CLAIMS

1. A modified cleavase, which is configured to cleave a peptide bond between a ~~terminally~~ labeled terminal amino acid residue and a penultimate terminal amino acid residue of a polypeptide, wherein the labeled terminal amino acid residue is produced by labeling an N-terminal amino acid residue of the polypeptide with a reagent, the modified cleavase is derived from a dipeptidyl aminopeptidase, which dipeptidyl aminopeptidase removes an unlabeled terminal dipeptide from a polypeptide, ~~wherein the dipeptide aminopeptidase comprises an amino acid sequence having at least 20 % sequence identity to the amino acid sequence of SEQ ID NO: 13 and also comprising an asparagine residue at a position corresponding to position 191 of SEQ ID NO: 13, a tryptophan residue or phenylalanine residue at a position corresponding to position 192 of SEQ ID NO: 13, an arginine residue at a position corresponding to position 196 of SEQ ID NO: 13, an asparagine residue at a position corresponding to position 306 of SEQ ID NO: 13, and an aspartate residue at a position corresponding to position 650 of SEQ ID NO: 13;~~ and wherein the modified cleavase comprises three~~two~~ or more amino acid substitutions in the residues corresponding to positions ~~N~~191, ~~W/F~~192, ~~R~~196, ~~N~~306, and ~~D~~650 of SEQ ID NO: 13 and comprises an amino acid sequence that exhibits at least 60% sequence identity to any one of SEQ ID NOs: 17-19, 23-28, and 31-39; and wherein a label of the labeled terminal amino acid residue comprises a chemical modification and no more than one exogenous amino acid.

2. The modified cleavase of claim 1, wherein the dipeptidyl aminopeptidase is a protein classified in MEROPS S46, or a functional homolog or fragment thereof.

3. The modified cleavase of claim 1, wherein the label of the labeled terminal amino acid residue consists of one exogenous amino acid and the chemical modification.~~comprises an amino acid and a chemical group.~~

4. The modified cleavase of claim 1, wherein the labeled terminal amino acid residue is labeled with the~~a chemical~~ reagent selected from the group consisting of a phenyl isothiocyanate (PITC), a nitro-PITC, a sulfo-PITC, a phenyl isocyanate (PIC), a nitro-PIC, a sulfo-PIC, Cbz-Cl (benzyl chloroformate) or Cbz-OSu (benzyloxycarbonyl N-succinimide), a carboxyl-activated amino-blocked amino acid, an anhydride, a 1-fluoro-2,4-dinitrobenzene (Sanger's reagent, DNFB), dansyl chloride (DNS-Cl, or 1-dimethylaminonaphthalene-5-sulfonyl chloride), 4-sulfonyl-2-nitrofluorobenzene (SNFB), 2-Pyridinecarboxaldehyde, 2-Formylphenylboronic acid,

2-Acetylphenylboronic acid, 1-Fluoro-2,4-dinitrobenzene, 4-Chloro-7-nitrobenzofurazan, Pentafluorophenylisothiocyanate, 4-(Trifluoromethoxy)-phenylisothiocyanate, 4-(Trifluoromethyl)-phenylisothiocyanate, 3-(Carboxylic acid)-phenylisothiocyanate, 3-(Trifluoromethyl)-phenylisothiocyanate, 1-Naphthylisothiocyanate, N-nitroimidazole-1-carboximidamide, N,N'-Bis(pivaloyl)-1H-pyrazole-1-carboxamidine, N,N'-Bis(benzyloxycarbonyl)-1H-pyrazole-1-carboxamidine, an acetylating reagent, a guanidinylation reagent, a thioacylation reagent, a thioacetylation reagent, a thiobenzylation reagent, an isatoic anhydride, an isonicotinic anhydride, an azaisatoic anhydride, a succinic anhydride, and a diheterocyclic methanimine reagent.

5. The modified cleavase of claim 1, wherein the modified cleavase comprises at least fourthree amino acid substitutions in the residues corresponding to positions ~~N~~191, ~~W/F~~192, ~~R~~196, ~~N~~306, and ~~D~~650 of SEQ ID NO: 13.

6. The modified cleavase of claim 1, wherein the modified cleavase comprises fiveat least four amino acid substitutions in the residues corresponding to positions ~~N~~191, ~~W/F~~192, ~~R~~196, ~~N~~306, and ~~D~~650 of SEQ ID NO: 13.

7. The modified cleavase of claim 1, wherein the length of the polypeptide is greater than 10 amino acids.

8. The modified cleavase of claim 1, wherein the modified cleavase comprises an amino acid sequence that is at least ~~30~~70 % identical to the amino acid sequence set forth in SEQ ID NO: 13.

9. The modified cleavase of claim 1, wherein the dipeptidyl aminopeptidase comprises an amino acid sequence that is at least ~~30~~70 % identical to the sequence set forth in SEQ ID NO: 13.

10. The modified cleavase of claim 9, wherein the threetwo or more amino acid substitutions are selected from the group consisting of: N191C, N191F, N191L, N191M, N191R, N191S, N191T, N191V, W192F, W192G, W192L, R196H, R196K, R196S, R196T, R196V, N306A, N306G, N306R, N306S, D650A, D650G.

