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(54) Title: ANTI-FACTOR D ANTIBODIES AND CONJUGATES

(57) Abstract: The invention provides anti-Factor D antibodies and conjugates and methods of using the same.

ANTI-FACTOR D ANTIBODIES AND CONJUGATES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of US Provisional Application No. 62/249,166, filed October 30, 2015, and US Provisional Application No. 62/250,995, filed November 4, 2015, each of which is incorporated by reference herein in its entirety for any purpose.

FIELD

[0002] The present invention relates to anti-Factor D antibodies and conjugates and methods of using the same.

BACKGROUND

[0003] The complement system plays a central role in the clearance of immune complexes and the immune response to infectious agents, foreign antigens, virus-infected cells and tumor cells. However, complement is also involved in pathological inflammation and in autoimmune diseases. Therefore, inhibition of excessive or uncontrolled activation of the complement cascade could provide clinical benefit to patients with such diseases and conditions.

[0004] The complement system encompasses three distinct activation pathways, designated the classical, mannose-binding lectin, and the alternative pathways. V.M. Holers In Clinical Immunology: Principles and Practice, ed. R.R. Rich, Mosby Press; 1996, 363-391. The classical pathway is a calcium/magnesium-dependent cascade which is normally activated by the formation of antigen-antibody complexes. The mannose-binding lectin (MBL) pathway is initiated by the binding of MBL to carbohydrate structures on pathogens, resulting in the activation of MBL protease (MASP) that cleaves C2 and C4 to form active C2a, C2b, C4a and C4b. The alternative pathway is a magnesium-dependent cascade which is activated by deposition and activation of C3 on certain susceptible surfaces (e.g. cell wall polysaccharides of yeast and bacteria, and certain biopolymer materials). Activation of the complement pathway generates biologically active fragments of complement proteins, e.g. C3a, C4a and C5a anaphylatoxins and C5b-9 membrane attack complexes (MAC), which mediate inflammatory activities involving leukocyte chemotaxis, activation of macrophages, neutrophils, platelets, mast cells and endothelial cells, vascular permeability, cytolysis, and tissue injury.

[0005] Factor D is a highly specific serine protease essential for activation of the alternative complement pathway. It cleaves factor B bound to C3b, generating the C3b/Bb enzyme which is the active component of the alternative pathway C3/C5 convertases. Factor D may be a suitable target for inhibition, since its plasma concentration in humans is very low (1.8 μg/ml), and it has been shown to be the limiting enzyme for activation of the alternative

complement pathway. P.H. Lesavre and H.J. Müller-Eberhard. (1978) J. Exp. Med. 148: 1498-1510; J.E. Volanakis et al. (1985) New Eng. J. Med. 312: 395-401.

[0006] The down-regulation of complement activation has been demonstrated to be effective in treating several disease indications in animal models and in ex vivo studies, e.g. systemic lupus erythematosus and glomerulonephritis, rheumatoid arthritis, cardiopulmonary bypass and hemodialysis, hyperacute rejection in organ transplantation, myocardial infarction, reperfusion injury, and adult respiratory distress syndrome. In addition, other inflammatory conditions and autoimmune/immune complex diseases are also closely associated with complement activation, including thermal injury, severe asthma, anaphylactic shock, bowel inflammation, urticaria, angioedema, vasculitis, multiple sclerosis, myasthenia gravis, membranoproliferative glomerulonephritis, and Sjögren's syndrome.

[0007] Age-related macular degeneration (AMD) is a progressive chronic disease of the central retina with significant consequences for visual acuity. Lim et al. (2012) Lancet 379:1728. Late forms of the disease are the leading cause of vision loss in industrialized countries. For the Caucasian population $\geq$ 40 years of age the prevalence of early AMD is estimated at 6.8% and advanced AMD at 1.5%. de Jong (2006) N. Engl. J. Med. 355: 1474. The prevalence of late AMD increases dramatically with age rising to 11.8% after 80 years of age. Two types of AMD exist, non-exudative (dry) and exudative (wet) AMD. The more common dry form AMD involves atrophic and hypertrophic changes in the retinal pigment epithelium (RPE) underlying the central retina (macula) as well as deposits (drusen) on the RPE. Advanced dry AMD can result in significant retinal damage, including geographic atrophy (GA), with irreversible vision loss. Moreover, patients with dry AMD can progress to the wet form, in which abnormal blood vessels called choroidal neovascular membranes (CNVMs) develop under the retina, leak fluid and blood, and ultimately cause a blinding disciform scar in and under the retina.

[0008] Drugs targeting new blood vessel formation (neovasculazation) have been the mainstay for treating wet AMD. Ranibizumab, which is an anti-VEGFA antibody fragment, has proven to be highly effective in improving vision for patients afflicted with wet AMD. Recent studies have implicated an association between AMD and key proteins in the complement cascade and a number of therapies targeting specific complement components are being developed to treat dry AMD. A humanized anti-Factor D Fab fragment (aFD.WT; lampalizumab; FCFD4514S) that potently inhibits Factor D and the alternative complement pathway, through binding to an exosite on factor D is currently in clinical development for the treatment of GA associated with dry AMD. Katschke et al. (2012) J. Biol. Chem. 287:12886. A recent phase II clinical trial has shown that monthly intravitreal injection of lampalizumab effectively slowed the progression of GA lesions in patients with advanced dry AMD.

[0009] Eyes have many unique biophysical and anatomic features that make the ocular drug delivery more challenging. For example, blood-ocular barriers are defense mechanisms to protect the eye from infection, but at the same time make it hard for drug to penetrate, especially for diseases in the posterior segments of the eye. Consequently, high-dose administration is often desired to achieve and maintain drug's onsite bioavailability (e.g., ocular residence time) in order to improve efficacy. Meanwhile, the limited space in the back of the eye restrains the drug volume to be delivered, which in turn demands drugs to be delivered in a high concentration formulation.

[0010] Patients with ocular diseases can also benefit from long acting/slow released delivery of therapeutics. Less frequent dosing would provide improved convenience to the patient, have potential benefits of decreased infection rate and increased clinical efficacy. Controlled release of high dose drugs could also minimize drug side effects. Two promising systems for long-acting delivery are PLGA-based solid implants and an implantable port delivery system (PDS). Both systems have the potential to provide near zero-order release kinetics for an extended period of time. For PLGA implants the protein drug is encapsulated in a hydrophobic polymer matrix and drug release is accomplished via slow hydrolysis of the polymer. The rate of release can be controlled by changing the drug loading, polymer hydrophobicity, or polymer molecular weight. The PDS is a refillable device where release into the vitreous is controlled by a porous metal membrane comprising a titanium frit. Since the reservoir has a low volume, a high protein concentration is required for effective delivery with the PDS.

[0011] In addition to or in lieu of high concentration and long acting delivery, increased bioavailability (e.g., ocular residence time) of the drug can be achieved, or facilitated, by post-translational modifications, wherein the protein drug is covalently conjugated with natural or synthetic polymers such as polysialylation, HESylation (conjugation with hydroxyethyl starch) and PEGylation. Chen et al (2011) Expert. Opin. Drug Deliv. 8:1221-36; Kontermann (2009) BioDrugs 23:93-109. PEGylation, the covalent attachment of polymer polyethylene glycol (PEG) to a protein, is a well-established technology especially useful for extending the half-life of antibody fragment therapeutics. Jevsevar et al. (2010) Biotech. J. 5:113-128.

[0012] The conditions that a drug is exposed to vary depending on the delivery system used. For incorporation into solid PLGA implants, lyophilized or spray-dried drug is used. Implants are produced using a hot-melt extrusion process such that the drug is briefly exposed to temperatures approaching 90°C. Although the drug remains in solid state for the duration of release, degradation of PLGA may expose the drug to a low pH environment. In contrast, drug delivered with the PDS is maintained at high concentration in liquid state and exposed to

vitreous which is characterized as a reducing environment at physiological ionic strength and pH.

[0013] Thus, there exists great needs for anti-factor D antibodies with improved stabilities, preferably suitable for high concentration formulation and/or long acting delivery.

SUMMARY

[0014] Lampalizumab is currently in phase III clinical trials for treatment of geographic atrophy (GA), an advanced form of dry AMD. While human clinical trials in GA indicate that a treatment effect is obtained with monthly intravitreal injection of lampalizumab, there exist incentives to use higher drug doses to achieve even better efficacy. Further, less frequent dosing would provide improved convenience to the patient, have potential benefits of decreased infection rate and increased clinical efficacy, and could facilitate treatment of patients with less advanced forms of dry AMD. In order to develop an anti-Factor D antibody with improved solubility and chemical stability suitable for higher drug doses or long-acting delivery, anti-Factor D variants have been investigated. Anti-Factor D variants have been identified with improved solubility and chemical stability. In order to further extend the effect of anti-Factor D antibodies, conjugation to a polyol, such as PEG, would be beneficial. The anti-Factor D variants with improved solubility and chemical stability were found to be very viscous when conjugated to PEG in high concentration solutions, however, making them less suitable for intravitreal injection. The present inventors have developed additional anti-Factor D antibodies that have comparable affinity for human Factor D as lampalizumab, improved affinity for cynomolgus Factor D, as well as improved solubility and chemical stability, and are less viscous when conjugated to PEG, making them more suitable as long-acting therapeutic agents.

[0015] The invention provides improved anti-Factor D antibodies and conjugates and methods of using the same.

[0016] In some embodiments, an isolated antibody that binds to Factor D, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence SYYMY (SEQ ID NO: 15); (b) HVR-H2 comprising the amino acid sequence X_4INPX$_5$X$_6$GX$_7$TNFNEKFKS (SEQ ID NO: 111), wherein X_4 is selected from E and W; X_5 is selected from T and Y; X_6 is selected from N, S, and Q; and X_7 is selected from G, D, and E; (c) HVR-H3 comprising the amino acid sequence EGGFAY (SEQ ID NO: 25); (d) HVR-L1 comprising the amino acid sequence KASQNVDTDVA (SEQ ID NO:9); (e) HVR-L2 comprising the amino acid sequence SASSRX$_1$S (SEQ ID NO: 108), wherein X_1 is selected from Y, K, and R; and (f) HVR-L3 comprising the amino acid sequence QQYX$_3$NYPLT (SEQ ID NO: 110), wherein X_3 is selected from N and E is provided.

[0017] In some aspects, the antibody comprises the sequence X$_2$SASSRX$_1$S (SEQ ID NO: 109), wherein X_1 is selected from Y, K, and R, and X_2 is selected from Y, R, S, K, and Q. In some

embodiments, X_2 is R. In other aspects, X_1 is Y.

[0018] In some embodiments, the antibody comprises HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-H2 comprising an amino acid sequence selected from SEQ ID NOs: 16 to 24, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, HVR-L2 comprising an amino acid sequence selected from SEQ ID NOs: 10 to 12, and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13 or 14.

[0019] In certain embodiments, the antibody comprises:

a. HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 16, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13;

b. HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 20, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13;

c. HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 17, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13;

d. HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 18, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13;

e. HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13;

f. HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 21, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25, HVR-L1 comprising the amino acid sequence of SEQ ID

NO: 9, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13;

g. HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13;

h. HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 23, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13;

i. HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 16, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 11, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13;

j. HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 16, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 12, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13;

k. HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 16, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 14; or

l. HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 24, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13.

[0020] In some embodiments, the amino acid at position 49 of the light chain is arginine (R).

[0021] In certain aspects, the antibody comprises a heavy chain variable region comprising an amino acid sequence selected from SEQ ID NOs: 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51,

53, 55, 57, 59, 61, and 63.

[0022] In other aspects, the antibody comprises a light chain variable region comprising an amino acid sequence selected from SEQ ID NOs: 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, and 62.

[0023] The antibody may comprise:

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 35 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 34;

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 39 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 38;

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 33 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 32;

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 45 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 44;

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 47 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 46;

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 49 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 48;

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 29 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 28;

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 31 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 30;

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 37 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 36;

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 41 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 40;

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 61 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 60;

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 43 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 42;

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 57 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 56;

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 51 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 50;

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 53 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 52;

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 55 and a

light chain variable region comprising the amino acid sequence of SEQ ID NO: 54;

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 59 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 58; or

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 63 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 62.

[0024] In some embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 35 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 34, or wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 39 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 38.

[0025] The antibody may be a monoclonal antibody, a humanized antibody, or a chimeric antibody, or any combination of monoclonal, humanized, and chimeric.

[0026] In some embodiments, the antibody binds Factor D.

[0027] In certain aspects the antibody is a fragment. In some embodiments, the fragment is a Fab fragment.

[0028] In some embodiments, the antibody light chain comprises a light chain constant region comprising the sequence of SEQ ID NO: 112.

[0029] In some embodiments, the antibody heavy chain comprises a heavy chain constant region comprising a sequence selected from SEQ ID NOs: 113, 128 to 132, 134 to 137, and 154-156.

[0030] In certain aspects, the antibody comprises:

a heavy chain comprising an amino acid sequence selected from SEQ ID NOs: 71, 118 to 122, and 140 to 146, and a light chain comprising the amino acid sequence of SEQ ID NO: 70;

a heavy chain comprising an amino acid sequence selected from SEQ ID NO: 75, 123 to 127, and 147 to 153 and a light chain comprising the amino acid sequence of SEQ ID NO: 74;

a heavy chain comprising the amino acid sequence of SEQ ID NO: 65 and a light chain comprising the amino acid sequence of SEQ ID NO: 64;

a heavy chain comprising the amino acid sequence of SEQ ID NO: 65 and a light chain comprising the amino acid sequence of SEQ ID NO: 64;

a heavy chain comprising the amino acid sequence of SEQ ID NO: 67 and a light chain comprising the amino acid sequence of SEQ ID NO: 66;

a heavy chain comprising the amino acid sequence of SEQ ID NO: 69 and a light chain comprising the amino acid sequence of SEQ ID NO: 68;

a heavy chain comprising the amino acid sequence of SEQ ID NO: 73 and a light chain comprising the amino acid sequence of SEQ ID NO: 72;

a heavy chain comprising the amino acid sequence of SEQ ID NO: 77 and a light chain comprising the amino acid sequence of SEQ ID NO: 76;

a heavy chain comprising the amino acid sequence of SEQ ID NO: 79 and a light chain comprising the amino acid sequence of SEQ ID NO: 78;

a heavy chain comprising the amino acid sequence of SEQ ID NO: 81 and a light chain comprising the amino acid sequence of SEQ ID NO: 80;

a heavy chain comprising the amino acid sequence of SEQ ID NO: 83 and a light chain comprising the amino acid sequence of SEQ ID NO: 82;

a heavy chain comprising the amino acid sequence of SEQ ID NO: 85 and a light chain comprising the amino acid sequence of SEQ ID NO: 84;

a heavy chain comprising the amino acid sequence of SEQ ID NO: 87 and a light chain comprising the amino acid sequence of SEQ ID NO: 86;

a heavy chain comprising the amino acid sequence of SEQ ID NO: 89 and a light chain comprising the amino acid sequence of SEQ ID NO: 88;

a heavy chain comprising the amino acid sequence of SEQ ID NO: 91 and a light chain comprising the amino acid sequence of SEQ ID NO: 90;

a heavy chain comprising the amino acid sequence of SEQ ID NO: 93 and a light chain comprising the amino acid sequence of SEQ ID NO: 92;

a heavy chain comprising the amino acid sequence of SEQ ID NO: 95 and a light chain comprising the amino acid sequence of SEQ ID NO: 94;

a heavy chain comprising the amino acid sequence of SEQ ID NO: 97 and a light chain comprising the amino acid sequence of SEQ ID NO: 96; or

a heavy chain comprising the amino acid sequence of SEQ ID NO: 99 and a light chain comprising the amino acid sequence of SEQ ID NO: 98.

[0031] In some aspects, the antibody comprises:

a heavy chain comprising an amino acid sequence selected from SEQ ID NOs: 71, 118 to 122, and 140 to 146, and a light chain comprising the amino acid sequence of SEQ ID NO: 70; or

a heavy chain comprising an amino acid sequence selected from SEQ ID NO: 75, 123 to 127, and 147 to 153 and a light chain comprising the amino acid sequence of SEQ ID NO: 74.

[0032] In some embodiments, the antibody comprises an engineered cysteine. The engineered cysteine may be selected from a T110C, A136C, L170C, L175C, T183C, or T205C mutation in the heavy chain, and I106C, R108C, R142C, K149C, and V205C mutation in the light chain, wherein the residue number is according to Kabat numbering.

[0033] In certain aspects, Factor D is human Factor D comprising the amino acid sequence of SEQ ID NO: 106.

[0034] In some embodiments, the antibody binds to cynomolgus monkey Factor D. The cynomolgus monkey Factor D may comprise the amino acid sequence of SEQ ID NO: 107.

[0035] In some aspects, the antibody binds to cynomolgus monkey Factor D with a K_D that

is less than 10-fold, or less than 7-fold, or less than 5-fold, or less than 3-fold higher than the K_D for human Factor D.

[0036] An isolated nucleic acid encoding any of the antibodies described herein is encompassed. A host cell comprising the nucleic acid is provided.

[0037] In some embodiments, the invention encompasses a method of producing any of the antibodies disclosed herein, comprising culturing the host cell so that the antibody is produced.

[0038] A pharmaceutical formulation comprising any of the antibodies disclosed herein and a pharmaceutically acceptable carrier is encompassed. The pharmaceutically acceptable carrier may comprise a buffer having a pH between about 5.5 and about 8.0. The antibody may be present in the pharmaceutical formulation at a concentration of at least 150 mg/ml, at least 160 mg/ml, at least 170 mg/ml, at least 180 mg/ml, at least 190 mg/ml, at least 200 mg/ml, or at least 210 mg/ml, or at least 220 mg/ml, or at least 230 mg/ml, or at least 240 mg/ml, or at least 250 mg/ml, or at least 260 mg/ml, or at least 270 mg/ml, or at least 280 mg/ml, or at least 290 mg/ml, or at least 300 mg/ml.

[0039] The antibody may be present in the pharmaceutical formulation at a concentration of between 150 mg/ml and 350 mg/ml, or between 150 mg/ml and 300 mg/ml, or between 170 mg/ml and 300 mg/ml, or between 200 mg/ml and 300 mg/ml.

[0040] In some embodiments, the pharmaceutical formulation comprises no visible precipitate after storage at 4°C for at least one week, at least two weeks, at least four weeks, at least six weeks, at least eight weeks, at least 12 weeks, at least 16 weeks, at least 20 weeks, at least 24 weeks, or at least 28 weeks.

[0041] In some embodiments, the viscosity of the composition at 25°C is less than 30cP, less than 25cP, less than 20cP, less than 15cP, or less than 10cP.

[0042] In some aspects, the concentration of the anti-Factor D antibody in the composition is between 100 mg/ml and 300 mg/ml, or between 150 mg/ml and 300 mg/ml.

[0043] In some embodiments, the pharmaceutical formulation is suitable for intravitreal administration through a narrow bore needle. The narrow bore needle may be about 30, 29, 28, 27, 26, 25, 24, 23, or 22 gauge.

[0044] The invention further comprises a conjugate comprising at least one antibody described herein covalently linked to one or more polyols. In some embodiments, the polyol is a multi-armed polyol. In some aspects, the conjugate comprises at least two, at least three, at least four, at least five, or at least six antibodies covalently linked to a multi-armed polyol.

[0045] The polyol of the conjugate may be covalently linked to at least one antibody through a free sulfhydryl group of a cysteine amino acid. The cysteine amino acid may be an engineered cysteine. The cysteine amino acid may be in a constant region of the antibody. The cysteine amino acid may be at the C-terminus of the heavy chain or light chain of the antibody.

[0046] In some embodiments, the conjugate comprises a polyol that is covalently linked to

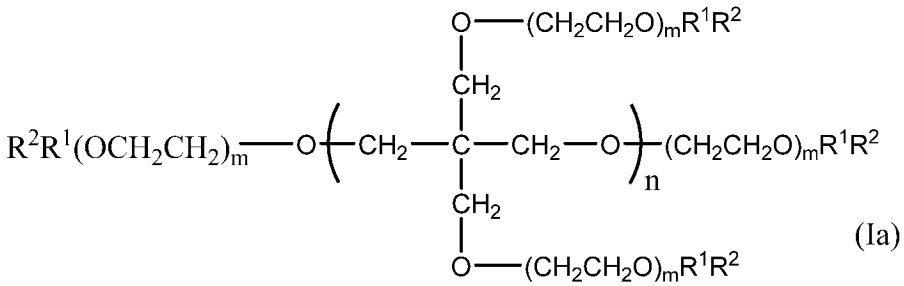
at least one antibody through a free amino group of a lysine amino acid. The lysine amino acid may be in a constant region of the antibody. The lysine amino acid may be at the C-terminus of the heavy chain or light chain of the antibody.

[0047] In some embodiments, the polyol is a multi-armed polyol selected from a dimer, a tetramer, a hexamer, and an octamer.

[0048] In some aspects, the multi-armed polyol is an octamer.

[0049] In some embodiments, the polyol is polyethylene glycol. In some aspects, the polyethylene glycol has a weight average molecular weight of from about 500 D to about 300,000 D. In other aspects the polyethylene glycol has a weight average molecular weight of from about 20,000 D to about 60,000 D. In other aspects the polyethylene glycol has a weight average molecular weight of about 40,000 D.

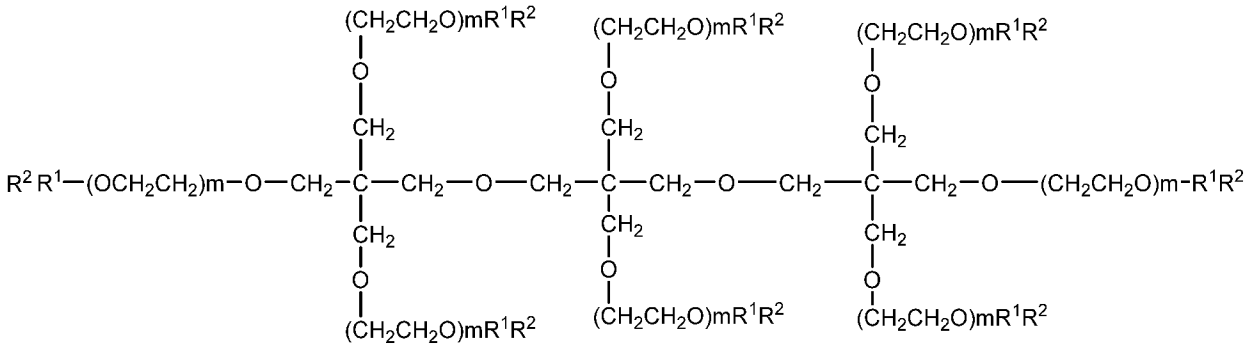
[0050] In some embodiments, the conjugate described herein has a polyethylene glycol having the structure of general formula (Ia):



(Ia)

wherein each m is independently an integer from 45-1000 or 3-250 or 50-200 or 100-250; n is an integer from 1-10; each R^1 is independently either absent, or is a linking group; and each R^2 is independently either hydrogen or a terminal reactive group; wherein at least one R^2 is a terminal reactive group and is covalently linked to the anti-Factor D antibody or the antibody variant.

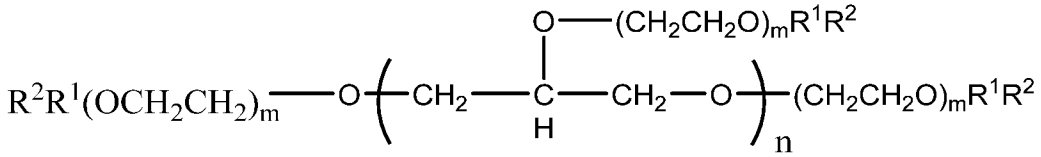
[0051] In some embodiments, the polyethylene glycol has the structure of general formula (Ib):



(Ib)

wherein each m is independently an integer from 45-1000 or 3-250 or 50-200 or 100-250; each R^1 is independently either absent, or is a linking group; and each R^2 is independently either hydrogen or a terminal reactive group; wherein at least one R^2 is a terminal reactive group and is covalently linked to the anti-Factor D antibody or the antibody variant.

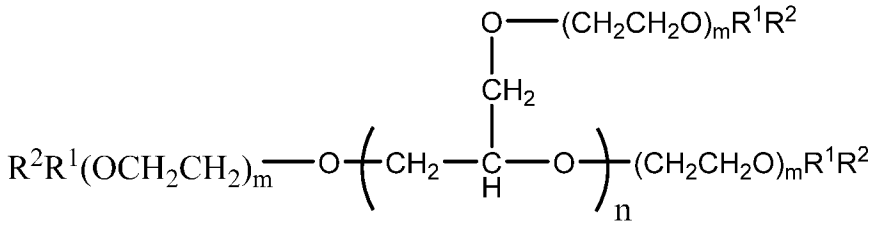
[0052] In some embodiments, the polyethylene glycol has the structure of general formula (IIa):



(IIa)

wherein each m is independently an integer of from 45-1000 or 3-250 or 50-200 or 100-250; n is an integer from 1-10; each R^1 is independently either absent, or is a linking group; and each R^2 is independently either hydrogen or a terminal reactive group; wherein at least one R^2 is a terminal reactive group and is covalently linked to the anti-Factor D antibody or the antibody variant. In some embodiments n is 4.

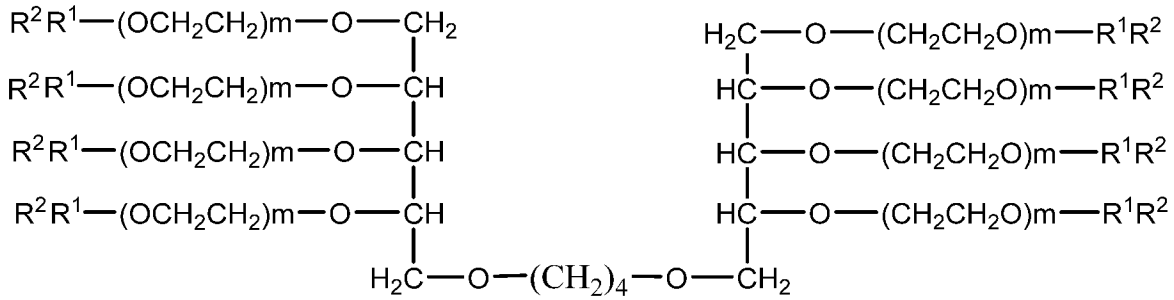
[0053] In some aspects, the polyethylene glycol has the structure of general formula (IIIa):



(IIIa)

wherein each m is independently an integer of from 45-1000 or 3-250 or 50-200 or 100-250; n is an integer from 1-10; each R^1 is independently either absent, or is a linking group; and each R^2 is independently either hydrogen or a terminal reactive group; wherein at least one R^2 is a terminal reactive group and is covalently linked to the anti-Factor D antibody or the antibody variant. In some embodiments, n is 4.

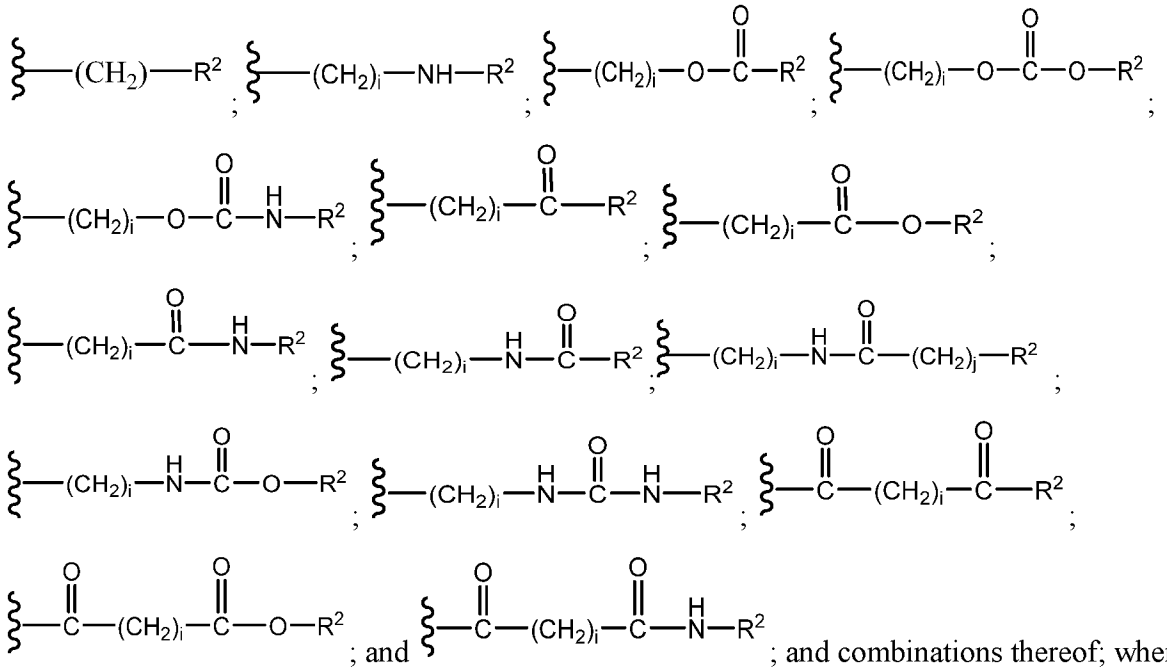
[0054] In some aspects, the polyethylene glycol has the structure of general formula (IVa):



(IVa)

wherein each m is independently an integer of from 45-1000 or 3-250 or 50-200 or 100-250; each R^1 is independently either absent, or is a linking group; and each R^2 is independently either hydrogen or a terminal reactive group; wherein at least one R^2 is a terminal reactive group and is covalently linked to the anti-Factor D antibody or the antibody variant. In some embodiments, m is an integer of 50-200. In other embodiments, m is an integer of 100-150.

[0055] In some embodiments, the at least one R^1 is a linking group, wherein R^1 and R^2 when taken together are selected from:



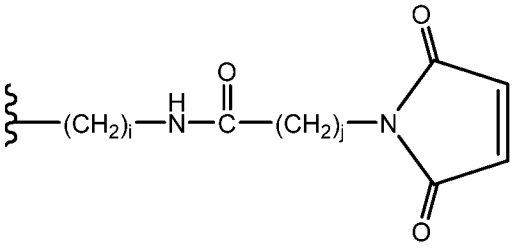
; and combinations thereof; wherein each i is independently an integer of 0-10; and j is an integer of 0-10.

[0056] In some embodiments, R^2 is independently selected from a thiol reactive group, an amino reactive group, and combinations thereof.

[0057] In some embodiments, each R^2 is independently selected from a maleimide, a sulfhydryl, a thiol, trifluoromethanesulfonate, tosylate, aziridine, epoxide, a pyridyl disulfide, succinimidyl ester, $-NH_2$, an aldehyde, a haloacetate, a haloacetamide, and a para-nitrophenyl carbonate.

[0058] In some aspects, R^2 is a maleimide.

[0059] The congjugate may have a R^1 and R^2, when taken together, are



, i is an integer of 0-10; and j is an integer of 0-10.

[0060] In some embodiments, at least seven of the R^2 groups are covalently linked to one of the anti-Factor D antibodies or the antibody variants.

[0061] In some embodiments, at least eight of the R^2 groups are covalently linked to one of the anti-Factor D antibodies or the antibody variants.

[0062] In some embodiments, the conjugate is prepared by covalently linking at least one antibody described herein to a multi-armed polyol.

[0063] A pharmaceutical formulation comprising the conjugate as described herein and a

pharmaceutically acceptable carrier is encompassed. The concentration of the antibody is at least 100 mg/ml, or at least 150 mg/ml, or at least 200 mg/ml, or at least 300 mg/ml. In other embodiments, the concentration of the anti-Factor D antibody or the antibody variant is from about 50 mg/ml to about 300 mg/ml.

[0064] In some embodiments, the viscosity of the pharmaceutical formulation or composition is at 25°C is less than 1000 cP, less than 900 cP, less than 800 cP, less than 700 cP, less than 600 cP, less than 500 cP, less than 400 cP, or less than 300 cP.

[0065] In some embodiments, the concentration of the anti-Factor D antibody in the formulation or composition is at least 100 mg/ml or at least 150 mg/ml.

[0066] Also encompassed is a delivery device for ocular delivery comprising the pharmaceutical formulation described herein and a means for delivering the formulation intravitreally to a patient.

[0067] In some embodiments, the delivery device formulation remains effective on site for a prolonged period of time.

[0068] In some embodiments, a method of treating a complement-mediated disorder in a subject comprising administering to the subject an effective amount of an antibody, the conjugate, or the pharmaceutical formulation described herein is encompassed.

[0069] In some embodiments, the complement-mediated disorder is systemic.

[0070] In another embodiment, the complement-mediated disorder is a complement-associated eye condition.

[0071] In some aspects, the complement-associated eye condition is selected from age-related macular degeneration (AMD), including dry and wet (non-exudative and exudative) forms, choroidal neovascularization (CNV), uveitis, diabetic retinopathy, ischemia-related retinopathy, diabetic macular edema, pathological myopia, von Hippel-Lindau disease, histoplasmosis of the eye, Central Retinal Vein Occlusion (CRVO), corneal neovascularization, and retinal neovascularization.

[0072] In some embodiments, the complement-associated eye condition is selected from intermediate dry form AMD or geographic atrophy (GA).

[0073] In some aspects, the method comprises administering the antibody, conjugate, or pharmaceutical formulation using an implantable port delivery system.

[0074] In some embodiments, the method comprises administering the antibody, conjugate, or pharmaceutical formulation by intravitreal administration. The intravitreal administration may be through a narrow bore needle. In some embodiments, the narrow bore needle is about 30, 29, 28, 27, 26, 25, 24, 23, or 22 gauge.

[0075] In some aspects, the method of treating further comprising administering an additional therapeutic agent to the individual. In some embodiments, the additional therapeutic agent is selected from an an ANG2 antagonist, a TIE2 antagonist, a VEGF antagonist, and a second

complement component antagonist.

[0076] In some embodiments, the additional therapeutic agent is an anti-ANG2 antibody.

[0077] In other embodiments, the additional therapeutic agent is an anti-TIE2 antibody.

[0078] In some aspects, the additional therapeutic agent is selected from a VEGF trap and an anti-VEGF antibody.

[0079] In other aspects, the additional therapeutic agent is a second complement component antagonist, wherein the second complement component antagonist inhibits a complement component selected from C1, C2, C3, C4, C5, C6, C7, C8 and C9.

[0080] Use of the antibody or the conjugate described herein for the preparation of a medicament for treating a complement-mediated disorder in a subject is encompassed. In some embodiments, the complement-mediated disorder is a complement-associated eye condition. In other embodiments, the complement-mediated disorder is systemic. In some aspects, the complement-associated eye condition is selected from age-related macular degeneration (AMD), including dry and wet (non-exudative and exudative) forms, choroidal neovascularization (CNV), uveitis, diabetic retinopathy, ischemia-related retinopathy, diabetic macular edema, pathological myopia, von Hippel-Lindau disease, histoplasmosis of the eye, Central Retinal Vein Occlusion (CRVO), corneal neovascularization, and retinal neovascularization. The complement-associated eye condition may be selected from intermediate dry form AMD or geographic atrophy (GA).

[0081] In some aspects, the antibody or the conjugate described herein is for use in therapy.

[0082] In some embodiments, the antibody or the conjugate described herein is for use in a method of treating a complement-mediated disorder in a subject.

[0083] In some embodiments, the antibody, conjugate, or formlation is for use in a method of treating a systemic complement-mediated disorder in a subject. In some embodiments, the complement-mediated disorder is a complement-associated eye condition. In some apsects, the complement-associated eye condition is selected from age-related macular degeneration (AMD), including dry and wet (non-exudative and exudative) forms, choroidal neovascularization (CNV), uveitis, diabetic retinopathy, ischemia-related retinopathy, diabetic macular edema, pathological myopia, von Hippel-Lindau disease, histoplasmosis of the eye, Central Retinal Vein Occlusion (CRVO), corneal neovascularization, and retinal neovascularization. In some embodiments, the complement-associated eye condition is selected from intermediate dry form AMD or geographic atrophy (GA).

BRIEF DESCRIPTION OF THE FIGURES

[0084] **FIGS. 1A-C** show an alignment of the murine 20D12 light chain variable region, human VL kappa I (VL_K) consensus sequence, and light chain variable regions of humanized variants of 20D12.

[0085] **FIGS. 2A-C** show an alignment of the murine 20D12 heavy chain variable

region, human VH subgroup I (VH_I) consensus sequence, and heavy chain variable regions of humanized variants of 20D12.

[0086] **FIGS. 3A-C** show overlays of portions of the hu20D12.v2.0-Factor D co-crystal structure and the lampalizumab-Factor D co-crystal structure. A) Overlay of the binding interface between the antibodies and Factor D. B) Detail of certain heavy chain contacts between the antibodies and Factor D. C) Detail of certain light chain contacts between the antibodies and Factor D.

[0087] **FIG. 4** shows alignments of the lampalizumab light chain (SEQ ID NO: 100) and heavy chain variable regions (SEQ ID NO: 101) and hu20D12.v2.0 ("hu20D12.v1.N54S") light chain (SEQ ID NO: 32) and heavy chain (SEQ ID NO: 33) variable regions.

[0088] **FIGS. 5A-B** show inhibition of Factor D by humanized 20D12 antibody Fabs and conjugates, as measured using a TR-FRET assay of factor B activation.

[0089] **FIGS. 6A-C** show that hu20D12.v2.0 (6A) and hu20D12.v2.1 (6C) are soluble at 292 mg/ml, and 260 mg/ml, respectively, while lampalizumab precipitates from solution at 227 mg/ml (6B).

[0090] **FIGS. 7A-C** show a typical SEC-MALS profile of PEG-octamer conjugated hu20D12.v2.1.C (7A), and SEC-QELS analysis to determine hydrodynamic radii (R_H) (7B, 7C).

[0091] **FIGS. 8A-C** shows the CEX chromatogram (8A), SEC-MALS profile (8B) of PEG-octamer conjugated hu20D12.v2.1.C fractions, and a table of the Mw (kDa), polydispersity (Mw/Mn), and R_H (nm) of the fractions (8C).

[0092] **FIGS. 9A-B** show the concentration-dependent viscosity of PEG-octamer conjugated antibodies. AFD.v14.C + TP-Oct; hu20D12.v2.1.C + TP-Oct; and hu20D12.v2.3.C + TP-Oct (9A), and AFD.v8.C + PEG tetramer; and hu20D12.v2.3 + PEG tetramer (9B).

[0093] **FIGS. 10A-B** show thermal stability of PEG-octamer conjugated Fabs by binding capacity (10A) and CE-SDS (10B).

[0094] **FIG. 11** shows various characteristics of humanized 20D12 antibodies, lampalizumab, and anti-Factor D variants AFD.v8 and AFD.v14.

[0095] **FIGS. 12A-B** show MALDI analysis of a multi-armed PEG comprising a hexaglycerol (HGEO) core (Sunbright® HGEO-400MA, NOF America, Corp.) and a tripentaerythritol (TP) core (8ARM (TP)-PEG-MAL, JenKem Technology, USA) (12A: HGEO core; 12B: TP core).

[0096] **FIGS. 13A-B** show percent systemic AP complement activity as measured by relative hemolysis of rRBCs over time in cynomolgus monkeys following intravitreal (IVT) injection of lampalizumab (13A) or unconjugated AFD.v14 (13B).

[0097] **FIGS. 14A-C** show percent systemic AP complement activity as measured by

relative hemolysis of rRBCs or ELISA following IVT injection of AFD.v14.C + TP-Oct (14A) or AFD.v14.C + HG-Oct (14B, 14C) in the eye of cynomolgus monkeys.

[0098] **FIGS. 15A-C** show serum PK for AFD.v14.C + TP-Oct compared to unconjugated AFD.v14 in a cynomolgus monkey Gyrolab XP assay.

[0099] **FIGS. 16A-B** show group mean (+/- SD) concentrations in serum (16A), and in vitreous humor, aqueous humor, or retinal homogenate (16B) following single ITV or IV administration of AFD.v14.C + TP-Oct in male cynomolgus monkeys.

[00100] **FIGS. 17A-B** show PK results from a Gyrolab XP assay with AFD.v14 and AFD.v14.C + TP-Oct in cynomolgus monkey vitreous humor.

[00101] **FIGS. 18A-B** show PK results from a Gyrolab XP assay with AFD.v14 and AFD.v14.C + TP-Oct in cynomolgus monkey acqueous humor.

[00102] **FIGS. 19A-B** show PK results from a Gyrolab XP assay with AFD.v14 and AFD.v14.C + TP-Oct in cynomolgus monkey retinal humor.

[00103] **FIG. 20** shows SEC analysis of hu20D12.v2.1.C+TP-Oct.

[00104] **FIG. 21** shows CE-SDS non-reduced (NR) electropherogram of hu20D12.v2.1.C+TP-Oct.

[00105] **FIG. 22** shows Fab concentration over time in vitreous humor of cynomolgus monkeys administered ^{125}I- hu20D12.v2.1.C+TP-Oct (Groups 1, 2, and 3) or ^{125}I-hu20D12.v2.1.C (Group 4) intravitreally.

[00106] **FIG. 23** shows Fab concentration over time in aqueous humor of cynomolgus monkeys administered ^{125}I- hu20D12.v2.1.C+TP-Oct (Groups 1, 2, and 3) or ^{125}I-hu20D12.v2.1.C (Group 4) intravitreally.

[00107] **FIG. 24** shows Fab concentration over time in retina of cynomolgus monkeys administered ^{125}I- hu20D12.v2.1.C+TP-Oct (Groups 1, 2, and 3) or ^{125}I- hu20D12.v2.1.C (Group 4) intravitreally.

DETAILED DESCRIPTION

[00108] Reference will now be made in detail to certain embodiments of the invention, examples of which are illustrated in the accompanying structures and formulas. While the invention will be described in conjunction with the enumerated embodiments, it will be understood that they are not intended to limit the invention to those embodiments. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents which may be included within the scope of the present invention as defined by the claims. One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. The present invention is in

no way limited to the methods and materials described.

[00109] All references cited throughout the disclosure are expressly incorporated by reference herein in their entirety. In the event that one or more of the incorporated literature, patents, and similar materials differs from or contradicts this application, including but not limited to defined terms, term usage, described techniques, or the like, this application controls.

I. DEFINITIONS

[00110] The words "comprise," "comprising," "include," "including," and "includes" when used in this specification and claims are intended to specify the presence of stated features, integers, components, or steps, but they do not preclude the presence or addition of one or more other features, integers, components, steps, or groups thereof.

[00111] An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In some embodiments, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

[00112] "Affinity" refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (*e.g.*, an antibody) and its binding partner (*e.g.*, an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (*e.g.*, antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (Kd). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

[00113] An "affinity matured" antibody refers to an antibody with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen.

[00114] The terms "anti-Factor D antibody", "aFD antibody", "AFD.Ab", and "an antibody that binds to Factor D" refer to an antibody that is capable of binding Factor D with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in

targeting Factor D. In some embodiments, the extent of binding of an anti-Factor D antibody to an unrelated, non-Factor D protein is less than about 10% of the binding of the antibody to Factor D as measured, *e.g.*, by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to Factor D has a dissociation constant (Kd) of ≤ 1μM, ≤ 100 nM, ≤ 10 nM, ≤ 5 nm, ≤ 4 nM, ≤ 3 nM, ≤ 2 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (*e.g.*, 10^{-8} M or less, *e.g.* from 10^{-8} M to 10^{-13} M, *e.g.*, from 10^{-9} M to 10^{-13} M). In certain embodiments, an anti-Factor D antibody binds to an epitope of Factor D that is conserved among Factor D from different species. The anti-Factor D antibodies include without limitation AFD.v# variants and hu20D12.v# variants with # being a number used to identify the particular variant.

[00115] The term "antibody" is used herein in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.

[00116] An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody and that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab-SH, Fab'-SH, Fab', Fab-C, Fab'-C, Fab'-C-SH, Fab-C-SH, scFv, diabody, or $F(ab')_2$; diabodies; linear antibodies; single-chain antibody molecules (*e.g.* scFv); and multispecific antibodies formed from antibody fragments.

[00117] As used herein, a "Fab" refers to an antibody that comprises a heavy chain constant region that comprises the CH1 domain, or a sufficient portion of the CH1 domain to form a disulfide bond with the light chain constant region, but does not contain a CH2 domain or a CH3 domain. As used herein, a Fab may comprise one or more amino acids of the hinge region. Thus, as used herein, the term "Fab" encompasses Fab' antibodies. A Fab may comprise additional non-native amino acids, such as a C-terminal cysteine, in which case it may be referred to as a Fab-C. As discussed below, the term Fab-C also encompasses Fabs comprising native amino acids of the hinge region, including a native cysteine at the C-terminus. In some embodiments, a Fab comprises an engineered cysteine (i.e., a Fab may be a THIOMAB).

[00118] A "Fab-C" refers to a Fab with a C-terminal cysteine, which may be a native cysteine that occurs at that residue position (such as a cysteine from the hinge region), or may be a cysteine added to the C-terminus that does not correspond to a native cysteine. The anti-Factor D antibodies include without limitation AFD.C antibodies and hu20D12.C antibodies, with "C" indicating that the antibody is a Fab with a C-terminal cysteine. Nonlimiting exemplary Fab-C heavy chain constant regions include the sequences of SEQ ID NOs: 129, 130, 132, 154, 155,

156, 157, and 158.

[00119] A "Fab-SH" refers to a Fab with a free thiol group. In some embodiments, the free thiol group is located in the last 10 amino acids of the C-terminus of the Fab. Fab-C antibodies are typically also Fab-SH antibodies. A further nonlimiting exemplary Fab-SH heavy chain constant region having the amino acid sequence of SEQ ID NO: 131. Typically, a Fab comprising an engineered cysteine (i.e., a Fab that is a THIOMAB) is a Fab-SH.

[00120] An "antibody that binds to the same epitope" as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more. An exemplary competition assay is provided herein.

[00121] The term "complement-associated disorder" is used in the broadest sense and includes disorders associated with excessive or uncontrolled complement activation. They include complement activation during cardiopulmonary bypass operations; complement activation due to ischemia-reperfusion following acute myocardial infarction, aneurysm, stroke, hemorrhagic shock, crush injury, multiple organ failure, hypobolemic shock, intestinal ischemia or other events causing ischemia. Complement activation has also been shown to be associated with inflammatory conditions such as severe burns, endotoxemia, septic shock, adult respiratory distress syndrome, hemodialysis; anaphylactic shock, severe asthma, angioedema, Crohn's disease, sickle cell anemia, poststreptococcal glomerulonephritis and pancreatitis. The disorder may be the result of an adverse drug reaction, drug allergy, IL-2 induced vascular leakage syndrome or radiographic contrast media allergy. It also includes autoimmune disease such as systemic lupus erythematosus, myasthenia gravis, rheumatoid arthritis, Alzheimer's disease and multiple sclerosis. Complement activation is also associated with transplant rejection. Complement activation is also associated with ocular diseases such as age-related macular degeneration, diabetic retinopathy and other ischemia-related retinopathies, choroidal neovascularization (CNV), uveitis, diabetic macular edema, pathological myopia, von Hippel-Lindau disease, histoplasmosis of the eye, Central Retinal Vein Occlusion (CRVO), corneal neovascularization, and retinal neovascularization.

[00122] The term "complement-associated eye condition" is used in the broadest sense and includes all eye conditions the pathology of which involves complement, including the classical and the alternative pathways, and in particular the alternative pathway of complement. Complement-associated eye conditions include, without limitation, macular degenerative diseases, such as all stages of age-related macular degeneration (AMD), including dry and wet (non-exudative and exudative) forms, choroidal neovascularization (CNV), uveitis, diabetic and

other ischemia-related retinopathies, and other intraocular neovascular diseases, such as diabetic macular edema, pathological myopia, von Hippel-Lindau disease, histoplasmosis of the eye, Central Retinal Vein Occlusion (CRVO), corneal neovascularization, and retinal neovascularization. In one example, complement-associated eye conditions includes age-related macular degeneration (AMD), including non-exudative (e.g. intermediate dry AMD or geographic atrophy (GA)) and exudative (e.g. wet AMD (choroidal neovascularization (CNV)) AMD, diabetic retinopathy (DR), endophthalmitis and uveitis. In a further example, nonexudative AMD may include the presence of hard drusen, soft drusen, geographic atrophy and/or pigment clumping. In one example, complement-associated eye conditions include age-related macular degeneration (AMD), including early AMD (e.g. includes multiple small to one or more non-extensive medium sized drusen), intermediate AMD (e.g. includes extensive medium drusen to one or more large drusen) and advanced AMD (e.g. includes geographic atrophy or advanced wet AMD (CNV). (Ferris et al., AREDS Report No. 18, ; Sallo et al., Eye Res., 34(3): 238-40 (2009); Jager et al., *New Engl. J. Med.,* 359(1): 1735 (2008)). In a further example, intermediate dry AMD may include large confluent drusen. In a further example, geographic atrophy may include photoreceptor and/or Retinal Pigmented Epithelial (RPE) loss. In a further example, the area of geographic atrophy may be small or large and/or may be in the macula area or in the peripheral retina. In one example, complement-associated eye condition is intermediate dry AMD. In one example, complement-associated eye condition is geographic atrophy. In one example, complement-associated eye condition is wet AMD (choroidal neovascularization (CNV)).

[00123] The term "chimeric" antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

[00124] The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: 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 domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively.

[00125] The term "polyol" when used herein refers broadly to polyhydric alcohol compounds. Polyols can be any water-soluble poly(alkylene oxide) polymer for example, and can have a linear or branched chain. Preferred polyols include those substituted at one or more hydroxyl positions with a chemical group, such as an alkyl group having between one and four carbons. Typically, the polyol is a poly(alkylene glycol), preferably polyethylene glycol (PEG). However, those skilled in the art recognize that other polyols, such as, for example, poly

(propylene glycol) and polyethylene-polypropylene glycol copolymers, can be employed using the techniques for conjugation described herein for PEG. The polyols of the disclosure include those well known in the art and those publicly available, such as from commercially available sources.

[00126] The term "conjugate" is used herein according to its broadest definition to mean joined or linked together. Molecules are "conjugated" when they act or operate as if joined. In particular embodiments, "conjugate" refers to an antibody (e.g., an antibody fragment, as detailed herein) covalently bound to a multi-armed polyol.

[00127] "Effector functions" refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.* B cell receptor); and B cell activation.

[00128] An "effective amount" of an agent, *e.g.*, a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result, such as a measurable improvement in the state, e.g. pathology, of the target disease or condition, such as, for example, a complement-associated eye condition.

[00129] The term "epitope" refers to the particular site on an antigen molecule to which an antibody binds.

[00130] "Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

[00131] The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

[00132] The term "glycosylated forms of Factor D" refers to naturally occurring forms of Factor D that are post-translationally modified by the addition of carbohydrate residues.

[00133] The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

[00134] A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

[00135] A "human consensus framework" is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., *Sequences of Proteins of Immunological Interest*, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3. In some embodiments, for the VL, the subgroup is subgroup kappa I as in Kabat et al., *supra.* In some embodiments, for the VH, the subgroup is subgroup III as in Kabat et al., *supra.*

[00136] A "humanized" antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (*e.g.*, CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, *e.g.*, a non-human antibody, refers to an antibody that has undergone humanization.

[00137] The term "hypervariable region" or "HVR," as used herein, refers to each of the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops ("hypervariable loops"). Generally, native four-chain antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). HVRs generally comprise amino acid residues from the hypervariable loops and/or from the "complementarity determining regions" (CDRs), the latter being of highest sequence variability and/or involved in antigen recognition. Exemplary hypervariable loops occur at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3). (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987).) Exemplary CDRs (CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3) occur at amino acid residues 24-34 of L1, 50-56 of L2, 89-97 of L3, 31-35B of H1, 50-65 of H2, and 95-102 of H3. (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991).) With the exception of CDR1 in VH, CDRs generally comprise the amino acid residues that form the hypervariable loops. CDRs also comprise "specificity determining

residues," or "SDRs," which are residues that contact antigen. SDRs are contained within regions of the CDRs called abbreviated-CDRs, or a-CDRs. Exemplary a-CDRs (a-CDR-L1, a-CDR-L2, a-CDR-L3, a-CDR-H1, a-CDR-H2, and a-CDR-H3) occur at amino acid residues 31-34 of L1, 50-55 of L2, 89-96 of L3, 31-35B of H1, 50-58 of H2, and 95-102 of H3. (See Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008).) Unless otherwise indicated, HVR residues and other residues in the variable domain (*e.g.*, FR residues) are numbered herein according to Kabat et al., *supra.*

[00138] A protein including an antibody is said to be "stable" if it essentially retains the intact conformational structure and biological activity. Various analytical techniques for measuring protein stability are available in the art and are reviewed in, e.g., Peptide and Protein Drug Delivery, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, N.Y., Pubs. (1991) and Jones (1993) *Adv. Drug Delivery Rev.* 10: 29-90. An antibody variant with "improved stability" refers to an antibody variant that is more stable comparing to the starting reference antibody. Preferably, antibody variants with improved stability are variants of the native (wild-type) antibodies in which specific amino acid residues are altered for the purpose of improving physical stability, and/or chemical stability, and/or biological activity, and/or reducing immunogenicity of the native antibodies. Walsh (2000) *Nat. Biotech.* 18:831-3.

[00139] The term "isomerization" refers generally to a chemical process by which a chemical compound is transformed into any of its isomeric forms, i.e., forms with the same chemical composition but with different structure or configuration and, hence, generally with different physical and chemical properties. Specifically used herein is aspartate isomerization, a process wherein one or more aspartic acid (D or Asp) residue(s) of a polypeptide have been transformed to isoaspartic acid residue(s). Geiger and Clarke (1987) *J. Biol. Chem.* 262:785-94.

[00140] The term "deamidation" refers generally to a chemical reaction wherein an amide functional group is removed from an organic compound. Specifically used herein is asparagine deamidation, a process wherein one or more asparagine (N or Asn) residue(s) of a polypeptide have been converted to aspartic acid (D or Asp), i.e., the neutral amide side chain has been converted to a residue with an overall acidic property. Xie and Schowen (1999) *J. Pharm. Sci.* 88:8-13.

[00141] A "conjugate" is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a polyol.

[00142] A "patient" or "individual" or "subject" is a mammal. Mammals include, but are not limited to, domesticated animals (*e.g.*, cows, sheep, cats, dogs, and horses), primates (*e.g.*, humans and non-human primates such as monkeys), rabbits, and rodents (*e.g.*, mice and rats). In certain embodiments, the patient, individual, or subject is a human.

[00143] By "monotherapy" is meant a therapeutic regimen that includes only a single therapeutic agent for the treatment of the target disease or condition, such as, for example, a complement-associated eye condition, during the course of the treatment period.

[00144] By "maintenance therapy" is meant a therapeutic regimen that is given to reduce the likelihood of disease recurrence or progression. Maintenance therapy can be provided for any length of time, including extended time periods up to the life-span of the subject. Maintenance therapy can be provided after initial therapy or in conjunction with initial or additional therapies. Dosages used for maintenance therapy can vary and can include diminished dosages as compared to dosages used for other types of therapy.

[00145] An "isolated antibody" is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (*e.g.*, SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (*e.g.*, ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, *see, e.g.*, Flatman et al., *J. Chromatogr. B* 848:79-87 (2007).

[00146] An "isolated nucleic acid" refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

[00147] "Isolated nucleic acid encoding an anti-Factor D antibody" refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

[00148] The terms "Factor D" and "fD" and "FD," as used herein, refers to any native, mature Factor D which results from processing of a Factor D precursor protein in a cell. The term includes Factor D from any vertebrate source, including mammals such as primates (*e.g.* humans and cynomolgus monkeys) and rodents (*e.g.*, mice and rats), unless otherwise indicated. The term also includes naturally occurring variants of Factor D, *e.g.*, splice variants or allelic variants. The amino acid sequence of an exemplary human Factor D prepropeptide is shown in SEQ ID NO: 104. The amino acid sequence of an exemplary mature human Factor D is amino acids 26-253 of SEQ ID NO: 104 (SEQ ID NO: 106).

[00149] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant

antibodies, *e.g.*, containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, 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 a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

[00150] A "naked antibody" refers to an antibody that is not conjugated to a heterologous moiety (*e.g.*, a cytotoxic moiety) or radiolabel. The naked antibody may be present in a pharmaceutical formulation.

[00151] "Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

[00152] A "vial" is a container suitable for holding a liquid or lyophilized preparation. In some embodiments, the vial is a single-use vial, e.g. a 20-cc single-use vial with a stopper.

[00153] A "port delivery system" or "PDS" is an implantable device for the eye with a refillable reservoir that allows delivery of a therapeutic agent over an extended period of time. The implant is constructed having a refill port in communication with a reservoir and a release control element that determines the rate of drug release into the eye. *See*, for example, US20100174272, 8,277,830; 8,399,006; 8,795,712; and 8,808,727.

[00154] A "small-bore needle" refers to a needle for injection of fluid composition of about 30, 29, 28, 27, 26, 25, 24, 23, or 22 gauge or higher, such as a 30 gauge needle. In some

embodiments, the small-bore needle has standard sized walls. In another embodiment, the small-bore needle has thin walls, which may be preferred for viscous solutions.

[00155] The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

[00156] "Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

[00157] In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will

not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

[00158] The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[00159] A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[00160] As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies of the invention are used to delay development of a disease or to slow the progression of a disease. In treatment of an immune related disease, a therapeutic agent may directly alter the magnitude of response of a component of the immune response, or render the disease more susceptible to treatment by other therapeutic agents, *e.g.*, antibiotics, antifungals, anti-inflammatory agents, chemotherapeutics, etc.

[00161] The "pathology" of a disease, such as a complement-associated eye condition, includes all phenomena that compromise the well-being of the patient. This includes, without limitation, abnormal or uncontrollable cell growth (neutrophilic, eosinophilic, monocytic, lymphocytic cells), antibody production, auto-antibody production, complement production, interference with the normal functioning of neighboring cells, release of cytokines or other secretory products at abnormal levels, suppression or aggravation of any inflammatory or immunological response, infiltration of inflammatory cells (neutrophilic, eosinophilic, monocytic, lymphocytic) into cellular spaces, *etc*.

[00162] Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

[00163] A drug that is administered "concurrently" with one or more other drugs is administered during the same treatment cycle, on the same day of treatment as the one or more

other drugs, and, optionally, at the same time as the one or more other drugs.

[00164] "Therapeutically effective amount" is the amount of a Factor D antagonist that is required to achieve a measurable improvement in the state, e.g., pathology, of the target disease or condition, such as, for example, a complement-associated eye condition.

[00165] The terms "long-acting delivery", "sustained-release" and "controlled release" are used generally to describe a delivery mechanism using formulation, dosage form, device or other types of technologies to achieve the prolonged or extended release or bioavailability of a therapeutic drug. It may refer to technologies that provide prolonged or extended release or bioavailability of the drug to the general systemic circulation or a subject or to local sites of action in a subject including, but not limited to, cells, tissues, organs, joints, regions, and the like. Furthermore, these terms may refer to a technology that is used to prolong or extend the release of the drug from a formulation or dosage form or they may refer to a technology used to extend or prolong the bioavailability or the pharmacokinetics or the duration of action of the drug to a subject or they may refer to a technology that is used to extend or prolong the pharmacodynamic effect elicited by a formulation. A "long-acting formulation," a "sustained release formulation," or a "controlled release formulation" is a pharmaceutical formulation, dosage form, or other technology that is used to provide long-acting delivery. In some embodiments, the controlled release is used to improve drug's local bioavailability, specifically ocular residence time in the context of ocular delivery. "Increased ocular residence time" refers to the post-delivery period during which the delivered ocular drug remains effective both in terms of quality (activity) and in terms of quantity (effective amount). In addition to or in lieu of high dose and controlled release, the drug can be modified post-translationally, such as via PEGylation, to achieve increased in vivo half-life.

[00166] The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (*See, e.g.*, Kindt et al. *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. *See, e.g.*, Portolano et al., *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

[00167] The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-

replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

II. COMPOSITIONS AND METHODS

[00168] In some embodiments, the invention is based, in part, on antibodies that bind to Factor D and conjugates comprising such antibodies. Antibodies and conjugates of the invention are useful, *e.g.*, for the treatment of complement-associated disorders, such as complement-associated eye conditions.

A. Exemplary Anti-Factor D Antibodies

[00169] Provided herein are isolated antibodies that bind to Factor D. In particular, provided herein are antibodies that bind Factor D with very high affinity, such as with a K_D of less than 100 pM, or less than 75 pM, or less than 50 pM, or less than 10 pM. Further, provided herein are antibodies, such as Fabs, that are highly soluble. For example, in some embodiments, a pharmaceutical formulation comprising a high concentration of an antibody provided herein contains no visible precipitate following storage at 4°C for at least 20 weeks. In some embodiments, a pharmaceutical formulation comprising a high concentration of an antibody provided herein comprises at least 200 mg/ml, or at least 230 mg/ml, or at least 250 mg/ml, or at least 270 mg/ml of the antibody (such as a Fab). In some embodiments, an antibody provided herein may be formulated at high concentration, such as at least 150 mg/ml, with a viscosity of less than 30 cP, or less than 20 cP, or less than 15 cP, or less than 10 cP at 25°C. In any of the embodiments described herein, the antibodies may be monoclonal antibodies. In some embodiments, the antibodies may be human antibodies, humanized antibodies, or chimeric antibodies. In any of the embodiments described herein, the antibodies may be Fab fragments.

[00170] In some embodiments, the invention provides an anti-Factor D antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence SYYMY (SEQ ID NO: 15); (b) HVR-H2 comprising the amino acid sequence X_4INPX_5X_6GX_7TNFNEKFKS (SEQ ID NO: 111), wherein X_4 is selected from E and W; X_5 is selected from T and Y; X_6 is selected from N, S, and Q; and X_7 is selected from G, D, and E; (c) HVR-H3 comprising the amino acid sequence EGGFAY (SEQ ID NO: 25); (d) HVR-L1 comprising the amino acid sequence KASQNVDTDVA (SEQ ID NO:9); (e) HVR-L2 comprising the amino acid sequence SASSRX_1S (SEQ ID NO: 108), wherein X_1 is selected from Y, K, and R; and (f) HVR-L3 comprising the amino acid sequence QQYX_3NYPLT (SEQ ID NO: 110), wherein X_3 is selected from N and E. In some

embodiments, the invention provides an anti-Factor D antibody comprising the sequence X_2SASSRX_1S (SEQ ID NO: 109; *i.e.*, comprises the amino acid X_2 immediately preceding HVR-L2), wherein X_1 is selected from Y, K, and R; X_2 is selected from Y, R, S, K, and Q. In some embodiments, X_2 is R. In some embodiments, X_1 is Y.

[00171] In some embodiments, the invention provides an anti-Factor D antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-H2 comprising an amino acid sequence selected from SEQ ID NOs: 16 to 24; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25, (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, (e) HVR-L2 comprising an amino acid sequence selected from SEQ ID NOs: 10 to 12, and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13 or 14.

