(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 November 2015 (05.11.2015)

WIPO | PCT

(10) International Publication Number
WO 2015/168643 A2

(51) International Patent Classification:
*A61K 47/48* (2006.01)

(21) International Application Number:
PCT/US2015/028926

(22) International Filing Date:
1 May 2015 (01.05.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/987,863 2 May 2014 (02.05.2014) US
62/081,923 19 November 2014 (19.11.2014) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

**Declarations under Rule 4.17**:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

**Published**:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: COMPOSITIONS AND METHODS RELATED TO ENGINEERED FC CONSTRUCTS

(57) Abstract: The present invention relates to compositions and methods of engineered IgG Fc constructs, wherein said Fc constructs include one or more Fc domains.

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# COMPOSITIONS AND METHODS RELATED TO ENGINEERED Fc CONSTRUCTS

## BACKGROUND

Therapeutic proteins, e.g., therapeutic antibodies and Fc-fusion proteins, have rapidly become a clinically important drug class for patients with immunological and inflammatory diseases.

## SUMMARY OF THE INVENTION

The present invention features biologically active Fc domain-containing therapeutic constructs. Such constructs may have desirable serum half-life and/or binding affinity and/or avidity for Fc receptors. These constructs are useful, e.g., to reduce inflammation in a subject, to promote clearance of autoantibodies in a subject, to suppress antigen presentation in a subject, to block an immune response, e.g., block an immune complex-based activation of the immune response in a subject, and to treat immunological and inflammatory diseases (e.g., autoimmune diseases) in a subject. The Fc constructs described herein can be used to treat patients having immunological and inflammatory diseases without significant stimulation of immune cells.

In general, the invention features Fc constructs having 2-10 Fc domains, e.g., Fc constructs having 2, 3, 4, 5, 6, 7, 8, 9, or 10 Fc domains. In some embodiments, the Fc construct includes 2-10 Fc domains, 2-5 Fc domains, 3-5 Fc domains, 2-8 Fc domains, or 2-6 Fc domains. The construct may include 2-6 (e.g., 2, 3, 4, 5, or 6) associated polypeptides, each polypeptide including at least one Fc domain monomer, wherein each Fc domain monomer of the construct is the same or differs by no more than 20 amino acids (e.g., no more than 15, 10 amino acids), e.g., no more than 20, 15, 10, 8, 7, 6, 5, 4, 3 or 2 amino acids, from another monomer of the construct. The Fc constructs described herein do not include an antigen-binding domain of an immunoglobulin. In some embodiments, the Fc construct (or an Fc domain within an Fc construct) is formed entirely or in part by association of Fc domain monomers that are present in different polypeptides. In certain embodiments, the Fc construct does not include an additional domain (e.g., an IgM tailpiece or an IgA tailpiece) that promotes association of two polypeptides. In other embodiments, covalent linkages are present in the Fc construct only between two Fc domain monomers that join to form an Fc domain. In other embodiments, the Fc construct does not include covalent linkages between Fc domains. In still other embodiments, the Fc construct provides for sufficient structural flexibility such that all or substantially all of the Fc domains in the Fc construct are capable of simultaneously interacting with an Fc receptor on a cell surface. In some embodiments, the Fc construct includes at least two Fc domains joined through a linker (e.g., a flexible amino acid spacer). In one embodiment, the domain monomers are different in primary sequence from wild-type or from each other in that they have dimerization selectivity modules.

An Fc construct of the invention can be in a pharmaceutical composition that includes a substantially homogenous population (e.g., at least 85%, 90%, 95%, 98%, or 99% homogeneous) of the Fc construct having 2-10 Fc domains, e.g., a construct having 2, 3, 4, 5, 6, 7, 8, 9, or 10 Fc domains, such as those described herein. Consequently, pharmaceutical compositions can be produced that do not have substantial aggregation or unwanted multimerization of Fc constructs.

In one aspect, the Fc construct includes three polypeptides that form two Fc domains. The first polypeptide has the formula A-L-B, wherein A includes a first Fc domain monomer; L is a linker; and B includes a second Fc domain monomer. The second polypeptide includes a third Fc domain monomer, and the third polypeptide includes a fourth Fc domain monomer. In this aspect, the first Fc domain monomer and the third Fc domain monomer combine to form a first Fc domain. Similarly, the second Fc domain monomer and the fourth Fc domain monomer combine to form a second Fc domain. Exemplary Fc constructs of this aspect of the invention are illustrated in Figs. 4 and 6.

In certain embodiments, the first Fc domain monomer and the third Fc domain monomer include complementary dimerization selectivity modules that promote dimerization between these Fc domain monomers. In other embodiments, the second Fc domain monomer and the fourth Fc domain monomer include complementary dimerization selectivity modules that promote dimerization between these Fc domain monomers.

In certain embodiments, one or more of A, B, the second polypeptide, and the third polypeptide consists of an Fc domain monomer. In one embodiment, each of A, B, the second polypeptide, and the third polypeptide consist of an Fc domain monomer.

In certain embodiments, the Fc construct can further include a heterologous moiety, e.g., a peptide, e.g., a peptide that binds a serum protein, e.g., an albumin-binding peptide. The moiety may be joined to the N-terminus or the carboxy-terminus of B or the third polypeptide, e.g., by way of a linker.

In certain embodiments, the Fc construct further includes an IgG $C_L$ antibody constant domain and an IgG $C_H1$ antibody constant domain. The IgG $C_H1$ antibody constant domain can be attached to the N-terminus of A or the second polypeptide, e.g., by way of a linker.

In other embodiments, the second and third polypeptides of the Fc construct have the same amino acid sequence.

In another aspect, the invention features an Fc construct that includes four polypeptides that form three Fc domains. The first polypeptide has the formula A-L-B, wherein A includes a first Fc domain monomer; L is a linker; and B includes a second Fc domain monomer. The second polypeptide has the formula A'-L'-B', wherein A' includes a third Fc domain monomer; L' is a linker; and B' includes a fourth Fc domain monomer. The third polypeptide includes a fifth Fc domain monomer, and the fourth polypeptide includes a sixth Fc domain monomer. In this aspect, A and A' combine to form a first Fc domain, B and fifth Fc domain monomer combine to form a second Fc domain, and B' and sixth Fc domain monomer combine to form a third Fc domain. An exemplary Fc construct of this aspect of the invention is illustrated in Fig. 5.

In certain embodiments, A and A' each include a dimerization selectivity module that promotes dimerization between these Fc domain monomers. In other embodiments, B and the fifth Fc domain monomer each include a dimerization selectivity module that promotes dimerization between these Fc domain monomers. In yet other embodiments, B' and the sixth Fc domain monomer each include a dimerization selectivity module that promotes dimerization between these Fc domain monomers.

In certain embodiments, one or more of A, B, A', B', the third polypeptide, and the fourth polypeptide consists of an Fc domain monomer. In one embodiment, each of A, B, A', B', the third polypeptide, and the fourth polypeptide consists of an Fc domain monomer.

In certain embodiments, the Fc construct further includes an IgG $C_L$ antibody constant domain and an IgG $C_H1$ antibody constant domain, wherein the IgG $C_L$ antibody constant domain is attached to the N-terminus of the IgG $C_H1$ antibody constant domain by way of a linker and the IgG $C_H1$ antibody constant domain is attached to the N-terminus of A, e.g., by way of a linker. In one embodiment, the Fc construct further includes a second IgG $C_L$ antibody constant domain and a second IgG $C_H1$ antibody constant domain, wherein the second IgG $C_L$ antibody constant domain is attached to the N-terminus of the second IgG $C_H1$ antibody constant domain, e.g., by way of a linker and the second IgG $C_H1$ antibody constant domain is attached to the N-terminus of A', e.g., by way of a linker.

In certain embodiments, the Fc construct further includes a heterologous moiety, e.g., a peptide, e.g., an albumin-binding peptide joined to the N-terminus or C-terminus of B or B', e.g., by way of a linker.

In other embodiments, the first and second polypeptides of the Fc construct have the same amino acid sequence and the third and fourth polypeptides of the Fc construct have the same amino acid sequence.

In another aspect, the invention features an Fc construct that includes two polypeptides. The first polypeptide has the formula A-L-B, wherein A includes a first Fc domain monomer; L is a linker; and B includes a serum protein-binding moiety, e.g., an albumin binding peptide. The second polypeptide includes a second Fc domain monomer. In this aspect, the first Fc domain monomer and the second Fc domain monomer combine to form an Fc domain.

In certain embodiments, the first Fc domain monomer and the second Fc domain monomer include complementary dimerization selectivity modules that promote dimerization between the first Fc domain monomer and the second Fc domain monomer.

In certain embodiments, A and the second polypeptide each consists of an Fc domain monomer.

In yet another aspect, the invention features an Fc construct that includes two polypeptides. The first polypeptide has the formula A-L1-B-L2-C, wherein A includes an IgG $C_L$ antibody constant domain; L1 and L2 are each a linker; B includes an IgG $C_H1$ antibody constant domain; and C includes a first Fc domain monomer. The second polypeptide has the formula A'-L1'-B'-L2'-C', wherein A' includes an IgG $C_L$ antibody constant domain; L1' and L2' are each a linker; B' includes an IgG $C_H1$ antibody constant domain; and C' includes a second Fc domain monomer. In this aspect, the first Fc domain monomer and the second Fc domain monomer combine to form an Fc domain. An exemplary Fc construct of this aspect of the invention is illustrated in Fig. 7A.

In certain embodiments, the first Fc domain monomer and the second Fc domain monomer include dimerization selectivity modules that promote dimerization between the first Fc domain monomer and the second Fc domain monomer.

In certain embodiments, C and C' each consist of an Fc domain monomer.

In certain embodiments, the Fc construct further includes a serum protein binding moiety, e.g., an albumin-binding peptide joined to the N-terminus or C-terminus of C or C' by way of a linker.

In yet another aspect, the invention features an Fc construct that includes four or more polypeptides. The first polypeptide has the formula A-L1-B-L2-C, wherein A includes an IgG $C_L$ antibody

constant domain; L1 and L2 are each a linker; B includes an IgG $C_H1$ antibody constant domain; and C includes a first Fc domain monomer. The second polypeptide has the formula A'-L1'-B'-L2'-C', wherein A' includes an IgG $C_L$ antibody constant domain; L1' and L2' are each a linker; B' includes an IgG $C_H1$ antibody constant domain; and C' includes a second Fc domain monomer. In this aspect, the first Fc domain monomer combines with a third Fc domain monomer to form a first Fc domain and the second Fc domain monomer combines with a fourth Fc domain monomer to form a second Fc domain. Additionally, the IgG $C_H1$ antibody constant domain of the first polypeptide combines with the IgG $C_L$ antibody constant domain of the second polypeptide and the IgG $C_H1$ antibody constant domain of the second polypeptide combines with the IgG $C_L$ antibody constant domain of the first polypeptide to form an Fc construct that includes two or more Fc domains. An exemplary Fc construct of this aspect of the invention is illustrated in Fig. 7B.

In another aspect, the invention features an Fc construct that includes two polypeptides. The first polypeptide includes a first Fc domain monomer and the second polypeptide includes a second Fc domain monomer. In this aspect, the first and second Fc domain monomers combine to form an Fc domain. An exemplary Fc construct of this aspect of the invention is illustrated in Fig. 1. Further in this aspect, the first Fc domain monomer and the second Fc domain monomer each include a dimerization selectivity module that promotes dimerization between the first Fc domain monomer and the second Fc domain monomer. Exemplary Fc constructs of this embodiment are illustrated in Figs. 2 and 3.

In certain embodiments, the first and second polypeptides each consist of an Fc domain monomer.

In certain embodiments, the Fc construct further includes a serum protein binding moiety, e.g., an albumin-binding peptide joined to the N-terminus or C-terminus of the first or second polypeptide, e.g., by way of a linker.

In another aspect, the invention features an Fc construct that includes two polypeptides. The first polypeptide has the formula A-L-B, wherein A includes a first Fc domain monomer; L is a linker; and B includes a second Fc domain monomer. The second polypeptide has the formula A'-L'-B', wherein A' includes a third Fc domain monomer; L' is a linker; and B' includes a fourth Fc domain monomer. In this aspect, the first and second Fc domain monomers each include an engineered cavity into their respective $C_H3$ antibody constant domains and the second and fourth Fc domain monomers each include an engineered protuberance into their respective $C_H3$ antibody constant domains, wherein the engineered cavity and the engineered protuberance are positioned to form a protuberance-into-cavity pair. Also in this aspect, the first Fc domain monomer and the third Fc domain monomer combine to form a first Fc domain and the second Fc domain monomer and the fourth Fc domain monomer combine to form a second Fc domain.

In certain embodiments, one or more of A, B, A', and B' consists of an Fc domain monomer. In one embodiment, each of A, B, A', and B' consists of an Fc domain monomer.

In certain embodiments, the Fc construct further includes a serum protein binding moiety, e.g., an albumin-binding peptide joined to the N-terminus or C-terminus of B or B', e.g., by way of a linker.

In certain embodiments, the Fc construct further includes an IgG $C_L$ antibody constant domain and an IgG $C_H1$ antibody constant domain, wherein the IgG $C_L$ antibody constant domain is attached to the N-terminus of the IgG $C_H1$ antibody constant domain, e.g., by way of a linker and the IgG $C_H1$ antibody constant domain is attached to the N-terminus of A by way of a linker. In one embodiment, the Fc construct further includes a second IgG $C_L$ antibody constant domain and a second IgG $C_H1$ antibody constant domain, wherein the second IgG $C_L$ antibody constant domain is attached to the N-terminus of the second IgG $C_H1$ antibody constant domain by way of a linker and the second IgG $C_H1$ antibody constant domain is attached to the N-terminus of A' by way of a linker.

In another aspect, the invention features an Fc construct consisting of a) a first polypeptide having the formula A-L-B; wherein A includes or consists of a first Fc domain monomer; L is a linker; and B includes or consists of a second Fc domain monomer; b) a second polypeptide having the formula A'-L'-B'; wherein A' includes or consists of a third Fc domain monomer; L' is a linker; and B' includes or consists of a fourth Fc domain monomer; c) a third polypeptide that includes or consists of a fifth Fc domain monomer; and d) a fourth polypeptide that includes or consists of a sixth Fc domain monomer. A of the first polypeptide and A' of the second polypeptide combine to form a first Fc domain; B of the first polypeptide and the fifth Fc domain monomer combine to form a second Fc domain; and B' of the second polypeptide and the sixth Fc domain monomer combine to form a third Fc domain. Each of the first and third Fc domain monomers includes complementary dimerization selectivity modules that promote dimerization between the first Fc domain monomer and the third Fc domain monomer, each of the second and fifth Fc domain monomers includes complementary dimerization selectivity modules that promote dimerization between the second Fc domain monomer and the fifth Fc domain monomer, and each of the fourth and sixth Fc domain monomers includes complementary dimerization selectivity modules that promote dimerization between the fourth Fc domain monomer and the sixth Fc domain monomer; wherein the Fc construct contains no more than three Fc domains.

In some embodiments of this aspect, either the first Fc domain monomer or the third Fc domain monomer includes a negatively-charged amino acid substitution, and the other Fc domain monomer includes a positively-charged amino acid substitution, either the second and fourth Fc domain monomers or the fifth and sixth Fc domain monomers include an engineered protuberance, and the other Fc domain monomers include an engineered cavity. In some embodiments, linker L1, L2, L1', and/or L2' is 3-200 amino acids in length. In some embodiments, linker L and/or L' consists of the sequence of any one of SEQ ID NOs: 1, 2, and 3.

In another aspect, the invention features an Fc construct consisting of a) a first polypeptide having the formula A-L1-B-L2-C; wherein A includes or consists of a first Fc domain monomer; L1 is a linker; B includes or consists of a second Fc domain monomer; L2 is a linker; and C includes or consists of a third Fc domain monomer; and b) a second polypeptide having the formula A'-L1'-B'-L2'-C'; wherein A' includes or consists of a fourth Fc domain monomer; L1' is a linker; B' includes or consists of a fifth Fc domain monomer; L2' is a linker; and C' includes or consists of a sixth Fc domain monomer; c) a third polypeptide that includes or consists of a seventh Fc domain monomer; d) a fourth polypeptide that includes or consists of a eighth Fc domain monomer; e) a fifth polypeptide that includes or consists of a

ninth Fc domain monomer; and f) a sixth polypeptide that includes or consists of a tenth Fc domain monomer. A of the first polypeptide and the seventh Fc domain monomer combine to form a first Fc domain; B of the first polypeptide and B' of the second polypeptide combine to form a second Fc domain; C of the first polypeptide and the eighth Fc domain monomer combine to form a third Fc domain, A' of the second polypeptide and the ninth Fc domain monomer combine to form a fourth Fc domain, and C' of the second polypeptide and the tenth Fc domain monomer combine to form a fifth Fc domain. Each of the first and seventh Fc domain monomers includes complementary dimerization selectivity modules that promote dimerization between the first Fc domain monomer and the seventh Fc domain monomer, each of the second and fifth Fc domain monomers includes complementary dimerization selectivity modules that promote dimerization between the second Fc domain monomer and the fifth Fc domain monomer, each of the third and eighth Fc domain monomers includes complementary dimerization selectivity modules that promote dimerization between the third Fc domain monomer and the eighth Fc domain monomer; each of the fourth and ninth Fc domain monomers includes complementary dimerization selectivity modules that promote dimerization between the fourth Fc domain monomer and the ninth Fc domain monomer; and each of the sixth and tenth Fc domain monomers includes complementary dimerization selectivity modules that promote dimerization between the sixth domain monomer and the tenth Fc domain monomer; wherein the Fc construct contains no more than five Fc domains.

In some embodiments of this aspect, each of the first, third, fourth, and sixth Fc domain monomers includes an engineered protuberance, the second Fc domain monomer includes a negatively-charged amino acid substitution, the fifth Fc domain monomer includes a positively-charged amino acid substitution, and each of the seventh, eighth, ninth, and tenth Fc domain monomers includes an engineered cavity. In some embodiments, linker L1, L2, L1', and/or L2' is 3-200 amino acids in length. In some embodiments, linker L1, L2, L1', and/or L2' consists of the sequence of any one of SEQ ID NOs: 1, 2, and 3.

In another aspect, the invention features an Fc construct consisting of a) a first polypeptide having the formula A-L1-B-L2-C; wherein A includes or consists of a first Fc domain monomer; L1 is a linker; B includes or consists of a second Fc domain monomer; L2 is a linker; and C includes or consists of a third Fc domain monomer; and b) a second polypeptide having the formula A'-L1'-B'-L2'-C'; wherein A' includes or consists of a fourth Fc domain monomer; L1' is a linker; B' includes or consists of a fifth Fc domain monomer; L2' is a linker; and C' includes or consists of a sixth Fc domain monomer; c) a third polypeptide that includes or consists of a seventh Fc domain monomer; d) a fourth polypeptide that includes or consists of a eighth Fc domain monomer; e) a fifth polypeptide that includes or consists of a ninth Fc domain monomer; f) a sixth polypeptide that includes or consists of a tenth Fc domain monomer. A of the first polypeptide and A' of the second polypeptide combine to form a first Fc domain; B of the first polypeptide and the seventh Fc domain monomer combine to form a second Fc domain; C of the first polypeptide and the eighth Fc domain monomer combine to form a third Fc domain, B' of the second polypeptide and the ninth Fc domain monomer combine to form a fourth Fc domain, and C' of the second polypeptide and the tenth Fc domain monomer combine to form a fifth Fc domain. Each of the first and fourth Fc domain monomers includes complementary dimerization selectivity modules that promote dimerization between the first Fc domain monomer and the fourth Fc domain monomer, each of the

second and seventh Fc domain monomers includes complementary dimerization selectivity modules that promote dimerization between the second Fc domain monomer and the seventh Fc domain monomer, each of the third and eighth Fc domain monomers includes complementary dimerization selectivity modules that promote dimerization between the third Fc domain monomer and the eighth Fc domain monomer; each of the fifth and ninth Fc domain monomers includes complementary dimerization selectivity modules that promote dimerization between the fifth Fc domain monomer and the ninth Fc domain monomer; and each of the sixth and tenth Fc domain monomers includes complementary dimerization selectivity modules that promote dimerization between the sixth domain monomer and the tenth Fc domain monomer; wherein the Fc construct contains no more than five Fc domains.

In some embodiments of this aspect, the first Fc domain monomer includes a negatively-charged amino acid substitution, the fourth Fc domain monomer includes a positively-charged amino acid substitution, each of the second, third, fifth, and sixth Fc domain monomers includes an engineered protuberance, and each of the seventh, eighth, ninth, and tenth Fc domain monomers includes an engineered cavity. In some embodiments, linker L1, L2, L1', and/or L2' is 3-200 amino acids in length. In some embodiments, linker L1, L2, L1', and/or L2' consists of the sequence of any one of SEQ ID NOs: 1, 2, and 3.

In another aspect, the invention features an Fc construct that includes one or more Fc domains, wherein the Fc construct is assembled from a single polypeptide sequence. The polypeptide has the formula A-L-B, wherein A includes a first Fc domain monomer; L is a linker (optionally a cleavable linker with, e.g., one, two or more cleavage sites); and B includes a second Fc domain monomer. The linker can be an amino acid spacer of sufficient length (e.g., at least 15 amino acids, preferably at least about 20 amino acid residues in length, e.g., 15-200 amino acids in length) and flexibility that the first Fc domain monomer and the second Fc domain monomer of the polypeptide combine to form an Fc domain. In certain embodiments, the first Fc domain monomer and the second Fc domain monomer include complementary dimerization selectivity modules that promote dimerization between the first Fc domain monomer and the second Fc domain monomer. Such a construct can be formed from expression of a single polypeptide sequence in a host cell. In one embodiment, the polypeptide has the formula A-L1-B-L2-C, wherein A includes a first Fc domain monomer; L1 is a linker (optionally a cleavable linker with, e.g., one, two, or more cleavage sites); B includes a second Fc domain monomer; L2 is a linker; and C is a third Fc domain monomer. The linker can be an amino acid spacer of sufficient length (e.g., at least 15 amino acids, preferably at least about 20 amino acid residues in length, e.g., 15-200 amino acids in length) and flexibility that the first Fc domain monomer and the second Fc domain monomer of the polypeptide combine to form an Fc domain. In certain embodiments, the first Fc domain monomer and the second Fc domain monomer include complementary dimerization selectivity modules that promote dimerization between the first Fc domain monomer and the second Fc domain monomer. An example of an Fc construct of this embodiment, including three Fc domains, is depicted in Fig. 10.

In any of the Fc constructs described herein, the Fc domain monomers of an Fc domain of the construct can have the same primary amino acid sequence. For example, the Fc domain monomers of an Fc domain may both be a wild-type sequence, or both Fc domain monomers of an Fc domain may

have the same dimerization selectivity module, e.g., both Fc domain monomers of an Fc domain may have identical reverse charge mutations in at least two positions within the ring of charged residues at the interface between $C_H3$ domains.

In any of the Fc constructs described herein, the Fc domain monomers of an Fc domain of a construct can have different sequences, e.g., sequences that differ by no more than 20 amino acids (e.g., no more than 15, 10 amino acids), e.g., no more than 20, 15, 10, 8, 7, 6, 5, 4, 3 or 2 amino acids, between two Fc monomers (i.e., between the Fc domain monomer and another monomer of the Fc construct). For example, Fc monomer sequences of a construct described herein may be different because complementary dimerization selectivity modules of any of the Fc constructs can include an engineered cavity in the $C_H3$ antibody constant domain of one of the domain monomers and an engineered protuberance in the $C_H3$ antibody constant domain of the other of the Fc domain monomers, wherein the engineered cavity and the engineered protuberance are positioned to form a protuberance-into-cavity pair of Fc domain monomers. Exemplary engineered cavities and protuberances are shown in Table 1. In other embodiments, the complementary dimerization selectivity modules include an engineered (substituted) negatively-charged amino acid in the $C_H3$ antibody constant domain of one of the domain monomers and an engineered (substituted) positively-charged amino acid in the $C_H3$ antibody constant domain of the other of the Fc domain monomers, wherein the negatively-charged amino acid and the positively-charged amino acid are positioned to promote formation of an Fc domain between complementary domain monomers. Exemplary complementary amino acid changes are shown in Table 2.

