

MODIFICATION OF PLANT MESSENGER PACKS

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in
 5 ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on June 13,
 2022, is named 51296-052WO2_Sequence_Listing_6_13_22_ST25 and is 1,718 bytes in size.

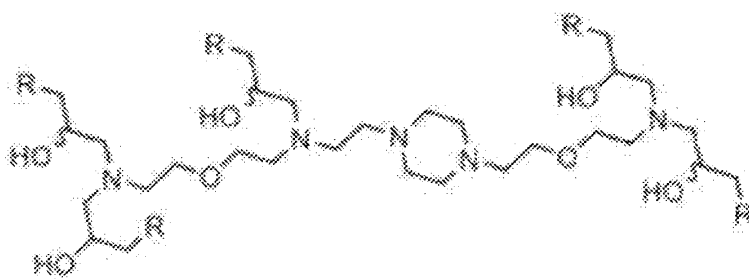
BACKGROUND

Delivery of heterologous functional agents (e.g., agricultural or therapeutic agents) can be limited
 10 by the degree to which the agent can penetrate cell barriers and thereby effectively act on an organism.
 For example, the barrier formed by the plant cell wall, bacterial cell wall, or fungal cell wall, or by the cell
 membrane and/or extracellular matrix of an animal cell, poses a challenge to cellular uptake of agents
 useful in agriculture or therapeutic applications. Therefore, there is a need in the art for methods and
 compositions promoting cellular uptake of agents and for methods of manufacturing plant messenger
 15 packs comprising heterologous functional agents.

SUMMARY OF THE INVENTION

In one aspect, provided herein is a composition comprising a plant messenger pack (PMP)
 modified to comprise a synthetic charged lipid, wherein the synthetic charged lipid has one or more of the
 20 following characteristics: (i) at least 2 ionizable amines; (ii) at least 3 lipid tails; wherein each of the lipid
 tails is at least 6 carbon atoms in length; (iii) a pKa of about 4.5 to about 7.5; (iv) an ionizable amine and a
 heteroorganic group separated by a chain of at least two atoms; and (v) an N:P ratio of at least 10;
 provided that the charged lipid is not selected from 1'-((2-(4-(2-(2-(bis(2-hydroxydodecyl)amino)ethyl) (2-
 hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethyl)azanediyl)bisdodecan-2-ol (C12-200), MD1 (cKK-E12),
 25 OF2, EPC, ZA3-Ep10, TT3, LP01, 5A2-SC8, Lipid 5 (Moderna), and 98N12-5.

In another aspect, provided herein is a composition comprising a PMP modified to comprise a
 synthetic charged lipid, wherein the PMP is modified by reconstituting a lipid film comprising purified PMP
 lipids in the presence of a synthetic charged lipid, thereby producing a modified PMP that comprises the
 synthetic charged lipid, wherein the synthetic charged lipid is represented by the following formula I:



(I)

30

where R is a C8-C14 alkyl group (e.g., a C12-14 alkyl group).

In some embodiments, the modified PMP further comprises a sterol.

In some embodiments, the modified PMP further comprises a PEGylated lipid.

In some embodiments, the modified PMP further comprises a sterol and a PEGylated lipid.

In some embodiments, the sterol is cholesterol or sitosterol.

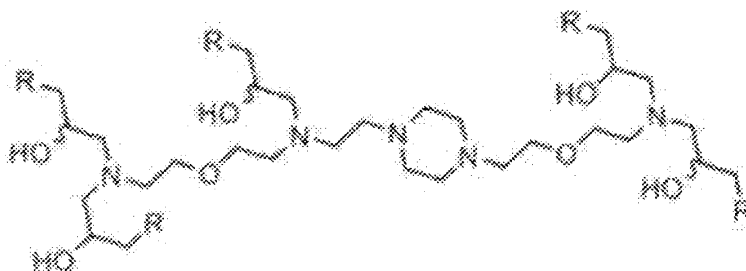
In some embodiments, the PEGylated lipid is C18-PEG2k, DMPE-PEG2k, or ALC-0159.

5 In some embodiments, the synthetic charged lipid, purified PMP lipids, sterol, and PEGylated lipid comprise about 30%-75%, about 35%-50%, about 10%-20%, and about 1%-3%, respectively, of the lipids in the modified PMP.

In some embodiments, the synthetic charged lipid, purified PMP lipids, sterol, and PEGylated lipid comprise about 25%-75%, about 35%-60%, about 10%-20%, and about 0.5%-5%, respectively, of the
10 lipids in the modified PMP.

In some embodiments, the synthetic charged lipid, purified PMP lipids, sterol, and PEGylated lipid are formulated at a molar ratio of about 35:50:12.5:2.5.

In some embodiments, the synthetic charged lipid is represented by the following formula I:



(I)

15 where R is a C8-C14 alkyl group (e.g., a C12-14 alkyl group).

In some embodiments, a lipid membrane of the modified PMPs comprises at least 35% of the lipid of formula I.

In some embodiments, the synthetic charged lipid is an ionizable lipid and the composition has a zeta potential of greater than -40 mV at pH 4 when in the absence of cargo. In some embodiments, the
20 composition has a zeta potential of greater than 0 mV at pH 4 when in the absence of cargo. In some embodiments, the composition has a zeta potential of greater than 20 mV at pH 4 when in the absence of cargo. In some embodiments, the composition has a zeta potential of greater than 30 mV at pH 4 when in the absence of cargo. In some embodiments, the composition has a zeta potential of about 40 mV at pH 4 when in the absence of cargo.

25 In some embodiments, the composition has a zeta potential of greater than -30 mV when in the absence of cargo. In some embodiments, the composition has a zeta potential of greater than -20 mV when in the absence of cargo. In some embodiments, the composition has a zeta potential of greater than -5 mV when in the absence of cargo. In some embodiments, the composition has a zeta potential of greater than 0 mV when in the absence of cargo. In some embodiments, the composition has a zeta
30 potential of about 30 mV when in the absence of cargo.

In some embodiments, the modified PMPs comprise a heterologous functional agent. In some embodiments, the heterologous functional agent is encapsulated by the modified PMPs. In some

embodiments, the heterologous functional agent is embedded on the surface of the modified PMPs. In some embodiments, the heterologous functional agent is conjugated to the surface of the modified PMPs.

In some embodiments, the heterologous functional agent is a polynucleotide. In some embodiments, the polynucleotide is chosen from an mRNA, an siRNA or siRNA precursor, a microRNA (miRNA) or miRNA precursor, a plasmid, a Dicer substrate small interfering RNA (dsiRNA), a short hairpin RNA (shRNA), an asymmetric interfering RNA (aiRNA), a peptide nucleic acid (PNA), a morpholino, a locked nucleic acid (LNA), a piwi-interacting RNA (piRNA), a ribozyme, a deoxyribozyme (DNAzyme), an aptamer, a circular RNA (circRNA), a guide RNA (gRNA), an ADAR targeting oligonucleotide, an antisense oligonucleotide, a long non-coding RNA, a ceDNA, a minicircle, a miniplasmid, or a DNA molecule encoding any of these RNAs. In some embodiments, the polynucleotide is chosen from an mRNA, an siRNA or siRNA precursor, a miRNA or miRNA precursor, a plasmid, a dsiRNA, a shRNA, an aiRNA, a LNA, a piRNA, a ribozyme, a DNAzyme, an aptamer, a circRNA, a gRNA, an ADAR targeting oligonucleotide, an antisense oligonucleotide, a long non-coding RNA, a ceDNA, a minicircle, or a miniplasmid, or a DNA molecule encoding any of these RNAs.

In some embodiments, the polynucleotide is an mRNA.

In some embodiments, the polynucleotide is an siRNA or a precursor thereof.

In some embodiments, the polynucleotide is a plasmid.

In some embodiments, the encapsulation efficiency of the polynucleotide by the modified PMP is at least 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or more than 99%.

In some embodiments, the modified PMPs are lipid reconstructed PMPs (LPMPs).

In some embodiments, the LPMP is produced by a method comprising lipid extrusion.

In some embodiments, the LPMP is produced by a method comprising processing a solution comprising a lipid extract of the PMPs in a microfluidics device comprising an aqueous phase, thereby producing the LPMPs.

In some embodiments, the aqueous phase comprises a heterologous functional agent.

In some embodiments, the modified PMPs have increased cell uptake. In some embodiments, the increased cell uptake is an increased cell uptake of at least 1%, 2%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% relative to the cell uptake of the unmodified PMP.

In some embodiments, the cell is a mammalian cell. In some embodiments, the mammalian cell is a human cell.

In some embodiments, the cell is a plant cell.

In some embodiments, the cell is a bacterial cell.

In some embodiments, the cell is a fungal cell.

In another aspect, provided herein is a method for delivering a PMP to a target cell, the method comprising contacting the target cell with a composition that comprises a PMP comprising a PMP modified to comprise a synthetic charged lipid, wherein the synthetic charged lipid has one or more of the following characteristics: (i) at least 2 ionizable amines; (ii) at least 3 lipid tails; wherein each of the lipid tails is at least 6 carbon atoms in length; (iii) a pKa of about 4.5 to about 7.5; (iv) an ionizable amine and a heteroorganic group separated by a chain of at least two atoms; and (v) an N:P ratio of at least 10;

provided that the charged lipid is not selected from 1'-((2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl) (2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethyl)azanediyl)bis(dodecan-2-ol) (C12-200), MD1 (cKK-E12), OF2, EPC, ZA3-Ep10, TT3, LP01, 5A2-SC8, Lipid 5 (Moderna), and 98N12-5.

In some embodiments, the PMP further comprises a sterol. In some embodiments, the PMP further comprises a PEGylated lipid. In some embodiments, the PMP further comprises a sterol and a PEGylated lipid. In some embodiments, the sterol is cholesterol or sitosterol. In some embodiments, the PEGylated lipid is C18-PEG2k, DMPE-PEG2k, or ALC-0159.

In some embodiments, the synthetic charged lipid, purified PMP lipids, sterol, and PEGylated lipid comprise about 30%-75%, about 35%-50%, about 10%-20%, and about 1%-3%, respectively, of the lipids in the modified PMP.

In some embodiments, the synthetic charged lipid, purified PMP lipids, sterol, and PEGylated lipid comprise about 25%-75%, about 35%-60%, about 10%-20%, and about 0.5%-5%, respectively, of the lipids in the modified PMP.

In some embodiments, the synthetic charged lipid, purified PMP lipids, sterol, and PEGylated lipid are formulated at a molar ratio of 35:50:12.5:2.5.

In some embodiments, the synthetic charged lipid is an ionizable lipid and the composition comprising the PMP has a zeta potential of greater than -40 mV at pH 4 when in the absence of cargo. In some embodiments, the composition comprising the PMP has a zeta potential of greater than 0 mV at pH 4 when in the absence of cargo. In some embodiments, the composition comprising the PMP has a zeta potential of greater than 20 mV at pH 4 when in the absence of cargo. In some embodiments, the composition comprising the PMP has a zeta potential of greater than 30 mV at pH 4 when in the absence of cargo. In some embodiments, the composition comprising the PMP has a zeta potential of about 40 mV at pH 4 when in the absence of cargo.

In some embodiments, the composition comprising the PMP has a zeta potential of greater than -30 mV when in the absence of cargo. In some embodiments, the composition comprising the PMP has a zeta potential of greater than -20 mV when in the absence of cargo. In some embodiments, the composition comprising the PMP has a zeta potential of greater than -5 mV when in the absence of cargo. In some embodiments, the composition comprising the PMP has a zeta potential of greater than 0 mV when in the absence of cargo. In some embodiments, the composition comprising the PMP has a zeta potential of about 30 mV when in the absence of cargo.

In some embodiments, the PMP comprises a heterologous functional agent. In some embodiments, the heterologous functional agent is encapsulated by the PMP. In some embodiments, the heterologous functional agent is embedded on the surface of the PMP. In some embodiments, the heterologous functional agent is conjugated to the surface of the PMP.

In some embodiments, the heterologous functional agent is a polynucleotide. In some embodiments, the polynucleotide is chosen from an mRNA, an siRNA or siRNA precursor, a miRNA or miRNA precursor, a plasmid, a dsRNA, a shRNA, an aiRNA, a PNA, a morpholino, a LNA, a piRNA, a ribozyme, a DNAzyme, an aptamer, a circRNA, a gRNA, an ADAR targeting oligonucleotide, an antisense oligonucleotide, a long non-coding RNA, a ceDNA, a minicircle, a miniplasmid, or a DNA molecule encoding any of these RNAs. In some embodiments, the polynucleotide is chosen from an mRNA, an

siRNA or siRNA precursor, a miRNA or miRNA precursor, a plasmid, a dsRNA, a shRNA, an aiRNA, a LNA, a piRNA, a ribozyme, a DNAzyme, an aptamer, a circRNA, a gRNA, an ADAR targeting oligonucleotide, an antisense oligonucleotide, a long non-coding RNA, a ceDNA, a minicircle, or a miniplasmid, or a DNA molecule encoding any of these RNAs.

5 In some embodiments, the polynucleotide is an mRNA.

In some embodiments, the polynucleotide is an siRNA or a precursor thereof.

In some embodiments, the polynucleotide is a plasmid.

In some embodiments, the encapsulation efficiency of the polynucleotide by the PMP is at least 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or more than 99%.

10 In some embodiments, the PMP is a lipid reconstructed PMP (LPMP).

In some embodiments, the PMP has increased cell uptake. In some embodiments, the increased cell uptake is an increased cell uptake of at least 1%, 2%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% relative to the cell uptake of the unmodified PMP.

15 In some embodiments, the cell is a mammalian cell. In some embodiments, the mammalian cell is a human cell.

In some embodiments, the cell is a plant cell.

In some embodiments, the cell is a bacterial cell.

In some embodiments, the cell is a fungal cell.

20 In another aspect, provided herein is a method of increasing the fitness of a plant, the method comprising delivering to the plant an effective amount of any one of the compositions provided herein, wherein the method increases the fitness of the plant relative to an untreated plant.

In some embodiments, the modified PMP comprises an agricultural agent.

In some embodiments, the plant is a plant of agricultural or horticultural importance.

In some embodiments, the plant is a soybean plant, a wheat plant, or a corn plant.

25 In another aspect, provided herein is a method of decreasing the fitness of a plant, the method comprising delivering to the plant an effective amount of any one of the compositions provided herein, wherein the method decreases the fitness of the plant relative to an untreated plant.

In some embodiments, the modified PMP comprises an agricultural agent.

In some embodiments, the plant is a weed.

30 In some embodiments, the composition is delivered to a leaf, seed, embryo, ovule, meristem, microspore, root, fruit, shoot, pollen, or flower of the plant.

In another aspect, provided herein is a method of increasing the fitness of a mammal, the method comprising delivering to the mammal an effective amount of any one of the compositions provided herein, wherein the method increases the fitness of the mammal relative to an untreated mammal.

35 In some embodiments, the modified PMP comprises a heterologous therapeutic agent. In some embodiments, the mammal is a human.

40 In another aspect, provided herein is a composition comprising a plurality of lipid reconstructed PMPs (LPMPs), wherein the LPMPs are produced by a process comprising the steps of: (a) providing a plurality of purified PMPs; (b) processing the plurality of PMPs to produce a lipid film; (c) reconstituting the lipid film in an organic solvent, wherein the organic solvent is dimethylformamide:methanol (DMF:MeOH),

- thereby producing a lipid solution; and (d) processing the lipid solution of step (c) in a microfluidics device comprising an aqueous phase, thereby producing the LPMPs; wherein the LPMPs comprise a synthetic charged lipid having one or more of the following characteristics: (i) at least ionizable amines; (ii) at least 3 lipid tails; wherein each of the lipid tails is at least 6 carbon atoms in length; (iii) a pKa of about 4.5 to about 7.5; (iv) an ionizable amine and a heteroorganic group separated by a chain of at least two atoms; and (v) an N:P ratio of at least 10; provided that the charged lipid is not selected from 1'-((2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl) (2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethyl)azanediyl)bisdodecan-2-ol) (C12-200), MD1 (cKK-E12), OF2, EPC, ZA3-Ep10, TT3, LP01, 5A2-SC8, Lipid 5 (Moderna), and 98N12-5.
- 15 In some embodiments, the charged lipid is added to the preparation prior to step (b).
 In some embodiments, the aqueous phase comprises a citrate buffer having a pH of about 3.2.
 In some embodiments, aqueous phase and the lipid solution are mixed at a 3:1 volumetric ratio.
 In some embodiments, the LPMPs comprise a heterologous functional agent. In some embodiments, the heterologous functional agent is a polynucleotide. In some embodiments, the
- 20 polynucleotide is chosen from an mRNA, an siRNA or siRNA precursor, a miRNA or miRNA precursor, a plasmid, a dsRNA, a shRNA, an aiRNA, a PNA, a morpholino, a LNA, a piRNA, a ribozyme, a DNAzyme, an aptamer, a circRNA, a gRNA, an ADAR targeting oligonucleotide, an antisense oligonucleotide, a long non-coding RNA, a ceDNA, a minicircle, a miniplasmid, or a DNA molecule encoding any of these RNAs. In some embodiments, the polynucleotide is chosen from an mRNA, an siRNA or siRNA precursor, a
- 25 miRNA or miRNA precursor, a plasmid, a dsRNA, a shRNA, an aiRNA, a LNA, a piRNA, a ribozyme, a DNAzyme, an aptamer, a circRNA, a gRNA, an ADAR targeting oligonucleotide, an antisense oligonucleotide, a long non-coding RNA, a ceDNA, a minicircle, or a miniplasmid, or a DNA molecule encoding any of these RNAs. In some embodiments, the heterologous functional agent is comprised by the aqueous phase.
- 30 In some embodiments, the LPMPs further comprise a sterol. In some embodiments, the LPMPs further comprise a PEGylated lipid. In some embodiments, the LPMPs further comprise a sterol and a PEGylated lipid.
 In some embodiments, the sterol is cholesterol or sitosterol.
 In some embodiments, the PEGylated lipid is C18-PEG2k, DMPE-PEG2k, or ALC-0159.
- 35 In another aspect, provided herein is a method for making LPMPs, the method comprising: (a) providing a plurality of purified PMPs; (b) processing the plurality of PMPs to produce a lipid film; (c) reconstituting the lipid film in an organic solvent, wherein the organic solvent is dimethylformamide:methanol (DMF:MeOH), thereby producing a lipid solution; and (d) processing the lipid solution of step (c) in a microfluidics device comprising an aqueous phase, thereby producing the LPMPs; wherein the LPMPs comprise a synthetic charged lipid having one or more of the following characteristics: (i) at least 2 ionizable amines; (ii) at least 3 lipid tails; wherein each of the lipid tails is at least 6 carbon atoms in length; (iii) a pKa of about 4.5 to about 7.5; (iv) an ionizable amine and a heteroorganic group separated by a chain of at least two atoms; and (v) an N:P ratio of at least 10; provided that the charged lipid is not selected from 1'-((2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl) (2-

hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethyl)azanediyl)bis(dodecan-2-ol) (C12-200), MD1 (cKK-E12), OF2, EPC, ZA3-Ep10, TT3, LP01, 5A2-SC8, Lipid 5 (Moderna), and 98N12-5.

In some embodiments, the charged lipid is added to the preparation prior to step (b).

In some embodiments, the aqueous phase comprises water, PBS, or a citrate buffer.

5 In some embodiments, the aqueous phase and the lipid solution are mixed at a 3:1 volumetric ratio.

In some embodiments, the LPMPs comprise a heterologous functional agent. In some embodiments, the heterologous functional agent is a polynucleotide. In some embodiments, the polynucleotide is chosen from an mRNA, an siRNA or siRNA precursor, a miRNA or miRNA precursor, a
10 plasmid, a dsRNA, a shRNA, an aiRNA, a PNA, a morpholino, a LNA, a piRNA, a ribozyme, a DNAzyme, an aptamer, a circRNA, a gRNA, an ADAR targeting oligonucleotide, an antisense oligonucleotide, a long non-coding RNA, a ceDNA, a minicircle, a miniplasmid, or a DNA molecule encoding any of these RNAs. In some embodiments, the polynucleotide is chosen from an mRNA, an siRNA or siRNA precursor, a
15 miRNA or miRNA precursor, a plasmid, a dsRNA, a shRNA, an aiRNA, a LNA, a piRNA, a ribozyme, a DNAzyme, an aptamer, a circRNA, a gRNA, an ADAR targeting oligonucleotide, an antisense oligonucleotide, a long non-coding RNA, a ceDNA, a minicircle, or a miniplasmid, or a DNA molecule encoding any of these RNAs. In some embodiments, the heterologous functional agent is comprised by the aqueous phase.

In some embodiments, the LPMPs further comprise a sterol. In some embodiments, the LPMPs
20 further comprise a PEGylated lipid. In some embodiments, the LPMPs further comprise a sterol and a PEGylated lipid.

In some embodiments, the sterol is cholesterol or sitosterol.

In some embodiments, the PEGylated lipid is C18-PEG2k, DMPE-PEG2k, or ALC-0159.

In some embodiments, the synthetic charged lipid is characterized as having at least 3 ionizable
25 amines. In some embodiments, the synthetic charged lipid is characterized as having at least 4 ionizable amines. In some embodiments, the synthetic charged lipid is characterized as having at least 5 ionizable amines. In some embodiments, the synthetic charged lipid is characterized as having at least 6 ionizable amines.

In some embodiments, the synthetic charged lipid is characterized as having at least 4 lipid tails.
30 In some embodiments, the synthetic charged lipid is characterized as having at least 5 lipid tails. In some embodiments, the synthetic charged lipid is characterized as having at least 6 lipid tails. In some embodiments, each lipid tail is independently 6-18 carbon atoms in length.

In some embodiments, the pKa is about 6.5 to about 7.5.

In some embodiments, the heteroorganic group is hydroxyl.

35 In some embodiments, the synthetic charged lipid has at least two of the characteristics. In some embodiments, the synthetic charged lipid has at least three of the characteristics. In some embodiments, the synthetic charged lipid has four or five of the characteristics. In some embodiments, the synthetic charged lipid has all of the characteristics.

In some embodiments, the heteroorganic group comprises a hydrogen bond donor.

40 In some embodiments, the heteroorganic group comprises a hydrogen bond acceptor.

In some embodiments, the heteroorganic group is -OH, -SH, -(CO)H, -CO₂H, -NH₂, -CONH₂, optionally substituted C₁-C₆ alkoxy, or fluorine.

In some embodiments, the synthetic charged lipid is a lipid presented in Table 18.

Other features and advantages of the invention will be apparent from the following Detailed Description and the Claims.

Definitions

As used herein, an "agriculturally acceptable" carrier or excipient is one that is suitable for use in agriculture, e.g., for use on plants. In certain embodiments, the agriculturally acceptable carrier or excipient does not have undue adverse side effects to the plants, the environment, or to humans or animals who consume the resulting agricultural products derived therefrom commensurate with a reasonable benefit/risk ratio.

As used herein, "delivering" or "contacting" refers to applying to a plant, animal, fungus, or bacterium, a PMP composition either directly on the plant, animal, fungus, or bacterium, or adjacent to the plant, animal, fungus, or bacterium, in a region where the composition is effective to alter the fitness of the plant, animal, fungus, or bacterium. In methods where the composition is directly contacted with a plant, animal, fungus, or bacterium, the composition may be contacted with the entire plant, animal, fungus, or bacterium or with only a portion of the plant, animal, fungus, or bacterium.

As used herein, "decreasing the fitness of a plant" refers to any disruption of the physiology of a plant (e.g., a weed) as a consequence of administration of a composition described herein (e.g., a PMP composition including modified PMPs, optionally including a heterologous functional agent), including, but not limited to, decreasing a population of a plant (e.g., a weed) by about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, 100% or more. A decrease in plant fitness can be determined in comparison to a plant to which the composition has not been administered.

As used herein, the term "effective amount," "effective concentration," or "concentration effective to" refers to an amount of a modified PMP, or a heterologous functional agent therein, sufficient to effect the recited result or to reach a target level (e.g., a predetermined or threshold level) in or on a target organism.

As used herein, "increasing the fitness of a plant" refers to an increase in the production of the plant, for example, an improved yield, improved vigor of the plant, or improved quality of the harvested product from the plant as a consequence of administration of a composition described herein (e.g., a PMP composition including modified PMPs, optionally including a heterologous functional agent). An improved yield of a plant relates to an increase in the yield of a product (e.g., as measured by plant biomass, grain, seed or fruit yield, protein content, carbohydrate or oil content or leaf area) of the plant by a measurable amount over the yield of the same product of the plant produced under the same conditions, but without the application of the instant compositions or compared with application of conventional agricultural agents. For example, yield can be increased by at least about 0.5%, about 1%, about 2%, about 3%, about 4%, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, or more than 100%. Yield can be expressed in terms of an amount by weight or volume of the plant or a product of the plant on some

basis. The basis can be expressed in terms of time, growing area, weight of plants produced, or amount of a raw material used. An increase in the fitness of plant can also be measured by other means, such as an increase or improvement of the vigor rating, increase in the stand (the number of plants per unit of area), increase in plant height, increase in stalk circumference, increase in plant canopy, improvement in appearance (such as greener leaf color as measured visually), improvement in root rating, increase in seedling emergence, protein content, increase in leaf size, increase in leaf number, fewer dead basal leaves, increase in tiller strength, decrease in nutrient or fertilizer requirements, increase in seed germination, increase in tiller productivity, increase in flowering, increase in seed or grain maturity or seed maturity, fewer plant verse (lodging), increased shoot growth, or any combination of these factors, by a measurable or noticeable amount over the same factor of the plant produced under the same conditions, but without the administration of the instant compositions or with application of conventional agricultural agents.

As used herein, the term “heterologous” refers to an agent that is either (1) exogenous to the plant (e.g., originating from a source that is not the plant from which the PMP is produced) or (2) endogenous to the plant from which the PMP is produced, but is present in the PMP (e.g., using loading, genetic engineering, *in vitro* or *in vivo* approaches) at a concentration that is higher than that found in nature (e.g., as found in a naturally-occurring plant extracellular vesicle).

As used herein, the term “functional agent” refers to an agent (e.g., an agricultural agent (e.g., pesticidal agent, fertilizing agent, herbicidal agent, plant-modifying agent) or a therapeutic agent (e.g., an antifungal agent, an antibacterial agent, a virucidal agent, an anti-viral agent, an insecticidal agent, a nematocidal agent, an antiparasitic agent, or an insect repellent)) that is or can be associated with PMPs (e.g., loaded into or onto PMPs (e.g., encapsulated by, embedded in, or conjugated to PMPs)) using *in vivo* or *in vitro* methods and is capable of effecting the recited result (e.g., increasing or decreasing the fitness of a plant, plant pest, plant symbiont, animal (e.g., human) pathogen, or animal pathogen vector) in accordance with the present compositions or methods. In some aspects, the functional agent is a polynucleotide.

As used herein, the term “agricultural agent” refers to an agent that can act on a plant, a plant pest, or a plant symbiont, such as a pesticidal agent, pest repellent, fertilizing agent, plant-modifying agent, or plant-symbiont modifying agent.

As used herein, the term “fertilizing agent” refers to an agent that is capable of increasing the fitness of a plant (e.g., a plant nutrient or a plant growth regulator) or a plant symbiont (e.g., a nucleic acid or a peptide).

As used herein, the term “pesticidal agent” refers to an agent, composition, or substance therein, that controls or decreases the fitness (e.g., kills or inhibits the growth, proliferation, division, reproduction, or spread) of an agricultural, environmental, or domestic/household pest, such as an insect, mollusk, nematode, fungus, bacterium, weed, or virus. Pesticides are understood to include naturally occurring or synthetic insecticides (larvicides or adulticides), insect growth regulators, acaricides (miticides), molluscicides, nematocides, ectoparasiticides, bactericides, fungicides, or herbicides. The term “pesticidal agent” may further encompass other bioactive molecules such as antibiotics, antivirals pesticides, antifungals, antihelminthics, nutrients, and/or agents that stun or slow insect movement.

As used herein, the term “plant-modifying agent” refers to an agent that can alter the genetic properties (e.g., increase gene expression, decrease gene expression, or otherwise alter the nucleotide sequence of DNA or RNA), epigenetic properties, or biochemical properties of a plant in a manner that results in a change (e.g., increase or decrease) in plant fitness.

5 As used herein, the term “therapeutic agent” refers to an agent that can act on an animal, e.g., a mammal (e.g., a human), an animal pathogen, or a pathogen vector, such as an antifungal agent, an antibacterial agent, a virucidal agent, an anti-viral agent, an insecticidal agent, a nematocidal agent, an antiparasitic agent, or an insect repellent.

As used herein, the term “formulated for delivery to a plant” refers to a PMP composition that includes an agriculturally acceptable carrier. As used herein, an “agriculturally acceptable” carrier or excipient is one that is suitable for use in agriculture without undue adverse side effects to the plants, the environment, or to humans or animals who consume the resulting agricultural products derived therefrom commensurate with a reasonable benefit/risk ratio. Non-limiting examples of agriculturally acceptable carriers or excipients are known in the art; see, e.g., the Compendium of Herbicide Adjuvants,
10 ppp[dot]purdue[dot]edu/wp-content/uploads/2016/11/PPP-115[dot]pdf.

As defined herein, the term “nucleic acid” and “polynucleotide” are interchangeable and refer to RNA or DNA that is linear or branched, single or double stranded, or a hybrid thereof, regardless of length (e.g., at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 150, 200, 250, 500, 1000, or more nucleic acids). The term also encompasses RNA/DNA hybrids. Nucleotides are typically linked in a nucleic acid
20 by phosphodiester bonds, although the term “nucleic acid” also encompasses nucleic acid analogs having other types of linkages or backbones (e.g., phosphoramidate, phosphorothioate, phosphorodithioate, O-methylphosphoroamidate, morpholino, locked nucleic acid (LNA), glycerol nucleic acid (GNA), threose nucleic acid (TNA), and peptide nucleic acid (PNA) linkages or backbones, among others). The nucleic acids may be single-stranded, double-stranded, or contain portions of both single-stranded and double-
25 stranded sequence. A nucleic acid can contain any combination of deoxyribonucleotides and ribonucleotides, as well as any combination of bases, including, for example, adenine, thymine, cytosine, guanine, uracil, and modified or non-canonical bases (including, e.g., hypoxanthine, xanthine, 7-methylguanine, 5,6-dihydrouracil, 5-methylcytosine, and 5 hydroxymethylcytosine).

As used herein, the term “pest” refers to organisms that cause damage to plants or other
30 organisms, are present where they are not wanted, or otherwise are detrimental to humans, for example, by negatively impacting human agricultural methods or products. Pests may include, for example, invertebrates (e.g., insects, nematodes, or mollusks), microorganisms (e.g., phytopathogens, endophytes, obligate parasites, facultative parasites, or facultative saprophytes), such as bacteria, fungi, or viruses; or weeds.

As used herein, the term “pesticidal agent” or “pesticide” refers to an agent, composition, or
35 substance therein, that controls or decreases the fitness (e.g., kills or inhibits the growth, proliferation, division, reproduction, or spread) of an agricultural, environmental, or domestic/household pest, such as an insect, mollusk, nematode, fungus, bacterium, weed, or virus. Pesticides are understood to encompass naturally occurring or synthetic insecticides (larvicides or adulticides), insect growth
40 regulators, acaricides (miticides), molluscicides, nematocides, ectoparasiticides, bactericides, fungicides,

or herbicides. The term "pesticidal agent" may further encompass other bioactive molecules such as antibiotics, antivirals pesticides, antifungals, antihelminthics, nutrients, and/or agents that stun or slow insect movement.

The pesticidal agent may be heterologous. As used herein, the term "heterologous" refers to an agent (e.g., a polynucleotide or a pesticidal agent) that is either (1) exogenous to the plant (e.g., originating from a source that is not the plant or plant part from which the PMP is produced) (e.g., added the PMP using loading approaches described herein) or (2) endogenous to the plant cell or tissue from which the PMP is produced, but present in the PMP (e.g., added to the PMP using loading approaches described herein, genetic engineering, *in vitro* or *in vivo* approaches) at a concentration that is higher than that found in nature (e.g., higher than a concentration found in a naturally-occurring plant extracellular vesicle).

As used herein, the term "repellent" refers to an agent, composition, or substance therein, that deters pests from approaching or remaining on a plant. A repellent may, for example, decrease the number of pests on or in the vicinity of a plant, but may not necessarily kill or decrease the fitness of the pest.

As used herein, the term "peptide," "protein," or "polypeptide" encompasses any chain of naturally or non-naturally occurring amino acids (either D- or L-amino acids), regardless of length (e.g., at least 2, 3, 4, 5, 6, 7, 10, 12, 14, 16, 18, 20, 25, 30, 40, 50, 100, or more amino acids), the presence or absence of post-translational modifications (e.g., glycosylation or phosphorylation), or the presence of, e.g., one or more non-amino acyl groups (for example, sugar, lipid, etc.) covalently linked to the peptide, and includes, for example, natural proteins, synthetic, or recombinant polypeptides and peptides, hybrid molecules, peptoids, or peptidomimetics.

As used herein, "percent identity" between two sequences is determined by the BLAST 2.0 algorithm, which is described in Altschul et al., (1990) *J. Mol. Biol.* 215:403-410. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information.

As used herein, the term "plant" refers to whole plants, plant organs, plant tissues, seeds, plant cells, seeds, and progeny of the same. Plant cells include, without limitation, cells from seeds, suspension cultures, embryos, meristematic regions, callus tissue, leaves, roots, shoots, gametophytes, sporophytes, pollen, and microspores. Plant parts include differentiated and undifferentiated tissues including, but not limited to the following: roots, stems, shoots, leaves, pollen, seeds, fruit, harvested produce, tumor tissue, sap (e.g., xylem sap and phloem sap), and various forms of cells and culture (e.g., single cells, protoplasts, embryos, and callus tissue). The plant tissue may be in a plant or in a plant organ, tissue, or cell culture.

As used herein, the term "modified PMPs" refers to a composition including a plurality of PMPs, wherein the PMPs include one or more heterologous agents (e.g., one or more exogenous lipids, such as a synthetic charged lipid (e.g., an ionizable and/or cationic lipid, e.g., a PMP comprising a synthetic charged lipid and a sterol and/or a PEGylated lipid) capable of increasing cell uptake (e.g., animal cell uptake, plant cell uptake, bacterial cell uptake, or fungal cell uptake) of the PMP, or a portion or component thereof (e.g., a heterologous functional agent carried by the PMP), relative to an unmodified PMP; capable of enabling or increasing delivery of a heterologous functional agent (e.g., an agricultural or

therapeutic agent) by the PMP to a cell, and/or capable of enabling or increasing loading (e.g., loading efficiency or loading capacity) of a heterologous functional agent (e.g., an agricultural or therapeutic agent). The PMPs may be modified *in vitro* or *in vivo*.

As used herein, the term “unmodified PMPs” refers to a composition including a plurality of PMPs that lack a heterologous cell uptake agent capable of increasing cell uptake (e.g., animal cell uptake, plant cell uptake, bacterial cell uptake, or fungal cell uptake) of the PMP.

As used herein, the term “cell uptake” refers to uptake of a PMP or a portion or component thereof (e.g., a heterologous functional agent carried by the PMP) by a cell, such as an animal cell, a plant cell, bacterial cell, or fungal cell. For example, uptake can involve transfer of the PMP or a portion of component thereof from the extracellular environment into or across the cell membrane, the cell wall, the extracellular matrix, or into the intracellular environment of the cell). Cell uptake of PMPs may occur via active or passive cellular mechanisms. Cell uptake includes aspects in which the entire PMP (e.g., LPMP) is taken up by a cell, e.g., taken up by endocytosis. In embodiments, one or more heterologous functional agents (e.g., polynucleotides) are exposed to the cytoplasm of the target cell following endocytosis and endosomal escape. In embodiments, a modified PMP (e.g., a PMP comprising a synthetic charged lipid (e.g., ionizable lipid and/or cationic lipid), e.g., a PMP comprising a synthetic charged lipid and a sterol and/or a PEGylated lipid) has an increased rate of endosomal escape relative to an unmodified PMP. Cell uptake also includes aspects in which the PMP (e.g., LPMP) fuses with the membrane of the target cell. In embodiments, one or more heterologous functional agents (e.g., polynucleotides) are exposed to the cytoplasm of the target cell following membrane fusion. In embodiments, a modified PMP (e.g., a PMP comprising a synthetic charged lipid (e.g., an ionizable lipid and/or cationic lipid), e.g., a PMP comprising a synthetic charged lipid and a sterol and/or a PEGylated lipid) has an increased rate of fusion with the membrane of the target cell (e.g., is more fusogenic) relative to an unmodified PMP.

As used herein, the term “cell-penetrating agent” refers to agents that alter properties (e.g., permeability) of the cell wall, extracellular matrix, or cell membrane of a cell (e.g., an animal cell, a plant cell, a bacterial cell, or a fungal cell) in a manner that promotes increased cell uptake relative to a cell that has not been contacted with the agent.

As used herein, the term “plant extracellular vesicle”, “plant EV”, or “EV” refers to an enclosed lipid-bilayer structure naturally occurring in a plant. Optionally, the plant EV includes one or more plant EV markers. As used herein, the term “plant EV marker” refers to a component that is naturally associated with a plant, such as a plant protein, a plant nucleic acid, a plant small molecule, a plant lipid, or a combination thereof. In some instances, the plant EV marker is an identifying marker of a plant EV but is not a pesticidal agent. In some instances, the plant EV marker is an identifying marker of a plant EV and also a pesticidal agent (e.g., either associated with or encapsulated by the plurality of PMPs, or not directly associated with or encapsulated by the plurality of PMPs).

As used herein, the term “plant messenger pack” or “PMP” refers to a lipid structure (e.g., a lipid bilayer, unilamellar, multilamellar structure; e.g., a vesicular lipid structure), that is about 5-2000 nm (e.g., at least 5-1000 nm, at least 5-500 nm, at least 400-500 nm, at least 25-250 nm, at least 50-150 nm, or at least 70-120 nm) in diameter that is derived from (e.g., enriched, isolated or purified from) a plant source

or segment, portion, or extract thereof, including lipid or non-lipid components (e.g., peptides, nucleic acids, or small molecules) associated therewith and that has been enriched, isolated or purified from a plant, a plant part, or a plant cell, the enrichment or isolation removing one or more contaminants or undesired components from the source plant. PMPs may be highly purified preparations of naturally occurring EVs. Preferably, at least 1% of contaminants or undesired components from the source plant are removed (e.g., at least 2%, 5%, 10%, 15%, 20%, 25%, 30%, 40%, 45%, 50%, 55%, 60%, 70%, 80%, 90%, 95%, 96%, 98%, 99%, or 100%) of one or more contaminants or undesired components from the source plant, e.g., plant cell wall components; pectin; plant organelles (e.g., mitochondria; plastids such as chloroplasts, leucoplasts or amyloplasts; and nuclei); plant chromatin (e.g., a plant chromosome); or plant molecular aggregates (e.g., protein aggregates, protein-nucleic acid aggregates, lipoprotein aggregates, or lipido-proteic structures). Preferably, a PMP is at least 30% pure (e.g., at least 40% pure, at least 50% pure, at least 60% pure, at least 70% pure, at least 80% pure, at least 90% pure, at least 99% pure, or 100% pure) relative to the one or more contaminants or undesired components from the source plant as measured by weight (w/w), spectral imaging (% transmittance), or conductivity (S/m).

In some instances, the PMP is a lipid reconstructed PMP (LPMP). As used herein, the terms "lipid reconstructed PMP" and "LPMP" refer to a PMP that has been derived from a lipid structure (e.g., a lipid bilayer, unilamellar, multilamellar structure; e.g., a vesicular lipid structure) derived from (e.g., enriched, isolated or purified from) a plant source, wherein the lipid structure is disrupted (e.g., disrupted by lipid extraction) and reassembled or reconstituted in a liquid phase (e.g., a liquid phase containing a cargo) using standard methods, e.g., reconstituted by a method comprising lipid film hydration and/or solvent injection, to produce the LPMP, as is described herein. The method may, if desired, further comprise sonication, freeze/thaw treatment, and/or lipid extrusion, e.g., to reduce the size of the reconstituted PMPs. Alternatively, LPMPs may be produced using a microfluidic device (such as a NANOASSEMBLER® IGNITE™ microfluidic instrument (Precision NanoSystems)). A PMP (e.g., a LPMP) may comprise between 10% and 100% lipids derived from the lipid structure from the plant source, e.g., may contain at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or 100% lipids derived from the lipid structure from the plant source. A PMP (e.g., a LPMP) may comprise all or a fraction of the lipid species present in the lipid structure from the plant source, e.g., it may contain at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100% of the lipid species present in the lipid structure from the plant source. A PMP (e.g., a LPMP) may comprise none, a fraction, or all of the protein species present in the lipid structure from the plant source, e.g., may contain 0%, less than 1%, less than 5%, less than 10%, less than 15%, less than 20%, less than 30%, less than 40%, less than 50%, less than 60%, less than 70%, less than 80%, less than 90%, less than 100%, or 100% of the protein species present in the lipid structure from the plant source. In some instances, the lipid bilayer of the PMP (e.g., LPMP) does not contain proteins. In some instances, the lipid structure of the PMP (e.g., LPMP) contains a reduced amount of proteins relative to the lipid structure from the plant source.

PMPs (e.g., LPMPs) may optionally include exogenous lipids, e.g., lipids that are exogenous to the plant (e.g., originating from a source that is not the plant or plant part from which the PMP is produced) (e.g., added the PMP using methods described herein). The lipid composition of the PMP may

include 0%, less than 1%, or at least 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more than 95% exogenous lipid. Exemplary exogenous lipids include synthetic charged lipids (e.g., ionizable and/or cationic lipids). The exogenous lipid may be a cell-penetrating agent, may be capable of increasing delivery of a heterologous functional agent (e.g., an agricultural or therapeutic agent) by the PMP to a cell, and/or may be capable of increasing loading (e.g., loading efficiency or loading capacity) of a heterologous functional agent (e.g., an agricultural or therapeutic agent). Further exemplary exogenous lipids include sterols and PEGylated lipids.

PMPs may optionally include additional agents, such as heterologous functional agents, e.g., cell-penetrating agents, pesticidal agents, fertilizing agents, plant-modifying agents, therapeutic agents, polynucleotides, polypeptides, or small molecules. The PMPs can carry or associate with additional agents (e.g., heterologous functional agents) in a variety of ways to enable delivery of the agent to a target plant, e.g., by encapsulating the agent, incorporation of the agent in the lipid bilayer structure, or association of the agent (e.g., by conjugation) with the surface of the lipid bilayer structure. Heterologous functional agents can be incorporated into the PMPs either in vivo (e.g., in planta) or in vitro (e.g., in tissue culture, in cell culture, or synthetically incorporated).

As used herein, the term “charged lipid” refers to an amphiphilic molecule (e.g., a lipid or a lipidoid, e.g., a synthetic lipid or lipidoid) containing a group (e.g., a head group) that is charged (e.g., is cationic) or that can be ionized under a given condition (e.g., pH) to produce one or more electrically charged species. Charged lipids include ionizable lipids and cationic lipids. In some embodiments, the charged lipid is a positively charged lipid.

As used herein, the term “ionizable lipid” refers to an amphiphilic molecule (e.g., a lipid or a lipidoid, e.g., a synthetic lipid or lipidoid) containing a group (e.g., a head group) that can be ionized, e.g., dissociated to produce one or more electrically charged species, under a given condition (e.g., pH).

As used herein, the term “cationic lipid” refers to an amphiphilic molecule (e.g., a lipid or a lipidoid, e.g., a synthetic lipid or lipidoid) containing a cationic group (e.g., a cationic head group).

As used herein, the term “lipidoid” refers to a molecule having one or more characteristics of a lipid.

As used herein, the term “stable PMP composition” (e.g., a composition including loaded or non-loaded PMPs) refers to a PMP composition that over a period of time (e.g., at least 24 hours, at least 48 hours, at least 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 30 days, at least 60 days, or at least 90 days) retains at least 5% (e.g., at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%) of the initial number of PMPs (e.g., PMPs per mL of solution) relative to the number of PMPs in the PMP composition (e.g., at the time of production or formulation) optionally at a defined temperature range (e.g., a temperature of at least 24°C (e.g., at least 24°C, 25°C, 26°C, 27°C, 28°C, 29°C, or 30°C), at least 20°C (e.g., at least 20°C, 21°C, 22°C, or 23°C), at least 4°C (e.g., at least 5°C, 10°C, or 15°C), at least -20°C (e.g., at least -20°C, -15°C, -10°C, -5°C, or 0°C), or -80°C (e.g., at least -80°C, -70°C, -60°C, -50°C, -40°C, or -30°C)); or retains at least 5% (e.g., at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%) of its activity (e.g., cell wall penetrating activity and/or pesticidal and/or repellent activity) relative to the initial activity of the PMP (e.g., at the time of production

or formulation) optionally at a defined temperature range (e.g., a temperature of at least 24°C (e.g., at least 24°C, 25°C, 26°C, 27°C, 28°C, 29°C, or 30°C), at least 20°C (e.g., at least 20°C, 21°C, 22°C, or 23°C), at least 4°C (e.g., at least 5°C, 10°C, or 15°C), at least -20°C (e.g., at least -20°C, -15°C, -10°C, -5°C, or 0°C), or -80°C (e.g., at least -80°C, -70°C, -60°C, -50°C, -40°C, or -30°C)).

5 As used herein, the term “formulated for delivery to an animal” refers to a PMP composition that includes a pharmaceutically acceptable carrier. As used herein, a “pharmaceutically acceptable” carrier or excipient is one that is suitable for administration to an animal (e.g., human), e.g., without undue adverse side effects to the animal (e.g., human).

10 As used herein, the term “untreated” refers to a plant, animal, fungus, or bacterium that has not been contacted with or delivered a PMP composition herein, including a separate plant, animal, fungus, or bacterium that has not been delivered the PMP composition, the same plant, animal, fungus, or bacterium undergoing treatment assessed at a time point prior to delivery of the PMP composition, or the same plant, animal, fungus, or bacterium undergoing treatment assessed at an untreated part of the plant, animal, fungus, or bacterium.

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BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1A is a graph showing the size (nm) and polydispersity index (PDI) of plant messenger packs (PMPs) comprising the lipid species 1'-((2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl) (2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethyl)azanediyl)bis(dodecan-2-ol) (C12-200), DLin-MC3-DMA (MC3), Lipid 5 (Moderna), or cKK-E12 (MD1) and an mRNA cargo encoding firefly luciferase (Fluc) and erythropoietin (EPO).

Fig. 1B is a graph showing the mRNA encapsulation efficiency (%) of PMPs comprising the lipid species C12-200, MC3, Lipid 5, or cKK-E12 and an mRNA cargo encoding Fluc and EPO.

25 **Fig. 2** is a set of photographs showing fluorescence of Fluc in whole mice that were intravenously injected with PMPs comprising the lipid species C12-200, MC3, Lipid 5, or cKK-E12 and an mRNA cargo encoding Fluc and EPO.

Fig. 3 is a set of photographs (1 second exposure) showing fluorescence of Fluc in dissected organs of mice that were intravenously injected with PMPs comprising the lipid species C12-200, MC3, Lipid 5, or cKK-E12 and an mRNA cargo encoding Fluc and EPO.

30 **Fig. 4** is a set of photographs (1 minute exposure) showing fluorescence of Fluc in dissected organs of mice that were intravenously injected with PMPs comprising the lipid species C12-200, MC3, Lipid 5, or cKK-E12 and an mRNA cargo encoding Fluc and EPO.

Fig. 5 is a set of photographs (5 minute exposure) showing fluorescence of Fluc in dissected organs of mice that were intravenously injected with PMPs comprising the lipid species C12-200, MC3, Lipid 5, or cKK-E12 and an mRNA cargo encoding Fluc and EPO.

35 **Fig. 6** is a set of photographs showing fluorescence of Fluc in whole mice that were intravenously injected with lipid nanoparticles (LNPs) or PMPs comprising PMP lipids derived from lemon, grapefruit (GF), lime, orange, or algae and an mRNA cargo encoding Fluc and EPO.

Fig. 7 is a set of photographs (1 second exposure) showing fluorescence of Fluc in dissected organs of mice that were intravenously injected with LNPs or PMPs comprising PMP lipids derived from lemon, grapefruit (GF), lime, orange, or algae and an mRNA cargo encoding Fluc and EPO.

Fig. 8 is a set of photographs (1 minute exposure) showing fluorescence of Fluc in dissected organs of mice that were intravenously injected with LNPs or PMPs comprising PMP lipids derived from lemon, grapefruit (GF), lime, orange, or algae and an mRNA cargo encoding Fluc and EPO.

Fig. 9 is a set of photographs (5 minute exposure) showing fluorescence of Fluc in dissected organs of mice that were intravenously injected with LNPs or PMPs comprising PMP lipids derived from lemon, grapefruit (GF), lime, orange, or algae and an mRNA cargo encoding Fluc and EPO.

Fig. 10 is a bar graph showing radiance (p/s/sq. cm/sr) of Fluc in organs of mice that were intravenously injected with LNPs or PMPs comprising PMP lipids derived from lemon, grapefruit (GF), lime, orange, or algae and an mRNA cargo encoding Fluc and EPO. MLN: mesenteric lymph nodes.

Fig. 11 is a bar graph showing transfection efficiency (relative luminescence units (RLU)) in HeLa cells treated with PMPs comprising an ionizable lipid (C12-200), a structural lipid (Lonestar grapefruit, Sunkist grapefruit, broccoli, ginger, or algae PMP lipids), a sterol (cholesterol), and a PEG-lipid (DMPE-PEG2k), wherein the structural lipids are at a 50% molar ratio. Compositions were delivered at 200 ng or 400 ng per well. Results for particles comprising DOPE as a structural lipid are also shown.

Fig. 12A is a bar graph showing viability of MCF-7 cells treated with the PMP and LNP compositions of Table 14 (shown as a percentage relative to untreated cells). Compositions were delivered at 200 ng per well. Cells treated with Lipofectamine (Thermo Scientific) are shown as a control.

Fig. 12B is a bar graph showing transfection efficiency (luciferase expression) in MCF-7 cells treated with the PMP and LNP compositions of Table 14. Compositions were delivered at 200 ng per well. Cells treated with Lipofectamine (Thermo Scientific) are shown as a control.

Fig. 13A is a graph showing the size (nm) and PDI of PMPs comprising PMP lipids from algae, Lonestar grapefruit, Sunkist grapefruit, broccoli, or ginger formulated according to Table 16.

Fig. 13B is a graph showing the cargo encapsulation efficiency (%) of PMPs and LNPs formulated according to Table 16.

Fig. 14 is a bar graph showing the size (nm) and PDI of PMPs comprising an ionizable lipid (C12-200 or MC3), broccoli PMP lipids (broccoli BiTS), a sterol (cholesterol), and a PEG-lipid (DMPE-PEG2k) formulated at the ratios shown.

Fig. 15 is a scatter plot showing the percent cholesterol included in PMP compositions comprising C12-200 or MC3 and the percent encapsulation of a cargo by the PMPs.

Fig. 16 is a bar graph showing the encapsulation efficiency of a cargo by PMPs comprising an ionizable lipid (C12-200 or MC3), broccoli PMP lipids (broccoli BiTS), a sterol (cholesterol), and a PEG-lipid (DMPE-PEG2k) formulated at the ratios shown.

Fig. 17 is a bar graph showing the level of luciferase expression (RLU) in HeLa cells contacted with PMPs comprising an ionizable lipid (C12-200 or MC3), broccoli PMP lipids (broccoli BiTS), a sterol (cholesterol), and a PEG-lipid (DMPE-PEG2k) formulated at the ratios shown, wherein the PMPs comprise a cargo encoding the luciferase.

Fig. 18 is a bar graph showing the level of luciferase expression (RLU) in HeLa cells that have been contacted with PMPs or LNPs formulated according to Table 16. GFL: Lonestar grapefruit; GFS: Sunkist grapefruit.

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DETAILED DESCRIPTION

Featured herein are modified plant messenger packs (PMPs). PMPs are lipid assemblies produced wholly or in part from plant extracellular vesicles (EVs), or segments, portions, or extracts thereof. PMPs can optionally include additional agents (e.g., heterologous functional agents, (e.g., a heterologous agricultural agent (e.g., pesticidal agent, fertilizing agent, herbicidal agent, plant-modifying agent) or a heterologous therapeutic agent (e.g., an antifungal agent, an antibacterial agent, a virucidal agent, an anti-viral agent, an insecticidal agent, a nematocidal agent, an antiparasitic agent, or an insect repellent)). The modified PMPs and related compositions and methods described herein can be used in a variety of agricultural and therapeutic methods.

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I. Modified Plant Messenger Pack Compositions

The PMP compositions described herein include a plurality of modified plant messenger packs (PMPs). A PMP is a lipid (e.g., lipid bilayer, unilamellar, or multilamellar structure) structure that includes a plant EV, or segment, portion, or extract (e.g., lipid extract) thereof. Plant EVs refer to an enclosed lipid-bilayer structure that naturally occurs in a plant and that is about 5-2000 nm in diameter. Plant EVs can originate from a variety of plant biogenesis pathways. In nature, plant EVs can be found in the intracellular and extracellular compartments of plants, such as the plant apoplast, the compartment located outside the plasma membrane and formed by a continuum of cell walls and the extracellular space. Alternatively, PMPs can be enriched plant EVs found in cell culture media upon secretion from plant cells. Plant EVs can be separated from plants, thereby providing PMPs, by a variety of methods further described herein. Further, the PMPs can optionally include a heterologous functional agent, (e.g., a heterologous agricultural agent (e.g., pesticidal agent, fertilizing agent, herbicidal agent, plant-modifying agent) or a heterologous therapeutic agent (e.g., a cell-penetrating agent, an antifungal agent, an antibacterial agent, a virucidal agent, an anti-viral agent, an insecticidal agent, a nematocidal agent, an antiparasitic agent, or an insect repellent)), which can be introduced *in vivo* or *in vitro*. In some aspects, the heterologous functional agent is at least one nucleic acid (e.g., a DNA or an RNA, e.g., a mRNA or an siRNA) or a small molecule.

PMPs can include plant EVs, or segments, portions, or extracts, thereof. Optionally, PMPs can also include exogenous lipids (e.g., sterols (e.g., cholesterol or sitosterol), synthetic charged lipids (e.g., ionizable and/or cationic lipids), and/or PEGylated lipids) in addition to lipids derived from plant EVs. In some embodiments, the plant EVs are about 5-1000 nm in diameter. For example, the PMP can include a plant EV, or segment, portion, or extract thereof, that has a mean diameter of about 5-50 nm, about 50-100 nm, about 100-150 nm, about 150-200 nm, about 200-250 nm, about 250-300 nm, about 300-350 nm, about 350-400 nm, about 400-450 nm, about 450-500 nm, about 500-550 nm, about 550-600 nm, about 600-650 nm, about 650-700 nm, about 700-750 nm, about 750-800 nm, about 800-850 nm, about 850-900 nm, about 900-950 nm, about 950-1000nm, about 1000-1250nm, about 1250-1500nm, about

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1500-1750nm, or about 1750-2000nm. In some instances, the PMP includes a plant EV, or segment, portion, or extract thereof, that has a mean diameter of about 5-950 nm, about 5-900 nm, about 5-850 nm, about 5-800 nm, about 5-750 nm, about 5-700 nm, about 5-650 nm, about 5-600 nm, about 5-550 nm, about 5-500 nm, about 5-450 nm, about 5-400 nm, about 5-350 nm, about 5-300 nm, about 5-250 nm, about 5-200 nm, about 5-150 nm, about 5-100 nm, about 5-50 nm, or about 5-25 nm. In certain instances, the plant EV, or segment, portion, or extract thereof, has a mean diameter of about 50-200 nm. In certain instances, the plant EV, or segment, portion, or extract thereof, has a mean diameter of about 50-300 nm. In certain instances, the plant EV, or segment, portion, or extract thereof, has a mean diameter of about 200-500 nm. In certain instances, the plant EV, or segment, portion, or extract thereof, has a mean diameter of about 30-150 nm.

In some instances, the PMP may include a plant EV, or segment, portion, or extract thereof, that has a mean diameter of at least 5 nm, at least 50 nm, at least 100 nm, at least 150 nm, at least 200 nm, at least 250 nm, at least 300 nm, at least 350 nm, at least 400 nm, at least 450 nm, at least 500 nm, at least 550 nm, at least 600 nm, at least 650 nm, at least 700 nm, at least 750 nm, at least 800 nm, at least 850 nm, at least 900 nm, at least 950 nm, or at least 1000 nm. In some instances, the PMP includes a plant EV, or segment, portion, or extract thereof, that has a mean diameter less than 1000 nm, less than 950 nm, less than 900 nm, less than 850 nm, less than 800 nm, less than 750 nm, less than 700 nm, less than 650 nm, less than 600 nm, less than 550 nm, less than 500 nm, less than 450 nm, less than 400 nm, less than 350 nm, less than 300 nm, less than 250 nm, less than 200 nm, less than 150 nm, less than 100 nm, or less than 50 nm. A variety of methods (e.g., a dynamic light scattering method) standard in the art can be used to measure the particle diameter of the plant EV, or segment, portion, or extract thereof.

In some instances, the PMP may include a plant EV, or segment, portion, or extract thereof, that has a mean surface area of 77 nm^2 to $3.2 \times 10^6 \text{ nm}^2$ (e.g., $77\text{-}100 \text{ nm}^2$, $100\text{-}1000 \text{ nm}^2$, $1000\text{-}1 \times 10^4 \text{ nm}^2$, $1 \times 10^4\text{-}1 \times 10^5 \text{ nm}^2$, $1 \times 10^5\text{-}1 \times 10^6 \text{ nm}^2$, or $1 \times 10^6\text{-}3.2 \times 10^6 \text{ nm}^2$). In some instances, the PMP may include a plant EV, or segment, portion, or extract thereof, that has a mean volume of 65 nm^3 to $5.3 \times 10^8 \text{ nm}^3$ (e.g., $65\text{-}100 \text{ nm}^3$, $100\text{-}1000 \text{ nm}^3$, $1000\text{-}1 \times 10^4 \text{ nm}^3$, $1 \times 10^4\text{-}1 \times 10^5 \text{ nm}^3$, $1 \times 10^5\text{-}1 \times 10^6 \text{ nm}^3$, $1 \times 10^6\text{-}1 \times 10^7 \text{ nm}^3$, $1 \times 10^7\text{-}1 \times 10^8 \text{ nm}^3$, $1 \times 10^8\text{-}5.3 \times 10^8 \text{ nm}^3$). In some instances, the PMP may include a plant EV, or segment, portion, or extract thereof, that has a mean surface area of at least 77 nm^2 , (e.g., at least 77 nm^2 , at least 100 nm^2 , at least 1000 nm^2 , at least $1 \times 10^4 \text{ nm}^2$, at least $1 \times 10^5 \text{ nm}^2$, at least $1 \times 10^6 \text{ nm}^2$, or at least $2 \times 10^6 \text{ nm}^2$). In some instances, the PMP may include a plant EV, or segment, portion, or extract thereof, that has a mean volume of at least 65 nm^3 (e.g., at least 65 nm^3 , at least 100 nm^3 , at least 1000 nm^3 , at least $1 \times 10^4 \text{ nm}^3$, at least $1 \times 10^5 \text{ nm}^3$, at least $1 \times 10^6 \text{ nm}^3$, at least $1 \times 10^7 \text{ nm}^3$, at least $1 \times 10^8 \text{ nm}^3$, at least $2 \times 10^8 \text{ nm}^3$, at least $3 \times 10^8 \text{ nm}^3$, at least $4 \times 10^8 \text{ nm}^3$, or at least $5 \times 10^8 \text{ nm}^3$).

In some instances, the PMP can have the same size as the plant EV or segment, extract, or portion thereof. Alternatively, the PMP may have a different size than the initial plant EV from which the PMP is produced. For example, the PMP may have a diameter of about 5-2000 nm in diameter. For example, the PMP can have a mean diameter of about 5-50 nm, about 50-100 nm, about 100-150 nm, about 150-200 nm, about 200-250 nm, about 250-300 nm, about 300-350 nm, about 350-400 nm, about 400-450 nm, about 450-500 nm, about 500-550 nm, about 550-600 nm, about 600-650 nm, about 650-

700 nm, about 700-750 nm, about 750-800 nm, about 800-850 nm, about 850-900 nm, about 900-950 nm, about 950-1000 nm, about 1000-1200 nm, about 1200-1400 nm, about 1400-1600 nm, about 1600 – 1800 nm, or about 1800 – 2000 nm. In some instances, the PMP may have a mean diameter of at least 5 nm, at least 50 nm, at least 100 nm, at least 150 nm, at least 200 nm, at least 250 nm, at least 300 nm, at least 350 nm, at least 400 nm, at least 450 nm, at least 500 nm, at least 550 nm, at least 600 nm, at least 650 nm, at least 700 nm, at least 750 nm, at least 800 nm, at least 850 nm, at least 900 nm, at least 950 nm, at least 1000 nm, at least 1200 nm, at least 1400 nm, at least 1600 nm, at least 1800 nm, or about 2000 nm. A variety of methods (e.g., a dynamic light scattering method) standard in the art can be used to measure the particle diameter of the PMPs. In some instances, the size of the PMP is determined following loading of heterologous functional agents, or following other modifications to the PMPs.

In some instances, the PMP may have a mean surface area of 77 nm^2 to $1.3 \times 10^7 \text{ nm}^2$ (e.g., $77\text{--}100 \text{ nm}^2$, $100\text{--}1000 \text{ nm}^2$, $1000\text{--}1 \times 10^4 \text{ nm}^2$, $1 \times 10^4\text{--}1 \times 10^5 \text{ nm}^2$, $1 \times 10^5\text{--}1 \times 10^6 \text{ nm}^2$, or $1 \times 10^6\text{--}1.3 \times 10^7 \text{ nm}^2$). In some instances, the PMP may have a mean volume of 65 nm^3 to $4.2 \times 10^9 \text{ nm}^3$ (e.g., $65\text{--}100 \text{ nm}^3$, $100\text{--}1000 \text{ nm}^3$, $1000\text{--}1 \times 10^4 \text{ nm}^3$, $1 \times 10^4\text{--}1 \times 10^5 \text{ nm}^3$, $1 \times 10^5\text{--}1 \times 10^6 \text{ nm}^3$, $1 \times 10^6\text{--}1 \times 10^7 \text{ nm}^3$, $1 \times 10^7\text{--}1 \times 10^8 \text{ nm}^3$, $1 \times 10^8\text{--}1 \times 10^9 \text{ nm}^3$, or $1 \times 10^9\text{--}4.2 \times 10^9 \text{ nm}^3$). In some instances, the PMP has a mean surface area of at least 77 nm^2 (e.g., at least 77 nm^2 , at least 100 nm^2 , at least 1000 nm^2 , at least $1 \times 10^4 \text{ nm}^2$, at least $1 \times 10^5 \text{ nm}^2$, at least $1 \times 10^6 \text{ nm}^2$, or at least $1 \times 10^7 \text{ nm}^2$). In some instances, the PMP has a mean volume of at least 65 nm^3 (e.g., at least 65 nm^3 , at least 100 nm^3 , at least 1000 nm^3 , at least $1 \times 10^4 \text{ nm}^3$, at least $1 \times 10^5 \text{ nm}^3$, at least $1 \times 10^6 \text{ nm}^3$, at least $1 \times 10^7 \text{ nm}^3$, at least $1 \times 10^8 \text{ nm}^3$, at least $1 \times 10^9 \text{ nm}^3$, at least $2 \times 10^9 \text{ nm}^3$, at least $3 \times 10^9 \text{ nm}^3$, or at least $4 \times 10^9 \text{ nm}^3$).

In some instances, the PMP may include an intact plant EV. Alternatively, the PMP may include a segment, portion, or extract of the full surface area of the vesicle (e.g., a segment, portion, or extract including less than 100% (e.g., less than 90%, less than 80%, less than 70%, less than 60%, less than 50%, less than 40%, less than 30%, less than 20%, less than 10%, less than 10%, less than 5%, or less than 1%) of the full surface area of the vesicle) of a plant EV. The segment, portion, or extract may be any shape, such as a circumferential segment, spherical segment (e.g., hemisphere), curvilinear segment, linear segment, or flat segment. In instances where the segment is a spherical segment of the vesicle, the spherical segment may represent one that arises from the splitting of a spherical vesicle along a pair of parallel lines, or one that arises from the splitting of a spherical vesicle along a pair of non-parallel lines. Accordingly, the plurality of PMPs can include a plurality of intact plant EVs, a plurality of plant EV segments, portions, or extracts, or a mixture of intact and segments of plant EVs. One skilled in the art will appreciate that the ratio of intact to segmented plant EVs will depend on the particular isolation method used. For example, grinding or blending a plant, or part thereof, may produce PMPs that contain a higher percentage of plant EV segments, portions, or extracts than a non-destructive extraction method, such as vacuum-infiltration.

In instances where, the PMP includes a segment, portion, or extract of a plant EV, the EV segment, portion, or extract may have a mean surface area less than that of an intact vesicle, e.g., a mean surface area less than 77 nm^2 , 100 nm^2 , 1000 nm^2 , $1 \times 10^4 \text{ nm}^2$, $1 \times 10^5 \text{ nm}^2$, $1 \times 10^6 \text{ nm}^2$, or $3.2 \times 10^6 \text{ nm}^2$. In some instances, the EV segment, portion, or extract has a surface area of less than 70 nm^2 , 60 nm^2 , 50 nm^2 , 40 nm^2 , 30 nm^2 , 20 nm^2 , or 10 nm^2 . In some instances, the PMP may include a plant EV,

or segment, portion, or extract thereof, that has a mean volume less than that of an intact vesicle, e.g., a mean volume of less than 65 nm³, 100 nm³, 1000 nm³, 1x10⁴ nm³, 1x10⁵ nm³, 1x10⁶ nm³, 1x10⁷ nm³, 1x10⁸ nm³, or 5.3x10⁸ nm³).

In instances where the PMP includes an extract of a plant EV, e.g., in instances where the PMP includes lipids extracted (e.g., with chloroform) from a plant EV, the PMP may include at least 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, or more than 99%, of lipids extracted (e.g., with chloroform) from a plant EV. The PMPs in the plurality may include plant EV segments and/or plant EV-extracted lipids or a mixture thereof.

Further outlined herein are details regarding methods of producing modified PMPs, plant EV markers that can be associated with PMPs, and formulations for compositions including PMPs.

A. Production Methods

PMPs may be produced from plant EVs, or a segment, portion or extract (e.g., lipid extract) thereof, that occur naturally in plants, or parts thereof, including plant tissues or plant cells. An exemplary method for producing PMPs includes (a) providing an initial sample from a plant, or a part thereof, wherein the plant or part thereof comprises EVs; and (b) isolating a crude PMP fraction from the initial sample, wherein the crude PMP fraction has a decreased level of at least one contaminant or undesired component from the plant or part thereof relative to the level in the initial sample. The method can further include an additional step (c) comprising purifying the crude PMP fraction, thereby producing a plurality of pure PMPs, wherein the plurality of pure PMPs have a decreased level of at least one contaminant or undesired component from the plant or part thereof relative to the level in the crude EV fraction. Each production step is discussed in further detail, below. Exemplary methods regarding the isolation and purification of PMPs is found, for example, in Rutter and Innes, *Plant Physiol.* 173(1): 728-741, 2017; Rutter et al, *Bio. Protoc.* 7(17): e2533, 2017; Regente et al, *J of Exp. Biol.* 68(20): 5485-5496, 2017; Mu et al, *Mol. Nutr. Food Res.*, 58, 1561–1573, 2014, and Regente et al, *FEBS Letters.* 583: 3363-3366, 2009, each of which is herein incorporated by reference.

In some instances, a plurality of PMPs may be isolated from a plant by a process which includes the steps of: (a) providing an initial sample from a plant, or a part thereof, wherein the plant or part thereof comprises EVs; (b) isolating a crude PMP fraction from the initial sample, wherein the crude PMP fraction has a decreased level of at least one contaminant or undesired component from the plant or part thereof relative to the level in the initial sample (e.g., a level that is decreased by at least 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 40%, 45%, 50%, 55%, 60%, 70%, 80%, 90%, 95%, 96%, 98%, 99%, or 100%); and (c) purifying the crude PMP fraction, thereby producing a plurality of pure PMPs, wherein the plurality of pure PMPs have a decreased level of at least one contaminant or undesired component from the plant or part thereof relative to the level in the crude EV fraction (e.g., a level that is decreased by at least 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 40%, 45%, 50%, 55%, 60%, 70%, 80%, 90%, 95%, 96%, 98%, 99%, or 100%).

The PMPs provided herein can include a plant EV, or segment, portion, or extract thereof, produced from a variety of plants. PMPs may be produced from any genera of plants (vascular or nonvascular), including but not limited to angiosperms (monocotyledonous and dicotyledonous plants),

gymnosperms, ferns, selaginellas, horsetails, psilophytes, lycophytes, algae (e.g., unicellular or multicellular, e.g., archaeplastida), or bryophytes. In certain instances, PMPs can be produced using a vascular plant, for example monocotyledons or dicotyledons or gymnosperms. For example, PMPs can be produced using alfalfa, apple, *Arabidopsis*, banana, barley, a *Brassica* species (e.g., *Arabidopsis* 5 *thaliana* or *Brassica napus*), canola, castor bean, chicory, chrysanthemum, clover, cocoa, coffee, cotton, cottonseed, corn, crambe, cranberry, cucumber, dendrobium, dioscorea, eucalyptus, fescue, flax, gladiolus, liliacea, linseed, millet, muskmelon, mustard, oat, oil palm, oilseed rape, papaya, peanut, pineapple, ornamental plants, *Phaseolus*, potato, rapeseed, rice, rye, ryegrass, safflower, sesame, sorghum, soybean, sugarbeet, sugarcane, sunflower, strawberry, tobacco, tomato, turfgrass, wheat or 10 vegetable crops such as lettuce, celery, broccoli, cauliflower, cucurbits; fruit and nut trees, such as apple, pear, peach, orange, grapefruit, lemon, lime, almond, pecan, walnut, hazel; vines, such as grapes, kiwi, hops; fruit shrubs and brambles, such as raspberry, blackberry, gooseberry; forest trees, such as ash, pine, fir, maple, oak, chestnut, poplar; with alfalfa, canola, castor bean, corn, cotton, crambe, flax, linseed, mustard, oil palm, oilseed rape, peanut, potato, rice, safflower, sesame, soybean, sugarbeet, 15 sunflower, tobacco, tomato, or wheat.

PMPs may be produced using a whole plant (e.g., a whole rosettes or seedlings) or alternatively from one or more plant parts (e.g., leaf, seed, root, fruit, vegetable, pollen, phloem sap, or xylem sap). For example, PMPs can be produced using shoot vegetative organs/structures (e.g., leaves, stems, or tubers), roots, flowers and floral organs/structures (e.g., pollen, bracts, sepals, petals, stamens, carpels, 20 anthers, or ovules), seed (including embryo, endosperm, or seed coat), fruit (the mature ovary), sap (e.g., phloem or xylem sap), plant tissue (e.g., vascular tissue, ground tissue, tumor tissue, or the like), and cells (e.g., single cells, protoplasts, embryos, callus tissue, guard cells, egg cells, or the like), or progeny of same. For instance, the isolation step may involve (a) providing a plant, or a part thereof. In some examples, the plant part is an *Arabidopsis* leaf. The plant may be at any stage of development. For 25 example, the PMPs can be produced using seedlings, e.g., 1 week, 2 week, 3 week, 4 week, 5 week, 6 week, 7 week, or 8 week old seedlings (e.g., *Arabidopsis* seedlings). Other exemplary PMPs can include PMPs produced using roots (e.g., ginger roots), fruit juice (e.g., grapefruit juice), vegetables (e.g., broccoli), pollen (e.g., olive pollen), phloem sap (e.g., *Arabidopsis* phloem sap), or xylem sap (e.g., tomato plant xylem sap).

30 PMPs can be produced using a plant, or part thereof, by a variety of methods. Any method that allows release of the EV-containing apoplastic fraction of a plant, or an otherwise extracellular fraction that contains PMPs comprising secreted EVs (e.g., cell culture media) is suitable in the present methods. EVs can be separated from the plant or plant part by either destructive (e.g., grinding or blending of a plant, or any plant part) or non-destructive (washing or vacuum infiltration of a plant or any plant part) 35 methods. For instance, the plant, or part thereof, can be vacuum-infiltrated, ground, blended, or a combination thereof to isolate EVs from the plant or plant part, thereby producing PMPs. For instance, the isolating step may involve vacuum infiltrating the plant (e.g., with a vesicle isolation buffer) to release and collect the apoplastic fraction. Alternatively, the isolating step may involve grinding or blending the plant to release the EVs, thereby producing PMPs.

Upon isolating the plant EVs, thereby producing PMPs, the PMPs can be separated or collected into a crude PMP fraction (e.g., an apoplastic fraction). For instance, the separating step may involve separating the plurality of PMPs into a crude PMP fraction using centrifugation (e.g., differential centrifugation or ultracentrifugation) and/or filtration to separate the plant PMP-containing fraction from large contaminants, including plant tissue debris or plant cells. As such, the crude PMP fraction will have a decreased number of large contaminants, including plant tissue debris or plant cells, as compared to the initial sample from the plant or plant part. Depending on the method used, the crude PMP fraction may additionally comprise a decreased level of plant cell organelles (e.g., nuclei, mitochondria or chloroplasts), as compared to the initial sample from the plant or plant part.

In some instances, the isolating step may involve separating the plurality of PMPs into a crude PMP fraction using centrifugation (e.g., differential centrifugation or ultracentrifugation) and/or filtration to separate the PMP-containing fraction from plant cells or cellular debris. In such instances, the crude PMP fraction will have a decreased number of plant cells or cellular debris, as compared to the initial sample from the source plant or plant part.

