CLAIMS

1. A method of making a protein comprising:

a. loading an affinity matrix with a mixture of multimeric proteins comprising (i) a first homodimer comprising two copies of a first polypeptide, (ii) a second homodimer comprising two copies of the second polypeptide, and (iii) a heterodimer comprising the first polypeptide and a second polypeptide, wherein the first polypeptide has greater affinity for the affinity matrix than does the second polypeptide, wherein the affinity matrix comprises a Protein A ligand affixed to a substrate comprising a multiplicity of particles having a mean diameter of 45 μm and comprising pores having a mean diameter of 1100 Å; and

b. eluting and collecting the heterodimer from the affinity matrix in a buffer comprising $CaCl_2$ or $MgCl_2$ and having a first pH range,

wherein the first homodimer elutes from the affinity matrix in the buffer at a second pH range and the second homodimer elutes from the affinity matrix in the buffer at a third pH range, wherein the third pH range comprises a higher pH than the first pH range, which comprises a higher pH than the second pH range.

2. The method according to claim 1, wherein the substrate comprises any one or more of agarose, poly(styrene divinylbenzene), polymethacrylate, cellulose, controlled pore glass, and spherical silica.

3. The method according to claim 1 or 2, wherein 5 to 50 grams of protein are loaded per liter of affinity matrix.

4. The method according to claim 1 comprising applying a pH gradient to the loaded affinity matrix of step (a).

5. The method according to claim 4 comprising washing the loaded affinity matrix of step (a) with a solution at pH 6-8 prior applying the pH gradient.

6. The method according to claim 4, wherein the pH gradient is run between pH 6 and pH 3.

7. The method according to any one of claims 4-6, wherein the first pH range is selected from a range within pH 5.5 and pH 3.6.

8. The method according to claim 7, wherein the buffer comprises acetate, optionally wherein the buffer comprises 40 mM acetate.

9. The method according to claim 1, wherein the buffer comprises $CaCl_2$.

10. The method according to claim 1, wherein the buffer comprises $MgCl_2$.

11. The method according to claim 9, wherein the buffer comprises 250-500 mM $CaCl_2$.

12. The method according to claim 10, wherein the buffer comprises 250-500 mM $MgCl_2$.

13. The method according to claim 1, 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.

14. The method according to claim 13, wherein the second polypeptide comprises a HY to RF substitution in its CH3 domain.

15. The method according to claim 13 or claim 14, wherein the heterodimer comprises a bispecific antibody.

16. The method according to claim 1, wherein the mixture of multimeric proteins is produced by a plurality of eukaryotic cells in a cell culture, optionally wherein the eukaryotic cells comprise Chinese hamster ovary (CHO) cells or derivatives thereof.

17. The method according to claim 1 comprising the steps of:

c. applying the collected heterodimer of step (b) to a multimodal chromatography resin in a buffer having an acidic pH;

d. eluting the heterodimer from the multimodal chromatography resin in a buffer having a more alkaline pH; and

e. collecting the heterodimer.