In some embodiments, in addition to the dimerization selectivity modules (e.g., the engineered cavities and protuberances, or the engineered positively and negatively-charged amino acids (see, e.g., exemplary amino acid changes in Tables 1 and 2)), an Fc construct described herein may also include additional amino acid substitutions from a wild type sequence in the Fc monomer sequences to, e.g., help to stabilize the Fc construct or to prevent protein aggregation.

In some embodiments, an Fc construct described herein includes 2-10 Fc domains (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10 domains), wherein at least two of the Fc domains of the construct have different dimerization selectivity modules. For example, constructs 5, 8, 9 and 10 have at least one Fc domain including engineered cavity and protuberance and at least one Fc domain including complementary reverse charge mutations.

In other embodiments, one or more linker in an Fc construct described herein is a bond.

In other embodiments, one or more linker in an Fc construct described herein is a spacer, e.g., an amino acid spacer of 2-200 amino acids.

In certain embodiments, the amino acid spacer is a glycine and/or serine rich spacer, e.g., the spacer includes two or more motifs of the sequence GS, GGS, GGGGS (SEQ ID NO: 1), GGSG (SEQ ID NO: 2), or SGGG (SEQ ID NO: 3).

In certain embodiments, when an Fc construct includes an albumin-binding peptide, the albumin-binding peptide has the sequence of DICLPRWGCLW (SEQ ID NO: 28).

In other embodiments, one or more of the Fc domain monomers in the Fc constructs described herein includes an IgG hinge domain, an IgG $C_H2$ antibody constant domain, and an IgG $C_H3$ antibody constant domain.

In certain embodiments, each of the Fc domain monomers in the foregoing Fc constructs includes an IgG hinge domain, an IgG $C_H2$ antibody constant domain, and an IgG $C_H3$ antibody constant domain.

In certain embodiments, the IgG is of a subtype selected from the group consisting of IgG1, IgG2a, IgG2b, IgG3, and IgG4.

In yet another aspect, the invention features a pharmaceutical composition that includes a substantially homogenous (e.g., at least 85%, 90%, 95%, 97%, 98%, 99% homogeneous) population of any Fc construct described herein. In one embodiment, a sterile syringe or vial qualified for pharmaceutical use contains a pharmaceutical composition wherein the only or primary active ingredient is a substantially homogenous (e.g., at least 85%, 90%, 95%, 98%, or 99% homogeneous) population of any one of the Fc constructs described herein. The pharmaceutical composition may include one or more inactive ingredients, e.g., selected from salts, detergents, surfactants, bulking agents, polymers, preservatives, and other pharmaceutical excipients, In another embodiment, the substantially homogenous pharmaceutical composition contains less than 10%, less than 5%, less than 4%, less than 3%, less than 2%, less than 1%, or less than 0.5% aggregates or unwanted multimers of the Fc construct.

In another aspect, the invention features a method of preparing any one of the foregoing Fc constructs. The method includes providing a host cell including a polynucleotide or polynucleotides encoding the polypeptides needed to assemble the Fc construct, expressing polypeptides in the host cell under conditions that allow for the formation of the Fc construct, and recovering (e.g., purifying) the Fc construct.

In some embodiments, the Fc construct is formed at least in part by association of Fc domain monomers that are present in different polypeptides. In certain embodiments, the Fc construct is formed by association of Fc domain monomers that are present in different polypeptides. In these embodiments, the Fc construct does not include an additional domain that promotes association of two polypeptides (e.g., an IgM tailpiece or an IgA tailpiece). In other embodiments, covalent linkages (e.g., disulfide bridges) are present only between two Fc domain monomers that join to form an Fc domain. In other embodiments, the Fc construct does not include covalent linkages (e.g., disulfide bridges) between Fc domains. In still other embodiments, the Fc construct provides for sufficient structural flexibility such that all or substantially all of the Fc domains in the Fc construct are capable of simultaneously interacting with an Fc receptor on a cell surface. In certain examples of any of these embodiments, the Fc construct includes at least two Fc domains joined through a linker (e.g., a flexible amino acid spacer).

In one embodiment, the Fc domain monomers of an Fc domain are found in different polypeptide chains that associate to form the Fc domain. For example, the constructs depicted in Fig. 4 and Fig. 6 have two Fc domains including three associated polypeptides. One of the three polypeptides includes two Fc domain monomers and the other two of the polypeptides each includes one Fc domain monomer. The construct depicted in Fig. 5 has three Fc domains including four associated polypeptides; two of the four polypeptides have two Fc domain monomers and the other two of the four polypeptides each has one Fc domain monomer. The Fc construct depicted in Fig. 7B can have n Fc domains (where n is 2-10)

including 2n polypeptides, each polypeptide including an Fc domain monomer, an IgG $C_L$ antibody constant domain, and an IgG $C_H1$ antibody constant domain. The constructs depicted in Figs. 8 and 9 each has five Fc domains including six associated polypeptides. Two of the six polypeptides have three Fc domain monomers and the other four of the six polypeptides each has one Fc domain monomer. The construct depicted in Fig 10. has three Fc domains including two associated polypeptides. Each of the two polypeptides contains three Fc domain monomers joined in a tandem series.

In another aspect, the invention features compositions and methods for promoting selective dimerization of Fc domain monomers. The invention includes an Fc domain wherein the two Fc domain monomers of the Fc domain include identical mutations in at least two positions within the ring of charged residues at the interface between $C_H3$ antibody constant domains. The invention also includes a method of making such an Fc domain, including introducing complementary dimerization selectivity modules having identical mutations in two Fc domain monomer sequences in at least two positions within the ring of charged residues at the interface between $C_H3$ antibody constant domains. The interface between $C_H3$ antibody constant domains consists of a hydrophobic patch surrounded by a ring of charged residues. When one $C_H3$ antibody constant domain comes together with another, these charged residues pair with residues of the opposite charge. By reversing the charge of both members of two or more complementary pairs of residues, mutated Fc domain monomers remain complementary to Fc domain monomers of the same mutated sequence, but have a lower complementarity to Fc domain monomers without those mutations. In this embodiment, the identical dimerization selectivity modules promotes homodimerization. Exemplary Fc domains include Fc monomers containing the double mutants K409D/D339K, K392D/D399K, E357K/K370E, D356K/K439D, K409E/D339K, K392E/D399K, E357K/K370D, or D356K/K439E. In another embodiment, an Fc domain includes Fc monomers including quadruple mutants combining any pair of the double mutants, e.g., K409D/D399K/E357K/K370E. In another embodiment, in addition to the identical dimerization selectivity modules, the Fc domain monomers of the Fc domain include complementary dimerization selectivity modules having non-identical mutations that promote specific association (e.g., engineered cavity and protuberance). As a result, the two Fc domain monomers include two dimerization selectivity modules and remain complementary to each other, but have a decreased complementarity to other Fc domain monomers. This embodiment promotes heterodimerization between a cavity-containing Fc domain and a protuberance-containing Fc domain monomer. In one example, the identical mutations in charged pair residues of both Fc domain monomers are combined with a protuberance on one Fc domain monomer and a cavity on the other Fc domain monomer.

In another aspect, the invention features a method of reducing inflammation in a subject in need thereof. In another aspect, the invention features a method of promoting clearance of autoantibodies in a subject in need thereof. In another aspect, the invention features a method of suppressing antigen presentation in a subject in need thereof. In another aspect, the invention features a method of reducing the immune response in a subject in need thereof, e.g., reducing immune complex-based activation of the immune response in a subject in need thereof. These methods include administering to the subject an Fc construct or pharmaceutical composition described herein.

In another aspect, the invention features a method of treating an inflammatory or autoimmune or immune disease in a subject by administering to the subject an Fc construct or pharmaceutical composition described herein (e.g., any one of constructs 1-10 and 5*). Exemplary diseases include: rheumatoid arthritis (RA); systemic lupus erythematosus (SLE); ANCA-associated vasculitis; antiphospholipid antibody syndrome; autoimmune hemolytic anemia; chronic inflammatory demyelinating neuropathy; clearance of anti-allo in transplant, anti-self in GVHD, anti-replacement, IgG therapeutics, IgG paraproteins; dermatomyositis; Goodpasture's Syndrome; organ system-targeted type II hypersensitivity syndromes mediated through antibody-dependent cell-mediated cytotoxicity, e.g., Guillain Barre syndrome, CIDP, dermatomyositis, Felty's syndrome, antibody-mediated rejection, autoimmune thyroid disease, ulcerative colitis, autoimmune liver disease; idiopathic thrombocytopenia purpura; Myasthenia Gravis, neuromyelitis optica; pemphigus and other autoimmune blistering disorders; Sjogren's Syndrome; autoimmune cytopenias and other disorders mediated through antibody-dependent phagocytosis; other FcR-dependent inflammatory syndromes, e.g., synovitis, dermatomyositis, systemic vasculitis, glomerulitis and vasculitis.

In another aspect, the invention features an Fc construct or pharmaceutical composition described herein (e.g., any one of constructs 1-10 and 5*) for use in reducing inflammation in a subject in need thereof. In another aspect, the invention features an Fc construct or pharmaceutical composition described herein (e.g., any one of constructs 1-10 and 5*) for use in promoting clearance of autoantibodies in a subject in need thereof. In another aspect, the invention features an Fc construct or pharmaceutical composition described herein (e.g., any one of constructs 1-10 and 5*) for use in suppressing antigen presentation in a subject in need thereof. In another aspect, the invention features an Fc construct or pharmaceutical composition described herein (e.g., any one of constructs 1-10 and 5*) for use in reducing the immune response in a subject in need thereof, e.g., reducing immune complex-based activation of the immune response in a subject in need thereof.

In another aspect, the invention features an Fc construct or pharmaceutical composition described herein (e.g., any one of constructs 1-10 and 5*) for use in treating an inflammatory or autoimmune or immune disease in a subject. Exemplary diseases include: rheumatoid arthritis (RA); systemic lupus erythematosus (SLE); ANCA-associated vasculitis; antiphospholipid antibody syndrome; autoimmune hemolytic anemia; chronic inflammatory demyelinating neuropathy; clearance of anti-allo in transplant, anti-self in GVHD, anti-replacement, IgG therapeutics, IgG paraproteins; dermatomyositis; Goodpasture's Syndrome; organ system-targeted type II hypersensitivity syndromes mediated through antibody-dependent cell-mediated cytotoxicity, e.g., Guillain Barre syndrome, CIDP, dermatomyositis, Felty's syndrome, antibody-mediated rejection, autoimmune thyroid disease, ulcerative colitis, autoimmune liver disease; idiopathic thrombocytopenia purpura; Myasthenia Gravis, neuromyelitis optica; pemphigus and other autoimmune blistering disorders; Sjogren's Syndrome; autoimmune cytopenias and other disorders mediated through antibody-dependent phagocytosis; other FcR-dependent inflammatory syndromes, e.g., synovitis, dermatomyositis, systemic vasculitis, glomerulitis and vasculitis.

In any of the Fc constructs described herein, it is understood that the order of the Fc domain monomers is interchangeable. For example, in a polypeptide having the formula A-L-B, the carboxy terminus of A can be joined to the amino terminus of L, which in turn is joined at its carboxy terminus to the amino terminus of B. Alternatively, the carboxy terminus of B can be joined to the amino terminus of L, which in turn is joined at its carboxy terminus to the amino terminus of C. Both of these configurations are encompassed by the formula A-L-B.

In a related aspect, the invention features a host cell that expresses any one of the foregoing Fc constructs. The host cell includes polynucleotides encoding the polypeptides needed to assemble the Fc construct, wherein the polynucleotides are expressed in the host cell.

Definitions:

As used herein, the term "Fc domain monomer" refers to a polypeptide chain that includes at least a hinge domain and second and third antibody constant domains ($C_H2$ and $C_H3$) or functional fragments thereof (e.g., fragments that that capable of (i) dimerizing with another Fc domain monomer to form an Fc domain, and (ii) binding to an Fc receptor. The Fc domain monomer can be any immunoglobulin antibody isotype, including IgG, IgE, IgM, IgA, or IgD. Additionally, the Fc domain monomer can be an IgG subtype (e.g., IgG1, IgG2a, IgG2b, IgG3, or IgG4). An Fc domain monomer does not include any portion of an immunoglobulin that is capable of acting as an antigen-recognition region, e.g., a variable domain or a complementarity determining region (CDR). Fc domain monomers can contain as many as ten changes from a wild-type Fc domain monomer sequence (e.g., 1-10, 1-8, 1-6, 1-4 amino acid substitutions, additions, or deletions) that alter the interaction between an Fc domain and an Fc receptor. Examples of suitable changes are known in the art.

As used herein, the term "Fc domain" refers to a dimer of two Fc domain monomers that is capable of binding an Fc receptor. In the wild-type Fc domain, the two Fc domain monomers dimerize by the interaction between the two $C_H3$ antibody constant domains, as well as one or more disulfide bonds that form between the hinge domains of the two dimerizing Fc domain monomers.

In the present invention, the term "Fc construct" refers to associated polypeptide chains forming between 2-10 Fc domains as described herein. Fc constructs described herein can include Fc domain monomers that have the same or different sequences. For example, an Fc construct can have two Fc domains, one of which includes IgG1 or IgG1-derived Fc domain monomers, and a second which includes IgG2 or IgG2-derived Fc domain monomers. In another example, an Fc construct can have two Fc domains, one of which comprises a "protuberance-into-cavity pair" and a second which does not comprise a "protuberance-into-cavity pair." In the present invention, an Fc domain does not include a variable region of an antibody, e.g., $V_H$, $V_L$, CDR, or HVR. An Fc domain forms the minimum structure that binds to an Fc receptor, e.g., FcγRI, FcγRIIa, FcγRIIb, FcγRIIIa, FcγRIIIb, FcγRIV.

As used herein, the term "antibody constant domain" refers to a polypeptide that corresponds to a constant region domain of an antibody (e.g., a $C_L$ antibody constant domain, a $C_H1$ antibody constant domain, a $C_H2$ antibody constant domain, or a $C_H3$ antibody constant domain).

As used herein, the term "promote" means to encourage and to favor, e.g., to favor the formation of an Fc domain from two Fc domain monomers which have higher binding affinity for each other than for other, distinct Fc domain monomers. As is described herein, two Fc domain monomers that combine to

form an Fc domain can have compatible amino acid modifications (e.g., engineered protuberances and engineered cavities) at the interface of their respective $C_H3$ antibody constant domains. The compatible amino acid modifications promote or favor the selective interaction of such Fc domain monomers with each other relative to with other Fc domain monomers which lack such amino acid modifications or with incompatible amino acid modifications. This occurs because, due to the amino acid modifications at the interface of the two interacting $C_H3$ antibody constant domains, the Fc domain monomers to have a higher affinity toward each other than to other Fc domain monomers lacking amino acid modifications.

As used herein, the term "a dimerization selectivity module" refers to a sequence of the Fc domain monomer that facilitates the favored pairing between two Fc domain monomers. "Complementary" dimerization selectivity modules are dimerization selectivity modules that promote or favor the selective interaction of two Fc domain monomers with each other. Complementary dimerization selectivity modules can have the same or different sequences. Exemplary complementary dimerization selectivity modules are described herein.

As used herein, the term "engineered cavity" refers to the substitution of at least one of the original amino acid residues in the $C_H3$ antibody constant domain with a different amino acid residue having a smaller side chain volume than the original amino acid residue, thus creating a three dimensional cavity in the $C_H3$ antibody constant domain. The term "original amino acid residue" refers to a naturally occurring amino acid residue encoded by the genetic code of a wild-type $C_H3$ antibody constant domain.

As used herein, the term "engineered protuberance" refers to the substitution of at least one of the original amino acid residues in the $C_H3$ antibody constant domain with a different amino acid residue having a larger side chain volume than the original amino acid residue, thus creating a three dimensional protuberance in the $C_H3$ antibody constant domain. The term "original amino acid residues" refers to naturally occurring amino acid residues encoded by the genetic code of a wild-type $C_H3$ antibody constant domain.

As used herein, the term "protuberance-into-cavity pair" describes an Fc domain including two Fc domain monomers, wherein the first Fc domain monomer includes an engineered cavity in its $C_H3$ antibody constant domain, while the second Fc domain monomer includes an engineered protuberance in its $C_H3$ antibody constant domain. In a protuberance-into-cavity pair, the engineered protuberance in the $C_H3$ antibody constant domain of the first Fc domain monomer is positioned such that it interacts with the engineered cavity of the $C_H3$ antibody constant domain of the second Fc domain monomer without significantly perturbing the normal association of the dimer at the inter-$C_H3$ antibody constant domain interface.

As used herein, the term "joined" is used to describe the combination or attachment of two or more elements, components, or protein domains, e.g., polypeptides, by means including chemical conjugation, recombinant means, and chemical bonds, e.g., disulfide bonds and amide bonds. For example, two single polypeptides can be joined to form one contiguous protein structure through chemical conjugation, a chemical bond, a peptide linker, or any other means of covalent linkage. In some embodiments, a first Fc domain monomer is joined to a second Fc domain monomer by way of a peptide linker, wherein the N-terminus of the peptide linker is joined to the C-terminus of the first Fc domain monomer through a chemical bond, e.g., a peptide bond, and the C-terminus of the peptide linker is

joined to the N-terminus of the second Fc domain monomer through a chemical bond, e.g., a peptide bond. In other embodiments, the N-terminus of an albumin-binding peptide is joined to the C-terminus of the $C_H3$ antibody constant domain of an Fc domain monomer by way of a linker in the same fashion as mentioned above.

As used herein, the term "associated" is used to describe the interaction, e.g., hydrogen bonding, hydrophobic interaction, or ionic interaction, between polypeptides (or sequences within one single polypeptide) such that the polypeptides (or sequences within one single polypeptide) are positioned to form an Fc construct that has at least one Fc domain. For example, two polypeptides, each including one Fc domain monomer, can associate to form an Fc construct (e.g., as depicted in Figs. 1-3). In some embodiments, three polypeptides, e.g., one polypeptide including two Fc domain monomers and two polypeptides each including one Fc domain monomer, associate to form an Fc construct that has two Fc domains (e.g., as is shown in Figs. 4 and 6). In some embodiments, four polypeptides, e.g., two polypeptides each including two Fc domain monomers and two polypeptides each including one Fc domain monomer, associate to form an Fc construct that has three Fc domains (e.g., as depicted in Fig. 5). In other embodiments, 2n polypeptides, e.g., each polypeptide including an Fc domain monomer, an IgG $C_L$ antibody constant domain, and an IgG $C_H1$ antibody constant domain associate to form an Fc construct that has n Fc domains (as is depicted in Fig. 7B). The two polypeptides can associate through their respective Fc domain monomers, or through other components of the polypeptide. For example, in Fig. 7B, polypeptide 708 associates with polypeptide 706 through its Fc domain monomer and associates with polypeptide 710 through association of its $C_L$ domain associating with the $C_H1$ domain of polypeptide 710. The association between polypeptides does not include covalent interactions. For example, in Fig. 10, Fc monomer sequences 1014 and 1012 within a single polypeptide associate to form an Fc domain, as do Fc monomer sequences 1004 and 1006.

As used herein, the term "linker" refers to a linkage between two elements, e.g., protein domains. A linker can be a covalent bond or a spacer. The term "bond" refers to a chemical bond, e.g., an amide bond or a disulfide bond, or any kind of bond created from a chemical reaction, e.g., chemical conjugation. The term "spacer" refers to a moiety (e.g., a polyethylene glycol (PEG) polymer) or an amino acid sequence (e.g., a 3-200 amino acid, 3-150 amino acid, or 3-100 amino acid sequence) occurring between two polypeptides or polypeptide domains to provide space and/or flexibility between the two polypeptides or polypeptide domains. An amino acid spacer is part of the primary sequence of a polypeptide (e.g., joined to the spaced polypeptides or polypeptide domains via the polypeptide backbone). The formation of disulfide bonds, e.g., between two hinge regions or two Fc domain monomers that form an Fc domain, is not considered a linker.

As used herein, the term "cleavable linker" refers to a linker containing one or more elements that can be selectively cleaved, e.g., after a construct is formed, e.g., a cleavable linker includes a polypeptide sequence that can be selectively cleaved by a protease.

As used herein, the term "albumin-binding peptide" refers to an amino acid sequence of 12 to 16 amino acids that has affinity for and functions to bind serum albumin. An albumin-binding peptide can be of different origins, e.g., human, mouse, or rat. In some embodiments of the present invention, an albumin-binding peptide is fused to the C-terminus of an Fc domain monomer to increase the serum half-

life of the Fc construct. An albumin-binding peptide can be fused, either directly or through a linker, to the N- or C-terminus of an Fc domain monomer.

As used herein, the term "multimer" refers to a molecule including at least two associated Fc constructs described herein.

As used herein, the term "polynucleotide" refers to an oligonucleotide, or nucleotide, and fragments or portions thereof, and to DNA or RNA of genomic or synthetic origin, which may be single- or double-stranded, and represent the sense or anti-sense strand. A single polynucleotide is translated into a single polypeptide.

As used herein, the term "polypeptide" describes a single polymer in which the monomers are amino acid residues which are joined together through amide bonds. A polypeptide is intended to encompass any amino acid sequence, either naturally occurring, recombinant, or synthetically produced.

As used herein, the term "amino acid positions" refers to the position numbers of amino acids in a protein or protein domain. The amino acid positions for antibody or Fc constructs are numbered using the Kabat numbering system (Kabat et al., *Sequences of Proteins of Immunological Interest,* National Institutes of Health, Bethesda, Md., ed 5, 1991).

As used herein, the term "host cell" refers to a vehicle that includes the necessary cellular components, e.g., organelles, needed to express proteins from their corresponding nucleic acids. The nucleic acids are typically included in nucleic acid vectors that can be introduced into the host cell by conventional techniques known in the art (transformation, transfection, electroporation, calcium phosphate precipitation, direct microinjection, etc.). A host cell may be a prokaryotic cell, e.g., a bacterial cell, or a eukaryotic cell, e.g., a mammalian cell (e.g., a CHO cell). As described herein, a host cell is used to express one or more polypeptides encoding desired domains which can then combine to form a desired Fc construct.

As used herein, the term "pharmaceutical composition" refers to a medicinal or pharmaceutical formulation that contains an active ingredient as well as one or more excipients and diluents to enable the active ingredient suitable for the method of administration. The pharmaceutical composition of the present invention includes pharmaceutically acceptable components that are compatible with the Fc construct. The pharmaceutical composition is typically in aqueous form for intravenous or subcutaneous administration.

As used herein, a "substantially homogenous population" of polypeptides or of an Fc construct is one in which at least 85% of the polypeptides or Fc constructs in a composition (e.g., a pharmaceutical composition) have the same number of Fc domains and the same Fc domain structure. In various embodiments, at least 90%, 92%, 95%, 97%, 98%, 99%, or 99.5% of the polypeptides or Fc constructs in the composition are the same. Accordingly, a pharmaceutical composition comprising a substantially homogenous population of an Fc construct is one in which at least 85% of the Fc constructs in the composition have the same number of Fc domains and the same structure. A substantially homogenous population of an Fc construct does not include more than 10% (e.g., not more than 8%, 5%, 2%, or 1%) multimers or aggregates of the Fc construct.

As used herein, the term "pharmaceutically acceptable carrier" refers to an excipient or diluent in a pharmaceutical composition. The pharmaceutically acceptable carrier must be compatible with the other ingredients of the formulation and not deleterious to the recipient. In the present invention, the

pharmaceutically acceptable carrier must provide adequate pharmaceutical stability to the Fc construct. The nature of the carrier differs with the mode of administration. For example, for oral administration, a solid carrier is preferred; for intravenous administration, an aqueous solution carrier (e.g., WFI, and/or a buffered solution) is generally used.

As used herein, "therapeutically effective amount" refers to an amount, e.g., pharmaceutical dose, effective in inducing a desired biological effect in a subject or patient or in treating a patient having a condition or disorder described herein. It is also to be understood herein that a "therapeutically effective amount" may be interpreted as an amount giving a desired therapeutic effect, either taken in one dose or in any dosage or route, taken alone or in combination with other therapeutic agents.

