CLAIMS

What is claimed is:

1. A split intein-based affinity chromatography system, comprising:

   a) a first fusion protein comprising a C-intein polypeptide joined to a target molecule by a peptide bond; andb) an affinity chromatography matrix comprising an N-intein polypeptide that is covalently attached to a solid support, wherein in the N-intein polypeptide, Cys at position 7 is substituted with Ala, Cys at position 65 is substituted with Thr and Cys at position 89 is substituted with Met or Lys wherein each position is corresponding to position in GP41-1 N-intein (SEQ ID NO:1).

2. The split intein-based affinity chromatography system of claim 1, wherein the N-intein polypeptide is attached to a solubilization partner to form a second fusion protein.

3. The split intein-based affinity chromatography system of claim 2, wherein the second fusion protein is attached to the solid support through a single site in the solubilization partner.

4. The split intein-based affinity chromatography system of claim 3, wherein oriented away from the solid support when the fusion protein is attached to the solid support

5. The split intein-based affinity chromatography system of claim 1, wherein the N-intein polypeptide comprises an amino acid sequence selected from SEQ ID NO: 6 or 8, or the N-intein polypeptide comprises an amino acid sequence sharing at least about 90% sequence identity with any one of SEQ ID NO: 6 or 8, and is capable of forming an active intein complex by associating with the first fusion protein comprising a C-intein polypeptide.

6. The split intein-based affinity chromatography system of claim 1, wherein the solid support is a chromatography resin.

7. The split intein-based affinity chromatography system of claim 6, wherein the chromatography resin includes a hydrophilic polyvinyl ether base.

8. The split intein-based affinity chromatography system of claim 1, wherein the solid support is a bead, a hollow fiber, a solid fiber, a pad, a gel, a membrane, a cassette, a column, a chip, a slide, a plate or a monolith.

9. The split intein-based affinity chromatography system of claim 1, wherein the solid support is a magnetic bead.

10. The split intein-based affinity chromatography system of claim 1, wherein the solid support comprises controlled pore glass, silica, zirconium oxide, titanium oxide, agarose, polymethacrylate, polyacrylate, polyacrylamide, polyvinyl alcohol, polystyrene or derivatives thereof.

11. The split intein-based affinity chromatography system of claim 1, wherein a spacer molecule is between the N-intein polypeptide and the solid support.

12. The split intein-based affinity chromatography system of claim 3, wherein a spacer molecule is between the second fusion protein and the solid support.

13. The split intein-based affinity chromatography system of claim 1, wherein the N-intein polypeptide is attached to the solid support at a single site in the N-intein polypeptide.

~~1.~~14. A method of affinity purifying a target molecule in a sample using the split intein-based affinity chromatography system of any one of claims 1-13, the method comprising:

a) providing a sample containing ~~a~~ the first fusion protein comprising a C-intein polypeptide joined to a target molecule by a peptide bond;

b) contacting the sample with an affinity chromatography matrix ~~that comprises a second fusion protein, wherein the second fusion protein comprises an N-intein polypeptide joined by a peptide bond to an N-intein solubilization partner that promotes solubility of~~ comprising the N-intein polypeptide, under conditions in which the C-intein polypeptide in the first fusion protein selectively binds to the N-intein polypeptide ~~in the second fusion protein~~ to form an intein complex that is inactive;

c) washing the affinity chromatography matrix containing the inactive intein complex to remove unbound contaminants;

d) exposing the intein complex to conditions under which the intein complex is active and cleaves the target molecule from the C-intein polypeptide; and

e) recovering the cleaved target molecule.

~~2. The method of Claim 1, wherein the sample is a crude protein preparation.~~

~~3. The method of Claim 1 or 2, wherein the target molecule is a monoclonal antibody to a therapeutic target.~~

~~4. The method of Claim 1 or 2, wherein the target molecule is a therapeutic target.~~

~~5.~~15. The method of ~~Claim any one of Claims 1-4~~ claim 14, wherein the conditions under which the C-intein polypeptide in the ~~first~~ fusion protein selectively binds to the N-intein polypeptide ~~in the second fusion protein~~ to form a catalytically-inactive intein complex include:

a) a temperature in the range of about 4-25 °C, and a buffer comprising 100 mM Tris-HCl, 25 mM NaCl, 0.1mM zinc chloride, pH=9;

b) a temperature in the range of about 4-25 °C, and a buffer comprising 50 mM NaAc, 0.5 M NaCl, pH=5; or

c) a temperature in the range of about 4-25 °C, and a buffer comprising 0.5 M NaCl, 10 mM Tris–HCl, pH=8.

~~6.~~16. The method of ~~any one of Claims 1-5~~ claim 14, wherein the conditions that promote catalytic activity of the intein complex include:

a) a buffer comprising 50 mM Tris-HCl, pH=7.0, 300 mM NaCl, 1 mM EDTA;

b) a buffer comprising 0.3 M L-arginine, 5 mM EDTA, 50 mM phosphate buffer, pH=6.5; or

c) a buffer comprising 0.5 M NaCl, 10 mM Tris–HCl, 50 mM DTT, pH=8.0.

17. The method of claim 14, wherein the target molecule is a monoclonal antibody to a therapeutic target or the target molecule is a therapeutic target.

18. The method of claim 14, further comprising cleaning, regenerating or storing the affinity chromatography matrix for subsequent use.

19. The method of claim 14, wherein the N-intein polypeptide is attached to a solubilization partner to form a second fusion protein.

20. The method of claim 19, wherein the second fusion protein is attached to the solid support through a single site in the solubilization partner

~~7. The method of Claim any one of Claims 1-6, wherein the N-intein solubilization partner has a molecular weight below 15 kDa, an Aliphatic Index value lower than 60, and a Grand Average Hydropathy value less than -1.~~

~~8. The method of any one of Claims 1-7, wherein the N-intein solubilization partner is N-intein solubilization partner 138 (SEQ ID NO:15) or a variant thereof.~~

~~9. The method of one of Claims 1-8, wherein the N-intein polypeptide is GP41-1 N-intein (SEQ ID NO:1) or a variant thereof, and the C-intein polypeptide is GP41-1 C-intein (SEQ ID NO:9).~~

~~10. The method of one of Claims 1-9, further comprising cleaning, regenerating or storing the affinity chromatography matrix for subsequent use.~~

~~11. A method of screening for an intein complex that is suitable for use in affinity purification, the method comprising:~~

~~a) contacting a first fusion protein that comprises a C-intein polypeptide joined to a target molecule by a peptide bond with a second fusion protein that comprises an N-intein polypeptide joined to an N-intein solubilization partner by a peptide bond, under conditions in which the C-intein polypeptide in the first fusion protein selectively binds to the N-intein polypeptide in the second fusion protein to form an intein complex; and~~

~~b) determining whether the target molecule is cleaved from the C-intein polypeptide under conditions which support intein activity, wherein the presence of the cleaved target molecule is indicative of a catalytically-active intein complex.~~