The crude PMP fraction can be further purified by additional purification methods to produce a plurality of pure PMPs. For example, the crude PMP fraction can be separated from other plant components by ultracentrifugation, e.g., using a density gradient (iodixanol or sucrose) and/or use of other approaches to remove aggregated components (e.g., precipitation or size-exclusion chromatography). The resulting pure PMPs may have a decreased level of contaminants or other undesired components from the source plant (e.g., one or more non-PMP components, such as protein aggregates, nucleic acid aggregates, protein-nucleic acid aggregates, free lipoproteins, lipido-proteic structures), nuclei, cell wall components, cell organelles, or a combination thereof) relative to one or more fractions generated during the earlier separation steps, or relative to a pre-established threshold level, e.g., a commercial release specification. For example, the pure PMPs may have a decreased level (e.g., by about 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%; or by about 2x fold, 4x fold, 5x fold, 10x fold, 20x fold, 25x fold, 50x fold, 75x fold, 100x fold, or more than 100x fold) of plant organelles or cell wall components relative to the level in the initial sample. In some instances, the pure PMPs are substantially free (e.g., have undetectable levels) of one or more non-PMP components, such as protein aggregates, nucleic acid aggregates, protein-nucleic acid aggregates, free lipoproteins, lipido-proteic structures), nuclei, cell wall components, cell organelles, or a combination thereof. Further examples of the releasing and separation steps can be found in Example 1. The PMPs may be at a concentration of, e.g., 1×10^9 , 5×10^9 , 1×10^{10} , 5×10^{10} , 5×10^{10} , 1×10^{11} , 2×10^{11} , 3×10^{11} , 4×10^{11} , 5×10^{11} , 6×10^{11} , 7×10^{11} , 8×10^{11} , 9×10^{11} , 1×10^{12} , 2×10^{12} , 3×10^{12} , 4×10^{12} , 5×10^{12} , 6×10^{12} , 7×10^{12} , 8×10^{12} , 9×10^{12} , 1×10^{13} , or more than 1×10^{13} PMPs/mL.

For example, protein aggregates may be removed from PMPs. For example, the PMPs can be taken through a range of pHs (e.g., as measured using a pH probe) to precipitate out protein aggregates in solution. The pH can be adjusted to, e.g., pH 3, pH 5, pH 7, pH 9, or pH 11 with the addition of, e.g., sodium hydroxide or hydrochloric acid. Once the solution is at the specified pH, it can be filtered to remove particulates. Alternatively, the PMPs can be flocculated using the addition of charged polymers, such as Polymin-P or Praestol 2640. Briefly, Polymin-P or Praestol 2640 is added to the solution and

mixed with an impeller. The solution can then be filtered to remove particulates. Alternatively, aggregates can be solubilized by increasing salt concentration. For example NaCl can be added to the PMPs until it is at, e.g., 1 mol/L. The solution can then be filtered to isolate the PMPs. Alternatively, aggregates are solubilized by increasing the temperature. For example, the PMPs can be heated under mixing until the solution has reached a uniform temperature of, e.g., 50°C for 5 minutes. The PMP mixture can then be filtered to isolate the PMPs. Alternatively, soluble contaminants from PMP solutions can be separated by size-exclusion chromatography column according to standard procedures, where PMPs elute in the first fractions, whereas proteins and ribonucleoproteins and some lipoproteins are eluted later. The efficiency of protein aggregate removal can be determined by measuring and comparing the protein concentration before and after removal of protein aggregates via BCA/Bradford protein quantification.

Any of the production methods described herein can be supplemented with any quantitative or qualitative methods known in the art to characterize or identify the PMPs at any step of the production process. PMPs may be characterized by a variety of analysis methods to estimate PMP yield, PMP concentration, PMP purity, PMP composition, or PMP sizes. PMPs can be evaluated by a number of methods known in the art that enable visualization, quantitation, or qualitative characterization (e.g., identification of the composition) of the PMPs, such as microscopy (e.g., transmission electron microscopy), dynamic light scattering, nanoparticle tracking, spectroscopy (e.g., Fourier transform infrared analysis), or mass spectrometry (protein and lipid analysis). In certain instances, methods (e.g., mass spectrometry) may be used to identify plant EV markers present on the PMP. To aid in analysis and characterization, of the PMP fraction, the PMPs can additionally be labelled or stained. For example, the PMPs can be stained with 3,3'-dihexyloxacarbocyanine iodide (DIOC₆), a fluorescent lipophilic dye, PKH67 (Sigma Aldrich); Alexa Fluor® 488 (Thermo Fisher Scientific), or DyLight™ 800 (Thermo Fisher). In the absence of sophisticated forms of nanoparticle tracking, this relatively simple approach quantifies the total membrane content and can be used to indirectly measure the concentration of PMPs (Rutter and Innes, *Plant Physiol.* 173(1): 728-741, 2017; Rutter et al, *Bio. Protoc.* 7(17): e2533, 2017). For more precise measurements, and to assess the size distributions of PMPs, nanoparticle tracking can be used.

During the production process, the PMPs can optionally be prepared such that the PMPs are at an increased concentration (e.g., by about 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%; or by about 2x fold, 4x fold, 5x fold, 10x fold, 20x fold, 25x fold, 50x fold, 75x fold, 100x fold, or more than 100x fold) relative to the EV level in a control or initial sample. The PMPs may make up about 0.1% to about 100% of the PMP composition, such as any one of about 0.01% to about 100%, about 1% to about 99.9%, about 0.1% to about 10%, about 1% to about 25%, about 10% to about 50%, about 50% to about 99%, or about 75% to about 100%. In some instances, the composition includes at least any of 0.1%, 0.5%, 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more PMPs, e.g., as measured by wt/vol, percent PMP protein composition, and/or percent lipid composition (e.g., by measuring fluorescently labelled lipids); See, e.g., Example 3). In some instances, the concentrated agents are used as commercial products, e.g., the final user may use diluted agents, which have a substantially lower concentration of active ingredient. In some embodiments, the

composition is formulated as an agricultural concentrate formulation, e.g., an ultra-low-volume concentrate formulation.

As illustrated by Example 1, PMPs can be produced using a variety of plants, or parts thereof (e.g., the leaf apoplast, seed apoplast, root, fruit, vegetable, pollen, phloem, or xylem sap). For example, PMPs can be released from the apoplastic fraction of a plant, such as the apoplast of a leaf (e.g., apoplast *Arabidopsis thaliana* leaves) or the apoplast of seeds (e.g., apoplast of sunflower seeds). Other exemplary PMPs are produced using roots (e.g., ginger roots), fruit juice (e.g., grapefruit juice), vegetables (e.g., broccoli), pollen (e.g., olive pollen), phloem sap (e.g., *Arabidopsis* phloem sap), xylem sap (e.g., tomato plant xylem sap), or cell culture supernatant (e.g. BY2 tobacco cell culture supernatant). This example further demonstrates the production of PMPs from these various plant sources.

As illustrated by Example 2, PMPs can be purified by a variety of methods, for example, by using a density gradient (iodixanol or sucrose) in conjunction with ultracentrifugation and/or methods to remove aggregated contaminants, e.g., precipitation or size-exclusion chromatography. For instance, Example 2 illustrates purification of PMPs that have been obtained via the separation steps outlined in Example 1.

Further, PMPs can be characterized in accordance with the methods illustrated in Example 3.

The PMP can be modified prior to use, as outlined further herein.

B. Modified PMPs and PMP compositions

Following production of the PMPs, the PMPs may be modified by loading with or formulating with a heterologous agent (e.g., a cell-penetrating agent) that is capable of increasing cell uptake (e.g., animal cell uptake (e.g., mammalian cell uptake, e.g., human cell uptake), plant cell uptake, bacterial cell uptake, or fungal cell uptake) relative to an unmodified PMP. For example, the modified PMPs may include (e.g., be loaded with, e.g., encapsulate or be conjugated to) or be formulated with (e.g., be suspended or resuspended in a solution comprising) a plant cell-penetrating agent, such as a synthetic charged lipid (e.g., an ionizable and/or cationic lipid). Each of the modified PMPs may comprise at least 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more than 90% synthetic charged lipid (e.g., ionizable and/or cationic lipid).

In some embodiments, the heterologous agent is a synthetic charged lipid, wherein the synthetic charged lipid has at least one (e.g., one, two, three, four or all five) of the characteristics listed below:

(i) at least 2 ionizable amines (e.g., at least 2, at least 3, at least 4, at least 5, at least 6, or more than 6 ionizable amines, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more than 12 ionizable amines);

(ii) at least 3 lipid tails (e.g., at least 3, at least 4, at least 5, at least 6, or more than 6 lipid tails, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more than 12 lipid tails), wherein each of the lipid tails is independently at least 6 carbon atoms in length (e.g., at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, or more than 18 carbon atoms in length, e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or more than 25 carbon atoms in length);

(iii) an acid dissociation constant (pKa) of between about 4.5 to about 7.5 (e.g., a pKa of about 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8,

6.9, 7.0, 7.1, 7.2, 7.3, 7.4, or 7.5 (e.g., a pKa of between about 6.5 and about 7.5 (e.g., a pKa of about 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, or 7.5));

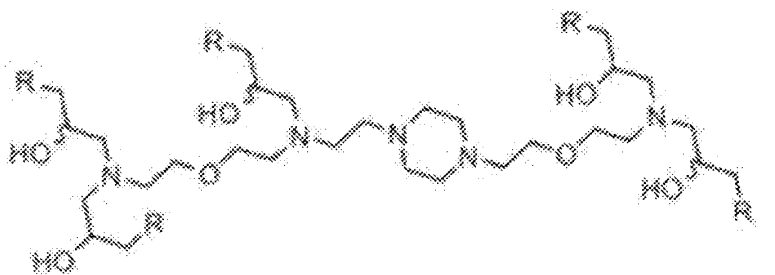
(iv) an ionizable amine and a heteroorganic group; and

(v) an N:P (amines of ionizable lipid:phosphates of mRNA) ratio of at least 10;

- 5 provided that the charged lipid is not selected from 1'-((2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethyl)azanediyl)bisdodecan-2-ol) (C12-200), MD1 (cKK-E12), OF2, EPC, ZA3-Ep10, TT3, LP01, 5A2-SC8, Lipid 5 (Moderna), and 98N12-5. C12-200 is described, e.g., in Whitehead et al., *Nature Communications*, 5: Article No. 4277, 2014. MD1 (cKK-E12), TT3, LP01, and Lipid 5 are described, e.g., in Miao et al., *Molecular Cancer*, 20(41), 2021. OF2 (OF-02)
- 10 is described, e.g., in Han et al., *Nature Communications*, 12: Article No. 7233, 2021. EPC is available from AVANTI® Polar Lipids. ZA3-Ep10 is described, e.g., in Miller et al., *Angew Chem Int Ed Engl*, 56(4): 1059-1063, 2017. 5A2-SC8 is described, e.g., in Zhou et al., *Proc Natl Acad Sci USA*, 113(3): 520-525, 2016. 98N12-5 is described, e.g., in Akinc et al., *Mol Ther*, 17(5): 872-879, 2009.

- In some embodiments, the heteroorganic group is hydroxyl. In some embodiments, the
- 15 heteroorganic group comprises a hydrogen bond donor. In some embodiments, the heteroorganic group comprises a hydrogen bond acceptor. In some embodiments, the heteroorganic group is -OH, -SH, - (CO)H, -CO₂H, -NH₂, -CONH₂, optionally substituted C₁-C₆ alkoxy, or fluorine.

- In some embodiments, the heterologous agent is a synthetic charged lipid, wherein the synthetic
- 20 charged lipid is represented by the following formula I:



(I)

where R is a C8-C14 alkyl group (e.g., a C12-14 alkyl group).

- In some embodiments, a lipid membrane of the modified PMPs comprises at least 35% of the lipid of formula I, e.g., at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or more
- 25 than 90% of the lipid of formula I, e.g., 35%-40%, 40%-50%, 50%-60%, 60%-70%, 70%-80%, or 80%-90% of the lipid of formula I.

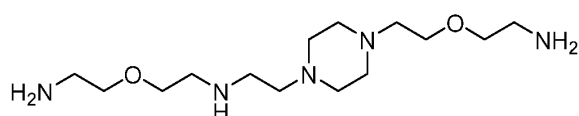
In some instances, the PMPs comprise at least 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more than 90% synthetic charged lipid.

- In some instances, the PMPs comprise a molar ratio of at least 0.1%, 1%, 5%, 10%, 15%, 20%,
- 30 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or more than 90% synthetic charged lipid, e.g., 1%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, 50%-60%, 60%-70%,

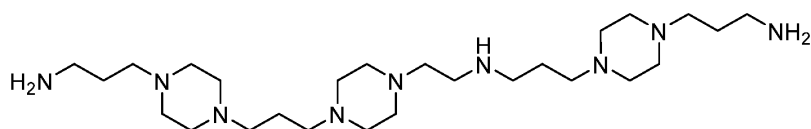
70%-80%, or 80%-90% synthetic charged lipid, e.g., about 25%-75% synthetic charged lipid (e.g., about 25%-75% synthetic charged lipid).

Synthetic charged lipids described herein may include an amine core described herein substituted with one or more (e.g., 1, 2, 3, 4, 5, or 6) lipid tails. Preferably, synthetic charged lipids described herein include at least 3 lipid tails. A lipid tail may be a C₈-C₁₈ hydrocarbon (e.g., C₆-C₁₈ alkyl or C₆-C₁₈ alkanoyl). An amine core may be substituted with one or more lipid tails at a nitrogen atom (e.g., one hydrogen atom attached to the nitrogen atom may be replaced with a lipid tail).

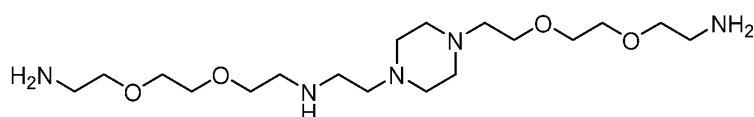
In some embodiments, the amine core has a structure of:



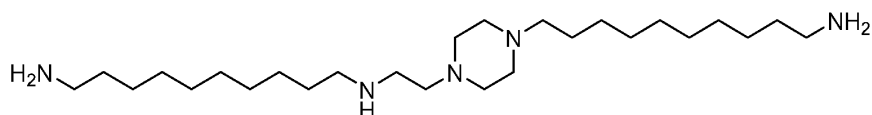
10 In some embodiments, the amine core has a structure of:



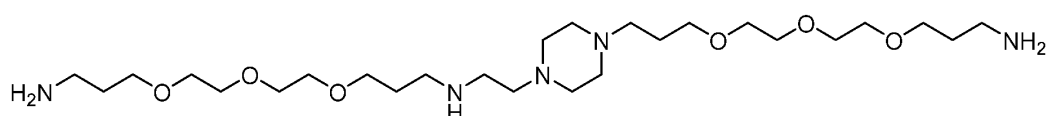
In some embodiments, the amine core has a structure of:



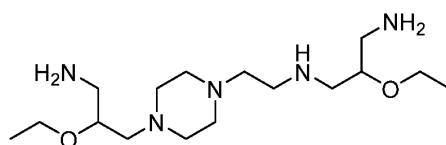
In some embodiments, the amine core has a structure of:



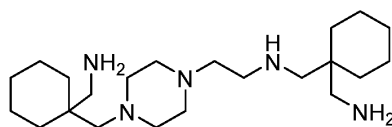
In some embodiments, the amine core has a structure of:



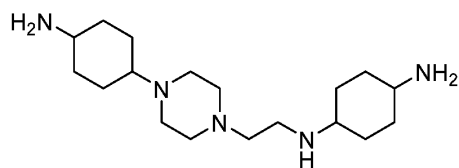
In some embodiments, the amine core has a structure of:



20 In some embodiments, the amine core has a structure of:

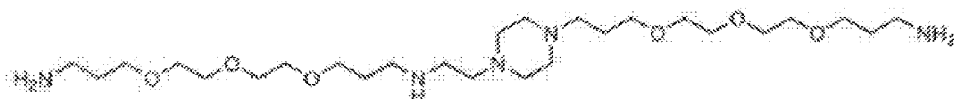
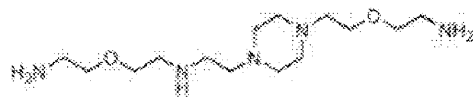
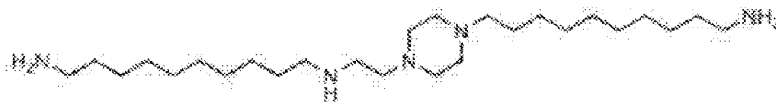


In some embodiments, the amine core has a structure of:

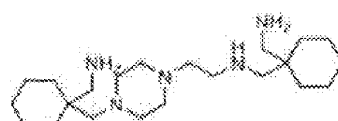


In some embodiments, the lipids have an amine core substituted with one or more (e.g., 1, 2, 3, 4, 5, or 6) lipid tails, for instance, at a nitrogen atom (e.g., one hydrogen atom attached to the nitrogen atom may be replaced with a lipid tail). The amine core may have one of the following structures:

5



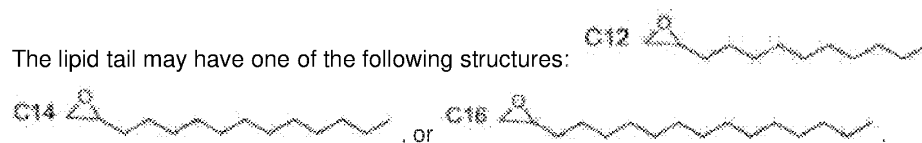
10



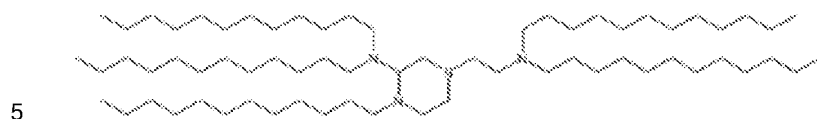
, or



The lipid tail may have one of the following structures:

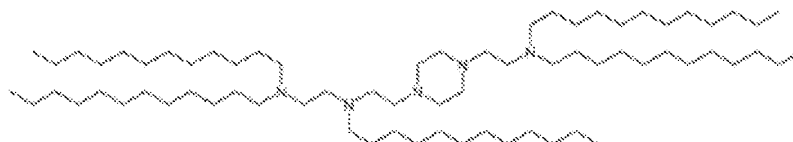


Other suitable cationic lipids for use in the compositions and methods of the invention include the cationic lipids as described in International Patent Publication WO 2016/118725, which is incorporated herein by reference in its entirety. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:



and pharmaceutically acceptable salts thereof.

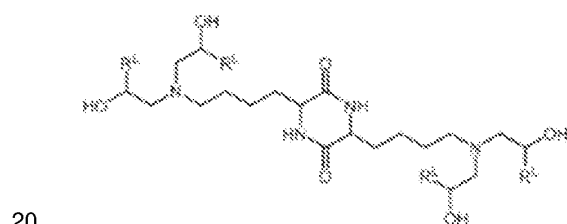
Other suitable cationic lipids for use in the compositions and methods of the invention include the cationic lipids as described in International Patent Publication WO 2016/118724, which is incorporated herein by reference in its entirety. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:



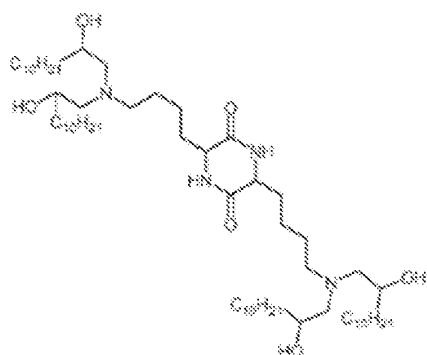
and pharmaceutically acceptable salts thereof.

Other suitable cationic lipids for use in the compositions and methods of the invention include a cationic lipid having the formula of 14,25-ditridecyl 15,18,21,24-tetraaza-octatriacontane, and pharmaceutically acceptable salts thereof.

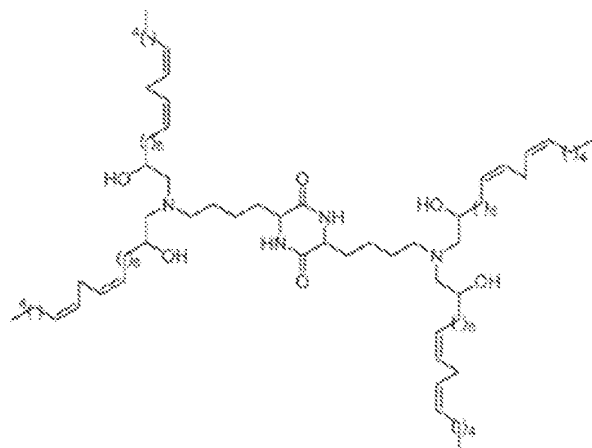
Other suitable cationic lipids for use in the compositions and methods of the invention include the cationic lipids as described in International Patent Publications WO 2013/063468 and WO 2016/205691, each of which is incorporated herein by reference in its entirety. In some embodiments, the compositions and methods of the present invention include a cationic lipid of the following formula:



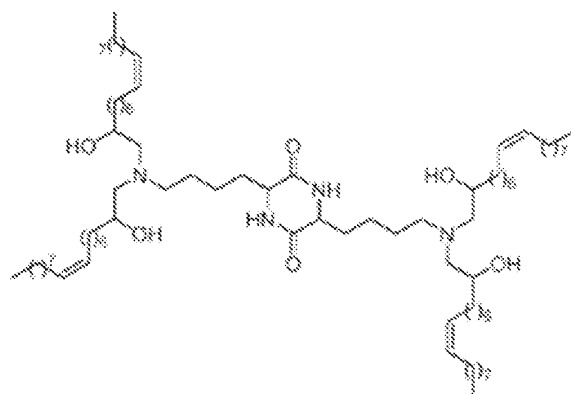
or pharmaceutically acceptable salts thereof, wherein each instance of R^L is independently optionally substituted C6-C40 alkenyl. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:



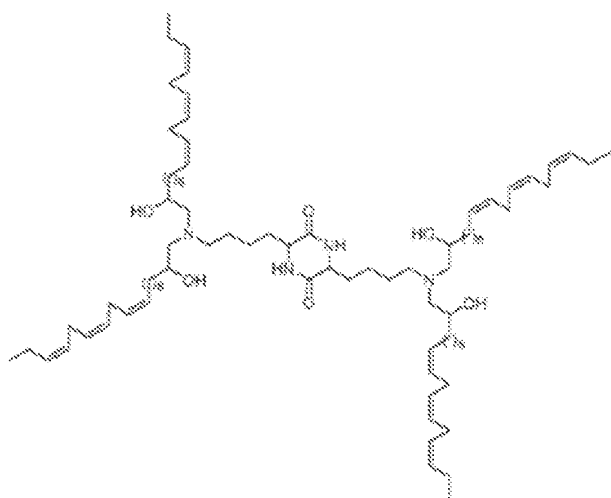
and pharmaceutically acceptable salts thereof. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:



- 5 and pharmaceutically acceptable salts thereof. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:

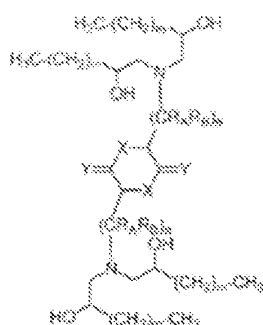


and pharmaceutically acceptable salts thereof. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:

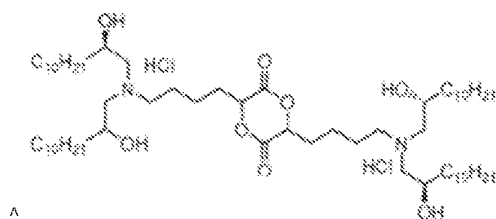


and pharmaceutically acceptable salts thereof.

Other suitable cationic lipids for use in the compositions and methods of the invention include the cationic lipids as described in International Patent Publication WO 2015/184256, which is incorporated
 5 herein by reference in its entirety. In some embodiments, the compositions and methods of the present invention include a cationic lipid of the following formula:

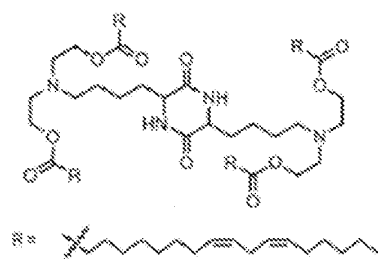


or a pharmaceutically acceptable salt thereof, wherein each X independently is O or S; each Y
 independently is O or S; each m independently is 0 to 20; each n independently is 1 to 6; each R_A is
 10 independently hydrogen, optionally substituted C1-50 alkyl, optionally substituted C2-50 alkenyl,
 optionally substituted C2-50 alkynyl, optionally substituted C3-10 carbocyclyl, optionally substituted 3-14
 membered heterocyclyl, optionally substituted C6-14 aryl, optionally substituted 5-14 membered
 heteroaryl or halogen; and each R_B is independently hydrogen, optionally substituted C1-50 alkyl,
 optionally substituted C2-50 alkenyl, optionally substituted C2-50 alkynyl, optionally substituted C3-10
 15 carbocyclyl, optionally substituted 3-14 membered heterocyclyl, optionally substituted C6-14 aryl,
 optionally substituted 5-14 membered heteroaryl or halogen. In certain embodiments, the compositions
 and methods of the present invention include a cationic lipid, "Target 23", having a compound structure of:



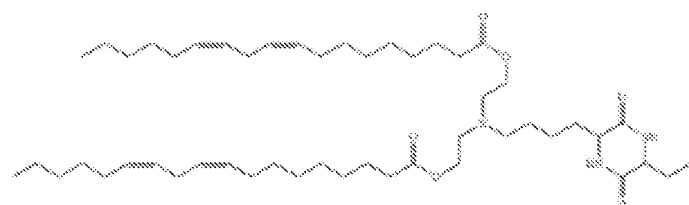
(Target 23) and pharmaceutically acceptable salts thereof.

- Other suitable cationic lipids for use in the compositions and methods of the invention include the cationic lipids as described in International Patent Publication WO 2016/004202, which is incorporated
 5 herein by reference in its entirety. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:

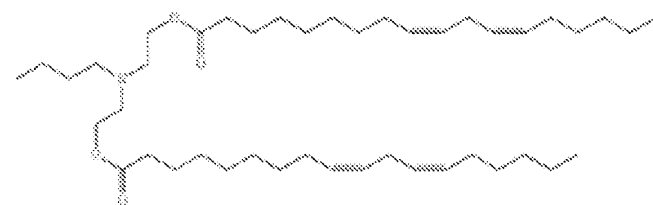


or a pharmaceutically acceptable salt thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:

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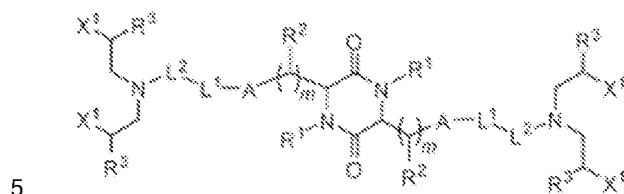


or a pharmaceutically acceptable salt thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



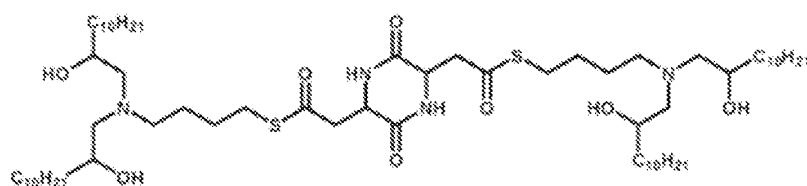
or a pharmaceutically acceptable salt thereof.

Other suitable cationic lipids for use in the compositions and methods of the present invention include cationic lipids as described in United States Provisional Patent Application Serial Number 62/758,179, which is incorporated herein by reference in its entirety. In some embodiments, the compositions and methods of the present invention include a cationic lipid of the following formula:

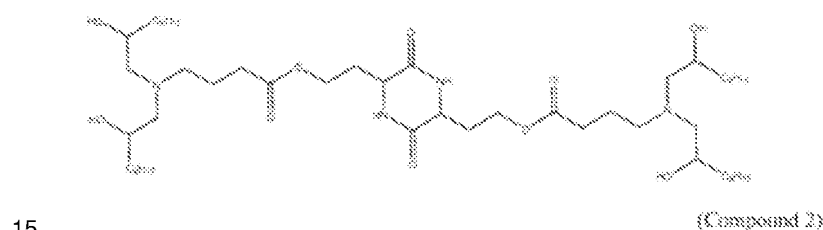


or a pharmaceutically acceptable salt thereof, wherein each R^1 and R^2 is independently H or C1-C6 aliphatic; each m is independently an integer having a value of 1 to 4; each A is independently a covalent bond or arylene; each L^1 is independently an ester, thioester, disulfide, or anhydride group; each L^2 is independently C2-C10 aliphatic; each X^1 is independently H or OH; and each R^3 is independently C6-C20 aliphatic. In some embodiments, the compositions and methods of the present invention include a cationic lipid of the following formula:

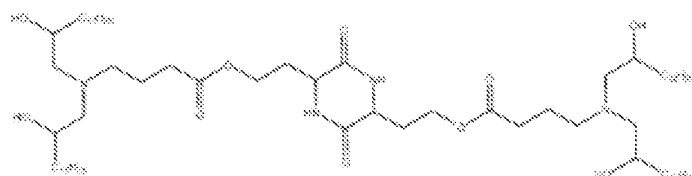
10



(Compound 1) or a pharmaceutically acceptable salt thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid of the following formula:

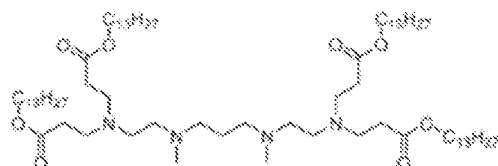


or a pharmaceutically acceptable salt thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid of the following formula:



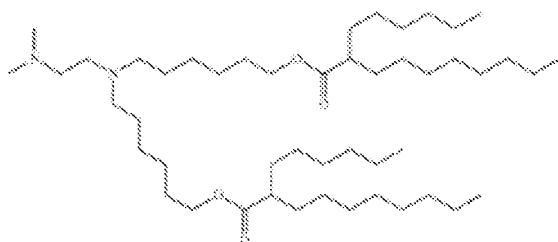
(Compound 3) or a pharmaceutically acceptable salt thereof.

- Other suitable cationic lipids for use in the compositions and methods of the present invention include the cationic lipids as described in J. McClellan, M. C. King, Cell 2010, 141, 210-217 and in Whitehead et al., Nature Communications (2014) 5:4277, which is incorporated herein by reference in its entirety. In certain embodiments, the cationic lipids of the compositions and methods of the present invention include a cationic lipid having a compound structure of:

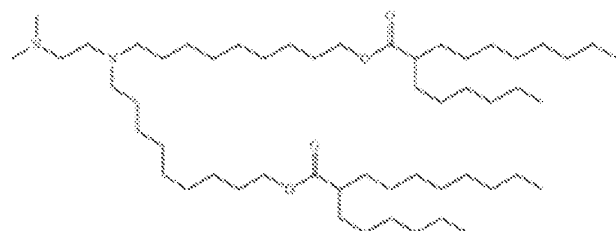


and pharmaceutically acceptable salts thereof.

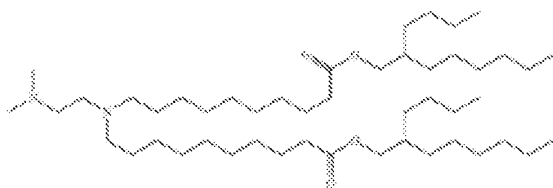
- Other suitable cationic lipids for use in the compositions and methods of the invention include the cationic lipids as described in International Patent Publication WO 2015/199952, which is incorporated herein by reference in its entirety. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



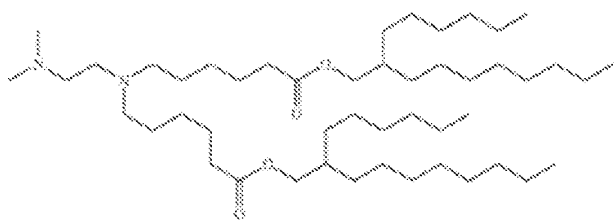
- and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



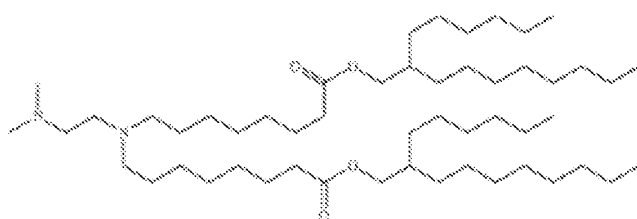
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



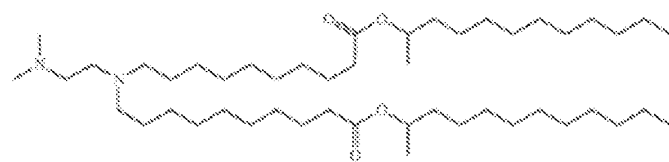
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



- 5 and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:

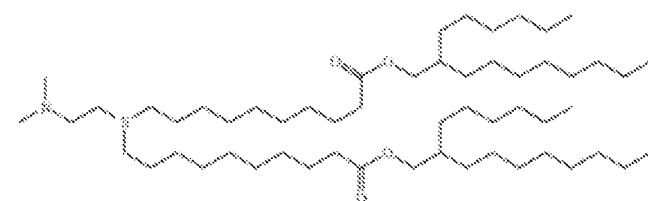


and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:

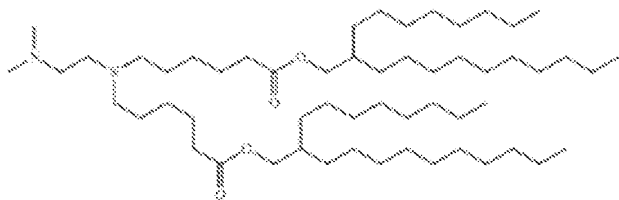


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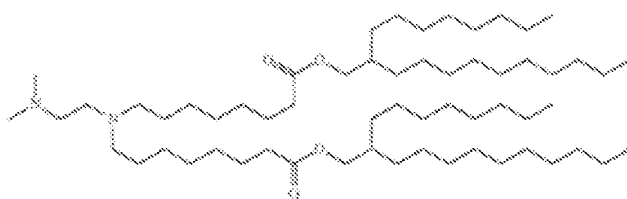
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



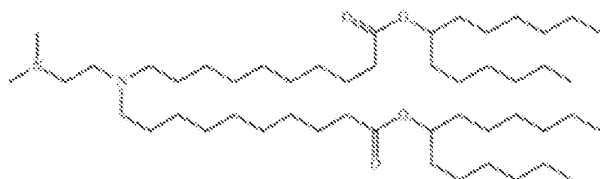
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



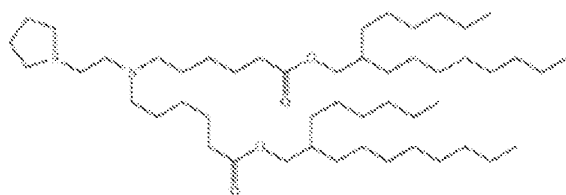
- 5 and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



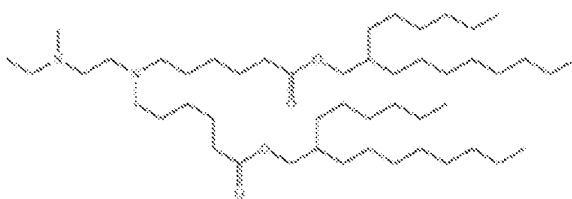
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



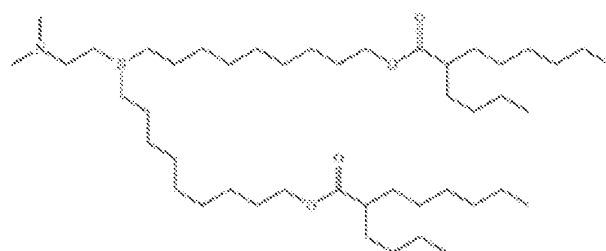
- 10 and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:

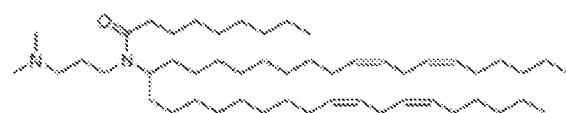


and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



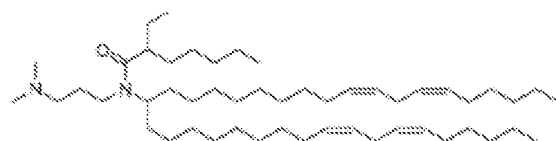
5 and pharmaceutically acceptable salts thereof.

Other suitable cationic lipids for use in the compositions and methods of the invention include the cationic lipids as described in International Patent Publication WO 2017/004143, which is incorporated herein by reference in its entirety. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



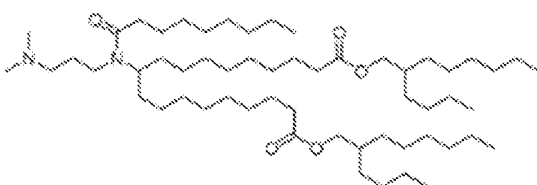
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and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:

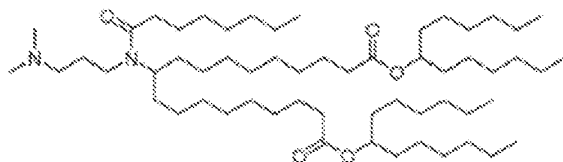


and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:

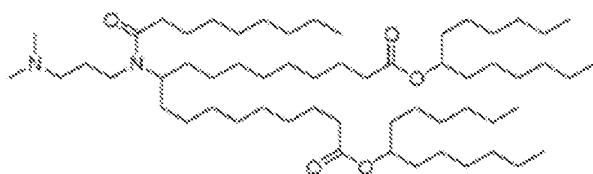
15



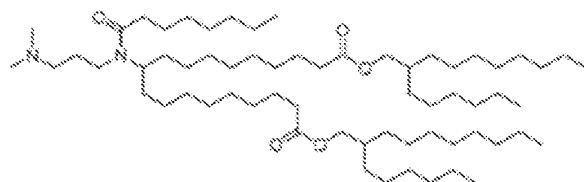
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



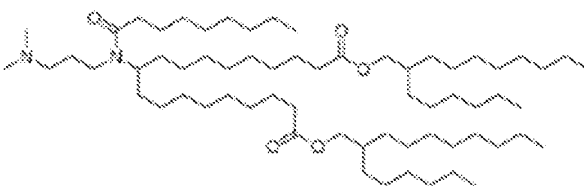
- 5 and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



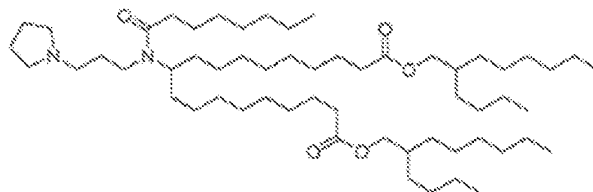
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



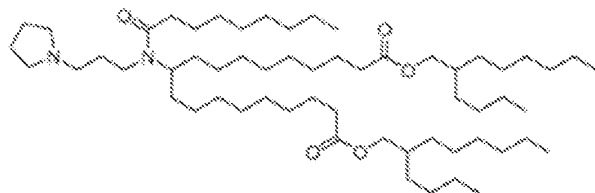
- 10 and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



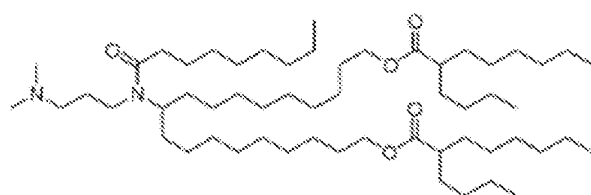
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



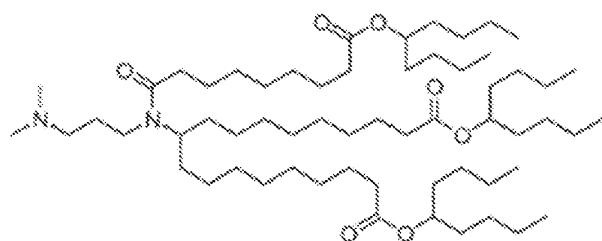
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



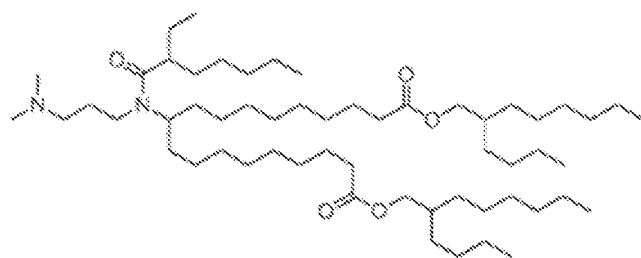
- 5 and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



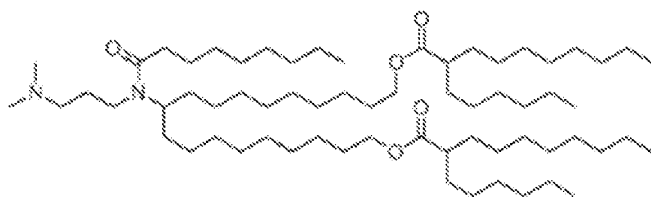
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



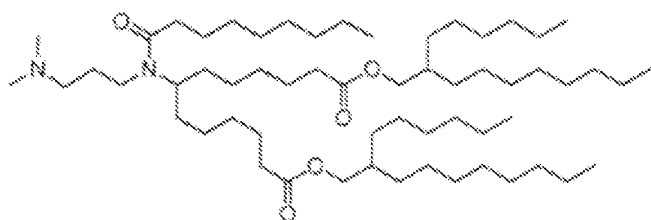
- 10 and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



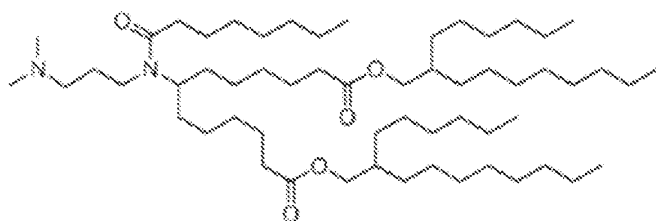
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



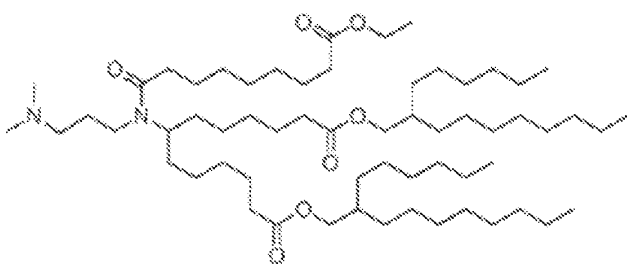
and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



- 5 and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:

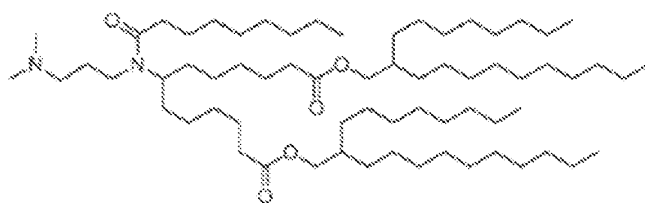


and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



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and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



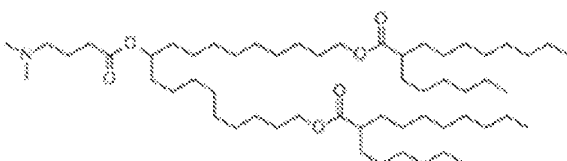
and pharmaceutically acceptable salts thereof.

Other suitable cationic lipids for use in the compositions and methods of the invention include the cationic lipids as described in International Patent Publication WO 2017/075531, which is incorporated
 5 herein by reference in its entirety. In some embodiments, the compositions and methods of the present invention include a cationic lipid of the following formula:

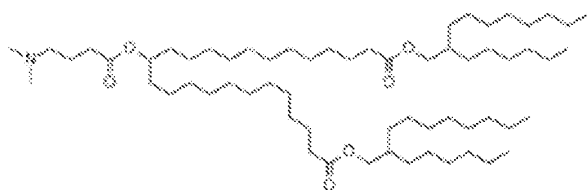


or a pharmaceutically acceptable salt thereof, wherein one of L¹ or L² is -O(C=O)-, -(C=O)O-, -C(=O)-, -O-,
 , -S(O)_x, -S-S-, -C(=O)S-, -SC(=O)-, -NR^aC(=O)-, -C(=O)NR^a-, NR^aC(=O)NR^a-, -OC(=O)NR^a-, or -
 10 NR^aC(=O)O-; and the other of L¹ or L² is -O(C=O)-, -(C=O)O-, -C(=O)-, -O-, -S(O)_x, -S-S-, -C(=O)S-,
 SC(=O)-, -NR^aC(=O)-, -C(=O)NR^a-, NR^aC(=O)NR^a-, -OC(=O)NR^a- or -NR^aC(=O)O- or a direct bond;
 G¹ and G² are each independently unsubstituted C₁-C₁₂ alkylene or C₁-C₁₂ alkenylene; G³ is C₁-
 C₂₄ alkylene, C₁-C₂₄ alkenylene, C₃-C₈ cycloalkylene, C₃-C₈ cycloalkenylene; R^a is H or C₁-C₁₂ alkyl;
 R¹ and R² are each independently C₆-C₂₄ alkyl or C₆-C₂₄ alkenyl; R³ is H, OR⁵, CN, -C(=O)OR⁴, -
 15 OC(=O)R⁴ or -NR⁵C(=O)R⁴; R⁴ is C₁-C₁₂ alkyl; R⁵ is H or C₁-C₆ alkyl; and x is 0, 1 or 2.

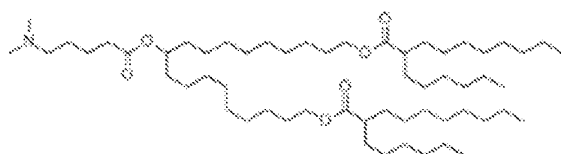
Other suitable cationic lipids for use in the compositions and methods of the invention include the cationic lipids as described in International Patent Publication WO 2017/117528, which is incorporated
 herein by reference in its entirety. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of
 the present invention include a cationic lipid having the compound structure:

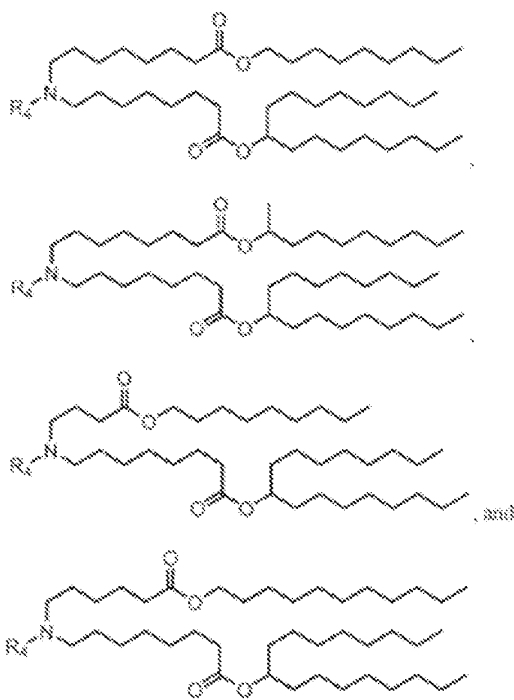


and pharmaceutically acceptable salts thereof. In some embodiments, the compositions and methods of the present invention include a cationic lipid having the compound structure:



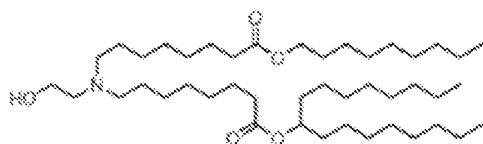
5 and pharmaceutically acceptable salts thereof.

Other suitable cationic lipids for use in the compositions and methods of the invention include the cationic lipids as described in International Patent Publication WO 2017/049245, which is incorporated herein by reference in its entirety. In some embodiments, the cationic lipids of the compositions and methods of the present invention include a compound of one of the following formulas:

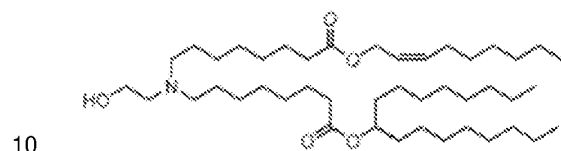


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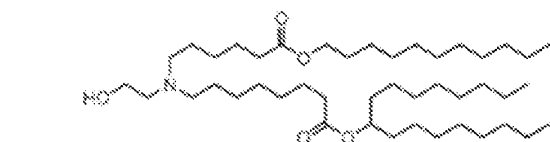
- and pharmaceutically acceptable salts thereof. For any one of these four formulas, R4 is independently selected from $-(CH_2)_nQ$ and $-(CH_2)_nCHQR$; Q is selected from the group consisting of $-OR$, $-OH$, $-O(CH_2)_nN(R)_2$, $-OC(O)R$, $-CX_3$, $-CN$, $-N(R)C(O)R$, $-N(H)C(O)R$, $-N(R)S(O)_2R$, $-N(H)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(H)C(O)N(R)_2$, $-N(H)C(O)N(H)(R)$, $-N(R)C(S)N(R)_2$, $-N(H)C(S)N(R)_2$, $-N(H)C(S)N(H)(R)$, and a heterocycle; and n is 1, 2, or 3. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:



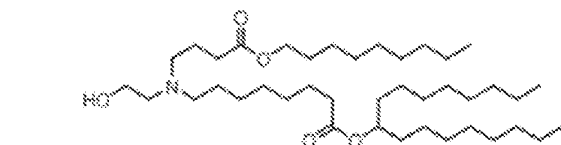
and pharmaceutically acceptable salts thereof. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:



and pharmaceutically acceptable salts thereof. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:

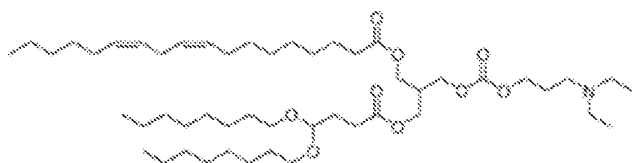


and pharmaceutically acceptable salts thereof. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:

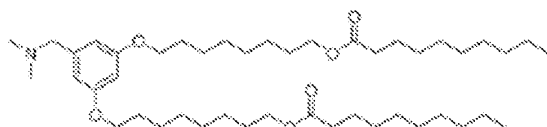


and pharmaceutically acceptable salts thereof.

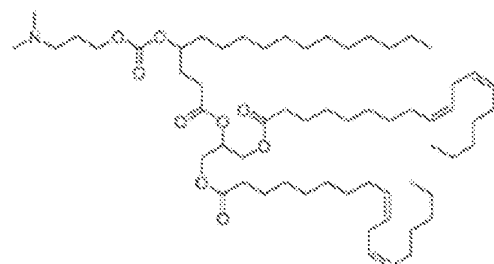
Other suitable cationic lipids for use in the compositions and methods of the invention include the cationic lipids as described in International Patent Publication WO 2017/173054 and WO 2015/095340, each of which is incorporated herein by reference in its entirety. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:



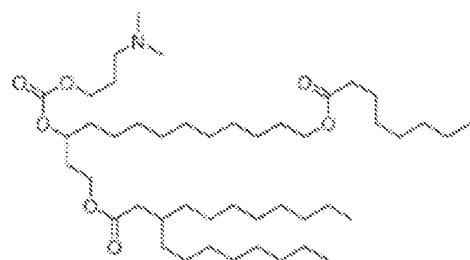
and pharmaceutically acceptable salts thereof. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:



- 5 and pharmaceutically acceptable salts thereof. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:



and pharmaceutically acceptable salts thereof. In certain embodiments, the compositions and methods of the present invention include a cationic lipid having a compound structure of:



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and pharmaceutically acceptable salts thereof.

- In some embodiments, the PMP compositions described herein may include a synthetic charged lipid (e.g., an ionizable and/or cationic lipid) as described in, may be formulated as described in, or may comprise or be comprised by a composition as described in WO2016118724, WO2016118725, WO2016187531, WO2017176974, WO2018078053, WO2019027999, WO2019036030, WO2019089828, WO2019099501, WO2020072605, WO2020081938, WO2020118041, WO2020146805, or WO2020219876, each of which is incorporated by reference herein in its entirety.
- 15

The agent may increase uptake of the PMP as a whole or may increase uptake of a portion or component of the PMP, such as a heterologous functional agent (e.g., a heterologous agricultural agent (e.g., pesticidal agent, fertilizing agent, herbicidal agent, plant-modifying agent) or a heterologous therapeutic agent (e.g., an antifungal agent, an antibacterial agent, a virucidal agent, an anti-viral agent, an insecticidal agent, a nematocidal agent, an antiparasitic agent, or an insect repellent)) carried by the PMP. The degree to which cell uptake (e.g., plant cell uptake, bacterial cell uptake, or fungal cell uptake) is increased may vary depending on the plant or plant part to which the composition is delivered, the PMP formulation, and other modifications made to the PMP. For example, the modified PMPs may have an increased cell uptake (e.g., animal cell uptake, plant cell uptake, bacterial cell uptake, or fungal cell uptake) of at least 1%, 2%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% relative to an unmodified PMP. In some instances, the increased cell uptake (e.g., animal cell uptake, plant cell uptake, bacterial cell uptake, or fungal cell uptake) is an increased cell uptake of at least 2x-fold, 4x-fold, 5x-fold, 10x-fold, 100x-fold, or 1000x-fold relative to an unmodified PMP.

In some embodiments, a PMP that has been modified with a synthetic charged lipid more efficiently encapsulates a negatively charged heterologous functional agent (e.g., a polynucleotide) than a PMP that has not been modified with a synthetic charged lipid. In some aspects, a PMP that has been modified with a synthetic charged lipid has altered biodistribution relative to a PMP that has not been modified with a synthetic charged lipid. In some aspects, a PMP that has been modified with a synthetic charged lipid has altered (e.g., increased) fusion with an endosomal membrane of a target cell relative to a PMP that has not been modified with a charged lipid.

In another aspect, the PMPs can be modified with other components (e.g., lipids, e.g., sterols, e.g., cholesterol; or small molecules) to further alter the functional and structural characteristics of the PMP. For example, the PMPs can be further modified with stabilizing molecules that increase the stability of the PMPs (e.g., for at least one day at room temperature, and/or stable for at least one week at 4°C).

In some embodiments, the PMP is modified with a sterol. In some embodiments, the sterol is cholesterol or sitosterol. In some instances, the PMPs comprise a molar ratio of at least 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, or more than 60% sterol (e.g., cholesterol or sitosterol), e.g., 1%-10%, 10%-20%, 20%-30%, 30%-40%, 40%-50%, or 50%-60% sterol. In some embodiments, the PMP comprises a molar ratio of about 35%-50% sterol (e.g., cholesterol or sitosterol), e.g., about 36%, 38.5%, 42.5%, or 46.5% sterol. In some embodiments, the PMP comprises a molar ratio of about 20%-40% sterol. In some embodiments, the PMP comprises a molar ratio of about 10%-20% sterol.

In some embodiments, a PMP that has been modified with a sterol has altered stability (e.g., increased stability) relative to a PMP that has not been modified with a sterol. In some aspects, a PMP that has been modified with a sterol has a greater rate of fusion with a membrane of a target cell relative to a PMP that has not been modified with a sterol.

In some embodiments, the PMP is modified with a PEGylated lipid. Polyethylene glycol (PEG) length can vary from 1kDa to 10kDa; in some aspects, PEG having a length of 2kDa is used. In some embodiments, the PEGylated lipid is C18-PEG2k, DMPE-PEG2k, or ALC-0159. In some instances, the PMPs comprise a molar ratio of at least 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%, 3.5%, 4%, 4.5%, 5%, 10%, 20%, 30%, 40%, 50%, or more than 50% PEGylated lipid (e.g., C18-PEG2k, DMPE-PEG2k, or ALC-0159), e.g., 0.1%-0.5%, 0.5%-1%, 1%-1.5%, 1.5%-2.5%, 2.5%-3.5%, 3.5%-5%, 5%-10%, 10%-20%, 20%-30%, 30%-40%, or 30%-50% PEGylated lipid. In some
 5 embodiments, the PMP comprises a molar ratio of about 0.1%-10% PEGylated lipid (e.g., C18-PEG2k, DMPE-PEG2k, or ALC-0159), e.g., about 1%-3% PEGylated lipid, e.g., about 1.5% or about 2.5% PEGylated lipid. In some embodiments, a PMP that has been modified with a PEGylated lipid has altered stability (e.g., increased stability) relative to a PMP that has not been modified with a PEGylated lipid. In some embodiments, a PMP that has been modified with a PEGylated lipid has altered particle
 10 size relative to a PMP that has not been modified with a PEGylated lipid. In some embodiments, a PMP that has been modified with a PEGylated lipid is less likely to be phagocytosed than a PMP that has not been modified with a PEGylated lipid. The addition of PEGylated lipids can also affect stability in GI tract and enhance particle migration through mucus. PEG may be used as a method to attach targeting moieties. In some embodiments, the PMP comprises a molar ratio of about 0.5%-5% PEGylated lipids.

15 In some embodiments, the PMPs are modified with a synthetic charged and one or both of a sterol (e.g., cholesterol or sitosterol) and a PEGylated lipid (e.g., C18-PEG2k, DMPE-PEG2k, or ALC-0159). In embodiments, the modified PMPs comprise a molar ratio of about 5%-60% PMP lipids (e.g., about 10%-20%, 20%-30%, 30%-40%, 40%-50%, or 50%-60% PMP lipids, e.g., about 10%, 12.5%, 16%, 20%, 30%, 40%, 50%, or 60% PMP lipids); about 25%-75% synthetic charged lipids (e.g., about 35% or
 20 about 50% synthetic charged lipids); about 10%-20% sterol (e.g., about 10%, 12%, 14%, 16%, 18%, or 20% sterol); and about 0.5%-5% PEGylated lipid (e.g., about 1%-3% PEGylated lipid, e.g., about 1.5% or about 2.5% PEGylated lipid).

In some embodiments, the synthetic charged lipid, purified PMP lipids, sterol, and PEGylated lipid comprise about 25%-75%, about 35%-60%, about 10%-20%, and about 0.5%-5%, respectively, of the
 25 lipids in the modified PMP. In some embodiments, the synthetic charged lipid, purified PMP lipids, sterol, and PEGylated lipid are formulated at a molar ratio of about 35:50:12.5:2.5.

In some embodiments, a PMP that has been modified with a synthetic charged lipid and a sterol and/or a PEGylated lipid more efficiently encapsulates a negatively charged cargo (e.g., a nucleic acid) than a PMP that has not been modified with a synthetic charged lipid and a sterol and/or a PEGylated
 30 lipid. The modified PMP may have an encapsulation efficiency for the cargo (e.g., nucleic acid, e.g., RNA or DNA) that is at least 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or more than 99%, e.g., may have an encapsulation efficiency of 5%-30%, 30%-50%, 50%-70%, 70%-80%, 80%-90%, 90%-95%, or 95%-100%.

Cell uptake of the modified PMPs can be measured by a variety of methods known in the art. For
 35 example, the PMPs, or a component thereof, can be labelled with a marker (e.g., a fluorescent marker) that can be detected in isolated cells to confirm uptake. For example, cell uptake can be detected based on measures of fitness, e.g., fitness of an animal, plant, bacterium, or fungus comprising the treated cell. For instance, efficacy of the present compositions and methods can be determined by comparing fitness changes in organisms treated with the presently modified PMPs relative to treatment of compositions
 40 lacking modified PMPs.

In some embodiments, a PMP composition provided herein comprises two or more different modified PMPs, e.g., comprises modified PMPs derived from different unmodified PMPs (e.g., unmodified PMPs from two or more different plant sources) and/or comprises modified PMPs comprising different species and/or different ratios of synthetic charged lipids, sterols, and/or PEGylated lipids.

5

C. Plant EV-Markers

The PMPs of the present compositions and methods may have a range of markers that identify the PMPs as being produced using a plant EV, and/or including a segment, portion, or extract thereof. As used herein, the term "plant EV-marker" refers to a component that is naturally associated with a plant and incorporated into or onto the plant EV *in planta*, such as a plant protein, a plant nucleic acid, a plant small molecule, a plant lipid, or a combination thereof. Examples of plant EV-markers can be found, for example, in Rutter and Innes, *Plant Physiol.* 173(1): 728-741, 2017; Raimondo et al., *Oncotarget.* 6(23): 19514, 2015; Ju et al., *Mol. Therapy.* 21(7):1345-1357, 2013; Wang et al., *Molecular Therapy.* 22(3): 522-534, 2014; and Regente et al, *J of Exp. Biol.* 68(20): 5485-5496, 2017; each of which is incorporated herein by reference in its entirety. Additional examples of plant EV-markers and are further outlined herein.

In some instances, the plant EV marker can include a plant lipid. Examples of plant lipid markers that may be found in the PMPs include phytosterol, campesterol, β -sitosterol, stigmasterol, avenasterol, glycosyl inositol phosphoryl ceramides (GIPCs), glycolipids (e.g., monogalactosyldiacylglycerol (MGDG) or digalactosyldiacylglycerol (DGDG)), or a combination thereof. For instance, the PMP may include GIPCs, which represent the main sphingolipid class in plants and are one of the most abundant membrane lipids in plants. Other plant EV markers may include lipids that accumulate in plants in response to abiotic or biotic stressors (e.g., bacterial or fungal infection), such as phosphatidic acid (PA) or phosphatidylinositol-4-phosphate (PI4P).

Alternatively, the plant EV marker may include a plant protein. In some instances, the protein plant EV marker may be an antimicrobial protein naturally produced by plants, including defense proteins that plants secrete in response to abiotic or biotic stressors (e.g., bacterial or fungal infection). Plant pathogen defense proteins include soluble *N*-ethylmaleimide-sensitive factor association protein receptor protein (SNARE) proteins (e.g., Syntaxin-121 (SYP121; GenBank Accession No.: NP_187788.1 or NP_974288.1), Penetration1 (PEN1; GenBank Accession No: NP_567462.1)) or ABC transporter Penetration3 (PEN3; GenBank Accession No: NP_191283.2). Other examples of plant EV markers includes proteins that facilitate the long-distance transport of RNA in plants, including phloem proteins (e.g., Phloem protein2-A1 (PP2-A1), GenBank Accession No: NP_193719.1), calcium-dependent lipid-binding proteins, or lectins (e.g., Jacalin-related lectins, e.g., *Helianthus annuus* jacalin (Helja; GenBank: AHZ86978.1). For example, the RNA binding protein may be Glycine-Rich RNA Binding Protein-7 (GRP7; GenBank Accession Number: NP_179760.1). Additionally, proteins that regulate plasmodesmata function can in some instances be found in plant EVs, including proteins such as Synap-Totgamin A A (GenBank Accession No: NP_565495.1). In some instances, the plant EV marker can include a protein involved in lipid metabolism, such as phospholipase C or phospholipase D. In some instances, the plant protein EV marker is a cellular trafficking protein in plants. In certain instances where the plant EV

marker is a protein, the protein marker may lack a signal peptide that is typically associated with secreted proteins. Unconventional secretory proteins seem to share several common features like (i) lack of a leader sequence, (ii) absence of post-translational modifications (PTMs) specific for ER or Golgi apparatus, and/or (iii) secretion not affected by brefeldin A which blocks the classical ER/Golgi-dependent secretion pathway. One skilled in the art can use a variety of tools freely accessible to the public (e.g., SecretomeP Database; SUBA3 (SUBcellular localization database for Arabidopsis proteins)) to evaluate a protein for a signal sequence, or lack thereof.

In instances where the plant EV marker is a protein, the protein may have an amino acid sequence having at least 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% sequence identity to a plant EV marker. For example, the protein may have an amino acid sequence having at least 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% sequence identity to PEN1 from *Arabidopsis thaliana* (GenBank Accession Number: NP_567462.1).

In some instances, the plant EV marker includes a nucleic acid encoded in plants, e.g., a plant RNA, a plant DNA, or a plant PNA. For example, the PMP may include dsRNA, mRNA, a viral RNA, a microRNA (miRNA) or miRNA precursor, or a small interfering RNA (siRNA) or siRNA precursor encoded by a plant. In some instances, the nucleic acid may be one that is associated with a protein that facilitates the long-distance transport of RNA in plants, as discussed herein. In some instances, the nucleic acid plant EV marker may be one involved in host-induced gene silencing (HIGS), which is the process by which plants silence foreign transcripts of plant pests (e.g., pathogens such as fungi). For example, the nucleic acid may be one that silences bacterial or fungal genes. In some instances, the nucleic acid may be a microRNA, such as miR159 or miR166, which target genes in a fungal pathogen (e.g., *Verticillium dahliae*). In some instances, the protein may be one involved in carrying plant defense compounds, such as proteins involved in glucosinolate (GSL) transport and metabolism, including Glucosinolate Transporter-1 -1 (GTR1; GenBank Accession No: NP_566896.2), Glucosinolate Transporter-2 (GTR2; NP_201074.1), or Epithiospecific Modifier 1 (ESM1; NP_188037.1).

In instances where the plant EV marker is a nucleic acid, the nucleic acid may have a nucleotide sequence having at least 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% sequence identity to a plant EV marker. For example, the nucleic acid may have a polynucleotide sequence having at least 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% sequence identity to miR159 or miR166.

In some instances, the plant EV marker includes a compound produced by plants. For example, the compound may be a defense compound produced in response to abiotic or biotic stressors, such as secondary metabolites. One such secondary metabolite that be found in PMPs are glucosinolates (GSLs), which are nitrogen and sulfur-containing secondary metabolites found mainly in Brassicaceae plants. Other secondary metabolites may include allelochemicals.

In some instances, the PMPs may also be identified as being produced using a plant EV based on the lack of certain markers (e.g., lipids, polypeptides, or polynucleotides) that are not typically produced by plants, but are generally associated with other organisms (e.g., markers of animal EVs, bacterial EVs, or fungal EVs). For example, in some instances, the PMP lacks lipids typically found in

animal EVs, bacterial EVs, or fungal EVs. In some instances, the PMP lacks lipids typical of animal EVs (e.g., sphingomyelin). In some instances, the PMP does not contain lipids typical of bacterial EVs or bacterial membranes (e.g., LPS). In some instances, the PMP lacks lipids typical of fungal membranes (e.g., ergosterol).

5 Plant EV markers can be identified using any approaches known in the art that enable identification of small molecules (e.g., mass spectroscopy, mass spectrometry), lipids (e.g., mass spectroscopy, mass spectrometry), proteins (e.g., mass spectroscopy, immunoblotting), or nucleic acids (e.g., PCR analysis). In some instances, a PMP composition described herein includes a detectable amount, e.g., a pre-determined threshold amount, of a plant EV marker described herein.

10

D. Loading of Agents

The PMPs can be modified to include a heterologous functional agent, e.g., a cell-penetrating agent and/or a heterologous agricultural agent (e.g., pesticidal agent, fertilizing agent, herbicidal agent, plant-modifying agent), a heterologous therapeutic agent (e.g., an antifungal agent, an antibacterial agent, a virucidal agent, an anti-viral agent, an insecticidal agent, a nematocidal agent, an antiparasitic agent, or an insect repellent)), such as those described herein. The PMPs can carry or associate with such agents by a variety of means to enable delivery of the agent to a target organism (e.g., a target animal, plant, bacterium, or fungus), e.g., by encapsulating the agent, incorporation of the component in the lipid bilayer structure, or association of the component (e.g., by conjugation) with the surface of the lipid bilayer structure of the PMP. In some instances, the heterologous functional agent (e.g., cell-penetrating agent) is included in the PMP formulation, as described in Section IB herein.

The heterologous functional agent can be incorporated or loaded into or onto the PMPs by any methods known in the art that allow association, directly or indirectly, between the PMPs and agent. Heterologous functional agent agents can be incorporated into the PMPs by an *in vivo* method (e.g., *in planta*, e.g., through production of PMPs from a transgenic plant that comprises the heterologous agent), or *in vitro* (e.g., in tissue culture, or in cell culture), or both *in vivo* and *in vitro* methods.

In instances where the PMPs are loaded with a heterologous functional agent (e.g., a heterologous agricultural agent (e.g., pesticidal agent, fertilizing agent, herbicidal agent, plant-modifying agent) or a heterologous therapeutic agent (e.g., an antifungal agent, an antibacterial agent, a virucidal agent, an anti-viral agent, an insecticidal agent, a nematocidal agent, an antiparasitic agent, or an insect repellent)) *in vivo*, PMPs may be produced using EVs, or a segments or portions thereof, or an extract containing EVs that has been loaded *in planta*. *In planta* methods include expression of the heterologous functional agent (e.g., a heterologous agricultural agent (e.g., pesticidal agent, fertilizing agent, herbicidal agent, plant-modifying agent) or a heterologous therapeutic agent (e.g., an antifungal agent, an antibacterial agent, a virucidal agent, an anti-viral agent, an insecticidal agent, a nematocidal agent, an antiparasitic agent, or an insect repellent)) in a plant that has been genetically modified to express the heterologous functional agent for loading into EVs. In some instances, the heterologous functional agent is exogenous to the plant. Alternatively, the heterologous functional agent may be naturally found in the plant, but engineered to be expressed at an elevated level relative to level of that found in a non-genetically modified plant.

In some instances, the PMPs can be loaded *in vitro*. The substance may be loaded onto or into (e.g., may be encapsulated by) the PMPs using, but not limited to, physical, chemical, and/or biological methods (e.g., in tissue culture or in cell culture). For example, the heterologous functional agent may be introduced into PMPs by one or more of electroporation, sonication, passive diffusion, stirring, lipid extraction, or extrusion. In some instances, the heterologous functional agent is incorporated into the PMP using a microfluidic device, e.g., using a method in which PMP lipids are provided in an organic phase, the heterologous functional agent is provided in an aqueous phase, and the organic and aqueous phases are combined in the microfluidics device to produce a PMP comprising the heterologous functional agent. Loaded PMPs can be assessed to confirm the presence or level of the loaded agent using a variety of methods, such as HPLC (e.g., to assess small molecules), immunoblotting (e.g., to assess proteins); and/or quantitative PCR (e.g., to assess nucleotides). However, it should be appreciated by those skilled in the art that the loading of a substance of interest into PMPs is not limited to the above-illustrated methods.

In some instances, the heterologous functional agent can be conjugated to the PMP, in which the heterologous functional agent is connected or joined, indirectly or directly, to the PMP. For instance, one or more heterologous functional agents can be chemically-linked to a PMP, such that the one or more heterologous functional agents are joined (e.g., by covalent or ionic bonds) directly to the lipid bilayer of the PMP. In some instances, the conjugation of various heterologous functional agents to the PMPs can be achieved by first mixing the one or more heterologous functional agents with an appropriate cross-linking agent (e.g., N-ethylcarbo-diimide ("EDC"), which is generally utilized as a carboxyl activating agent for amide bonding with primary amines and also reacts with phosphate groups) in a suitable solvent. After a period of incubation sufficient to allow the heterologous functional agent to attach to the cross-linking agent, the cross-linking agent/ heterologous functional agent mixture can then be combined with the PMPs and, after another period of incubation, subjected to a sucrose gradient (e.g., and 8, 30, 45, and 60% sucrose gradient) to separate the free heterologous functional agent and free PMPs from the heterologous functional agent conjugated to the PMPs. As part of combining the mixture with a sucrose gradient, and an accompanying centrifugation step, the PMPs conjugated to the heterologous functional agent are then seen as a band in the sucrose gradient, such that the conjugated PMPs can then be collected, washed, and dissolved in a suitable solution for use as described herein.

In some instances, the PMPs are stably associated with the heterologous functional agent prior to and following delivery of the PMP, e.g., to a plant. In other instances, the PMPs are associated with the heterologous functional agent such that the heterologous functional agent becomes dissociated from the PMPs following delivery of the PMP, e.g., to a plant.

The PMPs can be loaded or the PMP composition can be formulated with various concentrations of the heterologous functional agent, depending on the particular agent or use. For example, in some instances, the PMPs are loaded or the PMP composition is formulated such that the PMP composition disclosed herein includes about 0.001, 0.01, 0.1, 1.0, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, or 95 (or any range between about 0.001 and 95) or more wt% of a heterologous functional agent. In some instances, the PMPs are loaded or the PMP composition is formulated such that the PMP composition includes about 95, 90, 80, 70, 60, 50, 40, 30, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1.0, 0.1, 0.01,

0.001 (or any range between about 95 and 0.001) or less wt% of a heterologous functional agent. For example, the PMP composition can include about 0.001 to about 0.01 wt%, about 0.01 to about 0.1 wt%, about 0.1 to about 1 wt%, about 1 to about 5 wt%, or about 5 to about 10 wt%, about 10 to about 20 wt% of the heterologous functional agent. In some instances, the PMP can be loaded or the PMP composition is formulated with about 1, 5, 10, 50, 100, 200, or 500, 1,000, 2,000 (or any range between about 1 and 2,000) or more µg/ml of a heterologous functional agent. A PMP of the invention can be loaded or a PMP composition can be formulated with about 2,000, 1,000, 500, 200, 100, 50, 10, 5, 1 (or any range between about 2,000 and 1) or less µg/ml of a heterologous functional agent.

In some instances, the PMPs are loaded or the PMP composition is formulated such that the PMP composition disclosed herein includes at least 0.001 wt%, at least 0.01 wt%, at least 0.1 wt%, at least 1.0 wt%, at least 2 wt%, at least 3 wt%, at least 4 wt%, at least 5 wt%, at least 6 wt%, at least 7 wt%, at least 8 wt%, at least 9 wt%, at least 10 wt%, at least 15 wt%, at least 20 wt%, at least 30 wt%, at least 40 wt%, at least 50 wt%, at least 60 wt%, at least 70 wt%, at least 80 wt%, at least 90 wt%, or at least 95 wt% of a heterologous functional agent. In some instances, the PMP can be loaded or the PMP composition can be formulated with at least 1 µg/ml, at least 5 µg/ml, at least 10 µg/ml, at least 50 µg/ml, at least 100 µg/ml, at least 200 µg/ml, at least 500 µg/ml, at least 1,000 µg/ml, at least 2,000 µg/ml of a heterologous functional agent.