## DESCRIPTION OF THE DRAWINGS

Fig.1 is an illustration of an Fc construct (construct 1) containing a dimer of two wild-type (wt) Fc domain monomers (102 and 104).

Fig. 2 is an illustration of an Fc construct (construct 2) containing a dimer of two Fc domain monomers. The first Fc domain monomer (202) contains a protuberance in its $C_H3$ antibody constant domain, while the second Fc domain monomer (204) contains a cavity in the juxtaposed position in its $C_H3$ antibody constant domain.

Fig. 3 is an illustration of another Fc construct (construct 3). This Fc construct contains a dimer of two Fc domain monomers (302 and 304), wherein both Fc domain monomers contain different charged amino acids at their $C_H3$-$C_H3$ interface than the wt sequence to promote favorable electrostatic interaction between the two Fc domain monomers.

Fig. 4 is an illustration of an Fc construct (construct 4) containing two Fc domains. This construct is formed from three polypeptides. The first polypeptide (402) contains two wt Fc domain monomers (404 and 406) joined in a tandem series. Each of the second and third polypeptides (408 and 410, respectively) contains a wt Fc domain monomer.

Fig. 5 is an illustration of an Fc construct (construct 5 or construct 5*) containing three Fc domains formed from four polypeptides. The first polypeptide (502) contains one Fc domain monomer containing different charged amino acids at the $C_H3$-$C_H3$ interface than the wt sequence (506) joined in a tandem series with a protuberance-containing Fc domain monomer (504). The second polypeptide (508) contains an Fc domain monomer containing different charged amino acids at the $C_H3$-$C_H3$ interface than the wt sequence (512) joined in a tandem series with another protuberance-containing Fc domain monomer (510). The third and fourth polypeptides (514 and 516, respectively) each contain a cavity-containing Fc domain monomer.

Fig. 6 is an illustration of an Fc construct (construct 6) containing two Fc domains formed from three polypeptides. The first polypeptide (602) contains two protuberance-containing Fc domain monomers (604 and 606) joined in a tandem series, while the second and third polypeptides (608 and 610, respectively) each contain an Fc domain monomer engineered to contain a corresponding cavity.

Fig. 7A is an illustration of another Fc construct (construct 7). This Fc construct contains a dimer of two $C_L$-$C_H1$-Fc domain monomers (702 and 704). In this embodiment, the $C_L$ antibody constant domains have joined to the adjacent $C_H1$ antibody constant domains.

Fig. 7B is an illustration of an Fc construct (construct 8) containing multimers of $C_L$-$C_H1$-Fc domain monomers (e.g., 706, 708, and 710) containing multiple Fc domains. In this Fc construct, the constituent polypeptide can be the same as the constituent polypeptide in construct 7. The $C_L$ antibody constant domain of one Fc construct (e.g., 712) interacts with the $C_H1$ antibody constant domain of a second, neighboring Fc construct (e.g., 714).

Fig. 8 is an illustration of an Fc construct (construct 9) containing five Fc domains formed from six polypeptides. The first and second polypeptides (802 and 810) each contain three Fc domain monomers (804, 806, 808, and 812, 814, 816, respectively) joined in a tandem series. Specifically, in polypeptide 802 or 810, a first protuberance-containing Fc domain monomer (804 or 812) is connected to a second Fc domain monomer containing different charged amino acids at the $C_H3$-$C_H3$ interface than the wt sequence (806 or 814), which is connected to a third protuberance-containing Fc domain monomer (808 or 816). The third through sixth polypeptides (818, 820, 822, and 824) each contain a cavity-containing Fc domain monomer and form an Fc domain with each of Fc domain monomers 804, 808, 812 and 816, respectively.

Fig. 9 is an illustration of an Fc construct (construct 10) containing five Fc domains formed from six polypeptides. The first and second polypeptides (902 and 910) each contain three Fc domain monomers (904, 906, 908, and 912, 914, 916, respectively) joined in a tandem series. Specifically, in polypeptide 902 or 910, a first protuberance-containing Fc domain monomer (904 or 912) is connected to a second protuberance-containing Fc domain monomer (906 or 914), which is connected to a third Fc domain monomer containing different charged amino acids at the $C_H3$-$C_H3$ interface than the wt sequence (908 or 916). The third through sixth polypeptides (918, 920, 922, and 924) each contain a cavity-containing Fc domain monomer and form an Fc domain with each of Fc domain monomers 904, 906, 912 and 914, respectively.

Fig. 10 is an illustration of an Fc construct (construct 11) containing three Fc domains formed from two polypeptides of identical sequence. The two polypeptides (1002 and 1010) each contain three Fc domain monomers (1004, 1006, 1008, and 1012, 1014, 1016, respectively) joined in a tandem series. Specifically, each polypeptide contains a first protuberance-containing Fc domain monomer (1004 or 1012) connected to a second cavity-containing Fc domain monomer (1006 or 1014), which is connected to a third Fc domain monomer with different charged amino acids at the $C_H3$-$C_H3$ interface than the wt sequence (1008, or 1016). Fc domain monomers 1008 and 1016 associate to form a first Fc domain; Fc domain monomers 1004 and 1006 associate to form a second Fc domain; and Fc domain monomers 1012 and 1014 associate to form a third Fc domain. Construct 11 can be formed from expression of a single polypeptide sequence in a host cell.

Figs. 11A-11B show reducing and non-reducing SDS-PAGE of construct 4, respectively.

Figs. 12A-12B show reducing and non-reducing SDS-PAGE of construct 6, respectively.

Fig. 13 is an SDS-PAGE of construct 5 and a table showing the percentages of the expressed protein having three Fc domains (trimer), two Fc domains (dimer), or one Fc domain (monomer) before and after construct 5 purification.

Figs. 14A and 14B show THP-1 monocyte activation (Fig. 14A) and blocking (Fig. 14B) assays using constructs 1, 5, and 6.

Fig. 15 shows effects of IVIG and constructs 5 and 6 in a K/BxN model of rheumatoid arthritis.

Fig. 16 shows effects of IVIG and constructs 5 and 6 in a chronic ITP model.

Fig. 17 shows inhibition of phagocytosis by IVIg or Construct 5 in THP-1 monocytic cells.

## DETAILED DESCRIPTION OF THE INVENTION

Therapeutic proteins that include Fc domains of IgG can be used to treat inflammation and immunological and inflammatory diseases. The present invention features compositions and methods for preparing various Fc constructs containing two or more (e.g., 2-10) Fc domains.

### I. Fc domain monomers

An Fc domain monomer includes a hinge domain, a $C_H2$ antibody constant domain, and a $C_H3$ antibody constant domain. The Fc domain monomer can be of immunoglobulin antibody isotype IgG, IgE, IgM, IgA, or IgD. The Fc domain monomer may also be of any immunoglobulin antibody isotype (e.g., IgG1, IgG2a, IgG2b, IgG3, or IgG4). A dimer of Fc domain monomers is an Fc domain (further defined herein) that can bind to an Fc receptor, e.g., FcγRIIIa, which is a receptor located on the surface of leukocytes. In the present invention, the $C_H3$ antibody constant domain of an Fc domain monomer may contain amino acid substitutions at the interface of the $C_H3$-$C_H3$ antibody constant domains to promote their association with each other. In some embodiments, an Fc domain monomer includes two other constant domains, e.g., $C_L$ and $C_H1$ antibody constant domains, attached to the N-terminus (Fig.7). In other embodiments, an Fc domain monomer includes an additional moiety, e.g., an albumin-binding peptide, attached to the C-terminus. In the present invention, an Fc domain monomer does not contain any type of antibody variable region, e.g., $V_H$, $V_L$, a complementarity determining region (CDR), or a hypervariable region (HVR).

### II. Fc domains

As defined herein, an Fc domain includes two Fc domain monomers that are dimerized by the interaction between the $C_H3$ antibody constant domains. In the present invention, an Fc domain does not include a variable region of an antibody, e.g., $V_H$, $V_L$, CDR, or HVR. An Fc domain forms the minimum structure that binds to an Fc receptor, e.g., FcγRI, FcγRIIa, FcγRIIb, FcγRIIIa, FcγRIIIb, FcγRIV.

### III. Dimerization selectivity modules

In the present invention, a dimerization selectivity module is the part of the Fc domain monomer that facilitates the preferred pairing of two Fc domain monomers to form an Fc domain. Specifically, a dimerization selectivity module is that part of the $C_H3$ antibody constant domain of an Fc domain monomer which includes amino acid substitutions positioned at the interface between interacting $C_H3$ antibody constant domains of two Fc monomers. In a dimerization selectivity module, the amino acid substitutions make favorable the dimerization of the two $C_H3$ antibody constant domains as a result of the compatibility of amino acids chosen for those substitutions. The ultimate formation of the favored Fc domain is selective over other Fc domains which form from Fc domain monomers lacking dimerization selectivity modules or with incompatible amino acid substitutions in the dimerization selectivity modules. This type of amino acid substitution can be made using conventional molecular cloning techniques well-known in the art, such as QuikChange® mutagenesis.

In some embodiments, a dimerization selectivity module includes an engineered cavity (described further herein) in the $C_H3$ antibody constant domain. In other embodiments, a dimerization selectivity module includes an engineered protuberance (described further herein) in the $C_H3$ antibody constant domain. To selectively form an Fc domain, two Fc domain monomers with compatible dimerization selectivity modules, e.g., one $C_H3$ antibody constant domain containing an engineered cavity and the other $C_H3$ antibody constant domain containing an engineered protuberance, combine to form a protuberance-into-cavity pair of Fc domain monomers.

In other embodiments, an Fc domain monomer with a dimerization selectivity module containing positively-charged amino acid substitutions and an Fc domain monomer with a dimerization selectivity module containing negatively-charged amino acid substitutions may selectively combine to form an Fc domain through the favorable electrostatic steering (described further herein) of the charged amino acids. Specific dimerization selectivity modules are further listed, without limitation, in Tables 1 and 2 described further below.

In other embodiments, two Fc domain monomers include dimerization selectivity modules containing identical reverse charge mutations in at least two positions within the ring of charged residues at the interface between $C_H3$ domains. By reversing the charge of both members of two or more complementary pairs of residues in the two Fc domain monomers, mutated Fc domain monomers remain complementary to Fc domain monomers of the same mutated sequence, but have a lower complementarity to Fc domain monomers without those mutations. In one embodiment, an Fc domain includes Fc monomers including the double mutants K409D/D339K, K392D/D399K, E357K/K370E, D356K/K439D, K409E/D339K, K392E/D399K, E357K/K370D, or D356K/K439E. In another embodiment, an Fc domain includes Fc monomers including quadruple mutants combining any pair of the double mutants, e.g., K409D/D399K/E357K/K370E.

The formation of such Fc domains is promoted by the compatible amino acid substitutions in the $C_H3$ antibody constant domains. Two dimerization selectivity modules containing incompatible amino acid substitutions, e.g., both containing engineered cavities, both containing engineered protuberances, or both containing the same charged amino acids at the $C_H3$-$C_H3$ interface, will not promote the formation of an Fc domain.

Furthermore, other methods used to promote the formation of Fc domains with defined Fc domain monomers include, without limitation, the LUZ-Y approach (U.S. Patent Application Publication No. WO2011034605) which includes C-terminal fusion of a monomer α–helices of a leucine zipper to each of the Fc domain monomers to allow heterodimer formation, as well as strand-exchange engineered domain (SEED) body approach (Davis et al., *Protein Eng Des Sel.* 23:195-202, 2010) that generates Fc domain with heterodimeric Fc domain monomers each including alternating segments of IgA and IgG $C_H3$ sequences.

**IV. Engineered cavities and engineered protuberances**

The use of engineered cavities and engineered protuberances (or the “knob-into-hole” strategy) is described by Carter and co-workers (Ridgway et al., *Protein Eng.* 9:617-612, 1996; Atwell et al., *J Mol Biol.* 270:26-35, 1997; Merchant et al., *Nat Biotechnol.* 16:677-681, 1998). The knob and hole interaction favors heterodimer formation, whereas the knob-knob and the hole-hole interaction hinder homodimer

formation due to steric clash and deletion of favorable interactions. The "knob-into-hole" technique is also disclosed in U.S. Patent No. 5,731,168.

In the present invention, engineered cavities and engineered protuberances are used in the preparation of the Fc constructs described herein. An engineered cavity is a void that is created when an original amino acid in a protein is replaced with a different amino acid having a smaller side-chain volume. An engineered protuberance is a bump that is created when an original amino acid in a protein is replaced with a different amino acid having a larger side-chain volume. Specifically, the amino acid being replaced is in the $C_H3$ antibody constant domain of an Fc domain monomer and is involved in the dimerization of two Fc domain monomers. In some embodiments, an engineered cavity in one $C_H3$ antibody constant domain is created to accommodate an engineered protuberance in another $C_H3$ antibody constant domain, such that both $C_H3$ antibody constant domains act as dimerization selectivity modules (described above) that promote or favor the dimerization of the two Fc domain monomers. In other embodiments, an engineered cavity in one $C_H3$ antibody constant domain is created to better accommodate an original amino acid in another $C_H3$ antibody constant domain. In yet other embodiments, an engineered protuberance in one $C_H3$ antibody constant domain is created to form additional interactions with original amino acids in another $C_H3$ antibody constant domain.

An engineered cavity can be constructed by replacing amino acids containing larger side chains such as tyrosine or tryptophan with amino acids containing smaller side chains such as alanine, valine, or threonine. Specifically, some dimerization selectivity modules (described further above) contain engineered cavities such as Y407V mutation in the $C_H3$ antibody constant domain. Similarly, an engineered protuberance can be constructed by replacing amino acids containing smaller side chains with amino acids containing larger side chains. Specifically, some dimerization selectivity modules (described further above) contain engineered protuberances such as T366W mutation in the $C_H3$ antibody constant domain. In the present invention, engineered cavities and engineered protuberances are also combined with inter-$C_H3$ domain disulfide bond engineering to enhance heterodimer formation. Specifically, the cavity Fc contains an Y349C mutation, and the protuberance Fc contains an S354C mutation. Other engineered cavities and engineered protuberances, in combination with either disulfide bond engineering or structural calculations (mixed HA-TF) are included, without limitation, in Table 1.

Table 1

| Strategy | $CH_3$ antibody constant domain of Fc domain monomer 1 | $CH_3$ antibody constant domain of Fc domain monomer 2 | Reference |
|---|---|---|---|
| **Engineered cavities and protuberances ("knob-into-hole")** | Y407T | T366Y | US 8,216,805 |
| | Y407A | T366W | US 8,216,805 |
| | F405A | T394W | US 8,216,805 |
| | Y407T | T366Y | US 8,216,805 |
| | T394S | F405W | US 8,216,805 |
| | T394W:Y407T | T366Y:F405A | US 8,216,805 |
| | T394S:Y407A | T366W:F405W | US 8,216,805 |
| | T366W:T394S | F405W:Y407A | US 8,216,805 |
| **Engineered cavities and protuberances ("knob-into-hole"), S-S engineering** | T366S:L368A:Y407V:Y349C | T366W:S354C | Zeidler et al., *J Immunol.* 163:1246-52, 1999 |
| **Mixed HA-TF** | S364H:F405A | Y349T:T394F | WO2006106905 |

Replacing an original amino acid residue in the $C_H3$ antibody constant domain with a different amino acid residue can be achieved by altering the nucleic acid encoding the original amino acid residue. The upper limit for the number of original amino acid residues that can be replaced is the total number of residues in the interface of the $C_H3$ antibody constant domains, given that sufficient interaction at the interface is still maintained.

**V. Electrostatic steering**

Electrostatic steering is the utilization of favorable electrostatic interactions between oppositely charged amino acids in peptides, protein domains, and proteins to control the formation of higher ordered protein molecules. A method of using electrostatic steering effects to alter the interaction of antibody domains to reduce for formation of homodimer in favor of heterodimer formation in the generation of bi-specific antibodies is disclosed in U.S. Patent Application Publication No. 2014-0024111.

In the present invention, electrostatic steering is used to control the dimerization of Fc domain monomers and the formation of Fc constructs. In particular, to control the dimerization of Fc domain monomers using electrostatic steering, one or more amino acid residues that make up the $C_H3$-$C_H3$ interface are replaced with positively- or negatively-charged amino acid residues such that the interaction becomes electrostatically favorable or unfavorable depending on the specific charged amino acids introduced. In some embodiments, a positively-charged amino acid in the interface, such as lysine, arginine, or histidine, is replaced with a negatively-charged amino acid such as aspartic acid or glutamic acid. In other embodiments, a negatively-charged amino acid in the interface is replaced with a positively-charged amino acid. The charged amino acids may be introduced to one of the interacting $C_H3$ antibody constant domains, or both. By introducing charged amino acids to the interacting $C_H3$ antibody constant domains, dimerization selectivity modules (described further above) are created that can selectively form dimers of Fc domain monomers as controlled by the electrostatic steering effects resulting from the interaction between charged amino acids.

In one particular example, to create a dimerization selectivity module including reversed charges, amino acid Asp399 in the $C_H3$ antibody constant domain is replaced with Lys, and amino acid Lys409 is replaced with Asp. Heterodimerization of Fc domain monomers can be promoted by introducing different, but compatible, mutations in the two Fc domain monomers, such as the charge residue pairs included, without limitation, in Table 2, Homodimerization of Fc domain monomers can be promoted by introducing the same mutations in both Fc domain monomers in a symmetric fashion, such as the double mutants K409D/D339K or K392D/D399K.

Table 2

| $CH_3$ antibody constant domain of Fc domain monomer 1 | $CH_3$ antibody constant domain of Fc domain monomer 2 | Reference |
|---|---|---|
| K409D | D399K | US 2014/0024111 |
| K409D | D399R | US 2014/0024111 |
| K409E | D399K | US 2014/0024111 |
| K409E | D399R | US 2014/0024111 |
| K392D | D399K | US 2014/0024111 |
| K392D | D399R | US 2014/0024111 |
| K392E | D399K | US 2014/0024111 |
| K392E | D399R | US 2014/0024111 |
| K409D:K392D | D399K:E356K | Gunasekaran et al., *J Biol Chem.* 285:19637-46, 2010 |
| K370E:K409D:K439E | E356K:E357K:D399K | Martens et al., *Clin Cancer Res.* 12:6144-52, 2006 |

**VI. Linkers**

In the present invention, a linker is used to describe a linkage or connection between polypeptides or protein domains and/or associated non-protein moieties. In some embodiments, a linker is a linkage or connection between at least two Fc domain monomers, for which the linker connects the C-terminus of the $C_H3$ antibody constant domain of a first Fc domain monomer to the N-terminus of the hinge domain of a second Fc domain monomer, such that the two Fc domain monomers are joined to each other in tandem series. In other embodiments, a linker is a linkage between an Fc domain monomer and any other protein domains that are attached to it. For example, a linker can attach the C-terminus of the $C_H3$ antibody constant domain of an Fc domain monomer to the N-terminus of an albumin-binding peptide. In another example, a linker can connect the C-terminus of a $C_H1$ antibody constant domain to the N-terminus of the hinge domain of an Fc domain monomer. In yet other embodiments, a linker can connect two individual protein domains (not including an Fc domain), for example, the C-terminus of a $C_L$ antibody constant domain can be attached to the N-terminus of a $C_H1$ antibody constant domain by way of a linker.

A linker can be a simple covalent bond, e.g., a peptide bond, a synthetic polymer, e.g., a polyethylene glycol (PEG) polymer, or any kind of bond created from a chemical reaction, e.g. chemical conjugation. In the case that a linker is a peptide bond, the carboxylic acid group at the C-terminus of one protein domain can react with the amino group at the N-terminus of another protein domain in a condensation reaction to form a peptide bond. Specifically, the peptide bond can be formed from synthetic means through a conventional organic chemistry reaction well-known in the art, or by natural production from a host cell, wherein a polynucleotide sequence encoding the DNA sequences of both proteins, e.g., two Fc domain monomer, in tandem series can be directly transcribed and translated into a

contiguous polypeptide encoding both proteins by the necessary molecular machineries, e.g., DNA polymerase and ribosome, in the host cell.

In the case that a linker is a synthetic polymer, e.g., a PEG polymer, the polymer can be functionalized with reactive chemical functional groups at each end to react with the terminal amino acids at the connecting ends of two proteins.

In the case that a linker (except peptide bond mentioned above) is made from a chemical reaction, chemical functional groups, e.g., amine, carboxylic acid, ester, azide, or other functional groups commonly used in the art, can be attached synthetically to the C-terminus of one protein and the N-terminus of another protein, respectively. The two functional groups can then react to through synthetic chemistry means to form a chemical bond, thus connecting the two proteins together. Such chemical conjugation procedures are routine for those skilled in the art.

*Spacer*

In the present invention, a linker between two Fc domain monomers can be an amino acid spacer including 3-200 amino acids. Suitable peptide spacers are known in the art, and include, for example, peptide linkers containing flexible amino acid residues such as glycine and serine. In certain embodiments, a spacer can contain motifs, e.g., multiple or repeating motifs, of GS, GGS, GGGGS (SEQ ID NO: 1), GGSG (SEQ ID NO: 2), or SGGG (SEQ ID NO: 3). In certain embodiments, a spacer can contain 2 to 12 amino acids including motifs of GS, e.g., GS, GSGS (SEQ ID NO: 4), GSGSGS (SEQ ID NO: 5), GSGSGSGS (SEQ ID NO: 6), GSGSGSGSGS (SEQ ID NO: 7), or GSGSGSGSGSGS (SEQ ID NO: 8). In certain other embodiments, a spacer can contain 3 to 12 amino acids including motifs of GGS, e.g., GGS, GGSGGS (SEQ ID NO: 9), GGSGGSGGS (SEQ ID NO: 10), and GGSGGSGGSGGS (SEQ ID NO: 11). In yet other embodiments, a spacer can contain 4 to 12 amino acids including motifs of GGSG (SEQ ID NO: 12), e.g., GGSG (SEQ ID NO: 13), GGSGGGSG (SEQ ID NO: 14), or GGSGGGSGGGSG (SEQ ID NO: 15). In other embodiments, a spacer can contain motifs of GGGGS (SEQ ID NO: 16), e.g., GGGGSGGGGSGGGGS (SEQ ID NO: 17). In other embodiments, a spacer can also contain amino acids other than glycine and serine, e.g., GENLYFQSGG (SEQ ID NO: 18), SACYCELS (SEQ ID NO: 19), RSIAT (SEQ ID NO: 20), RPACKIPNDLKQKVMNH (SEQ ID NO: 21), GGSAGGSGSGSSGGSSGASGTGTAGGTGSGSGTGSG (SEQ ID NO: 22), AAANSSIDLISVPVDSR (SEQ ID NO: 23), or GGSGGGSEGGGSEGGGSEGGGSEGGGSEGGGSGGGS (SEQ ID NO: 24). In certain embodiments in the present invention, a 12- or 20-amino acid peptide spacer is used to connect two Fc domain monomers in tandem series (Figs. 4-6), the 12- and 20-amino acid peptide spacers consisting of sequences GGGSGGGSGGGS (SEQ ID NO: 25) and SGGGSGGGSGGGSGGGSGGG (SEQ ID NO: 26), respectively. In other embodiments, an 18-amino acid peptide spacer consisting of sequence GGSGGGSGGGSGGGSGGS (SEQ ID NO: 27) is used to connect $C_L$ and $C_H1$antibody constant domains (Fig. 7A-7B).

