For example, protease-based recovery using site-specific proteases or autoprotease fusion partners to separate the peptides from the carrier protein has been used with moderate success, but these strategies require large peptide fusions and are not compatible with concatemeric peptide production strategies.

[0006] Peptide production methods using onconase as an insoluble carrier protein have been developed and present a potential strategy to produce peptides in bacteria or yeast. One of these onconase-based strategies was recently described by Pane, K., *et al.* (2016) *PLoS One.* 11(1):e0146552 ("Pane, *et al.*"). Pane *et al*. used an onconase-peptide fusion construct to express and purify peptides from inclusion bodies in *E. coli*. Separation of the peptides from the onconase was performed using a chemical cleavage strategy, which can be performed using low-cost reagents and generally targets peptide bonds present at low frequency in proteins. Notably, though the protocol described by Pane *et al.* required the use of chaotropic agents but precluded the use of a chromatographic step. The protocol still resulted in low peptide yield and purity, which can potentially compromise downstream applications of peptides produced by this method.

[0007] Thus, there remains a need for methods of production of short peptides that avoid toxicity and proteolytic degradation, as well as allow for simple, inexpensive and efficient recovery of the peptides.

SUMMARY

[0008] In one aspect, the present disclosure provides for a method of producing oligopeptides in a bacteria cell, comprising: expressing a fusion polypeptide comprising an insoluble carrier polypeptide operably linked to two or more oligopeptides by a peptide bond, and releasing the two or more oligopeptides from the insoluble carrier polypeptide by sequence-specific chemical cleavage of the peptide bond; wherein the insoluble carrier polypeptide comprises a TAF12 polypeptide that consists of SEQ ID NO: 23, or a modified TAF12 polypeptide defined as having an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to SEQ ID NO: 23.

[0008a] Example 1: A method of producing oligopeptides comprising:

expressing a fusion polypeptide comprising an insoluble carrier polypeptide operably linked to two or more oligopeptides by a peptide bond, and

releasing the two or more oligopeptides from the insoluble carrier polypeptide by sequence-specific chemical cleavage of the peptide bond.

[0009] Example 2: The method of Example 1, wherein the insoluble carrier polypeptide comprises an onconase polypeptide.

[0010] Example 3: The method of Example 1, wherein the insoluble carrier polypeptide comprises a TAF12 polypeptide.

[0011] Example 4: The method of Example 1, wherein the insoluble carrier polypeptide comprises a trpΔLE polypeptide, a ketosteroid isomerase (KSI) polypeptide, a β-galactosidase polypeptide, a PagP polypeptide , a truncated *E. coli* PurF F4 fragment polypeptide, a *Pseudomonas aeruginosa* PaP3.30 polypeptide, a histone fold domain of the human transcription factor TAF12 (TAF12-HFD) polypeptide, a cleavable self-aggregating tag INTEIN-ELK16, a *E. coli* maltose-binding protein, an *E. coli* RNAse II polypeptide, an *E. coli* alkaline phosphatase polypeptide, an *E. coli* phospholipase A polypeptide, an *E. coli* β-lactamase polypeptide, a *Salmonella typhimurium* MalK protein, a *Clostridium thermocellum* endoglucanase D polypeptide, a *Bacillus thuringiensis subsp. aizawai* IPL7 insecticidal proteins, a human

procathepsin B polypeptide, a porcine interferon-γ polypeptide, a T5 DNA polymerase polypeptide, and an *E. coli* thioredoxin polypeptide.

[0012] Example 5. The method of any one of Examples 1-4, wherein the fusion polypeptide comprises three or more oligopeptides, four or more oligopeptides, five or more oligopeptides, six or more oligopeptides, eight or more oligopeptides, ten or more oligopeptides, fifteen or more oligopeptides, twenty or more oligopeptides.

[0013] Example 6: The method of any one of Examples 1-5, wherein the two or more oligopeptides are different.

[0014] Example 7: The method of any one of Examples 1-6, wherein all the oligopeptides are operably linked to the N-terminus of the insoluble carrier polypeptide or all the oligopeptides are operably linked to the C-terminus of the insoluble carrier polypeptide.

[0015] Example 8: The method of any one of Examples 1-6, wherein at least one oligopeptide is operably linked to the N-terminus of the insoluble carrier polypeptide and at least one oligopeptide is operably linked to the C-terminus of the insoluble carrier polypeptide.

[0016] Example 9. The method of Example 8, wherein the fusion polypeptide comprises two or more oligopeptides, three or more oligopeptides, four or more oligopeptides, five or more oligopeptides, six or more oligopeptides, eight or more oligopeptides, ten or more oligopeptides operably linked to the N-terminus of the insoluble carrier polypeptide and/or two or more oligopeptides, three or more oligopeptides, four or more oligopeptides, five or more oligopeptides, six or more oligopeptides, eight or more oligopeptides, ten or more oligopeptides operably linked to the C-terminus of the insoluble carrier polypeptide.

[0017] Example 10: The method of any one of Examples 1-9, wherein the peptide bond comprises (i) a methionine and the sequence-specific chemical cleavage is with cyanogen bromide, (ii) a tryptophan and the sequence-specific chemical cleavage is with BNPS-skatole, (iii) an aspartic acid-proline (Asp-Pro) bond and the sequence-specific chemical cleavage is with formic acid, (iv) an asparagine-glycine bond and the sequence-specific chemical cleavage is with hydroxylamine, or (v) a cysteine and the sequence-specific chemical cleavage is with NTCB.

[0018] Example 11: The method of any one of Examples 1-9, wherein the peptide bond comprises an Asp-Pro bond and the sequence-specific chemical cleavage is with acetic acid.

[0019] Example 12: The method of any one of Examples 1-11, wherein the oligopeptides are operably linked by the peptide bond and are released from each other when the oligopeptides are released from the insoluble carrier polypeptide.

[0020] Example 13: The method of any one of Examples 1-11, wherein the oligopeptides are operably linked by a different peptide bond and are released from each other by sequence-specific chemical cleavage of the different peptide bond after the oligopeptides are released from the insoluble carrier polypeptide.

[0021] Example 14: The method of Example 13, wherein the different peptide bond comprises (i) a methionine and the sequence-specific chemical cleavage is with cyanogen bromide, (ii) a tryptophan and the sequence-specific chemical cleavage is with BNPS-skatole, (iii) an aspartic acid-proline (Asp-Pro) bond and the sequence-specific chemical cleavage is with formic acid, (iv) an asparagine-glycine bond and the sequence-specific chemical cleavage is with hydroxylamine, or (v) a cysteine and the sequence-specific chemical cleavage is with NTCB.

[0022] Example 15: The method of Example 13, wherein the different peptide bond comprises an Asp-Pro bond and the sequence-specific chemical cleavage is with acetic acid.

[0023] Example 16: The method of any one of Examples 1-15, wherein the oligopeptides are at least four amino acids long, at least five amino acids long, at least six amino acids long, at least seven amino acids long, at least eight amino acids long, at least nine amino acids long, at least ten amino acids long, at least fifteen amino acids long, at least twenty amino acids long, or at least twenty-five amino acids long.

