

binding protein complexes are relatively simple compared to existing multi-specific antibodies in that only two or three polypeptide chains are used per complex, which is fewer than a standard human IgG molecule, which consists of four chains. In some embodiments, the positioning of the half Fab heavy and half Fab light regions on the two or three chains of the complexes according to the disclosure will favor efficient formation of heterodimer protein complexes that bind two different epitopes, or at least provide the public with a useful choice.

[0008] In some embodiments, the multi-specific antigen binding protein complexes can activate an immune cell in the presence of a target cell. In some embodiments, the multi-specific antigen binding protein complexes may provide one or more benefits, such as high specificity for activation of immune cells in the presence of cancer cells or a target tumor environment, and/or high efficacy in promoting immune cell-mediated target cell killing, or at least provide the public with a useful choice.

SUMMARY

[0009] The present disclosure provides various examples of a two-chain multi-specific antigen binding protein complex that can bind two different epitopes on the same target antigen or two different epitopes on different target antigens at the same time, where the protein complex comprises: two different Fab regions, an Fc region, and a first and a second linker.

[0009a] In one aspect, provided herein is an activatable multi-specific antigen binding protein complex, comprising a) a first polypeptide chain comprising (i) a first half Fab *heavy* region, (ii) a first linker, (iii) a second half Fab *heavy* region, and (iv) a first half Fc region, wherein the first half Fab *heavy* region comprises a first-variable region and first-constant region from a first Fab *heavy* chain, and wherein the second half Fab *heavy* region comprises a second-variable region and second-constant region from a second Fab *heavy* chain; and a second polypeptide chain comprising (i) a first half Fab *light* region, (ii) a second linker, (iii) a second half Fab *light* region, and (iv) a second half Fc region, wherein the first half Fab *light* region comprises a first-variable region and first-constant region from a first Fab *light* chain, and wherein the second half Fab *light* region comprises a second-variable region and second-constant region from a second Fab *light* chain, wherein the second linker is cleavable, and wherein the first and second polypeptide chains associate with each other to form the multi-specific antigen binding protein complex having a first full Fab domain that is capable of binding a first epitope, a second full

Fab domain that is capable of binding a second epitope that differs from the first epitope, a full Fc domain that is capable of binding an Fc receptor, and a first and second linker, wherein the first full Fab domain and the second full Fab domain are different and are arranged in tandem; **or** b) a first polypeptide chain comprising (i) a first half Fab *heavy* region, (ii) a first half Fc region, (iii) a first linker, and (iv) a second half Fab *heavy* region, wherein the first half Fab *heavy* region comprises a first-variable region and first-constant region from a first Fab *heavy* chain, and wherein the second half Fab *heavy* region comprises a second-variable region and second-constant region from a second Fab *heavy* chain; and a second polypeptide chain comprising (i) a first half Fab *light* region, (ii) a second half Fc region, (iii) a second linker, and (iv) a second half Fab *light* region, wherein the first half Fab *light* region comprises a first-variable region and first-constant region from a first Fab *light* chain, and wherein the second half Fab *light* region comprises a second-variable region and second-constant region from a second Fab *light* chain, wherein the second linker is cleavable, and wherein the first and second polypeptide chains associate with each other to form the multi-specific antigen binding protein complex having a first full Fab domain that is capable of binding a first epitope, a second full Fab domain that is capable of binding a second epitope that differs from the first epitope, a full Fc domain that is capable of binding an Fc receptor, and a first and second linker, wherein the first full Fab domain and the second full Fab domain are different and are arranged in a non-tandem manner.

[0009b] In one aspect, provided herein is a pharmaceutical composition comprising the multi-specific antigen binding protein complex as described herein and a pharmaceutically-acceptable excipient.

[0009c] In one aspect, provided herein is one or more nucleic acids encoding (i) the first polypeptide chain of the multi-specific antigen binding protein complex as described herein and (ii) the second polypeptide chain the multi-specific antigen binding protein complex as described herein.

[0009d] In one aspect, provided herein is one or more expression vectors comprising the one or more nucleic acids as described herein operably linked to one or more promoters.

[0009e] In one aspect, provided herein is a host cell harboring the one or more expression vectors as described herein.

[0009f] In one aspect, provided herein is a method for preparing a multi-specific antigen binding protein complex, comprising: a) culturing a population of the host cell as described herein under

conditions suitable for expressing the first and the second polypeptide chains and suitable for associating the first and second polypeptide chains with each other to form the multi-specific antigen binding protein complex; and b) recovering the multi-specific antigen binding protein complex from the population of the host cell.

[0009g] In one aspect, provided herein is the multi-specific antigen binding protein complex as described herein for use in therapy.

[0009h] In one aspect, provided herein is a use of a therapeutically effective amount of the multi-specific antigen binding protein complex as described herein in the manufacture of a medicament for treating a disease in a subject, wherein the disease comprises a cancer, comprising a prostate cancer, breast cancer, ovarian cancer, head and neck cancer, bladder cancer, skin cancer, colorectal cancer, anal cancer, rectum cancer, pancreatic cancer, lung cancer (including non-small cell lung and small cell lung cancers), leiomyoma cancer, brain cancer, glioma cancer, glioblastoma cancer, esophagus cancer, liver cancer, kidney cancer, stomach cancer, colon cancer, cervical cancer, uterine cancer, endometrial cancer, vulva cancer, larynx cancer, vaginal cancer, bone cancer, nasal cavity cancer, paranasal sinus cancer, nasopharynx cancer, oral cavity cancer, oropharynx cancer, larynx cancer, hypolarynx cancer, salivary gland cancer, ureter cancer, urethral cancer, penial cancer, or testicular cancer.

[0009i] In one aspect, provided herein is a method for binding a first and a second epitope, comprising: a) contacting the first epitope with the multi-specific antigen binding protein complex as described herein, wherein the second linker is in an un-cleaved state; and b)

[0010] binding the first epitope with the first Fab region, wherein the first Fab region binds the first epitope and the second Fab region exhibits a level of binding to the second epitope that is reduced relative to the level of binding to the second epitope exhibited when the second linker is in a cleaved state. In one example, the two different Fab regions and the Fc region are formed from the association of a first polypeptide chain with a second polypeptide chain, wherein each of the first and second polypeptide chains carries two different half Fab regions and a half Fc region, wherein the first polypeptide chain carries the first linker, the second polypeptide chain carries the second linker, and the second linker is cleavable.

[0011] In one example, the first and second polypeptide chains associate with each other to form the multi-specific antigen binding protein complex having a first Fab region that is

capable of binding a first epitope, a second Fab region that is capable of binding a second epitope that differs from the first epitope, a full Fc region that is capable of binding an Fc receptor, and a first and second linker.