**VII. Serum protein-binding peptides**

Binding to serum protein peptides can improve the pharmacokinetics of protein pharmaceuticals, and in particular the Fc constructs described here may be fused with serum protein-binding peptides

As one example, albumin-binding peptides that can be used in the methods and compositions described here are generally known in the art. In one embodiment, the albumin binding peptide includes the sequence DICLPRWGCLW (SEQ ID NO: 28)

In the present invention, albumin-binding peptides may be attached to the N- or C-terminus of certain polypeptides in the Fc construct. In one embodiment, an albumin-binding peptide may be attached to the C-terminus of one or more polypeptides in constructs 1, 2, 3, or 7A (Figs. 1, 2, 3, and 7A, respectively). In another embodiment, an albumin-binding peptide can be fused to the C-terminus of the polypeptide encoding two Fc domain monomers linked in tandem series in constructs 4, 5, and 6 (Figs. 4, 5, and 6, respectively). In yet another embodiment, an albumin-binding peptide can be attached to the C-terminus of Fc domain monomer which is joined to the second Fc domain monomer in the polypeptide encoding the two Fc domain monomers linked in tandem series, as shown in constructs 4 and 6 (Figs. 4 and 6, respectively). Albumin-binding peptides can be fused genetically to Fc constructs or attached to Fc constructs through chemical means, e.g., chemical conjugation. If desired, a spacer can be inserted between the Fc construct and the albumin-binding peptide. Without being bound to a theory, it is expected that inclusion of an albumin-binding peptide in an Fc construct of the invention may lead to prolonged retention of the therapeutic protein through its binding to serum albumin.

**VIII. Fc constructs**

In general, the invention features Fc constructs having 2-10 Fc domains. These may have greater binding affinity and/or avidity than a single wild-type Fc domain for an Fc receptor, e.g., FcγRIIIa. The invention discloses methods of engineering amino acids at the interface of two interacting $C_H3$ antibody constant domains such that the two Fc domain monomers of an Fc domain selectively form a dimer with each other, thus preventing the formation of unwanted multimers or aggregates. An Fc construct includes an even number of Fc domain monomers, with each pair of Fc domain monomers forming an Fc domain. An Fc construct includes, at a minimum, one functional Fc domain formed from a dimer of two Fc domain monomers.

In some embodiments, an Fc construct contains one Fc domain including a dimer of two Fc domain monomers (Figs. 1-3 and 7A). The interacting $C_H3$ antibody constant domains may be unmodified (Fig. 1) or may contain amino acid substitutions at their interface. Specifically, the amino acid substitutions can be engineered cavities (Fig. 2), engineered protuberances (Fig. 2), or charged amino acids (Fig. 3).

In other embodiments, an Fc construct contains two Fc domains (Figs. 4 and 6) formed from three polypeptides. The first polypeptide contains two Fc domain monomers joined in tandem series joined by way of a linker, and the second and third polypeptides contain one Fc domain monomer. The second and third polypeptides may be the same polypeptide or may be different polypeptides. Fig. 4 depicts an example of such an Fc construct. The first polypeptide contains two wild-type Fc domain monomers joined in tandem series by way of a linker, and the second and third polypeptides each contain one wild-type Fc domain monomer. One of the Fc domain monomers in the first polypeptide forms a first Fc domain with the second polypeptide, while the other Fc domain monomer in the first polypeptide forms a second Fc domain with the third polypeptide. The second and third polypeptides are not attached or linked to each other. Fig. 6 depicts a similar Fc construct to that of Fig. 4. In Fig. 6, the Fc domain

monomers in the first polypeptide both contain engineered protuberances in the $C_H3$ antibody constant domains, while the second and third polypeptides contain engineered cavities in the $C_H3$ antibody constant domains. The engineered protuberance-into-cavity $C_H3$- $C_H3$ interface favors the formation of heterodimers of Fc domain monomers and prevents the uncontrolled formation of unwanted multimers. As described further herein, in Example 4, dimerization selectivity modules including engineered $C_H3$ antibody constant domains prevent the formation of unwanted multimers that are seen in Example 3, which describes Fc construct formation from Fc domain monomers lacking dimerization selectivity modules.

Furthermore, in other embodiments, an Fc construct can contain three Fc domains formed from four polypeptides (Fig. 5). The first and second polypeptides can be the same or different, as can the third and fourth polypeptides. In this example, the first and second polypeptides both encode two Fc domain monomers connected by way of a linker in tandem series, wherein one Fc domain monomer contains charged amino acid substitutions in the $C_H3$ antibody constant domain while the other Fc domain monomer contains a protuberance in the $C_H3$ antibody constant domain. The third and fourth polypeptides both encode an Fc domain monomer with a cavity. The first and second polypeptides form a first Fc domain with each other through interaction of the reverse charges in their $C_H3$ antibody constant domains. The second and third Fc domains are formed from protuberance-into-cavity interactions between the protuberances in the first and second polypeptides and the cavities in the third and fourth polypeptides. Each Fc domain monomer in this Fc construct contains a dimerization selectivity module which promotes the formation of specific Fc domains.

In yet other embodiments, a single polypeptide can form dimers (e.g., construct 7A; Fig. 7A) or multimers (e.g., construct 7B; Fig. 7B), not through interaction between $C_H3$ antibody constant domains, but through interaction between $C_L$ constant domains and $C_H1$ constant domains. Fig. 7B depicts an Fc construct containing multiple Fc domains in which the $C_L$ domain of one Fc domain interacts with the $C_H1$ domain of a neighboring Fc domain.

In yet other embodiments, Fc constructs can contain five Fc domains formed from six polypeptides. Two examples are depicted in Figs. 8 and 9. While these depicted Fc constructs are comprised of six polypeptides, four of the polypeptides can be encoded by the same nucleic acid, and the remaining two polypeptides can also be encoded by the same nucleic acid. As a result, these Fc constructs can be produced by the expression of two nucleic acids in a suitable host cell.

In another embodiment, an Fc construct containing two or more Fc domains can be formed from two polypeptides having the same primary sequence. Such a construct can be formed from expression of a single polypeptide sequence in a host cell. An example is depicted in Fig. 10. In this example, a single nucleic acid is sufficient to encode an Fc construct containing three Fc domains. Two Fc domain monomers that are part of the same polypeptide are permitted to form an Fc domain by the inclusion of a flexible linker of a sufficient length and flexibility; this linker may be a cleavable linker. This same polypeptide also contains a third Fc domain monomer joined by way of an optional flexible linker. This third Fc domain monomer is capable of joining to another Fc domain monomer to produce the Y-shaped Fc construct depicted in Fig. 10. Formation of Fc domains can be controlled through the use of dimerization selectivity modules, as is also depicted in Fig. 10.

**IX. Host cells and protein production**

In the present invention, a host cell refers to a vehicle that includes the necessary cellular components, e.g., organelles, needed to express the polypeptides and constructs described herein from their corresponding nucleic acids. The nucleic acids may be included in nucleic acid vectors that can be introduced into the host cell by conventional techniques known in the art (transformation, transfection, electroporation, calcium phosphate precipitation, direct microinjection, etc.). Host cells can be of either mammalian or bacterial origin. Mammalian host cells include, but are not limited to, CHO (or CHO-derived cell strains, e.g., CHO-K1, CHO-DXB11 CHO-DG44), murine host cells (e.g., NS0, Sp2/0), VERY, HEK (e.g., HEK293), BHK, HeLa, COS, MDCK, 293, 3T3, W138, BT483, Hs578T, HTB2, BT20 and T47D, CRL7O3O and HsS78Bst cells. Host cells can also be chosen that modulate the expression of the protein constructs, or modify and process the protein product in the specific fashion desired. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of protein products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the protein expressed.

For expression and secretion of protein products from their corresponding DNA plasmid constructs, host cells may be transfected or transformed with DNA controlled by appropriate expression control elements known in the art, including promoter, enhancer, sequences, transcription terminators, polyadenylation sites, and selectable markers. Methods for expression of therapeutic proteins are known in the art. See, for example, Paulina Balbas, Argelia Lorence (eds.) *Recombinant Gene Expression: Reviews and Protocols (Methods in Molecular Biology)*, Humana Press; 2nd ed. 2004 edition (July 20, 2004); Vladimir Voynov and Justin A. Caravella (eds.) *Therapeutic Proteins: Methods and Protocols (Methods in Molecular Biology)* Humana Press; 2nd ed. 2012 edition (June 28, 2012).

**X. Purification**

An Fc construct can be purified by any method known in the art of protein purification, for example, by chromatography (e.g., ion exchange, affinity (e.g., Protein A affinity), and size-exclusion column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. For example, an Fc construct can be isolated and purified by appropriately selecting and combining affinity columns such as Protein A column with chromatography columns, filtration, ultra filtration, salting-out and dialysis procedures (see, e.g., *Process Scale Purification of Antibodies*, Uwe Gottschalk (ed.) John Wiley & Sons, Inc., 2009; and Subramanian (ed.) *Antibodies-Volume I-Production and Purification*, Kluwer Academic/Plenum Publishers, New York (2004)). In some instances, an Fc construct can be conjugated to marker sequences, such as a peptide to facilitate purification. An example of a marker amino acid sequence is a hexa-histidine peptide, which binds to nickel-functionalized agarose affinity column with micromolar affinity. Other peptide tags useful for purification include, but are not limited to, the hemagglutinin “HA” tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., 1984, Cell 37:767).

For the Fc constructs, Protein A column chromatography may be employed as a purification process. Protein A ligands interact with Fc constructs through the Fc region, making Protein A chromatography a highly selective capture process that is able to remove most of the host cell proteins.

In the present invention, Fc constructs may be purified using Protein A column chromatography as described in Example 2.

**XI. Pharmaceutical compositions/preparations**

The invention features pharmaceutical compositions that include one or more Fc constructs described herein. In one embodiment, a pharmaceutical composition includes a substantially homogenous population of Fc constructs that are identical or substantially identical in structure. In various examples, the pharmaceutical composition includes a substantially homogenous population of any one of constructs 1-10 and 5*.

A therapeutic protein construct, e.g., an Fc construct, of the present invention can be incorporated into a pharmaceutical composition. Pharmaceutical compositions including therapeutic proteins can be formulated by methods know to those skilled in the art. The pharmaceutical composition can be administered parenterally in the form of an injectable formulation including a sterile solution or suspension in water or another pharmaceutically acceptable liquid. For example, the pharmaceutical composition can be formulated by suitably combining the Fc construct with pharmaceutically acceptable vehicles or media, such as sterile water for injection (WFI), physiological saline, emulsifier, suspension agent, surfactant, stabilizer, diluent, binder, excipient, followed by mixing in a unit dose form required for generally accepted pharmaceutical practices. The amount of active ingredient included in the pharmaceutical preparations is such that a suitable dose within the designated range is provided.

The sterile composition for injection can be formulated in accordance with conventional pharmaceutical practices using distilled water for injection as a vehicle. For example, physiological saline or an isotonic solution containing glucose and other supplements such as D-sorbitol, D-mannose, D-mannitol, and sodium chloride may be used as an aqueous solution for injection, optionally in combination with a suitable solubilizing agent, for example, alcohol such as ethanol and polyalcohol such as propylene glycol or polyethylene glycol, and a nonionic surfactant such as polysorbate 80™, HCO-50, and the like commonly known in the art. Formulation methods for therapeutic protein products are known in the art, see e.g., Banga (ed.) *Therapeutic Peptides and Proteins: Formulation, Processing and Delivery Systems* (2d ed.) Taylor & Francis Group, CRC Press (2006).

**XII. Dosage**

The pharmaceutical compositions are administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective to result in an improvement or remediation of the symptoms. The pharmaceutical compositions are administered in a variety of dosage forms, e.g., intravenous dosage forms, subcutaneous dosage forms, oral dosage forms such as ingestible solutions, drug release capsules, and the like. The appropriate dosage for the individual subject depends on the therapeutic objectives, the route of administration, and the condition of the patient. Generally, recombinant proteins are dosed at 1-200 mg/kg, e.g., 1-100 mg/kg, e.g., 20-100 mg/kg. Accordingly, it will be necessary for a healthcare provider to tailor and titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect.

**XIII. Indications**

The pharmaceutical compositions and methods of the invention are useful to reduce inflammation in a subject, to promote clearance of autoantibodies in a subject, to suppress antigen presentation in a subject, to reduce the immune response, e.g., to block immune complex-based activation of the immune response in a subject, and to treat immunological and inflammatory conditions or diseases in a subject. Exemplary conditions and diseases include rheumatoid arthritis (RA); systemic lupus erythematosus (SLE); ANCA-associated vasculitis; antiphospholipid antibody syndrome; autoimmune hemolytic anemia; chronic inflammatory demyelinating neuropathy; clearance of anti-allo in transplant, anti-self in GVHD, anti-replacement, IgG therapeutics, IgG paraproteins; dermatomyositis; Goodpasture's Syndrome; organ system-targeted type II hypersensitivity syndromes mediated through antibody-dependent cell-mediated cytotoxicity, e.g., Guillain Barre syndrome, CIDP, dermatomyositis, Felty's syndrome, antibody-mediated rejection, autoimmune thyroid disease, ulcerative colitis, autoimmune liver disease; idiopathic thrombocytopenia purpura; Myasthenia Gravis, neuromyelitis optica; pemphigus and other autoimmune blistering disorders; Sjogren's Syndrome; autoimmune cytopenias and other disorders mediated through antibody-dependent phagocytosis; other FcR-dependent inflammatory syndromes e.g., synovitis, dermatomyositis, systemic vasculitis, glomerulitis and vasculitis..

## EXAMPLES

### Example 1 – Design and cloning of DNA plasmid constructs

A total of eight DNA plasmid constructs were used to assemble eight Fc constructs (Figs. 1-7B). The DNA plasmid constructs were transfected into human embryonic kidney (HEK) 293 cells for protein production. The eight encoded secreted polypeptides had the general structures as described below:

A. *wt Fc*: wild-type Fc domain monomer (Fig. 1: 102 and 104; Fig. 4: 408 and 410).

B. *protuberance Fc*: Fc domain monomer with engineered protuberance in $C_H3$ antibody constant domain (Fig. 2: 202).

C. *cavity Fc*: Fc domain monomer with engineered cavity in $C_H3$ antibody constant domain (Fig. 2: 204; Fig. 5: 514 and 516).

C*. *cavity Fc**: Fc domain monomer with engineered cavity in $C_H3$ antibody constant domain (Fig. 2: 204; Fig. 5: 514 and 516). Cavity Fc* also contains additional amino acid substitutions relative to cavity Fc.

D. *charges Fc*: Fc domain monomer with reversed charges in $C_H3$ antibody constant domain (Fig. 3: 302 and 304).

E. *wt-12-wt Fc2*: Two Fc domain monomers joined in series by way of a 12-amino acid GGGS peptide linker (Fig. 4: 402).

F. *protuberance-20-charges Fc2*: Fc domain monomer with reversed charges in $C_H3$ antibody constant domain and Fc domain monomer with engineered protuberance in $C_H3$ antibody constant domain joined in series by way of a 20-amino acid SGGG peptide linker (Fig. 5: 502 and 508).

F*. *protuberance-20-charges Fc2**: Fc domain monomer with reversed charges in $C_H3$ antibody constant domain and Fc domain monomer with engineered protuberance in $C_H3$ antibody constant domain joined in series by way of a 20-amino acid SGGG peptide linker (Fig. 5: 502 and 508). Protuberance-20-charges Fc2* also contains additional amino acid substitutions relative to protuberance Fc.

G. *protuberance-20-protuberance Fc2*: Two Fc domain monomers both with engineered protuberance in $C_H3$ antibody constant domain joined in series by way of a 20-amino acid GGGS peptide linker (Fig. 6: 602).

H. *$C_HC_L$ Fc+*: Fc domain monomer with $C_H1$ and $C_L$ constant domains attached to the hinge domain (Fig. 7A: 702 and 704; Fig. 7B: 706, 708, 710, 712, 714, and 716). The $C_L$ constant domain is attached by way of an 18 amino acid GGGS peptide linker to a $C_{H1}$ constant domain.

Fc DNA sequences were derived from human IgG1 Fc. Protuberance, cavity and charges mutations were substituted in the parental Fc sequence. DNA encoding a leader peptide derived from the human immunoglobulin Kappa Light chain was attached to the 5' region. All but one of the polypeptides ($C_HC_L$ Fc+) contained this encoded peptide on the amino terminus to direct protein translocation into the endoplasmic reticulum for assembly and secretion. It will be understood that any one of a variety of leader peptides may be used in connection with the present invention. The leader peptide is usually clipped off in the ER lumen. An 11 nucleotide sequence containing a 5' terminal EcoR1 site was added upstream of the ATG start codon. A 30 nucleotide sequence containing a 3' terminal Xho1 site was added downstream of the 3' terminal TGA translation termination codon. The DNA sequences were optimized for expression in mammalian cells and cloned into the pcDNA3.4 mammalian expression vector.

Mutations are denoted by the wild-type amino acid residue followed by the position using the EU Kabat numbering system (Kabat et al., *Sequences of Proteins of Immunological Interest*, National Institutes of Health, Bethesda, Md., ed. 5, 1991) and then the replacement residue in single-letter code. The nucleotide and amino acid sequences of secreted polypeptides A-H described above are provided below (except for cavity Fc* and protuberance-20-charges Fc2*, for which only the amino acid sequences are provided).

.

wt Fc

SEQ ID NO: 29:

```
1         10         20         30         40         50
|         |          |          |          |          |

GACAAGACCCACACCTGTCCGCCTTGCCCTGCCCCTGAGCTGCTGGGAGG
CCCCAGCGTGTTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATGATCA
GCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGAC
CCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGC
CAAGACCAAGCCCAGAGAGGAACAGTACAACAGCACCTACCGGGTGGTGT
CCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAG
TGCAAAGTCTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACCATCAG
CAAGGCCAAGGGCCAGCCCCGCGAGCCCCAGGTGTACACACTGCCCCCCA
GCCGGGACGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAA
GGCTTCTACCCCAGCGATATCGCCGTGGAATGGGAGAGCAACGGCCAGCC
CGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGACGGCTCAT
TCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCCGGTGGCAGCAGGGC
AACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACAC
CCAGAAGTCCCTGAGCCTGAGCCCCGGCAAG
```

SEQ ID NO: 30:

```
1         10         20         30         40         50
|         |          |          |          |          |
DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK
```

protuberance Fc

SEQ ID NO: 31:

```
1         10         20         30         40         50
|         |          |          |          |          |
GACAAGACCCACACCTGTCCGCCTTGCCCTGCCCCTGAGCTGCTGGGAGG
CCCCAGCGTGTTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATGATCA
GCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGAC
CCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGC
CAAGACCAAGCCCAGAGAGGAACAGTACAACAGCACCTACCGGGTGGTGT
CCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAG
TGCAAAGTCTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACCATCAG
CAAGGCCAAGGGCCAGCCCCGCGAGCCCCAGGTGTACACACTGCCCCCCT
GCCGGGACGAGCTGACCAAGAACCAGGTGTCCCTGTGGTGCCTGGTGAAA
GGCTTCTACCCCAGCGATATCGCCGTGGAATGGGAGAGCAACGGCCAGCC
CGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGACGGCTCAT
TCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCCGGTGGCAGCAGGGC
AACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACAC
CCAGAAGTCCCTGAGCCTGAGCCCCGGCAAG
```

SEQ ID NO: 32:

```
1         10         20         30         40         50
|         |          |          |          |          |
DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK
```

cavity Fc

SEQ ID NO: 33:

```
1         10         20         30         40         50
|         |          |          |          |          |

GACAAGACCCACACCTGTCCGCCTTGCCCTGCCCCTGAGCTGCTGGGAGG
CCCCAGCGTGTTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATGATCA
GCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGAC
CCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGC
CAAGACCAAGCCCAGAGAGGAACAGTACAACAGCACCTACCGGGTGGTGT
CCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAG
TGCAAAGTCTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACCATCAG
CAAGGCCAAGGGCCAGCCCCGCGAGCCCCAAGTGTGTACACTGCCCCCCA
GCCGGGACGAGCTGACCAAGAACCAGGTGTCCCTGAGCTGCGCCGTGAAA
GGCTTCTACCCCAGCGATATCGCCGTGGAATGGGAGAGCAACGGCCAGCC
CGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGACGGCTCAT
TCTTCCTGGTTAGCAAGCTGACCGTGGACAAGAGCCGGTGGCAGCAGGGC
AACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACAC
CCAGAAGTCCCTGAGCCTGAGCCCCGGCAAG
```

SEQ ID NO: 34:

```
1         10         20         30         40         50
|         |          |          |          |          |

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK
```

cavity Fc*SEQ ID NO: 45:

```
1         10         20         30         40         50
|         |          |          |          |          |

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVE
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK
```

charges Fc

SEQ ID NO: 35:

```
1         10         20         30         40         50
|         |          |          |          |          |
```

GACAAGACCCACACCTGTCCGCCTTGCCCTGCCCCTGAGCTGCTGGGAGG
CCCCAGCGTGTTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATGATCA
GCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGAC
CCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGC
CAAGACCAAGCCCAGAGAGGAACAGTACAACAGCACCTACCGGGTGGTGT
CCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAG
TGCAAAGTCTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACCATCAG
CAAGGCCAAGGGCCAGCCCCGCGAGCCCCAGGTGTACACACTGCCCCCCA
GCCGGGACGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAA
GGCTTCTACCCCAGCGATATCGCCGTGGAATGGGAGAGCAACGGCCAGCC
CGAGAACAACTACAAGACCACCCCCCCCTGTGCTGAAAAGCGACGGCTCAT
TCTTCCTGTACAGCGACCTGACCGTGGACAAGAGCCGGTGGCAGCAGGGC
AACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACAC
CCAGAAGTCCCTGAGCCTGAGCCCCGGCAAG

SEQ ID NO: 36:

1 10 20 30 40 50

| | | | | |

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSDLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGK

wt-12-wt Fc2

SEQ ID NO: 37:

1 10 20 30 40 50

| | | | | |

GACAAGACCCACACCTGTCCCCCTTGCCCTGCCCCTGAGCTGCTGGGAGG
CCCCAGCGTGTTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATGATCA
GCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGAC
CCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGC
CAAGACCAAGCCCAGAGAGGAACAGTACAACAGCACCTACCGGGTGGTGT
CCGTGCTGACCGTGCTGCACCAGGACTGGCTCAACGGCAAAGAGTACAAG
TGCAAGGTGTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACCATCAG
CAAGGCCAAGGGCCAGCCCCGCGAGCCCCAGGTCTACACACTGCCCCCCA
GCCGGGACGAGCTGACCAAGAACCAGGTCTCCCTGACCTGCCTGGTGAAA
GGCTTCTACCCCAGCGATATCGCCGTGGAATGGGAGAGCAACGGCCAGCC
CGAGAACAACTACAAGACCACCCCCCCCTGTGCTGGACAGCGACGGCTCAT
TCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCCGGTGGCAGCAGGGC
AACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACAC
CCAGAAGTCCCTGAGCCTGAGCCCCGGCAAAGGCGGGGGATCTGGGGGAG
GAAGCGGAGGCGGCAGCGATAAGACCCATACCTGCCCTCCCTGTCCCGCT
CCCGAACTGCTGGGGGGACCCTCCGTGTTTCTGTTTCCACCTAAGCCTAA
GGATACGCTCATGATCTCCAGAACCCCTGAAGTCACATGTGTGGTGGTCG
ATGTGTCTCATGAAGATCCCGAAGTCAAGTTTAACTGGTATGTGGATGGG
GTCGAGGTCCACAATGCCAAAACAAAGCCTCGGGAAGAACAGTATAACTC
CACCTACAGAGTCGTCAGCGTGCTGACAGTCCTTCATCAGGATTGGCTGA
ATGGGAAAGAGTACAAATGTAAAGTGTCTAACAAAGCTCTGCCCGCTCCT
ATCGAAAAGACCATCTCCAAAGCCAAAGGGCAGCCCAGAGAACCTCAGGT
GTACACCCTGCCACCCTCCAGAGATGAGCTGACAAAAAATCAGGTGTCAC
TGACATGTCTGGTGAAAGGGTTTTATCCCTCCGACATTGCTGTGGAATGG

GAATCCAATGGGCAGCCTGAAAACAATTATAAGACAACACCTCCCGTGCT
GGACTCCGATGGCTCATTTTTTCTGTACTCTAAACTGACAGTGGATAAGT
CCAGATGGCAGCAGGGAAATGTGTTTTCCTGCTCTGTGATGCATGAAGCT
CTGCATAATCACTATACACAGAAAAGCCTGTCCCTGTCCCCCGGCAAG

