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(71) Applicant *(for all designated States except AL, AT, BE, BG, CH, CN, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IN, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR)*: **GENENTECH, INC.** [US/US]; 1 DNA Way, South San Francisco, California 94080 (US).

(71) Applicant *(for AL, AT, BE, BG, CH, CN, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IN, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR only)*: **F. HOFFMANN-LA ROCHE AG** [CH/CH]; Grenzacherstrasse 124, 4070 Basel (CH).

(72) Inventors: **HASS, Philip E.**; Genentech, Inc., 1 DNA Way, South San Francisco, California 94080 (US). **KELLEY, Robert F.**; Genentech, Inc., 1 DNA Way, South San Francisco, California 94080 (US). **SCHEER, Justin M.**; Genentech, Inc., 1 DNA Way, South San Francisco, California 94080 (US). **SHATZ, Whitney**; Genentech, Inc., 1 DNA Way, South San Francisco, California 94080 (US). **TESAR, Devin**; Genentech, Inc., 1 DNA Way, South San Francisco, California 94080 (US). **VAN LOOKEREN CAMPAGNE, Menno**; Genentech, Inc., 1 DNA Way, South San Francisco, California 94080 (US).

(74) Agents: **ALLEN, Derick E.** et al.; Armstrong Teasdale LLP, 7700 Forsyth Boulevard, Suite 1800, St. Louis, Missouri 63105 (US).

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(54) Title: ANTI-FACTOR D ANTIBODY VARIANT CONJUGATES AND USES THEREOF

(57) Abstract: The disclosure relates to antibody-polymer conjugates comprising one or more anti-Factor D antibody variants, their production and their use in the preparation of compositions and medicaments for treatment of diseases and disorders associated with excessive or uncontrolled complement activation.

ANTI-FACTOR D ANTIBODY VARIANT CONJUGATES AND USES THEREOF

Cross-Reference to Related Applications

[0001] The instant application claims the benefit of priority to U.S. Provisional Application No. 62/249,020, which was filed on October 30, 2015, and to U.S. Provisional Application No. 62/250,965, which was filed on November 4, 2015, both of which are herein incorporated by reference in their entirety.

Sequence Listing

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on October 27, 2016, is named P33044-WO.txt and is 172,164 bytes in size.

Background of the Disclosure

[0003] The development of therapeutic antibodies represents a revolutionary era in the long history of human medicine. More than 30 antibodies have been approved for human therapy, and over 250 antibodies are in clinical development worldwide for a wide range of major diseases, including cancer, autoimmunity, inflammation, cardiovascular disease, infectious diseases and ocular disease. Over the past decade, the market for monoclonal antibody products has grown exponentially, propelled by the success of such blockbuster drugs as trastuzumab, bevacizumab, rituximab, infliximab and adalimumab. While these first-generation antibody therapeutics have benefited numerous patients, advances in antibody technology and a deeper understanding of the mechanism of action have paved the way for improved versions of antibodies with even better efficacy and fewer side effects.

[0004] Successful development and viable use of antibody therapeutics pose many unique challenges compared to traditional medicines that are small organic and inorganic molecules. The biophysical properties of antibodies, like all proteins, are important determinants of their behavior and have significant impacts for development of therapeutics relating to expression, purification, formulation, storage, delivery, pharmacokinetics, immunogenicity and dosing regimens. Among the many characteristics, protein stability is a main feature defining the quality of a candidate antibody and its desirability as a successful therapeutic.

[0005] Protein therapy often requires delivering high dose of the protein to patients in order to achieve the desired efficacy. Meanwhile, certain routes of administration are associated

with limitations such as delivery time, volume and physical force that require the high dose protein to be in a high-concentration formulation (e.g., at least 100 mg/ml). However, highly concentrated protein formulations pose particular challenges with respect to stability, solubility, viscosity and other protein properties.

[0006] Proteins can be unstable and become degraded via multiple physical and chemical degradation pathways. Physical instability occurs mainly via two pathways – denaturation and aggregation, whereas chemical instability can occur via many pathways, such as deamidation, isomerization, cross-linking, oxidation, and fragmentation. Antibody instability is undesirable for drug development, as it can lead to decreased amount of active drug and lower *in vivo* efficacy, increased variability among batches of the therapeutics, and perhaps most importantly, immunogenicity in patients against aggregates and degradants. Wang et al (2007) *J. Pharm. Sci.* 96:1-26; Moore et al (1980) *J Clin Endocrinology & Metabolism* 51: 691-697; Rosenberg et al (2006) AAPSJ 8:E501-7; Joubert et al (2011) *J Biol Chem* 286: 25118-25133; Joubert et al (2012) *J Biol Chem*(2012) 286:25266-79).

[0007] Antibodies are large multidomain proteins, and factors contributing to their stability and propensity to aggregate are complex, including many extrinsic conditions such as temperature, pH, concentration, ionic strength and physical stress. Equally critical is the protein's own primary sequence. Although by nature the Fc region is largely identical between antibodies of a particular isotype, the Fab region differs greatly. Thus, there are significant variations in stability and aggregation propensity between antibodies, largely due to Fab sequence differences and the particular antigen specificity of the antibody. Lowe et al. (2011) *Adv. Protein Chem. Struct Biol.* 84:41-61.

[0008] 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.

[0009] 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.

[0010] 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.

[0011] 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.

[0012] 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 ≥ 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.

[0013] 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, 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.

[0014] 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.

[0015] 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.

[0016] 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.

[0017] 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.

[0018] Thus, there exists a great need for anti-factor D antibodies, as well as conjugates thereof, having improved stabilities, and that in some embodiments, are suitable for high concentration formulation and/or long acting delivery.

Summary of the Invention

[0019] The present disclosure is based in part on the discovery that targeted amino acid substitutions of identified hot spots in an antibody can effectively improve the antibody's stability and overall potency as a therapeutic. Conjugation of such antibodies to multi-armed polymers, such as multi-armed polyols, can improve the vitreous humour half-life, the aqueous humour half-life, and/or the retinal half-life, as compared to the unconjugated antibodies.

[0020] In some aspects, the present disclosure relates to conjugates comprising one or more anti-Factor D antibodies or anti-Factor D antibody variants covalently linked to one or more multi-armed polyols. In certain embodiments, the polyol is an eight-armed polyol (i.e., an octamer). In some embodiments, the polyol is a polyethylene glycol (PEG). In certain embodiments, the PEG may have the structure of any of general formulas (Ia), (Ib), (IIa), (IIIa), or (IVa), as set forth hereinafter.

[0021] The anti-factor D antibody variants used in the conjugates of the present disclosure have improved stability. The anti-factor D antibody variants comprise substitution of at least one target aspartic acid (D or Asp) residue within a hypervariable region (HVR) of a reference anti-Factor D antibody, wherein the target Asp residue is identified as prone to isomerization and the substitution is Asp to Glutamic acid (E or Glu), and wherein the anti-Factor D antibody variant exhibits improved stability without significant loss of Factor D binding affinity when compared to the reference anti-Factor D antibody. In some aspects, the target Asp residue subject to substitution is within an Asp-Xaa motif, wherein Xaa is Asp, Gly, His, Ser or Thr. In some aspects, the target Asp residue is the first Asp of an Asp-Asp (DD) motif. In some aspects, the anti-factor D antibody variants comprise one or more substitutions at additional Asp sites within a HVR of a reference anti-Factor D antibody, wherein the substitution is Asp to Serine (S or Ser) in order to reduce the overall charges of the antibody, thereby improving the solubility of the antibody. In some aspects, the anti-factor D antibody variants comprise one or more substitutions at asparagine (N or Asn) sites identified as prone to deamidation, wherein the substitution is Asn to Ser in order to reduce or eliminate the antibody's deamidation.

[0022] In some embodiments, the anti-Factor D antibody variant is a Fab fragment, wherein the C-terminus of the heavy chain of the Fab fragment ends in the amino acids "CDKTHT," "CDKTHL," "CDKTH," "CDKT," "CDK," or "CD." In some embodiments, the C-terminus of the heavy chain of the Fab fragment ends in the sequence "CDKTHX," 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," "CDKTHTCPPC," "CDKTHTCPPS," "CDKTHTSPPC", "CDKTHTAPPC", "CDKTHTSGGC" or "CYGPPC". 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 domain amino acid sequence selected from the group consisting of SEQ ID NOs: 54 (ending in "CDKTHT"), 55-66 (ending in "CDKTHL," "CDKTHTC," "CPPC," "CPPS," "SPPC," "APPC," "SGGC," "CYGPPC," "CDKTH," "CDKT," "CDK," or "CD"), and 116 (ending in "CDKTHX"). In some embodiments, a Fab is an IgG2 Fab fragment comprising a heavy chain constant domain amino acid sequence of SEQ ID NOs: 67 (ending in "VERK") or an IgG2 Fab-C fragment comprising a heavy chain constant domain amino acid sequence of SEQ ID NO: 68 (ending in "VERKC"). In some embodiments, a Fab is an IgG4 Fab fragment comprising a

heavy chain constant domain amino acid sequence selected from the group consisting of SEQ ID NOs: 69-73 (ending in "KYGPP", "KYGP", "KYG", "KY", or "K") or an IgG4 Fab-C fragment comprising a heavy chain constant domain amino acid sequence of SEQ ID NO: 74 (ending in "KYGPPC"). 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.

[0023] In some aspects, the reference anti-factor D antibody used to generate the antibody variants used in the conjugates of the disclosure comprises the light chain variable domain sequence of SEQ ID NO:3, the heavy chain variable domain sequence of SEQ ID NO:4, or both. Subsequently, the resulting antibody variants may comprise a light chain HVR1 (HVR-L1) sequence of SEQ ID NO:11 and a heavy chain HVR2 (HVR-H2) sequence of SEQ ID NO:12, or may comprise a light chain HVR3 (HVR-L3) sequence of SEQ ID NO:13, or may comprise a light chain HVR1 (HVR-L1) sequence of SEQ ID NO:14 and a heavy chain HVR2 (HVR-H2) sequence of SEQ ID NO:12, or may comprise a heavy chain HVR3 (HVR-H3) sequence of SEQ ID NO:15.

[0024] In some aspects, the anti-factor D antibody variant used in the conjugates of the disclosure is a variant of a reference anti-factor D antibody, wherein the reference anti-factor D antibody comprises the light chain sequence of SEQ ID NO:1 and the heavy chain sequence of SEQ ID NO:2, and wherein the variant comprises the following sequence modifications over the reference anti-factor D antibody: a light chain HVR1 (HVR-L1) sequence of SEQ ID NO:11 and a heavy chain HVR2 (HVR-H2) sequence of SEQ ID NO:12. Such variant is referred to as the "TM" variant (AFD.v6) in the Examples herein below (see, e.g., Table 1).

[0025] In some aspects, the anti-factor D antibody variant used in the conjugates of the disclosure is a variant of a reference anti-factor D antibody, wherein the reference anti-factor D antibody comprises the light chain sequence of SEQ ID NO:1 and a heavy chain sequence selected from the group consisting of SEQ ID NOs: 34-53 and 115, and wherein the variant comprises the following sequence modifications over the reference anti-factor D antibody: a light chain HVR1 (HVR-L1) sequence of SEQ ID NO:11 and a heavy chain HVR2 (HVR-H2) sequence of SEQ ID NO:12. Such variants are referred to as "modified TM" variants. These modified TM variants comprise a heavy chain constant domain that differs from that of the TM variant, and which is selected from the group consisting of SEQ ID NOs: 55-74 and 116.

[0026] In some aspects, the anti-factor D antibody variant used in the conjugates of the disclosure is a variant of a reference anti-factor D antibody, wherein the reference anti-factor D antibody comprises the light chain sequence of SEQ ID NO:1 and the heavy chain sequence of

SEQ ID NO:2, and wherein the variant comprises the following sequence modifications over the reference anti-factor D antibody: a light chain HVR1 (HVR-L1) sequence of SEQ ID NO:11, a heavy chain HVR2 (HVR-H2) sequence of SEQ ID NO:12 and a light chain HVR3 (HVR-L3) sequence of SEQ ID NO:13. Such variant is referred to as the "TM.D92E" variant (AFD.v7) in Examples herein below (see, e.g., Table 1).

[0027] In some aspects, the anti-factor D antibody variant used in the conjugates of the disclosure is a variant of a reference anti-factor D antibody, wherein the reference anti-factor D antibody comprises the light chain sequence of SEQ ID NO:1 and a heavy chain sequence selected from the group consisting of SEQ ID NOs: 34-53 and 115, and wherein the variant comprises the following sequence modifications over the reference anti-factor D antibody: a light chain HVR1 (HVR-L1) sequence of SEQ ID NO:11, a heavy chain HVR2 (HVR-H2) sequence of SEQ ID NO:12 and a light chain HVR3 (HVR-L3) sequence of SEQ ID NO:13. Such variants are referred to as "modified TM.D92E" variants. These modified TM.D92E variants comprise a heavy chain constant domain that differs from that of the TM.D92E variant, and which is selected from the group consisting of SEQ ID NOs: 55-74 and 116.

[0028] In some aspects, the anti-factor D antibody variant used in the conjugates of the disclosure is a variant of a reference anti-factor D antibody, wherein the reference anti-factor D antibody comprises the light chain sequence of SEQ ID NO:1 and the heavy chain sequence of SEQ ID NO:2, and wherein the variant comprises the following sequence modifications over the reference anti-factor D antibody: a light chain HVR1 (HVR-L1) sequence of SEQ ID NO:14 and a heavy chain HVR2 (HVR-H2) sequence of SEQ ID NO:12. Such variant is referred to as the "SIESD" variant (AFD.v8) in Examples herein below (see, e.g., Table 1). In some embodiments, the "SIESD" variant (AFD.v8) comprises the light chain sequence of SEQ ID NO: 26 and the heavy chain sequence of SEQ ID NO: 27. In some embodiments, a Cys-modified version of the "SIESD" variant comprises the light chain sequence of SEQ ID NO: 26 and the heavy chain sequence of SEQ ID NO: 30. In some embodiments, a Cys-Pro-Pro-Cys-modified version of the "SIESD" variant comprises the light chain sequence of SEQ ID NO: 26 and the heavy chain sequence of SEQ ID NO: 31. In some embodiments, a modified version of the "SIESD" variant comprises the light chain sequence of SEQ ID NO: 26 and the heavy chain sequence selected from the group consisting of SEQ ID NOs: 75-92 and 117.

[0029] In some aspects, the anti-factor D antibody variant used in the conjugates of the disclosure is a variant of a reference anti-factor D antibody, wherein the reference anti-factor D antibody comprises the light chain sequence of SEQ ID NO:1 and a heavy chain sequence selected from the group consisting of SEQ ID NOs: 34-53 and 115, and wherein the variant

comprises the following sequence modifications over the reference anti-factor D antibody: a light chain HVR1 (HVR-L1) sequence of SEQ ID NO:14 and a heavy chain HVR2 (HVR-H2) sequence of SEQ ID NO:12. Such variants are referred to as "modified SIESD" variants. These modified SIESD variants comprise a heavy chain constant domain that differs from that of the SIESD variant, and which is selected from the group consisting of SEQ ID NOs: 55-74 and 116.

[0030] In some aspects, the anti-factor D antibody variant used in the conjugates of the disclosure is a variant of a reference anti-factor D antibody, wherein the reference anti-factor D antibody comprises the light chain sequence of SEQ ID NO:1 and the heavy chain sequence of SEQ ID NO:2, and wherein the variant comprises the following sequence modifications over the reference anti-factor D antibody: a light chain HVR1 (HVR-L1) sequence of SEQ ID NO:14, a heavy chain HVR2 (HVR-H2) sequence of SEQ ID NO:12 and a heavy chain HVR3 (HVR-H3) sequence of SEQ ID NO:15. Such variant is referred to as the "SIESD.N103S" variant (AFD.v14) in Examples herein below (see, e.g., Table 1). In some embodiments, the "SIESD.N103S" variant (AFD.v14) comprises the light chain sequence of SEQ ID NO: 28 and the heavy chain sequence of SEQ ID NO: 29. In some embodiments, a Cys-modified version of the "SIESD.N103S" variant comprises the light chain sequence of SEQ ID NO: 28 and the heavy chain sequence of SEQ ID NO: 32. In some embodiments, a Cys-Pro-Pro-Cys-modified version of the "SIESD.N103S" variant comprises the light chain sequence of SEQ ID NO: 28 and the heavy chain sequence of SEQ ID NO: 33. In some embodiments, a modified version of the "SIESD.N103S" variant comprises the light chain sequence of SEQ ID NO: 28 and a heavy chain sequence selected from the group consisting of SEQ ID NOs: 93-110 and 118.

[0031] In some aspects, the anti-factor D antibody variant used in the conjugates of the disclosure is a variant of a reference anti-factor D antibody, wherein the reference anti-factor D antibody comprises the light chain sequence of SEQ ID NO:1 and a heavy chain sequence selected from the group consisting of SEQ ID NOs: 34-53 and 115, and wherein the variant comprises the following sequence modifications over the reference anti-factor D antibody: a light chain HVR1 (HVR-L1) sequence of SEQ ID NO:14, a heavy chain HVR2 (HVR-H2) sequence of SEQ ID NO:12 and a heavy chain HVR3 (HVR-H3) sequence of SEQ ID NO:15. Such variants are referred to as "modified SIESD.N103S" variants. These modified SIESD.N103S variants comprise a heavy chain constant domain that differs from that of the SIESD.N103S variant, and which is selected from the group consisting of SEQ ID NOs: 55-74 and 116.

[0032] In some aspects, the present disclosure relates to conjugates comprising one or more anti-Factor D antibody variants comprising one or more substitutions within the HVRs of

a reference anti-Factor D antibody. In some aspects, the reference anti-Factor D antibody comprises the following HVR sequences:

HVR-L1: ITSTDIDDDMN (SEQ ID NO: 5);

HVR-L2: GGNTLRP (SEQ ID NO: 6);

HVR-L3: LQSDSLPYT (SEQ ID NO: 7);

HVR-H1: GYTFTNYGMN (SEQ ID NO: 8);

HVR-H2: WINTYTGETTYADDFKG (SEQ ID NO: 9); and

HVR-H3: EGGVNN (SEQ ID NO: 10).

The corresponding variants comprise one or more of the following substitutions:

(a) D5S in SEQ ID NO: 5;

(b) D7E in SEQ ID NO: 5;

(c) D8S in SEQ ID NO: 5 (a, b, and c disclosed in SEQ ID NO: 22);

(d) D13E in SEQ ID NO: 9 (SEQ ID NO: 23);

(e) D4E in SEQ ID NO: 7 (SEQ ID NO: 24); or

(f) N5S in SEQ ID NO: 10 (SEQ ID NO: 25).

In some embodiments, the reference anti-Factor D antibody comprises a heavy chain constant domain amino acid sequence selected from the group consisting of SEQ ID NOs: 54-74 and 116.

[0033] In some aspects, the variant combines the substitutions (b)-(d) above. In another aspect, the variant combines the substitutions (b)-(e) above. In another aspect, the variant combines the substitutions (a)-(d) above. In another aspect, the variant combines the substitutions (a)-(d) and (f) above. In another aspect, the variant comprises one or more of substitution (a), (b), (c), (d), (e), or (f) above, and further comprises a heavy chain constant domain amino acid sequence selected from the group consisting of SEQ ID NOs: 54-74 and 116. In another aspect the variant comprises substitutions selected from the group consisting of: the substitutions (b)-(d) above, the substitutions (b)-(e) above, the substitutions (a)-(d) above, and the substitutions (a)-(d) and (f) above, wherein the variant further comprises a heavy chain constant domain amino acid sequence selected from the group consisting of SEQ ID NOs: 54-74 and 116.

[0034] In some aspects, the present disclosure relates to a conjugate comprising one or more anti-Factor D antibody comprising a light chain variable domain amino acid sequence of SEQ ID NO:16, 18 or 19. In another aspect, the present disclosure relates to a conjugate comprising an anti-Factor D antibody comprising a heavy chain variable domain amino acid sequence of SEQ ID NO:17 or 20. In another aspect, the anti-Factor D antibody may comprise a

heavy chain constant domain amino acid sequence selected from the group consisting of SEQ ID NOs: 54-74 and 116. In another aspect, the anti-Factor D antibody comprises a light chain variable domain amino acid sequence of SEQ ID NO:16, 18 or 19 and a heavy chain variable domain amino acid sequence of SEQ ID NO:17 or 20. For example, the anti-Factor D antibody can be the "TM" variant (AFD.v6) that comprises the light chain variable domain amino acid sequence of SEQ ID NO:16 and the heavy chain variable domain amino acid sequence of SEQ ID NO:17; the "TM.D92E" variant (AFD.v7) that comprises the light chain variable domain amino acid sequence of SEQ ID NO:18 and the heavy chain variable domain amino acid sequence of SEQ ID NO:17; the "SIESD" variant (AFD.v8) that comprises the light chain variable domain amino acid sequence of SEQ ID NO:19 and the heavy chain variable domain amino acid sequence of SEQ ID NO:17; or the "SIESD.N103S" variant (AFD.v14) that comprises the light chain variable domain amino acid sequence of SEQ ID NO:19 and the heavy chain variable domain amino acid sequence of SEQ ID NO:20.

[0035] In another aspect, the anti-Factor D antibody comprises a light chain variable domain amino acid sequence of SEQ ID NO:16, 18 or 19, a heavy chain variable domain amino acid sequence of SEQ ID NO:17 or 20, and a heavy chain constant domain amino acid sequence selected from the group consisting of SEQ ID NOs: 54-74 and 116. For example, the anti-Factor D antibody can be a modified version of the "TM" variant (AFD.v6) that comprises the light chain variable domain amino acid sequence of SEQ ID NO:16, the heavy chain variable domain amino acid sequence of SEQ ID NO:17, and a heavy chain constant domain amino acid sequence selected from the group consisting of SEQ ID NOs: 55-74 and 116; a modified version of the "TM.D92E" variant (AFD.v7) that comprises the light chain variable domain amino acid sequence of SEQ ID NO:18, the heavy chain variable domain amino acid sequence of SEQ ID NO:17, and a heavy chain constant domain amino acid sequence selected from the group consisting of SEQ ID NOs: 55-74 and 116; a modified version of the "SIESD" variant (AFD.v8) that comprises the light chain variable domain amino acid sequence of SEQ ID NO:19, the heavy chain variable domain amino acid sequence of SEQ ID NO:17, and a heavy chain constant domain amino acid sequence selected from the group consisting of SEQ ID NOs: 55-74 and 116; or a modified version of the "SIESD.N103S" variant (AFD.v14) that comprises the light chain variable domain amino acid sequence of SEQ ID NO:19, the heavy chain variable domain amino acid sequence of SEQ ID NO:20, and a heavy chain constant domain amino acid sequence selected from the group consisting of SEQ ID NOs: 55-74 and 116.

[0036] In some embodiments, the anti-Factor D antibody is a modified version of the "SIESD" variant (AFD.v8) that comprises the light chain variable domain amino acid sequence

of SEQ ID NO:19, and a heavy chain sequence selected from the group consisting of SEQ ID NOs: 30, 31, 75-92, and 117. In some embodiments, the anti-Factor D antibody is a modified version of the "SIESD" variant (AFD.v8) that comprises the light chain sequence of SEQ ID NO: 26, and a heavy chain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 30, 31, 75-92, and 117. In another embodiment, the anti-Factor D antibody is a modified version of the "SIESD.N103S" variant (AFD.v14) that comprises the light chain variable domain amino acid sequence of SEQ ID NO:19 and a heavy chain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 32, 33, 93-110, and 118. In some embodiments, the anti-Factor D antibody is a modified version of the "SIESD.N103S" variant (AFD.v14) that comprises the light chain sequence of SEQ ID NO: 28, and a heavy chain sequence selected from the group consisting of SEQ ID NOs: 32, 33, 93-110, and 118.

[0037] In some aspects, the present disclosure relates to conjugates comprising one or more anti-Factor D antibody having a variable light chain comprising a HVR-L1 having the sequence of SEQ ID NO:11 or 14, a HVR-L2 having the sequence of SEQ ID NO:6, and a HVR-L3 having the sequence of SEQ ID NO:7 or 13; and a variable heavy chain comprising a HVR-H1 having the sequence of SEQ ID NO:8, a HVR-H2 having the sequence of SEQ ID NO:9 or 12, and a HVR-H3 having the sequence of SEQ ID NO:10 or 15. In another embodiment, the anti-Factor D antibody may further comprise a heavy chain constant domain amino acid sequence selected from the group consisting of SEQ ID NOs: 54-74 and 116. For example, the anti-Factor D antibody can be the "SIESD" variant (AFD.v8) comprising the following six HVR sequences: HVR-L1 (SEQ ID NO:14), HVR-L2 (SEQ ID NO:6), HVR-L3 (SEQ ID NO:7), HVR-H1 (SEQ ID NO:8), HVR-H2 (SEQ ID NO:12), and HVR-H3 (SEQ ID NO:10); or the "SIESD.N103S" variant (AFD.v14) comprising the following six HVR sequences: HVR-L1 (SEQ ID NO:14), HVR-L2 (SEQ ID NO:6), HVR-L3 (SEQ ID NO:7), HVR-H1 (SEQ ID NO:8), HVR-H2 (SEQ ID NO:12), and HVR-H3 (SEQ ID NO:15). In some embodiments, the anti-Factor D antibody can be a modified version of the "SIESD" variant (AFD.v8) comprising the following six HVR sequences: HVR-L1 (SEQ ID NO:14), HVR-L2 (SEQ ID NO:6), HVR-L3 (SEQ ID NO:7), HVR-H1 (SEQ ID NO:8), HVR-H2 (SEQ ID NO:12), and HVR-H3 (SEQ ID NO:10), and further comprising a heavy chain constant domain amino acid sequence selected from the group consisting of SEQ ID NOs: 55-74 and 116. In another embodiment, the anti-Factor D antibody can be a modified version of the "SIESD.N103S" variant (AFD.v14) comprising the following six HVR sequences: HVR-L1 (SEQ ID NO:14), HVR-L2 (SEQ ID NO:6), HVR-L3 (SEQ ID NO:7), HVR-H1 (SEQ ID NO:8), HVR-H2 (SEQ ID NO:12), and HVR-H3 (SEQ ID NO:15), and further comprising a

heavy chain constant domain amino acid sequence selected from the group consisting of SEQ ID NOs: 55-74 and 116.

[0038] In some aspects, the present disclosure relates to conjugates comprising one or more anti-Factor D antibody variants with no detectable Asp isomerization, wherein the variants are made by a method for removing or reducing isomerization, comprising: (a) identifying one or more Asp residues prone to Asp isomerization within HVRs of a reference anti-Factor D antibody; (b) substituting Glu for the Asp residue identified in step (a); (c) screening the resulting candidate variants for Asp isomerization; and (d) selecting those variants that have no detectable Asp isomerization. In some aspects, the method above is combined with a method for removing or reducing deamidation, comprising (a) identifying one or more Asn residues prone to deamidation within HVRs of the reference anti-Factor D antibody; (b) substituting Ser for the Asn residue identified in step (a); (c) screening the resulting candidate variants for deamidation; and (d) selecting those variants having reduced or eliminated deamidation. In another aspect, the method for removing or reducing isomerization is combined with a method for reducing overall charge of the antibody by: (a) selecting one or more negatively charged amino acid residues D or E within HVRs of the reference anti-Factor D antibody; (b) substituting Ser for the residue selected in step (a); (c) screening the resulting candidate variants for solubility; and (d) selecting those variants having improved solubility when compared to the reference anti-Factor D antibody.

[0039] In some aspects, the present 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 of the anti-Factor D antibodies or antibody variants disclosed herein 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), as set forth herein.

[0040] In some aspects, the conjugates of the present disclosure comprise anti-factor D antibody variants that have improved stability while maintaining the factor D binding affinity when compared to the reference anti-factor D antibody. In some aspects, the antibodies bind to Factor D with a binding affinity of at least about 10^{-9} to 10^{-12}M. In some aspects, the antibodies used in the conjugates of the present disclosure include human, humanized or chimeric antibodies.

[0041] In some aspects, the antibodies used in the conjugates of the present disclosure are antibody fragments (e.g., antigen-binding fragments). The antibody fragments may, for

example, be Fab, Fab′, $F(ab')_2$, scFv, $(scFv)_2$, dAb, complementarity determining region (CDR) fragments, linear antibodies, single-chain antibody molecules, minibodies, diabodies, or multispecific antibodies formed from antibody fragments.

[0042] In other aspects of the disclosure, the present disclosure includes compositions comprising a conjugate of the disclosure. In another aspect, the disclosure concerns a composition of matter comprising a conjugate of the disclosure, as described herein, in combination with a carrier. Optionally, the carrier is a pharmaceutically acceptable carrier.

[0043] In some aspects, the present disclosure includes pharmaceutical formulations comprising the conjugates described herein, at therapeutically effective concentrations. In some aspects, the pharmaceutical formulation comprises the antibody or antibody variant at a concentration of at least about 100 mg/mL, from about 100 to about 150 mg/ mL, from about 100 to about 200 mg/ mL, from about 100 to about 300 mg/ mL, from about 100 to about 400 mg/ mL, from about 100 to about 500 mg/ml; at least about 200 mg/ mL, at least about 300 mg/ mL, at least about 400 mg/ mL or at least about 500 mg/ mL. In some aspects, the concentration of the antibody or antibody variant in the formulation is about 200, 250, 300, 350, 400, 450 or 500 mg/ mL. In some aspects, the concentration of the antibody or antibody variant in the formulation is less than about 450 mg/ mL.

[0044] Another aspect of the present disclosure is the use of the conjugate or pharmaceutical formulation of the disclosure for treatment of disorders associated with excessive or uncontrolled complement activation. In one embodiment, the disclosure is directed to a method of treating a complement-associated disorder in a subject, the method comprising administering to the subject a conjugate or pharmaceutical formulation of the disclosure. The disorders 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. In one embodiment, the complement-associated disorder is systemic. It may also include autoimmune disease such as systemic lupus erythematosus, myasthenia gravis, rheumatoid arthritis, Alzheimer's disease and multiple sclerosis. In another embodiment, complement activation is also associated with

transplant rejection. In another embodiment, complement activation is also associated with ocular diseases (all ocular conditions and diseases the pathology of which involve complement, including the classical and the alternative pathway of complement) or complement-associated eye conditions, such as, for example, without limitation, macular degenerative disease, such as all stages of age-related macular degeneration (AMD), including dry and wet (non-exudative and exudative) forms, 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. In one example, complement-associated eye conditions include 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 another 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). 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, the complement-associated eye condition is intermediate dry AMD. In one example, the complement-associated eye condition is geographic atrophy. In one example, the complement-associated eye condition is wet AMD (choroidal neovascularization (CNV)). In one embodiment, the conjugate or pharmaceutical formulation is administered using an implantable port-delivery system. In one embodiment, the conjugate or pharmaceutical formulation is administered by intravitreal administration. In one embodiment, the method or use further comprises administering to the subject an additional therapeutic agent, such as a HTRA1 antagonist, an ANG2 antagonist, a TIE2 antagonist, a VEGF antagonist, or an antagonist of one or more of the C1, C2, C3, C4, C5, C6, C7, C8, or C9 complement components.

[0045] In another aspect, the disclosure provides a kit, comprising a conjugate of the disclosure. In some embodiments, the disclosure provides a kit, comprising a conjugate of the disclosure and instructions for use. In some embodiments, the disclosure concerns a kit

comprising a conjugate of the disclosure and instructions for administering said conjugate, to treat a complement-associated disorder. In some embodiments, the disclosure provides a kit comprising a first container comprising a composition comprising one or more one or more conjugate of the disclosure; and a second container comprising a buffer. In some embodiments, the buffer is pharmaceutically acceptable. In some embodiments, a composition comprising a conjugate of the disclosure further comprises a carrier, which in some embodiments is pharmaceutically acceptable. In some embodiments, a kit further comprises instructions for administering the composition (e.g., the conjugate comprising one or more antibody, or antibody fragment thereof (e.g., antigen-binding fragment) to a subject. In some embodiments, a kit further comprises instructions for use of the kit.

[0046] In some aspects, the disclosure concerns an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of complement-associated disorders. In some embodiments, the disclosure concerns an article of manufacture, comprising: (a) a container; (b) a label on the container; and (c) a composition of matter comprising a conjugate of the present disclosure, contained with the container, wherein the label on said container indicates that the composition can be used for treatment, prevention and/or diagnosis of complement-associated disorders.

[0047] In some aspects, the disclosure provides use of a conjugate of the disclosure in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disease, such as a complement-associated eye condition. In one embodiment, the disclosure is directed to a method of treating a complement-associated disorder, such as a complement-associated eye condition, in a subject, the method comprising administering to the subject a conjugate or pharmaceutical formulation of the disclosure. In some embodiments, the complement-associated eye condition is selected from 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 one example, the complement-associated eye condition is geographic atrophy. In one example, the complement-associated eye condition is wet AMD (choroidal neovascularization (CNV)).

[0048] In some aspects, the disclosure provides use of an article of manufacture of the disclosure in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disease, such as a complement-associated eye condition. In some embodiments, the complement-associated eye condition is selected from 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 one example, the complement-associated eye condition is geographic atrophy. In one example, the complement-associated eye condition is wet AMD (choroidal neovascularization (CNV)).

[0049] In some aspects, the disclosure provides use of a kit of the disclosure in the preparation of a medicament for the therapeutic and/or prophylactic treatment of a disease, such as a complement-associated eye condition. In some embodiments, the complement-associated eye condition is selected from 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 one example, the complement-associated eye condition is geographic atrophy. In one example, the complement-associated eye condition is wet AMD (choroidal neovascularization (CNV)).

[0050] In some aspects, the disclosure provides a formulation comprising a conjugate comprising one or more Factor D antagonist, and further comprises a HTRA1 antagonist, an ANG2 antagonist, a TIE2 antagonist, a VEGF antagonist, or an antagonist of one or more of the C1, C2, C3, C4, C5, C6, C7, C8, and C9 complement components. In some embodiments, the Factor D antagonist is an anti-Factor D antibody. In a further embodiment, the anti-Factor D antibody is an anti-Factor D antibody variant described herein. In some embodiments the HTRA1 antagonist is an anti-HTRA1 antibody. In another embodiment the ANG2 antagonist is an anti-ANG2 antibody. In another embodiment, the TIE2 antagonist is an anti-TIE2 antibody. In another embodiment, the VEGF antagonist is an anti-VEGF antibody. In another embodiment, the antagonist of the C2 and/or C4 and/or C5 complement components is an anti-C2 and/or anti-C4 and/or anti-C5 antibody.

[0051] In some aspects, the treatment of disorders associated with excessive or uncontrolled complement activation in a human subject with a disorder associated with excessive or uncontrolled complement activation comprises administering to the subject an effective amount of a therapeutic compound, such as conjugate comprising one or more Factor D antagonist, and further comprises administering to the subject an effective amount of a second therapeutic compound, such as a HTRA1 antagonist, an ANG2 antagonist, a TIE2 antagonist, a VEGF antagonist, or an antagonist of one or more of the C1, C2, C3, C4, C5, C6, C7, C8, and

C9 complement components. In some embodiments, the Factor D antagonist is an anti-Factor D antibody. In some embodiments, the anti-Factor D antibody is an anti-Factor D antibody variant described herein. In some embodiments, the HTRA1 antagonist is an anti-HTRA1 antibody. In another embodiment the ANG2 antagonist is an anti-ANG2 antibody. In another embodiment, the TIE2 antagonist is an anti-TIE2 antibody. In another embodiment, the VEGF antagonist is an anti-VEGF antibody. In another embodiment, the antagonist of the C2 and/or C4 and/or C5 complement components is an anti-C2 and/or anti-C4 and/or anti-C5 antibody.

[0052] In some aspects, the administration of the conjugate comprising the Factor D antagonist 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.

Brief Description of the Figures

[0053] Figures 1A-1D shows amino acid sequences of a reference anti-factor D antibody WT (aFD.WT) and its select variants (1A: light and heavy chain sequences of WT; 1B: alignment of light and heavy chain variable domains; 1C: light and heavy chain sequences of SIESD (AFD.v8) and heavy chain sequences of Cys-modified SIESD (AFD.v8) and Cys-Pro-Pro-Cys-modified SIESD (AFD.v8); 1D: light and heavy chain sequences of SIESD.N103S (AFD.v14) and heavy chain sequences of Cys-modified SIESD.N103S (AFD.v14) and Cys-Pro-Pro-Cys-modified SIESD.N103S (AFD.v14)). HVRs within the variable domains are underlined. Residue substitutions in the variants are shown in bold. Cys and Cys-Pro-Pro-Cys (SEQ ID NO: 21) modifications are shown in italics in Figure 1C and 1D.

[0054] Figures 2A-2C illustrates antigen binding capacity of various antibody Fab fragments over prolonged time under defined conditions (2A: Fab protein concentration of 10 mg/mL in pH 5.5 buffer; 2B: Fab protein concentration of 100 mg/ml in PBS; 2C: Fab protein concentration of 100 mg/ml in PBS).

[0055] Figures 3A-3B illustrates degradations of various antibody Fab fragments over time under defined conditions whereby main peak is determined by ion-exchange chromatography (IEC) (3A: Fab protein concentration of 10 mg/mL in pH 5.5 buffer; 3B: Fab protein concentration of 100 mg/ml in PBS).

[0056] Figures 4A-4B illustrates isomerization and deamidation of various antibody Fab fragments over time under defined conditions (4A: Fab protein concentration of 10 mg/mL in pH 5.5 buffer; 4B: Fab protein concentration of 100 mg/ml in PBS).

[0057] Figure 5 illustrates aggregation of various antibody Fab fragments over prolonged time under defined condition (Fab protein concentration of 100 mg/ml in PBS) as determined by measurements of monomer peak by size-exclusion chromatography (SEC).

[0058] Figure 6 illustrates solubility of aFD.WT, AFD.v2, AFD.v6 and AFD.v8 at pH 6 and low ionic strength (~100 mg/ml in 20 mM His-HCl, pH 6).

[0059] Figure 7 illustrates solubility of antibody Fab fragments at pH 6 and low ionic strength (~ 100 mg/ml in 20 mM His-HCl, pH 6). The insolubility of aFD.WT is reversed by the exchange into PBS, a salt (NaCl) containing buffer, via dialysis.

[0060] Figure 8 illustrates solubility of antibody Fab fragments in PBS (pH 7.3) at 227 mg/ml for aFD.WT, 269 mg/ml for AFD.v8 and 344 mg/ml for AFD.v14.

[0061] Figure 9 illustrates % aggregate as measured by size-exclusion chromatography (SEC) of SIESD.N103S (AFD.v14) in PBS prior to 3 week incubation at 2-8°C.

[0062] Figure 10A illustrates the antigen binding capacity for a high concentration (272 mg/mL) AFD.v8 formulation (20 mM His-HCl, pH 5.5) over prolonged time under thermal stress at 37°C. The hatched area denotes the ±10% standard error in the measurements.

[0063] Figure 10B illustrates the chemical and physical stability for a high concentration (272 mg/mL) AFD.v8 formulation (20 mM His-HCl, pH 5.5) over prolonged time under thermal stress at 37°C. The N101 and E95 are according to Kabat numbering.

[0064] Figure 11 illustrates pharmacokinetics of antibody Fab fragments upon intravitreal injection in rabbits.

[0065] Figure 12 illustrates protein concentration dependence of viscosity for antibody Fab fragments in pH 5.5 buffer.

[0066] Figures 13A and 13B show the 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) (13A: HGEO core; 13B: TP core).

[0067] Figures 14A – 14C show the results of a purification of the AFD.v14.C + TP octamer by 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) (14A: initial chromatogram of the SEC column; 14B: an expansion of the peak from 600 mL to 1100 mL; 14C: MALS profile of the chromatogram fractions collected during the purification shown in 14B).

[0068] Figures 15A – 15C show the results of a purification of the AFD.v14.C + HG octamer by SEC on a Sephacryl S-300 HR (GE Healthcare) column in 20 mM His-acetate, pH

5.5, 50 mM NaCl (isocratic gradient) (15A: initial chromatogram of the SEC column; 15B: enlargement of the chromatogram of 15A from 2900-3600 mL; 15C: MALS profile of the chromatogram fractions collected during the purification shown in Figure 15B).

[0069] Figures 16A – 16B show the results of an analysis of the AFD.v14.C + HG octamer using a Sephacryl S-400 HR (GE Healthcare) column in PBS, pH 7.4 (16A: initial chromatogram of the column; 16B: MALS profile of the chromatogram in Figure 16A).

[0070] Figures 17A – 17B show the results of a purification of the AFD.v14.C + HGEO octamer by 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 SEC-MALS characterization on Sephacryl S-400 HR at 0.25 mL/minute in PBS, pH 7.4 (17A: initial chromatogram of the SEC S-400 column; 17B: MALS profile of the chromatogram fractions in Figure 17A).

[0071] Figures 18A – 18B show the results of a purification of the AFD.v14.C + HGEO octamer by 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 SEC-MALS characterization on a Tosoh G3000PW column (18A: initial chromatogram of the SEC S-300 column; 18B: overlay of laser intensity for S-300 fractions from 18A using SEC-MALS with G3000PW column).

[0072] Figures 19A – 19C show further purification of select fractions in Example 9a (shown in Figure 14) by cation exchange chromatography (CEX) in Triton wash, 10-20% 1M NaCl gradient over 50CV (19A: initial chromatogram of the CEX column; 19B: SEC gel of the fractions from the CEX column; 19C: MALS profile of the chromatogram fractions in Figure 19A).

[0073] Figure 20 shows the final analytical run of the AFD.v14.C + TP octamer after CEX purification.

[0074] Figures 21A – 21B compare the results of the purification methods for the AFD.v14.C + TP octamer after cation exchange chromatography (CEX), SEC chromatography on the SEC S-400 HR column, or SEC chromatography on the SEC S-300 HR column (21A: stacked display of the chromatograms of the three different purification columns; 21B: SEC gel comparing the samples from the three different purification columns).

[0075] Figures 22A – 22B compare PEG-Fab conjugates prepared with PEGs having different cores (22A: SEC gel comparing the purified samples of the conjugates with the different cores; 22B: MALS profile of the conjugates prepared with the different cores).

[0076] Figure 23 shows the viscosity of the AFD.v14.C + HG octamer and a PEG-Fab conjugate prepared from Sunbright® PTE-400MA from NOF America Corp. (a tetramer) as a function of AFD.v14 concentration.

[0077] Figure 24 shows the viscosity of the AFD.v14.C + TP octamer and the AFD.v14.C + HGEO octamer as a function of AFD.v14 concentration in 20mL His-Ace, pH 6.5 and 50 mM NaCl at 20°C.

[0078] Figures 25A and 25B show the thermal stability of the AFD.v14.C + TP octamer in 10 mg/mL, PBS (25A) and 10 mg/mL of 20 mM histidine HCl, 50 mM NaCl, at pH 6.5 (25B) as a function of time.

[0079] Figures 26 and 27 show the slow release of the Fab fragment and dimerization during the course of the thermal stability study (26: SEC-MALS analysis of the conjugate over time; 27: CE-SDS analysis of the conjugate over time).

[0080] Figure 28 shows the maintenance of binding capacity of the AFD.v14.C + TP octamer to Factor D during the thermal stability study as measured by surface plasmon resonance.

[0081] Figures 29A and 29B show the concentration versus time of AFD.v14 in cynomolgus monkey vitreous humor following administration of AFD.v14 or AFD.v14.C + TP octamer in a pharmacokinetic study (29A: vitreous humor concentration; 29B: vitreous humor concentration data normalized for dosing strength).

[0082] Figures 30A and 30B show the concentration versus time of AFD.v14 in cynomolgus monkey eye aqueous humor following administration of AFD.v14 or AFD.v14.C + TP octamer in a pharmacokinetic study (30A: eye aqueous humor concentration; 30B: eye aqueous humor concentration data normalize for dosing strength).

[0083] Figures 31A and 31B show the concentration versus time of AFD.v14 in cynomolgus monkey retinal homogenate following administration of AFD.v14 or AFD.v14.C + TP octamer in a pharmacokinetic study (31A: retinal concentration; 31B: retinal concentration data normalize for dosing strength).

[0084] Figures 32A – 32C show the concentration versus time of AFD.v14 in cynomolgus monkey serum following administration of AFD.v14 or AFD.v14.C + TP octamer in a pharmacokinetic study for both intravitreal and intravenous injection administration (32A: serum concentration for intravitreal injection; 32B: serum concentration for intravitreal injection normalize for dosing strength; 32C: serum concentration for intravenous administration).

[0085] Figures 33A – 33B shows a comparison of the Factor D concentration and AFD.v14.C + TP octamer in a pharmacokinetic study following the administration of the AFD.v14.C + TP octamer by either an intravenous or intravitreal injection (33A: serum concentrations; 33B: ocular concentrations).

[0086] Figures 34A – 34B show inhibition curves for a time-resolved fluorescence energy transfer (TR-FRET) assay of Factor D-dependent factor B activation (34A: Fab-tetramer conjugates as compared to unconjugated Fab; 34B: AFD.v14.C + TP octamer as compared to unconjugated Fab).

[0087] Figures 35A – 35E show the systemic AP complement activity as compared to total Factor D and therapeutic agent concentration in cynomolgus monkey serum following intravitreal injection administration (35A: 10 mg/eye lampalizumab (comparative data); 35B: 25 mg/eye AFD.v14; 35C: 3.9 mg/eye AFD.v14.C + TP octamer; 35D: 7.1 mg/eye AFD.v14.C + HG octamer; 35E: 11.8 mg/eye AFD.v14.C + HG octamer).

Detailed Description of the Invention

Definitions

[0088] Terms used throughout this application are to be construed with ordinary and typical meaning to those of ordinary skill in the art. However, Applicants desire that the following terms be given the particular definition as defined below.

[0089] The term "antibody" is used in the broadest sense, and specifically covers full length monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies) and antibody fragments so long as they exhibit the desired biological activity such as antigen-binding activity. Antibodies (Abs) and immunoglobulins (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific target, immunoglobulins include both antibodies and other antibody-like molecules which lack target specificity. Native antibodies and immunoglobulins are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end. The term “Antibody” as used herein expressly encompasses antibody fragments retaining antigen-binding activity.

[0090] An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody 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.

[0091] As used herein, an "anti-factor D antibody" means an antibody which specifically binds to Factor D in such a manner so as to inhibit or substantially reduce complement activation.

[0092] The term "Factor D" is used herein to refer to native sequence and variant Factor D polypeptides.

[0093] As used herein, the term "AFD.Ab" refers to any anti-Factor D antibody.

[0094] 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).

[0095] 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, 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: 56, 57, 59, 60, 61, 62, 68, and 74.

[0096] 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: 58. Typically, a Fab comprising an engineered cysteine (i.e., a Fab that is a THIOMAB) is a Fab-SH.

[0097] 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).

[0098] The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called hypervariable regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FRs). The variable domains of native heavy and light chains each comprise four FRs, largely adopting a β-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β-sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC).

[0099] Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an F(ab′)2 fragment that has two antigen-binding sites and is still capable of cross-linking antigen.

[00100] The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments (including Fab-C) differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Antibody fragments with free thiol groups may be indicated with an "-SH." 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. F(ab′)2 antibody fragments originally were produced as pairs of Fab′ fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[00101] "Fv" is the minimum antibody fragment which contains a complete antigen-recognition and antigen-binding site. This region consists of a dimer of one heavy chain and one light chain variable domain in tight, non-covalent association. It is in this configuration that the

three hypervariable regions of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six hypervariable regions confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three hypervariable regions specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[00102] 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. HVR-H3 is believed to play a unique role in conferring fine specificity to antibodies. See, e.g., Xu et al. (2000) Immunity 13:37-45; Johnson and Wu (2003) in Methods in Molecular Biology 248:1-25 (Lo, ed., Human Press, Totowa, N.J.). "Framework Region" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined. An HVR region as used herein comprise any number of residues located within positions 24-36 (for L1), 46-56 (for L2), 89-97 (for L3), 26-35B (for H1), 47-65 (for H2), and 93-102 (for H3). Therefore, an HVR includes residues in positions described previously:

A) 24-34 (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);

B) 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).

C) 30-36 (L1), 46-55 (L2), 89-96 (L3), 30-35 (H1), 47-58 (H2), 93-100a-j (H3) (MacCallum et al. J. Mol. Biol. 262:732-745 (1996).

[00103] Hypervariable regions may comprise "extended hypervariable regions" as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2) and 89-97 (L3) in the VL and 26-35B (H1), 50-65, 47-65 or 49-65 (H2) and 93-102, 94-102 or 95-102 (H3) in the VH. The variable domain residues are numbered according to Kabat *et al.*, *supra* for each of these definitions.

[00104] 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).)

[00105] An "antibody variant" or "modified antibody" of a reference antibody (also referred to as "starting antibody" or "parent antibody") is an antibody that comprises an amino acid sequence different from that of the reference/starting antibody, wherein one or more of the amino acid residues of the reference antibody have been modified. Generally, an antibody variant will possess at least 80% sequence identity, preferably at least 90% sequence identity, more preferably at least 95% sequence identity, and most preferably at least 98% sequence identity with the reference antibody. Percentage sequence identity is determined for example, by the Fitch et al., *Proc. Natl. Acad. Sci. USA,* 80: 1382-1386 (1983), version of the algorithm described by Needleman et al., *J. Mol. Biol.*, 48: 443-453 (1970), after aligning the sequences of the reference antibody and the candidate antibody variant to provide for maximum homology. Identity or similarity is defined herein as the percentage of amino acid residues in the candidate variant sequence that are identical (i.e., same residue) or similar (i.e., amino acid residue from the same group based on common side-chain properties, see below) with the parent antibody residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Amino acid sequence variants of an antibody may be prepared by introducing appropriate nucleotide changes into DNA encoding the antibody, or by peptide synthesis. Such variants include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequence of the antibody of interest. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the antibody, such as changing the number or position of glycosylation sites. Methods for generating antibody sequence variants of antibodies are similar to those for generating amino acid sequence variants of polypeptides described in U.S. Pat. No. 5,534,615, expressly incorporated herein by reference, for example.

[00106] 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.

[00107] 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.

[00108] 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.

[00109] Amino acid residues "prone" to certain identified physical or chemical processes (e.g., isomerization or deamidation) refer to those residues within a specific protein molecule that have been identified to have the propensity to undergo the identified processes such as isomerization or deamidation. Their propensities are often determined by their relative positions within the primary and/or conformational structure of the protein. For example, it has been shown that the first Asp in an Asp-XXX motif (wherein XXX can be Asp, Gly, His, Ser or Thr) is prone to Asp isomerization due to the involvement of its adjacent residue, where some other Asp within the same protein may not possess such propensity. Assays for identifying residues to certain process within a specific protein molecule are known in the art. See, e.g., Cacia et al (1996) *Biochem.* 35:1897-1903.

[00110] "Active" or "activity" or "biological activity" in the context of an anti-factor D antibody of the present disclosure is the ability to antagonize (partially or fully inhibit) a biological activity of Factor D. One example of a biological activity of a Factor D antagonist is the ability to achieve a measurable improvement in the state, e.g., pathology, of a Factor D-associated disease or condition, such as, for example, a complement-associated eye condition. The activity can be determined in *in vitro* or *in vivo* tests, including binding assays, alternative pathway hemolysis assays (e.g., assays measuring inhibition of the alternative pathway complement activity or activation), using a relevant animal model, or human clinical trials.

[00111] 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.

[00112] 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)).

[00113] "Treatment" (and grammatical variations thereof such as "treat" or "treating") is an intervention performed with the intention of preventing the development or altering the pathology of a disorder. Accordingly, "treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented. 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, conjugates 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.

[00114] 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.*

[00115] The term "mammal" as used herein refers to any animal classified as a mammal, including, without limitation, humans, higher primates, domestic and farm animals, and zoo, sports or pet animals such horses, pigs, cattle, dogs, cats and ferrets, *etc.* In some embodiments of the disclosure, the mammal is a human.

[00116] Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

[00117] "Therapeutically effective amount" is the amount of a "Factor D antagonist" which 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.

[00118] An "amino acid substitution" refers to the replacement of at least one existing amino acid residue in a predetermined amino acid sequence with another different "replacement" amino acid residue. The replacement residue or residues may be "naturally occurring amino acid residues" (*i.e.*, encoded by the genetic code) and selected from the group consisting of: alanine (ala); arginine (Arg); asparagine (Asn); aspartic acid (Asp); cysteine (Cys); glutamine (Gln); glutamic acid (Glu); glycine (Gly), histidine (His); isoleucine (Ile); leucine (Leu); lysine (Lys); methionine (Met); phenylalanine (Phe); proline (Pro); serine (Ser); threonine (Thr); tryptophan (Trp); tyrosine (Tyr); and valine (Val). Substitution with one or more non-naturally occurring amino acid residues is also encompassed by the definition of an amino acid substitution herein. A "non-naturally occurring amino acid residue" refers to a residue, other than those naturally occurring amino acid residues listed above, which is able to covalently bind adjacent amino acid residue(s) in a polypeptide chain. Examples of non-naturally occurring amino acid residues include norleucine, ornithine, norvaline, homoserine and other amino acid residue analogues such as those described in Ellman et al., *Meth. Enzym,* 202: 301-336 (1991). To generate such non-naturally occurring amino acid residues, the procedures of Noren et al., *Science*, 244: 182 (1989) and Ellman et al., *supra*, can be used. Briefly, these procedures involve chemically activating a suppressor tRNA with a non-naturally occurring amino acid residue followed by *in vitro* transcription and translation of the RNA.

[00119] An "amino acid insertion" refers to the incorporation of at least one amino acid into a predetermined amino acid sequence. While the insertion will usually consist of the insertion of one or two amino acid residues, the present application contemplates larger "peptide insertions", *e.g.,* insertion of about three to about five or even up to about ten amino acid residues. The inserted residue(s) may be naturally occurring or non-naturally occurring as disclosed above.

[00120] An “amino acid deletion” refers to the removal of at least one amino acid residue from a predetermined amino acid sequence.

[00121] 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 aspects, 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.

[00122] The term “port delivery system” refers to an implantable device for the eye with a refillable reservoir that allows delivery of a therapeutic agent over an extended period of time. Exemplary port delivery systems are described, e.g., in U.S. Patent Application Serial No. 2010/0174272, and U.S. Patent Nos. 8,277,830; 8,399,006; 8,795,712; and 8,808,727, all of which are herein incorporated by reference.

[00123] 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.

[00124] 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.

[00125] A “small-bore needle” or a “narrow-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.

[00126] “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.

[00127] 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.

[00128] 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.

[00129] 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.

[00130] A drug that is administered "simultaneously" 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.

Anti-Factor D Antibodies and Variants thereof

[00131] In some aspects, the present disclosure is directed to the production and use of conjugates comprising one or more anti-Factor D antibodies or variants thereof. Anti-Factor D antibodies and variants thereof that are suitable for use in forming the conjugates of the disclosure are described in U.S. Patent Application Serial No. 14/700853 (filed April 30, 2015), which is herein incorporated by reference in its entirety.

[00132] In some aspects, the parent reference anti-Factor D antibody forming the base for creating the variants used in the conjugates of the disclosure is a humanized anti-Factor D antibody. Methods for humanizing non-human antibodies are well known in the art. Generally,

a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al. (1986) *Nature* 321:522-525; Riechmann et al. (1988) *Nature* 332:323-327; Verhoeyen et al. (1988) *Science* 239:1534-1536), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[00133] The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies can in some instances be important to reduce antigenicity and/or HAMA response (human anti-mouse antibody) when the antibody is intended for human therapeutic use. Reduction or elimination of a HAMA response is generally a significant aspect of clinical development of suitable therapeutic agents. See, e.g., Khaxzaeli et al. (1988) *J. Natl. Cancer Inst* 80:937; Jaffers et al. (1986) *Transplantation* 41:572; Shawler et al. (1985) *J. Immunol.* 135:1530; Sears et al. (1984) *J. Biol. Response Mod.* 3:138; Miller et al. (1983) *Blood* 62:988; Hakimi et al. (1991*) J. Immunol.* 147:1352; Reichmann et al. (1988) *Nature* 332:323; Junghans et al. (1990) *Cancer Res.* 50:1495. As described herein, in some aspects, the present disclosure provides conjugates comprising antibodies that are humanized such that HAMA response is reduced or eliminated. Variants of these antibodies can further be obtained using routine methods known in the art, some of which are further described below. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable domain sequences. The human V domain sequence which is closest to that of the rodent is identified and the human framework region (FR) within it accepted for the humanized antibody (Sims et al. (1993) *J. Immunol.* 151:2296; Chothia et al. (1987) *J. Mol. Biol.*196:901). Another method uses a particular framework region derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:4285; Presta et al. (1993) *J. Immunol.* 151:2623).

[00134] For example, an amino acid sequence from an antibody as described herein can serve as a starting (parent) sequence for diversification of the framework and/or hypervariable

sequence(s). A selected framework sequence to which a starting hypervariable sequence is linked is referred to herein as an acceptor human framework. While the acceptor human frameworks may be from, or derived from, a human immunoglobulin (the VL and/or VH regions thereof), the acceptor human frameworks may be from, or derived from, a human consensus framework sequence as such frameworks have been demonstrated to have minimal, or no, immunogenicity in human patients. An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a VL or VH framework derived from a human immunoglobulin framework, or from a human consensus framework. An acceptor human framework "derived from" a human immunoglobulin framework or human consensus framework may comprise the same amino acid sequence thereof, or may contain pre-existing amino acid sequence changes. Where pre-existing amino acid changes are present, preferably no more than 5 and preferably 4 or less, or 3 or less, pre-existing amino acid changes are present. In some embodiments, the VH acceptor human framework is identical in sequence to the VH human immunoglobulin framework sequence or human consensus framework sequence. In some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence. A "human consensus framework" is a framework which represents the most commonly occurring amino acid residue 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.* In some embodiments, for the VL, the subgroup is subgroup kappa I as in Kabat *et al.* In some embodiments, for the VH, the subgroup is subgroup III as in Kabat *et al.*

[00135] Where the acceptor is derived from a human immunoglobulin, one may optionally select a human framework sequence that is selected based on its homology to the donor framework sequence by aligning the donor framework sequence with various human framework sequences in a collection of human framework sequences, and select the most homologous framework sequence as the acceptor. The acceptor human framework may be from or derived from human antibody germline sequences available in the public databases.

[00136] In some embodiments, human consensus frameworks herein are from, or derived from, VH subgroup VII and/or VL kappa subgroup I consensus framework sequences.

[00137] In some embodiments, the human framework template used for generation of an anti-Factor D antibody may comprise framework sequences from a template comprising a combination of VI-4.1b+ (VH7 family) and JH4d for VH chain and/or a combination of DPK4 (VκI family) and JK2 for VL chain.

[00138] While the acceptor may be identical in sequence to the human framework sequence selected, whether that be from a human immunoglobulin or a human consensus framework, the present disclosure contemplates that the acceptor sequence may comprise pre-existing amino acid substitutions relative to the human immunoglobulin sequence or human consensus framework sequence. These pre-existing substitutions are preferably minimal; usually four, three, two or one amino acid differences only relative to the human immunoglobulin sequence or consensus framework sequence.

[00139] Hypervariable region residues of the non-human antibody are incorporated into the VL and/or VH acceptor human frameworks. For example, one may incorporate residues corresponding to the Kabat CDR residues, the Chothia hypervariable loop residues, the Abm residues, and/or contact residues. Optionally, the extended hypervariable region residues as follows are incorporated: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2) and 89-97 (L3), 26-35B (H1), 50-65, 47-65 or 49-65 (H2) and 93-102, 94-102, or 95-102 (H3).

[00140] In some aspects, the anti-Factor D antibody or antibody variants used in the conjugates comprise a light chain domain and a heavy chain variable domain. In some aspects, the reference anti-Factor D antibody comprises a light chain variable domain of SEQ ID NO:3. In some aspects, the reference anti-Factor D antibody comprises a heavy chain variable domain of SEQ ID NO:4.

[00141] Further, an anti-Factor D antibody may comprise any suitable constant domain sequence, provided that the antibody retains the ability to bind Factor D. For example, in some embodiments, anti-Factor D antibodies used in the conjugates of the disclosure comprise at least a portion of a heavy chain constant domain. In some embodiments, anti-Factor D antibodies comprise a heavy chain constant domain of either one or a combination of an α, δ, ε, γ, or μ heavy chain. Depending on the amino acid sequence of the constant domain of their heavy chains (C_H), immunoglobulins can be assigned to different classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, having heavy chains designated α, δ, ε, γ, and μ, respectively. The γ and α classes are further divided into subclasses on the basis of relatively minor differences in C_H sequence and function, e.g., humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. In some embodiments, anti-Factor D antibodies used in the conjugates of the disclosure comprise a heavy chain constant domain comprising substitutions at amino acid positions that results in a desired effect on effector function (e.g., binding affinity). In some embodiments, anti-Factor D antibodies used in the conjugates of the disclosure comprise a heavy chain constant domain comprising substitutions at amino acid positions that do not result in an effect on effector function (e.g., binding affinity).

In some embodiments, the anti-Factor D antibodies comprise a heavy chain constant domain of the IgG type (e.g., IgG1, IgG2, IgG3 or IgG4) and further comprise a substitution at position 114 (Kabat numbering; equivalent to 118 in EU numbering), 168 (Kabat numbering; equivalent to 172 in EU numbering), 172 (Kabat numbering; equivalent to 176 in EU numbering) and/or 228 (EU numbering). In some embodiments, the anti-Factor D antibodies comprise a heavy chain constant domain of the IgG (e.g., IgG1, IgG2, IgG3 or IgG4) type and further comprise a substitution at position 114 wherein position 114 is a cysteine (C) or alanine (A), position 168 is cysteine (C) or alanine (A), position 172 is a cysteine (C) or alanine (A) and/or position 228 is a proline (P), arginine (R) or serine (S).

[00142] Further, for example, in some embodiments, anti-Factor D antibodies used in the conjugates of the disclosure comprise at least a portion of a light chain constant domain. In some embodiments, the anti-Factor D antibodies comprise a light chain constant domain of either one or a combination of a kappa or a lambda light chain, as the light chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. In some embodiments, anti-Factor D antibodies used in the conjugates of the disclosure comprise a light chain constant domain comprising substitutions at amino acid positions that results in a desired effect on effector function (e.g., binding affinity). In some embodiments, anti-Factor D antibodies used in the conjugates of the disclosure comprise a light chain constant domain comprising substitutions at amino acid positions that do not result in an effect on effector function (e.g., binding affinity). In some embodiments, anti-Factor D antibodies used in the conjugates of the disclosure comprise a light chain constant domain of the kappa type and further comprise a substitution at position 110, 144, 146 and/or 168 (Kabat numbering). In some embodiments, anti-Factor D antibodies used in the conjugates of the disclosure comprise a light chain constant domain of the kappa type and further comprise a substitution at position 110 wherein 110 is a cysteine (C) or valine (V), at position 144 wherein 144 is a cysteine (C) or alanine (A), at position 146 wherein 146 is a isoleucine (I) or valine (V) and/or at position 168 wherein 168 is a cysteine (C) or serine (S).

[00143] A parent or reference anti-Factor D antibody, including a humanized anti-Factor D antibody, can be modified to generate modified anti-Factor D antibodies, or anti-Factor D antibody variants. In some embodiments, the modified anti-Factor D antibodies, and variants thereof, may have improved physical, chemical, biological or homogeneity properties over the parent antibody.

[00144] In some embodiments, an antibody used in the conjugates of the disclosure comprises one or more amino acid alterations (e.g., substitutions) into one or more of the hypervariable regions of the parent antibody. Alternatively, or in addition, one or more alterations (e.g., substitutions) of framework region residues may be introduced in the parent antibody. Examples of framework region residues to modify include those which non-covalently bind antigen directly (Amit et al., (1986) *Science*, 233: 747-753); interact with/effect the conformation of a CDR (Chothia et al. (1987) *J. Mol. Biol.*, 196: 901-917), and/or participate in the V_L-V_H interface (EP 239 400B1). In certain embodiments, modification of one or more of such framework region residues results in an enhancement of the binding affinity of the antibody for the antigen. For example, from about one to about 5 framework residues may be altered in this embodiment of the disclosure. Examples of framework or HVR region residues to modify include sites, wherein modifications at such sites result in the generation of deamidated variants (for example, asparagine (N or Asn) residue(s) modified to aspartate (D or Asp), oxidation variants (for example, methionine (M or Met) residue(s) and/or tryptophan (W or Trp) residue(s) modified to sulfone or sulfoxide) or pyroglutamate variants (for example, glutamine (Q or Gln) residue(s) modified to pyroglutamate). Examples of framework region residues or HVR region residues to modify include possible deamidation sites (i.e., asparagine (N or Asn)), oxidation sites (i.e., methionine (M or Met) or tryptophan (W or Trp)) or pyroglutamate conversion sites (i.e., glutamine (Q or Gln)), wherein modification at such sites prevent deamidation and/or oxidation and/or pyroglutamate conversion, respectively.

[00145] To prevent the formation of deamidated variants, asparagine (N or Asn) may be mutated to alanine (A or Ala), glutamine (Q or Gln) or serine (S or Ser). To prevent the formation of oxidated variants, methionine (Met) or tryptophan (W or Trp) may be mutated to leucine (L) or isoleucine (I). To prevent the formation of pyroglutamate variants, glutamine (Q or Gln) may be mutated to glutamate (E or Glu). (Amphlett, G. et al., *Pharm. Biotechnol.*, 9:1-140 (1996)). Alternatively, or in addition, one or more alterations (e.g., substitutions) of framework region residues may be in the Fc region in the parent antibody.

[00146] One useful procedure for generating such modified antibodies is called "alanine scanning mutagenesis" (Cunningham and Wells (1989) *Science* 244:1081-1085). Here, one or more of the hypervariable region residue(s) are replaced by alanine or polyalanine residue(s) to affect the interaction of the amino acids with the antigen. Those hypervariable region residue(s) demonstrating functional sensitivity to the substitutions then are refined by introducing further or other mutations at or for the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* need not be

predetermined. The ala-mutants produced this way are screened for their biological activity (i.e., binding affinity or hemolysis assay) as described herein.

[00147] Even more substantial modifications in the antibodies or fragments thereof (e.g., antigen-binding fragments) biological properties are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on 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; and

(6) aromatic: trp, tyr, phe.

[00148] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[00149] In another embodiment, the sites selected for modification are modified, and those modifications with improved binding affinity are selected by phage display.

[00150] Nucleic acid molecules encoding amino acid sequence mutants or modified amino acid sequences are prepared by a variety of methods known in the art. These methods include, but are not limited to, oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the parent antibody. One method for making mutants or variants or modified amino acid sequences is site directed mutagenesis (see, *e.g.*, Kunkel (1985) *Proc. Natl. Acad. Sci. USA* 82:488).

[00151] In certain embodiments, the modified antibody will only have a single hypervariable region residue substituted. In other embodiments, two or more of the hypervariable region residues of the parent antibody will have been substituted, *e.g.,* from about two to about ten hypervariable region substitutions. Ordinarily, the modified antibody will have an amino acid sequence having at least 75% amino acid sequence identity or similarity (defined above in Definition section) with the amino acid sequence of either the heavy or light chain variable domain of the parent antibody, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, and most preferably at least 95%.

[00152] Following production of the modified antibody, the biological activity of that molecule relative to the parent antibody is determined. As noted above, this may involve determining the binding affinity and/or other biological activities of the antibody variant, or fragment thereof (e.g., antigen-binding fragment). In some embodiments of the disclosure, a panel of modified antibodies is prepared and screened for binding affinity for the antigen such as Factor D or a fragment thereof. One or more of the antibody mutants or modified antibodies selected from this initial screen are optionally subjected to one or more further biological activity assays to confirm that the antibody variant(s), or fragments thereof (e.g., antigen-binding fragments) are indeed useful, *e.g.,* for preclinical studies.

[00153] The modified anti-Factor D antibodies described herein may be subjected to further modifications, oftentimes depending on the intended use of the modified antibody. Such modifications may involve further alteration of the amino acid sequence, fusion to heterologous polypeptide(s) and/or covalent modifications such as those elaborated below. With respect to amino acid sequence alterations, exemplary modifications are elaborated above. For example, any cysteine residue not involved in maintaining the proper conformation of the modified antibody also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant cross linking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability (particularly where the antibody is an antibody fragment such as an Fv fragment).

[00154] Another type of amino acid mutant has an altered glycosylation pattern. This may be achieved by deleting one or more carbohydrate moieties found in the antibody, and/or adding one or more glycosylation sites that are not present in the antibody. Glycosylation of antibodies, or antibody fragments (e.g., antigen-binding fragments) is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used. Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The

alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

Affinity and Biological Activity of Anti-Factor D Antibodies and Variants Thereof

[00155] Antibodies having characteristics identified herein as being desirable in an anti-Factor D antibody, may be screened for desirable properties such as factor D-binding affinity and factor D-inhibiting activity *in vitro* or *in vivo*.

a. Affinity

[00156] In some aspects, the anti-Factor D antibody variants used in the conjugates of the disclosure compete with the parent anti-Factor D antibody from which they are generated. Anti-Factor D antibody variants that bind to the same epitope as the parent anti-Factor D antibody are also provided.

[00157] To determine whether an anti-Factor D antibody variant bind to the same epitope on human Factor D bound by a reference anti-Factor D antibody, a cross-blocking assay may be performed (Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988)). Alternatively, epitope mapping may be performed to determine whether an anti-Factor D antibody binds an epitope of interest (Champe et al. (1995) *J. Biol. Chem.* 270: 1388-1394. Antibody affinities, for example for human Factor D, may be determined using standard methods, including the surface plasmon resonance (SPR) assay described in more details in the Examples.

[00158] In some aspects, the factor D binding affinity of the anti-Factor D antibody variant used in the conjugates of the disclosure is comparable to that of the parent anti-Factor D antibody from which it is generated. In some aspects, the factor D binding affinity of anti-Factor D antibody variant used in the conjugates of the disclosure is within 10-fold, 7-fold, 5-fold, 2-fold or 1-fold of that of the parent anti-Factor D antibody.

[00159] In some embodiments, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is 20 nM ($20x10^{-9}$ M) or better. In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is 10 nM ($10x10^{-9}$ M) or better. In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is 1.0 nM ($1.0x10^{-9}$ M) or better. In another embodiment, the

disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is 0.5 nM ($0.5x10^{-9}$ M) or better. In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is 1.0 pM ($1.0x10^{-12}$ M) or better. In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is 0.5 pM ($0.5x10^{-12}$ M) or better.

[00160] In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as an IgG to Factor D) is 10.0 nM ($10.0x10^{-9}$ M) or better. In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as an IgG to Factor D) is 5.0 nM ($5.0x10^{-9}$ M) or better. In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as an IgG to Factor D) is 1.0 nM ($1.0x10^{-9}$ M) or better. In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as an IgG to Factor D) is 0.5 nM ($0.5x10^{-9}$ M) or better. In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as an IgG to Factor D) is 5.0 pM ($5.0x10^{-12}$ M) or better. In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as an IgG to Factor D) is 2.0 pM ($2.0x10^{-12}$ M) or better. In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as an IgG to Factor D) is 1.0 pM ($1.0x10^{-12}$ M) or better. In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as an IgG to Factor D) is 0.5 pM ($0.5x10^{-12}$ M) or better.

[00161] In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is between 0.5 mM ($0.5x10^{-6}$ M) and 0.5

pM ($0.5x10^{-12}$ M). In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is between 15 nM ($15x10^{-9}$ M) and 0.1 nM ($0.1x10^{-9}$ M). In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is between 5.5 nM ($5.5x10^{-9}$ M) and 1 nM ($1x10^{-9}$ M). In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is between 0.5 pM ($0.5x10^{-12}$ M) and 50 pM ($5x10^{-11}$ M).

[00162] In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as an IgG to Factor D) is between 0.5 mM ($0.5x10^{-6}$ M) and 0.5 pM ($0.5x10^{-12}$ M). In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, or antibody variants thereof, wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as an IgG to Factor D) is between 10 nM ($10x10^{-9}$ M) and 0.05 nM ($0.05x10^{-9}$ M). In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as an IgG to Factor D) is between 5.5 nM ($5.5x10^{-9}$ M) and 1 nM ($1x10^{-9}$ M). In another embodiment the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as an IgG to Factor D) is between 0.5 pM ($0.5x10^{-12}$ M) and 50 pM ($5x10^{-11}$ M).

[00163] In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is about 1.4 pM ($1.4x10^{-12}$ M). In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as a IgG to Factor D) is about 1.1 pM ($1.1x10^{-12}$ M). In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is about 0.19 nM ($0.19x10^{-9}$ M). In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as a IgG to Factor D) is about 0.08 nM ($0.08x10^{-9}$ M). In

another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is about 12.3 nM ($12.3x10^{-9}$ M). In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as an IgG to Factor D) is about 9.0 nM ($9.0x10^{-9}$ M).

[00164] In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is about 1.4 pM ($1.4x10^{-12}$ M) +/- 0.5. In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as an IgG to Factor D) is about 1.1 pM ($1.1x10^{-12}$ M) +/- 0.6. In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is about 0.19 nM ($0.19x10^{-9}$ M) +/- .01. In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as a IgG to Factor D) is about 0.08 nM ($0.08x10^{-9}$ M) +/- 0.01. In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is about 12.3 nM ($12.3x10^{-9}$ M) +/- 2. In another embodiment, the disclosure provides a conjugate comprising an anti-Factor D antibody, wherein the affinity of the antibody in its bivalent form to Factor D (e.g., affinity of the antibody as a IgG to Factor D) is about 9.0 nM ($9.0x10^{-9}$ M) +/- 1.

[00165] In another embodiment, an anti-Factor D antibody used in the conjugates of the disclosure may have an affinity in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) of about 1.4 pM ($1.4x10^{-12}$ M) +/- 2. In another embodiment, an anti-Factor D antibody used in the conjugates of the disclosure may have an affinity in its bivalent form to Factor D (e.g., affinity of the antibody as a IgG to Factor D) of about 1.1 pM ($1.1x10^{-12}$ M) +/- 2. In another embodiment, an anti-Factor D antibody used in the conjugates of the disclosure may have an affinity in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is about 0.19 nM ($0.19x10^{-9}$ M) +/- 2. In another embodiment, an anti-Factor D antibody, or antibody variant thereof, used in the conjugates of the disclosure may have an affinity in its bivalent form to Factor D (e.g., affinity of the antibody as a IgG to Factor D) is about 0.08 nM ($0.08x10^{-9}$ M) +/- 2. In another embodiment, an anti-

Factor D antibody used in the conjugates of the disclosure may have an affinity in its monovalent form to Factor D (e.g., affinity of the antibody as a Fab fragment to Factor D) is about 12.3 nM ($12.3x10^{-9}$ M) +/- 2. In another embodiment, an anti-Factor D antibody used in the conjugates of the disclosure may have an affinity in its bivalent form to Factor D (e.g., affinity of the antibody as a IgG to Factor D) is about 9.0 nM ($9.0x10^{-9}$ M) +/- 2.

[00166] As is well-established in the art, binding affinity of a ligand to its receptor can be determined using any of a variety of assays, and expressed in terms of a variety of quantitative values. Accordingly, in some embodiments, the binding affinity is expressed as K_D values and reflects intrinsic binding affinity (e.g., with minimized avidity effects). Generally and preferably, binding affinity is measured *in vitro*, whether in a cell-free or cell-associated setting. As described in greater detail herein, fold difference in binding affinity can be quantified in terms of the ratio of the monovalent binding affinity value of a humanized antibody (e.g., in Fab form) and the monovalent binding affinity value of a reference/comparator antibody (e.g., in Fab form) (e.g., a murine antibody having donor hypervariable region sequences), wherein the binding affinity values are determined under similar assay conditions. Thus, in some embodiments, the fold difference in binding affinity is determined as the ratio of the K_D values of the humanized antibody in Fab form and said reference/comparator Fab antibody. For example, in some embodiments, if an antibody of the disclosure (A) has an affinity that is "3-fold lower" than the affinity of a reference antibody (M), then if the K_D value for A is 3x, the K_D value of M would be 1x, and the ratio of K_D of A to K_D of M would be 3:1. Conversely, in some embodiments, if an antibody of the disclosure (C) has an affinity that is "3-fold greater" than the affinity of a reference antibody (R), then if the K_D value for C is 1x, the K_D value of R would be 3x, and the ratio of K_D of C to K_D of R would be 1:3. Any of a number of assays known in the art, including those described herein, can be used to obtain binding affinity measurements, including, for example, Biacore, radioimmunoassay (RIA) and ELISA.

[00167] Further, K_D values for an antibody used in the conjugates of the disclosure may vary depending on conditions of the particular assay used. For example, in some embodiments, binding affinity measurements may be obtained in an assay wherein the Fab or antibody is immobilized and binding of the ligand, i.e., Factor D, is measured or alternatively, the ligand, i.e., Factor D, for the Fab or antibody is immobilized and binding of the Fab or antibody is measured. In some embodiments, the binding affinity measurements may be obtained in an assay wherein the regeneration conditions may comprise (1) 10mM glycine or 4M $MgCl_2$ at pH 1.5, and (2) pH between pH of 1.0 and pH of 7.5, including pH of 1.5, pH of 5.0, pH of 6.0 and pH of 7.2. In some embodiments, the binding affinity measurements may be obtained in an

assay wherein the binding conditions may comprise (1) PBS or HEPES-buffered saline and (2) Tween-20, i.e., 0.1% Tween-20. In some embodiments, the binding affinity measurements may be obtained in an assay wherein the source of the ligand, i.e., Factor D, may be from commercially available sources. In some embodiments, binding affinity measurements may be obtained in an assay wherein (1) the Fab or antibody is immobilized and binding of the ligand, i.e., Factor D is measured, (2) the regeneration conditions comprise 4M $MgCl_2$ at pH 7.2 and (3) the binding conditions comprise HEPES-buffered saline, pH 7.2 containing 0.1% Tween-20. In some embodiments, binding affinity measurements may be obtained in an assay wherein (1) the ligand, i.e., Factor D, is immobilized and binding of the Fab or antibody is measured, (2) the regeneration conditions comprise 10mM glycine at pH 1.5 and (3) the binding conditions comprise PBS buffer.

b. Biological Activity

[00168] 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, hemolytic inhibition assays using rabbit RBCs may be used, including those described in Example 2. 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)

[00169] In some embodiments, the present disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{50} values less than 30 nM. In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies

inhibits alternative pathway hemolysis with IC_{50} values less than 15 nM. In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{50} values less than 10 nM. In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{50} values less than 5 nM.

[00170] In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{50} values between 30 nM and 2 nM. In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{50} values between 25 nM and 7 nM. In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{50} values between 20 nM and 12 nM. In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{50} values between 30 nM and 15 nM. In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{50} values between 12 nM and 8 nM. In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{50} values between 7 nM and 2 nM. In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{50} values between 6 nM and 3 nM. In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{50} values between 8 nM and 5 nM. In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{50} values between 5 nM and 2 nM. In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{50} values between 10 nM and 5 nM. In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with $IC5_{50}$ values between 8 nM and 2 nM. In some embodiments, the disclosure is directed to conjugates

comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{50} values between 7 nM and 3 nM. In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{50} values between 6 nM and 4 nM. In another embodiment, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with an IC_{50} value of about 4.7 nM ± 0.6 nM. In another embodiment, the disclosure is directed to anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with an IC_{50} value of about 6.4 nM ± 0.6 nM. In another embodiment, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with an IC_{50} value of about 3.5 nM ± 0.5 nM. In another embodiment, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with an IC_{50} value of about 4.4 nM ± 1.5 nM. In another embodiment, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with an IC_{50} value of about 10.2 nM ± 0.8 nM. In another embodiment, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with an IC_{50} value of about 23.9 nM ± 5.0 nM.

[00171] In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{90} values less than 80 nM. In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{90} values less than 50 nM. In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{90} values less than 40 nM. In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{90} values less than 20 nM. In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{50} values less than 15 nM.

[00172] In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{90} values between 80 nM and 10 nM. In some embodiments, the disclosure is

directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{90} values between 75 nM and 15 nM. In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{90} values between 70 nM and 20 nM. In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{90} values between 65 nM and 25 nM. In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{90} values between 60 nM and 30 nM. In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{90} values between 55 nM and 35 nM. In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{90} values between 50 nM and 40 nM. In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{90} values between 80 nM and 70 nM. In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{90} values between 55 nM and 25 nM. In some embodiments, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with IC_{90} values between 16 nM and 12 nM. In another embodiment, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with an IC_{90} value of about 14.0 nM ± 1.0 nM. In another embodiment, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with an IC_{90} value of about 38.0 nM ± 11.0 nM. In another embodiment, the disclosure is directed to conjugates comprising anti-Factor D antibodies, wherein a Fab fragment of such antibodies inhibits alternative pathway hemolysis with an IC_{90} value of about 72.6 nM ± 4.8 nM.

[00173] In some embodiments, the disclosure is directed to conjugates comprising an anti-Factor D antibody 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).

[00174] In some embodiments, the disclosure is directed to 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 conjugates comprising a humanized anti-Factor D antibody fragment (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 conjugates comprising a humanized anti-Factor D antibody fragment (e.g. antigen-binding fragment) that is capable of penetrating throughout the entire thickness of the retina.

[00175] 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 another embodiment, 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.

Construction of Anti-Factor D Antibody or Antibody Variant–Polymer Conjugates

a. Multi-armed Polymers

[00176] In some aspects, the conjugates of the present disclosure can be made by derivatizing the anti-Factor D antibodies or antibody variants 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.

[00177] 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.

[00178] In some aspects, the anti-Factor D antibodies or antibody variants are derivatized 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.

[00179] 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.

[00180] 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.

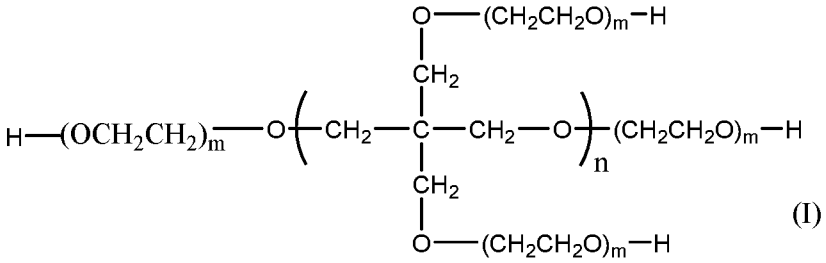
[00181] A variety of methods for PEGylating proteins are known in the art. Specific methods of producing proteins conjugated to PEG include the methods described in U.S. Pat. No. 4,179,337, U.S. Pat. No. 4,935,465, and U.S. Patent No. 5,849,535, all of which are herein incorporated by reference in their 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.

[00182] 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.

[00183] 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.

[00184] In some embodiments, the multi-armed PEG used to prepare the conjugates of the present disclosure has the structure of general formula (I):

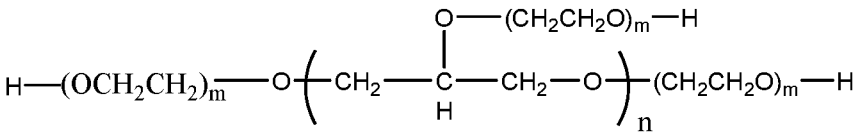


(I)

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, 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.

[00185] 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.

[00186] In another aspect, the multi-armed PEG used to prepare the conjugates of the present disclosure has the structure of general formula (II):

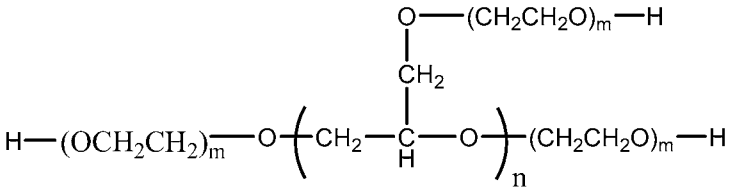


(II)

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, 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.

[00187] 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.

[00188] In another aspect, the multi-armed PEG used to prepare the conjugates of the present disclosure has the structure of general formula (III):

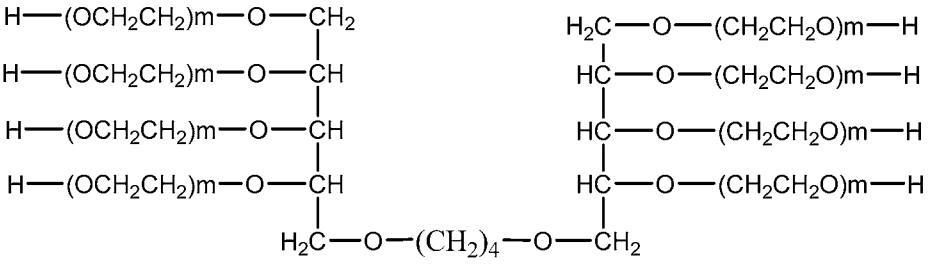


(III)

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, 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.

[00189] 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.

[00190] In another aspect, the multi-armed PEG used to prepare the conjugates of the present disclosure has the structure of general formula (IV):



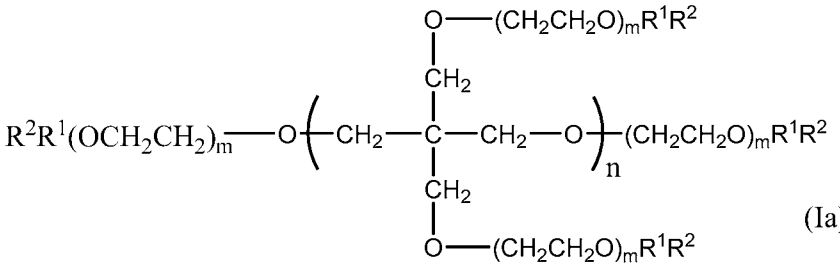
(IV)

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, or from about 3 to about 250, or from about 50 to about 200, or from about 100 to about 150.

[00191] 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 or antibody variants 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.

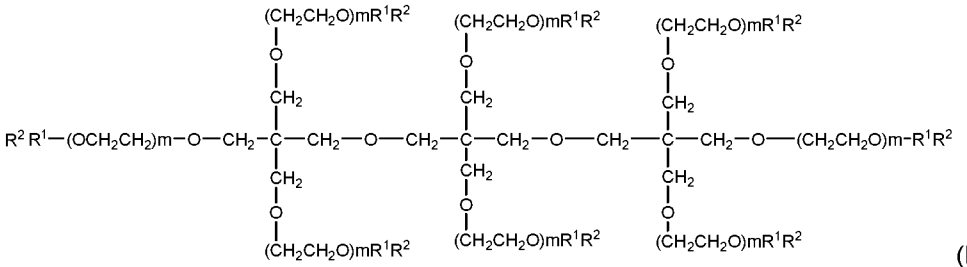
[00192] In other aspects, the multi-armed PEG used to prepare the conjugates of the present disclosure is a functionalized multi-armed PEG comprising at least one terminal reactive group. The terminal reactive group can conjugate directly to the anti-Factor D antibodies or antibody variants to form the conjugates of the present disclosure. In some embodiments, the functionalized multi-armed PEG has the structure of general formula (Ia):



(Ia)

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 the group consisting of a thiol reactive group, an amino reactive group, and combinations thereof.

[00193] 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):

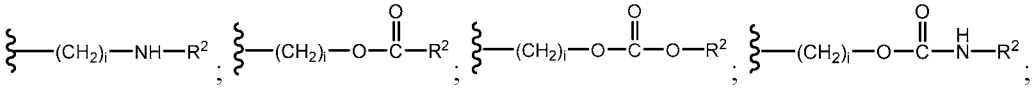


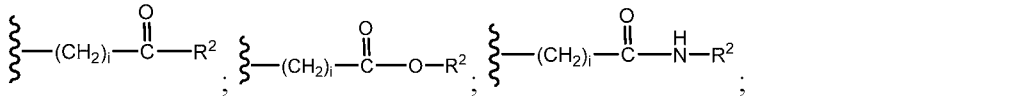
(Ib)

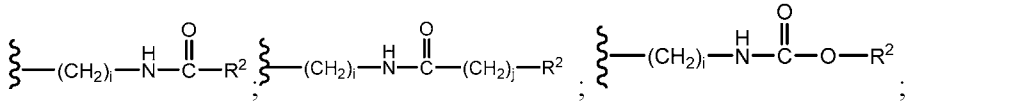
wherein m, R^1, and R^2 are as defined above. In particular, in one embodiment, each m denotes the length or size of the particular arm of the polyol (PEG) and is independently an integer of from about 45 to about 1000, or from about 3 to about 250, or from about 50 to about 200, or from about 100 to 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 the group consisting of a thiol reactive group, an amino reactive group, and combinations thereof.