[0012] In one example, the first Fab region exhibits binding to the first target epitope and the second Fab region exhibits reduced binding to the second target epitope when the second linker is un-cleaved compared to when the second linker is cleaved. In one example, the

second Fab region exhibits increased binding to the second target epitope when the second linker is cleaved compared to when the second linker is un-cleaved. In one example, upon cleavage of the second linker, the first Fab region and the second Fab region are capable of binding to the first and second target epitopes, respectively, at the same time.

[0013] In one example, the first linker is cleavable with a matrix metalloprotease, wherein the matrix metalloprotease is MMP1, MMP2, MMP3, MMP8, MMP9, MMP11, MMP13, MMP14, or MT1-MMP (membrane type 1 matrix metalloproteinase).

[0014] In one example, the first linker comprises the amino acid sequence of TSGSGGSGGSV (SEQ ID NO: 156), (SG)_n (SEQ ID NO: 157), (SGG)_n (SEQ ID NO: 158), (SGGG)_n (SEQ ID NO: 159), (SSG)_n (SEQ ID NO: 160), (GS)_n (SEQ ID NO: 161), (GGG)_n (SEQ ID NO: 162), (GSGGS)_n (SEQ ID NO: 163), (GSG)_n (SEQ ID NO: 164), (GGGGS)_n (SEQ ID NO: 165), (GGGS)_n (SEQ ID NO: 166), (GGGGSGS)_n (SEQ ID NO: 167), (GGGGSGGS)_n (SEQ ID NO: 168) or (GGS)_n (SEQ ID NO: 169), wherein n is an integer of 1-6.

[0015] In one example, the second linker comprises the amino acid sequence of GGSGSGSGGSSGGGSGGGGS (DP linker), TSGSGGSGGSV (EG or EH linker), TSGSGGSPLGMGGSGSV (EI or EU linker), TSGSGGSPLGVGGSGSV (EJ or EV linker), TSGSGGSPAALGGSGSV (EK or EW linker), TSGSGGSPAGLGGSGSV (EL or EX linker), TSGSGGSPLGMVGV (EM or EY linker), TSGSGGSPLGVVGV (EN or EZ linker), TSGSGGSPAALVGV (EO or FA linker), TSGSGGSPAGLVGV (EP or FB linker), TSGSGGSPLGMVLV (EQ or FC linker), TSGSGGSPLGVVLV (ER or FD linker), TSGSGGSPAALVLV (ES or FE linker) or TSGSGGSPAGLVVLV (ST or FF linker) (SEQ ID NOS; 11-24, respectively, or SEQ ID NOS:43-56, respectively).

[0016] In one example of the two-chain multi-specific antigen binding protein complex, the two different Fab regions are arranged in-tandem, and the protein complex comprises: (a) a first polypeptide chain comprising (i) a first half Fab *heavy* region, (ii) a first linker, (iii) a second half Fab *heavy* region, and (iv) a first half Fc region, wherein the first half Fab *heavy* region comprises a first-variable region and first-constant region from a first Fab *heavy* chain, and wherein the second half Fab *heavy* region comprises a second-variable region and second-constant region from a second Fab *heavy* chain; and (b) a second polypeptide chain comprising (i) a first half Fab *light* region, (ii) a second linker, (iii) a second half Fab *light* region, and (iv) a second half Fc region, wherein the first half Fab *light* region comprises a first-variable region

and first-constant region from a first Fab *light* chain, and wherein the second half Fab *light* region comprises a second-variable region and second-constant region from a second Fab *light* chain, and wherein the second linker is cleavable. Non-limiting examples of in-tandem protein complexes are shown in Figures 1, 5, 6 and 7.

[0017] In one example of the two-chain multi-specific antigen binding protein complex, the two different Fab regions are arranged in a non-tandem manner, and the protein complex comprises: (a) a first polypeptide chain comprising (i) a first half Fab *heavy* region, (ii) a first half Fc region, (iii) a first linker, and (iv) a second half Fab *heavy* region, wherein the first half Fab *heavy* region comprises a first-variable region and first-constant region from a first Fab *heavy* chain, and wherein the second half Fab *heavy* region comprises a second-variable region and second-constant region from a second Fab *heavy* chain; and (b) a second polypeptide chain comprising (i) a first half Fab *light* region, (ii) a second half Fc region, (iii) a second linker, and (iv) a second half Fab *light* region, wherein the first half Fab *light* region comprises a first-variable region and first-constant region from a first Fab *light* chain, and wherein the second half Fab *light* region comprises a second-variable region and second-constant region from a second Fab *light* chain, and wherein the second linker is cleavable. Non-limiting examples of non-tandem protein complexes are shown in Figures 3, 8, 9 and 10.

[0018] In one example, any of the two-chain multi-specific antigen binding protein complexes described herein comprise (i) a first polypeptide chain having a hole mutation in the first half Fc region and a second polypeptide chain having a knob mutation in the second half Fc region, or (ii) a first polypeptide chain having a knob mutation in the first half Fc region and a second polypeptide chain having a hole mutation in the second half Fc region.

[0019] In one example, any of the two-chain multi-specific antigen binding protein complexes described herein comprise first and second polypeptide chains having any one or any combination of two or more knob and hole mutations comprising T366Y, T366W, T366S, L368A, T394S, T394W, F405A, F405W, Y407A, Y407V and/or Y407T according to Kabat numbering.

[0020] In one example, any of the two-chain multi-specific antigen binding protein complexes described herein comprise a first Fab region that binds the first target epitope with a dissociation constant K_d of 10^{-5} M or less, or 10^{-6} M or less, or 10^{-7} M or less, or 10^{-8} M or less, or 10^{-9} M or less, or 10^{-10} M or less.

[0021] In one example, any of the two-chain multi-specific antigen binding protein complexes described herein comprise a second Fab region wherein upon cleavage of the second linker the second Fab region binds the second target epitope with a dissociation constant K_d of 10^{-5} M or less, or 10^{-6} M or less, or 10^{-7} M or less, or 10^{-8} M or less, or 10^{-9} M or less, or 10^{-10} M or less.

[0022] In one example of the two-chain multi-specific antigen binding protein complex, the two different Fab regions are arranged in-tandem, and the protein complex comprises: **(a)** a first polypeptide chain which comprises (e.g., in N- to C-terminal order): (i) a first heavy chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:1; (ii) a first heavy chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:2; (iii) a first linker comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:3; (iv) a second heavy chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:4; (v) a second heavy chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:5; (vi) a first hinge sequence comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:6; (vii) a first CH2 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:7; and (viii) a first CH3 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:8; and **(b)** a second polypeptide chain which comprises (e.g., in N- to C-terminal order): (i) a first light chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:9; (ii) a first light chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:10; (iii) a second linker comprising an amino acid sequence that is at least 95% identical to any one of SEQ ID NOS:11-24; (iv) a second light chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:25; (v) a second light chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:26; (vi) a second hinge sequence comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:27; (vii) a second CH2 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:28; and (viii) a second CH3 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:29 (e.g., Kv6.1, Figures 31A-C).