SEQ ID NO: 38:

1 10 20 30 40 50

| | | | | |

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGKGGGSGGGSGGGSDKTHTCPPCPA
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA
LHNHYTQKSLSLSPGK

protuberance-20-charges Fc2

SEQ ID NO: 39:

1 10 20 30 40 50

| | | | | |

GACAAGACCCACACCTGTCCCCCTTGCCCAGCCCCTGAGCTGCTGGGAGG
CCCCAGCGTGTTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATGATCA
GCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGAC
CCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGC
CAAGACCAAGCCCAGAGAGGAACAGTACAACAGCACCTACCGGGTGGTGT
CCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAGTACAAG
TGCAAGGTGTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACCATCAG
CAAGGCCAAGGGCCAGCCCCGCGAGCCCCAGGTGTACACCCTGCCCCCTT
GCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGTGGTGCCTGGTCAAG
GGCTTCTACCCCAGCGATATCGCCGTGGAATGGGAGAGCAACGGCCAGCC
CGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACAGCGACGGCTCAT
TCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCCGGTGGCAGCAGGGC
AACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACAC
CCAGAAGTCCCTGAGCCTGAGCCCCGGCAAGTCTGGGGGAGGATCAGGGG
GTGGAAGTGGCGGTGGATCTGGTGGTGGAAGCGGAGGCGGCGATAAGACA
CACACATGCCCCCCCTGTCCAGCTCCCGAACTGCTGGGGGGACCCTCCGT
GTTTCTGTTTCCACCTAAGCCTAAGGATACGCTCATGATCTCCAGAACCC
CTGAAGTCACATGTGTGGTGGTCGATGTGTCTCATGAAGATCCCGAAGTC
AAGTTTAATTGGTATGTCGATGGGGTCGAGGTGCACAATGCCAAAACAAA
ACCTCGGGAAGAACAGTATAACTCCACATACAGAGTGGTGTCTGTCCTCA
CAGTCCTGCATCAGGATTGGCTCAATGGGAAAGAGTACAAATGTAAAGTC
TCTAACAAGGCTCTCCCCGCTCCGATCGAAAAGACCATCTCCAAAGCCAA
AGGGCAGCCCAGAGAACCTCAGGTCTACACACTGCCTCCCAGCCGGGACG
AGCTGACAAAAAATCAAGTGTCTCTGACCTGCCTCGTGAAGGGCTTTTAT
CCCTCCGACATTGCCGTCGAGTGGGAGTCCAATGGACAGCCGGAAAACAA
TTATAAGACCACGCCTCCAGTGCTGAAGTCCGACGGCAGCTTCTTTCTGT
ACTCCGACCTGACAGTGGATAAGTCCAGATGGCAGCAAGGGAATGTGTTC
TCCTGTTCCGTGATGCATGAAGCCCTCCATAATCACTATACCCAGAAAAG
CCTGTCCCTGTCCCCTGGCAAG

SEQ ID NO: 40:

```
1         10         20         30         40         50
|         |          |          |          |          |
DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGKSGGGSGGGSGGGSGGGSGGGDKT
HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSDLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK
```

protuberance-20-charges Fc2*

SEQ ID NO: 46:

```
1         10         20         30         40         50
|         |          |          |          |          |
DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDKLTKNQVSLWCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGKSGGGSGGGSGGGSGGGSGGGDKT
HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSDLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK
```

protuberance-20-protuberance Fc2

SEQ ID NO: 41:

```
1         10         20         30         40         50
|         |          |          |          |          |
GACAAGACCCACACCTGTCCCCCTTGCCCTGCCCCTGAGCTGCTGGGAGG
CCCCAGCGTGTTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATGATCA
GCCGGACCCCCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGAC
CCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGC
CAAGACCAAGCCCAGAGAGGAACAGTACAACAGCACCTACCGGGTGGTGT
CCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAGTACAAG
TGCAAGGTGTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACCATCAG
CAAGGCCAAGGGCCAGCCCCGCGAGCCCCAGGTGTACACCCTGCCCCCTT
GCAGAGATGAACTGACCAAGAACCAGGTGTCCCTGTGGTGCCTGGTCAAG
GGCTTCTACCCCAGCGATATCGCCGTGGAATGGGAGAGCAACGGCCAGCC
CGAGAACAACTACAAGACCACCCCCCCCTGTGCTGGACAGCGACGGCTCAT
TCTTCCTGTACAGCAAGCTGACCGTGGACAAGAGCCGGTGGCAGCAGGGC
```

AACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACAC
CCAGAAGTCCCTGAGCCTGAGCCCCGGCAAGTCTGGGGGAGGATCAGGGG
GTGGAAGTGGCGGTGGATCTGGTGGTGGAAGCGGAGGCGGCGATAAGACA
CACACATGCCCCCCCTGTCCAGCTCCCGAACTGCTGGGGGGACCCTCCGT
GTTTCTGTTTCCACCTAAGCCTAAGGATACGCTCATGATCTCCAGAACCC
CTGAAGTCACATGTGTGGTGGTCGATGTGTCTCATGAAGATCCCGAAGTC
AAGTTTAACTGGTATGTGGATGGGGTCGAGGTCCACAATGCCAAAACAAA
GCCTCGGGAAGAACAGTATAACTCCACCTACAGAGTCGTCAGCGTGCTGA
CAGTCCTGCATCAAGATTGGCTCAATGGGAAAGAGTATAAGTGTAAAGTC
TCGAACAAAGCCCTCCCCGCTCCTATCGAAAAGACCATCTCCAAAGCCAA
AGGGCAGCCCAGAGAACCTCAGGTCTACACACTGCCTCCATGTCGGGACG
AGCTGACAAAAAATCAGGTGTCACTGTGGTGTCTGGTGAAGGGGTTTTAC
CCTTCCGACATTGCTGTGGAATGGGAATCCAATGGGCAGCCTGAAAACAA
TTATAAGACAACACCTCCCGTGCTGGACTCCGATGGCTCATTTTTTCTGT
ACTCTAAACTGACAGTGGATAAGTCCAGATGGCAGCAGGGAAATGTGTTT
TCCTGCTCTGTGATGCATGAAGCTCTGCATAATCACTATACACAGAAAAG
CCTGTCCCTGTCCCCTGGCAAG

SEQ ID NO: 42:

1 10 20 30 40 50

| | | | | |

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGKSGGGSGGGSGGGSGGGSGGGDKT
HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFY
PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK

$C_HC_L$ Fc+

SEQ ID NO: 43:

1 10 20 30 40 50

| | | | | |

AGGACAGTGGCCGCTCCCAGCGTGTTCATCTTCCCACCCAGCGACGAGCA
GCTGAAGTCCGGCACAGCCAGCGTGGTCTGCCTGCTGAACAACTTCTACC
CCCGCGAGGCCAAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGAGCGGC
AACAGCCAGGAAAGCGTCACCGAGCAGGACAGCAAGGACTCCACCTACAG
CCTGTCTAGCACCCTGACCCTGAGCAAGGCCGACTACGAGAAGCACAAGG
TGTACGCCTGCGAAGTGACCCACCAGGGCCTGTCCAGCCCCGTGACCAAG
AGCTTCAACAGAGGCGAGTGCGGCGGCTCTGGCGGAGGATCCGGGGGAGG
ATCAGGCGGCGGAAGCGGAGGCAGCGCTAGCACAAAGGGCCCCTCCGTGT
TCCCCCTGGCCCCCAGCAGCAAGAGCACATCTGGCGGAACAGCCGCCCTG
GGCTGCCTGGTGAAAGACTACTTCCCCGAGCCCGTGACCGTGTCCTGGAA
CTCTGGCGCCCTGACCAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGA
GCAGCGGCCTGTACTCCCTGAGCAGCGTGGTGACAGTGCCTAGCAGCAGC
CTGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCAGCAACAC
CAAAGTGGACAAGCGGGTGGAACCCAAGAGCTGCGACAAGACCCACACGT
GTCCCCCCTGCCCAGCCCCTGAACTGCTGGGCGGACCTAGCGTGTTCCTG
TTCCCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCCGAAGT

GACCTGCGTGGTGGTGGACGTGTCCCACGAGGACCCTGAAGTGAAGTTCA
ATTGGTACGTGGACGGCGTGGAAGTGCACAATGCCAAGACCAAGCCCAGA
GAGGAACAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCT
GCACCAGGACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACA
AGGCCCTGCCTGCCCCCATCGAGAAAACCATCAGCAAGGCCAAGGGCCAG
CCCCGCGAGCCCCAGGTGTACACACTGCCCCCCAGCCGGGACGAGCTGAC
CAAGAACCAGGTGTCCCTGACCTGTCTGGTGAAAGGCTTCTACCCCTCCG
ATATCGCCGTGGAATGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAG
ACCACCCCCCCCTGTGCTGGACTCCGACGGCTCATTCTTCCTGTACAGCAA
GCTGACCGTGGACAAGAGCCGGTGGCAGCAGGGCAACGTGTTCAGCTGCT
CCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGAGC
CTGAGCCCCGGCAAA

SEQ ID NO: 44:

1 10 20 30 40 50

| | | | | |

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG
NSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK
SFNRGECGGSGGGSGGGSGGGSGGSASTKGPSVFPLAPSSKSTSGGTAAL
GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS
LGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFL
FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR
EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

**Example 2 – Expression of Fc construct proteins**

For protein expression of the Fc constructs, two of the DNA plasmid constructs selected from A-H described in Example 1 were transfected into EXPI293 cells (LifeTechnologies). Liposome transfection was used to introduce plasmid DNA into EXPI293 cells. The total amount of transfected plasmid constructs was fixed whereas the ratio of different plasmid constructs was varied to maximize the yield of desired constructs (see Table 3 below). For each Fc construct, the ratio (by mass) of the two transfected DNA plasmid constructs is shown in Table 3. Illustrations of the constructs are shown Figs. 1-7B.

After protein expression, the expressed constructs were purified from the cell culture supernatant by Protein A-based affinity column chromatography. Media supernatants were loaded onto a Poros MabCapture A (LifeTechnologies) column using an AKTA Avant preparative chromatography system (GE Healthcare Life Sciences). Captured Fc constructs were then washed with phosphate buffered saline (low-salt wash) followed by phosphate buffered saline supplemented with 500mM NaCl (high-salt wash). Fc constructs are eluted with 100mM glycine, 150mM NaCl, pH 3 buffer. The protein solution emerging from the column is neutralized by addition of 1M TRIS pH 7.4 to a final concentration of 100mM. The Fc constructs were further fractionated by ion exchange chromatography using Poros® XS resin (Applied Biosciences Cat. # 4404336). The column was pre-equilibrated with 10 mM MES, pH 6 (buffer A), and the sample was eluted with a gradient against 10 mM MES, 500 mM sodium chloride, pH 6 (buffer B).

We obtained a total of seven Fc constructs (see Table 3 below and Figs. 1-7B). Purified Fc constructs were analyzed by SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis)

under both reducing and non-reducing conditions followed by Coomassie Blue staining to confirm the presence of protein bands of expected size.

Table 3

| Construct | Plasmids Transfected | Ratio of Plasmids A:B | Approx. MW in kDa (reducing SDS-Page) | Approx. MW in KDa (non-reducing SDS-Page) |
|---|---|---|---|---|
| 1 | A: Wt Fc | n/a | 25 | 50 |
| 2 | A: Protuberance Fc<br>B: Cavity Fc | 1:1 | 25 | 50 |
| 3 | A: Charges Fc | | 25 | 50 |
| 4 | A: Wt-12-Wt Fc2<br>B: Wt FC | 1:2 | 25, 50 | 100 |
| 5 | A: Protuberance-20-Charges Fc2<br>B: Cavity Fc | 2:1 | 25, 50 | 150 |
| 5* | A: Protuberance-20-Charges Fc2*<br>B: Cavity Fc* | 2:1 | 25, 50 | 150 |
| 6 | A: Protuberance-20-Protuberance Fc2<br>B: Cavity Fc | 1:1 | 25, 50 | 100 |
| 7 and 8 | A: Ch, Cl, Fc+ | n/a | 50 | 100 |

**Example 3 – Preparation and SDS-PAGE analysis of construct 4**

Two DNA plasmid constructs, wt-12-wt Fc2 (DNA plasmid construct E in Example 1) and wt Fc (DNA plasmid construct A in Example 1), were used to express construct 4 (Fig. 4). The two plasmid constructs were transfected into HEK 293 cells for protein expression and purification as described in Example 2. Fig. 11A-B shows the reducing and non-reducing SDS-PAGE of construct 4. On reducing SDS-PAGE (Fig. 11A), we observed a band at approximately 25 kDa (lanes 2 and 3, Fig.11A) corresponding to the wt Fc domain monomer and a band at 50 kDa corresponding to the wt-12-wt Fc2 tandem dimer (lanes 1-3, Fig. 11A). On non-reducing SDS-PAGE (Fig. 11B), lanes 2 and 3 each contain the final protein product of construct 4 in higher (1/2) and lower (1/3) protein amounts, respectively. We observed one major band at approximately 100 kDa corresponding to the association of wt-12-wt Fc2 tandem dimer with two wt Fc domain monomers to form construct 4, and another major band of approximately equal signal intensity at approximately 50 kDa corresponding to free wt-12-wt Fc2 tandem dimer that is not joined with wt Fc domain monomers.

In addition, we observed higher molecular weight bands at approximately 150 kDa, 200 kDa and 250 kDa (lanes 2 and 3, Fig. 11B) corresponding to multimers of wt-12-wt Fc2 and wt Fc domain monomer.

**Example 4 – Preparation and SDS-PAGE analysis of construct 6**

Two plasmid constructs, protuberance-20-protuberance Fc2 (DNA plasmid construct G in Example 1) and cavity Fc (DNA plasmid construct C in Example 1), were used to express construct 6 (Fig. 6). The two plasmid constructs were transfected into HEK 293 cells for protein expression and purification as described in Example 2. Figs. 12A-12B show the reducing and non-reducing SDS-PAGE of construct 6. On reducing SDS-PAGE (Fig. 12A), we observed a band at approximately 25 kDa (lanes 2 and 3, Fig. 12A) corresponding to the cavity Fc domain monomer and a band at 50 kDa corresponding to the protuberance-20-protuberance Fc2 tandem dimer (lanes 1-3, Fig. 12A). On non-reducing SDS-

PAGE (Fig. 12B), lanes 2 and 3 each contain the final protein product of construct 6 in higher (1/2) and lower (1/3) protein amounts. We observed one major band at approximately 100 kDa corresponding to the association of the protuberance-20-protuberance Fc2 tandem dimer with two cavity Fc domain monomers and a minor band of weaker signal intensity at approximately 50 kDa corresponding to free protuberance-20-protuberance Fc2 tandem dimer that was not combined with any cavity Fc domain monomer.

A similar experiment was performed with construct 5 (Fig. 13). Two plasmid constructs, protuberance-20-charges Fc2 (DNA plasmid construct F in Example 1) and cavity Fc (DNA plasmid construct C in Example 1), were used to express construct 5 (Fig. 5). The two plasmid constructs were transfected into EXPI293 cells at empirically determined ratios by cationic lipid transfection. The transfected cultures are incubated in cell culture media for 6-8 days. After this time, the cells were removed by centrifugation. The supernatant (media, lane 1 of Fig. 13) contains construct 5 which was secreted by the transfected cells into the media. There are also contaminating host cell proteins in the media. Construct 5 was purified from the media by Protein-A affinity chromatography. At this point, the media contained the desired construct 5 having three Fc domains (trimer) as well as a some proportion of misassembled proteins having two Fc domains (dimer, about 10-15%) and one Fc domain (monomer, 5-10%). There was also a small amount of contaminating host cell proteins still present. The Protein A column eluate was buffer exchanged, concentrated, and fractionated by Strong Cation Exchange (SCX) chromatography. Briefly, construct 5 was bound to the SCX column and then eluted with a salt and pH gradient. This step enabled separation of the desired construct 5 having three Fc domains from most of the misassembled proteins having two or one Fc domain, from construct 5 having unwanted post translational modifications, and from contaminating host cell proteins. After another round of concentration and buffer exchange, a pure, final protein product of construct 5 was obtained (pure, lane 2 of Fig. 13).

Fig. 13 depicts an SDS-PAGE of media obtained from cultured host cells engineered to express construct 5 (lane 1), and of purified construct 5 (lane 2). Also shown is a table showing the percentages of the major bands of the SDS-PAGE for each sample. In the media sample (lane 1), a major band at approximately 150 kDa was observed, corresponding to the final protein product of construct 5 having three Fc domains. The media sample also contained a minor band of weaker signal intensity at 100 kDa corresponding to a protein having two Fc domains, and a second minor band of weakest signal intensity at 50 kDa corresponding a protein having one Fc domain. After purification (lane 2), the major band at approximately 150 kDa, corresponding to the final protein product of construct 5 having three Fc domains is enriched. Quantification of the signal intensities of the protein bands on the SDS-PAGE of construct 5 showed that, in the culture media, before protein purification, about 79% of the total protein was the desired protein product of construct 5. After protein purification, a substantially homogenous population of construct 5 having about 95% purity was obtained.

These findings demonstrate that the selectivity dimerization module containing either an engineered protuberance or an engineered cavity in the $C_H3$ antibody constant domain reduces self-association and prevents uncontrolled Fc-mediated aggregate or multimer formation, indicating that the use of dimerization selectivity modules in the constructs described herein can be used to produce substantially homogenous preparations of the Fc constructs. This observation has significant implications

for advantages in manufacturing, yield, and purity of the constructs, e.g., in order to control biological activity and potency.

**Example 5 – Binding affinity and avidity**

The binding of constructs to multiple Fcγ receptors was assessed using cell-based FRET competition assays (Cisbio Bioassays). Constructs 5 and 6 showed at least a ten-fold decrease in IC50 (i.e. increased binding) to FcγRIIa, FcγRIIb, and FcγRIIIa relative to the wild type Fc domain (construct 1).

**Example 6 – Monocyte activation and blocking assays**

Three Fc constructs, constructs 1, 5, and 6, containing one, three, and two Fc domains, respectively, were tested for their ability to activate THP-1 monocytes on their own. IL-8 release was used as an indicator of monocyte activation. Constructs 1, 5, and 6 were expressed and purified as described in Examples 1 and 2. Each of the purified Fc constructs was added to THP-1 monocytes. No substantial IL-8 release was observed for any of the three constructs. The data are provided in Fig. 14A.

The same three Fc constructs were then tested for their ability to inhibit Fc receptor-mediated monocyte activation. IgG1 (100 μg/mL) was immobilized on a 96 well plate and used to induce IL-8 release by THP-1 monocytes. Serial dilutions of constructs 1, 5 and 6 or control substances (intravenous immunoglobulin (IVIg), human serum albumin (HSA), and glycine buffer) were subsequently performed in the tissue culture plate. THP-1 monocytes ($1.5 \times 10^5$ cells) were immediately added with thorough mixing. The cultures were incubated for 18 h and the supernatants analyzed for IL-8. Constructs 5 and 6 were found to inhibit IL-8 release more effectively than construct 1 at low doses. The data are provided in Fig. 14B.

**Example 7 – K/BxN arthritis model**

Fc constructs 1, 5, and 6 and IVIg were tested for their ability to protect mice from joint inflammation in the K/BxN serum transfer model using a method described in Anthony, Proc. Natl. Acad. Sci. U.S.A. 105:19571-19578 (2008). Twelve-week old K/BxN mice were generated/purchased from Jackson Laboratories. A total of thirty C57BLmice were separated into five groups of six mice each. Each group was injected intravenously (i.v.) with 200 µl construct 6 at 0.1 g/kg, 200 µl construct 5 at 0.1 g/kg, 200 µl IVIg at 0.1 g/kg, 230 µl IVIg at 1 g/kg, or 200 µl phosphate-buffered saline (PBS) one hour before injection of 200 µl K/BxN serum (an arthritis inducing serum) (Day 0). Inflammation was scored by clinical examination of paw swelling and ankle thickness. For paw swelling, each paw was scored 0-3 (0, no swelling; 3, maximal swelling). Scores of four paws were added for total clinical score per individual mouse. For ankle thickness, caliper measurement was used. Each mouse was scored daily from Day 0 to Day 10. The daily average clinical score for each group of six mice was plotted in Fig. 15.
As shown in Fig. 15, IVIg at 1 g/kg, construct 5 at 0.1 g/kg, and construct 6 at 0.1 g/kg provided similar level of inflammation protection. Given that constructs 5 and 6 were administered at ten-fold lower dose compared to the dose of IVIg, constructs 5 and 6 appear to be more potent than IVIg.

**Example 8 – Chronic ITP model**

Constructs 1 and 5, as well as IVIg, were tested for their ability to treat mice undergoing immune thrombocytopenia (ITP). ITP was induced by an anti-platelet Ab that causes platelet depletion. Forty five C57BL/6 mice (18-22 g, Charles Rivers Labs, MA) were injected i.p. with 1.5 µg/mouse of rat anti-CD41 antibody (Ab) (clone MWReg30 BioLegend cat#133910) once daily for 4 days (on days 1, 2, 3 and 4). Five mice were injected with 1.5 µg/mouse of a rat IgG1, k isotype control Ab (BioLegend cat# 400414) to determine normal platelet levels. Abs were injected in 100 µl of PBS. All mice were dosed once intravenously with 200 µl of either saline control, IVIg at 1 g/kg, construct 1 at 0.02, 0.03, 0.1, and 0.3 g/kg, and construct 5 at 0.004, 0.02, and 0.1 g/kg 2 h after the third anti-CD41 Ab injection on day 3. Mice were bled on day 5 (24 h after the forth anti-CD41 Ab injection) to quantitate total platelet levels by the VetScan Instrument. All procedures were performed in compliance with the Animal Welfare Act and with the Guide for the Care and Use of Laboratory Animals.

As shown in Fig. 16, platelet levels were significantly increased after therapeutic treatment with construct 5 at 0.02 and 0.1 g/kg when compared to saline control (**** $p < 0.0001$ by One-way ANOVA with multiple comparisons test). Platelet levels in these groups were similar to the levels in the normal, isotype treated-group. Therapeutic treatment with IVIg at 1 g/kg and construct 1 at 0.1 and 0.3 g/kg, also significantly increased platelet levels when compare to saline control (* $p < 0.05$; ** $p<0.01$ respectively by One-way ANOVA with multiple comparisons test) but platelet levels in these groups were lower than in the 0.02 and 0.1 g/kg construct 5 treated-groups. In this model, construct 5 appears to be about 50-fold more potent than IVIg.

**Example 9 – Construct 5* shows augmented binding and avidity to FcγR compared to IVIg**

Following the same protocol as described in Example 8, two plasmid constructs, encoding protuberance-20-charges Fc2* (construct F* in Example 1) and cavity Fc* (construct C* in Example 1), were used to express and purify construct 5*. The binding profile of this construct to various Fc receptors was compared to that of IVIG in a fluorescence resonance energy transfer (FRET) competitive binding assay.

Construct 5* displayed an overall binding profile to the different Fcγ-receptors similar to that of IVIg (with the lowest binding affinity observed for FcγRIIb) but with greatly enhanced binding to all low affinity FcγRs when compared to IVIg. Augmented binding to FcγR corresponds to higher avidity, which refers to the cumulative effect of the accumulated affinities of each individual binding interaction. IC50 values for construct 5* were consistently lower than those of IVIg, indicating striking increases in binding to low affinity FcγRs compared to individual IgG molecules. For example, compared to IVIg, construct 5* displayed approximately 170 fold increased affinity FcγRIIa (H131 variant), 55 fold increased affinity for FcγRIIb.

**Example 10 – Inhibition of Phagocytosis in THP-1 Monocytic cells**

Construct 5* and IVIg were tested in a model of phagocytosis.