[0024] Example 17: The method of any one of Examples 1-16, wherein the oligopeptides are less than fifty amino acids long, less than forty-five amino acids long, less than forty amino acids long, less than thirty-five amino acids long, less than thirty amino acids long, less than twenty-five amino acids long, or less than twenty amino acids long.

[0025] Example 18: The method of any one of Examples 1-15, wherein the oligopeptides are between four amino acids long and fifty amino acids long, between six amino acids long and forty amino acids long, between six amino acids long and thirty amino acids long, or between eight amino acids long and twenty-five amino acids long.

[0026] Example 19: The method of any one of Example 1-18, wherein the fusion peptide is expressed in bacteria or yeast.

[0027] Example 20: The method of Example 19, wherein the bacteria is *E. coli* or *Vibrio natriegens*

[0028] Example 21: The method of Example 19 or Example 20, wherein the yield of released oligopeptide is at least 10 mg/L of bacterial culture, at least 20 mg/L of bacterial culture, at least 30 mg/L of bacterial culture, at least 40 mg/L of bacterial culture, at least 50 mg/L of bacterial culture, at least 1 g/L or at least 5 g/L.

[0029] Example 22: A fusion polypeptide comprising an insoluble carrier polypeptide operably linked to two or more oligopeptides, wherein the operably linkage between the two or more oligopeptides and the

insoluble carrier polypeptide comprise a peptide bond that is capable of sequence-specific chemical cleavage.

[0030] Example 23: The fusion polypeptide of Example 22, wherein the insoluble carrier polypeptide comprises an onconase polypeptide.

[0031] Example 24: The fusion polypeptide of Example 22, wherein the insoluble carrier polypeptide comprises a TAF12 polypeptide.

[0032] Example 25: The fusion polypeptide of Example 22, wherein the insoluble carrier polypeptide comprises a trpΔLE polypeptide, a ketosteroid isomerase (KSI) polypeptide, a β-galactosidase polypeptide, a PagP polypeptide , a truncated *E. coli* PurF F4 fragment polypeptide, a *Pseudomonas aeruginosa* PaP3.30 polypeptide, a histone fold domain of the human transcription factor TAF12 (TAF12-HFD) polypeptide, a cleavable self-aggregating tag INTEIN-ELK16, a *E. coli* maltose-binding protein, an *E. coli* RNAse II polypeptide, an *E. coli* alkaline phosphatase polypeptide, an *E. coli* phospholipase A polypeptide, an *E. coli* β-lactamase polypeptide, a *Salmonella typhimurium* MalK protein, a *Clostridium thermocellum* endoglucanase D polypeptide, a *Bacillus thuringiensis subsp. aizawai* IPL7 insecticidal proteins, a human procathepsin B polypeptide, a porcine interferon-γ polypeptide, a T5 DNA polymerase polypeptide, and an *E. coli* thioredoxin polypeptide.

[0033] Example 26. The fusion polypeptide of any one of Examples 22-25, wherein the fusion polypeptide comprises three or more oligopeptides, four or more oligopeptides, five or more oligopeptides, six or more oligopeptides, eight or more oligopeptides, ten or more oligopeptides, fifteen or more oligopeptides, twenty or more oligopeptides.

[0034] Example 27: The fusion polypeptide of any one of Examples 22-26, wherein the two or more oligopeptides are different.

[0035] Example 28: The fusion polypeptide of any one of Examples 22-27, wherein all the oligopeptides are operably linked to the N-terminus of the insoluble carrier polypeptide or all the oligopeptides are operably linked to the C-terminus of the insoluble carrier polypeptide.

[0036] Example 29: The fusion polypeptide of any one of Examples 22-27, wherein at least one oligopeptide is operably linked to the N-terminus of the insoluble carrier polypeptide and at least one oligopeptide is operably linked to the C-terminus of the insoluble carrier polypeptide.

[0037] Example 30. The fusion polypeptide of Example 29, wherein the fusion polypeptide comprises two or more oligopeptides, three or more oligopeptides, four or more oligopeptides, five or more oligopeptides, six or more oligopeptides, eight or more oligopeptides, ten or more oligopeptides operably linked to the N-terminus of the insoluble carrier polypeptide and/or two or more oligopeptides, three or more oligopeptides, four or more oligopeptides, five or more oligopeptides, six or more oligopeptides, eight or

more oligopeptides, ten or more oligopeptides operably linked to the C-terminus of the insoluble carrier polypeptide.

[0038] Example 31: The fusion polypeptide of any one of Examples 22-30, wherein the peptide bond comprises (i) a methionine and the sequence-specific chemical cleavage is with cyanogen bromide, (ii) a tryptophan and the sequence-specific chemical cleavage is with BNPS-skatole, (iii) an aspartic acid-proline (Asp-Pro) bond and the sequence-specific chemical cleavage is with formic acid, (iv) an asparagine-glycine bond and the sequence-specific chemical cleavage is with hydroxylamine, or (v) a cysteine and the sequence-specific chemical cleavage is with NTCB.

[0039] Example 32: The fusion polypeptide of any one of Examples 22-30, wherein the peptide bond comprises an Asp-Pro bond and the sequence-specific chemical cleavage is with acetic acid.

[0040] Example 33: The fusion polypeptide of any one of Examples 22-32, wherein the oligopeptides are operably linked by the peptide bond and are capable of release from each other with the sequence-specific chemical cleavage.

[0041] Example 34: The fusion polypeptide of any one of Examples 22-32, wherein the oligopeptides are operably linked by a different peptide bond and are capable of release from each other by a different sequence-specific chemical cleavage.

[0042] Example 35: The fusion polypeptide of Example 34, wherein the different peptide bond comprises (i) a methionine and the different sequence-specific chemical cleavage is with cyanogen bromide, (ii) a tryptophan and the different sequence-specific chemical cleavage is with BNPS-skatole, (iii) an aspartic acid-proline (Asp-Pro) bond and the different sequence-specific chemical cleavage is with formic acid, (iv) an asparagine-glycine bond and the different sequence-specific chemical cleavage is with hydroxylamine, or (v) a cysteine and the different sequence-specific chemical cleavage is with NTCB.

[0043] Example 36: The fusion polypeptide of Example 34, wherein the different peptide bond comprises an Asp-Pro bond and the different sequence-specific chemical cleavage is with acetic acid.

[0044] Example 37: The fusion polypeptide of any one of Examples 22-36, wherein the oligopeptides are at least four amino acids long, at least five amino acids long, at least six amino acids long, at least seven amino acids long, at least eight amino acids long, at least nine amino acids long, at least ten amino acids long, at least fifteen amino acids long, at least twenty amino acids long, or at least twenty-five amino acids long.

[0045] Example 38: The fusion polypeptide of any one of Examples 22-37, wherein the oligopeptides are less than fifty amino acids long, less than forty-five amino acids long, less than forty amino acids long, less than thirty-five amino acids long, less than thirty amino acids long, less than twenty-five amino acids long, or less than twenty amino acids long.

[0046] Example 39: The fusion polypeptide of any one of Examples 22-36, wherein the oligopeptides are between four amino acids long and fifty amino acids long, between six amino acids long and forty amino acids long, between six amino acids long and thirty amino acids long, or between eight amino acids long and twenty-five amino acids long.

