ENGINEERED COMPLEMENT INHIBITOR PROTEINS

INCORPORATION BY REFERENCE TO ANY PRIORITY APPLICATIONS

[0001] Any and all applications for which a foreign or domestic priority claim is identified in the Application Data Sheet as filed with the present application are hereby incorporated by reference under 37 CFR 1.57. This Application claims priority to U.S. Provisional Application No. 63/559733, filed February 29, 2024. The entire contents of this related Application are incorporated herein by reference.

REFERENCE TO SEQUENCE LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled SequenceListingKDIAK.224WO.xml created on February 27, 2025, which is 227,012 bytes in size. The information in the electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

FIELD

[0003] The present invention relates to protein inhibitors engineered to inhibit the complement pathway.

BACKGROUND

[0004] The complement system is a component of the innate immune system that promotes inflammation and cell lysis in response to infections and tissue injury. Alterations in the balance of activation and regulation of the complement system have been associated with diseases including age-related macular degeneration (AMD). AMD is the leading cause of central vision loss in the population over age 50 years. It is classified into two types: dry and wet AMD. In the dry form, loss of photoreceptors and retinal pigment epithelium (RPE) cells in the macula results in tissue atrophy with the advanced stage of the disease termed geographic

atrophy (GA). Clinical trials have demonstrated a reduction in the growth of GA following the inhibition of complement pathways.

SUMMARY

[0005] Provided herein is a protein inhibitor comprising a first polypeptide, the first polypeptide comprising an oligomerization domain (e.g., dimerization domain) and a complement regulator comprising a Complement Receptor 1 (CR1) complement control protein (CCP) domain and a Factor H (FH) CCP domain, wherein the CCP domains comprise at least one C3b and/or C4b binding domain, and wherein the oligomerization domain is covalently linked to the complement regulator. Optionally, the oligomerization domain comprises an Fc domain. Optionally, the oligomerization domain comprises an anti-VEGF-VH-CH1 domain. Optionally, the oligomerization domain comprises an anti-VEGF-VL-CL domain.

[0006] In some embodiments a protein inhibitor which comprises a first polypeptide and a second polypeptide is provided. The first polypeptide comprises SEQ ID NO: 50 and the second polypeptide comprises SEQ ID NO: 50, wherein the first polypeptide is conjugated to the second polypeptide via disulfide bonds.

[0007] In some embodiments, a method of treatment or prophylaxis of a disease is provided. The method comprises identifying a subject in need of treatment and administering a protein inhibitor as described above. In some embodiments, the disease is selected from the group consisting of age-related macular degeneration (AMD), geographic atrophy (GA) paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), C3 glomerulopathy (C3G) or rheumatoid arthritis.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] **Fig. 1A**. depicts schematics of some embodiments of proteins that regulate the complement system.

[0009] **Fig. 1B** depicts schematics of engineered complement regulators.

[0010] **Fig. 2** depicts a schematic of some embodiments of an Fc domain (SEQ ID NO: 46).

[0011] **Fig. 3.** depicts a schematic of some embodiments of a protein inhibitor (KC010, SEQ ID NO: 59).

[0012] **Fig. 4.** depicts a schematic of some embodiments of a protein inhibitor (KC003, SEQ ID NO: 52).

[0013] **Fig. 5.** depicts a schematic of some embodiments of a protein inhibitor (KC001, SEQ ID NO: 50).

[0014] **Fig. 6.** depicts a schematic of some embodiments of a protein inhibitor (KC011, SEQ ID NO: 60).

[0015] **Fig. 7.** depicts a schematic of some embodiments of a protein inhibitor (KC021, SEQ ID NO: 70).

[0016] **Fig. 8.** depicts a schematic of some embodiments of an aVEGF-Fab (SEQ ID NO: 85).

[0017] **Fig. 9.** depicts a schematic of some embodiments of a protein inhibitor (KC032, SEQ ID NO: 81).

[0018] **Fig. 10.** depicts a schematic of some embodiments of a protein inhibitor (KC033, SEQ ID NO: 82).

[0019] **Fig. 11A** depicts a schematic of some embodiments of complement factors and engineered complement regulators used in this study.

[0020] **Fig. 11B** a schematic of some embodiments of a protein inhibitor (KC007, SEQ ID NO: 56).

[0021] **Fig. 11C** a schematic of some embodiments of a protein inhibitor (KC027, SEQ ID NO: 76).

[0022] **Fig. 11D** a schematic of some embodiments of a protein inhibitor (KC028, SEQ ID NO: 77).

[0023] **Fig. 11E** a schematic of some embodiments of a protein inhibitor (KC030, SEQ ID NO: 79).

[0024] **Fig. 12A** is a graph depicting some embodiments of protein inhibitors and FH inhibiting hemolysis mediated by the Alternative Pathway (AP).

[0025] **Fig. 12B** is a graph depicting some embodiments of protein inhibitors and FH inhibiting hemolysis mediated by the Classical Pathway (CP).

[0026] **Fig. 13A** is an SDS-PAGE gel depicting some embodiments of the effects of FH and a protein inhibitor (KC001) on C3b and its cleavage products by FI.

[0027] **Fig 13B** is an SDS-PAGE gel depicting some embodiments of the effects of FH and protein inhibitors (KC001, KC016, and KC003) on C4b degradation products produced by FI and cofactor FH.

[0028] **Figs. 14A-D** are surface plasmon resonance (SPR) sensorgrams depicting some embodiments of the C3b binding kinetics of FH (A) and protein inhibitors, KC001 (B), KC002 (C), and KC021 (D). The equilibrium dissociation constant KD is shown in the panes.

[0029] **Figs. 15A-C** are surface plasmon resonance (SPR) sensorgrams depicting some embodiments of the C3b binding kinetics of protein inhibitors, KC001 (A), KC010 (B), KC021(C), which include the Fc domain, captured on Protein A chip. The equilibrium dissociation constant KD and ligand levels are shown in the panes.

[0030] **Fig. 16** is a graph depicting some embodiments of FH and the protein inhibitors inhibiting hemolysis mediated by the Alternative Pathway (AP). KC032, KC033 and KC035 are bispecific protein inhibitors, which comprise anti-VEGF Fab and complement regulator modules. KC001 comprises a complement regulator module fused to Fc.

[0031] **Fig. 17A-D** are surface plasmon resonance (SPR) sensorgrams depicting some embodiments of the VEGF165 binding kinetics of bispecific protein inhibitors, KC032(A), KC033(B), and KC035 (C), which comprise anti-VEGF Fab and complement regulator modules, and the control anti-VEGF Fab (D).

[0032] **Fig. 18** is a graph depicting some embodiments of anti-VEGF activity of bispecific protein inhibitors and anti-VEGF Ab in a VEGF cell reporter assay. Anti-VEGF Ab (bivalent) is used as control (dark circle), KC032 (open diamond), KC033 (open square), and KC035 (open triangle) contains monovalent anti-VEGF Fab, which are fused to complement regulator modules.

[0033] **Fig. 19** depicts a schematic of some embodiments of a protein inhibitor (KC041, SEQ ID NO: 105).

[0034] **Fig. 20** is an SDS-PAGE gel depicting some embodiments of purified fusion proteins under reducing and non-reducing conditions. KC043: (lane 2) non-reducing condition and (lane 7) reducing conditions; KC044: (lane 3) non-reducing and (lane 8)

reducing conditions; KC045: (lane 4) non-reducing and (lane 9) reducing conditions; KC041: (lane 5) non-reducing and (lane 10) reducing conditions.

[0035] **Fig. 21** is a graph depicting some embodiments of protein inhibitors (KC038, KC041, KC043, FH, Eculizumab) inhibiting the alternative pathway. Inhibition of alternative pathways by complement inhibitor modules fused to IgG Fc fragment. The concentration listed next to the molecule ID represents the IC_{50} value determined from the hemolysis assay. IC_{50} value of FH has not been determined.

[0036] **Fig. 22** is a graph depicting some embodiments of protein inhibitors (KC038, KC041, KC043) inhibiting the classical pathway. Inhibition of classical pathways by complement inhibitor modules fused to IgG Fc fragment. The concentration listed next to the molecule ID represents the IC50 value determined from the hemolysis assay.

[0037] **Fig. 23** is an SDS-PAGE gel depicting some embodiments of a cofactor assay of C3b cleavage by Factor I (FI) demonstrating sCR1, KC041, C3b and its cleavage products by FI.

[0038] **Fig 24A-24B** are surface plasmon resonance (SPR) sensorgrams depicting some embodiments of C3b binding kinetics for KC038 (A) and KC041 (B). The equilibrium dissociation constant KD is shown in the panes.

[0039] **Fig. 25A-25B** are surface plasmon resonance (SPR) sensorgrams depicting some embodiments of C3b binding kinetics of C3b binding for KC038 (A), KC041 (B). The equilibrium dissociation constant KD and ligand levels are shown in the panes.

[0040] **Fig. 26** is a surface plasmon resonance (SPR) sensorgrams depicting some embodiments of a DAA assessment on C3 convertase by SPR.

[0041] **Fig. 27** is an SDS-PAGE gel depicting some embodiments of purified fusion proteins under reducing and non-reducing conditions. KC048: (lane 2) non-reducing condition and (lane 4) reducing conditions; KC049: (lane 3) non-reducing and (lane 5) reducing conditions.

[0042] **Fig. 28** is a graph depicting some embodiments of protein inhibitors (KC041, KC050, KC052) inhibiting the alternative pathway. The concentration listed next to the molecule ID represents the IC_{50} value determined from the hemolysis assay.

[0043] **Fig. 29** is a graph depicting some embodiments of protein inhibitors (KC050, KC052, Eculizumab) inhibiting the classical pathway. The concentration listed next to the molecule ID represents the IC_{50} value determined from the hemolysis assay.

[0044] **Fig. 30** is an SDS-PAGE gel depicting some embodiments of a cofactor assay of C3b cleavage by FI and the effects of protein inhibitors (KC049, KC050).

[0045] **Fig. 31** is an SDS-PAGE gel depicting some embodiments of a cofactor assay of C4b cleavage by FI and the effects of protein inhibitors (KC049, KC050).

[0046] **Fig. 32A- 32B** are surface plasmon resonance (SPR) sensorgrams depicting some embodiments of decay acceleration activity (DAA) assessments of C3 convertase by SPR. (A) Sensorgrams of DAA of bispecific inhibitors. (B) Sensorgrams of DAA in the presence of Factor P (FP).

[0047] **Fig. 33.** is a graph depicting some embodiments of a competitive ELISA assay demonstrating inhibition of IL-6 binding to IL-6Rα by bispecific inhibitors in an IL-6/IL-6Rα DuoSet ELISA.

DETAILED DESCRIPTION

[0048] Provided herein are protein inhibitors of the complement system and methods of use thereof. In some embodiments, the protein inhibitors can be used to reduce inflammation and tissue damage in conditions including macular degeneration.

[0049] In some embodiments, the protein inhibitors comprise oligomerization domains or complement receptor domains covalently linked to complement regulator domains wherein the complement regulator domains are comprised of combinations of CCP domains from Complement Regulator 1 (CR1) and Factor H (FH).

[0050] In some embodiments, the protein inhibitors inhibit the complement system by binding C3b and/or C4b and promoting dissociation of complement convertases and/or Factor I (FI) mediated digestion and inactivation of C3b and C4b.

[0051] These and additional embodiments are provided below, following the definition section.

[0052] The complement system, a major component of the innate immune system, comprehends a collection of proteins that operate in the response to infections and tissue injury. Activation of the complement system can occur through three pathways: i) the classical

pathway (CP), which is triggered by activators such as antigen-antibody complexes; ii) the lectin pathway (LP), which is similar to the classical pathway and is triggered by carbohydrates present on bacteria, fungi and other pathogens; and iii) the alternative pathway (AP), which recognizes the cell surfaces of pathogens and accounts for most of the complement activity. In contrast to the CP and LP, the AP is permanently active at a low level to quickly respond to infections.

[0053] All three pathways converge at the formation of the C3 and C5 convertases. C3 cleavage by C3b convertase results in the formation of anaphylatoxin C3a, which induces inflammation and C3b that opsonizes cells and targets them for phagocytosis. C5 cleavage by C5 convertase results in the anaphylatoxin C5a, a potent proinflammatory chemoattractant, and C5b. The latter triggers the terminal/lytic pathway characterized by the assembly of a large protein complex (C5b-C9), the membrane attack complex (MAC), on the membrane of the pathogen or host cell. MAC not only lyses cells, but also induces cell cycling, proliferation, protein synthesis, apoptosis and contributes to the activation of the NLRP3 inflammasome complex.

[0054] To protect cells and host tissues from unintended complement activation, cells express a myriad of regulators including soluble forms such as complement factor H (FH or CFH) 20, 24 **FIG. 1A**, C4b-binding protein (C4BP), and membrane associated proteins such as complement receptor 1 (CR1) 10, 14 **FIG. 1A**, membrane cofactor protein (MCP), decay-accelerating factor (DAF). These regulators may act through two inhibitory mechanisms, (1) decay accelerating activity (DAA) whereby regulator binding enhances the spontaneous decay of C3 convertases either C4b2a or C3bBb, or (2) Factor I (FI) cofactor activity (CA) whereby C3b and C4b are proteolytically inactivated by the serine protease FI. Here, a cofactor protein must first bind to substrates (C4b or C3b) 11, 12, 13, 21, 23 **FIG. 1A** before the enzyme can engage and digest molecules into fragments that cannot form convertases. Both FH 20, 24 **FIG. 1A** and CR1 10 can act through both mechanisms, while DAF and MCP operate exclusively via DAA and CA, respectively.

[0055] Regulators, including CR1 and FH 10, 20, 24 **FIG. 1A** are extended proteins composed of tandem complement control protein (CCP) domains, also known as short consensus repeat (SCR) or sushi domains, linked by short sequences of 3 to 8 amino acids. CCPs are autonomously folding globular domains composed of approximately 60 amino acid

residues, which includes four conserved cysteine residues forming two disulfide bridges and highly conserved tryptophan, glycine, proline, and hydrophobic residues at various positions. The number of CCP domains differ amongst the various regulators: FH (20 CCPs) 20, 24 **FIG 1A and FIG. 11**; CR1(30 CCPs) 10 **FIG 1A**; DAF and MCP (4 CCPs).

[0056] FH is a critical regulator of the AP pathway. It is a large (155 kDa) glycoprotein with primary site of synthesis in the hepatocytes, and secondary sites in a variety of cells including retinal pigment epithelium (RPE), fibroblasts, neurons, astrocytes, glia cells, and peripheral blood lymphocytes. Being soluble, FH plays an important protective role for surfaces lacking membrane-bound regulators (e.g. extracellular matrix). FH complement regulatory activities are performed by binding to C3b via CCPs 1-4/19-20, 21, 23 **FIG. 1A** and C3b degradation products iC3b, C3d(g) via CCPs 19-20, 23 **FIG. 1A**. While CCP regions 6-8, 22 **FIG. 1A** and 19-20, 23 **FIG. 1A** direct FH localization to self-surface areas by interactions with host-specific glycans, namely glycosaminoglycans (GAGs) or sialic acid-containing glycans (CCP 20) 23 **FIG. 1A**. FH belongs to a family of highly related proteins that includes five complement Factor H-related proteins 1-5 (CFHR1 50 **FIG. 11**, CFHR2, CFHR3, CFHR4, CFHR5 60 **FIG. 11** or FHR-1, FHR-2, FHR-3, FHR-4, FHR-5) and the spliced variant FH-like protein (FHL-1). The function for FHR proteins is less well understood. Similarly, they are composed of tandem CCP domains (FHR-1, 5 CCPs; FHR2, 4 CCPs; FHR-3, 5 CCPs; FHR-4, 9 CCPs; FHR-5, 9 CCPs). CCP 1-2 domains in FHR-1, FHR-2, FHR-5 present the ability for homo- and hetero-oligomerization that may increase avidity of these FHRs for their ligands.

[0057] CR1, 10 **FIG. 1A** is a large (190 – 280 kDa) type I transmembrane glycoprotein. It is expressed in all peripheral blood cells except for platelets, natural killer cells and most T cells. CR1 is a versatile inhibitor of both classical and alternative pathway due to its capability to bind C3b and C4b with high affinity 11-13, **FIG. 1A**. A soluble form (sCR1) can also be found, and it may play a protective role during inflammatory conditions. All except the two carboxy terminal CCPs can be clustered into four (A-D) long homologous repeat (LHR) regions 15-8 **FIG. 1A** each composed of seven CCPs. Structure-function studies demonstrated that LHRs A-C, 15-7 **FIG. 1A** present regulatory activity and functional activity is centered around the first three CCPs in each one of those LHR regions (CCP 1-3: high affinity for C4b, low affinity for C3b, DAA; CCP 8-10 and CCP15-17: high affinity for C3b,

low for C4b, CA). CR1 is the only co-factor protein able to degrade C3b further into C3dg. This degradation product binds to CR2, 70 **FIG. 11** which ends up facilitating the antibody response by decreasing the amount of antigen required for B-cell activation.

[0058] C3b can also interact with different receptors including complement receptor 2 (CR2, complement C3d receptor) 70 **FIG. 11** and complement receptor of the immunoglobulin superfamily (CRIg) 40 **FIG. 11**. CR2 is a large (approximately 145 kDa) type I transmembrane glycoprotein containing 14-16 CCP domains 70 **FIG. 11**. It is expressed in mature B lymphocytes, T lymphocytes, follicular dendritic cells, basophils, keratinocytes, astrocytes, and epithelial cells. It also exists in a soluble form. CR2 binds to various C3b degradation products, iC3b, C3dg, and C3d through interactions mediated by CCPs 1-2. CRIg is a type I transmembrane protein (28 – 44 kDa) consisting of two immunoglobulin (Ig)-like domains, a transmembrane region, and a cytoplasmic domain. It is expressed on tissue-resident macrophages. CRIg binds to C3b and iC3b molecules on particle surfaces with its N-terminal IgV domain and internalizes along with the phagocytosed cargo.

[0059] Minor alterations in the balance of activation and regulation due to inappropriate initiation and/or imbalanced production of components and regulators result in undesired complement responses and downstream inflammation, cell lysis and tissue injury. Complement misfunction has been linked to inflammatory, autoimmune, neurodegenerative, and infectious diseases. Dysregulation of the AP has been associated with age-related macular degeneration (AMD), paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), C3 glomerulopathy (C3G), rheumatoid arthritis and others.

[0060] AMD is the leading cause of central vision loss in the population over age 50 years. It is classified into two types dry and wet AMD. In the dry form, loss of photoreceptors and retinal pigment epithelium (RPE) cells in the macula results in tissue atrophy with the advanced stage of the disease termed geographic atrophy (GA). In the wet form, choroidal neovascularization (CNV), which can leak fluid and blood, develops under the retina and macula. Dry AMD is characterized by the presence of drusen (extracellular deposits of protein and lipid aggregates) between the basal lamina of the RPE and Bruch's membrane. Several findings indicate complement plays a role in the onset and progression of AMD. Complement proteins (e.g. C3, C5, C5b-9) and their regulators (e.g. FH) have been identified in drusen. Elevated C3b deposition has been observed in donor eyes which carry genetic

variations around the CFH gene. Complement system activation products have been found elevated systemically in AMD patients. Lastly, clinical trials have demonstrated a reduction in the growth of GA following the inhibition of C3 and C5.

Terms

[0061] "Domain" as used herein refers to a polypeptide that may comprise an engineered protein, a full-length natural protein, or a portion of a natural protein. As used herein, a domain may be a polypeptide that has a function or an activity or has been engineered to have a function and/or an activity. A domain may be a single polypeptide or a region within a larger polypeptide. A polypeptide or polypeptide region will still comprise a domain if one or more amino acid is added to or deleted from the domain.

[0062] "Protein inhibitor" as used herein refers to an engineered protein that can inhibit one or more activities or functions. As used herein, protein inhibitors can also refer without limitation to engineered proteins that can inhibit the complement system. Protein inhibitors can comprise one or more domains. Protein inhibitors can be considered modular proteins which can be constructed from multiple domains in multiple combinations and orders without limitation. As used here, protein inhibitors may comprise an oligomerization domain that can be covalently linked through peptide bonds or other mechanisms of associations to other domains including domains that regulate the complement system. In some embodiments, protein inhibitors may also comprise domains that inhibit activities other than the complement system. Protein inhibitors can inhibit through a direct or an indirect mechanism. Protein inhibitors can have one or more domains that inhibit a biological function. Protein inhibitors can also promote or stimulate a biological function or activity that has the effect of inhibiting a different biological function or activity. The term protein inhibitor can also encompass bispecific protein inhibitors and multi-specific protein inhibitors. Protein inhibitors can be a monomers, dimers, trimers or other multimers of polypeptides. A protein inhibitor will still comprise a protein inhibitor if one or more amino acids are added to or deleted from the protein inhibitor.

[0063] "Bispecific protein inhibitor" as used herein refers to a construct that has at least two domains that can inhibit two or more different activities in an environment or subject. In some embodiments, inhibition is selectively targeted to at least two known targets.

[0064] "Multi-specific inhibitor" refers to a construct that has more than two domains that can inhibit more than two different activities in an environment or subject. In some embodiments, inhibition is selectively targeted to more than two known targets.

[0065] "Oligomerization domain" as used herein refers without limitation to protein domains comprising properties that allow them to form macromolecular complexes. Oligomerization domains can assemble into macromolecular complexes with other proteins with the same oligomerization domain or with different oligomerization domains. Dimerization domains are examples of oligomerization domains. Macromolecular complexes, including dimers, trimers, and multimers, can be associated through covalent and non-covalent mechanisms. Oligomerization domains can comprise domains with unconjugated cysteines that permit conjugation through disulfide bond formation to one or more other proteins or protein domains that also include unconjugated cysteines. In some embodiments, oligomerization domains have functions, activities, and targeting capabilities in addition to their ability to form associations and/or disulfide bonds with other polypeptides. In some embodiments, domains from complement regulatory proteins comprise oligomerization domains. In some embodiments, the oligomerization domain promotes macromolecular complex formations with proteins in the complement system. In some embodiments, oligomerization domains increase the avidity of the protein inhibitors for their targets including but not limited to the complement system.

[0066] An "antibody" is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term encompasses not only intact polyclonal or monoclonal antibodies, but also, unless otherwise specified, any antigen binding portion thereof that competes with the intact antibody for specific binding, fusion proteins comprising an antigen binding portion, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site. Antigen binding portions include, for example, Fab, Fab', $F(ab')_2$, Fd, Fv, domain antibodies (dAbs, e.g., shark and camelid antibodies), fragments including complementarity determining regions (CDRs), single chain variable fragment antibodies (scFv), maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv, and polypeptides that contain at least a portion of an immunoglobulin that is

sufficient to confer specific antigen binding to the polypeptide. An antibody includes an antibody of any class, such as IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant region of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG_1, IgG_2, IgG_3, IgG_4, IgA_1 and IgA_2. The heavy-chain constant regions that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively.

[0067] A "variable region" of an antibody refers to the variable region of the antibody light chain or the variable region of the antibody heavy chain, either alone or in combination. The variable regions of the heavy and light chains each consist of four framework regions (FRs) connected by three complementarity determining regions (CDRs) also known as hypervariable regions, and contribute to the formation of the antigen binding site of antibodies. If variants of a subject variable region are desired, particularly with substitution in amino acid residues outside of a CDR region (i.e., in the framework region), appropriate amino acid substitution, preferably, conservative amino acid substitution, can be identified by comparing the subject variable region to the variable regions of other antibodies which contain CDR1 and CDR2 sequences in the same canonincal class as the subject variable region (Chothia and Lesk, J Mol Biol 196(4): 901-917, 1987).

[0068] In certain embodiments, definitive delineation of a CDR and identification of residues comprising the binding site of an antibody is accomplished by solving the structure of the antibody and/or solving the structure of the antibody-ligand complex. In certain embodiments, that can be accomplished by any of a variety of techniques known to those skilled in the art, such as X-ray crystallography. In certain embodiments, various methods of analysis can be employed to identify or approximate the CDR regions. In certain embodiments, various methods of analysis can be employed to identify or approximate the CDR regions. Examples of such methods include, but are not limited to, the Kabat definition, the Chothia definition, the IMGT approach (Lefranc et al., 2003) Dev Comp Immunol. 27:55-77), computational programs such as Paratome (Kunik et al., 2012, Nucl Acids Res. W521-4), the AbM definition, and the conformational definition.

[0069] The Kabat definition is a standard for numbering the residues in an antibody and is typically used to identify CDR regions. See, e.g., Johnson & Wu, 2000, Nucleic Acids Res., 28: 214-8. The Chothia definition is similar to the Kabat definition, but the Chothia definition takes into account positions of certain structural loop regions. See, e.g., Chothia et al., 1986, J. Mol. Biol., 196: 901-17; Chothia et al., 1989, Nature, 342: 877-83. The AbM definition uses an integrated suite of computer programs produced by Oxford Molecular Group that model antibody structure. See, e.g., Martin et al., 1989, Proc Natl Acad Sci (USA), 86:9268-9272; "AbM™, A Computer Program for Modeling Variable Regions of Antibodies," Oxford, UK; Oxford Molecular, Ltd. The AbM definition models the tertiary structure of an antibody from primary sequence using a combination of knowledge databases and ab initio methods, such as those described by Samudrala et al., 1999, "Ab Initio Protein Structure Prediction Using a Combined Hierarchical Approach," in PROTEINS, Structure, Function and Genetics Suppl., 3:194-198. The contact definition is based on an analysis of the available complex crystal structures. See, e.g., MacCallum et al., 1996, J. Mol. Biol., 5:732-45. In another approach, referred to herein as the "conformational definition" of CDRs, the positions of the CDRs may be identified as the residues that make enthalpic contributions to antigen binding. See, e.g., Makabe et al., 2008, Journal of Biological Chemistry, 283:1156-1166. Still other CDR boundary definitions may not strictly follow one of the above approaches, but will nonetheless overlap with at least a portion of the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues do not significantly impact antigen binding. As used herein, a CDR may refer to CDRs defined by any approach known in the art, including combinations of approaches. The methods used herein may utilize CDRs defined according to any of these approaches. For any given embodiment containing more than one CDR, the CDRs may be defined in accordance with any of Kabat, Chothia, extended, IMGT, Paratome, AbM, and/or conformational definitions, or a combination of any of the foregoing.

[0070] As known in the art, a "constant region" of an antibody refers to the constant region of the antibody light chain or the constant region of the antibody heavy chain, either alone or in combination.

[0071] As used herein, "monoclonal antibody" refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies

comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler and Milstein, 1975, Nature 256:495, or may be made by recombinant DNA methods such as described in U.S. Pat. No. 4,816,567. The monoclonal antibodies may also be isolated from phage libraries generated using the techniques described in McCafferty et al., 1990, Nature 348:552-554, for example. As used herein, "humanized" antibody refers to forms of non-human (e.g. murine) antibodies that are chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv, Fab, Fab', F(ab')2 or other antigen-binding subsequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. Preferably, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a CDR of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity. The humanized antibody may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences, but are included to further refine and optimize antibody performance.

[0072] A “human antibody” is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen binding residues.

[0073] The term “chimeric antibody” is intended to refer to antibodies in which the variable region sequences are derived from one species and the constant region sequences are derived from another species, such as an antibody in which the variable region sequences are

derived from a mouse antibody and the constant region sequences are derived from a human antibody.

[0074] As used herein, an antibody "interacts with" or "binds" its antigen when the equilibrium dissociation constant is equal to or less than 20 nM, preferably less than about 6 nM, more preferably less than about 1 nM, most preferably less than about 0.75 nM. In some embodiments, the affinity of the antibody is between 400 and 800 pM, e.g., 450-700, or 500-600 pM.

[0075] An antibody that "preferentially binds" or "specifically binds" (used interchangeably herein) to an epitope is a term well understood in the art, and methods to determine such specific or preferential binding are also well known in the art. A molecule is said to exhibit "specific binding" or "preferential binding" if it reacts or associates more frequently, and/or more rapidly, and/or with greater duration and/or with greater affinity with a particular cell or substance than it does with alternative cells or substances. An antibody "specifically binds" or "preferentially binds" to a target if it binds with greater affinity, and/or avidity, and/or more readily, and/or with greater duration than it binds to other substances. It is also understood by reading this definition that, for example, an antibody (or moiety or epitope) that specifically or preferentially binds to a first target may or may not specifically or preferentially bind to a second target. As such, "specific binding" or "preferential binding" does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means preferential binding.

[0076] "Fc domain" as used herein Fc domain refers to an IgG constant region. In some embodiments, Fc domain comprises SEQ ID. NO: 46 Table 1.10. In some embodiments, Fc domain comprises an oligomerization domain. In some embodiments, Fc domain comprises a modular component in a protein inhibitor, a bispecific protein inhibitor or a multi-specific protein inhibitor. As used herein, Fc domain can refer to a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. An Fc domain is still a Fc domain if one or more amino acids is added or deleted.

[0077] "Anti-VEGF-CH1" as used herein refers to the heavy chain component of the Fab fragment of the anti-VEGF IgG antibody. In some embodiments, anti-VEGF-CH1 comprise SEQ ID. NO: 47, Table 1.10. In some embodiments, anti-VEGF-CH1 comprises an oligomerization domain. In some embodiments, anti-VEGF-CH1 comprises a modular

component of a protein inhibitor, a bispecific protein inhibitor or a multi-specific protein inhibitor. An Anti-VEGF-CH1 domain is still an Anti-VEGF-CH1 domain if one or more amino acids is added or deleted.

[0078] "Anti-VEGF-CL" as used herein refers to the light chain component of the Fab fragment of an anti-VEGF IgG antibody. As used herein, anti-VEGF-CL comprises SEQ ID. NO: 48 Table 1.10. In some embodiments, anti-VEGF-CL comprises an oligomerization domain. In some embodiments, anti-VEGF-CL comprises a modular component of a protein inhibitor, a bispecific protein inhibitor or a multi-specific protein inhibitor. An anti-VEGF-CL is still an anti-VEGF-CL if one or more amino acids is added or deleted.

[0079] "Anti-VEGF-HC" as used herein refers to the heavy chain fragment component of the anti-VEGF IgG antibody. In some embodiments, Anti-VEGF-HC comprises SEQ ID. NO: 49 (Table 1.10). In some embodiments, anti-VEGF-HC comprises an oligomerization domain. In some embodiments, anti-VEGF-HC comprises modular component of a protein inhibitor, a bispecific protein inhibitor or a multi-specific protein inhibitor. An anti-VEGF-HC is still an anti-VEGF-HC if one or more amino acids is added or deleted.

[0080] "Complement receptor domain (CRD)" as used herein refers to a domain that in some embodiments is covalently linked to a complement regulator. In some embodiments, it is the N-terminal region of CRIg-CR1-FH. In some embodiments, it is the N-terminal region of CR2. Any full-length protein or region of a complement regulator protein can comprise a complement receptor domain. A complement receptor domain will still comprise a complement receptor domain if one or more amino acids are added to or deleted from the complement receptor domain.

[0081] "Signal Peptide" as used herein refers to short peptides located at the N-termini of proteins that provide signals for proteins to be secreted. In some embodiments, signal peptide comprises IL-2sp SEQ ID NO: 3, Table 1.2. In some embodiments, signal peptide comprises hCR1sp SEQ ID NO: 4, Table 1.2.

[0082] "His Tag" as used herein refers without limitation to polypeptides comprising two or more tandem histidine residues that are covalently linked to proteins and are used to purify the proteins. In some embodiments, His tag comprises H6, SEQ ID NO: 5 (Table 1.2). In some embodiments, His tag comprises H8_FLAG, SEQ ID NO: 6 (Table 1.2).