[0023] In one example of the two-chain multi-specific antigen binding protein complex, the two different Fab regions are arranged in a non-tandem manner, and the protein complex

comprises: **(a)** a first polypeptide chain which comprises (e.g., in N- to C-terminal order): (i) a first heavy chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:30; (ii) a first heavy chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:31; (iii) a first hinge comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:32; (iv) a first CH2 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:33; (v) a first CH3 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:34; (vi) a first linker sequence comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:35; (vii) a second heavy chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:36; and (viii) a second heavy chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:37; and **(b)** a second polypeptide chain which comprises (e.g., in N- to C-terminal order): (i) a first light chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:38; (ii) a first light chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:39; (iii) a second hinge comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:40; (iv) a second CH2 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:41; (v) a second CH3 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:42; (vi) a second linker sequence comprising an amino acid sequence that is at least 95% identical to any one of SEQ ID NOS:43-56; (vii) a second light chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:57; and (viii) a second light chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:58 (e.g., Kv6.2, Figures 32A-C).

[0024] The present disclosure provides a pharmaceutical composition comprising any one of the two-chain multi-specific antigen binding protein complexes described herein and a pharmaceutically-acceptable excipient.

[0025] In one example, any of the two-chain multi-specific antigen binding protein complexes described herein can be used as a medicament.

[0026] The present disclosure provides one or more nucleic acids encoding (i) the first polypeptide chain of any one of the two-chain multi-specific antigen binding protein complexes

described herein, and encoding (ii) the second polypeptide chain of any of the two-chain multi-specific antigen binding protein complexes described herein.

[0027] The present disclosure provides one or more vectors comprising any of the one or more nucleic acids described herein operably linked to one or more promoters.

[0028] The present disclosure provides a host cell harboring any of the one or more vectors described herein.

[0029] The present disclosure provides a method for preparing any of the two-chain multi-specific antigen binding protein complexes described herein, comprising: culturing a population of the host cell described herein under conditions suitable for expressing the first and second polypeptide chains. In one example, the method further comprises: isolating (e.g., recovering) the first and second polypeptide chains. In one example, the method further comprises subjecting the first and second polypeptide chains to conditions suitable for associating the first and second polypeptide chains with each other to form the two-chain multi-specific antigen binding protein complex. In one example, the formed two-chain multi-specific antigen binding protein complex comprises a hetero-dimeric molecule.

[0030] The present disclosure provides (i) a first nucleic acid encoding the first polypeptide chain of any one of the two-chain multi-specific antigen binding protein complexes described herein, and (ii) a second nucleic acid encoding the second polypeptide chain of any of the two-chain multi-specific antigen binding protein complexes described herein.

[0031] The present disclosure provides (i) a first vector operably linked to the first nucleic acid encoding the first polypeptide chain of any one of the two-chain multi-specific antigen binding protein complexes described herein, and (ii) a second vector operably linked to the second nucleic acid encoding the second polypeptide chain of any of the two-chain multi-specific antigen binding protein complexes described herein. In one example, the first vector is a first expression vector comprising at least one promoter which is operably linked to the first nucleic acid encoding the first polypeptide chain. In one example, the second vector is a second expression vector comprising at least one promoter which is operably linked to the second nucleic acid encoding the second polypeptide chain.

[0032] The present disclosure provides a host cell harboring any of the first and second vectors described herein.

[0033] The present disclosure provides method for preparing any of the two-chain multi-specific antigen binding protein complexes described herein, comprising: culturing a population of the host cell harboring the first and second vectors under conditions suitable for expressing the first and second polypeptide chains, wherein the first vector is operably linked to the first nucleic acid encoding the first polypeptide chain of any one of the two-chain multi-specific antigen binding protein complexes described herein, and wherein the second vector is operably linked to the second nucleic acid encoding the second polypeptide chain of any of the two-chain multi-specific antigen binding protein complexes described herein. In one example, the method further comprises: isolating (recovering) the first and second polypeptide chains. In one example, the method further comprises subjecting the first and second polypeptide chains to conditions suitable for associating the first and second polypeptide chains with each other to form the two-chain multi-specific antigen binding protein complex. In one example, the formed two-chain multi-specific antigen binding protein complex comprises a hetero-dimeric molecule.

[0034] The present disclosure provides (i) a first host cell harboring the first vector comprising a promoter operably linked to the first nucleic acid encoding the first polypeptide chain of any one of the two-chain multi-specific antigen binding protein complexes described herein, and (ii) a second host cell harboring the second vector comprising a promoter operably linked to the second nucleic acid encoding the second polypeptide chain of any one of the two-chain multi-specific antigen binding protein complexes described herein.

[0035] The present disclosure provides a method for preparing any of the two-chain multi-specific antigen binding protein complexes described herein, comprising: (a) culturing a population of the first host cell harboring the first vector under conditions suitable for expressing the first polypeptide chain, and (b) culturing a population of the second host cell harboring the second vector under conditions suitable for expressing the second polypeptide chain. In one example, the method further comprises: isolating (recovering) the first and second polypeptide chains. In one example, the method further comprises subjecting the first and second polypeptide chains to conditions suitable for associating the first and second polypeptide chains with each other to form the two-chain multi-specific antigen binding protein complex. In one example, the formed two-chain multi-specific antigen binding protein complex comprises a hetero-dimeric molecule.

[0036] The present disclosure provides a method for treating a disease in a subject, comprising: administering to the subject a therapeutically effective amount of any one of the two-chain multi-specific antigen binding protein complexes described herein.

[0037] In one example the disease comprises a cancer of the: prostate, breast, ovary, head and neck, bladder, skin, colorectal, anus, rectum, pancreas, lung (including non-small cell lung and small cell lung cancers), leiomyoma, brain, glioma, glioblastoma, esophagus, liver, kidney, stomach, colon, cervix, uterus, endometrium, vulva, larynx, vagina, bone, nasal cavity, paranasal sinus, nasopharynx, oral cavity, oropharynx, larynx, hypolarynx, salivary glands, ureter, urethra, penis, or testis.

