ORTHOGONAL MULTIMERIC PROTEINS

CROSS-REFERENCE

[0001] This application claims priority to U.S. Provisional Application No. 63/498,958, filed on April 28, 2023, which is incorporated herein by reference in its entirety.

SEQUENCE LISTING

[0002] This application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. The XML copy, created April 26, 2024, is named “51096_4013_SL.xml” and is 203,720 bytes in size.

BACKGROUND OF THE INVENTION

[0003] Production of bispecific antibodies in IgG format is challenging, as antibody heavy chains bind antibody light chains in a relatively promiscuous manner. As a result of this promiscuous pairing, concomitant expression of, e.g., two antibody heavy chains and two antibody light chains naturally leads to heavy chain homodimerization and/or scrambling of heavy chain/light chain pairings. While there are numerous approaches to circumvent the problem of heavy chain homodimerization, circumventing the scrambling of heavy chain/light chain pairing has been more difficult due to the complex multidomain heterodimeric interactions within antibody Fabs.

[0004] Similarly, the production of multimeric proteins that include two heavy chains and two light chains can result in heavy chain homodimerization and/or scrambling of heavy chain/light chain pairings. As such, there remains an unmet need for novel multimeric proteins with the ability to overcome the promiscuous pairing of two heavy chains and two light chains.

SUMMARY

[0005] In one example, the present disclosure provides a multimeric protein, comprising: (a) a first monomer comprising a VH1-CH1-hinge-CH2-CH3 monomer, wherein VH1 is a first variable heavy domain and CH2-CH3 is a first variant IgG Fc domain; (b) a second monomer comprising a VL1-CL1 monomer, wherein VL1 is a first variable light domain, and wherein the first variable heavy domain and the first variable light domain form a first antigen binding

domain; (c) a third monomer comprising a VH2-CH1-hinge-CH2-CH3 monomer, wherein VH2 is a second variable heavy domain and CH2-CH3 is a second variant IgG Fc domain; and (d) a fourth monomer comprising a VL2-CL2 monomer, wherein VL2 is a second variable light domain, and wherein the second variable heavy domain and the second variable light domain form a second antigen binding domain, wherein either the first monomer and the second monomer or the third monomer and the fourth monomer comprise a set of CH1:CL electrostatic variants, wherein the set of CH1:CL electrostatic variants comprises amino acid substitutions at amino acid residues K213/K218:D122/E123, wherein numbering is according to EU numbering.

[0006] In a further example and in accordance with the above, the set of CH1:CL electrostatic variants comprises amino acid substitutions selected from a group including: (i) K213E/K218D:D122K/E123K, (ii) K213E/K218E:D122K/E123K, (iii) K213D/K218E:D122K/E123K, (iv) K213D/K218D:D122K/E123K, (v) K213E/K218D:D122K/E123R, (vi) K213E/K218E:D122K/E123R, (vii) K213D/K218E:D122K/E123R, (viii) K213D/K218D:D122K/E123R, (ix) K213E/K218D:D122R/E123K, (x) K213E/K218E:D122R/E123K, (xi) K213D/K218E:D122R/E123K, (xii) K213D/K218D:D122R/E123K, (xiii) K213E/K218D:D122R/E123R, (xiv) K213E/K218E:D122R/E123R, (xv) K213D/K218E:D122R/E123R, and (xvi) K213D/K218D:D122R/E123R, wherein numbering is according to EU numbering.

[0007] In a further example and in accordance with any of the above, the set of CH1:CL electrostatic variants comprises amino acid substitutions K213E/K218D:D122K/E123K, wherein numbering is according to EU numbering.

[0008] In a further example and in accordance with any of the above, the first monomer and the second monomer do not comprise the set of CH1:CL electrostatic variants.

[0009] In a further example and in accordance with any of the above, the third monomer and the fourth monomer do not comprise the set of CH1:CL electrostatic variants.

[0010] In a further example and in accordance with any of the above, the first and/or second variant IgG Fc domains comprise one or more FcγRIIIA (CD16a) binding variant substitutions.

[0011] In a further example and in accordance with the above, the one or more FcγRIIIA (CD16a) binding variant substitutions are selected from a group including: (i) 236A, (ii) 239D, (iii) 239E, (iv) 243L, (v) 298A, (vi) 299T, (vii) 332E, (viii) 332D, (ix) 239D/332E, (x)

236A/332E, (xi) 239D/332E/330L, and (xii) 332E/330L, wherein numbering is according to EU numbering.

[0012] In a further example and in accordance with any of the above, the first and second variant IgG Fc domains comprise a set of FcγRIIIA (CD16a) binding variant substitutions selected from a group including: (i) S239D/I332E : S239D/I332E, (ii) S239D : S239D, (iii) I332E : I332E, (iv) WT : S239D/I332E, (v) WT : S239D, (vi) WT : I332E, (vii) S239D/I332E : WT, (viii) S239D : WT, (ix) I332E : WT, (x) S239D/I332E : S239D, (xi) S239D/I332E : I332E, (xii) S239D : S239D/I332E, (xiii) I332E : S239D/I332E, (xiv) S239D : I332E, and (xv) I332E : S239D, wherein numbering is according to EU numbering.

[0013] In a further example and in accordance with any of the above, the first and/or second variant IgG Fc domains comprise the FcγRIIIA (CD16a) binding variant substitutions of S239D/I332E, wherein numbering is according to EU numbering.

[0014] In a further example and in accordance with any of the above, the first and second variant IgG Fc domains comprise a set of heterodimerization variants selected from a group including those depicted in Figures 13A-13F, wherein numbering is according to EU numbering.

[0015] In a further example and in accordance with the above, the set of heterodimerization variants is selected from a group including: (i) S364K/E357Q : L368D/K370S, (ii) S364K : L368D/K370S, (iii) S364K : L368E/K370S, (iv) D401K : T411E/K360E/Q362E, and (v) T366W : T366S/L368A/Y407V, wherein numbering is according to EU numbering.

[0016] In a further example and in accordance with any of the above, the first and second variant IgG Fc domains further comprise one or more ablation variants.

[0017] In a further example and in accordance with the above, the one or more ablation variants are E233P/L234V/L235A/G236del/S267K, wherein numbering is according to EU numbering.

[0018] In a further example and in accordance with any of the above, one of the first or second variant IgG Fc domains comprise one or more pI variants.

[0019] In a further example and in accordance with the above, the one or more pI variants are N208D/Q295E/N384D/Q418E/N421D, wherein numbering is according to EU numbering.

[0020] In a further example and in accordance with any of the above, the first variant IgG Fc domain comprises amino acid variants

S364K/E357Q/E233P/L234V/L235A/G236del/S267K, wherein the second variant IgG Fc domain comprises amino acid variants L368D/K370S/N208D/Q295E/N384D/Q418E/N421D/E233P/L234V/L235A/G236del/S267K, and wherein numbering is according to EU numbering.

[0021] In a further example and in accordance with any of the above, the first and second variant IgG Fc domains each further comprise amino acid variants M428L/N434S or M428L/N434A, wherein numbering is according to EU numbering.

[0022] In a further example and in accordance with any of the above, the first variant IgG Fc domain is selected from a group including: (i) a first variant IgG1 Fc domain, (ii) a first variant IgG2 Fc domain, and (iii) a first variant IgG4 Fc domain.

[0023] In a further example and in accordance with any of the above, the second variant IgG Fc domain is selected from a group including: (i) a second variant IgG1 Fc domain, (ii) a second variant IgG2 Fc domain, and (iii) a second variant IgG4 Fc domain.

[0024] In another example, a nucleic acid composition comprising nucleic acids encoding the first monomer, the second monomer, the third monomer, and the fourth monomer of the multimeric protein (as described above) is provided.

[0025] In another example, an expression vector comprising the nucleic acids (as described above) is provided.

[0026] In another example, a host cell transformed with an expression vector (as described above) is provided.

[0027] In another example, a method of making a multimeric protein is provided, the method comprising: (a) culturing the host cell (as described above) under conditions wherein the multimeric protein is expressed; and (b) recovering the multimeric protein.

[0028] In one aspect, the present disclosure provides a multimeric protein, comprising: (a) a first monomer comprising a VH1-CH1-hinge-CH2-CH3 monomer, wherein VH1 is a first variable heavy domain and CH2-CH3 is a first variant IgG Fc domain; (b) a second monomer comprising a VL1-CL1 monomer, wherein VL1 is a first variable light domain, and wherein the first variable heavy domain and the first variable light domain form a first antigen binding domain; (c) a third monomer comprising a VH2-CH1-hinge-CH2-CH3 monomer, wherein VH2 is a second variable heavy domain and CH2-CH3 is a second variant IgG Fc domain; and (d) a fourth monomer comprising a VL2-CL2 monomer, wherein VL2 is a second variable light domain, and wherein the second variable heavy domain and the second variable light domain form a second antigen binding domain,

wherein either the third monomer and the fourth monomer or the first monomer and the second monomer comprise a set of CH1:CL steric variants, wherein the set of CH1:CL steric variants comprises amino acid substitutions at amino acid residues selected from the group consisting of: (i) A141:F118, (ii) A141:F116, and (iii) K147:S131, wherein numbering is according to EU numbering.

[0028a] In another example, the present disclosure provides a multimeric protein, comprising: (a) a first monomer comprising a VH1-CH1-hinge-CH2-CH3 monomer, wherein VH1 is a first variable heavy domain and CH2-CH3 is a first variant IgG Fc domain; (b) a second monomer comprising a VL1-CL1 monomer, wherein VL1 is a first variable light domain, and wherein the first variable heavy domain and the first variable light domain form a first antigen binding domain; (c) a third monomer comprising a VH2-CH1-hinge-CH2-CH3 monomer, wherein VH2 is a second variable heavy domain and CH2-CH3 is a second variant IgG Fc domain; and (d) a fourth monomer comprising a VL2-CL2 monomer, wherein VL2 is a second variable light domain, and wherein the second variable heavy domain and the second variable light domain form a second antigen binding domain, wherein either the first

monomer and the second monomer or the third monomer and the fourth monomer comprise a set of CH1:CL steric variants, wherein the set of CH1:CL steric variants comprises amino acid substitutions at amino acid residues selected from a group including: (i) A141:F118, (ii) A141:F116, and (iii) K147:S131, wherein numbering is according to EU numbering.

[0029] In a further example and in accordance with the above, the set of CH1:CL steric variants comprises amino acid substitutions selected from a group including: (i) A141F:F118A, (ii) A141F:F116A, and (iii) K147S:S131K, wherein numbering is according to EU numbering.

[0030] In a further example and in accordance with any of the above, the first monomer and the second monomer do not comprise the set of CH1:CL steric variants.

[0031] In a further example and in accordance with any of the above, the third monomer and the fourth monomer do not comprise the set of CH1:CL steric variants.

[0032] In a further example and in accordance with any of the above, the first and/or second variant IgG Fc domains comprise one or more FcγRIIIA (CD16a) binding variant substitutions.

[0033] In a further example and in accordance with the above, the one or more FcγRIIIA (CD16a) binding variant substitutions are selected from a group including: (i) 236A, (ii) 239D, (iii) 239E, (iv) 243L, (v) 298A, (vi) 299T, (vii) 332E, (viii) 332D, (ix) 239D/332E, (x) 236A/332E, (xi) 239D/332E/330L, and (xii) 332E/330L, wherein numbering is according to EU numbering.

[0034] In a further example and in accordance with any of the above, the first and second variant IgG Fc domains comprise a set of FcγRIIIA (CD16a) binding variant substitutions selected from a group including: (i) S239D/I332E : S239D/I332E, (ii) S239D : S239D, (iii) I332E : I332E, (iv) WT : S239D/I332E, (v) WT : S239D, (vi) WT : I332E, (vii) S239D/I332E : WT, (viii) S239D : WT, (ix) I332E : WT, (x) S239D/I332E : S239D, (xi) S239D/I332E : I332E, (xii) S239D : S239D/I332E, (xiii) I332E : S239D/I332E, (xiv) S239D : I332E, and (xv) I332E : S239D, wherein numbering is according to EU numbering.

[0035] In a further example and in accordance with any of the above, the first and/or second variant IgG Fc domains comprise the FcγRIIIA (CD16a) binding variant substitutions of S239D/I332E, wherein numbering is according to EU numbering.

[0036] In a further example and in accordance with any of the above, the first and second variant IgG Fc domains comprise a set of heterodimerization variants selected from a group

including those depicted in Figures 13A-13F, wherein numbering is according to EU numbering.

[0037] In a further example and in accordance with the above, the set of heterodimerization variants is selected from a group including: (i) S364K/E357Q : L368D/K370S, (ii) S364K : L368D/K370S, (iii) S364K : L368E/K370S, (iv) D401K : T411E/K360E/Q362E, and (v) T366W : T366S/L368A/Y407V, wherein numbering is according to EU numbering.

[0038] In a further example and in accordance with any of the above, the first and second variant IgG Fc domains further comprise one or more ablation variants.

[0039] In a further example and in accordance with the above, the one or more ablation variants are E233P/L234V/L235A/G236del/S267K, wherein numbering is according to EU numbering.

[0040] In a further example and in accordance with any of the above, one of the first or second variant IgG Fc domains comprise one or more pI variants.

[0041] In a further example and in accordance with the above, the one or more pI variants are N208D/Q295E/N384D/Q418E/N421D, wherein numbering is according to EU numbering.