[0083] "Linker" as used herein refers without limitation to a polypeptide comprising two or more amino acid residues joined by peptide bonds that is used to link two polypeptides. A linker as used herein permit protein domains to be connected by covalent bonds which in some embodiments may enhance the functions or activities of some protein domains by bringing the protein domains into proximity. In some embodiments, linkers may permit polypeptides with new and/or enhanced functions or activities to be created. A linker can be comprised of any combination of amino acids. In some embodiments a linker comprises combinations of glycine and serine. A linker can be comprised of any number of amino acids.

[0084] "Complement regulator" as used herein refers without limitation to combinations of protein domains from different native complement cofactors or regulator proteins that can inhibit the complement system. In some embodiments, complement regulator comprises combinations of CR1 and FH CCP domains that inhibit the complement system. In some embodiments, complement regulator comprises a CR1-FH(4) CCP combination domain. In some embodiments, complement regulator comprises a "CR1-FH(4) CCP combination domain" which includes one or more FH CCP domains covalently linked to its C-terminus. In some embodiments, a complement regulator comprises domains that bind to C3b and/or C4b wherein binding results in inhibition of the complement system and/or pathways. A complement regulator will still comprise a complement regulator if one or more amino acid is added to or deleted from the complement regulator.

[0085] "Factor H (FH)" as used herein refers to a regulator or cofactor of the complement system. FH can refer to, without limitation, human FH (Protein ID: P08603), mouse FH, rat FH, canine FH, chicken FH, bovine FH. In some embodiments, FH comprises human FH ("Hu FH", SEQ ID NO: 1, Table 1.1).

[0086] "Complement Receptor 1 (CR1)" as used herein refers to a regulator or cofactor of the complement system. CR1 can refer to, without limitation, human CR1 (Protein ID: P17927), mouse CR1, rat CR1, canine CR1, chicken CR1, bovine CR1. In some embodiments, CR1 comprises human CR1 ("HuCR1", SEQ ID NO: 2, **Table 1.1**).

[0087] "Complement Receptor 1 (CR1) Complement Control Protein (CCP) Domain" as used herein refers without limitation to CCP domains comprising CR1. In some embodiments, a CR1 CCP domain comprises a sequence in **Table 1.3,** SEQ ID NOs: 7-16 and

Table 9, SEQ ID NO: 89. A CR1 CCP domain will still comprise a CR1 CCP domain if one or more amino acid is added to or deleted from the CR1 CCP domain.

[0088] "Factor (FH) Complement Control Protein (CCP) Domain" as used herein refers without limitation to FH CCP domains comprising Factor H. In some embodiments, a FH CCP domain comprises a sequence in **Table 1.4,** SEQ. ID. NOs: 17-21 and Table 9, SEQ ID NO: 90. A FH CCP domain will still comprise a FH CCP domain if one or more amino acid is added to or deleted from the CR1 CCP domain.

[0089] "CFHR1" as used herein refers to a regulator of the complement system. CFHR1 can refer to, without limitation, human CFHR1 (Protein ID: Q03591), mouse CFHR1, rat CFHR1, canine CFHR1, chicken CFHR1, bovine CFHR1. CFHR1 can also refer to N-terminal domains of human CFHR1 (CFHR1(1-2), SEQ ID NO: 42, **Table 1.9**).

[0090] "CFHR3" as used herein refers to a regulator of the complemenet system. CFHR3 can refer to, without limitation, human CFHR3 (Protein ID: Q02985) and domains of human CFHR3 (SEQ ID NO: 91 and 92, Table 9 and CFHR3 (1-2), SEQ ID NO: 98).

[0091] "CFHR5" as used herein refers to a regulator of the complement system. CFHR5 can refer to, without limitation, human CFHR5 (Protein ID: Q9BXR6), mouse CFHR5, rat CFHR5, canine CFHR5, chicken CFHR5, bovine CFHR5. CFHR5 can also refer to N-terminal domains of human CFHR5 (CFHR5(1-2), SEQ ID NO: 43, **Table 1.9**). In some embodiments, a CFHR5 CCP domain comprises a sequence in Table 9, SEQ ID NOs: 93-94.

[0092] "CR2" as used herein refers to a regulator of the complement system. CR2 can refer to, without limitation, human CR2 (Protein ID: P20023), mouse CR2, rat CFHR1, canine CR2, chicken CR2, bovine CR2. CR2 can also refer to N-terminal domains of human and CR2(1-4), SEQ ID NO: 44, **Table 1.9**.

[0093] "CRIg" as used herein refers to a regulator of the complement system. CRIg can refer to, without limitation, human CRIg (Protein ID: Q9Y279), mouse CRIg, rat CRIg, canine CRIg, chicken CRIg, bovine CRIg. In some embodiments, CRIg refers to an N-terminal immunoglobulin domains of human CRIg containing IgV and IgC2 immunoglobulin domains (CRIg, SEQ ID NO: 45, **Table 1.9**).

[0094] "Complement Control Protein (CCP) Domain" as used herein refers without limitation to protein domains in complement regulatory proteins, including CR1 and FH. CCP domains are autonomously folding globular domains composed of approximately 60 amino

acid residues, which include four conserved cysteine residues forming two disulfide bridges and highly conserved tryptophan, glycine, proline, and hydrophobic residues at various positions. Tandem CCP domains in proteins that regulate complement are linked by short sequences of 3 to 8 amino acids. CCP domains are also known as short consensus repeats (SCRs) and sushi domains. In some embodiments, a CCP domain comprises a sequence in Table 1.3, SEQ ID NOs: 7-16, Table 1.4, SEQ ID NOs: 17-21, and Table 9, SEQ ID NOs: 89-94. A CCP domain will still comprise a CCP domain if one or more amino acid is added to or deleted from the CCP domain.

[0095] "CR1-CCP-Combination Domain" as used herein refers without limitation to polypeptides comprising multiple tandem CR1 CCP domains. In some embodiments, a CR1-CCP-Combination Domain comprises a sequence in **Table 1.5**, SEQ ID NOs: 22-26. A CR1-CCP-Combination Domain will still comprise a CR1-CCP-Combination Domain if one or more amino acid is added to or deleted from the CR1-CCP-Combination Domain.

[0096] "CR1-FH(4) CCP-Combination Domain" as used herein refers, without limitation, to a polypeptide comprising the FH(4) CCP domain fused to the C-terminus of combinations of tandem CR1 CCP domains. In some embodiments, a CR1-FH(4)-CCP-Combination Domain comprises a sequence listed in **Table 1.6**, SEQ ID NOs: 27-31. A CR1-FH(4) CCP-Combination Domain will still comprise a CR1-FH(4) CCP-Combination Domain if one or more amino acid is added to or deleted from the CR1-FH(4)-CCP-Combination Domain.

[0097] "FH-CCP Combination Domain" as used herein refers, without limitation, to a polypeptide comprising combinations of tandem FH CCP domains. In some embodiments, a FH-CCP Combination Domain comprises a sequence listed in **Table 1.8**, SEQ ID NOs: 37-41 and Table 10.2, SEQ ID NO: 97. A FH-CCP Combination Domain will still comprise a FH-CCP Combination Domain if one or more amino acid is added to or deleted from the FH-CCP Combination Domain.

[0098] The term "K_D", as used herein, refers without limitation to the equilibrium dissociation constant.

[0099] The term "subject" as used herein includes human and other mammalian subjects that receive either prophylactic or therapeutic treatment.

[0100] For purposes of classifying amino acids substitutions as conservative or nonconservative, amino acids are grouped as follows: Group I (hydrophobic side chains): met, ala, val, leu, ile; Group II (neutral hydrophilic side chains): cys, ser, thr; Group III (acidic side chains): asp, glu; Group IV (basic side chains): asn, gln, his, lys, arg; Group V (residues influencing chain orientation): gly, pro; and Group VI (aromatic side chains): trp, tyr, phe. Conservative substitutions involve substitutions between amino acids in the same class. Non-conservative substitutions constitute exchanging a member of one of these classes for a member of another.

[0101] The phrase "a" or "an" entity refers to one or more of that entity; for example, a compound refers to one or more compounds or at least one compound. As such, the terms "a" (or "an"), "one or more", and "at least one" can be used interchangeably herein.

[0102] "About" means variation one might see in measurements taken among different instruments, samples, and sample preparations.

[0103] Compositions or methods "comprising" one or more recited element may include other elements not specifically recited. For example, a protein inhibitor that comprises an oligomerization domain and a complement regulator domain can include other domains that have additional functions and/or affinities.

PROTEIN INHIBITORS

[0104] A protein inhibitor of the present disclosure can include a polypeptide (e.g., first polypeptide) that includes an oligomerization domain or complement receptor domain; and a complement regulator covalently linked to the oligomerization or complement receptor domain. The complement regulator can include a Complement Receptor 1 (CR1) complement control protein (CCP) domain comprising at least one C3b and/or C4b binding domain; and a Factor H (FH), a complement factor H related 3 (CFHR3), or a complement factor H related 5 (CFHR5) CCP domain, or a combination thereof. In some embodiments, the protein inhibitor includes a linker between the oligomerization/complement receptor domain and the complement regulator. In some embodiments, the protein inhibitor includes a linker (e.g., any one of GS1-GS5 in Table 1.7) between the CR1 CCP domain and the FH, CFHR3, or CFHR5 CCP domain. In some embodiments, the protein inhibitor of the present disclosure includes the following structure, from N- to C-terminus: (oligomerization/complement receptor domain)-(Linker)-(complement regulator domain), where the "-" indicates a covalent bond

(e.g., peptide bond) linking the C-terminus of the preceding domain with the N-terminus of the subsequent domain.. In some embodiments, the oligomerization domain includes an amino acid sequence of one or more of SEQ ID NOs: 42, 43, and 46-49. In some embodiments, the complement regulator domain includes an amino acid sequence of one or more of SEQ ID NOs: 44 and 45. In some embodiments, the complement regulator domain includes a CR1 CCP domain comprising at least one C3b and/or C4b binding domain. In some embodiments, the CR1 CCP domain includes an amino acid sequence of one or more of SEQ ID NOs: 22-26, 95, 96, and 137. In some embodiments, the complement regulator domain includes a CR1 CCP domain comprising at least one C3b and/or C4b binding domain and a Factor H (FH), a complement factor H related 3 (CFHR3), or a complement factor H related 5 (CFHR5) CCP domain, or a combination thereof. In some embodiments, the complement regulator domain includes a first domain containing the CR1 CCP domain linked by a linker (e.g., any one of GS1-GS5 in Table 1.7) to a second domain containing the FH, CFHR3, or the CFHR5 CCP domain, or a combination thereof. In some embodiments, the complement regulator domain includes the CR1 CCP domain and the FH domain (e.g., FH(4)). In some embodiments, the complement regulator domain includes the CR1 CCP domain and the CFHR5 CCP domain. In some embodiments, the first domain containing the CR1 CCP domain includes the CR1 CCP domain and a FH domain (e.g., FH(4)). In some embodiments, the first domain containing the CR1 CCP domain includes an amino acid sequence of one or more of SEQ ID NOs: 22-31, 95, 96, and 137 (or a functional variant thereof). In some embodiments, the second domain containing the FH, CFHR3, or the CFHR5 CCP domain, or a combination thereof includes an amino acid sequence of one or more of SEQ ID NOs: 37-41, and 97-99 (or a functional variant thereof). In some embodiments, the first domain containing the CR1 CCP domain includes an amino acid sequence of one or more of SEQ ID NOs: 22-31, 95, 96, and 137 (or a functional variant thereof), and the second domain containing the FH, CFHR3, or the CFHR5 CCP domain, or a combination thereof includes an amino acid sequence of one or more of SEQ ID NOs: 17-21, 37-41, and 97-99 (or a functional variant thereof).

[0105] In some embodiments, the complement regulator is or includes an engineered complement regulator 31-36 in **FIG. 1B.** In some embodiments, the complement regulator in **FIG. 1B,** 31, comprises FH CCP domain 4 covalently linked to CR1 CCP domain 15-17. In some embodiments the complement regulator in **FIG. 1B,** 31, comprises SEQ ID

NO: 29, Table 1.6. In some embodiments, the complement regulator in **FIG. 1B**, 31, comprises the complement regulator in protein inhibitor KC010, SEQ ID NO: 59 (Table 3.1). In some embodiments, the complement regulator in **FIG. 1B,** 32, comprises FH CCP domain 7 covalently linked to a combination of CR1 and FH CCP domains comprising FH CCP domain 4 covalently linked to CR1 CCP domain 15-17. In some embodiments, the complement regulator in **FIG. 1B**, 32, comprises the complement regulator in protein inhibitor KC011, SEQ ID NO: 60 (Table 3.1). In some embodiments, the complement regulator in **FIG. 1B,** 33, comprises FH CCP domains 18-20 covalently linked to a combination of CR1 and FH CCP domains comprising FH CCP domain 4 covalently linked to CR1 CCP domain 15-17. In some embodiments, the complement regulator in **FIG. 1B**, 33, comprises the complement regulator in protein inhibitor KC001, SEQ ID NO: 50 (Table 3.1). In some embodiments, the complement regulator in **FIG. 1B,** 34, comprises FH CCP domains 18-19 covalently linked to a combination of CR1 and FH CCP domains comprising FH CCP domain 4 covalently linked to CR1 CCP domain 15-17. In some embodiments, the complement regulator in **FIG. 1B**, 34, comprises the complement regulator in protein inhibitor KC017, SEQ ID NO: 66 (Table 3.1). In some embodiments, the complement regulator in **FIG. 1B,** 35, comprises FH CCP domains 18-20 covalently linked to a combination of CR1 and FH CCP domains comprising FH CCP domain 4 covalently linked to CR1 CCP domain 1-4 and 15-17. In some embodiments, the complement regulator in **FIG. 1B**, 35, comprises the complement regulator in protein inhibitor KC003, SEQ ID NO: 52 (Table 3.1). In some embodiments, the complement regulator in **FIG. 1B,** 36, comprises FH CCP domains 18-20 covalently linked to a combination of CR1 and FH CCP domains comprising FH CCP domain 4 covalently linked to CR1 CCP domain 1-3. In some embodiments, the complement regulator in **FIG. 1B**, 36, comprises the complement regulator in protein inhibitor KC013, SEQ ID NO: 62 or KC014, SEQ ID NO: 63 (Table 3.1).

[0106] In some embodiments, the protein inhibitor includes a complement regulator containing a CR1-FH(4) combination domain including a FH(4) CCP domain fused to the C-terminus of a CR1 CCP combination domain. In some embodiments, the CR1 CCP combination domain comprises CR1 CCP domains 1-3, 1-4, 8-10, 15-17, 15-18, or a combination thereof (e.g., 1-4 and 15-17, or 1-4 and 15-18, or 1-3 and 15-17). In some embodiments, the CR1-FH(4) CCP combination domain comprises any one of SEQ ID NOs: 27-31 (Tables 1.6). In some embodiments, the complement regulator includes a FH CCP

combination domain linked (e.g., via a linker such as, but not limited to, any one of GS1-GS5 in Table 1.7) to the C-terminus of the CR1-FH(4) combination domain. In some embodiments, the FH CCP combination domain includes FH domains 6-7, 18-20, 18-19 and 7, 18-19, 19 and 7, or 19-20. In some embodiments, the FH CCP combination domain includes any one of SEQ ID NOs: 37-41 (Table 1.8) and SEQ ID NO: 97, Table 10.2. In some embodiments, the complement regulator includes a FH domain (e.g., FH(7)) linked (e.g., via a linker) to the C-terminus of the CR1-FH(4) combination domain. In some embodiments, the protein inhibitor comprises a Fc domain covalently linked to the complement regulator by a linker (e.g., any one of GS1-GS5 in Table 1.7). In some embodiments, the protein inhibitor comprises a dimeric protein inhibitor comprising a first polypeptide and a second polypeptide conjugated through the formation of disulfide bonds between cysteine residues in the oligomerization domains.

[0107] With reference to FIG. 3, a non-limiting embodiment of a protein inhibitor 100 is provided. In some embodiments, the protein inhibitor in **FIG. 3** includes the amino acid sequence set forth in SEQ ID NO: 59 (Table 3.1). In some embodiments of the protein inhibitor 100 in **FIG. 3**, the protein inhibitor comprises a monomer 101, 102. In some embodiments of the protein inhibitor in **FIG. 3**, the protein inhibitor 100 comprises a dimer 103. In some embodiments of the protein inhibitor in **FIG. 3**, the protein inhibitor 100 comprises an oligomerization domain 200 covalently linked to a complement regulator 400. In some embodiments, the oligomerization domain 200 is covalently linked to the complement regulator 400, by a linker 300. In some embodiments in **FIG. 3**, the oligomerization domain 200 comprises the Fc domain 201. In some embodiments, the complement regulator 400, comprises combinations of CR1 and FH CCP domains. In some embodiments, the complement regulator in **FIG. 3** binds C3b or C4b resulting in inhibition of the complement system and or pathways. In some embodiments in **FIG. 3**, the complement regulator 400 comprises a CR1-FH(4) combination domain 700 comprising FH(4) CCP domain 604 fused to the C-terminus of a CR1 CCP combination domain 520. In some embodiments in **FIG. 3**, the CR1 CCP combination domain 520, comprises CR1 CCP domains 15-17, 515-517. In some embodiments, the CR1-FH(4) CCP combination domain 700 comprises SEQ ID NO: **29** (Table 1.6). In some embodiments in **FIG. 3**, the protein inhibitor 100 comprises the Fc domain 201 covalently linked to the complement regulator 400 by the GS5 linker 305 SEQ ID NO: 36, (Table 1.7). In some embodiments in **FIG. 3**, the protein inhibitor 100 comprises a monomer

comprising a first polypeptide 101 or 102. In some embodiments in **FIG. 3**, the protein inhibitor comprises a dimeric protein inhibitor 103 comprising a first polypeptide 101 and a second polypeptide 102 conjugated through the formation of disulfide bonds 205 between cysteine residues in the oligomerization domains 201.

[0108] With reference to FIG. 4, a non-limiting embodiment of a protein inhibitor 100 is provided. In some embodiments, the protein inhibitor in **FIG. 4** includes the amino acid sequence set forth in SEQ ID NO: 52(Table 3.1). In some embodiments of the protein inhibitor in **FIG. 4**, the protein inhibitor 100 comprises a monomer 101 or 102. In some embodiments of the protein inhibitor in **FIG. 4**, the protein inhibitor 100 comprises a dimer 103. In some embodiments of the protein inhibitor in **FIG. 4**, the protein inhibitor 100 comprises an oligomerization domain 200 covalently linked to a complement regulator 400. In some embodiments of the protein inhibitor in **FIG. 4**, the protein inhibitor 100 comprises an oligomerization domain 200 covalently linked to the complement regulator 400, by a linker 300. In some embodiments of the protein inhibitor in **FIG. 4**, the protein inhibitor 100 comprises an oligomerization domain 200 comprising the Fc domain 201. In some embodiments in **FIG. 4**, the protein inhibitor 100 comprises the Fc domain 201 covalently linked to the complement regulator 400 by the GS5 linker 305 SEQ ID NO: 36 (Table 1.7). In some embodiments of the protein inhibitor of **FIG. 4**, the protein inhibitor 100 comprises a complement regulator 400 comprising combinations of CR1 and FH CCP domains. In some embodiments, the complement regulator in FIG. 4 binds C3b or C4b resulting in inhibition of the complement system and or pathways. In some embodiments of the protein inhibitor in **FIG. 4**, the protein inhibitor comprises a complement regulator 400 comprising a CR1-FH(4) combination domain 700 covalently linked to a FH CCP combination domain 630. In some embodiments of **FIG. 4**, the complement regulator 400 comprises a CR1-FH(4) combination domain 700 comprising the FH(4) CCP domain 604 fused to the C-terminus of a CR1 CCP domain combination 520. In some embodiments of **FIG. 4**, the complement regulator 400 comprises a FH CCP domain combination 630 fused to the C-terminus of the FH(4) CCP domain in a CR1-FH(4) combination domain 700. In some embodiments in FIG. 4, the CR1 CCP combination domain 520, comprises CR1 CCP domains 1-4 and 15-17, 501-504 and 515-517. In some embodiments in **FIG. 4**, the complement regulator 400 comprises a CR1-FH(4) CCP combination domain 700 covalently linked to a FH CCP combination domain 630. In

some embodiments in **FIG. 4**, the complement regulator 400 comprises a FH CCP combination domain 630 comprising FH CCP domains 18-20, 618-620, SEQ ID NO: 37 (Table 1.8). In some embodiments in FIG. 4, the complement regulator 400 comprises the CR1-FH(4) CCP combination domain 700 covalently linked to the FH CCP combination domain 630 by a linker 300. In some embodiments of Fig. 4, the complement regulator comprises a CR1-FH(4) CCP combination domain 700 covalently linked to the FH CCP combination domain 630 by the GS3 linker 303. In some embodiments in FIG. 4, the inhibitor 100 comprises a monomer comprising a first polypeptide 101 or 102. In some embodiments in FIG, 4, the protein inhibitor comprises a dimeric protein inhibitor 103 comprising a first polypeptide 101 and a second polypeptide 102 conjugated through the formation of disulfide bonds 205 between cysteine residues in the oligomerization domains 201.

[0109] With reference to FIG. 5, a non-limiting embodiment of a protein inhibitor is provided. In some embodiments, the protein inhibitor in **FIG. 5** includes the amino acid sequence set forth in SEQ ID NO: 50 (Table 3.1). In some embodiments of the protein inhibitor in **FIG. 5**, the protein inhibitor comprises a monomer. In some embodiments of the protein inhibitor in **FIG. 5**, the protein inhibitor comprises a dimer 103. In some embodiments of the protein inhibitor in **FIG. 5**, the protein inhibitor 100 comprises an oligomerization domain 200 covalently linked to a complement regulator 400. In some embodiments of the protein inhibitor in **FIG. 5**, the protein inhibitor comprises the oligomerization domain 200 covalently linked to the complement regulator 400, by a linker 300. In some embodiments of the protein inhibitor in **FIG. 5**, the protein inhibitor comprises an oligomerization domain comprising the Fc domain 201. In some embodiments in **FIG. 5**, the protein inhibitor 100 comprises the Fc domain 201 covalently linked to the complement regulator 400 by the GS5 linker 305. In some embodiments, the complement regulator in FIG. 5 binds C3b or C4b resulting in inhibition of the complement system and or pathways. In some embodiments of the protein inhibitor of **FIG. 5**, the protein inhibitor comprises a complement regulator 400 comprising a combination of CR1 and FH CCP domains 700. In some embodiments of the protein inhibitor in **FIG. 5**, the protein inhibitor comprises a complement regulator 400 comprising a CR1-FH(4) combination domain 700 covalently linked to a FH CCP combination domain 630. In some embodiments of **FIG. 5**, a complement regulator 400 comprises a CR1-FH(4) combination domain 700 comprising the FH(4) CCP domain 604 fused to the C-terminus of a CR1 CCP domain

combination 520. In some embodiments of **FIG. 5**, a complement regulator 400 comprises a FH CCP combination domain 630 fused to the C-terminus of the FH(4) CCP domain in a CR1-FH(4) combination domain 700. In some embodiments in **FIG. 5**, the CR1 CCP combination domain 520, comprises CR1 CCP domains 15-17, 515-517. In some embodiments in FIG. 5, the complement regulator 400 comprises a FH CCP combination domain 630 comprising FH CCP domains 18-20, 618-620. In some embodiments in **FIG. 5**, the complement regulator 400 comprises the CR1-FH(4) CCP combination domain 700 covalently linked to the FH CCP combination domain 630. In some embodiments in **FIG. 5**, the complement regulator 400 comprises the CR1-FH(4) CCP combination domain 700 covalently linked to the FH CCP combination domain 630 by a linker 300. In some embodiments of **Fig. 5**, the complement regulator comprises a CR1-FH(4) CCP combination domain 700 covalently linked to the FH CCP combination domain 630 by the GS3 linker 303. In some embodiments in **FIG. 5**, the inhibitor 100 comprises a monomer comprising a first polypeptide 101 or 102. In some embodiments in **FIG, 5**, the protein inhibitor comprises a dimeric protein inhibitor 103 comprising a first polypeptide 101 and a second polypeptide 102 conjugated through the formation of disulfide bonds 205 between cysteine residues in the oligomerization domains 201.

[0110] With reference to FIG. 6, a non-limiting embodiment of a protein inhibitor 100 is provided. In some embodiments, the protein inhibitor in **FIG. 6** includes the amino acid sequence set forth in SEQ ID NO: 60 (Table 3.1). In some embodiments of the protein inhibitor 100 in **FIG. 6**, the protein inhibitor comprises a monomer 101 or 102. In some embodiments of the protein inhibitor 100 in **FIG. 6**, the protein inhibitor comprises a dimer 103. In some embodiments of the protein inhibitor 100 in **FIG. 6**, the protein inhibitor 100 comprises a oligomerization domain 200 covalently linked to a complement regulator 400. In some embodiments of the protein inhibitor in **FIG 6**, the protein inhibitor comprises the oligomerization domain 200 covalently linked to the complement regulator 400, by a linker 300. In some embodiments of the protein inhibitor in **FIG. 6**, the protein inhibitor 100 comprises an oligomerization domain 200 comprising the Fc domain 201. In some embodiments in **FIG. 6**, the protein inhibitor 100 comprises the Fc domain 201 covalently linked to the complement regulator 400 by the GS5 linker 305. In some embodiments, the complement regulator in FIG. 6 binds C3b or C4b resulting in inhibition of the complement

system and or pathways. In some embodiments of the protein inhibitor in **FIG. 6**, the protein inhibitor comprises a complement regulator 400 comprising a CR1-FH(4) combination domain 700 covalently linked to FH CCP domain 7 (607). In some embodiments of **FIG. 6**, a complement regulator 400 comprises a CR1-FH(4) combination domain 700 comprising the FH(4) CCP domain 604 fused to the C-terminus of a CR1 CCP domain combination 520. In some embodiments of **FIG. 6**, a complement regulator 400 comprises FH CCP domain 7, 607 fused to the C-terminus of the FH(4) CCP domain in a CR1-FH(4) combination domain 700. In some embodiments in FIG. 6, the CR1 CCP combination domain 520, comprises CR1 CCP domains 15-17, 515-517. In some embodiments in **FIG. 6**, the complement regulator 400 comprises the CR1-FH(4) CCP combination domain 700 covalently linked to FH CCP domain 7, 607. In some embodiments in **FIG. 6**, the CR1-FH(4) CCP combination domain comprises 32 **FIG. 1B**. In some embodiments in **FIG. 6**, the complement regulator 400 comprises the CR1-FH(4) CCP combination domain 700 covalently linked to FH CCP domain 7, 607 by a linker 300. In some embodiments of Fig. 6, the complement regulator comprises a CR1-FH(4) CCP combination domain 700 covalently linked to FH CCP domain 7, 607 by the GS3 linker 303. In some embodiments in **FIG. 6**, the inhibitor 100 comprises a monomer comprising a first polypeptide 101 or 102. In some embodiments in **FIG. 6**, the protein inhibitor comprises a dimeric protein inhibitor 103 comprising a first polypeptide 101 and a second polypeptide 102 conjugated through the formation of disulfide bonds 205 between cysteine residues in the oligomerization domains 201.

[0111] With reference to FIG. 7, a non-limiting embodiment of a protein inhibitor 100 is provided. In some embodiments, the protein inhibitor in **FIG. 7** includes the amino acid sequence set forth in SEQ ID NO: 70. In some embodiments of the protein inhibitor in **FIG. 7**, the protein inhibitor comprises a monomer. In some embodiments of the protein inhibitor in **FIG. 7**, the protein inhibitor comprises a dimer 103. In some embodiments of the protein inhibitor in **FIG. 7**, the protein inhibitor 100 comprises an oligomerization domain 200 covalently linked to a complement regulator 400. In some embodiments of the protein inhibitor in **FIG. 7**, the protein inhibitor comprises the oligomerization domain 200 covalently linked to the complement regulator 400, by a linker 300. In some embodiments of the protein inhibitor in **FIG. 7**, the protein inhibitor comprises an oligomerization domain comprising the Fc domain 201. In some embodiments in **FIG. 7**, the protein inhibitor 100 comprises the Fc

domain 201 covalently linked to the complement regulator 400 by the GS5 linker 305. In some embodiments, the complement regulator in FIG. 7 binds C3b or C4b resulting in inhibition of the complement system and or pathways. In some embodiments of the protein inhibitor in **FIG. 7**, the protein inhibitor 100 comprises a complement regulator 400 comprising a CR1-FH(4) combination domain 700 covalently linked to a FH CCP combination domain 630. In some embodiments of **FIG. 7**, the complement regulator 400 comprises a CR1-FH(4) combination domain 700 comprising the FH(4) CCP domain 604 fused to the C-terminus of a CR1 CCP combination domain 520. In some embodiments of **FIG. 7**, the complement regulator 400 comprises a FH CCP combination domain 630 fused to the C-terminus of the FH(4) CCP domain in a CR1-FH(4) combination domain 700. In some embodiments in **FIG. 7**, the CR1 CCP combination domain 520, comprises CR1 CCP domains 15-17, 515-517. In some embodiments in **FIG. 7**, the complement regulator 400 comprises a FH CCP combination domain 630 comprising FH CCP domains 19-20, 619-620. In some embodiments in **FIG. 7**, the complement regulator 400 comprises the CR1-FH(4) CCP combination domain 700 covalently linked to the FH CCP combination domain 630. In some embodiments in **FIG. 7**, the complement regulator 400 comprises the CR1-FH(4) CCP combination domain 700 covalently linked to the FH CCP combination domain 630 by a linker 300. In some embodiments of **FIG. 7**, the complement regulator comprises a CR1-FH(4) CCP combination domain 700 covalently linked to the FH CCP combination domain 630 by the GS3 linker 303. In some embodiments in FIG. 7, the inhibitor 100 comprises a monomer comprising a first polypeptide 101 or 102. In some embodiments in **FIG. 7**, the protein inhibitor comprises a dimeric protein inhibitor 103 comprising a first polypeptide 101 and a second polypeptide 102 conjugated through the formation of disulfide bonds 205 between cysteine residues in the oligomerization domains 201.

