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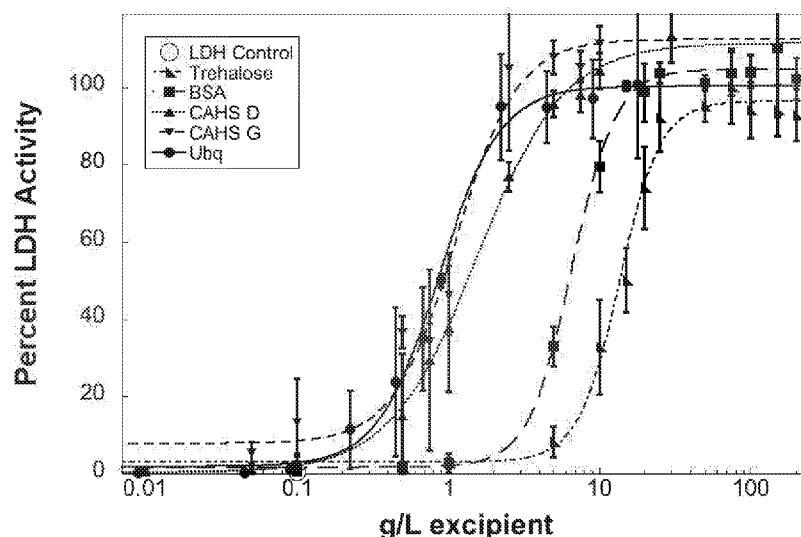
(71) Applicant: **THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL** [US/US]; 109 Church Street, Chapel Hill, North Carolina 27516 (US).(72) Inventors: **PISZKIEWICZ, Samantha**; 112 Misty Woods Circle, Apt. T, Chapel Hill, North Carolina 27514 (US). **PIELAK, Gary Joseph**; 612 Laurel Hill Road, Chapel Hill, North Carolina 27514 (US). **BOOTHBY,**

Thomas Christopher Clark; 707 White Cross Road, Chapel Hill, North Carolina 27516 (US).

(74) Agent: **BONNEN, Alice, M.**; MYERS BIGEL, P.A., PO Box 37428, Raleigh, North Carolina 27627 (US).(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,

(54) Title: COMPOSITIONS AND METHODS FOR STABILIZATION OF PROTEINS

Fig. 1



(57) **Abstract:** The present invention relates to methods and compositions for stabilizing proteins. Compositions are provided comprising at least one protective polypeptide and/or sugar polymer, and at least one heterologous polypeptide and/or peptide of interest. Further provided are methods for stabilizing proteins, methods for stabilizing cells, tissues and organs using the compositions and solutions of the invention. Also provided are methods for producing a cell and/or an organism having increased tolerance to desiccation and/or drought, comprising introducing the organism and/or cell at least one polynucleotide encoding a protective polypeptide.

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COMPOSITIONS AND METHODS FOR STABILIZATION OF PROTEINS

STATEMENT REGARDING ELECTRONIC FILING OF A SEQUENCE LISTING

5 A Sequence Listing in ASCII text format, submitted under 37 C.F.R. § 1.821, entitled 5470-831PR_ST25.txt, 645,186 bytes in size, generated on November 4, 2017 and filed via EFS-Web, is provided in lieu of a paper copy. This Sequence Listing is hereby incorporated herein by reference into the specification for its disclosures.

STATEMENT OF FEDERAL SUPPORT

10 This invention was made with Government support under Grant Numbers IOS-1410854 and CHE-1607359 awarded by the National Science Foundation. The United States Government has certain rights in the invention.

FIELD OF THE INVENTION

15 The invention relates to methods and compositions for stabilizing proteins using polypeptides such as ubiquitin, negative regulator of flagellin synthesis (Flg M), SRC Homology 3 (SH3) Domain, small ubiquitin-like modifier (SUMO), and/or serum albumin, and/or using sugar polymers.

BACKGROUND OF THE INVENTION

20 Many vaccines and protein based pharmaceuticals have limited shelf lives and are structurally and functionally unstable, requiring them to be produced, transported, and stored using a system of refrigerators and freezers known as the “cold-chain.” This makes many of these lifesaving drugs difficult and expensive to manufacture and deliver.

Although numerous molecules are used as crowding agents to stabilize pharmaceuticals in liquid formulations, these additives can be flawed. For example, non-reducing sugars like mannitol, sorbitol, and trehalose are effective in solution but are prone to crystallization and phase separation upon freezing. (Shire, S. J. *Curr. Opin. Biotechnol.* **20**, 708–714 (2009)).

30 Sucrose does not have this problem, but its hydrolysis results in unwanted glycosylation of pharmaceuticals (Shire, S. J. *Curr. Opin. Biotechnol.* **20**, 708–714 (2009)). Surfactants are also common additives; however, surfactants, such as polysorbate 20 and 80, produce peroxides that oxidize methionine groups (Shire, S. J. *Curr. Opin. Biotechnol.* **20**, 708–714 (2009)).

Recombunin®, human serum albumin heterologously expressed in and purified from yeast, is

also used as a stabilizer in drug formulations. However, formulations containing Recombum® still require refrigeration (AlbumedFix. RECOMBUMIN® FORMULATE WITH CONFIDENCE (2016)). These stabilizers and others have extended the half-lives of many pharmaceuticals, but none have eliminated the requirement of low-temperature storage for liquid formulations. Furthermore, many potential protein-based pharmaceuticals never make it to the market because they are deemed too unstable even with low-temperature storage and the addition of stabilizing additives.

Some protein-based pharmaceuticals can be stored at room temperature if they are lyophilized (freeze dried); however, most protein-based pharmaceuticals denature as a result of either the freezing or drying process. Sometimes crowding agents can protect protein-based pharmaceuticals during lyophilization, but none of these crowding agents work universally. The most effective additives for a given pharmaceutical is highly dependent on factors including the pI, β -sheet content, and melting temperature of the drug (Roughton et al. *Comput. Chem. Eng.* **58**, 369–377 (2013)). Even with the addition of stabilizers, many protein-based pharmaceuticals are too unstable to survive lyophilization (Roughton et al. *Comput. Chem. Eng.* **58**, 369–377 (2013)).

The present invention overcomes previous shortcomings in the art by providing new compositions and methods for stabilizing proteins and other biomedical material.

SUMMARY OF THE INVENTION

One aspect of the invention provides a liquid composition comprising: at least one heterologous polypeptide and/or peptide of interest and (a) at least one protective polypeptide selected from the group consisting of a ubiquitin polypeptide, a negative regulator of flagellin synthesis (Flg M) polypeptide, a SRC Homology 3 (SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide, and a serum albumin polypeptide, and any combination thereof; and/or (b) at least one sugar polymer.

A second aspect provides a solid composition comprising: at least one heterologous polypeptide and/or peptide of interest and (a) at least one protective polypeptide selected from the group consisting of a ubiquitin polypeptide, a negative regulator of flagellin synthesis (Flg M) polypeptide, a SRC Homology 3 (SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide, and a serum albumin polypeptide, and any combination thereof; and/or (b) at least one sugar polymer.

A third aspect of the invention provides a method of stabilizing at least one heterologous polypeptide and/or peptide of interest, comprising, contacting the at least one heterologous

polypeptide and/or peptide of interest with (a) at least one protective polypeptide selected from the group consisting of a ubiquitin polypeptide, a negative regulator of flagellin synthesis (Flg M) polypeptide, a SRC Homology 3 (SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide, and a serum albumin polypeptide, and any combination thereof; and/or
5 (b) at least one sugar polymer to produce a liquid composition comprising the at least one heterologous polypeptide and/or peptide of interest and the at least one protective polypeptide and/or at least one sugar polymer, thereby stabilizing the at least one heterologous polypeptide and/or peptide of interest.

In a fourth aspect, the invention provides a method of stabilizing a heterologous cell,
10 tissue or organ, comprising, contacting the heterologous cell, tissue or organ with a solution comprising: (a) at least one protective polypeptide selected from the group consisting of a ubiquitin polypeptide, a negative regulator of flagellin synthesis (Flg M) polypeptide, a SRC Homology 3 (SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide, and a serum albumin polypeptide, and any combination thereof; and/or (b) at least one sugar
15 polymer, thereby stabilizing the heterologous cell, tissue or organ.

In a fifth aspect, the present invention provides a method of increasing drought or desiccation tolerance in an organism, comprising introducing into the organism at least one heterologous nucleotide sequence encoding a protective polypeptide selected from the group consisting of a ubiquitin polypeptide, a negative regulator of flagellin synthesis (Flg M)
20 polypeptide, a SRC Homology 3 (SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide, and a serum albumin polypeptide, and any combination thereof; to produce a transgenic organism expressing the heterologous nucleotide sequence, thereby increasing the drought or desiccation tolerance of the transgenic organism.

In a sixth aspect, the present invention provides a method of producing a transgenic
25 organism having increased tolerance to drought or desiccation, comprising introducing into the organism at least one heterologous nucleotide sequence encoding a protective polypeptide selected from the group consisting of a ubiquitin polypeptide, a negative regulator of flagellin synthesis (Flg M) polypeptide, a SRC Homology 3 (SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide, and a serum albumin polypeptide, and any combination
30 thereof, thereby producing a transgenic organism having increased drought or desiccation tolerance.

In a seventh aspect, the present invention provides a method of increasing drought or desiccation tolerance in a seed, comprising coating the seed with (a) at least one protective polypeptide selected from the group consisting of a ubiquitin polypeptide, a negative regulator

of flagellin synthesis (Flg M) polypeptide, a SRC Homology 3 (SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide, and a serum albumin polypeptide, and any combination thereof; and/or (b) at least one sugar polymer, thereby increasing drought or desiccation tolerance of the seed.

5 In an eighth aspect, the invention provides a transgenic plant, fungus or bacterium having increased tolerance to drought or desiccation comprising in its genome at least one heterologous nucleotide sequence encoding (a) a protective polypeptide selected from the group consisting of a ubiquitin polypeptide, a negative regulator of flagellin synthesis (Flg M) polypeptide, a SRC Homology 3 (SH3) Domain polypeptide, and a serum albumin polypeptide, and any
10 combination thereof.

In a ninth aspect, the invention provides method of reactivating one or more desiccated and inactivated proteins, comprising resuspending the desiccated and inactivated protein in a solution comprising:(a) at least one protective polypeptide selected from the group consisting of a ubiquitin polypeptide, a negative regulator of flagellin synthesis (Flg M) polypeptide, a SRC
15 Homology 3 (SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide, a serum albumin polypeptide, and a tardigrade disordered polypeptide (TDP), and any combination thereof; and/or (b) at least one sugar polymer.

Further provided are transgenic organisms and/or transgenic cells comprising the heterologous nucleotide sequences or recombinant nucleic acid constructs of the invention.

20 These and other aspects of the invention are set forth in more detail in the description of the invention below.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows protection against desiccation-induced lactate dehydrogenase (LDH)
25 inactivation at room temperature.

Fig. 2 shows protection of LDH activity against desiccation-plus-heat (95°C).

Fig. 3 shows protection against desiccation-induced LDH inactivation at room temperature.

Fig. 4 shows protection against desiccation-induced LDH inactivation at room
30 temperature.

Fig. 5 shows protection against desiccation-induced LDH inactivation at room temperature.

Fig. 6 shows resurrection of desiccation-inactivated LDH

DETAILED DESCRIPTION

The present invention now will be described hereinafter with reference to the accompanying drawings and examples, in which embodiments of the invention are shown. This description is not intended to be a detailed catalog of all the different ways in which the invention may be implemented, or all the features that may be added to the instant invention. For example, features illustrated with respect to one embodiment may be incorporated into other embodiments, and features illustrated with respect to a particular embodiment may be deleted from that embodiment. Thus, the invention contemplates that in some embodiments of the invention, any feature or combination of features set forth herein can be excluded or omitted. In addition, numerous variations and additions to the various embodiments suggested herein will be apparent to those skilled in the art in light of the instant disclosure, which do not depart from the instant invention. Hence, the following descriptions are intended to illustrate some particular embodiments of the invention, and not to exhaustively specify all permutations, combinations and variations thereof.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The terminology used in the description of the invention herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention.

All publications, patent applications, patents and other references cited herein are incorporated by reference in their entireties for the teachings relevant to the sentence and/or paragraph in which the reference is presented.

Unless the context indicates otherwise, it is specifically intended that the various features of the invention described herein can be used in any combination. Moreover, the present invention also contemplates that in some embodiments of the invention, any feature or combination of features set forth herein can be excluded or omitted. To illustrate, if the specification states that a composition comprises components A, B and C, it is specifically intended that any of A, B or C, or a combination thereof, can be omitted and disclaimed singularly or in any combination.

As used in the description of the invention and the appended claims, the singular forms “a,” “an” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise.

Also as used herein, “and/or” refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (“or”).

5 The term “about,” as used herein when referring to a measurable value such as a dosage or time period and the like refers to variations of $\pm 20\%$, $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, $\pm 0.5\%$, or even $\pm 0.1\%$ of the specified amount.

As used herein, phrases such as “between X and Y” and “between about X and Y” should be interpreted to include X and Y. As used herein, phrases such as “between about X and Y” mean “between about X and about Y” and phrases such as “from about X to Y” mean “from
10 about X to about Y.”

The term “comprise,” “comprises” and “comprising” as used herein, specify the presence of the stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof.

15 As used herein, the transitional phrase “consisting essentially of” means that the scope of a claim is to be interpreted to encompass the specified materials or steps recited in the claim and those that do not materially affect the basic and novel characteristic(s) of the claimed invention. Thus, the term “consisting essentially of” when used in a claim of this invention is not intended to be interpreted to be equivalent to “comprising.”

20 As used herein, the terms “express,” “expresses,” “expressed” or “expression,” and the like, with respect to a nucleic acid molecule and/or a nucleotide sequence (e.g., RNA or DNA) indicates that the nucleic acid molecule and/or a nucleotide sequence is transcribed and, optionally, translated. Thus, a nucleic acid molecule and/or a nucleotide sequence may express a polypeptide of interest or a functional untranslated RNA.

25 As used herein, “contact,” “contacting,” “contacted,” and grammatical variations thereof, refers to placing the components of a desired reaction together under conditions suitable for carrying out the desired reaction (e.g., stabilizing the polypeptide, peptide, cell, tissue or organ). The term “contact” may comprise any method in which a polypeptide, peptide, cell, organ and/or tissue is exposed to, provided with, or in which a TDP is applied.

30 A “heterologous polypeptide and/or peptide of interest” as used herein, refers to a polypeptide and/or peptide for which protection against degradation or loss of activity is desired. The polypeptide and/or peptide of interest may be heterologous to the organism, to the genus or to the species from which the protective polypeptide (e.g., ubiquitin, Flg M, SH3, SUMO and serum albumin) and/or the sugar polymer is obtained.

A “heterologous cell, tissue or organ” as used herein, refers to a cell, tissue or organ that is heterologous to the organism, to the genus or to the species that naturally produces the polypeptide (e.g., ubiquitin, Flg M, SH3, SUMO and serum albumin) and/or sugar polymer used to protect the heterologous cell, tissue or organ.

5 As used herein, “stabilizing” a heterologous polypeptide and/or peptide (and/or the polypeptides and/or peptides in cells, tissues, and/or organs) means maintaining the structure (1°, 2°, 3° and/or 4° structure) and the function of the polypeptide and/or peptide under either aqueous conditions or dried conditions, or after being frozen and/or dried and then thawed and/or rehydrated as compared to the heterologous polypeptide and/or peptide that has not been
 10 frozen and/or dried and then thawed and/or rehydrated. In some embodiments, the at least one heterologous polypeptide and/or peptide of interest (and/or the polypeptides and/or peptides in cells, tissues, and/or organs) may be stable at a temperature from about -80°C to about 100°C once the at least one heterologous polypeptide and/or peptide of interest (and/or cell, tissue, and/or organ) is contacted with the at least one protective polypeptide and/or sugar polymer. In
 15 some embodiments, at least about 10% to about 100% (e.g., about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 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 %, or any range or value therein) of the structure and function of the
 20 stabilized polypeptide and/or peptide (and/or cell, tissue and/or organ) is maintained. Thus, in some embodiments, about 10% to about 90%, about 10 to about 85% about 10% to about 80%, about 10% to about 75%, about 10% to about 70%, about 10% to about 60%, about 10% to about 50%, about 20% to about 90%, about 20% to about 85%, about 20% to about 80%, about 20% to about 75%, about 20% to about 70%, about 20% to about 60%, about 20% to about
 25 50%, about 30% to about 90%, about 30 to about 85%, about 30% to about 80%, about 30% to about 75%, about 30% to about 70%, about 30% to about 60%, about 30% to about 50%, about 40% to about 90%, about 40 to about 85%, about 40% to about 80%, about 40% to about 75%, about 40% to about 70%, about 40% to about 60%, about 40% to about 50%, about 50% to about 90%, about 50 to about 85%, about 50% to about 80%, about 50% to about 75%, about
 30 50% to about 70%, about 50% to about 60%, and the like, of the structure and function of the stabilized polypeptide and/or peptide (and/or cell, tissue and/or organ) is maintained. In representative embodiments, when dried (e.g., solid compositions), the polypeptides and/or peptides (and/or the polypeptides and/or peptides in cells, tissues, and/or organs) may be stabilized over a range of temperature from about -80°C to about 100°C. In further

representative embodiments, the polypeptides and/or peptides (and/or the polypeptides and/or peptides in cells, tissues, and/or organs) in solution (liquid composition) may be stabilized over a range of temperatures from about -80°C to about 40°C.

As used herein, “stabilizing” a cell, organ or tissue means maintaining the structure and function of a cell, organ or tissue under either aqueous conditions or dried conditions, or after being frozen and/or dried and then thawed and/or rehydrated.

As used herein, “reactivating” or “reactivation” of a desiccated and inactivated protein means activating the desiccated and inactivated protein such that it has at least about 80% (e.g., about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100%, and any range or value therein) of its original level of activity (e.g., as compared to the same protein that has not been desiccated and inactivated).

As used herein, a “cell, organ and/or tissue” refers to any cell, organ or tissue from an organism useful with this invention (e.g., a fungus, a bacterium, an archaeon, a protist, a plant, an animal). In some embodiments, an organ and/or tissue may include, but is not limited to, lung, liver, bladder, kidney, heart, brain, stomach, intestines (large and small), eye or any part thereof (e.g., lens, cornea), ear or any part thereof (e.g., earlobe, cochlea), gallbladder, esophagus, salivary gland, tongue, teeth, pancreas, ureter, urethra, ovary, uterus, vagina, fallopian tube, testes, vas deferens, penis, pituitary gland, thyroid gland, adrenal gland, lymph node, spleen, thymus, bone marrow, skin (including subcutaneous skin), connective tissue, muscle tissue, nervous tissue, epithelial tissue, mineralized tissue, meristematic tissue, petal, sepal, stamen, pistil, anther, pollen, flower, fruit, flower bud, ovule, seed, embryo, petiole, stem, root, coleoptile, stalk, shoot, branch, apical meristem, axillary bud, cotyledon, hypocotyl, and leaf, callus tissue, protoplast, hyphae, and/or hymenium.

As used herein, the terms “increase,” “increasing,” “increased,” “enhance,” “enhanced,” “enhancing,” and “enhancement” (and grammatical variations thereof) describe an elevation of at least about 25%, 50%, 75%, 100%, 150%, 200%, 300%, 400%, 500% or more as compared to a control. For example, the activity of a desiccated and inactivated protein may be increased by contacting the desiccated/inactivated polypeptide with at least one protective polypeptide and/or sugar polymer of the invention (e.g., increase by at least about 25%, 50%, 75%, 100%, 150%, 200%, 300%, 400%, 500% or more as compared to a control (e.g., the desiccated/inactivated polypeptide)).

An “increased tolerance to drought or desiccation” as used herein refers to the ability of an organism or part thereof that has been either contacted with at least one protective polypeptide (e.g., ubiquitin, Flg M, SH3, SUMO and serum albumin) and/or sugar polymer, or

transformed with at least one heterologous nucleotide sequence encoding a protective polypeptide to withstand exposure to drought, or desiccation (e.g., water loss) better than a control organism or part thereof (e.g., an organism or part thereof that has been exposed to drought or desiccation but was not contacted with the at least one protective polypeptide and/or sugar polymer or transformed with at least one heterologous nucleotide sequence encoding a protective polypeptide as described herein). Increased tolerance to drought or desiccation can be measured using a variety of parameters including, but not limited to, survival, metabolic capacity, reproductive capacity, ability to germinate, developmental potential, structural integrity, functional integrity, viability, morphological integrity, decreased necrosis/apoptosis, time required to recover to pre-desiccation/drought levels of metabolism, cell division, reproduction, germination, development, and/or function as compared to an organism or part thereof exposed to the same stress but not having been contacted with the protective polypeptide and/or sugar polymer.