Phagocytosis is the process by which cells (phagocytes) engulf solid particles such as bacteria, to form an internal vesicle known as a phagosome. In the immune system, phagocytosis is a major mechanism used to remove pathogens and cell debris. Monocytes and macrophages are among the cells

specialized in clearing opsonized (antibody coated) particles from the immune system through phagocytosis, a mechanism largely dependent on FcγR mediated engagement. However, in autoimmune diseases, phagocytes can become activated leading to the detrimental release of pro-inflammatory cytokines or the phagocytosis of other critical cells in the body. IVIg, containing pooled, polyvalent, IgG antibodies extracted from the plasma of over one thousand blood donors, is used to treat autoimmune disease.

In this assay system, fluorescently labeled antibody-coated latex beads, a mimic of opsonized bacteria or viruses, were fed to THP-1 cells and allowed to be phagocytosed in the presence and absence of construct 5* and IVIg. At the end of the incubation period, any external fluorescence was quenched with trypan blue, and the amount of intracellular fluorescence quantified by flow cytometry. All groups were normalized to their non-treated control (THP-1 cells and latex beads only). Results are representative of two separate experiments.

As shown in Fig. 17, the phagocytosis of opsonized beads by THP-1 monocytic cells is inhibited by treatment with both IVIg and construct 5*, but the IC50 value for construct 5* is approximately 100-fold lower than for IVIg. This suggests that an Fc construct of the invention, e.g., construct 5*, can be used to treat autoimmune indications, as well as other indications that are treatable using IVIg.

## CLAIMS

1. An Fc construct comprising:
   a). a first polypeptide having the formula A-L-B; wherein
      i). A comprises a first Fc domain monomer;
      ii). L is a linker; and
      iii). B comprises a second Fc domain monomer;
   b). a second polypeptide comprising a third Fc domain monomer; and
   c). a third polypeptide comprising a fourth Fc domain monomer;
   wherein said first Fc domain monomer and said third Fc domain monomer combine to form a first Fc domain and said second Fc domain monomer and said fourth Fc domain monomer combine to form a second Fc domain.

2. The Fc construct of claim 1, wherein said first Fc domain monomer and said third Fc domain monomer comprise complementary dimerization selectivity modules that promote dimerization between said first Fc domain monomer and said third Fc domain monomer.

3. The Fc construct of claim 1 or 2, wherein said second Fc domain monomer and said fourth Fc domain monomer comprise complementary dimerization selectivity modules that promote dimerization between said second Fc domain monomer and said fourth Fc domain monomer.

4. The Fc construct of any one of claims 1-3, wherein A consists of an Fc domain monomer.

5. The Fc construct of any one of claims 1-4, wherein B consists of an Fc domain monomer.

6. The Fc construct of any one of claims 1-5, wherein said second polypeptide consists of an Fc domain monomer.

7. The Fc construct of any one of claims 1-6, wherein said third polypeptide consists of an Fc domain monomer.

8. The Fc construct of any one of claims 1-7, further comprising an albumin-binding peptide joined to the N-terminus or C-terminus of one or more of the polypeptides by way of a linker.

9. The Fc construct of any one of claims 1-8, further comprising an IgG $C_L$ antibody constant domain and an IgG $C_H1$ antibody constant domain, wherein said IgG $C_H1$ antibody constant domain is attached to the N-terminus of A or said second polypeptide by way of a linker.

10. The Fc construct of any one of claims 1-9, wherein said second polypeptide and said third polypeptide have the same amino acid sequence.

11. An Fc construct comprising:
   a). a first polypeptide having the formula A-L-B; wherein
      i). A comprises a first Fc domain monomer;
      ii). L is a linker; and
      iii). B comprises a second Fc domain monomer;

b). a second polypeptide having the formula A'-L'-B'; wherein

i). A' comprises a third Fc domain monomer;

ii). L' is a linker; and

iii). B' comprises a fourth Fc domain monomer;

c). a third polypeptide comprises a fifth Fc domain monomer; and

d). a fourth polypeptide comprises a sixth Fc domain monomer;

wherein said A of first polypeptide and said A' of second polypeptide combine to form a first Fc domain, said B of first polypeptide and fifth Fc domain monomer combine to form a second Fc domain, and said B' of second polypeptide and sixth Fc domain monomer combine to form a third Fc domain.

12. The Fc construct of claim 11, wherein said first Fc domain monomer and said third Fc domain monomer comprise complementary dimerization selectivity modules that promote dimerization between said first Fc domain monomer and said third Fc domain monomer.

13. The Fc construct of claim 11 or 12, wherein said second Fc domain monomer and said fifth Fc domain monomer comprise complementary dimerization selectivity modules that promote dimerization between said second Fc domain monomer and said fifth Fc domain monomer.

14. The Fc construct of any one of claims 11-13, wherein said fourth Fc domain monomer and said sixth Fc domain monomer comprise complementary dimerization selectivity modules that promote dimerization between said fourth Fc domain monomer and said sixth Fc domain monomer.

15. The Fc construct of any one of claims 11-14, wherein A consists of an Fc domain monomer.

16. The Fc construct of any one of claims 11-15, wherein B consists of an Fc domain monomer.

17. The Fc construct of any one of claims 11-16, wherein A' consists of an Fc domain monomer.

18. The Fc construct of any one of claims 11-17, wherein B' consists of an Fc domain monomer.

19. The Fc construct of any one of claims 11-18, wherein said third polypeptide consists of an Fc domain monomer.

20. The Fc construct of any one of claims 11-19, wherein said fourth polypeptide consists of an Fc domain monomer.

21. The Fc construct of any one of claims 11-20, further comprising an IgG $C_L$ antibody constant domain and an IgG $C_H1$ antibody constant domain, wherein said IgG $C_L$ antibody constant domain is attached to the N-terminus of said IgG $C_H1$ antibody constant domain by way of a linker and said IgG $C_H1$ antibody constant domain is attached to the N-terminus of A by way of a linker.

22. The Fc construct of claim 21, further comprising a second IgG $C_L$ antibody constant domain and a second IgG $C_H1$ antibody constant domain, wherein said second IgG $C_L$ antibody constant domain is attached to the N-terminus of said second IgG $C_H1$ antibody constant domain by way of a linker and said second IgG $C_H1$ antibody constant domain is attached to the N-terminus of A' by way of a linker.

said IgG $C_H1$ antibody constant domain is attached to the N-terminus of A or A' by way of a linker.

23. The Fc construct of any one of claims 11-21, further comprising an albumin-binding peptide.

24. The Fc construct of any one of claims 11-22, wherein said first polypeptide and said second polypeptide have the same amino acid sequence and wherein said third polypeptide and said fourth polypeptide have the same amino acid sequence.

25. An Fc construct comprising:

a). a first polypeptide having the formula A-L-B; wherein

i). A comprises a first Fc domain monomer;

ii). L is a linker; and

iii). B comprises an albumin binding peptide; and

b). a second polypeptide comprising a second Fc domain monomer;

wherein said first Fc domain monomer and said second Fc domain monomer combine to form an Fc domain.

26. The Fc construct of claim 25, wherein said first Fc domain monomer and said second Fc domain monomer comprise complementary dimerization selectivity modules that promote dimerization between said first Fc domain monomer and said second Fc domain monomer.

27. The Fc construct of claim 25 or 26, wherein A consists of an Fc domain monomer.

28. The Fc construct of any one of claims 25-27, wherein second polypeptide consists of an Fc domain monomer.

29. An Fc construct comprising:

a). a first polypeptide having the formula A-L1-B-L2-C; wherein

i). A comprises an IgG $C_L$ antibody constant domain;

ii). L1 and L2 are each a linker;

iii) B comprises an IgG $C_H1$ antibody constant domain; and

iv). C comprises a first Fc domain monomer; and

b). a second polypeptide having the formula A'-L1'-B'-L2'-C'; wherein

i). A' comprises an IgG $C_L$ antibody constant domain;

ii). L1' and L2' are each a linker;

iii) B' comprises an IgG $C_H1$ antibody constant domain; and

iv). C' comprises a second Fc domain monomer; wherein

said first Fc domain monomer and said second Fc domain monomer combine to form an Fc domain.

30. The Fc construct of claim 29, wherein said first Fc domain monomer and said second Fc domain monomer comprise complementary dimerization selectivity modules that promote dimerization between said first Fc domain monomer and said second Fc domain monomer.

31. The Fc construct of claim 29 or 30, wherein C consists of an Fc domain monomer.

32. The Fc construct of any one of claims 29-31, wherein C' consists of an Fc domain monomer.

33. The Fc construct of any one of claims 29-32, further comprising an albumin-binding peptide joined to the C-terminus of C or C' by way of a linker.

34. An Fc construct comprising:

a). a first polypeptide having the formula A-L1-B-L2-C; wherein

i). A comprises an IgG $C_L$ antibody constant domain;

ii). L1 and L2 are each a linker;

iii) B comprises an IgG $C_H1$ antibody constant domain; and

iv). C comprises a first Fc domain monomer; and

b). a second polypeptide having the formula A'-L1'-B'-L2'-C'; wherein

i). A' comprises an IgG $C_L$ antibody constant domain;

ii). L1' and L2' are each a linker;

iii) B' comprises an IgG $C_H1$ antibody constant domain; and

iv). C' comprises a second Fc domain monomer;

c).a third polypeptide comprising a third Fc domain monomer; and

d). a fourth polypeptide comprising a fourth Fc domain monomer; wherein

said first Fc domain monomer and said third Fc domain monomer combine to form a first Fc domain;

said second Fc domain monomer and fourth third Fc domain monomer combine to form a second Fc domain;

the IgG $C_H1$ antibody constant domain of said first polypeptide combines with the IgG $C_L$ antibody constant domain of said second polypeptide; and

the IgG $C_H1$ antibody constant domain of said second polypeptide combines with the IgG $C_L$ antibody constant domain of said first polypeptide.

35. An Fc construct comprising:

a). a first polypeptide comprising a first Fc domain monomer; and

b). a second polypeptide comprising a second Fc domain monomer; wherein

said first Fc domain monomer and said second Fc domain monomer combine to form an Fc domain, and said first Fc domain monomer and said second Fc domain monomer comprise complementary dimerization selectivity modules that promote dimerization between said first Fc domain monomer and said second Fc domain monomer.

36. The Fc construct of claim 35, wherein said first polypeptide consists of an Fc domain monomer.

37. The Fc construct of claim 35 or 36, wherein said second polypeptide consists of an Fc domain monomer.

38. The Fc construct of any one of claims 35-37, further comprising an albumin-binding peptide.

39. The Fc construct of any one of claims 35-38, further comprising an IgG $C_L$ antibody constant domain and an IgG $C_H1$ antibody constant domain, wherein said IgG $C_H1$ antibody constant domain is attached to the N-terminus of said first or second polypeptide by way of a linker.

40. An Fc construct comprising:

a). a first polypeptide having the formula A-L-B; wherein

i). A comprises a first Fc domain monomer;

ii). L is a linker; and

iii). B comprises a second Fc domain monomer;

b). a second polypeptide having the formula A'-L'-B'; wherein

i). A' comprises a third Fc domain monomer;

ii). L' is a linker; and

iii). B' comprises a fourth Fc domain monomer;

wherein said first and second Fc domain monomer comprise an engineered cavity into their respective $C_H3$ domains and said third and fourth Fc domain monomer comprise an engineered protuberance into their respective $C_H3$ domains, wherein said engineered cavity and said engineered protuberance are positioned to form a protuberance-into-cavity pair, wherein said first Fc domain monomer and said third Fc domain monomer combine to form a first Fc domain and said second Fc domain monomer and said fourth Fc domain monomer combine to form a second Fc domain.

41. The Fc construct of claim 40, wherein each of said A, B, A', or B' consists of an Fc domain monomer.

42. The Fc construct of claim 40 or 41, further comprising an albumin-binding peptide joined to the C-terminus of B or B' by way of a linker.

43. The Fc construct of any one of claims 40-42, further comprising an IgG $C_L$ antibody constant domain and an IgG $C_H1$ antibody constant domain, wherein said IgG $C_H1$ antibody constant domain is attached to the N-terminus of A or A' by way of a linker.

44. The Fc construct of any one of claims 2, 3, 12, 13, 14, 26, 30, or 35, wherein said dimerization selectivity modules comprise an engineered cavity into the $C_H3$ domain of one of said Fc domain monomers and an engineered protuberance into the $C_H3$ domain of the other of said Fc domain monomers, wherein said engineered cavity and said engineered protuberance are positioned to form a protuberance-into-cavity pair of Fc domain monomers.

45. The Fc construct of claim 44, wherein one of said Fc domain monomers comprises Y407V and Y349C and the other of said Fc domain monomers comprises T366W and S354C.

46. The Fc construct of any one of claims 2, 3, 12, 13, 14, 26, 30, or 35, wherein said dimerization selectivity modules comprise a negatively-charged amino acid into the $C_H3$ domain of one of said domain monomers and a positively-charged amino acid into the $C_H3$ domain of the other of said Fc domain monomers, wherein said negatively-charged amino acid and said positively-charged amino acid are positioned to promote formation of an Fc domain.

47. The Fc construct of claim 46, wherein one of said Fc domain monomers comprises D399K and the other of said Fc domain monomers comprises K409D.

48. The Fc construct of any one of claims 1-47, wherein one or more linker in said Fc construct is a bond.

49. The Fc construct of any one of claims 1-47, wherein one or more linker in said Fc construct is a spacer.

50. The Fc construct of claim 49, wherein said spacer comprises the sequence of any one of SEQ ID NOs: 1-27.

51. The Fc construct of claim 50, wherein said sequence consists of any one of SEQ ID NOs: 1, 2, and 3.

52. The Fc construct of any one of claims 8, 22, 25, 33, 38, and 42, wherein said albumin-binding peptide comprises the sequence of SEQ ID NO: 28.

53. The Fc construct of claim 52, wherein said albumin-binding peptide consists of the sequence of SEQ ID NO: 28.

54. An Fc construct comprising:

a). a first polypeptide having the formula A-L1-B-L2-C; wherein

i). A comprises a first Fc domain monomer;

ii). L1 is a linker;

iii). B comprises a second Fc domain monomer;

iv). L2 is a linker;

v). C comprises a third Fc domain monomer; and

b). a second polypeptide having the formula A'-L1'-B'-L2'-C'; wherein

i). A' comprises a fourth Fc domain monomer;

ii). L1' is a linker;

iii) B' comprises a fifth Fc domain monomer;

iv). L2' is a linker;

v). C' comprises a sixth Fc domain monomer; wherein

said first Fc domain monomer and said second Fc domain monomer combine to form a first Fc domain, said fourth Fc domain monomer and said fifth Fc domain monomer combine to form a second Fc domain, and said third Fc domain monomer and said sixth Fc domain monomer combine to form a third Fc domain.

55. The Fc construct of claim 54, wherein linkers L1 and L1' are cleavable linkers.

56. The Fc construct of any one of claims 1-55, wherein one or more of said Fc domain monomers comprises an IgG hinge domain, an IgG $C_H2$ antibody constant domain, and an IgG $C_H3$ antibody constant domain.

57. The Fc construct of claim 56, wherein each of said Fc domain monomers comprises an IgG hinge domain, an IgG $C_H2$ antibody constant domain, and an IgG $C_H3$ antibody constant domain.

58. The Fc construct of any claim 56 or 57, wherein said IgG is of a subtype selected from the group consisting of IgG1, IgG2a, IgG2b, IgG3, and IgG4.

59. A method of preparing an Fc construct of any one of claims 1-58, said method comprising:

a) providing a host cell comprising polynucleotides encoding said polypeptides;

b) expressing said first, second, and third polypeptides in said host cell under conditions that allow for the formation of said Fc construct; and

c) recovering said Fc construct.

60. A host cell that expresses an Fc construct of any one of claims 1-58, said host cell comprising polynucleotides encoding said polypeptides, wherein said polynucleotides are expressed in said host cell.

61. A pharmaceutical composition comprising a substantially homogenous population of an Fc construct having 2-10 Fc domains.

62. The pharmaceutical composition of claim 61, wherein a plurality of the 2-10 Fc domains are formed by association of two Fc domain monomers comprising complementary dimerization selectivity modules that promote dimerization between two Fc domain monomers.

63. The pharmaceutical composition of claim 62, wherein the two separate polypeptides of the plurality have the same amino acid sequence.

64. The pharmaceutical composition of claim 62, wherein the two separate polypeptides of the plurality have different amino acid sequences.

65. The pharmaceutical composition of claim 62, wherein the complementary Fc monomer sequences have complementary dimerization selectivity modules.

66. The pharmaceutical composition of claim 61, wherein the Fc construct has one or more (e.g., 2, 3, 4, 5 or 6) of the following characteristics:

a) is formed at least in part by association of Fc domain monomers that are present in different polypeptides;

b) does not include an additional domain that promotes association of two polypeptides;

c) comprises covalent linkages only between two Fc domain monomers that join to form Fc domains;

d) does not comprise covalent linkages between Fc domains;

e) provides for sufficient flexibility such that all or substantially all of the Fc domains in the Fc construct are capable of simultaneously interacting with an Fc receptor on a cell surface; and/or

f) comprises at least two Fc domains joined through a linker.

67. A pharmaceutical composition comprising a substantially homogenous population of Fc constructs of any one of claims 1-58.

68. The pharmaceutical composition of any one of claims 61-67, wherein the Fc construct comprises 2-5 associated polypeptides, each polypeptide comprising at least one Fc domain monomer, wherein each Fc domain monomer of the construct is the same or differs by no more than 10 amino acids.

69. The pharmaceutical composition of any one of claims 61-67, wherein the Fc construct comprises two Fc domains comprising three associated polypeptides, one of the three polypeptides comprising two Fc domain monomers and two of the three polypeptides each comprising one Fc domain monomer.

70. The pharmaceutical composition of any one of claims 61-67, wherein the Fc construct comprises three Fc domains comprising four associated polypeptides, two of the four polypeptides each comprising two Fc domain monomer and two of the four polypeptides each comprising one Fc domain monomer.

71. The pharmaceutical composition of any one of claims 61-67, wherein the Fc construct consists of n Fc domains comprising 2n polypeptides, each polypeptide comprising an Fc domain monomer, an IgG $C_L$ antibody constant domain, and an IgG $C_H1$ antibody constant domain.

72. A method of treating inflammation in a subject, said method comprising administering to said subject a pharmaceutical composition comprising therapeutically effective amount of an Fc construct of any one of claims 1-58 or a pharmaceutical composition of any one of claims 61-71.

73. A method of reducing immune complex-based activation of the immune response in a subject, said method comprising administering to said subject an Fc construct of any one of claims 1-58 or a pharmaceutical composition of any one of claims 61-71.

74. A method of claim 73, wherein said subject has an autoimmune disease.

75. An Fc construct consisting of

a). a first polypeptide having the formula A-L-B; wherein

i). A comprises a first Fc domain monomer;

ii). L is a linker; and

iii). B comprises a second Fc domain monomer;

b). a second polypeptide having the formula A'-L'-B'; wherein

i). A' comprises a third Fc domain monomer;

ii). L' is a linker; and

iii). B' comprises a fourth Fc domain monomer;

c). a third polypeptide that comprises a fifth Fc domain monomer; and

d). a fourth polypeptide that comprises a sixth Fc domain monomer;

wherein A and A' combine to form a first Fc domain, B and said fifth Fc domain monomer combine to form a second Fc domain, and B' and said sixth Fc domain monomer combine to form a third Fc domain;

wherein each of said first and third Fc domain monomers comprises a complementary dimerization selectivity module that promote dimerization between said first Fc domain monomer and said third Fc domain monomer,

each of said second and fifth Fc domain monomers comprises a complementary dimerization selectivity module that promote dimerization between said second Fc domain monomer and said fifth Fc domain monomer, and

each of said fourth and sixth Fc domain monomers comprises a complementary dimerization selectivity module that promote dimerization between said fourth Fc domain monomer and said sixth Fc domain monomer; and

wherein said Fc construct contains no more than three Fc domains.

76. An Fc construct consisting of

a). a first polypeptide having the formula A-L-B; wherein

i). A consists of a first Fc domain monomer;

ii). L is a linker; and

iii). B consists of a second Fc domain monomer;

b). a second polypeptide having the formula A'-L'-B'; wherein

i). A' consists of a third Fc domain monomer;

ii). L' is a linker; and

iii). B' consists of a fourth Fc domain monomer;

c). a third polypeptide that consists of a fifth Fc domain monomer; and

d). a fourth polypeptide that consists of a sixth Fc domain monomer;

wherein A and A' combine to form a first Fc domain, B and said fifth Fc domain monomer combine to form a second Fc domain, and B' and said sixth Fc domain monomer combine to form a third Fc domain;

wherein each of said first and third Fc domain monomers comprises a complementary dimerization selectivity module that promote dimerization between said first Fc domain monomer and said third Fc domain monomer,

each of said second and fifth Fc domain monomers comprises a complementary dimerization selectivity module that promote dimerization between said second Fc domain monomer and said fifth Fc domain monomer, and

each of said fourth and sixth Fc domain monomers comprises a complementary dimerization selectivity module that promote dimerization between said fourth Fc domain monomer and said sixth Fc domain monomer; and

wherein said Fc construct contains no more than three Fc domains.

77. The Fc construct of claim 75 or 76, wherein said complementary dimerization selectivity module of said first Fc domain monomer comprises a negatively-charged amino acid substitution, said complementary dimerization selectivity module of said third Fc domain monomer comprises a positively-charged amino acid substitution, said complementary dimerization selectivity module of said second Fc domain monomer comprises an engineered protuberance, said complementary dimerization selectivity module of said fifth Fc domain monomer comprises an engineered cavity, said complementary dimerization selectivity module of said fourth Fc domain monomer comprises an engineered protuberance, and said complementary dimerization selectivity module of said sixth Fc domain monomer comprises an engineered cavity.

78. The Fc construct of any one of claims 75-77, wherein said linker L and/or L' is 3-200 amino acids in length.

79. The Fc construct of claim 77, wherein said linker L and/or L' consists of the sequence of any one of SEQ ID NOs: 1, 2, and 3.

80. An Fc construct consisting of

a). a first polypeptide having the formula A-L1-B-L2-C; wherein

i). A comprises a first Fc domain monomer;

ii). L1 is a linker;

iii) B comprises a second Fc domain monomer; and

iv). L2 is a linker;

v). C comprises a third Fc domain monomer; and

b). a second polypeptide having the formula A'-L1'-B'-L2'-C'; wherein

i). A' comprises a fourth Fc domain monomer;

ii). L1' is a linker;

iii) B' comprises a fifth Fc domain monomer; and

iv). L2' is a linker;

v). C' comprises a sixth Fc domain monomer;

c). a third polypeptide that comprises a seventh Fc domain monomer;

d). a fourth polypeptide that comprises a eighth Fc domain monomer;

e). a fifth polypeptide that comprises a ninth Fc domain monomer;

d). a sixth polypeptide that comprises a tenth Fc domain monomer;

wherein A and said seventh Fc domain monomer combine to form a first Fc domain, B and B' combine to form a second Fc domain, C and said eighth Fc domain monomer combine to form a third Fc domain, A' and said ninth Fc domain monomer combine to form a fourth Fc domain, and C' and said tenth Fc domain monomer combine to form a fifth Fc domain;

wherein each of said first and seventh Fc domain monomers comprises a complementary dimerization selectivity module that promote dimerization between said first Fc domain monomer and said seventh Fc domain monomer,

each of said second and fifth Fc domain monomers comprises a complementary dimerization selectivity module that promote dimerization between said second Fc domain monomer and said fifth Fc domain monomer,

each of said third and eighth Fc domain monomers comprises a complementary dimerization selectivity module that promote dimerization between said third Fc domain monomer and said eighth Fc domain monomer;

each of said fourth and ninth Fc domain monomers comprises a complementary dimerization selectivity module that promote dimerization between said fourth Fc domain monomer and said ninth Fc domain monomer; and

each of said sixth and tenth Fc domain monomers comprises a complementary dimerization selectivity module that promote dimerization between said sixth domain monomer and said tenth Fc domain monomer; and

wherein said Fc construct contains no more than five Fc domains.

