What is claimed is:

Claims

1. An in-vitro method for expanding a population of lymphocytes, comprising: contacting a sample that contains a population of lymphocytes with a multimerizing reagent wherein a first agent and a second agent are reversibly bound; wherein the multimerizing reagent is in a soluble form and comprises an oligomer or polymer of streptavidin, avidin, streptavidin mutein, or avidin mutein; wherein the first agent (i) comprises at least one of a first streptavidin or a avidin binding partner capable of reversibly binding to a first binding site of the multimerizing reagent and is capable of forming a reversible bond between the first streptavidin or avidin binding partner and the first binding site, (ii) capable of binding to a receptor molecule on a surface of a lymphocyte in the population and stimulating a primary signal to the lymphocyte; and wherein the second agent (i) comprises at least one of a second streptavidin or avidin binding partner capable of reversibly binding to a second binding site of the multimerizing reagent and is capable of forming a reversible bond between the second streptavidin or avidin binding partner and the second binding site, (ii) capable of binding to an accessory molecule on a surface of the lymphocyte, capable of stimulating an accessory signal to the lymphocyte, and thereby capable of stimulating a lymphocyte in a population.

2. The method of claim 1, wherein the lymphocyte population is a B cell population, a T cell population, a natural killer cell population, or a mixture of these.

3. The method of claim 2, wherein the population of lymphocytes comprises a T cell population and the first agent, comprises an MHC I: peptide complex or stimulates a TCR/CD3 complex related signal in T cell, and/or comprises a binding reagent that binds to CD3.

4. The method of claim 3, wherein the accessory molecule is CD28 or CD137, and/or the second agent comprises a binding reagent that binds to CD28 or CD137, or both are a mixture of two different binding reagents that specifically binds CD28 or CD137.

5. The method of any one of claims 1 to 4, wherein the first agent specifically binds CD3.

6. The method of any one of claims 1 to 5, wherein the second agent specifically binds CD28 or CD137.

7. The method of any of claims 1 to 6, wherein the first agent is an anti-CD3 antibody, a bivalent antibody fragment of an anti-CD3 antibody, a monovalent antibody fragment of an anti-CD3 antibody, or a proteinaceous CD3 binding molecule that has an antibody-like binding characteristic.

8. The method of claim 7, wherein the monovalent antibody fragment is selected from a group consisting of a Fab fragment, a Fv fragment, and a single-chain Fv fragment (scFv).

9. The method of any one of claims 1 to 8, wherein the second agent is an anti-CD28 antibody, a bivalent antibody of an anti-CD28 antibody, a monovalent antibody fragment of an anti-CD28 antibody, or proteinaceous CD28 molecule having an antibody-like binding characteristic.

10. The method of claim 9, wherein the monovalent antibody fragment is selected from a group consisting of a Fab fragment, a Fv fragment, and a single-chain Fv fragment (scFv).

11. The method of any one of claims 1 to 10, wherein the first agent is an anti-CD3Fab and the second agent is an anti-CD28Fab.

12. The method of claim 3, wherein the population of lymphocytes comprises a B cell population, the receptor molecule is CD40 or CD137, and/or the first agent comprises a binding reagent that binds CD40 or CD137.

13. The method of any one of claims 1 to 12, wherein each subunit of the oligomer or polymer is cross-linked by a polysaccharide.

14. The method of any one of claims 1 to 12, wherein each subunit of the oligomer or polymer is cross-linked via a bifunctional linker.

15. The method of any one of claims 1 to 14, wherein the oligomer or polymer of streptavidin, avidin, streptavidin mutein, or avidin mutein comprises three or more monomers of streptavidin, avidin, streptavidin mutein, or avidin mutein.

16. The method of any one of claims 1 to 15, wherein the multimerizing reagent comprises an oligomer or a polymer of streptavidin mutein.