11. A method of treating a polypeptide, comprising the steps of:

(a) contacting the polypeptide with a reagent for labeling a N-terminal amino acid of the polypeptide to produce a labeled polypeptide comprising a labeled terminal amino acid residue; and

(b) contacting the labeled polypeptide with a modified cleavase,

which is configured to cleave a peptide bond between the ~~terminally~~ labeled terminal amino acid residue and a penultimate terminal amino acid residue of the polypeptide, wherein the modified cleavase is derived from a dipeptidyl aminopeptidase, which dipeptidyl aminopeptidase removes an unlabeled terminal dipeptide from a polypeptide, ~~wherein the dipeptide aminopeptidase comprises an amino acid sequence having at least 20 % sequence identity to the amino acid sequence of SEQ ID NO: 13 and also comprising an asparagine residue at a position corresponding to position 191 of SEQ ID NO: 13, a tryptophan residue or phenylalanine residue at a position corresponding to position 192 of SEQ ID NO: 13, an arginine residue at a position corresponding to position 196 of SEQ ID NO: 13, an asparagine residue at a position corresponding to position 306 of SEQ ID NO: 13, and an aspartate residue at a position corresponding to position 650 of SEQ ID NO: 13;~~ and wherein the modified cleavase comprises three~~two~~ or more amino acid substitutions in the residues corresponding to positions ~~N~~191, ~~W/F~~192, ~~R~~196, ~~N~~306, and ~~D~~650 of SEQ ID NO: 13, and comprises an amino acid sequence that exhibits at least 60 % sequence identity to any one of SEQ ID NOs: 17-19, 23-28, and 31-39; and wherein a label of the labeled terminal amino acid residue comprises a chemical modification and no more than one exogenous amino acid.

12. The method of claim 11, wherein the modified cleavase comprises at least four~~three~~ amino acid substitutions in the residues corresponding to positions ~~N~~191, ~~W/F~~192, ~~R~~196, ~~N~~306, and ~~D~~650 of SEQ ID NO: 13.

13. The method of claim 11, wherein the modified cleavase comprises an amino acid sequence that is at least ~~30~~70 % identical to the amino acid sequence set forth in SEQ ID NO: 13.

14. The method of claim 13, wherein the three~~two~~ or more amino acid substitutions are selected from the group consisting of: N191C, N191F, N191L, N191M, N191R, N191S, N191T, N191V, W192F, W192G, W192L, R196H, R196K, R196S, R196T, R196V, N306A, N306G, N306R, N306S, D650A, D650G, and D650S.

15. The method of claim 11, further comprising contacting the polypeptide with a binding agent configured to bind to the labeled terminal amino acid of the polypeptide, wherein: the contacting with the reagent for labeling the N-terminal amino acid is before the contacting with the binding agent; and the contacting with the binding agent is before the contacting of the polypeptide with the modified cleavase.

16. The method of claim 15, wherein the binding agent comprises a coding tag with identifying information regarding the binding agent.

~~17. The method of claim 15, wherein:~~

~~the contacting with the reagent for labeling the terminal amino acid is before the contacting with the binding agent; and~~

~~the contacting with the binding agent is before the contacting of the polypeptide with the modified cleavase.~~

17~~18~~. The method of claim 15~~17~~, wherein the steps of the contacting of the polypeptide with the binding agent, with the reagent for labeling the N-terminal amino acid, and with the modified cleavase, are repeated one or more times.

18~~19~~. The method of claim 11, wherein the polypeptide is directly or indirectly joined to a solid support.

19~~20~~. A kit for treating a polypeptide, comprising:

a reagent for labeling a terminal N-amino acid residue of the polypeptide configured to produce a ~~terminally~~ labeled terminal amino acid residue of the polypeptide; and

a modified cleavase, which is configured to cleave a peptide bond between the ~~terminally~~ labeled terminal amino acid residue and a penultimate terminal amino acid residue of the polypeptide, wherein the labeled terminal amino acid residue is produced by labeling the N-terminal amino acid residue with the reagent, the modified cleavase is derived from a dipeptidyl aminopeptidase, which dipeptidyl aminopeptidase removes an unlabeled terminal dipeptide from a polypeptide, ~~wherein the dipeptide aminopeptidase comprises an amino acid sequence having at least 20 % sequence identity to the amino acid sequence of SEQ ID NO: 13 and also comprising an asparagine residue at a position corresponding to position 191 of SEQ ID NO: 13, a tryptophan residue or phenylalanine residue at a position corresponding to position 192 of SEQ ID NO: 13, an arginine residue at a position corresponding to position 196 of SEQ ID NO: 13, an asparagine residue at a position corresponding to position 306 of SEQ ID NO: 13, and an aspartate residue at a position corresponding to position 650 of SEQ ID NO: 13;~~ and wherein the modified cleavase comprises three~~two~~ or more amino acid substitutions in the residues corresponding to positions ~~N~~191, ~~W/F~~192, ~~R~~196, ~~N~~306, and ~~D~~650 of SEQ ID NO: 13, and comprises an amino acid sequence that exhibits at least 60 % sequence identity to any one of

SEQ ID NOs: 17-19, 23-28, and 31-39; and wherein a label of the labeled terminal amino acid residue comprises a chemical modification and no more than one exogenous amino acid.