

OPTIMIZED MULTABODY CONSTRUCTS, COMPOSITIONS, AND METHODS

CROSS-REFERENCE TO RELATED APPLICATIONS

5 [0001] The present application claims the benefit of and priority to U.S. Provisional Patent Application Nos. 63/220,920, (filed July 12, 2021) and 63/289,016 (filed December 13, 2021), the entire contents of each of which are hereby incorporated by reference in its entirety for all purposes.

SEQUENCE LISTING

10 [0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on July 12, 2022, is named "Sequence Listing Jul-2022 3206-5068.txt" and is 38,881 bytes in size.

BACKGROUND

15 [0003] Therapeutics based on antibodies or antibody fragments are being developed for a variety of uses, e.g., for treating various diseases or conditions.

[0004] However, antibody-based therapeutics may need to be tuned so that they have
20 desirable characteristics (for example, desirable biodistribution, half-lives, etc.) after administration to a subject. Some antibody-based therapeutics have a format that differs from that of a native immunoglobulin molecule. For example, in some therapeutics, an antibody or antibody fragment is fused to another polypeptide; in some, the antibody or antibody fragment(s) is in a configuration or has a valence not found in nature. These antibody-based
25 therapeutics may also need to be tuned.

[0005] Thus, there remains a need for optimized antibody-based therapeutics.

SUMMARY

[0006] The present disclosure addresses this need with the provision of a suite of optimized self-assembled polypeptide complexes comprising antibody fragments. Depending on the
30 desired characteristics (e.g., pharmacokinetic characteristics), a particular self-assembled polypeptide complex or set of complexes can be chosen for use. For example, in certain embodiments, provided are self-assembled polypeptide complexes that have half-lives or other pharmacokinetic characteristics similar to that of IgG molecules.

[0006a] In one aspect, the present disclosure refers to a self-assembled polypeptide complex comprising: (a) a plurality of first fusion polypeptides, each first fusion polypeptide comprising (1) an Fc polypeptide linked to (2) a nanocage monomer or subunit thereof, wherein the Fc polypeptide comprises a Fc chain having one or more mutations relative to a reference Fc chain of the same Ig class, and (b) a plurality of second fusion polypeptides, each second fusion polypeptide comprising (1) an antigen-binding antibody fragment linked to (2) a nanocage monomer or subunit thereof.

[0006b] In another aspect, the present disclosure refers to a composition comprising the self-assembled polypeptide complex as disclosed herein for use in therapy.

[0006c] In another aspect, the present disclosure refers to a fusion polypeptide comprising (1) an Fc polypeptide linked to (2) a nanocage monomer or subunit thereof, wherein the Fc polypeptide comprises a Fc chain having one or more mutations relative to a reference Fc chain of the same Ig class, wherein the one or more mutations comprise a mutation or set of mutations associated with altered binding to FcRn and/or with altered effector function.

[0007] In accordance with an example, there is provided a self-assembled polypeptide complex comprising

(a) a plurality of first fusion polypeptides, each first fusion polypeptide comprising (1) an Fc polypeptide linked to (2) a nanocage monomer or subunit thereof, wherein the Fc polypeptide comprises a Fc chain having one or more mutations relative to a reference Fc chain of the same Ig class, and

(b) a plurality of second fusion polypeptides, each second fusion polypeptide comprising (1) an antigen-binding antibody fragment linked to (2) a nanocage monomer or subunit thereof.

[0008] In an example, (1) if the Fc polypeptide is an IgG1 Fc polypeptide, the antigen-binding fragment is not a Fab fragment that binds to SARS-CoV-2; and/or (2) if the nanocage monomer is a mouse ferritin monomer and the Fc polypeptide is a mouse IgG2a Fc polypeptide, the antigen-binding antibody fragment is not a Fab fragment that binds to CD19.

[0009] In an example, the nanocage monomer is a ferritin monomer.

[0010] In an example, the ferritin monomer is a ferritin light chain.

[0011] In an example, the self-assembled polypeptide complex does not comprise any ferritin heavy chains or subunits of ferritin heavy chains.

[0012] In an example, the ferritin monomer is a human ferritin.

[0013] In an example, the Fc polypeptide is an IgG1 Fc polypeptide.

