

[0006] All references cited herein, including patent applications, patent publications, non-patent literature, and UniProtKB/Swiss-Prot/GenBank Accession numbers are herein incorporated by reference in their entirety, as if each individual reference were specifically and individually indicated to be incorporated by reference.

SUMMARY

[0007] The present application provides heterodimeric proteins comprising CH3 domains having engineered residues that form disulfide bonds and/or salt bridges. Also provided are activatable antibodies targeting CD3 and/or HER2.

[0008] Accordingly, one example of the present application provides a heterodimeric protein comprising a first polypeptide comprising a first immunoglobulin heavy chain constant domain 3 (CH3 domain) and a second polypeptide comprising a second CH3 domain, wherein: i) the first CH3 domain comprises a cysteine (C) residue at position 390 and the second CH3 domain comprises a cysteine residue at position 400, or the first CH3 domain comprises a cysteine residue at position 400 and the second CH3 domain comprises a cysteine residue at position 390; or ii) the first CH3 domain comprises a cysteine residue at position 392 and the second CH3 domain comprises a cysteine residue at position 397, or the first CH3 domain comprises a cysteine residue at position 397 and the second CH3 domain comprises a cysteine residue at position 392; or iii) the first CH3 domain comprises a cysteine residue at position 392 and the second CH3 domain comprises a cysteine residue at position 400, or the first CH3 domain comprises a cysteine residue at position 400 and the second CH3 domain comprises a cysteine residue at position 392; and wherein the amino acid residue numbering is based on EU numbering. In some examples, i) the first CH3 domain comprises N390C substitution and the second CH3 domain comprises S400C substitution, or the first CH3 domain comprises S400C substitution and the second CH3 domain comprises N390C substitution; or ii) the first CH3 domain comprises K392C substitution and the second CH3 domain comprises V397C substitution, or the first CH3 domain comprises V397C substitution and the second CH3 domain comprises K392C substitution; or iii) the first CH3 domain comprises K392C substitution and the second CH3 domain comprises S400C substitution, or the first CH3 domain comprises S400C substitution and the second CH3 domain comprises K392C substitution.

[0008a] In one aspect, the present disclosure refers to a heterodimeric protein comprising a first polypeptide comprising a first immunoglobulin heavy chain constant domain 3 (CH3 domain) and a second polypeptide comprising a second CH3 domain, wherein: i) the first CH3 domain comprises a cysteine (C) residue at position 390 and the second CH3 domain comprises a cysteine residue at position 400, or the first CH3 domain comprises a cysteine residue at position 400 and the second CH3 domain comprises a cysteine residue at position 390; or ii) the first CH3 domain comprises a cysteine residue at position 392 and the second CH3 domain comprises a cysteine residue at position 397, or the first CH3 domain comprises a cysteine residue at position 397 and the second CH3 domain comprises a cysteine residue at position 392; or iii) the first CH3 domain comprises a cysteine residue at position 392 and the second CH3 domain comprises a cysteine residue at position 400, or the first CH3 domain comprises a cysteine residue at position 400 and the second CH3 domain comprises a cysteine residue at position 392; and wherein the amino acid residue numbering is based on EU numbering.

[0009] In some examples according to any one of the heterodimeric proteins described above, i) the first CH3 domain further comprises a positively charged residue at position 357 and

the second CH3 domain further comprises a negatively charged residue at position 351, or the first CH3 domain further comprises a negatively charged residue at position 351 and the second CH3 domain further comprises a positively charged residue at position 357; or ii) the first CH3 domain further comprises a positively charged residue at position 411 and the second CH3 domain further comprises a negatively charged residue at position 370, or the first CH3 domain further comprises a negatively charged residue at position 370 and the second CH3 domain further comprises a positively charged residue at position 411; or iii) the first CH3 domain further comprises a positively charged residue at position 364 and the second CH3 domain further comprises a negatively charged residue at position 370, or the first CH3 domain further comprises a negatively charged residue at position 370 and the second CH3 domain further comprises a positively charged residue at position 364; or a combination of i) and ii), or a combination of i) and iii), and wherein the amino acid residue numbering is based on EU numbering. In some examples, the first CH3 domain further comprises a positively charged residue at position 356 and the second CH3 domain further comprises a negatively charged residue at position 439, or first CH3 domain further comprises a negatively charged residue at position 439 and the second CH3 domain further comprises a positively charged residue at position 356; and wherein the amino acid residue numbering is based on EU numbering. In some examples, i) the positively charged residue is a lysine (K) residue, and the negatively charged residue is an aspartic acid (D) residue; or ii) the positively charged residue is a lysine (K) residue, and the negatively charged residue is a glutamic acid (E) residue; or iii) the positively charged residue is an arginine I residue, and the negatively charged residue is an aspartic acid (D) residue; or iv) the positively charged residue is an arginine I residue, and the negatively charged residue is a glutamic acid (E) residue. In some examples, i) the first CH3 domain comprises E357K and T411K substitutions and the second CH3 domain comprises L351D and K370D substitutions, or the first CH3 domain comprises L351D and K370D substitutions and the second CH3 domain comprises E357K and T411K substitutions; or ii) the first CH3 domain comprises E357K and S364K substitutions and the second CH3 domain comprises L351D and K370D substitutions, or the first CH3 domain comprises L351D and K370D substitutions and the second CH3 domain comprises E357K and S364K substitutions; or iii) the first CH3 domain comprises D356K, E357K and S364K substitutions and the second CH3 domain comprises L351D, K370D and K439D substitutions, or the first CH3 domain comprises L351D, K370D and K439D substitutions and the second CH3 domain comprises D356K, E357K and S364K substitutions.

[0009a] In one aspect, the present disclosure refers to a heterodimeric protein comprising a first polypeptide comprising a first CH3 domain and a second polypeptide comprising a second CH3 domain, wherein: i) the first CH3 domain comprises a positively charged residue at position 357 and the second CH3 domain comprises a negatively charged residue at position 351, or the first CH3 domain comprises a negatively charged residue at position 351 and the second CH3 domain comprises a positively charged residue at position 357; or ii) the first CH3 domain comprises a positively charged residue at position 411 and the second CH3 domain comprises a negatively charged residue at position 370, or the first CH3 domain comprises a negatively charged residue at position 370 and the second CH3 domain comprises a positively charged residue at position 411; or iii) the first CH3 domain comprises a positively charged residue at position 364 and the second CH3 domain comprises a negatively charged residue at position 370, or the first CH3 domain comprises a negatively charged residue at position 370 and the second CH3 domain comprises a positively charged residue at position 364; and wherein the amino acid residue numbering is based on EU numbering.