In some instances, the PMP composition is formulated with the heterologous functional agent by suspending the PMPs in a solution comprising or consisting of the heterologous functional agent, e.g., suspending or resuspending the PMPs by vigorous mixing. The heterologous functional agent (e.g., cell-penetrating agent, e.g., enzyme, detergent, ionic, fluoruous, or zwitterionic liquid, or synthetic charged lipid may comprise, e.g., less than 1% or at least 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the solution.

Examples of particular heterologous functional agents that can be loaded into the PMPs are further outlined in the section entitled "Heterologous Functional Agents."

E. Production of LPMPs using microfluidics

In some aspects, the PMP composition comprises a plurality of lipid reconstructed PMPs (LPMPs), wherein the LPMPs are produced by a process which comprises the steps of (a) providing a plurality of purified PMPs (e.g., PMPs purified as described in Section IA herein); (b) processing the plurality of PMPs to produce a lipid film; (c) reconstituting the lipid film in an organic solvent or solvent combination, thereby producing a lipid solution; and (d) processing the lipid solution of step (c) in a microfluidics device comprising an aqueous phase, thereby producing the LPMPs; wherein the LPMPs comprise a synthetic charged lipid, wherein the synthetic charged lipid has at least one (e.g., one, two, three, four or all five) of the characteristics listed below:

- (i) at least 2 ionizable amines (e.g., at least 2, at least 3, at least 4, at least 5, at least 6, or more than 6 ionizable amines, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more than 12 ionizable amines);
- (ii) at least 3 lipid tails (e.g., at least 3, at least 4, at least 5, at least 6, or more than 6 lipid tails, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more than 12 lipid tails), wherein each of the lipid tails is independently at least 6 carbon atoms in length (e.g., at least 6, at least 7, at least 8, at least 9, at least

10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, or more than 18 carbon atoms in length, e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or more than 25 carbon atoms in length);

(iii) an acid dissociation constant (pKa) of about 4.5 to about 7.5 (e.g., a pKa of about 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, or 7.5 (e.g., a pKa of between about 6.5 and about 7.5 (e.g., a pKa of about 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, or 7.5)));

(iv) an ionizable amine and a heteroorganic group separated by a chain of at least two atoms; and

(v) an N:P (amines of ionizable lipid:phosphates of mRNA) ratio of at least 10; provided that the charged lipid is not selected from 1'-((2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethyl)azanediyl)bis(dodecan-2-ol) (C12-200), MD1 (cKK-E12), OF2, EPC, ZA3-Ep10, TT3, LP01, 5A2-SC8, Lipid 5 (Moderna), and 98N12-5.

In some embodiments, the heteroorganic group is hydroxyl. In some embodiments, the heteroorganic group comprises a hydrogen bond donor. In some embodiments, the heteroorganic group comprises a hydrogen bond acceptor. In some embodiments, the heteroorganic group is -OH, -SH, - (CO)H, -CO₂H, -NH₂, -CONH₂, optionally substituted C₁-C₆ alkoxy, or fluorine.

In some instances, processing the plurality of PMPs to produce a lipid film includes extracting lipids from the plurality of PMPs, e.g., extracting lipids using the Bligh-Dyer method (Bligh and Dyer, *J Biolchem Physiol*, 37: 911-917, 1959). The extracted lipids may be provided as a stock solution, e.g., a solution in chloroform:methanol. Producing the lipid film may comprise, e.g., evaporation of the solvent with a stream of inert gas (e.g., nitrogen). In some examples, the exogenous lipid is added to the preparation prior to step (b), e.g., mixed with extracted PMP lipids prior to step (b). The exogenous lipids may be added to amount to, e.g., 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more than 90% (w/w) of total lipids in the preparation. In some examples, the exogenous lipid (e.g., charged lipid, e.g., ionizable lipid and/or cationic lipid) is added to amount to 25% or 40% (w/w) of total lipids in the preparation.

In some instances, the LPMPs comprise an exogenous sterol, e.g., sitosterol, sitostanol, β -sitosterol, 7 α -hydroxycholesterol, pregnenolone, cholesterol (e.g., ovine cholesterol or cholesterol isolated from plants), stigmasterol, campesterol, fucosterol, or an analog (e.g., a glycoside, ester, or peptide) of any sterol. In some examples, the exogenous sterol is added to the preparation prior to step (b), e.g., mixed with extracted PMP lipids prior to step (b). The exogenous sterol may be added to amount to, e.g., 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more than 90% (w/w) of total lipids and sterols in the preparation.

In some instances, the LPMPs comprise a synthetic charged lipid and an exogenous sterol.

In some instances, the organic solvent in which the lipid film is dissolved is dimethylformamide:methanol (DMF:MeOH). Alternatively, the organic solvent or solvent combination may be, e.g., acetonitrile, acetone, ethanol, methanol, dimethylformamide, tetrahydrofuran, 1-butanol, dimethyl sulfoxide, acetonitrile:ethanol, acetonitrile:methanol, acetone:methanol, methyl tert-butyl ether:propanol, tetrahydrofuran:methanol, dimethyl sulfoxide:methanol, or dimethylformamide:methanol.

The aqueous phase may be any suitable solution, e.g., a citrate buffer (e.g., a citrate buffer having a pH of about 3.2), water, or phosphate-buffered saline (PBS). The aqueous phase may further comprise a heterologous functional agent, e.g., an agent described in Section II herein, e.g., a nucleic acid (e.g., an siRNA or siRNA precursor (e.g., dsRNA), miRNA or miRNA precursor, mRNA, or plasmid (pDNA)) or a small molecule.

The lipid solution and the aqueous phase may be mixed in the microfluidics device at any suitable ratio. In some examples, aqueous phase and the lipid solution are mixed at a 3:1 volumetric ratio.

F. Zeta Potential

5 The PMP composition comprising a plurality of modified PMPs comprising a synthetic charged lipid may have, e.g., a zeta potential of greater than -30 mV when in the absence of cargo, greater than -20 mV, greater than -5mV, greater than 0 mV, or about 30 mv when in the absence of cargo. In some examples, the PMP composition has a negative zeta potential, e.g., a zeta potential of less than 0 mV, less than -10 mV, less than -20 mV, less than -30 mV, less than -40 mV, or less than -50 mV when in the absence of cargo. In some examples, the PMP composition has a positive zeta potential, e.g., a zeta potential of greater than 0 mV, greater than 10 mV, greater than 20 mV, greater than 30 mV, greater than 40 mV, or greater than 50 mV when in the absence of cargo. In some examples, the PMP composition has a zeta potential of about 0.

The zeta potential of the PMP composition may be measured using any method known in the art. Zeta potentials are generally measured indirectly, e.g., calculated using theoretical models from the data obtained using methods and techniques known in the art, e.g., electrophoretic mobility or dynamic electrophoretic mobility. Electrophoretic mobility is typically measured using microelectrophoresis, electrophoretic light scattering, or tunable resistive pulse sensing. Electrophoretic light scattering is based on dynamic light scattering. Typically, zeta potentials are accessible from dynamic light scattering (DLS) measurements, also known as photon correlation spectroscopy or quasi-elastic light scattering.

G. Formulations

i. Agricultural Formulations

To allow ease of application, handling, transportation, storage, and effective activity, PMPs (e.g., modified PMPs as described herein), can be formulated with other substances. PMPs can be formulated into, for example, baits, concentrated emulsions, dusts, emulsifiable concentrates, fumigants, gels, granules, microencapsulations, seed treatments, suspension concentrates, suspoemulsions, tablets, water soluble liquids, water dispersible granules or dry flowables, wettable powders, and ultra-low volume solutions. For further information on formulation types see "Catalogue of Pesticide Formulation Types and International Coding System" Technical Monograph n° 2, 5th Edition by CropLife International (2002).

PMP compositions can be applied as aqueous suspensions or emulsions prepared from concentrated formulations of such agents. Such water-soluble, water-suspendable, or emulsifiable formulations are either solids, usually known as wettable powders, or water dispersible granules, or liquids usually known as emulsifiable concentrates, or aqueous suspensions. Wettable powders, which may be compacted to form water dispersible granules, comprise an intimate mixture of the PMP

composition, a carrier, and surfactants. The carrier is usually selected from among the attapulgite clays, the montmorillonite clays, the diatomaceous earths, or the purified silicates. Effective surfactants, including from about 0.5% to about 10% of the wettable powder, are found among sulfonated lignins, condensed naphthalenesulfonates, naphthalenesulfonates, alkylbenzenesulfonates, alkyl sulfates, and non-ionic surfactants such as ethylene oxide adducts of alkyl phenols.

Emulsifiable concentrates can comprise a suitable concentration of PMPs, such as from about 50 to about 500 grams per liter of liquid dissolved in a carrier that is either a water miscible solvent or a mixture of water-immiscible organic solvent and emulsifiers. Useful organic solvents include aromatics, especially xylenes and petroleum fractions, especially the high-boiling naphthalenic and olefinic portions of petroleum such as heavy aromatic naphtha. Other organic solvents may also be used, such as the terpenic solvents including rosin derivatives, aliphatic ketones such as cyclohexanone, and complex alcohols such as 2-ethoxyethanol. Suitable emulsifiers for emulsifiable concentrates are selected from conventional anionic and non-ionic surfactants.

Aqueous suspensions comprise suspensions of water-insoluble PMP compositions dispersed in an aqueous carrier at a concentration in the range from about 5% to about 50% by weight. Suspensions are prepared by finely grinding the composition and vigorously mixing it into a carrier comprised of water and surfactants. Ingredients, such as inorganic salts and synthetic or natural gums may also be added, to increase the density and viscosity of the aqueous carrier.

PMP compositions may also be applied as granular compositions that are particularly useful for applications to the soil. Granular compositions usually contain from about 0.5% to about 10% by weight of the PMP composition, dispersed in a carrier that comprises clay or a similar substance. Such compositions are usually prepared by dissolving the formulation in a suitable solvent and applying it to a granular carrier which has been pre-formed to the appropriate particle size, in the range of from about 0.5 to about 3 mm. Such compositions may also be formulated by making a dough or paste of the carrier and compound and crushing and drying to obtain the desired granular particle size.

Dusts containing the present PMP formulation are prepared by intimately mixing PMPs in powdered form with a suitable dusty agricultural carrier, such as kaolin clay, ground volcanic rock, and the like. Dusts can suitably contain from about 1% to about 10% of the packets. They can be applied as a seed dressing or as a foliage application with a dust blower machine.

It is equally practical to apply the present formulation in the form of a solution in an appropriate organic solvent, usually petroleum oil, such as the spray oils, which are widely used in agricultural chemistry.

PMPs can also be applied in the form of an aerosol composition. In such compositions the packets are dissolved or dispersed in a carrier, which is a pressure-generating propellant mixture. The aerosol composition is packaged in a container from which the mixture is dispensed through an atomizing valve.

Another embodiment is an oil-in-water emulsion, wherein the emulsion comprises oily globules which are each provided with a lamellar liquid crystal coating and are dispersed in an aqueous phase, wherein each oily globule comprises at least one compound which is agriculturally active, and is individually coated with a monolamellar or oligolamellar layer including: (1) at least one non-ionic lipophilic

surface-active agent, (2) at least one non-ionic hydrophilic surface-active agent and (3) at least one ionic surface-active agent, wherein the globules having a mean particle diameter of less than 800 nanometers. Further information on the embodiment is disclosed in U.S. patent publication 20070027034 published Feb. 1, 2007. For ease of use, this embodiment will be referred to as "OIWE."

5 Additionally, generally, when the molecules disclosed above are used in a formulation, such formulation can also contain other components. These components include, but are not limited to, (this is a non-exhaustive and non-mutually exclusive list) wetters, spreaders, stickers, penetrants, buffers, sequestering agents, drift reduction agents, compatibility agents, anti-foam agents, cleaning agents, and emulsifiers. A few components are described forthwith.

10 A wetting agent is a substance that when added to a liquid increases the spreading or penetration power of the liquid by reducing the interfacial tension between the liquid and the surface on which it is spreading. Wetting agents are used for two main functions in agrochemical formulations: during processing and manufacture to increase the rate of wetting of powders in water to make concentrates for soluble liquids or suspension concentrates; and during mixing of a product with water in a spray tank to
15 reduce the wetting time of wettable powders and to improve the penetration of water into water-dispersible granules. Examples of wetting agents used in wettable powder, suspension concentrate, and water-dispersible granule formulations are: sodium lauryl sulfate; sodium dioctyl sulfosuccinate; alkyl phenol ethoxylates; and aliphatic alcohol ethoxylates.

 A dispersing agent is a substance which adsorbs onto the surface of particles and helps to
20 preserve the state of dispersion of the particles and prevents them from reaggregating. Dispersing agents are added to agrochemical formulations to facilitate dispersion and suspension during manufacture, and to ensure the particles redisperse into water in a spray tank. They are widely used in wettable powders, suspension concentrates and water-dispersible granules. Surfactants that are used as dispersing agents have the ability to adsorb strongly onto a particle surface and provide a charged or
25 steric barrier to reaggregation of particles. The most commonly used surfactants are anionic, non-ionic, or mixtures of the two types. For wettable powder formulations, the most common dispersing agents are sodium lignosulfonates. For suspension concentrates, very good adsorption and stabilization are obtained using polyelectrolytes, such as sodium naphthalene sulfonate formaldehyde condensates. Tristyrylphenol ethoxylate phosphate esters are also used. Non-ionics such as alkylarylethylene oxide
30 condensates and EO-PO block copolymers are sometimes combined with anionics as dispersing agents for suspension concentrates. In recent years, new types of very high molecular weight polymeric surfactants have been developed as dispersing agents. These have very long hydrophobic 'backbones' and a large number of ethylene oxide chains forming the 'teeth' of a 'comb' surfactant. These high molecular weight polymers can give very good long-term stability to suspension concentrates because
35 the hydrophobic backbones have many anchoring points onto the particle surfaces. Examples of dispersing agents used in agrochemical formulations are: sodium lignosulfonates; sodium naphthalene sulfonate formaldehyde condensates; tristyrylphenol ethoxylate phosphate esters; aliphatic alcohol ethoxylates; alkyl ethoxylates; EO-PO (ethylene oxide - propylene oxide) block copolymers; and graft copolymers.

An emulsifying agent is a substance which stabilizes a suspension of droplets of one liquid phase in another liquid phase. Without the emulsifying agent the two liquids would separate into two immiscible liquid phases. The most commonly used emulsifier blends contain alkylphenol or aliphatic alcohol with twelve or more ethylene oxide units and the oil-soluble calcium salt of dodecylbenzenesulfonic acid. A
5 range of hydrophile-lipophile balance ("HLB") values from 8 to 18 will normally provide good stable emulsions. Emulsion stability can sometimes be improved by the addition of a small amount of an EO-PO block copolymer surfactant.

A solubilizing agent is a surfactant which will form micelles in water at concentrations above the critical micelle concentration. The micelles are then able to dissolve or solubilize water-insoluble
10 materials inside the hydrophobic part of the micelle. The types of surfactants usually used for solubilization are non-ionics, sorbitan monooleates, sorbitan monooleate ethoxylates, and methyl oleate esters.

Surfactants are sometimes used, either alone or with other additives such as mineral or vegetable oils as adjuvants to spray-tank mixes to improve the biological performance of the PMP
15 composition on the target. The types of surfactants used for bioenhancement depend generally on the nature and mode of action of the PMP composition. However, they are often non-ionics such as: alkyl ethoxylates; linear aliphatic alcohol ethoxylates; aliphatic amine ethoxylates.

A carrier or diluent in an agricultural formulation is a material added to the PMP composition to give a product of the required strength. Carriers are usually materials with high absorptive capacities,
20 while diluents are usually materials with low absorptive capacities. Carriers and diluents are used in the formulation of dusts, wettable powders, granules, and water-dispersible granules.

Organic solvents are used mainly in the formulation of emulsifiable concentrates, oil-in-water emulsions, suspoemulsions, and ultra low volume formulations, and to a lesser extent, granular formulations. Sometimes mixtures of solvents are used. The first main groups of solvents are aliphatic
25 paraffinic oils such as kerosene or refined paraffins. The second main group (and the most common) comprises the aromatic solvents such as xylene and higher molecular weight fractions of C9 and C10 aromatic solvents. Chlorinated hydrocarbons are useful as cosolvents to prevent crystallization of PMP composition when the formulation is emulsified into water. Alcohols are sometimes used as cosolvents to increase solvent power. Other solvents may include vegetable oils, seed oils, and esters of vegetable
30 and seed oils.

Thickeners or gelling agents are used mainly in the formulation of suspension concentrates, emulsions, and suspoemulsions to modify the rheology or flow properties of the liquid and to prevent separation and settling of the dispersed particles or droplets. Thickening, gelling, and anti-settling agents generally fall into two categories, namely water-insoluble particulates and water-soluble polymers. It is
35 possible to produce suspension concentrate formulations using clays and silicas. Examples of these types of materials, include, but are not limited to, montmorillonite, bentonite, magnesium aluminum silicate, and attapulgite. Water-soluble polysaccharides have been used as thickening-gelling agents for many years. The types of polysaccharides most commonly used are natural extracts of seeds and seaweeds or are synthetic derivatives of cellulose. Examples of these types of materials include, but are
40 not limited to, guar gum; locust bean gum; carrageenan; alginates; methyl cellulose; sodium

carboxymethyl cellulose (SCMC); hydroxyethyl cellulose (HEC). Other types of anti-settling agents are based on modified starches, polyacrylates, polyvinyl alcohol, and polyethylene oxide. Another good anti-settling agent is xanthan gum.

Microorganisms can cause spoilage of formulated products. Therefore preservation agents are used to eliminate or reduce their effect. Examples of such agents include, but are not limited to: propionic acid and its sodium salt; sorbic acid and its sodium or potassium salts; benzoic acid and its sodium salt; p-hydroxybenzoic acid sodium salt; methyl p-hydroxybenzoate; and 1,2-benzisothiazolin-3-one (BIT).

The presence of surfactants often causes water-based formulations to foam during mixing operations in production and in application through a spray tank. In order to reduce the tendency to foam, anti-foam agents are often added either during the production stage or before filling into bottles. Generally, there are two types of anti-foam agents, namely silicones and non-silicones. Silicones are usually aqueous emulsions of dimethyl polysiloxane, while the non-silicone anti-foam agents are water-insoluble oils, such as octanol and nonanol, or silica. In both cases, the function of the anti-foam agent is to displace the surfactant from the air-water interface.

“Green” agents (e.g., adjuvants, surfactants, solvents) can reduce the overall environmental footprint of crop protection formulations. Green agents are biodegradable and generally derived from natural and/or sustainable sources, e.g., plant and animal sources. Specific examples are: vegetable oils, seed oils, and esters thereof, also alkoxylated alkyl polyglucosides.

In some instances, PMPs can be freeze-dried or lyophilized. See U.S. Pat. No. 4,311,712. The PMPs can later be reconstituted on contact with water or another liquid. Other components can be added to the lyophilized or reconstituted PMPs, for example, other heterologous functional agents, agriculturally acceptable carriers, or other materials in accordance with the formulations described herein.

Other optional features of the composition include carriers or delivery vehicles that protect the PMP composition against UV and/or acidic conditions. In some instances, the delivery vehicle contains a pH buffer. In some instances, the composition is formulated to have a pH in the range of about 4.5 to about 9.0, including for example pH ranges of about any one of 5.0 to about 8.0, about 6.5 to about 7.5, or about 6.5 to about 7.0.

For further information on agricultural formulations, see “Chemistry and Technology of Agrochemical Formulations” edited by D. A. Knowles, copyright 1998 by Kluwer Academic Publishers. Also see “Insecticides in Agriculture and Environment—Retrospects and Prospects” by A. S. Perry, I. Yamamoto, I. Ishaaya, and R. Perry, copyright 1998 by Springer-Verlag.

ii. Pharmaceutical Formulations

The modified PMPs described herein can be formulated into pharmaceutical compositions, e.g., for administration to an animal (e.g., a human). The pharmaceutical composition may be administered to an animal (e.g., human) with a pharmaceutically acceptable diluent, carrier, and/or excipient. Depending on the mode of administration and the dosage, the pharmaceutical composition of the methods described herein will be formulated into suitable pharmaceutical compositions to permit facile delivery. The single dose may be in a unit dose form as needed.

A PMP composition may be formulated for e.g., oral administration, intravenous administration (e.g., injection or infusion), or subcutaneous administration to an animal. For injectable formulations, various effective pharmaceutical carriers are known in the art (See, e.g., Remington: The Science and Practice of Pharmacy, 22nd ed., (2012) and ASHP Handbook on Injectable Drugs, 18th ed., (2014)).

5 Pharmaceutically acceptable carriers and excipients in the present compositions are nontoxic to recipients at the dosages and concentrations employed. Acceptable carriers and excipients may include buffers such as phosphate, citrate, HEPES, and TAE, antioxidants such as ascorbic acid and methionine, preservatives such as hexamethonium chloride, octadecyldimethylbenzyl ammonium chloride, resorcinol, and benzalkonium chloride, proteins such as human serum albumin, gelatin, dextran, and
10 immunoglobulins, hydrophilic polymers such as polyvinylpyrrolidone, amino acids such as glycine, glutamine, histidine, and lysine, and carbohydrates such as glucose, mannose, sucrose, and sorbitol. The compositions may be formulated according to conventional pharmaceutical practice. The concentration of the compound in the formulation will vary depending upon a number of factors, including the dosage of the active agent (e.g., PMP) to be administered, and the route of administration.

15 For oral administration to an animal, the PMP composition can be prepared in the form of an oral formulation. Formulations for oral use can include tablets, caplets, capsules, syrups, or oral liquid dosage forms containing the active ingredient(s) in a mixture with non-toxic pharmaceutically acceptable excipients. These excipients may be, for example, inert diluents or fillers (e.g., sucrose, sorbitol, sugar, mannitol, microcrystalline cellulose, starches including potato starch, calcium carbonate, sodium chloride,
20 lactose, calcium phosphate, calcium sulfate, or sodium phosphate); granulating and disintegrating agents (e.g., cellulose derivatives including microcrystalline cellulose, starches including potato starch, croscarmellose sodium, alginates, or alginic acid); binding agents (e.g., sucrose, glucose, sorbitol, acacia, alginic acid, sodium alginate, gelatin, starch, pregelatinized starch, microcrystalline cellulose, magnesium aluminum silicate, carboxymethylcellulose sodium, methylcellulose, hydroxypropyl methylcellulose,
25 ethylcellulose, polyvinylpyrrolidone, or polyethylene glycol); and lubricating agents, glidants, and antiadhesives (e.g., magnesium stearate, zinc stearate, stearic acid, silicas, hydrogenated vegetable oils, or talc). Other pharmaceutically acceptable excipients can be colorants, flavoring agents, plasticizers, humectants, buffering agents, and the like. Formulations for oral use may also be provided in unit dosage form as chewable tablets, non-chewable tablets, caplets, capsules (e.g., as hard gelatin capsules wherein
30 the active ingredient is mixed with an inert solid diluent, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium). The compositions disclosed herein may also further include an immediate-release, extended release or delayed-release formulation.

For parenteral administration to an animal, the PMP compositions may be formulated in the form of liquid solutions or suspensions and administered by a parenteral route (e.g., subcutaneous,
35 intravenous, or intramuscular). The pharmaceutical composition can be formulated for injection or infusion. Pharmaceutical compositions for parenteral administration can be formulated using a sterile solution or any pharmaceutically acceptable liquid as a vehicle. Pharmaceutically acceptable vehicles include, but are not limited to, sterile water, physiological saline, or cell culture media (e.g., Dulbecco's Modified Eagle Medium (DMEM), α -Modified Eagles Medium (α -MEM), F-12 medium). Formulation

methods are known in the art, see e.g., Gibson (ed.) *Pharmaceutical Preformulation and Formulation* (2nd ed.) Taylor & Francis Group, CRC Press (2009).

II. Heterologous Functional Agents

5 The PMPs manufactured herein can further include a heterologous functional agent (e.g., a heterologous agricultural agent (e.g., pesticidal agent, fertilizing agent, herbicidal agent, plant-modifying agent) or a heterologous therapeutic agent (e.g., an antifungal agent, an antibacterial agent, a virucidal agent, an anti-viral agent, an insecticidal agent, a nematicidal agent, an antiparasitic agent, or an insect repellent)). For example, the PMP may encapsulate the heterologous functional agent. Alternatively, the
10 heterologous functional agent can be embedded on or conjugated to the surface of the PMP. In some instances, the PMPs include two or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10) different heterologous functional agents. Heterologous functional agents may be added at any step during the manufacturing process effective to introduce the agent into the manufactured PMPs.

 In certain instances, the heterologous functional agent (e.g., a heterologous agricultural agent
15 (e.g., pesticidal agent, fertilizing agent, herbicidal agent, plant-modifying agent, a heterologous nucleic acid, a heterologous polypeptide, or a heterologous small molecule) or a heterologous therapeutic agent (e.g., an antifungal agent, an antibacterial agent, a virucidal agent, an anti-viral agent, a nematicidal agent, an antiparasitic agent, or an insect repellent)) can be modified. For example, the modification can be a chemical modification, e.g., conjugation to a marker, e.g., fluorescent marker or a radioactive
20 marker. In other examples, the modification can include conjugation or operational linkage to a moiety that enhances the stability, delivery, targeting, bioavailability, or half-life of the agent, e.g., a lipid, a glycan, a polymer (e.g., PEG), or a cation moiety.

 Examples of heterologous functional agents that can be loaded into the PMPs manufactured herein are outlined below.

25 A. Heterologous agricultural agents

 The PMPs manufactured herein can include a heterologous agricultural agent (e.g., an agent that effects a plant or an organism that associates with a plant and can be loaded into a PMP), such as a pesticidal agent, herbicidal agent, fertilizing agent, or a plant-modifying agent.

30 For example, in some instances, the PMPs may include a pesticidal agent. The pesticidal agent can be an antifungal agent, an antibacterial agent, an insecticidal agent, a molluscicidal agent, a nematicidal agent, a virucidal agent, or a combination thereof. The pesticidal agent can be a chemical agent, such as those well known in the art. Alternatively or additionally, the pesticidal agent can be a peptide, a polypeptide, a nucleic acid, a polynucleotide, or a small molecule. The pesticidal agent may be
35 an agent that can decrease the fitness of a variety of plant pests or can be one that targets one or more specific target plant pests (e.g., a specific species or genus of plant pests).

 In some instances, the PMPs may include one or more heterologous fertilizing agents. Examples of heterologous fertilizing agents include plant nutrients or plant growth regulators, such as those well known in the art. Alternatively, or additionally, the fertilizing agent can be a peptide, a polypeptide, a
40 nucleic acid, or a polynucleotide that can increase the fitness of a plant symbiont. The fertilizing agent may be an agent that can increase the fitness of a variety of plants or plant symbionts or can be one that

targets one or more specific target plants or plant symbionts (e.g., a specific species or genera of plants or plant symbionts).

In other instances, the PMPs may include one or more heterologous plant-modifying agents. In some instances, the plant-modifying agent can include a peptide or a nucleic acid.

5

i. Antibacterial agents

The PMP compositions described herein can further include an antibacterial agent. In some instances, the PMP compositions include two or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10) different antibacterial agents. For example, the antibacterial agent can decrease the fitness of (e.g., decrease growth or kill) a bacterial plant pest (e.g., a bacterial plant pathogen). A PMP composition including an antibiotic as described herein can be contacted with a target pest, or plant infested thereof, in an amount and for a time sufficient to: (a) reach a target level (e.g., a predetermined or threshold level) of antibiotic concentration inside or on the target pest; and (b) decrease fitness of the target pest. The antibacterials described herein may be formulated in a PMP composition for any of the methods described herein, and in certain instances, may be associated with the PMP thereof.

As used herein, the term “antibacterial agent” refers to a material that kills or inhibits the growth, proliferation, division, reproduction, or spread of bacteria, such as phytopathogenic bacteria, and includes bactericidal (e.g., disinfectant compounds, antiseptic compounds, or antibiotics) or bacteriostatic agents (e.g., compounds or antibiotics). Bactericidal antibiotics kill bacteria, while bacteriostatic antibiotics only slow their growth or reproduction.

Bactericides can include disinfectants, antiseptics, or antibiotics. The most used disinfectants can comprise: active chlorine (i.e., hypochlorites (e.g., sodium hypochlorite), chloramines, dichloroisocyanurate and trichloroisocyanurate, wet chlorine, chlorine dioxide etc.), active oxygen (peroxides, such as peracetic acid, potassium persulfate, sodium perborate, sodium percarbonate and urea perhydrate), iodine (iodopovidone (povidone-iodine, Betadine), Lugol's solution, iodine tincture, iodinated nonionic surfactants), concentrated alcohols (mainly ethanol, 1-propanol, called also n-propanol and 2-propanol, called isopropanol and mixtures thereof; further, 2-phenoxyethanol and 1- and 2-phenoxypropanols are used), phenolic substances (such as phenol (also called carbolic acid), cresols (called Lysole in combination with liquid potassium soaps), halogenated (chlorinated, brominated) phenols, such as hexachlorophene, triclosan, trichlorophenol, tribromophenol, pentachlorophenol, Dibromol and salts thereof), cationic surfactants, such as some quaternary ammonium cations (such as benzalkonium chloride, cetyl trimethylammonium bromide or chloride, didecyldimethylammonium chloride, cetylpyridinium chloride, benzethonium chloride) and others, non-quaternary compounds, such as chlorhexidine, glucoprotamine, octenidine dihydrochloride etc.), strong oxidizers, such as ozone and permanganate solutions; heavy metals and their salts, such as colloidal silver, silver nitrate, mercury chloride, phenylmercury salts, copper sulfate, copper oxide-chloride, copper hydroxide, copper octanoate, copper oxychloride sulfate, copper sulfate, copper sulfate pentahydrate, etc. Heavy metals and their salts are the most toxic, and environment-hazardous bactericides and therefore, their use is strongly oppressed or canceled; further, also properly concentrated strong acids (phosphoric, nitric, sulfuric, amidosulfuric, toluenesulfonic acids) and alkalis (sodium, potassium, calcium hydroxides).

As antiseptics (i.e., germicide agents that can be used on human or animal body, skin, mucoses, wounds and the like), few of the above mentioned disinfectants can be used, under proper conditions (mainly concentration, pH, temperature and toxicity toward man/animal). Among them, important are: properly diluted chlorine preparations (i.e., Daquin's solution, 0.5% sodium or potassium hypochlorite solution, pH-adjusted to pH 7-8, or 0.5-1% solution of sodium benzenesulfochloramide (chloramine B)), some iodine preparations, such as iodopovidone in various galenics (ointment, solutions, wound plasters), in the past also Lugol's solution, peroxides as urea perhydrate solutions and pH-buffered 0.1-0.25% peracetic acid solutions, alcohols with or without antiseptic additives, used mainly for skin antiseptics, weak organic acids such as sorbic acid, benzoic acid, lactic acid and salicylic acid some phenolic compounds, such as hexachlorophene, triclosan and Dibromol, and cation-active compounds, such as 0.05-0.5% benzalkonium, 0.5-4% chlorhexidine, 0.1-2% octenidine solutions.

The PMP composition described herein may include an antibiotic. Any antibiotic known in the art may be used. Antibiotics are commonly classified based on their mechanism of action, chemical structure, or spectrum of activity.

The antibiotic described herein may target any bacterial function or growth processes and may be either bacteriostatic (e.g., slow or prevent bacterial growth) or bactericidal (e.g., kill bacteria). In some instances, the antibiotic is a bactericidal antibiotic. In some instances, the bactericidal antibiotic is one that targets the bacterial cell wall (e.g., penicillins and cephalosporins); one that targets the cell membrane (e.g., polymyxins); or one that inhibits essential bacterial enzymes (e.g., rifamycins, lipiarmycins, quinolones, and sulfonamides). In some instances, the bactericidal antibiotic is an aminoglycoside (e.g., kasugamycin). In some instances, the antibiotic is a bacteriostatic antibiotic. In some instances the bacteriostatic antibiotic targets protein synthesis (e.g., macrolides, lincosamides, and tetracyclines). Additional classes of antibiotics that may be used herein include cyclic lipopeptides (such as daptomycin), glycolcyclines (such as tigecycline), oxazolidinones (such as linezolid), or lipiarmycins (such as fidaxomicin). Examples of antibiotics include rifampicin, ciprofloxacin, doxycycline, ampicillin, and polymyxin B. The antibiotic described herein may have any level of target specificity (e.g., narrow- or broad-spectrum). In some instances, the antibiotic is a narrow-spectrum antibiotic, and thus targets specific types of bacteria, such as gram-negative or gram-positive bacteria. Alternatively, the antibiotic may be a broad-spectrum antibiotic that targets a wide range of bacteria.

Other non-limiting examples of antibiotics are found in Table 1. One skilled in the art will appreciate that a suitable concentration of each antibiotic in the composition depends on factors such as efficacy, stability of the antibiotic, number of distinct antibiotics, the formulation, and methods of application of the composition.

Table 1. Examples of antibiotics

Antibiotics	Action
Penicillins, cephalosporins, vancomycin	Cell wall synthesis
Polymixin, gramicidin	Membrane active agent, disrupt cell membrane

Tetracyclines, macrolides, chloramphenicol, clindamycin, spectinomycin	Inhibit protein synthesis
Sulfonamides	Inhibit folate-dependent pathways
Ciprofloxacin	Inhibit DNA-gyrase
Isoniazid, rifampicin, pyrazinamide, ethambutol, (myambutol)l, streptomycin	Antimycobacterial agents

ii. Antifungal agents

The PMP compositions described herein can further include an antifungal agent. In some instances, the PMP compositions include two or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10) different antifungal agents. For example, the antifungal agent can decrease the fitness of (e.g., decrease growth or kill) a fungal plant pest. A PMP composition including an antifungal as described herein can be contacted with a target fungal pest, or plant infested therewith, in an amount and for a time sufficient to: (a) reach a target level (e.g., a predetermined or threshold level) of antibiotic concentration inside or on the target fungus; and (b) decrease fitness of the target fungus. The antifungals described herein may be formulated in a PMP composition for any of the methods described herein, and in certain instances, may be associated with the PMP thereof.

As used herein, the term "fungicide" or "antifungal agent" refers to a substance that kills or inhibits the growth, proliferation, division, reproduction, or spread of fungi, such as phytopathogenic fungi. Many different types of antifungal agent have been produced commercially. Non limiting examples of antifungal agents include: azoxystrobin, mancozeb, prothioconazole, folpet, tebuconazole, difenoconazole, captan, bupirimate, or fosetyl-AI. Further exemplary fungicides include, but are not limited to, strobilurins, azoxystrobin, dimoxystrobin, enestroburin, fluoxastrobin, kresoxim-methyl, metominostrobin, picoxystrobin, pyraclostrobin, trifloxystrobin, orysastrobin, carboxamides, carboxanilides, benalaxyl, benalaxyl-M, benodanil, carboxin, mebenil, mepronil, fenfuram, fenhexamid, flutolanil, furalaxyl, furcarbanil, furametpyr, metalaxyl, metalaxyl-M (mefenoxam), methfuroxam, metsulfosax, ofurace, oxadixyl, oxycarboxin, penthiopyrad, pyracarbolid, salicylanilide, tecloftalam, thifluzamide, tiadinil, N-biphenylamides, bixafen, boscalid, carboxylic acid morpholides, dimethomorph, flumorph, benzamides, flumetover, fluopicolid (picobenzamid), zoxamid, carboxamides, carpropamid, diclocymet, mandipropamid, silthiofam, azoles, triazoles, bitertanol, bromuconazole, cyproconazole, difenoconazole, diniconazole, enilconazole, epoxiconazole, fenbuconazole, flusilazol, fluquinconazole, flutriafol, hexaconazole, imibenconazole, ipconazole, metconazole, myclobutanil, penconazole, propiconazole, prothioconazole, simeconazole, tebuconazole, tetraconazole, triadimenol, triadimefon, triticonazole, Imidazoles, cyazofamid, imazalil, pefurazoate, prochloraz, triflumizole, benzimidazoles, benomyl, carbendazim, fuberidazole, thiabendazole, ethaboxam, etridiazole, hymexazol, nitrogen-containing heterocyclyl compounds, pyridines, fuazinam, pyrifenoxy, pyrimidines, bupirimate, cyprodinil, ferimzone, fenarimol, mepanipyrim, nuarimol, pyrimethanil, piperazines, triforine, pyrroles, fludioxonil, fenpiclonil, morpholines, aldimorph, dodemorph, fenpropimorph, tridemorph, dicarboximides, iprodione, procymidone, vinclozolin, acibenzolar-S-methyl, anilazine, captan, captafol, dazomet, diclomezin,

fenoxanil, folpet, fenpropidin, famoxadon, fenamidon, oclthilone, probenazole, proquinazid, pyroquilon, quinoxifen, tricyclazole, carbamates, dithiocarbamates, ferbam, mancozeb, maneb, metiram, metam, propineb, thiram, zineb, ziram, diethofencarb, flubenthiavdicarb, iprovalicarb, propamocarb, guanidines, dodine, iminoctadine, guazatine, kasugamycin, polyoxins, streptomycin, validamycin A, organometallic

5 compounds, fentin salts, sulfur-containing heterocyclcyl compounds, isoprothiolane, dithianone, organophosphorous compounds, edifenphos, fosetyl, fosetyl-aluminum, iprobenfos, pyrazophos, tolclofos-methyl, Organochlorine compounds, thiophanate-methyl, chlorothalonil, dichlofluanid, tolylfluanid, flusulfamide, phthalide, hexachlorobenzene, pencycuron, quitozene, nitrophenyl derivatives, binapacryl, dinocap, dinobuton, spiroxamine, cyflufenamid, cymoxanil, metrafenon, N-2-cyanophenyl-3,4-

10 dichloroisothiazol-5-carboxamide (isotianil), N-(3',4',5'-trifluorobiphenyl-2-yl)-3-difluoromethyl-1-methylpyrazole-4-carboxamide, 3-[5-(4-chlorophenyl)-2,3-dimethylisoxazolidin-3-yl]-pyridine, N-(3',4'-dichloro-4-fluorobiphenyl-2-yl)-3-difluoromethyl-1-methylpyrazol-e-4-carboxamide, 5-chloro-7-(4-methylpiperidin-1-yl)-6-(2,4,6-trifluorophenyl)-[1,2,4]triazolo[1,5-a]pyrimidine, 2-butoxy-6-iodo-3-propylchromen-4-one, N,N-dimethyl-3-(3-bromo-6-fluoro-2-methylindole-1-sulfonyl)-[1,2,4]triazole-1-

15 sulfonamide, methyl-(2-chloro-5-[1-(3-methylbenzyloxyimino)-ethyl]benzyl)carbamate, methyl-(2-chloro-5-[1-(6-methylpyridin-2-ylmethoxy-imino)ethyl]benzyl)carbamate, methyl 3-(4-chlorophenyl)-3-(2-isopropoxycarbonylamino-3-methylbutyl-amino)propionate, 4-fluorophenyl N-(1-(1-(4-cyanophenyl)ethanesulfonyl)but-2-yl)carbamate, N-(2-(4-[3-(4-chlorophenyl)prop-2-ynloxy]-3-methoxyphenyl)ethyl)-2-methanesulfonylamino-3-methylbutyramide, N-(2-(4-[3-(4-chlorophenyl)prop-2-

20 ynyloxy]-3-methoxyphenyl)ethyl)-2-ethanesulfonylamino-3-methylbutyramide, N-(4'-bromobiphenyl-2-yl)-4-difluoromethyl-2-methylthiazol-5-carboxamide, N-(4'-trifluoromethylbiphenyl-2-yl)-4-difluoromethyl-2-methylthiazol-5-carboxamide, N-(4'-chloro-3'-fluorobiphenyl-2-yl)-4-difluoromethyl-2-methylthiazol-5-carboxamide, or methyl 2-(ortho-((2,5-dimethylphenoxy-methylene)phenyl)-3-methoxyacrylate. One skilled in the art will appreciate that a suitable concentration of each antifungal in the composition

25 depends on factors such as efficacy, stability of the antifungal, number of distinct antifungals, the formulation, and methods of application of the composition.

iii. Insecticides

The PMP compositions described herein can further include an insecticide. In some instances,

30 the PMP compositions include two or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10) different insecticide agents. For example, the insecticide can decrease the fitness of (e.g., decrease growth or kill) an insect plant pest. A PMP composition including an insecticide as described herein can be contacted with a target insect pest, or plant infested therewith, in an amount and for a time sufficient to: (a) reach a target level (e.g., a predetermined or threshold level) of insecticide concentration inside or on the target

35 insect; and (b) decrease fitness of the target insect. The insecticides described herein may be formulated in a PMP composition for any of the methods described herein, and in certain instances, may be associated with the PMP thereof.

As used herein, the term "insecticide" or "insecticidal agent" refers to a substance that kills or inhibits the growth, proliferation, reproduction, or spread of insects, such as agricultural insect pests. Non

40 limiting examples of insecticides are shown in Table 2. Additional non-limiting examples of suitable

- insecticides include biologics, hormones or pheromones such as azadirachtin, *Bacillus* species, *Beauveria* species, codlemone, *Metarrhizium* species, *Paecilomyces* species, *thuringiensis*, and *Verticillium* species, and active compounds having unknown or non-specified mechanisms of action such as fumigants (such as aluminium phosphide, methyl bromide and sulphuryl fluoride) and selective feeding inhibitors (such as cryolite, flonicamid and pymetrozine). One skilled in the art will appreciate that a suitable concentration of each insecticide in the composition depends on factors such as efficacy, stability of the insecticide, number of distinct insecticides, the formulation, and methods of application of the composition.

10 **Table 2. Examples of insecticides**

Class	Compounds
chloronicotinyls/neonicotinoids	acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, nithiazine, thiacloprid, thiamethoxam, imidaclothiz, (2E)-1-[(2-chloro-1,3-thiazol-5-yl)methyl]-3,5-dimethyl-N-nitro-1,3,5-tri-azinan-2-imine, acetylcholinesterase (AChE) inhibitors (such as carbamates and organophosphates)
carbamates	alanycarb, aldicarb, aldoxycarb, allyxycarb, aminocarb, bendiocarb, benfuracarb, bufencarb, butacarb, butocarboxim, butoxycarboxim, carbaryl, carbofuran, carbosulfan, chloethocarb, dimetilan, ethiofencarb, fenobucarb, fenothiocarb, formetanate, furathiocarb, isoprocarb, metam-sodium, methiocarb, methomyl, metolcarb, oxamyl, phosphocarb, pirimicarb, promecarb, propoxur, thiodicarb, thiofanox, triazamate, trimethacarb, XMC, xylcarb
organophosphates	acephate, azamethiphos, azinphos (-methyl, -ethyl), bromophos-ethyl, bromfenvinfos (-methyl), butathiofos, cadusafos, carbophenothion, chlorethoxyfos, chlorfenvinphos, chlormephos, chlorpyrifos (-methyl/-ethyl), coumaphos, cyanofenphos, cyanophos, demeton-S-methyl, demeton-S-methylsulphon, dialifos, diazinon, dichlofenthion, dichlorvos/DDVP, dicrotophos, dimethoate, dimethylvinphos, dioxabenzofos, disulfoton, EPN, ethion, ethoprophos, etrimfos, famphur, fenamiphos, fenitrothion, fensulfothion, fenthion, flupyrzofos, fonofos, formothion, fosmethilan, fosthiazate, heptenophos, iodofenphos, iprobenfos, isazofos, isofenphos, isopropyl O-salicylate, isoxathion, malathion, mecarbam, methacrifos, methamidophos, methidathion, mevinphos, monocrotophos, naled, omethoate, oxydemeton-methyl, parathion (-methyl/-ethyl), phenthoate, phorate, phosalone, phosmet, phosphamidon, phosphocarb, phoxim, pirimiphos (-methyl/-ethyl), profenofos, propaphos, propetamphos, prothiofos, prothoate, pyraclofos, pyridaphenthion, pyridathion, quinalphos,

	sebufos, sulfotep, sulprofos, tebupirimfos, temephos, terbufos, tetrachlorvinphos, thiometon, triazophos, triclofon, vamidothion
pyrethroids	acrinathrin, allethrin (d-cis-trans, d-trans), cypermethrin (alpha-, beta-, theta-, zeta-), permethrin (cis-, trans-), beta-cyfluthrin, bifenthrin, bioallethrin, bioallethrin-S-cyclopentyl-isomer, bioethanomethrin, biopermethrin, bioresmethrin, chlovaporthrin, cis-cypermethrin, cis-resmethrin, cis-permethrin, clocythrln, cycloprothrin, cyfluthrin, cyhalothrin, cyphenothrin, DDT, deltamethrin, empenhrin (1R-isomer), esfenvalerate, etofenprox, fenfluthrin, fenpropathrin, fenpyrithrin, fenvalerate, flubrocyrhinate, flucythrinate, flufenprox, flumethrin, fluvalinate, fubfenprox, gamma-cyhalothrin, imiprothrin, kadethrin, lambda, cyhalothrin, metofluthrin, phenothrin (1R-trans isomer), prallethrin, profluthrin, protrifenbute, pyresmethrin, resmethrin, RU 15525, silafluofen, tau-fluvalinate, tefluthrin, terallethrin, tetramethrin (1R-isomer), tralocyrhtrin, tralomethrin, transfluthrin, ZXI 8901, pyrethrins (pyrethrum)
oxadiazines	indoxacarb, acetylcholine receptor modulators (such as spinosyns)
spinosyns	spinosad
cyclodiene	camphechlor, chlordane, endosulfan, gamma-HCH, HCH, heptachlor,
organochlorines	lindane, methoxychlor
fiproles	acetoprole, ethiprole, vaniliprole, fipronil
mectins	abamectin, avermectin, emamectin, emamectin-benzoate, fenoxycarb, hydroprene, kinoprene, methoprene, ivermectin, lepimectin, epofenonane, pyriproxifen, milbemectin, milbemycin, triprene
diacylhydrazines	chromafenozide, halofenozide, methoxyfenozide, tebufenozide
benzoylureas	bistrifluoron, chlorfluazuron, diflubenzuron, fluazuron, flucycloxuron, flufenoxuron, hexaflumuron, lufenuron, novaluron, noviflumuron, penfluoron, teflubenzuron, triflumuron
organotins	azocyclotin, cyhexatin, fenbutatin oxide
pyrroles	chlorfenapyr
dinitrophenols	binapacyrl, dinobuton, dinocap, DNOC
METIs	fenazaquin, fenpyroximate, pyrimidifen, pyridaben, tebufenpyrad, tolfenpyrad, rotenone, acequinocyl, fluacrypyrim, microbial disrupters of the intestinal membrane of insects (such as Bacillus thuringiensis strains), inhibitors of lipid synthesis (such as tetrionic acids and tetramic acids)
tetrionic acids	spirodiclofen, spiromesifen, spirotetramat

tetramic acids	cis-3-(2,5-dimethylphenyl)-8-methoxy-2-oxo-1-azaspiro[4.5]dec-3-en-4-yl ethyl carbonate (alias: carbonic acid, 3-(2,5-dimethylphenyl)-8-methoxy-2-oxo-1-azaspiro[4.5]dec-3-en-4-yl ethyl ester; CAS Reg. No.: 382608-10-8), carboxamides (such as flonicamid), octopaminergic agonists (such as amitraz), inhibitors of the magnesium-stimulated ATPase (such as propargite), ryanodin receptor agonists (such as phthalamides or rynaxapyr)
phthalamides	N2-[1,1-dimethyl-2-(methylsulphonyl)ethyl]-3-iodo-N1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]-1,2-benzenedicarboxamide (i.e., flubendiamide; CAS reg. No.: 272451-65-7)

iv. Nematicide

The PMP compositions described herein can further include a nematicide. In some instances, the PMP compositions include two or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10) different nematicides. For example, the nematicide can decrease the fitness of (e.g., decrease growth or kill) a nematode plant pest. A PMP composition including a nematicide as described herein can be contacted with a target nematode pest, or plant infested therewith, in an amount and for a time sufficient to: (a) reach a target level (e.g., a predetermined or threshold level) of nematicide concentration inside or on the target nematode; and (b) decrease fitness of the target nematode. The nematicides described herein may be formulated in a PMP composition for any of the methods described herein, and in certain instances, may be associated with the PMP thereof.

As used herein, the term "nematicide" or "nematicidal agent" refers to a substance that kills or inhibits the growth, proliferation, reproduction, or spread of nematodes, such as agricultural nematode pests. Non limiting examples of nematicides are shown in Table 3. One skilled in the art will appreciate that a suitable concentration of each nematicide in the composition depends on factors such as efficacy, stability of the nematicide, number of distinct nematicides, the formulation, and methods of application of the composition.

Table 3. Examples of nematicides

FUMIGANTS	D-D, 1,3-Dichloropropene, Ethylene Dibromide, 1,2-Dibromo-3-Chloropropane, Methyl Bromide, Chloropicrin, Metam Sodium, Dazomet, Methyl Isothiocyanate (MITC), Sodium Tetrathiocarbonate, Chloropicrin,
CARBAMATES	Aldicarb, Aldoxycarb, Carbofuran, Oxamyl, Cleothocarb
ORGANOPHOSPHATES	Ethoprophos, Fenamiphos, Cadusafos, Fosthiazate, Fensulfothion, Thionazin, Isazofos,
BIOCHEMICALS	DITERA®, CLANDOSAN®, SINCOCIN®

v. Molluscicide

The PMP compositions described herein can further include a molluscicide. In some instances, the PMP compositions include two or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10) different

molluscicides. For example, the molluscicide can decrease the fitness of (e.g., decrease growth or kill) a mollusk plant pest. A PMP composition including a molluscicide as described herein can be contacted with a target mollusk pest, or plant infested therewith, in an amount and for a time sufficient to: (a) reach a target level (e.g., a predetermined or threshold level) of molluscicide concentration inside or on the target mollusk; and (b) decrease fitness of the target mollusk. The molluscicides described herein may be formulated in a PMP composition for any of the methods described herein, and in certain instances, may be associated with the PMP thereof.

As used herein, the term "molluscicide" or "molluscicidal agent" refers to a substance that kills or inhibits the growth, proliferation, reproduction, or spread of mollusks, such as agricultural mollusk pests. A number of chemicals can be employed as a molluscicide, including metal salts such as iron(III) phosphate, aluminium sulfate, and ferric sodium EDTA,[3][4], metaldehyde, methiocarb, or acetylcholinesterase inhibitors. One skilled in the art will appreciate that a suitable concentration of each molluscicide in the composition depends on factors such as efficacy, stability of the molluscicide, number of distinct molluscicides, the formulation, and methods of application of the composition.

15

vi. Virucides

The PMP compositions described herein can further include a virucide. In some instances, the PMP compositions include two or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10) different virucides. For example, the virucide can decrease the fitness of (e.g., decrease or eliminate) a viral plant pathogen. A PMP composition including a virucide as described herein can be contacted with a target virus, or plant infested therewith, in an amount and for a time sufficient to: (a) reach a target level (e.g., a predetermined or threshold level) of virucide concentration; and (b) decrease or eliminate the target virus. The virucides described herein may be formulated in a PMP composition for any of the methods described herein, and in certain instances, may be associated with the PMP thereof.

As used herein, the term "virucide" or "antiviral" refers to a substance that kills or inhibits the growth, proliferation, reproduction, development, or spread of viruses, such as agricultural virus pathogens. A number of agents can be employed as a virucide, including chemicals or biological agents (e.g., nucleic acids, e.g., dsRNA). One skilled in the art will appreciate that a suitable concentration of each virucide in the composition depends on factors such as efficacy, stability of the virucide, number of distinct virucides, the formulation, and methods of application of the composition.

30

vii. Herbicides

The PMP compositions described herein can further include one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10) herbicide. For example, the herbicide can decrease the fitness of (e.g., decrease or eliminate) a weed. A PMP composition including an herbicide as described herein can be contacted with a target weed in an amount and for a time sufficient to: (a) reach a target level (e.g., a predetermined or threshold level) of herbicide concentration on the plant and (b) decrease the fitness of the weed. The herbicides described herein may be formulated in a PMP composition for any of the methods described herein, and in certain instances, may be associated with the PMP thereof.

35

As used herein, the term "herbicide" refers to a substance that kills or inhibits the growth, proliferation, reproduction, or spread of weeds. A number of chemicals can be employed as a herbicides, including Glufosinate, Propaquizafop, Metamitron, Metazachlor, Pendimethalin, Flufenacet, Diflufenican, Clomazone, Nicosulfuron, Mesotrione, Pinoxaden, Sulcotrione, Prosulfocarb, Sulfentrazone, Bifenox, 5 Quinmerac, Triallate, Terbutylazine, Atrazine, Oxyfluorfen, Diuron, Trifluralin, or Chlorotoluron. Further examples of herbicides include, but are not limited to, benzoic acid herbicides, such as dicamba esters, phenoxyalkanoic acid herbicides, such as 2,4-D, MCPA and 2,4-DB esters, aryloxyphenoxypropionic acid herbicides, such as clodinafop, cyhalofop, fenoxaprop, fluazifop, haloxyfop, and quizalofop esters, pyridinecarboxylic acid herbicides, such as aminopyralid, picloram, and clopyralid esters, 10 pyrimidinecarboxylic acid herbicides, such as aminocyclopyrachlor esters, pyridyloxyalkanoic acid herbicides, such as fluoroxyppyr and triclopyr esters, and hydroxybenzonitrile herbicides, such as bromoxynil and ioxynil esters, esters of the arylpyridine carboxylic acids, and arylpyrimidine carboxylic acids of the generic structures disclosed in U.S. Pat. No. 7,314,849, U.S. Pat. No. 7,300,907, and U.S. Pat. No. 7,642,220, each of which is incorporated by reference herein in its entirety. In certain 15 embodiments, the herbicide can be selected from the group consisting of 2,4-D, 2,4-DB, acetochlor, acifluorfen, alachlor, ametryn, amitrole, asulam, atrazine, azafenidin, benefin, bensulfuron, bensulide, bentazon, bromacil, bromoxynil, butylate, carfentrazone, chloramben, chlorimuron, chlorproham, chlorsulfuron, clethodim, clomazone, clopyralid, cloransulam, cyanazine, cycloate, DCPA, desmedipham, dichlobenil, diclofop, diclosulam, diethatyl, difenzoquat, diflufenzopyr, dimethenamid-p, diquat, diuron, 20 DSMA, endothall, EPTC, ethalfluralin, ethametsulfuron, ethofumesate, fenoxaprop, fluazifop-P, flucarbazone, flufenacet, flumetsulam, flumiclorac, flumioxazin, fluometuron, fluoroxyppyr, fluthiacet, fomesafen, foramsulfuron, glufosinate, glyphosate, halosulfuron, haloxyfop, hexazinone, imazamethabenz, imazamox, imazapic, imazaquin, imazethapyr, isoxaben, isoxaflutole, lactofen, linuron, MCPA, MCPB, mesotrione, methazole, metolachlor-s, metribuzin, metsulfuron, molinate, MSMA, 25 napropamide, naptalam, nicosulfuron, norflurazon, oryzalin, oxadiazon, oxasulfuron, oxyfluorfen, paraquat, pebulate, pelargonic acid, pendimethalin, phenmedipham, picloram, primisulfuron, prodiamine, prometryn, pronamide, propachlor, propanil, prosulfuron, pyrazon, pyridate, pyriithiobac, quinclorac, quizalofop, rimsulfuron, sethoxydim, siduron, simazine, sulfentrazone, sulfometuron, sulfosulfuron, tebuthiuron, terbacil, thiazopyr, thifensulfuron, thiobencarb, tralkoxydim, triallate, triasulfuron, tribenuron, 30 triclopyr, trifluralin, triflurosulfuron, vernolate. One skilled in the art will appreciate that a suitable concentration of each herbicide in the composition depends on factors such as efficacy, stability of the herbicide, number of distinct herbicides, the formulation, and methods of application of the composition.

viii. Repellents

35 The PMP compositions described herein can further include a repellent. In some instances, the PMP compositions include two or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10) different repellents. For example, the repellent can repel any of the pests described herein (e.g., insects, nematodes, or mollusks); microorganisms (e.g., phytopathogens or endophytes, such as bacteria, fungi, or viruses); or weeds. A PMP composition including a repellent as described herein can be contacted 40 with a target plant, or plant infested therewith, in an amount and for a time sufficient to: (a) reach a target

level (e.g., a predetermined or threshold level) of repellent concentration; and (b) decrease the levels of the pest on the plant relative to an untreated plant. The repellent described herein may be formulated in a PMP composition for any of the methods described herein, and in certain instances, may be associated with the PMP thereof.

5 In some instances, the repellent is an insect repellent. Some examples of well-known insect repellents include: benzil; benzyl benzoate; 2,3,4,5-bis(butyl-2-ene)tetrahydrofurfural (MGK Repellent 11); butoxypolypropylene glycol; N-butylacetanilide; normal-butyl-6,6-dimethyl-5,6-dihydro-1,4-pyrone-2-carboxylate (Indalone); dibutyl adipate; dibutyl phthalate; di-normal-butyl succinate (Tabatrex); N,N-diethyl-meta-toluamide (DEET); dimethyl carbate (endo,endo)-dimethyl bicyclo[2.2.1] hept-5-ene-2,3-dicarboxylate); dimethyl phthalate; 2-ethyl-2-butyl-1,3-propanediol; 2-ethyl-1,3-hexanediol (Rutgers 612);
10 di-normal-propyl isocinchomeronate (MGK Repellent 326); 2-phenylcyclohexanol; p-methane-3,8-diol, and normal-propyl N,N-diethylsuccinamate. Other repellents include citronella oil, dimethyl phthalate, normal-butylmesityl oxide oxalate and 2-ethyl hexanediol-1,3 (See, Kirk-Othmer Encyclopedia of Chemical Technology, 2nd Ed., Vol. 11: 724-728; and The Condensed Chemical Dictionary, 8th Ed., p
15 756).

An insect repellent may be a synthetic or nonsynthetic insect repellent. Examples of synthetic insect repellents include methyl anthranilate and other anthranilate-based insect repellents, benzaldehyde, DEET (N,N-diethyl-m-toluamide), dimethyl carbate, dimethyl phthalate, icaridin (i.e., picaridin, Bayrepel, and KBR 3023), indalone (e.g., as used in a "6-2-2" mixture (60% Dimethyl phthalate, 20% Indalone, 20% Ethylhexanediol), IR3535 (3-[N-Butyl-N-acetyl]-aminopropionic acid, ethyl ester),
20 metofluthrin, permethrin, SS220, or tricyclodecenylyl allyl ether. Examples of natural insect repellents include beautyberry (Callicarpa) leaves, birch tree bark, bog myrtle (Myrica Gale), catnip oil (e.g., nepetalactone), citronella oil, essential oil of the lemon eucalyptus (Corymbia citriodora; e.g., p-menthane-3,8-diol (PMD)), neem oil, lemongrass, tea tree oil from the leaves of Melaleuca alternifolia,
25 tobacco, or extracts thereof.

ix. Fertilizing agents

The PMP compositions described herein can further include a heterologous fertilizing agent. In some instances, the heterologous fertilizing agent is associated with the PMPs. For example, a PMP
30 may encapsulate the heterologous fertilizing agent. Additionally, or alternatively, the heterologous fertilizing agent can be embedded on or conjugated to the surface of the PMP.

Examples of heterologous fertilizing agents include plant nutrients or plant growth regulators, such as those well known in the art. Alternatively, or additionally, the fertilizing agent can be a peptide, a polypeptide, a nucleic acid, or a polynucleotide that can increase the fitness of a plant symbiont. The
35 fertilizing agent may be an agent that can increase the fitness of a variety of plants or plant symbionts or can be one that targets one or more specific target plants or plant symbionts (e.g., a specific species or genera of plants or plant symbionts).

In some instances, the heterologous fertilizing agent can be modified. For example, the modification can be a chemical modification, e.g., conjugation to a marker, e.g., fluorescent marker or a
40 radioactive marker. In other examples, the modification can include conjugation or operational linkage to

a moiety that enhances the stability, delivery, targeting, bioavailability, or half-life of the agent, e.g., a lipid, a glycan, a polymer (e.g., PEG), a cation moiety.

Examples of heterologous fertilizing agents that can be used in the presently disclosed PMP compositions and methods are outlined below.

5 In some instances, the heterologous fertilizing agent includes any material of natural or synthetic origin that is applied to soils or to plant tissues to supply one or more plant nutrients essential to the growth of plants. The plant nutrient may include a macronutrient, micronutrient, or a combination thereof. Plant macronutrients include nitrogen, phosphorus, potassium, calcium, magnesium, and/or sulfur. Plant micronutrients include copper, iron, manganese, molybdenum, zinc, boron, silicon, cobalt, and/or
10 vanadium. Examples of plant nutrient fertilizers include a nitrogen fertilizer including, but not limited to urea, ammonium nitrate, ammonium sulfate, non-pressure nitrogen solutions, aqua ammonia, anhydrous ammonia, ammonium thiosulfate, sulfur-coated urea, urea-formaldehydes, IBDU, polymer-coated urea, calcium nitrate, ureaform, or methylene urea, phosphorous fertilizers such as diammonium phosphate, monoammonium phosphate, ammonium polyphosphate, concentrated superphosphate and triple
15 superphosphate, or potassium fertilizers such as potassium chloride, potassium sulfate, potassium-magnesium sulfate, potassium nitrate. Such compositions can exist as free salts or ions within the composition. Fertilizers may be designated by the content of one or more of its components, such as nitrogen, phosphorous, or potassium. The content of these elements in a fertilizer may be indicated by the N—P—K value (where N=nitrogen content by weight percentage, P=phosphorous content by weight
20 percentage, and K=potassium content by weight percentage).

Inorganic fertilizers, on the other hand, are manufactured from non-living materials and include, for example, ammonium nitrate, ammonium sulfate, urea, potassium chloride, potash, ammonium phosphate, anhydrous ammonia, and other phosphate salts. Inorganic fertilizers are readily commercially available and contain nutrients in soluble form that are immediately available to the plant. Inorganic
25 fertilizers are generally inexpensive, having a low unit cost for the desired element. One skilled in the art will appreciate that the exact amount of a given element in a fertilizing agent may be calculated and administered to the plant or soil.

Fertilizers may be further classified as either organic fertilizers or inorganic fertilizers. Organic fertilizers include fertilizers having a molecular skeleton with a carbon backbone, such as in compositions derived from living matter. Organic fertilizers are made from materials derived from living things. Animal manures, compost, bonemeal, feather meal, and blood meal are examples of common organic fertilizers. Organic fertilizers, on the other hand, are typically not immediately available to plants and require soil microorganisms to break the fertilizer components down into simpler structures prior to use by the plants. In addition, organic fertilizers may not only elicit a plant growth response as observed with common
30 inorganic fertilizers, but natural organic fertilizers may also stimulate soil microbial population growth and activities. Increased soil microbial population (e.g., plant symbionts) may have significant beneficial effects on the physical and chemical properties of the soil, as well as increasing disease and pest resistance.

In one aspect, a PMP composition including a plant nutrient as described herein can be
40 contacted with the plant in an amount and for a time sufficient to: (a) reach a target level (e.g., a

predetermined or threshold level) of plant nutrient concentration inside or on the plant, and (b) increase the fitness of the plant relative to an untreated plant.

In another aspect, a PMP composition including a plant nutrient as described herein can be contacted with the plant symbiont in an amount and for a time sufficient to: (a) reach a target level (e.g., a
 5 predetermined or threshold level) of plant nutrient concentration inside or on the plant symbiont (e.g., a bacteria or fungal endosymbiont), and (b) increase the fitness of the plant symbiont relative to an untreated plant symbiont.

The heterologous fertilizing agent may include a plant growth regulator. Exemplary plant growth regulators include auxins, cytokinins, gibberellins, and abscisic acid. In some instances, the plant growth
 10 regulator is abscisic acid, amidochlor, ancymidol, 6-benzylaminopurine, brassinolide, butralin, chlormequat (chlormequat chloride), choline chloride, cyclanilide, daminozide, dikegulac, dimethipin, 2,6-dimethylpuridine, ethephon, flumetralin, flurprimidol, fluthiacet, forchlorfenuron, gibberellic acid, inabenfide, indole-3-acetic acid, maleic hydrazide, mefluidide, mepiquat (mepiquat chloride), naphthaleneacetic acid, N-6-benzyladenine, paclobutrazol, prohexadione (prohexadione-calcium),
 15 prohydrojasmon, thidiazuron, triapenthenol, tributyl phosphorotrithioate, 2,3,5-tri-iodobenzoic acid, trinexapac-ethyl and uniconazole. Other plant growth regulators that can be incorporated seed coating compositions are described in US 2012/0108431, which is incorporated by reference in its entirety.

x.Plant-modifying agents

20 The PMP compositions described herein include one or more heterologous plant-modifying agents. For example, the PMPs may encapsulate the heterologous plant-modifying agent. Alternatively or additionally, the heterologous plant-modifying agent can be embedded on or conjugated to the surface of the PMP.

In some instances, the plant-modifying agent can include a peptide or a nucleic acid. The plant-
 25 modifying agent may be an agent that increases the fitness of a variety of plants or can be one that targets one or more specific plants (e.g., a specific species or genera of plants). Additionally, in some instances, the PMP compositions include two or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10) different plant-modifying agents.

Further, in some instances, the heterologous plant-modifying agent (e.g., an agent including a
 30 nucleic acid molecule or peptide) can be modified. For example, the modification can be a chemical modification, e.g., conjugation to a marker, e.g., fluorescent marker or a radioactive marker. In other examples, the modification can include conjugation or operational linkage to a moiety that enhances the stability, delivery, targeting, bioavailability, or half-life of the agent, e.g., a lipid, a glycan, a polymer (e.g., PEG), a cation moiety.

35 Examples of heterologous plant-modifying agents (e.g., peptides or nucleic acids) that can be used in the presently disclosed PMP compositions and methods are outlined below.

B. Polypeptides

The PMP composition (e.g., PMPs) described herein may include a heterologous polypeptide. In
 40 some instances, the PMP composition described herein includes a polypeptide or functional fragments or

derivative thereof that modifies a plant (e.g., e.g., increases the fitness of the plant). For example, the polypeptide can increase the fitness of a plant. A PMP composition including a polypeptide as described herein can be contacted with a plant in an amount and for a time sufficient to: (a) reach a target level (e.g., a predetermined or threshold level) of polypeptide concentration; and (b) modify the plant (e.g., increase the fitness of the plant).

Examples of polypeptides that can be used herein can include an enzyme (e.g., a metabolic recombinase, a helicase, an integrase, a RNase, a DNase, or an ubiquitination protein), a pore-forming protein, a signaling ligand, a cell penetrating peptide, a transcription factor, a receptor, an antibody, a nanobody, a gene editing protein (e.g., CRISPR-Cas system, TALEN, or zinc finger), riboprotein, a protein aptamer, or a chaperone.

Polypeptides included herein may include naturally occurring polypeptides or recombinantly produced variants. In some instances, the polypeptide may be a functional fragments or variants thereof (e.g., an enzymatically active fragment or variant thereof). For example, the polypeptide may be a functionally active variant of any of the polypeptides described herein with at least 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%, or 99% identity, e.g., over a specified region or over the entire sequence, to a sequence of a polypeptide described herein or a naturally occurring polypeptide. In some instances, the polypeptide may have at least 50% (e.g., at least 50%, 60%, 70%, 80%, 90%, 95%, 97%, 99%, or greater) identity to a protein of interest.

The polypeptides described herein may be formulated in a composition for any of the uses described herein. The compositions disclosed herein may include any number or type (e.g., classes) of polypeptides, such as at least about any one of 1 polypeptide, 2, 3, 4, 5, 10, 15, 20, or more polypeptides. A suitable concentration of each polypeptide in the composition depends on factors such as efficacy, stability of the polypeptide, number of distinct polypeptides in the composition, the formulation, and methods of application of the composition. In some instances, each polypeptide in a liquid composition is from about 0.1 ng/mL to about 100 mg/mL. In some instances, each polypeptide in a solid composition is from about 0.1 ng/g to about 100 mg/g.

Methods of making a polypeptide are routine in the art. See, in general, Smales & James (Eds.), *Therapeutic Proteins: Methods and Protocols* (Methods in Molecular Biology), Humana Press (2005); and Crommelin, Sindelar & Meibohm (Eds.), *Pharmaceutical Biotechnology: Fundamentals and Applications*, Springer (2013).

Methods for producing a polypeptide involve expression in plant cells, although recombinant proteins can also be produced using insect cells, yeast, bacteria, mammalian cells, or other cells under the control of appropriate promoters. Mammalian expression vectors may comprise nontranscribed elements such as an origin of replication, a suitable promoter and enhancer, and other 5' or 3' flanking nontranscribed sequences, and 5' or 3' nontranslated sequences such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and termination sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the other genetic elements required for expression of a heterologous DNA sequence. Appropriate cloning and expression vectors for use with

bacterial, fungal, yeast, and mammalian cellular hosts are described in Green & Sambrook, *Molecular Cloning: A Laboratory Manual* (Fourth Edition), Cold Spring Harbor Laboratory Press (2012).

Various mammalian cell culture systems can be employed to express and manufacture a recombinant polypeptide agent. Examples of mammalian expression systems include CHO cells, COS
 5 cells, HeLA and BHK cell lines. Processes of host cell culture for production of protein therapeutics are described in, e.g., Zhou and Kantardjieff (Eds.), *Mammalian Cell Cultures for Biologics Manufacturing* (Advances in Biochemical Engineering/Biotechnology), Springer (2014). Purification of proteins is described in Franks, *Protein Biotechnology: Isolation, Characterization, and Stabilization*, Humana Press (2013); and in Cutler, *Protein Purification Protocols* (Methods in Molecular Biology), Humana Press
 10 (2010). Formulation of protein therapeutics is described in Meyer (Ed.), *Therapeutic Protein Drug Products: Practical Approaches to formulation in the Laboratory, Manufacturing, and the Clinic*, Woodhead Publishing Series (2012).

In some instances, the PMP composition includes an antibody or antigen binding fragment thereof. For example, an agent described herein may be an antibody that blocks or potentiates activity
 15 and/or function of a component of the plant. The antibody may act as an antagonist or agonist of a polypeptide (e.g., enzyme or cell receptor) in the plant. The making and use of antibodies against a target antigen is known in the art. See, for example, Zhiqiang An (Ed.), *Therapeutic Monoclonal Antibodies: From Bench to Clinic*, 1st Edition, Wiley, 2009 and also Greenfield (Ed.), *Antibodies: A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory Press, 2013, for methods of making
 20 recombinant antibodies, including antibody engineering, use of degenerate oligonucleotides, 5'-RACE, phage display, and mutagenesis; antibody testing and characterization; antibody pharmacokinetics and pharmacodynamics; antibody purification and storage; and screening and labeling techniques.

C. Nucleic acids

25 In some instances, the PMPs described herein include a heterologous nucleic acid (heterologous polynucleotide). Numerous nucleic acids are useful in the PMP compositions and methods described herein. The PMPs disclosed herein may include any number or type (e.g., classes) of heterologous nucleic acids (e.g., DNA molecule (e.g., plasmid) or RNA molecule, e.g., mRNA, guide RNA (gRNA), or inhibitory RNA molecule or precursor thereof (e.g., siRNA, shRNA, or miRNA or a precursor of any of
 30 these), or a hybrid DNA-RNA molecule), such as at least about 1 class or variant of a nucleic acid, or 2, 3, 4, 5, 10, 15, 20, or more classes or variants of nucleic acids. A suitable concentration of each nucleic acid in the composition depends on factors such as efficacy, stability of the nucleic acid, number of distinct nucleic acids, the formulation, and methods of application of the composition. Examples of nucleic acids useful herein include a DNA molecule (e.g., a plasmid), an mRNA, an siRNA, a Dicer
 35 substrate small interfering RNA (dsiRNA), an antisense oligonucleotide, an antisense RNA, a short interfering RNA (siRNA) or siRNA precursor (e.g., one or more strands of RNA that hybridize inter- or intra-molecularly to form at least partially double-stranded RNA having at least about 20 contiguous base-pairs), a short hairpin (shRNA), a microRNA (miRNA) or miRNA precursor, an asymmetric interfering RNA (aiRNA), a peptide nucleic acid (PNA), a morpholino, a locked nucleic acid (LNA), a piwi-interacting
 40 RNA (piRNA), a ribozyme, a deoxyribozymes (DNAzyme), an aptamer (DNA, RNA), a circular RNA

(circRNA), a guide RNA (gRNA), an ADAR (adenosine deaminases that act on RNA) targeting oligonucleotide, a long non-coding RNA, a closed-ended DNA (ceDNA), a minicircle, a miniplasmid, or a DNA molecule encoding any of the recited RNAs.

The composition of claim 28, wherein the polynucleotide is chosen from an mRNA, an siRNA or
 5 siRNA precursor, a miRNA or miRNA precursor, a plasmid, a dsRNA, a shRNA, an aiRNA, a LNA, a piRNA, a ribozyme, a DNzyme, an aptamer, a circRNA, a gRNA, an ADAR targeting oligonucleotide, an antisense oligonucleotide, a long non-coding RNA, ceDNA, minicircle, or miniplasmid, or a DNA molecule encoding any of these RNAs.

A PMP composition including a nucleic acid as described herein can be contacted with a plant in
 10 an amount and for a time sufficient to: (a) reach a target level (e.g., a predetermined or threshold level) of nucleic acid concentration; and (b) modify the plant (e.g., increase the fitness of the plant).

(a) Nucleic Acids Encoding Peptides

In some instances, the PMPs include a heterologous nucleic acid encoding a polypeptide.