[0112] With reference to FIG.9, a non-limiting embodiment of a protein inhibitor 100 is provided. In some embodiments, the protein inhibitor in **FIG. 9** includes the amino acid sequence set forth in SEQ ID NO: 81. In some embodiments of the protein inhibitor in FIG. 9, the protein inhibitor comprises a monomer. In some embodiments of the protein inhibitor in FIG. 9, the protein inhibitor comprises a dimer 103. In some embodiments of the protein inhibitor in FIG 9, the protein inhibitor 100 comprises an oligomerization domain 200 covalently linked to a complement regulator 400. In some embodiments of the protein inhibitor

in **FIG. 9**, the protein inhibitor comprises the oligomerization domain 200 covalently linked to the complement regulator 400, by a linker 300. In some embodiments of the protein inhibitor in FIG. 9, the protein inhibitor comprises an oligomerization domain comprising the VEGF-VH-CH1 domain 202. In some embodiments of the protein inhibitor in **FIG. 9**, the protein inhibitor comprises an oligomerization domain comprising the VEGF-VL-CL domain 203. In some embodiments in **FIG. 9**, the protein inhibitor 100 comprises the VEGF-VH-CH1 domain 202 and/or the VEGF-VL-CL domain 203 covalently linked to the complement regulator 400 by the GS5 linker 305. In some embodiments of the protein inhibitor in **FIG. 9**, the VEGF Fab 208 binds to VEGF and blocks VEGF signaling pathway. In some embodiments of the protein inhibitor in **FIG. 9**, the protein inhibitor comprises a complement regulator 400 comprising a CR1-FH(4) combination domain 700 covalently linked to a FH CCP combination domain 630. In some embodiments of **FIG. 9**, the complement regulator 400 comprises a CR1-FH(4) combination domain comprising the FH(4) CCP domain 604 fused to the C-terminus of a CR1 CCP domain combination 520. In some embodiments, the complement regulator in **FIG. 9** binds C3b or C4b resulting in inhibition of the complement system and or pathways. In some embodiments of **FIG. 9**, the complement regulator 400 comprises a FH CCP domain combination 630 fused to the C-terminus of the FH(4) CCP domain in a CR1-FH(4) combination domain 700. In some embodiments in **FIG. 9**, the CR1 CCP domain combination 520, comprises CR1 CCP domains 15-17, 515-517. In some embodiments in **FIG. 9**, the complement regulator 400 comprises a FH CCP domain combination 630 comprising FH CCP domains 18-20, 618-620. In some embodiments in **FIG. 9**, the complement regulator 400 comprises the CR1-FH(4) CCP combination domain 700 covalently linked to the FH CCP combination domain 630. In some embodiments in **FIG. 9**, the complement regulator 400 comprises the CR1-FH(4) CCP combination domain 700 covalently linked to the FH CCP combination domain 630 by a linker 300. In some embodiments of FIG. 7, the complement regulator comprises a CR1-FH(4) CCP combination domain 700 covalently linked to the FH CCP combination domain 630 by the GS3 linker 303. In some embodiments in **FIG. 9**, the inhibitor 100 comprises a monomer comprising a first polypeptide 101 or 102. In some embodiments in **FIG. 9**, the protein inhibitor comprises a dimeric protein inhibitor 103 comprising a first polypeptide 101 and a second polypeptide 102 conjugated through the

formation of disulfide bonds 206 between cysteine residues in the oligomerization domains 202 and 203.

[0113] With reference to FIG. 10, a non-limiting embodiment of a protein inhibitor 100 is provided. In some embodiments, the protein inhibitor in **FIG. 10** includes the amino acid sequence set forth in SEQ ID NO: 82. In some embodiments of the protein inhibitor in **FIG. 10**, the protein inhibitor comprises a monomer. In some embodiments of the protein inhibitor in **FIG. 10**, the protein inhibitor comprises a dimer 103. In some embodiments of the protein inhibitor in **FIG. 10**, the protein inhibitor 100 comprises an oligomerization domain 200 covalently linked to a complement regulator 400. In some embodiments of the protein inhibitor in **FIG. 10**, the protein inhibitor comprises the oligomerization domain 200 covalently linked to the complement regulator 400, by a linker 300. In some embodiments of the protein inhibitor in **FIG. 10**, the protein inhibitor comprises an oligomerization domain comprising the VEGF-VH-CH1 domain 202. In some embodiments of the protein inhibitor in **FIG. 10**, the protein inhibitor comprises an oligomerization domain comprising the VEGF-VL-CL domain 203. In some embodiments in **FIG. 10**, the protein inhibitor 100 comprises the VEGF-VH-CH1 domain 202 and/or the VEGF-VL-CL domain 203 covalently linked to a complement regulator 400 by the GS5 linker 305. In some embodiments of the protein inhibitor in **FIG. 10**, the VEGF Fab 208 binds to VEGF and blocks VEGF signaling pathway. In some embodiments of the protein inhibitor in **FIG. 10**, the protein inhibitor comprises a complement regulator 400 comprising a CR1-FH(4) combination domain 700 covalently linked to a FH CCP combination domain 630. In some embodiments of **FIG. 10**, the complement regulator 400 comprises a CR1-FH(4) combination domain comprising the FH(4) CCP domain 604 fused to the C-terminus of a CR1 CCP domain combination 520. In some embodiments, the complement regulator in **FIG. 10** binds C3b or C4b resulting in inhibition of the complement system and or pathways. In some embodiments of **FIG. 10**, the complement regulator 400 comprises a FH CCP domain combination 630 fused to the C-terminus of the FH(4) CCP domain in a CR1-FH(4) combination domain 700. In some embodiments in **FIG. 10**, the CR1 CCP domain combination 520, comprises CR1 CCP domains 15-17, 515-517. In some embodiments in FIG. 10, the CR1 CCP domain combination 520, comprises CR1 CCP domains 1-4 and 15-17, 501-504 and 515-517. In some embodiments in **FIG. 10**, the complement regulator 400 comprises a FH CCP domain combination 630 comprising FH CCP

domains 18-20, 618-620. In some embodiments in FIG. 10, the complement regulator 400 comprises the CR1-FH(4) CCP combination domain 700 covalently linked to the FH CCP combination domain 630. In some embodiments in FIG. 10, the complement regulator 400 comprises the CR1-FH(4) CCP combination domain 700 covalently linked to the FH CCP combination domain 630 by a linker 300. In some embodiments of **FIG. 10**, the complement regulator comprises a CR1-FH(4) CCP combination domain 700 covalently linked to the FH CCP combination domain 630 by the GS3 linker 303. In some embodiments in **FIG. 10**, the inhibitor 100 comprises a monomer comprising a first polypeptide 101 or 102. In some embodiments in **FIG. 10**, the protein inhibitor comprises a dimeric protein inhibitor 103 comprising a first polypeptide 101 and a second polypeptide 102 conjugated through the formation of disulfide bonds 206 between cysteine residues in the oligomerization domains 202 and 203.

[0114] With reference to FIGs. 11B-11E, non-limiting embodiments of protein inhibitors 100 are provided. In some embodiments, the protein inhibitors comprise complement regulators fused to domains of complement regulatory protein 81, 82, 83, 84 **FIG. 11A**. In some embodiments, the complement regulators 400 are fused to N-terminal domains of complement regulatory proteins 81, 82, 83, 84 **FIGs. 11B-11E**. In some embodiments, the protein inhibitor comprises an N-terminal domain from a complement regulatory protein that comprises an oligomerization domain 210, 211 **FIGs. 11C-11D**. In some embodiments, the N-terminal domains comprise complement receptor domains 800 **FIGs. 11B** and **11E**. In some embodiments, protein inhibitors comprising N-terminal domains from complement regulatory proteins are monomers. In some embodiments, protein inhibitors comprising N-terminal domains from complement regulatory proteins are dimers or multimers. In some embodiments, the oligomerization domain can be from any protein that regulates complement.

[0115] With reference to FIG. 11B, a non-limiting embodiment of a protein inhibitor 100 is provided. In some embodiments, the protein inhibitor in **FIG. 11B,** KC007, includes the amino acid sequence set forth in SEQID NO: 56 (Table 3.1), 81. In some embodiments, the protein inhibitor in **FIG. 11B** comprises a complement receptor domain 800 comprising the N-terminal domain of CRIg (CRIg(IgV-IgC2)) 802 SEQ ID NO: 45 (Table 1.9). In some embodiments, the protein inhibitor in **FIG. 11B,** 81, comprises the CRIg immunoglobulin domains 802 covalently linked to a combination of CR1 and FH CCP

domains comprising FH CCP domain 4 covalently linked to CR1 CCP domains 15-17. In some embodiments of the protein inhibitor in **FIG. 11B**, the protein inhibitor 100 comprises a complement receptor domain 800 covalently linked to N-terminal of complement regulator 400 by the GS5 linker 305. In some embodiments of the protein inhibitor in **FIG. 11B**, the protein inhibitor 100 comprises a complement regulator 400 comprising a CR1-FH(4) combination domain 700 covalently linked to a FH CCP combination domain 630. In some embodiments of **FIG. 11B**, the complement regulator 400 comprises a CR1-FH(4) combination domain 700 comprising the FH(4) CCP domain 604 fused to the C-terminus of a CR1 CCP combination domain 520. In some embodiments of **FIG. 11B**, the complement regulator 400 comprises a FH CCP combination domain 630 fused to the C-terminus of the FH(4) CCP domain in a CR1-FH(4) combination domain 700. In some embodiments in **FIG. 11B**, the CR1 CCP combination domain 520, comprises CR1 CCP domains 15-17, 515-517. In some embodiments in **FIG. 11B**, the complement regulator 400 comprises a FH CCP combination domain 630 comprising FH CCP domains 19-20, 619-620. In some embodiments in **FIG. 11B**, the complement regulator 400 comprises the CR1-FH(4) CCP combination domain 700 covalently linked to the FH CCP combination domain 630. In some embodiments in **FIG. 11B**, the complement regulator 400 comprises the CR1-FH(4) CCP combination domain 700 covalently linked to the FH CCP combination domain 630 by a linker 300. In some embodiments of **FIG. 11B**, the complement regulator 400 comprises a CR1-FH(4) CCP combination domain 700 covalently linked to the FH CCP combination domain 630 by the GS4 linker 304 SEQ ID NO: 35.

[0116] With reference to FIG. 11C, a non-limiting embodiment of a protein inhibitor 100 is provided. In some embodiments, the protein inhibitor in **FIG. 11C** KC027, includes the amino acid sequence set forth in SEQID NO: 76 (Table 3.1), 82. In some embodiments, the protein inhibitor in **FIG. 11C** comprises an oligomerization domain 200 comprising the N-terminal domain of CFHR1 (CFHR1(1-2)) 210 SEQ ID NO: 42 (Table 1.9). In some embodiments of the protein inhibitor in **FIG. 11C**, the protein inhibitor 100 comprises a monomer. In some embodiments of the protein inhibitor in **FIG. 11C**, the protein inhibitor comprises a dimer or a multimer. In some embodiments of the protein inhibitor in **FIG. 11C**, the protein inhibitor 100 comprises an oligomerization domain 200 covalently linked to a complement regulator 400. In some embodiments of the protein inhibitor in **FIG. 11C**, the

protein inhibitor 100 comprises a complement regulator 400 comprising a CR1-FH(4) combination domain 700 covalently linked to a FH CCP combination domain 630. In some embodiments of **FIG. 11C**, the complement regulator 400 comprises a CR1-FH(4) combination domain 700 comprising the FH(4) CCP domain 604 fused to the C-terminus of a CR1 CCP combination domain 520. In some embodiments of **FIG. 11C**, the complement regulator 400 comprises a FH CCP combination domain 630 fused to the C-terminus of the FH(4) CCP domain in a CR1-FH(4) combination domain 700. In some embodiments in **FIG. 11C**, the CR1 CCP combination domain 520, comprises CR1 CCP domains 15-17, 515-517. In some embodiments in **FIG. 11C**, the complement regulator 400 comprises a FH CCP combination domain 630 comprising FH CCP domains 19-20, 619-620. In some embodiments in **FIG. 11C**, the complement regulator 400 comprises the CR1-FH(4) CCP combination domain 700 covalently linked to the FH CCP combination domain 630. In some embodiments in **FIG. 11C**, the complement regulator 400 comprises the CR1-FH(4) CCP combination domain 700 covalently linked to the FH CCP combination domain 630 by a linker 300. In some embodiments of **FIG. 11C**, the complement regulator 400 comprises a CR1-FH(4) CCP combination domain 700 covalently linked to the FH CCP combination domain 630 by the GS4 linker 304 SEQ ID NO: 35.

[0117] With reference to FIG. 11D, a non-limiting embodiment of a protein inhibitor 100 is provided. In some embodiments, the protein inhibitor in **FIG. 11D** KC028, includes the amino acid sequence set forth in SEQ ID NO: 77 (Table 3.1), 83. In some embodiments, the protein inhibitor in **FIG. 11D** comprises an oligomerization domain 200 comprising the N-terminal domain of CRHR5 (CRHR5(1-2)) 211 SEQ ID NO: 43 (Table 1.9). In some embodiments of the protein inhibitor in **FIG. 11D**, the protein inhibitor 100 comprises a monomer. In some embodiments of the protein inhibitor in **FIG. 11D**, the protein inhibitor comprises a dimer or a multimer. In some embodiments of the protein inhibitor in **FIG. 11D**, the protein inhibitor 100 comprises an oligomerization domain 200 covalently linked to a complement regulator 400. In some embodiments of the protein inhibitor in **FIG. 11D**, the protein inhibitor 100 comprises a complement regulator 400 comprising a CR1-FH(4) combination domain 700 covalently linked to a FH CCP combination domain 630. In some embodiments of **FIG. 11D**, the complement regulator 400 comprises a CR1-FH(4) combination domain 700 comprising the FH(4) CCP domain 604 fused to the C-terminus of a

CR1 CCP combination domain 520. In some embodiments of **FIG. 11D**, the complement regulator 400 comprises a FH CCP combination domain 630 fused to the C-terminus of the FH(4) CCP domain in a CR1-FH(4) combination domain 700. In some embodiments in **FIG. 11D**, the CR1 CCP combination domain 520, comprises CR1 CCP domains 15-17, 515-517. In some embodiments in **FIG. 11D**, the complement regulator 400 comprises a FH CCP combination domain 630 comprising FH CCP domains 19-20, 619-620. In some embodiments in **FIG. 11D**, the complement regulator 400 comprises the CR1-FH(4) CCP combination domain 700 covalently linked to the FH CCP combination domain 630. In some embodiments in **FIG. 11D**, the complement regulator 400 comprises the CR1-FH(4) CCP combination domain 700 covalently linked to the FH CCP combination domain 630 by a linker 300. In some embodiments of **FIG. 11D**, the complement regulator 400 comprises a CR1-FH(4) CCP combination domain 700 covalently linked to the FH CCP combination domain 630 by the GS4 linker 304 SEQ ID NO: 35.

[0118] With reference to FIG. 11E, a non-limiting embodiment of a protein inhibitor 100 is provided. In some embodiments, the protein inhibitor in **FIG. 11E** KC030, includes the amino acid sequence set forth in SEQ ID NO: 79 (Table 3.1), 84. In some embodiments, the protein inhibitor 100 in **FIG. 11E** comprises a complement receptor domain 800 comprising the N-terminal domain of CR2 (CR2(1-4)) 801 SEQ ID NO: 44 (Table 1.9). In some embodiments of the protein inhibitor in **FIG. 11E**, the protein inhibitor 100 comprises a complement receptor domain 800 covalently linked to a complement regulator 400. In some embodiments of the protein inhibitor in **FIG. 11E**, the protein inhibitor 100 comprises a complement receptor domain 800 covalently linked to N-terminus of a complement regulator 400 by the GS5 linker 305. In some embodiments of the protein inhibitor in **FIG. 11E**, the protein inhibitor 100 comprises a complement regulator 400 comprising a CR1-FH(4) combination domain 700 covalently linked to a FH CCP combination domain 630. In some embodiments of **FIG. 11E**, the complement regulator 400 comprises a CR1-FH(4) combination domain 700 comprising the FH(4) CCP domain 604 fused to the C-terminus of a CR1 CCP combination domain 520. In some embodiments of **FIG. 11E**, the complement regulator 400 comprises a FH CCP combination domain 630 fused to the C-terminus of the FH(4) CCP domain in a CR1-FH(4) combination domain 700. In some embodiments in **FIG. 11E**, the CR1 CCP combination domain 520, comprises CR1 CCP domains 15-17, 515-517.

In some embodiments in **FIG. 11E**, the complement regulator 400 comprises a FH CCP combination domain 630 comprising FH CCP domains 19-20, 619-620. In some embodiments in **FIG. 11E**, the complement regulator 400 comprises the CR1-FH(4) CCP combination domain 700 covalently linked to the FH CCP combination domain 630. In some embodiments in **FIG. 11E**, the complement regulator 400 comprises the CR1-FH(4) CCP combination domain 700 covalently linked to the FH CCP combination domain 630 by a linker 300. In some embodiments of **FIG. 11E**, the complement regulator 400 comprises a CR1-FH(4) CCP combination domain 700 covalently linked to the FH CCP combination domain 630 by the GS4 linker 304 SEQ ID NO: 35.

[0119] In some embodiments, the protein inhibitor includes a complement regulator containing a CR1 CCP combination domain. In some embodiments, the CR1 CCP combination domain comprises CR1 CCP domains 1-3, 1-4, 8-10, 15-17, 15-18, or a combination thereof (e.g., 1-4 and 15-17, or 1-4 and 15-18, or 1-3 and 15-17). In some embodiments, the CR1 CCP combination domain comprises CR1 CCP domains 15-18. In some embodiments, the CR1 CCP combination domain comprises SEQ ID NO: 95 (Table 10.1). In some embodiments, the CR1 CCP combination domain comprises SEQ ID NO: 96 (Table 10.1). In some embodiments, the complement regulator includes a CFHR5 CCP combination domain linked (e.g., via a linker such as, but not limited to, any one of GS1-GS5 in Table 1.7) to the C-terminus of the CR1 CCP combination domain. In some embodiments, the CFHR5 CCP combination domain includes CFHR5 domains 8-9. In some embodiments, the CFHR5 CCP combination domain includes SEQ ID NO: 99 (Table 10.2). In some embodiments, the protein inhibitor comprises a Fc domain covalently linked to the complement regulator by a linker (e.g., any one of GS1-GS5 in Table 1.7). In some embodiments, the protein inhibitor comprises a dimeric protein inhibitor comprising a first polypeptide and a second polypeptide conjugated through the formation of disulfide bonds between cysteine residues in the oligomerization domains.

[0120] With reference to FIG. 19, a non-limiting embodiment of a protein inhibitor 100 is provided. In some embodiments, the protein inhibitor in **FIG. 19** includes the amino acid sequence set forth in SEQ ID NO: 105. In some embodiments of the protein inhibitor in **FIG. 19**, the protein inhibitor comprises a monomer. In some embodiments of the protein inhibitor in **FIG. 19**, the protein inhibitor comprises a dimer 103. In some embodiments of the

protein inhibitor in **FIG. 19**, the protein inhibitor 100 comprises an oligomerization domain 200 covalently linked to a complement regulator 400. In some embodiments of the protein inhibitor in **FIG. 19**, the protein inhibitor comprises the oligomerization domain 200 covalently linked to the complement regulator 400, by a linker 300. In some embodiments of the protein inhibitor in **FIG. 19**, the protein inhibitor comprises an oligomerization domain comprising the Fc domain 201. In some embodiments in **FIG. 19**, the protein inhibitor 100 comprises the Fc domain 201 covalently linked to the complement regulator 400 by the GS5 linker 305. In some embodiments, the complement regulator in FIG. 19 binds C3b or C4b resulting in inhibition of the complement system and or pathways. In some embodiments of the protein inhibitor in **FIG. 19**, the protein inhibitor 100 comprises a complement regulator 400 comprising a CR1 CCP combination domain 520 covalently linked (e.g., via a linker) to a CFHR5 CCP combination domain 920. In some embodiments of **FIG. 19**, the complement regulator 400 comprises a CFHR5 CCP combination domain 920 fused (e.g., via a linker) to the C-terminus of the CR1 CCP combination domain 520. In some embodiments in **FIG. 19**, the CR1 CCP combination domain 520, comprises CR1 CCP domains 15-18 (515-518, respectively). In some embodiments in **FIG. 19**, the complement regulator 400 comprises a CFHR5 CCP combination domain 920 comprising CFHR5 domains 8-9 (908-909, respectively). In some embodiments in **FIG. 19**, the complement regulator 400 comprises the CFHR5 CCP combination domain 920 covalently linked to the CR1 CCP combination domain 520 by a linker 300. In some embodiments of **FIG. 19**, the complement regulator comprises the CFHR5 CCP combination domain 920 covalently linked to the CR1 CCP combination domain 520 by the GS3 linker 303. In some embodiments in FIG. 19, the inhibitor 100 comprises a monomer comprising a first polypeptide 101 or 102. In some embodiments in **FIG. 19**, the protein inhibitor comprises a dimeric protein inhibitor 103 comprising a first polypeptide 101 and a second polypeptide 102 conjugated through the formation of disulfide bonds 205 between cysteine residues in the oligomerization domains 201.

[0121] In some embodiments, the protein inhibitor 100 comprises a first polypeptide 101 wherein the first polypeptide 101 comprises an oligomerization domain 200 and a complement regulator domain 400 **FIGS. 3-7, 9-10 and 11C-11D, and 19** (in all cases figures show non-limiting examples of protein inhibitors and components of protein inhibitors).

[0122] In some embodiments, the protein inhibitor 100 is a monomer 101 or 102 **FIGS. 3-7, 9-10 and 11B-11E, and 19**. In some embodiments, the protein inhibitor 100 comprises a first polypeptide 101 with at least one unpaired cysteine and a second polypeptide 102 with at least one unpaired cysteine **FIGS. 3-7 and 9-10, and 19**. In some embodiments, the protein inhibitor 100 comprises a first polypeptide 101 conjugated to a second polypeptide 102 through unpaired cysteines in the oligomerization domains 200 of the polypeptides **FIGS. 3-7 and 9-10, and 19**. In some embodiments, the protein inhibitor 100 is a dimer 103 that is a homodimer **FIGS. 3-7 and FIGs. 11C-11D, and FIG. 19.** As used herein, "homodimer" denotes a dimer that includes two identical or substantially identical monomer units. In some embodiments, the protein inhibitor 100 is a dimer 103 that is a heterodimer **FIGS. 9-10**. As used herein, "heterodimer" denotes a dimer that includes two different monomer units.

[0123] In some embodiments, the protein inhibitors comprising an oligomerization domain form dimers or multimers through disulfide bond formation between their oligomerization domains. In some embodiments, the protein inhibitors comprising an oligomerization domain form dimers or multimers through interactions between their oligomerization domains through mechanisms other than disulfide bond formation. In some embodiments, the protein inhibitor 100 is a monomer 200. In some embodiments, the protein inhibitor 100 is a monomer 211. In some embodiments, the protein inhibitor 100 is a dimer 103 that is a homodimer of 210. In some embodiments, the protein inhibitor is a dimer 103 that is a homodimer of 211. In some embodiments, the protein inhibitor is a dimer 103 that is a heterodimer of 210 and 211. In some embodiments, the protein inhibitor is a trimer. In some embodiments, the protein inhibitor is a multimer of polypeptides conjugated together through unpaired cysteines in their oligomerization domains. In some embodiments, the inhibitor can be comprised of any number of protein inhibitors.

[0124] In some embodiments, the conjugation of two or more inhibitors through their oligomerization domains provides superior stability to the inhibitors. In some embodiments, the conjugation of two or more inhibitors through their oligomerization domains makes the inhibitors more effective in promoting Factor I-mediated degradation of C3b and C4b. In some embodiments, conjugation of two or more protein inhibitors through their oligomerization domains provides a greater concentration of the protein inhibitors to sites of activity. In some embodiments, conjugations of two or more oligomerization domains of the

protein inhibitors results in an enhanced inhibitory or biological activity. In some embodiments, the oligomerization domain comprises any protein domain that can be conjugated to another protein domain through unpaired cysteines.

[0125] Any suitable oligomerization domain can be used in the protein inhibitors of the present disclosure. Suitable oligomerization domains include, without limitation, a Fc domain, antibody fragment (e.g., Fab fragment), a complement factor H-related protein domain (e.g., CFHR1(1-2) or CFHR5(1-2) domains), leucine zipper domain, coiled-coil domain, heterodimeric Fc domains ("knob-into-hole"), etc. In some embodiments, the oligomerization domain is an Fc domain. In some embodiments, the oligomerization domain 200 is an Fc domain 201 **FIGS. 3-7, and 19** having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 46, **FIGS. 2-7, and 19**. In some embodiments, the oligomerization domain includes the amino acid sequence of SEQ ID NO: 46. In some embodiments, the oligomerization domain is or includes the amino acid sequence of SEQ ID NO: 42 or 43.

[0126] In some embodiments, the oligomerization domain is an antibody fragment (e.g., a Fab fragment). Any suitable antibody fragment (e.g., a Fab fragment) can be used as the oligomerization domain. In some embodiments, the antibody fragment (e.g., a Fab fragment) binds to an antigen. The antibody fragment (e.g., a Fab fragment) can bind to any suitable antigen. Suitable antigens include, without limitation, VEGF, IL-6, PDGF, CFD, IL-1β, IL-1α, IL-18, TNF-α.

[0127] In some embodiments, the antibody fragment (e.g., a Fab fragment) binds to a VEGF molecule (e.g., VEGF-A,). In some embodiments, the antibody fragment (e.g., a Fab fragment) binds to human VEGF. In some embodiments, the antibody fragment (e.g., a Fab fragment) is an anti-VEGF antibody fragment (e.g., an anti-VEGF Fab fragment). In some embodiments, the anti-VEGF antibody fragment (e.g., an anti-VEGF Fab fragment is derived from a full-length anti-VEGF antibody. Any suitable full length anti-VEGF antibody or anti-VEGF antibody fragment can be used. For example, and without limitation, bevacizumab (AVASTIN, Genentech/Roche) is a humanized mouse monoclonal antibody that binds to and neutralizes human VEGF, in particular to all isoforms of VEGF-A and to bioactive proteolytic fragments of VEGF-A. For example, and without limitation, ranibizumab (LUCENTIS®(ranibizumab), Genentech/Roche) is an antibody fragment or Fab that targets

VEGF. In some embodiments, the oligomerization domain is a Fab fragment of an anti-VEGF-A antibody as described in, e.g., WO2017117464, which is incorporated herein by reference in its entirety. In some embodiments, the oligomerization domain includes an anti-VEGF-VH-CH1 domain. In some embodiments, the oligomerization domain 200 is or includes an anti-VEGF-VH-CH1 domain 202 having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 47, **FIGS. 8-10**. In some embodiments, the oligomerization domain 200 is or includes an anti-VEGF-VH-CH1 domain 202 having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 47 with variations, if any, outside a CDR of the anti-VEGF-VH-CH1 domain. In some embodiments, the oligomerization domain includes an anti-VEGF-VL-CL domain. In some embodiments, the oligomerization domain includes an anti-VEGF-HC. In some embodiments, the oligomerization domain includes an anti-VEGF-HC having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO:49. In some embodiments, the oligomerization domain includes an anti-VEGF-HC. In some embodiments, the oligomerization domain includes an anti-VEGF-HC having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO:49 with variations, if any, outside a CDR of the anti-VEGF-HC. In some embodiments, the oligomerization domain 200 is or includes an anti-VEGF-VL-CL domain 203 having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 48, **FIGS. 8-10**. In some embodiments, the oligomerization domain is or includes an anti-VEGF-VL-CL domain having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 48 with variations, if any, outside a CDR of the anti-VEGF-VL-CL domain. In some embodiments, the oligomerization domain includes an anti-VEGF-VH-CH1 domain and an anti-VEGF-VL-CL domain. In some embodiments, the oligomerization domain includes an anti-VEGF-VH-CH1 domain having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 47, and an anti-VEGF-VL-CL domain having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 48. In some embodiments, the oligomerization domain includes an anti-VEGF-VH-CH1 domain having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 47 with variations, if any, outside a CDR of the anti-VEGF-VH-CH1 domain, and an anti-VEGF-VL-CL domain having at least 80, 85, 90, 95, 96, 97, 98,

99, or 100% identity with the amino acid sequence of SEQ ID NO: 48 with variations, if any, outside a CDR of the anti-VEGF-VL-CL domain. In some embodiments, the oligomerization domain includes an anti-VEGF-HC and an anti-VEGF-VL-CL domain. In some embodiments, the oligomerization domain includes an anti-VEGF-HC having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 49, and an anti-VEGF-VL-CL domain having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 48. In some embodiments, the oligomerization domain includes an anti-VEGF-HC having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 49 with variations, if any, outside a CDR of the anti-VEGF-HC, and an anti-VEGF-VL-CL domain having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 48 with variations, if any, outside a CDR of the anti-VEGF-VL-CL domain.

[0128] In some embodiments, the antibody fragment (e.g., a Fab fragment) binds to an interleukin 6 (IL-6) molecule. In some embodiments, the antibody fragment (e.g., a Fab fragment) binds to human IL-6. In some embodiments, the antibody fragment (e.g., a Fab fragment) is an anti-IL-6 antibody fragment (e.g., an anti-IL-6 Fab fragment). In some embodiments, the anti-IL-6 antibody fragment (e.g., an anti-IL-6 Fab fragment is derived from a full-length anti-IL-6 antibody. Any suitable full length anti-IL-6 antibody or anti-IL-6 antibody fragment can be used. For example, and without limitation, the anti-IL-6 antibody or fragment is siltuximab, sirukumab, or clazakizumab. In some embodiments, the oligomerization domain is a Fab fragment of an anti-IL-6 antibody as described in, e.g., WO2019169341, which is incorporated herein by reference in its entirety. In some embodiments, the oligomerization domain includes an anti-IL-6-VH-CH1 domain. In some embodiments, the oligomerization domain includes an anti-IL-6-VH-CH1 domain having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 112. In some embodiments, the oligomerization domain includes an anti-IL-6-VH-CH1 domain having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 112 with variations, if any, outside a CDR of the anti-IL-6-VH-CH1 domain In some embodiments, the oligomerization domain includes an anti-IL-6-HC. In some embodiments, the oligomerization domain includes an anti-IL-6-HC having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 114.

In some embodiments, the oligomerization domain includes an anti-IL-6-HC. In some embodiments, the oligomerization domain includes an anti-IL-6-HC having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 114 with variations, if any, outside a CDR of the anti-IL-6-HC. In some embodiments, the oligomerization domain includes an anti-IL-6-LC domain. In some embodiments, the oligomerization domain includes an anti-IL-6-VL-CL domain having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 113. In some embodiments, the oligomerization domain includes an anti-IL-6-VL-CL domain having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 113 with variations, if any, outside a CDR of the anti-IL-6-VL-CL. In some embodiments, the oligomerization domain includes an anti-IL-6-VH-CH1 domain and an anti-IL-6-VL-CL domain. In some embodiments, the oligomerization domain includes an anti-IL-6-VH-CH1 domain having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 112, and an anti-IL-6-VL-CL domain having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 113. In some embodiments, the oligomerization domain includes an anti-IL-6-VH-CH1 domain having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 112 with variations, if any, outside a CDR of the anti-IL-6-VH-CH1, and an anti-IL-6-VL-CL domain having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 113 with variations, if any, outside a CDR of the anti-IL-6-VL-CL. In some embodiments, the oligomerization domain includes an anti-IL-6-HC and an anti-IL-6-VL-CL domain. In some embodiments, the oligomerization domain includes an anti-IL-6-HC having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 114 with variations, if any, outside a CDR of the anti-IL-6-HC, and an anti-IL-6-VL-CL domain having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 113 with variations, if any, outside a CDR of the anti-IL-6-VL-CL domain.

[0129] In some embodiments, the oligomerization domain is a CFHR1(1-2) domain. In some embodiments, the oligomerization domain 200 is CFHR1(1-2) domain having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 42, **FIG. 11C**.

[0130] In some embodiments, the oligomerization domain is a CFHR5(1-2) domain. In some embodiments, the oligomerization domain 200 is CFHR5(1-2) domain having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 43 and SEQ ID NO: 43, **FIG. 11D**.

[0131] In some embodiments, the protein inhibitor includes a complement receptor domain. Any suitable complement receptor domain can be used. In some embodiments, the complement receptor domain is a CR2(1-4) domain. In some embodiments, the complement receptor domain 800 is CR2(1-4) domain having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 44 **FIG. 11E**.

[0132] In some embodiments, the complement receptor domain is a CRIg(IgV-IgC2) domain. In some embodiments, the complement receptor domain 800 is a CRIg(IgV-IgC2) domain having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identity with the amino acid sequence of SEQ ID NO: 45, **FIG. 11B**.

[0133] In some embodiments, the protein inhibitor 100 comprises an oligomerization domain 200 covalently linked to a complement regulator 400, **FIGS. 3-7 9-10, 11C-11D, and 19**. In some embodiments, the protein inhibitor 100 comprises an oligomerization domain covalently linked to a complement regulator 400 by a linker 300. In some embodiments the linker 300 comprises of any combination of amino acids. In some embodiments, the linker 300 comprises glycine and serine. In some embodiments, the linker comprises between 2 and 20 amino acids. In some embodiments, the linker 300 connecting the oligomerization domain 200 to the complement regulator 400 comprises GGGGSGGGGS, SEQ ID NO: 36, 305, **FIGS. 3-7** and **9-10, and 19**.

