

CLAIMS

What is claimed:

1. A transthyretin (TTR) protein complex, wherein said TTR protein complex comprises TTR subunits A, B, C, and D;
wherein TTR subunits A and B dimerize to form TTR dimer AB;
wherein TTR subunits C and D dimerize to form TTR dimer CD;
wherein TTR dimers AB and CD further dimerize to form TTR tetramer ABCD; and
wherein each of A, B, C, and D comprise the amino acid sequence of SEQ ID NO: 1, except for the following mutation(s):
wherein each of A, B, C, and D comprise mutations C10A and K15A,
wherein each of A and B comprise at least one mutation selected from L17D/E, V20D/E, G22D/E, S112D/E, T119D/E, and V121D/E, and
wherein each of C and D comprise at least one mutation selected from L17R/K, V20R/K, G22R/K, S112R/K, T119R/K, and V121R/K.
2. The TTR protein complex according to claim 1, wherein: each of A and B comprise at least two mutations selected from: L17D/E, V20D/E, G22D/E, S112D/E, T119D/E, and V121D/E, and each of C and D comprise at least two mutations selected from L17R/K, V20R/K, G22R/K, S112R/K, T119R/K, and V121R/K.
3. The TTR protein complex according to claim 1 or 2, wherein each of A, B, C, and D comprise the amino acid sequence of SEQ ID NO: 1 with the following mutations:
both A and B comprise L17D, and both C and D comprise L17R;
both A and B comprise L17E, and both C and D comprise L17R;
both A and B comprise V20D, and both C and D comprise L17R;
both A and B comprise V20E, and both C and D comprise L17R;
both A and B comprise G22D, and both C and D comprise L17R;
both A and B comprise G22E, and both C and D comprise L17R;
both A and B comprise S112D, and both C and D comprise L17R;
both A and B comprise S112E, and both C and D comprise L17R;
both A and B comprise T119D, and both C and D comprise L17R;
both A and B comprise T119E, and both C and D comprise L17R;
both A and B comprise V121D, and both C and D comprise L17R;
both A and B comprise V121E, and both C and D comprise L17R;
both A and B comprise L17D, and both C and D comprise L17K;

both A and B comprise L17E, and both C and D comprise L17K;
 both A and B comprise V20D, and both C and D comprise L17K;
 both A and B comprise V20E, and both C and D comprise L17K;
 both A and B comprise G22D, and both C and D comprise L17K;
 both A and B comprise G22E, and both C and D comprise L17K;
 both A and B comprise S112D, and both C and D comprise L17K;
 both A and B comprise S112E, and both C and D comprise L17K;
 both A and B comprise T119D, and both C and D comprise L17K;
 both A and B comprise T119E, and both C and D comprise L17K;
 both A and B comprise V121D, and both C and D comprise L17K;
 both A and B comprise V121E, and both C and D comprise L17K;
 both A and B comprise L17D, and both C and D comprise V20R;
 both A and B comprise L17E, and both C and D comprise V20R;
 both A and B comprise V20D, and both C and D comprise V20R;
 both A and B comprise V20E, and both C and D comprise V20R;
 both A and B comprise G22D, and both C and D comprise V20R;
 both A and B comprise G22E, and both C and D comprise V20R;
 both A and B comprise S112D, and both C and D comprise V20R;
 both A and B comprise S112E, and both C and D comprise V20R;
 both A and B comprise T119D, and both C and D comprise V20R;
 both A and B comprise T119E, and both C and D comprise V20R;
 both A and B comprise V121D, and both C and D comprise V20R;
 both A and B comprise V121E, and both C and D comprise V20R;
 both A and B comprise L17D, and both C and D comprise V20K;
 both A and B comprise L17E, and both C and D comprise V20K;
 both A and B comprise V20D, and both C and D comprise V20K;
 both A and B comprise V20E, and both C and D comprise V20K;
 both A and B comprise G22D, and both C and D comprise V20K;
 both A and B comprise G22E, and both C and D comprise V20K;
 both A and B comprise S112D, and both C and D comprise V20K;
 both A and B comprise S112E, and both C and D comprise V20K;
 both A and B comprise T119D, and both C and D comprise V20K;
 both A and B comprise T119E, and both C and D comprise V20K;
 both A and B comprise V121D, and both C and D comprise V20K;
 both A and B comprise V121E, and both C and D comprise V20K;
 both A and B comprise L17D, and both C and D comprise G22R;
 both A and B comprise L17E, and both C and D comprise G22R;