[0042] In a further example and in accordance with any of the above, the first variant IgG Fc domain comprises amino acid variants S364K/E357Q/E233P/L234V/L235A/G236del/S267K, wherein the second variant IgG Fc domain comprises amino acid variants L368D/K370S/N208D/Q295E/N384D/Q418E/N421D/E233P/L234V/L235A/G236del/S267K, and wherein numbering is according to EU numbering.

[0043] In a further example and in accordance with any of the above, the first and second variant IgG Fc domains each further comprise amino acid variants M428L/N434S or M428L/N434A, wherein numbering is according to EU numbering.

[0044] In a further example and in accordance with any of the above, the first variant IgG Fc domain is selected from a group including: (i) a first variant IgG1 Fc domain, (ii) a first variant IgG2 Fc domain, and (iii) a first variant IgG4 Fc domain.

[0045] In a further example and in accordance with any of the above, the second variant IgG Fc domain is selected from a group including: (i) a second variant IgG1 Fc domain, (ii) a second variant IgG2 Fc domain, and (iii) a second variant IgG4 Fc domain.

[0046] In another example, a nucleic acid composition comprising nucleic acids encoding the first monomer, the second monomer, the third monomer, and the fourth monomer of the multimeric protein (as described above) is provided.

[0047] In another example, an expression vector comprising the nucleic acids (as described above) is provided.

[0048] In another example, a host cell transformed with an expression vector (as described above) is provided.

[0049] In another example, a method of making a multimeric protein is provided, the method comprising: (a) culturing the host cell (as described above) under conditions wherein the multimeric protein is expressed; and (b) recovering the multimeric protein.

[0050] In another example, the present disclosure provides a multimeric protein, comprising: (a) a first monomer comprising a VH1-CH1-hinge-CH2-CH3 monomer, wherein VH1 is a first variable heavy domain and CH2-CH3 is a first variant IgG Fc domain; (b) a second monomer comprising a VL1-CL1 monomer, wherein VL1 is a first variable light domain, and wherein the first variable heavy domain and the first variable light domain form a first antigen binding domain; (c) a third monomer comprising a VH2-CH1-hinge-CH2-CH3 monomer, wherein VH2 is a second variable heavy domain and CH2-CH3 is a second variant IgG Fc domain; and (d) a fourth monomer comprising a VL2-CL2 monomer, wherein VL2 is a second variable light domain, and wherein the second variable heavy domain and the second variable light domain form a second antigen binding domain, wherein (i) the first monomer and the second monomer comprise a first set of VH:VL electrostatic variants comprising amino acid substitutions at amino acid residues Q39:Q38, and (ii) the third monomer and the fourth monomer comprise a second set of VH:VL electrostatic variants, comprising amino acid substitutions at amino acid residues Q39:Q38, wherein numbering is according to Kabat numbering.

[0051] In a further example and in accordance with the above, the first set of VH:VL electrostatic variants and the second set of VH:VL electrostatic variants comprise amino acid substitutions selected from a group including: (i) Q39E:Q38K and Q39K:Q38E, respectively, (ii) Q39E:Q38K and Q39K:Q38D, respectively, (iii) Q39E:Q38K and Q39R:Q38E, respectively, (iv) Q39E:Q38K and Q39R:Q38D, respectively, (v) Q39E:Q38K and Q39E:Q38K, respectively, (vi) Q39E:Q38K and Q39E:Q38R, respectively, (vii) Q39E:Q38K and Q39D:Q38K, respectively, (viii) Q39E:Q38K and Q39D:Q38R, respectively, (ix) Q39E:Q38R and Q39K:Q38E, respectively, (x) Q39E:Q38R and Q39K:Q38D, respectively,

(xi) Q39E:Q38R and Q39R:Q38E, respectively, (xii) Q39E:Q38R and Q39R:Q38D, respectively, (xiii) Q39E:Q38R and Q39E:Q38K, respectively, (xiv) Q39E:Q38R and Q39E:Q38R, respectively, (xv) Q39E:Q38R and Q39D:Q38K, respectively, (xvi) Q39E:Q38R and Q39D:Q38R, respectively, (xvii) Q39D:Q38K and Q39K:Q38E, respectively, (xviii) Q39D:Q38K and Q39K:Q38D, respectively, (xix) Q39D:Q38K and Q39R:Q38E, respectively, (xx) Q39D:Q38K and Q39R:Q38D, respectively, (xxi) Q39D:Q38K and Q39E:Q38K, respectively, (xxii) Q39D:Q38K and Q39E:Q38R, respectively, (xxiii) Q39D:Q38K and Q39D:Q38K, respectively, (xxiv) Q39D:Q38K and Q39D:Q38R, respectively, (xxv) Q39D:Q38R and Q39K:Q38E, respectively, (xxvi) Q39D:Q38R and Q39K:Q38D, respectively, (xxvii) Q39D:Q38R and Q39R:Q38E, respectively, (xxviii) Q39D:Q38R and Q39R:Q38D, respectively, (xxix) Q39D:Q38R and Q39E:Q38K, respectively, (xxx) Q39D:Q38R and Q39E:Q38R, respectively, (xxxi) Q39D:Q38R and Q39D:Q38K, respectively, (xxxii) Q39D:Q38R and Q39D:Q38R, respectively, (xxxiii) Q39K:Q38E and Q39K:Q38E, respectively, (xxxiv) Q39K:Q38E and Q39K:Q38D, respectively, (xxxv) Q39K:Q38E and Q39R:Q38E, respectively, (xxxvi) Q39K:Q38E and Q39R:Q38D, respectively, (xxxvii) Q39K:Q38E and Q39E:Q38K, respectively, (xxxviii) Q39K:Q38E and Q39E:Q38R, respectively, (xxxix) Q39K:Q38E and Q39D:Q38K, respectively, (xl) Q39K:Q38E and Q39D:Q38R, respectively, (xli) Q39R:Q38E and Q39K:Q38E, respectively, (xlii) Q39R:Q38E and Q39K:Q38D, respectively, (xliii) Q39R:Q38E and Q39R:Q38E, respectively, (xliv) Q39R:Q38E and Q39R:Q38D, respectively, (xlv) Q39R:Q38E and Q39E:Q38K, respectively, (xlvi) Q39R:Q38E and Q39E:Q38R, respectively, (xlvii) Q39R:Q38E and Q39D:Q38K, respectively, (xlviii) Q39R:Q38E and Q39D:Q38R, respectively, (xlix) Q39K:Q38D and Q39K:Q38E, respectively, (l) Q39K:Q38D and Q39K:Q38D, respectively, (li) Q39K:Q38D and Q39R:Q38E, respectively, (lii) Q39K:Q38D and Q39R:Q38D, respectively, (liii) Q39K:Q38D and Q39E:Q38K, respectively, (liv) Q39K:Q38D and Q39E:Q38R, respectively, (lv) Q39K:Q38D and Q39D:Q38K, respectively, (lvi) Q39K:Q38D and Q39D:Q38R, respectively, (lvii) Q39R:Q38D and Q39K:Q38E, respectively, (lviii) Q39R:Q38D and Q39K:Q38D, respectively, (lix) Q39R:Q38D and Q39R:Q38E, respectively, (lx) Q39R:Q38D and Q39R:Q38D, respectively, (lxi) Q39R:Q38D and Q39E:Q38K, respectively, (lxii) Q39R:Q38D and Q39E:Q38R, respectively, (lxiii) Q39R:Q38D and Q39D:Q38K, respectively, and (lxiv) Q39R:Q38D and Q39D:Q38R, respectively, wherein numbering is according to Kabat numbering.

[0052] In a further example and in accordance with any of the above, the first set of VH:VL electrostatic variants comprises amino acid substitutions Q39E:Q38K, and the second set of VH:VL electrostatic variants comprises amino acid substitutions Q39K:Q38E, wherein numbering is according to Kabat numbering.

[0053] In a further example and in accordance with any of the above, the first set of VH:VL electrostatic variants comprises amino acid substitutions Q39K:Q38E, and the second set of VH:VL electrostatic variants comprises amino acid substitutions Q39E:Q38K, wherein numbering is according to Kabat numbering.

[0054] In a further example and in accordance with any of the above, the first and/or second variant IgG Fc domains comprise one or more FcγRIIIA (CD16a) binding variant substitutions.

[0055] In a further example and in accordance with the above, the one or more FcγRIIIA (CD16a) binding variant substitutions are selected from a group including: (i) 236A, (ii) 239D, (iii) 239E, (iv) 243L, (v) 298A, (vi) 299T, (vii) 332E, (viii) 332D, (ix) 239D/332E, (x) 236A/332E, (xi) 239D/332E/330L, and (xii) 332E/330L, wherein numbering is according to EU numbering.

[0056] In a further example and in accordance with any of the above, the first and second variant IgG Fc domains comprise a set of FcγRIIIA (CD16a) binding variant substitutions selected from a group including: (i) S239D/I332E : S239D/I332E, (ii) S239D : S239D, (iii) I332E : I332E, (iv) WT : S239D/I332E, (v) WT : S239D, (vi) WT : I332E, (vii) S239D/I332E : WT, (viii) S239D : WT, (ix) I332E : WT, (x) S239D/I332E : S239D, (xi) S239D/I332E : I332E, (xii) S239D : S239D/I332E, (xiii) I332E : S239D/I332E, (xiv) S239D : I332E, and (xv) I332E : S239D, wherein numbering is according to EU numbering.

[0057] In a further example and in accordance with any of the above, the first and/or second variant IgG Fc domains comprise the FcγRIIIA (CD16a) binding variant substitutions of S239D/I332E, wherein numbering is according to EU numbering.

[0058] In a further example and in accordance with any of the above, the first and second variant IgG Fc domains comprise a set of heterodimerization variants selected from a group including those depicted in Figures 13A-13F, wherein numbering is according to EU numbering.

[0059] In a further example and in accordance with the above, the set of heterodimerization variants is selected from a group including: (i) S364K/E357Q : L368D/K370S, (ii) S364K : L368D/K370S, (iii) S364K : L368E/K370S, (iv) D401K :

T411E/K360E/Q362E, and (v) T366W : T366S/L368A/Y407V, wherein numbering is according to EU numbering.

[0060] In a further example and in accordance with any of the above, the first and second variant IgG Fc domains further comprise one or more ablation variants.

[0061] In a further example and in accordance with the above, the one or more ablation variants are E233P/L234V/L235A/G236del/S267K, wherein numbering is according to EU numbering.

[0062] In a further example and in accordance with any of the above, one of the first or second variant IgG Fc domains comprise one or more pI variants.

[0063] In a further example and in accordance with the above, the one or more pI variants are N208D/Q295E/N384D/Q418E/N421D, wherein numbering is according to EU numbering.

[0064] In a further example and in accordance with any of the above, the first variant IgG Fc domain comprises amino acid variants S364K/E357Q/E233P/L234V/L235A/G236del/S267K, wherein the second variant IgG Fc domain comprises amino acid variants L368D/K370S/N208D/Q295E/N384D/Q418E/N421D/E233P/L234V/L235A/G236del/S267K, and wherein numbering is according to EU numbering.

[0065] In a further example and in accordance with any of the above, the first and second variant IgG Fc domains each further comprise amino acid variants M428L/N434S or M428L/N434A, wherein numbering is according to EU numbering.

[0066] In a further example and in accordance with any of the above, the first variant IgG Fc domain is selected from a group including: (i) a first variant IgG1 Fc domain, (ii) a first variant IgG2 Fc domain, and (iii) a first variant IgG4 Fc domain.

[0067] In a further example and in accordance with any of the above, the second variant IgG Fc domain is selected from a group including: (i) a second variant IgG1 Fc domain, (ii) a second variant IgG2 Fc domain, and (iii) a second variant IgG4 Fc domain.

[0068] In another aspect, the present disclosure provides a nucleic acid composition comprising nucleic acids encoding the first, second, third, and fourth monomers as described herein. In another example, a nucleic acid composition comprising nucleic acids encoding the first monomer, the second monomer, the third monomer, and the fourth monomer of the multimeric protein (as described above) is provided.

[0069] In another example, an expression vector comprising the nucleic acids (as described above) is provided.

[0070] In another aspect, the present disclosure provides a host cell transformed with the one or more expression vectors as described herein. In another example, a host cell transformed with an expression vector (as described above) is provided.

[0071] In another aspect, a method of making a multimeric protein is provided, the method comprising: (a) culturing the host cell (as described above) under conditions wherein the multimeric protein is expressed; and (b) recovering the multimeric protein.

[0072] In another example, the present disclosure provides a multimeric protein, comprising: (a) a first monomer comprising a VH1-CH1-hinge-CH2-CH3 monomer, wherein VH1 is a first variable heavy domain and CH2-CH3 is a first variant IgG Fc domain; (b) a second monomer comprising a VL1-CL1 monomer, wherein VL1 is a first variable light domain, and wherein the first variable heavy domain and the first variable light domain form a first antigen binding domain; (c) a third monomer comprising a VH2-CH1-hinge-CH2-CH3 monomer, wherein VH2 is a second variable heavy domain and CH2-CH3 is a second variant IgG Fc domain; and (d) a fourth monomer comprising a VL2-CL2 monomer, wherein VL2 is a second variable light domain, and wherein the second variable heavy domain and the second variable light domain form a second antigen binding domain, wherein (i) the first monomer and the second monomer comprise a first set of VH:VL electrostatic variants and a set of CH1:CL electrostatic variants, and (ii) the third monomer and the fourth monomer comprise a second set of VH:VL electrostatic variants and a set of CH1:CL steric variants.

