

with affinity for IgG or for serum albumin.

[0007] U.S. Patent No. 8,969,521 (Baker, King, Sheffler and, Yeates) discloses a general method for designing self-assembling protein nanomaterials, and an isolated polypeptide, comprising a specific 184 amino acid sequence, capable of forming a multimeric assembly.

[0008] U.S. Patent Application Publication No. 20070218547 (Yeates, Padilla, Yoshida, and Colovos) discloses self assembling proteins for producing extended materials, including a fusion protein comprising a first oligomerization domain that naturally associates into homodimeric structures and a second oligomerization domain that naturally associates into homotetrameric structures, wherein said first and second oligomerization domains are rigidly linked to each other.

SUMMARY OF THE INVENTION

[0009] The present invention provides for a protein cage polypeptide (or scaffolding protein) useful or capable of forming a hollow tetrahedral pyramid structure, wherein the protein cage polypeptide, or scaffolding protein, is capable of binding specifically to an antibody or part thereof, or any chimeric protein, molecule or compound comprising the antibody, or part thereof.

[0009a] In one aspect, provided herein is a protein cage polypeptide, or scaffolding protein, useful or capable of forming a hollow tetrahedral pyramid structure, wherein the protein cage polypeptide, or scaffolding protein, is capable of binding specifically to an antibody or part thereof; and wherein the protein cage polypeptide, or scaffolding protein, comprises (i) an amino acid sequence comprising at least 85% amino acid identity to any one of SEQ ID NOs: 20-39; and (ii) an amino acid sequence capable of specifically binding to an antibody or part thereof, wherein the amino acid sequence comprises DCAWHLGELVWCT (SEQ ID NO:41) or GCDCAWHLGELVWCTCG (SEQ ID NO:42); and wherein the protein cage polypeptide, or scaffolding protein, comprises the following structure: Polypeptide 1—AHL—Polypeptide 2—INSERT A— Polypeptide 3—INSERT B— Polypeptide 4; wherein AHL is an “alpha helical linker” and INSERT A and INSERT B designate a site at which the amino acid sequence capable of specifically binding an antibody or part thereof is introduced; and wherein the amino acid sequence is introduced into the INSERT A site of the protein cage polypeptide between positions corresponding to positions 318 and 322 of SEQ ID NO:40; or introduced into the INSERT B site of the protein cage polypeptide between positions corresponding to positions 442 and 449 of SEQ ID NO:40.

[0009b] In another aspect, provided herein is a hollow tetrahedral pyramid structure comprising twelve protein cage polypeptides as described herein assembled as the tetrahedral pyramid structure.

[0009c] In another aspect, provided herein is a “self-assembling protein nanoparticle decorated with antibodies” (SAPNA) which is a chimeric protein assembly comprising: (a) one or more antibodies and (b) a protein cage polypeptide that provides a scaffold upon which to array the antibodies, wherein the protein cage polypeptide comprises an amino acid sequence having at least 90% amino acid identity to any one of SEQ ID NOs: 20-39; and wherein the one or more antibodies are bound to an amino acid sequence capable of specifically binding to an antibody or part thereof, wherein the amino acid sequence comprises DCAWHLGELVWCT (SEQ ID NO:41) or GCDCAWHLGELVWCTCG (SEQ ID NO:42); inserted at an INSERT A site or an INSERT B site of the protein cage polypeptide, wherein INSERT A and INSERT B designate a site at which the amino acid sequence capable of specifically binding an antibody or part thereof can be inserted; and wherein the site of insertion for INSERT A is between position 318 and 322 with reference to SEQ ID NO:40; and the site of insertion for INSERT B is between position 442 and 449 with reference to SEQ ID NO:40.

[0009d] In yet another aspect, provided herein is a “self-assembling protein nanoparticle” (SAPNA) structure comprising: (1) one protein cage polypeptide or scaffolding protein as described herein, or a plurality thereof assembled into a 3-dimensional assembly, (2) optionally one or more human or rabbit IgG antibodies, (3) optionally an IgG binding loop, and (4) optionally, when the plurality of polypeptides or scaffolding proteins are assembled into a 3-dimensional assembly, a cargo of interest confined or enclosed by the 3-dimensional assembly.

[0009e] In yet another aspect, provided herein is a method for detecting or isolating a pathogenic biological agent, or part thereof, the method comprising: (a) providing a “self-assembling protein nanoparticle decorated with antibody” (SAPNA) as described herein wherein the antibody is capable of binding specifically to a pathogenic biological agent, or part thereof; (b) contacting the SAPNA with a sample comprising the pathogenic biological agent, or part thereof, such that the SAPNA binds the pathogenic biological agent, or part thereof; (c) detecting the SAPNA pathogenic biological agent, or part thereof via detection, and/or separating the SAPNA bound pathogenic biological agent, or part thereof, from the rest of the sample; and (d) determining the abundance of the pathogenic biological agent, or part thereof.