15 Nucleic acids encoding a polypeptide may have a length from about 10 to about 50,000 nucleotides (nts), about 25 to about 100 nts, about 50 to about 150 nts, about 100 to about 200 nts, about 150 to about 250 nts, about 200 to about 300 nts, about 250 to about 350 nts, about 300 to about 500 nts, about 10 to about 1000 nts, about 50 to about 1000 nts, about 100 to about 1000 nts, about 1000 to about 2000 nts, about 2000 to about 3000 nts, about 3000 to about 4000 nts, about 4000 to about 5000 nts, about 5000
 20 to about 6000 nts, about 6000 to about 7000 nts, about 7000 to about 8000 nts, about 8000 to about 9000 nts, about 9000 to about 10,000 nts, about 10,000 to about 15,000 nts, about 10,000 to about 20,000 nts, about 10,000 to about 25,000 nts, about 10,000 to about 30,000 nts, about 10,000 to about 40,000 nts, about 10,000 to about 45,000 nts, about 10,000 to about 50,000 nts, or any range therebetween.

25 The PMP composition may also include active variants of a nucleic acid sequence of interest. In some instances, the variant of the nucleic acids has at least 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%, or 99% identity, e.g., over a specified region or over the entire sequence, to a sequence of a nucleic acid of interest. In some instances, the invention includes an active polypeptide
 30 encoded by a nucleic acid variant as described herein. In some instances, the active polypeptide encoded by the nucleic acid variant has at least 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%, or 99% identity, e.g., over a specified region or over the entire amino acid sequence, to a sequence of a polypeptide of interest or the naturally derived polypeptide sequence.

35 Certain methods for expressing a nucleic acid encoding a protein may involve expression in cells, including insect, yeast, plant, bacteria, or other cells under the control of appropriate promoters. Expression vectors may include nontranscribed elements, such as an origin of replication, a suitable promoter and enhancer, and other 5' or 3' flanking nontranscribed sequences, and 5' or 3' nontranslated sequences such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor
 40 sites, and termination sequences. DNA sequences derived from the SV40 viral genome, for example,

SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the other genetic elements required for expression of a heterologous DNA sequence. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described in Green et al., *Molecular Cloning: A Laboratory Manual*, Fourth Edition, Cold Spring Harbor Laboratory Press, 2012.

Genetic modification using recombinant methods is generally known in the art. A nucleic acid sequence coding for a desired gene can be obtained using recombinant methods known in the art, such as, for example by screening libraries from cells expressing the gene, by deriving the gene from a vector known to include the same, or by isolating directly from cells and tissues containing the same, using standard techniques. Alternatively, a gene of interest can be produced synthetically, rather than cloned.

Expression of natural or synthetic nucleic acids is typically achieved by operably linking a nucleic acid encoding the gene of interest to a promoter, and incorporating the construct into an expression vector. Expression vectors can be suitable for replication and expression in bacteria. Expression vectors can also be suitable for replication and integration in eukaryotes. Typical cloning vectors contain transcription and translation terminators, initiation sequences, and promoters useful for expression of the desired nucleic acid sequence.

Additional promoter elements, e.g., enhancers, regulate the frequency of transcriptional initiation. Typically, these are located in the region 30-110 basepairs (bp) upstream of the start site, although a number of promoters have recently been shown to contain functional elements downstream of the start site as well. The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the thymidine kinase (tk) promoter, the spacing between promoter elements can be increased to 50 bp apart before activity begins to decline. Depending on the promoter, it appears that individual elements can function either cooperatively or independently to activate transcription.

One example of a suitable promoter is the immediate early cytomegalovirus (CMV) promoter sequence. This promoter sequence is a strong constitutive promoter sequence capable of driving high levels of expression of any polynucleotide sequence operatively linked thereto. Another example of a suitable promoter is Elongation Growth Factor-1 α (EF-1 α). However, other constitutive promoter sequences may also be used, including, but not limited to the simian virus 40 (SV40) early promoter, mouse mammary tumor virus (MMTV), human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus immediate early promoter, a Rous sarcoma virus promoter, as well as human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, the hemoglobin promoter, and the creatine kinase promoter.

Alternatively, the promoter may be an inducible promoter. The use of an inducible promoter provides a molecular switch capable of turning on expression of the polynucleotide sequence which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Examples of inducible promoters include, but are not limited to a metallothionein promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline promoter.

The expression vector to be introduced can also contain either a selectable marker gene or a reporter gene or both to facilitate identification and selection of expressing cells from the population of

cells sought to be transfected or infected through viral vectors. In other aspects, the selectable marker may be carried on a separate piece of DNA and used in a co-transfection procedure. Both selectable markers and reporter genes may be flanked with appropriate regulatory sequences to enable expression in the host cells. Useful selectable markers include, for example, antibiotic-resistance genes, such as
 5 neo and the like.

Reporter genes may be used for identifying potentially transformed cells and for evaluating the functionality of regulatory sequences. In general, a reporter gene is a gene that is not present in or expressed by the recipient source and that encodes a polypeptide whose expression is manifested by some easily detectable property, e.g., enzymatic activity. Expression of the reporter gene is assayed at a
 10 suitable time after the DNA has been introduced into the recipient cells. Suitable reporter genes may include genes encoding luciferase, beta-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green fluorescent protein gene (e.g., Ui-Tei et al., FEBS Letters 479:79-82, 2000). Suitable expression systems are well known and may be prepared using known techniques or obtained commercially. In general, the construct with the minimal 5' flanking region showing the highest
 15 level of expression of reporter gene is identified as the promoter. Such promoter regions may be linked to a reporter gene and used to evaluate agents for the ability to modulate promoter-driven transcription.

In some instances, an organism may be genetically modified to alter expression of one or more proteins. Expression of the one or more proteins may be modified for a specific time, e.g., development or differentiation state of the organism. In one instances, the invention includes a composition to alter
 20 expression of one or more proteins, e.g., proteins that affect activity, structure, or function. Expression of the one or more proteins may be restricted to a specific location(s) or widespread throughout the organism.

(b) Synthetic mRNA

The PMP composition may include a synthetic mRNA molecule, e.g., a synthetic mRNA molecule encoding a polypeptide. The synthetic mRNA molecule can be modified, e.g., chemically. The mRNA molecule can be chemically synthesized or transcribed in vitro. The mRNA molecule can be disposed on a plasmid, e.g., a viral vector, bacterial vector, or eukaryotic expression vector. In some examples, the mRNA molecule can be delivered to cells by transfection, electroporation, or transduction (e.g.,
 30 adenoviral or lentiviral transduction).

In some instances, the modified RNA agent of interest described herein has modified nucleosides or nucleotides. Such modifications are known and are described, e.g., in WO 2012/019168. Additional modifications are described, e.g., in WO 2015/038892; WO 2015/038892; WO 2015/089511; WO 2015/196130; WO 2015/196118 and WO 2015/196128 A2.

In some instances, the modified RNA encoding a polypeptide of interest has one or more terminal modification, e.g., a 5' cap structure and/or a poly-A tail (e.g., of between 100-200 nucleotides in length). The 5' cap structure may be selected from the group consisting of CapO, CapI, ARCA, inosine, NI-methyl-guanosine, 2'fluoro- guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, and 2-azido- guanosine. In some cases, the modified RNAs also contain a 5' UTR including
 40 at least one Kozak sequence, and a 3' UTR. Such modifications are known and are described, e.g., in

WO 2012/135805 and WO 2013/052523. Additional terminal modifications are described, e.g., in WO 2014/164253 and WO 2016/011306, WO 2012/045075, and WO 2014/093924. Chimeric enzymes for synthesizing capped RNA molecules (e.g., modified mRNA) which may include at least one chemical modification are described in WO 2014/028429.

5 In some instances, a modified mRNA may be cyclized, or concatemerized, to generate a translation competent molecule to assist interactions between poly-A binding proteins and 5'-end binding proteins. The mechanism of cyclization or concatemerization may occur through at least 3 different routes: 1) chemical, 2) enzymatic, and 3) ribozyme catalyzed. The newly formed 5'-/3'- linkage may be intramolecular or intermolecular. Such modifications are described, e.g., in WO 2013/151736.

10 Methods of making and purifying modified RNAs are known and disclosed in the art. For example, modified RNAs are made using only in vitro transcription (IVT) enzymatic synthesis. Methods of making IVT polynucleotides are known in the art and are described in WO 2013/151666, WO 2013/151668, WO 2013/151663, WO 2013/151669, WO 2013/151670, WO 2013/151664, WO 2013/151665, WO 2013/151671, WO 2013/151672, WO 2013/151667 and WO 2013/151736. Methods of purification include purifying an RNA transcript including a polyA tail by contacting the sample with a surface linked to a plurality of thymidines or derivatives thereof and/or a plurality of uracils or derivatives thereof (polyT/U) under conditions such that the RNA transcript binds to the surface and eluting the purified RNA transcript from the surface (WO 2014/152031); using ion (e.g., anion) exchange chromatography that allows for separation of longer RNAs up to 10,000 nucleotides in length via a scalable method (WO 2014/144767); and subjecting a modified mRNA sample to DNase treatment (WO 2014/152030).

Formulations of modified RNAs are known and are described, e.g., in WO 2013/090648. For example, the formulation may be, but is not limited to, nanoparticles, poly(lactic-co-glycolic acid)(PLGA) microspheres, lipidoids, lipoplex, liposome, polymers, carbohydrates (including simple sugars), cationic lipids, fibrin gel, fibrin hydrogel, fibrin glue, fibrin sealant, fibrinogen, thrombin, rapidly eliminated lipid nanoparticles (reLNPs) and combinations thereof.

Modified RNAs encoding polypeptides in the fields of human disease, antibodies, viruses, and a variety of in vivo settings are known and are disclosed in for example, Table 6 of International Publication Nos. WO 2013/151666, WO 2013/151668, WO 2013/151663, WO 2013/151669, WO 2013/151670, WO 2013/151664, WO 2013/151665, WO 2013/151736; Tables 6 and 7 International Publication No. WO 2013/151672; Tables 6, 178 and 179 of International Publication No. WO 2013/151671; Tables 6, 185 and 186 of International Publication No. WO 2013/151667. Any of the foregoing may be synthesized as an IVT polynucleotide, chimeric polynucleotide or a circular polynucleotide, and each may include one or more modified nucleotides or terminal modifications.

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(c) Inhibitory RNA

In some instances, the PMP composition includes an inhibitory RNA molecule, e.g., that acts via the RNA interference (RNAi) pathway. In some instances, the inhibitory RNA molecule decreases the level of gene expression in a plant and/or decreases the level of a protein in the plant. In some instances, the inhibitory RNA molecule inhibits expression of a plant gene. For example, an inhibitory RNA molecule

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may include a short interfering RNA or its precursor, short hairpin RNA, and/or a microRNA or its precursor that targets a gene in the plant. Certain RNA molecules can inhibit gene expression through the biological process of RNA interference (RNAi). RNAi molecules include RNA or RNA-like structures typically containing 15-50 base pairs (such as about 18-25 base pairs) and having a nucleobase sequence identical (or complementary) or nearly identical (or substantially complementary) to a coding sequence in an expressed target gene within the cell. RNAi molecules include, but are not limited to: short interfering RNAs (siRNAs), double-strand RNAs (dsRNA), short hairpin RNAs (shRNA), meroduplexes, dicer substrates, and multivalent RNA interference (U.S. Pat. Nos. 8,084,599 8,349,809, 8,513,207 and 9,200,276). A shRNA is a RNA molecule including a hairpin turn that decreases expression of target genes via RNAi. shRNAs can be delivered to cells in the form of plasmids, e.g., viral or bacterial vectors, e.g., by transfection, electroporation, or transduction). A microRNA is a non-coding RNA molecule that typically has a length of about 21 or 22 nucleotides. MiRNAs bind to target sites on mRNA molecules and silence the mRNA, e.g., by causing cleavage of the mRNA, destabilization of the mRNA, or inhibition of translation of the mRNA. In some instances, the inhibitory RNA molecule decreases the level and/or activity of a negative regulator of function. In other instances, the inhibitor RNA molecule decreases the level and/or activity of an inhibitor of a positive regulator of function. The inhibitory RNA molecule can be chemically synthesized or transcribed in vitro.

In some instances, the nucleic acid is a DNA, a RNA, or a PNA. In some instances, the RNA is an inhibitory RNA. In some instances, the inhibitory RNA inhibits gene expression in a plant. In some instances, the nucleic acid is an mRNA, a modified mRNA, or a DNA molecule that, in the plant, increases expression of an enzyme (e.g., a metabolic recombinase, a helicase, an integrase, a RNase, a DNase, or an ubiquitination protein), a pore-forming protein, a signaling ligand, a cell penetrating peptide, a transcription factor, a receptor, an antibody, a nanobody, a gene editing protein (e.g., CRISPR-Cas system, TALEN, or zinc finger), a riboprotein, a protein aptamer, or a chaperone. In some instances, the nucleic acid is an mRNA, a modified mRNA, or a DNA molecule that increases the expression of an enzyme (e.g., a metabolic enzyme, a recombinase enzyme, a helicase enzyme, an integrase enzyme, a RNase enzyme, a DNase enzyme, or an ubiquitination protein), a pore-forming protein, a signaling ligand, a cell penetrating peptide, a transcription factor, a receptor, an antibody, a nanobody, a gene editing protein (e.g., a CRISPR-Cas system, a TALEN, or a zinc finger), a riboprotein, a protein aptamer, or a chaperone. In some aspects, the nucleic acid encodes the enzyme, pore-forming protein, signaling ligand, cell penetrating peptide, transcription factor, receptor, antibody, nanobody, gene editing protein, riboprotein, protein aptamer, or chaperone. In some instances, the increase in expression in the plant is an increase in expression of about 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100% relative to a reference level (e.g., the expression in an untreated plant). In some instances, the increase in expression in the plant is an increase in expression of about 2x fold, about 4x fold, about 5x fold, about 10x fold, about 20x fold, about 25x fold, about 50x fold, about 75x fold, or about 100x fold or more, relative to a reference level (e.g., the expression in an untreated plant).

In some instances, the nucleic acid is an antisense RNA, a dsRNA, a siRNA, a shRNA, a miRNA, an aiRNA, a PNA, a morpholino, a LNA, a piRNA, a ribozyme, a DNAzyme, an aptamer (DNA, RNA), a circRNA, a gRNA, or a DNA molecules (e.g., a plasmid) that acts to reduce, in the plant,

expression of, e.g., an enzyme (a metabolic enzyme, a recombinase enzyme, a helicase enzyme, an integrase enzyme, a RNase enzyme, a DNase enzyme, a polymerase enzyme, a ubiquitination protein, a superoxide management enzyme, or an energy production enzyme), a transcription factor, a secretory protein, a structural factor (actin, kinesin, or tubulin), a riboprotein, a protein aptamer, a chaperone, a receptor, a signaling ligand, or a transporter. In some instances, the decrease in expression in the plant is a decrease in expression of about 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100% relative to a reference level (e.g., the expression in an untreated plant). In some instances, the decrease in expression in the plant is a decrease in expression of about 2x fold, about 4x fold, about 5x fold, about 10x fold, about 20x fold, about 25x fold, about 50x fold, about 75x fold, or about 100x fold or more, relative to a reference level (e.g., the expression in an untreated plant).

RNAi molecules include a sequence substantially complementary, or fully complementary, to all or a fragment of a target gene. RNAi molecules may complement sequences at the boundary between introns and exons to prevent the maturation of newly-generated nuclear RNA transcripts of specific genes into mRNA for transcription. RNAi molecules complementary to specific genes can hybridize with the mRNA for a target gene and prevent its translation. The antisense molecule can be DNA, RNA, or a derivative or hybrid thereof. Examples of such derivative molecules include, but are not limited to, peptide nucleic acid (PNA) and phosphorothioate-based molecules such as deoxyribonucleic guanidine (DNG) or ribonucleic guanidine (RNG).

RNAi molecules can be provided as ready-to-use RNA synthesized in vitro or as sense and antisense RNA sequences (or DNA encoding sense and antisense RNA sequences) transfected into cells which will yield RNAi molecules upon transcription. Hybridization of the RNA molecule with, e.g., the target mRNA results in degradation of the hybridized complex by RNase H and/or inhibition of the formation of translation complexes. Both result in a failure to produce the product of the original gene.

The length of the RNAi molecule that hybridizes to the transcript of interest may be around 10 nucleotides, between about 15 or 30 nucleotides, or about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more nucleotides. In embodiments, the RNAi molecule hybridizes to the transcript of interest to form a perfectly or near-perfectly double-stranded region of at least about 17 base pairs; in embodiments the double-stranded region includes at least about 10 contiguous base pairs. The degree of identity of the antisense sequence to the targeted transcript may be at least 75%, at least 80%, at least 85%, at least 90%, or at least 95.

RNAi molecules may also include overhangs, i.e., typically unpaired, overhanging nucleotides which are not directly involved in the double helical structure normally formed by the core sequences of the herein defined pair of sense strand and antisense strand. RNAi molecules may contain 3' and/or 5' overhangs of about 1-5 bases independently on each of the sense strands and antisense strands. In some instances, both the sense strand and the antisense strand contain 3' and 5' overhangs. In some instances, one or more of the 3' overhang nucleotides of one strand base pairs with one or more 5' overhang nucleotides of the other strand. In other instances, the one or more of the 3' overhang nucleotides of one strand base do not pair with the one or more 5' overhang nucleotides of the other strand. The sense and antisense strands of an RNAi molecule may or may not contain the same number of nucleotide bases. The antisense and sense strands may form a duplex wherein the 5' end only has a

blunt end, the 3' end only has a blunt end, both the 5' and 3' ends are blunt ended, or neither the 5' end nor the 3' end are blunt ended. In another instance, one or more of the nucleotides in the overhang contains a thiophosphate, phosphorothioate, deoxynucleotide inverted (3' to 3' linked) nucleotide or is a modified ribonucleotide or deoxynucleotide.

5 Small interfering RNA (siRNA) molecules include a nucleotide sequence that is identical to about 15 to about 25 contiguous nucleotides of the target mRNA. In some instances, the siRNA sequence commences with the dinucleotide AA, includes a GC-content of about 30-70% (about 30-60%, about 40-60%, or about 45%-55%), and does not have a high percentage identity to any nucleotide sequence other than the target in the genome in which it is to be introduced, for example as determined by standard
10 BLAST search.

siRNAs and shRNAs resemble intermediates in the processing pathway of the endogenous microRNA (miRNA) genes (Bartel, *Cell* 116:281-297, 2004). In some instances, siRNAs can function as miRNAs and vice versa (Zeng et al., *Mol. Cell* 9:1327-1333, 2002; Doench et al., *Genes Dev.* 17:438-442, 2003). Exogenous siRNAs downregulate mRNAs with seed complementarity to the siRNA (Birmingham
15 et al., *Nat. Methods* 3:199-204, 2006). Multiple target sites within a 3' UTR give stronger downregulation (Doench et al., *Genes Dev.* 17:438-442, 2003).

Known effective siRNA sequences and cognate binding sites are also well represented in the relevant literature. RNAi molecules are readily designed and produced by technologies known in the art. In addition, there are computational tools that increase the chance of finding effective and specific
20 sequence motifs (Pei et al., *Nat. Methods* 3(9):670-676, 2006; Reynolds et al., *Nat. Biotechnol.* 22(3):326-330, 2004; Khvorova et al., *Nat. Struct. Biol.* 10(9):708-712, 2003; Schwarz et al., *Cell* 115(2):199-208, 2003; Ui-Tei et al., *Nucleic Acids Res.* 32(3):936-948, 2004; Heale et al., *Nucleic Acids Res.* 33(3):e30, 2005; Chalk et al., *Biochem. Biophys. Res. Commun.* 319(1):264-274, 2004; and Amarzguoui et al., *Biochem. Biophys. Res. Commun.* 316(4):1050-1058, 2004).

25 The RNAi molecule modulates expression of RNA encoded by a gene. Because multiple genes can share some degree of sequence homology with each other, in some instances, the RNAi molecule can be designed to target a class of genes with sufficient sequence homology. In some instances, the RNAi molecule can contain a sequence that has complementarity to sequences that are shared amongst different gene targets or are unique for a specific gene target. In some instances, the RNAi molecule can
30 be designed to target conserved regions of an RNA sequence having homology between several genes thereby targeting several genes in a gene family (e.g., different gene isoforms, splice variants, mutant genes, etc.). In some instances, the RNAi molecule can be designed to target a sequence that is unique to a specific RNA sequence of a single gene.

An inhibitory RNA molecule can be modified, e.g., to contain modified nucleotides, e.g., 2'-fluoro,
35 2'-o-methyl, 2'-deoxy, unlocked nucleic acid, 2'-hydroxy, phosphorothioate, 2'-thiouridine, 4'-thiouridine, 2'-deoxyuridine. Without being bound by theory, it is believed that such modifications can increase nuclease resistance and/or serum stability, or decrease immunogenicity.

In some instances, the RNAi molecule or its precursor is linked to a delivery polymer via a physiologically labile bond or linker. The physiologically labile linker is selected such that it undergoes a
40 chemical transformation (e.g., cleavage) when present in certain physiological conditions, (e.g., disulfide

bond cleaved in the reducing environment of the cell cytoplasm). Release of the molecule from the polymer, by cleavage of the physiologically labile linkage, facilitates interaction of the molecule with the appropriate cellular components for activity.

The RNAi molecule-polymer conjugate may be formed by covalently linking the molecule to the polymer. The polymer is polymerized or modified such that it contains a reactive group A. The RNAi molecule is also polymerized or modified such that it contains a reactive group B. Reactive groups A and B are chosen such that they can be linked via a reversible covalent linkage using methods known in the art.

Conjugation of the RNAi molecule to the polymer can be performed in the presence of an excess of polymer. Because the RNAi molecule and the polymer may be of opposite charge during conjugation, the presence of excess polymer can reduce or eliminate aggregation of the conjugate. Alternatively, an excess of a carrier polymer, such as a polycation, can be used. The excess polymer can be removed from the conjugated polymer prior to administration of the conjugate. Alternatively, the excess polymer can be co-administered with the conjugate.

The making and use of inhibitory agents based on non-coding RNA such as ribozymes, RNase P, siRNAs, and miRNAs are also known in the art, for example, as described in Sioud, RNA Therapeutics: Function, Design, and Delivery (Methods in Molecular Biology). Humana Press (2010).

(d) Gene Editing

The PMP compositions described herein may include a component of a gene editing system. For example, the agent may introduce an alteration (e.g., insertion, deletion (e.g., knockout), translocation, inversion, single point mutation, or other mutation) in a gene in the plant. Exemplary gene editing systems include the zinc finger nucleases (ZFNs), Transcription Activator-Like Effector-based Nucleases (TALEN), and the clustered regulatory interspaced short palindromic repeat (CRISPR) system. ZFNs, TALENs, and CRISPR-based methods are described, e.g., in Gaj et al., Trends Biotechnol. 31(7):397-405, 2013.

In a typical CRISPR/Cas system, an endonuclease is directed to a target nucleotide sequence (e.g., a site in the genome that is to be sequence-edited) by sequence-specific, non-coding guide RNAs that target single- or double-stranded DNA sequences. Three classes (I-III) of CRISPR systems have been identified. The class II CRISPR systems use a single Cas endonuclease (rather than multiple Cas proteins). One class II CRISPR system includes a type II Cas endonuclease such as Cas9, a CRISPR RNA (crRNA), and a trans-activating crRNA (tracrRNA). The crRNA contains a guide RNA, i.e., typically an about 20-nucleotide RNA sequence that corresponds to a target DNA sequence. The crRNA also contains a region that binds to the tracrRNA to form a partially double-stranded structure which is cleaved by RNase III, resulting in a crRNA/tracrRNA hybrid. The RNAs serve as guides to direct Cas proteins to silence specific DNA/RNA sequences, depending on the spacer sequence. See, e.g., Horvath et al., Science 327:167-170, 2010; Makarova et al., Biology Direct 1:7, 2006; Pennisi, Science 341:833-836, 2013. The target DNA sequence must generally be adjacent to a protospacer adjacent motif (PAM) that is specific for a given Cas endonuclease; however, PAM sequences appear throughout a given genome. CRISPR endonucleases identified from various prokaryotic species have unique PAM sequence

requirements; examples of PAM sequences include 5'-NGG (SEQ ID NO: 1) (*Streptococcus pyogenes*), 5'-NNAGAA (SEQ ID NO: 2) (*Streptococcus thermophilus* CRISPR1), 5'-NGGNG (SEQ ID NO: 3) (*Streptococcus thermophilus* CRISPR3), and 5'-NNNGATT (SEQ ID NO: 4) (*Neisseria meningitidis*). Some endonucleases, e.g., Cas9 endonucleases, are associated with G-rich PAM sites, e.g., 5'-NGG (SEQ ID NO: 1), and perform blunt-end cleaving of the target DNA at a location 3 nucleotides upstream from (5' from) the PAM site. Another class II CRISPR system includes the type V endonuclease Cpf1, which is smaller than Cas9; examples include AsCpf1 (from *Acidaminococcus* sp.) and LbCpf1 (from *Lachnospiraceae* sp.). Cpf1-associated CRISPR arrays are processed into mature crRNAs without the requirement of a tracrRNA; in other words a Cpf1 system requires only the Cpf1 nuclease and a crRNA to cleave the target DNA sequence. Cpf1 endonucleases, are associated with T-rich PAM sites, e.g., 5'-TTN (SEQ ID NO: 5). Cpf1 can also recognize a 5'-CTA (SEQ ID NO: 6) PAM motif. Cpf1 cleaves the target DNA by introducing an offset or staggered double-strand break with a 4- or 5-nucleotide 5' overhang, for example, cleaving a target DNA with a 5-nucleotide offset or staggered cut located 18 nucleotides downstream from (3' from) from the PAM site on the coding strand and 23 nucleotides downstream from the PAM site on the complimentary strand; the 5-nucleotide overhang that results from such offset cleavage allows more precise genome editing by DNA insertion by homologous recombination than by insertion at blunt-end cleaved DNA. See, e.g., Zetsche et al., *Cell* 163:759-771, 2015.

For the purposes of gene editing, CRISPR arrays can be designed to contain one or multiple guide RNA sequences corresponding to a desired target DNA sequence; see, for example, Cong et al., *Science* 339:819-823, 2013; Ran et al., *Nature Protocols* 8:2281-2308, 2013. At least about 16 or 17 nucleotides of gRNA sequence are required by Cas9 for DNA cleavage to occur; for Cpf1 at least about 16 nucleotides of gRNA sequence is needed to achieve detectable DNA cleavage. In practice, guide RNA sequences are generally designed to have a length of between 17-24 nucleotides (e.g., 19, 20, or 21 nucleotides) and complementarity to the targeted gene or nucleic acid sequence. Custom gRNA generators and algorithms are available commercially for use in the design of effective guide RNAs. Gene editing has also been achieved using a chimeric single guide RNA (sgRNA), an engineered (synthetic) single RNA molecule that mimics a naturally occurring crRNA-tracrRNA complex and contains both a tracrRNA (for binding the nuclease) and at least one crRNA (to guide the nuclease to the sequence targeted for editing). Chemically modified sgRNAs have also been demonstrated to be effective in genome editing; see, for example, Hendel et al., *Nature Biotechnol.* 985-991, 2015.

Whereas wild-type Cas9 generates double-strand breaks (DSBs) at specific DNA sequences targeted by a gRNA, a number of CRISPR endonucleases having modified functionalities are available, for example: a nickase version of Cas9 generates only a single-strand break; a catalytically inactive Cas9 (dCas9) does not cut the target DNA but interferes with transcription by steric hindrance. dCas9 can further be fused with an effector to repress (CRISPRi) or activate (CRISPRa) expression of a target gene. For example, Cas9 can be fused to a transcriptional repressor (e.g., a KRAB domain) or a transcriptional activator (e.g., a dCas9-VP64 fusion). A catalytically inactive Cas9 (dCas9) fused to FokI nuclease (dCas9-FokI) can be used to generate DSBs at target sequences homologous to two gRNAs. See, e.g., the numerous CRISPR/Cas9 plasmids disclosed in and publicly available from the Addgene repository (Addgene, 75 Sidney St., Suite 550A, Cambridge, MA 02139; addgene.org/crispr/). A double nickase

Cas9 that introduces two separate double-strand breaks, each directed by a separate guide RNA, is described as achieving more accurate genome editing by Ran et al., Cell 154:1380-1389, 2013.

CRISPR technology for editing the genes of eukaryotes is disclosed in US Patent Application Publications US 2016/0138008 A1 and US 2015/0344912 A1, and in US Patents 8,697,359, 8,771,945, 8,945,839, 8,999,641, 8,993,233, 8,895,308, 8,865,406, 8,889,418, 8,871,445, 8,889,356, 8,932,814, 8,795,965, and 8,906,616. Cpf1 endonuclease and corresponding guide RNAs and PAM sites are disclosed in US Patent Application Publication 2016/0208243 A1.

In some instances, the desired genome modification involves homologous recombination, wherein one or more double-stranded DNA breaks in the target nucleotide sequence is generated by the RNA-guided nuclease and guide RNA(s), followed by repair of the break(s) using a homologous recombination mechanism (homology-directed repair). In such instances, a donor template that encodes the desired nucleotide sequence to be inserted or knocked-in at the double-stranded break is provided to the cell or subject; examples of suitable templates include single-stranded DNA templates and double-stranded DNA templates (e.g., linked to the polypeptide described herein). In general, a donor template encoding a nucleotide change over a region of less than about 50 nucleotides is provided in the form of single-stranded DNA; larger donor templates (e.g., more than 100 nucleotides) are often provided as double-stranded DNA plasmids. In some instances, the donor template is provided to the cell or subject in a quantity that is sufficient to achieve the desired homology-directed repair but that does not persist in the cell or subject after a given period of time (e.g., after one or more cell division cycles). In some instances, a donor template has a core nucleotide sequence that differs from the target nucleotide sequence (e.g., a homologous endogenous genomic region) by at least 1, at least 5, at least 10, at least 20, at least 30, at least 40, at least 50, or more nucleotides. This core sequence is flanked by homology arms or regions of high sequence identity with the targeted nucleotide sequence; in some instances, the regions of high identity include at least 10, at least 50, at least 100, at least 150, at least 200, at least 300, at least 400, at least 500, at least 600, at least 750, or at least 1000 nucleotides on each side of the core sequence. In some instances where the donor template is in the form of a single-stranded DNA, the core sequence is flanked by homology arms including at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, or at least 100 nucleotides on each side of the core sequence. In instances, where the donor template is in the form of a double-stranded DNA, the core sequence is flanked by homology arms including at least 500, at least 600, at least 700, at least 800, at least 900, or at least 1000 nucleotides on each side of the core sequence. In one instance, two separate double-strand breaks are introduced into the cell or subject's target nucleotide sequence with a double nickase Cas9 (see Ran et al., Cell 154:1380-1389, 2013), followed by delivery of the donor template.

In some instances, the composition includes a gRNA and a targeted nuclease, e.g., a Cas9, e.g., a wild type Cas9, a nickase Cas9 (e.g., Cas9 D10A), a dead Cas9 (dCas9), eSpCas9, Cpf1, C2C1, or C2C3, or a nucleic acid encoding such a nuclease. The choice of nuclease and gRNA(s) is determined by whether the targeted mutation is a deletion, substitution, or addition of nucleotides, e.g., a deletion, substitution, or addition of nucleotides to a targeted sequence. Fusions of a catalytically inactive endonuclease e.g., a dead Cas9 (dCas9, e.g., D10A; H840A) tethered with all or a portion of (e.g., biologically active portion of) an (one or more) effector domain create chimeric proteins that can be linked

to the polypeptide to guide the composition to specific DNA sites by one or more RNA sequences (sgRNA) to modulate activity and/or expression of one or more target nucleic acids sequences.

In instances, the agent includes a guide RNA (gRNA) for use in a CRISPR system for gene editing. In some instances, the agent includes a zinc finger nuclease (ZFN), or a mRNA encoding a ZFN, that targets (e.g., cleaves) a nucleic acid sequence (e.g., DNA sequence) of a gene in the plant. In some instances, the agent includes a TALEN, or an mRNA encoding a TALEN, that targets (e.g., cleaves) a nucleic acid sequence (e.g., DNA sequence) in a gene in the plant.

For example, the gRNA can be used in a CRISPR system to engineer an alteration in a gene in the plant. In other examples, the ZFN and/or TALEN can be used to engineer an alteration in a gene in the plant. Exemplary alterations include insertions, deletions (e.g., knockouts), translocations, inversions, single point mutations, or other mutations. The alteration can be introduced in the gene in a cell, e.g., in vitro, ex vivo, or in vivo. In some examples, the alteration increases the level and/or activity of a gene in the plant. In other examples, the alteration decreases the level and/or activity of (e.g., knocks down or knocks out) a gene in the plant. In yet another example, the alteration corrects a defect (e.g., a mutation causing a defect), in a gene in the plant.

In some instances, the CRISPR system is used to edit (e.g., to add or delete a base pair) a target gene in the plant. In other instances, the CRISPR system is used to introduce a premature stop codon, e.g., thereby decreasing the expression of a target gene. In yet other instances, the CRISPR system is used to turn off a target gene in a reversible manner, e.g., similarly to RNA interference. In some instances, the CRISPR system is used to direct Cas to a promoter of a gene, thereby blocking an RNA polymerase sterically.

In some instances, a CRISPR system can be generated to edit a gene in the plant, using technology described in, e.g., U.S. Publication No. 20140068797, Cong, Science 339: 819-823, 2013; Tsai, Nature Biotechnol. 32:6 569-576, 2014; U.S. Patent No.: 8,871,445; 8,865,406; 8,795,965; 8,771,945; and 8,697,359.

In some instances, the CRISPR interference (CRISPRi) technique can be used for transcriptional repression of specific genes in the plant. In CRISPRi, an engineered Cas9 protein (e.g., nuclease-null dCas9, or dCas9 fusion protein, e.g., dCas9-KRAB or dCas9-SID4X fusion) can pair with a sequence specific guide RNA (sgRNA). The Cas9-gRNA complex can block RNA polymerase, thereby interfering with transcription elongation. The complex can also block transcription initiation by interfering with transcription factor binding. The CRISPRi method is specific with minimal off-target effects and is multiplexable, e.g., can simultaneously repress more than one gene (e.g., using multiple gRNAs). Also, the CRISPRi method permits reversible gene repression.

In some instances, CRISPR-mediated gene activation (CRISPRa) can be used for transcriptional activation of a gene in the plant. In the CRISPRa technique, dCas9 fusion proteins recruit transcriptional activators. For example, dCas9 can be fused to polypeptides (e.g., activation domains) such as VP64 or the p65 activation domain (p65D) and used with sgRNA (e.g., a single sgRNA or multiple sgRNAs), to activate a gene or genes in the plant. Multiple activators can be recruited by using multiple sgRNAs – this can increase activation efficiency. A variety of activation domains and single or multiple activation domains can be used. In addition to engineering dCas9 to recruit activators, sgRNAs can also be

engineered to recruit activators. For example, RNA aptamers can be incorporated into a sgRNA to recruit proteins (e.g., activation domains) such as VP64. In some examples, the synergistic activation mediator (SAM) system can be used for transcriptional activation. In SAM, MS2 aptamers are added to the sgRNA. MS2 recruits the MS2 coat protein (MCP) fused to p65AD and heat shock factor 1 (HSF1).

5 The CRISPRi and CRISPRa techniques are described in greater detail, e.g., in Dominguez et al., Nat. Rev. Mol. Cell Biol. 17:5-15, 2016, incorporated herein by reference. In addition, dCas9-mediated epigenetic modifications and simultaneous activation and repression using CRISPR systems, as described in Dominguez et al., can be used to modulate a gene in the plant.

10 D. Heterologous therapeutic agents

The PMPs manufactured herein can include a heterologous therapeutic agent (e.g., an agent that affects an animal (e.g., a mammal, e.g., a human), an animal pathogen, or a pathogen vector thereof, and can be loaded into a PMP), such as a therapeutic peptide, a therapeutic nucleic acid (e.g., a therapeutic RNA), a therapeutic small molecule, or a pathogen control agent (e.g., antifungal agent, an antibacterial agent, a virucidal agent, an anti-viral agent, an insecticidal agent, a nematocidal agent, an antiparasitic agent, or an insect repellent). PMPs loaded with such agents can be formulated with a pharmaceutically acceptable carrier for delivery to an animal, an animal pathogen, or a pathogen vector thereof.

i. Antibacterial agents

20 The PMP compositions described herein can further include an antibacterial agent. For example, a PMP composition including an antibiotic as described herein can be administered to an animal in an amount and for a time sufficient to: reach a target level (e.g., a predetermined or threshold level) of antibiotic concentration inside or on the animal; and/or treat or prevent a bacterial infection in the animal. The antibacterials described herein may be formulated in a PMP composition for any of the methods described herein, and in certain instances, may be associated with the PMP thereof. In some instances, the PMP compositions includes two or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10) different antibacterial agents.

As used herein, the term "antibacterial agent" refers to a material that kills or inhibits the growth, proliferation, division, reproduction, or spread of bacteria, such as phytopathogenic bacteria, and includes bactericidal (e.g., disinfectant compounds, antiseptic compounds, or antibiotics) or bacteriostatic agents (e.g., compounds or antibiotics). Bactericidal antibiotics kill bacteria, while bacteriostatic antibiotics only slow their growth or reproduction.

Bactericides can include disinfectants, antiseptics, or antibiotics. The most used disinfectants can comprise: active chlorine (i.e., hypochlorites (e.g., sodium hypochlorite), chloramines, dichloroisocyanurate and trichloroisocyanurate, wet chlorine, chlorine dioxide etc.), active oxygen (peroxides, such as peracetic acid, potassium persulfate, sodium perborate, sodium percarbonate and urea perhydrate), iodine (iodopovidone (povidone-iodine, Betadine), Lugol's solution, iodine tincture, iodinated nonionic surfactants), concentrated alcohols (mainly ethanol, 1-propanol, called also n-propanol and 2-propanol, called isopropanol and mixtures thereof; further, 2-phenoxyethanol and 1- and 2- phenoxypropanols are used), phenolic substances (such as phenol (also called carbolic acid), cresols

(called Lysole in combination with liquid potassium soaps), halogenated (chlorinated, brominated) phenols, such as hexachlorophene, triclosan, trichlorophenol, tribromophenol, pentachlorophenol, Dibromol and salts thereof), cationic surfactants, such as some quaternary ammonium cations (such as benzalkonium chloride, cetyl trimethylammonium bromide or chloride, didecyl dimethylammonium chloride, cetylpyridinium chloride, benzethonium chloride) and others, non-quaternary compounds, such as chlorhexidine, glucoprotamine, octenidine dihydrochloride etc.), strong oxidizers, such as ozone and permanganate solutions; heavy metals and their salts, such as colloidal silver, silver nitrate, mercury chloride, phenylmercury salts, copper sulfate, copper oxide-chloride, copper hydroxide, copper octanoate, copper oxychloride sulfate, copper sulfate, copper sulfate pentahydrate, etc. Heavy metals and their salts are the most toxic, and environment-hazardous bactericides and therefore, their use is strongly oppressed or canceled; further, also properly concentrated strong acids (phosphoric, nitric, sulfuric, amidosulfuric, toluenesulfonic acids) and alkalis (sodium, potassium, calcium hydroxides). As antiseptics (i.e., germicide agents that can be used on human or animal body, skin, mucosae, wounds and the like), few of the above mentioned disinfectants can be used, under proper conditions (mainly concentration, pH, temperature and toxicity toward man/animal). Among them, important are: properly diluted chlorine preparations (i.e., Daquin's solution, 0.5% sodium or potassium hypochlorite solution, pH-adjusted to pH 7-8, or 0.5-1% solution of sodium benzenesulfochloramide (chloramine B)), some iodine preparations, such as iodopovidone in various galenics (ointment, solutions, wound plasters), in the past also Lugol's solution, peroxides as urea perhydrate solutions and pH-buffered 0.1-0.25% peracetic acid solutions, alcohols with or without antiseptic additives, used mainly for skin antiseptics, weak organic acids such as sorbic acid, benzoic acid, lactic acid and salicylic acid some phenolic compounds, such as hexachlorophene, triclosan and Dibromol, and cation-active compounds, such as 0.05-0.5% benzalkonium, 0.5-4% chlorhexidine, 0.1-2% octenidine solutions.

The PMP composition described herein may include an antibiotic. Any antibiotic known in the art may be used. Antibiotics are commonly classified based on their mechanism of action, chemical structure, or spectrum of activity.

The antibiotic described herein may target any bacterial function or growth processes and may be either bacteriostatic (e.g., slow or prevent bacterial growth) or bactericidal (e.g., kill bacteria). In some instances, the antibiotic is a bactericidal antibiotic. In some instances, the bactericidal antibiotic is one that targets the bacterial cell wall (e.g., penicillins and cephalosporins); one that targets the cell membrane (e.g., polymyxins); or one that inhibits essential bacterial enzymes (e.g., rifamycins, lipiarmycins, quinolones, and sulfonamides). In some instances, the bactericidal antibiotic is an aminoglycoside (e.g., kasugamycin). In some instances, the antibiotic is a bacteriostatic antibiotic. In some instances the bacteriostatic antibiotic targets protein synthesis (e.g., macrolides, lincosamides, and tetracyclines). Additional classes of antibiotics that may be used herein include cyclic lipopeptides (such as daptomycin), glycolcyclines (such as tigecycline), oxazolidinones (such as linezolid), or lipiarmycins (such as fidaxomicin). Examples of antibiotics include rifampicin, ciprofloxacin, doxycycline, ampicillin, and polymyxin B. The antibiotic described herein may have any level of target specificity (e.g., narrow- or broad-spectrum). In some instances, the antibiotic is a narrow-spectrum antibiotic, and thus targets

specific types of bacteria, such as gram-negative or gram-positive bacteria. Alternatively, the antibiotic may be a broad-spectrum antibiotic that targets a wide range of bacteria.

Examples of antibacterial agents suitable for the treatment of animals include Penicillins (Amoxicillin, Ampicillin, Bacampicillin, Carbenicillin, Cloxacillin, Dicloxacillin, Flucloxacillin, Mezlocillin, Nafcillin, Oxacillin, Penicillin G, Crysticillin 300 A.S., Pentids, Permapen, Pfizerpen, Pfizerpen-AS, Wycillin, Penicillin V, Piperacillin, Pivampicillin, Pivmecillinam, Ticarcillin), Cephalosporins (Cefacetrile (cephacetrile), Cefadroxil (cefadroxyl), Cefalexin (cephalexin), Cefaloglycin (cephaloglycin), Cefalonium (cephalonium), Cefaloridine (cephaloridine), Cefalotin (cephalothin), Cefapirin (cephapirin), Cefatrizine, Cefazaflur, Cefazedone, Cefazolin (cephazolin), Cefradine (cephradine), Cefroxadine, Ceftezole, Cefaclor, Cefamandole, Cefmetazole, Cefonicid, Cefotetan, Cefoxitin, Cefprozil (cefprozil), Cefuroxime, Cefuzonam, Cefcapene, Cefdaloxime, Cefdinir, Cefditoren, Cefetamet, Cefixime, Cefmenoxime, Cefodizime, Cefotaxime, Cefpimizole, Cefpodoxime, Cefteram, Ceftibuten, Ceftiofur, Ceftiolene, Ceftizoxime, Ceftriaxone, Cefoperazone, Ceftazidime, Cefclidine, Cefepime, Cefluprenam, Cefoselis, Cefozopran, Cefpirome, Cefquinome, Ceftobiprole, Ceftaroline, Cefaclomezine, Cefaloram, Cefaparole, Cefcanel, Cefedrolor, Cefempidone, Cefetizole, Cefivitril, Cefmatilen, Cefmepidium, Cefovecin, Cefoxazole, Cefrotil, Cefsumide, Cefuracetime, Ceftioxide, Combinations, Ceftazidime/Avibactam, Ceftolozane/Tazobactam), Monobactams (Aztreonam), Carbapenems (Imipenem, Imipenem/cilastatin, Doripenem, Ertapenem, Meropenem, Meropenem/vaborbactam), Macrolide (Azithromycin, Erythromycin, Clarithromycin, Dirithromycin, Roxithromycin, Telithromycin), Lincosamides (Clindamycin, Lincomycin), Streptogramins (Pristinamycin, Quinupristin/dalfopristin), Aminoglycoside (Amikacin, Gentamicin, Kanamycin, Neomycin, Netilmicin, Paromomycin, Streptomycin, Tobramycin), Quinolone (Flumequine, Nalidixic acid, Oxolinic acid, Piromidic acid, Pipemidic acid, Rosoxacin, Second Generation, Ciprofloxacin, Enoxacin, Lomefloxacin, Nadifloxacin, Norfloxacin, Ofloxacin, Pefloxacin, Rufloxacin, Balofloxacin, Gatifloxacin, Grepafloxacin, Levofloxacin, Moxifloxacin, Pazufloxacin, Sparfloxacin, Temafloxacin, Tosufloxacin, Besifloxacin, Delafloxacin, Clinafloxacin, Gemifloxacin, Prulifloxacin, Sitafloracin, Trovafloracin), Sulfonamides (Sulfamethizole, Sulfamethoxazole, Sulfisoxazole, Trimethoprim-Sulfamethoxazole), Tetracycline (Demeclocycline, Doxycycline, Minocycline, Oxytetracycline, Tetracycline, Tigecycline), Other (Lipopeptides, Fluoroquinolone, Lipoglycopeptides, Cephalosporin, Macrocyclics, Chloramphenicol, Metronidazole, Tinidazole, Nitrofurantoin, Glycopeptides, Vancomycin, Teicoplanin, Lipoglycopeptides, Telavancin, Oxazolidinones, Linezolid, Cycloserine 2, Rifamycins, Rifampin, Rifabutin, Rifapentine, Rifalazil, Polypeptides, Bacitracin, Polymyxin B, Tuberactinomycins, Viomycin, Capreomycin).

One skilled in the art will appreciate that a suitable concentration of each antibiotic in the composition depends on factors such as efficacy, stability of the antibiotic, number of distinct antibiotics, the formulation, and methods of application of the composition.

ii. Antifungal agents

The PMP compositions described herein can further include an antifungal agent. For example, a PMP composition including an antifungal as described herein can be administered to an animal in an amount and for a time sufficient to reach a target level (e.g., a predetermined or threshold level) of

antifungal concentration inside or on the animal; and/or treat or prevent a fungal infection in the animal. The antifungals described herein may be formulated in a PMP composition for any of the methods described herein, and in certain instances, may be associated with the PMP thereof. In some instances, the PMP compositions includes two or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10) different antifungal agents.

As used herein, the term "fungicide" or "antifungal agent" refers to a substance that kills or inhibits the growth, proliferation, division, reproduction, or spread of fungi, such as fungi that are pathogenic to animals. Many different types of antifungal agent have been produced commercially. Non limiting examples of antifungal agents include: Allylamines (Amorolfin, Butenafine, Naftifine, Terbinafine), Imidazoles ((Bifonazole, Butoconazole, Clotrimazole, Econazole, Fenticonazole, Ketoconazole, Isoconazole, Luliconazole, Miconazole, Omoconazole, Oxiconazole, Sertaconazole, Sulconazole, Tioconazole, Terconazole); Triazoles (Albaconazole, Efinaconazole, Fluconazole, Isavuconazole, Itraconazole, Posaconazole, Ravuconazole, Terconazole, Voriconazole), Thiazoles (Abafungin), Polyenes (Amphotericin B, Nystatin, Natamycin, Trichomycin), Echinocandins (Anidulafungin, Caspofungin, Micafungin), Other (Tolnaftate, Flucytosine, Butenafine, Griseofulvin, Ciclopirox, Selenium sulfide, Tavaborole). One skilled in the art will appreciate that a suitable concentration of each antifungal in the composition depends on factors such as efficacy, stability of the antifungal, number of distinct antifungals, the formulation, and methods of application of the composition.

iii. Insecticides

The PMP compositions described herein can further include an insecticide. For example, the insecticide can decrease the fitness of (e.g., decrease growth or kill) an insect vector of an animal pathogen. A PMP composition including an insecticide as described herein can be contacted with an insect, in an amount and for a time sufficient to: (a) reach a target level (e.g., a predetermined or threshold level) of insecticide concentration inside or on the insect; and (b) decrease fitness of the insect. In some instances, the insecticide can decrease the fitness of (e.g., decrease growth or kill) a parasitic insect. A PMP composition including an insecticide as described herein can be contacted with a parasitic insect, or an animal infected therewith, in an amount and for a time sufficient to: (a) reach a target level (e.g., a predetermined or threshold level) of insecticide concentration inside or on the parasitic insect; and (b) decrease the fitness of the parasitic insect. The insecticides described herein may be formulated in a PMP composition for any of the methods described herein, and in certain instances, may be associated with the PMP thereof. In some instances, the PMP compositions include two or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10) different insecticide agents.

As used herein, the term "insecticide" or "insecticidal agent" refers to a substance that kills or inhibits the growth, proliferation, reproduction, or spread of insects, such as insect vectors of animal pathogens or parasitic insects. Non limiting examples of insecticides are shown in Table 4. Additional non-limiting examples of suitable insecticides include biologics, hormones or pheromones such as azadirachtin, *Bacillus* species (e.g., *Bacillus thuringiensis*), *Beauveria* species, codlemone, *Metarrhizium* species, *Paecilomyces* species, *Saccharopolyspora* species, and *Verticillium* species, and active compounds having unknown or non-specified mechanisms of action such as fumigants (such as

aluminium phosphide, methyl bromide and sulphuryl fluoride) and selective feeding inhibitors (such as cryolite, flonicamid and pymetrozine). One skilled in the art will appreciate that a suitable concentration of each insecticide in the composition depends on factors such as efficacy, stability of the insecticide, number of distinct insecticides, the formulation, and methods of application of the composition.

5

Table 4. Examples of insecticides

Class	Compounds
chloronicotinyls/neonicotinoids	acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, nithiazine, thiacloprid, thiamethoxam, imidaclothiz, (2E)-1-[(2-chloro-1,3-thiazol-5-yl)methyl]-3,5-dimethyl-N-nitro-1,3,5-tri-azinan-2-imine, acetylcholinesterase (AChE) inhibitors (such as carbamates and organophosphates)
carbamates	alanycarb, aldicarb, aldoxycarb, allylxcarb, aminocarb, bendiocarb, benfuracarb, bufencarb, butacarb, butocarboxim, butoxycarboxim, carbaryl, carbofuran, carbosulfan, chloethocarb, dimetilan, ethiofencarb, fenobucarb, fenothiocarb, formetanate, furathiocarb, isoprocarb, metam-sodium, methiocarb, methomyl, metolcarb, oxamyl, phosphocarb, pirimicarb, promecarb, propoxur, thiodicarb, thiofanox, triazamate, trimethacarb, XMC, xylylcarb
organophosphates	acephate, azamethiphos, azinphos (-methyl, -ethyl), bromophos-ethyl, bromfenvinfos (-methyl), butathiofos, cadusafos, carbophenothion, chlorethoxyfos, chlorfenvinphos, chlormephos, chlorpyrifos (-methyl/-ethyl), coumaphos, cyanofenphos, cyanophos, demeton-S-methyl, demeton-S-methylsulphon, dialifos, diazinon, dichlofenthion, dichlorvos/DDVP, dicrotophos, dimethoate, dimethylvinphos, dioxabenzofos, disulfoton, EPN, ethion, ethoprophos, etrimfos, famphur, fenamiphos, fenitrothion, fensulfothion, fenthion, flupyrzofos, fonofos, formothion, fosmethilan, fosthiazate, heptenophos, iodofenphos, iprobenfos, isazofos, isofenphos, isopropyl O-salicylate, isoxathion, malathion, mecarbam, methacrifos, methamidophos, methidathion, mevinphos, monocrotophos, naled, omethoate, oxydemeton-methyl, parathion (-methyl/-ethyl), phenthoate, phorate, phosalone, phosmet, phosphamidon, phosphocarb, phoxim, pirimiphos (-methyl/-ethyl), profenofos, propaphos, propetamphos, prothiofos, prothoate, pyraclofos, pyridaphenthion, pyridathion, quinalphos, sebufos, sulfotep, sulprofos, tebupirimfos, temephos, terbufos, tetrachlorvinphos, thiometon, triazophos, triclofon, vamidothion
pyrethroids	acrinathrin, allethrin (d-cis-trans, d-trans), cypermethrin (alpha-, beta-, theta-, zeta-), permethrin (cis-, trans-), beta-cyfluthrin, bifenthrin, bioallethrin, bioallethrin-S-cyclopentyl-isomer, bioethanomethrin, biopermethrin, bioresmethrin, chlovaporthrin, cis-cypermethrin, cis-resmethrin, cis-permethrin, clocythrins, cycloprothrin, cyfluthrin, cyhalothrin, cyphenothrin, DDT, deltamethrin, empenhrin (1R-isomer), esfenvalerate, etofenprox, fenfluthrin, fenpropathrin, fenpyrithrin, fenvalerate, flubrocyrthinate, flucythrinate, flufenprox, flumethrin, fluvalinate, fubfenprox, gamma-cyhalothrin, imiprothrin, kadethrin, lambda, cyhalothrin, metofluthrin, phenothrin (1R-trans isomer), prallethrin, profluthrin, protrifenbute, pyresmethrin, resmethrin, RU 15525, silafluofen, tau-fluvalinate, tefluthrin, terallethrin, tetramethrin (1R-isomer), traloccythrins, tralomethrin, transfluthrin, ZXI 8901, pyrethrins (pyrethrum)
oxadiazines	indoxacarb, acetylcholine receptor modulators (such as spinosyns)
spinosyns	spinosad
cyclodiene	camphechlor, chlordane, endosulfan, gamma-HCH, HCH, heptachlor,

organochlorines	lindane, methoxychlor
fiproles	acetoprole, ethiprole, vaniliprole, fipronil
mectins	abamectin, avermectin, emamectin, emamectin-benzoate, fenoxycarb, hydroprene, kinoprene, methoprene, ivermectin, lepimectin, epofenonane, pyriproxifen, milbemectin, milbemycin, triprene
diacylhydrazines	chromafenozide, halofenozide, methoxyfenozide, tebufenozide
benzoylureas	bistrifluoron, chlorfluazuron, diflubenzuron, fluazuron, flucycloxuron, flufenoxuron, hexaflumuron, lufenuron, novaluron, noviflumuron, penfluoron, teflubenzuron, triflumuron
organotins	azocyclotin, cyhexatin, fenbutatin oxide
pyrroles	chlorfenapyr
dinitrophenols	binapacyrl, dinobuton, dinocap, DNOC
METIs	fenazaquin, fenpyroximate, pyrimidifen, pyridaben, tebufenpyrad, tolfenpyrad, rotenone, acequinocyl, fluacrypyrim, microbial disrupters of the intestinal membrane of insects (such as <i>Bacillus thuringiensis</i> strains), inhibitors of lipid synthesis (such as tetrionic acids and tetramic acids)
tetrionic acids	spirodiclofen, spiromesifen, spirotetramat
tetramic acids	cis-3-(2,5-dimethylphenyl)-8-methoxy-2-oxo-1-azaspiro[4.5]dec-3-en-4-yl ethyl carbonate (alias: carbonic acid, 3-(2,5-dimethylphenyl)-8-methoxy-2-oxo-1-azaspiro[4.5]dec-3-en-4-yl ethyl ester; CAS Reg. No.: 382608-10-8), carboxamides (such as flonicamid), octopaminergic agonists (such as amitraz), inhibitors of the magnesium-stimulated ATPase (such as propargite), ryanodin receptor agonists (such as phthalamides or rynaxapyr)
phthalamides	N2-[1,1-dimethyl-2-(methylsulphonyl)ethyl]-3-iodo-N1-[2-methyl-4-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl]phenyl]-1,2-benzenedicarboxamide (i.e., flubendiamide; CAS reg. No.: 272451-65-7)

iv. Nematicides

The PMP compositions described herein can further include a nematicide. In some instances, the PMP composition includes two or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10) different nematicides. For example, the nematicide can decrease the fitness of (e.g., decrease growth or kill) a parasitic nematode. A PMP composition including a nematicide as described herein can be contacted with a parasitic nematode, or an animal infected therewith, in an amount and for a time sufficient to: (a) reach a target level (e.g., a predetermined or threshold level) of nematicide concentration inside or on the target nematode; and (b) decrease fitness of the parasitic nematode. The nematicides described herein may be formulated in a PMP composition for any of the methods described herein, and in certain instances, may be associated with the PMP thereof.

As used herein, the term "nematicide" or "nematicidal agent" refers to a substance that kills or inhibits the growth, proliferation, reproduction, or spread of nematodes, such as a parasitic nematode. Non limiting examples of nematicides are shown in Table 5. One skilled in the art will appreciate that a suitable concentration of each nematicide in the composition depends on factors such as efficacy, stability of the nematicide, number of distinct nematicides, the formulation, and methods of application of the composition.

Table 5. Examples of nematicides

FUMIGANTS	D-D, 1,3-Dichloropropene, Ethylene Dibromide, 1,2-Dibromo-3-Chloropropane, Methyl Bromide, Chloropicrin, Metam Sodium, Dazomet, Methyl Isothiocyanate (MITC), Sodium Tetrathiocarbonate, Chloropicrin,
CARBAMATES	Aldicarb, Aldoxycarb, Carbofuran, Oxamyl, Cleothocarb
ORGANOPHOSPHATES	Ethoprophos, Fenamiphos, Cadusafos, Fosthiazate, Fensulfothion, Thionazin, Isazofos,
BIOCHEMICALS	DITERA®, CLANDOSAN®, SINOCIN®

v. Antiparasitic agent

The PMP compositions described herein can further include an antiparasitic agent. For example, the antiparasitic can decrease the fitness of (e.g., decrease growth or kill) a parasitic protozoan. A PMP composition including an antiparasitic as described herein can be contacted with a protozoan in an amount and for a time sufficient to: (a) reach a target level (e.g., a predetermined or threshold level) of antiparasitic concentration inside or on the protozoan, or animal infected therewith; and (b) decrease fitness of the protozoan. This can be useful in the treatment or prevention of parasites in animals. For example, a PMP composition including an antiparasitic agent as described herein can be administered to an animal in an amount and for a time sufficient to: reach a target level (e.g., a predetermined or threshold level) of antiparasitic concentration inside or on the animal; and/or treat or prevent a parasite (e.g., parasitic nematode, parasitic insect, or protozoan) infection in the animal. The antiparasitic described herein may be formulated in a PMP composition for any of the methods described herein, and in certain instances, may be associated with the PMP thereof. In some instances, the PMP composition includes two or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10) different antiparasitic agents.

As used herein, the term "antiparasitic" or "antiparasitic agent" refers to a substance that kills or inhibits the growth, proliferation, reproduction, or spread of parasites, such as parasitic protozoa, parasitic nematodes, or parasitic insects. Examples of antiparasitic agents include Anthelmintics (Bephenium, Diethylcarbamazine, Ivermectin, Niclosamide, Piperazine, Praziquantel, Pyrantel, Pyrvinium, Benzimidazoles, Albendazole, Flubendazole, Mebendazole, Thiabendazole, Levamisole, Nitazoxanide, Monopantel, Emodepside, Spiroindoles), Scabicides (Benzyl benzoate, Benzyl benzoate/disulfiram, Lindane, Malathion, Permethrin), Pediculicides (Piperonyl butoxide/pyrethrins, Spinosad, Moxidectin), Scabicides (Crotamiton), Anticestodes (Niclosamide, Pranziquantel, Albendazole), Antiamoebics (Rifampin, Apmphotericin B); or Antiprotozoals (Melarsoprol, Eflornithine, Metronidazole, Tinidazole, Miltefosine, Artemisinin). In certain instances, the antiparasitic agent may be used for treating or preventing infections in livestock animals, e.g., Levamisole, Fenbendazole, Oxfendazole, Albendazole, Moxidectin, Eprinomectin, Doramectin, Ivermectin, or Clorsulon. One skilled in the art will appreciate that a suitable concentration of each antiparasitic in the composition depends on factors such as efficacy, stability of the antiparasitic, number of distinct antiparasitics, the formulation, and methods of application of the composition.

vi. Antiviral agent

The PMP compositions described herein can further include an antiviral agent. A PMP composition including an antiviral agent as described herein can be administered to an animal in an amount and for a time sufficient to reach a target level (e.g., a predetermined or threshold level) of

antiviral concentration inside or on the animal; and/or to treat or prevent a viral infection in the animal. The antivirals described herein may be formulated in a PMP composition for any of the methods described herein, and in certain instances, may be associated with the PMP thereof. In some instances, the PMP composition includes two or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10) different antivirals.

As used herein, the term “antiviral” or “virucide” refers to a substance that kills or inhibits the growth, proliferation, reproduction, development, or spread of viruses, such as viral pathogens that infect animals. A number of agents can be employed as an antiviral, including chemicals or biological agents (e.g., nucleic acids, e.g., dsRNA). Examples of antiviral agents useful herein include Abacavir, Acyclovir (Aciclovir), Adefovir, Amantadine, Amprenavir (Agenerase), Ampligen, Arbidol, Atazanavir, Atripla, Balavir, Cidofovir, Combivir, Dolutegravir, Darunavir, Delavirdine, Didanosine, Docosanol, Edoxudine, Efavirenz, Emtricitabine, Enfuvirtide, Entecavir, Ecoliever, Famciclovir, Fomivirsen, Fosamprenavir, Foscarnet, Fosfonet, Fusion inhibitor, Ganciclovir, Ibacitabine, Immunovir, Idoxuridine, Imiquimod, Indinavir, Inosine, Integrase inhibitor, Interferon type III, Interferon type II, Interferon type I, Interferon, Lamivudine, Lopinavir, Loviride, Maraviroc, Moroxydine, Methisazone, Nelfinavir, Nevirapine, Nexavir, Nitazoxanide, Nucleoside analogues, Norvir, Oseltamivir (Tamiflu), Peginterferon alfa-2a, Penciclovir, Peramivir, Pleconaril, Podophyllotoxin, Raltegravir, Ribavirin, Rimantadine, Ritonavir, Pyrimidine, Saquinavir, Sofosbuvir, Stavudine, Synergistic enhancer (antiretroviral), Telaprevir, Tenofovir, Tenofovir disoproxil, Tipranavir, Trifluridine, Trizivir, Tromantadine, Truvada, Valaciclovir (Valtrex), Valganciclovir, Vicriviroc, Vidarabine, Viramidine, Zalcitabine, Zanamivir (Relenza), or Zidovudine. One skilled in the art will appreciate that a suitable concentration of each antiviral in the composition depends on factors such as efficacy, stability of the antivirals, number of distinct antivirals, the formulation, and methods of application of the composition.

25 *vii. Repellents*

The PMP compositions described herein can further include a repellent. For example, the repellent can repel a vector of animal pathogens, such as insects. The repellent described herein may be formulated in a PMP composition for any of the methods described herein, and in certain instances, may be associated with the PMP thereof. In some instances, the PMP composition includes two or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10) different repellents.

For example, a PMP composition including a repellent as described herein can be contacted with an insect vector or a habitat of the vector in an amount and for a time sufficient to: (a) reach a target level (e.g., a predetermined or threshold level) of repellent concentration; and/or (b) decrease the levels of the insect near or on nearby animals relative to a control. Alternatively, a PMP composition including a repellent as described herein can be contacted with an animal in an amount and for a time sufficient to: (a) reach a target level (e.g., a predetermined or threshold level) of repellent concentration; and/or (b) decrease the levels of the insect near or on the animal relative to an untreated animal.

Some examples of well-known insect repellents include: benzil; benzyl benzoate; 2,3,4,5-bis(butyl-2-ene)tetrahydrofurfural (MGK Repellent 11); butoxypolypropylene glycol; N-butylacetanilide; normal-butyl-6,6-dimethyl-5,6-dihydro-1,4-pyrone-2-carboxylate (Indalone); dibutyl adipate; dibutyl

phthalate; di-normal-butyl succinate (Tabatrex); N,N-diethyl-meta-toluamide (DEET); dimethyl carbate (endo,endo)-dimethyl bicyclo[2.2.1] hept-5-ene-2,3-dicarboxylate); dimethyl phthalate; 2-ethyl-2-butyl-1,3-propanediol; 2-ethyl-1,3-hexanediol (Rutgers 612); di-normal-propyl isocinchomeronate (MGK Repellent 326); 2-phenylcyclohexanol; p-methane-3,8-diol, and normal-propyl N,N-diethylsuccinamate. Other
 5 repellents include citronella oil, dimethyl phthalate, normal-butylmesityl oxide oxalate and 2-ethyl hexanediol-1,3 (See, Kirk-Othmer Encyclopedia of Chemical Technology, 2nd Ed., Vol. 11: 724-728; and The Condensed Chemical Dictionary, 8th Ed., p 756).

In some instances, the repellent is an insect repellent, including synthetic or nonsynthetic insect repellents. Examples of synthetic insect repellents include methyl anthranilate and other anthranilate-
 10 based insect repellents, benzaldehyde, DEET (N,N-diethyl-m-toluamide), dimethyl carbate, dimethyl phthalate, icaridin (i.e., picaridin, Bayrepel, and KBR 3023), indalone (e.g., as used in a "6-2-2" mixture (60% Dimethyl phthalate, 20% Indalone, 20% Ethylhexanediol), IR3535 (3-[N-Butyl-N-acetyl]-aminopropionic acid, ethyl ester), metofluthrin, permethrin, SS220, or tricyclodecenyl allyl ether. Examples of natural insect repellents include beautyberry (Callicarpa) leaves, birch tree bark, bog myrtle
 15 (Myrica Gale), catnip oil (e.g., nepetalactone), citronella oil, essential oil of the lemon eucalyptus (Corymbia citriodora; e.g., p-menthane-3,8-diol (PMD)), neem oil, lemongrass, tea tree oil from the leaves of Melaleuca alternifolia, tobacco, or extracts thereof.

III. Methods of Use

20 The PMPs herein are useful in a variety of agricultural or therapeutic methods. Examples of methods of using PMPs (e.g., including modified PMPs described herein) are described further below.

A. Delivery to a Plant

Provided herein are methods of delivering a PMP composition (e.g., including modified PMPs
 25 described herein) to a plant, e.g., by contacting the plant, or part thereof, with the PMP composition. In some instances, plants may be treated with PMPs not including a heterologous functional agent. In other instances, the PMPs include a heterologous functional agent, e.g., pesticidal agents (e.g., antibacterial agents, antifungal agents, nematicides, molluscicides, virucides, herbicides), pest control agents (e.g., repellents), fertilizing agents, or plant-modifying agents.

30 In one aspect, provided herein is a method of increasing the fitness of a plant, the method including delivering to the plant the PMP composition described herein (e.g., in an effective amount and duration) to increase the fitness of the plant relative to an untreated plant (e.g., a plant that has not been delivered the PMP composition).

An increase in the fitness of the plant as a consequence of delivery of a PMP composition can
 35 manifest in a number of ways, e.g., thereby resulting in a better production of the plant, for example, an improved yield, improved vigor of the plant (e.g., improved tolerance of abiotic or biotic stress or improved resistance to pests) or improved quality of the harvested product from the plant. An improved yield of a plant relates to an increase in the yield of a product (e.g., as measured by plant biomass, grain, seed or fruit yield, protein content, carbohydrate or oil content or leaf area) of the plant by a measurable amount
 40 over the yield of the same product of the plant produced under the same conditions, but without the

application of the instant compositions or compared with application of conventional agricultural agents. For example, yield can be increased by at least about 0.5%, about 1%, about 2%, about 3%, about 4%, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, or more than 100%. Yield can be expressed in terms of an amount by weight or volume of the plant or a product of the plant on some basis. The basis can be expressed in terms of time, growing area, weight of plants produced, or amount of a raw material used. For example, such methods may increase the yield of plant tissues including, but not limited to: seeds, fruits, kernels, bolls, tubers, roots, and leaves.

An increase in the fitness of a plant as a consequence of delivery of a PMP composition can also be measured by other methods, such as an increase or improvement of the vigor rating, the stand (the number of plants per unit of area), plant height, stalk circumference, stalk length, leaf number, leaf size, plant canopy, visual appearance (such as greener leaf color), root rating, emergence, protein content, increased tillering, bigger leaves, more leaves, less dead basal leaves, stronger tillers, less fertilizer needed, less seeds needed, more productive tillers, earlier flowering, early grain or seed maturity, less plant verse (lodging), increased shoot growth, earlier germination, or any combination of these factors, by a measurable or noticeable amount over the same factor of the plant produced under the same conditions, but without the administration of the instant compositions or with application of conventional agricultural agents.

Provided herein is a method of modifying or increasing the fitness of a plant, the method including delivering to the plant an effective amount of a PMP composition provided herein, wherein the method modifies the plant and thereby introduces or increases a beneficial trait in the plant (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%) relative to an untreated plant. In particular, the method may increase the fitness of the plant (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%) relative to an untreated plant.

In some instances, the increase in plant fitness is an increase (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%) in disease resistance, drought tolerance, heat tolerance, cold tolerance, salt tolerance, metal tolerance, herbicide tolerance, chemical tolerance, water use efficiency, nitrogen utilization, resistance to nitrogen stress, nitrogen fixation, pest resistance, herbivore resistance, pathogen resistance, yield, yield under water-limited conditions, vigor, growth, photosynthetic capability, nutrition, protein content, carbohydrate content, oil content, biomass, shoot length, root length, root architecture, seed weight, or amount of harvestable produce.

In some instances, the increase in fitness is an increase (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%) in development, growth, yield, resistance to abiotic stressors, or resistance to biotic stressors. An abiotic stress refers to an environmental stress condition that a plant or a plant part is subjected to that includes, e.g., drought stress, salt stress, heat stress, cold stress, and low nutrient stress. A biotic stress refers to an environmental stress condition that a plant or plant part is subjected to that includes, e.g. nematode stress, insect herbivory stress, fungal pathogen stress, bacterial pathogen stress, or viral pathogen

stress. The stress may be temporary, e.g. several hours, several days, several months, or permanent, e.g. for the life of the plant.

In some instances, the increase in plant fitness is an increase (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%) in quality of products harvested from the plant. For example, the increase in plant fitness may be an improvement in commercially favorable features (e.g., taste or appearance) of a product harvested from the plant. In other instances, the increase in plant fitness is an increase in shelf-life of a product harvested from the plant (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%).

Alternatively, the increase in fitness may be an alteration of a trait that is beneficial to human or animal health, such as a reduction in allergen production. For example, the increase in fitness may be a decrease (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%) in production of an allergen (e.g., pollen) that stimulates an immune response in an animal (e.g., human).

The modification of the plant (e.g., increase in fitness) may arise from modification of one or more plant parts. For example, the plant can be modified by contacting leaf, seed, pollen, root, fruit, shoot, flower, cells, protoplasts, or tissue (e.g., meristematic tissue) of the plant. As such, in another aspect, provided herein is a method of increasing the fitness of a plant, the method including contacting pollen of the plant with an effective amount of a PMP composition herein, wherein the method increases the fitness of the plant (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%) relative to an untreated plant.

In yet another aspect, provided herein is a method of increasing the fitness of a plant, the method including contacting a seed of the plant with an effective amount of a PMP composition disclosed herein, wherein the method increases the fitness of the plant (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%) relative to an untreated plant.

In another aspect, provided herein is a method including contacting a protoplast of the plant with an effective amount of a PMP composition herein, wherein the method increases the fitness of the plant (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%) relative to an untreated plant.

In a further aspect, provided herein is a method of increasing the fitness of a plant, the method including contacting a plant cell of the plant with an effective amount of a PMP composition herein, wherein the method increases the fitness of the plant (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%) relative to an untreated plant.

In another aspect, provided herein is a method of increasing the fitness of a plant, the method including contacting meristematic tissue of the plant with an effective amount of a PMP composition herein, wherein the method increases the fitness of the plant (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%) relative to an untreated plant.

In another aspect, provided herein is a method of increasing the fitness of a plant, the method including contacting an embryo of the plant with an effective amount of a PMP composition herein, wherein the method increases the fitness of the plant (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%) relative to an untreated plant.

In cases where an herbicide is included in the PMP, or compositions thereof, the methods may be further used to decrease the fitness of or kill weeds. In such instances, the method may be effective to decrease the fitness of the weed by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more in comparison to an untreated weed (e.g., a weed to which the PMP composition has not been administered). For example, the method may be effective to kill the weed, thereby decreasing a population of the weed by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more in comparison to an untreated weed. In some instances, the method substantially eliminates the weed. Examples of weeds that can be treated in accordance with the present methods are further described herein.

i. Plants

A variety of plants can be delivered or treated with a PMP composition described herein. Plants that can be delivered a PMP composition (i.e., "treated") in accordance with the present methods include whole plants and parts thereof, including, but not limited to, shoot vegetative organs/structures (e.g., leaves, stems and tubers), roots, flowers and floral organs/structures (e.g., bracts, sepals, petals, stamens, carpels, anthers and ovules), seed (including embryo, endosperm, cotyledons, and seed coat) and fruit (the mature ovary), plant tissue (e.g., meristematic tissue, vascular tissue, ground tissue, and the like) and cells (e.g., guard cells, egg cells, and the like), and progeny of same. Plant parts can further refer parts of the plant such as the shoot, root, stem, seeds, stipules, leaves, petals, flowers, ovules, bracts, branches, petioles, internodes, bark, pubescence, tillers, rhizomes, fronds, blades, pollen, stamen, and the like.