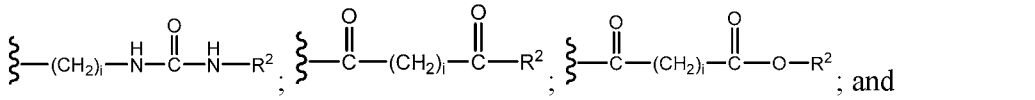
[00194] Multi-armed PEGs having the structure of general formula (Ib) have a tripentaerythritol (TP) core structure, and are also referred to herein as TP octamers.

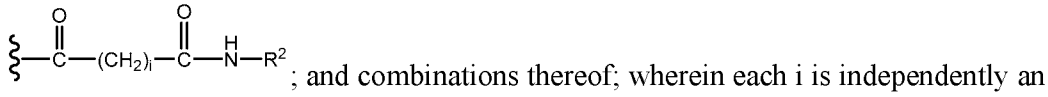
[00195] In some embodiments, the functionalized multi-armed PEG has the structure of general formula (Ia) or (Ib), wherein each R^1, when present, is the same or different, and R^1 and R^2 when taken together are selected from the group consisting of ;

 ;

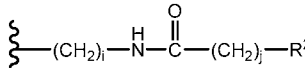
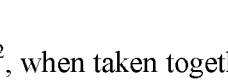
 ;

 ;

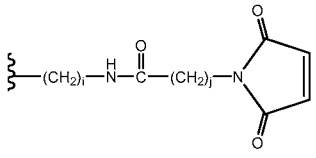
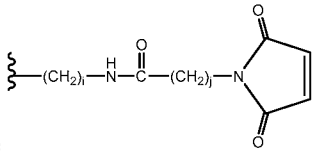
 ;

 ; and

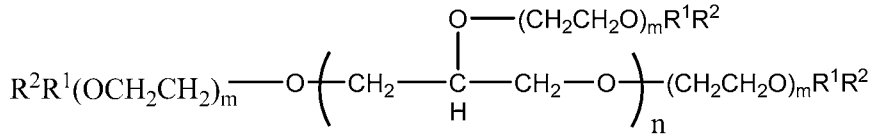
; and combinations thereof; wherein each i is independently an integer of 0-10; j is an integer of 0-10; and R^2 is as defined herein. In some embodiments, each R^1 is a linking group.

[00196] 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.

[00197] In some aspects, the functionalized multi-armed PEG has the structure of general formula (Ia) or (Ib), wherein each R^2 is independently selected from the group consisting of 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 the group consisting of bromoacetate, iodoacetate, chloroacetate, and combinations thereof. In some embodiments, each R^2 is independently a haloacetamide selected from the group consisting of bromoacetamide, iodoacetamide, chloroacetamide, and combinations thereof. In some embodiments, R^2 is a maleimide.

[00198] 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 one embodiment, 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.

[00199] 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)

wherein each m denotes the length or size of the particular arm of the polyol (PEG) and is independently an integer of 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 the group consisting of a thiol reactive group, an amino reactive group, and combinations thereof.

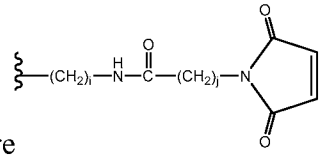
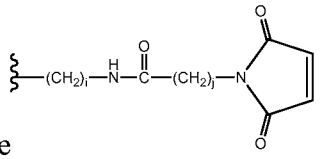
[00200] In some embodiments, the functionalized multi-armed PEG has the structure of general formula (IIa), wherein n is an integer from 2 to 6. In some embodiments, the functionalized multi-armed PEG has the structure of general formula (IIa), wherein n is 3. In another embodiment, the functionalized multi-armed PEG has the structure of general formula (IIa), wherein n is 2, and the multi-armed PEG is a tetramer. In 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.

[00201] 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 the group consisting of $\sim\!-(CH_2)-R^2$; $\sim\!-(CH_2)_i-NH-R^2$; $\sim\!-(CH_2)_i-O-C(=O)-R^2$; $\sim\!-(CH_2)_i-O-C(=O)-O-R^2$; $\sim\!-(CH_2)_i-O-C(=O)-NH-R^2$; $\sim\!-(CH_2)_i-C(=O)-R^2$; $\sim\!-(CH_2)_i-C(=O)-O-R^2$; $\sim\!-(CH_2)_i-C(=O)-NH-R^2$; $\sim\!-(CH_2)_i-NH-C(=O)-R^2$; $\sim\!-(CH_2)_i-NH-C(=O)-(CH_2)_j-R^2$; $\sim\!-(CH_2)_i-NH-C(=O)-O-R^2$; $\sim\!-(CH_2)_i-NH-C(=O)-NH-R^2$; $\sim\!-C(=O)-(CH_2)_i-C(=O)-R^2$; $\sim\!-C(=O)-(CH_2)_i-C(=O)-O-R^2$; and $\sim\!-C(=O)-(CH_2)_i-C(=O)-NH-R^2$; 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.

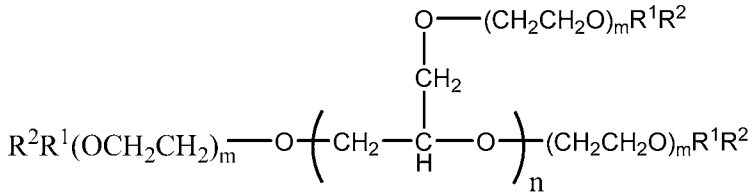
[00202] In some embodiments, the functionalized multi-armed PEG has the structure of general formula (IIa), wherein R^1 and R^2, when taken together, are $\sim\!-(CH_2)_i-NH-C(=O)-(CH_2)_j-R^2$, wherein i, j, and R^2 are as defined herein. In some embodiments, R^1 and R^2, when taken together, are $\sim\!-(CH_2)_i-NH-C(=O)-(CH_2)_j-R^2$, wherein i is 2; j is 2 or 3, and R^2 is as defined herein.

[00203] In some aspects, the functionalized multi-armed PEG has the structure of general formula (IIa), wherein each R^2 is independently selected from the group consisting of 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 the group consisting of bromoacetate, iodoacetate, chloroacetate, and combinations thereof. In some embodiments, each R^2 is independently a haloacetamide selected from the group consisting of bromoacetamide, iodoacetamide, chloroacetamide, and combinations thereof. In some embodiments, R^2 is a maleimide.

[00204] 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.

[00205] In another aspect, the functionalized multi-armed PEG has the structure of general formula (IIIa):

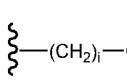
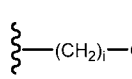
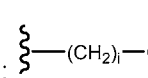
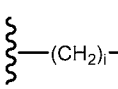
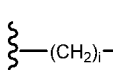
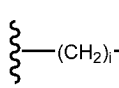
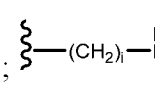
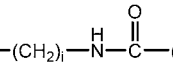
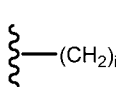
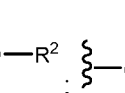
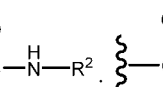
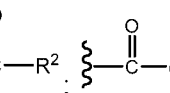
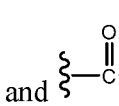
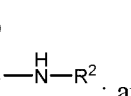
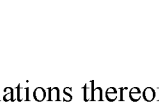
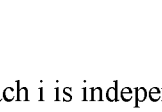
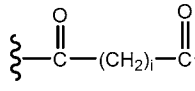


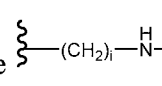
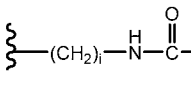
(IIIa)

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 the group consisting of a thiol reactive group, an amino reactive group, and combinations thereof.

[00206] 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

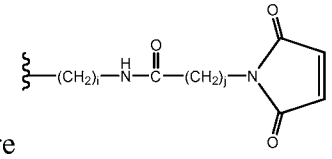
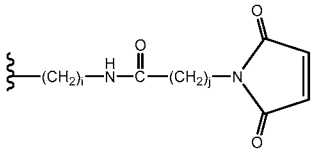
the multi-armed PEG is a tetramer. In another embodiment, the functionalized multi-armed PEG has the structure of general formula (IIIa), wherein n is 4, and the multi-armed PEG is a hexamer. In another embodiment, the functionalized multi-armed PEG has the structure of general formula (IIIa), wherein 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.

[00207] 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 the group consisting of ; ;  ;  ;  ;  ;  ;  ;  ;  ;  ;  ;  ;  ;  ;  ;  ;  ; 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.

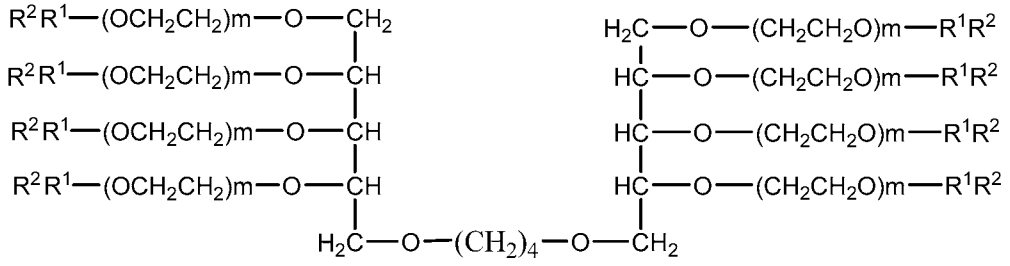
[00208] In some embodiments, the functionalized multi-armed PEG has the structure of general formula (IIIa), wherein R^1 and R^2, when taken together, are  , wherein i, j, and R^2 are as defined herein. In some embodiments, R^1 and R^2, when taken together, are  , wherein i is 2; j is 2 or 3, and R^2 is as defined herein.

[00209] In some aspects, the functionalized multi-armed PEG has the structure of general formula (IIIa), wherein each R^2 is independently selected from the group consisting of 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 the group consisting of bromoacetate, iodoacetate, chloroacetate, and combinations thereof. In some embodiments, each R^2 is independently a haloacetamide selected

from the group consisting of bromoacetamide, iodoacetamide, chloroacetamide, and combinations thereof. In some embodiments, R^2 is a maleimide.

[00210] 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.

[00211] In another aspect, the functionalized multi-armed PEG has the structure of general formula (IVa):

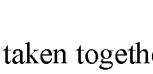
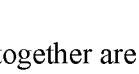
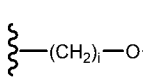
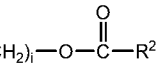
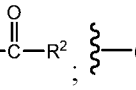
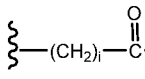
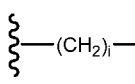
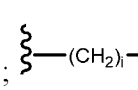
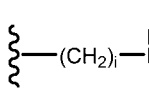
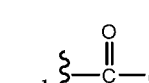


(IVa)

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 the group consisting of a thiol reactive group, an amino reactive group, and combinations thereof.

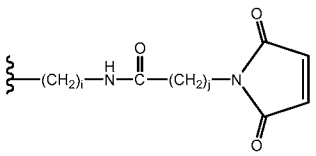
[00212] Multi-armed PEGs having the structure of general formula (IVa) have a butanediol core structure, and are also referred to herein as DX octamers.

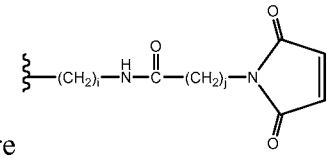
[00213] In some embodiments, the functionalized multi-armed PEG has the structure of general formula (IVa), wherein each R^1, when present, is the same or different, and R^1 and R^2

when taken together are selected from the group consisting of  ;  ;  ;  ;  ; ;  ;  ;  ; ;  ; ; ; ;  ; ; ; ; 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.

[00214] 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.

[00215] In some aspects, each R^2 is independently selected from the group consisting of 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 the group consisting of bromoacetate, iodoacetate, chloroacetate, and combinations thereof. In some embodiments, each R^2 is independently a haloacetamide selected from the group consisting of bromoacetamide, iodoacetamide, chloroacetamide, and combinations thereof. In some embodiments, R^2 is a maleimide.

[00216] 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.

[00217] The multi-armed PEGs used to prepare the conjugates of the disclosure preferably will have a low polydispersity of PEG chain (arm) length. In particular, 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.

[00218] 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.

[00219] 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 an average molecular weight of 40,000 D.

b. Conjugates

[00220] 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).

[00221] The conjugates of the present disclosure may be characterized by the number of anti-Factor D antibodies or antibody variants conjugated to each multi-armed PEG. This is referred to herein as "fabylation" or "degree of fabylation". The number of anti-Factor D

antibodies or antibody variants 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.

[00222] In one preferred embodiment, 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 or antibody variants 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 or antibody variants 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 or antibody variants 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 or antibody variants 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 or antibody variants are covalently linked to the PEG. In another embodiment, the conjugate comprises an eight-armed PEG, wherein at least seven anti-Factor D antibodies or antibody variants 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 or antibody variants 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 or antibody variants 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 or antibody variants are covalently linked to the PEG. In another embodiment, the conjugate of the disclosure comprises an eight-armed PEG, wherein from 7-8 anti-Factor D antibodies or antibody variants are covalently linked to the PEG.

[00223] 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.

[00224] In some aspects, 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 Figure 1).

[00225] 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.

[00226] 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.

[00227] 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 domain, 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.

[00228] In some aspects, 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.

[00229] 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: 21), 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 (SEQ ID NO:111, at the C'-terminal end for the purpose of providing one attachment site for polymer conjugation. In still another embodiment, an anti-Factor D antibody variant Fab fragment described herein is modified by adding four additional residues, Cys-Pro-Pro-Ser (SEQ ID NO: 112), at the C'-terminal end for the purpose of providing one attachment site for polymer conjugation. In still another embodiment, an anti-Factor D antibody variant Fab fragment described herein is modified by adding four additional residues, Ala-Pro-Pro-Cys (SEQ ID NO:113), at the C'-terminal end for the purpose of providing one attachment site 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-Gly-Gly-Cys (SEQ ID NO:114), at the C'-terminal end for the purpose of providing one attachment site for polymer conjugation. In still another embodiment, an anti-Factor D antibody variant Fab

fragment described herein has a C'-terminal end that has been modified to end in "CYGPPC", providing one attachment site for polymer conjugation.

[00230] 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.

[00231] 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.

c. Characterization and Activity

[00232] 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.

[00233] 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.

[00234] PEGylation changes the physical and chemical properties of the antibody drug, and may results in improved pharmacokinetic behaviors, including but not limited to: improved stability; decreased immunogenicity; increased hydrodynamic radius (RH); and/or extended circulating life, as well as increased ocular residence time.

[00235] 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 1.5 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.

[00236] In some embodiments, the conjugates of the present disclosure may have a hydrodynamic radius (RH), as determined using methods known in the art, of from about 3 nm to about 30 nm or more, or alternatively from about 5 nm to about 25 nm, and in some embodiments may be about 5 nm, about 10 nm, about 15 nm, about 20 nm, about 25 nm, or more.

[00237] In some embodiments, the conjugates of the present disclosure may exhibit a stability, characterized by the %loss of binding capacity (e.g., fD-binding capacity) per month measured using methods known in the art (e.g., when exposed to physiological conditions), of about 15%, about 12%, about 10%, about 8%, or even less.

[00238] In some embodiments, the conjugates of the present disclosure may exhibit an IC50 potency value, as determined using a time-resolved fluorescence energy transfer (TR-FRET) assay of Factor D-dependent Factor B activation, as detailed in the examples. In some embodiments, the conjugates inhibit Factor D-dependent Factor B activation with IC_{50} values of from about 25 pM to about 10 nM, or from about 25 pM to about 5 nM, or from about 25 pM to about 1 nM, or from about 25 pM to about 750 pM, or from about 25 pM to about 500 pM.

[00239] 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, less than 300 cP, less than 200 cP, less than 100 cP, less than 50 cP, or less than 30 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.

Pharmaceutical Formulations

[00240] Therapeutic formulations of the conjugates of the present disclosure thereof may be prepared for storage as lyophilized formulations or aqueous solutions by mixing the conjugate having the desired degree of purity with optional "pharmaceutically-acceptable" carriers, excipients or stabilizers typically employed in the art (all of which are termed "excipients"). For example, buffering agents, stabilizing agents, preservatives, isotonifiers, non-

ionic detergents, antioxidants and other miscellaneous additives. (See Remington's Pharmaceutical Sciences, 16th edition, A. Osol, Ed. (1980)). Such additives must be nontoxic to the recipients at the dosages and concentrations employed.

[00241] Buffering agents help to maintain the pH in the range which approximates physiological conditions. They are preferably present at concentration ranging from about 2 mM to about 50 mM. Suitable buffering agents for use with the present disclosure include both organic and inorganic acids and salts thereof such as citrate buffers (e.g., monosodium citrate-disodium citrate mixture, citric acid-trisodium citrate mixture, citric acid-monosodium citrate mixture, etc.), succinate buffers (e.g., succinic acid-monosodium succinate mixture, succinic acid-sodium hydroxide mixture, succinic acid-disodium succinate mixture, etc.), tartrate buffers (e.g., tartaric acid-sodium tartrate mixture, tartaric acid-potassium tartrate mixture, tartaric acid-sodium hydroxide mixture, etc.), fumarate buffers (e.g., fumaric acid-monosodium fumarate mixture, etc.), fumarate buffers (e.g., fumaric acid-monosodium fumarate mixture, fumaric acid-disodium fumarate mixture, monosodium fumarate-disodium fumarate mixture, etc.), gluconate buffers (e.g., gluconic acid-sodium glyconate mixture, gluconic acid-sodium hydroxide mixture, gluconic acid-potassium glyuconate mixture, etc.), oxalate buffer (e.g., oxalic acid-sodium oxalate mixture, oxalic acid-sodium hydroxide mixture, oxalic acid-potassium oxalate mixture, etc.), lactate buffers (e.g., lactic acid-sodium lactate mixture, lactic acid-sodium hydroxide mixture, lactic acid-potassium lactate mixture, etc.) and acetate buffers (e.g., acetic acid-sodium acetate mixture, acetic acid-sodium hydroxide mixture, etc.). Additionally, there may be mentioned phosphate buffers, histidine buffers and trimethylamine salts such as Tris.

[00242] Preservatives may be added to retard microbial growth, and may be added in amounts ranging from 0.2%-1% (w/v). Suitable preservatives for use with the present disclosure include phenol, benzyl alcohol, meta-cresol, methyl paraben, propyl paraben, octadecyldimethylbenzyl ammonium chloride, benzalconium halides (e.g., chloride, bromide, iodide), hexamethonium chloride, alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, and 3-pentanol.

[00243] Isotonicifiers sometimes known as "stabilizers" may be added to ensure isotonicity of liquid compositions of the present disclosure and include polyhydric sugar alcohols, preferably trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol.

[00244] Stabilizers refer to a broad category of excipients which can range in function from a bulking agent to an additive which solubilizes the therapeutic agent or helps to prevent denaturation or adherence to the container wall. Typical stabilizers can be polyhydric sugar

alcohols (enumerated above); amino acids such as arginine, lysine, glycine, glutamine, asparagine, histidine, alanine, ornithine, L-leucine, 2-phenylalanine, glutamic acid, threonine, etc., organic sugars or sugar alcohols, such as lactose, trehalose, stachyose, mannitol, sorbitol, xylitol, ribitol, myoinisitol, galactitol, glycerol and the like, including cyclitols such as inositol; polyethylene glycol; amino acid polymers; sulfur containing reducing agents, such as urea, glutathione, thioctic acid, sodium thioglycolate, thioglycerol, .alpha.-monothioglycerol and sodium thio sulfate; low molecular weight polypeptides (i.e., <10 residues); proteins such as human serum albumin, bovine serum albumin, gelatin or immunoglobulins; hydrophylic polymers, such as polyvinylpyrrolidone monosaccharides, such as xylose, mannose, fructose, glucose; disaccharides such as lactose, maltose, sucrose and trisaccacharides such as raffinose; polysaccharides such as dextran. Stabilizers may be present in the range from 0.1 to 10,000 weights per part of weight active protein.

[00245] Non-ionic surfactants or detergents (also known as "wetting agents") may be added to help solubilize the therapeutic agent as well as to protect the therapeutic protein against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stressed without causing denaturation of the protein. Suitable non-ionic surfactants include polysorbates (20, 80, etc.), polyoxamers (184, 188 etc.), Pluronic® polyols, polyoxyethylene sorbitan monoethers (Tween®-20, Tween®-80, etc.). Non-ionic surfactants may be present in a range of about 0.05 mg/ml to about 1.0 mg/ml, preferably about 0.07 mg/ml to about 0.2 mg/ml.

[00246] Additional miscellaneous excipients include bulking agents, (e.g. starch), chelating agents (e.g. EDTA), antioxidants (e.g., ascorbic acid, methionine, vitamin E), and cosolvents. 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. For example, it may be desirable to further provide an immunosuppressive agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended. The active ingredients may also be entrapped in microcapsule prepared, for example, by coascervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsule and poly-(methylmethacylate) microcapsule, respectively, in colloidal drug delivery systems (for example, liposomes, albumin micropheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington 's Pharmaceutical Sciences, 16th edition, A. Osal, Ed. (1980).

[00247] The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished, for example, by filtration through sterile filtration membranes. Sustained-

release preparations may be prepared. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing the antibody, or antibody variant or fragment (e.g. antigen-binding fragment) thereof, 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.

[00248] The conjugates of the disclosure 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 conjugates of the disclosure 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.

[00249] 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. In one embodiment, administration is intravitreal using a narrow bore needle. In one embodiment, the narrow bore needle is 30, 29, 28, 27, 26, 25, 24, 23, or 22 gauge.

[00250] 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.

[00251] The amount of antibody or antibody variant thereof which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. Where possible, it is desirable to determine the dose-response curve and the pharmaceutical compositions of the disclosure first *in vitro*, and then in useful animal model systems prior to testing in humans.

[00252] In some embodiments, the antibodies and conjugates described herein may be formulated to provide a concentration of antibody in the formulations of at least 50 mg/mL, at

least 75 mg/mL, at least 100 mg/mL, at least 125 mg/mL, at least 150 mg/mL, at least 175 mg/mL, at least 200 mg/mL, at least 225 mg/mL, at least 250 mg/mL, at least 275 mg/mL, at least 300 mg/mL, at least 325 mg/mL, at least 350 mg/mL, at least 375 mg/mL, at least 400 mg/mL, at least 425 mg/mL, at least 450 mg/mL, at least 475 mg/mL, or at least 500 mg/mL. In some embodiments, the antibody in the formulation is in an amount of at least 100 mg/mL. In some embodiments, the antibody in the formulation is in an amount of at least 200 mg/mL. In some embodiments, the antibody in the formulation is in an amount of at least 300 mg/mL. In some embodiments, the antibodies and conjugates described herein may be formulated to provide a concentration of antibody in the formulations of from about 50 mg/mL to about 500 mg/mL, about 50 mg/mL to about 300 mg/mL, about 100 mg/mL to about 500 mg/mL, about 100 mg/mL to about 300 mg/mL, about 200 mg/mL to about 500 mg/mL, about 200 mg/mL to about 400 mg/mL, about 200 mg/mL to about 300 mg/mL, or about 250 mg/mL to about 375 mg/mL.

[00253] In some embodiments, an aqueous solution of conjugate comprising therapeutic polypeptide, antibody, or antibody variant thereof, or fragment thereof (e.g. antigen-binding fragment), is administered by subcutaneous injection. In another embodiment, an aqueous solution of conjugate comprising therapeutic polypeptide, antibody, or antibody variant thereof, or fragment thereof (e.g. antigen-binding fragment) is administered by intravitreal injection. Each dose may range from about 0.3 mg to about 30 mg per eye.

[00254] The dosing schedule for subcutaneous 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.

Articles of Manufacture and Kits

[00255] Another embodiment of the disclosure is an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of conditions targeted by the antibodies of the disclosure, or variants thereof or fragments thereof (e.g. antigen-binding fragments). For example, the disclosure concerns an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of complement-associated disorders. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for treating, preventing and/or diagnosis of the complement-associated condition and may have a sterile access port (for example the container

may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an anti-Factor D antibody conjugate of the disclosure. The label or package insert indicates that the composition is useful for treatment, prevention and/or diagnosis of a particular condition.

[00256] Package insert refers 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. 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 will further comprise instructions for administering the antibody composition to the patient.

[00257] Additionally, the article of manufacture may further comprise a second 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.

[00258] 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.

Therapeutic Uses

[00259] The conjugates of the present disclosure may be used to treat a mammal. In some embodiments, the 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.

[00260] The conjugate is administered by any suitable means, including parenteral, subcutaneous, intraperitoneal, intrapulmonary, and intranasal, and, if desired for local immunosuppressive treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or 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. In one embodiment, administration is intravitreal using a narrow bore needle. In one embodiment, the narrow bore needle is 30, 29, 28, 27, 26, 25, 24, 23, or 22 gauge. In one embodiment, the dosing is administered using an implantable port delivery system.

[00261] For the prevention or treatment of disease, the appropriate dosage of the 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.

[00262] Depending on the type and severity of the disease, a sufficient amount of conjugate may be administered to provide from about 1 to about 25 mg/eye (i.e., from about 0.015 mg/kg to about 0.36 mg/kg, assuming one eye is treated) of the antibody to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage of the conjugate might be sufficient to provide the antibody in a range from about 1 to about 20 mg/eye or more, or from about 1 to about 15 mg/eye or more, depending on the factors mentioned above. 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.

[00263] 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.

[00264] The antibodies disclosed herein which recognize Factor D as their target and the conjugates comprising these antibodies may be used to treat complement-mediated (complement-associated) disorders in a subject. 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. The disorder may be systemic. It may also include 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. In one embodiment, the complement-associated disorder is a complement-associated eye condition. In one embodiment, the complement-associated eye condition is selected from the group consisting of 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 one embodiment, the complement-associated eye condition is selected from intermediate dry form AMD or geographic atrophy (GA).

[00265] A conjugate comprising Factor D antagonist 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 one example, the Factor D antagonist is an anti-Factor D antibody. In a further example, the anti-Factor D antibody is an anti-Factor D antibody variant described herein. 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), and a second complement component antagonist. In one example, the second therapeutic compound is a HTRA1 antagonist, an ANG2 antagonist, a TIE2 antagonist, or a VEGF antagonist. In a further example, the HTRA1 antagonist is an anti-HTRA1 antibody. In another embodiment, the ANG2 antagonist is an anti-ANG2 antibody. In another embodiment, the TIE2 antagonist is an anti-TIE2 antibody. In some embodiments, the VEGF antagonist is selected from a VEGF

trap (such as aflibercept (Eylea®) and an anti-VEGF antibody (such as bevacizumab (Avastin®) or ranabizumab (Lucentis®)).

[00266] Other therapeutic agents suitable for combined administration with the conjugates comprising an anti-Factor D antibody as disclosed herein are antagonists of various members of the classical or alternative complement pathway (complement inhibitors). Thus, the conjugates disclosed herein may be administered in combination with antagonists of one or more of the C1, C2, C3, C4, C5, C6, C7, C8, and C9 complement components. In some embodiments, the conjugates comprising anti-Factor D disclosed herein are combined with antagonists of the C2 and/or C4 and/or C5 complement components, such as anti-C2 and/or anti-C4 and/or anti-C5 antibodies. Such antibodies are known in the art and/or are commercially available. An anti-C5 antibody eculizumab (Alexion, Cheshire, CT, USA), has been approved for the treatment of Paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic uremic syndrome (aHUS). Other complement inhibitors are disclosed, for example, in US Publication No. 20050036991 A1. Thus, the conjugates comprising anti-Factor D antibody as disclosed herein may be administered in combination with an effective amount of one or more complement inhibitors, including, without limitation, anti-C2 and anti-C5 antibodies, optionally in combination with at least one additional Factor D antagonist/antibody.

[00267] 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 a Factor D antagonist or a conjugate comprising the Factor D antagonist, and further comprising administering to the subject an effective amount of a second therapeutic compound, that is a HTRA1 antagonist, an ANG2 antagonist, a TIE2 antagonist, a VEGF antagonist, and/or an antagonist of one or more of the C1, C2, C3, C4, C5, C6, C7, C8, and C9 complement components. In one example, the Factor D antagonist is an anti-Factor D antibody, and the conjugate comprises one or more anti-factor D antibody. In a further example, the anti-Factor D antibody is an anti-Factor D antibody variant described herein, and the conjugate comprises one or more anti-Factor D antibody variant. In one example, the HTRA antagonist is an anti-HTRA1 antibody. In another example, the ANG2 antagonist is an anti-ANG2 antibody. In another example, the TIE2 antagonist is an anti-TIE2 antibody. In another example, the VEGF antagonist is an anti-VEGF antibody. In another embodiment, the antagonist of the C2 and/or C4 and/or C5 complement components is as anti-C2 and/or anti-C4 and/or anti-C5 antibody. In one example, the complement-mediated disorder is an complement-associated eye condition. In one example, 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 one example, the complement-associated eye condition is geographic atrophy. In one example, the complement-associated eye condition is wet AMD (choroidal neovascularization (CNV)).

[00268] 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.

EXAMPLES

[00269] The following examples are offered by way of illustration and not by way of limitation. Commercially available reagents referred to in the examples were used according to manufacturer's instructions unless otherwise indicated. The source of those cells identified in the following examples, and throughout the specification, by ATCC accession numbers is the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209.

Example 1: Generation of Anti-Factor D Antibody Variants

[00270] Lampalizumab, 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 (FCFD4515S; hereinafter "aFD") is an antibody Fab fragment comprised of a 214 residue light chain (SEQ ID NO:1) and a 223 residue heavy chain (SEQ ID NO:2).

[00271] While results of a phase II human clinical trial in GA indicate that a treatment effect is obtained with monthly intravitreal injection of aFD, 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.

[00272] Efforts were made to further improve the wild type aFD (WT)'s physical and chemical stabilities, especially under low pH conditions and/or at high concentrations under neutral pH. Aspartic acid residues Asp-30 on the light chain and Asp-62 on the heavy chain

(Figure 1A) have been identified as prone to isomerization. Asp isomerization involves dehydration to form a cyclic imide intermediate (Asu) that is normally long-lived at pH<8 and detected as a basic peak upon ion exchange chromatography (IEC). Formation of the cyclic intermediate is accelerated at lower pH. Hydrolysis of the cyclic intermediate to form Asp or Iso-Asp, yielding the same charge state as the starting material and thus not detectable by IEC, is faster at higher pH. Isomerization of Asp-62 (Asp-61 according to Kabat numbering) does not appear to affect potency since it is not in contact with factor D in the crystal structure of the Fab:fD complex. Katschke et al. (2012) *J. Biol. Chem.* 287:12886. Asp-30, together with light chain residues Asp-32 and Asp-92, make an electrostatic contact with basic residues on factor D. Isomerization of Asp-30 is quite rapid and presumed to account for an observed loss in potency of the antibody. Isomerization of Asp residues 32 and 92 could also have an effect on fD-binding but the rates are known to be very slow. Formation of the cyclic imide, or its subsequent hydrolysis to iso-aspartic acid, at position 30 could negatively impact antigen binding through perturbation of the electrostatic interaction. Antigen-binding measurements on the isolated basic fraction suggest that the cyclic intermediate form is fully active, consistent with iso-asp formation as the cause of loss in binding.

[00273] Asn-103 (Asn-101 according to Kabat numbering) on the heavy chain is susceptible to deamidation, a reaction that proceeds with higher rate at neutral as compared with slightly acidic pH (6-7). Deamidation can be detected as the appearance of an acidic peak upon IEC. Asn deamidation, like Asp isomerization, proceeds through a cyclic Asu intermediate. However, since formation of Asu from Asn only occurs at higher pH where Asu is hydrolyzed to form Asp or Iso-Asp, usually only the acidic peak is detected. The side chain of Asn-103 forms a hydrogen bond with factor D residue Arg-172. The effect of deamidation at this site, or formation of the cyclic imide intermediate, Asu, on antigen binding is unknown.

[00274] The aFD.WT has a lower pI (7.1) than a typical humanized Fab (pI 8-9). The composition of CDR-L1 (Figure 1A) results in a negative charge cluster on the VL domain. These features may affect solubility of the molecule, especially at low pH and low ionic strength. In addition, high concentration formulations of aFD.WT, even at neutral pH and physiological ionic strength, may have the tendency to form non-covalent dimers at a faster rate at 37°C.

[00275] Several variants of aFD.WT were produced for the purpose of improving stabilities. 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. 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 Fabs, cell paste from 10 L fermentation of transformed cells was suspended in extraction buffer and homogenized using a microfluidizer. Fabs were captured by immunoaffinity chromatography on Protein G- Sepharose or kappa-select and eluted with a low pH buffer. The low pH eluate was adjusted to pH 5 and further purified by cation exchange chromatography on an S-Sepharose column. Identities of the purified proteins were confirmed by mass spectroscopy and the pooled fractions were concentrated to about 10 mg/mL, and exchanged into PBS buffer (pH 7.3) (also referred to herein as "PBS"; 8 mM dibasic sodium phosphate (Na_2HPO_4), 2 mM monobasic potassium phosphate (KH_2PO_4), 140 mM NaCl, 2.7 mM KCl), via diafiltration.

Example 2: Bioactivities of the Anti-Factor D Antibody Variants

[00276] Promising single and combination mutants were tested for factor D (fD) binding affinity and ability to inhibit factor D activities.

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

[00277] Kinetics and binding constant K_D for factor D binding to immobilized aFD.WT and variants thereof 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/minute, the running buffer was HBS-P+, 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. 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.

Table 1. Effect of mutations on affinity for factor D

Mutant	**SPR K_D (pM)**	**Variant #**
aFD.WT	≤10	
VL-D28S	≤10	AFD.v1
VL-D30E	≤10	AFD.v2
VL-D31S	≤10	AFD.v3
VL-D32S	26	AFD.v4
VL-D28S:D31S:D32S	280	AFD.v5
VL-D30E:D31S VH-D62E ("TM")	≤10	AFD.v6
VL-D30E:D31S VH-D62E VL-D92E ("TM.D92E")	≤10	AFD.v7
VL-D28S:D30E:D31S VH-D62E ("SIESD")	16.7±4.4	AFD.v8
VL-D28S:D30E:D31S VH-D62E VL-N34S	30	AFD.v9
VL-D28S:D30E:D31S VH-D62E VL-D92E	70	AFD.v10
VL-D28S:D30E:D31S:D92E VH-D62E:N103S	102	AFD.v15
VL-D28S:D30E:D31S VH-D62E VH-N52S	70	AFD.v11
VL-D28S:D30E:D31S VH-D62E VH-N103D	23	AFD.v12
VL-D28S:D30E:D31S VH-D62E VH-N103Q	60	AFD.v13
VL-D28S:D30E:D31S VH-D62E VH-N103S ("SIESD.N103S")	25.6±6.3	AFD.v14

[00278] Mutants are named and numbered based on location in aFD.WT's light chain variable domain (VL; SEQ ID NO:3) and heavy chain variable domain (VH; SEQ ID NO:4). Single letter code for the wild-type residue followed by sequence position followed by single letter code for the substituted amino acid. Multiple changes on the same domain are separated by a colon.

[00279] As shown in Table 1, aFD.WT has a high affinity for fD, at the limit (~10 pM KD) that can be determined with SPR technology. Aspartic acid residues 28, 30, and 31 in

CDR-L1 could be individually substituted with Ser, Glu, and Ser, respectively, without apparent effect on affinity for fD (Table 1). In contrast, replacement of CDR-L1 Asp32 with Ser resulted in a significant loss in fD-binding whether tested individually (AFD.v4) or in combination with D28S and D31S mutants (AFD.v5). fD affinities equivalent to the wild-type molecule were determined for a triple mutant ("TM" (AFD.v6)) combining VL-D30E, D31S and VH-D62E and for a quad mutant (TM.D92E (AFD.v7)) which adds VL-D92E to TM (AFD.v6). The VH-D62E is a replacement at a site that undergoes isomerization without apparent effect on fD-binding; VL-Asp92 is an antigen contact residue with a slow rate of isomerization. The quad mutant "SIESD" (AFD.v8) combining VL-D28S, D30E, D31S and VH-D62E shows a small (~ 2-fold) loss in affinity for fD. In the context of SIESD (AFD.v8), the VL-D92E replacement resulted in a further loss in affinity for fD (see AFD.v10 (SIESD.D92E in Table 1)).

[00280] Potential sites of deamidation were tested for replacement with other residues. Both VL-N34 and VH-N52 are in contact with fD in the co-crystal structure but neither of these sites show significant rates of deamidation under neutral pH conditions. Ser substitution at these sites resulted in a loss in affinity (Table 1; AFD.v9 and AFD.v11). VH residue Asn-103 does contact fD and has a measureable rate of deamidation in PBS. Substitution of Asn-103 with Asp or Ser in the context of SIESD (AFD.v8) resulted in small, acceptable losses in affinity for fD (see AFD.v12 (SIESD.N103D) and AFD.v14 (SIESD.N103S)) (Table 1). A Gln substitution for Asn-103 resulted in a larger decrease in binding affinity (see AFD.v13 (SIESD.N103Q) (Table 1). Similar to SIESD (AFD.v8), SIESD.N103S.D92E (AFD.v15) which added VL-D92E to the penta mutant SIESD.N103S (AFD.v14) resulted in a further 4-fold decrease in affinity for fD.

b. Factor D Inhibition Assays

[00281] aFD.WT and variants were tested for their abilities to inhibit Factor D-induced complement activation, using an alternative pathway (AP) hemolysis assay. 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 aFDs were prepared at a 2X concentration and added to 96-well polypropylene plates. Er suspension were mixed with GVB/0.1M EGTA/0.1M $MgCl_2$ 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.

Table 2. Inhibitory potency of AFD.Ab variants

Sample	IC50 (nM)* AP Hemolysis
WT (aFD.WT)	3.4
SIESD (AFD.v8)	4.2
SIESD.N103S (AFD.v14)	4.1
TM.D92E (AFD.v7)	3.8

*RSE= ±30%

[00282] As shown in Table 2, variants SIESD (AFD.v8), SIESD.N103S (AFD.v14), and TM.D92E (AFD.v7) have potencies for inhibiting fD-dependent complement activation activities that are equivalent to aFD.WT, given the standard error in IC50 measurement of ±30%.

c. Binding Capacity over Prolonged Time

[00283] SPR was also used to measure total binding of AFD.Ab variants to fD over time under defined conditions. The standard error in these measurements is ±10%. Figure 2A shows that at pH 5.5, loss in binding was about 40% in one month for aFD.WT and AFD.Ab variants, D30E (AFD.v2) and TM (AFD.v6), whereas loss of binding for SIESD (AFD.v8) and SIESD.N103S (AFD.v14) was smaller at about 15%, even for prolonged period of time (70 days). As a comparison, an anti-VEGF antibody Fab fragment (aVEGF) showed no loss in binding over 70 days. Addition of salt to the pH 7.4 condition seemed to enhance the rate of loss in binding for aFD.WT and aVEGF (data not shown). As shown in Figure 2B, in the presence of PBS (with Fab protein concentration at 100 mg/ml), D30E (AFD.v2) and TM (AFD.v6) had equivalent rates of loss in binding that was slower than observed for aFD.WT. The loss in binding after 10 weeks at 37 °C was about 30% for aFD.WT, and 20% for D30E (AFD.v2) and TM (AFD.v6) variants of anti-factor D. Loss in binding after 10 weeks at 37 °C was only 10% for SIESD.N103S (AFD.14; Figure 2B), no greater than the experimental error, and equivalent to that observed for aVEGF under the same conditions. The thermal stress in PBS experiment at 100 mg/mL Fab concentration was repeated for SIESD (AFD.v8) in order to collect data out to 70 days. As shown in Figure 2C, loss in binding at 70 days was less than 10% for SIESD (AFD.v8).

Example 3. Anti-Factor D Antibody Variants with Improved Stability

[00284] Based on the affinity assays above, several single and combination anti-Factor D antibody variants were selected for further stability analysis.

a. Solubility

[00285] Samples were initially tested for solubility at low ionic strength and pH 6. Samples were first prepared in 20 mM His-HCl pH 5 buffer by concentration to ~100 mg/mL using Amicon Centriprep YM-10 centrifugal filter units. These solutions at pH 5 and low ionic strength did not show turbidity upon visual inspection. Samples were centrifuged at 14,000xg for 10 minutes to pellet any insoluble material. No pellet was observed, and the protein concentration of the solution was determined by UV absorbance measurements. Samples (~1 mL) were placed in Slide-A-Lyzer cassettes of 10 K MWCO (Pierce) and dialyzed overnight at 4 °C versus 1 L of 20 mM His buffer, pH 6, followed by visual inspection for turbidity. Photographs of the solutions were taken and are provided in Figure 6. At pH 6 and low ionic strength conditions (~ 100 mg/ml in 20 mM His-HCl, pH 6), aFD.WT and D30E (AFD.v2) solutions were noticeably turbid, TM (AFD.v6) solution was less turbid and the solution of SIESD (AFD.v8) was clear (Figure 6). After centrifugation as above, whereby large pellets were visually observed for aFD.WT and AFD.v2, a smaller pellet for TM (AFD.v6), and no pellet for SIESD (AFD.v8), protein concentrations of the supernatants were determined by UV absorbance measurements (Table 3). aFD.WT and D30E (AFD.v2) showed solubilities of less than 50 mg/ml, TM (AFD.v6) showed solubility approaching 100 mg/mL and SIESD (AFD.v8) was fully soluble under these conditions. The small reduction in protein concentration for SIESD (AFD.v8) after pH 6 dialysis relative to the pH 5 starting concentration reflects a dilution effect upon dialysis rather than precipitation of AFD.v8 since no pellet was observed upon centrifugation.

Table 3. Solubility of AFD.Ab Variants (~ 100 mg/ml in 20 mM His-HCl, pH 6)

AFD.Ab Variant #	Concentration at pH 5, before pH 6 dialysis (mg/ml)	Concentration after pH 6 dialysis at 4°C and centrifugation (mg/ml)
aFD.WT	102	40
AFD.v2 (D30E)	102	14
AFD.v6 (TM)	102	92
AFD.v8 (SIESD)	100	94

[00286] Further variants AFD.v3, AFD.v12, AFD.v13 and AFD.v14 were tested in no salt solubility tests. After dialysis into pH 6 buffer at 4°C and incubation overnight at 37°C, all of the protein solutions except aFD.WT were clear (Figure 7). Measurements of protein concentration after 37 °C incubation and centrifugation (Table 4) indicate that all the variants were more soluble than aFD.WT. The turbid solution of aFD.WT (Figure 7, top row) became clear when subsequently dialyzed versus PBS (pH 7.3), a salt (NaCl) containing buffer, which suggests that the precipitation was reversible with salt addition and/or increase in pH (Figure 7, bottom row). The solubility data on AFD.v3 indicates that the single amino acid change D31S, removal of 1 negatively charged residue, can result in increased solubility. The further amino acid changes in AFD.v8, AFD.v12, AFD.v13 and AFD.v14 also result in increased solubility.

Table 4. Solubility of AFD.Ab Variants at pH 6, no salt

Condition	aFD. WT	AFD.v3 (D31S)	AFD.v8 (SIESD)	AFD.v12 (SIESD. N103D)	AFD.v13 (SIESD. N103Q)	AFD.v14 (SIESD. N103S)
Concentration at pH 5, before pH 6 dialysis (mg/ml)	112	106	120	118	109	103
Concentration after pH 6 dialysis at 4°C , incubation at 37 °C overnight and centrifugation (mg/ml)	63	97	99	94	96	80

[00287] aFD.WT, SIESD (AFD.v8) and SIESD.N103S (AFD.v14) were also tested for solubility under conditions of physiological pH (pH 7.3) and ionic strength. For solubility testing under physiological pH and ionic strength, samples were dialyzed overnight versus PBS,

and then concentrated to 227-372 mg/mL using Amicon Centriprep YM-10 centrifugal filter units. After overnight incubation at 4 °C, samples were visually inspected for turbidity, a portion was centrifuged to remove precipitated protein and the concentration of protein was determined by UV absorbance measurements and reported in Table 5. Prior to centrifugation, the aFD.WT sample was turbid whereas the solutions of SIESD (AFD.v8) and SIESD.N103S (AFD.v14) were clear (aFD.WT, AFD.v8 and AFD.v14 shown in Figure 8). The concentration of AFD.v14 was 344 mg/mL for the solution in the photograph (Figure 8) which was then further concentrated to 372 mg/mL. The concentration of AFD.v8 was 269 mg/ml for the solution in Figure 8. The concentration of aFD.WT was 227 mg/mL for the solution in Figure 8. After centrifugation, a pellet was observed with the aFD.WT solution, but no pellet was observed for the solutions of SIESD (AFD.v8) and SIESD.N103S (AFD.v14). The protein concentration data (Table 5) indicated that aFD.WT can only be concentrated to 227 mg/mL in PBS before precipitation is observed, whereas the solubility limits are higher for SIESD (AFD.v8) (≥ 269 mg/mL) and SIESD.N103S (AFD.v14) (≥ 372 mg/mL). Since no precipitate was observed for SIESD (AFD.v8) at 269 mg/mL, and no attempt was made to further concentrate the solution, this is the lower limit of solubility for this variant in PBS. Similarly, the lower limit of solubility for SIESD.N103S (AFD.v14) in PBS is 372 mg/mL. The 269 mg/mL solution of SIESD (AFD.v8) in PBS remained clear after 4 weeks of incubation at 2-8°C. Similarly, there was not any apparent increase in turbidity for the 372 mg/mL solution of SIESD.N103S (AFD.v14) in PBS after 3 weeks of incubation at 2-8°C. At this concentration, there was a very small change in % aggregate as measured using size-exclusion chromatography (SEC) (Figure 9), increasing from 0.9% to 2.1% in 3 weeks at 2-8°C (SEC data prior to 3 week incubation (0.9% aggregate) is shown in Figure 9; SEC data after 3 week incubation is data not shown).

Table 5. Solubility of AFD.Ab Variants (in PBS (pH 7.3))

AFD.Ab Variant #	Isoelectric point (pI)*	Concentration after centrifugation (mg/ml)
aFD.WT	7.1	227
SIESD (AFD.v8)	7.3	269
SIESD.N103S (AFD.v14)	7.4	372

* pI values were determined by imaged capillary isoelectric focusing (icIEF)

[00288] Solubilities of variants SIESD (AFD.v8) and SIESD.N103S (AFD.v14) were also compared in a buffer of pH 5.5 (20 mM HCl pH 5.5), and varied NaCl concentration, that may be representative of formulations used for drugs administered via intravitreal injection.

Solutions of about 100 mg/mL protein concentration were prepared and dialyzed against a test buffer. These solutions were then concentrated using Amicon Centriprep YM-10 centrifugal filter units. The concentrations obtained whereby the solutions remained visibly clear at ambient temperature are reported in Table 6. SIESD (AFD.v8) has high solubility, up to 314 mg/mL, at pH 5.5 and low NaCl concentration. High concentrations of SIESD.N103S (AFD.v14) were also achievable, up to 278 mg/mL with addition of 100 mM NaCl.

Table 6. Solubility of AFD.Ab variants at pH 5.5 (20 mM His-HCl pH 5.5) and varied NaCl concentration

NaCl Concentration, mM	SIESD(AFD.v8) mg/mL	SIESD.N103S(AFD.v14) mg/mL
0	314	NT
50	290	200
100	NT	278

NT=not tested

[00289] Although SIESD.N103S (AFD.v14) has two (2) fewer negatively charged residues in comparison to aFD.WT, these changes in charge do not result in a significant change in pI (Table 5), as measured by imaged capillary isoelectric focusing (iCIEF) (Salas-Solano et al, *J. Sep Sci*, 35(22): 3124 (2012)). Proteins are expected to have minimum solubility at pH values close to the pI (Green, A.A., *J. Biol. Chem.*, 93: 517-542 (1931)). For SIESD.N103S (AFD.v14) the increased solubility in PBS (pH 7.3) is not correlated with change in pI. Rather, the Asp to Ser amino acid changes in LC-CDR-L1 (VL-D28S and D31S) appear to alter the charge distribution on the surface of the molecule.

b. Isomerization and Deamidation

[00290] To simulate the exposure of variants to a variety of conditions that may be found in long-acting delivery systems, antibodies were stressed under varied pH and salt conditions for several weeks at 37°C. Specifically, antibodies were evaluated in the following five different formulations:

Formulation 1: 10 mg/mL, 10 mM phosphate buffer at pH 2.5,

Formulation 2: 10 mg/mL, 10 mM histidine HCl at pH 5.5,

Formulation 3: 10 mg/mL, 10 mM phosphate buffer at pH 7.4 (“low salt”),

Formulation 4: 10 mg/mL, pH 7.4 PBS (“high salt”; 10 mM phosphate, 137 mM NaCl); and,

Formulation 5: 100 mg/mL, pH 7.4 PBS.

[00291] All solutions had 0.02% PS20, were incubated at 37°C and were sampled every 2 weeks. The low salt conditions (pH 2.5, 5.5, and 7.4) were to evaluate the effect of chemical degradation in liquid formulations. PBS was used as a mimic of the pH and ionic strength of human vitreous. In addition, comparing the 10 mM phosphate, pH 7.4, to PBS condition should reveal ionic strength effects on chemical and physical stability. The PBS samples were buffer exchanged regularly during incubation to simulate the exchange of vitreous. The wild type AFD ("WT" or "aFD.WT") and aVEGF were evaluated in all 5 conditions. D30E (AFD.v2) and TM (AFD.v6) variants were tested in all formulations except number 4. SIESD (AFD.v8) and SIESD.N103S (AFD.v14) were tested in formulations 2 and 5.

[00292] The chemical degradation being quantified were deamidation, which is characterized by the formation of acidic peaks, and the dehydration step of isomerization, which is characterized by the formation of a long-lived succinimide (Asu) intermediate detected as basic peaks. Anionic exchange chromatography (Dionex ProPac SAX-10 columns) (IEC) was used to quantify the appearance of Asn deamidated and Asp dehydrated species within antibody samples in different formulations.

[00293] For all conditions tested, aFD.WT shows the greatest rate in loss of main peak and increase in basic peak amongst the tested antibodies. This is most noticeable at pH 5.5 where aVEGF, D30E (AFD.v2), TM (AFD.v6), SIESD (AFD.v8), and SIESD.N103S (AFD.v14) all show significantly lower rates of main peak loss than aFD.WT, see Figure 3A. SIESD.N103S (AFD.v14) shows the slowest rate of main peak loss with the rate similar to aVEGF at pH 5.5 (Figure 3A) and even slower than aVEGF in PBS (Figure 3B). As shown in Figure 4B (100 mg/ml of Fab in PBS (pH 7.3), D30E (AFD.v2) shows approximately half the rate of basic peak formation as aFD.WT whereas TM (AFD.v6), SIESD (AFD.v8), and SIESD.N103S (AFD.14) show negligible basic peak formation. In contrast, as shown in Figure 4B (100 mg/ml of Fab in PBS (pH 7.3), the rate of acidic peak generation for aFD.WT and D30E (AFD.v2) are equivalent for PBS condition and about 2-fold slower than determined for TM (AFD.v6) and SIESD (AFD.v8). Acidic peak formation for SIESD.N103S (AFD.v14) in PBS is essentially negligible.

c. Aggregation

[00294] Size exclusion chromatography (SEC) was utilized to quantify the formation of aggregates and monomers for tested antibodies. Column used was TSK-GEL Super SW2000 (Tosoh Biosci.). Materials and conditions were used based on the manufacturer's instructions (www.tskgel.com).

[00295] The % monomers over time based on SEC data for tested antibodies at 100 mg/ml of Fab formulated in PBS at 37°C are shown in Figure 5. aFD.WT shows a decrease in monomer peak fraction by 3-4% per month. AFD.v2 (D30E) which has no change in negative charge compared to aFD.WT shows a similar rate of monomer loss. AFD.v6, AFD.v8 and AFD.v14 show a decreased rate of monomer loss. The differences in monomer content at day zero reflect minor variations in the homogeneity of the purified preparations. The aggregation rate for D30E (AFD.v2) and TM (AFD.v6) is comparable at pH 5.5 and 7.4 (no salt) at 10 mg/ml protein concentration. The addition of salt at pH 7.4 does not affect the rate of aggregation for AFD.Ab variants but it doubles the rate of aggregation for aVEGF. Aggregation is protein concentration dependent since increasing the concentration from 10 mg/mL to 100 mg/mL in PBS increases the rate of aggregation for all samples tested (Table 7). Aggregation at 10 mg/mL concentration in 10 mM phosphate buffer pH 7.4 and no NaCl, and at 10 mg/mL concentration in PBS was minimal (Table 7). At 100 mg/mL concentration in PBS, the loss in monomer is much greater for aFD.WT and D30E (AFD.v2) (5.8% and 7.3% in 40 days, respectively) than for aVEGF, TM (AFD.v6), SIESD (AFD.v8), and SIESD.N103S (AFD.v14) (1.8%, 1.5%, 0.7%, and 1.5% in 40 days, respectively) at 100 mg/mL in PBS at 37 °C. These data suggest that AFD.v6, AFD.v8 and AFD.v14 have less aggregation than aFD.WT and AFD.v2 and may be more suitable as therapeutics as they may be less prone to *in vivo* immunogenicity.

Table 7. Effect of salt and protein concentration on aggregation of AFD.Ab variants and aVEGF as determined by SEC at 40 days

Formulation Conditions	Decrease in % Monomer after 40 Days					
	aFD. WT	D30E (AFD.v2)	TM (AFD.v6)	SIESD (AFD.v8)	SIESD.N103S (AFD.v14)	aVEGF
10 mg/mL in 10 mM sodium phosphate pH 7.4	1.6	2.1	.9			.36
10 mg/mL in PBS	1.5		.8			.63
100 mg/mL in PBS	5.8	7.3	1.5	0.7	1.5	1.78

[00296] To detect fragmentation formed as a function of pH, capillary electrophoresis sodium dodecyl sulfate (CE-SDS) was performed using a Beckman PA800 System with an uncoated fused-silica capillary with a 50 µm internal diameter (Polymicro Technologies, Inc.). Samples were prepared by a Beckman Coulter NXp Liquid Handling Robot with automation equivalent to Q12695. Samples were injected into the capillary at a voltage of 5 kV for 15 seconds and then mobilized at a voltage of 15 kV for 30 minutes. All samples were run at

ambient temperature. The electropherograms of all tested antibodies are similar to that of aFD.WT. Only at pH 2.5 was significant fragmentation observed. At no condition were higher molecular weight species observed, indicating that any aggregates formed are SDS-dissociable and not covalently linked.

[00297] The above stability results show that the triple (TM (AFD.v6)) and quad (SIESD (AFD.v8)) mutant variants of anti-Factor D have chemical stability that is significantly improved over aFD.WT or D30E (AFD.v2). In this series, SIESD.N103S (AFD.14) has the highest chemical stability at pH 5.5 and in PBS, similar to the stability of aVEGF. Both isomerization and deamidation sites have been removed and solubility at neutral pH has been increased while maintaining the fD binding affinity. Based on the above findings, the selected anti-Factor D variants described herein, particularly the SIESD (AFD.v8) and SIESD.N103S (AFD.v14) variants, are suitable for both high concentration formulation and for long acting delivery via, e.g., a port delivery system (PDS) device. For example, long acting delivery using a permanent, refillable device, such as a port delivery system may require high concentration formulation and low tendency to aggregate under physiological conditions of pH (~ 7.3) and ionic strength (~ 150 mM NaCl).

List of HVR Sequences (substitutions in variants are underlined)

SEQ ID NO:	Description	Sequence
5	HVR-L1 of WT	ITSTDIDDDMN
6	HVR-L2 of WT/TM (AFD.v6)/TM.92E (AFD.v7)/SIESD (AFD.v8)/SIESD.N103S (AFD.v14)	GGNTLRP
7	HVR-L3 of WT/TM (AFD.v6)/SIESD (AFD.v8)/SIESD.N103S (AFD.v14)	LQSDSLPYT
8	HVR-H1 of WT/TM (AFD.v6)/TM.92E (AFD.v7)/SIESD (AFD.v8)/SIESD.N103S (AFD.v14)	GYTFTNYGMN
9	HVR-H2 of WT	WINTYTGETTYADDFKG
10	HVR-H3 of WT/TM (AFD.v6)/TM.92E (AFD.v7)/SIESD (AFD.v8)	EGGVNN
11	HVR-L1 of TM (AFD.v6)/TM.D92E (AFD.v7)	ITSTDI<u>ES</u>DMN
12	HVR-H2 of TM (AFD.v6)/TM.92E (AFD.v7)/SIESD (AFD.v8)/SIESD.N103S (AFD.v14)	WINTYTGETTYA<u>E</u>DFKG
13	HVR-L3 of TM.D92E (AFD.v7)	LQS<u>E</u>SLPYT
14	HVR-L1 of SIESD (AFD.v8)/SIESD.N103S (AFD.v14)	ITST<u>SIES</u>DMN
15	HVR-H3 of SIESD.N103S (AFD.v14)	EGGV<u>S</u>N

Example 4: Stability of High Concentration Formulation of SIESD (AFD.v8)

[00298] Based on the stability studies above, AFD.v8 was assessed for suitability for high concentration formulations in low ionic strength, pH 5.5 buffer by performing a thermal (37 °C) stress test. A solution of about 100 mg/mL AFD.v8 was prepared and dialyzed versus 20 mM histidine hydrochloride (His-HCl), pH 5.5, then concentrated using Amicon YM-10 centrifugal filter units. Protein concentration upon removal from the filter units was determined to be 272 mg/mL using absorbance measurements at 280 nm. Aliquots of 100 µL were filtered through 0.22 µm cellulose acetate filters using sterilized Spin-X (Costar) centrifuge tube filters. The filtered solutions contained in snap-cap eppendorf tubes were sealed with parafilm and placed in a warm room maintained at 37 °C. At pre-determined times of 0, 1, 2, 4, and 8 months a tube was removed, diluted 10-fold by addition of 900 µL storage buffer (10 mM His-HCl, pH 5.5, 10% trehalose, 0.01% polysorbate 20) and stored frozen at -70 °C until analysis could be performed. Samples were analyzed for generation of charge variants by ion-exchange chromatography (IEC), presence of aggregates by size-exclusion chromatography (SEC), retention of antigen-binding capacity by surface plasmon resonance (SPR) measurements, and specific chemical changes in the variable domains by peptide mapping. IEC was performed as described in Example 3 (paragraph 00292), with SEC done as described in Example 3 (paragraph 00294). SPR and peptide mapping was done as described below.

a. Peptide Mapping

[00299] Samples of AFD.v8 were diluted with RCM buffer (6M guanidine HCl, 360mM Tris, 2mM EDTA, pH 8.6) to 1 mg/mL in a 1.5 mL eppendorf tube. The reduction reaction was initiated by adding 1M dithiothreitol (DTT) to a final concentration of 20 mM and then incubating at 37°C for 1 hour. Following reduction, the alkylation reaction was carried out by adding 1M iodo acetic acid (IAA) to a final concentration of 50mM and incubating in the dark at room temperature for 15 minutes. The reduced and alkylated samples were buffer exchanged into the digest buffer (25mM Tris, 2.0M $CaCl_2$, pH 8.2) using G-25 Minitrap columns. Trypsin was added in a 1:40 trypsin to protein ratio (by mass) and the digestion was allowed to go to completion by incubating at 37°C for 4 hours.

LC/MS-MS

[00300] A Waters H-Class Acquity connected to a Thermo Orbitrap Q Exactive mass spectrometer were used for LC/MS-MS analysis. 10 µg of trypsin digested samples were loaded onto the Waters Acquity UPLC CSH column and run using the following LC conditions:

Mobile phase A – 0.1% FA in H_20

Mobile phase B – 0.1% FA in ACN

Column temperature: 77C

Flow rate: 0.2mL/min

Time (minutes)	%B
0	1.0
2	1.0
7	13.0
42	35.0
44.1	95.0
46.0	95.0
46.1	1.0
64	1.0

[00301] For the mass spectral analysis, full MS1 scans were detected at 35K resolution using the FTMS (Fourier transform MS, Orbitrap). The top eight ions detected (dynamic exclusion off) in the full MS1 scans were selected for HCD fragmentation in data dependent MS2 scans, which were detected using the FTMS. Downstream data analysis including extracted ion chromatographs and quantification of native and post-translationally modified peptides were performed using Thermo Scientific's XCalibur software.

b. Binding Capacity by SPR Measurements

[00302] Functional activity for binding to immobilized human factor D was assessed by SPR measurements. A Series S, CM5 sensor chip was docked into a Biacore® T200 instrument[1] (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. On a flow cell of the sensor chip human factor D (fD) was covalently immobilized by injecting a solution containing 100 µg/mL fD prepared by dilution of fD (2.4 mg/mL) with 10 mM sodium acetate pH 5. The flow rate was 10 µL/minute 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.

[00303] Antigen-binding active concentrations of AFD were determined using the calibration-dependent concentration analysis routine of the Biacore® T200 evaluation software. A standard curve of unstressed AFD was prepared through gravimetric dilution of standards 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 (10 mM HEPES pH 7.4, 150 mM NaCl, 0.01% PS20). 60 µL aliquots were injected over the specific antigen surface using a flow rate of 10 µL/minute 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 starting material 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.

c. Results

[00304] Results of this thermal stress test indicate that AFD.v8 is a stable molecule in high concentration formulation. Greater than 80% of antigen-binding capacity (Figure 10A) was maintained after 4 months at 37 °C. Very little aggregate formation occurred at 4 months and the protein was nearly 100% monomer by SEC (Figure 10B). Some chemical changes took place by 4 months as indicated by the increase in acidic species (Figure 10B, %Acidic – IEC) to approximately 15% and basic species to approximately 20% (Figure 10B, %Basic – IEC). After 8 months at 37 °C there was an additional increase in acidic and basic species, a loss in monomer content, and a decrease in factor D-binding capacity. Peptide mapping suggests that the acidic species primarily arose from deamidation of CDR-H3 Asn-103 (Asn-101 according to Kabat numbering) (Figure 10B, CDR-H3 N101% deamindation) whereas basic variants were contributed by pyroglutamic acid formation at the N-terminus of the heavy chain (Figure 10B, HC-E1 % succinimide) and isomerization of CDR-H3 residue Glu-99 (Glu-95 according to Kabat numbering) (Figure 10B, CDR-H3 E97 % isomerization). Since Asn-103 and Glu-99 (Asn-101 and Glu-95, respectively, according to Kabat numbering) are in contact with factor D in the co-crystal structure of lamaplizumab and factor D (Katschke KJ, Jr., Wu P, Ganesan R,

Kelley RF, Mathieu MA, Hass PE, Murray J, Kirchhofer D, Weismann C, van Lookeren Campange M, "Inhibiting alternative pathway complement activation by targeting the factor D exosite", *J. Biol. Chem.* (2012) 287:12886-92), but the N-terminus of the heavy chain is not, it is likely that Asn-103 deamidation and Glu-99 isomerization, as well as the decrease in monomer content, directly contribute to the loss in factor D-binding at 8 months. Nonetheless, given the slow rate of these chemical and physical changes, coupled with the expected further decrease in rate at lower temperature, a high concentration liquid formulation of AFD.v8 stored at 2-8 °C or frozen at -20 °C would appear to give acceptable shelf-life.

Example 5: Rabbit pK for AFD.v8/v14

[00305] *In vivo* pK studies for AFD.v8 and AFD.v14 were performed in rabbits. pK parameters were determined from single dose experiments because humanized antibodies are immunogenic in rabbits upon repeat dosing or when exposure is increased through sustained delivery formulations.

[00306] The animals' care was in accordance with Genentech Institutional Animal Care and Use Committee guidelines. Naïve New Zealand White (NZW) rabbits (41 male animals; 3.1 kg to 4.1 kg and approximately 4 months of age at the time of dosing) were assigned to dose groups and dosed with the test items at Charles River Laboratories.

[00307] SIESD(AFD.v8), SIESD.N103S(AFD.v14) or ranibizumab were administered via a single bilateral intravitreal injection to rabbits and observed for up to 27 days. Topical antibiotic (tobramicin ophthalmic ointment) was applied to both eyes twice on the day before treatment, immediately following the injection, and twice on the day following the injection, with the exception of animals sent to necropsy on Days 1 and 2. Prior to dosing, mydriatic drops (1% tropicamide) were applied to each eye for full pupil dilation. Animals were sedated with isoflurane/oxygen gas prior to and during the procedure. Alcaine (0.5%) was also applied to each eye prior to injection. The conjunctivae was flushed with benzalkonium chloride (ZephiranTM) diluted in sterile water, U.S.P. to 1:10,000 (v/v)

[00308] Syringes were filled under a laminar flow hood immediately prior to dosing. Fabs were administered by a single 30 μL intravitreal injection (0.3 mg dose) to both eyes in all animals. Doses were administered by a board-certified veterinary ophthalmologist using sterilized 100 μL Hamilton Luer Lock syringes with a 30-gauge x 1/2" needle. In order to mimic clinical dosing, eyes were dosed in the infero-temporal quadrants, i.e., in 5 o'clock and 7 o'clock positions for the left and right eyes, respectively (when facing the animal). The eyes

were examined by slit-lamp biomicroscopy and/or indirect ophthalmoscopy immediately following treatment.

[00309] All animals underwent exsanguination by incision of the axillary or femoral arteries following anesthesia by intravenous injection of sodium pentobarbital. Aqueous humor, vitreous humor and retina tissue were collected, snap frozen in liquid nitrogen and stored at -80°C. Antibody Fab in retina was extracted by homogenization in 50 mM Tris-HCl pH 8.0, 1 M NaCl. Determination of vitreous and retinal concentrations of test articles was by GRIP ELISA as described below. Values below the LLOQ were not used in pharmacokinetic analysis or for graphical or summary purposes. Pharmacokinetic parameters were determined by non-compartmental analysis with nominal time and dose (Phoenix WinNonlin, Pharsight Corp, Mountain View, CA).

[00310] Analyses of SIESD (AFD.v8), SIESD.N103S (AFD.v14) and ranibizumab were done in the generic immunoglobulin pharmacokinetic (GRIP) ELISA, with the exceptions noted herein. Sheep anti-human-IgG (The Binding Site; San Diego, CA) was diluted to 1000 ng/mL in 0.5 M carbonate/bicarbonate, pH 9.6, and coated onto 384-well ELISA plates (Nunc; Neptune, NJ) during an overnight incubation at 4°C. Plates were washed with PBS plus 0.05% Tween-20 and blocked during a 1- to 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 standard curves were prepared by serially diluting AFD.v8, AFD.v14 or ranibizumab from 40 - 0.625 ng/mL in assay buffer (PBS, 0.5% BSA, 15 ppm Proclin, 0.05% Tween 20, 0.25% CHAPS, 5 mM EDTA, 0.35M NaCl, (pH 7.4)). The rabbit vitreous or retinal homogenate samples were diluted a minimum of 1:100 or 1:50, respectively, in assay buffer. The diluted standards, controls, and samples were then incubated on the washed plates for 1 – 2 hours. Following a wash step, plate-bound AFD.v8, AFD.v14 or ranibizumab was detected during a 1.5 hour incubation with HRP-conjugated sheep anti-human IgG mAb (Bethyl Laboratories Inc; Montgomery, TX) diluted to 83.3 ng/mL in assay diluent (PBS + 0.5% BSA + 0.05% Tween 20 + 10ppm Proclin). After a final wash, tetramethyl benzidine peroxidase substrate (Moss, Inc., Pasadena, MD) was added, 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 (Multiscan Ascent, Thermo Fischer; Waltham, MA). The concentrations of AFD.v8, AFD.v14 or ranibizumab were calculated from a four-parameter fit of the respective standard curve using in-house Excel-based software. Taking into account the minimum dilution in vitreous or retinal homogenate, the minimum quantifiable

concentration of AFD.v8, AFD.v14 or ranibizumab in rabbit vitreous or retinal homogenate was 62.5 ng/mL or 31.25 ng/mL, respectively.

[00311] Time-dependent concentration curves observed for intravitreal injection of 0.3 mg SIESD (AFD.v8), SIESD.N103S (AFD.v14), or a comparator dose of ranibizumab (anti-VEGF), are shown in Figure 11.

[00312] Analysis of the vitreal data using a non-compartmental model indicated that both SIESD (AFD.v8) and SIESD.N103S (AFD.v14) have clearance properties very similar to ranibizumab. All three proteins gave very similar exposure, as reflected in the AUC parameter, in the three ocular compartments: vitreous humor, aqueous humor, and retina. PK parameters calculated for ranibizumab were consistent with results of earlier studies in rabbits (Gaudrealt et al, *Retina*, 27:1260-6 (2007)). Both SIESD (AFD.v8) and SIESD.N103S (AFD.v14) show target-independent ocular clearance properties that render these molecules suitable for development.

Example 6: Viscosity for AFD.v8/v14

[00313] As low viscosity is important for intravitreal administration, viscosity for SIESD (AFD.v8) and SIESD.N103S (AFD.v14) was measured at different protein concentrations in a pH 5.5, low salt buffer. Viscosity measurements were performed on a TA Instruments cone and plate rheometer thermostatted at 25 °C using a shear rate of 1000 s^{-1}.

[00314] aFD.WT, SIESD (AFD.v8) and SIESD.N103S (AFD.v14) gave similar profiles of viscosity dependence on protein concentration with viscosities acceptable for intravitreal injection (<30 cP) even at concentrations exceeding 200 mg/mL (Figure 12).

Example 7. Anti-Factor D Antibody Variants Further Modified for Polymer Conjugation

[00315] The aFD.WT and variants described in the above Examples are Fab fragments. While the variable domains of their light and heavy chains (VL and VH) vary in sequences as shown in Figure 1B, their constant domains CL and CH1 remain the same. In particular, the CH1 domain of the heavy chain ends at the Threonine residue as shown in Figure 1A (SEQ ID NO:2) Figure 1C (SEQ ID NO: 27) and Figure 1D (SEQ ID NO: 29). In order to prepare the AFD.Ab variants for polymer conjugation such as PEGylation, the heavy chains of the Fab fragments were further modified by adding the first cysteine residue from the hinge region of the Fab' counterpart (e.g., Cys-modified HC (Fab-C) for AFD.v8 (SEQ ID NO: 30) and Cys-modified HC (Fab-C) for AFD.v14 (SEQ ID NO: 32)), so that the added cysteine serves as the attachment site of PEG polymer. The resulting fragment can therefore be conjugated with one

arm of the multi-arm PEG. The heavy chains of the Fab fragments were also modified by adding the first four residues from the hinge region of the Fab' counterpart, namely Cys-Pro-Pro-Cys (SEQ ID NO: 21) (e.g., Cys-Pro-Pro-Cys-modified HC for AFD.v8 (SEQ ID NO: 31) and Cys-Pro-Pro-Cys-modified HC for AFD.v14 (SEQ ID NO: 33)), so that the two added Cys both serve as attachment sites for PEG, resulting in a modified AFD.Ab Fab fragment capable of attaching two PEG molecules.

[00316] The Cys-modified and Cys-Pro-Pro-Cys-modified variants were prepared 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. For small scale expression, 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 Fab-C purified as described in Example 1. For larger scale production of Fab-C, cell paste from 10 L fermentation of transformed cells was suspended in extraction buffer and homogenized using a microfluidizer, and the Fab-C was purified as described in Example 8.

Example 8: Preparation of AFD.v14 Conjugates

[00317] The AFD.v14 variant containing the Cys-modified HC (SEQ ID NO: 32) prepared in Example 7 (referred to herein as the "Cys-modified AFD.v14 variant" or "AFD.v14.C") was conjugated with commercially available maleimide-functionalized multi-armed PEGs having varying core structures.

a. Maleimide-Functionalized Multi-Armed PEGs

[00318] The maleimide-functionalized multi-armed PEGs detailed in Table 8, below, were used in the conjugation reactions:

Table 8. Maleimide-functionalized multi-armed PEGs

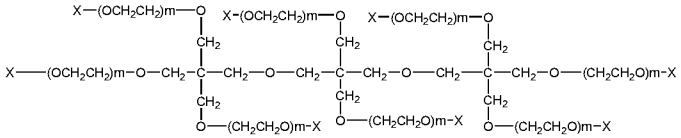
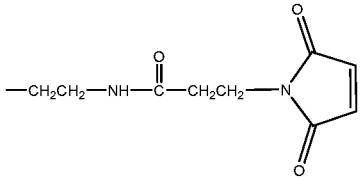
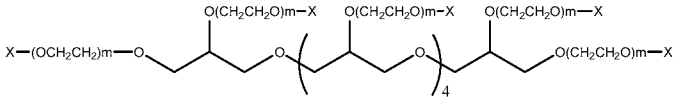
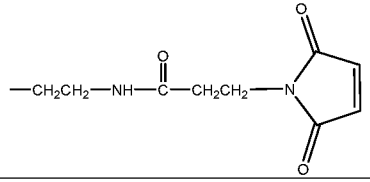
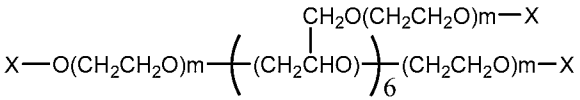
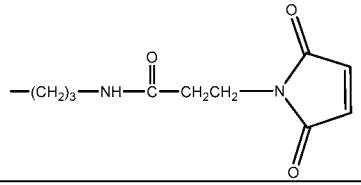
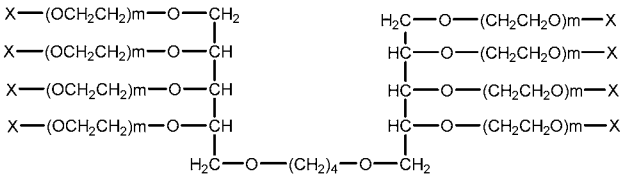
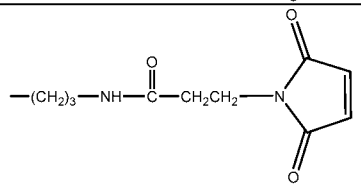
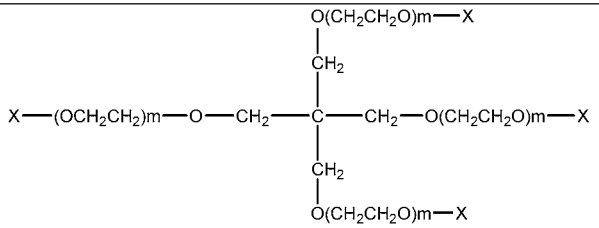
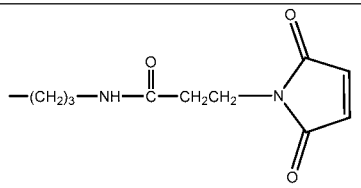
PEG	Vendor	Core structure	Functional Group (X)	* Poly-Dispersity	Average MW
8ARM (TP)-PEG-MAL	JenKem Technology, USA	Tripentaerythritol (TP) 		~1.04	40,000
8ARM-PEG-MAL	JenKem Technology, USA	Hexaglycerin (HG) 		~1.08	40,000
Sunbright® HGEO-400MA	NOF America Corp.	Hexaglycerol (HGEO) 		~1.33	40,000
Sunbright®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

Table 8. Maleimide-functionalized multi-armed PEGs

PEG	Vendor	Core structure	Functional Group (X)	* Poly-Dispersity	Average MW
Sunbright® PTE-400MA	NOF America Corp.			- -	40,000

* As provided by vendor. Note that this refers to the PEG chain length.

[00319] 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 set forth in Figures 13A and 13B.

[00320] As can be seen from Figures 13A and 13B, the 8ARM (TP)-PEG-MAL containing the TP core was more homogeneous than the Sunbright® HGEO-400MA containing the HGEO core.

b. Conjugation of Cys-Modified AFD.v14 Variant and Maleimide-Functionalized Multi-Armed PEGs

[00321] The Cys-modified AFD.v14 variant prepared in Example 7 was captured using Gamma Plus resin, with 6.5 mM 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 Cys-modified AFD.v14 monomer was further isolated using SP Sepharose High Performance strong cation exchange resin (from GE Healthcare) in 25 mM sodium acetate pH 5.0, with 0.05% Triton X-100 + 0.05% Triton X-114 for 19 hours for endotoxin removal. Elution was performed with gradient between 0-20% 25 mM sodium acetate pH 5.0 + 1 M NaCl over 20 column volumes. The monomeric Fab-C with deblocked c-terminal cysteine was then prepared for PEGylation by titrating to pH 6.5 using 1 M HEPES pH 7.2. The Cys-modified AFD.v14 Fab-C was then conjugated to the maleimide-functionalized multi-armed PEG in 25 mM sodium acetate pH 6.5, 150 mM NaCl, 4 mM EDTA, at a concentration around 5 mg/mL. The Cys-modified AFD.v14 variant was not further concentrated in order to minimize cysteine reactivity loss due to Fab-C dimerization. After equilibrating to room temperature, the maleimide-functionalized multi-armed PEG was resuspended in 25mM sodium acetate pH 5.0 to a concentration of 10 mg/mL. The pH was kept below pH 6 to avoid maleimide ring opening. Once the 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 (# Fab/PEG) was checked by SEC-MALS.

Example 9: Purification and Characterization of AFD.v14 Conjugates

[00322] The conjugates prepared in Example 8 were purified and analyzed using SEC-MALS to confirm PEGylation and determine conjugation efficiency for different PEG core structures. Unless otherwise indicated, conjugation efficiency was determined by Size Exclusion Chromatography (SEC) using a 300 x 8 mm Shodex OH pak SB-804 HQ run at 0.8 mL/minute 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. 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 99.0°, also by Wyatt Technology. Raw data was worked up using Wyatt's proprietary Astra software, where molar mass and RH constants were set using a rituximab standard.

a. Cys-Modified AFD.v14-8ARM (TP)-PEG-MAL Conjugate

[00323] The Cys-modified AFD.v14-8ARM (TP)-PEG-MAL conjugate (containing the TP core structure) (hereinafter the "AFD.v14 TP conjugate" or "AFD.v14.C + TP octamer") prepared in Example 8 was 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 and conjugation efficiency were determined using in-line static Multi-Angle laser Light Scattering (MALS) by Wyatt Technology and Shodex OH pak SB-804 (Figure 14C). Raw data was analyzed using Wyatt's proprietary Astra software, where molar mass constants were set using a rituximab standard. Molar mass was used to estimate the average number of AFD.v14 variants attached to each PEG. The results are shown in Figures 14A, 14B, and 14C and in Table 9.

Table 9

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; n/d = not determined
* B5-B7 are deemed to be 8 Fabs/PEG based on %error in the MALS measurements.

[00324] As can be seen from Table 9, conjugation of the Cys-modified AFD.v14 variant (AFD.v14.C) with a multi-armed PEG octamer having the TP core produced conjugates comprising 8 Fabs/PEG, demonstrating that good conjugation efficiency can be achieved with PEG octamers comprising a TP core (e.g., approximately 45% recovery of 8 Fabs/PEG from conjugation).

b. Cys-Modified AFD.v14-8ARM-PEG-MAL Conjugate

[00325] The Cys-modified AFD.v14-8ARM-PEG-MAL conjugate (containing the HG core structure (JenKem)) (hereinafter the "AFD.v14 HG conjugate" or the "AFD.v14.C + HG octamer") prepared in Example 8 was 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 and conjugation efficiency was determined using Tosoh G3000PW column and in-line static 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°, also by Wyatt Technology. Raw data was analyzed using Wyatt's proprietary Astra software, where molar mass and RH constants were set using a rituximab standard. Molar mass was used to estimate the number of AFD.v14 variants attached to each PEG. The results are shown in Figures 15A, 15B, and 15C and in Table 10.

Table 10

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

[00326] As can be seen from Table 10, conjugation of the Cys-modified AFD.v14 variant (AFD.v14.C) with a PEG octamer comprising the HG core produced conjugates comprising 8 Fabs/PEG. The recovery of conjugates comprising 8 Fabs/PEG (approximately 20% recovery), however, was about half the amount of conjugates comprising 8 Fabs/PEG that was recovered when using the conjugate with the TP core. Conjugation with the HG core also produced more conjugates comprising 5-7 Fabs/PEG, than was observed with the TP core, and significantly more aggregates.

[00327] In an effort to improve Fabylation estimate and RH measurement, the product containing fractions obtained following the S-300 purification were pooled and alternately analyzed using SEC-MALS on a 10/300 Sephacryl S-400 HR (GE Healthcare) column in PBS, pH 7.4, run at 0.25 mL/minute. Molar mass and RH were determined as described above. The SEC and MALS results are set forth in Figures 16A and 16B.

[00328] The conjugates prepared using the 8ARM-PEG-MAL (HG core) and analyzed using Sephacryl S-400 HR had 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 AFD.v14-HGEO-400MA Conjugate

[00329] The Cys-modified AFD.v14-HGEO-400MA conjugate (containing the Sunbright® HGEO-400MA PEG) (hereinafter the "AFD.v14 HGEO conjugate" or "AFD.v14.C + HGEO octamer") prepared in Example 8 was 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, conjugation efficiency, and RH were determined as described above using Sephacryl S-400 HR, run at 0.25 mL/minute in PBS, pH 7.4.

[00330] The results are set forth in Figures 17A and 17B. The conjugates prepared using the Sunbright® HGEO-400MA PEG (HGEO core) had 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.

[00331] Following purification of the AFD.v14 HGEO conjugates using SEC on the Sephacryl S-300 HR column, conjugation efficiency and molar mass were alternately determined using Tosoh G3000PW column and in-line static MALS by Wyatt Technology, as described above. Molar mass was used to estimate the number of AFD.v14 variants attached to each PEG. The results from this analysis are set forth in Figures 18A and 18B, and in Table 11.

Table 11

Fraction #	Molar Mass (g/mol)	Estimated Fabylation
B1	2,145,000 (± 0.8%)	agg
B2	665,800 (± 0.7%)	agg
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%)	n/d

agg = aggregates; n/d = not determined

[00332] As can be seen from Table 11, conjugation of the Cys-modified AFD.v14 variant (AFD.v14.C) with a PEG octamer comprising the HGEO core produced conjugates comprising 8 Fabs/PEG. Conjugation with the HGEO core also produced more conjugates comprising 5-6 Fabs/PEG, than was observed with the TP core. Finally, conjugation with the HEGO core resulted in more aggregates, and a lower conjugation efficiency, as compared to the TP core.

Example 10: Enrichment of AFD.v14 Conjugates

[00333] 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 was used to enrich for highly fabylated conjugates.

[00334] Fractions B4-B7 (estimated fabylation of 8 Fab/PEG) from the SEC purification of the AFD.v14.C + TP octamer described in Example 9a were pooled (about 45% recovery), and subjected to cation exchange chromatography (CEX) using SP Sepharose High Performance strong cation exchange resin (GE Healthcare) in 25 mM sodium acetate pH 5.0, 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% using 25 mM sodium acetate pH 5.0 plus 1 M NaCl over 50 column volumes (CV). Fractions were analyzed using SEC-MALS + QELS using Shodex OH pak SB-804 HQ, as described above. The results are set forth in Figures 19A, 19B, 19C, and Table 12.

Table 12

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

[00335] The conjugate containing fractions obtained following CEX on SP Sepharose resin were pooled and analyzed using a 300 x 8 mm Shodex OH pak SB-804 HQ, run at 0.8 mL/minute using phosphate buffered saline (PBS), pH 7.4, 150 mM NaCl, under isocratic conditions. Molar mass and RH were determined as described above. The MALS results are set forth in Figure 20.

[00336] Following enrichment, conjugates prepared using the 8ARM (TP)-PEG-MAL (TP core) were obtained that had an average RH of 10.5 nm (± 2.5%), an average molar mass of 407.1 kDa (± 0.2%), and an average of 7.8 Fabs/PEG.

[00337] The conjugate containing fractions obtained following cation exchange chromatography purification of the TP conjugate described above were pooled (CEX pool) and compared to pooled fractions obtained following SEC using Sephacryl S-300 HR (GE Healthcare) (see Example 9a) (S300 pool), and pooled fractions obtained following SEC using Sephacryl S-400 HR (GE Healthcare) in 20 mM His-acetate, pH 5.5, 50 mM NaCl (isocratic gradient) (data not shown) (S400 pool). The pooled fractions were subject to capillary SDS gel electrophoresis (CE-SDS), and the results are set forth in Figures 21A and 21B.

[00338] As can be seen from Figures 21A and 21B, purification on S-300 and S-400 resin gave similar results. Enrichment of the conjugates using CEX, however, visibly enriched the amount of conjugates comprising 8 Fabs/PEG, while removing lower and higher molecular weight contaminants.

Example 11: Comparison of PEG Cores

[00339] The properties of conjugates prepared in Example 8, comprising either the 8ARM-(TP)-PEG-MAL (containing the TP core structure), the 8ARM-PEG-MAL (containing the HG core structure (JenKem)), or the Sunbright®-DX-400MA PEG (containing the butanediol core structure, referred to herein as the "AFD.v14 DX conjugate" or the "AFD.v14.C + DX octamer"), were compared using SEC-MALS. The HG and DX conjugates were 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). For the TP conjugate, pooled fractions obtained following purification on Sephacryl S-300 HR as described in Example 9a ("CEX load") and pooled fractions obtained following the CEX enrichment as described in Example 10 ("TP final") were used. Molar mass and conjugation efficiency was determined using MALS by Wyatt Technology and a 300 x 8 mm Shodex OH pak SB-804 HQ run at 0.8 mL/minute using phosphate buffered saline (PBS) pH 7.2, 150 mM NaCl under isocratic conditions. RH was determined as described above. The results are set forth in Figures 22A, 22B, and Table 13.

Table 13

Conjugate	PEG Core Structure	M_W (kDa)	Polydispersity (Mw/Mn)	R_H (nm)	Gel Lane #
AFD.v14.C + TP octamer*	Tripentaerythritol (TP)	407.1	1.004	10.5	6, 12
AFD.v14.C + HG octamer	Hexaglycerol (HG, JenKem)	539.1	1.289	12.8	4, 10
AFD.v14.C + DX octamer	Butanediol (DX)	355.6	1.005	9.2	3, 9

*Data is for TP final. Gel lanes 5 and 11 (Figure 22A) were CEX load.

[00340] Polydispersity was determined using methods known in the art, and in particular was determined using Astra software commercially available from Wyatt Technology.

[00341] As can be observed from these results, although the AFD.v14 DX conjugate had a low polydispersity, it did not provide as high a conjugation efficiency as the AFD.v14.C + TP octamer.

Example 12: Viscosity of AFD.v14 Conjugates

[00342] As low viscosity is important for intravitreal administration, viscosity of the Cys-modified AFD.v14 variant (AFD.v14.C) conjugated to either a PEG octamer (8ARM (HG)-PEG-MAL from JenKem Technology, USA; the AFD.v14.C + HG octamer) or a PEG tetramer (Sunbright® PTE-400MA from NOF America Corp.), prepared in Example 8, was measured at different protein concentrations in a pH 7.4 phosphate buffered saline (PBS) solution. Viscosity measurements were performed on a TA Instruments cone and plate rheometer thermostatted at 40 °C using a shear rate of 1000 s^{-1}. The results are shown in Figure 23.

[00343] As can be seen from Figure 23, conjugation of the AFD.v14 variant to the HGEO octamer allowed for a greater protein concentration, as compared to conjugation to the tetramer, at comparable viscosity.

[00344] The viscosity of the AFD.v14 HGEO conjugate (AFD.v14.C + HGEO octamer) at different protein concentrations was compared to that of the AFD.v14.C + TP octamer. Viscosity was measured at different protein concentrations at pH 6.5 in 20mM His-Ace, 50 mM NaCl formulation. Viscosity measurements were performed on a TA Instruments cone and plate rheometer thermostatted at 20 °C using a shear rate of 1000 s^{-1}. The results are set forth in Figure 24.

[00345] As can be seen from Figure 24, the AFD.v14.C + TP octamer had a lower viscosity than the AFD.v14.C + HGEO octamer, at comparable protein concentrations.

Example 13: Thermal Stability of AFD.v14 Conjugates

[00346] To simulate the exposure of the AFD.v14 conjugates to conditions that may be found in long-acting delivery systems, samples of the AFD.v14.C + TP octamer (prepared in Example 8) were stressed under two different pH and salt conditions for several weeks at 37°C. Specifically, conjugates were evaluated in the following formulations:

[00347] Formulation 1: 10 mg/mL, PBS; and,

[00348] Formulation 2: 10 mg/mL, 20 mM histidine HCl, 50 mM NaCl, at pH 6.5.