[00172] In some embodiments, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence SYYMY (SEQ ID NO: 15); (b) HVR-H2 comprising the amino acid sequence X_4INPX_5X_6GX_7TNFNEKFKS (SEQ ID NO: 111), wherein X_4 is selected from E and W; X_5 is selected from T and Y; X_6 is selected from N, S, and Q; and X_7 is selected from G, D, and E; (c) HVR-H3 comprising the amino acid sequence EGGFAY (SEQ ID NO: 25). In some embodiments, the invention provides an anti-Factor D antibody comprising at least one, at least two, or all three HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-H2 comprising an amino acid sequence selected from SEQ ID NOs: 16 to 24; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25. In some embodiments, the invention provides an anti-Factor D antibody comprising at least one, at least two, or all three HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 16; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25. In some embodiments, the invention provides an anti-Factor D antibody comprising at least one, at least two, or all three HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 17; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25. In some embodiments, the invention provides an anti-Factor D antibody comprising at least one, at least two, or all three HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 18; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25. In some embodiments, the invention provides an anti-Factor D antibody comprising at least one, at least two, or all three HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-H2 comprising the amino acid sequence

of SEQ ID NO: 19; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25. In some embodiments, the invention provides an anti-Factor D antibody comprising at least one, at least two, or all three HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 20; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25. In some embodiments, the invention provides an anti-Factor D antibody comprising at least one, at least two, or all three HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 21; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25. In some embodiments, the invention provides an anti-Factor D antibody comprising at least one, at least two, or all three HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25. In some embodiments, the invention provides an anti-Factor D antibody comprising at least one, at least two, or all three HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 23; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25. In some embodiments, the invention provides an anti-Factor D antibody comprising at least one, at least two, or all three HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 24; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25.

[00173] In some embodiments, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence KASQNVDTDVA (SEQ ID NO:9); (b) HVR-L2 comprising the amino acid sequence SASSRX$_1$S (SEQ ID NO: 108), wherein X$_1$ is selected from Y, K, and R; and (c) HVR-L3 comprising the amino acid sequence QQYX$_3$NYPLT (SEQ ID NO: 110), wherein X$_3$ is selected from N and E. In some embodiments, the invention provides an anti-Factor D antibody comprising at least one, at least two, or all three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9; (b) HVR-L2 comprising an amino acid sequence selected from SEQ ID NOs: 10 to 12; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13 or 14. In some embodiments, the invention provides an anti-Factor D antibody comprising at least one, at least two, or all three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13. In some embodiments, the invention provides an anti-Factor D antibody comprising at least one, at least two, or all three HVRs selected from (a) HVR-L1 comprising the amino acid

sequence of SEQ ID NO: 9; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 11; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13. In some embodiments, the invention provides an anti-Factor D antibody comprising at least one, at least two, or all three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 12; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13. In some embodiments, the invention provides an anti-Factor D antibody comprising at least one, at least two, or all three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 14. In some embodiments, the invention provides an anti-Factor D antibody comprising at least one, at least two, or all three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13. In some embodiments, the invention provides an anti-Factor D antibody comprising at least one, at least two, or all three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 14. In any of the foregoing embodiments, the amino acid immediately preceding HVR-L2 may be selected from Y, R, S, K, and Q. In some embodiments, the amino acid immediately preceding HVR-L2 is R.

[00174] In some embodiments, an anti-Factor D antibody is provided, wherein the antibody comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence SYYMY (SEQ ID NO: 15), (ii) HVR-H2 comprising the amino acid sequence X_4INPX_5X_6GX_7TNFNEKFKS (SEQ ID NO: 111), wherein X_4 is selected from E and W; X_5 is selected from T and Y; X_6 is selected from N, S, and Q; and X_7 is selected from G, D, and E, and (iii) HVR-H3 comprising the amino acid sequence EGGFAY (SEQ ID NO: 25); and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence KASQNVDTDVA (SEQ ID NO:9), (ii) HVR-L2 comprising the amino acid sequence SASSRX_1S (SEQ ID NO: 108), wherein X_1 is selected from Y, K, and R, and (c) HVR-L3 comprising the amino acid sequence QQYX_3NYPLT (SEQ ID NO: 110), wherein X_3 is selected from N and E.

[00175] In some embodiments, an anti-Factor D antibody is provided, wherein the antibody comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence SYYMY (SEQ ID

NO: 15), (ii) HVR-H2 comprising the amino acid sequence X_4INPX_5X_6GX_7TNFNEKFKS (SEQ ID NO: 111), wherein X_4 is selected from E and W; X_5 is selected from T and Y; X_6 is selected from N, S, and Q; and X_7 is selected from G, D, and E, and (iii) HVR-H3 comprising the amino acid sequence EGGFAY (SEQ ID NO: 25); and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence KASQNVDTDVA (SEQ ID NO:9), (ii) HVR-L2 comprising the amino acid sequence SASSRX_1S (SEQ ID NO: 108), wherein X_1 is selected from Y, K, and R, and (c) HVR-L3 comprising the amino acid sequence QQYX_3NYPLT (SEQ ID NO: 110), wherein X_3 is selected from N and E. In some embodiments, the invention provides an anti-Factor D antibody comprising the sequence X_2SASSRX_1S (*i.e.*, comprises the amino acid X_2 immediately preceding HVR-L2), wherein X_1 is selected from Y, K, and R; X_2 is selected from Y, R, S, K, and Q. In some embodiments, X_2 is R. In some embodiments, X_1 is Y.

[00176] In some embodiments, an anti-Factor D antibody is provided, wherein the antibody comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 16, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 25; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13.

[00177] In some embodiments, an anti-Factor D antibody is provided, wherein the antibody comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 20, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 25; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13.

[00178] In some embodiments, an anti-Factor D antibody is provided, wherein the antibody comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 17, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 25; and (b) a VL domain

comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13.

[00179] In some embodiments, an anti-Factor D antibody is provided, wherein the antibody comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 18, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 25; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13.

[00180] In some embodiments, an anti-Factor D antibody is provided, wherein the antibody comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 25; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13.

[00181] In some embodiments, an anti-Factor D antibody is provided, wherein the antibody comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 21, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 25; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13.

[00182] In some embodiments, an anti-Factor D antibody is provided, wherein the antibody comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22, and (iii) HVR-H3

comprising an amino acid sequence selected from SEQ ID NO: 25; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13.

[00183] In some embodiments, an anti-Factor D antibody is provided, wherein the antibody comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 23, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 25; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13.

[00184] In some embodiments, an anti-Factor D antibody is provided, wherein the antibody comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 24, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 25; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13.

[00185] In some embodiments, an anti-Factor D antibody is provided, wherein the antibody comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 16, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 25; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 11, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13.

[00186] In some embodiments, an anti-Factor D antibody is provided, wherein the antibody comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15,

(ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 16, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 25; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 12, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13.

[00187] In some embodiments, an anti-Factor D antibody is provided, wherein the antibody comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 16, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 25; and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 14.

[00188] In some embodiments, an anti-Factor D antibody is provided, comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 16; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13.

[00189] In some embodiments, an anti-Factor D antibody is provided, comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 20; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13.

[00190] In some embodiments, an anti-Factor D antibody is provided, comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 17; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13.

[00191] In some embodiments, an anti-Factor D antibody is provided, comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-H2 comprising the

amino acid sequence of SEQ ID NO: 18; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13.

[00192] In some embodiments, an anti-Factor D antibody is provided, comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13.

[00193] In some embodiments, an anti-Factor D antibody is provided, comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 21; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13.

[00194] In some embodiments, an anti-Factor D antibody is provided, comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13.

[00195] In some embodiments, an anti-Factor D antibody is provided, comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 23; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13.

[00196] In some embodiments, an anti-Factor D antibody is provided, comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 16; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 11; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13.

[00197] In some embodiments, an anti-Factor D antibody is provided, comprising (a)

HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 16; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 12; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 14.

[00198] In some embodiments, an anti-Factor D antibody is provided, comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 16; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 14.

[00199] In some embodiments, an anti-Factor D antibody is provided, comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 24; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13.

[00200] In any of the embodiments herein, an anti-Factor D antibody may be humanized. In some embodiments, an anti-Factor D antibody comprises HVRs as in any of the above embodiments, and further comprises a human acceptor framework, *e.g.* a human immunoglobulin framework or a human consensus framework. In certain embodiments, the human acceptor framework is the human VL kappa I consensus (VL_{KI}) framework and/or the VH framework VH_1. In certain embodiments, the human acceptor framework is the human VL kappa I consensus (VL_{KI}) framework and/or the VH framework VH_1 comprising any one of the mutations described herein.

[00201] In another aspect, an anti-Factor D antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from SEQ ID NOs: 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, and 63. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to an amino acid sequence selected from SEQ ID NOs: 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, and 63 contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-Factor D antibody comprising that sequence retains the ability to bind to Factor D. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 27, 29, 31,

33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, or 63. In certain embodiments, a total of 1 to 5 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, or 63. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (*i.e.*, in the FRs). Optionally, the anti-Factor D antibody comprises the VH sequence of SEQ ID NO: 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, or 63, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two or three HVRs selected from: (a) HVR-H1 comprising the amino acid sequence SYYMY (SEQ ID NO: 15), (b) HVR-H2 comprising the amino acid sequence X_4INPX$_5$X$_6$GX$_7$TNFNEKFKS (SEQ ID NO: 111), wherein X_4 is selected from E and W; X_5 is selected from T and Y; X_6 is selected from N, S, and Q; and X_7 is selected from G, D, and E, and (c) HVR-H3 comprising the amino acid sequence EGGFAY (SEQ ID NO: 25). In some embodiments, the VH comprises one, two or three HVRs selected from: (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 16 or 20; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25.

[00202] In another aspect, an anti-Factor D antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from SEQ ID NOs: 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, and 62. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to an amino acid sequence selected from SEQ ID NOs: 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, and 62 contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-Factor D antibody comprising that sequence retains the ability to bind to Factor D. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, or 62. In certain embodiments, a total of 1 to 5 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, or 62. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (*i.e.*, in the FRs). Optionally, the anti-Factor D antibody comprises the VL sequence of SEQ ID NO: 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, or 62, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising the amino acid sequence KASQNVDTDVA (SEQ ID NO:9); (b) HVR-L2 comprising the amino acid sequence SASSRX1S (SEQ ID NO: 108), wherein X1 is selected from Y, K, and R; and (c) HVR-L3

comprising the amino acid sequence QQYX3NYPLT (SEQ ID NO: 110), wherein X3 is selected from N and E. In some embodiments, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:9; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13. In any of the foregoing embodiments, the amino acid immediately preceding HVR-L2 may be selected from Y, R, S, K, and Q. In some embodiments, the amino acid immediately preceding HVR-L2 is R.

[00203] In another aspect, an anti-Factor D antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above.

[00204] In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 35 and SEQ ID NO: 34, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 39 and SEQ ID NO: 38, respectively, including post-translational modifications of those sequences.

[00205] In a further aspect, provided herein are antibodies that bind to the same epitope as an anti-Factor D antibody. For example, in certain embodiments, an antibody is provided that binds to the same epitope as an anti-Factor D antibody comprising a VH sequence of SEQ ID NO: 35 and a VL sequence of SEQ ID NO: 34, respectively. In certain embodiments, an antibody is provided that binds to the same epitope as an anti-Factor D antibody comprising a VH sequence of SEQ ID NO: 39 and a VL sequence of SEQ ID NO: 38, respectively.

[00206] Provided herein are antibodies comprising a light chain variable domain comprising the HVR1-LC, HVR2-LC and HVR3-LC sequence according to Kabat numbering as depicted in Figures 1A-C and a heavy chain variable domain comprising the HVR1-HC, HVR2-HC and HVR3-HC sequence according to Kabat numbering as depicted in Figures 2A-C. In some embodiments, the antibody comprises a light chain variable domain comprising the HVR1-LC, HVR2-LC and/or HVR3-LC sequence, and the FR1-LC, FR2-LC, FR3-LC and/or FR4-LC sequence as depicted in Figures 1A-C. In some embodiments, the antibody comprises a heavy chain variable domain comprising the HVR1-HC, HVR2-HC and/or HVR3-HC sequence, and the FR1-HC, FR2-HC, FR3-HC and/or FR4-HC sequence as depicted in Figures 2A-C.

[00207] In a further aspect of the invention, an anti-Factor D antibody according to any of the above embodiments is a monoclonal antibody, including a human antibody. In some embodiments, an anti-Factor D antibody is an antibody fragment, *e.g.*, a Fv, Fab, Fab-SH, Fab'-SH, Fab', Fab-C, Fab'-C, Fab'-C-SH, Fab-C-SH, scFv, diabody, or $F(ab')_2$ fragment. In another embodiment, the antibody is a substantially full length antibody, *e.g.*, an IgG1 antibody, IgG2a

antibody or other antibody class or isotype as defined herein. In some embodiments, the anti-Factor D antibody is a Fab.

[00208] In a further aspect, an anti-Factor D antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described below.

1. Antibody Affinity

[00209] In certain embodiments, an antibody provided herein has a dissociation constant (Kd) of ≤ 1μM, ≤ 100 nM, ≤ 50 nM, ≤ 10 nM, ≤ 5 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM, and optionally is ≥ 10^{-13} M. (*e.g.* 10^{-8} M or less, *e.g.* from 10^{-8} M to 10^{-13} M, *e.g.*, from 10^{-9} M to 10^{-13} M).

[00210] In some embodiments, Kd is measured by a radiolabeled antigen binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen as described by the following assay. Solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (*see, e.g.*, Chen et al., *J. Mol. Biol.* 293:865-881(1999)). To establish conditions for the assay, MICROTITER® multi-well plates (Thermo Scientific) are coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest (*e.g.*, consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., *Cancer Res.* 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (*e.g.*, about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (*e.g.*, for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20®) in PBS. When the plates have dried, 150 μl/well of scintillant (MICROSCINT-20 ™; Packard) is added, and the plates are counted on a TOPCOUNT ™ gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

[00211] According to another embodiment, Kd is measured using surface plasmon resonance assays using a BIACORE®-2000 or a BIACORE ®-3000 (BIAcore, Inc., Piscataway, NJ) at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIACORE, Inc.) are activated with *N*-ethyl-*N'*- (3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate,

pH 4.8, to 5 μg/ml (~0.2 μM) before injection at a flow rate of 5 μl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25°C at a flow rate of approximately 25 μl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio k_{off}/k_{on}. *See, e.g.*, Chen et al., *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds 10^6 M^{-1} s^{-1} by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

2. Antibody Fragments

[00212] In certain embodiments, an antibody provided herein is an antibody fragment. Antibody fragments include, but are not limited to, Fv, Fab, Fab-SH, Fab'-SH, Fab', Fab-C, Fab'-C, Fab'-C-SH, Fab-C-SH, scFv, diabody, or $F(ab')_2$ fragments, and other fragments described herein. For a review of certain antibody fragments, see Hudson et al. *Nat. Med.* 9:129-134 (2003). For a review of scFv fragments, *see, e.g.*, Pluckthün, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and $F(ab')_2$ fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046. Antibody fragments with free thiol groups may be indicated with an "-SH." For example, Fab-SH (including Fab-C-SH) is the designation for Fab in which at least one cysteine residue of the constant domains bears a free thiol group.

[00213] In some embodiments, the C-terminus of the heavy chain of a Fab fragment ends in the amino acids "CDKTHT" (SEQ ID NO: 165), "CDKTHL" (SEQ ID NO: 166), "CDKTH" (SEQ ID NO: 167), "CDKT" (SEQ ID NO: 168), "CDK," or "CD." In some embodiments, the C-terminus of the heavy chain of the Fab fragment ends in the sequence CDKTH**X** (SEQ ID NO: 169), wherein **X** is any amino acid except T. Truncations and/or mutations at the C

terminus may be able to reduce or eliminate AHA-reactivity against the Fab, without compromising thermostability or expression. In some embodiments, the C-terminus of the heavy chain of a Fab fragment ends in the amino acids "CDKTHTC" (SEQ ID NO: 170), "CDKTHTCPPC" (SEQ ID NO: 171), "CDKTHTCPPS" (SEQ ID NO: 172), "CDKTHTSPPC" (SEQ ID NO: 173), "CDKTHTAPPC" (SEQ ID NO: 174), "CDKTHTSGGC" (SEQ ID NO: 175), or "CYGPPC" (SEQ ID NO: 176). In some such embodiments, a free cysteine in the C-terminal amino acids may be amenable to conjugation, for example, to a polymer such as PEG. In some embodiments, a Fab fragment comprises a heavy chain constant region selected from SEQ ID NOs: 113 (CDKTHT (SEQ ID NO: 165)), 128 to 132 (CDKTHL (SEQ ID NO: 166), CDKTHTC (SEQ ID NO: 170), CDKTHTCPPC (SEQ ID NO: 171), CDKTHTCPPS (SEQ ID NO: 172), CDKTHTSPPC (SEQ ID NO: 173)), 154 to 156 (APPC (SEQ ID NO: 177), SGGC (SEQ ID NO: 178), CYGPPC (SEQ ID NO: 176)), and 134 to 137 (CDKTH (SEQ ID NO: 167), CDKT (SEQ ID NO: 168), CDK ,CD). In some embodiments, a Fab is an IgG2 Fab fragment comprising a heavy chain constant region of SEQ ID NO: 138 (VERK (SEQ ID NO: 179)) or IgG2 Fab-C fragment comprising a heavy chain constant region of SEQ ID NO: 157 (VERKC (SEQ ID NO: 180)). In some embodiments, a Fab is an IgG4 Fab fragment comprising a heavy chain constant region selected from SEQ ID NOs: 139 (KYGPP (SEQ ID NO: 181)), SEQ ID NO: 163 (KYGP (SEQ ID NO: 182)), and 159-161 (KYG, KY, K), or an IgG4 Fab-C fragment comprising a heavy chain constant region of SEQ ID NO: 158 (KYGPPC (SEQ ID NO: 183)). As an alternative to truncating and/or mutation at the C terminus, to avoid pre-existing anti-hinge antibody (PE-AHA) responses, IgG2 or IgG4 Fab fragments may be used, since these do not show PE-AHA response.

[00214] Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. *See,* for example, EP 404,097; WO 1993/01161; Hudson et al., *Nat. Med.* 9:129-134 (2003); and Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., *Nat. Med.* 9:129-134 (2003).

[00215] Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; *see, e.g.*, U.S. Patent No. 6,248,516 B1).

[00216] Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (*e.g. E. coli* or phage), as described herein.

3. Chimeric and Humanized Antibodies

[00217] In certain embodiments, an antibody provided herein is a chimeric antibody.

Certain chimeric antibodies are described, *e.g.*, in U.S. Patent No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (*e.g.*, a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

[00218] In certain embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, *e.g.*, CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (*e.g.*, the antibody from which the HVR residues are derived), *e.g.*, to restore or improve antibody specificity or affinity.

[00219] Humanized antibodies and methods of making them are reviewed, *e.g.*, in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, *e.g.*, in Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Nat'l Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, *Methods* 36:25-34 (2005) (describing SDR (a-CDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., *Methods* 36:43-60 (2005) (describing "FR shuffling"); and Osbourn et al., *Methods* 36:61-68 (2005) and Klimka et al., *Br. J. Cancer*, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

[00220] Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (*see, e.g.*, Sims et al. *J. Immunol.* 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (*see, e.g.*, Carter et al. *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); and Presta et al. *J. Immunol.*, 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (*see, e.g.*, Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (*see, e.g.*, Baca et al., *J. Biol. Chem.* 272:10678-10684 (1997) and Rosok et al., *J. Biol. Chem.* 271:22611-22618 (1996)).

4. Human Antibodies

[00221] In certain embodiments, an antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001) and Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008).

[00222] Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, *Nat. Biotech.* 23:1117-1125 (2005). See also, *e.g.*, U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Patent No. 5,770,429 describing HUMAB® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VELOCIMOUSE® technology). Human variable regions from intact antibodies generated by such animals may be further modified, *e.g.*, by combining with a different human constant region.

[00223] Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (*See, e.g.*, Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., *J. Immunol.*, 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, *Xiandai Mianyixue*, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3):185-91 (2005).

[00224] Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for

selecting human antibodies from antibody libraries are described below.

5. Library-Derived Antibodies

[00225] Antibodies of the invention may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, *e.g.*, in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, *e.g.*, in the McCafferty et al., *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132(2004).

[00226] In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (*e.g.*, from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths et al., *EMBO J,* 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

[00227] Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

6. Multispecific Antibodies

[00228] In certain embodiments, an antibody provided herein is a multispecific antibody, *e.g.* a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain embodiments, one of the binding

specificities is for Factor D and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of Factor D. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express Factor D. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

[00229] Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, *Nature* 305: 537 (1983)), WO 93/08829, and Traunecker et al., *EMBO J.* 10: 3655 (1991)), and "knob-in-hole" engineering (*see, e.g.*, U.S. Patent No. 5,731,168). The term "knob-into-hole" or "KnH" technology as used herein refers to the technology directing the pairing of two polypeptides together in vitro or in vivo by introducing a protuberance (knob) into one polypeptide and a cavity (hole) into the other polypeptide at an interface in which they interact. For example, KnHs have been introduced in the Fc:Fc binding interfaces, CL:CH1 interfaces or VH/VL interfaces of antibodies (see, e.g., US 2011/0287009, US2007/0178552, WO 96/027011, WO 98/050431, Zhu et al., 1997, Protein Science 6:781-788, and WO2012/106587). In some embodiments, KnHs drive the pairing of two different heavy chains together during the manufacture of multispecific antibodies. For example, multispecific antibodies having KnH in their Fc regions can further comprise single variable domains linked to each Fc region, or further comprise different heavy chain variable domains that pair with similar or different light chain variable domains. KnH technology can be also be used to pair two different receptor extracellular domains together or any other polypeptide sequences that comprises different target recognition sequences (e.g., including affibodies, peptibodies and other Fc fusions).

[00230] The term "knob mutation" as used herein refers to a mutation that introduces a protuberance (knob) into a polypeptide at an interface in which the polypeptide interacts with another polypeptide. In some embodiments, the other polypeptide has a hole mutation.

[00231] The term "hole mutation" as used herein refers to a mutation that introduces a cavity (hole) into a polypeptide at an interface in which the polypeptide interacts with another polypeptide. In some embodiments, the other polypeptide has a knob mutation.

[00232] A brief nonlimiting discussion is provided below.

[00233] A "protuberance" refers to at least one amino acid side chain which projects from the interface of a first polypeptide and is therefore positionable in a compensatory cavity in the adjacent interface (i.e. the interface of a second polypeptide) so as to stabilize the heteromultimer, and thereby favor heteromultimer formation over homomultimer formation, for example. The protuberance may exist in the original interface or may be introduced synthetically (e.g., by altering nucleic acid encoding the interface). In some embodiments, nucleic acid

encoding the interface of the first polypeptide is altered to encode the protuberance. To achieve this, the nucleic acid encoding at least one "original" amino acid residue in the interface of the first polypeptide is replaced with nucleic acid encoding at least one "import" amino acid residue which has a larger side chain volume than the original amino acid residue. It will be appreciated that there can be more than one original and corresponding import residue. The side chain volumes of the various amino residues are shown, for example, in Table 1 of US2011/0287009. A mutation to introduce a "protuberance" may be referred to as a "knob mutation."

[00234] In some embodiments, import residues for the formation of a protuberance are naturally occurring amino acid residues selected from arginine (R), phenylalanine (F), tyrosine (Y) and tryptophan (W). In some embodiments, an import residue is tryptophan or tyrosine. In some embodiment, the original residue for the formation of the protuberance has a small side chain volume, such as alanine, asparagine, aspartic acid, glycine, serine, threonine or valine.

[00235] A "cavity" refers to at least one amino acid side chain which is recessed from the interface of a second polypeptide and therefore accommodates a corresponding protuberance on the adjacent interface of a first polypeptide. The cavity may exist in the original interface or may be introduced synthetically (e.g. by altering nucleic acid encoding the interface). In some embodiments, nucleic acid encoding the interface of the second polypeptide is altered to encode the cavity. To achieve this, the nucleic acid encoding at least one "original" amino acid residue in the interface of the second polypeptide is replaced with DNA encoding at least one "import" amino acid residue which has a smaller side chain volume than the original amino acid residue. It will be appreciated that there can be more than one original and corresponding import residue. In some embodiments, import residues for the formation of a cavity are naturally occurring amino acid residues selected from alanine (A), serine (S), threonine (T) and valine (V). In some embodiments, an import residue is serine, alanine or threonine. In some embodiments, the original residue for the formation of the cavity has a large side chain volume, such as tyrosine, arginine, phenylalanine or tryptophan. A mutation to introduce a "cavity" may be referred to as a "hole mutation."

[00236] The protuberance is "positionable" in the cavity which means that the spatial location of the protuberance and cavity on the interface of a first polypeptide and second polypeptide respectively and the sizes of the protuberance and cavity are such that the protuberance can be located in the cavity without significantly perturbing the normal association of the first and second polypeptides at the interface. Since protuberances such as Tyr, Phe and Trp do not typically extend perpendicularly from the axis of the interface and have preferred conformations, the alignment of a protuberance with a corresponding cavity may, in some instances, rely on modeling the protuberance/cavity pair based upon a three-dimensional

structure such as that obtained by X-ray crystallography or nuclear magnetic resonance (NMR). This can be achieved using widely accepted techniques in the art.

[00237] In some embodiments, a knob mutation in an IgG1 constant region is T366W (EU numbering). In some embodiments, a hole mutation in an IgG1 constant region comprises one or more mutations selected from T366S, L368A and Y407V (EU numbering). In some embodiments, a hole mutation in an IgG1 constant region comprises T366S, L368A and Y407V (EU numbering).

[00238] In some embodiments, a knob mutation in an IgG4 constant region is T366W (EU numbering). In some embodiments, a hole mutation in an IgG4 constant region comprises one or more mutations selected from T366S, L368A, and Y407V (EU numbering). In some embodiments, a hole mutation in an IgG4 constant region comprises T366S, L368A, and Y407V (EU numbering).

[00239] Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (*see, e.g.*, US Patent No. 4,676,980, and Brennan et al., *Science*, 229: 81 (1985)); using leucine zippers to produce bi-specific antibodies (*see, e.g.*, Kostelny et al., *J. Immunol.*, 148(5):1547-1553 (1992)); using "diabody" technology for making bispecific antibody fragments (*see, e.g.*, Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993)); and using single-chain Fv (sFv) dimers (*see,e.g.* Gruber et al., *J. Immunol.*, 152:5368 (1994)); and preparing trispecific antibodies as described, *e.g.*, in Tutt et al. *J. Immunol.* 147: 60 (1991).

[00240] Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (*see, e.g.* US 2006/0025576A1).

[00241] The antibody or fragment herein also includes a "Dual Acting FAb" or "DAF" comprising an antigen binding site that binds to Factor D as well as another, different antigen (*see,* US 2008/0069820, for example).

7. Antibody Variants

[00242] In certain embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, *e.g.*,

antigen-binding.

a) Substitution, Insertion, and Deletion Variants

[00243] In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in Table 1 under the heading of "preferred substitutions." More substantial changes are provided in Table 1 under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, *e.g.*, retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

TABLE 1

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

[00244] Amino acids may be grouped according to common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

[00245] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[00246] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.* a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (*e.g.*, improvements) in certain biological properties (*e.g.*, increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, *e.g.*, using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (*e.g.* binding affinity).

[00247] Alterations (*e.g.*, substitutions) may be made in HVRs, *e.g.*, to improve antibody affinity. Such alterations may be made in HVR "hotspots," *i.e.*, residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (*see, e.g.*, Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, *e.g.*, in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, (2001).) In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (*e.g.*, error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (*e.g.*, 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, *e.g.*, using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[00248] In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the

antibody to bind antigen. For example, conservative alterations (*e.g.*, conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR "hotspots" or SDRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[00249] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (*e.g.*, charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (*e.g.*, alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex is used to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[00250] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (*e.g.* for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

b) Glycosylation variants

[00251] In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[00252] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. *See, e.g.*, Wright et al. *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, *e.g.*, mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain

improved properties.

[00253] In some embodiments, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e. g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, *i.e.*, between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. *See, e.g.*, US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8,* knockout CHO cells (*see, e.g.*, Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

[00254] Antibodies variants are further provided with bisected oligosaccharides, *e.g.*, in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, *e.g.*, in WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana *et al.*). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, *e.g.*, in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

c) Fc region variants

[00255] In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (*e.g.*, a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (*e.g.* a substitution) at one or more amino acid positions.

[00256] In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express Fc(RIII only, whereas monocytes express Fc(RI, Fc(RII and Fc(RIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (*see, e.g.* Hellstrom, I. et al. *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (see Bruggemann, M. et al., *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (*see,* for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in an animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. *See, e.g.*, C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (*see,* for example, Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996); Cragg, M.S. et al., *Blood* 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half life determinations can also be performed using methods known in the art (*see, e.g.*, Petkova, S.B. et al., *Int'l. Immunol.* 18(12):1759-1769 (2006)).

[00257] In some embodiments, one or more amino acid modifications may be introduced

into the Fc portion of the antibody provided herein in order to increase IgG binding to the neonatal Fc receptor. In certain embodiments, the antibody comprises the following three mutations according to EU numbering: M252Y, S254T, and T256E (the "YTE mutation") (US Patent No. 8,697,650; see also Dall'Acqua et al., Journal of Biological Chemistry 281(33):23514-23524 (2006). In certain embodiments, the YTE mutation does not affect the ability of the antibody to bind to its cognate antigen. In certain embodiments, the YTE mutation increases the antibody's serum half-life compared to the native (i.e., non-YTE mutant) antibody. In some embodiments, the YTE mutation increases the serum half-life of the antibody by 3-fold compared to the native (i.e., non-YTE mutant) antibody. In some embodiments, the YTE mutation increases the serum half-life of the antibody by 2-fold compared to the native (i.e., non-YTE mutant) antibody. In some embodiments, the YTE mutation increases the serum half-life of the antibody by 4-fold compared to the native (i.e., non-YTE mutant) antibody. In some embodiments, the YTE mutation increases the serum half-life of the antibody by at least 5-fold compared to the native (i.e., non-YTE mutant) antibody. In some embodiments, the YTE mutation increases the serum half-life of the antibody by at least 10-fold compared to the native (i.e., non-YTE mutant) antibody. See, e.g., US Patent No. 8,697,650; see also Dall'Acqua et al., Journal of Biological Chemistry 281(33):23514-23524 (2006).

[00258] In certain embodiments, the YTE mutant provides a means to modulate antibody-dependent cell-mediated cytotoxicity (ADCC) activity of the antibody. In certain embodiments, the YTEO mutant provides a means to modulate ADCC activity of a humanized IgG antibody directed against a human antigen. See, e.g., US Patent No. 8,697,650; see also Dall'Acqua et al., Journal of Biological Chemistry 281(33):23514-23524 (2006).

[00259] In certain embodiments, the YTE mutant allows the simultaneous modulation of serum half-life, tissue distribution, and antibody activity (e.g., the ADCC activity of an IgG antibody). See, e.g., US Patent No. 8,697,650; see also Dall'Acqua et al., Journal of Biological Chemistry 281(33):23514-23524 (2006).

[00260] Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

[00261] In certain embodiments, the proline at position 329 (EU numbering) (P329) of a wild-type human Fc region is substituted with glycine or arginine or an amino acid residue large enough to destroy the proline sandwich within the Fc/Fcγ receptor interface, that is formed between the P329 of the Fc and tryptophane residues W87 and W110 of FcγRIII (Sondermann

et al.: Nature 406, 267-273 (20 July 2000)). In a further embodiment, at least one further amino acid substitution in the Fc variant is S228P, E233P, L234A, L235A, L235E, N297A, N297D, or P331S and still in another embodiment said at least one further amino acid substitution is L234A and L235A of the human IgG1 Fc region or S228P and L235E of the human IgG4 Fc region, all according to EU numbering (U.S. Patent No. 8,969,526 which is incorporated by reference in its entirety).

[00262] In certain embodiments, a polypeptide comprises the Fc variant of a wild-type human IgG Fc region wherein the polypeptide has P329 of the human IgG Fc region substituted with glycine and wherein the Fc variant comprises at least two further amino acid substitutions at L234A and L235A of the human IgG1 Fc region or S228P and L235E of the human IgG4 Fc region, and wherein the residues are numbered according to the EU numbering (U.S. Patent No. 8,969,526 which is incorporated by reference in its entirety). In certain embodiments, the polypeptide comprising the P329G, L234A and L235A (EU numbering) substitutions exhibit a reduced affinity to the human FcγRIIIA and FcγRIIA, for down-modulation of ADCC to at least 20% of the ADCC induced by the polypeptide comprising the wildtype human IgG Fc region, and/or for down-modulation of ADCP (U.S. Patent No. 8,969,526 which is incorporated by reference in its entirety).

[00263] In a specific embodiment the polypeptide comprising an Fc variant of a wildtype human Fc polypeptide comprises a triple mutation: an amino acid substitution at position Pro329, a L234A and a L235A mutation according to EU numbering (P329 / LALA) (U.S. Patent No. 8,969,526 which is incorporated by reference in its entirety). In specific embodiments, the polypeptide comprises the following amino acid substitutions: P329G, L234A, and L235A according to EU numbering.

[00264] Certain antibody variants with improved or diminished binding to FcRs are described. (*See, e.g.*, U.S. Patent No. 6,737,056; WO 2004/056312, and Shields et al., *J. Biol. Chem.* 9(2): 6591-6604 (2001).)

[00265] In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, *e.g.*, substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).

[00266] In some embodiments, alterations are made in the Fc region that result in altered (*i.e.*, either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), *e.g.*, as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie et al. *J. Immunol.* 164: 4178-4184 (2000).

[00267] Antibodies with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al.,

J. Immunol. 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, *e.g.*, substitution of Fc region residue 434 (US Patent No. 7,371,826).

[00268] See also Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

d) Cysteine engineered antibody variants

[00269] In certain embodiments, it may be desirable to create cysteine engineered antibodies, *e.g.*, a "THIOMAB™," in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at sites of the antibody that are available for conjugation. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties to create an conjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: K149 (Kabat numbering) of the light chain; V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; A140 (EU numbering) of the heavy chain; L174 (EU numbering) of the heavy chain; Y373 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. In specific embodiments, the antibodies described herein comprise the HC-A140C (EU numbering) cysteine substitution. In specific embodiments, the antibodies described herein comprise the LC-K149C (Kabat numbering) cysteine substitution. In specific embodiments, the antibodies described herein comprise the HC-A118C (EU numbering) cysteine substitution. Cysteine engineered antibodies may be generated as described, *e.g.*, in U.S. Patent No. 7,521,541.

[00270] In certain embodiments, the antibody comprises one of the following heavy chain cysteine substitutions:

Table 2A:

Chain (HC/LC)	Residue	EU Mutation Site #	Kabat Mutation Site #
HC	T	114	110
HC	A	140	136
HC	L	174	170
HC	L	179	175
HC	T	187	183

HC	T	209	205
HC	V	262	258
HC	G	371	367
HC	Y	373	369
HC	E	382	378
HC	S	424	420
HC	N	434	430
HC	Q	438	434

[00271] Suitable residues in the heavy chain for cysteine substitution in antibody Fab fragments include positions 110, 136, 170, 175, 183, and 205 (Kabat numbering).

[00272] In certain embodiments, the antibody comprises one of the following light chain cysteine substitutions:

Table 2B:

Chain (HC/LC)	Residue	EU Mutation Site #	Kabat Mutation Site #
LC	I	106	106
LC	R	108	108
LC	R	142	142
LC	K	149	149
LC	V	205	205

e) Antibody Derivatives

[00273] In certain embodiments, an antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the

particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

[00274] In another embodiment, conjugates of an antibody and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In some embodiments, the nonproteinaceous moiety is a carbon nanotube (Kam et al., *Proc. Natl. Acad. Sci. USA* 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

B. Recombinant Methods and Compositions

[00275] Antibodies may be produced using recombinant methods and compositions, *e.g.*, as described in U.S. Patent No. 4,816,567. In some embodiments, isolated nucleic acid encoding an anti-Factor D antibody described herein is provided. Such nucleic acid may encode an amino acid sequence comprising the VL and/or an amino acid sequence comprising the VH of the antibody (*e.g.*, the light and/or heavy chains of the antibody). In a further embodiment, one or more vectors (*e.g.*, expression vectors) comprising such nucleic acid are provided. In a further embodiment, a host cell comprising such nucleic acid is provided. In one such embodiment, a host cell comprises (*e.g.*, has been transformed with): (1) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and an amino acid sequence comprising the VH of the antibody, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the VH of the antibody. In some embodiments, the host cell is eukaryotic, *e.g.* a Chinese Hamster Ovary (CHO) cell or lymphoid cell (*e.g.*, Y0, NS0, Sp20 cell). In some embodiments, a method of making an anti-Factor D antibody is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

[00276] For recombinant production of an anti-Factor D antibody, nucleic acid encoding an antibody, *e.g.*, as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

[00277] Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For

expression of antibody fragments and polypeptides in bacteria, *see, e.g.*, U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523. (See also Charlton, *Methods in Molecular Biology, Vol. 248* (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003), pp. 245-254, describing expression of antibody fragments in *E. coli*.) After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

[00278] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern. See Gerngross, *Nat. Biotech.* 22:1409-1414 (2004), and Li et al., *Nat. Biotech.* 24:210-215 (2006).

[00279] Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells.

[00280] Plant cell cultures can also be utilized as hosts. *See, e.g.*, US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES™ technology for producing antibodies in transgenic plants).

[00281] Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, *e.g.*, in Graham et al., *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, *e.g.*, in Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, *e.g.*, in Mather et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including $DHFR^-$ CHO cells (Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, *see, e.g.*, Yazaki and Wu, *Methods in Molecular Biology, Vol. 248* (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003).

C. Assays

[00282] Anti-Factor D antibodies provided herein may be identified, screened for, or

characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

[00283] In some embodiments, an antibody of the invention is tested for its antigen binding activity, *e.g.*, by known methods such as ELISA, BIACore®, FACS, or Western blot.

[00284] In another aspect, competition assays may be used to identify an antibody that competes with any of the antibodies described herein for binding to Factor D. In certain embodiments, such a competing antibody binds to the same epitope (*e.g.*, a linear or a conformational epitope) that is bound by an antibody described herein. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in *Methods in Molecular Biology* vol. 66 (Humana Press, Totowa, NJ).

[00285] In an exemplary competition assay, immobilized Factor D is incubated in a solution comprising a first labeled antibody that binds to Factor D (*e.g.*, any of the antibodies described herein) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to Factor D. The second antibody may be present in a hybridoma supernatant. As a control, immobilized Factor D is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to Factor D, excess unbound antibody is removed, and the amount of label associated with immobilized Factor D is measured. If the amount of label associated with immobilized Factor D is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to Factor D. See Harlow and Lane (1988) *Antibodies: A Laboratory Manual* ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

[00286] To determine whether an anti-Factor D antibody, or variant or fragment thereof (e.g. antigen-binding fragment) is capable of binding to Factor D and exerting a biological effect, for example, inhibition of alternative pathway hemolysis, a hemolytic inhibition assay, e.g., using rabbit RBCs may be used. Such hemolytic inhibition may be determined using standard assays (Kostavasili et al. (1997) *J of Immunology* 158:1763-72; Wiesmann et al. (2006) *Nature* 444:159-60). Activation of complement in such assays may be initiated with serum or plasma. Appropriate concentrations of Factor D in serum or plasma (Pascual et al. (1998) *Kidney International* 34:529-536; Complement Facts Book, Bernard J. Morley and Mark J. Walport, editors, Academic Press (2000); Barnum et al. (1984) *J. Immunol. Methods*, 67: 303-309) can be routinely determined according to methods known in the art, including those that have been described in references such as Pascual et al. (1998) *Kidney International* 34:529-536

and Barnum et al. (1984) *J. Immunol. Methods* 67:303-309. The present disclosure relates generally to antibodies capable of inhibiting biological activities associated with Factor D. For example, at a concentration of 18 μg/ml (equivalent to about 1.5 times the molar concentration of human factor D in the blood; molar ratio of anti-Factor D antibody to Factor D of about 1.5:1), significant inhibition of the alternative complement activity by the antibody can be observed (see, e.g., US Patent No. 6,956,107)

[00287] In some embodiments, the present disclosure is directed to anti-Factor D antibodies and conjugates thereof, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{50} values less than 30 nM, or less than 15 nM, or less than 10 nM, or less than 5 nM. In some embodiments, the disclosure is directed to anti-Factor D antibodies and conjugates, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{50} values between 30 nM and 2 nM, or between 25 nM and 2 nM, or between 20 nM and 2 nM, or between 10 nM and 2 nM, or between 7 nM and 2 nM.

[00288] In some embodiments, the present disclosure is directed to anti-Factor D antibodies and conjugates thereof, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{90} values less than 80 nM, or less than 50 nM, or less than 40 nM, or less than 20 nM, or less than 15 nM. In some embodiments, the present disclosure is directed to anti-Factor D antibodies and conjugates thereof, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{90} values between 80 nM and 5 nM, or between 75 nM and 5 nM, or between 70 nM and 5 nM, or between 65 nM and 5 nM, or between 60 nM and 5 nM, or between 55 nM and 5 nM, or between 50 nM and 5 nM, or between 50 nM and 10 nM.

[00289] In some embodiments, the disclosure is directed to anti-Factor D antibodie and conjugates wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis in an antibody to Factor D molar ratio of about 0.05:1 (0.05) to about 10:1 (10), or about 0.09:1 (0.09) to about 8:1 (8), or about 0.1:1 (0.1) to about 6:1 (6), or about 0.15:1 (0.15) to about 5:1 (5), or about 0.19:1 (0.19) to about 4:1 (4), or about 0.2:1 (0.2) to about 3:1 (3), or about 0.3:1 (0.3) to about 2:1 (2), or about 0.4:1 (0.4) to about 1:1 (1), or about 0.5:1 (0.5) to about 1:2 (0.5), or about 0.6:1 (0.6) to about 1:3 (0.33), or about 0.7:1 (0.7) to about 1:4 (0.25), or about 0.8:1 (0.8) to about 1:5 (0.2) or about 0.9:1 (0.9) to about 1:6 (0.17).

[00290] In some embodiments, the disclosure is directed to anti-Factor D antibodies and conjugates comprising fragments of humanized anti-Factor D antibodies (e.g. antigen-binding fragments). The antibody fragments of the present disclosure may, for example, be Fv, Fab, Fab-SH, Fab'-SH, Fab', Fab-C, Fab'-C, Fab'-C-SH, Fab-C-SH, scFv, diabody, or $F(ab')_2$, dAb, complementarity determining region (CDR) fragments, linear antibodies, single-chain antibody

molecules, minibodies, diabodies, or multispecific antibodies formed from antibody fragments. In a further embodiment, the disclosure is directed to a humanized anti-Factor D antibody fragment or conjugate thereof (e.g. antigen-binding fragment) that is capable of penetrating substantially all of the retina. In an even further embodiment, the disclosure is directed to a humanized anti-Factor D antibody fragment or conjugate thereof (e.g. antigen-binding fragment) that is capable of penetrating throughout the entire thickness of the retina.

[00291] In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein an unconjugated Fab fragment of such antibodies has a half life of at least 3, 5, 7, 10 or 12 days after administration into a mammalian eye (e.g. human) via a single intravitreal injection. In some embodiments, the disclosure is directed to conjugates comprising humanized anti-Factor D antibodies, wherein an unconjugated Fab fragment of such antibodies inhibits alternative pathway (AP) complement activation for at least 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110 or 115 days after administration into a mammalian eye (e.g. human) via a single intravitreal injection. In another embodiment, the disclosure is directed to conjugates comprising humanized anti-Factor D antibodies, wherein the concentration of an unconjugated Fab fragment of such antibodies that inhibits alternative pathway (AP) complement activation is maintained in retinal tissue for at least 40, 45, 50, 55, 60, 65, 70, 75, 80 or 85 days after administration into a mammalian eye (e.g. human) via a single intravitreal injection. In another embodiment, the disclosure is directed to conjugates comprising humanized anti-Factor D antibodies, wherein the concentration of an unconjugated Fab fragment of such antibodies that inhibits alternative pathway (AP) complement activation is maintained in the vitreous humor for at least 80, 85, 90, 95, 100, 105, 110 or 115 days after administration into a mammalian eye (e.g. human) via a single intravitreal injection.

D. Conjugates

[00292] The invention also provides conjugates comprising any anti-Factor D antibody provided herein conjugated to one or more heterologous molecules, such as polyols.

1. Multi-armed Polymers

[00293] In some embodiments, the conjugates of the present disclosure can be made by derivatizing the anti-Factor D antibodies described herein by conjugating the antibodies or variants thereof with a multi-armed polymer. It will be appreciated that any multi-armed polymer that provides the conjugate with the desired size or that has the selected average molecular weight as described herein is suitable for use in constructing the antibody-polymer conjugates of the disclosure.

[00294] Many polymers are suitable for use in pharmaceuticals. *See, e.g.,* Davis et al., Biomedical Polymers: Polymeric Materials and Pharmaceuticals for Biomedical Use, pp. 441-

451 (1980). In all embodiments of the present disclosure, a non-proteinaceous polymer is used to form the conjugates of the disclosure. The non-proteinaceous polymer ordinarily is a hydrophilic synthetic polymer, i.e., a polymer not otherwise found in nature. However, polymers which exist in nature and are produced by recombinant or in vitro methods may also be useful, as are polymers which are isolated from native sources.

[00295] In some embodiments, the anti-Factor D antibodies are derivitized by conjugating (e.g., covalently linking) the antibodies or variants thereof to a multi-armed polyol. Thus, in some embodiments, the disclosure is directed to a conjugate comprising one or more anti-Factor D antibody or antibody variant disclosed herein covalently linked to one or more multi-armed polyol. The polyol employed can be any water-soluble poly (alkylene oxide) polymer and can have a linear or branched chain. Suitable polyols include those substituted at one or more hydroxyl positions with a chemical group, such as an alkyl group having between one and four carbons. Typically, the polyol is a poly(alkylene glycol), such as polyethylene glycol (PEG), and thus, for ease of description, the remainder of the discussion relates to an exemplary embodiment wherein the polyol employed is PEG, and the process of conjugating the polyol to a polypeptide is termed "PEGylation." However, those skilled in the art will recognize that other polyols, such as, for example, poly(propylene glycol) and polyethylene-polypropylene glycol copolymers, can be employed using the techniques for conjugation described herein for PEG.

[00296] The polyols used to form the conjugates of the present disclosure are multi-armed polyols. As used herein, “multi-armed polyol” refers to a polyol comprising a core structure to which at least two arms are attached. The multi-armed polyol may be, for example, a dimer (two arms), a tetramer (four arms), a hexamer (six arms), an octamer (eight arms), etc. In some aspects, the multi-armed polyol is a multi-armed PEG.

[00297] The weight average molecular weight of the multi-armed PEG used in the PEGylation of the anti-Factor D antibodies and antibody variants can vary, and typically may range from about 500 to about 300,000 daltons (D). In some embodiments, the weight average molecular weight of the multi-armed PEG is from about 1,000 to about 100,000 D, and, in some embodiments, from about 20,000 to about 60,000 D. In some embodiments, PEGylation is carried out with a multi-armed PEG having a weight average molecular weight of about 40,000 D.

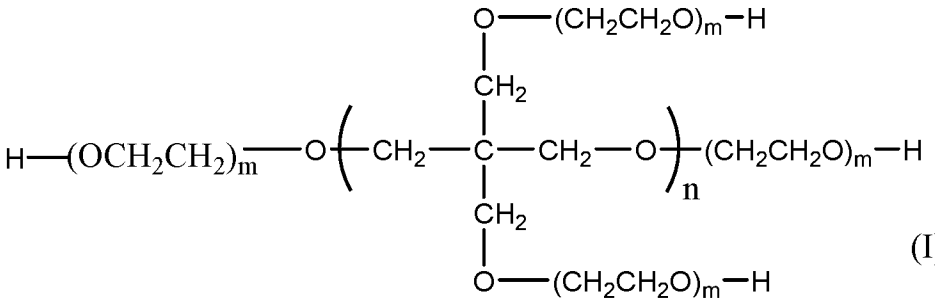
[00298] A variety of methods for PEGylating proteins are known in the art. Specific methods of producing proteins conjugated to PEG include the methods described in U.S. Pat. No. 4,179,337, U.S. Pat. No. 4,935,465, and U.S. Patent No. 5,849,535, all of which are herein incorporated by reference in their entirety. Typically the protein is covalently bonded via one or more of the amino acid residues of the protein to a terminal reactive group on the polymer. The

polymer with the reactive group(s) is designated herein as an activated or functionalized polymer (e.g., a functionalized PEG). The reactive group selectively reacts with free sulfhydryl or amino or other reactive groups on the antibody or antibody variant. The multi-armed PEG polymer can be coupled to the sulfhydryl or amino or other reactive group on the antibody or antibody variant in either a random or a site specific manner. It will be understood, however, that the type and amount of the reactive group chosen, as well as the type of polymer employed, to obtain optimum results, will depend on the particular antibody or antibody variant employed to limit, and preferably substantially prevent, having the reactive group react with too many active groups on the antibody. As it may not be possible to sufficiently limit or prevent this in some instances, typically from about 0.05 to about 1000 moles, or, in some embodiments, from about 0.05 to about 200 moles of functionalized polymer per mole of antibody, depending on antibody concentration, may be employed. The final amount of functionalized polymer per mole of antibody is a balance to maintain optimum activity, while at the same time optimizing, if possible, the vitreous humor, retina, and/or aqueous humor half-life of the antibody.

[00299] While the residues may be any reactive amino acids on the antibody or antibody variant, such as the N-terminal amino acid group, in some embodiments, the reactive amino acid is cysteine, which is linked to the reactive group of the functionalized polymer through its free thiol group as shown, for example, in WO 99/03887, WO 94/12219, WO 94/22466, U.S. Patent No. 5,206, 344, U.S. Patent No. 5,166,322, and U.S. Patent No. 5,206,344, all of which are herein incorporated by reference in their entirety. In such embodiments, the polymer may comprise at least one terminal reactive group that is capable of reacting specifically with the free sulfhydryl or thiol group(s) on the parental antibody. Such groups include, but are not limited to, maleimide, a sulfhydryl, a thiol, trifluoromethanesulfonate, tosylate, aziridine, epoxide, a pyridyl disulfide, succinimidyl ester, $-NH_2$, an aldehyde, a haloacetate, a haloacetamide, and a para-nitrophenyl carbonate, among others. The polymer can be coupled to the parental antibody using any protocol suitable for the chemistry of the coupling system selected, such as the protocols and systems described in U.S. Pat. No. 4,179,337, U.S. Pat. No. 7,122,636, and Jevsevar, et al., *Biotech J.*, Vol. 5, pp. 113-128 (2010). Alternatively, the reactive amino acid may be lysine, which is linked to the reactive group of the functionalized polymer through its free epsilon-amino group (*see, e.g.,* WO 93/00109, incorporated by reference herein), or glutamic or aspartic acid, which is linked to the polymer through an amide bond. The reactive group of the polymer can then react with, for example, the α (alpha) and ε (epsilon) amines or sulfhydryl groups of proteins to form a covalent bond. It will be appreciated that the present disclosure is not limited to conjugates utilizing any particular type of linkage between an antibody or antibody fragment and a polymer.

[00300] Suitable functionalized multi-armed PEGs for use in preparing the conjugates of the disclosure can be produced by a number of conventional reactions. For example, a N-hydroxysuccinimide ester of a PEG (M-NHS-PEG) can be prepared from a PEG-monomethyl ether by reaction with N,N'-dicyclohexylcarbodiimide (DCC) and N-hydroxysuccinimide (NHS), according to the method of Buckmann and Merr, *Makromol. Chem.*, Vol. 182, pp. 1379-1384 (1981). In addition, a PEG terminal hydroxy group can be converted to an amino group, for example, by reaction with thionyl bromide to form PEG-Br, followed by aminolysis with excess ammonia to form PEG-NH_2. The PEG-NH_2 can then be conjugated to the antibody or antibody variant of interest using standard coupling reagents, such as Woodward's Reagent K. Furthermore, a PEG terminal-CH_2OH group can be converted to an aldehyde group, for example, by oxidation with MnO_2. The aldehyde group can be conjugated to the antibody or antibody variant by reductive alkylation with a reagent such as cyanoborohydride.

[00301] In some embodiments, the multi-armed PEG used to prepare the conjugates of the present disclosure has the structure of general formula (I):

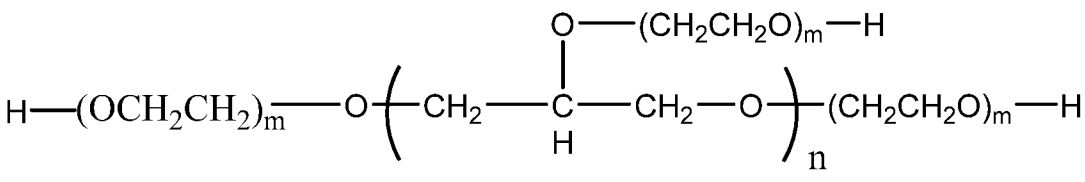


(I)

wherein each m denotes the length or size of the particular arm of the polyol (PEG) and is independently an integer of from about 45 to about 1000, about 3 to about 250, or from about 50 to about 200, or from about 100 to about 150; and n is an integer from about 1 to about 10.

[00302] In some embodiments, the multi-armed PEG has the structure of general formula (I), wherein n is 1, and the multi-armed PEG is a tetramer. In another embodiment, the multi-armed PEG has the structure of general formula (I), wherein n is 2, and the multi-armed PEG is a hexamer. In another embodiment, the multi-armed PEG has the structure of general formula (I), wherein n is 3, and the multi-armed PEG is an octamer.

[00303] In another aspect, the multi-armed PEG used to prepare the conjugates of the present disclosure has the structure of general formula (II):

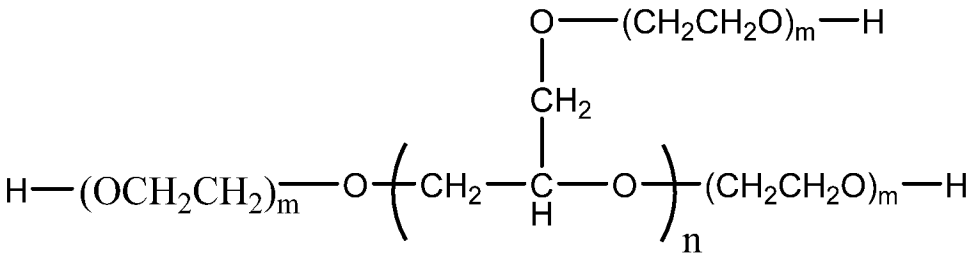


(II)

wherein each m denotes the length or size of the particular arm of the polyol (PEG) and is independently an integer of from about 45 to about 1000, about 3 to about 250, or from about 50 to about 200, or from about 100 to about 150; and n is an integer from about 1 to about 10.

[00304] In some embodiments, the multi-armed PEG has the structure of general formula (II), wherein n is 2, and the multi-armed PEG is a tetramer. In another embodiment, the multi-armed PEG has the structure of general formula (II), wherein n is 4, and the multi-armed PEG is a hexamer. In another embodiment, the multi-armed PEG has the structure of general formula (II), wherein n is 6, and the multi-armed PEG is an octamer.

[00305] In another aspect, the multi-armed PEG used to prepare the conjugates of the present disclosure has the structure of general formula (III):

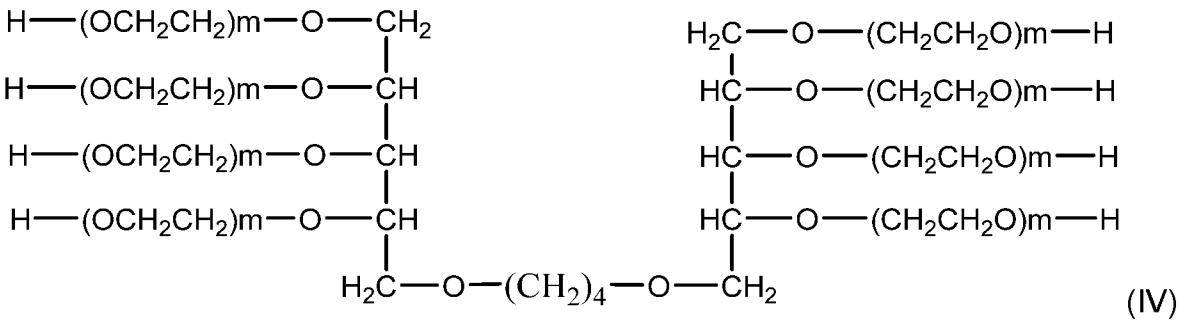


(III)

wherein each m denotes the length or size of the particular arm of the polyol (PEG) and is independently an integer of from about 45 to about 1000, about 3 to about 250, or from about 50 to about 200, or from about 100 to about 150; and n is an integer from about 1 to about 10.

[00306] In some embodiments, the multi-armed PEG has the structure of general formula (III), wherein n is 2, and the multi-armed PEG is a tetramer. In another embodiment, the multi-armed PEG has the structure of general formula (III), wherein n is 4, and the multi-armed PEG is a hexamer. In another embodiment, the multi-armed PEG has the structure of general formula (III), wherein n is 6, and the multi-armed PEG is an octamer.

[00307] In another aspect, the multi-armed PEG used to prepare the conjugates of the present disclosure has the structure of general formula (IV):

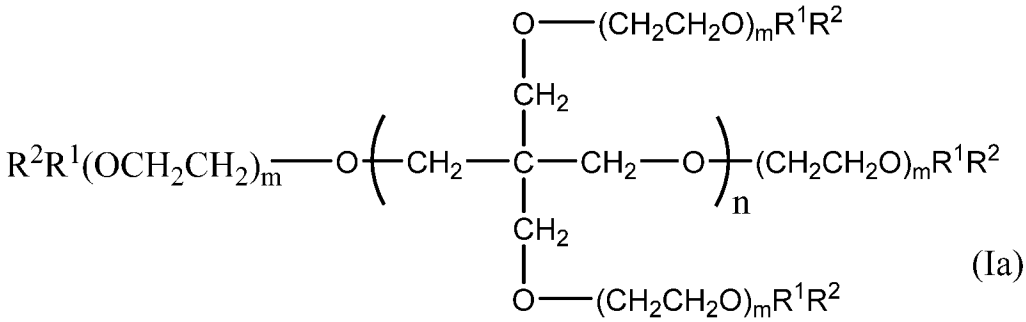


(IV)

wherein each m denotes the length or size of the particular arm of the polyol (PEG) and is independently an integer of from about 45 to about 1000, about 3 to about 250, or from about 50 to about 200, or from about 100 to about 150.

[00308] The multi-armed PEG having the structure of any of general formulas (I) to (IV) may be functionalized to, for example, attach a terminal reactive group suitable for reacting with or conjugating to the antibody (e.g., antibody fragment) using any of the techniques described above to produce a functionalized multi-armed PEG. In other embodiments, however, the multi-armed PEG can be covalently linked to the anti-Factor D antibodies through a multifunctional crosslinking agent which reacts with the PEG and one or more amino acid residues of the antibody or antibody variant to be linked, as described in, for example, U.S. Pat. No. 7,122,636, which is herein incorporated by reference in its entirety.

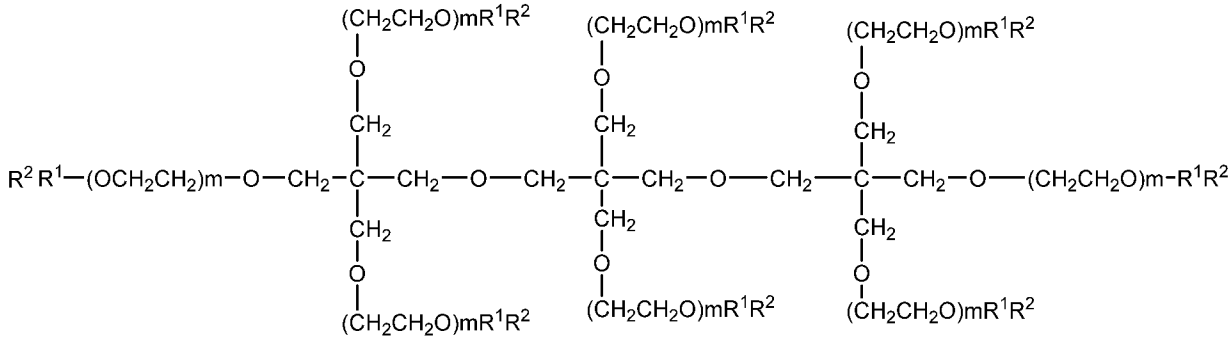
[00309] In other aspects, the multi-armed PEG used to prepare the conjugates of the present disclosure is a functionalized multi-armed PEG comprising at least one terminal reactive group. The terminal reactive group can conjugate directly to the anti-Factor D antibodies to form the conjugates of the present disclosure. In some embodiments, the functionalized multi-armed PEG has the structure of general formula (Ia):



(Ia)

[00310] wherein each m denotes the length or size of the particular arm of the polyol (PEG) and is independently an integer of from about 45 to about 1000, about 3 to about 250, or from about 50 to about 200, or from about 100 to about 150; and n is an integer from about 1 to about 10; each R^1 is independently either absent, or is a linking group; and each R^2 is independently either hydrogen or a terminal reactive group; wherein at least one R^2 is a terminal reactive group. In some embodiments, R^2 is independently selected form a thiol reactive group, an amino reactive group, and combinations thereof.

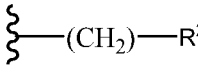
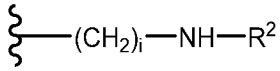
[00311] In some embodiments, the functionalized multi-armed PEG has the structure of general formula (Ia), wherein n is an integer from 1 to 3. In some embodiments, the functionalized multi-armed PEG has the structure of general formula (Ia), wherein n is 1, and the multi-armed PEG is a tetramer. In another embodiment, the functionalized multi-armed PEG has the structure of general formula (Ia), wherein n is 2, and the multi-armed PEG is a hexamer. In another embodiment, the functionalized multi-armed PEG has the structure of general formula (Ia), wherein n is 3, and the multi-armed PEG is an octamer. In such embodiments, the octamer has the structure of general formula (Ib):

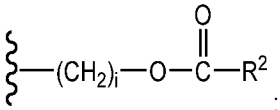
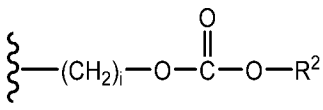
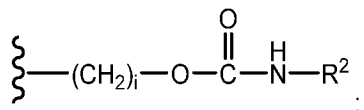


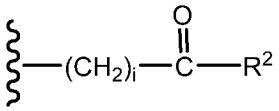
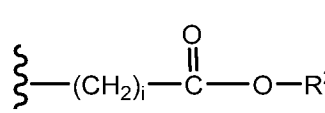
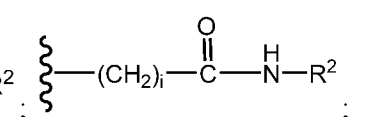
(Ib)

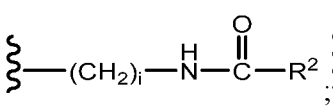
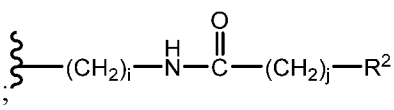
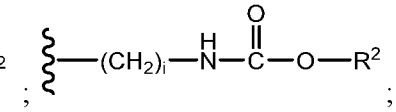
wherein m, R^1, and R^2 are as defined above.

