

anchoring protein anchoring domain interactions) (Goldenberg et al., *Journal of Nuclear Medicine*, 49(1):158-163 (2008)).

[0007] However, there remains a need for multimerized proteins, such as multimerized whole antibodies and antibody fragments (e.g., Fabs), which demonstrate enhanced biological and therapeutic properties. For example, there remains a need for multimerized proteins which have increased antibody avidity and enhanced antigen clustering compared to their non-multimerized counterparts.

SUMMARY

[0008] In one aspect, the present disclosure relates to a homodimer fusion protein comprising two antigen binding proteins, wherein the antigen binding proteins are linked to a protein complex. In a particular example, the protein complex is a TTR protein complex. In a particular example, the antigen binding protein is an antibody. In another particular example, the antigen binding proteins are directly fused without a linker to the protein complex. In another particular example, the C-terminus of the antigen binding protein is directly fused to an N-terminus present in the TTR protein complex. In some examples, the antigen binding proteins are fused to the protein complex via a linker. In other examples, the C-terminus of the antigen binding protein is linked to an N-terminus present in the TTR protein complex. The linker may be an amino acid linker, such as an amino acid linker that is 1-20 amino acids in length. In particular examples, the amino acid linker is GGGGS, (GGGGS)₂, (GGGGS)₃, (GGGGS)₄, (GGGGS)₅, or (GGGGS)₆.

[0009] In another aspect, the present disclosure relates to a homotetramer fusion protein comprising four antigen binding proteins, wherein the antigen binding proteins are linked to a protein complex. In one example, the protein complex is a TTR protein complex. In another example, the antigen binding protein is an antibody. The antigen binding protein may be a Fab. In some examples, the antigen binding proteins are directly fused without a linker to said protein complex. In particular examples, the C-terminus of the antigen binding protein is directly fused to an N-terminus present in the TTR protein complex. In other examples, the antigen binding proteins are fused to said protein complex via a linker. The C-terminus of the antigen binding protein may be linked to an N-terminus present in the TTR protein complex. In some examples, the linker is an amino acid linker, such as an amino acid linker that is 1-20 amino acids in length. In particular examples, the amino acid linker is GGGGS, (GGGGS)₂, (GGGGS)₃, (GGGGS)₄, (GGGGS)₅, or (GGGGS)₆.

[0010] In another aspect, the present disclosure also relates to a pharmaceutical composition comprising any of the homodimer or homotetramer fusion proteins discussed above.

[0011] In another aspect, the present disclosure relates to a use of the homodimer fusion protein as disclosed herein in the manufacture of a medicament for treating cancer. In yet another aspect, the present disclosure refers to a use of the homotetramer fusion protein as disclosed herein in the manufacture of a medicament for treating cancer. In addition, the present disclosure relates to a method of treating cancer using any of the homodimer or homotetramer fusion proteins discussed above. Moreover, the present disclosure relates to uses of any of the homodimer or homotetramer fusion proteins discussed above in the treatment of cancer. In another example, the present disclosure relates to any of the homodimer or homotetramer fusion proteins discussed above for use in the treatment of cancer.

[0012] In some aspects, the present disclosure relates to one or more isolated nucleic acid(s) encoding any of the homodimer or homotetramer fusion proteins discussed above. Expression vectors comprising such nucleic acids are also contemplated, as are recombinant host cells comprising the nucleic acids and/or vectors discussed herein. In particular examples, the recombinant host cell is a Chinese hamster ovary (CHO) cell, E5 cell, baby hamster kidney (BHK) cell, monkey kidney (COS) cell, human hepatocellular carcinoma cell, or human embryonic kidney 293 (HEK 293) cell.

[0013] Methods of making any of the homodimer or homotetramer fusion proteins discussed above are also part of the present disclosure. Such methods may comprise a) culturing the recombinant host cell of claim 32 or 33; and b) isolating the homodimer or homotetramer fusion protein from said culture.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] FIG. 1. Figure 1 is a schematic representation of the homomultimer constructs of the present invention. Figure 1a is an exemplary TTR antibody homodimer fusion protein, where the C-terminus of both antibody heavy chains is linked to the N-terminus of each TTR subunit. Figure 1b is an exemplary TTR antibody homotetramer fusion protein, where one of the two heavy chains of each antibody C-terminus is linked to the N-terminus of each TTR subunit. The “+” and “-” signs indicate Fc charge pairs which allow for consistent attachment of one TTR subunit per whole antibody. Figure 1c is an exemplary TTR Fab homotetramer fusion protein, where the C-terminus of each Fab fragment is linked to the N-terminus of each TTR subunit. Each of Figures 1a-1c shows the optional linker between the heavy chain and TTR.

[0015] FIG. 2. Figure 2 is a series of SDS-PAGE gels that demonstrate that the anti-CB1 TTR antibody homodimer with no linker and varying linker lengths (Figure 2a), anti-CB1 TTR antibody homotetramer with no linker (Figure 2b), and anti-CB1 TTR Fab homotetramer proteins with no linker (Figure 2c), respectively, are robustly expressed in HEK 293 cells. Figure 2 is further discussed in Example 2.

