CLAIMS

What is claimed is:

1. A recombinant influenza neuraminidase (NA) tetramer, comprising four copies of a fusion protein, said fusion protein comprising, in N- to C-terminal order, a DA4B tetramerization domain and an influenza NA head domain,

   wherein the DA4B tetramerization domain comprises, consists essentially of, or consists of a DA4B polypeptide sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to any one of SEQ ID NOs: 1-5.

2. The tetramer of claim 1, further comprising a linker between said DA4B tetramerization domain and said influence NA head domain, wherein the linker consists of GGSGGSGG (SEQ ID NO: 11) or comprises the polypeptide sequence GGSGGSGG (SEQ ID NO: 11), the linker consists of ATPRGG (SEQ ID NO: 12) or comprises the polypeptide sequence ATPRGG (SEQ ID NO: 12), the linker consists of ATPRGSGG (SEQ ID NO: 13) or comprises the polypeptide sequence ATPRGSGG (SEQ ID NO: 13), or the linker consists of GGATPRGSGG (SEQ ID NO: 14) or comprises the polypeptide sequence GGATPRGSGG (SEQ ID NO: 14).

3. The tetramer of any one of claims 1 to 2, wherein the influenza NA head domain comprises an NA polypeptide sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to any one of SEQ ID NOs: 15-31 or 423.

4. The tetramer of any one of claim 1 to 3, wherein the influenza NA head domain comprises three or more amino acid substitutions selected from the group consisting of I99P, Y100L, T131Q, C161V, E165S, S172A, V177I, S196T, V205I, Q408M, R419V, and V453T, numbered relative SEQ ID NO: 15;

   optionally, wherein the influenza NA head domain comprises amino acid substitutions, numbered relative to SEQ ID NO: 15, selected from.

   a) I99P, C161V, E165S, S172A, S196T;
   b) I99P, T131Q, C161V, E165S, S172A, S196T;
   c) I99P, T131Q, C161V, E165S, S172A, V177I, S196T;
   d) I99P, T131Q, C161V, E165S, S172A, S196T, V453T;
   e) I99P, T131Q, C161V, E165S, S172A, V177I, S196T, V205I;
   f) I99P, Y100L, T131Q, C161V, E165S, S172A, V177I, S196T, V205I, Q408M, R419V;

g) I99P, C161V, E165S, S172A, V177I, S196T;
h) I99P, C161V, S172A, S196T; or
i) I99P, C161V, S172A, V177I, S196T.

5. The tetramer of any one of claim 1 to 4, wherein:
   a) the NA head domains are in a closed conformation;
   b) the tetramer has increased thermal stability in the presence of chelating agent compared to a tetramer with tetrabrachion tetramerization domain;
   c) the tetramer has increased interaction to neuraminidase inhibitor compared to a tetramer with tetrabrachion tetramerization domain; and/or
   d) the tetramer is expressed in recombinant cell culture at increased yield compared to a tetramer with a tetrabrachion tetramerization domain.
6. A fusion protein comprised by the tetramer of any one of claims 1 to 5.
7. A polynucleotide encoding the fusion protein of claim 6, optionally wherein said polynucleotide is an mRNA.
8. A pharmaceutical composition, comprising the tetramer of any one of claims 1 to 5 and a pharmaceutically acceptable carrier.
9. Use of an effective amount of the pharmaceutical composition of claim 8 in the manufacture of a medicament for immunizing a subject in need thereof against infection by influenza or for treating and/or preventing influenza in a subject in need thereof.
10. The use of claim 9, wherein the medicament generates an antibody response to the tetramer equivalent to or greater than the antibody response to administration of a tetramer with a tetrabrachion tetramerization domain and/or at least as effectively as administering a tetramer with a tetrabrachion tetramerization domain.
11. A method of producing a recombinant influenza neuraminidase (NA) tetramer, comprising culturing a host cell comprising the polynucleotide of claim 7 in cell culture media, and harvesting the recombinant influenza neuraminidase (NA) tetramer from the cell culture media or from the host cell.
12. A recombinant influenza neuraminidase (NA) polypeptide, comprising an influenza NA head domain comprising one or more amino acid substitutions selected from the group consisting of I99P, Y100L, T131Q, C161V, E165S, S172A, V177I, S196T, V205I, Q408M, R419V, and V453T, numbered relative SEQ ID NO: 15.
13. A pharmaceutical composition, comprising a recombinant influenza neuraminidase (NA) tetramer according to any one of claims 1 to 5 and an

influenza HA vaccine antigen, wherein the influenza HA vaccine antigen is a protein nanostructure comprising an influenza HA ectodomain, optionally, wherein the protein nanostructure comprises a trimeric component comprising a trimer protein comprising a fusion protein, wherein the fusion protein comprises, in N- to C-terminal order, the influenza HA ectodomain, a polypeptide linker, and an assembly domain.

14.The pharmaceutical composition of claim 13,

wherein the assembly domain comprises a polypeptide sequence that is at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to any one of SEQ ID NOs: 458-460;

wherein the influenza HA ectodomain comprises a polypeptide sequence that is at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to any one of SEQ ID NOs: 452-455, or 465;

wherein the pentamer protein comprises a polypeptide sequence that is at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to any one of SEQ ID NOs: 461-463;

wherein the trimer protein comprises a polypeptide sequence that is at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to any one of SEQ ID NOs: 448-451, or 464; and/or

wherein the polypeptide linker comprises a polypeptide sequence that is at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 456 or 457.

15. A protein nanostructure comprising a trimeric component comprising a trimer polypeptide and a pentameric component comprising a pentamer polypeptide, wherein the trimer polypeptide is a fusion protein comprising, in N- to C-terminal order, an influenza HA ectodomain, a polypeptide linker, and an assembly domain,

wherein the assembly domain comprises a polypeptide sequence at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to any one of SEQ ID NOs: 458-460;

wherein the influenza HA ectodomain comprises a polypeptide sequence that is at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to any one of SEQ ID NOs: 452-455, or 465;

wherein the pentamer protein comprises a polypeptide sequence that is at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to any one of SEQ ID NOs: 461-463;

wherein the trimer protein comprises a polypeptide sequence that is at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to any one of SEQ ID NOs: 448-451, or 464; and/or

wherein the polypeptide linker comprises a polypeptide sequence that is at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 456 or 457.