CLAIMS

1. A vaccine composition comprising:

(i) a nanoparticle comprising a non-ionic detergent core and a viral glycoprotein, wherein the viral glycoprotein is associated with the core and the detergent is present at about 0.03% to about 0.05%; and

(ii) a pharmaceutically acceptable buffer; and wherein the non-ionic detergent is selected from the group consisting of PS20, PS40, PS60, PS65, and PS80.

2. The vaccine composition of claim 1, wherein the viral glycoprotein is selected from the group consisting of an RSV F protein, an Ebola Glycoprotein, a Rabies Virus G Protein, an influenza HA protein, an influenza NA protein, and combinations thereof.

3. The vaccine composition of claim 2, wherein the viral glycoprotein is an RSV F protein and the molar ratio of non-ionic detergent to viral glycoprotein is about 30:1 to about 60:1.

4. The vaccine composition of claim 3, wherein the RSV F protein comprises a deletion of to 10 amino acids corresponding to amino acids 137-146 of SEQ ID NO:2 and an inactivated primary furin cleavage site corresponding to amino acids 131 to 136 of SEQ ID NO:2, wherein the primary furin cleavage site is inactivated by mutation.

5. The vaccine composition of claim 4, wherein the composition is substantially free of any other detergent.

6. The vaccine composition of claim 4, wherein the RSV-F protein is selected from the group consisting of SEQ ID NOS:1-13 and variants of SEQ ID NOS:1-13 lacking part or all of the N-terminal signal peptide.

7. The vaccine composition of claim 6, wherein the RSV F protein comprises SEQ ID NO:19.

8. The vaccine composition of claim 6, wherein the RSV F protein consists of SEQ ID NO:19.

9. The vaccine composition of claim 8, wherein the non-ionic detergent is PS80.

10. The vaccine composition of claim 9, wherein the molar ratio is about 50.

11. The vaccine composition of claim 1, wherein the pharmaceutically acceptable buffer comprises

(i) sodium phosphate at 15 mM to 25 mM;

(ii) NaCl at about 150 mM;

(iii) histidine at 0.25% to 2% w/v;

wherein the composition pH is between 5.8 and 6.4.

12. The vaccine composition of claim 11, wherein the pharmaceutically acceptable buffer comprises

(i) sodium phosphate at about 22 mM;

(ii) NaCl at about 150 mM;

(iii) histidine at about 1%;

wherein the composition pH is about 6.2.

13. The vaccine composition of any of claims 1-12, wherein the RSV F protein further comprises fatty acids linked to the protein.

14. The vaccine composition of claim 1, wherein the nanoparticles have a Z-ave diameter of about 20 nm to about 60 nm.

15. The vaccine composition of claim 2, wherein the vaccine composition comprises RSV F protein at a concentration of between about 60 μg/mL and about 290 μg/mL.

16. The vaccine composition of claim 1, wherein the shape of the nanoparticle is a spherical oligomer or an anisotropic rod.

17. The vaccine composition of any of claims 1-16, wherein the vaccine composition is substantially free of any other detergent as determined by analytical ultracentrifugation.

18. The vaccine composition of claim 10, wherein each nanoparticle comprises between 4 and 7 RSV F protein trimers.

19. A method of preventing a viral infection comprising administering the vaccine composition of any of claims 1-18 to a subject.

20. The method of claim 19, wherein composition is administered intramuscularly.

21. The method of claim 19, wherein each dose consists of between about 30 μg and about 150 μg of the RSV F protein.

22. The method of claim 19, wherein a single dose of the composition is administered.

23. The method of claim 19, wherein the subject is selected from the group consisting of an adult, a senior adult, a pregnant female adult, a child, a neonate, and an infant.

24. The method of claim 19, wherein the composition comprises an adjuvant.

25. The method of claim 24, wherein the adjuvant is an alum adjuvant and at least 80% of the adjuvant is bound to the nanoparticle.