[00349] PBS was used as a mimic of the pH and ionic strength of human vitreous. Aliquots (100 µL) of solutions of AFD.v14-TP conjugate, formulated at 10 mg/mL in PBS or 20 mM His-acetate 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 (T0, T2w, T4w, or T8w, respectively). Incubations were terminated by freezing at -70 °C. After thawing, samples were analyzed by SEC-MALS using a Shodex OH pak SB-804 HQ as described above, CE-SDS and by biacore to assess fD-binding capacity, as described below. The relative peak area for the conjugate determined by CE-SDS as a function of incubation time is shown in Figures 25A and 25B, suggesting a 1%/week decrease in conjugate at 37 °C. A similar change in conjugate, with increase in free Fab and dimer species, is observed by SEC-MALS (Figure 26). No change in binding capacity greater than the standard error in the measurements (±10%) was determined for incubation of the conjugate at 37 °C (Figure 28). The binding capacity remained steady even after 8 weeks at 37 °C in phosphate buffered saline (PBS) and after 4 weeks at pH 6.5.

a. CE-SDS Analysis

[00350] Material and Reagents: AFD.v14.C + TP octamer samples were thawed from –70 °C before use. Potassium cyanide (KCN) and 3-(2-furoyl)quinoline-2-carboxaldeyde (FQ) reagents were purchased from Molecular Probes (Eugene, OR, USA). Monobasic and dibasic sodium phosphate, dimethyl sulfoxide (DMSO), dithiothreitol (DTT) and N-ethylmaleimide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium dodecyl sulfate (SDS), 0.1 M sodium hydroxide (NaOH) and 0.1 M hydrochloric acid (HCl) reagents were purchased from J.T. Baker (Phillipsburg, NJ, USA). Replaceable sieving gel was purchased from Beckman Coulter, Inc. (Fullerton, CA, USA).

[00351] Solutions: Aqueous solutions were prepared with deionized 18.2 MΩ water from a Millipore purification system (Billerica, MA, USA). Solutions of 0.1M sodium phosphate, pH 6.7 reaction buffer and 4% SDS were filtered through a 0.2 µm membrane filter (Millipore,

Bedford, MA, USA) and diluted before use. Stock solutions of 20 mM fluorogenic FQ were prepared in Dimethyl sulfoxide (DMSO) and stored in the dark at -20 °C. Aliquots were thawed and diluted with water before use.

[00352] FQ Labeling Procedure: Solutions of AFD.v14.C + TP octamer (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 minutes at 70°C to control disulfide reshuffling under denaturing conditions (*see, e.g.*, Michels, D.A., Brady, L.J., Guo, A., Balland, A., *Anal Chem* 2007, 79, 5963-5971). Ten microliters of each 2.5 mM FQ and 30 mM KCN reagents were added to the SDS-AFD.v14 solution, and the final solution was incubated for 10 minutes 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 minutes at 70°C.

[00353] CE-SDS Analysis: Separation of AFD.v14.C + TP octamer 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 minutes, 1 minute, 1 minute and 10 minutes, respectively. (see, e.g., Michels, et al., *Anal Chem* 2007, 79, 5963-5971; Michels, et al., *Electrophoresis* 2012, 33, 815-826.)

b. Binding Capacity

[00354] The following materials were purchased from GE Healthcare: Series S CM5 Sensor Chips (cat#BR-1005-30); 10 X Biacore® running buffer (cat#BR-1006-71): 0.1 M Hepes pH 7.4, 1.5 M NaCl, 0.5% Polysorbate® 20; regeneration solution (cat#BR-1003-55): 10 mM Gly-HCl pH 2.1; and, amine coupling kit (cat#BR-1000-50). 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/minute 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. 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 AFD.v14.C + TP octamer 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/minute 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 AFD.v14.C + TP octamer 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 14: Cynomolgus Monkey PK for AFD.v14 Conjugate

[00355] *In vivo* pK studies for the AFD.v14.C + TP octamer, prepared in Example 8, and purified as described in Examples 9a and 10, 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

[00356] 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 a single bilateral intravitreal dose 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 octamer through a 30-gauge needle (2 injections in each eye of 50 µl; 100 µl total 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 octamer 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 octamer at 0.4 mg/animal. For IV administration, the AFD.v14.C + TP octamer was formulated as 10 mM sodium succinate, 10% trehalose, and 0.05% Tween-20 (pH 5.0).

[00357] Ocular tissues were collected from Groups 1, 2, and 3. One animal (2 eyes) from Group 1 and two animals (4 eyes) from each of Groups 2 and 3 were euthanized 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 vitreous humor, aqueous humor, and retinal tissue were collected from both eyes. The entire retinal layer was collected using filter paper days after flash freezing of the eyes. The AFD.v14 and AFD.v14.C + TP octamer concentrations were determined in the vitreous and aqueous humor and retinal tissues.

[00358] 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.

[00359] Details of the study protocol are set forth in Table 14.

Table 14. 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

Group	Dose	Route	Number of Animals	Ocular time points (days)	Serum time points
3	4 mg/eye	IVT (bilateral)	10	1, 4, 8, 12, 20	6 hr; 1, 2, 4, 6, 8, 12, 20 days
4	0.4 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 octamer

[00360] A Gyrolab XP assay was used to quantify AFD.v14 and AFD.v14.C + TP octamer 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 octamer standard curves were prepared by serially diluting AFD.v14 or AFD.v14.C + TP 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 and AFD.v14.C + TP 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 and AFD.v14.C + TP octamer in cynomolgus monkey serum, vitreous humor, aqueous humor and retinal homogenate.

[00361] The vitreous humor, aqueous humor, and retinal pK results are set forth in Figures 29A (vitreous) and 29B (vitreous, normalized), Figures 30A (aqueous) and 30B (aqueous, normalized), and Figures 31A (retina) and 31B (retina, normalized) and in Tables 15-17 below.

Table 15. Vitreous PK for AFD.v14 control (Group 1) and AFD.v14.C + TP octamer (Groups 2 and 3)

Group	Dose (µg/eye)	$T_{1/2}$ (days)	AUC (Day*µg/mL)	AUC/dose (Day*µg/mL/mg dose)	$T_{1/2}$ ext*	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

*increase compared to control

Table 16. Aqueous PK for AFD.v14.C + TP octamer

Group	Dose (µg/eye)	$T_{1/2}$ (days)	AUC_{last} (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 17. Retinal PK for AFD.v14.C + TP octamer

Group	Dose (µg/eye)	$T_{1/2}$ (days)	AUC_{last} (Day*µg/mL)	Vz (mL)	Cl/F (mL/Day)
2	1000	3.6	31	196	38
3	4000	5.9	98	309	36

[00362] As can be seen from Table 15, the vitreal terminal half-life for both Group 2 (3.5 days) and Group 3 (5 days) 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 16 and 17, and Figures 30 and 31, 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.

[00363] The serum pK results for Groups 1-3 are set forth in Figures 32A and 32B (normalized), and the serum pK results for Group 4 are set forth in Figure 32C.

[00364] As can be seen from Figures 32A and 32B, the serum pK curves for Groups 2 and 3 (AFD.v14.C + TP octamer) are parallel to each other (Figure 32A), and overlap after dose normalization (Figure 32B). The serum AUC for Groups 2 and 3 is dose proportional, up until the last measured time point.

[00365] The terminal half-life for Group 4 (AFD.v14.C + TP octamer; 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

[00366] 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).

[00367] The average serum fD and AFD.v14.C + TP octamer concentrations for Groups 2, 3, and 4 are set forth in Figure 33A. As can be seen from Figure 33A, 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.

[00368] The average ocular fD and AFD.v14.C + TP octamer concentrations for Groups 2 and 3 are set forth in Figure 33B. As can be seen from Figure 33B, the AFD.Ab concentration in the vitreous humor, aqueous humor, and retinal homogenate exceeded the fD concentration at all time points tested.

Example 15: Potency of Anti-Factor D Antibody Variants and Conjugates for Inhibition of Factor D

[00369] The potency of AFD.Ab variants or conjugates comprising a Cys-modified Fab variant for inhibition of Factor D are determined in a time-resolved fluorescence energy transfer (TR-FRET) assay of Factor D-dependent factor B activation.

[00370] The Cys-modified AFD.v14 variant (AFD.v14.C), and the AFD.v8 variant containing the Cys-modified HC (SEQ ID NO: 30) prepared in Example 7 (the "Cys-modified AFD.v8 variant" or "AFD.v8.C") were each conjugated with a maleimide-functionalized multi-

armed PEG tetramer (Sunbright® PTE-400MA from NOF America Corp.) according to the procedure set forth in Example 8, to form conjugates (referred to hereinafter as the "AFD.v14 tetramer" or the "AFD.v8 tetramer", respectively).

[00371] Dilutions of the AFD.Ab Fab variant, conjugate, or Fab 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 (125 pM or 50 pM, respectively) (fD, Complement Technology; Tyler, TX) or ERB (no enzyme control). Ranibizumab (anti-VEGF) was used as the negative control. The Factor D/AFD.Ab or Factor D/conjugate 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 AFD.Ab Fab or conjugate, enzyme, cofactor, and substrate were incubated for 45 minutes 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 minutes. 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 or conjugate concentrations causing half-maximal inhibition (IC50) were determined by nonlinear regression analysis using a four-parameter fit model (KaleidaGraph Synergy Software; Reading, PA).

[00372] Inhibition curves for the TR-FRET assay are shown in Figure 34A (Table 18). Lampalizumab has an IC50 for inhibition of Factor D-dependent fB activation of 24 pM, and the standard error in IC50 is ± 25%. The IC50 for AFD.v8 and AFD.v14 is comparable to that measured for lampalizumab. See Figure 34A (Table 18). The difference in IC50 for the conjugated Cys-modified AFD.Ab versions (AFD.v8 tetramer and AFD.v14 tetramer) compared to the unconjugated Fab is likely due to difficulty in handling more viscous PEGylated molecules (Figure 34A, Table 18).

Table 18: IC50 of Factor D-dependent Factor B Activation (50 pM fD)

Molecule	Average IC50 fB Activation (pM)
AFD.v8	27.65
AFD.v14	34.03
AFD.v8 tetramer	11.03
AFD.v14 tetramer	14.77
Ranibizumab	n/a
Lampalizumab	24.38

[00373] The potency of the AFD.v14.C + TP octamer for inhibition of Factor D was also determined in a TR-FRET assay of Factor D-dependent factor B activation using the procedure described above, with addition of 125 pM fD. The IC50 for the AFD.v14.C + TP octamer (AFD.v14.C + TP octamer) was compared to AFD.v14, Cys-modified AFD.v14 ("AFD.v14.C"), and lampalizumab. Ranibizumab was used as the negative control. The results are set forth in Figure 34B and Table 19.

Table 19: IC50 of Factor D-dependent Factor B Activation (125 pM fD)

Molecule	Average IC50 fB Activation (pM)
Lampalizumab	72
AFD.v14	87
AFD.v14 TP octamer (S200 pool)*	104
AFD.v14 TP octamer (CEX pool)^	77
AFD.v14.C	72
Ranibizumab	n/a

* Obtained following purification using SEC on a Sephacryl S-200 HR (GE Healthcare) column.
^Obtained following CEX enrichment (Example 10).

[00374] The IC50 of the AFD.v14 TP octamer (both S300 pool and CEX pool) is potent, and is comparable to that measured for unconjugated Fab (lampalizumab, AFD.v14, AFD.v14.C). Enrichment of the AFD.v14 TP octamer using cation exchange chromatography resulted in a more potent product.

Example 16: Effect of Anti-Factor D Antibody Variants and Conjugates on Systemic Alternative Complement Pathway Activity

[00375] 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

[00376] 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.

[00377] 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.

[00378] 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.

[00379] 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.

[00380] 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.

[00381] 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

[00382] 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

[00383] 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

[00384] 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.

[00385] 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

[00386] 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 35 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 35 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.

[00387] Erythrocytes were prepared, and the assay was performed, as described above, 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.

f. Results

[00388] The percent relative hemolysis in comparison to total fD and the therapeutic active is shown in Figures 35A (lampalizumab, 10 mg/eye), 35B (AFD.v14, 25 mg/eye), and 35C (AFD.v14.C + TP octamer, 3.9 mg/eye). The lampalizumab data (Figure 35A) 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 35B, 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 35A). In comparison, no systemic AP inhibition was observed following administration of 3.9 mg/eye of the AFD.v14.C + TP octamer (Figure 35C). 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.

[00389] The percent relative AP complement activity in comparison to total fD and total conjugate is shown in Figures 35D (AFD.v14.C + HG octamer, 7.1 mg/eye) and 35E (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.

[00390] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the disclosure described herein. Such equivalents are intended to be encompassed by the following claims.

[00391] Although the foregoing disclosure 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 disclosure. The disclosures of all patent and scientific literatures cited herein are expressly incorporated in their entirety by reference.

[00392] The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the disclosure. The present disclosure is not to be limited in scope by the construct deposited, since the deposited embodiment is intended as a single illustration of certain aspects of the disclosure and any constructs that are functionally equivalent are within the scope of this disclosure. Indeed, various modifications of the disclosure in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

WE CLAIM:

1. A conjugate comprising one or more anti-Factor D antibody variant covalently linked to one or more polyols, wherein:

at least one anti-Factor D antibody variant in the conjugate comprises substitution of at least one target aspartic acid (D or Asp) residue within a hypervariable region (HVR) of a reference anti-Factor D antibody, wherein the target Asp residue is identified as prone to isomerization and the substitution is Asp to Glutamic acid (E or Glu), and wherein each antibody variant exhibits improved stability without significant loss of Factor D binding affinity when compared to the reference anti-Factor D antibody; and

the polyol is a multi-armed polyol.

2. The conjugate according to claim 1, wherein the Asp residue is within an Asp-Xaa motif, wherein Xaa is Asp, Gly, His, Ser or Thr.

3. The conjugate according to claim 2, wherein the target Asp residue is the first Asp of an Asp-Asp motif.

4. The conjugate according to claim 1, wherein at least one antibody variant in the conjugate further comprises substitution of serine (S or Ser) for additional at least one Asp residue within a HVR of a reference anti-Factor D antibody, wherein the resulting antibody variant has lower negative charge and exhibits improved solubility when compared to the reference anti-Factor D antibody.

5. The conjugate according to claim 1, wherein at least one antibody variant in the conjugate further comprises one or more Ser substitution for deamidation prone asparagine (N or Asn) residue within a HVR of a reference anti-Factor D antibody.

6. The conjugate according to claim 1, wherein the reference anti-Factor D antibody comprises the light chain variable domain sequence of SEQ ID NO:3.

7. The conjugate according to claim 6, wherein the reference anti-Factor D antibody further comprises the heavy chain variable domain sequence of SEQ ID NO:4.

8. The conjugate according to claim 7, wherein the reference anti-Factor D antibody comprises the light chain sequence of SEQ ID NO:1 and the heavy chain sequence of SEQ ID NO:2.

9. The conjugate according to claim 7, wherein the reference anti-Factor D antibody comprises the light chain sequence of SEQ ID NO:1 and a heavy chain sequence selected from the group consisting of SEQ ID NOs: 34-53 and 115.

10. The conjugate according to claim 8 or claim 9, wherein at least one antibody variant in the conjugate comprises a light chain HVR1 (HVR-L1) sequence of SEQ ID NO:11 and a heavy chain HVR2 (HVR-H2) sequence of SEQ ID NO:12.

11. The conjugate according to claim 10, wherein the antibody variant further comprises a light chain HVR3 (HVR-L3) sequence of SEQ ID NO:13.

12. The conjugate according to claim 8 or claim 9, wherein at least one antibody variant in the conjugate comprises a light chain HVR1 (HVR-L1) sequence of SEQ ID NO:14 and a heavy chain HVR2 (HVR-H2) sequence of SEQ ID NO:12.

13. The conjugate according to claim 12, wherein the antibody variant further comprises a heavy chain HVR3 (HVR-H3) sequence of SEQ ID NO:15.

14. A conjugate comprising one or more anti-Factor D antibody variant covalently linked to one or more polyols, wherein:

at least one anti-Factor D antibody variant in the conjugate comprises substitution at one or more positions within the HVRs of a reference anti-Factor D antibody, wherein said reference anti-Factor D antibody comprises light chain HVR-1 comprising the sequence ITSTDIDDDMN (SEQ ID NO: 5), light chain HVR-2 comprising the sequence GGNTLRP (SEQ ID NO: 6), light chain HVR-3 comprising the sequence LQSDSLPYT (SEQ ID NO: 7), heavy chain HVR-1 comprising the sequence GYTFTNYGMN (SEQ ID NO: 8), heavy chain HVR-2 comprising the sequence WINTYTGETTYADDFKG (SEQ ID NO: 9), and heavy chain HVR-3 comprising the sequence EGGVNN (SEQ ID NO: 10), and wherein said substitution is one or more of the following: (a) amino acid at position 5 of SEQ ID NO: 5 is S (a, b, and c disclosed in SEQ ID NO: 22); (b) amino acid at position 7 of SEQ ID NO: 5 is E; (c) amino acid at position 8 of SEQ ID NO: 5 is S; (d) amino acid at position 13 of SEQ ID NO: 9 is E (SEQ ID NO: 23); (e) amino acid at position 4 of SEQ ID NO: 7 is E (SEQ ID NO: 24); or (f) amino acid at position 5 of SEQ ID NO: 10 is S (SEQ ID NO: 25); and

the polyol is a multi-armed polyol.

15. The conjugate according to claim 14, wherein said substitution is selected from the group consisting of:

(i) substitutions (b)-(d);

(ii) substitutions (b)-(e);

(iii) substitutions (a)-(d); and

(iv) substitutions (a)-(d) and (f).

16. The conjugate according to claim 14 or claim 15, wherein the variant comprises a heavy chain constant domain amino acid sequence selected from the group consisting of SEQ ID NOs: 54-74 and 116.

17. A conjugate comprising one or more anti-Factor D antibody covalently linked to one or more polyols, wherein:

at least one anti-Factor D antibody in the conjugate is independently selected from the group consisting of:

a) an anti-Factor D antibody comprising a light chain variable domain amino acid sequence of SEQ ID NO:16, 18 or 19; and

b) an anti-Factor D antibody comprising a heavy chain variable domain amino acid sequence of SEQ ID NO:17 or 20; and

the polyol is a multi-armed polyol.

18. The conjugate according to claim 17, wherein at least one anti-factor D antibody in the conjugate comprises a light chain variable domain amino acid sequence of SEQ ID NO:16, 18 or 19 and a heavy chain variable domain amino acid sequence of SEQ ID NO:17 or 20.

19. The conjugate according to claim 18, wherein the light chain variable domain amino acid sequence is according to SEQ ID NO:19 and the heavy chain variable domain amino acid sequence is according to SEQ ID NO:17.

20. The conjugate according to claim 18, wherein the light chain variable domain amino acid sequence is according to SEQ ID NO:19 and the heavy chain variable domain amino acid sequence is according to SEQ ID NO:20.

21. The conjugate according to any one of claims 17-20, wherein at least one anti-Factor D antibody comprises a heavy chain constant domain amino acid sequence selected from the group consisting of SEQ ID NOs: 55-74 and 116.

22. A conjugate comprising one or more anti-Factor D antibody covalently linked to one or more polyols, wherein:

at least one anti-Factor D antibody in the conjugate has a variable light chain comprising a HVR-L1 having the sequence of SEQ ID NO:11 or 14, a HVR-L2 having the sequence of SEQ ID NO:6, and a HVR-L3 having the sequence of SEQ ID NO:7 or 13; and a variable heavy chain comprising a HVR-H1 having the sequence of SEQ ID NO:8, a HVR-H2 having the sequence of SEQ ID NO:9 or 12, and a HVR-H3 having the sequence of SEQ ID NO:10 or 15; and

the polyol is a multi-armed polyol.

23. The conjugate according to claim 22, wherein each anti-Factor D antibody in the conjugate is independently selected from the group consisting of:

(i) an anti-Factor D antibody having a variable light chain comprising a HVR-L1 having the sequence of SEQ ID NO:14, a HVR-L2 having the sequence of SEQ ID NO:6, and a HVR-L3 having the sequence of SEQ ID NO:7; and a variable heavy chain comprising a HVR-H1 having the sequence of SEQ ID NO:8, a HVR-H2 having the sequence of SEQ ID NO:12, and a HVR-H3 having the sequence of SEQ ID NO:10; and

(ii) an anti-Factor D antibody having a variable light chain comprising a HVR-L1 sequence of SEQ ID NO: HVR-L1 having the sequence of SEQ ID NO: 14, a HVR-L2 having the sequence of SEQ ID NO:6, and a HVR-L3 having the sequence of SEQ ID NO:7; and a variable heavy chain comprising a HVR-H1 having the sequence of SEQ ID NO:8, a HVR-H2 having the sequence of SEQ ID NO:12, and a HVR-H3 having the sequence of SEQ ID NO:15.

24. The conjugate according to claim 22 or claim 23, wherein at least one anti-Factor D antibody has a heavy chain constant domain amino acid sequence selected from the group consisting of SEQ ID NOs: 55-74 and 116.

25. A conjugate comprising one or more anti-Factor D antibody covalently linked to one or more polyols, wherein:

at least one anti-Factor D antibody in the conjugate is selected from the group consisting of:

(i) an anti-Factor D antibody having a light chain having the amino acid sequence of SEQ ID NO: 26 and a heavy chain having the amino acid sequence of SEQ ID NO: 27;

(ii) an anti-Factor D antibody having a light chain having the amino acid sequence of SEQ ID NO: 28 and a heavy chain having the amino acid sequence of SEQ ID NO: 29;

(iii) an anti-Factor D antibody having a light chain having the amino acid sequence of SEQ ID NO: 26 and a heavy chain having the amino acid sequence of SEQ ID NO: 30;

(iv) an anti-Factor D antibody having a light chain having the amino acid sequence of SEQ ID NO: 26 and a heavy chain having the amino acid sequence of SEQ ID NO: 31;

(v) an anti-Factor D antibody having a light chain having the amino acid sequence of SEQ ID NO: 26 and a heavy chain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 75-92 and 117;

(vi) an anti-Factor D antibody having a light chain having the amino acid sequence of SEQ ID NO: 28 and a heavy chain having the amino acid sequence of SEQ ID NO: 32;

(vii) an anti-Factor D antibody having a light chain having the amino acid sequence of SEQ ID NO: 28 and a heavy chain having the amino acid sequence of SEQ ID NO: 33; and,

(vii) an anti-Factor D antibody having a light chain having the amino acid sequence of SEQ ID NO: 28 and a heavy chain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 93-110 and 118; wherein

the polyol is a multi-armed polyol.

26. The conjugate according to claim 25, wherein at least one anti-Factor D antibody in the conjugate is an anti-Factor D antibody having a light chain having the amino acid sequence of SEQ ID NO: 28 and a heavy chain having the amino acid sequence of SEQ ID NO: 32.

27. The conjugate according to claim 25, wherein at least one anti-Factor D antibody in the conjugate is an anti-Factor D antibody having a light chain having the amino acid sequence of SEQ ID NO: 26 and a heavy chain having the amino acid sequence of SEQ ID NO: 30.

28. The conjugate according to any one of claims 1-27, wherein the anti-Factor D antibody or the antibody variant is an antibody fragment selected from the group consisting of Fab, Fab', Fab'-SH, Fab-C, Fab-C-SH, and combinations thereof.

29. The conjugate according to claim 28, wherein at least one antibody fragment in the conjugate comprises one cysteine residue at the C'-terminal end.

30. The conjugate according to claim 28, wherein at least one antibody fragment in the conjugate comprises an amino acid sequence of SEQ ID NO: 21 at the C'-terminal end.

31. The conjugate according to claim 28, wherein at least one antibody fragment in the conjugate comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 111-114 at the C'-terminal end.

32. The conjugate according to any one of claims 1-31, wherein the polyol is covalently linked to at least one of the anti-Factor D antibodies or the antibody variants at a free sulfhydryl group of a cysteine residue.

33. The conjugate of claim 32, wherein the cysteine residue is an engineered cysteine.

34. The conjugate according to claim 32 or claim 33, wherein the cysteine residue is in a constant domain of the anti-Factor D antibody or the antibody variant.

35. The conjugate according to any one of claims 32 to 34, wherein the cysteine residue is at the C'-terminal end of the anti-Factor D antibody or the antibody variant.

36. The conjugate according to any one of claims 32 to 35, wherein the conjugate comprises at least two of the anti-Factor D antibodies or the antibody variants, and the polyol is covalently linked to each anti-Factor D antibody or antibody variant at a free sulfhydryl group of a cysteine residue.

37. The conjugate according to any one of claims 1-16, wherein at least one of the antibody variants comprises substitution of at least one amino acid residue within a constant domain of the reference anti-Factor D antibody, wherein the amino acid residue is substituted with Cys.

38. The conjugate according to claim 37, wherein the polyol is covalently linked to the antibody variant at a free sulfhydryl group of the substituted cysteine residue.

39. The conjugate according to any one of claims 1-38, wherein the polyol is covalently linked to at least one of the anti-Factor D antibodies or the antibody variants at a free amino group of a lysine residue.

40. The conjugate according to claim 39, wherein the lysine residue is within a constant domain of the anti-Factor D antibody or the antibody variant.

41. The conjugate according to any one of claims 1-40, wherein at least two of the anti-Factor D antibodies or the antibody variants are covalently linked to the polyol.

42. The conjugate according to claim 41, wherein at least three of the anti-Factor D antibodies or the antibody variants are covalently linked to the polyol.

43. The conjugate according to claim 42, wherein at least four of the anti-Factor D antibodies or the antibody variants are covalently linked to the polyol.

44. The conjugate according to claim 43, wherein at least five of the anti-Factor D antibodies or the antibody variants are covalently linked to the polyol.

45. The conjugate according to claim 44, wherein at least six of the anti-Factor D antibodies or the antibody variants are covalently linked to the polyol.

46. The conjugate according to claim 45, wherein at least seven of the anti-Factor D antibodies or the antibody variants are covalently linked to the polyol.

47. The conjugate according to claim 46, wherein eight of the anti-Factor D antibodies or the antibody variants are covalently linked to the polyol.

48. The conjugate according to any one of claims 1-47, wherein the multi-armed polyol is selected from the group consisting of a dimer, a tetramer, a hexamer, and an octamer.

49. The conjugate according to claim 48, wherein the multi-armed polyol is an octamer.

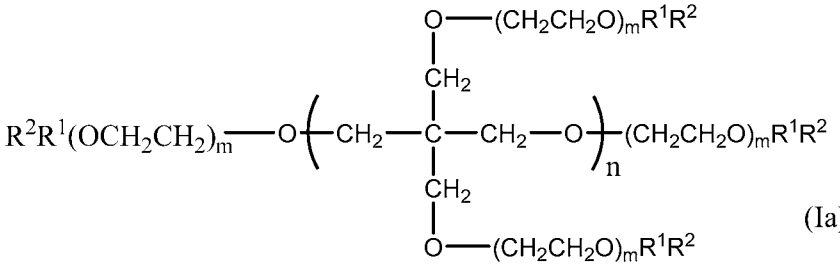
50. The conjugate according to any one of claims 1-49, wherein the polyol is polyethylene glycol.

51. The conjugate according to claim 50, wherein the polyethylene glycol has a weight average molecular weight of from about 500 D to about 300,000 D.

52. The conjugate according to claim 51, wherein the polyethylene glycol has a weight average molecular weight of from about 20,000 D to about 60,000 D.

53. The conjugate according to claim 52, wherein the polyethylene glycol has a weight average molecular weight of about 40,000 D.

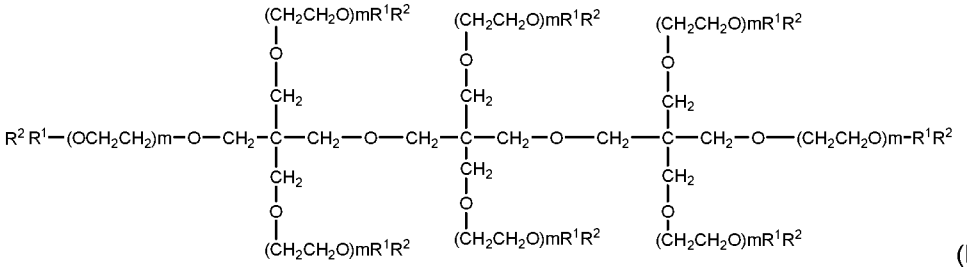
54. The conjugate according to any one of claims 50-53, wherein the polyethylene glycol has the structure of general formula (Ia):



(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 anti-Factor D antibody or the antibody variant.

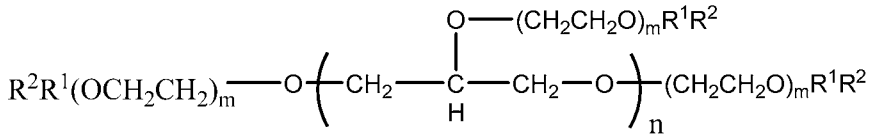
55. The conjugate according to any one of claims 50-53, wherein the polyethylene glycol has the structure of general formula (Ib):



(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 anti-Factor D antibody or the antibody variant.

56. The conjugate according to any one of claims 50-53, wherein the polyethylene glycol has the structure of general formula (IIa):

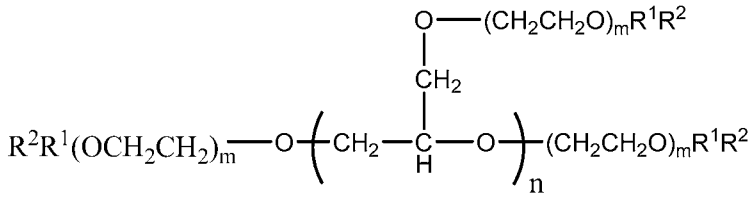


(IIa)

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 anti-Factor D antibody or the antibody variant.

57. The conjugate according to claim 56, wherein n is 6.

58. The conjugate according to any one of claims 50-53, wherein the polyethylene glycol has the structure of general formula (IIIa):



(IIIa)

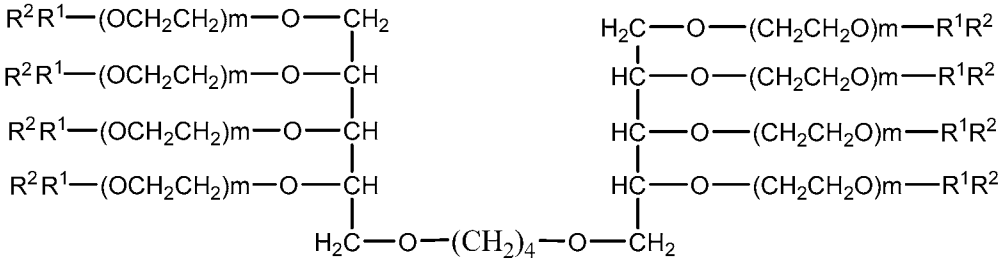
wherein each m is independently an integer of from 3-250; n is an integer from 1-10; each R^1 is independently either absent, or is a linking group; and each R^2 is independently either hydrogen or a terminal reactive group; wherein at least one R^2 is a terminal reactive group and is covalently linked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

59. The conjugate according to claim 58, wherein n is 2.

60. The conjugate according to claim 58, wherein n is 4.

61. The conjugate according to claim 58, wherein n is 6.

62. The conjugate according to any one of claims 50-53, wherein the polyethylene glycol has the structure of general formula (IVa):

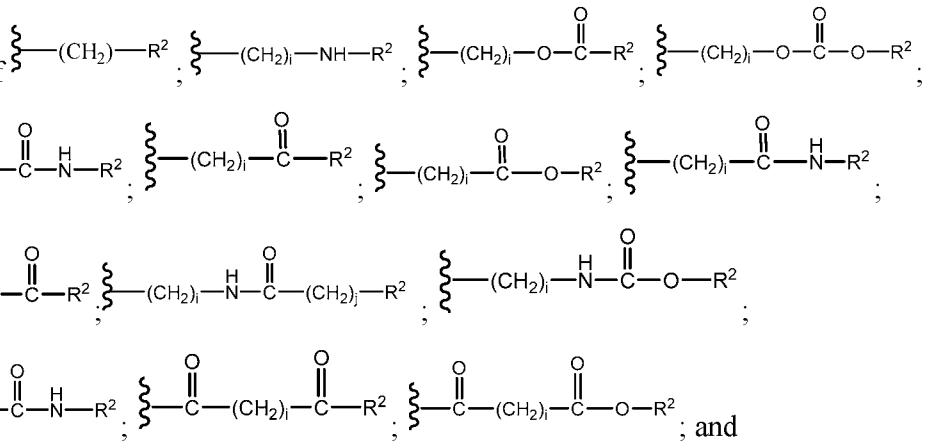


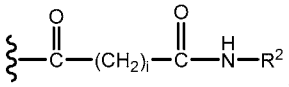
(IVa)

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 anti-Factor D antibody or the antibody variant.

63. The conjugate according to any one of claims 54-62, wherein m is an integer of 50-200.

64. The conjugate according to claim 63, wherein m is an integer of 100-150.

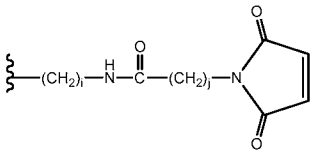
65. The conjugate according to any one of claims 54-64, wherein at least one R^1 is a linking group, wherein R^1 and R^2 when taken together are selected from the group consisting of  ; and


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

66. The conjugate according to any one of claims 54-65, wherein each R^2 is independently selected from the group consisting of a thiol reactive group, an amino reactive group, and combinations thereof.

67. The conjugate according to claim 66, wherein each R^2 is independently selected from the group consisting of 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.

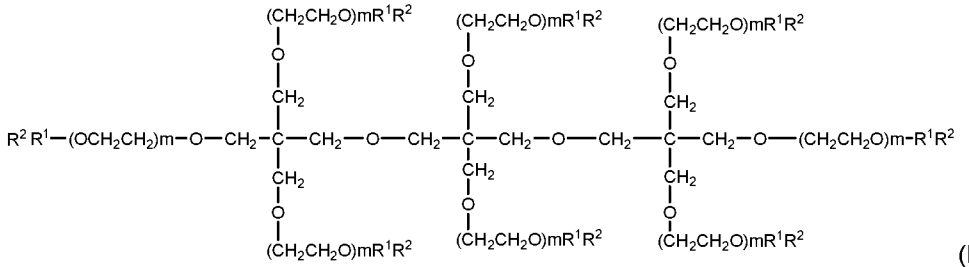
68. The conjugate according to claim 67, wherein R^2 is a maleimide.

69. The conjugate according to any one of claims 54-68, 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.

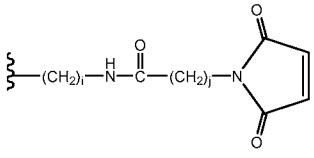
70. The conjugate according to any one of claims 54-58 or 61-69, wherein at least seven of the R^2 groups are covalently linked to one of the anti-Factor D antibodies or the antibody variants.

71. The conjugate according to claim 70, wherein eight of the R^2 groups are covalently linked to one of the anti-Factor D antibodies or the antibody variants.

72. The conjugate according to claim 26 or claim 27, 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 anti-Factor D antibody or the antibody variant.

73. The conjugate according to claim 72, 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.

74. The conjugate according to claim 73, wherein i is 2 and j is 2.

75. A conjugate according to any one of claims 1-74, wherein the conjugate is prepared by covalently linking at least one anti-Factor D antibody or antibody variant to the multi-armed polyol.

76. The conjugate of claim 75, wherein the multi-armed polyol is selected from the group consisting of a dimer, a tetramer, a hexamer, and an octamer.

77. The conjugate according to claim 75 or claim 76, wherein the polyol is a polyethylene glycol.

78. A pharmaceutical formulation comprising the conjugate according to any one of claims 1-77.

79. The pharmaceutical formulation according to claim 78, wherein the concentration of the anti-Factor D antibody or the antibody variant is at least 100 mg/ml.

80. The pharmaceutical formulation according to claim 79, wherein the concentration of the anti-Factor D antibody or the antibody variant is at least 200 mg/ml.

81. The pharmaceutical formulation according to claim 80, wherein the concentration of the anti-Factor D antibody or the antibody variant is at least 300 mg/ml.

82. The pharmaceutical formulation according to claim 78, wherein the concentration of the anti-Factor D antibody or the antibody variant is from about 50 mg/mL to about 300 mg/ml.

83. A long acting delivery device for ocular delivery comprising the pharmaceutical formulation according to any one of claims 78-82 and a means for delivering the formulation intravitreally to a patient, whereby the formulation remains effective on site for a prolonged period of time.

84. Use of the formulation according to any one of claims 78-82 or device of claim 83 in the manufacture of a medicament for treating a complement-associated disorder in a subject.

85. Use according to claim 84, wherein the complement-associated disorder is systemic.

86. Use according to claim 85, wherein the complement-associated disorder is an ocular disease.

87. Use according to claim 86, wherein the ocular disease is selected from the group consisting of age-related macular degeneration (AMD), including dry and wet (non-exudative and exudative) forms, diabetic retinopathy, ischemia-related retinopathy, 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.

88. Use according to claim 87, wherein the ocular disease is selected from the group consisting of intermediate dry form AMD and geographic atrophy (GA).

89. The conjugate of any one of claims 1 to 77 for use in therapy.

90. The conjugate of any one of claims 1 to 77 for use in a method of treating a complement-associated disorder in a subject.

91. The conjugate of any one of claims 1 to 77 for use in a method of treating a systemic complement-associated disorder in a subject.

92. The conjugate of claim 91, wherein the complement-associated disorder is a complement-associated eye condition.

93. The conjugate of claim 92, wherein the complement-associated eye condition is selected from the group consisting of 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.

94. The conjugate of claim 92, wherein the complement-associated eye condition is selected from the group consisting of intermediate dry form AMD and geographic atrophy (GA).

95. A method of treating a complement-associated disorder in a subject comprising administering to the subject an effective amount of the conjugate of any one of claims 1 to 77, or the pharmaceutical formulation of any one of claims 78 to 82.

96. The method of claim 95, wherein the complement-associated disorder is systemic.

97. The method of claim 95, wherein the complement-associated disorder is a complement-associated eye condition.

98. The method of claim 97, wherein the complement-associated eye condition is selected from the group consisting of 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.

99. The method of claim 97, wherein the complement-associated eye condition is selected from the group consisting of intermediate dry form AMD and geographic atrophy (GA).

100. The method of any one of claims 95 to 99, wherein the method comprises administering the conjugate or pharmaceutical formulation using an implantable port delivery system.

101. The method of any one of claims 95 to 100, wherein the method comprises administering the conjugate or pharmaceutical formulation by intravitreal administration.

102. The method of claim 101, wherein the intravitreal administration is through a narrow bore needle.

103. The method of claim 102, wherein the narrow bore needle is about 30, 29, 28, 27, 26, 25, 24, 23, or 22 gauge.

104. The method of any one of claims 95 to 103, further comprising administering an additional therapeutic agent to the individual.

105. The method of claim 104, wherein the additional therapeutic agent is selected from the group consisting of an ANG2 antagonist, a TIE2 antagonist, a VEGF antagonist, and a second complement component antagonist.

106. The method of claim 104, wherein the additional therapeutic agent is an anti-ANG2 antibody.

107. The method of claim 104, wherein the additional therapeutic agent is an anti-TIE2 antibody.

108. The method of claim 104, wherein the additional therapeutic agent is selected from the group consisting of a VEGF trap and an anti-VEGF antibody.

109. The method of claim 104, wherein the additional therapeutic agent is a second complement component antagonist, wherein the second complement component antagonist inhibits a complement component selected from the group consisting of C1, C2, C3, C4, C5, C6, C7, C8 and C9.

WT Light Chain

```
DIQVTQSPSS LSASVGDRVT ITCITSTDID DDMNWYQQKP GKVPKLLISG GNTLRPGVPS   60
RFSGSGSGTD FTLTISSLQP EDVATYYCLQ SDSLPYTFGQ GTKVEIKRTV AAPSVFIFPP  120
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT  180
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC  (SEQ ID NO:1)               214
```

WT Heavy Chain

```
EVQLVQSGPE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW INTYTGETTY   60
ADDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCEREG GVNNWGQGTL VTVSSASTKG  120
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL  180
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT  (SEQ ID NO:2)      223
```

FIG. 1A

VL Domain

```
WT          DIQVTQSPSSLSASVGDRVTITCITSTDIDDDMNWYQQKPGKVPKLLISGGNTLRPGVPSRFSGSGSGTDFTLTISSLQP
TM          DIQVTQSPSSLSASVGDRVTITCITSTDIESDMNWYQQKPGKVPKLLISGGNTLRPGVPSRFSGSGSGTDFTLTISSLQP
TM.D92E     DIQVTQSPSSLSASVGDRVTITCITSTDIESDMNWYQQKPGKVPKLLISGGNTLRPGVPSRFSGSGSGTDFTLTISSLQP
SIESD        DIQVTQSPSSLSASVGDRVTITCITSTSIESDMNWYQQKPGKVPKLLISGGNTLRPGVPSRFSGSGSGTDFTLTISSLQP
SIESD.N103S DIQVTQSPSSLSASVGDRVTITCITSTSIESDMNWYQQKPGKVPKLLISGGNTLRPGVPSRFSGSGSGTDFTLTISSLQP

WT          EDVATYYCLQSDSLPYTFGQGTKVEIK     (SEQ ID NO:3)
TM          EDVATYYCLQSDSLPYTFGQGTKVEIK     (SEQ ID NO:16)
TM.D92E     EDVATYYCLQSESLPYTFGQGTKVEIK     (SEQ ID NO:18)
SIESD       EDVATYYCLQSDSLPYTFGQGTKVEIK     (SEQ ID NO:19)
SIESD.N103S EDVATYYCLQSDSLPYTFGQGTKVEIK     (SEQ ID NO:19)
```

VH Domain

```
WT          EVQLVQSGPELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTGETTYADDFKGRFVFSLDTSVSTAY
TM          EVQLVQSGPELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTGETTYAEDFKGRFVFSLDTSVSTAY
TM.D92E     EVQLVQSGPELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTGETTYAEDFKGRFVFSLDTSVSTAY
SIESD       EVQLVQSGPELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTGETTYAEDFKGRFVFSLDTSVSTAY
SIESD.N103S EVQLVQSGPELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTGETTYAEDFKGRFVFSLDTSVSTAY

WT          LQISSLKAEDTAVYYCEREGGVNNWGQGTLVTVSS     (SEQ ID NO:4)
TM          LQISSLKAEDTAVYYCEREGGVNNWGQGTLVTVSS     (SEQ ID NO:17)
TM.D92E     LQISSLKAEDTAVYYCEREGGVNNWGQGTLVTVSS     (SEQ ID NO:17)
SIESD       LQISSLKAEDTAVYYCEREGGVNNWGQGTLVTVSS     (SEQ ID NO:17)
SIESD.N103S LQISSLKAEDTAVYYCEREGGVSNWGQGTLVTVSS     (SEQ ID NO:20)
```

FIG. 1B

SIESD (AFD.v8) Light Chain

```
DIQVTQSPSS LSASVGDRVT ITCITSTSIE SDMNWYQQKP GKVPKLLISG GNTLRPGVPS     60
RFSGSGSGTD FTLTISSLQP EDVATYYCLQ SDSLPYTFGQ GTKVEIKRTV AAPSVFIFPP    120
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT    180
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC  (SEQ ID NO:26)                214
```

SIESD (AFD.v8) Heavy Chain

```
EVQLVQSGPE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW INTYTGETTY     60
AEDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCEREG GVNNWGQGTL VTVSSASTKG    120
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL    180
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT   (SEQ ID NO:27)     223
```

Cys-Modified SIESD (AFD.v8) Heavy Chain

```
EVQLVQSGPE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW INTYTGETTY     60
AEDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCEREG GVNNWGQGTL VTVSSASTKG    120
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL    180
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTC   (SEQ ID NO:30)    223
```

Cys-Pro-Pro-Cys-Modified SIESD (AFD.v8) Heavy Chain

```
EVQLVQSGPE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW INTYTGETTY     60
AEDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCEREG GVNNWGQGTL VTVSSASTKG    120
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL    180
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTCPPC (SEQ ID NO:31)   223
```

FIG. 1C

SIESD.N103S (AFD.v14) Light Chain

```
DIQVTQSPSS LSASVGDRVT ITCITSTSIE SDMNWYQQKP GKVPKLLISG GNTLRPGVPS     60
RFSGSGSGTD FTLTISSLQP EDVATYYCLQ SDSLPYTFGQ GTKVEIKRTV AAPSVFIFPP    120
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT    180
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC  (SEQ ID NO:28)                214
```

SIESD.N103S (AFD.v14) Heavy Chain

```
EVQLVQSGPE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW INTYTGETTY     60
AEDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCEREG GVSNWGQGTL VTVSSASTKG    120
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL    180
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT   (SEQ ID NO:29)     223
```

Cys-Modified SIESD.N103S (AFD.v14) Heavy Chain

```
EVQLVQSGPE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW INTYTGETTY     60
AEDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCEREG GVSNWGQGTL VTVSSASTKG    120
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL    180
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTC   (SEQ ID NO:32)    223
```

Cys-Pro-Pro-Cys-Modified SIESD.N103S (AFD.v14) Heavy Chain

```
EVQLVQSGPE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW INTYTGETTY     60
AEDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCEREG GVSNWGQGTL VTVSSASTKG    120
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL    180
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTCPPC (SEQ ID NO:33)   223
```

FIG. 1D

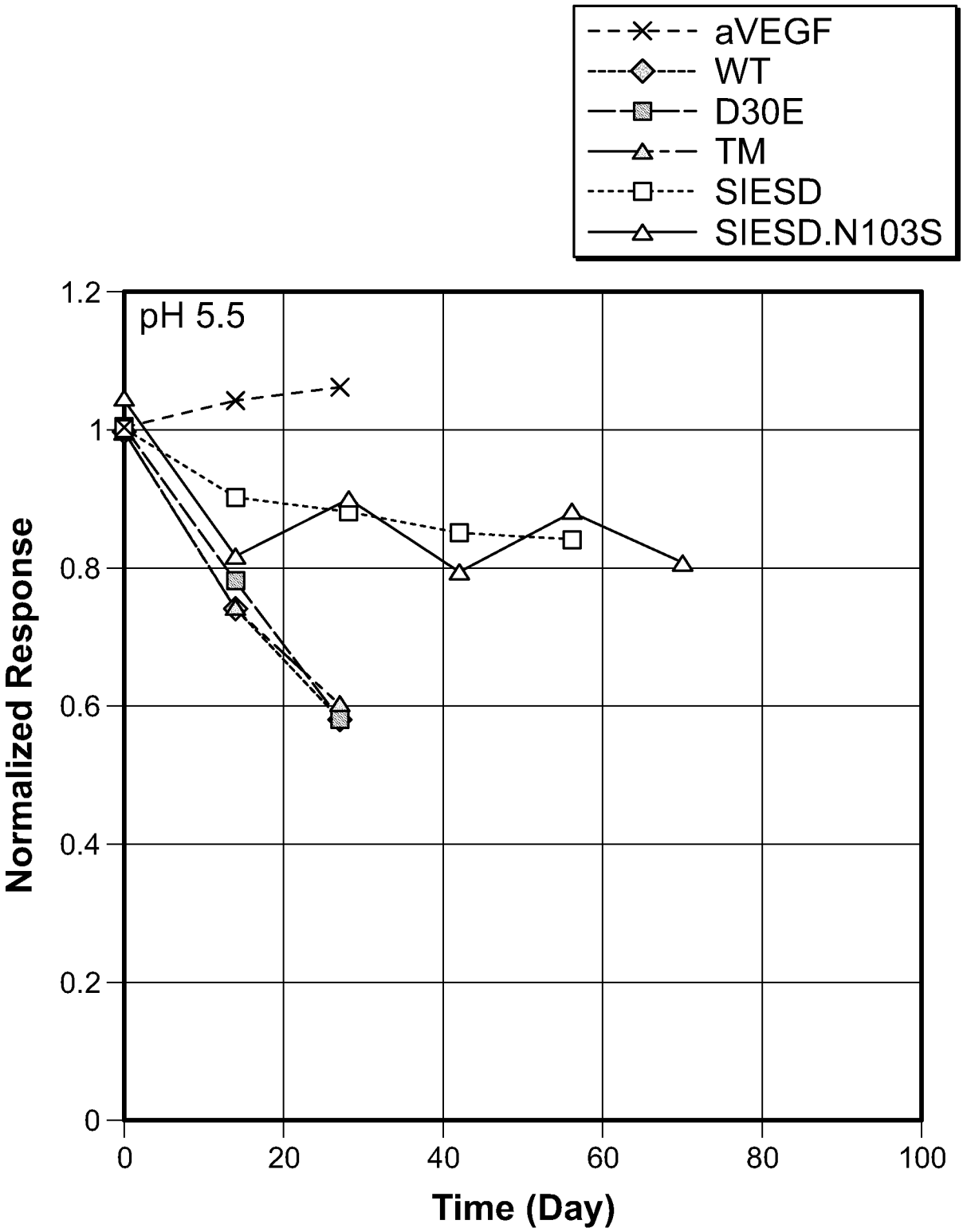

aVEGF
WT
D30E
TM
SIESD
SIESD.N103S
pH 5.5
Normalized Response
Time (Day)
0
0.2
0.4
0.6
0.8
1
1.2
0
20
40
60
80
100

FIG. 2A

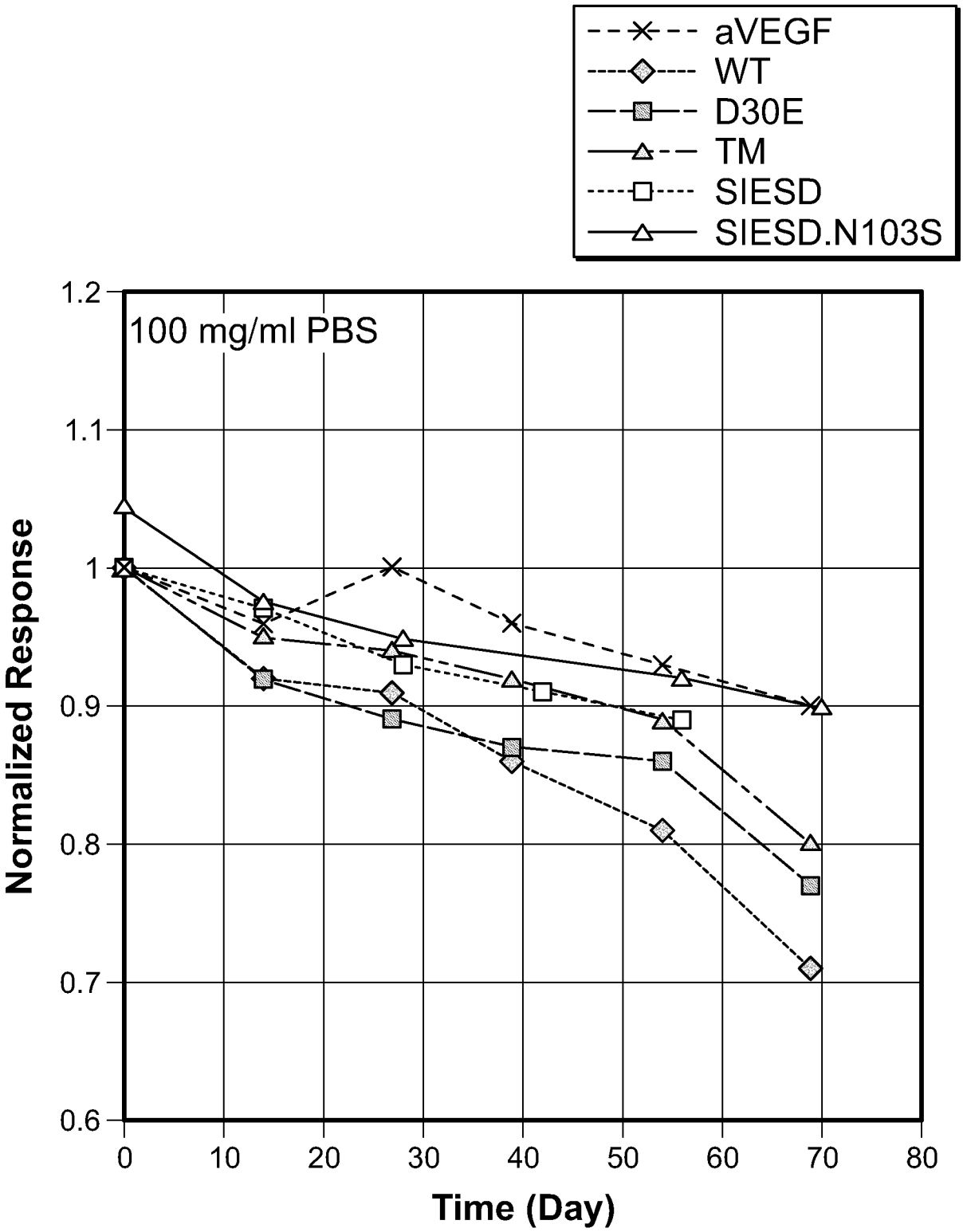

aVEGF
WT
D30E
TM
SIESD
SIESD.N103S
100 mg/ml PBS
Normalized Response
Time (Day)
1.2
1.1
1
0.9
0.8
0.7
0.6
0
10
20
30
40
50
60
70
80

FIG. 2B

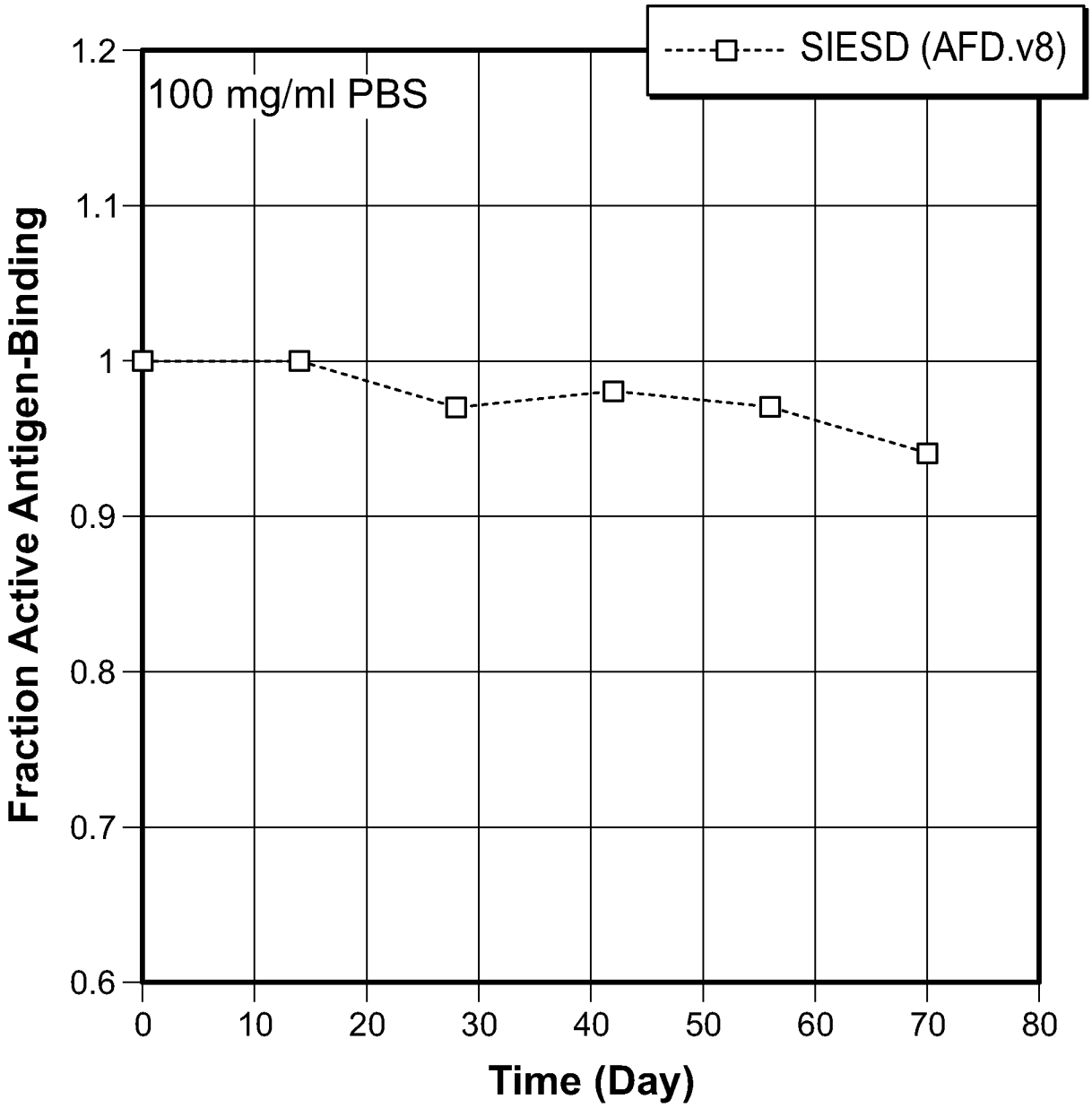

SIESD (AFD.v8)
100 mg/ml PBS
Fraction Active Antigen-Binding
1.2
1.1
1
0.9
0.8
0.7
0.6
0
10
20
30
40
50
60
70
80
Time (Day)

FIG. 2C

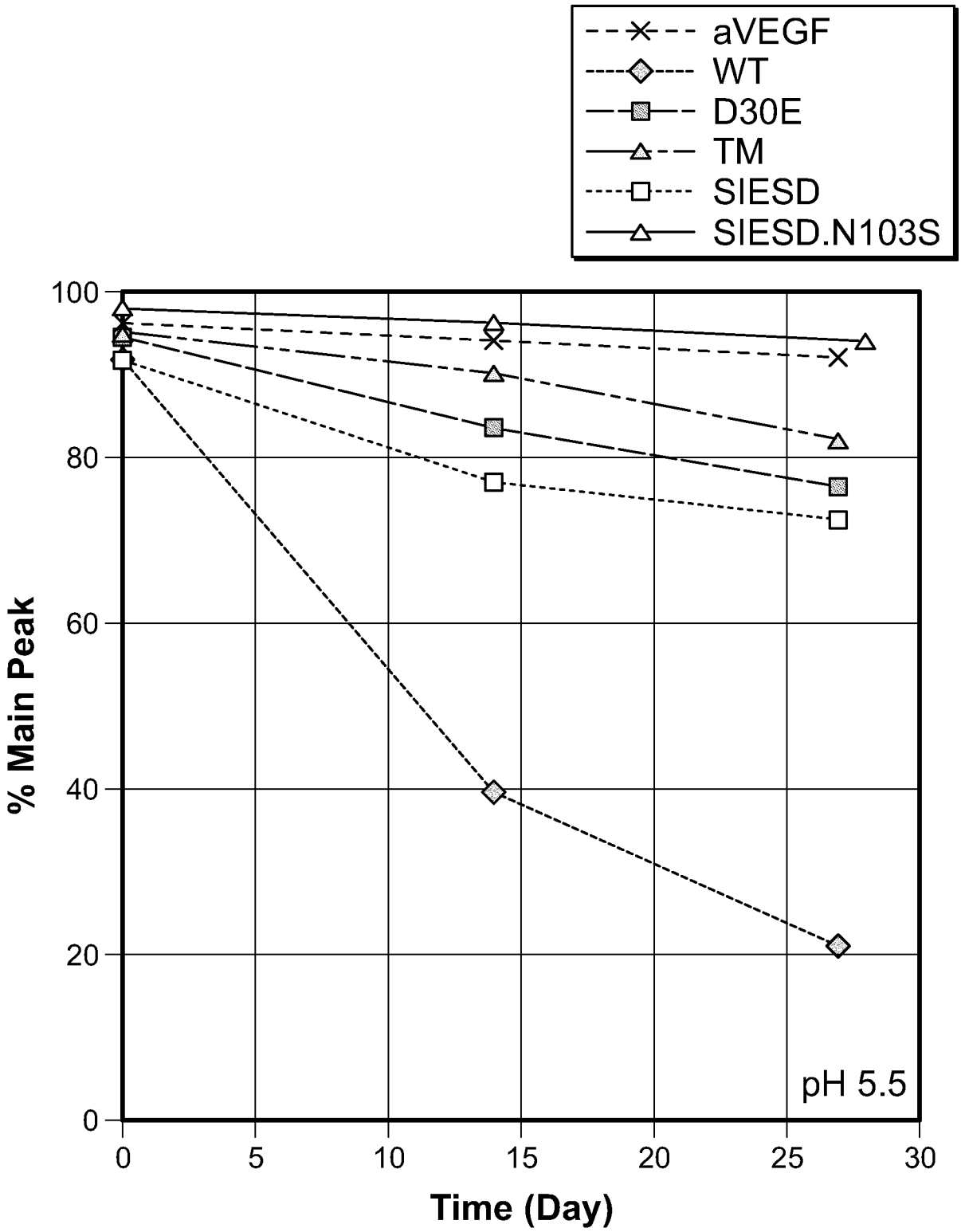

aVEGF
WT
D30E
TM
SIESD
SIESD.N103S
100
80
60
40
20
0
% Main Peak
0
5
10
15
20
25
30
Time (Day)
pH 5.5

FIG. 3A

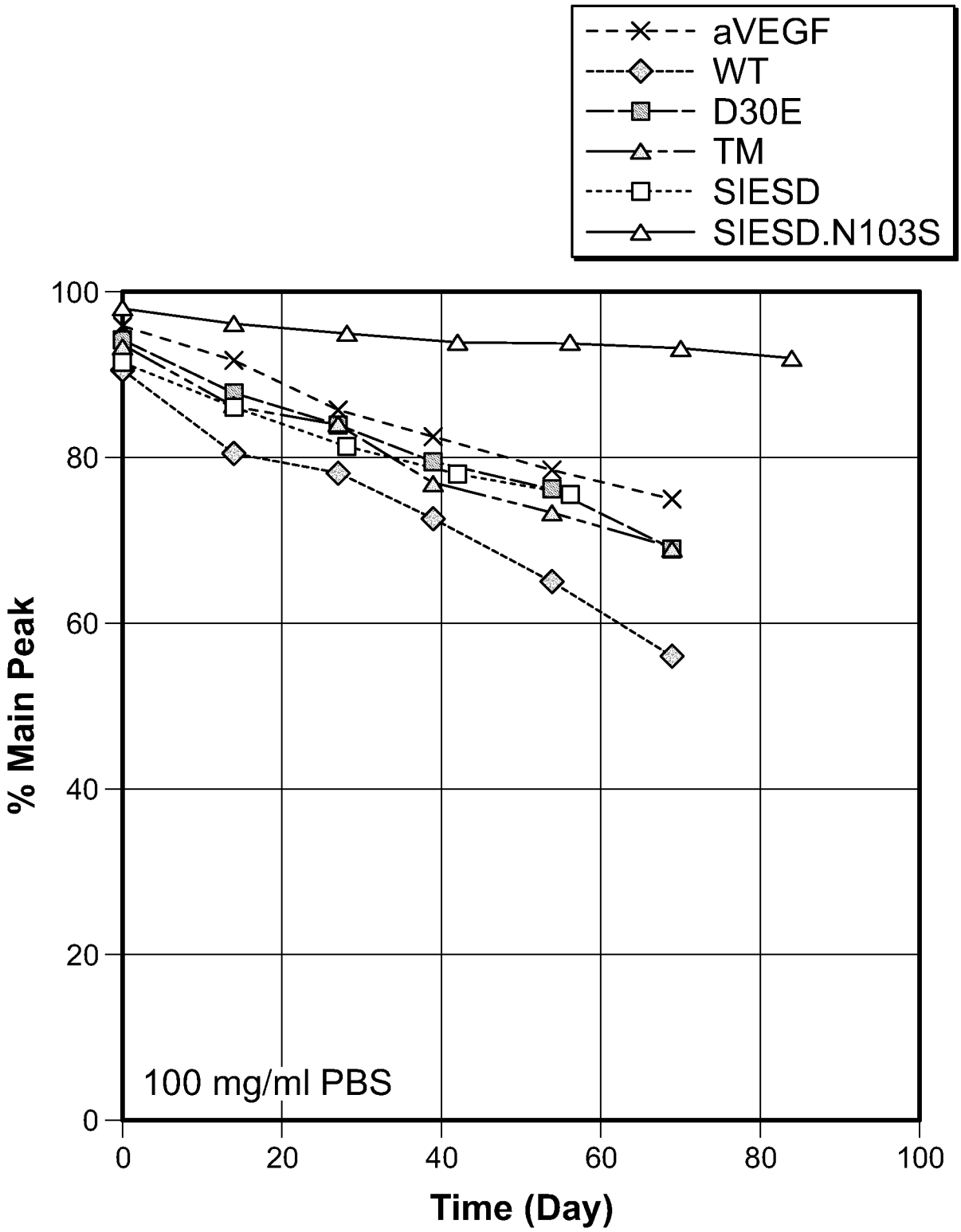

aVEGF
WT
D30E
TM
SIESD
SIESD.N103S
100
80
60
40
20
0
% Main Peak
100 mg/ml PBS
0
20
40
60
80
100
Time (Day)

FIG. 3B

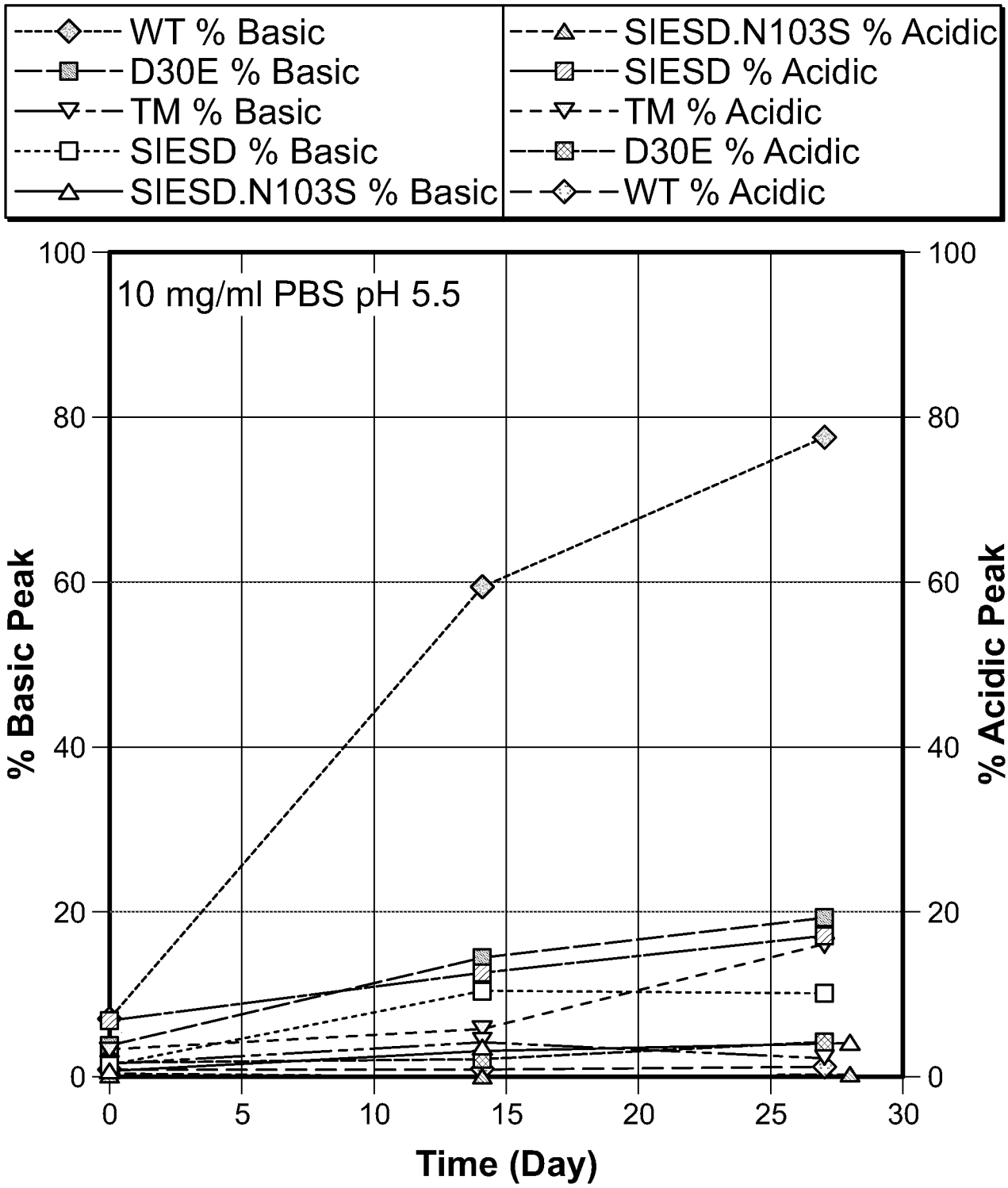

WT % Basic
D30E % Basic
TM % Basic
SIESD % Basic
SIESD.N103S % Basic
SIESD.N103S % Acidic
SIESD % Acidic
TM % Acidic
D30E % Acidic
WT % Acidic
10 mg/ml PBS pH 5.5
% Basic Peak
% Acidic Peak
Time (Day)
0
20
40
60
80
100
0
5
10
15
20
25
30

FIG. 4A

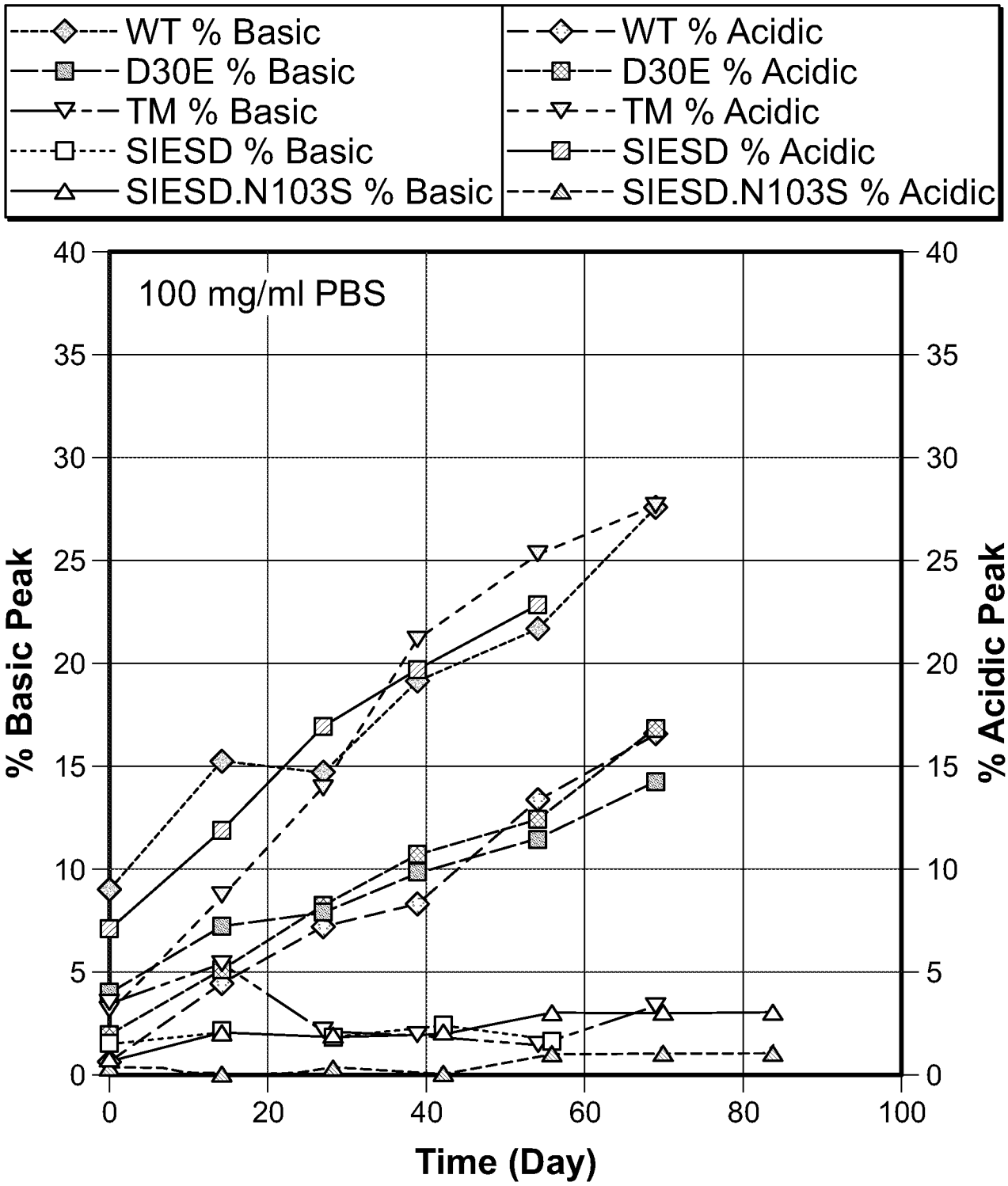

WT % Basic
D30E % Basic
TM % Basic
SIESD % Basic
SIESD.N103S % Basic
WT % Acidic
D30E % Acidic
TM % Acidic
SIESD % Acidic
SIESD.N103S % Acidic
100 mg/ml PBS
% Basic Peak
% Acidic Peak
Time (Day)
0
5
10
15
20
25
30
35
40
0
20
40
60
80
100

FIG. 4B

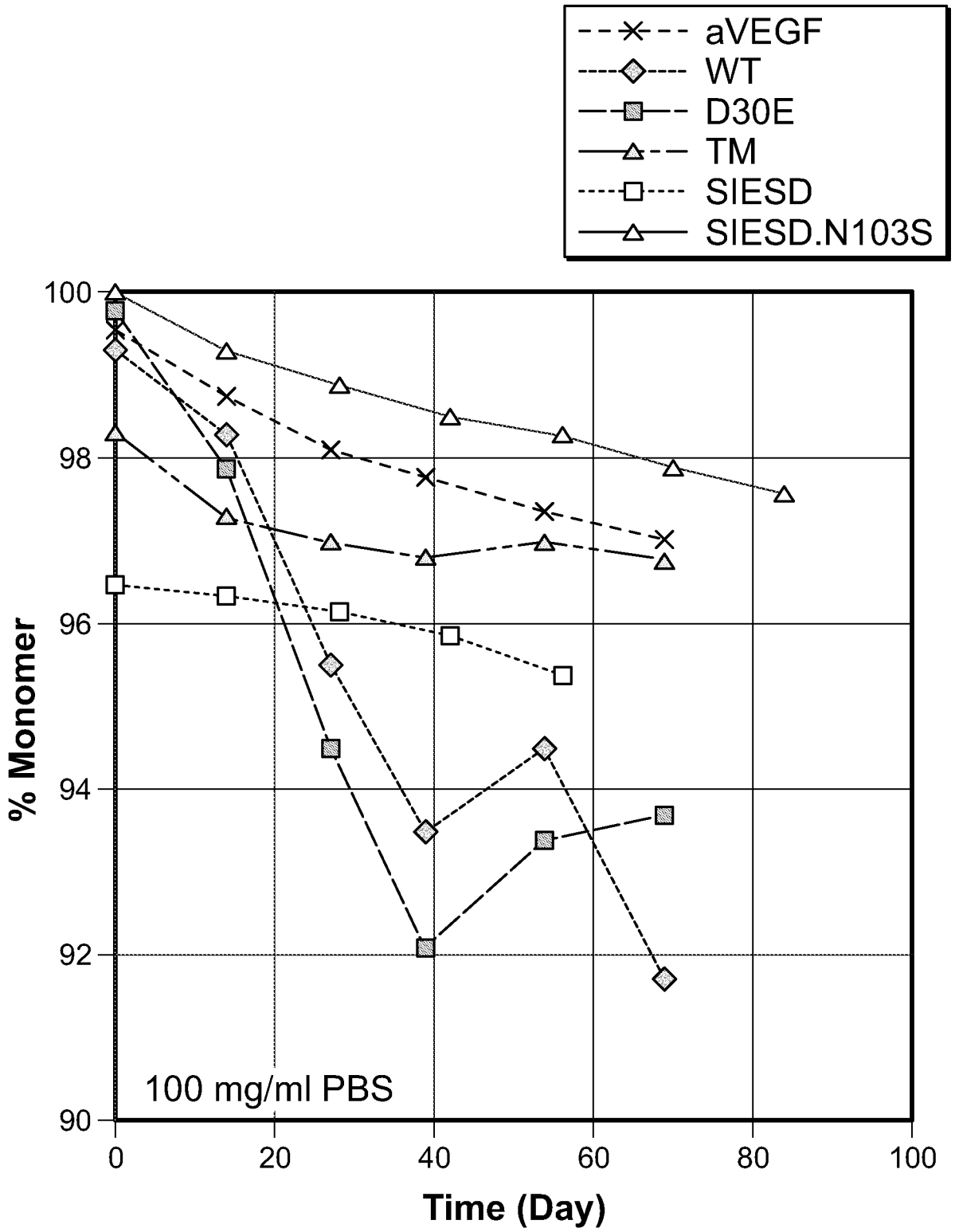

aVEGF
WT
D30E
TM
SIESD
SIESD.N103S
100
98
96
94
92
90
% Monomer
100 mg/ml PBS
0
20
40
60
80
100
Time (Day)

FIG. 5

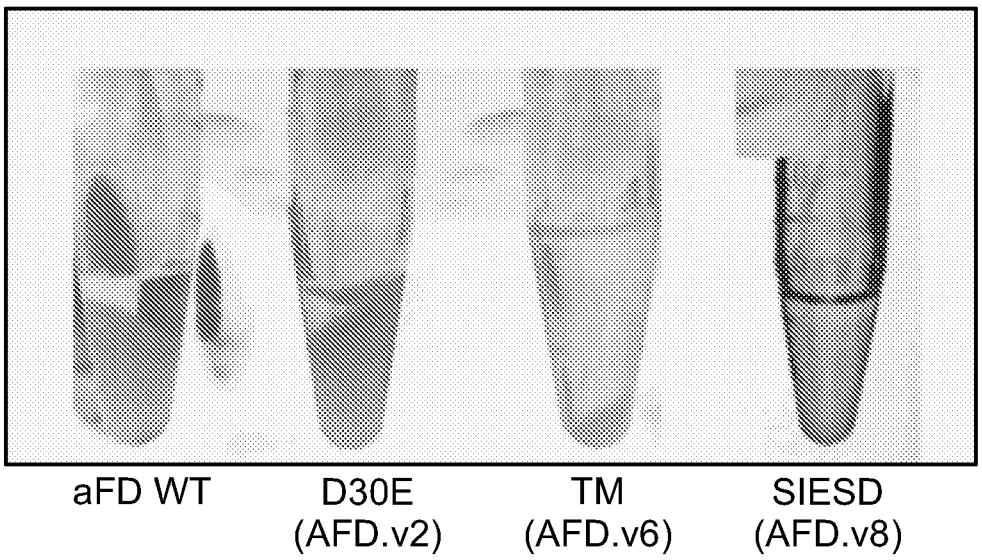

aFD WT
D30E
(AFD.v2)
TM
(AFD.v6)
SIESD
(AFD.v8)

FIG. 6

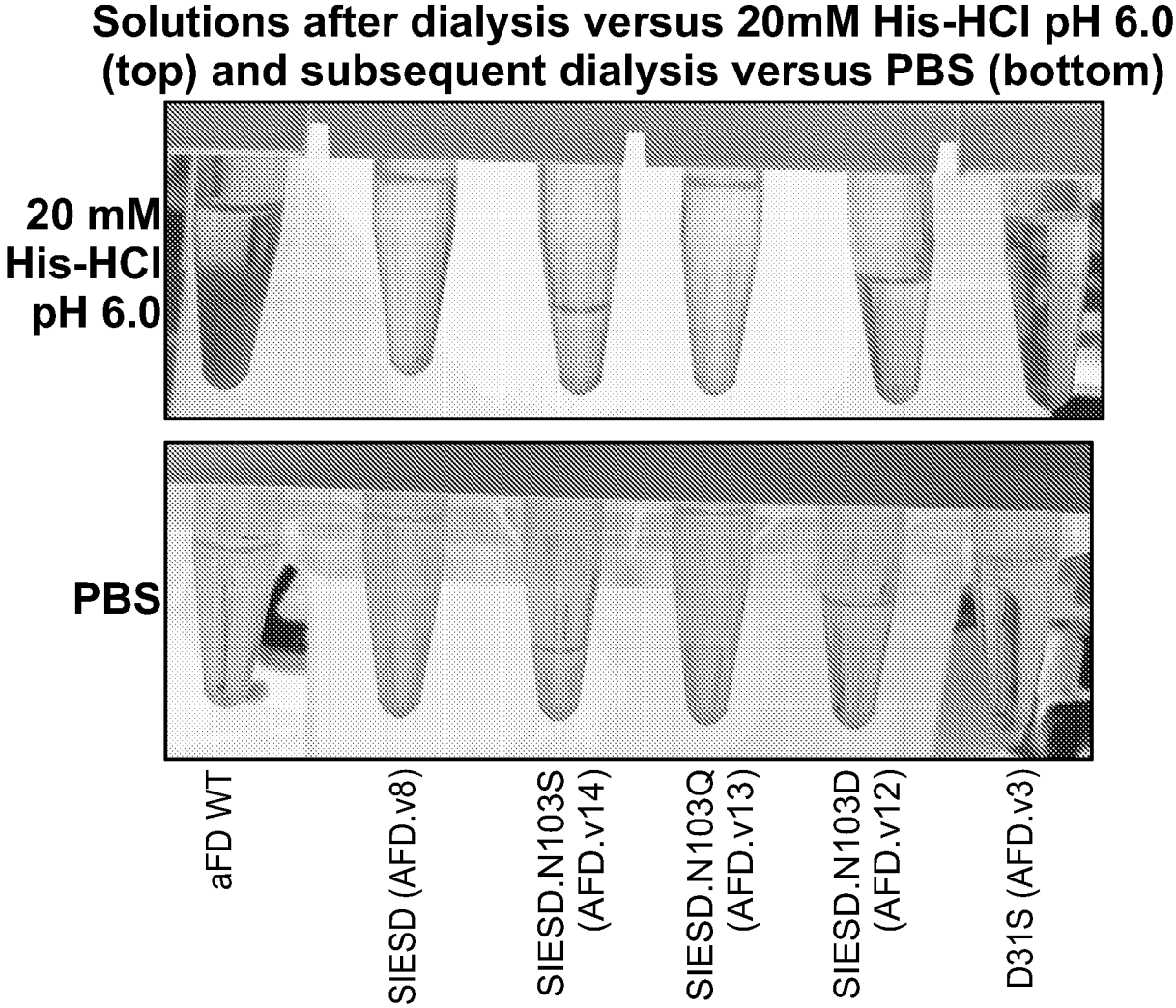

Solutions after dialysis versus 20mM His-HCl pH 6.0 (top) and subsequent dialysis versus PBS (bottom)
20 mM His-HCl pH 6.0
PBS
aFD WT
SIESD (AFD.v8)
SIESD.N103S (AFD.v14)
SIESD.N103Q (AFD.v13)
SIESD.N103D (AFD.v12)
D31S (AFD.v3)

FIG. 7

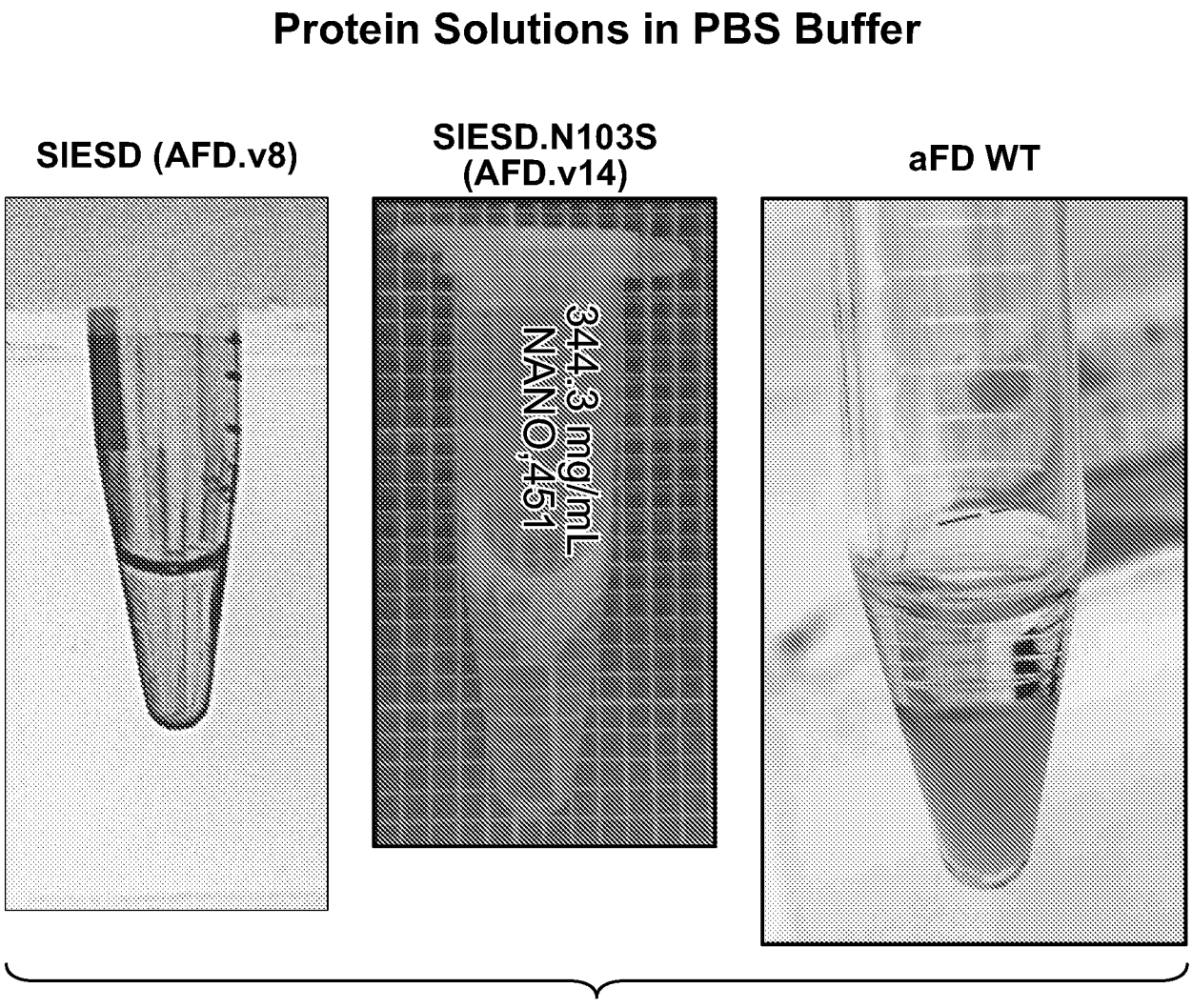
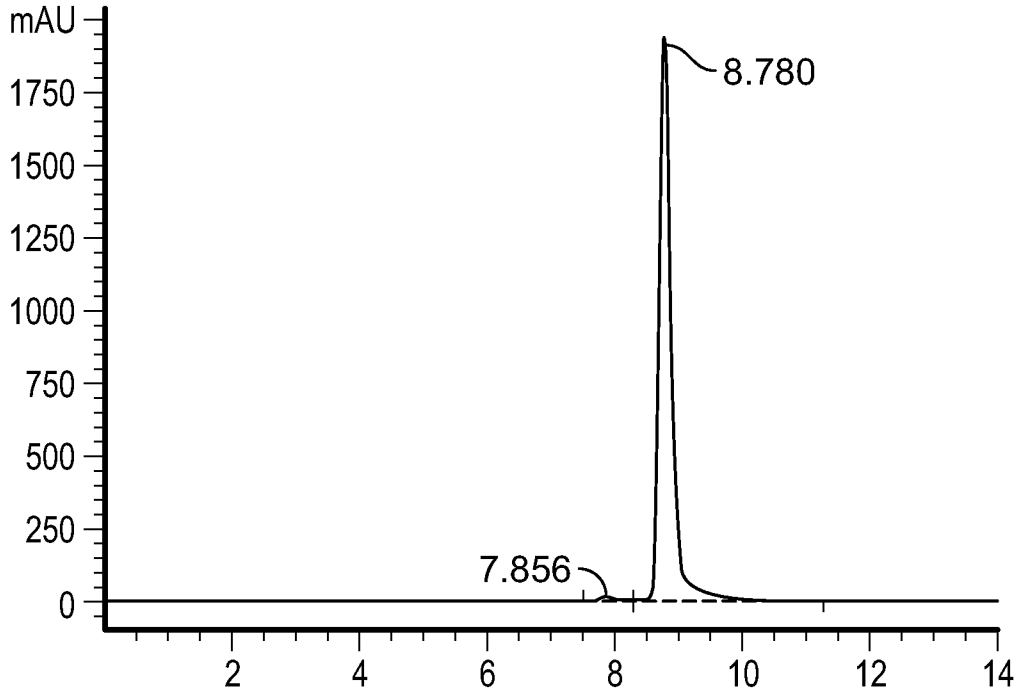

Protein Solutions in PBS Buffer
SIESD (AFD.v8)
SIESD.N103S
(AFD.v14)
aFD WT
344.3 mg/mL
NANO,451

FIG. 8

SEC Profile for 372 mg/mL SIESD.N103S (AFD.v14) in PBS

DAD1 A, Sig=280, 2 Ref=off



Area Percent Report

Sorted by : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=280, 2 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.856	VV	0.2537	241.13916	12.69158	0.94195
2	8.780	VB	0.1986	2.53587e4	1939.25708	99.05805
Totals:				2.55999e4	1951.94866	

FIG. 9

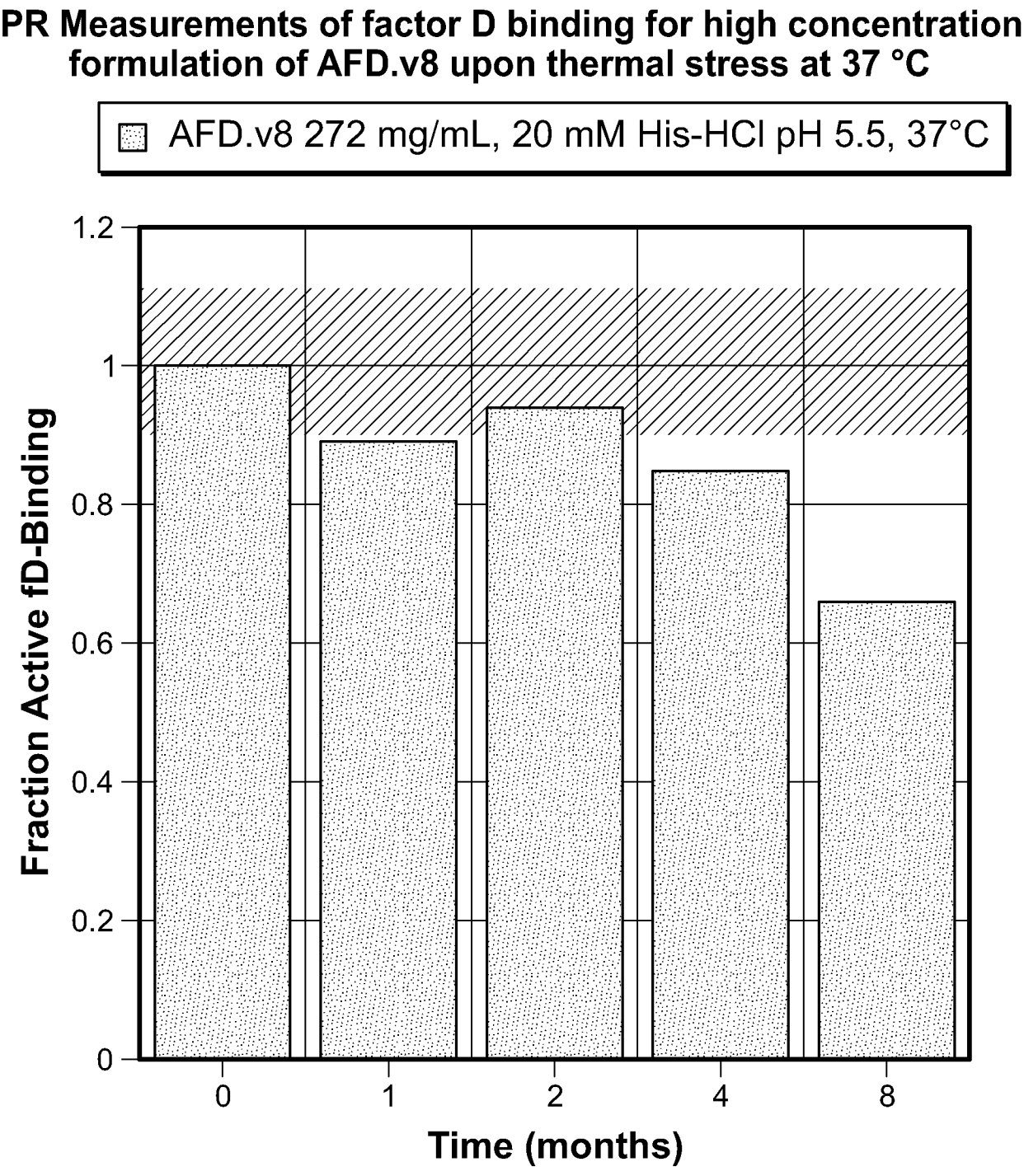

SPR Measurements of factor D binding for high concentration formulation of AFD.v8 upon thermal stress at 37 °C
AFD.v8 272 mg/mL, 20 mM His-HCl pH 5.5, 37°C
Fraction Active fD-Binding
1.2
1
0.8
0.6
0.4
0.2
0
0
1
2
4
8
Time (months)

FIG. 10A

Assessment of chemical and physical stability for high concentration (272 mg/mL) formulation (20 mM His-HCL pH 5.5) of AFD.v8 stressed at 37 ºC.