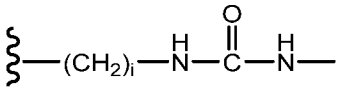
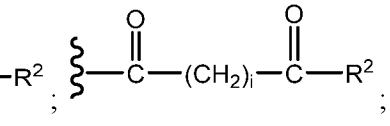
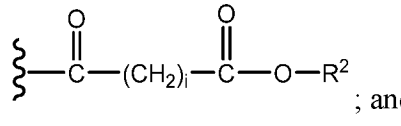
[00312] Multi-armed PEGs having the structure of general formula (Ib) have a tripentaerythritol (TP) core structure, and are also referred to herein as TP octamers.

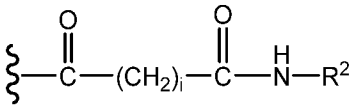
[00313] In some embodiments, the functionalized multi-armed PEG has the structure of general formula (Ia) or (Ib), wherein each R^1, when present, is the same or different, and R^1 and R^2 when taken together are selected from  ;  ;

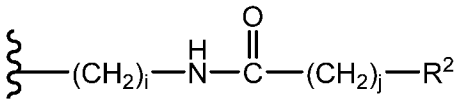
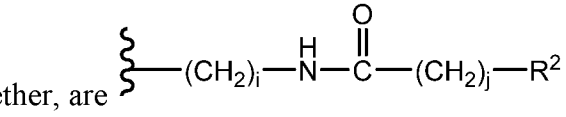
 ;  ;  ;

 ;  ;  ;

 ;  ;  ;

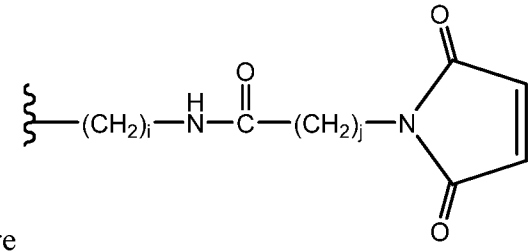
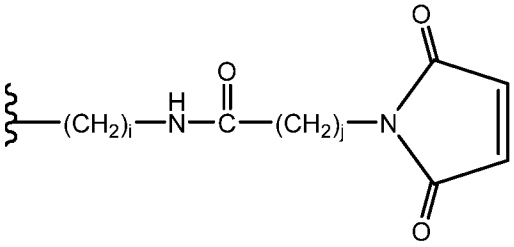
 ;  ;  ; and

 ; and combinations thereof; wherein each i is independently an integer of 0-10; j is an integer of 0-10; and R^2 is as defined herein. In some embodiments, each R^1 is a linking group.

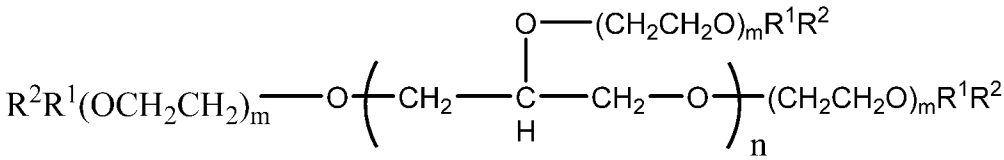
[00314] In some embodiments, the functionalized multi-armed PEG has the structure of general formula (Ia) or (Ib), wherein R^1 and R^2, when taken together, are  , wherein i, j, and R^2 are as defined herein. In some embodiments, R^1 and R^2, when taken together, are  , wherein i

is 2; j is 2 or 3, and R^2 is as defined herein.

[00315] In some embodiments, the functionalized multi-armed PEG has the structure of general formula (Ia) or (Ib), wherein each R^2 is independently selected from a maleimide, a sulfhydryl, a thiol, trifluoromethanesulfonate, tosylate, aziridine, epoxide, a pyridyl disulfide, succinimidyl ester, $-NH_2$, an aldehyde, a haloacetate, a haloacetamide, and a para-nitrophenyl carbonate. In some embodiments, each R^2 is independently a haloacetate selected from bromoacetate, iodoacetate, chloroacetate, and combinations thereof. In some embodiments, each R^2 is independently a haloacetamide selected from bromoacetamide, iodoacetamide, chloroacetamide, and combinations thereof. In some embodiments, R^2 is a maleimide.

[00316] In some embodiments, the functionalized multi-armed PEG has the structure of general formula (Ia) or (Ib), wherein each R^2 is a maleimide. In some embodiments, the functionalized multi-armed PEG has the structure of general formula (Ia) or (Ib), wherein R^1 and R^2, when taken together, are  , wherein i and j are as defined above. In some embodiments, the functionalized multi-armed PEG has the structure of general formula (Ia) or (Ib), wherein R^1 and R^2, when taken together, are  , wherein i is 2 and j is 2.

[00317] In another aspect, the functionalized multi-armed PEG used to prepare the conjugates of the present disclosure has the structure of general formula (IIa):

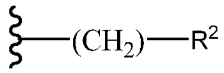
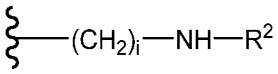


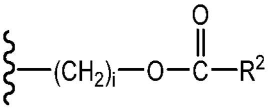
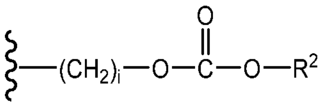
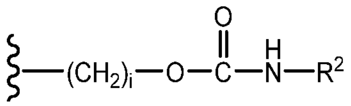
(IIa)

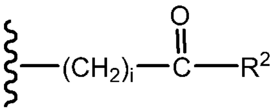
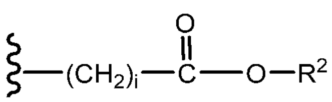
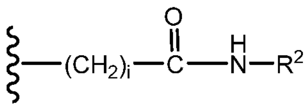
[00318] wherein each m denotes the length or size of the particular arm of the polyol (PEG) and is independently an integer of from about 45 to about 1000, about 3 to about 250, or from about 50 to about 200, or from about 100 to about 150; and n is an integer from about 1 to about 10; each R^1 is independently either absent, or is a linking group; and each R^2 is independently either hydrogen or a terminal reactive group; wherein at least one R^2 is a terminal

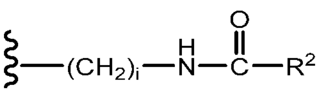
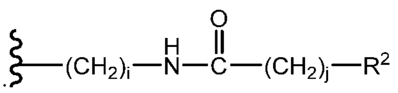
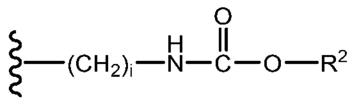
reactive group. In some embodiments, R^2 is independently selected form a thiol reactive group, an amino reactive group, and combinations thereof.

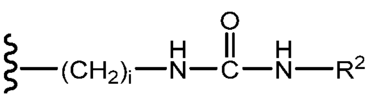
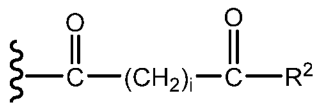
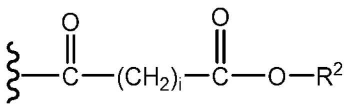
[00319] In some embodiments, the functionalized multi-armed PEG has the structure of general formula (IIa), wherein n is an integer from 2 to 6. In some embodiments, the functionalized multi-armed PEG has the structure of general formula (IIa), wherein n is 2, and the multi-armed PEG is a tetramer. In some embodiments, the functionalized multi-armed PEG has the structure of general formula (IIa), wherein n is 3. In another embodiment, the functionalized multi-armed PEG has the structure of general formula (IIa), wherein n is 4, and the multi-armed PEG is a hexamer. In another embodiment, the functionalized multi-armed PEG has the structure of general formula (IIa), wherein n is 6, and the multi-armed PEG is an octamer. Octamers having the structure of general formula (IIa) have a hexaglycerin (HG) core structure, and are also referred to herein as HG octamers.

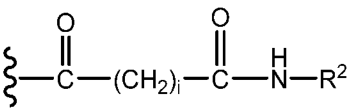
[00320] In some embodiments, the functionalized multi-armed PEG has the structure of general formula (IIa), wherein each R^1, when present, is the same or different, and R^1 and R^2 when taken together are selected from  ;  ;

 ;  ;  ;

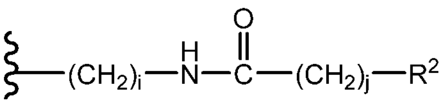
 ;  ;  ;

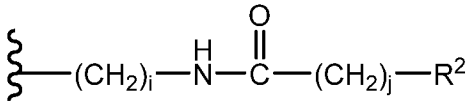
 ;  ;  ;

 ;  ;  ; and

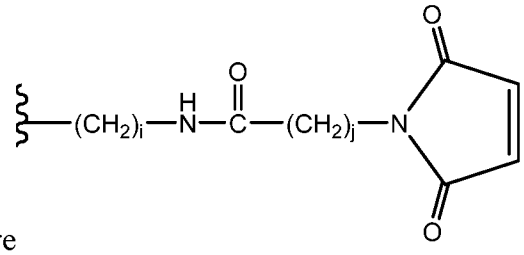
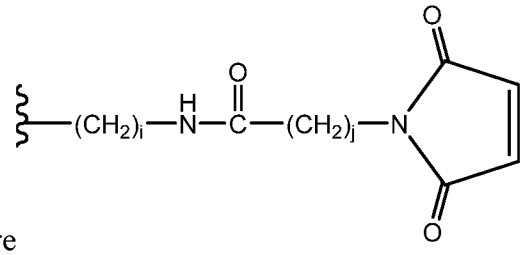
 ; and combinations thereof; wherein each i is independently an integer of 0-10; j is an integer of 0-10; and R^2 is as defined herein. In some embodiments, each R^1 is a linking group.

[00321] In some embodiments, the functionalized multi-armed PEG has the structure of general formula (IIa), wherein R^1 and R^2, when taken together, are

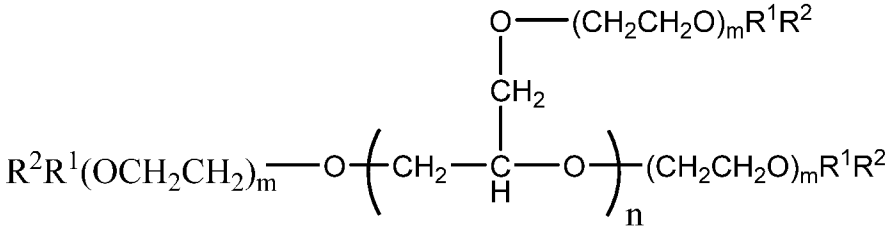
 , wherein i, j, and R^2 are as defined herein. In some

embodiments, R^1 and R^2, when taken together, are  , wherein i is 2; j is 2 or 3, and R^2 is as defined herein.

[00322] In some embodiments, the functionalized multi-armed PEG has the structure of general formula (IIa), wherein each R^2 is independently selected from a maleimide, a sulfhydryl, a thiol, trifluoromethanesulfonate, tosylate, aziridine, epoxide, a pyridyl disulfide, succinimidyl ester, $-NH_2$, an aldehyde, a haloacetate, a haloacetamide, and a para-nitrophenyl carbonate. In some embodiments, each R^2 is independently a haloacetate selected from bromoacetate, iodoacetate, chloroacetate, and combinations thereof. In some embodiments, each R^2 is independently a haloacetamide selected from bromoacetamide, iodoacetamide, chloroacetamide, and combinations thereof. In some embodiments, R^2 is a maleimide.

[00323] In some embodiments, the functionalized multi-armed PEG has the structure of general formula (IIa), wherein each R^2 is a maleimide. In some embodiments, the functionalized multi-armed PEG has the structure of general formula (IIa), wherein R^1 and R^2, when taken together, are  , wherein i and j are as defined above. In some embodiments, the functionalized multi-armed PEG has the structure of general formula (IIa), wherein R^1 and R^2, when taken together, are  , wherein i is 2 and j is 2.

[00324] In another aspect, the functionalized multi-armed PEG has the structure of general formula (IIIa):

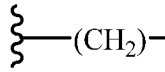


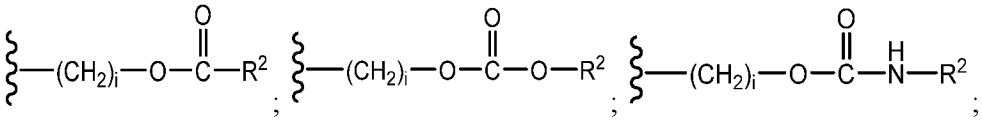
(IIIa)

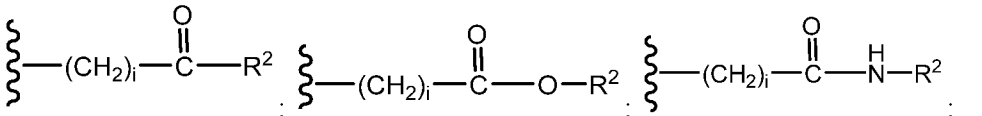
[00325] wherein each m denotes the length or size of the particular arm of the

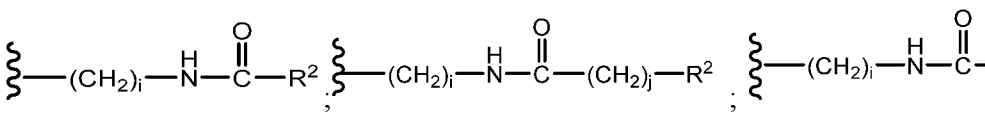
polyol (PEG) and is independently an integer of from about 45 to about 1000, or from about 3 to about 250, or from about 50 to about 200, or from about 100 to about 150; and n is an integer from about 1 to about 10; each R^1 is independently either absent, or is a linking group; and each R^2 is independently either hydrogen or a terminal reactive group; wherein at least one R^2 is a terminal reactive group. In some embodiments, R^2 is independently selected form a thiol reactive group, an amino reactive group, and combinations thereof.

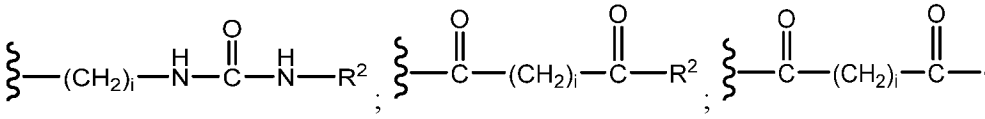
[00326] In some embodiments, the functionalized multi-armed PEG has the structure of general formula (IIIa), wherein n is an integer from 2 to 6. In some embodiments, the functionalized multi-armed PEG has the structure of general formula (IIIa), wherein n is 2, and the multi-armed PEG is a tetramer. In another embodiment, the functionalized multi-armed PEG has the structure of general formula (IIIa), wherein n is 4, and the multi-armed PEG is a hexamer. In another embodiment, the functionalized multi-armed PEG has the structure of general formula (IIIa), wherein n is 6, and the multi-armed PEG is an octamer. Octamers having the structure of general formula (IIIa) have a hexaglycerol (HGEO) core structure, and are also referred to herein as HGEO octamers.

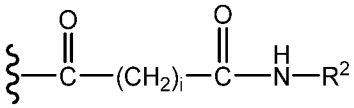
[00327] In some embodiments, the functionalized multi-armed PEG has the structure of general formula (IIIa), wherein each R^1, when present, is the same or different, and R^1 and R^2 when taken together are selected from  ; ;

 ;

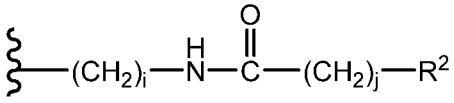
 ;

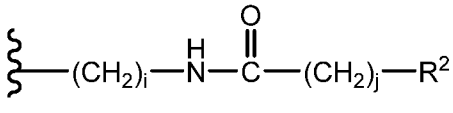
 ;

 ; and

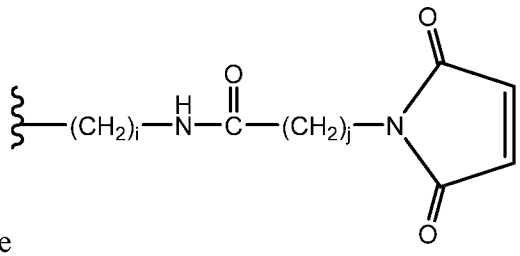
 ; and combinations thereof; wherein each i is independently an integer of 0-10; j is an integer of 0-10; and R^2 is as defined herein. In some embodiments, each R^1 is a linking group.

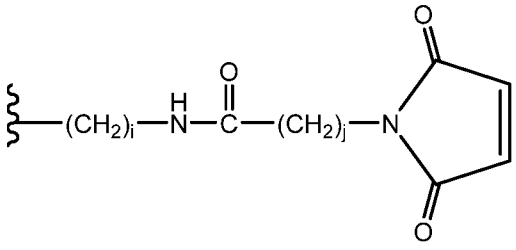
[00328] In some embodiments, the functionalized multi-armed PEG has the structure of general formula (IIIa), wherein R^1 and R^2, when taken together, are



, wherein i, j, and R^2 are as defined herein. In some embodiments, R^1 and R^2, when taken together, are  , wherein i is 2; j is 2 or 3, and R^2 is as defined herein.

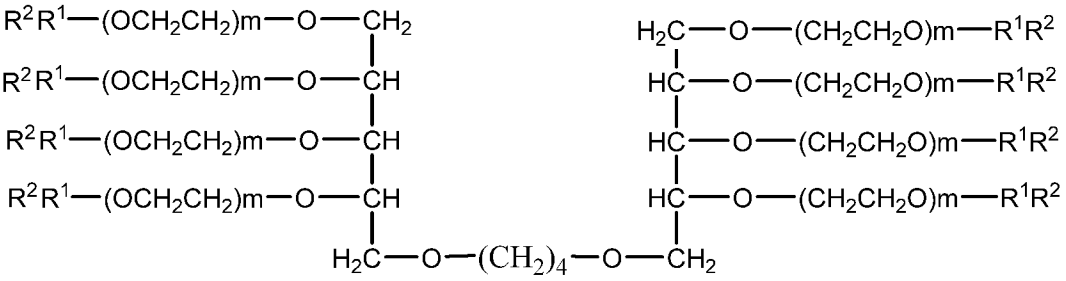
[00329] In some embodiments, the functionalized multi-armed PEG has the structure of general formula (IIIa), wherein each R^2 is independently selected from a maleimide, a sulfhydryl, a thiol, trifluoromethanesulfonate, tosylate, aziridine, epoxide, a pyridyl disulfide, succinimidyl ester, $-NH_2$, an aldehyde, a haloacetate, a haloacetamide, and a para-nitrophenyl carbonate. In some embodiments, each R^2 is independently a haloacetate selected from bromoacetate, iodoacetate, chloroacetate, and combinations thereof. In some embodiments, each R^2 is independently a haloacetamide selected from bromoacetamide, iodoacetamide, chloroacetamide, and combinations thereof. In some embodiments, R^2 is a maleimide.

[00330] In some embodiments, the functionalized multi-armed PEG has the structure of general formula (IIIa), wherein each R^2 is a maleimide. In some embodiments, the functionalized multi-armed PEG has the structure of general formula (IIIa), wherein R^1 and R^2, when taken together, are  , wherein i and j are as defined above. In some embodiments, the functionalized multi-armed PEG has the structure of general formula (IIIa), wherein R^1 and R^2, when taken together, are



, wherein i is 3 and j is 2.

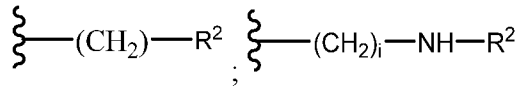
[00331] In another aspect, the functionalized multi-armed PEG has the structure of general formula (IVa):

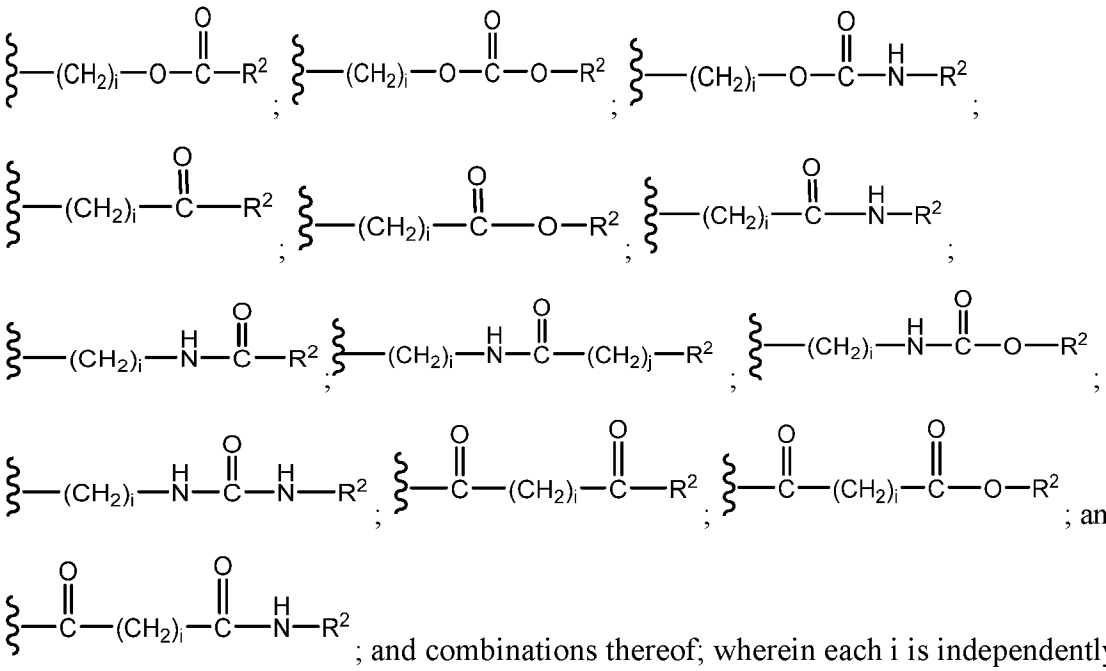


(IVa)

wherein each m denotes the length or size of the particular arm of the polyol (PEG) and is independently an integer of from about 45 to about 1000, or from about 3 to about 250, or from about 50 to about 200, or from about 100 to about 150; each R^1 is independently either absent, or is a linking group; and each R^2 is independently either hydrogen or a terminal reactive group; wherein at least one R^2 is a terminal reactive group. In some embodiments, R^2 is independently selected form a thiol reactive group, an amino reactive group, and combinations thereof.

[00332] Multi-armed PEGs having the structure of general formula (IVa) have a butanediol core structure, and are also referred to herein as DX octamers.

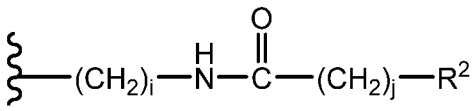
[00333] In some embodiments, the functionalized multi-armed PEG has the structure of general formula (IVa), wherein each R^1, when present, is the same or different, and R^1 and R^2 when taken together are selected from  ; ;

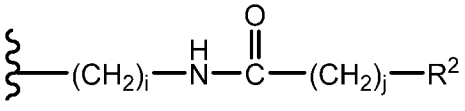


; and ; and combinations thereof; wherein each i is independently an integer of 0-10; j is an integer of 0-10; and R^2 is as defined herein. In some embodiments, each R^1 is a linking group.

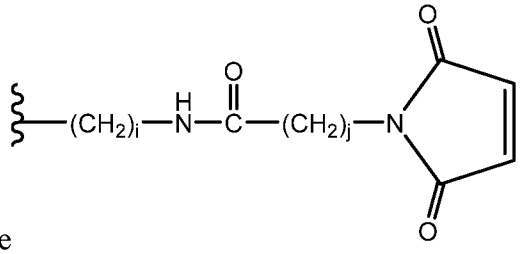
[00334] In some embodiments, the functionalized multi-armed PEG has the structure of

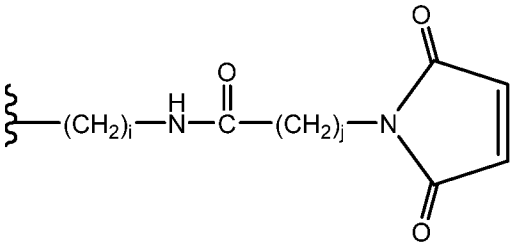
general formula (IVa), wherein R^1 and R^2, when taken together, are



, wherein i, j, and R^2 are as defined herein. In some embodiments, R^1 and R^2, when taken together, are  , wherein i is 2; j is 2 or 3, and R^2 is as defined herein.

[00335] In some embodiments, each R^2 is independently selected from a maleimide, a sulfhydryl, a thiol, trifluoromethanesulfonate, tosylate, aziridine, epoxide, a pyridyl disulfide, succinimidyl ester, $-NH_2$, an aldehyde, a haloacetate, a haloacetamide, and a para-nitrophenyl carbonate. In some embodiments, each R^2 is independently a haloacetate selected from bromoacetate, iodoacetate, chloroacetate, and combinations thereof. In some embodiments, each R^2 is independently a haloacetamide selected from bromoacetamide, iodoacetamide, chloroacetamide, and combinations thereof. In some embodiments, R^2 is a maleimide.

[00336] In some embodiments, the functionalized multi-armed PEG has the structure of general formula (IVa), wherein each R^2 is a maleimide. In some embodiments, the functionalized multi-armed PEG has the structure of general formula (IVa), wherein R^1 and R^2, when taken together, are  , wherein i and j are as defined above. In some embodiments, the functionalized multi-armed PEG has the structure of general formula (IVa), wherein R^1 and R^2, when taken together, are



, wherein i is 3 and j is 2.

[00337] Other functionalized multi-armed PEGs suitable for use in the present disclosure are described in U.S. Pat. App. Publ. No. 2011/0286956, and U.S. Pat. App. Publ. No. 2015/0073155, both of which are herein incorporated by reference in their entirety.

[00338] Functionalized multi-armed PEGs suitable for use in the present disclosure can also be purchased from a number of vendors. For example, JenKem Technology, USA sells maleimide-functionalized PEG octamers (e.g., 8ARM (TP)-PEG-MAL and 8ARM (HG)-PEG-

MAL) and tetramers. NOF America Corp. also sells maleimide functionalized PEG octamers (e.g., Sunbright® HGEO-400MA; Sunbright® DX-400MA) and tetramers (e.g., Sunbright® PTE-400MA). Such octamers and tetramers are available in a variety of molecular weights, including a weight average molecular weight of 40,000 D.

2. Conjugates

[00339] In some embodiments, the disclosure is directed to a conjugate comprising one or more anti-Factor D antibody or antibody variant disclosed herein and one or more multi-armed polyol, wherein the conjugate is prepared by covalently linking at least one anti-Factor D antibody or antibody variant to the polyol. In some embodiments, the multi-armed polyol is a PEG. In some embodiments, the PEG is an octamer. In some embodiments, the PEG has the structure of general formula (Ia), (Ib), (IIa), (IIIa), or (IVa).

[00340] The conjugates of the present disclosure may be characterized by the number of anti-Factor D antibodies conjugated to each multi-armed PEG. This is referred to herein as "fabylation" or "degree of fabylation". The number of anti-Factor D antibodies conjugated to each PEG may vary depending on a variety of factors, including: 1) the number of arms in the PEG; 2) the number and/or reactivity of the terminal reactive groups on the PEG; 3) the core structure of the PEG; and/or, 4) PEGylation reaction conditions. A high polydispersity of the multi-armed PEG used to prepare the conjugate may in some instances complicate the analysis of the final conjugate, in particular making an accurate determination of the number of antibodies (e.g., Fabs) per PEG more difficult and uncertain. Accordingly, the PEG used to form the conjugate will typically have a polydispersity (determined using methods known in the art) within a range of about 1 to about 1.35, and in various embodiments will have a polydispersity of about 1 to about 1.25, about 1 to about 1.2, about 1 to about 1.15, about 1 to about 1.1, about 1.05, or even about 1.

[00341] In some embodiments, the conjugate of the disclosure comprises an eight-armed PEG, wherein at least one anti-Factor D antibody or antibody variant is covalently linked to the PEG. In another embodiment, the conjugate of the disclosure comprises an eight-armed PEG, wherein at least two anti-Factor D antibodies are covalently linked to the PEG. In another embodiment, the conjugate of the disclosure comprises an eight-armed PEG, wherein at least three anti-Factor D antibodies are covalently linked to the PEG. In another embodiment, the conjugate of the disclosure comprises an eight-armed PEG, wherein at least four anti-Factor D antibodies are covalently linked to the PEG. In another embodiment, the conjugate of the disclosure comprises an eight-armed PEG, wherein at least five anti-Factor D antibodies are covalently linked to the PEG. In another embodiment, the conjugate of the disclosure comprises an eight-armed PEG, wherein at least six anti-Factor D antibodies are covalently linked to the

PEG. In another embodiment, the conjugate comprises an eight-armed PEG, wherein at least seven anti-Factor D antibodies are covalently linked to the PEG. In another embodiment, the conjugate of the disclosure comprises an eight-armed PEG, wherein eight anti-Factor D antibodies are covalently linked to the PEG. In some embodiments, the conjugate of the disclosure comprises an eight-armed PEG, wherein from 5-8 anti-Factor D antibodies are covalently linked to the PEG. In another embodiment, the conjugate of the disclosure comprises an eight-armed PEG, wherein from 6-8 anti-Factor D antibodies are covalently linked to the PEG. In another embodiment, the conjugate of the disclosure comprises an eight-armed PEG, 7-8 anti-Factor D antibodies are covalently linked to the PEG.

[00342] In some embodiments, the conjugate of the disclosure comprises a multi-armed PEG having the structure of any one of general formulas (Ia), (Ib), (IIa), (IIIa), or (IVa). In such embodiments, at least one R^2 is covalently linked to an anti-Factor D antibody or antibody variant described herein. In some embodiments, the multi-armed PEG having the structure of any one of general formulas (Ia), (Ib), (IIa), (IIIa), or (IVa) is an octamer, and at least two, at least three, at least four, at least five, at least six, at least seven, or all eight R^2 groups are covalently linked to an anti-Factor D antibody or antibody variant described herein.

[00343] In some embodiments, the conjugates of the present disclosure include species wherein the multi-armed polyol is covalently attached to a specific site or specific sites on the parental antibody; i.e., polymer attachment is targeted to a particular region or a particular amino acid residue or residues in the parental antibody or antibody fragments. Standard mutagenesis techniques can be used to alter the number and/or location of potential PEGylation sites in the parental antibody or antibody fragments. Thus, to the extent that amino acid substitutions introduce or replace amino acids such as cysteine and lysine, the anti-Factor D antibodies and variants thereof of the present disclosure can contain a greater or lesser number of potential PEGylation sites than a native sequence anti-Factor D (shown in Figures 1A-C).

[00344] As discussed above, site specific conjugation of polymers is most commonly achieved by attachment to cysteine residues in the parental antibody or antibody fragment. In such embodiments, the coupling chemistry can, for example, utilize the free sulfhydryl group of a cysteine residue not in a disulfide bridge in the parental antibody.

[00345] In some embodiments, one or more cysteine residue(s) naturally present in the parental antibody is (are) used as attachment site(s) for polymer conjugation. In other embodiments, free amino groups on the antibody or antibody variant can be thiolated with 2-imino-thiolane (Traut's reagent) and then coupled to, e.g., a maleimide-functionalized PEG, as described in Pedley, et al., *Br. J. Cancer*, Vol. 70, pp. 1126-1130 (1994). In another embodiment, one or more cysteine residue(s) is (are) engineered into a selected site or sites in

the parental antibody for the purpose of providing a specific attachment site or sites for polymer.

[00346] Cysteine engineered antibodies have been described previously (U.S. Pat. Pub. No. 2007/0092940 and Junutula, J. R., et al, *J. Immunol Methods*, Vol. 332(1-2), pp. 41-52 (2008), all herein incorporated by reference in their entirety). In some embodiments, cysteine engineered antibodies can be parental antibodies. These are useful for generating antibody fragments having a free cysteine in a particular location, typically in a constant region, e.g., C_L or C_H1. A parent antibody engineered to contain a cysteine is referred to herein as a "ThioMab" and Fab fragments produced from such cysteine engineered antibodies, regardless of the method of production, are referred to herein as "ThioFabs." As described previously (*see, e.g.,* U.S. Pat. Pub. No. 2007/0092940 and Junutula, J. R., et al, *J. Immunol Methods*, Vol. 332(1-2), pp. 41-52 (2008)), mutants with replaced ("engineered") cysteine (Cys) residues are evaluated for the reactivity of the newly introduced, engineered cysteine thiol groups. The thiol reactivity value is a relative, numerical term in the range of 0 to 1.0 and can be measured for any cysteine engineered antibody. In addition to having a reactive thiol group, ThioMabs should be selected such that they retain antigen binding capability. The design, selection, and preparation of cysteine engineered antibodies were described in detail previously (*see, e.g.*, WO 2011/069104, which is herein incorporated by reference). In some embodiments, engineered cysteines are introduced into the constant domains of heavy or light chains. As such, the cysteine engineered antibodies retain the antigen binding capability of their wild type, parent antibody counterparts and, as such, are capable of binding specifically, to antigens.

[00347] In some embodiments, the present disclosure relates to antibody fragment-polymer conjugates, wherein the antibody fragment is a Fab, and the polymer is attached to one or more cysteine residue in the light or heavy chain of the Fab fragment that would ordinarily form the inter-chain disulfide bond linking the light and heavy chains.

[00348] In another aspect, the present disclosure relates to antibody fragment-polymer conjugates, wherein the antibody fragment is a Fab-C, and the polymer attachment is targeted to the hinge region of the Fab-C fragment. In some embodiments, one or more cysteine residue(s) naturally present in the hinge region of the antibody fragment is (are) used to attach the polymer. In another embodiment, one or more cysteine residues is (are) engineered into the hinge region of the Fab-C fragment for the purpose of providing a specific attachment site or sites for polymer. In some embodiments, an anti-Factor D antibody variant Fab fragment disclosed herein is modified by adding one cysteine at the C'-terminal end for the purpose of providing one attachment site for polymer conjugation. In another embodiment, an anti-Factor D antibody variant Fab fragment described herein is modified by adding four additional residues, Cys-Pro-Pro-Cys (SEQ ID NO: 162), at the C'-terminal end for the purpose of providing two attachment

sites for polymer conjugation. In still another embodiment, an anti-Factor D antibody variant Fab fragment described herein is modified by adding four additional residues, Ser-Pro-Pro-Cys (see, e.g., SEQ ID NO: 121), at the C'-terminal end for the purpose of providing one attachment sites for polymer conjugation.

[00349] The degree and sites of PEGylation can also be manipulated by adjusting reaction conditions, such as the relative concentrations of the functionalized PEG and the protein as well as the pH. Suitable conditions for a desired degree of PEGylation can be determined empirically by varying the parameters of standard PEGylation reactions.

[00350] PEGylation of anti-Factor D antibodies and antibody variants is carried out by any convenient method. Suitable PEGylation conditions are set forth in WO 2011/069104 and WO 03/029420, both of which are herein incorporated by reference in their entirety.

3. Characterization

[00351] The PEGylated proteins can be characterized by SDS-PAGE, gel filtration, NMR, peptide mapping, liquid chromatography-mass spectrophotometry, and in vitro biological assays. The extent of fabylation is typically first shown by SDS-PAGE. Polyacrylamide gel electrophoresis in 10% SDS is typically run in 10 mM Tris-HC1 pH 8.0, 100 mM NaCl as elution buffer. To demonstrate which residue is PEGylated, peptide mapping using proteases such as trypsin and Lys-C protease can be performed. Thus, samples of PEGylated and non-PEGylated antibodies can be digested with a protease such as Lys-C protease and the resulting peptides separated by a technique such as reverse phase HPLC. The chromatographic pattern of peptides produced can be compared to a peptide map previously determined for the anti-Factor D polypeptide.

[00352] Each peak can then be analyzed by mass spectrometry to verify the size of the conjugate in the peak. Depending on the PEG used in the conjugation, and the size of the conjugate in the peak, the number of antibodies or variants thereof conjugated to the PEG can be estimated. The fragment(s) that conjugated to PEG groups are usually not retained on the HPLC column after injection and disappear from the chromatograph. Such disappearance from the chromatograph is an indication of PEGylation on that particular fragment that should contain at least one PEGylatable amino acid residue. PEGylated anti-Factor D antibodies and antibody variants may further be assayed for ability to interact with Factor D and other biological activities using known methods in the art.

[00353] PEGylation changes the physical and chemical properties of the antibody drug, and may results in improved pharmacokinetic behaviors such as improved stability, decreased immunogenicity, extended circulating life as well as increased ocular residence time.

[00354] In some embodiments, the conjugates of the present disclosure have an increased

half-life after administration into a mammalian eye (e.g. human) via a single intravitreal injection, as compared to the corresponding unconjugated anti-Factor D antibody or antibody variant. In some embodiments, the increase in half-life is at least 1.4 times, or at least 1.8 times, or at least 2 times the half-life of the corresponding unconjugated anti-Factor D antibody or antibody variant.

[00355] In some embodiments, the conjugate is stable over an extended period of time, with loss of Factor D binding capacity of less than 20%, less than 15%, or less than 10% per month at physiological conditions.

[00356] In some embodiments, the conjugate has a viscosity that makes it suitable for administration through a narrow bore needle. In some embodiments, the viscosity of the conjugate is less than 800 cP, less than 700 cP, less than 600 cP, less than 500 cP, less than 400 cP, or less than 300 cP at a concentration of 150-250 mg/ml. In some embodiments, the viscosity of the conjugate is less than 300 cP at a concentration of 200 mg/ml.

[00357] In some embodiments, the R_H ranges from 3-30 nM.

E. Methods and Compositions for Diagnostics and Detection

[00358] In certain embodiments, any of the anti-Factor D antibodies provided herein is useful for detecting the presence of Factor D in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. A "biological sample" comprises, *e.g.*, a cell or tissue.

[00359] In some embodiments, an anti-Factor D antibody for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of Factor D in a biological sample is provided. In certain embodiments, the method comprises contacting the biological sample with an anti- Factor D antibody as described herein under conditions permissive for binding of the anti-Factor D antibody to Factor D, and detecting whether a complex is formed between the anti- Factor D antibody and Factor D in the biological sample. Such method may be an *in vitro* or *in vivo* method. In some embodiments, an anti-Factor D antibody is used to select subjects eligible for therapy with an anti-Factor D antibody, *e.g.* where Factor D is a biomarker for selection of patients. In a further embodiment, the biological sample is a cell or tissue.

[00360] In further embodiments, a method of diagnosis or detection comprises contacting a first anti-Factor D antibody immobilized to a substrate with a biological sample to be tested for the presence of Factor D, exposing the substrate to a second anti-Factor D antibody, and detecting whether the second anti-Factor D is bound to a complex between the first anti-Factor D antibody and Factor D in the biological sample. A substrate may be any supportive medium, *e.g.*, glass, metal, ceramic, polymeric beads, slides, chips, and other substrates. In certain

embodiments, a biological sample comprises a cell or tissue. In certain embodiments, the first or second anti-Factor D antibody is any of the antibodies described herein.

[00361] In certain embodiments, labeled anti-Factor D antibodies are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, *e.g.*, through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes ^{32}P, ^{14}C, ^{125}I, ^{3}H, and ^{131}I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luceriferases, *e.g.*, firefly luciferase and bacterial luciferase (U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, *e.g.*, glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like. In another embodiment, a label is a positron emitter. Positron emitters include but are not limited to ^{68}Ga, ^{18}F, ^{64}Cu, ^{86}Y, ^{76}Br, ^{89}Zr, and ^{124}I. In a particular embodiment, a positron emitter is ^{89}Zr.

F. Pharmaceutical Formulations

[00362] Pharmaceutical formulations of an anti-Factor D antibody or conjugate as described herein are prepared by mixing such antibody or conjugate having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-

protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include insterstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In some embodiments, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

[00363] Exemplary lyophilized antibody or conjugate formulations are described in US Patent No. 6,267,958. Aqueous antibody or conjugate formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

[00364] The formulation herein may also contain more than one active ingredient as necessary for the particular indication being treated. In some embodiments, the active ingredients have complementary activities that do not adversely affect each other.

[00365] Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

[00366] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody or conjugate, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D- (-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the

aggregation mechanism is discovered to be intermolecular S--S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

[00367] The formulations to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, *e.g.*, by filtration through sterile filtration membranes.

[00368] The antibodies and conjugates described herein for prevention or treatment of an ocular disease or condition are typically administered by ocular, intraocular, and/or intravitreal injection, and/or juxtascleral injection, and/or subtenon injection, and/or superchoroidal injection and/or topical administration in the form of eye drops and/or ointment. Such compounds of the invention may be delivered by a variety of methods, e.g. intravitreally as a device and/or a depot that allows for slow release of the compound into the vitreous, including those described in references such as Intraocular Drug Delivery, Jaffe, Jaffe, Ashton, and Pearson, editors, Taylor & Francis (March 2006). In one example, a device may be in the form of a min pump and/or a matrix and/or a passive diffusion system and/or encapsulated cells that release the compound for a prolonged period of time (Intraocular Drug Delivery, Jaffe, Jaffe, Ashton, and Pearson, editors, Taylor & Francis (March 2006). Other methods of administration may also be used, which includes but is not limited to, topical, parenteral, subcutaneous, intraperitoneal, intrapulmonary, intranasal, and intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration.

[00369] Formulations for ocular, intraocular or intravitreal administration can be prepared by methods and using ingredients known in the art. A main requirement for efficient treatment is proper penetration through the eye. Unlike diseases of the front of the eye, where drugs can be delivered topically, retinal diseases require a more site-specific approach. Eye drops and ointments rarely penetrate the back of the eye, and the blood-ocular barrier hinders penetration of systemically administered drugs into ocular tissue. Accordingly, usually the method of choice for drug delivery to treat retinal disease, such as AMD and CNV, is direct intravitreal injection. Intravitrial injections are usually repeated at intervals which depend on the patient's condition, and the properties and half-life of the drug delivered. For intraocular (e.g. intravitreal) penetration, usually molecules of smaller size are preferred.

[00370] For ocular administration, in some embodiments, the antibodies and conjugates described herein may be formulated in a pharmaceutically acceptable carrier at pH5.5.

[00371] In some embodiments, the antibodies and conjugates described herein may be formulated for delivery using an implantable port delivery system (PDS). As noted previously,

the PDS is a refillable device where release into the vitreous is controlled by a porous metal membrane comprising a titanium frit. Since the reservoir has a low volume, in some embodiments, a high protein concentration is required for effective delivery with the PDS. Accordingly, in some embodiments, the antibodies and conjugates described herein are formulated at high concentration. In some embodiments, the antibodies and conjugates described herein may be formulated at a concentration of at least 150 mg/ml, at least 160 mg/ml, at least 170 mg/ml, at least 180 mg/ml, at least 190 mg/ml, at least 200 mg/ml, or at least 210 mg/ml, or at least 220 mg/ml, or at least 230 mg/ml, or at least 240 mg/ml, or at least 250 mg/ml, or at least 260 mg/ml, or at least 270 mg/ml, or at least 280 mg/ml, or at least 290 mg/ml, or at least 300 mg/ml. In some embodiments, the antibodies and conjugates described herein may be formulated at a concentration of between 150 mg/ml and 350 mg/ml, or between 150 mg/ml and 300 mg/ml, or between 170 mg/ml and 300 mg/ml, or between 200 mg/ml and 300 mg/ml.

[00372] The efficacy of the treatment of complement-associated eye conditions, such as AMD or CNV, can be measured by various endpoints commonly used in evaluating intraocular diseases. For example, vision loss can be assessed. Vision loss can be evaluated by, but not limited to, e.g., measuring by the mean change in best correction visual acuity (BCVA) from baseline to a desired time point (e.g., where the BCVA is based on Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity chart and assessment at a test distance of 4 meters), measuring the proportion of subjects who lose fewer than 15 letters in visual acuity at a desired time point compared to baseline, measuring the proportion of subjects who gain greater than or equal to 15 letters in visual acuity at a desired time point compared to baseline, measuring the proportion of subjects with a visual-acuity Snellen equivalent of 20/2000 or worse at a desired time point, measuring the NEI Visual Functioning Questionnaire, measuring the size of CNV and amount of leakage of CNV at a desired time point, e.g., by fluorescein angiography, etc. Ocular assessments can be done, e.g., which include, but are not limited to, e.g., performing eye exam, measuring intraocular pressure, assessing visual acuity, measuring slitlamp pressure, assessing intraocular inflammation, etc.

[00373] In some embodiments, an anti-Factor D antibody of conjugate thereof is administered intravitreally at a dose of about 0.3 mg to about 30 mg per eye.

[00374] The dosing schedule for administration may vary form once a month to daily depending on a number of clinical factors, including the type of disease, severity of disease, and the subject's sensitivity to the therapeutic agent.

G. Therapeutic Methods and Compositions

[00375] Any of the anti-Factor D antibodies or conjugates provided herein may be used in

methods, *e.g.*, therapeutic methods.

[00376] An "individual," "patient," or "subject" according to any of the embodiments herein may be a human.

[00377] The anti-Factor D antibodies and conjugates of the present disclosure may be used to treat a mammal. In some embodiments, the anti-Factor D antibody or conjugate is administered to a nonhuman mammal for the purposes of obtaining preclinical data, for example. Exemplary nonhuman mammals to be treated include nonhuman primates, dogs, cats, rodents and other mammals in which preclinical studies are performed. Such mammals may be established animal models for a disease to be treated with the antibody, or may be used to study toxicity of the antibody of interest. In each of these embodiments, dose escalation studies may be performed on the mammal.

[00378] The anti-Factor D antibody or conjugate may be administered by any suitable means, including parenteral, subcutaneous, intraperitoneal, intravitreal, intrapulmonary, and intranasal, and, if desired for local immunosuppressive treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, intravitreal, and subcutaneous administration. In addition, the conjugate is suitably administered by pulse infusion, particularly with declining doses of the antibody, or antibody variant thereof or fragment thereof (e.g. antigen-binding fragment). In some embodiments, the dosing is given by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic.

[00379] For the prevention or treatment of disease, the appropriate dosage of the anti-Factor D antibody or conjugate will depend on the type of disease to be treated, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody and the discretion of the attending physician.

[00380] Depending on the type and severity of the disease, about 1-25 mg/eye (0.015 mg/kg – 0.36 mg/kg per eye) of the antibody is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays. An exemplary dosing regimen is disclosed in WO 94/04188.

[00381] The conjugate compositions may be formulated, dosed and administered in a manner consistent with good medical practice. Factors for consideration in this context include

the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The "therapeutically effective amount" of the conjugate to be administered will be governed by such considerations, and is the minimum amount necessary to prevent, ameliorate, or treat a disease or disorder. The conjugate need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody, or antibody variant thereof or fragment thereof (e.g. antigen-binding fragment), present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as used hereinbefore or about from 1 to 99% of the heretofore employed dosages.

[00382] The antibodies disclosed herein which recognize Factor D as their target and the conjugates comprising these antibodies may be used to treat complement-mediated disorders. These disorders are associated with excessive or uncontrolled complement activation. They include: complement activation during cardiopulmonary bypass operations; complement activation due to ischemia-reperfusion following acute myocardial infarction, aneurysm, stroke, hemorrhagic shock, crush injury, multiple organ failure, hypobolemic shock and intestinal ischemia. These disorders can also include disease or condition is an inflammatory condition such as severe burns, endotoxemia, septic shock, adult respiratory distress syndrome, hemodialysis, anaphylactic shock, severe asthma, angioedema, Crohn's disease, sickle cell anemia, poststreptococcal glomerulonephritis and pancreatitis. The disorder may be the result of an adverse drug reaction, drug allergy, IL-2 induced vascular leakage syndrome or radiographic contrast media allergy. It also includes autoimmune disease such as systemic lupus erythematosus, myasthenia gravis, rheumatoid arthritis, Alzheimer's disease and multiple sclerosis. Complement activation is also associated with transplant rejection. Recently there has been a strong correlation shown between complement activation and ocular diseases such as age-related macular degeneration, diabetic retinopathy and other ischemia-related retinopathies, choroidal neovascularization (CNV), uveitis, diabetic macular edema, pathological myopia, von Hippel-Lindau disease, histoplasmosis of the eye, Central Retinal Vein Occlusion (CRVO), corneal neovascularization, and retinal neovascularization.

[00383] An anti-Factor D antibody of conjugate can be administered alone or in combination with at least a second therapeutic compound. Administration of the conjugate and any second therapeutic compound can be done simultaneously, e.g., as a single composition or as two or more distinct compositions using the same or different administration routes.

Alternatively, or additionally the administration can be done sequentially, in any order. In certain embodiments, intervals ranging from minutes to days, to weeks to months, can be present between the administrations of the two or more compositions. For example, the conjugate comprising the Factor D antagonist may be administered first, followed by the second therapeutic compound. However, simultaneous administration or administration of the second therapeutic compound prior to the conjugate is also contemplated. In some embodiments, the second therapeutic compound is selected from an HTRA1 antagonist, an ANG2 antagonist (such as anti-ANG2 antibodies as disclosed, for example, in US20090304694 A1), a TIE2 antagonist (such as anti-TIE2 antibodies as disclosed, for example, in US Patent No. 6,376,653), a VEGF antagonist (such as VEGF antagonists as disclosed, for example, in US Patent No. 6,884,879 issued Feb. 26, 2015 and WO98/45331 (bevacizumab and other humanized anti-VEGF antibodies); WO2005/012359 and WO2005/044853 (G6 or B20 series antibodies (e.g. G6-31, B20-4.1); WO98/45331 (ranabizumab), and a second complement component antagonist. In some embodiments, the HTRA1 antagonist is an anti-HTRA1 antibody. In some embodiments, the ANG2 antagonist is an anti-ANG2 antibody. In some embodiments, the TIE2 antagonist is an anti-TIE2 antibody. In some embodiments, a VEGF antagonist is selected from a VEGF trap (such as aflibercept (Eylea®) and an anti-VEGF antibody (such as bevacizumab (Avastin®) or ranabizumab (Lucentis®)). In some embodiments, the second complement component antagonist inhibits various members of the classical or alternative complement pathway (complement inhibitors), selected from from C1, C2, C3, C4, C5, C6, C7, C8, C9 complement components.

[00384] In some embodiments, the treatment of the present disclosure for complement-mediated disorders in a human subject with a complement-mediated disorder comprises administering to the subject an effective amount of a therapeutic compound, such as an anti-Factor D antibody of conjugate, and further comprising administering to the subject an effective amount of a second therapeutic compound. In some embodiments, the second therapeutic compound is an HTRA1 antagonist. In some embodiments, the second therapeutic compound is an ANG2 antagonist. In some embodiments, the second therapeutic compound is a TIE2 antagonist. In some embodiments, the second therapeutic compound is a VEGF antagonist. In some embodiments, the second therapeutic compound is a second complement component antagonist. In some embodiments, the complement-mediated disorder is an complement-associated eye condition. In some embodiments, the ocular disorder is age-related macular degeneration (AMD), including non-exudative (e.g. intermediate dry AMD or geographic atrophy (GA)) and exudative (e.g. wet AMD (choroidal neovascularization (CNV)) AMD, diabetic retinopathy (DR), endophthalmitis and uveitis. In one example, the complement-

associated eye condition is intermediate dry AMD. In some embodiments, the complement-associated eye condition is geographic atrophy. In some embodiments, the complement-associated eye condition is wet AMD (choroidal neovascularization (CNV)).

[00385] Combined administration herein includes co-administration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein generally there is a time period while both (or all) active agents simultaneously exert their biological activities.

[00386] In a further aspect, the invention provides pharmaceutical formulations comprising any of the anti-Factor D antibodies or conjugates provided herein, *e.g.*, for use in any of the above therapeutic methods. In some embodiments, a pharmaceutical formulation comprises any of the anti-Factor D antibodies or conjugates provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical formulation comprises any of the anti-Factor D antibodies or conjugates provided herein and at least one additional therapeutic agent.

[00387] It is understood that any of the above formulations or therapeutic methods may be carried out using either or both a conjugate of the invention and/or an anti-Factor D antibody.

H. Articles of Manufacture

[00388] Articles of manufacture, or "kits", containing an anti-Factor D antibody described herein useful for the treatment methods herein are provided. In some embodiments, the kit comprises a container comprising an anti-Factor D antibody. The kit may further comprise a label or package insert, on or associated with the container. The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products. Suitable containers include, for example, bottles, vials, syringes, blister pack, etc. The container may be formed from a variety of materials such as glass or plastic. The container may hold an anti-Factor D antibody described herein or a formulation thereof which is effective for use in a treatment method herein, and may have a sterile access port (for example, the container may be a vial having a stopper pierceable by a hypodermic injection needle). The label or package insert indicates that the composition is used in a treatment method as described and claimed herein. The article of manufacture may also contain a further container comprising a pharmaceutically acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[00389] The kit may further comprise directions for the administration of the anti-Factor

D antibody described herein. In some embodiments, the label or package insert indicates that the composition is used for treating complement-associated disorders, such as, for example, any of the conditions listed before, including eye disorders e.g. age-related macular degeneration (AMD). The label or package insert may further comprise instructions for administering the antibody composition to the patient. In some embodiments, if the kit comprises a first composition comprising the anti-Factor D antibody and a second pharmaceutical formulation, the kit may further comprise directions for the simultaneous, sequential or separate administration of the first and second pharmaceutical compositions to a patient in need thereof.

[00390] According to some embodiments, a kit may comprise the anti-Factor D antibody described herein and a container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[00391] In some embodiments, a kit comprises an implantable port delivery system (PDS) and a composition comprising the anti-Factor D antibody or conjugate described herein. In some embodiments, the kit comprises instructions for implanting the PDS and filling the reservoir with the antibody or conjugate. In some embodiments, a kit comprises a composition comprising the anti-Factor D antibody or conjugate formulated for refilling a PDS.

[00392] In another embodiment, kits are also provided that are useful for various purposes, e.g., for treatment, prevention and/or diagnosis of complement-associated disorders, for complement-associated hemolysis assays, for purification or immunoprecipitation of Factor D polypeptide from cells. For isolation and purification of Factor D polypeptide, the kit can contain an anti-Factor D antibody coupled to beads (e.g., sepharose beads). Kits can be provided which contain the antibodies for detection and quantitation of Factor D polypeptide *in vitro*, e.g., in an ELISA or a Western blot. As with the article of manufacture, the kit comprises a container and a label or package insert on or associated with the container. The container holds a composition comprising a conjugate of the disclosure comprising at least one anti-Factor antibody. Additional containers may be included that contain, e.g., diluents and buffers, control antibodies. The label or package insert may provide a description of the composition as well as instructions for the intended in vitro or detection use. The label or package insert may provide instructions for the administration (e.g. the antibody, or antibody fragment thereof (e.g. antigen-binding fragment) to a subject.

III. EXAMPLES

[00393] The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description

provided above.

Example 1: Generation of Anti-Factor D Humanized Antibodies

[00394] Lampalizumab (sometimes also referred to as "aFD.WT" or "FCFD4515S"), a humanized anti-Factor D Fab fragment that potently inhibits Factor D and the alternative complement pathway, through binding to an exosite on factor D is currently in clinical development for the treatment of geographic atrophy (GA), an advanced form of dry AMD. Lampalizumab comprises a 214 residue light chain (SEQ ID NO: 102) and a 223 residue heavy chain (SEQ ID NO: 103).

[00395] While results of a phase II human clinical trial in GA indicate that a treatment effect is obtained with monthly intravitreal injection of aFD.WT, there exist incentives to use higher drug doses to achieve even better efficacy. Meanwhile, less frequent dosing would provide improved convenience to the patient, have potential benefits of decreased infection rate and increased clinical efficacy, and could facilitate treatment of patients with less advanced forms of dry AMD.

[00396] In order to develop a Factor D inhibitor that may be formulated at high concentration, with lower viscosity, and which could be stored without precipitation, a new humanized anti-Factor D antibody was developed based on anti-Factor D murine antibody 20D12. *See* PCT Publication No. 2008/147883 A1. Briefly, the VL and VH domains from murine 20D12 (VH of SEQ ID NO: 8 and VL of SEQ ID NO: 7) were aligned with the human VL kappa I (VL_{KI}) and human VH subgroup I (VH_I) consensus sequences. Hypervariable regions (HVR) from the murine 20D12 antibody were engineered into VL_{KI} and VH_I acceptor frameworks to generate CDR-grafted variants, as discussed below.

[00397] From the 20D12 VL domain, positions 24-34 (L1), 50-56 (L2) and 89-97 (L3) were grafted into VL_{KI}. From the 20D12 VH domain, positions 31-35 (H1), 50-65 (H2) and 95-102 (H3) were grafted into VH_I. The HVR definitions are defined by their sequence hypervariability (Wu, T. T. & Kabat, E. A. (1970)), their structural location (Chothia, C. & Lesk, A. M. (1987)) and their involvement in antigen-antibody contacts (MacCallum et al. J. Mol. Biol. 262: 732-745 (1996)). Certain vernier positions were mutated back to the murine sequence, including positions 36 and 46 in VL and positions 67, 69, and 71 in VH. *See* Figures 1A-C and 2A-C.

[00398] Synthetic genes encoding the variable domains of the initial humanized version of antibody 20D12, hu20D12.v1 (Figures 1A-C and 2A-C; VH of SEQ ID NO: 29, VL of SEQ ID NO: 28), were produced and cloned into vectors for expression as full length IgG. Antibody was expressed transiently in CHO cells and purified as previously described (Kelley & Meng (2012) Methods Mol Biol 901:277-93). Binding affinity to human factor D was determined by

surface plasmon resonance (SPR) measurements on a Biacore®T200 instrument using anti-Fc capture for murine 20D12 (capture kit #BR-1008-38) and hu20D12.v1 IgG (capture kit #BR-1008-39). As shown below in Table 3, murine 20D12 and hu20D12.v1 have comparable affinities for human factor D.

[00399] The antibodies were also assayed for Factor D inhibition in the hemolysis assay, as follows. The AP hemolysis assay using rabbit erythrocytes (Er) has been previously described (Pangburn (1998) Methods. Enzymol. 162:639; Katschke et al. (2009) J. Biol. Chem. 284:10473). Er (Colorado Serum) were washed three times with 0.5% bovine skin gelatin in veronal buffer (GVB) and re-suspended. Dilutions of anti-factor D antibodies were prepared at a 2X concentration and added to 96-well polypropylene plates. Er suspension were mixed with GVB/0.1M EGTA/0.1M MgCl2 and added to the plates. Complement activation was initiated by the addition of C1q-depleted human serum to avoid any complement activation through the classical pathway (CompTech; diluted 1:3 in GVB). After a 30 minute incubation at room temperature, the reaction stopped by adding 10 mM EDTA in GVB. The plates were centrifuged and the supernatants transferred. The absorbance of the supernatant was read at 412 nm. The AFD.Ab concentrations causing half-maximal inhibition (IC50) were determined by a nonlinear regression analysis. The results of that analysis are shown in Table 3.

Table 3: Human Factor D binding kinetics data for IgG-formateed mouse and humanized 20D12

Antibody	**On-rate ($M^{-1}s^{-1}$)**	**Off-Rate (s^{-1})**	**KD (pM)**	**Hemolysis IC50 (nM)**
Mu20D12	5.9e6	3.9e-4	66	4.0
Hu20D12.v1	1.5e7	6.7e-4	46	4.3

[00400] As shown in Table 3, the affinity of humanized antibody hu20D12.v1 is comparable to the affinity of the parental murine antibody. Hu20D12.v1 also retained inhibitory activity against Factor D in the hemolysis assay.

Example 2: Production and characterization of hu20D12 Fab fragments

[00401] The portion of the gene encoding the Fab fragment of hu20D12.v1 was subcloned into an *E. coli* expression vector similar to that previously described (Carter et al. (1992) BioTechnology 10:163). For small scale expression and purification, DNA was transformed into E. coli strain 64B4. Single colonies were picked into 5 mL LB media (media prep code A2008) containing 50 µg/mL carbenecillin (media prep code A3232) and grown overnight in 14 mL culture tubes with shaking at 200 RPM in an Innova incubator at 37°C. These cultures were used to inoculate 250 mLs of complete soy crap media (media prep code A4564), 50 µg/mL carbenecillin, in a 1 L baffled shake flask. Cultures were grown overnight at 30°C with shaking

at 200 RPM and then harvested by centrifugation. The cell pellet was lysed using PopCulture media (invitrogen), and Fabs purified on Gravitrap Protein G columns (GE Healthcare), following protocols supplied by the manufacturers. For larger scale production of Fab, cell paste from 10 L fermentation of transformed cells was suspended in extraction buffer and homogenized using a microfluidizer. Fab was captured by immunoaffinity chromatography on kappa-select and eluted with a low pH buffer. The eluate was immediately neutralized with 1M TRIS (pH 8.0) and buffer exchanged into PBS using an Amicon centricon filtration device (EMD Millipore). This pool was further purified using hydrophobic interaction chromatography (HIC). The solution was prepared for HIC by adjusting the pH to 6.5 through acetic acid addition and adding ammonium sulfate to a final concentration of 2.5 M. HIC was on a 3 mL ProPac HIC-10 column (Thermo Scientific) that had been equilibrated with 25mM $NaPO_4$, 2.5M Ammonium Sulfate (pH 6.5). A two-step gradient with 25mM $NaPO_4$, 25% Isopropyl Alcohol (pH 6.5) elution buffer was used to elute the bound protein. Fractions were analyzed using intact Fab liquid chromatography mass spectrometry (LCMS) to determine peak identity. Fractions containing Fab protein were buffer exchanged into PBS.

Example 3: Stability and molecular assessment of hu20D12

[00402] To simulate the exposure of hu20D12.v1 to conditions that may be found in long-acting delivery systems, the antibody Fab at 100 mg/mL in PBS was stressed for four weeks at 37°C. PBS was used as a mimic of the pH and ionic strength of human vitreous. Hu20D12.v1 showed good resistance to aggregation for this stress condition, with only a 0.6% loss of monomer, but a deamidation of Asn-54 (CDR-H2) was revealed by using tryptic peptide mapping.

[00403] As noted above, hu20D12.v1 includes an NG deamidation site in HVR-H2. N54 in HVR-H2 was therefore mutated to S (N54S) or Q (N54Q) to remove the NG deamidation site. Point mutations were introduced by site-directed mutagenesis using the QuikChangeII® (Agilent) mutagenesis kit following the protocol supplied with the kit. Oligonucleotide primers specifying the required codon changes were synthesized. Plasmids with designed changes were identified and confirmed by DNA sequencing. Variant Fabs were expressed in E. coli and purified as described above for hu20D12.v1. In Figures 1A-C and 2A-C, hu20D12.v1 (VH of SEQ ID NO: 29, VL of SEQ ID NO: 28) shows the CDR grafted antibody without the N54 mutation and hu20D12.v2.0 shows the CDR grafted antibody with the N54S mutation (VH of SEQ ID NO: 33, VL of SEQ ID NO: 32) and hu20D12.v1.1 shows the CDR grafted antibody with the N54Q mutation (VH of SEQ ID NO: 31, VL of SEQ ID NO: 30). The binding affinities (K_D) of the two variant Fabs and parental hu20D12.v1 Fab for Factor D were assessed by surface plasmon resonance (SPR) measurements using the anti-huFab capture protocol

described herein (*see, e.g.*, Example 5). The Fabs were also assayed for Factor D inhibition in the hemolysis assay. Table 4 shows the affinities of the N54S and N54Q variants of hu20D12.v1 and IC50 of each antibody in the hemolysis assay.

