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- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

(54) Title: DISELENIDE-STABILIZED FEED ENZYMES

(57) Abstract: Provided herein are stabilized feed enzymes such as stabilized phytase, stabilized xylanase, and stabilized mannanase polypeptides containing one or more non-standard amino acids, functional fragments thereof, or variants thereof that maintain enzymatic activity even under harsh conditions, such as reducing environments as found in the gastrointestinal tract of an animal. The present disclosure also relates to methods of contacting these stabilized polypeptides with their respective substrates in an environment.



DISELENIDE-STABILIZED FEED ENZYMES

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/780,452, filed on December 17, 2018, the contents of which are incorporated by reference herein in their entirety.

BACKGROUND

[0002] As the demand for animal products continues to rise on a global scale, improving feed conversion and efficiency has been a focus for the agriculture industry. For certain nutrients, animal feed conversion and efficiency may be limited due to an insufficient production by the animals of the necessary enzymes that facilitate the digestion and adsorption of the nutrients. Thus, for certain animals and animal feed, feed conversion and efficiency can be improved by supplementing the necessary enzymes. For example, phytase, an enzyme that breaks down phytate, has been used as an animal feed supplement to improve the animal's adsorption of dietary phosphorus, and carbohydrases such as xylanase and mannanase have been used to improve animal adsorption of plant fibers.

[0003] However, like many other enzymes, the activities of phytase, xylanase, and mannanase depend on the environment in which they catalyze reactions. Often, enzymes rely on certain structural features to maintain their activity and these structural features may be compromised by certain conditions. For example, in reducing environments such as found in certain parts of the gastrointestinal tract of an animal, the intermolecular and/or intramolecular disulfide bridges of enzymes may be reduced, leading to structural changes in the enzyme and ultimately, reduction or loss of activity. Thus, there remains a need for feed enzymes such as phytase, xylanase, and mannanase polypeptides that are compatible with the reducing environment of an animal's gastrointestinal tract.

SUMMARY

[0004] Provided herein is phytase polypeptide containing a non-standard amino acid, a functional fragment thereof, or a variant thereof that maintains enzymatic activity even under harsh conditions, such as reducing environments as found in certain parts of a gastrointestinal tract of an animal. Provided herein is xylanase polypeptide containing a non-standard amino acid, a functional fragment thereof, or a variant thereof that maintains enzymatic activity even under harsh conditions, such as reducing environments. Provided herein is mannanase

polypeptide containing a non-standard amino acid, a functional fragment thereof, or a variant thereof that maintains enzymatic activity even under harsh conditions, such as reducing environments. Further provided herein are methods of contacting the phytase, xylanase, and mannanase with their respective substrates in an environment.

[0005] In one aspect, provided herein is a composition comprising a stabilized phytase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof.

[0006] In one aspect, provided herein is a composition comprising a stabilized phytase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized phytase polypeptide has a higher hydrolytic activity for a phytate substrate in an environment than a hydrolytic activity for the phytate substrate of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids in the environment.

[0007] In another aspect, provided herein is a composition comprising a stabilized phytase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized phytase polypeptide does not destabilize in an environment that a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids does destabilize.

[0008] In some embodiments, the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, has a melting temperature (T_m) that is at least 5°C higher than a T_m of a corresponding recombinant enzyme, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0009] In yet another aspect, provided herein is a composition comprising a stabilized phytase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized phytase polypeptide has a melting temperature (T_m) that is at least 5°C higher than a T_m of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0010] In some embodiments, the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, has a higher hydrolytic activity for a phytate substrate in an environment than a hydrolytic activity for the phytate substrate of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0011] In some embodiments, the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, does not destabilize in an environment that a corresponding phytase

polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids does destabilize.

[0012] In some embodiments, at least one, two, three, four or more of the one or more non-standard amino acids is selenocysteine.

[0013] In some embodiments, at least two of the one or more non-standard amino acids are directly linked by a bond.

[0014] In some embodiments, at least four of the one or more non-standard amino acids are directly linked by a bond, wherein a first pair of the at least four of the one or more non-standard amino acids is directly linked by a bond, and a second pair of the at least four of the one or more non-standard amino acids is directly linked by a bond.

[0015] In some embodiments, the bond is a diselenide bond or a selenyl-sulfhydryl bond.

[0016] In some embodiments, the diselenide bond or the selenyl-sulfhydryl bond is an intermolecular or an intramolecular bond.

[0017] In some embodiments, the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, has a half-life that is at least 1.1 fold higher than a half-life of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0018] In some embodiments, the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, has at least 70%, 75%, 80%, 85%, 90%, 95% sequence identity to a sequence from Table 1.

[0019] In some embodiments, the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, comprises a sequence with at least 70%, 75%, 80%, 85%, 90%, 95% sequence identity to at least 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, or 433 contiguous amino acids of the sequence from Table 1.

[0020] In some embodiments, the sequence from Table 1 is SEQ ID NO: 1.

[0021] In some embodiments, the one or more non-standard amino acids is at (a) position 15 of SEQ ID NO:1, (b) position 53 of SEQ ID NO:1, (c) position 74 of SEQ ID NO:1, (d) position 99 of SEQ ID NO:1, (e) position 121 of SEQ ID NO:1, (f) position 130 of SEQ ID NO:1, (g) position 163 of SEQ ID NO:1, (h) position 155 of SEQ ID NO:1, (i) position 199 of SEQ ID NO:1, (j) position 200 of SEQ ID NO:1, (k) position 209 of SEQ ID NO:1, (l) position 221 of SEQ ID NO:1, (m) position 403 of SEQ ID NO:1, (n) position 412 of SEQ ID NO:1, or (o) position 429 of SEQ ID NO:1.

[0022] In some embodiments, the one or more non-standard amino acids is at (a) position 15 of SEQ ID NO:1, (b) position 99 of SEQ ID NO:1, (c) position 130 of SEQ ID NO:1, (d) position

155 of SEQ ID NO:1, (e) position 200 of SEQ ID NO:1, (f) position 209 of SEQ ID NO:1, (g) position 403 of SEQ ID NO:1, (h) position 412 of SEQ ID NO:1, or (i) position 429 of SEQ ID NO:1.

[0023] In some embodiments, a non-standard amino acid at position 99 is directly linked by a bond to a non-standard amino acid at position 130.

[0024] In some embodiments, a non-standard amino acid at position 155 is directly linked by a bond to a non-standard amino acid at position 429.

[0025] In some embodiments, a non-standard amino acid at position 200 is directly linked by a bond to a non-standard amino acid at position 209.

[0026] In some embodiments, a non-standard amino acid at position 403 is directly linked by a bond to a non-standard amino acid at position 412. In some embodiments, a non-standard amino acid at position 53 is directly linked by a bond to a non-standard amino acid at position 199.

[0027] In some embodiments, a non-standard amino acid at position 74 is directly linked by a bond to a non-standard amino acid at position 121.

[0028] In some embodiments, a non-standard amino acid at position 163 is directly linked by a bond to a non-standard amino acid at position 221.

[0029] In some embodiments, the sequence from Table 1 is SEQ ID NO:2 or SEQ ID NO:98.

[0030] In some embodiments, the one or more non-standard amino acids is at (a) position 165 of SEQ ID NO: 2 or SEQ ID NO: 98, (b) position 227 of SEQ ID NO: 2 or SEQ ID NO: 98, (c) position 279 of SEQ ID NO: 2 or SEQ ID NO: 98, (d) position 281 of SEQ ID NO: 2 or SEQ ID NO: 98, (e) position 284 of SEQ ID NO: 2 or SEQ ID NO: 98, (f) position 286 of SEQ ID NO: 2 or SEQ ID NO: 98, (g) position 331 of SEQ ID NO: 2 or SEQ ID NO: 98, or (h) position 334 of SEQ ID NO: 2 or SEQ ID NO: 98.

[0031] In some embodiments, a non-standard amino acid at position 165 is directly linked by a bond to a non-standard amino acid at position 284.

[0032] In some embodiments, a non-standard amino acid at position 281 is directly linked by a bond to a non-standard amino acid at position 334.

[0033] In some embodiments, a non-standard amino acid at position 227 is directly linked by a bond to a non-standard amino acid at position 279.

[0034] In some embodiments, a non-standard amino acid at position 286 is directly linked by a bond to a non-standard amino acid at position 331.

[0035] In some embodiments, the sequence from Table 1 is a sequence selected from SEQ ID NOs:99-110.

[0036] In some embodiments, the one or more non-standard amino acids is at position 77, position 108, position 133, position 178, position 188, position 382, position 391, or position 408 of an amino acid sequence selected from SEQ ID NOs:99-110.

[0037] In some embodiments, a non-standard amino acid at position 77 is directly linked by a bond to a non-standard amino acid at position 108.

[0038] In some embodiments, a non-standard amino acid at position 133 is directly linked by a bond to a non-standard amino acid at position 408.

[0039] In some embodiments, a non-standard amino acid at position 178 is directly linked by a bond to a non-standard amino acid at position 188.

[0040] In some embodiments, a non-standard amino acid at position 382 is directly linked by a bond to a non-standard amino acid at position 391.

[0041] In some embodiments, the sequence from Table 1 is a sequence selected from SEQ ID NOs:111-114.

[0042] In some embodiments, the one or more non-standard amino acids is at position 124, position 155, position 180, position 225, position 235, position 429, position 438, or position 455 of an amino acid sequence selected from SEQ ID NOs:111-114.

[0043] In some embodiments, a non-standard amino acid at position 124 is directly linked by a bond to a non-standard amino acid at position 155.

[0044] In some embodiments, a non-standard amino acid at position 180 is directly linked by a bond to a non-standard amino acid at position 455.

[0045] In some embodiments, a non-standard amino acid at position 225 is directly linked by a bond to a non-standard amino acid at position 235.

[0046] In some embodiments, a non-standard amino acid at position 429 is directly linked by a bond to a non-standard amino acid at position 43.

[0047] In some embodiments, the sequence from Table 1 is SEQ ID NO:115.

[0048] In some embodiments, the one or more non-standard amino acids is at position 77, position 79, position 110, position 135, position 180, position 190, position 206, position 384, position 393, or position 410 of SEQ ID NO:115.

[0049] In some embodiments, a non-standard amino acid at position 77 is directly linked by a bond to a non-standard amino acid at position 206.

[0050] In some embodiments, a non-standard amino acid at position 79 is directly linked by a bond to a non-standard amino acid at position 110.

[0051] In some embodiments, a non-standard amino acid at position 135 is directly linked by a bond to a non-standard amino acid at position 410.

[0052] In some embodiments, a non-standard amino acid at position 180 is directly linked by a bond to a non-standard amino acid at position 190.

[0053] In some embodiments, a non-standard amino acid at position 384 is directly linked by a bond to a non-standard amino acid at position 393.

[0054] In some embodiments, the bond is a diselenide bond or a selenyl-sulfhydryl bond.

[0055] In some embodiments, the diselenide bond or the selenyl-sulfhydryl bond is in a location of a disulfide bond in a corresponding recombinant enzyme without the one or more non-standard amino acids.

[0056] In some embodiments, the T_m of the corresponding stabilized phytase polypeptide, functional fragment thereof, or variant thereof, is less than 37°C.

[0057] In some embodiments, the T_m of the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, is greater than 37°C, 40°C, 45°C, 50°C, 55°C, 60°C, or 65°C.

[0058] In some embodiments, the T_m of the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, is at least 10°C higher than the T_m of the corresponding recombinant enzyme, functional fragment thereof, or variant thereof.

[0059] In some embodiments, the T_m of the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, is at least 15°C higher than the T_m of the corresponding recombinant enzyme, functional fragment thereof, or variant thereof.

[0060] In some embodiments, the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, has a half-life in an environment that is at least 1.1 fold higher than a half-life of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids in the environment.

[0061] In some embodiments, the half-life of the phytase polypeptide, functional fragment thereof, or variant thereof in the environment, is greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or more hours.

[0062] In some embodiments, the half-life of the phytase polypeptide, functional fragment thereof, or variant thereof in the environment, is greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or more days.

[0063] In some embodiments, the stabilized phytase polypeptide has at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 fold higher hydrolytic activity for a phytate substrate in an environment than an hydrolytic activity for the phytate substrate of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0064] In some embodiments, a loss of hydrolytic activity of the stabilized phytase polypeptide in a reducing environment as compared to an activity of the stabilized phytase polypeptide in a non-reducing environment is at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% less than a loss of hydrolytic activity of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids under the same conditions.

[0065] In some embodiments, the stabilized phytase polypeptide has at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 fold higher hydrolytic activity for a phytate substrate after being present in an environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or 50 minutes than a hydrolytic activity for the phytate substrate of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids after being present in the environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or 50 minutes.

[0066] In some embodiments, the stabilized phytase polypeptide has at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 fold higher hydrolytic activity for a phytate substrate after being present in an environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, or 24 hours than a hydrolytic activity for the phytate substrate of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids after being present in the environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, or 24 hours.

[0067] In some embodiments, the phytate substrate is in an animal feedstock.

[0068] In some embodiments, the environment is an environment with a temperature of from 4 °C to 98 °C.

[0069] In some embodiments, the environment is a reducing environment.

[0070] In some embodiments, the environment is an environment with an acidic pH.

[0071] In some embodiments, the environment is a stomach environment.

[0072] In some embodiments, the environment is a rumen environment.

[0073] In some embodiments, the environment comprises a reducing agent at a concentration of from 0.01 mM to 100 mM.

[0074] In some embodiments, the environment has a pH of from 1-7.

[0075] In some embodiments, the environment has a pH of from 1-5.

[0076] In some embodiments, the environment has a pH of from 1-3.

[0077] In some embodiments, the environment has a salt concentration of from 10 mM to 1 M.

[0078] In some embodiments, the environment has a reduction potential that is less than 200 mV, 150 mV, 100 mV, 50 mV, 0 mV, -50 mV, -100 mV, -150 mV, -160 mV, -170 mV, -180 mV, -190 mV, -200 mV, -210 mV, -220 mV, -230 mV, -240 mV, -250 mV, -260 mV, -270 mV, -280 mV, -290 mV, -300 mV, -310 mV, -320 mV, -330 mV, -340 mV, -350 mV, -360 mV, -370 mV, -380 mV, -390 mV, -400 mV, -410 mV, -420 mV, -430 mV, -440 mV, -450 mV, -460 mV, -470 mV, -480 mV, -490 mV, -500 mV, -510 mV, -520 mV, -530 mV, -540 mV, -550 mV, -560 mV, -570 mV, -580 mV, -590 mV, or -600 mV.

[0079] In some embodiments, the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, is recombinant.

[0080] In some embodiments, the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, is an animal, a plant, a fungi, or a bacterial phytase.

[0081] In some embodiments, the composition further comprises a xylanase polypeptide.

[0082] In some embodiments, the xylanase is a stabilized xylanase comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof.

[0083] In some embodiments, provided herein is a composition comprising a polynucleotide encoding the composition provided herein.

[0084] In some embodiments, the polynucleotide is a vector.

[0085] In some embodiments, a bond directly linking two of the one or more non-standard amino acids of the stabilized phytase polypeptide does not break in an environment, wherein the bond directly linking two of the one or more standard amino acids of the corresponding phytase polypeptide does break in the same environment.

[0086] In one aspect, provided herein is a method of making the herein-described composition, the method comprising expressing an amino acid sequence of the stabilized phytase polypeptide.

[0087] In some embodiments, expressing comprises expressing in a cell or in vitro.

[0088] In some embodiments, the cell is a bacterial cell.

[0089] In some embodiments, the cell is a genomically recoded cell.

[0090] In some embodiments, the cell comprises a reassigned codon recognized by a stabilizing non-standard amino acid tRNA comprising an anticodon corresponding to the reassigned codon.

[0091] In some embodiments, the amino acid sequence of the stabilized phytase polypeptide is encoded by a polynucleotide sequence comprising at least one codon of a natural amino acid that has been replaced by the reassigned codon.

[0092] In some embodiments, the stabilizing non-standard amino acid tRNA is a selenocysteine tRNA.

[0093] In some embodiments, the method comprises culturing the cell under conditions in which the amino acid sequence of the stabilized phytase polypeptide is expressed.

[0094] In some embodiments, the reassigned codon is UAG, UAA, UGA, or a combination thereof.

[0095] In one aspect, provided herein is a method comprising contacting a phytate substrate in an environment to a stabilized phytase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof; wherein the stabilized phytase polypeptide, functional fragment thereof, or variant thereof catalyzes hydrolysis of the phytate substrate at a higher rate than a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0096] In some embodiments, the stabilized phytase polypeptide, functional fragment thereof, or variant thereof is the stabilized phytase polypeptide, functional fragment thereof, or variant thereof described herein,

[0097] In some embodiments, the phytate substrate is in an animal feedstock.

[0098] In some embodiments, the phytate substrate is from an animal feedstock.

[0099] In some embodiments, the method further comprises providing an animal feedstock comprising the phytate substrate.

[0100] In some embodiments, the method further comprises contacting the animal feedstock with the stabilized phytase polypeptide.

[0101] In some embodiments, the method further comprises feeding the animal feedstock to an animal.

[0102] In some embodiments, the environment is an environment with a temperature of from 4 °C to 50 °C.

[0103] In some embodiments, the environment is a reducing environment.

[0104] In some embodiments, the environment is an environment with an acidic pH.

[0105] In some embodiments, the environment is a stomach environment.

[0106] In some embodiments, the environment is a rumen environment.

[0107] In some embodiments, the environment comprises a reducing agent at a concentration of from 0.01 mM to 100 mM.

[0108] In some embodiments, the environment has a pH of from 1-7.

[0109] In some embodiments, the environment has a pH of from 1-5.

[0110] In some embodiments, the environment has a pH of from 1-3.

[0111] In some embodiments, the environment has a salt concentration of from 10 mM to 1 M.

[0112] In some embodiments, the environment has a reduction potential that is less than 200 mV, 150 mV, 100 mV, 50 mV, 0 mV, -50 mV, -100 mV, -150 mV, -160 mV, -170 mV, -180 mV, -190 mV, -200 mV, -210 mV, -220 mV, -230 mV, -240 mV, -250 mV, -260 mV, -270 mV, -280 mV, -290 mV, -300 mV, -310 mV, -320 mV, -330 mV, -340 mV, -350 mV, -360 mV, -370 mV, -380 mV, -390 mV, -400 mV, -410 mV, -420 mV, -430 mV, -440 mV, -450 mV, -460 mV, -470 mV, -480 mV, -490 mV, -500 mV, -510 mV, -520 mV, -530 mV, -540 mV, -550 mV, -560 mV, -570 mV, -580 mV, -590 mV, or -600 mV.

[0113] In one aspect, provided herein is a composition comprising a stabilized carbohydrase such as a stabilized xylanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof.

[0114] In one aspect, provided herein is a composition comprising a stabilized xylanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized xylanase polypeptide has a higher hydrolytic activity for a polysaccharide substrate in an environment than a hydrolytic activity for the polysaccharide substrate of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0115] In another aspect, provided herein is a composition comprising a stabilized xylanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized xylanase polypeptide does not destabilize in an environment that a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids does destabilize.

[0116] In some embodiments, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, has a melting temperature (T_m) that is at least 5°C higher than a T_m of a corresponding recombinant enzyme, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0117] In yet another aspect, provided herein is a composition comprising a stabilized xylanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized xylanase polypeptide has a melting temperature (T_m) that is at least 5°C higher than a T_m of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0118] In some embodiments, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, has a higher hydrolytic activity for a polysaccharide substrate in an environment than a hydrolytic activity for the polysaccharide substrate of a corresponding

xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0119] In some embodiments, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, does not destabilize in an environment that a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids does destabilize.

[0120] In some embodiments, at least one, two, three, four or more of the one or more non-standard amino acids is selenocysteine.

[0121] In some embodiments, at least two of the one or more non-standard amino acids are directly linked by a bond.

[0122] In some embodiments, at least four of the one or more non-standard amino acids are directly linked by a bond, wherein a first pair of the at least four of the one or more non-standard amino acids is directly linked by a bond, and a second pair of the at least four of the one or more non-standard amino acids is directly linked by a bond.

[0123] In some embodiments, the bond is a diselenide bond or a selenyl-sulfhydryl bond.

[0124] In some embodiments, the diselenide bond or the selenyl-sulfhydryl bond is an intermolecular or an intramolecular bond.

[0125] In some embodiments, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, has a half-life that is at least 1.1 fold higher than a half-life of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0126] In some embodiments, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, has at least 70%, 75%, 80%, 85%, 90%, 95% sequence identity to a sequence selected from Table 2.

[0127] In some embodiments, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, comprises a sequence with at least 70%, 75%, 80%, 85%, 90%, 95% sequence identity to at least 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, or 396 contiguous amino acids of the sequence the sequence selected from Table 2.

[0128] In some embodiments, the sequence selected from Table 2 is SEQ ID NO: 71.

[0129] In some embodiments, the one or more non-standard amino acids is at position 110 of SEQ ID NO: 71, or at position 154 of SEQ ID NO: 71.

[0130] In some embodiments, a non-standard amino acid at position 110 is directly linked by a bond to a non-standard amino acid at position 154.

[0131] In some embodiments, the bond is a diselenide bond or a selenyl-sulfhydryl bond.

[0132] In some embodiments, the diselenide bond or the selenyl-sulfhydryl bond is in a location of a disulfide bond in a corresponding recombinant enzyme without the one or more non-standard amino acids.

[0133] In some embodiments, the T_m of the corresponding stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, is less than 37°C.

[0134] In some embodiments, the T_m of the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, is greater than 37°C, 40°C, 45°C, 50°C, 55°C, 60°C, or 65°C.

[0135] In some embodiments, the T_m of the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, is at least 10°C higher than the T_m of the corresponding recombinant enzyme, functional fragment thereof, or variant thereof.

[0136] In some embodiments, the T_m of the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, is at least 15°C higher than the T_m of the corresponding recombinant enzyme, functional fragment thereof, or variant thereof.

[0137] In some embodiments, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, has a half-life in an environment that is at least 1.1 fold higher than a half-life of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids in the environment.

[0138] In some embodiments, the half-life of the xylanase polypeptide, functional fragment thereof, or variant thereof in the environment, is greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or more hours.

[0139] In some embodiments, the half-life of the xylanase polypeptide, functional fragment thereof, or variant thereof in the environment, is greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or more days.

[0140] In some embodiments, the stabilized xylanase polypeptide has at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 fold higher hydrolytic activity for a polysaccharide substrate in an environment than an hydrolytic activity for the polysaccharide substrate of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0141] In some embodiments, a loss of hydrolytic activity of the stabilized xylanase polypeptide in a reducing environment as compared to an activity of the stabilized xylanase polypeptide in a non-reducing environment is at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% less than a loss of hydrolytic

activity of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids under the same conditions.

[0142] In some embodiments, the stabilized xylanase polypeptide has at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 fold higher hydrolytic activity for a polysaccharide substrate after being present in an environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or 50 minutes than a hydrolytic activity for the polysaccharide substrate of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids after being present in the environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or 50 minutes.

[0143] In some embodiments, the stabilized xylanase polypeptide has at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 fold higher hydrolytic activity for a polysaccharide substrate after being present in an environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, or 24 hours than a hydrolytic activity for the polysaccharide substrate of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids after being present in the environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, or 24 hours.

[0144] In some embodiments, the polysaccharide substrate is in an animal feedstock.

[0145] In some embodiments, the environment is an environment with a temperature of from 4 °C to 98 °C.

[0146] In some embodiments, the environment is a reducing environment.

[0147] In some embodiments, the environment is an environment with an acidic pH.

[0148] In some embodiments, the environment is a stomach environment.

[0149] In some embodiments, the environment is a rumen environment.

[0150] In some embodiments, the environment comprises a reducing agent at a concentration of from 0.01 mM to 100 mM.

[0151] In some embodiments, the environment has a pH of from 1-7.

[0152] In some embodiments, the environment has a pH of from 1-5.

[0153] In some embodiments, the environment has a pH of from 1-3.

[0154] In some embodiments, the environment has a salt concentration of from 10 mM to 1 M.

[0155] In some embodiments, the environment has a reduction potential that is less than 200 mV, 150 mV, 100 mV, 50 mV, 0 mV, -50 mV, -100 mV, -150 mV, -160 mV, -170 mV, -180 mV, -190 mV, -200 mV, -210 mV, -220 mV, -230 mV, -240 mV, -250 mV, -260 mV, -270 mV, -280 mV, -290 mV, -300 mV, -310 mV, -320 mV, -330 mV, -340 mV, -350 mV, -360 mV, -370

mV, -380 mV, -390 mV, -400 mV, -410 mV, -420 mV, -430 mV, -440 mV, -450 mV, -460 mV, -470 mV, -480 mV, -490 mV, -500 mV, -510 mV, -520 mV, -530 mV, -540 mV, -550 mV, -560 mV, -570 mV, -580 mV, -590 mV, or -600 mV.

[0156] In some embodiments, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, is recombinant.

[0157] In some embodiments, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, is an animal, a plant, a fungi, or a bacterial xylanase.

[0158] In some embodiments, the composition further comprises a phytase polypeptide.

[0159] In some embodiments, the phytase is a stabilized phytase comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof.

[0160] In one aspect, provided herein is a composition comprising a polynucleotide encoding the composition described herein.

[0161] In some embodiments, the polynucleotide is a vector.

[0162] In some embodiments, a bond directly linking two of the one or more non-standard amino acids of the stabilized xylanase polypeptide does not break in an environment, wherein the bond directly linking two of the one or more standard amino acids of the corresponding xylanase polypeptide does break in the same environment.

[0163] In some embodiments, the method comprises expressing an amino acid sequence of the stabilized xylanase polypeptide.

[0164] In some embodiments, expressing comprises expressing in a cell or in vitro.

[0165] In some embodiments, the cell is a bacterial cell.

[0166] In some embodiments, the cell is a genomically recoded cell.

[0167] In some embodiments, the cell comprises a reassigned codon recognized by a stabilizing non-standard amino acid tRNA comprising an anticodon corresponding to the reassigned codon.

[0168] In some embodiments, the amino acid sequence of the stabilized xylanase polypeptide is encoded by a polynucleotide sequence comprising at least one codon of a natural amino acid that has been replaced by the reassigned codon.

[0169] In some embodiments, the stabilizing non-standard amino acid tRNA is a selenocysteine tRNA.

[0170] In some embodiments, the method comprises culturing the cell under conditions in which the amino acid sequence of the stabilized xylanase polypeptide is expressed.

[0171] In some embodiments, the reassigned codon is UAG, UAA, UGA, or a combination thereof.

[0172] In another aspect, provided herein is a method comprising contacting a polysaccharide substrate in an environment to a stabilized xylanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof; wherein the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof catalyzes hydrolysis of the polysaccharide substrate at a higher rate than a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0173] In some embodiments, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof is the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof.

[0174] In some embodiments, the polysaccharide substrate is in an animal feedstock.

[0175] In some embodiments, the polysaccharide substrate is from an animal feedstock.

[0176] In some embodiments, the method further comprises providing an animal feedstock comprising the polysaccharide substrate.

[0177] In some embodiments, the method further comprises contacting the animal feedstock with the stabilized xylanase polypeptide.

[0178] In some embodiments, the method further comprises feeding the animal feedstock to an animal.

[0179] In some embodiments, the environment is an environment with a temperature of from 4 °C to 50 °C.

[0180] In some embodiments, the environment is a reducing environment.

[0181] In some embodiments, the environment is an environment with an acidic pH.

[0182] In some embodiments, the environment is a stomach environment.

[0183] In some embodiments, the environment is a rumen environment.

[0184] In some embodiments, the environment comprises a reducing agent at a concentration of from 0.01 mM to 100 mM.

[0185] In some embodiments, the environment has a pH of from 1-7.

[0186] In some embodiments, the environment has a pH of from 1-5.

[0187] In some embodiments, the environment has a pH of from 1-3.

[0188] In some embodiments, the environment has a salt concentration of from 10 mM to 1 M.

[0189] In some embodiments, the environment has a reduction potential that is less than 200 mV, 150 mV, 100 mV, 50 mV, 0 mV, -50 mV, -100 mV, -150 mV, -160 mV, -170 mV, -180 mV, -190 mV, -200 mV, -210 mV, -220 mV, -230 mV, -240 mV, -250 mV, -260 mV, -270 mV, -280 mV, -290 mV, -300 mV, -310 mV, -320 mV, -330 mV, -340 mV, -350 mV, -360 mV, -370

mV, -380 mV, -390 mV, -400 mV, -410 mV, -420 mV, -430 mV, -440 mV, -450 mV, -460 mV, -470 mV, -480 mV, -490 mV, -500 mV, -510 mV, -520 mV, -530 mV, -540 mV, -550 mV, -560 mV, -570 mV, -580 mV, -590 mV, or -600 mV.

[0190] In one aspect, provided herein is a feedstock comprising the composition described herein, where a concentration of the phytase in the final feed mixture is less than 1200 units of the phytase per kilogram of the final feed mixture.

[0191] In some embodiments, the feedstock further comprises any of the herein-described compositions.

[0192] In one aspect, provided herein is a feedstock comprising the herein-described composition, where a concentration of the xylanase in the final feed mixture is less than 1200 units of the xylanase per kilogram of the final feed mixture.

[0193] In one aspect, provided herein is a feedstock comprising a bacterial cell, which bacterial cell expresses or secretes a stabilized phytase of any herein-described composition.

[0194] In some embodiments, the feedstock further comprises an additional bacterial cell, which additional bacterial cell expresses or secretes a stabilized xylanase of any herein-described composition.

[0195] In one aspect, provided herein is a feedstock comprising a bacterial cell, which bacterial cell expresses or secretes a stabilized xylanase of any herein-described composition.

[0196] In one aspect, provided herein is a method comprising feeding an animal with a feedstock comprising a stabilized phytase polypeptide and/or a stabilized xylanase polypeptide.

[0197] In one aspect, provided herein is a method comprising feeding an animal with a feedstock comprising a bacterial cell, which bacterial cell expresses or secretes a stabilized phytase polypeptide and/or a stabilized xylanase polypeptide.

[0198] In some embodiments, the animal extracts the stabilized phytase polypeptide and/or the stabilized xylanase polypeptide from the bacterial cell.

[0199] In one aspect, provided herein is a composition comprising a stabilized mannanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof.

[0200] In one aspect, provided herein is a composition comprising a stabilized mannanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized mannanase polypeptide has a higher hydrolytic activity for a beta-D-mannoside substrate in an environment than a hydrolytic activity for the beta-D-mannoside substrate of a corresponding mannanase polypeptide, functional fragment thereof, or

variant thereof that does not comprise the one or more non-standard amino acids in the environment.

[0201] In another aspect, provided herein is a composition comprising a stabilized mannanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized mannanase polypeptide does not destabilize in an environment that a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids does destabilize.

[0202] In some embodiments, the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, has a melting temperature (T_m) that is at least 5°C higher than a T_m of a corresponding recombinant enzyme, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0203] In yet another aspect, provided herein is a composition comprising a stabilized mannanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized mannanase polypeptide has a melting temperature (T_m) that is at least 5°C higher than a T_m of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0204] In some embodiments, the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, has a higher hydrolytic activity for a beta-D-mannoside substrate in an environment than a hydrolytic activity for the beta-D-mannoside substrate of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0205] In some embodiments, the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, does not destabilize in an environment that a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids does destabilize.

[0206] In some embodiments, at least one, two, three, four or more of the one or more non-standard amino acids is selenocysteine.

[0207] In some embodiments, at least two of the one or more non-standard amino acids are directly linked by a bond.

[0208] In some embodiments, at least four of the one or more non-standard amino acids are directly linked by a bond, wherein a first pair of the at least four of the one or more non-standard

amino acids is directly linked by a bond, and a second pair of the at least four of the one or more non-standard amino acids is directly linked by a bond.

[0209] In some embodiments, the bond is a diselenide bond or a selenyl-sulfhydryl bond.

[0210] In some embodiments, the diselenide bond or the selenyl-sulfhydryl bond is an intermolecular or an intramolecular bond.

[0211] In some embodiments, the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, has a half-life that is at least 1.1 fold higher than a half-life of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0212] In some embodiments, the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, has at least 70%, 75%, 80%, 85%, 90%, 95% sequence identity to a sequence from Table 3.

[0213] In some embodiments, the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, comprises a sequence with at least 70%, 75%, 80%, 85%, 90%, 95% sequence identity to at least 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, or 302 contiguous amino acids of the sequence from Table 3.

[0214] In some embodiments, the sequence from Table 3 is SEQ ID NO:85.

[0215] In some embodiments, the one or more non-standard amino acids is at position 74 or position 81 of SEQ ID NO: 85.

[0216] In some embodiments, a non-standard amino acid at position 74 is directly linked by a bond to a non-standard amino acid at position 81.

[0217] In some embodiments, the bond is a diselenide bond or a selenyl-sulfhydryl bond.

[0218] In some embodiments, the diselenide bond or the selenyl-sulfhydryl bond is in a location of a disulfide bond in a corresponding recombinant enzyme without the one or more non-standard amino acids.

[0219] In some embodiments, the T_m of the corresponding stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, is less than 37°C.

[0220] In some embodiments, the T_m of the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, is greater than 37°C, 40°C, 45°C, 50°C, 55°C, 60°C, or 65°C.

[0221] In some embodiments, the T_m of the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, is at least 10°C higher than the T_m of the corresponding recombinant enzyme, functional fragment thereof, or variant thereof.

[0222] In some embodiments, the T_m of the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, is at least 15°C higher than the T_m of the corresponding recombinant enzyme, functional fragment thereof, or variant thereof.

[0223] In some embodiments, the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, has a half-life in an environment that is at least 1.1 fold higher than a half-life of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids in the environment.

[0224] In some embodiments, the half-life of the mannanase polypeptide, functional fragment thereof, or variant thereof in the environment, is greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or more hours.

[0225] In some embodiments, the half-life of the mannanase polypeptide, functional fragment thereof, or variant thereof in the environment, is greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or more days.

[0226] In some embodiments, the stabilized mannanase polypeptide has at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 fold higher hydrolytic activity for a beta-D-mannoside substrate in an environment than an hydrolytic activity for the beta-D-mannoside substrate of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0227] In some embodiments, a loss of hydrolytic activity of the stabilized mannanase polypeptide in a reducing environment as compared to an activity of the stabilized mannanase polypeptide in a non-reducing environment is at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% less than a loss of hydrolytic activity of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids under the same conditions.

[0228] In some embodiments, the stabilized mannanase polypeptide has at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 fold higher hydrolytic activity for a beta-D-mannoside substrate after being present in an environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or 50 minutes than a hydrolytic activity for the beta-D-mannoside substrate of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids after being present in the environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or 50 minutes.

[0229] In some embodiments, the stabilized mannanase polypeptide has at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 fold higher hydrolytic activity for a beta-D-mannoside substrate after being present in an environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, or 24 hours than a hydrolytic activity for the beta-D-mannoside substrate of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids after being present in the environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, or 24 hours.

[0230] In some embodiments, the beta-D-mannoside substrate is in an animal feedstock.

[0231] In some embodiments, the environment is an environment with a temperature of from 4 °C to 98 °C.

[0232] In some embodiments, the environment is a reducing environment.

[0233] In some embodiments, the environment is an environment with an acidic pH.

[0234] In some embodiments, the environment is a stomach environment.

[0235] In some embodiments, the environment is a rumen environment.

[0236] In some embodiments, the environment comprises a reducing agent at a concentration of from 0.01 mM to 100 mM.

[0237] In some embodiments, the environment has a pH of from 1-7.

[0238] In some embodiments, the environment has a pH of from 1-5.

[0239] In some embodiments, the environment has a pH of from 1-3.

[0240] In some embodiments, the environment has a salt concentration of from 10 mM to 1 M.

[0241] In some embodiments, the environment has a reduction potential that is less than 200 mV, 150 mV, 100 mV, 50 mV, 0 mV, -50 mV, -100 mV, -150 mV, -160 mV, -170 mV, -180 mV, -190 mV, -200 mV, -210 mV, -220 mV, -230 mV, -240 mV, -250 mV, -260 mV, -270 mV, -280 mV, -290 mV, -300 mV, -310 mV, -320 mV, -330 mV, -340 mV, -350 mV, -360 mV, -370 mV, -380 mV, -390 mV, -400 mV, -410 mV, -420 mV, -430 mV, -440 mV, -450 mV, -460 mV, -470 mV, -480 mV, -490 mV, -500 mV, -510 mV, -520 mV, -530 mV, -540 mV, -550 mV, -560 mV, -570 mV, -580 mV, -590 mV, or -600 mV.

[0242] In some embodiments, the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, is recombinant.

[0243] In some embodiments, the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, is an animal, a plant, a fungi, or a bacterial mannanase.

[0244] In some embodiments, the composition further comprises a phytase polypeptide, a xylanase polypeptide or a combination thereof.

[0245] In some embodiments, the phytase polypeptide is a stabilized phytase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof.

[0246] In some embodiments, the xylanase polypeptide is a stabilized xylanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof.

[0247] In one aspect, provided herein is a composition comprising a polynucleotide encoding the herein-described composition.

[0248] In some embodiments, the polynucleotide is a vector.

[0249] In some embodiments, a bond directly linking two of the one or more non-standard amino acids of the stabilized mannanase polypeptide does not break in an environment, wherein the bond directly linking two of the one or more standard amino acids of the corresponding mannanase polypeptide does break in the same environment.