[0014] In an example, the Fc polypeptide is an IgG2 Fc polypeptide.

[0015] In an example, the Fc polypeptide is a single-chain Fc (scFc).

[0016] In an example, the Fc polypeptide is an Fc monomer.

[0017] In an example, the antigen-binding antibody fragment comprises a light chain variable domain and a heavy chain variable domain.

[0018] In an example, the antigen-binding antibody fragment is a Fab fragment.

[0019] In an example, each second fusion polypeptide does not comprise any CH2 or CH3 domains.

[0020] In an example, the one or more mutations comprise a mutation or set of mutations associated with altered binding to FcRn.

[0021] In an example, the mutation or set of mutations comprises a mutation at one or more of the following residues: M252, I253, S254, T256, K288, M428, and N434, or combinations thereof, wherein numbering is according to the EU index.

[0022] In an example, the mutation or set of mutations comprises mutations at M428 and N434, wherein numbering is according to the EU index.

[0023] In an example, the mutation or set of mutations comprises M428L and N434S mutations, wherein numbering is according to the EU index.

[0024] In an example, the altered binding to FcRn is decreased binding to FcRn.

[0025] In an example, the mutation or set of mutations associated with decreased binding to FcRn is selected from the group consisting of I253A, I253V, and K288A, and combinations thereof, wherein numbering is according to the EU index.

[0026] In an example, the one or more mutations comprise a mutation or set of mutations associated with altered effector function.

[0027] In an example, the Fc polypeptide is an IgG1 Fc polypeptide, and wherein the mutation or set of mutations comprises a mutation at one or more the following residues: L234, L235, G236, G237, P329, and A330, or combinations thereof, wherein numbering is according to the EU index.

[0028] In an example, the altered effector function is decreased effector function.

[0029] In an example, the mutation or set of mutations associated with decreased effector function is selected from the group consisting of LALA (L234A/L235A), LALAP (L234A/L235A/P329G), G236R, G237A, and A330L, wherein numbering is according to the EU index.

[0030] In an example, the nanocage monomer or subunit thereof is a ferritin monomer subunit, and

a. each first fusion polypeptide comprises a ferritin monomer subunit which is C-half-ferritin and each second fusion polypeptide comprises a ferritin monomer subunit which is N-half-ferritin; or

b. each first fusion polypeptide comprises a ferritin monomer subunit which is N-half-ferritin and each second fusion polypeptide comprises a ferritin monomer subunit which is C-half-ferritin.

[0031] In an example, the self-assembled polypeptide complex is characterized by a 1:1 ratio of first fusion polypeptides to second fusion polypeptides.

[0032] In an example, within each first fusion polypeptide, the Fc polypeptide is linked to the nanocage monomer or subunit thereof via an amino acid linker.

[0033] In an example, within each first fusion polypeptide, the Fc polypeptide is linked to the nanocage monomer or subunit thereof at the N-terminus of the nanocage monomer or subunit thereof.

[0034] In an example, within each second fusion polypeptide, the antigen-binding antibody fragment is linked to the nanocage monomer or subunit thereof via an amino acid linker.

[0035] In an example, within each second fusion polypeptide, the antigen-binding antibody fragment is linked to the nanocage monomer or subunit thereof at the N-terminus of the nanocage monomer or subunit thereof.

[0036] In an example, the self-assembled polypeptide complex further comprises a plurality of third fusion polypeptides, each third fusion polypeptide comprising (1) an antigen-binding antibody fragment linked to (2) a nanocage monomer or a subunit thereof, wherein the third fusion polypeptide is different than the second fusion polypeptide.

[0037] In an example, the antigen-binding antibody fragment within the third fusion polypeptide comprises a light chain variable domain and a heavy chain variable domain.

[0038] In an example, the antigen-binding antibody fragment within the third fusion polypeptide is a Fab fragment.

[0039] In an example, each third fusion polypeptide does not comprise any CH2 or CH3 domains.

[0040] In an example, the antigen-binding antibody fragment of the second fusion polypeptide is capable of binding a first epitope, the antigen-binding fragment of the third fusion polypeptide is capable of binding a second epitope, and the first epitopes and second epitopes are distinct and non-overlapping.