A “part of an organism” (e.g., part thereof) refers to a multicellular organism and includes but is not limited to a cell, an organ, and other tissues from the organism. A “part of an organism” may also include, but is not limited to, nucleic acids, proteins, lipids, carbohydrates, and the like, that are present in an organism.

An isolated cell refers to a cell that is separated from other components with which it is normally associated in its natural state. For example, an isolated cell can be a cell in culture medium and/or a cell in a pharmaceutically acceptable carrier.

As used herein, the terms “reduce,” “reduced,” “reducing,” “reduction,” “diminish,” and “decrease” (and grammatical variations thereof), describe, for example, a decrease of at least about 5%, 10%, 15%, 20%, 25%, 35%, 50%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% as compared to a control. In particular embodiments, the reduction can result in no or essentially no (*i.e.*, an insignificant amount, *e.g.*, less than about 10% or even 5%) detectable activity or amount. For example, the activity a polypeptide or peptide of interest may be reduced by about 10% to 100% when desiccated as compared to a non-desiccated control. Thus, in some embodiments, the activity of a polypeptide may be reduced by desiccation to about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 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% of its original activity (prior to desiccation), and any range or value therein.

A "native" or "wild type" nucleic acid, nucleotide sequence, polypeptide or amino acid sequence refers to a naturally occurring or endogenous nucleic acid, nucleotide sequence, polypeptide or amino acid sequence. Thus, for example, a "wild type mRNA" is an mRNA that is naturally occurring in or endogenous to the organism. A "homologous" nucleic acid sequence is a nucleotide sequence naturally associated with a host cell into which it is introduced.

As used herein, "nucleic acid," "nucleotide sequence," and "polynucleotide" are used interchangeably and encompass both RNA and DNA, including cDNA, genomic DNA, mRNA, synthetic (*e.g.*, chemically synthesized) DNA or RNA and chimeras of RNA and DNA. The term polynucleotide, nucleotide sequence, or nucleic acid refers to a chain of nucleotides without regard to length of the chain. The nucleic acid can be double-stranded or single-stranded. Where single-stranded, the nucleic acid can be a sense strand or an antisense strand. The nucleic acid can be synthesized using oligonucleotide analogs or derivatives (*e.g.*, inosine or phosphorothioate nucleotides). Such oligonucleotides can be used, for example, to prepare nucleic acids that have altered base-pairing abilities or increased resistance to nucleases. The present invention further provides a nucleic acid that is the complement (which can be either a full complement or a partial complement) of a nucleic acid, nucleotide sequence, or polynucleotide of this invention.

As used herein, the term "gene" refers to a nucleic acid molecule capable of being used to produce mRNA, antisense RNA, miRNA, anti-microRNA antisense oligodeoxyribonucleotide (AMO) and the like. Genes may or may not be capable of being used to produce a functional protein or gene product. Genes can include both coding and non-coding regions (*e.g.*, introns, regulatory elements, promoters, enhancers, termination sequences and/or 5' and 3' untranslated regions). A gene may be "isolated" by which is meant a nucleic acid that is substantially or essentially free from components normally found in association with the nucleic acid in its natural state. Such components include other cellular material, culture medium from recombinant production, and/or various chemicals used in chemically synthesizing the nucleic acid.

The terms "complementary" or "complementarity," as used herein, refer to the natural binding of polynucleotides under permissive salt and temperature conditions by base-pairing. For example, the sequence "A-G-T" binds to the complementary sequence "T-C-A." Complementarity between two single-stranded molecules may be "partial," in which only some of the nucleotides bind, or it may be complete when total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands.

The term “isolated” can refer to a nucleic acid, nucleotide sequence or polypeptide that is substantially free of cellular material, viral material, and/or culture medium (when produced by recombinant DNA techniques), or chemical precursors or other chemicals (when chemically synthesized). Moreover, an “isolated fragment” is a fragment of a nucleic acid, nucleotide
5 sequence or polypeptide that is not naturally occurring as a fragment and would not be found in the natural state. “Isolated” does not mean that the preparation is technically pure (homogeneous), but it is sufficiently pure to provide the polypeptide or nucleic acid in a form in which it can be used for the intended purpose.

In some embodiments, the recombinant nucleic acid molecules, nucleotide sequences
10 and polypeptides of the invention are “isolated.” An “isolated” nucleic acid molecule, an “isolated” nucleotide sequence or an “isolated” polypeptide is a nucleic acid molecule, nucleotide sequence or polypeptide that, by the hand of man, exists apart from its native environment and is therefore not a product of nature. An isolated nucleic acid molecule, nucleotide sequence or polypeptide may exist in a purified form that is at least partially
15 separated from at least some of the other components of the naturally occurring organism or virus, for example, the cell or viral structural components or other polypeptides or nucleic acids commonly found associated with the polynucleotide. In representative embodiments, the isolated nucleic acid molecule, the isolated nucleotide sequence and/or the isolated polypeptide is at least about 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more
20 pure.

In other embodiments, an isolated nucleic acid molecule, nucleotide sequence or polypeptide may exist in a non-native environment such as, for example, a recombinant host cell. Thus, for example, with respect to nucleotide sequences, the term “isolated” means that it is separated from the chromosome and/or cell in which it naturally occurs. A polynucleotide is
25 also isolated if it is separated from the chromosome and/or cell in which it naturally occurs in and is then inserted into a genetic context, a chromosome and/or a cell in which it does not naturally occur (e.g., a different host cell, different regulatory sequences, and/or different position in the genome than as found in nature). Accordingly, the recombinant nucleic acid molecules, nucleotide sequences and their encoded polypeptides are “isolated” in that, by the
30 hand of man, they exist apart from their native environment and therefore are not products of nature, however, in some embodiments, they can be introduced into and exist in a recombinant host cell.

In some embodiments, the nucleotide sequences and/or recombinant nucleic acid molecules of the invention can be operatively associated with a variety of promoters for

expression in soybean plant cells. Thus, in representative embodiments, a recombinant nucleic acid of this invention can further comprise one or more promoters operably linked to one or more nucleotide sequences.

By “operably linked” or “operably associated” as used herein, it is meant that the indicated elements are functionally related to each other, and are also generally physically related. Thus, the term “operably linked” or “operably associated” as used herein, refers to nucleotide sequences on a single nucleic acid molecule that are functionally associated. Thus, a first nucleotide sequence that is operably linked to a second nucleotide sequence means a situation when the first nucleotide sequence is placed in a functional relationship with the second nucleotide sequence. For instance, a promoter is operably associated with a nucleotide sequence if the promoter effects the transcription or expression of said nucleotide sequence. Those skilled in the art will appreciate that the control sequences (*e.g.*, promoter) need not be contiguous with the nucleotide sequence to which it is operably associated, as long as the control sequences function to direct the expression thereof. Thus, for example, intervening untranslated, yet transcribed, sequences can be present between a promoter and a nucleotide sequence, and the promoter can still be considered “operably linked” to the nucleotide sequence.

A “promoter” is a nucleotide sequence that controls or regulates the transcription of a nucleotide sequence (*i.e.*, a coding sequence) that is operably associated with the promoter. The coding sequence may encode a polypeptide and/or a functional RNA. Typically, a “promoter” refers to a nucleotide sequence that contains a binding site for RNA polymerase II and directs the initiation of transcription. In general, promoters are found 5', or upstream, relative to the start of the coding region of the corresponding coding sequence. The promoter region may comprise other elements that act as regulators of gene expression. These include a TATA box consensus sequence, and often a CAAT box consensus sequence (Breathnach and Chambon, (1981) *Annu. Rev. Biochem.* 50:349). Promoters can include, for example, constitutive, inducible, temporally regulated, developmentally regulated, chemically regulated, tissue-preferred and/or tissue-specific promoters for use in the preparation of recombinant nucleic acid molecules, *i.e.*, “chimeric genes” or “chimeric polynucleotides.” In particular aspects, a “promoter” useful with the invention is a promoter capable of initiating transcription of a nucleotide sequence in a cell of interest. The choice of promoter will vary depending on the temporal and spatial requirements for expression, and also depending on the host cell to be transformed. Accordingly, in some embodiments, a nucleic acid encoding a protective polypeptide may be operably linked to a promoter. In some embodiments, a nucleic acid encoding a protective polypeptide may be operably linked to a heterologous promoter.

The terms "coding region" and "coding sequence" are used interchangeably and refer to a polynucleotide region that encodes a polypeptide or functional RNA and, when placed under the control of appropriate regulatory sequences, expresses the encoded polypeptide or functional RNA. The boundaries of a coding region are generally determined by a translation start codon at its 5' end and a translation stop codon at its 3' end. A coding region can encode one or more polypeptides or functional RNAs. For instance, a coding region can encode a polypeptide or functional RNA that is subsequently processed into two or more polypeptides or functional RNAs. A regulatory sequence or regulatory region is a nucleotide sequence that regulates expression of a coding region to which it is operably linked. Nonlimiting examples of regulatory sequences include promoters, transcription initiation sites, translation start sites, internal ribosome entry sites, translation stop sites, and terminators. "Operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. A regulatory sequence is "operably linked" to a coding region when it is joined in such a way that expression of the coding region is achieved under conditions compatible with the regulatory sequence.

The term "fragment," as applied to a polynucleotide, will be understood to mean a nucleotide sequence of reduced length relative to a reference nucleic acid or nucleotide sequence and comprising, consisting essentially of, and/or consisting of a nucleotide sequence of contiguous nucleotides identical or almost identical (*e.g.*, 90%, 92%, 95%, 98%, 99% identical) to the reference nucleic acid or nucleotide sequence. Such a nucleic acid fragment according to the invention may be, where appropriate, included in a larger polynucleotide of which it is a constituent. In some embodiments, such fragments can comprise, consist essentially of, and/or consist of oligonucleotides having a length of at least about 8, 10, 12, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200, or more consecutive nucleotides of a nucleic acid or nucleotide sequence according to the invention.

The term "fragment," as applied to a polypeptide, will be understood to mean an amino acid sequence of reduced length relative to a reference polypeptide or amino acid sequence and comprising, consisting essentially of, and/or consisting of an amino acid sequence of contiguous amino acids identical or almost identical (*e.g.*, 90%, 92%, 95%, 98%, 99% identical) to the reference polypeptide or amino acid sequence. Such a polypeptide fragment according to the invention may be, where appropriate, included in a larger polypeptide of which it is a constituent. In some embodiments, such fragments can comprise, consist essentially of, and/or consist of peptides having a length of at least about 4, 6, 8, 10, 12, 15, 20, 25, 30, 35, 40, 45, 50,

75, 100, 150, 200, or more consecutive amino acids of a polypeptide or amino acid sequence according to the invention.

As used herein, a “functional” polypeptide or “functional fragment” is one that substantially retains at least one biological activity normally associated with that polypeptide (e.g., target protein binding). In particular embodiments, the “functional” polypeptide or “functional fragment” substantially retains all of the activities possessed by the unmodified peptide. By “substantially retains” biological activity, it is meant that the polypeptide retains at least about 20%, 30%, 40%, 50%, 60%, 75%, 85%, 90%, 95%, 97%, 98%, 99%, or more, of the biological activity of the native polypeptide (and can even have a higher level of activity than the native polypeptide). A “non-functional” polypeptide is one that exhibits little or essentially no detectable biological activity normally associated with the polypeptide (e.g., at most, only an insignificant amount, e.g., less than about 10% or even 5%). Biological activities such as protein binding can be measured using assays that are well known in the art and as described herein.

Polypeptides and fragments thereof of the invention may be modified for use by the addition, at the amino- and/or carboxyl-terminal ends, of a blocking agent. Such blocking agents can include, without limitation, additional related or unrelated peptide sequences that can be attached to the amino and/or carboxyl terminal residues of the peptide to be administered. For example, one or more non-naturally occurring amino acids, such as D-alanine, can be added to the termini. Alternatively, blocking agents such as pyroglutamic acid or other molecules known in the art can be attached to the amino and/or carboxyl terminal residues, or the amino group at the amino terminus or carboxyl group at the carboxyl terminus can be replaced with a different moiety. Additionally, the peptide terminus can be modified, e.g., by acetylation of the N-terminus and/or amidation of the C-terminus. Likewise, the peptides can be covalently or noncovalently coupled to pharmaceutically acceptable “carrier” proteins prior to use.

In particular embodiments, nucleic acids of the present invention may encode any suitable epitope tag, including, but not limited to, poly-Arg tags (e.g., RRRRR (SEQ ID NO:1) and RRRRRR (SEQ ID NO:2) and poly-His tags (e.g., HHHHHH (SEQ ID NO:3)). In some embodiments, the nucleic acid may comprise a nucleotide sequence encoding a poly-Arg tag, a poly-His tag, a FLAG tag (i.e., DYKDDDDK (SEQ ID NO:4)), a Strep-tag II™ (GE Healthcare, Pittsburgh, PA, USA) (i.e., WSHYPQFEK (SEQ ID NO:5)), and/or a c-myc tag (i.e., EQKLISEEDL (SEQ ID NO:6)).

Similarly, in some embodiments, proteins of the present invention may comprise any suitable epitope tag, including, but not limited to, poly-Arg tags (e.g., RRRRR (SEQ ID NO:1)

and RRRRRR (SEQ ID NO:2) and poly-His tags (e.g., HHHHHH (SEQ ID NO:3)). In some embodiments, the polypeptide may comprise a poly-Arg tag, a poly-His tag, a FLAG tag (i.e., DYKDDDDK (SEQ ID NO:4)), a Strep-tag II™ (GE Healthcare, Pittsburgh, PA, USA) (i.e., WSHYPQFEK (SEQ ID NO:5)), and/or a c-myc tag (i.e., EQKLISEEDL (SEQ ID NO:6)).

5 Different nucleic acids or proteins having homology are referred to herein as “homologues.” The term homologue includes homologous sequences from the same and other species and orthologous sequences from the same and other species. “Homology” refers to the level of similarity between two or more nucleic acid and/or amino acid sequences in terms of percent of positional identity (i.e., sequence similarity or identity). Homology also refers to the
 10 concept of similar functional properties among different nucleic acids or proteins. Thus, the compositions and methods of the invention further comprise homologues to the nucleotide sequences and polypeptide sequences of this invention. “Orthologous,” as used herein, refers to homologous nucleotide sequences and/ or amino acid sequences in different species that arose from a common ancestral gene during speciation. A homologue of a nucleotide sequence of this
 15 invention has a substantial sequence identity (e.g., at least about 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and/or 100%) to said nucleotide sequence of the invention.

As used herein “sequence identity” refers to the extent to which two optimally aligned polynucleotide or peptide sequences are invariant throughout a window of alignment of
 20 components, e.g., nucleotides or amino acids. “Identity” can be readily calculated by known methods including, but not limited to, those described in: *Computational Molecular Biology* (Lesk, A. M., ed.) Oxford University Press, New York (1988); *Biocomputing: Informatics and Genome Projects* (Smith, D. W., ed.) Academic Press, New York (1993); *Computer Analysis of Sequence Data, Part I* (Griffin, A. M., and Griffin, H. G., eds.) Humana Press, New Jersey
 25 (1994); *Sequence Analysis in Molecular Biology* (von Heinje, G., ed.) Academic Press (1987); and *Sequence Analysis Primer* (Gribskov, M. and Devereux, J., eds.) Stockton Press, New York (1991).

As used herein, the term “percent sequence identity” or “percent identity” refers to the percentage of identical nucleotides in a linear polynucleotide sequence of a reference (“query”)
 30 polynucleotide molecule (or its complementary strand) as compared to a test (“subject”) polynucleotide molecule (or its complementary strand) when the two sequences are optimally aligned. In some embodiments, “percent identity” can refer to the percentage of identical amino acids in an amino acid sequence.

As used herein, the phrase “substantially identical,” in the context of two nucleic acid molecules, nucleotide sequences or protein sequences, refers to two or more sequences or subsequences that have at least about 80%, least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% nucleotide
5 or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection.

For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated if necessary, and
10 sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

An “identity fraction” for aligned segments of a test sequence and a reference sequence is the number of identical components which are shared by the two aligned sequences divided by
15 the total number of components in reference sequence segment, *i.e.*, the entire reference sequence or a smaller defined part of the reference sequence. As used herein, the term “percent sequence identity” or “percent identity” refers to the percentage of identical nucleotides in a linear polynucleotide sequence of a reference (“query”) polynucleotide molecule (or its complementary strand) as compared to a test (“subject”) polynucleotide molecule (or its
20 complementary strand) when the two sequences are optimally aligned (with appropriate nucleotide insertions, deletions, or gaps totaling less than 20 percent of the reference sequence over the window of comparison). In some embodiments, “percent identity” can refer to the percentage of identical amino acids in an amino acid sequence.

Optimal alignment of sequences for aligning a comparison window are well known to
25 those skilled in the art and may be conducted by tools such as the local homology algorithm of Smith and Waterman, the homology alignment algorithm of Needleman and Wunsch, the search for similarity method of Pearson and Lipman, and optionally by computerized implementations of these algorithms such as GAP, BESTFIT, FASTA, and TFASTA available as part of the GCG® Wisconsin Package® (Accelrys Inc., San Diego, CA). An “identity fraction” for aligned
30 segments of a test sequence and a reference sequence is the number of identical components which are shared by the two aligned sequences divided by the total number of components in the reference sequence segment, *i.e.*, the entire reference sequence or a smaller defined part of the reference sequence. Percent sequence identity is represented as the identity fraction multiplied by 100. The comparison of one or more polynucleotide sequences may be to a full-length

polynucleotide sequence or a portion thereof, or to a longer polynucleotide sequence. For purposes of this invention “percent identity” may also be determined using BLASTX version 2.0 for translated nucleotide sequences and BLASTN version 2.0 for polynucleotide sequences.

The percent of sequence identity can be determined using the “Best Fit” or “Gap”
5 program of the Sequence Analysis Software Package™ (Version 10; Genetics Computer Group, Inc., Madison, Wis.). “Gap” utilizes the algorithm of Needleman and Wunsch (Needleman and Wunsch, *J Mol. Biol.* 48:443-453, 1970) to find the alignment of two sequences that maximizes the number of matches and minimizes the number of gaps. “BestFit” performs an optimal
10 alignment of the best segment of similarity between two sequences and inserts gaps to maximize the number of matches using the local homology algorithm of Smith and Waterman (Smith and Waterman, *Adv. Appl. Math.* 2:482 (1981); Smith *et al.*, *Nucleic Acids Res.* 11:2205 (1983)).

Useful methods for determining sequence identity are also disclosed in Guide to Huge Computers (Martin J. Bishop, ed., Academic Press, San Diego (1994)), and Carillo, H., and Lipton, D., *Applied Math* 48:1073(1988)). More particularly, preferred computer programs for
15 determining sequence identity include but are not limited to the Basic Local Alignment Search Tool (BLAST) programs which are publicly available from National Center Biotechnology Information (NCBI) at the National Library of Medicine, National Institute of Health, Bethesda, Md. 20894; see BLAST Manual, Altschul *et al.*, NCBI, NLM, NIH; (Altschul *et al.*, *J. Mol. Biol.* 215:403 (1990)); version 2.0 or higher of BLAST programs allows the introduction of gaps
20 (deletions and insertions) into alignments; for peptide sequence BLASTX can be used to determine sequence identity; and, for polynucleotide sequence BLASTN can be used to determine sequence identity.

Two nucleotide sequences can be considered to be substantially complementary when the two sequences hybridize to each other under stringent conditions. In some representative
25 embodiments, two nucleotide sequences considered to be substantially complementary hybridize to each other under highly stringent conditions.

“Stringent hybridization conditions” and “stringent hybridization wash conditions” in the context of nucleic acid hybridization experiments such as Southern and Northern hybridizations are sequence dependent, and are different under different environmental parameters. An
30 extensive guide to the hybridization of nucleic acids is found in Tijssen *Laboratory Techniques in Biochemistry and Molecular Biology-Hybridization with Nucleic Acid Probes* part I chapter 2 “Overview of principles of hybridization and the strategy of nucleic acid probe assays” Elsevier, New York (1993). Generally, highly stringent hybridization and wash conditions are selected to

be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH.

The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Very stringent conditions are selected to be equal to the T_m for a particular probe. An example of stringent hybridization conditions for hybridization of complementary nucleotide sequences which have more than 100 complementary residues on a filter in a Southern or northern blot is 50% formamide with 1 mg of heparin at 42°C, with the hybridization being carried out overnight. An example of highly stringent wash conditions is 0.1 5M NaCl at 72°C for about 15 minutes. An example of stringent wash conditions is a 0.2x SSC wash at 65°C for 15 minutes (*see*, Sambrook, *infra*, for a description of SSC buffer). Often, a high stringency wash is preceded by a low stringency wash to remove background probe signal. An example of a medium stringency wash for a duplex of, e.g., more than 100 nucleotides, is 1x SSC at 45°C for 15 minutes. An example of a low stringency wash for a duplex of, e.g., more than 100 nucleotides, is 4-6x SSC at 40°C for 15 minutes. For short probes (e.g., about 10 to 50 nucleotides), stringent conditions typically involve salt concentrations of less than about 1.0 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3, and the temperature is typically at least about 30°C. Stringent conditions can also be achieved with the addition of destabilizing agents such as formamide. In general, a signal to noise ratio of 2x (or higher) than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization. Nucleotide sequences that do not hybridize to each other under stringent conditions are still substantially identical if the proteins that they encode are substantially identical. This can occur, for example, when a copy of a nucleotide sequence is created using the maximum codon degeneracy permitted by the genetic code.