17. The method of any one of claims 1 to 16, wherein the first streptavidin or avidin binding partner and/or the second streptavidin or avidin binding partner comprises streptavidin or avidin binding peptide.

18. The method of any one of claims 1 to 17, wherein the first streptavidin or avidin binding partner and/or the second streptavidin or avidin binding partner independently comprises a streptavidin binding peptide Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ ID NO. 1) or a streptavidin binding peptide SAWSHPQFEK$(GGGS)_2$GGSAWSHPQFEK (SEQ. ID NO. 7).

19. The method of any one of claims 1 to 18, wherein the multimerizing reagent comprises streptavidin mutein comprising amino acid sequence $Val1^{44}$-Thr^{45}-Ala^{46}-Arg^{47} in sequence positions 44 to 47 of a wild type streptavidin, or streptavidin mutein comprising amino acid sequence Ile^{44}-Gly^{45}-Ala^{46}-Arg^{47} in sequence positions 44 to 47 of a wild type streptavidin.

20. The method of claim 19, wherein an N-terminal amino acid residue of the streptavidin mutein is within a region of amino acids 10 to 16 of a wild type streptavidin amino acid sequence, and a C-terminal amino acid residue of the streptavidin mutein is within a region of amino acids 133 to 142 of a wild type streptavidin amino acid sequence.

21. The method of any of claims 1 to 20, wherein the first or second streptavidin or avidin binding partners are identical.

22. The method of any of claims 1 to 20, wherein the first and second streptavidin or avidin binding partner are different.

23. The method of any of claims 1 to 22, further comprising separating a bond between the first streptavidin or avidin binding partner of the first agent and the first binding site of the multimerizing reagent, and/or separating a bond between the second streptavidin or avidin binding partner of the second agent and the second binding site of the multimerizing reagent.

24. The method of claim 23, wherein the separating causes the stimulation of a primary signal and/or an accessory signal to a lymphocyte to end.

25. The method of any of claims 1 to 24, wherein dissociation constant (K_d) of a reversible bond between the first streptavidin or avidin binding partner and the first binding site and/or a dissociation constant (K_d) of reversible bond between the second streptavidin or avidin binding partner and the second binding site is in a range of 10^{-2}M to 10^{-13}M.

26. The method of any one of claims 1 to 25, wherein a reversible bond between the first streptavidin or avidin binding partner and the first binding site and/or a reversible bond between the second streptavidin or avidin binding partner and the second binding site is separated by contacting a population of lymphocytes with biotin or a biotin analog.

27. The method of any of claims 1 to 26, wherein contacting the sample with the multimerizing reagent causes a specific binding property of a T cell towards the multimerizing reagent.

28. The method of claim 27, further comprising separating the T cell bound to the multimerizing reagent from a non-bound cell of the cell population.

29. The method of claim 27 or 28, wherein a T cell receptor or a chimera antigen receptor is introduced to a T cell either after stimulation or during stimulation.

30. The method of claim 29, wherein the T cell is stimulated by a third agent that binds to the introduced T cell receptor of the chimera antigen receptor.

31. The method of any one of claims 1 to 30, wherein the population of lymphocytes is a population of peripheral blood mononuclear cells (PBMC) or a population of concentrated or purified T cells.

32. The multimerizing reagent that reversibly binds to the first agent and the second agent, wherein the multimerizing reagent is in a soluble form and comprises an oligomer or polymer of streptavidin, avidin, streptavidin mutein, or avidin mutein, wherein; the first agent comprises (i) a first streptavidin or avidin binding peptide capable of reversibly binding to a first binding site of the multimerizing reagent, and (ii) a first antibody or an antigen-binding fragment thereof that binds to a receptor molecule on a surface of a lymphocyte and stimulates a signal to the lymphocyte; and the second agent comprises (i) a second streptavidin or avidin binding peptide capable of reversibly binding to a second binding site of the multimerizing reagent, and (ii) a second antibody or an antigen binding fragment thereof that binds to an accessory molecule on a surface of a lymphocyte and provides a co-stimulating signal to the lymphocyte.