26. The method of claim 19, wherein the composition contains no added adjuvant.

27. The method of 22, wherein the dose is in a volume of about 0.5 mL.

28. A composition comprising a heterologous population of at least three RSV F nanoparticles types, wherein each nanoparticle comprises at least one RSV F protein trimer surrounding a detergent-containing core that comprises PS80; and

wherein the first RSV F nanoparticle type comprises anisotropic rods,

wherein the second RSV F nanoparticle type comprises spherical oligomers,

and wherein the third RSV F nanoparticle type comprises intermediates of anisotropic rods and spherical oligomers.

29. The composition of claim 28, wherein each RSV F trimer is selected from the group consisting of RSV F proteins having a deletion of 1 to 10 amino acids corresponding to residues 137-146 of SEQ ID NO:2, and mixtures thereof.

30. The composition of claim 28 wherein each RSV F trimer comprises RSV F proteins having (i) a deletion of 1 to 10 amino acids corresponding to residues 137-146 of SEQ ID NO:2 and (ii) an inactivated primary fusion cleavage site, and mixtures thereof.

31. The composition of claim 29 wherein the RSV F protein is selected from SEQ ID NOS:1-13 and variants of SEQ ID NOS:1-13 lacking part or all of the N-terminal signal peptide.

32. The composition of claim 31 wherein the RSV F protein comprises SEQ ID NO:19.

33. The composition of claim 31 wherein the RSV F protein consists of SEQ ID NO:19.

34. A method of manufacturing an RSV F protein nanoparticle comprising preparing an RSV F protein extract from a host cell using a first detergent and exchanging the first detergent for a second detergent, wherein the second detergent is PS80, whereby the nanoparticle exhibits enhanced stability when maintained in an amount of PS80 about 0.03% to about 0.05%

35. The method of claim 34, wherein the first detergent is NP-9.

36 The method of claim 34 wherein the enhanced stability is selected from one or more of protease resistance, oxidative stress resistance, thermal stress resistance, and resistance to agitation.

37. The method of claim 34 wherein the PS80 exists in a molar ratio to the RSV F protein at about 30 to about 60.

38. A nanoparticle comprising a viral protein trimer associated with a PS80 detergent core, wherein the molar ratio of the PS80: viral protein trimer is about 30 to about 60, preferably about 50.

39. The nanoparticle of claim 38 wherein the viral protein trimer is an RSV F protein trimer.

40. The nanoparticle of claim 38, wherein the nanoparticle has an average diameter (Z-ave) about 20 to about 60 nm as measured by dynamic light scattering.

41. The nanoparticle of claim 38 wherein each RSV F protein trimer contains an RSV F protein selected from the group consisting of RSV F proteins having a deletion of 1 to 10 amino acids corresponding to residues 137-146 of SEQ ID NO:2.

42. The nanoparticle of claim 41 further comprising an inactivated primary fusion cleavage site.

43. The nanoparticle of claim 39 wherein the RSV F protein has the sequence of any of SEQ ID NOS: 1-13 or SEQ ID NOS:1-13 lacking part or all of the N-terminal signal peptide.

44. The nanoparticle of claim 39 wherein the RSV F protein comprises a deletion of ten amino acids corresponding to residues 137-146 of SEQ ID NO:2 and inactivation of the primary furin cleavage site by mutation of arginine residues at positions 133, 135, and 136 to glutamine.

45. The nanoparticle of claim 44 wherein the RSV F protein comprises SEQ ID NO:19.

46. The nanoparticle of claim 44 wherein the RSV F protein consists of SEQ ID NO:19.

47. A method of producing a nanoparticle comprising protein antigens surrounding a non-ionic detergent core comprising

(i) binding a protein extract comprising a first detergent to a protein purification column;

(ii) performing a detergent exchange by substantially replacing the first detergent with a second detergent; and

(iii) eluting the protein extract from the column in the presence of the second detergent to provide the nanoparticle;

wherein the molar ratio of the second detergent:protein is about 30 to about 60; and wherein the second detergent is selected from the group consisting of PS20, PS40, PS60, PS65, and PS80.