[0250] In some embodiments, the method comprises expressing an amino acid sequence of the stabilized mannanase polypeptide.

[0251] In some embodiments, expressing comprises expressing in a cell or in vitro.

[0252] In some embodiments, the cell is a bacterial cell.

[0253] In some embodiments, the cell is a genomically recoded cell.

[0254] In some embodiments, the cell comprises a reassigned codon recognized by a stabilizing non-standard amino acid tRNA comprising an anticodon corresponding to the reassigned codon.

[0255] In some embodiments, the amino acid sequence of the stabilized mannanase polypeptide is encoded by a polynucleotide sequence comprising at least one codon of a natural amino acid that has been replaced by the reassigned codon.

[0256] In some embodiments, the stabilizing non-standard amino acid tRNA is a selenocysteine tRNA.

[0257] In some embodiments, the method further comprises culturing the cell under conditions in which the amino acid sequence of the stabilized mannanase polypeptide is expressed.

[0258] In some embodiments, the reassigned codon is UAG, UAA, UGA, or a combination thereof.

[0259] In one aspect, provided herein is a method comprising contacting a beta-D-mannoside substrate in an environment to a stabilized mannanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof; wherein the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof catalyzes hydrolysis of the beta-D-mannoside substrate at a higher rate than a corresponding mannanase polypeptide,

functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0260] In some embodiments, the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof is the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof described herein.

[0261] In some embodiments, the beta-D-mannoside substrate is in an animal feedstock.

[0262] In some embodiments, the beta-D-mannoside substrate is from an animal feedstock.

[0263] In some embodiments, the method further comprises providing an animal feedstock comprising the beta-D-mannoside substrate.

[0264] In some embodiments, the method further comprises contacting the animal feedstock with the stabilized mannanase polypeptide.

[0265] In some embodiments, the method further comprises feeding the animal feedstock to an animal.

[0266] In some embodiments, the environment is an environment with a temperature of from 4 °C to 50 °C.

[0267] In some embodiments, the environment is a reducing environment.

[0268] In some embodiments, the environment is an environment with an acidic pH.

[0269] In some embodiments, the environment is a stomach environment.

[0270] In some embodiments, the environment is a rumen environment.

[0271] In some embodiments, the environment comprises a reducing agent at a concentration of from 0.01 mM to 100 mM.

[0272] In some embodiments, the environment has a pH of from 1-7.

[0273] In some embodiments, the environment has a pH of from 1-5.

[0274] In some embodiments, the environment has a pH of from 1-3.

[0275] In some embodiments, the environment has a salt concentration of from 10 mM to 1 M.

[0276] In some embodiments, the environment has a reduction potential that is less than 200 mV, 150 mV, 100 mV, 50 mV, 0 mV, -50 mV, -100 mV, -150 mV, -160 mV, -170 mV, -180 mV, -190 mV, -200 mV, -210 mV, -220 mV, -230 mV, -240 mV, -250 mV, -260 mV, -270 mV, -280 mV, -290 mV, -300 mV, -310 mV, -320 mV, -330 mV, -340 mV, -350 mV, -360 mV, -370 mV, -380 mV, -390 mV, -400 mV, -410 mV, -420 mV, -430 mV, -440 mV, -450 mV, -460 mV, -470 mV, -480 mV, -490 mV, -500 mV, -510 mV, -520 mV, -530 mV, -540 mV, -550 mV, -560 mV, -570 mV, -580 mV, -590 mV, or -600 mV.

[0277] In one aspect, provided herein is a method of manufacturing an animal feedstock by combining one or more stabilized feed enzymes, functional fragments, or variants thereof with one or more substrates of the feed enzymes.

[0278] In some embodiments, the one or more stabilized feed enzymes, functional fragments, or variants thereof comprise a stabilized phytase, a stabilized carbohydrase, or a combination thereof.

[0279] In some embodiments, the one or more stabilized feed enzymes, functional fragments, or variants thereof comprise a stabilized phytase, a stabilized xylanase, a stabilized mannanase, or a combination thereof.

[0280] In some embodiments, the one or more stabilized feed enzymes are combined with the one or more substrates before feeding the feedstock to an animal, or they may be pre-combined.

INCORPORATION BY REFERENCE

[0281] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

DETAILED DESCRIPTION

[0282] The following descriptions illustrate embodiments of the present disclosure in detail. It is to be understood that this present disclosure is not limited to the particular embodiments described herein and as such can vary. Those of skill in the art will recognize that there are numerous variations and modifications of this present disclosure, which are encompassed within its scope.

[0283] Although various features of the present disclosure may be described in the context of a single embodiment, the features may also be provided separately or in any suitable combination. Conversely, although the present disclosure may be described herein in the context of separate embodiments for clarity, the present disclosure may also be implemented in a single embodiment.

[0284] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0285] All terms are intended to be understood as they would be understood by a person skilled in the art. Unless defined otherwise, all technical and scientific terms used herein have the same

meaning as commonly understood by one of ordinary skill in the art to which the disclosure pertains.

[0286] The following definitions supplement those in the art and are directed to the current application and are not to be imputed to any related or unrelated case, *e.g.*, to any commonly owned patent or application. Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the present disclosure, the preferred materials and methods are described herein. Accordingly, the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

I. Definitions

[0287] The term “encoding” refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (*e.g.*, rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene, cDNA, or RNA, encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

[0288] The term “endogenous” refers to any material from or produced inside an organism, cell, tissue or system.

[0289] The term “exogenous” refers to any material introduced from or produced outside an organism, cell, tissue or system.

[0290] The term “expression” refers to the transcription and/or translation of a particular nucleotide sequence driven by a promoter.

[0291] The term “homologous” or “identity” refers to the subunit sequence identity between two polymeric molecules, *e.g.*, between two nucleic acid molecules, such as, two DNA molecules or two RNA molecules, or between two polypeptide molecules. When a subunit position in both of the two molecules is occupied by the same monomeric subunit; *e.g.*, if a position in each of two DNA molecules is occupied by adenine, then they are homologous or identical at that position. The homology between two sequences is a direct function of the number of matching or homologous positions; *e.g.*, if half (*e.g.*, five positions in a polymer ten subunits in length) of the positions in two sequences are homologous, the two sequences are 50% homologous; if 90% of

the positions (e.g., 9 of 10), are matched or homologous, the two sequences are 90% homologous.

[0292] The term “isolated” means altered or removed from the natural state. For example, a nucleic acid or a peptide naturally present in a living animal is not “isolated,” but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is “isolated.” An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell.

[0293] In the context of the present invention, the following abbreviations for the commonly occurring nucleic acid bases are used. “A” refers to adenosine, “C” refers to cytosine, “G” refers to guanosine, “T” refers to thymidine, and “U” refers to uridine.

[0294] The term “operably linked” or “transcriptional control” refers to functional linkage between a regulatory sequence and a heterologous nucleic acid sequence resulting in expression of the latter. For example, a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Operably linked DNA sequences can be contiguous with each other and, e.g., where necessary to join two protein coding regions, are in the same reading frame.

[0295] The term “nucleic acid” or “polynucleotide” refers to deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., *Nucleic Acid Res.* 19:5081 (1991); Ohtsuka et al., *J. Biol. Chem.* 260:2605-2608 (1985); and Rossolini et al., *Mol. Cell. Probes* 8:91-98 (1994)).

[0296] The term “amino acid” as used herein refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, gamma-

carboxyglutamate, and O-phosphoserine. The term “amino acid analogs” as used herein refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an alpha carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. The term “amino acid mimetics” as used herein refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

[0297] The term “non-standard amino acid” refers to any amino acid other than the 20 standard amino acids (alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine). Selenocysteine is a non-standard amino acid (NSAA).

[0298] The terms “peptide,” “polypeptide,” and “protein” are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein’s or peptide’s sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. “Polypeptides” include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. A polypeptide includes a natural peptide, a recombinant peptide, or a combination thereof.

[0299] The term “promoter” refers to a DNA sequence recognized by the transcription machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a polynucleotide sequence.

[0300] The term “constitutive” promoter refers to a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell under most or all physiological conditions of the cell.

[0301] The term “inducible” promoter refers to a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product

to be produced in a cell substantially only when an inducer which corresponds to the promoter is present in the cell.

[0302] The term “transfected” or “transformed” or “transduced” refers to a process by which exogenous nucleic acid is transferred or introduced into the host cell. A “transfected” or “transformed” or “transduced” cell is one which has been transfected, transformed or transduced with exogenous nucleic acid. The cell includes the primary subject cell and its progeny.

[0303] Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. As another example, a range such as 95-99% identity, includes something with 95%, 96%, 97%, 98% or 99% identity, and includes subranges such as 96-99%, 96-98%, 96-97%.

[0304] The term “about” or “approximately” can mean within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, “about” can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, “about” can mean a range of up to 20%, up to 10%, up to 5%, or up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, within 5-fold, or within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated the term “about” meaning within an acceptable error range for the particular value should be assumed.

[0305] As used in this specification and claim(s), the words “comprising” (and any form of comprising, such as “comprise” and “comprises”), “having” (and any form of having, such as “have” and “has”), “including” (and any form of including, such as “includes” and “include”) or “containing” (and any form of containing, such as “contains” and “contain”) are inclusive or open-ended and do not exclude additional, unrecited elements or method steps. It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the present disclosure, and vice versa. Furthermore, compositions of the present disclosure can be used to achieve methods of the present disclosure.

[0306] Reference in the specification to “some embodiments,” “an embodiment,” “one embodiment” or “other embodiments” means that a particular feature, structure, or characteristic described in connection with the embodiments is included in at least some embodiments, but not necessarily all embodiments, of the present disclosures. To facilitate an understanding of the present disclosure, a number of terms and phrases are defined below.

[0307] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the claimed subject matter belongs. It is to be understood that the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of any subject matter claimed. In this application, the use of the singular includes the plural unless specifically stated otherwise.

II. Non-Standard Amino Acid Containing Polypeptides

[0308] The use of non-standard amino acids in proteins offers the possibility of polypeptides having greatly expanded functionality that could be exploited for wide range of applications. For example, by incorporation of selenocysteine into polypeptides it may be possible to develop enzymes having enhanced levels of stability or activity and to produce highly active polypeptides. However, these approaches have, to date, been hampered by the inability to produce organisms that stably retain translation pathways that predictable and reliably incorporate selenocysteine into encoded polypeptides. Studies detailed herein demonstrate a stable system for use of tRNA molecules that can incorporate selenocysteine and for production of polypeptides that incorporate selenocysteine positions. Importantly, this system can be easily moved from one organism to another without the need of re-engineering.

[0309] Over 100 NSAAs with diverse chemistries have been synthesized and co-translationally incorporated into proteins using evolved orthogonal aminoacyl-tRNA synthetase (aaRSs)/tRNA pairs. Non-standard amino acids have been designed based on tyrosine or pyrrolysine. An aaRS/tRNA may be provided on a plasmid or into the genome of the genomically recoded organism. An orthogonal aaRS/tRNA pair will be used to bioorthogonally incorporate NSAAs into proteins. Vector-based over-expression systems may be used to outcompete natural codon function with its reassigned function. If one completely abolishes natural UAG translation function, far lower aaRS/tRNA function may be sufficient to achieve efficient NSAA incorporation. Genomically recoded organism (GRO)-based NSAA incorporation can use either vector- and/or genome-based aaRS/tRNA pairs. Genome-based aaRS/tRNA pairs have been used to reduce the mis-incorporation of standard amino acids in the absence of available

NSAAs. Since the UAG codon function has been completely reassigned in the genomically recoded organism, NSAAs, such as selenocysteine, can be incorporated in the genomically recoded organism without any phenotypic consequences. NSAA incorporation in the genomically recoded organism may involve supplementing the growth media with the non-standard amino acid, such as selenocysteine, and an inducer for the aaRS. Alternatively, the aaRS may be expressed constitutively. Alternatively, as in the present disclosure, the endogenous seryl-tRNA synthetase may be used to seryl-ate selenocysteine tRNA, which tRNA is acted upon by enzymes comprising SslA to produce tRNA^{Sec} (selenocysteine charged tRNA). Media may be supplemented with a selenium source like sodium selenite to improve production of tRNA^{Sec}. The desired protein can be overexpressed using any desired protein overexpression system (e.g., T7-RNAP, constitutive incorporation, or inducible expression based on IPTG/allolactose, anhydrotetracycline, arabinose, rhamnose, or other inducible systems). The protein cross-link (diselenide bond) may form spontaneously based on proximity-based geometric catalysis during protein folding, and the protein can be handled as any other over-expressed product.

[0310] The inventors have developed polypeptides and methods to produce polypeptides in genomically recoded organisms (GRO) that fold into biologics that, for example, are stabilized by diselenide bonds between selenocysteine amino acids. Whereas disulfide bonds between cysteine amino acids have a redox potential of about -220 mV, diselenide bonds have a redox potential of about -380 mV. Since the bacterial cytosol typically has a redox potential of about -280 to -300 mV, diselenides but not disulfides avoid reduction so that they form and persist in the cytosol. Since diselenides have the same geometric bond angles and torsions as disulfides, as well as very similar bond lengths, they can be substituted into polypeptides without disrupting the three-dimensional structure of the polypeptide. Further, since intended in vivo environments like blood contain reducing agents like glutathione, albumin, and thioredoxin, disulfides in polypeptides can be reduced, causing the polypeptide to unfold and, in the case of multiple disulfides, “scramble” the disulfides so that incorrect cysteines are bonded to each other. Both of these result in abrogation of the intended biological activity of the polypeptide. The lower redox potential of diselenides renders them resistant to reduction when exposed to blood serum or purified reducing components of blood serum, endowing them with a longer blood serum half-life than disulfide-bearing counterparts.

[0311] While peptides bearing diselenide-forming selenocysteines may be produced in vitro by solid phase peptide synthesis, the process does not scale tractably to the yields necessary for animal feed applications. However, in vivo production of recombinant seleno-proteins is limited

by strict sequence requirements on where selenocysteine may appear in proteins. In particular, a selenocysteine insertion sequence (SECIS) element must appear in the coding DNA sequence at the selenocysteine incorporation site in order to recruit endogenous selenocysteine translation machinery, comprising a specialized elongation factor (SelB). Instead, a recoded strain of *E. coli* can be used, which has an unassigned codon, such as an amber stop codon, together with an engineered selenocysteine tRNA with an anti-amber anticodon that permits targeted placement of selenocysteine into polypeptides by introduction of the amber stop codon into the corresponding DNA coding sequence. The modified tRNA interacts with the endogenous elongation factor EF-Tu. Other codons can be recoded, typically rare codons, as is known in the art. A codon on an mRNA and an anti-codon on a tRNA are typically triplets of complementary base sequences.

[0312] Recoded proteins may be synthesized in bacteria, such as *E. coli* cells, or in vitro, in translation or linked transcription-translation systems. Genes or mRNA encoding such recoded proteins are non-naturally occurring, and are variants of naturally occurring coding sequences. Although many of the proteins that we show in the associated sequence listing have all cysteine residues which participate in disulfide bonds replaced with selenocysteine residues, all cysteine residues need not be replaced to gain the benefits of the substitution. Even one diselenide bond may improve the stability of a protein. Any number of diselenide bonds (selenocysteine pairs) may be substituted for disulfide bonds in the proteins. If a protein has N disulfide bonds, the protein may have anywhere from N, N minus 1, N minus 2, N minus 3, N minus 4, ...down to 1 such bond. It is also possible to form a bond between cysteine and selenocysteine residues called a selenylsulfide. This bond has a lower redox potential (~ -270 mv) than a disulfide (-220 mv) but not than bacterial cytoplasm (-280 mv). The selenylsulfide bond may be used to increase resistance to reduction in certain redox environments. Selenylsulfides may be used in place of diselenides using methods described here by substituting selenocysteine for a single disulfide bonded cysteine, or by substituting cysteine for a single diselenide bonded selenocysteine.

[0313] Further, one or more diselenide bonds or selenylsulfide bonds may replace one or more engineered disulfide bonds. Thus, in some embodiments, one or more selenocysteines may be at positions where amino acids at those positions are not cysteines. The positions of engineered disulfide are available, e.g., in Sanchez-Romero et al., (2013) Mechanism of Protein Kinetic Stabilization by Engineered Disulfide Crosslinks. PLoS ONE 8(7): e70013, which is hereby incorporated by reference in its entirety.

[0314] Sequences of disulfide-stabilized biologics with substituted selenocysteines can be produced in the cytosol of *E. coli* using our method at the mg/L scale in standard laboratory shaker flasks, and scaled to g/L production in microbial fermenters.

[0315] Enzymes with different combinations of diselenide bonds and disulfides include, but are not limited to, nucleases, polymerases, ligases, reverse transcriptases, restriction endonucleases, carbon fixing enzymes (e.g., carbon capturing enzymes), phytases, and carbohydrases. Any cysteine in an enzyme disclosed herein may be maintained as a selenocysteine so long as the presence of the selenocysteine does not interfere with the expression, folding, or intended function of the polypeptide. Methods are provided herein for producing and verifying the presence of selenocysteines participating in the intended diselenide bonds for various enzymes, including, but not limited to, nucleases, polymerases, ligases, reverse transcriptases, restriction endonucleases, carbon fixing enzymes (e.g., carbon capturing enzymes), phytases, and carbohydrases (e.g., xylanase and mannanase).

[0316] Stabilized enzymes may be made and used according to the invention with diselenide bonds between two selenocysteine residues. This technique and modification can be useful for producing enzymes that maintain activity even in harsh conditions such as reducing environments. Provided herein are stabilized enzymes containing non-standard amino acids that have enzymatic activity in harsh conditions, such as reducing buffers or lysis buffers, that is higher than a corresponding enzyme without the non-standard amino acids under the same conditions. The stabilized enzymes can comprise a stabilized phytase, xylanase, or mannanase polypeptide. Also provided herein are polynucleotides encoding these stabilized enzymes, cells for expressing and/or producing these stabilized enzymes, and methods of use of these stabilized enzymes.

[0317] Enzymes with different combinations of diselenide bonds and disulfides include, but are not limited to, nucleases, polymerases, ligases, reverse transcriptases, restriction endonucleases, and carbon fixing enzymes (e.g., carbon capturing enzymes), phytases, and carbohydrases (e.g., xylanase and mannanase).

Phytases

[0318] In some embodiments, an enzyme containing one or more catalytic cysteine residues (i.e. a cysteine involved in a catalysis reaction, e.g., an active site cysteine) may be made and used according to the invention with one or more selenocysteine residue substitutions for these one or more catalytic cysteine residues. The one or more selenocysteine substitutions can increase or alter the enzyme activity in the reaction environment.

[0319] In some embodiments, a phytase may be made and used according to the invention with diselenide bonds between two selenocysteine residues. For example, a phytase may have one, two, three, or four disulfide bonds. In some embodiments, a phytase enzyme comprises at least 2, 4, 6, or 8 selenocysteine residues. In some embodiments, a phytase enzyme comprises at least 1, 2, 3, or 4 diselenide bonds.

[0320] In some embodiments, a phytase can comprise one or more non-standard amino acids. In some embodiments, a phytase can comprise one or more selenocysteine residues. In some embodiments, a phytase can comprise a diselenide bond between two selenocysteine residues. The diselenide bonds may be intramolecular or intermolecular. In some embodiments, a phytase can comprise one or more diselenide bonds. In some embodiments, a phytase comprising one or more non-standard amino acids has enzymatic activity in harsh conditions, such as reducing buffers or the digestive tract of an animal, that is higher than a corresponding phytase without the non-standard amino acids under the same conditions.

[0321] For example, a phytase provided herein comprising one or more non-standard amino acids, such as one or more selenocysteine residues, can cleave a bond of its substrate (e.g., a phytate or phytic acid) with a hydrolytic activity that is at least 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 times higher than a corresponding phytase without the one or more non-standard amino acids.

[0322] For example, a phytase provided herein comprising one or more non-standard amino acids, such as one or more selenocysteine residues, can cleave a bond of its substrate (e.g., a phytate) in an environment with a hydrolytic activity that is at least 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 times higher than a corresponding phytase without the one or more non-standard amino acids in the same environment.

[0323] For example, a phytase provided herein comprising one or more non-standard amino acids, such as one or more selenocysteine residues, can cleave a bond of its substrate (e.g., a phytate) in an environment comprising a detergent, a reducing reagent, and/or a reducing enzyme (e.g., a reductase) with a hydrolytic activity that is at least 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 times higher than a corresponding phytase

without the one or more non-standard amino acids in the same environment comprising the detergent, the reducing reagent, and/or the reducing enzyme (e.g., a reductase).

[0324] For example, a phytase provided herein comprising one or more non-standard amino acids, such as one or more selenocysteine residues, can cleave a bond of its substrate (e.g., a phytate) in an environment with a redox potential of less than about -150 mV, with a hydrolytic activity that is at least 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 times higher than a corresponding phytase without the one or more non-standard amino acids in an environment with the same redox potential. For example, a phytase provided herein comprising one or more non-standard amino acids, such as one or more selenocysteine residues, can cleave a bond of its substrate (e.g., a phytate) in an environment with a redox potential of less than about -160 mV, less than about -170 mV, less than about -180 mV, less than about -190 mV, less than about -200 mV, less than about -210 mV, less than about -220 mV, less than about -230 mV, less than about -240 mV, or less than about -250 mV, less than about -260 mV, less than about -270 mV, less than about -280 mV, less than about -290 mV, less than about -300 mV, less than about -310 mV, less than about -320 mV, less than about -330 mV, less than about -340 mV, or less than about -350 mV, less than about -360 mV, less than about -370 mV, less than about -380 mV, less than about -390 mV, less than about -400 mV, less than about -410 mV, less than about -420 mV, less than about -430 mV, less than about -440 mV, or less than about -450 mV, less than about -460 mV, less than about -470 mV, less than about -480 mV, less than about -490 mV, less than about -500 mV, less than about -510 mV, less than about -520 mV, less than about -530 mV, less than about -540 mV, or less than about -550 mV, less than about -560 mV, less than about -570 mV, less than about -580 mV, less than about -590 mV, or less than about -600 mV, with a hydrolytic activity that is at least 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 times higher than a corresponding phytase without the one or more non-standard amino acids in an environment with the same redox potential.

[0325] In some aspects, provided herein is a composition comprising a stabilized phytase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof. In some embodiments, the stabilized phytase polypeptide may be made and used according to the invention with diselenide bonds between two selenocysteine residues. In

some embodiments, the stabilized phytase polypeptide can comprise one or more non-standard amino acids. In some embodiments, the stabilized phytase polypeptide can comprise one or more selenocysteine residues. In some embodiments, the stabilized phytase polypeptide can comprise a diselenide bond between two selenocysteine residues. The diselenide bonds may be intramolecular or intermolecular. In some embodiments, the stabilized phytase polypeptide can comprise one or more diselenide bonds. In some embodiments, the stabilized phytase polypeptide can comprise one or more catalytic selenocysteine substitutions.

[0326] In some aspects, provided herein is a composition comprising a stabilized phytase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized phytase polypeptide has a higher hydrolytic activity for a phytate substrate in an environment than a hydrolytic activity for the phytate substrate of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids. For example, the stabilized phytase polypeptide can have at least 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 or greater fold higher hydrolytic activity for a phytate substrate in an environment than a hydrolytic activity for the phytate substrate of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0327] In some aspects, provided herein is a composition comprising a stabilized phytase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized phytase polypeptide does not destabilize in an environment that a corresponding phytases polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids does destabilize. The destabilization can be obtained by contacting the corresponding phytase polypeptide with one or more destabilization agents. The destabilization can be obtained by placing the corresponding phytase polypeptide in a destabilization environment. The environment to destabilize the corresponding phytase polypeptide is described elsewhere herein. In some embodiments, the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, has a melting temperature (T_m) that is at least 5°C higher than a T_m of a corresponding recombinant enzyme, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0328] In some aspects, provided herein is a composition comprising a stabilized phytase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized phytase polypeptide has a melting temperature (T_m) that is at least 5°C higher than a T_m of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0329] In some embodiments, the composition can comprise a stabilized phytase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized phytase polypeptide can have a melting temperature (T_m) that can be at least 1°C, 2°C, 3°C, 4°C, 5°C, 6°C, 7°C, 8°C, 9°C, 10°C, 11°C, 12°C, 13°C, 14°C, 15°C, 16°C, 17°C, 18°C, 19°C, 20°C, 21°C, 22°C, 23°C, 24°C, 25°C, 26°C, 27°C, 28°C, 29°C, 30°C, 31°C, 32°C, 33°C, 34°C, 35°C or 36°C higher than a T_m of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids. In some embodiments, the composition can comprise a stabilized phytase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized phytase polypeptide can have a melting temperature (T_m) that can be less than 1°C higher than a T_m of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0330] In some embodiments, at least one, two, three, four or more of the one or more non-standard amino acids is selenocysteine. In some embodiments, at least two of the one or more non-standard amino acids are directly linked by a bond.

[0331] In some embodiments, at least four of the one or more non-standard amino acids can be directly linked by a bond, wherein a first pair of the at least four of the one or more non-standard amino acids can be directly linked by a bond, and a second pair of the at least four of the one or more non-standard amino acids can be directly linked by a bond. In some embodiments, the bond is a diselenide bond or selenyl-sulfhydryl bond. In some embodiments, the bond is a diselenide bond. In some embodiments, the diselenide bond can be an intermolecular or an intramolecular bond. In some embodiments, the bond is a selenyl-sulfhydryl bond between a cysteine and a selenocysteine. In some embodiments, the selenyl-sulfhydryl bond can be an intermolecular or an intramolecular bond.

[0332] In some embodiments, the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, can have a half-life that can be at least a 1.1 fold higher than a half-life of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids. In some embodiments, the stabilized

phytase polypeptide, functional fragment thereof, or variant thereof, can have a half-life that can be at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 or greater fold higher than a half-life of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids. In some embodiments, the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, can have a half-life that can be less than 1.1 fold higher than a half-life of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0333] In some embodiments, the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, can have at least 70%, 75%, 80%, 85%, 90%, 95% sequence identity to a sequence from Table 1. In some embodiments, the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, can have at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% or greater sequence identity to a sequence from Table 1. In some embodiments, the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, can have less than 10% sequence identity to a sequence from Table 1.

Table 1. Phytase Sequences

SEQ ID	Accession ID in NCBI Protein Database	Sequence
1	Q2VY22	MSTFIIRLLIFSLLCGSFSIHAEQNGMKLERVVIVSRHGVRAPTKFTPIMKDVTPDQWPQWDVP LGWLTTPRGGELVSELGQYQRLWFTSKGLLNNQTCPSPGQVAVIADTDQRTRKTGEAFLAGLAP KCQIQVHYQKDEEKNDPLFNPVKMGKCSFNTLKVKNAILERAGGNIELYTQRYQSSFRTLNV LNFSQSETCKTTEKSTKCTLPEALPSEFKVTPDNVSLPGAWSLSSTLTEIFLLQEAQGMPPQVAW GRITGEKEWRDLLSLHNAQFDLLQRTPEVARSRATPLLDIMDTALLTNGTTENRYGIKLPVSL FIAGHDTNLANLSGALDLKWSLPGQPDNTPPGGELVFEKWKRTSDNTDWVQVSFVYQTLRDM RDIQPLSLEKPAGKVLDKLIACEEKNSQGMCSLKSFSRLIKEIRVPECAVTE
2	P07102	MKAILIPFLSLLIPLTPQSAFAQSEPELKLESVVIVSRHGVRAPTKATQLMQDVTPDAWPTWPVK LGWLTTPRGGELIAYLGHYQRQRLVADGLLAKKGCPQSGQVAIIADVDERTRKTGEAFAAGLA PDCAITVHTQADTSSPDPLFNPLKTGVCQLDNANVTDAILSRAGGSIAFTGHRQTAFRELERV LNFPQSNLCLKREKQDESCSLTQALPSELKVSADNVSLTGAVSLASMLTEIFLLQQAQGMPEPG WGRITDSHQWNTLLSLHNAQFYLLQRTPEVARSRATPLLDLIKALTTPHPQKQAYGVTLPSTV LFIAGHDTNLANLGGALELNWTLPGQPDNTPPGGELVFERWRRLSDNSQWIVQVSLVFQTLQQ

		MRDKTPLSLNTPPGEVKLTLAGCEERNAQGMCSLAGFTQIVNEARIPACSL
3	40549124	MKAILIPFLSLLIPLTPQSAFAQSEPELKLESVVIVSRHGVRAPTKATQLMQDVTPDAWPTWPK LGWLTPRGELIAYLGHYQRQRLVADGLLAKKGCPQPGQVAIIADVDERTRKTGEAFAAGLA PDCAITVHTQADTSSPDPLFNPLKTGVCQLDNANVTDAILSRAGGSIADFTGHRQTAFRELERV LNFSQLNLCLNREKQDESCSLTQALPSELKVSADNVSLTGAVSLASMLTEIFLLQQAQGMPEPG WGRITDSHQWNTLLSLHNAQFYLLQRTPEVARSRATPLLDLIMAALTPHPPQKQAYGVTLP TSVLFIAGHDTNLANLGGALELNWTLPGQPDNTPPGELVFERWRRLSDNSQWIVSLVFQTLQQ MRDKTPLSLNTPPGEVKLTLAGCEERNAQGMCSLAGFTQIVNEARIPACSL
4	304097	MPRTSLLTLACALATGASAFSYGAAIPQSTQEKQFSQEFRDGYSLKHYGGNGPYSERVSYGIA RDPPTGCEVDQVIMVKRHGERYSPSAGKSIEEALAKVYSINTTEYKGDLAFLNDWTYYVPNE CYYNAETTSGPYAGLLDAYNHGNDYKARYGHLWNGETVVPFFSSGYGRVIETARKFGEGFFG YNYSTNAALNIISESEVMGADSLTPTCDTDNDQTTCDNLTYQLPQFKVAAARLNSQNPGMNL TASDVYNLIVMASFELNARFNSWINAFTQDEWVSFGYVEDLNYYYYCAGPGDKNMAAVGAVY ANASLTLLNQGPKEAGPLFFNF AHD TNITPIL AALGVLIPNEDLPLDRVAFGNPYSIGNIVPMGG HLTIERLSCQATALSDKGTIVRLVLNEAVLPFNDCTSGPGYSCPLANYTSILNKNLPDYTTTCN VSASYPQYLSFWWNYNNTTTELNYRSSPIACQEGDAMD
5	Q96VH9	MVSSAFAPSILLSLMSLALSTQFSFVAAQLPIPAQNTSNWGPYDPFFPVEPYAAPPEGCTVTQV NLIQRHGARWPTSGARSRQVA AVAKIQMARPFTDPKYEFLNDFVYKFGVADLLPFGANQSHQ TGTDMYTRYSTLFEGGDVPFVRAAGDQRRVDSSTNWTAGFGDASGETVLPTLQVVLQEEGNC TLCNNMCPNEVDGDESTTWLGVFAPNITARLNAAAPSANLSDSDALTLMDMCPFDLSSGNAS PFCDLFTAEEYVSYEYYYDLKYYGTGPGNALGPVQGVGYVNELLARLTGQAVRDETQTNRT LDSDPATFPLNRTFYADFSHDNTMVPIFAALGLFNATALDPLKPDENRLWVDSKLVFSGHMT VEKLACSGKEAVRVLVND AVQPLEFCGGVDGVCELSAFVESQTYARENGQGDFAKCGFVPSE
6	P34752	MGVSAVLLPLYLLSGVTSGLAVPASRNQSSCDTVDQGYQCFSETSHLWGQYAPFFSLANESVIS PEVPAGCRVTF AQVLSRHGARYPTDSKGKKYKYSALIEEIQQNATTFDGKYAFLKTYNYSLGADD LTPFGEQELVNSGIKFYQRYESLTRNIVPFIRSSGSSRVIASGKKFIEGFQSTKLKDPRAQPGQSSP KIDVVISSEASSNNTLDPGTCTVFEDSELADTVEANFTATFVPSIRQRLNDLSGVTLTDTEVTY LMDMCSFDTISTSTVDTKLSPFCDLFTHDEWYNDYLSLKKYYGHGAGNPLGPTQGVGYANE LIARLTHSPVHDDTSSNHTLDSSPATFPLNSTLYADFSHDNGIISILFALGLYNGTKPLSTTTVENI TQTDGFSSAWTVPFASRLYVEMMQCAEQEPLVRVLVNDRVVPLHGPCVDALGRCTRDSFVR GLSFARSGGDWAECEFA
7	37605066	MLKLYVAACL VVAGVSIPTDPTVTQVPDYFQTSYGPYAGATKAGGAPFLAQTNPISQPTYVA NTPLVTTLPISGEPHDGNIFGWMGTLSPYQPSPDGFGVDEYPLPPGANITQIHMVHRHGSRYPTS

		NSAISDWAKKIMQYRSNGTVFSGELEFLNAWNYQLGQAELTARGRQELFDSGILHWFNYGKL YDPASKIIARTTTMVRMLQSAENFLNGFFGPNWNTNATLEVIIESTGFNNSLAGNDMCRNAKN TSGGDAVNEWALYLQKATNRFRSEISGSLNWTVDDTYNAQSMCPYETVALGYSPFCTLFSW EEWQGFQYVNDLNLGYNYGMGSPVGRAIGLGFVEELIARLQGQIPNPEDSIGFNQSLDDSAAT FPLNQTIYFDFSHDNEMFSMLTALGLTQFGDYLSPTKPSADRSIGSHIVPFSATFVFEIHKAPGL VRENRSKYCGESVYENTSEETTYIHLVINQRTVPLGQSSISACGQRDDGWCEISAFIQAQKENIVK ANYEESCFGNWSIPAYGEIRDGAIPKNATS
8	255940650	MSLLGTFALVVYFAKGTQCNPPPRATTQPDLPSTPVNMSKRSDCTTVDDGGYQCNSLSHKW GQYSPYFSLSEESSISNEVPHDCQITFAQVISRHGARFPSAKKSKVYAKLIENIQANATAYNGNT KFLRSYKTYMGGDDLVPFGVNQMVDSGTKFYQRYEALAKKAVPFIRSSDSGRVVASGVNFIK GFQQAKLDDKNANHRQPSPKTNVIIEESGTNNLNLHSEICPKFEDNELGDKVEEKYMKIFVPPI RARLEADLPGVKLEDIDVVSMDICPFETVSSSDAAELSPFCDLFTPTESQYDYLQSLSKYY GYGAGNPLGPTQGVGFVNELIARLTRHPVRDHTSTNRALDAPGAATFPLNYTMYADFTHDNG MIPFFFALGLYNGTAPLSLTHVQSPSQTDGFSSAWTVPFGARAYVEMMQCRREPEPLVRVLVN DRVIPLHGCPVDKLGRCRRRDFVKGLTFARSGGDWARCYK
9	B2KSJ7	MTISAFERQKLTLRNGLLIAIGTACSLGSTVYASETPASGYQTEKVVILSRHGVRAPTKMTQTM RDVTPNTWPEWPVKLGYITPRGEQLIRVMGGFYREKFQQQGILSQENCPAPNSVYVWADVDQ RTLKTGEAFLAGLAPQCGLTIHHQQNLEKADPLFHPVKAGICSMKQIQQAQVEKEAQNPIDN LNQHYLPSLALMNTTLNFANSAWCQKHSVDKSCDLGQSMPSKLSIQENGKITLDGAIGLSS LAEIFLLEYAQGMPKAAWGNHSEQEWAALLKLHNTQFDLMARTPYIARHNGTPLLQAISNAL DPNATASKLPDISPDNKILFIAGHDTNIANISGMLNISWTLPGQPDNTPPGGALVFERLADKSGK QYVSVSMVYQTLEQLHAQTPLSLKEPAGSVQLKIPGCSQDQTAEGYCPLPTFTRMVSQSVEPGC QLQ
10	A0A1B7HL 36	MTISAFKRQKLTLRNGLLVAIGTACSLSSTVYASETPASGYQVEKVVILSRHGVRAPTKMTQT MRDVTPTNTWPEWPVKLGYITPRGEHLIKLMGGFYREKFQQQGILSQENCPAPNSVYVWADVD QRTLKTGEAFLAGLAPQCGLTIHHQQNLEKADPLFHPVKAGICSMKQIQQAQVEKEAQTPID NLNQHLYLPSLALMNTTLNFANSAWCQKHSVDKSCDLGQSMPSKLSIQENGKITLDGAIGLSS TLAEIFLLEYAQGMPKAAWGNHSEQEWAALLKLHNTQFDLMARTPYIARHNGTPLLQAISNA LDPNATASKLPDISPDNKILFIAGHDTNIANISGMLNISWTLPGQPDNTPPGGALVFERLADKAG KQYVSVSMVYQTLEQLHAQTPLSLKEPAGSVQLKIPGCSQDQTAEGYCPLPTFTRVVSQSVEPGC QLQ
11	A0A1B7I2D 3	MTIPAFKRQKLTLHPGLFVALSAIFSLGSTAYANDTPASGYQVEKVVILSRHGVRAPTKMTQT MRDVTPTNTWPEWPVKLGYITPRGEHLISLMGGFYREKFQQQGILSQENCPTNSVYVWADVD QRTLKTGEAFLAGLAPQCGLTIHHQQNLEKADPLFHPVKAGTCSMDKTQIQQAQVEKEAQPIE

