CLAIMS

What is claimed is:

1. An affinity chromatography ligand comprising one or more C domains of *Staphylococcus* Protein A (SpA), wherein at least one C domain comprises a deletion of at least 3 consecutive amino acids from the N-terminus, starting at position 1 or position 2.
2. An affinity chromatography ligand comprising one or more B domains of *Staphylococcus* Protein A (SpA), wherein at least one B domain comprises a deletion of at least 3 consecutive amino acids from the N-terminus, starting at position 1 or position 2.
3. An affinity chromatography ligand comprising one or more Z domains of *Staphylococcus* Protein A (SpA), wherein at least one Z domain comprises a deletion of at least 3 consecutive amino acids from the N-terminus, starting at position 1 or position 2.
4. The affinity chromatography ligand according to claim 1, 2 or 3, wherein the ligand exhibits reduced fragmentation, relative to a wt counterpart, following exposure to 0.5M NaOH for at least 5 hours.
5. An affinity chromatography matrix comprising an affinity chromatography ligand according to claim 1, 2 or 3 attached to a solid support.
6. The affinity chromatography matrix of claim 5, wherein the ligand exhibits reduced fragmentation when attached to the solid support, relative to a wt counterpart, following exposure to 0.5M NaOH for 5 hours.
7. The affinity chromatography ligand according to claim 1, 2, or 3, wherein one or more domains of the ligand further comprise an amino acid mutation at position 29 to reduce Fab binding.
8. The affinity chromatography ligand of claim 7, wherein the amino acid mutation comprises replacing a glycine amino acid residue or an alanine amino acid residue with a lysine amino acid residue.
9. The affinity chromatography matrix of claim 5, wherein affinity chromatography ligand is attached to the solid support *via* multipoint attachment.
10. The affinity chromatography ligand according to claim 1, wherein the affinity chromatography ligand comprises two, three, four, five, six, seven or more C domains of *Staphylococcus* Protein A (SpA), wherein at least one C domain

comprises a deletion of three consecutive amino acids from the N-terminus or a deletion of four consecutive amino acids from the N-terminus or a deletion of five consecutive amino acids from the N-terminus, starting at position 1 or at position 2.

11. The affinity chromatography ligand according to claim 3, wherein the affinity chromatography ligand comprises two, three, four, five, six, seven or more Z domains of *Staphylococcus* Protein A (SpA), wherein at least one Z domain comprises a deletion of three consecutive amino acids from the N-terminus or a deletion of four amino acids from the N-terminus or a deletion of five consecutive amino acids from the N-terminus, starting at position 1 or at position 2.

12. The affinity chromatography ligand according to claim 2, wherein the affinity ligand comprises two, three, four, five, six, seven or more B domains of *Staphylococcus* Protein A (SpA), wherein at least one B domain comprises a deletion of three consecutive amino acids from the N-terminus or a deletion of four amino acids from N-terminus or a deletion of five consecutive amino acids from the N-terminus, starting at position 1 or at position 2.

13. A method of affinity purifying one or more target molecules from a sample, the method comprising the steps of:

    (a) providing a sample comprising one or more target molecules;

    (b) contacting the sample with the matrix of claim 5 under conditions such that the one or more target molecules bind to the matrix; and

    (c) recovering the one or more bound target molecules by elution.

14. The affinity chromatography matrix of claim 5, wherein the matrix retains at least 95% of its initial binding capacity after 5 hours incubation in 0.5M NaOH.

15. The affinity chromatography matrix of claim 5, wherein the matrix retains at least 95% of its initial binding capacity after 25 hours incubation in 0.1M NaOH.

16. An affinity ligand comprising the structure:

$$[(X)_n\ (Y)_m]_{n+m},$$

where X represents a B domain, a Z domain or a C domain of SpA,

~~n represents the number of domains ranging from zero through (m-1);~~

Y represents a B domain or a Z domain or a C domain of SpA having at least 3 consecutive amino acids deleted from the N-terminus, and

m represents the number of Y domains ranging from one through eight, where the ligand is attached to a solid support *via* multipoint attachment.