both A and B comprise V20D, and both C and D comprise G22R;
 both A and B comprise V20E, and both C and D comprise G22R;
 both A and B comprise G22D, and both C and D comprise G22R;
 both A and B comprise G22E, and both C and D comprise G22R;
 both A and B comprise S112D, and both C and D comprise G22R;
 both A and B comprise S112E, and both C and D comprise G22R;
 both A and B comprise T119D, and both C and D comprise G22R;
 both A and B comprise T119E, and both C and D comprise G22R;
 both A and B comprise V121D, and both C and D comprise G22R;
 both A and B comprise V121E, and both C and D comprise G22R;
 both A and B comprise L17D, and both C and D comprise G22K;
 both A and B comprise L17E, and both C and D comprise G22K;
 both A and B comprise V20D, and both C and D comprise G22K;
 both A and B comprise V20E, and both C and D comprise G22K;
 both A and B comprise G22D, and both C and D comprise G22K;
 both A and B comprise G22E, and both C and D comprise G22K;
 both A and B comprise S112D, and both C and D comprise G22K;
 both A and B comprise S112E, and both C and D comprise G22K;
 both A and B comprise T119D, and both C and D comprise G22K;
 both A and B comprise T119E, and both C and D comprise G22K;
 both A and B comprise V121D, and both C and D comprise G22K;
 both A and B comprise V121E, and both C and D comprise G22K;
 both A and B comprise L17D, and both C and D comprise S112R;
 both A and B comprise L17E, and both C and D comprise S112R;
 both A and B comprise V20D, and both C and D comprise S112R;
 both A and B comprise V20E, and both C and D comprise S112R;
 both A and B comprise G22D, and both C and D comprise S112R;
 both A and B comprise G22E, and both C and D comprise S112R;
 both A and B comprise S112D, and both C and D comprise S112R;
 both A and B comprise S112E, and both C and D comprise S112R;
 both A and B comprise T119D, and both C and D comprise S112R;
 both A and B comprise T119E, and both C and D comprise S112R;
 both A and B comprise V121D, and both C and D comprise S112R;
 both A and B comprise V121E, and both C and D comprise S112R;
 both A and B comprise L17D, and both C and D comprise S112K;
 both A and B comprise L17E, and both C and D comprise S112K;
 both A and B comprise V20D, and both C and D comprise S112K;

both A and B comprise V20E, and both C and D comprise S112K;
 both A and B comprise G22D, and both C and D comprise S112K;
 both A and B comprise G22E, and both C and D comprise S112K;
 both A and B comprise S112D, and both C and D comprise S112K;
 both A and B comprise S112E, and both C and D comprise S112K;
 both A and B comprise T119D, and both C and D comprise S112K;
 both A and B comprise T119E, and both C and D comprise S112K;
 both A and B comprise V121D, and both C and D comprise S112K;
 both A and B comprise V121E, and both C and D comprise S112K;
 both A and B comprise L17D, and both C and D comprise T119R;
 both A and B comprise L17E, and both C and D comprise T119R;
 both A and B comprise V20D, and both C and D comprise T119R;
 both A and B comprise V20E, and both C and D comprise T119R;
 both A and B comprise G22D, and both C and D comprise T119R;
 both A and B comprise G22E, and both C and D comprise T119R;
 both A and B comprise S112D, and both C and D comprise T119R;
 both A and B comprise S112E, and both C and D comprise T119R;
 both A and B comprise T119D, and both C and D comprise T119R;
 both A and B comprise T119E, and both C and D comprise T119R;
 both A and B comprise V121D, and both C and D comprise T119R;
 both A and B comprise V121E, and both C and D comprise T119R;
 both A and B comprise L17D, and both C and D comprise T119K;
 both A and B comprise L17E, and both C and D comprise T119K;
 both A and B comprise V20D, and both C and D comprise T119K;
 both A and B comprise V20E, and both C and D comprise T119K;
 both A and B comprise G22D, and both C and D comprise T119K;
 both A and B comprise G22E, and both C and D comprise T119K;
 both A and B comprise S112D, and both C and D comprise T119K;
 both A and B comprise S112E, and both C and D comprise T119K;
 both A and B comprise T119D, and both C and D comprise T119K;
 both A and B comprise T119E, and both C and D comprise T119K;
 both A and B comprise V121D, and both C and D comprise T119K;
 both A and B comprise V121E, and both C and D comprise T119K;
 both A and B comprise L17D, and both C and D comprise V121R;
 both A and B comprise L17E, and both C and D comprise V121R;
 both A and B comprise V20D, and both C and D comprise V121R;
 both A and B comprise V20E, and both C and D comprise V121R;