[0047] Example 40: A method of releasing an oligopeptide fused to an insoluble carrier polypeptide, which forms inclusion bodies in a cell comprising

a) expressing a fusion polypeptide comprising the oligopeptide operably linked to the insoluble carrier polypeptide, wherein the operable linkage is a peptide bond that is capable of sequence-specific chemical cleavage by acetic acid,

b) purifying the inclusion bodies, and

c) incubating the inclusion bodies with acid at a temperature of greater than 50°C for at least one hour, wherein the oligopeptide is released from the fusion polypeptide by sequence-specific cleavage of the peptide bond.

[0048] Example 41: The method of Example 40, wherein the insoluble carrier polypeptide comprises an onconase polypeptide.

[0049] Example 42: The method of Example 40, wherein the insoluble carrier polypeptide comprises a TAF12 polypeptide.

[0050] Example 43: The method of Example 40, wherein the insoluble carrier polypeptide comprises a trpΔLE polypeptide, a ketosteroid isomerase (KSI) polypeptide, a β-galactosidase polypeptide, a PagP polypeptide , a truncated *E. coli* PurF F4 fragment polypeptide, a *Pseudomonas aeruginosa* PaP3.30 polypeptide, a histone fold domain of the human transcription factor TAF12 (TAF12-HFD) polypeptide, a cleavable self-aggregating tag INTEIN-ELK16, a *E. coli* maltose-binding protein, an *E. coli* RNAse II polypeptide, an *E. coli* alkaline phosphatase polypeptide, an *E. coli* phospholipase A polypeptide, an *E. coli* β-lactamase polypeptide, a *Salmonella typhimurium* MalK protein, a *Clostridium thermocellum* endoglucanase D polypeptide, a *Bacillus thuringiensis subsp. aizawai* IPL7 insecticidal proteins, a human procathepsin B polypeptide, a porcine interferon-γ polypeptide, a T5 DNA polymerase polypeptide, and an *E. coli* thioredoxin polypeptide.

[0051] Example 44: The method of any one of Examples 40-43, wherein the temperature of step c) is greater than 60°C, greater than 70°C, greater than 80°C, or greater than 90°C.

[0052] Example 45: The method of any one of Examples 40-44, wherein the temperature of step c) is less than 100°C, less than 95°C, less than 90°C, or less than 85°C.

[0053] Example 46: The method of any one of Examples 40-45, wherein the pH in step c) is below 3.0.

[0054] Example 47: The method of any one of Examples 40-45, wherein the pH in step c) is from 2.6 to 2.8.

[0055] Example 48: The method of any one of Examples 40-47, wherein the acid is a strong acid optionally selected from hydrochloric acid and sulfuric acid.

[0056] Example 49: The method of any one of Examples 40-47, wherein the acid is a weak acid.

[0057] Example 50: The method of Example 49, wherein the weak acid is acetic acid.

[0058] Example 51: The method of Example 50, wherein the acetic acid concentration is at least two percent by weight, at least three percent by weight, at least four percent by weight, or at least five percent by weight.

[0059] Example 52: The method of Example 50 or Example 51, wherein the acetic acid concentration is less than fifty percent by weight, less than forty-five percent by weight, less than forty percent by weight, less than thirty-five percent by weight, or less than thirty percent by weight.

[0060] Example 53: The method of any one of Examples 40-52, wherein the insoluble carrier polypeptide is solubilized by the incubating of step c).

[0061] Example 54: The method of any one of Examples 40-52, wherein the insoluble carrier polypeptide is not solubilized by the incubating of step c).

[0062] Example 55. The method of any one of Examples 40-54, wherein the fusion polypeptide comprises three or more oligopeptides, four or more oligopeptides, five or more oligopeptides, six or more oligopeptides, eight or more oligopeptides, ten or more oligopeptides, fifteen or more oligopeptides, twenty or more oligopeptides.

[0063] Example 56: The method of any one of Examples 40-55, wherein the two or more oligopeptides are different.

[0064] Example 57: The method of any one of Examples 40-56, wherein all the oligopeptides are operably linked to the N-terminus of the insoluble carrier polypeptide or all the oligopeptides are operably linked to the C-terminus of the insoluble carrier polypeptide.

[0065] Example 58: The method of any one of Examples 40-56, wherein at least one oligopeptide is operably linked to the N-terminus of the insoluble carrier polypeptide and at least one oligopeptide is operably linked to the C-terminus of the insoluble carrier polypeptide.

[0066] Example 59. The method of Example 58, wherein the fusion polypeptide comprises two or more oligopeptides, three or more oligopeptides, four or more oligopeptides, five or more oligopeptides, six or more oligopeptides, eight or more oligopeptides, ten or more oligopeptides operably linked to the N-terminus of the insoluble carrier polypeptide and/or two or more oligopeptides, three or more oligopeptides, four or more oligopeptides, five or more oligopeptides, six or more oligopeptides, eight or more oligopeptides, ten or more oligopeptides operably linked to the C-terminus of the insoluble carrier polypeptide.

[0067] Example 60: The method of any one of Examples 40-59, wherein the peptide bond comprises (i) a methionine and the sequence-specific chemical cleavage is with cyanogen bromide, (ii) a tryptophan and the sequence-specific chemical cleavage is with BNPS-skatole, (iii) an aspartic acid-proline (Asp-Pro) bond and the sequence-specific chemical cleavage is with formic acid, (iv) an asparagine-glycine bond and the sequence-specific chemical cleavage is with hydroxylamine, or (v) a cysteine and the sequence-specific chemical cleavage is with NTCB.

[0068] Example 61: The method of any one of Examples 40-60, wherein the peptide bond comprises an Asp-Pro bond and the sequence-specific chemical cleavage is acid cleavage.

[0069] Example 62: The method of any one of Examples 40-61, wherein the oligopeptides are operably linked by the peptide bond and are released from each other when the oligopeptides are released from the insoluble carrier polypeptide.

[0070] Example 63: The method of any one of Examples 40-62, wherein the oligopeptides are operably linked by a different peptide bond and are released from each other by sequence-specific chemical cleavage of the different peptide bond after the oligopeptides are released from the insoluble carrier polypeptide.

[0071] Example 64: The method of Example 63, wherein the different peptide bond comprises (i) a methionine and the sequence-specific chemical cleavage is with cyanogen bromide, (ii) a tryptophan and the sequence-specific chemical cleavage is with BNPS-skatole, (iii) an aspartic acid-proline (Asp-Pro) bond and the sequence-specific chemical cleavage is with formic acid, (iv) an asparagine-glycine bond and the sequence-specific chemical cleavage is with hydroxylamine, or (v) a cysteine and the sequence-specific chemical cleavage is with NTCB.

[0072] Example 65: The method of Example 64, wherein the different peptide bond comprises an Asp-Pro bond and the sequence-specific chemical cleavage is acid cleavage.

[0073] Example 66: The method of any one of Examples 40-65, wherein the oligopeptides are at least four amino acids long, at least five amino acids long, at least six amino acids long, at least seven amino acids long, at least eight amino acids long, at least nine amino acids long, at least ten amino acids long, at least fifteen amino acids long, at least twenty amino acids long, or at least twenty-five amino acids long.