[0073] In a further example and in accordance with the above, wherein: (i) the set of CH1:CL electrostatic variants comprises amino acid substitutions at amino acid residues K213/K218:D122/E123, wherein numbering is according to EU numbering; (ii) the set of CH1:CL steric variants comprises amino acid substitutions at amino acid residues selected from a group including: (a) A141:F116, (b) A141:F118, and (c) K147:S131, wherein numbering is according to EU numbering; and (iii) the first set of VH:VL electrostatic variants and the second set of VH:VL electrostatic variants each comprise amino acid substitutions at amino acid residues Q39:Q38, wherein numbering is according to Kabat numbering.

[0074] In a further example and in accordance with any of the above, wherein: (a) the set of CH1:CL electrostatic variants comprises amino acid substitutions selected from a group including: (i) K213E/K218D:D122K/E123K, (ii) K213E/K218E:D122K/E123K, (iii) K213D/K218E:D122K/E123K, (iv) K213D/K218D:D122K/E123K, (v) K213E/K218D:D122K/E123R, (vi) K213E/K218E:D122K/E123R, (vii) K213D/K218E:D122K/E123R, (viii) K213D/K218D:D122K/E123R, (ix)

K213E/K218D:D122R/E123K, (x) K213E/K218E:D122R/E123K, (xi) K213D/K218E:D122R/E123K, (xii) K213D/K218D:D122R/E123K, (xiii) K213E/K218D:D122R/E123R, (xiv) K213E/K218E:D122R/E123R, (xv) K213D/K218E:D122R/E123R, and (xvi) K213D/K218D:D122R/E123R, wherein numbering is according to EU numbering; (b) the set of CH1:CL steric variants comprises amino acid substitutions selected from a group including: (i) A141F:F118A, (ii) A141F:F116A, and (iii) K147S:S131K, wherein numbering is according to EU numbering; and (c) the first set of VH:VL electrostatic variants and the second set of VH:VL electrostatic variants comprise amino acid substitutions selected from a group including: (i) Q39E:Q38K and Q39K:Q38E, respectively, (ii) Q39E:Q38K and Q39K:Q38D, respectively, (iii) Q39E:Q38K and Q39R:Q38E, respectively, (iv) Q39E:Q38K and Q39R:Q38D, respectively, (v) Q39E:Q38K and Q39E:Q38K, respectively, (vi) Q39E:Q38K and Q39E:Q38R, respectively, (vii) Q39E:Q38K and Q39D:Q38K, respectively, (viii) Q39E:Q38K and Q39D:Q38R, respectively, (ix) Q39E:Q38R and Q39K:Q38E, respectively, (x) Q39E:Q38R and Q39K:Q38D, respectively, (xi) Q39E:Q38R and Q39R:Q38E, respectively, (xii) Q39E:Q38R and Q39R:Q38D, respectively, (xiii) Q39E:Q38R and Q39E:Q38K, respectively, (xiv) Q39E:Q38R and Q39E:Q38R, respectively, (xv) Q39E:Q38R and Q39D:Q38K, respectively, (xvi) Q39E:Q38R and Q39D:Q38R, respectively, (xvii) Q39D:Q38K and Q39K:Q38E, respectively, (xviii) Q39D:Q38K and Q39K:Q38D, respectively, (xix) Q39D:Q38K and Q39R:Q38E, respectively, (xx) Q39D:Q38K and Q39R:Q38D, respectively, (xxi) Q39D:Q38K and Q39E:Q38K, respectively, (xxii) Q39D:Q38K and Q39E:Q38R, respectively, (xxiii) Q39D:Q38K and Q39D:Q38K, respectively, (xxiv) Q39D:Q38K and Q39D:Q38R, respectively, (xxv) Q39D:Q38R and Q39K:Q38E, respectively, (xxvi) Q39D:Q38R and Q39K:Q38D, respectively, (xxvii) Q39D:Q38R and Q39R:Q38E, respectively, (xxviii) Q39D:Q38R and Q39R:Q38D, respectively, (xxix) Q39D:Q38R and Q39E:Q38K, respectively, (xxx) Q39D:Q38R and Q39E:Q38R, respectively, (xxxi) Q39D:Q38R and Q39D:Q38K, respectively, (xxxii) Q39D:Q38R and Q39D:Q38R, respectively, (xxxiii) Q39K:Q38E and Q39K:Q38E, respectively, (xxxiv) Q39K:Q38E and Q39K:Q38D, respectively, (xxxv) Q39K:Q38E and Q39R:Q38E, respectively, (xxxvi) Q39K:Q38E and Q39R:Q38D, respectively, (xxxvii) Q39K:Q38E and Q39E:Q38K, respectively, (xxxviii) Q39K:Q38E and Q39E:Q38R, respectively, (xxxix) Q39K:Q38E and Q39D:Q38K, respectively, (xl) Q39K:Q38E and Q39D:Q38R, respectively, (xli) Q39R:Q38E and Q39K:Q38E, respectively, (xlii) Q39R:Q38E and Q39K:Q38D, respectively, (xliii)

Q39R:Q38E and Q39R:Q38E, respectively, (xliv) Q39R:Q38E and Q39R:Q38D, respectively, (xlv) Q39R:Q38E and Q39E:Q38K, respectively, (xlvi) Q39R:Q38E and Q39E:Q38R, respectively, (xlvii) Q39R:Q38E and Q39D:Q38K, respectively, (xlviii) Q39R:Q38E and Q39D:Q38R, respectively, (xlix) Q39K:Q38D and Q39K:Q38E, respectively, (l) Q39K:Q38D and Q39K:Q38D, respectively, (li) Q39K:Q38D and Q39R:Q38E, respectively, (lii) Q39K:Q38D and Q39R:Q38D, respectively, (liii) Q39K:Q38D and Q39E:Q38K, respectively, (liv) Q39K:Q38D and Q39E:Q38R, respectively, (lv) Q39K:Q38D and Q39D:Q38K, respectively, (lvi) Q39K:Q38D and Q39D:Q38R, respectively, (lvii) Q39R:Q38D and Q39K:Q38E, respectively, (lviii) Q39R:Q38D and Q39K:Q38D, respectively, (lix) Q39R:Q38D and Q39R:Q38E, respectively, (lx) Q39R:Q38D and Q39R:Q38D, respectively, (lxi) Q39R:Q38D and Q39E:Q38K, respectively, (lxii) Q39R:Q38D and Q39E:Q38R, respectively, (lxiii) Q39R:Q38D and Q39D:Q38K, respectively, and (lxiv) Q39R:Q38D and Q39D:Q38R, respectively, wherein numbering is according to Kabat numbering.

[0075] In a further example and in accordance with any of the above, the set of CH1:CL electrostatic variants comprises amino acid substitutions K213E/K218D:D122K/E123K, wherein numbering is according to EU numbering.

[0076] In a further example and in accordance with any of the above, the first set of VH:VL electrostatic variants comprises amino acid substitutions Q39E:Q38K, and the second set of VH:VL electrostatic variants comprises amino acid substitutions Q39K:Q38E, wherein numbering is according to Kabat numbering.

[0077] In a further example and in accordance with any of the above, the first set of VH:VL electrostatic variants comprises amino acid substitutions Q39K:Q38E, and the second set of VH:VL electrostatic variants comprises amino acid substitutions Q39E:Q38K, wherein numbering is according to Kabat numbering.

[0078] In a further example and in accordance with any of the above, the first and/or second variant IgG Fc domains comprise one or more FcγRIIIA (CD16a) binding variant substitutions.

[0079] In a further example and in accordance with the above, the one or more FcγRIIIA (CD16a) binding variant substitutions are selected from a group including: (i) 236A, (ii) 239D, (iii) 239E, (iv) 243L, (v) 298A, (vi) 299T, (vii) 332E, (viii) 332D, (ix) 239D/332E, (x) 236A/332E, (xi) 239D/332E/330L, and (xii) 332E/330L, wherein numbering is according to EU numbering.

[0080] In a further example and in accordance with any of the above, the first and second variant IgG Fc domains comprise a set of FcγRIIIA (CD16a) binding variant substitutions selected from a group including: (i) S239D/I332E : S239D/I332E, (ii) S239D : S239D, (iii) I332E : I332E, (iv) WT : S239D/I332E, (v) WT : S239D, (vi) WT : I332E, (vii) S239D/I332E : WT, (viii) S239D : WT, (ix) I332E : WT, (x) S239D/I332E : S239D, (xi) S239D/I332E : I332E, (xii) S239D : S239D/I332E, (xiii) I332E : S239D/I332E, (xiv) S239D : I332E, and (xv) I332E : S239D, wherein numbering is according to EU numbering.

[0081] In a further example and in accordance with any of the above, the first and/or second variant IgG Fc domains comprise the FcγRIIIA (CD16a) binding variant substitutions of S239D/I332E, wherein numbering is according to EU numbering.

[0082] In a further example and in accordance with any of the above, the first and second variant IgG Fc domains comprise a set of heterodimerization variants selected from a group including those depicted in Figures 13A-13F, wherein numbering is according to EU numbering.

[0083] In a further example and in accordance with the above, the set of heterodimerization variants is selected from a group including: (i) S364K/E357Q : L368D/K370S, (ii) S364K : L368D/K370S, (iii) S364K : L368E/K370S, (iv) D401K : T411E/K360E/Q362E, and (v) T366W : T366S/L368A/Y407V, wherein numbering is according to EU numbering.

[0084] In a further example and in accordance with any of the above, the first and second variant IgG Fc domains further comprise one or more ablation variants.

[0085] In a further example and in accordance with the above, the one or more ablation variants are E233P/L234V/L235A/G236del/S267K, wherein numbering is according to EU numbering.

[0086] In a further example and in accordance with any of the above, one of the first or second variant IgG Fc domains comprise one or more pI variants.

[0087] In a further example and in accordance with the above, the one or more pI variants are N208D/Q295E/N384D/Q418E/N421D, wherein numbering is according to EU numbering.

[0088] In a further example and in accordance with any of the above, the first variant IgG Fc domain comprises amino acid variants S364K/E357Q/E233P/L234V/L235A/G236del/S267K, wherein the second variant IgG Fc domain comprises amino acid variants

L368D/K370S/N208D/Q295E/N384D/Q418E/N421D/E233P/L234V/L235A/G236del/S267K, and wherein numbering is according to EU numbering.

[0089] In a further example and in accordance with any of the above, the first and second variant IgG Fc domains each further comprise amino acid variants M428L/N434S or M428L/N434A, wherein numbering is according to EU numbering.

[0090] In a further example and in accordance with any of the above, the first variant IgG Fc domain is selected from a group including: (i) a first variant IgG1 Fc domain, (ii) a first variant IgG2 Fc domain, and (iii) a first variant IgG4 Fc domain.

[0091] In a further example and in accordance with any of the above, the second variant IgG Fc domain is selected from a group including: (i) a second variant IgG1 Fc domain, (ii) a second variant IgG2 Fc domain, and (iii) a second variant IgG4 Fc domain.

[0092] In another example, a nucleic acid composition comprising nucleic acids encoding the first monomer, the second monomer, the third monomer, and the fourth monomer of the multimeric protein (as described above) is provided.

[0093] In another example, an expression vector comprising the nucleic acids (as described above) is provided.

[0094] In another example, a host cell transformed with an expression vector (as described above) is provided.

[0095] In another example, a method of making a multimeric protein is provided, the method comprising: (a) culturing the host cell (as described above) under conditions wherein the multimeric protein is expressed; and (b) recovering the multimeric protein.

[0096] In another example, the present disclosure provides a multimeric protein, comprising: (a) a first monomer comprising a VH1-CH1-hinge-CH2-CH3 monomer, wherein VH1 is a first variable heavy domain and CH2-CH3 is a first variant IgG Fc domain; (b) a second monomer comprising a VL1-CL1 monomer, wherein VL1 is a first variable light domain, and wherein the first variable heavy domain and the first variable light domain form a first antigen binding domain; (c) a third monomer comprising a VH2-CH1-hinge-CH2-CH3 monomer, wherein VH2 is a second variable heavy domain and CH2-CH3 is a second variant IgG Fc domain; and (d) a fourth monomer comprising a VL2-CL2 monomer, wherein VL2 is a second variable light domain, and wherein the second variable heavy domain and the second variable light domain form a second antigen binding domain, wherein (i) the VH:VL interface of the first monomer and the second monomer comprises a first set of VH:VL electrostatic variants comprising amino acid substitutions at amino acid residues Q39:Q38,

and the VH:VL interface of the third monomer and the fourth monomer comprises a second set of VH:VL electrostatic variants comprising amino acid substitutions at amino acid residues Q39:Q38, wherein numbering is according to Kabat numbering, (ii) the CH1:CL interface of the first monomer and the second monomer and/or the CH1:CL interface of the third monomer and the fourth monomer comprises one or more amino acid substitutions, wherein the one or more amino acid substitutions comprises (1) a set of CH1:CL electrostatic variants, or (2) a set of CH1:CL steric variants, wherein numbering is according to EU numbering, and (iii) the multimeric protein has antigen binding affinity substantially equivalent to a corresponding multimeric protein lacking the amino acid substitutions at the VH:VL interfaces and the CH1:CL interface(s).

[0097] In a further example and in accordance with the above, the CH1:CL interface of the first monomer and the second monomer does not comprise the one or more amino acid substitutions.

[0098] In a further example and in accordance with the above, the CH1:CL interface of the third monomer and the fourth monomer does not comprise the one or more amino acid substitutions.

[0099] In a further example and in accordance with any of the above, the CH1:CL interface comprises a set of CH1:CL electrostatic variants.

[0100] In a further example and in accordance with the above, the set of CH1:CL electrostatic variants comprises amino acid substitutions at amino acid residues K213/K218:D122/E123, wherein numbering is according to EU numbering.