[0038] In one example, the disease comprises a hematologic cancer, wherein the hematologic cancer is B chronic lymphocytic leukemia (B-CLL), B and T acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), hairy cell leukemia (HCL), myeloproliferative disorder/neoplasm (MPDS), myelodysplasia syndrome, non-Hodgkin's lymphoma (NHL) including Burkitt's lymphoma (BL), Waldenstrom's Macroglobulinemia, mantle cell lymphoma, AIDS-related lymphoma, Hodgkin's Lymphoma (HL), T cell lymphoma (TCL), multiple myeloma (MM), plasma cell myeloma, plasmacytoma, giant cell myeloma, heavy-chain myeloma, or light chain or Bence-Jones myeloma.

[0039] The present disclosure provides a method for binding a first and a second target epitope, comprising: (a) contacting the first target epitope with any of the two-chain multi-specific antigen binding protein complexes described herein, wherein the second linker is in an un-cleaved state and the protein complex is inactive; and (b) binding the first target epitope with the first Fab region, wherein the first Fab region binds the first target epitope and the second Fab region exhibits reduced binding to the second target epitope when the second linker is in the un-cleaved state. In one example, the method further comprises: (c) cleaving the second linker to generate an activated two-chain multi-specific antigen binding protein complex wherein the second Fab region can bind to the second target epitope. In one example, the method further comprises: (d) contacting the second target epitope with the activated two-chain multi-specific antigen binding protein complex; and (e) binding the second target epitope with the second Fab region.

[0040] In one example, the first target epitope comprises a cell surface antigen expressed by a tumor or cancer cell.

[0041] In one example, second epitope comprises a cell surface antigen expressed by an effector T cell. In one example, the activated two-chain multi-specific antigen binding protein complex forms a cell synapse by binding the cell surface antigen expressed by the tumor or cancer cell and by binding the cell surface antigen expressed by the effector T cell.

[0042] In one example, the effector T cell in the cell synapse kills the tumor or cancer cell by mediating cytotoxic cell killing.

[0043] The present disclosure provides various examples of a three-chain multi-specific antigen binding protein complex that can bind two different epitopes on the same target antigen or two different epitopes on different target antigens at the same time, where the protein complex comprises: two different Fab regions, an Fc region, and a first and a second linker.

[0044] In one example, the two different Fab regions and the Fc region are formed from the association of a first, second and third polypeptide chains.

[0045] In one example, the first, second and third polypeptide chains associate with each other to form the multi-specific antigen binding protein complex having a first Fab region that is capable of binding a first epitope, a second Fab region that is capable of binding a second epitope that differs from the first epitope, a full Fc region that is capable of binding an Fc receptor, and a first and second linker.

[0046] In one example of the three-chain multi-specific antigen binding protein complex, the two different Fab regions are arranged in-tandem, and the protein complex comprises: (a) a first polypeptide chain comprising (i) a first half Fab *heavy* region, (ii) a first linker, (iii) a second half Fab *heavy* region, and (iv) a first half Fc region, wherein the first half Fab *heavy* region comprises a first-variable region and first-constant region from a first Fab *heavy* chain, and wherein the second half Fab *heavy* region comprises a second-variable region and second-constant region from a second Fab *heavy* chain; (b) a second polypeptide chain comprising (i) a first half Fab *light* region, wherein the first half Fab *light* region comprises a first-variable region and first-constant region from a first Fab *light* chain; and (c) a third polypeptide chain comprising (i) a second half Fab *light* region, and (ii) a second half Fc region, wherein the second half Fab *light* region comprises a second-variable region and second-constant region from

a second Fab *light* chain. Non-limiting examples of in-tandem protein complexes are shown in Figures 2, 11, 12 and 13.

[0047] In one example of the three-chain multi-specific antigen binding protein complex, the two different Fab regions are arranged in a non-tandem manner, and the protein complex comprises: (a) a first polypeptide chain comprising (i) a first half Fab *heavy* region, (ii) a first half Fc region, (iii) a first linker, and (iv) a second half Fab *heavy* region, wherein the first half Fab *heavy* region comprises a first-variable region and first-constant region from a first Fab *heavy* chain, and wherein the second half Fab *heavy* region comprises a second-variable region and second-constant region from a second Fab *heavy* chain; and (b) a second polypeptide chain comprising (i) a first half Fab *light* region, (ii) a second half Fc region, wherein the first half Fab *light* region comprises a first-variable region and first-constant region from a first Fab *light* chain; and (c) a third polypeptide chain comprising (i) a second half Fab *light* region, wherein the second half Fab *light* region comprises a second-variable region and second-constant region from a second Fab *light* chain. Non-limiting examples of non-tandem protein complexes are shown in Figures 4, 14, 15 and 16.

[0048] In one example, any of the three-chain multi-specific antigen binding protein complexes having the two different Fab regions are arranged in-tandem as described herein comprise (i) a first polypeptide chain having a hole mutation in the first half Fc region and a third polypeptide chain having a knob mutation in the second half Fc region, or (ii) a first polypeptide chain having a knob mutation in the first half Fc region and a third polypeptide chain having a hole mutation in the second half Fc region.

[0049] In one example, any of the three-chain multi-specific antigen binding protein complexes having the two different Fab regions are arranged in-tandem as described herein comprise first and third polypeptide chains having any one or any combination of two or more knob and hole mutations comprising T366Y, T366W, T366S, L368A, T394S, T394W, F405A, F405W, Y407A, Y407V and/or Y407T according to Kabat numbering.

[0050] In one example, any of the three-chain multi-specific antigen binding protein complexes having the two different Fab regions are arranged in a non-tandem manner as described herein comprise (i) a first polypeptide chain having a hole mutation in the first half Fc region and a second polypeptide chain having a knob mutation in the second half Fc region, or

(ii) a first polypeptide chain having a knob mutation in the first half Fc region and a second polypeptide chain having a hole mutation in the second half Fc region.

[0051] In one example, any of the three-chain multi-specific antigen binding protein complexes having the two different Fab regions are arranged in a non-tandem manner as described herein comprise first and second polypeptide chains having any one or any combination of two or more knob and hole mutations comprising T366Y, T366W, T366S, L368A, T394S, T394W, F405A, F405W, Y407A, Y407V and/or Y407T according to Kabat numbering.

[0052] In one example, any of the three-chain multi-specific antigen binding protein complexes described herein comprise a first Fab region that binds the first target epitope with a dissociation constant K_d of 10^{-5} M or less, or 10^{-6} M or less, or 10^{-7} M or less, or 10^{-8} M or less, or 10^{-9} M or less, or 10^{-10} M or less.

[0053] In one example, any of the three-chain multi-specific antigen binding protein complexes described herein comprise a second Fab region that binds the second target epitope with a dissociation constant K_d of 10^{-5} M or less, or 10^{-6} M or less, or 10^{-7} M or less, or 10^{-8} M or less, or 10^{-9} M or less, or 10^{-10} M or less.