both A and B comprise G22D, and both C and D comprise V121R;
 both A and B comprise G22E, and both C and D comprise V121R;
 both A and B comprise S112D, and both C and D comprise V121R;
 both A and B comprise S112E, and both C and D comprise V121R;
 both A and B comprise T119D, and both C and D comprise V121R;
 both A and B comprise T119E, and both C and D comprise V121R;
 both A and B comprise V121D, and both C and D comprise V121R;
 both A and B comprise V121E, and both C and D comprise V121R;
 both A and B comprise L17D, and both C and D comprise V121K;
 both A and B comprise L17E, and both C and D comprise V121K;
 both A and B comprise V20D, and both C and D comprise V121K;
 both A and B comprise V20E, and both C and D comprise V121K;
 both A and B comprise G22D, and both C and D comprise V121K;
 both A and B comprise G22E, and both C and D comprise V121K;
 both A and B comprise S112D, and both C and D comprise V121K;
 both A and B comprise S112E, and both C and D comprise V121K;
 both A and B comprise T119D, and both C and D comprise V121K;
 both A and B comprise T119E, and both C and D comprise V121K;
 both A and B comprise V121D, and both C and D comprise V121K; or
 both A and B comprise V121E, and both C and D comprise V121K.

4. The TTR protein complex according to any one of claims 1 or 2, wherein:

both A and B comprise L17D, and both C and D comprise V121R;
 both A and B comprise L17D, and both C and D comprise V121K;
 both A and B comprise L17E, and both C and D comprise V121R;
 both A and B comprise V20D, and both C and D comprise V20R;
 both A and B comprise V20D, and both C and D comprise V20K;
 both A and B comprise V20E, and both C and D comprise V20R;
 both A and B comprise V20E, and both C and D comprise V20K;
 both A and B comprise T119D, and both C and D comprise L17R;
 both A and B comprise T119D, and both C and D comprise L17K; or
 both A and B comprise V121E, and both C and D comprise L17K.

5. The TTR protein complex according to claim 1 or 2, wherein each of A, B, C, and D comprise the amino acid sequence of SEQ ID NO: 1 with the following mutations:

both A and B comprise L17D/V20D, and both C and D comprise L17K/V20K;
 both A and B comprise L17D/V20E, and both C and D comprise L17K/V20R;

both A and B comprise L17E/V20D, and both C and D comprise L17R/V20K;
both A and B comprise L17E/V20E, and both C and D comprise L17R/V20R;
both A and B comprise L17D/T119D, and both C and D comprise L17K/V121K;
both A and B comprise L17D/V121E, and both C and D comprise L17K/V121R;
both A and B comprise L17E/T119D, and both C and D comprise L17R/V121K;
both A and B comprise L17E/V121E, and both C and D comprise L17R/V121R;
both A and B comprise V20D/T119D, and both C and D comprise V20K/V121K;
both A and B comprise V20D/V121E, and both C and D comprise V20K/V121R;
both A and B comprise V20E/T119D, and both C and D comprise V20R/V121K; or
both A and B comprise V20E/V121E, and both C and D comprise V20R/V121R.