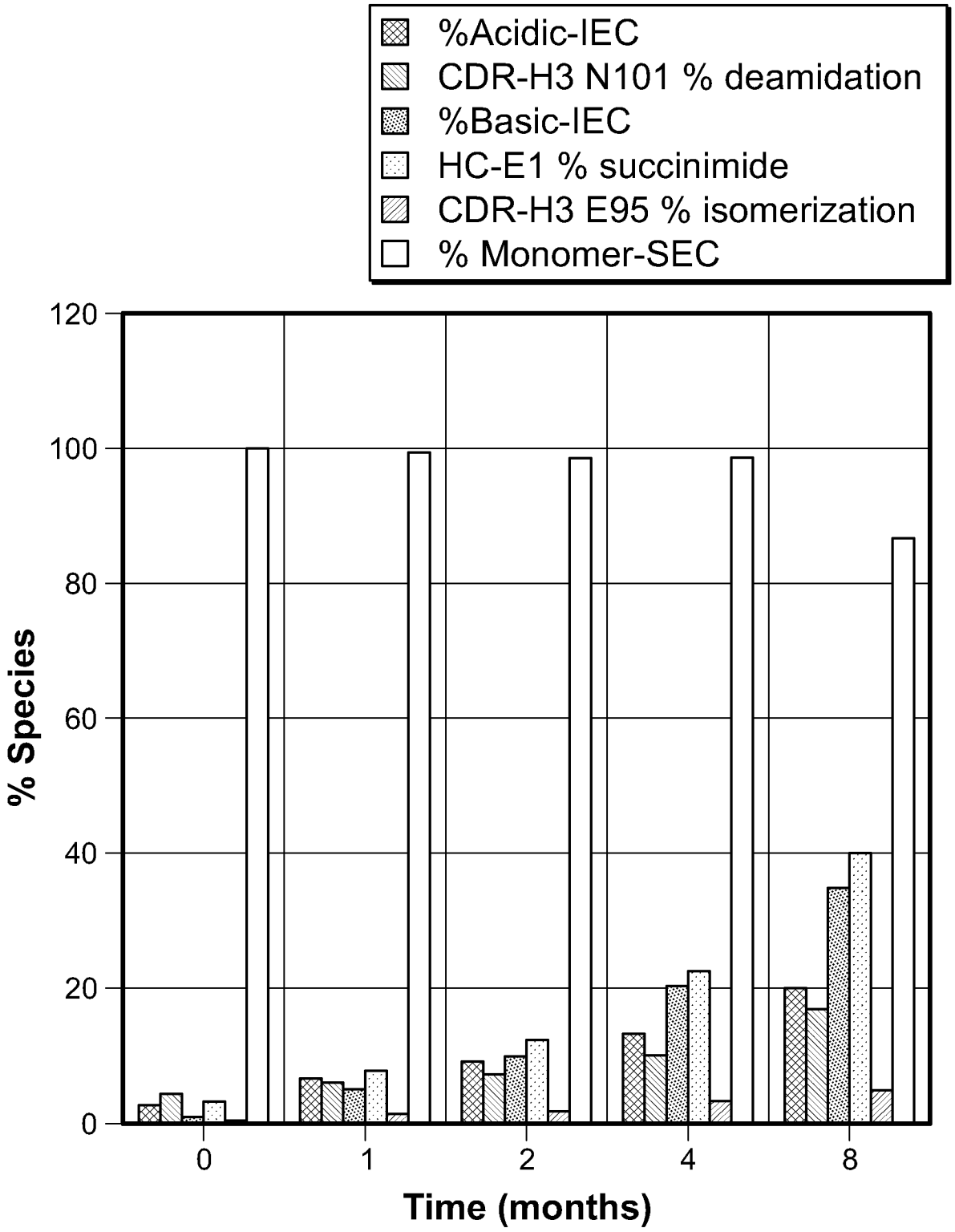
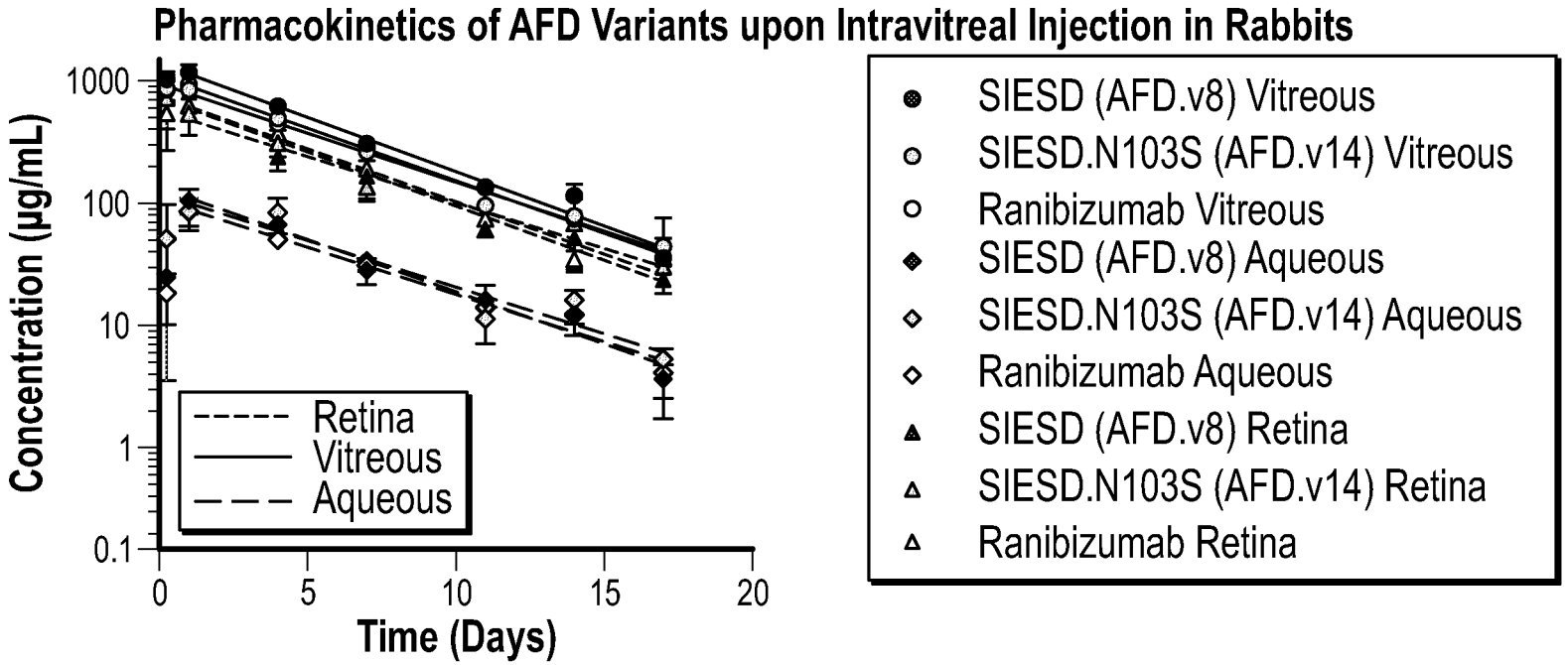


FIG. 10B



	Vitreous (Liquid)				Aqueous		Retina	
Molecule*	Half-life (Days)	AUC_{all} (Day*µg/mL)	CL (mL/Day)	V_{ss} (mL)	Half-life (Days)	AUC_{all} (Day*µg/mL)	Half-life (Days)	AUC_{all} (Day*µg/mL)
SIESD (AFD.v8)	3.4	4460	0.22	1.0	3.5	411	3.4	2489
SIESD.N103S (AFD.v14)	3.7	3450	0.28	1.4	4.0	432	4.0	2199
Ranibizumab	3.5	3600	0.27	1.3	3.9	353	3.5	2453

*1 mg/eye dose, study 14-0995

FIG. 11

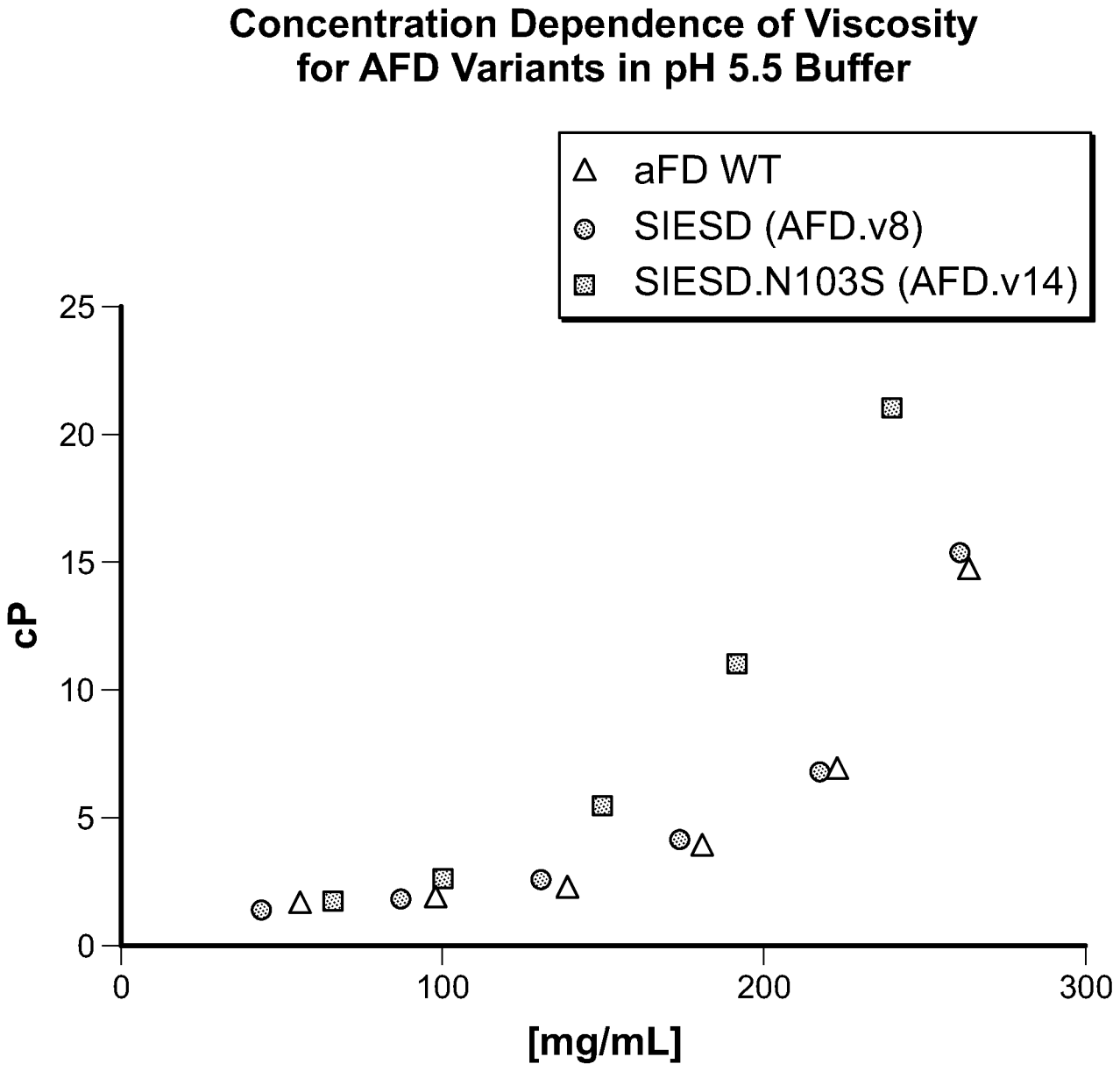

Concentration Dependence of Viscosity
for AFD Variants in pH 5.5 Buffer
aFD WT
SIESD (AFD.v8)
SIESD.N103S (AFD.v14)
cP
25
20
15
10
5
0
0
100
200
300
[mg/mL]

FIG. 12

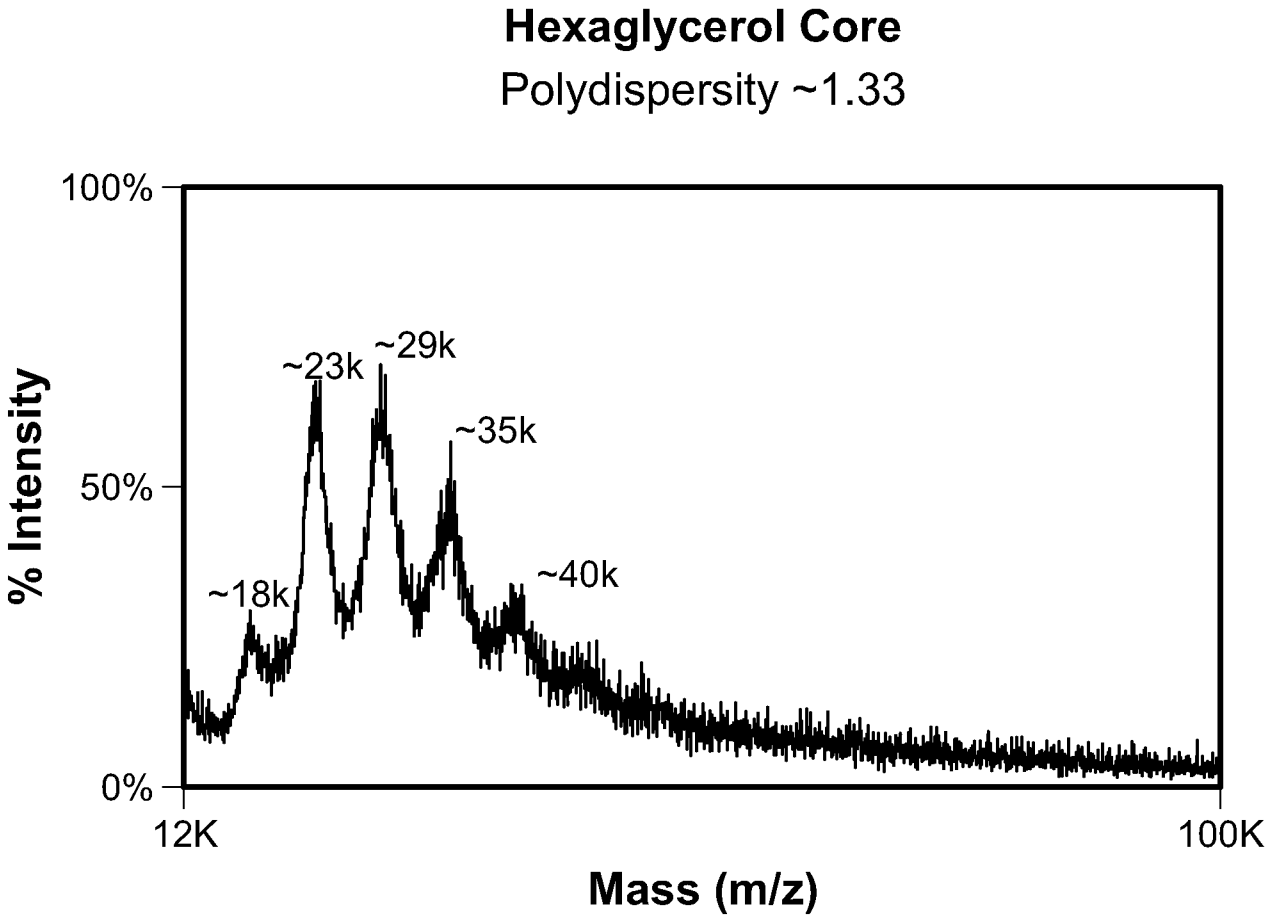

Hexaglycerol Core
Polydispersity ~1.33
100%
50%
0%
% Intensity
~18k
~23k
~29k
~35k
~40k
12K
100K
Mass (m/z)

FIG. 13A

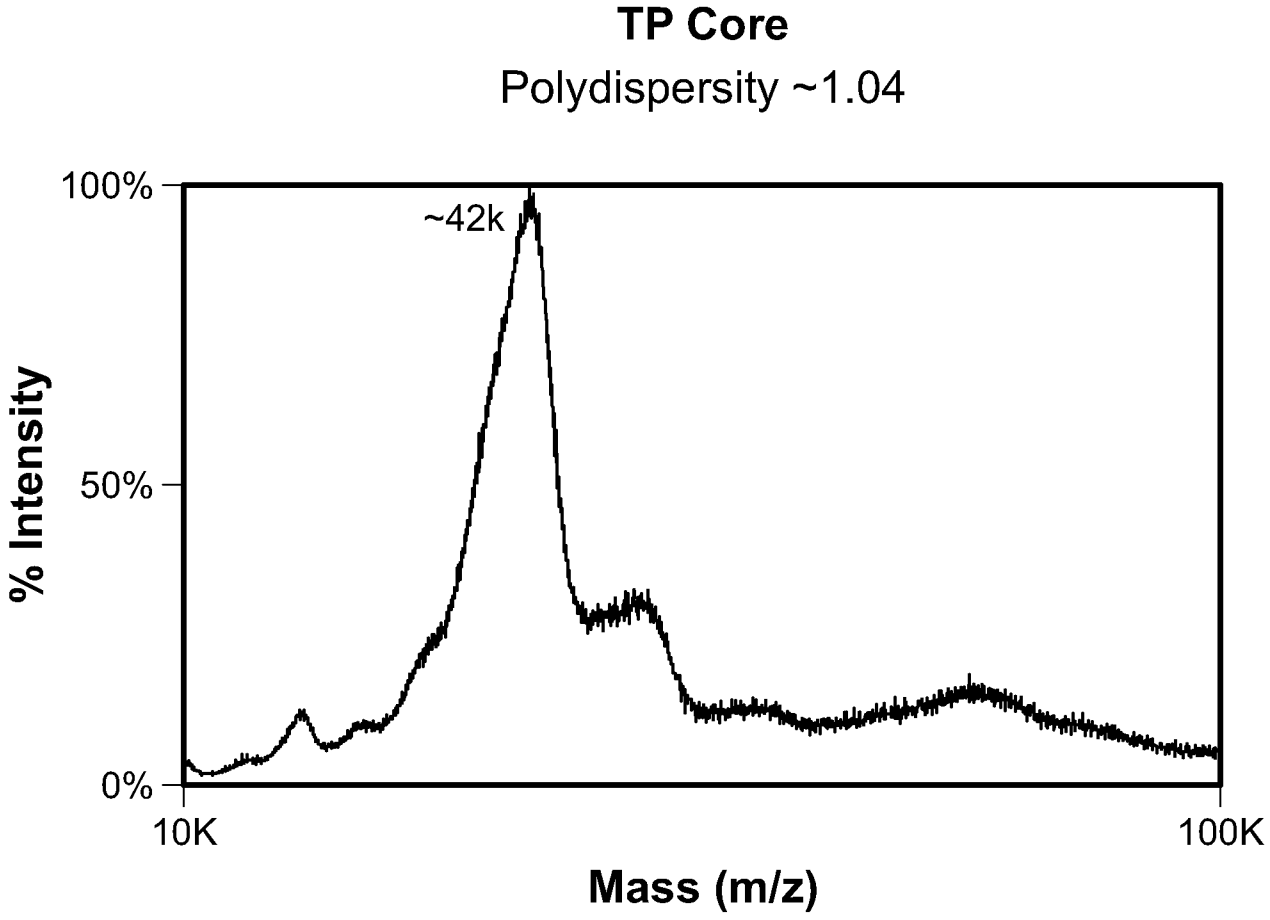

TP Core
Polydispersity ~1.04
100%
50%
0%
~42k
% Intensity
10K
100K
Mass (m/z)

FIG. 13B

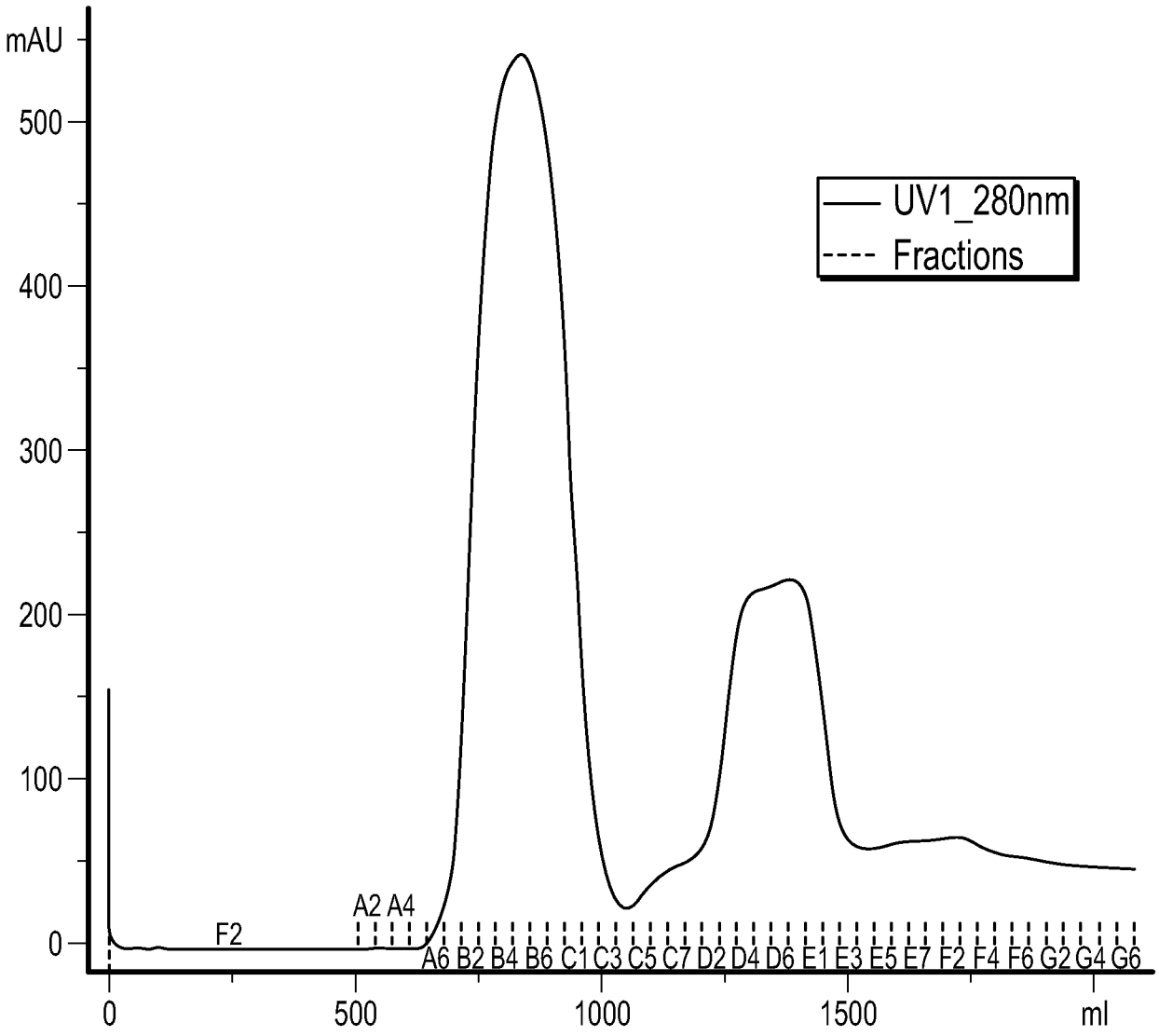

mAU
500
400
300
200
100
0
UV1_280nm
Fractions
F2
A2 A4
A6 B2 B4 B6 C1 C3 C5 C7 D2 D4 D6 E1 E3 E5 E7 F2 F4 F6 G2 G4 G6
0
500
1000
1500
ml

FIG. 14A

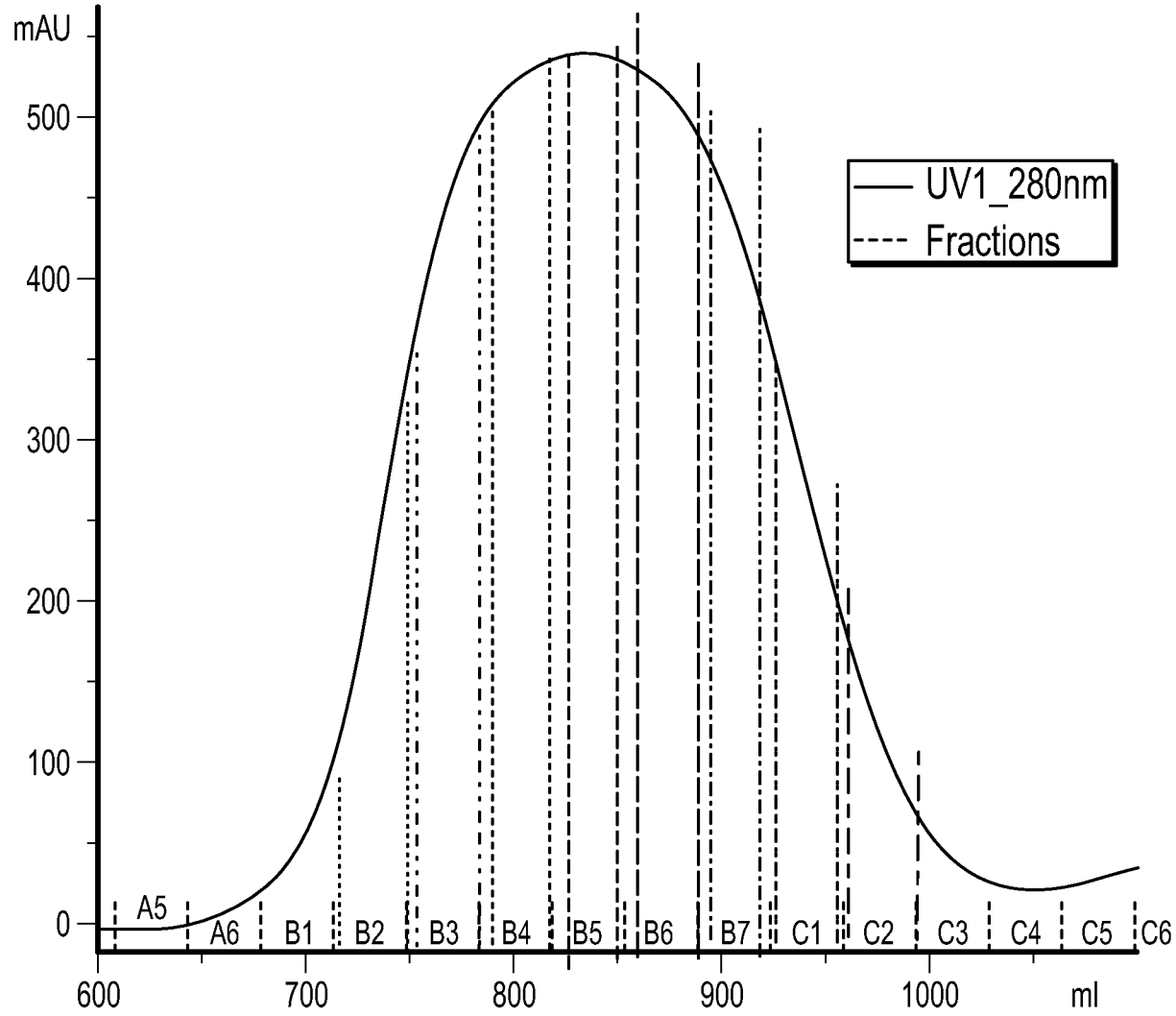


FIG. 14B

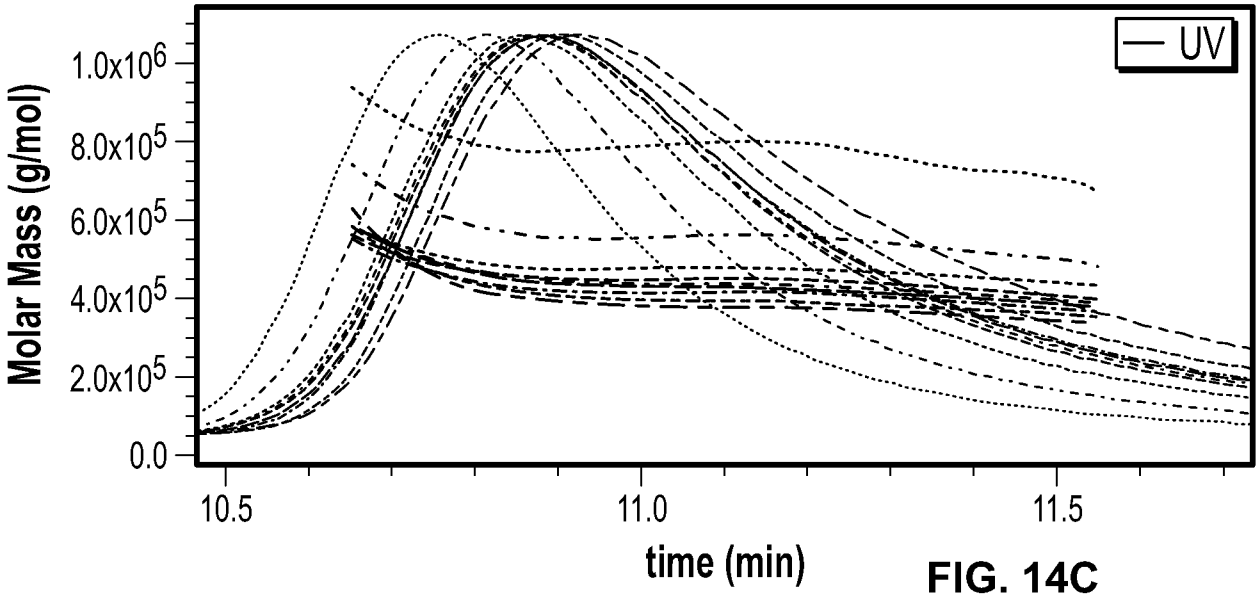


FIG. 14C

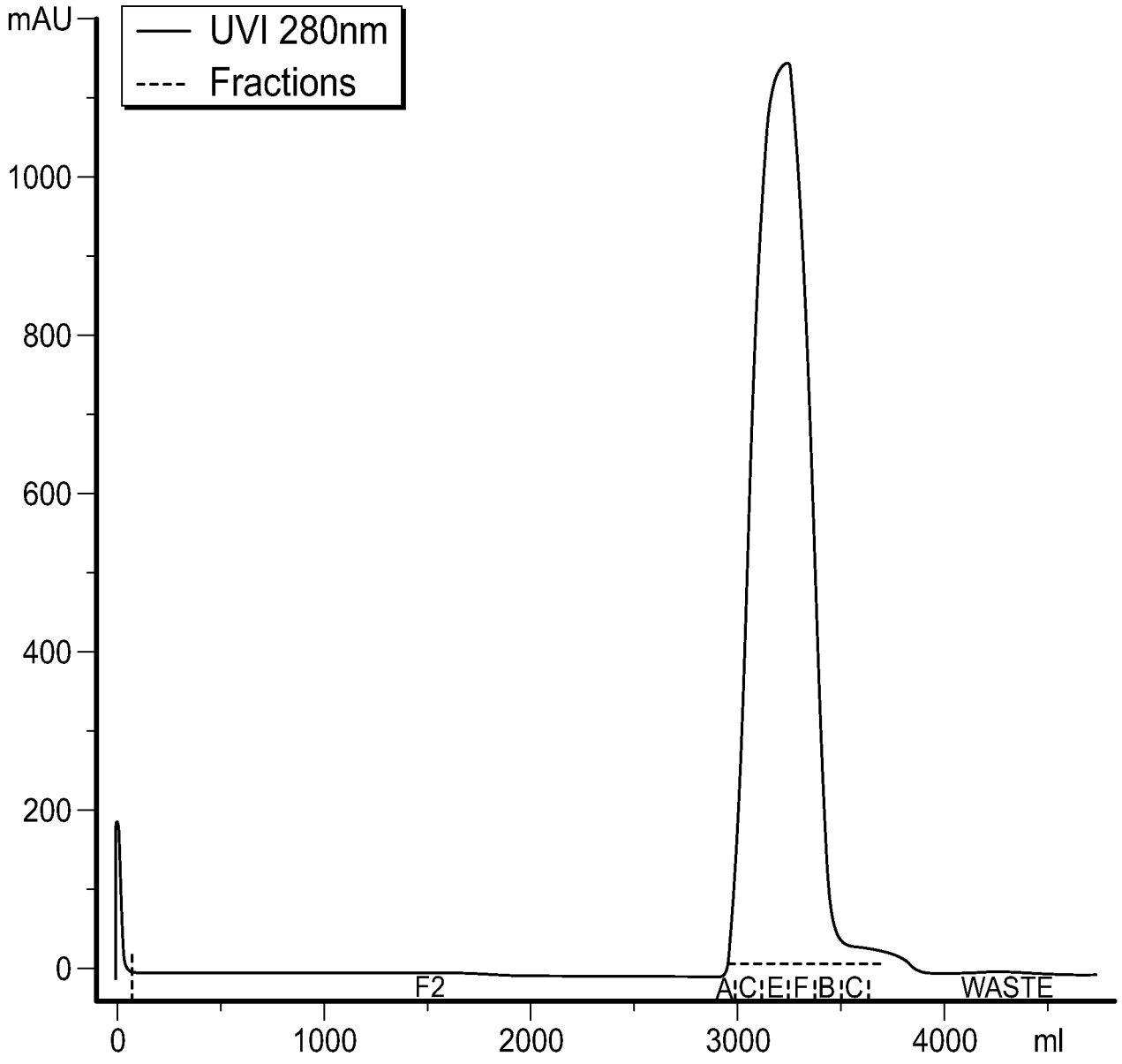

mAU
UVI 280nm
Fractions
1000
800
600
400
200
0
F2
A
C
E
F
B
C
WASTE
0
1000
2000
3000
4000
ml

FIG. 15A

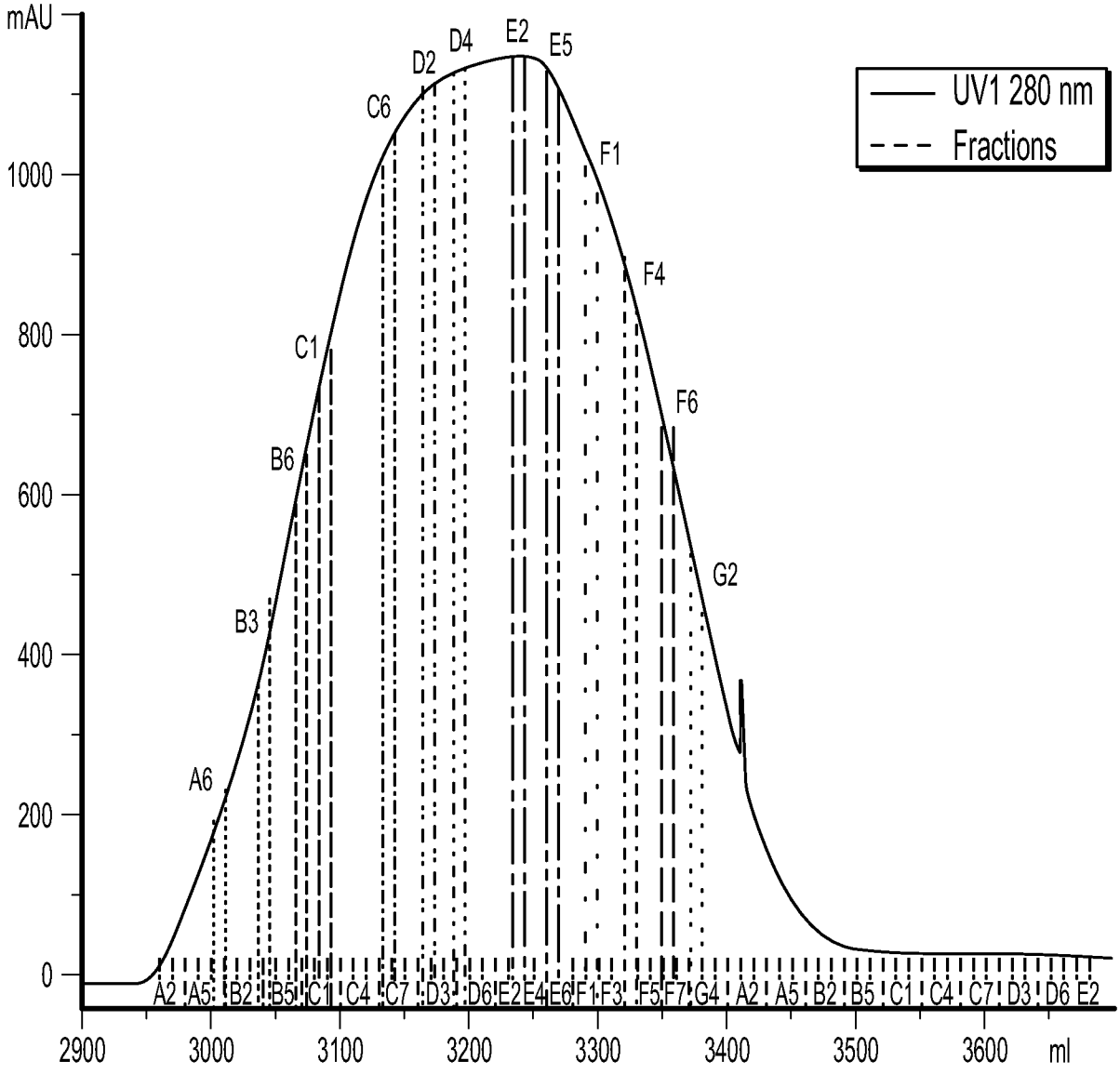


FIG. 15B

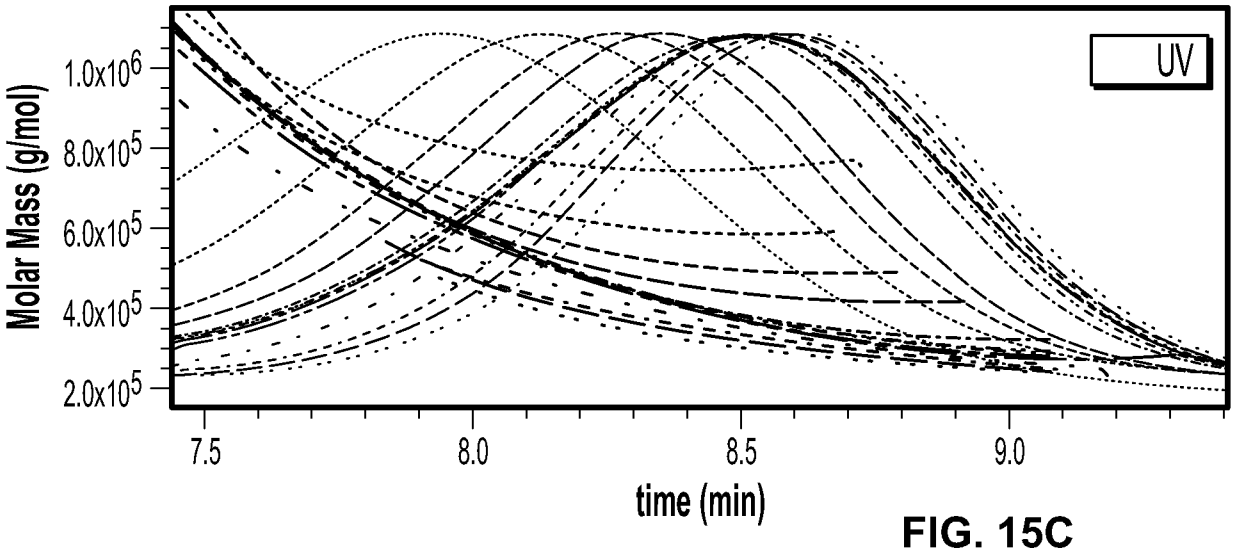


FIG. 15C

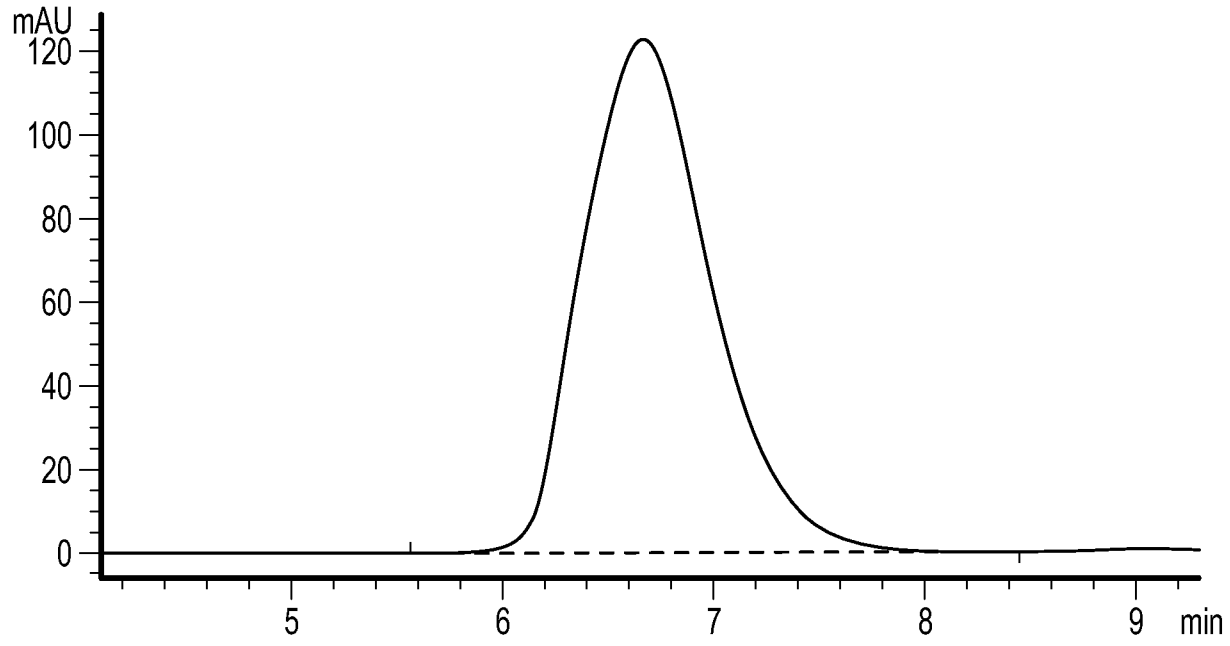


FIG. 16A

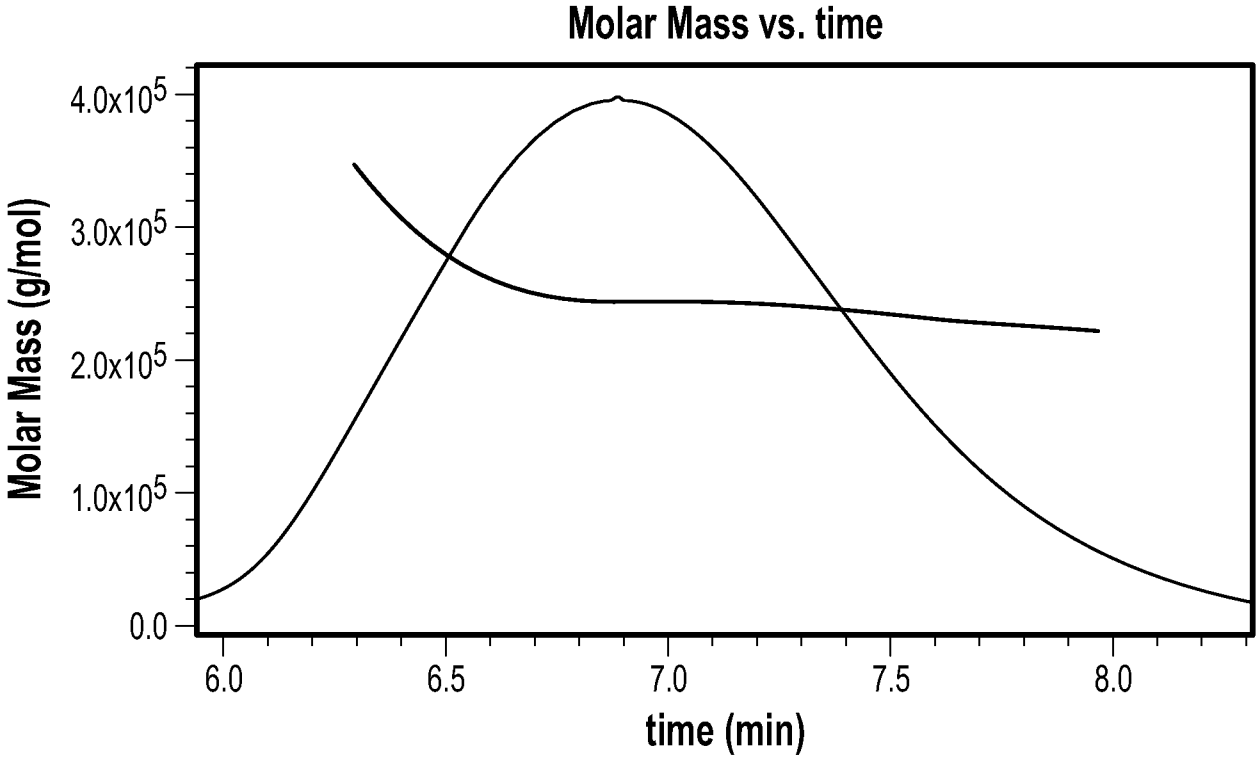


FIG. 16B

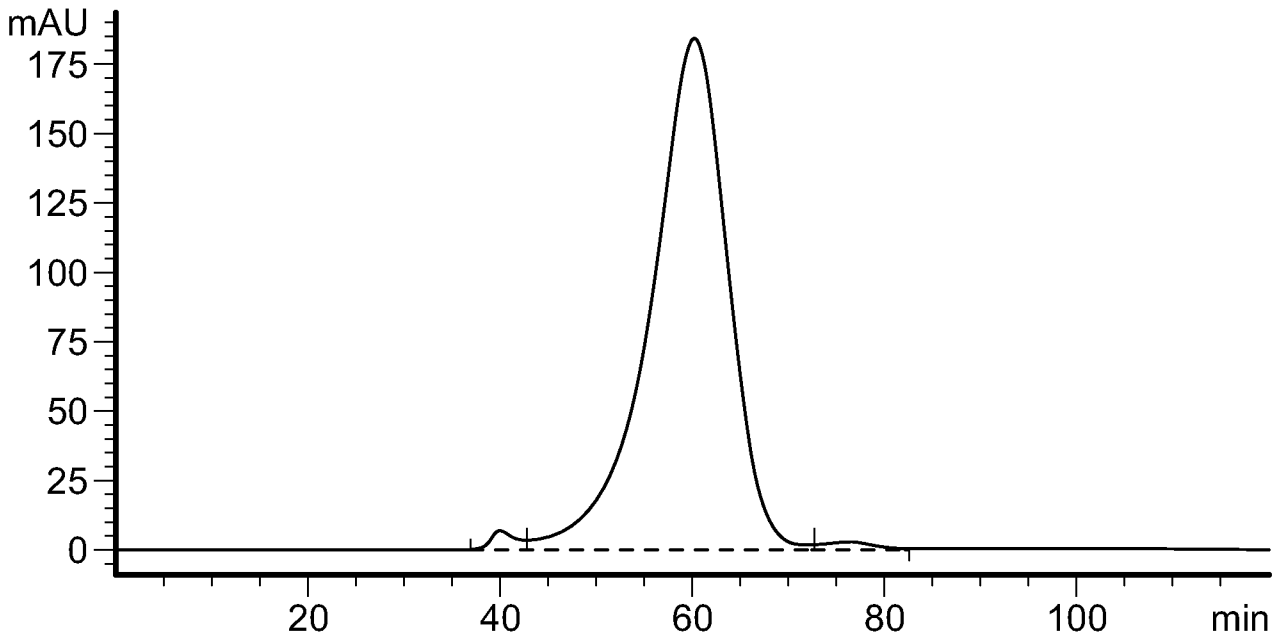


FIG. 17A

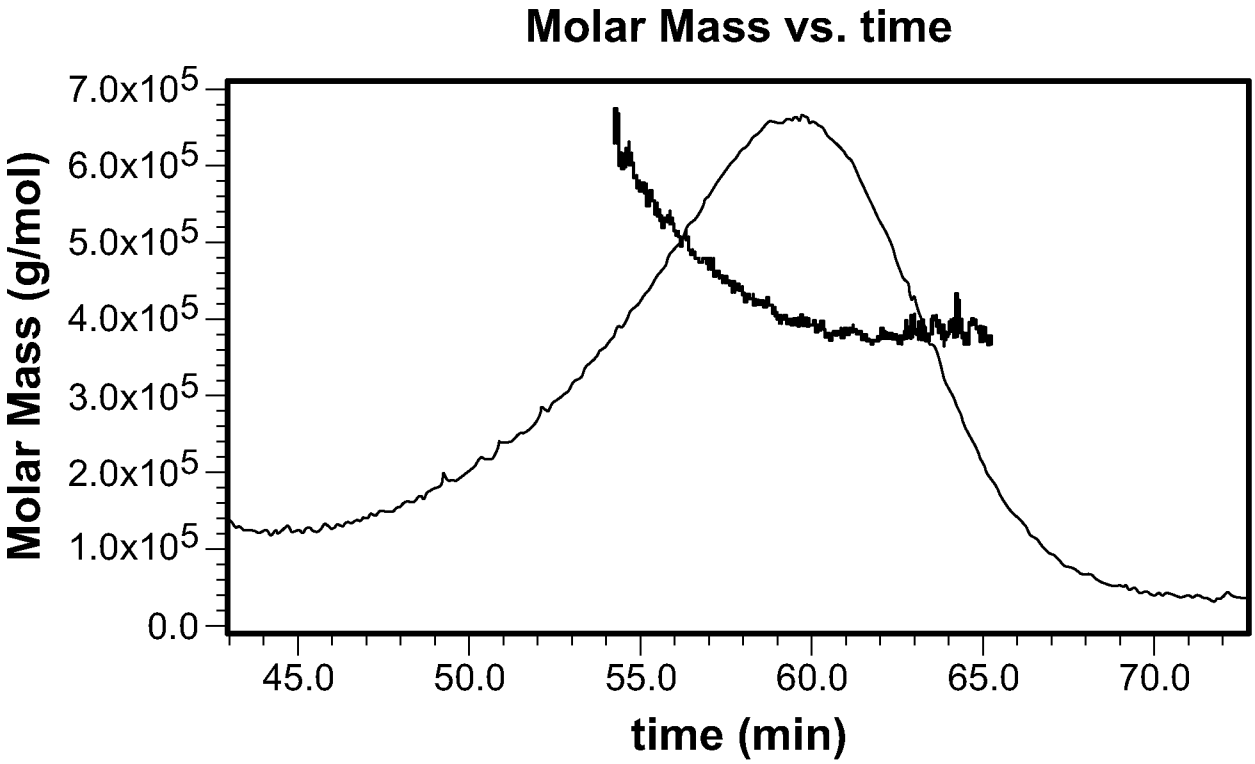

Molar Mass vs. time
Molar Mass (g/mol)
7.0x10^5
6.0x10^5
5.0x10^5
4.0x10^5
3.0x10^5
2.0x10^5
1.0x10^5
0.0
45.0
50.0
55.0
60.0
65.0
70.0
time (min)

FIG. 17B

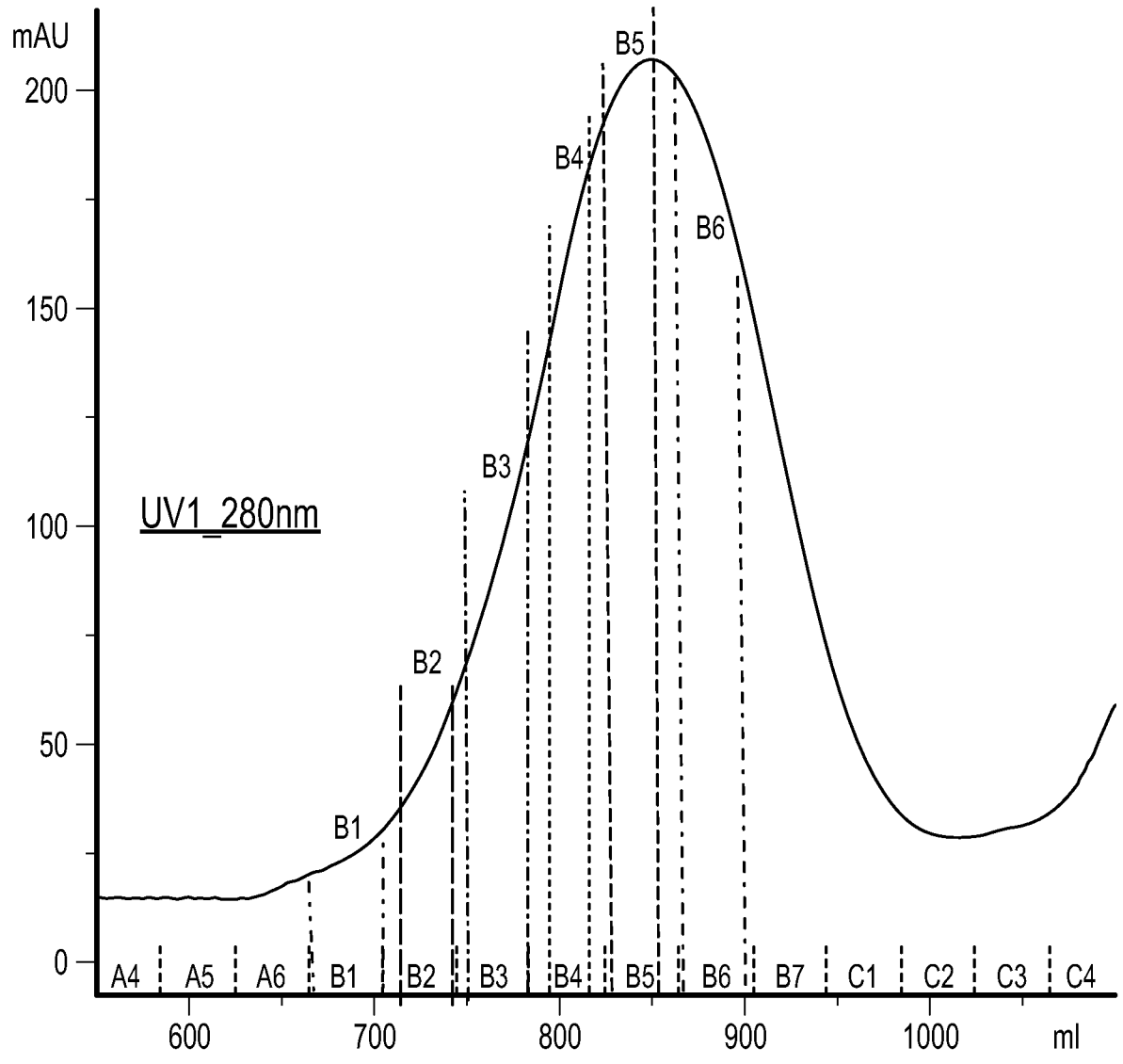

mAU
200
150
100
50
0
UV1_280nm
B1
B2
B3
B4
B5
B6
A4
A5
A6
B1
B2
B3
B4
B5
B6
B7
C1
C2
C3
C4
600
700
800
900
1000
ml

FIG. 18A

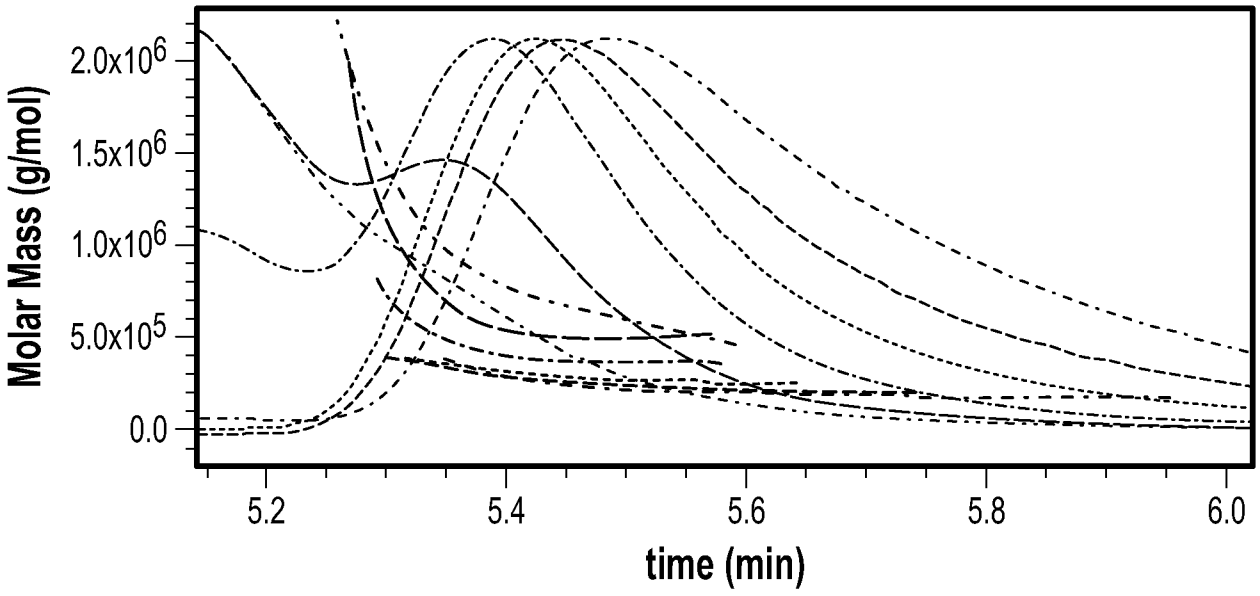

Molar Mass (g/mol)
2.0x10^6
1.5x10^6
1.0x10^6
5.0x10^5
0.0
5.2
5.4
5.6
5.8
6.0
time (min)

FIG. 18B

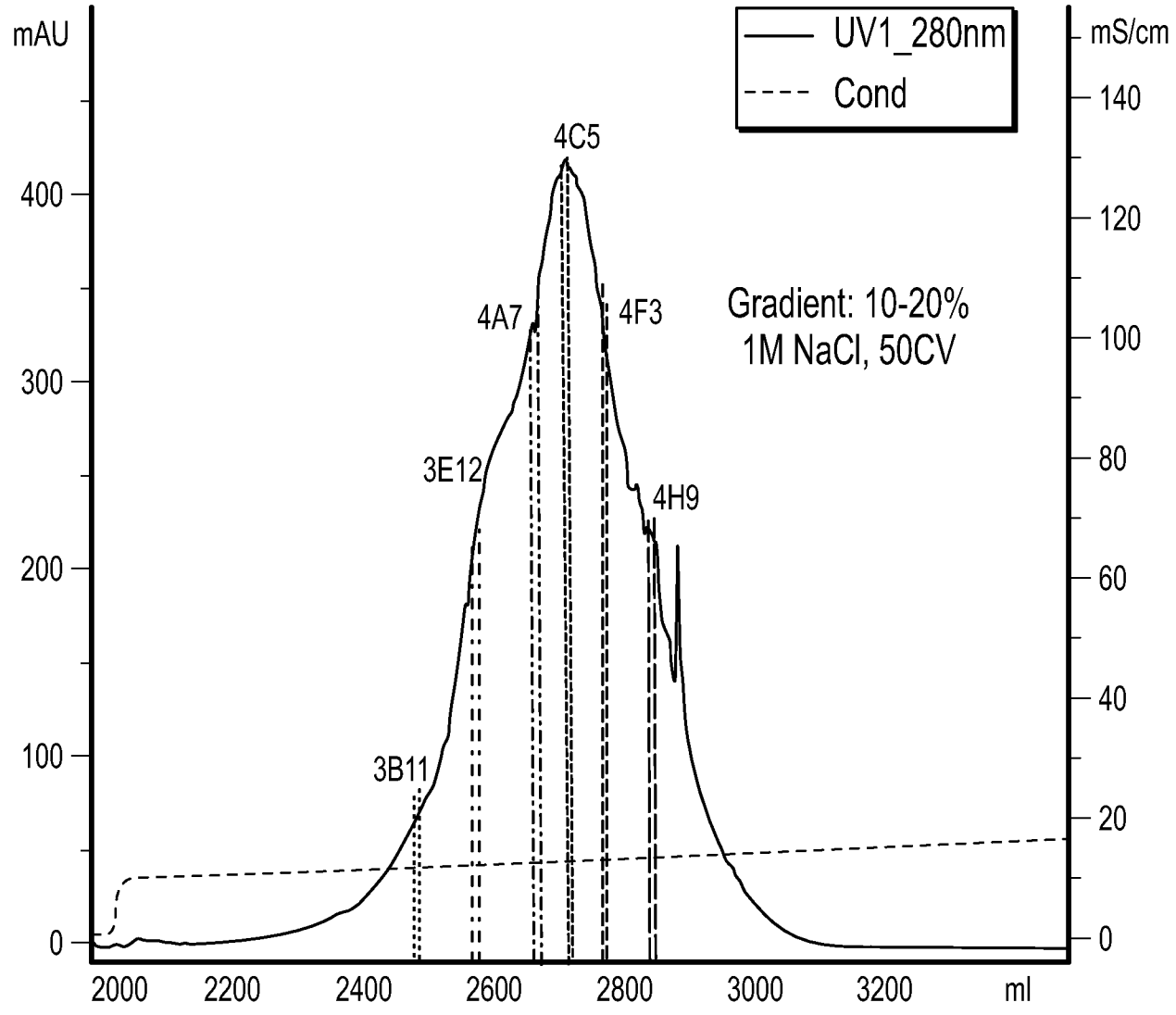

mAU
mS/cm
UV1_280nm
Cond
4C5
4A7
4F3
3E12
4H9
3B11
Gradient: 10-20%
1M NaCl, 50CV
0
100
200
300
400
0
20
40
60
80
100
120
140
2000
2200
2400
2600
2800
3000
3200
ml

FIG. 19A

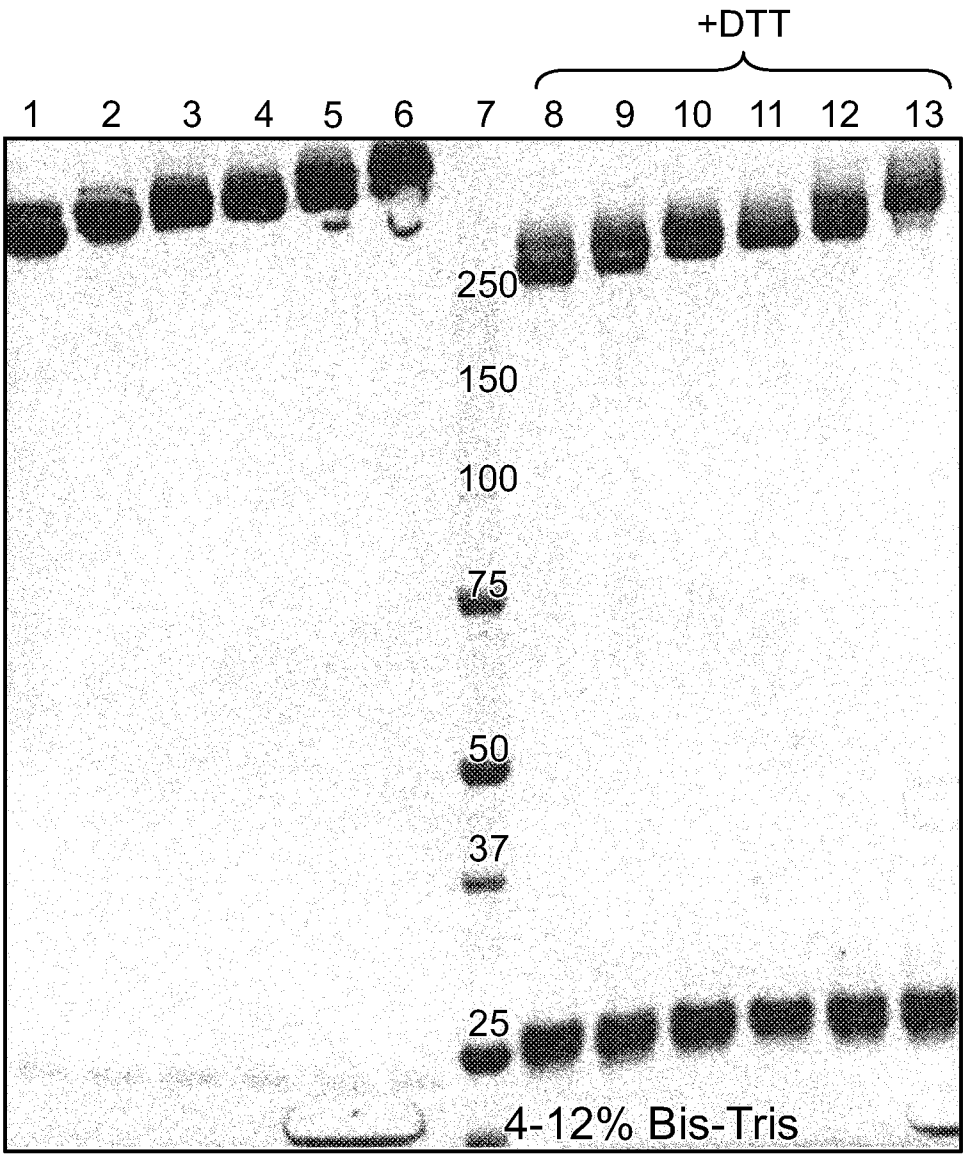


FIG. 19B

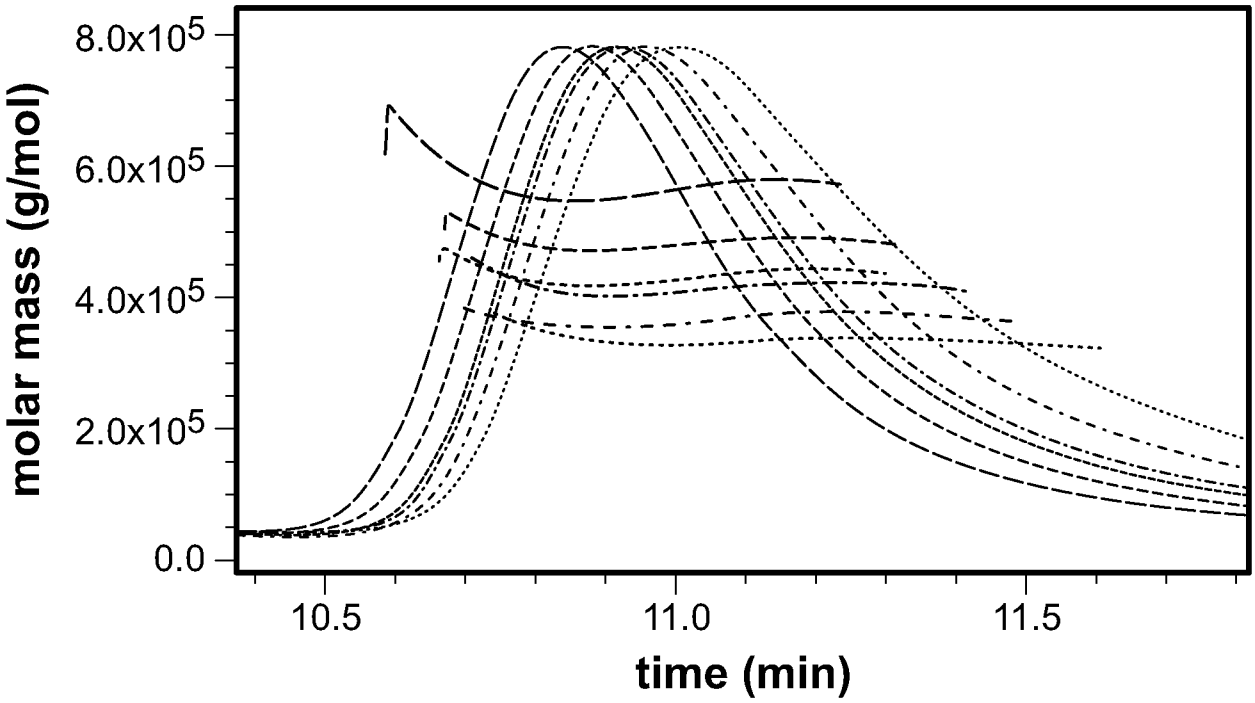

molar mass (g/mol)
8.0x10^5
6.0x10^5
4.0x10^5
2.0x10^5
0.0
10.5
11.0
11.5
time (min)

FIG. 19C

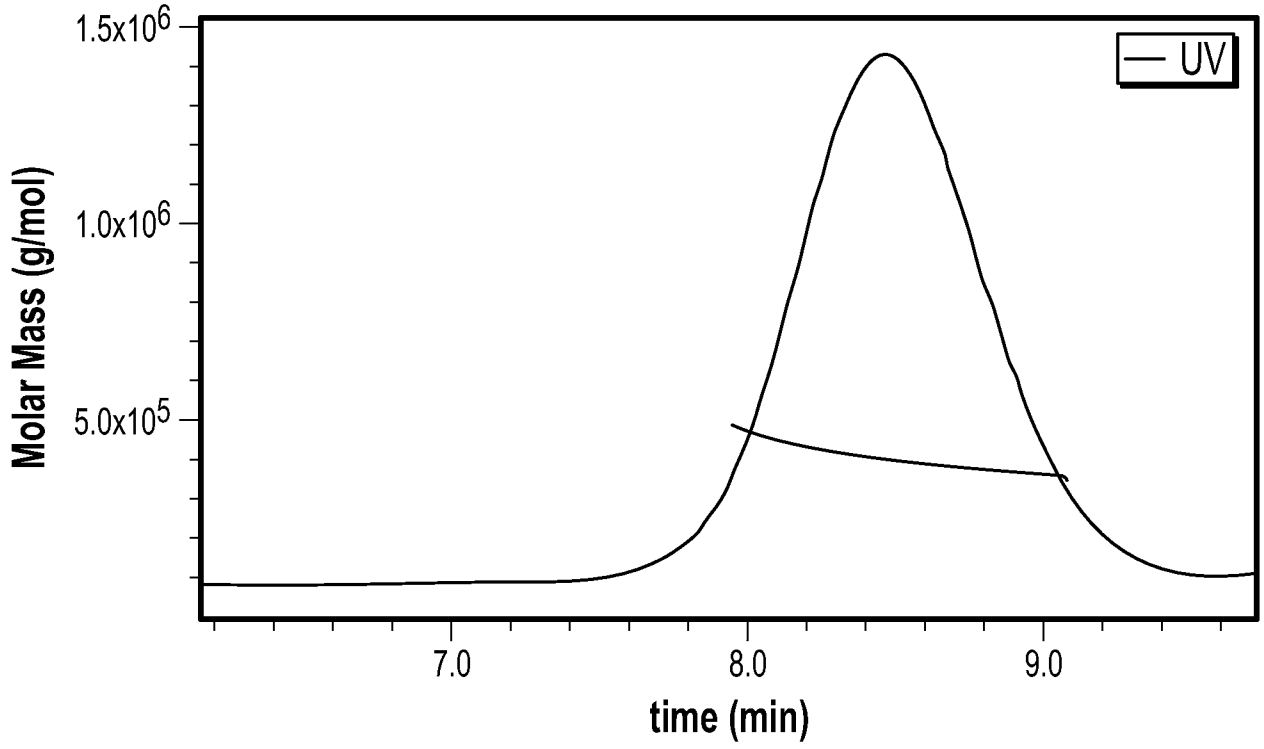


FIG. 20

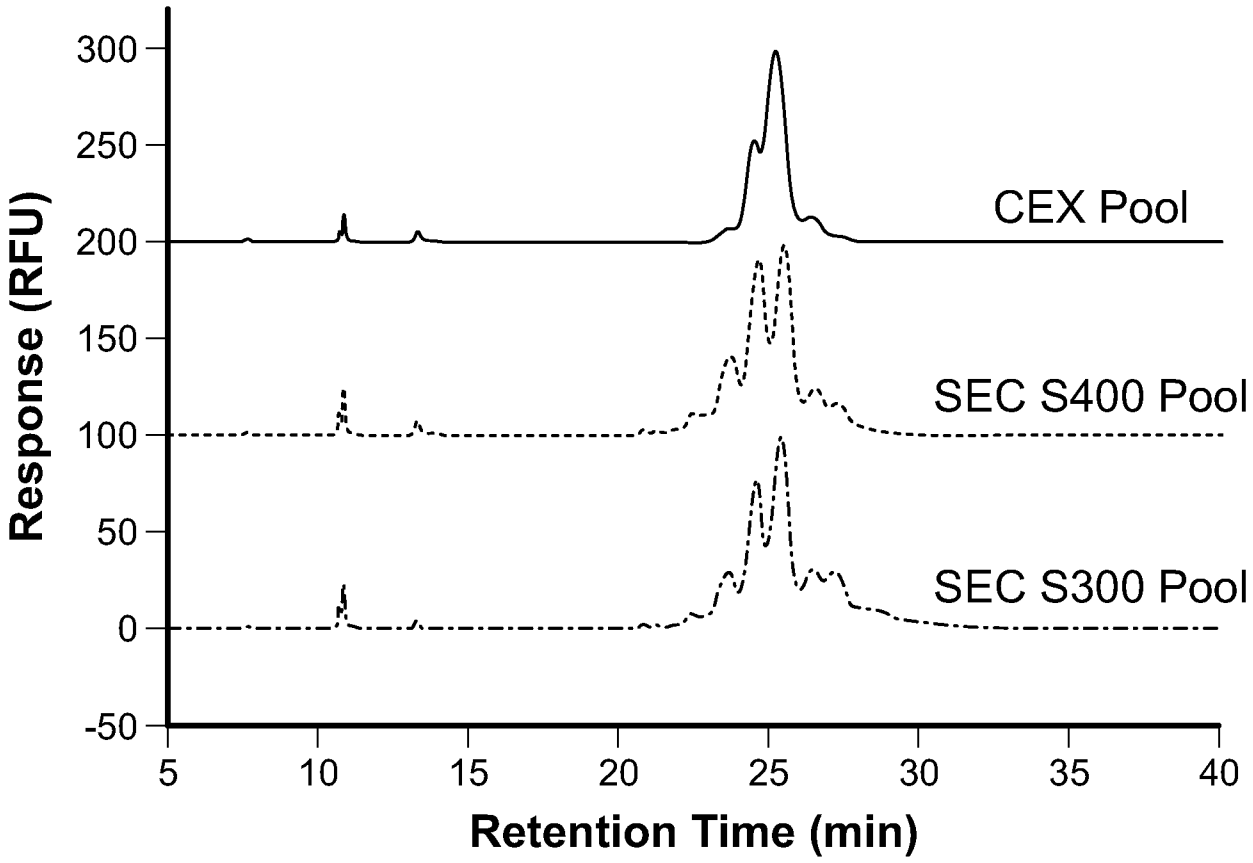

300
250
200
150
100
50
0
-50
Response (RFU)
5
10
15
20
25
30
35
40
Retention Time (min)
CEX Pool
SEC S400 Pool
SEC S300 Pool

FIG. 21A

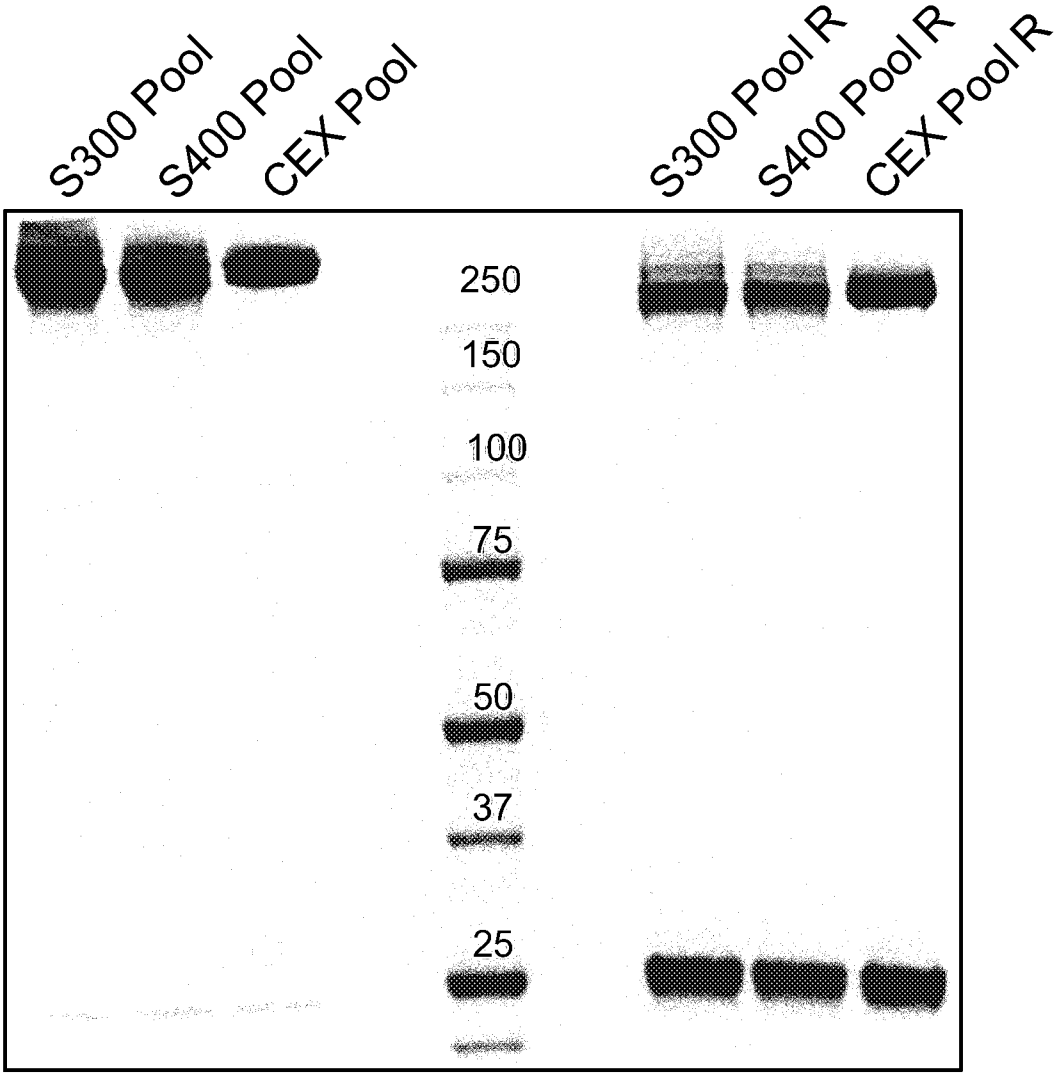

S300 Pool
S400 Pool
CEX Pool
S300 Pool R
S400 Pool R
CEX Pool R
250
150
100
75
50
37
25

FIG. 21B

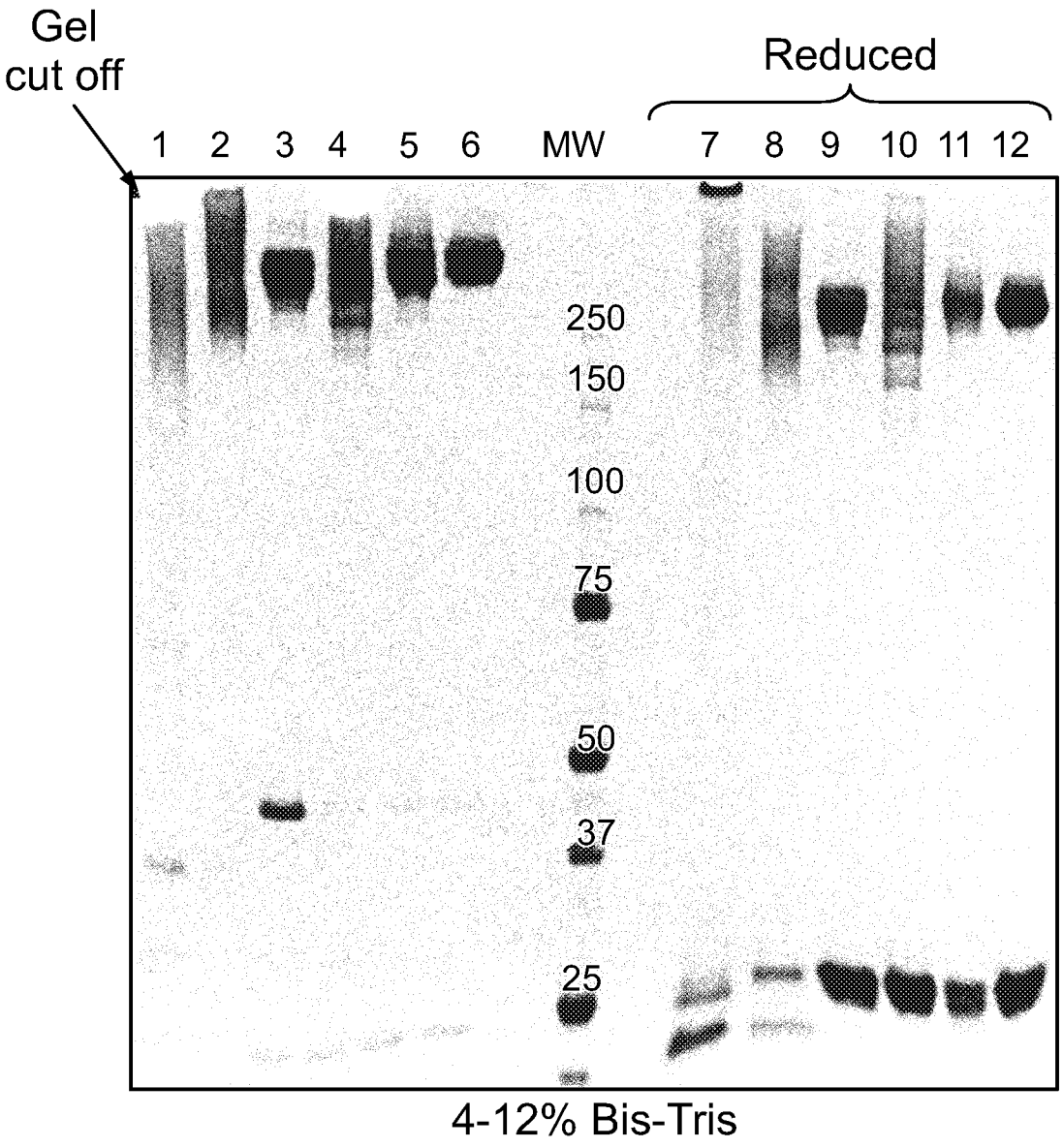

Gel
cut off
Reduced
1 2 3 4 5 6 MW 7 8 9 10 11 12
250
150
100
75
50
37
25
4-12% Bis-Tris