[0010] In some embodiments, the antibody is an IgG antibody. In some embodiments, the part of the antibody is an Fc region of an antibody, such as an IgG, IgA, IgD, IgE, or IgM antibody. In some embodiments, the antibody is a human, chicken, mice, rabbit, sheep, or goat antibody. In some embodiments, the antibody is a humanized antibody. In some embodiments, the IgG antibody is a human IgG antibody. In some embodiments, the antibody is part of a chimeric protein, molecule or compound, comprising the antibody, or part thereof. In some embodiments, the chimeric protein, or other molecule or compound, comprises an Fc

What is claimed is:

1. A protein cage polypeptide, or scaffolding protein, useful or capable of forming a hollow tetrahedral pyramid structure, wherein the protein cage polypeptide, or scaffolding protein, is capable of binding specifically to an antibody or part thereof; and wherein the protein cage polypeptide, or scaffolding protein, comprises
 - (i) an amino acid sequence comprising at least 85% amino acid identity to any one of SEQ ID NOs: 20-39; and
 - (ii) an amino acid sequence capable of specifically binding to an antibody or part thereof, wherein the amino acid sequence comprises DCAWHLGELVWCT (SEQ ID NO:41) or GCDCAWHLGELVWCTCG (SEQ ID NO:42); andwherein the protein cage polypeptide, or scaffolding protein, comprises the following structure: Polypeptide 1—AHL—Polypeptide 2—INSERT A—Polypeptide 3—INSERT B—Polypeptide 4; wherein AHL is an “alpha helical linker” and INSERT A and INSERT B designate a site at which the amino acid sequence capable of specifically binding an antibody or part thereof is introduced; and wherein the amino acid sequence is introduced into the INSERT A site of the protein cage polypeptide between positions corresponding to positions 318 and 322 of SEQ ID NO:40; or introduced into the INSERT B site of the protein cage polypeptide between positions corresponding to positions 442 and 449 of SEQ ID NO:40.
2. The protein cage polypeptide, or scaffolding protein, of claim 1, wherein the amino acid sequence inserted into the INSERT A site has a length of 17 to 25 amino acids and the amino acid sequence inserted into the INSERT B site has a length of 28 to 85 amino acids.
3. The protein cage polypeptide of claim 1 or 2, wherein Polypeptide 1 comprises an amino acid sequence comprising at least 85% amino acid identity to the amino acid sequence from the N-terminus to up to the AQEAQKQK sequence of any one of SEQ ID NO:20-39.
4. The protein cage polypeptide of any one of claims 1-3, wherein Polypeptide 1 comprises an amino acid sequence comprising the following: YGTAR, TDD, LXENLGTR, IDV, TGXRT, and/or SA; wherein X is any charged amino acid

residue.

5. The protein cage polypeptide of any one of claims 1-4, wherein Polypeptide 1 comprises 278 to 303 amino acid residues.
6. The protein cage polypeptide of any one of claims 1-5, wherein AHL comprises an amino acid sequence comprising: AQEAQKQK.
7. The protein cage polypeptide of claim of any one of claims 1-5, wherein AHL comprises 5, 6, 7, 8, 9, 10, or 11 amino acid residues.
8. The protein cage polypeptide of any one of claims 1-7, wherein Polypeptide 2 comprises an amino acid sequence comprising at least 85% amino acid identity to the amino acid sequence from the C-end of the AQEAQKQK sequence to the N-end of the INSERT A site.
9. The protein cage polypeptide of any one of claims 1-8, wherein Polypeptide 2 comprises an amino acid sequence comprising the following: LTEVETYVLS (SEQ ID NO:43).
10. The protein cage polypeptide of any one of claims 1-9, wherein Polypeptide 2 comprises 30 to 36 amino acid residues.
11. The protein cage polypeptide of claim 10, wherein Polypeptide 2 comprises 33 amino acid residues.
12. The protein cage polypeptide of any one of claims 1-11, wherein Polypeptide 3 comprises an amino acid sequence comprising at least 85% amino acid identity to the amino acid sequence from the C-end of the INSERT A site to the N-end of the INSERT B site
13. The protein cage polypeptide of any one of claims 1-12, wherein Polypeptide 3 comprises an amino acid sequence comprising the following: FTLTVPSERGLQR (SEQ ID NO:44) and/or CATCEQIAD (SEQ ID NO:45).
14. The protein cage polypeptide of any one of claims 1-13, wherein Polypeptide 3 comprises 110 to 130 amino acid residues.