		NLNQRYIPSLALMNRALNFSTSAWCQKHSADKSCDLAQSMPSKLSIKDNGNKVALDGAIGLSS TLAEIFLLEYAQGMPQAAWGNHSEQEWASLLKLHNTQFDLMARTPYIAAHNGTPLLQTISNA LEPKADVSKLPDISPNNKILFIAGHDTNIANIAGMLNMRWTLPGQPDNTPPGGALVFERLADKS GKQYVSVSMVYQTLVQLRAQTPLSLKEPAGSVQLKIPGCSDQTAEGYCPLPTFKRVVSQSEEP GCQLQ
12	A0A1B7IJE 9	MTISAFNHKKLTLHPGLFVALSAIFSLGSTAYANDTPASGYQVEKVVLRSRHGVRAPTGMTQT MRDVTPTNTWPEWPVKLGYYITPRGEHLISLMGGFYREKFQQQGILSQGSCPTNSIYVWADVQ RTLKTGEAFLAGLAPQCGLTIHHQQNLEKADPLFHPVKAGTCSMDKTQIQQAVEKEAQTPIEN LNQHYIPSLALMNTALNFSTSAWCQKHSADKSCDLAQSMPSKLSIKDNGNKVALDGAIGLSS LAEIFLLEYAQGMPQAAWGNHSEQEWASLLKLHNAQFDLMARTPYIATHNGTPLLQTISHAL EPKADVSKLPDISPDNKILFIAGHDTNIANIAGMLNMRWTLPGQPDNTPPGGALVFERLADKSG KQYVSVSMVYQTLEQLRSQTPLSLNQPAGSVQLKIPGCNDQTAEGYCPLSTFTRVVSQSVEPG CQLQ
13	A0A1B7IID 8	MTISAFNRKKLTLHPGLFVALSAIFSLGSTAYANDTPASGYQVEKVVLRSRHGVRAPTGMTQTI RDVTPTNTWPEWPVKLGYYITPRGEHLISLMGGFYREKFQQQGILSQGSCPTNSIYVWADVQ RTLKTGEAFLAGLAPQCGLTIHHQQNLEKADPLFHPVKAGTCSMDKTQVQQAVEKEAQTPIEN NQHYIPSLALMNTTALNFSTSAWCQKHSADKSCDLAQSMPSKLSIKDNGNKVALDGAIGLSS AEIFLLEYAQGMPQAAWGNHSEQEWASLLKLHNAQFDLMARTPYIAAHNGTPLLQTISNALE PKADVSKLPGISPDKILFLAGHDTNIANIAGMLNMRWTLPGQPDNTPPGGALVFERLADKSG KQYVSVSMVYQTLEQLRSQTPLSLNQPAGSVQLKIPGCNDQTAEGYCPLSTFTRVVSQSVEPG CQLQ
14	Q000T0	MTITVDSLRLSVLTLILNSYAIAPVAIQPTGYTLERVVILSRHGVRSPKQTQLMNDVTPDTW PQWPVAAGYLTPRGAQLVTLMGGFYGDYFRSQGLLAAGCPTDAVIYAQADVDQRTLTGQA FLDGIAPGCGLVHYQADLKKVDPLFHPVDAGVCKLDSTQTHKAVERLGGPLSELSKRYAKP FAQMGEILNFAASPYCKSLQQGKTCDFANFAANKITVKNPGTKVSLSGPLALSSTLGEIFLLQ NSQAMPDVAWHRLTGEDNWISLLSLHNAQFDLMAKTPYIARHKGTPLLQQIETALVLQRDAQ GQTLPLSPQTKILFLGGHDTNIANIAGMLGANWQLPQQPDNTPPGGGLVFELWQNPNDNHQRYV AVKMFYQTMGQLRNAEKLDLKNNPAGRVPVAIDGCENSGDDKLCQLDTFQKKVAQAIEPAC HI
15	B4X9S4	MTVASYRLRLPALALMLSSFALGAAPVITAPAGYTLERVVILSRHGVRSPKQTQLMNEVTPD KWPQWPVKAGYLTPRGAQLVTLLGAFYGEYFRSQGLLPAGCPPEGTVYAQADIDQRTLTGQ AFLDGVAPGCGLEVHYQADLKKTDPLFHPVEAGVCKVDLAQTRQAVEQRLGGPLTTLNQRYA KPFAQMGEVLNFAESPFCKSLQQGKTCDFATFAANEIDVNKDGTKISLTGPLALSSTLAEIFLL QNSQAMPDVAWHRLSGAENWVSLSLHNAQFDLMAKTPYIARHKGTPLLQQINTALVLQRD

		AQGQTLPLSPQTKVLFLGGHDTNIANIAGMLGANWQLPQQPDNTPPGGGLVFELWQHPDNHQ RYVAVKMFYQTMQDLRNVEKLNLTNNPAGIPIAVEGCENMGDDKLCQLETFEKKIAQVIEPA CHI
16	A0A0U1QT W3	MSVLENRVRLSGLVLMLSGLAAITAPVAAEPSGYTLERVVILSRHGVRSPKQTQLMNDVTPD KWPQWPVKAGYLTTPRGAEVLTMGGFYGDYFRSLGLLAAGCPAEGVVYAQADIDQRTTLTG QAFLDGVAPGCGLTVHNQADLKKTDPLFHPVEAGVCKLDAAQTDKAIEEQLGGLDTSVQRY AKPFAQMMDVNLFAASPYCKSLQQGKTCDFAHFAANEVNVNKEGTKVTLSGPLALSSTLGEI FLLQNAQAMPEVAWQRLKGAENWVSLLSLHNAQFNLMKTPYIARHKGTPLLQQIDTALTQ LDAQQGKLPIAQNRLVFLGGHDTNIANIAGMLGADWQLPEQPDNTPPGGGLVFELWQNPND HQRVAVKMFYQTMQDLRNAEKLDLKNNPAGIISVAVAGCENNGDDKLCQLDTFQKKVAKV IEPACHI
17	A0A2G4U4 U3	MTIAINRMRLSTMALMLASFAINAAPIAAPVTGYTLERVVILSRHGVRSPKQTQLMNDVTPDK WPQWPVQAGYLTTPRGAQLVTLTMGGFYGDYFRSQGLLPAGCPADGAIYAQADVDQRTTLTGQ AFLDGIAPGCGLKVHYQADLKKVDPLFHPVEAGVCKLDSAQSQAIEARLGGPLSELSQRYAK PFAQMGEILNFAASPYCNSLQQGKTCDFATFAANEVKVNKQGTKVSLSGPLALSSTLGEIFLL QNSQGMVDVAWNRLSGAENWVSLLSLHNAQFDLMKTPYIARHKGTPLLQQIDTALVLQRD GQGQTLPLSAQTKLLFLGGHDTNIANVAGMLGANWQLPQQPDNTPPGGGLVFELWQNPNDH QQYVAVKMFYQTMQDLRNSEKLDLKIHPAGIVAIEIAGCENNGADKLCQLDTFQKRVAQIIEP ACHI
18	C4SNF9	MTVAKNRLRLVALTLILSSFTISATPVVAQPGGYTLERVVILSRHGVRSPKQTQLMNDVTPDK WPQWPVKAGYLTTPRGAQLVTLTMGGFYGDYFRSQGLLPAGCPAEGEIIYAQADIDQRTTLTGQA FLDGVAPTCGLKVHYQADLKKIDPLFHPVEAGICKDLAQTRKAVEERLGGPLSTLSQRYAKS FAQMSEVLNFAASPYCQSLQQNGKTCDFATFATNEIEMNNEGTKVSLNGPLALSSTLGEVFL QNSQAMPDVAVHRLSGIENWFSLLSLHNQVFDLMKTPYIARSKGTALLQQIDTALVLHRDG QGQKLPLSPQTKVLFLGGHDSNIANIAGMLGANWQLPQQPDNTPPGGGLVFELWQNPKNHQ RYVAVKMFYQTMQDLRNADKLDLKNNPAGIVSVAIDGCENNGDNKLCQLDTFQKKVAQVIEP ACHI
19	C4S833	MRLTALGLMLGSFAISAAPVAAAPVTGYTLERVVILSRHGVRSPKQTELMNDVTPDKWPQWP VPAGYLTTPRGAQLVTLTMGGFYGDYFRNQGLLPAGCPADGTLYAQADIDQRTTLTGQAFLDGI APGCGLKVHYQADLKKVDPLFHPVEAGVCQLDSTQTHRAIEAQLGAPLSELSQRYAKPFAQM GEILNFTASPYCKSLQQGKSCDFATFAANEVKVNQQGTKVSLSGPLALSSTLGEIFLLQNSQG MPDVAVHRLSGAENWVSLLSLHNAQFDLMKTPYIARHKGTPLLQQIVTALVLQRKGQGQTL PLSEQTKLLFLGGHDTNIANIGGMLGANWQLPQQPDNTPPGGGLVFELWQNPNDHQQYVAVK MFYQTMQDLRNSEKLDLKSHPAGIVPIEIEGCENIGTDKLCQLDTFQKRVAQVIEPACHI

20	A0A2R4NN M2	MARAIKNLRLMALLMLGSFASHAAPIVSPPTGYTLERVVILSRHGVRSPKQTQLMNDVTPD KWPQWPVQAGYLTPRGAQLVALMGAFYGDYFRHRGLIPMGCPPSGAIYAQADVDQRTLTG EAFLEGIAPDCGLKMHYQADLKRIDPLFHPVDAGVCKLDDVQTHQAIEARLGGPLSELSQRYA KPFAQMGEILNFAGSPYCKLLQQGKTCDFATFAANKVEVNQAGTKVSLSGPLALSSTLGEIFL LQNSQGMSDVAWHRLTGEENWRSLLSLHNAQFDLMAKTPYIARHKGTPLLQQIDTALVLERK GQGQTLPLSEQTKILFLGGHDTNIANIGGMLGADWQLPQQPDNTPPGGGLVFELWQDPMNHQ RYVAVKMFYQTMQDLRNSEKLDLNNNPAGVVPIEIDGCENIGVNKLCQLATFQNKVAKAIEP ACHI
21	D9D7K9	MSVAKRNLHLSALTLIMGCFTAGAAPIATPPASYTLERVVILSRHGVRSPKQTQLMNDVTPDK WPLWPVKAGYLTPRRAELVTLMGGFYGDYFRSQGLLSAGCPVDGSVYAQADVDQRTLTGQ AFLDGIAPDCGLKVHYQADLKKVDPLFHTVEAGVCKLDSAKTHQAVEERLGGPLSDLSQRYA KPFAQMGEVLNFAASPYCKSLQKNGKTCDFATFTANEIKVNEEGTKVSLSGPLALSSTLGEIFL LQDSQAMPDVAWHRLSGEENWVSLSLHNAQFDLMAKTPYIARHKGTPLLQQIDTALVLRD AQGQTLPLSPQTKLLFLGGHDTNIANIAGMLGANWQLPQQPDNTPPGGGLVFELWQNPNDHQ RYVAVKMFYQTMQDLRNAEKLDKNNPAKIVPITIEGCENEGDNKLCQLETFQKKVAQVIEP ACHI
22	B6RGT1	MTIAKEYLRLSILTLVSSFTLSAAPLAAQSTGYTLERVVILSRHGVRSPKQTQLMNDVTPDK WPQWPVKAGYLTPRGAGLVTLMGFFYGDYFRSYGLLPAGCPADESIYVQADVDQRTLTGQ AFLDGIAPDCGLKVHYQADLKKIDPLFHTVEAGVCKLDPEKTHQAVEKRLGGPLNELSQRYAK PFALMGEVLNFSASPYCNSLQQGKTCDFATFAANEIEVNKEGTKVSLSGPLALSSTLGEIFLLQ NSQAMPDVAWNRLSGEENWISLLSLHNAQFDLMAKTPYIARHKGTPLLQQIDTALVLRDAQ GQTLPLSPQTKLLFLGGHDTNIANIAGMLGANWQLPQQPDNTPPGGGLVFELWQNPNDHQRY VAVKMFYQTMQDLRNADKLDLKNNPARIPIAIEGCENEGDNKLCQLETFQKKVAQVIEPACH I
23	Q7CIZ7	MSVLENRVRLSGLVLMGLAAITAPVAAEPSGYTLERVVILSRHGVRSPKQTQLMNDVTPD KWPQWPVKAGYLTPRGAELVTLMGGFYGDYFRSLGLLAAGCPAEGGVYAQADIDQRTLTG QAFLDGVAPGCGLTVHNQADLKKTDPLFHPVEAGVCKLDAAQTDKAIEEQLGGPLDTVSQRY AKPFAQMGDVLNFAASPYCKSLQQGKTCDFAHFAANEVNVNKEGTKVTLSGPLALSSTLGEI FLLQNAQAMPEVAWQRLKGAENWVSLSLHNAQFNLMAKTPYIARHKGTPLLQQIDTALTQ LDAQGQKLPIAQNRVFLGGHDTNIANIAGMLGADWQLPEQPDNTPPGGGLVFELWQNPND HQRYVAVKMFYQTMQDLRNAEKLDLKNNPAGIISVAVAGCENNGDDKLCELDFTQKKVAKV IEPACHI
24	Q003Y3	MNTLLFRLIMFIFMFGSFPLQAEVPDDMKLERVVIVSRHGVRAPTKFTPLMQEITPYYPQWD VPLGWL TARGGELVTEMGRYQQKVLIDNGVLESNVCPSPEQVAVIADTDQRTKTGEAFLAG

		FAPGCKNKVHYQKDHDKKDPLFNPVKMGVCAFNVQKTQEAILTRAEGNIERYTQRYDSAFRT LEQVLNFSRSAACRSASQSGCTLPGLPSELRVSA DTVSLSGAWSLSSMLTEIFLLQEAQGMPE VAWGRIHGEKEWTALLSLHNAQFDLLQRTPEVARSRATPLLDLISEALVSNGSTENHYGIKLPV SLLFIAGHDTNLANLSGVFDLNLWSLPGQPDNTPPGGELVFERWTRVSDNTDWIQISFVFQTLQQ MRKFKPFSSSSLPNKIVLTLPSCQDKNPEGMCPLKHFIDIVQTARIPQCAVMADVNR
25	A0A1C6YW 96	MTISLFTHCPTRLLKRIPLALIAASILTTTSYASETVPSTGYQLEKVVILSRHGVRAPTKMTQTM DVTDAWPEWPVKLG YITPRGEHLVSLMGGFYRQTFQQLGILSKDRCPTANDVYVWADVDQ RTRKTGEAFLAGLAPECHLSIHHQQDIKQADPLFHPVKAGVCSMEKTQVQQAVEQQAGMPIA QLNQHYRPALALMSRVLNFPKSAYCQQHSADQTCDFQAQAMPSKLSIKDDGNKVALDGAVGLS STLAEIFLLEHAQGM PNAAWGKIHSEQDWNALLALHNAQFDLMSRTPYIAKHNGTPLLQTIVS AINSQTGTRELPELSADNKILFLAGHDTNIANIAGMLGLSWTLPQPDNTPPGGALVFERWSDK AGKKYVSVQMMYQTLAQLRNQTPLTLDEPAGSVALKIPGCDDQTAQGYCPLDTFTRLAKQNE LAECQ
26	A0A085GU 77	MTISLFAHSPTRLLKRMPLAFIAASMLTTASYASETEPSGYQLEKVVILSRHGVRAPTKMTQTM RDVTPNAWPEWPVNLGYITPRGEHLVSLMGGFYRQKFQQLGILSKDRCPTANDVYVWADVD QRTRKTGEAFLAGLAPECHLSIHHQQDLKQADPLFHPVKAGVCTMEKTQVQQAVEQQAGMPI AQLNQHYRPALALMSSVLNFPKSTY CQQHSADQTCDFQAQAMPSKLSIKDDGNKVALDGAVGL SSTLAEIFLLEHAQGM PNAAWGKIHSEQDWITLLSLHNAQFDLMSRTSYVAKHNGTPLLQTIVS AINSQTSARDLPELSADNKILFLAGHDTNIANIAGMLGMSWTLPQPDNTPPGGALVFERWSD NAGKKYVSVQMMYQTLAQLRNQTPLTLDPAGSVALKIPGCDDQTAEGYCPLDTFTRLAKQN ELVECQ
27	H9TUK5	MTISLFNRNKPAIAQRILCPLIVALFSGLPAYASDTAPAGFQLEKVVILSRHGVRAPTKMTQTM DVTPHQWPEWPVKLG YITPRGEHLISLMGGFYRERFQQGLLPKDNCPTDAVYVWADVDQR TRKTGEAFLAGLAPQCDLAIHHQQNTQQADPLFHPVKAGICSMDKSQVHAAVEKQAGTPIETL NQRYQASLALMSSVLDFPKSPYCQQH NIGKLCDFSQAMPSRLAINDDGNKVALEGAVGLASTL AEIFLLEHAQGM PKVAWGNIHTEQQWNSLLKLHNAQFDLMSRTPYIAKHNGTPLLQTIAHALG SNITSRPLDISPDNKILFIAGHDTNIANISGMLGMTWTLPQPDNTPPGGALVFERWVDNAGKP YVSVNMVYQTLAQLHDQAPLTLQHPAGSVRLNIPGSDQTPDGYCPLSTFSRLVSHSVEPACQ LP
28	A0A2A2MH T5	MTISLFNRNKPAIAQRILCPLIVALFSSLPAYASDTAPSGFQLEKVVILSRHGVRAPTKMTQTM DVTPHQWPEWPVKLG YITPRGEHLISLMGGFYRERLQQGLLSKDNCPTDAVYVWADVDQR TRKTGEAFLAGLAPQCDLAIHHQQNIQADPLFHPVKAGVCSMDKSQVRTAVEKQAGAPIETL NQRYQASLALMSSVLDFPKSLYCQQH NIGKLCDFSQAMPSRLAIKDDGNKAALEGAVGLSSTL AEIFLLEHAQGM PKVAWGNIHSEQWNSLLKLHNAQFDLMSRTPYIAKHNGTPLLQTITHALG

		ADIASHPDPDISPDNKILFIAGHDTNIANISGMLGMTWTLPGQPDNTPPGGALVFERWVDKEGK PYVSVNMVYQTLAQLHDQTPLTLQHPAGSVRLNIPGCSDQTPDGYCPLSTFSRLVSHSVEPACQ LP
29	A0A2J9H2 M2	MTISLFTRSPTRLLKCMPLAFIAASMLTTASYASETEPSGYQLEKVVILSRHGVRAPTKMTQTM RDVTPNAWPEWPVKLGYYITPRGEHLVSLMGGFYRQKFQQLGILSKDRCPTANDVYVWADVD QRTRKTGEAFLAGLAPECHLSIIHQQDIKQADPLFHPVKAGVCTMEKTQVQQAVEQQAGMPI DQLNQHYRPALALMSSVLNFPKSTYCCQHSADQTCDFFAQAMPSKLSIKDNGNKVALDGAVGL SSTLAEIFLLEYAQGMPDTAWGKIHSEQDWNALLTLHNAQFDLMSRTPYIAKHNGTPLLQTIVS AINSQPSSRELPELSADNKILFLAGHDTNIANIAGMFGMSWTLPGQPDNTPPGGALVFERWSDK TGKKYVSVQMMYQTLAQLRNQTPLTLDKPAGSVALKIPGCDDQTAQGYCPLDTFTRLAKQNE LVECQKL
30	A0A0C5VZ Q7	MTISLFNRNKPAIAQRILCPLIVALFSGLPAYASDTAPAGLQLEKVVILSRHGVRAPTKMTQTM DVTPHQWPEWPVKLGYYITPRGEHLISLMGGFYRERFQQGGLPKDNCPTPDVYVWADVDQQR TRKTGEAFLAGLAPQCDLAIHHQQNIQADPLFHPVKAGICSMDKSQVHAAVEKQAGAPIETL NQRYQASLALMNSVLDFPKSPYCCQHNIQKLCDFSQAMPSRLAINDDGNKVALEGAVGLSSTL AEIFLLEHAQGMPKVAWGNHTKQQWDSLLKLHNAQFDLMSRTPYIAKHNGTPLLQTIAHAL GSNITSRPLDISPDNKILFIAGHDTNIANISGMLGMTWTLPGQPDNTPPGGALVFERWVDNAG KPYVSVNMVYQTLAQLHDQTPLTLQHPAGSVRLNIPGCSDQTPDGYCPLSTFSRLVSHSVEPAC QLP
98	N/A	QSEPELKLES VVIVSRHGVR APTKFTQLMQ DVTPDAWPTW PVKLGELTPR GCELIAYLGH YWRQRLVADE LLPKCGCPQS GQVAIIADVD ERTRKTGECF AAGLAPDCAI TVHHQADTSS PDPLFNPLKT GVCQLDVANV TRAILERAGG SIADFTGHYQ TAFRELERVL NFPQSNLCLK REKQDESCSL TQALPSELKV SADNVSLTGA VSLASMLTEI FLLQQAQGMP EPGWGRITDS HQWNTLLSLH NAQFDLLQRT PEVARSRATP LLDLIKALT PHPPQKQAYG VTLPTSVLFI AGHDTNLANL GGALELNWTL PGQPDNTPPG GELVFERWRR LSDNSQWIVQ SLVFQTLQQM RDKTPLSLNT PPGEVKLTLA GCEERNAQGM CSLAGFTQIV NEARIPACSL
99	N/A	QSEPELKLESVVIVSRHGVRAPTKATQLMQDVTPDAWPTWPVKLGWLTTPRGGELIAYLGHYQ RQRLVADGLLAKKGCPQSGQVAIIADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLF NPLKTGVCQLDNARVTEAILSRAAGSIADFTGHRQTAFRELERVLNFPQSNLCLKREKQDESCS LTQALPSELKYSADNVSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQWNTLLSLHNA QFYLLQRTPEVARSRATPLLDLIKALTTPHPPQKQAYGVTLPSTSVLFIAGHDTNLANLGALEL NWTLPQPDNTPPGGELVFERWRRSLDNSQWIVQSLVFQTLQQMRDKTPLSLNTPPGEVKLTL

		AGCEERNAQGMCSLAGFTQIVNEARIPACSL
100	N/A	QSEPELKLESVVIVSRHGVRAPTKATQLMQDVTPDAWPTWPVKLGWLTTPRGGELIAYLGHYQ RQRLVADGLLAKKGCPQSGQVAIIADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLF NPLKTGVCQLDNANVTDAILSRAGGSIADFTGHRQTAFRELERVLNFPQSNLCLKREKQDESCS LTQALPSELKPSADNVSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQWNTLLSLHNA QFYLLQRTPEVARSRATPLLDLIKALTTPHPPQKQAYGVTLPSTVLFIAGHDTNLANLGGALEL NWTLPGQPDNTPPGGELVFERWRRLSDNSQWIVSLVFQTLQQMRDKTPLSLNTPPGEVKLTL AGCEERNAQGMCSLAGFTQIVNEARIPACSL
101	N/A	QSEPELKLESVVIVSRHGVRAPTKATQLMQDVTPDAWPTWPVKLGWLTTPRGGELIAYLGHYQ RQRLVADGLLADKGCPQSGQVAIIADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLF NPLKTGVCQLDNANVTDAILSRAGGSIADFTGHRQTAFRELERVLNFPQSNLCLKREKQDESCS LTQALPSELKYSADNVSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQWNTLLSLHNA QFYLLQRTPEVARSRATPLLDLIKALTTPHPPQKQAYGVTLPSTVLFIAGHDTNLANLGGALEL NWTLPGQPDNTPPGGELVFERWRRLSDNSQWIVSLVFQTLQQMRDKTPLSLNTPPGEVKLTL AGCEERNAQGMCSLAGFTQIVNEARIPACSL
102	N/A	QSEPELKLESVVIVSRHGVRAPTKATQLMQDVTPDAWPTWPVKLGWLTTPRGGELIAYLGHYQ RQRLVADGLLAKKGCPQSGQVAIIADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLF NPLKTGVCQLDNANVTDAIISRAGGSIADFTGHRQTAFRELERVLNFPQSNLCLKREKQDESCS LTQALPSEIKVSADNVSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQWNTLLSLHNA QFYLLQRTPEVARSRATPLLDLIKALTTPHPPQKQAYGVTLPSTVLFIAGHDTNLANLGGALEL NWTLPGQPDNTPPGGELVFERWRRLSDNSQWIVSLVFQTLQQMRDKTPLSLNTPPGEVKLTL AGCEERNAQGMCSLAGFTQIVNEARIPACSL
103	N/A	QSEPELKLESVVIVSRHGVRAPTKATQLMQDVTPDAWPTWPVKLGWLTTPRGGELIAYLGHYQ RQRLVADGLLADKGCPQSGQVAIIADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLF NPLKTGVCQLDNARVTEAILSRAGGSIADFTGHRQTAFRELERVLNFPQSNLCLKREKQDESCS LTQALPSELKPSADNVSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQWNTLLSLHNA QFYLLQRTPEVARSRATPLLDLIKALTTPHPPQKQAYGVTLPSTVLFIAGHDTNLANLGGALEL NWTLPGQPDNTPPGGELVFERWRRLSDNSQWIVSLVFQTLQQMRDKTPLSLNTPPGEVKLTL AGCEERNAQGMCSLAGFTQIVNEARIPACSL
104	N/A	QSEPELKLESVVIVSRHGVRAPTKATQLMQDVTPDAWPTWPVKLGWLTTPRGGELIAYLGHYQ RQRLVADGLLAKKGCPQSGQVAIIADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLF NPLKTGVCQLDNANVTDAILSRAGGSIADFTGHRQTAFRELERVLNFPQSNLCLKREKQDESCS LTQALPSELKVSADNVSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQWNTLLSLHNA

		QFYLLQRTPEVARSRATPLLDLIKALTTPHPPQKQAYGVTLPSTSVLFIAGHDTNLANLGGALEL NWTLPGQPDNTPPGGELVFERWRRLSDNSQWIVSLVFQTLQQMRDKTPLSLNTPPGEVKLTL AGCEERNAQGMCSLAGFTQIVNEARIPACSL
105	N/A	QSEPELKLESVVIVSRHGVRAPTKATQLMQDVTPDAWPTWPVKLGWLTTPRGGELIAYLGHYQ RQRLVADGLLAKKGCPQSGQVAIIADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLF NPLKTGVCQLDNANVTDAILSRAGGSIADFTGHRQTAFRELERVLNFPQSNLCLKREKQDESCS LTQALPSELKYSADNVSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQWNTLLSLHNA QFYLLQRTPEVARSRATPLLDLIKALTTPHPPQKQAYGVTLPSTSVLFIAGHDTNLANLGGALEL NWTLPGQPDNTPPGGELVFERWRRLSDNSQWIVSLVFQTLQQMRDKTPLSLNTPPGEVKLTL AGCEERNAQGMCSLAGFTQIVNEARIPACSL
106	N/A	QSEPELKLESVVIVSRHGVRAPTKATQLMQDVTPDAWPTWPVKLGWLTTPRGGELIAYLGHYQ RQRLVADGLLAKKGCPQSGQVAIIADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLF NPLKTGVCQLDNARVTEAILSRAGGSIADFTGHRQTAFRELERVLNFPQSNLCLKREKQDESCS LTQALPSELKVSADNVSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQWNTLLSLHNA QFYLLQRTPEVARSRATPLLDLIKALTTPHPPQKQAYGVTLPSTSVLFIAGHDTNLANLGGALEL NWTLPGQPDNTPPGGELVFERWRRLSDNSQWIVSLVFQTLQQMRDKTPLSLNTPPGEVKLTL AGCEERNAQGMCSLAGFTQIVNEARIPACSL
107	N/A	QSEPELKLESVVIVSRHGVRAPTKATQLMQDVTPDAWPTWPVKLGWLTTPRGGELIAYLGHYQ RQRLVADGLLAKKGCPQSGQVAIIADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLF NPLKTGVCQLDNANVTDAILSRAGGSIADFTGHRQTAFRELERVLNFPQSNLCLKREKQDESCS LTQALPSELKPSADNVSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQWNTLLSLHNA QFYLLQRTPEVARSRATPLLDLIKALTTPHPPQKQAYGVTLPSTSVLFIAGHDTNLANLGGALEL NWTLPGQPDNTPPGGELVFERWRRLSDNSQWIVSLVFQTLQQMRDKTPLSLNTPPGEVKLTL AGCEERNAQGMCSLAGFTQIVNEARIPACSL
108	N/A	QSEPELKLESVVIVSRHGVRAPTKATQLMQDVTPDAWPTWPVKLGWLTTPRGGELIAYLGHYQ RQRLVADGLLADKGCPQSGQVAIIADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLF NPLKTGVCQLDNANVTDAILSRAGGSIADFTGHRQTAFRELERVLNFPQSNLCLKREKQDESCS LTQALPSELKVSADNVSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQWNTLLSLHNA QFYLLQRTPEVARSRATPLLDLIKALTTPHPPQKQAYGVTLPSTSVLFIAGHDTNLANLGGALEL NWTLPGQPDNTPPGGELVFERWRRLSDNSQWIVSLVFQTLQQMRDKTPLSLNTPPGEVKLTL AGCEERNAQGMCSLAGFTQIVNEARIPACSL
109	N/A	QSEPELKLESVVIVSRHGVRAPTKATQLMQDVTPDAWPTWPVKLGWLTTPRGGELIAYLGHYQ RQRLVADGLLAKKGCPQSGQVAIIADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLF

		NPLKTGVCQLDNANVTDAIISRAGGSIADFTGHRQTAFRELERVLNFPQSNLCLKREKQDESCS LTQALPSEIKVSADNVSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQWNTLLSLHNA QFYLLQRTPEVARSRATPLLDLIKALTTPHPPQKQAYGVTLPSTSVLFIAGHDTNLANLGGALEL NWTLPGQPDNTPPGGELVFERWRRLSDNSQWIVSLVFQTLQQMRDKTPLSLNTPPGEVKLT AGCEERNAQGMCSLAGFTQIVNEARIPACSL
110	N/A	QSEPELKLESVVIVSRHGVRAPTKATQLMQDVTPDAWPTWPVKLGWLTPRGGELIAYLGHYQ RQRLVADGLLADKGCPQSGQVAIIADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLF NPLKTGVCQLDNARVTEAILSRAGGSIADFTGHRQTAFRELERVLNFPQSNLCLKREKQDESCS LTQALPSELKPSADNVSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQWNTLLSLHNA QFYLLQRTPEVARSRATPLLDLIKALTTPHPPQKQAYGVTLPSTSVLFIAGHDTNLANLGGALEL NWTLPGQPDNTPPGGELVFERWRRLSDNSQWIVSLVFQTLQQMRDKTPLSLNTPPGEVKLT AGCEERNAQGMCSLAGFTQIVNEARIPACSL
111	N/A	FSYGAAIPQSTQEKQFSQEFRDGYSLKHYYGNGPYSERVSYGIARDPPTSELKLESVVIVSRHG VRAPTKATQLMQDVTPDAWPTWPVKLGWLTPRGGELIAYLGHYQRQRLVADGLLAKKGCPQ SGQVAIIADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLFNPLKTGVCQLDNANVTDA AILSRAGGSIADFTGHRQTAFRELERVLNFPQSNLCLKREKQDESCSLTQALPSELKVSADNVSL TGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQWNTLLSLHNAQFYLLQRTPEVARSRATP LLDLIKALTTPHPPQKQAYGVTLPSTSVLFIAGHDTNLANLGGALELNWTLPGQPDNTPPGGELV FERWRRLSDNSQWIVSLVFQTLQQMRDKTPLSLNTPPGEVKLTLAGCEERNAQGMCSLAGFT QIVNEARIPACSL
112	N/A	FSYGAAIPQSTQEKQFSQEFRDGYSLKHYYGNGPYSERVSYGIARDPPTSELKLESVVIVSRHG VRAPTKATQLMQDVTPDAWPTWPVKLGWLTPRGGELIAYLGHYQRQRLVADGLLAKKGCPQ SGQVAIIADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLFNPLKTGVCQLDNANVTDA AILSRAGGSIADFTGHRQTAFRELERVLNFPQSNLCLKREKQDESCSLTQALPSELKVSADNVSL TGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQWNTLLSLHNAQFYLLQRTPEVARSRATP LLDLIKALTTPHPPQKQAYGVTLPSTSVLFIAGHDTNLANLGGALELNWTLPGQPDNTPPGGELV FERWRRLSDNSQWIVSLVFQTLQQMRDKTPLSLNTPPGEVKLTLAGCEERNAQGMCSLAGFT QIVNEARIPACSLLSFWWNYNTTTELNYRSSPIACQEGDAMD
113	N/A	FSYGAAIPQSTQEKQFSQEFRDGYSLKHYYGNGPYSERVSYGIARDPPTSELKLESVVIVSRHG VRAPTKATQLMQDVTPDAWPTWPVKLGWLTPRGGELIAYLGHYQRQRLVADGLLAKKGCPQ SGQVAIIADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLFNPLKTGVCQLDNANVTDA AILSRAGGSIADFTGHRQTAFRELERVLNFPQSNLCLKREKQDESCSLTQALPSELKVSADNVSL TGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQWNTLLSLHNAQFYLLQRTPEVARSRATP LLDLIKALTTPHPPQKQAYGVTLPSTSVLFIAGHDTNLANLGGALELNWTLPGQPDNTPPGGELV

		FERWRRLSDNSQWIVSLVFQTLQQMRDKTPLSLNTPPGEVKLTLAGCEERNAQGMCSLAGFT QIVNEARIPACSL
114	N/A	FSYGAAIPQSTQEKQFSQEFRDGYSILKHYGGNGPYSERVSYGIARDPPTSELKLESVVIVSRHG VRAPTKATQLMQDVTPDAWPTWPVKLGWLTTPRGGELIAYLGHYQRQRLVADGLLAKKGCPQ SGQVAIIADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLFNPLKTGVCQLDNANVTD AILSRAGGSIADFTGHRQTAFRELERVLNFPQSNLCLKREKQDESCSLTQALPSELKVSADNVSL TGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQWNTLLSLHNAQFYLLQRTPEVARSRATP LLDLIKTALTPHPQKQAYGVTLPSTVLFIAGHDTNLANLGGALELNWTLPGQPDNTPPGGELV FERWRRLSDNSQWIVSLVFQTLQQMRDKTPLSLNTPPGEVKLTLAGCEERNAQGMCSLAGFT QIVNEARIPACSLLSFWWNNTTTELNYRSSPIACQEGDAMD
115	N/A	MAQSEPELKLESVVIVSRHGVRAPTKATQLMQDVTPDAWPTWPVKLGELTPRGGELIAYLGH YWRQRLVADGLLPKCGCPQSGQVAIIADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPD LFNPLKTGVCQLDNANVTDAILERAGGSIADFTGHYQTAFRELERVLNFPQSNLCLKREKQDES CSLTQALPSELKVSADCVSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQWNTLLSLH NAQFDLLQRTPEVARSRATPLLDLIKALTPHPQKQAYGVTLPSTVLFIAGHDTNLANLGGAL ELNWTLPGQPDNTPPGGELVFERWRRLSDNSQWIVSLVFQTLQQMRDKTPLSLNTPPGEVKL TLAGCEERNAQGMCSLAGFTQIVNEARIPACSL

[0334] In some embodiments, the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, can comprise a sequence with at least 70%, 75%, 80%, 85%, 90%, 95% sequence identity to at least 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, or 433 contiguous amino acids of a sequence from Table 1. In some embodiments, the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, can comprise a sequence with at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% or greater sequence identity to at least 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, or 433 contiguous amino acids of a sequence from Table 1. In some embodiments, the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, can comprise a sequence with less than 10% sequence identity to at least 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, or 433 contiguous amino acids of a sequence from Table 1.

[0335] In some embodiments, the sequence from Table 1 is SEQ ID NO: 1. In some embodiments, the sequence from Table 1 is SEQ ID NO: 2 or SEQ ID NO: 98. In some embodiments, the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, can comprise a sequence with at least 70%, 75%, 80%, 85%, 90%, 95% sequence identity to at

least 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, or 433 contiguous amino acids of SEQ ID NO: 1.

[0336] In some embodiments, the phytase comprises an amino acid sequence with at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to at least 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, or 433 contiguous amino acids of SEQ ID NO: 1, where one or more non-standard amino acids such as selenocysteine are at positions 15, 53, 74, 99, 121, 130, 155, 163, 199, 200, 209, 221, 403, 412, and/or 429.

[0337] In some embodiments, the phytase further comprises at least one affinity tag. In some embodiments, an affinity tag of a phytase is a C-terminal affinity tag. In some embodiments, an affinity tag of a phytase is an N-terminal affinity tag. In some embodiments, a first affinity tag of a phytase is an N-terminal affinity tag and a second affinity tag of a phytase is a C-terminal affinity tag. In some embodiments, a first affinity tag of a phytase is a first N-terminal affinity tag and a second affinity tag of a phytase is a second N-terminal affinity tag. In some embodiments, a first affinity tag of a phytase is a first C-terminal affinity tag and a second affinity tag of a phytase is a second C-terminal affinity tag.