33. The multimerizing reagent of claim 32, capable of stimulating a lymphocyte population.

34. The multimerizing reagent of claim 33, wherein the lymphocyte population to be stimulated is a B cell population, T cell population, or a natural killer cell population.

35. The multimerizing reagent of any one of claims 32 to 34, wherein the first agent comprises an MHC I: peptide complex or the first agent stimulates a TCR/CD3 complex related signal in the T cell.

36. The multimerizing reagent of any one of claims 32 to 35, wherein the first agent specifically binds CD3.

37. The multimerizing reagent of any one of claims 32 to 36, wherein the second agent that binds the accessory molecule is a binding agent that specifically binds CD28 or CD137.

38. The multimerizing reagent of any one of claims 32 to 37, wherein the first agent specifically binds CD3 and is selected from a group consisting of an anti-CD3 antibody, a bivalent antibody fragment of an anti-CD3 antibody, and a monovalent antibody fragment of an anti-CD3 antibody; and the second agent specifically binds CD28 or CD137 and is selected from a group consisting of an anti-CD28 antibody, a bivalent antibody fragment of an anti-CD28 antibody, and a monovalent antibody fragment of an anti-CD28 antibody, an anti-CD137 antibody, a bivalent antibody fragment of an anti-CD137 antibody, and a monovalent antibody fragment of an anti-CD137 antibody.

39. The multimerizing reagent of claim 38, wherein the bivalent antibody fragment is a $F(ab')_2$ fragment or a bivalent single-chain Fv fragment.

40. The multimerizing reagent of claim 38, wherein the monovalent antibody fragment is selected from a group consisting of a Fab fragment, a Fv fragment, and a single-chain Fv fragment (scFv).

41. The multimerizing reagent of claim 40, wherein the first agent is a monovalent antibody fragment of an anti-CD3 antibody that is Fab, and the second agent is a monovalent antibody fragment of an anti-CD28 antibody that is Fab.

42. The multimerizing reagent any one of claims 32 to 34, wherein the first agent is a binding reagent that specifically binds CD40 or CD137, and a population of lymphocytes comprises a B cell.

43. The multimerizing reagent of any one of claims 32 to 42, wherein the oligomer or polymer of streptavidin, avidin, streptavidin mutein, or avidin mutein is cross-linked by a polysaccharide or via a bifunctional linker.

44. The multimerizing reagent of any one of claims 32 to 43, wherein the oligomer or polymer of streptavidin, avidin, streptavidin mutein, or avidin mutein comprises three or more monomers of streptavidin, avidin, streptavidin mutein, or avidin mutein.

45. The multimerizing reagent of any one of claims 32 to 44, wherein the first streptavidin or avidin binding peptide and second streptavidin or avidin binding peptide independently comprises streptavidin binding peptide Trp-Ser-His-Pro-Gln-Phe-Glu-Lys (SEQ. ID NO. 1) or streptavidin binding peptide SAWSHPQFEK$(GGGS)_2$GGSAWSHPQFEK (SEQ. ID NO. 7).

46. The multimerizing reagent of any one of claims 32 to 45, wherein the multimerizing reagent comprises streptavidin mutein comprising amino acid sequence $Va1^{44}$-Thr^{45}-Ala^{46}-Arg^{47} in sequence positions 44 to 47 of a wild type streptavidin, or streptavidin mutein comprising amino acid sequence Ile^{44}-Gly^{45}-Ala^{46}-Arg^{47} in sequence positions 44 to 47 of a wild type streptavidin.

47. The multimerizing reagent of claim 46, wherein the N-terminal amino acid residue of the streptavidin mutein is within a region of amino acids 10 to 16 of a wild type streptavidin amino acid sequence, and the C-terminal amino acid residue of the streptavidin mutein is within a region of amino acids 133 to 142 of a wild type streptavidin amino acid sequence.