[0074] Example 67: The method of any one of Examples 40-66, wherein the oligopeptides are less than fifty amino acids long, less than forty-five amino acids long, less than forty amino acids long, less than thirty-five amino acids long, less than thirty amino acids long, less than twenty-five amino acids long, or less than twenty amino acids long.

[0075] Example 68: The method of any one of Examples 40-65, wherein the oligopeptides are between four amino acids long and fifty amino acids long, between six amino acids long and forty amino acids long,

between six amino acids long and thirty amino acids long, or between eight amino acids long and twenty-five amino acids long.

[0076] Example 69: The method of any one of Example 40-68, wherein the fusion peptide is expressed in bacteria or yeast.

[0077] Example 70: The method of Example 69, wherein the bacteria is *E. coli* or *Vibrio natriegens*

[0078] Example 71: A method of purifying inclusion bodies comprising a fusion protein comprising

a) expressing a fusion protein comprising an oligopeptide operably linked to an insoluble carrier polypeptide, which forms inclusion bodies in a cell, wherein the operable linkage is a chemically cleavable amino acid sequence,

b) lysing the cell to form a cell lysate,

c) centrifuging the cell lysate to form a pellet,

d) washing the pellet in a surfactant buffer comprising a nonionic surfactant at least once, at least twice, or at least three times,

e) washing the pellet in a salt buffer comprising at least 0.5M NaCl at least once, at least twice, or at least three times, and

f) washing the pellet in water at least once, at least twice, or at least three times, and thereby producing purified inclusion bodies.

[0079] Example 72: The method of Example 71, wherein the insoluble carrier polypeptide comprises a TAF12 polypeptide.

[0080] Example 73: The method of Example 71, wherein the insoluble carrier polypeptide comprises an onconase polypeptide.

[0081] Example 74: The method of Example 71, wherein the insoluble carrier polypeptide comprises a trpΔLE polypeptide, a ketosteroid isomerase (KSI) polypeptide, a β-galactosidase polypeptide, a PagP polypeptide , a truncated *E. coli* PurF F4 fragment polypeptide, a *Pseudomonas aeruginosa* PaP3.30 polypeptide, a histone fold domain of the human transcription factor TAF12 (TAF12-HFD) polypeptide, a cleavable self-aggregating tag INTEIN-ELK16, a *E. coli* maltose-binding protein, an *E. coli* RNAse II polypeptide, an *E. coli* alkaline phosphatase polypeptide, an *E. coli* phospholipase A polypeptide, an *E. coli* β-lactamase polypeptide, a *Salmonella typhimurium* MalK protein, a *Clostridium thermocellum* endoglucanase D polypeptide, a *Bacillus thuringiensis subsp. aizawai* IPL7 insecticidal proteins, a human procathepsin B polypeptide, a porcine interferon-γ polypeptide, a T5 DNA polymerase polypeptide, and an *E. coli* thioredoxin polypeptide.

[0082] Example 75: The method of any one of Examples 71-74, wherein the salt buffer is at least 0.6M NaCl, at least 0.7M NaCl, or at least 0.75M NaCl.

[0083] Example 76. The method of any one of Examples 71-75, wherein the fusion polypeptide comprises three or more oligopeptides, four or more oligopeptides, five or more oligopeptides, six or more oligopeptides, eight or more oligopeptides, ten or more oligopeptides, fifteen or more oligopeptides, twenty or more oligopeptides.

[0084] Example 77: The method of any one of Examples 71-76, wherein the two or more oligopeptides are different.

[0085] Example 78: The method of any one of Examples 71-77, wherein all the oligopeptides are operably linked to the N-terminus of the insoluble carrier polypeptide or all the oligopeptides are operably linked to the C-terminus of the insoluble carrier polypeptide.

[0086] Example 79: The method of any one of Examples 71-77, wherein at least one oligopeptide is operably linked to the N-terminus of the insoluble carrier polypeptide and at least one oligopeptide is operably linked to the C-terminus of the insoluble carrier polypeptide.

[0087] Example 80. The method of Example 79, wherein the fusion polypeptide comprises two or more oligopeptides, three or more oligopeptides, four or more oligopeptides, five or more oligopeptides, six or more oligopeptides, eight or more oligopeptides, ten or more oligopeptides operably linked to the N-terminus of the insoluble carrier polypeptide and/or two or more oligopeptides, three or more oligopeptides, four or more oligopeptides, five or more oligopeptides, six or more oligopeptides, eight or more oligopeptides, ten or more oligopeptides operably linked to the C-terminus of the insoluble carrier polypeptide.

[0088] Example 81: The method of any one of Examples 71-80, wherein the peptide bond comprises (i) a methionine and the sequence-specific chemical cleavage is with cyanogen bromide, (ii) a tryptophan and the sequence-specific chemical cleavage is with BNPS-skatole, (iii) an aspartic acid-proline (Asp-Pro) bond and the sequence-specific chemical cleavage is with formic acid, (iv) an asparagine-glycine bond and the sequence-specific chemical cleavage is with hydroxylamine, or (v) a cysteine and the sequence-specific chemical cleavage is with NTCB.

[0089] Example 82: The method of any one of Examples 71-80, wherein the peptide bond comprises an Asp-Pro bond and the sequence-specific chemical cleavage is acid cleavage.

[0090] Example 83: The method of any one of Examples 71-82, wherein the oligopeptides are operably linked by the peptide bond and are released from each other when the oligopeptides are released from the insoluble carrier polypeptide.

[0091] Example 84: The method of any one of Examples 71-82, wherein the oligopeptides are operably linked by a different peptide bond and are released from each other by sequence-specific chemical

cleavage of the different peptide bond after the oligopeptides are released from the insoluble carrier polypeptide.

[0092] Example 85: The method of Example 84, wherein the different peptide bond comprises (i) a methionine and the sequence-specific chemical cleavage is with cyanogen bromide, (ii) a tryptophan and the sequence-specific chemical cleavage is with BNPS-skatole, (iii) an aspartic acid-proline (Asp-Pro) bond and the sequence-specific chemical cleavage is with formic acid, (iv) an asparagine-glycine bond and the sequence-specific chemical cleavage is with hydroxylamine, or (v) a cysteine and the sequence-specific chemical cleavage is with NTCB.

[0093] Example 86: The method of Example 84, wherein the different peptide bond comprises an Asp-Pro bond and the sequence-specific chemical cleavage is with acetic acid.

[0094] Example 87: The method of any one of Examples 71-86, wherein the oligopeptides are at least four amino acids long, at least five amino acids long, at least six amino acids long, at least seven amino acids long, at least eight amino acids long, at least nine amino acids long, at least ten amino acids long, at least fifteen amino acids long, at least twenty amino acids long, or at least twenty-five amino acids long.

[0095] Example 88: The method of any one of Examples 71-87, wherein the oligopeptides are less than fifty amino acids long, less than forty-five amino acids long, less than forty amino acids long, less than thirty-five amino acids long, less than thirty amino acids long, less than twenty-five amino acids long, or less than twenty amino acids long.

[0096] Example 89: The method of any one of Examples 71-86, wherein the oligopeptides are between four amino acids long and fifty amino acids long, between six amino acids long and forty amino acids long, between six amino acids long and thirty amino acids long, or between eight amino acids long and twenty-five amino acids long.