The class of plants that can be treated in a method disclosed herein includes the class of higher and lower plants, including angiosperms (monocotyledonous and dicotyledonous plants), gymnosperms, ferns, horsetails, psilophytes, lycophytes, bryophytes, and algae (e.g., multicellular or unicellular algae). Plants that can be treated in accordance with the present methods further include any vascular plant, for example monocotyledons or dicotyledons or gymnosperms, including, but not limited to alfalfa, apple, Arabidopsis, banana, barley, canola, castor bean, chrysanthemum, clover, cocoa, coffee, cotton, cottonseed, corn, crambe, cranberry, crucifers, cucumber, dendrobium, dioscorea, eucalyptus, fescue, flax, gladiolus, liliacea, linseed, millet, muskmelon, mustard, oat, oil palm, canola or oilseed rape, papaya, peanut, pineapple, ornamental plants, Phaseolus, potato, rapeseed, rice, rye, ryegrass, safflower, sesame, sorghum, soybean, sugarbeet, sugarcane, sunflower, strawberry, tobacco, tomato, turfgrass, wheat, and vegetable crops such as lettuce, celery, broccoli, cauliflower, cucurbits; fruit and nut trees, such as apple, pear, peach, orange, grapefruit, lemon, lime, almond, pecan, walnut, hazel; vines, such as grapes (e.g., a vineyard), kiwi, hops; cannabis, fruit shrubs and brambles, such as raspberry, blackberry, gooseberry; forest trees, such as ash, pine, fir, maple, oak, chestnut, popular; with alfalfa, canola, castor bean, corn, cotton, crambe, flax, linseed, mustard, oil palm, oilseed rape, peanut, potato, rice, safflower, sesame, soybean, sugarbeet, sunflower, tobacco, tomato, and wheat. Plants that can be treated in accordance with the methods of the present invention include any crop plant, for example, forage crop, oilseed crop, grain crop, fruit crop, vegetable crop, fiber crop, spice crop, nut crop, turf crop, sugar crop, beverage crop, and forest crop. In certain instances, the crop plant that is treated in the method is a

soybean plant. In other certain instances, the crop plant is wheat. In certain instances, the crop plant is corn. In certain instances, the crop plant is cotton. In certain instances, the crop plant is alfalfa. In certain instances, the crop plant is sugarbeet. In certain instances, the crop plant is rice. In certain instances, the crop plant is potato. In certain instances, the crop plant is tomato.

- 5 In certain instances, the plant is a crop. Examples of such crop plants include, but are not limited to, monocotyledonous and dicotyledonous plants including, but not limited to, fodder or forage legumes, ornamental plants, food crops, trees, or shrubs selected from *Acer* spp., *Allium* spp., *Amaranthus* spp., *Ananas comosus*, *Apium graveolens*, *Arachis* spp., *Asparagus officinalis*, *Beta vulgaris*, *Brassica* spp. (e.g., *Brassica napus*, *Brassica rapa* ssp. (canola, oilseed rape, turnip rape), *Camellia sinensis*, *Canna*
- 10 *indica*, *Cannabis sativa*, *Capsicum* spp., *Castanea* spp., *Cichorium endivia*, *Citrullus lanatus*, *Citrus* spp., *Cocos* spp., *Coffea* spp., *Coriandrum sativum*, *Corylus* spp., *Crataegus* spp., *Cucurbita* spp., *Cucumis* spp., *Daucus carota*, *Fagus* spp., *Ficus carica*, *Fragaria* spp., *Ginkgo biloba*, *Glycine* spp. (e.g., *Glycine max*, *Soja hispida* or *Soja max*), *Gossypium hirsutum*, *Helianthus* spp. (e.g., *Helianthus annuus*), *Hibiscus* spp., *Hordeum* spp. (e.g., *Hordeum vulgare*), *Ipomoea batatas*, *Juglans* spp., *Lactuca sativa*, *Linum*
- 15 *usitatissimum*, *Litchi chinensis*, *Lotus* spp., *Luffa acutangula*, *Lupinus* spp., *Lycopersicon* spp. (e.g., *Lycopersicon esculentum*, *Lycopersicon lycopersicum*, *Lycopersicon pyriforme*), *Malus* spp., *Medicago sativa*, *Mentha* spp., *Miscanthus sinensis*, *Morus nigra*, *Musa* spp., *Nicotiana* spp., *Olea* spp., *Oryza* spp. (e.g., *Oryza sativa*, *Oryza latifolia*), *Panicum miliaceum*, *Panicum virgatum*, *Passiflora edulis*, *Petroselinum crispum*, *Phaseolus* spp., *Pinus* spp., *Pistacia vera*, *Pisum* spp., *Poa* spp., *Populus* spp.,
- 20 *Prunus* spp., *Pyrus communis*, *Quercus* spp., *Raphanus sativus*, *Rheum rhabarbarum*, *Ribes* spp., *Ricinus communis*, *Rubus* spp., *Saccharum* spp., *Salix* sp., *Sambucus* spp., *Secale cereale*, *Sesamum* spp., *Sinapis* spp., *Solanum* spp. (e.g., *Solanum tuberosum*, *Solanum integrifolium* or *Solanum lycopersicum*), *Sorghum bicolor*, *Sorghum halepense*, *Spinacia* spp., *Tamarindus indica*, *Theobroma cacao*, *Trifolium* spp., *Triticosecale rimpaui*, *Triticum* spp. (e.g., *Triticum aestivum*, *Triticum durum*,
- 25 *Triticum turgidum*, *Triticum hybernum*, *Triticum macha*, *Triticum sativum* or *Triticum vulgare*), *Vaccinium* spp., *Vicia* spp., *Vigna* spp., *Viola odorata*, *Vitis* spp., and *Zea mays*. In certain embodiments, the crop plant is rice, oilseed rape, canola, soybean, corn (maize), cotton, sugarcane, alfalfa, *sorghum*, or wheat.

- In certain instance, the compositions and methods can be used to treat post-harvest plants or plant parts, food, or feed products. In some instances, the food or feed product is a non-plant food or feed product (e.g., a product edible for humans, veterinary animals, or livestock (e.g., mushrooms)).
- 30

- The plant or plant part for use in the present invention include plants of any stage of plant development. In certain instances, the delivery can occur during the stages of germination, seedling growth, vegetative growth, and reproductive growth. In certain instances, delivery to the plant occurs during vegetative and reproductive growth stages. Alternatively, the delivery can occur to a seed. The stages of vegetative and reproductive growth are also referred to herein as "adult" or "mature" plants.
- 35

ii. Weeds

- In cases where an herbicide is included in the PMP, or compositions thereof, the methods may be further used to decrease the fitness of or kill weeds. In such instances, the method may be effective to decrease the fitness of the weed by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%,
- 40

100%, or more in comparison to an untreated weed (e.g., a weed to which the PMP composition has not been administered). For example, the method may be effective to kill the weed, thereby decreasing a population of the weed by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more in comparison to an untreated weed. In some instances, the method substantially eliminates the weed. Examples of weeds that can be treated in accordance with the present methods are further described herein.

As used herein, the term weed refers to a plant that grows where it is not wanted. Such plants are typically invasive and, at times, harmful, or have the risk of becoming so. Weeds may be treated with the present PMP compositions to reduce or eliminate the presence, viability, or reproduction of the plant.

For example, and without being limited thereto, the methods can be used to target weeds known to damage plants. For example, and without being limited thereto, the weeds can be any member of the following group of families: Gramineae, Umbelliferae, Papilionaceae, Cruciferae, Malvaceae, Eufhorbiaceae, Compositae, Chenopodiaceae, Fumariaceae, Charyophyllaceae, Primulaceae, Geraniaceae, Polygonaceae, Juncaceae, Cyperaceae, Aizoaceae, Asteraceae, Convolvulaceae, Cucurbitaceae, Euphorbiaceae, Polygonaceae, Portulacaceae, Solanaceae, Rosaceae, Simaroubaceae, Lardizabalaceae, Liliaceae, Amaranthaceae, Vitaceae, Fabaceae, Primulaceae, Apocynaceae, Araliaceae, Caryophyllaceae, Asclepiadaceae, Celastraceae, Papaveraceae, Onagraceae, Ranunculaceae, Lamiaceae, Commelinaceae, Scrophulariaceae, Dipsacaceae, Boraginaceae, Equisetaceae, Geraniaceae, Rubiaceae, Cannabaceae, Hypericaceae, Balsaminaceae, Lobeliaceae, Caprifoliaceae, Nyctaginaceae, Oxalidaceae, Vitaceae, Urticaceae, Polypodiaceae, Anacardiaceae, Smilacaceae, Araceae, Campanulaceae, Typhaceae, Valerianaceae, Verbenaceae, Violaceae. For example, and without being limited thereto, the weeds can be any member of the group consisting of *Lolium rigidum*, *Amaramthus palmeri*, *Abutilon theopratsi*, *Sorghum halepense*, *Conyza Canadensis*, *Setaria verticillata*, *Capsella pastoris*, and *Cyperus rotundas*. Additional weeds include, for example, *Mimosapigra*, *salvinia*, *hyptis*, *senna*, *noogoora*, *burr*, *Jatropha gossypifolia*, *Parkinsonia aculeate*, *Chromolaena odorata*, *Cryptoslegia grandiflora*, or *Andropogon gayanus*. Weeds can include monocotyledonous plants (e.g., *Agrostis*, *Alopecurus*, *Avena*, *Bromus*, *Cyperus*, *Digitaria*, *Echinochloa*, *Lolium*, *Monochoria*, *Rottboellia*, *Sagittaria*, *Scirpus*, *Setaria*, *Sida* or *Sorghum*) or dicotyledonous plants (*Abutilon*, *Amaranthus*, *Chenopodium*, *Chrysanthemum*, *Conyza*, *Galium*, *Ipomoea*, *Nasturtium*, *Sinapis*, *Solanum*, *Stellaria*, *Veronica*, *Viola* or *Xanthium*).

The compositions and related methods can be used to prevent infestation by or reduce the numbers of pathogens or pathogen vectors in any habitats in which they reside (e.g., outside of animals, e.g., on plants, plant parts (e.g., roots, fruits and seeds), in or on soil, water, or on another pathogen or pathogen vector habitat. Accordingly, the compositions and methods can reduce the damaging effect of pathogen vectors by for example, killing, injuring, or slowing the activity of the vector, and can thereby control the spread of the pathogen to animals. Compositions disclosed herein can be used to control, kill, injure, paralyze, or reduce the activity of one or more of any pathogens or pathogen vectors in any developmental stage, e.g., their egg, nymph, instar, larvae, adult, juvenile, or desiccated forms. The details of each of these methods are described further below.

40

B. Delivery to a Plant Pest

Provided herein are methods of delivering a PMP composition (e.g., including modified PMPs described herein) to a plant pest, e.g., by contacting the plant pest with the PMP composition. In some instances, the plant pests may be treated with PMPs not including a heterologous functional agent. In other instances, the PMPs include a heterologous functional agent, e.g., pesticidal agents (e.g.,
5 antibacterial agents, antifungal agents, nematicides, molluscicides, virucides, or herbicides) or pest control agents (e.g., repellents). For example, the methods can be useful for decreasing the fitness of a pest, e.g., to prevent or treat a pest infestation as a consequence of delivery of a PMP composition.

In one aspect, provided herein is a method of decreasing the fitness of a pest, the method
10 including delivering to the pest the PMP composition described herein (e.g., in an effective amount and for an effective duration) to decrease the fitness of the pest relative to an untreated pest (e.g., a pest that has not been delivered the PMP composition).

In one aspect, provided herein is a method of decreasing a fungal infection in (e.g., treating) a plant having a fungal infection, wherein the method includes delivering to the plant pest a PMP
15 composition including a plurality of PMPs (e.g., a PMP composition described herein).

In another aspect, provided herein is a method of decreasing a fungal infection in (e.g., treating) a plant having a fungal infection, wherein the method includes delivering to the plant pest a PMP composition including a plurality of PMPs (e.g., a PMP composition described herein), and wherein the plurality of PMPs include an antifungal agent. In some instances, the antifungal agent is a nucleic acid
20 that inhibits expression of a gene (e.g., *dcl1* and *dcl2* (i.e., *dcl1/2*) in a fungus that causes the fungal infection. In some instances, the fungal infection is caused by a fungus belonging to a *Sclerotinia* spp. (e.g., *Sclerotinia sclerotiorum*), a *Botrytis* spp. (e.g., *Botrytis cinerea*), an *Aspergillus* spp., a *Fusarium* spp., or a *Penicillium* spp. In some instances, the composition includes a PMP produced from an *Arabidopsis* apoplast EV. In some instances, the method decreases or substantially eliminates the fungal
25 infection.

In another aspect, provided herein is a method of decreasing a bacterial infection in (e.g., treating) a plant having a bacterial infection, wherein the method includes delivering to the plant pest a PMP composition including a plurality of PMPs (e.g., a PMP composition described herein).

In another aspect, provided herein is a method of decreasing a bacterial infection in (e.g.,
30 treating) a plant having a bacterial infection, wherein the method includes delivering to the plant pest a PMP composition including a plurality of PMPs, and wherein the plurality of PMPs include an antibacterial agent. In some instances, the antibacterial agent is streptomycin. In some instances, the bacterial infection is caused by a bacterium belonging to a *Pseudomonas* spp (e.g., *Pseudomonas syringae*). In some instances, the composition includes a PMP produced from an *Arabidopsis* apoplast EV. In some
35 instances, the method decreases or substantially eliminates the bacterial infection.

In another aspect, provided herein is a method of decreasing the fitness of an insect plant pest, wherein the method includes delivering to the insect plant pest a PMP composition including a plurality of PMPs (e.g., a PMP composition described herein).

In another aspect, provided herein is a method of decreasing the fitness of an insect plant pest,
40 wherein the method includes delivering to the insect plant pest a PMP composition including a plurality of

PMPs (e.g., a PMP composition described herein), and wherein the plurality of PMPs includes an insecticidal agent. In some instances, the insecticidal agent is a peptide nucleic acid. In some instances, the insect plant pest is an aphid. In some instances, the insect plant pest is a lepidopteran (e.g., *Spodoptera frugiperda*). In some instances, the insect plant pest is an arachnid, e.g., a mite. In some instances, the method decreases the fitness of the insect plant pest relative to an untreated insect plant pest

In another aspect, provided herein is a method of decreasing the fitness of a nematode plant pest, wherein the method includes delivering to the nematode plant pest a PMP composition including a plurality of PMPs (e.g., a PMP composition described herein).

In another aspect, provided herein is a method of decreasing the fitness of a nematode plant pest, wherein the method includes delivering to the nematode plant pest a PMP composition including a plurality of PMPs (e.g., a PMP composition described herein), and wherein the plurality of PMPs include a nematocidal agent. In some instances, the nematocidal agent is a neuropeptide (e.g., Mi-NLP-15b). In some instances, the nematode plant pest is a root-knot nematode. In some instances, the method decreases the fitness of the nematode plant pest relative to an untreated nematode plant pest.

In another aspect, provided herein is a method of decreasing the fitness of a weed, wherein the method includes delivering to the weed a PMP composition including a plurality of PMPs (e.g., a PMP composition described herein).

In another aspect, provided herein is a method of decreasing the fitness of a weed, wherein the method includes delivering to the weed a PMP composition including a plurality of PMPs (e.g., a PMP composition described herein), and wherein the plurality of PMPs include an herbicidal agent (e.g. Glufosinate). In some instances, the weed is an Indian goosegrass (*Eleusine indica*). In some instances, the method decreases the fitness of the weed relative to an untreated weed.

A decrease in the fitness of the pest as a consequence of delivery of a PMP composition can manifest in a number of ways. In some instances, the decrease in fitness of the pest may manifest as a deterioration or decline in the physiology of the pest (e.g., reduced health or survival) as a consequence of delivery of the PMP composition. In some instances, the fitness of an organism may be measured by one or more parameters, including, but not limited to, reproductive rate, fertility, lifespan, viability, mobility, fecundity, pest development, body weight, metabolic rate or activity, or survival in comparison to a pest to which the PMP composition has not been administered. For example, the methods or compositions provided herein may be effective to decrease the overall health of the pest or to decrease the overall survival of the pest. In some instances, the decreased survival of the pest is about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or greater than 100% greater relative to a reference level (e.g., a level found in a pest that does not receive a PMP composition). In some instances, the methods and compositions are effective to decrease pest reproduction (e.g., reproductive rate, fertility) in comparison to a pest to which the PMP composition has not been administered. In some instances, the methods and compositions are effective to decrease other physiological parameters, such as mobility, body weight, life span, fecundity, or metabolic rate, by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or greater than 100% relative to a reference level (e.g., a level found in a pest that does not receive a PMP composition).

In some instances, the decrease in pest fitness may manifest as a decrease in the production of one or more nutrients in the pest (e.g., vitamins, carbohydrates, amino acids, or polypeptides) in comparison to a pest to which the PMP composition has not been administered. In some instances, the methods or compositions provided herein may be effective to decrease the production of nutrients in the pest (e.g., vitamins, carbohydrates, amino acids, or polypeptides) by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or greater than 100% relative to a reference level (e.g., a level found in a pest that does not receive a PMP composition).

In some instances, the decrease in pest fitness may manifest as an increase in the pest's sensitivity to a pesticidal agent and/or a decrease in the pest's resistance to a pesticidal agent in comparison to a pest to which the PMP composition has not been administered. In some instances, the methods or compositions provided herein may be effective to increase the pest's sensitivity to a pesticidal agent by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or greater than 100% relative to a reference level (e.g., a level found in a pest that does not receive a PMP composition). The pesticidal agent may be any pesticidal agent known in the art, including insecticidal agents. In some instances, the methods or compositions provided herein may increase the pest's sensitivity to a pesticidal agent by decreasing the pest's ability to metabolize or degrade the pesticidal agent into usable substrates in comparison to a pest to which the PMP composition has not been administered.

In some instances, the decrease in pest fitness may manifest as an increase in the pest's sensitivity to an allelochemical agent and/or a decrease in the pest's resistance to an allelochemical agent in comparison to a pest to which the PMP composition has not been administered. In some instances, the methods or compositions provided herein may be effective to decrease the pest's resistance to an allelochemical agent by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or greater than 100% relative to a reference level (e.g., a level found in a pest that does not receive a PMP composition). In some instances, the allelochemical agent is caffeine, soyacystatin, fenitrothion, monoterpenes, diterpene acids, or phenolic compounds (e.g., tannins, flavonoids). In some instances, the methods or compositions provided herein may increase the pest's sensitivity to an allelochemical agent by decreasing the pest's ability to metabolize or degrade the allelochemical agent into usable substrates in comparison to a pest to which the PMP composition has not been administered.

In some instances, the methods or compositions provided herein may be effective to decrease the pest's resistance to parasites or pathogens (e.g., fungal, bacterial, or viral pathogens or parasites) in comparison to a pest to which the PMP composition has not been administered. In some instances, the methods or compositions provided herein may be effective to decrease the pest's resistance to a pathogen or parasite (e.g., fungal, bacterial, or viral pathogens; or parasitic mites) by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or greater than 100% relative to a reference level (e.g., a level found in a pest that does not receive a PMP composition).

In some instances, the methods or compositions provided herein may be effective to decrease the pest's ability to carry or transmit a plant pathogen (e.g., plant virus (e.g., TYLCV) or a plant bacterium (e.g., *Agrobacterium* spp.)) in comparison to a pest to which the PMP composition has not been administered. For example, the methods or compositions provided herein may be effective to decrease the pest's ability to carry or transmit a plant pathogen (e.g., a plant virus (e.g., TYLCV) or plant bacterium

(e.g., *Agrobacterium* spp.)) by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or greater than 100% relative to a reference level (e.g., a level found in a pest that does not receive a PMP composition).

Additionally or alternatively, in cases where an herbicide is included in the PMP, or compositions thereof, the methods may be further used to decrease the fitness of or kill weeds. In such instances, the method may be effective to decrease the fitness of the weed by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more in comparison to an untreated weed (e.g., a weed to which the PMP composition has not been administered). For example, the method may be effective to kill the weed, thereby decreasing a population of the weed by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more in comparison to an untreated weed. In some instances, the method substantially eliminates the weed. Examples of weeds that can be treated in accordance with the present methods are further described herein.

In some instances, the decrease in pest fitness may manifest as other fitness disadvantages, such as a decreased tolerance to certain environmental factors (e.g., a high or low temperature tolerance), a decreased ability to survive in certain habitats, or a decreased ability to sustain a certain diet in comparison to a pest to which the PMP composition has not been administered. In some instances, the methods or compositions provided herein may be effective to decrease pest fitness in any plurality of ways described herein. Further, the PMP composition may decrease pest fitness in any number of pest classes, orders, families, genera, or species (e.g., 1 pest species, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 500, or more pest species). In some instances, the PMP composition acts on a single pest class, order, family, genus, or species.

Pest fitness may be evaluated using any standard methods in the art. In some instances, pest fitness may be evaluated by assessing an individual pest. Alternatively, pest fitness may be evaluated by assessing a pest population. For example, a decrease in pest fitness may manifest as a decrease in successful competition against other insects, thereby leading to a decrease in the size of the pest population.

i. Fungi

The PMP compositions and related methods can be useful for decreasing the fitness of a fungus, e.g., to prevent or treat a fungal infection in a plant. Included are methods for delivering a PMP composition to a fungus by contacting the fungus with the PMP composition. Additionally or alternatively, the methods include delivering the PMP composition to a plant at risk of or having a fungal infection, by contacting the plant with the PMP composition.

The PMP compositions and related methods are suitable for delivery to fungi that cause fungal diseases in plants, including diseases caused by powdery mildew pathogens, for example *Blumeria* species, for example *Blumeria graminis*; *Podosphaera* species, for example *Podosphaera leucotricha*; *Sphaerotheca* species, for example *Sphaerotheca fuliginea*; *Uncinula* species, for example *Uncinula necator*; diseases caused by rust disease pathogens, for example *Gymnosporangium* species, for example *Gymnosporangium sabinae*; *Hemileia* species, for example *Hemileia vastatrix*; *Phakopsora* species, for example *Phakopsora pachyrhizi* and *Phakopsora meibomia*; *Puccinia* species, for example

Puccinia recondite, *P. triticina*, *P. graminis* or *P. striiformis* or *P. hordei*; *Uromyces* species, for example *Uromyces appendiculatus*; diseases caused by pathogens from the group of the *Oomycetes*, for example *Albugo* species, for example *Albugo candida*; *Bremia* species, for example *Bremia lactucae*; *Peronospora* species, for example *Peronospora pisi*, *P. parasitica* or *P. brassicae*; *Phytophthora* species, for example
5 *Phytophthora infestans*; *Plasmopara* species, for example *Plasmopara viticola*; *Pseudoperonospora* species, for example *Pseudoperonospora humuli* or *Pseudoperonospora cubensis*; *Pythium* species, for example *Pythium ultimum*; leaf blotch diseases and leaf wilt diseases caused, for example, by *Alternaria* species, for example *Alternaria solani*; *Cercospora* species, for example *Cercospora beticola*; *Cladosporium* species, for example *Cladosporium cucumerinum*; *Cochliobolus* species, for example
10 *Cochliobolus sativus* (conidia form: *Drechslera*, Syn: *Helminthosporium*), *Cochliobolus miyabeanus*; *Colletotrichum* species, for example *Colletotrichum lindemuthianum*; *Cycloconium* species, for example *Cycloconium oleaginum*; *Diaporthe* species, for example *Diaporthe citri*; *Elsinoe* species, for example *Elsinoe fawcettii*; *Gloeosporium* species, for example *Gloeosporium laeticolor*; *Glomerella* species, for example *Glomerella cingulata*; *Guignardia* species, for example *Guignardia bidwelli*; *Leptosphaeria*
15 species, for example *Leptosphaeria maculans*, *Leptosphaeria nodorum*; *Magnaporthe* species, for example *Magnaporthe grisea*; *Microdochium* species, for example *Microdochium nivale*; *Mycosphaerella* species, for example *Mycosphaerella graminicola*, *M. arachidicola* and *M. fijiensis*; *Phaeosphaeria* species, for example *Phaeosphaeria nodorum*; *Pyrenophora* species, for example *Pyrenophora teres*, *Pyrenophora tritici repentis*; *Ramularia* species, for example *Ramularia collo-cygni*, *Ramularia areola*;
20 *Rhynchosporium* species, for example *Rhynchosporium secalis*; *Septoria* species, for example *Septoria apii*, *Septoria lycopersii*; *Typhula* species, for example *Typhula incarnata*; *Venturia* species, for example *Venturia inaequalis*; root and stem diseases caused, for example, by *Corticium* species, for example *Corticium graminearum*; *Fusarium* species, for example *Fusarium oxysporum*; *Gaeumannomyces* species, for example *Gaeumannomyces graminis*; *Rhizoctonia* species, such as, for example *Rhizoctonia*
25 *solani*; *Sarocladium* diseases caused for example by *Sarocladium oryzae*; *Sclerotium* diseases caused for example by *Sclerotium oryzae*; *Tapesia* species, for example *Tapesia acuformis*; *Thielaviopsis* species, for example *Thielaviopsis basicola*; ear and panicle diseases (including corn cobs) caused, for example, by *Alternaria* species, for example *Alternaria* spp.; *Aspergillus* species, for example *Aspergillus flavus*; *Cladosporium* species, for example *Cladosporium cladosporioides*; *Claviceps* species, for
30 example *Claviceps purpurea*; *Fusarium* species, for example *Fusarium culmorum*; *Gibberella* species, for example *Gibberella zeae*; *Monographella* species, for example *Monographella nivalis*; *Septoria* species, for example *Septoria nodorum*; diseases caused by smut fungi, for example *Sphacelotheca* species, for example *Sphacelotheca reiliana*; *Tilletia* species, for example *Tilletia caries*, *T. controversa*; *Urocystis* species, for example *Urocystis occulta*; *Ustilago* species, for example *Ustilago nuda*, *U. nuda tritici*; fruit
35 rot caused, for example, by *Aspergillus* species, for example *Aspergillus flavus*; *Botrytis* species, for example *Botrytis cinerea*; *Penicillium* species, for example *Penicillium expansum* and *P. purpurogenum*; *Sclerotinia* species, for example *Sclerotinia sclerotiorum*; *Verticillium* species, for example *Verticillium albo-atrum*; seed and soilborne decay, mould, wilt, rot and damping-off diseases caused, for example, by *Alternaria* species, caused for example by *Alternaria brassicicola*; *Aphanomyces* species, caused for
40 example by *Aphanomyces euteiches*; *Ascochyta* species, caused for example by *Ascochyta lentis*;

Aspergillus species, caused for example by *Aspergillus flavus*; *Cladosporium* species, caused for example by *Cladosporium herbarum*; *Cochliobolus* species, caused for example by *Cochliobolus sativus*; (Conidiaform: *Drechslera*, *Bipolaris* Syn: *Helminthosporium*); *Colletotrichum* species, caused for example by *Colletotrichum coccodes*; *Fusarium* species, caused for example by *Fusarium culmorum*; *Gibberella* species, caused for example by *Gibberella zeae*; *Macrophomina* species, caused for example by *Macrophomina phaseolina*; *Monographella* species, caused for example by *Monographella nivalis*; *Penicillium* species, caused for example by *Penicillium expansum*; *Phoma* species, caused for example by *Phoma lingam*; *Phomopsis* species, caused for example by *Phomopsis sojiae*; *Phytophthora* species, caused for example by *Phytophthora cactorum*; *Pyrenophora* species, caused for example by *Pyrenophora graminea*; *Pyricularia* species, caused for example by *Pyricularia oryzae*; *Pythium* species, caused for example by *Pythium ultimum*; *Rhizoctonia* species, caused for example by *Rhizoctonia solani*; *Rhizopus* species, caused for example by *Rhizopus oryzae*; *Sclerotium* species, caused for example by *Sclerotium rolfsii*; *Septoria* species, caused for example by *Septoria nodorum*; *Typhula* species, caused for example by *Typhula incarnata*; *Verticillium* species, caused for example by *Verticillium dahliae*; cancers, galls and witches' broom caused, for example, by *Nectria* species, for example *Nectria galligena*; wilt diseases caused, for example, by *Monilinia* species, for example *Monilinia laxa*; leaf blister or leaf curl diseases caused, for example, by *Exobasidium* species, for example *Exobasidium vexans*; *Taphrina* species, for example *Taphrina deformans*; decline diseases of wooden plants caused, for example, by Esca disease, caused for example by *Phaemoniella clamydospora*, *Phaeoacremonium aleophilum* and *Fomitiporia mediterranea*; *Eutypa* dyebark, caused for example by *Eutypa lata*; *Ganoderma* diseases caused for example by *Ganoderma boninense*; *Rigidoporus* diseases caused for example by *Rigidoporus lignosus*; diseases of flowers and seeds caused, for example, by *Botrytis* species, for example *Botrytis cinerea*; diseases of plant tubers caused, for example, by *Rhizoctonia* species, for example *Rhizoctonia solani*; *Helminthosporium* species, for example *Helminthosporium solani*; Club root caused, for example, by *Plasmodiophora* species, for example *Plasmodiophora brassicae*; diseases caused by bacterial pathogens, for example *Xanthomonas* species, for example *Xanthomonas campestris* pv. *oryzae*; *Pseudomonas* species, for example *Pseudomonas syringae* pv. *lachrymans*; *Erwinia* species, for example *Erwinia amylovora*.

Fungal diseases on leaves, stems, pods and seeds caused, for example, by *Alternaria* leaf spot (*Alternaria spec. atrans tenuissima*), Anthracnose (*Colletotrichum gloeosporoides dematium* var. *truncatum*), brown spot (*Septoria glycines*), cercospora leaf spot and blight (*Cercospora kikuchii*), choanephora leaf blight (*Choanephora infundibulifera trispora* (Syn.)), dactuliophora leaf spot (*Dactuliophora glycines*), downy mildew (*Peronospora manshurica*), drechslera blight (*Drechslera glycini*), frog-eye leaf spot (*Cercospora sojina*), leptosphaerulina leaf spot (*Leptosphaerulina trifolii*), phyllosticta leaf spot (*Phyllosticta sojaecola*), pod and stem blight (*Phomopsis sojiae*), powdery mildew (*Microsphaera diffusa*), pyrenochaeta leaf spot (*Pyrenochaeta glycines*), rhizoctonia aerial, foliage, and web blight (*Rhizoctonia solani*), rust (*Phakopsora pachyrhizi*, *Phakopsora meibomia*), scab (*Sphaceloma glycines*), stemphylium leaf blight (*Stemphylium botryosum*), target spot (*Corynespora cassicola*).

Fungal diseases on roots and the stem base caused, for example, by black root rot (*Calonectria crotalariae*), charcoal rot (*Macrophomina phaseolina*), fusarium blight or wilt, root rot, and pod and collar

rot (*Fusarium oxysporum*, *Fusarium orthoceras*, *Fusarium semitectum*, *Fusarium equiseti*), mycoleptodiscus root rot (*Mycoleptodiscus terrestris*), neocosmospora (*Neocosmospora vasinfecta*), pod and stem blight (*Diaporthe phaseolorum*), stem canker (*Diaporthe phaseolorum* var. *caulivora*), phytophthora rot (*Phytophthora megasperma*), brown stem rot (*Phialophora gregata*), pythium rot

5 (*Pythium aphanidermatum*, *Pythium irregulare*, *Pythium debaryanum*, *Pythium myriotylum*, *Pythium ultimum*), rhizoctonia root rot, stem decay, and damping-off (*Rhizoctonia solani*), sclerotinia stem decay (*Sclerotinia sclerotiorum*), sclerotinia southern blight (*Sclerotinia rolfsii*), thielaviopsis root rot (*Thielaviopsis basicola*).

In certain instances, the fungus is a *Sclerotinia* spp (*Sclerotinia sclerotiorum*). In certain

10 instances, the fungus is a *Botrytis* spp (e.g., *Botrytis cinerea*). In certain instances, the fungus is an *Aspergillus* spp. In certain instances, the fungus is a *Fusarium* spp. In certain instances, the fungus is a *Penicillium* spp.

Compositions of the present invention are useful in various fungal control applications. The above-described compositions may be used to control fungal phytopathogens prior to harvest or post-

15 harvest fungal pathogens. In one embodiment, any of the above-described compositions are used to control target pathogens such as *Fusarium* species, *Botrytis* species, *Verticillium* species, *Rhizoctonia* species, *Trichoderma* species, or *Pythium* species by applying the composition to plants, the area surrounding plants, or edible cultivated mushrooms, mushroom spawn, or mushroom compost. In another embodiment, compositions of the present invention are used to control post-harvest pathogens

20 such as *Penicillium*, *Geotrichum*, *Aspergillus niger*, or *Colletotrichum* species.

Table 6 provides further examples of fungi, and plant diseases associated therewith, that can be treated or prevented using the PMP composition and related methods described herein.

Table 6. Fungal pests

Disease	Causative Agent
Alternaria leaf blight of wheat	<i>Alternaria trititica</i>
Alternaria leaf spot of cole crops	<i>Alternaria japonica</i>
American soybean rust	<i>Phakopsora meibomia</i>
Ampelopsis rust	<i>Phakopsora ampelopsidis</i>
Anemone	<i>Ochropsora ariae</i>
Angular leaf spot of Citrus	<i>Pseudocercospora angolensis</i>
Arctic Rubus rust	<i>Phragmidium arcticum</i>
Ascochyta blight of broad beans	<i>Didymella fabae</i>
Ash dieback	<i>Chalara fraxinea</i>
Asia mountain Rosa rust	<i>Phragmidium butleri</i>
Asian filbert rust	<i>Pucciniastrum coryli</i>
Asian Kuehneola rose rust	<i>Kuehneola japonica</i>
Asian Mountain Rubus rust	<i>Phragmidium assamense</i>
Asian Phragmidium Rubus rust	<i>Phragmidium arisanense</i>
Asian pistacio rust	<i>Pileolaria pistaciae</i>

Asian rose rust	<i>Gerwasia rosae</i>
Asian Rubus rust	<i>Hamaspota hashiokai</i>
Asian soybean rust	<i>Phakopsora pachyrhizi</i>
Asian sugarcane smut	<i>Sporisorium sacchari</i>
Asian Wart bark, blister canker, ring rot, Physalospora canker of pear and apple	<i>Botryosphaeria berengeriana f. sp. pyricola</i>
Asian/European brown rot of rosaceae	<i>Monilinia fructigena</i>
Asiatic brown fruit rot	<i>Monilia polystroma</i>
Barclay's Asian Rubus rust	<i>Phragmidium barclayi</i>
Black leaf blight of soybean	<i>Arkoala nigra</i>
Blister blight of tea	<i>Exobasidium vexans</i>
Blue stain of Mongolian oak	<i>Ophiostoma longicollum</i>
Box Rust or Boxwood Rust	<i>Puccinia buxi</i>
Brown rust of sugarcane	<i>Puccinia melanocephala</i>
Cherry leaf scorch	<i>Apiognomonina erythrostoma</i>
Chocolate spot of Ya Li pears	<i>Alternaria yaliinficiens</i>
Chrysanthemum White Rust	<i>Puccinia horiana</i>
Coffee Leaf Rust	<i>Hemileia vastatrix</i>
Common Asian Rubus Rust	<i>Hamaspota acutissima</i>
Common larch	<i>Melampsora capraearum</i>
Common potato and tomato rust	<i>Puccinia pittieriana</i>
Crumenulopsis pine dieback	<i>Crumenulopsis sororia</i>
Daylily Rust	<i>Puccinia hemerocallidis</i>
Digitalis Downy Mildew	<i>Peronospora digitalis</i>
Downy mildew (Plasmopara) of Impatiens	<i>Plasmopara obducens</i>
Eggplant	<i>Puccinia substriata var. substriata</i>
Ergot of pearl millet	<i>Claviceps fusiformis</i>
European Larch canker	<i>Lachnellula willkommii</i>
Few-loculed Asian Rubus rust	<i>Phragmidium pauciloculare</i>
Flag smut of wheat	<i>Urocystis agropyri</i>
Gladiolus Rust	<i>Uromyces transversalis</i>
Goplane dioscoreae	<i>Goplane dioscoreae</i>
Grape leaf rust	<i>Phakopsora euveitis</i>
Gray Rubus rust	<i>Phragmidium griseum</i>
Himalayan rhododendron spruce rust	<i>Chrysomyxa himalensis</i>

Hiratsuka Rubus rust	<i>Phragmidium hiratsukanum</i>
Horse's tooth or ergot of maize	<i>Claviceps gigantea</i>
Japanese apple rust	<i>Gymnosporangium yamadae</i>
Japanese Chamaecyparis	<i>Gymnosporangium miyabei</i>
Japanese ergot of sorghum	<i>Claviceps sorghicola</i>
Kamtschatka rose rust	<i>Phragmidium kamtschatkae</i>
Late wilt of maize	<i>Harpophora maydis</i>
Long-Spored Asian Rubus rust	<i>Hamaspora longissima</i>
Mal secco disease of Citrus	<i>Phoma tracheiphila</i>
Miscanthus	<i>Puccinia miscanthi</i>
Mulberry rust	<i>Aecidium mori</i>
Nambu Rubus rust	<i>Phragmidium nambuianum</i>
Neck rot of onion	<i>Ciborinia allii</i>
New Zealand Rubus Rust	<i>Hamaspora australis</i>
Northern blue stain of pine	<i>Leptographium wingfieldii</i>
Northern spruce	<i>Chrysomyxa rhododendri</i>
Oak Wilt	<i>Ceratocystis fagacearum</i>
Orange rust of sugarcane	<i>Puccinia kuehnii</i>
Peronospora radii	<i>Peronospora radii</i>
Pistachio Rust	<i>Pileolaria terebinthi</i>
Poinsettia scab	<i>Sphaceloma poinsettiae</i>
Potato smut	<i>Thecaphora solani</i>
Puccinia gladioli on Gladiolus	<i>Puccinia gladioli</i>
Puccinia glyceriae (anam. Aecidium hydrangea)	<i>Puccinia glyceriae</i>
Puccinia mcleanii on Gladiolus	<i>Puccinia mcleanii</i>
Puccinia psidii	<i>Puccinia psidii</i>
Pucciniastrum actinidiae on Actinidia spp.	<i>Pucciniastrum actinidiae</i>
Red Miscanthus rust	<i>Puccinia erythropus</i>
Rust of European blackberry	<i>Phragmidium bulbosum</i>
Rust of Rubus saxatilis	<i>Phragmidium acuminatum</i>
Rust on Asian Rubus	<i>Gerwasia rubi</i>
Rust on South American Rubus	<i>Gerwasia imperialis</i>
Scots stem pine rust	<i>Cronartium flaccidum</i>
Shoot blight of boxwood	<i>Calonectria pseudonaviculata</i>
Sirex wasp fungus	<i>Amylostereum areolatum</i>
Solanum	<i>Puccinia agrophila</i>
South American Rubus rust	<i>Gerwasia mayorii</i>

Sporisorium smut of wild Saccharum	<i>Sporisorium pulverulentum</i>
Spruce needle rust	<i>Chrysomyxa abietis</i>
Stackburn, seedling blight, leaf spot of rice	<i>Alternaria padwickii</i>
Sudden needle drop of Spruce (SNEED)	<i>Setomelanomma holmii</i>
Sugary disease or Asian ergot of sorghum	<i>Claviceps sorghi</i>
Sweet potato rust	<i>Endophyllum kaernbachii</i>
Taiwan Rubus rust	<i>Phragmidium formosanum</i>
Tar spot of corn	<i>Phyllachora maydis</i>
Teak Rust	<i>Olivea tectonae</i>
Thekopsora areolate	<i>Thekopsora areolata</i>
Tip over disease of eggplant	<i>Diaporthe vexans</i>
Tropical American Kuehneola rust of Rubus	<i>Kuehneola loeseneriana</i>
Tropical American Mainsia Rubus rust	<i>Mainsia rubi</i>
Tropical Soybean Rust	<i>Aecidium glycines</i>
Uromyces gladioli on Gladiolus	<i>Uromyces gladioli</i>
Uromyces nyikensis on Gladiolus	<i>Uromyces nyikensis</i>
Uromycladium tepperianum on Acacia spp.	<i>Uromycladium tepperianum</i>
Variable Rubus	<i>Gerwasia variabilis</i>
Wineberry Rubus rust	<i>Hamaspora sinica</i> var. <i>sinica</i>
Yamada Rubusrust	<i>Phragmidium yamadatum</i>
Anthraco nose leaf blight and stalk rot	<i>Colletotrichum graminicola</i> anthracnose (teleomorph: <i>Glomerella graminicola</i>), <i>Glomerella tucumanensis</i> (anamorph: <i>Glomerella falcatum</i>)
<i>Aspergillus</i> ear and kernel rot	<i>Aspergillus flavus</i>
Banded leaf and sheath spot	<i>Rhizoctonia solani</i> = <i>Rhizoctonia microsclerotia</i> (teleomorph: <i>Thanatephorus cucumeris</i>)
Bean rust	<i>Uromyces appendiculatus</i>
Black bundle disease	<i>Acremonium strictum</i> = <i>Cephalosporium acremonium</i>
Black kernel rot	<i>Lasiodiplodia theobromae</i> = <i>Botryodiplodia theobromae</i>
Borde blanco	<i>Marasmiellus</i> sp.
Brown spot (black spot, stalk rot)	<i>Physoderma maydis</i>

Brown stripe downy mildew	<i>Sclerophthora rayssiae</i> var. <i>zeae</i>
<i>Cephalosporium</i> kernel rot	<i>Acremonium strictum</i> = <i>Cephalosporium acremonium</i>
Charcoal rot	<i>Macrophomina phaseolina</i>
Corn common rust	<i>Puccinia sorghi</i>
Corn southern rust	<i>Puccinia polysora</i>
Corn tropical rust	<i>Physopella pallescens</i> , <i>P. zeae</i> = <i>Angiospora zeae</i>
<i>Corticium</i> ear rot	<i>Thanatephorus cucumeris</i> = <i>Corticium sasakii</i>
Cotton rust	<i>Puccinia schedonnardi</i>
Cotton southwestern rust	<i>Puccinia cacabata</i>
Cotton tropical rust	<i>Phakopsora gossypii</i>
Crazy top downy mildew	<i>Sclerophthora macrospora</i> = <i>S. macrospora</i>
<i>Curvularia</i> leaf spot	<i>Curvularia clavata</i> , <i>C. eragrostidis</i> , = <i>C. maculans</i> (teleomorph: <i>Cochliobolus eragrostidis</i>), <i>Curvularia inaequalis</i> , <i>C. intermedia</i> (teleomorph: <i>Cochliobolus intermedius</i>), <i>Curvularia lunata</i> (teleomorph: <i>Cochliobolus lunatus</i>), <i>Curvularia pallescens</i> (teleomorph: <i>Cochliobolus pallescens</i>), <i>Curvularia senegalensis</i> , <i>C. tuberculata</i> (teleomorph: <i>Cochliobolus tuberculatus</i>)
<i>Didymella</i> leaf spot	<i>Didymella exitialis</i>
<i>Diplodia</i> ear rot and stalk rot	<i>Diplodia frumenti</i> (teleomorph: <i>Botryosphaeria festucae</i>)
<i>Diplodia</i> ear rot, stalk rot, seed rot and seedling blight	<i>Diplodia maydis</i> = <i>Stenocarpella maydis</i>
<i>Diplodia</i> leaf spot or leaf streak	<i>Stenocarpella macrospora</i> = <i>Diplodia macrospore</i>
Grape leaf Downey mildew	<i>Plasmopara viticola</i>
Dry ear rot (cob, kernel and stalk rot)	<i>Nigrospora oryzae</i> (teleomorph: <i>Khuskia oryzae</i>)
Ear rots, minor	<i>Aspergillus glaucus</i> , <i>A. niger</i> , <i>Aspergillus</i> spp., <i>Cunninghamella</i> sp., <i>Curvularia pallescens</i> , <i>Doratomyces stemonitis</i> = <i>Cephalotrichum stemonitis</i> , <i>Fusarium culmorum</i> , <i>Gonatobotrys simplex</i> , <i>Pithomyces maydici</i> , <i>Rhizopus microsporus</i> , <i>R. stolonifer</i> = <i>R. nigricans</i> , <i>Scopulariopsis brumptii</i>
epitea	<i>Melampsora larici</i>
Ergot (horse's tooth, diente del caballo)	<i>Claviceps gigantea</i> (anamorph: <i>Sphacelia</i> sp.)
Eyespot	<i>Aureobasidium zeae</i> = <i>Kabatiella zeae</i>
<i>Fusarium</i> ear and stalk rot	<i>Fusarium subglutinans</i> = <i>F. moniliforme</i> var. <i>subglutinans</i>
<i>Fusarium</i> kernel, root and stalk rot, seed rot and seedling blight	<i>Fusarium moniliforme</i> (teleomorph: <i>Gibberella fujikuroi</i>)
<i>Fusarium</i> stalk rot, seedling root rot	<i>Fusarium avenaceum</i> (teleomorph: <i>Gibberella avenacea</i>)

<i>Gibberella</i> ear and stalk rot	<i>Gibberella zeae</i> (anamorph: <i>Fusarium graminearum</i>)
Gray ear rot	<i>Botryosphaeria zeae</i> = <i>Physalospora zeae</i> (anamorph: <i>Macrophoma zeae</i>)
Gray leaf spot (<i>Cercospora</i>	<i>Cercospora sorghi</i> = <i>C. sorghi</i> var. <i>maydis</i> , <i>C. zeae-maydis</i> leaf spot)
Green ear downy mildew	<i>Sclerospora graminicola</i>
<i>Helminthosporium</i> ear rot (race 1)	<i>Bipolaris zeicola</i> = <i>Helminthosporium carbonum</i>
<i>Helminthosporium</i> root rot	<i>Exserohilum pedicellatum</i> = <i>Helminthosporium pedicellatum</i> (teleomorph: <i>Setosphaeria</i>)
<i>Hormodendrum</i> ear rot (<i>Cladosporium</i> rot)	<i>Cladosporium cladosporioides</i> = <i>Hormodendrum cladosporioides</i> , <i>C. herbarum</i> (teleomorph: <i>Mycosphaerella tassiana</i>)
<i>Hyalothyridium</i> leaf spot	<i>Hyalothyridium maydis</i>
Java downy mildew	<i>Peronosclerospora maydis</i> = <i>Sclerospora maydis</i>
Late wilt	<i>Cephalosporium maydis</i>
Leaf (brown) rust	<i>Puccinia recondita</i> (anamorph: <i>Aecidium clematidis</i>)
Leaf spots, minor	<i>Alternaria alternata</i> , <i>Ascochyta maydis</i> , <i>A. tritici</i> , <i>A. zeicola</i> , <i>Bipolaris victoriae</i> = <i>Helminthosporium victoriae</i> (teleomorph: <i>Cochliobolus victoriae</i>), <i>C. sativus</i> (anamorph: <i>Bipolaris sorokiniana</i> = <i>H. Exserohilum maydis</i> , <i>Leptothyrium zeae</i> , <i>Ophiosphaerella herpotricha</i> , <i>Setosphaeria prolata</i>) <i>Graphium penicilliioides</i> , <i>Leptosphaeria prolata</i> = <i>Drechslera prolata</i> (teleomorph: <i>sorokiniana</i> = <i>H. sativum</i>), <i>Epicoccum nigrum</i> , (anamorph: <i>Scolecosporella</i> sp.), <i>Paraphaeosphaeria michotii</i> , <i>Phoma</i> sp., <i>Septoria zeae</i> , <i>S. zeicola</i> , <i>S. zeina</i>
Rust fungi	<i>Puccinia veronicae-longifoliae</i>
Musk rose rust	<i>Phragmidium rosae-moschatae</i>
Multiflora rose rust	<i>Phragmidium rosae-multiflorae</i>
Northern corn leaf blight	<i>Exaerohilum turcicum</i> = <i>Helminthosporium turcicum</i> , <i>Setosphaeria turcica</i>
Northern corn leaf spot	<i>Cochliobolus carbonum</i>
Oat crown rust	<i>Puccinia coronata</i>
Oat stem Rust	<i>Puccinia graminis</i>
Peanut rust	<i>Puccinia arachidis</i>
<i>Penicillium</i> ear rot (blue eye, blue mold)	<i>Penicillium</i> spp., <i>P. chrysogenum</i> , <i>P. expansum</i> , <i>P. oxalicum</i>
Bay willow-larch rust	<i>Melampsora larici-pentandrae</i>
<i>Phaeocystostroma</i> stalk rot and root rot	<i>Phaeocystostroma ambiguum</i> , <i>Phaeocystosporella zeae</i>

Phaeosphaeria leaf spot	<i>Phaeosphaeria maydis</i> , <i>Sphaerulina maydis</i>
Philippine downy mildew	<i>Peronosclerospora philippinensis</i> = <i>Sclerospora philippinensis</i>
<i>Physalospora</i> ear rot	<i>Botryosphaeria Botryosphaeria festucae</i> = <i>Physalospora zeicola</i> , (anamorph: <i>Diplodia frumenti</i>)
Potato common rust	<i>Puccinia pittierianap</i>
Potato deforming rust	<i>Aecidium cantensis</i>
Cereals and grasses Powdery mildew	<i>Erysiphe graminis</i>
Rose Powdery mildew	<i>Sphaerotheca pannosa</i>
Wheat Powdery mildew	<i>Blumeria graminis</i> f. sp. <i>tritici</i> ,
Barley Powdery mildew	<i>Blumeria graminis</i> f. sp. <i>hordei</i>
Grape Powdery mildew	<i>Microsphaera diffusa</i>
Legume Powdery mildew	<i>Erysiphe necator</i> (or <i>Uncinula necator</i>)
Grape Powdery mildew	<i>Leveillula taurica</i> , or <i>Oidiopsis taurica</i>
Onion Powdery mildew	<i>Podosphaera leucotricha</i>
Apple Powdery mildew	<i>Podosphaera xanthii</i> , <i>Erysiphe cichoracearum</i> , <i>Podosphaera fusca</i> , <i>Leveillula taurica</i>
Cucurbits Powdery mildew	<i>Microsphaera syringae</i>
Lilacs Powdery mildew	<i>Podosphaera aphanis</i> , <i>Geum rivale</i>
Strawberry Powdery mildew	<i>Erysiphe berberidis</i>
Hawthorn Powdery mildew	<i>Podosphaera oxyacanthae</i>
Gooseberry Powdery mildew	<i>Sphaerotheca mors-uvae</i>
Purple leaf sheath	Hemiparasitic bacteria and fungi
<i>Pyrenochaeta</i> stalk rot and root rot	<i>Phoma terrestris</i> , <i>Pyrenochaeta terrestris</i>
<i>Pythium</i> root rot	<i>Pythium</i> spp., <i>P. arrhenomanes</i> , <i>P. graminicola</i>
<i>Pythium</i> stalk rot	<i>Pythium aphanidermatum</i> = <i>P. butleri</i> L.
Red kernel disease (ear mold, leaf and seed rot)	<i>Epicoccum nigrum</i>
<i>Rhizoctonia</i> ear rot	<i>Rhizoctonia zeae</i> (teleomorph: <i>Waitea circinata</i>)
<i>Rhizoctonia</i> root rot and stalk rot	<i>Rhizoctonia solani</i> , <i>Rhizoctonia zeae</i>
Root rots, minor	<i>Alternaria alternata</i> , <i>Cercospora sorghi</i> , <i>Dictyochoeta fertilis</i> , <i>Fusarium acuminatum</i> (teleomorph: <i>Gibberella acuminata</i>), <i>F. equiseti</i> (teleomorph: <i>G. intricans</i>), <i>F. oxysporum</i> , <i>F. pallidoroseum</i> , <i>F. poae</i> , <i>F. roseum</i> , <i>F. cyanogena</i> , (anamorph: <i>F. sulphureum</i>), <i>Microdochium bolleyi</i> , <i>Mucor</i> sp., <i>Periconia circinata</i> , <i>Phytophthora cactorum</i> , <i>P. drechsleri</i> , <i>P. nicotianae</i> var. <i>parasitica</i> , <i>Rhizopus arrhizus</i>

<i>Rostratum</i> leaf spot (leaf disease, ear and, stalk rot)	<i>Setosphaeria rostrata</i> , <i>Helminthosporium</i> (anamorph: <i>Exserohilum rostratum</i> = <i>Helminthosporium rostratum</i>)
rugosae	<i>Phragmidium rosae</i>
Rust, common corn	<i>Puccinia sorghi</i>
Rust, southern corn	<i>Puccinia polysora</i>
Rust, tropical corn	<i>Physopella pallescens</i> , <i>P. zeae</i> = <i>Angiospora zeae</i>
sativae	<i>Balansia oryzae</i>
<i>Sclerotium</i> ear rot (southern blight)	<i>Sclerotium rolfsii</i> (teleomorph: <i>Athelia rolfsii</i>)
Seed rot-seedling blight	<i>Bipolaris sorokiniana</i> , <i>B. zeicola</i> = <i>Helminthosporium carbonum</i> , <i>Diplodia maydis</i> , <i>Exserohilum pedicellatum</i> , <i>Exserohilum turcicum</i> = <i>Helminthosporium turcicum</i> , <i>Fusarium avenaceum</i> , <i>F. culmorum</i> , <i>F. moniliforme</i> , <i>Gibberella zeae</i> (anamorph: <i>F. graminearum</i>), <i>Macrophomina phaseolina</i> , <i>Penicillium</i> spp., <i>Phomopsis</i> sp., <i>Pythium</i> spp., <i>Rhizoctonia solani</i> , <i>R. zeae</i> , <i>Sclerotium rolfsii</i> , <i>Spicaria</i> sp.
<i>Selenophoma</i> leaf spot	<i>Selenophoma</i> sp.
Sheath rot	<i>Gaeumannomyces graminis</i>
Shuck rot	<i>Myrothecium gramineum</i>
sieboldii	<i>Hamaspora rubi</i>
Silage mold	<i>Monascus purpureus</i> , <i>M. rubber</i>
Smut, common	<i>Ustilago zeae</i> = <i>U. maydis</i>
Smut, false	<i>Ustilaginoidea virens</i>
Smut, head	<i>Sphacelotheca reiliana</i> = <i>Sporisorium holci-sorghi</i>
<i>Sorghum</i> downy mildew	<i>Peronosclerospora sorghi</i> = <i>Sclerospora sorghi</i>
Southern corn leaf blight and stalk rot	<i>Cochliobolus heterostrophus</i> (anamorph: <i>Bipolaris maydis</i> = <i>Helminthosporium maydis</i>)
Southern leaf spot	<i>Stenocarpella macrospora</i> = <i>Diplodia macrospora</i>
Soybean rust	<i>Phakopsora pachyrhizi</i>
<i>Spontaneum</i> downy mildew	<i>Peronosclerospora spontanea</i> = <i>Sclerospora spontanea</i>
Stalk rots, minor	<i>Cercospora sorghi</i> , <i>Fusarium episphaeria</i> , <i>F. merismoides</i> , <i>F. oxysporum</i> , <i>F. poae</i> , <i>F. roseum</i> , <i>F. solani</i> (teleomorph: <i>Nectria haematococca</i>), <i>F. tricinctum</i> , <i>Mariannaea elegans</i> , <i>Mucor</i> sp., <i>Rhopoglyphus zeae</i> , <i>Spicaria</i> sp.
Stem rust	<i>Puccinia graminis</i> = <i>P. graminis</i> f. sp. <i>secalis</i>
Storage rots	<i>Aspergillus</i> spp., <i>Penicillium</i> spp. and other fungi
Sugarcane common rust	<i>Puccinia melanocephala</i> = <i>P. eriantha</i>
Sugarcane downy mildew	<i>Peronosclerospora sacchari</i> = <i>Sclerospora sacchari</i>

Tar spot	<i>Phyllachora maydis</i>
thunbergii	<i>Phragmidium rubi</i>
<i>Trichoderma</i> ear rot and root rot	<i>Trichoderma viride</i> = <i>T. lignorum</i> (teleomorph: <i>Hypocrea</i> sp.)
Wheat leaf (brown) rust	<i>Puccinia triticina</i> = <i>P. Recondita</i> f. <i>Sp. tritici</i> = <i>P. tritici-duri</i>
Wheat stem (black) rust	<i>Puccinia graminis</i> = <i>P. graminis</i> f. <i>sp. tritici</i>
Wheat stripe (yellow) rust	<i>Puccinia striiformis</i> (anamorph: <i>P. uredoglumarum</i>)
White ear rot, root and stalk rot	<i>Stenocarpella maydis</i> = <i>Diplodia zeae</i>
Yellow leaf blight	<i>Ascochyta ischaemi</i> , <i>Phyllosticta maydis</i> (teleomorph: <i>Mycosphaerella zeae-maydis</i>)
Zonate leaf spot	<i>Gloeocercospora sorghi</i>

ii. Bacteria

The PMP compositions and related methods can be useful for decreasing the fitness of a bacterium, e.g., to prevent or treat a bacterial infection in a plant. Included are methods for delivering a PMP composition to a bacterium by contacting the bacteria with the PMP composition. Additionally or alternatively, the methods include delivering the biopesticide to a plant at risk of or having a bacterial infection, by contacting the plant with the PMP composition.

The PMP compositions and related methods are suitable for delivery to bacteria, or a plant infected therewith, including any bacteria described further below. For example, the bacteria may be one belonging to Actinobacteria or Proteobacteria, such as bacteria in the families of the *Burkholderiaceae*, *Xanthomonadaceae*, *Pseudomonadaceae*, *Enterobacteriaceae*, *Microbacteriaceae*, and *Rhizobiaceae*.

In some instances the bacteria is a bacterial species capable of genetic transformation (e.g., *Agrobacterium tumefaciens* or other *Agrobacterium* spp., *Sinorhizobium* (= *Ensifer*) sp., *Mesorhizobium*, sp., *Bradyrhizobium* sp., *Azobacter* sp., *Phyllobacter* sp.), or capable of plant rhizosphere colonization or root nodulation (e.g., *Sinorhizobium* (= *Ensifer*) sp., *Mesorhizobium*, sp., *Azorhizobium* sp., *Bradyrhizobium* sp., *Rhizobium* sp.).

In some instances, the bacteria is an *Acidovorax avenae* subsp., including e.g., *Acidovorax avenae* subsp. *avenae* (= *Pseudomonas avenae* subsp. *avenae*), *Acidovorax avenae* subsp. *cattleyae* (= *Pseudomonas cattleyae*), or *Acidovorax avenae* subsp. *citrulli* (= *Pseudomonas pseudoalcaligenes* subsp. *citrulli*, *Pseudomonas avenae* subsp. *citrulli*).

In some instances, the bacterium is a *Burkholderia* spp., including e.g., *Burkholderia andropogonis* (= *Pseudomonas andropogonis*, *Pseudomonas woodsii*), *Burkholderia caryophylli* (= *Pseudomonas caryophylli*), *Burkholderia cepacia* (= *Pseudomonas cepacia*), *Burkholderia gladioli* (= *Pseudomonas gladioli*), *Burkholderia gladioli* pv. *agaricola* (= *Pseudomonas gladioli* pv. *agaricola*), *Burkholderia gladioli* pv. *alliiicola* (i.e., *Pseudomonas gladioli* pv. *alliiicola*), *Burkholderia gladioli* pv. *gladioli* (i.e., *Pseudomonas gladioli*, *Pseudomonas gladioli* pv. *gladioli*), *Burkholderia glumae* (i.e., *Pseudomonas glumae*), *Burkholderia plantarii* (i.e., *Pseudomonas plantarii*), *Burkholderia solanacearum* (i.e., *Ralstonia solanacearum*), or *Ralstonia* spp.

In some instances, the bacterium is a *Liberibacter* spp., including *Candidatus Liberibacter* spec., including e.g., *Candidatus Liberibacter asiaticus*, *Liberibacter africanus* (Laf), *Liberibacter americanus*

(Lam), *Liberibacter asiaticus* (Las), *Liberibacter europaeus* (Leu), *Liberibacter psyllae*, or *Liberibacter solanacearum* (Lso).

In some instances, the bacterium is a *Corynebacterium* spp. including e.g., *Corynebacterium fascians*, *Corynebacterium flaccumfaciens* pv. *flaccumfaciens*, *Corynebacterium michiganensis*,
 5 *Corynebacterium michiganense* pv. *tritici*, *Corynebacterium michiganense* pv. *nebraskense*, or *Corynebacterium sepedonicum*.

In some instances, the bacterium is a *Erwinia* spp. including e.g., *Erwinia amylovora*, *Erwinia ananas*, *Erwinia carotovora* (i.e., *Pectobacterium carotovorum*), *Erwinia carotovora* subsp. *atroseptica*,
 10 *Erwinia carotovora* subsp. *carotovora*, *Erwinia chrysanthemi*, *Erwinia chrysanthemi* pv. *zeae*, *Erwinia dissolvens*, *Erwinia herbicola*, *Erwinia rhapontic*, *Erwinia stewartii*, *Erwinia tracheiphila*, or *Erwinia uredovora*.

In some instances, the bacterium is a *Pseudomonas syringae* subsp., including e.g.,
Pseudomonas syringae pv. *actinidiae* (Psa), *Pseudomonas syringae* pv. *atropis*, *Pseudomonas syringae* pv. *coronafaciens*, *Pseudomonas syringae* pv. *glycinea*, *Pseudomonas syringae* pv. *lachrymans*,
 15 *Pseudomonas syringae* pv. *maculicola*, *Pseudomonas syringae* pv. *papulans*, *Pseudomonas syringae* pv. *striafaciens*, *Pseudomonas syringae* pv. *syringae*, *Pseudomonas syringae* pv. *tomato*, or *Pseudomonas syringae* pv. *tabaci*.

In some instances, the bacterium is a *Streptomyces* spp., including e.g., *Streptomyces acidiscabies*, *Streptomyces albidoflavus*, *Streptomyces candidus* (i.e., *Actinomyces candidus*),
 20 *Streptomyces caviscabies*, *Streptomyces collinus*, *Streptomyces europaeiscabiei*, *Streptomyces intermedius*, *Streptomyces ipomoeae*, *Streptomyces luridiscabiei*, *Streptomyces niveiscabiei*, *Streptomyces puniscabiei*, *Streptomyces reticuliscabiei*, *Streptomyces scabiei*, *Streptomyces scabies*, *Streptomyces setonii*, *Streptomyces stelliscabiei*, *Streptomyces turgidiscabies*, or *Streptomyces wedmorensis*.

In some instances, the bacterium is a *Xanthomonas axonopodis* subsp., including e.g.,
Xanthomonas axonopodis pv. *alfalfae* (= *Xanthomonas alfalfae*), *Xanthomonas axonopodis* pv. *aurantifolii*
 (= *Xanthomonas fuscans* subsp. *aurantifolii*), *Xanthomonas axonopodis* pv. *allii* (= *Xanthomonas campestris* pv. *allii*), *Xanthomonas axonopodis* pv. *axonopodis*, *Xanthomonas axonopodis* pv. *bauhiniae*
 (= *Xanthomonas campestris* pv. *bauhiniae*), *Xanthomonas axonopodis* pv. *begoniae* (= *Xanthomonas campestris* pv. *begoniae*), *Xanthomonas axonopodis* pv. *betlicola* (= *Xanthomonas campestris* pv. *betlicola*),
 30 *Xanthomonas axonopodis* pv. *biophyti* (= *Xanthomonas campestris* pv. *biophyti*), *Xanthomonas axonopodis* pv. *cajani* (= *Xanthomonas campestris* pv. *cajani*), *Xanthomonas axonopodis* pv. *cassavae*
 (= *Xanthomonas cassavae*, *Xanthomonas campestris* pv. *cassavae*), *Xanthomonas axonopodis* pv. *cassiae* (= *Xanthomonas campestris* pv. *cassiae*), *Xanthomonas axonopodis* pv. *citri* (= *Xanthomonas citri*),
 35 *Xanthomonas axonopodis* pv. *citrumelo* (= *Xanthomonas alfalfae* subsp. *citrumelonis*), *Xanthomonas axonopodis* pv. *clitoriae* (= *Xanthomonas campestris* pv. *clitoriae*), *Xanthomonas axonopodis* pv. *coracanae*
 (= *Xanthomonas campestris* pv. *coracanae*), *Xanthomonas axonopodis* pv. *cyamopsidis* (= *Xanthomonas campestris* pv. *cyamopsidis*), *Xanthomonas axonopodis* pv. *desmodii* (= *Xanthomonas campestris* pv. *desmodii*),
 40 *Xanthomonas axonopodis* pv. *desmodiigangetici* (= *Xanthomonas campestris* pv. *desmodiigangetici*), *Xanthomonas axonopodis* pv. *desmodiilaxiflori* (= *Xanthomonas campestris* pv. *desmodiilaxiflori*).

desmodiilaxiflori), *Xanthomonas axonopodis* pv. *desmodirotundifolii* (= *Xanthomonas campestris* pv. *desmodirotundifolii*), *Xanthomonas axonopodis* pv. *dieffenbachiae* (= *Xanthomonas campestris* pv. *dieffenbachiae*), *Xanthomonas axonopodis* pv. *erythrinae* (= *Xanthomonas campestris* pv. *erythrinae*), *Xanthomonas axonopodis* pv. *fascicularis* (= *Xanthomonas campestris* pv. *fasciculari*), *Xanthomonas axonopodis* pv. *glycines* (= *Xanthomonas campestris* pv. *glycines*), *Xanthomonas axonopodis* pv. *khayae* (= *Xanthomonas campestris* pv. *khayae*), *Xanthomonas axonopodis* pv. *lespedezae* (= *Xanthomonas campestris* pv. *lespedezae*), *Xanthomonas axonopodis* pv. *maculifoliigardeniae* (= *Xanthomonas campestris* pv. *maculifoliigardeniae*), *Xanthomonas axonopodis* pv. *malvacearum* (= *Xanthomonas citri* subsp. *malvacearum*), *Xanthomonas axonopodis* pv. *manihotis* (= *Xanthomonas campestris* pv. *manihotis*), *Xanthomonas axonopodis* pv. *martyiicola* (= *Xanthomonas campestris* pv. *martyiicola*), *Xanthomonas axonopodis* pv. *melhusii* (= *Xanthomonas campestris* pv. *melhusii*), *Xanthomonas axonopodis* pv. *nakataecorchori* (= *Xanthomonas campestris* pv. *nakataecorchori*), *Xanthomonas axonopodis* pv. *passiflorae* (= *Xanthomonas campestris* pv. *passiflorae*), *Xanthomonas axonopodis* pv. *patellii* (= *Xanthomonas campestris* pv. *patellii*), *Xanthomonas axonopodis* pv. *pedalii* (= *Xanthomonas campestris* pv. *pedalii*), *Xanthomonas axonopodis* pv. *phaseoli* (= *Xanthomonas campestris* pv. *phaseoli*), *Xanthomonas phaseoli*, *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* (= *Xanthomonas fuscans*), *Xanthomonas axonopodis* pv. *phyllanthi* (= *Xanthomonas campestris* pv. *phyllanthi*), *Xanthomonas axonopodis* pv. *physalidicola* (= *Xanthomonas campestris* pv. *physalidicola*), *Xanthomonas axonopodis* pv. *poinsettiicola* (= *Xanthomonas campestris* pv. *poinsettiicola*), *Xanthomonas axonopodis* pv. *punicae* (= *Xanthomonas campestris* pv. *punicae*), *Xanthomonas axonopodis* pv. *rhynchosiae* (= *Xanthomonas campestris* pv. *rhynchosiae*), *Xanthomonas axonopodis* pv. *ricini* (= *Xanthomonas campestris* pv. *ricini*), *Xanthomonas axonopodis* pv. *sesbaniae* (= *Xanthomonas campestris* pv. *sesbaniae*), *Xanthomonas axonopodis* pv. *tamarindi* (= *Xanthomonas campestris* pv. *tamarindi*), *Xanthomonas axonopodis* pv. *vasculorum* (= *Xanthomonas campestris* pv. *vasculorum*), *Xanthomonas axonopodis* pv. *vesicatoria* (= *Xanthomonas campestris* pv. *vesicatoria*, *Xanthomonas vesicatoria*), *Xanthomonas axonopodis* pv. *vignaeradiatae* (= *Xanthomonas campestris* pv. *vignaeradiatae*), *Xanthomonas axonopodis* pv. *vignicola* (= *Xanthomonas campestris* pv. *vignicola*), or *Xanthomonas axonopodis* pv. *vitians* (= *Xanthomonas campestris* pv. *vitians*).

In some instances, the bacterium is *Xanthomonas campestris* pv. *musacearum*, *Xanthomonas campestris* pv. *pruni* (= *Xanthomonas arboricola* pv. *pruni*), or *Xanthomonas fragariae*.

In some instances, the bacteria is a *Xanthomonas translucens* supsp. (= *Xanthomonas campestris* pv. *hordei*) including e.g., *Xanthomonas translucens* pv. *arrhenatheri* (= *Xanthomonas campestris* pv. *arrhenatheri*), *Xanthomonas translucens* pv. *cerealis* (= *Xanthomonas campestris* pv. *cerealis*), *Xanthomonas translucens* pv. *graminis* (= *Xanthomonas campestris* pv. *graminis*), *Xanthomonas translucens* pv. *phlei* (= *Xanthomonas campestris* pv. *phlei*), *Xanthomonas translucens* pv. *phleipratensis* (= *Xanthomonas campestris* pv. *phleipratensis*), *Xanthomonas translucens* pv. *poae* (= *Xanthomonas campestris* pv. *poae*), *Xanthomonas translucens* pv. *secalis* (= *Xanthomonas campestris* pv. *secalis*), *Xanthomonas translucens* pv. *translucens* (= *Xanthomonas campestris* pv. *translucens*), or *Xanthomonas translucens* pv. *undulosa* (= *Xanthomonas campestris* pv. *undulosa*).

In some instances, the bacterium is a *Xanthomonas oryzae* subsp., *Xanthomonas oryzae* pv. *oryzae* (= *Xanthomonas campestris* pv. *oryzae*), or *Xanthomonas oryzae* pv. *oryzicola* (= *Xanthomonas campestris* pv. *oryzicola*).

In some instances, the bacterium is a *Xylella fastidiosa* from the family of Xanthomonadaceae.

- 5 Table 7 shows further examples of bacteria, and diseases associated therewith, that can be treated or prevented using the PMP composition and related methods described herein.

Table 7. Bacterial pests

Disease	Causative Agent
Bacterial leaf blight and stalk rot	<i>Pseudomonas avenae</i> subsp. <i>avenae</i>
Bacterial leaf spot	<i>Xanthomonas campestris</i> pv. <i>holcicola</i>
Bacterial stalk rot	<i>Enterobacter dissolvens</i> = <i>Erwinia dissolvens</i>
Bacterial stalk and top rot	<i>Erwinia carotovora</i> subsp. <i>carotovora</i> , <i>Erwinia chrysanthemi</i> pv. <i>Zee</i>
Bacterial stripe	<i>Pseudomonas andropogonis</i>
Chocolate spot	<i>Pseudomonas syringae</i> pv. <i>Coronafaciens</i>
Goss's bacterial wilt blight (leaf freckles and wilt)	<i>Clavibacter michiganensis</i> subsp. <i>nebraskensis</i> = <i>Cornebacterium michiganense</i> pv. <i>Nebraskense</i>
<i>Holcus</i> spot	<i>Pseudomonas syringae</i> pv. <i>Syringae</i>
Purple leaf sheath	Hemiparasitic bacteria
Seed rot-seedling blight	<i>Bacillus subtilis</i>
Stewart's disease (bacterial wilt)	<i>Pantoea stewartii</i> = <i>Erwinia stewartii</i>
Corn stunt (Mesa Central or Rio Grande stunt)	Achapparramiento, stunt, <i>Spiroplasma kunkelii</i>
Soft rot	<i>Dickeya dianthicola</i>
Soft rot	<i>Dickeya solani</i>
Fire blight	<i>Erwinia amylovora</i>
Soft rot	<i>P. atrosepticum</i>
Soft rot	<i>Pectobacterium carotovorum</i> ssp. <i>carotovorum</i>
Soft rot	<i>Pectobacterium wasabiae</i>
Bacterial blight	<i>Pseudomonas syringae</i> pv. <i>Porri</i> and pv. <i>Tomato</i>
Brown blotch Disease	<i>Pseudomonas tolaasii</i>
Bacterial wilt	<i>Ralstonia solanacearum</i>
Bacteria wilt	<i>Ralstonia solanacearum</i>
Common scab	<i>Streptomyces scabies</i>
Common scab	<i>Streptomyces scabies</i>
<i>Xanthomonas</i> leaf blight of onion	<i>Xanthomonas axonopodis</i> pv. <i>allii</i>
Asiatic citrus canker	<i>Xanthomonas axonopodis</i> pv. <i>citri</i>

Citrus bacterial spot	<i>Xanthomonas axonopodis</i> pv. <i>citrumelo</i>
Bacterial spot	<i>Xanthomonas campestris</i> pv. <i>vesicatoria</i>
Pierce's Disease	<i>Xylella fastidiosa</i>

iii. Arthropods

The PMP compositions and related methods can be useful for decreasing the fitness of an insect, e.g., to prevent or treat an insect infestation in a plant. The term "insect" includes any organism
5 belonging to the phylum Arthropoda and to the class Insecta or the class Arachnida, in any stage of development, i.e., immature and adult insects. Included are methods for delivering a PMP composition to an insect by contacting the insect with the PMP composition. Additionally or alternatively, the methods include delivering the biopesticide to a plant at risk of or having an insect infestation, by contacting the plant with the PMP composition.

10 The PMP compositions and related methods are suitable for preventing or treating infestation by an insect, or a plant infested therewith, including insects belonging to the following orders: Acari, Araneae, Anoplura, Coleoptera, Collembola, Dermaptera, Dictyoptera, Diplura, Diptera (e.g., spotted-wing *Drosophila*), Embioptera, Ephemeroptera, Grylloblatodea, Hemiptera (e.g., aphids, Greenhouse whitefly), Homoptera, Hymenoptera, Isoptera, Lepidoptera, Mallophaga, Mecoptera, Neuroptera,
15 Odonata, Orthoptera, Phasmida, Plecoptera, Protura, Psocoptera, Siphonaptera, Siphunculata, Thysanura, Strepsiptera, Thysanoptera, Trichoptera, or Zoraptera.