Table 4: Relative binding affinity of Fab-formatted antibodies for human Factor D

Variant	Name	KD (pM)	IC50 Hemolysis (nM)
hu20D12.v1	hu20D12.v1	33	2.8
Hu20D12.v1.N54Q	hu20D12.v1.1	45	6.7
Hu20D12.v1.N54S	hu20D12.v2.0	40	4.7

[00404] As shown in Table 4, the N54S variant of hu20D12.v1 (hu20D12.v2.0) had comparable affinities for Factor D. The N54S and N54Q variants also retained inhibitory activity against Factor D in the hemolysis assay. Hu20D12.v2.0 was selected for further analysis and affinity improvement.

[00405] Molecular assessment (MA) was performed on hu20D12.v2.0 to determine suitability of the antibody for further development. In these experiments, the molecule is tested in accelerated stability tests involving incubation at 37°C or 40°C. Chemical stability is evaluated using peptide mapping, SEC is used to determine susceptibility to aggregation, and for some conditions SPR-measurements of antigen-binding are used to assess retention of activity. These tests indicate if the molecule poses any challenges to standard methods of manufacture, formulation, or delivery. Table 5 shows the results of the stress test.

Table 5: Stress test of hu20D12.v2.0

Category	Parameter	hu20D12.v2.0
Stability	Thermal Stress: 40°C, 2 weeks	DT (CDR-L1) Stable NN (CDR-L3) Stable SEC monomer loss: 0 % IEC main peak loss: 10 %
	Trp/Met Oxidation Assay	M (CDR-H1) Stable
Ocular Specific TCP Tests	Sustained delivery stress: 37°C, 4 weeks	DT (CDR-L1): 3 % isomerization NN (CDR-L3): 3 % deamidation M (CDR-H1): 4 % increase in oxidation 1 % monomer loss No loss in antigen binding relative to control via Biacore

[00406] Similar to hu20D12.v1, hu20D12.v2.0 did not aggregate when stressed at 100

mg/mL in PBS at 37°C for 4 weeks. Unlike hu20D12.v1, hu20D12.v2.0 did not show a deamidation site under this stress condition, suggesting that the N54S substitution abrogated the deamidation and that the variant would have higher stability at physiological pH and ionic strength. Hu20D12.v2.0 also showed good stability in a pH 5.5 formulation suitable for ocular drugs.

Example 4: Structure determination of hu20D12.v2.0:Factor D complex

[00407] To better understand how hu20D12.v2.0 binds human factor D, the structure of hu20D12.v2.0 Fab in complex with Factor D was determined. Human factor D protein and hu20D12.v2.0 Fab were mixed in 1:1 molar ratio and purified over a Superdex 200 column pre-equilibrated with 20 mM Hepes pH 7.2 and 150 mM NaCl. The peak fractions containing the complex were pooled, concentrated to 32 mg/ml and used in crystallization trial. Crystals were grown at 4°C using vapor diffusion method by mixing protein in 2:1 (v/v) with a reservoir solution containing 0.1 M sodium cacodylate pH 6.5 and 1M sodium citrate. The crystals were cryo-protected in artificial mother liquor containing 20% ethylene glycol and flash frozen in liquid nitrogen. A 2.9 Å data set was collected at ALS 5.0.2 and the structure solved by molecular replacement method. A comparison to another anti-Factor D Fab:Factor D complex previously solved (Katschke et al. (2012) J. Biol. Chem. 287, 12886) is shown in the overlay of Figures 3A- C. Although these two antibodies have different CDR sequences, they bind to nearly identical epitopes on Factor D. An alignment of the prior Factor D antibody (lampalizumab) with hu20D12.v2.0 ("hu20D12.v1.N54S") is shown in Figure 4.

Example 5: Design of Higher Affinity Anti-Factor D Humanized Antibody Variants

[00408] Improving the affinity of an antibody with a pM affinity by conventional screening methods, such as phage display, is typically very difficult. Thus, a structure-based analysis of the binding of 20D12 to Factor D using the crystal structure described above was used to identify specific amino acid residues that could be mutated to improve affinity.

[00409] Certain contacts between hu20D12.v2.0 and Factor D were identified as potentially sub-optimal and those residues were selected for mutation to the corresponding lampalizumab amino acid, which may provide greater affinity for the antigen. Examination of fD:hu20D12.v2.0 complex structure indicates that although the side chain of Asp-173 is in contact with hu20D12.v2.0, the fD Asp does not make a salt bridge or complete hydrogen bonds with the antibody Fab. *See* Figure 3C. Introduction of a positively charged residue on the antibody, such as Arg or Lys, could satisfy ion pairing requirements for the Asp. A position on hu20D12.v2.0 that appears to be close enough to build such an interaction is position 49 in FR2 of the light chain. This position is a Tyr in hu20D12.v2.0 so we tested whether Lys or Arg substitution at this position would increase the fD-binding affinity. In addition, lampalizumab

binds with higher affinity to fD than hu20D12.v2.0 (< 10 pM versus 39 pM, *see* Figure 11) and has different residues at key contacts within the interface. For example, hu20D12.v2.0 CDR-H2 residues Glu-50, Thr-53, Gly56 are Trp-50, Tyr-53 and Glu-56 in lampalizumab. *See* Figure 3B. The tryptophan (W) at position 50 of the lampalizumab heavy chain may form a hydrophobic interaction with Factor D, while hu20D12.v2.0 has a glutamic acid (E) at that position. The tyrosine at position 53 of the lampalizumab heavy chain may also pack better with the residues nearby in Factor D than the threonine (T) at that position in hu20D12.v2.0. In the light chain, CDR-L2 Tyr-55 and CDR-L3-Asn92 in hu20D12.v2.0 are CDR-L2 Arg-55 and CDR-L3-Asp92 in lampalizumab. Each of these residues appears to contact Factor D in the Factor D:lampalizumab complex structure, with CDR-L-Asp92 making a charged interaction with Lys223a of Factor D. *See* Katschke et al. (2012) for Lys223a numbering.

[00410] Based on these observations, and others, additional 20D12 variants were designed. The light chain and heavy chain sequences for 20D12 variants are shown in Figures 1A-C and 2A-C, respectively. The variants were tested for the effect on binding to fD. Mutations were introduced into the *E. coli* Fab expression plasmid, and variant Fabs were expressed and purified as described above.

Factor D Binding Affinity by Surface Plasmon Resonance (SPR) Measurements

[00411] Kinetics and binding constant K_D for Factor D binding to immobilized hu20D12 versions was determined by surface plasmon resonance (SPR) measurements on a Biacore®T200 instrument. Antibody Fab fragments were immobilized on a Series S CM5 sensor chip using the anti-huFab capture kit (GE healthcare Cat. # 28-9583-25) following a protocol described by the manufacturer. Kinetics of binding were calculated from sensorgrams recorded for injection of 60 µL aliquots of solutions of human Factor D varied in concentration from 0.39 nM to 25 nM in 2-fold increments. The flow rate was 30 µL/min, the running buffer was HBS-P+ (GE Healthcare cat#BR-1006-71), the temperature of analysis was 25°C, real-time reference cell subtraction was employed, and dissociation following factor D injection was followed for 10 minutes. For some variants with high affinity and slow off-rate, dissociation was monitored for 2 hours to obtain a more accurate measure of the dissociation rate. After subtraction of the sensorgram observed for injection of running buffer, data were analyzed according to a 1:1 model using BiaEval software v4.1 (GE Healthcare) to extract the kinetics and affinity constants.

[00412] Replacement of light chain residue Tyr-49 with Arg (hu20D12.v2.1) results in a ≥4-fold improvement in binding to fD. *See* Figure 11. The increased affinity is the result of a slower off-rate for dissociation. Replacement of Tyr-49 with Ser (hu20D12.v2.6), Lys (hu20D12.v2.7) or Gln (hu20D12.v2.8) does not increase affinity for fD. Substitution of CDR-

H2 Glu-50 with Trp (hu20D12.v2.9), or CDR-L2 Tyr-55 with Lys (hu20D12.v2.10) or Arg (hu20D12.v2.11), results in large decreases in affinity for fD. Individual replacement of CDR-L2 Gly-56 with Asp (hu20D12.v2.2) or Glu (hu20D12.v2.4), or Thr-53 with Tyr (hu20D12.v2.5), does not appear to result in increased affinity; however, combination of these changes with Y49R in variants (hu20D12.v2.12, v2.14, v2.15, v2.3) does appear to further increase affinity. Comparing sequences of hu20D12 with lampalizumab implicates that replacing Tyr-55 on CDR-L2 would result in a variant with increased affinity due to its expected interaction with fD Asp-173. However, crystal structure indicates that replacement of Tyr-49 with Arg (Y49R) is a better choice for increasing affinity. Although not tested individually, the CDR-L3 substitution N92E does appear to combine with Y49R in variant (hu20D12.v2.13) to produce a favorable increase in binding-affinity.

[00413] The Factor D-binding affinities to human Factor D (hFD) of hu20D12 variants v2.1, v2.3, v2.12-v2.15, and also lampalizumab, are at the limits of detection (KD~10 pM) for current SPR technology. Several of the hu20D12 variants v2.1, v2.3, v2.14 and v2.15 bind to cynomolgus monkey factor D (cyfD), with hu20D12.v2.1 having the highest affinity (KD=17 pM). In addition, binding affinity of hu20D12.v2.1 to cynomolgus monkey Factor D is improved almost 10-fold relative to hu20D12.v2.0 and improved 2-fold relative to lampalizumab. Cynomolgus monkeys are often used in pre-clinical safety and efficacy assessments of therapeutic candidates so high binding affinity to cFD is a desirable property. Fabs were tested for thermal stability by differential scanning calorimetry (DSC). Table 6 shows the results of the thermal stability analysis.

Table 6: Melting temperatures by DSC

Fab	Tm (°C)
AFD.v8	70.5
AFD.v14	70.3
Hu20D12.v2.0	79.8
Hu20D12.v2.3	80.3
Hu20D12.v2.13	73.6
Hu20D12.v2.14	81.0
Hu20D12.v2.15	81.2

[00414] All of the hu20D12 variants tested showed high thermal stability with melting points higher than observed for AFD.v8 and AFD.v14, two variants of lampalizumab.

Example 6: Potency of hu20D12 variants for inhibition of Factor D

[00415] Potency of hu20D12 variants and conjugated hu20D12 Fab-C versions for inhibition of Factor D was determined in a time-resolved fluorescence energy transfer (TR-FRET) assay of Factor D-dependent factor B activation. Dilutions of hu20D12 Fab, conjugated hu20D12 Fab-C versions, or control were prepared in enzymatic reaction buffer (ERB; 75 mM

NaCl, 1 mM MgCl2, 25 mM Tris, 0.005% polysorbate 20, pH 7.3) at a 4x concentration and combined in equal volumes with 0.5 nM or 0.2 nM factor D (fD, Complement Technology; Tyler, TX) or ERB (no enzyme control). Ranibizumab (anti-VEGF) was used as the negative control. The Factor D/AFD.Ab mixtures (7 µl/well) were added to 364-well Proxiplate F plus black plates (Perkin Elmer Health Sciences; Waltham, MA) followed by 7 µl/well of substrate. The substrate consisted of a mixture of C3b (Complement Technology) at 7 µg/mL (40 nM) and factor B (Complement Technology) at 1 µg/mL (15 nM). The Fab or conjugated Fab-C versions, enzyme, cofactor, and substrate were incubated for 45 min at room temperature with gentle agitation. The reaction was stopped with 7 µl/well of a detection reagent cocktail mixture consisting of biotinylated anti-factor Bb (2F12, GNE PRO282909) at 8 nM, Europium-conjugated anti-factor Ba (custom conjugation of 1C3, GNE PRO282908 by Life Technologies; Madison, WI) at 4 nM, and streptavidin-Alexa 647 at 25 nM. The plate was incubated at room temperature in the dark for 30 min. Time-resolved fluorescence energy transfer was detected with a PHERAstar FS microplate reader (BMG LabTech; Cary, NC) by exciting at 337 nm and detecting Europium emission at 620 nm and Alexa fluor emission at 665 nm. The AFD.Ab concentrations causing half-maximal inhibition (IC50) were determined by nonlinear regression analysis using a four-parameter fit model (KaleidaGraph Synergy Software; Reading, PA).

[00416] Inhibition curves for the TR-FRET assay are shown in Figure 5A, Figure 5B (and Table 7) and Figure 11 (conditions for this assay were 50 pM Factor D, 15 nM Factor B (fB), and 40 nM Factor C3b). Lampalizumab has an IC50 for inhibition of Factor D-dependent fB activation of 24 pM, and the standard error in IC50 is ± 25% (Figure 5B (Table 7) and Figure 11). The sensitivity of the assay precludes testing at Factor D concentrations lower than 50 pM. Thus, the lowest IC50 that can be measured, equivalent to the concentration where 50% of the Factor D is inhibited, and assuming a 1:1 interaction of inhibitor and factor D, is 25 pM. The IC50 for hu20D12.v2.0 is about 2-fold higher than measured for lampalizumab. *See* Figure 5B (Table 7) and Figure 11. All of the variants tested (v2.1, 2.14, 2.15, 2.3) have increased potency relative to hu20D12.v2.0 with IC50 values the same as, or approaching, the value measured for lampalizumab. *See* Figure 5A, 5B (Table 7) and Figure 11.

Table 7: IC50

Molecule	**AVG IC50 (pM)**
hu20D12.v2.0	59.22
AFD.v8	27.65
AFD.v14	34.03

hu20D12.v2.3	31.20
AFD.v8.C + PEG tetramer	11.03
AFD.v14.C + PEG tetramer	14.77
hu20D12.v2.3.C + PEG tetramer	35.22
RANIBIZUMAB	N/A
LAMPALIZUMAB	24.38

Example 7: Stability and molecular assessment of affinity matured hu20D12

[00417] Molecular assessment was performed on hu20D12.v2.1 and hu20D12.v2.3. No issues requiring non-standard practices were identified for hu20D12.v2.1. In contrast, lampalizumab has several risks for formulation and long acting delivery (results of stress test for lampalizumab in Table 8). The concentration-dependence of the viscosity for hu20D12.v2.1 was determined, and indicated that this Fab has low viscosity in a buffer used for ocular formulations and is suitable for high concentration formulation. Hu20D12.v2.0 is soluble in PBS at concentrations at least as high as tested, 292 mg/ml, whereas lampalizumab shows precipitation at 227 mg/ml. *See* Figures 6A and 6B. In addition, the hu20D12.v2.0 solution remained clear with no evidence of precipitate after 27 weeks at 4°C. This indicates that a high concentration formulation of the Fab, which is useful for less frequent dosing with improved efficacy, is more readily obtainable for the humanized variants of 20D12 than for lampalizumab. Table 8 shows the results of the stress test of hu20D12.v2.1.

Table 8: Stress test of lampalizumab

Category	**Parameter**	**Lampalizumab**
Stability	Thermal Stress: 40°C, 2 weeks	CDR-L1 ^{30}DD unstable CDR-H2 ^{61}DD unstable
	Trp/Met Oxidation Assay	CDR-H2 Trp-50 > 35%
Ocular Specific TCP Tests	High Conc. Thermal Stress: 40°C, 2 weeks	IEC main peak loss >16%
	Sustained delivery stress: 37°C, 4 weeks	High conc. 5.8% loss of monomer at 40 days 30% loss in antigen binding capacity at 70 days

Table 9: Stress test of hu20D12.v2.1

Category	Parameter	hu20D12.v2.1
Stability	Thermal Stress: 40°C, 2 weeks	NP (CDR-H2) Stable DT (CDR-L1) Stable NNY (CDR-L3) Stable SEC monomer loss: 0.3 % IEC main peak loss: 0.5 %
	Thermal Stress: 40°C, 2 weeks	NP (CDR-H2): no observed hydrolysis DT (CDR-L1): 2.3 % increase in isomerization NNY (CDR-L3): 0.0 % increase in deamidation SEC monomer loss: 0.3 % IEC main peak loss: 5.3 %
	Trp/Met Oxidation Assay	M (CDR-H1) Stable
Ocular Specific TCP Tests	Viscosity in pH 5.5 buffer	7 cP at 177 mg/mL
	High Conc. Thermal Stress: 40°C, 2 weeks	SEC monomer loss: 1.7 % IEC main peak loss: 7.1 %
	Solubility in PBS, pH 7.4	No insoluble particulates
		Low turbidity at 150 mg/mL
	Sustained delivery stress: 37°C, 4 weeks	No loss in antigen binding 1.6% loss of monomer by SEC

[00418] To simulate conditions that may be experienced by hu20D12.v2.1 Fab in long-acting delivery systems, high concentration samples of hu20D12.v2.1 Fab were held at 37°C for a month in two formulation conditions: (1) ~200mg/mL, PBS, pH 7.4, and (2) ~170mg/mL, 20mM histidine hydrochloride, pH 5.5. PBS was chosen to mimic the pH and ionic strength of the human vitreous humor. Histidine hydrochloride was chosen as a representative formulation for liquid ophthalmology therapeutics. The formulation concentration (170mg/mL-200mg/mL) was chosen to reflect a clinically relevant dose formulation for long-acting delivery of anti-Factor D. Molecule stability was assessed via ion-exchange chromatography (IEC) for chemical stability and size exclusion chromatography (SEC) for aggregation propensity. Surface plasmon resonance (SPR) measurements of antigen binding were used to assess activity retention.

[00419] Hu20D12.v2.1, formulated at 200mg/mL in PBS pH 7.4 or at 170mg/mL in 20mM HisHCl pH 5.5, was sterile filtered using 0.22µm SteriFlip units (EMD Millipore), aliquoted (100µL), and incubated at 37°C for 0, 2 or 4 weeks. Upon incubation completion, samples were diluted in a sucrose-containing formulation buffer and frozen at -20°C. After thawing, samples were analyzed by IEC, SEC and SPR, as described below.

[00420] *Chemical Stability Assessment by Ion Exchange Chromatography.* Chemical

stability of hu20D12.v2.1 was monitored using Ion Exchange Chromatography (IEC). IEC was performed on an Agilent 1200 HPLC with an in-line diode array detector (DAD). Thawed protein samples were prepared for IEC by being diluted to 1mg/mL in PBS. Samples were held at 2-8°C prior to injection on column to maintain stability. Separation of a 20µg protein injection was performed using a ProPac SAX-10 2x250mm column (Dionex) at 40°C. Solvent A was 20mM Tris pH 8.2 and Solvent B was 250mM sodium chloride in Solvent A. A PBS buffer blank was included for subtraction of buffer contribution to UV signal. The salt gradient used for separation of the chemical degradants is shown in Table 10.

Table 10. Ion Exchange Chromatography Gradient for hu20D12.v2.1

Time (min)	% Solvent A	% Solvent B
0	100	0
45	20	80
45.5	0	100
50	0	100
50.1	100	0
60	100	0

[00421] Data were processed using Chromeleon 6.8 software (Thermo Scientific) to integrate all chromatogram peaks attributed to protein. The percent peak area was calculated using Equation 1 and was reported for all peaks of interest, termed acid variants, main peak, and basic variants. If no peak for acid or basic variant was detected, the peak area percentage was reported as zero.

$$\%\ Peak\ Area = \frac{area\ of\ peak}{total\ area\ of\ protein\ peaks} \times 100 \qquad (Equation\ 1)$$

[00422] The IEC results for the 37°C high concentration stability samples of hu20D12.v2.1 are reported in Table 11A-B.

Table 11A. Chemical Stability of 37°C High Concentration Stability Samples (PBS) of hu20D12.v2.1 by Ion Exchange Chromatography

Time (weeks)	% Acidic Variants	%Main Peak	%Basic Variants
0	11.0	78.0	11.1
2	14.0	75.5	10.5
4	15.8	71.3	12.9

Table 11B. Chemical Stability of 37°C High Concentration Stability Samples (His-HCl pH 5.5) of hu20D12.v2.1 by Ion Exchange Chromatography

Time (weeks)	% Acidic Variants	%Main Peak	%Basic Variants
0	11.1	76.3	12.7
2	13.6	75.9	10.6
4	15.5	71.7	12.8

[00423] *Molecule Size Distribution Assessment by Size Exclusion Chromatography.*

Molecule size distribution of hu20D12.v2.1 was monitored using Size Exclusion Chromatography (SEC). SEC was performed on an Agilent 1200 HPLC with an in-line diode array detector (DAD). Thawed protein samples were prepared for SEC by being diluted to 1mg/mL in PBS. Samples were held at 2-8°C prior to injection on column to maintain stability. Separation of a 100µg protein injection was performed using a TSKgel G3000SWxl column (Tosoh Biosciences, part no. 08541) at ambient temperature. Mobile phase consisted of 0.20 M potassium phosphate, 0.25 M potassium chloride, pH 6.2 ± 0.1. UV sample signal was monitored at 280nm. A PBS buffer blank was included for subtraction of buffer contribution to UV signal.

[00424] Data were processed using Chromeleon 6.8 software (Thermo Scientific) to integrate all chromatogram peaks attributed to protein. The percent peak area was calculated using equation 1, above, and was reported for all peaks of interest, termed high molecular weight species (HMWS), main peak (monomer), and low molecular weight species (LMWS). If no peak for HMWS or LMWS was detected, the peak area percentage was reported as zero. The SEC results for the 37°C high concentration stability samples of hu20D12.v2.1 are reported in Table 12A-B.

Table 12A. Molecule Size Distribution of 37°C High Concentration Stability Samples (PBS) of hu20D12.v2.1 by Size Exclusion Chromatography

Time (weeks)	%LMWS	%Monomer	%HMWS
0	0	98.3	1.7
2	0	96.7	3.3
4	0	96.0	4.0

Table 12B. Molecule Size Distribution of 37°C High Concentration Stability Samples (His-HCl pH 5.5) of hu20D12.v2.1 by Size Exclusion Chromatography

Time (weeks)	%LMWS	%Monomer	%HMWS
0	0	98.7	1.2
2	0	97.4	2.6
4	0	97.1	2.9

[00425] *Factor D Binding Capacity by Surface Plasmon Resonance Measurements.* Surface plasmon resonance (SPR) measurements were carried out using a BIAcore®T200 instrument (GE Healthcare) to determine the binding capacity of stressed hu20D12.v2.1 samples. A Series S carboxymethylated dextran (CM5) sensor chip was prepared by immobilizing human Factor D (target RU ~3,000) using an amine coupling kit (GE Healthcare) following a protocol described by the manufacturer. Standard solutions of unstressed hu20D12.v2.1 were prepared in HBS-P+ running buffer (GE Healthcare) from 106.5nM to

3.31nM in two-fold dilutions and were injected at 10μL/min for 180s to generate a standard curve. Stressed samples were diluted to ~40nM in HBS-P+ buffer and injected at 10μL/min for 180s. After each injection, the sensor chip surface was regenerated with 10mM Glycine-HCl, pH 2.1, at 30μL/min for 30s. Concentration analysis was carried out using the BIAcore®T200 analysis software (GE Healthcare). This type of analysis has a standard error of ±10% such that samples with binding capacities of 90-110% are considered to be fully active. The SPR Factor D binding capacity results for the 37°C high concentration stability samples of hu20D12.v2.1 are shown in Table 13A-B.

Table 13A. Factor D Binding Capacity of 37°C High Concentration Stability Samples (PBS) of hu20D12.v2.1 by Surface Plasmon Resonance Measurements

Time (weeks)	%Factor D Binding
0	90.0
2	100.0
4	90.1

Table 13B. Factor D Binding Capacity of 37°C High Concentration Stability Samples (His-HCl pH 5.5) of hu20D12.v2.1 by Surface Plasmon Resonance Measurements

Time (weeks)	%Factor D Binding
0	91.0
2	92.2
4	88.3

Overall, these results indicate that hu20D12.v2.1 is well suited for high concentration formulation in both PBS and 20 mM His-HCl pH 5.5. There is only a slow rate of loss of main peak by both SEC and IEC, and very little decrease in factor D-binding, relative to the initial sample, for at least 4 weeks of thermal stress at 37°C.

Example 8: Polymer Conjugation of hu20D12 variants

[00426] Vitreal half-life can be extended by increasing the hydrodynamic radius of the antibody Fab. Hydrodynamic radius of a Fab can be increased by covalent attachment of a polymer such as polyethylene glycol (PEG). It can be beneficial for this modification to be done in a site-specific manner, at a site removed from the antigen-binding region, so as to minimize the effect of conjugation on biological activity. Availability of a free cysteine residue for modification is desirable for this purpose. Single or multiple free cysteines can be employed to enable single or multiple sites of attachment. A single cysteine at the C-terminus of the heavy chain was included in the Fabs for modification. The Fab-C versions of the antibodies were made by extending the Fab heavy chain by one residue to include Cys-226 (EU numbering). In

the IgG1 molecule, Cys-226 forms the first inter-heavy chain disulfide bond of the hinge. Oligonucleotide-directed mutagenesis was performed as described above to produce gene constructs for the Fab-C versions of hu20D12.v2.1.C (SEQ ID NO: 119) and hu20D12.v2.3.C (SEQ ID NO: 124). Similar mutagenesis procedures can be used to generate additional Fab-C constructs, for example, SEQ ID NOs: 71, 118 to 122, and 140 to 146 for hu20D12.v2.1.C, and SEQ ID NOs: 75, 123, 125 to 127, and 147 to 153 for hu20D12.v2.3.C.

[00427] Fab-C versions of hu20D12.v2.1 ("hu20D12.v2.1.C") and hu20D12.v2.3 ("hu20D12.v2.3.C") were expressed in E. coli and purified substantially as follows. The Fab-C variants were captured using Gamma Plus resin, with a 6.5mM GSH pH 8.5 wash for 5 column volumes to deblock c-terminal cysteine and disrupt Fab-C dimer formation, followed by elution into 0.1M acetic acid pH 2.9. The Fab-C monomers were further isolated using SP Sepharose High Performance strong cation exchange resin from GE in 25mM Sodium Acetate pH 5.0, with a 0.05% Triton X-100+0.05% Triton X-114 was for 19 hours for endotoxin removal. Elution was performed with gradient between 0-20% 25mM Sodium Acetate pH 5.0+1M NaCl over 20 column volumes. Hu20D12.v2.1.C or hu20D12.v2.3.C were conjugated to PEG octamer in 25mM NaAcetate pH 6.5, 150mM NaCl, 4mM EDTA, at a concentration around 5 mg/mL. The Fab-C's were not further concentrated in order to minimize cysteine reactivity loss due to Fab-C dimerization. After equilibrating to room temperature, 40K TP PEG powder from JenKem was resuspended in 25mM NaAcetate pH 5.0 to a concentration of 10 mg/mL. The pH was kept below pH 6 to avoid maleimide ring opening. Once PEG was solubilized, it was added to the Fab-C pool at a molar ratio of 0.1125:1 PEG to Fab-C. The mixture was then left at room temperature with gentle shaking overnight. The following day, the conjugation efficiency was checked by SEC-MALS. Following conjugation, hu20D12.v2.1.C + TP-Oct was purified using Size Exclusion Chromatography (SEC) on a Sephacryl S-300 HR (GE Healthcare) column in 20 mM His-acetate, pH 5.5, 50 mM NaCl (isocratic gradient) followed by Cation Exchange (CEX) using SP Sepharose High Performance strong cation exchange resin from GE to enrich for 8 Fabs/PEG. The CEX step was run in 25mM Sodium Acetate pH 5.0 and eluted using a 10-20% 1M NaCl gradient over 50CV. An example of CEX chromatogram (Figure 8A) and Fab distribution in the S300 pool (Figure 8B) is presented. Data for different fractions are shown in Figure 8C.

[00428] For all processes, the ratio of Fab/PEG was determined by Size Exclusion Chromatography (SEC) using a 300 x 8 mm Shodex OH pak SB-804 HQ run at 0.8 mL/min using phosphate buffered saline (PBS) pH 7.2, 150 mM NaCl under isocratic conditions. Molar mass was determined using in-line static Multi-Angle laser Light Scattering (MALS) by Wyatt Technology. A typical SEC-MALS profile for conjugated hu20D12.v2.1.C + TP-Oct is shown

in Figure 7A. Photon correlation spectroscopy was used to determine hydrodynamic radii (R_H), using Quasi-Elastic Light Scattering (QELS), a single photon counting module with detection at a 99.0°, also by Wyatt Technology. *See* Figure 7B. Raw data was analyzed using Wyatt's proprietary Astra software, where molar mass and R_H constants were set using a rituximab standard. The R_H value for hu20D12.v2.1.C + TP-Oct is 10.3 nm. *See* Figure 7C.

[00429] Size Exclusion Chromatography (SEC) is also used to determine the size variant distribution of anti-factor D conjugates. Solutions of anti-factor D conjugates (300 µg) are diluted to 2 mg/ml with 20 mM Sodium Phosphate, 300 mM Sodium Chloride, pH 7.0 buffer. For the analysis, an HPLC system capable of gradient flow with UV detection at 280 nm is used. The chromatographic column is a Tosoh G4000 SWxl (8 µm, 450 Å, 7.8 mmID x 30 cm), operated at 0.5 ml/min. Fifty microliters of 2 mg/ml anti-factor D diluted in mobile phase are injected into the HPLC system. The column temperature is ambient, the autosampler is at 2-8°C the mobile phase is in isocratic mode. The column is equilibrated until a stable baseline is obtained and reference material is injected until a consistent chromatographic profile is observed for a minimum of two consecutive injections. This size variant based assay is capable of resolving the unconjugated Fab forms from the conjugate and the aggregate forms (Figure 20).

[00430] Both hu20D12.v2.1.C + TP-Oct and hu20D12.v2.3.C + TP-Oct retained full activity in the TR-FRET inhibition assay after conjugation to octamer-PEG. *See* Figure 5A. Site-specific conjugation appeared to preserve activity. Further, arranging the Fab on a multimeric scaffold does not significantly affect inhibitor potency.

[00431] Viscosity of a formulation is an important parameter for determining suitability for injection. Therefore, viscosity measurements were carried out for the PEGylated Fab-C. Viscosity measurements were performed on a TA Instruments cone and plate rheometer thermostatted at 25°C using a shear rate of 1000 s^{-1}. For measurements on Fabs, proteins were prepared in a pH 5.5, low salt buffer. Viscosity measurements on PEGylated Fab-C materials employed a buffer of 20 mM His-acetate, 50 mM NaCl, pH 6.5 and the temperature of measurement was 20°C. PEG-octamer conjugates of both hu20D12.v2.1.C ("hu20D12.v2.1.C + TP-Oct") and hu20D12.v2.3.C ("hu20D12.v2.3.C + TP-Oct") show low viscosity and a concentration dependence of viscosity that is suitable for injection of a high concentration formulation. *See* Figure 9A. In contrast, the PEG-octamer conjugate of AFD.v14.C ("AFD.v14.C + TP-Oct"), which is a variant of lampalizumab, shows higher viscosity and a concentration dependence of viscosity that poses more challenge for injection of a high concentration formulation. *See* Figure 9A. Viscosity measurements were also done on Fab-C proteins conjugated to 40K-tetramer-PEG (Sunbright®PTE-400 MA, *see* Table 15). The PEG tetramer conjugate of hu20D12.v2.3.C ("hu20D12.v2.3.C + PEG tetramer") shows low viscosity

and a concentration dependence of viscosity that is suitable for injection of a high concentration formulation. *See* Figure 9B. In contrast, the PEG-tetramer conjugate of AFD.v8.C ("AFD.v8.C + PEG tetramer"), which is a variant of lampalizumab, shows higher viscosity and a concentration dependence of viscosity that poses more challenge for injection of a high concentration formulation. *See* Figure 9B.

[00432] Viscosity measurements were also performed on hu20D12.v2.1.C+TP-Oct using an AR-G2 Advanced Rheometer with anodized aluminum geometry 20 mm diameter, 1° angle (part #513204.905). Measurements were carried out on solutions of varied protein concentration in a buffer comprised of 20 mM His-HCl, pH 5.5, 0.02% polysorbate-20. The temperature was 25°C and the shear rate was 1000 s^{-1}. As shown in Table 14 this formulation had a very good profile for dependence of viscosity on protein concentration and at the highest Fab concentration tested (235 mg/mL) the viscosity was only 203 centiPoise (cP).

Table 14. Viscosity of hu20D12.v2.1-C + TP-Oct formulated in 20 mM His-HCl, pH 5.5, 0.02% polysorbate-20, at varied protein concentration

Protein Concentration (mg/mL)	Viscosity (cP)
50	2.83
125	15.14
235	203.22

[00433] PEG Preparation: PEG powders were reconstituted to 10mg/mL with water. A 10mM Sodium trifluoroacetic acid solution was used as a cationizing agent. A 20mg/ml α-Cyano-4-hydroxycinnaminic acid matrix solution dissolved in 50% acetonitrile: 0.1% triflououacetic acid was employed. PEG solution, sodium trifluoroacetic acid and 50% acetonitrile: 0.1% trifluoroacetic acid were mixed at a 3:3:4 ratio. One microliter of the PEG mixture was deposited onto a MALDI target plate. One microliter of matrix was added to the spot and allowed to dry at ambient temperature. Analysis was performed on a 4800 MALDI TOF/TOF instrument (Sciex, Framingham, MA) equipped with a 355 nm, 200 Hz Nd:Yag Laser in linear mode with m/z range from 5000-100000 and a laser power of 5000.

[00434] External calibrations were performed using PEG standards ranging in size from 2000 to 40000 daltons (Sigma, St. Louis, MO) under similar sample conditions. Mass readings were reported as the highest point on the unresolved spectrum, in cases where more than one peak existed, readings were reported as the apex of each peak. Peaks were visualized using Data Explorer software (Sciex, Framingham, MA).

[00435] Reconstituted PEG solutions were also analyzed by direct infusion *via* a Nanomate (Advion, Ithaca, NY) onto Exactive Plus EMR (Extended Mass Range) orbitrap

instrument (EMR, Thermo, San Jose, CA). A 1% (10 mg/1 ml) 1,8-Bis(tetramethylguanidino)naphthalene (Sigma, St. Louis, MO) solution dissolved in 70% H2O: 30% Dimethyl sulfoxide (DMSO) was used as charge reducing reagent. 10 ul of 10mg/mL PEG solution was combined with 10 ul 1% 1,8-Bis(tetramethylguanidino)naphthalene, and direct infused onto the EMR at the following acquisition parameters: Spray Gas pressure,1.0 psi; Spray voltage to apply 1.50 kV; Capillary temp, 325 °C; S-lens RF level,100; Scan rage,1000 to 20000 m/z; Fragmentation, in-source CID 200 Ev, CE 200; Resolution, 17500; Polarity, positive; Microscans,10; AGC targe, 3e6; Maximum injection time, 50; AGC mode, fixed; Averaging, 100; Source DC offset, 25V; Injection Flatapole DC, 8V; Inter Flatapole lens, 7V; Bent Flatapole DC, 6V, Transfer multipole DC tune offset, 0V; C-Trap entrance lens tune offset, 0V; Trapping gas pressure setting, 8. Spectra were visualized using Thermo Xcalibur Qual Browser then annotated manually.

[00436] PEG-Fab conjugates, in which core PEG structure and number of conjugates varied by sample type, may be analyzed on a 4800 MALDI TOF/TOF, in linear mode. Four microliters of PEG-Fab conjugate are mixed with four microliters of sodium trifluoroacetic acid and two microliters of 50% acetonitrile: 0.1% trifluoroacetic acid. Equal volumes of PEG-Fab solution and 20mg/mL sinapinic acid (in 50% acetonitrile: 0.1% trifluoroacetic acid, Agilent, Santa Clara, CA) matrix are deposited onto a MALDI target plate for analysis. The m/z range is set from 100000 to 500000, with target mass set to 400000. Spectra are visualized using Data Explorer then annotated manually.

Example 9: Thermal Stability of Polymer Conjugates

[00437] To simulate the exposure of the hu20D12 conjugates to conditions that may be found in long-acting delivery systems, samples of the hu20D12.v2.1.C and hu20D12.v2.3.C TP conjugates (prepared as above) were stressed under two different pH and salt conditions for several weeks at 37°C. Specifically, conjugates were evaluated in the following formulations:

Formulation 1: ~25 mg/mL, PBS,

Formulation 2: ~25 mg/mL, 20 mM histidine HCl, 50 mM NaCl, at pH 6.5.

[00438] PBS was used as a mimic of the pH and ionic strength of human vitreous. Aliquots (100 µL) of solutions of hu20D12.v2.1 or hu20D12.v2.3 conjugate, formulated at 25 mg/mL in PBS or 20 mM His-Ac pH 6.5, 50 mM NaCl, were sterile filtered by centrifugal filtration using 0.22 µm Costar® Spin-X centrifuge tubes (Corning) and then incubated at 37°C for 0, 2, 4, or 8 weeks. Incubations were terminated by freezing at -70°C. After thawing, samples were analyzed by SEC-MALS, CE-SDS, and by biacore to assess Factor D-binding capacity (methods described below). No change in binding capacity greater than the standard error in the measurements (±10%) was determined for incubation of the conjugate at 37°C. *See*

Figure 10A, showing thermal stability (37°C) of hu20D12.v2.1.C + TP-Oct analyzed by binding capacity (SPR). Cross-hatched region shows standard error of ± 10 %. The binding capacity remained steady even after 4 weeks at 37°C in PBS and after 4 weeks at pH 6.5. Analysis of hu20D12.v2.1.C +TP-Oct conjugate by CE-SDS showed good thermal stability (37 °C) in both formulations. *See* Figure 10B. A loss in % peak area of the conjugate of only ~1%/week was observed.

a. FQ labeling procedure

[00439] Samples were labeled according to the following procedure. Solutions of hu20D12.2.1-C + TP-Oct (300 µg) were exchanged into 0.5 mL sodium phosphate reaction buffer using NAP-5 gel filtration columns (GE Healthcare, Piscataway, NJ, USA) to remove potentially competing formulation constituents. A 250 µL aliquot of the desalted conjugate was mixed with 30 µL of 150 mM N-ethylmaleimide dissolved in 4% SDS and incubated for 5 min at 70°C to control disulfide reshuffling under denaturing conditions. Ten microliters of each 2.5 mM FQ and 30 mM KCN reagents were added to the SDS- hu20D12.2.1-C + TP-Oct solution, and the final solution was incubated for 10 min at 50°C before diluted threefold with 1% SDS to quench the reaction. For reducing analysis, aliquots of the diluted samples were incubated with 50 mM DTT for 10 min at 70°C.

b. CE-SDS Analysis

[00440] Separation of PEGylated Fab samples was performed with 31.2 cm (21 cm effective length) fused-silica capillaries of 50 µm ID (Polymicro technologies, Phoenix, AZ, USA) encased in 40°C thermal controlled cartridges. Fully automated Beckman PA800+ systems (Beckman Coulter, Brea, CA, USA) were equipped with LIF detection and used 32 Karat version 9.1 to control the instrument. The LIF detector used a 3.5 mW argon-ion laser having an excitation at 488 nm; emission was collected through a 600 ± 20 nm bandpass filter (Edmund Optics, Barrington, NJ, USA). Voltage was applied in the negative mode (reverse polarity). Sample solutions were introduced electrokinetically at 5 kV for 25 s and separated at 17 kV. Between runs, the capillary was washed with 0.1 M NaOH, 0.1 M HCl and Beckman gel buffer for 5 min, 1 min, 1 min and 10 min, respectively. *See, e.g.*, Michels et al., 2007, *Anal. Chem.*, 79: 5963-71; Michels et al., 2012, *Electrophoresis*, 33: 815-26. An example electropherogram of a non-reduced sample of hu20D12.2.1-C + TP-Oct is shown in Figure 21.

c. Surface Plasmon Resonance Analysis

[00441] A Series S, CM5 sensor chip was docked into a Biacore® T200 instrument (GE Healthcare), primed with 1X running buffer and normalized with 70% glycerol following a protocol supplied by the manufacturer. The sensor chip surface was activated for amine-coupling of antigens using the amine coupling kit with the materials provided and the protocol

suggested by the manufacturer. Human factor D (fD) was covalently immobilized by injecting a solution containing 100 µg/mL antigen prepared by dilution of fD (PUR#20491, 2.4 mg/mL) with 10 mM sodium acetate pH 5. The flow rate was 10 µL/min and an injection volume of 70 µL was used. This yielded a typical coupling density across multiple experiments of about 5000 Resonance Units (RU) for fD. Unreacted amine coupling sites were blocked by injection of 70 µL 1 M ethanolamine.

[00442] Antigen-binding active concentrations of antibody Fab were determined using the calibration-dependent concentration analysis routine of the Biacore® T200 evaluation software. A standard curve of hu20D12.v2.1.C + TP-Oct was prepared through gravimetric dilution of a stock solution to 5 µg/mL followed by serial 2-fold dilutions to produce samples of 2.5, 1.25, 0.625, 0.313, 0.156, and 0.078 µg/mL. Test samples were prepared by gravimetric dilution to obtain protein concentrations of about 0.5, 1.0, or 1.5 µg/mL. All samples (200 µL volume) were prepared using 1X running buffer. 60 µL aliquots were injected over the specific antigen surface using a flow rate of 10 µL/min with the sensor chip maintained at 25°C and primed with 1X running buffer. Antibody bound to specific antigen was determined from the SPR signal near the end of the sample injection. Bound antibody was eluted at the end of each binding cycle through injection of 30 µL of 10 mM Gly-HCl pH 2.1 to cause dissociation of the antibody-antigen complex. The standard curve of hu20D12.2.1-C + TP-Oct was used to determine the relationship of SPR signal to antibody concentration using a four-parameter function to analyze the data. Parameters calculated from the standard curve were used to calculate the antigen-binding concentration of test samples based on the observed SPR signal. The ratio of this concentration to the protein concentration determined by absorbance measurements gives the fraction or percent binding.

Example 10: Additional Anti-Factor D Antibody Variants for Polymer Conjugation

[00443] As discussed above, including a free cysteine in the constant region of the anti-Factor D Fab can improve conjugation and minimize interference with binding to antigen. In addition to the heavy chain Fab C-terminus "CDKTHTC," the heavy chains of the Fab fragments may also be modified by adding the first four residues from the hinge region of the Fab-C counterpart, to give the C-terminus "CDKTHTCPPC." The C-terminus "CDKTHTCPPS," "CDKTHTSPPC," "CDKTH," "CDKT," "CDK," "CD," "APPC," "SGGC," or "CYGPPC" may also be used. In some embodiments, the "CDKTHTCPPC" terminus allows attachment of two PEG molecules. The resulting Fabs can be conjugated with a multi-arm PEG.

Example 11: Preparation of Anti-Factor D Antibody Conjugates

[00444] Humanized anti-Factor D Fab variants with the heavy chain Fab terminus

"CDKTHTC," "CDKTHTCPPC," "CDKTHTSPPC," "CDKTHTCPPS," "CDKTH," "CDKT," "CDK," "CD," "APPC," "SGGC," or "CYGPPC" are conjugated with commercially available maleimide-functionalized multi-armed PEGs having varying core structures.

a. Maleimide-Functionalized Multi-Armed PEGs

[00445] The maleimide-functionalized multi-armed PEGs detailed in Table 15, below, are used in the conjugation reactions:

Table 15: Maleimide-functionalized multi-armed PEGs

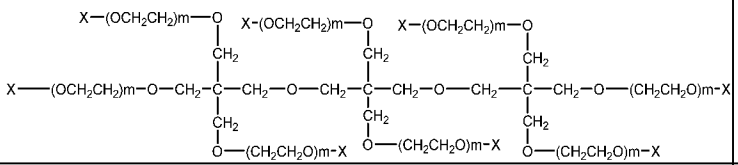
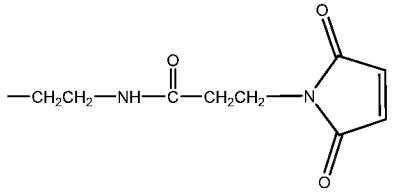
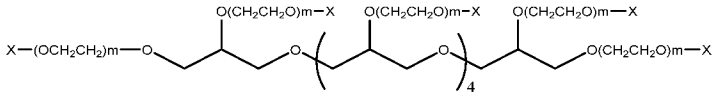
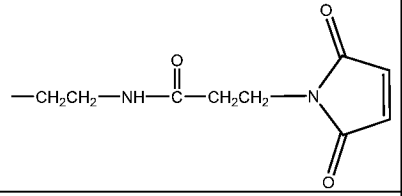
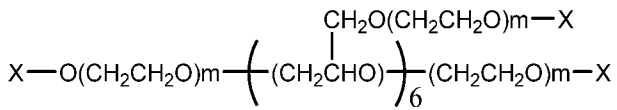
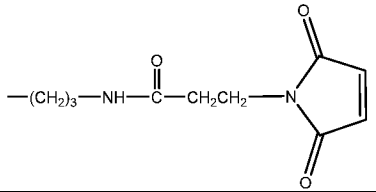
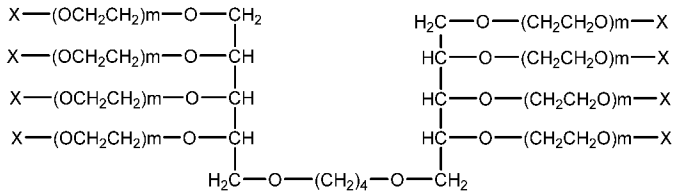
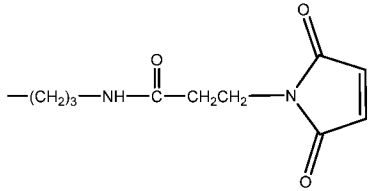
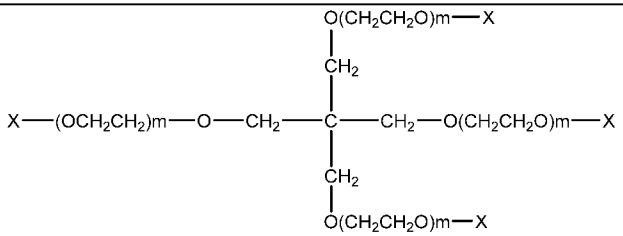
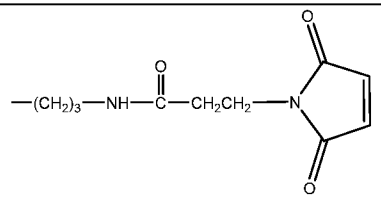
PEG	Vendor	Core structure	Functional Group (X)	Poly-dispersity	Average MW
8ARM (TP)-PEG-MAL	JenKem Technology, USA	Tripentaerythritol (TP) 		~1.04	40,000
8ARM-PEG-MAL	JenKem Technology, USA	Hexaglycerin (HG) 		~1.08	40,000
Sunbright ® HGEO-400MA	NOF America Corp.	Hexaglycerol (HGEO) 		~1.33	40,000
Sunbright ® DX-400MA	NOF America Corp.	Butanediol 		~1.01	40,000

Table 15: Maleimide-functionalized multi-armed PEGs

PEG	Vendor	Core structure	Functional Group (X)	Poly-dispersity	Average MW
Sunbright ® PTE-400MA	NOF America Corp.			*Not provided*	40,000

[00446] The 8ARM (TP)-PEG-MAL (JenKem Technology, USA) and Sunbright® HGEO-400MA (NOF America, Corp.) were analyzed using MALDI to compare the homogeneity of PEG octamers containing either the TP core or the HGEO core. The results are shown in Figures 12A and 12B. As can be seen from those figures, the 8ARM (TP)-PEG-MAL containing the TP core was more homogeneous than the Sunbright® HGEO-400MA containing the HGEO core.

b. Conjugation of Fabs with Maleimide-Functionalized Multi-Armed PEGs

[00447] The cysteine-modified Fab is captured using Gamma Plus resin, with 6.5mM GSH pH 8.5 wash for 5 column volumes to deblock C-terminal cysteine and disrupt Fab-C dimer formation, followed by elution into 0.1M acetic acid pH 2.9. The cysteine-modified Fab monomer is further isolated using SP Sepharose High Performance strong cation exchange resin from GE in 25mM Sodium Acetate pH 5.0, with 0.05% Triton X-100+0.05% Triton X-114 was for 19 hours for endotoxin removal. Elution is performed with gradient between 0-20% 25mM Sodium Acetate pH 5.0+1M NaCl over 20 column volumes. The monomeric Fab-C with deblocked c-terminal cysteine is then prepared for PEGylation by being titrated to pH 6.5 using 1M HEPES pH 7.2. Fab-C is then conjugated to PEG octamer in 25mM NaAcetate pH 6.5, 150mM NaCl, 4mM EDTA, at a concentration around 5 mg/mL. Fab-C is not further concentrated in order to minimize cysteine reactivity loss due to Fab-C dimerization. After equilibrating to room temperature, 40K TP PEG powder from JenKem is resuspended in 25mM NaAcetate pH 5.0 to a concentration of 10 mg/mL. The pH is kept below pH 6 to avoid maleimide ring opening. Once PEG is solubilized, it is added to the Fab-C pool at a molar ratio of 0.1125:1 PEG to Fab-C. The mixture is then left at room temperature with gentle shaking overnight. The following day, the conjugation efficiency is checked by SEC-MALS.

Example 12: Exemplary Purification and Characterization of Conjugates

[00448] The conjugates prepared in Example 10 are purified and analyzed using SEC-MALS to confirm PEGylation and determine conjugation efficiency for different PEG core structures. Conjugation efficiency is determined by Size Exclusion Chromatography (SEC) using a 300 x 8 mm Shodex OH pak SB-804 HQ run at 0.8 mL/min using phosphate buffered saline (PBS) pH 7.2, 150 mM NaCl under isocratic conditions. Molar mass is determined using in-line static Multi-Angle laser Light Scattering (MALS) by Wyatt Technology. Photon correlation spectroscopy was used to determine hydrodynamic radii (RH), using Quasi-Elastic Light Scattering (QELS), a single photon counting module with detection at a 99.0o, also by Wyatt Technology. Raw data is analyzed using Wyatt's proprietary Astra software, where molar mass and RH constants are set using a rituximab standard.

a. Cys-Modified Fab-8ARM (TP)-PEG-MAL Conjugate ("Fab TP conjugate")

[00449] The conjugates are purified using Size Exclusion Chromatography (SEC) on a Sephacryl S-300 HR (GE Healthcare) column in 20 mM His-acetate, pH 5.5, 50 mM NaCl (isocratic gradient). Molar mass is determined using in-line static Multi-Angle laser Light Scattering (MALS) by Wyatt Technology and Shodex OHpak SB804 (13C). Raw data is analyzed using Wyatt's proprietary Astra software, where molar mass constants were set using a rituximab standard. Molar mass is used to estimate the average number of Fabs attached to each PEG. Fabylation is determined by subtracting 40 g/mol (average mass of PEG octamer) from the MALS measured mass, then dividing by the average mass of cysteine-modified Fab. The theoretical molar mass for 8 Fab's + 40K PEG octamer is 416,056 g/mol. Exemplary data is shown in Table 16.

Table 16

Fraction #	Molar Mass (g/mol)	Estimated Fabylation
B2	502,000	agg
B3	470,200	N/D
B4	453,200	N/D
B5	444,300	8 Fabs/PEG
B6	430,400	8 Fabs/PEG
B7	410,900	8 Fabs/PEG
C1	388,100	7 Fabs/PEG
C2	349,100	6-7 Fabs/PEG

agg = aggregates

[00450] Conjugation of cysteine-modified Fab with a multi-armed PEG octamer having the TP core produces conjugates comprising 8 Fabs/PEG, demonstrating that good conjugation efficiency can be achieved with PEG octamers comprising a TP core.

b. Cys-Modified Fab-8ARM-PEG-MAL Conjugate ("Fab HG conjugate")

[00451] The Cys-modified Fab-8ARM-PEG-MAL conjugate (containing the HG core structure (JenKem); hereinafter the "Fab HG conjugate") prepared in Example 10 is purified using SEC on a Sephacryl S-300 HR (GE Healthcare) column in 20 mM His-acetate, pH 5.5, 50 mM NaCl (isocratic gradient). The conjugate containing fractions are pooled, and further purified using SEC on a Sephacryl S-300 HR (GE Healthcare) column in 20 mM His-acetate, pH 5.5, 50 mM NaCl (isocratic gradient). Molar mass is determined using Tosoh G3000PW column and in-line static MALS by Wyatt Technology. Photon correlation spectroscopy is used to determine hydrodynamic radii (RH), using Quasi-Elastic Light Scattering (QELS), a single photon counting module with detection at a 99°, also by Wyatt Technology. Raw data is analyzed using Wyatt's proprietary Astra software, where molar mass and RH constants are set using a rituximab standard. Molar mass is used to estimate the number of Fabs attached to each

PEG. Exemplary results are shown in Table 17.

Table 17

Fraction #	Mw (kDa)	Estimated Fabylation	R_H (nm)
A6	1146.6 (± 0.1%)	Agg	16.0 (± 4.9%)
B3	861.6 (± 0.1%)	Agg	14.5 (± 4.0%)
B6	758.3 (± 0.1%)	Agg	13.7 (± 3.8%)
C1	649.3 (± 3.8%)	n/d	13.5 (± 3.8%)
C6	562.6 (± 0.1%)	n/d	12.8 (± 3.6%)
D2	546.7 (± 0.1%)	n/d	12.8 (± 3.7%)
D4	536.6 (± 0.1%)	n/d	12.6 (± 3.6%)
E2	525.4 (± 0.1%)	n/d	12.5 (± 3.5%)
E5	489.2 (± 0.2%)	8 Fab/PEG	12.3 (± 3.7%)
F1	409.2 (± 0.2%)	7-8 Fab/PEG	10.8 (± 3.7%)
F4	342.2 (± 0.1%)	6-7 Fab/PEG	9.7 (± 2.9%)
F6	325.5 (± 0.2%)	6 Fab/PEG	9.5 (± 0.3%)
G2	302.4 (± 0.2%)	5-6 Fab/PEG	9.3 (± 3.1%)

agg = aggregates
n/d = not determined

[00452] As can be seen from Table 17, conjugation of the Cys-modified Fab with a PEG octamer comprising the HG core produces conjugates comprising 8 Fabs/PEG. Conjugation with the HG core also produced more conjugates comprising 5-7 Fabs/PEG, than was observed with the TP core.

[00453] In an effort to improve Fabylation estimate and RH measurement, the HG final pool prepared above may be alternately analyzed using SEC-MALS on a 10/300 Sephacryl S-400 HR (GE Healthcare) column in PBS, pH 7.4, run at 0.25mL/min. Molar mass and RH are determined as described above. In an exemplary experiment, the conjugates prepared using the 8ARM-PEG-MAL (HG core) and purified on Sephacryl S-400 HR have an average RH of 12.2 nm (± 4.5%), an average molar mass of 340.3 kDa (± 8.9%), and an average of 6.4 Fabs/PEG.

c. Cys-Modified Fab-HGEO-400MA Conjugate ("Fab-HGEO1 conjugate")

[00454] The Cys-modified Fab-HGEO-400MA conjugate (containing the Sunbright® HGEO-400MA PEG; hereinafter the "Fab HGEO1 conjugate") prepared in Example 4 is purified using SEC on a Sephacryl S-400 HR (GE Healthcare) column in 20 mM His-acetate, pH 5.5, 50 mM NaCl (isocratic gradient). Molar mass and RH are determined using Sephacryl S-400 HR, run at 0.25mL/min in PBS pH 7.4.

[00455] In an exemplary experiment, the conjugates prepared using the Sunbright® HGEO-400MA PEG (HGEO core) have an average RH of 15.2 nm (± 4.5%), an average molar mass of 423.8 kDa (± 10.6%), and an average of 8.2 Fabs/PEG.

d. Cys-Modified Fab-8ARM (HGEO)-PEG-MAL Conjugate ("Fab HGEO2 conjugate")

[00456] The Cys-modified Fab-8ARM(HGEO)-PEG-MAL conjugate (containing the

HGEO core structure (JenKem); hereinafter the "Fab HGEO2 conjugate") prepared as above is purified using SEC on a Sephacryl S-300 HR (GE Healthcare) column in 20 mM His-acetate, pH 5.5, 50 mM NaCl (isocratic gradient). Molar mass is determined as described above. Molar mass is used to estimate the number of Fabs attached to each PEG. Exemplary results are set forth in Table 18.

Table 18

Fraction #	Molar Mass (g/mol)	Estimated Fabylation
B1	2,145,000 (± 0.8%)	aggregate
B2	665,800 (± 0.7%)	aggregate
B3	426,400 (± 0.8%)	8 Fabs/PEG
B4	296,400 (± 0.8%)	6 Fabs/PEG
B5	246,200 (± 0.8%)	5 Fabs/PEG
B6	215,000 (± 0.8%)	

[00457] As can be seen from Table 18, conjugation of the Cys-modified Fab with a PEG octamer comprising the HGEO2 core produces conjugates comprising 8 Fabs/PEG. Conjugation with the HGEO2 core also produces more conjugates comprising 5-6 Fabs/PEG, than was observed with the TP core.

Example 13: Enrichment of Conjugates

[00458] One way to increase the Fab concentration in an intravitreal formulation without significantly increasing formulation viscosity, is to increase the percentage of highly fabylated conjugates in the formulation. In this example, cation exchange chromatography is used to enrich for highly fabylated conjugates.

[00459] Fractions containing estimated fabylation of 8 Fab/PEG from the SEC purification of the Fab TP conjugate described above are pooled and subjected to cation exchange chromatography using, e.g., SP Sepharose High Performance strong cation exchange resin from GE, with 0.05% Triton X-100+0.05% Triton X-114 wash for 19 hours to remove endotoxin, followed by gradient elution between 10-20% 1M NaCl over 50 column volumes (CV). Molar mass is determined as described above. Exemplary results are set forth in Table 19.

Table 19

Fraction #	Molar mass (g/mol)	Estimated Fabylation	GEL Lane #
3B11	335,000	6 Fabs/PEG	1, 8
3E12	367,100	7 Fabs/PEG	2, 9
4A7	414,200	8 Fabs/PEG	3, 10
4C5	430,000	8 Fabs/PEG	4, 11
4F3	483,900	n/d	5, 12

4H9	567,400	n/d	6, 13

[00460] The conjugate containing fractions are pooled, and further purified using a 300 x 8 mm Shodex OH pak SB-804 HQ, run at 0.8 mL/min using phosphate buffered saline (PBS), pH 7.4, 150 mM NaCl, under isocratic conditions. Molar mass and RH are determined as described above.

[00461] Following enrichment, conjugates prepared using the 8ARM (TP)-PEG-MAL (TP core) are obtained that have an average R_H of 10.5 nm (± 2.5%), an average molar mass of 407.1 kDa (± 0.2%), and an average of 7.8 Fabs/PEG.

Example 14: Measurement of Systemic Alternative Complement Pathway Activity in Cynomolgus Monkeys

[00462] Lampalizumab has previously been shown to transiently inhibit systemic complement function in cynomolgus monkeys (*see* Loyet, et al., *J. Pharmacol. Exp. Ther.*, 2014, Vol. 351, pp. 527-537). In the current example, the effect of intravitreal administration of an anti-Factor D antibody variant or an AFD.Ab conjugate on systemic alternative complement pathway (AP) activity was evaluated in cynomolgus monkeys.

a. Pharmacokinetic/Pharmacodynamic Studies in Cynomolgus Monkeys

[00463] The AFD.Ab variant and conjugate were administered by a single-dose IVT or intravenous injection to male cynomolgus monkeys (M. fascicularis) of Chinese origin to assess the pharmacokinetics (PK) and pharmacodynamics (PD) of the molecules. These studies were conducted at Covance Laboratories (Madison, WI). All procedures were conducted in compliance with the US Department of Agriculture Animal Welfare Act Regulations (9 CFR 3), Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Animal Welfare.

[00464] Four studies were performed. In the first (control) study (Study 1, n=10), lampalizumab was administered to both eyes, in two 50 µL IVT doses, separated by 15 minutes. These animals received 10 mg/eye for a total of 20 mg/animal. Blood was collected predose (day -2) and post dose at the following time points: 45 minutes, and 2, 6, 10, 24, 34, 48, 96, 120, 154, 192, 288, and 384 hours. After blood collections at 24, 48, 120, 192, and 384 hours, two animals per group were removed from the study and euthanized to collect ocular matrix. The lampalizumab control Study has previously been described in Loyet, et al., *J. Pharmacol. Exp. Ther.*, 2014, 351:527-537.

[00465] In Study 2 (n=3), AFD.v14 was administered to both eyes, in two 50 µL IVT doses, separated by 15 minutes. These animals received 25 mg/eye for a total of 50 mg/animal. Blood was collected predose (day -1 and -3) and post dose at the following time points: 30

minutes, and 2, 8, 24, 48, and 96 hours.

[00466] In Study 3 (n=10), the AFD.v14.C + TP octamer was administered to both eyes, in two 50 µL IVT doses, separated by 15 minutes, to provide 3.9 mg/eye of AFD.v14, for a total of 7.8 mg/animal of AFD.v14. Blood was collected predose (week -1 and week -2) and post dose at the following time points: 1, 6, 24, 48, 72, 96, 144, 192, 288, and 480 hours. Two animals per group at each time point (at 24, 96, 192, 288, and 480 hours) were removed from the study and euthanized to collect ocular matrix.

[00467] In Study 4, the AFD.v14.C + HG octamer was administered to both eyes in two 50 µL IVT doses, separated by 15 minutes, to provide either 7.1 mg/eye of AFD.v14 (n=2) or 11.8 mg/eye of AFD.v14 (n=1) , for a total of 14.2 mg/animal or 23.6 mg/animal of AFD.v14. Blood was collected predose (day -7 and -1) and post dose at the following time points: 1, 6, 24, 96, and 168 hours.

