chromatography columns has been only moderately successful.

[0007] Organic multivalent cations have been indicated for the precipitation of acidic proteins (Farhner et al., U.S. Patent Application No. 20080193981; Ma *et al.*, *J. Chromatogr. B* (2010) 878:798-806; Peram *et al.*, *Biotechnol. Progr.*, (2010) 26:1322-1326; Glynn, in U. Gottschalk (ed.), *Process Scale Purification of Antibodies*, J. T. Wiley and Sons, (2009) Hoboken, 309-324), as well as for precipitation of DNA and endotoxins (Glynn *supra*; Cordes *et al.*, Biotechnol. Progr., (1990) 6:283-285; Dissing *et al.*, *Bioseparation*, (1999) 7 221:9-11) and inactivation of virus (Bernhardt, U.S. Patent. 5,559,250). Multivalent metal cations have also been indicated to remove DNA and endotoxin from some protein preparations (Akcasu *et al.*, *Nature*, (1960) 187:323-324; Matsuzawa *et al.*, *Nucl. Acids Res.*, (2003) 3(3):163-164; Christensen *et al.*, *Prot. Expr. Purif.*, (2004) 37:468-471; Kejnovsky *et al.*, *Nucl. Acids Res.*, (1997) 25:1870-1871; Ongkudon *et al.*, *Anal. Chem.*, (2011) 83 391:13-17).

[0008] Immobilized TREN (tris(2-aminoethyl)amine) is known and commercially available for the purpose of conducting immobilized metal affinity chromatography (IMAC), where the immobilized TREN is initially loaded with a metal ion, and biomolecules are captured by contact with the TREN-associated metal ion, then subsequently recovered by dissociating the target biomolecule from the metal ion. IMAC ligands other than TREN, such as iminodiacetic acid (IDA) and nitriloacetic acid (NTA), are also known and commercially available for the purpose of conducting IMAC, where the ligand is initially loaded with a metal ion, and biomolecules are captured by contact with the ligand-associated metal ion, then subsequently recovered by dissociating the target biomolecule from the metal ion.

SUMMARY

[0009] In one aspect, the present invention refers to a method of reducing aggregate and product-contaminant complex content in a protein preparation comprising a desired protein, the method comprising (i) contacting the protein preparation with allantion at a supersaturated concentration selected from the group consisting of (a) from about 0.6 to about 1%; (b) from about 1 to about 2%; (c) from about 2 to about 5%; (d) from about 5 to about 10%; (e) from about 10 to about 25%; and (f) from about 25 to about 50%, (ii) contacting the protein preparation with an organic multivalent anion selected from heptanoic acid, octanoic acid, nonanoic acid, nonenoic acid, and decanoic acid at a concentration ranging from 0.05% to 5%,

(iii) contacting the protein preparation with at least one solid surface comprising at least one surface-bound ligand capable of binding a metal, wherein the surface-bound ligand capable of binding a metal is initially substantially devoid of a metal, wherein operating conditions are selected to substantially prevent the binding of the desired protein to the at least one solid surface and (iv) separating the protein preparation from the at least one surface-bound ligand; wherein when more than one surface-bound ligand is present, each surface-bound ligand is independently either of the same net charge or charge neutral. In another aspect, the present invention refers to a kit providing for the convenient practice of the present invention. Also disclosed herein are methods of reducing aggregate and product-contaminant complex content in a protein preparation comprising a desired protein, the method comprising (i) contacting the protein preparation with at least one solid surface comprising at least one surface-bound ligand capable of binding a metal, wherein the surface-bound ligand capable of binding a metal is initially substantially devoid of a metal, wherein operating conditions are selected to substantially prevent the binding of the desired protein to the at least one solid surface and (ii) separating the protein preparation from the at least one surface-bound ligand, wherein when more than one surface-bound ligand is present,

What is claimed is:

1. A method of reducing aggregate and product-contaminant complex content in a protein preparation comprising a desired protein, the method comprising (i) contacting the protein preparation with allantion at a supersaturated concentration selected from the group consisting of (a) from about 0.6 to about 1%; (b) from about 1 to about 2%; (c) from about 2 to about 5%; (d) from about 5 to about 10%; (e) from about 10 to about 25%; and (f) from about 25 to about 50%, (ii) contacting the protein preparation with an organic multivalent anion selected from heptanoic acid, octanoic acid, nonanoic acid, nonenoic acid, and decanoic acid at a concentration ranging from 0.05% to 5%, (iii) contacting the protein preparation with at least one solid surface comprising at least one surface-bound ligand capable of binding a metal, wherein the surface-bound ligand capable of binding a metal is initially substantially devoid of a metal, wherein operating conditions are selected to substantially prevent the binding of the desired protein to the at least one solid surface and (iv) separating the protein preparation from the at least one surface-bound ligand; wherein when more than one surface-bound ligand is present, each surface-bound ligand is independently either of the same net charge or charge neutral.

2. The method of claim 1, wherein the surface-bound ligand capable of binding a metal provides further chemical functionality selected from the group consisting of electrostatic interactions, hydrophobic interactions, pi-pi interactions, hydrogen bonding, and combinations thereof.