The following are examples of sets of hybridization/wash conditions that may be used to clone homologous nucleotide sequences that are substantially identical to reference nucleotide sequences of the invention. In one embodiment, a reference nucleotide sequence hybridizes to the “test” nucleotide sequence in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 2X SSC, 0.1% SDS at 50°C. In another embodiment, the reference nucleotide sequence hybridizes to the “test” nucleotide sequence in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 1X SSC, 0.1% SDS at 50°C or in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 0.5X SSC, 0.1% SDS at 50°C. In still further embodiments, the reference nucleotide sequence hybridizes to the “test” nucleotide sequence in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄,

1 mM EDTA at 50°C with washing in 0.1X SSC, 0.1% SDS at 50°C, or in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 0.1X SSC, 0.1% SDS at 65°C.

In some embodiments, a recombinant nucleic acid molecule of the invention can be an “expression cassette” or can be comprised within an expression cassette. As used herein, “expression cassette” means a recombinant nucleic acid molecule comprising a nucleotide sequence of interest (e.g., the nucleotide sequences of the invention; e.g., a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NO:7-209**, a nucleotide sequence having at least about 70% identity to any of **SEQ ID NOs:210-314**, or a nucleotide sequence having at least about 80% identity to any of **SEQ ID NOs:315-419**, and/or fragments thereof), wherein said nucleotide sequence is operably associated with at least a control sequence (e.g., a promoter, a heterologous promoter). Thus, some embodiments of the invention provide expression cassettes designed to express the nucleotide sequences of the invention in a cell.

An expression cassette comprising a nucleotide sequence of interest may be chimeric, meaning that at least one of its components is heterologous with respect to at least one of its other components. An expression cassette may also be one that is naturally occurring but has been obtained in a recombinant form useful for heterologous expression.

An expression cassette also can optionally include a transcriptional and/or translational termination region (*i.e.*, termination region) that is functional in the cell in which the nucleotide sequence of interest is to be expressed. A variety of transcriptional terminators are available for use in expression cassettes and are responsible for the termination of transcription beyond the heterologous nucleotide sequence of interest and correct mRNA polyadenylation. The termination region may be native to the transcriptional initiation region, may be native to the operably linked nucleotide sequence of interest, may be native to the host organism, or may be derived from another source (*i.e.*, foreign or heterologous to the promoter, the nucleotide sequence of interest, the host organism, or any combination thereof). In addition, in some embodiments, a coding sequence's native transcription terminator can be used.

An expression cassette of the invention also can include a nucleotide sequence for a selectable marker, which can be used to select a transformed organism and/or cell. As used herein, “selectable marker” means a nucleotide sequence that when expressed imparts a distinct phenotype to the transformed organism or cell expressing the marker and thus allows such transformed organisms or cells to be distinguished from those that do not have the marker. Such a nucleotide sequence may encode either a selectable or screenable marker, depending on

whether the marker confers a trait that can be selected for by chemical means, such as by using a selective agent (*e.g.*, an antibiotic, herbicide, or the like), or on whether the marker is simply a trait that one can identify through observation or testing, such as by screening. Of course, many examples of suitable selectable markers useful in various organisms are known in the art and can be used in the expression cassettes described herein.

In addition to expression cassettes, the nucleic acid molecules and nucleotide sequences described herein can be used in connection with vectors. The term “vector” refers to a composition for transferring, delivering or introducing a nucleic acid (or nucleic acids) into a cell. A vector comprises a nucleic acid molecule comprising the nucleotide sequence(s) to be transferred, delivered or introduced. Vectors for use in transformation of animals, plants and other organisms are well known in the art. Non-limiting examples of general classes of vectors including but not limited to a viral vector, a plasmid vector, a phage vector, a phagemid vector, a cosmid vector, a fosmid vector, a bacteriophage, an artificial chromosome, or an *Agrobacterium* binary vector in double or single stranded linear or circular form which may or may not be self transmissible or mobilizable. A vector as defined herein can transform prokaryotic or eukaryotic host either by integration into the cellular genome or exist extrachromosomally (*e.g.*, an autonomous replicating plasmid with an origin of replication). Additionally included are shuttle vectors by which is meant a DNA vehicle capable, naturally or by design, of replication in two different host organisms, which may be selected from prokaryotic and eukaryotic organisms. In some representative embodiments, the nucleic acid in the vector is under the control of, and operably linked to, an appropriate promoter or other regulatory elements for transcription in a host cell such as a microbial, *e.g.* bacterial, or an animal or a plant cell. The vector may be a bi-functional expression vector which functions in multiple hosts. In the case of genomic DNA, this may contain its own promoter or other regulatory elements and in the case of cDNA this may be under the control of an appropriate promoter or other regulatory elements for expression in the host cell.

The present inventors have discovered that heterologous polypeptides and/or peptides may be stabilized in the presence of protective polypeptides, in both aqueous (liquid) and (solid) compositions.

Accordingly, in some embodiments, a liquid composition is provided comprising, consisting essentially of, or consisting of: at least one heterologous polypeptide and/or peptide of interest and (a) at least one protective polypeptide selected from the group consisting of a ubiquitin polypeptide, a negative regulator of flagellin synthesis (Flg M) polypeptide, a SRC Homology 3 (SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide,

and a serum albumin polypeptide, and any combination thereof; and/or (b) at least one sugar polymer.

In some embodiments, a solid composition is provided comprising, consisting essentially of, or consisting of: at least one heterologous polypeptide and/or peptide of interest and (a) at least one protective polypeptide selected from the group consisting of a ubiquitin polypeptide, a negative regulator of flagellin synthesis (Flg M) polypeptide, a SRC Homology 3 (SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide, and a serum albumin polypeptide, and any combination thereof; and/or (b) at least one sugar polymer. In some embodiments, a solid composition may be produced by drying or partially drying a liquid composition of the invention. In some embodiments, a solid composition of the invention may comprise about 0% to about 5% by weight water (e.g., about 0.001, 0.002, 0.003, 0.004, 0.005, 0.006, 0.007, 0.008, 0.009, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 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, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5% by weight water, or any range or value therein).

In some embodiments, a solution is provided comprising, consisting essentially of, or consisting of: (a) at least one protective polypeptide selected from the group consisting of a ubiquitin polypeptide, a negative regulator of flagellin synthesis (Flg M) polypeptide, a SRC Homology 3 (SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide, and a serum albumin polypeptide, and any combination thereof; and/or (b) at least one sugar polymer; and/or (c) any combination of (a) and (b).

Further provided is a method of stabilizing at least one heterologous polypeptide and/or peptide of interest, comprising: contacting the at least one heterologous polypeptide and/or peptide of interest with (a) at least one protective polypeptide selected from the group consisting of a ubiquitin polypeptide, a negative regulator of flagellin synthesis (Flg M) polypeptide, a SRC Homology 3 (SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide, and a serum albumin polypeptide, and any combination thereof; and/or (b) at least one sugar polymer to produce a liquid composition comprising the at least one heterologous polypeptide and/or peptide of interest and the at least one protective polypeptide and/or at least one sugar polymer, thereby stabilizing the at least one heterologous polypeptide and/or peptide of interest as compared to a control (e.g., the heterologous polypeptide and or peptide of interest that has not been contacted with the protective polypeptide). In some embodiments, the method further comprises at least partially drying the liquid composition comprising the at least one

heterologous polypeptide of interest and the at least one protective polypeptide and/or at least one sugar polymer.

In some embodiments, the invention provides a method of stabilizing a heterologous cell, tissue or organ, comprising: contacting the heterologous cell, tissue or organ with a solution or liquid composition of the invention comprising: (a) at least one protective polypeptide selected from the group consisting of a ubiquitin polypeptide, a negative regulator of flagellin synthesis (Flg M) polypeptide, a SRC Homology 3 (SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide, and a serum albumin polypeptide, and any combination thereof; and/or (b) at least one sugar polymer, thereby stabilizing the heterologous cell, tissue or organ as compared to a control (e.g., a heterologous cell, tissue or organ that has not been contacted with the liquid composition). Exemplary heterologous cells, tissues or organs may include, but are not limited to, those from a fungus, a bacterium, an archaeon, a plant, a protist, or an animal. In some embodiments, an animal may include, but is not limited to, a mammal, a bird, a fish, an insect, a reptile, a nematode, or an amphibian. A mammal includes, but is not limited to, a human, a non-human primate, a dog, a cat, a goat, a horse, a pig, a cow, a sheep, a rat, a guinea pig, a mouse, a gerbil, or a hamster.

The inventors have also discovered that the protective polypeptides described herein and polypeptides from tardigrades (e.g., tardigrade disordered polypeptides (TDPs)) have the surprising ability to reactivate desiccated and inactivate proteins. Thus, in some embodiments, the invention provides a method of reactivating one or more desiccated and inactivated proteins, comprising resuspending the one or more desiccated and inactivated proteins in a solution comprising: (a) at least one protective polypeptide selected from the group consisting of a ubiquitin polypeptide, a negative regulator of flagellin synthesis (Flg M) polypeptide, a SRC Homology 3 (SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide, a serum albumin polypeptide, and a TDP, and/or any combination thereof.

Further provided is a method of increasing drought or desiccation tolerance in a seed, the method comprising: coating the seed with a solution of the present invention (e.g., a solution comprising at least one ubiquitin polypeptide, a negative regulator of flagellin synthesis (Flg M) polypeptide, a SRC Homology 3 (SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide, and a serum albumin polypeptide; and/or (b) at least one sugar polymer) to produce a coated seed, thereby increasing drought or desiccation tolerance of the seed. In some embodiments, the coating may be dried or partially dried on the seed.

Drying of a liquid composition or solution of the invention may commence any time following the contacting or coating with the liquid composition or solution. Any method of

drying a liquid composition or solution may be used including, but not limited to, freeze-drying, air-drying, spray-drying, spray-freeze-drying, vacuum drying, and/or foam drying.

As used herein, “partially drying” refers to drying a composition or solution such that it comprises less water than when the drying process began. Thus, for example, “partially drying” can mean removing about 0.001 % to about 100% of the water that was present in the composition or solution prior to initiating the drying process. (e.g., about 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008%, 0.009%, 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.5% or 100% (or any range or value therein). Thus, in some embodiments the amount of water removed when a composition or solution is partially dried can be from about 0.001 to about 0.01%, about 0.001 to about 0.1%, about 0.001 to about 1%, about 0.01 to about 1%, about 0.1 to about 1%, about 0.001 to about 10%, about 0.01 to about 10%, about 0.1 to about 10%, about 10% to about 90%, about 20% to about 90%, about 30% to about 90%, about 40% to about 90%, about 50% to about 90%, about 60% to about 90%, about 10% to about 80%, about 20% to about 80%, about 30% to about 80%, about 40% to about 80%, about 50% to about 80%, about 60% to about 80%, about 70% to about 80%, about 10% to about 70%, about 20% to about 70%, about 30% to about 70%, about 40% to about 70%, about 50% to about 70%, about 10% to about 50%, about 20% to about 50%, about 30% to about 50%, about 40% to about 50% (or any range or value therein) of the water that was present in the composition or solution prior to initiating the drying process. Of course, a partially dried composition may be dried further such that it contains less water than when the further drying began.

In some embodiments, a solid composition of the invention may comprise a hydration level of about 0 to about 10 g water per gram of dried protein (e.g., up to about 10 g water per gram of dried protein; e.g., about 0.001, 0.002, 0.003, 0.004, 0.005, 0.006, 0.007, 0.008, 0.009, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.5, 3, 3.5, 4, 4.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, and any range or value therein). In representative embodiments, a solid composition of the invention may comprise a hydration level of about 0g to about 0.005g, about 0g to about 0.01g, about 0.005g to about 0.01g, about 0.005g to about 0.1g, about 0.01g to about 0.1g water per gram of dried protein, and any value or range therein.

The amount of a protective polypeptide (e.g., ubiquitin, FlgM, SH3 domain, SUMO serum albumin) and/or a sugar polymer in a liquid composition, solid composition, and/or

solution of the invention can vary depending on the heterologous polypeptide and/or peptide of interest, whether it is a liquid or a solid, and/or whether the composition is a liquid composition or solution that will be dried. Thus, in some embodiments, the concentration of a protective polypeptide and/or a sugar polymer in a liquid composition and/or solution of the invention may be about 1 g/L to about 300 g/L or any range or value therein (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 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, 110, 120, 130, 140, 150, 160, 170, 180, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300 g/L, or any range or value therein). In some embodiments, the protective polypeptide and/or sugar polymer concentration in a liquid composition or solution of the invention may be about 1 g/L to about 250 g/L, about 1 g/L to about 200 g/L, about 1 g/L to about 100 g/L, about 1 g/L to about 50 g/L, about 1 g/L to about 20 g/L, about 1 g/L to about 10 g/L, about 5 g/L to about 300 g/L, about 5 g/L to about 250 g/L, about 5 g/L to about 200 g/L, about 5 g/L to about 100 g/L, about 5 g/L to about 50 g/L, about 5 g/L to about 20 g/L, about 5 g/L to about 10 g/L, 10 g/L to about 300 g/L, about 10 g/L to about 250 g/L, about 10 g/L to about 200 g/L, about 10 g/L to about 100 g/L, about 10 g/L to about 50 g/L, about 10 g/L to about 20 g/L, about 25 g/L to about 300 g/L, about 25 g/L to about 250 g/L, about 25 g/L to about 200 g/L, about 25 g/L to about 100 g/L, about 25 g/L to about 50 g/L, about 25 g/L to about 40 g/L, about 25 g/L to about 30 g/L, about 50 g/L to about 300 g/L, about 50 g/L to about 250 g/L, about 50 g/L to about 200 g/L, about 50 g/L to about 100 g/L, about 50 g/L to about 75 g/L, about 50 g/L to about 60 g/L, about 100 g/L to about 300 g/L, about 100 g/L to about 250 g/L, about 100 g/L to about 200 g/L, about 100 g/L to about 150 g/L, about 100 g/L to about 125 g/L. In some embodiments, the concentration of the at least one protective polypeptide may be greater than 1 g/L, optionally greater than 5 g/L.

In some embodiments, a liquid composition, solid composition, and/or solution may comprise about 50% to about 99.9% of protective polypeptide and/or sugar polymer (total weight) (e.g., about 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, 99.99% total weight, and any range or value therein). In some embodiments, a liquid composition, solid composition, and/or solution may comprise about 90% to 99.99% of protective polypeptide and/or sugar polymer (total weight) (e.g., about 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.1, 99.15, 99.2, 99.25, 99.3, 99.35, 99.4, 99.45, 99.5, 99.55, 99.6, 99.65, 99.7, 99.75, 99.8, 99.85, 99.9, 99.95, 99.99% total weight, and any range or value therein).

In some embodiments, the mass ratio of the at least one heterologous polypeptide and/or peptide of interest, or the one or more desiccated and inactivated proteins to the at least one protective polypeptide and/or sugar polymer in a liquid or a solid composition may be about 1:1000 to about 1:1 (e.g., about 1:1000, 1:950, 1:900, 1:850, 1:800, 1:750, 1:700, 1:650, 1:600, 1:550, 1:500, 1:450, 1:400, 1:350, 1:300, 1:250, 1:200, 1:150, 1:125, 1:100, 1:95, 1:90, 1:85, 1:80, 1:75, 1:70, 1:65, 1:60, 1:55, 1:50, 1:45, 1:40, 1:35, 1:30, 1:25, 1:20, 1:15, 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1; and any range or value therein). In representative embodiments, the at least one heterologous polypeptide and/or peptide of interest to the at least one protective polypeptide and/or sugar polymer in a liquid or a solid composition may be about 1:100 to about 1:10, about 1:20 to about 1:10, or about 1:20 to about 1:1.

The liquid compositions, solid compositions, and/or solutions of this invention may comprise any number or combination of protective polypeptides and/or sugar polymers. Thus, in some embodiments, the liquid compositions, solid compositions, and/or solutions can comprise, consist essentially of, or consist of at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 or more different protective polypeptides and/or sugar polymers (e.g., from about 1 to about 25, about 1 to about 20, about 1 to about 15, about 1 to about 10, about 1 to about 5, about 2 to about 10, about 2 to about 5, about 4 to about 10, about 6 to about 10 different protective polypeptides and/or sugar polymers and the like).

Any ubiquitin polypeptide may be used as protective polypeptide as described herein. Exemplary ubiquitin polypeptides include, but are not limited to, those from a mammal, a fish, an insect, a bird, a plant, a bacterium, a protist, a fungus, a plant, a nematode, or an archaeon. Exemplary mammals include, a laboratory animal (e.g., a rat, mouse, guinea pig, rabbit, etc.), a farm or commercial animal (e.g., a bovine, pig, horse, goat, donkey, sheep, etc.), or a domestic animal (e.g., cat, dog, ferret, etc.), primates such as a human or a non-human primate (e.g., a chimpanzee, baboon, monkey, orangutan, gorilla, etc.). In some embodiments, the protective polypeptide may be a ubiquitin polypeptide having at least 70% identity (e.g., 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, 99.9, 100%) to an amino acid sequence of any of **SEQ ID NOs:7-30**; and/or may be a ubiquitin polypeptide encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:7-30**, or any combination thereof.

Any SRC Homology 3 (SH3) Domain polypeptide may be used as protective polypeptide as described herein. Exemplary SH3 domain polypeptides include, but are not limited to, those obtained from a eukaryote polypeptide or a prokaryote polypeptide comprising an SH3 domain. In some embodiments, a SH3 domain polypeptide may be obtained from, for

example, a polypeptide from a primate such as a human or a non-human primate (e.g., a chimpanzee, baboon, monkey, orangutan, gorilla, etc.), a farm or commercial animal (e.g., a bovine, pig, horse, goat, donkey, sheep, etc.), or a domestic animal (e.g., cat, dog, ferret, etc.), a laboratory animal (e.g., a rat, mouse, guinea pig, rabbit, etc.), a reptile (e.g., a lizard, turtle, snake, etc.), a bacterium (e.g., *Escherichia*), a plant, e.g., *Arabidopsis*), a fungus (e.g., yeast), protist (e.g., *Monosiga*), insect (e.g., *Drosophila*), fish (e.g., salmon, danio), amphibian (e.g., *Xenopus*), nematode (e.g., *C. elegans*), and/or avian (e.g., chicken, parakeet). In some embodiments, the protective polypeptide may be a SH3 domain polypeptide having at least 70% identity to an amino acid sequence of any of **SEQ ID NOs:31-53**; and/or may be a SH3 domain polypeptide encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:31-53**, or any combination thereof.

Any negative regulator of flagellin synthesis (Flg M) polypeptide may be used as protective polypeptide as described herein. Exemplary Flg M polypeptides include, but are not limited to, those obtained from a monotrichous bacterium or a lophotrichous bacterium. In some embodiments, a Flg M polypeptide may be obtained from *Escherichia*, *Pseudomonas*, *Bacillus*, *Vibrio*, *Helicobacter*, *Klebsiella*, *Burkholderia*, *Salmonella*, *Campylobacter*, *Enterobacter*, *Kluyvera*, *Proteobacteria*, *Shigella*, *Citrobacter*, *Diaporthe*, *Beauveria* and/or *Caulobacter*. In some embodiments, the protective polypeptide may be a Flg M polypeptide having at least 70% identity to an amino acid sequence of any of **SEQ ID NOs:54-64**; and/or may be a Flg M polypeptide encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:54-64**, or any combination thereof.

Any small ubiquitin-like modifier (SUMO) polypeptide may be used as a protective polypeptide as described herein. Exemplary SUMO polypeptides include those from a eukaryote or a prokaryote. In some embodiments, a SUMO polypeptide may include, but is not limited to, those from a human or a non-human primate (e.g., monkey, gorilla, orangutan, chimpanzee), a farm or commercial animal (e.g., a bovine, pig, horse, goat, donkey, sheep, etc.), or a domestic animal (e.g., cat, dog, ferret, etc.), a laboratory animal (e.g., a rat, mouse, guinea pig, rabbit, etc.), a reptile (e.g., a lizard, turtle, snake, etc.), a bacterium (e.g., *Escherichia*), a plant, e.g., *Arabidopsis*), a fungus (e.g., yeast), protist (e.g., *Monosiga*), insect (e.g., *Drosophila*), fish (e.g., salmon, danio), amphibian (e.g., *Xenopus*), nematode (e.g., *C. elegans*), and/or avian (e.g., chicken, parakeet). In some embodiments, the protective polypeptide may be a SUMO polypeptide having at least 70% identity to an amino acid sequence of any of **SEQ ID NOs:65-86**; and/or may be a SUMO polypeptide encoded by a nucleotide sequence encoding an amino

acid sequence having at least about 70% identity to any of **SEQ ID NOs:65-86**, or any combination thereof.