[0054] In one example of the three-chain multi-specific antigen binding protein complex, the two different Fab regions are arranged in-tandem, and the protein complex comprises: **(a)** a first polypeptide chain which comprises (e.g., in N- to C-terminal order): (i) a first heavy chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:59; (ii) a first heavy chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:60; (iii) a first linker comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:61; (iv) a second heavy chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:62; (v) a second heavy chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:63; (vi) a first hinge sequence comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:64; (vii) a first CH2 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:65; and (viii) a first CH3 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:66; and **(b)** a second polypeptide chain which comprises (e.g., in N- to C-terminal order): (i) a first light chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID

NO:67; (ii) a first light chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:68; and (c) a third polypeptide chain which comprises (e.g., in N- to C-terminal order): (i) a second light chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:69; (ii) a second light chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:70; (iii) a second hinge sequence comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:71; (iv) a second CH2 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:72; and (v) a second CH3 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:73 (e.g., Kv5.1, Figures 33A-B).

[0055] In one example of the three-chain multi-specific antigen binding protein complex, the two different Fab regions are arranged in-tandem, and the protein complex comprises: (a) a first polypeptide chain which comprises (e.g., in N- to C-terminal order): (i) a first heavy chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:105; (ii) a first heavy chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:106; (iii) a first linker comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:107; (iv) a second heavy chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:108; (v) a second heavy chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:109; (vi) a first hinge sequence comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:110; (vii) a first CH2 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:111; and (viii) a first CH3 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:112; and (b) a second polypeptide chain which comprises (e.g., in N- to C-terminal order): (i) a first light chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:113; (ii) a first light chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:114; and (c) a third polypeptide chain which comprises (e.g., in N- to C-terminal order): (i) a second light chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:115; (ii) a second light chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:116; (iii) a second hinge sequence comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:117; (iv) a second CH2 domain comprising an amino acid sequence that is at least

95% identical to SEQ ID NO:118; and (v) a second CH3 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:119 (e.g., Kv5.1, Figures 36A-B).

[0056] In one example of the three-chain multi-specific antigen binding protein complex, the two different Fab regions are arranged in-tandem, and the protein complex comprises: **(a)** a first polypeptide chain which comprises (e.g., in N- to C-terminal order): (i) a first heavy chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:135; (ii) a first heavy chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:136; (iii) a first linker comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:137; (iv) a second heavy chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:138; (v) a second heavy chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:139; (vi) a first hinge sequence comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:140; (vii) a first CH2 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:141; and (viii) a first CH3 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:142; and **(b)** a second polypeptide chain which comprises (e.g., in N- to C-terminal order): (i) a first light chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:143; (ii) a first light chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:144; and **(c)** a third polypeptide chain which comprises (e.g., in N- to C-terminal order): (i) a second light chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:145; (ii) a second light chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:146; (iii) a second hinge sequence comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:147; (iv) a second CH2 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:148; and (v) a second CH3 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:149 (e.g., Kv5.1, Figures 38A-B).

[0057] In one example of the three-chain multi-specific antigen binding protein complex, the two different Fab regions are arranged in a non-tandem manner, and the protein complex comprises: **(a)** a first polypeptide chain which comprises (e.g., in N- to C-terminal order): (i) a first heavy chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:74; (ii) a first heavy chain constant domain comprising an amino acid

sequence that is at least 95% identical to SEQ ID NO:75; (iii) a first hinge comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:76; (iv) a first CH2 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:77; (v) a first CH3 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:78; (vi) a first linker sequence comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:79 or 80; (vii) a second heavy chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:81; and (viii) a second heavy chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:82; and **(b)** a second polypeptide chain which comprises (e.g., in N- to C-terminal order): (i) a first light chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:83; (ii) a first light chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:84; (iii) a second hinge comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:85; (iv) a second CH2 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:86; (v) a second CH3 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:87; and **(c)** (i) a second light chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:88; and (ii) a second light chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:89 (e.g., Kv4.33, Figures 34A-B).

[0058] In one example of the three-chain multi-specific antigen binding protein complex, the two different Fab regions are arranged in a non-tandem manner, and the protein complex comprises: **(a)** a first polypeptide chain which comprises (e.g., in N- to C-terminal order): (i) a first heavy chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:90; (ii) a first heavy chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:91; (iii) a first hinge comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:92; (iv) a first CH2 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:93; (v) a first CH3 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:94; (vi) a first linker sequence comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:95; (vii) a second heavy chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:96; and (viii) a second heavy chain constant domain

comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:97; and **(b)** a second polypeptide chain which comprises (e.g., in N- to C-terminal order): (i) a first light chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:98 (ii) a first light chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:99; (iii) a second hinge comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:100; (iv) a second CH2 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:101; (v) a second CH3 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:102; and **(c)** (i) a second light chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:103; and (ii) a second light chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:104 (e.g., Kv4.33, Figures 35A-B).

[0059] In one example of the three-chain multi-specific antigen binding protein complex, the two different Fab regions are arranged in a non-tandem manner, and the protein complex comprises: **(a)** a first polypeptide chain which comprises (e.g., in N- to C-terminal order): (i) a first heavy chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:120; (ii) a first heavy chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:121; (iii) a first hinge comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:122; (iv) a first CH2 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:123; (v) a first CH3 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:124; (vi) a first linker sequence comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:125 (vii) a second heavy chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:126; and (viii) a second heavy chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:127; and **(b)** a second polypeptide chain which comprises (e.g., in N- to C-terminal order): (i) a first light chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:128 (ii) a first light chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:129; (iii) a second hinge comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:130; (iv) a second CH2 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:131; (v) a second CH3 domain comprising an amino acid sequence that is at least 95% identical to SEQ ID

NO:132; and (c) (i) a second light chain variable domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:133; and (ii) a second light chain constant domain comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:134 (e.g., Kv4.33, Figures 37A-B).

[0060] The present disclosure provides a pharmaceutical composition comprising any one of the three-chain multi-specific antigen binding protein complexes described herein and a pharmaceutically-acceptable excipient.

[0061] In one example, any of the three-chain multi-specific antigen binding protein complexes described herein can be used as a medicament.

[0062] The present disclosure provides one or more nucleic acids encoding (i) the first polypeptide chain of any one of the three-chain multi-specific antigen binding protein complexes described herein, and encoding (ii) the second polypeptide chain of any of the three-chain multi-specific antigen binding protein complexes described herein, and encoding (iii) the third polypeptide chain of any of the three-chain multi-specific antigen binding protein complexes described herein.