[0041] In an example, first epitopes and second epitopes are from the same protein.

[0042] In an example, the self-assembled polypeptide complex comprises a total of 24 to 48 fusion polypeptides.

[0043] In an example, the self-assembled polypeptide complex comprises a total of at least 24 fusion polypeptides.

[0044] In an example, the self-assembled polypeptide complex comprises a total of at least 32 fusion polypeptides.

[0045] In an example, the self-assembled polypeptide complex has a total of about 32 fusion polypeptides.

[0046] In an example, the half-life of the self-assembled polypeptide complex is at least 3 days, at least 5 days, at least 7 days, at least 10 days, at least 14 days, at least 21 days, or at least 28 days when administered to a subject in need thereof.

[0047] In an example, the self-assembled polypeptide complex is characterized in that, after administration of a composition comprising the self-assembled polypeptide complex, the self-assembled polypeptide complex has a half-life substantially similar to that of a reference IgG molecule administered by the same route of administration and in a similar composition.

[0048] In an example, the reference IgG molecule is an antibody from which the antigen-binding antibody fragment within the second fusion polypeptide is derived or is an antibody from which the antigen-binding antibody fragment within the third fusion polypeptide is derived.

5 **[0049]** In an example, the half-life of the self-assembled polypeptide complex is from about 3 to about 35 days when administered to a subject in need thereof.

[0050] In an example, the self-assembled polypeptide complex is detectable in serum after at least 3 days, at least 5 days, at least 7 days, at least 10 days, at least 14 days, at least 21 days, or at least 28 days after administration to a subject in need thereof.

10 **[0051]** In an example, the area-under-the-curve (AUC) of the self-assembled polypeptide complex is at least 10 day·µg/mL, at least 25 day·µg/mL, at least 50 day·µg/mL, at least 100 day·µg/mL, at least 200 day·µg/mL, at least 300 day·µg/mL, at least 400 day·µg/mL, at least 500 day·µg/mL, at least 750 day·µg/mL, at least 1000 day·µg/mL, at least 1500 day·µg/mL, at least 2000 day·µg/mL, at least 2500 day·µg/mL, at least 3000 day·µg/mL, at least 4000
15 day·µg/mL, at least 5000 day·µg/mL, at least 6000 day·µg/mL, at least 7000 day·µg/mL, or at least 8000 day·µg/mL when administered to a subject in need thereof.

[0052] In an example, the area-under-the-curve (AUC) of the self-assembled polypeptide complex is from about 10 to about 8000 day·µg/mL when administered to a subject in need thereof.

20 **[0053]** In an example, the maximum concentration ($C_{\max}$) of the self-assembled polypeptide complex is at least 10 µg/mL, at least 25 µg/mL, at least 50 µg/mL, at least 100 µg/mL, at least 250 µg/mL, at least 500 µg/mL, at least 750 µg/mL, at least 1 mg/mL, at least 10 mg/mL, at least 25 mg/mL, at least 50 mg/mL, at least 75 mg/mL, at least 100 mg/mL, at least 250 mg/mL, at least 500 mg/mL, or at least 750 mg/mL when administered to a subject
25 in need thereof.

[0054] In an example, the maximum concentration ($C_{\max}$) of the self-assembled polypeptide complex is from about 10 µg/mL to about 750 mg/mL when administered to a subject in need thereof.

[0055] In an example, the subject is human.

30 **[0056]** In an example, administration to the subject is by parenteral administration.

[0057] In an example, administration to the subject is by subcutaneous administration, intravenous administration, intramuscular administration, intranasal administration, or by inhalation.

[0058] In an example, the self-assembled polypeptide complex is characterized in that the self-assembled polypeptide complex induces antibody-dependent cellular phagocytosis (ADCP) in an *in vitro* model of ADCP.

[0059] In an example, the ADCP is induced at a level of at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, or at least 60% internalization of target.

[0060] In accordance with an example, there is provided a method comprising administering a composition comprising the self-assembled polypeptide complex described herein to a mammalian subject.

[0061] In an example, the subject is human.

[0062] In an example, the method comprises administration by a systemic route.

[0063] In an example, the systemic route comprises subcutaneous, intravenous, or intramuscular injection, inhalation, or intranasal administration.