FIG. 22A

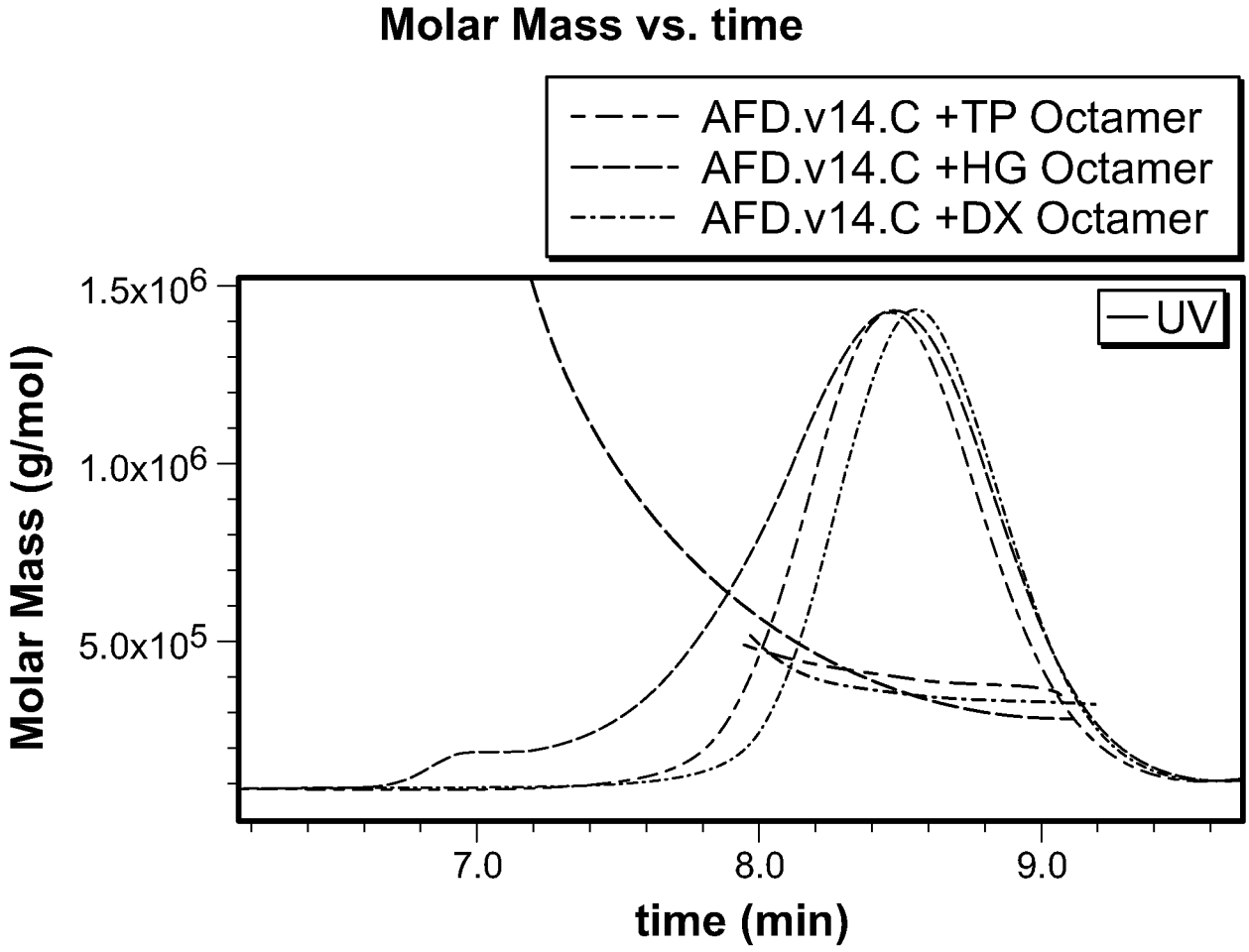

Molar Mass vs. time
AFD.v14.C +TP Octamer
AFD.v14.C +HG Octamer
AFD.v14.C +DX Octamer
UV
Molar Mass (g/mol)
1.5x10^6
1.0x10^6
5.0x10^5
7.0
8.0
9.0
time (min)

FIG. 22B

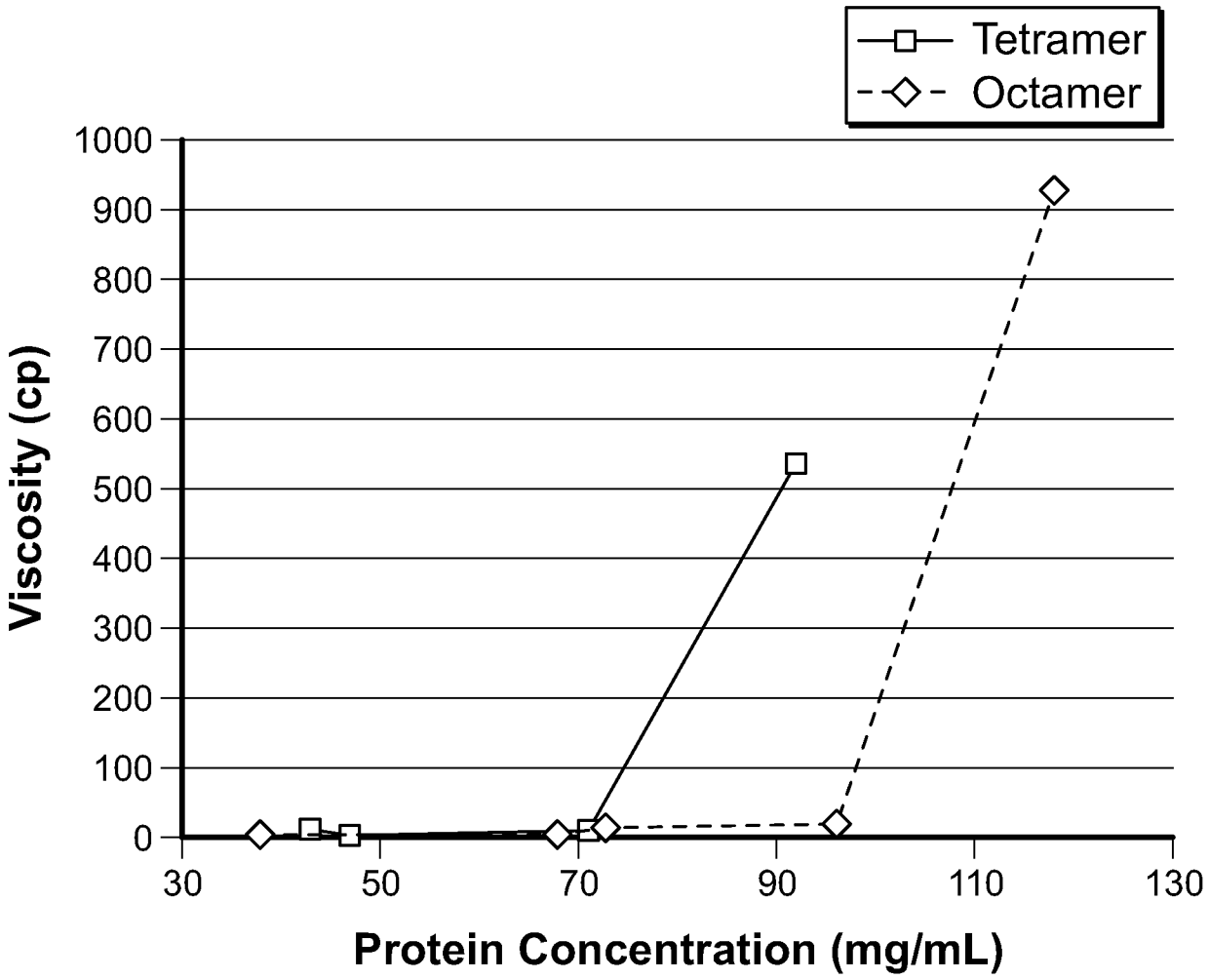

Tetramer
Octamer
1000
900
800
700
600
500
400
300
200
100
0
Viscosity (cp)
30
50
70
90
110
130
Protein Concentration (mg/mL)

FIG. 23

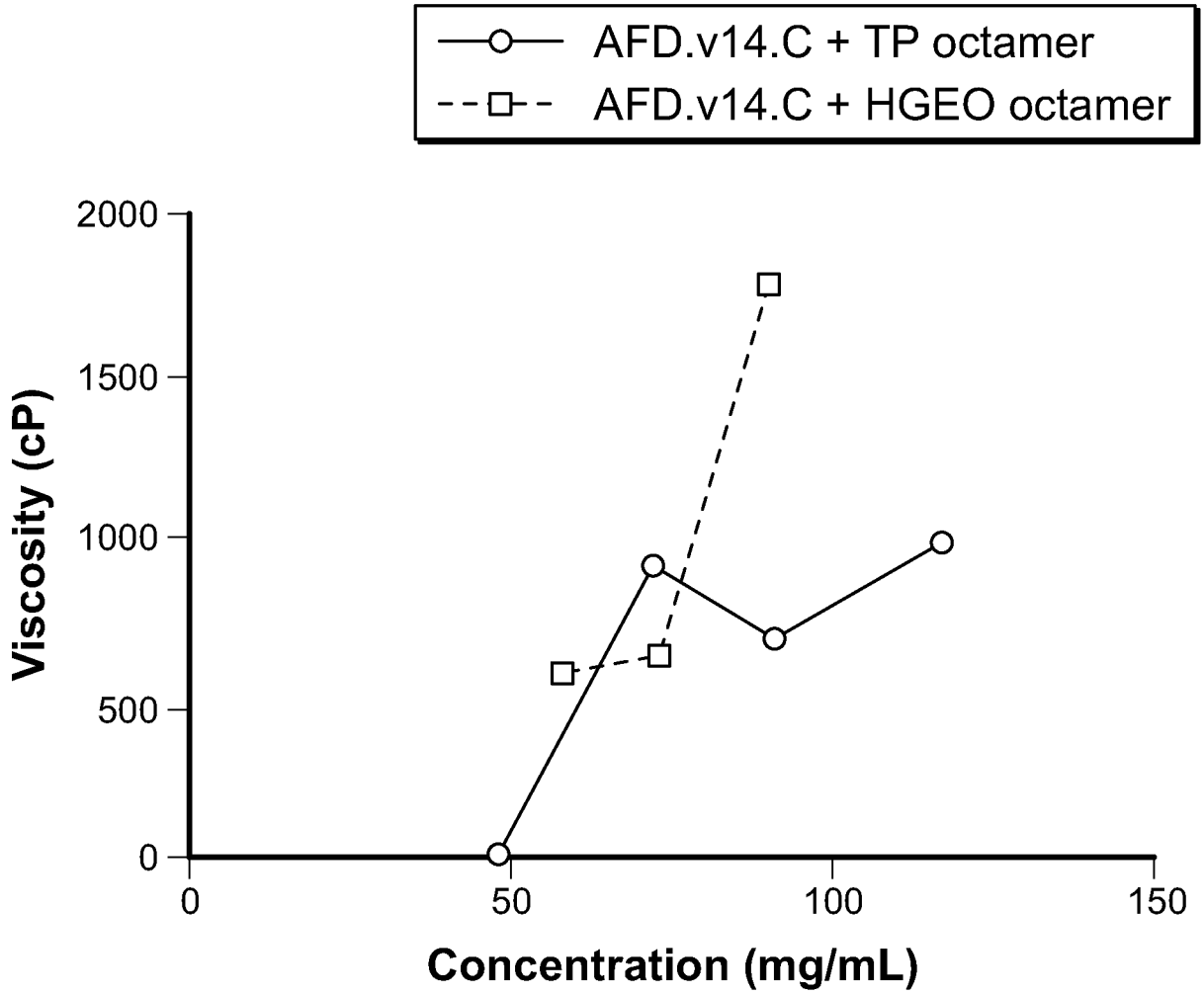

AFD.v14.C + TP octamer
AFD.v14.C + HGEO octamer
Viscosity (cP)
2000
1500
1000
500
0
0
50
100
150
Concentration (mg/mL)

FIG. 24

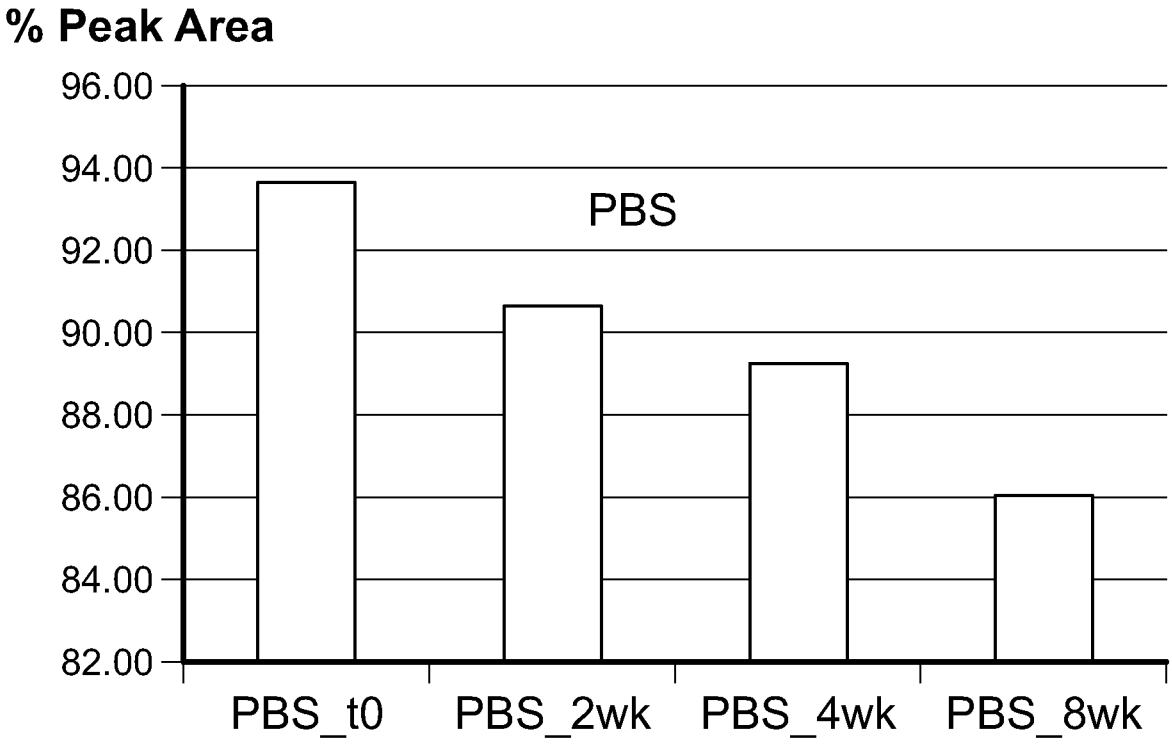


FIG. 25A

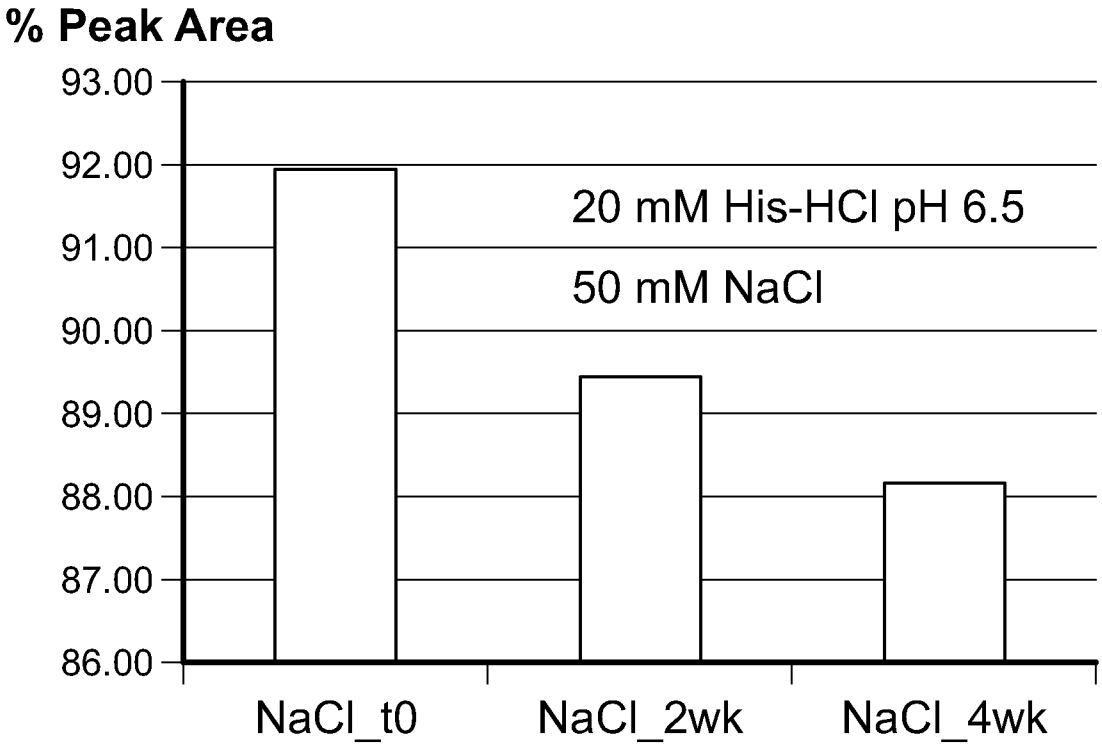
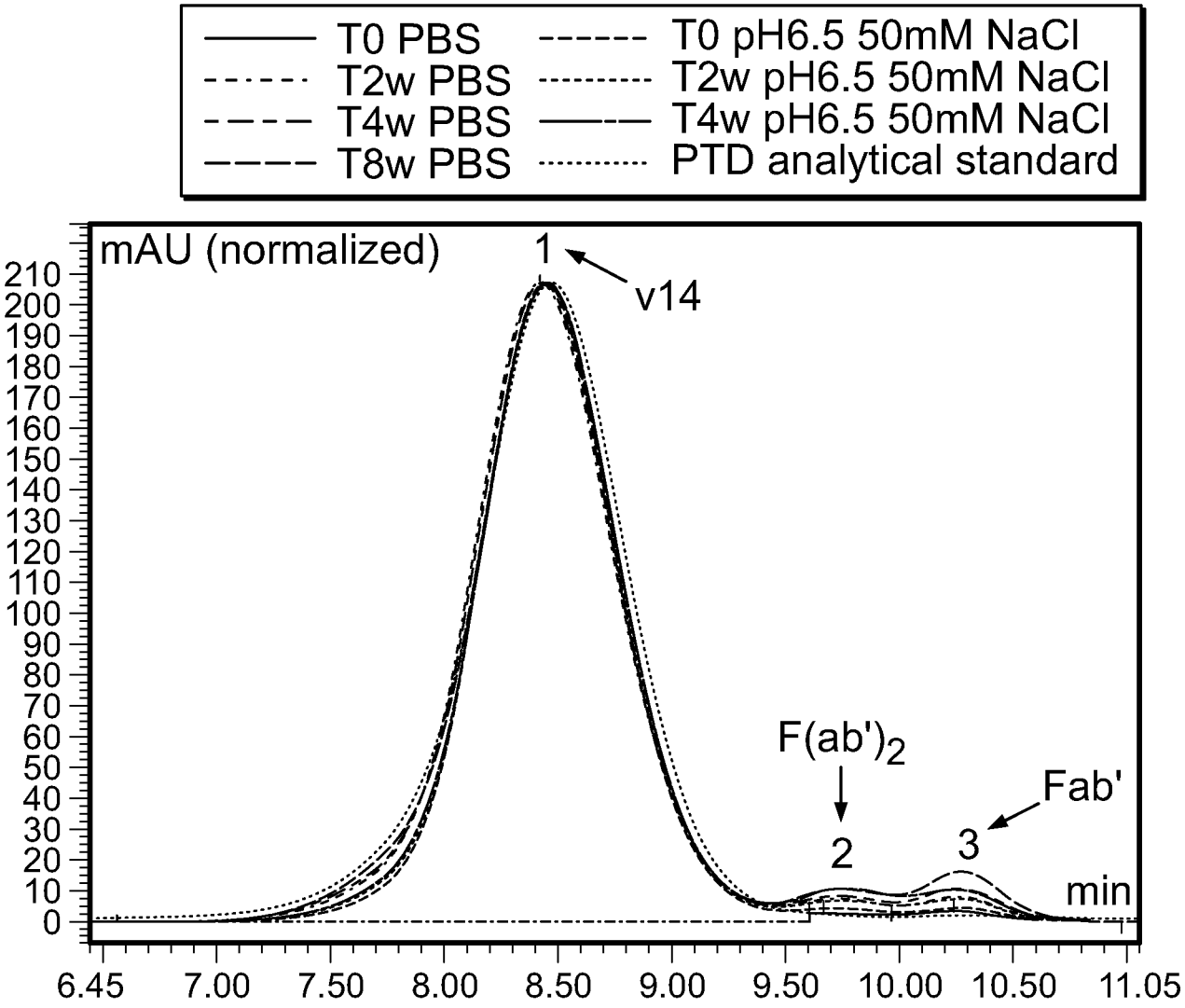


FIG. 25B



	% Total		
Sample ID	**Peak 1**	**Peak 2**	**Peak 3**
T0 PBS	98.48	0.58	0.94
T2w PBS	95.82	2.02	2.16
T4w PBS	94.85	2.32	2.84
T8w PBS	93.00	2.77	4.23
T0 pH6.5, 50mM NaCl	97.53	1.21	1.26
T2w pH6.5, 50mM NaCl	95.99	2.00	2.02
T4w pH6.5, 50mM NaCl	93.94	3.22	2.84
PTD std	100.00	0.00	0.00

FIG. 26

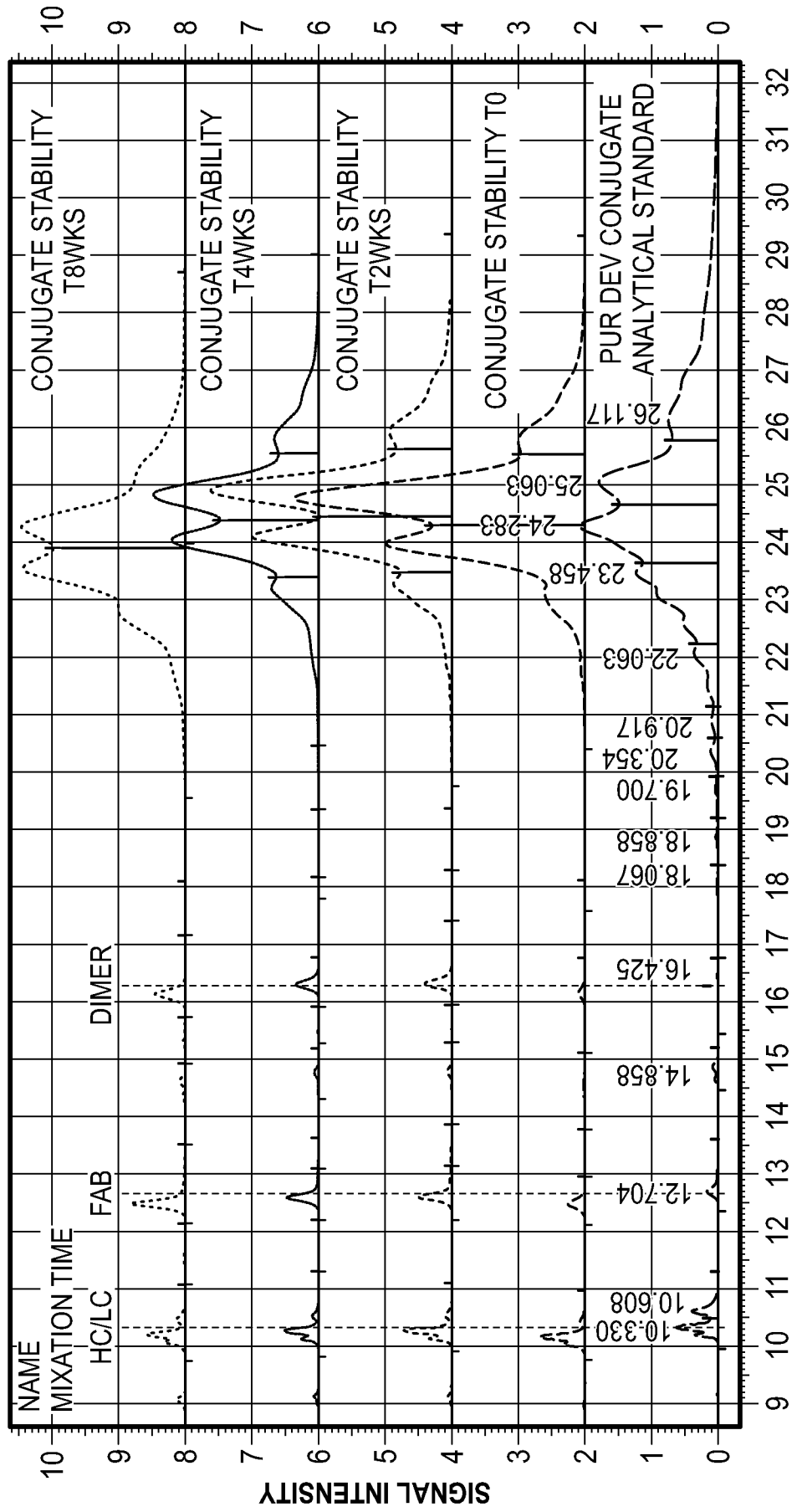

SIGNAL INTENSITY
TIME (MINUTES)
NAME
MIXATION TIME
HC/LC
FAB
DIMER
CONJUGATE STABILITY T8WKS
CONJUGATE STABILITY T4WKS
CONJUGATE STABILITY T2WKS
CONJUGATE STABILITY T0
PUR DEV CONJUGATE ANALYTICAL STANDARD
10.330
10.608
12.704
14.858
16.425
18.067
18.858
19.700
20.354
20.917
22.063
23.458
24.283
25.063
26.117

FIG. 27

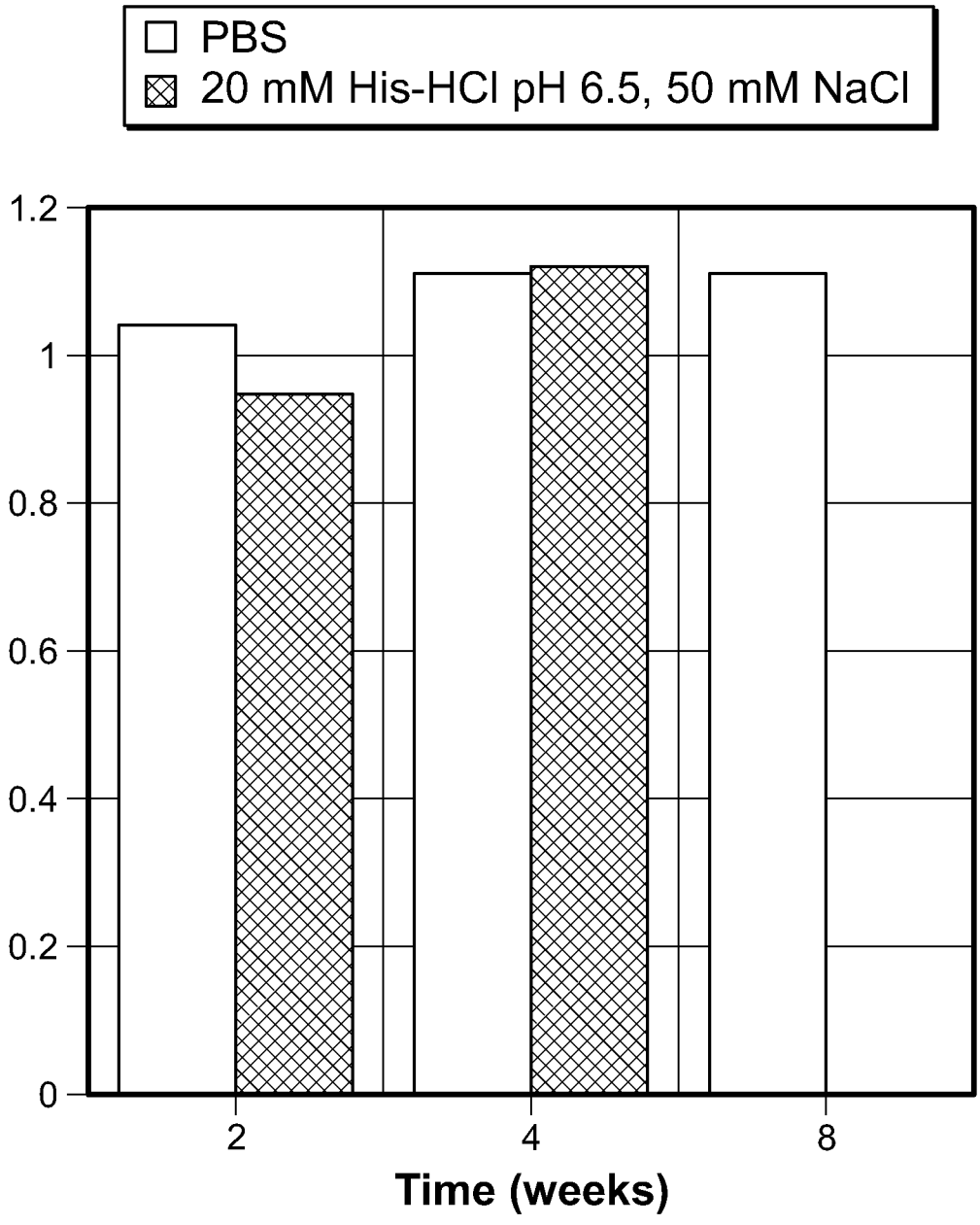


FIG. 28

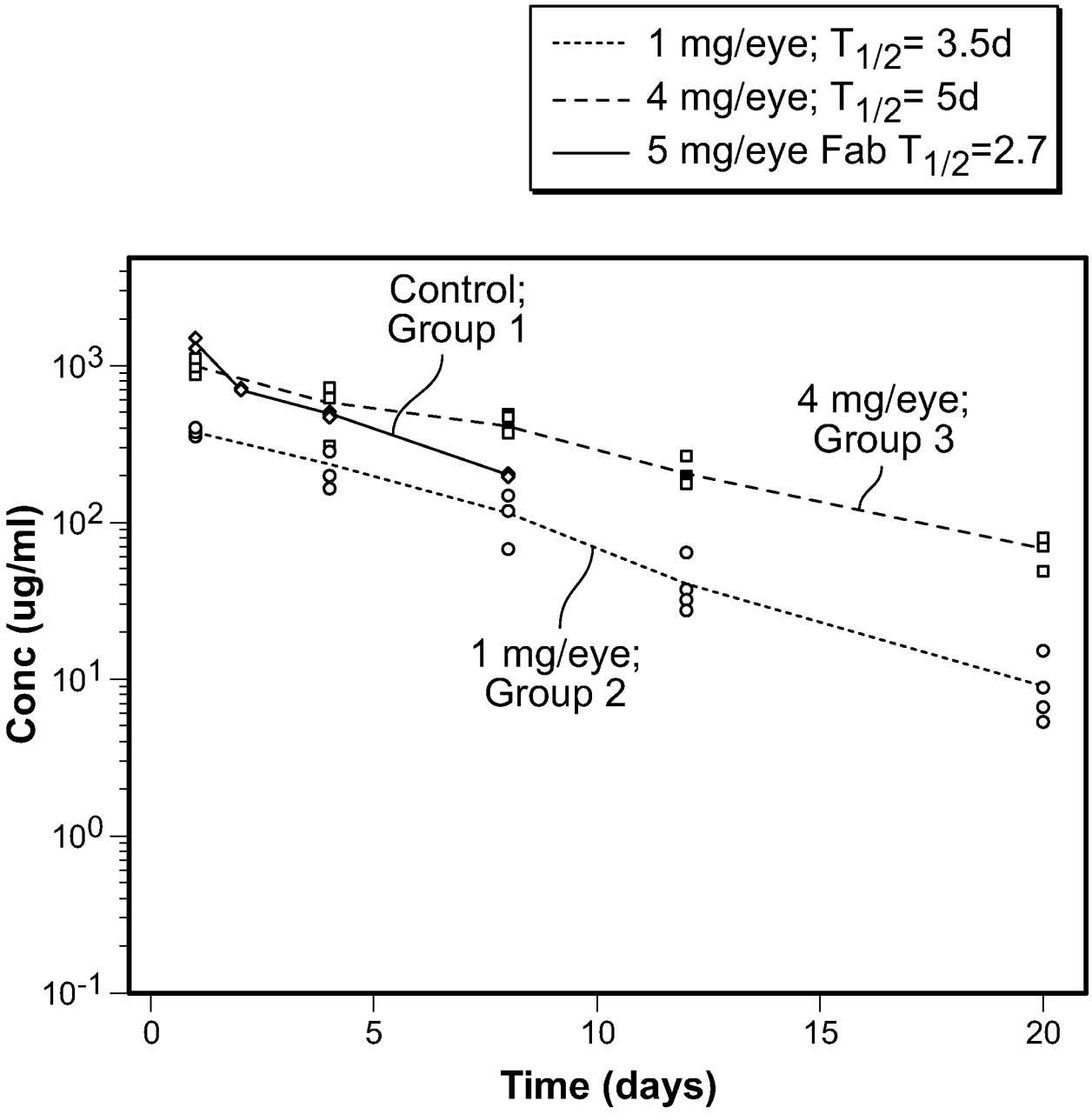

1 mg/eye; T1/2= 3.5d
4 mg/eye; T1/2= 5d
5 mg/eye Fab T1/2=2.7
Control;
Group 1
4 mg/eye;
Group 3
1 mg/eye;
Group 2
Conc (ug/ml)
10^3
10^2
10^1
10^0
10^-1
0
5
10
15
20
Time (days)

FIG. 29A

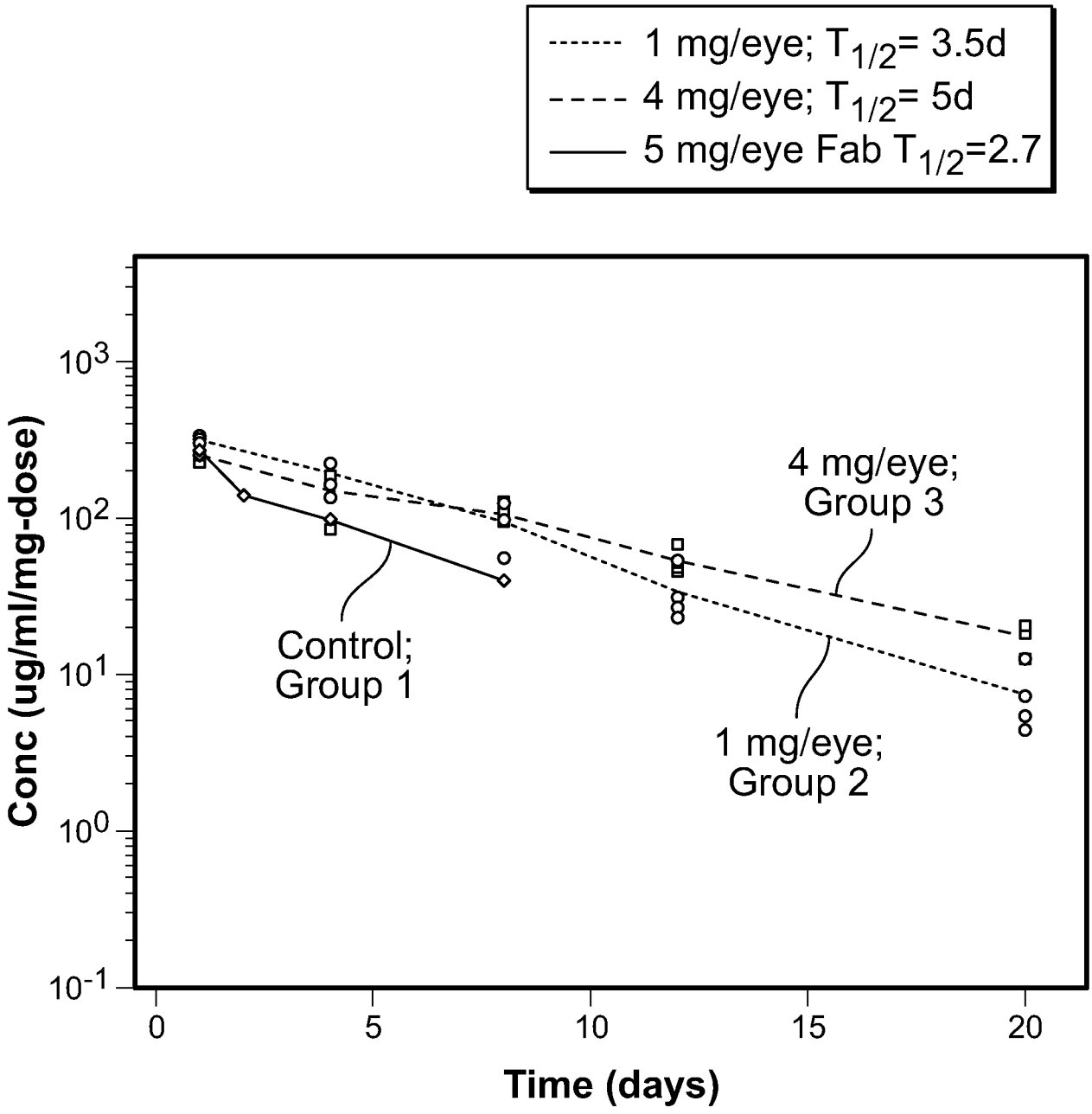

1 mg/eye; T1/2= 3.5d
4 mg/eye; T1/2= 5d
5 mg/eye Fab T1/2=2.7
Conc (ug/ml/mg-dose)
10^3
10^2
10^1
10^0
10^-1
0
5
10
15
20
Time (days)
4 mg/eye;
Group 3
Control;
Group 1
1 mg/eye;
Group 2

FIG. 29B

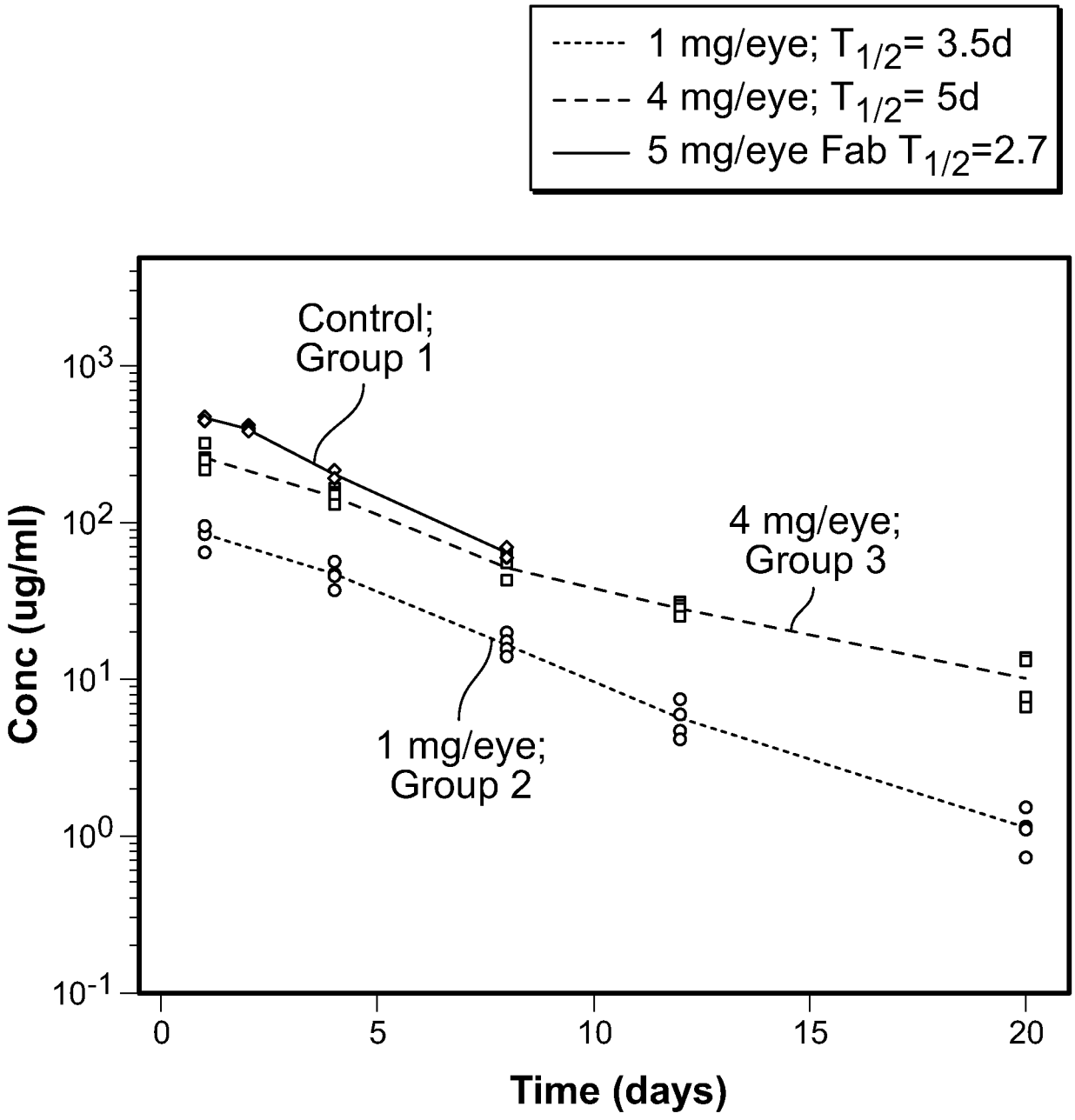

1 mg/eye; T1/2= 3.5d
4 mg/eye; T1/2= 5d
5 mg/eye Fab T1/2=2.7
Control;
Group 1
4 mg/eye;
Group 3
1 mg/eye;
Group 2
Conc (ug/ml)
10^3
10^2
10^1
10^0
10^-1
0
5
10
15
20
Time (days)

FIG. 30A

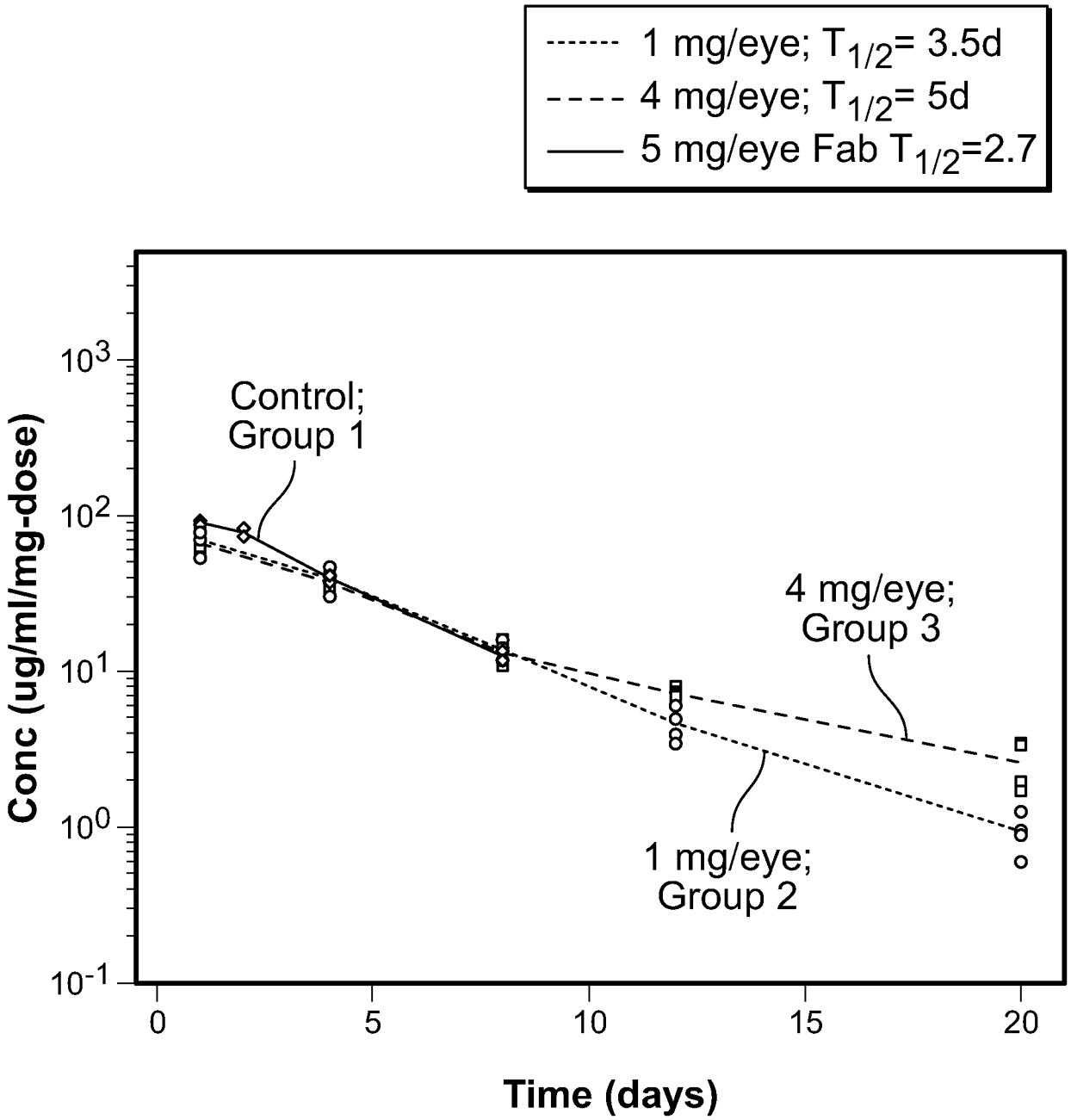

1 mg/eye; T1/2= 3.5d
4 mg/eye; T1/2= 5d
5 mg/eye Fab T1/2=2.7
Control;
Group 1
4 mg/eye;
Group 3
1 mg/eye;
Group 2
Conc (ug/ml/mg-dose)
Time (days)
10^3
10^2
10^1
10^0
10^-1
0
5
10
15
20

FIG. 30B

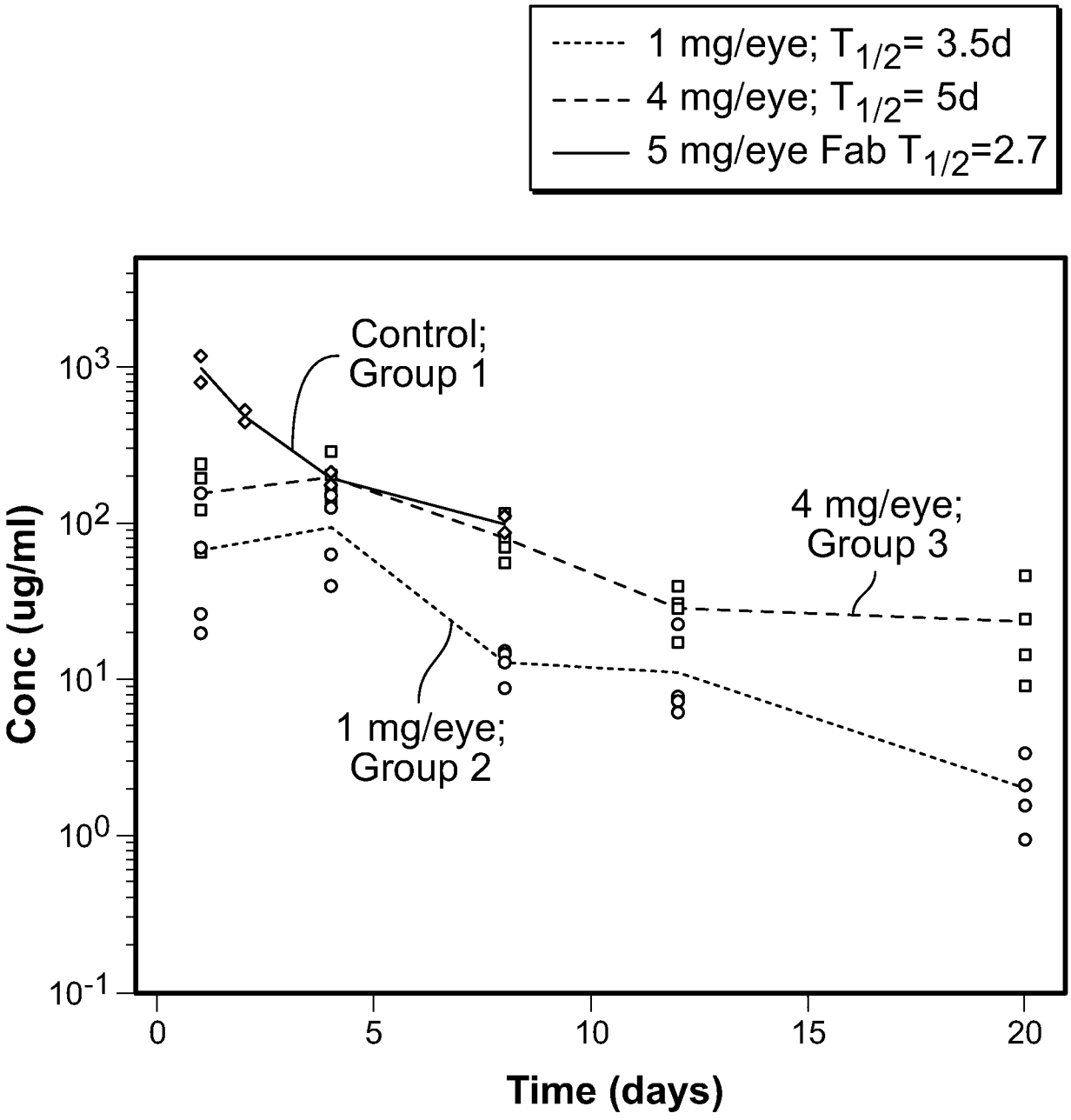

1 mg/eye; $T_{1/2}$= 3.5d
4 mg/eye; $T_{1/2}$= 5d
5 mg/eye Fab $T_{1/2}$=2.7
Control; Group 1
4 mg/eye; Group 3
1 mg/eye; Group 2
Conc (ug/ml)
10^3
10^2
10^1
10^0
10^{-1}
0
5
10
15
20
Time (days)

FIG. 31A

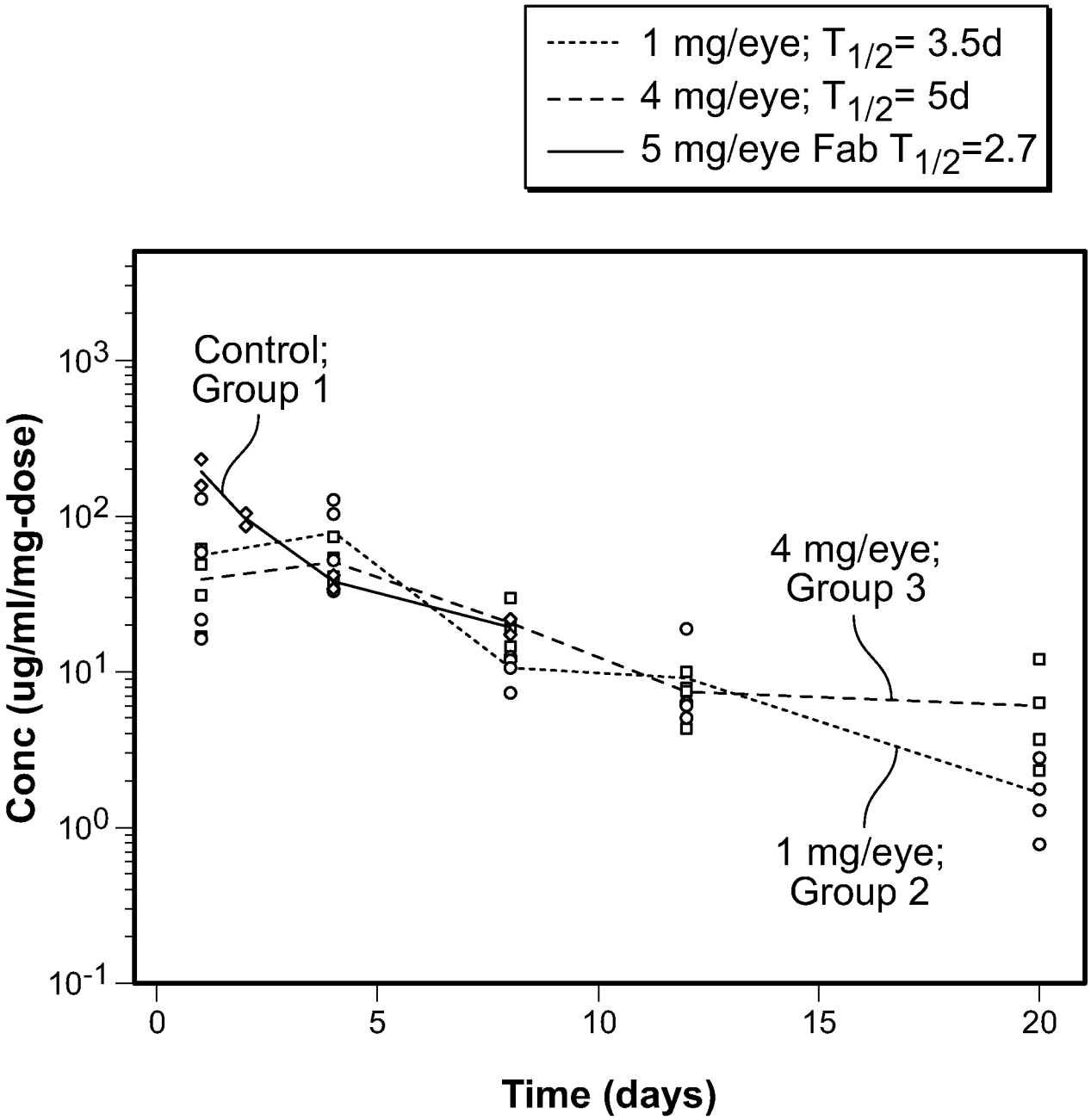

1 mg/eye; T1/2= 3.5d
4 mg/eye; T1/2= 5d
5 mg/eye Fab T1/2=2.7
Control;
Group 1
4 mg/eye;
Group 3
1 mg/eye;
Group 2
Conc (ug/ml/mg-dose)
Time (days)
10^3
10^2
10^1
10^0
10^-1
0
5
10
15
20

FIG. 31B

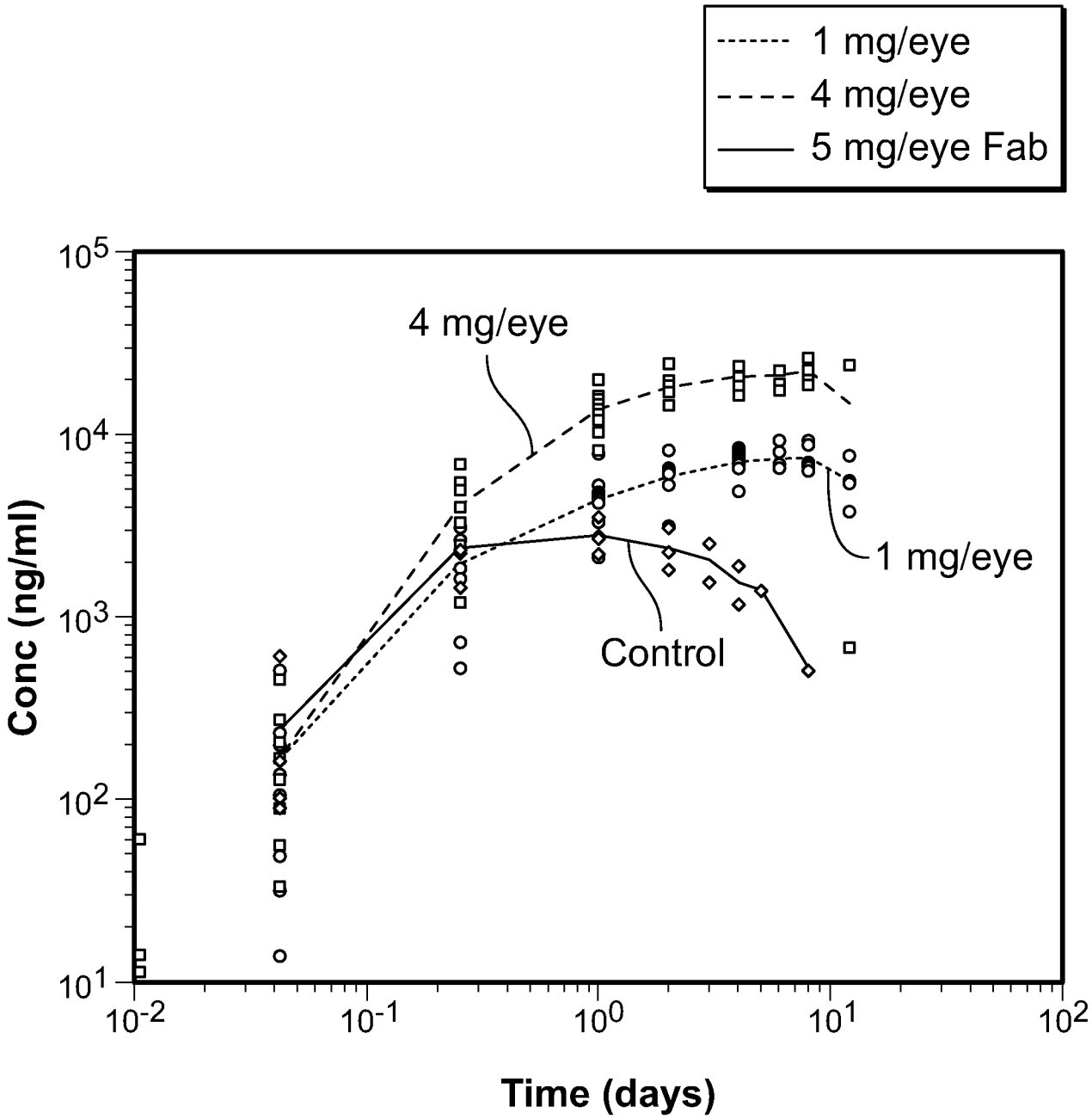

1 mg/eye
4 mg/eye
5 mg/eye Fab
4 mg/eye
1 mg/eye
Control
Conc (ng/ml)
Time (days)
10^5
10^4
10^3
10^2
10^1
10^-2
10^-1
10^0
10^1
10^2

FIG. 32A

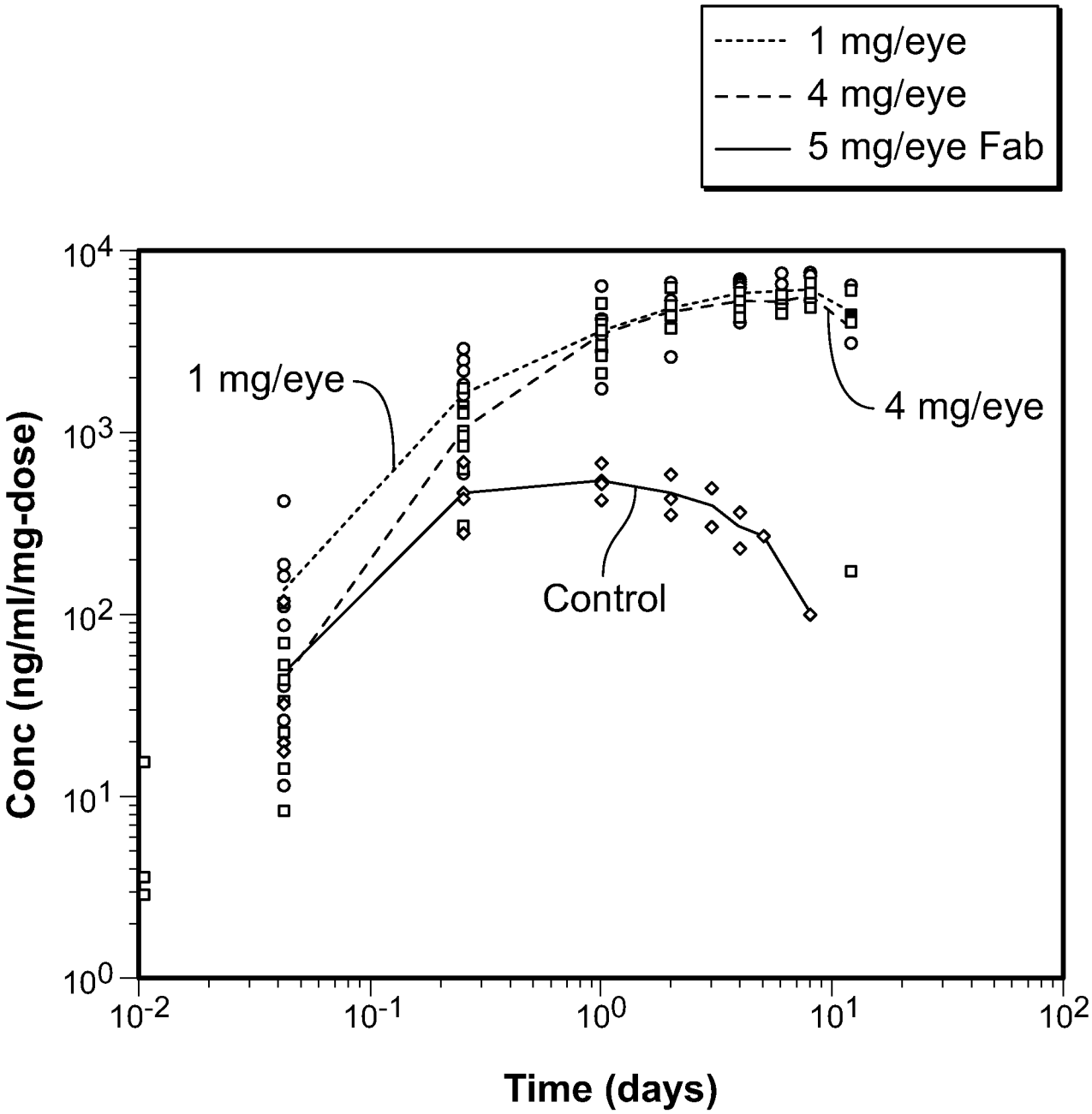

1 mg/eye
4 mg/eye
5 mg/eye Fab
Conc (ng/ml/mg-dose)
Time (days)
1 mg/eye
4 mg/eye
Control
10^4
10^3
10^2
10^1
10^0
10^-2
10^-1
10^0
10^1
10^2

FIG. 32B

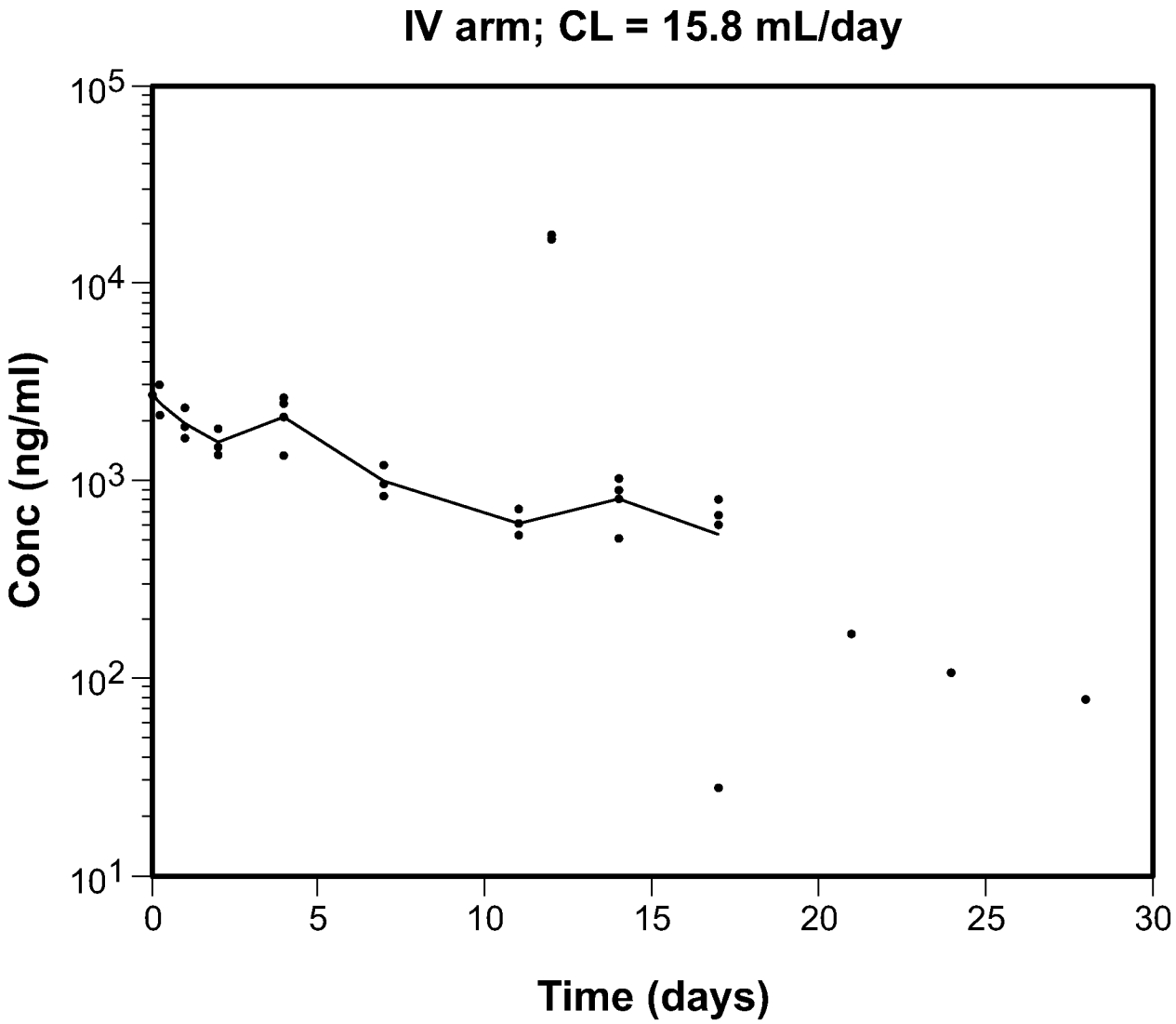

IV arm; CL = 15.8 mL/day
Conc (ng/ml)
10^5
10^4
10^3
10^2
10^1
0
5
10
15
20
25
30
Time (days)

FIG. 32C

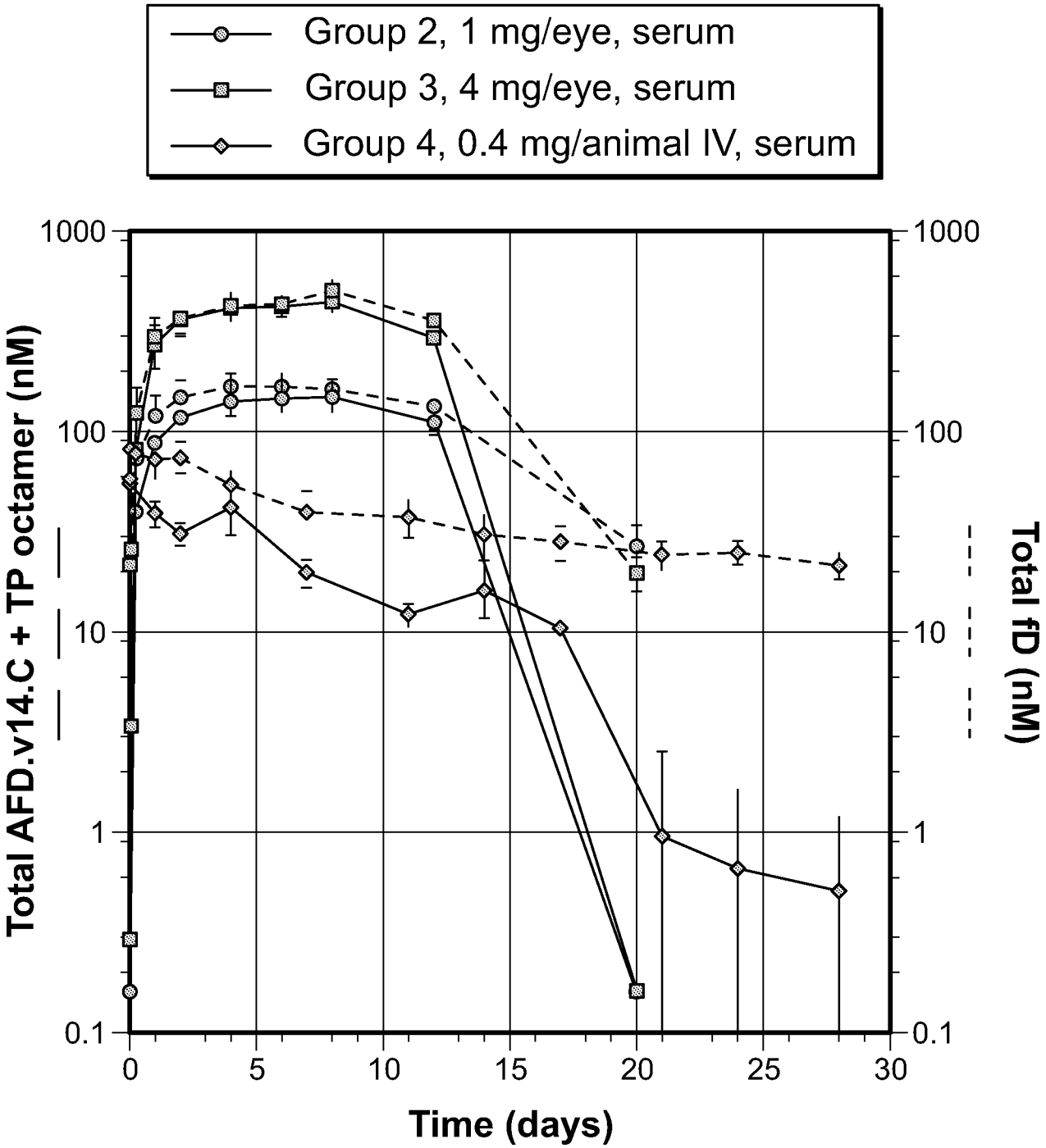

Group 2, 1 mg/eye, serum
Group 3, 4 mg/eye, serum
Group 4, 0.4 mg/animal IV, serum
Total AFD.v14.C + TP octamer (nM)
Total fD (nM)
1000
100
10
1
0.1
0
5
10
15
20
25
30
Time (days)

FIG. 33A

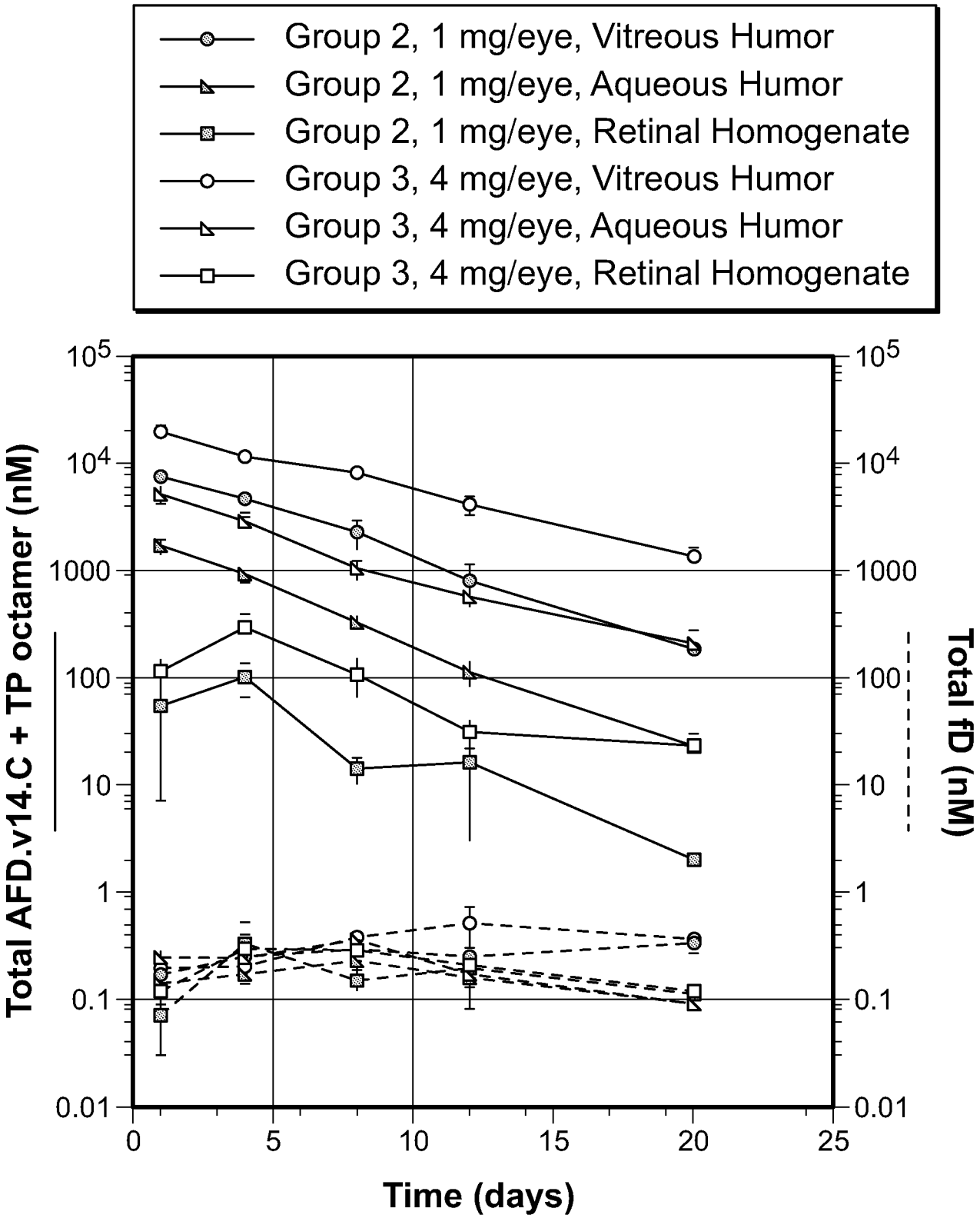

Group 2, 1 mg/eye, Vitreous Humor
Group 2, 1 mg/eye, Aqueous Humor
Group 2, 1 mg/eye, Retinal Homogenate
Group 3, 4 mg/eye, Vitreous Humor
Group 3, 4 mg/eye, Aqueous Humor
Group 3, 4 mg/eye, Retinal Homogenate
Total AFD.v14.C + TP octamer (nM)
Total fD (nM)
Time (days)
10^5
10^4
1000
100
10
1
0.1
0.01
0
5
10
15
20
25

FIG. 33B

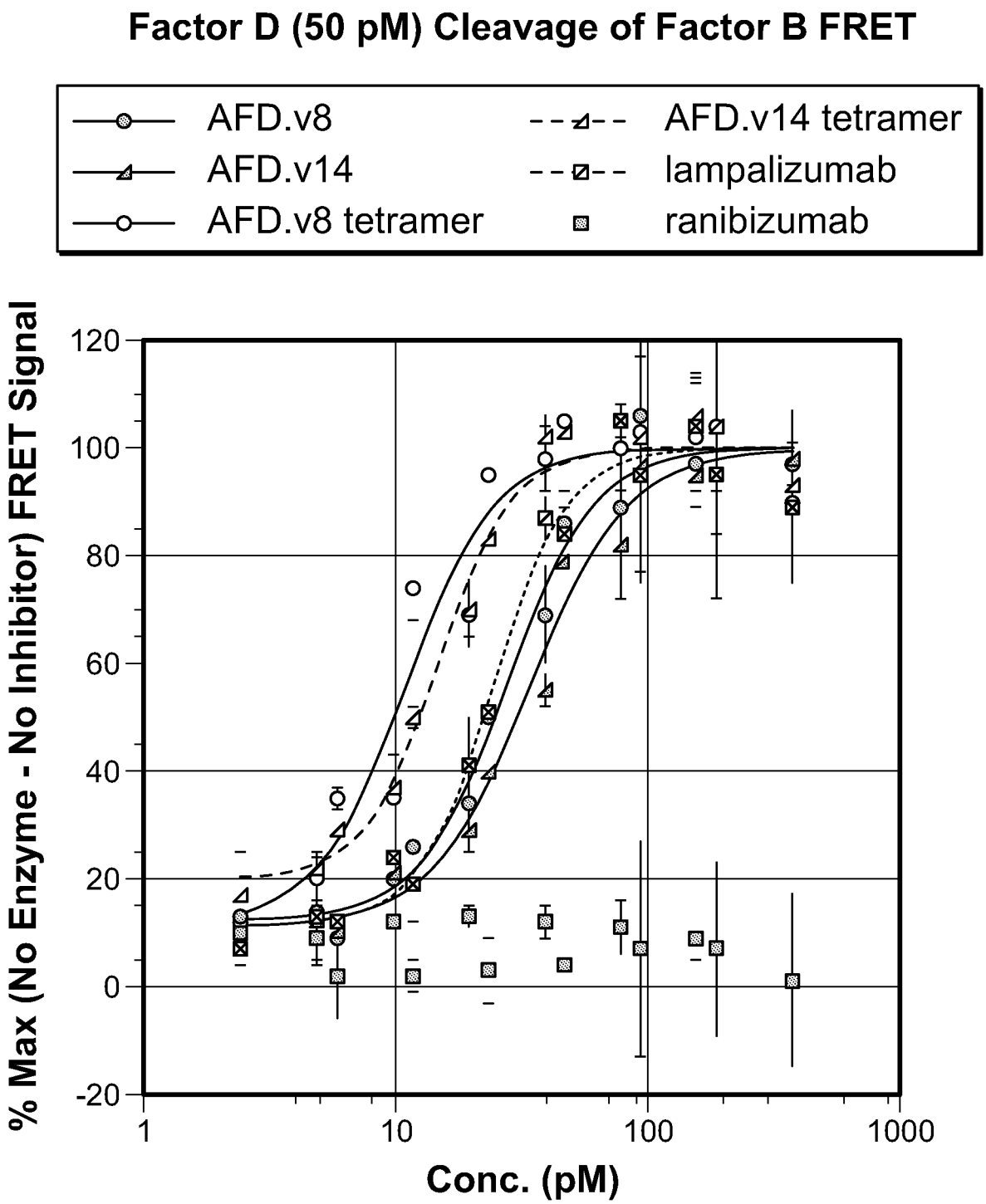

Factor D (50 pM) Cleavage of Factor B FRET
AFD.v8
AFD.v14
AFD.v8 tetramer
AFD.v14 tetramer
lampalizumab
ranibizumab
% Max (No Enzyme - No Inhibitor) FRET Signal
120
100
80
60
40
20
0
-20
1
10
100
1000
Conc. (pM)

FIG. 34A

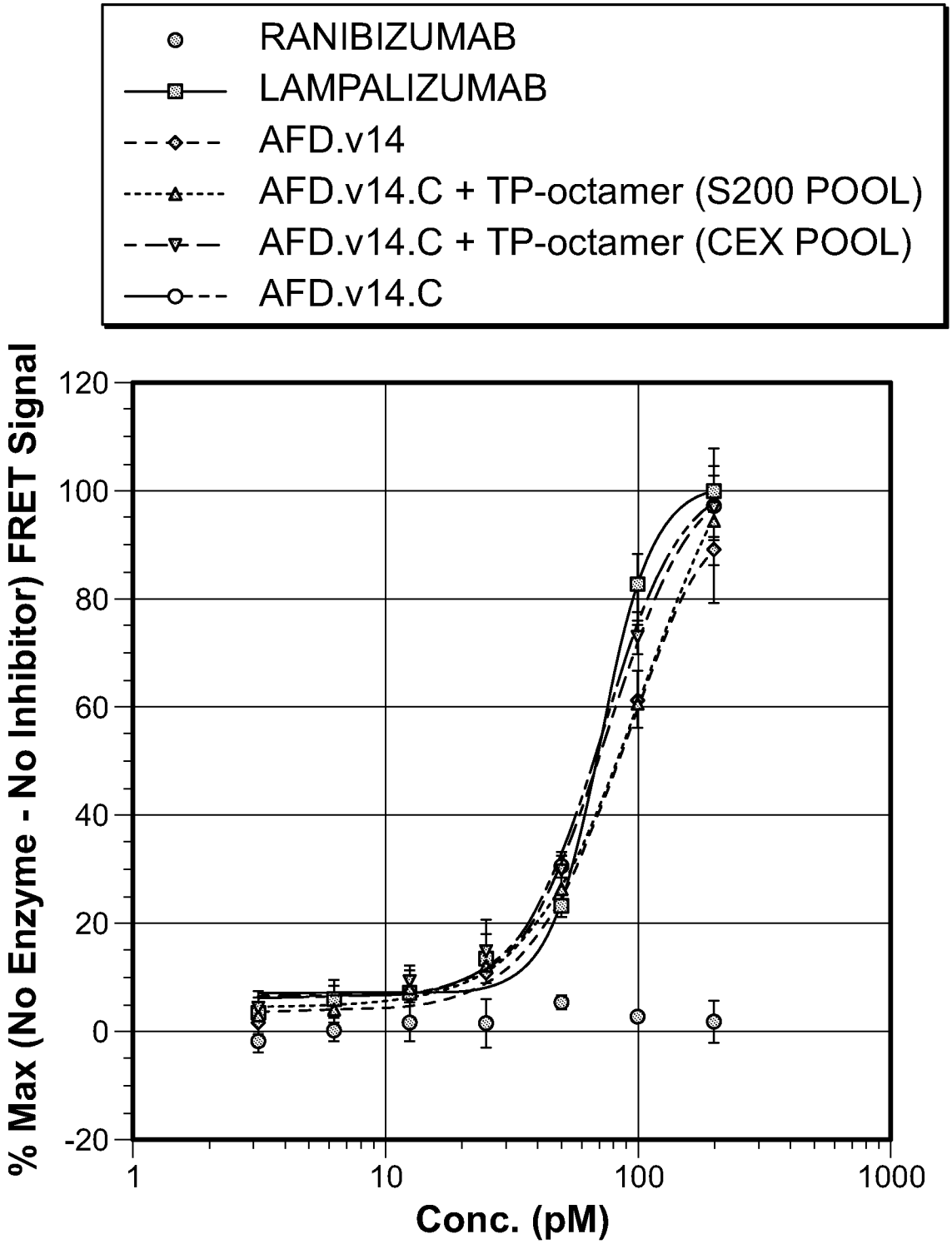

RANIBIZUMAB
LAMPALIZUMAB
AFD.v14
AFD.v14.C + TP-octamer (S200 POOL)
AFD.v14.C + TP-octamer (CEX POOL)
AFD.v14.C
% Max (No Enzyme - No Inhibitor) FRET Signal
120
100
80
60
40
20
0
-20
1
10
100
1000
Conc. (pM)

FIG. 34B

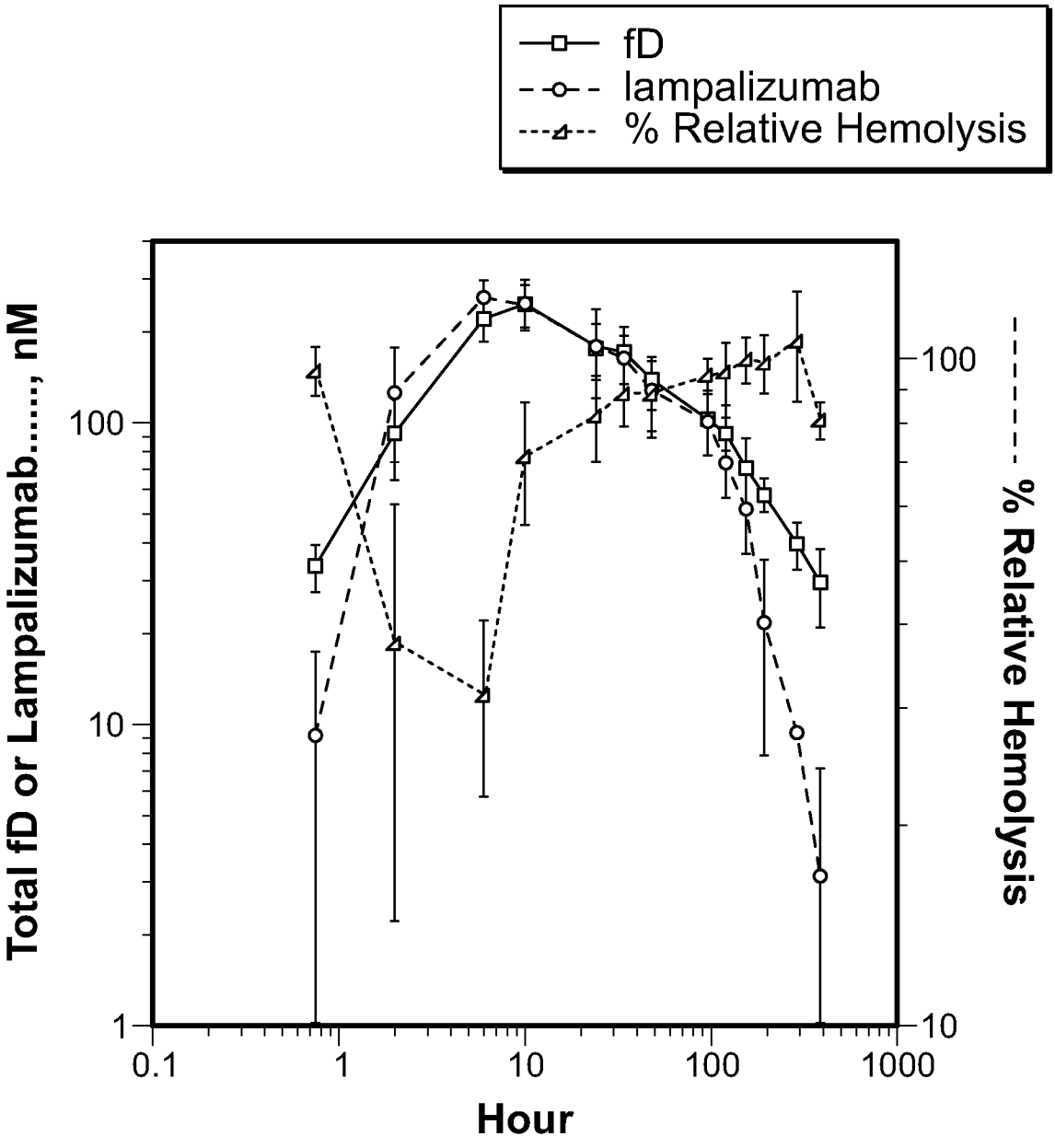

fD
lampalizumab
% Relative Hemolysis
Total fD or Lampalizumab....., nM
100
10
1
0.1
1
10
100
1000
Hour
100
10
% Relative Hemolysis

FIG. 35A

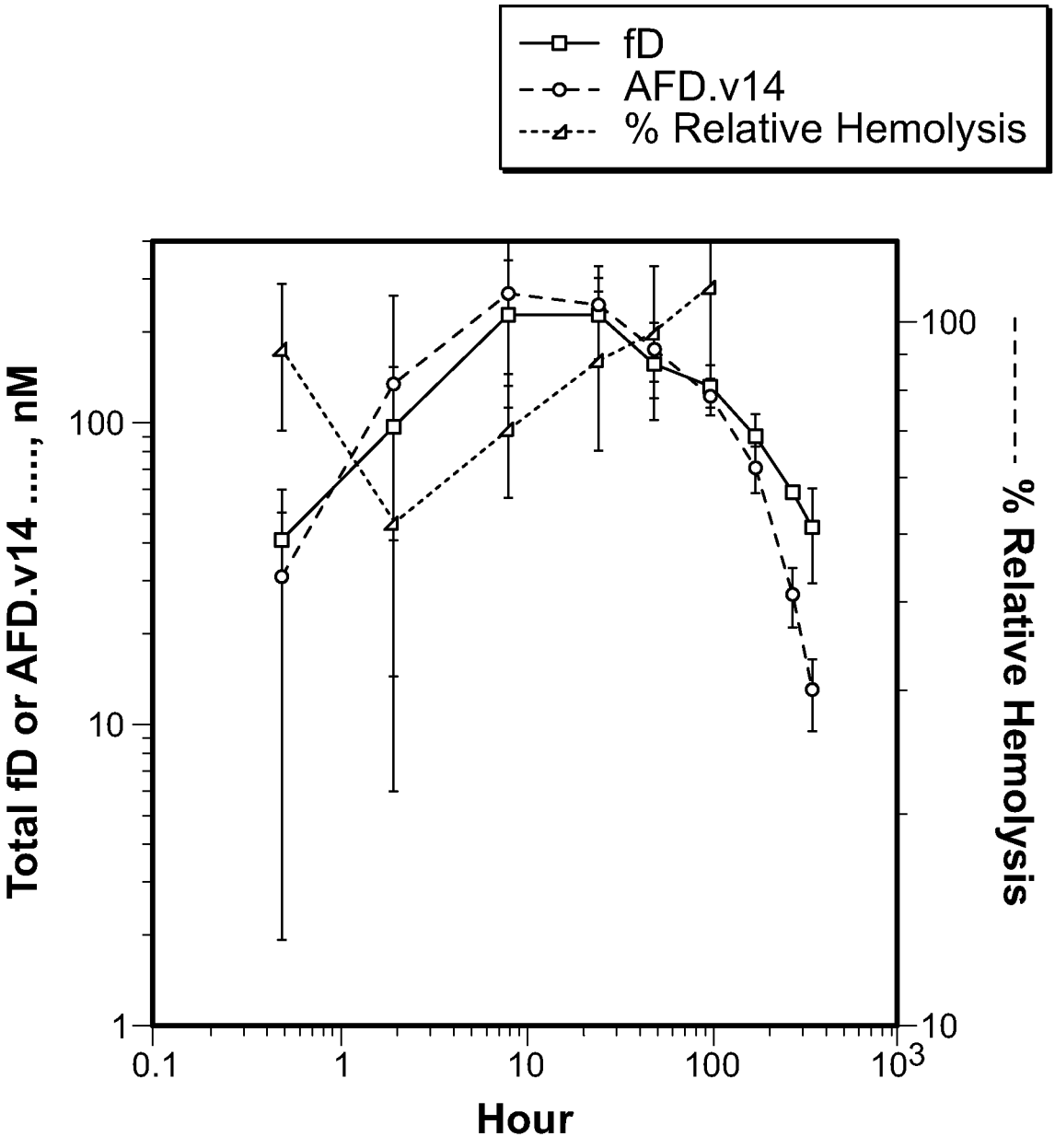

fD
AFD.v14
% Relative Hemolysis
Total fD or AFD.v14 ....., nM
100
10
1
0.1
1
10
100
10^3
Hour
100
10
% Relative Hemolysis