[0010] In some examples according to any one of the heterodimeric proteins described above, i) the first CH3 domain further comprises K392D and K409D substitutions and the second CH3 domain further comprises D356K, and D399K substitutions, or the first CH3 domain further comprises D356K and D399K substitutions and the second CH3 domain further comprises K392D and K409D substitutions; or ii) the first CH3 domain further comprises L368D and K370S substitutions and the second CH3 domain further comprises E357Q and S364K substitutions, or the first CH3 domain further comprises E357Q and S364K substitutions and the second CH3 domain further comprises L368D and K370S substitutions; or iii) the first CH3 domain further comprises L351K and T366K substitutions and the second CH3 domain further comprises L351D and L368E substitutions, or the first CH3 domain further comprises L351D and L368E substitutions and the second CH3 domain further comprises L351K and T366K substitutions; or (iv) the first CH3 domain further comprises P395K, P396K and V397K substitutions and the second CH3 domain comprises T394D, P395D and P396D substitutions, or the first CH3 domain further comprises T394D, P395D and P396D substitutions and the second CH3 domain further comprises P395K, P396K and V397K substitutions; or (v) the first CH3 domain further comprises F405E, Y407E and K409E substitutions and the second CH3 domain comprises F405K and Y407K substitutions, or the first CH3 domain further comprises F405K and Y407K substitutions and the second CH3 domain further comprises F405E, Y407E and K409E substitutions.

[0011] In some examples according to any one of the heterodimeric proteins described above, i) the first CH3 domain comprises E357K, S364K and N390C substitutions and the second CH3 domain comprises L351D, K370D, and S400C substitutions, or the first CH3 domain comprises L351D, K370D, and S400C substitutions and the second CH3 domain comprises E357K, S364K and N390C substitutions; or ii) the first CH3 domain comprises E357K, S364K and S400C substitutions and the second CH3 domain comprises L351D, K370D, and N390C substitutions, or the first CH3 domain comprises L351D, K370D, and N390C substitutions and the second CH3 domain comprises E357K, S364K and S400C substitutions; or iii) the first CH3 domain comprises D356K, E357K, S364K and S400C substitutions and the second CH3 domain comprises L351D, K370D, N390C and K439D substitutions, or the first CH3 domain comprises L351D, K370D, N390C and K439D substitutions and the second CH3 domain comprises D356K, E357K, S364K and S400C substitutions; or iv) the first CH3 domain comprises D356K, E357K, S364K and N390C substitutions and the second CH3 domain comprises L351D, K370D, K439D and S400C substitutions, or the first CH3 domain comprises L351D, K370D, K439D and S400C

substitutions and the second CH3 domain comprises D356K, E357K, S364K and N390C substitutions.

[0012] In some examples according to any one of the heterodimeric proteins described above, the first CH3 domain and the second CH3 domain further comprise knob-into-hole residues. In some examples, i) the first CH3 domain comprises T336S, L368A and Y407V substitutions and the second CH3 domain comprises T366W substitution, or the first CH3 domain comprises T366W substitution and the second CH3 domain comprises T336S, L368A and Y407V substitutions; or ii) the first CH3 domain comprises L368V and Y407V substitutions and the second CH3 domain comprises T366W substitution, or the first CH3 domain comprises T366W substitution and the second CH3 domain comprises L368V and Y407V substitutions.

[0013] Another example of the present application provides a heterodimeric protein comprising a first polypeptide comprising a first CH3 domain and a second polypeptide comprising a second CH3 domain, wherein: i) the first CH3 domain comprises a positively charged residue at position 357 and the second CH3 domain comprises a negatively charged residue at position 351, or the first CH3 domain comprises a negatively charged residue at position 351 and the second CH3 domain comprises a positively charged residue at position 357; or ii) the first CH3 domain comprises a positively charged residue at position 411 and the second CH3 domain comprises a negatively charged residue at position 370, or the first CH3 domain comprises a negatively charged residue at position 370 and the second CH3 domain comprises a positively charged residue at position 411; or iii) the first CH3 domain comprises a positively charged residue at position 364 and the second CH3 domain comprises a negatively charged residue at position 370, or the first CH3 domain comprises a negatively charged residue at position 370 and the second CH3 domain comprises a positively charged residue at position 364; and wherein the amino acid residue numbering is based on EU numbering. In some examples, the first CH3 domain comprises a positively charged residue at position 356 and the second CH3 domain comprises a negatively charged residue at position 439, or first CH3 domain comprises a negatively charged residue at position 439 and the second CH3 domain comprises a positively charged residue at position 356; and wherein the amino acid residue numbering is based on EU numbering. In some examples, i) the positively charged residue is a lysine (K) residue, and the negatively charged residue is an aspartic acid (D) residue; or ii) the positively charged residue is a lysine (K) residue, and the negatively charged residue is a glutamic acid (E) residue; or iii) the positively charged residue is an arginine (R) residue, and the negatively charged residue is an aspartic acid (D) residue; or iv) the

positively charged residue is an arginine (R) residue, and the negatively charged residue is a glutamic acid (E) residue. In some examples, i) the first CH3 domain comprises E357K and T411K substitutions and the second CH3 domain comprises L351D and K370D substitutions, or the first CH3 domain comprises L351D and K370D substitutions and the second CH3 domain comprises E357K and T411K substitutions; or ii) the first CH3 domain comprises E357K and S364K substitutions and the second CH3 domain comprises L351D and K370D substitutions, or the first CH3 domain comprises L351D and K370D substitutions and the second CH3 domain comprises E357K and S364K substitutions; or iii) the first CH3 domain comprises D356K, E357K and S364K substitutions and the second CH3 domain comprises L351D, K370D and K439D substitutions, or the first CH3 domain comprises L351D, K370D and K439D substitutions and the second CH3 domain comprises D356K, E357K and S364K substitutions.

