CLAIMS

What is claimed is:

1. A method of purifying a charge-shielded fusion protein from a cell lysate or periplasmic releasate, wherein the charge-shielded fusion protein comprises a biologically active domain and a charge-shielding domain, and wherein the method comprises hydrophobic interaction chromatography as a first chromatography step.

2. A method for producing a charge-shielded fusion protein from a cell lysate or periplasmic releasate wherein the charge-shielded fusion protein comprises a biologically active domain and a charge-shielding domain, wherein the method comprises

i) culturing cells comprising a nucleic acid encoding the charge-shielded fusion protein; and

ii) purifying the charge-shielded fusion protein, wherein the charge-shielded protein is purified from the cell lysate or periplasmic releasate using hydrophobic interaction chromatography as a first chromatography step.

3. The method of claim 1 or 2, wherein

(a) the charge-shielded fusion protein is at least 45% pure after the first chromatography step;

(b) the method further comprises an anion exchange chromatography; optionally wherein the anion exchange interaction chromatography is selected from the group consisting of a POROS 50HQ resin, a POROS XQ resin, and a Gigacap Q-650M resin; preferably wherein the anion exchange interaction chromatography is a Gigacap Q-650M resin;

(c) the method further comprises a cation exchange chromatography; optionally wherein

i) the cation exchange interaction chromatography is a strong cation exchanger;

ii) the cation exchange interaction chromatography is a mixed mode resin; or

iii) the cation exchange interaction chromatography is selected from the group consisting of a Capto MMC resin, a CMM Hypercel resin, a Capto SP impres resin, a

Fracto gel SO_3- resin, a GigaCap S-650S resin, and a POROS XS resin; preferably wherein the cation exchange interaction chromatography is a POROS XS resin; and/or

(d) the method comprises a sequence of chromatography steps comprising in order:

i) hydrophobic interaction chromatography;

ii) anion exchange chromatography; and

iii) cation exchange chromatography.

4. The method of any one of any preceding claim, wherein

(a) the biologically active domain is charged at pH of about 7.0, wherein the charge-shielding domain increases the hydrodynamic radius of the protein, and/or wherein the charge-shielding domain does not have a charge at pH of about 7.0;

(b) the molecular weight of the biologically active domain is less than the molecular weight of the charge-shielding domain;

(c) the molecular weight of the charge-shielding domain is between 10 kDa and 60 kDa;

(d) the molecular weight of the charge-shielding domain is between 10 kDa and 20 kDa; and/or

(e) the molecular weight of the biologically active domain is between 30 kDa and 40 kDa.

5. The method of any one of any preceding claim, wherein the molecular weight of the charge-shielding domain is sufficient to increase the *in vivo* half-life of the charge-shielded fusion protein or a multimer of the charge-shielded fusion protein; optionally wherein the *in vivo* half-life of the charge-shielded fusion protein or a multimer of the charge-shielded fusion protein is increased compared to the half-life of a protein comprising the biologically active domain without the charge-shielding domain or a multimeric protein comprising the biologically active domain without the charge-shielding domain.

6. The method of any one of any preceding claim, wherein

(a) the charge-shielding domain has a random coil or disordered structure; and/or

(b) the charge-shielding domain is a polypeptide consisting of one or more of alanine, serine and proline residues; optionally

wherein the charge-shielding domain is a polypeptide consisting of proline and alanine residues.

7. A method for producing a PASylated biologically active fusion protein from a cell lysate or periplasmic releasate comprising

i) culturing cells comprising a nucleic acid encoding the PASylated biologically active protein; and

ii) purifying the PASylated biologically active protein,

wherein the PASylated biologically active protein is purified from the cell lysate or periplasmic releasate using hydrophobic interaction chromatography as a first chromatography step.

8. A method for purifying a charge-shielded fusion protein comprising a biologically active domain and a charge-shielding domain from a cell lysate or periplasmic releasate, the method comprising the following steps in order

i) applying a load solution comprising the charge-shielded fusion protein to a hydrophobic interaction chromatography column;

ii) applying a wash solution to the hydrophobic interaction chromatography column;

iii) applying an elution solution to the hydrophobic interaction column to elute the charge-shielded protein;

iv) applying the eluted charge-shielded fusion protein in iii) as a load solution to an anion exchange chromatography column;

v) eluting the charge-shielded fusion protein from the anion exchange chromatography column;

vi) applying the eluted charge-shielded fusion protein in vi) as a load solution to a cation exchange chromatography column;

vii) applying a wash solution to the cation exchange chromatography column;

viii) applying an elution solution to the cation exchange chromatography column to elute the charge-shielded fusion protein.

9. The method of claim 8, wherein

(a) the load solution in step i) comprises 0.25 – 3 M Na_2SO_4 or 0.25- 0.6 M NH_4SO_4 and a pH of 5.5 to 6.5;

(b) the elution solution in step iii) comprises 0.3-0.5 M NH_4SO_4 and has a pH of 5.5 to 6.5;

(c) the load solution in step iv) has a conductivity of 0.7- 4.0 mS/cm and a pH of 7.0 to 9.1;

(d) the load solution in step vi) has a pH of 5.9 to 7.0 and a conductivity of 0.7 to 2.5 mS/cm; and/or

(e) the elution solution in step viii) has a pH of 6.0 to 7.0 and a conductivity of 0.7 to 4.0 mS/cm.

10. The method of any one of any preceding claim, wherein the hydrophobic interaction chromatography is selected from the group consisting of a POROS Benzyl ultra resin, a Hexyl-650C resin, and a Phenyl-600M resin; preferably wherein the hydrophobic interaction chromatography is a
Phenyl-600M resin.

11. The method any one of any preceding claim, wherein the biologically active domain is an asparaginase subunit; optionally wherein

(a) the asparaginase is selected from the group consisting of an *E. coli* asparaginase and an *Erwinia* asparaginase;

(b) the asparaginase comprises the amino acid sequence set forth in SEQ ID NO:1, SEQ ID NO:5, or SEQ ID NO:7; or

(c) the charge-shielded fusion protein comprises the amino acid sequence set forth in SEQ ID NO: 9 or SEQ ID NO:10.

12. The method of claim 2 or claim 7, wherein the cell is a bacterial cell; optionally

wherein the cell is an *E. coli* cell or a *Pseudomonas* cell.

13. A charge-shielded protein produced by the method of any preceding claim.

14. A pharmaceutical composition comprising the charge-shielded protein of claim 13 and a pharmaceutically acceptable carrier.

15. The charge-shielded protein of claim 13 or the pharmaceutical composition of claim 14 for use as a medicament.

16. A composition comprising a PASylated asparaginase, wherein the PASylated asparaginase is at least 45% pure.