CLAIMS

1. A bispecific protein complex having the formula A-X:Y-B wherein:

   A-X is a first fusion protein;

   Y-B is a second fusion protein;

   X:Y is a heterodimeric-tether;

   : is a binding interaction between X and Y;

   A is a first protein component of the bispecific protein complex selected from a Fab or Fab' fragment;

   B is a second protein component of the bispecific protein complex selected from a Fab or Fab' fragment;

   wherein X is independently selected from a scFv, a VHH and a peptide, with the proviso that when X is a peptide Y is an antibody or an antigen-binding fragment thereof, such as a scFv or VHH and when X is a scFv or VHH then Y is an antigen, such as a peptide and wherein Y is independently selected from a scFv, a VHH and a peptide, with the proviso that when Y is a peptide X is an antibody or an antigen-binding fragment, such as a scFv or VHH and when Y is a scFv or a VHH then X is an antigen, such as a peptide,

   wherein X or Y is a scFv or VHH specific to the peptide GCN4 (SEQ ID NO:1 or amino acids 1 to 38 of SEQ ID NO:1) or wherein X or Y is a peptide GCN4 (SEQ ID NO:1 or amino acids 1 to 38 of SEQ ID NO:1).
2. A bispecific protein complex according to claim 1, wherein A is a Fab fragment.
3. A bispecific protein complex according to claim 1 or 2, wherein B is a Fab fragment.
4. A bispecific protein complex according to any one of claims 1 to 3, wherein X is fused, optionally via a linker, to the C-terminal of the heavy chain in the Fab or Fab' fragment.
5. A bispecific protein complex according to any one of claims 1 to 4, wherein Y is fused, optionally via a linker, to the C-terminal of the heavy chain in the Fab or Fab' fragment.
6. A bispecific protein complex according to any one of claims 1 to 5, wherein the peptide is in the range 5 to 25 amino acids in length.
7. A bispecific protein complex according to any one of claims 1 to 6, wherein the binding affinity between X and Y is 5 nM or stronger.
8. A bispecific protein complex according to claim 7, wherein the binding affinity between X and Y is 900pM or stronger, such as 800, 700, 600, 500, 400 or 300pM.
9. A bispecific protein complex according to any one of claims 1 to 8, wherein when X or Y is a scFv specific to the peptide GCN4 (SEQ ID NO:1 or amino acids 1 to 38 of SEQ ID NO:1), the scFv is 52SR4 (SEQ ID NO:3 or amino acids 1 to 243 of SEQ ID NO:3).

10. The bispecific protein complex according to any one of claims 1 to 9, wherein A and/or B is specific for an antigen selected from the group comprising: cell surface receptors such as T cell or B cell signalling receptors, co-stimulatory molecules , checkpoint inhibitors, natural killer cell receptors, Immunoglobulin receptors, TNFR family receptors, B7 family receptors, adhesion molecules, integrins, cytokine/chemokine receptors, GPCRs, growth factor receptors, kinase receptors, tissue-specific antigens, cancer antigens, pathogen recognition receptors, complement receptors, hormone receptors or soluble molecules such as cytokines, chemokines, leukotrienes, growth factors, hormones, enzymes, and ion channels.
11. A composition comprising one or more bispecific protein complexes defined in any one of claims 1 to 10.
12. A bispecific protein complex according to any one of claims 1 to 10 or a composition according to claim 11 for use in therapy.
13. A method of detecting synergistic biological function in a bispecific protein complex according to any one of claims 1 to 10 comprising testing the bispecific protein complex in one or more functional assays.
14. A method for detecting synergistic biological function in a heterodimerically-tethered bispecific protein complex of formula A-X:Y-B
wherein X:Y is a heterodimeric-tether
: is a binding interaction between X and Y,
A and B are protein components of the bispecific in the form of fusion proteins with X and Y respectively, said method comprising the steps of:

(i) testing for activity in a functional assay for part or all of a multiplex comprising at least one heterodimerically-tethered bispecific protein complex; and

(ii) analysing the readout(s) from the functional assay to detect synergistic biological function in the heterodimerically-tethered bispecific protein complex,

wherein X is independently selected from a scFv, a VHH and a peptide, with the proviso that when X is a peptide Y is an antibody or an antigen-binding fragment thereof, such as a scFv or VHH and when X is a scFv or VHH then Y is an antigen, such as a peptide and wherein Y is independently selected from a scFv, a VHH and a peptide, with the proviso that when Y is a peptide X is an antibody or an antigen-binding fragment, such as a scFv or VHH and when Y is a scFv or a VHH then X is an antigen, such as a peptide,

wherein X or Y is a scFv or VHH specific to the peptide GCN4 (SEQ ID NO:1 or amino acids 1 to 38 of SEQ ID NO:1) or wherein X or Y is a peptide GCN4 (SEQ ID NO:1 or amino acids 1 to 38 of SEQ ID NO:1).

15. A method according to claim 14 wherein the multiplex comprises at least two heterodimerically-tethered bispecific protein complexes.
16. A method according to any one of claims 14 to 15 wherein the heterodimerically tethered bispecific protein complex(es) do not contain an Fc region.
17. A method according to any one of claims 14 or 16, wherein A is independently selected from a Fab fragment, a Fab' fragment, a VHH, a VH, a VL and a scFv.
18. A method according to claim 17, wherein A is a Fab or Fab' fragment, such as a Fab.
19. A method according to any one of claims 14 to 18, wherein B is independently selected from an antibody, a Fab fragment, a Fab' fragment, a VHH, a VH, a VL and a scFv.
20. A method according to claim 19, wherein B is a Fab or Fab' fragment, such as a Fab fragment.
21. A method according to any one of claims 14 to 20, wherein X is fused, optionally via a linker, to the C-terminal of a heavy chain in the antibody, Fab or Fab' fragment.
22. A method according to any one of claims 14 to 21, wherein Y is fused, optionally via a linker to the C-terminal of a heavy chain in the antibody, Fab or Fab' fragment.
23. A method according to any one of claims 14 to 22, wherein the peptide is in the range 5 to 25 amino acids in length.
24. A method according to any one of claims 14 to 23 wherein the binding affinity between X and Y is 5 nM or stronger.
25. A method according to claim 24, wherein the binding affinity of between X and Y is 900pM or stronger, such as 800, 700, 600, 500, 400 or 300pM.
26. A method according to any one of claims 14 to 25, wherein when X or Y is a scFv specific to the peptide GCN4 (SEQ ID NO:1 or amino acids 1 to 38 of SEQ ID NO:1), the scFv is 52SR4 (SEQ ID NO: 3 or amino acids 1-243 of SEQ ID NO: 3).
27. A method according to any one of claims 14 to 26, wherein A and/or B is specific for an antigen selected from the group comprising: cell surface receptors such as T cell or B cell signalling receptors, co-stimulatory molecules , checkpoint inhibitors, natural killer cell receptors, Immunoglobulin receptors, TNFR family receptors, B7 family receptors, adhesion molecules, integrins, cytokine/chemokine receptors, GPCRs, growth factor receptors, kinase receptors, tissue-specific antigens, cancer antigens, pathogen recognition receptors, complement receptors, hormone receptors or soluble molecules

such as cytokines, chemokines, leukotrienes, growth factors, hormones or enzymes or ion channels.

28. A method according to any one of claims 14 to 27 wherein the heterodimerically tethered bispecific protein complexes are not purified prior to testing.
29. A method according to claim 28 wherein the A-X and Y-B fusion proteins are expressed transiently and not purified before being mixed in a 1:1 molar ratio to generate each heterodimerically tethered bispecific protein complex.