In some instances, the insect is from the class Arachnida, for example, *Acarus* spp., *Aceria sheldoni*, *Aculops* spp., *Aculus* spp., *Amblyomma* spp., *Amphitetranynchus viennensis*, *Argas* spp., *Boophilus* spp., *Brevipalpus* spp., *Bryobia graminum*, *Bryobia praetiosa*, *Centruroides* spp., *Choriotopes* spp., *Dermanyssus gallinae*, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Dermacentor* spp., *Eotetranychus* spp., *Epitrimerus pyri*, *Eutetranychus* spp., *Eriophyes* spp., *Glycyphagus domesticus*,
20 *Halotydeus destructor*, *Hemitarsonemus* spp., *Hyalomma* spp., *Ixodes* spp., *Latrodectus* spp., *Loxosceles* spp., *Metatetranychus* spp., *Neutrombicula autumnalis*, *Nuphessa* spp., *Oligonychus* spp., *Ornithodoros* spp., *Ornithonyssus* spp., *Panonychus* spp., *Phyllocoptruta oleivora*, *Polyphagotarsonemus latus*,
25 *Psoroptes* spp., *Rhipicephalus* spp., *Rhizoglyphus* spp., *Sarcoptes* spp., *Scorpio maurus*, *Steneotarsonemus* spp., *Steneotarsonemus spinki*, *Tarsonemus* spp., *Tetranychus* spp., *Trombicula alfreddugesi*, *Vaejovis* spp., or *Vasates lycopersici*.

In some instances, the insect is from the class Chilopoda, for example, *Geophilus* spp. or *Scutigera* spp.

30 In some instances, the insect is from the order Collembola, for example, *Onychiurus armatus*.

In some instances, the insect is from the class Diplopoda, for example, *Blaniulus guttulatus*; from the class Insecta, e.g. from the order Blattodea, for example, *Blattella asahinai*, *Blattella germanica*, *Blatta orientalis*, *Leucophaea maderae*, *Panchlora* spp., *Parcoblatta* spp., *Periplaneta* spp., or *Supella longipalpa*.

35 In some instances, the insect is from the order Coleoptera, for example, *Acalymma vittatum*, *Acanthoscelides obtectus*, *Adoretus* spp., *Agelastica alni*, *Agriotes* spp., *Alphitobius diaperinus*, *Amphimallon solstitialis*, *Anobium punctatum*, *Anoplophora* spp., *Anthonomus* spp., *Anthrenus* spp.,

- Apion* spp., *Apogonia* spp., *Atomaria* spp., *Attagenus* spp., *Bruchidius obtectus*, *Bruchus* spp., *Cassida* spp., *Cerotoma trifurcata*, *Ceutorrhynchus* spp., *Chaetocnema* spp., *Cleonus mendicus*, *Conoderus* spp., *Cosmopolites* spp., *Costelytra zealandica*, *Ctenicera* spp., *Curculio* spp., *Cryptolestes ferrugineus*, *Cryptorhynchus lapathi*, *Cylindrocopturus* spp., *Dermestes* spp., *Diabrotica* spp. (e.g., corn rootworm),
- 5 *Dichocrocis* spp., *Dicladispa armigera*, *Diloboderus* spp., *Epilachna* spp., *Epitrix* spp., *Faustinus* spp., *Gibbium psyllodes*, *Gnathocerus cornutus*, *Hellula undalis*, *Heteronychus arator*, *Heteronyx* spp., *Hylamorpha elegans*, *Hylotrupes bajulus*, *Hypera postica*, *Hypomeces squamosus*, *Hypothenemus* spp., *Lachnosterna consanguinea*, *Lasioderma serricorne*, *Latheticus oryzae*, *Lathridius* spp., *Lema* spp., *Leptinotarsa decemlineata*, *Leucoptera* spp., *Lissorhoptrus oryzophilus*, *Lixus* spp., *Luperodes* spp.,
- 10 *Lyctus* spp., *Megascelis* spp., *Melanotus* spp., *Meligethes aeneus*, *Melolontha* spp., *Migdolus* spp., *Monochamus* spp., *Naupactus xanthographus*, *Necrobia* spp., *Niptus hololeucus*, *Oryctes rhinoceros*, *Oryzaephilus surinamensis*, *Oryzaphagus oryzae*, *Otiorrhynchus* spp., *Oxycetonia jucunda*, *Phaedon cochleariae*, *Phyllophaga* spp., *Phyllophaga helleri*, *Phyllotreta* spp., *Popillia japonica*, *Premnotrypes* spp., *Prostephanus truncatus*, *Psylliodes* spp., *Ptinus* spp., *Rhizobius ventralis*, *Rhizopertha dominica*,
- 15 *Sitophilus* spp., *Sitophilus oryzae*, *Sphenophorus* spp., *Stegobium paniceum*, *Sternechus* spp., *Symphyletes* spp., *Tanymecus* spp., *Tenebrio molitor*, *Tenebrioides mauretanicus*, *Tribolium* spp., *Trogoderma* spp., *Tychius* spp., *Xylotrechus* spp., or *Zabrus* spp.

- In some instances, the insect is from the order Diptera, for example, *Aedes* spp., *Agromyza* spp., *Anastrepha* spp., *Anopheles* spp., *Asphondylia* spp., *Bactrocera* spp., *Bibio hortulanus*, *Calliphora erythrocephala*, *Calliphora vicina*, *Ceratitis capitata*, *Chironomus* spp., *Chrysomyia* spp., *Chrysops* spp.,
- 20 *Chrysozona pluvialis*, *Cochliomyia* spp., *Contarinia* spp., *Cordylobia anthropophaga*, *Cricotopus sylvestris*, *Culex* spp., *Culicoides* spp., *Culiseta* spp., *Cuterebra* spp., *Dacus oleae*, *Dasyneura* spp., *Delia* spp., *Dermatobia hominis*, *Drosophila* spp., *Echinocnemus* spp., *Fannia* spp., *Gasterophilus* spp., *Glossina* spp., *Haematopota* spp., *Hydrellia* spp., *Hydrellia griseola*, *Hylemya* spp., *Hippobosca* spp.,
- 25 *Hypoderma* spp., *Liriomyza* spp., *Lucilia* spp., *Lutzomyia* spp., *Mansonella* spp., *Musca* spp. (e.g., *Musca domestica*), *Oestrus* spp., *Oscinella frit*, *Paratanytarsus* spp., *Paralauterborniella subcincta*, *Pegomyia* spp., *Phlebotomus* spp., *Phorbia* spp., *Phormia* spp., *Piophilina casei*, *Prodiptosis* spp., *Psila rosae*, *Rhagoletis* spp., *Sarcophaga* spp., *Simulium* spp., *Stomoxys* spp., *Tabanus* spp., *Tetanops* spp., or *Tipula* spp.

- 30 In some instances, the insect is from the order Heteroptera, for example, *Anasa tristis*, *Antestiopsis* spp., *Boisea* spp., *Blissus* spp., *Calocoris* spp., *Campylomma livida*, *Cavelerius* spp., *Cimex* spp., *Collaria* spp., *Creontiades dilutus*, *Dasynus piperis*, *Dichelops furcatus*, *Diconocoris hewetti*, *Dysdercus* spp., *Euschistus* spp., *Eurygaster* spp., *Heliopeltis* spp., *Horcias nobilellus*, *Leptocoris* spp., *Leptocoris varicornis*, *Leptoglossus phyllopus*, *Lygus* spp., *Macropes excavatus*, *Miridae*, *Monalonion atratum*, *Nezara* spp., *Oebalus* spp., *Pentomidae*, *Piesma quadrata*, *Piezodorus* spp., *Psallus* spp.,
- 35 *Pseudacysta persea*, *Rhodnius* spp., *Sahlbergella singularis*, *Scaptocoris castanea*, *Scotinophora* spp., *Stephanitis nashi*, *Tibraca* spp., or *Triatoma* spp.

- In some instances, the insect is from the order Homiptera, for example, *Acizzia acaciaebaileyanae*, *Acizzia dodonaeae*, *Acizzia uncatoides*, *Acrida turrata*, *Acyrtosipon* spp., *Acrogonia*
- 40 spp., *Aeneolamia* spp., *Agonoscena* spp., *Aleyrodes proletella*, *Aleurolobus barodensis*, *Aleurothrix*

- floccosus*, *Allocaridara malayensis*, *Amrasca* spp., *Anuraphis cardui*, *Aonidiella* spp., *Aphanostigma pini*, *Aphis* spp. (e.g., *Apis gossypii*), *Arboridia apicalis*, *Arytainilla* spp., *Aspidiella* spp., *Aspidiotus* spp., *Atanus* spp., *Aulacorthum solani*, *Bemisia tabaci*, *Blastopsylla occidentalis*, *Boreioglycaspis melaleucae*, *Brachycaudus helichrysi*, *Brachycolus* spp., *Brevicoryne brassicae*, *Cacopsylla* spp., *Calligypona*
- 5 *marginata*, *Carneiocephala fulgida*, *Ceratovacuna lanigera*, *Cercopidae*, *Ceroplastes* spp., *Chaetosiphon fragaefolii*, *Chionaspis tegalensis*, *Chlorita onukii*, *Chondracris rosea*, *Chromaphis juglandicola*, *Chrysomphalus ficus*, *Cicadulina mbila*, *Coccoxymylus halli*, *Coccus* spp., *Cryptomyzus ribis*, *Cryptoneossa* spp., *Ctenarytaina* spp., *Dalbulus* spp., *Dialeurodes citri*, *Diaphorina citri*, *Diaspis* spp., *Drosicha* spp., *Dysaphis* spp., *Dysmicoccus* spp., *Empoasca* spp., *Eriosoma* spp., *Erythroneura* spp.,
- 10 *Eucalyptolyma* spp., *Euphyllura* spp., *Euscelis bilobatus*, *Ferrisia* spp., *Geococcus coffeae*, *Glycaspis* spp., *Heteropsylla cubana*, *Heteropsylla spinulosa*, *Homalodisca coagulata*, *Homalodisca vitripennis*, *Hyalopterus arundinis*, *Icerya* spp., *Idiocerus* spp., *Idioscopus* spp., *Laodelphax striatellus*, *Lecanium* spp., *Lepidosaphes* spp., *Lipaphis erysimi*, *Macrosiphum* spp., *Macrosteles facifrons*, *Mahanarva* spp., *Melanaphis sacchari*, *Metcalfiella* spp., *Metopolophium dirhodum*, *Monellia costalis*, *Monelliopsis pecanis*,
- 15 *Myzus* spp., *Nasonovia ribisnigri*, *Nephotettix* spp., *Nettigoniclla spectra*, *Nilaparvata lugens*, *Oncometopia* spp., *Orthezia praelonga*, *Oxya chinensis*, *Pachypsylla* spp., *Parabemisia myricae*, *Paratrioza* spp., *Parlatoria* spp., *Pemphigus* spp., *Pentatomidae* spp. (e.g., *Halyomorpha halys*), *Peregrinus maidis*, *Phenacoccus* spp., *Phloeomyzus passerinii*, *Phorodon humuli*, *Phylloxera* spp., *Pinnaspis aspidistrae*, *Planococcus* spp., *Prosopidopsylla flava*, *Protopulvinaria pyriformis*,
- 20 *Pseudaulacaspis pentagona*, *Pseudococcus* spp., *Psyllopsis* spp., *Psylla* spp., *Pteromalus* spp., *Pyrilla* spp., *Quadrapsidiotus* spp., *Quesada gigas*, *Rastrococcus* spp., *Rhopalosiphum* spp., *Saissetia* spp., *Scaphoideus titanus*, *Schizaphis graminum*, *Selenaspidus articulatus*, *Sogata* spp., *Sogatella furcifera*, *Sogatodes* spp., *Stictocephala festina*, *Siphoninus phillyrae*, *Tenalaphara malayensis*, *Tetragonocephala* spp., *Tinocallis caryaefoliae*, *Tomaspis* spp., *Toxoptera* spp., *Trialeurodes*
- 25 *vaporariorum*, *Trioza* spp., *Typhlocyba* spp., *Unaspis* spp., *Viteus vitifolii*, *Zygina* spp.; from the order Hymenoptera, for example, *Acromyrmex* spp., *Athalia* spp., *Atta* spp., *Diprion* spp., *Hoplocampa* spp., *Lasius* spp., *Monomorium pharaonis*, *Sirex* spp., *Solenopsis invicta*, *Tapinoma* spp., *Urocerus* spp., *Vespa* spp., or *Xeris* spp.

In some instances, the insect is from the order Isopoda, for example, *Armadillidium vulgare*,

30 *Oniscus asellus*, or *Porcellio scaber*.

In some instances, the insect is from the order Isoptera, for example, *Coptotermes* spp., *Cornitermes cumulans*, *Cryptotermes* spp., *Incisitermes* spp., *Microtermes obesi*, *Odontotermes* spp., or *Reticulitermes* spp.

In some instances, the insect is from the order Lepidoptera, for example, *Achroia grisella*,

35 *Acronicta major*, *Adoxophyes* spp., *Aedia leucomelas*, *Agrotis* spp., *Alabama* spp., *Amyelois transitella*, *Anarsia* spp., *Anticarsia* spp., *Argyroplote* spp., *Barathra brassicae*, *Borbo cinnara*, *Bucculatrix thurberiella*, *Bupalus piniarius*, *Busseola* spp., *Cacoecia* spp., *Caloptilia theivora*, *Capua reticulana*, *Carpocapsa pomonella*, *Carpocapsa niponensis*, *Cheimatobia brumata*, *Chilo* spp., *Choristoneura* spp., *Clysia ambiguella*, *Cnaphalocerus* spp., *Cnaphalocrocis medinalis*, *Cnephasia* spp., *Conopomorpha* spp.,

40 *Conotrachelus* spp., *Copitarsia* spp., *Cydia* spp., *Dalaca noctuides*, *Diaphania* spp., *Diatraea saccharalis*,

Earias spp., *Ecdytoplopha aurantium*, *Elasmopalpus lignosellus*, *Eldana saccharina*, *Ephestia* spp., *Epinotia* spp., *Epiphyas postvittana*, *Etiella* spp., *Eulia* spp., *Eupoecilia ambiguella*, *Euproctis* spp., *Euxoa* spp., *Feltia* spp., *Galleria mellonella*, *Gracillaria* spp., *Grapholitha* spp., *Hedylepta* spp., *Helicoverpa* spp., *Heliothis* spp., *Hofmannophila pseudospretella*, *Homoeosoma* spp., *Homona* spp., *Hyponomeuta padella*,
5 *Kakivoria flavofasciata*, *Laphygma* spp., *Laspeyresia molesta*, *Leucinodes orbonalis*, *Leucoptera* spp., *Lithocolletis* spp., *Lithophane antennata*, *Lobesia* spp., *Loxagrotis albicosta*, *Lymantria* spp., *Lyonetia* spp., *Malacosoma neustria*, *Maruca testulalis*, *Mamstra brassicae*, *Melanitis leda*, *Mocis* spp., *Monopis obviella*, *Mythimna separata*, *Nemapogon cloacellus*, *Nymphula* spp., *Oiketicus* spp., *Oria* spp., *Orthaga* spp., *Ostrinia* spp., *Oulema oryzae*, *Panolis flammea*, *Parnara* spp., *Pectinophora* spp., *Perileucoptera*
10 spp., *Phthorimaea* spp., *Phyllocnistis citrella*, *Phyllonorycter* spp., *Pieris* spp., *Platynota stultana*, *Plodia interpunctella*, *Plusia* spp., *Plutella xylostella*, *Prays* spp., *Prodenia* spp., *Protoparce* spp., *Pseudaletia* spp., *Pseudaletia unipuncta*, *Pseudoplusia includens*, *Pyrausta nubilalis*, *Rachiplusia nu*, *Schoenobius* spp., *Scirpophaga* spp., *Scirpophaga innotata*, *Scotia segetum*, *Sesamia* spp., *Sesamia inferens*, *Sparganothis* spp., *Spodoptera* spp., *Spodoptera praefica*, *Stathmopoda* spp., *Stomopteryx subsecivella*,
15 *Synanthedon* spp., *Tecia solanivora*, *Thermesia gemmatilis*, *Tinea cloacella*, *Tinea pellionella*, *Tineola bisselliella*, *Tortrix* spp., *Trichophaga tapetzella*, *Trichoplusia* spp., *Tryporyza incertulas*, *Tuta absoluta*, or *Virachola* spp.

In some instances, the insect is from the order Orthoptera or Saltatoria, for example, *Acheta domesticus*, *Dichroplus* spp., *Gryllotalpa* spp., *Hieroglyphus* spp., *Locusta* spp., *Melanoplus* spp., or
20 *Schistocerca gregaria*.

In some instances, the insect is from the order Phthiraptera, for example, *Damalinia* spp., *Haematopinus* spp., *Linognathus* spp., *Pediculus* spp., *Ptirus pubis*, *Trichodectes* spp.

In some instances, the insect is from the order Psocoptera for example *Lepinatus* spp., or *Liposcelis* spp.

25 In some instances, the insect is from the order Siphonaptera, for example, *Ceratophyllus* spp., *Ctenocephalides* spp., *Pulex irritans*, *Tunga penetrans*, or *Xenopsylla cheopsis*.

In some instances, the insect is from the order Thysanoptera, for example, *Anaphothrips obscurus*, *Baliothrips biformis*, *Drepanothrips reuteri*, *Enneothrips flavens*, *Frankliniella* spp., *Heliothrips* spp., *Hercinothrips femoralis*, *Rhipiphorothrips cruentatus*, *Scirtothrips* spp., *Taeniothrips cardamomi*, or
30 *Thrips* spp.

In some instances, the insect is from the order Zygentoma (=Thysanura), for example, *Ctenolepisma* spp., *Lepisma saccharina*, *Lepismodes inquilinus*, or *Thermobia domestica*.

In some instances, the insect is from the class Symphyla, for example, *Scutigera* spp.

In some instances, the insect is a mite, including but not limited to, Tarsonemid mites, such as
35 *Phytonemus pallidus*, *Polyphagotarsonemus latus*, *Tarsonemus bilobatus*, or the like; Eupodid mites, such as *Penthaleus erythrocephalus*, *Penthaleus major*, or the like; Spider mites, such as *Oligonychus shinkajii*, *Panonychus citri*, *Panonychus mori*, *Panonychus ulmi*, *Tetranychus kanzawai*, *Tetranychus urticae*, or the like; Eriophyid mites, such as *Acaphylla theavagrans*, *Aceria tulipae*, *Aculops lycopersici*, *Aculops pelekassi*, *Aculus schlechtendali*, *Eriophyes chibaensis*, *Phyllocoptruta oleivora*, or the like;
40 Acarid mites, such as *Rhizoglyphus robini*, *Tyrophagus putrescentiae*, *Tyrophagus similis*, or the like;

- Bee brood mites, such as *Varroa jacobsoni*, *Varroa destructor* or the like; Ixodides, such as *Boophilus microplus*, *Rhipicephalus sanguineus*, *Haemaphysalis longicornis*, *Haemaphysalis flava*, *Haemaphysalis campanulata*, *Ixodes ovatus*, *Ixodes persulcatus*, *Amblyomma* spp., *Dermacentor* spp., or the like; Cheyletidae, such as *Cheyletiella yasguri*, *Cheyletiella blakei*, or the like; Demodicidae, such as
- 5 *Demodex canis*, *Demodex cati*, or the like; Psoroptidae, such as *Psoroptes ovis*, or the like; Sarcoptidae, such as *Sarcoptes scabiei*, *Notoedres cati*, *Knemidocoptes* spp., or the like.

Table 8 shows further examples of insects that cause infestations that can be treated or prevented using the PMP compositions and related methods described herein.

10 **Table 8. Insect pests**

Common Name	Latin name
European corn borer	<i>Ostrinia nubilalis</i>
Corn earworm	<i>Helicoverpa zea</i>
Beet armyworm	<i>Spodoptera exigua</i>
Fall armyworm	<i>Spodoptera frugiperda</i>
Southwestern corn borer	<i>Diatraea grandiosella</i>
Lesser cornstalk borer	<i>Elasmopalpus lignosellus</i>
Stalk borer	<i>Papaipema nebris</i>
Common armyworm	<i>Pseudaletia unipuncta</i>
Black cutworm	<i>Agrotis ipsilon</i>
Western bean cutworm	<i>Striacosta albicosta</i>
Yellowstriped armyworm	<i>Spodoptera ornithogalli</i>
Western yellowstriped armyworm	<i>Spodoptera praefica</i>
Southern armyworm	<i>Spodoptera eridania</i>
Southern armyworm	<i>Spodoptera eridania</i>
Variegated cutworm	<i>Peridroma saucia</i>
Stalk borer	<i>Papaipema nebris</i>
Cabbage looper	<i>Trichoplusia ni</i>
Tomato pinworm	<i>Keiferia lycopersicella</i>
Tobacco hornworm	<i>Manduca sexta</i>
Tomato hornworm	<i>Manduca quinquemaculata</i>
Imported cabbageworm	<i>Artogeia rapae</i>
Cabbage butterfly	<i>Pieris brassicae</i>
Cabbage looper	<i>Trichoplusia ni</i>
Diamondback moth	<i>Plutella xylostella</i>
Beet armyworm	<i>Spodoptera exigua</i>
Common cutworm	<i>Agrotis segetum</i>
Potato tuberworm	<i>Phthorimaea operculella</i>
Diamondback moth	<i>Plutella xylostella</i>

Sugarcane borer	<i>Diatraea saccharalis</i>
Glassy cutworm	<i>Crymodes devastator</i>
Dingy cutworm	<i>Feltia ducens</i>
Claybacked cutworm	<i>Agrotis gladiaria</i>
Green cloverworm	<i>Plathypena scabra</i>
Soybean looper	<i>Pseudoplusia includes</i>
Velvetbean caterpillar	<i>Anticarsia gemmatilis</i>
Northern corn rootworm	<i>Coleoptera Diabrotica barberi</i>
Southern corn rootworm	<i>Diabrotica undecimpunctata</i>
Western corn rootworm	<i>Diabrotica virgifera</i>
Maize weevil	<i>Sitophilus zeamais</i>
Colorado potato beetle	<i>Leptinotarsa decemlineata</i>
Tobacco flea beetle	<i>Epitrix hirtipennis</i>
Crucifer flea beetle	<i>Phyllotreta cruciferae</i>
Western black flea beetle	<i>Phyllotreta pusilla</i>
Pepper weevil	<i>Anthonomus eugenii</i>
Colorado potato beetle	<i>Leptinotarsa decemlineata</i>
Potato flea beetle	<i>Epitrix cucumeris</i>
Wireworms <i>Melanotus</i> spp.	<i>Hemicrepidus memnonius</i>
Wireworms	<i>Ceutorhynchus assimilis</i>
Cabbage seedpod weevil	<i>Phyllotreta cruciferae</i>
Crucifer flea beetle	<i>Melanolus</i> spp.
Wireworm	<i>Aeolus mellillus</i>
Wheat wireworm	<i>Aeolus mancus</i>
Sand wireworm	<i>Horistonotus uhlerii</i>
Maize billbug	<i>Sphenophorus maidis</i>
Timothy billbug	<i>Sphenophorus zae</i>
Bluegrass billbug	<i>Sphenophorus parvulus</i>
Southern corn billbug	<i>Sphenophorus callosus</i>
White grubs	<i>Phyllophaga</i> spp.
Corn flea beetle	<i>Chaetocnema pulicaria</i>
Japanese beetle	<i>Popillia japonica</i>
Mexican bean beetle	<i>Epilachna varivestis</i>
Bean leaf beetle	<i>Cerotoma trifurcate</i>
Blister beetles	<i>Epicauta pestifera</i> <i>Epicauta lemniscata</i>
Corn leaf aphid	<i>Homoptera Rhopalosiphum maidis</i>
Corn root aphid	<i>Anuraphis maidiradicis</i>
Green peach aphid	<i>Myzus persicae</i>

Potato aphid	<i>Macrosiphum euphorbiae</i>
Greenhouse whitefly	<i>Trialeurodes vaporariorum</i>
Sweetpotato whitefly	<i>Bemisia tabaci</i>
Silverleaf whitefly	<i>Bemisia argentifolii</i>
Cabbage aphid	<i>Brevicoryne brassicae</i>
Green peach aphid	<i>Myzus persicae</i>
Potato leafhopper	<i>Empoasca fabae</i>
Potato psyllid	<i>Paratrioza cockerelli</i>
Silverleaf whitefly	<i>Bemisia argentifolii</i>
Sweetpotato whitefly	<i>Bemisia tabaci</i>
Carrot aphid	<i>Cavariella aegopodii</i>
Cabbage aphid	<i>Brevicoryne brassicae</i>
West Indian canefly	<i>Saccharosydne saccharivora</i>
Yellow sugarcane aphid	<i>Sipha flava</i>
Threecornered alfalfa hopper	<i>Spissistilus festinus</i>
<i>Lygus Hesperus</i>	<i>Hemiptera Lygus lineolaris</i>
<i>Lygus</i> bug	<i>Lygus rugulipennis</i>
Green stink bug	<i>Acrosternum hilare</i>
Brown stick bug	<i>Euschistus servus</i>
Chinch bug	<i>Blissus leucopterus leucopterus</i>
Leafminer	<i>Diptera Liriomyza trifolii</i>
Vegetable leafminer	<i>Liriomyza sativae</i>
Tomato leafminer	<i>Scrobipalpula absoluta</i>
Seedcorn maggot	<i>Delia platura</i>
Cabbage maggot	<i>Delia brassicae</i>
Cabbage root fly	<i>Delia radicum</i>
Carrot rust fly	<i>Psilia rosae</i>
Sugarbeet root maggot	<i>Tetanops myopaeformis</i>
Differential grasshopper	<i>Orthoptera Melanoplus differentialis</i>
Redlegged grasshopper	<i>Melanoplus femurrubrum</i>
Two-striped grasshopper	<i>Melanoplus bivittatus</i>

iv. Mollusks

The PMP compositions and related methods can be useful for decreasing the fitness of a mollusk, e.g., to prevent or treat a mollusk infestation in a plant. The term "mollusk" includes any organism belonging to the phylum Mollusca. Included are methods for delivering a PMP composition to a mollusk by contacting the mollusk with the PMP composition. Additionally or alternatively, the methods include delivering the biopesticide to a plant at risk of or having a mollusk infestation, by contacting the plant with the PMP composition.

The PMP compositions and related methods are suitable for preventing or treating infestation by terrestrial Gastropods (e.g., slugs and snails) in agriculture and horticulture. They include all terrestrial slugs and snails which mostly occur as polyphagous pests on agricultural and horticultural crops. For example, the mollusk may belong to the family Achatinidae, Agriolimacidae, Ampullariidae, Arionidae, 5 Bradybaenidae, Helicidae, Hydromiidae, Lymnaeidae, Milacidae, Urocyclidae, or Veronicellidae.

For example, in some instances, the mollusk is *Achatina* spp., *Archachatina* spp. (e.g., *Archachatina marginata*), *Agriolimax* spp., *Arion* spp. (e.g., *A. ater*, *A. circumscriptus*, *A. distinctus*, *A. fasciatus*, *A. hortensis*, *A. intermedius*, *A. rufus*, *A. subfuscus*, *A. silvaticus*, *A. lusitanicus*), *Arliomax* spp. (e.g., *Ariolimax columbianus*), *Biomphalaria* spp., *Bradybaena* spp. (e.g., *B. fruticum*), *Bulinus* spp., 10 *Cantareus* spp. (e.g., *C. asperses*), *Cepaea* spp. (e.g., *C. hortensis*, *C. nemoralis*, *C. hortensis*), *Cernuella* spp., *Cochlicella* spp., *Cochlodina* spp. (e.g., *C. laminata*), *Deroceas* spp. (e.g., *D. agrestis*, *D. empiricorum*, *D. laeve*, *D. panornimatum*, *D. reticulatum*), *Discus* spp. (e.g., *D. rotundatus*), *Euomphalia* spp., *Galba* spp. (e.g., *G. trunculata*), *Helicella* spp. (e.g., *H. itala*, *H. obvia*), *Helicigona* spp. (e.g., *H. arbustorum*), *Helicodiscus* spp., *Helix* spp. (e.g., *H. aperta*, *H. aspersa*, *H. pomatia*), *Limax* spp. (e.g., *L. cinereoniger*, *L. flavus*, *L. marginatus*, *L. maximus*, *L. tenellus*), *Limicolaria* spp. (e.g., *Limicolaria aurora*), 15 *Lymnaea* spp. (e.g., *L. stagnalis*), *Mesodon* spp. (e.g., *Meson thyroidus*), *Monadenia* spp. (e.g., *Monadenia fidelis*), *Milax* spp. (e.g., *M. gagates*, *M. marginatus*, *M. sowerbyi*, *M. budapestensis*), *Oncomelania* spp., *Neohelix* spp. (e.g., *Neohelix albolabris*), *Opeas* spp., *Otala* spp. (e.g., *Otala lactea*), *Oxyloma* spp. (e.g., *O. pfeifferi*), *Pomacea* spp. (e.g., *P. canaliculata*), *Succinea* spp., *Tandonia* spp. 20 (e.g., *T. budapestensis*, *T. sowerbyi*), *Theba* spp., *Vallonia* spp., or *Zonitoides* spp. (e.g., *Z. nitidus*).

v. Nematodes

The PMP compositions and related methods can be useful for decreasing the fitness of a nematode, e.g., to prevent or treat a nematode infestation in a plant. The term “nematode” includes any 25 organism belonging to the phylum Nematoda. Included are methods for delivering a PMP composition to a nematode by contacting the nematode with the PMP composition. Additionally or alternatively, the methods include delivering the biopesticide to a plant at risk of or having a nematode infestation, by contacting the plant with the PMP composition.

The PMP compositions and related methods are suitable for preventing or treating infestation by 30 nematodes that cause damage plants including, for example, *Meloidogyne* spp. (root- knot), *Heterodera* spp., *Globodera* spp., *Pratylenchus* spp., *Helicotylenchus* spp., *Radopholus similis*, *Ditylenchus dipsaci*, *Rotylenchulus reniformis*, *Xiphinema* spp., *Aphelenchoides* spp. and *Belonolaimus longicaudatus*. In some instances, the nematode is a plant parasitic nematodes or a nematode living in the soil. Plant parasitic nematodes include, but are not limited to, ectoparasites such as *Xiphinema* spp., *Longidorus* 35 spp., and *Trichodorus* spp.; semiparasites such as *Tylenchulus* spp.; migratory endoparasites such as *Pratylenchus* spp., *Radopholus* spp., and *Scutellonema* spp.; sedentary parasites such as *Heterodera* spp., *Globodera* spp., and *Meloidogyne* spp., and stem and leaf endoparasites such as *Ditylenchus* spp., *Aphelenchoides* spp., and *Hirshmaniella* spp. Especially harmful root parasitic soil nematodes are such as cystforming nematodes of the genera *Heterodera* or *Globodera*, and/or root knot nematodes of the 40 genus *Meloidogyne*. Harmful species of these genera are for example *Meloidogyne incognita*, *Heterodera*

glycines (soybean cyst nematode), *Globodera pallida* and *Globodera rostochiensis* (potato cyst nematode), which species are effectively controlled with the PMP compositions described herein. However, the use of the PMP compositions described herein is in no way restricted to these genera or species, but also extends in the same manner to other nematodes.

- 5 Other examples of nematodes that can be targeted by the methods and compositions described herein include but are not limited to e.g. *Aglenchus agricola*, *Anguina tritici*, *Aphelenchoides arachidis*, *Aphelenchoides fragaria* and the stem and leaf endoparasites *Aphelenchoides* spp. in general, *Belonolaimus gracilis*, *Belonolaimus longicaudatus*, *Belonolaimus nortoni*, *Bursaphelenchus cocophilus*, *Bursaphelenchus eremus*, *Bursaphelenchus xylophilus*, *Bursaphelenchus mucronatus*, and
- 10 *Bursaphelenchus* spp. in general, *Cacopaurus pestis*, *Criconemella curvata*, *Criconemella onoensis*, *Criconemella ornata*, *Criconemella rusium*, *Criconemella xenoplax* (= *Mesocriconema xenoplax*) and *Criconemella* spp. in general, *Criconemoides femiae*, *Criconemoides onoense*, *Criconemoides ornatum* and *Criconemoides* spp. in general, *Ditylenchus destructor*, *Ditylenchus dipsaci*, *Ditylenchus myceliophagus* and the stem and leaf endoparasites *Ditylenchus* spp. in general, *Dolichodorus*
- 15 *heterocephalus*, *Globodera pallida* (= *Heterodera pallida*), *Globodera rostochiensis* (potato cyst nematode), *Globodera solanacearum*, *Globodera tabacum*, *Globodera virginia* and the sedentary, cyst forming parasites *Globodera* spp. in general, *Helicotylenchus digonicus*, *Helicotylenchus dihystra*, *Helicotylenchus erythrine*, *Helicotylenchus multicinctus*, *Helicotylenchus nannus*, *Helicotylenchus pseudorobustus* and *Helicotylenchus* spp. in general, *Hemicriconemoides*, *Hemicycliophora arenaria*,
- 20 *Hemicycliophora nudata*, *Hemicycliophora parvana*, *Heterodera avenae*, *Heterodera cruciferae*, *Heterodera glycines* (soybean cyst nematode), *Heterodera oryzae*, *Heterodera schachtii*, *Heterodera zae* and the sedentary, cyst forming parasites *Heterodera* spp. in general, *Hirschmaniella gracilis*, *Hirschmaniella oryzae*, *Hirschmaniella spinicaudata* and the stem and leaf endoparasites *Hirschmaniella* spp. in general, *Hoplolaimus aegyptii*, *Hoplolaimus californicus*, *Hoplolaimus columbus*, *Hoplolaimus*
- 25 *galeatus*, *Hoplolaimus indicus*, *Hoplolaimus magnistylus*, *Hoplolaimus pararobustus*, *Longidorus africanus*, *Longidorus breviannulatus*, *Longidorus elongatus*, *Longidorus laevicapitatus*, *Longidorus vineacola* and the ectoparasites *Longidorus* spp. in general, *Meloidogyne acronea*, *Meloidogyne africana*, *Meloidogyne arenaria*, *Meloidogyne arenaria thamesi*, *Meloidogyne artiella*, *Meloidogyne chitwoodi*, *Meloidogyne coffeicola*, *Meloidogyne ethiopica*, *Meloidogyne exigua*, *Meloidogyne fallax*, *Meloidogyne*
- 30 *graminicola*, *Meloidogyne graminis*, *Meloidogyne hapla*, *Meloidogyne incognita*, *Meloidogyne incognita acrita*, *Meloidogyne javanica*, *Meloidogyne kikuyensis*, *Meloidogyne minor*, *Meloidogyne naasi*, *Meloidogyne paranaensis*, *Meloidogyne thamesi* and the sedentary parasites *Meloidogyne* spp. in general, *Meloinema* spp., *Nacobbus aberrans*, *Neotylenchus vigissi*, *Paraphelenchus pseudoparietinus*, *Paratrachodorus allius*, *Paratrachodorus lobatus*, *Paratrachodorus minor*, *Paratrachodorus nanus*,
- 35 *Paratrachodorus porosus*, *Paratrachodorus teres* and *Paratrachodorus* spp. in general, *Paratylenchus hamatus*, *Paratylenchus minutus*, *Paratylenchus projectus* and *Paratylenchus* spp. in general, *Pratylenchus agilis*, *Pratylenchus allenii*, *Pratylenchus andinus*, *Pratylenchus brachyurus*, *Pratylenchus cerealis*, *Pratylenchus coffeae*, *Pratylenchus crenatus*, *Pratylenchus delattrei*, *Pratylenchus giibbicaudatus*, *Pratylenchus goodeyi*, *Pratylenchus hamatus*, *Pratylenchus hexincisus*, *Pratylenchus*
- 40 *loosi*, *Pratylenchus neglectus*, *Pratylenchus penetrans*, *Pratylenchus pratensis*, *Pratylenchus scribneri*,

Pratylenchus teres, *Pratylenchus thornei*, *Pratylenchus vulnus*, *Pratylenchus zae* and the migratory endoparasites *Pratylenchus* spp. in general, *Pseudohalenchus minutus*, *Psilenchus magnidens*, *Psilenchus tumidus*, *Punctodera chalcoensis*, *Quinisulcius acutus*, *Radopholus citrophilus*, *Radopholus similis*, the migratory endoparasites *Radopholus* spp. in general, *Rotylenchulus borealis*, *Rotylenchulus parvus*, *Rotylenchulus reniformis* and *Rotylenchulus* spp. in general, *Rotylenchus laurentinus*, *Rotylenchus macrodoratus*, *Rotylenchus robustus*, *Rotylenchus uniformis* and *Rotylenchus* spp. in general, *Scutellonema brachyurum*, *Scutellonema bradys*, *Scutellonema clathricaudatum* and the migratory endoparasites *Scutellonema* spp. in general, *Subanguina radiciola*, *Tetylenchus nicotianae*, *Trichodorus cylindricus*, *Trichodorus minor*, *Trichodorus primitivus*, *Trichodorus proximus*, *Trichodorus similis*, *Trichodorus sparsus* and the ectoparasites *Trichodorus* spp. in general, *Tylenchorhynchus agri*, *Tylenchorhynchus brassicae*, *Tylenchorhynchus clarus*, *Tylenchorhynchus claytoni*, *Tylenchorhynchus digitatus*, *Tylenchorhynchus ebriensis*, *Tylenchorhynchus maximus*, *Tylenchorhynchus nudus*, *Tylenchorhynchus vulgaris* and *Tylenchorhynchus* spp. in general, *Tylenchulus semipenetrans* and the semiparasites *Tylenchulus* spp. in general, *Xiphinema americanum*, *Xiphinema brevicolle*, *Xiphinema dimorphicaudatum*, *Xiphinema index* and the ectoparasites *Xiphinema* spp. in general.

Other examples of nematode pests include species belonging to the family *Criconeematidae*, *Belonolaimidae*, *Hoploaimidae*, *Heteroderidae*, *Longidoridae*, *Pratylenchidae*, *Trichodoridae*, or *Anguinidae*.

Table 9 shows further examples of nematodes, and diseases associated therewith, that can be treated or prevented using the PMP compositions and related methods described herein.

Table 9. Nematode pests

Disease	Causative Agent
Awl	<i>Dolichoderus</i> spp., <i>D. heterocephalus</i>
Bulb and stem (Europe)	<i>Ditylenchus dipsaci</i>
Burrowing	<i>Radopholus similis</i> <i>R. similis</i>
Cyst	<i>Heterodera avenae</i> , <i>H. zae</i> , <i>H. schachtii</i> ; <i>Globodera rostochiensis</i> , <i>G. pallida</i> , and <i>G. tabacum</i> ; <i>Heterodera trifolii</i> , <i>H. medicaginis</i> , <i>H. ciceri</i> , <i>H. mediterranea</i> , <i>H. cyperi</i> , <i>H. salixophila</i> , <i>H. zae</i> , <i>H. goettingiana</i> , <i>H. riparia</i> , <i>H. humuli</i> , <i>H. latipons</i> , <i>H. sorghi</i> , <i>H. fici</i> , <i>H. litoralis</i> , and <i>H. turcomanica</i> ; <i>Punctodera chalcoensis</i>
Dagger	<i>Xiphinema</i> spp., <i>X. americanum</i> , <i>X. Mediterraneum</i>

False root-knot	<i>Nacobbus dorsalis</i>
Lance	<i>Hoplolaimus</i> spp., <i>H. galeatus</i>
Lance, Columbia	<i>Hoplolaimus</i> <i>Columbus</i>
Lesion	<i>Pratylenchus</i> spp., <i>P. brachyurus</i> , <i>P. coffeae</i> , <i>P. crenatus</i> , <i>P. hexincisus</i> , <i>P. neglectus</i> , <i>P. penetrans</i> , <i>P. scribneri</i> , <i>P. magnica</i> , <i>P. neglectus</i> , <i>P. thornei</i> , <i>P. vulnus</i> , <i>P. zae</i>
Needle	<i>Longidorus</i> spp., <i>L. breviannulatus</i>
Others	<i>Hirschmanniella</i> species, <i>Pratylenchoid magnicauda</i>
Ring	<i>Criconemella</i> spp., <i>C. ornata</i>
Root-knot	<i>Meloidogyne</i> spp., <i>M. arenaria</i> , <i>M. chitwoodi</i> , <i>M. artiellia</i> , <i>M. fallax</i> , <i>M. hapla</i> , <i>M. javanica</i> , <i>M. incognita</i> , <i>M. microtyla</i> , <i>M. partityla</i> , <i>M. panyuensis</i> , <i>M. paranaensis</i>
Spiral	<i>Helicotylenchus</i> spp.
Sting	<i>Belonolaimus</i> spp., <i>B. longicaudatus</i>
Stubby-root	<i>Paratrichodorus</i> spp., <i>P. christiei</i> , <i>P. minor</i> , <i>Quinisulcius acutus</i> , <i>Trichodorus</i> spp.
Stunt	<i>Tylenchorhynchus dubius</i>

vi. Viruses

The PMP compositions and related methods can be useful for decreasing the fitness of a virus, e.g., to prevent or treat a viral infection in a plant. Included are methods for delivering a PMP composition to a virus by contacting the virus with the PMP composition. Additionally or alternatively, the methods include delivering the PMP composition to a plant at risk of or having a viral infection, by contacting the plant with the PMP composition.

The PMP compositions and related methods are suitable for delivery to a virus that causes viral diseases in plants, including the viruses and diseases listed in Table 10.

Table 10. Viral plant pathogens

Disease	Causative Agent
Alfamoviruses:	Alfalfa mosaic alfamovirus
Bromoviridae	
Alphacryptoviruses:	Alfalfa 1 alphacryptovirus, Beet 1 alphacryptovirus, Beet 2

Partitiviridae	alphacryptovirus, Beet 3 alphacryptovirus, Carnation 1
	alphacryptovirus, Carrot temperate 1 alphacryptovirus, Carrot
	temperate 3 alphacryptovirus, Carrot temperate 4 alphacryptovirus,
	Cocksfoot alphacryptovirus, Hop trefoil 1 alphacryptovirus, Hop
	trefoil 3 alphacryptovirus, Radish yellow edge alphacryptovirus,
	Ryegrass alphacryptovirus, Spinach temperate alphacryptovirus,
	<i>Vicia</i> alphacryptovirus, White clover 1 alphacryptovirus, White
	clover 3 alphacryptovirus
Badnaviruses	Banana streak badnavirus, <i>Cacao</i> swollen shoot badnavirus, <i>Canna</i>
	yellow mottle badnavirus, <i>Commelina</i> yellow mottle badnavirus,
	<i>Dioscorea</i> bacilliform badnavirus, Kalanchoe top-spotting
	badnavirus, Rice tungro bacilliform badnavirus, <i>Schefflera</i> ringspot
	badnavirus, Sugarcane bacilliform badnavirus
Betacryptoviruses:	Carrot temperate 2 betacryptovirus, Hop trefoil 2 betacryptovirus,
Partitiviridae	Red clover 2 betacryptovirus, White clover 2 betacryptovirus
Bigeminiviruses:	<i>Abutilon</i> mosaic bigeminivirus, <i>Ageratum</i> yellow vein
Geminiviridae	bigeminivirus, Bean calico mosaic bigeminivirus, Bean golden
	mosaic bigeminivirus, Bhendi yellow vein mosaic bigeminivirus,
	Cassava African mosaic bigeminivirus, Cassava Indian mosaic
	bigeminivirus, Chino del tomate bigeminivirus, Cotton leaf crumple
	bigeminivirus, Cotton leaf curl bigeminivirus, Croton yellow vein
	mosaic bigeminivirus, <i>Dolichos</i> yellow mosaic bigeminivirus,
	<i>Euphorbia</i> mosaic bigeminivirus, Horsegum yellow mosaic
	bigeminivirus, <i>Jatropha</i> mosaic bigeminivirus, Lima bean golden
	mosaic bigeminivirus, Melon leaf curl bigeminivirus, Mung bean
	yellow mosaic bigeminivirus, Okra leaf-curl bigeminivirus, Pepper
	hausteco bigeminivirus, Pepper Texas bigeminivirus, Potato yellow
	mosaic bigeminivirus, <i>Rhynchosia</i> mosaic bigeminivirus, Serrano
	golden mosaic bigeminivirus, Squash leaf curl bigeminivirus,
	Tobacco leaf curl bigeminivirus, Tomato Australian leafcurl
	bigeminivirus, Tomato golden mosaic bigeminivirus, Tomato
	Indian leafcurl bigeminivirus, Tomato leaf crumple bigeminivirus,
	Tomato mottle bigeminivirus, Tomato yellow leaf curl
	bigeminivirus, Tomato yellow mosaic bigeminivirus, Watermelon
	chlorotic stunt bigeminivirus, Watermelon curly mottle
	bigeminivirus
Bromoviruses:	Broad bean mottle bromovirus, Brome mosaic bromovirus, <i>Cassia</i>
Bromoviridae	yellow blotch bromovirus, Cowpea chlorotic mottle bromovirus,
	<i>Melandrium</i> yellow fleck bromovirus, Spring beauty latent

	bromovirus
Bymoviruses:	Barley mild mosaic bymovirus, Barley yellow mosaic bymovirus,
Potyviridae	Oat mosaic bymovirus, Rice necrosis mosaic bymovirus, Wheat
	spindle streak mosaic bymovirus, Wheat yellow mosaic bymovirus
Capilloviruses	Apple stem grooving capillovirus, Cherry A capillovirus, Citrus
	tatter leaf capillovirus, Lilac chlorotic leafspot capillovirus
Carlaviruses	Blueberry scorch carlavirus, Cactus 2 carlavirus, Caper latent
	carlavirus, Carnation latent carlavirus, <i>Chrysanthemum</i> B
	carlavirus, Dandelion latent carlavirus, Elderberry carlavirus, Fig S
	carlavirus, <i>Helenium</i> S carlavirus, Honeysuckle latent carlavirus,
	Hop American latent carlavirus, Hop latent carlavirus, Hop mosaic
	carlavirus, <i>Kalanchoe</i> latent carlavirus, Lilac mottle carlavirus, Lily
	symptomless carlavirus, Mulberry latent carlavirus, Muskmelon
	vein necrosis carlavirus, <i>Nerine</i> latent carlavirus, <i>Passiflora</i> latent
	carlavirus, Pea streak carlavirus, Poplar mosaic carlavirus, Potato M
	carlavirus, Potato S carlavirus, Red clover vein mosaic carlavirus,
	Shallot latent carlavirus, Strawberry pseudo mild yellow edge
	carlavirus
Carmoviruses:	Bean mild mosaic carmovirus, <i>Cardamine</i> chlorotic fleck
Tombusviridae	carmovirus, Carnation mottle carmovirus, Cucumber leaf spot
	carmovirus, Cucumber soil-borne carmovirus, <i>Galinsoga</i> mosaic
	carmovirus, <i>Hibiscus</i> chlorotic ringspot carmovirus, Melon necrotic
	spot carmovirus, <i>Pelargonium</i> flower break carmovirus, Turnip
	crinkle carmovirus
Caulimoviruses	Blueberry red ringspot caulimovirus, Carnation etched ring
	caulimovirus, Cauliflower mosaic caulimovirus, <i>Dahlia</i> mosaic
	caulimovirus, Figwort mosaic caulimovirus, Horseradish latent
	caulimovirus, <i>Mirabilis</i> mosaic caulimovirus, Peanut chlorotic
	streak caulimovirus, Soybean chlorotic mottle caulimovirus, Sweet
	potato caulimovirus, Thistle mottle caulimovirus
Closteroviruses	Beet yellow stunt closterovirus, Beet yellows closterovirus, Broad
	bean severe chlorosis closterovirus, Burdock yellows closterovirus,
	Carnation necrotic fleck closterovirus, <i>Citrus</i> tristeza closterovirus,
	Clover yellows closterovirus, Grapevine stem pitting associated
	closterovirus, Wheat yellow leaf closterovirus
Comoviruses:	Bean pod mottle comovirus, Bean rugose mosaic comovirus, Broad
Comoviridae	bean stain comovirus, Broad bean true mosaic comovirus, Cowpea
	mosaic comovirus, Cowpea severe mosaic comovirus, Glycine
	mosaic comovirus, Pea mild mosaic comovirus, Potato Andean

	mottle comovirus, Quail pea mosaic comovirus, Radish mosaic
	comovirus, Red clover mottle comovirus, Squash mosaic
	comovirus, <i>Ullucus</i> C comovirus
Cucumoviruses:	Cucumber mosaic cucumovirus, Peanut stunt cucumovirus, Tomato
Bromoviridae	aspermy cucumovirus
Cytorhabdoviruses:	Barley yellow striate mosaic cytorhabdovirus, Broad bean yellow
Rhabdoviridae	vein cytorhabdovirus, Broccoli necrotic yellows cytorhabdovirus,
	Cereal northern mosaic cytorhabdovirus, <i>Festuca</i> leaf streak
	cytorhabdovirus, Lettuce necrotic yellows cytorhabdovirus,
	<i>Sonchus</i> cytorhabdovirus, Strawberry crinkle cytorhabdovirus
Dianthoviruses	Carnation ringspot dianthovirus, Red clover necrotic mosaic
	dianthovirus, Sweet clover necrotic mosaic dianthovirus
Enamoviruses	Pea enation mosaic enamovirus
Fijiviruses:	Maize rough dwarf fijivirus, Oat sterile dwarf fijivirus, Pangola
Reoviridae	stunt fijivirus, Rice black-streaked dwarf fijivirus, Sugarcane Fiji
	disease fijivirus
Furoviruses	Beet necrotic yellow vein furovirus, Beet soil-borne furovirus,
	Broad bean necrosis furovirus, Oat golden stripe furovirus, Peanut
	clump furovirus, Potato mop-top furovirus, <i>Sorghum</i> chlorotic spot
	furovirus, Wheat soil-borne mosaic furovirus
Hordeiviruses	<i>Anthoxanthum</i> latent blanching hordeivirus, Barley stripe mosaic
	hordeivirus, <i>Lychnis</i> ringspot hordeivirus, <i>Poa</i> semilatifolia
	hordeivirus
Hybrigeminiviruses:	Beet curly top hybrigeminivirus, Tomato pseudo curly top
Geminiviridae	hybrigeminivirus
Idaeoviruses	Raspberry bushy dwarf idaeovirus
Illaviruses:	Apple mosaic illavirus, <i>Asparagus</i> 2 illavirus, Blueberry necrotic
Bromoviridae	shock illavirus, Citrus leaf rugose illavirus, <i>Citrus</i> variegation
	illavirus, Elm mottle illavirus, <i>Humulus japonicus</i> illavirus,
	<i>Hydrangea</i> mosaic illavirus, Lilac ring mottle illavirus, <i>Parietaria</i>
	mottle illavirus, Plum American line pattern illavirus, Prune dwarf
	illavirus, <i>Prunus</i> necrotic ringspot illavirus, Spinach latent illavirus,
	Tobacco streak illavirus, Tulare apple mosaic illavirus
Ipomoviruses:	Sweet potato mild mottle ipomovirus, Sweet potato yellow dwarf
Potyviridae	ipomovirus
Luteoviruses	Barley yellow dwarf luteovirus, Bean leaf roll luteovirus, Beet mild
	yellowing luteovirus, Beet western yellows luteovirus, Carrot red
	leaf luteovirus, Groundnut rosette assistor luteovirus, Potato leafroll
	luteovirus, <i>Solanum</i> yellows luteovirus, Soybean dwarf luteovirus,

	Soybean Indonesian dwarf luteovirus, Strawberry mild yellow edge
	luteovirus, Subterranean clover red leaf luteovirus, Tobacco
	necrotic dwarf luteovirus
Machlomoviruses	Maize chlorotic mottle machlomovirus
Macluraviruses	<i>Maclura</i> mosaic macluravirus, <i>Narcissus</i> latent macluravirus
Marafiviruses	Bermuda grass etched-line marafivirus, Maize rayado fino
	marafivirus, Oat blue dwarf marafivirus
Monogeminiviruses:	<i>Chloris</i> striate mosaic monogeminivirus, <i>Digitaria</i> striate mosaic
Geminiviridae	monogeminivirus, <i>Digitaria</i> streak monogeminivirus, Maize streak
	monogeminivirus, <i>Miscanthus</i> streak monogeminivirus, <i>Panicum</i>
	streak monogeminivirus, <i>Paspalum</i> striate mosaic
	monogeminivirus, Sugarcane streak monogeminivirus, Tobacco
	yellow dwarf monogeminivirus, Wheat dwarf monogeminivirus
Nanaviruses	Banana bunchy top nanavirus, Coconut foliar decay nanavirus,
	Faba bean necrotic yellows nanavirus, Milk vetch dwarf nanavirus,
	Subterranean clover stunt nanavirus
Necroviruses	Tobacco necrosis necrovirus, Carnation yellow stripe necrovirus,
	<i>Lisianthus</i> necrosis necrovirus
Nepoviruses:	<i>Arabis</i> mosaic nepovirus, Arracacha A nepovirus, Artichoke Italian
Comoviridae	latent nepovirus, Artichoke yellow ringspot nepovirus, Blueberry
	leaf mottle nepovirus, <i>Cacao</i> necrosis nepovirus, Cassava green
	mottle nepovirus, Cherry leaf roll nepovirus, Cherry rasp leaf
	nepovirus, Chicory yellow mottle nepovirus, Crimson clover latent
	nepovirus, <i>Cycas</i> necrotic stunt nepovirus, Grapevine Bulgarian
	latent nepovirus, Grapevine chrome mosaic nepovirus, Grapevine
	fanleaf nepovirus, <i>Hibiscus</i> latent ringspot nepovirus, Lucerne
	Australian latent nepovirus, Mulberry ringspot nepovirus,
	Myrobalan latent ringspot nepovirus, Olive latent ringspot
	nepovirus, Peach rosette mosaic nepovirus, Potato black ringspot
	nepovirus, Potato U nepovirus, Raspberry ringspot nepovirus,
	Tobacco ringspot nepovirus, Tomato black ring nepovirus, Tomato
	ringspot nepovirus
Nucleorhabdoviruses:	Carrot latent nucleorhabdovirus, Coriander feathery red vein
Rhabdoviridae	nucleorhabdovirus, Cow parsnip mosaic nucleorhabdovirus,
	<i>Cynodon</i> chlorotic streak nucleorhabdovirus, <i>Datura</i> yellow vein
	nucleorhabdovirus, Eggplant mottled dwarf nucleorhabdovirus,
	Maize mosaic nucleorhabdovirus, <i>Pittosporum</i> vein yellowing
	nucleorhabdovirus, Potato yellow dwarf nucleorhabdovirus,
	<i>Sonchus</i> yellow net nucleorhabdovirus, Sowthistle yellow vein

	nucleorhabdovirus, Tomato vein clearing nucleorhabdovirus, Wheat
	American striate mosaic nucleorhabdovirus
Oryzaviruses:	<i>Echinochloa</i> ragged stunt oryzavirus, Rice ragged stunt oryzavirus
Reoviridae	
Ourmiaviruses	Cassava Ivorian bacilliform ourmiavirus, Epirus cherry
	ourmiavirus, Melon Ourmia ourmiavirus, <i>Pelargonium</i> zonate spot
	ourmiavirus
Phytoreoviruses:	Clover wound tumor phytoreovirus, Rice dwarf phytoreovirus, Rice
Reoviridae	gall dwarf phytoreovirus, Rice bunchy stunt phytoreovirus, Sweet
	potato phytoreovirus
Potexviruses	<i>Asparagus</i> 3 potexvirus, Cactus X potexvirus, Cassava X
	potexvirus, Chicory X potexvirus, Clover yellow mosaic
	potexvirus, <i>Commelina</i> X potexvirus, <i>Cymbidium</i> mosaic
	potexvirus, <i>Daphne</i> X potexvirus, Foxtail mosaic potexvirus,
	<i>Hydrangea</i> ringspot potexvirus, Lily X potexvirus, <i>Narcissus</i>
	mosaic potexvirus, <i>Nerine</i> X potexvirus, Papaya mosaic potexvirus,
	Pepino mosaic potexvirus, <i>Plantago asiatica</i> mosaic potexvirus,
	Plantain X potexvirus, Potato <i>aucuba</i> mosaic potexvirus, Potato X
	potexvirus, Tulip X potexvirus, <i>Viola</i> mottle potexvirus, White
	clover mosaic potexvirus
Potyvirus:	<i>Alstroemeria</i> mosaic potyvirus, <i>Amaranthus</i> leaf mottle potyvirus,
Potyviridae	<i>Araujia</i> mosaic potyvirus, Arracacha Y potyvirus, Artichoke latent
	potyvirus, <i>Asparagus</i> 1 potyvirus, Banana bract mosaic potyvirus,
	Bean common mosaic necrosis potyvirus, Bean common mosaic
	potyvirus, Bean yellow mosaic potyvirus, Beet mosaic potyvirus,
	<i>Bidens</i> mosaic potyvirus, <i>Bidens</i> mottle potyvirus, Cardamom
	mosaic potyvirus, Carnation vein mottle potyvirus, Carrot thin leaf
	potyvirus, Cassava brown streak potyvirus, <i>Cassia</i> yellow spot
	potyvirus, Celery mosaic potyvirus, Chickpea bushy dwarf
	potyvirus, Chickpea distortion mosaic potyvirus, Clover yellow
	vein potyvirus, <i>Commelina diffusa</i> potyvirus, <i>Commelina</i> mosaic
	potyvirus, Cowpea green vein-banding potyvirus, Cowpea
	Moroccan aphid-borne mosaic potyvirus, Cowpea rugose mosaic
	potyvirus, <i>Crinum</i> mosaic potyvirus, <i>Daphne</i> Y potyvirus, Dasheen
	mosaic potyvirus, <i>Datura</i> Colombian potyvirus, <i>Datura</i> distortion
	mosaic potyvirus, <i>Datura</i> necrosis potyvirus, <i>Datura</i> shoestring
	potyvirus, <i>Dendrobium</i> mosaic potyvirus, <i>Desmodium</i> mosaic
	potyvirus, <i>Dioscorea alata</i> potyvirus, <i>Dioscorea</i> green banding
	mosaic potyvirus, Eggplant green mosaic potyvirus, <i>Euphorbia</i>

	ringspot potyvirus, <i>Freesia</i> mosaic potyvirus, Groundnut eyespot
	potyvirus, Guar symptomless potyvirus, Guinea grass mosaic
	potyvirus, <i>Helenium</i> Y potyvirus, Henbane mosaic potyvirus,
	<i>Hippeastrum</i> mosaic potyvirus, <i>Hyacinth</i> mosaic potyvirus, <i>Iris</i>
	<i>fulva</i> mosaic potyvirus, <i>Iris</i> mild mosaic potyvirus, <i>Iris</i> severe
	mosaic potyvirus, Johnsongrass mosaic potyvirus, <i>Kennedya</i> Y
	potyvirus, Leek yellow stripe potyvirus, Lettuce mosaic potyvirus,
	Lily mottle potyvirus, Maize dwarf mosaic potyvirus, <i>Malva</i> vein
	clearing potyvirus, Marigold mottle potyvirus, <i>Narcissus</i> yellow
	stripe potyvirus, <i>Nerine</i> potyvirus, Onion yellow dwarf potyvirus,
	<i>Ornithogalum</i> mosaic potyvirus, Papaya ringspot potyvirus, Parsnip
	mosaic potyvirus, <i>Passiflora</i> ringspot potyvirus, <i>Passiflora</i> South
	African potyvirus, Passionfruit woodiness potyvirus, Patchouli
	mosaic potyvirus, Pea mosaic potyvirus, Pea seed-borne mosaic
	potyvirus, Peanut green mosaic potyvirus, Peanut mottle potyvirus,
	Pepper Indian mottle potyvirus, Pepper mottle potyvirus, Pepper
	severe mosaic potyvirus, Pepper veinal mottle potyvirus, Plum pox
	potyvirus, Pokeweed mosaic potyvirus, Potato A potyvirus, Potato
	V potyvirus, Potato Y potyvirus, <i>Primula</i> mosaic potyvirus,
	<i>Ranunculus</i> mottle potyvirus, <i>Sorghum</i> mosaic potyvirus, Soybean
	mosaic potyvirus, Statice Y potyvirus, Sugarcane mosaic potyvirus,
	Sweet potato feathery mottle potyvirus, Sweet potato G potyvirus,
	Swordbean distortion mosaic potyvirus, Tamarillo mosaic
	potyvirus, <i>Telfairia</i> mosaic potyvirus, Tobacco etch potyvirus,
	Tobacco vein-banding mosaic potyvirus, Tobacco vein mottling
	potyvirus, Tobacco wilt potyvirus, Tomato Peru potyvirus,
	<i>Tradescantia-Zebrina</i> potyvirus, <i>Tropaeolum</i> 1 potyvirus,
	<i>Tropaeolum</i> 2 potyvirus, Tuberose potyvirus, Tulip band-breaking
	potyvirus, Tulip breaking potyvirus, Tulip chlorotic blotch
	potyvirus, Turnip mosaic potyvirus, <i>Ullucus</i> mosaic potyvirus,
	<i>Vallota</i> mosaic potyvirus, Vanilla mosaic potyvirus, Vanilla
	necrosis potyvirus, <i>Voandzeia</i> distortion mosaic potyvirus,
	Watermelon mosaic 1 potyvirus, Watermelon mosaic 2 potyvirus,
	Wild potato mosaic potyvirus, <i>Wisteria</i> vein mosaic potyvirus, Yam
	mosaic potyvirus, Zucchini yellow fleck potyvirus, Zucchini yellow
	mosaic potyvirus
Rymoviruses:	<i>Hordeum</i> mosaic rymovirus, Oat necrotic mottle
Potyviridae	
Agropyron mosaic	

rymovirus	rymovirus, Ryegrass mosaic rymovirus, Wheat streak mosaic
	rymovirus
Satellite RNAs	<i>Arabis</i> mosaic satellite RNA, Chicory yellow mottle satellite RNA,
	Cucumber mosaic satellite RNA, Grapevine fanleaf satellite RNA,
	Strawberry latent ringspot satellite RNA, Tobacco ringspot satellite
	RNA, Tomato black ring satellite RNA, Velvet tobacco mottle
	satellite RNA
Satelliviruses	Maize white line mosaic satellivirus, <i>Panicum</i> mosaic satellivirus,
	Tobacco mosaic satellivirus, Tobacco necrosis satellivirus
Sequiviruses:	Dandelion yellow mosaic sequivirus, Parsnip yellow fleck
Sequiviridae	sequivirus
Sobemoviruses	Bean southern mosaic sobemovirus, Blueberry shoestring
	sobemovirus, Cocksfoot mottle sobemovirus, Lucerne transient
	streak sobemovirus, Rice yellow mottle sobemovirus, <i>Rottboellia</i>
	yellow mottle sobemovirus, <i>Solanum nodiflorum</i> mottle
	sobemovirus, Sowbane mosaic sobemovirus, Subterranean clover
	mottle sobemovirus, Turnip rosette sobemovirus, Velvet tobacco
	mottle, sobemovirus
Tenuiviruses	Maize stripe tenuivirus, Rice grassy stunt tenuivirus, Rice hoja
	blanca tenuivirus, Rice stripe tenuivirus
Tobamoviruses	Cucumber green mottle mosaic tobamovirus, Frangipani mosaic
	tobamovirus, Kyuri green mottle mosaic tobamovirus,
	<i>Odontoglossum</i> ringspot tobamovirus, Paprika mild mottle
	tobamovirus, Pepper mild mottle tobamovirus, Ribgrass mosaic
	tobamovirus, <i>Opuntia</i> Sammons' tobamovirus, Sunn-hemp mosaic
	tobamovirus, Tobacco mild green mosaic tobamovirus, Tobacco
	mosaic tobamovirus, Tomato mosaic tobamovirus, <i>Ullucus</i> mild
	mottle tobamovirus
Tobraviruses	Pea early browning tobravirus, Pepper ringspot tobravirus, Tobacco
	rattle tobravirus
Tombusviruses:	Artichoke mottled crinkle tombusvirus, Carnation Italian ringspot
Tombusviridae	tombusvirus, Cucumber necrosis tombusvirus, <i>Cymbidium</i> ringspot
	tombusvirus, Eggplant mottled crinkle tombusvirus, Grapevine
	Algerian latent tombusvirus, Lato River tombusvirus, Neckar River
	tombusvirus, <i>Pelargonium</i> leaf curl tombusvirus, Pepper Moroccan
	tombusvirus, <i>Petunia</i> asteroid mosaic tombusvirus, Tomato bushy
	stunt tombusvirus
Tospoviruses:	<i>Impatiens</i> necrotic spot tospovirus, Peanut yellow spot tospovirus,
Bunyaviridae	Tomato spotted wilt tospovirus

Trichoviruses	Apple chlorotic leaf spot trichovirus, <i>Heracleum</i> latent trichovirus,
	Potato T trichovirus
Tymoviruses	<i>Abelia</i> latent tymovirus, <i>Belladonna</i> mottle tymovirus, <i>Cacao</i>
	yellow mosaic tymovirus, <i>Clitoria</i> yellow vein tymovirus,
	<i>Desmodium</i> yellow mottle tymovirus, <i>Dulcamara</i> mottle tymovirus,
	Eggplant mosaic tymovirus, <i>Erysimum</i> latent tymovirus, <i>Kennedya</i>
	yellow mosaic tymovirus, Melon rugose mosaic tymovirus, Okra
	mosaic tymovirus, <i>Ononis</i> yellow mosaic tymovirus, Passionfruit
	yellow mosaic tymovirus, <i>Physalis</i> mosaic tymovirus, <i>Plantago</i>
	mottle tymovirus, Potato Andean latent tymovirus, <i>Scrophularia</i>
	mottle tymovirus, Turnip yellow mosaic, tymovirus, <i>Voandzeia</i>
	necrotic mosaic tymovirus, Wild cucumber mosaic tymovirus
Umbraviruses	Bean yellow vein banding umbravirus, Carrot mottle mimic
	umbravirus, Carrot mottle umbravirus, Carrot mottle mimic
	umbravirus, Groundnut rosette umbravirus, Lettuce speckles mottle
	umbravirus, Tobacco mottle umbravirus
Varicosaviruses	<i>Freesia</i> leaf necrosis varicosavirus, Lettuce big-vein varicosavirus,
	Tobacco stunt varicosavirus
Waikaviruses:	<i>Anthriscus</i> yellows waikavirus, Maize chlorotic dwarf waikavirus,
Sequiviridae	Rice tungro spherical waikavirus
Putative	Alsike clover vein mosaic virus, <i>Alstroemeria</i> streak potyvirus,
Ungrouped	<i>Amaranthus</i> mosaic potyvirus, Amazon lily mosaic potyvirus,
Viruses	<i>Anthoxanthum</i> mosaic potyvirus, Apple stem pitting virus,
	<i>Aquilegia</i> potyvirus, <i>Asclepias</i> rhabdovirus, <i>Atropa belladonna</i>
	rhabdovirus, Barley mosaic virus, Barley yellow streak mosaic
	virus, Beet distortion mosaic virus, Beet leaf curl rhabdovirus, Beet
	western yellows ST9-associated RNA virus, Black raspberry
	necrosis virus, Bramble yellow mosaic potyvirus, Brinjal mild
	mosaic potyvirus, Broad bean B virus, Broad bean V potyvirus,
	Broad bean yellow ringspot virus, <i>Bryonia</i> mottle potyvirus,
	Burdock mosaic virus, Burdock mottle virus, <i>Callistephus chinensis</i>
	chlorosis rhabdovirus, Canary reed mosaic potyvirus, <i>Canavalia</i>
	<i>maritima</i> mosaic potyvirus, Carnation rhabdovirus, Carrot mosaic
	potyvirus, Cassava symptomless rhabdovirus, <i>Cassia</i> mosaic virus,
	<i>Cassia</i> ringspot virus, Celery yellow mosaic potyvirus, Celery
	yellow net virus, Cereal flame chlorosis virus, Chickpea filiform
	potyvirus, Chilli veinal mottle potyvirus, <i>Chrysanthemum</i> spot
	potyvirus, <i>Chrysanthemum</i> vein chlorosis rhabdovirus, <i>Citrus</i>
	leprosis rhabdovirus, <i>Citrus</i> ringspot virus, Clover mild mosaic

	virus, Cocksfoot streak potyvirus, <i>Colocasia</i> bobone disease
	rhabdovirus, Cucumber toad-skin rhabdovirus, Cucumber vein
	yellowing virus, <i>Cypripedium calceolus</i> potyvirus, <i>Datura innoxia</i>
	Hungarian mosaic potyvirus, <i>Dioscorea trifida</i> potyvirus, Dock
	mottling mosaic potyvirus, <i>Dodonaea</i> yellows-associated virus,
	Eggplant severe mottle potyvirus, <i>Euonymus</i> fasciation
	rhabdovirus, <i>Euonymus</i> rhabdovirus, Fern potyvirus, Fig potyvirus,
	<i>Gerbera</i> symptomless rhabdovirus, Grapevine fleck virus,
	Grapevine stunt virus, Guar top necrosis virus, <i>Habenaria</i> mosaic
	potyvirus, <i>Holcus lanatus</i> yellowing rhabdovirus, <i>Holcus</i> streak
	potyvirus, <i>Iris germanica</i> leaf stripe rhabdovirus, <i>Iris</i> Japanese
	necrotic ring virus, <i>Isachne</i> mosaic potyvirus, <i>Kalanchoe</i> isometric
	virus, Kenaf vein-clearing rhabdovirus, <i>Launaea</i> mosaic potyvirus,
	Lupin yellow vein rhabdovirus, Maize eyespot virus, Maize line
	virus, Maize mottle/chlorotic stunt virus, Maize white line mosaic
	virus, <i>Malvastrum</i> mottle virus, <i>Melilotus</i> mosaic potyvirus, Melon
	vein-banding mosaic potyvirus, <i>Melothria</i> mottle potyvirus,
	<i>Mimosa</i> mosaic virus, Mung bean mottle potyvirus, <i>Narcissus</i>
	degeneration potyvirus, <i>Narcissus</i> late season yellows potyvirus,
	<i>Nerine</i> Y potyvirus, <i>Nothoscordum</i> mosaic potyvirus, Oak ringspot
	virus, Orchid fleck rhabdovirus, Palm mosaic potyvirus, Parsley
	green mottle potyvirus, Parsley rhabdovirus, Parsnip leafcurl virus,
	Passionfruit Sri Lankan mottle potyvirus, Passionfruit vein-clearing
	rhabdovirus, Patchouli mottle rhabdovirus, Pea stem necrosis virus,
	Peanut top paralysis potyvirus, Peanut veinal chlorosis rhabdovirus,
	<i>Pecteilis</i> mosaic potyvirus, Pepper mild mosaic potyvirus, <i>Perilla</i>
	mottle potyvirus, Pigeonpea proliferation rhabdovirus, Pigeonpea
	sterility mosaic virus, Plantain 7 potyvirus, Plantain mottle
	rhabdovirus, <i>Pleioblastus</i> chino potyvirus, Poplar decline potyvirus,
	<i>Primula</i> mottle potyvirus, Purple granadilla mosaic virus,
	<i>Ranunculus repens</i> symptomless rhabdovirus, Rice yellow stunt
	virus, <i>Saintpaulia</i> leaf necrosis rhabdovirus, <i>Sambucus</i> vein
	clearing rhabdovirus, <i>Sarracenia purpurea</i> rhabdovirus, Shamrock
	chlorotic ringspot potyvirus, Soybean mild mosaic virus, Soybean
	rhabdovirus, Soybean spherical virus, Soybean yellow vein virus,
	Soybean Z potyvirus, Strawberry latent C rhabdovirus, Strawberry
	mottle virus, Strawberry pallidosis virus, Sunflower mosaic
	potyvirus, Sweet potato latent potyvirus, Teasel mosaic potyvirus,
	Thimbleberry ringspot virus, Tomato mild mottle potyvirus,

	<i>Trichosanthes</i> mottle potyvirus, Tulip halo necrosis virus, Tulip
	mosaic virus, Turnip vein-clearing virus, Urd bean leaf crinkle
	virus, <i>Vigna sinensis</i> mosaic rhabdovirus, Watercress yellow spot
	virus, Watermelon Moroccan mosaic potyvirus, Wheat chlorotic
	spot rhabdovirus, White bryony potyvirus, Wineberry latent virus,
	<i>Zinnia</i> mild mottle potyvirus, <i>Zoysia</i> mosaic potyvirus

C. Delivery to a Plant Symbiont

Provided herein are methods of delivering to a plant symbiont a PMP composition (e.g., including modified PMPs described herein) disclosed herein. Included are methods for delivering a PMP composition to a symbiont (e.g., a bacterial endosymbiont, a fungal endosymbiont, or an insect) by contacting the symbiont with a PMP composition. The methods can be useful for increasing the fitness of plant symbiont, e.g., a symbiont that is beneficial to the fitness of a plant. In some instances, plant symbionts may be treated with PMPs not including a heterologous functional agent. In other instances, the PMPs include a heterologous functional agent, e.g., fertilizing agents.

As such, the methods can be used to increase the fitness of a plant symbiont. In one aspect, provided herein is a method of increasing the fitness of a symbiont, the method including delivering to the symbiont the PMP composition described herein (e.g., in an effective amount and for an effective duration) to increase the fitness of the symbiont relative to an untreated symbiont (e.g., a symbiont that has not been delivered the PMP composition).

In one aspect, provided herein is a method of increasing the fitness of a fungus (e.g., a fungal endosymbiont of a plant), wherein the method includes delivering to the endosymbiont a PMP composition including a plurality of PMPs (e.g., a PMP composition described herein). For example, the plant symbiont may be an endosymbiotic fungus, such as a fungus of the genus *Aspergillaceae*, *Ceratobasidiaceae*, *Coniochaetaceae*, *Cordycipitaceae*, *Corticaceae*, *Cystofilobasidiaceae*, *Davidiellaceae*, *Debaryomycetaceae*, *Dothioraceae*, *Erysiphaceae*, *Filobasidiaceae*, *Glomerellaceae*, *Hydnaceae*, *Hypocreaceae*, *Leptosphaeriaceae*, *Montagnulaceae*, *Mortierellaceae*, *Mycosphaerellaceae*, *Nectriaceae*, *Orbiliaceae*, *Phaeosphaeriaceae*, *Pleosporaceae*, *Pseudeurotiaceae*, *Rhizopodaceae*, *Sclerotiniaceae*, *Stereaceae*, or *Trichocomaceae*.