FIG. 35B

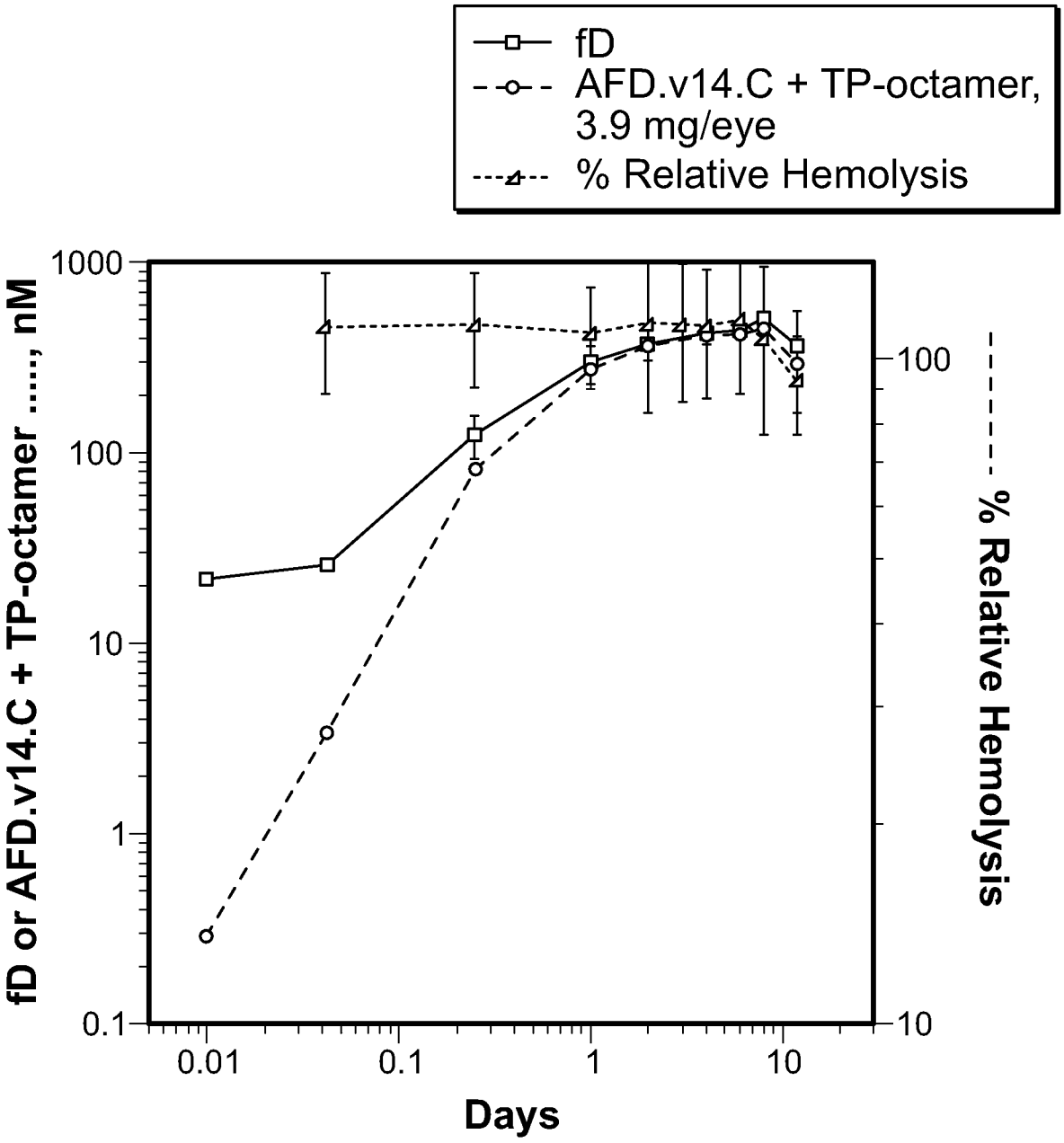

fD
AFD.v14.C + TP-octamer, 3.9 mg/eye
% Relative Hemolysis
fD or AFD.v14.C + TP-octamer ....., nM
1000
100
10
1
0.1
0.01
0.1
1
10
Days
100
10
% Relative Hemolysis

FIG. 35C

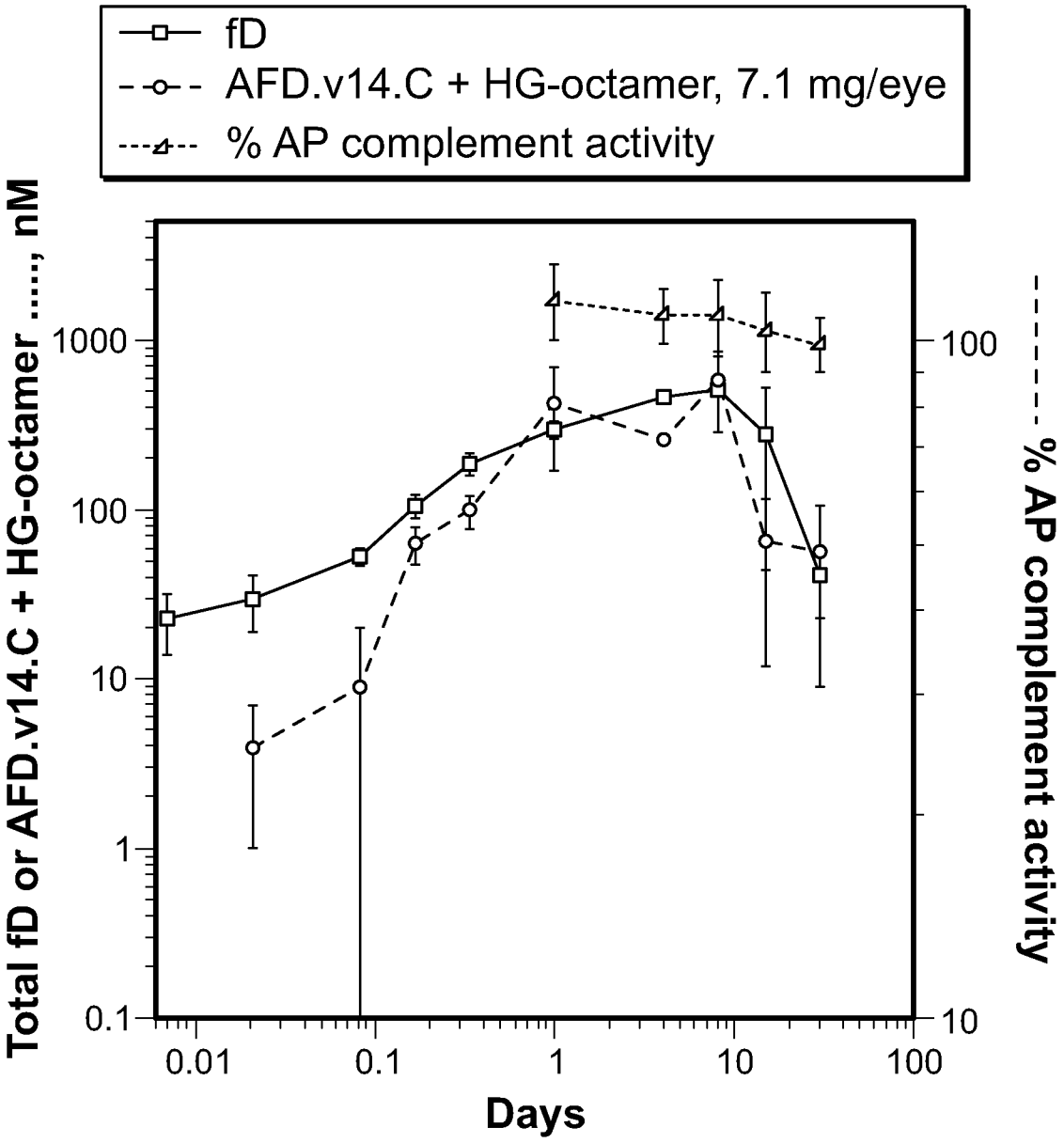

fD
AFD.v14.C + HG-octamer, 7.1 mg/eye
% AP complement activity
Total fD or AFD.v14.C + HG-octamer ....., nM
% AP complement activity
Days
1000
100
10
1
0.1
100
10
0.01
0.1
1
10
100

FIG. 35D

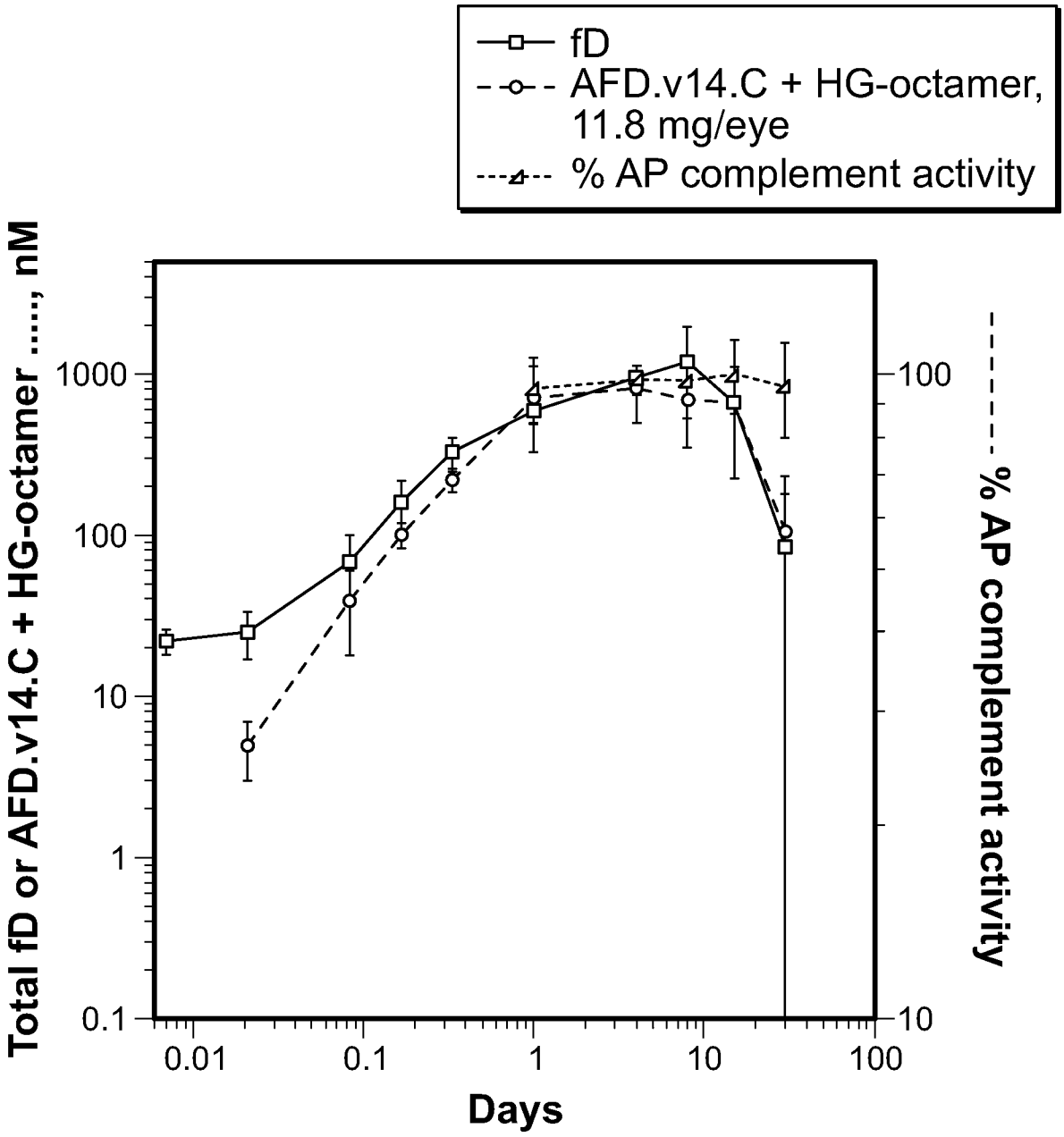

fD
AFD.v14.C + HG-octamer, 11.8 mg/eye
% AP complement activity
Total fD or AFD.v14.C + HG-octamer ....., nM
% AP complement activity
Days
1000
100
10
1
0.1
100
10
0.01
0.1
1
10
100

FIG. 35E

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/059179

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K47/60 A61P27/02
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, EMBASE, BIOSIS, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2009/134711 A1 (GENENTECH INC [US]; HUANG ARTHUR J [US]; KELLEY ROBERT F [US]; LOWMAN) 5 November 2009 (2009-11-05) paragraphs [0154], [0159]; claims 1-10; figures 4, 10; sequences 47, 63 -----	1-109
Y	RYAN S M ET AL: "Advances in PEGylation of important biotech molecules: Delivery aspects", EXPERT OPINION ON DRUG DELIVERY, INFORMA HEALTHCARE, GB, vol. 5, no. 4, 1 April 2008 (2008-04-01), pages 371-383, XP008106736, ISSN: 1742-5247, DOI: 10.1517/17425247.5.4.371 the whole document ----- -/--	1-109

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 January 2017	31/01/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Langer, Miren

3

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/059179

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 99/01556 A2 (GENENTECH INC [US]) 14 January 1999 (1999-01-14) -----	1-109
A	K. J. KATSCHKE ET AL: "Inhibiting Alternative Pathway Complement Activation by Targeting the Factor D Exosite", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 287, no. 16, 13 April 2012 (2012-04-13), pages 12886-12892, XP055040644, ISSN: 0021-9258, DOI: 10.1074/jbc.M112.345082 -----	1-109
A	WO 2015/032776 A1 (HOFFMANN LA ROCHE [CH]; HOFFMANN LA ROCHE [US]) 12 March 2015 (2015-03-12) -----	1-109
A	WO 2008/055206 A2 (GENENTECH INC [US]; WU HERREN [US]; SINGH SANJAYA [US]; FUNG SEK CHUNG) 8 May 2008 (2008-05-08) -----	1-109
A	US 2013/302333 A1 (HASS PHILIP [US] ET AL) 14 November 2013 (2013-11-14) -----	1-109
X,P	WO 2015/168468 A1 (GENENTECH INC [US]; HOFFMANN LA ROCHE [CH]) 5 November 2015 (2015-11-05) example 4 claim 1 paragraph [0113] -----	1-109

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2016/059179

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2009134711 A1	05-11-2009	AR 071510 A1	23-06-2010
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		EP 0996728 A2	03-05-2000
		ES 2210778 T3	01-07-2004
		HK 1027833 A1	27-02-2004

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(71)申请人 豪夫迈·罗氏有限公司
地址 瑞士巴塞尔

(72)发明人 菲利普·E·哈斯
罗伯特·F·凯利
贾斯廷·M·希尔 惠特尼·沙茨
德温·特萨
门诺·凡卢克伦康帕涅

(74)专利代理机构 中科专利商标代理有限责任公司 11021
代理人 吴小明 柳春琦

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(54)发明名称

抗-因子D抗体变体缀合物及其用途

(57)摘要

本发明涉及包含一个或多个抗-因子D抗体变体的抗体-聚合物缀合物、它们的制备及其在制备用于治疗与过度的或不受控的补体激活有关的疾病或病症的组合物和药物的用途。

CN 108472382 A

1.一种缀合物,所述缀合物包含与一个或多个多元醇共价连接的一个或多个抗-因子D抗体变体,其中:

所述缀合物中的至少一个抗-因子D抗体变体包含在参比抗-因子D抗体的高变区(HVR)内的至少一个靶天冬氨酸(D或Asp)残基的置换,其中所述靶Asp残基被鉴定为倾向于异构化并且所述置换是Asp至谷氨酸(E或Glu),并且其中当与所述参比抗-因子D抗体相比时,每个抗体变体显示改善的稳定性而不显著丧失因子D结合亲和力;并且

所述多元醇是多臂多元醇。

2.根据权利要求1所述的缀合物,其中所述Asp残基在Asp-Xaa基序内,其中Xaa是Asp、Gly、His、Ser或Thr。

3.根据权利要求2所述的缀合物,其中靶Asp残基是Asp-Asp基序中的第一个Asp。

4.根据权利要求1所述的缀合物,其中所述缀合物中的至少一个抗体变体还包含丝氨酸(S或Ser)对参比抗-因子D抗体的HVR内的另外的至少一个Asp残基的置换,其中当与所述参比抗-因子D抗体相比时,所得抗体变体具有较低的负电荷并且展现改善的溶解性。

5.根据权利要求1所述的缀合物,其中所述缀合物中的至少一个抗体变体还包含对参比抗-因子D抗体的HVR内的具有脱酰胺倾向的天冬酰胺(N或Asn)残基的一个或多个Ser置换。

6.根据权利要求1所述的缀合物,其中所述参比抗-因子D抗体包含SEQ ID NO:3的轻链可变结构域序列。

7.根据权利要求6所述的缀合物,其中所述参比抗-因子D抗体还包含SEQ ID NO:4的重链可变结构域序列。

8.根据权利要求7所述的缀合物,其中所述参比抗-因子D抗体包含SEQ ID NO:1的轻链序列和SEQ ID NO:2的重链序列。

9.根据权利要求7所述的缀合物,其中所述参比抗-因子D抗体包含SEQ ID NO:1的轻链序列和选自由以下组成的组的重链序列:SEQ ID NO:34-53和115。

10.根据权利要求8或权利要求9所述的缀合物,其中所述缀合物中的至少一个抗体变体包含SEQ ID NO:11的轻链HVR1(HVR-L1)序列和SEQ ID NO:12的重链HVR2(HVR-H2)序列。

11.根据权利要求10所述的缀合物,其中所述抗体变体还包含SEQ ID NO:13的轻链HVR3(HVR-L3)序列。

12.根据权利要求8或权利要求9所述的缀合物,其中所述缀合物中的至少一个抗体变体包含SEQ ID NO:14的轻链HVR1(HVR-L1)序列和SEQ ID NO:12的重链HVR2(HVR-H2)序列。

13.根据权利要求12所述的缀合物,其中所述抗体变体还包含SEQ ID NO:15的重链HVR3(HVR-H3)序列。

14.一种缀合物,所述缀合物包含与一个或多个多元醇共价连接的一个或多个抗-因子D抗体变体,其中:

所述缀合物中的至少一个抗-因子D抗体变体包含参比抗-因子D抗体的HVR内的一个或多个位置处的置换,其中所述参比抗-因子D抗体包含:包含序列ITSTDIDDDMN(SEQ ID NO:5)的轻链HVR-1,包含序列GGNTLRP(SEQ ID NO:6)的轻链HVR-2,包含序列LQSDSLPYT(SEQ ID NO:7)的轻链HVR-3,包含序列GYTFTNYGMN(SEQ ID NO:8)的重链HVR-1,包含序列WINTYTGETTYADDFKG(SEQ ID NO:9)的重链HVR-2,和包含序列EGGVNN(SEQ ID NO:10)的重

链HVR-3，并且其中所述置换为以下中的一个或多个：(a)SEQ ID NO:5的位置5处的氨基酸是S(SEQ ID NO:22中公开的a、b、c)；(b)SEQ ID NO:5的位置7处的氨基酸是E；(c)SEQ ID NO:5的位置8处的氨基酸是S；(d)SEQ ID NO:9的位置13处的氨基酸是E(SEQ ID NO:23)；(e)SEQ ID NO:7的位置4处的氨基酸是E(SEQ ID NO:24)；或(f)SEQ ID NO:10的位置5处的氨基酸是S(SEQ ID NO:25)；并且

所述多元醇是多臂多元醇。

15.根据权利要求14所述的缀合物，其中所述置换选自由以下组成的组：

(i)置换(b)-(d)；

(ii)置换(b)-(e)；

(iii)置换(a)-(d)；和

(iv)置换(a)-(d)和(f)。

16.根据权利要求14或权利要求15所述的缀合物，其中所述变体包含选自由以下组成的组的重链恒定结构域氨基酸序列：SEQ ID NO:54-74和116。

17.一种缀合物，所述缀合物包含与一个或多个多元醇共价连接的一个或多个抗-因子D抗体，其中：

所述缀合物中的至少一个抗-因子D抗体独立地选自由以下组成的组：

a)包含SEQ ID NO:16、18或19的轻链可变结构域氨基酸序列的抗-因子D抗体；并且

b)包含SEQ ID NO:17或20的重链可变结构域氨基酸序列的抗-因子D抗体；和

所述多元醇是多臂多元醇。

18.根据权利要求17所述的缀合物，其中所述缀合物中的至少一个抗-因子D抗体包含SEQ ID NO:16、18或19的轻链可变结构域氨基酸序列和SEQ ID NO:17或20的重链可变结构域氨基酸序列。

19.根据权利要求18所述的缀合物，其中所述轻链可变结构域氨基酸序列为根据SEQ ID NO:19并且所述重链可变结构域氨基酸序列为根据SEQ ID NO:17。

20.根据权利要求18所述的缀合物，其中所述轻链可变结构域氨基酸序列为根据SEQ ID NO:19并且所述重链可变结构域氨基酸序列为根据SEQ ID NO:20。

21.根据权利要求17-20中任一项所述的缀合物，其中至少一个抗-因子D抗体包含选自由以下组成的组的重链恒定结构域氨基酸序列：SEQ ID NO:55-74和116。

22.一种缀合物，所述缀合物包含与一个或多个多元醇共价连接的一个或多个抗-因子D抗体，其中：

所述缀合物中的至少一个抗-因子D抗体具有可变轻链和可变重链，所述可变轻链包含具有SEQ ID NO:11或14的序列的HVR-L1，具有SEQ ID NO:6的序列的HVR-L2，和具有SEQ ID NO:7或13的序列的HVR-L3；所述可变重链包含具有SEQ ID NO:8的序列的HVR-H1，具有SEQ ID NO:9或12的序列的HVR-H2，和具有SEQ ID NO:10或15的序列的HVR-H3；并且

所述多元醇是多臂多元醇。

23.根据权利要求22所述的缀合物，其中所述缀合物中的每个抗-因子D抗体独立地选自由以下组成的组：

(i)具有可变轻链和可变重链的抗-因子D抗体，所述可变轻链包含具有SEQ ID NO:14的序列的HVR-L1，具有SEQ ID NO:6的序列的HVR-L2，和具有SEQ ID NO:7的序列的HVR-L3；

所述可变重链包含具有SEQ ID NO:8的序列的HVR-H1,具有SEQ ID NO:12的序列的HVR-H2,和具有SEQ ID NO:10的序列的HVR-H3;和

(ii)具有可变轻链和可变重链的抗-因子D抗体,所述可变轻链包含具有SEQ ID NO:14的序列的SEQ ID NO:HVR-L1的HVR-L1序列,具有SEQ ID NO:6的序列的HVR-L2,和具有SEQ ID NO:7的序列的HVR-L3;所述可变重链包含具有SEQ ID NO:8的序列的HVR-H1,具有SEQ ID NO:12的序列的HVR-H2,和具有SEQ ID NO:15的序列的HVR-H3。

24.根据权利要求22或权利要求23所述的缀合物,其中至少一个抗-因子D抗体具有选自由以下组成的组的重链恒定结构域氨基酸序列:SEQ ID NO:55-74和116。

25.一种缀合物,所述缀合物包含与一个或多个多元醇共价连接的一个或多个抗-因子D抗体,其中:

所述缀合物中的至少一个抗-因子D抗体选自由以下组成的组:

(i)具有以下的抗-因子D抗体:具有SEQ ID NO:26的氨基酸序列的轻链,和具有SEQ ID NO:27的氨基酸序列的重链;

(ii)具有以下的抗-因子D抗体:具有SEQ ID NO:28的氨基酸序列的轻链,和具有SEQ ID NO:29的氨基酸序列的重链;

(iii)具有以下的抗-因子D抗体:具有SEQ ID NO:26的氨基酸序列的轻链,和具有SEQ ID NO:30的氨基酸序列的重链;

(iv)具有以下的抗-因子D抗体:具有SEQ ID NO:26的氨基酸序列的轻链,和具有SEQ ID NO:31的氨基酸序列的重链;

(v)具有以下的抗-因子D抗体:具有SEQ ID NO:26的氨基酸序列的轻链,和具有选自由SEQ ID NO:75-92和117组成的组的氨基酸序列的重链;

(vi)具有以下的抗-因子D抗体:具有SEQ ID NO:28的氨基酸序列的轻链,和具有SEQ ID NO:32的氨基酸序列的重链;

(vii)具有以下的抗-因子D抗体:具有SEQ ID NO:28的氨基酸序列的轻链,和具有SEQ ID NO:33的氨基酸序列的重链;和

(vii)具有以下的抗-因子D抗体:具有SEQ ID NO:28的氨基酸序列的轻链,和具有选自由SEQ ID NO:93-110和118组成的组的氨基酸序列的重链;其中

所述多元醇是多臂多元醇。

26.根据权利要求25所述的缀合物,其中所述缀合物中的至少一个抗-因子D抗体是具有以下的抗-因子D抗体:具有SEQ ID NO:28的氨基酸序列的轻链,和具有SEQ ID NO:32的氨基酸序列的重链。

27.根据权利要求25所述的缀合物,其中所述缀合物中的至少一个抗-因子D抗体是具有以下的抗-因子D抗体:具有SEQ ID NO:26的氨基酸序列的轻链,和具有SEQ ID NO:30的氨基酸序列的重链。

28.根据权利要求1-27中任一项所述的缀合物,其中所述抗-因子D抗体或所述抗体变体是选自由以下组成的组的抗体片段:Fab、Fab'、Fab'-SH、Fab-C、Fab-C-SH及其组合。

29.根据权利要求28所述的缀合物,其中所述缀合物中的至少一个抗体片段在C'-末端包含一个半胱氨酸残基。

30.根据权利要求28所述的缀合物,其中所述缀合物中的至少一个抗体片段在C'-末端

包含SEQ ID NO:21的氨基酸序列。

31.根据权利要求28所述的缀合物，其中所述缀合物中的至少一个抗体片段在C'-末端包含选自由以下组成的组的氨基酸序列:SEQ ID NO:111-114。

32.根据权利要求1-31中任一项所述的缀合物，其中所述多元醇在半胱氨酸残基的游离巯基处与至少一个所述抗-因子D抗体或所述抗体变体共价连接。

33.根据权利要求32所述的缀合物，其中所述半胱氨酸残基是工程改造的半胱氨酸。

34.根据权利要求32或权利要求33所述的缀合物，其中所述半胱氨酸残基在所述抗-因子D抗体或所述抗体变体的恒定结构域中。

35.根据权利要求32至34中任一项所述的缀合物，其中所述半胱氨酸残基处于所述抗-因子D抗体或所述抗体变体的C'-末端。

36.根据权利要求32至35中任一项所述的缀合物，其中所述缀合物包含至少两个所述抗-因子D抗体或所述抗体变体，并且所述多元醇在半胱氨酸残基的游离巯基处与每个抗-因子D抗体或抗体变体共价连接。

37.根据权利要求1-16中任一项所述的缀合物，其中至少一个所述抗体变体包含所述参比抗-因子D抗体的恒定结构域内的至少一个氨基酸残基的置换，其中所述氨基酸残基用Cys置换。

38.根据权利要求37所述的缀合物，其中所述多元醇在置换的半胱氨酸残基的游离巯基处与所述抗体变体共价连接。

39.根据权利要求1-38中任一项所述的缀合物，其中所述多元醇在赖氨酸残基的游离氨基处与至少一个所述抗-因子D抗体或所述抗体变体共价连接。

40.根据权利要求39所述的缀合物，其中所述赖氨酸残基在所述抗-因子D抗体或所述抗体变体的恒定结构域内。

41.根据权利要求1-40中任一项所述的缀合物，其中至少两个所述抗-因子D抗体或所述抗体变体与所述多元醇共价连接。

42.根据权利要求41所述的缀合物，其中至少三个所述抗-因子D抗体或所述抗体变体与所述多元醇共价连接。

43.根据权利要求42所述的缀合物，其中至少四个所述抗-因子D抗体或所述抗体变体与所述多元醇共价连接。

44.根据权利要求43所述的缀合物，其中至少五个所述抗-因子D抗体或所述抗体变体与所述多元醇共价连接。

45.根据权利要求44所述的缀合物，其中至少六个所述抗-因子D抗体或所述抗体变体与所述多元醇共价连接。

46.根据权利要求45所述的缀合物，其中至少七个所述抗-因子D抗体或所述抗体变体与所述多元醇共价连接。

47.根据权利要求46所述的缀合物，其中至少八个所述抗-因子D抗体或所述抗体变体与所述多元醇共价连接。

48.根据权利要求1-47中任一项所述的缀合物，其中所述多臂多元醇选自由以下组成的组:二聚体、四聚体、六聚体和八聚体。

49.根据权利要求48所述的缀合物，其中所述多臂多元醇是八聚体。

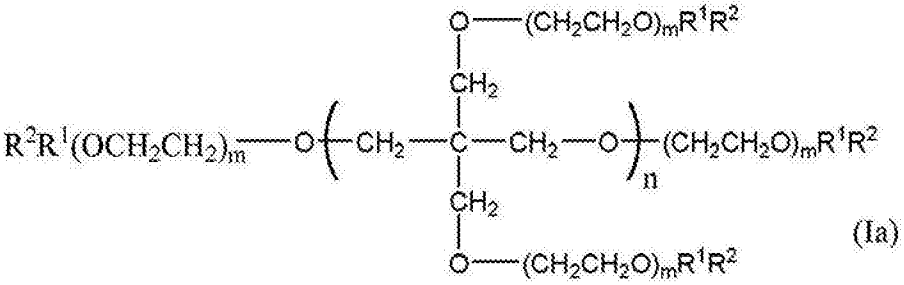
50.根据权利要求1-49中任一项所述的缀合物，其中所述多元醇是聚乙二醇。

51.根据权利要求50所述的缀合物，其中所述聚乙二醇的重均分子量为约500D至300,000D。

52.根据权利要求51所述的缀合物，其中所述聚乙二醇的重均分子量为约20,000D至60,000D。

53.根据权利要求52所述的缀合物，其中所述聚乙二醇的重均分子量为约40,000D。

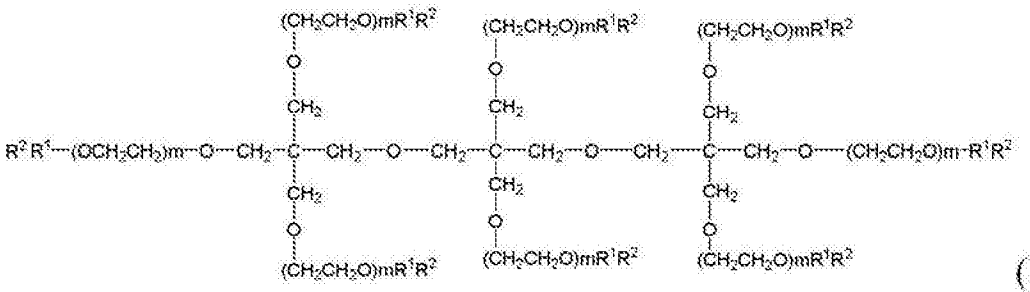
54.根据权利要求50-53中任一项所述的缀合物，其中所述聚乙二醇具有通式(Ia)的结构：



(Ia)

其中各m独立地是3-250的整数；n是1-10的整数；各R^1独立地是不存在或是连接基团；并且各R^2独立地是氢或末端反应性基团；其中至少一个R^2是末端反应性基团并且与所述抗-因子D抗体或所述抗体变体共价连接。

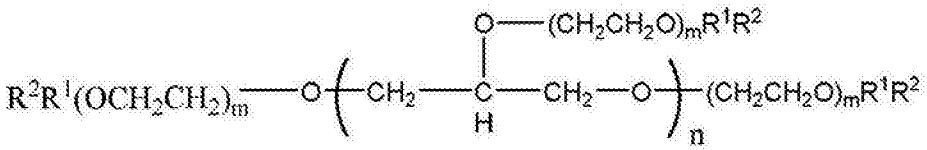
55.根据权利要求50-53中任一项所述的缀合物，其中所述聚乙二醇具有通式(Ib)的结构：



(Ib)

其中各m独立地是3-250的整数；各R^1独立地是不存在或是连接基团；并且各R^2独立地是氢或末端反应性基团；其中至少一个R^2是末端反应性基团并且与所述抗-因子D抗体或所述抗体变体共价连接。

56.根据权利要求50-53中任一项所述的缀合物，其中所述聚乙二醇具有通式(IIa)的结构：

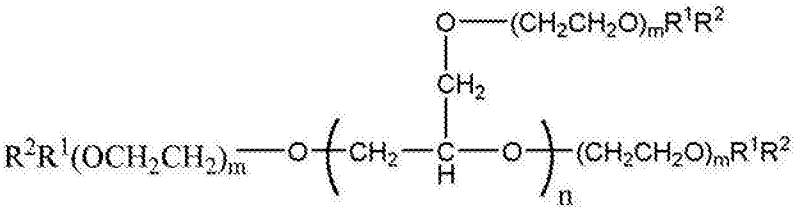


(IIa)

其中各m独立地是3-250的整数；n是1-10的整数；各R^1独立地是不存在或是连接基团；并且各R^2独立地是氢或末端反应性基团；其中至少一个R^2是末端反应性基团并且与所述抗-因子D抗体或所述抗体变体共价连接。

57.根据权利要求56所述的缀合物，其中n是6。

58.根据权利要求50-53中任一项所述的缀合物，其中所述聚乙二醇具有通式(IIIa)的结构：



(IIIa)

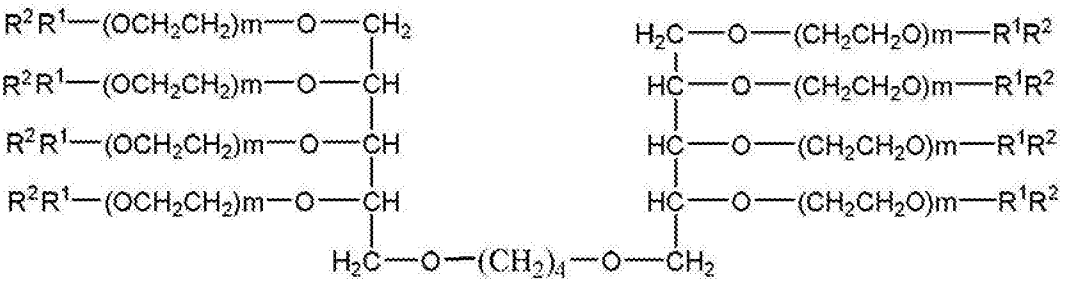
其中各m独立地是3-250的整数；n是1-10的整数；各R^1独立地是不存在或是连接基团；并且各R^2独立地是氢或末端反应性基团；其中至少一个R^2是末端反应性基团并且与所述抗-因子D抗体或所述抗体变体共价连接。

59.根据权利要求58所述的缀合物，其中n是2。

60.根据权利要求58所述的缀合物，其中n是4。

61.根据权利要求58所述的缀合物，其中n是6。

62.根据权利要求50-53中任一项所述的缀合物，其中所述聚乙二醇具有通式(IVa)的结构：

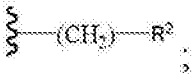


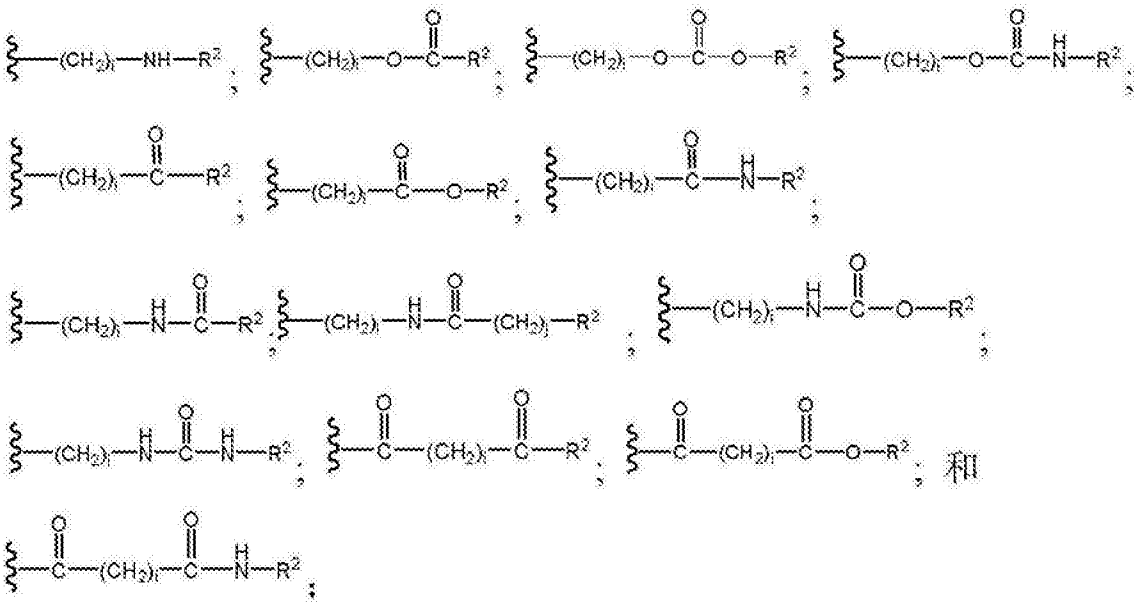
(IVa)

其中各m独立地是3-250的整数；各R^1独立地是不存在或是连接基团；并且各R^2独立地是氢或末端反应性基团；其中至少一个R^2是末端反应性基团并且与所述抗-因子D抗体或所述抗体变体共价连接。

63.根据权利要求54-62中任一项所述的缀合物，其中m是50-200的整数。

64.根据权利要求63所述的缀合物，其中m是100-150的整数。

65.根据权利要求54-64中任一项所述的缀合物，其中至少一个R^1是连接基团，其中R^1和R^2当连接在一起时选自由以下组成的组：  。



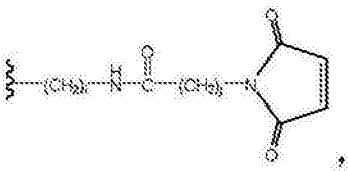
及其组合，其中各i独立地是0-10的整数；并且j是0-10的整数。

66.根据权利要求54-65中任一项所述的缀合物，其中各R^2独立地选自由以下组成的组：硫醇反应性基团、氨基反应性基团及其组合。

67.根据权利要求66所述的缀合物，其中各R^2独立地选自由以下组成的组：马来酰亚胺、巯基、硫醇、三氟甲磺酸酯、甲苯磺酸酯、氮丙啶、环氧化物、吡啶基二硫化物、琥珀酰亚胺酯、$-NH_2$、醛、卤代乙酸酯、卤代乙酰胺和碳酸对-硝基苯酯。

68.根据权利要求67所述的缀合物，其中R^2是马来酰亚胺。

69.根据权利要求54-68中任一项所述的缀合物，其中R^1和R^2，当连接在一起时，为

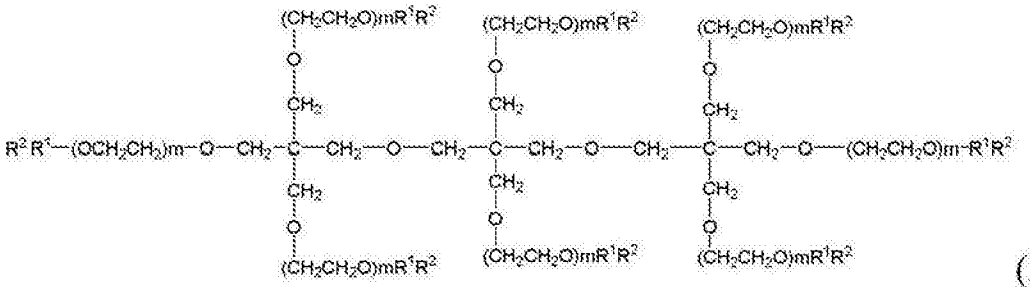


，i是0-10的整数；并且j是0-10的整数。

70.根据权利要求54-58或61-69中任一项所述的缀合物，其中所述R^2基团中的至少七个与所述抗-因子D抗体或所述抗体变体中的一个共价连接。

71.根据权利要求70所述的缀合物，其中所述R^2基团中的八个与所述抗-因子D抗体或所述抗体变体中的一个共价连接。

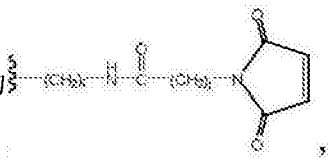
72.根据权利要求26或权利要求27所述的缀合物，其中所述聚乙二醇具有通式(Ib)的结构：



(Ib)

其中各m独立地是3-250的整数；各R^1独立地是不存在或是连接基团；并且各R^2独立地是氢或末端反应性基团；其中至少一个R^2是末端反应性基团并且与所述抗-因子D抗体或所述

抗体变体共价连接。

73.根据权利要求72所述的缀合物，其中R^1和R^2，当连接在一起时，为  ，

i是0-10的整数；并且j是0-10的整数。

74.根据权利要求73所述的缀合物，其中i是2并且j是2。

75.根据权利要求1-74中任一项所述的缀合物，其中通过将至少一个抗-因子D抗体或抗体变体共价连接至所述多臂多元醇来制备所述缀合物。

76.根据权利要求75所述的缀合物，其中所述多臂多元醇选自由以下组成的组：二聚体、四聚体、六聚体和八聚体。

77.根据权利要求75或权利要求76所述的缀合物，其中所述多元醇是聚乙二醇。

78.一种药物制剂，其包括根据权利要求1-77中任一项所述的缀合物。

79.根据权利要求78所述的药物制剂，其中所述抗-因子D抗体或所述抗体变体的浓度为至少100mg/ml。

80.根据权利要求79所述的药物制剂，其中所述抗-因子D抗体或所述抗体变体的浓度为至少200mg/ml。

81.根据权利要求80所述的药物制剂，其中所述抗-因子D抗体或所述抗体变体的浓度为至少300mg/ml。

82.根据权利要求78所述的药物制剂，其中所述抗-因子D抗体或所述抗体变体的浓度为约50mg/mL至约300mg/ml。

83.一种用于眼部递送的长效递送装置，其包含：根据权利要求78-82中任一项所述的药物制剂，和用于将所述制剂玻璃体内递送至患者的工具，由此所述制剂原位保持有效达延长的时间段。

84.根据权利要求78-82中任一项所述的制剂或根据权利要求83所述的装置在制备药物中的用途，所述药物用于治疗受试者中的补体相关性病症。

85.根据权利要求84所述的用途，其中所述补体相关性病症是系统性的。

86.根据权利要求85所述的用途，其中所述补体相关性病症是眼病。

87.根据权利要求86所述的用途，其中所述眼病选自由以下组成的组：年龄相关性黄斑变性(AMD)(包括干性和湿性(非渗出性和渗出性)形式)、糖尿病性视网膜病、缺血相关性视网膜病、脉络膜新血管形成(CNV)、葡萄膜炎、糖尿病性黄斑水肿、病理性近视、希佩尔-林道病、眼的组织胞浆菌病、视网膜中央静脉阻塞(CRVO)、角膜新血管形成和视网膜新血管形成。

88.根据权利要求87所述的用途，其中所述眼病选自由以下组成的组：中期干性形式AMD和地图状萎缩(GA)。

89.根据权利要求1至77中任一项所述的缀合物，其用于治疗。

90.根据权利要求1至77中任一项所述的缀合物，其用于治疗受试者中的补体相关性病症的方法中。

91.根据权利要求1至77中任一项所述的缀合物，其用于治疗受试者中的系统性补体相

关性病症的方法中。

92.根据权利要求91所述的缀合物，其中所述补体相关性病症是补体相关性眼部病症。

93.根据权利要求92所述的缀合物，其中所述补体相关性眼部病症选自由以下组成的组：年龄相关性黄斑变性(AMD)(包括干性和湿性(非渗出性和渗出性)形式)、脉络膜新血管形成(CNV)、葡萄膜炎、糖尿病性视网膜病、缺血相关性视网膜病、糖尿病性黄斑水肿、病理性近视、希佩尔-林道病、眼的组织胞浆菌病、视网膜中央静脉阻塞(CRVO)、角膜新血管形成和视网膜新血管形成。

94.根据权利要求92所述的缀合物，其中所述补体相关性眼部病症选自由以下组成的组：中期干性形式AMD和地图状萎缩(GA)。

95.一种治疗受试者中补体相关性病症的方法，所述方法包括向所述受试者施用有效量的根据权利要求1至77中任一项所述的缀合物或根据权利要求78至82中任一项所述的药物制剂。

96.根据权利要求95所述的方法，其中所述补体相关性病症是系统性的。

97.根据权利要求95所述的方法，其中所述补体相关性病症是补体相关性眼部病症。

98.根据权利要求97所述的方法，其中所述补体相关性眼部病症选自由以下组成的组：年龄相关性黄斑变性(AMD)(包括干性和湿性(非渗出性和渗出性)形式)、脉络膜新血管形成(CNV)、葡萄膜炎、糖尿病性视网膜病、缺血相关性视网膜病、糖尿病性黄斑水肿、病理性近视、希佩尔-林道病、眼的组织胞浆菌病、视网膜中央静脉阻塞(CRVO)、角膜新血管形成和视网膜新血管形成。

99.根据权利要求97所述的方法，其中所述补体相关性眼部病症选自由以下组成的组：中期干性形式AMD或地图状萎缩(GA)。

100.根据权利要求95至99中任一项所述的方法，其中所述方法包括使用可植入端口递送系统施用所述缀合物或药物制剂。

101.根据权利要求95至100中任一项所述的方法，其中所述方法包括通过玻璃体内施用来施用所述缀合物或药物制剂。

102.根据权利要求101所述的方法，其中所述玻璃体内施用是经由窄孔径针。

103.根据权利要求102所述的方法，其中所述窄孔径针是约30、29、28、27、26、25、24、23或22号。

104.根据权利要求95至103中任一项所述的方法，其进一步包括将另外的治疗剂施用至个体。

105.根据权利要求104所述的方法，其中所述另外的治疗剂选自由以下组成的组：ANG2拮抗剂、TIE2拮抗剂、VEGF拮抗剂和第二补体组分拮抗剂。

106.根据权利要求104所述的方法，其中所述另外的治疗剂是抗-ANG2抗体。

107.根据权利要求104所述的方法，其中所述另外的治疗剂是抗-TIE2抗体。

108.根据权利要求104所述的方法，其中所述另外的治疗剂选自由以下组成的组：VEGF捕获剂和抗-VEGF抗体。

109.根据权利要求104所述的方法，其中所述另外的治疗剂是第二补体组分拮抗剂，其中所述第二补体组分拮抗剂抑制选自由以下组成的组的补体组分：C1、C2、C3、C4、C5、C6、C7、C8和C9。

抗-因子D抗体变体缀合物及其用途

[0001] 对相关申请的交叉引用

[0002] 本申请要求2015年10月30日提交的美国临时专利申请号62/249,020和2015年11月4日提交的美国临时专利申请号62/250,965的优先权利益，所述两个临时专利申请都通过引用整体并入本文。

[0003] 序列表

[0004] 本申请包含已经以ASCII格式电子提交的序列表，并且该序列表通过引用整体结合于此。所述ASCII副本创建于2016年10月27日，名称为P33044-WO.txt并且大小为172,164字节。

背景技术

[0005] 治疗性抗体的开发代表着人类医药的悠久历史中的革命性纪元。超过30种抗体已被批准用于人类治疗，并且在世界范围内超过250种抗体处于临床开发阶段以用于多种重大疾病，包括癌症、自身免疫、炎症、心血管病、传染病和眼病。在过去十年，单克隆抗体产品的市场呈指数增长，其受轰动性药物如曲妥珠单抗、贝伐单抗、利妥昔单抗、英夫利昔单抗和阿达木单抗的成功的推进。这些第一代抗体治疗剂已经使众多患者受益，同时抗体技术的进步和对作用机制的更深理解为具有甚至更佳效力和更少副作用的改进版本的抗体铺平了道路。

[0006] 与作为小的有机和无机分子的常规药物相比，抗体治疗剂的成功开发和可行的利用具有许多独特的挑战。与所有蛋白质一样，抗体的生物物理性质对于其行为是重要的决定因素并且显著影响涉及表达、纯化、制剂、存储、递送、药物动力学、免疫原性和用药方案的治疗剂的开发。在众多性质中，蛋白质稳定性是决定候选抗体品质及其作为成功治疗剂的合意性的主要特征。

[0007] 蛋白质疗法通常需要将高剂量的蛋白质递送至患者以便实现所需的效力。同时，某些给药途径与限制如递送时间、体积和体力有关，其需要高剂量蛋白质在高浓度制剂(例如，至少100mg/ml)中。然而，高浓度蛋白质制剂提出了关于稳定性、溶解性、粘性及其他蛋白质性质的特别挑战。

[0008] 蛋白质可以是不稳定的并且经由多种物理和化学分解途径而被分解。物理不稳定性主要经由两种途径发生-变性和聚集，而化学不稳定性可以经由多种途径发生，如脱酰胺、异构化、交联、氧化和片段化。抗体不稳定性对于药物开发是不利的，因为其可能导致活性药物量的减小和较低的体内效力，治疗剂的各批次间的可变性增加，并且可能是最重要的，患者中针对聚集物和降解物的免疫原性。Wang等(2007)J.Pharm.Sci.96:1-26;Moore等(1980)J Clin Endocrinology&Metabolism 51:691-697;Rosenberg等(2006)AAPSJ 8:E501-7;Joubert等(2011)J Biol Chem 286:25118-25133;Joubert等(2012)J Biol Chem (2012)286:25266-79)。

[0009] 抗体是大的多结构域蛋白质，并且对其稳定性和聚集倾向性有作用的因素是复杂的，包括许多外在条件如温度、pH、浓度、离子强度和物理应力。同样重要的是蛋白质本身的

一级序列。虽然在特定同种型的抗体之间Fc区域的性质在很大程度上相同，但是Fab区有很大不同。因此，很大程度上由于Fab序列差异和抗体的特别的抗原特异性，在抗体之间在稳定性和聚集倾向方面存在显著差异。Lowe等(2011)Adv.Protein Chem.Struct Biol.84:41-61。

[0010] 补体系统在免疫复合物的清除和对传染剂、外部抗原、病毒感染的细胞和肿瘤细胞的免疫反应方面发挥中心作用。然而，补体也参与病理炎症和自体免疫病。因此，抑制过度的或不受控的补体级联激活可以为患有此种疾病和病症的患者提供临床益处。

[0011] 补体系统包括三种不同的激活途径，其被指定为经典途径、结合甘露糖的凝集素途径和旁路途径。V.M.Holers于Clinical Immunology:Principles and Practice,ed.R.R.Rich,Mosby Press;1996,363-391。经典途径是钙/镁依赖性级联，其通常通过形成抗原-抗体复合物而激活。结合甘露糖的凝集素(MBL)途径通过MBL结合病原体上的糖类结构而引发，导致切割C2和C4以形成活性C2a、C2b、C4a和C4b的MBL蛋白酶(MASP)的活化。旁路途径是镁依赖性级联，其通过C3在某些易感表面(例如酵母和细菌的细胞壁多糖，和某些生物聚合材料)上的沉积和活化而激活。补体途径的激活产生补体蛋白质的生物活性片段，例如C3a、C4a和C5a过敏毒素和C5b-9膜攻击复合物(MAC)，其介导涉及白细胞趋化性、巨噬细胞、嗜中性粒细胞、血小板、肥大细胞和内皮细胞的激活、血管通透性、细胞溶解和组织损伤的炎性活动。

[0012] 因子D是对于备选补体途径的激活必要的高度特异性的丝氨酸蛋白酶。其切割与C3b结合的因子B，产生作为旁路途径C3/C5转化酶的活性组分的C3b/Bb酶。因子D可以作为抑制的合适靶标，因为其在人中的血浆浓度非常低(1.8μg/ml)，并且已经证明其是旁路补体途径的激活的限速酶。P.H.Lesavre和H.J.Müller-Eberhard.(1978)J.Exp.Med.148:1498-1510;J.E.Volanakis等人(1985)New Eng.J.Med.312:395-401。

[0013] 已经证明，在动物模型中以及在离体研究中，补体激活的下调在治疗若干疾病适应证方面是有效的，所述疾病适应证例如是系统性红斑狼疮和肾小球肾炎、类风湿性关节炎、心肺分流术(cardiopulmonary bypass)和血液透析、器官移植中的超急性排斥、心肌梗死、再灌注损伤和成人呼吸窘迫综合征。另外，其它炎性病症和自体免疫/免疫复合物疾病也与补体激活密切相关，所述疾病包括热损伤、严重哮喘、过敏性休克、肠炎、荨麻疹、血管性水肿、血管炎、多发性硬化、重症肌无力、膜性增生性肾小球肾炎和舍格伦(Sjögren)综合征。

[0014] 年龄相关性黄斑变性(AMD)是中央视网膜的进行性的慢性疾病，其对视觉敏锐性有显著后果。Lim等人(2012)Lancet 379:1728。在工业化国家中，该疾病的晚期形式是视力丧失的主要原因。对于≥40岁的高加索人群，早期AMD的患病率估计在6.8％而晚期AMD在1.5％。de Jong(2006)N.Engl.J.Med.355:1474。在80岁后，晚期AMD的患病率随年龄增长显著增加至11.8％。存在两种类型的AMD，非渗出性(干性)AMD和渗出性(湿性)AMD。更常见的干性形式AMD包括中央视网膜(黄斑)下的视网膜色素上皮(RPE)的萎缩和肥大性改变以及RPE上的沉积物(玻璃疣)。晚期干性AMD可以导致显著的视网膜损伤，包括地图状萎缩(GA)，以及不可逆的视力丧失。此外，患有干性AMD的患者可以进展至湿性形式，其中在视网膜下发展出被称为脉络膜新生血管膜(CNVM)的异常血管，渗液和渗血，并且最终导致视网膜中和视网膜下的致盲性盘状瘢痕。

[0015] 靶向新血管形成(新血管生成)的药物已经是治疗湿性AMD的主要依靠。雷珠单抗(Ranibizumab),一种抗-VEGFA抗体片段,已被证明可以高效地改善患有湿性AMD的患者的视力。最近的研究暗示了AMD与补体级联中的关键蛋白质之间的关联性,并且正在开发多种靶向特定补体组分的疗法用以治疗干性AMD。通过结合因子D上的外部位点而有效抑制因子D及补体旁路途径的人源化抗-因子D Fab片段(aFD,lampalizumab;FCFD4514S)目前处于临床开发中以用于治疗与干性AMD相关的GA。Katschke等人(2012)J.Biol.Chem.287:12886。最近的II期临床试验显示每月玻璃体内注射lampalizumab有效地减慢患有晚期干性AMD的患者中的GA病变的进展。

[0016] 眼睛具有许多独特的生物物理和解剖学特征,其使得眼部药物递送更具有挑战性。例如,血-眼屏障是保护眼睛免于感染的防御机制,但是同时使得药物难于渗入,尤其是对于眼睛后段中的疾病。因此,通常需要高剂量施用来实现和保持药物的原位生物利用度(例如,眼部停留时间)以便改善效力。同时,眼睛后部中的有限空间限制了递送的药物体积,其又要求药物在高浓度制剂中递送。

[0017] 患有眼病的患者也可以受益于治疗剂的长效/缓释递送。较低频率的给药将为患者提供改善的便利性,具有降低感染率和增加临床效力的潜在益处。高剂量药物的受控释放还可以使药物副作用最小化。两种有希望的用于长效递送的系统是基于PLGA的固体植入物和可植入端口递送系统(PDS)。两种系统都有可能提供接近零级的释放动力学用于延长的时间。对于PLGA植入物,蛋白质药物被包封在疏水聚合物基质中并且药物释放经由聚合物的缓慢水解实现。释放速率可以通过改变药物载量、聚合物疏水性或聚合物分子量来控制。PDS是一种可再填充的装置,其中向玻璃体内的释放通过包含钛玻璃料的多孔金属膜来控制。因为储库具有小的体积,所以利用PDS进行有效递送需要高的蛋白浓度。

[0018] 药物所暴露的条件根据使用的递送系统而变化。对于在固体PLGA植入物中的结合,使用冻干的或喷雾干燥的药物。使用热熔挤出法来制备植入物,使得药物短暂地暴露于接近90℃的温度。虽然药物在释放过程中保持固态,但是PLGA的降解可以将药物暴露于低pH环境。相反,利用PDS递送的药物被保持在液态下的高浓度并被暴露于玻璃体,玻璃体被表征为一种具有减小的在生理离子强度和pH下还原的环境。

[0019] 除了高浓度和长效递送以外或作为高浓度和长效递送的替代,药物的增加的生物利用度(例如,眼部停留时间)可以通过翻译后修饰实现或受其促进,其中蛋白质药物与天然的或合成的聚合物共价缀合,如聚唾液酸化、HES化(与羟乙基淀粉缀合)和PEG化。Chen等人(2011)Expert.Opin.Drug Deliv.8:1221-36;Kontermann(2009)BioDrugs 23:93-109。PEG化,即将聚合物聚乙二醇(PEG)共价连接至蛋白质,是一种尤其可用于延长抗体片段治疗剂的半衰期的确立已久的技术。Jevsevar等人(2010)Biotech.J.5:113-128。

[0020] 因此,对于具有改善的稳定性以及在某些实施方案中适合于高浓度制剂和/或长效递送的抗-因子D抗体及其缀合物存在强烈的需求。

[0021] 发明概述

[0022] 本发明部分基于这样的发现,即抗体中对鉴定的热点的靶向氨基酸置换可以有效地提高抗体的稳定性和作为治疗剂的整体效力。如与未缀合的抗体相比,此种抗体与多臂聚合物(诸如多臂多元醇)的缀合可以提高玻璃体液半衰期、房水半衰期和/或视网膜半衰期。

[0023] 在一些方面，本发明涉及包含与一种或多种多臂多元醇共价连接的一种或多种抗-因子D抗体或抗-因子D抗体变体的缀合物。在某些实施方案中，多元醇是八臂多元醇(即，八聚体)。在一些实施方案中，多元醇是聚乙二醇(PEG)。在某些实施方案中，PEG可以具有通式(Ia)、(Ib)、(IIa)、(IIIa)或(IVa)中任一种的结构(如下文所示)。

[0024] 用于本发明的缀合物中的抗-因子D抗体变体具有改善的稳定性。抗-因子D抗体变体包含在参比抗-因子D抗体的高变区(HVR)内的至少一个靶天冬氨酸(D或Asp)残基的置换，其中所述靶Asp残基被鉴定为倾向于异构化并且所述置换是Asp至谷氨酸(E或Glu)，并且其中当与所述参比抗-因子D抗体相比时，所述抗-因子D抗体变体显示改善的稳定性而不显著丧失因子D结合亲和力。在一些方面，进行置换的靶Asp残基在Asp-Xaa基序内，其中Xaa是Asp、Gly、His、Ser或Thr。在一些方面，靶Asp残基是Asp-Asp(DD)基序中的第一个Asp。在一些方面，抗-因子D抗体变体包含在参比抗-因子D抗体的HVR内的另外的Asp位点处的一个或多个置换，其中所述置换是Asp至丝氨酸(S或Ser)以减少抗体的整体电荷，由此提高抗体的溶解性。在一些方面，抗-因子D抗体变体包含在被鉴定为倾向于脱酰胺的天冬酰胺(N或Asn)位点处的一个或多个置换，其中所述置换是Asn至Ser以减少或消除抗体的脱酰胺。

[0025] 在一些实施方案中，抗-因子D抗体变体是Fab片段，其中所述Fab片段的重链的C-末端以氨基酸“CDKTHT”、“CDKTHL”、“CDKTH”、“CDKT”、“CDK”或“CD”结束。在一些实施方案中，Fab片段的重链的C-末端以序列“CDKTHX”结束，其中X是除T以外的任何氨基酸。C-末端处的截短和/或突变能够减少或消除针对Fab的AHA反应性，而不危害热稳定性或表达。在一些实施方案中，Fab片段的重链的C-末端以氨基酸“CDKTHTC”、“CDKTHTCPPC”、“CDKTHTCPPS”、“CDKTHTSPPC”、“CDKTHTAPPC”、“CDKTHTSGGC”或“CYGPPC”结束。在一些这样的实施方案中，C-末端氨基酸中的游离半胱氨酸可以接受缀合至例如聚合物(诸如PEG)。在一些实施方案中，Fab片段包含选自由以下组成的组的重链恒定结构域氨基酸序列：SEQ ID NO:54(以“CDKTHT”结束)、55-66(以“CDKTHL”、“CDKTHTC”、“CPPC”、“CPPS”、“SPPC”、“APPC”、“SGGC”、“CYGPPC”、“CDKTH”、“CDKT”、“CDK”或“CD”结束)、和116(以“CDKTHX”结束)。在一些实施方案中，Fab是包含SEQ ID NO:67(以“VERK”结束)的重链恒定结构域氨基酸序列的IgG2Fab片段，或包含SEQ ID NO:68(以“VERKC”结束)的重链恒定结构域氨基酸序列的IgG2Fab-C片段。在一些实施方案中，Fab是包含选自由以下组成的组的重链恒定结构域氨基酸序列的IgG4Fab片段：SEQ ID NO:69-73(以“KYGPP”、“KYGP”、“KYG”、“KY”或“K”结束)，或包含SEQ ID NO:74(以“KYGPPC”结束)的重链恒定结构域氨基酸序列的IgG4Fab-C片段。作为C末端处的截短和/或突变的替代方式，为了避免预先存在的抗铰链抗体(PE-AHA)应答，可以使用IgG2或IgG4Fab片段，因为这些片段不显示PE-AHA应答。

[0026] 在一些方面，用于产生用于本发明的缀合物的抗体变体的参比抗-因子D抗体包含SEQ ID NO:3的轻链可变结构域序列，SEQ ID NO:4的重链可变结构域序列，或两者。随后，所得的抗体变体可以包含SEQ ID NO:11的轻链HVR1(HVR-L1)序列和SEQ ID NO:12的重链HVR2(HVR-H2)序列，或可以包含SEQ ID NO:13的轻链HVR3(HVR-L3)序列，或可以包含SEQ ID NO:14的轻链HVR1(HVR-L1)序列和SEQ ID NO:12的重链HVR2(HVR-H2)序列，或可以包含SEQ ID NO:15的重链HVR3(HVR-H3)序列。

[0027] 在一些方面，用于本发明的缀合物的抗-因子D抗体变体是参比抗-因子D抗体的变体，其中所述参比抗-因子D抗体包含SEQ ID NO:1的轻链序列和SEQ ID NO:2的重链序列，

并且其中所述变体相对于参比抗-因子D抗体包含以下序列修饰:SEQ ID NO:11的轻链HVR1(HVR-L1)序列和SEQ ID NO:12的重链HVR2(HVR-H2)序列。此种变体在本文以下的实施例中被称为“TM”变体(AFD.v6)(参见,例如,表1)。

[0028] 在一些方面,用于本发明的缀合物的抗-因子D抗体变体是参比抗-因子D抗体的变体,其中所述参比抗-因子D抗体包含SEQ ID NO:1的轻链序列和选自由以下组成的组的重链序列:SEQ ID NO:34-53和115,并且其中所述变体相对于参比抗-因子D抗体包含以下序列修饰:SEQ ID NO:11的轻链HVR1(HVR-L1)序列和SEQ ID NO:12的重链HVR2(HVR-H2)序列。此种变体被称为“修饰的TM”变体。这些修饰的TM变体包含不同于TM变体的重链恒定结构域,并且其选自由以下组成的组:SEQ ID NO:55-74和116。

[0029] 在一些方面,用于本发明的缀合物的抗-因子D抗体变体是参比抗-因子D抗体的变体,其中所述参比抗-因子D抗体包含SEQ ID NO:1的轻链序列和SEQ ID NO:2的重链序列,并且其中所述变体相对于参比抗-因子D抗体包含以下序列修饰:SEQ ID NO:11的轻链HVR1(HVR-L1)序列、SEQ ID NO:12的重链HVR2(HVR-H2)序列和SEQ ID NO:13的轻链HVR3(HVR-L3)序列。此种变体在本文以下的实施例中被称为“TM.D92E”变体(AFD.v7)(参见,例如,表1)。

[0030] 在一些方面,用于本发明的缀合物的抗-因子D抗体变体是参比抗-因子D抗体的变体,其中所述参比抗-因子D抗体包含SEQ ID NO:1的轻链序列和选自由以下组成的组的重链序列:SEQ ID NO:34-53和115,并且其中所述变体相对于参比抗-因子D抗体包含以下序列修饰:SEQ ID NO:11的轻链HVR1(HVR-L1)序列、SEQ ID NO:12的重链HVR2(HVR-H2)序列和SEQ ID NO:13的轻链HVR3(HVR-L3)序列。此种变体被称为“修饰的TM.D92E”变体。这些修饰的TM.D92E变体包含不同于TM.D92E变体的重链恒定结构域,并且其选自由以下组成的组:SEQ ID NO:55-74和116。

[0031] 在一些方面,用于本发明的缀合物的抗-因子D抗体变体是参比抗-因子D抗体的变体,其中所述参比抗-因子D抗体包含SEQ ID NO:1的轻链序列和SEQ ID NO:2的重链序列,并且其中所述变体相对于参比抗-因子D抗体包含以下序列修饰:SEQ ID NO:14的轻链HVR1(HVR-L1)序列和SEQ ID NO:12的重链HVR2(HVR-H2)序列。此种变体在本文以下的实施例中被称为“SIESD”变体(AFD.v8)(参见,例如,表1)。在一些实施方案中,“SIESD”变体(AFD.v8)包含SEQ ID NO:26的轻链序列和SEQ ID NO:27的重链序列。在一些实施方案中,Cys修饰版本的“SIESD”变体包含SEQ ID NO:26的轻链序列和SEQ ID NO:30的重链序列。在一些实施方案中,Cys-Pro-Pro-Cys修饰版本的“SIESD”变体包含SEQ ID NO:26的轻链序列和SEQ ID NO:31的重链序列。在一些实施方案中,修饰版本的“SIESD”变体包含SEQ ID NO:26的轻链序列和选自由以下组成的组的重链序列:SEQ ID NO:75-92和117。

[0032] 在一些方面,用于本发明的缀合物的抗-因子D抗体变体是参比抗-因子D抗体的变体,其中所述参比抗-因子D抗体包含SEQ ID NO:1的轻链序列和选自由以下组成的组的重链序列:SEQ ID NO:34-53和115,并且其中所述变体相对于参比抗-因子D抗体包含以下序列修饰:SEQ ID NO:14的轻链HVR1(HVR-L1)序列和SEQ ID NO:12的重链HVR2(HVR-H2)序列。此种变体被称为“修饰的SIESD”变体。这些修饰的SIESD变体包含不同于SIESD变体的重链恒定结构域,并且其选自由以下组成的组:SEQ ID NO:55-74和116。

[0033] 在一些方面,用于本发明的缀合物的抗-因子D抗体变体是参比抗-因子D抗体的变

体，其中所述参比抗-因子D抗体包含SEQ ID NO:1的轻链序列和SEQ ID NO:2的重链序列，并且其中所述变体相对于参比抗-因子D抗体包含以下序列修饰：SEQ ID NO:14的轻链HVR1(HVR-L1)序列、SEQ ID NO:12的重链HVR2(HVR-H2)序列和SEQ ID NO:15的重链HVR3(HVR-H3)序列。此种变体在本文以下的实施例中被称为“SIESD.N103S”变体(AFD.v14)(参见，例如，表1)。在一些实施方案中，“SIESD.N103S”变体(AFD.v14)包含SEQ ID NO:28的轻链序列和SEQ ID NO:29的重链序列。在一些实施方案中，Cys修饰版本的“SIESD.N103S”变体包含SEQ ID NO:28的轻链序列和SEQ ID NO:32的重链序列。在一些实施方案中，Cys-Pro-Pro-Cys修饰版本的“SIESD.N103S”变体包含SEQ ID NO:28的轻链序列和SEQ ID NO:33的重链序列。在一些实施方案中，修饰版本的“SIESD.N103S”变体包含SEQ ID NO:28的轻链序列和选自由以下组成的组的重链序列：SEQ ID NO:93-110和118。

[0034] 在一些方面，用于本发明的缀合物的抗-因子D抗体变体是参比抗-因子D抗体的变体，其中所述参比抗-因子D抗体包含SEQ ID NO:1的轻链序列和选自由以下组成的组的重链序列：SEQ ID NO:34-53和115，并且其中所述变体相对于参比抗-因子D抗体包含以下序列修饰：SEQ ID NO:14的轻链HVR1(HVR-L1)序列、SEQ ID NO:12的重链HVR2(HVR-H2)序列和SEQ ID NO:15的重链HVR3(HVR-H3)序列。此种变体被称为“修饰的SIESD.N103S”变体。这些修饰的SIESD.N103S变体包含不同于SIESD.N103S变体的重链恒定结构域，并且其选自由以下组成的组：SEQ ID NO:55-74和116。

[0035] 在一些方面，本发明涉及包含一个或多个抗-因子D抗体变体的缀合物，所述抗-因子D抗体变体包含在参比抗-因子D抗体的HVR内的一个或多个置换。在一些方面，参比抗-因子D抗体包含以下HVR序列：

[0036] HVR-L1：ITSTDIDDDMN(SEQ ID NO:5)；

[0037] HVR-L2：GGNTLRP(SEQ ID NO:6)；

[0038] HVR-L3：LQSDSLPYT(SEQ ID NO:7)；

[0039] HVR-H1：GYTFTNYGMN(SEQ ID NO:8)；

[0040] HVR-H2：WINTYTGETTYADDFKG(SEQ ID NO:9)；和

[0041] HVR-H3：EGGVNN(SEQ ID NO:10)。

[0042] 相应的变体包含一个或多个以下置换：

[0043] (a)SEQ ID NO:5中的D5S；

[0044] (b)SEQ ID NO:5中的D7E；

[0045] (c)SEQ ID NO:5中的D8S(SEQ ID NO:22中公开的a、b和c)；

[0046] (d)SEQ ID NO:9中的D13E(SEQ ID NO:23)；

[0047] (e)SEQ ID NO:7中的D4E(SEQ ID NO:24)；或

[0048] (f)SEQ ID NO:10中的N5S(SEQ ID NO:25)。

[0049] 在一些实施方案中，参比抗-因子D抗体包含选自由以下组成的组的重链恒定结构域氨基酸序列：SEQ ID NO:54-74和116。

[0050] 在一些方面，变体结合以上置换(b)-(d)。在另一个方面，变体结合以上置换(b)-(e)。在另一个方面，变体结合以上置换(a)-(d)。在另一个方面，变体结合以上置换(a)-(d)和(f)。在另一个方面，变体包含以上置换(a)，(b)，(c)，(d)，(e)，or(f)中的一个或多个，并且进一步包含选自由以下组成的组的重链恒定结构域氨基酸序列：SEQ ID NO:54-74和

116。在另一个方面，变体包含选自由以下组成的组的置换：以上置换(b)-(d)、以上置换(b)-(e)、以上置换(a)-(d)以及以上置换(a)-(d)和(f)，其中所述变体进一步包含选自由以下组成的组的重链恒定结构域氨基酸序列：SEQ ID NO:54-74和116。

[0051] 在一些方面，本发明涉及包含一个或多个抗-因子D抗体的缀合物，所述抗-因子D抗体包含SEQ ID NO:16、18或19的轻链可变结构域氨基酸序列。在另一个方面，本发明涉及包含抗-因子D抗体的缀合物，所述抗-因子D抗体包含SEQ ID NO:17或20的重链可变结构域氨基酸序列。在另一个方面，抗-因子D抗体可以包含选自由以下组成的组的重链恒定结构域氨基酸序列：SEQ ID NO:54-74和116。在另一个方面，抗-因子D抗体包含SEQ ID NO:16、18或19的轻链可变结构域氨基酸序列和SEQ ID NO:17或20的重链可变结构域氨基酸序列。例如，抗-因子D抗体可以是包含SEQ ID NO:16的轻链可变结构域氨基酸序列和SEQ ID NO:17的重链可变结构域氨基酸序列的“TM”变体(AFD.v6)；包含SEQ ID NO:18的轻链可变结构域氨基酸序列和SEQ ID NO:17的重链可变结构域氨基酸序列的“TM.D92E”变体(AFD.v7)；包含SEQ ID NO:19的轻链可变结构域氨基酸序列和SEQ ID NO:17的重链可变结构域氨基酸序列的“SIESD”变体(AFD.v8)；或包含SEQ ID NO:19的轻链可变结构域氨基酸序列和SEQ ID NO:20的重链可变结构域氨基酸序列的“SIESD.N103S”变体(AFD.v14)。

[0052] 在另一个方面，抗-因子D抗体包含SEQ ID NO:16、18或19的轻链可变结构域氨基酸序列、SEQ ID NO:17或20的重链可变结构域氨基酸序列和选自由以下组成的组的重链恒定结构域氨基酸序列：SEQ ID NO:54-74和116。例如，抗-因子D抗体可以是修饰版本的“TM”变体(AFD.v6)，其包含SEQ ID NO:16的轻链可变结构域氨基酸序列、SEQ ID NO:17的重链可变结构域氨基酸序列和选自由以下组成的组的重链恒定结构域氨基酸序列：SEQ ID NO:55-74和116；修饰版本的“TM.D92E”变体(AFD.v7)，其包含SEQ ID NO:18的轻链可变结构域氨基酸序列、SEQ ID NO:17的重链可变结构域氨基酸序列和选自由以下组成的组的重链恒定结构域氨基酸序列：SEQ ID NO:55-74和116；修饰版本的“SIESD”变体(AFD.v8)，其包含SEQ ID NO:19的轻链可变结构域氨基酸序列、SEQ ID NO:17的重链可变结构域氨基酸序列和选自由以下组成的组的重链恒定结构域氨基酸序列：SEQ ID NO:55-74和116；修饰版本的“SIESD.N103S”变体(AFD.v14)，其包含SEQ ID NO:19的轻链可变结构域氨基酸序列、SEQ ID NO:20的重链可变结构域氨基酸序列和选自由以下组成的组的重链恒定结构域氨基酸序列：SEQ ID NO:55-74和116。

[0053] 在一些实施方案中，抗-因子D抗体是修饰版本的“SIESD”变体(AFD.v8)，其包含SEQ ID NO:19的轻链可变结构域氨基酸序列和选自由以下组成的组的重链序列：SEQ ID NO:30、31、75-92和117。在一些实施方案中，抗-因子D抗体是修饰版本的“SIESD”变体(AFD.v8)，其包含SEQ ID NO:26的轻链序列和具有选自由以下组成的组的氨基酸序列的重链：SEQ ID NO:30、31、75-92和117。在另一个实施方案中，抗-因子D抗体是修饰版本的“SIESD.N103S”变体(AFD.v14)，其包含SEQ ID NO:19的轻链可变结构域氨基酸序列和具有选自由以下组成的组的氨基酸序列的重链序列：SEQ ID NO:32、33、93-110和118。在一些实施方案中，抗-因子D抗体是修饰版本的“SIESD.N103S”变体(AFD.v14)，其包含SEQ ID NO:28的轻链序列和选自由以下组成的组的重链序列：SEQ ID NO:32、33、93-110和118。

[0054] 在一些方面，本发明涉及包含一个或多个抗-因子D抗体的缀合物，所述抗-因子D抗体具有可变轻链和可变重链，所述可变轻链包含具有SEQ ID NO:11或14的序列的HVR-

L1,具有SEQ ID NO:6的序列的HVR-L2,和具有SEQ ID NO:7或13的序列的HVR-L3;所述可变重链包含具有SEQ ID NO:8的序列的HVR-H1,具有SEQ ID NO:9或12的序列的HVR-H2,和具有SEQ ID NO:10或15的序列的HVR-H3。在另一个实施方案中,抗-因子D抗体还可以包含选自由以下组成的组的重链恒定结构域氨基酸序列:SEQ ID NO:54-74和116。例如,抗-因子D抗体可以是包含以下六个HVR序列的"SIESD"变体(AFD.v8):HVR-L1(SEQ ID NO:14),HVR-L2(SEQ ID NO:6),HVR-L3(SEQ ID NO:7),HVR-H1(SEQ ID NO:8),HVR-H2(SEQ ID NO:12),和HVR-H3(SEQ ID NO:10);或包含以下六个HVR序列的"SIESD.N103S"变体(AFD.v14):HVR-L1(SEQ ID NO:14),HVR-L2(SEQ ID NO:6),HVR-L3(SEQ ID NO:7),HVR-H1(SEQ ID NO:8),HVR-H2(SEQ ID NO:12),和HVR-H3(SEQ ID NO:15)。在一些实施方案中,抗-因子D抗体可以是包含以下六个HVR序列的修饰版本的"SIESD"变体(AFD.v8):HVR-L1(SEQ ID NO:14),HVR-L2(SEQ ID NO:6),HVR-L3(SEQ ID NO:7),HVR-H1(SEQ ID NO:8),HVR-H2(SEQ ID NO:12),和HVR-H3(SEQ ID NO:10),并且其进一步包含选自由以下组成的组的重链恒定结构域氨基酸序列:SEQ ID NO:55-74和116。在另一个实施方案中,抗-因子D抗体可以是包含以下六个HVR序列的修饰版本的"SIESD.N103S"变体(AFD.v14):HVR-L1(SEQ ID NO:14),HVR-L2(SEQ ID NO:6),HVR-L3(SEQ ID NO:7),HVR-H1(SEQ ID NO:8),HVR-H2(SEQ ID NO:12),和HVR-H3(SEQ ID NO:15),并且其进一步包含选自由以下组成的组的重链恒定结构域氨基酸序列:SEQ ID NO:55-74和116。

[0055] 在一些方面,本发明涉及包含不具有可检测的Asp异构化的抗-因子D抗体变体的缀合物,其中所述变体通过用于除去或减少异构化的方法制备,所述方法包括:(a)鉴定在参比抗-因子D抗体的HVR内的一个或多个倾向于Asp异构化的Asp残基;(b)用Glu置换步骤(a)中鉴定的Asp残基;(c)针对Asp异构化筛选所得的候选变体;和(d)选择不具有可检测的Asp异构化的那些变体。在一些方面,将以上方法与用于除去或减少脱酰胺的方法相结合,所述方法包括(a)鉴定在参比抗-因子D抗体的HVR内的一个或多个倾向于脱酰胺的Asn残基;(b)用Ser置换步骤(a)中鉴定的Asn残基;(c)针对脱酰胺筛选所得的候选变体;和(d)选择具有减少的或消除的脱酰胺的那些变体。在另一个方面,将用于除去或减少异构化的方法与用于减少抗体总体电荷的方法相结合,所述方法是通过:(a)选择在参比抗-因子D抗体的HVR内的一个或多个带负电的氨基酸残基D或E;(b)用Ser置换步骤(a)中选择的残基;(c)针对溶解性筛选所得的候选变体;和(d)选择与所述参比抗-因子D抗体相比具有改善的溶解性的那些变体。

[0056] 在一些方面,本发明涉及包含本文公开的一种或多种抗-因子D抗体或抗体变体和一种或多种多臂多元醇的缀合物,其中所述缀合物通过将至少一个本文中公开的抗-因子D抗体或抗体变体共价连接至多元醇来制备。在一些实施方案中,多臂多元醇是PEG。在一些实施方案中,PEG是八聚体。在一些实施方案中,PEG具有通式(Ia)、(Ib)、(IIa)、(IIIa)或(IVa)的结构(如本文中所示):

[0057] 在一些方面,当与所述参比抗-因子D抗体相比时,本发明的缀合物包含在保持因子D结合亲和力的同时具有改善的稳定性的抗-因子D抗体变体。在一些方面,抗体以至少约10^{-9}至10^{-12}M的结合亲和力结合因子D。在一些方面,用于本发明的缀合物的抗体包括人抗体、人源化抗体或嵌合抗体。

[0058] 在一些方面,用于本发明的缀合物的抗体是抗体片段(例如抗原结合片段)。本发

明的抗体片段可以是例如Fab、Fab'、F(ab')$_2$、scFv、(scFv)$_2$、dAb、互补决定区(CDR)片段、线性抗体、单链抗体分子、微抗体、双抗体、或由抗体片段形成的多特异性抗体。

[0059] 在本发明的其他方面，本发明包括组合物，所述组合物包含本发明的缀合物。在另一个方面，本发明涉及物质的组合物，所述物质的组合物包含与载体结合的如本文所述的本发明的缀合物。任选地，所述载体是药用载体。

[0060] 在一些方面，本发明包括药物制剂，所述药物制剂包含治疗有效浓度的本文所述的缀合物。在一些方面，所述药物制剂包含至少约100mg/mL、约100至约150mg/mL、约100至约200mg/mL、约100至约300mg/mL、约100至约400mg/mL、约100至约500mg/ml；至少约200mg/mL、至少约300mg/mL、至少约400mg/mL或至少约500mg/mL的浓度的抗体或抗体变体。在一些方面，制剂中抗体或抗体变体的浓度为约200、250、300、350、400、450或500mg/mL。在一些方面，制剂中抗体或抗体变体的浓度小于约450mg/mL。

[0061] 本发明的另一个方面是本发明的缀合物或药物制剂用于治疗与过度或不受控的补体激活相关的病症的用途。在一个实施方案中，本发明涉及治疗受试者中的补体相关性病症的方法，所述方法包括向所述受试者施用本发明的缀合物或药物制剂。所述病症包括心肺分流术期间的补体激活；由急性心肌梗死、动脉瘤、卒中、出血性休克、挤压伤、多器官衰竭、低血容量性休克、肠缺血或其它引起缺血的事件之后的缺血-再灌注引起的补体激活。补体激活还已经被证明与炎症病症相关，所述炎症病症诸如严重烧伤、内毒素血症、脓毒性休克、成人呼吸窘迫综合征、血液透析；过敏性休克、严重哮喘、血管性水肿、克罗恩病(Crohn's disease)、镰状细胞性贫血、链球菌感染后肾小球肾炎和胰腺炎。所述病况可以是不良药物反应、药物变态反应、IL-2诱导的血管渗漏综合征或放射摄影造影剂变态反应的结果。在一个实施方案中，补体相关性病症是系统性的。其还可以包括自身免疫疾病，诸如系统性红斑狼疮、重症肌无力、类风湿性关节炎、阿尔茨海默病(Alzheimer's disease)和多发性硬化。在另一个实施方案中，补体激活还与移植排斥相关。在另一个实施方案中，补体激活还与眼病(病理学涉及补体(包括补体的经典和旁路途径)的所有眼部病况和疾病)或补体相关性眼部病况相关，诸如，例如，不限于，黄斑变性病，诸如所有阶段的年龄相关性黄斑变性(AMD)，包括干性和湿性(非渗出性和渗出性)形式，糖尿病性视网膜病和其它缺血相关性视网膜病，脉络膜新血管形成(CNV)，葡萄膜炎，糖尿病性黄斑水肿，病理性近视，希佩尔-林道病(von Hippel-Lindau disease)，眼的组织胞浆菌病，视网膜中央静脉阻塞(CRVO)，角膜新血管形成和视网膜新血管形成。在一个实例中，补体相关性眼部病况包括年龄相关性黄斑变性(AMD)，包括非渗出性(例如中期干性AMD或地图状萎缩(GA))和渗出性(例如湿性AMD(脉络膜新血管形成(CNV))AMD，糖尿病性视网膜病(DR)，眼内炎和葡萄膜炎。在另外的实例中，非渗出性AMD可以包括硬玻璃疣、软玻璃疣、地图状萎缩和/或色素凝集的存在。在另一个实例中，补体相关性眼部病况包括年龄相关性黄斑变性(AMD)，包括早期AMD(例如包括多个小的至一个或多个非渗出性的中等大小的玻璃疣)、中期AMD(例如包括大量的中等玻璃疣至一个或多个大玻璃疣)和晚期AMD(例如包括地图状萎缩或晚期湿性AMD(CNV)。在另外的实例中，中期干性AMD可以包括大的汇合的玻璃疣。在另外的实例中，地图状萎缩可以包括光感受器和/或视网膜色素上皮(RPE)损失。在另外的实例中，地图状萎缩的面积可以是小的或大的和/或可以是在黄斑区域中或在外围视网膜中。在一个实例中，补体相关性眼部病况是中期干性AMD。在一个实例中，补体相关性眼部病况是地图状萎缩。在

一个实例中，补体相关性眼部病况是湿性AMD(脉络膜新血管形成(CNV))。在一个实施方案中，使用可植入端口递送系统施用缀合物或药物制剂。在一个实施方案中，通过玻璃体内施用来施用缀合物或药物制剂。在一个实施方案中，所述方法或用途还包括向受试者施用另外的治疗剂，诸如HTRA1拮抗剂，ANG2拮抗剂，TIE2拮抗剂，VEGF拮抗剂，或C1、C2、C3、C4、C5、C6、C7、C8或C9补体组分中一个或多个的拮抗剂。

[0062] 在另一个方面，本发明提供试剂盒，所述试剂盒包含本发明的缀合物。在一些实施方案中，本发明提供试剂盒，所述试剂盒包含本发明的缀合物及使用说明。在一些实施方案中，本发明涉及试剂盒，所述试剂盒包含本发明的缀合物以及用于施用所述缀合物以治疗补体相关性病症的说明。在一些实施方案中，本发明提供试剂盒，所述试剂盒包含第一容器，所述第一容器包含组合物，所述组合物包含一种或多种本发明的缀合物；和第二容器，所述第二容器包含缓冲剂。在一些实施方案中，所述缓冲剂是药用的。在一些实施方案中，包含本发明的缀合物的组合物还包含载体，在一些实施方案中，所述载体是药用的。在一些实施方案中，所述试剂盒还包含用于将所述组合物(例如，包含一个或多个抗体或其抗体片段(例如抗原结合片段)的缀合物)施用于受试者的说明。在一些实施方案中，所述试剂盒还包含使用所述试剂盒的说明。

[0063] 在一些方面，本发明涉及制品，所述制品含有可用于治疗、预防和/或诊断补体相关性病症的材料。在一些实施方案中，本发明涉及制品，所述制品包含：(a)容器；(b)容器上的标签；和(c)用所述容器盛放的物质组合物，所述物质组合物包含本发明的缀合物，其中所述容器上的标签指示所述组合物可以用于治疗、预防和/或诊断补体相关性病症。

[0064] 在一些方面，本发明提供本发明的缀合物在制备药物中的用途，所述药物用于疾病(诸如补体相关性眼部病况)的治疗性和/或预防性治疗。在一个实施方案中，本发明涉及治疗受试者中的补体相关性病症(诸如补体相关性眼部病况)的方法，所述方法包括向所述受试者施用本发明的缀合物或药物制剂。在一些实施方案中，补体相关性眼部病况选自年龄相关性黄斑变性(AMD)，包括非渗出性(例如中期干性AMD或地图状萎缩(GA))和渗出性(例如湿性AMD(脉络膜新血管形成(CNV))AMD，糖尿病性视网膜病(DR)，眼内炎和葡萄膜炎。在一个实例中，补体相关性眼部病况是中期干性AMD。在一个实例中，补体相关性眼部病况是地图状萎缩。在一个实例中，补体相关性眼部病况是湿性AMD(脉络膜新血管形成(CNV))。