[0101] In a further example and in accordance with any of the above, the set of CH1:CL electrostatic variants comprises amino acid substitutions selected from a group including: (i) K213E/K218D:D122K/E123K, (ii) K213E/K218E:D122K/E123K, (iii) K213D/K218E:D122K/E123K, (iv) K213D/K218D:D122K/E123K, (v) K213E/K218D:D122K/E123R, (vi) K213E/K218E:D122K/E123R, (vii) K213D/K218E:D122K/E123R, (viii) K213D/K218D:D122K/E123R, (ix) K213E/K218D:D122R/E123K, (x) K213E/K218E:D122R/E123K, (xi) K213D/K218E:D122R/E123K, (xii) K213D/K218D:D122R/E123K, (xiii) K213E/K218D:D122R/E123R, (xiv) K213E/K218E:D122R/E123R, (xv) K213D/K218E:D122R/E123R, and (xvi) K213D/K218D:D122R/E123R, wherein numbering is according to EU numbering.

[0102] In a further example and in accordance with any of the above, the set of CH1:CL electrostatic variants comprises amino acid substitutions K213E/K218D:D122K/E123K, wherein numbering is according to EU numbering.

[0103] In a further example and in accordance with any of the above, the CH1:CL interface comprises a set of CH1:CL steric variants.

[0104] In a further example and in accordance with the above, the set of CH1:CL steric variants comprises amino acid substitutions at amino acid residues selected from a group including: (i) A141:F118, (ii) A141:F116, and (iii) K147:S131, wherein numbering is according to EU numbering.

[0105] In a further example and in accordance with any of the above, the set of CH1:CL steric variants comprises amino acid substitutions selected from a group including: (i) A141F:F118A, (ii) A141F:F116A, and (iii) K147S:S131K, wherein numbering is according to EU numbering.

[0106] In a further example and in accordance with any of the above, wherein: (i) the CH1:CL interface of the first monomer and the second monomer comprises a set of CH1:CL electrostatic variants; and (ii) the CH1:CL interface of the third monomer and the fourth monomer comprises a set of CH1:CL steric variants.

[0107] In a further example and in accordance with any of the above, wherein: (i) the CH1:CL interface of the first monomer and the second monomer comprises a set of CH1:CL steric variants; and (ii) the CH1:CL interface of the third monomer and the fourth monomer comprises a set of CH1:CL electrostatic variants.

[0108] In a further example and in accordance with any of the above, wherein: (i) the set of CH1:CL electrostatic variants comprises amino acid substitutions at amino acid residues K213/K218:D122/E123, wherein numbering is according to EU numbering; and (ii) the set of CH1:CL steric variants comprises amino acid substitutions at amino acid residues selected from a group including: (i) A141:F118, (ii) A141:F116, and (iii) K147:S131, wherein numbering is according to EU numbering.

[0109] In a further example and in accordance with any of the above, wherein: (i) the set of CH1:CL electrostatic variants comprises amino acid substitutions selected from a group including: (i) K213E/K218D:D122K/E123K, (ii) K213E/K218E:D122K/E123K, (iii) K213D/K218E:D122K/E123K, (iv) K213D/K218D:D122K/E123K, (v) K213E/K218D:D122K/E123R, (vi) K213E/K218E:D122K/E123R, (vii) K213D/K218E:D122K/E123R, (viii) K213D/K218D:D122K/E123R, (ix)

K213E/K218D:D122R/E123K, (x) K213E/K218E:D122R/E123K, (xi) K213D/K218E:D122R/E123K, (xii) K213D/K218D:D122R/E123K, (xiii) K213E/K218D:D122R/E123R, (xiv) K213E/K218E:D122R/E123R, (xv) K213D/K218E:D122R/E123R, and (xvi) K213D/K218D:D122R/E123R, wherein numbering is according to EU numbering; and (ii) the set of CH1:CL steric variants comprises amino acid substitutions selected from a group including: (i) A141F:F118A, (ii) A141F:F116A, and (iii) K147S:S131K, wherein numbering is according to EU numbering.

[0110] In a further example and in accordance with any of the above, the set of CH1:CL electrostatic variants comprises amino acid substitutions K213E/K218D:D122K/E123K, wherein numbering is according to EU numbering.

[0111] In a further example and in accordance with any of the above, the first set of VH:VL electrostatic variants and the second set of VH:VL electrostatic variants comprise amino acid substitutions selected from a group including: (i) Q39E:Q38K and Q39K:Q38E, respectively, (ii) Q39E:Q38K and Q39K:Q38D, respectively, (iii) Q39E:Q38K and Q39R:Q38E, respectively, (iv) Q39E:Q38K and Q39R:Q38D, respectively, (v) Q39E:Q38K and Q39E:Q38K, respectively, (vi) Q39E:Q38K and Q39E:Q38R, respectively, (vii) Q39E:Q38K and Q39D:Q38K, respectively, (viii) Q39E:Q38K and Q39D:Q38R, respectively, (ix) Q39E:Q38R and Q39K:Q38E, respectively, (x) Q39E:Q38R and Q39K:Q38D, respectively, (xi) Q39E:Q38R and Q39R:Q38E, respectively, (xii) Q39E:Q38R and Q39R:Q38D, respectively, (xiii) Q39E:Q38R and Q39E:Q38K, respectively, (xiv) Q39E:Q38R and Q39E:Q38R, respectively, (xv) Q39E:Q38R and Q39D:Q38K, respectively, (xvi) Q39E:Q38R and Q39D:Q38R, respectively, (xvii) Q39D:Q38K and Q39K:Q38E, respectively, (xviii) Q39D:Q38K and Q39K:Q38D, respectively, (xix) Q39D:Q38K and Q39R:Q38E, respectively, (xx) Q39D:Q38K and Q39R:Q38D, respectively, (xxi) Q39D:Q38K and Q39E:Q38K, respectively, (xxii) Q39D:Q38K and Q39E:Q38R, respectively, (xxiii) Q39D:Q38K and Q39D:Q38K, respectively, (xxiv) Q39D:Q38K and Q39D:Q38R, respectively, (xxv) Q39D:Q38R and Q39K:Q38E, respectively, (xxvi) Q39D:Q38R and Q39K:Q38D, respectively, (xxvii) Q39D:Q38R and Q39R:Q38E, respectively, (xxviii) Q39D:Q38R and Q39R:Q38D, respectively, (xxix) Q39D:Q38R and Q39E:Q38K, respectively, (xxx) Q39D:Q38R and Q39E:Q38R, respectively, (xxxi) Q39D:Q38R and Q39D:Q38K, respectively, (xxxii) Q39D:Q38R and Q39D:Q38R, respectively, (xxxiii) Q39K:Q38E and Q39K:Q38E, respectively, (xxxiv) Q39K:Q38E and Q39K:Q38D, respectively, (xxxv) Q39K:Q38E and Q39R:Q38E, respectively, (xxxvi)

Q39K:Q38E and Q39R:Q38D, respectively, (xxxvii) Q39K:Q38E and Q39E:Q38K, respectively, (xxxviii) Q39K:Q38E and Q39E:Q38R, respectively, (xxxix) Q39K:Q38E and Q39D:Q38K, respectively, (xl) Q39K:Q38E and Q39D:Q38R, respectively, (xli) Q39R:Q38E and Q39K:Q38E, respectively, (xlii) Q39R:Q38E and Q39K:Q38D, respectively, (xliii) Q39R:Q38E and Q39R:Q38E, respectively, (xliv) Q39R:Q38E and Q39R:Q38D, respectively, (xlv) Q39R:Q38E and Q39E:Q38K, respectively, (xlvi) Q39R:Q38E and Q39E:Q38R, respectively, (xlvii) Q39R:Q38E and Q39D:Q38K, respectively, (xlviii) Q39R:Q38E and Q39D:Q38R, respectively, (xlix) Q39K:Q38D and Q39K:Q38E, respectively, (l) Q39K:Q38D and Q39K:Q38D, respectively, (li) Q39K:Q38D and Q39R:Q38E, respectively, (lii) Q39K:Q38D and Q39R:Q38D, respectively, (liii) Q39K:Q38D and Q39E:Q38K, respectively, (liv) Q39K:Q38D and Q39E:Q38R, respectively, (lv) Q39K:Q38D and Q39D:Q38K, respectively, (lvi) Q39K:Q38D and Q39D:Q38R, respectively, (lvii) Q39R:Q38D and Q39K:Q38E, respectively, (lviii) Q39R:Q38D and Q39K:Q38D, respectively, (lix) Q39R:Q38D and Q39R:Q38E, respectively, (lx) Q39R:Q38D and Q39R:Q38D, respectively, (lxi) Q39R:Q38D and Q39E:Q38K, respectively, (lxii) Q39R:Q38D and Q39E:Q38R, respectively, (lxiii) Q39R:Q38D and Q39D:Q38K, respectively, and (lxiv) Q39R:Q38D and Q39D:Q38R, respectively, wherein numbering is according to Kabat numbering.

[0112] In a further example and in accordance with the above, the first set of VH:VL electrostatic variants comprises amino acid substitutions Q39E:Q38K, and the second set of VH:VL electrostatic variants comprises amino acid substitutions Q39K:Q38E, wherein numbering is according to Kabat numbering.

[0113] In a further example and in accordance with the above, the first set of VH:VL electrostatic variants comprises amino acid substitutions Q39K:Q38E, and the second set of VH:VL electrostatic variants comprises amino acid substitutions Q39E:Q38K, wherein numbering is according to Kabat numbering.

[0114] In a further example and in accordance with any of the above, the first and/or second variant IgG Fc domains comprise one or more FcγRIIIA (CD16a) binding variant substitutions.

[0115] In a further example and in accordance with the above, the one or more FcγRIIIA (CD16a) binding variant substitutions are selected from a group including: (i) 236A, (ii) 239D, (iii) 239E, (iv) 243L, (v) 298A, (vi) 299T, (vii) 332E, (viii) 332D, (ix) 239D/332E, (x)

236A/332E, (xi) 239D/332E/330L, and (xii) 332E/330L, wherein numbering is according to EU numbering.

[0116] In a further example and in accordance with any of the above, the first and second variant IgG Fc domains comprise a set of FcγRIIIA (CD16a) binding variant substitutions selected from a group including: (i) S239D/I332E : S239D/I332E, (ii) S239D : S239D, (iii) I332E : I332E, (iv) WT : S239D/I332E, (v) WT : S239D, (vi) WT : I332E, (vii) S239D/I332E : WT, (viii) S239D : WT, (ix) I332E : WT, (x) S239D/I332E : S239D, (xi) S239D/I332E : I332E, (xii) S239D : S239D/I332E, (xiii) I332E : S239D/I332E, (xiv) S239D : I332E, and (xv) I332E : S239D, wherein numbering is according to EU numbering.

[0117] In a further example and in accordance with any of the above, the first and/or second variant IgG Fc domains comprise the FcγRIIIA (CD16a) binding variant substitutions of S239D/I332E, wherein numbering is according to EU numbering.

[0118] In a further example and in accordance with any of the above, the first and second variant IgG Fc domains comprise a set of heterodimerization variants selected from a group including those depicted in Figures 13A-13F, wherein numbering is according to EU numbering.

[0119] In a further example and in accordance with the above, the set of heterodimerization variants is selected from a group including: (i) S364K/E357Q : L368D/K370S, (ii) S364K : L368D/K370S, (iii) S364K : L368E/K370S, (iv) D401K : T411E/K360E/Q362E, and (v) T366W : T366S/L368A/Y407V, wherein numbering is according to EU numbering.

[0120] In a further example and in accordance with any of the above, the first and second variant IgG Fc domains further comprise one or more ablation variants.

[0121] In a further example and in accordance with the above, the one or more ablation variants are E233P/L234V/L235A/G236del/S267K, wherein numbering is according to EU numbering.

[0122] In a further example and in accordance with any of the above, one of the first or second variant IgG Fc domains comprise one or more pI variants.

[0123] In a further example and in accordance with the above, the one or more pI variants are N208D/Q295E/N384D/Q418E/N421D, wherein numbering is according to EU numbering.

[0124] In a further example and in accordance with any of the above, the first variant IgG Fc domain comprises amino acid variants

S364K/E357Q/E233P/L234V/L235A/G236del/S267K, wherein the second variant IgG Fc domain comprises amino acid variants L368D/K370S/N208D/Q295E/N384D/Q418E/N421D/E233P/L234V/L235A/G236del/S267K, and wherein numbering is according to EU numbering.

[0125] In a further example and in accordance with any of the above, the first and second variant IgG Fc domains each further comprise amino acid variants M428L/N434S or M428L/N434A, wherein numbering is according to EU numbering.

[0126] In a further example and in accordance with any of the above, the first variant IgG Fc domain is selected from a group including: (i) a first variant IgG1 Fc domain, (ii) a first variant IgG2 Fc domain, and (iii) a first variant IgG4 Fc domain.

[0127] In a further example and in accordance with any of the above, the second variant IgG Fc domain is selected from a group including: (i) a second variant IgG1 Fc domain, (ii) a second variant IgG2 Fc domain, and (iii) a second variant IgG4 Fc domain.

[0128] In another example, a nucleic acid composition comprising nucleic acids encoding the first monomer, the second monomer, the third monomer, and the fourth monomer of the multimeric protein (as described above) is provided.

[0129] In another example, an expression vector comprising the nucleic acids (as described above) is provided.

[0130] In another example, a host cell transformed with an expression vector (as described above) is provided.

[0131] In another example, a method of making a multimeric protein is provided, the method comprising: (a) culturing the host cell (as described above) under conditions wherein the multimeric protein is expressed; and (b) recovering the multimeric protein.

