significant cost associated with Protein A chromatography and current limitations related to throughput, scale-up and ligand leeching, alternative purification methods are necessary.

SUMMARY

[0004] The present disclosure is directed to a composition and methods for increasing the binding capacity of protein A chromatography by covalent attachment of a protein A domain (E, D, A, B, C) or a synthetic protein Z, for example, a synthetic Z domain, to *Drosophila melanogaster* transcription factor Ultrabithorax (Ubx) materials. The inventors have discovered that Ubx materials incorporate about 1,000-fold greater SpA protein per gram of material than commercial chromatography resins crosslinked to the same SpA protein. The compositions provided herein include fusion proteins containing *Drosophila melanogaster* transcription factor Ultrabithorax (Ubx) or a fragment thereof and an immunoglobulin binding protein. In some examples, the immunoglobulin binding protein is a protein A domain, a protein Z domain or a fragment thereof.

[0005] An affinity chromatography matrix comprising from about 2 to about 18 immunoglobulin binding domains, for example, SpA or Z domain repeats, is also provided. By covalently attaching multiple immunoglobulin binding domains, for example, from about 2 to about 18 SpA or Z domain repeats, to a Ubx protein or a fragment thereof, binding capacity can be increased. A common problem associated with other SpA applications is that the chemical fixation of SpA domains to solid phase matrices is susceptible to degradation leading to leeching of protein A. See, for example, US Patent Application Publication No. 20050038231. The compositions and methods provided herein allow for increased immunoglobulin purity by preventing SpA contamination through ligand leaching. Covalent attachment of an SpA domain repeat(s), a Z domain(s) or a fragment thereof to Ubx materials prevents ligand leaching or disassociation from the matrix. Thus, the disclosure provides an antibody affinity separation matrix with increased capacity that allows for isolation of immunoglobulins with increased purity. Methods of using any of the affinity separation matrices provided herein to isolate or purify antibodies or immunoglobulin-containing proteins are also provided.

[0005a] In one aspect, the present disclosure relates to a fusion protein comprising at least one Ubx protein and an immunoglobulin binding protein, wherein the immunoglobulin binding protein comprises (a) a single domain of staphylococcal Protein A (SpA) or a functional variant thereof; or, (b) domain Z or a functional variant thereof.

[0005b] In another aspect, the present disclosure relates to a solid matrix comprising the fusion protein of the disclosure.

[0005c] In another aspect, the present disclosure relates to a method for isolating an immunoglobulin from an immunoglobulin-containing solution comprising: a) contacting the immunoglobulin-containing solution with the solid matrix of the disclosure under conditions wherein immunoglobulin contained in the solution is adsorbed to the matrix; b) washing the adsorbed immunoglobulins; and c) eluting the immunoglobulin from the matrix.

What is claimed is:

1. A fusion protein comprising at least one Ubx protein and an immunoglobulin binding protein, wherein the immunoglobulin binding protein comprises (a) a single domain of staphylococcal Protein A (SpA) or a functional variant thereof; or, (b) domain Z or a functional variant thereof.
2. The fusion protein of Claim 1, wherein the domain of the staphylococcal Protein A (SpA) is an E, D, A, B or C domain.
3. The fusion protein of claim 1, wherein a functional variant is protein G, protein L, or protein A/G.
4. The fusion protein of Claim 1, wherein the immunoglobulin binding protein contains two or more different monomeric domains of staphylococcal Protein A (SpA) or a functional variant thereof, or domain Z or a functional variant thereof.
5. The fusion protein of Claim 4, wherein the two or more different monomeric domains of the staphylococcal Protein A (SpA) are the E, D, A, B or C domains.
6. The fusion protein of Claim 1, wherein the immunoglobulin binding protein contains two or more monomeric domains of staphylococcal Protein A (SpA) or a functional variant thereof, or domain Z or a functional variant thereof, wherein the monomeric domains are the same.
7. The fusion protein of Claim 6, wherein the two or more different monomeric domains of the staphylococcal Protein A (SpA) are the E, D, A, B, C or Z domains.
8. The fusion protein of any one of claims 1-7, wherein the Ubx protein comprises SEQ ID NO: 20 or a fragment thereof, and wherein the immunoglobulin binding protein comprises SEQ ID NO: 1 or a fragment thereof.
9. A solid matrix comprising the fusion protein of any one of Claim 1-8.
10. The matrix of Claim 9, wherein leaching of the immunoglobulin binding protein from the solid matrix is prevented or reduced.
11. The matrix of Claim 9 or 10, wherein the binding capacity of 1 mL of material is greater than 1 g/mL.
12. A method for isolating an immunoglobulin from an immunoglobulin-containing solution comprising:

    a) contacting the immunoglobulin-containing solution with the solid matrix of any one of claims 9-11 under conditions wherein immunoglobulin contained in the solution is adsorbed to the matrix;

b) washing the adsorbed immunoglobulins; and

c) eluting the immunoglobulin from the matrix.