[0063] The present disclosure provides a vector operably linked to the one or more nucleic acids encoding the first polypeptide chain of any one of the three-chain multi-specific antigen binding protein complexes described herein, and encoding the second polypeptide chain of any of the three-chain multi-specific antigen binding protein complexes described herein, and encoding the third polypeptide chain of any of the three-chain multi-specific antigen binding protein complexes described herein.

[0064] The present disclosure provides a host cell harboring the vector which is operably linked to the one or more nucleic acids encoding the first polypeptide chain of any one of the three-chain multi-specific antigen binding protein complexes described herein, and encoding the second polypeptide chain of any of the three-chain multi-specific antigen binding protein complexes described herein, and encoding the third polypeptide chain of any of the three-chain multi-specific antigen binding protein complexes described herein.

[0065] The present disclosure provides a method for preparing any of the three-chain multi-specific antigen binding protein complexes described herein, comprising: culturing a population of the host cell harboring the vector under conditions suitable for expressing the first, second and third polypeptide chains, wherein the vector is operably linked to the one or more nucleic acids

encoding the first polypeptide chain of any one of the three-chain multi-specific antigen binding protein complexes described herein, and encoding the second polypeptide chain of any of the three-chain multi-specific antigen binding protein complexes described herein, and encoding the third polypeptide chain of any of the three-chain multi-specific antigen binding protein complexes described herein. In one example, the method further comprises: isolating (recovering) the first, second and third polypeptide chains. In one example, the method further comprises subjecting the first, second and third polypeptide chains to conditions suitable for associating the first, second and third polypeptide chains with each other to form/assemble the three-chain multi-specific antigen binding protein complex. In one example, the formed/assembled three-chain multi-specific antigen binding protein complex comprises a hetero-dimeric molecule.

[0066] The present disclosure provides (i) a first nucleic acid encoding the first polypeptide chain of any one of the three-chain multi-specific antigen binding protein complexes described herein, and (ii) a second nucleic acid encoding the second polypeptide chain of any of the three-chain multi-specific antigen binding protein complexes described herein, (ii) a third nucleic acid encoding the third polypeptide chain of any of the three-chain multi-specific antigen binding protein complexes described herein.

[0067] The present disclosure provides a vector system comprising a first vector operably linked to the first nucleic acid and optionally operably linked to any one or any combination of the second and/or third nucleic acid(s), and a second vector operably linked to any one or any combination of the second and/or third nucleic acid(s), wherein the first nucleic acid encodes the first polypeptide chain of any one of the three-chain multi-specific antigen binding protein complexes described herein, wherein the second nucleic acid encodes the second polypeptide chain of any of the three-chain multi-specific antigen binding protein complexes described herein, and wherein the third nucleic acid encodes the third polypeptide chain of any of the three-chain multi-specific antigen binding protein complexes described herein.

[0068] In one example, (i) a first vector is operably linked to the first nucleic acid encoding the first polypeptide chain of any one of the three-chain multi-specific antigen binding protein complexes described herein, and (ii) a second vector operably linked to the second nucleic acid encoding the second polypeptide chain of any of the three-chain multi-specific antigen binding protein complexes described herein, and the second vector operably linked to the third nucleic

acid encoding the third polypeptide chain of any of the three-chain multi-specific antigen binding protein complexes described herein. In one example, the first vector is a first expression vector comprising at least one promoter which is operably linked to the first nucleic acid encoding the first polypeptide chain. In one example, the second vector is a second expression vector comprising at least one promoter which is operably linked to the second nucleic acid encoding the second polypeptide chain and is operably linked to the third nucleic acid encoding the third polypeptide chain.

[0069] The present disclosure provides a host cell harboring the vector system, wherein the vector system comprises a first vector operably linked to the first nucleic acid and optionally operably linked to any one or any combination of the second and/or third nucleic acid(s), and a second vector operably linked to any one or any combination of the second and/or third nucleic acid(s), wherein the first nucleic acid encodes the first polypeptide chain of any one of the three-chain multi-specific antigen binding protein complexes described herein, wherein the second nucleic acid encodes the second polypeptide chain of any of the three-chain multi-specific antigen binding protein complexes described herein, and wherein the third nucleic acid encodes the third polypeptide chain of any of the three-chain multi-specific antigen binding protein complexes described herein.

[0070] In one example, the host cell harbors the first and second vectors, wherein (i) the first vector is operably linked to the first nucleic acid encoding the first polypeptide chain of any one of the three-chain multi-specific antigen binding protein complexes described herein, and (ii) the second vector operably linked to the second nucleic acid encoding the second polypeptide chain of any of the three-chain multi-specific antigen binding protein complexes described herein, and the second vector is operably linked to the third nucleic acid encoding the third polypeptide chain of any of the three-chain multi-specific antigen binding protein complexes described herein.

[0071] The present disclosure provides method for preparing any of the three-chain multi-specific antigen binding protein complexes described herein, comprising: culturing a population of the host cell harboring the first and second vectors under conditions suitable for expressing the first, second and third polypeptide chains, wherein (i) the first vector is operably linked to the first nucleic acid encoding the first polypeptide chain of any one of the three-chain multi-specific antigen binding protein complexes described herein, and (ii) the second vector operably linked to

the second nucleic acid encoding the second polypeptide chain of any of the three-chain multi-specific antigen binding protein complexes described herein, and the second vector is operably linked to the third nucleic acid encoding the third polypeptide chain of any of the three-chain multi-specific antigen binding protein complexes described herein. In one example, the method further comprises: isolating (recovering) the first, second and third polypeptide chains. In one example, the method further comprises subjecting the first, second and third polypeptide chains to conditions suitable for associating the first, second and third polypeptide chains with each other to form/assemble the three-chain multi-specific antigen binding protein complex. In one example, the formed/assembled three-chain multi-specific antigen binding protein complex comprises a hetero-dimeric molecule.

[0072] The present disclosure provides a method for treating a disease in a subject, comprising: administering to the subject a therapeutically effective amount of any one of the three-chain multi-specific antigen binding protein complexes described herein.

[0073] In one example the disease comprises a cancer of the: prostate, breast, ovary, head and neck, bladder, skin, colorectal, anus, rectum, pancreas, lung (including non-small cell lung and small cell lung cancers), leiomyoma, brain, glioma, glioblastoma, esophagus, liver, kidney, stomach, colon, cervix, uterus, endometrium, vulva, larynx, vagina, bone, nasal cavity, paranasal sinus, nasopharynx, oral cavity, oropharynx, larynx, hypolarynx, salivary glands, ureter, urethra, penis, or testis.