A serum albumin polypeptide useful as a protective polypeptide may be any serum albumin polypeptide. Exemplary serum albumin polypeptides include those from any animal including but not limited to a human, a non-human primate (e.g., monkey, gorilla, orangutan, chimpanzee), a farm or commercial animal (e.g., a bovine, pig, horse, goat, donkey, sheep, etc.), or a domestic animal (e.g., cat, dog, ferret, etc.), a laboratory animal (e.g., a rat, mouse, guinea pig, rabbit, etc.), a fish (e.g., salmon, danio), an amphibian (e.g., frog), reptile (e.g., turtle, lizard, snake) and/or an avian (e.g., chicken, parakeet, parrot). In some embodiments, the protective polypeptide may be a serum albumin polypeptide having at least 70% identity to an amino acid sequence of any of **SEQ ID NOs:87-104**; and/or may be a serum albumin polypeptide encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:87-104**, or any combination thereof.

In some embodiments, a sugar polymer may be used to protect a heterologous polypeptide of interest. Exemplary sugar polymers include, but are not limited to, neutral, highly branched, hydrophilic polysaccharides (e.g., FICOLL[®]), branched glucose polymers with reducing linkages (e.g., dextran), branched glucose polymers with non-reducing linkages (glycogen), branched or linear glucose polymers with non-reducing linkages (alpha glycosidic linkages, e.g., starch (e.g., amylose, amylopectin)), and/or glucose polymers with beta glycosidic linkages (e.g., cellulose).

Any number or combination of tardigrade polypeptides (TDPs) obtained from any tardigrade genus or species may be used as a protective polypeptide alone or in combination with any other protective polypeptides as described herein for reactivating a desiccated and inactivated protein. Tardigrades are a phylum of microscopic animals renowned for their ability to survive a vast array of environmental extremes, including essentially complete desiccation for up to a decade (Goldstein and Blaxter, *Curr. Biol.* 12, R475 (2002)). Exemplary tardigrade genera from which a TDP may be obtained include *Macrobiotus* spp., *Isohypsibius* spp., *Diphascon* spp., *Echiniscus* spp., *Minibiotus* spp., *Doryphoribius* spp., *Paramacrobiotus* spp., *Hypsibius* spp., *Milnesium* spp., *Pseudechiniscus* spp., *Ramazzottius* spp., *Batillipes* spp., *Bryodelphax* spp., *Dactylobiotus* spp., *Echiniscoides* spp., *Calcarobiotus* spp., *Tenuibiotus* spp., *Itaquascon* spp., *Cornechiniscus* spp., and/or *Halechiniscus* spp. In some embodiments, the at least one TDP may be obtained from the tardigrade genera of *Hypsibius* spp., *Paramacrobiotus* spp., *Milnesium* spp. and/or *Ramazzottius* spp. In some embodiments, the TDP may be obtained from one or more of the exemplary tardigrade species provided in **Table 1**. In some

embodiments, a TDP may be obtained from *Hypsibius dujardini*, *Paramacrobiotus richters*, *Milnesium tardigradum* and/or *Ramazzottius varieornatus*.

Accordingly, in some embodiments, a solution may comprise, consist essentially of, or consist of a TDP comprising an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:104-208**; an amino acid sequence encoded by a nucleotide sequence having at least about 80% identity to any one of **SEQ ID NOs:210-314**; or an amino acid sequence encoded by a nucleotide sequence having at least about 80% identity to any one of **SEQ ID NOs:315-419**; or any combination thereof. In representative embodiments, a solution may comprise, consist essentially, or consist of a TDP comprising an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:121, 123, 136, 139, and/or 148**; an amino acid sequence encoded by a nucleotide sequence having at least about 70% identity to any one of **SEQ ID NOs:226, 228, 241, 244, and/or 247**; or an amino acid sequence encoded by a nucleotide sequence having at least about 70% identity to any one of **SEQ ID NOs:331, 333, 346, 349 and/or 352**; or any combination thereof.

A liquid composition or a solid composition of the invention may further comprise one or more excipients. In some embodiments, an excipient includes, but is not limited to, trehalose, sucrose, maltose, mannitol, sorbitol, polysorbate, a buffer, a salt, an antioxidant, a preservative, a colorant, and/or a flavorant.

In some embodiments, the at least one heterologous polypeptide and/or peptide of interest may be a therapeutic agent or it may be part of a protein-based food. The at least one heterologous polypeptide and/or peptide of interest may be in purified form or it may be in a mixture (unpurified or partially purified). Thus, for example, the at least one heterologous polypeptide and/or peptide of interest may be obtained from, for example, an organism (bacteria, fungi, animals, plants, archaea, protists, etc.), the cells of an organism (either isolated or cultured), from serum and/or from *in vitro* expression systems. The heterologous polypeptides and/or peptides so produced may then be protected (stabilized) by contacting them with at least one protective polypeptide (e.g., ubiquitin polypeptide, Flg M polypeptide, SH3 Domain polypeptide, SUMO polypeptide, and/or a serum albumin polypeptide) immediately without any further isolation or purification or they may be contacted with the at least one protective polypeptide (e.g., ubiquitin polypeptide, Flg M polypeptide, SH3 Domain polypeptide, SUMO polypeptide, and/or a serum albumin polypeptide) after they are purified or partially purified. Thus, a mixture may include, for example, serum, cell culture, and/or one or more constituents of an organism or cell thereof, and/or of an *in vitro* expression system, and the like. In addition,

a protein based-food may have multiple additional components (e.g., a mixture), which additional components may or may not be proteinaceous.

A therapeutic protein may be any protein based molecule (e.g., a biologic) including, but not limited to, a vaccine, an antibody, an enzyme, hormone, and/or a globular protein.

5 The term "antibody" or "antibodies" as used herein refers to all types of immunoglobulins, including IgG, IgM, IgA, IgD, and IgE. The antibody can be monoclonal or polyclonal and can be of any species of origin, including (for example) mouse, rat, rabbit, horse, goat, sheep, camel, or human, or can be a chimeric antibody. *See, e.g., Walker et al., Molec. Immunol.* 26:403 (1989). The antibodies can be recombinant monoclonal antibodies produced
10 according to the methods disclosed in U.S. Patent No. 4,474,893 or U.S. Patent No. 4,816,567. The antibodies can also be chemically constructed according to the method disclosed in U.S. Patent No. 4,676,980. As used herein, "antibody" also refers to antibody fragments, for example, Fab, Fab', F(ab')₂, and Fv fragments; domain antibodies, diabodies; vaccibodies, linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody
15 fragments. Also included within the scope of the present invention are antibodies, which are altered or mutated for compatibility with species other than the species in which the antibody was produced. For example, antibodies may be humanized or camelized. Humanized forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of
20 antibodies) which contain minimal sequence derived from non-human immunoglobulin.

 A "protein-based food" is any food that comprises protein including, but not limited to, meat, seafood, a food comprised of plant based proteins (tofu, tempeh), and/or fungal based proteins (tempeh, meat-substitutes) and the like. Thus, in some embodiments, a protective polypeptide (e.g., ubiquitin polypeptide, Flg M polypeptide, SH3 Domain polypeptide, SUMO
25 polypeptide, and/or a serum albumin polypeptide) may be used as a food additive to stabilize proteins in food products.

 In some embodiments, when a liquid composition, solid composition and/or solution of the invention comprises a salt, the concentration of the salt can be about 0.01 mM to about 100 mM or any range or value therein (e.g., 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1,
30 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 mM and any range or value therein). In some embodiments, the salt concentration can be about 0.1 mM to 50 mM and any value or range therein). Any appropriate physiologically compatible salt may be used, for example, NaCl.

The pH of a liquid composition, solid composition and/or solution of the invention may be about 5 to about 9, or any range or value therein (e.g., about 5, 5.1, 5.2, 5.3, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.5, 8.6, 8.7, 8.8, 8.9, 9, and the like). In representative embodiments, the pH of a liquid composition, solid composition and/or solution of the invention may be, for example, about pH 6 to about pH 8, about pH 6.5 to about pH 7.5, optionally about pH 7.

In some embodiments, the liquid compositions, solid compositions and/or solutions of the invention may comprise a buffer. Any buffer may be used provided the buffer is within the pH range of about pH 5 to about pH 9, and within the salt concentration of about 0 to 100 mM.

The present invention further provides methods of producing transgenic cells or transgenic organism having increased tolerance to drought or desiccation. Thus, in some embodiments, a method of producing a transgenic cell having increased tolerance to drought or desiccation is provided, comprising: introducing into a cell at least one heterologous polynucleotide encoding a ubiquitin polypeptide, a negative regulator of flagellin synthesis (Flg M) polypeptide, a SRC Homology 3 (SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide, a serum albumin polypeptide, or any combination thereof; to produce a transgenic organism expressing the heterologous polynucleotide, thereby producing a transgenic cell having increased tolerance to drought or desiccation as compared to a control (e.g., the cell in which the at least one heterologous polynucleotide encoding a ubiquitin polypeptide, a Flg M polypeptide, a SH3 Domain polypeptide, a SUMO polypeptide, or a serum albumin polypeptide is not introduced).

In some embodiments, a cell may be from an animal, a fungus, a bacterium, an archaeon, a protist, or a plant. Non-limiting examples of animals include a mammal, a bird, a fish, an insect, a reptile, a nematode, or an amphibian. Exemplary mammals include a human, a non-human primate (e.g., a monkey, a gorilla, a chimpanzee, a baboon, and the like), a cow (cattle), a dog, a cat, a ferret, a horse, a donkey, a goat, a sheep, a rabbit, a guinea pig, a hamster, a mouse, and/or a rat. The transformation may be performed *in vitro* (e.g., single cells, cell masses, or tissues) or *in vivo* (e.g., one or more cells in an organism). In some embodiments, a transformed cell may be used to regenerate a transgenic multicellular organism.

Thus, in some embodiments, wherein the cell is a plant cell, the method further comprising regenerating a transgenic plant from the transgenic cell, the regenerated transgenic plant comprising the heterologous nucleotide sequence encoding a protective polypeptide (e.g., a ubiquitin polypeptide, a Flg M polypeptide, a SH3 Domain polypeptide, a SUMO polypeptide, a serum albumin polypeptide, or any combination thereof) in its genome. In some embodiments, a

transgenic plant of the invention may produce transgenic seed, the genome of the seed comprising a heterologous nucleotide sequence encoding a protective polypeptide.

In some embodiments, a method of producing a transgenic organism having increased tolerance to drought or desiccation is provided, comprising: introducing into the organism at
5 least one heterologous polynucleotide encoding a ubiquitin polypeptide, a negative regulator of flagellin synthesis (Flg M) polypeptide, a SRC Homology 3 (SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide, a serum albumin polypeptide, or any combination thereof, thereby producing a transgenic organism having increased drought or desiccation tolerance as compared to a control (e.g., the organism in which the at least one heterologous
10 polynucleotide encoding a ubiquitin polypeptide, a Flg M polypeptide, a SH3 Domain polypeptide, a SUMO polypeptide, or a serum albumin polypeptide is not introduced).

In some embodiments, a method of increasing drought or desiccation tolerance in an organism is provided, the method comprising: introducing into the organism at least one
15 heterologous polynucleotide encoding a ubiquitin polypeptide, a negative regulator of flagellin synthesis (Flg M) polypeptide, a SRC Homology 3 (SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide, a serum albumin polypeptide, or any combination thereof, to produce a transgenic organism expressing the heterologous polynucleotide, thereby increasing the drought or desiccation tolerance of the transgenic organism.

An organism useful with this invention may be any organism that can be transformed
20 and includes, but is not limited to, an animal, a fungus, a bacterium, an archaeon, a protist, or a plant. Non-limiting examples of an animals includes a mammal, a bird, a fish, an insect, a reptile, a nematode, or an amphibian. Exemplary mammals include a human, a non-human primate (e.g., a monkey, a gorilla, a chimpanzee, a baboon, and the like) a bovine, a dog, a cat, a ferret, a horse, a donkey, a goat, a sheep, a rabbit, a guinea pig, a hamster, a mouse, and/or a rat.
25 In some embodiments, the organism that can be transformed is not a human.

Any polynucleotide encoding a ubiquitin polypeptide may be introduced into a cell or organism as described herein. Exemplary ubiquitin encoding polynucleotides include, but are not limited to, those from a mammal, a fish, an insect, a bird, a plant, a bacterium, a protist, a fungus, a plant, a nematode, or an archaeon. Exemplary mammals include, a laboratory animal
30 (e.g., a rat, mouse, guinea pig, rabbit, etc.), a farm or commercial animal (e.g., a bovine, pig, horse, goat, donkey, sheep, etc.), or a domestic animal (e.g., cat, dog, ferret, etc.), primates such as a human or a non-human primate (e.g., a chimpanzee, baboon, monkey, orangutan, gorilla, etc.). In some embodiments, a ubiquitin polypeptide may be encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity (e.g., 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, 99.9, 100%) to any of **SEQ ID NOs:7-30**; and/or any combination thereof.

Any polynucleotide encoding a SRC Homology 3 (SH3) Domain may be introduced into a cell or organism as described herein. Exemplary SH3 domain encoding polynucleotides include, but are not limited to, those obtained from a eukaryote polypeptide or a prokaryote polynucleotide comprising an SH3 domain. In some embodiments, a SH3 domain encoding polynucleotide may be obtained from, for example, from a primate such as a human or a non-human primate (e.g., a chimpanzee, baboon, monkey, orangutan, gorilla, etc.), a farm or commercial animal (e.g., a bovine, pig, horse, goat, donkey, sheep, etc.), or a domestic animal (e.g., cat, dog, ferret, etc.), a laboratory animal (e.g., a rat, mouse, guinea pig, rabbit, etc.), a reptile (e.g., a lizard, turtle, snake, etc.), a bacterium (e.g., *Escherichia*), a plant, e.g., *Arabidopsis*), a fungus (e.g., yeast), protist (e.g., *Monosiga*), insect (e.g., *Drosophila*), fish (e.g., salmon, danio), amphibian (e.g., *Xenopus*), nematode (e.g., *C. elegans*), and/or avian (e.g., chicken, parakeet). In some embodiments, a SH3 domain may be encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:31-53**; and/or any combination thereof.

Any polynucleotide encoding a negative regulator of flagellin synthesis (Flg M) polypeptide may be introduced into a cell or organism as described herein. Exemplary Flg M encoding polynucleotides include, but are not limited to, those obtained from a monotrichous bacterium or a lophotrichous bacterium. In some embodiments, a Flg M polypeptide may be obtained from *Escherichia*, *Pseudomonas*, *Bacillus*, *Vibrio*, *Helicobacter*, *Klebsiella*, *Burkholderia*, *Salmonella*, *Campylobacter*, *Enterobacter*, *Kluyvera*, *Proteobacteria*, *Shigella*, *Citrobacter*, *Diaporthe*, *Beauveria* and/or *Caulobacter*. In some embodiments, a Flg M polypeptide may be encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:54-64**; and/or any combination thereof.

Any polynucleotide encoding a small ubiquitin-like modifier (SUMO) polypeptide may be introduced into a cell or organism as described herein. Exemplary SUMO encoding polynucleotides include those from a eukaryote or a prokaryote. In some embodiments, a SUMO encoding polynucleotide may be obtained from a polypeptide from, for example, a human or a non-human primate (e.g., monkey, gorilla, orangutan, chimpanzee), a farm or commercial animal (e.g., a bovine, pig, horse, goat, donkey, sheep, etc.), or a domestic animal (e.g., cat, dog, ferret, etc.), a laboratory animal (e.g., a rat, mouse, guinea pig, rabbit, etc.), a reptile (e.g., a lizard, turtle, snake, etc.), a bacterium (e.g., *Escherichia*), a plant, e.g., *Arabidopsis*), a fungus (e.g., yeast), protist (e.g., *Monosiga*), insect (e.g., *Drosophila*), fish (e.g., salmon, danio),

amphibian (e.g., *Xenopus*), nematode (e.g., *C. elegans*), and/or avian (e.g., chicken, parakeet). In some embodiments, a SUMO polypeptide may be encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:65-86**; and/or any combination thereof.

5 Any polynucleotide encoding a serum albumin polypeptide useful as a protective polypeptide may be introduced into a cell or organism as described herein. Exemplary serum albumin encoding polynucleotides include those from any animal including, but not limited to, a human, a non-human primate (e.g., monkey, gorilla, orangutan, chimpanzee), a farm or commercial animal (e.g., a bovine, pig, horse, goat, donkey, sheep, etc.), or a domestic animal
10 (e.g., cat, dog, ferret, etc.), a laboratory animal (e.g., a rat, mouse, guinea pig, rabbit, etc.), a fish (e.g., salmon, danio), an amphibian (e.g., frog), reptile (e.g., turtle, lizard, snake) and/or an avian (e.g., chicken, parakeet, parrot). In some embodiments, a serum albumin polypeptide may be encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:87-104**; and/or any combination thereof.

15 In some embodiments, the at least one heterologous polynucleotide may be operably linked to a promoter. In some embodiments, the promoter may be heterologous to the polynucleotide and/or heterologous to the organism or cell into which the polynucleotide is introduced. In some embodiments, the promoter may be endogenous to the polynucleotide that is introduced but heterologous to the organism or cell into which the polynucleotide is
20 introduced. In some embodiments, the heterologous polynucleotide may be heterologous to the organism or cell into which the heterologous polynucleotide is being introduced. In other embodiments, the heterologous polynucleotide may be endogenous to the organism or cell into which the heterologous polynucleotide is being introduced but may be heterologous to the promoter that is operably linked to the polynucleotide.

25 The present invention further provides transgenic cells produced by the methods of the invention comprising at least one heterologous polynucleotide encoding a protective polypeptide (e.g., ubiquitin, SH3 Domain, Flg M, SUMO, serum albumin) and having increased tolerance to drought or desiccation as compared to a control (e.g., a cell that does not comprise a heterologous polynucleotide encoding a ubiquitin polypeptide, a negative regulator of flagellin
30 synthesis (Flg M) polypeptide, a SRC Homology 3 (SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide, and/or a serum albumin polypeptide). In some embodiments, the cell can be, but is not limited to, an animal cell (e.g., a mammalian cell, an avian cell, a reptile cell, an amphibian cell, an insect cell, or a fish cell, a sperm cell, an egg cell,

a stem cell, a red blood cell, muscle cell, and the like), a fungal cell, a bacterial cell, or a plant cell.

In some embodiments, a transgenic organism (e.g., a transgenic animal, plant, fungus, protist, archaeon, or bacterium) is provided having increased tolerance to drought or desiccation produced by the methods of the invention, wherein the transgenic organism comprises in its genome at least one heterologous polynucleotide encoding a protective polypeptide. In some embodiments, the invention provides a seed of a transgenic plant produced by the methods of the invention, wherein the seed comprises in its genome at least one heterologous polynucleotide encoding a protective polypeptide. In further embodiments, the invention provides a crop comprising a plurality of transgenic plants of the invention, planted together in an agricultural field, a golf course, a residential lawn, a road side, an athletic field, and /or a recreational field.

“Introducing,” in the context of a heterologous polynucleotide encoding a protective polypeptide means presenting the polynucleotide to the cell of an organism in such a manner that the polynucleotide gains access to the interior of the cell. The methods of the invention do not depend on a particular method for introducing one or more polynucleotides into an organism, only that they gain access to the interior of at least one cell of the organism. Where more than one polynucleotide is to be introduced, these polynucleotides can be assembled as part of a single polynucleotide or nucleic acid construct, or as separate polynucleotides or nucleic acid constructs, and can be located on the same or different expression constructs or transformation vectors. Accordingly, these polynucleotides may be introduced into cells in a single transformation event, in separate transformation events, or, for example, they may be incorporated into an organism as part of a breeding protocol.

The term “transformation” as used herein refers to the introduction of a heterologous nucleic acid into a cell. Transformation of a cell may be stable or transient. Thus, in some embodiments, a cell of the invention may be stably transformed with a nucleotide sequence of the invention. In other embodiments, a cell may be transiently transformed with a nucleotide sequence of the invention.

“Transient transformation” in the context of a polynucleotide means that a polynucleotide is introduced into the cell and does not integrate into the genome of the cell.

By “stably introducing” or “stably introduced” in the context of a polynucleotide introduced into a cell is intended that the introduced polynucleotide is stably incorporated into the genome of the cell, and thus the cell is stably transformed with the polynucleotide.