[0134] In some embodiments, linker 300 **FIGS. 3-7, 9-10, and 11B-11E, and 19** refers to GS1 (SEQ ID NO: 32, Table 1.7). In some embodiments, linker 300 refers to GS2 (SEQ ID NO: 33, Table 1.7). In some embodiments, linker 300 refers to GS3 (SEQ ID NO: 34, Table 1.7). In some embodiments, linker 300 refers to GS4 (SEQ ID NO: 35, Table 1.7). In some embodiments, linker 300 refers to GS5 (SEQ ID NO: 36, Table 1.7).

[0135] In some embodiments, the protein inhibitor 100 **FIGS. 3-7,9-10, and 11B-11E** comprises a complement regulator 400 comprising at least one CCP domain from CR1 and at least one CCP domain from FH **FIGS. 3-7** and **9-10**. In some embodiments, the complement regulator comprises any CCP domain from CR1 and FH.

[0136] In some embodiments, the complement regulator 400 **FIGS. 3-7, 9-10, and FIGS. 11B-11E, and 19** comprises a CR1 CCP domain from the sequences in Table 1.3, SEQ ID NOs: 7-16. In some embodiments, the CR1 CCP domain comprises CR1(01) SEQ ID NO: 7 (Table 1.3). In some embodiments, the CR1 CCP domain comprises CR1(02) SEQ ID NO: 8 (Table 1.3). In some embodiments, the CR1 CCP domain comprises CR1(03) SEQ ID NO: 9 (Table 1.3). In some embodiments, the CR1 CCP domain comprises CR1(04) SEQ ID NO: 10 (Table 1.3). In some embodiments, the CR1 CCP domain comprises CR1(08) SEQ ID NO: 11 (Table 1.3). In some embodiments, the CR1 CCP domain comprises CR1(09) SEQ ID NO: 12 (Table 1.3). In some embodiments, the CR1 CCP domain comprises CR1(10) SEQ ID NO: 13 (Table 1.3). In some embodiments, the CR1 CCP domain comprises CR1(15) SEQ ID NO: 14 (Table 1.3). In some embodiments, the CR1 CCP domain comprises CR1(16) SEQ ID NO: 15 (Table 1.3). In some embodiments, the CR1 CCP domain comprises CR1(17) SEQ ID NO: 16 (Table 1.3).

[0137] In some embodiments, the complement regulator 400 **FIGS. 3-7, 9-10, and 11B-11E** comprises a FH CCP domain from the sequences in Table 1.4, SEQ ID NOs: 17-21. In some embodiments, the FH CCP domain comprises FH(04) SEQ ID NO: 17 (Table 1.4). In some embodiments, the FH CCP domain comprises FH(07) SEQ ID NO: 18 (Table 1.4). In some embodiments, the FH CCP domain comprises FH(18) SEQ ID NO: 19 (Table 1.4). In some embodiments, the FH CCP domain comprises FH(19) SEQ ID NO: 20 (Table 1.4). In some embodiments, the FH CCP domain comprises FH(20) SEQ ID NO: 21 (Table 1.4).

[0138] In some embodiments, the complement regulator 400 comprises a combination of CR1 CCP domains that form a CR1-CCP-combination domain 520 **FIGS. 3-7, 9-10 and 11B-11E, and 19**. In some embodiments, the CR1-CCP-combination domain 520 **FIGS. 3-7, 9-10 and 11B-11E, and 19** comprises any combination of CCP domains from CR1. In some combinations, the CR1-CCP-combination domain 520 comprises CR1 CCP domains 1-3, CR1(1-3), SEQ ID NO: 22 (Table 1.5). In some embodiments, the CR1-CCP-combination domain 520 comprises CR1 CCP domains 1-4, CR1(1-4), SEQ ID NO: 23 (Table 1.5). In some embodiments, the CR1-CCP-combination domain 520 comprises CR1 CCP domains 8-10, CR1(8-10), SEQ ID NO: 24 (Table 1.5). In some embodiments, the CR1-CCP-combination domain 520 comprises CR1 CCP domains 15-17, CR1(15-17), SEQ ID NO: 25 (Table 1.5). In some embodiments, the CR1-CCP-combination domain 520 comprises CR1 CCP domains 1-

4 and 15-17, CR1(1-4,15-17), SEQ ID NO: 26 (Table 1.5). In some embodiments, the CR1-CCP-combination domain 520 comprises CR1 CCP domains 1-3 and 15-17 (e.g., SEQ ID NO:137).

[0139] In some embodiments, the complement regulator 400 **FIGS. 3-7, 9-10 and 11B-11E** comprises a FH CCP domain fused to the C-terminus of two or more CR1 CCP domains. In some embodiments, the FH CCP domain covalently linked to the C-terminus of two or more CR1 CCP domains comprises any FH CCP domain. In some embodiments, the complement regulator 400 comprises FH(4) SEQ ID NO: 17 604, FIG. 3 covalently linked to the C-terminus of two or more CR1 CCP domains.

[0140] In some embodiments, the complement regulator comprises the FH(4) CCP domain covalently linked to the C-terminus of two or more CR1 CCP domains to form a CR1-FH(4) CCP combination domain 700, **FIGS. 3-7, 9-10 and 11B-11E**. In some embodiments, the CR1-FH(4) CCP-combination domain 700 comprises FH(4) fused to the C-terminus of CR1 CCP domains 1-3, SEQ ID NO: 27. In some embodiments, the CR1-FH(4) CCP-combination domain 700 comprises FH(4) fused to the C-terminus of CR1 CCP domains 8-10 CR1(8-10)-FH(4), SEQ ID NO: 28 (Table 1.6). In some embodiments, the CR1-FH(4) CCP-combination domain 700 comprises FH(4) fused to the C-terminus of CR1 CCP domains 15-17 (515-517) CR1(15-7)-FH(4), SEQ ID NO: 29 (Table 1.6) FIGS. 3, 5-7, 9-10, 11B-11E. In some embodiments, the CR1-FH(4) CCP-combination domain 700 comprises FH(4) fused to the C-terminus of CR1 CCP domains 1-3 and 15-17, CR1(1-3,15-17)-FH(4), SEQ ID NO: 30, Table 1.6. In some embodiments, the CR1-FH(4) CCP-combination domain 700 comprises FH(4) fused to the C-terminus of CR1 CCP domains 1-4 and 15-17, CR1(1-4, 15-17)-FH(4), SEQ ID NO: 31, Table 1.6 **FIG. 4**.

[0141] In some embodiments, the complement regulator 400 comprises a combination of FH CCP domains comprising a FH-CCP-combination domain 630, **FIGS. 4-5**, 7, **9-10, 11B-11E**. In some embodiments, the FH-CCP-combination domain comprises combinations of any FH CCP domains. In some embodiments, the FH-CCP-combination domain 630 comprises FH CCP domains 18-20 FH(18-20), SEQ ID NO: 37 (Table 1.8) **FIGS. 4-5** and **9-10**. In some embodiments, FH-CCP-combination domain comprises FH CCP domains 18-19 and 7 FH(18-19, 7), SEQ ID NO: 38 (Table 1.8). In some embodiments, the FH-CCP-combination domain comprises FH CCP domains 18 and 19, FH(18-19), SEQ ID

NO: 39 (Table 1.8). In some embodiments, the FH-CCP-combination domain comprises FH CCP domains 19 and 7, FH(19, 7), SEQ ID NO: 40 (Table 1.8). In some embodiments, FH-CCP-combination domain 630 comprises FH CCP domains 19-20 FH(19-20), SEQ ID NO: 41 (Table 1.8) **FIGS. 7 and 11B-11E**.

[0142] In some embodiments, the complement regulator 400 comprises one or more FH CCP domain covalently linked to the C-terminus of a CR1-FH(4) CCP-combination domain 700, **FIGS. 4-7, 9-10 and 11B-11E**. In some embodiments, the FH CCP domain or domains covalently linked to the C-terminus of a CR1-FH(4) CCP-combination domain comprises any FH CCP domain. In some embodiments, the complement regulator 400 comprises FH(18-20), SEQ ID NO: 37, Table 1.8, covalently linked to the C-terminus of a CR1-FH(4) CCP combination domain 700, **FIGS. 4-5** and **9-10**. In some embodiments, the complement regulator 400 comprises FH(18-19, 7), SEQ ID NO: 38, Table 1.8, covalently linked to the C-terminus of a CR1-FH(4) CCP combination domain. In some embodiments, the complement regulator comprises FH(18-19), SEQ ID NO: 39, Table 1.8, covalently linked to the C-terminus of a CR1-FH(4) CCP combination domain FIG. 1B, 34. In some embodiments, the complement regulator comprises FH(19, 7), SEQ ID NO: 40, Table 1.8, covalently linked to the C-terminus of a CR1-FH(4) CCP combination domain. In some embodiments, the complement regulator comprises FH(19-20), SEQ ID NO: 41, Table 1.8, covalently linked to the C-terminus of a CR1-FH(4) CCP combination domain.

[0143] In some embodiments, the complement regulator comprises a single FH CCP domain covalently linked to the C-terminus of a CR1-FH(4) CCP combination domain. In some embodiments, the FH CCP domain covalently linked to the to the C-terminus of a CR1-FH(4) CCP combination domain comprises any FH CCP domain. In some embodiments, the complement regulator 400 comprises FH(7) SEQ ID NO: 18, 607 covalently linked to the C-terminus of a CR1-FH(4) CCP combination domain 700, **FIG. 6**. In some embodiments, the single FH CCP domain is linked to the C-terminus of a CR1-FH(4) CCP combination domain via a linker. In some embodiments, the linker comprises any amino acid. In some embodiments, the linker comprises any number of amino acids. In some embodiments, the linker comprises glycine and serine. In some embodiments, the single FH CCP domain is linked to the CR1-FH(4) CCP combination domain via a linker selected from SEQ ID Nos. 32-

36 (Table 1.7). In some embodiments, a complement regulator comprises FH CCP domain 7 linked to a CR1-FH(4) CCP combination domain via linker GS3 SEQ ID NO: 34 (Table 1.7).

[0144] In some embodiments, the complement regulator comprises two or more CR1 and FH CCP domain covalently linked via linkers. In some embodiments, the complement regulator 400 comprises two or more FH CCP domains covalently linked via linkers 300, **FIGS. 4-7, 9-10 and 11B-11E**. In some embodiments, the linker comprises any amino acid. In some embodiments, the linker comprises any number of amino acids. In some embodiments, the linker comprises glycine and serine. In some embodiments, the linker comprises GS, SEQ ID NO: 32, Table 1.7. In some embodiments, the linker comprises GGGGS, SEQ ID NO: 33, Table 1.7. In some embodiments, the linker comprises GSGGGGS, SEQ ID NO: 34 (Table 1.7), 303, **FIGS. 4-7 and 9-10**. In some embodiments, the linker comprises GSGGGGSGGGGS (SEQ ID NO: 35) (Table 1.7). 304.

[0145] In some embodiments, the protein inhibitor comprises KC001 (SEQ ID NO: 50, Tables 2 and 3.1), or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC002 (SEQ ID NO: 51, Tables 2 and 3.1), or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC003 (SEQ ID NO: 52, Tables 2 and 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC004 (SEQ ID NO: 53, Table 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC005 (SEQ ID NO: 54, Table 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC006 (SEQ ID NO: 55, Table 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC007 (SEQ ID NO: 56, Table 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC008 SEQ ID NO: 57 (Table 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC009 SEQ ID NO: 58 (Table 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC010 SEQ ID NO: 59 (Table 3.1) , or a

sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC011 SEQ ID NO: 60 (Table 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC012 SEQ ID NO: 61 (Table 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC013 SEQ ID NO: 62 (Table 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC014 SEQ ID NO: 63 (Table 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC015 SEQ ID NO: 64 (Table 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC016 SEQ ID NO: 65 (Table 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC017 SEQ ID NO: 66 (Table 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC018 SEQ ID NO: 67 (Table 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC019 SEQ ID NO: 68 (Table 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC020 SEQ ID NO: 69 (Table 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC021 SEQ ID NO: 70 (Table 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC022 SEQ ID NO: 71 (Table 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC023 SEQ ID NO: 72 (Table 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC024 SEQ ID NO: 73 (Table 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC025 SEQ ID NO: 74 (Table 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some

embodiments, the protein inhibitor comprises KC026 SEQ ID NO: 75 (Table 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC027 SEQ ID NO: 76 (Table 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC028 SEQ ID NO: 77 (Table 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC029 SEQ ID NO: 78 (Table 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC030 SEQ ID NO: 79 (Table 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor comprises KC031 SEQ ID NO: 80 (Table 3.1) , or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto.

[0146] In some embodiments, the protein inhibitor is capable of binding C3b. In some embodiments, the protein inhibitor is capable of promoting C3b degradation. In some embodiments, the protein inhibitors promotes at least 70, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% of C3b degradation including any range above any one of the preceding values and any range between any two of the preceding values As an example of such superior promotion of Factor I-mediated C3b degradation see SDS-PAGE in **FIG. 13A**.

[0147] In some embodiments, the protein inhibitor is capable of binding C4b. In some embodiments, the protein inhibitor is capable of promoting C4b degradation. In some embodiments, the protein inhibitors promote at least 70, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% of C4b degradation including any range above any one of the preceding values and any range between any two of the preceding values. As an example of such superior promotion of Factor I-mediated C4b degradation see SDS-PAGE in **FIG. 13B**.

[0148] In some embodiments, the protein inhibitor includes a complement regulator that includes one or more CFHR domains (e.g., one or more domains of CFHR3 or CFHR5). In some embodiments, the complement regulator includes a CR1 CCP combination domain and a CFHR5 CCP combination domain.

[0149] In some embodiments, the protein inhibitor is a bispecific protein inhibitor. In some embodiments, the bispecific protein inhibitor includes an oligomerization domain that is an antibody fragment (e.g., a Fab fragment). Any suitable antibody fragment (e.g., a Fab

fragment) can be used as the oligomerization domain, as described herein. In some embodiments, the antibody fragment (e.g., a Fab fragment) binds to, without limitation, VEGF, IL-6, PDGF, CFD, IL-1β, IL-1α, IL-18, TNF-α. In some embodiments, the antibody fragment (e.g., a Fab fragment) binds to VEGF. In some embodiments, the bispecific protein inhibitor includes an oligomerization domain that is an anti-VEGF Fab fragment, as described herein. In some embodiments, the antibody fragment (e.g., a Fab fragment) binds to IL-6. In some embodiments, the bispecific protein inhibitor includes an oligomerization domain that is an anti-IL-6 Fab fragment, as described herein.

[0150] In some embodiments, the protein inhibitor is a multi-specific protein inhibitor.

[0151] In some embodiments, the protein inhibitor (e.g., bispecific protein inhibitor) includes a complement regulator containing a CR1-FH(4) combination domain including a FH(4) CCP domain fused to the C-terminus of a CR1 CCP combination domain (e.g., a CR1-FH(4) combination domain). In some embodiments, the CR1 CCP combination domain comprises CR1 CCP domains 1-3, 1-4, 8-10, 15-17, 15-18, or a combination thereof (e.g., 1-4 and 15-17, or 1-4 and 15-18, or 1-3 and 15-17). In some embodiments, the CR1-FH(4) CCP combination domain comprises any one of SEQ ID NOs: 27-31 and (Tables 1.6). In some embodiments, the CR1 CCP combination domain comprises CR1 CCP domains 8-10. In some embodiments, the CR1-FH(4) CCP combination domain comprises SEQ ID NO: 29. In some embodiments, the CR1 CCP combination domain comprises CR1 CCP domains 1-4 and 15-17. In some embodiments, the CR1-FH(4) CCP combination domain comprises SEQ ID NO: 31. In some embodiments, the complement regulator includes a FH CCP combination domain linked (e.g., via a linker such as, but not limited to, any one of GS1-GS5 in Table 1.7) to the C-terminus of the CR1-FH(4) combination domain. In some embodiments, the FH CCP combination domain includes FH domains 6-7, 18-20, 18-19 and 7, 18-19, 19 and 7, or 19-20. In some embodiments, the FH CCP combination domain includes FH domains 18-20. In some embodiments, the FH CCP combination domain includes SEQ ID NO: 37. In some embodiments, the FH CCP combination domain includes FH domains 6-7. In some embodiments, the FH CCP combination domain includes SEQ ID NO: 97. In some embodiments, the protein inhibitor (e.g., bispecific protein inhibitor) comprises a Fab fragment covalently linked to the complement regulator by a linker (e.g., any one of GS1-GS5 in Table

1.7). In some embodiments, the protein inhibitor comprises a dimeric protein inhibitor comprising a first polypeptide and a second polypeptide conjugated through the formation of disulfide bonds between cysteine residues in the oligomerization domains (e.g., the Fab fragment). The complement regulator can be linked to the oligomerization domain in any suitable manner. In some embodiments, the oligomerization domain that is a Fab fragment includes a light chain (e.g., a VL-CL domain) and a heavy chain (e.g., a VH-CH1 domain). In some embodiments, the C-terminus of the light chain is linked (e.g., via the linker) to the N-terminus of CR1 CCP domains 8-10, which in some embodiments may be further linked (e.g., via another linker) to FH domains 18-20. In some embodiments, the C-terminus of the light chain is linked (e.g., via the linker) to the N-terminus of CR1 CCP domains 1-4 and 15-17. In some embodiments, the C-terminus of the heavy chain is linked (e.g., via the linker) to the N-terminus of CR1 CCP domains 8-10, which in some embodiments may be further linked (e.g., via another linker) to FH domains 18-20. In some embodiments, the C-terminus of the heavy chain is linked (e.g., via the linker) to the N-terminus of CR1 CCP domains 1-4 and 15-17. In some embodiments, the C-terminus of the light chain is linked (e.g., via the linker) to the N-terminus of CR1 CCP domains 8-10, which in some embodiments may be further linked (e.g., via another linker) to FH domains 18-20, and the C-terminus of the heavy chain is linked (e.g., via the linker) to the N-terminus of CR1 CCP domains 8-10, which in some embodiments may be further linked (e.g., via another linker) to FH domains 18-20. In some embodiments, the C-terminus of the light chain is linked (e.g., via the linker) to the N-terminus of CR1 CCP domains 8-10, which in some embodiments may be further linked (e.g., via another linker) to FH domains 18-20, and the C-terminus of the heavy chain is linked (e.g., via the linker) to the N-terminus of CR1 CCP domains 1-4 and 15-17. In some embodiments, the C-terminus of the heavy chain is linked (e.g., via the linker) to the N-terminus of CR1 CCP domains 8-10, which in some embodiments may be further linked (e.g., via another linker) to FH domains 18-20, and the C-terminus of the light chain is linked (e.g., via the linker) to the N-terminus of CR1 CCP domains 1-4 and 15-17. In some embodiments, the C-terminus of the light chain is linked (e.g., via the linker) to the N-terminus of CR1 CCP domains 1-4 and 15-17, and the C-terminus of the heavy chain is linked (e.g., via the linker) to the N-terminus of CR1 CCP domains 1-4 and 15-17.

[0152] In some embodiments, the protein inhibitor comprises KC032 (SEQ ID NOs: 81 and 133, Tables 3.2 and 3.3). In some embodiments, the protein inhibitor comprises KC033 (SEQ ID NOs: 82 and 134, Table 3.2 and 3.3). In some embodiments, the protein inhibitor comprises KC034 (SEQ ID NOs: 83 and 135, Table 3.2 and 3.3). In some embodiments, the protein inhibitor comprises KC035 (SEQ ID NOs: 84 and 136, Table 3.2 and 3.3).

[0153] In some embodiments, the protein inhibitor includes a first polypeptide that contains any one of the amino acid sequences set forth in SEQ ID NOs: 81-84, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto; and a second polypeptide that contains any one of the amino acid sequences set forth in SEQ ID NOs: 133-136, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes a first polypeptide that contains an amino acid sequence set forth in SEQ ID NO: 81, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto; and a second polypeptide that contains an amino acid sequence set forth in SEQ ID NO: 133, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes a first polypeptide that contains an amino acid sequence set forth in SEQ ID NO: 82, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto; and a second polypeptide that contains an amino acid sequence set forth in SEQ ID NO: 133, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes a first polypeptide that contains an amino acid sequence set forth in SEQ ID NO: 83, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto; and a second polypeptide that contains an amino acid sequence set forth in SEQ ID NO: 135, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes a first polypeptide that contains an amino acid sequence set forth in SEQ ID NO: 84, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto; and a second polypeptide that contains an amino acid sequence set forth in SEQ ID NO: 136, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto.

[0154] In some embodiments, with reference to FIG. 5, the protein inhibitor 103 comprises a first polypeptide comprising: SEQ ID NO: 50; and a second polypeptide comprising: SEQ ID NO: 50. In some embodiments, the first polypeptide is conjugated to the

second polypeptide via disulfide bonds 205. In some embodiments, the protein inhibitor includes a first polypeptide having, having about, or having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% sequence identity to SEQ ID NO: 50; and a second polypeptide having, having about, or having at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% sequence identity to SEQ ID NO: 50.

[0155] In some embodiments, the protein inhibitor comprises a first polypeptide, the first polypeptide comprising a complement receptor domain (CRD); and a complement regulator comprising a Complement Receptor 1 (CR1) complement control protein (CCP) domain and a Factor H (FH) CCP domain, wherein the CCP domains comprise at least one C3b and/or C4b binding domain, and wherein the CRD is covalently linked to the complement regulator.

[0156] Herein, any arrangement that recites oligomerization domain can be modified by adding complement receptor domain as shown in embodiments in Figures 11B and 11E.

[0157] In some embodiments, the protein inhibitor includes any one of the amino acid sequences set forth in Tables 1.1-1.10, 2, 3.1, 3.2, 3.3, 3.4, 8, 9, 10.1, 10.2, 11, 12, 13.1, 13.2, 13.3, and 14, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes any one or more of the amino acid sequences set forth in SEQ ID NOs: 100-111 and 115-132, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 100, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 101, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 102, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 103, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 104, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 105, or a sequence at least 80, 85,

90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 106, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 107, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 108, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 109, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 110, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 111, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 115, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 116, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 117, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 118, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 119, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 120, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 121, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 122, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 123, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor

includes an amino acid sequence set forth in SEQ ID NO: 124, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 125, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 126, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 127, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 128, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 129, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 130, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 131, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes an amino acid sequence set forth in SEQ ID NO: 132, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto.

[0158] In some embodiments, the protein inhibitor (e.g., bispecific protein inhibitor) includes a complement regulator containing a CR1 CCP domain. In some embodiments, the protein inhibitor (e.g., bispecific protein inhibitor) includes a complement regulator containing a CR1-CHFR3 combination domain including a CFHR3 domain fused to the C-terminus of a CR1 CCP combination domain (e.g., a CR1-CFHR3 combination domain). In some embodiments, the CR1 CCP combination domain comprises CR1 CCP domains 1-3, 1-4, 8-10, 15-17, 15-18, or a combination thereof (e.g., 1-4 and 15-17, or 1-4 and 15-18, or 1-3 and 15-17). In some embodiments, the CR1 CCP domain comprises CR1 CCP domains 1-4. In some embodiments, the CR1 CCP combination domain comprises SEQ ID NO: 23, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the CR1-CFHR3 combination domain comprises CR1 CCP domains 15-18. In some embodiments, the CR1-CFHR3 combination domain comprises SEQ ID NO: 95, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some

embodiments, the complement regulator includes a CFHR CPP domain linked (e.g., via a linker such as, but not limited to, any one of GS1-GS5 in Table 1.7) to the C-terminus of the CR1 CCP domain or the CR1-CFHR3 combination domain. In some embodiments, the CFHR CPP domain includes CFHR3 CPP domains 1-2 or CFHR5 CPP domains 8-9. In some embodiments, the CFHR CPP domain includes SEQ ID NO: 98, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the CFHR CPP domain includes SEQ ID NO: 99, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto.

[0159] In some embodiments, the protein inhibitor (e.g., bispecific protein inhibitor) comprises a Fab fragment covalently linked to the complement regulator by a linker (e.g., any one of GS1-GS5 in Table 1.7). In some embodiments, the protein inhibitor comprises a dimeric protein inhibitor comprising a first polypeptide and a second polypeptide conjugated through the formation of disulfide bonds between cysteine residues in the oligomerization domains (e.g., the Fab fragment). The complement regulator can be linked to the oligomerization domain in any suitable manner. In some embodiments, the oligomerization domain that is a Fab fragment includes a light chain (e.g., a VL-CL domain) and a heavy chain (e.g., a VH-CH1 domain). In some embodiments, the C-terminus of the light chain is linked (e.g., via the linker) to the N-terminus of a CR1 CCP domains (e.g., CR1 CCP domains 1-4), which in some embodiments may be further linked (e.g., via another linker) to CFHR3 CPP domains (e.g., CFHR3 CPP domains 1-2) or CFHR5 CPP domains (e.g., CFHR5 CPP domains 8-9). In some embodiments, the C-terminus of the light chain is linked (e.g., via the linker) to the N-terminus of a CR1-CFHR3 combination domain (e.g., CR1 CCP domains 15-18 and a CFHR3 domain), which in some embodiments may be further linked (e.g., via another linker) to CFHR3 CPP domains (e.g., CFHR3 CPP domains 1-2) or CFHR5 CPP domains (e.g., CFHR5 CPP domains 8-9). In some embodiments, the C-terminus of the heavy chain is linked (e.g., via the linker) to the N-terminus of a CR1 CCP domains (e.g., CR1 CCP domains 1-4), which in some embodiments may be further linked (e.g., via another linker) to CFHR3 CPP domains (e.g., CFHR3 CPP domains 1-2) or CFHR5 CPP domains (e.g., CFHR5 CPP domains 8-9). In some embodiments, the C-terminus of the heavy chain is linked (e.g., via the linker) to the N-terminus of a CR1-CFHR3 combination domain (e.g., CR1 CCP domains 15-18 and a CFHR3 domain), which in some embodiments may be further linked (e.g., via another linker)

to CFHR3 CPP domains (e.g., CFHR3 CPP domains 1-2) or CFHR5 CPP domains (e.g., CFHR5 CPP domains 8-9). In some embodiments, the C-terminus of the light chain is linked (e.g., via the linker) to the N-terminus of a CR1 CCP domains (e.g., CR1 CCP domains 1-4), which in some embodiments may be further linked (e.g., via another linker) to CFHR3 CPP domains (e.g., CFHR3 CPP domains 1-2) or CFHR5 CPP domains (e.g., CFHR5 CPP domains 8-9), and the C-terminus of the heavy chain is linked (e.g., via the linker) to the N-terminus of a CR1 CCP domains (e.g., CR1 CCP domains 1-4), which in some embodiments may be further linked (e.g., via another linker) to CFHR3 CPP domains (e.g., CFHR3 CPP domains 1-2) or CFHR5 CPP domains (e.g., CFHR5 CPP domains 8-9). In some embodiments, the C-terminus of the light chain is linked (e.g., via the linker) to the N-terminus of a CR1 CCP domains (e.g., CR1 CCP domains 1-4), which in some embodiments may be further linked (e.g., via another linker) to CFHR3 CPP domains (e.g., CFHR3 CPP domains 1-2) or CFHR5 CPP domains (e.g., CFHR5 CPP domains 8-9), and the C-terminus of the heavy chain is linked (e.g., via the linker) to the N-terminus of a CR1-CFHR3 combination domain (e.g., CR1 CCP domains 15-18 and a CFHR3 domain), which in some embodiments may be further linked (e.g., via another linker) to CFHR3 CPP domains (e.g., CFHR3 CPP domains 1-2) or CFHR5 CPP domains (e.g., CFHR5 CPP domains 8-9). In some embodiments, the C-terminus of the light chain is linked (e.g., via the linker) to the N-terminus of a CR1-CFHR3 combination domain (e.g., CR1 CCP domains 15-18 and a CFHR3 domain), which in some embodiments may be further linked (e.g., via another linker) to CFHR3 CPP domains (e.g., CFHR3 CPP domains 1-2) or CFHR5 CPP domains (e.g., CFHR5 CPP domains 8-9), and the C-terminus of the heavy chain is linked (e.g., via the linker) to the N-terminus of a CR1 CCP domains (e.g., CR1 CCP domains 1-4), which in some embodiments may be further linked (e.g., via another linker) to CFHR3 CPP domains (e.g., CFHR3 CPP domains 1-2) or CFHR5 CPP domains (e.g., CFHR5 CPP domains 8-9). In some embodiments, the C-terminus of the light chain is linked (e.g., via the linker) to the N-terminus of a CR1-CFHR3 combination domain (e.g., CR1 CCP domains 15-18 and a CFHR3 domain), which in some embodiments may be further linked (e.g., via another linker) to CFHR3 CPP domains (e.g., CFHR3 CPP domains 1-2) or CFHR5 CPP domains (e.g., CFHR5 CPP domains 8-9), and the C-terminus of the heavy chain is linked (e.g., via the linker) to the N-terminus of a CR1-CFHR3 combination domain (e.g., CR1 CCP domains 15-18 and a CFHR3 domain), which in some embodiments may be

further linked (e.g., via another linker) to CFHR3 CPP domains (e.g., CFHR3 CPP domains 1-2) or CFHR5 CPP domains (e.g., CFHR5 CPP domains 8-9).

[0160] In some embodiments, the protein inhibitor includes a first polypeptide that contains any one of the amino acid sequences set forth in SEQ ID NOs: 115-123, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto; and a second polypeptide that contains any one of the amino acid sequences set forth in SEQ ID NOs: 124-132, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes a first polypeptide that contains an amino acid sequence set forth in SEQ ID NO: 115, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto; and a second polypeptide that contains an amino acid sequence set forth in SEQ ID NO: 124, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes a first polypeptide that contains an amino acid sequence set forth in SEQ ID NO: 116, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto; and a second polypeptide that contains an amino acid sequence set forth in SEQ ID NO: 125, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes a first polypeptide that contains an amino acid sequence set forth in SEQ ID NO: 117, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto; and a second polypeptide that contains an amino acid sequence set forth in SEQ ID NO: 126, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes a first polypeptide that contains an amino acid sequence set forth in SEQ ID NO: 118, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto; and a second polypeptide that contains an amino acid sequence set forth in SEQ ID NO: 127, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes a first polypeptide that contains an amino acid sequence set forth in SEQ ID NO: 119, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto; and a second polypeptide that contains an amino acid sequence set forth in SEQ ID NO: 128, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes a first polypeptide that contains an amino acid sequence set forth in SEQ ID NO: 120, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto; and a second polypeptide that contains an amino acid sequence

set forth in SEQ ID NO: 129, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes a first polypeptide that contains an amino acid sequence set forth in SEQ ID NO: 121, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto; and a second polypeptide that contains an amino acid sequence set forth in SEQ ID NO: 130, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes a first polypeptide that contains an amino acid sequence set forth in SEQ ID NO: 122, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto; and a second polypeptide that contains an amino acid sequence set forth in SEQ ID NO: 131, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the protein inhibitor includes a first polypeptide that contains an amino acid sequence set forth in SEQ ID NO: 123, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto; and a second polypeptide that contains an amino acid sequence set forth in SEQ ID NO: 132, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto.

[0161] In any of the protein inhibitors of the present disclosure, in some embodiments, any sequence variation relative to a reference sequence is or includes a conservative substitution.