[0097] Example 90: The method of any one of Example 71-89, wherein the fusion peptide is expressed in bacteria or yeast.

[0098] Example 91: The method of Example 90, wherein the bacteria is *E. coli* or *Vibrio natriegens*

[0099] Example 92: A method of producing a fusion polypeptide comprising expressing the fusion polypeptide comprising an oligopeptide operably linked to the C-terminus of an onconase polypeptide, wherein the onconase polypeptide comprises one or more amino acid substitutions in the 11 N-terminal amino acids as compared to SEQ ID NO: 1, wherein the onconase-oligopeptide fusion protein comprising the onconase polypeptide is expressed at a higher level than a fusion protein comprising an onconase of SEQ ID NO: 1 when expressed under the same conditions.

[0100] Example 93: The method of Example 92, wherein the fusion polypeptide comprises three or more oligopeptides, four or more oligopeptides, five or more oligopeptides, six or more oligopeptides, eight or

more oligopeptides, ten or more oligopeptides, fifteen or more oligopeptides, twenty or more oligopeptides.

[0101] Example 94: The method of Example 92 or Example 93, wherein the two or more oligopeptides are different.

[0102] Example 95: The method of any one of Examples 92-94, wherein all the oligopeptides are operably linked to the N-terminus of the onconase polypeptide or all the oligopeptides are operably linked to the C-terminus of the onconase polypeptide.

[0103] Example 96: The method of any one of Examples 92-94, wherein at least one oligopeptide is operably linked to the N-terminus of the onconase polypeptide and at least one oligopeptide is operably linked to the C-terminus of the onconase polypeptide.

[0104] Example 97. The method of Example 96, wherein the fusion polypeptide comprises two or more oligopeptides, three or more oligopeptides, four or more oligopeptides, five or more oligopeptides, six or more oligopeptides, eight or more oligopeptides, ten or more oligopeptides operably linked to the N-terminus of the onconase polypeptide and/or two or more oligopeptides, three or more oligopeptides, four or more oligopeptides, five or more oligopeptides, six or more oligopeptides, eight or more oligopeptides, ten or more oligopeptides operably linked to the C-terminus of the onconase polypeptide.

[0105] Example 98: The method of any one of Examples 92-97, wherein the peptide bond comprises (i) a methionine and the sequence-specific chemical cleavage is with cyanogen bromide, (ii) a tryptophan and the sequence-specific chemical cleavage is with BNPS-skatole, (iii) an aspartic acid-proline (Asp-Pro) bond and the sequence-specific chemical cleavage is with formic acid, (iv) an asparagine-glycine bond and the sequence-specific chemical cleavage is with hydroxylamine, or (v) a cysteine and the sequence-specific chemical cleavage is with NTCB.

[0106] Example 99: The method of any one of Examples 92-97, wherein the peptide bond comprises an Asp-Pro bond and the sequence-specific chemical cleavage is with acetic acid.

[0107] Example 100: The method of any one of Examples 92-99, wherein the oligopeptides are operably linked by the peptide bond and are released from each other when the oligopeptides are released from the onconase.

[0108] Example 101: The method of any one of Examples 92-99, wherein the oligopeptides are operably linked by a different peptide bond and are released from each other by sequence-specific chemical cleavage of the different peptide bond after the oligopeptides are released from the onconase.

[0109] Example 102: The method of Example 101, wherein the different peptide bond comprises (i) a methionine and the sequence-specific chemical cleavage is with cyanogen bromide, (ii) a tryptophan and the sequence-specific chemical cleavage is with BNPS-skatole, (iii) an aspartic acid-proline (Asp-Pro)

bond and the sequence-specific chemical cleavage is with formic acid, (iv) an asparagine-glycine bond and the sequence-specific chemical cleavage is with hydroxylamine, or (v) a cysteine and the sequence-specific chemical cleavage is with NTCB.

[0110] Example 103: The method of Example 101, wherein the different peptide bond comprises an Asp-Pro bond and the sequence-specific chemical cleavage is acid cleavage.

[0111] Example 104: The method of any one of Examples 92-103, wherein the oligopeptides are at least four amino acids long, at least five amino acids long, at least six amino acids long, at least seven amino acids long, at least eight amino acids long, at least nine amino acids long, at least ten amino acids long, at least fifteen amino acids long, at least twenty amino acids long, or at least twenty-five amino acids long.

[0112] Example 105: The method of any one of Examples 92-104, wherein the oligopeptides are less than fifty amino acids long, less than forty-five amino acids long, less than forty amino acids long, less than thirty-five amino acids long, less than thirty amino acids long, less than twenty-five amino acids long, or less than twenty amino acids long.

[0113] Example 106: The method of any one of Examples 92-103, wherein the oligopeptides are between four amino acids long and fifty amino acids long, between six amino acids long and forty amino acids long, between six amino acids long and thirty amino acids long, or between eight amino acids long and twenty-five amino acids long.

[0114] Example 107: The method of any one of Example 92-106, wherein the fusion peptide is expressed in bacteria or yeast.

[0115] Example 108: The method of Example 107, wherein the bacteria is *E. coli* or *Vibrio natriegens*

[0116] Example 109: An onconase polypeptide comprising one or more amino acid substitutions in the 11 N-terminal amino acids of the onconase polypeptide as compared to SEQ ID NO: 1, wherein the onconase polypeptide is expressed at a higher level than an onconase protein of SEQ ID NO: 1 when expressed under the same conditions.

[0117] Example 110: The onconase polypeptide of Example 109, wherein the onconase polypeptide comprises the amino acid sequence of one of SEQ ID NOs: 2-10 and 15-22, optionally with one, two, three, four, five or all six C-terminal histidine residues deleted as shown in FIG. 20A.

[0118] Example 111: A fusion polypeptide comprising an onconase polypeptide operably linked to one or more oligopeptides, wherein the operably linkage between the one or more oligopeptides and the onconase polypeptide comprise a peptide bond that is capable of sequence-specific chemical cleavage, wherein the onconase polypeptide comprises one or more amino acid substitutions in the 11 N-terminal amino acids of the onconase polypeptide as compared to SEQ ID NO: 1, wherein the onconase polypeptide is

expressed at a higher level than an onconase protein of SEQ ID NO: 1 when expressed under the same conditions.

[0119] Example 112: The fusion polypeptide of Example 111, wherein the onconase polypeptide comprises the amino acid sequence of one of SEQ ID NOs: 2-10 and 15-22, optionally with one, two, three, four, five or all six C-terminal histidine residues deleted as shown in FIG. 20A.

[0120] Example 113. The fusion polypeptide of Example 111 or Example 112, wherein the fusion polypeptide comprises three or more oligopeptides, four or more oligopeptides, five or more oligopeptides, six or more oligopeptides, eight or more oligopeptides, ten or more oligopeptides, fifteen or more oligopeptides, twenty or more oligopeptides.

[0121] Example 114: The fusion polypeptide of any one of Examples 111-113, wherein the two or more oligopeptides are different.

[0122] Example 115: The fusion polypeptide of any one of Examples 111-114, wherein all the oligopeptides are operably linked to the N-terminus of the onconase polypeptide or all the oligopeptides are operably linked to the C-terminus of the onconase polypeptide.