[0338] For example, the phytase can comprise a poly-histidine tag, poly-histidine-glycine tag, poly-arginine tag, poly-aspartate tag, poly-cysteine tag, poly-phenylalanine, c-myc tag, Herpes simplex virus glycoprotein D (gD) tag, FLAG tag, KT3 epitope tag, tubulin epitope tag, T7 gene 10 protein peptide tag, streptavidin tag, streptavidin binding peptide (SPB) tag, Strep-tag, Strep-tag II, albumin-binding protein (ABP) tag, alkaline phosphatase (AP) tag, bluetongue virus tag (B-tag), calmodulin binding peptide (CBP) tag, chloramphenicol acetyl transferase (CAT) tag, choline-binding domain (CBD) tag, chitin binding domain (CBD) tag, cellulose binding domain (CBP) tag, dihydrofolate reductase (DHFR) tag, galactose-binding protein (GBP) tag, maltose binding protein (MBP), glutathione-S-transferase (GST), Glu-Glu (EE) tag, human influenza hemagglutinin (HA) tag, horseradish peroxidase (HRP) tag, NE-tag, HSV tag, ketosteroid isomerase (KSI) tag, KT3 tag, LacZ tag, luciferase tag, NusA tag, PDZ domain tag, AviTag, Calmodulin-tag, E-tag, S-tag, SBP-tag, Softag 1, Softag 3, TC tag, VSV-tag, Xpress tag, Isopeptag, SpyTag, SnoopTag, Profinity eXact tag, Protein C tag, S1-tag, S-tag, biotin-carboxy carrier protein (BCCP) tag, green fluorescent protein (GFP) tag, small ubiquitin-like modifier (SUMO) tag, tandem affinity purification (TAP) tag, HaloTag, Nus-tag, Thioredoxin-tag, Fc-tag, CYD tag, HPC tag, TrpE tag, ubiquitin tag, VSV-G epitope tag, V5 tag, or a combination thereof.

[0339] In some embodiments, the phytase further comprises at least two affinity tags. For example, the phytase can comprise at least two affinity tags selected from a poly-histidine tag, poly-histidine-glycine tag, poly-arginine tag, poly-aspartate tag, poly-cysteine tag, poly-phenylalanine, c-myc tag, Herpes simplex virus glycoprotein D (gD) tag, FLAG tag, KT3 epitope tag, tubulin epitope tag, T7 gene 10 protein peptide tag, streptavidin tag, streptavidin binding peptide (SPB) tag, Strep-tag, Strep-tag II, albumin-binding protein (ABP) tag, alkaline phosphatase (AP) tag, bluetongue virus tag (B-tag), calmodulin binding peptide (CBP) tag, chloramphenicol acetyl transferase (CAT) tag, choline-binding domain (CBD) tag, chitin binding domain (CBD) tag, cellulose binding domain (CBP) tag, dihydrofolate reductase (DHFR) tag, galactose-binding protein (GBP) tag, maltose binding protein (MBP), glutathione-S-transferase (GST), Glu-Glu (EE) tag, human influenza hemagglutinin (HA) tag, horseradish peroxidase (HRP) tag, NE-tag, HSV tag, ketosteroid isomerase (KSI) tag, KT3 tag, LacZ tag, luciferase tag, NusA tag, PDZ domain tag, AviTag, Calmodulin-tag, E-tag, S-tag, SBP-tag, Softag 1, Softag 3, TC tag, VSV-tag, Xpress tag, Isopeptag, SpyTag, SnoopTag, Profinity eXact tag, Protein C tag, S1-tag, S-tag, biotin-carboxy carrier protein (BCCP) tag, green fluorescent protein (GFP) tag, small ubiquitin-like modifier (SUMO) tag, tandem affinity purification (TAP)

tag, HaloTag, Nus-tag, Thioredoxin-tag, Fc-tag, CYD tag, HPC tag, TrpE tag, ubiquitin tag, VSV-G epitope tag, and V5 tag.

[0340] In some embodiments, the phytase comprises an affinity tag that is GST. In some embodiments, the phytase comprises an affinity tag that is a poly-histidine tag, such as a 6x-His tag. In some embodiments, the phytase comprises an affinity tag that is MBP. In some embodiments, the phytase comprises an affinity tag that is a strep-tag, such as two strep tags.

[0341] In some embodiments, the phytase comprises a first affinity tag that is GST and a second affinity tag that is a poly-histidine tag, such as a 6x-His tag. In some embodiments, the phytase comprises a first affinity tag that is GST and a second affinity tag that is a strep tag. In some embodiments, the phytase comprises a first affinity tag that is a strep tag, such as two strep tags, and a second affinity tag that is a poly-histidine tag, such as a 6x-His tag. In some embodiments, the phytase comprises a first affinity tag that is MBP and a second affinity tag that is a poly-histidine tag, such as a 6x-His tag. In some embodiments, the phytase comprises a first affinity tag that is MBP and a second affinity tag that is a strep tag, such as two strep tags.

[0342] In some embodiments, the phytase comprises a first affinity tag that is GST, a second affinity tag that is a poly-histidine tag, such as a 6x-His tag, and a third affinity tag that is a strep tag, such as two strep tags. In some embodiments, the phytase comprises a GST tag, a His tag, and two strep tags. In some embodiments, the phytase comprises a first affinity tag that is MBP, a second affinity tag that is a poly-histidine tag, such as a 6x-His tag, and a third affinity tag that is a strep tag, such as two strep tags. In some embodiments, the phytase comprises a MBP tag, a His tag, and two strep tags.

[0343] In some embodiments, the phytase comprises an affinity tag, wherein the phytase and affinity tag are separated by a linker. In some embodiments, the phytase comprises a first affinity tag and a second affinity tag, wherein the phytase and the first affinity tag are separated by a linker, and wherein the phytase and the second affinity tag are separated by a linker. In some embodiments, the phytase comprises a first affinity tag and a second affinity tag, wherein the first and second affinity tags are separated by a linker. In some embodiments, the phytase comprises a first affinity tag, a second affinity tag and a third affinity tag, wherein the first, second and third affinity tags are each separated by a linker. In some embodiments, the phytase comprises a first affinity tag, a second affinity tag, a third affinity tag and a fourth affinity tag, wherein the first, second, third and fourth affinity tags are each separated by a linker. In some embodiments, a linker comprises an amino acid sequence of (GS)_n, (GGS)_n, or (GGGS)_n or a combination thereof, where n is an integer of from 1-10.

[0344] In some embodiments, the one or more non-standard amino acids can be at position 15 of SEQ ID NO:1, position 53 of SEQ ID NO:1, position 74 of SEQ ID NO:1, position 99 of SEQ ID NO:1, position 121 of SEQ ID NO:1, position 130 of SEQ ID NO:1, position 163 of SEQ ID NO:1, position 155 of SEQ ID NO:1, position 199 of SEQ ID NO:1, position 200 of SEQ ID NO:1, position 209 of SEQ ID NO:1, position 221 of SEQ ID NO:1, position 403 of SEQ ID NO:1, position 412 of SEQ ID NO:1, or position 429 of SEQ ID NO:1. In some embodiments, the one or more non-standard amino acids can be at position 15 of SEQ ID NO:1, position 99 of SEQ ID NO:1, position 130 of SEQ ID NO:1, position 155 of SEQ ID NO:1, position 200 of SEQ ID NO:1, position 209 of SEQ ID NO:1, position 403 of SEQ ID NO:1, position 412 of SEQ ID NO:1, and position 429 of SEQ ID NO:1.

[0345] In some embodiments, the phytase comprises one non-standard amino acid that is at position 15 of SEQ ID NO:1, position 53 of SEQ ID NO:1, position 74 of SEQ ID NO:1, position 99 of SEQ ID NO:1, position 121 of SEQ ID NO:1, position 130 of SEQ ID NO:1, position 163 of SEQ ID NO:1, position 155 of SEQ ID NO:1, position 199 of SEQ ID NO:1, position 200 of SEQ ID NO:1, position 209 of SEQ ID NO:1, position 221 of SEQ ID NO:1, position 403 of SEQ ID NO:1, position 412 of SEQ ID NO:1, or position 429 of SEQ ID NO:1. In some embodiments, the phytase comprises two non-standard amino acids, the positions of which are selected from position 15 of SEQ ID NO:1, position 53 of SEQ ID NO:1, position 74 of SEQ ID NO:1, position 99 of SEQ ID NO:1, position 121 of SEQ ID NO:1, position 130 of SEQ ID NO:1, position 163 of SEQ ID NO:1, position 155 of SEQ ID NO:1, position 199 of SEQ ID NO:1, position 200 of SEQ ID NO:1, position 209 of SEQ ID NO:1, position 221 of SEQ ID NO:1, position 403 of SEQ ID NO:1, position 412 of SEQ ID NO:1, and position 429 of SEQ ID NO:1. In some embodiments, the phytase comprises three non-standard amino acids, the positions of which are selected from position 15 of SEQ ID NO:1, position 53 of SEQ ID NO:1, position 74 of SEQ ID NO:1, position 99 of SEQ ID NO:1, position 121 of SEQ ID NO:1, position 130 of SEQ ID NO:1, position 163 of SEQ ID NO:1, position 155 of SEQ ID NO:1, position 199 of SEQ ID NO:1, position 200 of SEQ ID NO:1, position 209 of SEQ ID NO:1, position 221 of SEQ ID NO:1, position 403 of SEQ ID NO:1, position 412 of SEQ ID NO:1, and position 429 of SEQ ID NO:1. In some embodiments, the phytase comprises four non-standard amino acids, the positions of which are selected from position 15 of SEQ ID NO:1, position 53 of SEQ ID NO:1, position 74 of SEQ ID NO:1, position 99 of SEQ ID NO:1, position 121 of SEQ ID NO:1, position 130 of SEQ ID NO:1, position 163 of SEQ ID NO:1, position 155 of SEQ ID NO:1, position 199 of SEQ ID NO:1, position 200 of SEQ ID NO:1, position 209 of SEQ ID NO:1, position 221 of SEQ ID NO:1, position 403 of SEQ ID NO:1, position 412 of SEQ ID NO:1, and position 429 of SEQ ID NO:1.

position 412 of SEQ ID NO:1, and position 429 of SEQ ID NO:1. In some embodiments, the phytase comprises five non-standard amino acids, the positions of which are selected from position 15 of SEQ ID NO:1, position 53 of SEQ ID NO:1, position 74 of SEQ ID NO:1, position 99 of SEQ ID NO:1, position 121 of SEQ ID NO:1, position 130 of SEQ ID NO:1, position 163 of SEQ ID NO:1, position 155 of SEQ ID NO:1, position 199 of SEQ ID NO:1, position 200 of SEQ ID NO:1, position 209 of SEQ ID NO:1, position 221 of SEQ ID NO:1, position 403 of SEQ ID NO:1, position 412 of SEQ ID NO:1, and position 429 of SEQ ID NO:1. In some embodiments, the phytase comprises six non-standard amino acids, the positions of which are selected from position 15 of SEQ ID NO:1, position 53 of SEQ ID NO:1, position 74 of SEQ ID NO:1, position 99 of SEQ ID NO:1, position 121 of SEQ ID NO:1, position 130 of SEQ ID NO:1, position 163 of SEQ ID NO:1, position 155 of SEQ ID NO:1, position 199 of SEQ ID NO:1, position 200 of SEQ ID NO:1, position 209 of SEQ ID NO:1, position 221 of SEQ ID NO:1, position 403 of SEQ ID NO:1, position 412 of SEQ ID NO:1, and position 429 of SEQ ID NO:1. In some embodiments, the phytase comprises seven non-standard amino acids, the positions of which are selected from position 15 of SEQ ID NO:1, position 53 of SEQ ID NO:1, position 74 of SEQ ID NO:1, position 99 of SEQ ID NO:1, position 121 of SEQ ID NO:1, position 130 of SEQ ID NO:1, position 163 of SEQ ID NO:1, position 155 of SEQ ID NO:1, position 199 of SEQ ID NO:1, position 200 of SEQ ID NO:1, position 209 of SEQ ID NO:1, position 221 of SEQ ID NO:1, position 403 of SEQ ID NO:1, position 412 of SEQ ID NO:1, and position 429 of SEQ ID NO:1. In some embodiments, the phytase comprises eight non-standard amino acids, the positions of which are selected from position 15 of SEQ ID NO:1, position 53 of SEQ ID NO:1, position 74 of SEQ ID NO:1, position 99 of SEQ ID NO:1, position 121 of SEQ ID NO:1, position 130 of SEQ ID NO:1, position 163 of SEQ ID NO:1, position 155 of SEQ ID NO:1, position 199 of SEQ ID NO:1, position 200 of SEQ ID NO:1, position 209 of SEQ ID NO:1, position 221 of SEQ ID NO:1, position 403 of SEQ ID NO:1, position 412 of SEQ ID NO:1, and position 429 of SEQ ID NO:1. In some embodiments, the phytase comprises nine non-standard amino acids, the positions of which are selected from position 15 of SEQ ID NO:1, position 53 of SEQ ID NO:1, position 74 of SEQ ID NO:1, position 99 of SEQ ID NO:1, position 121 of SEQ ID NO:1, position 130 of SEQ ID NO:1, position 163 of SEQ ID NO:1, position 155 of SEQ ID NO:1, position 199 of SEQ ID NO:1, position 200 of SEQ ID NO:1, position 209 of SEQ ID NO:1, position 221 of SEQ ID NO:1, position 403 of SEQ ID NO:1, position 412 of SEQ ID NO:1, and position 429 of SEQ ID NO:1. In some embodiments, the phytase comprises ten non-standard amino acids, the positions of which are selected from position 15 of SEQ ID NO:1, position 53 of SEQ ID NO:1, position

74 of SEQ ID NO:1, position 99 of SEQ ID NO:1, position 121 of SEQ ID NO:1, position 130 of SEQ ID NO:1, position 163 of SEQ ID NO:1, position 155 of SEQ ID NO:1, position 199 of SEQ ID NO:1, position 200 of SEQ ID NO:1, position 209 of SEQ ID NO:1, position 221 of SEQ ID NO:1, position 403 of SEQ ID NO:1, position 412 of SEQ ID NO:1, and position 429 of SEQ ID NO:1.

[0346] In some embodiments, a non-standard amino acid at position 99 can be directly linked by a bond to a non-standard amino acid at position 130. In some embodiments, a non-standard amino acid at position 155 can be directly linked by a bond to a non-standard amino acid at position 429. In some embodiments, a non-standard amino acid at position 200 can be directly linked by a bond to a non-standard amino acid at position 209. In some embodiments, a non-standard amino acid at position 403 can be directly linked by a bond to a non-standard amino acid at position 412. In some embodiments, a non-standard amino acid at position 126 can be directly linked by a bond to a non-standard amino acid at position 195. In some embodiments, a non-standard amino acid at position 126 can be directly linked by a bond to a non-standard amino acid at position 231. In some embodiments, a non-standard amino acid at position 53 can be directly linked by a bond to a non-standard amino acid at position 199. In some embodiments, a non-standard amino acid at position 74 can be directly linked by a bond to a non-standard amino acid at position 121. In some embodiments, a non-standard amino acid at position 163 can be directly linked by a bond to a non-standard amino acid at position 221.

[0347] In some embodiments, any two non-standard amino acids located at any two of the positions selected from position 15 of SEQ ID NO:1, position 99 of SEQ ID NO:1, position 130 of SEQ ID NO:1, position 155 of SEQ ID NO:1, position 200 of SEQ ID NO:1, position 209 of SEQ ID NO:1, position 403 of SEQ ID NO:1, position 412 of SEQ ID NO:1, and position 429 of SEQ ID NO:1 can be directly linked by a bond. In some embodiments, any two non-standard amino acids located at any two of the positions selected from position 53 of SEQ ID NO:1, position 74 of SEQ ID NO:1, position 121 of SEQ ID NO:1, position 163 of SEQ ID NO:1, position 199 of SEQ ID NO:1, and position 221 of SEQ ID NO:1 can be directly linked by a bond.

[0348] In some embodiments, the one or more non-standard amino acids can be at one or more of the positions selected from 165 of SEQ ID NO:2, position 227 of SEQ ID NO:2, position 279 of SEQ ID NO:2, position 281 of SEQ ID NO:2, position 284 of SEQ ID NO:2, position 286 of SEQ ID NO:2, position 331 of SEQ ID NO:2, and position 334 of SEQ ID NO:2.

[0349] In some embodiments, a non-standard amino acid at position 165 of SEQ ID NO:2 can be directly linked by a bond to a non-standard amino acid at position 284 of SEQ ID NO:2. In

some embodiments, a non-standard amino acid at position 281 of SEQ ID NO:2 can be directly linked by a bond to a non-standard amino acid at position 334 of SEQ ID NO:2. In some embodiments, a non-standard amino acid at position 227 of SEQ ID NO:2 can be directly linked by a bond to a non-standard amino acid at position 279 of SEQ ID NO:2. In some embodiments, a non-standard amino acid at position 286 of SEQ ID NO:2 can be directly linked by a bond to a non-standard amino acid at position 331 of SEQ ID NO:2. In some embodiments, any two non-standard amino acids located at any two of the positions selected from 165 of SEQ ID NO:2, position 227 of SEQ ID NO:2, position 279 of SEQ ID NO:2, position 281 of SEQ ID NO:2, position 284 of SEQ ID NO:2, position 286 of SEQ ID NO:2, position 331 of SEQ ID NO:2, and position 334 of SEQ ID NO:2 can be directly linked by a bond.

[0350] In some embodiments, the one or more non-standard amino acids can be at one or more of the positions selected from 165 of SEQ ID NO:98, position 227 of SEQ ID NO:98, position 279 of SEQ ID NO: 98, position 281 of SEQ ID NO: 98, position 284 of SEQ ID NO:98, position 286 of SEQ ID NO:98, position 331 of SEQ ID NO:98, and position 334 of SEQ ID NO:98.

[0351] In some embodiments, a non-standard amino acid at position 165 of SEQ ID NO:98 can be directly linked by a bond to a non-standard amino acid at position 284 of SEQ ID NO:98. In some embodiments, a non-standard amino acid at position 281 of SEQ ID NO:98 can be directly linked by a bond to a non-standard amino acid at position 334 of SEQ ID NO:98. In some embodiments, a non-standard amino acid at position 227 of SEQ ID NO:98 can be directly linked by a bond to a non-standard amino acid at position 279 of SEQ ID NO:98. In some embodiments, a non-standard amino acid at position 286 of SEQ ID NO:98 can be directly linked by a bond to a non-standard amino acid at position 331 of SEQ ID NO:98. In some embodiments, any two non-standard amino acids located at any two of the positions selected from 165 of SEQ ID NO:98, position 227 of SEQ ID NO:98, position 279 of SEQ ID NO:98, position 281 of SEQ ID NO:98, position 284 of SEQ ID NO:98, position 286 of SEQ ID NO:98, position 331 of SEQ ID NO:98, and position 334 of SEQ ID NO:98 can be directly linked by a bond.

[0352] In some embodiments, the one or more non-standard amino acids can be at one or more of the positions selected from 77 of SEQ ID NOs:99-110, position 108 of SEQ ID NOs:99-110, position 133 of SEQ ID NOs:99-110, position 178 of SEQ ID NOs:99-110, position 188 of SEQ ID NOs:99-110, position 382 of SEQ ID NOs:99-110, position 391 of SEQ ID NOs:99-110, and position 408 of SEQ ID NOs:99-110.

[0353] In some embodiments, any two non-standard amino acids located at any two of the positions selected from positions 77, 108, 133, 178, 188, 382, 391, and 408 of an amino acid sequence selected from SEQ ID NOs:99-110 can be directly linked by a bond. In some embodiments, a non-standard amino acid at position 77 of an amino acid sequence can be directly linked by a bond to a non-standard amino acid at position 108 of the amino acid sequence. In some embodiments, a non-standard amino acid at position 133 of an amino acid sequence can be directly linked by a bond to a non-standard amino acid at position 408 of the amino acid sequence. In some embodiments, a non-standard amino acid at position 178 of an amino acid sequence can be directly linked by a bond to a non-standard amino acid at position 188 of the amino acid sequence. In some embodiments, a non-standard amino acid at position 382 of an amino acid sequence can be directly linked by a bond to a non-standard amino acid at position 391 of the amino acid sequence. In some embodiments, the amino acid sequence is selected from SEQ ID NOs:99-110.

[0354] In some embodiments, the one or more non-standard amino acids can be at one or more of the positions selected from 124 of SEQ ID NOs:111-114, position 155 of SEQ ID NOs:111-114, position 180 of SEQ ID NOs:111-114, position 225 of SEQ ID NOs:111-114, position 235 of SEQ ID NOs:111-114, position 429 of SEQ ID NOs:111-114, position 438 of SEQ ID NOs:111-114, and position 455 of SEQ ID NOs:111-114.

[0355] In some embodiments, any two non-standard amino acids located at any two of the positions selected from positions 124, 155, 180, 225, 235, 429, 438, and 455 of an amino acid sequence selected from SEQ ID NOs:111-114 can be directly linked by a bond. In some embodiments, a non-standard amino acid at position 124 of an amino acid sequence can be directly linked by a bond to a non-standard amino acid at position 155 of the amino acid sequence. In some embodiments, a non-standard amino acid at position 180 of an amino acid sequence can be directly linked by a bond to a non-standard amino acid at position 455 of the amino acid sequence. In some embodiments, a non-standard amino acid at position 225 of an amino acid sequence can be directly linked by a bond to a non-standard amino acid at position 235 of the amino acid sequence. In some embodiments, a non-standard amino acid at position 429 of an amino acid sequence can be directly linked by a bond to a non-standard amino acid at position 438 of the amino acid sequence. In some embodiments, the amino acid sequence is selected from SEQ ID NOs:111-114.

[0356] In some embodiments, the one or more non-standard amino acids can be at one or more of the positions selected from 77 of SEQ ID NO:115, position 79 of SEQ ID NO:115, position 110 of SEQ ID NO:115, position 135 of SEQ ID NO:115, position 180 of SEQ ID NO:115,

position 190 of SEQ ID NO:115, position 206 of SEQ ID NO:115, position 384 of SEQ ID NO:115, position 393 of SEQ ID NO:115, and position 410 of SEQ ID NO:115.

[0357] In some embodiments, a non-standard amino acid at position 77 of SEQ ID NO:115 can be directly linked by a bond to a non-standard amino acid at position 206 of SEQ ID NO:115. In some embodiments, a non-standard amino acid at position 79 of SEQ ID NO:115 can be directly linked by a bond to a non-standard amino acid at position 110 of SEQ ID NO:115. In some embodiments, a non-standard amino acid at position 135 of SEQ ID NO:115 can be directly linked by a bond to a non-standard amino acid at position 410 of SEQ ID NO:115. In some embodiments, a non-standard amino acid at position 180 of SEQ ID NO:115 can be directly linked by a bond to a non-standard amino acid at position 190 of SEQ ID NO:115. In some embodiments, a non-standard amino acid at position 384 of SEQ ID NO:115 can be directly linked by a bond to a non-standard amino acid at position 393 of SEQ ID NO:115.

[0358] In some embodiments, the bond can be a diselenide bond or selenyl-sulfhydryl bond. In some embodiments, the bond is a diselenide bond. In some embodiments, the bond is a selenyl-sulfhydryl bond. In some embodiments, the diselenide bond or selenyl-sulfhydryl bond can be in a location of a disulfide bond in a corresponding recombinant enzyme without the one or more non-standard amino acids.

[0359] In some embodiments, the T_m of the corresponding stabilized phytase polypeptide, functional fragment thereof, or variant thereof, can be less than 37°C. In some embodiments, the T_m of the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, can be greater than 37°C, 40°C, 45°C, 50°C, 55°C, 60°C, or 65°C. In some embodiments, the T_m of the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, can be at least 37°C, 40°C, 45°C, 50°C, 55°C, 60°C, 65°C or greater.

[0360] In some embodiments, the T_m of the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, can be at least 10°C higher than the T_m of the corresponding recombinant enzyme, functional fragment thereof, or variant thereof. In some embodiments, the T_m of the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, can be at least 15°C higher than the T_m of the corresponding recombinant enzyme, functional fragment thereof, or variant thereof. In some embodiments, the T_m of the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, can be at least 15°C, 20°C, 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, 65°C or greater higher than the T_m of the corresponding recombinant enzyme, functional fragment thereof, or variant thereof. In some embodiments, the T_m of the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, can be

less than 10°C higher than the T_m of the corresponding recombinant enzyme, functional fragment thereof, or variant thereof.

[0361] In some embodiments, the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, can have a half-life in an environment that can be at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 or greater fold higher than a half-life of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids in the environment. In some embodiments, the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, can have a half-life in an environment that is at least a 1.1 fold higher than a half-life of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids in the environment.

[0362] In some embodiments, the half-life of the phytase polypeptide, functional fragment thereof, or variant thereof in the environment, can be greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or more hours. In some embodiments, the half-life of the phytase polypeptide, functional fragment thereof, or variant thereof in the environment, can be less than 1 hour.

[0363] In some embodiments, the half-life of the phytase polypeptide, functional fragment thereof, or variant thereof in the environment, can be greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or more days. In some embodiments, the half-life of the phytase polypeptide, functional fragment thereof, or variant thereof in the environment, can be less than 1 day.

[0364] In some embodiments, the stabilized phytase polypeptide can have at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 fold higher hydrolytic activity for a phytate substrate in an environment than a hydrolytic activity for the phytate substrate of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids. In some embodiments, the stabilized phytase polypeptide can have less than a 1.1 fold higher hydrolytic activity for a phytate substrate in an environment than a hydrolytic activity for the phytate substrate of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0365] In some embodiments, the stabilized phytase polypeptide can have at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 fold

higher hydrolytic activity for a phytate substrate after being present in an environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or 50 minutes than a hydrolytic activity for the phytate substrate of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids after being present in the environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or 50 minutes. In some embodiments, the stabilized phytase polypeptide can have less than a 1.1 fold higher hydrolytic activity for a phytate substrate after being present in an environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or 50 minutes than a hydrolytic activity for the phytate substrate of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids after being present in the environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or 50 minutes.

[0366] In some embodiments, the stabilized phytase polypeptide can have at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 fold higher hydrolytic activity for a phytate substrate after being present in an environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, or 24 hours than a hydrolytic activity for the phytate substrate of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids after being present in the environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, or 24 hours. In some embodiments, the stabilized phytase polypeptide can have less than a 1.1 fold higher hydrolytic activity for a phytate substrate after being present in an environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, or 24 hours than a hydrolytic activity for the phytate substrate of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids after being present in the environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, or 24 hours.

[0367] In some embodiments, the (i) stabilized phytase polypeptide, (ii) a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids, or (iii) both may lose hydrolytic activity in a reducing environment, as compared to its activity in a non-reducing environment. For example, the loss of hydrolytic activity of a stabilized phytase polypeptide in a reducing environment, as compared to its activity in a non-reducing environment may be at least or at most 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30%, 40%, 50%, or 60%. For another example, the loss of hydrolytic activity of the corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids in a reducing environment, as compared to its activity in a non-reducing environment may be at least or at

most 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 99.9%. In some embodiments, the loss of hydrolytic activity in a reducing environment, as compared to the hydrolytic activity in a non-reducing environment is less for the stabilized phytase polypeptide, for example at least or at most 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% less, than for a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids. Under the same conditions, for example, when comparing an enzyme's activity under a reducing environment and the enzyme's activity under a non-reducing environment, the activity difference such as a loss of activity under the reducing environment is less significant for a stabilized phytase than a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids, e.g., at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% less.

[0368] In some embodiments, the phytate substrate comprises phytic acid. In some embodiments, the phytate substrate comprises a phytate in a salt form. In some embodiments, the phytate substrate comprises both phytic acid and phytate in a salt form. In some embodiments, the phytate substrate is in an animal feedstock, such as the feedstock described in the present disclosure.

[0369] In some embodiments, the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, can be recombinant. In some embodiments, the recombinant can be generated using recombinant DNA technology, such as, for example, the stabilized phytase polypeptide, functional fragment thereof, or variant thereof expressed by a bacteriophage or yeast expression system. In other embodiments, the recombinant can be generated by the synthesis of a DNA molecule encoding the stabilized phytase polypeptide, functional fragment thereof, or variant thereof.

[0370] In some embodiments, the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, can be an animal phytase, a plant phytase, a fungi phytase, or a bacterial phytase. The stabilized phytase polypeptide, functional fragment thereof, or variant thereof, can be any kinds of phytase, including, but not limited to, *E. coli* phytase, *Microcella alkaliphila* phytase, *Lactobacillus algidus* phytase, *Vibrio cholerae* phytase, *Bifidobacterium longum* phytase, *Homo sapiens* phytase, and *Raoultella ornithinolytica* phytase. In some embodiments, a composition can comprise stabilized animal phytases, stabilized plant phytases, stabilized fungi phytases, stabilized bacterial phytases, or any combination thereof.

[0371] In some embodiments, the composition further comprises a carbohydrase, such as a xylanase polypeptide or a mannanase polypeptide. In some embodiments, the composition further comprises one or more carbohydrases comprising xylanases, mannanases, or both. In some embodiments, the xylanase is a stabilized xylanase comprising one or more non-standard amino acid, a functional fragment thereof, or a variant thereof. In some embodiments, the mannanase is a stabilized mannanase comprising one or more non-standard amino acid, a functional fragment thereof, or a variant thereof.

[0372] In some embodiments, a composition can comprise a polynucleotide encoding the composition disclosed herein. In some embodiments, the polynucleotide can be a vector. The vector can be a fragment of nucleic acid molecules. The vector can be taken from a virus, a plasmid, or the cell of a higher organism. The vector can be stably maintained in an organism. The vector can be inserted with a foreign nucleic acid fragment for cloning purposes. The vector can comprise features that allow for the convenient insertion or removal of a nucleic acid fragment to or from a vector. The vector can be genetically engineered plasmids.

[0373] In some embodiments, a bond directly linking two of the one or more non-standard amino acids of the stabilized phytase polypeptide may not break in an environment, when the bond directly linking two of the one or more standard amino acids of the corresponding phytase polypeptide may break in the same environment.

[0374] In some embodiments, the method of making the composition disclosed herein can comprise expressing an amino acid sequence of the stabilized phytase polypeptide. In some embodiments, expressing can comprise expressing in a cell or *in vitro*.

[0375] In some embodiments, the cell can be a bacterial cell. In some embodiments, the cell can be a genomically recoded cell. In some embodiments, the cell may not be a bacterial cell. The cell can be obtained or isolated from a subject. The cell can be obtained or isolated from a tissue. The subject may be an animal such as a human, a mouse, a rat, a pig, a dog, a rabbit, a sheep, a horse, a chicken or other animal. A cell may be a neuron. The cell may be one of the cells of a blood-brain barrier system. The cell may be a cell line, such as a neuronal cell line. The cell may be a primary cell, such as cells obtained from a brain of a subject. The cell may be a population of cells that may be isolated from a subject, such as a tissue biopsy, a cytology specimen, a blood sample, a fine needle aspirate (FNA) sample, or any combination thereof. The cell may be obtained from a bodily fluid such as urine, milk, sweat, lymph, blood, sputum, amniotic fluid, aqueous humour, vitreous humour, bile, cerebrospinal fluid, chyle, chyme, exudates, endolymph, perilymph, gastric acid, mucus, pericardial fluid, peritoneal fluid, pleural fluid, pus, rheum, saliva, sebum, serous fluid, smegma, sputum, tears, vomit, or other bodily fluid. The cell

may comprise cancerous cells, non-cancerous cells, tumor cells, non-tumor cells, healthy cells, or any combination thereof.

[0376] In some embodiments, the cell can comprise a reassigned codon recognized by a stabilizing non-standard amino acid tRNA comprising an anticodon corresponding to the reassigned codon.

[0377] In some embodiments, the amino acid sequence of the stabilized phytase polypeptide can be encoded by a polynucleotide sequence comprising at least one codon of a natural amino acid that can have been replaced by the reassigned codon. In some embodiments, the amino acid sequence of the stabilized phytase polypeptide can be encoded by a polynucleotide sequence comprising at least one, two, or three stop codons or codons of a natural amino acid that can be replaced by the reassigned codon.

[0378] In some embodiments, the stabilizing non-standard amino acid tRNA can be a selenocysteine tRNA.

[0379] In some embodiments, the method can comprise culturing the cell under conditions in which the amino acid sequence of the stabilized phytase polypeptide can be expressed. In some embodiments, the reassigned codon can be UAG, UAA, UGA, or a combination thereof.

[0380] In some aspects, provided herein is a method comprising contacting a phytate substrate in an environment to a stabilized phytase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized phytase polypeptide, functional fragment thereof, or variant thereof catalyzes hydrolysis of the phytate substrate at a higher rate than a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids. In some embodiments, the stabilized phytase polypeptide, functional fragment thereof, or variant thereof can catalyze hydrolysis of a phytate substrate at a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 or greater fold higher rate than a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0381] In some embodiments, the stabilized phytase polypeptide, functional fragment thereof, or variant thereof can be the stabilized phytase polypeptide, functional fragment thereof, or variant thereof disclosed elsewhere herein. In some embodiments, the phytate substrate is from an animal feedstock. In some embodiments, the phytate substrate is in an animal feedstock. In some embodiments, the phytate substrate is all or a part of the phytates in an animal feedstock.

[0382] The enzymes described herein (e.g., stabilized phytases, stabilized xylanases, and stabilized mannanases) can be made in a host cell, or in vitro, in cell-free synthetic systems. Host cells may be any that can be robustly recoded. These can be bacterial cells that have well developed genetic systems, of which *E. coli* is exemplary. Other bacterial species can also be used. Cell-free systems for producing the proteins may be coupled transcription/translation systems or only translation systems. A notable aspect of the methods of the invention is the use of biological syntheses rather than chemical synthesis means.

[0383] Culturing of recoded cells with the constructed nucleic acid sequences may be by any means known in the art. The culturing may be batch or continuous, in shaker flasks or in fermenters or immobilized on solid surfaces, such as small particles contained in larger vessels. Typically the culture medium will be supplemented with a source of selenium, such as Na_2SeO_3 . As is known in the art, production of the desired protein variant may be under the control of an inducer or a repressor. Any such systems which are known in the art may be selected for convenience of construction and protein production.

Xylanase

[0384] In some aspects, provided herein is a composition comprising a stabilized xylanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof. In some embodiments, the stabilized xylanase polypeptide may be made and used according to the invention with diselenide bonds between two selenocysteine residues. In some embodiments, the stabilized xylanase polypeptide can comprise one or more non-standard amino acids. In some embodiments, the stabilized xylanase polypeptide can comprise one or more selenocysteine residues. In some embodiments, the stabilized xylanase polypeptide can comprise a diselenide bond between two selenocysteine residues. The diselenide bonds may be intramolecular or intermolecular. In some embodiments, the stabilized xylanase polypeptide can comprise one or more diselenide bonds. In some embodiments, the stabilized xylanase polypeptide can comprise one or more catalytic selenocysteine substitutions.

[0385] In some aspects, provided herein is a composition comprising a stabilized xylanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized xylanase polypeptide has a higher hydrolytic activity for a polysaccharide substrate in an environment than a hydrolytic activity for the polysaccharide substrate of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids. For example, the stabilized xylanase polypeptide can have at least 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 12, 13, 14, 15, 16,

17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 or greater fold higher hydrolytic activity for a polysaccharide substrate in an environment than a hydrolytic activity for the polysaccharide substrate of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0386] In some aspects, provided herein is a composition comprising a stabilized xylanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized xylanase polypeptide does not destabilize in an environment that a corresponding xylanases polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids does destabilize. The destabilization can be obtained by contacting the corresponding xylanase polypeptide with one or more destabilization agents. The destabilization can be obtained by placing the corresponding xylanase polypeptide in a destabilization environment. The environment to destabilize the corresponding xylanase polypeptide is described elsewhere herein. In some embodiments, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, has a melting temperature (T_m) that is at least 5°C higher than a T_m of a corresponding recombinant enzyme, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0387] In some aspects, provided herein is a composition comprising a stabilized xylanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized xylanase polypeptide has a melting temperature (T_m) that is at least 5°C higher than a T_m of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0388] In some embodiments, the composition can comprise a stabilized xylanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized xylanase polypeptide can have a melting temperature (T_m) that can be at least 1°C, 2°C, 3°C, 4°C, 5°C, 6°C, 7°C, 8°C, 9°C, 10°C, 11°C, 12°C, 13°C, 14°C, 15°C, 16°C, 17°C, 18°C, 19°C, 20°C, 21°C, 22°C, 23°C, 24°C, 25°C, 26°C, 27°C, 28°C, 29°C, 30°C, 31°C, 32°C, 33°C, 34°C, 35°C or 36°C higher than a T_m of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids. In some embodiments, the composition can comprise a stabilized xylanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized xylanase polypeptide can have a

melting temperature (T_m) that can be less than 1°C higher than a T_m of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0389] In some embodiments, at least one, two, three, four or more of the one or more non-standard amino acids is selenocysteine. In some embodiments, at least two of the one or more non-standard amino acids are directly linked by a bond.

[0390] In some embodiments, at least four of the one or more non-standard amino acids can be directly linked by a bond, wherein a first pair of the at least four of the one or more non-standard amino acids can be directly linked by a bond, and a second pair of the at least four of the one or more non-standard amino acids can be directly linked by a bond. In some embodiments, the bond is a diselenide bond or selenyl-sulfhydryl bond. In some embodiments, the bond is a diselenide bond. In some embodiments, the diselenide bond can be an intermolecular or an intramolecular bond. In some embodiments, the bond is a selenyl-sulfhydryl bond between a cysteine and a selenocysteine. In some embodiments, the selenyl-sulfhydryl bond can be an intermolecular or an intramolecular bond.