6. The TTR protein complex according to claim 1 or 2, wherein each of A, B, C, and D comprise the amino acid sequence of SEQ ID NO: 1 with the following mutations:

both A and B comprise V20E/T119D, and both C and D comprise V20R/V121K;
both A and B comprise L17D/T119D, and both C and D comprise L17K/V121K;
both A and B comprise L17E/T119D, and both C and D comprise L17R/V121K;
both A and B comprise L17E/V20D, and both C and D comprise L17R/V20K;
both A and B comprise L17D/V20D, and both C and D comprise L17K/V20K; or
both A and B comprise L17E/V121E, and both C and D comprise L17R/V121R.

7. The TTR protein complex according to any one of claims 1-6, wherein said TTR protein complex is attached to 1, 2, 3, 4, 5, 6, 7, or 8 antigen binding proteins or peptides.

8. The TTR protein complex according to claim 7, wherein said TTR protein complex is attached to 1, 2, 3, or 4 antigen binding proteins or peptides.

9. The TTR protein complex according to claim 7 or 8, wherein said antigen binding protein or peptide is attached to the TTR protein complex at the C-terminus of a TTR subunit.

10. The TTR protein complex according to claim 7 or 8, wherein said antigen binding protein or peptide is attached to the TTR protein complex at the N-terminus of a TTR subunit.

11. The TTR protein complex according to any one of claims 7-10, wherein said TTR protein complex is

directly attached to the antigen binding proteins.

12. The TTR protein complex according to any one of claims 7-10, wherein said TTR protein complex is

directly attached to the antigen binding proteins via a linker.

13. The TTR protein complex according to claim 12, wherein said linker is an amino acid-based linker comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60 amino acids.

14. The TTR protein complex according to claim 12, wherein said linker is an amino acid-based linker comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 amino acids.

15. The TTR protein complex according to claim 12, wherein said linker is an amino acid-based linker comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids.

16. The TTR protein complex according to claim 12, wherein said linker is an amino acid-based linker comprising 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids.

17. The TTR protein complex according to claim 12, wherein said linker is G, GG, GGG, GGGG, GGGGG, GGGGGG, GGGGGGG, GGGGGGGG, GGGGGGGGG, or GGGGGGGGGG.

18. The TTR protein complex according to claim 12, wherein said linker is $G(G_xB_y)_rG_z$ and wherein:

G = glycine;
B = any amino acid;
x = 1-15;
y = 1-5;
z = 1-15; and
r = 1-20.

19. The TTR protein complex according to claim 18, wherein:

B = Q, S, A, E, P, T, K, R, D or N;
x = 4;
y = 1;
z = 4; and
r = 1.

20. The TTR protein complex according to claim 12, wherein said linker is selected from the list comprising: GG, GGGG, GGGSGG, GGGSGGGG, and GGAGGGAGGG.

21. The TTR protein complex according to any one of claims 7-20, wherein said TTR protein complex is attached to two antigen binding proteins, wherein said antigen binding proteins bind different antigens.

22. The TTR protein complex according to any one of claims 7-20, wherein said TTR protein complex is attached to four antigen binding proteins, wherein said antigen binding proteins bind to at least two different antigens.

23. The TTR protein complex according to any one of claims 7-22, wherein the antigen binding proteins are antibodies.

24. The TTR protein complex according to any one of claims 7-22, wherein the antigen binding proteins are Fabs or scFvs.

25. The TTR protein complex according to claim 24, wherein the antigen binding proteins are Fabs.

26. The TTR protein complex according to any one of claims 7-22, wherein the antigen binding proteins are a mixture of antibodies and Fabs.

27. The TTR protein complex according to any one of claims 21-26, wherein said antigens are antigens on tumor cells.

28. A pharmaceutical composition comprising a TTR protein complex according to any one of claims 1-27.

29. A TTR protein complex according to claim 27 for use in therapy.

30. One or more isolated nucleic acid(s) encoding a TTR protein complex according to any one of claims 1-27.

31. An expression vector comprising a nucleic acid of claim 30.

32. A recombinant host cell comprising a nucleic acid of claim 30 or a vector of claim 31.

33. The recombinant host cell of claim 32, 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.

34. A method of making a TTR protein complex according to any one of claims 1-27, said method comprising:

culturing the recombinant host cell of claim 32 or 33; and
isolating the TTR protein complex from said culture.