[0123] Example 116: The fusion polypeptide of any one of Examples 111-114, wherein at least one oligopeptide is operably linked to the N-terminus of the onconase polypeptide and at least one oligopeptide is operably linked to the C-terminus of the onconase polypeptide.

[0124] Example 117. The fusion polypeptide of Example 116, wherein the fusion polypeptide comprises two or more oligopeptides, three or more oligopeptides, four or more oligopeptides, five or more oligopeptides, six or more oligopeptides, eight or more oligopeptides, ten or more oligopeptides operably linked to the N-terminus of the onconase polypeptide and/or two or more oligopeptides, three or more oligopeptides, four or more oligopeptides, five or more oligopeptides, six or more oligopeptides, eight or more oligopeptides, ten or more oligopeptides operably linked to the C-terminus of the onconase polypeptide.

[0125] Example 118: The fusion polypeptide of any one of Examples 111-117, wherein the peptide bond comprises (i) a methionine and the sequence-specific chemical cleavage is with cyanogen bromide, (ii) a tryptophan and the sequence-specific chemical cleavage is with BNPS-skatole, (iii) an aspartic acid-proline (Asp-Pro) bond and the sequence-specific chemical cleavage is with formic acid, (iv) an asparagine-glycine bond and the sequence-specific chemical cleavage is with hydroxylamine, or (v) a cysteine and the sequence-specific chemical cleavage is with NTCB.

[0126] Example 119: The fusion polypeptide of any one of Examples 111-117, wherein the peptide bond comprises an Asp-Pro bond and the sequence-specific chemical cleavage is acid cleavage.

[0127] Example 120: The fusion polypeptide of any one of Examples 111-119, wherein the oligopeptides are operably linked by the peptide bond and are capable of release from each other with the sequence-specific chemical cleavage.

[0128] Example 121: The fusion polypeptide of any one of Examples 105-119, wherein the oligopeptides are operably linked by a different peptide bond and are capable of release from each other by a different sequence-specific chemical cleavage.

[0129] Example 122: The fusion polypeptide of Example 121, wherein the different peptide bond comprises (i) a methionine and the different sequence-specific chemical cleavage is with cyanogen bromide, (ii) a tryptophan and the different sequence-specific chemical cleavage is with BNPS-skatole, (iii) an aspartic acid-proline (Asp-Pro) bond and the different sequence-specific chemical cleavage is with formic acid, (iv) an asparagine-glycine bond and the different sequence-specific chemical cleavage is with hydroxylamine, or (v) a cysteine and the different sequence-specific chemical cleavage is with NTCB.

[0130] Example 123: The fusion polypeptide of Example 121, wherein the different peptide bond comprises an Asp-Pro bond and the different sequence-specific chemical cleavage is acid cleavage.

[0131] Example 124: The fusion polypeptide of any one of Examples 111-123, wherein the oligopeptides are at least four amino acids long, at least five amino acids long, at least six amino acids long, at least seven amino acids long, at least eight amino acids long, at least nine amino acids long, at least ten amino acids long, at least fifteen amino acids long, at least twenty amino acids long, or at least twenty-five amino acids long.

[0132] Example 125: The fusion polypeptide of any one of Examples 111-124, wherein the oligopeptides are less than fifty amino acids long, less than forty-five amino acids long, less than forty amino acids long, less than thirty-five amino acids long, less than thirty amino acids long, less than twenty-five amino acids long, or less than twenty amino acids long.

[0133] Example 126: The fusion polypeptide of any one of Examples 111-125, wherein the oligopeptides are between four amino acids long and fifty amino acids long, between six amino acids long and forty amino acids long, between six amino acids long and thirty amino acids long, or between eight amino acids long and twenty-five amino acids long.

[0134] Example 127. An oligopeptide comprising an active amino acid sequence and an N-terminal proline, a C-terminal aspartic acid, or both the N-terminal proline and the C-terminal aspartic acid, wherein the active amino acid sequence is a miPEP and the oligopeptide regulates an miRNA or the active amino acid sequence is a peptide microbial inhibitor and the oligopeptide inhibits a microbe.

[0135] Example 128: A nucleic acid encoding a fusion polypeptide of any one of Examples 22-39, the onconase of Example 109 or Example 110, or the fusion polypeptide of any one of Examples 111-126.

[0136] Example 129: A cell comprising the nucleic acid of Example 128.

[0137] Example 130: The cell of Example 129, wherein the cell is a bacterial cell or a yeast cell.

[0138] Example 131: The cell of Example 129, wherein the bacteria is *E. coli* or *Vibrio natriegens*.

[0139] Example 132: The cell of Example 131, wherein the cell is a BL21 bacterial cell.

[0140] Example 133: The cell of Example 131, wherein the cell does not express Lon and ompT proteases.

[0141] Example 134: The nucleic acid of Example 128, wherein the nucleic acid is an isolated nucleic acid.

[0142] Example 135: The method of any one of Examples 1-108, wherein the fusion polypeptide or fusion peptide is expressed in *E. coli*.

[0143] Example 136: The method of any one of Examples 1-108 and 135, wherein the fusion polypeptide or fusion protein is expressed in cells grown in a fermentation bioreactor.

[0144] Example 137: The method of any one of Examples 1-108, wherein the cleavage takes place for about 1-24 hours at a pH of about 2-3.5 and a temperature of about 70-90°C.

[0145] Example 138: A method of producing a fusion polypeptide comprising: expressing the fusion polypeptide comprising an oligopeptide operably linked to the C-terminus or the C-terminus of a modified TAF polypeptide, wherein modified TAF polypeptide comprises an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 23.

[0146] Example 139: The method of Example 138, wherein the fusion polypeptide comprises three or more oligopeptides, four or more oligopeptides, five or more oligopeptides, six or more oligopeptides, eight or more oligopeptides, ten or more oligopeptides, fifteen or more oligopeptides, twenty or more oligopeptides.

[0147] Example 140: The method of Example 138 or Example 139, wherein the two or more oligopeptides are different.

[0148] Example 141: The method of any one of Examples 138-140, wherein all the oligopeptides are operably linked to the N-terminus of the modified TAF polypeptide or all the oligopeptides are operably linked to the C-terminus of the modified TAF polypeptide.

[0149] Example 142: The method of any one of Examples 138-140, wherein at least one oligopeptide is operably linked to the N-terminus of the modified TAF polypeptide and at least one oligopeptide is operably linked to the C-terminus of the modified TAF polypeptide.

[0150] Example 143. The method of Example 142, wherein the fusion polypeptide comprises two or more oligopeptides, three or more oligopeptides, four or more oligopeptides, five or more oligopeptides, six or more oligopeptides, eight or more oligopeptides, ten or more oligopeptides operably linked to the N-terminus of the modified TAF polypeptide and/or two or more oligopeptides, three or more oligopeptides,

four or more oligopeptides, five or more oligopeptides, six or more oligopeptides, eight or more oligopeptides, ten or more oligopeptides operably linked to the C-terminus of the modified TAF polypeptide.