[0014] In some examples according to any one of the heterodimeric proteins described above, i) the first CH3 domain further comprises K392C substitution and the second CH3 domain further comprises D399C substitution, or the first CH3 domain further comprises D399C substitution and the second CH3 domain further comprises K392C substitution; or ii) the first CH3 domain further comprises Y394C substitution and the second CH3 domain further comprises S354C substitution, or the first CH3 domain further comprises S354C substitution and the second CH3 domain further comprises Y394C substitution; or iii) the first CH3 domain further comprises D356C substitution and the second CH3 domain further comprises Y349C substitution, or the first CH3 domain further comprises Y349C substitution and the second CH3 domain further comprises D356C substitution.

[0015] In some examples according to any one of the heterodimeric proteins described above, the first CH3 domain and the second CH3 domain are human CH3 domains.

[0016] In some examples according to any one of the heterodimeric proteins described above, the first polypeptide and the second polypeptide each comprises from the N-terminus to the C-terminus at least a portion of an immunoglobulin hinge region, an immunoglobulin heavy chain constant domain 2 (CH2 domain), and the CH3 domain. In some examples, the CH2 domains and the CH3 domains form an IgG Fc region. In some examples, the Fc region is of the human IgG1 subclass. In some examples, the Fc region is of the human IgG4 subclass. In some examples, the Fc region further comprises S228P substitution. In some examples, the Fc region further comprises N297A substitution.

[0017] In some examples according to any one of the heterodimeric proteins described above, the first polypeptide and the second polypeptide are antibody heavy chains. In some examples, the heterodimeric protein further comprises the heterodimeric protein further comprises one or more antibody light chains. In some examples, the heterodimeric protein is a multispecific antibody.

[0018] In some examples according to any one of the heterodimeric proteins described above, the heterodimeric protein further comprises a third polypeptide and a fourth polypeptide, wherein:

(i) the first polypeptide comprises a structure represented by the formula:

VH1-CH1-hinge-CH2-first CH3-L1-scFv1 (Ia);

(ii) the second polypeptide comprises a structure represented by the formula:

VH2-CH1-hinge-CH2-second CH3-L2-scFv2 (IIa);

(iii) the third polypeptide comprises a structure represented by the formula:

VL1-CL (Ib); and

(iv) the fourth polypeptide comprises a structure represented by the formula:

VL2-CL (IIb);

wherein VL1 is a first immunoglobulin light chain variable domain; VH1 is a first immunoglobulin heavy chain variable domain; VL2 is a second immunoglobulin light chain variable domain; VH2 is a second immunoglobulin heavy chain variable domain; scFv1 is a first single-chain variable fragment; scFv2 is a second single-chain variable fragment; CL is an immunoglobulin light chain constant domain; CH1 is an immunoglobulin heavy chain constant domain 1; CH2 is an immunoglobulin heavy chain constant domain 2; hinge is an immunoglobulin hinge region connecting the CH1 and CH2 domains; and L1 and L2 is each independently a bond or a peptide linker; wherein VL1 and VH1 associate to form a first Fv that specifically binds to a first target; wherein VL2 and VH2 associate to form a second Fv that specifically binds to a second target; wherein scFv1 specifically binds to a third target; and wherein scFv2 specifically binds to a fourth target. In some examples, scFv1 and scFv2 are identical. In some examples, the first Fv and the second Fv are identical. In some examples, the first Fv and the second Fv are different. In some examples, the first Fv specifically binds PDL1, the second Fv specifically binds CD137, and scFv1 and scFv2 specifically bind CTLA-4. In some examples, scFv1 and/or scFv2 comprises from the N-terminus to the C-terminus: VH-L-VL, wherein L is a peptide linker. In some examples, scFv1 and/or scFv2 comprises a first cysteine residue at position 44 in the VH and a

second cysteine residue at position 100 in the VL, wherein the first cysteine residue and the second cysteine residue form a disulfide bond. In some examples, L1 and/or L2 is a peptide linker comprising the amino acid sequence of SEQ ID NO: 80 or SEQ ID NO: 81. In some examples, VL1 and VL2 are identical. In some examples, VL1 and VL2 are different.

[0019] In some examples according to any one of the heterodimeric proteins described above, the heterodimeric protein comprises a first polypeptide, a second polypeptide and a third polypeptide, wherein:

(i) the first polypeptide comprises a structure represented by the formula:

VH-CH1-hinge-CH2-first CH3 (IIIa);

(ii) the second polypeptide comprises a structure represented by the formula:

scFv-hinge-CH2-second CH3 (IVa); and

(iii) the third polypeptide comprises a structure represented by the formula:

VL-CL (IIIb);

wherein VL is an immunoglobulin light chain variable domain; VH is an immunoglobulin heavy chain variable domain; scFv is a single-chain variable fragment; CL is an immunoglobulin light chain constant domain; CH1 is an immunoglobulin heavy chain constant domain 1; CH2 is an immunoglobulin heavy chain constant domain 2; and hinge is an immunoglobulin hinge region connecting the CH1 and CH2 domains; wherein VL and VH associate to form an Fv that specifically binds to a first target; and wherein the scFv specifically binds to a second target. In some examples, the first target is a tumor antigen, and the second target is CD3. In some examples, the first target is HER2. In some examples, the first target is a first immune checkpoint molecule, and the second target is a second immune checkpoint molecule. In some examples, the first target is PDL1 and the second target is CD137. In some examples, the first target is CD137 and the second target is PDL1. In some examples, the scFv comprises from the N-terminus to the C-terminus: VH-L-VL, wherein L is a peptide linker. In some examples, the scFv comprises a first cysteine residue at position 44 in the VH and a second cysteine residue at position 100 in the VL, wherein the first cysteine residue and the second cysteine residue form a disulfide bond. In some examples, the scFv is fused to the hinge in the second polypeptide via a peptide linker comprising the amino acid sequence of SEQ ID NO: 80 or SEQ ID NO: 81.