[0074] In one example, the disease comprises a hematologic cancer, wherein the hematologic cancer is B chronic lymphocytic leukemia (B-CLL), B and T acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), hairy cell leukemia (HCL), myeloproliferative disorder/neoplasm (MPDS), myelodysplasia syndrome, non-Hodgkin's lymphoma (NHL) including Burkitt's lymphoma (BL), Waldenstrom's Macroglobulinemia, mantle cell lymphoma, AIDS-related lymphoma, Hodgkin's Lymphoma (HL), T cell lymphoma (TCL), multiple myeloma (MM), plasma cell myeloma, plasmacytoma, giant cell myeloma, heavy-chain myeloma, or light chain or Bence-Jones myeloma.

[0075] The present disclosure provides a method for binding a first and a second target epitope, comprising: (a) contacting the first target epitope and the second target epitope with any of the three-chain multi-specific antigen binding protein complexes described herein, and (b)

binding the first target epitope with the first Fab region and binding the second target epitope with the second Fab region.

[0076] In one example, the first target epitope comprises a cell surface antigen expressed by a tumor or cancer cell.

[0077] In one example, second epitope comprises a cell surface antigen expressed by an effector T cell. In one example, the three-chain multi-specific antigen binding protein complex forms a cell synapse by binding the cell surface antigen expressed by the tumor or cancer cell and by binding the cell surface antigen expressed by the effector T cell.

[0078] In one example, the effector T cell in the cell synapse kills the tumor or cancer cell by mediating cytotoxic cell killing.

BRIEF DESCRIPTION OF THE FIGURES

[0079] **Figure 1** is a schematic showing a non-limiting embodiment of a protein complex comprising 2 polypeptide chains with two different Fab regions arranged in an in-tandem manner (e.g., Kv6.1).

[0080] **Figure 2** is a schematic showing a non-limiting embodiment of a protein complex comprising 3 polypeptide chains with two different Fab regions arranged in an in-tandem manner (e.g., Kv5.1).

[0081] **Figure 3** is a schematic showing a non-limiting embodiment of a protein complex comprising 2 polypeptide chains with two different Fab regions arranged in a non-tandem manner (e.g., Kv6.2).

[0082] **Figure 4** is a schematic showing a non-limiting embodiment of a protein complex comprising 3 polypeptide chains with two different Fab regions arranged in a non-tandem manner (e.g., Kv4.33).

[0083] **Figure 5** is a schematic showing a non-limiting embodiment of a protein complex comprising 2 polypeptide chains with two different Fab regions arranged in an in-tandem manner (e.g., an alternative arrangement).

[0084] **Figure 6** is a schematic showing a non-limiting embodiment of a protein complex comprising 2 polypeptide chains with two different Fab regions arranged in an in-tandem manner (e.g., an alternative arrangement).

ACTIVATABLE MULTI-SPECIFIC

What is claimed:

1. An activatable multi-specific antigen binding protein complex, comprising
 - a) a first polypeptide chain comprising (i) a first half Fab *heavy* region, (ii) a first linker, (iii) a second half Fab *heavy* region, and (iv) a first half Fc region, wherein the first half Fab *heavy* region comprises a first-variable region and first-constant region from a first Fab *heavy* chain, and wherein the second half Fab *heavy* region comprises a second-variable region and second-constant region from a second Fab *heavy* chain; and a second polypeptide chain comprising (i) a first half Fab *light* region, (ii) a second linker, (iii) a second half Fab *light* region, and (iv) a second half Fc region, wherein the first half Fab *light* region comprises a first-variable region and first-constant region from a first Fab *light* chain, and wherein the second half Fab *light* region comprises a second-variable region and second-constant region from a second Fab *light* chain, wherein the second linker is cleavable, and wherein the first and second polypeptide chains associate with each other to form the multi-specific antigen binding protein complex having a first full Fab domain that is capable of binding a first epitope, a second full Fab domain that is capable of binding a second epitope that differs from the first epitope, a full Fc domain that is capable of binding an Fc receptor, and a first and second linker, wherein the first full Fab domain and the second full Fab domain are different and are arranged in tandem; **or**
 - b) a first polypeptide chain comprising (i) a first half Fab *heavy* region, (ii) a first half Fc region, (iii) a first linker, and (iv) a second half Fab *heavy* region, wherein the first half Fab *heavy* region comprises a first-variable region and first-constant region from a first Fab *heavy* chain, and wherein the second half Fab *heavy* region comprises a second-variable region and second-constant region from a second Fab *heavy* chain; and a second polypeptide chain comprising (i) a first half Fab *light* region, (ii) a second half Fc region, (iii) a second linker, and (iv) a second half Fab *light* region, wherein the first half Fab *light* region comprises a first-variable region and first-constant region from a first Fab *light* chain, and wherein the second half

Fab *light* region comprises a second-variable region and second-constant region from a second Fab *light* chain, wherein the second linker is cleavable, and wherein the first and second polypeptide chains associate with each other to form the multi-specific antigen binding protein complex having a first full Fab domain that is capable of binding a first epitope, a second full Fab domain that is capable of binding a second epitope that differs from the first epitope, a full Fc domain that is capable of binding an Fc receptor, and a first and second linker, wherein the first full Fab domain and the second full Fab domain are different and are arranged in a non-tandem manner.

2. The protein complex of claim 1, wherein: the first full Fab domain exhibits binding to the first epitope; the second full Fab domain exhibits a first level of binding to the second epitope when the second linker is un-cleaved; the second full Fab domain exhibits a second level of binding to the second epitope when the second linker is cleaved, the second level being increased relative to the first level; and upon cleavage of the second linker the first full Fab domain and the second full Fab domain are capable of binding to the first and second target epitopes, respectively, at the same time.
3. The protein complex of claim 1 or 2, wherein the second linker is cleavable with a matrix metalloprotease (MMP), and wherein the matrix metalloprotease is MMP1, MMP2, MMP3, MMP8, MMP9, MMP11, MMP13, MMP14, or MT1-MMP (membrane type 1 matrix metalloproteinase).
4. The protein complex of any one of claims 1-3, wherein the second linker comprises the amino acid sequence of
 - a) GSGSGSGGSSGGSGGGGS (DP linker, SEQ ID NO:11);
 - b) TSGSGGSGGSV (EG or EH linker, SEQ ID NOS:12 or 44);
 - c) TSGSGGSPLGMGGSGSV (EI or EU linker, SEQ ID NOS:13 or 45);
 - d) TSGSGGSPLGVGGSGSV (EJ or EV linker, SEQ ID NOS:14 or 46);
 - e) TSGSGGSPAALGGSGSV (EK or EW linker, SEQ ID NOS:15 or 47);
 - f) TSGSGGSPAGLGGSGSV (EL or EX linker, SEQ ID NOS:16 or 48);

- g) TSGSGGSPLGMVGV (EM or EY linker, SEQ ID NOS:17 or 49);
- h) TSGSGGSPLGVVGV (EN or EZ linker, SEQ ID NOS:18 or 50);
- i) TSGSGGSPAALVGV (EO or FA linker, SEQ ID NOS:19 or 51);
- j) TSGSGGSPAGLVGV (EP or FB linker, SEQ ID NOS:20 or 52);
- k) TSGSGGSPLGMVLV (EQ or FC linker, SEQ ID NOS:21 or 53);
- l) TSGSGGSPLGVVLV (ER or FD linker, SEQ ID NOS:22 or 54);
- m) TSGSGGSPAALVLV (ES or FE linker, SEQ ID NOS:23 or 55); or
- n) TSGSGGSPAGLVLV (ST or FF linker, SEQ ID NOS:24 or 56).