[0391] In some embodiments, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, can have a half-life that can be at least a 1.1 fold higher than a half-life of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids. In some embodiments, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, can have a half-life that can be at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 or greater fold higher than a half-life of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids. In some embodiments, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, can have a half-life that can be less than 1.1 fold higher than a half-life of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0392] In some embodiments, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, can have at least 70%, 75%, 80%, 85%, 90%, 95% sequence identity to a sequence selected from Table 2. In some embodiments, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, can have at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% or greater sequence identity to a sequence selected from

Table 2. In some embodiments, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, can have less than 10% sequence identity to a sequence selected from Table 2.

Table 2. Xylanase Sequences

SEQ ID	Protein Data Bank Accession Code	Sequence
31	3M4F	MKFTLTIAGLLAVGSTAAPTTEKRNPGGIDYVQNYNGDVADFQYNEGAGTYTCGWGSDTF VVGLGWSTGAARDITYSATYNAGGSGSYLAVYGWVNSPQAEYYIVESYGDYNPCSNAEGLG TLESDGSTYTVCTDTRTNEPSITGTSTFTQYWSVRQSERTSGTVTVGNHFNywaAQHGFGDSY NFQVMAVEAFSGSGSASVS
32	5MRJ	MHTRALALALALAAAPAAVFAQSPIWGQCGNGWPGPYTCVSGTVCKVVKSNPWLTPPSD WYHQCVPGTGDPDPDPEDPEDPTDPEDPGTNGELHDKFVAKGKLFFGTEIDHYHLNNQPLT NIVKSSFGQVTHENSMKWDAIEPSRGRFSWGNADAVVDFAVANGKMIRGHTLVWHSQLPD WVKQINDRNTLTQVIEDHVTAMVTRYKGIHVWDVVNEIFSEDGNLRDSVFSRVLGEDFVGI AFRAARAADPNKLYINDYNLDNANYAKVTRGMVEKVNRWVSQGVPIDGIGSQCHLAAPG GWNPASQVPGALRALAGANVKEIAVTELDIAGAAANDYLTVMNACLQIEKCVGITVWGVSD KDSWRQGDNPPLFDNNYQPKAAYHALVNAL
33	2COV	MGTEPPENCQDDFNFNYSVDQEIEVYHVDKGWSAGWNYVCLNDYCLPGNKSNGAFRKT FN AVLGQDYKLTfKVEDRYGQGQQLDRNITFTTQVCNLEHHHHHHH
34	1TA3	QSASLNDLFVAAGKSYFGTCSQALLQNSQNEAIVASQFGVITPENS MKWDALEPSQGNFGW SGADYLV DYATQHKKVRGHTLVWHSQLP SWVSSIGDANTLRSVMTNHINEVVG RYKGI MHWDVVNEIFNEDGTFRNSVFYNLLGEDFVRIAFETARAADPAKLYINDYNLDSASYAKTQ AMASYVKKWLAEGVPIDGIGSQAHYSSSHWSSTEAGALSSLANTGVSEVAITELDIAGAASS DYLNLLNACLNEQKCVGITVWGVSDKDSWRASDSPLFDGNYQPKDAYNAIVNALS
35	1T6G	SAGINYVQNYNGNLGDFTYDESAGTFSMYWEDGVSSDFVVG LGWTTGSSNAITYSAEYSAS GSSSYLAVYGWVNYPQAEYYIVEDYGDYNPCSSATSLGT VYSDGSTYQVCTDTRTNEPSITG TSTFTQYFSVRESTRSGTVTVANHFNFWAQHGFGNSDFNYQVMAVEAWSGAGSASVTISS
36	4XUY	EASVSIDTKFKAHGKKYLG NIGDQYTLTKNSKTPAIKADFGALTPENS MKWDATEPSRGQFS FSGSDYLVNFAQSNNKLIRGHTLVWHSQLP SWVQSITDKNTLIEVMKNHITVMQHYKGIY AWDVVNEIFNEDGSLRDSVFYK VIGEDYVRIAFETARAADPNKLYINDYNLDSASYPKL TG MVSHVKKWIAAGIPIDGIGSQTHLSAGGGAGISGALNALAGAGTKEIAVTELDIAGASSTDYV EVVEACL NQPKCIGITVWGVADPDSWRSSSTPLFD SNYNPKPAYTAIANAL

37	1XNC	ASTDYWQNWTDGGGIVNAVNGSGGNYSVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWA PNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTGTYKGTVKCDGGTYDIYTTTRYNAPSIDGD RTTFTQYWSVRQSKRPTGSNATITFTCHVNAWKSHGMNLGSNWAYQVMATEGYQSSGSSN VTVW
38	1AXK	FDCAEYRSTNIYGYGLYEVSMPAKNTGIVSSFFTYTGPAHGTQWDEIDIEFLGKDDTKVQFN YYTNGVGGHEKVISLGFDAASKGFHTYAFDWQPGYIKWYVDGVLKHTATANIPSTPGKIMMN LWNGTGVDWLGSYNGANPLYAEYDWVKYTSNASTDYWQNWTDGGGIVNAVNGSGGNY SVNWSNTGNFVVGKGWTTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWG TYRPTGTYKGTVKSDGGTYDIYTTTRYNAPSIDGDRTTFTQYWSVRQSKRPTGSNATITFSNH VNAWKSHGMNLGSNWAYQVMATEGYQSSGSSNVTWGSVFWEPKSYFNPSTWEKADGYS NGGVFNCTWRANNVNFTNDGKLKLGLTSSA
39	5XZO	WGLNNAARADGKLWFGTAADIPGLEQDDRYYMKEYNNTHDFGGTTPANIMKFMFTEPEQN VFNTGAQEFLDIAFASHKLVRCHNLIWQSELPTWVTNPTTNWTNETLSKVLQNHVYTLVSH FGDQCYSWDVVNEALSDDPAGSYQNNIWFDTIGPEYVAMAFEYAEKAVKDHKLVNKLYYN DYNIEYPGPKSTAAQNIVKELKARNIQIDGVGLESHFIAGETPSQATQITNMADFTSLDIDVAV TELDVRLYLPPNATSEAQQVADYYATVAACAATERCIGITVWDFDDTYSWVPSTFAGQGYA DLFFQPDGPNTPLVKKAAYDGCLQALQH
40	IPVX	GTPNSEGWHGYYYSWWSDDGGGDDSTYTNNSSGGTYEITWGNNGNLVGGKGWNPGLNARA IHFTGVYQPNGTSYLSVYGWTRNPLVEYYIVENFGSSNPSSGSTDLGTVSCDGSTYTLGQSTR YNAPSIDGTQTFNQYWSVRQDKRSSGTVQTGCHFDAWASAGLNVTDHYYQIVATEGYFSS GYARITVADVG
41	IE5B	TGCSVTATRAEEWSDGFNVITYSVSGSSAWTVNLALNGSQTIQASWVANVTGSGSTRVTPN GSGNTFGVTVMKNGSSTTPAATCAGS
42	1EXG	ASSGPAGCQVLWGVNQWNTGFTANVTVKNTSSAPVDGWTLTFSFSGQQVTQAWSSTVTQS GSAVTVRNAPWNGSIPAGGTAQFGFNGSHTGTNAAPTAFSLNGTPCTVG
43	IHEH	TGSCSVSAVRGEEWADRFNVITYSVSGSSSWVTLGLNGGQSVQSSWNAALTGSSGTVTARP NGSGNSFGVTFYKNGSSATPGATCATG
44	3CUI	ATTLKEAADGAGRDFGFALDPNRLSEAQYKAIADSEFNLVVAENAMKWDATEPSQNSFSFG AGDRVASYAADTGKELYGHTLVWHSQLPDWAKNLNGSAFESAMVNHVTKVADHFEGKVA SWDVVNEAFADGGGRRQDSAFQQLGNGYIETAFRAARAADPTAKLCINDYNVEGINAKSN SLYDLVKDFKARGVPLDCVGFQSHLIVGQVPGDFRQNLQRFADLGVDVRITELDIRMRTPSD ATKLATQAADYKKVVQACMQVTRCQGVTVWGITDKYSWVPDVFPGEAALVWDASYAKK PAYAAVMEAFGAS

45	1V6Y	AESTLGAAAAQSGRYFGTAIASGKLGDSAYTTIASREFNMVTAENEMKIDATEPQRGQFNFSA GDRVYNWAVQNGKQVRGHTLAWHSQQPGWMQSLSGSTLRQAMIDHINGVMGHYKGKIAQ WDVVNEAFSDDGSGGRRDSNLQRTGNDWIEVAFRTARAADPAAKLCYNDYNIENWTWAKT QGVYNMVRDFKQRGVPIDCVGFQSHLIVGQVPGDFRQNLQRFADLGVDVRITELDIRMRTPS DATKLATQAADYKKVVQACMQVTRCQGVTVWGITDKYSWVPDVFPGEGAALVWDASYAK KPAYAAVMEAFGSRSHHHHHH
46	5M0K	MITTSRHRARRRVVSLTTAGLVATAAVALAAPAHAGTTLGQSAERGRYFGVAIAAGRM NDGYTMGIVDREFDSIVAENEMKMDATEPNRNQFNFSNGDRIVNYALGKGKKVRGHTLAW HAQQPGWMQNMMSGQSLRDALLNHVSRVASYYRGKIHSDVVNEAFADDGRGSRDRSNLQR TGNDWIEAAAFRAARSADPGAKLCYNDYNTDGVNAKSTGVYNMVRDFKARGVPIDCVGFQS HLGTTVPSDYQANLQRFADLGVDVQITELDIQQGSNQANAYRQVVQACLAVSRCTGITVWG VRDTSWRTGANPLLFDGSGNKKAAYA AVLEALNAGGGNGGGNNGGSSSVDTNAWYV
47	1GNY	GNVVIEVDMANGWRGNASGSTSHSGITYSADGVTFAALGDGVGAVFDIARPTTLEDAVIAM VVNVSAEFKASEANLQIFAQLKEDWSKGEWDCLAGSSELTADTDLTLTCTIDEDDDKFNQTA RDVQVGIAKGTTPAGTITIKSVTITLAQEA
48	1US3	MVAASEGNVVIEVDMANGWRGNASGSTSHSGITYSADGVTFAALGDGVGAVFDIARPTTLE DAVIAMVVNVSAEFKASEANLQIFAQLKEDWSKGEWDCLAASSELTADTDLTLTCTIDEDDD KFNQTARDVQVGIAKGTTPAGTITIKSVTITLAQEAYSANVDHLRDLAPSDFIGVAVSNTDSA TYNLLTNSREQAVVKKHFNHLTAGNIMKMSYMQPTEGNFNFTNADAFVDWATENNMTVHG HALVWHSDYQVPNFMKNWAGSAEDFLAALDTHITTIVDHYEAKGNLVSWDVVNEAIDDNSP ANFRTTDSAFYVKSGNSSVYIERAFQTARAADPAVILYYNDYNIENQNAKTTKMVDMVKDF QARSIPIDGVGFQMHVCMNYPISIANISAAMKKVVDLGLLVKITELDVAVNQPHCDAYPANKI NPLTEAAQLAQKKRYCDVVKAYLDTVPVNQRGGISVWGTTDANTWLDGLYREQFEDEKIS WPLLFNNYNDKPALRGFADALIGTQCTNTHLHHHHHH
49	1W32	MGLASLADFIGVAVAASGGNADIFTSSARQNIVRAEFNQITAENIMKMSYMYSGSNFSFTNS DRLVSWAAQNGQTVHGHALVWHPSYQLPNWASDSNANFRQDFARHIDTVAAHFAGQVKS WDVVNEALFDSADDPDGRGSANGYRQSVFYRQFGGPEYIDEAFRRARAADPTAELYYNDFN TEENGAKTTALVNLVQRLNNGVPIDGVGFQMHVMNDYPSIANIRQAMQKIVALSPCLKIKIT ELDVRLLNPNYDGNSSNNYTNRNDCAVSCAGLDRQKARYKEIVQAYLEVPPGRRGGITVWG IADPDSWLYTHQNLDPDWPLLFNDNLQPKPAYQGVVEALSGR
50	2VUL	MISLKRVAALLCVAGLGMSAANAQTCLTSPQTGFHNGFFYSFWKDSPGTVNFCLLEGGRYTS NWSGINNWVGKGWQTGSRRNITYSGSFNTPGNGYLALYGWTTNPLVEYYVDSWGSWRP PGSDGTFLGTVNSDGGTYDIYRAQRVNAPSIIGNATFYQYWSVRQSKRVGGTITGNHFDW

		ASVGLNLGTHNYQIMATEGYQSSGSSDITVSS
51	3U7B	AASGLEAAMKAAGKQYFGTALTVRNDQGEIDIINNKNEIGSITPENAMKWEAIQPNRGQFNW GPADQHAAAATSRGYELRCHTLVWHSQLPSPWVANGNWNQTLQAVMRDHINAVMGRYRG KCTHWDVVNEALNEDGTYRDSVFLRVIGEAYIPIAFRMALAADPTTKLYYNDYNLEYGNAK TEGAKRIARLVKSYGLRIDGIGLQAHMTSESTPTQNTPTPSRAKLASVLQGLADLGVDVAYTE LDIRMNTPATQQKLQTNADAYARIVGSCMDVKRCVGITVWGISDKYSWVPGTFPGEGSALL WNDNFQKKPSYTSTLNTINRR
52	4XX6	PTSPFETLRAAAAPRYFGAALGVPHLLNFTHDPLFDVTAVLQFNGATPENEMKWAYIEPERN QFNFTGGDIVAAAFSAANDYVLRGHNLVWYQELAPWVETLTGEDLWNATVNHITVMTHYK ESFNIYAWDVVNEAFNDNGTYRENVWYTQLGPDYIPNAYAVARSVNTPSKLYINDYNTEGIN NKSDALLAVVQSMKAHNLVDGVGFQCHFFVGELPPDLEQNFAFVAAGVEIAVTELDIRMN LPPSQADIEQQARDYATVVNACKAQGAACVGITTWGITDLYSWIPSTYPGEGYALLFDDNYV PHPAFNATIQUALLA
53	3WP4	AEFQSFCSASHSGQSVKVTGNKVGTTGGVGYELWADSGNNSATFYSDSFSCTFQNAAGDYL CRSGLSFDSTKTPSQIGRMKADFKLVKQNSSNVGYSYVGVGWTRSPLEVEYIVDNWLSPPF PGDWVGNKKHGSFTIDGAQYTVYENTRTGPSIDGDTTFNQYFSIRQQARDCGTIDISAHFDQW EKLGMTMGKLHEAKVLGEAGNVNGGASGTADFPYAKVYIGD
54	4F8X	DIDLNKLAQRRGKHWFGTAAADIPGTAETDAAYLKVLKQNFGEITPANAMKFMYTETEQNV FNFTEGEQFLEVAERFGSKVRCHNLVWASQVSDFVTSKTWTAKELTAVMKNHIFKTVQHFG RRCYSWDVVNEALNGDGTFFSSSVWYDTIGEEFYLAFKYAQEALAQIGANDVKLYYNDYGI ENPGTKSTAVLQLVSNLRKRGIRIDGVGLESHFIVGETPSLADQLATKQAYIKANLDVAVTEL DVRFSTVPYYTAAQKQQAEDYYVSASCMNAGPRCIGVVWDFDDAYSWVPSAFAGQGG ACLFNNTLEAKPAYYAVADALEGKPCSV
55	1BG4	EASVSIKAFKAHGGKYLGTIGDQYTLTKNTKNPAIKADFGQLTPENSMKWDATEPNRGQF TFSGSDYLVNFAQSNGKLIRGHTLVWHSQLPGWVSSITDKNTLISVLKNHITVMTRYKGIY AWDVLNEIFNEDGSLRNSVFYNVIGEDYVRIAFETARSVDPNAKLYINDYNLDSAGYSKVN MVSHVKKWLAAGIPIDIGSQTHLGAGAGSAVAGALNALASAGTKEIAITELDIAGASSTDYV NVVNACLNQAKCVGITVWGVADPDSWRSSSSPLLFDGNYNPKAAYNAIANAL
56	1H12	AFNNNPSSVGAYSSGTYRNLAQEMGKTNIQKVNSTFDNMFGYNNTQQLYYPYTENGVIYA HYIKAINPDEGDDIRTEGQSWGMTAAVMLNKQEEFDNLWRFKAYQKNPDNHPDAKKQGV YAWKLKLNQNGFVYKVDEGPAPDGEEYFAFALLNASARWGNSGEFNYYNDAITMLNTIKNK LMENQIIRFSPYIDNLTDPSYHIPAFYDYFANNVTNQADKNYWRQVATKSRTLLKNHFTKVS SPHWNLPFTLSRLDGSPVIGYIFNGQANPGQWYEFDAWRVIMNVGLDAHLMGAQAWHKS

		VNKALGFLSYAKTNNKNCYEQVYSYGGAQNRGCAGEGQKAANAVALLASTNAGQANEFF NEFWLSQPTGDYRYYNGSLYMLAMLHVSGNFKFYNNFTN
57	1E8R	MGNQQCNWYGTL YPLCVTTNGWGWEDQRSCARSTCAAQPAPFGIVGSG
58	3MU7	GSLDIAVYWGQSFDER SLEATCDSGNYAYVIIGFLNTFGGGQTPALDISGHSPKGLEPQIKHCQ SKNVKVLLSIGGPAGPYSLDSRNDANDLAVYLHKNFLLPPAGTSES RPFNAVLGDIDFHIEH GGPSQYQLLANILSSFRLSGSEFALTAAPQCVYPDPNLGTVINSATFDAIWVQFYNNPQCSYSA SNASALMNAWKEWSMKARTDKVFLGFPAPHPDAAGSGYMPPTKVKFSVFPNAQDSTKFGGI MLWDSYWDTVSQFSNKILGKGV
59	3OIH	ANLDIAVYWGQNFDER SLEATCDTGNYAYVIIGFLNTFGGGQTPALDISGHSPSGLEPQIKHC QSKNVKVLLSIGGPKGPYSLDSRSDANDLAVYLFNNFLLPPGHSEN RPFNAVLGDIDFHIEH GGPSQYQLLANILSSFRLAGTEFALTAAPQCVYPDPNLGTVINSATFDAIWVQFYNNPQCSYSS GNAEALMNAWREWSMKARTKKVFLGFPAPHPDAAGSGYMPPEKVKFHVFPAAKKS YKFGGI MLWDSYWDTVSNFSSKILGEGW
60	1NQ6	AGALGDAAAAGRYFGAAVAANHLGEAAYASTLDAQFGSVTPENEMKWDAVESSRNSFSF SAADRIVSHAQSKGMKVRGHTLVWHSQLPGWVSPLAATDLRSAMNNHITQVMTHYKGGIHS WDVVNEAFQDGGSGARRSSPFQDKLGNGFIEEAFRTARTVDADAKLCYNDYNTDGQNAKSN AVYEMVKDFKQRGVPIDCVGFQSHFNSNSPVP SDFQANLQRFADLGVDVQITELDIEGSGSAQ AANYTKVVNACLAVTRCTGITVWGVTDKYSWRSGGTPLLFDGDYNKKPAYDAVLAAL
61	1KNM	EPPADGGQIKGVGSGRCLDVPDASTSDGTQLQLWDCHSGTNQQWAATDAGELRVYGDKCL DAAGTSNGSKVQIYSCWGGDNQKWRLNSDGSVVGVSGLCLDAVGNGTANGTLIQLYTCS NGSNQRWTRT
62	1V0L	AESTLGAAAAQSGRYFGTAIASGRLSDSTYTSIAGREFNMVTAENEMKIDATEPQRGQFNFS ADRVYNWAVQNGKQVRGHTLAWHSQQPGWMQSLSGSALRQAMIDHINGVMAHYKGGKIVQ WDVVNEAFADGSSGARRDSNLQRSGNDWIEVAFRTARAADPSAKLCYNDYNVENWTWAKT QAMYNMVRDFKQRGVPIDCVGFQSHFNSGSPYNSNFRTTLQNFAALGVDVAITELDIQGAPA STYANVTNDCLAVSRCLGITVWGVDRSDSWRSEQTPLLFNNDGSKKAAYTAVLDALNGGDS SEPPADGG
63	2D1Z	AESTLGAAAAQSGRYFGTAIASGKLGD SAYTTIASREFNMVTAENEMKIDATEPQRGQFNFS GDRVYNWAVQNGKQVRGHTLAWHSQQPGWMQSLSGSTLRQAMIDHINGVMGHYKGGKIAQ WDVVSHAFSDDGSGGRRDSNLQRTGNDWIEVAFRTARAADPAKLCYNDYNIENWTWAKT QGVYNMVRDFKQRGVPIDCVGFQSHFNSGSPYNSNFRTTLQNFAALGVDVAITELDIQGASS TYAAVTNDCLAVSRCLGITVWGVDRDTSWRSGDTPLLFGDGSKKAAYTAVLNALNGGSST PPPSGGGQIKGVGSGRCLDVPNASTTDGTQVQLYDCHSATNQWQTYTDAGELRVYGDKCLD

		AAGTGNGTKVQIYSCWGGDNQKWRLNSDGSIVGVQSGLCCLDAVGGGTANGTLIQLYSCSNG SNQRWTRT
64	2G3I	AESTLGAAAAQSGRYFGTAIASGKLGDSAYTTIASREFNMVTAENEMKIDATEPQRGQFNFS GDRVYNWAVQNGKQVRGHTLAWHSAQPGWMQSLSGSTLRQAMIDHINGVMGHYKGKIAQ WDVVNEAFSDDGSGGRRDSNLQRTGNDWIEVAFRTARAADPAAKLCYNDYNIENWTWAKT QGVYNMVRDFKQRGVPIDCVGFQSHFNSGSPYNSNFRITLQNFALGVDVAITELDIQGASS TYAAVTNDCLAVSRCLGITVWGVDRDTSWASGDTPLLFGDGSKKAAYTAVLNALNGGGS RSHHHHHH
65	3WUG	DTATLGELAEAKGRYFGSATDNPELPDTQYTQILGSEFSQITPENTMKWQYTEPSRGRFDYTA AEEIVDLAESNGQSVRGHTLVWHNQLPSWVDDVPAGELLGVMRDHITHEVDHFKGRLIHWD VVNEAFEEDGSRRQSVFQQKIGDSYIAEAFKAARAADPDVKLYYNDYNIIEGIGPKSDAVYEM VKSFKAQGIPIDGVGMQAHLIAGQVPASLQENIRRFADLGVDVALTELDIRMTLPRTAAKDAQ QATDYGAVVEACL VVSRCVGITVWDYTDKYSWVPSVFPQGGAALPWDEDFAKKPAYHAIA AALN
66	5HXV	MCITTSQTGTHNGYYSFWTNGGGEVTMCLGPGGEYSVTWVNCGDFTSGKGWNPANAQTV TYSGEFNPNGNAYLAVYGWTTDPLVEYYILESYGTYNPSSGLTLLGQVTSDDGGTYDIYSTQR VDQPSIEGTSTFNQYWSVRTEKRVGGTVTTANHF AAWKALGLEMGTYNMIVSTEGYESSG SSTITVS
67	5GYC	MGEQVVKPTDERIIDPSTANTQLTGGVCYNTTSGGNKPLAGSPYGYETWIDTGGGVCSLCWY GADQGGGAAFRATWTNPHDFLGR LGYFWNENKPYSHYENIYCGFNYTRSGRKTAGDYSYIG IYGWSRNPSASNSNERLIEYYIVEDWFGNQWQADTSPMGINTTGGTVMGSFTVDGSSYQIRN TRVNQPSIEGDKTFVQYFSIRQSPRKS GTISITEHFKKWEKLG MKLGDNMYECKFLIEAGAGE GFFDARLIQFYRADNEGNILQITPLEHHHHHH
68	IIIW	EAAQSVDQLIKARGKVYFGVATDQNRLTTGKNAAIIQANFGQVTPENSMKWDATEPSQGNF NFAGADYLVNWAQQNGKLIRGHTLVWHSQ LPSWVSSITDKNTLTNVMKNHITLMTRYKG KIRAWD VVNEAFNEDGSLRQTVFLNVIGEDYIPIAFQTARAADPNAKLYINDYNLDSASYPKT QAIVNRVKKWRAAGVPIDGIGSQTHLSAGQGASVLQALPLLASAGTPEVAITELDVAGASSTD YVNVNACLNVSSCVGITVWGVADPDSWRASTTPLLFDGNFNPKPAYNAIVQNLQQ
69	ITUX	AAAQSVDQLIDARGKVYFGVATDQNRLTTGKNAAIIQADFGQVTPENSMKWDATEPSQGNF NFAGADYLVNWAQQNGKLIRGHTLVWHSQ LPSWVVSITDKNTLTNVMKNHITTIMTRYIGKI RAWDVVNEAFNEDGSLRQTVFNNVIGEDYIPIAFRTARAADPNAKLYINDYNLDSASKPKTSA IVKRKVKWRAAGVPIDGIGSQTHLSAGQGASIDAALPNLASAGTPEVAITELDIAGATSTDYV DVVNACLVDVSCIGITVWGVADPDSWRASTTPLLFDGNFNPKPAYNAIVQLL

70	4BS0	MAEAAQSIDQLIKARGKVYFGVATDQNRLTTGKNAAIKADFGMVWPENSMQWDATEPSQG NFNFAGADYLVNWAQQNGKLIGAGCLVWHNFLPSWVSSITDKNTLINVMKNHITTLMTRYK GKIRTWDVVGEAFNEDGSLRQNVFLNVIGEDYIPIAFQTARAADPNKLYIMDYNLDSASYPK TQAIVNRVKQWRAAGVPIDGIGSQMHL SAGQGAGVLQALPLLASAGTPEVSILMLDVAGASP TDYVNVVNACLVNQSCVGITVMGVADPDSAFASSTPLLFDGNFNPKPAYNAIVQDLQQGSIE GRGHHHHHHH
71	1YNA	ETTPNSEGWHHDGYYYSWWSDGGAQATYTNLEGGTYEISWGDGGNLVGKGWNPGLNARAI HFEGVYQPNGNSYLA VYGWTRNPLVEYYIVENFGTYDPSSGATDLGTVECDGSIYRLGKTTR VNAPSIDGTQTFDQYWSVRQDKRTSGTVQTGCHFDAWARAGLVNNGDHYYQIVATEGYFSS GYARITVADV G
72	4XV0	EASQSIDQLIKRKGLYFGTATDRGLLQREKNAAIIQADLGQVTPENSMKWQSLENNQGQLN WGDADYLVNFAQQNGKSIRGHTLIWHSQLPWVNNINNADTLRQVIRTHVSTVVGRYKGI RAWDVVNEIFNEDGTLRSSVFSRLLGEEFVSIAFRAARDADPSARLYINDYNLDRANYGKVN GLKTYVSKWISQGVPI D GIGSQSHLSGGGGSGTLGALQQLATVPVTELAITELDIQGAPTTDYT QVVQACLSVSKCVGITVWGISDKDSWRASTNPLLFDANFNPKPAYNSIVGILQ
73	1T6E	LPVLAPVTKDPATSLYTI PFHDGASLVLDVAGPLVWSTCDGGQPPAEIPCSSPTCLLANAYPAP GCPAPSCGSDKHDKPCTAYPYNPVSGACAAGSLSHTRFVANTTDGSKPVSKVNVGVLAACAP SKLLASLPRGSTGVAGLANGLALPAQVASAQKVANRFLCLPTGGPGVAIFGGGPVPWPQF TQSMPYTPLVTKGGSPAHYISARSIVVGDRVPVPEGALATGGVMLSTRLPYVLLRPDVYRPL MDAFTKALAAQHANGAPVARAVEAVAPFGVCYDTKTLGNNLGGYAVPNVQLGLDGGSDW TMTGKNSMVDVKQGTACVAFVEMKGVAAGDGRAPAVILGGAQMEDFVLDFDMEKKRLGF SRLPHFTGCGGL
74	2DDX	LDGVLVPESGILVSVGQDVDSVNDYASALGTIPAGVTNYVGIVNLDGLNSDADAGAGRNNIA ELANAYPTSALVVGVS MNGEVDASGRYNANIDTLLNTLAGYDRPVYLRWAYEVDGPWN GHSPSGIVTSFYVHDRIIALGHQAKISLVWQVASYCPTPGGQLDQWWPGSEYVDWVGLSYF APQDCNWD RVNEAAQFARSKGKPLFLNESTPQRYQVADLTYSADPAKG TNRQSKTSQQLWD EWFAPYFQFMSDNSDIVKGFTYINADWDSQWRWAAPYNEGYWGDSRVQANALIKSNWQQE IAKGQYINHSETLFETLGYGSTHHHHHHH
75	N/A	MCSFTTAFVAATLAVGILASPAARPSALTKRNTPNSSGTNNGYFYQFWDGSSGTTTTYTNGP AGEYSVNWQNVGDFTAGKGWSQAEPRNISFSGSVNCGGNFYLA VYTWSTQGENYILEDYGT YNPCSGGTSGSLYSDGSEYQVCLVDRGNLYLQNW SVRQNKRRSSGTVTTANHYNYYQSHG MTHNPLSSAA YQIVSTEGYGSSGSADITVSEASPSTSSPTSTTSSTSPATTSSSSGTGAH WGQC GGTGWSGPTTCVSPYTCQQINPYYSQCL

76	N/A	MKVTAAGFAGLLATVLAAPAADSALVERASGINYVQNYNGNLGDFTYNESAGTYSMYWENG VNGDFVVGLGWSTGAARSITYSANYNAASSGSYLSVYGWINSPQAEYYIVENYGDYNPCSGA QSLGTLTSDGGTYQVCTDTRYNQPSITGTSTFTQFFSVRQSKRSSGTVTTGNHFNFWAQHGFG NSYNFQVMAVEAFSGAGSATVTVS
77	N/A	MVGFTPVALAALAATGALAFPAGNATELEKRQTPNSEGWHDGYYYSWWSDGGAQATYTN LEGGTYEISWGDGGNLVGGKGWNPGLNARAIHFEGVYQPNGNSYLAVYGWTRNPLVEYYIV ENFGTYDPSSGATDLGTVECDGSIYRLGKTTRVNAPSIDGTQTFDQYWSVRQDKRTSGTVQT GCHFDAWARAGLNVNGDHYYQIVATEGYFSSGYARITVADVG
78	N/A	MVGFTPVALAALAATGALAFPAGNATELEKRQTPNSEGWHDGYYYSWWSDGGAQATYTN LEGGTYEISWGDGGNLVGGKGWNPGLNARAVRPQDRNRIVRPRSFESEKLAFLTPTSLTCTQ IHFEGVYQPNGNSYLAVYGWTRNPLVEYYIVENFGTYDPSSGATDLGTVECDGSIYRLGKTTR VNAPSIDGTQTFDQYWSVRQDKRTSGTVQTGCHFDAWARAGLNVNGDHYYQIVATEGYFSS GYARITVADVG
79	N/A	MNEPLTITQARRRRRLGLRRIVTSAFALALAIAGALLPGTAHADTTITQNQTGYDNGYFYSFW TDAPGTVSMTLHSGGSYSTSWRNTGNFVAGKGWSTGGRRTVTYNASFNPSGNAYLTLYGWT RNPLVEYYIVESWGTYRPTGTYKGTVTDDGGTYDIYETWRYNAPSIEGTRTFQQFWSVRQQK RTSGTITIGNHFDAWARAGMNLGSHDYQIMATEGYQSSGSSTVSISEGGNPGNPGNPGNPGNP GNPGNPGGGCVATLSAGQQWSDRYNLNVSVSGSNNWTVRMDVPYPARIATWNIHAQWPES QVLIARPNGNGNNGWGTIQHNGNWTWPTVTCTAN
80	N/A	MGVNAFPRPGARRFTGGLYRALAAATVSVVGVVTALTVTQPASAAASTLAEGAAQHNRIFYG VAIAANRLNDSVYTNIANREFNSVTAENEMKIDATEPQQGRFDFTQADRIYNWARQNGKQVR GHTLAWHSQQPQWMQNLSGQALRQAMINHIQGVMSYYRGKIPIDVNEAFEDGNSGRRR DSNLQRTGNDWIEVAFRTARQADPSAKLCYNDYNIENWNAAKTQAVYNMVRDFKSRGVPID CVGFQSHFNSGNPYNPNFRITLQQFAALGVDVEVTELDIENAPAQTYASVIRDCLAVDRCTGI TVWGVSDSDSWRSYQNPLFDNNGNKKQAYYAVLDALNEGSDDDGGGPSNPPVSPPPGGGSG QIRGVASNRVIDVPNGNTADGTQVQLYDCHSGSNQQWYTYTSSGEFRIFGNKCLDAGGSSNGA VVQIYSCWGGANQKWELRADGTIVGVQSGLCCLDAVGGGTGNGTRLQLYSCWGGNNQKWS YNA
81	N/A	MVNFSTLFLAASTAALAAAAPSIKRQTLTSSATGTHNGYYSFWTDGQGNIRFNLESGGQYS VTWSGNGNWVGGKGWNPGLTDNRVINYTADYRPNNGNSYLAVYGWTRNPLIEYYVVEFSGTY DPSTGATRMGSVTTDGGTYNIYRTQRVNAPSIEGKTFYQYWSVRTSKRTGGTVTMANHFN AWRQAGLQLGSHDYQIVATEGYSSGSATVNVGGSTTGGNNGGNNGGNNGGNTGGSN VSISRPRKMGSLASTRS

82	N/A	MVRLSPVLLASIAGSGLPLAQAAGLNTAAKAIGLKYFGTATDNPELSDTAYETQLNNTQDFG QLTPANSKMWDATEPEQNVFTFSAGDQIANLAKANGQMLRCHNLVWYNQLPSWVTSGSWT NETLLAAMKNHITNVVTHYKGQCYAWDVVNEALNDDGTYRSNVFYQYIGEAYIPIAFATAA AADPNAKLYYNDYNEIEYPGAKATAAQNVLKLVQSYGARIDGVGLQSHFIVGETPSTSSQQQN MAAFTALGVEVAITELDIRMQLPETEALLTQQATDYQSTVQACANTKGCVGITVWDWTDKY SWVPSTFSGYGDACPWDANYQKKPAYEGILTGLGQTVTSTTYIISPTTSVGTGTTTSSGGSGG TTGVAQHWECQCGGLGWTGPTVCASGYTCTVINEEYYSQCL
83	N/A	MISFLTCQFEYTEPSPNVFNYSGGDTILAIENHGKRVCHNLIWVSQLPDWVNGSWTAASL TAVMKTHITNLITHWGGRCYSWDVVNEALAANGSWASSIWYDTIGPEYFFLAYRFAQEAVE KTGQDIKLYYNDYGIEAPGPKTTAAYNLVKELQARGIRIDGVGLESHEFVGATPSKDAQVEA KQGFLDLGVDVVTELDVRFPEGPFTYAAGEKQQAQDYDTVASCVEVGPRCVGITVWDFD DAYSWVPSSFPQGGAADLYNGTLQRKPAYYAVAEALQGVSCSVC
84	N/A	MRPSFLRYVSLAPLLWSAAASAEPVRVLDTFDPCLISTNGKYAFATTGNGVNVQIAQSD DFVTWERLAGTDALPGPFPSWVASSPTVWAPDVIQRLDGTVMYYSALSSTDPKHKCFGAAT STSITGPYPEDNYIACPLDQGGAIIDPDGFIDDDGTMYVVYKVDGSNLDGDGTHPTPIMLQA LEPDGITPTGDIKLLDRDASDGILIEAPSLVRSVVSQTYFLSYSSHYYASLHYDVGATGPAV KGPFTKAQAPLLVTGDNTNAGPLGGPGGADFSVDGTRIVFHAFENGRNLNTEGRALYTSQIV LEGDVIRLV

[0393] In some embodiments, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, can comprise a sequence with at least 70%, 75%, 80%, 85%, 90%, 95% sequence identity to at least 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, or 396 contiguous amino acids of a sequence selected from Table 2. In some embodiments, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, can comprise a sequence with at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% or greater sequence identity to at least 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, or 396 contiguous amino acids of a sequence selected from Table 2. In some embodiments, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, can comprise a sequence with less than 10% sequence identity to at least 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, or 396 contiguous amino acids of a sequence selected from Table 2.

[0394] In some embodiments, the sequence selected from Table 2 is SEQ ID NO: 71. In some embodiments, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, can comprise a sequence with at least 70%, 75%, 80%, 85%, 90%, 95% sequence

identity to at least 25, 50, 75, 100, 125, 150, 175, or 196 contiguous amino acids of SEQ ID NO: 71.

[0395] In some embodiments, the xylanase comprises an amino acid sequence with at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to at least 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, or 196 contiguous amino acids of SEQ ID NO: 71, where one or more non-standard amino acids such as selenocysteine are at positions 110 and/or 154.

[0396] In some embodiments, the xylanase further comprises at least one affinity tag. In some embodiments, an affinity tag of a xylanase is a C-terminal affinity tag. In some embodiments, an affinity tag of a xylanase is an N-terminal affinity tag. In some embodiments, a first affinity tag of a xylanase is an N-terminal affinity tag and a second affinity tag of a xylanase is a C-terminal affinity tag. In some embodiments, a first affinity tag of a xylanase is a first N-terminal affinity tag and a second affinity tag of a xylanase is a second N-terminal affinity tag. In some embodiments, a first affinity tag of a xylanase is a first C-terminal affinity tag and a second affinity tag of a xylanase is a second C-terminal affinity tag.