15. The protein cage polypeptide of claim 14, wherein Polypeptide 3 comprises 121 amino acid residues.
16. The protein cage polypeptide of any one of claims 1-15, wherein Polypeptide 4 comprises an amino acid sequence comprising at least 85% amino acid identity to the amino acid sequence from the C-end of the INSERT B site.
17. The protein cage polypeptide of any one of claims 1-17, wherein Polypeptide 4 comprises an amino acid sequence comprising: EHHHHHHH.
18. The protein cage polypeptide of any one of claims 1-5 or 7-16, wherein Polypeptide 4 comprises 5 to 13 amino acid residues.
19. The protein cage polypeptide of claim 18, wherein Polypeptide 4 comprises 8 amino acid residues.
20. The protein cage polypeptide of any one of claims 1-19, wherein the protein cage polypeptide comprises an amino acid sequence comprising any one or more, or all, stretches of or individual amino acid residues indicated by an asterisk in Fig. 6.
21. The protein cage polypeptide of any one of claims 1-20, wherein the protein cage polypeptide comprises an amino acid sequence comprising any one or more, or all, charged amino acids stretches in the corresponding position(s) indicated by “#” in Fig. 6.
22. The protein cage polypeptide of any one of claims 1-21, wherein the protein cage polypeptide comprises a polypeptide of from 400 to 700 amino acid residues.
23. The protein cage polypeptide of claim 22, wherein the protein cage polypeptide comprises a polypeptide of from 450 to 650 amino acid residues.
24. The protein cage polypeptide of any one of claims 1-23, wherein the antibody is an IgG antibody.
25. The protein cage polypeptide of any one of claims 1-24, wherein the part of the antibody is a Fc region of an IgG antibody.
26. The protein cage polypeptide of claim 24 or 25, wherein the IgG antibody is a human

IgG antibody.

27. The protein cage polypeptide of claim 24 or 25, wherein the IgG antibody is part of an Fc chimeric protein.
28. The protein cage polypeptide, or scaffolding protein, of any one of claims 1-27, wherein the binding affinity K_a of the protein cage polypeptide, or scaffolding protein, to the antibody or part thereof, is equal to or more than 10^7 M^{-1} , 10^8 M^{-1} , or 10^9 M^{-1} .
29. The protein cage polypeptide, or scaffolding protein, of any of claims 1-28, wherein the protein cage polypeptide, or scaffolding protein, is binding specifically to the antibody or part thereof, or any chimeric protein, molecule or compound comprising the antibody, or part thereof; wherein the antibody or part thereof is binding specifically to a pathogenic biological agent, or part thereof.
30. A hollow tetrahedral pyramid structure comprising twelve protein cage polypeptides of one of claims 1-29 assembled as the tetrahedral pyramid structure.
31. A “self-assembling protein nanoparticle decorated with antibodies” (SAPNA) which is a chimeric protein assembly comprising: (a) one or more antibodies and (b) a protein cage polypeptide that provides a scaffold upon which to array the antibodies, wherein the protein cage polypeptide comprises an amino acid sequence having at least 90% amino acid identity to any one of SEQ ID NOs: 20-39; and wherein the one or more antibodies are bound to an amino acid sequence capable of specifically binding to an antibody or part thereof, wherein the amino acid sequence comprises DCAWHLGELVWCT (SEQ ID NO:41) or GCDCAWHLGELVWCTCG (SEQ ID NO:42); inserted at an INSERT A site or an INSERT B site of the protein cage polypeptide, wherein INSERT A and INSERT B designate a site at which the amino acid sequence capable of specifically binding an antibody or part thereof can be inserted; and wherein the site of insertion for INSERT A is between position 318 and 322 with reference to SEQ ID NO:40; and the site of insertion for INSERT B is between position 442 and 449 with reference to SEQ ID NO:40.
32. The SAPNA of claim 31, wherein the antibody is capable of binding specifically to a pathogenic biological agent, or part thereof.

33. A “self-assembling protein nanoparticle” (SAPNA) structure comprising: (1) one protein cage polypeptide or scaffolding protein of one of claims 1-30, or a plurality thereof assembled into a 3-dimensional assembly, (2) optionally one or more human or rabbit IgG antibodies, (3) optionally an IgG binding loop, and (4) optionally, when the plurality of polypeptides or scaffolding proteins are assembled into a 3-dimensional assembly, a cargo of interest confined or enclosed by the 3-dimensional assembly.
34. The SAPNA structure of claim 33, wherein the 3-dimensional assembly is a tetrahedral pyramid.
35. A method for detecting or isolating a pathogenic biological agent, or part thereof, the method comprising: (a) providing a “self-assembling protein nanoparticle decorated with antibody” (SAPNA) of claim 34 wherein the antibody is capable of binding specifically to a pathogenic biological agent, or part thereof; (b) contacting the SAPNA with a sample comprising the pathogenic biological agent, or part thereof, such that the SAPNA binds the pathogenic biological agent, or part thereof; (c) detecting the SAPNA pathogenic biological agent, or part thereof via detection, and/or separating the SAPNA bound pathogenic biological agent, or part thereof, from the rest of the sample; and (d) determining the abundance of the pathogenic biological agent, or part thereof.