[00468] For all studies, predose and postdose serum samples were collected from each animal via the femoral vein for PK and PD analyses. At each time point, whole blood was collected into serum separator tubes, allowed to clot at ambient temperature for at least 20 minutes, then centrifuged in a refrigerated centrifuge set at a temperature range of 2°C–8°C. The serum was harvested within 20 minutes of centrifugation and stored between −60°C and −80°C until analysis.

b. Total AFD.v14/conjugate analysis

[00469] A Gyrolab XP assay was used to quantify AFD.v14, AFD.v14.C + TP octamer, and AFD.v14.C + HG octamer in cynomolgus monkey serum. Samples were diluted 1:4 – 1:3000 in sample buffer (phosphate buffered saline (PBS), 0.5% bovine serum albumin (BSA), 15 ppm Proclin (Sigma-Aldrich), 0.05% Tween 20, 0.25% CHAPS, 50 µg/mL muIgG (Equitech Bio, Cat. #SLM66), 5 mM EDTA (pH 7.4)). The AFD.v14 and AFD.v14 TP and HG conjugate standard curves were prepared by serially diluting AFD.v14, AFD.v14.C + TP octamer, or AFD.v14.C + HG octamer from 2.06 - 1500ng/mL in sample buffer. Capture and detection reagents were applied at 100 µg/mL of biotin-conjugated goat anti-human IgG (HC + LC, Bethyl, Cat#A80-319B) in PBS/0.01% Tween 20/0.02% NaN_3 and Alexa-anti-CDR (clone 234, Genentech) at 25 nM in Rexxip F (Gyrolab). The assay was run on a Gyrolab Bioaffy 200 CD, and wash steps used PBS/0.01% Tween 20/0.02% NaN_3 followed by Gyros pH 11 wash buffer. The instrument was run and data analyzed as described by the manufacturer with a 1% PMT setting. The concentrations of AFD.v14, AFD.v14.C + TP octamer, and AFD.v14.C + HG octamer were determined from a five-parameter fit of its standard curve. The minimum quantifiable concentration was 8.24 ng/mL (0.16 nM) for AFD.v14, AFD.v14.C + TP octamer, and AFD.v14.C + HG octamer in cynomolgus monkey serum.

c. Pharmacodynamics Assay for factor D in Cynomolgus Monkey Serum

[00470] A sandwich ELISA was used to quantify factor D (fD) in cynomolgus monkey serum. Mouse anti-human factor D clone 4676 (Genentech) was diluted to 1 μg/mL in coating buffer (0.05M Sodium Carbonate, pH 9.6) and incubated overnight at 4°C on 384-well Maxisorp plates (Thermo Scientific, Cat#. 464718). Plates were washed with PBS plus 0.05% Tween 20 and blocked during a 2 hour incubation with PBS plus 0.5% bovine serum albumin (BSA). This and all subsequent incubations were performed at room temperature with gentle agitation. The cynomolgus monkey fD standard curve was prepared by serially diluting fD from 0.04 - 5 ng/mL in sample buffer (assay buffer supplemented with 500 ng/mL of the AFD.v14 therapeutic and 50 μg/mL mouse IgG). The serum samples and controls were diluted to a minimum of 1:100 in sample buffer. The diluted standards, controls, and samples were then incubated on the plates for 2 hours, and plate-bound fD/AFD.Ab complex was detected using biotin-conjugated mouse-anti-CDR mAb to AFD.Ab (clone 242, 1μg/mL) for one hour followed by High Sensitivity SA-HRP (3 ng/mL, Pierce Cat.#21130) also for one hour. After a final wash, tetramethyl benzidine (Moss, Cat.# TMBE-1000) was added and color was developed for 10-15 minutes, and the reaction was stopped with 1 M phosphoric acid. The plates were read at 450 nm with a 620 nm reference using a microplate reader. The concentrations of fD were determined from a four parameter fit of the standard curve. The minimum quantifiable concentration in cynomolgus monkey serum was 3.9 ng/mL (0.16 nM).

d. AP Hemolysis Assay

[00471] The ability of AFD.v14 and AFD.v14.C + TP octamer to inhibit AP activity was evaluated in a hemolytic assay in which serum (either human or monkey) was combined with rabbit erythrocytes, as designed and described by Pangburn (*Methods Enzymol*, 1988, 162:639–653) and Katschke et al. (*J. Biol. Chem.*, 2009, 284:10473–10479). To ensure complement activation did not occur through the classic complement pathway (CP), C1q-depleted human serum (Complement Technologies, Tyler, TX) was used, and the buffer included EGTA to chelate calcium, a cation essential for CP activity.

[00472] C1q-depleted human serum was used to activate the AP. The concentration of fD present in 10% C1q-depleted human serum was 9.6 nM in-well, a value in agreement with previously reported fD levels in serum (Barnum, et al., *J. Immunol. Methods*, 1984, 67:303–309; Loyet et al., *Invest. Ophthalmol. Vis. Sci.*, 2012, 53:6628–6637).

e. Determination of Inhibition of Systemic AP Activity in AFD.v14.C + HG octamer -Treated Cynomolgus Monkey Serum

[00473] To evaluate the time course and dose dependency of any potential inhibition of systemic AP activity subsequent to dosing with AFD.v14.C + HG octamer or AFD.v14.C + TP

octamer, either a plate-based WIESLAB Complement System AP ELISA or an *ex vivo* assay was performed.

[00474] To evaluate the time course and dose dependency of any potential inhibition of systemic AP activity subsequent to dosing with AFD.v14.C + HG octamer or AFD.v14.C + TP octamer, either a plate-based WIESLAB Complement System AP ELISA (the data from this assay are referred to in Figure 13 and 14 as "% AP complement activity") or an *ex vivo* assay similar to the in vitro AP hemolysis assay described above was performed (the data from this assay are referred to in Figure 14 as "% relative hemolysis." In this assay, however, instead of adding a dilution curve of exogenous AFD.v14.C + HG octamer or AFD.v14.C + TP octamer to the serum samples, the samples themselves were serially diluted, with any inhibition of hemolytic activity attributed to the injected dose of AFD.v14.C + HG octamer or AFD.v14.C + TP octamer.

[00475] Erythrocytes were prepared, and the assay was performed, as described herein, for the AP hemolysis assay with the following modifications. To determine the absorbance corresponding to maximum lysis, total lysis controls were prepared with sterile water (80 µl/well), whereas GVB was added to all other wells (50 µl). Cynomolgus monkey serum samples were serially diluted 1:1.5 over six points and added along with a negative control (buffer only) to 96-well U-bottom polypropylene plates (30 µl/well). The total lysis controls represented maximum (100%) hemolysis. Data points were collected in triplicate, and the mean percent maximum hemolysis was plotted against the reciprocal of the final serum dilution in the assay. The 50% maximal hemolysis (AH50) values, defined as 50% maximal hemolysis, were determined by nonlinear regression analysis using a four-parameter fit model. For those curves that did not reach saturation, the AH50 was estimated using a curve fit in which the upper asymptote was fixed at 100%. The percent relative hemolysis was calculated for each individual time point as [(postdose AH50 for the individual time point)/(predose AH50)] × 100. The AH50 value for serum from each individual normal cynomolgus monkey can vary as much as 2-fold from the overall average of AH50 values. Therefore, the predose and postdose samples from each study animal were run on the same assay plate to ensure that postdose changes in AP activity were directly compared with the individual animal's baseline complement activity.

[00476] The percent relative hemolysis in comparison to total fD and the therapeutic active is shown in Figures 13A (lampalizumab, 10 mg/eye), 13B (AFD.v14, 25 mg/eye), and 14A (AFD.v14.C + TP octamer, 3.9 mg/eye). The lampalizumab data (Figure 33A) is comparative data obtained following IVT administration of 10 mg/eye of lampalizumab, as described in Loyet, et al., *J. Pharmacol. Exp. Ther.*, 2014, 351:527-537). As can be seen from Figure 13B, administration of 25 mg/eye of AFD.v14 transiently inhibited systemic AP activity,

with activity returning to baseline by 24 hours post administration, similar to results previously observed for lampalizumab (Figure 13A). In comparison, no systemic AP inhibition was observed following administration of 3.9 mg/eye of the AFD.v14.C + TP octamer (Figure 14A). Without wishing to be bound to any particular theory, it is believed that the slower clearance from the eye obtained with the conjugate compared to Fab (e.g., lampalizumab and AFD.v14) allows fD to saturate the AFD.Ab at earlier time points, preventing systemic complement inhibition.

[00477] The percent relative AP complement activity in comparison to total fD and total conjugate is shown in Figures 14B (AFD.v14.C + HG octamer, 7.1 mg/eye) and 14C (AFD.v14.C + HG octamer, 11.8 mg/eye). As can be seen from these figures, negligible systemic complement inhibition was observed for the AFD.v14.C + HG octamer for IVT dosage up to 11.8 mg/eye. Due to the slower clearance from the eye, the conjugate concentration remains below the molar concentration of fD, in particular at time points earlier than 10 hours. This is in contrast to similar eye-dosed concentrations of the AFD.Ab Fab in which at these early time points the molar concentration exceeds the molar fD concentration and leads to systemic AP inhibition.

[00478] PEG octamers of 20D12.v2.1, 20D12.v2.1.C, 20D12.v2.3, and 20D12.v2.3.C would be expected to behave similarly as AFD.v14.C + TP-Oct and AFD.v14.C + HG-Oct conjugates by showing decreased levels of systemic complement inhibition compared to unconjugated versions of 20D12.v2.1, 20D12.v2.1.C, 20D12.v2.3 and 20D12.v2.3.C.

Example 15: Cynomolgus Monkey PK for AFD.Ab Variants and Conjugates

[00479] One way to assess *in vivo* PK profiles of AFD.Ab variants or conjugates is single dose experiments performed in cynomolgus monkeys. In vivo PK studies for the AFD.v14 + TP-Oct conjugate were performed in Cynomolgus monkey. PK parameters were determined from single dose experiments. Unconjugated, unmodified AFD.v14 (SIESD.N103S) was used as a control. The animals' care was in accordance with Genentech Institutional Animal Care and Use Committee guidelines.

a. Study Parameters

[00480] Cynomolgus monkeys (28 male animals; 2 kg to 4 kg and approximately 2-7 years in age at the time of dosing) were assigned to one of four dosing groups. Group 1 (control) animals (4 animals) received bilateral intravitreal doses of 5 mg/eye (10 mg/animal) of AFD.v14 through a 30 gauge needle (100 µl dose volume). Group 2 and 3 animals (10 animals in each group) received a bilateral intravitreal dose of 1 or 4 mg/eye (2 or 8 mg/animal), respectively based on Fab weight, of the AFD.v14.C + TP-Oct conjugate through a 30-gauge

needle (100 µl dose volume). Animals were sedated (10 mg/kg ketamine HCl, 0.5 mg/kg diazepam) and treated with topical proparacaine prior to injection. The AFD.v14 or AFD.v14.C+TP-Oct conjugate was then administered through the sclera and pars plana, 4 mm posterior to the limbus, with the needle directed posterior to the lens into the midvitreous. The Group 4 animals (4 animals) received a single IV bolus (1 mL) of the AFD.v14.C + TP-Oct conjugate at 0.4 mg/animal. For IV administration, the AFD.v14.C + TP-Oct conjugate was formulated as 10 mM sodium succinate, 10% trehalose, and 0.05% Tween-20 (pH 5.0).

[00481] Ocular tissues were collected from all Groups. One animal (2 eyes) per group was euthanized for Group 1 and two animals (4 eyes) were euthanized for Groups 2 and 3 at the following times after dosing: Group 1 – days 1 (24 hours), 2, 4, and 8; Groups 2 and 3 – days 1 (24 hours), 4, 8, 12, and 20. After euthanasia, both eyes were enucleated, and the AFD.v14 and AFD.v14.C + TP-Oct conjugate concentrations were determined in the vitreous and aqueous humor and retinal tissues. After flash freezing of the eyes, filter paper was later used to collect the entire retinal layer.

[00482] All blood samples (approximately 1 mL) were collected via a femoral or cephalic vein. Samples were drawn at the following times after IVT or IV dosing: Group 1 – 1 hour, 6 hours, and days 1 (24 hours), 2, 3, 4, 5, and 7; Groups 2 and 3 – 1 hour, 6 hours, and days 1 (24 hours), 2, 4, 6, 8, 12, and 20; Group 4 – 1 hour, 6 hours, and days 1 (24 hours), 2, 4, 7, 11, 14, 17, 21, 24, and 28. Within one hour of blood collection, samples were clotted at room temperature, and serum was separated by centrifugation and stored at -60°C to -80°C. Details of the study protocol are set forth in Table 20.

Table 20: Cynomolgus Monkey PK Study Parameters

Group	**Dose**	**Route**	**Number of Animals**	**Ocular time points (days)**	**Serum time points**
1	5 mg/eye	IVT (bilateral)	4	1, 2, 4, 8	1 and 6 hr; 1, 2, 3, 4, 5, 7 days
2	1 mg/eye	IVT (bilateral)	10	1, 4, 8, 12, 20	6 hr; 1, 2, 4, 5, 8, 12, 20 days
3	4.0 mg/eye	IVT (bilateral)	10	1, 4, 8, 12, 20	6 hr; 1, 2, 4, 6, 8, 12, 20 days
4	0.3 mg/animal	IV	4	n/a	6 hr; 1, 2, 4, 7, 11, 14, 17, 21, 24, 28 days

b. Pharmacokinetics Assay for AFD.v14 and AFD.v14.C + TP-Oct Conjugate

[00483] A Gyrolab XP assay was used to quantify AFD.v14 and AFD.v14.C + TP-Oct conjugate in cynomolgus monkey serum, vitreous humor, aqueous humor, and retinal homogenate. Samples were diluted 1:4 – 1:3000 in sample buffer (phosphate buffered saline (PBS), 0.5% bovine serum albumin (BSA), 15 ppm Proclin (Sigma-Aldrich), 0.05% Tween 20,

0.25% CHAPS, 50 µg/mL muIgG (Equitech Bio, Cat. #SLM66), 5 mM EDTA (pH 7.4)). The AFD.v14 and AFD.v14.C + TP-Oct conjugate standard curves were prepared by serially diluting AFD.v14 or AFD.v14.C + TP-Oct conjugate from 2.06 - 1500ng/mL in sample buffer. Capture and detection reagents were applied at 100 µg/mL of biotin-conjugated goat anti-human IgG (HC + LC, Bethyl, Cat#A80-319B) in PBS/0.01% Tween 20/0.02% NaN_3 and Alexa-anti-CDR (clone 234, Genentech) at 25 nM in Rexxip F (Gyrolab). The assay was run on a Gyrolab Bioaffy 200 CD, and wash steps used PBS/0.01% Tween 20/0.02% NaN_3 followed by Gyros pH 11 wash buffer. The instrument was run and data analyzed as described by the manufacturer with a 1% PMT setting. The concentrations of AFD.v14 and AFD.v14.C + TP-Oct conjugate were determined from a five-parameter fit of its standard curve. The minimum quantifiable concentration was 8.24 ng/mL (0.16 nM) for AFD.v14 and AFD.v14.C + TP-Oct conjugate in cynomolgus monkey serum, vitreous humor, aqueous humor and retinal homogenate.

[00484] The vitreous humor, aqueous humor, and retinal PK results are set forth in Tables 21-23 below, and Figures 17A (vitreous), 17B (vitreous normalized), 18A (acqueous), 18B (acqueous normalized), 19A (retina), and 19B (retina, normalized).

Table 21: Vitreous PK for AFD.v14 control (Group 1) and AFD.v14.C + TP-Octconjugate (Group 2 & 3)

Group	Dose (µg/eye)	T1/2 (days)	AUC (Day*µg/mL)	AUC/dose (Day*µg/mL/mg dose)	T1/2 ext (increase compared to control)	Vss (mL)	Cl (mL/day)
1	5000	2.7	--	--	--	3	0.79
2	1000	3.5	2530	2100	1.3	2.3	0.47
3	4000	5	7730	1980	1.9	3.4	0.46

Table 22: Aqueous PK for AFD.v14.C + TP-Oct conjugate

Group	Dose (µg/eye)	T1/2 (days)	AUClast (Day*µg/mL)	Vz (mL)	Cl/F (mL/Day)
2	1000	3	434	12	2.73
3	4000	5.2	1430	20	2.58

Table 23: Retinal PK AFD.v14.C + TP-Oct conjugate

Group	Dose (µg/eye)	T1/2 (days)	AUClast (Day*µg/mL)	Vz (mL)	Cl/F (mL/Day)
2	1000	3.6	31	196	38
3	4000	5.9	98	309	36

[00485] As can be seen from Table 21 and Figures 17A-B, the vitreal terminal half-life for both the conjugated AFD.v14 groups (Groups 2 and 3) was longer than that of the unconjugated AFD.v14 control (Group 1), and longer than the average half-life of unconjugated lampalizumab and ranibizumab Fabs (about 2.34 days). The average AUC/mg-dose for

conjugated AFD.v14 Groups 2 and 3 (about 2040) was higher than the average AUC/mg-dose for the unconjugated lampalizumab Fab (about 1733). Based on vitreal terminal half-life, the 4.0 mg/eye dose cleared more slowly than the 1.0 mg/eye dose. As can be seen from Tables 22 and 23, and Figures 18 and 19, a longer terminal half-life was also observed in aqueous humor and retina for Groups 2 and 3 (conjugated AFD.v14), as compared to unconjugated Fab.

[00486] The serum PK results for Groups 2 and 3 are set forth in Figures 15A and 15B (normalized), and the serum PK results for Group 4 are set forth in Figure 15C. The serum PK curves for Groups 2 and 3 (AFD.v14.C + TP-Oct conjugate) are parallel to each other, and overlap after dose normalization. *See* Figures 15A and 15 B. The serum AUC for Groups 2 and 3 is dose proportional, up until the last measured time point.

[00487] The terminal half-life for Group 4 (AFD.v14.C + TP-Oct conjugate; IV dose) was 7.5 days, and the clearance was 15.8 mL/day (5.64 mL/kg/day (average weight of Group 4 monkeys was 2.8 kg)). On measurement days 21, 24, and 28, the serum concentration dropped below the limit of detection for 3 out of the 4 Group 4 monkeys.

c. Pharmacodynamics Assay for Factor D in Cynomolgus Monkey Serum

[00488] A sandwich ELISA was used to quantify factor D (fD) in cynomolgus monkey serum, vitreous humor, aqueous humor and retinal homogenate. Mouse anti-human factor D clone 4676 (Genentech) was diluted to 1 µg/mL in coating buffer (0.05M Sodium Carbonate, pH 9.6) and incubated overnight at 4°C on 384-well Maxisorp plates (Thermo Scientific, Cat#. 464718). Plates were washed with PBS plus 0.05% Tween 20 and blocked during a 2 hour incubation with PBS plus 0.5% bovine serum albumin (BSA). This and all subsequent incubations were performed at room temperature with gentle agitation. The cynomolgus monkey fD standard curve was prepared by serially diluting fD from 0.04 - 5 ng/mL in sample buffer (assay buffer supplemented with 500 ng/mL of the AFD.v14 therapeutic and 50 µg/mL mouse IgG). The serum samples and controls were diluted to a minimum of 1:100 in sample buffer. The vitreous humor, aqueous humor, and retinal homogenate samples and controls were diluted to a minimum of 1:10 in sample buffer. The diluted standards, controls, and samples were then incubated on the plates for 2 hours, and plate-bound fD/AFD.Ab complex was detected using biotin-conjugated mouse-anti-CDR mAb to AFD.Ab (clone 242, 1µg/mL) for one hour followed by High Sensitivity SA-HRP (3 ng/mL, Pierce Cat.#21130) also for one hour. After a final wash, tetramethyl benzidine (Moss, Cat.# TMBE-1000) was added and color was developed for 10-15 minutes, and the reaction was stopped with 1 M phosphoric acid. The plates were read at 450 nm with a 620 nm reference using a microplate reader. The concentrations of fD were determined from a four parameter fit of the standard curve. The minimum quantifiable concentration in cynomolgus monkey serum was 3.9 ng/mL (0.16 nM). The minimum quantifiable concentration

in cynomolgus monkey vitreous humor, aqueous humor and retinal homogenate was 0.39 ng/ml (0.016nM).

[00489] The average serum fD and AFD.v14.C + TP-Oct conjugate concentrations for Groups 2, 3, and 4 are set forth in Figure 16. Figure 16A shows that the serum fD concentration was higher than the AFD.Ab concentration at all time points tested. These results indicate that systemic AP complement activity is maintained in all groups.

[00490] The average ocular fD and AFD.v14.C + TP-Oct conjugate concentrations for Groups 2 and 3 are set forth in Figure 16B, which shows that the AFD.Ab concentration in the vitreous humor, aqueous humor, and retinal homogenate exceeded the fD concentration at all time points tested.

[00491] Based on the data set forth in this example for the PK parameters following IVT administration of AFD.v14.C +TP-Oct conjugates, conjugation of AFD.v14 resulted in sustained levels of conjugates in the vitreous humor and aqueous humor of the eye. However, while AFD.Ab concentrations in the vitreous humor, aqueous humor, and retinal homogenate exceeded the fD concentration at all timepoints tested, this was not seen for any timepoint in serum samples with IVT dosing. Similar methods as those described in this example can be used to evaluate PEG octamers of 20D12.v2.1, 20D12.v2.1.C, 20D12.v2.3, and 20D12.v2.3.C. It is expected that when dosed IVT conjugated 20D12.v2.1, 20D12.v2.1.C, 20D12.v2.3 and 20D12.v2.3.C would behave similarly to conjugated AFD.v14.C + TP-Oct.

Example 16: Cynomolgus Monkey PK for hu20D12.v2.1.C+TP-Oct and hu20D12.v2.1.C

[00492] To assess *in vivo* pharmacokinetic (PK) profiles of hu20D12.v2.1.C+TP-Oct and hu20D12.v2.1.C, an *in vivo* PK study was performed in cynomolgus monkeys using radioiodinated hu20D12.v2.1.C+TP-Oct (^{125}I- hu20D12.v2.1.C+TP-Oct) and hu20D12.v2.1.C (^{125}I-20D12.v2.1.C). PK parameters were determined from single dose experiments following conversion of the radioactive signal to µg-equivalents/mL or gram concentrations in each tissue collected. The animals' care was in accordance with Genentech Institutional Animal Care and Use Committee guidelines.

a. Study Parameters

[00493] Cynomolgus monkeys (43 female animals; 2 kg to 4 kg and approximately 3-4 years in age at the time of dosing) were assigned to one of five dosing groups. Group 1, 2, and 3 animals (n=10/group) received bilateral intravitreal doses of 1 mg/eye (2 mg/animal), 7.5 mg/eye (15 mg/animal), or 15 mg/eye (30 mg/animal), respectively, of ^{125}I-hu20D12.v2.1.C+TP-Oct through a 27 gauge needle (100 µl dose volume). Group 4 animals (n=10) received a bilateral intravitreal dose of 15 mg/eye (30 mg/animal of unconjugated ^{125}I-hu20D12.v2.1.C through a 27-gauge needle (100 µl dose volume). Animals were sedated (5-15

mg/kg ketamine HCl and isoflurane to inhalation effect) and treated with topical 0.5% propoaracaine HCl, 2.5% phenylephrine HCl, and 1% Tropicamide prior to injection. ^{125}I-hu20D12.v2.1.C+TP-Oct or unconjugated ^{125}I-hu20D12.v2.1.C was then administered through the sclera, with the needle directed towards the posterior axis of the globe and into the midvitreous.

[00494] Ocular tissues were collected from all groups. Two animals (4 eyes) per group were euthanized at the following times after dosing: Days 1 (4 hours post-dose), 3, 8, 13, and 29. After euthanasia, both eyes were enucleated, and the ^{125}I- hu20D12.v2.1.C+TP-Oct and unconjugated ^{125}I-hu20D12.v2.1.C radioactivity levels were determined in the vitreous humor, aqueous humor, and retina. Details of the study protocol are set forth in Table 24.

Table 24: Cynomolgus Monkey Radioactive PK Study Parameters

Group	**Dose**	**Route**	**Animal #**	**Ocular time points (days)**	**Serum time points**
1	1 mg/ eye	IVT (bilateral)	10	Day 1 (4 hours post-dose), Day 3 (48 hours post-dose) and Days 8, 13, and 29	Predose, Day 1 (1, 4, 6, 8, 12 hours post-dose), Day 2 (24 hours post-dose), Day 3 (48 hours post-dose) and Days 5, 8, 11, 13, 15, 22 and 29
2	7.5 mg/ eye	IVT (bilateral)	10		
3	15 mg/ eye	IVT (bilateral)	10		
4	15 mg/ eye	IVT (bilateral)	10		

b. Pharmacokinetics RadioAssay for ^{125}I- hu20D12.v2.1.C+TP-Oct and unconjugated ^{125}I-hu20D12.v2.1.C

[00495] A radiodection assay was used to detect the amount of radioactivity in cynomolgus monkey vitreous humor, aqueous humor, and retinal homogenate. The vitreous was homogenized using cermanic beads and then triplicate weighed aliquots of 0.025-0.05g were removed from the homogenate for gamma counting. The aqueous humor was counted for radioactivity as is. The retina was homogenized and then counted for radioactivity in its entirety. Radioativity in all matrices were detected using a Wallac Wizard 1470 Gamma Counter. Following radioactivity detection, the concentrations of ^{125}I- hu20D12.v2.1.C+TP-Oct and unconjugated ^{125}I-hu20D12.v2.1.C were calculated by converting the radioactivity per mL of tissue into µg-equivalents per mL of tissue using the specific activity of the dose solution for each arm (in units of µCi/mL). An assumption of 1g equaling 1mL was applied during the conversion.

[00496] The vitreous humor, aqueous humor, and retina PK results are set forth in Tables 25-27 below, and Figures 22 (vitreous), 23 (aqueous), and 24 (retina).

Table 25: Vitreous humor PK for hu20D12.v2.1.C+TP-Oct and unconjugated hu20D12.v2.1.C

Group	Dose (µg/eye)	$T_{1/2}$ (days)	AUC (Day*µg/mL)	AUC/dose (Day*µg/mL/mg dose)	Vss (mL)	CL (mL/day)
1	1000	4.62	3770	3770	1.64	0.265
2	7500	4.64	15700	2093	2.89	0.478
3	15000	5.01	36300	2420	2.89	0.413
4	15000	2.70	31000	2067	1.82	0.484

Table 26: Aqueous humor PK for hu20D12.v2.1.C+TP-Oct and unconjugated hu20D12.v2.1.C

Group	Dose (µg/eye)	$T_{1/2}$ (days)	AUC (Day*µg/mL)	Vz/F (mL)	CL/F (mL/Day)
1	1000	4.31	768	8.10	1.30
2	7500	4.30	3950	11.8	1.90
3	15000	4.89	7420	14.3	2.02
4	15000	2.47	12400	4.31	1.21

Table 27: Retina PK for hu20D12.v2.1.C+TP-Oct and unconjugated hu20D12.v2.1.C following IVT administration

Group	Dose (µg/eye)	$T_{1/2}$ (days)	AUC (Day*µg/mL)	Vz/F (mL)	CL/F (mL/Day)
1	1000	5.26	520	14.6	1.92
2	7500	5.44	2130	27.7	3.53
3	15000	5.51	6970	17.1	2.15
4	15000	3.31	6620	10.8	2.27

[00497] As can be seen from Table 25 and Figure 22, the vitreal terminal half-life for the hu20D12.v2.1.C+TP-Oct groups (Groups 1, 2 and 3) was statistically significantly longer than that of the unconjugated hu20D12.v2.1.C control (Group 4), and longer than the average half-life of unconjugated lampalizumab and ranibizumab Fabs (about 2.34 days). The average AUC/mg-dose for hu20D12.v2.1.C+TP-Oct groups was higher than the average AUC/mg-dose for the unconjugated hu20D12.v2.1.C control (about 2067 Day*µg/mL/mg dose) and the unconjugated lampalizumab Fab (about 1733). As can be seen from Tables 26 and 27, and Figures 23 and 24, a longer terminal half-life was also observed in aqueous humor and retina for hu20D12.v2.1.C+TP-Oct groups, as compared to unconjugated hu20D12.v2.1.C.

[00498] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

Table of Sequences

SEQ ID NO	Description	Sequence
1	mu20D12 HVR-L1	KASQNVDTDVA
2	mu20D12 HVR-L2	SASSRYS
3	mu20D12 HVR-L3	QQYNNYPLT
4	mu20D12 HVR-H1	SYYMY
5	mu20D12 HVR-H2	EINPTNGGTNFNEKFKS
6	mu20D12 HVR-H3	EGGFAY
7	mu20D12 light chain variable region (VL)	DIVMTQSQKF MSTSVGDRVS VTCKASQNVD TDVAWFQQKP GQSPRGLIYS ASSRYSGVPD RFTGSGSGTD FTLTISNVQS EDLAEYFCQQ YNNYPLTFGS GTKVEIK
8	murine 20D12 (mu20D12) heavy chain variable region (VH)	QVQLQQSGAE LVKPGASVKL SCKASGYTFT SYYMYWVKER PGQGLEWIGE INPTNGGTNF NEKFKSKATL TVDTSSNTAY MQLSSLTSED SAVYYCAREG GFAYWGQGTL VTVSA
9	HVR-L1 of antibodies: hu20D12.v1 hu20D12.v1.1 hu20D12.v2.0 hu20D12.v2.1 hu20D12.v2.2 hu20D12.v2.3 hu20D12.v2.4 hu20D12.v2.5 hu20D12.v2.6 hu20D12.v2.7 hu20D12.v2.8 hu20D12.v2.9 hu20D12.v2.10 hu20D12.v2.11 hu20D12.v2.12 hu20D12.v2.13 hu20D12.v2.14 hu20D12.v2.15	KASQNVDTDVA
10	HVR-L2 of antibodies: hu20D12.v1 hu20D12.v1.1 hu20D12.v2.0 hu20D12.v2.1 hu20D12.v2.2 hu20D12.v2.3 hu20D12.v2.4 hu20D12.v2.5 hu20D12.v2.6 hu20D12.v2.7 hu20D12.v2.8 hu20D12.v2.9 hu20D12.v2.12 hu20D12.v2.13 hu20D12.v2.14 hu20D12.v2.15	SASSRYS
11	hu20D12.v2.10 HVR-L2	SASSRKS
12	hu20D12.v2.11 HVR-L2	SASSRRS
13	HVR-L3 of antibodies: hu20D12.v1	QQYNNYPLT

	hu20D12.v1.1 hu20D12.v2.0 hu20D12.v2.1 hu20D12.v2.2 hu20D12.v2.3 hu20D12.v2.4 hu20D12.v2.5 hu20D12.v2.6 hu20D12.v2.7 hu20D12.v2.8 hu20D12.v2.9 hu20D12.v2.10 hu20D12.v2.11 hu20D12.v2.12 hu20D12.v2.14 hu20D12.v2.15	
14	hu20D12.v2.13 HVR-L3	QQYENYPLT
15	HVR-H1 of antibodies: hu20D12.v1 hu20D12.v1.1 hu20D12.v2.0 hu20D12.v2.1 hu20D12.v2.2 hu20D12.v2.3 hu20D12.v2.4 hu20D12.v2.5 hu20D12.v2.6 hu20D12.v2.7 hu20D12.v2.8 hu20D12.v2.9 hu20D12.v2.10 hu20D12.v2.11 hu20D12.v2.12 hu20D12.v2.13 hu20D12.v2.14 hu20D12.v2.15	SYYMY
16	HVR-H2 of antibodies: hu20D12.v2.0 hu20D12.v2.1 hu20D12.v2.6 hu20D12.v2.7 hu20D12.v2.8 hu20D12.v2.10 hu20D12.v2.11 hu20D12.v2.13	EINPTSGGTNFNEKFKS
17	hu20D12.v1 HVR-H2	EINPTNGGTNFNEKFKS
18	hu20D12.v1.1 HVR-H2	EINPTQGGTNFNEKFKS
19	hu20D12.v2.2 HVR-H2	EINPTSGDTNFNEKFKS
20	hu20D12.v2.3 HVR-H2	EINPYSGDTNFNEKFKS
21	hu20D12.v2.4 HVR-H2 hu20D12.v2.14 HVR-H2	EINPTSGETNFNEKFKS
22	hu20D12.v2.5 HVR-H2 hu20D12.v2.12 HVR-H2	EINPYSGGTNFNEKFKS
23	hu20D12.v2.9 HVR-H2	WINPTSGGTNFNEKFKS
24	hu20D12.v2.15 HVR-H2	EINPYSGETNFNEKFKS
25	HVR-H3 of antibodies:	EGGFAY

	hu20D12.v1 hu20D12.v1.1 hu20D12.v2.0 hu20D12.v2.1 hu20D12.v2.2 hu20D12.v2.3 hu20D12.v2.4 hu20D12.v2.5 hu20D12.v2.6 hu20D12.v2.7 hu20D12.v2.8 hu20D12.v2.9 hu20D12.v2.10 hu20D12.v2.11 hu20D12.v2.12 hu20D12.v2.13 hu20D12.v2.14 hu20D12.v2.15	
26	human VL kappa I (VL_{KI})	DIQMTQSPSS LSASVGDRVT ITCRASQGIS SYLAWYQQKP GKAPKLLIYA ASSLQSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YYSYPFTFGQ GTKVEIK
27	human VH subgroup I	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYIHWVRQA PGQGLEWIGW INPGSGNTNY AQKFQGRVTI TRDTSTSTAY LELSSLRSED TAVYYCARFD YWGQGTLVTV SS
28	hu20D12.v1 VL	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIYS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIK
29	hu20D12.v1 VH	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTNGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSS
30	hu20D12.v1.1 VL	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIYS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIK
31	hu20D12.v1.1 VH	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTQGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSS
32	hu20D12.v2.0 VL	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIYS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIK
33	hu20D12.v2.0 VH	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSS
34	hu20D12.v2.1 VL	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIRS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIK
35	hu20D12.v2.1 VH	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSS
36	hu20D12.v2.2 VL	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIYS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIK
37	hu20D12.v2.2 VH	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGDTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSS
38	hu20D12.v2.3 VL	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIRS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIK

39	hu20D12.v2.3 VH	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPYSGDTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSS
40	hu20D12.v2.4 VL	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIYS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIK
41	hu20D12.v2.4 VH	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGETNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSS
42	hu20D12.v2.5 VL	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIYS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIK
43	hu20D12.v2.5 VH	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPYSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSS
44	hu20D12.v2.6 VL	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLISS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIK
45	hu20D12.v2.6 VH	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSS
46	hu20D12.v2.7 VL	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIKS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIK
47	hu20D12.v2.7 VH	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSS
48	hu20D12.v2.8 VL	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIQS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIK
49	hu20D12.v2.8 VH	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSS
50	hu20D12.v2.9 VL	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIYS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIK
51	hu20D12.v2.9 VH	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGW INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSS
52	hu20D12.v2.10 VL	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIYS ASSRKSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIK
53	hu20D12.v2.10 VH	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSS
54	hu20D12.v2.11 VL	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIYS ASSRRSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIK
55	hu20D12.v2.11 VH	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSS
56	hu20D12.v2.12 VL	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIRS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIK
57	hu20D12.v2.12 VH	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPYSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSS

58	hu20D12.v2.13 VL	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIRS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YENYPLTFGQ GTKVEIK
59	hu20D12.v2.13 VH	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSS
60	hu20D12.v2.14 VL	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIRS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIK
61	hu20D12.v2.14 VH	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGETNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSS
62	hu20D12.v2.15 VL	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIRS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIK
63	hu20D12.v2.15 VH	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPYSGETNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSS
64	hu20D12.v1 light chain (LC)	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIYS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
65	hu20D12.v1 heavy chain Fab (HC Fab)	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTNGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT
66	hu20D12.v1.1 LC	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIYS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
67	hu20D12.v1.1 HC Fab	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTQGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT
68	hu20D12.v2.0 LC	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIYS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
69	hu20D12.v2.0 HC Fab	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT
70	hu20D12.v2.1 LC	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIRS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIKRTV AAPSVFIFPP

		SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
71	hu20D12.v2.1 HC Fab ("CDKTHT")	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT
72	hu20D12.v2.2 LC	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIYS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
73	hu20D12.v2.2 HC Fab	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGDTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT
74	hu20D12.v2.3 LC	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIRS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
75	hu20D12.v2.3 HC Fab ("CDKTHT")	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPYSGDTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT
76	hu20D12.v2.4 LC	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIYS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
77	hu20D12.v2.4 HC Fab	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGETNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT
78	hu20D12.v2.5 LC	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIYS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
79	hu20D12.v2.5 HC Fab	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPYSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT

80	hu20D12.v2.6 LC	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLISS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
81	hu20D12.v2.6 HC Fab	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT
82	hu20D12.v2.7 LC	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIKS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
83	hu20D12.v2.7 HC Fab	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT
84	hu20D12.v2.8 LC	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIQS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
85	hu20D12.v2.8 HC Fab	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT
86	hu20D12.v2.9 LC	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIYS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
87	hu20D12.v2.9 HC Fab	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGW INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT
88	hu20D12.v2.10 LC	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIYS ASSRKSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
89	hu20D12.v2.10 HC Fab	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG

		PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT
90	hu20D12.v2.11 LC	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIYS ASSRRSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
91	hu20D12.v2.11 HC Fab	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT
92	hu20D12.v2.12 LC	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIRS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
93	hu20D12.v2.12 HC Fab	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPYSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT
94	hu20D12.v2.13 LC	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIRS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YENYPLTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
95	hu20D12.v2.13 HC Fab	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT
96	hu20D12.v2.14 LC	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIRS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
97	hu20D12.v2.14 HC Fab	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGETNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT
98	hu20D12.v2.15 LC	DIQMTQSPSS LSASVGDRVT ITCKASQNVD TDVAWFQQKP GKAPKGLIRS ASSRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPLTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC

99	hu20D12.v2.15 HC Fab	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPYSGETNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT
100	Lampalizumab VL	DIQVTQSPSS LSASVGDRVT ITCITSTDID DDMNWYQQKP GKVPKLLISG GNTLRPGVPS RFSGSGSGTD FTLTISSLQP EDVATYYCLQ SDSLPYTFGQ GTKVEIK
101	Lampalizumab VH	EVQLVQSGPE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW INTYTGETTY ADDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCEREG GVNNWGQGTL VTVSS
102	Lampalizumab LC	DIQVTQSPSS LSASVGDRVT ITCITSTDID DDMNWYQQKP GKVPKLLISG GNTLRPGVPS RFSGSGSGTD FTLTISSLQP EDVATYYCLQ SDSLPYTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC
103	Lampalizumab HC Fab	EVQLVQSGPE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW INTYTGETTY ADDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCEREG GVNNWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT
104	Human complement Factor D, prepropeptide (UniProtKB/Swiss-Prot: P00746.5, 14-OCT-2015)	MHSWERLAVL VLLGAAACAA PPRGRILGGR EAEAHARPYM ASVQLNGAHL CGGVLVAEQW VLSAAHCLED AADGKVQVLL GAHSLSQPEP SKRLYDVLRA VPHPDSQPDT IDHDLLLLQL SEKATLGPAV RPLPWQRVDR DVAPGTLCDV AGWGIVNHAG RRPDSLQHVL LPVLDRATCN RRTHHDGAIT ERLMCAESNR RDSCKGDSGG PLVCGGVLEG VVTSGSRVCG NRKKPGIYTR VASYAAWIDS VLA
105	Human complement Factor D, propeptide	APPRGRILGGR EAEAHARPYM ASVQLNGAHL CGGVLVAEQW VLSAAHCLED AADGKVQVLL GAHSLSQPEP SKRLYDVLRA VPHPDSQPDT IDHDLLLLQL SEKATLGPAV RPLPWQRVDR DVAPGTLCDV AGWGIVNHAG RRPDSLQHVL LPVLDRATCN RRTHHDGAIT ERLMCAESNR RDSCKGDSGG PLVCGGVLEG VVTSGSRVCG NRKKPGIYTR VASYAAWIDS VLA
106	Human complement Factor D, mature, aa26-253	LGGREAEAHA RPYMASVQLN GAHLCGGVLV AEQWVLSAAH CLEDAADGKV QVLLGAHSLS QPEPSKRLYD VLRAVPHPDS QPDTIDHDLL LLQLSEKATL GPAVRPLPWQ RVDRDVAPGT LCDVAGWGIV NHAGRRPDSL QHVLLPVLDR ATCNRRTHHD GAITERLMCA ESNRRDSCKG DSGGPLVCGG VLEGVVTSGS RVCGNRKKPG IYTRVASYAA WIDSVLA
107	Cynomolgus monkey complement Factor D, precursor, predicted (NCBI Reference Sequence: XP_005587397.1, 18-SEP-2013)	MHSWEHLAVL VLLGVAACAA QPRGRILGGR EAEAHARPYM ASVQVNGEHL CGGVLVAEQW VLSAAHCLED AADGKVQVLL GAHSLSQPEP SKRLYDVLRA VPHPDSRPDT IDHDLLLLQL SEKATLGPAV RPLPWQRVDR DVEPGTLCDV AGWGIVSHAG RRPDRLQHVL LPVLDRATCN RRTHHDGAIT QRMMCAESNR RDSCKGDSGG PLVCGGVLEG VVTSGSRVCG NRKKPGIYTR VASYAAWIDS VLA
108	hu20D12 consensus HVR-L2	SASSRX$_1$S, wherein X$_1$ is selected from Y, K, and R
109	hu20D12 consensus extended HVR-L2	X$_2$SASSRX$_1$S, wherein X$_1$ is selected from Y, K, and R; X$_2$ is selected from Y, R, S, K,

		and Q
110	hu20D12 consensus HVR-L3	QQYX$_3$NYPLT, wherein X$_3$ is selected from N and E
111	hu20D12 consensus HVR-H2	X$_4$INPX$_5$X$_6$GX$_7$TNFNEKFKS, wherein X$_4$ is selected from E and W; X$_5$ is selected from T and Y; X$_6$ is selected from N, S, and Q; and X$_7$ is selected from G, D, and E
112	Fab light chain constant region	RTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
113	Fab heavy chain constant region ("CDKTHT")	ASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT
128	Fab heavy chain constant region ("CDKTHL")	ASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THL
129	Fab heavy chain constant region ("CDKTHTC"); "Fab-C" in Examples refers to this sequence	ASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTC
130	Fab heavy chain constant region ("CDKTHTCPPC")	ASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTCPPC
131	Fab heavy chain constant region ("CDKTHTCPPS")	ASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTCPPS
132	Fab heavy chain constant region ("CDKTHTSPPC")	ASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTSPPC
154	Fab heavy chain constant region ("CDKTHTAPPC")	ASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTAPPC
155	Fab heavy chain constant region ("CDKTHTSGGC")	ASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTSGGC
156	Fab heavy chain constant region ("CYGPPC")	ASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCYGPPC
134	Fab heavy chain constant region ("CDKTH")	ASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK TH
135	Fab heavy chain constant region ("CDKT")	ASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK T
136	Fab heavy chain constant region ("CDK")	ASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK
137	Fab heavy chain constant region ("CD")	ASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCD
138	Fab heavy chain constant region (IgG2)	ASTKG PSVFPLAPCS RSTSESTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSN FGTQTYTCNV DHKPSNTKVD KTVERK
139	Fab heavy chain constant region (IgG4) ("KYGPP (SEQ ID NO: 181)")	ASTKG PSVFPLAPCS RSTSESTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTKTYTCNV DHKPSNTKVD KRVESKYGPP

157	Fab heavy chain constant region (IgG2 Fab-C) (“VERKC”)	ASTKG PSVFPLAPCS RSTSESTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSN FGTQTYTCNV DHKPSNTKVD KTVERKC
158	Fab heavy chain constant region (IgG4 Fab-C) (“KYGPPC”)	ASTKG PSVFPLAPCS RSTSESTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTKTYTCNV DHKPSNTKVD KRVESKYGPPC
159	Fab heavy chain constant region (IgG4) (“KYG”)	ASTKG PSVFPLAPCS RSTSESTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTKTYTCNV DHKPSNTKVD KRVESKYG
160	Fab heavy chain constant region (IgG4) (“KY”)	ASTKG PSVFPLAPCS RSTSESTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTKTYTCNV DHKPSNTKVD KRVESKY
161	Fab heavy chain constant region (IgG4) (“K”)	ASTKG PSVFPLAPCS RSTSESTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTKTYTCNV DHKPSNTKVD KRVESK
114	AFD.v8 LC	DIQVTQSPSS LSASVGDRVT ITCITSTSIE SDMNWYQQKP GKVPKLLISG GNTLRPGVPS RFSGSGSGTD FTLTISSLQP EDVATYYCLQ SDSLPYTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
115	AFD.v8 HC Fab	EVQLVQSGPE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW INTYTGETTY AEDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCEREG GVNNWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT
116	AFD.v14 LC	DIQVTQSPSS LSASVGDRVT ITCITSTSIE SDMNWYQQKP GKVPKLLISG GNTLRPGVPS RFSGSGSGTD FTLTISSLQP EDVATYYCLQ SDSLPYTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
117	AFD.v14 HC Fab	EVQLVQSGPE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW INTYTGETTY AEDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCEREG GVSNWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT
133	AFD.v14 HC Fab (“CDKTHTC”; also referred to as AFD.v14.C)	EVQLVQSGPE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW INTYTGETTY AEDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCEREG GVSNWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTC
118	hu20D12.v2.1 HC Fab (“CDKTHL”)	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THL
119	hu20D12.v2.1 HC Fab (“CDKTHTC”; also referred to as hu20D12.v2.1.C)	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG

		PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTC
120	hu20D12.v2.1 HC Fab ("CDKTHTCPPC")	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTCPPC
121	hu20D12.v2.1 HC Fab ("CDKTHTSPPC")	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTSPPC
122	hu20D12.v2.1 HC Fab ("CDKTHTCPPS")	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTCPPS
140	hu20D12.v2.1 HC Fab ("CDKTH")	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK TH
141	hu20D12.v2.1 HC Fab ("CDKT")	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK T
142	hu20D12.v2.1 HC Fab ("CDK")	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK
143	hu20D12.v2.1 HC Fab ("CD")	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCD
144	hu20D12.v2.1 HC Fab ("APPC")	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSAPP C
145	hu20D12.v2.1 HC Fab ("SGGC")	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSSGG C

146	hu20D12.v2.1 HC Fab ("CYGPPC")	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPTSGGTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCYG PPC
123	hu20D12.v2.3 HC Fab ("CDKTHL")	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPYSGDTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THL
124	hu20D12.v2.3 HC Fab ("CDKTHTC")	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPYSGDTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTC
125	hu20D12.v2.3 HC Fab ("CDKTHTCPPC")	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPYSGDTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTCPPC
126	hu20D12.v2.3 HC Fab ("CDKTHTSPPC")	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPYSGDTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTSPPC
127	hu20D12.v2.3 HC Fab ("CDKTHTCPPS")	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPYSGDTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTCPPS
147	hu20D12.v2.3 HC Fab ("CDKTH")	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPYSGDTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK TH
148	hu20D12.v2.3 HC Fab ("CDKT")	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPYSGDTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK T
149	hu20D12.v2.3 HC Fab ("CDK")	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPYSGDTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK
150	hu20D12.v2.3 HC Fab ("CD")	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPYSGDTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG

		PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCD
151	hu20D12.v2.3 HC Fab ("APPC")	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPYSGDTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSAPP C
152	hu20D12.v2.3 HC Fab ("SGGC")	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPYSGDTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSSGG C
153	hu20D12.v2.3 HC Fab ("CYGPPC")	EVQLVQSGAE VKKPGASVKV SCKASGYTFT SYYMYWVRQA PGQGLEWIGE INPYSGDTNF NEKFKSRATL TVDTSTSTAY LELSSLRSED TAVYYCAREG GFAYWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCYG PPC
162	CPPC	CPPC
163	Fab heavy chain constant region (IgG4) ("KYGP")	ASTKG PSVFPLAPCS RSTSESTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTKTYTCNV DHKPSNTKVD KRVESKYGP
164	Fab heavy chain constant region ("CDKTHX")	ASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THX$_8$, wherein X$_8$ is selected from any amino acid except T
165	C-terminus of heavy chain of a Fab fragment	CDKTHT
166	C-terminus of heavy chain of a Fab fragment	CDKTHL
167	C-terminus of heavy chain of a Fab fragment	CDKTH
168	C-terminus of heavy chain of a Fab fragment	CDKT
169	C-terminus of heavy chain of a Fab fragment	CDKTHX; wherein X is selected from any amino acid except T
170	C-terminus of heavy chain of a Fab fragment	CDKTHTC
171	C-terminus of heavy chain of a Fab fragment	CDKTHTCPPC
172	C-terminus of heavy chain of a Fab fragment	CDKTHTCPPS
173	C-terminus of heavy chain of a Fab fragment	CDKTHTSPPC
174	C-terminus of heavy chain of a Fab fragment	CDKTHTAPPC
175	C-terminus of heavy chain of a Fab fragment	CDKTHTSGGC
176	C-terminus of heavy chain of a Fab fragment	CYGPPC
177	C-terminus of heavy chain of	APPC

	a Fab fragment	
178	C-terminus of heavy chain of a Fab fragment	SGGC
179	C-terminus of heavy chain of a Fab fragment	VERK
180	C-terminus of heavy chain of a Fab fragment	VERKC
181	C-terminus of heavy chain of a Fab fragment	KYGPP
182	C-terminus of heavy chain of a Fab fragment	KYGP
183	C-terminus of heavy chain of a Fab fragment	KYGPPC

WHAT IS CLAIMED IS:

1. An isolated antibody that binds to Factor D, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence SYYMY (SEQ ID NO: 15); (b) HVR-H2 comprising the amino acid sequence X_4INPX$_5$X$_6$GX$_7$TNFNEKFKS (SEQ ID NO: 111), wherein X_4 is selected from E and W; X_5 is selected from T and Y; X_6 is selected from N, S, and Q; and X_7 is selected from G, D, and E; (c) HVR-H3 comprising the amino acid sequence EGGFAY (SEQ ID NO: 25); (d) HVR-L1 comprising the amino acid sequence KASQNVDTDVA (SEQ ID NO:9); (e) HVR-L2 comprising the amino acid sequence SASSRX$_1$S (SEQ ID NO: 108), wherein X_1 is selected from Y, K, and R; and (f) HVR-L3 comprising the amino acid sequence QQYX$_3$NYPLT (SEQ ID NO: 110), wherein X_3 is selected from N and E.

2. The antibody of claim 1, wherein the antibody comprises the sequence X$_2$SASSRX$_1$S (SEQ ID NO: 109), wherein X_1 is selected from Y, K, and R; X_2 is selected from Y, R, S, K, and Q.

3. The antibody of claim 2, wherein X_2 is R.

4. The antibody of any one of the preceding claims, wherein X_1 is Y.

5. The antibody of any one of the preceding claims, wherein the antibody comprises HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-H2 comprising an amino acid sequence selected from SEQ ID NOs: 16 to 24, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, HVR-L2 comprising an amino acid sequence selected from SEQ ID NOs: 10 to 12, and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13 or 14.

6. The antibody of any one of the preceding claims, wherein the antibody comprises:

a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 16, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13;

b) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 20, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13;

c) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 17, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13;

d) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 18, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13;

e) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13;

f) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 21, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13;

g) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13;

h) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 23, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13;

i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 16, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 11, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13;

j) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 16, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 12, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13;

k) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 16, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 14; or

l) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 15, HVR-H2 comprising the amino acid sequence of SEQ ID NO: 24, HVR-H3 comprising the amino acid sequence of SEQ ID NO: 25, HVR-L1 comprising the amino acid sequence of SEQ ID NO: 9, HVR-L2 comprising the amino acid sequence of SEQ ID NO: 10, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 13.

7. The antibody of any one of the preceding claims, wherein the amino acid at position 49 of the light chain is arginine (R).

8. The antibody of any one of the preceding claims, wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence selected from SEQ ID NOs: 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, and 63.

9. The antibody of any one of the preceding claims, wherein the antibody comprises a light chain variable region comprising an amino acid sequence selected from SEQ ID NOs: 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, and 62.

10. The antibody of any one of the preceding claims, wherein the antibody comprises:

a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 35 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 34;

b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 39 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 38;

c) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 33 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 32;

d) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 45 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 44;

e) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 47 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 46;

f) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 49 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 48;

g) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 29 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 28;

h) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 31 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 30;

i) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 37 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 36;

j) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 41 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 40;

k) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 61 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 60;

l) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 43 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 42;

m) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 57 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 56;

n) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 51 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 50;

o) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 53 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 52;

p) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 55 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 54;

q) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 59 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 58; or

r) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 63 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 62.

11. The antibody of claim 1, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 35 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 34, or wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 39 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 38.

12. The antibody of any one of the preceding claims, which is a monoclonal antibody.

13. The antibody of any one of the preceding claims, which is a humanized or chimeric antibody.

14. The antibody of any one of the preceding claims, which is an antibody fragment that binds Factor D.

15. The antibody of claim 14, which is a Fab fragment.

16. The antibody of claim 15, wherein the light chain comprises a light chain constant region comprising the sequence of SEQ ID NO: 112.

17. The antibody of claim 15 or claim 16, wherein the heavy chain comprises a heavy chain constant region comprising a sequence selected from SEQ ID NOs: 113, 128 to 132, 134 to 137, and 154-156.

18. The antibody of any one of claims 14 to 17, wherein the antibody comprises:

a) a heavy chain comprising an amino acid sequence selected from SEQ ID NOs: 71, 118 to 122, and 140 to 146, and a light chain comprising the amino acid sequence of SEQ ID NO: 70;

b) a heavy chain comprising an amino acid sequence selected from SEQ ID NO: 75, 123 to 127, and 147 to 153 and a light chain comprising the amino acid sequence of SEQ ID NO: 74;

c) a heavy chain comprising the amino acid sequence of SEQ ID NO: 65 and a light chain comprising the amino acid sequence of SEQ ID NO: 64;

d) a heavy chain comprising the amino acid sequence of SEQ ID NO: 65 and a light chain comprising the amino acid sequence of SEQ ID NO: 64;

e) a heavy chain comprising the amino acid sequence of SEQ ID NO: 67 and a light chain comprising the amino acid sequence of SEQ ID NO: 66;

f) a heavy chain comprising the amino acid sequence of SEQ ID NO: 69 and a light chain comprising the amino acid sequence of SEQ ID NO: 68;

g) a heavy chain comprising the amino acid sequence of SEQ ID NO: 73 and a light chain comprising the amino acid sequence of SEQ ID NO: 72;

h) a heavy chain comprising the amino acid sequence of SEQ ID NO: 77 and a light chain comprising the amino acid sequence of SEQ ID NO: 76;

i) a heavy chain comprising the amino acid sequence of SEQ ID NO: 79 and a light chain comprising the amino acid sequence of SEQ ID NO: 78;

j) a heavy chain comprising the amino acid sequence of SEQ ID NO: 81 and a light chain comprising the amino acid sequence of SEQ ID NO: 80;

k) a heavy chain comprising the amino acid sequence of SEQ ID NO: 83 and a light chain comprising the amino acid sequence of SEQ ID NO: 82;

l) a heavy chain comprising the amino acid sequence of SEQ ID NO: 85 and a light chain comprising the amino acid sequence of SEQ ID NO: 84;

m) a heavy chain comprising the amino acid sequence of SEQ ID NO: 87 and a light chain comprising the amino acid sequence of SEQ ID NO: 86;

n) a heavy chain comprising the amino acid sequence of SEQ ID NO: 89 and a light chain comprising the amino acid sequence of SEQ ID NO: 88;

o) a heavy chain comprising the amino acid sequence of SEQ ID NO: 91 and a light chain comprising the amino acid sequence of SEQ ID NO: 90;

p) a heavy chain comprising the amino acid sequence of SEQ ID NO: 93 and a light chain comprising the amino acid sequence of SEQ ID NO: 92;

q) a heavy chain comprising the amino acid sequence of SEQ ID NO: 95 and a light chain comprising the amino acid sequence of SEQ ID NO: 94;

r) a heavy chain comprising the amino acid sequence of SEQ ID NO: 97 and a light chain comprising the amino acid sequence of SEQ ID NO: 96; or

s) a heavy chain comprising the amino acid sequence of SEQ ID NO: 99 and a light chain comprising the amino acid sequence of SEQ ID NO: 98.

19. The antibody of any one of claims 14 to 17, wherein the antibody comprises:

a) a heavy chain comprising an amino acid sequence selected from SEQ ID NOs: 71, 118 to 122, and 140 to 146, and a light chain comprising the amino acid sequence of SEQ ID NO: 70; or

b) a heavy chain comprising an amino acid sequence selected from SEQ ID NO: 75, 123 to 127, and 147 to 153 and a light chain comprising the amino acid sequence of SEQ ID NO: 74.

20. The antibody of any one of the preceding claims, wherein the antibody comprises an engineered cysteine.

21. The antibody of claim 20, wherein the engineered cysteine is selected from a T110C, A136C, L170C, L175C, T183C, or T205C mutation in the heavy chain, and I106C, R108C, R142C, K149C, and V205C mutation in the light chain, wherein the residue number is according to Kabat numbering.

22. The antibody of claim 19, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 119 and a light chain comprising the amino acid sequence of SEQ ID NO: 70.

23. An isolated antibody that binds to Factor D, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 119 and a light chain comprising the amino acid sequence of SEQ ID NO: 70.

24. An isolated antibody that binds to Factor D, wherein the antibody comprises a heavy chain consisting of the amino acid sequence of SEQ ID NO: 119 and a light chain consisting of the amino acid sequence of SEQ ID NO: 70.

25. The antibody of any one of the preceding claims, wherein Factor D is human Factor D comprising the amino acid sequence of SEQ ID NO: 106.

26. The antibody of any one of the preceding claims, wherein the antibody binds to cynomolgus monkey Factor D.

27. The antibody of claim 26, wherein the cynomolgus monkey Factor D comprises the amino acid sequence of SEQ ID NO: 107.

28. The antibody of claim 26 or claim 27, wherein the antibody binds to cynomolgus monkey Factor D with a K_D that is less than 10-fold, or less than 7-fold, or less than 5-fold, or less than 3-fold higher than the K_D for human Factor D.

29. An isolated nucleic acid encoding the antibody of any one of the preceding claims.

30. A host cell comprising the nucleic acid of claim 29.

31. A method of producing the antibody of any one of claims 1 to 28 comprising culturing the host cell of claim 30 so that the antibody is produced.

32. A pharmaceutical formulation comprising the antibody of any one of claims 1 to 28 and a pharmaceutically acceptable carrier.

33. The pharmaceutical formulation of claim 32, wherein the pharmaceutically acceptable carrier comprises about a buffer having a pH between about 5.5 and about 8.0.

34. The pharmaceutical formulation of claim 32 or claim 33, wherein the antibody is present at a concentration of at least 150 mg/ml, at least 160 mg/ml, at least 170 mg/ml, at least 180 mg/ml, at least 190 mg/ml, at least 200 mg/ml, or at least 210 mg/ml, or at least 220 mg/ml, or at least 230 mg/ml, or at least 240 mg/ml, or at least 250 mg/ml, or at least 260 mg/ml, or at least 270 mg/ml, or at least 280 mg/ml, or at least 290 mg/ml, or at least 300 mg/ml.

35. The pharmaceutical formulation of any one of claims 32 to 34, wherein the antibody is present at a concentration of between 150 mg/ml and 350 mg/ml, or between 150 mg/ml and 300 mg/ml, or between 170 mg/ml and 300 mg/ml, or between 200 mg/ml and 300 mg/ml.

36. The pharmaceutical formulation of any one of claims 32 to 35, wherein the composition comprises no visible precipitate after storage at 4°C for at least one week, at least two weeks, at least four weeks, at least six weeks, at least eight weeks, at least 12 weeks, at least 16 weeks, at least 20 weeks, at least 24 weeks, or at least 28 weeks.

37. The pharmaceutical formulation of any one of claims 32 to 36, wherein the viscosity of the composition at 25°C is less than 30cP, less than 25cP, less than 20cP, less than 15cP, or less than 10cP.

38. The pharmaceutical formulation of claim 37, wherein the concentration of the anti-Factor D antibody in the composition is between 100 mg/ml and 300 mg/ml, or between 150 mg/ml and 300 mg/ml.

39. The pharmaceutical formulation of any one of claims 32 to 38, wherein the pharmaceutical formulation is suitable for intravitreal administration through a narrow bore needle.

40. The pharmaceutical formulation of claim 36, wherein the narrow bore needle is about 30, 29, 28, 27, 26, 25, 24, 23, or 22 gauge.

41. A conjugate comprising at least one antibody of any one of claims 1 to 28 covalently linked to one or more polyols.

42. The conjugate of claim 41, wherein the polyol is a multi-armed polyol.

43. The conjugate of claim 41 or claim 42, wherein the conjugate comprises at least two, at least three, at least four, at least five, or at least six antibodies covalently linked to a multi-armed polyol.

44. The conjugate of any one of claims 41 to 43, wherein the polyol is covalently linked to at least one antibody through a free sulfhydryl group of a cysteine amino acid.

45. The conjugate of claim 44, wherein the cysteine amino acid is an engineered cysteine.

46. The conjugate of claim 44 or claim 45, wherein the cysteine amino acid is in a constant region of the antibody.

47. The conjugate of any one of claims 44 to 46, wherein the cysteine amino acid is at the C-terminus of the heavy chain or light chain of the antibody.

48. The conjugate of any one of claims 41 to 43, wherein the polyol is covalently linked to at least one antibody through a free amino group of a lysine amino acid.

49. The conjugate of claim 48, wherein the lysine amino acid is in a constant region of the antibody.

50. The conjugate of claim 48, wherein the lysine amino acid is at the C-terminus of the heavy chain or light chain of the antibody.

51. The conjugate of any one of claims 41 to 50, wherein the polyol is a multi-armed polyol selected from a dimer, a tetramer, a hexamer, and an octamer.

52. The conjugate of claim 51, wherein the multi-armed polyol is an octamer.

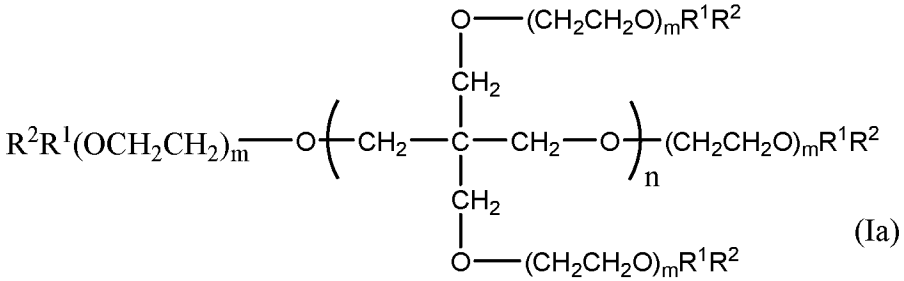
53. The conjugate of any one of claims 38 to 49, wherein the polyol is polyethylene glycol.

54. The conjugate of claim 53, wherein the polyethylene glycol has a weight average molecular weight of from about 500 D to about 300,000 D.

55. The conjugate of claim 53, wherein the polyethylene glycol has a weight average molecular weight of from about 20,000 D to about 60,000 D.

56. The conjugate of claim 53, wherein the polyethylene glycol has a weight average molecular weight of about 40,000 D.

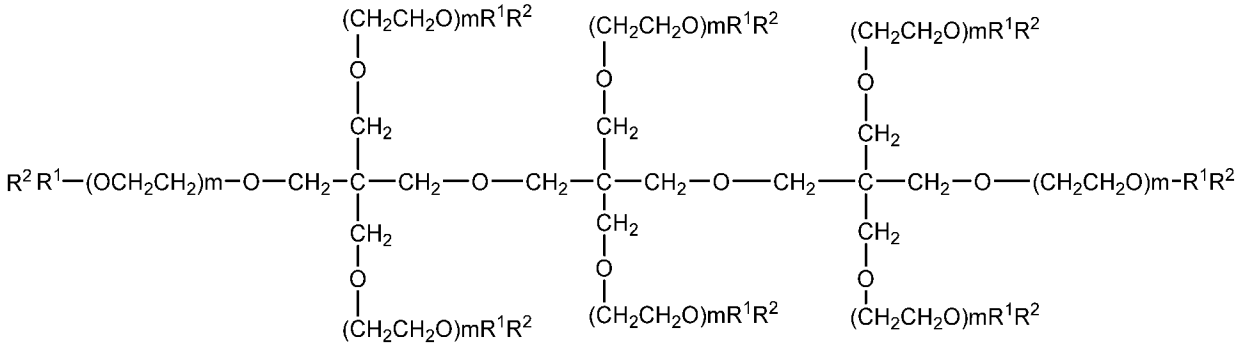
57. The conjugate of any one of claims 53 to 56, wherein the polyethylene glycol has the structure of general formula (Ia):



(Ia)

wherein each m is independently an integer from 3-250; n is an integer from 1-10; each R^1 is independently either absent, or is a linking group; and each R^2 is independently either hydrogen or a terminal reactive group; wherein at least one R^2 is a terminal reactive group and is covalently linked to the antibody.