[0020] In some examples according to any one of the heterodimeric proteins described above, the heterodimeric protein is an activatable antibody, wherein the heterodimeric protein comprises a first polypeptide, a second polypeptide and a third polypeptide, and wherein:

(i) the first polypeptide comprises a structure represented by the formula:

VH-CH1-hinge-CH2-first CH3 (Va);

(ii) the second polypeptide comprises a structure represented by the formula:

MM1-CM1-scFv-hinge-CH2-second CH3 (VIa); and

(iii) the third polypeptide comprises a structure represented by the formula:

MM2-CM2-VL-CL (IVb);

wherein VL is an immunoglobulin light chain variable domain; VH is an immunoglobulin heavy chain variable domain; scFv is a single-chain variable fragment; CL is an immunoglobulin light chain constant domain; CH1 is an immunoglobulin heavy chain constant domain 1; CH2 is an immunoglobulin heavy chain constant domain 2; hinge is an immunoglobulin hinge region connecting the CH1 and CH2 domains; MM1 is a first masking peptide; MM2 is a second masking peptide; CM1 is a first cleavable peptide; and CM2 is a second cleavable peptide; wherein VL and VH associate to form a first Fv that specifically binds to a first target; wherein the scFv specifically binds to a second target; wherein MM1 inhibits the binding of the first Fv to the first target when CM1 is not cleaved; and wherein MM2 inhibits the binding of the scFv to the second target when CM2 is not cleaved. In some examples, the first target is a tumor antigen, and the second target is CD3. In some examples, MM1 comprises the amino acid sequence of SEQ ID NO: 35. In some examples, the first Fv comprises a VH comprising an CDR-H1 comprising the amino acid sequence of SEQ ID NO: 61, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 62, and/or a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 63. In some examples, the TBM comprises a VL comprising a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 64, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 65, and/or a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 66. In some examples, the first target is HER2. In some examples, MM2 comprises the amino acid sequence of SEQ ID NO: 36. In some examples, the scFv comprises a VH comprising an CDR-H1 comprising the amino acid sequence of SEQ ID NO: 69, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 70, and/or a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 71. In some examples, the TBM comprises a VL comprising a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 72, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 73, and/or a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 74.

[0021] One example of the present application provides an activatable antibody comprising: a first polypeptide comprising, from N-terminus to C-terminus, a masking moiety (MM), a cleavable

moiety (CM), and a target binding moiety (TBM), wherein the MM comprises the amino acid sequence of SEQ ID NO: 35; wherein the MM inhibits binding of the activatable antibody to human CD3 when the CM is not cleaved; wherein the CM comprises at least a first cleavage site; and wherein: a) the TBM comprises a VL and the activatable antibody further comprises a second polypeptide comprising a VH; b) the TBM comprises a VH and the activatable antibody further comprises a second polypeptide comprising a VL; c) the TBM comprises from the N-terminus to the C-terminus, a VL and a VH; or d) the TBM comprise from the N-terminus to the C-terminus, a VH and a VL; and wherein the activatable antibody binds to human CD3 via the VH and VL when the CM is cleaved. In some examples, the TBM comprises a VH comprising an CDR-H1 comprising the amino acid sequence of SEQ ID NO: 61, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 62, and/or a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 63. In some examples, the TBM comprises a VL comprising a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 64, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 65, and/or a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 66.

[0021a] In one aspect, the present disclosure refers to an activatable antibody comprising: a first polypeptide comprising, from N-terminus to C-terminus, a masking moiety (MM), a cleavable moiety (CM), and a target binding moiety (TBM), wherein the MM comprises the amino acid sequence of SEQ ID NO: 35; wherein the MM inhibits binding of the activatable antibody to human CD3 when the CM is not cleaved; wherein the CM comprises at least a first cleavage site; and wherein: a) the TBM comprises a VL and the activatable antibody further comprises a second polypeptide comprising a VH; b) the TBM comprises a VH and the activatable antibody further comprises a second polypeptide comprising a VL; c) the TBM comprises from the N-terminus to the C-terminus, a VL and a VH; or d) the TBM comprise from the N-terminus to the C-terminus, a VH and a VL; and wherein the activatable antibody binds to human CD3 via the VH and VL when the CM is cleaved.

[0022] One example of the present application provides an activatable antibody comprising: a first polypeptide comprising, from N-terminus to C-terminus, a masking moiety (MM), a cleavable moiety (CM), and a target binding moiety (TBM), wherein the MM comprises the amino acid sequence of SEQ ID NO: 36; wherein the MM inhibits binding of the activatable antibody to human HER2 when the CM is not cleaved; wherein the CM comprises at least a first cleavage site; and wherein a) the TBM comprises a VL and the activatable antibody further comprises a second polypeptide comprising a VH; b) the TBM comprises a VH and the activatable antibody further comprises a second polypeptide comprising a VL; c) the TBM comprises from the N-terminus to the C-terminus, a VL and a VH; or d) the TBM comprise from the N-terminus to the C-terminus, a VH and a VL; and wherein the activatable antibody binds to human HER2 via the VH and VL when the CM is cleaved. In some examples, the TBM comprises a VH comprising an CDR-H1 comprising the amino acid sequence of SEQ ID NO: 69, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 70, and/or a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 71. In some examples, the TBM comprises a VL comprising a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 72, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 73, and/or a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 74.

[0022a] In one aspect, the present disclosure refers to an activatable antibody comprising: a first polypeptide comprising, from N-terminus to C-terminus, a masking moiety (MM), a cleavable moiety (CM), and a target binding moiety (TBM), wherein the MM comprises the amino acid sequence of SEQ ID NO: 36; wherein the MM inhibits binding of the activatable antibody to human HER2 when the CM is not cleaved; wherein the CM comprises at least a first cleavage site; and wherein: a) the TBM comprises a VL and the activatable antibody further comprises a second polypeptide comprising a VH; b) the TBM comprises a VH and the activatable antibody further comprises a second polypeptide comprising a VL; c) the TBM comprises from the N-terminus to the C-terminus, a VL and a VH; or d) the TBM comprise from the N-terminus to the C-terminus, a VH and a VL; and wherein the activatable antibody binds to human HER2 via the VH and VL when the CM is cleaved.

[0023] In some examples according to any one of activatable antibodies described above, the activatable antibody comprises a first polypeptide, a second polypeptide and a third polypeptide, wherein:

(i) the first polypeptide comprises a structure represented by the formula:

VH-CH1-hinge-CH2-first CH3 (Va);

(ii) the second polypeptide comprises a structure represented by the formula:

MM1-CM1-scFv-hinge-CH2-second CH3 (VIa); and

(iii) the third polypeptide comprises a structure represented by the formula:

MM2-CM2-VL-CL (IVb);

wherein:

VL is an immunoglobulin light chain variable domain;

VH is an immunoglobulin heavy chain variable domain;

scFv is a single-chain variable fragment;

CL is an immunoglobulin light chain constant domain;

CH1 is an immunoglobulin heavy chain constant domain 1;

CH2 is an immunoglobulin heavy chain constant domain 2;

hinge is an immunoglobulin hinge region connecting the CH1 and CH2 domains;

MM1 is a first masking peptide;

MM2 is a second masking peptide;

CM1 is a first cleavable peptide; and

CM2 is a second cleavable peptide;

wherein VL and VH associate to form a first Fv that specifically binds to a first target; wherein the scFv specifically binds to a second target; and wherein MM is MM1 or MM2.