[0065] 在一些方面，本发明提供本发明的制品在制备药物中的用途，所述药物用于疾病(诸如补体相关性眼部病况)的治疗性和/或预防性治疗。在一些实施方案中，补体相关性眼部病况选自年龄相关性黄斑变性(AMD)，包括非渗出性(例如中期干性AMD或地图状萎缩(GA))和渗出性(例如湿性AMD(脉络膜新血管形成(CNV))AMD，糖尿病性视网膜病(DR)，眼内炎和葡萄膜炎。在一个实例中，补体相关性眼部病况是中期干性AMD。在一个实例中，补体相关性眼部病况是地图状萎缩。在一个实例中，补体相关性眼部病况是湿性AMD(脉络膜新血管形成(CNV))。

[0066] 在一些方面，本发明提供本发明的试剂盒在制备药物中的用途，所述药物用于疾病(诸如补体相关性眼部病况)的治疗性和/或预防性治疗。在一些实施方案中，补体相关性眼部病况选自年龄相关性黄斑变性(AMD)，包括非渗出性(例如中期干性AMD或地图状萎缩(GA))和渗出性(例如湿性AMD(脉络膜新血管形成(CNV))AMD，糖尿病性视网膜病(DR)，眼内炎和葡萄膜炎。在一个实例中，补体相关性眼部病况是中期干性AMD。在一个实例中，补体相

关性眼部病况是地图状萎缩。在一个实例中，补体相关性眼部病况是湿性AMD(脉络膜新血管形成(CNV))。

[0067] 在一些方面，本发明提供包含缀合物的制剂，所述缀合物包含一个或多个因子D拮抗剂，并且所述制剂还包含HTRA1拮抗剂，ANG2拮抗剂，TIE2拮抗剂，VEGF拮抗剂，或C1、C2、C3、C4、C5、C6、C7、C8和C9补体组分中一个或多个的拮抗剂。在一些实施方案中，因子D拮抗剂是抗-因子D抗体。在另外的实施方案中，抗-因子D抗体是本文所述的抗-因子D抗体变体。在一些实施方案中，HTRA1拮抗剂是抗-HTRA1抗体。在另一个实施方案中，ANG2拮抗剂是抗-ANG2抗体。在另一个实施方案中，TIE2拮抗剂是抗-TIE2抗体。在另一个实施方案中，VEGF拮抗剂是抗-VEGF抗体。在另一个实施方案中，C2和/或C4和/或C5补体组分的拮抗剂是抗-C2和/或抗-C4和/或抗-C5抗体。

[0068] 在一些方面，治疗患有与过度或不受控的补体激活相关的病症的人受试者中的与过度或不受控的补体激活相关的病症包括向所述受试者施用有效量的治疗化合物，诸如包含一个或多个因子D拮抗剂的缀合物，并且还包括向所述受试者施用有效量的第二治疗化合物，诸如HTRA1拮抗剂，ANG2拮抗剂，TIE2拮抗剂，VEGF拮抗剂，或C1、C2、C3、C4、C5、C6、C7、C8和C9补体组分中一个或多个的拮抗剂。在一些实施方案中，因子D拮抗剂是抗-因子D抗体。在一些实施方案中，抗-因子D抗体是本文所述的抗-因子D抗体变体。在一些实施方案中，HTRA1拮抗剂是抗-HTRA1抗体。在另一个实施方案中，ANG2拮抗剂是抗-ANG2抗体。在另一个实施方案中，TIE2拮抗剂是抗-TIE2抗体。在另一个实施方案中，VEGF拮抗剂是抗-VEGF抗体。在另一个实施方案中，C2和/或C4和/或C5补体组分的拮抗剂是抗-C2和/或抗-C4和/或抗-C5抗体。

[0069] 在一些方面，包含因子D拮抗剂的缀合物和任何第二治疗化合物的施用可以同时进行，例如，作为单个组合物或者作为两种以上的不同组合物，使用相同或不同的施用途径。备选地或另外地，所述施用可以以任何顺序相继进行。

[0070] 附图简述

[0071] 图1A-1D显示了参比抗-因子D抗体WT(aFD.WT)及其选择变体的氨基酸序列(1A:WT的轻链和重链序列;1B:轻链和重链可变结构域的比对;1C:SIESD(AFD.v8)的轻链和重链序列，以及Cys修饰的SIESD(AFD.v8)和Cys-Pro-Pro-Cys-修饰的SIESD(AFD.v8)的重链序列;1D:SIESD.N103S(AFD.v14)的轻链和重链序列，以及Cys修饰的SIESD.N103S(AFD.v14)和Cys-Pro-Pro-Cys-修饰的SIESD.N103S(AFD.v14))的重链序列)。可变结构域内的HVR是加下划线的。变体中的残基置换以粗体显示。在图1C和1D中，Cys和Cys-Pro-Pro-Cys(SEQ ID NO:21)修饰以斜体显示。

[0072] 图2A-AC显示在延长的时间内在限定的条件下不同抗体Fab片段的抗原结合能力(2A:10mg/mL的Fab蛋白浓度，在pH 5.5缓冲剂中;2B:100mg/ml的Fab蛋白浓度，在PBS中;2C:100mg/ml的Fab蛋白浓度，在PBS中)。

[0073] 图3A-3B显示在限定的条件下不同抗体Fab片段随时间的降解，由此通过离子交换色谱法(IEC)确定主峰(3A:10mg/mL的Fab蛋白浓度，在pH 5.5缓冲剂中;3B:100mg/ml的Fab蛋白浓度，在PBS中)。

[0074] 图4A-4B显示在限定的条件下不同抗体Fab片段随时间的异构化和脱酰胺(4A:10mg/mL的Fab蛋白浓度，在pH 5.5缓冲剂中;4B:100mg/ml的Fab蛋白浓度，在PBS中)。

[0075] 图5显示在限定的条件下在延长的时间内不同抗体Fab片段的聚集(100mg/ml的Fab蛋白浓度,在PBS中),如通过分子排阻色谱法(SEC)测量单体峰所确定的。

[0076] 图6显示aFD WT、AFD.v2、AFD.v6和AFD.v8在pH 6以及低离子强度(～100mg/ml,在20mM His-HCl,pH 6中)的溶解性。

[0077] 图7显示抗体Fab片段在pH 6和低离子强度(～100mg/ml,在20mM His-HCl,pH 6中)的溶解性。aFD.WT的不溶性通过经由透析交换到PBS(一种含盐(NaCl)的缓冲剂)中而转变。

[0078] 图8显示227mg/ml(aFD.WT),269mg/ml(AFD.v8)和344mg/ml(AFD.v14)的抗体Fab片段在PBS(pH 7.3)中的溶解性。

[0079] 图9显示在2-8℃温育3周前通过分子排阻色谱法(SEC)测量的SIESD.N103S(AFD.v14)在PBS中的%聚集。

[0080] 图10A显示在37℃在热应力下在延长的时间内高浓度(272mg/mL)AFD.v8制剂(20mM His-HCl,pH 5.5)的抗原结合能力。画有阴影线的面积表示测量中±10%的标准误差。

[0081] 图10B显示在37℃在热应力下在延长的时间内高浓度(272mg/mL)AFD.v8制剂(20mM His-HCl,pH 5.5)的化学和物理稳定性。N101和E95根据Kabat编号。

[0082] 图11显示在兔中进行玻璃体内注射后抗体Fab片段的药物动力学。

[0083] 图12显示在pH 5.5缓冲剂中的抗体Fab片段的粘度的蛋白浓度依赖性。

[0084] 图13A和13B显示了包含六甘油(HGEO)核心(Sunbright®HGEO-400MA,NOF America,Corp.)和三季戊四醇(TP)核心(8ARM(TP)-PEG-MAL,JenKem Technology,USA)的多臂PEG的MALDI分析(13A:HGEO核心;13B:TP核心)。

[0085] 图14A-14C显示了在20mM His-乙酸盐,pH 5.5,50mM NaCl(等度梯度)在Sephacryl S-300HR(GE Healthcare)柱上通过尺寸排阻色谱法(SEC)纯化AFD.v14.C+TP八聚体的结果(14A:SEC柱的初始色谱图;14B:600mL至1100mL的峰的放大;14C:14B所显示的纯化期间收集的色谱级分的MALS谱)。

[0086] 图15A-14C显示了在20mM His-乙酸盐,pH 5.5,50mM NaCl(等度梯度)在Sephacryl S-300HR(GE Healthcare)柱上通过SEC纯化AFD.v14.C+HG八聚体的结果(15A:SEC柱的初始色谱图;15B:2900-3600mL的15A色谱图的放大;15C:图15B所显示的纯化期间收集的色谱级分的MALS谱)。

[0087] 图16A-16B显示了在PBS(pH 7.4)中使用Sephacryl S-400HR(GE Healthcare)柱分析AFD.v14.C+HG八聚体的结果(16A:柱的初始色谱图;16B:图16A色谱图的MALS谱)。

[0088] 图17A-17B显示了在20mM His-乙酸盐,pH 5.5,50mM NaCl中(等度梯度)在Sephacryl S-300HR(GE Healthcare)柱上通过SEC纯化AFD.v14.C+HGEO八聚体的结果,随后是PBS,pH 7.4中以0.25mL/分钟在Sephacryl S-400HR上的SEC-MALS表征(17A:SEC S-400柱的初始色谱图;17B:图17A色谱级分的MALS谱)。

[0089] 图18A-18B显示了在20mM His-乙酸盐,pH 5.5,50mM NaCl中(等度梯度)在Sephacryl S-300HR(GE Healthcare)柱上通过SEC纯化AFD.v14.C+HGEO八聚体的结果,随后是Tosoh G3000PW柱上的SEC-MALS表征(18A:SEC S-300柱的初始色谱图;18B:采用G3000PW柱使用SEC-MALS对18A的S-300级分的激光强度的重叠)。

[0090] 图19A-19C显示了在50CV内，在Triton洗液、10-20％1M NaCl梯度中，通过阳离子交换色谱法(CEX)进一步纯化实施例9a中的选择级分(图14所示)(19A:CEX柱的初始色谱图;19B:来自CEX柱的级分的SEC凝胶;19C:图19A中色谱级分的MALS谱)。

[0091] 图20显示了CEX纯化后AFD.v14.C+TP八聚体的最终分析运行。

[0092] 图21A-21B比较了阳离子交换色谱(CEX)、SEC S-400HR柱上的SEC色谱或SEC S-300HR柱上的SEC色谱之后，AFD.v14.C+TP八聚体的纯化方法的结果(21A:三种不同纯化柱的色谱的叠加展示;21B:比较来自三种不同纯化柱的样品的SEC凝胶)。

[0093] 图22A-22B比较了使用具有不同核心的PEG制备的PEG-Fab缀合物(22A:比较了具有不同核心的缀合物的纯化样品的SEC凝胶;22B:使用不同核心制备的缀合物的MALS谱)。

[0094] 图23显示了AFD.v14.C+HG八聚体和由来自NOF America Corp.的Sunbright® PTE-400MA(一种四聚体)制备的PEG-Fab缀合物的粘度作为AFD.v14浓度的函数。

[0095] 图24显示了在20℃，在20mL His-Ace,pH 6.5和50mM NaCl中，AFD.v14.C+TP八聚体和AFD.v14.C+HGEO八聚体的粘度作为AFD.v14浓度的函数。

[0096] 图25A和25B显示了在10mg/mL,PBS(25A)和10mg/mL的20mM组氨酸HCl,50mM NaCl,于pH 6.5(25B)，AFD.v14.C+TP八聚体的热稳定性作为时间的函数。

[0097] 图26和27显示了Fab片段的缓慢释放和热稳定性研究过程中的二聚化(26:随时间的缀合物的SEC-MALS分析;27:随时间的缀合物的CE-SDS分析)。

[0098] 图28显示了热稳定性期间AFD.v14.C+TP八聚体与因子D的结合能力的维持，如通过表面等离子共振所测量的。

[0099] 图29A和29B显示了在药代动力学研究中，在施用AFD.v14或AFD.v14.C+TP八聚体之后，AFD.v14在食蟹猴玻璃体液中的浓度(相对于时间)(29A:玻璃体液浓度;29B:针对给药强度标准化的玻璃体液浓度数据)。

[0100] 图30A和30B显示了在药代动力学研究中，在施用AFD.v14或AFD.v14.C+TP八聚体之后，AFD.v14在食蟹猴眼睛房水中的浓度(相对于时间)(30A:眼睛房水浓度;30B:针对给药强度标准化的眼睛房水浓度数据)。

[0101] 图31A和31B显示了在药代动力学研究中，在施用AFD.v14或AFD.v14.C+TP八聚体之后，AFD.v14在食蟹猴视网膜匀浆中的浓度(相对于时间)(31A:视网膜浓度;31B:针对给药强度标准化的视网膜浓度数据)。

[0102] 图32A-32C显示了在玻璃体内和静脉内注射施用的药代动力学研究中，在施用AFD.v14或AFD.v14.C+TP八聚体之后，AFD.v14在食蟹猴血浆中的浓度(相对于时间)(32A:玻璃体内注射的血浆浓度;32B:针对给药强度标准化的玻璃体内注射血浆浓度;32C:静脉内施用的血浆浓度)。

[0103] 图33A-33B显示了通过静脉内或玻璃体内注射施用AFD.v14.C+TP八聚体之后，在药代动力学研究中因子D浓度和AFD.v14.C+TP八聚体的比较(33A:血浆浓度;33B:眼部浓度)。

[0104] 图34A-34B显示了因子D依赖性因子B激活的时间分辨荧光能量转移(TR-FRET)测量的抑制曲线(34A:Fab-四聚体缀合物，与未缀合的Fab相比;34B:AFD.v14.C+TP八聚体，与未缀合的Fab相比)。

[0105] 图35A-35E显示了在玻璃体内注射施用之后，相比于食蟹猴血浆中的总因子D和治

疗剂浓度的系统性AP补体活性(35A:10mg/眼lampalizumab(对比数据);35B:25mg/眼AFD.v14;35C:3.9mg/眼AFD.v14.C+TP八聚体;35D:7.1mg/眼AFD.v14.C+HG八聚体;35E:11.8mg/眼AFD.v14.C+HG八聚体)。

[0106] 发明详述

[0107] 定义

[0108] 贯穿本申请使用的术语被解释为具有对本领域技术人员来说普通的和典型的含义。然而,申请人希望的是,以下术语被给予以下限定的具体定义。

[0109] 术语"抗体"以最广涵义使用,并且具体地涵盖全长单克隆抗体、多克隆抗体、多特异性抗体(例如,双特异性抗体)和抗体片段,只要它们显示所需的生物学活性(诸如抗原结合活性)即可。抗体(Ab)和免疫球蛋白(Ig)是具有相同结构特性的糖蛋白。抗体显示对特定靶标的结合特异性,而免疫球蛋白包括抗体和其它缺少靶标特异性的抗体样分子。天然抗体和免疫球蛋白通常是约150,000道尔顿的异源四聚体糖蛋白,由两条相同的轻(L)链和两条相同的重(H)链组成。每条重链在一端具有可变结构域(V_H),之后是若干个恒定结构域。每条轻链在一端具有可变结构域(V_L)而在其另一端具有恒定结构域。如本文所使用的术语"抗体"清楚地涵盖保持抗原结合活性的抗体片段。

[0110] "抗体片段"是指不同于完整抗体的包含完整抗体的一部分的分子,所述部分结合与完整抗体结合的抗原。抗体片段的实例包括但不限于Fv、Fab、Fab-SH、Fab'-SH、Fab'、Fab-C、Fab'-C、Fab'-C-SH、Fab-C-SH、scFv、双抗体(diabody)或$F(ab')_2$;双抗体;线性抗体;单链抗体分子(例如scFv);和由抗体片段形成的多特异性抗体。

[0111] 如本文所使用的,"抗-因子D抗体"意指以这样的方式特异性结合因子D的抗体,从而抑制或基本上减少补体激活。

[0112] 术语"因子D"用在本文中指天然序列和变体因子D多肽。

[0113] 如本文所使用的,术语"AFD.Ab"是指任何抗-因子D抗体。

[0114] 如本文所使用的,"Fab"是指这样的抗体:所述抗体包含重链恒定区,所述重链恒定区包含CH1结构域或CH1结构域的足够的部分以与轻链恒定区形成二硫键,但是不含有CH2结构域或CH3结构域。如本文所使用的,Fab可以包含铰链区的一个或多个氨基酸。因此,如本文所使用的,术语"Fab"涵盖Fab'抗体。Fab可以包含另外的非天然氨基酸,诸如C-末端半胱氨酸,在该情况下其可以称为Fab-C。如下讨论的,术语Fab-C还涵盖包含铰链区的天然氨基酸(包括C-末端处的天然半胱氨酸)的Fab。在一些实施方案中,Fab包含工程改造的半胱氨酸(即,Fab可以是THIOMAB)。

[0115] "Fab-C"是指具有C-末端半胱氨酸的Fab,所述半胱氨酸可以是在该残基位置出现的天然半胱氨酸(诸如来自铰链区的半胱氨酸),或者可以是添加到C-末端的半胱氨酸(其不对应于天然半胱氨酸)。抗-因子D抗体包括但不限于AFD.C抗体,其中"C"表示抗体是具有C-末端半胱氨酸的Fab。非限制性的示例性Fab-C重链恒定区包括SEQ ID NO:56、57、59、60、61、62、68和74的序列。

[0116] "Fab-SH"是指具有游离硫醇基团的Fab。在一些实施方案中,游离硫醇基团位于Fab的C-末端的最后10个氨基酸。Fab-C抗体通常也是Fab-SH抗体。另外的非限制性的示例性Fab-SH重链恒定区具有SEQ ID NO:58的氨基酸序列。通常,包含工程改造的半胱氨酸的Fab(即,其为THIOMAB的Fab)是Fab-SH。

[0117] 术语“可变区”或“可变结构域”是指参与抗体结合至抗原的抗体重链或轻链的结构域。天然抗体的重链和轻链的可变结构域(分别是VH和VL)通常具有相似的结构,各结构域包含四个保守的框架区(FR)和三个高变区(HVR)。(参见,例如Kindt等人Kuby Immunology,第6版,W.H.Freeman&Co.,第91页(2007))。单个VH结构域或VL结构域可以足以赋予抗原结合特异性。另外,结合特定抗原的抗体可以使用来自结合该抗原的抗体的VH或VL结构域进行分离,以分别筛选互补VL结构域或VH结构域的文库。参见,例如Portolano等人,J.Immunol.150:880-887(1993);Clarkson等人,Nature 352:624-628(1991)。

[0118] 术语“可变”是指这样的事实,即在抗体之间可变结构域的某些部分在序列上差异很大并且被用于各特定抗体对其特定抗原的结合和特异性。然而,可变性在抗体的可变结构域上并不是均匀分布的。其集中于在轻链和重链可变结构域中的三个被称为高变区的区段。可变结构域的更高度保守的部分被称为框架区(FR)。天然重链和轻链的可变结构域各自包含四个FR,其大多采取β折叠构型,通过三个高变区连接,所述三个高变区形成环连接,并且在一些情况下形成β折叠结构的部分。每条链中的高变区通过FR非常接近地保持在一起,并与另一条链的高变区一起促成抗体的抗原结合位点的形成(参见Kabat等人,Sequences of Proteins of Immunological Interest,第5版,Public Health Service,National Institutes of Health,Bethesda,Md.(1991))。恒定结构域不直接参与抗体与抗原的结合,但是展现出多种效应子功能,诸如在抗体依赖性细胞毒性(ADCC)中抗体的参与。

[0119] 用木瓜蛋白酶消化抗体产生称作“Fab”片段的两个相同的抗原结合片段(各自具有单个抗原结合位点)和一个残余“Fc”片段,其名称反映了它易于结晶的能力。木瓜蛋白酶处理产生具有两个抗原结合位点并且仍然能够交联抗原的F(ab′)2片段。

[0120] Fab片段还含有轻链的恒定结构域和重链的第一恒定结构域(CH1)。Fab’片段(包括Fab-C)与Fab片段的不同之处在于在重链CH1结构域的羧基末端添加了几个残基,包括来自抗体铰链区的一个或多个半胱氨酸。具有游离硫醇基团的抗体片段可以表示为具有“-SH”。Fab’-SH(包括Fab-C-SH)是其中恒定结构域的至少一个半胱氨酸残基携带游离硫醇基团的Fab的名称。F(ab′)2抗体片段最初是作为在它们之间具有铰链半胱氨酸的Fab’片段对而产生的。抗体片段的其它化学偶联也是已知的。

[0121] “Fv”是含有完整抗原识别和抗原结合位点的最小抗体片段。该区由一个重链可变结构域和一个轻链可变结构域以紧密的、非共价缔合的二聚体组成。在这种构型中,每个可变结构域的三个高变区相互作用从而限定在VH-VL二聚体的表面上的抗原结合位点。总而言之,六个高变区将抗原结合特异性赋予抗体。然而,即使单个可变结构域(或只包含对抗原具有特异性的三个高变区的Fv的一半)也具有识别和结合抗原的能力,虽然亲和性低于完整结合位点。

[0122] 如本文所使用的,术语“高变区”或“HVR”是指抗体可变结构域中序列上高度可变和/或形成结构确定的环(“高变环”)的各区域。通常,天然的四链抗体包含六个HVR;三个在VH中(H1、H2、H3)和三个在VL中(L1、L2、L3)。HVR通常包含来自高变环和/或来自“互补决定区”(CDR)的氨基酸残基,后者具有最高序列可变性和/或参与抗原识别。据信HVR-H3在赋予抗体优良的特异性方面发挥独特的作用。参见,例如,Xu等人(2000)Immunity 13:37-45;Johnson和Wu(2003)于Methods in Molecular Biology 248:1-25(Lo编,Human Press,

Totowa,N.J.)。“框架区”或“FR”残基是不同于如本文所限定的高变区残基的那些可变结构域残基。如本文所使用的,HVR区包含位于位置24-36(对于L1)、46-56(对于L2)、89-97(对于L3)、26-35B(对于H1)、47-65(对于H2)和93-102(对于H3)内的任意数目的残基。因此,HVR包括在前述位置中的残基:

[0123] A)24-34(L1),50-52(L2),91-96(L3),26-32(H1),53-55(H2),和96-101(H3)(Chothia和Lesk,J.Mol.Biol.196:901-917(1987);

[0124] B)L1的24-34,L2的50-56,L3的89-97,H1的31-35B,H2的50-65,和H3的95-102(Kabat等人,Sequences of Proteins of Immunological Interest,第5版,Public Health Service,National Institutes of Health,Bethesda,MD(1991)。

[0125] C)30-36(L1),46-55(L2),89-96(L3),30-35(H1),47-58(H2),93-100a-j(H3)(MacCallum等人J.Mol.Biol.262:732-745(1996)。

[0126] 高变区可以包含以下“延长的高变区”:在VL中的24-36或24-34(L1),46-56或50-56(L2)以及89-97(L3),和在VH中的26-35B(H1),50-65、47-65或49-65(H2)以及93-102、94-102或95-102(H3)。对于这些定义中的每个,可变结构域残基根据Kabat等人(见上)进行编号。

[0127] 除了VH中的CDR1以外,CDR通常包含形成高变环的氨基酸残基。CDR还包含“特异性决定残基”或“SDR”,其是接触抗原的残基。SDR被包含在CDR的被称为简短CDR(abbreviated-CDR)或a-CDR的区域内。示例性a-CDR(a-CDR-L1、a-CDR-L2、a-CDR-L3、a-CDR-H1、a-CDR-H2和a-CDR-H3)出现于L1的氨基酸残基31-34处、L2的氨基酸残基50-55处、L3的氨基酸残基89-96处、H1的氨基酸残基31-35B处、H2的氨基酸残基50-58处和H3的氨基酸残基95-102处。(参见Almagro和Fransson,Front.Biosci.13:1619-1633(2008))。

[0128] 参比抗体(也称为“起始抗体”或“亲本抗体”)的“抗体变体”或“修饰的抗体”是包含与参比/起始抗体的氨基酸序列不同的氨基酸序列的抗体,其中参比抗体的一个或多个氨基酸残基已被修饰。通常,抗体变体将与参比抗体具有至少80%序列同一性,优选地至少90%序列同一性,更优选地至少95%序列同一性,并且最优选地至少98%序列同一性。百分比序列同一性例如通过Fitch等人,Proc.Natl.Acad.Sci.USA,80:1382-1386(1983),Needleman等人,J.Mol.Biol.,48:443-453(1970)描述的算法版本,在将参比抗体的序列与候选抗体变体比对以提供最大同源性后确定。同一性或相似性在本文中被限定为,在对序列进行比对并且在需要的情况下引入间隙(gap)以实现最大百分比序列同一性后,候选变体序列中与亲本抗体残基相同(即相同的残基)或相似(即来自基于共有侧链性质的相同组的氨基酸残基,见下)的氨基酸残基的百分比。可以通过将适合的核苷酸改变引入到编码抗体的DNA或者通过肽合成来制备抗体的氨基酸序列变体。这种变体包括,例如,从目的抗体的氨基酸序列内的残基缺失,和/或向目的抗体的氨基酸序列内的残基插入,和/或目的抗体的氨基酸序列内的残基置换。可以产生缺失、插入和置换的任意组合以实现最终构建体,条件是所述最终构建体拥有所需特征。氨基酸变化也可以改变抗体的翻译后加工,诸如改变糖基化位点的数量或位置。用于产生抗体的抗体序列变体的方法类似于例如美国专利号5,534,615中描述的用于产生多肽的氨基酸序列变体的那些方法,所述专利通过引用清楚地结合于此。

[0129] 如果包括抗体在内的蛋白质基本上保持完整的构象结构和生物学活性,则称其为

“稳定的”。多种用于测量蛋白质稳定性的分析技术是本领域中可获得的并且综述于例如Peptide and Protein Drug Delivery,247-301,Vincent Lee Ed.,Marcel Dekker,Inc.,New York,N.Y.,Pubs.(1991)和Jones(1993)Adv.Drug Delivery Rev.10:29-90中。具有“改善的稳定性”的抗体变体是指与起始参比抗体相比更稳定的抗体变体。优选地,具有改善的稳定性的抗体变体是天然(野生型)抗体的变体,其中特定的氨基酸残基被改变以用于改善天然抗体的物理稳定性、和/或化学稳定性、和/或生物学活性、和/或降低其免疫原性。Walsh(2000)Nat.Biotech.18:831-3。

[0130] 术语“异构化”通常是指这样一种化学过程,通过所述化学过程化学化合物被转化为其异构形式,即,具有相同的化学组成但是具有不同的结构或构型并且因此通常具有不同的物理和化学性质的形式。本文中具体使用的是天冬氨酸异构化,其是这样的过程,其中多肽的一个或多个天冬氨酸(D或Asp)残基被转化为异天冬氨酸残基。Geiger和Clarke(1987)J.Biol.Chem.262:785-94。

[0131] 术语“脱酰胺”通常是指这样的化学反应,其中酰胺官能团被从有机化合物除去。本文中具体使用的是天冬酰胺脱酰胺,其是这样的过程,其中多肽的一个或多个天冬酰胺(N或Asn)残基被转化为天冬氨酸(D或Asp),即中性酰胺侧链被转化为整体具有酸性的残基。Xie和Schowen(1999)J.Pharm.Sci.88:8-13。

[0132] 氨基酸残基“倾向”于某些鉴定的物理或化学过程(例如,异构化或脱酰胺)是指特定蛋白质分子内被鉴定为具有进行鉴定的过程(诸如异构化或脱酰胺)的倾向的那些残基。它们的倾向通常通过它们在蛋白质的一级和/或构象结构内的相对位置确定。例如,已经证明,Asp-XXX基序(其中XXX可以是Asp、Gly、His、Ser或Thr)中的第一个Asp由于其相邻残基的参与而倾向于Asp异构化,其中相同蛋白质内的一些其它Asp可能不具有这种倾向。用于在具体蛋白质分子内鉴定残基对某些过程的倾向性的测定是本领域中已知的。参见,例如,Cacia等人(1996)Biochem.35:1897-1903。

[0133] 在本发明的抗-因子D抗体的语境中,“活性的”或“活性”或“生物活性”是对抗(部分或完全抑制)因子D的生物活性的能力。因子D拮抗剂的生物活性的一个实例是实现可测量的因子D相关疾病或病症(诸如例如补体相关性眼部病症)的状态(例如病理学)的改善的能力。可以在体外或体内测试中使用相关动物模型或人临床试验来确定活性,所述测试包括结合测定、备选途径溶血测定(例如测量备选途径补体活性或激活的抑制的测定)。

[0134] 术语“补体相关性病症”以最广意义使用并且包括与过度或不受控的补体激活相关的病症。其包括心肺分流术期间的补体激活;由急性心肌梗死、动脉瘤、卒中、出血性休克、挤压伤、多器官衰竭、低血容量性休克、肠缺血或其它引起缺血的事件之后的缺血-再灌注引起的补体激活。补体激活还已经被证明与炎症病症相关,所述炎症病症诸如严重烧伤、内毒素血症、脓毒性休克、成人呼吸窘迫综合征、血液透析;过敏性休克、严重哮喘、血管性水肿、克罗恩病(Crohn’s disease)、镰状细胞性贫血、链球菌感染后肾小球肾炎和胰腺炎。所述病况可以是不良药物反应、药物变态反应、IL-2诱导的血管渗漏综合征或放射摄影造影剂变态反应的结果。其还包括自身免疫疾病,诸如系统性红斑狼疮、重症肌无力、类风湿性关节炎、阿尔茨海默病(Alzheimer’s disease)和多发性硬化。补体激活还与移植排斥相关。补体激活还与眼病相关,所述眼病诸如年龄相关性黄斑变性、糖尿病性视网膜病和其它缺血相关性视网膜病、脉络膜新血管形成(CNV)、葡萄膜炎、糖尿病性黄斑水肿、病理性近

视、希佩尔-林道病、眼的组织胞浆菌病、视网膜中央静脉阻塞(CRVO)、角膜新血管形成和视网膜新血管形成。

[0135] 术语“补体相关性眼部病症”以最广意义使用并且包括病理学涉及补体(包括经典和旁路途径,并且特别是补体的旁路途径)的所有眼部病症。补体相关性眼部病症包括,但不限于,黄斑变性病(诸如所有阶段的年龄相关性黄斑变性(AMD),包括干性和湿性(非渗出性和渗出性)形式),脉络膜新血管形成(CNV),葡萄膜炎,糖尿病性和其它缺血相关性视网膜病,以及其它眼内新生血管性疾病,诸如糖尿病性黄斑水肿、病理性近视、希佩尔-林道病、眼的组织胞浆菌病、视网膜中央静脉阻塞(CRVO),角膜新血管形成和视网膜新血管形成。在一个实例中,补体相关性眼部病症包括年龄相关性黄斑变性(AMD),包括非渗出性(例如中期干性AMD或地图状萎缩(GA))和渗出性(例如湿性AMD(脉络膜新血管形成(CNV))AMD,糖尿病性视网膜病(DR),眼内炎和葡萄膜炎。在另外的实例中,非渗出性AMD可以包括硬玻璃疣、软玻璃疣、地图状萎缩和/或色素凝集的存在。在一个实例中,补体相关性眼部病症包括年龄相关性黄斑变性(AMD),包括早期AMD(例如包括多个小的至一个或多个非渗出性的中等大小的玻璃疣)、中期AMD(例如包括大量的中等玻璃疣至一个或多个大玻璃疣)和晚期AMD(例如包括地图状萎缩或晚期湿性AMD(CNV)。(Ferris等人,AREDS Report No.18;Sallo等人,Eye Res.,34(3):238-40(2009);Jager等人,New Engl.J.Med.,359(1):1735(2008))。在另外的实例中,中期干性AMD可以包括大的汇合的玻璃疣。在另外的实例中,地图状萎缩可以包括光感受器和/或视网膜色素上皮(RPE)损失。在另外的实例中,地图状萎缩的面积可以是小的或大的和/或可以是在黄斑区域中或在外围视网膜中。在一个实例中,补体相关性眼部病症是中期干性AMD。在一个实例中,补体相关性眼部病症是地图状萎缩。在一个实例中,补体相关性眼部病症是湿性AMD(脉络膜新血管形成(CNV))。

[0136] “治疗(treatment)”(及其语法上的变体,诸如“treat”或“treating”)是以阻止病症的病理学的发展或改变病症的病理学为目的而进行的干预。因此,“治疗”是指治疗性治疗和预防性或防预性的措施。需要治疗的那些包括已经患有病症的那些以及要预防病症的那些。所需的治疗效果包括,但不限于,防止疾病出现或复发,减轻症状,减小疾病的任何直接或间接病理学后果,降低病情进展速率,改善或缓和疾病状态,以及缓解或预后改善。在一些实施方案中,本发明的缀合物是用来延缓疾病发展或用来减慢疾病的进展。在免疫相关疾病的治疗中,治疗剂可以直接改变免疫反应的组分的反应程度,或者通过其它治疗剂(例如抗生素、抗真菌剂、消炎剂、化疗剂等)使疾病对治疗更敏感。

[0137] 疾病(诸如补体相关性眼部病症)的“病理学”包括危及患者健康的所有现象。这包括,但不限于,异常或不受控的细胞生长(嗜中性粒细胞、嗜酸性粒细胞、单核细胞、淋巴细胞)、抗体产生、自身抗体产生、补体产生、干扰邻近细胞的正常功能、以异常水平释放细胞因子或其它分泌性产物、抑制或加重任何炎症反应或免疫反应、炎症细胞(嗜中性粒细胞、嗜酸性粒细胞、单核细胞、淋巴细胞)渗入到细胞空间中等。

[0138] 如本文所使用的,术语“哺乳动物”是指被分类为哺乳动物的任何动物,包括但不限于人、高等灵长类、家养和农场动物,以及动物园动物、竞技动物或宠物,诸如马、猪、牛、狗、猫和雪貂等。在本发明的一些实施方案中,哺乳动物是人。

[0139] 与一种或多种另外的治疗剂“组合”施用包括同时(并行)施用和以任何次序的连续施用。

[0140] “治疗有效量”是实现可测量的目标疾病或病症(诸如例如补体相关性眼部病症)的状态(例如病理学)的改善所需要的“因子D拮抗剂”的量。

[0141] “氨基酸置换”是指将预先确定的氨基酸序列中的至少一个现有的氨基酸残基替换成另一种不同的“替代”氨基酸残基。替代残基可以是“天然存在的氨基酸残基”(即,由遗传密码编码的)并且选自由以下组成的组:丙氨酸(ala);精氨酸(Arg);天冬酰胺(Asn);天冬氨酸(Asp);半胱氨酸(Cys);谷氨酰胺(Gln);谷氨酸(Glu);甘氨酸(Gly),组氨酸(His);异亮氨酸(Ile);亮氨酸(Leu);赖氨酸(Lys);甲硫氨酸(Met);苯丙氨酸(Phe);脯氨酸(Pro);丝氨酸(Ser);苏氨酸(Thr);色氨酸(Trp);酪氨酸(Tyr);和缬氨酸(Val)。用一个或多个非天然存在的氨基酸残基进行的置换也被包括在本文中氨基酸置换的定义中。“非天然存在的氨基酸残基”是指不同于以上列出的那些天然存在的氨基酸残基的残基,其能够共价结合多肽链中的邻近氨基酸残基。非天然存在的氨基酸残基的实例包括正亮氨酸、鸟氨酸、正缬氨酸、高丝氨酸和其他氨基酸残基类似物如Ellman等人,Meth.Enzym,202:301-336(1991)中所述的那些。为了产生此种非天然存在的氨基酸残基,可以使用Noren等人,Science,244:182(1989)和Ellman等人(见上)的方法。简言之,这些方法包括化学活化具有非天然存在的氨基酸残基的抑制tRNA,之后体外转录和翻译所述RNA。

[0142] “氨基酸插入”是指将至少一个氨基酸结合到预先确定的氨基酸序列中。虽然插入通常由一个或两个氨基酸残基的插入组成,但是本申请预期更大的“肽插入”,例如插入约三至约五个或甚至多达约十个氨基酸残基。插入的残基可以是如以上公开的天然存在的或非天然存在的氨基酸残基。

[0143] “氨基酸缺失”是指从预先确定的氨基酸序列除去至少一个氨基酸残基。

[0144] 术语“长效递送”、“持续释放”和“受控释放”通常用于描述利用制剂、剂型、装置或其它类型的技术以实现治疗性药物的延长的或延展的释放或生物利用度的递送机制。其可以指向全身体循环或受试者或向受试者中的局部作用位点(包括但不限于细胞、组织、器官、关节、区域等)提供药物的延长的或延展的释放或生物利用度的技术。此外,这些术语可以指用于延长或延展药物从制剂或剂型的释放的技术,或者它们可以指用于延展或延长药物的生物利用度或药物动力学或对受试者的作用持续时间的技术,或者它们可以指用于延展或延长制剂引发的药物动力学效应的技术。“长效制剂”、“持续释放制剂”或“受控释放制剂”是用于提供长效递送的药物制剂、剂型或其它技术。在一些方面,受控释放用于改善药物的局部生物利用度,特别是在眼部递送的情况中,其用于改善眼部停留时间。“增加的眼部停留时间”是指递送后的时间段,在这期间递送的眼部药物在质量(活性)和数量(有效量)方面都保持有效。除了高剂量和受控释放以外或作为高剂量和受控释放的替代,药物可以被翻译后修饰(诸如经由PEG化),以实现增加的体内半衰期。

[0145] 术语“端口递送系统”是指具有可再填充的储器的用于眼睛的可植入装置,其允许在延长的时间段内递送治疗剂。示例性的端口递送系统描述于例如美国专利申请序列号2010/0174272,以及美国专利号8,277,830;8,399,006;8,795,712;和8,808,727中,所有这些都通过引用结合于此。

[0146] 当在本文中使用时,术语“多元醇”在广义上是指多羟基醇化合物。多元醇可以是例如任何水溶性聚(氧化烯)聚合物,并且能够具有直链或支链。优选的多元醇包括在一个或多个羟基位置处被化学基团(诸如,具有一至四个碳的烷基)取代的那些多元醇。典型地,

多元醇是聚(亚烷基二醇),优选聚乙二醇(PEG)。然而,本领域技术人员认识到可以使用本文描述的用于PEG的缀合技术来利用其它多元醇,诸如例如聚(丙二醇)和聚乙二醇-聚丙二醇共聚物。本发明公开内容的多元醇包括本领域熟知的那些和公开可获得的那些,诸如来自可商购的来源的那些。

[0147] 根据其最宽泛的定义,术语"缀合"在本文中用于意指结合或连接在一起。当分子如同结合在一起而起作用或操作时,分子是"缀合的"。在特别的实施方案中,"缀合物"是指与多臂多元醇共价结合的抗体(例如,如本文详述的抗体片段)。

[0148] "小孔径针"或"窄孔径针"是指用于注射流体组合物的约30、29、28、27、26、25、24、23或22号针或更高规格的针,诸如30号针。在一些实施方案中,小孔径针具有标准尺寸的壁。在另一个实施方案中,小孔径针具有薄壁,这对于粘稠溶液可能是优选的。

[0149] 相对于参比多肽序列的"百分比(%)氨基酸序列同一性"定义为在对序列进行比对并且在需要的情况下引入空位以实现最大百分比的序列同一性并且不考虑任何保守性置换作为序列同一性的一部分之后,候选序列中与参比多肽序列中的氨基酸残基相同的氨基酸残基的百分比。为了确定氨基酸序列同一性百分比的比对可以以本领域能力范围内的多种方式实现,例如,使用可公开获得的计算机软件诸如BLAST、BLAST-2、ALIGN或Megalign(DNASTAR)软件。本领域技术人员可以确定用于比对序列的适当参数,包括实现正在比较的全长序列范围内最大比对所需要的任何算法。然而,出于本文目的,使用序列比较计算机程序ALIGN-2产生氨基酸序列同一性%值。ALIGN-2序列比较计算机程序由Genentech,Inc.创作,并且源代码已经随用户文档提交至U.S.Copyright Office,Washington D.C.,20559(美国版权办公室华盛顿特区20559),其中它以美国版权登记号TXU510087登记。ALIGN-2程序从Genentech,Inc.,South San Francisco(南旧金山),California(加利福尼亚州)可公开获得或可以从源代码汇编。应当将ALIGN-2程序汇编用以在UNIX操作系统(包括数字式UNIX V4.0D)上使用。全部序列比较参数通过ALIGN-2程序设定并且不变动。

[0150] 在使用ALIGN-2进行氨基酸序列比较的情况下,如下计算给定的氨基酸序列A与、同或针对给定的氨基酸序列B的%氨基酸序列同一性(这可以备选地描述为给定的氨基酸序列A具有或包含与、同或针对给定的氨基酸序列B的某一%氨基酸序列同一性):

[0151] 100乘以分数X/Y,

[0152] 其中X是通过序列比对程序ALIGN-2在该程序的A和B比对中评定为相同匹配的氨基酸残基的数目,并且其中Y是B中氨基酸残基的总数。将可以理解的是,在氨基酸序列A的长度不等于氨基酸序列B的长度时,A相对于B的%氨基酸序列同一性将不等于B相对于A的%氨基酸序列同一性。除非另外特别声明,否则本文所用的全部%氨基酸序列同一性值如紧接前段中所述的使用ALIGN-2计算机程序获得。

[0153] 术语"药物制剂"是指这样的制备物,其处于这样的形式从而允许包含于其中的活性成分的生物活性有效,并且其不含有对于将施用该制剂的受试者不可接受地有毒的额外组分。

[0154] "药用载体"是指除活性成分之外,药物制剂中对受试者无毒的成分。药用载体包括但不限于缓冲液、赋形剂、稳定剂或防腐剂。

[0155] 与一种或多种其它药物"同时"施用的药物在相同的治疗周期期间施用,在与一种或多种其它药物的治疗的同一天施用,和任选地在与一种或多种其它药物的同一时间施

用。

[0156] 抗-因子D抗体及其变体

[0157] 在一些方面，本发明涉及包括一个或多个抗-因子D抗体或其变体的缀合物的制备和使用。适用于形成本发明的缀合物的抗-因子D抗体及其变体描述于美国专利申请序列号14/700853(2015年4月30日提交)中，其通过引用整体结合于此。

[0158] 在一些方面，形成用于产生用于本发明的缀合物的变体的碱基的亲本参比抗-因子D抗体是人源化抗-因子D抗体。用于将非人抗体人源化的方法是本领域公知的。通常，人源化抗体具有从非人来源引入其中的一个或多个氨基酸残基。这些非人氨基酸残基通常被称为“进口”残基，其典型地取自“进口”可变结构域。人源化可以基本上遵循Winter及同事(Jones等人(1986)Nature 321:522-525;Riechmann等人(1988)Nature 332:323-327;Verhoeyen等人(1988)Science 239:1534-1536)的方法，通过用啮齿动物CDR或CDR序列置换人抗体的相应序列进行。因此，这种”人源化”抗体是嵌合抗体(美国专利号4,816,567)，其中基本上少于完整人可变结构域被来自非人物种的相应序列置换。实际上，人源化抗体典型地是这样的人抗体，其中一些CDR残基和可能的一些FR残基被来自啮齿动物抗体中的类似位点的残基置换。

[0159] 在欲将抗体用于人治疗用途时，在一些情况下，用于制备人源化抗体的人可变结构域(轻链和重链两者)的选择可能对于降低抗原性和/或HAMA反应(人抗小鼠抗体)是重要的。减少或消除HAMA反应通常是临床开发合适的治疗剂的重要方面。参见，例如，Khaxzaeli等人(1988)J.Natl.Cancer Inst 80:937;Jaffers等人(1986)Transplantation 41:572;Shawler等人(1985)J.Immunol.135:1530;Sears等人(1984)J.Biol.Response Mod.3:138;Miller等人(1983)Blood 62:988;Hakimi等人(1991)J.Immunol.147:1352;Reichmann等人(1988)Nature 332:323;Junghans等人(1990)Cancer Res.50:1495。如本文所述，在一些方面，本发明提供缀合物，其包含被人源化从而减少或消除了HAMA反应的抗体。这些抗体的变体可以进一步使用本领域已知的常规方法获得，所述方法中的一些在下文中被进一步描述。根据所谓的“最佳-配合(best-fit)”方法，针对已知的人可变结构域序列的整个文库来筛选啮齿动物抗体的可变结构域的序列。鉴定与啮齿动物的V结构域序列最接近的人V结构域序列并且其中的人框架区(FR)被接受用于人源化抗体(Sims等人(1993)J.Immunol.151:2296;Chothia等人(1987)J.Mol.Biol.196:901)。另一种方法使用来源于所有特定轻链或重链亚组的人抗体的共有序列的特定框架区。相同的框架可以用于若干不同的人源化抗体(Carter等人(1992)Proc.Natl.Acad.Sci.USA 89:4285;Presta等人(1993)J.Immunol.151:2623)。

[0160] 例如，来自如本文描述的抗体的氨基酸序列可以充当用于框架和/或高变序列的多样化的起始(亲本)序列。选择的与起始高变序列相连的框架序列在本文中被称为受体人框架。虽然受体人框架可以来自或来源于人免疫球蛋白(其VL和/或VH区)，但是当这种框架被证明在人类患者中具有最小免疫原性或没有免疫原性时，受体人框架可以来自或来源于人共有框架序列。出于本文目的，“受体人框架”是这样的框架，其包含来源于人免疫球蛋白框架或来自人共有框架的VL或VH框架的氨基酸序列。“来源于”人免疫球蛋白框架或人共有框架的受体人框架可以包含其相同的氨基酸序列，或可以含有预先存在的氨基酸序列改变。当存在预先存在的氨基酸改变时，优选地存在不超过5个并且优选地4以下或3个以下预

先存在的氨基酸改变。在一些实施方案中，VH受体人框架在序列上与VH人免疫球蛋白框架序列或人共有框架序列相同。在一些实施方案中，VL受体人框架在序列上与VL人免疫球蛋白框架序列或人共有框架序列相同。“人共有框架”是这样的框架，其代表在人免疫球蛋白VL或VH框架序列的选择中最常出现的氨基酸残基。通常，人免疫球蛋白VL或VH框架序列的选项来自可变结构域序列的亚组。通常，所述序列亚组是如在Kabat等人中的亚组。在一些实施方案中，对于VL，所述亚组是如在Kabat等人中的亚组κI。在一些实施方案中，对于VH，所述亚组是如在Kabat等人中的亚组III。

[0161] 当受体来源于人免疫球蛋白时，可以在人框架序列的集合中任选地选择基于其与供体框架序列的同源性(通过将供体框架序列与不同人框架序列比对)选择的人框架序列，并且选择同源性最高的框架序列作为受体。受体人框架可以来自或来源于公众数据库中可获得的人抗体种系序列。

[0162] 在一些实施方案中，本文中的人共有框架来自或来源于VH亚组VII和/或VLκ亚组I共有框架序列。

[0163] 在一些实施方案中，用于产生抗-因子D抗体的人框架模板可以包含来自包含对于VH链的VI-4.1b+(VH7家族)和对于VL链的DPK4(VκI家族)和JK2的组合的模板的框架序列。

[0164] 虽然受体可以在序列上与选择的人框架序列相同而不论其是来自人免疫球蛋白还是人共有框架，但是本发明预计受体序列相对于所述人免疫球蛋白序列或人共有框架序列可以包含预先存在的氨基酸置换。这些预先存在的置换优选地是最少的；通常是相对于所述人免疫球蛋白序列或共有框架序列的四个、三个、两个或一个氨基酸差异。

[0165] 非人抗体的高变区残基被并入到VL和/或VH受体人框架中。例如，可以并入对应于Kabat CDR残基、Chothia高变环残基、Abm残基和/或接触残基的残基。任选地，并入如下的延长的高变区残基：24-36或24-34(L1)、46-56或50-56(L2)和89-97(L3)，26-35B(H1)，50-65、47-65或49-65(H2)和93-102、94-102或95-102(H3)。

[0166] 在一些方面，用于缀合物的抗-因子D抗体或抗体变体包含轻链结构域和重链可变结构域。在一些方面，参比抗-因子D抗体包含SEQ ID NO:3的轻链可变结构域。在一些方面，参比抗-因子D抗体包含SEQ ID NO:4的重链可变结构域。

[0167] 此外，抗-因子D抗体可以包含任何合适的恒定结构域序列，前提是抗体保持结合因子D的能力。例如，在一些实施方案中，用于本发明的缀合物的抗-因子D抗体包含至少部分的重链恒定结构域。在一些实施方案中，抗-因子D抗体包含α、δ、ε、γ或μ重链中的一个或其组合的重链恒定结构域。根据其重链恒定结构域(C_H)的氨基酸序列，可以将免疫球蛋白分配为不同的类别或同种型。存在五类免疫球蛋白：IgA、IgD、IgE、IgG和IgM，其重链分别被指定为α、δ、ε、γ和μ。基于在C_H序列和功能上相对小的差异，γ和α类被进一步划分为亚类，例如，人表达以下亚类：IgG1、IgG2、IgG3、IgG4、IgA1和IgA2。在一些实施方案中，用于本发明的缀合物的抗-因子D抗体包含重链恒定结构域，所述重链恒定结构域包含在对效应子功能(例如结合亲和力)产生所需影响的氨基酸位置处的置换。在一些实施方案中，用于本发明的缀合物的抗-因子D抗体包含重链恒定结构域，所述重链恒定结构域包含在对效应子功能(例如结合亲和力)不产生影响的氨基酸位置处的置换。在一些实施方案中，抗-因子D抗体包含IgG型(例如IgG1、IgG2、IgG3或IgG4)的重链恒定结构域并且进一步包含位置114(Kabat编号；相当于EU编号中的118)、168(Kabat编号；相当于EU编号中的172)、172(Kabat

编号;相当于EU编号中的176)和/或228(EU编号)处的置换。在一些实施方案中,抗-因子D抗体包含IgG(例如IgG1、IgG2、IgG3或IgG4)型的重链恒定结构域并且进一步包含位置114处的置换,其中位置114是半胱氨酸(C)或丙氨酸(A),位置168是半胱氨酸(C)或丙氨酸(A),位置172是半胱氨酸(C)或丙氨酸(A)和/或位置228是脯氨酸(P)、精氨酸(R)或丝氨酸(S)。

[0168] 此外,例如,在一些实施方案中,用于本发明的缀合物的抗-因子D抗体包含至少部分的轻链恒定结构域。在一些实施方案中,抗-因子D抗体包含κ或λ轻链中的一个或其组合的轻链恒定结构域,因为来自任何脊椎动物物种的轻链都可以基于其恒定结构域的氨基酸序列而被分配至两个明显不同的类别(称为κ和λ)中的一个。在一些实施方案中,用于本发明的缀合物的抗-因子D抗体包含轻链恒定结构域,所述轻链恒定结构域包含在对效应子功能(例如结合亲和力)产生所需影响的氨基酸位置处的置换。在一些实施方案中,用于本发明的缀合物的抗-因子D抗体包含轻链恒定结构域,所述轻链恒定结构域包含在对效应子功能(例如结合亲和力)不产生影响的氨基酸位置处的置换。在一些实施方案中,用于本发明的缀合物的抗-因子D抗体包含κ型的轻链恒定结构域并且进一步包含位置110、144、146和/或168(Kabat编号)处的置换。在一些实施方案中,用于本发明的缀合物的抗-因子D抗体包含κ型的轻链恒定结构域并且进一步包含位置110、位置144、位置146和/或位置168处的置换,其中110是半胱氨酸(C)或缬氨酸(V),144是半胱氨酸(C)或丙氨酸(A),146是异亮氨酸(I)或缬氨酸(V),168是半胱氨酸(C)或丝氨酸(S)。

[0169] 可以修饰包括人源化抗-因子D抗体在内的亲本或参比抗-因子D抗体以产生修饰的抗-因子D抗体或抗-因子D抗体变体。在一些实施方案中,修饰的抗-因子D抗体及其变体相比于亲本抗体可以具有改善的物理、化学、生物学或均一性性质。

[0170] 在一些实施方案中,用于本发明的缀合物的抗体的一个或多个氨基酸改变(例如置换)成亲本抗体高变区的一个或多个氨基酸。备选地或另外地,可以将框架区的一个或多个改变(例如置换)引入亲本抗体中。修饰的框架区残基的实例包括直接与抗原非共价结合(Amit等人,(1986)Science,233:747-753);与CDR相互作用/影响CDR的构象(Chothia等人(1987)J.Mol.Biol.,196:901-917)和/或参与V_L-V_H界面(EP 239 400B1)的那些。在某些实施方案中,一个或多个此种框架区残基的修饰导致抗体对抗原的结合亲和力增强。例如,在本发明的该实施方案中,约一个至约5个框架残基可以被改变。修饰的框架或HVR区残基的实例包括这样的位点,其中在所述位点处的修饰导致产生脱酰胺的变体(例如,天冬酰胺(N或Asn)残基被修饰成天冬氨酸(D或Asp),氧化变体(例如,甲硫氨酸(M或Met)残基和/或色氨酸(W或Trp)残基被修饰成砜或亚砜)或焦谷氨酸变体(例如,谷氨酰胺(Q或Gln)残基被修饰成焦谷氨酸)。修饰的框架区残基或HVR区残基的实例包括可能的脱酰胺位点(即,天冬酰胺(N或Asn))、氧化位点(即,甲硫氨酸(M或Met)或色氨酸(W或Trp))或焦谷氨酸转化位点(即,谷氨酰胺(Q或Gln)),其中这些位点处的修饰分别阻止脱酰胺作用和/或氧化和/或焦谷氨酸转化。

[0171] 为了防止形成脱酰胺变体,可以将天冬酰胺(N或Asn)突变成丙氨酸(A或Ala)、谷氨酰胺(Q或Gln)或丝氨酸(S或Ser)。为了防止形成氧化变体,可以将甲硫氨酸(Met)或色氨酸(W或Trp)突变成亮氨酸(L)或异亮氨酸(I)。为了防止形成焦谷氨酸变体,可以将谷氨酰胺(Q或Gln)突变成谷氨酸(E或Glu)。(Amphlett,G.等人,Pharm.Biotechnol.,9:1-140(1996))。备选地或另外地,框架区残基的一个或多个改变(例如置换)可以在亲本抗体的Fc

区中。

[0172] 一种可用于产生此种修饰抗体的方法被称为“丙氨酸扫描诱变”(Cunningham和Wells(1989)Science 244:1081-1085)。这里,一个或多个高变区残基被丙氨酸或聚丙氨酸残基代替从而影响氨基酸与抗原的相互作用。然后,通过在置换位点处或者针对置换位点引入另外的或其它突变来精修对置换显示功能敏感性的那些高变区残基。因此,虽然用于引入氨基酸序列变化的位点是预先确定的,但是突变本身的性质不需要预先确定。筛选以此方式产生的ala突变体的生物学活性(即结合亲和力或溶血测定),如本文所述。

[0173] 即使在抗体或其片段(例如抗原结合片段)中有更多的修饰,也可以通过选择对保持以下方面的影响显著不同的置换来实现生物学性质:(a)置换区域中多肽主链的结构,例如,呈折叠或螺旋构型,(b)分子在靶位点处的电荷或疏水性,或(c)侧链的大小。基于共有的侧链性质,天然存在的残基被划分成多组:

[0174] (1)疏水:正亮氨酸、met、ala、val、leu、ile;

[0175] (2)中性亲水:cys、ser、thr、asn、gln;

[0176] (3)酸性:asp、glu;

[0177] (4)碱性:his、lys、arg;

[0178] (5)影响链取向的残基:gly、pro;和

[0179] (6)芳香性:trp、tyr、phe。

[0180] 非保守性置换将需要将这些类别之一的成员交换为另一个类别的成员。

[0181] 在另一个实施方案中,修饰被选择用于修饰的位点,并且通过噬菌体展示选择具有提高的结合亲和力的那些修饰。

[0182] 通过本领域已知的多种方法来制备编码氨基酸序列突变体或修饰的氨基酸序列的核酸分子。这些方法包括但不限于对早期制备的变体或非变体形式的亲本抗体进行寡核苷酸介导的(或定点)诱变、PCR诱变和盒式诱变。一种用于制备突变体或变体或修饰的氨基酸序列的方法是定点诱变(参见,例如,Kunkel(1985)Proc.Natl.Acad.Sci.USA 82:488)。

[0183] 在某些实施方案中,修饰的抗体将仅具有单个置换的高变区残基。在其它实施方案中,亲本抗体的高变区残基中的两个或更多个将被置换,例如约两个至约十个高变区置换。通常,修饰的抗体的氨基酸序列与亲本抗体的重链可变结构域或轻链可变结构域的氨基酸序列具有至少75%,更优选至少80%,更优选至少85%,更优选至少90%,并且最优选至少95%的氨基酸序列同一性或相似性(以上在定义部分被定义)。

[0184] 在制备修饰的抗体后,确定所述分子相对于亲本抗体的生物学活性。如上所述,这可以包括确定抗体变体或其片段(例如抗原结合片段)的结合亲和力和/或其它生物学活性。在本发明的一些实施方案中,制备一系列修饰的抗体并筛选其对抗原如因子D或其片段的结合亲和力。任选地对选自该初筛的一个或多个抗体突变体或修饰的抗体进行一种或多种进一步的生物学活性测定以确认所述抗体变体或其片段(例如抗原结合片段)确实可用于例如临床前研究。

[0185] 可以对本文所述的修饰的抗-因子D抗体进行进一步修饰,通常这取决于修饰的抗体的目的用途。所述修饰可以包括氨基酸序列的进一步改变,与异源多肽融合和/或共价修饰如以下详述的那些。关于氨基酸序列改变,以上详述了示例性修饰。例如,也可以置换任何不涉及保持修饰的抗体的正确构象的半胱氨酸残基(通常用丝氨酸置换),以改善分子的

氧化稳定性和防止异常交联。相反地，可以向抗体中添加半胱氨酸键以改善其稳定性(特别是当抗体是抗体片段诸如Fv片段时)。

[0186] 另一类氨基酸突变体具有改变的糖基化模式。这可以通过以下实现：删除存在于抗体中的一个或多个碳水化合物部分，和/或添加抗体中不存在的一个或多个糖基化位点。抗体或抗体片段(例如抗原结合片段)的糖基化通常是N-连接的或O-连接的。N-连接是指碳水化合物部分连接于天冬酰胺残基的侧链。三肽序列天冬酰胺-X-丝氨酸和天冬酰胺-X-苏氨酸(其中X是除脯氨酸以外的任何氨基酸)是用于将碳水化合物部分酶促连接于天冬酰胺侧链的识别序列。因此，在多肽中存在这些三肽序列中的任一个产生了潜在的糖基化位点。O-连接的糖基化是指将糖N-乙酰半乳糖胺、半乳糖或木糖之一连接于羟基氨基酸，最常见的是丝氨酸或苏氨酸，但是也可以使用5-羟脯氨酸或5-羟赖氨酸。向抗体中添加糖基化位点可以通过改变氨基酸序列使得其含有一个或多个上述三肽序列而便利地完成(用于N-连接的糖基化位点)。还可以通过向原始抗体的序列中添加或置换一个或多个丝氨酸或苏氨酸残基来进行所述改变(用于O-连接的糖基化位点)。

[0187] 抗-因子D抗体及其变体的亲和力和生物活性

[0188] 针对理想性质如因子D-结合亲和力和因子D-抑制活性，可以在体外或体内筛选具有在本文中被鉴定为对于抗-因子D抗体来说理想的特性的抗体。

[0189] a.亲和力

[0190] 在一些方面，用于本发明的缀合物的抗-因子D抗体变体与其所来源于的亲本抗-因子D抗体竞争。还提供结合与亲本抗-因子D抗体相同的表位的抗-因子D抗体变体。

[0191] 为了确定抗-因子D抗体变体是否结合人因子D上的参比抗-因子D抗体所结合的相同表位，可以进行交叉阻断测定(Antibodies,ALaboratory Manual,Cold Spring Harbor Laboratory,Ed Harlow and David Lane(1988))。备选地，可以进行表位定位(mapping)以确定抗-因子D抗体是否结合目标表位(Champe等人(1995)J.Biol.Chem.270:1388-1394。抗体对例如人因子D的亲和力可以使用标准方法确定，所述方法包括实施例中更为详细描述的表面等离子共振(SPR)测定。

[0192] 在一些方面，用于本发明的缀合物的抗-因子D抗体变体的因子D结合亲和力与其所来源于的亲本抗-因子D抗体的因子D结合亲和力相当。在一些方面，用于本发明的缀合物的抗-因子D抗体变体的因子D结合亲和力在亲本抗-因子D抗体的因子D结合亲和力的10倍、7倍、5倍、2倍或1倍内。

[0193] 在一些实施方案中，本发明提供包含抗-因子D抗体的缀合物，其中单价形式的抗体对因子D的亲和力(例如，作为Fab片段的抗体对因子D的亲和力)为20nM($20x10^{-9}M$)或更好。在另一个实施方案中，本发明提供包含抗-因子D抗体的缀合物，其中单价形式的抗体对因子D的亲和力(例如，作为Fab片段的抗体对因子D的亲和力)为10nM($10x10^{-9}M$)或更好。在另一个实施方案中，本发明提供包含抗-因子D抗体的缀合物，其中单价形式的抗体对因子D的亲和力(例如，作为Fab片段的抗体对因子D的亲和力)为1.0nM($1.0x10^{-9}M$)或更好。在另一个实施方案中，本发明提供包含抗-因子D抗体的缀合物，其中单价形式的抗体对因子D的亲和力(例如，作为Fab片段的抗体对因子D的亲和力)为0.5nM($0.5x10^{-9}M$)或更好。在另一个实施方案中，本发明提供包含抗-因子D抗体的缀合物，其中单价形式的抗体对因子D的亲和力(例如，作为Fab片段的抗体对因子D的亲和力)为1.0pM($1.0x10^{-12}M$)或更好。在另一个实施

方案中，本发明提供包含抗-因子D抗体的缀合物，其中单价形式的抗体对因子D的亲和力(例如，作为Fab片段的抗体对因子D的亲和力)为0.5pM(0.5x10^{-12}M)或更好。

[0194] 在另一个实施方案中，本发明提供包含抗-因子D抗体的缀合物，其中二价形式的抗体对因子D的亲和力(例如，作为IgG的抗体对因子D的亲和力)为10.0nM(10.0x10^{-9}M)或更好。在另一个实施方案中，本发明提供包含抗-因子D抗体的缀合物，其中二价形式的抗体对因子D的亲和力(例如，作为IgG的抗体对因子D的亲和力)为5.0nM(5.0x10^{-9}M)或更好。在另一个实施方案中，本发明提供包含抗-因子D抗体的缀合物，其中二价形式的抗体对因子D的亲和力(例如，作为IgG的抗体对因子D的亲和力)为1.0nM(1.0x10^{-9}M)或更好。在另一个实施方案中，本发明提供包含抗-因子D抗体的缀合物，其中二价形式的抗体对因子D的亲和力(例如，作为IgG的抗体对因子D的亲和力)为0.5nM(0.5x10^{-9}M)或更好。在另一个实施方案中，本发明提供包含抗-因子D抗体的缀合物，其中二价形式的抗体对因子D的亲和力(例如，作为IgG的抗体对因子D的亲和力)为5.0pM(5.0x10^{-12}M)或更好。在另一个实施方案中，本发明提供包含抗-因子D抗体的缀合物，其中二价形式的抗体对因子D的亲和力(例如，作为IgG的抗体对因子D的亲和力)为2.0pM(2.0x10^{-12}M)或更好。在另一个实施方案中，本发明提供包含抗-因子D抗体的缀合物，其中二价形式的抗体对因子D的亲和力(例如，作为IgG的抗体对因子D的亲和力)为1.0pM(1.0x10^{-12}M)或更好。在另一个实施方案中，本发明提供包含抗-因子D抗体的缀合物，其中二价形式的抗体对因子D的亲和力(例如，作为IgG的抗体对因子D的亲和力)为0.5pM(0.5x10^{-12}M)或更好。

[0195] 在另一个实施方案中，本发明提供包含抗-因子D抗体的缀合物，其中单价形式的抗体对因子D的亲和力(例如，作为Fab片段的抗体对因子D的亲和力)为0.5mM(0.5x10^{-6}M)至0.5pM(0.5x10^{-12}M)。在另一个实施方案中，本发明提供包含抗-因子D抗体的缀合物，其中单价形式的抗体对因子D的亲和力(例如，作为Fab片段的抗体对因子D的亲和力)为15nM(15x10^{-9}M)至0.1nM(0.1x10^{-9}M)。在另一个实施方案中，本发明提供包含抗-因子D抗体的缀合物，其中单价形式的抗体对因子D的亲和力(例如，作为Fab片段的抗体对因子D的亲和力)为5.5nM(5.5x10^{-9}M)至1nM(1x10^{-9}M)。在另一个实施方案中，本发明提供包含抗-因子D抗体的缀合物，其中单价形式的抗体对因子D的亲和力(例如，作为Fab片段的抗体对因子D的亲和力)为0.5pM(0.5x10^{-12}M)至50pM(5x10^{-11}M)。

[0196] 在另一个实施方案中，本发明提供包含抗-因子D抗体的缀合物，其中二价形式的抗体对因子D的亲和力(例如，作为IgG的抗体对因子D的亲和力)为0.5mM(0.5x10^{-6}M)至0.5pM(0.5x10^{-12}M)。在另一个实施方案中，本发明提供包含抗-因子D抗体或其抗体变体的缀合物，其中二价形式的抗体对因子D的亲和力(例如，作为IgG的抗体对因子D的亲和力)为10nM(10x10^{-9}M)至0.05nM(0.05x10^{-9}M)。在另一个实施方案中，本发明提供包含抗-因子D抗体的缀合物，其中二价形式的抗体对因子D的亲和力(例如，作为IgG的抗体对因子D的亲和力)为5.5nM(5.5x10^{-9}M)至1nM(1x10^{-9}M)。在另一个实施方案中，本发明提供包含抗-因子D抗体的缀合物，其中二价形式的抗体对因子D的亲和力(例如，作为IgG的抗体对因子D的亲和力)为0.5pM(0.5x10^{-12}M)至50pM(5x10^{-11}M)。

[0197] 在另一个实施方案中，本发明提供包含抗-因子D抗体的缀合物，其中单价形式的抗体对因子D的亲和力(例如，作为Fab片段的抗体对因子D的亲和力)为约1.4pM(1.4x10^{-12}M)。在另一个实施方案中，本发明提供包含抗-因子D抗体的缀合物，其中二价形式的抗体

对因子D的亲和力(例如,作为IgG的抗体对因子D的亲和力)为约1.1pM(1.1x10^{-12}M)。在另一个实施方案中,本发明提供包含抗-因子D抗体的缀合物,其中单价形式的抗体对因子D的亲和力(例如,作为Fab片段的抗体对因子D的亲和力)为约0.19nM(0.19x10^{-9}M)。在另一个实施方案中,本发明提供包含抗-因子D抗体的缀合物,其中二价形式的抗体对因子D的亲和力(例如,作为IgG的抗体对因子D的亲和力)为约0.08nM(0.08x10^{-9}M)。在另一个实施方案中,本发明提供包含抗-因子D抗体的缀合物,其中单价形式的抗体对因子D的亲和力(例如,作为Fab片段的抗体对因子D的亲和力)为约12.3nM(12.3x10^{-9}M)。在另一个实施方案中,本发明提供包含抗-因子D抗体的缀合物,其中二价形式的抗体对因子D的亲和力(例如,作为IgG的抗体对因子D的亲和力)为约9.0nM(9.0x10^{-9}M)。

[0198] 在另一个实施方案中,本发明提供包含抗-因子D抗体的缀合物,其中单价形式的抗体对因子D的亲和力(例如,作为Fab片段的抗体对因子D的亲和力)为约1.4pM(1.4x10^{-12}M)+/-0.5。在另一个实施方案中,本发明提供包含抗-因子D抗体的缀合物,其中二价形式的抗体对因子D的亲和力(例如,作为IgG的抗体对因子D的亲和力)为约1.1pM(1.1x10^{-12}M)+/-0.6。在另一个实施方案中,本发明提供包含抗-因子D抗体的缀合物,其中单价形式的抗体对因子D的亲和力(例如,作为Fab片段的抗体对因子D的亲和力)为约0.19nM(0.19x10^{-9}M)+/-.01。在另一个实施方案中,本发明提供包含抗-因子D抗体的缀合物,其中二价形式的抗体对因子D的亲和力(例如,作为IgG的抗体对因子D的亲和力)为约0.08nM(0.08x10^{-9}M)+/-0.01。在另一个实施方案中,本发明提供包含抗-因子D抗体的缀合物,其中单价形式的抗体对因子D的亲和力(例如,作为Fab片段的抗体对因子D的亲和力)为约12.3nM(12.3x10^{-9}M)+/-2。在另一个实施方案中,本发明提供包含抗-因子D抗体的缀合物,其中二价形式的抗体对因子D的亲和力(例如,作为IgG的抗体对因子D的亲和力)为约9.0nM(9.0x10^{-9}M)+/-1。

[0199] 在另一个实施方案中,单价形式的用于本发明的缀合物的抗-因子D抗体对因子D的亲和力(例如,作为Fab片段的抗体对因子D的亲和力)可以为约1.4pM(1.4x10^{-12}M)+/-2。在另一个实施方案中,二价形式的用于本发明的缀合物的抗-因子D抗体对因子D的亲和力(例如,作为IgG的抗体对因子D的亲和力)可以为约1.1pM(1.1x10^{-12}M)+/-2。在另一个实施方案中,单价形式的用于本发明的缀合物的抗-因子D抗体对因子D的亲和力(例如,作为Fab片段的抗体对因子D的亲和力)可以为约0.19nM(0.19x10^{-9}M)+/-2。在另一个实施方案中,二价形式的用于本发明的缀合物的抗-因子D抗体或其抗体变体对因子D的亲和力(例如,作为IgG的抗体对因子D的亲和力)可以为约0.08nM(0.08x10^{-9}M)+/-2。在另一个实施方案中,单价形式的用于本发明的缀合物的抗-因子D抗体对因子D的亲和力(例如,作为Fab片段的抗体对因子D的亲和力)可以为约12.3nM(12.3x10^{-9}M)+/-2。在另一个实施方案中,二价形式的用于本发明的缀合物的抗-因子D抗体对因子D的亲和力(例如,作为IgG的抗体对因子D的亲和力)可以为约9.0nM(9.0x10^{-9}M)+/-2。

[0200] 如本领域中已知的,配体对其受体的结合亲和力可以使用多种测定中的任一种来确定,并且可以表示为多种数量值。因此,在一些实施方案中,结合亲和力以K_D值表示并且反映固有结合亲和力(例如,在亲合力作用最小的情况下)。通常并且优选地,在体外(在无细胞情况下或在细胞相关情况下)测量结合亲和力。如本文中更为详细地描述的,结合亲和力的倍数差异可以被量化为人源化抗体(例如,Fab形式的)的单价结合亲和力值与参比/比较抗体(例如,Fab形式的)(例如,具有供体高变区序列的鼠抗体)的单价结合亲和力值之

比，其中在相似的测定条件下测定结合亲和力值。因此，在一些实施方案中，结合亲和力的倍数差异被确定为Fab形式的人源化抗体与所述参比/比较Fab抗体的K_D值之比。例如，在一些实施方案中，如果本发明的抗体(A)的亲和力比参比抗体(M)的亲和力“3倍低”，那么如果A的K_D值是3x，则M的K_D值将是1x，并且A的K_D与M的K_D之比将是3:1。相反，在一些实施方案中，如果本发明的抗体(C)的亲和力比参比抗体(R)的亲和力“3倍高”，那么如果C的K_D值是1x，则R的K_D值将是3x，并且C的K_D与R的K_D之比将是1:3。包括本文中所述的那些在内的本领域中已知的多种测定中的任一种可以用于获得结合亲和力量度，所述测定包括例如Biacore、放射性免疫测定(RIA)和ELISA。

[0201] 此外，用于本发明的缀合物的抗体的K_D值可以变化，这取决于使用的具体测定的条件。例如，在一些实施方案中，结合亲和力量度可以在这样的测定中获得，其中将Fab或抗体固定并且测量配体(即因子D)的结合，或备选地，将Fab或抗体的配体(即因子D)固定并且测量Fab或抗体的结合。在一些实施方案中，结合亲和力量度可以在这样的测定中获得，其中再生条件可以包含(1)10mM甘氨酸或4M $MgCl_2$，pH 1.5，以及(2)pH 1.0至pH 7.5之间的pH，包括pH 1.5、pH 5.0、pH 6.0和pH 7.2。在一些实施方案中，结合亲和力量度可以在这样的测定中获得，其中结合条件可以包含(1)PBS或HEPES-缓冲盐水和(2)Tween-20，即0.1％Tween-20。在一些实施方案中，结合亲和力量度可以在这样的测定中获得，其中配体(即因子D)的来源可以来自可商购的来源。在一些实施方案中，结合亲和力量度可以在这样的测定中获得，其中(1)将Fab或抗体固定并且测量配体(即因子D)的结合，(2)再生条件包含4M $MgCl_2$，pH 7.2，并且(3)结合条件包含HEPES-缓冲盐水，pH 7.2，含0.1％Tween-20。在一些实施方案中，结合亲和力量度可以在这样的测定中获得，其中(1)将配体(即因子D)固定并测量Fab或抗体的结合，(2)再生条件包含10mM甘氨酸，pH 1.5，并且(3)结合条件包含PBS缓冲剂。

[0202] b.生物活性

[0203] 为了确定抗因子D抗体或其变体或片段(例如抗原结合片段)是否能够与因子D结合并发挥生物效应(例如抑制旁路途径溶血)，可以使用利用兔RBC进行的溶血抑制测定，包括实施例2中所述的那些。这种溶血抑制可以使用标准测定进行确定(Kostavasili等人(1997)J of Immunology158:1763-72;Wiesmann等人(2006)Nature 444:159-60)。这种测定中的补体激活可以用血清或血浆引发。因子D在血清或血浆中的适合浓度(Pascual等人(1998)Kidney International 34:529-536;Complement Facts Book,Bernard J.Morley和Mark J.Walport,编辑,Academic Press(2000);Barnum等人(1984)J.Immunol.Methods,67:303-309)可以根据本领域已知的方法常规地确定，包括已经在参考文献中描述的那些，诸如Pascual等人(1998)Kidney International 34:529-536和Barnum等人(1984)J.Immunol.Methods 67:303-309。本发明通常涉及能够抑制与因子D有关的生物活性的抗体。例如，在18μg/ml的浓度下(相当于血液中人因子D的摩尔浓度的约1.5倍;抗-因子D抗体与因子D的摩尔比为约1.5:1)，可以观察到抗体对旁路补体活性的显著抑制(参见例如美国专利号6,956,107)。

[0204] 在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{50}值小于30nM。在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{50}值小于15nM。在一些

实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{50}值小于10nM。在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{50}值小于5nM。

[0205] 在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{50}值为30nM至2nM。在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{50}值为25nM至7nM。在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{50}值为20nM至12nM。在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{50}值为30nM至15nM。在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{50}值为12nM至8nM。在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{50}值为7nM至2nM。在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{50}值为6nM至3nM。在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{50}值为8nM至5nM。在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{50}值为5nM至2nM。在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{50}值为10nM至5nM。在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中$IC5_{50}$值为8nM至2nM。在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{50}值为7nM至3nM。在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{50}值为6nM至4nM。在另一个实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{50}值为约4.7nM±0.6nM。在另一个实施方案中，本发明涉及抗-因子D抗体，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{50}值为约6.4nM±0.6nM。在另一个实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{50}值为约3.5nM±0.5nM。在另一个实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{50}值为约4.4nM±1.5nM。在另一个实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{50}值为约10.2nM±0.8nM。在另一个实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{50}值为约23.9nM±5.0nM。

[0206] 在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{90}值小于80nM。在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{90}值小于50nM。在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{90}值小于40nM。在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{90}值小于20nM。在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{50}

值小于15nM。

[0207] 在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{90}值为80nM至10nM。在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{90}值为75nM至15nM。在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{90}值为70nM至20nM。在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{90}值为65nM至25nM。在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{90}值为60nM至30nM。在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{90}值为55nM至35nM。在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{90}值为50nM至40nM。在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{90}值为80nM至70nM。在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{90}值为55nM至25nM。在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{90}值为16nM至12nM。在另一个实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{90}值为约14.0nM±1.0nM。在另一个实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{90}值为约38.0nM±11.0nM。在另一个实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段抑制旁路途径溶血，其中IC_{90}值为约72.6nM±4.8nM。

[0208] 在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的Fab片段以如下抗体与因子D摩尔比抑制旁路途径溶血：约0.05:1(0.05)至约10:1(10)、或约0.09:1(0.09)至约8:1(8)、或约0.1:1(0.1)至约6:1(6)、或约0.15:1(0.15)至约5:1(5)、或约0.19:1(0.19)至约4:1(4)、或约0.2:1(0.2)至约3:1(3)、或约0.3:1(0.3)至约2:1(2)、或约0.4:1(0.4)至约1:1(1)、或约0.5:1(0.5)至约1:2(0.5)、或约0.6:1(0.6)至约1:3(0.33)、或约0.7:1(0.7)至约1:4(0.25)、或约0.8:1(0.8)至约1:5(0.2)、或约0.9:1(0.9)至约1:6(0.17)。

[0209] 在一些实施方案中，本发明涉及包含人源化抗-因子D抗体的片段(例如抗原结合片段)的缀合物。本发明的抗体片段可以是例如Fv、Fab、Fab-SH、Fab'-SH、Fab'、Fab-C、Fab'-C、Fab'-C-SH、Fab-C-SH、scFv、双抗体、或$F(ab')_2$、dAb、互补决定区(CDR)片段、线性抗体、单链抗体分子、微抗体(minibody)、双抗体、或由抗体片段形成的多特异性抗体。在另外的实施方案中，本发明涉及包含能够基本上穿透全部视网膜的人源化抗-因子D抗体片段(例如抗原结合片段)的缀合物。在甚至另外的实施方案中，本发明涉及包含能够穿透贯穿整个视网膜厚度的人源化抗-因子D抗体片段(例如抗原结合片段)的缀合物。

[0210] 在一些实施方案中，本发明涉及包含抗-因子D抗体的缀合物，其中所述抗体的未缀合的Fab片段在经由单次玻璃体内注射施用至哺乳动物眼睛(例如人)后具有至少3、5、7、10或12天的半衰期。在另一个实施方案中，本发明涉及包含人源化抗-因子D抗体的缀合物，其中所述抗体的未缀合的Fab片段在经由单次玻璃体内注射施用至哺乳动物眼睛(例如人)

后抑制旁路途径(AP)补体激活达至少40、45、50、55、60、65、70、75、80、85、90、95、100、105、110或115天。在另一个实施方案中，本发明涉及包含人源化抗-因子D抗体的缀合物，其中所述抗体的未缀合的Fab片段抑制旁路途径(AP)补体激活的浓度在经由单次玻璃体内注射施用至哺乳动物眼睛(例如人)后在视网膜组织中维持至少40、45、50、55、60、65、70、75、80或85天。在另一个实施方案中，本发明涉及包含人源化抗-因子D抗体的缀合物，其中所述抗体的未缀合的Fab片段抑制旁路途径(AP)补体激活的浓度在经由单次玻璃体内注射施用至哺乳动物眼睛(例如人)后在玻璃体液中维持至少80、85、90、95、100、105、110或115天。

[0211] 抗-因子D抗体或抗体变体-聚合物缀合物的构建

[0212] a.多臂聚合物

[0213] 在一些方面，能够通过将本文描述的抗-因子D抗体或抗体变体通过缀合抗体或其变体与多臂聚合物进行衍生化来制备本发明的缀合物。应当理解，提供具有所需尺寸的缀合物或具有如本文描述的所选平均分子量的任何多臂聚合物适用于构建本发明的抗体-聚合物缀合物。

[0214] 许多聚合物适用于药物。参见，例如，Davis等人，Biomedical Polymers: Polymeric Materials and Pharmaceuticals for Biomedical Use,pp.441-451(1980)。在本发明的所有实施方案中，将非蛋白质聚合物用于形成本发明的缀合物。非蛋白质聚合物通常是亲水性合成聚合物，即在自然界中不以其它方式发现的聚合物。然而，天然存在并且通过重组或体外方法产生的聚合物也可以是有用的，如从天然来源分离的聚合物也是有用的。

[0215] 在一些方面，抗-因子D抗体或抗体变体通过将抗体或其变体缀合(例如，共价连接)至多臂多元醇来进行衍生化。因此，在一些实施方案中，本发明涉及包含与一种或多种多臂多元醇共价连接的本文公开的一种或多种抗-因子D抗体或抗体变体的缀合物。采用的多元醇可以是任何水溶性聚(氧化烯)聚合物，并且能够具有直链或支链。合适的多元醇包括在一个或多个羟基位置处被化学基团(诸如，具有一至四个碳的烷基)取代的那些多元醇。典型地，多元醇是聚(亚烷基二醇)，诸如聚乙二醇(PEG)，并且因此为了便于描述，其余的讨论涉及其中采用的多元醇是PEG的示例性实施方案，并且将多元醇缀合至多肽的过程称为“PEG化”。然而，本领域技术人员将认识到可以使用本文描述的用于PEG的缀合技术来利用其它多元醇，诸如例如聚(丙二醇)和聚乙二醇-聚丙二醇共聚物。

[0216] 用于形成本发明的缀合物的多元醇是多臂多元醇。如本文所使用的，“多臂多元醇”是指包含与至少两个臂连接的核心结构的多元醇。多臂多元醇可以是例如二聚体(两个臂)，四聚体(四个臂)，六聚体(六个臂)，八聚体(八个臂)等。在一些方面，多臂多元醇是多臂PEG。

[0217] 用于抗-因子D抗体和抗体变体的PEG化中的多臂PEG的重均分子量可以变化，并且通常可以在约500至约300,000道尔顿(D)的范围内。在一些实施方案中，多臂PEG的重均分子量是约1,000至约100,000D，并且在一些实施方案中是约20,000至约60,000D。在一些实施方案中，使用重均分子量为约40,000D的多臂PEG进行PEG化。

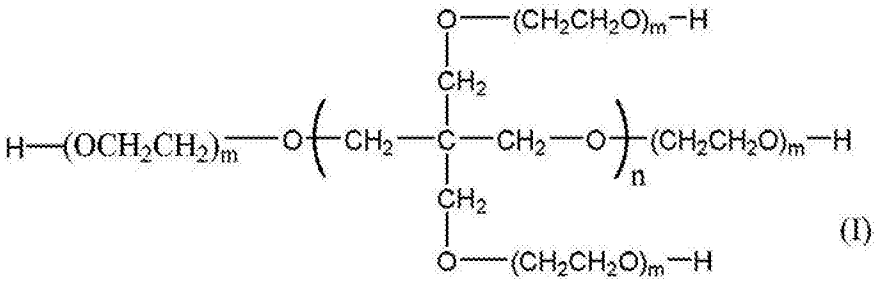
[0218] 用于PEG化蛋白质的多种方法在本领域中是已知的。产生与PEG缀合的蛋白质的具体方法包括美国专利号4,179,337、美国专利号4,935,465和美国专利号5,849,535中描述的方法，这些文献全部通过引用整体结合于此。通常，蛋白质经由蛋白质的一个或多个氨基

酸残基与聚合物上的末端反应性基团共价结合。具有一个或多个反应性基团的聚合物在本文中被称为活化或官能化聚合物(例如,官能化PEG)。反应性基团选择性地与抗体或抗体变体上的游离巯基或氨基或其它反应性基团反应。多臂PEG聚合物可以以随机方式或位点特异性方式偶联至抗体或抗体变体上的巯基或氨基或其它反应性基团。然而,应当理解,为了获得最佳结果,所选择的反应性基团的类型和量以及所采用的聚合物的类型将取决于用于限制并且优选基本上防止反应性基团与抗体上的过多活性基团反应的特定抗体或抗体变体。由于在一些情况下不可能充分地限制或防止这种情形,取决于抗体浓度,可以采用通常约0.05至约1000摩尔或者在一些实施方案中约0.05至约200摩尔的官能化聚合物/摩尔抗体。每摩尔抗体的官能化聚合物的最终量是保持最佳活性的平衡,而如果可能的话,同时优化抗体的玻璃体液、视网膜和/或房水半衰期。

[0219] 虽然残基可以是抗体或抗体变体上的任何反应性氨基酸,诸如N-末端氨基酸基团,但是在一些实施方案中,反应性氨基酸是半胱氨酸,其经由其游离硫醇基团与官能化聚合物的反应性基团连接,如例如在WO 99/03887、WO 94/12219、WO 94/22466、美国专利号5,206,344、美国专利号5,166,322和美国专利号5,206,344中所示的,这些文献全部通过引用整体结合于此。在这样的实施方案中,聚合物可以包含至少一个末端反应性基团,所述反应性基团能够与亲本抗体上的一个或多个游离巯基或硫醇基团特异性反应。其中,这种基团包括但不限于,马来酰亚胺、巯基、硫醇、三氟甲磺酸酯、甲苯磺酸酯、氮丙啶、环氧化物、吡啶基二硫化物、琥珀酰亚胺酯、-NH_2、醛、卤代乙酸酯、卤代乙酰胺和碳酸对-硝基苯酯。可以使用适用于所选的偶联系统的化学的任何方案将聚合物偶联至亲本抗体,诸如美国专利号4,179,337、美国专利号7,122,636以及Jevsevar等人,Biotech J.,Vol.5,pp.113-128(2010)中所描述的方案和系统。备选地,反应性氨基酸可以是赖氨酸,其经由其游离ε-氨基连接至官能化聚合物的反应性基团(参见例如WO 93/00109,其通过引用结合于此),或者谷氨酸或天冬氨酸,其经由酰胺键与聚合物连接。然后,聚合物的反应性基团可以与例如蛋白质的α(alpha)和ε(epsilon)胺或巯基反应以形成共价键。应当理解,本发明不限于利用抗体或抗体片段与聚合物之间的任何特定类型的连接的缀合物。

[0220] 用于制备本发明缀合物的合适的官能化多臂PEG可以通过许多常规反应产生。例如,PEG的N-羟基琥珀酰亚胺酯(M-NHS-PEG)可以根据Buckmann和Merr,Makromol.Chem.,Vol.182,pp.1379-1384(1981)的方法,通过与N,N'-二环己基碳二亚胺(DCC)和N-羟基琥珀酰亚胺(NHS)反应由PEG-单甲基醚制备。另外,例如,通过与亚硫酰溴反应形成PEG-Br,然后用过量氨进行氨解以形成PEG-NH_2,可以将PEG末端羟基转化为氨基。然后,可以使用标准偶联剂(诸如伍德沃德氏试剂K(Woodward's Reagent K))将PEG-NH_2缀合至感兴趣的抗体或抗体变体。此外,可以例如通过用MnO_2进行氧化将PEG末端-CH_2OH基团转化为醛基。醛基可以通过用试剂(诸如氰基硼氢化物)进行还原性烷基化而与抗体或抗体变体缀合。

[0221] 在一些实施方案中,用于制备本发明缀合物的多臂PEG具有通式(I)的结构:

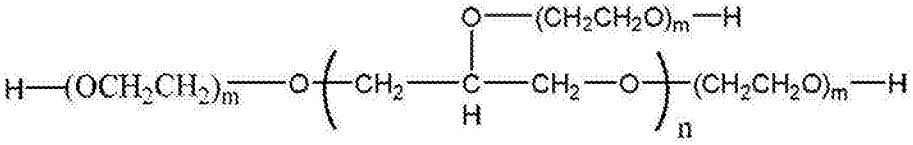
[0222] 

(I)

[0223] 其中各m表示多元醇(PEG)的特定臂的长度或尺寸，并且独立地是约45至约1000、约3至约250、或约50至约200、或约100至约150的整数；并且n是约1至约10的整数。

[0224] 在一些实施方案中，多臂PEG具有通式(I)的结构，其中n是1，并且多臂PEG是四聚体。在另一个实施方案中，多臂PEG具有通式(I)的结构，其中n是2，并且多臂PEG是六聚体。在另一个实施方案中，多臂PEG具有通式(I)的结构，其中n是3，并且多臂PEG是八聚体。

[0225] 在另一个方面，用于制备本发明缀合物的多臂PEG具有通式(II)的结构：

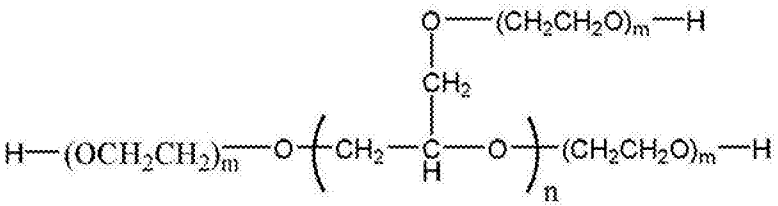
[0226] 