5. The protein complex of any one of claims 1-4, wherein the first polypeptide chain comprises a knob or hole mutation in the first half Fc region; the second polypeptide chain comprises a hole mutation in the second half Fc region if the first polypeptide chain comprises a knob mutation in the first half Fc region; and the second polypeptide chain comprises a knob mutation in the second half Fc region if the first polypeptide chain comprises a hole mutation in the first half Fc region.
6. The protein complex of any one of claims 1-5, wherein
 - a) the first full Fab domain and the second full Fab domain are arranged in-tandem and: (i) the first polypeptide chain having the first half Fab *heavy* region comprises the amino acid sequences of SEQ ID NO: 1 and SEQ ID NO: 2, and the second half Fab *heavy* region comprises the amino acid sequences of SEQ ID NO: 4 and SEQ ID NO: 5, and (ii) the second polypeptide chain having the first half Fab *light* region comprises the amino acid sequences of SEQ ID NO: 9 and SEQ ID NO: 10, and the second half Fab *light* region comprises the amino acid sequences of SEQ ID NO: 25 and SEQ ID NO: 26; **or**
 - b) the first full Fab domain and the second full Fab domain are arranged in a non-tandem manner and: (i) the first polypeptide chain having the first half Fab *heavy* region comprises the amino acid sequences of SEQ ID NO: 30 and SEQ ID NO: 31, and the second half Fab *heavy* region comprises the amino acid sequences of SEQ ID NO: 36 and SEQ ID NO: 37, and (ii) the second polypeptide chain having the first half Fab *light* region comprises the amino acid sequences of SEQ ID NO: 38

and SEQ ID NO: 39, and the second half Fab *light* region comprises the amino acid sequences of SEQ ID NO: 57 and SEQ ID NO: 58.

7. A pharmaceutical composition comprising the multi-specific antigen binding protein complex of any one of claims 1-6 and a pharmaceutically-acceptable excipient.
8. The multi-specific antigen binding protein complex of any one of claims 1-6 or the pharmaceutical composition of claim 7 for use as a medicament.
9. One or more nucleic acids encoding (i) the first polypeptide chain of the multi-specific antigen binding protein complex of claim 1 and (ii) the second polypeptide chain the multi-specific antigen binding protein complex of any one of claims 1-8.
10. One or more expression vectors comprising the one or more nucleic acids of claim 9 operably linked to one or more promoters.
11. A host cell harboring the one or more expression vectors of claim 10.
12. A method for preparing a multi-specific antigen binding protein complex, comprising:
 - a) culturing a population of the host cell of claim 11 under conditions suitable for expressing the first and the second polypeptide chains and suitable for associating the first and second polypeptide chains with each other to form the multi-specific antigen binding protein complex; and
 - b) recovering the multi-specific antigen binding protein complex from the population of the host cell.
13. The multi-specific antigen binding protein complex of any one of claims 1-6 for use in therapy.
14. Use of a therapeutically effective amount of the multi-specific antigen binding protein complex of any one of claims 1-6 in the manufacture of a medicament for treating a disease in a subject, wherein the disease comprises a cancer, comprising a prostate cancer, breast cancer, ovarian cancer, head and neck cancer, bladder cancer, skin cancer, colorectal

cancer, anal cancer, rectum cancer, pancreatic cancer, lung cancer (including non-small cell lung and small cell lung cancers), leiomyoma cancer, brain cancer, glioma cancer, glioblastoma cancer, esophagus cancer, liver cancer, kidney cancer, stomach cancer, colon cancer, cervical cancer, uterine cancer, endometrial cancer, vulva cancer, larynx cancer, vaginal cancer, bone cancer, nasal cavity cancer, paranasal sinus cancer, nasopharynx cancer, oral cavity cancer, oropharynx cancer, larynx cancer, hypolarynx cancer, salivary gland cancer, ureter cancer, urethral cancer, penial cancer, or testicular cancer.

15. The use of claim 14, wherein the disease comprises a hematologic cancer, including a B chronic lymphocytic leukemia (B-CLL), B and T acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), hairy cell leukemia (HCL), myeloproliferative disorder/neoplasm (MPDS), myelodysplasia syndrome, non-Hodgkin's lymphoma (NHL) including Burkitt's lymphoma (BL), Waldenstrom's Macroglobulinemia, mantle cell lymphoma, AIDS-related lymphoma, Hodgkin's Lymphoma (HL), T cell lymphoma (TCL), multiple myeloma (MM), plasma cell myeloma, plasmacytoma, giant cell myeloma, heavy-chain myeloma, or light chain or Bence-Jones myeloma.
16. A method for binding a first and a second epitope, comprising:
 - a) contacting the first epitope with the multi-specific antigen binding protein complex of any one of claims 1-6, wherein the second linker is in an un-cleaved state; and
 - b) binding the first epitope with the first Fab region, wherein the first Fab region binds the first epitope and the second Fab region exhibits a level of binding to the second epitope that is reduced relative to the level of binding to the second epitope exhibited when the second linker is in a cleaved state.
17. The method of claim 16 further comprising: cleaving the second linker to generate an activated multi-specific antigen binding protein complex wherein the second Fab region exhibits a level of binding to the second epitope that is increased relative to the level of binding to the second epitope exhibited when the second linker is in the un-cleaved state.
18. The method of claim 17 further comprising:

- a) contacting the second target epitope with the activated multi-specific antigen binding protein complex; and
 - b) binding the second target epitope with the second Fab region.
19. The method of any one of claims 16-18, wherein the first target epitope comprises a cell surface antigen expressed by a tumor or cancer cell, and wherein the second epitope comprises a cell surface antigen expressed by an effector T cell.
20. The method of claim 19, wherein the activated multi-specific antigen binding protein complex forms a cell synapse by binding the cell surface antigen expressed by the tumor or cancer cell and by binding the cell surface antigen expressed by the effector T cell.
21. The method of claim 20, wherein the effector T cell in the cell synapse kills the tumor or cancer cell by mediating cytotoxic cell killing.