81. An Fc construct consisting of

a). a first polypeptide having the formula A-L1-B-L2-C; wherein

i). A consists of a first Fc domain monomer;

ii). L1 is a linker;

iii) B consists of a second Fc domain monomer; and

iv). L2 is a linker;

v). C consists of a third Fc domain monomer; and

b). a second polypeptide having the formula A'-L1'-B'-L2'-C'; wherein

i). A' consists of a fourth Fc domain monomer;

ii). L1' is a linker;

iii) B' consists of a fifth Fc domain monomer; and

iv). L2' is a linker;

v). C' consists of a sixth Fc domain monomer;

c). a third polypeptide that consists of a seventh Fc domain monomer;

d). a fourth polypeptide that consists of a eighth Fc domain monomer;

e). a fifth polypeptide that consists of a ninth Fc domain monomer;

d). a sixth polypeptide that consists of a tenth Fc domain monomer;

wherein A and said seventh Fc domain monomer combine to form a first Fc domain, B and B' combine to form a second Fc domain, C and said eighth Fc domain monomer combine to form a third Fc domain, A' and said ninth Fc domain monomer combine to form a fourth Fc domain, and C' and said tenth Fc domain monomer combine to form a fifth Fc domain;

wherein each of said first and seventh Fc domain monomers comprises a complementary dimerization selectivity module that promote dimerization between said first Fc domain monomer and said seventh Fc domain monomer,

each of said second and fifth Fc domain monomers comprises a complementary dimerization selectivity module that promote dimerization between said second Fc domain monomer and said fifth Fc domain monomer,

each of said third and eighth Fc domain monomers comprises a complementary dimerization selectivity module that promote dimerization between said third Fc domain monomer and said eighth Fc domain monomer;

each of said fourth and ninth Fc domain monomers comprises a complementary dimerization selectivity module that promote dimerization between said fourth Fc domain monomer and said ninth Fc domain monomer; and

each of said sixth and tenth Fc domain monomers comprises a complementary dimerization selectivity module that promote dimerization between said sixth domain monomer and said tenth Fc domain monomer; and

wherein said Fc construct contains no more than five Fc domains.

82. The Fc construct of claim 80 or 81, wherein said complementary dimerization selectivity module of said second Fc domain monomer comprises a negatively-charged amino acid substitution, said complementary dimerization selectivity module of said fifth Fc domain monomer comprises a positively-charged amino acid substitution, said complementary dimerization selectivity module of each of said first, third, fourth, and sixth Fc domain monomers comprises an engineered protuberance, and complementary dimerization selectivity module of each of said seventh, eighth, ninth, and tenth Fc domain monomers comprises an engineered cavity.

83. An Fc construct consisting of

a). a first polypeptide having the formula A-L1-B-L2-C; wherein

i). A comprises a first Fc domain monomer;

ii). L1 is a linker;

iii) B comprises a second Fc domain monomer; and

iv). L2 is a linker;

v). C comprises a third Fc domain monomer; and

b). a second polypeptide having the formula A'-L1'-B'-L2'-C'; wherein

i). A' comprises a fourth Fc domain monomer;

ii). L1' is a linker;

iii) B' comprises a fifth Fc domain monomer; and

iv). L2' is a linker;

v). C' comprises a sixth Fc domain monomer;

c). a third polypeptide that comprises a seventh Fc domain monomer;

d). a fourth polypeptide that comprises a eighth Fc domain monomer;

e). a fifth polypeptide that comprises a ninth Fc domain monomer;

d). a sixth polypeptide that comprises a tenth Fc domain monomer;

wherein A and A' combine to form a first Fc domain, B and said seventh Fc domain monomer combine to form a second Fc domain, C and said eighth Fc domain monomer combine to form a third Fc domain, B' and said ninth Fc domain monomer combine to form a fourth Fc domain, and C' and said tenth Fc domain monomer combine to form a fifth Fc domain;

wherein each of said first and fourth Fc domain monomers comprises complementary dimerization selectivity modules that promote dimerization between said first Fc domain monomer and said fourth Fc domain monomer,

each of said second and seventh Fc domain monomers comprises complementary dimerization selectivity modules that promote dimerization between said second Fc domain monomer and said seventh Fc domain monomer,

each of said third and eighth Fc domain monomers comprises complementary dimerization selectivity modules that promote dimerization between said third Fc domain monomer and said eighth Fc domain monomer;

each of said fifth and ninth Fc domain monomers comprises complementary dimerization selectivity modules that promote dimerization between said fifth Fc domain monomer and said ninth Fc domain monomer; and

each of said sixth and tenth Fc domain monomers comprises complementary dimerization selectivity modules that promote dimerization between said sixth domain monomer and said tenth Fc domain monomer; and

wherein said Fc construct contains no more than five Fc domains.

84. An Fc construct consisting of

a). a first polypeptide having the formula A-L1-B-L2-C; wherein

i). A consists of a first Fc domain monomer;

ii). L1 is a linker;

iii) B consists of a second Fc domain monomer; and

iv). L2 is a linker;

v). C consists of a third Fc domain monomer; and

b). a second polypeptide having the formula A'-L1'-B'-L2'-C'; wherein

i). A' consists of a fourth Fc domain monomer;

ii). L1' is a linker;

iii) B' consists of a fifth Fc domain monomer; and

iv). L2' is a linker;

v). C' consists of a sixth Fc domain monomer;

c). a third polypeptide that consists of a seventh Fc domain monomer;

d). a fourth polypeptide that consists of a eighth Fc domain monomer;

e). a fifth polypeptide that consists of a ninth Fc domain monomer;

d). a sixth polypeptide that consists of a tenth Fc domain monomer;

wherein A and A' combine to form a first Fc domain, B and said seventh Fc domain monomer combine to form a second Fc domain, C and said eighth Fc domain monomer combine to form a third Fc domain, B' and said ninth Fc domain monomer combine to form a fourth Fc domain, and C' and said tenth Fc domain monomer combine to form a fifth Fc domain;

wherein each of said first and fourth Fc domain monomers comprises complementary dimerization selectivity modules that promote dimerization between said first Fc domain monomer and said fourth Fc domain monomer,

each of said second and seventh Fc domain monomers comprises complementary dimerization selectivity modules that promote dimerization between said second Fc domain monomer and said seventh Fc domain monomer,

each of said third and eighth Fc domain monomers comprises complementary dimerization selectivity modules that promote dimerization between said third Fc domain monomer and said eighth Fc domain monomer;

each of said fifth and ninth Fc domain monomers comprises complementary dimerization selectivity modules that promote dimerization between said fifth Fc domain monomer and said ninth Fc domain monomer; and

each of said sixth and tenth Fc domain monomers comprises complementary dimerization selectivity modules that promote dimerization between said sixth domain monomer and said tenth Fc domain monomer; and

wherein said Fc construct contains no more than five Fc domains.

85. The Fc construct of claim 83 or 84, wherein said complementary dimerization selectivity module of said first Fc domain monomer comprises a negatively-charged amino acid substitution, said complementary dimerization selectivity module of said fourth Fc domain monomer comprises a positively-charged amino acid substitution, said complementary dimerization selectivity module of each of said second, third, fifth, and sixth Fc domain monomers comprises an engineered protuberance, and complementary dimerization selectivity module of each of said seventh, eighth, ninth, and tenth Fc domain monomers comprises an engineered cavity.

86. The Fc construct of any one of claims 80-85, wherein said linker L1, L2, L1', and/or L2' is 3-200 amino acids in length.

87. The Fc construct of claim 86, wherein said linker L1, L2, L1', and/or L2' consists of the sequence of any one of SEQ ID NOs: 1, 2, and 3.

88. An Fc construct of any one of claims 1-58 or 75-87 for use in promoting clearance of autoantibodies, suppressing antigen presentation, reducing the immune response in a subject in need thereof, or reducing immune complex-based activation of the immune response.

89. An Fc construct of any one of claims 1-58 or 75-87 for use in treating an inflammatory or autoimmune or immune disease (e.g., rheumatoid arthritis (RA); systemic lupus erythematosus (SLE); ANCA-associated vasculitis; antiphospholipid antibody syndrome; autoimmune hemolytic anemia; chronic inflammatory demyelinating neuropathy; clearance of anti-allo in transplant, anti-self in GVHD, anti-replacement, IgG therapeutics, IgG paraproteins; dermatomyositis; Goodpasture's Syndrome; organ system-targeted type II hypersensitivity syndromes mediated through antibody-dependent cell-mediated cytotoxicity, e.g., Guillain Barre syndrome, CIDP, dermatomyositis, Felty's syndrome, antibody-mediated rejection, autoimmune thyroid disease, ulcerative colitis, autoimmune liver disease; idiopathic thrombocytopenia purpura; Myasthenia Gravis, neuromyelitis optica; pemphigus and other autoimmune blistering disorders; Sjogren's Syndrome; autoimmune cytopenias and other disorders mediated through antibody-dependent phagocytosis; other FcR-dependent inflammatory syndromes, e.g., synovitis, dermatomyositis, systemic vasculitis, glomerulitis or vasculitis).

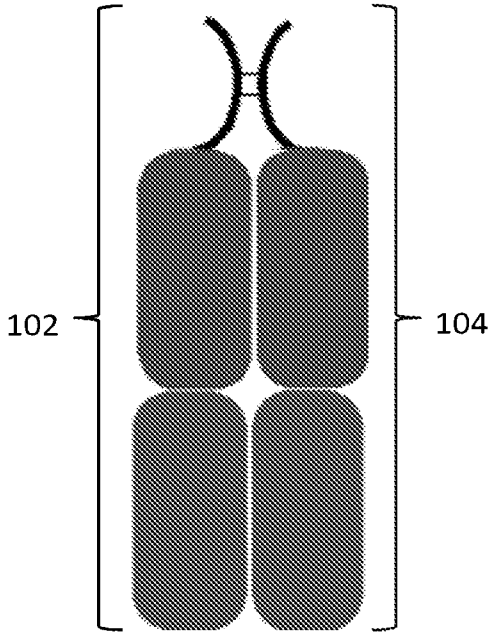



**Fig. 1 – construct 1**

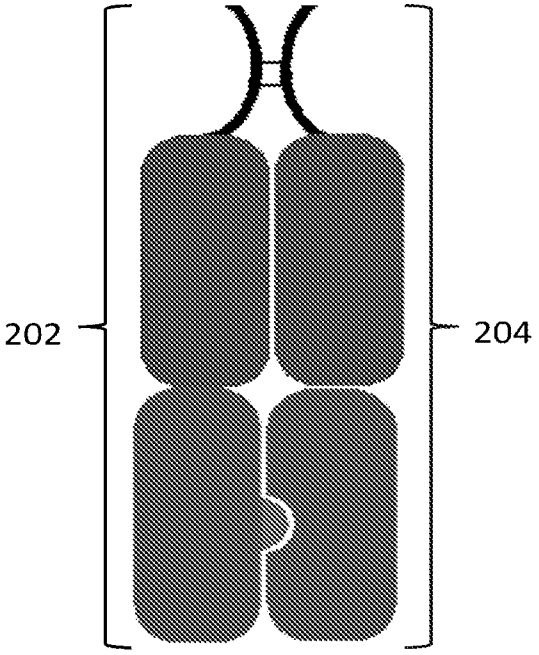



**Fig.2 – construct 2**

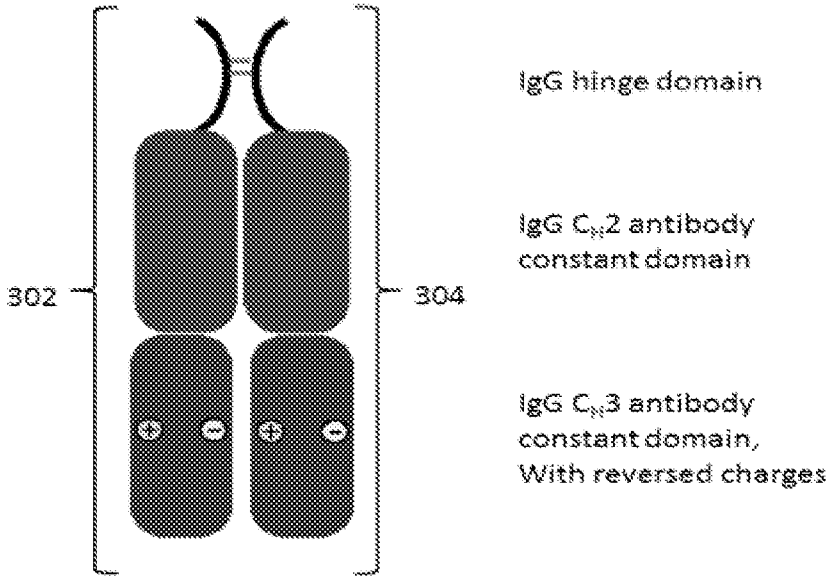



**Fig. 3 – construct 3**

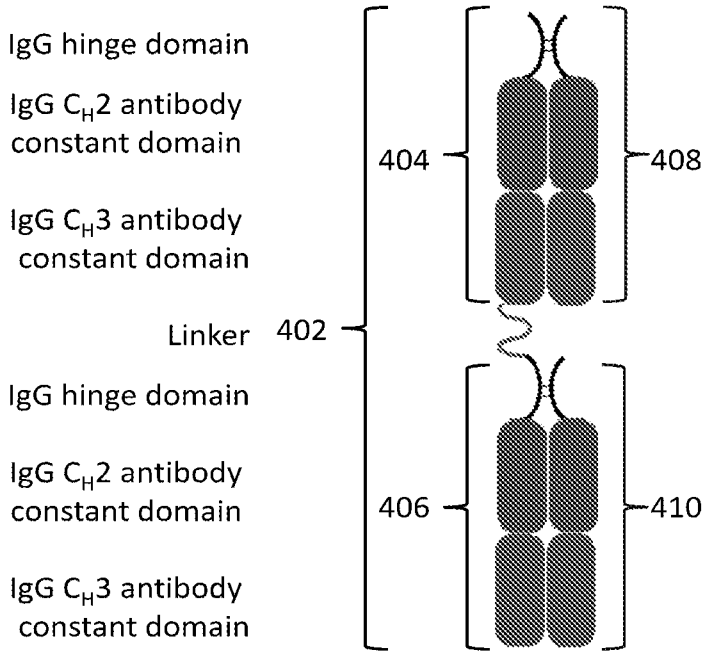



**Fig. 4 – construct 4**

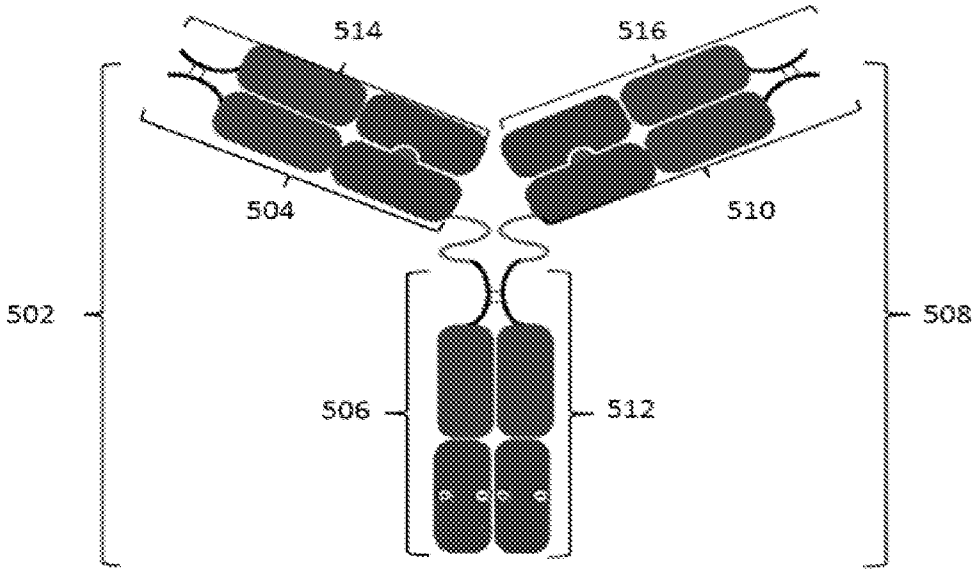



Fig. 5 – constructs 5, 5*

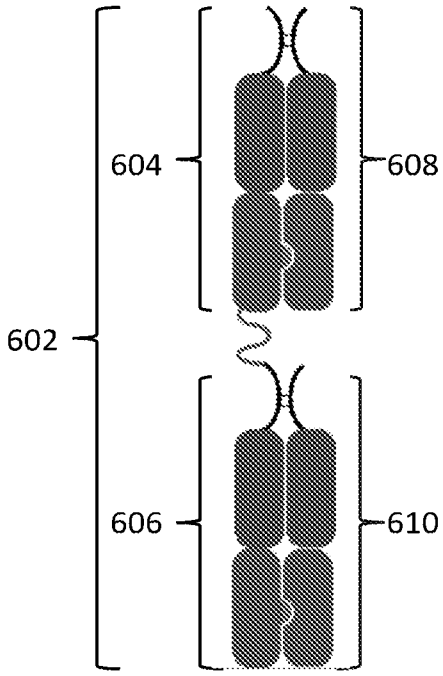



Fig. 6 – construct 6

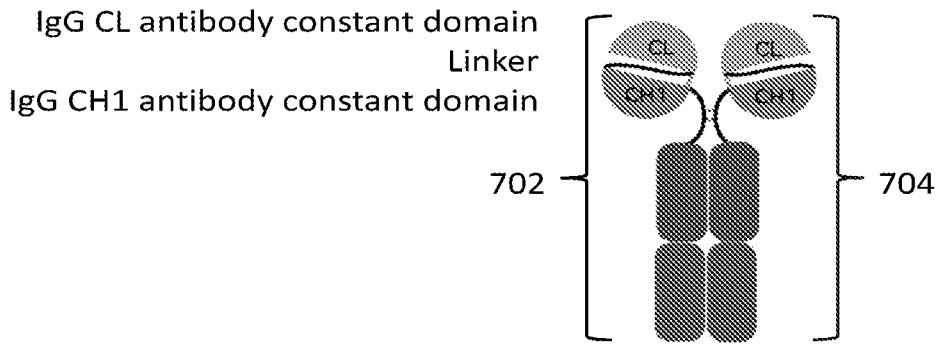



Fig. 7A – construct 7

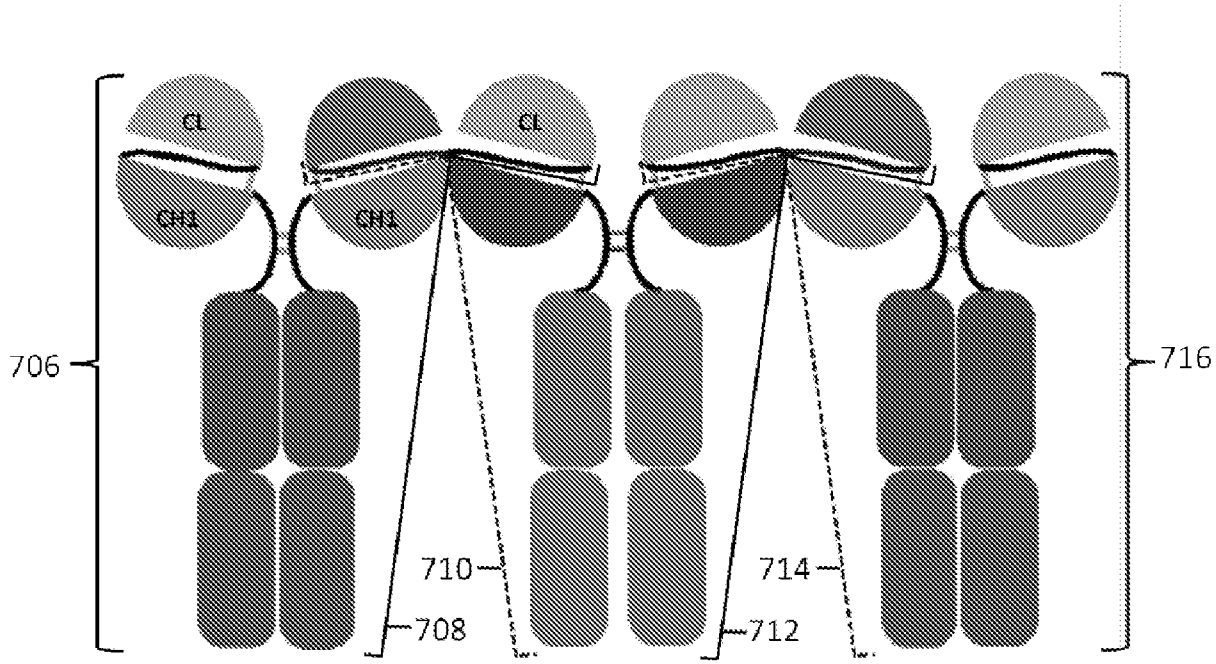



Fig. 7B – construct 8 (multimers of construct 7)