[0132] In another example, the present disclosure provides a multimeric protein, comprising: (a) a first monomer comprising a VH1-CH1-hinge-CH2-CH3 monomer, wherein VH1 is a first variable heavy domain and CH2-CH3 is a first variant IgG Fc domain; (b) a second monomer comprising a VL1-CL1 monomer, wherein VL1 is a first variable light domain, and wherein the first variable heavy domain and the first variable light domain form a first antigen binding domain; (c) a third monomer comprising a VH2-CH1-hinge-CH2-CH3 monomer, wherein VH2 is a second variable heavy domain and CH2-CH3 is a second variant IgG Fc domain; and (d) a fourth monomer comprising a VL2-CL2 monomer, wherein VL2 is a second variable light domain, and wherein the second variable heavy domain and the second variable light domain form a second antigen binding domain, wherein (i) the first

monomer and the second monomer comprise a first set of VH:VL electrostatic variants comprising amino acid substitutions Q39E:Q38K, and a set of CH1:CL electrostatic variants comprising amino acid substitutions K213E/K218D:D122K/E123K, (ii) the third monomer and the fourth monomer comprise a second set of VH:VL electrostatic variants comprising amino acid substitutions Q39K:Q38E, and a set of CH1:CL steric variants comprising amino acid substitutions A141F:F116A, (iii) numbering of the first set and second set of VH:VL electrostatic variants is according to Kabat numbering, and (iv) numbering of the set of CH1:CL electrostatic variants and the set of CH1:CL steric variants is according to EU numbering.

[0133] In another example, a nucleic acid composition comprising nucleic acids encoding the first monomer, the second monomer, the third monomer, and the fourth monomer of the multimeric protein (as described above) is provided.

[0134] In another example, an expression vector comprising the nucleic acids (as described above) is provided.

[0135] In another example, a host cell transformed with an expression vector (as described above) is provided.

[0136] In another example, a method of making a multimeric protein is provided, the method comprising: (a) culturing the host cell (as described above) under conditions wherein the multimeric protein is expressed; and (b) recovering the multimeric protein.

[0137] In another example, the present disclosure provides a multimeric protein, comprising: (a) a first monomer comprising a VH1-CH1-hinge-CH2-CH3 monomer, wherein VH1 is a first variable heavy domain and CH2-CH3 is a first variant IgG Fc domain; (b) a second monomer comprising a VL1-CL1 monomer, wherein VL1 is a first variable light domain, and wherein the first variable heavy domain and the first variable light domain form a first antigen binding domain; (c) a third monomer comprising a VH2-CH1-hinge-CH2-CH3 monomer, wherein VH2 is a second variable heavy domain and CH2-CH3 is a second variant IgG Fc domain; and (d) a fourth monomer comprising a VL2-CL2 monomer, wherein VL2 is a second variable light domain, and wherein the second variable heavy domain and the second variable light domain form a second antigen binding domain, wherein (i) the first monomer and the second monomer comprise a first set of VH:VL electrostatic variants comprising amino acid substitutions Q39E:Q38K, and a set of CH1:CL electrostatic variants comprising amino acid substitutions K213E/K218D:D122K/E123K, (ii) the third monomer and the fourth monomer comprise a second set of VH:VL electrostatic variants comprising

amino acid substitutions Q39K:Q38E, and a set of CH1:CL steric variants comprising amino acid substitutions A141F:F118A, (iii) numbering of the first set and second set of VH:VL electrostatic variants is according to Kabat numbering, and (iv) numbering of the set of CH1:CL electrostatic variants and the set of CH1:CL steric variants is according to EU numbering.

[0138] In another example, a nucleic acid composition comprising nucleic acids encoding the first monomer, the second monomer, the third monomer, and the fourth monomer of the multimeric protein (as described above) is provided.

[0139] In another example, an expression vector comprising the nucleic acids (as described above) is provided.

[0140] In another example, a host cell transformed with an expression vector (as described above) is provided.

[0141] In another example, a method of making a multimeric protein is provided, the method comprising: (a) culturing the host cell (as described above) under conditions wherein the multimeric protein is expressed; and (b) recovering the multimeric protein.

[0142] In another example, the present disclosure provides a multimeric protein, comprising: (a) a first monomer comprising a VH1-CH1-hinge-CH2-CH3 monomer, wherein VH1 is a first variable heavy domain and CH2-CH3 is a first variant IgG Fc domain; (b) a second monomer comprising a VL1-CL1 monomer, wherein VL1 is a first variable light domain, and wherein the first variable heavy domain and the first variable light domain form a first antigen binding domain; (c) a third monomer comprising a VH2-CH1-hinge-CH2-CH3 monomer, wherein VH2 is a second variable heavy domain and CH2-CH3 is a second variant IgG Fc domain; and (d) a fourth monomer comprising a VL2-CL2 monomer, wherein VL2 is a second variable light domain, and wherein the second variable heavy domain and the second variable light domain form a second antigen binding domain, wherein (i) the first monomer and the second monomer comprise a first set of VH:VL electrostatic variants comprising amino acid substitutions Q39E:Q38K, and a set of CH1:CL electrostatic variants comprising amino acid substitutions K213E/K218D:D122K/E123K, (ii) the third monomer and the fourth monomer comprise a second set of VH:VL electrostatic variants comprising amino acid substitutions Q39K:Q38E, and a set of CH1:CL steric variants comprising amino acid substitutions K147S:S131K, (iii) numbering of the first set and second set of VH:VL electrostatic variants is according to Kabat numbering, and (iv) numbering of the set of

CH1:CL electrostatic variants and the set of CH1:CL steric variants is according to EU numbering.

[0143] In another example, a nucleic acid composition comprising nucleic acids encoding the first monomer, the second monomer, the third monomer, and the fourth monomer of the multimeric protein (as described above) is provided.

[0144] In another example, an expression vector comprising the nucleic acids (as described above) is provided.

[0145] In another example, a host cell transformed with an expression vector (as described above) is provided.

[0146] In another example, a method of making a multimeric protein is provided, the method comprising: (a) culturing the host cell (as described above) under conditions wherein the multimeric protein is expressed; and (b) recovering the multimeric protein.

[0147] In another example, the present disclosure provides a multimeric protein, comprising: (a) a first monomer comprising a VH-CH1 domain, wherein VH is a variable heavy domain; and (b) a second monomer comprising a VL-CL domain, wherein VL is a variable light domain, wherein: (i) the variable heavy domain and the variable light domain form an antigen binding domain, and (ii) the first monomer and the second monomer comprise a set of CH1:CL electrostatic variants comprising amino acid substitutions at amino acid residues K213/K218:D122/E123, wherein numbering is according to EU numbering.

[0148] In a further example and in accordance with the above, the set of CH1:CL electrostatic variants comprises amino acid substitutions selected from a group including: (i) K213E/K218D:D122K/E123K, (ii) K213E/K218E:D122K/E123K, (iii) K213D/K218E:D122K/E123K, (iv) K213D/K218D:D122K/E123K, (v) K213E/K218D:D122K/E123R, (vi) K213E/K218E:D122K/E123R, (vii) K213D/K218E:D122K/E123R, (viii) K213D/K218D:D122K/E123R, (ix) K213E/K218D:D122R/E123K, (x) K213E/K218E:D122R/E123K, (xi) K213D/K218E:D122R/E123K, (xii) K213D/K218D:D122R/E123K, (xiii) K213E/K218D:D122R/E123R, (xiv) K213E/K218E:D122R/E123R, (xv) K213D/K218E:D122R/E123R, and (xvi) K213D/K218D:D122R/E123R, wherein numbering is according to EU numbering.

[0149] In a further example and in accordance with any of the above, the set of CH1:CL electrostatic variants comprises amino acid substitutions K213E/K218D:D122K/E123K, wherein numbering is according to EU numbering.

[0150] In a further example and in accordance with any of the above, further comprising a set of VH:VL electrostatic variants, wherein the set of VH:VL electrostatic variants comprises amino acid substitutions at amino acid residues Q39:Q38, wherein numbering is according to Kabat numbering.

[0151] In a further example and in accordance with the above, the set of VH:VL electrostatic variants comprise amino acid substitutions selected from a group including: (i) Q39E:Q38K, (ii) Q39E:Q38R, (iii) Q39D:Q38K, (iv) Q39D:Q38R, (v) Q39K:Q38E, (vi) Q39R:Q38E, (vii) Q39K:Q38D, and (viii) Q39R:Q38D, wherein numbering is according to Kabat numbering.

[0152] In a further example and in accordance with any of the above, the set of VH:VL electrostatic variants comprise amino acid substitutions Q39E:Q38K, wherein numbering is according to Kabat numbering.

[0153] In a further example and in accordance with any of the above, further comprising a set of CH1:CL steric variants, wherein the set of CH1:CL steric variants comprises amino acid substitutions at amino acid residues selected from a group including: (i) A141:F118, (ii) A141:F116, and (iii) K147:S131, wherein numbering is according to EU numbering.

[0154] In a further example and in accordance with the above, the set of CH1:CL steric variants comprises amino acid substitutions selected from a group including: (i) A141F:F118A, (ii) A141F:F116A, and (iii) K147S:S131K, wherein numbering is according to EU numbering.

[0155] In another example, a nucleic acid composition comprising nucleic acids encoding the first monomer and the second monomer of the multimeric protein (as described above) is provided.

[0156] In another example, an expression vector comprising the nucleic acids (as described above) is provided.

[0157] In another example, a host cell transformed with an expression vector (as described above) is provided.

[0158] In another example, a method of making a multimeric protein is provided, the method comprising: (a) culturing the host cell (as described above) under conditions wherein the multimeric protein is expressed; and (b) recovering the multimeric protein.

[0159] In another aspect, the present disclosure provides a multimeric protein, comprising: (a) a first monomer comprising a VH-CH1 domain, wherein VH is a variable heavy domain; and (b) a second monomer comprising a VL-CL domain, wherein VL is a variable light domain, wherein: (1) the variable heavy domain and the variable light domain form an antigen binding domain, and (2) the first monomer and the second monomer comprise a set of CH1:CL steric variants comprising amino acid substitutions at amino acid residues selected from the group consisting of: (i) A141:F118, (ii) A141:F116, and (iii) K147:S131, wherein numbering is according to EU numbering.

[0159a] In another example, the present disclosure provides a multimeric protein, comprising: (a) a first monomer comprising a VH-CH1 domain, wherein VH is a variable heavy domain; and (b) a second monomer comprising a VL-CL domain, wherein VL is a variable light

domain, wherein: (i) the variable heavy domain and the variable light domain form an antigen binding domain, and (ii) the first monomer and the second monomer comprise a set of CH1:CL steric variants comprising amino acid substitutions at amino acid residues selected from a group including: (i) A141:F118, (ii) A141:F116, and (iii) K147:S131, wherein numbering is according to EU numbering.

[0160] In a further example and in accordance with the above, the set of CH1:CL steric variants comprises amino acid substitutions selected from a group including: (i) A141F:F118A, (ii) A141F:F116A, and (iii) K147S:S131K, wherein numbering is according to EU numbering.

[0161] In a further example and in accordance with any of the above, further comprising a set of VH:VL electrostatic variants, wherein the set of VH:VL electrostatic variants comprises amino acid substitutions at amino acid residues Q39:Q38, wherein numbering is according to Kabat numbering.

[0162] In a further example and in accordance with the above, the set of VH:VL electrostatic variants comprise amino acid substitutions selected from a group including: (i) Q39E:Q38K, (ii) Q39E:Q38R, (iii) Q39D:Q38K, (iv) Q39D:Q38R, (v) Q39K:Q38E, (vi) Q39R:Q38E, (vii) Q39K:Q38D, and (viii) Q39R:Q38D, wherein numbering is according to Kabat numbering.

[0163] In a further example and in accordance with any of the above, the set of VH:VL electrostatic variants comprise amino acid substitutions Q39E:Q38K, wherein numbering is according to Kabat numbering.

[0164] In a further example and in accordance with any of the above, further comprising a set of CH1:CL electrostatic variants, wherein the set of CH1:CL electrostatic variants comprises amino acid substitutions at amino acid residues K213/K218:D122/E123, wherein numbering is according to EU numbering.

[0165] In a further example and in accordance with the above, the set of CH1:CL electrostatic variants comprises amino acid substitutions selected from a group including: (i) K213E/K218D:D122K/E123K, (ii) K213E/K218E:D122K/E123K, (iii) K213D/K218E:D122K/E123K, (iv) K213D/K218D:D122K/E123K, (v) K213E/K218D:D122K/E123R, (vi) K213E/K218E:D122K/E123R, (vii) K213D/K218E:D122K/E123R, (viii) K213D/K218D:D122K/E123R, (ix) K213E/K218D:D122R/E123K, (x) K213E/K218E:D122R/E123K, (xi) K213D/K218E:D122R/E123K, (xii) K213D/K218D:D122R/E123K, (xiii)

K213E/K218D:D122R/E123R, (xiv) K213E/K218E:D122R/E123R, (xv) K213D/K218E:D122R/E123R, and (xvi) K213D/K218D:D122R/E123R, wherein numbering is according to EU numbering.

[0166] In a further example and in accordance with any of the above, the set of CH1:CL electrostatic variants comprises amino acid substitutions K213E/K218D:D122K/E123K, wherein numbering is according to EU numbering.

[0167] In another example, a nucleic acid composition comprising nucleic acids encoding the first monomer and the second monomer of the multimeric protein (as described above) is provided.

[0168] In another example, an expression vector comprising the nucleic acids (as described above) is provided.