48. The method of claim 47 wherein the protein antigen is a viral glycoprotein.

49. The method of claim 48 wherein the non-ionic detergent is PS80.

50. The method of claim 47 wherein the first detergent is NP-9.

51. The method of claim 47 wherein the protein antigen is an RSV-F glycoprotein.

52. The method of claim 47 wherein the molar ratio is about 45 to about 60.

53. The method of claim 52 wherein the molar ratio is about 45.

54. The method of claim 47 further comprising step (iv) combining the nanoparticle with an alum adjuvant to provide a composition comprising a nanoparticle having at least 80% of the nanoparticle in the composition bound to the alum.

55. The method of claim 54 wherein at least 90% of the nanoparticle is bound to the alum.

56. The method of claim 47 wherein the protein extract is prepared by steps comprising recombinantly expressing the protein in an Sf9 host cell and treating the host cell with the first detergent to provide a protein extract, wherein the Sf9 cells is a single knock-out for a cathepsin L or a chitinase, or a double knock-out cell for both a cathepsin L and chitinase.

57. The method of claim 47 wherein the protein purification column is a lentil lectin column.

58. A combination nanoparticle comprising a non-ionic detergent core and at least two viral proteins, wherein each viral protein is associated with the core and wherein the molar ratio of non-ionic detergent: at least one viral protein is about 30 to about 60.

59. The combination nanoparticle of claim 58 wherein the nanoparticle comprises an influenza HA protein and an RSV F protein.

60. The combination nanoparticle of claim 58 wherein the nanoparticle comprises an influenza HA protein and an influenza NA protein.

61. The combination nanoparticle of claim 60 wherein the HA and NA protein are from the same influenza sub-type.

62. The combination nanoparticle of claim 60 wherein the HA and NA protein are from different influenza sub-types.

63. The combination nanoparticle of claim 60 wherein at least one of the HA and NA protein is from a Group 1 or Group 2 influenza.

64. The combination nanoparticle of claim 60 wherein at least one of the HA and NA protein is from a B type influenza.

65. A method of inducing an immune response comprising administering a composition of claim 1 to a subject.

66. The method of claim 65 wherein the subject is a human female.

67. The method of claim 66 wherein the human female is pregnant.

68. A method of boosting an immune response in an infant comprising administering to an infant harboring anti-RSV F antibodies a composition of claim 1.

69. A method of preparing a recombinant trypsin-resistant influenza HA nanoparticle comprising the steps of

(i) binding a protein extract comprising a first detergent to a protein purification column;

(ii) performing a detergent exchange by substantially replacing the first detergent with a second detergent; and

(iii) eluting the protein extract from the column in the presence of the second detergent to provide the nanoparticle;

wherein the transition midpoint (Tm) of the nanoparticle, as measured by differential scanning calorimetry is at least about 60, wherein no buffer used during preparation has a pH of below 7.0.

70. A recombinant trypsin-resistant HA nanoparticle prepared according to the method of claim 69.

71. A vaccine composition comprising a nanoparticle of claim 70 and a pharmaceutically acceptable carrier.

72. The vaccine composition comprising a nanoparticle of claim 71 comprising an adjuvant.

73. The method of claim 35 wherein the exchange of the first detergent is substantially complete such that the amount of NP-9 detected by HPLC is less than about 0.1%, less than about 0.01%, less than about 0.001%, or is undetectable.

74. An Ebola virus glycoprotein (GP) nanoparticle, comprising an Ebola virus GP trimer associated with a non-ionic detergent core.

75. The nanoparticle of claim 74 wherein the non-ionic detergent in the core comprises polysorbate-80 (PS80).

76. The nanoparticle of claim 74 wherein the nanoparticle has an average diameter about 20 to about 40 nm.

77. The nanoparticle of claim 74 wherein the nanoparticle contains multiple copies of the Ebola virus GP trimer.

78. The nanoparticle of claim 77 wherein the nanoparticle contains up to 15 trimers.

79. The nanoparticle of claim 74 wherein the nanoparticle is produced in an Sf9 cell.

80. The nanoparticle of claim 74 wherein the Ebola virus GP amino acid sequence is about 85% identical, about 90% identical, about 95% identical, about 97% identical, or about 98% identical to SEQ ID NO:29.