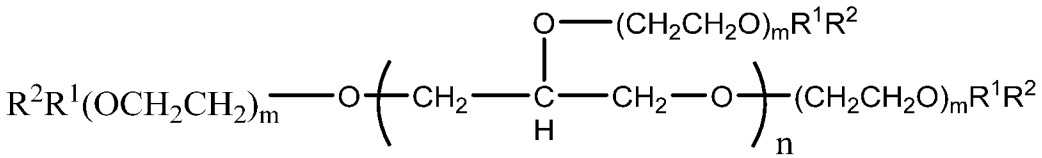
58. The conjugate of any one of claims 53 to 56, wherein the polyethylene glycol has the structure of general formula (Ib):



(Ib)

wherein each m is independently an integer from 3-250; each R^1 is independently either absent, or is a linking group; and each R^2 is independently either hydrogen or a terminal reactive group; wherein at least one R^2 is a terminal reactive group and is covalently linked to the antibody.

59. The conjugate of any one of claims 53 to 56, wherein the polyethylene glycol has the structure of general formula (IIa):

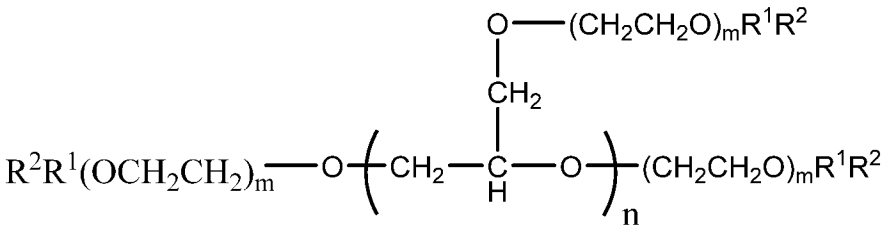


(IIa)

wherein each m is independently an integer of from 3-250; n is an integer from 1-10; each R^1 is independently either absent, or is a linking group; and each R^2 is independently either hydrogen or a terminal reactive group; wherein at least one R^2 is a terminal reactive group and is covalently linked to the antibody.

60. The conjugate of claim 59, wherein n is 4.

61. The conjugate of any one of claims 53 to 56, wherein the polyethylene glycol has the structure of general formula (IIIa):

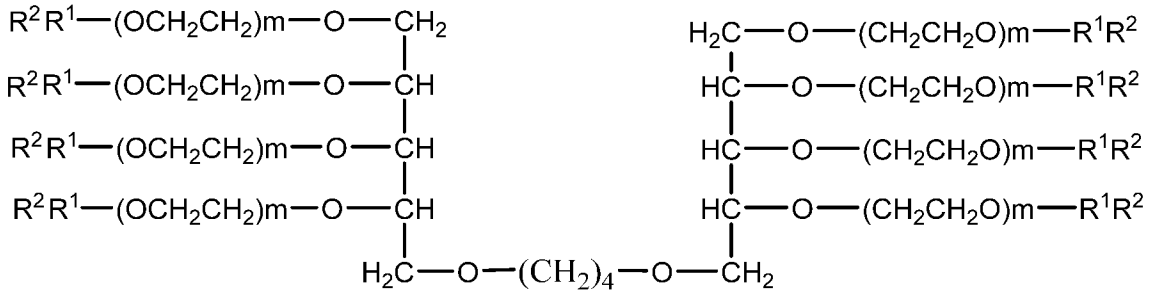


(IIIa)

wherein each m is independently an integer of from 3-250; n is an integer from 1-10; each R^1 is independently either absent, or is a linking group; and each R^2 is independently either hydrogen or a terminal reactive group; wherein at least one R^2 is a terminal reactive group and is covalently linked to the antibody.

62. The conjugate of claim 61, wherein n is 4.

63. The conjugate of any one of claims 53 to 56, wherein the polyethylene glycol has the structure of general formula (IVa):

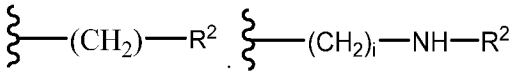
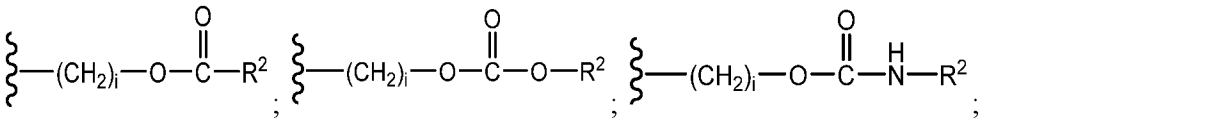
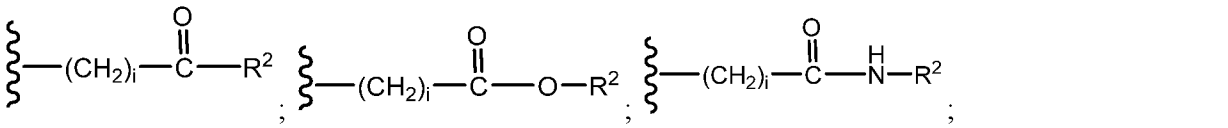
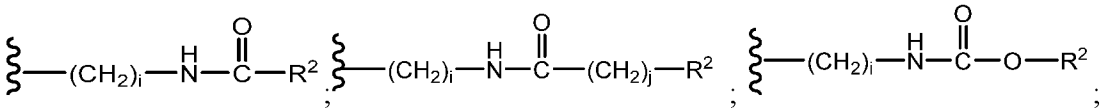
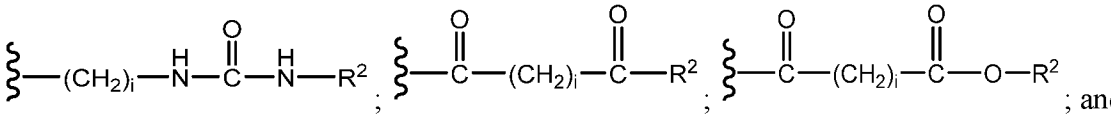
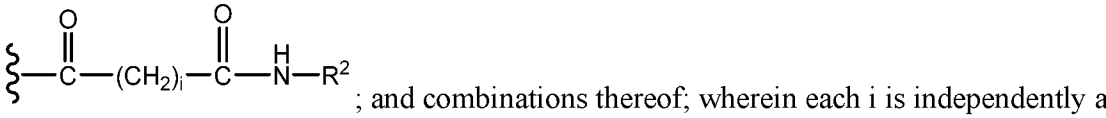
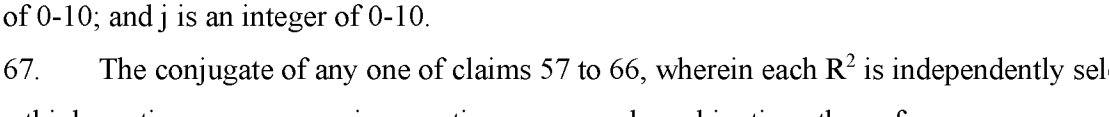


(IVa)

wherein each m is independently an integer of from 3-250; each R^1 is independently either absent, or is a linking group; and each R^2 is independently either hydrogen or a terminal reactive group; wherein at least one R^2 is a terminal reactive group and is covalently linked to the antibody.

64. The conjugate of any one of claims 57 to 63, wherein m is an integer of 50-200.

65. The conjugate of claim 64, wherein m is an integer of 100-150.

66. The conjugate of any one of claims 57 to 65, wherein at least one R^1 is a linking group, wherein R^1 and R^2 when taken together are selected from  ;  ;  ;  ;  ;  ;  ; and ; and combinations thereof; wherein each i is independently an integer of 0-10; and j is an integer of 0-10.

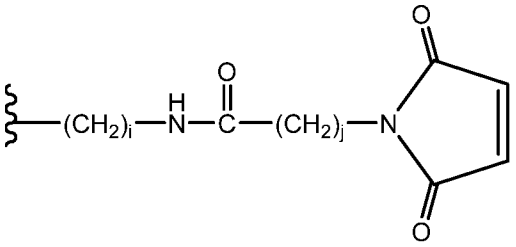
67. The conjugate of any one of claims 57 to 66, wherein each R^2 is independently selected from a thiol reactive group, an amino reactive group, and combinations thereof.

68. The conjugate of claim 67, wherein each R^2 is independently selected from a maleimide, a sulfhydryl, a thiol, trifluoromethanesulfonate, tosylate, aziridine, epoxide, a pyridyl disulfide,

succinimidyl ester, -NH_2, an aldehyde, a haloacetate, a haloacetamide, and a para-nitrophenyl carbonate.

69. The conjugate of claim 68, wherein R^2 is a maleimide.

70. The conjugate of any one of claims 57 to 69, wherein R^1 and R^2, when taken together, are



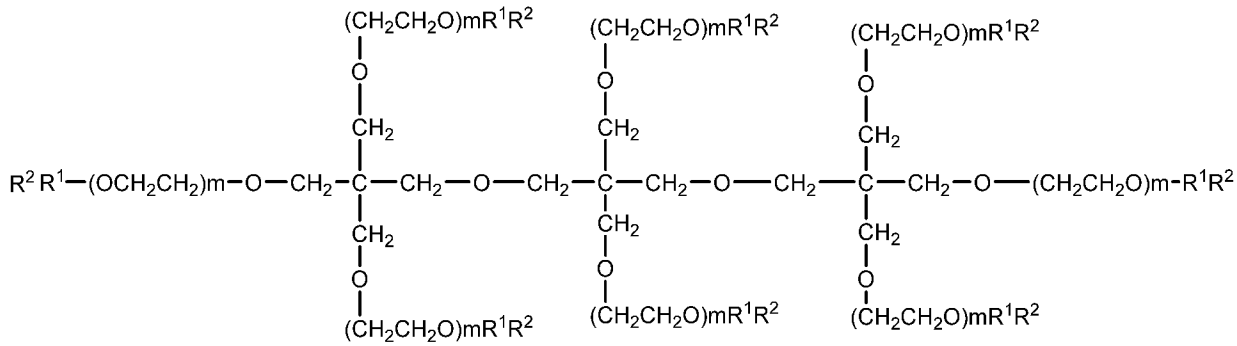
, i is an integer of 0-10; and j is an integer of 0-10.

71. The conjugate of any one of claims 57 to 69, wherein at least seven of the R^2 groups are covalently linked to one of the antibodies.

72. The conjugate of claim 71, wherein eight of the R^2 groups are covalently linked to one of the antibodies.

73. The conjugate of any one of claims 41 to 72, comprising at least one antibody of any one of claims 22 to 24 covalently linked to one or more polyols.

74. A conjugate comprising at least one antibody of any one of claims 22 to 24 covalently linked to a polyethylene glycol having the structure of general formula (Ib):



(Ib)

wherein each m is independently an integer from 3-250; each R^1 is independently either absent, or is a linking group; and each R^2 is independently either hydrogen or a terminal reactive group; wherein at least one R^2 is a terminal reactive group and is covalently linked to the antibody.

75. The conjugate of claim 74, wherein m is an integer of 50-200.

76. The conjugate of claim 74, wherein m is an integer of 100-150.

77. A conjugate of any one of claims 41 to 76, wherein the conjugate is prepared by covalently linking at least one antibody of any one of claims 1 to 28 to a multi-armed polyol.

78. A pharmaceutical formulation comprising the conjugate according to any one of claims 41 to 77 and a pharmaceutically acceptable carrier.

79. The pharmaceutical formulation of claim 78, wherein the concentration of the antibody is at least 100 mg/ml, or at least 150 mg/ml, or at least 200 mg/ml, or at least 300 mg/ml.

80. The pharmaceutical formulation of claim 79, wherein the concentration of the antibody is from about 50 mg/ml to about 300 mg/ml.

81. The pharmaceutical formulation of any one of claims 78 to 80, wherein the viscosity of the composition at 25°C is less than 1000 cP, less than 900 cP, less than 800 cP, less than 700 cP, less than 600 cP, less than 500 cP, less than 400 cP, or less than 300 cP.

82. The pharmaceutical formulation of claim 81, wherein the concentration of the anti-Factor D antibody in the composition is at least 100 mg/ml or at least 150 mg/ml.

83. A delivery device for ocular delivery comprising the pharmaceutical formulation of any one of claims 32 to 40 and 78 to 82 and a means for delivering the formulation intravitreally to a patient.

84. The delivery device of claim 83, wherein the formulation remains effective on site for a prolonged period of time.

85. A method of treating a complement-mediated disorder in a subject comprising administering to the subject an effective amount of the antibody of any one of claims 1 to 28, the conjugate of any one of claims 41 to 75, or the pharmaceutical formulation of any one of claims 32 to 40 and 78 to 82.

86. The method of claim 85, wherein the complement-mediated disorder is systemic.

87. The method of claim 85, wherein the complement-mediated disorder is a complement-associated eye condition.

88. The method of claim 87, wherein the complement-associated eye condition is selected from age-related macular degeneration (AMD), including dry and wet (non-exudative and exudative) forms, choroidal neovascularization (CNV), uveitis, diabetic retinopathy, ischemia-related retinopathy, diabetic macular edema, pathological myopia, von Hippel-Lindau disease, histoplasmosis of the eye, Central Retinal Vein Occlusion (CRVO), corneal neovascularization, and retinal neovascularization.

89. The method of claim 87, wherein the complement-associated eye condition is selected from intermediate dry form AMD or geographic atrophy (GA).

90. The method of any one of claims 85 to 89, wherein the method comprises administering the antibody, conjugate, or pharmaceutical formulation using an implantable port delivery system.

91. The method of any one of claims 85 to 89, wherein the method comprises administering the antibody, conjugate, or pharmaceutical formulation by intravitreal administration.

92. The method of claim 91, wherein the intravitreal administration is through a narrow bore needle.

93. The method of claim 92, wherein the narrow bore needle is about 30, 29, 28, 27, 26, 25, 24, 23, or 22 gauge.

94. The method of any one of claims 85 to 93, further comprising administering an additional therapeutic agent to the individual.

95. The method of claim 94, wherein the additional therapeutic agent is selected from an an ANG2 antagonist, a TIE2 antagonist, a VEGF antagonist, and a second complement component antagonist.

96. The method of claim 94, wherein the additional therapeutic agent is an anti-ANG2 antibody.

97. The method of claim 94, wherein the additional therapeutic agent is an anti-TIE2 antibody.

98. The method of claim 94, wherein the additional therapeutic agent is selected from a VEGF trap and an anti-VEGF antibody.

99. The method of claim 94, wherein the additional therapeutic agent is a second complement component antagonist, wherein the second complement component antagonist inhibits a complement component selected from C1, C2, C3, C4, C5, C6, C7, C8 and C9.

100. Use of the antibody of any one of claims 1 to 28 or the conjugate of any one of claims 43 to 77 for the preparation of a medicament for treating a complement-mediated disorder in a subject.

101. The use of claim 100, wherein the complement-mediated disorder is a complement-associated eye condition.

102. The use of claim 100, wherein the complement-mediated disorder is systemic.

103. The use of claim 102, wherein the complement-associated eye condition is selected from age-related macular degeneration (AMD), including dry and wet (non-exudative and exudative) forms, choroidal neovascularization (CNV), uveitis, diabetic retinopathy, ischemia-related retinopathy, diabetic macular edema, pathological myopia, von Hippel-Lindau disease, histoplasmosis of the eye, Central Retinal Vein Occlusion (CRVO), corneal neovascularization, and retinal neovascularization.

104. The use of claim 102, wherein the complement-associated eye condition is selected from intermediate dry form AMD or geographic atrophy (GA).

105. The antibody of any one of claims 1 to 28 or the conjugate of any one of claims 41 to 77 for use in therapy.

106. The antibody of any one of claims 1 to 28 or the conjugate of any one of claims 41 to 77 for use in a method of treating a complement-mediated disorder in a subject.

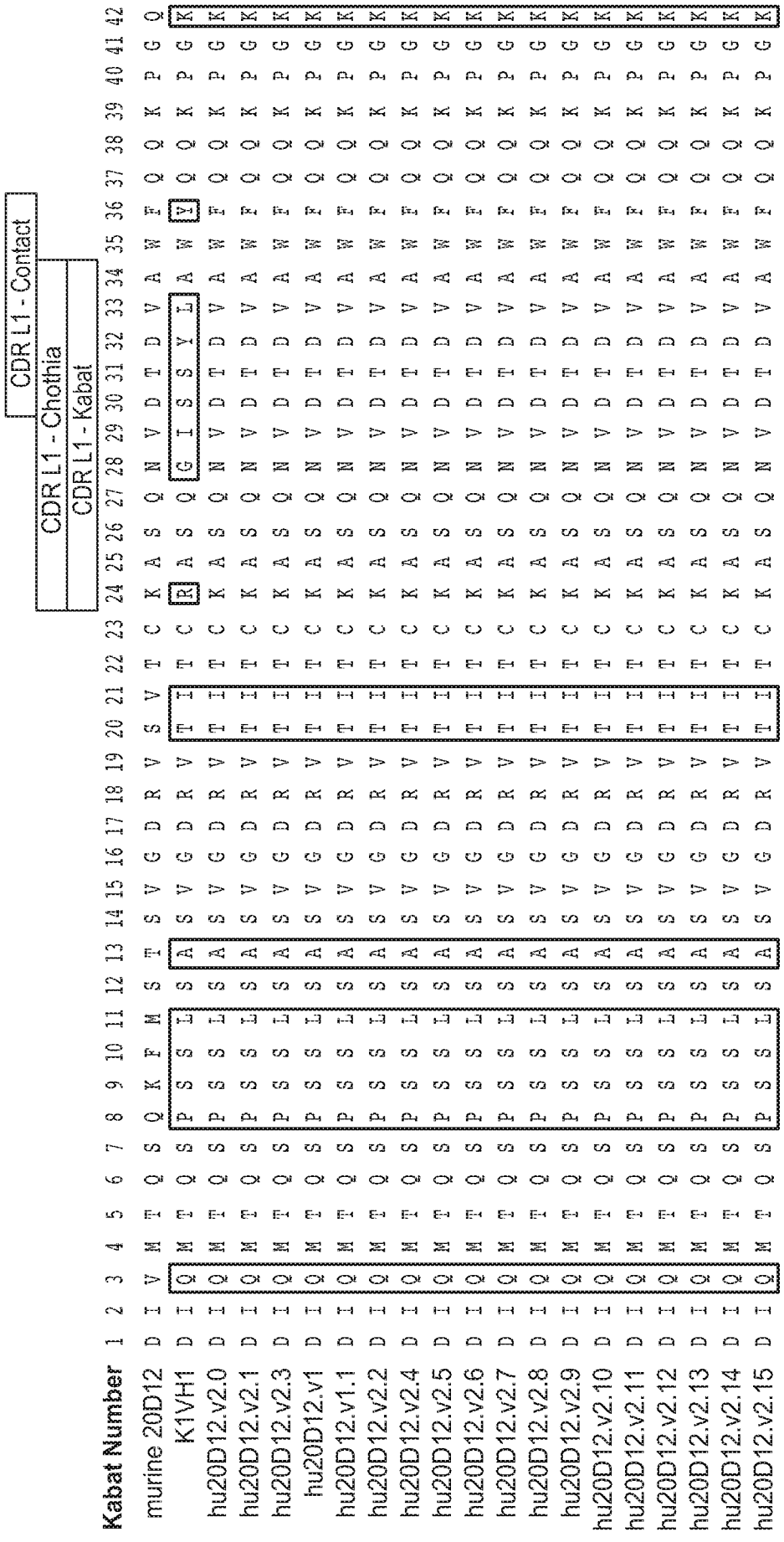
107. The antibody of any one of claims 1 to 28 or the conjugate of any one of claims 41 to 77 for use in a method of treating a systemic complement-mediated disorder in a subject.

108. The antibody or conjugate of claim 107, wherein the complement-mediated disorder is a complement-associated eye condition.

109. The antibody or conjugate of claim 108, wherein the complement-associated eye condition is selected from age-related macular degeneration (AMD), including dry and wet (non-exudative and exudative) forms, choroidal neovascularization (CNV), uveitis, diabetic retinopathy, ischemia-related retinopathy, diabetic macular edema, pathological myopia, von Hippel-Lindau disease,

histoplasmosis of the eye, Central Retinal Vein Occlusion (CRVO), corneal neovascularization, and retinal neovascularization.

110. The antibody or conjugate of claim 108, wherein the complement-associated eye condition is selected from intermediate dry form AMD or geographic atrophy (GA).


CDR L1 - Contact
CDR L1 - Chothia
CDR L1 - Kabat
Kabat Number
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42
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hu20D12.v2.3 D I Q M T Q S P S S L S A S V G D R V T I T C K A S Q N V D T D V A W F Q Q K P G K
hu20D12.v1 D I Q M T Q S P S S L S A S V G D R V T I T C K A S Q N V D T D V A W F Q Q K P G K
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hu20D12.v2.4 D I Q M T Q S P S S L S A S V G D R V T I T C K A S Q N V D T D V A W F Q Q K P G K
hu20D12.v2.5 D I Q M T Q S P S S L S A S V G D R V T I T C K A S Q N V D T D V A W F Q Q K P G K
hu20D12.v2.6 D I Q M T Q S P S S L S A S V G D R V T I T C K A S Q N V D T D V A W F Q Q K P G K
hu20D12.v2.7 D I Q M T Q S P S S L S A S V G D R V T I T C K A S Q N V D T D V A W F Q Q K P G K
hu20D12.v2.8 D I Q M T Q S P S S L S A S V G D R V T I T C K A S Q N V D T D V A W F Q Q K P G K
hu20D12.v2.9 D I Q M T Q S P S S L S A S V G D R V T I T C K A S Q N V D T D V A W F Q Q K P G K
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hu20D12.v2.14 D I Q M T Q S P S S L S A S V G D R V T I T C K A S Q N V D T D V A W F Q Q K P G K
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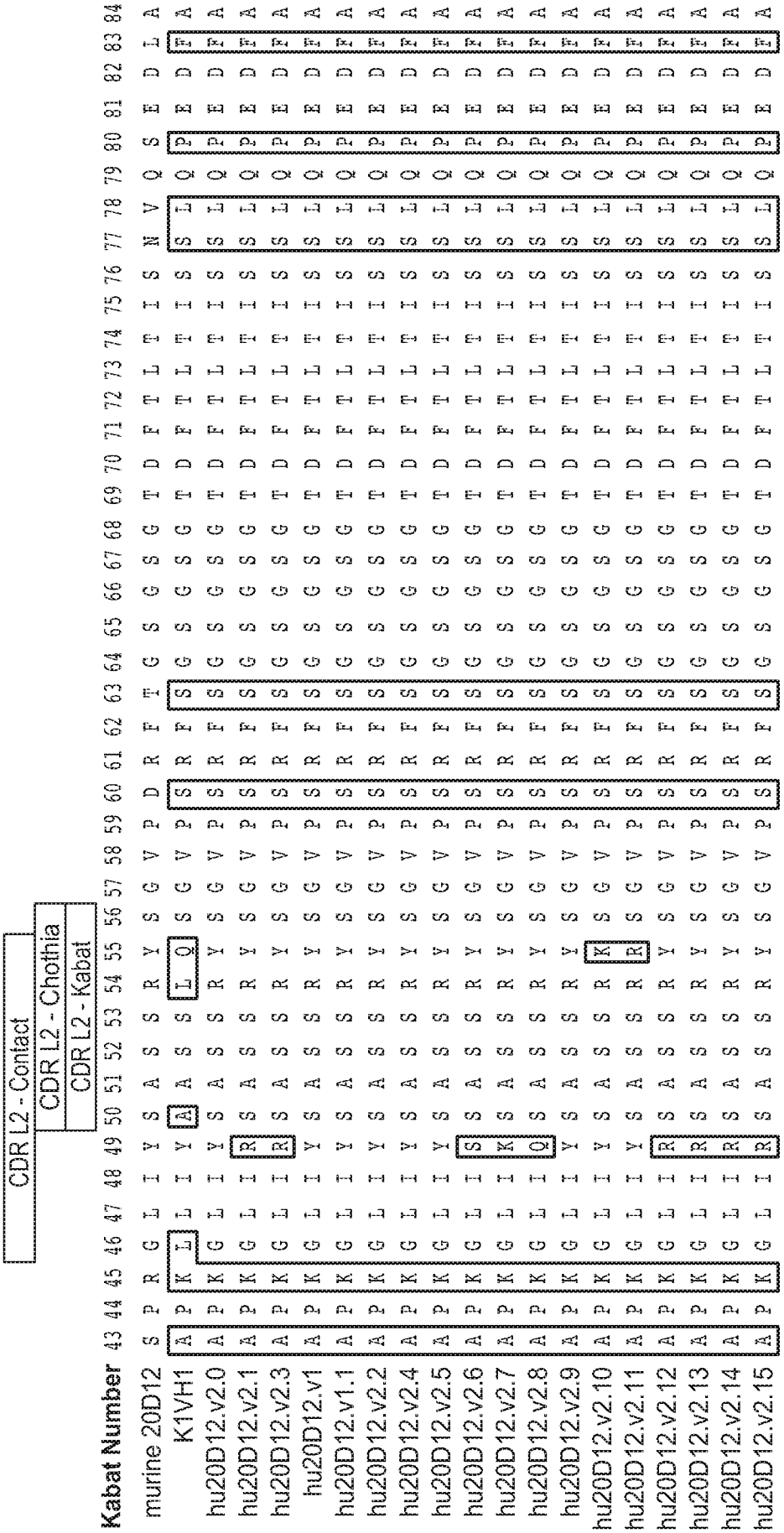
FIG. 1A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CDR L2 - Chothia
CDR L2 - Kabat
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hu20D12.v2.3 A P K G L I R S A S S R Y S G V P S R F S G S G S G T D F T L T I S S L Q P E D F A
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hu20D12.v2.14 A P K G L I R S A S S R Y S G V P S R F S G S G S G T D F T L T I S S L Q P E D F A
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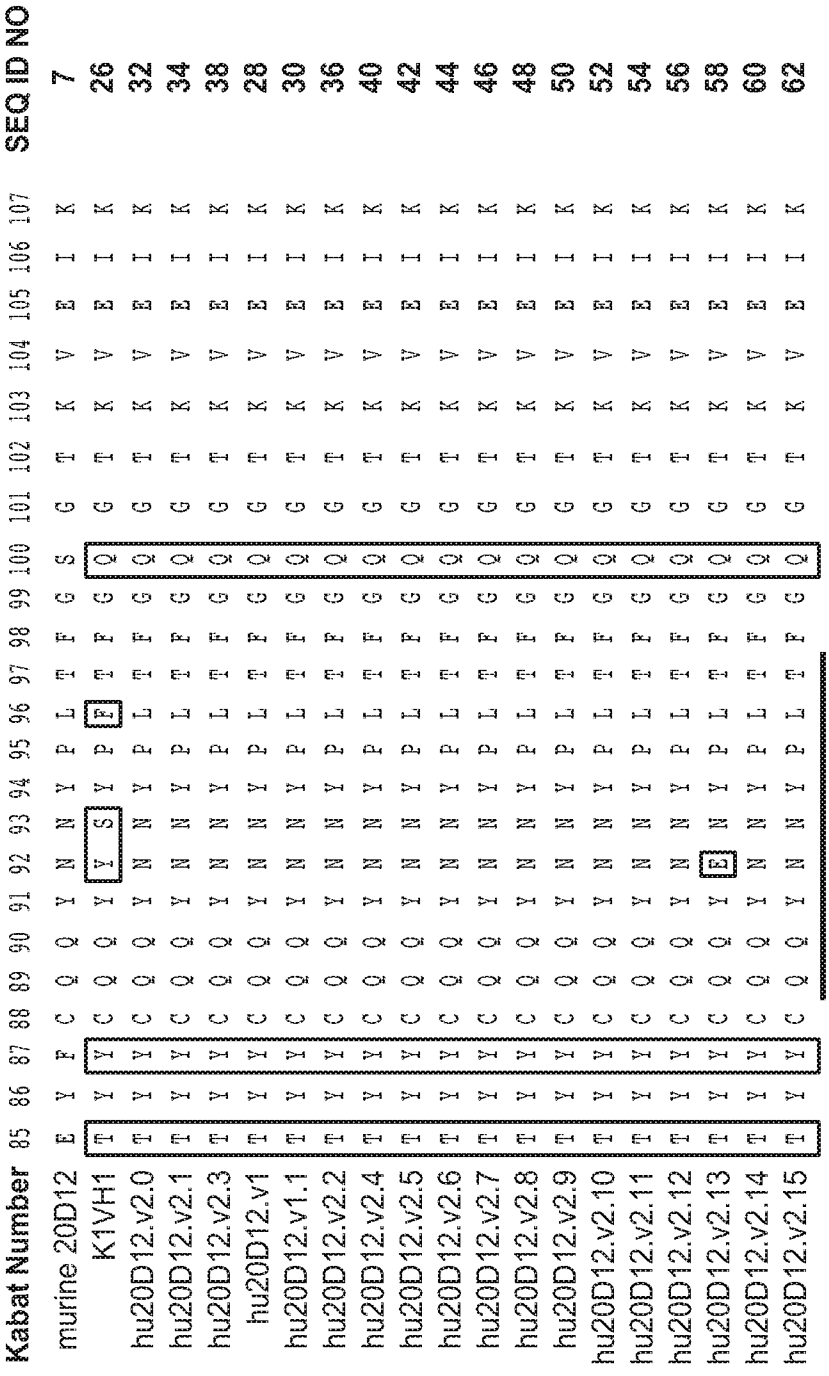
FIG. 1B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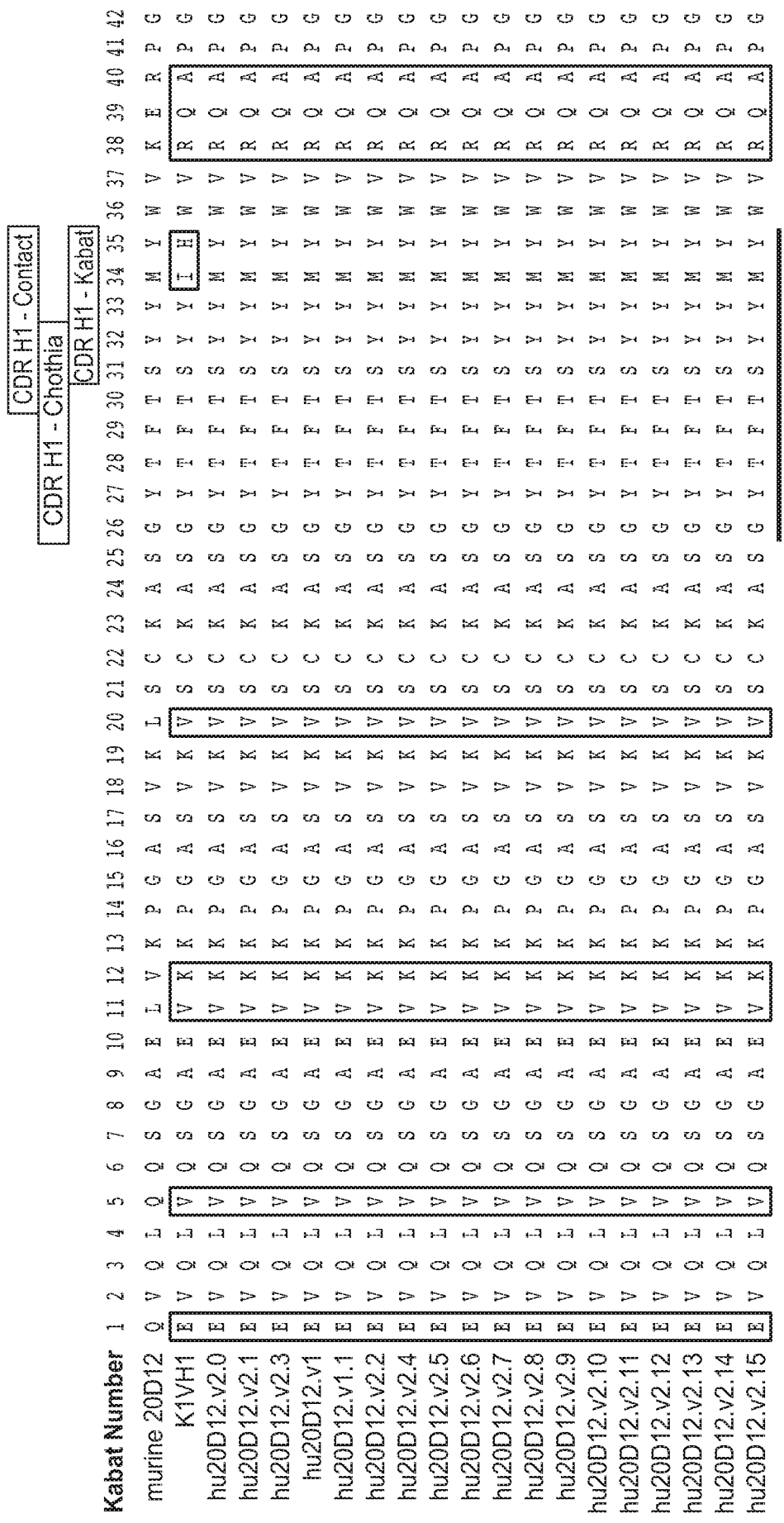
hu20D12.v2.0 T Y Y C Q Q Y N N Y P L T F G Q G T K V E I K 32
hu20D12.v2.1 T Y Y C Q Q Y N N Y P L T F G Q G T K V E I K 34
hu20D12.v2.3 T Y Y C Q Q Y N N Y P L T F G Q G T K V E I K 38
hu20D12.v1 T Y Y C Q Q Y N N Y P L T F G Q G T K V E I K 28
hu20D12.v1.1 T Y Y C Q Q Y N N Y P L T F G Q G T K V E I K 30
hu20D12.v2.2 T Y Y C Q Q Y N N Y P L T F G Q G T K V E I K 36
hu20D12.v2.4 T Y Y C Q Q Y N N Y P L T F G Q G T K V E I K 40
hu20D12.v2.5 T Y Y C Q Q Y N N Y P L T F G Q G T K V E I K 42
hu20D12.v2.6 T Y Y C Q Q Y N N Y P L T F G Q G T K V E I K 44
hu20D12.v2.7 T Y Y C Q Q Y N N Y P L T F G Q G T K V E I K 46
hu20D12.v2.8 T Y Y C Q Q Y N N Y P L T F G Q G T K V E I K 48
hu20D12.v2.9 T Y Y C Q Q Y N N Y P L T F G Q G T K V E I K 50
hu20D12.v2.10 T Y Y C Q Q Y N N Y P L T F G Q G T K V E I K 52
hu20D12.v2.11 T Y Y C Q Q Y N N Y P L T F G Q G T K V E I K 54
hu20D12.v2.12 T Y Y C Q Q Y N N Y P L T F G Q G T K V E I K 56
hu20D12.v2.13 T Y Y C Q Q Y E N Y P L T F G Q G T K V E I K 58
hu20D12.v2.14 T Y Y C Q Q Y N N Y P L T F G Q G T K V E I K 60
hu20D12.v2.15 T Y Y C Q Q Y N N Y P L T F G Q G T K V E I K 62

FIG. 1C


CDR H1 - Contact
CDR H1 - Chothia
CDR H1 - Kabat
Kabat Number
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K1VH1 E V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F T S Y Y M Y W V R Q A P G G
hu20D12.v2.0 E V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F T S Y Y M Y W V R Q A P G G
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hu20D12.v1.1 E V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F T S Y Y M Y W V R Q A P G G
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hu20D12.v2.4 E V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F T S Y Y M Y W V R Q A P G G
hu20D12.v2.5 E V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F T S Y Y M Y W V R Q A P G G
hu20D12.v2.6 E V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F T S Y Y M Y W V R Q A P G G
hu20D12.v2.7 E V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F T S Y Y M Y W V R Q A P G G
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hu20D12.v2.9 E V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F T S Y Y M Y W V R Q A P G G
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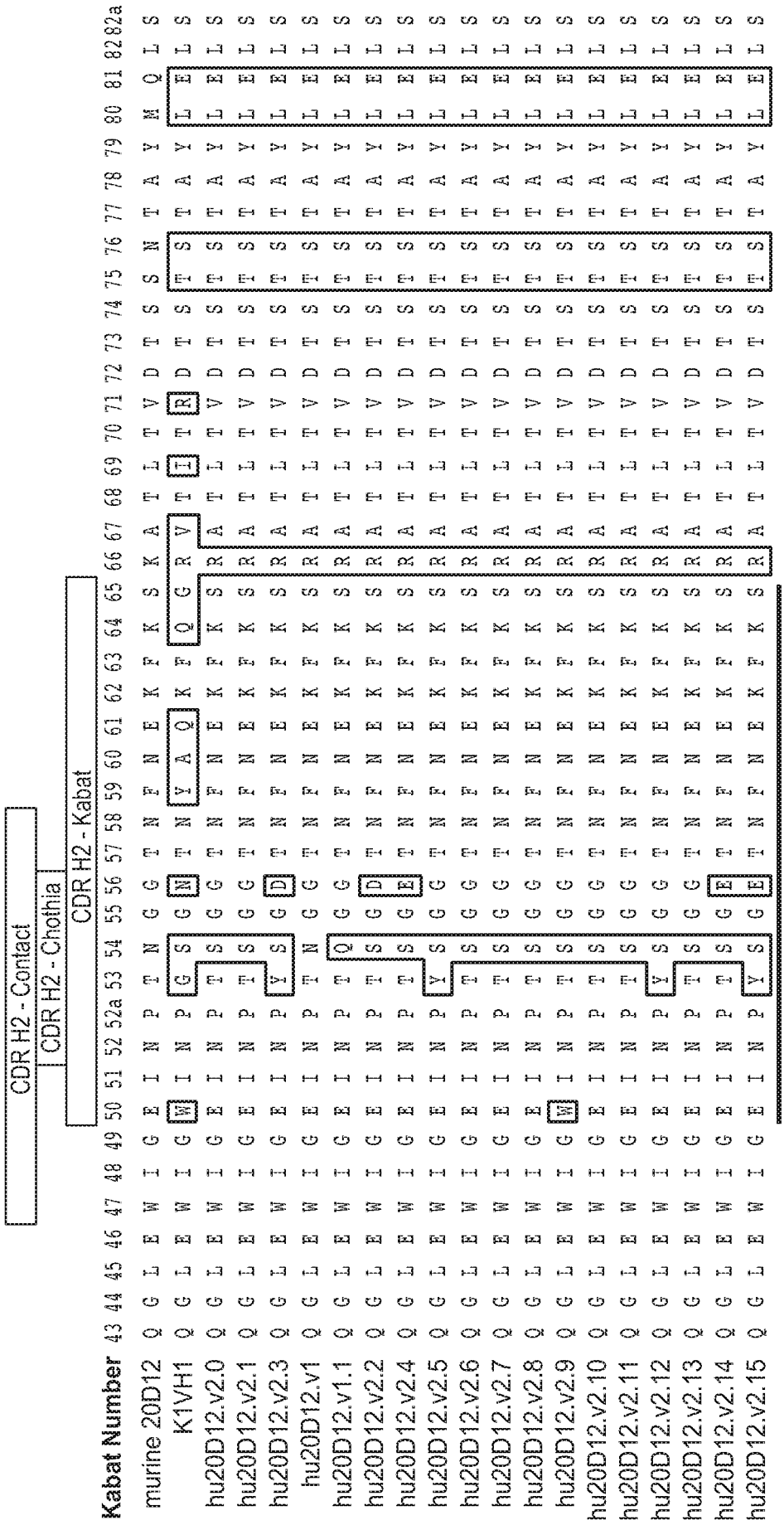
hu20D12.v2.12 E V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F T S Y Y M Y W V R Q A P G G
hu20D12.v2.13 E V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F T S Y Y M Y W V R Q A P G G
hu20D12.v2.14 E V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F T S Y Y M Y W V R Q A P G G
hu20D12.v2.15 E V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F T S Y Y M Y W V R Q A P G G

FIG. 2A


CDR H2 - Contact
CDR H2 - Chothia
CDR H2 - Kabat
Kabat Number
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K1VH1 Q G L E W I G E I N P G S G N T N Y A Q K F Q G R V T I T R D T S T S T A Y L E L S
hu20D12.v2.0 Q G L E W I G E I N P T S G G T N F N E K F K S R A T L T V D T S T S T A Y L E L S
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hu20D12.v1 Q G L E W I G E I N P T N G G T N F N E K F K S R A T L T V D T S T S T A Y L E L S
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hu20D12.v2.4 Q G L E W I G E I N P T S G E T N F N E K F K S R A T L T V D T S T S T A Y L E L S
hu20D12.v2.5 Q G L E W I G E I N P Y S G G T N F N E K F K S R A T L T V D T S T S T A Y L E L S
hu20D12.v2.6 Q G L E W I G E I N P T S G G T N F N E K F K S R A T L T V D T S T S T A Y L E L S
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hu20D12.v2.8 Q G L E W I G E I N P T S G G T N F N E K F K S R A T L T V D T S T S T A Y L E L S
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hu20D12.v2.13 Q G L E W I G E I N P T S G G T N F N E K F K S R A T L T V D T S T S T A Y L E L S
hu20D12.v2.14 Q G L E W I G E I N P T S G E T N F N E K F K S R A T L T V D T S T S T A Y L E L S
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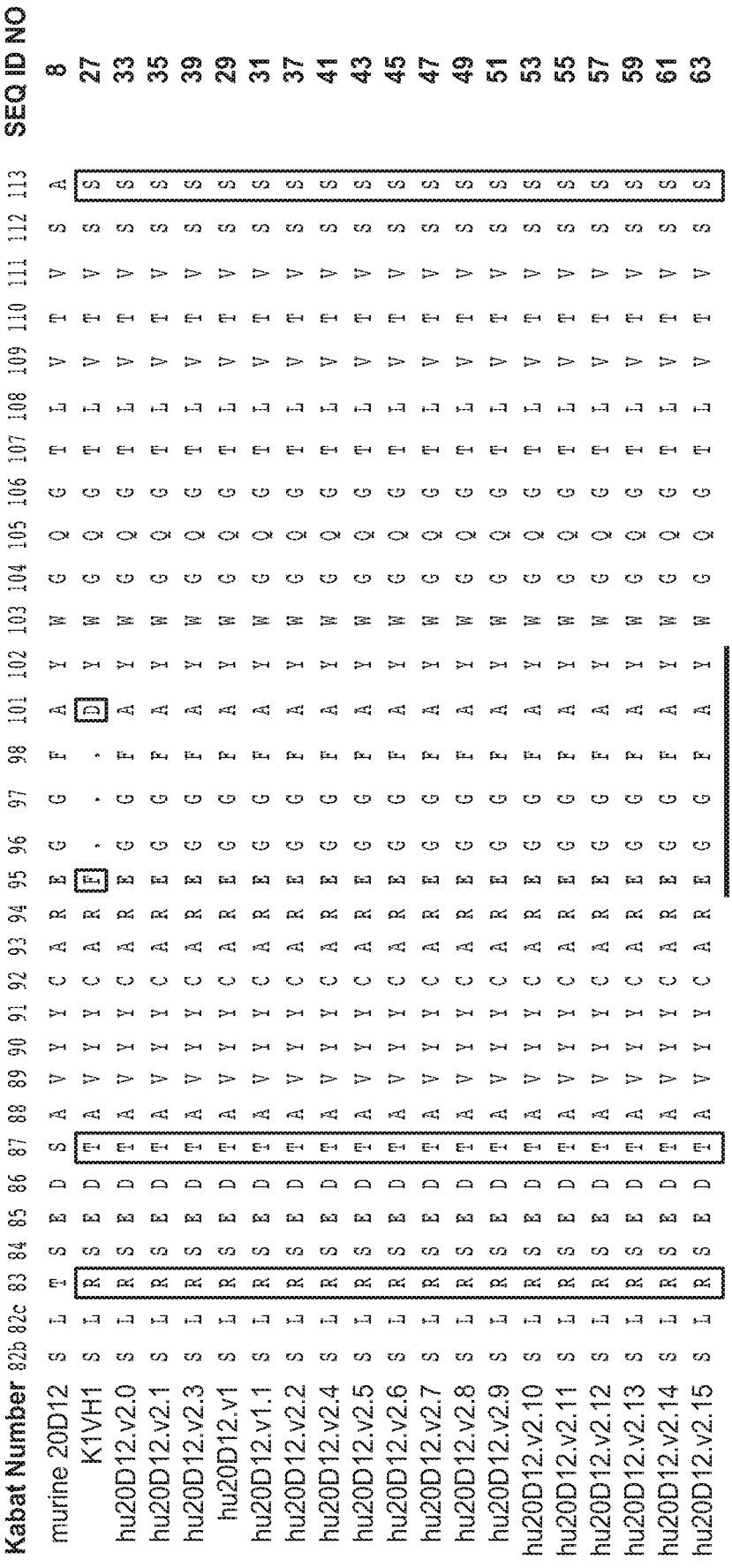
FIG. 2B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hu20D12.v2.0 S L R S E D T A V Y Y C A R E G G F A Y W G Q G T L V T V S S 33
hu20D12.v2.1 S L R S E D T A V Y Y C A R E G G F A Y W G Q G T L V T V S S 35
hu20D12.v2.3 S L R S E D T A V Y Y C A R E G G F A Y W G Q G T L V T V S S 39
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hu20D12.v2.10 S L R S E D T A V Y Y C A R E G G F A Y W G Q G T L V T V S S 53
hu20D12.v2.11 S L R S E D T A V Y Y C A R E G G P A Y W G Q G T L V T V S S 55
hu20D12.v2.12 S L R S E D T A V Y Y C A R E G G F A Y W G Q G T L V T V S S 57
hu20D12.v2.13 S L R S E D T A V Y Y C A R E G G P A Y W G Q G T L V T V S S 59
hu20D12.v2.14 S L R S E D T A V Y Y C A R E G G F A Y W G Q G T L V T V S S 61
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FIG. 2C

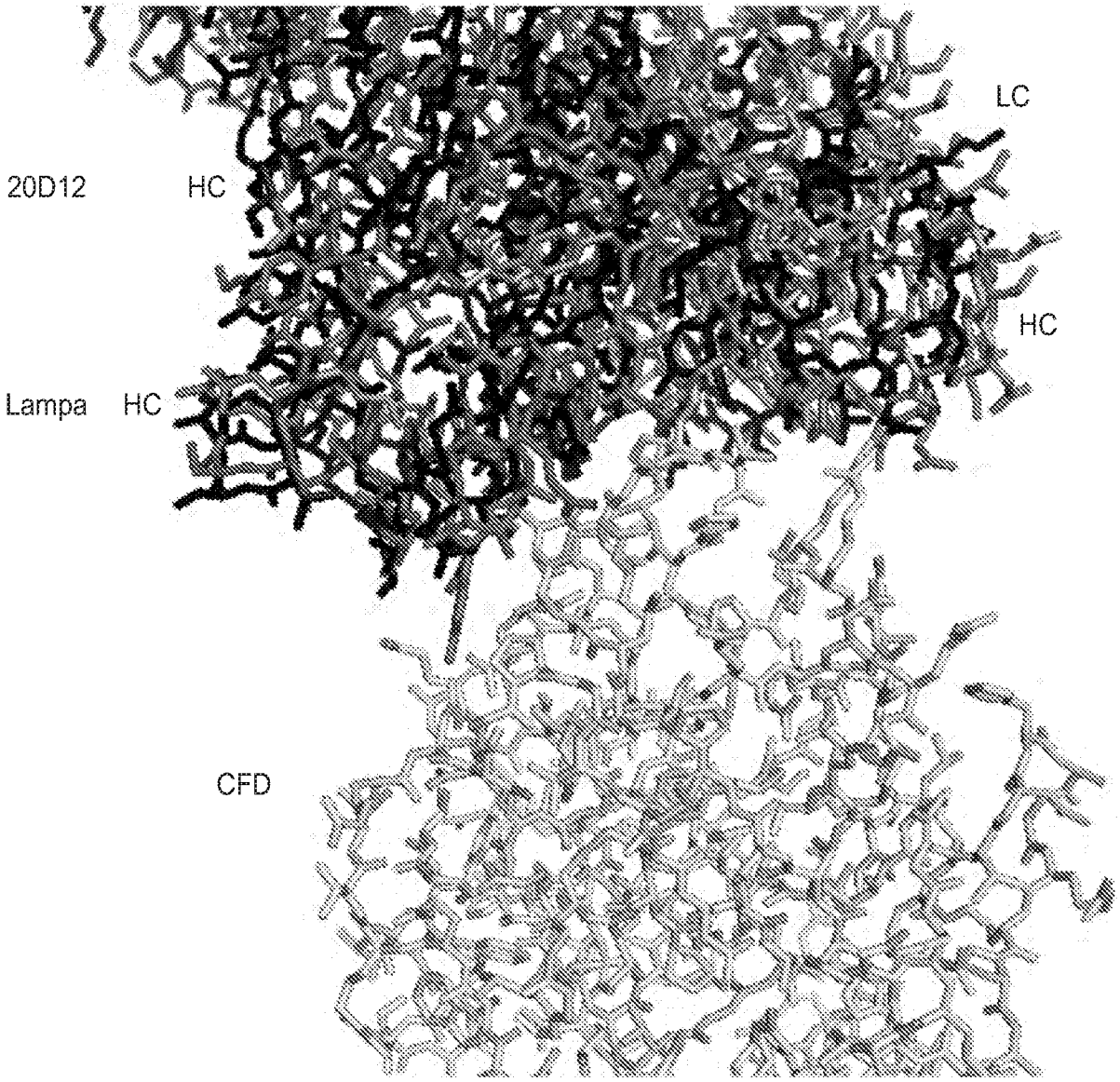
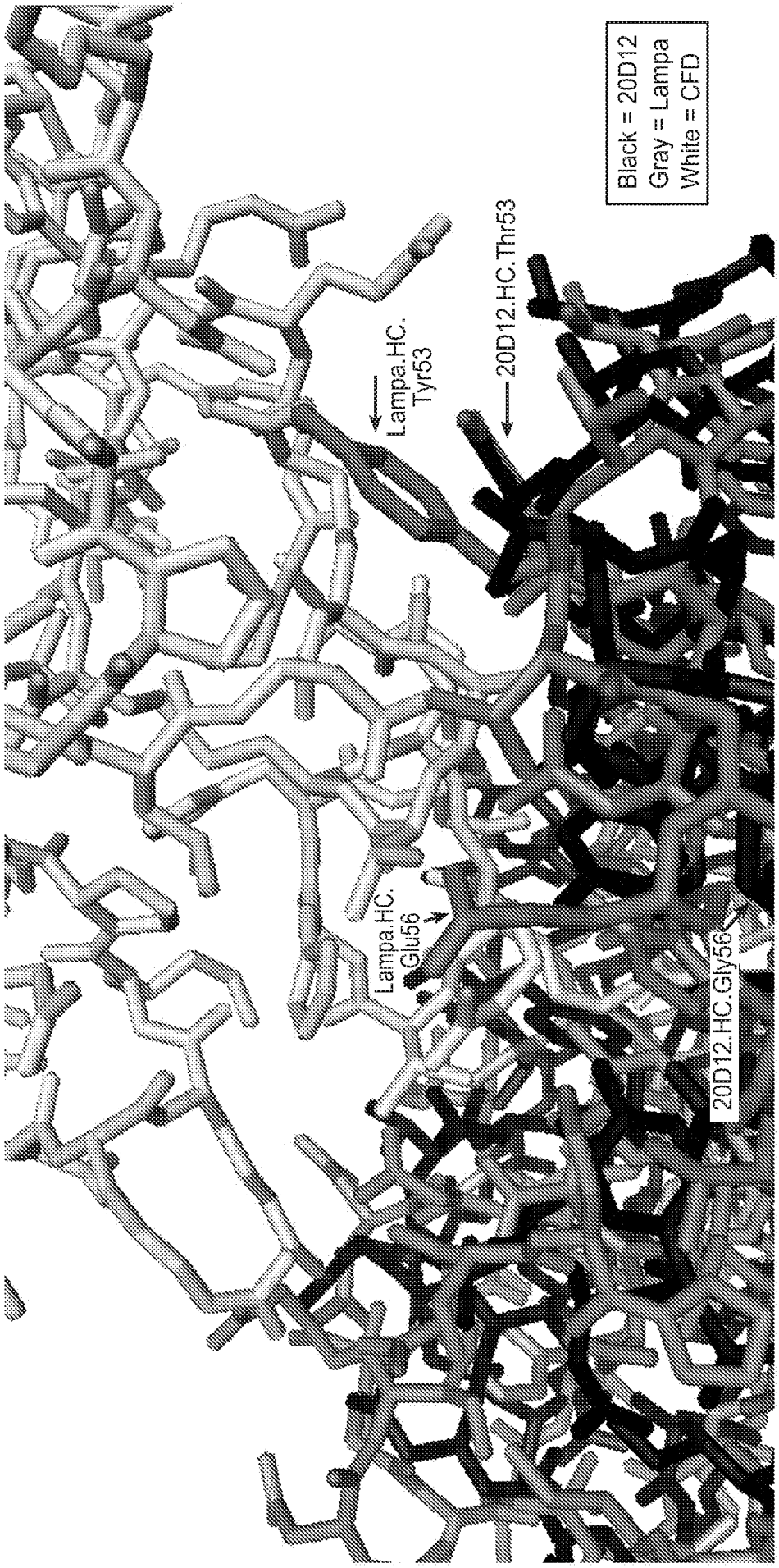


FIG. 3A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Gray = Lampa
White = CFD
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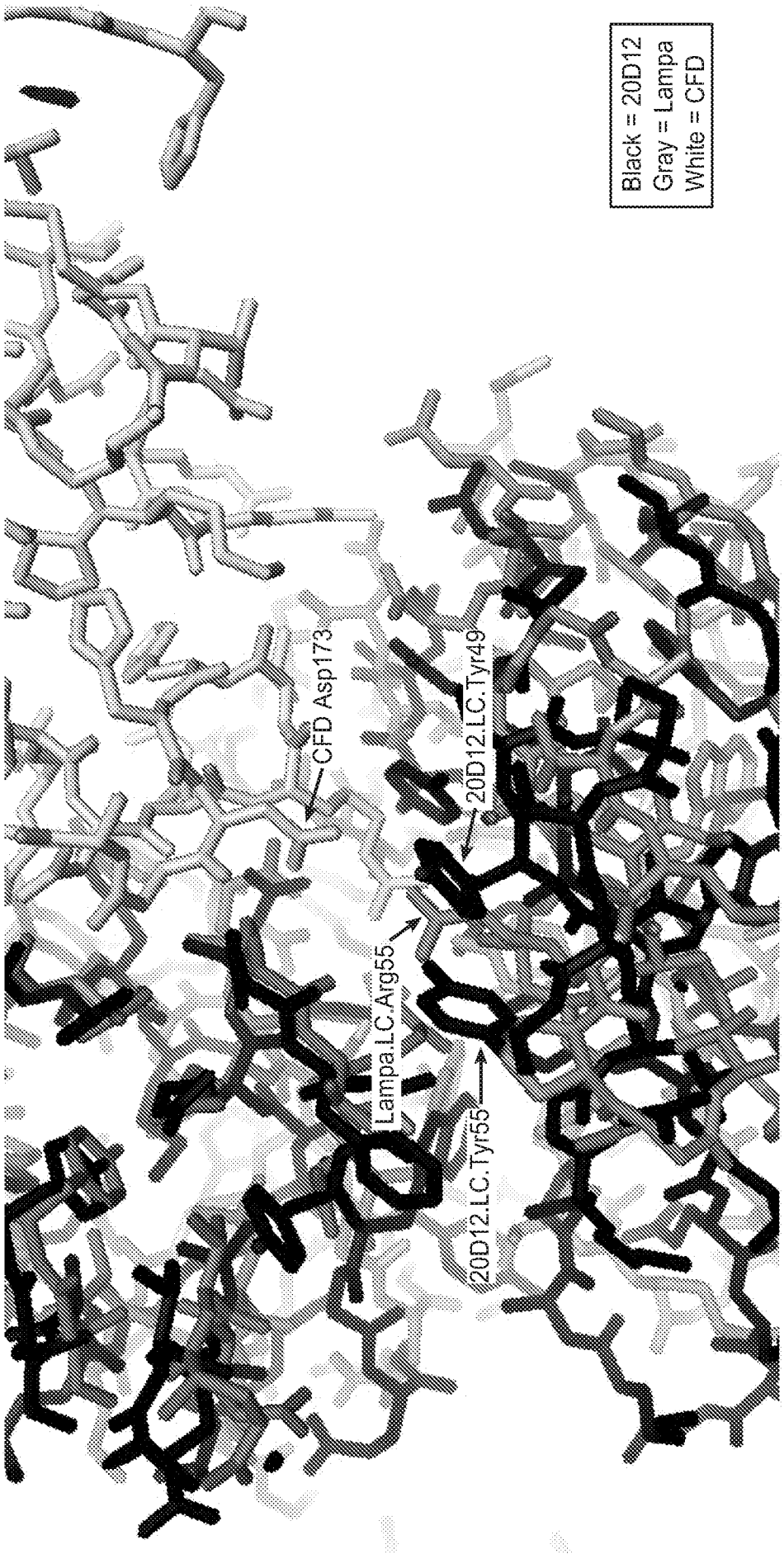
20D12.HC.Thr53
Lampa.HC.
Glu56
20D12.HC.Gly56

FIG. 3B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White = CFD
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Lampa.LC.Arg55
20D12.LC.Tyr55

FIG. 3C

Light Chain, Kappa: Humanized Antibody Aligned to lampalizumab

CDR L1

Kabat Number 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36
lampalizumab D I Q V T Q S P S S L S A S V G D R V T I T C I T S T . . . . . . D I D D D M N W Y
hu20D12.v1.N54S D I Q M T Q S P S S L S A S V G D R V T I T C K A S Q . . . . . . N V D T D V A W F

CDR L2

Kabat Number 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71
lampalizumab Q Q K P G K V P K L L I S G G N T L . . . . . R P G V P S R F S G S G S G T D F
hu20D12.v1.N54S Q Q K P G K A P K G L I Y S A S S R . . . . . Y S G V P S R F S G S G S G T D F

CDR L3

Kabat Number 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107
lampalizumab T L T I S S L Q P E D V A T Y Y C L Q S D S L P . . . . . . Y T F G Q G T K V E I K
hu20D12.v1.N54S T L T I S S L Q P E D F A T Y Y C Q Q Y N N Y P . . . . . . L T F G Q G T R L E I K

Heavy Chain: Humanized Antibody Aligned to lampalizumab

CDR H1

Kabat Number 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44
lampalizumab E V Q L V Q S G P E L K K P G A S V K V S C K A S G Y T F T N Y G M N . . W V R Q A P G Q G
hu20D12.v1.N54S E V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F T S Y Y M Y . . W V R Q A P G Q G

CDR H2

Kabat Number 45 46 47 48 49 50 51 52 a 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 a b c 83 84
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hu20D12.v1.N54S L E W I G E I N P . . T S G G T N F N E K F K S R A T L T V D T S T S T A Y L E L S S L R S

CDR H3

Kabat Number 85 86 87 88 89 90 91 92 93 94 95 96 97 98 101 102 103 104 105 106 107 108 109 110 111 112 113
lampalizumab E D T A V Y Y C E R E G G . . . . V N N W G Q G T L V T V S S
hu20D12.v1.N54S E D T A V Y Y C A R E G G . . . . F A Y W G Q G T L V T V S S

FIG. 4

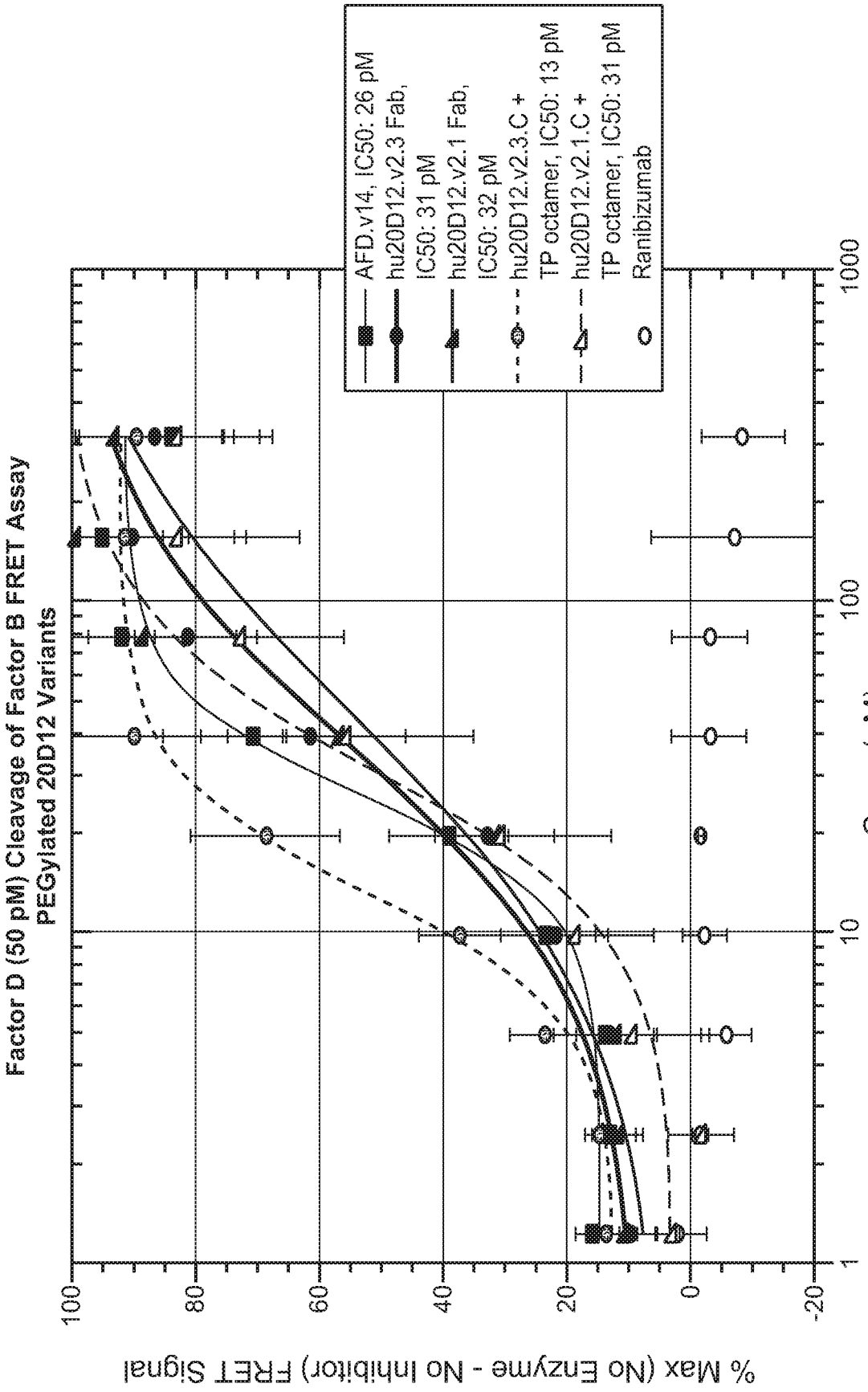
Factor D (50 pM) Cleavage of Factor B FRET Assay
PEGylated 20D12 Variants
AFD.v14, IC50: 26 pM
hu20D12.v2.3 Fab, IC50: 31 pM
hu20D12.v2.1 Fab, IC50: 32 pM
hu20D12.v2.3.C + TP octamer, IC50: 13 pM
hu20D12.v2.1.C + TP octamer, IC50: 31 pM
Ranibizumab
% Max (No Enzyme - No Inhibitor) FRET Signal
Conc. (pM)
100
80
60
40
20
0
-20
1
10
100
1000

