CLAIMS

~~1.~~ 1. A method of ~~manufacturing a heterodimer~~ making a protein comprising:

~~a. contacting a protein A matrix comprising a Z-domain tetramer attached to a substrate with a mixture comprising a heterodimer protein and a homodimer protein;~~

~~b. washing said protein A matrix with a wash buffer having a pH > 6; and~~

~~c. eluting said heterodimer protein from said protein A matrix with an elute buffer comprising a salt that is more chaotropic than NaCl and a pH of about 3.8 - 5.5.~~

~~2. The method of claim 1, wherein said substrate comprises poly(styrene divinylbenzene) or agarose.~~

~~3. The method of claim 1 or 2, wherein said substrate comprises a particle with a mean diameter of ≤ 45 µm or ≤ 85 µm.~~

~~4. The method of claim 1, wherein said heterodimer protein is a bispecific antibody.~~

~~5. The method of claim 4, wherein said bispecific antibody comprises one unsubstituted CH3 domain and one substituted CH3 domain that comprises a modification that eliminates or reduces binding to protein A.~~

~~6. The method of claim 5, wherein said modification comprises H435R/Y436F.~~

~~7. The method of claim 5, wherein said bispecific antibody comprises a VH3 sequence of an IgG.~~

~~8. The method of claim 1, wherein said salt that is more chaotropic than NaCl is $CaCl_2$ or $MgCl_2$.~~

~~9. The method of claim 7, wherein said elute buffer comprises ≥ 250 mM $CaCl_2$ and has a pH of about 4.0 – 5.5.~~

~~10. The method of claim 1, wherein said mixture is harvested from a CHO cell culture.~~

~~11. The method of claim 1 comprising subjecting harvested CHO cell culture medium to a unit operation prior to step (a) to obtain said mixture.~~

~~12. The method of claim 12, wherein said unit operation is capture chromatography.~~

~~13. A method of purifying a bispecific antibody from a mixture comprising the bispecific antibody and a monospecific antibody, said method comprising:~~

~~contacting the mixture with an engineered protein A chromatography matrix~~ 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

~~a.~~ b. eluting and collecting the heterodimer from the affinity matrix in a buffer comprising ~~a Z-domain tetramer;~~

~~b. washing said mixture with a low salt wash buffer having a pH of about 5 to about 8.5; and~~

~~c. eluting said bispecific antibody from said engineered protein A matrix with an elute buffer comprising ≥ 250 mM~~ $CaCl_2$ or $MgCl_2$ and having a ~~pH of 3.6 – 5.5, 3.8 – 5.0, 4.5 – 5.5, or 5.0-5.1~~first pH range,

~~wherein said bispecific antibody comprises one unsubstituted CH3 domain and one substituted CH3 domain that comprises a modification that eliminates or reduces binding to protein A.~~

~~14. The method of claim 13, wherein said modification comprises H435R/Y436F.~~

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.

~~15.~~ 3. The method ~~of~~according to claim ~~13, wherein said eluting step (c) is isocratic.~~1 or 2, wherein 5 to 50 grams of protein are loaded per liter of affinity matrix.

~~16.~~ 4. The method ~~of~~according to claim ~~13, wherein said low salt wash buffer comprises 20 mM phosphate at about pH 7.2.~~1 comprising applying a pH gradient to the loaded affinity matrix of step (a).

5. The method ~~of~~according to claim ~~13~~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 ~~said elute~~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 $MgCl_2$.

~~17.~~ 11. The method according to claim 9, wherein the buffer comprises 250-500 mM $CaCl_2$~~, pH 3.8 – 5.0~~.

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.

~~18.~~ 14. The method according to claim 13, wherein ~~said bispecific antibody~~the second polypeptide comprises a ~~VH3 sequence of an IgG~~HY to RF substitution in its CH3 domain.

15. The method ~~of~~according to claim 13 or claim 14, wherein ~~said Z-domain tetramer~~the heterodimer comprises a bispecific antibody.

16. The method according to claim 1, wherein the mixture of multimeric proteins is ~~attached to a polystyrene or agarose bead with~~produced by a ~~mean diameter~~plurality of ~~about ≤ 45 μm~~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

~~19.~~ e. collecting the heterodimer.