[0024] In some examples according to any one of activatable antibodies described above, the activatable antibody comprises an Fc region comprising a first CH3 domain and a second CH3 domain, wherein the first CH3 domain comprises D356K, E357K, S364K and S400C substitutions and the second CH3 domain comprises L351D, K370D, N390C and K439D substitutions, or the first CH3 domain comprises L351D, K370D, N390C and K439D substitutions and the second CH3 domain comprises D356K, E357K, S364K and S400C substitutions.

[0025] One example of the present application provides one or more nucleic acid(s) encoding the heterodimeric protein according to any one of the heterodimeric proteins described above or the activatable antibody according to any one of the activatable antibodies described above, vector(s)

comprising the one or more nucleic acids, and a host cell comprising the one or more nucleic acids or the vector. In some examples, there is provided a method for preparing a heterodimeric protein or an activatable antibody, comprising: (a) culturing the host cell according to any one of the host cells described above under conditions that allow expression of the one or more nucleic acid(s) or vector; and (b) recovering the heterodimeric protein or the activatable antibody from the host cell culture.

[0025a] In one aspect, the present disclosure refers to one or more nucleic acid(s) encoding the heterodimeric protein disclosed herein or the activatable antibody disclosed herein.

[0025b] In one aspect, the present disclosure refers to a vector comprising the one or more nucleic acid(s) disclosed herein.

[0025c] In one aspect, the present disclosure refers to a host cell comprising the one or more nucleic acid(s) disclosed herein or the vector disclosed herein.

[0025d] In one aspect, the present disclosure refers to a method for preparing a heterodimeric protein or an activatable antibody, comprising: (a) culturing the host cell disclosed herein under conditions that allow expression of the one or more nucleic acid(s) or vector; and (b) recovering the heterodimeric protein or the activatable antibody from the host cell culture.

[0026] One example of the present application provides a pharmaceutical composition comprising the heterodimeric protein according to any one of the heterodimeric proteins described above or the activatable antibodies according to any one of the activatable antibodies described above, and a pharmaceutically acceptable carrier.

[0026a] In one aspect, the present disclosure refers to a pharmaceutical composition comprising the heterodimeric protein disclosed herein or the activatable antibody disclosed herein, and a pharmaceutically acceptable carrier.

[0027] One example of the present application provides a method for treating a disease or condition in a subject in need thereof, comprising administering to the subject an effective amount of the pharmaceutical composition according to any one of the pharmaceutical compositions described above. In some examples, the disease or condition is cancer. In some examples, the cancer is lung cancer. In some examples, the cancer is a HER-2 positive cancer. In some examples, the cancer is ovarian cancer. In some examples, the cancer is prostate cancer or melanoma. In some examples, the cancer is an advanced-stage cancer.

[0027a] In one aspect, the present disclosure refers to use of the pharmaceutical composition disclosed herein in the manufacture of a medicament for treating a disease or condition in a subject in need thereof.

[0028] Compositions, uses, kits and articles of manufacture comprising any one of the heterodimeric proteins described above are also provided.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] FIGs. 1-5 provide schematic diagrams of exemplary antibody designs of the present application.

[0030] FIG. 1A shows a Fab-Fc/Fc one-armed scaffold schematic. FIG. 1B shows a common light chain scaffold schematic.

[0031] FIG. 2 shows a Morrison format bispecific scaffold schematic. Shown at right are PD-L1 X CD137 and CD137 X PD-L1 bispecific antibodies in the Morrison format.

[0032] FIG. 3 shows trispecific scaffold schematics, including, at right, a trispecific antibody to PD-L1, CD137, and CTLA4.

[0033] FIG. 4 shows ScFv bispecific scaffold schematics, including, at right, a HER2 and CD3 bispecific antibody schematic.

CLAIMS

What is claimed is:

1. A heterodimeric protein comprising a first polypeptide comprising a first immunoglobulin heavy chain constant domain 3 (CH3 domain) and a second polypeptide comprising a second CH3 domain, wherein:
 - i) the first CH3 domain comprises a cysteine (C) residue at position 390 and the second CH3 domain comprises a cysteine residue at position 400, or the first CH3 domain comprises a cysteine residue at position 400 and the second CH3 domain comprises a cysteine residue at position 390; or
 - ii) the first CH3 domain comprises a cysteine residue at position 392 and the second CH3 domain comprises a cysteine residue at position 397, or the first CH3 domain comprises a cysteine residue at position 397 and the second CH3 domain comprises a cysteine residue at position 392; or
 - iii) the first CH3 domain comprises a cysteine residue at position 392 and the second CH3 domain comprises a cysteine residue at position 400, or the first CH3 domain comprises a cysteine residue at position 400 and the second CH3 domain comprises a cysteine residue at position 392; andwherein the amino acid residue numbering is based on EU numbering.
2. The heterodimeric protein of claim 1, wherein:
 - i) the first CH3 domain comprises N390C substitution and the second CH3 domain comprises S400C substitution, or the first CH3 domain comprises S400C substitution and the second CH3 domain comprises N390C substitution; or
 - ii) the first CH3 domain comprises K392C substitution and the second CH3 domain comprises V397C substitution, or the first CH3 domain comprises V397C substitution and the second CH3 domain comprises K392C substitution; or
 - iii) the first CH3 domain comprises K392C substitution and the second CH3 domain comprises S400C substitution, or the first CH3 domain comprises S400C substitution and the second CH3 domain comprises K392C substitution.
3. The heterodimeric protein of claim 1 or claim 2, wherein:
 - i) the first CH3 domain further comprises a positively charged residue at position 357 and the second CH3 domain further comprises a negatively charged residue at position 351, or

the first CH3 domain further comprises a negatively charged residue at position 351 and the second CH3 domain further comprises a positively charged residue at position 357; or
 ii) the first CH3 domain further comprises a positively charged residue at position 411 and the second CH3 domain further comprises a negatively charged residue at position 370, or the first CH3 domain further comprises a negatively charged residue at position 370 and the second CH3 domain further comprises a positively charged residue at position 411; or
 iii) the first CH3 domain further comprises a positively charged residue at position 364 and the second CH3 domain further comprises a negatively charged residue at position 370, or the first CH3 domain further comprises a negatively charged residue at position 370 and the second CH3 domain further comprises a positively charged residue at position 364; or
 a combination of i) and ii), or a combination of i) and iii); and
 wherein the amino acid residue numbering is based on EU numbering.