FIG. 5A

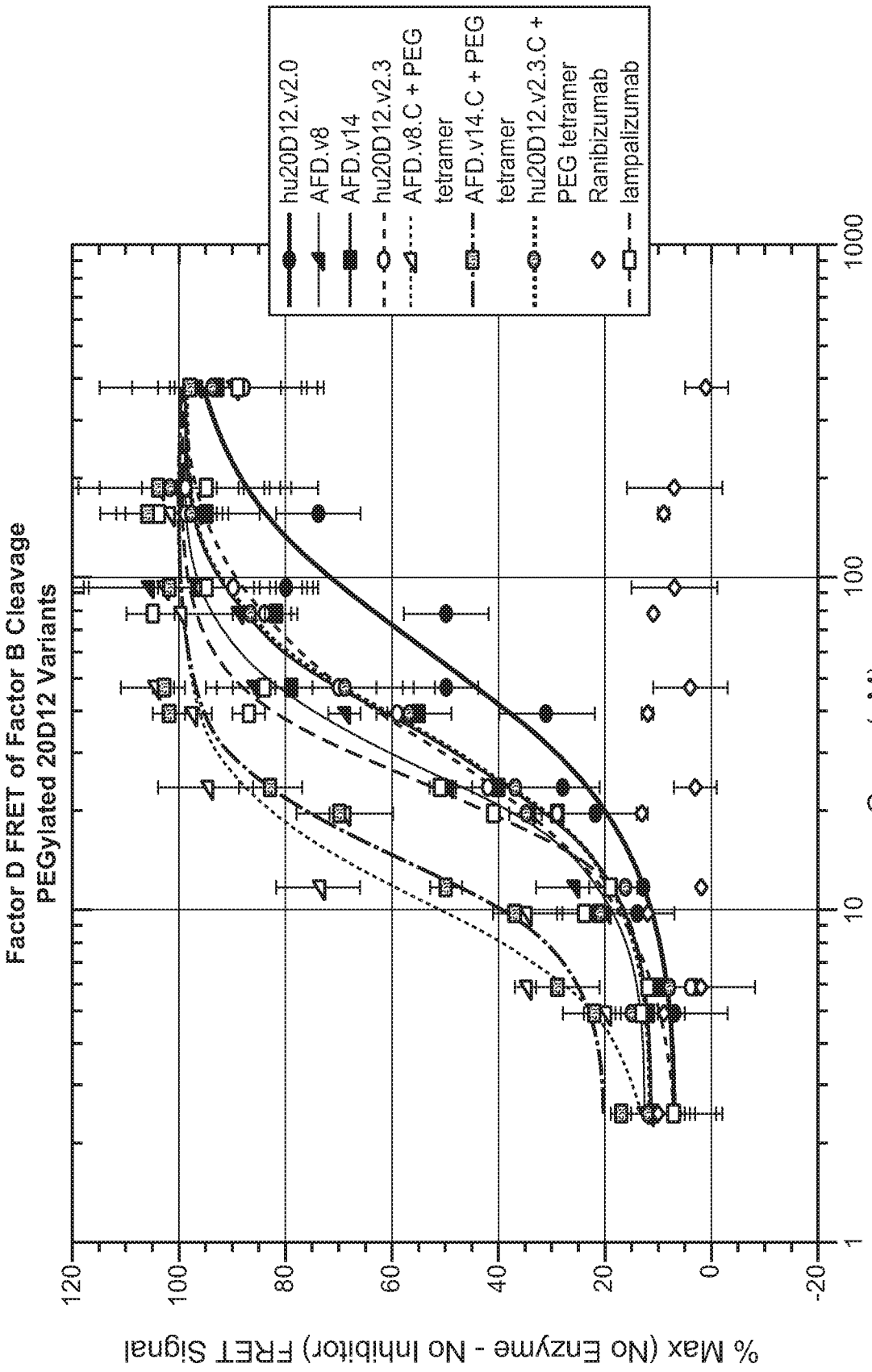
Factor D FRET of Factor B Cleavage
PEGylated 20D12 Variants
hu20D12.v2.0
AFD.v8
AFD.v14
hu20D12.v2.3
AFD.v8.C + PEG tetramer
AFD.v14.C + PEG tetramer
hu20D12.v2.3.C + PEG tetramer
Ranibizumab
lampalizumab
% Max (No Enzyme - No Inhibitor) FRET Signal
120
100
80
60
40
20
0
-20
1
10
100
1000
Conc. (pM)

FIG. 5B

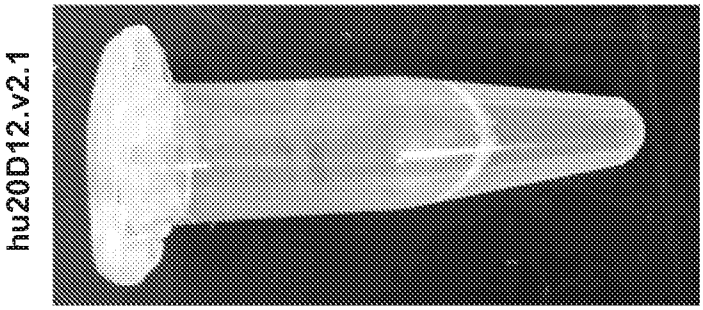

FIG. 6C

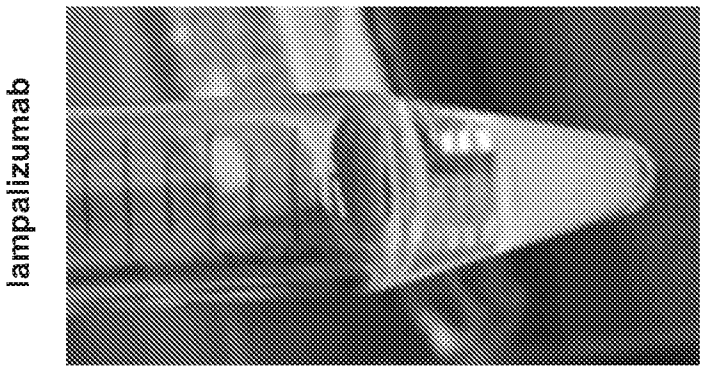

FIG. 6B

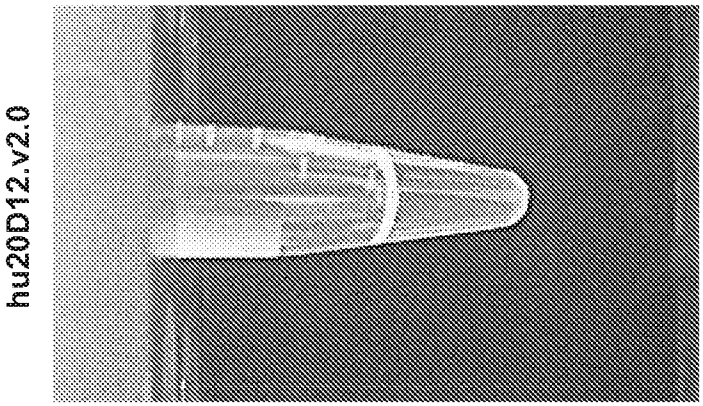

FIG. 6A

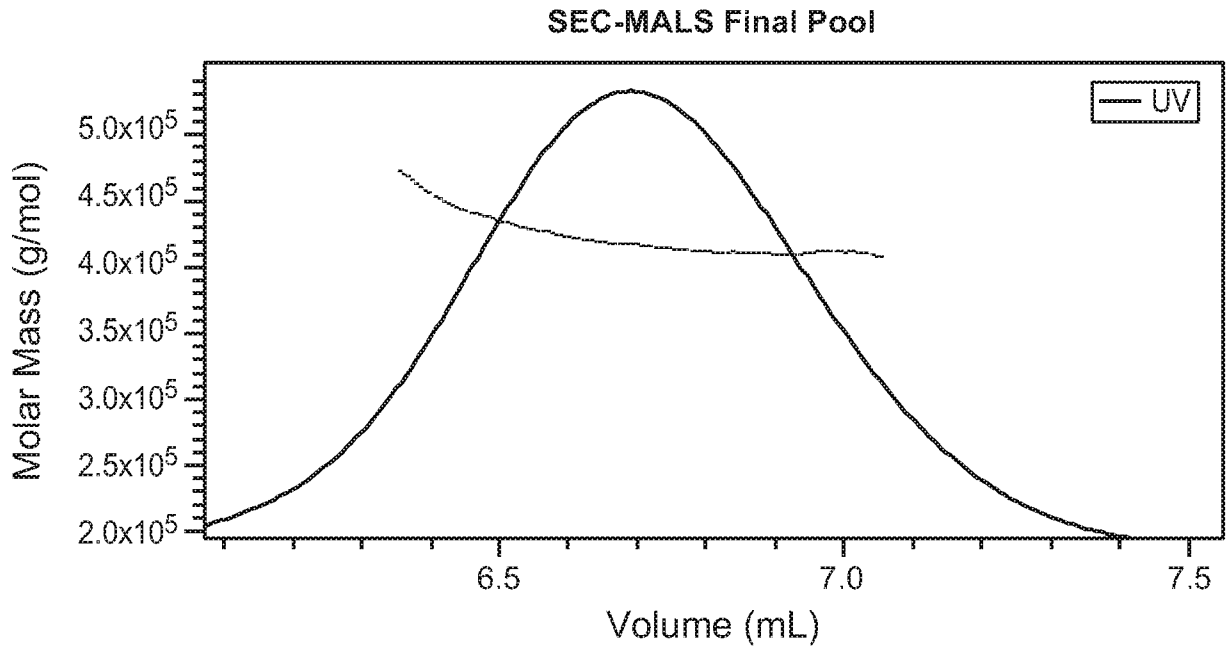

FIG. 7A

	Theoretical Mw (kDa)	Measured Mw (kDa)	Polydispersity (Mw/Mn)	R_h (nm)
V3.2.1	422.1 (±0.2%)	411.7	1.001 (±0.24%)	10.3 (±3.2%)

FIG. 7C

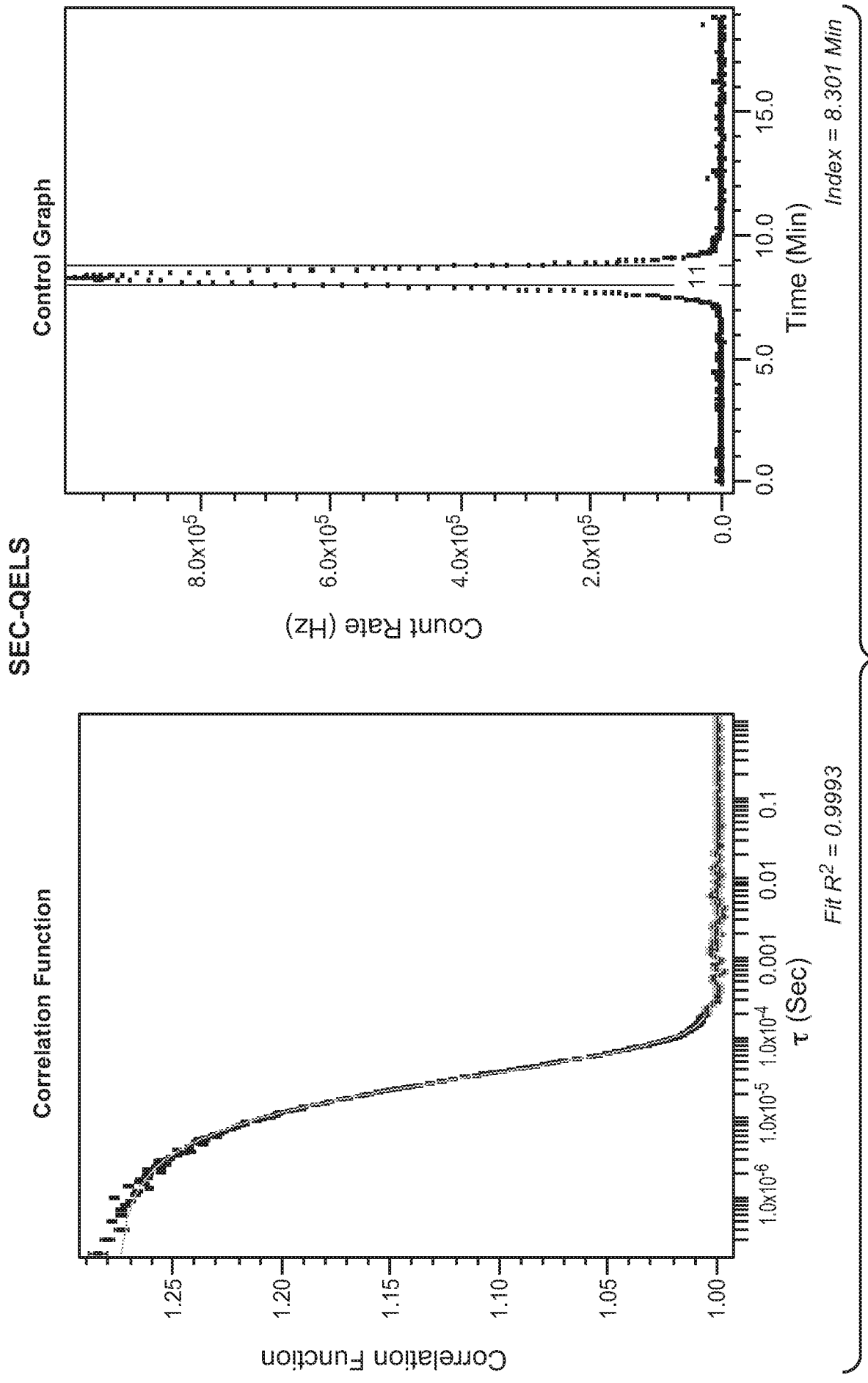
SEC-QELS
Correlation Function
Correlation Function
1.25
1.20
1.15
1.10
1.05
1.00
1.0x10-6
1.0x10-5
1.0x10-4
0.001
0.01
0.1
τ (Sec)
Fit R2 = 0.9993
Control Graph
Count Rate (Hz)
8.0x105
6.0x105
4.0x105
2.0x105
0.0
0.0
5.0
10.0
15.0
Time (Min)
11
Index = 8.301 Min

FIG. 7B

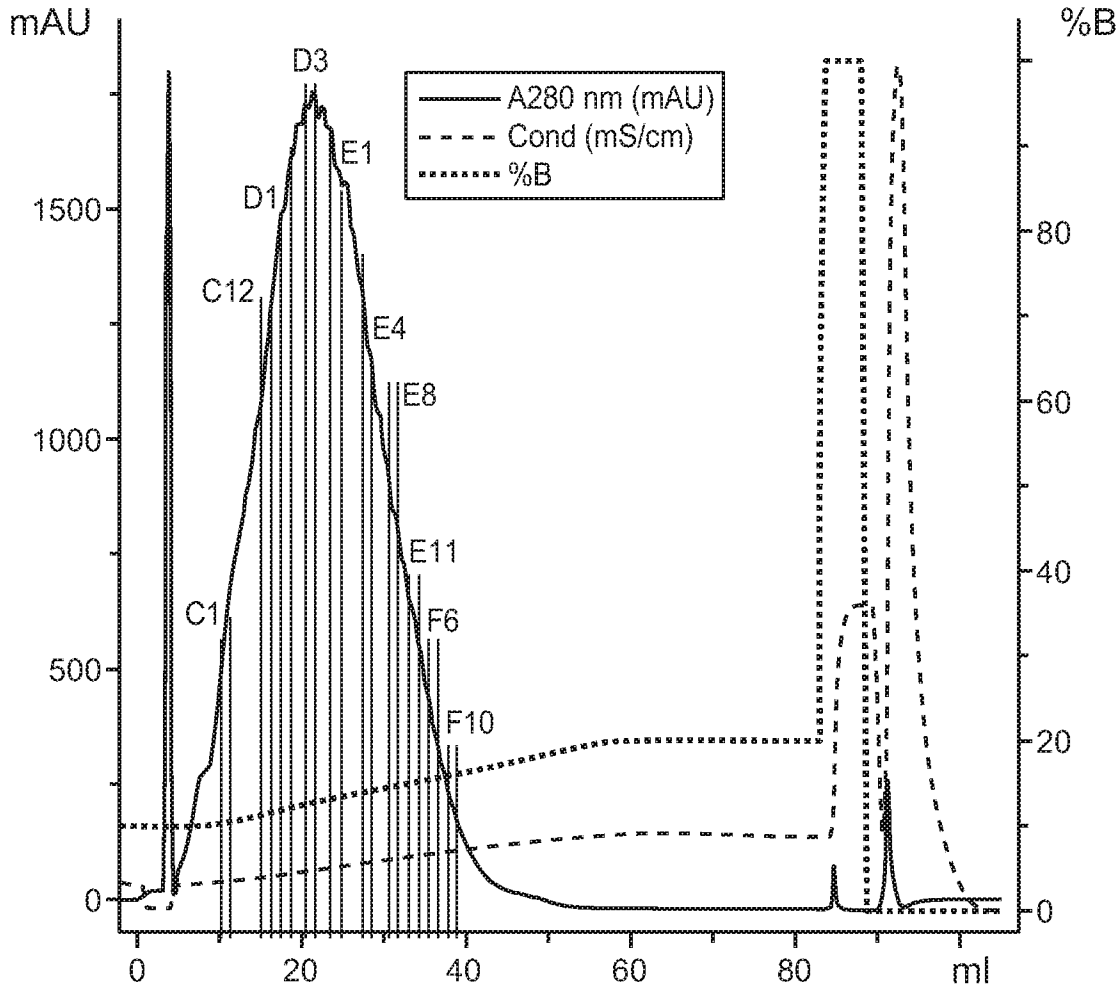
mAU
%B
A280 nm (mAU)
Cond (mS/cm)
%B
D3
E1
D1
C12
E4
E8
E11
C1
F6
F10
1500
1000
500
0
80
60
40
20
0
0
20
40
60
80
ml

FIG. 8A

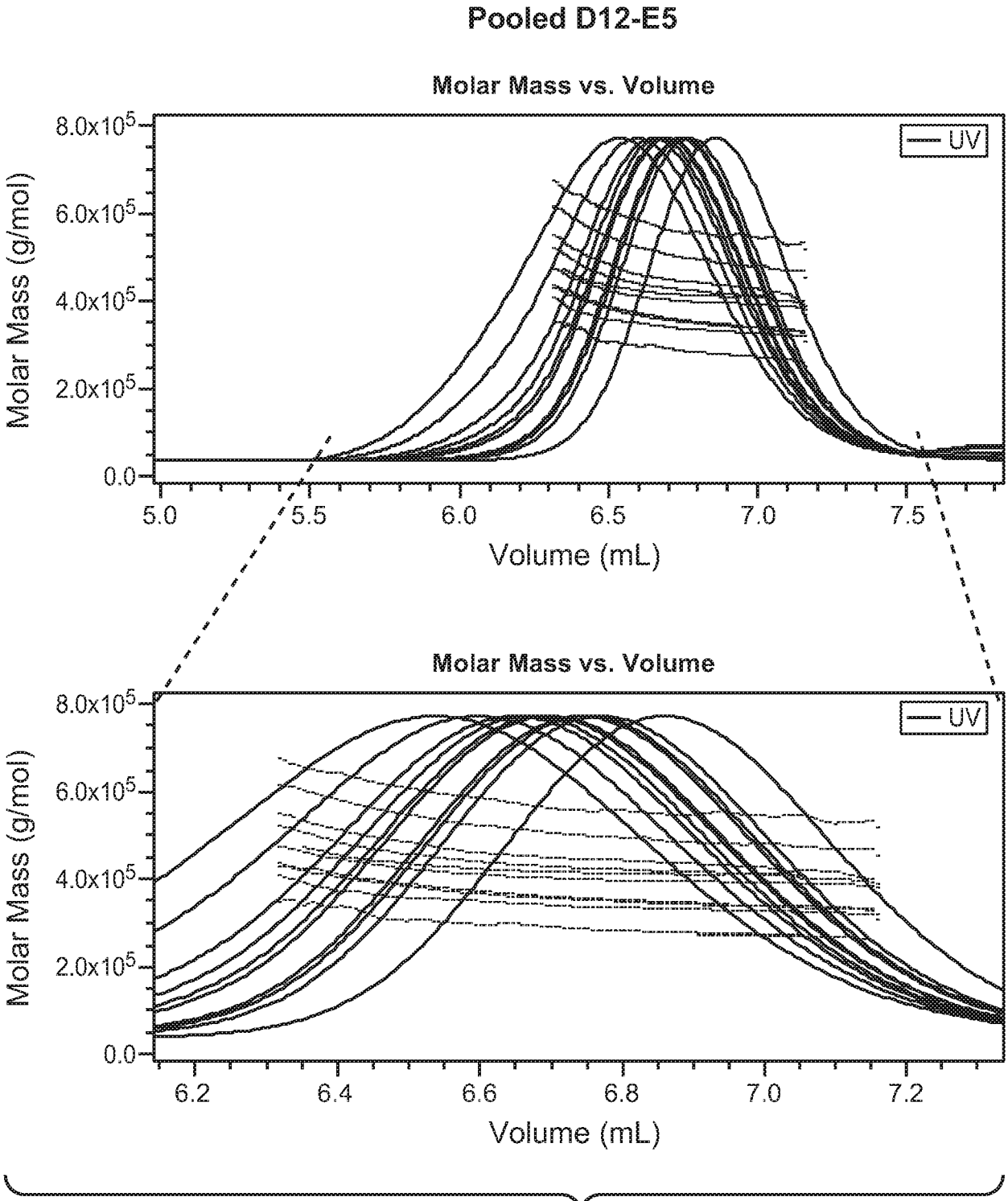
Pooled D12-E5
Molar Mass vs. Volume
Molar Mass (g/mol)
8.0x10^5
6.0x10^5
4.0x10^5
2.0x10^5
0.0
UV
5.0
5.5
6.0
6.5
7.0
7.5
Volume (mL)
Molar Mass vs. Volume
Molar Mass (g/mol)
8.0x10^5
6.0x10^5
4.0x10^5
2.0x10^5
0.0
UV
6.2
6.4
6.6
6.8
7.0
7.2
Volume (mL)

FIG. 8B

Fraction #	Mw (kDa)	Polydispersity (Mw/Mn)	R_h (nm)
CI	283.3 (±0.5%)	1.002	8.3
C12	339.8 (±0.2%)	1.002	9.4
DI	356.8 (±0.2%)	1.003	9.6
D3	361.5 (±0.2%)	1.003	9.7
E1	407.8 (±0.2%)	1.002	10.1
E4	421.8 (±0.2%)	1.002	10.2
E8	437.0 (±0.2%)	1.003	10.4
E11	460.0 (±0.2%)	1.004	10.5
F6	523.2 (±0.2%)	1.005	10.9
F10	587.5 (±0.2%)	1.004	11.2

FIG. 8C

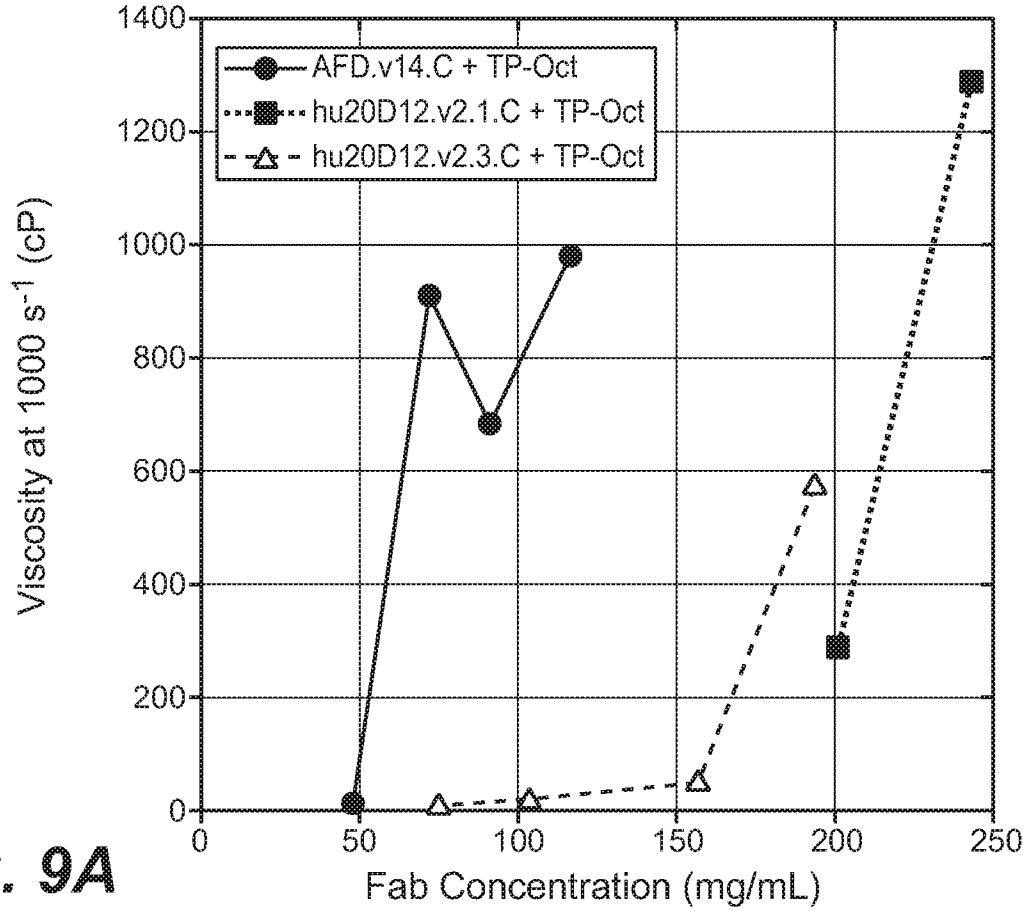
AFD.v14.C + TP-Oct
hu20D12.v2.1.C + TP-Oct
hu20D12.v2.3.C + TP-Oct
Viscosity at 1000 s-1 (cP)
Fab Concentration (mg/mL)
0
200
400
600
800
1000
1200
1400
0
50
100
150
200
250

FIG. 9A

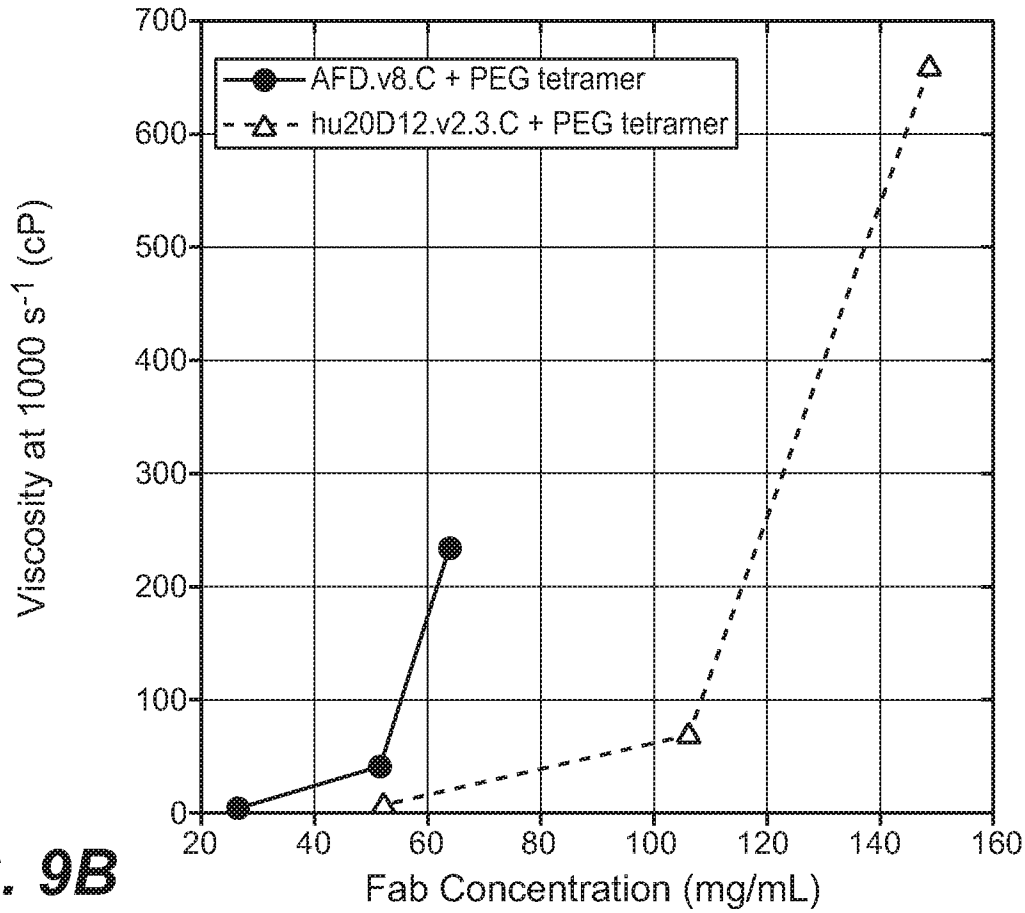
AFD.v8.C + PEG tetramer
hu20D12.v2.3.C + PEG tetramer
Viscosity at 1000 s-1 (cP)
Fab Concentration (mg/mL)
0
100
200
300
400
500
600
700
20
40
60
80
100
120
140
160

FIG. 9B

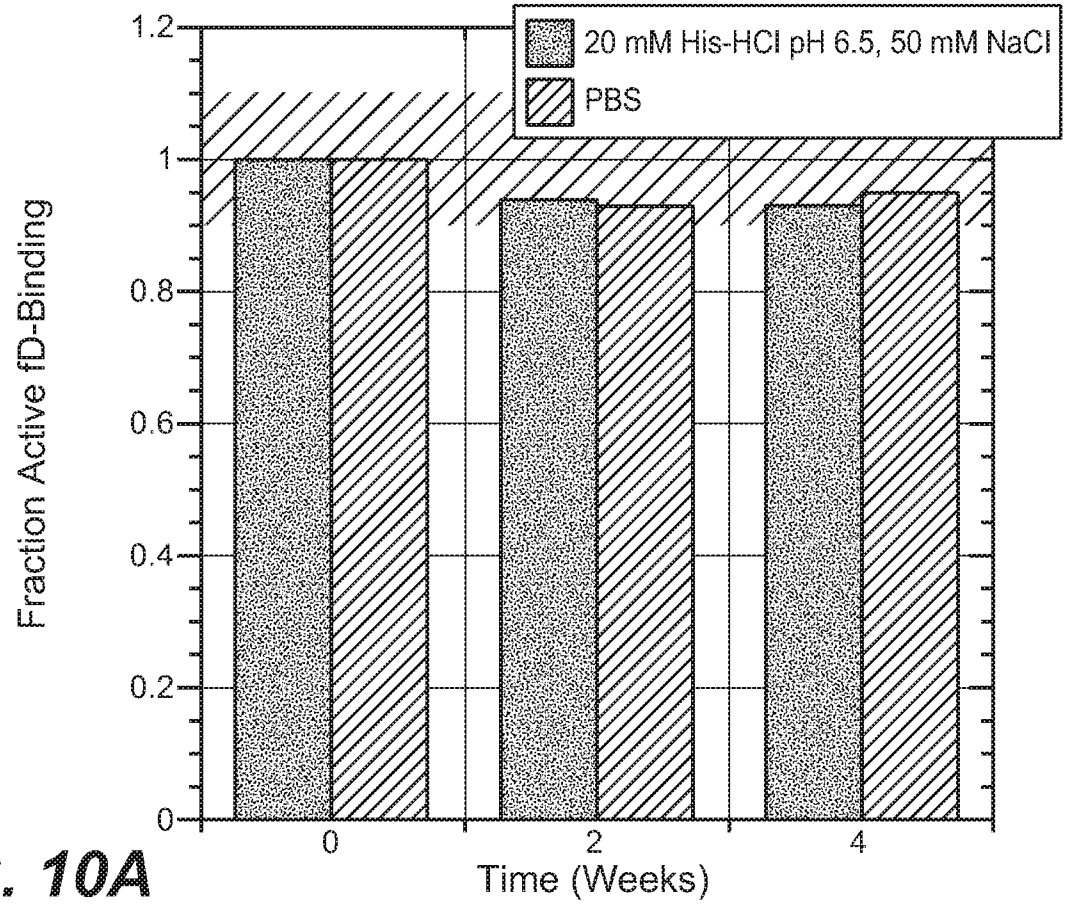

FIG. 10A

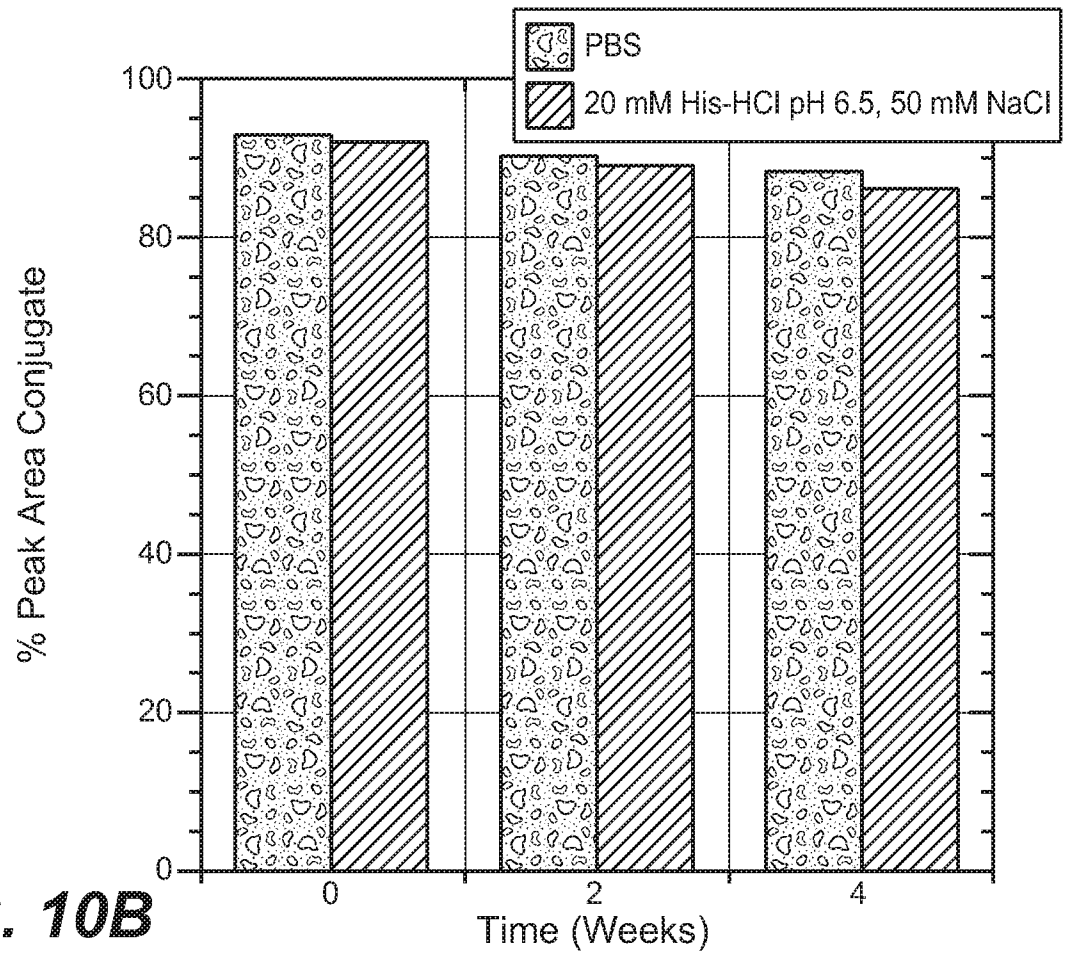

FIG. 10B

Antibody	Name	On-Rate ($M^{-1}s^{-1}$)	Off-Rate (s^{-1})	KD (pM) hFD (Human)	KD (pM) cFD (cyno)	FRET IC50 (pM)	Viscosity on PEG-octamer	Viscosity on (Fab; No PEG)	Solubility (PBS pH 7.4)	MA Solubility (PBS pH 7.4)
hu20D12.v1.N54S	hu20D12.v2.0	1.4e7	5.5e-4	39	152	51, 59		12 cP @ 280 mg/ml	Clear @ 292 mg/ml	
hu20D12.v1.N54S.G56D	hu20D12.v2.2	1.5e7	9.5e-4	65						
hu20D12.v1.N54S.G56E	hu20D12.v2.4	1.7e7	4.9e-4	28						
hu20D12.v1.N54S.T53Y	hu20D12.v2.5	1.7e7	7.9e-4	45						
hu20D12.v1.N54S.Y49S	hu20D12.v2.6	2.1e6	3.0e-4	143						
hu20D12.v1.N54S.Y49K	hu20D12.v2.7	1.1e7	5.5e-4	49						
hu20D12.v1.N54S.Y49Q	hu20D12.v2.8	6.6e7	4e-3	61						
hu20D12.v1.N54S.Y49R	hu20D12.v2.1	1.2e7	8.1e-5	<10(6.8)	17	33, 32	288 cP @ 201 mg/ml	7.1 cP @ 177 mg/ml	Clear @ 260 mg/ml	0.443 OD @ 150 mg/ml
hu20D12.v1.N54S.E50W	hu20D12.v2.9	3.4e7	6.3e-3	185						
hu20D12.v1.N54S.Y55K	hu20D12.v2.10	5e6	3e-2	6000						
hu20D12.v1.N54S.Y55R	hu20D12.v2.11	3e6	3e-2	10000						
hu20D12.v1.N54S.Y49R.T53Y	hu20D12.v2.12	1.8e7	1.5e-4	<10(8.3)						
hu20D12.v1.N54S.N92E.Y49R	hu20D12.v2.13	4.1e7	1.3e-4	<10(3.1)						
hu20D12.v1.N54S.Y49R.G56E	hu20D12.v2.14	1.5e7	8e-5	<10(5.3)	50	25				
hu20D12.v1.N54S.Y49R.T53Y.G56E	hu20D12.v2.15	1.9e7	6e-5	<10(3.2)	27	24				
hu20D12.v1. N54S, Y49R, T53Y, G56D	hu20D12.v2.3	1.2e7	2.9e-5	<10(2.4)	24	25, 31, 31	577 cP @194 mg/ml			
AFD.v8		2.0e7	2.1e-4	10.5	24	21, 28		14.4 cP @ 261 mg/ml		
AFD.v14		0.9e7	2.1e-4	23.3	56	25, 34, 26	979 cP @ 117 mg/ml	21 cP @ 240 mg/ml		
lampalizumab		8.2e7	3.3e-4	<10(4.0)	30	24		14.8 cP @ 264 mg/ml	Precipitates @ 227 mg/ml	Precipitates @ 150 mg/ml; no OD value

FIG. 11

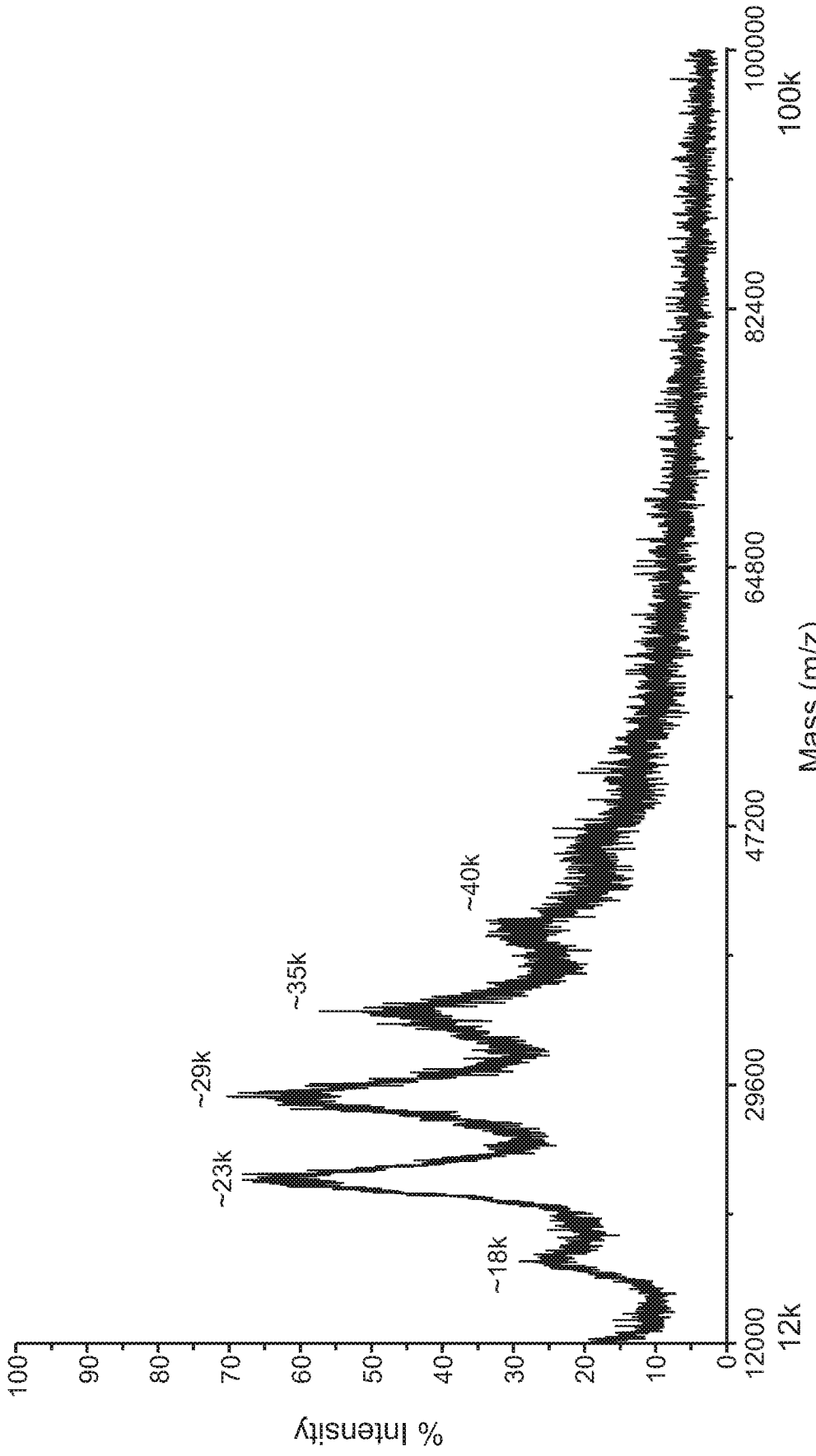
% Intensity
100
90
80
70
60
50
40
30
20
10
0
12000
12k
29600
47200
64800
82400
100000
100k
Mass (m/z)
~18k
~23k
~29k
~35k
~40k

FIG. 12A

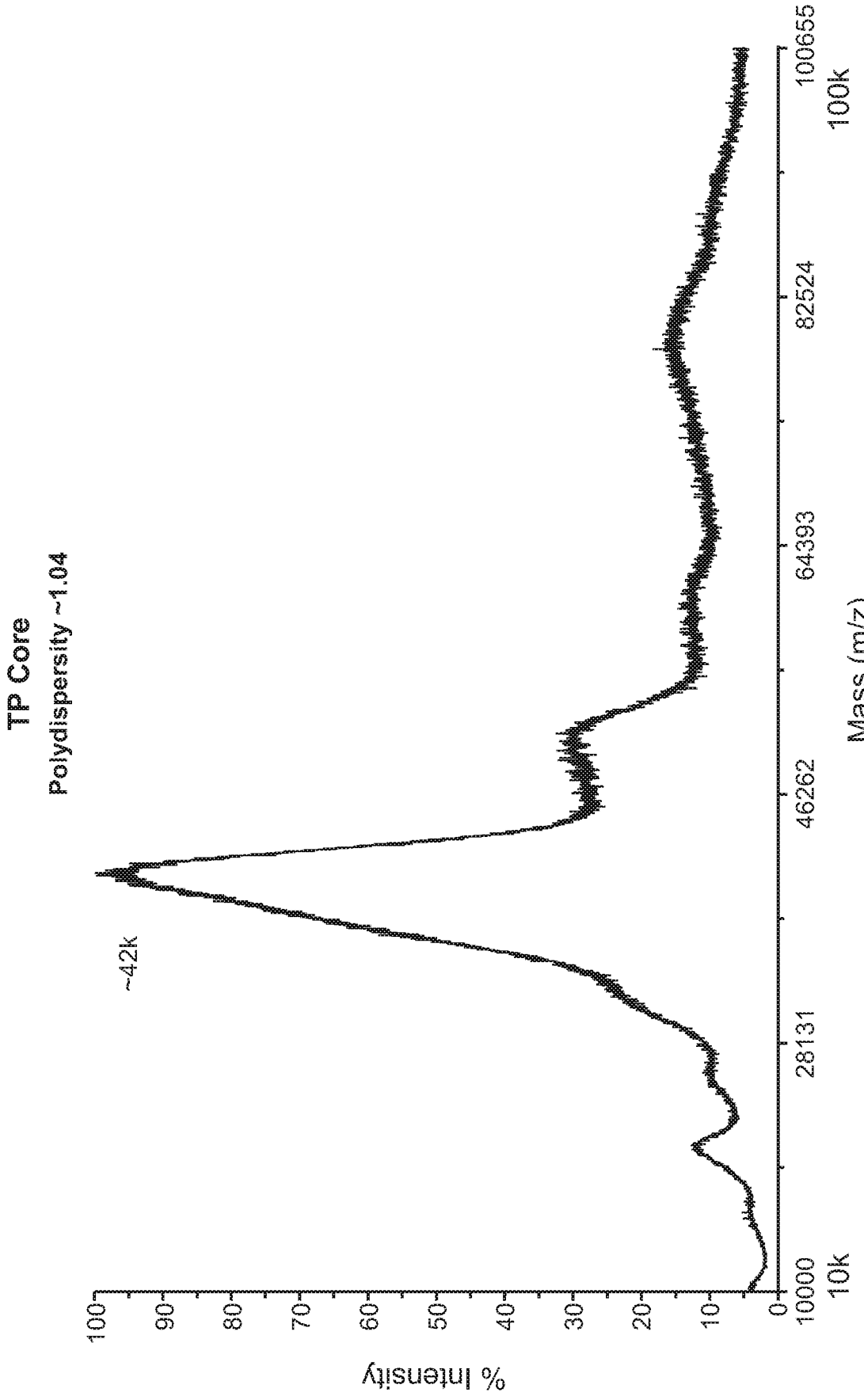
TP Core
Polydispersity ~1.04
~42k
% Intensity
100
90
80
70
60
50
40
30
20
10
0
10000
10k
28131
46262
64393
82524
100655
100k
Mass (m/z)

FIG. 12B

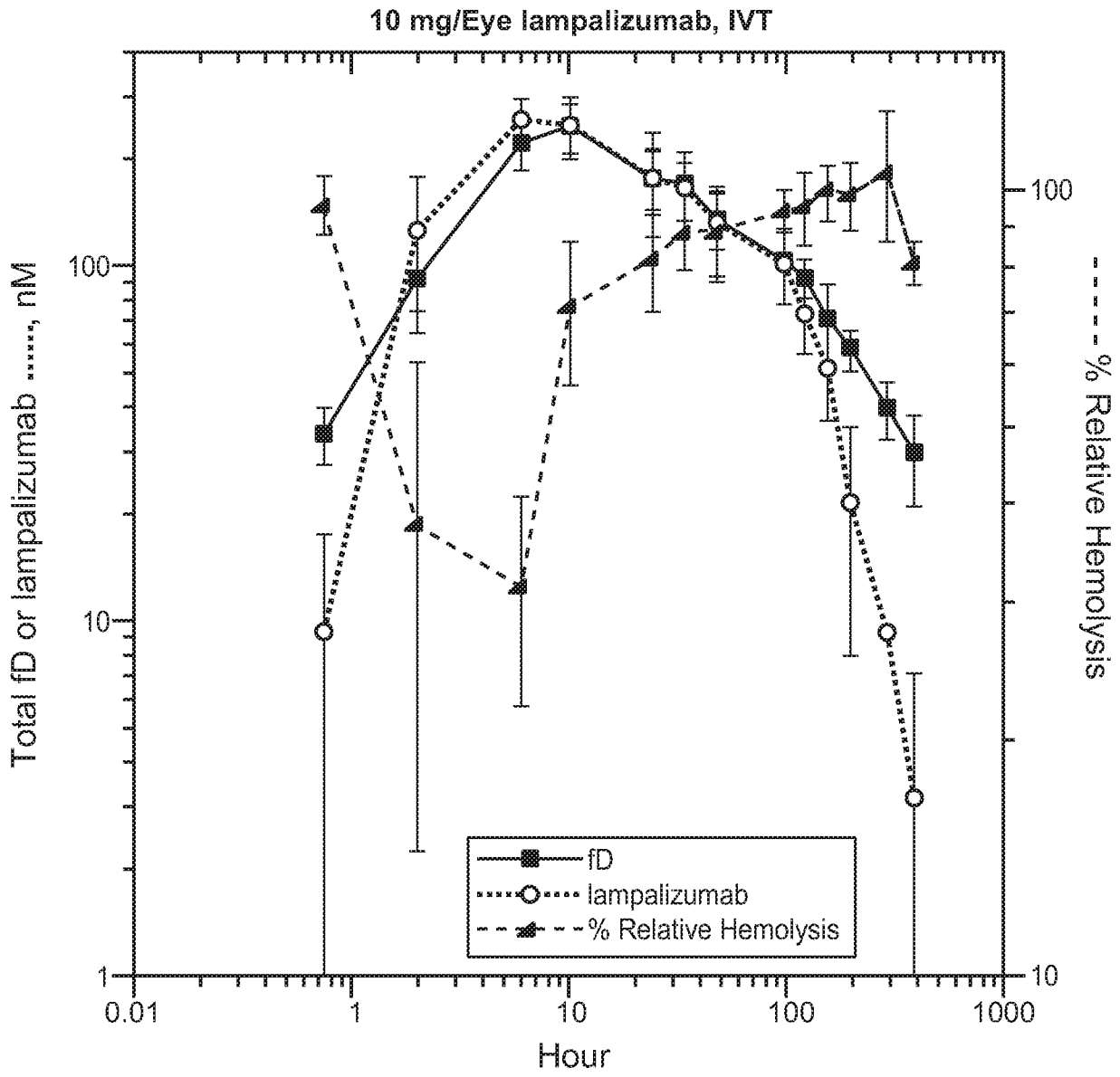
10 mg/Eye lampalizumab, IVT
Total fD or lampalizumab ……, nM
100
10
1
0.01
1
10
100
1000
Hour
100
10
- - - - - % Relative Hemolysis
fD
lampalizumab
% Relative Hemolysis

FIG. 13A

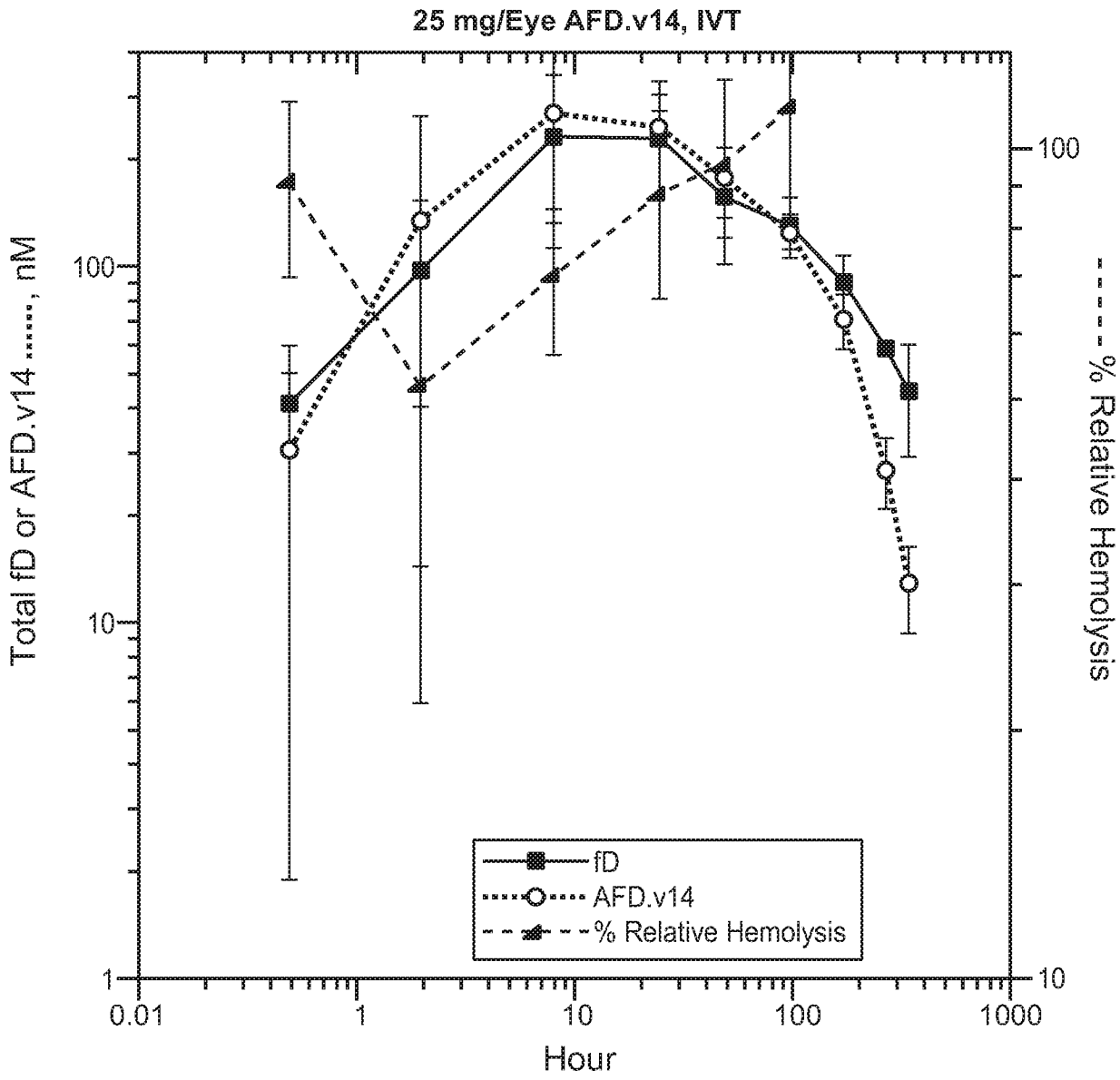
25 mg/Eye AFD.v14, IVT
Total fD or AFD.v14 ....., nM
% Relative Hemolysis
Hour
100
10
1
0.01
1
10
100
1000
fD
AFD.v14
% Relative Hemolysis

FIG. 13B

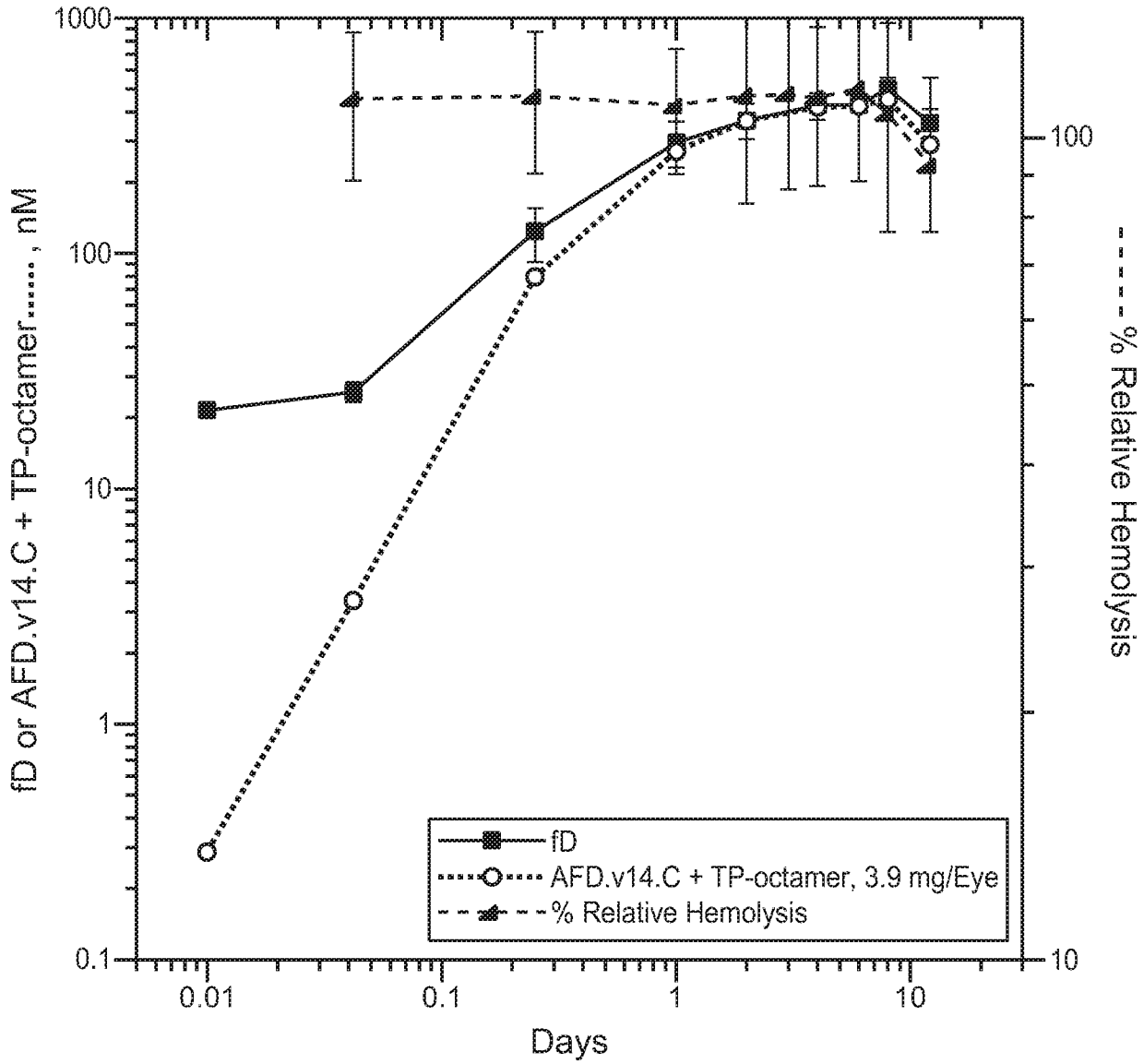
1000
100
10
1
0.1
fD or AFD.v14.C + TP-octamer..... , nM
100
10
0.01
0.1
1
10
Days
- - - - - % Relative Hemolysis
fD
AFD.v14.C + TP-octamer, 3.9 mg/Eye
% Relative Hemolysis

FIG. 14A

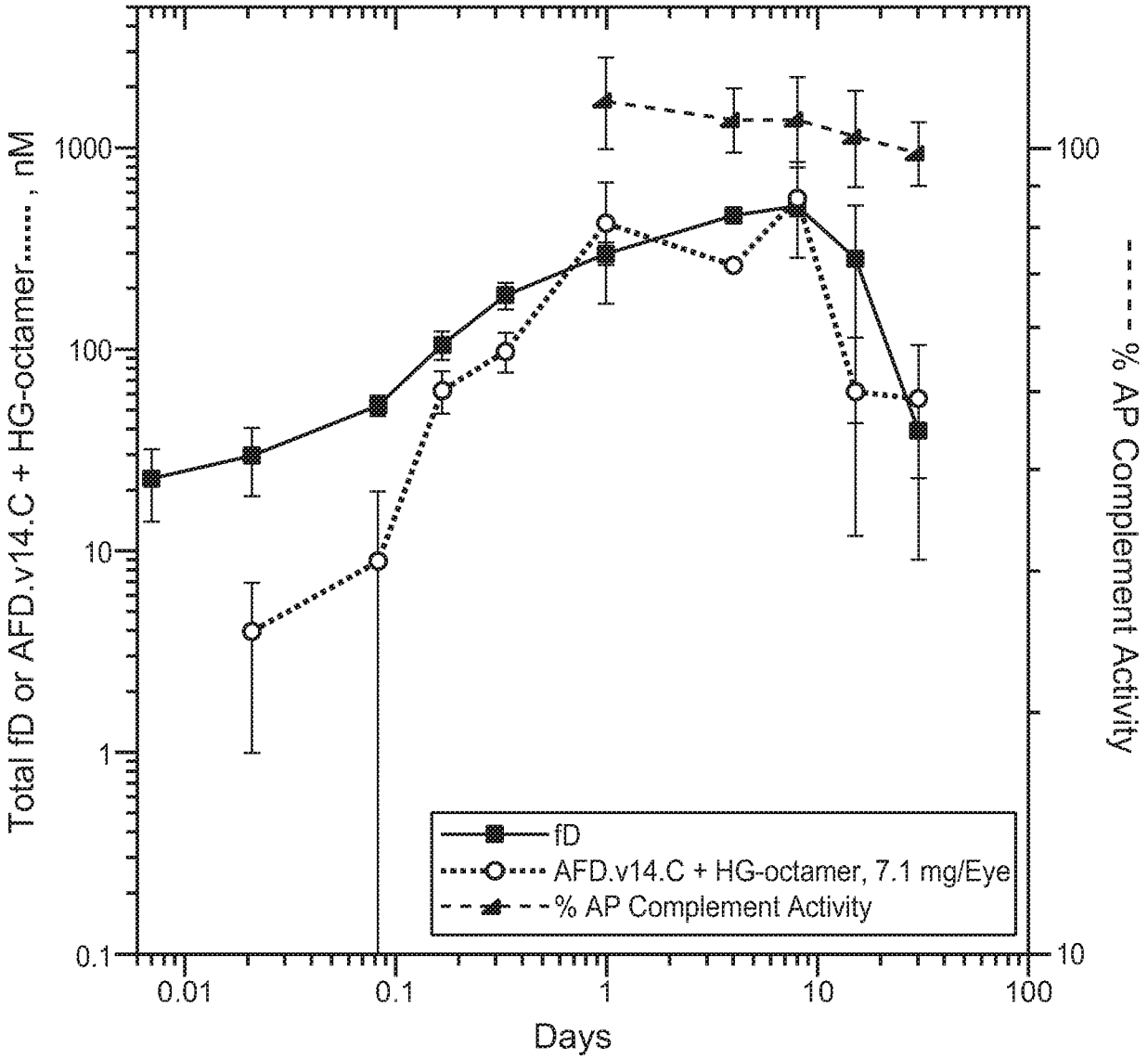
Total fD or AFD.v14.C + HG-octamer..... , nM
1000
100
10
1
0.1
0.01
0.1
1
10
100
Days
100
10
----- % AP Complement Activity
fD
AFD.v14.C + HG-octamer, 7.1 mg/Eye
% AP Complement Activity

