CLAIMS

What is claimed is:

1. A method of stabilizing an N-Intein Ligand during expression and purification, the method comprising:

    a. forming an intein complex via assembly of an N-Intein Ligand and a Cognate Binding Partner;

    b. purifying the intein complex; and

    c. immobilizing the intein complex to a solid support.

2. The method of claim 1, further comprising the steps of:

    d. subjecting the intein complex to conditions that disrupt association between the N-Intein Ligand and the Cognate Binding Partner; and

    e. providing conditions that allow the N-Intein Ligand to fold into an active state while remaining immobilized.

3. The method of claim 1, wherein the Cognate Binding Partner comprises a C-terminal intein segment.

4. The method of claim 1, wherein, in step a), the N-Intein Ligand and the Cognate Binding Partner are co-expressed *in vivo*.

5. The method of claim 4, wherein the N-Intein Ligand and the Cognate Binding Partner are expressed in a single cell from a single plasmid or two-plasmid system.

6. The method of claim 1, wherein, in step a), the N-Intein Ligand is exposed to the Cognate Binding Partner *in trans*, after expression of the N-Intein Ligand.

7. The method of claim 1, wherein, in step c), the N-terminal intein segment is covalently immobilized to the solid support.

8. The method of claim 1, wherein the solid support is a conventional chromatographic media, including a porous resin, a membrane, a monolith or a magnetic bead.

9. The method of claim 8, wherein the chromatographic media is a solid chromatographic resin backbone.

10. The method of claim 7, wherein N-Intein Ligand density on a solid support is greater than 10 mg of N-Intein Ligand/mL resin volume.

11. The method of claim 1, wherein a chaotropic agent or a basic or acidic solution can be used to create conditions that disrupt association between the N-Intein Ligand and the Cognate Binding Partner.

12. The method of claim 2, wherein disrupting association between the N-Intein Ligand and the Cognate Binding Partner is followed by a condition that causes the N-Intein Ligand to revert to an active state wherein the N-Intein Ligand can accept a new binding partner.

13. The method of claim 12, wherein the disrupting conditions include one of the following: a chaotropic agent such as guanidine hydrochloride, an acid such as phosphoric acid, or a base such as sodium hydroxide.

14. The method of claim 1, wherein the N-Intein Ligand has been derived from a native intein.

15. The method of claim 14, wherein N-Intein Ligand is derived from an *Npu* DnaE intein.

16. The method of claim 14, wherein the Cognate Binding Partner is derived from an *Npu* DnaE intein.

17. The method of claim 1, wherein the N-Intein Ligand comprises a purification tag and an INT_N segment.

18. The method of claim 17, wherein the N-Intein Ligand does not comprise any cysteine residues within the INT_N portion of the N-Intein Ligand.

19. The method of claim 17, wherein an N-Intein Ligand comprising a naturally occurring INT_N segment has been modified so that at least one internal cysteine residue has been mutated to at least one serine residue.

20. The method of claim 17, wherein the purification tag comprises one or more histidine residues.

21. The method of claim 1, wherein the N-Intein Ligand comprises one or more amino acids constituting an immobilization moiety.

22. The method of claim 21, wherein the amino acids are encoded to be expressed in direct fusion to or operably linked to the C-terminus of the INT_N segment, thereby allowing for covalent immobilization of the N-Intein Ligand.

23. The method of claim 21, wherein the one or more amino acids within the immobilization moiety are cysteine residues.

24. The method of claim 1, wherein the N-Intein Ligand further comprises a sensitivity-enhancing motif, which renders it highly sensitive to extrinsic conditions.

25. The method of claim 24, wherein the sensitivity-enhancing motif is in the N-terminus region of the N-Intein Ligand.

26. The method of claim 24, wherein the extrinsic condition is pH, temperature, zinc, or a combination of these.

27. The method of claim 1, wherein the N-Intein Ligand comprises SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 9, or 18.

28. The method of claim 1, wherein the Cognate Binding Partner comprises SEQ ID NO: 10, 11, 12, 13, 14, 15, or 16.