[0162] Provided herein are protein inhibitors of the complement system. In some embodiments, the protein inhibitors prevent and inhibit unintended complement activation. In some embodiments, the protein inhibitors inhibit the classical pathway. In some embodiments, the protein inhibitors inhibit the alternative pathway. In some embodiments, the protein inhibitors inhibit the alternative pathway and the classical pathway. Inhibition of complement activity by the protein inhibitors can be assayed using any suitable option, e.g., using a hemolytic assay specific to the alternative pathway as described herein and/or a hemolytic assay for the classical pathway (CH50) as described herein. In some embodiments, the protein inhibitors inhibit hemolysis due to complement activation (e.g., via the alternative pathway and/or the classical pathway). In some embodiments, the protein inhibitors provide superior results in protecting cells and host tissue from damage and cell lysis caused by unintended complement activation. In some embodiments, the complement inhibitors bind C3b and/or C4b and promote their degradation by Factor I. In some embodiments, the protein inhibitor inhibits complement activity (e.g., via the alternative pathway or the classical pathway) with an IC_{50}

of, of about, or of at most 10, 5, 2.5, 2, 1.5, 1, 0.8, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 nM or less, or optionally, inhibits complement activity with an IC_{50} in a range defined by any two of the preceding values (e.g., 10-0.1 nM, 5-0.1nM, 2.5-0.2 nM, 1-0.1 nM, etc.). In some embodiments, the protein inhibitor inhibits complement activity (e.g., via the alternative pathway or the classical pathway) with an IC_{50} of at most 1 nM, or of about 0.5 nM. In some embodiments, the protein inhibitor inhibits complement activity (e.g., via the alternative pathway or the classical pathway) with an IC_{50} in a range of 1-0.1nM. In some embodiments, the protein inhibitor of the present disclosure exhibits cofactor activity to facilitate CFI-mediated C3b degradation. In some embodiments, the protein inhibitor exhibits cofactor activity for CFI-mediated C3b degradation that is greater than the cofactor activity by soluble form of CR1 (sCR1). In some embodiments, the protein inhibitor binds to complement protein C3b (e.g., human C3b) with a K_D of, of about, or of at most $1x10^{-6}$, $1x10^{-7}$, $0.5x10^{-7}$, $1x10^{-8}$, $0.5x10^{-8}$, $1x10^{-9}$, $0.5x10^{-9}$, $1x10^{-10}$, $0.5x10^{-10}$, $1x10^{-11}$ M, or optionally, the protein inhibitor binds to complement protein C3b with a K_D in a range defined by any two of the preceding values (e.g., $1x10^{-6}$-$1x10^{-11}$ M, $1x10^{-7}$-$1x10^{-11}$ M, $1x10^{-8}$-$1x10^{-11}$ M, $1x10^{-7}$-$1x10^{-10}$ M, or $1x10^{-9}$-$1x10^{-11}$ M, etc.). The binding equilibrium dissociation constant between the protein inhibitor and complement protein C3b can be measured using any suitable option, e.g., using a surface plasmon resonance (SPR)-based assay as described herein. In some embodiments, the protein inhibitor binds to complement protein C3b with a K_D of at most $1x10^{-7}$ M. In some embodiments, the protein inhibitor binds to complement protein C3b with a K_D of at most $1x10^{-8}$ M. In some embodiments, the protein inhibitor binds to complement protein C3b with a K_D of at most $1x10^{-9}$ M. In some embodiments, the protein inhibitor binds to complement protein C3b with a K_D in a range of $1x10^{-7}$ to $1x10^{-11}$ M. In some embodiments, the protein inhibitor accelerates decay of the C3 convertase complex. Decay accelerating activity (DAA) of the protein inhibitor on the C3 convertase complex can be assayed using any suitable option, e.g., using a SPR-based assay as described herein.

[0163] In some embodiments, a method for treatment or prophylaxis of a disease is presented. The method comprises identifying a subject in need of treatment and administering an effective dose of any of the inhibitors described herein to a subject thereof. In some embodiments the disease can be age-related macular degeneration (AMD). In some embodiments, the disease can be Geographic atrophy (GA). In some embodiments the disease

can be paroxysmal nocturnal hemoglobinuria (PNH). In some embodiments the disease can be atypical hemolytic uremic syndrome (aHUS). In some embodiments the disease can be C3 glomerulopathy (C3G). In some embodiments the disease can be rheumatoid arthritis. In some embodiments, the therapy is achieved by administering any one or more of the constructs provided herein.

[0164] Some nonlimiting embodiments of the various domains/components provided herein are shown in the following tables.

Table 1.1. Human Factor H (FH) and soluble human Complement receptor 1 (CR1) amino acid sequences.

Protein Name	SEQ ID NO:	Amino Acid Sequence
FH	1	DCNELPPRRNTEILTGSWSDQTYPEGTQAIYKCRPGYRSLGNVIMV CRKGEWVALNPLRKCQKRPCGHPGDTPFGTFTLTGGNVFEYGVKAV YTCNEGYQLLGEINYRECDTDGWTNDIPICEVVKCLPVTAPENGKI VSSAMEPDREYHFGQAVRFVCNSGYKIEGDEEMHCSDDGFWSKEKP KCVEISCKSPDVINGSPISQKIIYKENERFQYKCNMGYEYSERGDA VCTESGWRPLPSCEEKSCDNPYIPNGDYSPLRIKHRTGDEITYQCR NGFYPATRGNTAKCTSTGWIPAPRCTLKPCDYPDIKHGGLYHENMR RPYFPVAVGKYYSYYCDEHFETPSGSYWDHIHCTQDGWSPAVPCLR KCYFPYLENGYNQNYGRKFVQGKSIDVACHPGYALPKAQTTVTCME NGWSPTPRCIRVKTCSKSSIDIENGFISESQYTYALKEKAKYQCKL GYVTADGETSGSITCGKDGWSAQPTCIKSCDIPVFMNARTKNDFTW FKLNDTLDYECHDGYESNTGSTTGSIVCGYNGWSDLPICYERECEL PKIDVHLVPDRKKDQYKVGEVLKFSCKPGFTIVGPNSVQCYHFGLS PDLPICKEQVQSCGPPPELLNGNVKEKTKEEYGHSEVVEYYCNPRF LMKGPNKIQCVDGEWTTLPVCIVEESTCGDIPELEHGWAQLSSPPY YYGDSVEFNCSESFTMIGHRSITCIHGVWTQLPQCVAIDKLKKCKS SNLIILEEHLKNKKEFDHNSNIRYRCRGKEGWIHTVCINGRWDPEV NCSMAQIQLCPPPPQIPNSHNMTTTLNYRDGEKVSVLCQENYLIQE GEEITCKDGRWQSIPLCVEKIPCSQPPQIEHGTINSSRSSQESYAH GTKLSYTCEGGFRISEENETTCYMGKWSSPPQCEGLPCKSPPEISH GVVAHMSDSYQYGEEVTYKCFEGFGIDGPAIAKCLGEKWSHPPSCI KTDCLSLPSFENAIPMGEKKDVYKAGEQVTYTCATYYKMDGASNVT CINSRWTGRPTCRDTSCVNPPTVQNAYIVSRQMSKYPSGERVRYQC RSPYEMFGDEEVMCLNGNWTEPPQCKDSTGKCGPPPPIDNGDITSF PLSVYAPASSVEYQCQNLYQLEGNKRITCRNGQWSEPPKCLHPCVI SREIMENYNIALRWTAKQKLYSRTGESVEFVCKRGYRLSSRSHTLR TTCWDGKLEYPTCAKR

CR1	2	GQCNAPEWLPFARPTNLTDEFEFPIGTYLNYECRPGYSGRPFSIIC LKNSVWTGAKDRCRRKSCRNPPDPVNGMVHVIKGIQFGSQIKYSCT KGYRLIGSSSATCIISGDTVIWDNETPICDRIPCGLPPTITNGDFI STNRENFHYGSVVTYRCNPGSGGRKVFELVGEPSIYCTSNDDQVGI WSGPAPQCIIPNKCTPPNVENGILVSDNRSLFSLNEVVEFRCQPGF VMKGPRRVKCQALNKWEPELPSCSRVCQPPPDVLHAERTQRDKDNF SPGQEVFYSCEPGYDLRGAASMRCTPQGDWSPAAPTCEVKSCDDFM GQLLNGRVLFPVNLQLGAKVDFVCDEGFQLKGSSASYCVLAGMESL WNSSVPVCEQIFCPSPPVIPNGRHTGKPLEVFPFGKTVNYTCDPHP DRGTSFDLIGESTIRCTSDPQGNGVWSSPAPRCGILGHCQAPDHFL FAKLKTQTNASDFPIGTSLKYECRPEYYGRPFSITCLDNLVWSSPK DVCKRKSCKTPPDPVNGMVHVITDIQVGSRINYSCTTGHRLIGHSS AECILSGNAAHWSTKPPICQRIPCGLPPTIANGDFISTNRENFHYG SVVTYRCNPGSGGRKVFELVGEPSIYCTSNDDQVGIWSGPAPQCII PNKCTPPNVENGILVSDNRSLFSLNEVVEFRCQPGFVMKGPRRVKC QALNKWEPELPSCSRVCQPPPDVLHAERTQRDKDNFSPGQEVFYSC EPGYDLRGAASMRCTPQGDWSPAAPTCEVKSCDDFMGQLLNGRVLF PVNLQLGAKVDFVCDEGFQLKGSSASYCVLAGMESLWNSSVPVCEQ IFCPSPPVIPNGRHTGKPLEVFPFGKAVNYTCDPHPDRGTSFDLIG ESTIRCTSDPQGNGVWSSPAPRCGILGHCQAPDHFLFAKLKTQTNA SDFPIGTSLKYECRPEYYGRPFSITCLDNLVWSSPKDVCKRKSCKT PPDPVNGMVHVITDIQVGSRINYSCTTGHRLIGHSSAECILSGNTA HWSTKPPICQRIPCGLPPTIANGDFISTNRENFHYGSVVTYRCNLG SRGRKVFELVGEPSIYCTSNDDQVGIWSGPAPQCIIPNKCTPPNVE NGILVSDNRSLFSLNEVVEFRCQPGFVMKGPRRVKCQALNKWEPEL PSCSRVCQPPPEILHGEHTPSHQDNFSPGQEVFYSCEPGYDLRGAA SLHCTPQGDWSPEAPRCAVKSCDDFLGQLPHGRVLFPLNLQLGAKV SFVCDEGFRLKGSSVSHCVLVGMRSLWNNSVPVCEHIFCPNPPAIL NGRHTGTPSGDIPYGKEISYTCDPHPDRGMTFNLIGESTIRCTSDP HGNGVWSSPAPRCELSVRAGHCKTPEQFPFASPTIPINDFEFPVGT SLNYECRPGYFGKMFSISCLENLVWSSVEDNCRRKSCGPPPEPFNG MVHINTDTQFGSTVNYSCNEGFRLIGSPSTTCLVSGNNVTWDKKAP ICEIISCEPPPTISNGDFYSNNRTSFHNGTVVTYQCHTGPDGEQLF ELVGERSIYCTSKDDQVGVWSSPPPRCISTNKCTAPEVENAIRVPG NRSFFSLTEIIRFRCQPGFVMVGSHTVQCQTNGRWGPKLPHCSRVC QPPPEILHGEHTLSHQDNFSPGQEVFYSCEPSYDLRGAASLHCTPQ GDWSPEAPRCTVKSCDDFLGQLPHGRVLLPLNLQLGAKVSFVCDEG FRLKGRSASHCVLAGMKALWNSSVPVCEQIFCPNPPAILNGRHTGT PFGDIPYGKEISYACDTHPDRGMTFNLIGESSIRCTSDPQGNGVWS SPAPRCELSVPAACPHPPKIQNGHYIGGHVSLYLPGMTISYICDPG YLLVGKGFIFCTDQGIWSQLDHYCKEVNCSFPLFMNGISKELEMKK VYHYGDYVTLKCEDGYTLEGSPWSQCQADDRWDPPLAKCTSRTHDA LIVGTLSGTIFFILLIIFLSWIILKHRKGNNAHENPKEVAIHLHSQ GGSSVHPRTLQTNEENSRVLP

Table 1.2. Signal peptide and his tag sequences

Signal Peptide and His Tag Names	SEQ ID NO:	Amino Acid Sequence
IL-2sp	3	MYRMQLLSCIALSLALVTNS
hCR1sp	4	MGASSPRSPEPVGPPAPGLPFCCGGSLLAVVVLLALPVAWG
H6	5	HHHHHH
H8_FLAG	6	GSHHHHHHHHDYKDDDDK

Table 1.3. Selected complement Receptor 1 (CR1) CCP domain sequences

CR1 CCP Domain Names	SEQ ID NO:	Amino Acid Sequence
CR1(01)	7	GQCNAPEWLPFARPTNLTDEFEFPIGTYLNYECRPGYSGRPFSIIC LKNSVWTGAKDRCRR
CR1(02)	8	KSCRNPPDPVNGMVHVIKGIQFGSQIKYSCTKGYRLIGSSSATCII SGDTVIWDNETPICDR
CR1(03)	9	IPCGLPPTITNGDFISTNRENFHYGSVVTYRCNPGSGGRKVFELVG EPSIYCTSNDDQVGIWSGPAPQCI
CR1(04)	10	IPNKCTPPNVENGILVSDNRSLFSLNEVVEFRCQPGFVMKGPRRVK CQALNKWEPELPSCSR
CR1(08)	11	GHCQAPDHFLFAKLKTQTNASDFPIGTSLKYECRPEYYGRPFSITC LDNLVWSSPKDVCKR
CR1(09)	12	KSCKTPPDPVNGMVHVITDIQVGSRINYSCTTGHRLIGHSSAECIL SGNAAHWSTKPPICQR
CR1(10)	13	IPCGLPPTIANGDFISTNRENFHYGSVVTYRCNPGSGGRKVFELVG EPSIYCTSNDDQVGIWSGPAPQCI
CR1(15)	14	GHCQAPDHFLFAKLKTQTNASDFPIGTSLKYECRPEYYGRPFSITC LDNLVWSSPKDVCKR
CR1(16)	15	KSCKTPPDPVNGMVHVITDIQVGSRINYSCTTGHRLIGHSSAECIL SGNTAHWSTKPPICQR
CR1(17)	16	IPCGLPPTIANGDFISTNRENFHYGSVVTYRCNLGSRGRKVFELVG EPSIYCTSNDDQVGIWSGPAPQCI

Table 1.4. Selected complement Factor H (FH) CCP domain sequences

FH CCP Domain Names	SEQ ID NO:	Amino Acid Sequence
FH(4)	17	EISCKSPDVINGSPISQKIIYKENERFQYKCNMGYEYSERGDAVCT ESGWRPLPSCEEK

FH(7)	18	RKCYFPYLENGYNQNYGRKFVQGKSIDVACHPGYALPKAQTTVTCM ENGWSPTPRCIR
FH(18)	19	TSCVNPPTVQNAYIVSRQMSKYPSGERVRYQCRSPYEMFGDEEVMC LNGNWTEPPQCKDST
FH(19)	20	GKCGPPPPIDNGDITSFPLSVYAPASSVEYQCQNLYQLEGNKRITC RNGQWSEPPKCLH
FH(20)	21	PCVISREIMENYNIALRWTAKQKLYSRTGESVEFVCKRGYRLSSRS HTLRTTCWDGKLEYPTCAKR

Table 1.5. Sequences of CR1 CCP Domain Combinations used in this study.

CR1 CCP Combination Domain Names	SEQ ID NO.	Domain 1 (SEQ ID NO:)	Domain 2 (SEQ ID NO:)	Domain 3 (SEQ ID NO:)	Domain 4 (SEQ ID NO:)	Domain 5 (SEQ ID NO:)	Domain 6 (SEQ ID NO:)	Domain 7 (SEQ ID NO:)
CR1(1-3)	22	7	8	9				
CR1(1-4)	23	7	8	9	10			
CR1(8-10)	24	11	12	13				
CR1(15-17)	25	14	15	16				
CR1(1-4, 15-17)	26	7	8	9	10	14	15	16
CR1(1-3, 15-17)	137	7	8	9	14	15	16	

Table 1.6 Sequences of CR1 and FH(4) CCP Domain Combinations used in this study.

SEQ ID NO.	SEQ Name	Domain 1 (SEQ ID NO:)	Domain 2 (SEQ ID NO:)
27	CR1(1-3)-FH(4)	22	17
28	CR1(8-10)-FH(4)	23	17
29	CR1(15-17)-FH(4)	24	17
30	CR1(1-3,15-17)-FH(4)	137	17
31	CR1(1-4,15-17)-FH(4)	26	17

Table 1.7. Linker sequences

Linker Names	SEQ ID NO:	Amino Acid Sequence
(GS1)	32	GS
(GS2)	33	GGGGS
(GS3)	34	GSGGGGS
(GS4)	35	GSGGGGSGGGGS
(GS5)	36	GGGGSGGGGS

Table 1.8. Sequences of FH CCP Domain Combinations used in this study.

Names of FH CCP Combinations Domains	SEQ ID NO:	Domain 1 (SEQ ID NO:)	Domain 2 (SEQ ID NO:)	Domain 3 (SEQ ID NO:)
FH(18-20)	37	19	20	21
FH(18-19, 7)	38	19	20	18
FH(18-19)	39	19	20	
FH(19, 7)	40	20	18	
FH(19-20)	41	20	21	

Table 1.9. Other complement factor protein sequences used in this study.

Names of Complement Factor Protein Domains	SEQ ID NO:	Amino Acid Sequence
CFHR1(1-2)	42	FCDFPKINHGILYDEEKYKPFSQVPTGEVFYYSCEYNFVSPS KSFWTRITCTEEGWSPTPKCLRLCFFPFVENGHSESSGQTHL EGDTVQIICNTGYRLQNNENNISCVERGWSTPPKCRS
CFHR5(1-2)	43	TLCDFPKIHHGFLYDEEDYNPFSQVPTGEVFYYSCEYNFVSP SKSFWTRITCTEEGWSPTPKCLRMCSFPFVKNGHSESSGLIH LEGDTVQIICNTGYSLQNNEKNISCVERGWSTPPICSFTD
CR2(1-4)	44	ISCGSPPPILNGRISYYSTPIAVGTVIRYSCSGTFRLIGEKS LLCITKDKVDGTWDKPAPKCEYFNKYSSCPEPIVPGGYKIRG STPYRHGDSVTFACKTNFSMNGNKSVWCQANNMWGPTRLPTC VSVFPLECPALPMIHNGHHTSENVGSIAPGLSVTYSCESGYL LVGEKIINCLSSGKWSAVPPTCEEARCKSLGRFPNGKVKEPP ILRVGVTANFFCDEGYRLQGPPSSRCVIAGQGVAWTKMPVCE EI
CRIg (IgV-IgC2)	45	GRPILEVPESVTGPWKGDVNLPCTYDPLQGYTQVLVKWLVQR GSDPVTIFLRDSSGDHIQQAKYQGRLHVSHKVPGDVSLQLST LEMDDRSHYTCEVTWQTPDGNQVVRDKITELRVQKLS

Table 1.10. IgG constant and variable domain sequences used in this study.

Protein Domain Name	SEQ ID NO:	Amino Acid Sequence
Fc	46	SCDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK

anti-VEGF-VH-CH1	47	EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPG KGLEWVGWINTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMN SLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCDKTHT
anti-VEGF-VL-CL	48	DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGK APKVLIYFTSSLHSGVPSRFSGSGSGTDFTLTISSLQPEDFA TYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC
anti-VEGF-HC	49	EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPG KGLEWVGWINTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMN SLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGAPSVFLXPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSCSP

Table 2. Amino acid sequences of representative protein inhibitors. Fc sequences are underlined, CR1 components are double underlined, FH sequences are in bold, and Gly-Ser linker is italicized.

Protein Inhibitor Name	**SEQ ID NO:**	**Amino Acid Sequence**
KC001	50	SCDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*GGGGSGGGGSG* HCQAPDHFLFAKLKTQTNASDFPIGTSLKYECRPEYYGRPFSITCLDN LVWSSPKDVCKRKSCKTPPDPVNGMVHVITDIQVGSRINYSCTTGHRL IGHSSAECILSGNTAHWSTKPPICQRIPCGLPPTIANGDFISTNRENF HYGSVVTYRCNLGSRGRKVFELVGEPSIYCTSNDDQVGIWSGPAPQCI **EISCKSPDVINGSPISQKSLFSLNERFQYKCNMGYEYSERGDAVCTES** **GWRPLPSCEEK***GSGGGGS***TSCVNPPTVQNAYIVSRQMSKYPSGERVRY** **QCRSPYEMFGDEEVMCLNGNWTEPPQCKDSTGKCGPPPPIDNGDITSF** **PLSVYAPASSVEYQCQNLYQLEGNKRITCRNGQWSEPPKCLHPCVISR**

		EIMENYNIALRWTAKQKLYSRTGESVEFVCKRGYRLSSRSHTLRTTCW DGKLEYPTCAKR
KC002	51	SCDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*GGGGSGGGGSG HCQAPDHFLFAKLKTQTNASDFPIGTSLKYECRPEYYGRPFSITCLDN LVWSSPKDVCKRKSCKTPPDPVNGMVHVITDIQVGSRINYSCTTGHRL IGHSSAECILSGNAAHWSTKPPICQRIPCGLPPTIANGDFISTNRENF HYGSVVTYRCNPGSGGRKVFELVGEPSIYCTSNDDQVGIWSGPAPQCI* **EISCKSPDVINGSPISQKSLFSLNERFQYKCNMGYEYSERGDAVCTES GWRPLPSCEEK*GSGGGGS*TSCVNPPTVQNAYIVSRQMSKYPSGERVRY QCRSPYEMFGDEEVMCLNGNWTEPPQCKDSTGKCGPPPPIDNGDITSF PLSVYAPASSVEYQCQNLYQLEGNKRITCRNGQWSEPPKCLHPCVISR EIMENYNIALRWTAKQKLYSRTGESVEFVCKRGYRLSSRSHTLRTTCW DGKLEYPTCAKR**
KC003	52	SCDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*GGGGSGGGGSG QCNAPEWLPFARPTNLTDEFEFPIGTYLNYECRPGYSGRPFSIICLKN SVWTGAKDRCRRKSCRNPPDPVNGMVHVIKGIQFGSQIKYSCTKGYRL IGSSSATCIISGDTVIWDNETPICDRIPCGLPPTITNGDFISTNRENF HYGSVVTYRCNPGSGGRKVFELVGEPSIYCTSNDDQVGIWSGPAPQCI IPNKCTPPNVENGILVSDNRSLFSLNEVVEFRCQPGFVMKGPRRVKCQ ALNKWEPELPSCSR*GHCQAPDHFLFAKLKTQTNASDFPIGTSLKYECR PEYYGRPFSITCLDNLVWSSPKDVCKRKSCKTPPDPVNGMVHVITDIQ VGSRINYSCTTGHRLIGHSSAECILSGNTAHWSTKPPICQRIPCGLPP TIANGDFISTNRENFHYGSVVTYRCNLGSRGRKVFELVGEPSIYCTSN DDQVGIWSGPAPQCI**EISCKSPDVINGSPISQKIIYKENERFQYKCNM **GYEYSERGDAVCTESGWRPLPSCEEK*GSGGGGS*TSCVNPPTVQNAYIV SRQMSKYPSGERVRYQCRSPYEMFGDEEVMCLNGNWTEPPQCKDSTGK CGPPPPIDNGDITSFPLSVYAPASSVEYQCQNLYQLEGNKRITCRNGQ WSEPPKCLHPCVISREIMENYNIALRWTAKQKLYSRTGESVEFVCKRG YRLSSRSHTLRTTCWDGKLEYPTCAKR**

Table 2.1 Amino acid sequences of representative protein inhibitors. The first 20 amino acid sequences are signal peptide, Fc sequences are underlined, CR1 components are double underlined, FH sequences are in bold, and Gly-Ser linker is italicized.

Protein Inhibitor Name	**SEQ ID NO:**	**Amino Acid Sequence**

KC001+ signal peptide	138	MYRMQLLSCIALSLALVTNSSCDKTHTCPPCPAPEAAGAPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK*GGGGSGGGGS*GHCQAPDHFLFAKLKTQTNASDFPIGTSL KYECRPEYYGRPFSITCLDNLVWSSPKDVCKRKSCKTPPDPVNGMVHV ITDIQVGSRINYSCTTGHRLIGHSSAECILSGNTAHWSTKPPICQRIP CGLPPTIANGDFISTNRENFHYGSVVTYRCNLGSRGRKVFELVGEPSI YCTSNDDQVGIWSGPAPQCI**EISCKSPDVINGSPISQKSLFSLNERFQ YKCNMGYEYSERGDAVCTESGWRPLPSCEEK***GSGGGGS***TSCVNPPTVQ NAYIVSRQMSKYPSGERVRYQCRSPYEMFGDEEVMCLNGNWTEPPQCK DSTGKCGPPPPIDNGDITSFPLSVYAPASSVEYQCQNLYQLEGNKRIT CRNGQWSEPPKCLHPCVISREIMENYNIALRWTAKQKLYSRTGESVEF VCKRGYRLSSRSHTLRTTCWDGKLEYPTCAKR
KC002+ signal peptide	139	MYRMQLLSCIALSLALVTNSSCDKTHTCPPCPAPEAAGAPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK*GGGGSGGGGSGHCQAPDHFLFAKLKTQTNASDFPIGTSL KYECRPEYYGRPFSITCLDNLVWSSPKDVCKRKSCKTPPDPVNGMVHV ITDIQVGSRINYSCTTGHRLIGHSSAECILSGNAAHWSTKPPICQRIP CGLPPTIANGDFISTNRENFHYGSVVTYRCNPGSGGRKVFELVGEPSI YCTSNDDQVGIWSGPAPQCI**EISCKSPDVINGSPISQKSLFSLNERFQ YKCNMGYEYSERGDAVCTESGWRPLPSCEEK***GSGGGGS***TSCVNPPTVQ NAYIVSRQMSKYPSGERVRYQCRSPYEMFGDEEVMCLNGNWTEPPQCK DSTGKCGPPPPIDNGDITSFPLSVYAPASSVEYQCQNLYQLEGNKRIT CRNGQWSEPPKCLHPCVISREIMENYNIALRWTAKQKLYSRTGESVEF VCKRGYRLSSRSHTLRTTCWDGKLEYPTCAKR
KC003+ signal peptide	140	MYRMQLLSCIALSLALVTNSSCDKTHTCPPCPAPEAAGAPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK*GGGGSGGGGSGQCNAPEWLPFARPTNLTDEFEFPIGTYL NYECRPGYSGRPFSIICLKNSVWTGAKDRCRRKSCRNPPDPVNGMVHV IKGIQFGSQIKYSCTKGYRLIGSSSATCIISGDTVIWDNETPICDRIP CGLPPTITNGDFISTNRENFHYGSVVTYRCNPGSGGRKVFELVGEPSI YCTSNDDQVGIWSGPAPQCIIPNKCTPPNVENGILVSDNRSLFSLNEV VEFRCQPGFVMKGPRRVKCQALNKWEPELPSCSR*GHCQAPDHFLFAKL KTQTNASDFPIGTSLKYECRPEYYGRPFSITCLDNLVWSSPKDVCKRK SCKTPPDPVNGMVHVITDIQVGSRINYSCTTGHRLIGHSSAECILSGN TAHWSTKPPICQRIPCGLPPTIANGDFISTNRENFHYGSVVTYRCNLG SRGRKVFELVGEPSIYCTSNDDQVGIWSGPAPQCI**EISCKSPDVINGS PISQKIIYKENERFQYKCNMGYEYSERGDAVCTESGWRPLPSCEEK***GS GGGGS***TSCVNPPTVQNAYIVSRQMSKYPSGERVRYQCRSPYEMFGDEE

		VMCLNGNWTEPPQCKDSTGKCGPPPPIDNGDITSFPLSVYAPASSVEY QCQNLYQLEGNKRITCRNGQWSEPPKCLHPCVISREIMENYNIALRWT AKQKLYSRTGESVEFVCKRGYRLSSRSHTLRTTCWDGKLEYPTCAKR
KC041+ signal peptide	141	MYRMQLLSCIALSLALVTNSSCDKTHTCPPCPAPEAAGAPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*GGGGSGGGGS*G HCQAPDHFLFAKLKTQTNASDFPIGTSLKYECRPEYYGRPFSITCLDNLVWS SPKDVCKRKSCKTPPDPVNGMVHVITDIQVGSRINYSCTTGHRLIGHSSAEC ILSGNTAHWSTKPPICQRIPCGLPPTIANGDFISTNRENFHYGSVVTYRCNL GSRGRKVFELVGEPSIYCTSNDDQVGIWSGPAPQCIIPNKCTPPNVENGILV SDNRSLFSLNEVVEFRCQPGFVMKGPRRVKCQALNKWEPELPSCSRVGSGGG SGSGGGGS**TAYCGPPPSINNGDTTSFPLSVYPPGSTVTYRCQSFYKLQGSVT VTCRNKQWSEPPRCLDPCVVSEENMNKNNIQLKWRNDGKLYAKTGDAVEFQC KFPHKAMISSPPFRAICQEGKFEYPICE**

Table 2.2. DNA sequences of representative protein inhibitors.

Protein inhibitor name	SEQ ID NO:
KC001-DNA	142
KC002-DNA	143
KC003-DNA	144
KC041-DNA	145
KC051-HC-DNA	146
KC051-LC-DNA	147
KC054-HC-DNA	148
KC054-LC-DNA	**149**

Table 2.3. DNA sequences of representative protein inhibitors encoding signal peptides.

Protein inhibitor name	SEQ ID NO:
KC001 + Signal peptide-DNA	150
KC002 + Signal peptide-DNA	151
KC003 + Signal peptide-DNA	152
KC041 + Signal peptide-DNA	153
KC051 + Signal peptide-HC-DNA	154
KC051 + Signal peptide-LC-DNA	155
KC054 + Signal peptide-HC-DNA	156
KC054 + Signal peptide-LC-DNA	**157**

Table 3.1. Sequence composition of protein inhibitors of complement pathway expressed and used in this study.

Molecule ID	SEQ ID NO:	Domain 1 (SEQ ID NO:)	Domain 2 (SEQ ID NO:)	Domain 3 (SEQ ID NO:)	Domain 4 (SEQ ID NO:)	Domain 5 (SEQ ID NO:)	Domain 6 (SEQ ID NO:)
KC001	50	46	36	29	34	37	
KC002	51	46	36	28	34	37	
KC003	52	46	36	31	34	37	
KC004	53	46	36	30			
KC005	54	6	45	36	29		
KC006	55	6	45	36	29	34	18
KC007	56	6	45	36	29	35	37
KC008	57	46	36	29	33	37	
KC009	58	46	36	29	32	37	
KC010	59	46	36	29	35	41	
KC011	60	46	36	29	34	18	
KC012	61	46	36	30	34	18	
KC013	62	46	36	27	34	37	
KC014	63	46	36	27	33	37	
KC015	64	46	36	27			
KC016	65	46	36	31			
KC017	66	46	36	29	35	39	
KC018	67	46	36	29	35	38	
KC019	68	46	36	29	35	20	
KC020	69	46	36	29	35	40	
KC021	70	46	36	29	35	41	
KC022	71	46	36	29	34	41	
KC023	72	46	36	29	32	41	
KC024	73	46	36	30	35	41	
KC025	74	29	35	41	5		
KC026	75	6	29	35	41		
KC027	76	6	42	29	35	41	
KC028	77	6	43	29	35	41	
KC029	78	6	43	30	35	41	
KC030	79	6	44	36	29	35	41
KC031	80	6	44	36	29		

Table 3.2. Sequence composition of Heavy chains of bispecific inhibitors comprising complement regulators fused to anti-VEGF-VH-CH1 and anti-VEGF-VL-CL.