[0064] In an example, after administration, the half-life of the self-assembled polypeptide complex in the mammalian subject is at least 3 days, at least 5 days, at least 7 days, at least 10 days, at least 14 days, at least 21 days, or at least 28 days.

[0065] In an example, after administration, the half-life of the self-assembled polypeptide complex in the mammalian subject is from 3 to 35 days.

[0066] In an example, after administration, the area-under-the-curve (AUC) of the self-assembled polypeptide complex in the mammalian subject is at least 10 day·µg/mL, at least 25 day·µg/mL, at least 50 day·µg/mL, at least 100 day·µg/mL, at least 200 day·µg/mL, at least 300 day·µg/mL, at least 400 day·µg/mL, at least 500 day·µg/mL, at least 750 day·µg/mL, at least 1000 day·µg/mL, at least 1500 day·µg/mL, at least 2000 day·µg/mL, at least 2500 day·µg/mL, at least 3000 day·µg/mL, at least 4000 day·µg/mL, at least 5000 day·µg/mL, at least 6000 day·µg/mL, at least 7000 day·µg/mL, or at least 8000 day·µg/mL .

[0067] In an example, after administration, the area-under-the-curve (AUC) of the self-assembled polypeptide complex in the mammalian subject is from about 10 to about 8000 day·µg/mL.

[0068] In an example, after administration, the maximum concentration (C_{max}) of the self-assembled polypeptide complex in the mammalian subject is at least 10 µg/mL, at least 25 µg/mL, at least 50 µg/mL, at least 100 µg/mL, at least 250 µg/mL, at least 500 µg/mL, at least 750 µg/mL, at least 1 mg/mL, at least 10 mg/mL, at least 25 mg/mL, at least 50 mg/mL, at least 75 mg/mL, at least 100 mg/mL, at least 250 mg/mL, at least 500 mg/mL, or at least 750 mg/mL.

[0069] In an example, after administration, the maximum concentration (C_{max}) of the self-assembled polypeptide complex in the mammalian subject is from about 10 µg/mL to about 750 mg/mL.

[0070] In accordance with an example, there is provided a fusion polypeptide comprising (1) an Fc polypeptide linked to (2) a nanocage monomer or subunit thereof, wherein the Fc polypeptide comprises a Fc chain having one or more mutations relative to a reference Fc chain of the same Ig class, wherein the one or more mutations comprise a mutation or set of mutations associated with altered binding to FcRn and/or with altered effector function.

[0071] In an example, the nanocage monomer is a ferritin monomer.

[0072] In an example, the ferritin monomer is a ferritin light chain.

[0073] In an example, the fusion polypeptide does not comprise any ferritin heavy chains or subunits of ferritin heavy chains.

[0074] In an example, the ferritin monomer is a human ferritin.

[0075] In an example, the Fc polypeptide is an IgG1 Fc polypeptide.

[0076] In an example, the Fc polypeptide is an IgG2 Fc polypeptide.

[0077] In an example, the Fc polypeptide is a single-chain Fc (scFc).

[0078] In an example, the mutation or set of mutations comprises a mutation at one or more of the following residues: M252, I253, S254, T256, K288, M428, and N434, or combinations thereof, wherein numbering is according to the EU index.

[0079] In an example, the altered binding to FcRn is decreased binding to FcRn.

[0080] In an example, the mutation or set of mutations associated with decreased binding to FcRn is selected from the group consisting of I253A, I253V, and K288A, and combinations thereof, wherein numbering is according to the EU index.

[0081] In an example, the Fc polypeptide is an IgG1 Fc polypeptide, and wherein the mutation or set of mutations comprises a mutation at one or more the following residues: L234, L235, G236, G237, P329, and A330, or combinations thereof, wherein numbering is according to the EU index.

[0082] In an example, the altered effector function is decreased effector function.

[0083] In an example, the mutation or set of mutations associated with decreased effector function is selected from the group consisting of LALA (L234A/L235A), LALAP (L234A/L235A/P329G), G236R, G237A, and A330L, wherein numbering is according to the EU index.