4. The heterodimeric protein of any one of claims 1-3, wherein the first CH3 domain further comprises a positively charged residue at position 356 and the second CH3 domain further comprises a negatively charged residue at position 439, or first CH3 domain further comprises a negatively charged residue at position 439 and the second CH3 domain further comprises a positively charged residue at position 356; and wherein the amino acid residue numbering is based on EU numbering.
5. The heterodimeric protein of claim 3 or claim 4, wherein:
 - i) the positively charged residue is a lysine (K) residue, and the negatively charged residue is an aspartic acid (D) residue; or
 - ii) the positively charged residue is a lysine (K) residue, and the negatively charged residue is a glutamic acid (E) residue; or
 - iii) the positively charged residue is an arginine (R) residue, and the negatively charged residue is an aspartic acid (D) residue; or
 - iv) the positively charged residue is an arginine (R) residue, and the negatively charged residue is a glutamic acid (E) residue.
6. The heterodimeric protein of claim 5, wherein:

- i) the first CH3 domain comprises E357K and T411K substitutions and the second CH3 domain comprises L351D and K370D substitutions, or the first CH3 domain comprises L351D and K370D substitutions and the second CH3 domain comprises E357K and T411K substitutions; or
 - ii) the first CH3 domain comprises E357K and S364K substitutions and the second CH3 domain comprises L351D and K370D substitutions, or the first CH3 domain comprises L351D and K370D substitutions and the second CH3 domain comprises E357K and S364K substitutions; or
 - iii) the first CH3 domain comprises D356K, E357K and S364K substitutions and the second CH3 domain comprises L351D, K370D and K439D substitutions, or the first CH3 domain comprises L351D, K370D and K439D substitutions and the second CH3 domain comprises D356K, E357K and S364K substitutions.
7. The heterodimeric protein of any one of claims 1-5, wherein:
- i) the first CH3 domain further comprises K392D and K409D substitutions and the second CH3 domain further comprises D356K and D399K substitutions, or the first CH3 domain further comprises D356K and D399K substitutions and the second CH3 domain further comprises K392D and K409D substitutions; or
 - ii) the first CH3 domain further comprises L368D and K370S substitutions and the second CH3 domain further comprises E357Q and S364K substitutions, or the first CH3 domain further comprises E357Q and S364K substitutions and the second CH3 domain further comprises L368D and K370S substitutions; or
 - iii) the first CH3 domain further comprises L351K and T366K substitutions and the second CH3 domain further comprises L351D and L368E substitutions, or the first CH3 domain further comprises L351D and L368E substitutions and the second CH3 domain further comprises L351K and T366K substitutions; or
 - (iv) the first CH3 domain further comprises P395K, P396K and V397K substitutions and the second CH3 domain comprises T394D, P395D and P396D substitutions, or the first CH3 domain further comprises T394D, P395D and P396D substitutions and the second CH3 domain further comprises P395K, P396K and V397K substitutions; or
 - (v) the first CH3 domain further comprises F405E, Y407E and K409E substitutions and the second CH3 domain comprises F405K and Y407K substitutions, or the first CH3 domain

further comprises F405K and Y407K substitutions and the second CH3 domain further comprises F405E, Y407E and K409E substitutions.

8. The heterodimeric protein of claim 6, wherein
 - i) the first CH3 domain comprises E357K, S364K and N390C substitutions and the second CH3 domain comprises L351D, K370D, and S400C substitutions, or the first CH3 domain comprises L351D, K370D, and S400C substitutions and the second CH3 domain comprises E357K, S364K and N390C substitutions; or
 - ii) the first CH3 domain comprises E357K, S364K and S400C substitutions and the second CH3 domain comprises L351D, K370D, and N390C substitutions, or the first CH3 domain comprises L351D, K370D, and N390C substitutions and the second CH3 domain comprises E357K, S364K and S400C substitutions; or
 - iii) the first CH3 domain comprises D356K, E357K, S364K and S400C substitutions and the second CH3 domain comprises L351D, K370D, N390C and K439D substitutions, or the first CH3 domain comprises L351D, K370D, N390C and K439D substitutions and the second CH3 domain comprises D356K, E357K, S364K and S400C substitutions; or
 - iv) the first CH3 domain comprises D356K, E357K, S364K and N390C substitutions and the second CH3 domain comprises L351D, K370D, K439D and S400C substitutions, or the first CH3 domain comprises L351D, K370D, K439D and S400C substitutions and the second CH3 domain comprises D356K, E357K, S364K and N390C substitutions.
9. The heterodimeric protein of any one of claims 1-8, wherein the first CH3 domain and the second CH3 domain further comprise knob-into-hole residues.
10. The heterodimeric protein of claim 9, wherein:
 - i) the first CH3 domain comprises T336S, L368A and Y407V substitutions and the second CH3 domain comprises T366W substitution, or the first CH3 domain comprises T366W substitution and the second CH3 domain comprises T336S, L368A and Y407V substitutions; or
 - ii) the first CH3 domain comprises L368V and Y407V substitutions and the second CH3 domain comprises T366W substitution, or the first CH3 domain comprises T366W substitution and the second CH3 domain comprises L368V and Y407V substitutions.