[0397] For example, the xylanase can comprise a poly-histidine tag, poly-histidine-glycine tag, poly-arginine tag, poly-aspartate tag, poly-cysteine tag, poly-phenylalanine, c-myc tag, Herpes simplex virus glycoprotein D (gD) tag, FLAG tag, KT3 epitope tag, tubulin epitope tag, T7 gene 10 protein peptide tag, streptavidin tag, streptavidin binding peptide (SPB) tag, Strep-tag, Strep-tag II, albumin-binding protein (ABP) tag, alkaline phosphatase (AP) tag, bluetongue virus tag (B-tag), calmodulin binding peptide (CBP) tag, chloramphenicol acetyl transferase (CAT) tag, choline-binding domain (CBD) tag, chitin binding domain (CBD) tag, cellulose binding domain (CBP) tag, dihydrofolate reductase (DHFR) tag, galactose-binding protein (GBP) tag, maltose binding protein (MBP), glutathione-S-transferase (GST), Glu-Glu (EE) tag, human influenza hemagglutinin (HA) tag, horseradish peroxidase (HRP) tag, NE-tag, HSV tag, ketosteroid isomerase (KSI) tag, KT3 tag, LacZ tag, luciferase tag, NusA tag, PDZ domain tag, AviTag, Calmodulin-tag, E-tag, S-tag, SBP-tag, Softag 1, Softag 3, TC tag, VSV-tag, Xpress tag,

Isopeptag, SpyTag, SnoopTag, Profinity eXact tag, Protein C tag, S1-tag, S-tag, biotin-carboxy carrier protein (BCCP) tag, green fluorescent protein (GFP) tag, small ubiquitin-like modifier (SUMO) tag, tandem affinity purification (TAP) tag, HaloTag, Nus-tag, Thioredoxin-tag, Fc-tag, CYD tag, HPC tag, TrpE tag, ubiquitin tag, VSV-G epitope tag, V5 tag, or a combination thereof.

[0398] In some embodiments, the xylanase further comprises at least two affinity tags. For example, the xylanase can comprise at least two affinity tags selected from a poly-histidine tag, poly-histidine-glycine tag, poly-arginine tag, poly-aspartate tag, poly-cysteine tag, poly-phenylalanine, c-myc tag, Herpes simplex virus glycoprotein D (gD) tag, FLAG tag, KT3 epitope tag, tubulin epitope tag, T7 gene 10 protein peptide tag, streptavidin tag, streptavidin binding peptide (SPB) tag, Strep-tag, Strep-tag II, albumin-binding protein (ABP) tag, alkaline phosphatase (AP) tag, bluetongue virus tag (B-tag), calmodulin binding peptide (CBP) tag, chloramphenicol acetyl transferase (CAT) tag, choline-binding domain (CBD) tag, chitin binding domain (CBD) tag, cellulose binding domain (CBP) tag, dihydrofolate reductase (DHFR) tag, galactose-binding protein (GBP) tag, maltose binding protein (MBP), glutathione-S-transferase (GST), Glu-Glu (EE) tag, human influenza hemagglutinin (HA) tag, horseradish peroxidase (HRP) tag, NE-tag, HSV tag, ketosteroid isomerase (KSI) tag, KT3 tag, LacZ tag, luciferase tag, NusA tag, PDZ domain tag, AviTag, Calmodulin-tag, E-tag, S-tag, SBP-tag, Softag 1, Softag 3, TC tag, VSV-tag, Xpress tag, Isopeptag, SpyTag, SnoopTag, Profinity eXact tag, Protein C tag, S1-tag, S-tag, biotin-carboxy carrier protein (BCCP) tag, green fluorescent protein (GFP) tag, small ubiquitin-like modifier (SUMO) tag, tandem affinity purification (TAP) tag, HaloTag, Nus-tag, Thioredoxin-tag, Fc-tag, CYD tag, HPC tag, TrpE tag, ubiquitin tag, VSV-G epitope tag, and V5 tag.

[0399] In some embodiments, the xylanase comprises an affinity tag that is GST. In some embodiments, the xylanase comprises an affinity tag that is a poly-histidine tag, such as a 6x-His tag. In some embodiments, the xylanase comprises an affinity tag that is MBP. In some embodiments, the xylanase comprises an affinity tag that is a strep-tag, such as two strep tags.

[0400] In some embodiments, the xylanase comprises a first affinity tag that is GST and a second affinity tag that is a poly-histidine tag, such as a 6x-His tag. In some embodiments, the xylanase comprises a first affinity tag that is GST and a second affinity tag that is a strep tag. In some embodiments, the xylanase comprises a first affinity tag that is a strep tag, such as two strep tags, and a second affinity tag that is a poly-histidine tag, such as a 6x-His tag. In some embodiments, the xylanase comprises a first affinity tag that is MBP and a second affinity tag

that is a poly-histidine tag, such as a 6x-His tag. In some embodiments, the xylanase comprises a first affinity tag that is MBP and a second affinity tag that is a strep tag, such as two strep tags.

[0401] In some embodiments, the xylanase comprises a first affinity tag that is GST, a second affinity tag that is a poly-histidine tag, such as a 6x-His tag, and a third affinity tag that is a strep tag, such as two strep tags. In some embodiments, the xylanase comprises a GST tag, a His tag, and two strep tags. In some embodiments, the xylanase comprises a first affinity tag that is MBP, a second affinity tag that is a poly-histidine tag, such as a 6x-His tag, and a third affinity tag that is a strep tag, such as two strep tags. In some embodiments, the xylanase comprises a MBP tag, a His tag, and two strep tags.

[0402] In some embodiments, the xylanase comprises an affinity tag, wherein the xylanase and affinity tag are separated by a linker. In some embodiments, the xylanase comprises a first affinity tag and a second affinity tag, wherein the xylanase and the first affinity tag are separated by a linker, and wherein the xylanase and the second affinity tag are separated by a linker. In some embodiments, the xylanase comprises a first affinity tag and a second affinity tag, wherein the first and second affinity tags are separated by a linker. In some embodiments, the xylanase comprises a first affinity tag, a second affinity tag and a third affinity tag, wherein the first, second and third affinity tags are each separated by a linker. In some embodiments, the xylanase comprises a first affinity tag, a second affinity tag, a third affinity tag and a fourth affinity tag, wherein the first, second, third and fourth affinity tags are each separated by a linker. In some embodiments, a linker comprises an amino acid sequence of (GS)_n, (GGS)_n, or (GGGS)_n or a combination thereof, where n is an integer of from 1-10.

[0403] In some embodiments, the one or more non-standard amino acids can be at position 110 of SEQ ID NO: 71 or position 154 of SEQ ID NO: 71. In some embodiments, the one or more non-standard amino acids can be at position 110 of SEQ ID NO: 71 and position 154 of SEQ ID NO: 71. In some embodiments, a non-standard amino acid at position 110 can be directly linked by a bond to a non-standard amino acid at position 154.

[0404] In some embodiments, the bond can be a diselenide bond or selenyl-sulfhydryl bond. In some embodiments, the bond is a diselenide bond. In some embodiments, the bond is a selenyl-sulfhydryl bond. In some embodiments, the diselenide bond or selenyl-sulfhydryl bond can be in a location of a disulfide bond in a corresponding recombinant enzyme without the one or more non-standard amino acids.

[0405] In some embodiments, the T_m of the corresponding stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, can be less than 37°C. In some embodiments, the T_m of the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, can be

greater than 37°C, 40°C, 45°C, 50°C, 55°C, 60°C, or 65°C. In some embodiments, the T_m of the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, can be at least 37°C, 40°C, 45°C, 50°C, 55°C, 60°C, 65°C or greater.

[0406] In some embodiments, the T_m of the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, can be at least 10°C higher than the T_m of the corresponding recombinant enzyme, functional fragment thereof, or variant thereof. In some embodiments, the T_m of the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, can be at least 15°C higher than the T_m of the corresponding recombinant enzyme, functional fragment thereof, or variant thereof. In some embodiments, the T_m of the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, can be at least 15°C, 20°C, 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, 65°C or greater higher than the T_m of the corresponding recombinant enzyme, functional fragment thereof, or variant thereof. In some embodiments, the T_m of the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, can be less than 10°C higher than the T_m of the corresponding recombinant enzyme, functional fragment thereof, or variant thereof.

[0407] In some embodiments, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, can have a half-life in an environment that can be at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 or greater fold higher than a half-life of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids in the environment. In some embodiments, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, can have a half-life in an environment that is at least a 1.1 fold higher than a half-life of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids in the environment.

[0408] In some embodiments, the half-life of the xylanase polypeptide, functional fragment thereof, or variant thereof in the environment, can be greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or more hours. In some embodiments, the half-life of the xylanase polypeptide, functional fragment thereof, or variant thereof in the environment, can be less than 1 hour.

[0409] In some embodiments, the half-life of the xylanase polypeptide, functional fragment thereof, or variant thereof in the environment, can be greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or more days. In some embodiments, the half-

life of the xylanase polypeptide, functional fragment thereof, or variant thereof in the environment, can be less than 1 day.

[0410] In some embodiments, the stabilized xylanase polypeptide can have at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 fold higher hydrolytic activity for a polysaccharide substrate in an environment than a hydrolytic activity for the polysaccharide substrate of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids. In some embodiments, the stabilized xylanase polypeptide can have less than a 1.1 fold higher hydrolytic activity for a polysaccharide substrate in an environment than a hydrolytic activity for the polysaccharide substrate of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0411] In some embodiments, the stabilized xylanase polypeptide can have at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 fold higher hydrolytic activity for a polysaccharide substrate after being present in an environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or 50 minutes than a hydrolytic activity for the polysaccharide substrate of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids after being present in the environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or 50 minutes. In some embodiments, the stabilized xylanase polypeptide can have less than a 1.1 fold higher hydrolytic activity for a polysaccharide substrate after being present in an environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or 50 minutes than a hydrolytic activity for the polysaccharide substrate of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids after being present in the environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or 50 minutes.

[0412] In some embodiments, the stabilized xylanase polypeptide can have at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 fold higher hydrolytic activity for a polysaccharide substrate after being present in an environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, or 24 hours than a hydrolytic activity for the polysaccharide substrate of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids after being present in the environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, or 24 hours. In some embodiments, the stabilized xylanase polypeptide can have less than a 1.1 fold higher hydrolytic

activity for a polysaccharide substrate after being present in an environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, or 24 hours than a hydrolytic activity for the polysaccharide substrate of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids after being present in the environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, or 24 hours.

[0413] In some embodiments, the (i) stabilized xylanase polypeptide, (ii) a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids, or (iii) both may lose hydrolytic activity in a reducing environment, as compared to its activity in a non-reducing environment. For example, the loss of hydrolytic activity of a stabilized xylanase polypeptide in a reducing environment, as compared to its activity in a non-reducing environment may be at least or at most 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30%, 40%, 50%, or 60%. For another example, the loss of hydrolytic activity of the corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids in a reducing environment, as compared to its activity in a non-reducing environment may be at least or at most 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 99.9%. In some embodiments, the loss of hydrolytic activity in a reducing environment, as compared to the hydrolytic activity in a non-reducing environment is less for the stabilized xylanase polypeptide, for example at least or at most 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% less, than for a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids. Under the same conditions, for example, when comparing an enzyme's activity under a reducing environment and the enzyme's activity under a non-reducing environment, the activity difference such as a loss of activity under the reducing environment is less significant for a stabilized xylanase than a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids, e.g., at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% less.

[0414] In some embodiments, the polysaccharide substrate comprises linear and/or branched polysaccharide. In some embodiments, the polysaccharide substrate comprises plant fibers such as cellulose and hemicellulose. In some embodiments, the polysaccharide substrate comprises starch. In some embodiments, the polysaccharide substrate comprises structural polysaccharides such as arabinoxylans, cellulose, chitins, and pectins. In some embodiments, the polysaccharide substrate is in an animal feedstock, such as the feedstock described in the present disclosure.

[0415] In some embodiments, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, can be recombinant. In some embodiments, the recombinant can be generated using recombinant DNA technology, such as, for example, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof expressed by a bacteriophage or yeast expression system. In other embodiments, the recombinant can be generated by the synthesis of a DNA molecule encoding the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof.

[0416] In some embodiments, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, can be an animal xylanase, a plant xylanase, a fungi xylanase, or a bacterial xylanase. The stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, can be any kinds of xylanase, including, but not limited to, *E.coli* xylanase, *Microcella alkaliphila* xylanase, *Lactobacillus algidus* xylanase, *Vibrio cholerae* xylanase, *Bifidobacterium longum* xylanase, *Homo sapiens* xylanase, and *Raoultella ornithinolytica* xylanase. In some embodiments, a composition can comprise stabilized animal xylanases, stabilized plant xylanases, stabilized fungi xylanases, stabilized bacterial xylanases, or any combination thereof.

[0417] In some embodiments, the composition further comprises a phytase. In some embodiments, the composition further comprises other carbohydrase such as mannanase. In some embodiments, the phytase is a stabilized phytase comprising one or more non-standard amino acid, a functional fragment thereof, or a variant thereof. In some embodiments, the mannanase is a stabilized mannanase comprising one or more non-standard amino acid, a functional fragment thereof, or a variant thereof.

[0418] In some embodiments, a composition can comprise a polynucleotide encoding the composition disclosed herein. In some embodiments, the polynucleotide can be a vector. The vector can be a fragment of nucleic acid molecules. The vector can be taken from a virus, a plasmid, or the cell of a higher organism. The vector can be stably maintained in an organism. The vector can be inserted with a foreign nucleic acid fragment for cloning purposes. The vector can comprise features that allow for the convenient insertion or removal of a nucleic acid fragment to or from a vector. The vector can be genetically engineered plasmids.

[0419] In some embodiments, a bond directly linking two of the one or more non-standard amino acids of the stabilized xylanase polypeptide may not break in an environment, when the bond directly linking two of the one or more standard amino acids of the corresponding xylanase polypeptide may break in the same environment.

[0420] In some embodiments, the method of making the composition disclosed herein can comprise expressing an amino acid sequence of the stabilized xylanase polypeptide. In some embodiments, expressing can comprise expressing in a cell or *in vitro*.

[0421] In some embodiments, the cell can be a bacterial cell. In some embodiments, the cell can be a genomically recoded cell. In some embodiments, the cell may not be a bacterial cell. The cell can be obtained or isolated from a subject. The cell can be obtained or isolated from a tissue. The subject may be an animal such as a human, a mouse, a rat, a pig, a dog, a rabbit, a sheep, a horse, a chicken or other animal. A cell may be a neuron. The cell may be one of the cells of a blood-brain barrier system. The cell may be a cell line, such as a neuronal cell line. The cell may be a primary cell, such as cells obtained from a brain of a subject. The cell may be a population of cells that may be isolated from a subject, such as a tissue biopsy, a cytology specimen, a blood sample, a fine needle aspirate (FNA) sample, or any combination thereof. The cell may be obtained from a bodily fluid such as urine, milk, sweat, lymph, blood, sputum, amniotic fluid, aqueous humour, vitreous humour, bile, cerebrospinal fluid, chyle, chyme, exudates, endolymph, perilymph, gastric acid, mucus, pericardial fluid, peritoneal fluid, pleural fluid, pus, rheum, saliva, sebum, serous fluid, smegma, sputum, tears, vomit, or other bodily fluid. The cell may comprise cancerous cells, non-cancerous cells, tumor cells, non-tumor cells, healthy cells, or any combination thereof.

[0422] In some embodiments, the cell can comprise a reassigned codon recognized by a stabilizing non-standard amino acid tRNA comprising an anticodon corresponding to the reassigned codon.

[0423] In some embodiments, the amino acid sequence of the stabilized xylanase polypeptide can be encoded by a polynucleotide sequence comprising at least one codon of a natural amino acid that can have been replaced by the reassigned codon. In some embodiments, the amino acid sequence of the stabilized xylanase polypeptide can be encoded by a polynucleotide sequence comprising at least one, two, or three stop codons or codons of a natural amino acid that can be replaced by the reassigned codon.

[0424] In some embodiments, the stabilizing non-standard amino acid tRNA can be a selenocysteine tRNA.

[0425] In some embodiments, the method can comprise culturing the cell under conditions in which the amino acid sequence of the stabilized xylanase polypeptide can be expressed. In some embodiments, the reassigned codon can be UAG, UAA, UGA, or a combination thereof.

[0426] In some aspects, provided herein is a method comprising contacting a polysaccharide substrate in an environment to a stabilized xylanase polypeptide comprising one or more non-

standard amino acids, a functional fragment thereof, or a variant thereof; wherein the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof catalyzes hydrolysis of the polysaccharide substrate at a higher rate than a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids. In some embodiments, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof can catalyze hydrolysis of a polysaccharide substrate at a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 or greater fold higher rate than a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0427] In some embodiments, the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof can be the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof disclosed elsewhere herein. In some embodiments, the polysaccharide substrate is from an animal feedstock. In some embodiments, the polysaccharide substrate is in an animal feedstock. In some embodiments, the polysaccharide substrate is all or a part of the polysaccharides in an animal feedstock.

Mannanase

[0428] In some aspects, provided herein is a composition comprising a stabilized mannanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof. In some embodiments, the stabilized mannanase polypeptide may be made and used according to the invention with diselenide bonds between two selenocysteine residues. In some embodiments, the stabilized mannanase polypeptide can comprise one or more non-standard amino acids. In some embodiments, the stabilized mannanase polypeptide can comprise one or more selenocysteine residues. In some embodiments, the stabilized mannanase polypeptide can comprise a diselenide bond between two selenocysteine residues. The diselenide bonds may be intramolecular or intermolecular. In some embodiments, the stabilized mannanase polypeptide can comprise one or more diselenide bonds. In some embodiments, the stabilized mannanase polypeptide can comprise one or more catalytic selenocysteine substitutions. In some embodiments, the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, has a melting temperature (T_m) that is at least 5°C higher than a T_m of a corresponding recombinant enzyme, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0429] In some aspects, provided herein is a composition comprising a stabilized mannanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized mannanase polypeptide has a higher hydrolytic activity for a beta-D-mannoside substrate in an environment than a hydrolytic activity for the beta-D-mannoside substrate of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids. For example, the stabilized mannanase polypeptide can have at least 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 or greater fold higher hydrolytic activity for a beta-D-mannoside substrate in an environment than a hydrolytic activity for the beta-D-mannoside substrate of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0430] In some aspects, provided herein is a composition comprising a stabilized mannanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized mannanase polypeptide does not destabilize in an environment that a corresponding mannanases polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids does destabilize. The destabilization can be obtained by contacting the corresponding mannanase polypeptide with one or more destabilization agents. The destabilization can be obtained by placing the corresponding mannanase polypeptide in a destabilization environment. The environment to destabilize the corresponding mannanase polypeptide is described elsewhere herein.

[0431] In some aspects, provided herein is a composition comprising a stabilized mannanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized mannanase polypeptide has a melting temperature (T_m) that is at least 5°C higher than a T_m of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0432] In some embodiments, the composition can comprise a stabilized mannanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized mannanase polypeptide can have a melting temperature (T_m) that can be at least 1°C, 2°C, 3°C, 4°C, 5°C, 6°C, 7°C, 8°C, 9°C, 10°C, 11°C, 12°C, 13°C, 14°C, 15°C, 16°C, 17°C, 18°C, 19°C, 20°C, 21°C, 22°C, 23°C, 24°C, 25°C, 26°C, 27°C, 28°C, 29°C, 30°C,

31°C, 32°C, 33°C, 34°C, 35°C or 36°C higher than a T_m of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids. In some embodiments, the composition can comprise a stabilized mannanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized mannanase polypeptide can have a melting temperature (T_m) that can be less than 1°C higher than a T_m of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0433] In some embodiments, at least one, two, three, four or more of the one or more non-standard amino acids is selenocysteine. In some embodiments, at least two of the one or more non-standard amino acids are directly linked by a bond.

[0434] In some embodiments, at least four of the one or more non-standard amino acids can be directly linked by a bond, wherein a first pair of the at least four of the one or more non-standard amino acids can be directly linked by a bond, and a second pair of the at least four of the one or more non-standard amino acids can be directly linked by a bond. In some embodiments, the bond is a diselenide bond or selenyl-sulfhydryl bond. In some embodiments, the bond is a diselenide bond. In some embodiments, the diselenide bond can be an intermolecular or an intramolecular bond. In some embodiments, the bond is a selenyl-sulfhydryl bond between a cysteine and a selenocysteine. In some embodiments, the selenyl-sulfhydryl bond can be an intermolecular or an intramolecular bond.

[0435] In some embodiments, the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, can have a half-life that can be at least a 1.1 fold higher than a half-life of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids. In some embodiments, the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, can have a half-life that can be at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 or greater fold higher than a half-life of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids. In some embodiments, the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, can have a half-life that can be less than 1.1 fold higher than a half-life of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0436] In some embodiments, the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, can have at least 70%, 75%, 80%, 85%, 90%, 95% sequence identity to a sequence from Table 3. In some embodiments, the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, can have at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% or greater sequence identity to a sequence from Table 3. In some embodiments, the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, can have less than 10% sequence identity to a sequence from Table 3.

Table 3. Mannanase Sequence

SEQ ID	Protein Data Bank Accession Code	Sequence
85	1BQC	ATGLHVKNGRLYEANGQEFIIRGVSHPHNWYPQHTQAFADIKSHGANTVRVVLNNGVVRWSKN GPSDVANVISLCKQNRILCMLEVHDTTGYGEQSGASTLDQAVDYWIELKSVLQGEEDYVLINI GNEPYGND SATVAAWATDTSAAIQLRLAAGFEHTLVVDAPNWGQDWTNTMRNNADQVYAS DPTGNTVFSIHMYGVYSQASTITSYLEHFVNAGLPLIIGEFHSDHSDGNPDEDTIMAEERLKL YIGWSWSGNGGGVEYLDMMVYNFDGDNLSWPGERIFYGPNGIASTAKEAVIFG
86	1QNR	ASSFVTISGTQFNIDGKVG YFAGTNCYWC SFLT NHADVDSTF SHISSGLKVV RVWGFNDVNT QPSPGQIWFQKLSATGSTINTGADGLQTL DYVVQSAEQHNKLIIPFVN NWS DYGGINAYVNAF GGNATTWYTNTAAQTQYRK YVQAVVSRYANST AIFAWELGNEPRCNGCSTDVIVQWATSVS QYVKSLDSNHLVTLGDEGLGLSTGDGAYPYTYGEGTDFAKNVQIKSLDFGTFLYPDSWGTN YTWGNGWIQTHAAACLAAGKPCVFEEYGAQQNPCTNEAPWQTSLTTRGMGGDMFWQWG DTFANGAQSN SDPYTVWYNSSNWQCLVKNHVDAIN
87	1RH9	FSNNNFVYTDGTHFALNGKSLYINGFNAYWLMYIAYDPSTRIKVTNTFQQASKYKMN VARTW AFSHGGSRLQ SAPGVYNEQMFQGLDFVISEAKKYGIHLIMSLVNNWDAFGGKKQYVEWAVQ RGQKLTSDDDFFTNP MVKGFYKNNVKVVLTRVNTITKVAYKDDPTILSWELINEPRCPSDL SG KTFQNWVLEMAGYLKSIDSNHLL EIGLEGFYGNDMRQYNPNSYIFGTNFISNNQVQGIDFTTIH MYPNQWLPGLTQEAQDKWASQW IQVHIDDSKMLKKPLLIAEFGKSTKTPGYTVAKRDNYFEK IYGTIFNCAKSGGPCGGGLFWQVLGQGMSSFDDGYQVVLQESPSTSRVILLQSLRLSKLS
88	3CBW	MSLAKPIEAHTVSPVNPNAQQTTKTVMNWL AHLNRTENRVL SGAFGGYSHDTFSMAEADRI RSATGQSPA IYGCDYARGWLETANIEDSIDVSCNGDLMSYWKNGGIPQISLHLANPAFQSGHFK TPITNDQYKKILDSSTVEGKRLNAML SKIADGLQELNQGV PVLFRPLHEMNGEWFWWGLTS YNQKDNERISLYKQLYKKIYHYMTDTRGLDHLI WVYSPDANRDFKTD FYPGASYVDIVGLDA YFQDAYSINGYDQLTALNKPF AFTEVGPQTANGSFDYSLFINAIKQKYPKTIYFLAWNDEWSA AVNKGASALYHDSWTLNKGEIWN GDSLTPIVEEGHHHHHHH

89	3VUP	RLHIQNGHFVLNGQRVFLSGGNLPWMSYAYDFGDGQWQRNKNRIEPEFKKLHDAGGNSMRL WIHIQGETTPAFNDQGFVTGPDKQGTMLDDMKDLLDTAKKYNILVFPCLWNAAVNQDSHNRL DGLIKDQHKLQSYIDKALKPIVNHVKGHVALGGWDLMNEPEGMMIPDKHNAEKCYPDTTALK NSGAGWAGNKYLYQDILRFLNWQADAIKTTDPGALVTMGVWNPKSNTDHFNMNNHYSDBC LRLAGGKQKGVDFYQFHSYSWQGWDEVAPFTHQASDYGLHKPIVVGEFWEQDGGGMTIT QMFNYVYNHGYAGAWSWHLVQRGDNQRKGITNIKDKTSNGKIPISL
90	3WFL	DTFPGTNGLDFTIDGTAGYFAGSNAYWLAFLTNNDVDKVLGDAESSGLRIMRVWGFNDVNT VPSSGTVYFQLLQDGTATINTGSDGLERLDYVVSAAEQHNVKLIINFVNNWSDYGGIPAYVNAF GGSTTSWYTDEASQAAYRNYIKTVVSRYIDSPAVFAWELANEPRCHGCDTSVIYNWVAATSSF IKSIDSQHLVCIGDEGLGLDIDSDGSYPYSYEGTNFTLNLGVDTIDFGTFHLYPSSWGVNSFG SPWVTAHGAACAAAGKPCLFEEYGVTSDKCSVEGGWQQTALDTRGIGADSFQFGDTLSTG QSPNDGYTIYYGTDDYTCLVTDRVASI
91	3WH9	SFASTSGLQFTIDGETGYFAGTNSYWIGFLTDNADVDLVMGHLKSSGLKILRVWGFNDVTSQP SSGTVWYQLHQDGKSTINTGADGLQRLDYVVSAAEQHDIKLIINFVNYWTDYGGMSAYVSAY GGSETDFYTSDTMQSAYQTYIKTVVERYSNSSAVFAWELANEPRCPCDTSVLYNWIEKTSK FIKGLDADMVCIGDEGFGLNIDSDGSYPYQFSEGLNFTMNLIDIDTIDFGTLHLYPDSWGTSD WGNGWITAHGAACKAAGKPCLLEEYGVTSNHCSVEGAWQKTALSTTGVGADLFWQYGDDL STGKSPDDGNTIYYGTSYQCLVTDHVAAGISA
92	3ZIZ	MGFLPQAQGGGAAASAKVSGTRFVIDGKTGYFAGTNSYWIGFLTNNRDVDTTLDHIASSGLKI LRVWGFNDVNNQPSGNTVWFQRLASSGSQINTGPNGLQRLDYLVSAETRGIKLIILVNYWD DFGGMKAYVNAFGGTKEWYTNAQAQEQYKRYIQAVVSRYVNSPAIFAWELANEPRCKGCN TNVIFNWATQISDYIRSLDKDHLITLGDEGFGLPGQTTYPYQYGEGETDFVKNLQKNLDFGTFH MYPGHWGVPTSFGPGWIKDHAAACRAAGKPCLLEEYGYESDRCNVQKGWQQASRELSRDG MSGDLFWQWGDQLSTGQTHNDGFTIYYGSSLATCLVTDHVRINALPAGDEQKLISEEDLNSA VDHHHHHHH
93	4AWE	KVPKGFVTTEGDHFKLDGKDFYFAGSNAYYFPFNDQPDIEKGMTAARAAGLTVFRTWGFNDK NRTYIPTGLPQYGNAGDPTNTVFQWFEADGTQTIDVSPFDKVVDSATKTGIKLIVALTNNW ADYGGMDVYTVNLGGKYHDDFYTPKIKEAFKRYVKAMVTRYRDSEAILAWELANEARCGA DGTRNLPRSEKGCTTETVTGWIEEMSAYVKSLEDGNHLVTWGEGGFNRGEDEEDGFYNGAD GGDFDRELGLRNVDFGTMHLYPDWWSKSIEWSNQWIHDHAASGRAANKPVVLEEYGWMTD KGRDLQLGQVKNETRLEVVGWQKIAIQEKLADMYWQFGYGGYSYGRNHDDSFTIYLEDD EAKELVYKHAKKVQKLNER
94	4OOU	MVKLFSFLLLWVVASPAFSSEFLKASGSNFYGGQKVFLSGVNFAWRSYGSDFGNGQYASNG

		PALKDWINKVKASGGNTARVWVHVEGQVSPAFDSHGFTSTDSKKTLLINDLSDLLDYANGQN VFLILVLFNGALQNNNSVQNLFWDESKLNSYINNALTTPMVNALKSKPSLAWEVLNEPEGTLO PGSDQNSCYDTSTLAAQAGAGWGGKKFPMKQILKTINWISSAIHNADSKALVTVGSWSELTQTD SFGYRNHYKDSCLTGAGGKSNGIINFYQMHTYSHSGKWNQNAPFKVNRWAYNVNDKPLLIGE FASVCSQNEGIQONLYKYAYNNGYNGALTWQFNSGGDCSDTYSNQMYGMQALKGQNDQSGG KGGMVSVNINHHHHHH
95	4QP0	ASSFVQTSGPQFTLDGKPFYFEGTNAYYLMTSDQSNVKQVFSDMKSLGLPVVRTWLFNLGSDS VWFQQWDSSSNKMVINDNSDTGLGRIDYIIQQAASQDIKLIFTLNNNWEDYGGMDYYVKNFG GTYHDDFYTNTEMIDSFKEYISHVLNRENSLTGVKYKDDPTIFGWEIANEPRCVGSGDFPASSN CSTTVTTAWIKEISEYIKSIDS NHL VAVGDEGFFNRKGESDYEYNGGSGMDFDAILALSSIDFGT FHLYPEAWSKGTDSWSVQWIKDHAAAQADADKPVIMEEYGLSTDALRVAQYPVWQGTVED EDLAADAFWQIAVPCSTMDGFGICASDNDIATTVTNHADAMAKK
96	5JUG	SACPSGATCGSYTVGGLGSRKQVRNAGGSSDLAVAMLQTERMDTAYPYGDNKSGDAANF GIFKQNWLMRLSACAQFGGQAGQYDNGAALNSSLGQDVSLHQSQSHYGLDAWFAGHRN GASGLSSPNTADIAAYKAAVYWIKAQLDADSANLGNDTRFWVQVPAI
97	5Y6T	EAFEQQFLDVQNGQLRLNGEKVFMSGMNIAWQNYGRDFGNGQYDCCTGNALEDYIVRIKAE GGNSLRIVHCDGGYTPEFDGNGYVVGTD AQNTMTSDLAQFLDVAYANNVLVFIVLWNGAT TPTSRYRDLIYDDSKLQTYIDQALVPMVSALSGKVALGGWEVMNEPEGIVSAGVSDGNPCFDT QPLAGSGAGWADSIPMQRLOSFINKQTAAIKRADPKVIVTLGWSERAQTDQFGWRNYITDN CLIDAGGDSLGVDFYQMHTYAWEGAYTSSSPLLVPNSQYNLDKPNNIGEFSSQSGGDRSITDQ FDWAYTQGYCGAWSWQANGGDNADSFATQAQGLNHLRGRNDQNAGGRIDIILQLEQKLIS EEDLNSAVDHHHHHH

[0437] In some embodiments, the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, can comprise a sequence with at least 70%, 75%, 80%, 85%, 90%, 95% sequence identity to at least 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, or 302 contiguous amino acids of a sequence from Table 3. In some embodiments, the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, can comprise a sequence with at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% or greater sequence identity to at least 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, or 302 contiguous amino acids of a sequence from Table 3. In some embodiments, the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, can comprise a sequence with less than 10% sequence identity to at least 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, or 302 contiguous amino acids of a sequence from Table 3.

[0438] In some embodiments, the sequence selected from Table 3 is SEQ ID NO: 85. In some embodiments, the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, can comprise a sequence with at least 70%, 75%, 80%, 85%, 90%, 95% sequence identity to at least 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, or 302 contiguous amino acids of SEQ ID NO: 85.

[0439] In some embodiments, the mannanase comprises an amino acid sequence with at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to at least 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, or 302 contiguous amino acids of SEQ ID NO: 85, where one or more non-standard amino acids such as selenocysteine are at positions 74 and/or 81.

[0440] In some embodiments, the mannanase further comprises at least one affinity tag. In some embodiments, an affinity tag of a mannanase is a C-terminal affinity tag. In some embodiments, an affinity tag of a mannanase is an N-terminal affinity tag. In some embodiments, a first affinity tag of a mannanase is an N-terminal affinity tag and a second affinity tag of a mannanase is a C-terminal affinity tag. In some embodiments, a first affinity tag of a mannanase is a first N-terminal affinity tag and a second affinity tag of a mannanase is a second N-terminal affinity tag. In some embodiments, a first affinity tag of a mannanase is a first C-terminal affinity tag and a second affinity tag of a mannanase is a second C-terminal affinity tag.

[0441] For example, the mannanase can comprise a poly-histidine tag, poly-histidine-glycine tag, poly-arginine tag, poly-aspartate tag, poly-cysteine tag, poly-phenylalanine, c-myc tag, Herpes simplex virus glycoprotein D (gD) tag, FLAG tag, KT3 epitope tag, tubulin epitope tag, T7 gene 10 protein peptide tag, streptavidin tag, streptavidin binding peptide (SPB) tag, Strep-

tag, Strep-tag II, albumin-binding protein (ABP) tag, alkaline phosphatase (AP) tag, bluetongue virus tag (B-tag), calmodulin binding peptide (CBP) tag, chloramphenicol acetyl transferase (CAT) tag, choline-binding domain (CBD) tag, chitin binding domain (CBD) tag, cellulose binding domain (CBP) tag, dihydrofolate reductase (DHFR) tag, galactose-binding protein (GBP) tag, maltose binding protein (MBP), glutathione-S-transferase (GST), Glu-Glu (EE) tag, human influenza hemagglutinin (HA) tag, horseradish peroxidase (HRP) tag, NE-tag, HSV tag, ketosteroid isomerase (KSI) tag, KT3 tag, LacZ tag, luciferase tag, NusA tag, PDZ domain tag, AviTag, Calmodulin-tag, E-tag, S-tag, SBP-tag, Softag 1, Softag 3, TC tag, VSV-tag, Xpress tag, Isopeptag, SpyTag, SnoopTag, Profinity eXact tag, Protein C tag, S1-tag, S-tag, biotin-carboxy carrier protein (BCCP) tag, green fluorescent protein (GFP) tag, small ubiquitin-like modifier (SUMO) tag, tandem affinity purification (TAP) tag, HaloTag, Nus-tag, Thioredoxin-tag, Fc-tag, CYD tag, HPC tag, TrpE tag, ubiquitin tag, VSV-G epitope tag, V5 tag, or a combination thereof.

[0442] In some embodiments, the mannanase further comprises at least two affinity tags. For example, the mannanase can comprise at least two affinity tags selected from a poly-histidine tag, poly-histidine-glycine tag, poly-arginine tag, poly-aspartate tag, poly-cysteine tag, poly-phenylalanine, c-myc tag, Herpes simplex virus glycoprotein D (gD) tag, FLAG tag, KT3 epitope tag, tubulin epitope tag, T7 gene 10 protein peptide tag, streptavidin tag, streptavidin binding peptide (SPB) tag, Strep-tag, Strep-tag II, albumin-binding protein (ABP) tag, alkaline phosphatase (AP) tag, bluetongue virus tag (B-tag), calmodulin binding peptide (CBP) tag, chloramphenicol acetyl transferase (CAT) tag, choline-binding domain (CBD) tag, chitin binding domain (CBD) tag, cellulose binding domain (CBP) tag, dihydrofolate reductase (DHFR) tag, galactose-binding protein (GBP) tag, maltose binding protein (MBP), glutathione-S-transferase (GST), Glu-Glu (EE) tag, human influenza hemagglutinin (HA) tag, horseradish peroxidase (HRP) tag, NE-tag, HSV tag, ketosteroid isomerase (KSI) tag, KT3 tag, LacZ tag, luciferase tag, NusA tag, PDZ domain tag, AviTag, Calmodulin-tag, E-tag, S-tag, SBP-tag, Softag 1, Softag 3, TC tag, VSV-tag, Xpress tag, Isopeptag, SpyTag, SnoopTag, Profinity eXact tag, Protein C tag, S1-tag, S-tag, biotin-carboxy carrier protein (BCCP) tag, green fluorescent protein (GFP) tag, small ubiquitin-like modifier (SUMO) tag, tandem affinity purification (TAP) tag, HaloTag, Nus-tag, Thioredoxin-tag, Fc-tag, CYD tag, HPC tag, TrpE tag, ubiquitin tag, VSV-G epitope tag, and V5 tag.

[0443] In some embodiments, the mannanase comprises an affinity tag that is GST. In some embodiments, the mannanase comprises an affinity tag that is a poly-histidine tag, such as a 6x-

His tag. In some embodiments, the mannanase comprises an affinity tag that is MBP. In some embodiments, the mannanase comprises an affinity tag that is a strep-tag, such as two strep tags.