“Stable transformation” or “stably transformed” as used herein means that a polynucleotide is introduced into a cell and integrates into the genome of the cell. As such, the

integrated polynucleotide is capable of being inherited by the progeny thereof, more particularly, by the progeny of multiple successive generations. "Genome" as used herein also includes the nuclear, mitochondrial, and plastid genome, and therefore includes integration of the nucleic acid into, for example, the chloroplast or mitochondrial genome. Stable transformation as used
5 herein can also refer to a transgene that is maintained extrachromasomally, for example, as a minichromosome.

Transient transformation may be detected by, for example, an enzyme-linked immunosorbent assay (ELISA) or Western blot, which can detect the presence of a peptide or polypeptide encoded by one or more transgene introduced into an organism. Stable
10 transformation of a cell can be detected by, for example, a Southern blot hybridization assay of genomic DNA of the cell with nucleic acid sequences which specifically hybridize with a nucleotide sequence of a transgene introduced into an organism (e.g., a plant). Stable transformation of a cell can be detected by, for example, a Northern blot hybridization assay of RNA of the cell with nucleic acid sequences, which specifically hybridize with a nucleotide
15 sequence of a transgene introduced into an organism. Stable transformation of a cell can also be detected by, e.g., a polymerase chain reaction (PCR) or other amplification reactions as are well known in the art, employing specific primer sequences that hybridize with target sequence(s) of a transgene, resulting in amplification of the transgene sequence, which can be detected according to standard methods. Transformation can also be detected by direct sequencing
20 and/or hybridization protocols well known in the art.

A polynucleotide of the invention (e.g., a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to of any of **SEQ ID NO:7-209**, a nucleotide sequence having at least about 70% identity to of any of **SEQ ID NOs:210-314**, or a nucleotide sequence having at least about 80% identity to any of **SEQ ID NOs:315-419**, and/or fragments
25 thereof) can be introduced into a cell by any method known to those of skill in the art. In some embodiments of the invention, transformation of a cell comprises nuclear transformation. In other embodiments, transformation of a cell comprises mitochondrial or chloroplast transformation.

Polynucleotides encoding protective polypeptides can be delivered directly into a cell
30 by any method known in the art, e.g., by transfection or microinjection. Those skilled in the art will appreciate that the isolated polynucleotides encoding the protective polypeptides will typically be associated with appropriate expression control sequences, e.g., transcription/translation control signals and polyadenylation signals.

It will further be appreciated that a variety of promoter/enhancer elements can be used depending on the level and tissue-specific expression desired. The promoter can be constitutive or inducible, depending on the pattern of expression desired. The promoter can be native or foreign and can be a natural or a synthetic sequence. By foreign, it is intended that the transcriptional initiation region is not found in the wild-type host into which the transcriptional initiation region is introduced. The promoter is chosen so that it will function in the target cell(s) of interest. A promoter or other regulatory element may be heterologous to the organism into which it is being introduced and/or it may be heterologous to the polynucleotide to which it is operably linked.

The polynucleotides encoding protective polypeptides can be incorporated into an expression vector. Expression vectors compatible with various host cells are well known in the art and contain suitable elements for transcription and translation of nucleic acids. Typically, an expression vector contains an "expression cassette," which includes, in the 5' to 3' direction, a promoter, a coding sequence encoding a double stranded RNA operatively associated with the promoter, and, optionally, a termination sequence including a stop signal for RNA polymerase and a polyadenylation signal for polyadenylase.

Non-limiting examples of animal and mammalian promoters known in the art include, but are not limited to, the SV40 early (SV40e) promoter region, the promoter contained in the 3' long terminal repeat (LTR) of Rous sarcoma virus (RSV), the promoters of the E1A or major late promoter (MLP) genes of adenoviruses (Ad), the cytomegalovirus (CMV) early promoter, the herpes simplex virus (HSV) thymidine kinase (TK) promoter, baculovirus IE1 promoter, elongation factor 1 alpha (EF1) promoter, phosphoglycerate kinase (PGK) promoter, ubiquitin (Ubc) promoter, an albumin promoter, the regulatory sequences of the mouse metallothionein-L promoter and transcriptional control regions, the ubiquitous promoters (HPRT, vimentin, α -actin, tubulin and the like), the promoters of the intermediate filaments (desmin, neurofilaments, keratin, GFAP, and the like), the promoters of therapeutic genes (of the MDR, CFTR or factor VIII type, and the like), mitochondrial-specific promoters, and/or pathogenesis and/or disease-related promoters. In addition, any of these expression sequences of this invention can be modified by addition of enhancer and/or regulatory sequences and the like.

Non-limiting examples of plant promoters include the promoter of the RubisCo small subunit gene 1 (PrbcS1), the promoter of the actin gene (Pactin), the promoter of the nitrate reductase gene (Pnr) and the promoter of duplicated carbonic anhydrase gene 1 (Pdca1). PrbcS1 and Pactin are constitutive promoters and Pnr and Pdca1 are inducible promoters. Pnr is induced by nitrate and repressed by ammonium and Pdca1 is induced by salt. Other constitutive

plant promoters include but are not limited to cestrum virus promoter (cmp) (U.S. Patent No. 7,166,770), the rice actin 1 promoter, CaMV 35S promoter, CaMV 19S promoter, nos promoter, Adh promoter, sucrose synthase promoter (, and the ubiquitin promoter. Non-limiting examples of tissue-specific promoters for plants include those associated with genes encoding the seed storage proteins (such as β -conglycinin, cruciferin, napin and phaseolin), zein or oil body proteins (such as oleosin), or proteins involved in fatty acid biosynthesis (including acyl carrier protein, stearyl-ACP desaturase and fatty acid desaturases (fad 2-1)), and other nucleic acids expressed during embryo development (such as Bce4). Non-limiting examples of promoters functional in chloroplasts include the bacteriophage T3 gene 9 5' UTR, the S-E9 small subunit RuBP carboxylase promoter, the Kunitz trypsin inhibitor gene promoter (Kti3).and other promoters disclosed in U.S. Patent No. 7,579,516.

A further aspect of the invention relates to kits for use in the methods of the invention. The kit can comprise one or more protective polypeptides of the invention in a form suitable for stabilizing vaccines, antibodies, a heterologous cell, tissue, organ and/or other biologics or in a form suitable for introducing into an organism. The kit can further comprise other components, such as therapeutic agents, carriers, buffers, containers, devices for administration/contacting, compositions for transformation, and the like. The kit can be designed for therapeutic use, diagnostic use, and/or research use and the additional components can be those suitable for the intended use. The kit can further comprise labels and/or instructions, e.g., for stabilizing a heterologous polypeptide, cell, tissue, or organ, or for, e.g., imparting drought or desiccation resistance/tolerance to an organism. Such labeling and/or instructions can include, for example, information concerning the amount, frequency and method of administration of the one or more TDPs.

Table 1. Exemplary tardigrade species

<i>Macrobiotus almadai</i>	<i>Macrobiotus insularis</i>	<i>Macrobiotus ragonesei</i>
<i>Macrobiotus altitudinalis</i>	<i>Macrobiotus islandicus</i>	<i>Macrobiotus ramoli</i>
<i>Macrobiotus alvaroi</i>	<i>Macrobiotus joannae</i>	<i>Macrobiotus rawsoni</i>
<i>Macrobiotus anderssoni</i>	<i>Macrobiotus kazmierskii</i>	<i>Macrobiotus recens</i>
<i>Macrobiotus andinus</i>	<i>Macrobiotus kirghizicus</i>	<i>Macrobiotus reinhardtii</i>
<i>Macrobiotus annae</i>	<i>Macrobiotus kollerii</i>	<i>Macrobiotus rigidus</i>
<i>Macrobiotus aradasi</i>	<i>Macrobiotus komareki</i>	<i>Macrobiotus rollei</i>
<i>Macrobiotus arguei</i>	<i>Macrobiotus kovalevi</i>	<i>Macrobiotus rubens</i>
<i>Macrobiotus ariekammensis</i>	<i>Macrobiotus krynauwi</i>	<i>Macrobiotus sandrae</i>
<i>Macrobiotus armatus</i>	<i>Macrobiotus kurasi</i>	<i>Macrobiotus santoroi</i>
<i>Macrobiotus artipharyngis</i>	<i>Macrobiotus lazzaroi</i>	<i>Macrobiotus sapiens</i>
<i>Macrobiotus ascensionis</i>	<i>Macrobiotus lissostomus</i>	<i>Macrobiotus semmelweisi</i>
<i>Macrobiotus australis</i>	<i>Macrobiotus liviae</i>	<i>Macrobiotus serratus</i>
<i>Macrobiotus baltatus</i>	<i>Macrobiotus longipes</i>	<i>Macrobiotus seychellensis</i>
<i>Macrobiotus barabanovi</i>	<i>Macrobiotus lusitanicus</i>	<i>Macrobiotus shennongensis</i>
<i>Macrobiotus binieki</i>	<i>Macrobiotus macocalix</i>	<i>Macrobiotus siamensis</i>
<i>Macrobiotus barbarae</i>	<i>Macrobiotus madegassus</i>	<i>Macrobiotus sicheli</i>
<i>Macrobiotus biserovi</i>	<i>Macrobiotus mandalaeae</i>	<i>Macrobiotus simulans</i>

<i>Macrobiotus blocki</i>	<i>Macrobiotus marlenae</i>	<i>Macrobiotus sklodowskiae</i>
<i>Macrobiotus brevipes</i>	<i>Macrobiotus martini</i>	<i>Macrobiotus snaresensis</i>
<i>Macrobiotus caelicola</i>	<i>Macrobiotus mauccii</i>	<i>Macrobiotus spectabilis</i>
<i>Macrobiotus carsicus</i>	<i>Macrobiotus meridionalis</i>	<i>Macrobiotus spertii</i>
<i>Macrobiotus caymanensis</i>	<i>Macrobiotus modestus</i>	<i>Macrobiotus stellaris</i>
<i>Macrobiotus contii</i>	<i>Macrobiotus montanus</i>	<i>Macrobiotus striatus</i>
<i>Macrobiotus coronatus</i>	<i>Macrobiotus mottai</i>	<i>Macrobiotus submorulatus</i>
<i>Macrobiotus creber</i>	<i>Macrobiotus nelsonae</i>	<i>Macrobiotus szeptyckii</i>
<i>Macrobiotus crenulatus</i>	<i>Macrobiotus neuquensis</i>	<i>Macrobiotus tehuelchensis</i>
<i>Macrobiotus danielisae</i>	<i>Macrobiotus norvegicus</i>	<i>Macrobiotus terminalis</i>
<i>Macrobiotus dariae</i>	<i>Macrobiotus nuragicus</i>	<i>Macrobiotus terricola</i>
<i>Macrobiotus denticulus</i>	<i>Macrobiotus occidentalis</i>	<i>Macrobiotus tetraplacoides</i>
<i>Macrobiotus diffusus</i>	<i>Macrobiotus ocotensis</i>	<i>Macrobiotus topali</i>
<i>Macrobiotus diguensis</i>	<i>Macrobiotus orcadensis</i>	<i>Macrobiotus trunovae</i>
<i>Macrobiotus dimentmani</i>	<i>Macrobiotus ovidii</i>	<i>Macrobiotus virgatus</i>
<i>Macrobiotus divergens</i>	<i>Macrobiotus ovostriatus</i>	<i>Macrobiotus vladimiri</i>
<i>Macrobiotus diversus</i>	<i>Macrobiotus ovovillosus</i>	<i>Macrobiotus wauensis</i>
<i>Macrobiotus drakensbergi</i>	<i>Macrobiotus pallarii</i>	<i>Macrobiotus wuzhishanensis</i>
<i>Macrobiotus echinogenitus</i>	<i>Macrobiotus papillosus</i>	<i>Macrobiotus yunshanensis</i>
<i>Macrobiotus erminiae</i>	<i>Macrobiotus patagonicus</i>	<i>Macrobiotus zhejiangensis</i>
<i>Macrobiotus evelinae</i>	<i>Macrobiotus patiens</i>	<i>Isohypsibius altai</i>
<i>Macrobiotus furciger</i>	<i>Macrobiotus perfidus</i>	<i>Isohypsibius annulatus</i>
<i>Macrobiotus gemmatus</i>	<i>Macrobiotus persimilis</i>	<i>Isohypsibius arbiter</i>
<i>Macrobiotus glebkai</i>	<i>Macrobiotus personatus</i>	<i>Isohypsibius archangajensis</i>
<i>Macrobiotus grandis</i>	<i>Macrobiotus peterseni</i>	<i>Isohypsibius arcuatus</i>
<i>Macrobiotus halei</i>	<i>Macrobiotus pilatoi</i>	<i>Isohypsibius asper</i>
<i>Macrobiotus hapukuensis</i>	<i>Macrobiotus polaris</i>	<i>Isohypsibius austriacus</i>
<i>Macrobiotus harmsworthi</i>	<i>Macrobiotus polonicus</i>	<i>Isohypsibius baicalensis</i>
<i>Macrobiotus hibiscus</i>	<i>Macrobiotus polyopus</i>	<i>Isohypsibius baldii</i>
<i>Macrobiotus hieronimi</i>	<i>Macrobiotus porteri</i>	<i>Isohypsibius baldioides</i>
<i>Macrobiotus hufelandi</i>	<i>Macrobiotus potockii</i>	<i>Isohypsibius barbarae</i>
<i>Macrobiotus humilis</i>	<i>Macrobiotus primitivae</i>	<i>Isohypsibius bartosi</i>
<i>Macrobiotus hyperboreus</i>	<i>Macrobiotus privitera</i>	<i>Isohypsibius basalovoi</i>
<i>Macrobiotus iharosi</i>	<i>Macrobiotus psephus</i>	<i>Isohypsibius belliformis</i>
<i>Macrobiotus insignis</i>	<i>Macrobiotus pseudocoronatus</i>	<i>Isohypsibius bellus</i>
<i>Macrobiotus insignis</i>	<i>Macrobiotus pseudofurcatus</i>	<i>Isohypsibius borkini</i>
<i>Macrobiotus insularis</i>	<i>Macrobiotus pseudoliviae</i>	<i>Isohypsibius brevispinosus</i>
<i>Macrobiotus islandicus</i>	<i>Macrobiotus pseudonuragicus</i>	<i>Isohypsibius brevitubulatus</i>
<i>Macrobiotus joannae</i>	<i>Macrobiotus punctillus</i>	<i>Isohypsibius brulloi</i>
<i>Macrobiotus kazmierskii</i>	<i>Macrobiotus radiatus</i>	<i>Isohypsibius bulbifer</i>
<i>Isohypsibius cameruni</i>	<i>Isohypsibius neoundulatus</i>	<i>Echiniscus barbarae</i>
<i>Isohypsibius campbellensis</i>	<i>Isohypsibius nipponicus</i>	<i>Echiniscus batramiae</i>
<i>Isohypsibius canadensis</i>	<i>Isohypsibius nodosus</i>	<i>Echiniscus becki</i>
<i>Isohypsibius ceciliae</i>	<i>Isohypsibius novaeguineae</i>	<i>Echiniscus bigranulatus</i>
<i>Isohypsibius changbaiensis</i>	<i>Isohypsibius palmai</i>	<i>Echiniscus bisculptus</i>
<i>Isohypsibius chiarae</i>	<i>Isohypsibius panovi</i>	<i>Echiniscus blumi</i>
<i>Isohypsibius costatus</i>	<i>Isohypsibius papillifer</i>	<i>Echiniscus calcaratus</i>
<i>Isohypsibius cyrilli</i>	<i>Isohypsibius pappi</i>	<i>Echiniscus calvus</i>
<i>Isohypsibius damxungensis</i>	<i>Isohypsibius pauper</i>	<i>Echiniscus canadensis</i>
<i>Isohypsibius dastychi</i>	<i>Isohypsibius pilatoi</i>	<i>Echiniscus canedoi</i>
<i>Isohypsibius deconincki</i>	<i>Isohypsibius pratensis</i>	<i>Echiniscus capillatus</i>
<i>Isohypsibius deflexus</i>	<i>Isohypsibius prosostomus</i>	<i>Echiniscus carsicus</i>
<i>Isohypsibius dudichi</i>	<i>Isohypsibius pseudoundulatus</i>	<i>Echiniscus carusoi</i>
<i>Isohypsibius duranteae</i>	<i>Isohypsibius pulcher</i>	<i>Echiniscus cavagnaroi</i>
<i>Isohypsibius effusus</i>	<i>Isohypsibius pushkini</i>	<i>Echiniscus cervicornis</i>
<i>Isohypsibius elegans</i>	<i>Isohypsibius qinlingensis</i>	<i>Echiniscus charrua</i>
<i>Isohypsibius eplenyiensis</i>	<i>Isohypsibius rahmi</i>	<i>Echiniscus cheonyoungi</i>
<i>Isohypsibius franzi</i>	<i>Isohypsibius reticulatus</i>	<i>Echiniscus cirnoi</i>
<i>Isohypsibius fuscus</i>	<i>Isohypsibius roberti</i>	<i>Echiniscus clavispinosus</i>
<i>Isohypsibius gilvus</i>	<i>Isohypsibius ronsisvallei</i>	<i>Echiniscus clevelandi</i>
<i>Isohypsibius glaber</i>	<i>Isohypsibius rudescui</i>	<i>Echiniscus columinis</i>
<i>Isohypsibius glazovi</i>	<i>Isohypsibius rugosus</i>	<i>Echiniscus corrugicaudatus</i>
<i>Isohypsibius gracilis</i>	<i>Isohypsibius sabellai</i>	<i>Echiniscus crassispinosus</i>
<i>Isohypsibius granditintinus</i>	<i>Isohypsibius sattleri</i>	<i>Echiniscus curiosus</i>
<i>Isohypsibius granulifer</i>	<i>Isohypsibius schaudinni</i>	<i>Echiniscus dariae</i>