Molecule ID	SEQ ID NO: (Excluding signal peptide)	Domain 1 (SEQ ID NO:)	Linker (SEQ ID NO:)	Domain 2 (SEQ ID NO:)	Linker (SEQ ID NO:)	Domain 3 (SEQ ID NO:)
KC032-HC	81	47	36	29	34	37
KC033-HC	82	47	36	29	34	37
KC034-HC	83	47	36	31		
KC035-HC	84	47	36	31		

Table 3.3. Sequence composition of Light Chains of bispecific inhibitors comprising complement regulators fused to anti-VEGF-VH-CH1 and anti-VEGF-VL-CL.

Molecule ID	SEQ ID NO: (Excluding signal peptide)	Domain 1 (SEQ ID NO:)	Linker (SEQ ID NO:)	Domain 2 (SEQ ID NO:)	Linker (SEQ ID NO:)	Domain 3 (SEQ ID NO:)
KC032-LC	133	48	36	29	34	37
KC033-LC	134	48	36	31		
KC034-LC	135	48	36	29	34	37
KC035-LC	136	48	36	31		

Table 3.4 Sequence composition of anti-VEGF-Fab and anti-VEGF Antibody (Ab)

Molecule ID	Heavy Chain SEQ ID NO:	Light Chain SEQ ID NO:
aVEGF-Ab	49	48
aVEGF-Fab	47	48

Molecule ID	Heavy Chain SEQ ID NO:	Light Chain SEQ ID NO:
aVEGF-Ab	49	48
aVEGF-Fab	47	48

Table 8. Sequence of human Complement Factor H related protein 3 (CFHR3) and human Complement Factor H related protein 5 (CFHR5) amino acid.

Molecule Name	SEQ ID NO.	Amino Acid Sequence
CFHR3	87	CDFPDIKHGGLFHENMRRPYFPVAVGKYYSYYCDEHFETPSGSYWDYIHCTQNGWSPA VPCLRKCYFPYLENGYNQNYGRKFVQGNSTEVACHPGYGLPKAQTTVTCTEKGWSPTP RCIRVRTCSKSDIEIENGFISESSSIYILNKEIQYKCKPGYATADGNSSGSITCLQNG WSAQPICINSSEKCGPPPPISNGDTTSFLLKVYVPQSRVEYQCQPYYELQGSNYVTCS NGEWSEPPRCIHPCIITEENMNKNNIKLKGRSDRKYYAKTGDTIEFMCKLGYNANTSI LSFQAVCREGIVEYPRCE
CFHR5	88	LCDFPKIHHGFLYDEEDYNPFSQVPTGEVFYYSCEYNFVSPSKSFWTRITCTEEGWSP TPKCLRMCSFPFVKNGHSESSGLIHLEGDTVQIICNTGYSLQNNEKNISCVERGWSTP PICSFTKGECHVPILEANVDAQPKKESYKVGDVLKFSCRKNLIRVGSDSVQCYQFGWS PNFPTCKGQVRSCGPPPQLSNGEVKEIRKEEYGHNEVVEYDCNPNFIINGPKKIQCVD GEWTTLPTCVEQVKTCGYIPELEYGYVQPSVPPYQHGVSVEVNCRNEYAMIGNNMITC INGIWTELPMCVATHQLKRCKIAGVNIKTLLKLSGKEFNHNSRIRYRCSDIFRYRHSV CINGKWNPEVDCTEKREQFCPPPPQIPNAQNMTTTVNYQDGEKVAVLCKENYLLPEAK EIVCKDGRWQSLPRCVESTAYCGPPPSINNGDTTSFPLSVYPPGSTVTYRCQSFYKLQ GSVTVTCRNKQWSEPPRCLDPCVVSEENMNKNNIQLKWRNDGKLYAKTGDAVEFQCKF PHKAMISSPPFRAICQEGKFEYPICE

Table 9. Sequences of selected complement control protein (CCP) domains from complement regulator proteins; CR1, FH, CFHR3, and CFHR5.

Domain Names	SEQ ID NO:	Amino Acid Sequence
CR1(18)	89	IPNKCTPPNVENGILVSDNRSLFSLNEVVEFRCQPGFVMKGPRRVKCQALNKWEPEL PSCSRV
FH (6)	90	LKPCDYPDIKHGGLYHENMRRPYFPVAVGKYYSYYCDEHFETPSGSYWDHIHCTQDG WSPAVPCL
CFHR3(1)	91	PCDFPDIKHGGLFHENMRRPYFPVAVGKYYSYYCDEHFETPSGSYWDYIHCTQNGWS PAVPCL
CFHR3(2)	92	RKCYFPYLENGYNQNYGRKFVQGNSTEVACHPGYGLPKAQTTVTCTEKGWSPTPRCI RVSFTL
CFHR5(8)	93	TAYCGPPPSINNGDTTSFPLSVYPPGSTVTYRCQSFYKLQGSVTVTCRNKQWSEPPR CLDP
CFHR5(9)	94	CVVSEENMNKNNIQLKWRNDGKLYAKTGDAVEFQCKFPHKAMISSPPFRAICQEGKF EYPICE

Table 10.1 Sequences of CR1 CCP domain combinations used in this study

Molecule ID	SEQ ID NO:	Domain 1 (SEQ ID NO:)	Domain 2 (SEQ ID NO:)	Domain 3 (SEQ ID NO:)	Domain 4 (SEQ ID NO:)	Domain 5 (SEQ ID NO:)	Domain 6 (SEQ ID NO:)	Domain 7 (SEQ ID NO:)	Domain 8 (SEQ ID NO:)

CR1(15-18)	95	14	15	16	89				
CR1(1-4,15-18)	96	7	8	9	10	14	15	16	89

Table 10.2 Sequence of complement regulator CCP domain combinations used in this study.

Molecule ID	**SEQ ID NO:**	Domain 1 (SEQ ID NO:)	Domain 2 (SEQ ID NO:)
FH(6-7)	97	90	18
CFHR3(1-2)	98	91	92
CFHR5(8-9)	99	93	94

Table 11. Sequence composition of engineered complement inhibitors expressed and used in this study.

Molecule ID	SEQ ID NO:	Domain 1 (SEQ ID NO:)	Domain 2 (SEQ ID NO:)	Domain 3 (SEQ ID NO:)	Domain 4 (SEQ ID NO:)	Domain 5 (SEQ ID NO:)
KC036	100	46	36	96	34	18
KC037	101	46	36	96	34	97
KC038	102	46	36	95	34	37
KC039	103	46	36	95	34	18
KC040	104	46	36	95	34	97
KC041	105	46	36	95	34	99
KC042	106	46	36	95	34	98
KC043	107	46	36	23	34	37
KC044	108	46	36	23	34	18
KC045	109	46	36	23	34	97
KC046	110	46	36	23	34	99
KC047	111	46	36	23	34	98

Table 12. Sequence of IgG constant and variable domain sequences used in this study.

Molecule Name	**SEQ ID NO:**	**Amino Acid Sequence**
anti-IL-6-VH-CH1	112	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAMHWVRQAPGKGLEWVAKISPGGS WTYYSDTVTDRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCARQAWGYYALDIWGQG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDK THT

anti-IL-6-VL-CL	113	DIQLTQSPSSLSASVGDRVTITCSASISVSYLYWYQQKPGKAPKLLIYDDSSLASG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSGYPYTFGQGTKVEIKRTVAAP SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
anti-IL-6-CH	114	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAMHWVRQAPGKGLEWVAKISPGGS WTYYSDTVTDRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCARQAWGYYALDIWGQG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDK THTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSCSPG K

Table 13.1. Sequence composition of bispecific inhibitors comprising complement regulators fused to various antibody IgG Fab fragments (heavy chain).

Molecule ID	SEQ ID NO:	Domain 1 (SEQ ID NO:)	Linker (SEQ ID NO:)	Domain 2 (SEQ ID NO:)	Linker (SEQ ID NO:)	Domain 3 (SEQ ID NO:)
KC048-HC	115	112	36	95	34	99
KC049-HC	116	112	36	95	34	99
KC050-HC	117	112	36	95	34	99
KC051-HC	118	112	36	23	34	99
KC052-HC	119	112	36	23	34	99
KC053-HC	120	47	36	95	34	99
KC054-HC	121	47	36	95	34	99
KC055-HC	122	47	36	23	34	99
KC056-HC	123	47	36	23	34	99

Table 13.2. Sequence composition of bispecific inhibitors comprising complement regulators fused to various antibody IgG Fab fragments (light chain).

Molecule ID	SEQ ID NO:	Domain 1 (SEQ ID NO:)	Linker (SEQ ID NO:)	Domain 2 (SEQ ID NO:)	Linker (SEQ ID NO:)	Domain 3 (SEQ ID NO:)
KC048-LC	124	113	36	23	34	98
KC049-LC	125	113	36	95	34	99
KC050-LC	126	113	36	23	34	99

KC051-LC	127	113	36	95	34	99
KC052-LC	128	113	36	23	34	99
KC053-LC	129	48	36	95	34	99
KC054-LC	130	48	36	23	34	99
KC055-LC	131	48	36	95	34	99
KC056-LC	132	48	36	23	34	99

Table 13.3. Sequence composition of anti-IL-6-Fab and anti-IL-6 antibody (Ab)

Molecule ID	Heavy Chain SEQ ID NO:	Light Chain SEQ ID NO:
aIL-6-Fab	112	113
aIL-6-Ab	114	113

Table 14. Amino acid sequence representing a complement inhibitor. Fc sequences are underlined, CR1 components are highlighted in grey. CFHR5 sequences are in bold, and Gly-Ser linker is italicized.

Protein Inhibitor Name	SEQ ID NO:	Amino Acid Sequence
KC041	105	SCDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGK*GGGGSGGGGS*GHCQAPDHFLFAKLKTQTNASDFPIG TSLKYECRPEYYGRPFSITCLDNLVWSSPKDVCKRKSCKTPPDPVNGMVHVIT DIQVGSRINYSCTTGHRLIGHSSAECILSGNTAHWSTKPPICQRIPCGLPPTI ANGDFISTNRENFHYGSVVTYRCNLGSRGRKVFELVGEPSIYCTSNDDQVGIW SGPAPQCIIPNKCTPPNVENGILVSDNRSLFSLNEVVEFRCQPGFVMKGPRRV KCQALNKWEPELPSCSRVGSGGGSGSGGGGS**TAYCGPPPSINNGDTTSFPLSV** **YPPGSTVTYRCQSFYKLQGSVTVTCRNKQWSEPPRCLDPCVVSEENMNKNNIQ** **LKWRNDGKLYAKTGDAVEFQCKFPHKAMISSPPFRAICQEGKFEYPICE**

[0165] Also provided are polynucleotides (or nucleic acids containing a nucleotide sequence) encoding any of the protein inhibitors or a portion thereof described herein. In another aspect, the present disclosure provides a method of making any of the polynucleotides described herein. Polynucleotides (or nucleic acids containing a nucleotide sequence) can be made using any suitable option. Accordingly, polynucleotides or compositions, including

pharmaceutical compositions, comprising polynucleotides, encoding any of the protein inhibitors are provided. In some embodiments, the nucleic acid includes a nucleotide sequence of any one of SEQ ID NO: 141-148 or 149-156, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto. In some embodiments, the nucleic acid includes a nucleotide sequence of any one of SEQ ID NO: 141-148 or 149-156, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto that encodes the polypeptide sequence encoded by any one of SEQ ID NO: 141-148 or 149-156, respectively.

[0166] Polynucleotides complementary to any such sequences are also encompassed by the present disclosure. Polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. RNA molecules include hnRNA molecules, which contain introns and correspond to a DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain introns. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present disclosure, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

[0167] Polynucleotides may comprise a native sequence (i.e., an endogenous sequence that encodes a protein inhibitor or a fragment thereof) or may comprise a variant of such a sequence. Polynucleotide variants contain one or more substitutions, additions, deletions and/or insertions such that the immunoreactivity of the encoded polypeptide is not diminished, relative to a native immunoreactive molecule. The effect on the immunoreactivity of the encoded polypeptide may generally be assessed as described herein. Variants preferably exhibit at least about 70% identity, more preferably, at least about 80% identity, yet more preferably, at least about 90% identity, and most preferably, at least about 95% identity to a polynucleotide sequence that encodes a protein inhibitor or a fragment thereof.

[0168] Two polynucleotide or polypeptide sequences are said to be "identical" if the sequence of nucleotides or amino acids in the two sequences is the same when aligned for maximum correspondence as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, or 40 to about

50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

[0169] Optimal alignment of sequences for comparison may be conducted using the MegAlign® program in the Lasergene® suite of bioinformatics software (DNASTAR®, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O., 1978, A model of evolutionary change in proteins - Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J., 1990, Unified Approach to Alignment and Phylogenes pp. 626-645 Methods in Enzymology vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M., 1989, CABIOS 5:151-153; Myers, E.W. and Muller W., 1988, CABIOS 4:11-17; Robinson, E.D., 1971, Comb. Theor. 11:105; Santou, N., Nes, M., 1987, Mol. Biol. Evol. 4:406-425; Sneath, P.H.A. and Sokal, R.R., 1973, Numerical Taxonomy the Principles and Practice of Numerical Taxonomy, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman, D.J., 1983, Proc. Natl. Acad. Sci. USA 80:726-730.

[0170] Preferably, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (i.e., gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (i.e. the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

[0171] Variants may also, or alternatively, be substantially homologous to a native gene, or a portion or complement thereof.

[0172] Suitable “moderately stringent conditions” include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5 X SSC,

overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1 % SDS.

[0173] As used herein, "highly stringent conditions" or "high stringency conditions" are those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 μg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

[0174] It will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present disclosure. Further, alleles of the genes comprising the polynucleotide sequences provided herein are within the scope of the present disclosure. Alleles are endogenous genes that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides. The resulting mRNA and protein may, but need not, have an altered structure or function. Alleles may be identified using standard techniques (such as hybridization, amplification and/or database sequence comparison).

[0175] The polynucleotides of this disclosure can be obtained using chemical synthesis, recombinant methods, or PCR. Any suitable options for chemical polynucleotide synthesis can be used. A desired DNA sequence can be produced using the sequences provided herein and any suitable DNA synthesizer.

[0176] For preparing polynucleotides using recombinant methods, a polynucleotide comprising a desired sequence can be inserted into a suitable vector, and the vector in turn can be introduced into a suitable host cell for replication and amplification, as further discussed herein. Polynucleotides may be inserted into host cells using any suitable option. Cells are transformed by introducing an exogenous polynucleotide by direct uptake, endocytosis, transfection, F-mating or electroporation. Once introduced, the exogenous polynucleotide can be maintained within the cell as a non-integrated vector (such as a plasmid) or integrated into the host cell genome. The polynucleotide so amplified can be isolated from the host cell by methods well known within the art. See, e.g., Sambrook et al., 1989.

[0177] Alternatively, PCR allows reproduction of DNA sequences. PCR technology is well known in the art and is described in U.S. Patent Nos. 4,683,195, 4,800,159, 4,754,065 and 4,683,202, as well as PCR: The Polymerase Chain Reaction, Mullis et al. eds., Birkauswer Press, Boston, 1994.

[0178] RNA can be obtained by using the isolated DNA in an appropriate vector and inserting it into a suitable host cell. When the cell replicates and the DNA is transcribed into RNA, the RNA can then be isolated using any suitable option, as set forth in Sambrook et al., 1989, supra, for example.

[0179] Suitable cloning vectors may be constructed according to standard techniques, or may be selected from a large number of cloning vectors available in the art. While the cloning vector selected may vary according to the host cell intended to be used, useful cloning vectors will generally have the ability to self-replicate, may possess a single target for a particular restriction endonuclease, and/or may carry genes for a marker that can be used in selecting clones containing the vector. Suitable examples include plasmids and bacterial viruses, e.g., pUC18, pUC19, Bluescript (e.g., pBS SK+) and its derivatives, mp18, mp19, pBR322, pMB9, ColE1, pCR1, RP4, phage DNAs, and shuttle vectors such as pSA3 and pAT28. These and many other cloning vectors are available from commercial vendors such as BioRad, Strategene, and Invitrogen.

[0180] Expression vectors are further provided. Expression vectors generally are replicable polynucleotide constructs that contain a polynucleotide according to the present disclosure. It is implied that an expression vector must be replicable in the host cells either as episomes or as an integral part of the chromosomal DNA. Suitable expression vectors include

but are not limited to plasmids, viral vectors, including adenoviruses, adeno-associated viruses, retroviruses, cosmids, and expression vector(s) disclosed in PCT Publication No. WO 87/04462. Vector components may generally include, but are not limited to, one or more of the following: a signal sequence; an origin of replication; one or more marker genes; suitable transcriptional controlling elements (such as promoters, enhancers and terminator). For expression (i.e., translation), one or more translational controlling elements are also usually required, such as ribosome binding sites, translation initiation sites, and stop codons.

[0181] The vectors containing the polynucleotides of interest can be introduced into the host cell by any of a number of appropriate means, including electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection (e.g., where the vector is an infectious agent such as vaccinia virus). The choice of introducing vectors or polynucleotides will often depend on features of the host cell.

[0182] The present disclosure also provides host cells comprising any of the polynucleotides described herein. Any host cells capable of over-expressing heterologous DNAs can be used for the purpose of isolating the genes encoding the polypeptide or protein of interest. Non-limiting examples of mammalian host cells include but not limited to COS, HeLa, and CHO cells. See also PCT Publication No. WO 87/04462. Suitable non-mammalian host cells include prokaryotes (such as *E. coli* or *B. subtillis*) and yeast (such as *S. cerevisae*, *S. pombe*; or *K. lactis*). A cell overexpressing the protein of interest can be identified.

[0183] Also provided are compositions that include one or more of the protein inhibitors described herein. Provided herein is a pharmaceutical composition that includes any one or more of the protein inhibitors described herein and a pharmaceutically acceptable excipient. As used herein, "pharmaceutically acceptable carrier" or "pharmaceutical acceptable excipient" includes any material which, when combined with an active ingredient, allows the ingredient to retain biological activity and causes no significant adverse toxicological effect on the patient. Any suitable pharmaceutically acceptable excipient may be included in the pharmaceutical composition. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, NaCl, normal saline solutions, lactated Ringer's, normal sucrose, normal glucose and the like, emulsions such as oil/water emulsion, various types of wetting agents, detergents such as

polysorbate 20 to prevent aggregation, and sugars such as sucrose as cryoprotectant. Non-limiting examples of diluents for aerosol or parenteral administration are phosphate buffered saline (PBS) or normal (0.9%) saline. In some embodiments, the pharmaceutically acceptable excipient is approved or approvable by the FDA for therapeutic use, particularly in humans.

[0184] The protein inhibitors described herein can be produced by recombinant expression including (i) the production of recombinant DNA by genetic engineering, (ii) introducing recombinant DNA into prokaryotic or eukaryotic cells by, for example and without limitation, transfection, electroporation or microinjection, (iii) cultivating the transformed cells, (iv) expressing protein inhibitors, *e.g.* constitutively or on induction, and (v) isolating the protein inhibitors, *e.g.* from the culture medium or by harvesting the transformed cells, in order to (vi) obtain purified protein inhibitors.

[0185] Protein inhibitors can be produced by expression in a suitable prokaryotic or eukaryotic host system characterized by producing a pharmacologically acceptable protein inhibitor molecule. Examples of eukaryotic cells are mammalian cells, such as CHO, COS, HEK 293, BHK, SK-Hip, and HepG2. Other suitable expression systems are prokaryotic (e.g., E. coli with pET/BL21 expression system), yeast (Saccharomyces cerevisiae and/or Pichia pastoris systems), and insect cells. In some embodiments, an isolated cell line that produces any of the protein inhibitors disclosed herein is provided. In some embodiments, the isolated cell line is selected, without limitations, from one or more of CHO, k1SV, XCeed, CHOK1SV, GS-KO.

[0186] In some embodiments, an isolated nucleic acid encoding any of the protein inhibitors disclosed herein is provided. In some embodiments, a recombinant expression vector comprising the isolated nucleic acid is provided. In some embodiments, a host cell comprises the expression vector.

[0187] A wide variety of vectors can be used for the preparation of the protein inhibitors and may be selected from eukaryotic and prokaryotic expression vectors. Examples of vectors for prokaryotic expression include plasmids such as, and without limitation, preset, pet, and pad, wherein the promoters used in prokaryotic expression vectors include one or more of, and without limitation, lac, trc, trp, recA, or araBAD. Examples of vectors for eukaryotic expression include, without limitation: (i) for expression in yeast, vectors such as, and without limitation, pAO, pPIC, pYES, or pMET, using promoters such as, and without limitation,

AOX1, GAP, GAL1, or AUG1; (ii) for expression in insect cells, vectors such as and without limitation, pMT, pAc5, pIB, pMIB, or pBAC, using promoters such as and without limitation PH, p10, MT, Ac5, OpIE2, gp64, or polh, and (iii) for expression in mammalian cells, vectors such as, and without limitation, pSVL, pCMV, pRc/RSV, pcDNA3, or pBPV, and vectors derived from, in one aspect, viral systems such as and without limitation vaccinia virus, adeno-associated viruses, herpes viruses, or retroviruses, using promoters such as and without limitation CMV, SV40, EF-1, UbC, RSV, ADV, BPV, and beta-actin.

[0188] Additional non-limiting embodiments are provided by the following numbered embodiments.

1. A protein inhibitor comprising a first polypeptide, the first polypeptide comprising:

an oligomerization domain; and

a complement regulator covalently linked to the oligomerization domain, the complement regulator comprising:

a Complement Receptor 1 (CR1) complement control protein (CCP) domain comprising at least one C3b and/or C4b binding domain; and

a Factor H (FH), a complement factor H related 3 (CFHR3), or a complement factor H related 5 (CFHR5) CCP domain.

2. The protein inhibitor of embodiment 1, wherein the inhibitor comprises:

the first polypeptide; and

a second polypeptide comprising:

a second oligomerization domain; and

a second complement regulator.

3. The protein inhibitor of embodiment 1 or 2, wherein the inhibitor is a homodimer.

4. The protein inhibitor of embodiment 1 or 2, wherein the inhibitor is a heterodimer.

5. The protein inhibitor of any one of the preceding embodiments, that is bispecific.

6. The protein inhibitor of any one of the preceding embodiments, wherein the oligomerization domain is selected from a Fc domain, a Fab fragment, or a complement factor H-related protein domain.

7. The protein inhibitor of any one of the preceding embodiments, wherein the oligomerization domain is selected from the group consisting of: an Fc domain that is SEQ ID NO: 46; an anti-VEGF-VH-CH1 domain that is SEQ ID NO: 47; an anti-VEGF-VL-CL domain that is SEQ ID NO: 48; an anti-VEGF-HC that is SEQ ID NO:49; a CFHR1(1-2) domain that is SEQ ID NO: 42; a CFHR5(1-2) domain that is SEQ ID NO: 43; an anti-IL-6-VH-CH1 domain that is SEQ ID NO: 112; an anti-IL-6-VL-VL domain that is SEQ ID NO: 113; or an anti-IL-6-CH domain that is SEQ ID NO: 114 .

8. The protein inhibitor of any one of the preceding embodiments, wherein the oligomerization domain comprises an Fc domain comprising SEQ ID NO: 46.

9. The protein inhibitor of any one of the preceding embodiments, wherein the oligomerization domain comprises an anti-VEGF-VH-CH1 comprising SEQ ID NO: 47.

10. The protein inhibitor of any one of the preceding embodiments, wherein the oligomerization domain comprises an anti-VEGF-VL-CL comprising SEQ ID NO: 48.

11. The protein inhibitor of any one of the preceding embodiments, wherein the oligomerization domain comprises an anti-VEGF-HC comprising SEQ ID NO: 49.

12. The protein inhibitor of any one of the preceding embodiments, wherein the oligomerization domain comprises an anti-IL-6-VH-CH1 comprising SEQ ID NO: 112.

13. The protein inhibitor of any one of the preceding embodiments, wherein the oligomerization domain comprises an anti-IL-6-LC comprising SEQ ID NO: 113.

14. The protein inhibitor of any one of the preceding embodiments, wherein the oligomerization domain comprises an anti-IL-6-HC comprising SEQ ID NO: 114.

15. The protein inhibitor of any one of the preceding embodiments, wherein the oligomerization domain is covalently linked to the complement regulator via a glycine-serine linker selected from sequences comprising SEQ ID Nos: 32-36.

16. The protein inhibitor of embodiment 15, wherein the glycine-serine linker comprises SEQ ID NO: 34.

17. The protein inhibitor of any one of the preceding embodiments, wherein the complement regulator is covalently linked to the oligomerization domain via GGGGSGGGGS (SEQ ID NO: 36).

18. The protein inhibitor of any one of the preceding embodiments, wherein the CR1 CCP domain comprises:

CR1 CCP domains 1-3, comprising SEQ ID NO: 22;

CR1 CCP domains 8-10, comprising SEQ ID NO: 24;

CR1 CCP domains 15-17, comprising SEQ ID NO: 25;

CR1 CCP domains 1-4 and 15-17, comprising SEQ ID NO: 26; or

CR1 CCP domains 1-3 and 15-17, comprising SEQ ID NO: 137.

19. The protein inhibitor of any one of the preceding embodiments, wherein the factor H CCP domain 4 is covalently linked to the C-terminus of the CR1 CCP domain.

20. The protein inhibitor of any one of the preceding embodiments, wherein the CR1 CCP domain comprises CR1 CCP domains 1-4 comprising SEQ ID NO: 23; CR1 CCP domains 15-18 comprising SEQ ID NO: 95; or CR1 CCP domains 1-4 and 15-18 comprising SEQ ID NO: 96.

21. The protein inhibitor of any one of embodiments 18-20, wherein the FH CCP domain comprises FH CCP domain 7 comprising SEQ ID NO: 18; FH CCP domains 6-7 comprising SEQ ID NO: 97; FH CCP domains 18-20 comprising SEQ ID NO: 37; FH CCP domains 18-19 and 7 comprising SEQ ID NO: 38; FH CCP domains 18-19 comprising SEQ ID NO: 39; FH CCP domains 19 and 7 comprising SEQ ID NO: 40; or FH CCP domains 19-20 comprising SEQ ID NO: 41.

22. The protein inhibitor of any one of the preceding embodiments, wherein the complement regulator comprises factor H CCP domain 4 covalently linked to the C-terminus of a subgroup of CR1 CCP domains.

23. The protein inhibitor of embodiment 22, wherein the CR1 CCP domains comprise CR1 CCP domains 1-3, comprising SEQ ID NO: 22.

24. The protein inhibitor of embodiment 22, wherein the CR1 CCP domains comprise CR1 CCP domains 8-10, comprising SEQ ID NO: 24.

25. The protein inhibitor of embodiment 22, wherein the CR1 CCP domains comprise CR1 CCP domains 15-17, comprising SEQ ID NO: 25.

26. The protein inhibitor of embodiment 22, wherein the CR1 CCP domains comprise CR1 CCP domains 1-4, and 15-17, comprising SEQ ID NO: 26.

27. The protein inhibitor of embodiment 22, wherein the CR1 CCP domains comprise CR1 CCP domains 1-3, and 15-17, comprising SEQ ID NO: 137.

28. The protein inhibitor of any of any one of the preceding embodiments, wherein a subgroup of FH CCP domains selected from SEQ ID NOs: 18-21 are covalently linked to the C terminus of FH CCP domain 4 via a glycine-serine linker.

29. The protein inhibitor of any one of the preceding embodiments, wherein the glycine serine linker comprises GGGGS (SEQ ID NO: 33).

30. The protein inhibitor of any one of embodiments 1-28, wherein the glycine serine linker comprises GSGGGGS (SEQ ID NO: 34).

31. The protein inhibitor of any one of embodiments 1-28, wherein the glycine serine linker comprises GSGGGGSGGGGS (SEQ ID NO: 35).

32. The protein inhibitor of any one of embodiments 1-28, wherein the glycine serine linker comprises GGGGSGGGGS (SEQ ID NO: 36).

33. The protein inhibitor of any one of the preceding embodiments, wherein the FH CCP domains comprise FH CCP domains 18-20 comprising SEQ ID NO: 37.

34. The protein inhibitor of any one of embodiments 1-32, wherein the FH CCP domains comprise FH CCP domains 18-19 and 7 comprising SEQ ID NO: 38.

35. The protein inhibitor of any one of embodiments 1-32, wherein the FH CCP domains comprise FH CCP domains 18-19 comprising SEQ ID NO: 39.

36. The protein inhibitor of any one of embodiments 1-32, wherein the FH CCP domains comprise FH CCP domains 19 and 7 comprising SEQ ID NO: 40.

37. The protein inhibitor of any one of embodiments 1-32, wherein the FH CCP domains comprise FH CCP domains 19-20 comprising SEQ ID NO: 41.

38. A protein inhibitor comprising:

a first polypeptide comprising:

SEQ ID NO: 50; and

a second polypeptide comprising:

SEQ ID NO: 50; and

wherein the first polypeptide is conjugated to the second polypeptide via disulfide bonds.

39. A protein inhibitor comprising a first polypeptide, the first polypeptide comprising:

a complement receptor domain (CRD);

a complement regulator comprising:

a Complement Receptor 1 (CR1) complement control protein (CCP) domain; and

a Factor H (FH) CCP domain;

wherein the CCP domains comprise at least one C3b and/or C4b binding domain; and

wherein the CRD is covalently linked to the complement regulator.

40. A protein inhibitor comprising an amino acid sequence of at least one of the amino acid sequences set forth in Tables 1.1-1.10, 2, 3.1, 3.2, 3.3, 3.4, 8, 9, 10.1, 10.2, 11, 12, 13.1, 13.2, 13.3, and 14, or a sequence at least 80% identical thereto.

41. A protein inhibitor comprising an amino acid sequence of at least one of SEQ ID NOs: 50- 84, 100-111, and 115-136, or a sequence at least 80% identical thereto.

42. A protein inhibitor comprising: (1) a first polypeptide comprising any one of the amino acid sequences set forth in SEQ ID NOs: 115-123, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto; and a second polypeptide comprising any one of the amino acid sequences set forth in SEQ ID NOs: 124-132, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto, optionally wherein the first and second polypeptides are paired as they are arranged in Tables 13.1 and 13.2; or (2) a first polypeptide comprising any one of the amino acid sequences set forth in SEQ ID NOs: 81-84, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto; and a second polypeptide comprising any one of the amino acid sequences set forth in SEQ ID NOs: 133-136, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto, optionally wherein the first and second polypeptides are paired as they are arranged in Tables 3.2 and 3.3.

43. A method of treatment comprising:

identifying a subject in need of treatment for age-related macular degeneration (AMD), paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), C3 glomerulopathy (C3G) or rheumatoid arthritis; and

administering to the subject the protein inhibitor of any one of the preceding embodiments.

[0189] All patents and other publications; including literature references, issued patents, published patent applications, and co-pending patent applications; cited throughout this application are expressly incorporated herein by reference for the purpose of describing and disclosing, for example, the methodologies described in such publications that might be used in connection with the technology described herein. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.

[0190] The description of embodiments of the disclosure is not intended to be exhaustive or to limit the disclosure to the precise form disclosed. While specific embodiments of, and examples for, the disclosure are described herein for illustrative purposes, various equivalent modifications are possible within the scope of the disclosure, as those skilled in the relevant art will recognize. For example, while method steps or functions are presented in a given order, alternative embodiments may perform functions in a different order, or functions may be performed substantially concurrently. The teachings of the disclosure provided herein can be applied to other procedures or methods as appropriate. The various embodiments described herein can be combined to provide further embodiments. Aspects of the disclosure can be modified, if necessary, to employ the compositions, functions and concepts of the above references and application to provide yet further embodiments of the disclosure. Moreover, due to biological functional equivalency considerations, some changes can be made in protein structure without affecting the biological or chemical action in kind or amount. These and other changes can be made to the disclosure in light of the detailed description. All such modifications are intended to be included within the scope of the appended claims.