[0016] FIG. 3. Figure 3 is a series of HPLC size exclusion chromatography (SEC) analyses of the anti-CB1 TTR antibody homodimer fusion protein with no linker, a (G₄S) linker, a (G₄S)₂ linker, a (G₄S)₃ linker, or a (G₄S)₄ linker. Figure 3 is further discussed in Example 2.

[0017] FIG. 4. Figure 4 shows that the TTR anti-CB1 antibody homotetramer and TTR anti-CB1 Fab homotetramer fusion proteins improve EC₅₀ compared to parental CB1 Ab. Figure 4 is further discussed in Example 3.

[0001] FIG. 5. Figure 5 is an SDS-PAGE gel that demonstrates that the anti-GITR TTR antibody homodimer (Lane 1), anti-GITR TTR antibody homotetramer (Lane 2), and anti-GITR TTR Fab homotetramer proteins (Lane 3), respectively, are expressed in HEK 293 cells. Lanes 4-7 are anti-dinitrophenyl (anti-DNP) antibody. Figure 5 is further discussed in Example 4.

CLAIMS

What is claimed:

1. A homodimer fusion protein comprising two antigen binding proteins, wherein said antigen binding proteins are linked to a protein complex.
2. The homodimer fusion protein of claim 1, wherein said protein complex is a TTR protein complex.
3. The homodimer fusion protein of claim 1 or 2, wherein the antigen binding protein is an antibody.
4. The homodimer fusion protein of any one of claims 1-3, wherein said antigen binding proteins are directly fused without a linker to said protein complex.
5. The homodimer fusion protein of claim 4, wherein said C-terminus of the antigen binding protein is directly fused to an N-terminus present in the TTR protein complex.
6. The homodimer fusion protein of any one of claims 1-3, wherein said antigen binding proteins are fused to said protein complex via a linker.
7. The homodimer fusion protein of claim 6, wherein said C-terminus of the antigen binding protein is linked to an N-terminus present in the TTR protein complex.
8. The homodimer fusion protein of claim 7, wherein said linker is an amino acid linker.
9. The amino acid linker of claim 8, wherein said amino acid linker is 1-40 amino acids in length.
10. The amino acid linker of claim 9, wherein said amino acid linker is GGGGS, (GGGS)₂, (GGGS)₃, (GGGS)₄, (GGGS)₅, or (GGGS)₆.
11. A homotetramer fusion protein comprising four antigen binding proteins, wherein said antigen binding proteins are linked to a protein complex.
12. The homotetramer fusion protein of claim 11, wherein said protein complex is a TTR protein complex.
13. The homotetramer fusion protein of claim 11 or 12, wherein the antigen binding protein is an antibody.

14. The homotetramer fusion protein of claim 11 or 12, wherein the antigen binding protein is a Fab.
15. The homotetramer fusion protein of any one of claims 11-14, wherein said antigen binding proteins are directly fused without a linker to said protein complex.
16. The homotetramer fusion protein of claim 15, wherein said C-terminus of the antigen binding protein is directly fused to an N-terminus present in the TTR protein complex.
17. The homotetramer fusion protein of any one of claims 11-14, wherein said antigen binding proteins are fused to said protein complex via a linker.
18. The homotetramer fusion protein of claim 17, wherein said C-terminus of the antigen binding protein is linked to an N-terminus present in the TTR protein complex.
19. The homotetramer fusion protein of claim 18, wherein said linker is an amino acid linker.
20. The amino acid linker of claim 19, wherein said amino acid linker is 1-40 amino acids in length.
21. The amino acid linker of claim 20, wherein said amino acid linker is GGGGS, (GGGGS)₂, (GGGGS)₃, (GGGGS)₄, (GGGGS)₅, or (GGGGS)₆.
22. A pharmaceutical composition comprising the homodimer fusion protein of any one of claims 1-10.
23. A pharmaceutical composition comprising the homotetramer fusion protein of any one of claims 11-22.
24. Use of the homodimer fusion protein of any one of claims 1-10 in the manufacture of a medicament for treating cancer.
25. Use of the homotetramer fusion protein of any one of claims 11-22 in the manufacture of a medicament for treating cancer.
26. One or more isolated nucleic acid(s) encoding a homodimer fusion protein of any one of claims 1-10.

27. An expression vector comprising a nucleic acid of claim 26.

28. A recombinant host cell comprising a nucleic acid of claim 26 or the vector of claim 27.

29. The recombinant host cell of claim 28, wherein said host cell is a Chinese hamster ovary (CHO) cell, E5 cell, baby hamster kidney (BHK) cell, monkey kidney (COS) cell, human hepatocellular carcinoma cell, or human embryonic kidney 293 (HEK 293) cell.

30. A method of making a homodimer fusion protein of any one of claims 1-10 or a homotetramer fusion protein of any one of claims 11-22, said method comprising:

- a) culturing the recombinant host cell of claim 28 or 29; and
- b) isolating the homodimer or homotetramer fusion protein from said culture.