17. An affinity chromatography ligand comprising two or more B domains of SpA or two or more C domains of SpA or two or more Z domains of SpA, or any combinations of B, Z or C domains in any order, wherein at least one of B, C or Z domains comprises a deletion of at least 3 amino acids from the N-terminus, starting at position 1 or at position 2.
18. The affinity chromatography ligand of claim 17, wherein the ligand exhibits reduced fragmentation relative to a ligand without any deletions, following exposure to 0.5M NaOH for 5 hours.
19. The affinity chromatography matrix according to claim 5 or claim 9, wherein the solid support is selected from the group consisting of controlled pore glass, silica, zirconium oxide, titanium oxide, agarose, polymethacrylate, polyacrylate, polyacrylamide, polyvinylether, polyvinyl alcohol and polystyrene and derivatives thereof .
20. An alkaline stable affinity chromatography ligand comprising at least five C domains, wherein each of the domains comprises a mutation to reduce Fab binding as well as deletion of 4 amino acids from the N-terminus, starting at position 1.

1. A method of purifying one or more immunoglobulins from a sample the method comprising the steps of:
    a. providing a sample comprising one or more immunoglobulins:
    b. contacting the sample with a matrix under conditions such that the one or more immunoglobulins bind to the matrix, wherein the matrix comprises an affinity chromatography ligand attached to a solid support and wherein the ligand is based on two or more B domains or two or more *Z* domains or two or more C domains of *Staphylococcus aureus* Protein A, each domain having a deletion of at least 3 or 4 consecutive amino acids from the N-terminus starting at position 1 or 2 corresponding to wild-type B, Z or C domain positions and further having a mutation to reduce Fab binding; and
    c. recovering the one or more bound immunoglobulins by elution.
2. The method of claim 1, wherein the sample is selected from the group consisting of a cell culture liquid, a cell culture supernatant and a fermentation broth.
3. The method of claim 1, wherein the mutation to reduce Fab binding comprises the replacement of glycine amino acid residue at position 29 with a lysine amino acid residue in case of the B domain and the C domain and the replacement of alanine amino acid residue at position 29 with a lysine amino acid residue in case of the *Z* domain.
4. The method of claim 1, wherein the ligand exhibits reduced fragmentation compared to its wild-type counterpart following exposure to 0.5M NaOH for at least 5 hours.
5. The method of claim 1, wherein the matrix retains at least 95% of its initial binding capacity after 5 hours incubation in 0.5M NaOH.
6. The method of claim 1, wherein the matrix retains at least 95% of its initial binding capacity after 25 hours incubation in 0.1M NaOH.

7. A method of separating an immunoglobulin from one or more of host cell proteins (HCPs), DNA, viruses, endotoxins, nutrients, one or more components of a cell culture medium and product related impurities, the method comprising the steps of:

    a. providing a sample comprising an immunoglobulin and one or more of host cell proteins (HCPs), DNA, viruses, endotoxins, nutrients, one or more components of a cell culture medium and product related impurities;

    b. contacting the sample with a matrix under conditions such that the immunoglobulin binds to the matrix, wherein the matrix comprises an affinity chromatography ligand attached to a solid support and wherein the ligand is based on two or more B domains or two or more Z domains or two or more C domains of *Staphylococcus aureus* Protein A, each domain having a deletion of at least 3 or 4 consecutive amino acids from the N-terminus starting at position 1 or 2 corresponding to wild-type B, Z or C domain positions and further having a mutation to reduce Fab binding; and

    c. recovering the bound immunoglobulin by elution,

    thereby to separate the immunoglobulin from one or more of host cell proteins (HC:Ps), DNA, viruses, endotoxins, nutrients, one or more components of a cell culture medium and product related impurities.

8. The method of claim 7, wherein the sample is a fermentation broth.

9. The method of claim 7, wherein the mutation to reduce Fab binding comprises the replacement of glycine amino acid residue at position 29 with a lysine amino add residue in case of the B domain and the C domain and the replacement of alanine amino acid residue at position 29 with a lysine amino acid residue in case of the Z domain.

10. The method of claim 7, wherein one or more components of a cell culture medium are selected from the group consisting of antifoam agents and antibiotics.

11. The method of claim 7, wherein the product related impurities comprise misfolded species and aggregates.

12. The method of claim 1, wherein the sample is a clarified cell culture broth.

13. The method of claim 7, wherein the sample is a clarified cell culture broth.
14. The method of da1m 7, wherein the matrix retains at least 95% of its initial binding capacity after 5 hours incubation in 0.5M NaOH.
15. The method of claim 7, wherein the matrix retains at least 95% of its initial binding capacity after 25 hours incubation in 0.1M NaOH.
16. The method of claim 7, wherein the ligand exhibits reduced fragmentation compared to its wild-type counterpart following exposure to 0.5M NaOH for at least 5 hours.