[0191] Specific elements of any of the foregoing embodiments can be combined or substituted for elements in other embodiments. Furthermore, while advantages associated with certain embodiments of the disclosure have been described in the context of these

embodiments, other embodiments may also exhibit such advantages, and not all embodiments need necessarily exhibit such advantages to fall within the scope of the disclosure.

[0192] The technology described herein is further illustrated by the following examples which in no way should be construed as being further limiting.

EXAMPLES

Example 1. *Construction and expression of complement regulator fusion proteins.*

[0193] This non-limiting example describes the development and expression of embodiments of the protein inhibitors.

[0194] *Construction*: **Figure 1A** is a schematic diagram of the domains of Complement Receptor 1 (CR1) and Factor H (FH). Each oval with a number refers to a complement control protein (CCP) domain in each protein. **Figure 1B** shows examples of engineered complement regulators. The functional CCP domains of CR1 are fused to FH CCP domains to create novel complement regulators (e.g., complement regulator fusion proteins). Linkers connecting CCP domains are indicated as straight lines. The amino acid sequence of human FH (accession no. P08603) and human CR1 (accession no. P17927) are listed in **Table 1.1**. Complement control protein (CCP) domains of CR1 involved in C3b / C4b binding as well as additional CR1 CCP domains adjacent to the C3b/C4B binding domains were selected to construct novel complement regulators.

[0195] Amino acid sequences of signal peptides and HIS tags used in the study are shown in **Table 1.2.** Histidine tags (**Table 1.2**) were utilized to purify CR1-FH regulator proteins.

[0196] The amino acid sequences of each CR1 CCP domain used in the study are shown in **Table 1.3.**

[0197] The amino acid sequences of each FH CCP domain used in the study are shown in **Table 1.4.**

[0198] The combinations of CR1 CCP domains included in the complement regulator domains of the protein inhibitors are shown in **Table 1.5**. Numbers in parenthesis following abbreviations of protein names indicate the numbers of the CCP domains included

in the CR1 CCP combination domain. For example, CR1(1-3) indicates use of CCP domains 1 to 3 of CR1.

[0199] The CR1 CCP domain combinations (**Table 1.5**) were then fused to the FH(4) CCP domain, which also binds to C3b, thereby providing the basic design of the CR1-FH fusion complement regulators or CR1-FH(4)-combination domains, which are listed in **Table 1.6**.

[0200] Glycine-Serine linkers (Sequences in **Table 1.7**) were used to 1) covalently link the oligomerization domains (**Table 1.10**) to the complement regulators and 2) covalently link selected FH CCP domains, which bind GAGs, (**Table 1.4**) and FH CCP domain combinations (**Table 1.8**) to the C-termini of the CR1-FH(4) combination domains **(Table 1.6)** in order to add additional function to the regulators.

[0201] Non-limiting schematic diagrams of a few protein inhibitor fusion proteins are drawn in **FIGS. 3-7** and **9-10.**

[0202] **Figure 11** is a schematic diagram representing the protein domains of CRIg (accession number Q9Y279), FH, CFHR1 (Q03591), CFHR5(Q9BXR6), and CR2 (P20023). CFHR1 and CFHR5 are aligned with equivalent modules of the full-length FH protein.

[0203] The engineered complement regulators were also fused to N-terminal domains of other human complement factors, such as CFHR1(1-2) (accession no. Q03591), CFHR5(1-2) (accession no. Q9BXR6), CR2(1-4) (accession no. P20023), and CRIg immunoglobulin domains (accession no. Q9Y279). The amino acid sequences of the N-terminal domains of the complement factors are listed in **Table 1.9**. Examples of engineered protein inhibitors, which include the N-terminal domains of the complement factors listed in **Table 1.9,** are depicted at the bottom of **Figure 11**.

[0204] Glycine-Serine linkers (Sequences in **Table 1.7**) were used to 1) covalently link the oligomerization domains (**Table 1.9**, SEQ ID NO: 42-43) to the complement regulators, 2) covalently link the complement receptor domains (**Table 1.9**, SEQ ID NO: 44-45), and 3) covalently link selected FH CCP domains, which bind GAGs, (**Table 1.4**) and FH CCP domain combinations (**Table 1.8**) to the C-termini of the CR1-FH(4) combination domains (**Table 1.6**) in order to add additional function to the regulators.

[0205] Schematic diagrams of a few protein inhibitor fusion proteins are drawn in **FIGS. 11B-11E**.

[0206] The complement regulators were also fused to Fab or Fc of IgG1 using a double repeat of a Gly-Gly-Gly-Gly-Ser linker (GS(5)). By strategically fusing complement regulators with different moieties, we can control their assembly as monomers or dimers, enabling the design of customized regulators for therapeutic needs. IgG heavy chain and light chain components sequences are listed in **Table 1.10**.

[0207] Amino acid sequences of representative protein inhibitors are shown in **Table 2**.

[0208] *Expression.* **Table 3.1** presents the sequence composition of the protein inhibitors expressed and tested in this study. **Tables 3.2 and 3.3** present the sequence composition of the protein inhibitors that comprise complement regulators fused to anti-VEGF-Fab and anti-VEGF Fab, anti-VEGF Ab to form bispecific protein inhibitors. The codon-optimized DNA sequences were cloned into the pCDND3.3 backbone. The human embryonic kidney (HEK293) cells or Chinese hamster ovary (CHO) cells were transfected with a construct plasmid. The secreted regulators were purified using MabSelect PrismA column or HisTrap HP column using AKTA Pure (Cytiva). Thus, the above shows that the constructed protein inhibitors can be expressed and purified.

Example 2. *Inhibition of the complement activated hemolysis.*

[0209] In this non-limiting example the functional activity of embodiments of the engineered protein inhibitors in inhibition of complement activation was evaluated through hemolytic assays for the alternative and classical pathways.

[0210] For evaluating the alternative pathway (AP), unsensitized sheep erythrocytes (Es) were used. Hemolysis of Es cells is triggered in the presence of magnesium ions, FH-depleted serum, and EGTA, which preferentially chelates calcium ion. The Es cells were washed three times with GVB° buffer (0.1 % gelatin, 5 mM Veronal, 145 mM $MgCl_2$, 0.025 % NaN_3, pH 7.3, CompTech, USA) at 4 °C. The protein inhibitors were serially diluted in FH-depleted human serum (Factor H-Dpl, CompTech, USA) to concentrations between 0.01 nM and 600 nM, then added to the washed Es cells. To trigger hemolysis, 5 mM MgEGTA (CompTech, USA) was added. The same amount of Es cells mixed with 10 % FH depleted human serum mixture without MgEGTA was used as a background subtraction. A hundred percent hemolysis was defined by mixing Es cells, 10 % FH depleted human serum,

and 5 mM MgEGTA. The reactions were incubated at 37 °C for 30 minutes, then quenched by adding the equal volume of ice chilled GVBE with EDTA (0.1 % gelatin, 5 mM Veronal, 145 mM NaCl, 0.025 % NaN_3, 10 mM EDTA, pH 7.3, CompTech, USA). The reaction plates were centrifuged at 800 x g for 10 minutes. Supernatant was transferred to a 96-well clear bottom plate and absorbance at 412 nm was recorded by iD3 plate reader (Molecular Devices). AH50 curves were generated using GraphPad PRISM v10.0 by subtracting the background value and normalizing to lysis of untreated control values. Average % lysis values with standard error for replicates were plotted against log concentration of inhibitors and fitted with a non-linear regression curve. As shown in **Figure 12A**, the protein inhibitors demonstrated a higher inhibitory effect than FH. IC_{50} values of the protein inhibitors for the alternative pathway activation are shown in **Table 4**.

Table 4. Inhibition of the complement activated hemolysis.

Molecule ID	AP IC_{50} [nM]	CP IC_{50} [nM]
FH	45.3 ± 1.78	684.4 ± 86.7
KC021	0.40 ± 0.067	0.63 ± 0.043
KC025	5.06 ± 2.02	2.65
KC026	1.70	0.62
KC028	1.69	1.44
KC030	4.74	n/a
KC031	26.76	n/a
KC005	2.45	11.84
KC006	3.22	10.55
KC001	0.38 ± 0.083	0.41
KC008	0.30 ± 0.001	0.41
KC010	0.26 ± 0.057	n/a
KC011	2.50	n/a
KC017	10.06	n/a
KC018	3.12	n/a
KC019	4.88	22.45
KC020	6.03	n/a
KC004	0.76	1.11
KC024	0.17	0.15
KC012	0.19	0.77
KC002	0.87	n/a
KC014	0.13	0.1

KC007	1.19	n/a

n/a indicates the assay was not performed. Samples with SD shown are performed 3 times independently, otherwise data with duplicated assay are recorded.

[0211] For the classical pathway, sheep erythrocytes coated with rabbit anti-sheep erythrocyte antiserum ((EA), CompTech, USA) were washed with GVB^{++} buffer (0.1 % gelatin, 5 mM Veronal, 145 mM NaCl, 0.15 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.025 % NaN_3, pH 7.3, CompTech) three times at 4 °C. The protein inhibitors were serially diluted in 2x normal human serum (NHS, CompTech) to concentrations between 0.01 nM and 600 nM (for FH, 1200 nM), then added to the equal volume of EA. A mixture of NHS in GVB^{++} buffer, and a mixture of EA mixed with NHS in GVB^{++} buffer was prepared as background and 100 % lysis condition, respectively. The reactions were incubated at 37 °C for 30 minutes, then quenched by adding the same volume of ice chilled GVBE with EDTA. The reaction plates were centrifuged at 800 x g for 10 minutes. Supernatant was transferred to a 96-well clear bottom plate and absorbance at 412 nm was measured by iD3 plate reader (Molecular Devices). CH50 curves were generated using GraphPad PRISM v10.0 by subtracting the background value and normalizing to lysis of untreated control values. Average % lysis values with standard error for replicates were plotted against log concentration of protein inhibitors and fitted with a non-linear regression curve (**Figure 12B**). Results demonstrated that the protein inhibitors also inhibit the classical pathway effectively. IC_{50} values of various protein inhibitors for classical pathway activation are shown in **Table 4.**

[0212] Thus, the results show that embodiments of the protein inhibitors tested inhibit both the alternative and classical complement pathways.

Example 3. *Cofactor activity assay in CFI-mediated C3b and C4b degradation*

[0213] This non-limiting example evaluates the function of embodiments of the protein inhibitors as cofactors for CFI-mediated C3b and C4b degradation

[0214] The cofactor activity assay was performed to evaluate the function of the protein inhibitors as cofactors for CFI-mediated C3b degradation. The reaction mixture of C3b (0.6 μM), FI (0.05 μM), and either FH or the protein inhibitors at 0.06 or 0.15 μM in 20 mM HEPES buffer, 150 mM $NaCl_2$. 2 mM $MgCl_2$ were prepared on ice, followed by incubation at 37 °C for 20 minutes. The negative control was prepared containing the same concentration

of C3b and FI without the protein inhibitors and incubated at 37 °C for 20 minutes. The reactions were stopped by adding NuPAGE™ LDS Sample buffer and NuPAGE™ Sample Reducing Agent (Thermo Fisher Scientific, USA) followed by incubation at 90 °C for 5 minutes, immediately. Samples were analyzed for degradation product of C3b alpha fragments on a Novex™ Tris-Glycine mini gel stained with SimplyBlue™ SafeStain (Thermo Fisher Scientific, USA). The samples without any protein inhibitors did not produce any degraded fragment while addition of FH produced 68 kDa and 43 kDa fragments. The protein inhibitor KC001(Seq ID. No. 050) cleaved C3b alpha fragment into 68 kDa, 43 kDa, and further degraded 40 kDa and C3dg fragments (**Figure 13A**). Lane 1 contains molecular weight markers. Lane 2 contains uncleaved C3b and FI (negative control). Lane 3 contains FH only. Lanes 4 and 5 contain FI, FH, C3b and C3b cleaved products. 68 kDa C3b α^1 fragment is right below of C3b β fragment and indicated with an arrow. Lanes 6 and 7 contain the protein inhibitor, KC001, FI, C3b, and C3b degraded fragments. Lane 8 contains only the protein inhibitor KC001.

[0215] Cofactor activity assay for C4b degradation was carried out as described above, except replacing C3b to C4b (0.6 μM) and incubation for 40 minutes. The negative control and FH as a cofactor in C4b, FI mixture did not generate any degraded fragments. However, the protein inhibitors, KC001 and KC003, acted as a cofactor for FI and generated C4d (45 kDa) and 25 kDa fragments (**Figure 13B**). Lane 1 contains molecular weight markers. Lane 2 contains FI and C4b (negative control). Lanes 3, 6, 9 and 12 contain FH, KC001, KC016, and KC003, respectively. Lanes 4 and 5 contain FI, FH, and uncleaved C4b. Lanes 7 and 8 contain FI, KC001, C4b, and C4b degraded products (45 kDa and 25 kDa). Lanes 10 and 11 contain FI, KC016, and C4b, and C4b cleaved products (45 kDa and 25 kDa). Lanes 13 and 14 contain FI, KC003, and C4b, and C4b cleaved products (45 kDa and 25 kDa).

[0216] Thus, the results show that embodiments of the protein inhibitors tested in this assay function as effective cofactors for CFI-mediated C3b and C4b degradation.

Example 4. *Binding kinetics for complement protein C3b*

[0217] This non-limiting example evaluates the binding kinetics of embodiments of the protein inhibitors for the complement protein C3b.

[0218] A surface plasmon resonance (SPR)-based assay was used to determine the binding kinetics of the protein inhibitors for the complement protein C3b using Biacore™ T200 system (Cytiva, USA). Flow cells 2, 3, and 4 of the CM5 chip were coated with human C3b (CompTech, USA) with ligand levels ranging between 300 to 400 response unit (RU) levels using an amine coupling protocol according to the manufacturer's instruction. The protein inhibitors were diluted in HBS-EP+ running buffer in concentration series ranging between 0-50 nM and injected over surface bound C3b at a flow rate of 30 µL/min for 50 seconds followed by dissociation in running buffer for 120 seconds. Surfaces were regenerated using 3 M magnesium chloride for 60 seconds at 50 µL/min. Binding kinetics were analyzed by Biacore™ T200 evaluation software. All sensorgrams were double reference subtracted and fitted using Langmuir 1:1 binding model. Protein inhibitors represented increased binding affinity. Sensorgrams of selected protein inhibitors are shown in **Figure 14B-D.**

[0219] **Figure 14A-D** show surface plasmon resonance (SPR) sensorgrams for C3b binding of FH (A), KC001 (B), KC002 (C), and KC021 (D). C3b was immobilized on the CM5 chip using amine coupling with ligand level between 300 to 400 response units (RU). The equilibrium dissociation constant KD is shown in the panes. The kinetic values from this assay are shown in **Table 5**.

Table 5. C3b binding kinetics. C3b was immobilized on the CM5 chip using amine coupling. Different concentrations of protein inhibitors were injected as soluble phase analyte.

	ka (1/Ms)	kd (1/s)	K_D (M)
FH	1.86E+06	3.40E-02	1.82E-08
KC026	1.17E+08	5.49E-02	4.70E-10
KC006	7.15E+06	1.15E-01	1.61E-08
KC021	2.96E+07	7.94E-03	2.68E-10
KC001	1.26E+07	5.29E-03	4.21E-10
KC008	1.88E+07	8.51E-03	4.53E-10
KC002	1.25E+07	6.18E-03	4.95E-10
KC015	1.11E+06	3.96E-03	3.58E-09

[0220] The protein inhibitors comprising the Fc domain were evaluated for C3b binding using SPR on Biacore™ T200 system (Cytiva, MA, USA) (**Fig. 15A-C**). protein inhibitors comprising the Fc domain were diluted to 5 nM in HBS-EP+ buffer and captured on Protein A sensor chip (Cytiva, MA, USA) at 10 µL/min for 20 seconds. The ligand level of the

protein regulators ranged from 18 to 45 RU levels. Complement protein C3b (Cytiva) was diluted in HBS-EP+ running buffer in concentration series ranging between 0-135 nM. Increasing concentrations of the analytes were then injected at 30 µL/min for 60 seconds followed by a 180 second dissociation step. The sensor chip surface was regenerated by 60 seconds injection of 10 mM Glycine, pH 1.7 at a flow rate of 50 µL/min. All sensorgrams were double reference subtracted and fitted using Langmuir 1:1 binding model in Biacore™ T200 evaluation software (Cytiva). **Table 6** summarizes the binding kinetics to C3b. Sensorgrams and Kinetic values are shown in **Figure 15A-C** and **Table 6**. **Figure 15A-C** shows surface plasmon resonance (SPR) sensorgrams for C3b binding of FH (A), KC001 (B), KC002 (C), and KC021 (D). C3b was immobilized on the CM5 chip using amine coupling with ligand level between 300 to 400 response units (RU). The equilibrium dissociation constant KD is shown in the panes.

Table 6. C3b Binding kinetics. Protein inhibitors were captured on Protein A coated chips. Different concentrations of C3b were injected as a solution phase analyte.

Sample ID	Ligand Level (RU)	ka (1/Ms)	kd (1/s)	K_D (M)
KC010	42.0	2.79E+05	1.22E-01	4.37E-07
KC021	26.0	1.77E+05	1.67E-02	9.60E-08
KC001	18.9	1.75E+05	2.05E-02	1.08E-07
KC008	22.1	1.70E+05	2.50E-02	1.50E-07
KC009	20.8	7.08E+04	4.55E-03	6.40E-08
KC022	21.9	2.48E+05	1.68E-02	6.76E-08
KC023	24.7	2.26E+05	2.04E-02	9.05E-08
KC002	40.5	1.28E+05	1.74E-02	1.36E-07
KC014	40.3	4.50E+06	2.90E+00	6.46E-07

[0221] Thus, the results show that embodiments of the protein inhibitors tested in the surface plasmon resonance (SPR)-based assay have a higher affinity for the complement protein C3b than factor H.

Example 5*. Construction and expression of protein inhibitors (e.g. bispecific inhibitors) comprising complement regulators and anti-VEGF Fab*

[0222] This non-limiting example describes the development and expression of embodiments of bispecific protein inhibitors.

[0223] To test the complement regulators as an additive module to another functional group, anti-VEGF-Fab was used in this study. Complement regulator modules were connected to the heavy chain and light chain of anti-VEGF Fab with a GS(5) linker (SEQ ID. 36) (**Table 1.7**) to create protein inhibitors that are bispecific inhibitors. Fusion to Fab moieties enables the toolkit for designing protein inhibitors to be expanded. This approach allows protein inhibitors to be created as homodimeric or heterodimeric scaffolds, depending on the specific targeting or functional needs.

[0224] The bispecific protein inhibitors were expressed by transfecting the HEK293 cells or CHO cells with equal amount of heavy chain and light chain constructs. The secreted anti-VEGF-Fab-CR1-FH fusion proteins were purified using CaptureSelect CH1 XL column using AKTA Pure (Cytiva). The list of expressed proteins is shown in **Tables 3.2 and 3.3.**

[0225] Thus, the above shows that embodiments of the constructed bispecific protein inhibitors can be expressed and purified.

Example 6. *Complement activated hemolysis inhibition by the bispecific protein inhibitors targeting complement activity regulation and VEGF pathways.*

[0226] This non-limiting example evaluates the ability of embodiments of the bispecific protein inhibitors to inhibit the alternative pathway (AP).

[0227] To determine the ability of the bispecific protein inhibitors to inhibit the AP activation pathway, a hemolysis assay for Alternative Pathway (AP) was conducted as described in the previous section. Es cells were incubated with FH-depleted serum and serially diluted bispecific protein inhibitors (0.01 nM and 300 nM), 5 mM MgEGTA at 37 °C for 30 minutes. AH50 curves are shown in **Figure 16** and IC_{50} values of the bispecific protein inhibitors for AP activation are shown in **Table 7.** The results indicated the bispecific protein inhibitors are capable of inhibiting complement AP activation about 100-fold higher than the natural regulator, FH.

[0228] **Figure 16** shows inhibition of the alternative pathway by the bispecific protein inhibitors, comprising anti-VEGF Fab and complement regulator modules. KC001 is

a protein inhibitor comprising a complement regulator covalently linked to an Fc domain and is used as a positive control (dark diamond).

Table 7. Inhibition of alternative complement pathway by bispecific protein inhibitors.

Molecule ID	AP IC_{50} [nM]
KC001	0.38
KC032	0.45
KC033	0.47
KC035	0.44

[0229] Thus, the results show that embodiments of the bispecific protein inhibitors inhibit the alternative complement pathway.

***Example** 7. Bispecific Protein Inhibitor Binding Kinetics to VEGF*

[0230] This non-limiting example evaluates the ability of embodiments of the bispecific protein inhibitors to bind VEGF.

[0231] The bispecific protein inhibitors comprising the complement regulators and the anti-VEGF Fab were evaluated for VEGF binding using SPR on Biacore™ T200 system (Cytiva). CM5 chip was coated with human Fab binder at around 1800 RU using amine coupling method with Human Fab Capture Kit (Cytiva, USA). Bispecific protein inhibitors were diluted to 5 nM in HBS-EP+ buffer and captured at 10 µL/min for 20 seconds. Serial dilution of VEGF165 (R&D systems, USA) ranging between 0-150 nM were flowed over captured bispecific protein inhibitors comprising the Fab domains for 120 seconds at 30 µL/min and dissociated for 70 min. All sensorgrams were double reference subtracted and fitted using a 1:1 Langmuir binding model. All tested embodiments of the bispecific protein inhibitors exhibited tight binding to VEGF165. The sensorgrams of the bispecific protein inhibitors with ligand level are shown in **Figure 17**.

[0232] Thus, the results show that embodiments of the bispecific protein inhibitors exhibited tight binding to VEGF165.

***Example** 8. VEGF cell reporter assay of the bispecific protein inhibitors*

[0233] This non-limiting example evaluates the ability of embodiments of the bispecific protein inhibitors to inhibit VEGF activity.

[0234] The efficacy of VEGF inhibition of the bispecific protein inhibitors comprising anti-VEGF Fab was assessed using VEGF bioassay kit utilizing Bio-Glo luciferase assay system from Promega (USA). Engineered HEK293 cells expressing both KDR (VEGF receptor) and a luminescent reporter under NFAT control enable sensitive detection of VEGF-induced signaling. Inhibition of VEGF binding to KDR by anti-VEGF antibodies leads to decreased signaling activation and resulted luminescence. The assay was performed according to the manufacturer's instruction. KDR/NFAT-RE HEK298 cells (40,000 cells per reaction) were incubated with serial dilution of bispecific protein inhibitors comprising anti-VEGF Fab and complement regulators (10 to 0.016 nM) in the presence of recombinant VEGF165 (0.5 nM, R&D Systems, USA). After a 6-hour incubation in 5% CO2 humidified incubator, equal volume of Bio-Glo reagent was added and luminescent was measured by iD3 plate reader (Molecular Devices). Average relative luminescence units (RLUs) with standard error for replicates were plotted against log concentrations of bispecific protein inhibitors using GraphPad Prism. The plots were fitted with a non-linear regression curve (**Figure 18**). The IC_{50} values of bispecific protein inhibitors were around 50% of the bivalent anti-VEGF Ab. Therefore, we can conclude that fusing anti-VEGF Fab to complement regulators does not affect VEGF inhibition efficacy of the bispecific protein inhibitors.

[0235] **Figure 18** is a graph of the anti-VEGF activity of bispecific inhibitors and anti-VEGF Ab in a VEGF cell reporter assay. Anti-VEGF Ab (bivalent) is used as control (dark circle). KC032 (open diamond), KC033 (open square), and KC035 (open triangle) contain monovalent anti-VEGF Fab fused to engineered complement regulators.

[0236] Thus, the results show that fusing anti-VEGF Fab to complement regulators does not affect the VEGF inhibition efficacy of embodiments of the bispecific protein inhibitors.

***Example 9**: Development of enhanced complement regulators for the treatment of Geographic Atrophy*

[0237] Geographic atrophy (GA) is a complex multifactorial disease. Multiple studies indicate complement cascade overactivation as a key contributor to the onset and progression of GA. Complement activation is downregulated by protease and associated cofactor proteins. The "recyclable" nature and domain modularity of these cofactor proteins make them promising targets for therapeutic development. Here, we present a novel class of

potent complement inhibitors or regulators based on protein domains from cofactor proteins Factor H (FH) and complement receptor 1 (CR1). These complement inhibitors or regulators are fusion proteins that are modular and can be combined or covalently linked with other therapeutic proteins to generate multispecific drugs or multispecific protein inhibitors.

[0238] **Methods:** Novel cofactor proteins or protein inhibitors of the complement pathway were designed by combining domains of FH and CR1. Binding affinity and biological activity were investigated using surface plasmon resonance (SPR), complement cleavage activity, and hemolysis assays for alternative and classical pathways. The modularity of the engineered protein inhibitors of the complement pathway was assessed by fusing them with an anti-VEGF Fab. These engineered proteins are bispecific protein inhibitors, and they were investigated for their affinities for C3b and VEGF-A165 using SPR, and inhibitory activities using hemolysis assay and VEGF cell reporter bioassay.

[0239] **Results:** The engineered bispecific protein inhibitors exhibited enhanced binding to C3b compared to the parent cofactors and promoted cleavage of C3b by FI into iC3b and further downstream degradation products C3c and C3dg. Hemolysis assays showed these bispecific protein inhibitors were up to 140-fold more effective than native human FH in inhibiting the alternative pathway. These molecules also showed potent inhibitory activity for the classical pathway. Fusion of these complement inhibitors or regulators to anti-VEGF Fab demonstrated that engineered complement regulators can be used as modules to generate multi-specific molecules. This bispecific protein inhibitor exhibited tight binding to both C3b and VEGF-A165, and inhibitory activities were comparable to their respective monospecific moieties.

[0240] **Conclusion:** We have engineered potent complement regulators by combining domains of FH and CR1. The novel complement regulators are modular and can be linked to other protein modules, such as antibody fragments to generate multispecific drugs.

Example 10. *Construction and expression of complement regulator fusion proteins*

[0241] This non-limiting example describes the development and expression of embodiments of the protein inhibitors.

[0242] *Construction*: The engineered complement inhibitors were synthesized through the fusion of DNA fragments encoding complement control protein (CCP) domains.

The functional CCP domains of CR1 are fused to complement regulatory domains that specifically bind to C3b and/or glycosaminoglycans (GAGs) present on host cell surfaces.

[0243] The amino acid sequence of human CFHR3 (accession no. Q02985) and human CFHR5 (accession no. Q9BXR6) are listed in **Table 8**.

[0244] The amino acid sequences of domains used in these constructs from CR1, FH, complement factor H-related protein 3 (CFHR3), and complement factor H-related protein 5 (CFHR5), are shown in **Table 9**.

[0245] The combinations of CR1 CCP domains in the complement regulator domains of the engineered complement inhibitors are shown in **Table 1.5** and **Table 10.1**. Numbers in parenthesis following abbreviations of protein names indicate the numbers of the CCP domains included in the CR1 CCP combination domain. For example, CR1(1-4) indicates use of CCP domains 1 to 4 of CR1. The combination of FH, CFHR3, CFHR5 domains included in the complement regulator domains of the protein inhibitors are shown in **Table 10.2**.

[0246] Glycine-Serine linkers (Sequences in **Table 1.7**) were used to covalently link the various FH CCP domain combinations, CFHR3 CCP domains, and CFHR5 CCP domains as delineated in **Table 1.8** and **Table 10.2**, to the C-termini of the CR1 combination domains specified in **Table 1.5** and **Table 10.1**. Additionally, CFHR3 and CFHR5 CCP domains, which also demonstrated GAG binding properties (**Table 10.2**) were covalently linked to the C-termini of the CR1 combination domains (**Table 1.5** and **Table 10.1**) through glycine-serine linker sequences. Such covalent linkage is designed to add additional functional attributes to the regulatory inhibitors.

[0247] The complement regulators were also fused to Fab or Fc of IgG1 using a double repeat of a Gly-Gly-Gly-Gly-Ser linker (GS(5) (SEQ ID NO: 036) to enhance their functionality. Complement regulators are strategically fused with various moieties to facilitate controlled assembly as either monomers or dimers. This approach enables the design and development of tailored complement regulators to address specific therapeutic applications. The sequences of the IgG heavy chain, light chain and Fc component utilized in this invention are provided in **Table 1.10** and **Table 12**.

[0248] The amino acid sequence of an embodiment of a protein inhibitor is shown in **Table 14** (KC041).

[0249] *Expression.* **Table 11** presents the sequence composition of protein inhibitors expressed and tested in this study. The codon optimized DNA sequences were cloned into the pCDNA3.3 backbone. Human embryonic kidney (HEK293) cells or Chinese hamster ovary (CHO) cells were transfected with a construct plasmid. The secreted inhibitors were harvested, and each inhibitor was purified using MabSelect PrismA column (Cytiva, USA) using AKTA Pure (Cytiva, USA). Eluted samples were buffer exchanged to phosphate buffered saline (PBS) using Vivaspin® 20 30 kDa MWCO (Cytiva, USA). A 2 ug sample of KC041 (lane 5), KC043 (lane 2), KC043 (lane 3), and KC044 (lane 4) was loaded onto an SDS-PAGE gel under non-reducing condition (**Figure 20**). The other complement inhibitors listed in **Table 11** were constructed, expressed, and purified in a similar manner.

[0250] The present disclosure demonstrated that embodiments of the protein inhibitors are capable of being expressed and purified.

Example 11. *Inhibition of the alternative complement pathway by engineered complement inhibitors*

[0251] This non-limiting example evaluates the ability of embodiments of the bispecific protein inhibitors to inhibit the alternative pathway (AP).

[0252] The functional activity of the engineered protein inhibitors in suppressing complement activation was assessed using hemolytic assays specific to the alternative pathway. The assays were conducted to evaluate the efficacy of the engineered inhibitors in preventing complement-mediated lysis of unsensitized sheep erythrocytes (Es, CompTech, USA), thereby demonstrating their ability to modulate alternative pathway activation.

[0253] Hemolysis of Es cells is triggered in the presence of FH-depleted serum mixed with $MgCl_2$ and EGTA (MgEGTA). The Es cells were washed three times with GVB° buffer (0.1 % gelatin, 5 mM Veronal, 145 mM $MgCl_2$, 0.025 % NaN_3, pH 7.3, CompTech, USA) at 4 °C. The amount of FH-depleted human serum (Factor H-Dpl, CompTech, USA) required to lyse 50% of 2 x 10^8 Es cells at 37 °C for 30 minutes was determined. The protein inhibitors were serially diluted in FH-depleted human serum to concentrations between 0.01 nM and 300 nM, then added to the washed Es cells. To trigger hemolysis, 5 mM MgEGTA (CompTech, USA) was added. The same amount of Es cells mixed with FH-depleted human serum mixture without MgEGTA was used as background subtraction. A

hundred percent hemolysis was defined by mixing Es cells, FH depleted human serum, and 5 mM MgEGTA. The reactions were incubated at 37 °C for 30 minutes, then quenched by adding the equal volume of ice chilled GVBE (0.1 % gelatin, 5 mM Veronal, 145 mM NaCl, 0.025 % NaN_3, 10 mM EDTA, pH 7.3, CompTech, USA). The reaction plates were centrifuged at 800 x g for 10 minutes. Supernatant was transferred to a 96-well clear bottom plate and absorbance at 412 nm was recorded by iD3 plate reader (Molecular Devices). Average % lysis values with standard error for replicates were plotted against the log concentration of inhibitors and fitted with a non-linear regression curve.

[0254] The results are shown in **Figure 21** and **Table 15**.

Table 15: Inhibition of the alternative pathway of complement activated hemolysis by engineered complement inhibitors.