[0084] In an example, the nanocage monomer or subunit thereof is a ferritin monomer subunit, and

a. each first fusion polypeptide comprises a ferritin monomer subunit which is C-half-ferritin and each second fusion polypeptide comprises a ferritin monomer subunit which is N-half-ferritin; or

b. each first fusion polypeptide comprises a ferritin monomer subunit which is N-half-ferritin and each second fusion polypeptide comprises a ferritin monomer subunit which is C-half-ferritin.

[0085] In an example, within each first fusion polypeptide, the Fc polypeptide is linked to the nanocage monomer or subunit thereof via an amino acid linker.

[0086] In an example, within each first fusion polypeptide, the Fc polypeptide is linked to the nanocage monomer or subunit thereof at the N-terminus of the nanocage monomer or subunit thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0087] FIG. 1A is a diagrammatic representation of human ferritin light chain (hFTL) and exemplary N-half ferritin and C-half ferritin molecules.

[0088] FIGS. 1B, 1C, and 1D are diagrammatic representations of exemplary Multabodies formation of the disclosure.

[0089] FIG. 2A are negative stain electron micrographs of T-01 MB, T-02 MB, and T-01 MB.v2, each containing wild-type IgG1 Fc.

[0090] FIG. 2B are negative stain electron micrographs of T-01 MB containing various Fc.

[0091] FIGS. 3A and 3B provide biolayer interferometry (BLI) concentration-response curves for the binding of T-01 MB containing various Fc to human FcRn at pH 5.6 and pH 7.4, respectively.

[0092] FIGS. 3C, 3D, and 3E provide BLI concentration-response curves for the binding of T-01 MB containing various Fc to human FcγRIIa, human FcγRIIb, and FcγRI, respectively.

[0093] FIG. 3F provides BLI concentration-response curves for the binding of T-01 MB.v2 containing wild-type IgG1 Fc or Fc IgG1 with M428L/N434S (LS) mutations to human Fc receptors.

[0094] FIG. 4A provides BLI concentration-response curves for the binding of T-01 MB, T-02 MB, and T-01 MB.v2 to target/epitope of PGDM1400, N49P7, 10E8v4, or iMab, as included as Fab in the Multabodies.

[0095] FIGS. 4B, 4C, and 4D provide BLI concentration-response curves for the binding of T-01 MB containing various Fc to the target/epitope of PGDM1400 (BG5050 SOSIP.664 D368R), N49P7 (93TH057), and 10E8v4 (gp41 MPER), respectively.

[0096] FIG. 4E provides BLI concentration-response curves for the binding of

CLAIMS

1. A self-assembled polypeptide complex comprising:
 - (a) a plurality of first fusion polypeptides, each first fusion polypeptide comprising (1) an Fc polypeptide linked to (2) a nanocage monomer or subunit thereof, wherein the Fc polypeptide comprises a Fc chain having one or more mutations relative to a reference Fc chain of the same Ig class, and
 - (b) a plurality of second fusion polypeptides, each second fusion polypeptide comprising (1) an antigen-binding antibody fragment linked to (2) a nanocage monomer or subunit thereof.
2. The self-assembled polypeptide complex of claim 1, wherein
 - (1) if the Fc polypeptide is an IgG1 Fc polypeptide, the antigen-binding fragment is not a Fab fragment that binds to SARS-CoV-2; and/or
 - (2) if the nanocage monomer is a mouse ferritin monomer and the Fc polypeptide is a mouse IgG2a Fc polypeptide, the antigen-binding antibody fragment is not a Fab fragment that binds to CD19.
3. The self-assembled polypeptide complex of claim 1 or 2, wherein the nanocage monomer is a ferritin monomer, optionally a human ferritin.
4. The self-assembled polypeptide complex of claim 3, wherein the ferritin monomer is a ferritin light chain.
5. The self-assembled polypeptide complex of claim 4, which does not comprise any ferritin heavy chains or subunits of ferritin heavy chains.
6. The self-assembled polypeptide complex of any one of claims 1-5, wherein the Fc polypeptide is an IgG1 Fc polypeptide.