[0151] Example 144: The method of any one of Examples 138-143, wherein the peptide bond comprises (i) a methionine and the sequence-specific chemical cleavage is with cyanogen bromide, (ii) a tryptophan and the sequence-specific chemical cleavage is with BNPS-skatole, (iii) an aspartic acid-proline (Asp-Pro) bond and the sequence-specific chemical cleavage is with formic acid, (iv) an asparagine-glycine bond and the sequence-specific chemical cleavage is with hydroxylamine, or (v) a cysteine and the sequence-specific chemical cleavage is with NTCB.

[0152] Example 145: The method of any one of Examples 138-143, wherein the peptide bond comprises an Asp-Pro bond and the sequence-specific chemical cleavage is with acetic acid or with sulfuric acid.

[0153] Example 146: The method of any one of Examples 138-145, wherein the oligopeptides are operably linked by the peptide bond and are released from each other when the oligopeptides are released from the modified TAF polypeptide.

[0154] Example 147: The method of any one of Examples 138-145, wherein the oligopeptides are operably linked by a different peptide bond and are released from each other by sequence-specific chemical cleavage of the different peptide bond after the oligopeptides are released from the modified TAF polypeptide.

[0155] Example 148: The method of Example 147, wherein the different peptide bond comprises (i) a methionine and the sequence-specific chemical cleavage is with cyanogen bromide, (ii) a tryptophan and the sequence-specific chemical cleavage is with BNPS-skatole, (iii) an aspartic acid-proline (Asp-Pro) bond and the sequence-specific chemical cleavage is with formic acid, (iv) an asparagine-glycine bond and the sequence-specific chemical cleavage is with hydroxylamine, or (v) a cysteine and the sequence-specific chemical cleavage is with NTCB.

[0156] Example 149: The method of Example 147, wherein the different peptide bond comprises an Asp-Pro bond and the sequence-specific chemical cleavage is acid cleavage.

[0157] Example 150: The method of any one of Examples 138-149, wherein the oligopeptides are at least four amino acids long, at least five amino acids long, at least six amino acids long, at least seven amino acids long, at least eight amino acids long, at least nine amino acids long, at least ten amino acids long, at least fifteen amino acids long, at least twenty amino acids long, or at least twenty-five amino acids long.

[0158] Example 151: The method of any one of Examples 138-150, wherein the oligopeptides are less than fifty amino acids long, less than forty-five amino acids long, less than forty amino acids long, less than

thirty-five amino acids long, less than thirty amino acids long, less than twenty-five amino acids long, or less than twenty amino acids long.

[0159] Example 152: The method of any one of Examples 138-149, wherein the oligopeptides are between four amino acids long and fifty amino acids long, between six amino acids long and forty amino acids long, between six amino acids long and thirty amino acids long, or between eight amino acids long and twenty-five amino acids long.

[0160] Example 153: The method of any one of Examples 138-152, wherein the fusion peptide is expressed in bacteria or yeast.

[0161] Example 154: The method of Example 153, wherein the bacteria is *E. coli* or *Vibrio natriegens*

[0162] Example 155: A modified TAF polypeptide comprising an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 23.

[0163] Example 156: The modified TAF polypeptide of Example 155, wherein the modified TAF polypeptide comprises the amino acid sequence of SEQ ID NO: 23.

[0164] In another aspect, the present disclosure provides for a fusion polypeptide comprising an insoluble carrier polypeptide operably linked to two or more oligopeptides, wherein the operably linkage between the two or more oligopeptides and the insoluble carrier polypeptide comprise a peptide bond that is capable of sequence-specific chemical cleavage, wherein the insoluble carrier polypeptide comprises a TAF12 polypeptide that consists of SEQ ID NO: 23, or a modified TAF12 polypeptide defined as having an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to SEQ ID NO: 23.

[0164a] Example 157: A fusion polypeptide comprising a modified TAF polypeptide operably linked to one or more oligopeptides, wherein the operably linkage between the one or more oligopeptides and the modified TAF polypeptide comprise a peptide bond that is capable of sequence-specific chemical cleavage, wherein the modified TAF polypeptide comprises comprising an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 23.

[0165] Example 158: The fusion polypeptide of Example 157, wherein the modified TAF polypeptide comprises the amino acid sequence of SEQ ID NO: 23.

[0166] Example 159. The fusion polypeptide of Example 157 or Example 158, wherein the fusion polypeptide comprises three or more oligopeptides, four or more oligopeptides, five or more oligopeptides, six or more oligopeptides, eight or more oligopeptides, ten or more oligopeptides, fifteen or more oligopeptides, twenty or more oligopeptides.

[0167] Example 160: The fusion polypeptide of any one of Examples 157-159, wherein the two or more oligopeptides are different.

[0168] Example 161: The fusion polypeptide of any one of Examples 157-160, wherein all the oligopeptides are operably linked to the N-terminus of the modified TAF polypeptide or all the oligopeptides are operably linked to the C-terminus of the modified TAF polypeptide.

[0169] Example 162: The fusion polypeptide of any one of Examples 157-160, wherein at least one oligopeptide is operably linked to the N-terminus of the modified TAF polypeptide and at least one oligopeptide is operably linked to the C-terminus of the modified TAF polypeptide.

[0170] Example 163. The fusion polypeptide of Example 162, wherein the fusion polypeptide comprises two or more oligopeptides, three or more oligopeptides, four or more oligopeptides, five or more oligopeptides, six or more oligopeptides, eight or more oligopeptides, ten or more oligopeptides operably linked to the N-terminus of the modified TAF polypeptide and/or two or more oligopeptides, three or more oligopeptides, four or more oligopeptides, five or more oligopeptides, six or more oligopeptides, eight or more oligopeptides, ten or more oligopeptides operably linked to the C-terminus of the modified TAF polypeptide.

[0171] Example 164: The fusion polypeptide of any one of Examples 157-163, wherein the peptide bond comprises (i) a methionine and the sequence-specific chemical cleavage is with cyanogen bromide, (ii) a tryptophan and the sequence-specific chemical cleavage is with BNPS-skatole, (iii) an aspartic acid-proline (Asp-Pro) bond and the sequence-specific chemical cleavage is with formic acid, (iv) an asparagine-glycine bond and the sequence-specific chemical cleavage is with hydroxylamine, or (v) a cysteine and the sequence-specific chemical cleavage is with NTCB.

[0172] Example 165: The fusion polypeptide of any one of Examples 157-163, wherein the peptide bond comprises an Asp-Pro bond and the sequence-specific chemical cleavage is acid cleavage.

[0173] Example 166: The fusion polypeptide of any one of Examples 157-165, wherein the oligopeptides are operably linked by the peptide bond and are capable of release from each other with the sequence-specific chemical cleavage.

[0174] Example 167: The fusion polypeptide of any one of Examples 157-165, wherein the oligopeptides are operably linked by a different peptide bond and are capable of release from each other by a different sequence-specific chemical cleavage.

[0175] Example 168: The fusion polypeptide of Example 167, wherein the different peptide bond comprises (i) a methionine and the different sequence-specific chemical cleavage is with cyanogen bromide, (ii) a tryptophan and the different sequence-specific chemical cleavage is with BNPS-skatole, (iii) an aspartic acid-proline (Asp-Pro) bond and the different sequence-specific chemical cleavage is with formic acid, (iv) an asparagine-glycine bond and the different sequence-specific chemical cleavage is with hydroxylamine, or (v) a cysteine and the different sequence-specific chemical cleavage is with NTCB.