Molecule ID	AP IC_{50} [nM]
KC036	0.28
KC037	0.83
KC039	0.99
KC040	1.14
KC045	0.16
KC042	0.35
KC046	0.24
KC049	0.69
KC051	0.24

[0255] **Figure 21:** Inhibition of alternative pathways by complement inhibitor modules fused to IgG Fc fragment. The concentration listed next to the molecule ID in the figure represents the IC50 value determined from the hemolysis assay. The IC50 values of FH and Eculizumab have not been determined.

[0256] The results indicated that embodiments of the protein inhibitors inhibit the activation of the alternative pathway.

Example 12. *Inhibition of the classical complement pathway by engineered complement inhibitors*

[0257] This non-limiting example evaluates the ability of embodiments of the bispecific protein inhibitors to inhibit the classical pathway (CP).

[0258] The hemolytic assay for the classical pathway (CH50) was employed to evaluate inhibition of classical complement pathway by the engineered complement inhibitors.

[0259] For the assay, sheep erythrocytes coated with rabbit anti-sheep erythrocyte antiserum (EA, CompTech, USA) were washed with ice cold GVB++ buffer (0.1 % gelatin, 5 mM Veronal, 145 mM NaCl, 0.15 mM CaCl2, 0.5 mM MgCl2, 0.025 % NaN3, pH 7.3, CompTech) three times. The protein inhibitors were serially diluted in normal human serum (NHS, CompTech) to concentrations between 0.01 nM and 300 nM (for FH, 1200 nM), then added to the equal volume of EA. A mixture of NHS in GVB++ buffer and a mixture of EA mixed with NHS in GVB++ buffer were prepared as background and 100 % lysis condition, respectively. The reactions were incubated at 37 °C for 30 minutes, then quenched by adding the same volume of ice chilled GVBE. The reaction plates were centrifuged at 800 x g for 10 minutes. Supernatant was transferred to a 96-well clear bottom plate and absorbance at 412 nm was measured by iD3 plate reader (Molecular Devices). Average % lysis values with standard error for replicates were plotted against log concentration of protein inhibitors and fitted with a non-linear regression curve using GraphPad prism V10.0.

[0260] Results demonstrated that embodiments of the protein inhibitors also inhibit the classical pathway. IC50 values of various protein inhibitors for classical pathway activation are shown in **Table 16** and **Figure 22**.

Table 16. Inhibition of the classical pathway of complement activated hemolysis by engineered complement inhibitors.

Molecule ID	CP IC_{50} [nM]
KC036	0.10
KC037	0.13
KC039	4.10
KC040	1.15
KC044	5.24
KC045	3.74
KC046	0.45
KC047	3.69
KC049	1.27

KC051	0.26

[0261] **Figure 22:** Inhibition of the classical pathway by complement inhibitor modules fused to the IgG Fc fragment. The concentration listed next to the molecule ID represents the IC50 value determined from the hemolysis assay.

[0262] The results demonstrate that embodiments of the protein inhibitors suppress the classical complement pathway.

Example 13. *Cofactor activity assay in CFI-mediated C3b degradation*

[0263] This non-limiting example assesses the role of embodiments of the protein inhibitors as cofactors in facilitating CFI-mediated C3b degradation.

[0264] The reaction mixture of C3b (0.6 mM), FI (0.05 mM), and either sCR1(R&D Systems) or the protein inhibitors at 25 or 12.5 nM in 20 mM HEPES buffer, 150 mM NaCl2, 2 mM MgCl2 were prepared on ice, followed by incubation at 37 °C for 30 minutes. The negative control was prepared containing the same concentration of C3b and protein inhibitors without the FI and incubated at 37 °C for 0 or 30 minutes. The reactions were stopped by adding NuPAGE™ LDS Sample buffer and NuPAGE™ Sample Reducing Agent (Thermo Fisher Scientific, USA) followed by incubation at 90 °C for 5 minutes, immediately. Samples were analyzed for degradation product of C3b alpha fragments on a NovexTM Tris-Glycine mini gel stained with SimplyBlue™ SafeStain (Thermo Fisher Scientific, USA).

[0265] **Figure 23** shows a cofactor assay of C3b cleavage by Factor I (FI). In the absence of FI, neither sCR1 nor KC041 generated any C3b cleavage fragments after 30 minutes of incubation. The protein inhibitor KC041 demonstrated greater cofactor activity, cleaving the C3b alpha chain into fragments of 68 kDa, 43 kDa, which were further degraded into 40 kDa and C3dg fragments within 30 minutes. In comparison sCR1 produced 68 kDa and 43 kDa fragments. (**Figure 23**).

[0215] The results demonstrate that embodiments of the protein inhibitors act as co-factors of FI-mediated C3b cleavage.

Example 14. *Binding kinetics of engineered complement inhibitors for complement protein C3b*

[0266] This non-limiting example assesses the binding kinetics of embodiments of the protein inhibitors for the complement protein C3b.

[0267] A surface plasmon resonance (SPR)-based assay was used to determine the binding kinetics of the protein inhibitors for the complement protein C3b using Biacore™ T200 system (Cytiva, USA).

[0268] Flow cells 2, 3, and 4 of the CM5 chip (Cytiva, USA) were coated with human C3b (CompTech, USA) with ligand levels ranging between 300 to 400 response unit (RU) using an amine coupling protocol according to the manufacturer's instruction. The protein inhibitors were diluted in HBS-EP+ running buffer in concentration series ranging between 0-50 nM and injected over surface bound C3b at a flow rate of 30 µL/min for 90 seconds followed by dissociation in running buffer for 180 seconds. Binding kinetics were analyzed by Biacore™ T200 evaluation software. All sensorgrams were subtracted with double reference and fitted using Langmuir 1:1 binding model in the evaluation software (Cytiva, USA). Protein inhibitors represented increased binding affinity. Sensorgrams of selected protein inhibitors are shown in **Figures 24A-24B.**

[0269] **Figures 24A and 24B:** C3b binding kinetics of complement regulators. Surface plasmon resonance (SPR) sensorgrams for C3b binding of KC038 (FIG. 24A), KC041 (FIG. 24B). The equilibrium dissociation constant KD is shown in the panes.

[0270] In an alternative assay format, the inhibitors were diluted to 5 nM in HBS-EP+ buffer and captured on Protein A sensor chip (Cytiva, MA, USA) at 10 µL/min for 20 seconds. The ligand level of the protein regulators ranged from 35 to 70 RU levels. Complement protein C3b (Cytiva) was diluted in HBS-EP+ running buffer in concentration series ranging between 0-500 nM. Increasing concentrations of the analytes were then injected at 30 µL/min for 60 seconds followed by a 300 second dissociation step. All sensorgrams were double reference subtracted and fitted using Langmuir 1:1 binding model in Biacore™ T200 evaluation software (Cytiva, USA). Sensorgrams and Kinetic values are shown in **Figures 25A-25B.**

[0271] **Figures 25A and 25B:** C3b binding kinetics of Fc fused complement regulators, captured on Protein A chip. SPR sensorgrams of C3b binding for KC038 (FIG. 25A), KC041 (FIG. 25B). The equilibrium dissociation constant KD and ligand levels are shown in the panes.

[0272] The results demonstrate that embodiments of the protein inhibitors tested in the surface plasmon resonance (SPR)-based assay have affinity for the complement protein C3b.

Example 15. *Decay acceleration activity assay for engineered complement inhibitors*

[0273] This non-limiting example evaluates the functional characteristics of embodiments of the protein inhibitors and their decay accelerating activity (DAA) on the C3 convertase using SPR.

[0274] C3b molecules were immobilized on a CM5 sensor chip (Cytiva) using an amine coupling protocol as described above. A mixture comprising 600 nM Factor B (FB) and 100 nM Factor D (FD) in HBS-P+ buffer was injected for 120 seconds over the C3b-coated surface to generate the convertase complex on the chip (**Figure 26, step 1**). Following a dissociation phase of 30 seconds (**Figure 26, step 2**), an analyte of either FH, KC041, or KC046 was injected for 60 seconds (**Figure 26, step 3**). The regeneration of the chip surface is achieved by injecting a 1 M NaCl in acetate buffer pH 4. For a comparative assessment of the decay acceleration activity (DAA) response, the SPR-binding signals of the analytes measured in the absence of the convertase were subtracted from the corresponding signals obtained during convertase decay cycle.

[0275] The inhibitor, KC041 (SEQ ID. NO: 105), exhibited minimal decay activity, whereas FH destabilized the convertase complex. The inhibitor KC046 (Seq. ID. No. 110) demonstrated the highest potency in promoting decay acceleration activity (**Figure 26**).

[0276] **Figure 26:** DAA activity assessment on C3 convertase using SPR.

[0277] The engineered protein inhibitors exhibit binding affinity to C3b, as demonstrated in assays utilizing both immobilized C3b and C3b in solution.

[0278] The results demonstrate that embodiments of the protein inhibitors promote decay of the convertase complex.

Example 16. *Expression of bispecific inhibitors*

[0279] This non-limiting example describes the development and expression of embodiments of bispecific protein inhibitors.

[0280] **Tables 13.1 and 13.2** provide the sequence composition of the bispecific inhibitors, including complement regulator and Fab fusion inhibitors, that were expressed and evaluated in this study. The codon optimized DNA sequences were cloned into the pCDNA3.3 backbone. The human embryonic kidney (HEK293) cells or Chinese hamster ovary (CHO) cells were transfected with the construct plasmids. The secreted inhibitors were harvested, and each inhibitor was purified using CaptureSelect CH1-XL (Thermo Fisher, USA) using AKTA Pure (Cytiva, USA). Eluted samples were buffer exchanged as described above. This study demonstrated that the engineered bispecific inhibitors can be expressed and purified**. Figure 27** illustrates an example of SDS-PAGE gel analysis of purified bispecific inhibitors, KC048 and KC049.

[0281] **Figure 27:** SDS-PAGE gel of purified fusion proteins. Purified fusion protein KC048 under non-reducing condition (lane 2) and reducing conditions (lane 4) ; KC049 under non-reducing condition (lane 3) and reducing conditions (lane 5).

Example 17. *Inhibition of the alternative complement pathway by bispecific inhibitors*

[0282] This non-limiting example examines the inhibitory effect of embodiments of bispecific inhibitors on the alternative pathway (AP) using the assay method described in Example 11.

[0283] The bispecific inhibitors were serially diluted in FH-depleted human serum (CompTech, USA) to concentrations between 0.01 nM and 300 nM, then added to the Es cells pre-washed with GVB° buffer (0.1 % gelatin, 5 mM Veronal, 145 mM $MgCl_2$, 0.025 % NaN_3, pH 7.3, CompTech, USA). MgEGTA solution was added to trigger complement activation. The reactions were incubated at 37 °C for 30 minutes, then quenched by adding the equal volume of ice chilled GVBE with EDTA (0.1 % gelatin, 5 mM Veronal, 145 mM NaCl, 0.025 % NaN_3, 10 mM EDTA, pH 7.3, CompTech, USA). Average % lysis values with standard error for replicates were plotted against the log concentration of inhibitors and fitted with a non-linear regression curve. The results are shown in **Figure 28** and in **Table 17**. The results demonstrated that the embodiments of bispecific inhibitors effectively suppressed the activation of alternative pathway, achieving inhibition comparable to that of Fc-fused inhibitor (KC041).

Table 17. Alternative pathway inhibition of the complement activated hemolysis.

Molecule ID	AP IC_{50} [nM]
KC049	0.69
KC051	0.24
KC054	0.68
KC056	0.42

[0284] **Figure 28:** Inhibition of alternative pathways by bispecific inhibitors in comparison to Fc-fusion inhibitor KC041. The concentration listed next to the molecule ID represents the IC_{50} value determined from the hemolysis assay.

[0285] The results indicate that embodiments of the protein inhibitors inhibit the activation of the alternative pathway (AP).

Example 18. *Inhibition of the classical complement pathway by bispecific inhibitors*

[0286] This non-limiting example examines the inhibitory effects of embodiments of the bispecific inhibitors on the classical pathway using the assay method described in Example 12.

[0287] The assay was performed in GVB^{++} buffer. The protein inhibitors were serially diluted in normal human serum (NHS, CompTech) to concentrations between 0.01 nM and 300 nM, then added to the equal volume of Ab-sensitized sheep erythrocyte (EA). The reactions were incubated at 37 °C for 30 minutes, then quenched by adding the same volume of ice chilled GVBE containing EDTA. Average % lysis values with standard errors for replicates were plotted against the log concentration of protein inhibitors and fitted with a non-linear regression curve (**Figure 29**). Results demonstrated that the bispecific inhibitors also inhibit the classical pathway effectively. IC_{50} values of various protein inhibitors for classical pathway activation are shown in **Table 18.**

Table 18. Classical pathway inhibition of the complement activated hemolysis

Molecule ID	CP IC_{50} [nM]
KC049	0.26
KC051	0.24
KC053	0.81
KC054	0.22

KC055	0.25
KC056	0.42

[0288] Fig. 29 shows inhibition of classical pathways by bispecific inhibitors fused to IgG Fab.

[0289] The results demonstrate that embodiments of the bispecific inhibitors effectively suppress the classical complement pathways.

Example 19. *Cofactor activity assay of bispecific inhibitors in CFI-mediated C3b and C4b degradation*

[0290] This non-limiting example examines the cofactor activity of embodiments of the bispecific inhibitors in CFI-mediated C3b and C4b degradation.

[0291] To assess the cofactor activity of bispecific inhibitors, time course cofactor assay was performed, similar to the assay described in Example 11. The reaction mixture of C3b (0.6 mM), FI (0.05 mM), and the protein inhibitors at 10 nM in HEPES buffer, 50 mM $NaCl_2$, 2 mM $MgCl_2$ were prepared on ice and aliquoted into 20 μL prior to incubation at 37 °C for an increasing amount of time (1, 5, 10, 30, 60, and 90 min). The negative controls, containing the same concentration of C3b and FI, were incubated at 37 °C for either 1 minute or 90 minutes. Samples were analyzed for degradation product of C3b alpha fragments on a NovexTM Tris-Glycine mini gel stained with SimplyBlue™ SafeStain. The samples without bispecific inhibitors did not produce any degraded fragments while the protein inhibitor KC049 and KC050 cleaved C3b alpha fragment into 68 kDa, 43 kDa, 40 kDa and C3dg fragments (**Figure 30**).

[0292] Cofactor activity assay for C4b degradation was carried out as described above, except replacing C3b to C4b (0.6 μM) and incubation for 60 and 90 minutes. The negative control, C4b and FI mixture, did not generate any degraded fragments at 90 min incubation. However, the bispecific inhibitors, KC049 and KC050, acted as a cofactor for FI and generated C4d (45 kDa) and 25 kDa fragments (**Figure 31**, lanes 4-7).

[0293] **Figure 31.** Cofactor assay of C4b cleavage by FI.

[0294] Thus, the results show that embodiments of the bispecific inhibitors tested in these assays function as effective cofactors for CFI-mediated C3b and C4b degradation.

Example 20. *Decay acceleration activity assay for bispecific inhibitors*

[0295] This non-limiting example examines the decay accelerating activity (DAA) of the bispecific inhibitors using SPR-based assay as described in Example 15.

[0296] A mixture of 600 nM FB and FD was flowed over the C3b-coated surface for 120 seconds (**Figure 32A, step 1**), facilitating convertase complex formation on the chip. Following a dissociation phase of 30 seconds (**Figure32A, step 2**), an analyte of either KC046, KC050, KC052, or buffer as negative control was injected for 60 seconds (**Figure 32A, step 3**). For a comparative assessment of the DDA response, the SPR-binding signals of the analytes measured in the absence of the convertase were subtracted from the corresponding signals obtained during convertase decay cycle. While the C3 convertase decays intrinsically at a slow rate, exposure to bispecific inhibitors resulted in a significant acceleration of C3 convertase decay (**Figure 32A**). This observed increase in decay rate demonstrates enhanced decay acceleration activity, thereby confirming the efficacy of the bispecific inhibitors in modulating the stability of C3 convertases.

[0297] The decay accelerating activity was also evaluated in the presence of C3 and C5 convertase stabilizing agent, Factor P (FP, Properdin). To create C3 convertase : FP complex, 5 nM of FP in HBS-P+ buffer was injected for 30 seconds after C3 convertase complex is generated (**Figure 32B, step 3**). After letting it dissociate for 15 seconds (**Figure32B, step 4**), an analyte of either KC046, KC050, KC052, or buffer as negative control was injected for 60 seconds (**Figure 32B, step 5**). For a comparative assessment of the DAA response, the SPR-binding signals of the analytes measured in the absence of the convertase and FP were subtracted from the corresponding signals obtained during convertase decay cycle. A slight reduction in the dissociation rate of the C3 convertase was observed, demonstrating that Factor P plays a role in stabilizing the C3 convertase complex. Upon contact with bispecific inhibitors, the C3 convertase complex rapidly dissociated (**Figure 32B**).

[0298] **Figure 32**: DAA assessment of C3 convertase by SPR. (A) Sensorgrams of DAA of bispecific inhibitors. (B) Sensorgram of DAA in the presence of Factor P (FP).

[0299] This demonstrates that embodiments of the bispecific inhibitors possess decay accelerating activity (DAA) with respect to the C3 convertase complex even in the presence of FP.

Example 21. *Competitive ELISA assay for inhibition of IL-6/IL-6R alpha binding interaction by bispecific inhibitors*

[0300] This non-limiting example examines the ability of some embodiments of the bispecific inhibitors to inhibit the IL-6/IL6Rα interaction.

[0301] The inhibitory capacity of anti-IL-6-Fab bispecific inhibitors in preventing IL-6 from binding to the IL-6Rα receptor was evaluated using a competitive ELISA with the Human IL-6/IL-6Rα Complex DuoSet® ELISA (DY8139-05, R&D Systems). Nunc MaxiSorp flat-bottom 96-well plates (Thermo Fisher, USA) were coated with 50 uL of IL-6/IL-6Rα DuoSet Capture antibody at 2 μg/mL in PBS (pH7.4) and incubated overnight at 4 °C. Following the wash steps with 1 x PBST (0.05% Tween 20 in PBS), the plates were blocked with 2% BSA in PBS buffer for 2 hours at room temperature. A mixture of IL-6 (1 nM) and IL-6Rα (6 nM) (227-SR-025/CF, R&D Systems) was prepared and incubated with either parental anti-IL-6 Fab (SEQ ID NOs: 112 and 113), anti-IL-6 antibody (SEQ ID NOs: 114 and 113), or bispecific inhibitors fused to anti-IL-6-Fab. The inhibitors were serially diluted threefold from 300 nM to 5 pM. A 50 μL aliquot of the IL-6/IL-6Rα/bispecific-Fab mixture was added to the blocked plates and incubated for 1 hour at room temperature. Unbound IL-6/bispecific-Fab complexes were removed by three washes with 1x PBST. The IL-6/IL-6Rα complex captured on the DuoSet capture antibody was detected by incubating with 50 μL of IL-1β DuoSet detection antibody, diluted to 0.5 μg/mL in blocking buffer, for 1 hour. After washing, the plates were incubated for 30 minutes with 50 μL of DuoSet Streptavidin-HRP diluted 1:40 in blocking buffer. The binding signal was developed using SureBlue™ TMB substrate (Cat. #52-00-02, KPL), and the reaction was terminated by the addition of 2 N sulfuric acid. Luminescence values corresponding to varying concentrations of anti-IL-6 antibody, anti-IL-6 Fab, and bispecific inhibitors were recorded using iD3 plate reader (Molecular Devices). These values were plotted against the respective concentrations of the inhibitors using GraphPad Prism v10.0 to generate an inhibition curve, thereby quantifying the inhibitory activity of the bispecific inhibitors. **Figure 33** demonstrates that the bispecific inhibitors, KC050 and KC052, inhibits IL-6 binding to IL-6Rα with efficiency comparable to their parental anti-IL-6 Fab.

[0302] **Figure 33** is a competitive ELISA assay demonstrating inhibition of IL-6 binding to IL-6Rα by bispecific inhibitors in an IL-6/IL-6Rα DuoSet ELISA. The concentration listed next to the molecule ID represents the IC_{50} value.

[0303] Thus, these results demonstrate that embodiments of the bispecific inhibitors inhibit IL-6 binding to IL-6Rα comparably to their parental anti-IL-6 Fab.

Example 21: *Development of Potent Bispecific Inhibitors Targeting Complement Pathways and Cytokines for the Treatment of Geographic Atrophy*

[0304] **Purpose**: Geographic atrophy (GA) is characterized by chronic inflammation and dysregulated immune responses. Elevated levels of cytokines and an unbalanced complement response are known to be key drivers of disease progression. Here, we present bispecific inhibitors combining complement regulator modules with anti-cytokine antibody fragments, demonstrating significant downregulation of complement activation and inhibition of cytokine activity.

[0305] **Methods**: Enhanced complement regulators were engineered by combining domains from complement receptor 1 (CR1) and other complement regulators that bind to either C3b and/or heparin. These complement regulator modules were fused to anti-cytokine Fab fragments to generate bispecific inhibitors. Binding affinities to C3b and cytokines, as well as decay accelerating activity (DAA) were assessed by surface plasmon resonance (SPR). The function of complement regulators was assessed by Factor I (FI) cofactor assay as well as hemolysis assays. Cytokine inhibition was quantified using competitive ELISA.

[0306] **Results**: The bispecific inhibitors demonstrated tight binding to C3b and facilitated C3b degradation by FI into iC3b and further downstream degradation products, C3c and C3dg. These inhibitors also exhibited potent DAA activity. In the hemolysis assay, the inhibitors were effective in blocking both the alternative pathway and classical pathways. The bispecific molecule inhibits cytokine in potency assays.

[0307] **Conclusions**: We have developed highly potent, bispecific inhibitors that were effective in downregulating both the alternative and classical complement pathways while suppressing targeted cytokine activities. These inhibitors offer a promising and comprehensive mechanism of action that addresses multiple pathological processes. By

addressing key drivers of disease progression, these inhibitors offer significant potential to achieve a more effective treatment approach for GA.

[0308] All patent filings, websites, other publications, accession numbers and the like cited above or below are incorporated by reference in their entirety for all purposes to the same extent as if each individual item were specifically and individually indicated to be so incorporated by reference. If different versions of a sequence are associated with an accession number at different times, the version associated with the accession number at the effective filing date of this application is meant. The effective filing date means the earlier of the actual filing date or filing date of a priority application referring to the accession number if applicable. Likewise if different versions of a publication, website or the like are published at different times, the version most recently published at the effective filing date of the application is meant unless otherwise indicated. Any feature, step, element, embodiment, or aspect disclosed herein can be used in combination with any other unless specifically indicated otherwise. Although some embodiments have been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A protein inhibitor comprising a first polypeptide, the first polypeptide comprising:

an oligomerization domain; and

a complement regulator covalently linked to the oligomerization domain, the complement regulator comprising:

a Complement Receptor 1 (CR1) complement control protein (CCP) domain comprising at least one C3b and/or C4b binding domain; and

a Factor H (FH), a complement factor H related 3 (CFHR3), or a complement factor H related 5 (CFHR5) CCP domain.

2. The protein inhibitor of claim 1, wherein the inhibitor comprises:

the first polypeptide; and

a second polypeptide comprising:

a second oligomerization domain; and

a second complement regulator.

3. The protein inhibitor of claim 2, wherein the inhibitor is a homodimer.

4. The protein inhibitor of claim 2, wherein the inhibitor is a heterodimer.

5. The protein inhibitor of claim 1, that is bispecific.

6. The protein inhibitor of claim 1, wherein the oligomerization domain is selected from a Fc domain, a Fab fragment, or a complement factor H-related protein domain.

7. The protein inhibitor of claim 1, wherein the oligomerization domain is selected from the group consisting of: an Fc domain that is SEQ ID NO: 46; an anti-VEGF-VH-CH1 domain that is SEQ ID NO: 47; an anti-VEGF-VL-CL domain that is SEQ ID NO: 48; an anti-VEGF-HC that is SEQ ID NO:49; a CFHR1(1-2) domain that is SEQ ID NO: 42; a CFHR5(1-2) domain that is SEQ ID NO: 43; an anti-IL-6-VH-CH1 domain that is SEQ ID NO: 112; an

anti-IL-6-VL-VL domain that is SEQ ID NO: 113; or an anti-IL-6-CH domain that is SEQ ID NO: 114.

8. The protein inhibitor of claim 7, wherein the oligomerization domain comprises an Fc domain comprising SEQ ID NO: 46.

9. The protein inhibitor of claim 7, wherein the oligomerization domain comprises an anti-VEGF-VH-CH1 comprising SEQ ID NO: 47.

10. The protein inhibitor of claim 7, wherein the oligomerization domain comprises an anti-VEGF-VL-CL comprising SEQ ID NO: 48.

11. The protein inhibitor of claim 7, wherein the oligomerization domain comprises an anti-VEGF-HC comprising SEQ ID NO: 49.

12. The protein inhibitor of claim 7, wherein the oligomerization domain comprises an anti-IL-6-VH-CH1 comprising SEQ ID NO: 112.

13. The protein inhibitor of claim 7, wherein the oligomerization domain comprises an anti-IL-6-LC comprising SEQ ID NO: 113.

14. The protein inhibitor of claim 7, wherein the oligomerization domain comprises an anti-IL-6-HC comprising SEQ ID NO: 114.

15. The protein inhibitor of claim 1, wherein the oligomerization domain is covalently linked to the complement regulator via a glycine-serine linker selected from sequences comprising SEQ ID Nos: 32-36.

16. The protein inhibitor of claim 15, wherein the glycine-serine linker comprises SEQ ID NO: 34.

17. The protein inhibitor of claim 1, wherein the complement regulator is covalently linked to the oligomerization domain via GGGGSGGGGS (SEQ ID NO: 36).

18. The protein inhibitor of claim 8, wherein the CR1 CCP domain comprises:
CR1 CCP domains 1-3, comprising SEQ ID NO: 22;
CR1 CCP domains 8-10, comprising SEQ ID NO: 24;
CR1 CCP domains 15-17, comprising SEQ ID NO: 25;
CR1 CCP domains 1-4 and 15-17, comprising SEQ ID NO: 26; or
CR1 CCP domains 1-3 and 15-17, comprising SEQ ID NO: 137.

19. The protein inhibitor of claim 18, wherein factor H CCP domain 4 is covalently linked to the C-terminus of the CR1 CCP domain.

20. The protein inhibitor of claim 8, wherein the CR1 CCP domain comprises CR1 CCP domains 1-4 comprising SEQ ID NO: 23; CR1 CCP domains 15-18 comprising SEQ ID NO: 95; or CR1 CCP domains 1-4 and 15-18 comprising SEQ ID NO: 96.

21. The protein inhibitor of any one of claims 18-20, wherein the FH CCP domain comprises FH CCP domain 7 comprising SEQ ID NO: 18; FH CCP domains 6-7 comprising SEQ ID NO: 97; FH CCP domains 18-20 comprising SEQ ID NO: 37; FH CCP domains 18-19 and 7 comprising SEQ ID NO: 38; FH CCP domains 18-19 comprising SEQ ID NO: 39; FH CCP domains 19 and 7 comprising SEQ ID NO: 40; or FH CCP domains 19-20 comprising SEQ ID NO: 41.

22. The protein inhibitor of claim 1, wherein the complement regulator comprises factor H CCP domain 4 covalently linked to the C-terminus of a subgroup of CR1 CCP domains.

23. The protein inhibitor of claim 22, wherein the CR1 CCP domains comprise CR1 CCP domains 1-3, comprising SEQ ID NO: 22.

24. The protein inhibitor of claim 22, wherein the CR1 CCP domains comprise CR1 CCP domains 8-10, comprising SEQ ID NO: 24.

25. The protein inhibitor of claim 22, wherein the CR1 CCP domains comprise CR1 CCP domains 15-17, comprising SEQ ID NO: 25.

26. The protein inhibitor of claim 22, wherein the CR1 CCP domains comprise CR1 CCP domains 1-4, and 15-17, comprising SEQ ID NO: 26.

27. The protein inhibitor of claim 22, wherein the CR1 CCP domains comprise CR1 CCP domains 1-3, and 15-17, comprising SEQ ID NO: 137.

28. The protein inhibitor of any of claims 23-25, wherein a subgroup of FH CCP domains selected from SEQ ID NOs: 18-21 are covalently linked to the C terminus of FH CCP domain 4 via a glycine-serine linker.

29. The protein inhibitor of claim 28, wherein the glycine serine linker comprises GGGGS (SEQ ID NO: 33).

30. The protein inhibitor of claim 28, wherein the glycine serine linker comprises GSGGGGS (SEQ ID NO: 34).

31. The protein inhibitor of claim 28, wherein the glycine serine linker comprises GSGGGGSGGGGS (SEQ ID NO: 35).

32. The protein inhibitor of claim 28, wherein the glycine serine linker comprises GGGGSGGGGS (SEQ ID NO: 36).

33. The protein inhibitor of claim 28, wherein the FH CCP domains comprise FH CCP domains 18-20 comprising SEQ ID NO: 37.

34. The protein inhibitor of claim 28, wherein the FH CCP domains comprise FH CCP domains 18-19 and 7 comprising SEQ ID NO: 38.

35. The protein inhibitor of claim 28, wherein the FH CCP domains comprise FH CCP domains 18-19 comprising SEQ ID NO: 39.

36. The protein inhibitor of claim 28, wherein the FH CCP domains comprise FH CCP domains 19 and 7 comprising SEQ ID NO: 40.

37. The protein inhibitor of claim 28, wherein the FH CCP domains comprise FH CCP domains 19-20 comprising SEQ ID NO: 41.

38. A protein inhibitor comprising:

a first polypeptide comprising:

SEQ ID NO: 50; and

a second polypeptide comprising:

SEQ ID NO: 50; and

wherein the first polypeptide is conjugated to the second polypeptide via disulfide bonds.

39. A protein inhibitor comprising a first polypeptide, the first polypeptide comprising:

a complement receptor domain (CRD);

a complement regulator comprising:

a Complement Receptor 1 (CR1) complement control protein (CCP) domain; and

a Factor H (FH) CCP domain;

wherein the CCP domains comprise at least one C3b and/or C4b binding domain; and

wherein the CRD is covalently linked to the complement regulator.

40. A protein inhibitor comprising an amino acid sequence of at least one of the amino acid sequences set forth in Tables 1.1-1.10, 2, 3.1, 3.2, 3.3, 3.4, 8, 9, 10.1, 10.2, 11, 12, 13.1, 13.2, 13.3, and 14, or a sequence at least 80% identical thereto.

41. A protein inhibitor comprising an amino acid sequence of at least one of SEQ ID NOs: 50-84, 100-111, and 115-136, or a sequence at least 80% identical thereto.

42. A protein inhibitor comprising: (1) a first polypeptide comprising any one of the amino acid sequences set forth in SEQ ID NOs: 115-123, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto; and a second polypeptide comprising any one of the amino acid sequences set forth in SEQ ID NOs: 124-132, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto, optionally wherein the first and second polypeptides are paired as they are arranged in Tables 13.1 and 13.2; or (2) a first polypeptide comprising any one of the amino acid sequences set forth in SEQ ID NOs: 81-84, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto; and a second polypeptide comprising any one of the amino acid sequences set forth in SEQ ID NOs: 133-136, or a sequence at least 80, 85, 90, 95, 96, 97, 98, 99% or more identical thereto, optionally wherein the first and second polypeptides are paired as they are arranged in Tables 3.2 and 3.3.

43. A method of treatment comprising:

identifying a subject in need of treatment for age-related macular degeneration (AMD), paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), C3 glomerulopathy (C3G) or rheumatoid arthritis; and

administering to the subject the protein inhibitor of any one of the preceding claims.