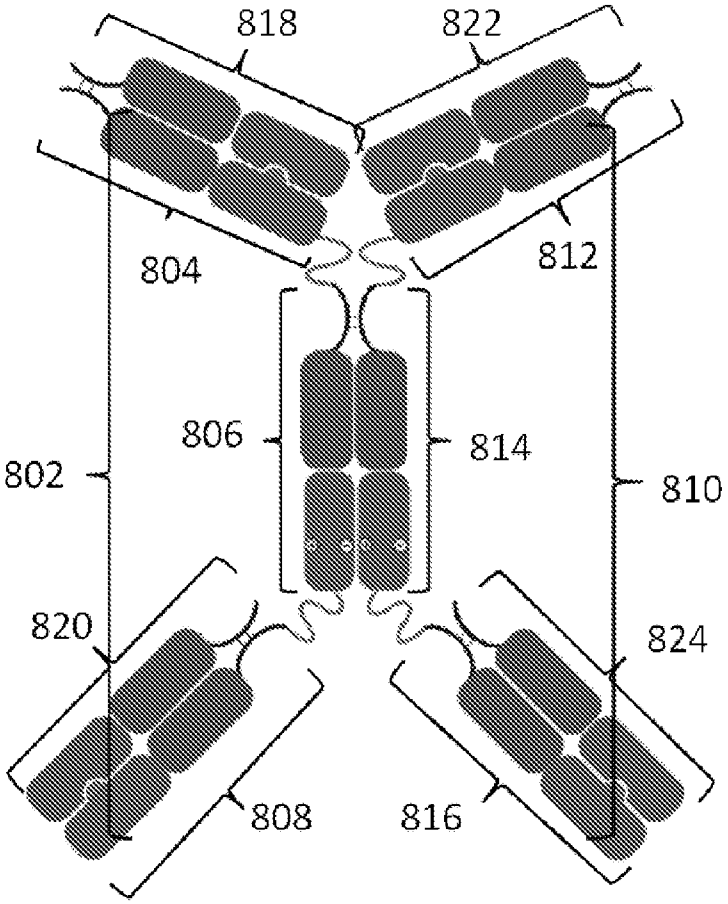



Fig. 8

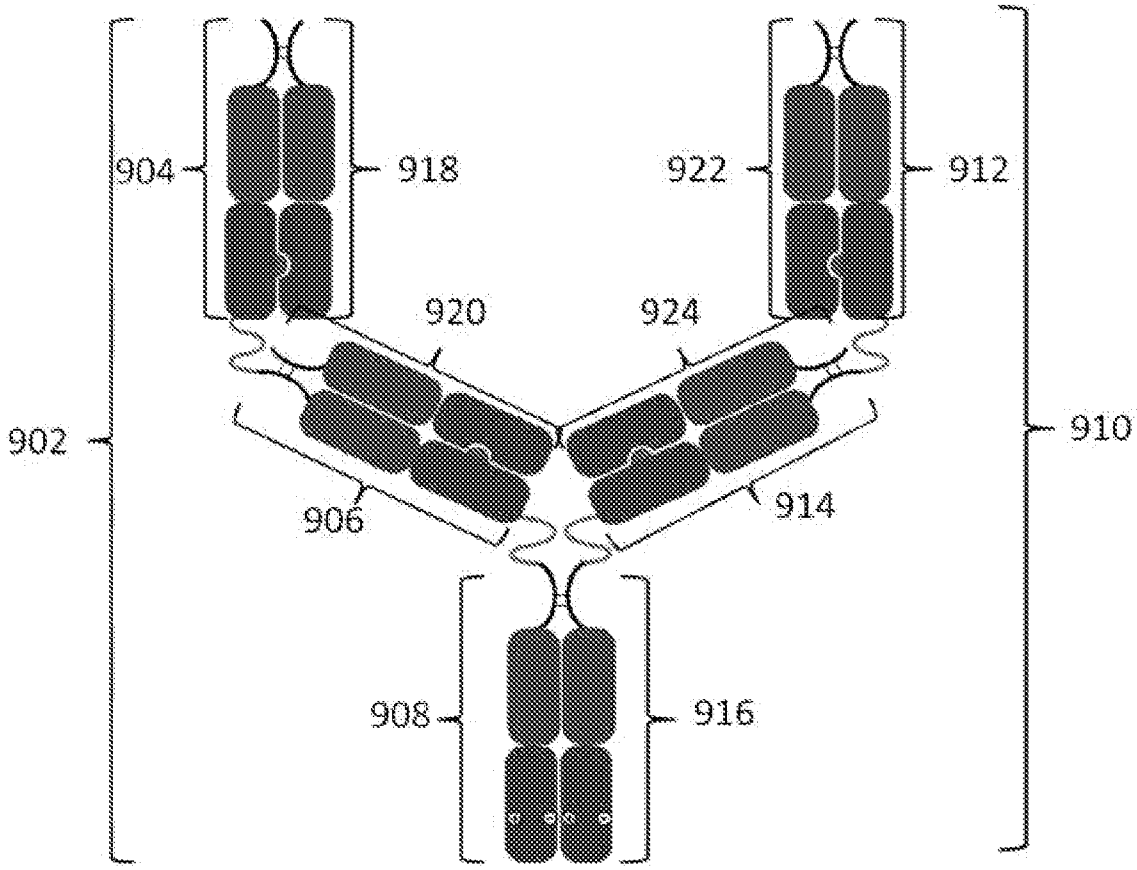



Fig. 9

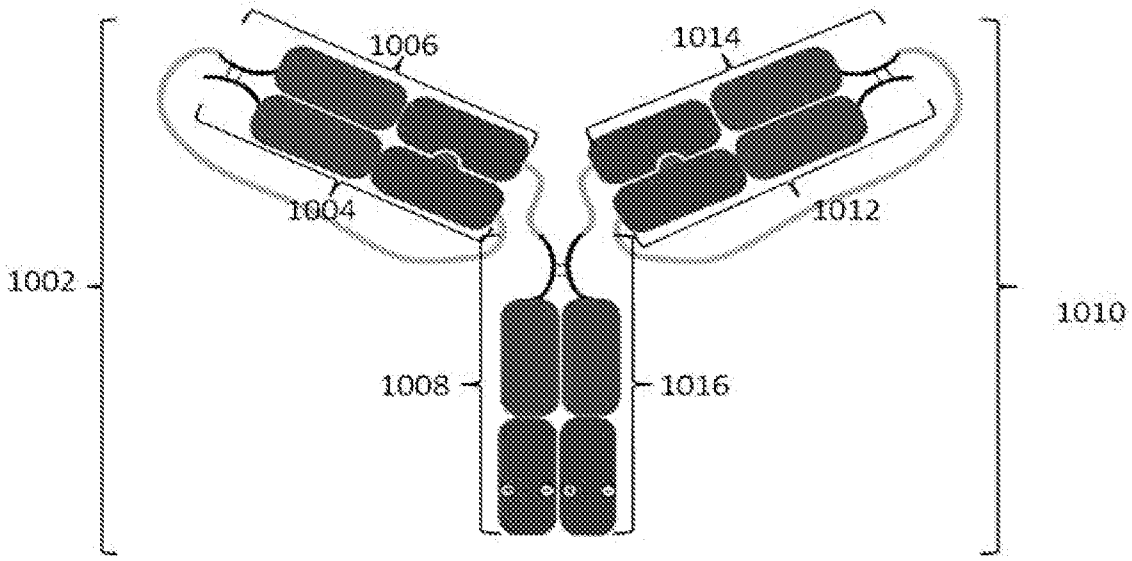



Fig. 10

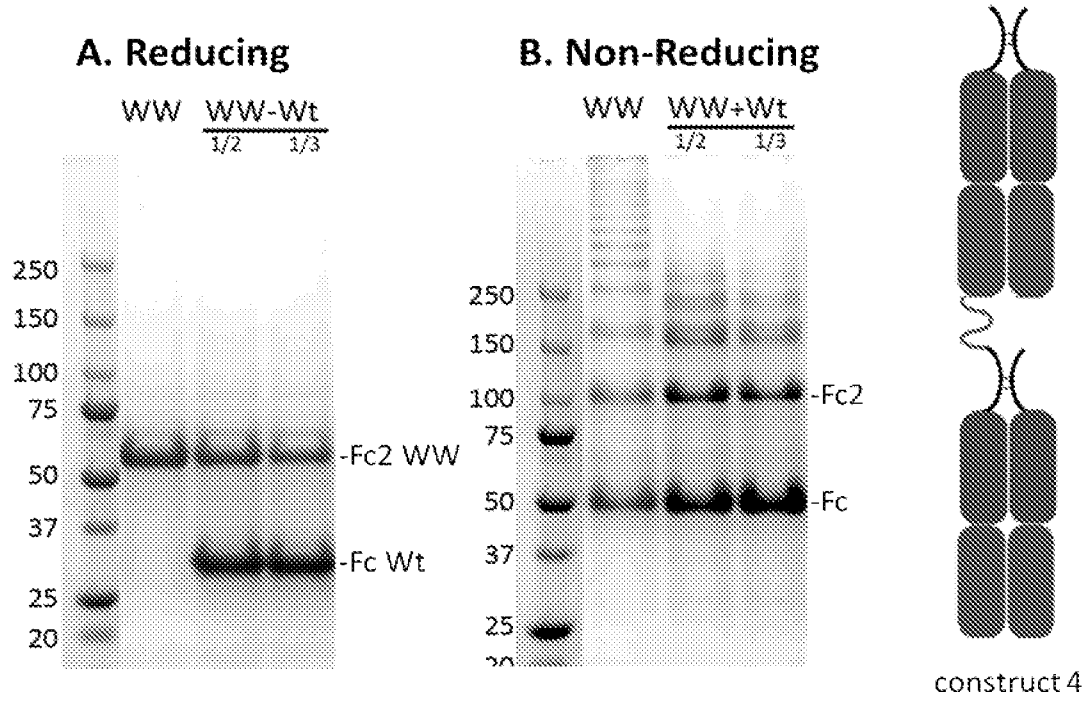

A. Reducing
WW
WW-Wt
1/2
1/3
250
150
100
75
50
37
25
20
-Fc2 WW
-Fc Wt
B. Non-Reducing
WW
WW+Wt
1/2
1/3
250
150
100
75
50
37
25
-Fc2
-Fc
construct 4

Fig. 11A-B

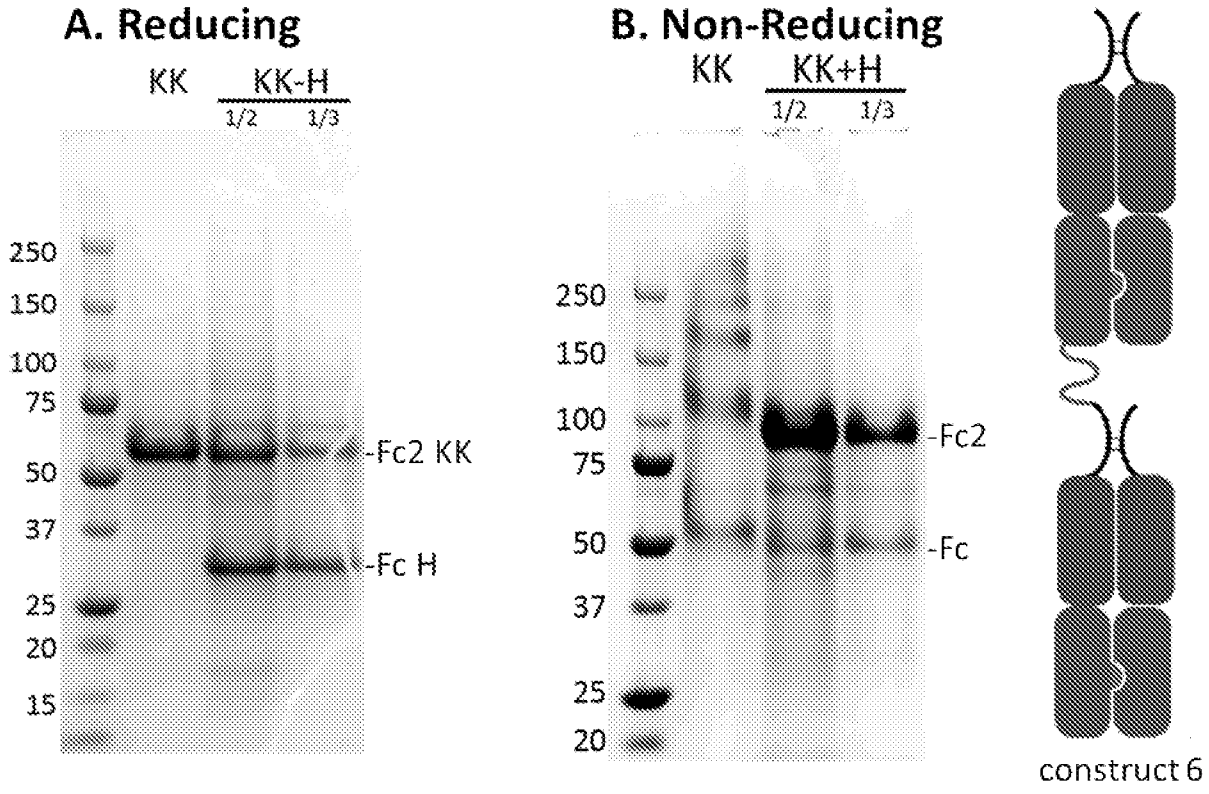

A. Reducing
KK
KK-H
1/2
1/3
250
150
100
75
50
37
25
20
15
-Fc2 KK
-Fc H
B. Non-Reducing
KK
KK+H
1/2
1/3
250
150
100
75
50
37
25
20
-Fc2
-Fc
construct 6

Fig. 12A-B

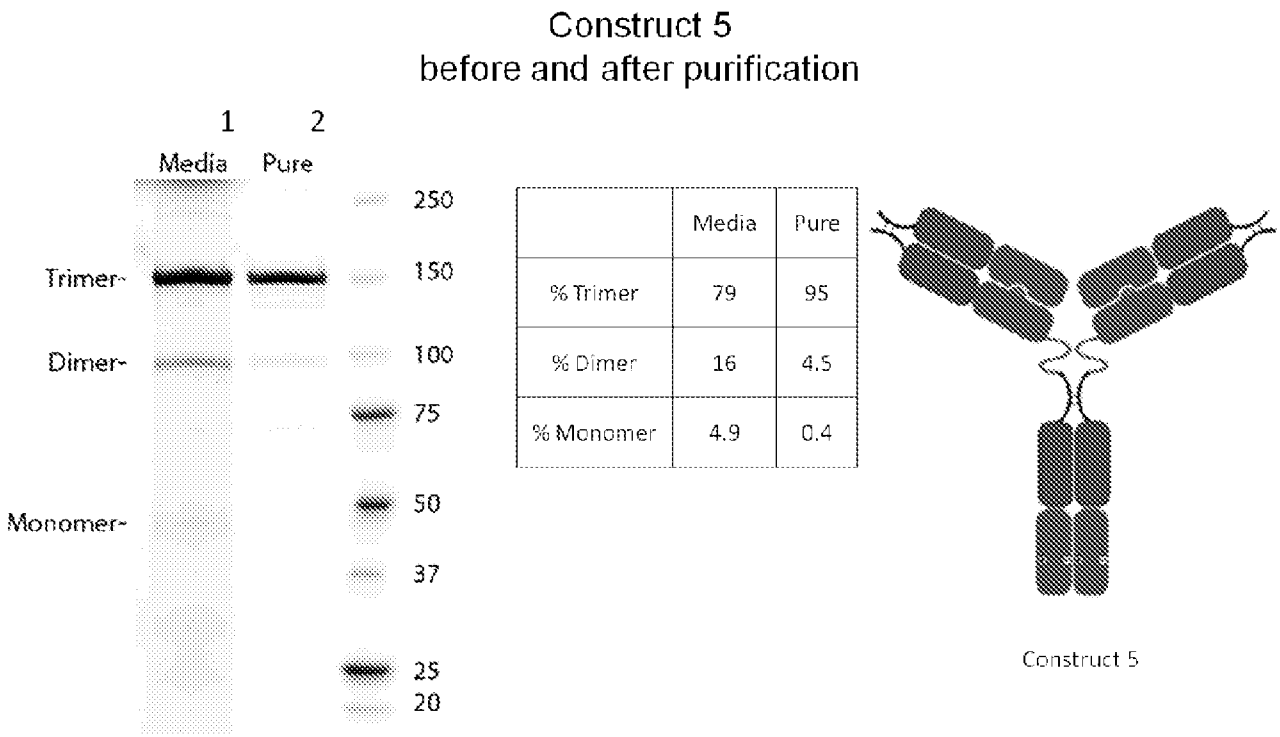

Construct 5
before and after purification
1
2
Media
Pure
Trimer
Dimer
Monomer
250
150
100
75
50
37
25
20
Media
Pure
% Trimer
79
95
% Dimer
16
4.5
% Monomer
4.9
0.4
Construct 5

**Fig. 13**

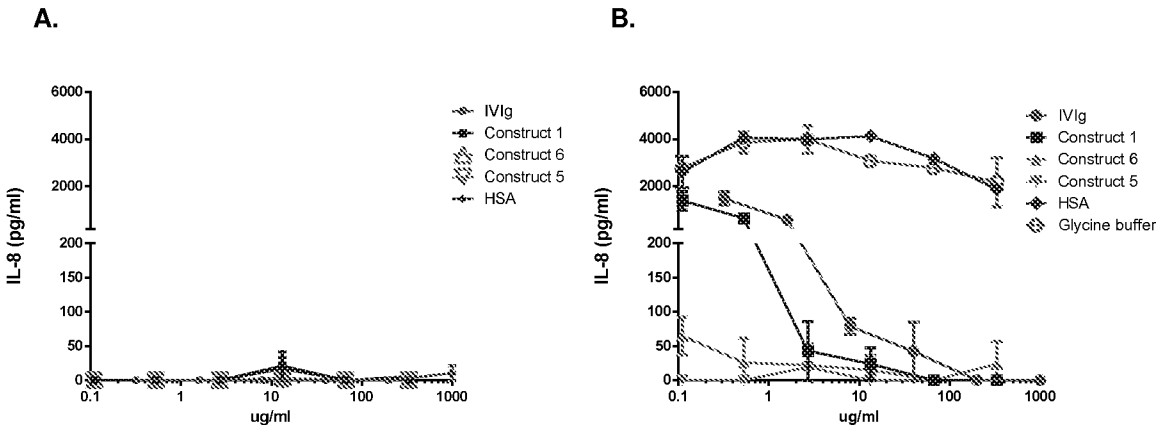

A.
B.
IL-8 (pg/ml)
6000
4000
2000
200
150
100
50
0
0.1
1
10
100
1000
ug/ml
IVIg
Construct 1
Construct 6
Construct 5
HSA
Glycine buffer

Fig. 14

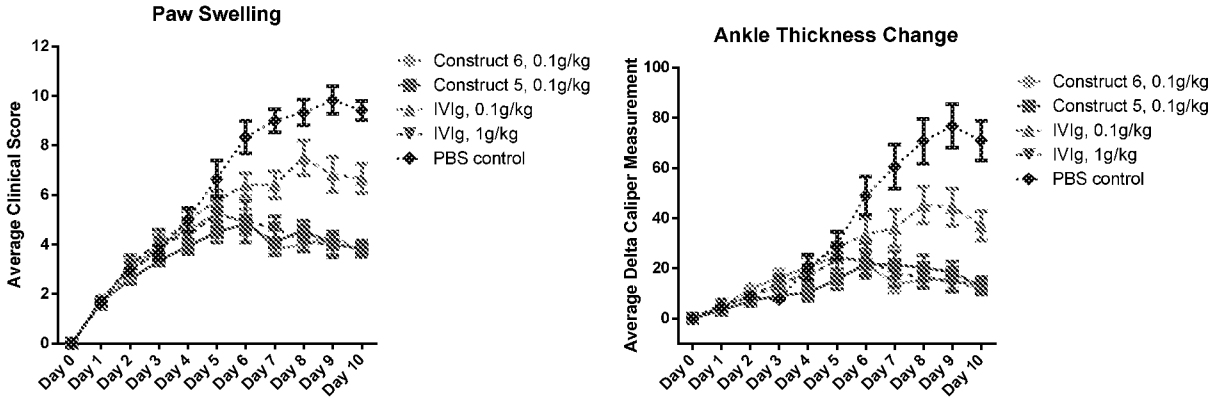

Paw Swelling
Average Clinical Score
0
2
4
6
8
10
12
Day 0
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Day 9
Day 10
Construct 6, 0.1g/kg
Construct 5, 0.1g/kg
IVIg, 0.1g/kg
IVIg, 1g/kg
PBS control
Ankle Thickness Change
Average Delta Caliper Measurement
0
20
40
60
80
100
Day 0
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Day 9
Day 10
Construct 6, 0.1g/kg
Construct 5, 0.1g/kg
IVIg, 0.1g/kg
IVIg, 1g/kg
PBS control

Fig. 15

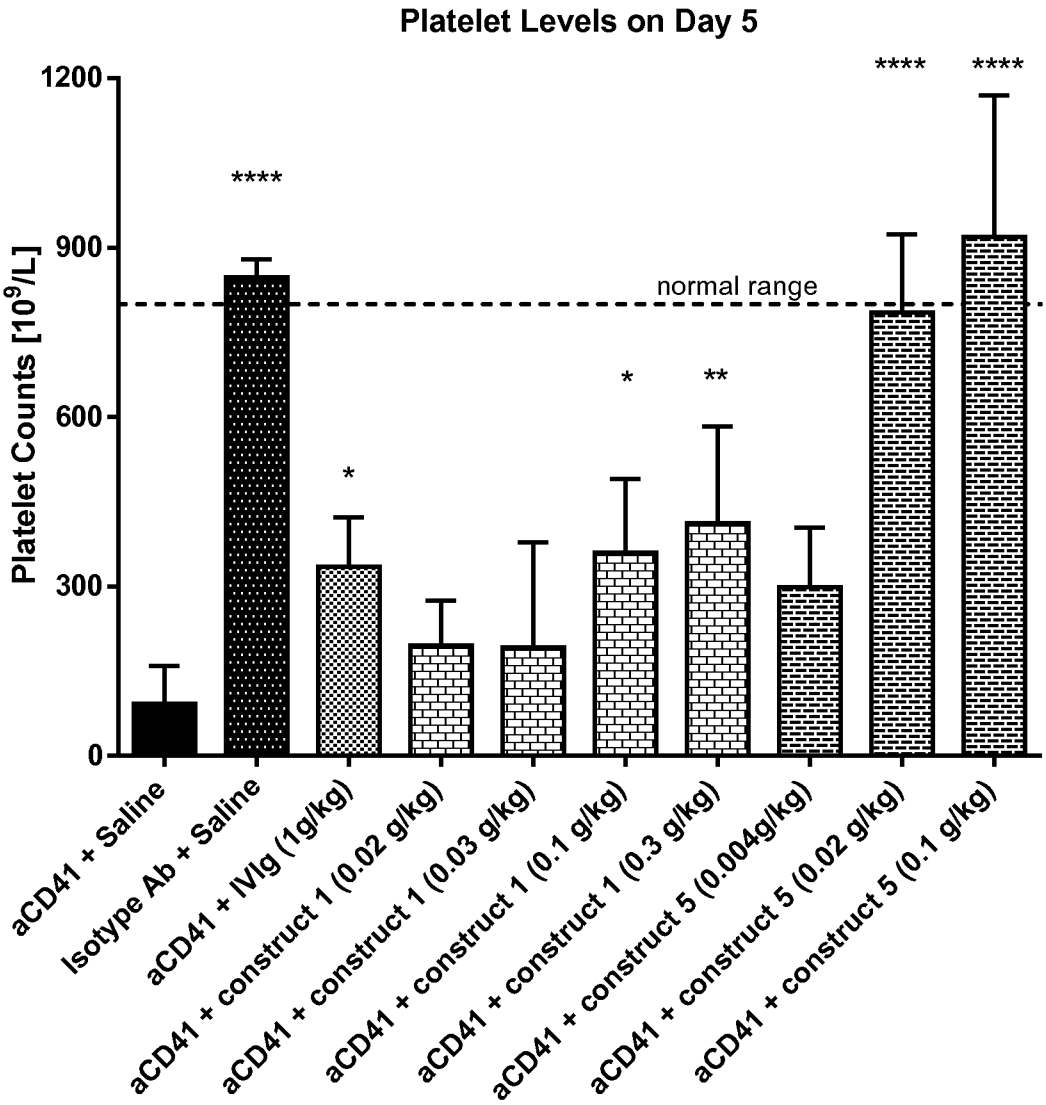

Platelet Levels on Day 5
Platelet Counts [10^9/L]
1200
900
600
300
0
normal range
****
****
****
*
*
**
aCD41 + Saline
Isotype Ab + Saline
aCD41 + IVIg (1g/kg)
aCD41 + construct 1 (0.02 g/kg)
aCD41 + construct 1 (0.03 g/kg)
aCD41 + construct 1 (0.1 g/kg)
aCD41 + construct 1 (0.3 g/kg)
aCD41 + construct 5 (0.004g/kg)
aCD41 + construct 5 (0.02 g/kg)
aCD41 + construct 5 (0.1 g/kg)

Fig. 16

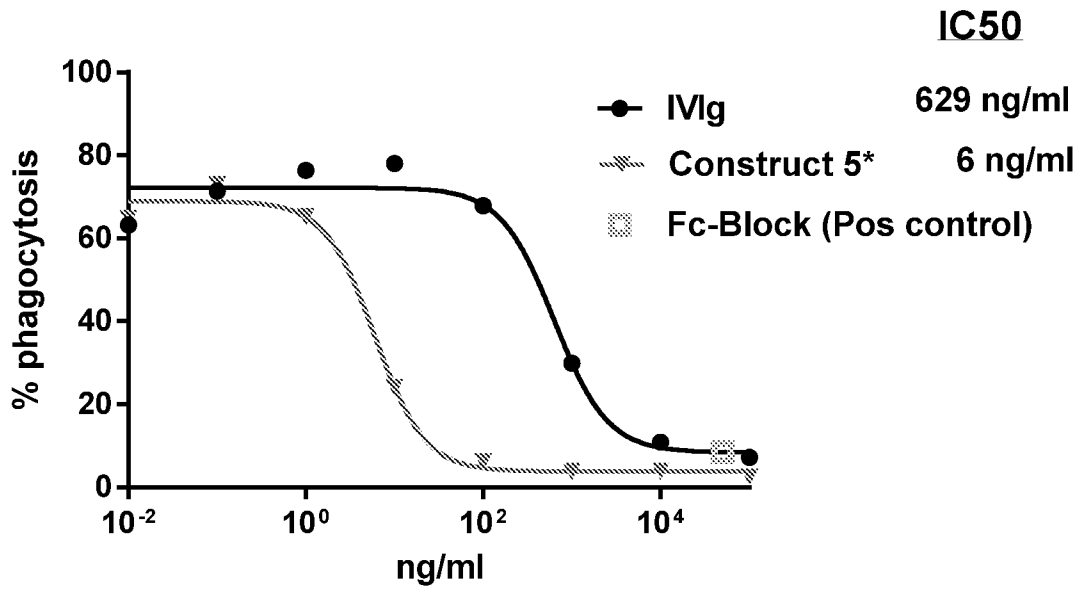

IC50
IVIg 629 ng/ml
Construct 5* 6 ng/ml
Fc-Block (Pos control)
% phagocytosis
100
80
60
40
20
0
10-2
100
102
104
ng/ml

Fig. 17