3. The method of any one of claims 1 or 2, wherein the contacting step further comprises contacting the protein preparation with at least one surface-bound ligand that provides a chemical functionality that does not comprise binding a metal.

4. The method of claim 3, wherein the at least one surface-bound ligand that provides a chemical functionality that does not comprise binding a metal is on the same solid surface as the at least one surface-bound ligand capable of binding a metal.

5. The method of claim 3, wherein the at least one surface-bound ligand that provides a chemical functionality that does not comprise binding a metal is on a separate solid surface from the at least one surface-bound ligand capable of binding a metal.

6. The method of any one of claims 3 to 5, wherein the chemical functionality that does not comprise binding a metal provides a chemical functionality selected from the group consisting of electrostatic interactions, hydrophobic interactions, pi-pi binding, hydrogen bonding, and combinations thereof.

7. The method of any one of claims 1 to 6, wherein the net charge on the surface-bound ligand capable of binding a metal is positive.

8. The method of claim 7, wherein surface-bound ligand capable of binding a metal comprises one selected from the group consisting of tris(2-aminoethyl)amine (TREN), diethylenetriamine, triethylenetetramine, tetraethylenepentamine, polypropylenimine tetraamine, deferoxamine (desferrioxamine), histidine, histamine, polyhistidine, arginine, polyarginine, lysine, polylysine, polyallylamine, and combinations thereof.

9. The method of any one of claims 1 to 6, wherein the net charge on the surface-bound ligand capable of binding a metal is negative.

10. The method of claim 9, wherein surface-bound ligand capable of binding a metal comprises one selected from the group consisting of iminodiacetic acid (2-(carboxymethylamino)acetic acid), ethylene glycol (aminoethylether) diacetic acid, diethyleaminetriaminepentaacetic acid, nitriloacetic acid (2,2',2''-nitrilotriacetic acid), aspartic acid, glutamic acid, polyaspartic acid, and combinations thereof.

11. The method of any of one of claims 1 to 10, wherein the supersaturated concentration of allantoin is from about 0.6 to about 1%, or from about 1 to about 2%.

12. The method of any one of claims 1 to 11, wherein a conductivity of the protein preparation comprises a range of from about 0.1 mS/cm to about 40 mS/cm.

13. The method of any one of claims 1 to 11, wherein a conductivity of the protein preparation comprises a range from about 40 mS/cm to about 200 mS/cm.

14. The method of any one of claims 1 to 13, wherein a further solid surface does not

substantially interact with the desired protein.

15. The method of any one of claims 1 to 14, wherein the operating conditions selected to substantially prevent the binding of the desired protein to the at least one solid surface comprises selecting a conductivity of the protein preparation sufficiently high to prevent substantial binding of the desired protein to each of the at least one surface-bound ligands.

16. The method of any one of claims 1 to 14, wherein the operating conditions selected to substantially prevent the binding of the desired protein to the at least one solid surface comprises selecting a conductivity of the protein preparation that is from about 0.001% to about 400% higher than the value required to prevent substantial binding of the desired protein to each of the at least one surface bound ligands.

17. The method of any one of claims 1 to 16, wherein the pH operating conditions are selected to substantially prevent the binding of the desired protein to the at least one solid surface.

18. The method of any one of claims 1 to 17, wherein the at least one solid surface comprises a particle or composite of particles.

19. The method of any one of claims 1 to 17, wherein the at least one solid surface comprises a fiber or composite of fibers.

20. The method of any one of claims 1 to 17, wherein the at least one solid surface comprises a membrane or composite of membranes.

21. The method of any one of claims 1 to 17, wherein the at least one solid surface comprises a monolith or composite of monoliths.

22. The method of any one of claims 1 to 21, wherein different surface-bound ligands are present on structurally similar but distinct solid surfaces from the at least one solid surface comprising the at least one surface-bound ligand capable of binding a metal.

23. The method of any one of claims 1 to 21, wherein different surface-bound ligands

are present on structurally distinct solid surfaces from the at least one solid surface comprising the at least one surface-bound ligand capable of binding a metal.

24. The method of any one of claims 1 to 23, wherein the protein preparation comprises a cell culture harvest.

25. The method of any one of claims 1 to 23, wherein the protein preparation comprises a clarified cell culture harvest.

26. The method of any one of claims 1 to 23, wherein the protein preparation comprises a recombinant protein.

27. The method of any one of claims 1 to 23, wherein the protein preparation comprises an antibody.

28. The method of any one of claims 1 to 27, wherein after the method the protein preparation has a reduced aggregate content.

29. The method of claim 28, wherein the aggregate content is reduced by an amount in a range selected from the group consisting of (a) from about 50% to about 99%; (b) from about 25% to about 50%; (c) from about 10% to about 25%; (d) from about 5% to about 10%; (e) from about 1% to about 5%; and (f) from about 0.1% to about 1%.

30. The method of any one of claims 1 to 29, further comprising contacting the protein preparation with at least one organic additive to reduce aggregate contaminants in the preparation, wherein the organic additive is selected from the group consisting of an electropositive ion, an electronegative ion, an organic solvent, an organic polymer, and a surfactant.

31. A kit providing for the convenient practice of any one of claims 1 to 30.