81. The nanoparticle of claim 74 wherein the Ebola virus GP amino acid sequence comprises SEQ ID NO:29.

82. The nanoparticle of claim 74 wherein the Ebola virus GP amino acid sequence consists of SEQ ID NO:29.

83. A vaccine composition comprising the nanoparticle of any of claims 74 to 82 and a saponin adjuvant, wherein the saponin adjuvant consists of Matrix A and Matrix C.

84. The vaccine composition of claim 83 wherein the Matrix A and Matrix C are present in a ratio of 85:15.

85. A method of inducing an immune response against Ebola Virus in a human comprising administering to the human a composition comprising an Ebola virus glycoprotein (GP) nanoparticle and a saponin adjuvant,

wherein the saponin adjuvant consists of Matrix A and Matrix C, and

wherein the nanoparticle comprises an Ebola virus GP trimer attached to a non-ionic detergent core.

86. The method of claim 85 wherein the Matrix A and Matrix C saponin adjuvants are present in a ratio of 85:15.

87. The method of claim 85 wherein the amino acid sequence of the Ebola virus GP is about 85% identical, about 90% identical, about 95% identical, about 97% identical, or about 98% identical to SEQ ID NO:29.

88. The method of claim 85 wherein the composition is administered intramuscularly.

89. The method of claim 85 wherein the immune response comprises one or more of: induction of IgG1 antibodies, induction of IgG2a antibodies, formation of long-lived plasma B cells, a follicular helper T cell (T_{FH}) response, a CD4+ T-cell response, and a CD8+ T-cell response.

90. The method of claim 85 wherein the composition comprises a heterogeneous population of GP nanoparticles having 2 to 6 trimers per nanoparticle.

91. A method of preventing Ebola Virus infection or disease in a human comprising intramuscularly administering to the human a vaccine composition of claim 83.

92. A nucleic acid encoding an Ebola protein comprising or consisting of SEQ ID NO:29.

93. An Sf9 host cell comprising the nucleic acid of claim 92.

CLAIMS

1. A composition comprising:

(i) a nanoparticle comprising a non-ionic detergent core and a viral glycoprotein, wherein the viral glycoprotein consists of a trimeric influenza HA glycoprotein, wherein the influenza HA glycoprotein contains a head region that projects outward from the non-ionic detergent core and a transmembrane domain that is associated with the non-ionic detergent core, wherein the amino acid sequence of the influenza HA glycoprotein has 100% identity to the amino acid sequence of the native influenza HA protein; wherein the non-ionic detergent is PS80;

and

(ii) a pharmaceutically acceptable buffer.

2. The composition of claim 1, wherein the influenza HA protein sub-type is selected from the group consisting of H1, H3, H4, H5, and H7.

3. The composition of claim 1, wherein the pharmaceutically acceptable buffer comprises (i) sodium phosphate at 15 mM to 50 mM; (ii) NaCl at about 150 to about 250 mM; wherein the composition pH is between 7.0 and 7.5.

4. The composition of claim 1, wherein the pharmaceutically acceptable buffer comprises (i) sodium phosphate at about 25 mM; (ii) NaCl at about 150 mM; wherein the composition pH is about 7.5.

5. A vaccine composition comprising:

(i) a nanoparticle comprising a non-ionic detergent core and a viral glycoprotein, wherein the viral glycoprotein consists of a trimeric influenza HA glycoprotein, and wherein the non-ionic detergent is PS80, wherein the influenza HA glycoprotein contains a head region that projects outward from the non-ionic detergent core and a transmembrane domain that is associated with the non-ionic detergent core, wherein the amino acid sequence of the influenza

HA glycoprotein has 100% identity to the amino acid sequence of the native influenza HA protein;

(ii) a buffer comprising 25 mM sodium phosphate, pH 7.5, and 150 mM sodium chloride; and

(iii) an ISCOM matrix adjuvant.

6. The vaccine composition of claim 5, wherein the ISCOM matrix adjuvant comprises a first ISCOM matrix containing fraction A, and a second ISCOM matrix containing fraction C.

7. The vaccine composition of claim 6, wherein the first ISCOM matrix comprises 70% to 95% by weight of the total ISCOM matrix, and the second ISCOM matrix comprises the remainder.

