 - (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.
11. The method of claim 10, wherein the third wash buffer comprises less than 50 mM salt.
12. The method of claim 10 or 11, wherein the impurities comprise homodimeric species of the first and second polypeptides.
13. The method of any one of claims 10-12, wherein the protein-binding ligand is Protein A, and the affinity matrix comprises the Protein A ligand affixed to a substrate.
14. The method of claim 13, 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.

15. The method of any one of claims 10-12, wherein the protein-binding ligand is Protein G, and the affinity matrix comprises the Protein G ligand affixed to a substrate.

16. The method of any one of claims 13-15, 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 .

17. The method of claim 16, wherein the particles comprise a mean diameter of from 40 μm to 60 μm .

18. The method of claim 17, wherein the particles comprise a mean diameter of from 45 μm to 55 μm .

19. The method of claim 18, wherein the particles comprise a mean diameter of about 50 μm .

20. The method of any one of claims 13-19, wherein the substrate comprises any one or more of agarose, poly(styrene divinylbenzene), polymethacrylate, cellulose, controlled pore glass, and spherical silica.

21. The method of any one of claims 13-20, wherein the particles comprise pores having a mean diameter of about 1100Å.

22. The method of any one of claims 10-21, wherein the first elution buffer comprises a salt at a concentration of greater than 250 mM.

23. The method of claim 22, wherein the salt concentration is greater than 300 mM or greater than 400 mM.

24. The method of claim 23, wherein the salt concentration is about 500 mM.

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

26. The method of any one of claims 10-25, wherein the second eluate comprises less than 50 mM of the salt.

27. The method of claim 26, wherein the second eluate comprises less than 10 mM of the salt.

28. The method of any one of claims 10-27, wherein the purified heterodimeric protein is eluted and collected in the second eluate via the third wash buffer.

29. The method of any one of claims 10-28, 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.

30. The method of any one of claims 10-29, 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.

31. The method of any one of claims 10-29, 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.

32. The method of claim 30, wherein the second polypeptide comprises a HY to RF substitution in its CH3 domain.

33. The method of any one of claims 10-32, wherein the first pH is from 6 to 8.

34. The method of any one of claims 10-33, wherein the second pH is from 4.0 to 4.25.

35. The method of claim 34, wherein the second pH is 4.10 ± 0.05 .

36. The method of any one of claims 10-35, wherein the third pH is from 2.8 to 3.5.

37. The method of any one of claims 10-36, wherein the fourth pH is from 6 to 8.

38. The method of any one of claims 10-37, further comprising an additional chromatography step or viral inactivation step following step (h).

39. The method of claim 38, wherein the additional chromatography step or viral inactivation step are performed under conditions with less than 100 mM salt.

40. The method of claim 39, wherein the additional chromatography step includes ion exchange chromatography.

41. The method of claim 40, wherein the ion exchange chromatography is anion exchange chromatography and is performed under conditions with less than 50 mM salt.

42. The method of any one of claims 10-41, wherein the heterodimeric protein is a bispecific antigen-binding protein.

43. The method of claim 42, wherein the bispecific antigen-binding protein is a bispecific antibody.