[0169] In another example, a host cell transformed with an expression vector (as described above) is provided.

[0170] In another example, a method of making a multimeric protein is provided, the method comprising: (a) culturing the host cell (as described above) under conditions wherein the multimeric protein is expressed; and (b) recovering the multimeric protein.

[0171] In another example, the present disclosure provides a multimeric protein, comprising: (a) a first monomer comprising a VH-CH1 domain, wherein VH is a variable heavy domain; and (b) a second monomer comprising a VL-CL domain, wherein VL is a variable light domain, wherein: (i) the variable heavy domain and the variable light domain form an antigen binding domain, and (ii) the first monomer and the second monomer comprise a set of VH:VL electrostatic variants comprising amino acid substitutions at amino acid residues Q39:Q38, wherein numbering is according to Kabat numbering.

[0172] In a further example and in accordance with the above, the set of VH:VL electrostatic variants comprise amino acid substitutions selected from a group including: (i) Q39E:Q38K, (ii) Q39E:Q38R, (iii) Q39D:Q38K, (iv) Q39D:Q38R, (v) Q39K:Q38E, (vi) Q39R:Q38E, (vii) Q39K:Q38D, and (viii) Q39R:Q38D, wherein numbering is according to Kabat numbering.

[0173] In a further example and in accordance with any of the above, the set of VH:VL electrostatic variants comprise amino acid substitutions Q39E:Q38K, wherein numbering is according to Kabat numbering.

[0174] In a further example and in accordance with any of the above, further comprising a set of CH1:CL electrostatic variants, wherein the set of CH1:CL electrostatic variants

comprises amino acid substitutions at amino acid residues K213/K218:D122/E123, wherein numbering is according to EU numbering.

[0175] In a further example and in accordance with the above, the set of CH1:CL electrostatic variants comprises amino acid substitutions selected from a group including: (i) K213E/K218D:D122K/E123K, (ii) K213E/K218E:D122K/E123K, (iii) K213D/K218E:D122K/E123K, (iv) K213D/K218D:D122K/E123K, (v) K213E/K218D:D122K/E123R, (vi) K213E/K218E:D122K/E123R, (vii) K213D/K218E:D122K/E123R, (viii) K213D/K218D:D122K/E123R, (ix) K213E/K218D:D122R/E123K, (x) K213E/K218E:D122R/E123K, (xi) K213D/K218E:D122R/E123K, (xii) K213D/K218D:D122R/E123K, (xiii) K213E/K218D:D122R/E123R, (xiv) K213E/K218E:D122R/E123R, (xv) K213D/K218E:D122R/E123R, and (xvi) K213D/K218D:D122R/E123R, wherein numbering is according to EU numbering.

[0176] In a further example and in accordance with any of the above, the set of CH1:CL electrostatic variants comprises amino acid substitutions K213E/K218D:D122K/E123K, wherein numbering is according to EU numbering.

[0177] In a further example and in accordance with any of the above, further comprising a set of CH1:CL steric variants, wherein the set of CH1:CL steric variants comprises amino acid substitutions at amino acid residues selected from a group including: (i) A141:F118, (ii) A141:F116, and (iii) K147:S131, wherein numbering is according to EU numbering.

[0178] In a further example and in accordance with the above, the set of CH1:CL steric variants comprises amino acid substitutions selected from a group including: (i) A141F:F118A, (ii) A141F:F116A, and (iii) K147S:S131K, wherein numbering is according to EU numbering.

[0179] In another example, a nucleic acid composition comprising nucleic acids encoding the first monomer and the second monomer of the multimeric protein (as described above) is provided.

[0180] In another example, an expression vector comprising the nucleic acids (as described above) is provided.

[0181] In another example, a host cell transformed with an expression vector (as described above) is provided.

[0182] In another example, a method of making a multimeric protein is provided, the method comprising: (a) culturing the host cell (as described above) under conditions wherein the multimeric protein is expressed; and (b) recovering the multimeric protein.

[0183] In another example, the present disclosure provides a multimeric protein, comprising: (a) a first monomer comprising a VH1-CH1-hinge-CH2-CH3 monomer, wherein VH1 is a first variable heavy domain and CH2-CH3 is a first variant IgG Fc domain; (b) a second monomer comprising a VL1-CL1 monomer, wherein VL1 is a first variable light domain, and wherein the first variable heavy domain and the first variable light domain form a first antigen binding domain; (c) a third monomer comprising a VH2-CH1-hinge-CH2-CH3 monomer, wherein VH2 is a second variable heavy domain and CH2-CH3 is a second variant IgG Fc domain; and (d) a fourth monomer comprising a VL2-CL2 monomer, wherein VL2 is a second variable light domain, and wherein the second variable heavy domain and the second variable light domain form a second antigen binding domain, wherein: (1) the first monomer and the second monomer comprise a set of CH1:CL electrostatic variants and the third monomer and the fourth monomer comprise a set of CH1:CL steric variants, or the first monomer and the second monomer comprise a set of CH1:CL steric variants and the third monomer and the fourth monomer comprise a set of CH1:CL electrostatic variants, (2) the set of CH1:CL electrostatic variants comprises a set of amino acid substitutions selected from the group including: (i) K213E/K218D:D122K/E123K, (ii) K213E/K218E:D122K/E123K, (iii) K213D/K218E:D122K/E123K, (iv) K213D/K218D:D122K/E123K, (v) K213E/K218D:D122K/E123R, (vi) K213E/K218E:D122K/E123R, (vii) K213D/K218E:D122K/E123R, (viii) K213D/K218D:D122K/E123R, (ix) K213E/K218D:D122R/E123K, (x) K213E/K218E:D122R/E123K, (xi) K213D/K218E:D122R/E123K, (xii) K213D/K218D:D122R/E123K, (xiii) K213E/K218D:D122R/E123R, (xiv) K213E/K218E:D122R/E123R, (xv) K213D/K218E:D122R/E123R, and (xvi) K213D/K218D:D122R/E123R, (3) the set of CH1:CL steric variants comprises a set of amino acid substitutions selected from the group including: (i) A141F:F118A, (ii) A141F:F116A, and (iii) K147S:S131K, (4) the first monomer and the second monomer comprise a first set of VH:VL electrostatic variants, and the third monomer and the fourth monomer comprise a second set of VH:VL electrostatic variants, (5) the first set of VH:VL electrostatic variants and the second set of VH:VL electrostatic variants comprise amino acid substitutions selected from the group including: (i) Q39E:Q38K and Q39K:Q38E, respectively, (ii) Q39K:Q38E and Q39E:Q38K, respectively,

(iii) Q39E:Q38K and Q39R:Q38E, respectively, (iv) Q39E:Q38K and Q39R:Q38D, respectively, (v) Q39E:Q38K and Q39E:Q38K, respectively, (vi) Q39E:Q38K and Q39E:Q38R, respectively, (vii) Q39E:Q38K and Q39D:Q38K, respectively, (viii) Q39E:Q38K and Q39D:Q38R, respectively, (ix) Q39E:Q38R and Q39K:Q38E, respectively, (x) Q39E:Q38R and Q39K:Q38D, respectively, (xi) Q39E:Q38R and Q39R:Q38E, respectively, (xii) Q39E:Q38R and Q39R:Q38D, respectively, (xiii) Q39E:Q38R and Q39E:Q38K, respectively, (xiv) Q39E:Q38R and Q39E:Q38R, respectively, (xv) Q39E:Q38R and Q39D:Q38K, respectively, (xvi) Q39E:Q38R and Q39D:Q38R, respectively, (xvii) Q39D:Q38K and Q39K:Q38E, respectively, (xviii) Q39D:Q38K and Q39K:Q38D, respectively, (xix) Q39D:Q38K and Q39R:Q38E, respectively, (xx) Q39D:Q38K and Q39R:Q38D, respectively, (xxi) Q39D:Q38K and Q39E:Q38K, respectively, (xxii) Q39D:Q38K and Q39E:Q38R, respectively, (xxiii) Q39D:Q38K and Q39D:Q38K, respectively, (xxiv) Q39D:Q38K and Q39D:Q38R, respectively, (xxv) Q39D:Q38R and Q39K:Q38E, respectively, (xxvi) Q39D:Q38R and Q39K:Q38D, respectively, (xxvii) Q39D:Q38R and Q39R:Q38E, respectively, (xxviii) Q39D:Q38R and Q39R:Q38D, respectively, (xxix) Q39D:Q38R and Q39E:Q38K, respectively, (xxx) Q39D:Q38R and Q39E:Q38R, respectively, (xxxi) Q39D:Q38R and Q39D:Q38K, respectively, (xxxii) Q39D:Q38R and Q39D:Q38R, respectively, (xxxiii) Q39K:Q38E and Q39K:Q38E, respectively, (xxxiv) Q39K:Q38E and Q39K:Q38D, respectively, (xxxv) Q39K:Q38E and Q39R:Q38E, respectively, (xxxvi) Q39K:Q38E and Q39R:Q38D, respectively, (xxxvii) Q39E:Q38K and Q39K:Q38D, respectively, (xxxviii) Q39K:Q38E and Q39E:Q38R, respectively, (xxxix) Q39K:Q38E and Q39D:Q38K, respectively, (xl) Q39K:Q38E and Q39D:Q38R, respectively, (xli) Q39R:Q38E and Q39K:Q38E, respectively, (xlii) Q39R:Q38E and Q39K:Q38D, respectively, (xliii) Q39R:Q38E and Q39R:Q38E, respectively, (xliv) Q39R:Q38E and Q39R:Q38D, respectively, (xlv) Q39R:Q38E and Q39E:Q38K, respectively, (xlvi) Q39R:Q38E and Q39E:Q38R, respectively, (xlvii) Q39R:Q38E and Q39D:Q38K, respectively, (xlviii) Q39R:Q38E and Q39D:Q38R, respectively, (xlix) Q39K:Q38D and Q39K:Q38E, respectively, (l) Q39K:Q38D and Q39K:Q38D, respectively, (li) Q39K:Q38D and Q39R:Q38E, respectively, (lii) Q39K:Q38D and Q39R:Q38D, respectively, (liii) Q39K:Q38D and Q39E:Q38K, respectively, (liv) Q39K:Q38D and Q39E:Q38R, respectively, (lv) Q39K:Q38D and Q39D:Q38K, respectively, (lvi) Q39K:Q38D and Q39D:Q38R, respectively, (lvii) Q39R:Q38D and Q39K:Q38E, respectively, (lviii) Q39R:Q38D and Q39K:Q38D, respectively, (lix) Q39R:Q38D and

Q39R:Q38E, respectively, (lx) Q39R:Q38D and Q39R:Q38D, respectively, (lxi) Q39R:Q38D and Q39E:Q38K, respectively, (lxii) Q39R:Q38D and Q39E:Q38R, respectively, (lxiii) Q39R:Q38D and Q39D:Q38K, respectively, and (lxiv) Q39R:Q38D and Q39D:Q38R, respectively, and (6) numbering of the set of CH1:CL electrostatic variants, numbering of the set of CH1:CL steric variants, numbering of the first set of VH:VL electrostatic variants, and numbering of the second set of VH:VL electrostatic variants are all according to EU numbering.

[0184] In a further example and in accordance with the above, the set of CH1:CL electrostatic variants comprises amino acid substitutions K213E/K218D:D122K/E123K.

[0185] In a further example and in accordance with any of the above, the set of CH1:CL electrostatic variants comprises amino acid substitutions A141F:F118A.

[0186] In a further example and in accordance with any of the above, the first set of VH:VL electrostatic variants comprises amino acid substitutions Q39E:Q38K, and the second set of VH:VL electrostatic variants comprises amino acid substitutions Q39K:Q38E. In other examples, the first set of VH:VL electrostatic variants comprises amino acid substitutions Q39K:Q38E, and the second set of VH:VL electrostatic variants comprises amino acid substitutions Q39E:Q38K.

[0187] In a further example and in accordance with any of the above, the first and/or second variant IgG Fc domains comprise one or more FcγRIIIA (CD16a) binding variant substitutions.

[0188] In a further example and in accordance with the above, the one or more FcγRIIIA (CD16a) binding variant substitutions are selected from the group including: (i) 236A, (ii) 239D, (iii) 239E, (iv) 243L, (v) 298A, (vi) 299T, (vii) 332E, (viii) 332D, (ix) 239D/332E, (x) 236A/332E, (xi) 239D/332E/330L, and (xii) 332E/330L, wherein numbering is according to EU numbering. In some further examples, the first and second variant IgG Fc domains comprise a set of FcγRIIIA (CD16a) binding variant substitutions selected from the group including: (i) S239D/I332E : S239D/I332E, (ii) S239D : S239D, (iii) I332E : I332E, (iv) WT : S239D/I332E, (v) WT : S239D, (vi) WT : I332E, (vii) S239D/I332E : WT, (viii) S239D : WT, (ix) I332E : WT, (x) S239D/I332E : S239D, (xi) S239D/I332E : I332E, (xii) S239D : S239D/I332E, (xiii) I332E : S239D/I332E, (xiv) S239D : I332E, and (xv) I332E : S239D, wherein numbering is according to EU numbering.

[0189] In a further example and in accordance with any of the above, the first and/or second variant IgG Fc domains comprise the FcγRIIIA (CD16a) binding variant substitutions of S239D/I332E, wherein numbering is according to EU numbering.

[0190] In a further example and in accordance with any of the above, the first and second variant IgG Fc domains comprise a set of heterodimerization variants selected from the group including those depicted in Figures 13A-13F, wherein numbering is according to EU numbering.