<i>Isohypsibius gyulai</i>	<i>Isohypsibius sculptus</i>	<i>Echiniscus darienae</i>
<i>Isohypsibius hadzii</i>	<i>Isohypsibius sellnicki</i>	<i>Echiniscus dearmatus</i>
<i>Isohypsibius helenae</i>	<i>Isohypsibius septentrionalis</i>	<i>Echiniscus dikenli</i>
<i>Isohypsibius hydrogogianus</i>	<i>Isohypsibius silvicola</i>	<i>Echiniscus diploglyptus</i>
<i>Isohypsibius hypostomoides</i>	<i>Isohypsibius sismicus</i>	<i>Echiniscus divergens</i>
<i>Isohypsibius improvisus</i>	<i>Isohypsibius solidus</i>	<i>Echiniscus dreyfusi</i>
<i>Isohypsibius indicus</i>	<i>Isohypsibius taibaiensis</i>	<i>Echiniscus duboisi</i>
<i>Isohypsibius irregibilis</i>	<i>Isohypsibius tetradactyloides</i>	<i>Echiniscus egnatie</i>
<i>Isohypsibius jakieli</i>	<i>Isohypsibius theresiae</i>	<i>Echiniscus ehrenbergi</i>
<i>Isohypsibius jingshanensis</i>	<i>Isohypsibius torulosus</i>	<i>Echiniscus elaeinae</i>
<i>Isohypsibius jinhouensis</i>	<i>Isohypsibius truncorum</i>	<i>Echiniscus elegans</i>
<i>Isohypsibius josephi</i>	<i>Isohypsibius tuberculatus</i>	<i>Echiniscus evelinae</i>
<i>Isohypsibius kenodontis</i>	<i>Isohypsibius tuberculoides</i>	<i>Echiniscus filamentosus</i>
<i>Isohypsibius kotovae</i>	<i>Isohypsibius tubereticulatus</i>	<i>Echiniscus ganczareki</i>
<i>Isohypsibius kristenseni</i>	<i>Isohypsibius tucumanensis</i>	<i>Echiniscus glaber</i>
<i>Isohypsibius ladogensis</i>	<i>Isohypsibius undulatus</i>	<i>Echiniscus granulatus</i>
<i>Isohypsibius laevis</i>	<i>Isohypsibius vej dovskyi</i>	<i>Echiniscus heterospinosus</i>
<i>Isohypsibius latunguis</i>	<i>Isohypsibius verae</i>	<i>Echiniscus hexacanthus</i>
<i>Isohypsibius leithaicus</i>	<i>Isohypsibius verrucosus</i>	<i>Echiniscus hoonsooi</i>
<i>Isohypsibius liae</i>	<i>Isohypsibius gibbus</i>	<i>Echiniscus homingi</i>
<i>Isohypsibius lineatus</i>	<i>Isohypsibius wilsoni</i>	<i>Echiniscus inocelatus</i>
<i>Isohypsibius longiunguis</i>	<i>Isohypsibius woodsae</i>	<i>Echiniscus insuetus</i>
<i>Isohypsibius lunulatus</i>	<i>Isohypsibius yunnanensis</i>	<i>Echiniscus jagodici</i>
<i>Isohypsibius macrodactylus</i>	<i>Echiniscus africanus</i>	<i>Echiniscus jamesi</i>
<i>Isohypsibius malawiensis</i>	<i>Echiniscus aliquantillus</i>	<i>Echiniscus japonicus</i>
<i>Isohypsibius mammillosus</i>	<i>Echiniscus angolensis</i>	<i>Echiniscus jenningsi</i>
<i>Isohypsibius marcellinoi</i>	<i>Echiniscus apuanus</i>	<i>Echiniscus kerguelensis</i>
<i>Isohypsibius marii</i>	<i>Echiniscus arcangelii</i>	<i>Echiniscus knowltoni</i>
<i>Isohypsibius mihelcici</i>	<i>Echiniscus arctomys</i>	<i>Echiniscus kofordi</i>
<i>Isohypsibius monoicus</i>	<i>Echiniscus arthuri</i>	<i>Echiniscus kosickii</i>
<i>Isohypsibius monstrosus</i>	<i>Echiniscus azoricus</i>	<i>Echiniscus lapponicus</i>
<i>Isohypsibius montanus</i>	<i>Echiniscus baius</i>	<i>Echiniscus laterosetosus</i>
<i>Isohypsibius myrops</i>	<i>Echiniscus baloghi</i>	<i>Echiniscus laterospinosus</i>
<i>Echiniscus latifasciatus</i>	<i>Echiniscus scabrospinosus</i>	<i>Minibiotus keppelensis</i>
<i>Echiniscus lichenorum</i>	<i>Echiniscus semifoveolatus</i>	<i>Minibiotus maculatus</i>
<i>Echiniscus limai</i>	<i>Echiniscus shaanxiensis</i>	<i>Minibiotus marcus</i>
<i>Echiniscus lineatus</i>	<i>Echiniscus siegristi</i>	<i>Minibiotus milleri</i>
<i>Echiniscus longispinosus</i>	<i>Echiniscus simba</i>	<i>Minibiotus orthofasciatus</i>
<i>Echiniscus loxophthalmus</i>	<i>Echiniscus speciosus</i>	<i>Minibiotus pilatus</i>
<i>Echiniscus madonnae</i>	<i>Echiniscus spiculifer</i>	<i>Minibiotus poricinctus</i>
<i>Echiniscus maesi</i>	<i>Echiniscus spiniger</i>	<i>Minibiotus pustulatus</i>
<i>Echiniscus malpighii</i>	<i>Echiniscus spinulosus</i>	<i>Minibiotus ramazzottii</i>
<i>Echiniscus manuelae</i>	<i>Echiniscus storkani</i>	<i>Minibiotus scopulus</i>
<i>Echiniscus marcus</i>	<i>Echiniscus sylvanus</i>	<i>Minibiotus sidereus</i>
<i>Echiniscus marginatus</i>	<i>Echiniscus taibaiensis</i>	<i>Minibiotus stuckenbergi</i>
<i>Echiniscus marginoporus</i>	<i>Echiniscus tamus</i>	<i>Minibiotus subintermedius</i>
<i>Echiniscus markezi</i>	<i>Echiniscus tardus</i>	<i>Minibiotus taiti</i>
<i>Echiniscus marleyi</i>	<i>Echiniscus tenuis</i>	<i>Minibiotus vinciguerrae</i>
<i>Echiniscus maucii</i>	<i>Echiniscus tessellatus</i>	<i>Minibiotus weglarskae</i>
<i>Echiniscus mediantus</i>	<i>Echiniscus testudo</i>	<i>Minibiotus weinerorum</i>
<i>Echiniscus merokensis</i>	<i>Echiniscus trisetosus</i>	<i>Minibiotus wuzhishanensis</i>
<i>Echiniscus migiurtinus</i>	<i>Echiniscus trojanus</i>	<i>Minibiotus xavieri</i>
<i>Echiniscus mihelcici</i>	<i>Echiniscus tropicalis</i>	<i>Doryphoribius amazonicus</i>
<i>Echiniscus militaris</i>	<i>Echiniscus tympanista</i>	<i>Doryphoribius bertolanii</i>
<i>Echiniscus molluscorum</i>	<i>Echiniscus velaminis</i>	<i>Doryphoribius bindae</i>
<i>Echiniscus moniliatus</i>	<i>Echiniscus vinculus</i>	<i>Doryphoribius dawkinsi</i>
<i>Echiniscus montanus</i>	<i>Echiniscus virginicus</i>	<i>Doryphoribius doryphorus</i>
<i>Echiniscus mosaicus</i>	<i>Echiniscus viridianus</i>	<i>Doryphoribius dupliglobulatus</i>
<i>Echiniscus multispinosus</i>	<i>Echiniscus viridis</i>	<i>Doryphoribius evelinae</i>
<i>Echiniscus murrayi</i>	<i>Echiniscus viridissimus</i>	<i>Doryphoribius flavus</i>
<i>Echiniscus nelsonae</i>	<i>Echiniscus walteri</i>	<i>Doryphoribius gibber</i>
<i>Echiniscus nepalensis</i>	<i>Echiniscus weisseri</i>	<i>Doryphoribius huangguoshuensis</i>
<i>Echiniscus nigripustulus</i>	<i>Echiniscus wendti</i>	<i>Doryphoribius koreanus</i>
<i>Echiniscus nobilis</i>	<i>Echiniscus zetotrymus</i>	<i>Doryphoribius korganovae</i>
<i>Echiniscus oihonnae</i>	<i>Minibiotus acadianus</i>	<i>Doryphoribius longistipes</i>

<i>Echiniscus ollantaytamboensis</i>	<i>Minibiotus acontistus</i>	<i>Doryphoribius macrodon</i>
<i>Echiniscus osellai</i>	<i>Minibiotus aculeatus</i>	<i>Doryphoribius maranguensis</i>
<i>Echiniscus pajstunensis</i>	<i>Minibiotus africanus</i>	<i>Doryphoribius mariae</i>
<i>Echiniscus palmai</i>	<i>Minibiotus allani</i>	<i>Doryphoribius mexicanus</i>
<i>Echiniscus perarmatus</i>	<i>Minibiotus aquatilis</i>	<i>Doryphoribius minimus</i>
<i>Echiniscus peruvianus</i>	<i>Minibiotus asteris</i>	<i>Doryphoribius neglectus</i>
<i>Echiniscus perviridis</i>	<i>Minibiotus bisoctus</i>	<i>Doryphoribius picoensis</i>
<i>Echiniscus phocae</i>	<i>Minibiotus claxtonae</i>	<i>Doryphoribius pilatoi</i>
<i>Echiniscus polygonalis</i>	<i>Minibiotus constellatus</i>	<i>Doryphoribius polynetae</i>
<i>Echiniscus pooensis</i>	<i>Minibiotus continuus</i>	<i>Doryphoribius qinlingense</i>
<i>Echiniscus porabrus</i>	<i>Minibiotus crassidens</i>	<i>Doryphoribius quadrituberculatus</i>
<i>Echiniscus postojnensis</i>	<i>Minibiotus deprecens</i>	<i>Doryphoribius smokiensis</i>
<i>Echiniscus pseudodelegans</i>	<i>Minibiotus diphasconides</i>	<i>Doryphoribius solidunguis</i>
<i>Echiniscus pseudowendti</i>	<i>Minibiotus eichhomi</i>	<i>Doryphoribius taiwanus</i>
<i>Echiniscus punctus</i>	<i>Minibiotus ethelae</i>	<i>Doryphoribius tergumrudis</i>
<i>Echiniscus pusae</i>	<i>Minibiotus fallax</i>	<i>Doryphoribius tessellatus</i>
<i>Echiniscus quadrispinosus</i>	<i>Minibiotus floriparus</i>	<i>Doryphoribius turkmenicus</i>
<i>Echiniscus quitensis</i>	<i>Minibiotus furcatus</i>	<i>Doryphoribius vietnamensis</i>
<i>Echiniscus rackae</i>	<i>Minibiotus granatai</i>	<i>Doryphoribius zappalai</i>
<i>Echiniscus ranzii</i>	<i>Minibiotus gumersindoi</i>	<i>Doryphoribius zyxiglobus</i>
<i>Echiniscus reticulatus</i>	<i>Minibiotus harrylewisi</i>	<i>Paramacrobiosus alekseevi</i>
<i>Echiniscus reymondi</i>	<i>Minibiotus hispidus</i>	<i>Paramacrobiosus areolatus</i>
<i>Echiniscus robertsi</i>	<i>Minibiotus hufelandioides</i>	<i>Paramacrobiosus beotiae</i>
<i>Echiniscus rodnae</i>	<i>Minibiotus intermedius</i>	<i>Paramacrobiosus centesimus</i>
<i>Echiniscus rufoviridis</i>	<i>Minibiotus jonesorum</i>	<i>Paramacrobiosus chieregoi</i>
<i>Echiniscus rugospinosus</i>	<i>Minibiotus julietae</i>	<i>Paramacrobiosus corgatensis</i>
<i>Paramacrobiosus crenatus</i>	<i>Hypsibius thaleri</i>	<i>Pseudechiniscus victor</i>
<i>Paramacrobiosus csotiensis</i>	<i>Milnesium alabamiae</i>	<i>Pseudechiniscus yunnanensis</i>
<i>Paramacrobiosus danielae</i>	<i>Milnesium almatyense</i>	<i>Ramazzottius affinis</i>
<i>Paramacrobiosus derkai</i>	<i>Milnesium antarcticum</i>	<i>Ramazzottius agannae</i>
<i>Paramacrobiosus garynahii</i>	<i>Milnesium asiaticum</i>	<i>Ramazzottius andreevi</i>
<i>Paramacrobiosus gerlachae</i>	<i>Milnesium brachyungue</i>	<i>Ramazzottius anomalus</i>
<i>Paramacrobiosus huziori</i>	<i>Milnesium dujiangensis</i>	<i>Ramazzottius baumanni</i>
<i>Paramacrobiosus kenianus</i>	<i>Milnesium eurytostomum</i>	<i>Ramazzottius belubellus</i>
<i>Paramacrobiosus lorenae</i>	<i>Milnesium granulatum</i>	<i>Ramazzottius bunikowskiae</i>
<i>Paramacrobiosus magdalenae</i>	<i>Milnesium jacobi</i>	<i>Ramazzottius cataphractus</i>
<i>Paramacrobiosus palaui</i>	<i>Milnesium katarzynae</i>	<i>Ramazzottius caucasicus</i>
<i>Paramacrobiosus peteri</i>	<i>Milnesium krzysztofi</i>	<i>Ramazzottius edmondabouti</i>
<i>Paramacrobiosus richtersi</i>	<i>Milnesium longiungue</i>	<i>Ramazzottius homingi</i>
<i>Paramacrobiosus rioplatensis</i>	<i>Milnesium minutum</i>	<i>Ramazzottius ljudmilae</i>
<i>Paramacrobiosus savai</i>	<i>Milnesium reductum</i>	<i>Ramazzottius montivagus</i>
<i>Paramacrobiosus tonollii</i>	<i>Milnesium reticulatum</i>	<i>Ramazzottius nivalis</i>
<i>Paramacrobiosus vanescens</i>	<i>Milnesium sandrae</i>	<i>Ramazzottius novemcinctus</i>
<i>Paramacrobiosus walteri</i>	<i>Milnesium swolenskyi</i>	<i>Ramazzottius oberhaeuseri</i>
<i>Hypsibius allisoni</i>	<i>Milnesium tardigradum</i>	<i>Ramazzottius rupeus</i>
<i>Hypsibius antonovae</i>	<i>Milnesium tetralamellatum</i>	<i>Ramazzottius saltensis</i>
<i>Hypsibius arcticus</i>	<i>Milnesium zsalakoe</i>	<i>Ramazzottius semisculptus</i>
<i>Hypsibius biscuitiformis</i>	<i>Pseudechiniscus alberti</i>	<i>Ramazzottius subanomalus</i>
<i>Hypsibius calcaratus</i>	<i>Pseudechiniscus asper</i>	<i>Ramazzottius szepteycki</i>
<i>Hypsibius camelopardalis</i>	<i>Pseudechiniscus bartkei</i>	<i>Ramazzottius theroni</i>
<i>Hypsibius choucoutiensis</i>	<i>Pseudechiniscus beasleyi</i>	<i>Ramazzottius thulini</i>
<i>Hypsibius conifer</i>	<i>Pseudechiniscus bidenticulatus</i>	<i>Ramazzottius tribulosus</i>
<i>Hypsibius convergens</i>	<i>Pseudechiniscus bispinosus</i>	<i>Ramazzottius valaamis</i>
<i>Hypsibius dujardini</i>	<i>Pseudechiniscus brevimontanus</i>	<i>Ramazzottius varieornatus</i>
<i>Hypsibius fuhmanni</i>	<i>Pseudechiniscus clavatus</i>	<i>Batillipes acaudatus</i>
<i>Hypsibius giusepperamazzotti</i>	<i>Pseudechiniscus conifer</i>	<i>Batillipes adriaticus</i>
<i>Hypsibius heardensis</i>	<i>Pseudechiniscus dicrani</i>	<i>Batillipes africanus</i>
<i>Hypsibius hypostomus</i>	<i>Pseudechiniscus distinctus</i>	<i>Batillipes annulatus</i>
<i>Hypsibius iskandarovi</i>	<i>Pseudechiniscus facettalis</i>	<i>Batillipes bullacaudatus</i>
<i>Hypsibius janetscheki</i>	<i>Pseudechiniscus goedeni</i>	<i>Batillipes camonensis</i>
<i>Hypsibius klebelsbergi</i>	<i>Pseudechiniscus gullii</i>	<i>Batillipes crassipes</i>
<i>Hypsibius kunmingensis</i>	<i>Pseudechiniscus insolitus</i>	<i>Batillipes dicrocercus</i>
<i>Hypsibius macrocalcaratus</i>	<i>Pseudechiniscus islandicus</i>	<i>Batillipes friauffi</i>
<i>Hypsibius maculatus</i>	<i>Pseudechiniscus jiroveci</i>	<i>Batillipes gilmartini</i>
<i>Hypsibius marcelli</i>	<i>Pseudechiniscus juanitae</i>	<i>Batillipes lesteri</i>

<i>Hypsibius microps</i>	<i>Pseudechiniscus jubatus</i>	<i>Batillipes littoralis</i>
<i>Hypsibius montanus</i>	<i>Pseudechiniscus megacephalus</i>	<i>Batillipes longispinosus</i>
<i>Hypsibius morikawai</i>	<i>Pseudechiniscus nataliae</i>	<i>Batillipes marcelli</i>
<i>Hypsibius multituberculatus</i>	<i>Pseudechiniscus novaezeelandiae</i>	<i>Batillipes mirus</i>
<i>Hypsibius novaezeelandiae</i>	<i>Pseudechiniscus occultus</i>	<i>Batillipes noerrevangi</i>
<i>Hypsibius pachyunguis</i>	<i>Pseudechiniscus papillosus</i>	<i>Batillipes orientalis</i>
<i>Hypsibius pallidus</i>	<i>Pseudechiniscus pilato</i>	<i>Batillipes pennaki</i>
<i>Hypsibius pallidoides</i>	<i>Pseudechiniscus pseudoconifer</i>	<i>Batillipes philippinensis</i>
<i>Hypsibius pedrottii</i>	<i>Pseudechiniscus pulcher</i>	<i>Batillipes phreaticus</i>
<i>Hypsibius pradellii</i>	<i>Pseudechiniscus quadrilobatus</i>	<i>Batillipes roscoffensis</i>
<i>Hypsibius ragonesei</i>	<i>Pseudechiniscus ramazzottii</i>	<i>Batillipes rotundiculus</i>
<i>Hypsibius roanensis</i>	<i>Pseudechiniscus raneyi</i>	<i>Batillipes similis</i>
<i>Hypsibius runae</i>	<i>Pseudechiniscus santomensis</i>	<i>Batillipes spinicauda</i>
<i>Hypsibius scaber</i>	<i>Pseudechiniscus scortecii</i>	<i>Batillipes tridentatus</i>
<i>Hypsibius scabropygus</i>	<i>Pseudechiniscus shilinensis</i>	<i>Batillipes tubernatis</i>
<i>Hypsibius septulatus</i>	<i>Pseudechiniscus sinensis</i>	<i>Bryodelphax aaseae</i>
<i>Hypsibius seychellensis</i>	<i>Pseudechiniscus spinerectus</i>	<i>Bryodelphax alzirae</i>
<i>Hypsibius shaanxiensis</i>	<i>Pseudechiniscus suillus</i>	<i>Bryodelphax amphoterus</i>
<i>Hypsibius stiliferus</i>	<i>Pseudechiniscus transsylvanicus</i>	<i>Bryodelphax asiaticus</i>
<i>Bryodelphax atlantis</i>	<i>Itaquascon pawlowskii</i>	<i>Diphascon gerdæ</i>
<i>Bryodelphax brevidentatus</i>	<i>Itaquascon pisoniae</i>	<i>Diphascon granifer</i>
<i>Bryodelphax crossotus</i>	<i>Itaquascon simplex</i>	<i>Diphascon halapiense</i>
<i>Bryodelphax dominicanus</i>	<i>Itaquascon umbellinae</i>	<i>Diphascon higginsi</i>
<i>Bryodelphax iohannis</i>	<i>Itaquascon unguiculum</i>	<i>Diphascon humicus</i>
<i>Bryodelphax lijiangensis</i>	<i>Cornechiniscus brachycomutus</i>	<i>Diphascon hydrophilum</i>
<i>Bryodelphax mateusi</i>	<i>Cornechiniscus ceratophorus</i>	<i>Diphascon harosi</i>
<i>Bryodelphax meronensis</i>	<i>Cornechiniscus comutus</i>	<i>Diphascon iltisi</i>
<i>Bryodelphax ortholineatus</i>	<i>Cornechiniscus holmeni</i>	<i>Diphascon langhovdense</i>
<i>Bryodelphax parvulus</i>	<i>Cornechiniscus lobatus</i>	<i>Diphascon latipes</i>
<i>Bryodelphax sinensis</i>	<i>Cornechiniscus madagascariensis</i>	<i>Diphascon mirabilis</i>
<i>Bryodelphax tatrensis</i>	<i>Cornechiniscus schrammi</i>	<i>Diphascon mitrense</i>
<i>Bryodelphax weglarskae</i>	<i>Cornechiniscus subcomutus</i>	<i>Diphascon nelsonae</i>
<i>Dactylobiotus ambiguus</i>	<i>Cornechiniscus tibetanus</i>	<i>Diphascon nobilei</i>
<i>Dactylobiotus ampullaceus</i>	<i>Halechiniscus chafarinensis</i>	<i>Diphascon nodulosum</i>
<i>Dactylobiotus aquatilis</i>	<i>Halechiniscus greveni</i>	<i>Diphascon nonbullatum</i>
<i>Dactylobiotus caldarellai</i>	<i>Halechiniscus gutteli</i>	<i>Diphascon oculatum</i>
<i>Dactylobiotus dervizi</i>	<i>Halechiniscus jejuensis</i>	<i>Diphascon ongulense</i>
<i>Dactylobiotus dispar</i>	<i>Halechiniscus macrocephalus</i>	<i>Diphascon opisthoglyptum</i>
<i>Dactylobiotus grandipes</i>	<i>Halechiniscus paratuleari</i>	<i>Diphascon patanei</i>
<i>Dactylobiotus haplonyx</i>	<i>Halechiniscus perfectus</i>	<i>Diphascon pingue</i>
<i>Dactylobiotus henanensis</i>	<i>Halechiniscus remanei</i>	<i>Diphascon pinguiforme</i>
<i>Dactylobiotus kansae</i>	<i>Halechiniscus tuleari</i>	<i>Diphascon platyungue</i>
<i>Dactylobiotus lombardoi</i>	<i>Diphascon arduifrons</i>	<i>Diphascon polare</i>
<i>Dactylobiotus luci</i>	<i>Diphascon behanae</i>	<i>Diphascon puniceum</i>
<i>Dactylobiotus macronyx</i>	<i>Diphascon belgicae</i>	<i>Diphascon ramazzottii</i>
<i>Dactylobiotus octavi</i>	<i>Diphascon carolae</i>	<i>Diphascon recamieri</i>
<i>Dactylobiotus parthenogeneticus</i>	<i>Diphascon clavatum</i>	<i>Diphascon rugocaudatum</i>
<i>Dactylobiotus selenicus</i>	<i>Diphascon gordonense</i>	<i>Diphascon rugosum</i>
<i>Echiniscoides andamanensis</i>	<i>Diphascon greveni</i>	<i>Diphascon sanae</i>
<i>Echiniscoides bruni</i>	<i>Diphascon linzhienensis</i>	<i>Diphascon secchii</i>
<i>Echiniscoides higginsi</i>	<i>Diphascon maucii</i>	<i>Diphascon serratum</i>
<i>Echiniscoides hoepneri</i>	<i>Diphascon modestum</i>	<i>Diphascon sexbullatum</i>
<i>Echiniscoides homingi</i>	<i>Diphascon montigenum</i>	<i>Diphascon stappersi</i>
<i>Echiniscoides pollocki</i>	<i>Diphascon onorei</i>	<i>Diphascon tenue</i>
<i>Echiniscoides sigismundi</i>	<i>Diphascon prorsirostre</i>	<i>Diphascon trachydorsatum</i>
<i>Echiniscoides travei</i>	<i>Diphascon scoticum</i>	<i>Diphascon victoriae</i>
<i>Tenuibiotus bondavallii</i>	<i>Diphascon tricuspidatum</i>	<i>Diphascon zaniwii</i>
<i>Tenuibiotus bozhkae</i>	<i>Diphascon triodon</i>	<i>Diphascon bicorne</i>
<i>Tenuibiotus ciprianoi</i>	<i>Diphascon aculeatum</i>	<i>Diphascon coniferens</i>
<i>Tenuibiotus danilovi</i>	<i>Diphascon alpinum</i>	<i>Diphascon marcuzzii</i>
<i>Tenuibiotus higginsi</i>	<i>Diphascon australianum</i>	<i>Diphascon mariae</i>
<i>Tenuibiotus hyperonyx</i>	<i>Diphascon bidropion</i>	<i>Diphascon punctatum</i>
<i>Tenuibiotus hystricogenitus</i>	<i>Diphascon birklehofii</i>	<i>Diphascon rivulare</i>
<i>Tenuibiotus kozharai</i>	<i>Diphascon bisbullatum</i>	<i>Diphascon speciosum</i>

<i>Tenuibiotus mongolicus</i>	<i>Diphascon boreale</i>	<i>Calcarobiotus digeronimoi</i>
<i>Tenuibiotus tenuiformis</i>	<i>Diphascon brevipes</i>	<i>Calcarobiotus filmeri</i>
<i>Tenuibiotus tenuis</i>	<i>Diphascon bullatum</i>	<i>Calcarobiotus gildae</i>
<i>Tenuibiotus voronkovi</i>	<i>Diphascon burti</i>	<i>Calcarobiotus hainanensis</i>
<i>Tenuibiotus willardi</i>	<i>Diphascon chilense</i>	<i>Calcarobiotus imperialis</i>
<i>Itaquascon biserovi</i>	<i>Diphascon claxtonae</i>	<i>Calcarobiotus longinoi</i>
<i>Itaquascon cambewarrese</i>	<i>Diphascon dastychi</i>	<i>Calcarobiotus occultus</i>
<i>Itaquascon enckelli</i>	<i>Diphascon dolmiticum</i>	<i>Calcarobiotus parvicar</i>
<i>Itaquascon globuliferum</i>	<i>Diphascon elongatum</i>	<i>Calcarobiotus polygonatus</i>
<i>Itaquascon mongolicus</i>	<i>Diphascon faialense</i>	<i>Calcarobiotus tetrannulatus</i>

The following examples are not intended to be a detailed catalog of all the different ways in which the present invention may be implemented or of all the features that may be added to the present invention. Persons skilled in the art will appreciate that numerous variations and additions to the various embodiments may be made without departing from the present invention. Hence, the following descriptions are intended to illustrate some particular embodiments of the invention, and not to exhaustively specify all permutations, combinations and variations thereof.