11. A heterodimeric protein comprising a first polypeptide comprising a first CH3 domain and a second polypeptide comprising a second CH3 domain, wherein:
- i) the first CH3 domain comprises a positively charged residue at position 357 and the second CH3 domain comprises a negatively charged residue at position 351, or the first CH3 domain comprises a negatively charged residue at position 351 and the second CH3 domain comprises a positively charged residue at position 357; or
 - ii) the first CH3 domain comprises a positively charged residue at position 411 and the second CH3 domain comprises a negatively charged residue at position 370, or the first CH3 domain comprises a negatively charged residue at position 370 and the second CH3 domain comprises a positively charged residue at position 411; or
 - iii) the first CH3 domain comprises a positively charged residue at position 364 and the second CH3 domain comprises a negatively charged residue at position 370, or the first CH3 domain comprises a negatively charged residue at position 370 and the second CH3 domain comprises a positively charged residue at position 364; and wherein the amino acid residue numbering is based on EU numbering.
12. The heterodimeric protein of claim 11, wherein the first CH3 domain comprises a charged residue at position 356 and the second CH3 domain comprises a negatively charged residue at position 439, or first CH3 domain comprises a negatively charged residue at position 439 and the second CH3 domain comprises a positively charged residue at position 356; and wherein the amino acid residue numbering is based on EU numbering.
13. The heterodimeric protein of claim 11 or claim 12, wherein:
- i) the positively charged residue is a lysine (K) residue, and the negatively charged residue is an aspartic acid (D) residue; or
 - ii) the positively charged residue is a lysine (K) residue, and the negatively charged residue is a glutamic acid (E) residue; or
 - iii) the positively charged residue is an arginine (R) residue, and the negatively charged residue is an aspartic acid (D) residue; or
 - iv) the positively charged residue is an arginine (R) residue, and the negatively charged residue is a glutamic acid (E) residue.

14. The heterodimeric protein of claim 13, wherein:
- i) the first CH3 domain comprises E357K and T411K substitutions and the second CH3 domain comprises L351D and K370D substitutions, or the first CH3 domain comprises L351D and K370D substitutions and the second CH3 domain comprises E357K and T411K substitutions; or
 - ii) the first CH3 domain comprises E357K and S364K substitutions and the second CH3 domain comprises L351D and K370D substitutions, or the first CH3 domain comprises L351D and K370D substitutions and the second CH3 domain comprises E357K and S364K substitutions; or
 - iii) the first CH3 domain comprises D356K, E357K and S364K substitutions and the second CH3 domain comprises L351D, K370D and K439D substitutions, or the first CH3 domain comprises L351D, K370D and K439D substitutions and the second CH3 domain comprises D356K, E357K and S364K substitutions.
15. The heterodimeric protein of any one of claims 11-14, wherein:
- i) the first CH3 domain further comprises K392C substitution and the second CH3 domain further comprises D399C substitution, or the first CH3 domain further comprises D399C substitution and the second CH3 domain further comprises K392C substitution; or
 - ii) the first CH3 domain further comprises Y394C substitution and the second CH3 domain further comprises S354C substitution, or the first CH3 domain further comprises S354C substitution and the second CH3 domain further comprises Y394C substitution; or
 - iii) the first CH3 domain further comprises D356C substitution and the second CH3 domain further comprises Y349C substitution, or the first CH3 domain further comprises Y349C substitution and the second CH3 domain further comprises D356C substitution.
16. The heterodimeric protein of any one of claims 1-15, wherein the first CH3 domain and the second CH3 domain are human CH3 domains.
17. The heterodimeric protein of any one of claims 1-16, wherein the first polypeptide and the second polypeptide each comprises from the N-terminus to the C-terminus at least a portion of an immunoglobulin hinge region, an immunoglobulin heavy chain constant domain 2 (CH2 domain), and the CH3 domain.

18. The heterodimeric protein of claim 17, wherein the CH2 domains and the CH3 domains form an IgG Fc region.
19. The heterodimeric protein of claim 18, wherein the Fc region is of the human IgG1 subclass.
20. The heterodimeric protein of claim 18, wherein the Fc region is of the human IgG4 subclass.
21. The heterodimeric protein of claim 20, wherein the Fc region further comprises S228P substitution.
22. The heterodimeric protein of any one of claims 18-20, wherein the Fc region further comprises N297A substitution.
23. The heterodimeric protein of any one of claims 1-22, the first polypeptide and the second polypeptide are antibody heavy chains, and wherein the heterodimeric protein further comprises one or more antibody light chains.
24. The heterodimeric protein of claim 23, wherein the heterodimeric protein is a multispecific antibody.
25. The heterodimeric protein of claim 24, further comprising a third polypeptide and a fourth polypeptide, wherein:
 - (i) the first polypeptide comprises a structure represented by the formula:
$$\text{VH1-CH1-hinge-CH2-first CH3-L1-scFv1 (Ia);}$$
 - (ii) the second polypeptide comprises a structure represented by the formula:
$$\text{VH2-CH1-hinge-CH2-second CH3-L2-scFv2 (IIa);}$$
 - (iii) the third polypeptide comprises a structure represented by the formula:
$$\text{VL1-CL (Ib); and}$$
 - (iv) the fourth polypeptide comprises a structure represented by the formula:
$$\text{VL2-CL (IIb);}$$wherein:

VL1 is a first immunoglobulin light chain variable domain;

VH is an immunoglobulin heavy chain variable domain;
 scFv is a single-chain variable fragment;
 CL is an immunoglobulin light chain constant domain;
 CH1 is an immunoglobulin heavy chain constant domain 1;
 CH2 is an immunoglobulin heavy chain constant domain 2;
 and hinge is an immunoglobulin hinge region connecting the CH1 and CH2 domains;

wherein VL and VH associate to form an Fv that specifically binds to a first target; and
 wherein the scFv specifically binds to a second target.

30. The heterodimeric protein of claim 29, wherein the Fv specifically binds CD137 and the scFv specifically binds PDL1.

31. The heterodimeric protein of claim 24, wherein the heterodimeric protein is an activatable antibody, wherein the heterodimeric protein comprises a third polypeptide, and wherein:

(i) the first polypeptide comprises a structure represented by the formula:

VH-CH1-hinge-CH2-first CH3 (Va);

(ii) the second polypeptide comprises a structure represented by the formula:

MM1-CM1-scFv-hinge-CH2-second CH3 (VIa); and

(iii) the third polypeptide comprises a structure represented by the formula:

MM2-CM2-VL-CL (IVb);

wherein:

VL is an immunoglobulin light chain variable domain;
 VH is an immunoglobulin heavy chain variable domain;
 scFv is a single-chain variable fragment;
 CL is an immunoglobulin light chain constant domain;
 CH1 is an immunoglobulin heavy chain constant domain 1;
 CH2 is an immunoglobulin heavy chain constant domain 2;
 hinge is an immunoglobulin hinge region connecting the CH1 and CH2 domains;
 MM1 is a first masking peptide;
 MM2 is a second masking peptide;
 CM1 is a first cleavable peptide; and

CM2 is a second cleavable peptide;
wherein VL and VH associate to form a first Fv that specifically binds to a first target;
wherein the scFv specifically binds to a second target;
wherein MM1 inhibits the binding of the scFv to the first target when CM1 is not cleaved;
and
wherein MM2 inhibits the binding of the first Fv to the second target when CM2 is not cleaved.