8. The vaccine composition of claim 7, wherein the first ISCOM matrix comprises 85% by weight of the total ISCOM matrix, and the second ISCOM matrix comprises the remainder.

9. The vaccine composition of claim 5, wherein the influenza HA protein sub-type is selected from the group consisting of H1, H3, H4, H5, and H7.

10. A method of preparing a recombinant influenza nanoparticle comprising steps of:

(i) binding a protein extract comprising a first detergent and influenza HA glycoprotein to a protein purification column, wherein the column binds the influenza glycoprotein;

(ii) performing a detergent exchange by substantially replacing the first detergent with a second detergent; and

(iii) eluting the bound influenza glycoprotein from the column in the presence of the second detergent to provide the nanoparticle, wherein the second detergent is PS80;

wherein the transition midpoint (Tm) of the nanoparticle, as measured by differential scanning calorimetry is at least about 60, wherein no buffer used during preparation has a pH of below 7.0, and wherein the amino acid sequence of the influenza HA glycoprotein has 100%

identity to the amino acid sequence of the native influenza HA glycoprotein, and wherein the nanoparticle consists of a viral glycoprotein and a non-ionic detergent core, wherein the viral glycoprotein is a trimeric HA glycoprotein, and wherein the HA glycoprotein contains a head region that projects outward from the non-ionic detergent core and a transmembrane domain that is associated with the non-ionic detergent core, and wherein the non-ionic detergent is PS80.

11. The method of claim 10, wherein the first detergent is NP-9.

12. The composition of claim 1, wherein the nanoparticle has a transition midpoint (Tm) peak in a range of about 60°C to about 75°C and wherein the Tm is measured by Differential Scanning Calorimetry.

13. The vaccine composition of claim 5, wherein the nanoparticle has a transition midpoint (Tm) peak in a range of about 60°C to about 75°C and wherein the Tm is measured by Differential Scanning Calorimetry.

14. The composition of claim 1, further comprising a second nanoparticle comprising a second influenza HA glycoprotein, wherein the HA glycoprotein contains a head region, which projects outward from the non-ionic detergent core, and a transmembrane domain, which is associated with the non-ionic detergent core, wherein the second influenza HA glycoprotein comprises an HA1 polypeptide and an HA2 polypeptide connected by a disulfide bond, wherein the non-ionic detergent is PS80.

15. The vaccine composition of claim 5, further comprising a second nanoparticle comprising a second influenza HA glycoprotein, wherein the HA glycoprotein contains a head region, which projects outward from the non-ionic detergent core, and a transmembrane domain, which is associated with the non-ionic detergent core, wherein the second influenza HA glycoprotein comprises an HA1 polypeptide and an HA2 polypeptide connected by a disulfide bond, wherein the non-ionic detergent is PS80.

16. The composition of claim 1, comprising a second nanoparticle, wherein the HA glycoprotein in the first nanoparticle is from a different influenza strain than the second nanoparticle.

17. The composition of claim 1, comprising three nanoparticles, wherein the HA glycoprotein in each nanoparticle is from a different influenza strain than the other two nanoparticles.

18. The composition of claim 1, comprising four nanoparticles, wherein the HA glycoprotein in each nanoparticle is from a different influenza strain than the other three nanoparticles.

19. The vaccine composition of claim 5, comprising a second nanoparticle, wherein the HA glycoprotein in the first nanoparticle is from a different influenza strain than the second nanoparticle.

20. The vaccine composition of claim 5, comprising three nanoparticles, wherein the HA glycoprotein in each nanoparticle is from a different influenza strain than the other two nanoparticles.

21. The vaccine composition of claim 5, comprising four nanoparticles, wherein the HA glycoprotein in each nanoparticle is from a different influenza strain than the other three nanoparticles.

22. The composition of claim 1, wherein the HA glycoprotein is a HA0 glycoprotein.

23. The vaccine composition of claim 5, wherein the HA glycoprotein is a HA0 glycoprotein.

24. The method of claim 10, wherein the HA glycoprotein is a HA0 glycoprotein.