

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 refers to a homodimer fusion protein comprising two antigen binding proteins, wherein said antigen binding proteins are linked to a transthyretin (TTR) protein tetramer, and wherein said antigen binding proteins are antibodies. In one example, 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 one aspect, the present disclosure refers to a homotetramer fusion protein comprising four antigen binding proteins, wherein said antigen binding proteins are linked to a transthyretin (TTR) protein tetramer, and wherein said antigen binding proteins are antibodies. In another example, 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 a homodimer fusion protein comprising two antigen binding proteins, wherein said antigen binding proteins are linked to a TTR protein tetramer, wherein said antigen binding proteins are antibodies, , and wherein said homodimer fusion protein comprises antigen binding proteins which bind to antigens on tumor cells. In another aspect, the present disclosure relates to a pharmaceutical composition comprising a homodimer fusion protein comprising two antigen binding proteins, wherein said antigen binding proteins are linked to a TTR protein tetramer, wherein said antigen binding proteins are antibodies, and wherein said antibody binds glucocorticoid-induced TNFR-related protein (GITR), tumor necrosis factor-related apoptosis-inducing ligand Receptor-2 (TRAILR2), or anti-cannabinoid receptor-1 (CB1R). In another aspect, the present disclosure also relates to a pharmaceutical composition comprising a homotetramer fusion protein comprising four antigen binding proteins, wherein said antigen binding proteins are linked to a TTR

protein tetramer, wherein said antigen binding proteins are antibodies, and wherein said homotetramer fusion protein comprises antigen binding proteins which bind to antigens on tumor cells. In yet another aspect, the present disclosure also relates to a pharmaceutical composition comprising a homotetramer fusion protein comprising four antigen binding proteins, wherein said antigen binding proteins are linked to a TTR protein tetramer, wherein said antigen binding proteins are antibodies, and wherein said antibody binds glucocorticoid-induced TNFR-related protein (GITR), TRAILR2, or anti-cannabinoid receptor-1 (CB1R).

[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, wherein said homodimer fusion protein comprises antigen binding proteins which bind to antigens on tumor cells. In another aspect, the present disclosure relates to a use of a homodimer fusion protein comprising two antigen binding proteins in the manufacture of a medicament for treating cancer, wherein said antigen binding proteins are linked to a TTR protein tetramer, wherein said antigen binding proteins are antibodies, and wherein said antibody binds glucocorticoid-induced TNFR-related protein (GITR), TRAILR2, or anti-cannabinoid receptor-1 (CB1R). 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, wherein said homotetramer fusion protein comprises antigen binding proteins which bind to antigens on tumor cells. In yet another aspect, the present disclosure refers to a use of a homotetramer fusion protein comprising four antigen binding proteins in the manufacture of a medicament for treating cancer, wherein said antigen binding proteins are linked to a TTR protein tetramer, wherein said antigen binding proteins are antibodies, and wherein said antibody binds glucocorticoid-induced TNFR-related protein (GITR), TRAILR2, or anti-cannabinoid receptor-1 (CB1R). 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.

What is claimed:

1. A homodimer fusion protein comprising two antigen binding proteins, wherein said antigen binding proteins are linked to a transthyretin (TTR) protein tetramer, and wherein said antigen binding proteins are antibodies.
2. The homodimer fusion protein of claim 1, wherein said antigen binding proteins are directly fused without a linker to said TTR protein tetramer.
3. The homodimer fusion protein of claim 2, wherein said C-terminus of the antigen binding protein is directly fused to an N-terminus present in the TTR protein tetramer.
4. The homodimer fusion protein of claim 1, wherein said antigen binding proteins are fused to said TTR protein tetramer via a linker.
5. The homodimer fusion protein of claim 4, wherein said C-terminus of the antigen binding protein is linked to an N-terminus present in the TTR protein tetramer.
6. The homodimer fusion protein of claim 5, wherein said linker is an amino acid linker.
7. The homodimer fusion protein of claim 6, wherein said amino acid linker is 1-40 amino acids in length.
8. The homodimer fusion protein of claim 7, wherein said amino acid linker is GGGGS, (GGGGS)₂, (GGGGS)₃, (GGGGS)₄, (GGGGS)₅, or (GGGGS)₆.
9. The homodimer fusion protein of any one of claims 1-8, wherein said antibody binds glucocorticoid-induced TNFR-related protein (GITR), tumor necrosis factor-related apoptosis-inducing ligand Receptor-2 (TRAILR2), or anti-cannabinoid receptor-1 (CB1R).
10. The homodimer fusion protein of claim 9, wherein said antibody binds TRAILR2.
11. The homodimer fusion protein of any one of claims 1-10, wherein each subunit of said TTR protein tetramer comprises the amino acid sequence of SEQ ID NO: 1.