[0444] In some embodiments, the mannanase comprises a first affinity tag that is GST and a second affinity tag that is a poly-histidine tag, such as a 6x-His tag. In some embodiments, the mannanase comprises a first affinity tag that is GST and a second affinity tag that is a strep tag. In some embodiments, the mannanase comprises a first affinity tag that is a strep tag, such as two strep tags, and a second affinity tag that is a poly-histidine tag, such as a 6x-His tag. In some embodiments, the mannanase comprises a first affinity tag that is MBP and a second affinity tag that is a poly-histidine tag, such as a 6x-His tag. In some embodiments, the mannanase comprises a first affinity tag that is MBP and a second affinity tag that is a strep tag, such as two strep tags.

[0445] In some embodiments, the mannanase comprises a first affinity tag that is GST, a second affinity tag that is a poly-histidine tag, such as a 6x-His tag, and a third affinity tag that is a strep tag, such as two strep tags. In some embodiments, the mannanase comprises a GST tag, a His tag, and two strep tags. In some embodiments, the mannanase comprises a first affinity tag that is MBP, a second affinity tag that is a poly-histidine tag, such as a 6x-His tag, and a third affinity tag that is a strep tag, such as two strep tags. In some embodiments, the mannanase comprises a MBP tag, a His tag, and two strep tags.

[0446] In some embodiments, the mannanase comprises an affinity tag, wherein the mannanase and affinity tag are separated by a linker. In some embodiments, the mannanase comprises a first affinity tag and a second affinity tag, wherein the mannanase and the first affinity tag are separated by a linker, and wherein the mannanase and the second affinity tag are separated by a linker. In some embodiments, the mannanase comprises a first affinity tag and a second affinity tag, wherein the first and second affinity tags are separated by a linker. In some embodiments, the mannanase comprises a first affinity tag, a second affinity tag and a third affinity tag, wherein the first, second and third affinity tags are each separated by a linker. In some embodiments, the mannanase comprises a first affinity tag, a second affinity tag, a third affinity tag and a fourth affinity tag, wherein the first, second, third and fourth affinity tags are each separated by a linker. In some embodiments, a linker comprises an amino acid sequence of (GS)_n, (GGS)_n, or (GGGS)_n or a combination thereof, where n is an integer of from 1-10.

[0447] In some embodiments, the one or more non-standard amino acids can be at position 74 of SEQ ID NO: 85 or position 81 of SEQ ID NO: 85. In some embodiments, the one or more non-standard amino acids can be at position 74 of SEQ ID NO: 85 and position 81 of SEQ ID

NO:85. In some embodiments, a non-standard amino acid at position 74 can be directly linked by a bond to a non-standard amino acid at position 81.

[0448] In some embodiments, the bond can be a diselenide bond or selenyl-sulfhydryl bond. In some embodiments, the bond is a diselenide bond. In some embodiments, the bond is a selenyl-sulfhydryl bond. In some embodiments, the diselenide bond or selenyl-sulfhydryl bond can be in a location of a disulfide bond in a corresponding recombinant enzyme without the one or more non-standard amino acids.

[0449] In some embodiments, the T_m of the corresponding stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, can be less than 37°C. In some embodiments, the T_m of the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, can be greater than 37°C, 40°C, 45°C, 50°C, 55°C, 60°C, or 65°C. In some embodiments, the T_m of the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, can be at least 37°C, 40°C, 45°C, 50°C, 55°C, 60°C, 65°C or greater.

[0450] In some embodiments, the T_m of the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, can be at least 10°C higher than the T_m of the corresponding recombinant enzyme, functional fragment thereof, or variant thereof. In some embodiments, the T_m of the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, can be at least 15°C higher than the T_m of the corresponding recombinant enzyme, functional fragment thereof, or variant thereof. In some embodiments, the T_m of the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, can be at least 15°C, 20°C, 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, 65°C or greater higher than the T_m of the corresponding recombinant enzyme, functional fragment thereof, or variant thereof. In some embodiments, the T_m of the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, can be less than 10°C higher than the T_m of the corresponding recombinant enzyme, functional fragment thereof, or variant thereof.

[0451] In some embodiments, the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, can have a half-life in an environment that can be at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 or greater fold higher than a half-life of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids in the environment. In some embodiments, the stabilized mannanase polypeptide, functional fragment thereof, or

variant thereof, can have a half-life in an environment that is at least a 1.1 fold higher than a half-life of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids in the environment.

[0452] In some embodiments, the half-life of the mannanase polypeptide, functional fragment thereof, or variant thereof in the environment, can be greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or more hours. In some embodiments, the half-life of the mannanase polypeptide, functional fragment thereof, or variant thereof in the environment, can be less than 1 hour.

[0453] In some embodiments, the half-life of the mannanase polypeptide, functional fragment thereof, or variant thereof in the environment, can be greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or more days. In some embodiments, the half-life of the mannanase polypeptide, functional fragment thereof, or variant thereof in the environment, can be less than 1 day.

[0454] In some embodiments, the stabilized mannanase polypeptide can have at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 fold higher hydrolytic activity for a beta-D-mannoside substrate in an environment than a hydrolytic activity for the beta-D-mannoside substrate of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids. In some embodiments, the stabilized mannanase polypeptide can have less than a 1.1 fold higher hydrolytic activity for a beta-D-mannoside substrate in an environment than a hydrolytic activity for the beta-D-mannoside substrate of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0455] In some embodiments, the stabilized mannanase polypeptide can have at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 fold higher hydrolytic activity for a beta-D-mannoside substrate after being present in an environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or 50 minutes than a hydrolytic activity for the beta-D-mannoside substrate of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids after being present in the environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or 50 minutes. In some embodiments, the stabilized mannanase polypeptide can have less than a 1.1 fold higher hydrolytic activity for a beta-D-mannoside substrate after being present in an environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or 50 minutes than a hydrolytic activity for the beta-D-mannoside substrate of a

corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids after being present in the environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or 50 minutes.

[0456] In some embodiments, the stabilized mannanase polypeptide can have at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 fold higher hydrolytic activity for a beta-D-mannoside substrate after being present in an environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, or 24 hours than a hydrolytic activity for the beta-D-mannoside substrate of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids after being present in the environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, or 24 hours. In some embodiments, the stabilized mannanase polypeptide can have less than a 1.1 fold higher hydrolytic activity for a beta-D-mannoside substrate after being present in an environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, or 24 hours than a hydrolytic activity for the beta-D-mannoside substrate of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids after being present in the environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, or 24 hours.

[0457] In some embodiments, the (i) stabilized mannanase polypeptide, (ii) a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids, or (iii) both may lose hydrolytic activity in a reducing environment, as compared to its activity in a non-reducing environment. For example, the loss of hydrolytic activity of a stabilized mannanase polypeptide in a reducing environment, as compared to its activity in a non-reducing environment may be at least or at most 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30%, 40%, 50%, or 60%. For another example, the loss of hydrolytic activity of the corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids in a reducing environment, as compared to its activity in a non-reducing environment may be at least or at most 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 99.9%. In some embodiments, the loss of hydrolytic activity in a reducing environment, as compared to the hydrolytic activity in a non-reducing environment is less for the stabilized mannanase polypeptide, for example at least or at most 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% less, than for a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids. Under the same conditions, for

example, when comparing an enzyme's activity under a reducing environment and the enzyme's activity under a non-reducing environment, the activity difference such as a loss of activity under the reducing environment is less significant for a stabilized mannanase than a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids, e.g., at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% less.

[0458] In some embodiments, the beta-D-mannoside substrate comprises plant fibers such as cellulose and hemicellulose. In some embodiments, the beta-D-mannoside substrate comprises starch. In some embodiments, the beta-D-mannoside substrate comprises structural polysaccharides such as arabinoxylans, cellulose, chitins, and pectins. In some embodiments, the beta-D-mannoside substrate is in an animal feedstock, such as the feedstock described in the present disclosure.

[0459] In some embodiments, the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, can be recombinant. In some embodiments, the recombinant can be generated using recombinant DNA technology, such as, for example, the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof expressed by a bacteriophage or yeast expression system. In other embodiments, the recombinant can be generated by the synthesis of a DNA molecule encoding the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof.

[0460] In some embodiments, the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, can be an animal mannanase, a plant mannanase, a fungi mannanase, or a bacterial mannanase. The stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, can be any kinds of mannanase, including, but not limited to, *E. coli* mannanase, *Microcella alkaliophila* mannanase, *Lactobacillus algidus* mannanase, *Vibrio cholerae* mannanase, *Bifidobacterium longum* mannanase, *Homo sapiens* mannanase, and *Raoultella ornithinolytica* mannanase. In some embodiments, a composition can comprise stabilized animal mannanases, stabilized plant mannanases, stabilized fungi mannanases, stabilized bacterial mannanases, or any combination thereof.

[0461] In some embodiments, the composition further comprises a phytase polypeptide. In some embodiments, the composition further comprises other carbohydrase such as xylanase. In some embodiments, the phytase polypeptide is a stabilized phytase polypeptide comprising one or more non-standard amino acid, a functional fragment thereof, or a variant thereof. In some embodiments, the xylanase is a stabilized xylanase comprising one or more non-standard amino

acid, a functional fragment thereof, or a variant thereof. In some embodiments, the composition further comprises a phytase polypeptide, a xylanase polypeptide, or a combination thereof.

[0462] In some embodiments, a composition can comprise a polynucleotide encoding the composition disclosed herein. In some embodiments, the polynucleotide can be a vector. The vector can be a fragment of nucleic acid molecules. The vector can be taken from a virus, a plasmid, or the cell of a higher organism. The vector can be stably maintained in an organism. The vector can be inserted with a foreign nucleic acid fragment for cloning purposes. The vector can comprise features that allow for the convenient insertion or removal of a nucleic acid fragment to or from a vector. The vector can be genetically engineered plasmids.

[0463] In some embodiments, a bond directly linking two of the one or more non-standard amino acids of the stabilized mannanase polypeptide may not break in an environment, when the bond directly linking two of the one or more standard amino acids of the corresponding mannanase polypeptide may break in the same environment.

[0464] In some embodiments, the method of making the composition disclosed herein can comprise expressing an amino acid sequence of the stabilized mannanase polypeptide. In some embodiments, expressing can comprise expressing in a cell or *in vitro*.

[0465] In some embodiments, the cell can be a bacterial cell. In some embodiments, the cell can be a genomically recoded cell. In some embodiments, the cell may not be a bacterial cell. The cell can be obtained or isolated from a subject. The cell can be obtained or isolated from a tissue. The subject may be an animal such as a human, a mouse, a rat, a pig, a dog, a rabbit, a sheep, a horse, a chicken or other animal. A cell may be a neuron. The cell may be one of the cells of a blood-brain barrier system. The cell may be a cell line, such as a neuronal cell line. The cell may be a primary cell, such as cells obtained from a brain of a subject. The cell may be a population of cells that may be isolated from a subject, such as a tissue biopsy, a cytology specimen, a blood sample, a fine needle aspirate (FNA) sample, or any combination thereof. The cell may be obtained from a bodily fluid such as urine, milk, sweat, lymph, blood, sputum, amniotic fluid, aqueous humour, vitreous humour, bile, cerebrospinal fluid, chyle, chyme, exudates, endolymph, perilymph, gastric acid, mucus, pericardial fluid, peritoneal fluid, pleural fluid, pus, rheum, saliva, sebum, serous fluid, smegma, sputum, tears, vomit, or other bodily fluid. The cell may comprise cancerous cells, non-cancerous cells, tumor cells, non-tumor cells, healthy cells, or any combination thereof.

[0466] In some embodiments, the cell can comprise a reassigned codon recognized by a stabilizing non-standard amino acid tRNA comprising an anticodon corresponding to the reassigned codon.

[0467] In some embodiments, the amino acid sequence of the stabilized mannanase polypeptide can be encoded by a polynucleotide sequence comprising at least one codon of a natural amino acid that can have been replaced by the reassigned codon. In some embodiments, the amino acid sequence of the stabilized mannanase polypeptide can be encoded by a polynucleotide sequence comprising at least one, two, or three stop codons or codons of a natural amino acid that can be replaced by the reassigned codon.

[0468] In some embodiments, the stabilizing non-standard amino acid tRNA can be a selenocysteine tRNA.

[0469] In some embodiments, the method can comprise culturing the cell under conditions in which the amino acid sequence of the stabilized mannanase polypeptide can be expressed. In some embodiments, the reassigned codon can be UAG, UAA, UGA, or a combination thereof.

[0470] In some aspects, provided herein is a method comprising contacting a beta-D-mannoside substrate in an environment to a stabilized mannanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof; wherein the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof catalyzes hydrolysis of the beta-D-mannoside substrate at a higher rate than a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids. In some embodiments, the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof can catalyze hydrolysis of a beta-D-mannoside substrate at a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 or greater fold higher rate than a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

[0471] In some embodiments, the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof can be the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof disclosed elsewhere herein. In some embodiments, the beta-D-mannoside substrate is from an animal feedstock. In some embodiments, the beta-D-mannoside substrate is in an animal feedstock. In some embodiments, the beta-D-mannoside substrate is all or a part of the polysaccharides in an animal feedstock.

III. Environments

[0472] In some embodiments, the environment is a reducing environment. In some embodiments, the reducing environment may or may not be in a gastrointestinal tract of an

animal. In one variation, the environment is any environment in an animal's gastrointestinal tract that is a reducing environment.

[0473] In some embodiments, the environment is an environment with a temperature. In some embodiments, the temperature is no less than about -20 °C, -10 °C, 0 °C, 1 °C, 2 °C, 3 °C, 4 °C, 5 °C, 10 °C, 20 °C, 30 °C, 40 °C, 50 °C, 60 °C, 70 °C, 80 °C, 90 °C, 95 °C, or 100 °C. In some embodiments, the temperature is no more than about 0 °C, 5 °C, 10 °C, 20 °C, 30 °C, 40 °C, 50 °C, 60 °C, 70 °C, 80 °C, 90 °C, 100 °C, 150 °C, or 200 °C. In some embodiments, the temperature is from 4 °C to 50 °C. In some embodiments, the temperature is from 4 °C to 100 °C. In some embodiments, the temperature is from 4 °C to 98 °C.

[0474] In some embodiments, the environment is an environment with a neutral, alkaline, or acidic pH. In certain embodiments, the environment is an environment with an acidic pH. In some embodiments, the environment has a pH of from 1 to 7. In some embodiments, the environment has a pH of from 1 to 5. In some embodiments, the environment has a pH of from 1 to 3. In other embodiments, the environment has a pH no less than 7. In some embodiments, the environment has a pH from 7 to 9 or 7 to 8. In certain embodiments, the environment has a pH about 7, for example, from 6.5 to 7.5 or 6 to 8.

[0475] In some embodiments, the environment has a salt concentration. In some embodiments, the salt concentration is no less than 10 mM, 50 mM, 100 mM, 200 mM, 300 mM, 400 mM, 500 mM, 600 mM, 700 mM, 800 mM, 900 mM, or 1 M. In some embodiments, the salt concentration is no higher than 50 mM, 100 mM, 200 mM, 300 mM, 400 mM, 500 mM, 600 mM, 700 mM, 800 mM, 900 mM, 1 M, or 2M. In some embodiments, the salt concentration is from 10 mM to 1 M.

[0476] In some embodiments, the environment comprises a reducing agent. In some embodiments, the reducing agent is a reducing reagent (such as an antioxidant), a reducing enzyme, or other reducing agent that lowers the reduction potential (i.e., redox potential) of the environment. In some embodiments, the reducing agent is present in the environment at a concentration of no less than about 0.01 mM, 0.05 mM, 0.1 mM, 0.5 mM, 1 mM, 10 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60 mM, 70 mM, 80 mM, 90 mM, or 100 mM. In some embodiments, the reducing agent is present in the environment at a concentration of no more than about 0.05 mM, 0.1 mM, 0.5 mM, 1 mM, 10 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60 mM, 70 mM, 80 mM, 90 mM, 100 mM, or 200mM. In some embodiments, the reducing agent is present in the environment at a concentration of from 0.01 mM to 100 mM.

[0477] In some embodiments, the environment has a reduction potential that is less than about 200 mV, 150 mV, 100 mV, 50 mV, 0 mV, -50 mV, -100 mV, -150 mV, -160 mV, -170 mV, -

180 mV, -190 mV, -200 mV, -210 mV, -220 mV, -230 mV, -240 mV, -250 mV, -260 mV, -270 mV, -280 mV, -290 mV, -300 mV, -310 mV, -320 mV, -330 mV, -340 mV, -350 mV, -360 mV, -370 mV, -380 mV, -390 mV, -400 mV, -410 mV, -420 mV, -430 mV, -440 mV, -450 mV, -460 mV, -470 mV, -480 mV, -490 mV, -500 mV, -510 mV, -520 mV, -530 mV, -540 mV, -550 mV, -560 mV, -570 mV, -580 mV, -590 mV, or -600 mV. In certain embodiments, the environment has a reduction potential that is less than about -100 mV, -110 mV, -120 mV, -130 mV, -140 mV, -150 mV, -160 mV, -170 mV, -180 mV, -190 mV, -200 mV, -210 mV, -220 mV, -230 mV, -240 mV, -250 mV, -260 mV, -270 mV, -280 mV, -290 mV, -300 mV, -310 mV, -320 mV, -330 mV, -340 mV, -350 mV, -360 mV, -370 mV, -380 mV, -390 mV, or -400 mV.

[0478] In certain embodiments, the environment is any environment in a gastrointestinal tract of an animal that comprises any of the characteristics described here.

IV. Methods of Contacting a Substrate with the Feed Enzyme

Method of Providing Feedstock

[0479] In some aspects, provided herein is a method comprising contacting a substrate in an environment to a stabilized feed enzyme (e.g., phytase, xylanase, and mannanase) comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof. Particularly, in some embodiments, the method comprises contacting an animal feedstock with the stabilized phytase, the stabilized carbohydrase (such as stabilized xylanase and stabilized mannanase), or any combination thereof. In some embodiments, the method comprises contacting the animal feedstock with the stabilized phytase polypeptide. In some embodiments, the method comprises contacting the animal feedstock with the stabilized xylanase polypeptide. In some embodiments, the method comprises contacting the animal feedstock with the stabilized mannanase polypeptide.

[0480] In some embodiments, the method comprises feeding the animal feedstock to an animal. In some embodiments, the method comprises providing an animal feedstock comprising one or more substrates of the herein-described feed enzymes. For example, in some embodiments, the method comprises providing an animal feedstock comprising the phytate substrate, the polysaccharide substrate, the beta-D-mannoside substrate, or any combination thereof.

[0481] In some aspects, provided herein is a feedstock comprising a composition described herein. For example, the feedstock may comprise any one or more of the substrates of feed enzymes. For another example, the feedstock may comprise any one or more of the feed enzymes. For yet another example, the feedstock may comprise one or more bacterial cells that express or secrete a stabilized enzyme.

[0482] In some aspects, provided herein is a method comprising feeding an animal with a feedstock comprising a stabilized feed enzyme, a functional fragment, or a variant thereof. In some embodiments, the method comprises feeding an animal with a feedstock comprising a stabilized phytase, a stabilized xylanase, a stabilized mannanase, or a combination thereof.

[0483] In some embodiments, the feedstock comprises a naturally occurring carbohydrate source and/or phytate source. In some embodiments, the feedstock comprises a carbohydrate source and/or phytate source that is derived from: seeds, roots, tubers, corn, tapioca, arrowroot, wheat, rice, potatoes, sweet potato, sago, beans (e.g., fava, lentils, mung beans, peas, and chickpeas), maize, cassava, or other starchy foods (e.g., acorns, arrowroot, arracacha, bananas, barley, breadfruit, buckwheat, canna, colacasia, katakuri, kudzu, malanga, millet, oats, oca, polynesian arrowroot, sorghum, rye, taro, chestnuts, water chestnuts, and yams). In further embodiments, the feedstock comprises a carbohydrate sources and/or phytate source that is derived from: legumes (e.g., peas, soybeans, lupins, green beans, and other beans), oats, rye, chia, barley, fruits (e.g., figs, avocados, plums, prunes, berries, bananas, apple skin, quinces, and pears), vegetables (e.g., broccoli, carrots, cauliflower, zucchini, celery, nopal, and Jerusalem artichokes), root tubers, root vegetables (e.g., sweet potatoes and onions), psyllium seed husks, seeds (e.g., flax seeds), nuts (e.g., almonds), whole grain foods, wheat, corn bran, lignans, or any combination thereof. In some embodiments, the source of the phytate substrate is derived from soy bean.

[0484] In certain embodiments, the feedstock is suitable for a variety of different animals such as swine, poultry and, cattle. A person skilled in the art would understand that the composition of the feedstock may depend on the type and age of an animal. In some embodiments, the feedstock may further comprise proteins, minerals (such as copper, calcium, and zinc), salts, essential amino acids, vitamins, and/or antibiotics. In some embodiments, the feedstock is a starter feed or nursery feed, wherein the nutritional content of the feedstock is optimized for the nutritional needs of the animal during the starter phase of growth. In some embodiments, the feedstock is a grower feed, which may be provided any time during the second week of growth through the final productive lifetime of the animal. In some embodiments, the feedstock is a finisher feed, which is generally provided during the final period of the productive lifetime of the animal.

[0485] In some aspects, provided herein is a method of manufacturing an animal feedstock by combining an animal feed with one or more of the herein-described stabilized feed enzymes, functional fragments, or variants thereof. In further aspects, provided herein is a method of manufacturing an animal feedstock by combining one or more substrates of the herein-described

stabilized enzymes with the stabilized enzymes, functional fragments, or variants thereof. In some embodiments, the stabilized feed enzymes (such as the stabilized phytase, stabilized xylanase, and the stabilized mannanase) may be combined with the animal feed or with the substrates before feeding the feedstock to an animal, or they may be pre-combined. In some embodiments, the stabilized feed enzymes (such as the stabilized phytase, stabilized xylanase, and the stabilized mannanase) are combined with the animal feed or with the substrates at least 1 minute, 30 minutes, 1 hour, 12 hours, 1 day, 1 week, a month, 6 months, or a year before feeding the feedstock to an animal. In some embodiments, the stabilized feed enzymes (such as the stabilized phytase, stabilized xylanase, and the stabilized mannanase) are combined with the animal feed or with the substrates at most 10 minute, 30 minutes, 1 hour, 12 hours, 1 day, 1 week, a month, 6 months, a year, or 2 years before feeding the feedstock to an animal. In some embodiments, the stabilized feed enzymes are combined with the animal feed or with the substrates to produce a final feed mixture. In some embodiments, the animal feedstock is a final feed mixture.

[0486] In some embodiments, the feedstock comprises the stabilized phytase polypeptide, a functional fragment, or a variant thereof. In some embodiments, a concentration of the phytase, a functional fragment, or a variant thereof in the final feed mixtures is less than about 10,000 units, 5000 units, 2500 units, 2000 units, 1500 units, 1200 units, 1000 units, 900 units, 800 units, 700 units, 600 units, 500 units, 400 units, 300 units, 200 units, 100 units, or 50 units of the phytase per kilogram of the final feed mixture. In some embodiments, a concentration of the phytase, a functional fragment, or a variant thereof in the final feed mixtures is more than about 2500 units, 2000 units, 1500 units, 1200 units, 1000 units, 900 units, 800 units, 700 units, 600 units, 500 units, 400 units, 300 units, 200 units, 100 units, 50 units, 25 units, 10 units, or 5 units of the phytase per kilogram of the final feed mixture. In some embodiments, a concentration of the phytase in the final feed mixture is less than 1200 units of the phytase per kilogram of the final feed mixture.

[0487] In some embodiments, the feedstock comprises the stabilized xylanase polypeptide, a functional fragment, or a variant thereof. In some embodiments, a concentration of the xylanase, a functional fragment, or a variant thereof in the final feed mixtures is less than about 10,000 units, 5000 units, 2500 units, 2000 units, 1500 units, 1200 units, 1000 units, 900 units, 800 units, 700 units, 600 units, 500 units, 400 units, 300 units, 200 units, 100 units, or 50 units of the xylanase per kilogram of the final feed mixture. In some embodiments, a concentration of the xylanase, a functional fragment, or a variant thereof in the final feed mixtures is more than about 2500 units, 2000 units, 1500 units, 1200 units, 1000 units, 900 units, 800 units, 700 units, 600

units, 500 units, 400 units, 300 units, 200 units, 100 units, 50 units, 25 units, 10 units, or 5 units of the xylanase per kilogram of the final feed mixture. In some embodiments, a concentration of the xylanase in the final feed mixture is less than 1200 units of the xylanase per kilogram of the final feed mixture.

[0488] In some embodiments, the feedstock comprises the stabilized mannanase polypeptide, a functional fragment, or a variant thereof. In some embodiments, a concentration of the mannanase, a functional fragment, or a variant thereof in the final feed mixtures is less than about 10,000 units, 5000 units, 2500 units, 2000 units, 1500 units, 1200 units, 1000 units, 900 units, 800 units, 700 units, 600 units, 500 units, 400 units, 300 units, 200 units, 100 units, or 50 units of the mannanase per kilogram of the final feed mixture. In some embodiments, a concentration of the mannanase, a functional fragment, or a variant thereof in the final feed mixtures is more than about 2500 units, 2000 units, 1500 units, 1200 units, 1000 units, 900 units, 800 units, 700 units, 600 units, 500 units, 400 units, 300 units, 200 units, 100 units, 50 units, 25 units, 10 units, or 5 units of the mannanase per kilogram of the final feed mixture. In some embodiments, a concentration of the mannanase in the final feed mixture is less than 1200 units of the mannanase per kilogram of the final feed mixture.

[0489] In some embodiments, the feedstock comprises a bacterial cell. In some embodiments, the bacterial cell expresses or secretes a stabilized feed enzyme, a functional fragment, or a variant thereof. In some embodiments, the bacterial cell expresses or secretes two or more stabilized feed enzymes, functional fragments, or variants thereof. In some embodiments, the bacterial cell expresses or secretes a stabilized phytase. In some embodiments, the bacterial cell expresses or secretes a stabilized xylanase. In some embodiments, the bacterial cell expresses or secretes a stabilized mannanase.

[0490] In some embodiments, the feedstock comprises an additional bacterial cell. In some embodiments, the additional bacterial cell expresses or secretes a stabilized feed enzyme, a functional fragment, or a variant thereof. In some embodiments, the additional bacterial cell expresses or secretes two or more stabilized feed enzymes, functional fragments, or variants thereof. In some embodiments, the additional bacterial cell expresses or secretes a stabilized phytase. In some embodiments, the additional bacterial cell expresses or secretes a stabilized xylanase. In some embodiments, the additional bacterial cell expresses or secretes a stabilized mannanase.

[0491] In some aspects, disclosed herein is a method of feeding an animal with a feedstock comprising a bacterial cell. In some embodiments, the bacterial cell expresses or secretes a stabilized enzyme. In some embodiments, the bacterial cell expresses or secretes a herein-

described stabilized phytase and/or a herein-described stabilized xylanase polypeptide. In some embodiments, the stabilized enzymes such as stabilized phytase and stabilized carbohydrase are extracted from the bacterial cell by the animal. Accordingly, in some embodiments, the method of feeding an animal with a feedstock comprising a bacterial cell that expresses or secretes a stabilized phytase, a stabilized xylanase, and/or a stabilized mannanase, which are extracted by the animal from the bacterial cell.

Suitable Animals

[0492] The animal feedstock may be provided to any suitable animal. In some embodiments, the animal is monogastric. It is generally understood that a monogastric animal has a single-chambered stomach. In other embodiments, the animal is a ruminant. It is generally understood that a ruminant has a multi-chambered stomach. In some embodiments, the animal is a ruminant in the pre-ruminant phase. Examples of such ruminants in the pre-ruminant phase include nursery calves.

[0493] In some embodiments, the animal is poultry. Examples of poultry include chicken, duck, turkey, goose, quail, or Cornish game hen. In one variation, the animal is a chicken. In some embodiments, the poultry is a layer hen, a broiler chicken, or a turkey.

[0494] In some embodiments, the animal is a mammal, including, for example, a cow, a pig, a goat, a sheep, a deer, a bison, a rabbit, an alpaca, a llama, a mule, a horse, a reindeer, a water buffalo, a yak, a guinea pig, a rat, a mouse, an alpaca, a dog, or a cat. In one variation, the animal is a cow. In another variation, the animal is a pig.

[0495] In some embodiments, the animal is a companion animal, which is suitable to have a close relationship with humans. For example, in some embodiments, a companion animal may be a dog, cat, horse, rabbit, ferret, hamster, mouse, bird, guinea pig, other small mammal, small reptile, or fish.

[0496] The animal feedstock may also be used in aquaculture. In some embodiments, the animal is an aquatic animal. Examples of aquatic animals may include a trout, a salmon, a bass, a tilapia, a shrimp, an oyster, a mussel, a clam, a lobster, or a crayfish. In one variation, the animal is a fish.

Gastrointestinal Tract Environment

[0497] The feedstock may be provided to an animal having any type of digestive system such as monogastric, avian, ruminant, and pseudo-ruminant digestive system.

[0498] In some embodiments, the animal has a monogastric digestive system. In some embodiments, the environment comprises all or a part of a gastrointestinal tract of a monogastric animal that comprises esophagus, stomach, small intestine, large intestine, anus, rectum, or any

combination thereof. In some embodiments, the environment in a gastrointestinal tract of a monogastric animal comprises stomach, small intestine, large intestine, or a combination thereof. In some embodiments, the environment comprises small and large intestines. In some embodiments, the environment comprises a stomach, i.e., a stomach environment.

[0499] In some embodiments, the animal has an avian digestive system. In some embodiments, the environment comprises all or a part of a gastrointestinal tract of an avian animal that comprises esophagus, crop, proventriculus, gizzard, small intestine, large intestine, cloaca, or any combination thereof. In some embodiments, the environment in a gastrointestinal tract of an avian animal comprises gizzard, small intestine, large intestine, or any combination thereof. In some embodiments, the environment comprises small and large intestines. In some embodiments, the environment comprises a stomach, i.e., a stomach environment.

[0500] In some embodiments, the animal has a ruminant digestive system. In some embodiments, the environment comprises all or a part of a gastrointestinal tract of a ruminant animal that comprises esophagus, rumen, reticulum, omasum, abomasum, small intestine, large intestine, or any combination thereof. In some embodiments, the environment in a gastrointestinal tract of a ruminant animal comprises rumen, reticulum, omasum, abomasum, small intestine, large intestine, or any combination thereof. In some embodiments, the environment comprises rumen, reticulum, omasum, abomasum, and small intestine. In some embodiments, the environment comprises rumen, i.e., a rumen environment.

[0501] In some embodiments, the animal has a pseudo-ruminant digestive system. In some embodiments, the environment comprises all or a part of a gastrointestinal tract of a pseudo-ruminant animal that comprises esophagus, stomach, small intestine, large intestine, cecum, rectum, anus, or any combination thereof. In some embodiments, the environment in a gastrointestinal tract of a pseudo-ruminant animal comprises small intestine, large intestine, cecum, or any combination thereof. In some embodiments, the animal may have digestive system features from more than one of the aforementioned types. In some embodiments, the animal may have digestive system features that are different from the aforementioned types.

[0502] In some embodiments, the environment is any environment in an animal's gastrointestinal tract where fermentation process occurs. Environments in an animal's gastrointestinal tract where fermentation occurs include, but are not limited to, stomach, rumen, cecum, and colon.

[0503] The above disclosure generally describes the present invention. All references disclosed herein are expressly incorporated by reference. A more complete understanding can be obtained

by reference to the following specific examples which are provided herein for purposes of illustration only, and are not intended to limit the scope of the invention.

CLAIMS

What is claimed is:

1. A composition comprising a stabilized phytase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof.
2. A composition comprising a stabilized phytase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized phytase polypeptide has a higher hydrolytic activity for a phytate substrate in an environment than a hydrolytic activity for the phytate substrate of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids in the environment.
3. A composition comprising a stabilized phytase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized phytase polypeptide does not destabilize in an environment that a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids does destabilize.
4. The composition of any one of claims 1-3, wherein the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, has a melting temperature (T_m) that is at least 5°C higher than a T_m of a corresponding recombinant enzyme, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.
5. A composition comprising a stabilized phytase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized phytase polypeptide has a melting temperature (T_m) that is at least 5°C higher than a T_m of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.
6. The composition of any one of claims 1, 3, or 5, wherein the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, has a higher hydrolytic activity for a phytate substrate in an environment than a hydrolytic activity for the phytate substrate of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.
7. The composition of any one of claims 1, 2, or 5, wherein the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, does not destabilize in an environment that a corresponding phytase polypeptide, functional fragment thereof, or

- variant thereof that does not comprise the one or more non-standard amino acids does destabilize.
8. The composition of any one of claims 1-7, wherein at least one, two, three, four or more of the one or more non-standard amino acids is selenocysteine.
 9. The composition of any one of claims 1-8, wherein at least two of the one or more non-standard amino acids are directly linked by a bond.
 10. The composition of claim 9, wherein at least four of the one or more non-standard amino acids are directly linked by a bond, wherein a first pair of the at least four of the one or more non-standard amino acids is directly linked by a bond, and a second pair of the at least four of the one or more non-standard amino acids is directly linked by a bond.
 11. The composition of claim 9 or 10, wherein the bond is a diselenide bond or a selenyl-sulfhydryl bond.
 12. The composition of claim 11, wherein the diselenide bond or the selenyl-sulfhydryl bond is an intermolecular or an intramolecular bond.
 13. The composition of any one of claims 1-12, wherein the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, has a half-life that is at least 1.1 fold higher than a half-life of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.
 14. The composition of any one of claims 1-13, wherein the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, has at least 70%, 75%, 80%, 85%, 90%, 95% sequence identity to a sequence from Table 1.
 15. The composition of any one of claims 1-14, wherein the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, comprises a sequence with at least 70%, 75%, 80%, 85%, 90%, 95% sequence identity to at least 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, or 433 contiguous amino acids of the sequence from Table 1.
 16. The composition of claim 15, wherein the sequence from Table 1 is SEQ ID NO:1.
 17. The composition of any one of claims 1-16, wherein the one or more non-standard amino acids is at
 - (a) position 15 of SEQ ID NO:1,
 - (b) position 99 of SEQ ID NO:1,
 - (c) position 130 of SEQ ID NO:1,
 - (d) position 155 of SEQ ID NO:1,

- (e) position 200 of SEQ ID NO:1,
 - (f) position 209 of SEQ ID NO:1,
 - (g) position 403 of SEQ ID NO:1,
 - (h) position 412 of SEQ ID NO:1, or
 - (i) position 429 of SEQ ID NO:1.
18. The composition of claim 17, wherein a non-standard amino acid at position 99 is directly linked by a bond to a non-standard amino acid at position 130.
19. The composition of claim 17 or 18, wherein a non-standard amino acid at position 155 is directly linked by a bond to a non-standard amino acid at position 429.
20. The composition of any one of claims 17-19, wherein a non-standard amino acid at position 200 is directly linked by a bond to a non-standard amino acid at position 209.
21. The composition of any one of claims 17-20, wherein a non-standard amino acid at position 403 is directly linked by a bond to a non-standard amino acid at position 412.
22. The composition of any one of claims 17-21, wherein a non-standard amino acid at position 53 is directly linked by a bond to a non-standard amino acid at position 199.
23. The composition of any one of claims 17-22, wherein a non-standard amino acid at position 74 is directly linked by a bond to a non-standard amino acid at position 121.
24. The composition of any one of claims 17-23, wherein a non-standard amino acid at position 163 is directly linked by a bond to a non-standard amino acid at position 221.
25. The composition of claim 15, wherein the sequence from Table 1 is SEQ ID NO:2 or SEQ ID NO:98.
26. The composition of any one of claims 1-15 or 25, wherein the one or more non-standard amino acids is at
- (a) position 165 of SEQ ID NO: 2 or SEQ ID NO: 98,
 - (b) position 227 of SEQ ID NO: 2 or SEQ ID NO: 98,
 - (c) position 279 of SEQ ID NO: 2 or SEQ ID NO: 98,
 - (d) position 281 of SEQ ID NO: 2 or SEQ ID NO: 98,
 - (e) position 284 of SEQ ID NO: 2 or SEQ ID NO: 98,
 - (f) position 286 of SEQ ID NO: 2 or SEQ ID NO: 98,
 - (g) position 331 of SEQ ID NO: 2 or SEQ ID NO: 98, or
 - (h) position 334 of SEQ ID NO: 2 or SEQ ID NO: 98.
27. The composition of any one of claims 25-26, wherein a non-standard amino acid at position 165 is directly linked by a bond to a non-standard amino acid at position 284.