EXAMPLES

Example 1. Protection against desiccation-induced inactivation at room temperature using ubiquitin polypeptides

Protection against desiccation-induced inactivation of lactate dehydrogenase (LDH) activity at room temperature was studied. Protective polypeptides (bovine serum albumin (BSA), tardigrade polypeptides (TDP)(produced in the cytosol (Cytosolic Abundant Heat Soluble (CAHS)) CAHS D and CAHS G and ubiquitin were used at concentrations of between 0.01 g/L to 200 g/L. Trehalose is a positive control. The results are provided in **Fig. 1** and show that at room temperature, ubiquitin protects against desiccation-induced inactivation as well as the CAHS protein.

Example 2. Protection against desiccation-induced inactivation in the presence of heat using ubiquitin polypeptides

Protection against desiccation-induced inactivation of lactate dehydrogenase (LDH) at 95 °C was studied. Protective polypeptides (TDP polypeptide CAHS G and ubiquitin were present at a concentration of 20 g/L. Trehalose is a positive control. The results are provided in **Fig. 2**, which shows that ubiquitin protects against desiccation-induced inactivation in the presence of heat (95 °C) better than the CAHS protein.

Example 3. Protection against desiccation-induced inactivation at room temperature using Flg M polypeptides

Protection against desiccation-induced inactivation of lactate dehydrogenase (LDH) at room temperature was studied. Protective polypeptides (bovine albumin serum, TDP CAHS G and ubiquitin) were present at various concentrations between 0.01 g/L to 200 g/L. Trehalose is a positive control. The results are provided in **Fig. 3** and show that at room temperature, Flg M protects against desiccation-induced inactivation better than the CAHS protein.

Example 4. Protection against desiccation-induced inactivation at room temperature using SH3 Domain polypeptides

Protection against desiccation-induced inactivation of lactate dehydrogenase (LDH) at 95 °C was studied. Protective polypeptides (bovine albumin serum, TDPs CAHS D and CAHS G, and ubiquitin) were present at concentrations of between 0.01 g/L to 200 g/L. Trehalose is a positive control. The results are provided in **Fig. 4**, which shows that the ability of SH3 Domain

polypeptides to protect against desiccation-induced inactivation in the presence of heat (95 °C) is between that of the CAHS proteins and BSA.

Example 5. Protection against desiccation-induced inactivation at room temperature using a sugar polymer

Protection against desiccation-induced inactivation of lactate dehydrogenase (LDH) activity at room temperature was studied. Protective polypeptides (bovine albumin serum, TDPs CAHS D and CAHS G, and Ficoll (e.g., Ficoll 70)) were present at concentrations of between 0.01 g/L to 200 g/L. Trehalose is a positive control. The results are provided in **Fig. 5**, which shows that the ability of Ficoll to protect against desiccation-induced inactivation at room temperature is similar to trehalose.

Example 6. Reactivation of desiccation-induced inactivated polypeptides

Reactivation of lactate dehydrogenase (LDH) activity was studied. The LDH was inactivated by desiccation at room temperature. The inactivated LDH was contacted with a solution comprising a protective polypeptides (bovine albumin serum and TDP CAHS G and the activity tested. The concentrations of the protective polypeptides in the solution were between 0.01 g/L to 200 g/L. Trehalose is a positive control. The results are provided in **Fig. 6**, which shows that the TDP CAHS G was more effective than the BSA at reactivating the inactive LDH.

It is believed that the protective polypeptides may function by facilitating proper refolding upon rehydration rather than protecting against misfolding during desiccation. These data show that an alternative approach to desiccating a polypeptide of interest in a composition comprising a protective polypeptide, the polypeptide of interest could be resuspended in a solution comprising the protective polypeptide prior to use.

The foregoing is illustrative of the present invention, and is not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

IN THE CLAIMS:

1. A liquid composition comprising:
at least one heterologous polypeptide and/or peptide of interest and
(a) at least one protective polypeptide selected from the group consisting of a ubiquitin
5 polypeptide, a negative regulator of flagellin synthesis (Flg M) polypeptide, a SRC Homology 3
(SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide, and a serum
albumin polypeptide, and any combination thereof; and/or
(b) at least one sugar polymer.
- 10 2. A solid composition produced by drying the liquid composition of claim 1.
3. A solid composition comprising:
at least one heterologous polypeptide and/or peptide of interest and
(a) at least one protective polypeptide selected from the group consisting of a ubiquitin
15 polypeptide, a negative regulator of flagellin synthesis (Flg M) polypeptide, a SRC Homology 3
(SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide, and a serum
albumin polypeptide and any combination thereof; and/or
(b) at least one sugar polymer.
- 20 4. The liquid composition of claim 1, or the solid composition of claim 2 or 3, wherein the
ubiquitin is from a mammal, a fish, an insect, a bird, a plant, a bacterium, a protist, a fungus, a
plant, a nematode, and/or an archaeon.
5. The liquid composition of claim 1 or claim 4, or the solid composition of any one of
25 claims 2 to 4, wherein the Flg M polypeptide is from a monotrichous bacterium and/or a
lophotrichous bacterium.
6. The liquid composition of any one of claims 1, 4 or 5, or the solid composition of any
one of claims 2 to 5, wherein the SH3 domain polypeptide and/or the SUMO polypeptide is
30 from a eukaryote and/or a prokaryote.
7. The liquid composition of any one of claims 1 or 4 to 6, or the solid composition of any
one of claims 2 to 6, wherein the serum albumin is from a human, a non-human primate (e.g., a

monkey, a gorilla, a chimpanzee, a baboon, and the like), a bovine, a dog, a cat, a ferret, a horse, a donkey, a goat, a sheep, a rabbit, a guinea pig, a hamster, a mouse, and/or a rat.

8. The liquid composition of any one of claims 1 or 4 to 7, or the solid composition of any one of claims 2 to 7, wherein the sugar polymer is a neutral, highly branched, high-mass, hydrophilic polysaccharide, a branched glucose polymer with reducing linkages, a branched glucose polymer with non-reducing linkages, a branched glucose polymer with non-reducing linkages, a linear glucose polymer with non-reducing linkages, and/or a glucose polymer with beta glycosidic linkages.

9. The liquid composition of any one of claims 1, or 4 to 8, or the solid composition of any one of claims 2 to 8, further comprising one or more excipients.

10. The liquid composition of any one of claims 1, or 4 to 9, wherein the concentration of the at least one protective polypeptide and/or the sugar polymer in the composition is about 1 g/L to about 300 g/L.

11. The liquid composition of any one of claims 1 or 4 to 10, or the solid composition of any one of claims 2 to 10, wherein the mass ratio of the at least one heterologous polypeptide and/or peptide of interest to the at least one protective polypeptide or sugar polymer is about 1:1000 to about 1:1, optionally about 1:100 to about 1:10 or about 1:20 to about 1:1.

12. The liquid composition of any one of claims 1 or 4 to 11, or the solid composition of any one of claims 2 to 11, wherein the ubiquitin polypeptide comprises an amino acid sequence having at least about 70% identity to any one of **SEQ ID NOs:7-30**; or the ubiquitin polypeptide is encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:7-30**; and/or any combination thereof.

13. The liquid composition of any one of claims 1 or 4 to 12, or the solid composition of any one of claims 2 to 12, wherein the SH3 domain comprises an amino acid sequence having at least about 70% identity to any one of **SEQ ID NOs:31-53**; or the SH3 domain is encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:31-53**; and/or any combination thereof.

14. The liquid composition of any one of claims 1, or 4 to 13, or the solid composition of any one of claims 2 to 12, wherein the Flg M polypeptide comprises an amino acid sequence having at least about 70% identity to any one of **SEQ ID NOs:54-64**; or the Flg M polypeptide is encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:54-64**; and/or any combination thereof.

15. The liquid composition of any one of claims 1, or 4 to 14, or the solid composition of any one of claims 2 to 14, wherein the SUMO polypeptide comprises an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:65-86**; or the SUMO polypeptide is encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:65-86**; and/or any combination thereof.

16. The liquid composition of any one of claims 1, or 4 to 15, or the solid composition of any one of claims 2 to 15, wherein the is serum albumin polypeptide comprises an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:87-104**; r the serum albumin polypeptide is encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:87-104**; and/or any combination thereof.

17. The liquid composition of any one of claims 1, or 4 to 16, or the solid composition of any one of claims 2 to 16, wherein the at least one heterologous polypeptide and/or peptide of interest is a therapeutic agent.

18. The composition of claim 17, wherein the therapeutic agent is a protein based vaccine, an antibody, an enzyme, hormone, and/or a globular protein.

19. The composition of claim 17 or claim 18, wherein the therapeutic agent is in a mixture.

20. The composition of any one of claims 1 to 22, wherein the at least one heterologous polypeptide and/or peptide of interest is part of a protein-based food.

21. A method of stabilizing at least one heterologous polypeptide and/or peptide of interest, comprising:

contacting the at least one heterologous polypeptide and/or peptide of interest with

(a) at least one protective polypeptide selected from the group consisting of a ubiquitin polypeptide, a negative regulator of flagellin synthesis (Flg M) polypeptide, a SRC Homology 3 (SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide, and a serum albumin polypeptide, and any combination thereof; and/or

(b) at least one sugar polymer to produce a liquid composition comprising the at least one heterologous polypeptide and/or peptide of interest and the at least one protective polypeptide and/or at least one sugar polymer, thereby stabilizing the at least one heterologous polypeptide and/or peptide of interest.

22. The method of claim 21, further comprising at least partially drying the liquid composition comprising the at least one heterologous polypeptide of interest and the at least one protective polypeptide and/or at least one sugar polymer.

23. The method of claim 21 or claim 22, wherein the at least one heterologous polypeptide and/or peptide of interest is a therapeutic agent or is part of a protein-based food.

24. The method of claim 23, wherein the therapeutic agent is a protein based vaccine, an antibody, an enzyme, hormone, and/or a globular protein.

25. The method of claim 23 or claim 24, wherein the therapeutic agent is in a mixture.

26. A method of stabilizing a heterologous cell, tissue or organ, comprising:
contacting the heterologous cell, tissue or organ with a solution comprising:

(a) at least one protective polypeptide selected from the group consisting of a ubiquitin polypeptide, a negative regulator of flagellin synthesis (Flg M) polypeptide, a SRC Homology 3 (SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide, and a serum albumin polypeptide, and any combination thereof; and/or

(b) at least one sugar polymer, thereby stabilizing the heterologous cell, tissue or organ.

27. The method of claim 26, wherein the concentration of the solution comprising the at least one protective polypeptide and/or the at least one sugar polymer is about 1 g/L to about 300 g/L.

28. The method of any one of claim 26 or claim 27, further comprising desiccating the heterologous cell, tissue or organ that is contacted with the at least one protective polypeptide and/or the at least one sugar polymer.

29. The method of any one of claims 26 to 28, wherein the heterologous cell, tissue or organ is from a fungus, a bacterium, an archaeon, a plant, a protist, or an animal.

30. The method of claim 29, wherein the animal is a mammal, a bird, a fish, an insect, a reptile, a nematode, or an amphibian.

31. The method of claim 30, wherein the mammal is a human, a non-human primate, a dog, a cat, a goat, a horse, a pig, a cow, a sheep, a rat, a guinea pig, a mouse, a gerbil, or a hamster.

32. A method of producing a transgenic cell having increased tolerance to drought or desiccation, comprising:

introducing into a cell at least one heterologous polynucleotide encoding a polypeptide selected from the group consisting of a ubiquitin polypeptide, a negative regulator of flagellin synthesis (Flg M) polypeptide, a SRC Homology 3 (SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide, and a serum albumin polypeptide, and any combination thereof; to produce a transgenic organism expressing the heterologous polynucleotide, thereby producing a transgenic cell having increased tolerance to drought or desiccation.

33. The method of claim 32, wherein the cell is from an animal, a fungus, a bacterium, an archaeon, a protist, or a plant.

34. The method of claim 33, wherein the animal is a mammal, a bird, a fish, an insect, a reptile, a nematode, or an amphibian.

35. The method of claim 34, wherein the mammal is a human, a non-human primate (e.g., a monkey, a gorilla, a chimpanzee, a baboon, and the like), a cow, a dog, a cat, a ferret, a horse, a donkey, a goat, a sheep, a rabbit, a guinea pig, a hamster, a mouse, and/or a rat.

36. A transgenic cell having increased tolerance to drought or desiccation produced by the method of any one of claims 32 to 35.

37. The transgenic cell of claim 36, wherein the cell is fungal cell, a bacterial cell, a plant cell, a protist cell, an archaeon cell, or an animal cell.

38. A method of increasing drought or desiccation tolerance in an organism, comprising introducing into the organism at least one heterologous polynucleotide encoding a polypeptide selected from the group consisting of a ubiquitin polypeptide, a negative regulator of flagellin synthesis (Flg M) polypeptide, a SRC Homology 3 (SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide, and a serum albumin polypeptide, and any combination thereof; to produce a transgenic organism expressing the heterologous polynucleotide, thereby increasing the drought or desiccation tolerance of the transgenic organism.

39. A method of producing a transgenic organism having increased tolerance to drought or desiccation, comprising introducing into the organism at least one heterologous polynucleotide encoding a polypeptide selected from the group consisting of a ubiquitin polypeptide, a negative regulator of flagellin synthesis (Flg M) polypeptide, a SRC Homology 3 (SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide, and a serum albumin polypeptide, and any combination thereof, thereby producing a transgenic organism having increased drought or desiccation tolerance.

40. The method of claim 38 or claim 39, wherein the organism is an animal, a bacterium, an archaeon, a protist, a fungus, or a plant.

41. The method of any one of claims 21 to 31, wherein the sugar polymer is a neutral, highly branched, high-mass, hydrophilic polysaccharide (e.g., FICOLL[®]), a branched glucose polymer with reducing linkages (e.g., dextran), a branched glucose polymer with non-reducing linkages (glycogen), a branched glucose polymer with non-reducing linkages (alpha glycosidic linkages, e.g., starch (e.g., amylose, amylopectin)), and/or a glucose polymer with beta glycosidic linkages (e.g., cellulose).

42. The method of any one of claims 21 to 41, wherein the ubiquitin polypeptide comprises an amino acid sequence having at least about 70% identity to any one of SEQ ID NOs:7-30; or

the ubiquitin polypeptide is encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:7-30**; and/or any combination thereof.

43. The method of any one of claims 21 to 42, wherein the SH3 domain comprises an amino acid sequence having at least about 70% identity to any one of **SEQ ID NOs:31-53**; or the SH3 domain is encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:31-53**; and/or any combination thereof.

44. The method of any one of claims 21 to 43, wherein the Flg M polypeptide comprises an amino acid sequence having at least about 70% identity to any one of **SEQ ID NOs:54-64**; or the Flg M polypeptide is encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:54-64**; and/or any combination thereof.

45. The method of any one of claims 21 to 44, wherein the SUMO polypeptide comprises an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:65-86**; or the SUMO polypeptide is encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:65-86**; and/or any combination thereof.

46. The method of any one of claims 21 to 45, wherein the serum albumin polypeptide comprises an amino acid sequence having at least about 70% identity to any of the amino acid sequences of **SEQ ID NOs:87-104**; or the serum albumin polypeptide is encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:87-104**; and/or any combination thereof.

47. A transgenic plant, fungus or bacterium having increased tolerance to drought or desiccation comprising at least one heterologous polynucleotide encoding (a) at least one protective polypeptide selected from the group consisting of a ubiquitin polypeptide, a negative regulator of flagellin synthesis (Flg M) polypeptide, a SRC Homology 3 (SH3) Domain polypeptide, and a serum albumin polypeptide.

48. The transgenic plant, fungus or bacterium of claim 47, wherein the at least one heterologous polynucleotide is operably linked to a heterologous promoter.

49. The transgenic plant, fungus or bacterium of any one of claims 47 to 48, wherein the ubiquitin polypeptide comprises an amino acid sequence having at least about 70% identity to any one of **SEQ ID NOs:7-30**; or the ubiquitin polypeptide is encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:7-30**; and/or any combination thereof.

50. The transgenic plant, fungus or bacterium of any one of claims 47 to 49, wherein the SH3 domain comprises an amino acid sequence having at least about 70% identity to any one of **SEQ ID NOs:31-53**; or the SH3 domain is encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:31-53**; and/or any combination thereof.

51. The transgenic plant, fungus or bacterium of any one of claims 47 to 50, wherein the Flg M polypeptide comprises an amino acid sequence having at least about 70% identity to any one of **SEQ ID NOs:54-64**; or the Flg M polypeptide is encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:54-64**; and/or any combination thereof.

52. The transgenic plant, fungus or bacterium of any one of claims 47 to 51, wherein the SUMO polypeptide comprises an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:65-86**; or the SUMO polypeptide is encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:65-86**; and/or any combination thereof.

53. The transgenic plant, fungus or bacterium of any one of claims 47 to 52, wherein the serum albumin polypeptide comprises an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:87-104**; or the serum albumin polypeptide is encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:87-104**; and/or any combination thereof.

54. A method of increasing drought or desiccation tolerance in a seed, comprising coating the seed with a solution comprising: (a) at least one protective polypeptide selected from the group consisting of a ubiquitin polypeptide, a negative regulator of flagellin synthesis (Flg

M) polypeptide, a SRC Homology 3 (SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide, and a serum albumin polypeptide, and any combination thereof; and/or (b) at least one sugar polymer; and/or (c) any combination of (a) and/or (b) to produce a coated seed, thereby increasing drought or desiccation tolerance of the seed.

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55. A method of reactivating one or more desiccated and inactivated proteins, comprising resuspending the desiccated and inactivated protein in a solution comprising:

(a) at least one protective polypeptide selected from the group consisting of a ubiquitin polypeptide, a negative regulator of flagellin synthesis (Flg M) polypeptide, a SRC Homology 3 (SH3) Domain polypeptide, small ubiquitin-like modifier (SUMO) polypeptide, a serum albumin polypeptide, and a tardigrade disordered polypeptide (TDP), and any combination thereof.

