

CLAIMS

What is claimed:

1. A method for purifying a target molecule in a sample, the method comprising the steps of
 - 5 (a) providing a sample containing a fusion protein comprising an intein-C polypeptide joined to a target molecule by a peptide bond (intein-C tagged target molecule);
 - (b) contacting the sample with a chromatography resin comprising a covalently-linked N-terminal intein polypeptide, under conditions in which the intein-C polypeptide in the fusion protein binds to the intein-N polypeptide in the resin to form an intein
10 complex;
 - (c) optionally washing the resin containing the intein complex to remove unbound contaminants;
 - (d) exposing the intein complex to conditions sufficient to release the target molecule from the intein-C polypeptide;
 - 15 (e) regenerating the chromatographic resin by contacting the resin with one or more compositions selected from the group consisting of:
 - (i) a composition having a pH in aqueous solution of about 1 to about 4;
 - (ii) a composition comprising at least one detergent; and
 - (iii) a composition comprising at least one kosmotropic agent and/or chaotropic
20 agent;so as to disrupt the intein-N intein-C complex and release the intein-C polypeptide from the chromatography resin; and
 - (f) optionally, performing at least one additional purification cycles by repeating steps (a) to (e) at least once,
 - 25 wherein the regenerated chromatography resin obtained from step (e) or optional step (f) retains at least about 60%, preferably at least about 70% and more preferably at least about 80% of its C-terminal intein binding capacity after each purification cycle.
2. The method according to claim 1, wherein step (e) comprises regenerating the
30 chromatographic resin by contacting the resin with a composition having a pH in aqueous solution of about 1 to about 4, preferably wherein the composition has a pH in aqueous solution between about 1 and about 3.5, more preferably between about 1 and about 2.

3. The method according to any one of the preceding claims, wherein step (e) comprises regenerating the chromatographic resin by contacting the resin with at least one detergent.
4. The method according to any one of the preceding claims, wherein step (e) comprises regenerating the chromatographic resin by contacting the resin with a composition
5 comprising at least one kosmotropic agent.
5. The method according to any one of the preceding claims, wherein step (e) comprises regenerating the chromatographic resin by contacting the resin with a composition comprising at least one chaotropic agent.
6. The method according to any one of the preceding claims, further comprising the step of
10 performing at least one purification cycle wherein step (e) is replaced by step (e)(iv), or performing at least one purification cycle which further comprises step (e)(iv):
(e)(iv) regenerating the chromatographic resin by contacting the resin with a basic composition having a pH in aqueous solution of about 9 or higher in at least one of the purification cycles.
- 15 7. The method according to claim 6, wherein steps (e)(i), (e)(ii), (e) (iii) and/or (e)(iv) are each independently of each other performed in at least 10% of the purification cycles, preferably in at least about 25% of the purification cycles, and more preferably in at least about 50% of the purification cycles.
8. The method according to claim 6 or 7, comprising multiple purification cycles, wherein
20 (A) step (e)(i) and (e)(ii) are independently performed, concurrently or sequentially, in any order; or
(B) step (e)(i) and (e)(iii) are independently performed, concurrently or sequentially, in any order; or
(C) step (e)(ii) and (e)(iii) are independently performed, concurrently or sequentially, in
25 any order; or
(D) step (e)(i) and (e)(ii) and (e)(iii) are independently performed, concurrently or sequentially, in any order;
(E) step (e)(i) and (e)(iv) are independently performed, concurrently or sequentially, in any order; or

(F) step (e)(ii) and (e)(iv) are independently performed, concurrently or sequentially, in any order; or

(G) step (e)(iii) and (e)(iv) are independently performed, concurrently or sequentially, in any order; or

5 (H) step (e)(i), (e)(ii) and (e)(iv) are independently performed, concurrently or sequentially, in any order; or

(I) step (e)(i), (e)(iii) and (e)(iv) are independently performed, concurrently or sequentially, in any order; or

10 (J) step (e)(ii), (e)(iii) and (e)(iv) are independently performed, concurrently or sequentially, in any order; or

(K) step (e)(i), (e)(ii), (e)(iii) and (e)(iv) are independently performed, concurrently or sequentially, in any order;

15 wherein, when any of steps (e)(i), (e)(ii), (e)(iii) and/or (e)(iv) are performed, such step or steps are performed in at least 10% of the purification cycles, preferably in at least about 25% of the purification cycles, and more preferably in at least about 50% of the purification cycles.

9. The method according to any one of the preceding claims, wherein the intein-C tagged target molecule is prepared by attaching an intein-C polypeptide to a target molecule to obtain a fusion protein, and expressing the fusion protein in an expression system.

20 10. The method according to any one of the preceding claims, wherein step (b) comprises contacting the chromatography resin with a cell culture supernatant comprising the intein-C tagged target molecule; or

wherein step (b) comprises loading the intein-C tagged target molecule in a saline buffer having a pH of about 8 to about 10.

25 11. The method according to any one of the preceding claims, wherein step (c) is performed, and comprises washing the chromatography resin with a washing buffer prior to releasing the target molecule from the intein-C polypeptide; preferably wherein the washing buffer comprises a detergent, a salt, a chaotropic agent, preferably urea or arginine, or a combination thereof.

12. The method according to any one of the preceding claims, wherein step (d) comprises contacting the resin with a cleavage buffer having a pH of about 6 to about 8, so as to release the target molecule from the intein-C polypeptide.
13. The method according to any one of the preceding claims, wherein the composition
5 having a pH in aqueous solution of about 1 to about 4 comprises an acidic buffer comprising an acid selected from the group consisting of phosphoric acid, glycine, hydrochloric acid, hydrobromic acid, citric acid, acetic acid, formic acid, lactic acid, carbonic acid, succinic acid, nitric acid, malic acid, oxalic acid, salicylic acid, formic acid, and any combinations thereof.
- 10 14. The method according to any one of the preceding claims,
wherein the chaotropic agent is selected from the group consisting of guanidinium chloride, arginine, n-butanol, ethanol, lithium perchlorate, lithium acetate, magnesium chloride, phenol, 2-propanol, sodium dodecyl sulfate, thiourea, urea, and any combination thereof; or
15 wherein the kosmotropic agent is an ionic kosmotropic agent or a nonionic kosmotropic agent selected from the group consisting of carbohydrates, amino acids and alcohols; or
wherein the basic composition comprises at least one of sodium hydroxide, caustic reagent, arginine, calcium hydroxide or potassium hydroxide; or
20 wherein the detergent is selected from the group consisting of anionic detergents, cationic detergents, non-ionic detergents and zwitter ionic detergents; preferably wherein the detergent is selected from the group consisting of polysorbates, polyethylene glycols, glycosides, poloxamers, CHAPS, CHAPSO, alkylbenzenesulfonates, quaternary ammonium salts and bile acids.
- 25 15. The method according to any one of the preceding claims, further comprising the step of isolating the target molecule obtained from step (d).
16. The method according to any one of the preceding claims, wherein the chromatography resin is polymer based or includes a polymer, preferably wherein the chromatography resin includes a hydrophilic polyvinyl ether base or a polymethacrylate.

17. The method of claim 16, wherein the chromatography resin is formulated on a solid support, wherein the solid support is a bead or a membrane.
18. The method according to any one of the preceding claims, wherein the target molecule is a protein, preferably wherein the sample is a clarified protein preparation.
- 5 19. The method according to any one of the preceding claims, wherein the intein-N polypeptide is attached to the chromatography resin through a functional group selected from the group consisting of hydroxyl, thiol, epoxide, amino, carbonyl epoxide and carboxylic acid.