12. A homotetramer fusion protein comprising four antigen binding proteins, wherein said antigen binding proteins are linked to a transthyretin (TTR) protein tetramer, and wherein said antigen binding proteins are antibodies.

13. The homotetramer fusion protein of claim 12, wherein said antigen binding proteins are directly fused without a linker to said protein tetramer.

14. The homotetramer fusion protein of claim 13, wherein said C-terminus of the antigen binding protein is directly fused to an N-terminus present in the TTR protein tetramer.

15. The homotetramer fusion protein of claim 12, wherein said antigen binding proteins are fused to said protein tetramer via a linker.

16. The homotetramer fusion protein of claim 15, wherein said C-terminus of the antigen binding protein is linked to an N-terminus present in the TTR protein tetramer.

17. The homotetramer fusion protein of claim 16, wherein said linker is an amino acid linker.

18. The homotetramer fusion protein of claim 17, wherein said amino acid linker is 1-40 amino acids in length.

19. The homotetramer fusion protein of claim 18, wherein said amino acid linker is GGGGS, (GGGGS)₂, (GGGGS)₃, (GGGGS)₄, (GGGGS)₅, or (GGGGS)₆.

20. The homotetramer fusion protein of any one of claims 12-19, wherein said antibody binds glucocorticoid-induced TNFR-related protein (GITR), tumor necrosis factor-related apoptosis-inducing ligand Receptor-2 (TRAILR2), or anti-cannabinoid receptor-1 (CB1R).

21. The homotetramer fusion protein of claim 20, wherein said antibody binds TRAILR2.

22. The homotetramer fusion protein of any one of claims 12-21, wherein each subunit of said TTR protein tetramer comprises the amino acid sequence of SEQ ID NO: 1.

23. A pharmaceutical composition comprising the homodimer fusion protein of any one of claims 1-8 or 11, wherein said homodimer fusion protein comprises antigen binding proteins which bind to antigens on tumor cells.

24. A pharmaceutical composition comprising the homodimer fusion protein of claim 9 or 10.

25. A pharmaceutical composition comprising the homotetramer fusion protein of any one of claims 12-19 or 22, wherein said homotetramer fusion protein comprises antigen binding proteins which bind to antigens on tumor cells.

26. A pharmaceutical composition comprising the homotetramer fusion protein of claim 20 or 21.

27. Use of the homodimer fusion protein of any one of claims 1-8 or 11 in the manufacture of a medicament for treating cancer, wherein said homodimer fusion protein comprises antigen binding proteins which bind to antigens on tumor cells.

28. Use of the homodimer fusion protein of claim 9 or 10 in the manufacture of a medicament for treating cancer.

29. Use of the homotetramer fusion protein of any one of claims 12-19 or 22 in the manufacture of a medicament for treating cancer, wherein said homotetramer fusion protein comprises antigen binding proteins which bind to antigens on tumor cells.

30. Use of the homotetramer fusion protein of claim 20 or 21 in the manufacture of a medicament for treating cancer.

31. One or more isolated nucleic acid(s) encoding [a] a homodimer fusion protein of any one of claims 1-11; or [b] a homotetramer fusion protein of any one of claims 12-22.

32. An expression vector comprising a nucleic acid of claim 31.

33. A recombinant host cell comprising a nucleic acid of claim 31 or the vector of claim 32.

34. The recombinant host cell of claim 33, wherein said host cell is a Chinese hamster ovary (CHO) cell, E5 cell, baby hamster kidney (BHK) cell, COS monkey kidney cell, human hepatocellular carcinoma cell, or human embryonic kidney 293 (HEK 293) cell.

35. A method of making a homodimer fusion protein of any one of claims 1-11 or a homotetramer fusion protein of any one of claims 12-22, said method comprising:

- a) culturing the recombinant host cell of claim 33 or 34; and
- b) isolating the homodimer or homotetramer fusion protein from said culture.