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56. The method of claim 54 or claim 55, wherein the concentration of the at least one protective polypeptide is about 1 g/L to about 300 g/L, optionally greater than 1 g/L, greater than 5 g/L.

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57. The method of claim 54 or claim 55, wherein the mass ratio of the one or more desiccated and inactivated proteins to the at least one protective polypeptide is about 1:1000 to about 1:1, optionally about 1:100 to about 1:10 or about 1:20 to about 1:10

20

58. The method of any one of claims 54 to 57, wherein the ubiquitin polypeptide comprises an amino acid sequence having at least about 70% identity to any one of **SEQ ID NOs:7-30**; or the ubiquitin polypeptide is encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:7-30**; and/or any combination thereof.

25

59. The method of any one of claims 54 to 58, wherein the SH3 domain comprises an amino acid sequence having at least about 70% identity to any one of **SEQ ID NOs:31-53**; or the SH3 domain is encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:31-53**; and/or any combination thereof.

30

60. The method of any one of claims 54 to 59, wherein the Flg M polypeptide comprises an amino acid sequence having at least about 70% identity to any one of **SEQ ID NOs:54-64**; or the Flg M polypeptide is encoded by a nucleotide sequence encoding an amino acid sequence

having at least about 70% identity to any of **SEQ ID NOs:54-64**; and/or any combination thereof.

61. The method of any one of claims 54 to 60, wherein the SUMO polypeptide comprises an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:65-86**; or the SUMO polypeptide is encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:65-86**; and/or any combination thereof.

62. The method of any one of claims 54 to 61, wherein the is serum albumin polypeptide comprises an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:87-104**; or the serum albumin polypeptide is encoded by a nucleotide sequence encoding an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:87-104**; and/or any combination thereof.

63. The method of any one of claims 55 to 62, wherein the at least one TDP is selected from the group consisting of amino acid sequences having at least about 70% identity to any of **SEQ ID NOs:104-208**; amino acid sequences encoded by a nucleotide sequence having at least about 70% identity to any of **SEQ ID NOs:209-313**; amino acid sequences encoded by a nucleotide sequence having at least about 70% identity to any of **SEQ ID NO:314-418**; and any combination thereof.

64. The method of any one of claims 55 to 62, wherein the at least one TDP is selected from the group of amino acid sequences consisting or an amino acid sequence having at least about 70% identity to any of **SEQ ID NOs:121, 123, 136, 139, and/or 148**; an amino acid sequence encoded by a nucleotide sequence having at least about 70% identity to any one of **SEQ ID NOs:226, 228, 241, 244, and/or 247**; or an amino acid sequence encoded by a nucleotide sequence having at least about 70% identity to any one of **SEQ ID NOs:331, 333, 346, 349 and/or 352**; or any combination thereof.

65. The method of any one of claims 55 to 64, wherein at least one of the one or more desiccated and inactivated proteins is a therapeutic agent.

66. The method of claim 65, wherein the therapeutic agent is a protein based vaccine, an antibody, an enzyme, hormone, and/or a globular protein.

67. The method of claim 65 or claim 66, wherein the therapeutic agent is in a mixture.

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68. The method of any one of claims 55 to 67, wherein the one or more desiccated and inactivated proteins are part of a protein-based food.

Fig. 1

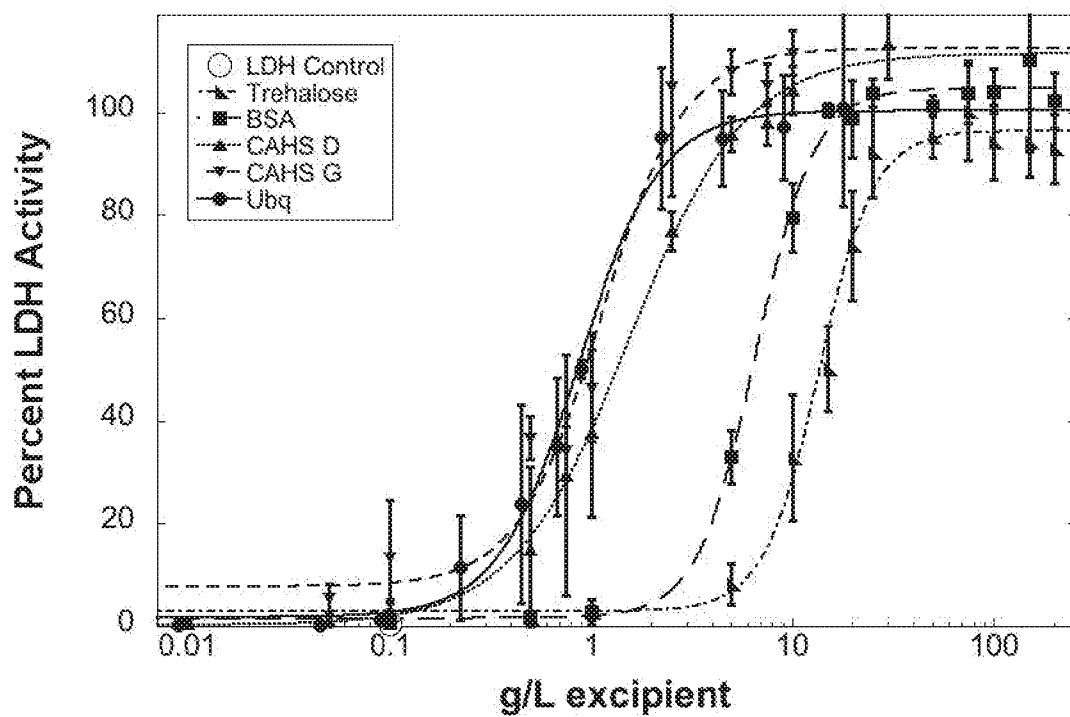


Fig. 2

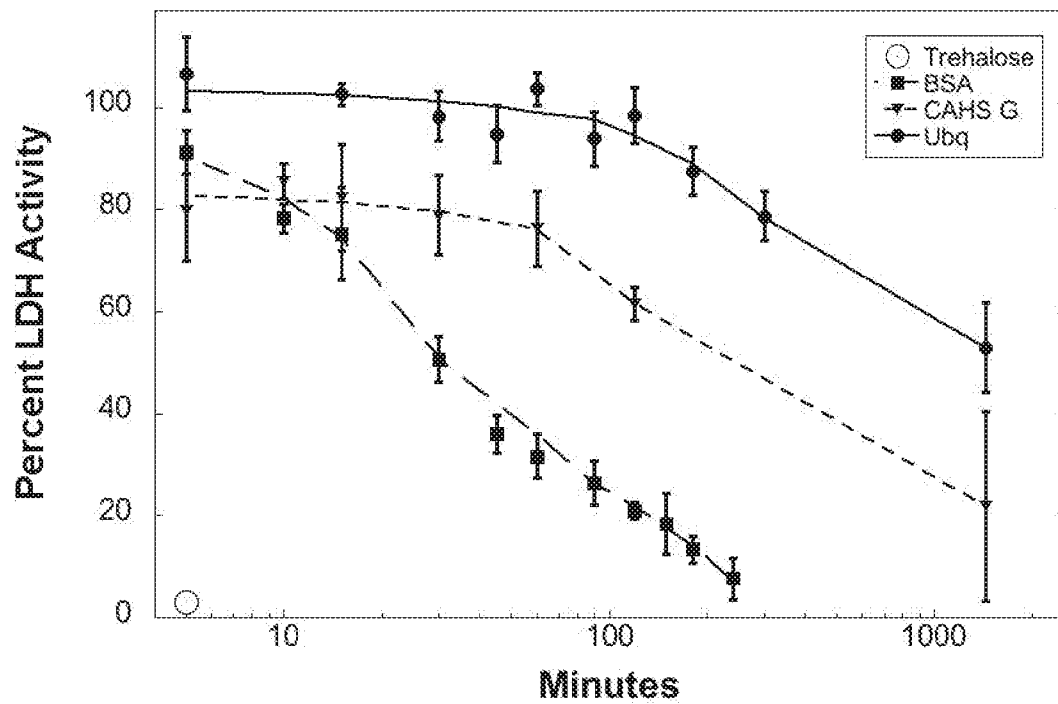


Fig. 3

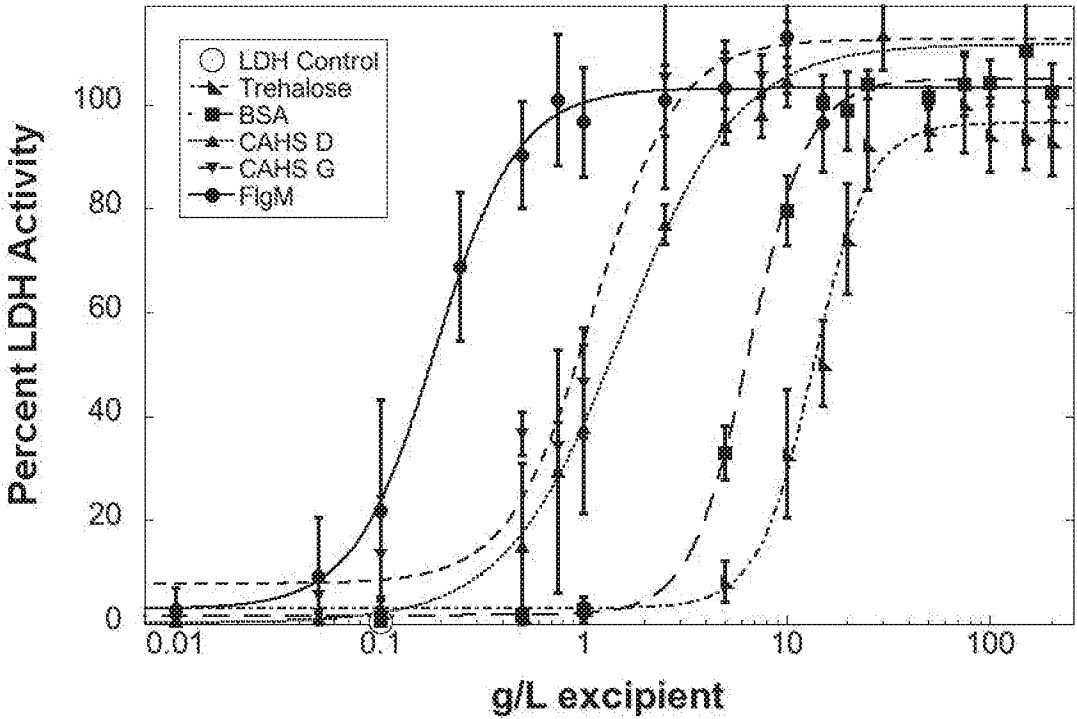


Fig. 4

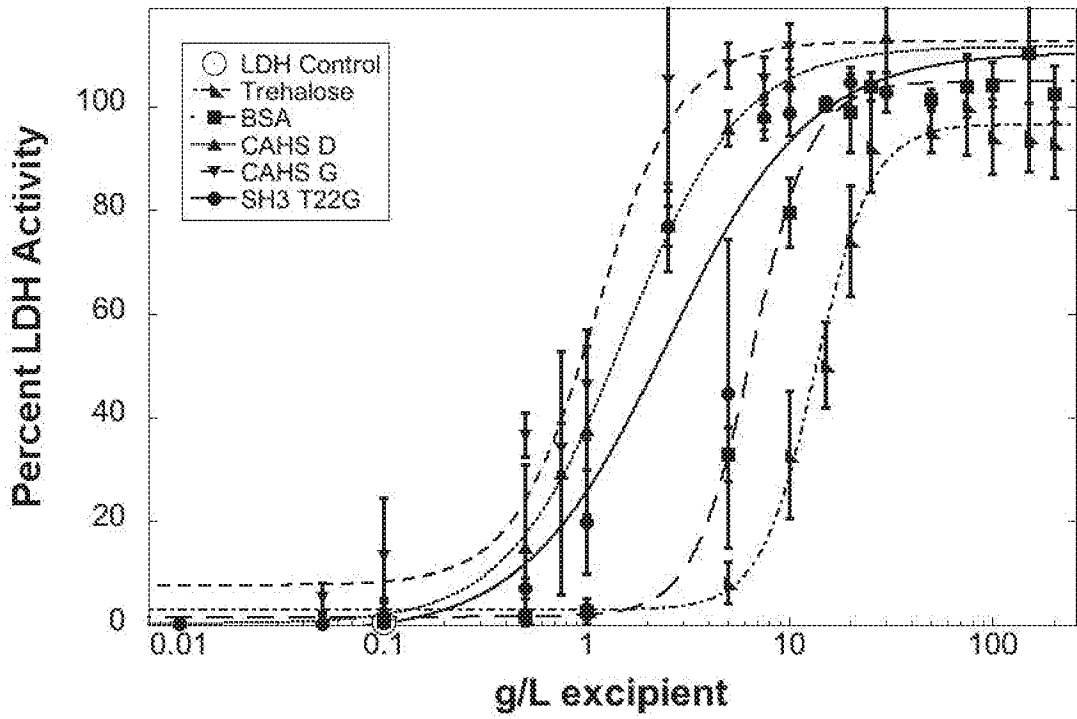


Fig. 5

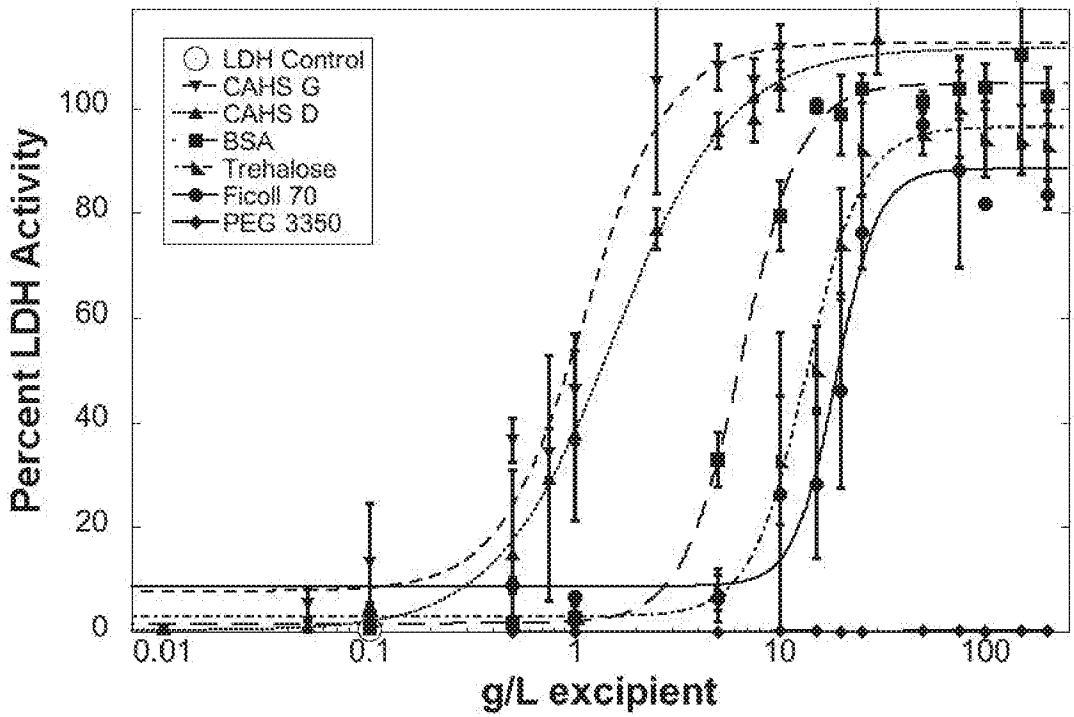
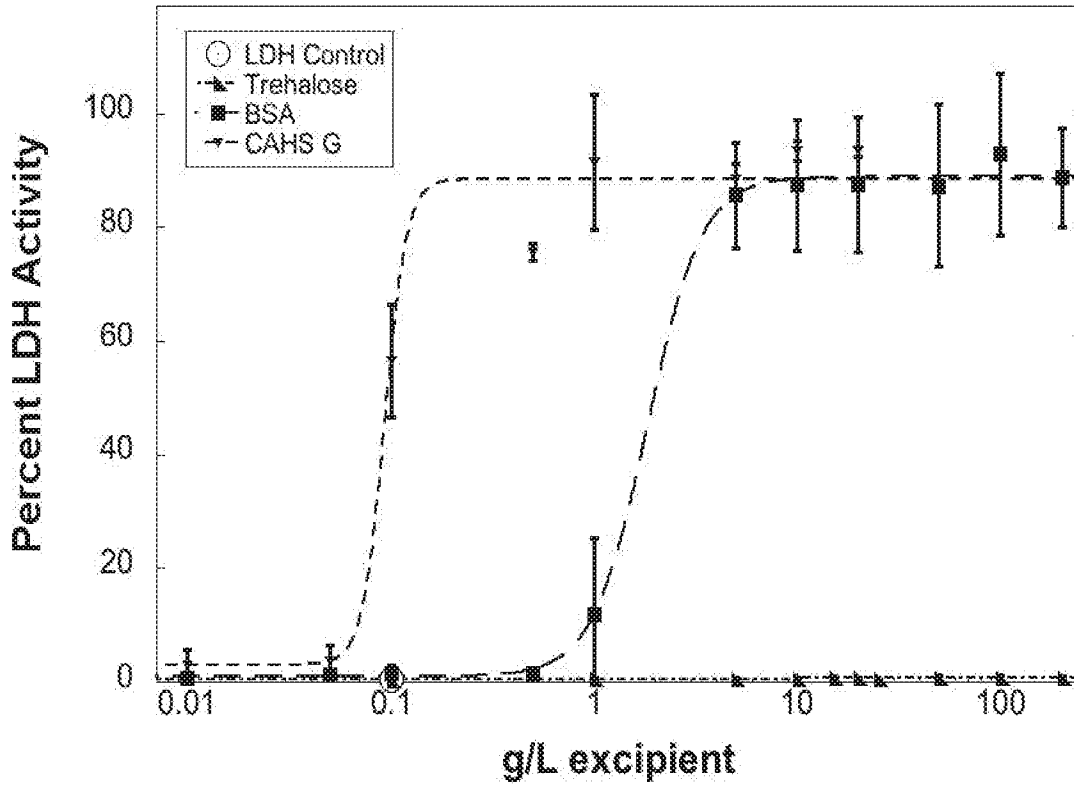


Fig. 6



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2018/060879

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A01N 1/02; A61K 9/08; A61K 9/19; A61K 35/12; A61K 47/26; A61K 47/42; C12N 1/04 (2019.01)

CPC - A01N 1/02; A01N 1/0205; A01N 1/021; A01N 1/0221; A61K 35/12; A61K 39/39591 (2019.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 34/285; 424/400; 424/489; 424/499; 435/260; 435/374; 435/1.3; 435/1.1; 436/8 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2017/0198058 A1 (AMGEN INC.) 13 July 2017 (13.07.2017) entire document	1-3, 21-24, 55-57
X	WO 1997/01627 A1 (IGEN, INC.) 16 January 1997 (16.01.1997) entire document	1, 4
X	US 2014/0356948 A1 (TEGO SCIENCE INC.) 04 December 2014 (04.12.2014) entire document	26-28
X	CN 103356706 A (STEMNION LLC) 23 October 2013 (23.10.2013) entire document; see machine translation	32-35
X	Li et al. "Heterologous expression of OsSIZ1, a rice SUMO E3 ligase, enhances broad abiotic stress tolerance in transgenic creeping bentgrass," Plant Biotechnology Journal, 11 December 2012 (11.12.2012), Vol. 11, Pgs. 432-445. entire document	32, 33, 36-40
X	US 2008/0148432 A1 (ABAD) 19 June 2008 (19.06.2008) entire document	47-49
A	BOOTHBY et al. "Tardigrades Use Intrinsically Disordered Proteins to Survive Desiccation," Mol Cell, 16 March 2017 (16.03.2017), Vol. 65, Pgs. 975-984. entire document	1-4, 21-24, 26-28, 32-40, 47-49, 54-57
A	US 2013/0281314 A1 (GENENTECH, INC.) 24 October 2013 (24.10.2013) entire document	1-4, 21-24, 26-28, 32-40, 47-49, 54-57
A	US 2011/0197316 A1 (LUO et al) 11 August 2011 (11.08.2011) entire document	1-4, 21-24, 26-28, 32-40, 47-49, 54-57

☒ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

24 January 2019

Date of mailing of the international search report

06 FEB 2019

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450

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Blaine R. Copenheaver

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2018/060879

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 5-20, 25, 29-31, 41-46, 50-53, 58-68
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2018/060879

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,773,705 A (VIERSTRA et al) 30 June 1998 (30.06.1998) entire document	1-4, 21-24, 26-28, 32-40, 47-49, 54-57