In another aspect, provided herein is a method of increasing the fitness of a bacterium (e.g., a bacterial endosymbiont of a plant), wherein the method includes delivering to the bacteria a PMP composition including a plurality of PMPs (e.g., a PMP composition described herein). For example, the plant symbiont may be an endosymbiotic bacteria, such as a bacteria of the genus *Acetobacteraceae*, *Acidobacteriaceae*, *Acidothermaceae*, *Aerococcaceae*, *Alcaligenaceae*, *Alicyclobacillaceae*, *Alteromonadaceae*, *Anaerolineaceae*, *Aurantimonadaceae*, *Bacillaceae*, *Bacteriovoraceae*, *Bdellovibrionaceae*, *Bradyrhizobiaceae*, *Brevibacteriaceae*, *Brucellaceae*, *Burkholderiaceae*, *Carboxydooellaceae*, *Caulobacteraceae*, *Cellulomonadaceae*, *Chitinophagaceae*, *Chromatiaceae*, *Chthoniobacteraceae*, *Chthonomonadaceae*, *Clostridiaceae*, *Comamonadaceae*, *Corynebacteriaceae*, *Coxiellaceae*, *Cryomorphaceae*, *Cyclobacteriaceae*, *Cytophagaceae*, *Deinococcaceae*, *Dermabacteraceae*, *Dermacoccaceae*, *Enterobacteriaceae*, *Enterococcaceae*, *Erythrobacteraceae*,

- Fibrobacteraceae, Flammeovirgaceae, Flavobacteriaceae, Frankiaceae, Fusobacteriaceae, Gaiellaceae, Gemmatimonadaceae, Geodermatophilaceae, Gly corny cetaceae, Haliangiaceae, Halomonadaceae, Holosporaceae, Hyphomicrobiaceae, Iamiaceae, Intraporangiaceae, Kineosporiaceae, Koribacteraceae, Lachnospiraceae, Lactobacillaceae, Legionellaceae, Leptospiraceae, Leuconostocaceae,*
- 5 *Methylobacteriaceae, Methylocystaceae, Methylophilaceae, Microbacteriaceae, Micrococcaceae, Micromonosporaceae, Moraxellaceae, Mycobacteriaceae, Mycoplasmataceae, Myxococcaceae, Nakamurellaceae, Neisseriaceae, Nitrosomonadaceae, Nocardaceae, Nocardoidaceae, Oceanospirillaceae, Opatutaceae, Oxalobacteraceae, Paenibacillaceae, Parachlamydiaceae, Pasteurellaceae, Patulibacteraceae, Peptostreptococcaceae, Phyllobacteriaceae, Piscirickettsiaceae,*
- 10 *Planctomycetaceae, Planococcaceae, Polyangiaceae, Porphyromonadaceae, Prevotellaceae, Promicromonosporaceae, Pseudomonadaceae, Pseudonocardiaceae, Rhizobiaceae, Rhodobacteraceae, Rhodospirillaceae, Roseiflexaceae, Rubrobacteriaceae, Sandaracinaceae, Sanguibacteraceae, Saprospiraceae, Segniliparaceae, Shewanellaceae, Sinobacteraceae, Solibacteraceae, Solimonadaceae, Solirubrobacteraceae, Sphingobacteriaceae, Sphingomonadaceae, Spiroplasmataceae,*
- 15 *Sporichthyaceae, Sporolactobacillaceae, Staphylococcaceae, Streptococcaceae, Streptomycetaceae, Syntrophobacteraceae, Veillonellaceae, Verrucomicrobiaceae, Weeksellaceae, Xanthobacteraceae, or Xanthomonadaceae.*

In yet another aspect, provided herein is a method of increasing the fitness of an insect (e.g., an insect symbiont of a plant), wherein the method includes delivering to the insect a PMP composition

20 including a plurality of PMPs (e.g., a PMP composition described herein). In some instances, the insect is a plant pollinator. For example, the insect may be of the genus Hymenoptera or Diptera. In some instances, the insect of the genus Hymenoptera is a bee. In other instances, the insect of the genus Diptera is a fly.

In some instances, the increase in symbiont fitness may manifest as an improvement in the

25 physiology of the symbiont (e.g., improved health or survival) as a consequence of administration of the PMP composition. In some instances, the fitness of an organism may be measured by one or more parameters, including, but not limited to, reproductive rate, lifespan, mobility, fecundity, body weight, metabolic rate or activity, or survival in comparison to a symbiont to which the PMP composition has not been delivered. For example, the methods or compositions provided herein may be effective to improve

30 the overall health of the symbiont or to improve the overall survival of the symbiont in comparison to a symbiont organism to which the PMP composition has not been administered. In some instances, the improved survival of the symbiont is about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or greater than 100% greater relative to a reference level (e.g., a level found in a symbiont that does not receive a PMP composition). In some instances, the methods and compositions are effective to

35 increase symbiont reproduction (e.g., reproductive rate) in comparison to a symbiont organism to which the PMP composition has not been administered. In some instances, the methods and compositions are effective to increase other physiological parameters, such as mobility, body weight, life span, fecundity, or metabolic rate, by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or greater than 100% relative to a reference level (e.g., a level found in a symbiont that does not receive a PMP

40 composition).

In some instances, the increase in symbiont fitness may manifest as an increase in the frequency or efficacy of a desired activity carried out by the symbiont (e.g., pollination, predation on pests, seed spreading, or breakdown of waste or organic material) in comparison to a symbiont organism to which the PMP composition has not been administered. In some instances, the methods or compositions provided
5 herein may be effective to increase the frequency or efficacy of a desired activity carried out by the symbiont (e.g., pollination, predation on pests, seed spreading, or breakdown of waste or organic material) by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or greater than 100% relative to a reference level (e.g., a level found in a symbiont that does not receive a PMP composition).

10 In some instances, the increase in symbiont fitness may manifest as an increase in the production of one or more nutrients in the symbiont (e.g., vitamins, carbohydrates, amino acids, or polypeptides) in comparison to a symbiont organism to which the PMP composition has not been administered. In some instances, the methods or compositions provided herein may be effective to increase the production of nutrients in the symbiont (e.g., vitamins, carbohydrates, amino acids, or
15 polypeptides) by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or greater than 100% relative to a reference level (e.g., a level found in a symbiont that does not receive a PMP composition). In some instances, the methods or compositions provided herein may increase nutrients in an associated plant by increasing the production or metabolism of nutrients by one or more microorganisms (e.g., endosymbiont) in the symbiont.

20 In some instances, the increase in symbiont fitness may manifest as a decrease in the symbiont's sensitivity to a pesticidal agent and/or an increase in the symbiont's resistance to a pesticidal agent in comparison to a symbiont organism to which the PMP composition has not been administered. In some instances, the methods or compositions provided herein may be effective to decrease the symbiont's sensitivity to a pesticidal agent by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%,
25 100%, or greater than 100% relative to a reference level (e.g., a level found in a symbiont that does not receive a PMP composition).

In some instances, the increase in symbiont fitness may manifest as a decrease in the symbiont's sensitivity to an allelochemical agent and/or an increase in the symbiont's resistance to an allelochemical agent in comparison to a symbiont organism to which the PMP composition has not been administered.
30 In some instances, the methods or compositions provided herein may be effective to increase the symbiont's resistance to an allelochemical agent by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or greater than 100% relative to a reference level (e.g., a level found in a symbiont that does not receive a PMP composition). In some instances, the allelochemical agent is caffeine, soyacystatin N, monoterpenes, diterpene acids, or phenolic compounds. In some instances, the
35 methods or compositions provided herein may decrease the symbiont's sensitivity to an allelochemical agent by increasing the symbiont's ability to metabolize or degrade the allelochemical agent into usable substrates.

In some instances, the methods or compositions provided herein may be effective to increase the symbiont's resistance to parasites or pathogens (e.g., fungal, bacterial, or viral pathogens; or parasitic
40 mites (e.g., Varroa destructor mite in honeybees)) in comparison to a symbiont organism to which the

PMP composition has not been administered. In some instances, the methods or compositions provided herein may be effective to increase the symbiont's resistance to a pathogen or parasite (e.g., fungal, bacterial, or viral pathogens; or parasitic mites (e.g., *Varroa destructor* mite in honeybees)) by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or greater than 100% relative to a
 5 reference level (e.g., a level found in a symbiont that does not receive a PMP composition).

In some instances, the increase in symbiont fitness may manifest as other fitness advantages, such as improved tolerance to certain environmental factors (e.g., a high or low temperature tolerance), improved ability to survive in certain habitats, or an improved ability to sustain a certain diet (e.g., an improved ability to metabolize soy vs corn) in comparison to a symbiont organism to which the PMP
 10 composition has not been administered. In some instances, the methods or compositions provided herein may be effective to increase symbiont fitness in any plurality of ways described herein. Further, the PMP composition may increase symbiont fitness in any number of symbiont classes, orders, families, genera, or species (e.g., 1 symbiont species, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 500, or more symbiont species). In some instances, the PMP composition acts
 15 on a single symbiont class, order, family, genus, or species.

Symbiont fitness may be evaluated using any standard methods in the art. In some instances, symbiont fitness may be evaluated by assessing an individual symbiont. Alternatively, symbiont fitness may be evaluated by assessing a symbiont population. For example, an increase in symbiont fitness may manifest as an increase in successful competition against other insects, thereby leading to an increase in
 20 the size of the symbiont population.

Examples of plant symbionts that can be treated with the present compositions or related methods are further described herein.

i. Fungi

25 The PMP compositions and related methods can be useful for increasing the fitness of a fungus, e.g., a fungus that is an endosymbiont of a plant (e.g., mycorrhizal fungus).

In some instances, the fungus is of the family *Aspergillaceae*, *Ceratobasidiaceae*, *Coniochaetaceae*, *Cordycipitaceae*, *Corticaceae*, *Cystofilobasidiaceae*, *Davidiellaceae*, *Debaryomycetaceae*, *Dothioraceae*, *Erysiphaceae*, *Filobasidiaceae*, *Glomerellaceae*, *Hydnaceae*,
 30 *Hypocreaceae*, *Leptosphaeriaceae*, *Montagnulaceae*, *Mortierellaceae*, *Mycosphaerellaceae*, *Nectriaceae*, *Orbiliaceae*, *Phaeosphaeriaceae*, *Pleosporaceae*, *Pseudeurotiaceae*, *Rhizopodaceae*, *Sclerotiniaceae*, *Stereaceae*, or *Trichocomaceae*.

In some instances, the fungus is a fungus having a mycorrhizal (e.g., ectomycorrhizal or endomycorrhizal) association with the roots of a plant, including fungi belonging to Glomeromycota, Basidiomycota, Ascomycota, or Zygomycota.
 35

ii. Bacteria

The PMP compositions and related methods can be useful for increasing the fitness of a bacterium, e.g., a bacterium that is an endosymbiont of a plant (e.g., nitrogen-fixing bacteria).

40 For example, the bacterium may be of the genus *Acidovorax*, *Agrobacterium*, *Bacillus*, *Burkholderia*, *Chryseobacterium*, *Curtobacterium*, *Enterobacter*, *Escherichia*, *Methylobacterium*,

Paenibacillus, Pantoea, Pseudomonas, Ralstonia, Rhizobium, Saccharibacillus, Sphingomonas, or Stenotrophomonas.

- In some instances, the bacteria is of the family : *Acetobacteraceae*, *Acidobacteriaceae*, *Acidothermaceae*, *Aerococcaceae*, *Alcaligenaceae*, *Alicyclobacillaceae*, *Alteromonadaceae*,
 5 *Anaerolineaceae*, *Aurantimonadaceae*, *Bacillaceae*, *Bacteriovoracaceae*, *Bdellovibrionaceae*,
Bradyrhizobiaceae, *Brevibacteriaceae*, *Brucellaceae*, *Burkholderiaceae*, *Carboxydocellaceae*,
Caulobacteraceae, *Cellulomonadaceae*, *Chitinophagaceae*, *Chromatiaceae*, *Chthoniobacteraceae*,
Chthonomonadaceae, *Clostridiaceae*, *Comamonadaceae*, *Corynebacteriaceae*, *Coxiellaceae*,
Cryomorphaceae, *Cyclobacteriaceae*, *Cytophagaceae*, *Deinococcaceae*, *Dermabacteraceae*,
 10 *Dermacoccaceae*, *Enterobacteriaceae*, *Enterococcaceae*, *Erythrobacteraceae*, *Fibrobacteraceae*,
Flammeovirgaceae, *Flavobacteriaceae*, *Frankiaceae*, *Fusobacteriaceae*, *Gaiellaceae*,
Gemmatimonadaceae, *Geodermatophilaceae*, *Gly corny cetaceae*, *Haliangiaceae*, *Halomonadaceae*,
Holospiraceae, *Hyphomicrobiaceae*, *Iamiaceae*, *Intrasporangiaceae*, *Kineosporiaceae*, *Koribacteraceae*,
Lachnospiraceae, *Lactobacillaceae*, *Legionellaceae*, *Leptospiraceae*, *Leuconostocaceae*,
 15 *Methylobacteriaceae*, *Methylocystaceae*, *Methylophilaceae*, *Microbacteriaceae*, *Micrococcaceae*,
Micromonosporaceae, *Moraxellaceae*, *Mycobacteriaceae*, *Mycoplasmataceae*, *Myxococcaceae*,
Nakamurellaceae, *Neisseriaceae*, *Nitrosomonadaceae*, *Nocardiaceae*, *Nocardiodaceae*,
Oceanospirillaceae, *Opitutaceae*, *Oxalobacteraceae*, *Paenibacillaceae*, *Parachlamydiaceae*,
Pasteurellaceae, *Patulibacteraceae*, *Peptostreptococcaceae*, *Phyllobacteriaceae*, *Piscirickettsiaceae*,
 20 *Planctomycetaceae*, *Planococcaceae*, *Polyangiaceae*, *Porphyromonadaceae*, *Prevotellaceae*,
Promicromonosporaceae, *Pseudomonadaceae*, *Pseudonocardiaceae*, *Rhizobiaceae*, *Rhodobacteraceae*,
Rhodospirillaceae, *Roseiflexaceae*, *Rubrobacteriaceae*, *Sandaracinaceae*, *Sanguibacteraceae*,
Saprospiraceae, *Segniliparaceae*, *Shewanellaceae*, *Sinobacteraceae*, *Solibacteraceae*, *Solimonadaceae*,
Solirubrobacteraceae, *Sphingobacteriaceae*, *Sphingomonadaceae*, *Spiroplasmataceae*,
 25 *Sporichthyaceae*, *Sporolactobacillaceae*, *Staphylococcaceae*, *Streptococcaceae*, *Streptomycetaceae*,
Syntrophobacteraceae, *Veillonellaceae*, *Verrucomicrobiaceae*, *Weeksellaceae*, *Xanthobacteraceae*, or
Xanthomonadaceae.

- In some instances, the endosymbiotic bacterium is of a family selected from the group consisting of: *Bacillaceae*, *Burkholderiaceae*, *Comamonadaceae*, *Enterobacteriaceae*, *Flavobacteriaceae*,
 30 *Methylobacteriaceae*, *Microbacteriaceae*, *Paenibacillaceae*, *Pseudomonaceae*, *Rhizobiaceae*,
Sphingomonadaceae, and *Xanthomonadaceae*.

- In some instances, the endosymbiotic bacterium is of a genus selected from the group consisting of: *Acidovorax*, *Agrobacterium*, *Bacillus*, *Burkholderia*, *Chryseobacterium*, *Curtobacterium*, *Enterobacter*,
Escherichia, *Methylobacterium*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Ralstonia*,
 35 *Saccharibacillus*, *Sphingomonas*, and *Stenotrophomonas*.

iii. Insects

- The PMP compositions and related methods can be useful for increasing the fitness of an insect, e.g., an insect that is beneficial to plant. The term insect includes any organism belonging to the phylum
 40 Arthropoda and to the class Insecta or the class Arachnida, in any stage of development, i.e., immature

and adult insects. For example, the host may include insects that are used in agricultural applications, including insects that aid in the pollination of crops, spreading seeds, or pest control.

In some instances, the host aids in pollination of a plant (e.g., bees, beetles, wasps, flies, butterflies, or moths). In some instances, the host aiding in pollination of a plant is a bee. In some instances, the bee is in the family Andrenidae, Apidae, Colletidae, Halictidae, or Megachilidae. In some examples, the host aiding in pollination of a plant is beetle. In particular instances, the PMP composition may be used to increase the fitness of a honeybee.

In some instances, the host aiding in pollination of a plant is a beetle, e.g., a species in the family Buprestidae, Cantharidae, Cerambycidae, Chrysomelidae, Cleridae, Coccinellidae, Elateridae, Melandryidae, Meloidae, Melyridae, Mordellidae, Nitidulidae, Oedemeridae, Scarabaeidae, or Staphylinidae.

In some instances, the host aiding in pollination of a plant is a butterfly or moth (e.g., Lepidoptera). In some instances, the butterfly or moth is a species in the family Geometridae, Hesperidae, Lycaenidae, Noctuidae, Nymphalidae, Papilionidae, Pieridae, or Sphingidae.

In some instances, the host aiding in pollination of a plant is a fly (e.g., Diptera). In some instances, the fly is in the family Anthomyiidae, Bibionidae, Bombyliidae, Calliphoridae, Cecidomyiidae, Certopogonidae, Chironomidae, Conopidae, Culicidae, Dolichopodidae, Empididae, Ephydriidae, Lonchopteridae, Muscidae, Mycetophilidae, Phoridae, Simuliidae, Stratiomyidae, or Syrphidae.

In some instances, the host aiding in pollination is an ant (e.g., Formicidae), sawfly (e.g., Tenthredinidae), or wasp (e.g., Sphecidae or Vespidae).

D. Delivery to an Animal Pathogen

Provided herein are methods of delivering a PMP composition (e.g., including modified PMPs described herein) to an animal (e.g., human) pathogen, such as one disclosed herein, by contacting the pathogen with a PMP composition. As used herein the term "pathogen" refers to an organism, such as a microorganism or an invertebrate, which causes disease or disease symptoms in an animal by, e.g., (i) directly infecting the animal, (ii) by producing agents that causes disease or disease symptoms in an animal (e.g., bacteria that produce pathogenic toxins and the like), and/or (iii) that elicit an immune (e.g., inflammatory response) in animals (e.g., biting insects, e.g., bedbugs). As used herein, pathogens include, but are not limited to bacteria, protozoa, parasites, fungi, nematodes, insects, viroids and viruses, or any combination thereof, wherein each pathogen is capable, either by itself or in concert with another pathogen, of eliciting disease or symptoms in animals, such as humans.

In some instances, animal (e.g., human) pathogen may be treated with PMPs not including a heterologous functional agent. In other instances, the PMPs include a heterologous functional agent, e.g., a heterologous therapeutic agent (e.g., antibacterial agent, antifungal agent, insecticide, nematicide, antiparasitic agent, antiviral agent, or a repellent). The methods can be useful for decreasing the fitness of an animal pathogen, e.g., to prevent or treat a pathogen infection or control the spread of a pathogen as a consequence of delivery of the PMP composition.

Examples of pathogens that can be targeted in accordance with the methods described herein include bacteria (e.g., *Streptococcus* spp., *Pneumococcus* spp., *Pseudomonas* spp., *Shigella* spp.,

Salmonella spp., *Campylobacter* spp., or an *Escherichia* spp), fungi (*Saccharomyces* spp. or a *Candida* spp), parasitic insects (e.g., *Cimex* spp), parasitic nematodes (e.g., *Heligmosomoides* spp), or parasitic protozoa (e.g., *Trichomoniasis* spp).

For example, provided herein is a method of decreasing the fitness of a pathogen, the method including delivering to the pathogen a PMP composition described herein, wherein the method decreases the fitness of the pathogen relative to an untreated pathogen. In some embodiments, the method includes delivering the composition to at least one habitat where the pathogen grows, lives, reproduces, feeds, or infests. In some instances of the methods described herein, the composition is delivered as a pathogen comestible composition for ingestion by the pathogen. In some instances of the methods described herein, the composition is delivered (e.g., to a pathogen) as a liquid, a solid, an aerosol, a paste, a gel, or a gas.

Also provided herein is a method of decreasing the fitness of a parasitic insect, wherein the method includes delivering to the parasitic insect a PMP composition including a plurality of PMPs. In some instances, the method includes delivering to the parasitic insect a PMP composition including a plurality of PMPs, wherein the plurality of PMPs includes an insecticidal agent. For example, the parasitic insect may be a bedbug. Other non-limiting examples of parasitic insects are provided herein. In some instances, the method decreases the fitness of the parasitic insect relative to an untreated parasitic insect

Additionally provided herein is a method of decreasing the fitness of a parasitic nematode, wherein the method includes delivering to the parasitic nematode a PMP composition including a plurality of PMPs. In some instances, the method includes delivering to the parasitic nematode a PMP composition including a plurality of PMPs, wherein the plurality of PMPs includes a nematicidal agent. For example, the parasitic nematode is *Heligmosomoides polygyrus*. Other non-limiting examples of parasitic nematodes are provided herein. In some instances, the method decreases the fitness of the parasitic nematode relative to an untreated parasitic nematode.

Further provided herein is a method of decreasing the fitness of a parasitic protozoan, wherein the method includes delivering to the parasitic protozoan a PMP composition including a plurality of PMPs. In some instances, the method includes delivering to the parasitic protozoan a PMP composition including a plurality of PMPs, wherein the plurality of PMPs includes an antiparasitic agent. For example, the parasitic protozoan may be *T. vaginalis*. Other non-limiting examples of parasitic protozoans are provided herein. In some instances, the method decreases the fitness of the parasitic protozoan relative to an untreated parasitic protozoan.

A decrease in the fitness of the pathogen as a consequence of delivery of a PMP composition can manifest in a number of ways. In some instances, the decrease in fitness of the pathogen may manifest as a deterioration or decline in the physiology of the pathogen (e.g., reduced health or survival) as a consequence of delivery of the PMP composition. In some instances, the fitness of an organism may be measured by one or more parameters, including, but not limited to, reproductive rate, fertility, lifespan, viability, mobility, fecundity, pathogen development, body weight, metabolic rate or activity, or survival in comparison to a pathogen to which the PMP composition has not been administered. For example, the methods or compositions provided herein may be effective to decrease the overall health of the pathogen or to decrease the overall survival of the pathogen. In some instances, the decreased

survival of the pathogen is about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or greater than 100% greater relative to a reference level (e.g., a level found in a pathogen that does not receive a PMP composition. In some instances, the methods and compositions are effective to decrease pathogen reproduction (e.g., reproductive rate, fertility) in comparison to a pathogen to which the PMP composition has not been administered. In some instances, the methods and compositions are effective to decrease other physiological parameters, such as mobility, body weight, life span, fecundity, or metabolic rate, by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or greater than 100% relative to a reference level (e.g., a level found in a pathogen that does not receive a PMP composition).

In some instances, the decrease in pest fitness may manifest as an increase in the pathogen's sensitivity to an antipathogen agent and/or a decrease in the pathogen's resistance to an antipathogen agent in comparison to a pathogen to which the PMP composition has not been delivered. In some instances, the methods or compositions provided herein may be effective to increase the pathogen's sensitivity to a pesticidal agent by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or greater than 100% relative to a reference level (e.g., a level found in a pest that does not receive a PMP composition).

In some instances, the decrease in pathogen fitness may manifest as other fitness disadvantages, such as a decreased tolerance to certain environmental factors (e.g., a high or low temperature tolerance), a decreased ability to survive in certain habitats, or a decreased ability to sustain a certain diet in comparison to a pathogen to which the PMP composition has not been delivered. In some instances, the methods or compositions provided herein may be effective to decrease pathogen fitness in any plurality of ways described herein. Further, the PMP composition may decrease pathogen fitness in any number of pathogen classes, orders, families, genera, or species (e.g., 1 pathogen species, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 500, or more pathogen species). In some instances, the PMP composition acts on a single pest class, order, family, genus, or species.

Pathogen fitness may be evaluated using any standard methods in the art. In some instances, pest fitness may be evaluated by assessing an individual pathogen. Alternatively, pest fitness may be evaluated by assessing a pathogen population. For example, a decrease in pathogen fitness may manifest as a decrease in successful competition against other pathogens, thereby leading to a decrease in the size of the pathogen population.

The PMP compositions and related methods described herein are useful to decrease the fitness of an animal pathogen and thereby treat or prevent infections in animals. Examples of animal pathogens, or vectors thereof, that can be treated with the present compositions or related methods are further described herein.

i. Fungi

The PMP compositions and related methods can be useful for decreasing the fitness of a fungus, e.g., to prevent or treat a fungal infection in an animal. Included are methods for delivering a PMP composition to a fungus by contacting the fungus with the PMP composition. Additionally or alternatively,

the methods include preventing or treating a fungal infection (e.g., caused by a fungus described herein) in an animal at risk of or in need thereof, by administering to the animal a PMP composition.

The PMP compositions and related methods are suitable for treatment or preventing of fungal infections in animals, including infections caused by fungi belonging to *Ascomycota* (*Fusarium oxysporum*, *Pneumocystis jirovecii*, *Aspergillus* spp., *Coccidioides immitis/posadasii*, *Candida albicans*),
 5 *Basidiomycota* (*Filobasidiella neoformans*, *Trichosporon*), *Microsporidia* (*Encephalitozoon cuniculi*, *Enterocytozoon bieneusi*), *Mucoromycotina* (*Mucor circinelloides*, *Rhizopus oryzae*, *Lichtheimia corymbifera*).

In some instances, the fungal infection is one caused by a belonging to the phylum *Ascomycota*,
 10 *Basidiomycota*, *Chytridiomycota*, *Microsporidia*, or *Zygomycota*. The fungal infection or overgrowth can include one or more fungal species, e.g., *Candida albicans*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, *C. auris*, *C. krusei*, *Saccharomyces cerevisiae*, *Malassezia globosa*, *M. restricta*, or *Debaryomyces hansenii*, *Gibberella moniliformis*, *Alternaria brassicicola*, *Cryptococcus neoformans*, *Pneumocystis carinii*, *P. jirovecii*, *P. murina*, *P. oryctolagi*, *P. wakefieldiae*, and *Aspergillus clavatus*. The fungal species may be
 15 considered a pathogen or an opportunistic pathogen.

In some instances, the fungal infection is caused by a fungus in the genus *Candida* (i.e., a *Candida* infection). For example, a *Candida* infection can be caused by a fungus in the genus *Candida* that is selected from the group consisting of *C. albicans*, *C. glabrata*, *C. dubliniensis*, *C. krusei*, *C. auris*, *C. parapsilosis*, *C. tropicalis*, *C. orthopsilosis*, *C. guilliermondii*, *C. rugosa*, and *C. lusitanae*. *Candida*
 20 infections that can be treated by the methods disclosed herein include, but are not limited to candidemia, oropharyngeal candidiasis, esophageal candidiasis, mucosal candidiasis, genital candidiasis, vulvovaginal candidiasis, rectal candidiasis, hepatic candidiasis, renal candidiasis, pulmonary candidiasis, splenic candidiasis, otomycosis, osteomyelitis, septic arthritis, cardiovascular candidiasis (e.g., endocarditis), and invasive candidiasis.

25 ii. Bacteria

The PMP compositions and related methods can be useful for decreasing the fitness of a bacterium, e.g., to prevent or treat a bacterial infection in an animal. Included are methods for administering a PMP composition to a bacterium by contacting the bacteria with the PMP composition.
 30 Additionally or alternatively, the methods include preventing or treating a bacterial infection (e.g., caused by a bacteria described herein) in an animal at risk of or in need thereof, by administering to the animal a PMP composition.

The PMP compositions and related methods are suitable for preventing or treating a bacterial infection in animals caused by any bacteria described further below. For example, the bacteria may be
 35 one belonging to *Bacillales* (*B. anthracis*, *B. cereus*, *S. aureus*, *L. monocytogenes*), *Lactobacillales* (*S. pneumoniae*, *S. pyogenes*), *Clostridiales* (*C. botulinum*, *C. difficile*, *C. perfringens*, *C. tetani*), *Spirochaetales* (*Borrelia burgdorferi*, *Treponema pallidum*), *Chlamydiales* (*Chlamydia trachomatis*, *Chlamydophila psittaci*), *Actinomycetales* (*C. diphtheriae*, *Mycobacterium tuberculosis*, *M. avium*), *Rickettsiales* (*R. prowazekii*, *R. rickettsii*, *R. typhi*, *A. phagocytophilum*, *E. chaffeensis*), *Rhizobiales*
 40 (*Brucella melitensis*), *Burkholderiales* (*Bordetella pertussis*, *Burkholderia mallei*, *B. pseudomallei*),

Neisseriales (*Neisseria gonorrhoeae*, *N. meningitidis*), *Campylobacterales* (*Campylobacter jejuni*, *Helicobacter pylori*), *Legionellales* (*Legionella pneumophila*), *Pseudomonadales* (*A. baumannii*, *Moraxella catarrhalis*, *P. aeruginosa*), *Aeromonadales* (*Aeromonas* sp.), *Vibrionales* (*Vibrio cholerae*, *V. parahaemolyticus*), *Thiotrichales*, *Pasteurellales* (*Haemophilus influenzae*), *Enterobacteriales* (*Klebsiella pneumoniae*, *Proteus mirabilis*, *Yersinia pestis*, *Y. enterocolitica*, *Shigella flexneri*, *Salmonella enterica*, *E. coli*).

iii. Parasitic Insects

The PMP compositions and related methods can be useful for decreasing the fitness of a parasitic insect, e.g., to prevent or treat a parasitic insect infection in an animal. The term "insect" includes any organism belonging to the phylum Arthropoda and to the class Insecta or the class Arachnida, in any stage of development, i.e., immature and adult insects. Included are methods for delivering a PMP composition to an insect by contacting the insect with the PMP composition. Additionally or alternatively, the methods include preventing or treating a parasitic insect infection (e.g., caused by a parasitic insect described herein) in an animal at risk of or in need thereof, by administering to the animal a PMP composition.

The PMP compositions and related methods are suitable for preventing or treating infection in animals by a parasitic insect, including infections by insects belonging to *Phthiraptera*: *Anoplura* (*Sucking lice*), *Ischnocera* (*Chewing lice*), *Amblycera* (*Chewing lice*). *Siphonaptera*: *Pulicidae* (*Cat fleas*), *Ceratophyllidae* (*Chicken-fleas*). *Diptera*: *Culicidae* (*Mosquitoes*), *Ceratopogonidae* (*Midges*), *Psychodidae* (*Sandflies*), *Simuliidae* (*Blackflies*), *Tabanidae* (*Horse-flies*), *Muscidae* (*House-flies, etc.*), *Calliphoridae* (*Blowflies*), *Glossinidae* (*Tsetse-flies*), *Oestridae* (*Bot-flies*), *Hippoboscidae* (*Louse-flies*). *Hemiptera*: *Reduviidae* (*Assassin-bugs*), *Cimicidae* (*Bed-bugs*). *Arachnida*: *Sarcoptidae* (*Sarcoptic mites*), *Psoroptidae* (*Psoroptic mites*), *Cytoditidae* (*Air-sac mites*), *Laminosioptes* (*Cyst-mites*), *Analgidae* (*Feather-mites*), *Acaridae* (*Grain-mites*), *Demodicidae* (*Hair-follicle mites*), *Cheyletiellidae* (*Fur-mites*), *Trombiculidae* (*Trombiculids*), *Dermanyssidae* (*Bird mites*), *Macronyssidae* (*Bird mites*), *Argasidae* (*Soft-ticks*), *Ixodidae* (*Hard-ticks*).

iv. Protozoa

The PMP compositions and related methods can be useful for decreasing the fitness of a parasitic protozoa, e.g., to prevent or treat a parasitic protozoa infection in an animal. The term "protozoa" includes any organism belonging to the phylum Protozoa. Included are methods for delivering a PMP composition to a parasitic protozoan by contacting the parasitic protozoa with the PMP composition. Additionally or alternatively, the methods include preventing or treating a protozoal infection (e.g., caused by a protozoan described herein) in an animal at risk of or in need thereof, by administering to the animal a PMP composition.

The PMP compositions and related methods are suitable for preventing or treating infection by parasitic protozoa in animals, including protozoa belonging to *Euglenozoa* (*Trypanosoma cruzi*, *Trypanosoma brucei*, *Leishmania* spp.), *Heterolobosea* (*Naegleria fowleri*), *Diplomonadida* (*Giardia intestinalis*), *Amoebozoa* (*Acanthamoeba castellanii*, *Balamuthia mandrillaris*, *Entamoeba histolytica*),

Blastocystis (*Blastocystis hominis*), *Apicomplexa* (*Babesia microti*, *Cryptosporidium parvum*, *Cyclospora cayetanensis*, *Plasmodium spp.*, *Toxoplasma gondii*).

v. *Nematodes*

5 The PMP compositions and related methods can be useful for decreasing the fitness of a parasitic nematode, e.g., to prevent or treat a parasitic nematode infection in an animal. Included are methods for delivering a PMP composition to a parasitic nematode by contacting the parasitic nematode with the PMP composition. Additionally or alternatively, the methods include preventing or treating a parasitic nematode infection (e.g., caused by a parasitic nematode described herein) in an animal at risk
10 of or in need thereof, by administering to the animal a PMP composition.

The PMP compositions and related methods are suitable for preventing or treating infection by parasitic nematodes in animals, including nematodes belonging to *Nematoda* (roundworms): *Angiostrongylus cantonensis* (rat lungworm), *Ascaris lumbricoides* (human roundworm), *Baylisascaris procyonis* (raccoon roundworm), *Trichuris trichiura* (human whipworm), *Trichinella spiralis*, *Strongyloides stercoralis*, *Wuchereria bancrofti*, *Brugia malayi*, *Ancylostoma duodenale* and *Necator americanus*
15 (human hookworms), *Cestoda* (tapeworms): *Echinococcus granulosus*, *Echinococcus multilocularis*, *Taenia solium* (pork tapeworm).

vi. *Viruses*

20 The PMP compositions and related methods can be useful for decreasing the fitness of a virus, e.g., to prevent or treat a viral infection in an animal. Included are methods for delivering a PMP composition to a virus by contacting the virus with the PMP composition. Additionally or alternatively, the methods include preventing or treating a viral infection (e.g., caused by a virus described herein) in an animal at risk of or in need thereof, by administering to the animal a PMP composition.

25 The PMP compositions and related methods are suitable for preventing or treating a viral infection in animals, including infections by viruses belonging to *DNA viruses*: *Parvoviridae*, *Papillomaviridae*, *Polyomaviridae*, *Poxviridae*, *Herpesviridae*; *Single-stranded negative strand RNA viruses*: *Arenaviridae*, *Paramyxoviridae* (*Rubulavirus*, *Respirovirus*, *Pneumovirus*, *Morbillivirus*), *Filoviridae* (*Marburgvirus*, *Ebolavirus*), *Bornaoviridae*, *Rhabdoviridae*, *Orthomyxoviridae*, *Bunyaviridae*,
30 *Nairovirus*, *Hantaviruses*, *Orthobunyavirus*, *Phlebovirus*. *Single-stranded positive strand RNA viruses*: *Astroviridae*, *Coronaviridae*, *Caliciviridae*, *Togaviridae* (*Rubivirus*, *Alphavirus*), *Flaviviridae* (*Hepacivirus*, *Flavivirus*), *Picornaviridae* (*Hepatovirus*, *Rhinovirus*, *Enterovirus*); or *dsRNA and Retro-transcribed Viruses*: *Reoviridae* (*Rotavirus*, *Coltivirus*, *Seadornavirus*), *Retroviridae* (*Deltaretrovirus*, *Lentivirus*), *Hepadnaviridae* (*Orthohepadnavirus*).

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E. Delivery to a Pathogen Vector

Provided herein are methods of delivering a PMP composition (e.g., including modified PMPs described herein) to pathogen vector, such as one disclosed herein, by contacting the pathogen vector with a PMP composition. As used herein, the term “vector” refers to an insect that can carry or transmit
40 an animal pathogen from a reservoir to an animal. Exemplary vectors include insects, such as those with

piercing-sucking mouthparts, as found in Hemiptera and some Hymenoptera and Diptera such as mosquitoes, bees, wasps, midges, lice, tsetse fly, fleas and ants, as well as members of the Arachnidae such as ticks and mites.

In some instances, the vector of the animal (e.g., human) pathogen may be treated with PMPs not including a heterologous functional agent. In other instances, the PMPs include a heterologous functional agent, e.g., a heterologous therapeutic agent (e.g., antibacterial agent, antifungal agent, insecticide, nematicide, antiparasitic agent, antiviral agent, or a repellent). The methods can be useful for decreasing the fitness of a pathogen vector, e.g., to control the spread of a pathogen as a consequence of delivery of the PMP composition. Examples of pathogen vectors that can be targeted in accordance with the present methods include insects, such as those described herein.

For example, provided herein is a method of decreasing the fitness of an animal pathogen vector, the method including delivering to the vector an effective amount of the PMP compositions described herein, wherein the method decreases the fitness of the vector relative to an untreated vector. In some instances, the method includes delivering the composition to at least one habitat where the vector grows, lives, reproduces, feeds, or infests. In some instances, the composition is delivered as a comestible composition for ingestion by the vector. In some instances, the vector is an insect. In some instances, the insect is a mosquito, a tick, a mite, or a louse. In some instances, the composition is delivered (e.g., to the pathogen vector) as a liquid, a solid, an aerosol, a paste, a gel, or a gas.

For example, provided herein is a method of decreasing the fitness of an insect vector of an animal pathogen, wherein the method includes delivering to the vector a PMP composition including a plurality of PMPs. In some instances, the method includes delivering to the vector a PMP composition including a plurality of PMPs, wherein the plurality of PMPs includes an insecticidal agent. For example, the insect vector may be a mosquito, tick, mite, or louse. Other non-limiting examples of pathogen vectors are provided herein. In some instances, the method decreases the fitness of the vector relative to an untreated vector.

In some instances, the decrease in vector fitness may manifest as a deterioration or decline in the physiology of the vector (e.g., reduced health or survival) as a consequence of administration of a composition. In some instances, the fitness of an organism may be measured by one or more parameters, including, but not limited to, reproductive rate, lifespan, mobility, fecundity, body weight, metabolic rate or activity, or survival in comparison to a vector organism to which the composition has not been delivered. For example, the methods or compositions provided herein may be effective to decrease the overall health of the vector or to decrease the overall survival of the vector. In some instances, the decreased survival of the vector is about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or greater than 100% greater relative to a reference level (e.g., a level found in a vector that does not receive a composition). In some instances, the methods and compositions are effective to decrease vector reproduction (e.g., reproductive rate) in comparison to a vector organism to which the composition has not been delivered. In some instances, the methods and compositions are effective to decrease other physiological parameters, such as mobility, body weight, life span, fecundity, or metabolic rate, by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or greater than 100% relative to a reference level (e.g., a level found in a vector that is not delivered the composition).

In some instances, the decrease in vector fitness may manifest as an increase in the vector's sensitivity to a pesticidal agent and/or a decrease in the vector's resistance to a pesticidal agent in comparison to a vector organism to which the composition has not been delivered. In some instances, the methods or compositions provided herein may be effective to increase the vector's sensitivity to a
5 pesticidal agent by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or greater than 100% relative to a reference level (e.g., a level found in a vector that does not receive a composition). The pesticidal agent may be any pesticidal agent known in the art, including insecticidal agents. In some instances, the methods or compositions provided herein may increase the vector's sensitivity to a pesticidal agent by decreasing the vector's ability to metabolize or degrade the pesticidal
10 agent into usable substrates in comparison to a vector to which the composition has not been delivered.

In some instances, the decrease in vector fitness may manifest as other fitness disadvantages, such as decreased tolerance to certain environmental factors (e.g., a high or low temperature tolerance), decreased ability to survive in certain habitats, or a decreased ability to sustain a certain diet in comparison to a vector organism to which the composition has not been delivered. In some instances,
15 the methods or compositions provided herein may be effective to decrease vector fitness in any plurality of ways described herein. Further, the composition may decrease vector fitness in any number of vector classes, orders, families, genera, or species (e.g., 1 vector species, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 500, or more vector species). In some instances, the composition acts on a single vector class, order, family, genus, or species.

20 Vector fitness may be evaluated using any standard methods in the art. In some instances, vector fitness may be evaluated by assessing an individual vector. Alternatively, vector fitness may be evaluated by assessing a vector population. For example, a decrease in vector fitness may manifest as a decrease in successful competition against other vectors, thereby leading to a decrease in the size of the vector population.

25 By decreasing the fitness of vectors that carry animal pathogens, the compositions provided herein are effective to reduce the spread of vector-borne diseases. The composition may be delivered to the insects using any of the formulations and delivery methods described herein, in an amount and for a duration effective to reduce transmission of the disease, e.g., reduce vertical or horizontal transmission between vectors and/or reduce transmission to animals. For example, the composition described herein
30 may reduce vertical or horizontal transmission of a vector-borne pathogen by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more in comparison to a vector organism to which the composition has not been delivered. As another example, the composition described herein may reduce vectorial competence of an insect vector by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more in comparison to a vector organism to which the composition has not been
35 delivered.

Non-limiting examples of diseases that may be controlled by the compositions and methods provided herein include diseases caused by Togaviridae viruses (e.g., Chikungunya, Ross River fever, Mayaro, Onyon-nyong fever, Sindbis fever, Eastern equine encephalomyelitis, Western equine encephalomyelitis, Venezuelan equine encephalomyelitis, or Barmah forest); diseases caused by
40 Flaviviridae viruses (e.g., Dengue fever, Yellow fever, Kyasanur Forest disease, Omsk haemorrhagic

fever, Japaenese encephalitis, Murray Valley encephalitis, Rocio, St. Louis encephalitis, West Nile encephalitis, or Tick-borne encephalitis); diseases caused by Bunyaviridae viruses (e.g., Sandly fever, Rift Valley fever, La Crosse encephalitis, California encephalitis, Crimean-Congo haemorrhagic fever, or Oropouche fever); disease caused by Rhabdoviridae viruses (e.g., Vesicular stomatitis); disease caused
 5 by Orbiviridae (e.g., Bluetongue); diseases caused by bacteria (e.g., Plague, Tularaemia, Q fever, Rocky Mountain spotted fever, Murine typhus, Boutonneuse fever, Queensland tick typhus, Siberian tick typhus, Scrub typhus, Relapsing fever, or Lyme disease); or diseases caused by protozoa (e.g., Malaria, African trypanosomiasis, Nagana, Chagas disease, Leishmaniasis, Piroplasmosis, Bancroftian filariasis, or
 Brugian filariasis).

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i. Pathogen Vectors

The methods and compositions provided herein may be useful for decreasing the fitness of a vector for an animal pathogen. In some instances, the vector may be an insect. For example, the insect vector may include, but is not limited to those with piercing-sucking mouthparts, as found in Hemiptera
 15 and some Hymenoptera and Diptera such as mosquitoes, bees, wasps, midges, lice, tsetse fly, fleas and ants, as well as members of the Arachnidae such as ticks and mites; order, class or family of Acarina (ticks and mites) e.g. representatives of the families Argasidae, Dermanyssidae, Ixodidae, Psoroptidae or Sarcoptidae and representatives of the species *Amblyomma* spp., *Anocenton* spp., *Argas* spp., *Boophilus* spp., *Cheyletiella* spp., *Chorioptes* spp., *Demodex* spp., *Dermacentor* spp., *Dermanyssus* spp.,
 20 *Haemophysalis* spp., *Hyalomma* spp., *Ixodes* spp., *Lynxacarus* spp., *Mesostigmata* spp., *Notoednes* spp., *Ornithodoros* spp., *Ornithonyssus* spp., *Otobius* spp., *otodectes* spp., *Pneumonyssus* spp., *Psoroptes* spp., *Rhipicephalus* spp., *Sarcoptes* spp., or *Trombicula* spp.; Anoplura (sucking and biting lice) e.g. representatives of the species *Bovicola* spp., *Haematopinus* spp., *Linognathus* spp., *Menopon* spp., *Pediculus* spp., *Pemphigus* spp., *Phylloxera* spp., or *Solenopotes* spp.; Diptera (flies) e.g.
 25 representatives of the species *Aedes* spp., *Anopheles* spp., *Calliphora* spp., *Chrysomyia* spp., *Chrysops* spp., *Cochliomyia* spp., *Cw/ex* spp., *Culicoides* spp., *Cuterebra* spp., *Dermatobia* spp., *Gastrophilus* spp., *Glossina* spp., *Haematobia* spp., *Haematopota* spp., *Hippobosca* spp., *Hypoderma* spp., *Lucilia* spp., *Lyperosia* spp., *Melophagus* spp., *Oestrus* spp., *Phaenicia* spp., *Phlebotomus* spp., *Phormia* spp., Acari (sarcoptic mange) e.g., *Sarcoptidae* spp., *Sarcophaga* spp., *Simulium* spp., *Stomoxys* spp., *Tabanus* spp., *Tannia* spp. or *Zzpu/alpha* spp.; Mallophaga (biting lice) e.g. representatives of the species
 30 *Damalina* spp., *Felicola* spp., *Heterodoxus* spp. or *Trichodectes* spp.; or Siphonaptera (wingless insects) e.g. representatives of the species *Ceratophyllus* spp., *Xenopsylla* spp; Cimicidae (true bugs) e.g. representatives of the species *Cimex* spp., *Tritominae* spp., *Rhodnius* spp., or *Triatoma* spp.

In some instances, the insect is a blood-sucking insect from the order Diptera (e.g., suborder
 35 Nematocera, e.g., family Colicidae). In some instances, the insect is from the subfamilies Culicinae, Corethrinae, Ceratopogonidae, or Simuliidae. In some instances, the insect is of a *Culex* spp.,

Theobaldia spp., *Aedes* spp., *Anopheles* spp., *Aedes* spp., *Forciponiyia* spp., *Culicoides* spp., or *Helea* spp.

In certain instances, the insect is a mosquito. In certain instances, the insect is a tick. In certain instances, the insect is a mite. In certain instances, the insect is a biting louse.

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F. Delivery to an Animal

Provided herein are methods of delivering a PMP composition (e.g., including modified PMPs described herein) to an animal cell, tissue or subject (e.g., a mammal, e.g., a human), e.g., by contacting the animal cell, tissue, subject, or a part thereof, with the PMP composition. In some instances, animals may be treated with PMPs not including a heterologous functional agent. In other instances, the PMPs include a heterologous functional agent, e.g., a heterologous therapeutic agent (e.g., a therapeutic protein or peptide nucleic acid, or small molecule, an antibacterial agent, antifungal agent, insecticide, nematocide, antiparasitic agent, antiviral agent, or a repellent).

In one aspect, provided herein is a method of increasing the fitness of an animal (e.g., a human), the method including delivering to the animal the PMP composition described herein (e.g., in an effective amount and duration) to increase the fitness of the animal relative to an untreated animal (e.g., an animal that has not been delivered the PMP composition).

An increase in the fitness of the animal as a consequence of delivery of a PMP composition can be determined by any method of assessing animal fitness (e.g., fitness of a mammal, e.g., fitness (e.g., health) of a human).

Provided herein is a method of modifying or increasing the fitness of an animal (e.g., a human), the method including delivering to the animal an effective amount of a PMP composition provided herein, wherein the method modifies the animal and thereby introduces or increases a beneficial trait in the animal (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%) relative to an untreated animal. In particular, the method may increase the fitness of the animal, e.g., a mammal, e.g., a human (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%) relative to an untreated animal.

In a further aspect, provided herein is a method of increasing the fitness of an animal (e.g., a human), the method including contacting a cell of the animal with an effective amount of a PMP composition herein, wherein the method increases the fitness of the animal, e.g., mammal, e.g., human (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%) relative to an untreated animal.

In certain instances, the animal is a mammal, e.g., a human. In certain instances, the animal is a livestock animal or a veterinary animal. In certain instances, the animal is a mouse.

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G. Application Methods

A plant described herein can be exposed to a PMP composition (e.g., including modified PMPs described herein) in any suitable manner that permits delivering or administering the composition to the plant. The PMP composition may be delivered either alone or in combination with other active (e.g., fertilizing agents) or inactive substances and may be applied by, for example, spraying, injection (e.g.,

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microinjection), through plants, pouring, dipping, in the form of concentrated liquids, gels, solutions, suspensions, sprays, powders, pellets, briquettes, bricks and the like, formulated to deliver an effective concentration of the PMP composition. Amounts and locations for application of the compositions described herein are generally determined by the habitat of the plant, the lifecycle stage at which the plant can be targeted by the PMP composition, the site where the application is to be made, and the physical and functional characteristics of the PMP composition.

In some instances, the composition is sprayed directly onto a plant e.g., crops, by e.g., backpack spraying, aerial spraying, crop spraying/dusting etc. In instances where the PMP composition is delivered to a plant, the plant receiving the PMP composition may be at any stage of plant growth. For example, formulated PMP compositions can be applied as a seed-coating or root treatment in early stages of plant growth or as a total plant treatment at later stages of the crop cycle. In some instances, the PMP composition may be applied as a topical agent to a plant.

Further, the PMP composition may be applied (e.g., in the soil in which a plant grows, or in the water that is used to water the plant) as a systemic agent that is absorbed and distributed through the tissues of a plant. In some instances, plants or food organisms may be genetically transformed to express the PMP composition.

Delayed or continuous release can also be accomplished by coating the PMP composition or a composition with the PMP composition(s) with a dissolvable or bioerodable coating layer, such as gelatin, which coating dissolves or erodes in the environment of use, to then make the PMP composition available, or by dispersing the agent in a dissolvable or erodable matrix. Such continuous release and/or dispensing devices may be advantageously employed to consistently maintain an effective concentration of one or more of the PMP compositions described herein.

In some instances, the PMP composition is delivered to a part of the plant, e.g., a leaf, seed, pollen, root, fruit, shoot, or flower, or a tissue, cell, or protoplast thereof. In some instances, the PMP composition is delivered to a cell of the plant. In some instances, the PMP composition is delivered to a protoplast of the plant. In some instances, the PMP composition is delivered to a tissue of the plant. For example, the composition may be delivered to meristematic tissue of the plant (e.g., apical meristem, lateral meristem, or intercalary meristem). In some instances, the composition is delivered to permanent tissue of the plant (e.g., simple tissues (e.g., parenchyma, collenchyma, or sclerenchyma) or complex permanent tissue (e.g., xylem or phloem)). In some instances, the composition is delivered to a plant embryo.

In some instances, the PMP composition may be recommended for field application as an amount of PMPs per hectare (g/ha or kg/ha) or the amount of active ingredient (e.g., PMP with or without a heterologous functional agent) or acid equivalent per hectare (kg a.i./ha or g a.i./ha). In some instances, a lower amount of heterologous functional agent in the present compositions may be required to be applied to soil, plant media, seeds plant tissue, or plants to achieve the same results as where the heterologous functional agent is applied in a composition lacking PMPs. For example, the amount of heterologous functional agent may be applied at levels about 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 50, or 100- fold (or any range between about 2 and about 100-fold, for example about 2- to 10- fold; about 5- to 15-fold, about 10- to 20-fold; about 10- to 50-fold) less than the same heterologous functional agent

applied in a non-PMP composition, e.g., direct application of the same heterologous functional agent without PMPs. PMP compositions of the invention can be applied at a variety of amounts per hectare, for example at about 0.0001, 0.001, 0.005, 0.01, 0.1, 1, 2, 10, 100, 1,000, 2,000, 5,000 (or any range between about 0.0001 and 5,000) kg/ha. For example, about 0.0001 to about 0.01, about 0.01 to about 10, about 10 to about 1,000, about 1,000 to about 5,000 kg/ha.

H. Therapeutic Methods

The PMP compositions (e.g., including modified PMPs described herein) can also be useful in a variety of therapeutic methods. For example, the methods and composition may be used for the prevention or treatment of pathogen infections in animals (e.g., humans). As used herein, the term “treatment” refers to administering a pharmaceutical composition to an animal for prophylactic and/or therapeutic purposes. To “prevent an infection” refers to prophylactic treatment of an animal who is not yet ill, but who is susceptible to, or otherwise at risk of, a particular disease. To “treat an infection” refers to administering treatment to an animal already suffering from a disease to improve or stabilize the animal's condition. The present methods involve delivering the PMP compositions described herein to an animal, such as a human.

For example, provided herein is a method of treating an animal having a fungal infection, wherein the method includes administering to the animal an effective amount of a PMP composition including a plurality of PMPs. In some instances, the method includes administering to the animal an effective amount of a PMP composition including a plurality of PMPs, wherein the plurality of PMPs includes an antifungal agent. In some instances, the antifungal agent is a nucleic acid that inhibits expression of a gene in a fungus that causes the fungal infection (e.g., Enhanced Filamentous Growth Protein (EFG1)). In some instances, the fungal infection is caused by *Candida albicans*. In some instances, composition includes a PMP produced from an *Arabidopsis* apoplast EV. In some instances, the method decreases or substantially eliminates the fungal infection.

In another aspect, provided herein is a method of treating an animal having a bacterial infection, wherein the method includes administering to the animal an effective amount of a PMP composition including a plurality of PMPs. In some instances, the method includes administering to the animal an effective amount of a PMP composition including a plurality of PMPs, and wherein the plurality of PMPs includes an antibacterial agent (e.g., Amphotericin B). In some instances, the bacterium is a *Streptococcus* spp., *Pneumococcus* spp., *Pseudomonas* spp., *Shigella* spp., *Salmonella* spp., *Campylobacter* spp., or an *Escherichia* spp. In some instances, the composition includes a PMP produced from an *Arabidopsis* apoplast EV. In some instances, the method decreases or substantially eliminates the bacterial infection. In some instances, the animal is a human, a veterinary animal, or a livestock animal.

The present methods are useful to treat an infection (e.g., as caused by an animal pathogen) in an animal, which refers to administering treatment to an animal already suffering from a disease to improve or stabilize the animal's condition. This may involve reducing colonization of a pathogen in, on, or around an animal by one or more pathogens (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%) relative to a starting amount and/or allow benefit to the individual (e.g.,

reducing colonization in an amount sufficient to resolve symptoms). In such instances, a treated infection may manifest as a decrease in symptoms (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%). In some instances, a treated infection is effective to increase the likelihood of survival of an individual (e.g., an increase in likelihood of survival by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%) or increase the overall survival of a population (e.g., an increase in likelihood of survival by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%). For example, the compositions and methods may be effective to "substantially eliminate" an infection, which refers to a decrease in the infection in an amount sufficient to sustainably resolve symptoms (e.g., for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months) in the animal.

The present methods are useful to prevent an infection (e.g., as caused by an animal pathogen), which refers to preventing an increase in colonization in, on, or around an animal by one or more pathogens (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100% relative to an untreated animal) in an amount sufficient to maintain an initial pathogen population (e.g., approximately the amount found in a healthy individual), prevent the onset of an infection, and/or prevent symptoms or conditions associated with infection. For example, individuals may receive prophylaxis treatment to prevent a fungal infection while being prepared for an invasive medical procedure (e.g., preparing for surgery, such as receiving a transplant, stem cell therapy, a graft, a prosthesis, receiving long-term or frequent intravenous catheterization, or receiving treatment in an intensive care unit), in immunocompromised individuals (e.g., individuals with cancer, with HIV/AIDS, or taking immunosuppressive agents), or in individuals undergoing long term antibiotic therapy.

The PMP composition can be formulated for administration or administered by any suitable method, including, for example, intravenously, intramuscularly, subcutaneously, intradermally, percutaneously, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intrathecally, intranasally, intravaginally, intrarectally, topically, intratumorally, peritoneally, subconjunctivally, intravesicularly, mucosally, intrapericardially, intraumbilically, intraocularly, intraorbitally, orally, topically, transdermally, intravitreally (e.g., by intravitreal injection), by eye drop, by inhalation, by injection, by implantation, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, by catheter, by lavage, in cremes, or in lipid compositions. The compositions utilized in the methods described herein can also be administered systemically or locally. The method of administration can vary depending on various factors (e.g., the compound or composition being administered and the severity of the condition, disease, or disorder being treated). In some instances, PMP composition is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. Dosing can be by any suitable route, e.g., by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

For the prevention or treatment of an infection described herein (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to

be treated, the severity and course of the disease, whether the is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the PMP composition. The PMP composition can be, e.g., administered to the patient at one time or over a series of treatments. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs or the infection is no longer detectable. Such doses may be administered intermittently, e.g., every week or every two weeks (e.g., such that the patient receives, for example, from about two to about twenty, doses of the PMP composition. An initial higher loading dose, followed by one or more lower doses may be administered. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

In some instances, the amount of the PMP composition administered to individual (e.g., human) may be in the range of about 0.01 mg/kg to about 5 g/kg (e.g., about 0.01 mg/kg – 0.1 mg/kg, about 0.1 mg/kg – 1 mg/kg, about 1 mg/kg-10 mg/kg, about 10 mg/kg-100 mg/kg, about 100 mg/kg – 1 g/kg, or about 1 g/kg- 5 g/kg), of the individual's body weight. In some instances, the amount of the PMP composition administered to individual (e.g., human) is at least 0.01 mg/kg (e.g., at least 0.01 mg/kg, at least 0.1 mg/kg, at least 1 mg/kg, at least 10 mg/kg, at least 100 mg/kg, at least 1 g/kg, or at least 5 g/kg), of the individual's body weight. The dose may be administered as a single dose or as multiple doses (e.g., 2, 3, 4, 5, 6, 7, or more than 7 doses). In some instances, the PMP composition administered to the animal may be administered alone or in combination with an additional therapeutic agent. The dose of the antibody administered in a combination treatment may be reduced as compared to a single treatment. The progress of this therapy is easily monitored by conventional techniques.

IV. Kits

The present invention also provides a kit including a container having a PMP composition described herein. The kit may further include instructional material for applying or delivering the PMP composition to a plant in accordance with a method of the present invention. The skilled artisan will appreciate that the instructions for applying the PMP composition in the methods of the present invention can be any form of instruction. Such instructions include, but are not limited to, written instruction material (such as, a label, a booklet, a pamphlet), oral instructional material (such as on an audio cassette or CD) or video instructions (such as on a video tape or DVD).

EXAMPLES

The following are examples of the methods of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

35

Example 1: Isolation of Plant Messenger Packs from plants

This example describes the isolation of crude plant messenger packs (PMPs) from various plant sources, including the leaf apoplast, seed apoplast, root, fruit, vegetable, pollen, phloem, xylem sap and plant cell culture medium.

40

*Experimental design:**a) PMP isolation from the apoplast of Arabidopsis thaliana leaves*

Arabidopsis (*Arabidopsis thaliana* Col-0) seeds are surface sterilized with 50% bleach and plated on 0.53 Murashige and Skoog medium containing 0.8% agar. The seeds are vernalized for 2 d at 4°C before being moved to short-day conditions (9-h days, 22°C, 150 μEm^{-2}). After 1 week, the seedlings are transferred to Pro-Mix PGX. Plants are grown for 4-6 weeks before harvest.

PMPs are isolated from the apoplastic wash of 4-6-week old Arabidopsis rosettes, as described by Rutter and Innes, *Plant Physiol.* 173(1): 728-741, 2017. Briefly, whole rosettes are harvested at the root and vacuum infiltrated with vesicle isolation buffer (20mM MES, 2mM CaCl₂, and 0.1 M NaCl, pH6). Infiltrated plants are carefully blotted to remove excess fluid, placed inside 30-mL syringes, and centrifuged in 50 mL conical tubes at 700g for 20min at 2°C to collect the apoplast extracellular fluid containing EVs. Next, the apoplast extracellular fluid is filtered through a 0.85 μm filter to remove large particles, and PMPs are purified as described in Example 2.

b) PMP isolation from the apoplast of sunflower seeds

Intact sunflower seeds (*H. annuus* L.), and are imbibed in water for 2 hours, peeled to remove the pericarp, and the apoplastic extracellular fluid is extracted by a modified vacuum infiltration-centrifugation procedure, adapted from Regente et al, *FEBS Letters.* 583: 3363-3366, 2009. Briefly, seeds are immersed in vesicle isolation buffer (20mM MES, 2mM CaCl₂, and 0.1 M NaCl, pH6) and subjected to three vacuum pulses of 10s, separated by 30s intervals at a pressure of 45 kPa. The infiltrated seeds are recovered, dried on filter paper, placed in fritted glass filters and centrifuged for 20 min at 400g at 4°C. The apoplast extracellular fluid is recovered, filtered through a 0.85 μm filter to remove large particles, and PMPs are purified as described in Example 2.

c) PMP isolation from ginger roots

Fresh ginger (*Zingiber officinale*) rhizome roots are purchased from a local supplier and washed 3x with PBS. A total of 200 grams of washed roots is ground in a mixer (Osterizer 12-speed blender) at the highest speed for 10 min (pause 1 min for every 1 min of blending), and PMPs are isolated as described in Zhuang *et al.*, *J Extracellular Vesicles.* 4(1):28713, 2015. Briefly, ginger juice is sequentially centrifuged at 1,000g for 10 min, 3,000g for 20 min and 10,000g for 40 min to remove large particles from the PMP-containing supernatant. PMPs are purified as described in Example 2.

d) PMP isolation from grapefruit juice

Fresh grapefruits (*Citrus × paradisi*) are purchased from a local supplier, their skins are removed, and the fruit is manually pressed, or ground in a mixer (Osterizer 12-speed blender) at the highest speed for 10 min (pause 1 min for every minute of blending) to collect the juice, as described by Wang *et al.*, *Molecular Therapy.* 22(3): 522-534, 2014 with minor modifications. Briefly, juice/juice pulp is sequentially centrifuged at 1,000g for 10 min, 3,000g for 20 min, and 10,000g for 40 min to remove large particles from the PMP-containing supernatant. PMPs are purified as described in Example 2.

40

e) *PMP isolation from broccoli heads*

Broccoli (*Brassica oleracea* var. *italica*) PMPs are isolated as previously described (Deng *et al.*, *Molecular Therapy*, 25(7): 1641-1654, 2017). Briefly, fresh broccoli is purchased from a local supplier, washed three times with PBS, and ground in a mixer (Osterizer 12-speed blender) at the highest speed
 5 for 10 min (pause 1 min for every minute of blending). Broccoli juice is then sequentially centrifuged at 1,000g for 10 min, 3,000g for 20 min, and 10,000g for 40 min to remove large particles from the PMP-containing supernatant. PMPs are purified as described in Example 2.

f) *PMP isolation from olive pollen*

10 Olive (*Olea europaea*) pollen PMPs are isolated as previously described in Prado *et al.*, *Molecular Plant*. 7(3):573-577, 2014. Briefly, olive pollen (0.1 g) is hydrated in a humid chamber at room temperature for 30 min before transferring to petri dishes (15 cm in diameter) containing 20 ml germination medium: 10% sucrose, 0.03% Ca(NO₃)₂, 0.01% KNO₃, 0.02% MgSO₄, and 0.03% H₃BO₃. Pollen is germinated at 30°C in the dark for 16 h. Pollen grains are considered germinated only when the
 15 tube is longer than the diameter of the pollen grain. Cultured medium containing PMPs is collected and cleared of pollen debris by two successive filtrations on 0.85 µm filters by centrifugation. PMPs are purified as described in Example 2.

g) *PMP isolation from Arabidopsis phloem sap*

20 Arabidopsis (*Arabidopsis thaliana* Col-0) seeds are surface sterilized with 50% bleach and plated on 0.53 Murashige and Skoog medium containing 0.8% agar. The seeds are vernalized for 2 d at 4°C before being moved to short-day conditions (9-h days, 22°C, 150 µEm⁻²). After 1 week, the seedlings are transferred to Pro-Mix PGX. Plants are grown for 4-6 weeks before harvest.

Phloem sap from 4-6-week old Arabidopsis rosette leaves is collected as described by Tetyuk *et al.*, *JoVE*. 80, 2013. Briefly, leaves are cut at the base of the petiole, stacked, and placed in a reaction
 25 tube containing 20 mM K₂-EDTA for one hour in the dark to prevent sealing of the wound. Leaves are gently removed from the container, washed thoroughly with distilled water to remove all EDTA, put in a clean tube, and phloem sap is collected for 5-8 hours in the dark. Leaves are discarded, phloem sap is filtered through a 0.85 µm filter to remove large particles, and PMPs are purified as described in
 30 Example 2.

h) *PMP isolation from tomato plant xylem sap*

Tomato (*Solanum lycopersicum*) seeds are planted in a single pot in an organic-rich soil, such as Sunshine Mix (Sun Gro Horticulture, Agawam, MA) and maintained in a greenhouse between 22°C and
 35 28°C. About two weeks after germination, at the two true-leaf stage, the seedlings are transplanted individually into pots (10 cm diameter and 17 cm deep) filled with sterile sandy soil containing 90% sand and 10% organic mix. Plants are maintained in a greenhouse at 22-28°C for four weeks.

Xylem sap from 4-week old tomato plants is collected as described by Kohlen *et al.*, *Plant Physiology*. 155(2):721-734, 2011. Briefly, tomato plants are decapitated above the hypocotyl, and a
 40 plastic ring is placed around the stem. The accumulating xylem sap is collected for 90 min after

decapitation. Xylem sap is filtered through a 0.85 µm filter to remove large particles, and PMPs are purified as described in Example 2.

i) PMP isolation from tobacco BY-2 cell culture medium

5 Tobacco BY-2 (*Nicotiana tabacum* L cv. Bright Yellow 2) cells are cultured in the dark at 26°C, on a shaker at 180 rpm in MS (Murashige and Skoog, 1962) BY-2 cultivation medium (pH 5.8) comprised MS salts (Duchefa, Haarlem, Netherlands, at#M0221) supplemented with 30 g/L sucrose, 2.0 mg/L potassium dihydrogen phosphate, 0.1 g/L myo-inositol, 0.2 mg/L 2,4-dichlorophenoxyacetic acid, and 1 mg/L thiamine HCl. The BY-2 cells are subcultured weekly by transferring 5% (v/v) of a 7-day-old cell
10 culture into 100mL fresh liquid medium. After 72-96 hours, BY-2 cultured medium is collected and centrifuged at 300 *g* at 4°C for 10 minutes to remove cells. The supernatant containing PMPs is collected and cleared of debris by filtration on 0.85 µm filter. PMPs are purified as described in Example 2.

Example 2: Production of purified Plant Messenger Packs (PMPs)

15 This example describes the production of purified PMPs from crude PMP fractions as described in Example 1, using ultrafiltration combined with size-exclusion chromatography, a density gradient (iodixanol or sucrose), and the removal of aggregates by precipitation or size-exclusion chromatography.

Experimental design:

20 *a) Production of purified grapefruit PMPs using ultrafiltration combined with size-exclusion chromatography*

The crude grapefruit PMP fraction from Example 1a is concentrated using 100-kDa molecular weight cut-off (MWCO) Amicon spin filter (Merck Millipore). Subsequently, the concentrated crude PMP solution is loaded onto a PURE-EV size exclusion chromatography column (HansaBioMed Life Sciences
25 Ltd) and isolated according to the manufacturer's instructions. The purified PMP-containing fractions are pooled after elution. Optionally, PMPs can be further concentrated using a 100-kDa MWCO Amicon spin filter, or by Tangential Flow Filtration (TFF). The purified PMPs are analyzed as described in Example 3.

b) Production of purified Arabidopsis apoplast PMPs using an iodixanol gradient

30 Crude Arabidopsis leaf apoplast PMPs are isolated as described in Example 1a, and purified PMPs are produced by using an iodixanol gradient as described in Rutter and Innes, *Plant Physiol.* 173(1): 728-741, 2017. To prepare discontinuous iodixanol gradients (OptiPrep; Sigma-Aldrich), solutions of 40% (v/v), 20% (v/v), 10% (v/v), and 5% (v/v) iodixanol are created by diluting an aqueous 60% OptiPrep stock solution in vesicle isolation buffer (VIB; 20mM MES, 2mM CaCl₂, and 0.1 M NaCl, pH6). The gradient is formed by layering 3 ml of 40% solution, 3 mL of 20% solution, 3 mL of 10%
35 solution, and 2 mL of 5% solution. The crude apoplast PMP solution from Example 1a is centrifuged at 40,000g for 60 min at 4°C. The pellet is resuspended in 0.5 ml of VIB and layered on top of the gradient. Centrifugation is performed at 100,000g for 17 h at 4°C. The first 4.5 ml at the top of the gradient is discarded, and subsequently 3 volumes of 0.7 ml that contain the apoplast PMPs are collected, brought
40 up to 3.5 mL with VIB and centrifuged at 100,000g for 60 min at 4°C. The pellets are washed with 3.5 ml

of VIB and repelleted using the same centrifugation conditions. The purified PMP pellets are combined for subsequent analysis, as described in Example 3.

c) Production of purified grapefruit PMPs using a sucrose gradient

5 Crude grapefruit juice PMPs are isolated as described in Example 1d, centrifuged at 150,000g for 90 min, and the PMP-containing pellet is resuspended in 1 ml PBS as described (Mu *et al.*, *Molecular Nutrition & Food Research*. 58(7):1561-1573, 2014). The resuspended pellet is transferred to a sucrose step gradient (8%/15%/30%/45%/60%) and centrifuged at 150,000g for 120 min to produce purified PMPs. Purified grapefruit PMPs are harvested from the 30%/45% interface, and subsequently analyzed,
10 as described in Example 3.

d) Removal of aggregates from grapefruit PMPs

In order to remove protein aggregates from produced grapefruit PMPs as described in Example 1d or purified PMPs from Example 2a-c, an additional purification step can be included. The produced
15 PMP solution is taken through a range of pHs to precipitate protein aggregates in solution. The pH is adjusted to 3, 5, 7, 9, or 11 with the addition of sodium hydroxide or hydrochloric acid. pH is measured using a calibrated pH probe. Once the solution is at the specified pH, it is filtered to remove particulates. Alternatively, the isolated PMP solution can be flocculated using the addition of charged polymers, such as Polymix-P or Praestol 2640. Briefly, 2-5 g per L of Polymix-P or Praestol 2640 is added to the solution
20 and mixed with an impeller. The solution is then filtered to remove particulates. Alternatively, aggregates are solubilized by increasing salt concentration. NaCl is added to the PMP solution until it is at 1 mol/L. The solution is then filtered to purify the PMPs. Alternatively, aggregates are solubilized by increasing the temperature. The isolated PMP mixture is heated under mixing until it has reached a uniform temperature of 50°C for 5 minutes. The PMP mixture is then filtered to isolate the PMPs. Alternatively,
25 soluble contaminants from PMP solutions are separated by size-exclusion chromatography column according to standard procedures, where PMPs elute in the first fractions, whereas proteins and ribonucleoproteins and some lipoproteins are eluted later. The efficiency of protein aggregate removal is determined by measuring and comparing the protein concentration before and after removal of protein aggregates via BCA/Bradford protein quantification. The produced PMPs are analyzed as described in
30 Example 3.

Example 3: Plant Messenger Pack characterization

This example describes the characterization of PMPs produced as described in Example 1 or Example 2.

35

Experimental design:

a) Determining PMP concentration

PMP particle concentration is determined by Nanoparticle Tracking Analysis (NTA) using a Malvern NanoSight, or by Tunable Resistive Pulse Sensing (TRPS) using an iZon qNano, following the
40 manufacturer's instructions. The protein concentration of purified PMPs is determined by using the DC

Protein assay (Bio-Rad). The lipid concentration of purified PMPs is determined using a fluorescent lipophilic dye, such as DiOC6 (ICN Biomedicals) as described by Rutter and Innes, *Plant Physiol.* 173(1): 728-741, 2017. Briefly, purified PMP pellets from Example 2 are resuspended in 100 ml of 10 mM DiOC6 (ICN Biomedicals) diluted with MES buffer (20 mM MES, pH 6) plus 1% plant protease inhibitor cocktail (Sigma-Aldrich) and 2 mM 2,29-dipyridyl disulfide. The resuspended PMPs are incubated at 37°C for 10 min, washed with 3mL of MES buffer, repelleted (40,000g, 60 min, at 4°C), and resuspended in fresh MES buffer. DiOC6 fluorescence intensity is measured at 485 nm excitation and 535 nm emission.

b) Biophysical and molecular characterization of PMPs

PMPs are characterized by electron and cryo-electron microscopy on a JEOL 1010 transmission electron microscope, following the protocol from Wu *et al.*, *Analyst.* 140(2):386-406, 2015. The size and zeta potential of the PMPs are also measured using a Malvern Zetasizer or iZon qNano, following the manufacturer's instructions. Lipids are isolated from PMPs using chloroform extraction and characterized with LC-MS/MS as demonstrated in Xiao *et al.* *Plant Cell.* 22(10): 3193-3205, 2010. Glycosyl inositol phosphorylceramides (GIPCs) lipids are extracted and purified as described by Cacas *et al.*, *Plant Physiology.* 170: 367-384, 2016, and analyzed by LC-MS/MS as described above. Total RNA, DNA, and protein are characterized using Quant-It kits from Thermo Fisher according to instructions. Proteins on the PMPs are characterized by LC-MS/MS following the protocol in Rutter and Innes, *Plant Physiol.* 173(1): 728-741, 2017. RNA and DNA are extracted using Trizol, prepared into libraries with the TruSeq Total RNA with Ribo-Zero Plant kit and the Nextera Mate Pair Library Prep Kit from Illumina, and sequenced on an Illumina MiSeq following manufacturer's instructions.

Example 4: Characterization of Plant Messenger Pack stability

This example describes measuring the stability of PMPs under a wide variety of storage and physiological conditions.