[0191] In a further example and in accordance with the above, the set of heterodimerization variants is selected from the group including: (i) S364K/E357Q : L368D/K370S, (ii) S364K : L368D/K370S, (iii) S364K : L368E/K370S, (iv) D401K : T411E/K360E/Q362E, and (v) T366W : T366S/L368A/Y407V, wherein numbering is according to EU numbering.

[0192] In a further example and in accordance with any of the above, the first and second variant IgG Fc domains further comprise one or more ablation variants.

[0193] In a further example and in accordance with the above, the one or more ablation variants are E233P/L234V/L235A/G236del/S267K, wherein numbering is according to EU numbering.

[0194] In a further example and in accordance with any of the above, one of the first or second variant IgG Fc domains comprise one or more pI variants.

[0195] In a further example and in accordance with the above, the one or more pI variants are N208D/Q295E/N384D/Q418E/N421D, wherein numbering is according to EU numbering.

[0196] In a further example and in accordance with any of the above, the first variant IgG Fc domain comprises amino acid variants S364K/E357Q/E233P/L234V/L235A/G236del/S267K, wherein the second variant IgG Fc domain comprises amino acid variants L368D/K370S/N208D/Q295E/N384D/Q418E/N421D/E233P/L234V/L235A/G236del/S267K, and wherein numbering is according to EU numbering.

[0197] In a further example and in accordance with any of the above, the first and second variant IgG Fc domains each further comprise amino acid variants M428L/N434S or M428L/N434A, wherein numbering is according to EU numbering.

[0198] In a further example and in accordance with any of the above, the first variant IgG Fc domain is selected from the group including: (i) a first variant IgG1 Fc domain, (ii) a first variant IgG2 Fc domain, and (iii) a first variant IgG4 Fc domain.

[0199] In a further example and in accordance with any of the above, the second variant IgG Fc domain is selected from the group including: (i) a second variant IgG1 Fc domain, (ii) a second variant IgG2 Fc domain, and (iii) a second variant IgG4 Fc domain.

[0200] In another example, a nucleic acid composition comprising nucleic acids encoding the first monomer, the second monomer, the third monomer, and the fourth monomer of the multimeric protein (as described above) is provided.

[0201] In another example, an expression vector comprising the nucleic acids (as described above) is provided.

[0202] In another example, a host cell transformed with an expression vector (as described above) is provided.

[0203] In another example, a method of making a multimeric protein is provided, the method comprising: (a) culturing the host cell (as described above) under conditions wherein the multimeric protein is expressed; and (b) recovering the multimeric protein.

[0204] In another example, the present disclosure provides a multimeric protein, comprising: (a) a variant human IgG1 CH1 domain (vCH1); and (b) a variant human kappa CL domain (vCL), wherein said CH1 domain and said CL domain together have a set of amino acid substitutions selected from the group including: (i) K213E/K218D:D122K/E123K, (ii) K213E/K218E:D122K/E123K, (iii) K213D/K218E:D122K/E123K, (iv) K213D/K218D:D122K/E123K, (v) K213E/K218D:D122K/E123R, (vi) K213E/K218E:D122K/E123R, (vii) K213D/K218E:D122K/E123R, (viii) K213D/K218D:D122K/E123R, (ix) K213E/K218D:D122R/E123K, (x) K213E/K218E:D122R/E123K, (xi) K213D/K218E:D122R/E123K, (xii) K213D/K218D:D122R/E123K, (xiii) K213E/K218D:D122R/E123R, (xiv) K213E/K218E:D122R/E123R, (xv) K213D/K218E:D122R/E123R, and (xvi) K213D/K218D:D122R/E123R, wherein numbering is according to EU numbering.

[0205] In another example, a nucleic acid composition comprising nucleic acids encoding the variant human IgG1 CH1 domain and the variant human kappa CL domain of the multimeric protein (as described above) is provided.

[0206] In another example, an expression vector comprising the nucleic acids (as described above) is provided.

[0207] In another example, a host cell transformed with an expression vector (as described above) is provided.

[0208] In another example, a method of making a multimeric protein is provided, the method comprising: (a) culturing the host cell (as described above) under conditions wherein the multimeric protein is expressed; and (b) recovering the multimeric protein.

[0209] In another example the present disclosure provides a multimeric protein, comprising: (a) a variant human IgG1 CH1 domain (vCH1); and (b) a variant human kappa CL domain (vCL), wherein: (1) said CH1 domain and said CL domain together have a first set of amino acid substitutions selected from the group including: (i) K213E/K218D:D122K/E123K, (ii) K213E/K218E:D122K/E123K, (iii) K213D/K218E:D122K/E123K, (iv) K213D/K218D:D122K/E123K, (v) K213E/K218D:D122K/E123R, (vi) K213E/K218E:D122K/E123R, (vii) K213D/K218E:D122K/E123R, (viii) K213D/K218D:D122K/E123R, (ix) K213E/K218D:D122R/E123K, (x) K213E/K218E:D122R/E123K, (xi) K213D/K218E:D122R/E123K, (xii) K213D/K218D:D122R/E123K, (xiii) K213E/K218D:D122R/E123R, (xiv) K213E/K218E:D122R/E123R, (xv) K213D/K218E:D122R/E123R, and (xvi) K213D/K218D:D122R/E123R, (2) said CH1 domain and said CL domain together have a second set of amino acid substitutions selected from the group including: (i) A141F:F118A, (ii) A141F:F116A, and (iii) K147S:S131K, and (3) numbering of said first set of amino acid substitutions and numbering of said second set of amino acid substitutions are both according to EU numbering.

[0210] In another example, a nucleic acid composition comprising nucleic acids encoding the variant human IgG1 CH1 domain and the variant human kappa CL domain of the multimeric protein (as described above) is provided.

[0211] In another example, an expression vector comprising the nucleic acids (as described above) is provided.

[0212] In another example, a host cell transformed with an expression vector (as described above) is provided.

[0213] In another example, a method of making a multimeric protein is provided, the method comprising: (a) culturing the host cell (as described above) under conditions wherein the multimeric protein is expressed; and (b) recovering the multimeric protein.

CLAIMS

WHAT IS CLAIMED IS:

1. A multimeric protein, comprising:

(a) a first monomer comprising a VH1-CH1-hinge-CH2-CH3 monomer, wherein VH1 is a first variable heavy domain and CH2-CH3 is a first variant IgG Fc domain;

(b) a second monomer comprising a VL1-CL1 monomer, wherein VL1 is a first variable light domain, and wherein the first variable heavy domain and the first variable light domain form a first antigen binding domain;

(c) a third monomer comprising a VH2-CH1-hinge-CH2-CH3 monomer, wherein VH2 is a second variable heavy domain and CH2-CH3 is a second variant IgG Fc domain; and

(d) a fourth monomer comprising a VL2-CL2 monomer, wherein VL2 is a second variable light domain, and wherein the second variable heavy domain and the second variable light domain form a second antigen binding domain,

wherein either the third monomer and the fourth monomer or the first monomer and the second monomer comprise a set of CH1:CL steric variants, wherein the set of CH1:CL steric variants comprises amino acid substitutions at amino acid residues selected from the group consisting of: (i) A141:F118, (ii) A141:F116, and (iii) K147:S131, wherein numbering is according to EU numbering.

2. The multimeric protein of claim 1, wherein the set of CH1:CL steric variants comprises amino acid substitutions selected from the group consisting of: (i) A141F:F118A, (ii) A141F:F116A, and (iii) K147S:S131K.

3. The multimeric protein of claim 2, wherein the set of CH1:CL steric variants comprises amino acid substitutions A141F:F118A.

4. The multimeric protein of claim 2, wherein the set of CH1:CL steric variants comprises amino acid substitutions K147S:S131K.

5. The multimeric protein of any one of claims 1-4, wherein:

(1) the third monomer and the fourth monomer comprise the set of CH1:CL steric variants and the first monomer and the second monomer do not comprise the set of CH1:CL steric variants; or

(2) the first monomer and the second monomer comprise the set of CH1:CL steric variants and the third monomer and the fourth monomer do not comprise the set of CH1:CL steric variants.

6. The multimeric protein of claim 1, wherein:

(1) (i) the first monomer and the second monomer comprise a set of CH1:CL electrostatic variants and the third monomer and the fourth monomer comprise the set of CH1:CL steric variants, or (ii) the first monomer and the second monomer comprise the set of CH1:CL steric variants and the third monomer and the fourth monomer comprise a set of CH1:CL electrostatic variants;

(2) the set of CH1:CL electrostatic variants comprises a set of amino acid substitutions selected from the group consisting of: (i) K213E/K218D:D122K/E123K, (ii) K213E/K218E:D122K/E123K, (iii) K213D/K218E:D122K/E123K, (iv) K213D/K218D:D122K/E123K, (v) K213E/K218D:D122K/E123R, (vi) K213E/K218E:D122K/E123R, (vii) K213D/K218E:D122K/E123R, (viii) K213D/K218D:D122K/E123R, (ix) K213E/K218D:D122R/E123K, (x) K213E/K218E:D122R/E123K, (xi) K213D/K218E:D122R/E123K, (xii) K213D/K218D:D122R/E123K, (xiii) K213E/K218D:D122R/E123R, (xiv) K213E/K218E:D122R/E123R, (xv) K213D/K218E:D122R/E123R, and (xvi) K213D/K218D:D122R/E123R;

(3) the set of CH1:CL steric variants comprises a set of amino acid substitutions selected from the group consisting of: (i) A141F:F118A, (ii) A141F:F116A, and (iii) K147S:S131K;

(4) the first monomer and the second monomer comprise a first set of VH:VL electrostatic variants, and the third monomer and the fourth monomer comprise a second set of VH:VL electrostatic variants;

(5) the first set of VH:VL electrostatic variants and the second set of VH:VL electrostatic variants comprise amino acid substitutions selected from the group consisting of: (i) Q39E:Q38K and Q39K:Q38E, respectively, (ii) Q39K:Q38E and

Q39E:Q38K, respectively; (iii) Q39E:Q38K and Q39R:Q38E, respectively, (iv) Q39E:Q38K and Q39R:Q38D, respectively, (v) Q39E:Q38K and Q39E:Q38K, respectively, (vi) Q39E:Q38K and Q39E:Q38R, respectively, (vii) Q39E:Q38K and Q39D:Q38K, respectively, (viii) Q39E:Q38K and Q39D:Q38R, respectively, (ix) Q39E:Q38R and Q39K:Q38E, respectively, (x) Q39E:Q38R and Q39K:Q38D, respectively, (xi) Q39E:Q38R and Q39R:Q38E, respectively, (xii) Q39E:Q38R and Q39R:Q38D, respectively, (xiii) Q39E:Q38R and Q39E:Q38K, respectively, (xiv) Q39E:Q38R and Q39E:Q38R, respectively, (xv) Q39E:Q38R and Q39D:Q38K, respectively, (xvi) Q39E:Q38R and Q39D:Q38R, respectively, (xvii) Q39D:Q38K and Q39K:Q38E, respectively, (xviii) Q39D:Q38K and Q39K:Q38D, respectively, (xix) Q39D:Q38K and Q39R:Q38E, respectively, (xx) Q39D:Q38K and Q39R:Q38D, respectively, (xxi) Q39D:Q38K and Q39E:Q38K, respectively, (xxii) Q39D:Q38K and Q39E:Q38R, respectively, (xxiii) Q39D:Q38K and Q39D:Q38K, respectively, (xxiv) Q39D:Q38K and Q39D:Q38R, respectively, (xxv) Q39D:Q38R and Q39K:Q38E, respectively, (xxvi) Q39D:Q38R and Q39K:Q38D, respectively, (xxvii) Q39D:Q38R and Q39R:Q38E, respectively, (xxviii) Q39D:Q38R and Q39R:Q38D, respectively, (xxix) Q39D:Q38R and Q39E:Q38K, respectively, (xxx) Q39D:Q38R and Q39E:Q38R, respectively, (xxxi) Q39D:Q38R and Q39D:Q38K, respectively, (xxxii) Q39D:Q38R and Q39D:Q38R, respectively, (xxxiii) Q39K:Q38E and Q39K:Q38E, respectively, (xxxiv) Q39K:Q38E and Q39K:Q38D, respectively, (xxxv) Q39K:Q38E and Q39R:Q38E, respectively, (xxxvi) Q39K:Q38E and Q39R:Q38D, respectively, (xxxvii) Q39E:Q38K and Q39K:Q38D, respectively, (xxxviii) Q39K:Q38E and Q39E:Q38R, respectively, (xxxix) Q39K:Q38E and Q39D:Q38K, respectively, (xl) Q39K:Q38E and Q39D:Q38R, respectively, (xli) Q39R:Q38E and Q39K:Q38E, respectively, (xlii) Q39R:Q38E and Q39K:Q38D, respectively, (xliii) Q39R:Q38E and Q39R:Q38E, respectively, (xliv) Q39R:Q38E and Q39R:Q38D, respectively, (xlv) Q39R:Q38E and Q39E:Q38K, respectively, (xlvi) Q39R:Q38E and Q39E:Q38R, respectively, (xlvii) Q39R:Q38E and Q39D:Q38K, respectively, (xlviii) Q39R:Q38E and Q39D:Q38R, respectively, (xlix) Q39K:Q38D and Q39K:Q38E, respectively, (l) Q39K:Q38D and Q39K:Q38D, respectively, (li) Q39K:Q38D and Q39R:Q38E, respectively, (lii) Q39K:Q38D and Q39R:Q38D, respectively, (liii) Q39K:Q38D and Q39E:Q38K, respectively, (liv) Q39K:Q38D and Q39E:Q38R, respectively, (lv) Q39K:Q38D and Q39D:Q38K, respectively, (lvi) Q39K:Q38D and Q39D:Q38R, respectively, (lvii) Q39R:Q38D and Q39K:Q38E, respectively, (lviii) Q39R:Q38D and Q39K:Q38D, respectively, (lix) Q39R:Q38D and

Q39R:Q38E, respectively, (lx) Q39R:Q38D and Q39R:Q38D, respectively, (lxi) Q39R:Q38D and Q39E:Q38K, respectively, (lxii) Q39R:Q38D and Q39E:Q38R, respectively, (lxiii) Q39R:Q38D and Q39D:Q38K, respectively, and (lxiv) Q39R:Q38D and Q39D:Q38R, respectively; and

(6) numbering of the set of CH1:CL electrostatic variants and the set of CH1:CL steric variants is according to EU numbering, and numbering of the first set of VH:VL electrostatic variants and the second set of VH:VL electrostatic variants is according to Kabat numbering.