32. The heterodimeric protein of claim 29 or 31, wherein the first target is a tumor antigen, and the second target is CD3.
33. The heterodimeric protein of claim 31 or 32, wherein MM1 comprises the amino acid sequence of SEQ ID NO: 35.
34. The heterodimeric protein of claim 32 or 33, wherein the first target is HER2.
35. The heterodimeric protein of claim 34, wherein MM2 comprises the amino acid sequence of SEQ ID NO: 36.
36. An activatable antibody comprising: a first polypeptide comprising, from N-terminus to C-terminus, a masking moiety (MM), a cleavable moiety (CM), and a target binding moiety (TBM), wherein the MM comprises the amino acid sequence of SEQ ID NO: 35; wherein the MM inhibits binding of the activatable antibody to human CD3 when the CM is not cleaved; wherein the CM comprises at least a first cleavage site; and wherein:
- a) the TBM comprises a VL and the activatable antibody further comprises a second polypeptide comprising a VH;
 - b) the TBM comprises a VH and the activatable antibody further comprises a second polypeptide comprising a VL;
 - c) the TBM comprises from the N-terminus to the C-terminus, a VL and a VH; or
 - d) the TBM comprise from the N-terminus to the C-terminus, a VH and a VL; and
- wherein the activatable antibody binds to human CD3 via the VH and VL when the CM is cleaved.

37. An activatable antibody comprising: a first polypeptide comprising, from N-terminus to C-terminus, a masking moiety (MM), a cleavable moiety (CM), and a target binding moiety (TBM), wherein the MM comprises the amino acid sequence of SEQ ID NO: 36; wherein the MM inhibits binding of the activatable antibody to human HER2 when the CM is not cleaved; wherein the CM comprises at least a first cleavage site; and wherein:

- a) the TBM comprises a VL and the activatable antibody further comprises a second polypeptide comprising a VH;
- b) the TBM comprises a VH and the activatable antibody further comprises a second polypeptide comprising a VL;
- c) the TBM comprises from the N-terminus to the C-terminus, a VL and a VH; or
- d) the TBM comprise from the N-terminus to the C-terminus, a VH and a VL; and

wherein the activatable antibody binds to human HER2 via the VH and VL when the CM is cleaved.

38. The activatable antibody of claim 36 or 37, comprising a first polypeptide, a second polypeptide and a third polypeptide, wherein:

(i) the first polypeptide comprises a structure represented by the formula:

VH-CH1-hinge-CH2-first CH3 (Va);

(ii) the second polypeptide comprises a structure represented by the formula:

MM1-CM1-scFv-hinge-CH2-second CH3 (VIa); and

(iii) the third polypeptide comprises a structure represented by the formula:

MM2-CM2-VL-CL (IVb);

wherein:

VL is an immunoglobulin light chain variable domain;

VH is an immunoglobulin heavy chain variable domain;

scFv is a single-chain variable fragment;

CL is an immunoglobulin light chain constant domain;

CH1 is an immunoglobulin heavy chain constant domain 1;

CH2 is an immunoglobulin heavy chain constant domain 2;

hinge is an immunoglobulin hinge region connecting the CH1 and CH2 domains;

MM1 is a first masking peptide;

MM2 is a second masking peptide;

CM1 is a first cleavable peptide; and
CM2 is a second cleavable peptide;
wherein VL and VH associate to form a first Fv that specifically binds to a first target;
wherein the scFv specifically binds to a second target; and wherein MM is MM1 or MM2.

39. The heterodimeric protein of any one of claims 32-35 or the activatable antibody of claim 36 or 38, wherein the first Fv or the TBM comprises a VH comprising an CDR-H1 comprising the amino acid sequence of SEQ ID NO: 61, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 62, and/or a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 63. In some embodiments, the TBM comprises a VL comprising a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 64, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 65, and/or a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 66.
40. The heterodimeric protein of claim 34 or 35, or the activatable antibody of any one of claims 37-39, wherein the scFv or the TBM comprises a VH comprising an CDR-H1 comprising the amino acid sequence of SEQ ID NO: 69, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 70, and/or a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 71. In some embodiments, the TBM comprises a VL comprising a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 72, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 73, and/or a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 74
41. The heterodimeric protein of any one of claims 31-35 or the activatable antibody of any one of claims 36-40, wherein the activatable antibody comprises an Fc region comprising a first CH3 domain and a second CH3 domain, wherein the first CH3 domain comprises D356K, E357K, S364K and S400C substitutions and the second CH3 domain comprises L351D, K370D, N390C and K439D substitutions, or the first CH3 domain comprises L351D, K370D, N390C and K439D substitutions and the second CH3 domain comprises D356K, E357K, S364K and S400C substitutions.
42. One or more nucleic acid(s) encoding the heterodimeric protein of any one of claims 1-35 and 39-41 or the activatable antibody of any one of claims 36-41.

43. A vector comprising the one or more nucleic acid(s) of claim 42.
44. A host cell comprising the one or more nucleic acid(s) of claim 42 or the vector of claim 43.
45. A method for preparing a heterodimeric protein or an activatable antibody, comprising:
- (a) culturing the host cell of claim 44 under conditions that allow expression of the one or more nucleic acid(s) or vector; and
 - (b) recovering the heterodimeric protein or the activatable antibody from the host cell culture.
46. A pharmaceutical composition comprising the heterodimeric protein of any one of claims 1-35 and 39-41 or the activatable antibody of any one of claims 36-41, and a pharmaceutically acceptable carrier.
47. Use of the pharmaceutical composition of claim 46 in the manufacture of a medicament for treating a disease or condition in a subject in need thereof.
48. The use of claim 47, wherein the disease or condition is cancer.
49. The use of claim 48, wherein the cancer is lung cancer.
50. The use of claim 48, wherein the cancer is ovarian cancer.