Experimental design:

PMPs produced as described in Examples 1 and 2 are subjected to various conditions. PMPs are suspended in water, 5% sucrose, or PBS and left for 1, 7, 30, and 180 days at -20°C, 4°C, 20°C, and 37°C. PMPs are also suspended in water and dried using a rotary evaporator system and left for 1, 7, and 30, and 180 days at 4°C, 20°C, and 37°C. PMPs are also suspended in water or 5% sucrose solution, flash-frozen in liquid nitrogen and lyophilized. After 1, 7, 30, and 180 days, dried and lyophilized PMPs are then resuspended in water. The previous three experiments with conditions at temperatures above 0°C are also exposed to an artificial sunlight simulator in order to determine content stability in simulated outdoor UV conditions. PMPs are also subjected to temperatures of 37°C, 40°C, 45°C, 50°C, and 55°C for 1, 6, and 24 hours in buffered solutions with a pH of 1, 3, 5, 7, and 9 with or without the addition of 1 unit of trypsin or in other simulated gastric fluids.

After each of these treatments, PMPs are brought back to 20°C, neutralized to pH 7.4, and characterized using some or all of the methods described in Example 3.

Example 5. Loading PMPs with cargo

This example describes methods of loading PMPs with small molecules, proteins, and nucleic acids to use as probes to determine PMP uptake efficiency in plants.

5 *a) Loading small molecules into PMPs*

PMPs are produced as described in Example 1 and Example 2. To load small molecules into PMPs, PMPs are placed in PBS solution with the small molecule either in solid form or solubilized. The solution is left for 1 hour at 22°C, according to the protocol in Sun, Mol. Ther., 2010. Alternatively, the solution is sonicated to induce poration and diffusion into the exosomes according to the protocol from
10 Wang et al, Nature Comm., 4: Article number: 1867, 2013. Alternatively, PMPs are electroporated according to the protocol from Wahlgren et al, Nucl. Acids. Res., 40(17): e130, 2012.

Alternatively, PMP lipids are isolated by adding 3.75 ml 2:1 (v/v) MeOH:CHCl₃ to 1 ml of PMPs in PBS and are vortexed. CHCl₃ (1.25 ml) and ddH₂O (1.25 ml) are added sequentially and vortexed. The mixture is then centrifuged at 2,000 r.p.m. for 10 min at 22°C in glass tubes to separate the mixture into
15 two phases (aqueous phase and organic phase). The organic phase sample containing the PMP lipids is dried by heating under nitrogen (2 psi). To produce small molecule-loaded PMPs, the isolated PMP lipids are mixed with the small molecule solution and passed through a lipid extruder according to the protocol from Haney et al, J Contr. Rel., 2015.

Before use, the loaded PMPs are purified using methods as described in Example 2 to remove
20 unbound small molecules. Loaded PMPs are characterized as described in Example 3, and their stability is tested as described in Example 4.

b) Loading proteins or peptides into PMPs

PMPs are produced as described in Example 1 and Example 2. To load proteins or peptides into
25 PMPs, PMPs are placed in solution with the protein or peptide in PBS. If the protein or peptide is insoluble, pH is adjusted until it is soluble. If the protein or peptide is still insoluble, the insoluble protein or peptide is used. The solution is then sonicated to induce poration and diffusion into the PMPs according to the protocol from Wang et al, Nature Comm., 4: Article number: 1867, 2013. Alternatively, PMPs are electroporated according to the protocol from Wahlgren et al, Nucl. Acids. Res., 40(17): e130,
30 2012.

Alternatively, PMP lipids are isolated by adding 3.75 ml 2:1 (v/v) MeOH:CHCl₃ to 1 ml of PMPs in PBS and are vortexed. CHCl₃ (1.25 ml) and ddH₂O (1.25 ml) are added sequentially and vortexed. The mixture is then centrifuged at 2,000 r.p.m. for 10 min at 22°C in glass tubes to separate the mixture into
35 two phases (aqueous phase and organic phase). The organic phase sample containing the PMP lipids is dried by heating under nitrogen (2 psi). To produce small molecule-loaded PMPs, the isolated PMP lipids are mixed with the small molecule solution and passed through a lipid extruder according to the protocol from Haney et al, J Contr. Rel., 2015.

Before use, the loaded PMPs are purified using the methods as described in Example 2 to
remove unbound peptides and protein. Loaded PMPs are characterized as described in Example 3, and
40 their stability is tested as described in Example 4. To measure loading of the protein or peptide, the

Pierce Quantitative Colorimetric Peptide Assay is used on a small sample of the loaded and unloaded PMPs.

c) Loading nucleic acids into PMPs

5 PMPs are produced as described in Example 1 and Example 2. To load nucleic acids into PMPs, PMPs are placed in solution with the nucleic acid in PBS. The solution is then sonicated to induce poration and diffusion into the PMPs according to the protocol from Wang et al, Nature Comm., 4: Article number: 1867, 2013. Alternatively, PMPs are electroporated according to the protocol from Wahlgren et al, Nucl. Acids. Res., 40(17): e130, 2012.

10 Alternatively, PMP lipids are isolated by adding 3.75 ml 2:1 (v/v) MeOH:CHCl₃ to 1 ml of PMPs in PBS and are vortexed. CHCl₃ (1.25 ml) and ddH₂O (1.25 ml) are added sequentially and vortexed. The mixture is then centrifuged at 2,000 r.p.m. for 10 min at 22°C in glass tubes to separate the mixture into two phases (aqueous phase and organic phase). The organic phase sample containing the PMP lipids is dried by heating under nitrogen (2 psi). To produce small molecule-loaded PMPs, the isolated PMP lipids
15 are mixed with the small molecule solution and passed through a lipid extruder according to the protocol from Haney et al, J Contr. Rel., 2015.

Before use, the PMPs are purified using the methods as described in Example 2 to remove unbound nucleic acids. Loaded PMPs are characterized as described in Example 3, and their stability is tested as described in Example 4. Nucleic acids that are loaded in the PMPs are quantified using either a
20 Quant-It assay from Thermo Fisher following manufacturer's instructions, or fluorescence is quantified with a plate reader if the nucleic acids are fluorescently labeled.

Example 6. Intravenous delivery of PMPs comprising synthetic charged lipids

Plant messenger packs (PMPs) comprising the lipid species 1'-((2-(4-(2-((2-(bis(2-
25 hydroxydodecyl)amino)ethyl) (2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethyl)azanediyl)bisdodecan-2-ol) (C12-200), DLin-MC3-DMA (MC3), Lipid 5 (Moderna), or cKK-E12 (MD1) and an mRNA cargo encoding firefly luciferase (Fluc) and erythropoietin (EPO) were delivered to mice by intravenous (IV) injection. 3 mice per group were tested (12 mice total; Balb/c, females).

Compositions were delivered at a dose/volume of 10 µg total (1:1 :: FLuc:EPO)/100 µL.

30 Samples were assessed using an IVIS® imaging system. Takedown was 4 hours post-injection.

Size and polydispersity index (PDI) of the PMP formulations comprising C12-200, MC3, Lipid 5, or cKK-E12 are shown in Fig. 1A. mRNA encapsulation efficiency is shown in Fig. 1B.

Auto-exposed images of whole mice treated with the PMP compositions are shown in Fig. 2. The fluorescent signal was strongest in mice treated with the PMP compositions comprising C12-200.

35 Fluorescence levels were comparable in mice treated with the PMP compositions comprising Lipid 5 and cKK-E12, and were lower in mice treated with the PMP compositions comprising MC3. Figs. 3, 4, and 5 show images of dissected mouse organs using an exposure of 1 second, 1 minute, and 5 minutes, respectively.

Example 7. Lipid reconstructed PMP (LPMP) formulation

This Example provides methods for producing, purifying, and formulating lipid reconstructed PMPs (LPMPs).

5 *A. Lipid mix*

25 mM lipid stocks are thawed at 37 °C for 5 minutes. Stocks are fully dissolved prior to mixing the lipid components. Stocks are heated and sonicated in a bath sonicator until dissolved.

Empty test tubes are weighted and PMP lipid stock (in chloroform) is aliquoted into test. Chloroform is removed using Turbovac. Dry powder weight of the lipids is measured, and lipids are
10 resolubilized in an appropriate solvent (ethanol or DMF:Methanol :: 4:1) at 25 mM concentration assuming total molecular weight = 720 g/mol.

B. mRNA solution

mRNA is thawed at room temperature (RT) and is not heated, vortexed, or sonicated.
15 mRNA solution is prepared with RNase-free water and 100 mM Citrate buffer pH 3.

C. Formulation

LPMPs are formulated using a NANOASSEMBLER® IGNITE™ microfluidic instrument (Precision NanoSystems) using the following settings:

- 20
 - Total volume = Change according to formulation
 - flow rate = 3:1
 - total flow rate = 9 mL/min
 - sample switching volume (Start) = 0.200 mL
 - sample switching volume (end) = 0.100 mL.

25 Syringes are loaded in the holders with mRNA solution in the left inlet and lipid mix in the right inlet. Samples are collected in a 15 mL conical tube.

D. Dialysis

Dialysis cassettes are prepared for formulated LPMP samples as follows:

- 30
 - Clean several 5 L glass beakers using RNase-free water and RNaseZap™ cleaning spray. Wash with water. Fill one 5 L beaker with 4 L of 1x phosphate-buffered saline (PBS) solution for every 10 samples produced.
 - Place a number of 3- or 15-mL SLIDE-A-LYZER™ G2 10K dialysis cassettes equal to the number of LPMP samples in these buckets. Allow cassettes to soak for at least 3 minutes.
 - 35
 - Using a syringe with 18G needle, take up the formulated LPMP solution and inject it into the dialysis cassette through the side port. Use one cassette per formulated LPMP sample. For best results, it is recommended this be done within 15 minutes of formulation.
 - Remove all remaining air from cassettes. Be sure not to puncture dialysis membrane.
 - Return filled dialysis cassettes to 5 L beakers with PBS solution. Allow cassettes to
40 exchange for about 4 hours.

○ After 4 hours, dispose dialysis buffer and replace with fresh 1x PBS solution. Replace the dialysis cassettes back in the buckets. Seal the buckets with PARAFILM® or foil and place in a 4°C fridge. Overnight dialysis is recommended; however a minimum of 8 hours with buffer agitation is effective.

- 5 ○ When dialysis is complete, carefully transfer each sample from dialysis cassettes to RNase-free tubes.

E. Concentrating the formulation

The entire sample is transferred to an appropriately sized AMICON® Ultra centrifugal filtration unit and the tube is spun at 3000 G for 30 minutes at 4°C. This step is repeated until the desired final concentration is reached. Sample concentration time can be increased as needed while making sure that gel formation does not happen, and the filter is not dried. The filter is gently washed with 200 µL PBS to collect particles stuck to the filter.

The sample is sterilely filtered through 0.22 µm pore size filter (preferably one with low retention volume) and stored in an Eppendorf tube.

F. Characterization

LPMPs may be characterized using, e.g., dynamic light scattering (DLS), Quant-iT™ RiboGreen® analysis; and/or Endosafe® nexgen-PTS™ endotoxin measurement.

20

Example 8. IV delivery of LNP and reconstructed PMP formulations

A. LNP formulation

A modified PMP formulation was composed of ionizable lipid:structural lipids:sterol:PEG-lipid (C12-200:DOPE:cholesterol:14:0 PEG2000 PE) at a molar ratio of 35:16:46.5:2.5, respectively. Lipids were solubilized in ethanol. Lipids were mixed at the indicated molar ratios and diluted in ethanol (organic phase) to 5.5 mM total lipid concentration and the mRNA solution (aqueous phase, TRILINK® Biotechnologies firefly luciferase (FLuc) mRNA) was prepared with RNase-free water and 100 mM citrate buffer pH 3 (Teknova) for a final concentration of 50 mM citrate buffer and 0.147 mg/mL mRNA. Formulations were maintained at ionizable lipid to mRNA N:P ratio of 15:1. The lipid mix and mRNA solution were mixed at a 1:3 ratio by volume, respectively, on the NANOASSEMBLER® IGNITE™ microfluidic instrument (Precision NanoSystems) at a total flow rate of 9 mL/minute. The resulting formulations were then loaded into SLIDE-A-LYZER™ G2 dialysis cassettes (10k MWCO) and dialyzed in 200 times sample volume of 1x PBS for 4 hours at room temperature with gentle stirring. The PBS was refreshed, and the formulations were further dialyzed for at least 14 hours at 4°C with gentle stirring. The dialyzed formulations were then collected and concentrated by centrifugation at 3000 x g for 30 minutes using AMICON® Ultra centrifugation filters (100k MWCO). The concentrated formulations were sterilized using 0.2 µm pore size PES syringe filters (MilliporeSigma) and characterized for size, polydispersity, and particle concentration using Zetasizer Ultra (Malvern Panalytical) and for mRNA encapsulation efficiency using the QUANT-IT™ RIBOGREEN® RNA Assay Kit (ThermoFisher Scientific) according to the manufacturer's protocol.

40

Molar ratios of lipids in LNPs comprising C12-200, DOPE, cholesterol, and DMPE-PEG2k (C14-PEG2k) are shown in Table 11.

Table 11. LNP formulation

Lipid	Molar ratio
C12-200	35
DOPE	16
Cholesterol	46.5
DMPE-PEG2k	2.5

5

B. mRNA loading of Ionizable Lipid Modified PMPs (Reconstructed PMPs or recPMPs)

A modified PMP formulation was composed of ionizable lipid:PMP lipids:sterol:PEG-lipid (C12-200:PMP lipids:cholesterol:14:0 PEG2000 PE) at a molar ratio of 35:x:46.5-x:2.5, respectively. PMP lipids were solubilized into 4:1 DMF:Methanol or ethanol while the ionizable lipid, cholesterol, and PEG-lipid were solubilized in ethanol. These lipids were mixed at the indicated molar ratios and diluted in ethanol (organic phase) to 5.5 mM total lipid concentration and the mRNA solution (aqueous phase, TRILINK® Biotechnologies FLuc mRNA) was prepared with RNase-free water and 100 mM citrate buffer pH 3 (Teknova) for a final concentration of 50 mM citrate buffer and 0.147 mg/mL mRNA concentration. PMP formulations were maintained at ionizable lipid to mRNA N:P ratio of 15:1. The lipid mix and mRNA solution were mixed at a 1:3 ratio by volume, respectively, on the NANOASSEMBLER® IGNITE™ microfluidic instrument (Precision NanoSystems) at a total flow rate of 9 mL/min. The resulting formulations were then loaded into SLIDE-A-LYZER™ G2 dialysis cassettes (10k MWCO) and dialyzed in 200 times the sample volume of 1x PBS for 4 hours at room temperature with gentle stirring. The PBS was refreshed, and the formulations were further dialyzed for at least 14 hours at 4°C with gentle stirring. The dialyzed formulations were then collected and concentrated by centrifugation at 3000 x g for 30 minutes using AMICON® Ultra centrifugation filters (100k MWCO). The concentrated formulations were sterilized using 0.2 µm pore size PES syringe filters (MilliporeSigma) and characterized for size, polydispersity, and particle concentration using Zetasizer Ultra (Malvern Panalytical) and for mRNA encapsulation efficiency using the QUANT-IT™ RIBOGREEN® RNA Assay Kit (ThermoFisher Scientific) according to the manufacturer's protocol.

25

Molar ratios of lipids for PMP compositions comprising C12-200, PMP lipids, cholesterol, and DMPE-PEG2k are shown in Table 12.

Table 12. PMP formulation

Lipid	Molar ratio
C12-200	35
PMP lipids	16
Cholesterol	46.5
DMPE-PEG2k	2.5

30

C. IV delivery of LNP and PMP formulations

Images of whole mice treated with the LNP composition or PMP compositions comprising PMP lipids derived from lemon, grapefruit (GF), lime, orange, or algae are shown in Fig. 6. Figs. 7, 8, and 9 show fluorescence in dissected organs from treated mice. Images were taken using an IVIS® imaging system.

Levels of biodistribution of LNPs and PMPs to the liver, spleen, mesenteric lymph nodes (MLN), pancreas, small intestine, large intestine, cecum, kidney, stomach, heart, lung, and thymus, quantified as radiance (p/s/sq. cm/sr) are shown in Fig. 10,

Example 9. mRNA, pDNA, and nanoplasmid formulation screens with varying levels of PMP lipids

A. PMP compositions comprising 50% structural lipids

PMPs comprising an ionizable lipid (C12-200), a structural lipid (Lonestar grapefruit, Sunkist grapefruit, broccoli, ginger, or algae PMP lipids), a sterol (cholesterol), and a PEG-lipid (DMPE-PEG2k) at the ratios shown in Table 13 were produced according to the methods described in Example 8. Particles comprising the structural lipid DOPE were also produced. Size, polydispersity index (PDI), and encapsulation efficiency of an mRNA cargo (FLuc mRNA) are shown for each composition.

Table 13. PMP and LNP formulations comprising 50% structural lipids

Formulation	Content	Ionizable Lipid		Structural Lipid		Sterol		PEG-lipid		Size	PDI	Encapsulation
		Name	mol %	Name	mol %	Name	mol %	Name	mol %	nm	-	%
Control	Fluc mRNA	C12-200	35	DOPE	50	Cholesterol	11.5	DMPE-PEG2k	3.5	62.6	0.202	97.3
Grapefruit Lonestar	Fluc mRNA	C12-200	35	Lonestar	50	Cholesterol	11.5	DMPE-PEG2k	3.5	61.2	0.236	98.5
Grapefruit Sunkist	Fluc mRNA	C12-200	35	Sunkist	50	Cholesterol	11.5	DMPE-PEG2k	3.5	61.5	0.181	48.5
Broccoli	Fluc mRNA	C12-200	35	Broccoli	50	Cholesterol	11.5	DMPE-PEG2k	3.5	83.3	0.050	60.3
Ginger	Fluc mRNA	C12-200	35	Ginger	50	Cholesterol	11.5	DMPE-PEG2k	3.5	59.8	0.125	40.7
Algae	Fluc mRNA	C12-200	35	Algae	50	Cholesterol	11.5	DMPE-PEG2k	3.5	62.4	0.150	56.4

Cells were contacted *in vitro* with the PMP and LNP compositions of Table 13. Transfection efficiency of HeLa cells is shown in Fig. 11.

B. PMP and LNP compositions comprising 20% or less structural lipids

PMPs and LNPs comprising an ionizable lipid (C12-200 or MC3), a structural lipid (DOPE or DSPC (LNPs) or broccoli PMP lipids (broccoli BITs) (PMPs)), a sterol (cholesterol or sitosterol), and a PEG-lipid (DMPE-PEG2k) at the ratios shown in Table 14 were produced according to the methods described in Example 8. Size, polydispersity index (PDI), and encapsulation efficiency of a plasmid DNA (pDNA) cargo (gWiz™-Luc, a plasmid encoding luciferase) are shown for each composition.

Table 14. PMP and LNP formulations comprising 10-20% structural lipids

Formulation	Cargo	Ionizable Lipid		Structural Lipid		Cholesterol		PEG		Size	PDI	Encapsulation
		Name	mol %	Name	mol %	Name	mol %	Name	mol %	nm	-	%
A	Luc pDNA	C12-200 N:P (w:w) 10:1	35	DOPE	16	Cholesterol	46.5	DMPE-PEG2k	2.5	80.2	0.190	51.1
B	Luc pDNA	C12-200 N:P (w:w) 20:1	35	DOPE	16	Cholesterol	46.5	DMPE-PEG2k	2.5	73.8	0.180	75.0
C	Luc pDNA	C12-200 N:P (w:w) 20:1	35	Broccoli BITs	16	Sitosterol	46.5	DMPE-PEG2k	2.5	73.1	0.110	5.0
D	Luc pDNA	C12-200 N:P (w:w) 20:1	35	Broccoli BITs	20	Sitosterol	42.5	DMPE-PEG2k	2.5	73.4	0.124	8.2
E	Luc pDNA	MC3	50	DSPC	12.5	Sitosterol	36	DMPE-PEG2k	1.5	83.2	0.110	96.5
F	Luc pDNA	MC3	50	Broccoli BITs	10	Cholesterol	38.5	DMPE-PEG2k	1.5	96.3	0.130	89.3
G	Luc pDNA	MC3	50	Broccoli BITs	10	Sitosterol	38.5	DMPE-PEG2k	1.5	100.3	0.200	82.3
H	Luc pDNA	MC3	50	Broccoli BITs	12.5	Cholesterol	36	DMPE-PEG2k	1.5	96.0	0.122	91.4
I	Luc pDNA	MC3	50	Broccoli BITs	12.5	Sitosterol	36	DMPE-PEG2k	1.5	3826	1.670	15.3

MCF7 (breast cancer) cells (7,000-10,000 per well) were contacted *in vitro* with the PMP and LNP compositions of Table 14. Viability of treated MCF-7 cells (shown as a percentage relative to untreated cells) is shown in Fig. 12A. Level of luciferase expression (transfection) is shown in Fig. 12B. Compositions were delivered at 200 ng per well. Measurements were taken 48 hours after treating. Cells treated with Lipofectamine (Thermo Scientific) are shown as a control.

Additional PMP and LNP compositions comprising an ionizable lipid (C12-200 or MC3), a structural lipid (DOPE (LNPs) or broccoli PMP lipids (broccoli BITs) or grapefruit PMP lipids (grapefruit BITs) (PMPs)), a sterol (cholesterol), and a PEG-lipid (DMPE-PEG2k) at the ratios shown in Table 15 were produced according to the methods described in Example 8. Size, polydispersity index (PDI), and encapsulation efficiency of a plasmid DNA (pDNA) cargo (gWiz™-Luc, a plasmid encoding luciferase) are shown for each composition.

Table 15. Additional PMP and LNP formulations comprising 10-20% structural lipids

Cargo	Ionizable Lipid:mRNA	Ionizable Lipid		Structural Lipid		Cholesterol		PEG		Size	PDI	Encapsulation
		Name	mol %	Name	mol %	Name	mol %	Name	mol %	nm	-	%
Fluc pDNA	20:1	C12-200	35	DOPE	16	Cholesterol	46.5	DMPE-PEG2k	2.5	104.6	0.158	91.3
Fluc pDNA	30:1	C12-200	35	Broccoli BITS	16	Cholesterol	46.5	DMPE-PEG2k	2.5	105.5	0.123	39.5
Fluc pDNA	20:1	C12-200	50	DOPE	10	Cholesterol	38.5	DMPE-PEG2k	1.5	125.8	0.099	53.0
Fluc pDNA	20:1	C12-200	50	Broccoli BITS	10	Cholesterol	38.5	DMPE-PEG2k	1.5	124.7	0.081	30.1
Fluc pDNA	30:1	C12-200	50	Broccoli BITS	10	Cholesterol	38.5	DMPE-PEG2k	1.5	134.3	0.076	39.4
Fluc pDNA	20:1	C12-200	50	Grapefruit BITS	10	Cholesterol	38.5	DMPE-PEG2k	1.5	130.5	0.185	43.6
Fluc pDNA	N:P 6:1	MC3	50	DSPC	12.5	Cholesterol	36	DMPE-PEG2k	1.5	75.2	0.098	97.1
Fluc pDNA		MC3	50	Grapefruit BITS	12.5	Cholesterol	36	DMPE-PEG2k	1.5	87.0	0.104	96.8

C. PMP and LNP compositions loaded with mRNA, pDNA, and nanoplastids

PMPs and LNPs comprising an ionizable lipid (C12-200), a structural lipid (DOPE (LNPs) or algae, Lonestar grapefruit, Sunkist grapefruit, broccoli, or ginger (PMPs)), a sterol (cholesterol), and a PEG-lipid (DMPE-PEG2k) at the ratios shown in Table 16 were produced according to the methods described in Example 8. Size, polydispersity index (PDI), concentration, and encapsulation efficiency (particles/mL) of an mRNA cargo (FLuc mRNA), a pDNA cargo (gWiz™-Luc, a plasmid encoding luciferase), or a nanoplastid cargo (NP: nanoplastid encoding luciferase) are shown for each composition.

Table 16. PMP and LNP formulations comprising mRNA, pDNA, and NP cargoes

Formulation	Content	Ionizable lipid		Structural lipid		Cholesterol		PEG		Size	PDI	Encapsulation	Concentration
		Name	mol %	Name	mol %	Name	mol %	Name	mol %	nm	-	%	Particles/mL
Algae	Fluc mRNA	C12-200	35	Algae	50	Cholesterol	11.5	DMP E-PEG 2k	3.5	69.0	0.194	67.8	2.39x10 ¹³

Lones tar	Fluc mRN A	C12 - 200	35	Lones tar	50	Cholest erol	11. 5	DMP E- PEG 2k	3. 5	73. 3	0.3 10	99.5	1.09x1 0 ¹⁵
Sunki st	Fluc mRN A	C12 - 200	35	Sunki st	50	Cholest erol	11. 5	DMP E- PEG 2k	3. 5	146 .4	0.2 99	96.9	1.43x1 0 ¹⁵
Brocc oli	Fluc mRN A	C12 - 200	35	Brocc oli	50	Cholest erol	11. 5	DMP E- PEG 2k	3. 5	88. 3	0.1 13	44.9	1.48x1 0 ¹³
Ginge r	Fluc mRN A	C12 - 200	35	Ginge r	50	Cholest erol	11. 5	DMP E- PEG 2k	3. 5	56. 9	0.1 97	40.8	1.18x1 0 ¹⁴
Contr ol	Fluc mRN A	C12 - 200	35	DOPE	16	Cholest erol	46. 5	DMP E- PEG 2k	2. 5	72. 3	0.1 86	99.0	1.28x1 0 ¹⁵
NP	Fluc NP	C12 - 200	35	DOPE	16	Cholest erol	46. 5	DMP E- PEG 2k	2. 5	83. 7	0.2 25	91.2	1.36x1 0 ¹³
pDNA	Fluc pDNA	C12 - 200	35	DOPE	16	Cholest erol	46. 5	DMP E- PEG 2k	2. 5	93. 8	0.2 18	60.0	5.18x1 0 ¹⁴

Size and polydispersity index (PDI) of the PMP and LNP formulations of Table 16 are shown in Fig. 13A. Cargo encapsulation efficiency is shown in Fig. 13B. Level of luciferase expression in HeLa cells contacted with the PMP and LNP formulations is shown in Fig. 18.

5

Example 10. MC3 and C12-200 formulation screens with varying levels of PMP lipids

Fig. 14 shows the size (nm) and PDI of PMPs comprising an ionizable lipid (C12-200 or MC3), broccoli PMP lipids (broccoli BiTS), a sterol (cholesterol), and a PEG-lipid (DMPE-PEG2k) formulated at the ratios shown. PMPs were produced according to the methods described in Example 8.

10 Fig. 15 shows the percent cholesterol included in PMP compositions comprising C12-200 or MC3 and the percent encapsulation of a cargo by the PMPs.

Fig. 16 shows the encapsulation efficiency of a cargo by PMPs comprising an ionizable lipid (C12-200 or MC3), broccoli PMP lipids (broccoli BiTS), a sterol (cholesterol), and a PEG-lipid (DMPE-PEG2k) formulated at the ratios shown.

15 Fig. 17 shows the level of luciferase expression (RLU) in HeLa cells contacted with PMPs comprising an ionizable lipid (C12-200 or MC3), broccoli PMP lipids (broccoli BiTS), a sterol (cholesterol), and a PEG-lipid (DMPE-PEG2k) formulated at the ratios shown, wherein the PMPs comprise a cargo encoding the luciferase.

20 Example 11. Preparation of Modified PMP Compositions with or without a Cargo

Exemplary Modified PMP Compositions.

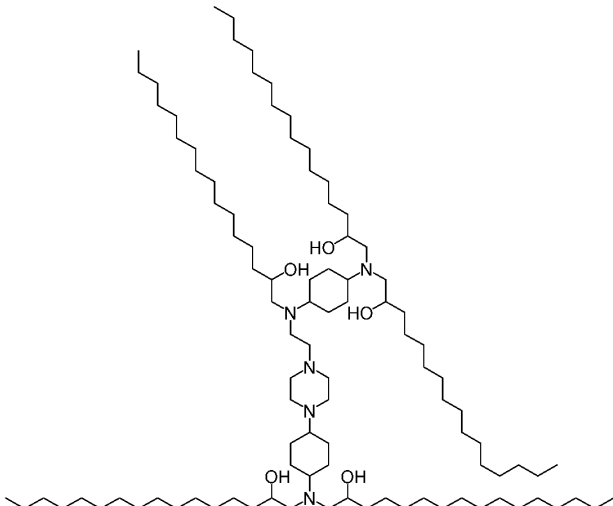
Exemplary modified PMP compositions were prepared to result in an ionizable lipid:natural lipid:sterol:PEG-lipid molar ratio of 35:50:12.5:2.5, respectively. For instance, exemplary modified PMP compositions in this example are shown in Table 17, below.

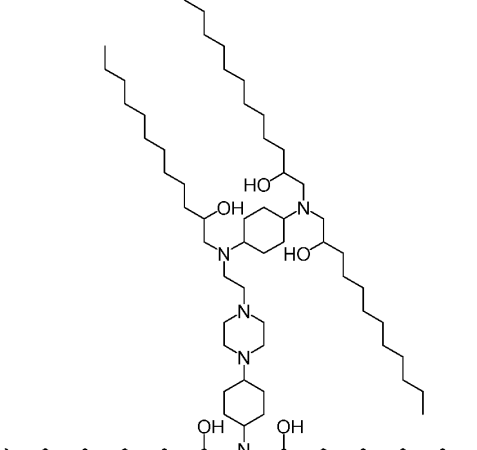
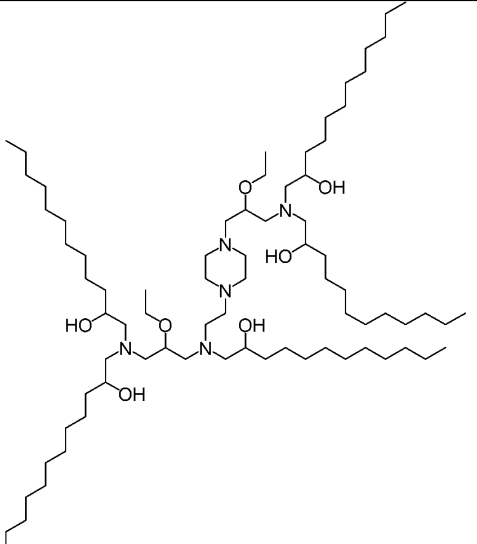
Table 17. Lipid ratiosLipids	Molar ratios
Exemplary ionizable lipid	35
Lemon PMP lipids	50
Plant cholesterol	12.5
DMPE-PEG2k	2.5

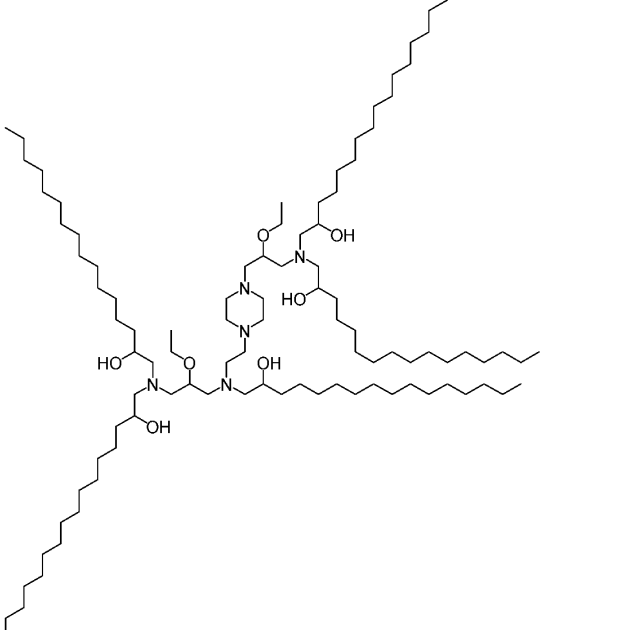
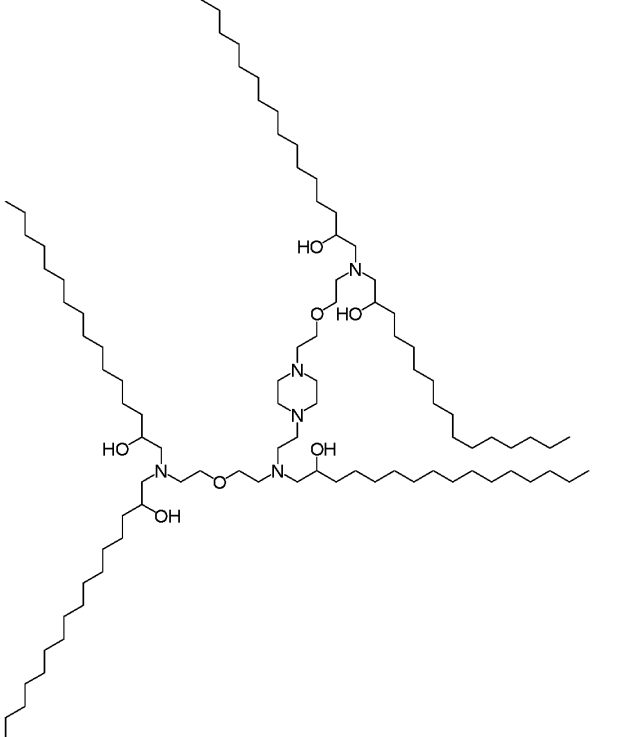
5 The exemplary ionizable lipids used for each exemplary modified PMP composition were Lipid Nos. 2254, 2206, 2255, 2256, 2207, 2257, 2258, 2208, and 2259, respectively (shown in Table 18), resulting in PMP Composition Nos. 2254, 2206, 2255, 2256, 2207, 2257, 2258, 2208, and 2259, respectively).

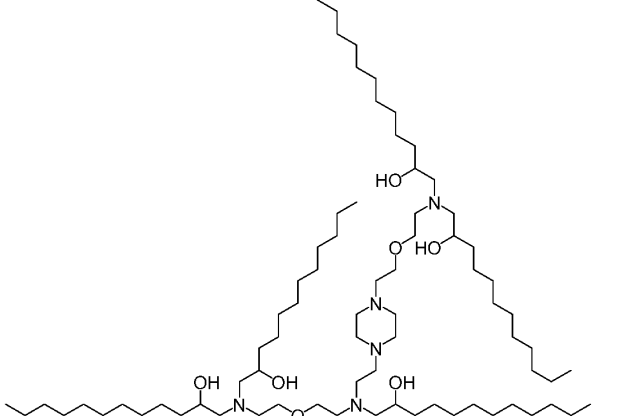
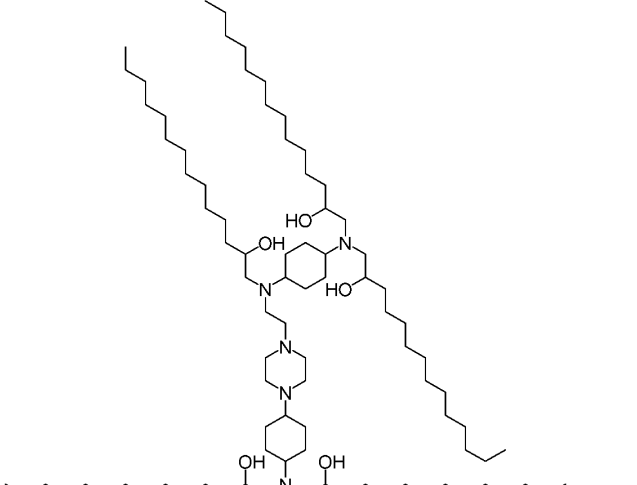
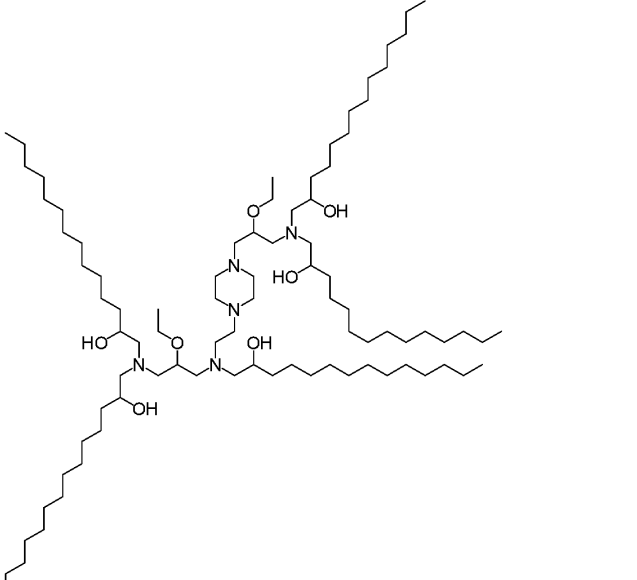
10 To prepare these modified PMP compositions, the lipids according to the above chart were solubilized in ethanol, except for lemon PMP lipids, which were solubilized in 4:1 DMF:methanol. The lipids were then mixed at the above molar ratios, and diluted in ethanol (organic phase) to obtain a total lipid concentration of 5.5 mM.

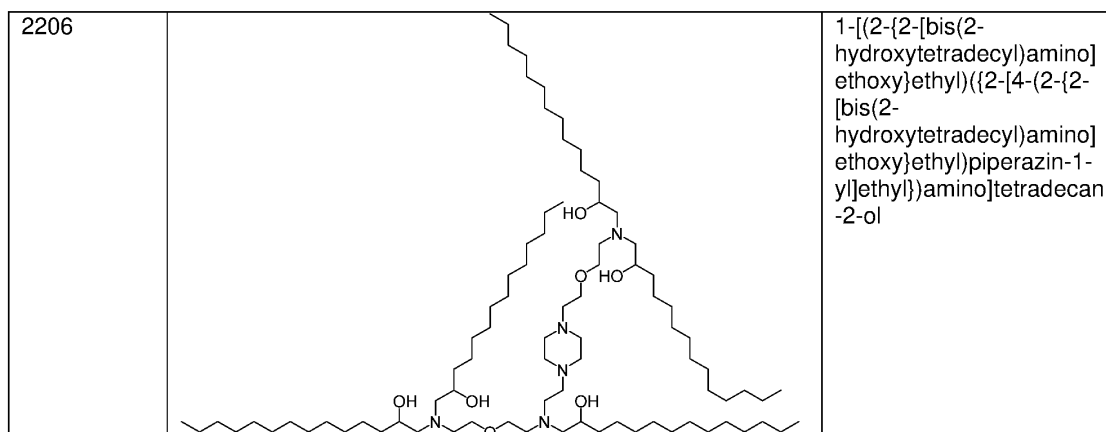
15 **Table 18. Ionizable lipids used for exemplary modified PMP compositions**

Lipid No.	Structure	IUPAC name
2259		1-({4-[bis(2-hydroxyhexadecyl)amino]cyclohexyl}[2-(4-[bis(2-hydroxyhexadecyl)amino]cyclohexyl)piperazin-1-yl)ethyl]amino)hexadecan-2-ol

2258		1-({4-[bis(2-hydroxydodecyl)amino]cyclohexyl}[2-(4-{4-[bis(2-hydroxydodecyl)amino]cyclohexyl}piperazin-1-yl)ethyl]amino)dodecan-2-ol
2256		1-({3-[bis(2-hydroxydodecyl)amino]-2-ethoxypropyl}[2-(4-{3-[bis(2-hydroxydodecyl)amino]-2-ethoxypropyl}piperazin-1-yl)ethyl]amino)dodecan-2-ol

2257		1-({3-[bis(2-hydroxyhexadecyl)amino]-2-ethoxypropyl}[2-(4-{3-[bis(2-hydroxyhexadecyl)amino]-2-ethoxypropyl})piperazin-1-yl]ethyl)amino]hexadecan-2-ol
2255		1-[(2-{2-[bis(2-hydroxyhexadecyl)amino]ethoxy}ethyl)(({2-[4-(2-{2-[bis(2-hydroxyhexadecyl)amino]ethoxy}ethyl)piperazin-1-yl]ethyl})amino)]hexadecan-2-ol

2254		1-[(2-{2-[bis(2-hydroxydodecyl)amino]ethoxy}ethyl){2-[4-(2-{2-[bis(2-hydroxydodecyl)amino]ethoxy}ethyl)piperazin-1-yl]ethyl})amino]dodecan-2-ol
2208		1-({4-[bis(2-hydroxytetradecyl)amino]cyclohexyl}[2-(4-{4-[bis(2-hydroxytetradecyl)amino]cyclohexyl)piperazin-1-yl]ethyl]amino)tetradecan-2-ol
2207		1-({3-[bis(2-hydroxytetradecyl)amino]-2-ethoxypropyl}[2-(4-{3-[bis(2-hydroxytetradecyl)amino]-2-ethoxypropyl)piperazin-1-yl]ethyl]amino)tetradecan-2-ol



Modified PMP Compositions Encapsulating mRNA.

An mRNA solution (aqueous phase, fluc:EPO mRNA) was prepared with RNase-free water and 100 mM citrate buffer pH 3 for a final concentration of 50 mM citrate buffer and 0.167 mg/mL mRNA (1:1 Fluc:EPO). The formulations were maintained at an ionizable lipid nitrogen:mRNA phosphate (N:P) ratio of 6:1.

For each modified PMP composition, the lipid mix and mRNA solution were mixed at a 1:3 ratio by volume, respectively, on a NANOASSEMBLER® IGNITE™ (Precision Nanosystems) at a total flow rate of 9 mL/min. The resulting compositions were then loaded into SLIDE-A-LYZER™ G2 dialysis cassettes (10k MWCO) and dialyzed in 200 times sample volume of 1x PBS for 2 hours at room temperature with gentle stirring. The PBS was refreshed, and the compositions were further dialyzed for at least 14 hours at 4 °C with gentle stirring. The dialyzed compositions were then collected and concentrated by centrifugation at 3000xg using AMICON® Ultra centrifugation filters (100k MWCO). The concentrated particles were characterized for size, polydispersity, and particle concentration using a Zetasizer Ultra (Malvern Panalytical) and for mRNA encapsulation efficiency using a QUANT-IT™ RIBOGREEN® RNA Assay Kit (ThermoFisher Scientific).

For pKa measurement, a TNA assay was conducted according to those described in Sabnis et al., *Molecular Therapy*, 26(6):1509-19, 2018), which is incorporated herein by reference in its entirety. Briefly, 20 buffers (10 mM sodium phosphate, 10 mM sodium borate, 10 mM sodium citrate, and 150 mM sodium chloride, in distilled water) of unique pH values ranging from 3.0 -12.0 were prepared using 1M sodium hydroxide and 1M hydrochloric acid. 3.25 µL of a modified PMP composition (0.04 mg/mL mRNA, in PBS) was incubated with 2 µL of TNS reagent (0.3 mM, in DMSO) and 90 µL of buffer for each pH value (described above) in a 96-well black-walled plate. Each pH condition was performed in triplicate wells. The TNS fluorescence was measured using a Biotek Cytation plate reader at excitation/emission wavelengths of 321/445 nm. The fluorescence values were then plotted and fit using a 4-parameter sigmoid curve. From the fit, the pH value yielding the half-maximal fluorescence was calculated and reported as the apparent modified PMP pKa value.

The particle characterization data for each exemplary modified PMP composition are shown in Table 19, below.

Table 19. Particle characterization data

Modified PMP Composition	Size (nm)	PDI	% entrapment efficiency	pKa
2254	63.93	0.15	90.2	6.57
2206	95.81	0.13	92.8	6.29
2255	120.13	0.08	92.5	5.85
2256	90.48	0.13	83.6	5.94
2207	114.63	0.18	89.6	5.61
2257	161.77	0.18	86.1	5.42
2258	114.57	0.18	75.7	6.74
2208	107.03	0.12	79.7	6.48
2259	134.97	0.17	40.7	6.32

Example 12. *In vivo* bioluminescent imaging

The exemplary modified PMP compositions prepared according to Example 11, with an encapsulated mRNA (EPO), were used in this example.

Bioluminescence screening.

8-9 week old female Balb/c mice were utilized for bioluminescence-based ionizable lipid screening efforts. Mice were obtained from Jackson Laboratories (JAX Stock: 000651) and allowed to acclimate for one week prior to manipulations. Animals were placed under a heat lamp for a few minutes before introducing them to a restraining chamber. The tail was wiped with alcohol pads (Fisher Scientific) and, for each modified PMP composition described above, 100 μ L of a modified PMP composition containing 10 μ g total mRNA (5 μ g Fluc + 5 μ g EPO) was injected intravenously using a 29G insulin syringe (Covidien). 4-6 hours post-dose, animals were injected with 200 μ L of 15mg/mL D-Luciferin (GoldBio), and placed in set nose cones inside the IVIS® Lumina LT imager (PerkinElmer). LivingImage software was utilized for imaging. Whole body bio-luminescence was captured at auto-exposure after which animals are removed from the IVIS® and placed into a CO₂ chamber for euthanasia. Cardiac puncture was performed on each animal after placing it in dorsal recumbency, and blood collection was performed using a 25G insulin syringe (BD). Once all blood samples were collected, tubes were spun at 2000G for 10 minutes using a tabletop centrifuge and plasma was aliquoted into individual Eppendorf tubes (Fisher Scientific) and stored at -80 °C for subsequent EPO quantification. EPO levels in plasma were determined using EPO MSD kit (Meso Scale Diagnostics).

hEPO MSD Measurement.

The reagents used for measuring hEPO levels included:

- MSD wash buffer (#R61AA-1)
- MSD EPO Kit (#K151VXK-2)
 - MSD GOLD 96 Small Spot Streptavidin Plate
 - Diluent 100

- Diluent 3
- Diluent 43
- Calibrator 9
- Capture Ab
- 5 ○ Detection Ab
- MSD GOLD Read Buffer B

General procedure.

The plate was coated. 200 µL of biotinylated capture antibody was added to 3.3 mL of Diluent 100 and was mixed by vortexing. 25 µL of the above solution was added to each well of the provided
 10 MSD GOLD Small Spot Streptavidin Plate. The plate was sealed with an adhesive plate seal and incubated with shaking at room temperature for 1 hour or at 2–8°C overnight. The plate was washed 3 times with at least 150 µL/well of 1X MSD Wash Buffer.

Preparation of Calibrator Standards.

The Calibrator vial(s) were brought to room temperature. Each vial of Calibrator was
 15 reconstituted by adding 250 µL of Diluent 43 to the glass vial, resulting in a 5× concentrated stock of the Calibrator. The reconstituted Calibrator was inverted at least 3 times, and equilibrated at room temperature for 15–30 minutes and then was vortexed briefly. Calibrator Standard 1 was prepared by adding 50 µL of the reconstituted Calibrator to 200 µL of Diluent 43 and vortexing. Calibrator Standard 2 was prepared by adding 75 µL of Calibrator Standard 1 to 225 µL of Diluent 43 and vortexing. The four-
 20 fold serial dilutions were repeated 5 additional times to generate a total of 7 Calibrator Standards. Vials were mixed by vortexing between each serial dilution. Diluent 43 was used as Calibrator Standard 8 (zero Calibrator).

Samples and Calibrators additions.

25 µL of Diluent 43 was added to each well. 25 µL of the prepared Calibrator Standard or sample
 25 was added to each well. The plate was sealed with an adhesive plate seal and incubated at room temperature with shaking for 1 hour.

Preparation and addition of the Detection Antibody Solution.

The detection antibody solution was provided as a 100× stock solution. The working solution was 1×. 60 µL of the supplied 100× detection antibody was added to 5940 µL of Diluent 3. The plate was
 30 washed 3 times with at least 150 µL/well of 1× MSD Wash Buffer. 50 µL of the Detection Antibody Solution prepared above was added to each well. The plate was sealed with an adhesive plate seal, and incubated at room temperature with shaking for 1 hour.

Sample reading.

The plate was washed 3 times with at least 150 µL/well of 1× MSD Wash Buffer. 150 µL of MSD
 35 GOLD Read Buffer B was added to each well. The plate was analyzed on an MSD instrument to read the EPO level.

The EPO levels determined by the *in-vivo* bioluminescent imaging for each exemplary modified PMP composition are shown in the table below. The spleen: liver ratio of average radiance for each exemplary modified PMP composition was also determined and shown in Table 20, below.

40

Table 20. Bioluminescence

	Bioluminescence				
PMP Composition	Whole body	Liver	Spleen	hEPO	Spleen:Liver Ratio
2254	1.60E+08	2.10E+07	4.70E+05	9.80E+04	0.02
2206	3.16E+08	7.04E+07	9.36E+06	1.69E+06	0.13
2255	6.50E+08	1.10E+08	3.80E+06	2.82E+06	0.04
2256	3.60E+07	3.90E+06	2.50E+05	1.70E+04	0.07
2207	5.51E+07	1.13E+07	2.70E+06	3.29E+05	0.24
2257	3.90E+08	1.60E+08	1.40E+06	3.20E+05	0.01
2258	1.60E+08	2.50E+07	1.00E+06	7.80E+04	0.04
2208	8.31E+07	1.55E+07	5.99E+06	1.42E+06	0.67
2259	-	-	-	-	-

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein

5 are expressly incorporated in their entirety by reference.

Other embodiments are within the claims.

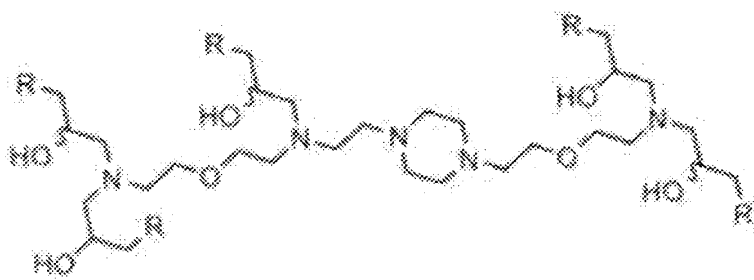
What is claimed is:

1. A composition comprising a plant messenger pack (PMP) modified to comprise a synthetic charged lipid, wherein the synthetic charged lipid has one or more of the characteristics listed below:

- (i) at least 2 ionizable amines;
- (ii) at least 3 lipid tails; wherein each of the lipid tails is at least 6 carbon atoms in length;
- (iii) a pKa of about 4.5 to about 7.5;
- (iv) an ionizable amine and a heteroorganic group separated by a chain of at least two atoms; and
- (v) an N:P ratio of at least 10;

provided that the charged lipid is not selected from 1'-((2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethyl)azanediyl)bis(dodecan-2-ol) (C12-200), MD1 (cKK-E12), OF2, EPC, ZA3-Ep10, TT3, LP01, 5A2-SC8, Lipid 5 (Moderna), and 98N12-5.

2. A composition comprising a PMP modified to comprise a synthetic charged lipid, wherein the PMP is modified by reconstituting a lipid film comprising purified PMP lipids in the presence of a synthetic charged lipid, thereby producing a modified PMP that comprises the synthetic charged lipid, wherein the synthetic charged lipid is represented by the following formula I:



(I)

where R is a C8-14 alkyl group.

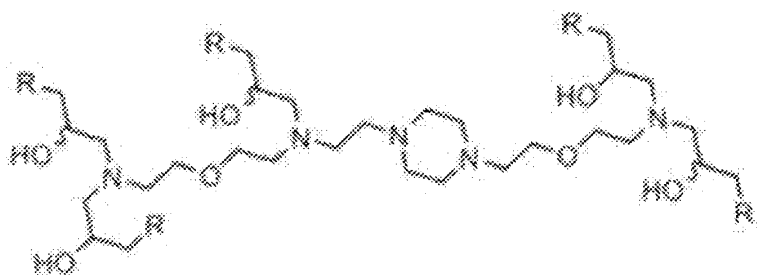
- 3. The composition of claim 1 or 2, wherein the modified PMP further comprises a sterol.
- 4. The composition of any one of claims 1-3, wherein the modified PMP further comprises a PEGylated lipid.
- 5. The composition of any one of claims 1-4, wherein the modified PMP further comprises a sterol and a PEGylated lipid.
- 6. The composition of claim 3 or 5, wherein the sterol is cholesterol or sitosterol.
- 7. The composition of any one of claims 4-6, wherein the PEGylated lipid is C18-PEG2k, DMPE-PEG2k, or ALC-0159.

8. The composition of claim 7, wherein the synthetic charged lipid, purified PMP lipids, sterol, and PEGylated lipid comprise about 30%-75%, about 35%-50%, about 10%-20%, and about 1%-3%, respectively, of the lipids in the modified PMP.

9. The composition of claim 7, wherein the synthetic charged lipid, purified PMP lipids, sterol, and PEGylated lipid comprise about 25%-75%, about 35%-60%, about 10%-20%, and about 0.5%-5%, respectively, of the lipids in the modified PMP.

10. The composition of claim 8, wherein the synthetic charged lipid, purified PMP lipids, sterol, and PEGylated lipid are formulated at a molar ratio of about 35:50:12.5:2.5.

11. The composition of any one of claims 1 and 3-10, wherein the synthetic charged lipid is represented by the following formula I:



(I)

where R is a C8-14 alkyl group.

12. The composition of claim 2 or 11, wherein a lipid membrane of the modified PMPs comprises at least 35% of the lipid of formula I.

13. The composition of any one of claims 1-12, wherein the synthetic charged lipid is an ionizable lipid and the composition has a zeta potential of greater than -40 mV at pH 4 when in the absence of cargo.

14. The composition of claim 13, wherein the composition has a zeta potential of greater than 0 mV at pH 4 when in the absence of cargo.

15. The composition of claim 14, wherein the composition has a zeta potential of greater than 20 mV at pH 4 when in the absence of cargo.

16. The composition of claim 15, wherein the composition has a zeta potential of greater than 30 mV at pH 4 when in the absence of cargo.

17. The composition of claim 16, wherein the composition has a zeta potential of about 40 mV at pH 4 when in the absence of cargo.

18. The composition of any one of claims 1-17, wherein the composition has a zeta potential of greater than -30 mV when in the absence of cargo.

19. The composition of claim 18, wherein the composition has a zeta potential of greater than -20 mV when in the absence of cargo.

20. The composition of claim 19, wherein the composition has a zeta potential of greater than -5 mV when in the absence of cargo.

21. The composition of claim 20, wherein the composition has a zeta potential of greater than 0 mV when in the absence of cargo.

22. The composition of claim 21, wherein the composition has a zeta potential of about 30 mV when in the absence of cargo.

23. The composition of any one of claims 1-22, wherein the modified PMPs comprise a heterologous functional agent.

24. The composition of claim 23, wherein the heterologous functional agent is encapsulated by the modified PMPs.

25. The composition of claim 24, wherein the heterologous functional agent is embedded on the surface of the modified PMPs.

26. The composition of claim 24, wherein the heterologous functional agent is conjugated to the surface of the modified PMPs.

27. The composition of any one of claims 23-26, wherein the heterologous functional agent is a polynucleotide.

28. The composition of claim 27, wherein the polynucleotide is chosen from an mRNA, an siRNA or siRNA precursor, a microRNA (miRNA) or miRNA precursor, a plasmid, a Dicer substrate small interfering RNA (dsiRNA), a short hairpin RNA (shRNA), an asymmetric interfering RNA (aiRNA), a peptide nucleic acid (PNA), a morpholino, a locked nucleic acid (LNA), a piwi-interacting RNA (piRNA), a ribozyme, a deoxyribozyme (DNAzyme), an aptamer, a circular RNA (circRNA), a guide RNA (gRNA), an ADAR targeting oligonucleotide, an antisense oligonucleotide, a long non-coding RNA, a ceDNA, a minicircle, a miniplasmid, or a DNA molecule encoding any of these RNAs.

29. The composition of claim 28, wherein the polynucleotide is chosen from an mRNA, an siRNA or siRNA precursor, a miRNA or miRNA precursor, a plasmid, a dsRNA, a shRNA, an aiRNA, a LNA, a piRNA, a ribozyme, a DNAzyme, an aptamer, a circRNA, a gRNA, an ADAR targeting oligonucleotide, an antisense oligonucleotide, a long non-coding RNA, a ceDNA, a minicircle, or a miniplasmid, or a DNA molecule encoding any of these RNAs.

30. The composition of claim 28 or 29, wherein the polynucleotide is an mRNA.

31. The composition of claim 28 or 29, wherein the polynucleotide is an siRNA or a precursor thereof.

32. The composition of claim 28 or 29, wherein the polynucleotide is a plasmid.

33. The composition of any one of claims 27-32, wherein the encapsulation efficiency of the polynucleotide by the modified PMP is at least 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or more than 99%.

34. The composition of any one of claims 1-33, wherein the modified PMPs are lipid reconstructed PMPs (LPMPs).

35. The composition of claim 34, wherein the LPMP is produced by a method comprising lipid extrusion.

36. The composition of claim 33, wherein the LPMP is produced by a method comprising processing a solution comprising a lipid extract of the PMPs in a microfluidics device comprising an aqueous phase, thereby producing the LPMPs.

37. The composition of claim 36, wherein the aqueous phase comprises a heterologous functional agent.

38. The composition of any one of claims 1-37, wherein the modified PMPs have increased cell uptake.

39. The composition of claim 38, wherein the increased cell uptake is an increased cell uptake of at least 1%, 2%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% relative to the cell uptake of the unmodified PMP.

40. The composition of claim 38 or 39, wherein the cell is a mammalian cell.

41. The composition of claim 40, wherein the mammalian cell is a human cell.

42. The composition of claim 38 or 39, wherein the cell is a plant cell.

43. The composition of claim 38 or 39, wherein the cell is a bacterial cell.

44. The composition of claim 38 or 39, wherein the cell is a fungal cell.

45. A method for delivering a PMP to a target cell, the method comprising contacting the target cell with a composition that comprises a PMP comprising a PMP modified to comprise a synthetic charged lipid, wherein the synthetic charged lipid has one or more of the characteristics listed below:

- (i) at least 2 ionizable amines;
- (ii) at least 3 lipid tails; wherein each of the lipid tails is at least 6 carbon atoms in length;
- (iii) a pKa of about 4.5 to about 7.5;
- (iv) an ionizable amine and a heteroorganic group separated by a chain of at least two atoms; and
- (v) an N:P ratio of at least 10;

provided that the charged lipid is not selected from 1'-((2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethyl)azanediyl)bis(dodecan-2-ol) (C12-200), MD1 (cKK-E12), OF2, EPC, ZA3-Ep10, TT3, LP01, 5A2-SC8, Lipid 5 (Moderna), and 98N12-5.

46. The method of claim 45, wherein the PMP further comprises a sterol.

47. The method of claim 45 or 46, wherein the PMP further comprises a PEGylated lipid.

48. The method of any one of claims 45-47, wherein the PMP further comprises a sterol and a PEGylated lipid.

49. The method of claim 46 or 48, wherein the sterol is cholesterol or sitosterol.

50. The method of claim 45, wherein the PEGylated lipid is C18-PEG2k, DMPE-PEG2k, or ALC-0159.

51. The method of claim 45, wherein the synthetic charged lipid, purified PMP lipids, sterol, and PEGylated lipid comprise about 30%-75%, about 35%-50%, about 10%-20%, and about 1%-3%, respectively, of the lipids in the modified PMP.

52. The method of claim 45, wherein the synthetic charged lipid, purified PMP lipids, sterol, and PEGylated lipid comprise about 25%-75%, about 35%-60%, about 10%-20%, and about 0.5%-5%, respectively, of the lipids in the modified PMP.

53. The method of claim 51, wherein the synthetic charged lipid, purified PMP lipids, sterol, and PEGylated lipid are formulated at a molar ratio of 35:50:12.5:2.5.

54. The method of any one of claims 45-53, wherein the synthetic charged lipid is an ionizable lipid and the composition comprising the PMP has a zeta potential of greater than -40 mV at pH 4 when in the absence of cargo.

55. The method of claim 54, wherein the composition comprising the PMP has a zeta potential of greater than 0 mV at pH 4 when in the absence of cargo.

56. The method of claim 55, wherein the composition comprising the PMP has a zeta potential of greater than 20 mV at pH 4 when in the absence of cargo.

57. The method of claim 56, wherein the composition comprising the PMP has a zeta potential of greater than 30 mV at pH 4 when in the absence of cargo.

58. The method of claim 57, wherein the composition comprising the PMP has a zeta potential of about 40 mV at pH 4 when in the absence of cargo.

59. The method of any one of claims 45-53, wherein the composition comprising the PMP has a zeta potential of greater than -30 mV when in the absence of cargo.

60. The method of claim 59, wherein the composition comprising the PMP has a zeta potential of greater than -20 mV when in the absence of cargo.

61. The method of claim 60, wherein the composition comprising the PMP has a zeta potential of greater than -5 mV when in the absence of cargo.

62. The method of claim 61, wherein the composition comprising the PMP has a zeta potential of greater than 0 mV when in the absence of cargo.

63. The method of claim 62, wherein the composition comprising the PMP has a zeta potential of about 30 mV when in the absence of cargo.

64. The method of any one of claims 45-63, wherein the PMP comprises a heterologous functional agent.

65. The method of claim 64, wherein the heterologous functional agent is encapsulated by the PMP.

66. The method of claim 64, wherein the heterologous functional agent is embedded on the surface of the PMP.

67. The method of claim 64, wherein the heterologous functional agent is conjugated to the surface of the PMP.

68. The method of any one of claims 64-67, wherein the heterologous functional agent is a polynucleotide.

69. The method of claim 68, wherein the polynucleotide is chosen from an mRNA, an siRNA or siRNA precursor, a miRNA or miRNA precursor, a plasmid, a dsRNA, a shRNA, an aiRNA, a PNA, a morpholino, a LNA, a piRNA, a ribozyme, a DNAzyme, an aptamer, a circRNA, a gRNA, an ADAR targeting oligonucleotide, an antisense oligonucleotide, a long non-coding RNA, a ceDNA, a minicircle, a miniplasmid, or a DNA molecule encoding any of these RNAs.

70. The method of claim 65, wherein the polynucleotide is chosen from an mRNA, an siRNA or siRNA precursor, a miRNA or miRNA precursor, a plasmid, a dsRNA, a shRNA, an aiRNA, a LNA, a piRNA, a ribozyme, a DNAzyme, an aptamer, a circRNA, a gRNA, an ADAR targeting oligonucleotide, an antisense oligonucleotide, a long non-coding RNA, a ceDNA, a minicircle, or a miniplasmid, or a DNA molecule encoding any of these RNAs.

71. The method of claim 69 or 70, wherein the polynucleotide is an mRNA.

72. The method of claim 69 or 70, wherein the polynucleotide is an siRNA or a precursor thereof.

73. The method of claim 69 or 70, wherein the polynucleotide is a plasmid.

74. The method of any one of claims 68-73, wherein the encapsulation efficiency of the polynucleotide by the PMP is at least 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or more than 99%.

75. The method of any one of claims 45-74, wherein the PMP is a lipid reconstructed PMP (LPMP).

76. The method of any one of claims 45-75, wherein the PMP has increased cell uptake.

77. The method of claim 76, wherein the increased cell uptake is an increased cell uptake of at least 1%, 2%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% relative to the cell uptake of the unmodified PMP.

78. The method of claim 76 or 77, wherein the cell is a mammalian cell.

79. The method of claim 78, wherein the mammalian cell is a human cell.

80. The method of claim 76 or 77, wherein the cell is a plant cell.

81. The method of claim 76 or 77, wherein the cell is a bacterial cell.

82. The method of claim 76 or 77, wherein the cell is a fungal cell.

83. A method of increasing the fitness of a plant, the method comprising delivering to the plant an effective amount of the composition of any one of claims 1-44, wherein the method increases the fitness of the plant relative to an untreated plant.

84. The method of claim 83, wherein the modified PMP comprises an agricultural agent.

85. The method of claim 83 or 84, wherein the plant is a plant of agricultural or horticultural importance.

86. The method of claim 85, wherein the plant is a soybean plant, a wheat plant, or a corn plant.

87. A method of decreasing the fitness of a plant, the method comprising delivering to the plant an effective amount of the composition of any one of claims 1-44, wherein the method decreases the fitness of the plant relative to an untreated plant.

88. The method of claim 87, wherein the modified PMP comprises an agricultural agent.

89. The method of claim 88, wherein the plant is a weed.

90. The method of any one of claims 83-89, wherein the composition is delivered to a leaf, seed, embryo, ovule, meristem, microspore, root, fruit, shoot, pollen, or flower of the plant.

91. A method of increasing the fitness of a mammal, the method comprising delivering to the mammal an effective amount of the composition of any one of claims 1-44, wherein the method increases the fitness of the mammal relative to an untreated mammal.

92. The method of claim 91, wherein the modified PMP comprises a heterologous therapeutic agent.

93. The method of claim 91 or 92, wherein the mammal is a human.

94. A composition comprising a plurality of lipid reconstructed PMPs (LPMPs), wherein the LPMPs are produced by a process comprising the steps of:

- (a) providing a plurality of purified PMPs;
- (b) processing the plurality of PMPs to produce a lipid film;
- (c) reconstituting the lipid film in an organic solvent, wherein the organic solvent is dimethylformamide:methanol (DMF:MeOH), thereby producing a lipid solution; and
- (d) processing the lipid solution of step (c) in a microfluidics device comprising an aqueous phase, thereby producing the LPMPs;

wherein the LPMPs comprise a synthetic charged lipid having one or more of the characteristics listed below:

- (i) at least ionizable amines;

- (ii) at least 3 lipid tails; wherein each of the lipid tails is at least 6 carbon atoms in length;
- (iii) a pKa of about 4.5 to about 7.5;
- (iv) an ionizable amine and a heteroorganic group separated by a chain of at least two atoms; and
- (v) an N:P ratio of at least 10;

provided that the charged lipid is not selected from 1'-((2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethyl)azanediyl)bis(dodecan-2-ol) (C12-200), MD1 (cKK-E12), OF2, EPC, ZA3-Ep10, TT3, LP01, 5A2-SC8, Lipid 5 (Moderna), and 98N12-5.

95. The composition of claim 94, wherein the charged lipid is added to the preparation prior to step (b).

96. The composition of any one of claims 94 or 95, wherein the aqueous phase comprises a citrate buffer having a pH of about 3.2.

97. The composition of any one of claims 94-96, wherein the aqueous phase and the lipid solution are mixed at a 3:1 volumetric ratio.

98. The composition of any one of claims 94-97, wherein the LPMPs comprise a heterologous functional agent.

99. The composition of claim 98, wherein the heterologous functional agent is a polynucleotide.

100. The composition of claim 99, wherein the polynucleotide is chosen from an mRNA, an siRNA or siRNA precursor, a miRNA or miRNA precursor, a plasmid, a dsRNA, a shRNA, an aiRNA, a PNA, a morpholino, a LNA, a piRNA, a ribozyme, a DNzyme, an aptamer, a circRNA, a gRNA, an ADAR targeting oligonucleotide, an antisense oligonucleotide, a long non-coding RNA, a ceDNA, a minicircle, or a miniplasmid, or a DNA molecule encoding any of these RNAs.

101. The composition of claim 100, wherein the polynucleotide is chosen from an mRNA, an siRNA or siRNA precursor, a miRNA or miRNA precursor, a plasmid, a dsRNA, a shRNA, an aiRNA, a LNA, a piRNA, a ribozyme, a DNzyme, an aptamer, a circRNA, a gRNA, an ADAR targeting oligonucleotide, an antisense oligonucleotide, a long non-coding RNA, a ceDNA, a minicircle, a miniplasmid, or a DNA molecule encoding any of these RNAs.

102. The composition of claim 100 or 101, wherein the heterologous functional agent is comprised by the aqueous phase.

103. The composition of any one of claims 94-102, wherein the LPMPs further comprise a sterol.

104. The composition of any one of claims 94-103, wherein the LPMPs further comprise a PEGylated lipid.

105. The composition of any one of claims 94-104, wherein the LPMPs further comprise a sterol and a PEGylated lipid.

106. The composition of claim 103 or 105, wherein the sterol is cholesterol or sitosterol.

107. The composition of claim 105 or 106, wherein the PEGylated lipid is C18-PEG2k, DMPE-PEG2k, or ALC-0159.

108. A method for making LPMPs, the method comprising:

- (a) providing a plurality of purified PMPs;
- (b) processing the plurality of PMPs to produce a lipid film;
- (c) reconstituting the lipid film in an organic solvent, wherein the organic solvent is dimethylformamide:methanol (DMF:MeOH), thereby producing a lipid solution; and
- (d) processing the lipid solution of step (c) in a microfluidics device comprising an aqueous phase, thereby producing the LPMPs;

wherein the LPMPs comprise a synthetic charged lipid having one or more of the characteristics listed below:

- (i) at least 2 ionizable amines;
- (ii) at least 3 lipid tails; wherein each of the lipid tails is at least 6 carbon atoms in length;
- (iii) a pKa of about 4.5 to about 7.5;
- (iv) an ionizable amine and a heteroorganic group separated by a chain of at least two atoms; and
- (v) an N:P ratio of at least 10;

provided that the charged lipid is not selected from 1'-((2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethyl)azanediyl)bisdodecan-2-ol (C12-200), MD1 (cKK-E12), OF2, EPC, ZA3-Ep10, TT3, LP01, 5A2-SC8, Lipid 5 (Moderna), and 98N12-5.

109. The method of claim 108, wherein the charged lipid is added to the preparation prior to step (b).

110. The method of claim 108 or 109, wherein the aqueous phase comprises water, PBS, or a citrate buffer.

111. The method of any one of claims 108-110, wherein the aqueous phase and the lipid solution are mixed at a 3:1 volumetric ratio.

112. The method of any one of claims 108-111, wherein the LPMPs comprise a heterologous functional agent.

113. The method of claim 112, wherein the heterologous functional agent is a polynucleotide.

114. The method of claim 113, wherein the polynucleotide is chosen from an mRNA, an siRNA or siRNA precursor, a miRNA or miRNA precursor, a plasmid, a dsRNA, a shRNA, an aiRNA, a PNA, a

morpholino, a LNA, a piRNA, a ribozyme, a DNAzyme, an aptamer, a circRNA, a gRNA, an ADAR targeting oligonucleotide, an antisense oligonucleotide, a long non-coding RNA, a ceDNA, a minicircle, a miniplasmid, or a DNA molecule encoding any of these RNAs.

115. The method of claim 114, wherein the polynucleotide is chosen from an mRNA, an siRNA or siRNA precursor, a miRNA or miRNA precursor, a plasmid, a dsRNA, a shRNA, an aiRNA, a LNA, a piRNA, a ribozyme, a DNAzyme, an aptamer, a circRNA, a gRNA, an ADAR targeting oligonucleotide, an antisense oligonucleotide, a long non-coding RNA, a ceDNA, a minicircle, or a miniplasmid, or a DNA molecule encoding any of these RNAs

116. The method of any one of claims 112-115, wherein the heterologous functional agent is comprised by the aqueous phase.

117. The method of any one of claims 112-116, wherein the LPMPs further comprise a sterol.

118. The method of any one of claims 112-117, wherein the LPMPs further comprise a PEGylated lipid.

119. The method of any one of claims 112-118, wherein the LPMPs further comprise a sterol and a PEGylated lipid.

120. The method of claim 117 or 119, wherein the sterol is cholesterol or sitosterol.

121. The method of claim 114, wherein the PEGylated lipid is C18-PEG2k, DMPE-PEG2k, or ALC-0159.

122. The composition of any one of claims 1-44 and 94-107 or the method of any one of claims 45-93 and 108-121, wherein the synthetic charged lipid is characterized as having at least 3 ionizable amines.

123. The composition of any one of claims 1-44 and 94-107 or the method of any one of claims 45-93 and 108-121, wherein the synthetic charged lipid is characterized as having at least 4 ionizable amines.

124. The composition of any one of claims 1-44 and 94-107 or the method of any one of claims 45-93 and 108-121, wherein the synthetic charged lipid is characterized as having at least 5 ionizable amines.

125. The composition of any one of claims 1-44 and 94-107 or the method of any one of claims 45-93 and 108-121, wherein the synthetic charged lipid is characterized as having at least 6 ionizable amines.

126. The composition of any one of claims 1-44 and 94-107 or the method of any one of claims 45-93 and 108-121, wherein the synthetic charged lipid is characterized as having at least 4 lipid tails.

127. The composition of any one of claims 1-44 and 94-107 or the method of any one of claims 45-93 and 108-121, wherein the synthetic charged lipid is characterized as having at least 5 lipid tails.

128. The composition of any one of claims 1-44 and 94-107 or the method of any one of claims 45-93 and 108-121, wherein the synthetic charged lipid is characterized as having at least 6 lipid tails.

129. The composition of any one of claims 1-44 and 94-107 or the method of any one of claims 45-93 and 108-121, wherein each lipid tail is independently 6-18 carbon atoms in length.

130. The composition of any one of claims 1-44 and 94-107 or the method of any one of claims 45-93 and 108-121, wherein the pKa is about 6.5 to about 7.5.

131. The composition of any one of claims 1-44 and 94-107 or the method of any one of claims 45-93 and 108-121, wherein the heteroorganic group is hydroxyl.

132. The composition of any one of claims 1-44 and 94-107 or the method of any one of claims 45-93 and 108-121, wherein the synthetic charged lipid has at least two of the characteristics.

133. The composition of any one of claims 1-44 and 94-107 or the method of any one of claims 45-93 and 108-121, wherein the synthetic charged lipid has at least three of the characteristics.

134. The composition of any one of claims 1-44 and 94-107 or the method of any one of claims 45-93 and 108-121, wherein the synthetic charged lipid has four or five of the characteristics.

135. The composition of any one of claims 1-44 and 94-107 or the method of any one of claims 45-93 and 108-121, wherein the synthetic charged lipid has all of the characteristics.

136. The composition of any one of claims 1-44, 94-107, and 122-135 or the method of any one of claims 45-93 and 108-135, wherein the heteroorganic group comprises a hydrogen bond donor.

137. The composition of any one of claims 1-44, 94-107, and 122-135 or the method of any one of claims 45-93 and 108-135, wherein the heteroorganic group comprises a hydrogen bond acceptor.

138. The composition of any one of claims 1-44, 94-107, and 122-135 or the method of any one of claims 45-93 and 108-135, wherein the heteroorganic group is -OH, -SH, -(CO)H, -CO₂H, -NH₂, -CONH₂, optionally substituted C₁-C₆ alkoxy, or fluorine.