7. The multimeric protein of claim 6, wherein the set of CH1:CL electrostatic variants comprises amino acid substitutions K213E/K218D:D122K/E123K.

8. The multimeric protein of claim 6 or 7, wherein the set of CH1:CL electrostatic variants comprises amino acid substitutions A141F:F118A.

9. The multimeric protein of claim 6 or 7, wherein the set of CH1:CL electrostatic variants comprises amino acid substitutions K147S:S131K.

10. The multimeric protein of any one of claims 6-9, wherein:

(1) the first set of VH:VL electrostatic variants comprises amino acid substitutions Q39E:Q38K, and the second set of VH:VL electrostatic variants comprises amino acid substitutions Q39K:Q38E; or

(2) the first set of VH:VL electrostatic variants comprises amino acid substitutions Q39K:Q38E, and the second set of VH:VL electrostatic variants comprises amino acid substitutions Q39E:Q38K.

11. The multimeric protein of any one of claims 1-10, wherein the first and/or second variant IgG Fc domains comprise one or more FcγRIIIA (CD16a) binding variant substitutions, wherein the one or more FcγRIIIA (CD16a) binding variant substitutions are selected from the group consisting of: (i) 236A, (ii) 239D, (iii) 239E, (iv) 243L, (v) 298A, (vi) 299T, (vii) 332E, (viii) 332D, (ix) 239D/332E, (x) 236A/332E, (xi) 239D/332E/330L, and (xii) 332E/330L, wherein numbering is according to EU numbering.

12. The multimeric protein of claim 11, wherein the first and second variant IgG Fc domains comprise a set of FcγRIIIA (CD16a) binding variant substitutions selected from the group consisting of: (i) S239D/I332E : S239D/I332E, (ii) S239D : S239D, (iii) I332E : I332E, (iv) WT : S239D/I332E, (v) WT : S239D, (vi) WT : I332E, (vii) S239D/I332E : WT, (viii) S239D : WT, (ix) I332E : WT, (x) S239D/I332E : S239D, (xi) S239D/I332E : I332E, (xii) S239D : S239D/I332E, (xiii) I332E : S239D/I332E, (xiv) S239D : I332E, and (xv) I332E : S239D, wherein numbering is according to EU numbering.

13. The multimeric protein of claim 11 or 12, wherein the first and/or second variant IgG Fc domains comprise the FcγRIIIA (CD16a) binding variant substitutions of S239D/I332E, wherein numbering is according to EU numbering.

14. The multimeric protein of any one of claims 1-13, wherein the first and second variant IgG Fc domains comprise a set of heterodimerization variants selected from the group consisting of: (i) L368D/K370S : S364K/E357Q, (ii) L368D/K370S : S364K, (iii) L368E/K370S : S364K, (iv) T411E/K360E/Q362E : D401K, and (v) T366S/L368A/Y407V : T366W, wherein numbering is according to EU numbering.

15. The multimeric protein of any one of claims 1-14, wherein the first and second variant IgG Fc domains further comprise one or more ablation variants, wherein the one or more ablation variants are E233P/L234V/L235A/G236del/S267K, wherein numbering is according to EU numbering.

16. The multimeric protein of any one of claims 1-15, wherein one of the first or second variant IgG Fc domains comprises one or more pI variants, wherein the one or more pI variants are N208D/Q295E/N384D/Q418E/N421D, wherein numbering is according to EU numbering.

17. The multimeric protein of any one of claims 1-16, wherein:

(1) the first variant IgG Fc domain comprises amino acid variants L368D/K370S/N208D/Q295E/N384D/Q418E/N421D/E233P/L234V/L235A/G236del/S267K, and the second variant IgG Fc domain comprises amino acid variants S364K/E357Q/E233P/L234V/L235A/G236del/S267K, wherein numbering is according to EU numbering; or

(2) the first variant IgG Fc domain comprises amino acid variants S364K/E357Q/E233P/L234V/L235A/G236del/S267K, and the second variant IgG Fc domain comprises amino acid variants L368D/K370S/N208D/Q295E/N384D/Q418E/N421D/E233P/L234V/L235A/G236del/S267K, wherein numbering is according to EU numbering.

18. The multimeric protein of any one of claims 1-17, wherein the first and second variant IgG Fc domains each further comprise amino acid variants M428L/N434S M428L/N434A, or M252Y/S243T/T256E, wherein numbering is according to EU numbering.

19. The multimeric protein of any one of claims 1-18, wherein:

(1) the first variant IgG Fc domain is selected from the group consisting of: (i) a first variant IgG1 Fc domain, (ii) a first variant IgG2 Fc domain, and (iii) a first variant IgG4 Fc domain; and

(2) the second variant IgG Fc domain is selected from the group consisting of: (i) a second variant IgG1 Fc domain, (ii) a second variant IgG2 Fc domain, and (iii) a second variant IgG4 Fc domain.

20. The multimeric protein of claim 1, wherein:

(1) the third monomer and the fourth monomer or the first monomer and the second monomer comprise the set of CH1:CL steric variants, wherein the set of CH1:CL steric variants comprises amino acid substitutions A141F:F118A;

(2) the first monomer and the second monomer comprise a set of CH1:CL electrostatic variants comprising amino acid substitutions K213E/K218D:D122K/E123K, wherein numbering is according to EU numbering; and

(3) the first monomer and the second monomer comprise a first set of VH:VL electrostatic variants comprising amino acid substitutions Q39E:Q38K, and the third monomer and the fourth monomer comprise a second set of VH:VL electrostatic variants comprising amino acid substitutions Q39K:Q38E, wherein numbering is according to Kabat numbering.

21. The multimeric protein of claim 1, wherein:

(1) the third monomer and the fourth monomer or the first monomer and the second monomer comprise the set of CH1:CL steric variants, wherein the set of CH1:CL steric variants comprises amino acid substitutions K147S:S131K;

(2) the first monomer and the second monomer comprise a set of CH1:CL electrostatic variants comprising amino acid substitutions K213E/K218D:D122K/E123K, wherein numbering is according to EU numbering; and

(3) the first monomer and the second monomer comprise a first set of VH:VL electrostatic variants comprising amino acid substitutions Q39E:Q38K, and the third monomer and the fourth monomer comprise a second set of VH:VL electrostatic variants comprising amino acid substitutions Q39K:Q38E, wherein numbering is according to Kabat numbering.

22. The multimeric protein of claim 1, wherein:

(1) the third monomer and the fourth monomer or the first monomer and the second monomer comprise the set of CH1:CL steric variants, wherein the set of CH1:CL steric variants comprises amino acid substitutions A141F:F118A;

(2) the first monomer and the second monomer comprise a set of CH1:CL electrostatic variants comprising amino acid substitutions K213E/K218D:D122K/E123K, wherein numbering is according to EU numbering;

(3) the first monomer and the second monomer comprise a first set of VH:VL electrostatic variants comprising amino acid substitutions Q39E:Q38K, and the third monomer and the fourth monomer comprise a second set of VH:VL electrostatic variants comprising amino acid substitutions Q39K:Q38E, wherein numbering is according to Kabat numbering;

(4) the first and second variant IgG Fc domains comprise a set of heterodimerization variants comprising amino acid substitutions L368D/K370S : S364K/E357Q, respectively, wherein numbering is according to EU numbering;

(5) the first and second variant IgG Fc domains each comprise one or more ablation variants, wherein the one or more ablation variants are E233P/L234V/L235A/G236del/S267K, wherein numbering is according to EU numbering;

(6) the first variant IgG Fc domain comprises one or more pI variants, wherein the one or more pI variants are N208D/Q295E/N384D/Q418E/N421D, wherein numbering is according to EU numbering; and

(7) the first and second variant IgG Fc domains each comprise amino acid variants M428L/N434S, wherein numbering is according to EU numbering.

23. The multimeric protein of claim 1, wherein:

(1) the third monomer and the fourth monomer or the first monomer and the second monomer comprise the set of CH1:CL steric variants, wherein the set of CH1:CL steric variants comprises amino acid substitutions K147S:S131K;

(2) the first monomer and the second monomer comprise a set of CH1:CL electrostatic variants comprising amino acid substitutions K213E/K218D:D122K/E123K, wherein numbering is according to EU numbering;

(3) the first monomer and the second monomer comprise a first set of VH:VL electrostatic variants comprising amino acid substitutions Q39E:Q38K, and the third monomer and the fourth monomer comprise a second set of VH:VL electrostatic variants comprising amino acid substitutions Q39K:Q38E, wherein numbering is according to Kabat numbering;

(4) the first and second variant IgG Fc domains comprise a set of heterodimerization variants comprising amino acid substitutions L368D/K370S : S364K/E357Q, respectively, wherein numbering is according to EU numbering;

(5) the first and second variant IgG Fc domains each comprise one or more ablation variants, wherein the one or more ablation variants are E233P/L234V/L235A/G236del/S267K, wherein numbering is according to EU numbering;

(6) the first variant IgG Fc domain comprises one or more pI variants, wherein the one or more pI variants are N208D/Q295E/N384D/Q418E/N421D, wherein numbering is according to EU numbering; and

(7) the first and second variant IgG Fc domains each comprise amino acid variants M428L/N434S, wherein numbering is according to EU numbering.

24. A nucleic acid composition comprising nucleic acids encoding the first, second, third, and fourth monomers of any one of claims 1-23.

25. The nucleic acid composition of claim 24, wherein the nucleic acids are in one or more expression vectors.

26. A host cell transformed with the one or more expression vectors of claim 25.

27. A method of making a multimeric protein, comprising:

(a) culturing the host cell of claim 26 under conditions wherein the multimeric protein is expressed; and

(b) recovering the multimeric protein.

28. A multimeric protein, comprising:

(a) a first monomer comprising a VH-CH1 domain, wherein VH is a variable heavy domain; and

(b) a second monomer comprising a VL-CL domain, wherein VL is a variable light domain, wherein:

(1) the variable heavy domain and the variable light domain form an antigen binding domain; and

(2) the first monomer and the second monomer comprise a set of CH1:CL steric variants comprising amino acid substitutions at amino acid residues selected from the group consisting of: (i) A141:F118, (ii) A141:F116, and (iii) K147:S131, wherein numbering is according to EU numbering.

29. The multimeric protein of claim 28, wherein the set of CH1:CL steric variants comprises amino acid substitutions selected from the group consisting of: (i) A141F:F118A, (ii) A141F:F116A, and (iii) K147S:S131K.

30. The multimeric protein of claim 29, wherein the set of CH1:CL steric variants comprises amino acid substitutions A141F:F118A.

31. The multimeric protein of claim 29, wherein the set of CH1:CL steric variants comprises amino acid substitutions K147S:S131K.

32. The multimeric protein of any one of claims 28-31, further comprising a set of VH:VL electrostatic variants, wherein the set of VH:VL electrostatic variants comprises amino acid substitutions at amino acid residues Q39:Q38, wherein numbering is according to Kabat numbering.

33. The multimeric protein of claim 32, wherein the set of VH:VL electrostatic variants comprise amino acid substitutions selected from the group consisting of: (i) Q39K:Q38E, (ii) Q39R:Q38E, (iii) Q39K:Q38D, (iv) Q39R:Q38D, (v) Q39E:Q38K, (vi) Q39E:Q38R, (vii) Q39D:Q38K, and (viii) Q39D:Q38R, , wherein numbering is according to Kabat numbering.

34. The multimeric protein of claim 32 or 33, wherein the set of VH:VL electrostatic variants comprise amino acid substitutions Q39K:Q38E, wherein numbering is according to Kabat numbering.

35. The multimeric protein of claim 28 or 29, wherein the set of CH1:CL steric variants comprises amino acid substitutions A141F:F118A, wherein numbering is according to EU numbering, and

wherein the multimeric protein further comprises a set of VH:VL electrostatic variants comprising amino acid substitutions Q39K:Q38E, wherein numbering is according to Kabat numbering.

36. The multimeric protein of claim 28 or 29, wherein the set of CH1:CL steric variants comprises amino acid substitutions K147S:S131K, wherein numbering is according to EU numbering, and

wherein the multimeric protein further comprises a set of VH:VL electrostatic variants comprising amino acid substitutions Q39K:Q38E, wherein numbering is according to Kabat numbering.

37. The multimeric protein of any one of claims 28-36, wherein the first monomer comprises a VH-CH1-hinge-CH2-CH3 monomer, and wherein the first monomer further comprises:

(1) one or more ablation variants, wherein the one or more ablation variants are E233P/L234V/L235A/G236del/S267K, wherein numbering is according to EU numbering; and/or

(2) amino acid variants M428L/N434S, wherein numbering is according to EU numbering.