[0176] Example 169: The fusion polypeptide of Example 167, wherein the different peptide bond comprises an Asp-Pro bond and the different sequence-specific chemical cleavage is acid cleavage.

[0177] Example 170: The fusion polypeptide of any one of Examples 157-169, wherein the oligopeptides are at least four amino acids long, at least five amino acids long, at least six amino acids long, at least seven amino acids long, at least eight amino acids long, at least nine amino acids long, at least ten amino acids long, at least fifteen amino acids long, at least twenty amino acids long, or at least twenty-five amino acids long.

[0178] Example 171: The fusion polypeptide of any one of Examples 157-170, wherein the oligopeptides are less than fifty amino acids long, less than forty-five amino acids long, less than forty amino acids long, less than thirty-five amino acids long, less than thirty amino acids long, less than twenty-five amino acids long, or less than twenty amino acids long.

[0179] Example 172: The fusion polypeptide of any one of Examples 157-171, wherein the oligopeptides are between four amino acids long and fifty amino acids long, between six amino acids long and forty amino acids long, between six amino acids long and thirty amino acids long, or between eight amino acids long and twenty-five amino acids long.

[0180] In a further aspect, the present disclosure provides for a nucleic acid encoding the fusion polypeptide as disclosed herein.

[0180a] Example 173: A nucleic acid encoding a fusion polypeptide of any one of Examples 22-39, the modified RAF polypeptide of Example 155 or Example 156, or the fusion polypeptide of any one of Examples 157-172.

[0181] In a further aspect, the present disclosure provides for a bacterial cell comprising the nucleic acid as disclosed herein, preferably wherein the bacterial cell is an E. coli cell, still preferably a BL21 E.coli cell.

[0181a] Example 174: A cell comprising the nucleic acid of Example 173.

[0182] Example 175: The cell of Example 174, wherein the cell is a bacterial cell or a yeast cell.

[0183] Example 176: The cell of Example 174, wherein the bacteria is *E. coli* or *Vibrio natriegens*.

[0184] Example 177: The cell of Example 176, wherein the cell is a BL21 bacterial cell.

[0185] Example 178: The cell of Example 176, wherein the cell does not express Lon and ompT proteases.

[0186] Example 179: The nucleic acid of Example 173, wherein the nucleic acid is an isolated nucleic acid.

BRIEF DESCRIPTION OF THE DRAWINGS

[0187] FIG. 1A is a schematic representation of the onconase-based fusion construct used for strategies for peptide bioproduction as exemplary means for production of peptides with insoluble carrier polypeptides. FIG. 1B shows the RP-HPLC chromatograms for the four pHs tested in the first experiment of Example 1 overlapped with the peaks for the peptide fraction and the protein fraction highlighted.

[0188] FIGs. 2A-2B compare the quantities of peptide and fusion protein released for four pHs tested from pH 2 to pH 5. FIG 2A shows the quantity of peptide released by cleavage at the four pHs tested. FIG 2B shows the quantity of fusion protein solubilized at the four pHs tested.

CLAIMS

1. A method of producing oligopeptides in a bacteria cell, comprising:

expressing a fusion polypeptide comprising an insoluble carrier polypeptide operably linked to two or more oligopeptides by a peptide bond, and

releasing the two or more oligopeptides from the insoluble carrier polypeptide by sequence-specific chemical cleavage of the peptide bond;

wherein the insoluble carrier polypeptide comprises a TAF12 polypeptide that consists of SEQ ID NO: 23, or a modified TAF12 polypeptide defined as having an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to SEQ ID NO: 23.

2. The method of claim 1, wherein the different sequence-specific chemical cleavage is an acidic cleavage, preferably wherein the acid is a strong acid that is optionally sulfuric acid.

3. The method of any of claims 1 or 2, wherein the peptide bond is an Asp-Pro bond; whereby the released oligopeptides optionally comprise an N-terminal proline or a C- terminal aspartic acid or comprise both an N-terminal proline and the C-terminal aspartic acid.

4. The method of any one of claims 1-3, wherein the fusion polypeptide comprises three or more oligopeptides, four or more oligopeptides, five or more oligopeptides, six or more oligopeptides, eight or more oligopeptides, ten or more oligopeptides, fifteen or more oligopeptides, twenty or more oligopeptides.

5. The method of any one of claims 1-4, wherein all the oligopeptides are operably linked to the C-terminus of the insoluble carrier polypeptide.

6. The method of any one of claims 1-5, wherein the bacteria is *E. coli*, optionally wherein the bacteria is a BL21 E. coli.

7. The method of any one of claims 1-6, comprising a step of purifying the inclusion bodies formed by the fusion polypeptide in the bacteria cell, wherein the step of purifying the inclusion bodies comprises

- lysing the cell to form a cell lysate,
- centrifuging the cell lysate to form a pellet,

- washing the pellet in a surfactant buffer comprising a nonionic surfactant at least once, at least twice, or at least three times,
- washing the pellet in a salt buffer comprising at least 0.5M NaCl at least once, at least twice, or at least three times, and
- washing the pellet in water at least once, at least twice, or at least three times, and thereby producing purified inclusion bodies.

8. The method of claim 7, comprising a step of purifying the inclusion bodies formed by the fusion polypeptide in the bacteria cell and a subsequent step of incubating the inclusion bodies with acid at a temperature of greater than 50°C, preferably greater than 80°C, for at least one hour, wherein the oligopeptide is released from the fusion polypeptide by sequence-specific cleavage of the peptide bond, optionally wherein the incubating step is conducted at a pH below 3.0, preferably from 2.6 to 2.8.

9. The method of any one of claims 1-7, wherein the cleavage takes place for 1-24 hours at a pH of 2-3.5 and a temperature of 70-90°C.

10. A fusion polypeptide comprising an insoluble carrier polypeptide operably linked to two or more oligopeptides, wherein the operably linkage between the two or more oligopeptides and the insoluble carrier polypeptide comprise a peptide bond that is capable of sequence-specific chemical cleavage, wherein the insoluble carrier polypeptide comprises a TAF12 polypeptide that consists of SEQ ID NO: 23, or a modified TAF12 polypeptide defined as having an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to SEQ ID NO: 23.

11. The fusion polypeptide of claim 10, wherein the different sequence-specific chemical cleavage is an acidic cleavage, and wherein the peptide bond is preferably an Asp-Pro bond.

12. The fusion polypeptide of any of claims 10 or 11, wherein the fusion polypeptide comprises three or more oligopeptides, four or more oligopeptides, five or more oligopeptides, six or more oligopeptides, eight or more oligopeptides, ten or more oligopeptides, fifteen or more oligopeptides, twenty or more oligopeptides.

13. The fusion polypeptide of any one of claims 10 to 12, wherein all the oligopeptides are operably linked to the C-terminus of the insoluble carrier polypeptide.

14. A nucleic acid encoding the fusion polypeptide as defined in any one of claims 10 to 13.

15. A bacterial cell comprising the nucleic acid of claim 14, preferably wherein the bacterial cell is an E. coli cell, still preferably a BL21 E.coli cell.