28. The composition of any one of claims 25-27, wherein a non-standard amino acid at position 281 is directly linked by a bond to a non-standard amino acid at position 334.
29. The composition of any one of claims 25-28, wherein a non-standard amino acid at position 227 is directly linked by a bond to a non-standard amino acid at position 279.
30. The composition of any one of claims 25-29, wherein a non-standard amino acid at position 286 is directly linked by a bond to a non-standard amino acid at position 331.
31. The composition of claim 15, wherein the sequence from Table 1 is a sequence selected from SEQ ID NOs:99-110.
32. The composition of any one of claims 1-15 or 31, wherein the one or more non-standard amino acids is at position 77, position 108, position 133, position 178, position 188, position 382, position 391, or position 408 of an amino acid sequence selected from SEQ ID NOs:99-110.
33. The composition of any one of claims 31-32, wherein a non-standard amino acid at position 77 is directly linked by a bond to a non-standard amino acid at position 108.
34. The composition of any one of claims 31-33, wherein a non-standard amino acid at position 133 is directly linked by a bond to a non-standard amino acid at position 408.
35. The composition of any one of claims 31-34, wherein a non-standard amino acid at position 178 is directly linked by a bond to a non-standard amino acid at position 188.
36. The composition of any one of claims 31-35, wherein a non-standard amino acid at position 382 is directly linked by a bond to a non-standard amino acid at position 391.
37. The composition of claim 15, wherein the sequence from Table 1 is a sequence selected from SEQ ID NOs:111-114.
38. The composition of any one of claims 1-15 or 37, wherein the one or more non-standard amino acids is at position 124, position 155, position 180, position 225, position 235, position 429, position 438, or position 455 of an amino acid sequence selected from SEQ ID NOs:111-114.
39. The composition of any one of claims 37-38, wherein a non-standard amino acid at position 124 is directly linked by a bond to a non-standard amino acid at position 155.
40. The composition of any one of claims 37-39, wherein a non-standard amino acid at position 180 is directly linked by a bond to a non-standard amino acid at position 455.
41. The composition of any one of claims 37-40, wherein a non-standard amino acid at position 225 is directly linked by a bond to a non-standard amino acid at position 235.
42. The composition of any one of claims 37-41, wherein a non-standard amino acid at position 429 is directly linked by a bond to a non-standard amino acid at position 438.

43. The composition of claim 15, wherein the sequence from Table 1 is SEQ ID NO:115.
44. The composition of any one of claims 1-15 or 43, wherein the one or more non-standard amino acids is at position 77, position 79, position 110, position 135, position 180, position 190, position 206, position 384, position 393, or position 410 of SEQ ID NO:115.
45. The composition of any one of claims 43-44, wherein a non-standard amino acid at position 77 is directly linked by a bond to a non-standard amino acid at position 206.
46. The composition of any one of claims 43-45, wherein a non-standard amino acid at position 79 is directly linked by a bond to a non-standard amino acid at position 110.
47. The composition of any one of claims 43-46, wherein a non-standard amino acid at position 135 is directly linked by a bond to a non-standard amino acid at position 410.
48. The composition of any one of claims 43-47, wherein a non-standard amino acid at position 180 is directly linked by a bond to a non-standard amino acid at position 190.
49. The composition of any one of claims 43-48, wherein a non-standard amino acid at position 384 is directly linked by a bond to a non-standard amino acid at position 393.
50. The composition of any one of claims 18-24, 27-30, 33-36, 39-42, or 45-49 wherein the bond is a diselenide bond or a selenyl-sulfhydryl bond.
51. The composition of claim 50, wherein the diselenide bond or the selenyl-sulfhydryl bond is in a location of a disulfide bond in a corresponding recombinant enzyme without the one or more non-standard amino acids.
52. The composition of any one of claims 4, 5, and 8-51, wherein the T_m of the corresponding stabilized phytase polypeptide, functional fragment thereof, or variant thereof, is less than 37°C.
53. The composition of any one of claims 4, 5, and 8-52, wherein the T_m of the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, is greater than 37°C, 40°C, 45°C, 50°C, 55°C, 60°C, or 65°C.
54. The composition of any one of claims 4, 5, and 8-53, wherein the T_m of the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, is at least 10°C higher than the T_m of the corresponding recombinant enzyme, functional fragment thereof, or variant thereof.
55. The composition of claim 54, wherein the T_m of the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, is at least 15°C higher than the T_m of the corresponding recombinant enzyme, functional fragment thereof, or variant thereof.

56. The composition of any one of claims 1-55, wherein the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, has a half-life in an environment that is at least 1.1 fold higher than a half-life of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids in the environment.
57. The composition of claim 56, wherein the half-life of the phytase polypeptide, functional fragment thereof, or variant thereof in the environment, is greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or more hours.
58. The composition of claim 56 or 57, wherein the half-life of the phytase polypeptide, functional fragment thereof, or variant thereof in the environment, is greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or more days.
59. The composition of any one of claims 2 and 4-58, wherein the stabilized phytase polypeptide has at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 fold higher hydrolytic activity for a phytate substrate in an environment than an hydrolytic activity for the phytate substrate of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.
60. The composition of any one of claims 2 and 4-59, wherein a loss of hydrolytic activity of the stabilized phytase polypeptide in a reducing environment as compared to an activity of the stabilized phytase polypeptide in a non-reducing environment is at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% less than a loss of hydrolytic activity of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids under the same conditions.
61. The composition of any one of claims 2 and 4-60, wherein the stabilized phytase polypeptide has at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 fold higher hydrolytic activity for a phytate substrate after being present in an environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or 50 minutes than a hydrolytic activity for the phytate substrate of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids after being present in the environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or 50 minutes.
62. The composition of any one of claims 2 and 4-61, wherein the stabilized phytase polypeptide has at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 10, 20,

- 30, 40, 50, 60, 70, 80, 90 or 100 fold higher hydrolytic activity for a phytate substrate after being present in an environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, or 24 hours than a hydrolytic activity for the phytate substrate of a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids after being present in the environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, or 24 hours.
63. The composition of any one of claims 2 and 4-62, wherein the phytate substrate is in an animal feedstock.
 64. The composition of any one of claims 2, 3, and 6-63, wherein the environment is an environment with a temperature of from 4 °C to 98 °C.
 65. The composition of any one of claims 2, 3, and 6-64, wherein the environment is a reducing environment.
 66. The composition of any one of claims 2, 3, and 6-65, wherein the environment is an environment with an acidic pH.
 67. The composition of any one of claims 2, 3, and 6-66, wherein the environment is a stomach environment.
 68. The composition of any one of claims 2, 3, and 6-67, wherein the environment is a rumen environment.
 69. The composition of any one of claims 2, 3, and 6-68, wherein the environment comprises a reducing agent at a concentration of from 0.01 mM to 100 mM.
 70. The composition of any one of claims 2, 3, and 6-69, wherein the environment has a pH of from 1-7.
 71. The composition of any one of claims 2, 3, and 6-70, wherein the environment has a pH of from 1-5.
 72. The composition of any one of claims 2, 3, and 6-71, wherein the environment has a pH of from 1-3.
 73. The composition of any one of claims 2, 3, and 6-72, wherein the environment has a salt concentration of from 10 mM to 1 M.
 74. The composition of any one of claims 2, 3, and 6-73, wherein the environment has a reduction potential that is less than 200 mV, 150 mV, 100 mV, 50 mV, 0 mV, -50 mV, -100 mV, -150 mV, -160 mV, -170 mV, -180 mV, -190 mV, -200 mV, -210 mV, -220 mV, -230 mV, -240 mV, -250 mV, -260 mV, -270 mV, -280 mV, -290 mV, -300 mV, -310 mV, -320 mV, -330 mV, -340 mV, -350 mV, -360 mV, -370 mV, -380 mV, -390 mV, -400 mV, -410 mV, -420 mV, -430 mV, -440 mV, -450 mV, -460 mV, -470 mV, -

480 mV, -490 mV, -500 mV, -510 mV, -520 mV, -530 mV, -540 mV, -550 mV, -560 mV, -570 mV, -580 mV, -590 mV, or -600 mV.

75. The composition of any one of claims 1-74, wherein the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, is recombinant.
76. The composition of any one of claims 1-75, wherein the stabilized phytase polypeptide, functional fragment thereof, or variant thereof, is an animal, a plant, a fungi, or a bacterial phytase.
77. The composition of any one of claims 1-76, further comprising a xylanase polypeptide.
78. The composition of claim 77, wherein the xylanase is a stabilized xylanase comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof.
79. A composition comprising a polynucleotide encoding the composition of any one of claims 1-76.
80. The composition of claim 79, wherein the polynucleotide is a vector.
81. The composition of any one of claims 1-80, wherein a bond directly linking two of the one or more non-standard amino acids of the stabilized phytase polypeptide does not break in an environment, wherein the bond directly linking two of the one or more standard amino acids of the corresponding phytase polypeptide does break in the same environment.
82. A method of making the composition of any one of claims 1-81, wherein the method comprises expressing an amino acid sequence of the stabilized phytase polypeptide.
83. The method of claim 82, wherein expressing comprises expressing in a cell or in vitro.
84. The method of claim 83, wherein the cell is a bacterial cell.
85. The method of claim 83 or 84, wherein the cell is a genomically recoded cell.
86. The method of any one of claims 83-85, wherein the cell comprises a reassigned codon recognized by a stabilizing non-standard amino acid tRNA comprising an anticodon corresponding to the reassigned codon.
87. The method of claim 86, wherein the amino acid sequence of the stabilized phytase polypeptide is encoded by a polynucleotide sequence comprising at least one codon of a natural amino acid that has been replaced by the reassigned codon.
88. The method of claim 86 or 87, wherein the stabilizing non-standard amino acid tRNA is a selenocysteine tRNA.

89. The method of any one of claims 83-88, wherein the method comprises culturing the cell under conditions in which the amino acid sequence of the stabilized phytase polypeptide is expressed.
90. The method of any one of claims 86-89, wherein the reassigned codon is UAG, UAA, UGA, or a combination thereof.
91. A method comprising contacting a phytate substrate in an environment to a stabilized phytase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof; wherein the stabilized phytase polypeptide, functional fragment thereof, or variant thereof catalyzes hydrolysis of the phytate substrate at a higher rate than a corresponding phytase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.
92. The method of claim 91, wherein the stabilized phytase polypeptide, functional fragment thereof, or variant thereof is the stabilized phytase polypeptide, functional fragment thereof, or variant thereof of any one of claims 1-81.
93. The method of claim 91 or 92, wherein the phytate substrate is in an animal feedstock.
94. The method of any one of claims 91-93, wherein the phytate substrate is from an animal feedstock.
95. The method of any one of claims 91-94, further comprising providing an animal feedstock comprising the phytate substrate.
96. The method of claim 95, further comprising contacting the animal feedstock with the stabilized phytase polypeptide.
97. The method of claim 95 or 96, further comprising feeding the animal feedstock to an animal.
98. The method of any one of claims 91-97, wherein the environment is an environment with a temperature of from 4 °C to 50 °C.
99. The method of any one of claims 91-98, wherein the environment is a reducing environment.
100. The method of any one of claims 91-99, wherein the environment is an environment with an acidic pH.
101. The method of any one of claims 91-100, wherein the environment is a stomach environment.
102. The method of any one of claims 91-101, wherein the environment is a rumen environment.

103. The method of any one of claims 91-102, wherein the environment comprises a reducing agent at a concentration of from 0.01 mM to 100 mM.
104. The method of any one of claims 91-103, wherein the environment has a pH of from 1-7.
105. The method of any one of claims 91-104, wherein the environment has a pH of from 1-5.
106. The method of any one of claims 91-105, wherein the environment has a pH of from 1-3.
107. The method of any one of claims 91-106, wherein the environment has a salt concentration of from 10 mM to 1 M.
108. The method of any one of claims 91-107, wherein the environment has a reduction potential that is less than 200 mV, 150 mV, 100 mV, 50 mV, 0 mV, -50 mV, -100 mV, -150 mV, -160 mV, -170 mV, -180 mV, -190 mV, -200 mV, -210 mV, -220 mV, -230 mV, -240 mV, -250 mV, -260 mV, -270 mV, -280 mV, -290 mV, -300 mV, -310 mV, -320 mV, -330 mV, -340 mV, -350 mV, -360 mV, -370 mV, -380 mV, -390 mV, -400 mV, -410 mV, -420 mV, -430 mV, -440 mV, -450 mV, -460 mV, -470 mV, -480 mV, -490 mV, -500 mV, -510 mV, -520 mV, -530 mV, -540 mV, -550 mV, -560 mV, -570 mV, -580 mV, -590 mV, or -600 mV.
109. A composition comprising a stabilized xylanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof.
110. A composition comprising a stabilized xylanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized xylanase polypeptide has a higher hydrolytic activity for a polysaccharide substrate in an environment than a hydrolytic activity for the polysaccharide substrate of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.
111. A composition comprising a stabilized xylanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized xylanase polypeptide does not destabilize in an environment that a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids does destabilize.
112. The composition of any one of claims 109-111, wherein the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, has a melting temperature (T_m) that is at least 5°C higher than a T_m of a corresponding recombinant enzyme, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

113. A composition comprising a stabilized xylanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized xylanase polypeptide has a melting temperature (T_m) that is at least 5°C higher than a T_m of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.
114. The composition of any one of claims 109, 111, and 113 wherein the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, has a higher hydrolytic activity for a polysaccharide substrate in an environment than a hydrolytic activity for the polysaccharide substrate of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.
115. The composition of any one of claims 109, 110, and 113, wherein the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, does not destabilize in an environment that a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids does destabilize.
116. The composition of any one of claims 109-115, wherein at least one, two, three, four or more of the one or more non-standard amino acids is selenocysteine.
117. The composition of any one of claims 1-116, wherein at least two of the one or more non-standard amino acids are directly linked by a bond.
118. The composition of claim 117, wherein at least four of the one or more non-standard amino acids are directly linked by a bond, wherein a first pair of the at least four of the one or more non-standard amino acids is directly linked by a bond, and a second pair of the at least four of the one or more non-standard amino acids is directly linked by a bond.
119. The composition of claim 117 or 118, wherein the bond is a diselenide bond or a selenyl-sulfhydryl bond.
120. The composition of claim 119, wherein the diselenide bond or the selenyl-sulfhydryl bond is an intermolecular or an intramolecular bond.
121. The composition of any one of claims 109-120, wherein the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, has a half-life that is at least 1.1 fold higher than a half-life of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

122. The composition of any one of claims 109-121, wherein the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, has at least 70%, 75%, 80%, 85%, 90%, 95% sequence identity to a sequence selected from Table 2.
123. The composition of any one of claims 109-122, wherein the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, comprises a sequence with at least 70%, 75%, 80%, 85%, 90%, 95% sequence identity to at least 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, or 396 contiguous amino acids of the sequence the sequence selected from Table 2.
124. The composition of claim 122 or 123, wherein the sequence selected from Table 2 is SEQ ID NO:71.
125. The composition of any one of claims 109-123, wherein the one or more non-standard amino acids is at position 110 of SEQ ID NO:71, or at position 154 of SEQ ID NO:71.
126. The composition of claim 125, wherein a non-standard amino acid at position 110 is directly linked by a bond to a non-standard amino acid at position 154.
127. The composition of claim 126, wherein the bond is a diselenide bond or a selenyl-sulfhydryl bond.
128. The composition of claim 127, wherein the diselenide bond or the selenyl-sulfhydryl bond is in a location of a disulfide bond in a corresponding recombinant enzyme without the one or more non-standard amino acids.
129. The composition of any one of claims 112, 113, and 116-128, wherein the T_m of the corresponding stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, is less than 37°C.
130. The composition of any one of claims 112, 113, and 116-129, wherein the T_m of the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, is greater than 37°C, 40°C, 45°C, 50°C, 55°C, 60°C, or 65°C.
131. The composition of any one of claims 112, 113, and 116-130, wherein the T_m of the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, is at least 10°C higher than the T_m of the corresponding recombinant enzyme, functional fragment thereof, or variant thereof.
132. The composition of claim 131, wherein the T_m of the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, is at least 15°C higher than the T_m of the corresponding recombinant enzyme, functional fragment thereof, or variant thereof.
133. The composition of any one of claims 109-132, wherein the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, has a half-life in an

- environment that is at least 1.1 fold higher than a half-life of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids in the environment.
134. The composition of claim 133, wherein the half-life of the xylanase polypeptide, functional fragment thereof, or variant thereof in the environment, is greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or more hours.
135. The composition of claim 133 or 134, wherein the half-life of the xylanase polypeptide, functional fragment thereof, or variant thereof in the environment, is greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or more days.
136. The composition of any one of claims 110, and 112-135, wherein the stabilized xylanase polypeptide has at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 fold higher hydrolytic activity for a polysaccharide substrate in an environment than an hydrolytic activity for the polysaccharide substrate of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.
137. The composition of any one of claims 110 and 112-136, wherein a loss of hydrolytic activity of the stabilized xylanase polypeptide in a reducing environment as compared to an activity of the stabilized xylanase polypeptide in a non-reducing environment is at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% less than a loss of hydrolytic activity of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids under the same conditions.
138. The composition of any one of claims 110 and 112-137, wherein the stabilized xylanase polypeptide has at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 fold higher hydrolytic activity for a polysaccharide substrate after being present in an environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or 50 minutes than a hydrolytic activity for the polysaccharide substrate of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids after being present in the environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or 50 minutes.
139. The composition of any one of claims 110 and 112-137, wherein the stabilized xylanase polypeptide has at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 fold higher hydrolytic activity for a polysaccharide substrate after being present in an environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12,

- 18, or 24 hours than a hydrolytic activity for the polysaccharide substrate of a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids after being present in the environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, or 24 hours.
140. The composition of any one of claims 110 and 112-139, wherein the polysaccharide substrate is in an animal feedstock.
 141. The composition of any one of claims 110, 111, and 114-140, wherein the environment is an environment with a temperature of from 4 °C to 98 °C.
 142. The composition of any one of claims 110, 111, and 114-141, wherein the environment is a reducing environment.
 143. The composition of any one of claims 110, 111, and 114-142, wherein the environment is an environment with an acidic pH.
 144. The composition of any one of claims 110, 111, and 114-143, wherein the environment is a stomach environment.
 145. The composition of any one of claims 110, 111, and 114-144, wherein the environment is a rumen environment.
 146. The composition of any one of claims 110, 111, and 114-145, wherein the environment comprises a reducing agent at a concentration of from 0.01 mM to 100 mM.
 147. The composition of any one of claims 110, 111, and 114-146, wherein the environment has a pH of from 1-7.
 148. The composition of any one of claims 110, 111, and 114-147, wherein the environment has a pH of from 1-5.
 149. The composition of any one of claims 110, 111, and 114-148, wherein the environment has a pH of from 1-3.
 150. The composition of any one of claims 110, 111, and 114-149, wherein the environment has a salt concentration of from 10 mM to 1 M.
 151. The composition of any one of claims 110, 111, and 114-150, wherein the environment has a reduction potential that is less than 200 mV, 150 mV, 100 mV, 50 mV, 0 mV, -50 mV, -100 mV, -150 mV, -160 mV, -170 mV, -180 mV, -190 mV, -200 mV, -210 mV, -220 mV, -230 mV, -240 mV, -250 mV, -260 mV, -270 mV, -280 mV, -290 mV, -300 mV, -310 mV, -320 mV, -330 mV, -340 mV, -350 mV, -360 mV, -370 mV, -380 mV, -390 mV, -400 mV, -410 mV, -420 mV, -430 mV, -440 mV, -450 mV, -460 mV, -470 mV, -480 mV, -490 mV, -500 mV, -510 mV, -520 mV, -530 mV, -540 mV, -550 mV, -560 mV, -570 mV, -580 mV, -590 mV, or -600 mV.

152. The composition of any one of claims 109-151, wherein the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, is recombinant.
153. The composition of any one of claims 109-152, wherein the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof, is an animal, a plant, a fungi, or a bacterial xylanase.
154. The composition of any one of claims 109-153, further comprising a phytase polypeptide.
155. The composition of claim 154, wherein the phytase is a stabilized phytase comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof.
156. A composition comprising a polynucleotide encoding the composition of any one of claims 109-155.
157. The composition of claim 156, wherein the polynucleotide is a vector.
158. The composition of any one of claims 109-157, wherein a bond directly linking two of the one or more non-standard amino acids of the stabilized xylanase polypeptide does not break in an environment, wherein the bond directly linking two of the one or more standard amino acids of the corresponding xylanase polypeptide does break in the same environment.
159. A method of making the composition of any one of claims 109-153, wherein the method comprises expressing an amino acid sequence of the stabilized xylanase polypeptide.
160. The method of claim 159, wherein expressing comprises expressing in a cell or in vitro.
161. The method of claim 160, wherein the cell is a bacterial cell.
162. The method of claim 160 or 161, wherein the cell is a genomically recoded cell.
163. The method of any one of claims 160-162, wherein the cell comprises a reassigned codon recognized by a stabilizing non-standard amino acid tRNA comprising an anticodon corresponding to the reassigned codon.
164. The method of claim 163, wherein the amino acid sequence of the stabilized xylanase polypeptide is encoded by a polynucleotide sequence comprising at least one codon of a natural amino acid that has been replaced by the reassigned codon.
165. The method of claim 163 or 164, wherein the stabilizing non-standard amino acid tRNA is a selenocysteine tRNA.
166. The method of any one of claims 160-165, wherein the method comprises culturing the cell under conditions in which the amino acid sequence of the stabilized xylanase polypeptide is expressed.

167. The method of any one of claims 163-166, wherein the reassigned codon is UAG, UAA, UGA, or a combination thereof.
168. A method comprising contacting a polysaccharide substrate in an environment to a stabilized xylanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof; wherein the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof catalyzes hydrolysis of the polysaccharide substrate at a higher rate than a corresponding xylanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.
169. The method of claim 168, wherein the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof is the stabilized xylanase polypeptide, functional fragment thereof, or variant thereof of any one of claims 109-153.
170. The method of claim 168 or 169, wherein the polysaccharide substrate is in an animal feedstock.
171. The method of any one of claims 168-170, wherein the polysaccharide substrate is from an animal feedstock.
172. The method of any one of claims 168-171, further comprising providing an animal feedstock comprising the polysaccharide substrate.
173. The method of claim 172, further comprising contacting the animal feedstock with the stabilized xylanase polypeptide.
174. The method of claim 172 or 173, further comprising feeding the animal feedstock to an animal.
175. The method of any one of claims 168-174, wherein the environment is an environment with a temperature of from 4 °C to 50 °C.
176. The method of any one of claims 168-175, wherein the environment is a reducing environment.
177. The method of any one of claims 168-176, wherein the environment is an environment with an acidic pH.
178. The method of any one of claims 168-177, wherein the environment is a stomach environment.
179. The method of any one of claims 168-178, wherein the environment is a rumen environment.
180. The method of any one of claims 168-179, wherein the environment comprises a reducing agent at a concentration of from 0.01 mM to 100 mM.

181. The method of any one of claims 168-180, wherein the environment has a pH of from 1-7.
182. The method of any one of claims 168-181, wherein the environment has a pH of from 1-5.
183. The method of any one of claims 168-182, wherein the environment has a pH of from 1-3.
184. The method of any one of claims 168-183, wherein the environment has a salt concentration of from 10 mM to 1 M.
185. The method of any one of claims 168-184, wherein the environment has a reduction potential that is less than 200 mV, 150 mV, 100 mV, 50 mV, 0 mV, -50 mV, -100 mV, -150 mV, -160 mV, -170 mV, -180 mV, -190 mV, -200 mV, -210 mV, -220 mV, -230 mV, -240 mV, -250 mV, -260 mV, -270 mV, -280 mV, -290 mV, -300 mV, -310 mV, -320 mV, -330 mV, -340 mV, -350 mV, -360 mV, -370 mV, -380 mV, -390 mV, -400 mV, -410 mV, -420 mV, -430 mV, -440 mV, -450 mV, -460 mV, -470 mV, -480 mV, -490 mV, -500 mV, -510 mV, -520 mV, -530 mV, -540 mV, -550 mV, -560 mV, -570 mV, -580 mV, -590 mV, or -600 mV.
186. A feedstock comprising the composition of any one of claims 1-76, wherein a concentration of the phytase in the final feed mixture is less than 1200 units of the phytase per kilogram of the final feed mixture.
187. The feedstock of claim 186, further comprising the composition of any one of claims 109-153.
188. A feedstock comprising the composition of any one of claims 109-153, wherein a concentration of the xylanase in the final feed mixture is less than 1200 units of the xylanase per kilogram of the final feed mixture.
189. The feedstock of claim 188, further comprising the composition of any one of claims 1-76.
190. A feedstock comprising a bacterial cell, wherein the bacterial cell expresses or secretes a stabilized phytase of the composition of any one of claims 1-76.
191. The feedstock of claim 190, further comprising an additional bacterial cell, wherein the additional bacterial cell expresses or secretes a stabilized xylanase of the composition of any one of claims 109-153.
192. A feedstock comprising a bacterial cell, wherein the bacterial cell expresses or secretes a stabilized xylanase of the composition of any one of claims 109-153.

193. A method comprising feeding an animal with a feedstock comprising a stabilized phytase polypeptide of the composition of any one of claims 1-76, and/or a stabilized xylanase polypeptide of the composition of any one of claims 109-153.
194. A method comprising feeding an animal with a feedstock comprising a bacterial cell, wherein the bacterial cell expresses or secretes a stabilized phytase polypeptide of the composition of any one of claims 1-76, and/or a stabilized xylanase polypeptide of the composition of any one of claims 109-153.
195. The method of 194, wherein the animal extracts the stabilized phytase polypeptide and/or the stabilized xylanase polypeptide from the bacterial cell.
196. A composition comprising a stabilized mannanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof.
197. A composition comprising a stabilized mannanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized mannanase polypeptide has a higher hydrolytic activity for a beta-D-mannoside substrate in an environment than a hydrolytic activity for the beta-D-mannoside substrate of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids in the environment.
198. A composition comprising a stabilized mannanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized mannanase polypeptide does not destabilize in an environment that a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids does destabilize.
199. The composition of any one of claims 196-198, wherein the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, has a melting temperature (T_m) that is at least 5°C higher than a T_m of a corresponding recombinant enzyme, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.
200. A composition comprising a stabilized mannanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized mannanase polypeptide has a melting temperature (T_m) that is at least 5°C higher than a T_m of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.

201. The composition of any one of claims 196, 198, or 200, wherein the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, has a higher hydrolytic activity for a beta-D-mannoside substrate in an environment than a hydrolytic activity for the beta-D-mannoside substrate of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.
202. The composition of any one of claims 196, 197, or 200, wherein the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, does not destabilize in an environment that a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids does destabilize.
203. The composition of any one of claims 196-202, wherein at least one, two, three, four or more of the one or more non-standard amino acids is selenocysteine.
204. The composition of any one of claims 196-203, wherein at least two of the one or more non-standard amino acids are directly linked by a bond.
205. The composition of claim 204, wherein at least four of the one or more non-standard amino acids are directly linked by a bond, wherein a first pair of the at least four of the one or more non-standard amino acids is directly linked by a bond, and a second pair of the at least four of the one or more non-standard amino acids is directly linked by a bond.
206. The composition of claim 204 or 205, wherein the bond is a diselenide bond or a selenyl-sulfhydryl bond.
207. The composition of claim 206, wherein the diselenide bond or the selenyl-sulfhydryl bond is an intermolecular or an intramolecular bond.
208. The composition of any one of claims 196-207, wherein the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, has a half-life that is at least 1.1 fold higher than a half-life of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.
209. The composition of any one of claims 196-208, wherein the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, has at least 70%, 75%, 80%, 85%, 90%, 95% sequence identity to a sequence from Table 3.
210. The composition of any one of claims 196-209, wherein the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, comprises a sequence with at least 70%, 75%, 80%, 85%, 90%, 95% sequence identity to at least 25, 50, 75, 100,

- 125, 150, 175, 200, 225, 250, 275, 300, or 302 contiguous amino acids of the sequence from Table 3.
211. The composition of claim 210, wherein the sequence from Table 3 is SEQ ID NO:85.
212. The composition of any one of claims 196-211, wherein the one or more non-standard amino acids is at position 74 or position 81 of SEQ ID NO:85.
213. The composition of claim 212, wherein a non-standard amino acid at position 74 is directly linked by a bond to a non-standard amino acid at position 81.
214. The composition of any one of claims 204-213, wherein the bond is a diselenide bond or a selenyl-sulfhydryl bond.
215. The composition of claim 214, wherein the diselenide bond or the selenyl-sulfhydryl bond is in a location of a disulfide bond in a corresponding recombinant enzyme without the one or more non-standard amino acids.
216. The composition of any one of claims 199, 200, and 203-215, wherein the T_m of the corresponding stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, is less than 37°C.
217. The composition of any one of claims 199, 200, and 203-216, wherein the T_m of the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, is greater than 37°C, 40°C, 45°C, 50°C, 55°C, 60°C, or 65°C.
218. The composition of any one of claims 199, 200, and 203-217, wherein the T_m of the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, is at least 10°C higher than the T_m of the corresponding recombinant enzyme, functional fragment thereof, or variant thereof.
219. The composition of claim 218, wherein the T_m of the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, is at least 15°C higher than the T_m of the corresponding recombinant enzyme, functional fragment thereof, or variant thereof.
220. The composition of any one of claims 196-219, wherein the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, has a half-life in an environment that is at least 1.1 fold higher than a half-life of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids in the environment.
221. The composition of claim 220, wherein the half-life of the mannanase polypeptide, functional fragment thereof, or variant thereof in the environment, is greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or more hours.

222. The composition of claim 220 or 221, wherein the half-life of the mannanase polypeptide, functional fragment thereof, or variant thereof in the environment, is greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or more days.
223. The composition of any one of claims 197 and 199-222, wherein the stabilized mannanase polypeptide has at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 fold higher hydrolytic activity for a beta-D-mannoside substrate in an environment than an hydrolytic activity for the beta-D-mannoside substrate of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.
224. The composition of any one of claims 197 and 199-223, wherein a loss of hydrolytic activity of the stabilized mannanase polypeptide in a reducing environment as compared to an activity of the stabilized mannanase polypeptide in a non-reducing environment is at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% less than a loss of hydrolytic activity of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids under the same conditions.
225. The composition of any one of claims 197 and 199-224, wherein the stabilized mannanase polypeptide has at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 fold higher hydrolytic activity for a beta-D-mannoside substrate after being present in an environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or 50 minutes than a hydrolytic activity for the beta-D-mannoside substrate of a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids after being present in the environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or 50 minutes.
226. The composition of any one of claims 197 and 199-224, wherein the stabilized mannanase polypeptide has at least a 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 fold higher hydrolytic activity for a beta-D-mannoside substrate after being present in an environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, or 24 hours than a hydrolytic activity for the beta-D-mannoside substrate of a corresponding mannanase polypeptide, functional fragment thereof, or variant

- thereof that does not comprise the one or more non-standard amino acids after being present in the environment for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, or 24 hours.
227. The composition of any one of claims 197 and 199-226, wherein the beta-D-mannoside substrate is in an animal feedstock.
 228. The composition of any one of claims 197, 198, and 201-227, wherein the environment is an environment with a temperature of from 4 °C to 98 °C.
 229. The composition of any one of claims 197, 198, and 201-228, wherein the environment is a reducing environment.
 230. The composition of any one of claims 197, 198, and 201-229, wherein the environment is an environment with an acidic pH.
 231. The composition of any one of claims 197, 198, and 201-230, wherein the environment is a stomach environment.
 232. The composition of any one of claims 197, 198, and 201-231, wherein the environment is a rumen environment.
 233. The composition of any one of claims 197, 198, and 201-232, wherein the environment comprises a reducing agent at a concentration of from 0.01 mM to 100 mM.
 234. The composition of any one of claims 197, 198, and 201-233, wherein the environment has a pH of from 1-7.
 235. The composition of any one of claims 197, 198, and 201-234, wherein the environment has a pH of from 1-5.
 236. The composition of any one of claims 197, 198, and 201-235, wherein the environment has a pH of from 1-3.
 237. The composition of any one of claims 197, 198, and 201-236, wherein the environment has a salt concentration of from 10 mM to 1 M.
 238. The composition of any one of claims 197, 198, and 201-237, wherein the environment has a reduction potential that is less than 200 mV, 150 mV, 100 mV, 50 mV, 0 mV, -50 mV, -100 mV, -150 mV, -160 mV, -170 mV, -180 mV, -190 mV, -200 mV, -210 mV, -220 mV, -230 mV, -240 mV, -250 mV, -260 mV, -270 mV, -280 mV, -290 mV, -300 mV, -310 mV, -320 mV, -330 mV, -340 mV, -350 mV, -360 mV, -370 mV, -380 mV, -390 mV, -400 mV, -410 mV, -420 mV, -430 mV, -440 mV, -450 mV, -460 mV, -470 mV, -480 mV, -490 mV, -500 mV, -510 mV, -520 mV, -530 mV, -540 mV, -550 mV, -560 mV, -570 mV, -580 mV, -590 mV, or -600 mV.
 239. The composition of any one of claims 196-238, wherein the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, is recombinant.

240. The composition of any one of claims 196-239, wherein the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof, is an animal, a plant, a fungi, or a bacterial mannanase.
241. The composition of any one of claims 196-240, further comprising a phytase polypeptide, a xylanase polypeptide or a combination thereof.
242. The composition of claim 241, wherein the phytase polypeptide is a stabilized phytase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof.
243. The composition of claim 241, wherein the xylanase polypeptide is a stabilized xylanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof.
244. A composition comprising a polynucleotide encoding the composition of any one of claims 196-240.
245. The composition of claim 244, wherein the polynucleotide is a vector.
246. The composition of any one of claims 196-245, wherein a bond directly linking two of the one or more non-standard amino acids of the stabilized mannanase polypeptide does not break in an environment, wherein the bond directly linking two of the one or more standard amino acids of the corresponding mannanase polypeptide does break in the same environment.
247. A method of making the composition of any one of claims 196-246, wherein the method comprises expressing an amino acid sequence of the stabilized mannanase polypeptide.
248. The method of claim 247, wherein expressing comprises expressing in a cell or in vitro.
249. The method of claim 248, wherein the cell is a bacterial cell.
250. The method of claim 248 or 249, wherein the cell is a genomically recoded cell.
251. The method of any one of claims 248-250, wherein the cell comprises a reassigned codon recognized by a stabilizing non-standard amino acid tRNA comprising an anticodon corresponding to the reassigned codon.
252. The method of claim 251, wherein the amino acid sequence of the stabilized mannanase polypeptide is encoded by a polynucleotide sequence comprising at least one codon of a natural amino acid that has been replaced by the reassigned codon.
253. The method of claim 251 or 252, wherein the stabilizing non-standard amino acid tRNA is a selenocysteine tRNA.

254. The method of any one of claims 248-253, further comprising culturing the cell under conditions in which the amino acid sequence of the stabilized mannanase polypeptide is expressed.
255. The method of any one of claims 251-254, wherein the reassigned codon is UAG, UAA, UGA, or a combination thereof.
256. A method comprising contacting a beta-D-mannoside substrate in an environment to a stabilized mannanase polypeptide comprising one or more non-standard amino acids, a functional fragment thereof, or a variant thereof, wherein the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof catalyzes hydrolysis of the beta-D-mannoside substrate at a higher rate than a corresponding mannanase polypeptide, functional fragment thereof, or variant thereof that does not comprise the one or more non-standard amino acids.
257. The method of claim 256, wherein the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof is the stabilized mannanase polypeptide, functional fragment thereof, or variant thereof of any one of claims 196-240.
258. The method of claim 256 or 257, wherein the beta-D-mannoside substrate is in an animal feedstock.
259. The method of any one of claims 256-258, wherein the beta-D-mannoside substrate is from an animal feedstock.
260. The method of any one of claims 256-259, further comprising providing an animal feedstock comprising the beta-D-mannoside substrate.
261. The method of claim 260, further comprising contacting the animal feedstock with the stabilized mannanase polypeptide.
262. The method of claim 260 or 261, further comprising feeding the animal feedstock to an animal.
263. The method of any one of claims 256-262, wherein the environment is an environment with a temperature of from 4 °C to 50 °C.
264. The method of any one of claims 256-263, wherein the environment is a reducing environment.
265. The method of any one of claims 256-264, wherein the environment is an environment with an acidic pH.
266. The method of any one of claims 256-265, wherein the environment is a stomach environment.

267. The method of any one of claims 256-266, wherein the environment is a rumen environment.
268. The method of any one of claims 256-267, wherein the environment comprises a reducing agent at a concentration of from 0.01 mM to 100 mM.
269. The method of any one of claims 256-268, wherein the environment has a pH of from 1-7.
270. The method of any one of claims 256-269, wherein the environment has a pH of from 1-5.
271. The method of any one of claims 256-270, wherein the environment has a pH of from 1-3.
272. The method of any one of claims 256-271, wherein the environment has a salt concentration of from 10 mM to 1 M.
273. The method of any one of claims 256-272, wherein the environment has a reduction potential that is less than 200 mV, 150 mV, 100 mV, 50 mV, 0 mV, -50 mV, -100 mV, 150 mV, -160 mV, -170 mV, -180 mV, -190 mV, -200 mV, -210 mV, -220 mV, -230 mV, -240 mV, -250 mV, -260 mV, -270 mV, -280 mV, -290 mV, -300 mV, -310 mV, -320 mV, -330 mV, -340 mV, -350 mV, -360 mV, -370 mV, -380 mV, -390 mV, -400 mV, -410 mV, -420 mV, -430 mV, -440 mV, -450 mV, -460 mV, -470 mV, -480 mV, -490 mV, -500 mV, -510 mV, -520 mV, -530 mV, -540 mV, -550 mV, -560 mV, -570 mV, -580 mV, -590 mV, or -600 mV.
274. A method of manufacturing an animal feedstock by combining one or more stabilized feed enzymes, functional fragments, or variants thereof with one or more substrates of the feed enzymes.
275. The method of claim 274, wherein the one or more stabilized feed enzymes, functional fragments, or variants thereof comprise a stabilized phytase, a stabilized xylanase, a stabilized mannanase, or a combination thereof.
276. The method of claim 274 or 275, wherein the one or more stabilized feed enzymes are combined with the one or more substrates before feeding the feedstock to an animal, or they may be pre-combined.