FIG. 14B

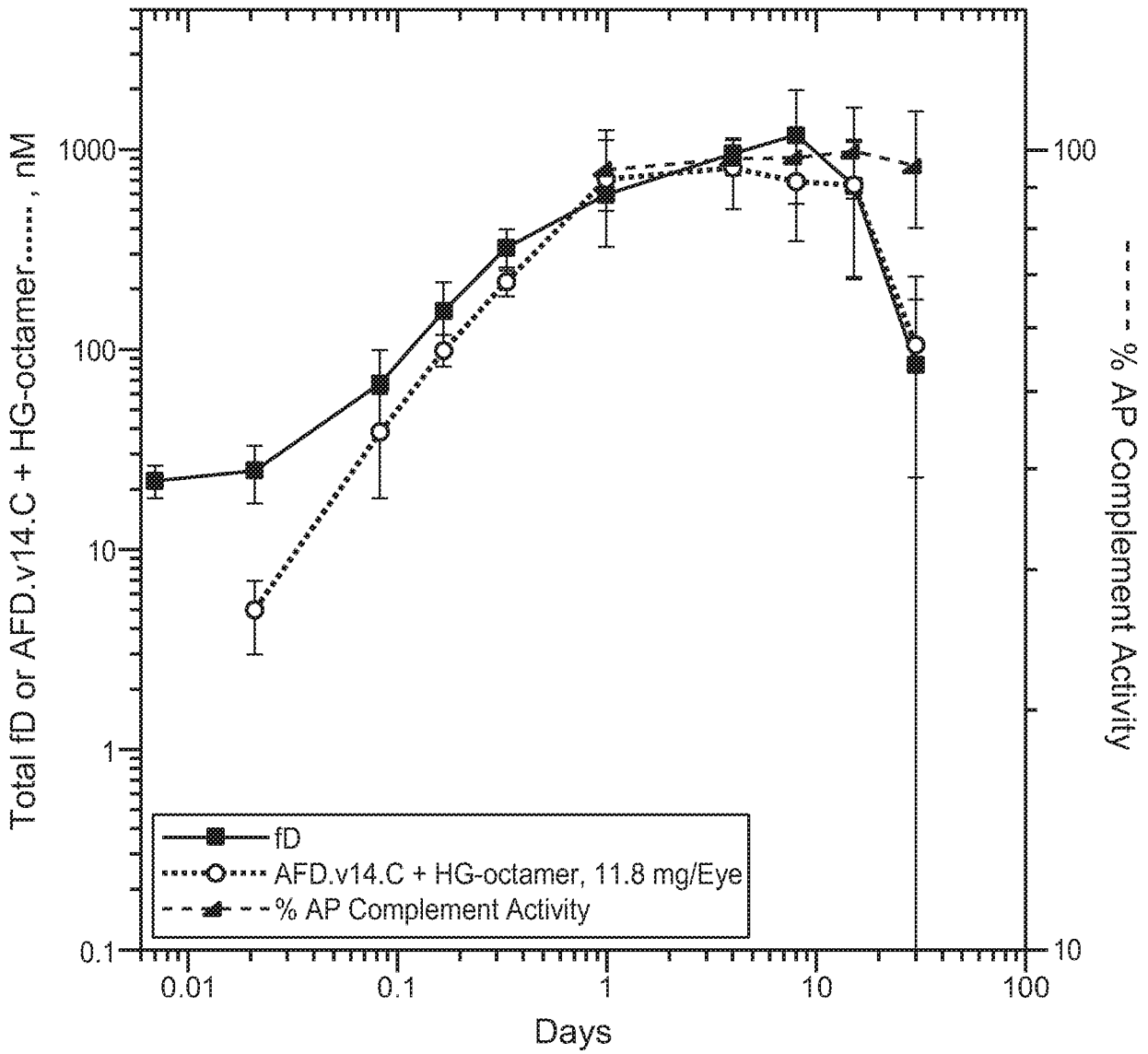
Total fD or AFD.v14.C + HG-octamer..... , nM
1000
100
10
1
0.1
0.01
0.1
1
10
100
Days
100
10
----- % AP Complement Activity
fD
AFD.v14.C + HG-octamer, 11.8 mg/Eye
% AP Complement Activity

FIG. 14C

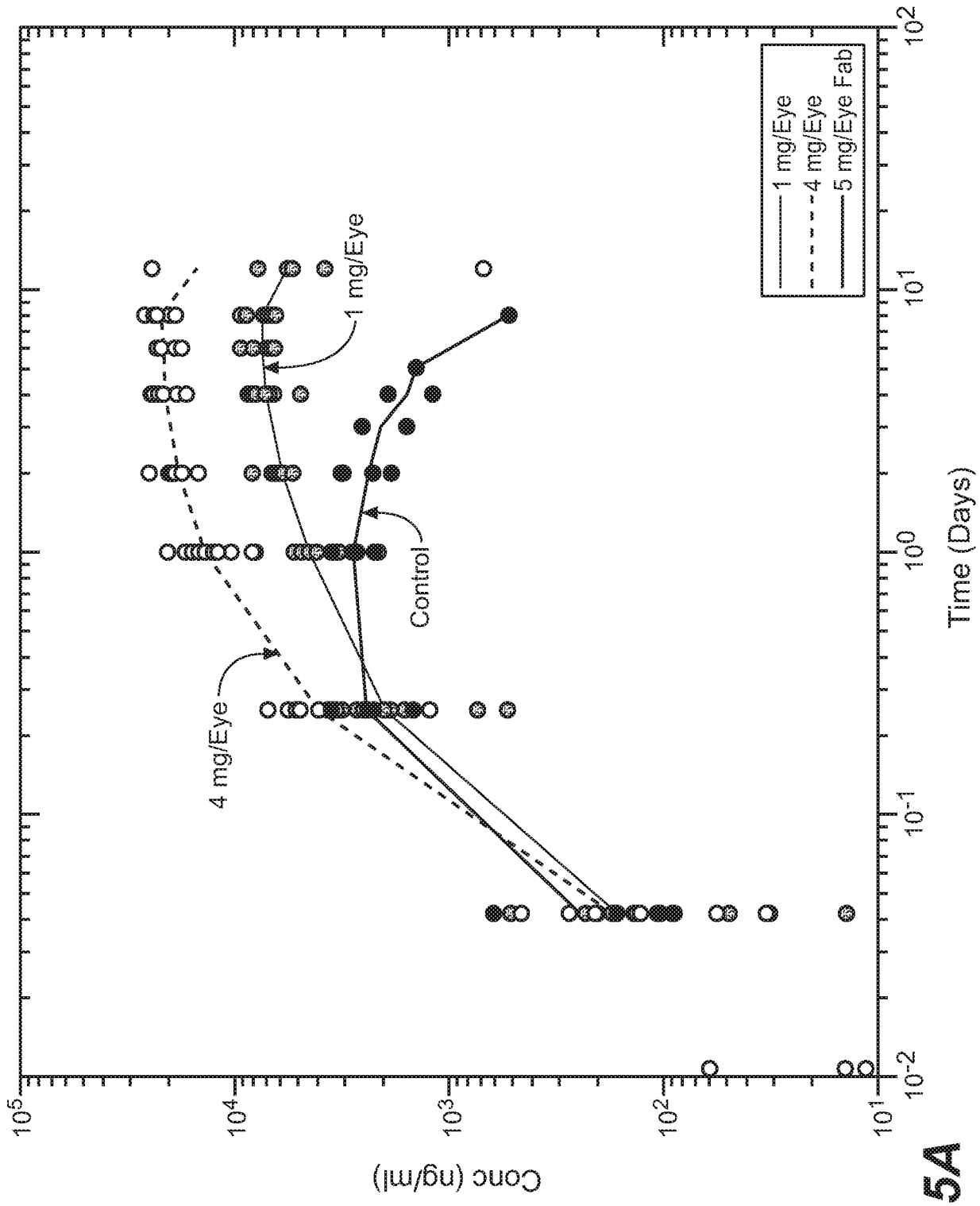
Conc (ng/ml)
Time (Days)
1 mg/Eye
4 mg/Eye
5 mg/Eye Fab
1 mg/Eye
Control
4 mg/Eye

FIG. 15A

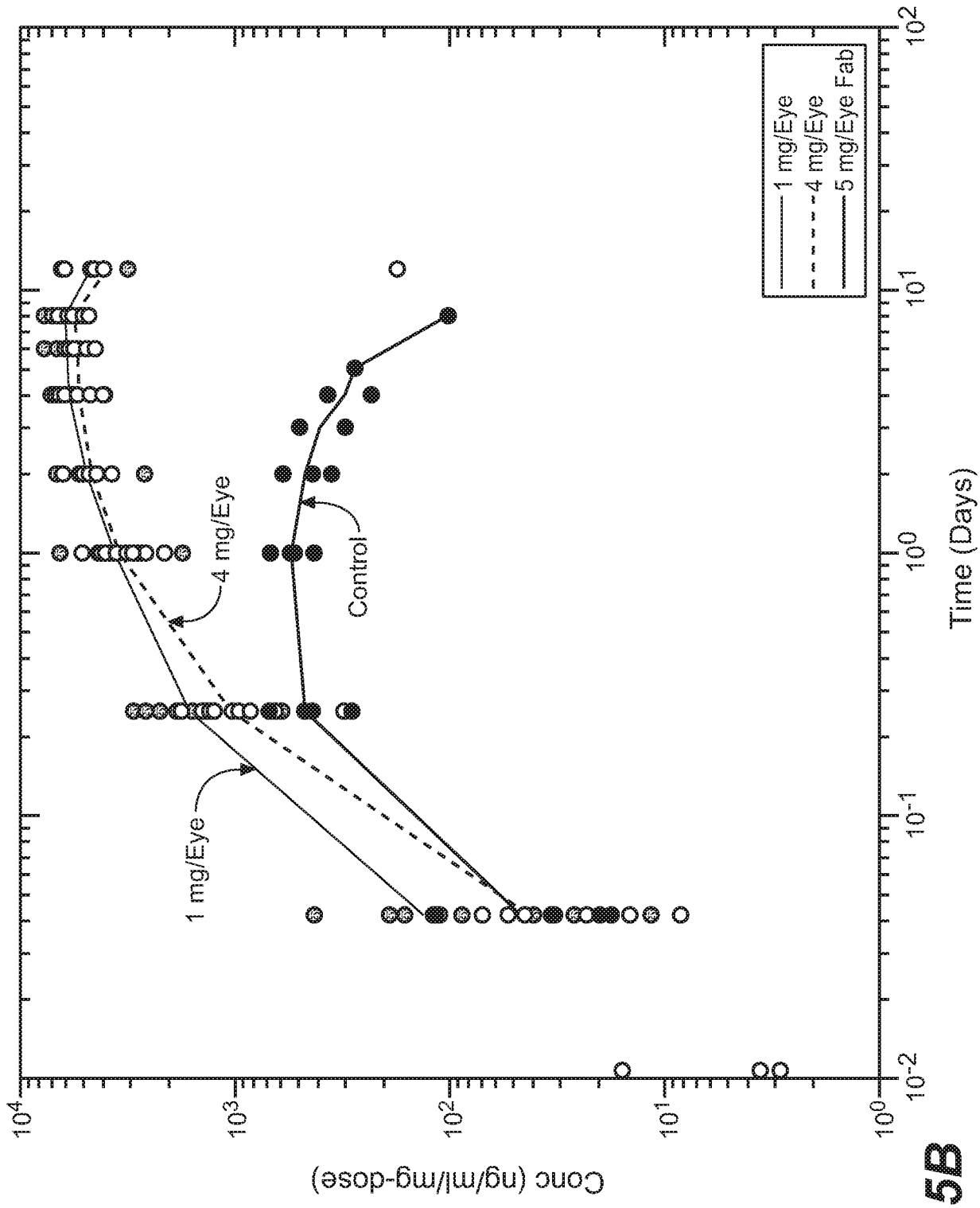
Conc (ng/ml/mg-dose)
Time (Days)
1 mg/Eye
4 mg/Eye
5 mg/Eye Fab
1 mg/Eye
4 mg/Eye
Control

FIG. 15B

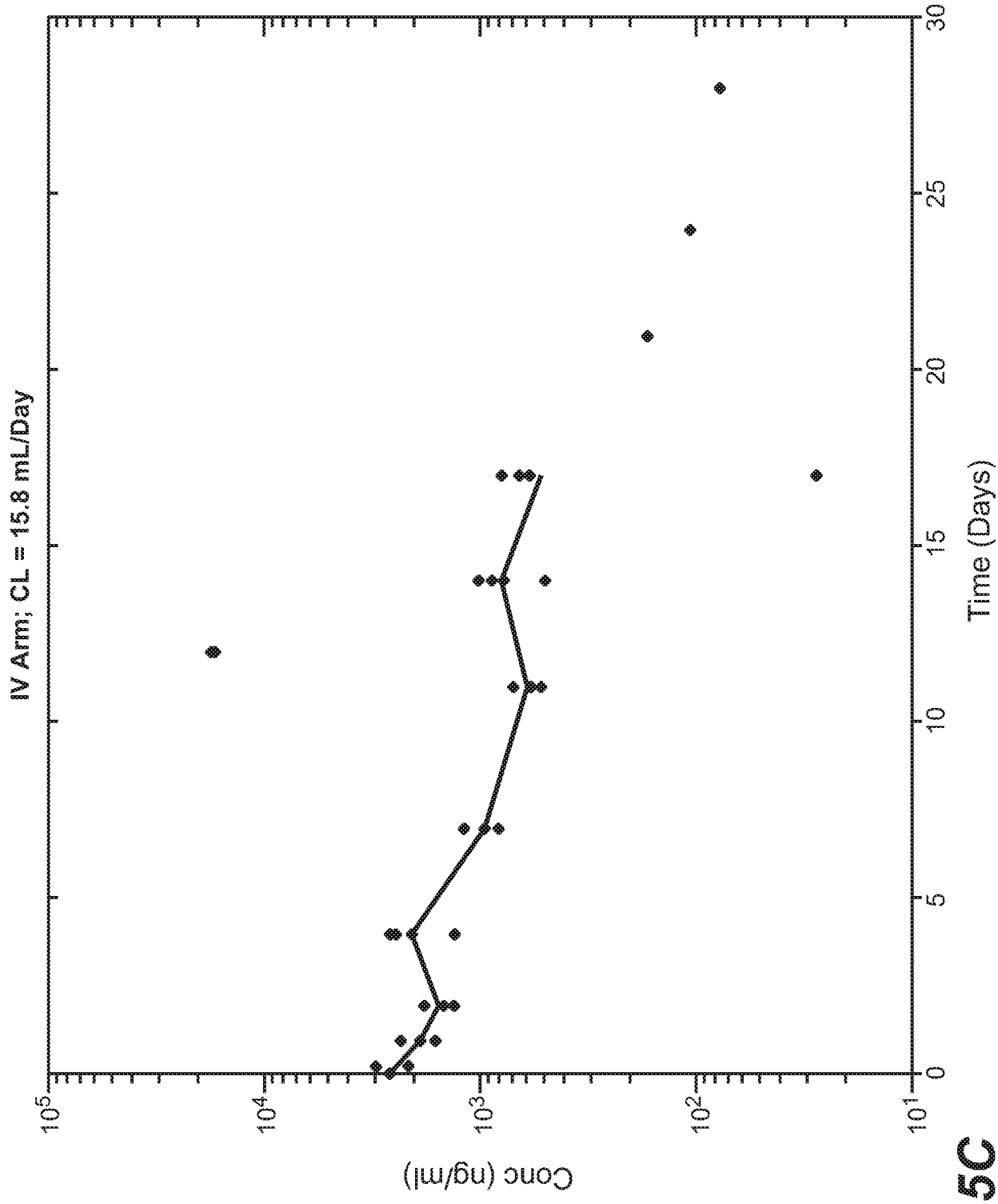
IV Arm; CL = 15.8 mL/Day
Conc (ng/ml)
10^5
10^4
10^3
10^2
10^1
0
5
10
15
20
25
30
Time (Days)

FIG. 15C

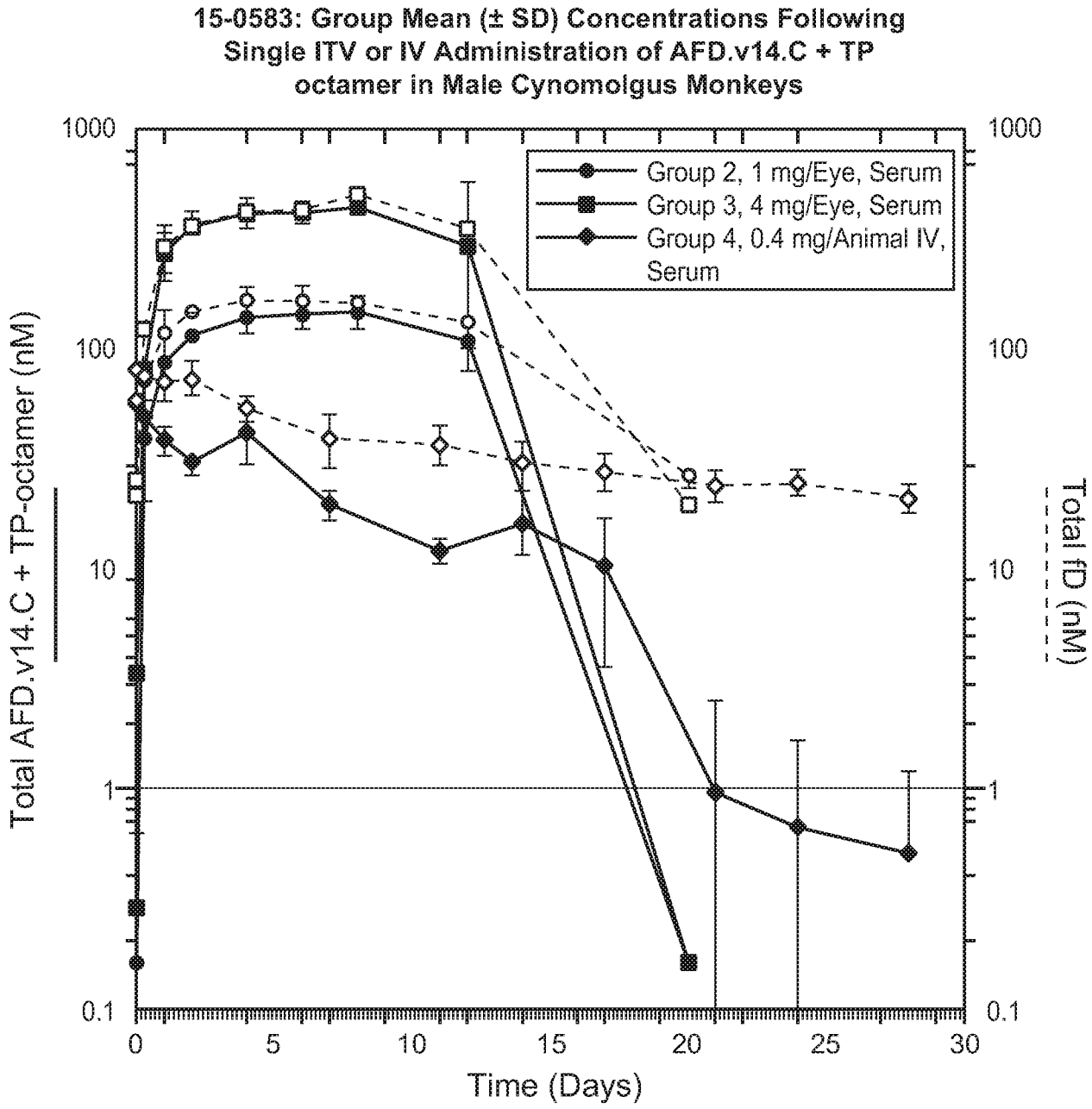
15-0583: Group Mean (± SD) Concentrations Following Single ITV or IV Administration of AFD.v14.C + TP octamer in Male Cynomolgus Monkeys
Group 2, 1 mg/Eye, Serum
Group 3, 4 mg/Eye, Serum
Group 4, 0.4 mg/Animal IV, Serum
Total AFD.v14.C + TP-octamer (nM)
Total fD (nM)
Time (Days)
1000
100
10
1
0.1
0
5
10
15
20
25
30

FIG. 16A

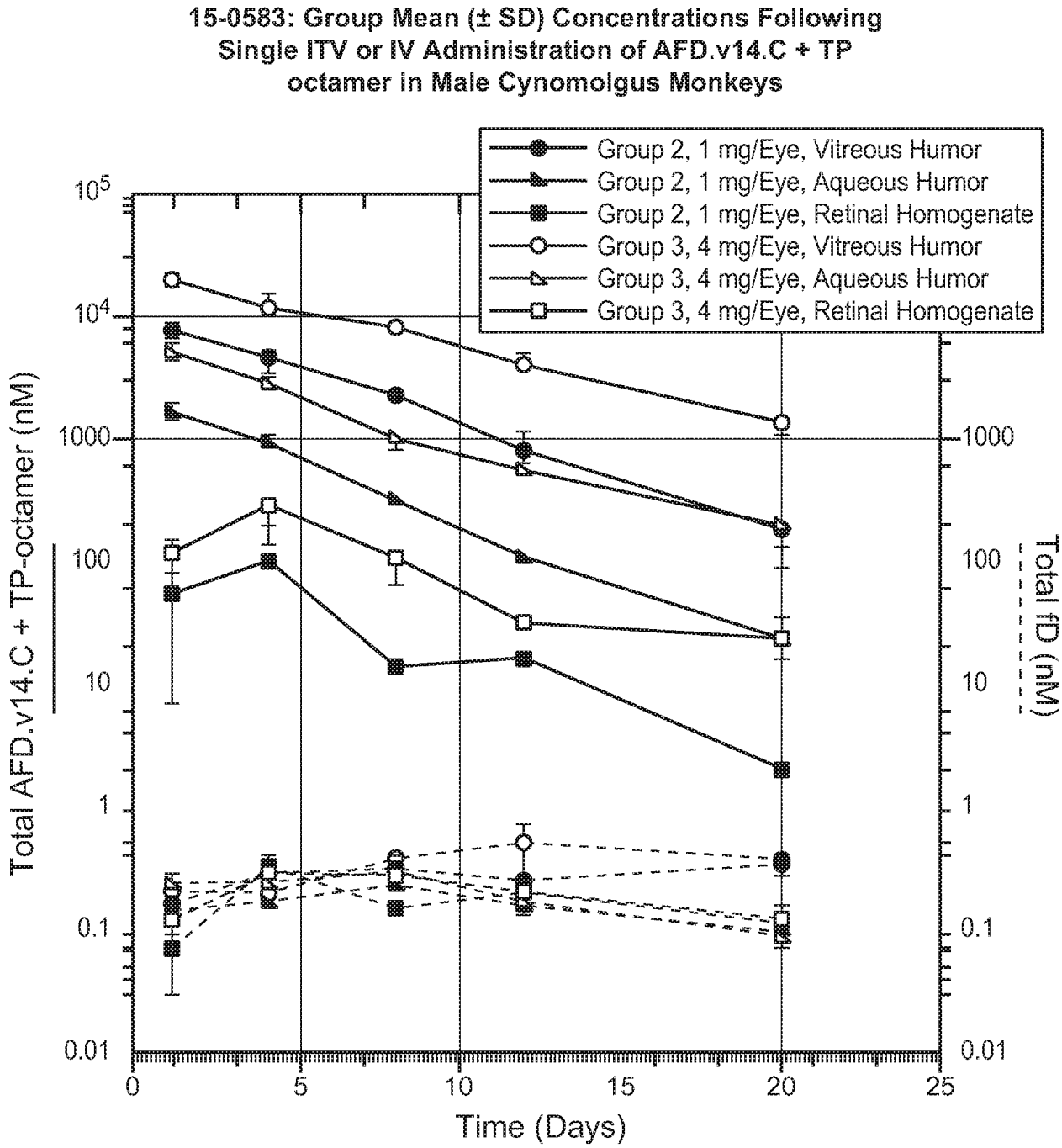
15-0583: Group Mean (± SD) Concentrations Following Single ITV or IV Administration of AFD.v14.C + TP octamer in Male Cynomolgus Monkeys
Group 2, 1 mg/Eye, Vitreous Humor
Group 2, 1 mg/Eye, Aqueous Humor
Group 2, 1 mg/Eye, Retinal Homogenate
Group 3, 4 mg/Eye, Vitreous Humor
Group 3, 4 mg/Eye, Aqueous Humor
Group 3, 4 mg/Eye, Retinal Homogenate
Total AFD.v14.C + TP-octamer (nM)
Total fD (nM)
Time (Days)
10^5
10^4
1000
100
10
1
0.1
0.01
0
5
10
15
20
25

FIG. 16B

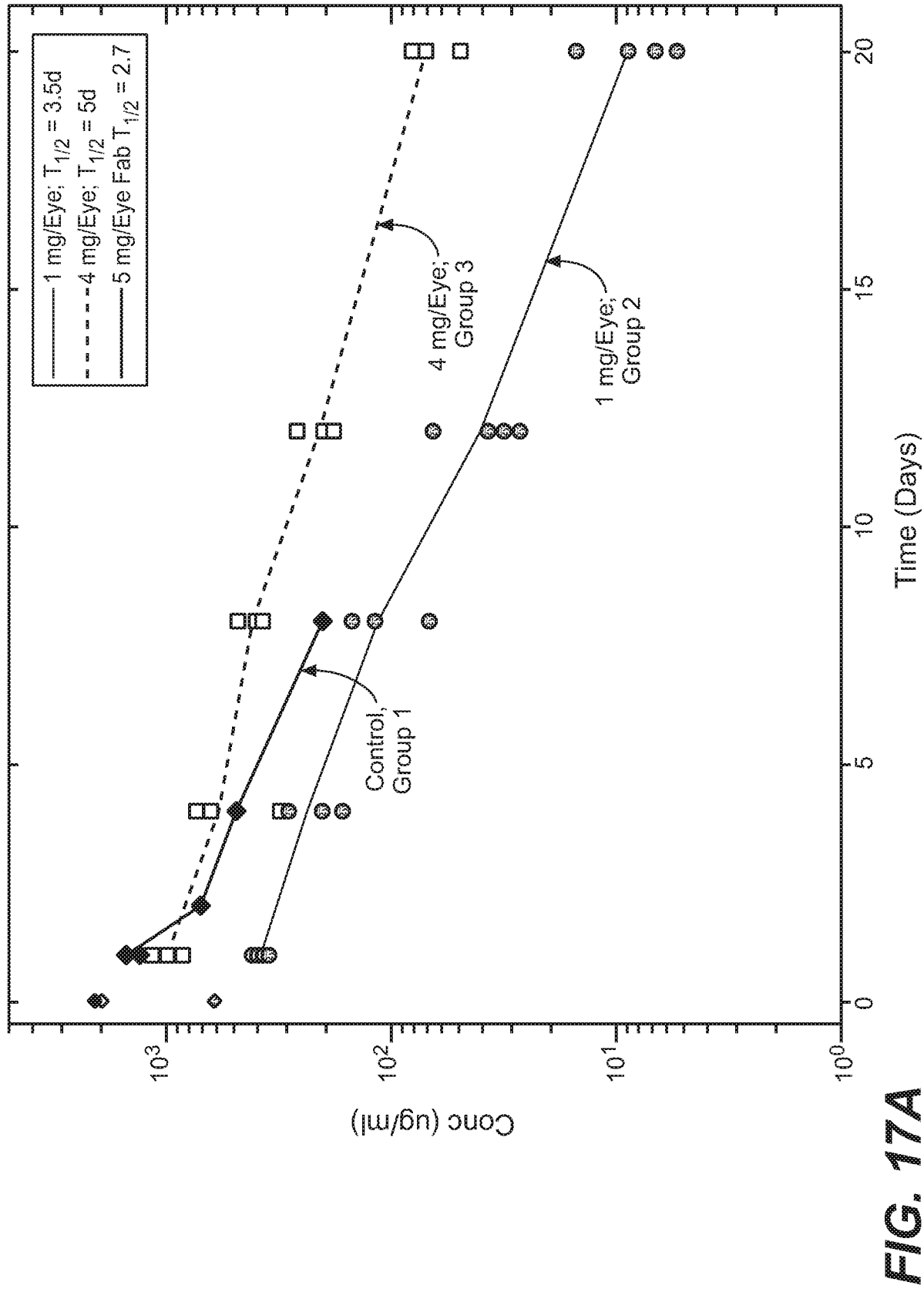
1 mg/Eye; $T_{1/2}$ = 3.5d
4 mg/Eye; $T_{1/2}$ = 5d
5 mg/Eye Fab $T_{1/2}$ = 2.7
4 mg/Eye; Group 3
1 mg/Eye; Group 2
Control, Group 1
Conc (ug/ml)
Time (Days)
10^3
10^2
10^1
10^0
0
5
10
15
20

FIG. 17A

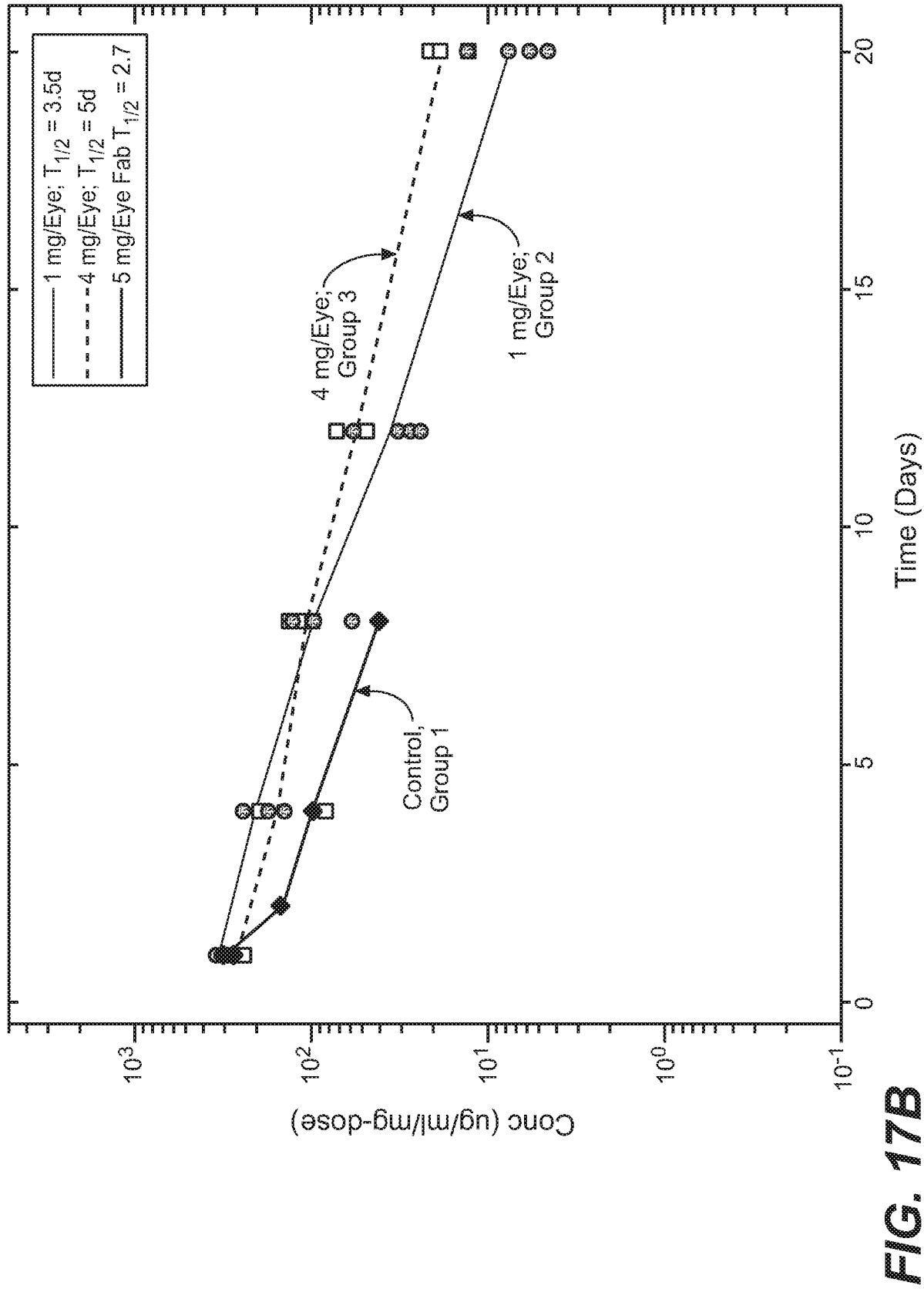
1 mg/Eye; $T_{1/2}$ = 3.5d
4 mg/Eye; $T_{1/2}$ = 5d
5 mg/Eye Fab $T_{1/2}$ = 2.7
4 mg/Eye; Group 3
1 mg/Eye; Group 2
Control, Group 1
Conc (ug/ml/mg-dose)
Time (Days)
10^3
10^2
10^1
10^0
10^{-1}
0
5
10
15
20

FIG. 17B

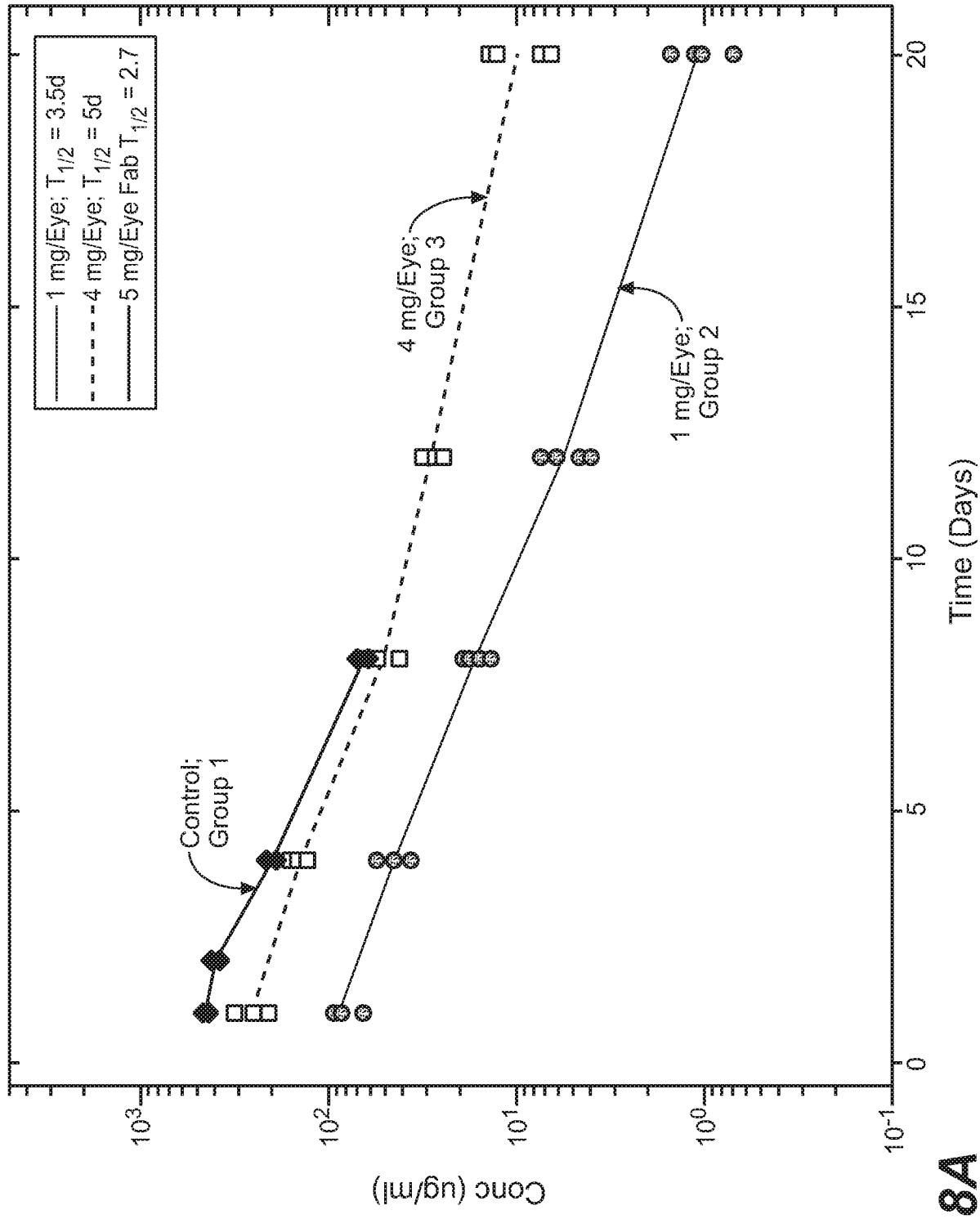
1 mg/Eye; $T_{1/2}$ = 3.5d
4 mg/Eye; $T_{1/2}$ = 5d
5 mg/Eye Fab $T_{1/2}$ = 2.7
Control; Group 1
4 mg/Eye; Group 3
1 mg/Eye; Group 2
Conc (ug/ml)
10^{3}
10^{2}
10^{1}
10^{0}
10^{-1}
0
5
10
15
20
Time (Days)

FIG. 18A

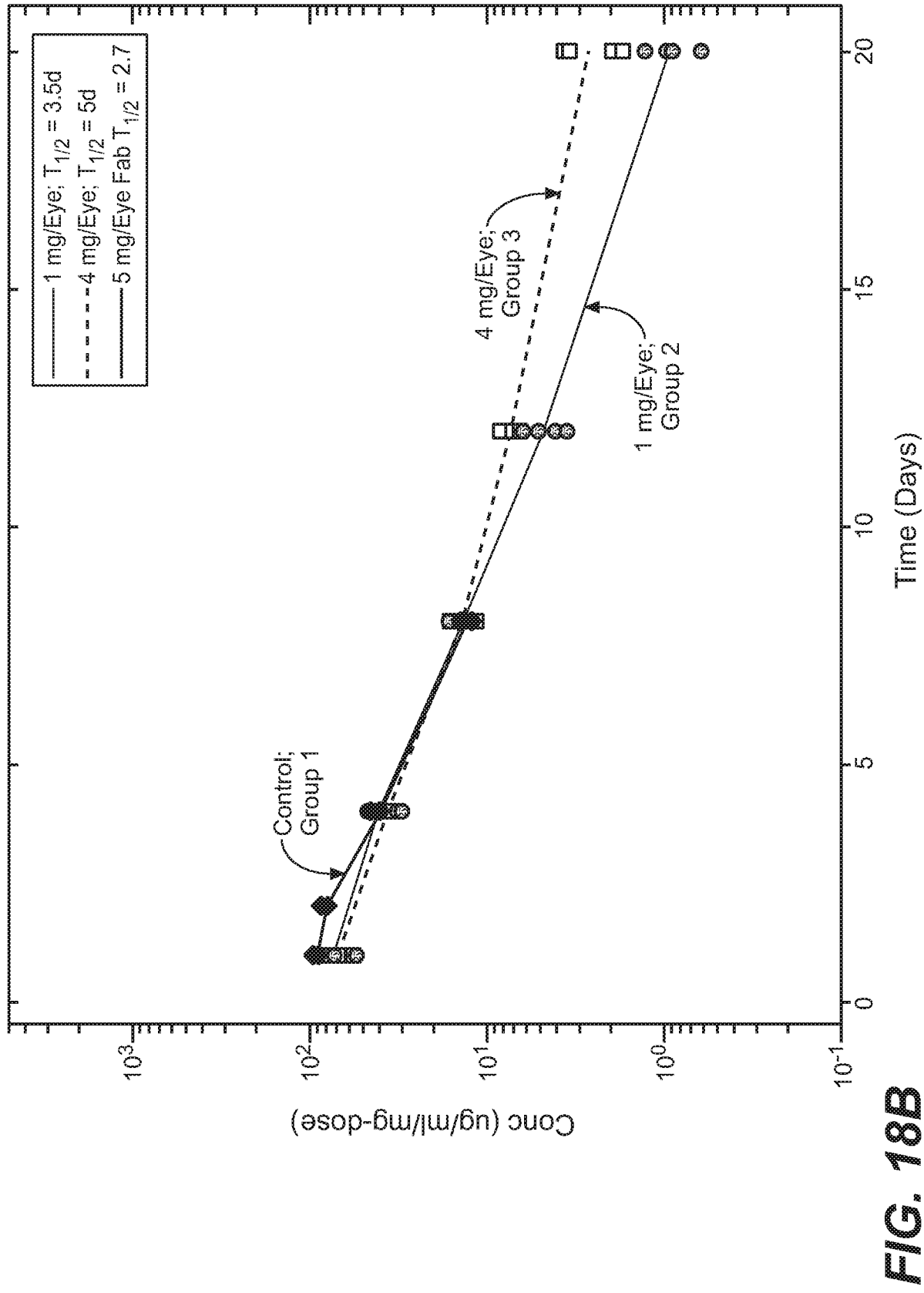
1 mg/Eye; $T_{1/2}$ = 3.5d
4 mg/Eye; $T_{1/2}$ = 5d
5 mg/Eye Fab $T_{1/2}$ = 2.7
Control; Group 1
4 mg/Eye; Group 3
1 mg/Eye; Group 2
Conc (ug/ml/mg-dose)
Time (Days)
10^{3}
10^{2}
10^{1}
10^{0}
10^{-1}
0
5
10
15
20

FIG. 18B

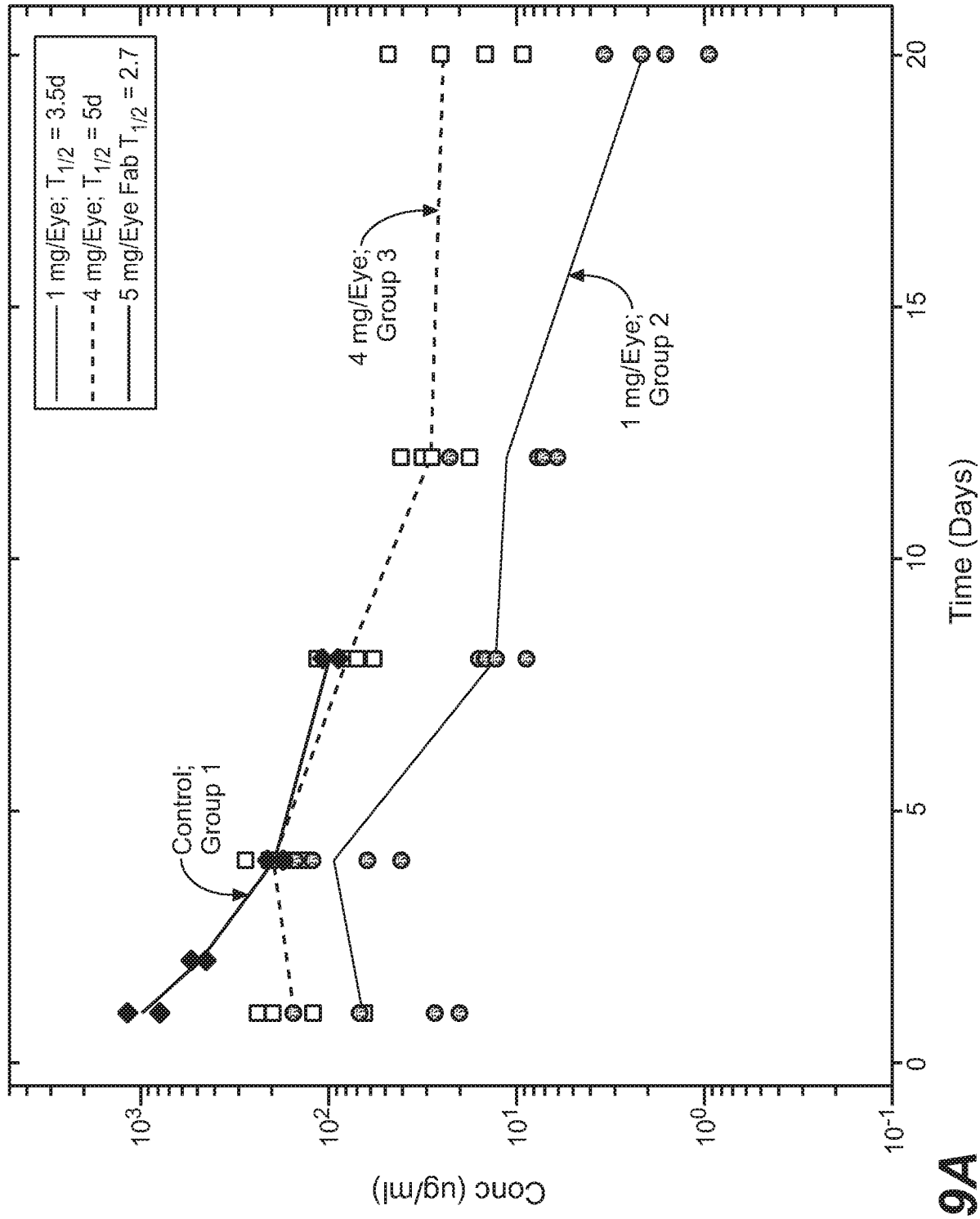
1 mg/Eye; $T_{1/2}$ = 3.5d
4 mg/Eye; $T_{1/2}$ = 5d
5 mg/Eye Fab $T_{1/2}$ = 2.7
Control; Group 1
4 mg/Eye; Group 3
1 mg/Eye; Group 2
Conc (ug/ml)
10^{3}
10^{2}
10^{1}
10^{0}
10^{-1}
0
5
10
15
20
Time (Days)

FIG. 19A

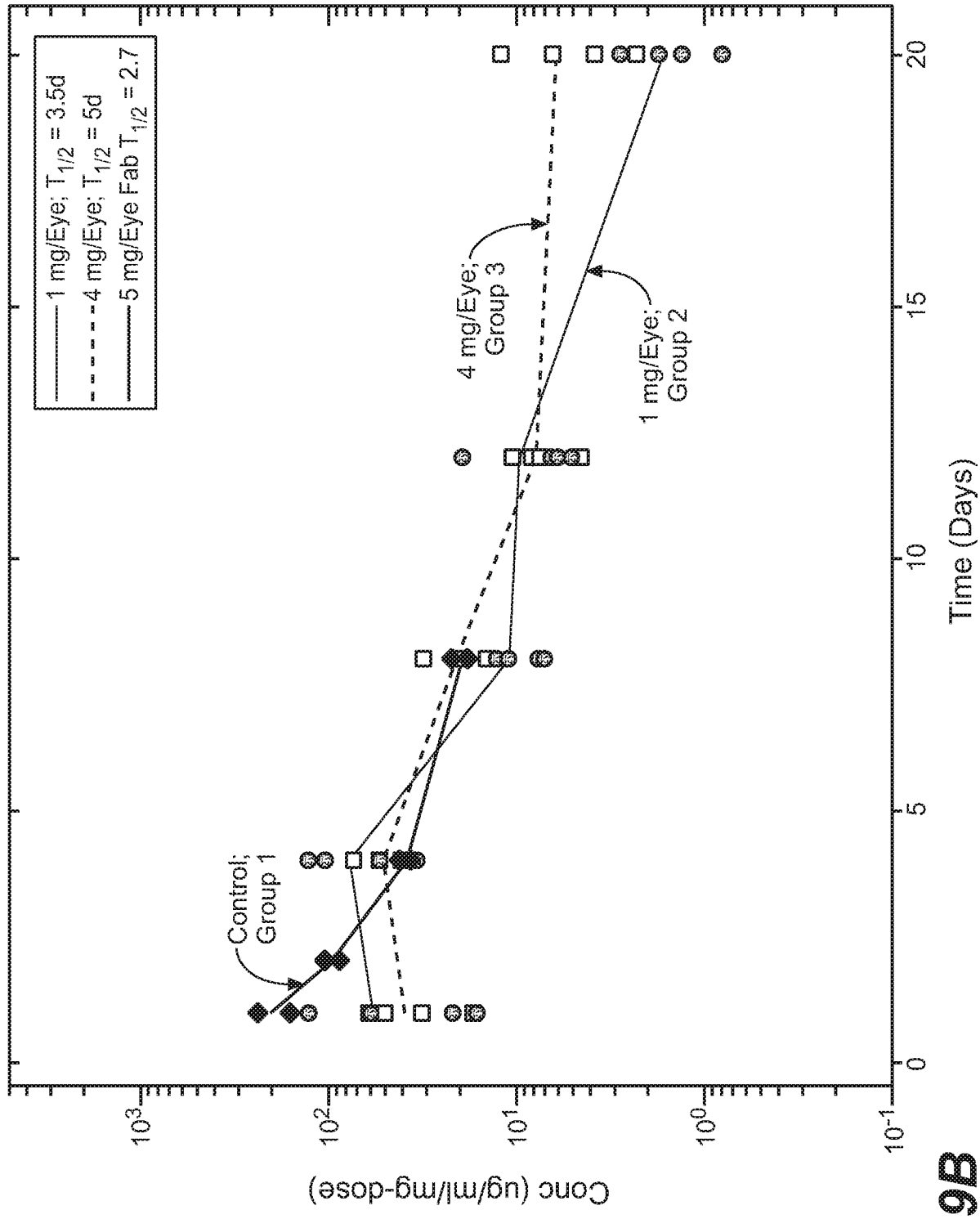
1 mg/Eye; $T_{1/2}$ = 3.5d
4 mg/Eye; $T_{1/2}$ = 5d
5 mg/Eye Fab $T_{1/2}$ = 2.7
Control; Group 1
4 mg/Eye; Group 3
1 mg/Eye; Group 2
Conc (ug/ml/mg-dose)
Time (Days)
10^{3}
10^{2}
10^{1}
10^{0}
10^{-1}
0
5
10
15
20

FIG. 19B

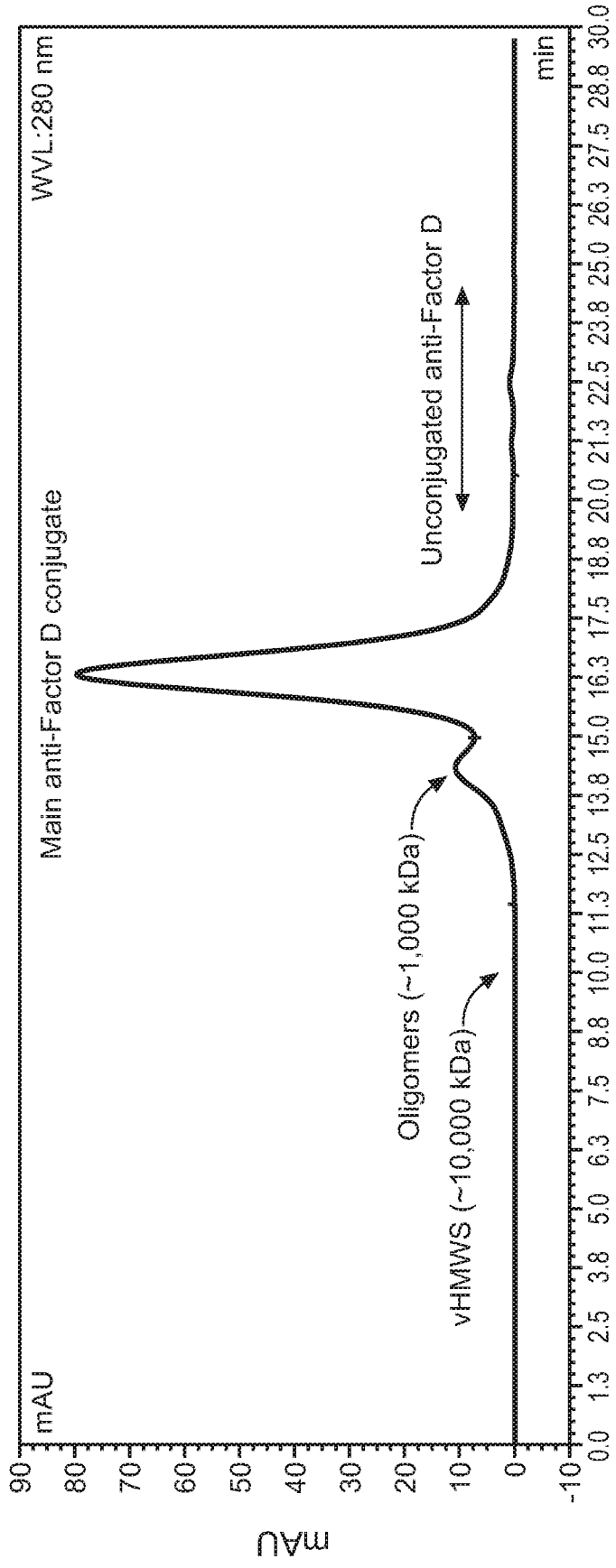
mAU
WVL:280 nm
Main anti-Factor D conjugate
Unconjugated anti-Factor D
Oligomers (~1,000 kDa)
vHMWS (~10,000 kDa)
min
Retention Time [min]
90
80
70
60
50
40
30
20
10
0
-10
0.0 1.3 2.5 3.8 5.0 6.3 7.5 8.8 10.0 11.3 12.5 13.8 15.0 16.3 17.5 18.8 20.0 21.3 22.5 23.8 25.0 26.3 27.5 28.8 30.0

FIG. 20

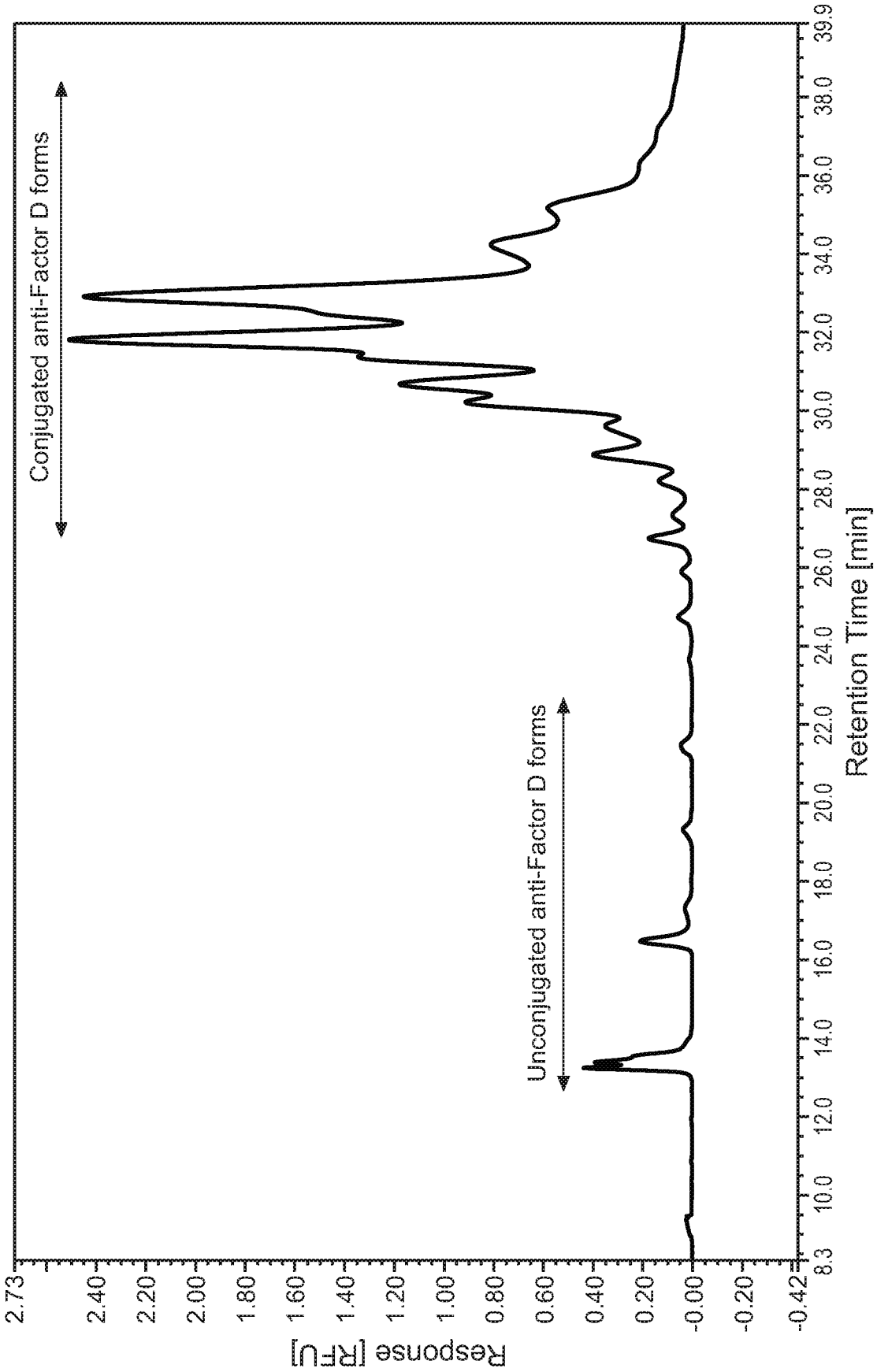
Conjugated anti-Factor D forms
Unconjugated anti-Factor D forms
Response [RFU]
Retention Time [min]
2.73
2.40
2.20
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
-0.00
-0.20
-0.42
8.3
10.0
12.0
14.0
16.0
18.0
20.0
22.0
24.0
26.0
28.0
30.0
32.0
34.0
36.0
38.0
39.9

FIG. 21

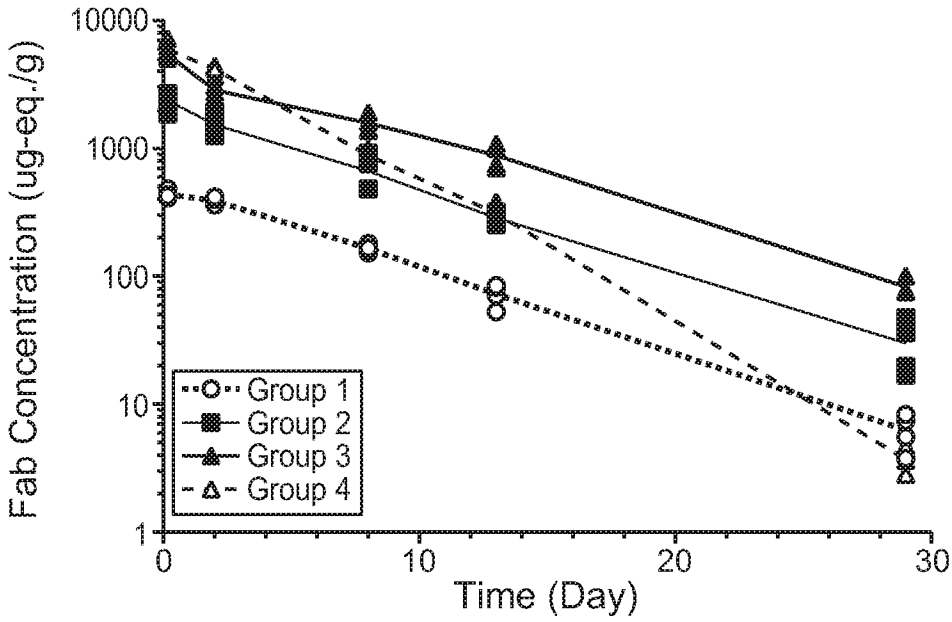

FIG. 22

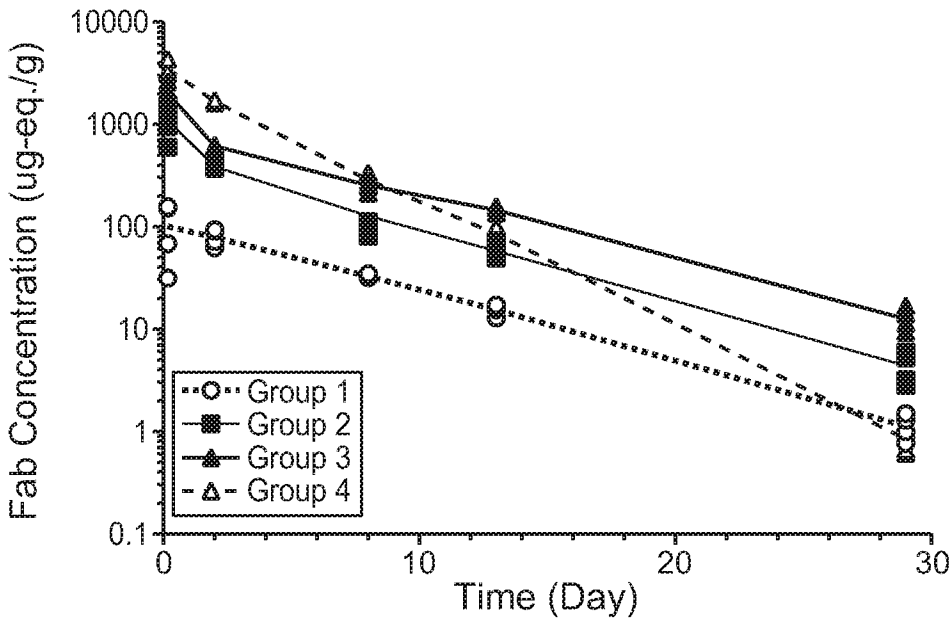

FIG. 23

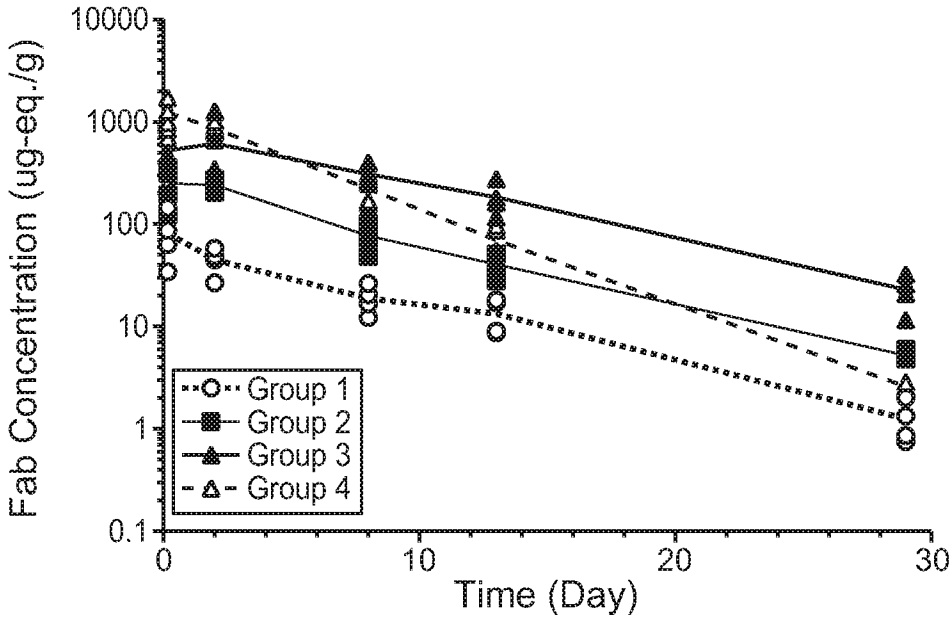

FIG. 24