7. The self-assembled polypeptide complex of any one of claims 1-6, wherein the Fc polypeptide is a single-chain Fc (scFc).
8. The self-assembled polypeptide complex of any one of claims 1-7, wherein the one or more mutations comprise a mutation or set of mutations associated with altered binding to FcRn.
9. The self-assembled polypeptide complex of claim 8, wherein the mutation or set of mutations comprises a mutation at one or more of the following residues: M252, I253, S254, T256, K288, M428, and N434, or combinations thereof, wherein numbering is according to the EU index.
10. The self-assembled polypeptide complex of claim 9, wherein the mutation or set of mutations comprises mutations at M428 and N434, wherein numbering is according to the EU index.
11. The self-assembled polypeptide complex of claim 10, wherein the mutation or set of mutations comprises M428L and N434S mutations, wherein numbering is according to the EU index.
12. The self-assembled polypeptide complex of claim 8 or 9, wherein the altered binding to FcRn is decreased binding to FcRn.
13. The self-assembled polypeptide complex of claim 9, wherein the mutation or set of mutations associated with decreased binding to FcRn is selected from the group consisting of I253A, I253V, and K288A, and combinations thereof, wherein numbering is according to the EU index.
14. The self-assembled polypeptide complex of any one of claims 1-13, wherein the one or more mutations comprise a mutation or set of mutations associated with altered effector function.

15. The self-assembled polypeptide complex of claim 14, wherein the Fc polypeptide is an IgG1 Fc polypeptide, and wherein the mutation or set of mutations comprises a mutation at one or more the following residues: L234, L235, G236, G237, P329, and A330, or combinations thereof, wherein numbering is according to the EU index.
16. The self-assembled polypeptide complex of claim 14, wherein the altered effector function is decreased effector function.
17. The self-assembled polypeptide complex of claim 16, wherein the mutation or set of mutations associated with decreased effector function is selected from the group consisting of LALA (L234A/L235A), LALAP (L234A/L235A/P329G), G236R, G237A, and A330L, wherein numbering is according to the EU index.
18. A composition comprising the self-assembled polypeptide complex of any one of claims 1-17 for use in therapy.
19. A fusion polypeptide comprising (1) an Fc polypeptide linked to (2) a nanocage monomer or subunit thereof, wherein the Fc polypeptide comprises a Fc chain having one or more mutations relative to a reference Fc chain of the same Ig class, wherein the one or more mutations comprise a mutation or set of mutations associated with altered binding to FcRn and/or with altered effector function.
20. The fusion polypeptide of claim 19, wherein the nanocage monomer is a ferritin monomer, optionally a human ferritin.
21. The fusion polypeptide of claim 20, wherein the ferritin monomer is a ferritin light chain.
22. The fusion polypeptide of claim 21, which does not comprise any ferritin heavy chains or subunits of ferritin heavy chains.

23. The fusion polypeptide of any one of claims 19-22, wherein the Fc polypeptide is an IgG1 Fc polypeptide.
24. The fusion polypeptide of any one of claims 19-23, wherein the Fc polypeptide is a single-chain Fc (scFc).
25. The fusion polypeptide of any one of claims 19-24, wherein the mutation or set of mutations comprises a mutation at one or more of the following residues: M252, I253, S254, T256, K288, M428, and N434, or combinations thereof, wherein numbering is according to the EU index.
26. The fusion polypeptide of any one of claims 19-25, wherein the altered binding to FcRn is decreased binding to FcRn.
27. The fusion polypeptide of claim 26, wherein the mutation or set of mutations associated with decreased binding to FcRn is selected from the group consisting of I253A, I253V, and K288A, and combinations thereof, wherein numbering is according to the EU index.
28. The fusion polypeptide of any one of claims 19-24, wherein the Fc polypeptide is an IgG1 Fc polypeptide, and wherein the mutation or set of mutations comprises a mutation at one or more the following residues: L234, L235, G236, G237, P329, and A330, or combinations thereof, wherein numbering is according to the EU index.
29. The fusion polypeptide of any one of claims 19-24 and 28, wherein the altered effector function is decreased effector function.
30. The fusion polypeptide of claim 29, wherein the mutation or set of mutations associated with decreased effector function is selected from the group consisting of LALA (L234A/L235A), LALAP (L234A/L235A/P329G), G236R, G237A, and A330L, wherein numbering is according to the EU index.