(II)

[0227] 其中各m表示多元醇(PEG)的特定臂的长度或尺寸，并且独立地是约45至约1000、约3至约250、或约50至约200、或约100至约150的整数；并且n是约1至约10的整数。

[0228] 在一些实施方案中，多臂PEG具有通式(II)的结构，其中n是2，并且多臂PEG是四聚体。在另一个实施方案中，多臂PEG具有通式(II)的结构，其中n是4，并且多臂PEG是六聚体。在另一个实施方案中，多臂PEG具有通式(II)的结构，其中n是6，并且多臂PEG是八聚体。

[0229] 在另一个方面，用于制备本发明缀合物的多臂PEG具有通式(III)的结构：

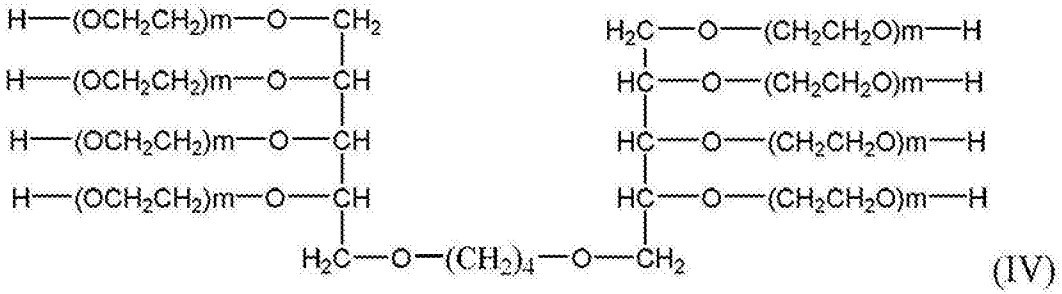
[0230] 

(III)

[0231] 其中各m表示多元醇(PEG)的特定臂的长度或尺寸，并且独立地是约45至约1000、约3至约250、或约50至约200、或约100至约150的整数；并且n是约1至约10的整数。

[0232] 在一些实施方案中，多臂PEG具有通式(III)的结构，其中n是2，并且多臂PEG是四聚体。在另一个实施方案中，多臂PEG具有通式(III)的结构，其中n是4，并且多臂PEG是六聚体。在另一个实施方案中，多臂PEG具有通式(III)的结构，其中n是6，并且多臂PEG是八聚体。

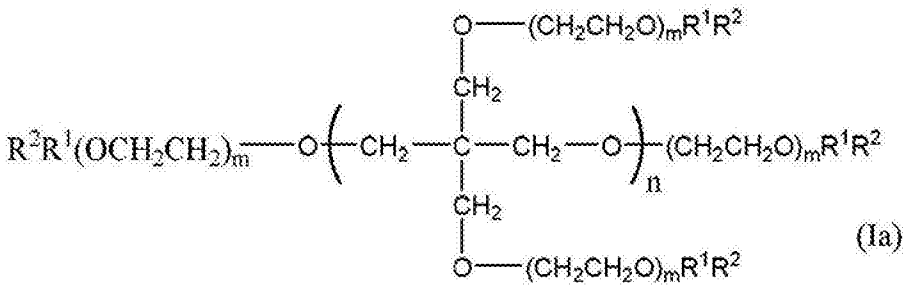
[0233] 在另一个方面，用于制备本发明缀合物的多臂PEG具有通式(IV)的结构：

[0234] 

[0235] 其中各m表示多元醇(PEG)的特定臂的长度或尺寸，并且独立地是约45至约1000、约3至约250、或约50至约200、或约100至约150的整数。

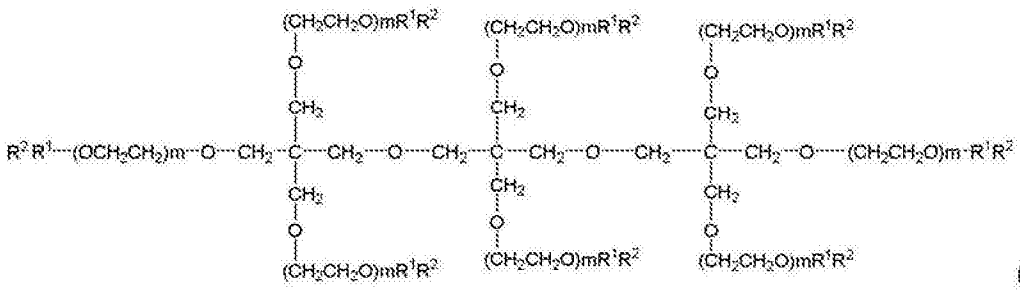
[0236] 具有通式(I)至(IV)中任一个的结构的多臂PEG可以被官能化以例如使用如上描述的任一个技术连接适于与抗体(例如抗体片段)反应或缀合的末端反应性基团，以产生官能化多臂PEG。然而，在其它实施方案中，多臂PEG可以经由多官能交联剂共价连接至抗-因子D抗体或抗体变体，所述多官能交联剂与PEG以及待连接的抗体或抗体片段的一个或多个氨基酸残基反应，如例如，美国专利号7,122,636中所描述的，该文献通过引用整体结合于此。

[0237] 在其它方面，用于制备本发明缀合物的多臂PEG是包含至少一个末端反应性基团的官能化多臂PEG。末端反应性基团可以直接缀合至抗-因子D抗体或抗体变体以形成本发明的缀合物。在一些实施方案中，官能化多臂PEG具有通式(Ia)的结构：

[0238] 

[0239] 其中各m表示多元醇(PEG)的特定臂的长度或尺寸，并且独立地是约45至约1000、约3至约250、或约50至约200、或约100至约150的整数；并且n是约1至约10的整数；各R^1独立地是不存在或是连接基团；并且各R^2独立地是氢或末端反应性基团；其中至少一个R^2是末端反应性基团。在一些实施方案中，R^2独立地选自由以下组成的组：硫醇反应性基团、氨基反应性基团及其组合。

[0240] 在一些实施方案中，官能化多臂PEG具有通式(Ia)的结构，其中n是1至3的整数。在一些实施方案中，官能化多臂PEG具有通式(Ia)的结构，其中n是1，并且多臂PEG是四聚体。在另一个实施方案中，官能化多臂PEG具有通式(Ia)的结构，其中n是2，并且多臂PEG是六聚体。在另一个实施方案中，官能化多臂PEG具有通式(Ia)的结构，其中n是3，并且多臂PEG是八聚体。在这样的实施方案中，八聚体具有通式(Ib)的结构：

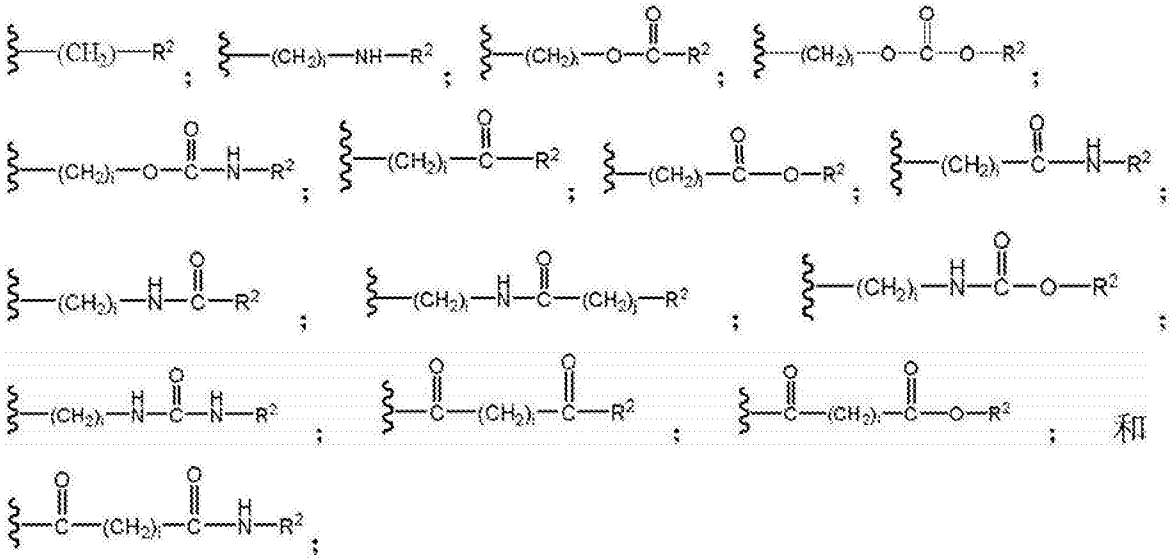
[0241] 

(Ib)

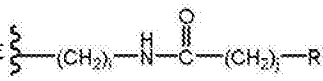
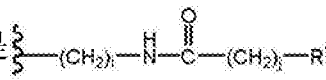
[0242] 其中m、R^1、R^2是如本文中所定义的。特别地，在一个实施方案中，各m表示多元醇(PEG)的特定臂的长度或尺寸，并且独立地是约45至约1000、约3至约250、或约50至约200、或约100至约150的整数；各R^1独立地是不存在或是连接基团；并且各R^2独立地是氢或末端反应性基团；其中至少一个R^2是末端反应性基团。在一些实施方案中，R^2独立地选自由以下组成的组：硫醇反应性基团、氨基反应性基团及其组合。

[0243] 具有通式(Ib)的结构的多臂PEG具有三季戊四醇(TP)核心结构，并且在本文中也称为TP八聚体。

[0244] 在一些实施方案中，官能化多臂PEG具有通式(Ia)或(Ib)的结构，其中各R^1当存在时是相同或不同的，并且R^1和R^2当连接一起时选自由以下组成的组：

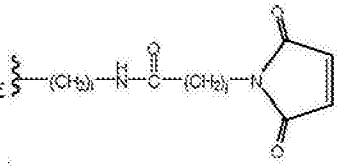
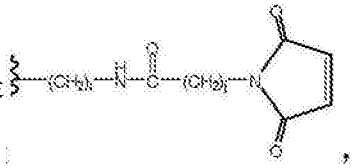


及其组合；其中各i独立地是0-10的整数；j是0-10的整数；并且R^2是如本文定义的。在一些实施方案中，各R^1是连接基团。

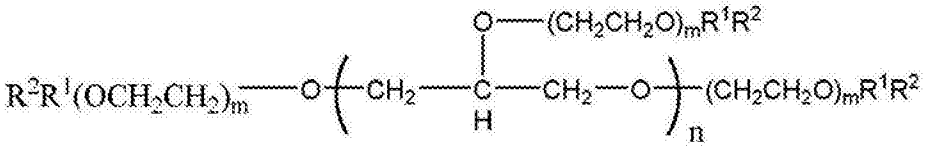
[0245] 在一些实施方案中，官能化多臂PEG具有通式(Ia)或(Ib)的结构，其中R^1和R^2当连接一起时是  ，其中i、j和R^2是如本文定义的。在一些实施方案中，R^1和R^2当连接一起时是  ，其中i是2；j是2或3，并且R^2是如本文定义的。

[0246] 在一些方面，官能化多臂PEG具有通式(Ia)或(Ib)的结构，各R^2独立地选自由以下组成的组：马来酰亚胺、巯基、硫醇、三氟甲磺酸酯、甲苯磺酸酯、氮丙啶、环氧化物、吡啶基二硫化物、琥珀酰亚胺酯、$-NH_2$、醛、卤代乙酸酯、卤代乙酰胺和碳酸对-硝基苯酯。在一些实施方案中，各R^2独立地是选自由以下组成的组的卤代乙酸酯：溴代乙酸酯、碘代乙酸酯、氯

代乙酸酯及其组合。在一些实施方案中，各R^2独立地是选自由以下组成的组的卤代乙酰胺：溴代乙酰胺、碘代乙酰胺、氯代乙酰胺及其组合。在一些实施方案中，R^2是马来酰亚胺。

[0247] 在一些实施方案中，官能化多臂PEG具有通式(Ia)或(Ib)的结构，其中各R^2是马来酰亚胺。在一些实施方案中，官能化多臂PEG具有通式(Ia)或(Ib)的结构，其中R^1和R^2当连接一起时是  ，其中i和j是如本文定义的。在一个实施方案中，官能化多臂PEG具有通式(Ia)或(Ib)的结构，其中R^1和R^2当连接一起时是  ，其中i是2并且j是2。

[0248] 在另一个方面，用于制备本发明缀合物的官能化多臂PEG具有通式(IIa)的结构：

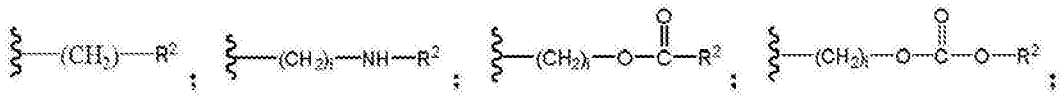
[0249] 

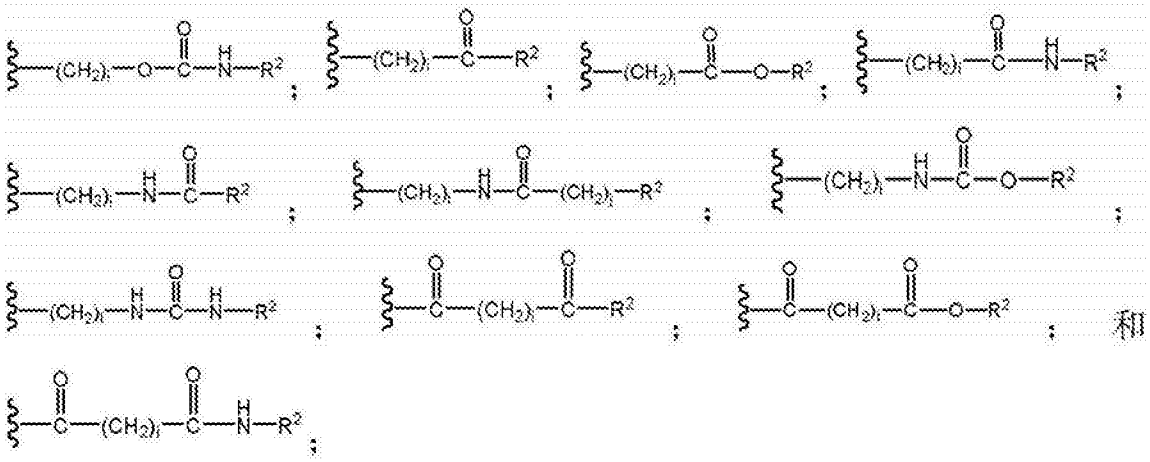
(IIa)

[0250] 其中各m表示多元醇(PEG)的特定臂的长度或尺寸，并且独立地是约45至约1000、约3至约250、或约50至约200、或约100至约150的整数；并且n是约1至约10的整数；各R^1独立地是不存在或是连接基团；并且各R^2独立地是氢或末端反应性基团；其中至少一个R^2是末端反应性基团。在一些实施方案中，R^2独立地选自由以下组成的组：硫醇反应性基团、氨基反应性基团及其组合。

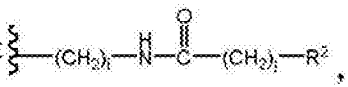
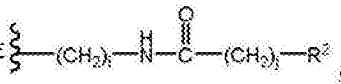
[0251] 在一些实施方案中，官能化多臂PEG具有通式(IIa)的结构，其中n是2至6的整数。在一些实施方案中，官能化多臂PEG具有通式(IIa)的结构，其中n是3。在一个实施方案中，官能化多臂PEG具有通式(IIa)的结构，其中n是2，并且多臂PEG是四聚体。在另一个实施方案中，官能化多臂PEG具有通式(IIa)的结构，其中n是4，并且多臂PEG是六聚体。在另一个实施方案中，官能化多臂PEG具有通式(IIa)的结构，其中n是6，并且多臂PEG是八聚体。具有通式(IIa)的结构的八聚体具有六甘油(HG)核心结构，并且在本文中也称为HG八聚体。

[0252] 在一些实施方案中，官能化多臂PEG具有通式(IIa)的结构，其中各R^1当存在时是相同或不同的，并且R^1和R^2当连接一起时选自由以下组成的组：

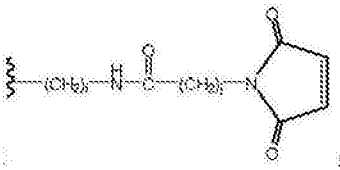
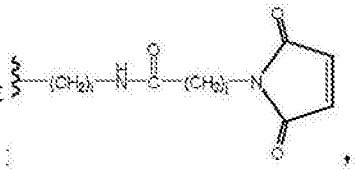




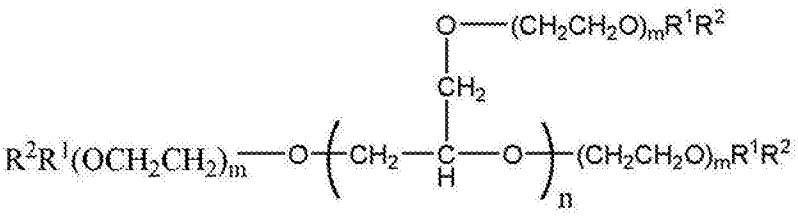
及其组合;其中各i独立地是0-10的整数;j是0-10的整数;并且R^2是如本文定义的。在一些实施方案中,各R^1是连接基团。

[0253] 在一些实施方案中,官能化多臂PEG具有通式(IIa)的结构,其中R^1和R^2当连接一起时是  ,其中i、j和R^2是如本文定义的。在一些实施方案中,R^1和R^2当连接一起时是  ,其中i是2;j是2或3,并且R^2是如本文定义的。

[0254] 在一些方面,官能化多臂PEG具有通式(IIa)的结构,各R^2独立地选自由以下组成的组:马来酰亚胺、巯基、硫醇、三氟甲磺酸酯、甲苯磺酸酯、氮丙啶、环氧化物、吡啶基二硫化物、琥珀酰亚胺酯、-NH_2、醛、卤代乙酸酯、卤代乙酰胺和碳酸对-硝基苯酯。在一些实施方案中,各R^2独立地是选自由以下组成的组的卤代乙酸酯:溴代乙酸酯、碘代乙酸酯、氯代乙酸酯及其组合。在一些实施方案中,各R^2独立地是选自由以下组成的组的卤代乙酰胺:溴代乙酰胺、碘代乙酰胺、氯代乙酰胺及其组合。在一些实施方案中,R^2是马来酰亚胺。

[0255] 在一些实施方案中,官能化多臂PEG具有通式(IIa)的结构,其中各R^2是马来酰亚胺。在一些实施方案中,官能化多臂PEG具有通式(IIa)的结构,其中R^1和R^2当连接一起时是  ,其中i和j是如本文定义的。在一些实施方案中,官能化多臂PEG具有通式(IIa)的结构,其中R^1和R^2当连接一起时是  ,其中i是2并且j是2。

[0256] 在另一个实施方案中,官能化多臂PEG具有通式(IIIa)的结构:

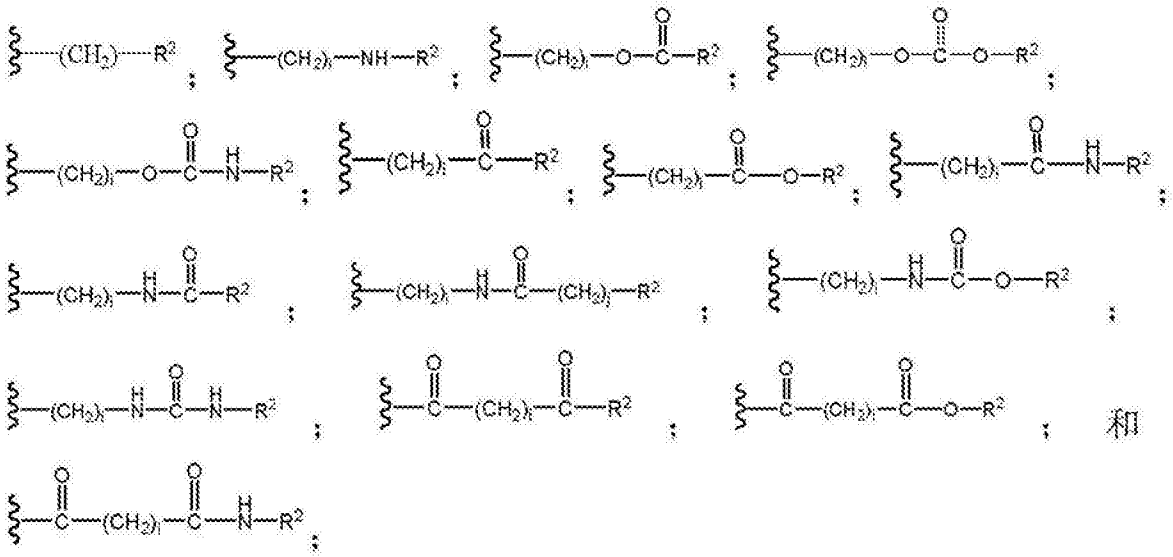
[0257] 

(IIIa)

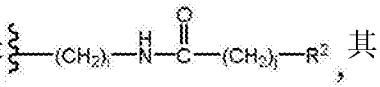
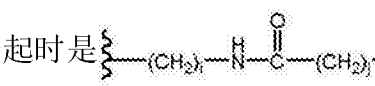
[0258] 其中各m表示多元醇(PEG)的特定臂的长度或尺寸，并且独立地是约45至约1000、约3至约250、或约50至约200、或约100至约150的整数；并且n是约1至约10的整数；各R^1独立地是不存在或是连接基团；并且各R^2独立地是氢或末端反应性基团；其中至少一个R^2是末端反应性基团。在一些实施方案中，R^2独立地选自由以下组成的组：硫醇反应性基团、氨基反应性基团及其组合。

[0259] 在一些实施方案中，官能化多臂PEG具有通式(IIIa)的结构，其中n是2至6的整数。在一些实施方案中，官能化多臂PEG具有通式(IIIa)的结构，其中n是2，并且多臂PEG是四聚体。在另一个实施方案中，官能化多臂PEG具有通式(IIIa)的结构，其中n是4，并且多臂PEG是六聚体。在另一个实施方案中，官能化多臂PEG具有通式(IIIa)的结构，其中n是6，并且多臂PEG是八聚体。具有通式(IIIa)的结构的八聚体具有六甘油(HGEO)核心结构，并且在本文中也称为HGEO八聚体。

[0260] 在一些实施方案中，官能化多臂PEG具有通式(IIIa)的结构，其中各R^1当存在时是相同或不同的，并且R^1和R^2当连接一起时选自由以下组成的组：



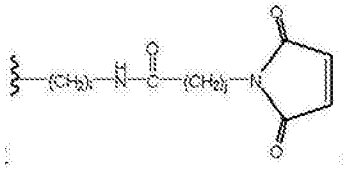
及其组合；其中各i独立地是0-10的整数；j是0-10的整数；并且R^2是如本文定义的。在一些实施方案中，各R^1是连接基团。

[0261] 在一些实施方案中，官能化多臂PEG具有通式(IIIa)的结构，其中R^1和R^2当连接一起时是  ，其中i、j和R^2是如本文定义的。在一些实施方案中，R^1和R^2当连接一起时是  ，其中i是2；j是2或3，并且R^2是如本文定义的。

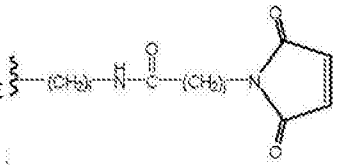
[0262] 在一些方面，官能化多臂PEG具有通式(IIIa)的结构，各R^2独立地选自由以下组成

的组：马来酰亚胺、巯基、硫醇、三氟甲磺酸酯、甲苯磺酸酯、氮丙啶、环氧化物、吡啶基二硫化物、琥珀酰亚胺酯、-NH_2、醛、卤代乙酸酯、卤代乙酰胺和碳酸对-硝基苯酯。在一些实施方案中，各R^2独立地是选自由以下组成的组的卤代乙酸酯：溴代乙酸酯、碘代乙酸酯、氯代乙酸酯及其组合。在一些实施方案中，各R^2独立地是选自由以下组成的组的卤代乙酰胺：溴代乙酰胺、碘代乙酰胺、氯代乙酰胺及其组合。在一些实施方案中，R^2是马来酰亚胺。

[0263] 在一些实施方案中，官能化多臂PEG具有通式(IIIa)的结构，其中各R^2是马来酰亚胺。在一些实施方案中，官能化多臂PEG具有通式(IIIa)的结构，其中R^1和R^2当连接一起时是



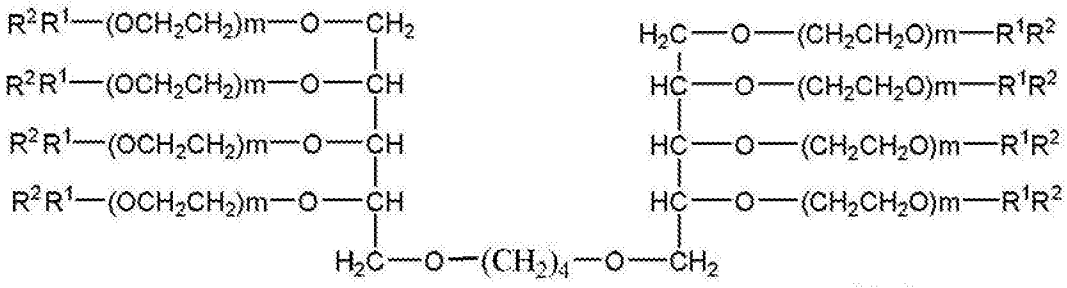
其中i和j是如本文定义的。在一些实施方案中，官能化多臂PEG具有通式(IIIa)的结构，其中R^1和R^2当连接一起时是



其中i是3并且j是2。

[0264] 在另一个实施方案中，官能化多臂PEG具有通式(IVa)的结构：

[0265]

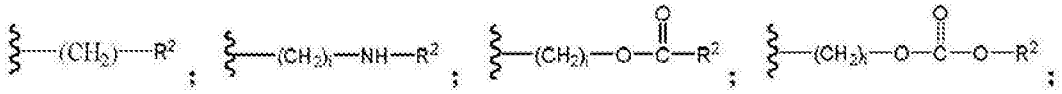


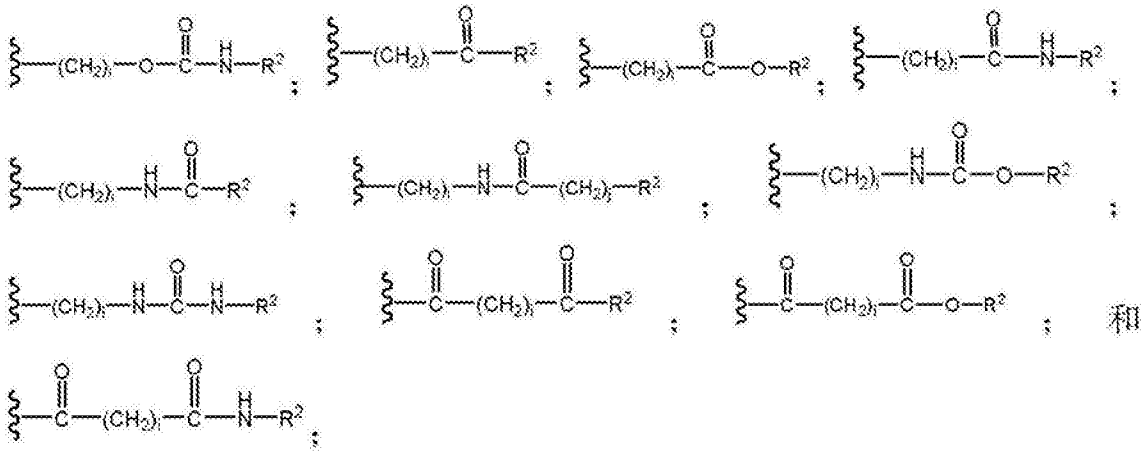
(IVa)

[0266] 其中各m表示多元醇(PEG)的特定臂的长度或尺寸，并且独立地是约45至约1000、约3至约250、或约50至约200、或约100至约150的整数；各R^1独立地是不存在或是连接基团；并且各R^2独立地是氢或末端反应性基团；其中至少一个R^2是末端反应性基团。在一些实施方案中，R^2独立地选自由以下组成的组：硫醇反应性基团、氨基反应性基团及其组合。

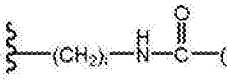
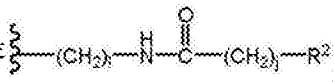
[0267] 具有通式(IVa)的结构的多臂PEG具有丁二醇核心结构，并且在本文中也称为DX八聚体。

[0268] 在一些实施方案中，官能化多臂PEG具有通式(IVa)的结构，其中各R^1当存在时是相同或不同的，并且R^1和R^2当连接一起时选自由以下组成的组：

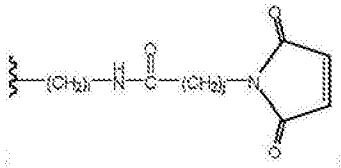
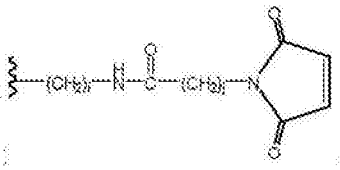




及其组合;其中各i独立地是0-10的整数;j是0-10的整数;并且R^2是如本文定义的。在一些实施方案中,各R^1是连接基团。

[0269] 在一些实施方案中,官能化多臂PEG具有通式(IVa)的结构,其中R^1和R^2当连接一起时是  ,其中i、j和R^2是如本文定义的。在一些实施方案中,R^1和R^2当连接一起时是  ,其中i是2;j是2或3,并且R^2是如本文定义的。

[0270] 在一些方面,各R^2独立地选自由以下组成的组:马来酰亚胺、巯基、硫醇、三氟甲磺酸酯、甲苯磺酸酯、氮丙啶、环氧化物、吡啶基二硫化物、琥珀酰亚胺酯、$-NH_2$、醛、卤代乙酸酯、卤代乙酰胺和碳酸对-硝基苯酯。在一些实施方案中,各R^2独立地是选自由以下组成的组的卤代乙酸酯:溴代乙酸酯、碘代乙酸酯、氯代乙酸酯及其组合。在一些实施方案中,各R^2独立地是选自由以下组成的组的卤代乙酰胺:溴代乙酰胺、碘代乙酰胺、氯代乙酰胺及其组合。在一些实施方案中,R^2是马来酰亚胺。

[0271] 在一些实施方案中,官能化多臂PEG具有通式(IVa)的结构,其中各R^2是马来酰亚胺。在一些实施方案中,官能化多臂PEG具有通式(IVa)的结构,其中R^1和R^2当连接一起时是  ,其中i和j是如本文定义的。在一些实施方案中,官能化多臂PEG具有通式(IVa)的结构,其中R^1和R^2当连接一起时是  ,其中i是3并且j是2。

[0272] 用于制备本发明的缀合物的多臂PEG将优选具有低的PEG链(臂)长度多分散性。特别地,用于制备缀合物的多臂PEG的高多分散性可以使最终缀合物的分析变得复杂,特别是使得准确确定抗体(例如Fab)数量/PEG变得更加困难和不确定。因此,用于形成缀合物的PEG将通常具有在约1至约1.35的范围内的多分散性(使用本领域已知的方法确定),并且在各种实施方案中将具有约1至约1.25、约1至约1.2、约1至约1.15、约1至约1.1、约1.05、或甚至约1的多分散性。

[0273] 适用于本发明的其它官能化多臂PEG在美国专利申请公开号2011/0286956和美国专利申请公开号2015/0073155中描述，这两篇文献通过引用整体结合于此。

[0274] 适用于本发明的官能化多臂PEG也可以购自多个供应商。例如，JenKem Technology，USA销售马来酰亚胺官能化的PEG八聚体(例如，8ARM(TP)-PEG-MAL和8ARM(HG)-PEG-MAL)和四聚体。NOF America Corp.也销售马来酰亚胺官能化的PEG八聚体(例如，Sunbright®HGEO-400MA；Sunbright®DX-400MA)和四聚体(例如，Sunbright®PTE-400MA)。这种八聚体或四聚体可以以各种分子量获得，包括40,000D的平均分子量。

[0275] b.缀合物

[0276] 在一些实施方案中，本发明涉及包含本文公开的一种或多种抗-因子D抗体或抗体变体和一种或多种多臂多元醇的缀合物，其中所述缀合物通过将至少一个抗-因子D抗体或抗体变体共价连接至多元醇来制备。在一些实施方案中，多臂多元醇是PEG。在一些实施方案中，PEG是八聚体。在一些实施方案中，PEG具有通式(Ia)、(Ib)、(IIa)、(IIIa)或(IVa)的结构：

[0277] 本发明的缀合物的特征可以在于与各个多臂PEG缀合的抗-因子D抗体或抗体变体的数量。这在本文中称为“fab化”或“fab化的程度”。与各个PEG缀合的抗-因子D抗体或抗体变体的数量可以取决于各种因素而变化，包括：1)PEG中臂的数量；2)PEG上的末端反应性基团的数量和/或反应性；3)PEG的核心结构；和/或4)PEG化反应条件。

[0278] 在一个优选的实施方案中，本发明的缀合物包含八臂PEG，其中至少一个抗-因子D抗体或抗体变体与PEG共价连接。在另一个实施方案中，本发明的缀合物包含八臂PEG，其中至少两个所述抗-因子D抗体或所述抗体变体与PEG共价连接。在另一个实施方案中，本发明的缀合物包含八臂PEG，其中至少三个抗-因子D抗体或抗体变体与PEG共价连接。在另一个实施方案中，本发明的缀合物包含八臂PEG，其中至少四个抗-因子D抗体或抗体变体与PEG共价连接。在另一个实施方案中，本发明的缀合物包含八臂PEG，其中至少五个抗-因子D抗体或抗体变体与PEG共价连接。在另一个实施方案中，本发明的缀合物包含八臂PEG，其中至少六个抗-因子D抗体或抗体变体与PEG共价连接。在另一个实施方案中，缀合物包含八臂PEG，其中至少七个抗-因子D抗体或抗体变体与PEG共价连接。在另一个实施方案中，本发明的缀合物包含八臂PEG，其中至少八个抗-因子D抗体或抗体变体与PEG共价连接。在一些实施方案中，本发明的缀合物包含八臂PEG，其中5-8个抗-因子D抗体或抗体变体与PEG共价连接。在另一个实施方案中，本发明的缀合物包含八臂PEG，其中6-8个抗-因子D抗体或抗体变体与PEG共价连接。在另一个实施方案中，本发明的缀合物包含八臂PEG，其中7-8个抗-因子D抗体或抗体变体与PEG共价连接。

[0279] 在一些实施方案中，本发明的缀合物包含多臂PEG，所述多臂PEG具有通式(Ia)、(Ib)、(IIa)、(IIIa)或(IVa)中任一个的结构。在这种实施方案中，至少一个R^2与本文描述的抗-因子D抗体或抗体变体共价连接。在一些实施方案中，具有通式(Ia)、(Ib)、(IIa)、(IIIa)或(IVa)中任一个的结构的多臂PEG是八聚体，并且至少两个、至少三个、至少四个、至少五个、至少六个、至少七个或全部八个R^2基团与本文描述的抗-因子D抗体或抗体变体共价连接。

[0280] 在一些方面，本发明的缀合物包括这样的种类，其中多臂多元醇共价连接至亲本抗体上的一个或多个特定位点；即，聚合物连接靶向亲本抗体或抗体片段中的特定区域或

一个或多个特定氨基酸残基。标准诱变技术可以用于改变亲本抗体或抗体片段中潜在的PEG化位点的数量和/或位置。因此，就氨基酸置换引入或替代氨基酸(诸如半胱氨酸和赖氨酸)的程度而言，本发明的抗-因子D抗体及其变体可以含有比天然序列抗-因子D更多或更少数量的潜在PEG化位点(如图1所示)。

[0281] 如上文所讨论的，聚合物的位点特异性缀合最常见地是通过与亲本抗体或抗体片段中的半胱氨酸残基连接来实现的。在这样的实施方案中，偶联化学可以例如利用不在亲本抗体的二硫桥键中的半胱氨酸残基的游离巯基。

[0282] 在一些实施方案中，天然存在于亲本抗体中的一个或多个半胱氨酸残基被用作用于聚合物缀合的一个或多个连接位点。在其它实施方案中，抗体或抗体变体上的游离氨基可以用2-亚氨基-四氢噻吩(thiolane)(Traut's试剂)硫醇化，并且然后与例如马来酰亚胺官能化的PEG偶联，如在Pedley等人，Br.J.Cancer，Vol.70，pp.1126-1130(1994)中描述的。在另一个实施方案中，将一个或多个半胱氨酸残基工程改造到亲本抗体中的所选择的一个或多个位点中以用于为聚合物提供一个或多个特定连接位点。

[0283] 先前已经描述了半胱氨酸工程改造的抗体(美国专利公开号2007/0092940和Junutula，J.R.等人，J.Immunol Methods，Vol.332(1-2)，pp.41-52(2008)，全部通过引用整体结合于此)。在一些实施方案中，半胱氨酸工程改造的抗体可以是亲本抗体。这些可用于产生在特定位置(通常在恒定结构域，例如C_L或C_H1)具有游离半胱氨酸的抗体片段。经工程改造以含有半胱氨酸的亲本抗体在本文中称为“ThioMab”，并且由这种半胱氨酸工程改造的抗体制备的Fab片段(不论制备方法)在本文中称为“ThioFab”。如先前所描述的(参见例如美国专利公开号2007/0092940和Junutula，J.R.等人，J.Immunol Methods，Vol.332(1-2)，pp.41-52(2008))，对于具有替代的(“工程改造的”)半胱氨酸(Cys)残基的突变体，评价新引入的工程改造的半胱氨酸硫醇基团的反应性。硫醇反应性值是范围为0至1.0的相对数值项，并且可以对任何半胱氨酸改造的抗体进行测量。除具有反应性硫醇基团之外，应该选择ThioMab以便它们保持抗原结合能力。先前详细描述了半胱氨酸工程改造的抗体的设计、选择和制备(参见例如WO 2011/069104，其通过引用结合于此)。在一些实施方案中，工程改造的半胱氨酸被引入到重链或轻链的恒定结构域中。同样地，半胱氨酸工程改造的抗体保持它们的野生型(亲本抗体对应物)的抗原结合能力，并且同样地能够与抗原特异性结合。

[0284] 在一些方面，本发明涉及抗体片段-聚合物缀合物，其中所述抗体片段是Fab，并且所述聚合物连接至通常将形成连接轻链和重链的链间二硫键的Fab片段的轻链或重链中的一个或多个半胱氨酸残基。

[0285] 在另一个方面，本发明涉及抗体片段-聚合物缀合物，其中所述抗体片段是Fab-C，并且聚合物连接靶向Fab-C片段的铰链区。在一些实施方案中，天然存在于抗体片段的铰链区中的一个或多个半胱氨酸残基被用于连接聚合物。在另一个实施方案中，将一个或多个半胱氨酸残基工程改造到Fab-C片段的铰链区中以用于为聚合物提供一个或多个特定连接位点。在一些实施方案中，本文公开的抗-因子D抗体变体Fab片段通过在C'-末端添加一个半胱氨酸进行修饰，以为聚合物缀合提供一个连接位点。在另一个实施方案中，本文描述的抗-因子D抗体变体Fab片段通过在C'-末端添加四个另外的半胱氨酸Cys-Pro-Pro-Cys(SEQ ID NO:21)进行修饰，以为聚合物缀合提供两个连接位点。在又一个实施方案中，本文描述

的抗-因子D抗体变体Fab片段通过在C'-末端添加四个另外的半胱氨酸Ser-Pro-Pro-Cys(SEQ ID NO:111进行修饰,以为聚合物缀合提供一个连接位点。在又一个实施方案中,本文描述的抗-因子D抗体变体Fab片段通过在C'-末端添加四个另外的半胱氨酸Cys-Pro-Pro-Ser(SEQ ID NO:112)进行修饰,以为聚合物缀合提供一个连接位点。在又一个实施方案中,本文描述的抗-因子D抗体变体Fab片段通过在C'-末端添加四个另外的半胱氨酸Ala-Pro-Pro-Cys(SEQ ID NO:113)进行修饰,以为聚合物缀合提供一个连接位点。在又一个实施方案中,本文描述的抗-因子D抗体变体Fab片段通过在C'-末端添加四个另外的半胱氨酸Ser-Gly-Gly-Cys(SEQ ID NO:114)进行修饰,以为聚合物缀合提供一个连接位点。在又一个实施方案中,本文描述的抗-因子D抗体变体Fab片段具有已经修饰为以"CYGPPC"结束的C'-末端,这为聚合物缀合提供一个连接位点。

[0286] PEG化的程度和位点还可以通过调节反应条件来操纵,诸如官能化PEG和蛋白质的相对浓度,以及pH。所需PEG化程度的合适的条件可以通过改变标准PGE化反应的参数来经验地确定。

[0287] 通过任何常规方法进行抗-因子D抗体和抗体变体的PEG化。合适的PEG化条件在WO 2011/069104和WO 03/029420中陈述,这两者通过引用整体结合于此。

[0288] c.表征和活性

[0289] PEG化蛋白质可以通过SDS-PAGE、凝胶过滤、NMR、肽作图(peptide mapping)、液相色谱-质谱分光光度法和体外生物学测定来表征。通常首先通过SDS-PAGE显示fab化的程度。10%SDS中的聚丙烯酰胺凝胶电泳通常在10mM Tris-HClpH 8.0、100mM NaCl作为洗脱缓冲液中进行。为了证明哪种残基是PEG化的,可以使用蛋白酶(诸如胰蛋白酶和Lys-C蛋白酶)进行肽作图。因此,PEG化和非PEG化抗体的样品可以用蛋白酶(诸如Lys-C蛋白酶)消化,并且通过诸如反相HPLC的技术分离所获得的肽。所产生的肽的色谱图可以与先前针对抗-因子D多肽确定的肽图谱进行比较。

[0290] 然后可以通过质谱分析每个峰来验证峰中缀合物的大小。取决于缀合中使用的PEG和在峰中缀合物的大小,可以估计缀合至PEG的抗体或其变体的数量。与PEG基团缀合的一个或多个片段通常在注射后不保留在HPLC柱上并从色谱中消失。这种从色谱中的消失表明该特定片段上的PEG化应该含有至少一个可PEG化的氨基酸残基。可以使用本领域已知的方法进一步测定PEG化的抗-因子D抗体和抗体变体与因子D相互作用的能力和其它生物活性。

[0291] PEG化改变抗体药物的物理和化学性质,并且可以导致改善的药物动力学表现,包括但不限于:改善的稳定性;降低的免疫原性;增加的流体动力学半径(RH);和/或延长的循环寿命,以及增加的眼部停留时间。

[0292] 在一些实施方案中,与相应的未缀合的抗-因子D抗体或抗体变体相比,本发明的缀合物在经由单次玻璃体内注射施用至哺乳动物眼睛(例如人)后具有增加的半衰期。在一些实施方案中,半衰期的增加是相应的未缀合的抗-因子D抗体或抗体变体的半衰期的至少1.4倍、或至少1.5倍、或至少1.8倍、或至少2倍。

[0293] 在一些实施方案中,本发明的缀合物可以具有以下的流体动力学半径(RH):约3nm至约30nm或更大,或备选地约5nm至约25nm,并且在一些实施方案中可以是约5nm、约10nm、约15nm、约20nm、约25nm或更大,如使用本领域已知的方法确定的。

[0294] 在一些实施方案中，本发明的缀合物可以展示的稳定性(通过使用本领域已知的方法测量的结合能力(例如，结合fD的能力)损失%/月来表征)(例如，当暴露于生理条件时)为约15%、约12%、约10%、约8%、或甚至更小。

[0295] 在一些实施方案中，本发明的缀合物可以展示IC50效力值，如使用因子D依赖性因子B激活的时间分辨荧光能量转移(TR-FRET)测定所确定的，如实施例中详细描述的。在一些实施方案中，缀合物抑制因子D依赖性因子B激活，其中IC_{50}值为约25pM至约10nM、或约25pM至约5nM、或约25pM至约1nM、或约25pM至约750pM、或约25pM至约500pM。

[0296] 在一些实施方案中，缀合物具有使其适于通过窄孔径针施用的粘度。在一些实施方案中，在150-250mg/ml的浓度下，缀合物的粘度小于800cP，小于700cP，小于600cP，小于500cP，小于400cP，小于300cP，小于200cP，小于100cP，小于50cP，或小于30cP。在一些实施方案中，在200mg/ml的浓度下，缀合物的粘度小于300cP。

[0297] 药物制剂

[0298] 可以通过将具有所需纯度的缀合物与通常在本领域中使用的任选的“药用”载体、赋形剂或稳定剂(所有这些都被称为”赋形剂”)混合来制备本发明的缀合物的治疗制剂以作为冻干制剂或水溶液储存。例如，缓冲剂、稳定剂、防腐剂、等渗剂、非离子去垢剂、抗氧化剂和其它混合添加剂。(参见Remington's Pharmaceutical Sciences，第16版，A.Osol编著(1980))。这些添加剂在所用的剂量和浓度对于接受体必须是无毒性的。

[0299] 缓冲剂有助于将pH维持在接近生理条件的范围内。它们优选地以范围为约2mM至约50mM的浓度存在。适用于本发明的缓冲剂包括有机和无机酸及其盐，诸如柠檬酸盐缓冲剂(例如，柠檬酸单钠-柠檬酸二钠混合物、柠檬酸-柠檬酸三钠混合物、柠檬酸-柠檬酸单钠混合物等)、琥珀酸盐缓冲剂(例如，琥珀酸-琥珀酸单钠混合物、琥珀酸-氢氧化钠混合物、琥珀酸-琥珀酸二钠混合物等)、酒石酸盐缓冲剂(例如，酒石酸-酒石酸钠混合物、酒石酸-酒石酸钾混合物、酒石酸-氢氧化钠混合物等)、富马酸盐缓冲剂(例如，富马酸-富马酸单钠混合物等)、富马酸盐缓冲剂(例如，富马酸-富马酸单钠混合物、富马酸-富马酸二钠混合物、富马酸单钠-富马酸二钠混合物等)、葡萄糖酸盐缓冲剂(例如，葡萄糖酸-葡萄糖酸钠混合物、葡萄糖酸-氢氧化钠混合物、葡萄糖酸-葡萄糖酸钾混合物等)、草酸盐缓冲剂(例如，草酸-草酸钠混合物、草酸-氢氧化钠混合物、草酸-草酸钾混合物等)、乳酸盐缓冲剂(例如，乳酸-乳酸钠混合物、乳酸-氢氧化钠混合物、乳酸-乳酸钾混合物等)以及乙酸缓冲剂(例如，乙酸-乙酸钠混合物、乙酸-氢氧化钠混合物等)。另外，可以提及的有磷酸盐缓冲剂、组氨酸缓冲剂和三甲胺盐诸如Tris。

[0300] 可以添加防腐剂以阻止微生物生长，并且其可以以范围为0.2%-1%(w/v)的量添加。适用于本发明的防腐剂包括苯酚、苄醇、间甲酚、对羟基苯甲酸甲酯、对羟基苯甲酸丙酯、十八烷基二甲基苄基氯化铵、苯扎卤铵(例如，苯扎氯铵、苯扎溴铵、苯扎碘铵)、六甲氯铵、对羟基苯甲酸烷基酯(诸如对羟基苯甲酸甲酯或对羟基苯甲酸丙酯)、儿茶酚、间苯二酚、环己醇和3-戊醇。

[0301] 可以添加等渗剂(有时也被称为“稳定剂”)以保证本发明的液体组合物的等渗性，并且其包括多元糖醇，优选三元或更高元糖醇，诸如甘油、赤藓醇、阿糖醇、木糖醇、山梨糖醇和甘露糖醇。

[0302] 稳定剂是指宽的赋形剂种类，其在功能上覆盖的范围可以是从填充剂到添加剂，

所述添加剂使治疗剂溶解或者有助于防止变性或粘附到容器壁上。典型的稳定剂可以是多元糖醇(以上例举的);氨基酸,诸如精氨酸、赖氨酸、甘氨酸、谷氨酰胺、天冬酰胺、组氨酸、丙氨酸、鸟氨酸、L-亮氨酸、2-苯丙氨酸、谷氨酸、苏氨酸等;有机糖或糖醇,诸如乳糖、海藻糖、水苏糖、甘露糖醇、山梨糖醇、木糖醇、核糖醇、肌醇(myoinisitol),半乳糖醇、甘油等,包括环醇诸如肌醇(inositol);聚乙二醇;氨基酸聚合物;含硫还原剂,诸如尿素、谷胱甘肽、硫辛酸、硫代乙酸钠、硫代甘油、α-单硫代甘油和硫代硫酸钠;低分子量多肽(即<10残基);蛋白质,诸如人血清白蛋白、牛血清白蛋白、明胶或免疫球蛋白;亲水聚合物,诸如聚乙烯吡咯烷酮;单糖,诸如木糖、甘露糖、果糖、葡萄糖;二糖,诸如乳糖、麦芽糖、蔗糖;和三糖,诸如棉子糖;多糖,诸如葡聚糖。稳定剂可以以范围为0.1至10,000重量/重量份的活性蛋白质存在。

[0303] 可以添加非离子表面活性剂或去垢剂(也称为"湿润剂")以促进治疗剂的溶解以及保护治疗性蛋白质免于搅拌导致的聚集,其也允许制剂暴露于剪切表面应力而不导致蛋白质的变性。合适的非离子表面活性剂包括聚山梨醇酯(20、80等)、泊洛沙姆(184、188等)、Pluronic®多元醇、聚氧乙烯山梨糖醇酐单酯(Tween®-20、Tween®-80等)。非离子表面活性剂可以以约0.05mg/ml至约1.0mg/ml(优选约0.07mg/ml至约0.2mg/ml)的范围存在。

[0304] 另外的混合赋形剂包括填充剂(例如淀粉)、螯合剂(例如EDTA)、抗氧化剂(例如,抗坏血酸、甲硫氨酸、维生素E)和助溶剂。本文中的制剂也可以含有多于一种活性成分,所述活性成分是被治疗的特定适应证所需的。在一些实施方案中,活性成分具有彼此不产生不利影响的互补活性。例如,可能需要进一步提供免疫抑制剂。这种分子以对预期目的有效的量适当地存在于组合中。活性成分还可以包埋于例如分别通过凝聚技术或界面聚合制备的微胶囊(例如,羟甲基纤维素微胶囊或明胶微胶囊和聚(甲基丙烯酸甲酯)微胶囊)、胶质药物递送系统(例如,脂质体、白蛋白微球体、微乳液、纳米粒子和纳米胶囊)或巨乳液(macroemulsion)中。这种技术在Remington's Pharmaceutical Sciences,第16版,A.Osal编著(1980)中公开。

[0305] 用于体内施用的制剂必须是无菌的。例如,这可通过借助无菌滤膜的过滤而轻易地实现。可以制备持续释放制备物。持续释放制备物的合适实例包括含有抗体或其抗体变体或片段(例如抗原结合片段)的固体疏水性聚合物的半透性基质,所述基质处于成形制品(例如,薄膜或微胶囊)形式。缓释基质的实例包括聚酯、水凝胶(例如,聚(2-羟乙基-甲基丙烯酸)或聚(乙烯醇))、聚交酯(美国专利号3,773,919)、L-谷氨酸和L-谷氨酸乙酯的共聚物、不可降解的乙烯-乙酸乙烯酯、可降解的乳酸-乙醇酸共聚物诸如LUPRON DEPOT™(可注射微球,其由乳酸-乙醇酸共聚物和乙酸亮丙瑞林组成)以及聚-D-(-)-3-羟基丁酸。聚合物诸如乙烯-乙酸乙烯酯和乳酸-乙醇酸能够释放分子超过100天,而某些水凝胶在更短的时间段内释放蛋白质。当被包封的抗体留在体内很长时间时,它们可能由于在37℃暴露于湿气而变性或聚集,从而导致生物活性丧失以及可能的免疫原性的改变。可以根据所涉及的机制设计用于稳定化的合理策略。例如,如果发现聚集机制是通过硫-二硫互换的分子间S--S键形成,则可以通过以下方法实现稳定化:修饰巯基残基、从酸性溶液中冷冻干燥、控制水分含量、使用合适的添加剂以及开发特异性聚合物基质组合物。

[0306] 用于预防或治疗眼部疾病或病症的本发明的缀合物通常通过以下方式使用:眼部、眼内和/或玻璃体内注射,和/或巩膜旁注射,和/或筋膜下(subtenon)注射,和/或脉络

膜上注射，和/或以滴眼液和/或软膏的形式局部施用。本发明的这种缀合物可以通过多种方法递送，例如作为允许化合物缓慢释放到玻璃体内的装置和/或贮藏物经玻璃体内递送，包括在参考文献诸如Intraocular Drug Delivery,Jaffe,Jaffe,Ashton,和Pearson,编辑,Taylor&Francis(2006年3月)中描述的那些。在一个实例中，装置可以呈微型泵和/或基质和/或被动扩散系统和/或包封的小室(cell)的形式，其在延长的时间段内释放化合物(Intraocular Drug Delivery,Jaffe,Jaffe,Ashton,和Pearson,编辑,Taylor&Francis(2006年3月)。也可以使用其它施用方法，其包括但不限于局部、肠胃外、皮下、腹膜内、肺内、鼻内和病变内施用。肠胃外输注包括肌肉内、静脉内、动脉内、腹膜内或皮下施用。

[0307] 可以通过本领域中已知的方法和使用本领域中已知的成分来制备用于眼部、眼内或玻璃体内施用的制剂。对有效治疗的主要要求是适当穿透通过眼睛。与其中可以局部递送药物的眼睛前部疾病不同，视网膜疾病需要更加具有位点特异性的方法。滴眼液和软膏很少穿透眼睛的后部，并且血眼屏障妨碍被系统性施用的药物穿透到眼组织中。因此，通常，所选择的用于药物递送以治疗视网膜疾病(诸如AMD和CNV)的方法是直接的玻璃体内注射。通常以一定间隔重复玻璃体内注射，所述间隔取决于患者的状态以及被递送的药物的性质和半衰期。对于眼内(例如玻璃体内)穿透，通常优选的是具有较小尺寸的分子。在一个实施方案中，使用窄孔径针进行玻璃体内施用。在一个实施方案中，窄孔径针是30、29、28、27、26、25、24、23或22号。

[0308] 可以通过通常用于评价眼内疾病的各种终点来测量补体相关性眼睛病症(诸如AMD或CNV)的治疗功效。例如，可以评价视力丧失。可以通过但不限于例如以下方法来评价视力丧失：通过从基线到所需时间点的最佳校正视敏度(BCVA)的平均改变来测量(例如，其中BCVA是基于糖尿病性视网膜病早期治疗研究(ETDRS)视力表并且在4米的测试距离进行评价)，测量相比于基线在所需时间点在视敏度方面失去少于15个字母的受试者的比例，测量相比于基线在所需时间点在视敏度方面得到多于或等于15个字母的受试者的比例，测量在所需时间点视敏度Snellen等效值为20/2000以下的受试者的比例，测量NEI视功能问卷，测量所需时间点的CNV的尺寸和CNV的渗漏量，例如通过荧光血管造影术等。可以进行眼部评价，例如，其包括但不限于例如进行眼部检查、测量眼内压、评价视敏度、测量裂隙灯压力、评价眼内炎症等。

[0309] 将有效治疗特定病症或病况的抗体或其抗体变体的量将取决于病症或病况的性质，并且可以通过标准临床技术来确定。在可能的情况下，首先需要在体外测定剂量-反应曲线和本发明的药物组合物，然后在对人进行测试之前在有用的动物模型系统中进行测定。

[0310] 在一些实施方案中，可以将本文描述的抗体和缀合物配制成抗体在制剂中的浓度为：至少50mg/mL、至少75mg/mL、至少100mg/mL、至少125mg/mL、至少150mg/mL、至少175mg/mL、至少200mg/mL、至少225mg/mL、至少250mg/mL、至少275mg/mL、至少300mg/mL、至少325mg/mL、至少350mg/mL、至少375mg/mL、至少400mg/mL、至少425mg/mL、至少450mg/mL、至少475mg/mL或至少500mg/mL。在一些实施方案中，抗体在制剂中的量为至少100mg/mL。在一些实施方案中，抗体在制剂中的量为至少200mg/mL。在一些实施方案中，抗体在制剂中的量为至少300mg/mL。在一些实施方案中，可以将本文描述的抗体和缀合物配制成抗体在制剂中的浓度为：约50mg/mL至约500mg/mL、约50mg/mL至约300mg/mL、约100mg/mL至约500mg/

mL、约100mg/mL至约300mg/mL、约200mg/mL至约500mg/mL、约200mg/mL至约400mg/mL、约200mg/mL至约300mg/mL、或约250mg/mL至约375mg/mL。

[0311] 在一些实施方案中，通过皮下注射来施用包含治疗性多肽、抗体或其抗体变体或其片段(例如抗原结合片段)的缀合物的水溶液。在另一个实施方案中，通过玻璃体注射来施用包含治疗性多肽、抗体或其抗体变体或其片段(例如抗原结合片段)的缀合物的水溶液。每剂可以是范围为约0.3mg至约30mg/眼。

[0312] 用于皮下施用的给药时间表可以从每月一次到每天一次变化，这取决于许多临床因素，包括疾病类型、疾病的严重度以及受试者对治疗剂的敏感性。

[0313] 制品或试剂盒

[0314] 本发明的另一个实施方案是含有可用于治疗、预防和/或诊断本发明的抗体或其变体或其片段(例如抗原结合片段)所靶向的病症的物质。例如，本发明涉及含有可用于治疗、预防和/或诊断补体相关性病症的物质的制品。所述制品包括容器和在所述容器上或与所述容器相结合的标签或包装插页。合适的容器包括，例如，瓶、小药瓶、注射器等。容器可以由多种材料诸如玻璃或塑料制成。所述容器容纳了可有效用于治疗、预防和/或诊断补体相关性病症的组合物并且可以具有无菌接入口(例如所述容器可以是静脉内输液袋或是具有可由皮下注射针头刺透的瓶塞的小药瓶)。组合物中的至少一种活性剂是本发明的抗-因子D抗体缀合物。所述标签或包装插页指示所述组合物可用于治疗、预防和/或诊断特定病症。

[0315] 包装插页是指通常包含在治疗产品的商品包装中的使用说明，其含有关于涉及此类治疗产品的使用的适应症、用法、剂量、施用、禁忌症和/或警告的信息。在一些实施方案中，所述标签或包装插页指示所述组合物用于治疗补体相关性病症，诸如例如之前列出的任何病症，包括眼部病症，例如年龄相关性黄斑变性(AMD)。所述标签或包装插页还将包含用于将抗体组合物施用至患者的使用说明。

[0316] 因此，制品还可以含有第二容器，其包含药用缓冲液，诸如注射用抑菌水(BWFI)、磷酸盐缓冲盐水、林格氏(Ringer)溶液和葡萄糖溶液。它可以进一步包括从商业和用户观点来看所需的其它材料，包括其它缓冲液、稀释剂、滤器、针和注射器。

[0317] 在另一个实施方案中，还提供这样的试剂盒，所述试剂盒可用于各种目的，例如，用于治疗、预防和/或诊断补体相关性病症，用于补体相关性溶血测定，用于从细胞纯化或免疫沉淀因子D多肽。对于因子D多肽的分离和纯化，所述试剂盒可以含有与小珠(例如，琼脂糖小珠)偶联的抗-因子D抗体。可以提供这样的试剂盒，所述试剂盒包含用于在体外(例如在ELISA或蛋白质印迹法中)检测和定量因子D多肽的抗体。与制品一样，所述试剂盒包括容器和在所述容器上或与所述容器相结合的标签或包装插页。所述容器盛有包含本发明的缀合物的组合物，所述缀合物包含至少一种抗-因子抗体。可以包括另外的容器，其含有例如稀释剂和缓冲液、对照抗体。所述标签或包装插页可以提供组合物的描述以及用于预期的体外或检测用途的使用说明。所述标签或包装插页可以提供用于向受试者施用(例如抗体或其抗体片段(例如抗原结合片段)的使用说明。

[0318] 治疗用途

[0319] 本发明的缀合物可以用于治疗哺乳动物。在一些实施方案中，缀合物被施用于非人哺乳动物以例如用于获得临床前数据。示例性的待治疗的非人哺乳动物包括非人灵长

类、狗、猫、啮齿动物和进行临床前研究的其它哺乳动物。所述哺乳动物可以是用于要用所述抗体治疗的疾病的建立的动物模型，或者可以用于研究目的抗体的毒性。在这些实施方案中的每一个中，可以在哺乳动物上进行剂量逐步增大研究。

[0320] 可以通过任何合适的手段施用缀合物，包括肠胃外施用、皮下施用、腹膜内施用、肺内施用和鼻内施用以及如有需要用于局部免疫抑制治疗、病灶内施用。肠胃外输注包括肌肉内、静脉内、动脉内、腹膜内或皮下施用。另外，缀合物适合通过脉冲输注(pulse infusion)使用，特别是用递减剂量的抗体或其抗体变体或其片段(例如抗原结合片段)。在一些实施方案中，给药可以通过注射(诸如静脉内或皮下注射)进行，这部分地取决于施用是否是短暂的或长期的。在一个实施方案中，使用窄孔径针进行玻璃体内施用。在一个实施方案中，窄孔径针是30、29、28、27、26、25、24、23或22号。在一个实施方案中，使用可植入端口递送系统施用给药。

[0321] 为了预防或治疗疾病，缀合物的合适剂量将取决于所治疗的疾病的类型、疾病的严重度和进程，抗体是否出于预防目的或治疗目的而施用、先前的疗法、患者的临床史以及对抗体的反应和主治医师的判断。

[0322] 取决于疾病的类型和严重度，可以施用足够量的缀合物以向患者提供约1至约25mg/眼(即，约0.015mg/kg至约0.36mg/kg，假设治疗一只眼睛)，不论是例如通过一次或多次单独的施用还是通过连续输注。缀合物的典型的日剂量可以足以提供范围为约1至约20mg/眼或更多，或者约1至约15mg/眼或更多的抗体，这取决于以上提及的因素。对于数天或更长时间的重复施用，取决于病况，持续治疗直至所需的疾病症状的抑制出现。然而，其它剂量方案可以是有用的。该疗法的过程可以容易地通过常规技术和测定监测。示例性的给药方案在WO 94/04188中公开。

[0323] 缀合物组合物可以以与良好医学实践相一致的方式来配制、给药和施用。在这种情况下考虑的因素包括所治疗的具体病症、所治疗的具体哺乳动物、个体患者的临床状况、病症的病因、药剂的递送位点、施用方法、施用时间表以及医学从业者已知的其它因素。待施用的缀合物的“治疗有效量”将由这样的考虑因素决定，并且是预防、改善或治疗疾病或病症所需的最小量。所述缀合物不需要但是任选地与目前用于预防或治疗所讨论病症的一种或多种药剂进行配制。所述其它药剂的有效量取决于制剂中存在的抗体或其抗体变体或其片段(例如抗原结合片段)的量、病症或治疗的类型以及以上讨论的其它因素。这些通常以如前文所使用的相同剂量以及施用途径使用，或以迄今为止采用的剂量的约1％至99％使用。

[0324] 识别因子D作为其靶标的本文公开的抗体和包含这些抗体的缀合物可以用于治疗个体中的补体介导的(补体相关性)病症。这些病症与过度或不受控的补体激活相关。其包括：心肺分流术(cardiopulmonary bypass operation)期间的补体激活；由急性心肌梗死、动脉瘤、卒中、出血性休克、挤压伤、多器官衰竭、低血容量性休克和肠缺血后的缺血-再灌注引起的补体激活。这些病症还可以包括以下疾病或病况：炎性病况，诸如严重烧伤、内毒素血症、脓毒性休克、成人呼吸窘迫综合征、血液透析、过敏性休克、严重哮喘、血管性水肿、克罗恩氏病(Crohn's disease)、镰状细胞性贫血、链球菌感染后肾小球肾炎和胰腺炎。所述病况可以是不良药物反应、药物变态反应、IL-2诱导的血管渗漏综合征或放射摄影造影剂变态反应的结果。病症可以是系统性的。其还可以包括自身免疫疾病，诸如系统性红斑狼

疮、重症肌无力、类风湿性关节炎、阿尔茨海默病(Alzheimer's disease)和多发性硬化。补体激活还与移植排斥相关。近来,补体激活和眼病之间显示强的相关性,所述眼病诸如年龄相关性黄斑变性、糖尿病性视网膜病和其它缺血相关性视网膜病、脉络膜新血管形成(CNV)、葡萄膜炎、糖尿病性黄斑水肿、病理性近视、希佩尔-林道病、眼的组织胞浆菌病、视网膜中央静脉阻塞(CRVO)、角膜新血管形成和视网膜新血管形成。在一个实施方案中,补体相关性病症是补体相关性眼部病症。在一个实施方案中,补体相关性眼部病症选自由以下组成的组:年龄相关性黄斑变性(AMD)(包括干性和湿性(非渗出性和渗出性)形式)、脉络膜新血管形成(CNV)、葡萄膜炎、糖尿病性视网膜病、缺血相关性视网膜病、糖尿病性黄斑水肿、病理性近视、希佩尔-林道病、眼的组织胞浆菌病、视网膜中央静脉阻塞(CRVO)、角膜新血管形成和视网膜新血管形成。在一个实施方案中,补体相关性眼部病症选自中期干性形式AMD或地图状萎缩(GA)。

[0325] 包含因子D拮抗剂的缀合物可以单独施用或与至少第二治疗化合物组合施用。缀合物和任何第二治疗化合物的施用可以同时进行,例如,作为单个组合物或者作为两种以上不同的组合物,使用相同或不同的施用途径。备选地或另外地,所述施用可以以任何顺序相继进行。在某些实施方案中,在两种以上组合物的施用之间可以存在数分钟至数日至数周至数月的间隔。例如,可以首先施用包含因子D拮抗剂的缀合物,然后施用第二治疗化合物。然而,还考虑第二治疗化合物与缀合物同时施用或在缀合物之前施用。在一个实例中,因子D拮抗剂是抗-因子D抗体。在另外的实例中,抗-因子D抗体是本文所述的抗-因子D抗体变体。在一些实施方案中,第二治疗化合物选自HTRA1拮抗剂、ANG2拮抗剂(诸如例如US20090304694 A1中公开的抗-ANG2抗体)、TIE2拮抗剂(诸如例如美国专利号6,376,653中公开的抗-TIE2抗体)、VEGF拮抗剂(诸如例如以下各项中公开的VEGF拮抗剂:2015年2月26日公布的美国专利号6,884,879,和WO98/45331(贝伐单抗(bevacizumab)和其它人源化抗-VEGF抗体);WO2005/012359和WO2005/044853(G6或B20系列抗体(例如G6-31、B20-4.1),以及第二补体组分拮抗剂。在一个实例中,第二治疗化合物是HTRA1拮抗剂、ANG2拮抗剂、TIE2拮抗剂、或VEGF拮抗剂。在另外的实例中,HTRA1拮抗剂是抗-HTRA1抗体。在另一个实施方案中,ANG2拮抗剂是抗-ANG2抗体。在另一个实施方案中,TIE2拮抗剂是抗-TIE2抗体。在一些实施方案中,VEGF拮抗剂选自VEGF捕获剂(诸如aflibercept(Eylea®)和抗-VEGF抗体(诸如贝伐单抗(Avastin®)或ranabizumab(Lucentis®))。

[0326] 适用于与如本文所公开的包含抗-因子D抗体的缀合物组合施用的其它治疗剂是经典或备选补体途径的各种成员的拮抗剂(补体抑制剂)。因此,本文公开的缀合物可以与C1、C2、C3、C4、C5、C6、C7、C8和C9补体组分中的一种或多种的拮抗剂组合施用。在一些实施方案中,本文公开的包含抗-因子D的缀合物与C2和/或C4和/或C5补体组分的拮抗剂(诸如抗-C2和/或抗-C4和/或抗-C5抗体)组合。这种抗体是本领域中已知的和/或是可商购的。抗-C5抗体eculizumab(Alexion,Cheshire,CT,USA)已被批准用于治疗阵发性夜间血红蛋白尿(Paroxysmal nocturnal hemoglobinuria)(PNH)和非典型溶血性尿毒症综合征(aHUS)。其它补体抑制剂在例如美国公开号20050036991A1中公开。因此,本文公开的包含抗-因子D抗体的缀合物可以与有效量的一种或多种补体抑制剂(包括但不限于抗-C2和抗-C5抗体)组合施用,任选地与至少一种另外的因子D拮抗剂/抗体组合施用。

[0327] 在一些实施方案中,本发明中治疗患有补体介导的病症的人受试者中的补体介导

的病症包括向所述受试者施用有效量的治疗化合物，诸如因子D拮抗剂或包含因子D拮抗剂的缀合物，并且还包括向所述受试者施用有效量的第二治疗化合物，所述第二治疗化合物为HTRA1拮抗剂，ANG2拮抗剂，TIE2拮抗剂，VEGF拮抗剂，或C1、C2、C3、C4、C5、C6、C7、C8和C9补体组分中一个或多个的拮抗剂。在一个实例中，因子D拮抗剂是抗-因子D抗体，并且缀合物包含一个或多个抗-因子D抗体。在另外的实例中，抗-因子D抗体是本文描述的抗-因子D抗体变体，并且缀合物包含一个或多个抗-因子D抗体变体。在一个实例中，HTRA拮抗剂是抗-HTRA1抗体。在另一个实例中，ANG2拮抗剂是抗-ANG2抗体。在另一个实例中，TIE2拮抗剂是抗-TIE2抗体。在另一个实例中，VEGF拮抗剂是抗-VEGF抗体。在另一个实施方案中，C2和/或C4和/或C5补体组分的拮抗剂是抗-C2和/或抗-C4和/或抗-C5抗体。在一个实例中，补体介导的病症是补体相关性眼部病症。在一个实例中，眼部病症是年龄相关性黄斑变性(AMD)，包括非渗出性(例如中期干性AMD或地图状萎缩(GA))和渗出性(例如湿性AMD(脉络膜新血管形成(CNV))AMD，糖尿病性视网膜病(DR)，眼内炎和葡萄膜炎。在一个实例中，补体相关性眼部病况是中期干性AMD。在一个实例中，补体相关性眼部病况是地图状萎缩。在一个实例中，补体相关性眼部病况是湿性AMD(脉络膜新血管形成(CNV))。

[0328] 本文中的组合施用包括使用分开的制剂或单个药物制剂的共同施用，以及以任何顺序的连续施用，其中两种(或全部)活性剂同时发挥其生物学活性通常需要一段时间。

实施例

[0329] 以下实施例是以说明性而非限制性的方式提供的。除非另有说明，实施例中提到的市售试剂根据制造商的说明书使用。以下实施例以及整个说明书中以ATCC登录号识别的那些细胞的来源是美国典型培养物保藏中心(American Type Culture Collection，10801University Boulevard，Manassas，VA 20110-2209)。

[0330] 实施例1：产生抗-因子D抗体变体

[0331] Lampalizumab是一种通过结合至因子D上的外部位点而有效抑制因子D和补体旁路途径的人源化抗-因子D Fab片段，其目前处于临床开发中以用于治疗地图状萎缩(GA)(干性AMD的晚期形式)。Lampalizumab(FCFD4515S；下文中“aFD”)是由214个残基的轻链(SEQ ID NO:1)和223个残基的重链(SEQ ID NO:2)组成的抗体Fab片段。

[0332] 虽然GA中的人II期临床试验的结果表明在每月玻璃体内注射aFD的情况下获得了治疗效果，但是存在使用更高的药物剂量以实现甚至更好的效力的动机。同时，较低的给药频率将给患者提供改善的便利性，具有降低感染率和增加临床效力的潜在益处，并且可以有助于治疗患有较早期形式的干性AMD的患者。

[0333] 进行努力以进一步改善野生型aFD(WT)的物理和化学稳定性，尤其是在低pH条件下和/或在高浓度在中性pH下的稳定性。轻链上的天冬氨酸残基Asp-30和重链上的天冬氨酸残基Asp-62(图1A)被鉴定为倾向于异构化。Asp异构化涉及脱水形成环状的亚酰胺中间体(Asu)，其通常可以在pH<8下长期存在并且在离子交换色谱法(IEC)时作为基础峰被检测到。环状中间体的形成在较低的pH下被加速。环状中间体水解以形成Asp或Iso-Asp，产生与起始材料相同的电荷状态并因此是无法通过IEC检测的，以上过程在较高的pH下更快。Asp-62(根据Kabat编号的Asp-61)的异构化似乎不影响潜能因为在Fab:fD复合物的晶体结构中其不与因子D接触。Katschke等人(2012)J.Biol.Chem.287:12886。Asp-30与轻链残基Asp-

32和Asp-92一起与因子D上的碱性残基进行静电接触。Asp-30的异构化相当快速并且推测其导致观察到的抗体潜能的损失。Asp残基32和92的异构化也可能影响fD结合但是已知的是速率非常慢。位置30处的环状亚酰胺的形成及其随后水解为异天冬氨酸可能通过扰乱静电相互作用而不利地影响抗原结合。分离的碱性部分上的抗原结合测量表明环状中间体形式是有完全活性的,与作为结合损失的原因的异-asp形成一致。

[0334] 重链上的Asn-103(根据Kabat编号的Asn-101)易于脱酰胺,该反应在中性pH下比在微酸性pH(6-7)下以更高的速率进行。脱酰胺可以根据IEC时酸性峰的出现来进行检测。Asn脱酰胺,与Asp异构化一样,经由环状Asu中间体进行。然而,因为由Asn形成Asu仅在较高的pH发生(其中Asu被水解以形成Asp或异-Asp),所以通常仅检测到酸性峰。Asn-103的侧链与因子D残基Arg-172形成氢键。该位点处的脱酰胺或环状亚酰胺中间体Asu的形成对抗原结合的影响是未知的。

[0335] aFD.WT的pI(7.1)比典型的人源化Fab(pI 8-9)低。CDR-L1的组成(图1A)导致VL结构域上的负电荷簇。这些特征可能影响分子的溶解性,尤其是在低pH和低离子强度。此外,在37℃,aFD.WT的高浓度制剂即使在中性pH和生理离子强度也可能倾向于以更快的速率形成非共价二聚体。

[0336] 制备aFD.WT的若干变体以用于提高稳定性。遵循随试剂盒提供的方案,通过使用QuikChangeII®(Agilent)诱变试剂盒进行定点诱变来引入点突变。合成载有所需的密码子改变的寡核苷酸引物。通过DNA测序鉴定和确定具有设计的改变的质粒。对于小规模的表达和纯化,将DNA转化到大肠杆菌菌株64B4中。将单菌落挑取到含有50μg/mL羧苄青霉素(carbenecillin)(培养基制备代码A3232)的5mL LB培养基(培养基制备编码A2008)中,并且在14mL培养管中在以200RPM进行振荡的情况下在Innova培养箱中在37℃培养过夜。将这些培养物用于接种在1L带挡板的摇瓶中的250mL的完全豆粕(soy crap)培养基(培养基制备编码A4564)(50μg/mL羧苄青霉素)中。将培养物在30℃在以200RPM进行振荡的情况下培养过夜,然后通过离心收集。将细胞沉淀用PopCulture介质(Invitrogen)裂解,并且在Gravitrap Protein G柱(GE Healthcare)上遵循制造商提供的方案纯化Fab。对于较大规模的Fab制备,将来自10L转化细胞发酵的细胞糊悬浮在提取缓冲液中并且使用微型流化仪将其匀浆化。通过免疫亲和层析在Protein G-Sepharose或κ-select上捕获Fab并且用低pH缓冲液洗脱。将低pH洗脱物调至pH 5并且进一步通过阳离子交换色谱在S-Sepharose柱上进行纯化。纯化的蛋白质的身份通过质谱确认并且将汇集的级分浓缩至约10mg/mL,并且经由透析交换到PBS缓冲剂(pH 7.3)(本文中也被称为"PBS";8mM磷酸氢二钠(Na_2HPO_4),2mM磷酸二氢钾(KH2PO4),140mM NaCl,2.7mM KCl)中。

[0337] 实施例2:抗-因子D抗体变体的生物活性

[0338] 测试有希望的单个及组合突变体的因子D(fD)结合亲和力及抑制因子D活性的能力。

[0339] a.通过表面等离子共振(SPR)测量的因子D结合亲和力

[0340] 通过在Biacore®T200仪器上进行表面等离子共振(SPR)测量来确定因子D结合固定的aFD.WT及其变体的动力学和结合常数K_D。遵循制造商描述的方案,使用抗-huFab捕获试剂盒(GE healthcare目录号28-9583-25)将抗体Fab片段固定在S系列CM5传感器芯片上。由针对浓度以2倍增量从0.39nM变化至25nM的人因子D溶液的60μL等分试样的注射记录的

传感图，计算结合的动力学。流速为30μL/min，运行缓冲液为HBS-P+，分析温度为25℃，采用实时参比室减除，并且跟踪因子D注射后的解离达10分钟。在减除对于注射运行缓冲液所观察到的传感图后，使用BiaEval软件v4.1(GE Healthcare)根据1:1模型对数据进行分析以提取动力学和亲和力常数。

[0341] 表1:突变在对因子D的亲和力方面的影响

[0342]

突变体	SPR K_D (pM)	变体#
aFD.WT	≤10	
VL-D28S	≤10	AFD.v1
VL-D30E	≤10	AFD.v2
VL-D31S	≤10	AFD.v3
VL-D32S	26	AFD.v4
VL-D28S:D31S:D32S	280	AFD.v5
VL-D30E:D31S VH-D62E (“TM”)	≤10	AFD.v6
VL-D30E:D31S VH-D62E VL-D92E (“TM.D92E”)	≤10	AFD.v7

[0343]

VL-D28S:D30E:D31S VH-D62E (“SIESD”)	16.7±4.4	AFD.v8
VL-D28S:D30E:D31S VH-D62E VL-N34S	30	AFD.v9
VL-D28S:D30E:D31S VH-D62E VL-D92E	70	AFD.v10
VL-D28S:D30E:D31S:D92E VH-D62E:N103S	102	AFD.v15
VL-D28S:D30E:D31S VH-D62E VH-N52S	70	AFD.v11
VL-D28S:D30E:D31S VH-D62E VH-N103D	23	AFD.v12
VL-D28S:D30E:D31S VH-D62E VH-N103Q	60	AFD.v13
VL-D28S:D30E:D31S VH-D62E VH-N103S (“SIESD.N103S”)	25.6±6.3	AFD.v14

[0344] 基于在aFD.WT的轻链可变结构域(VL;SEQ ID NO:3)和重链可变结构域(VH;SEQ ID NO:4)中的位置来对突变体进行命名和编号。野生型残基的单字母代号之后是序列位置，之后是置换的氨基酸的单字母代号。通过冒号将相同结构域上的多个变化分开。

[0345] 如表1中所示，aFD.WT对fD具有高的亲和力，其为可通过SPR技术测定的极限(~10pM KD)。CDR-L1中的天冬氨酸残基28、30和31可以单独地分别用Ser、Glu和Ser置换，而不明显影响对fD的亲和力(表1)。相反，用Ser替代CDR-L1Asp32导致fD-结合的明显损失，不论

是单独地测试(AFD.v4)还是与D28S和D31S突变体(AFD.v5)组合地测试。确定将VL-D30E、D31S和VH-D62E组合的三重突变体("TM"(AFD.v6))以及向TM(AFD.v6)添加VL-D92E的四重突变体(TM.D92E(AFD.v7))的fD亲和力与野生型分子相当。VH-D62E是在进行异构化而不明显影响fD-结合的位点处的置换;VL-Asp92是具有缓慢异构化速率的抗原接触残基。将VL-D28S、D30E、D31S和VH-D62E组合的四重突变体"SIESD"(AFD.v8)显示对fD的亲和力的小的(～2倍)损失。在SIESD(AFD.v8)的情况下,VL-D92E置换导致对fD的亲和力的进一步损失(参见AFD.v10(表1中的SIESD.D92E))。

[0346] 针对利用其它残基进行的置换对脱酰胺的潜在位点进行测试。在共晶结构中,VL-N34和VH-N52都与fD接触,但是这些位点在中性pH条件下都不显示显著的脱酰胺速率。这些位点处的Ser置换导致亲和力的损失(表1;AFD.v9和AFD.v11)。VH残基Asn-103接触fD并且在PBS中具有可测量的脱酰胺速率。在SIESD(AFD.v8)的情况中,将Asn-103置换成Asp或Ser导致对fD的亲和力的小的可接受的损失(参见AFD.v12(SIESD.N103D)和AFD.v14(SIESD.N103S))(表1)。用Gln置换Asn-103导致结合亲和力的较大下降(参见AFD.v13(SIESD.N103Q)(表1)。与SIESD(AFD.v8)类似,向五重突变体SIESD.N103S(AFD.v14)添加VL-D92E的SIESD.N103S.D92E(AFD.v15)进一步导致对fD亲和力的4倍下降。

[0347] b.因子D抑制测定

[0348] 使用备选途径(AP)溶血测定测试aFD.WT和变体抑制因子D诱导的补体激活的能力。使用兔红血球(Er)的AP溶血测定之前已被描述。Pangburn(1998),Methods.Enzymol.162:639;Katschke等人(2009)J.Biol.Chem.284:10473。将Er(Colorado Serum)用佛罗那(verona1)缓冲液(GVB)中的0.5%牛皮明胶洗涤三次并重悬。以2X浓度制备aFD的稀释物并将其添加到96孔聚丙烯板中。将Er悬浮液与GVB/0.1M EGTA/0.1M $MgCl_2$混合并添加到板中。通过添加C1q耗尽的人血清来启动补体激活,以避免经由经典途径的任何补体激活(CompTech;1:3稀释在GVB中)。室温下温育30分钟后,通过添加GVB中的10mM EDTA来终止反应。将所述板离心并将上清液转移。在412nm处读取上清液的吸光度。通过非线性回归分析确定引起半最大抑制(IC50)的AFD.Ab浓度。

[0349] 表2:AFD.Ab变体的抑制效力

[0350]

样品	IC50 (nM)* AP 溶血
WT (aFD.WT)	3.4
SIESD (AFD.v8)	4.2
SIESD.N103S (AFD.v14)	4.1
TM.D92E (AFD.v7)	3.8

[0351] *RSE=±30%

[0352] 如表2中所示,变体SIESD(AFD.v8)、SIESD.N103S(AFD.v14)和TM.D92E(AFD.v7)具有抑制与aFD WT相当的fD依赖性补体激活活性的潜力,IC50测量中的标准差为±30%。

[0353] c.在延长的时间内的结合能力

[0354] 还使用SPR来测量在限定的条件下AFD.Ab变体与fD的随时间的总的结合。这些测

量中的标准差为±10%。图2A显示在pH 5.5,对于aFD.WT和AFD.Ab变体D30E(AFD.v2)和TM(AFD.v6),在一个月内,结合的损失约为40%,而对于SIESD(AFD.v8)和SIESD.N103S(AFD.v14),即使在延长的时间内(70天),结合的损失也较小,为约15%。作为比较,抗-VEGF抗体Fab片段(aVEGF)在70天内未显示结合的损失。对于aFD.WT和aVEGF,向pH 7.4条件添加盐似乎加快结合损失的速率(数据未显示)。如图2B中所示,在存在PBS的情况下(Fab蛋白浓度为100mg/ml),D30E(AFD.v2)和TM(AFD.v6)具有相当的结合损失速率,其慢于对于aFD.WT所观察到的。在37℃ 10周后的结合损失为约30%(对于aFD.WT)和20%(对于抗-因子D变体D30E(AFD.v2)和TM(AFD.v6))。对于SIESD.N103S,在37℃ 10周后的结合损失仅为10%(AFD.14;图2B),不大于实验误差,并且与在相同条件下对于aVEGF所观察到的相当。对SIESD(AFD.v8),重复在PBS实验中在100mg/mL Fab浓度的热应激以收集达70天的数据。如图2C中所示,对于SIESD(AFD.v8),70天时的结合损失小于10%。

[0355] 实施例3.具有改善的稳定性的抗-因子D抗体变体

[0356] 基于以上亲和力测定,选择若干单个或组合抗-因子D抗体变体用于进一步的稳定性分析。

[0357] a.溶解性

[0358] 首先测试样品在低离子强度和pH 6的溶解性。首先通过使用Amicon Centriprep YM-10离心过滤单元浓缩至～100mg/mL而在20mM His-HCl pH 5缓冲液中制备样品。在肉眼观察时,这些溶液在pH 5和低离子强度未显示混浊。将样品以14,000xg离心10分钟以使任何不溶性物质沉淀。没有观察到沉淀,并且通过UV吸光度测量确定溶液的蛋白浓度。将样品(～1mL)置于10K MWCO的Slide-A-Lyzer盒(Pierce)中并在4℃对1L的20mM His缓冲液,pH 6进行透析过夜,之后肉眼观察浊度。拍摄溶液的照片并提供在图6中。在pH 6和低离子强度条件(～100mg/ml,在20mM His-HCl,pH 6中),aFD.WT和D30E(AFD.v2)溶液明显是混浊的,TM(AFD.v6)溶液没那么混浊,而SIESD(AFD.v8)的溶液是清的(图6)。在如以上那样离心后,对于aFD.WT和AFD.v2,肉眼观察到大的沉淀,对于TM(AFD.v6),肉眼观察到大的沉淀,而对于SIESD(AFD.v8),肉眼没有观察到沉淀,通过UV吸光度测量确定上清液的蛋白浓度(表3)。aFD.WT和D30E(AFD.v2)显示小于50mg/ml的溶解性,TM(AFD.v6)显示接近100mg/mL的溶解性而SIESD(AFD.v8)在这些条件下可全溶。对于SIESD(AFD.v8),在pH 6透析后,蛋白浓度相对于pH 5起始浓度的小幅减小反映了透析后的稀释作用而不是AFD.v8的沉淀,因为在离心后未观察到沉淀。

[0359] 表3.AFD.Ab变体的溶解性(～100mg/ml,在20mM His-HCl,pH 6中)

[0360]

AFD.Ab 变体#	在 pH 6 透析前，在 pH 5 的浓度(mg/ml)	在 4°C pH 6 透析和离心后的浓度(mg/ml)
aFD.WT	102	40
AFD.v2 (D30E)	102	14
AFD.v6 (TM)	102	92
AFD.v8 (SIESD)	100	94

[0361] 在无盐溶解性测试中测试另外的变体AFD.v3、AFD.v12、AFD.v13和AFD.v14。在4℃

透析到pH 6缓冲液中并在37℃孵育过夜后，除了aFD.WT以往的所有蛋白质溶液都是清的(图7)。37℃孵育和离心后对蛋白浓度的测量(表4)显示，所有变体的可溶性都比aFD.WT更好。在随后对PBS(pH7.3)，一种含有盐(NaCl)的缓冲液透析时，aFD.WT的混浊溶液(图7，顶行)变清，这暗示在添加盐和/或升高pH的情况下，沉淀是可逆的(图7，底行)。关于AFD.v3的溶解性数据指示，单个氨基酸变化D31S(除去1个带负电荷的残基)可以导致增加的溶解性。AFD.v8、AFD.v12、AFD.v13和AFD.v14中进一步的氨基酸变化也导致增加的溶解性。

[0362] 表4.AFD.Ab变体在pH 6、无盐情况下的溶解性

[0363]

条件	aFD.WT	AFD.v3 (D31S)	AFD.v8 (SIESD)	AFD.v12 (SIESD.N103D)	AFD.v13 (SIESD.N103Q)	AFD.v14 (SIESD.N103S)
在pH 6透析前，在pH 5的浓度(mg/ml)	112	106	120	118	109	103
在4°C pH 6透析，在37 °C孵育过夜以及离心后的浓度(mg/ml)	63	97	99	94	96	80

[0364] 还测试了aFD.WT、SIESD(AFD.v8)和SIESD.N103S(AFD.v14)在生理pH(pH 7.3)和离子强度的条件下的溶解性。对于在生理pH和离子强度下的溶解性测试，将样品对PBS过夜透析，然后使用Amicon Centriprep YM-10离心过滤单元浓缩至227-372mg/mL。在4℃过夜孵育后，肉眼观察样品的浊度，将一部分浓缩以除去沉淀的蛋白质并通过UV吸光度测量确定蛋白浓度，并在表5中进行报告。在离心之前，aFD.WT样品是混浊的，而SIESD(AFD.v8)和SIESD.N103S(AFD.v14)的溶液是清的(图8中显示的aFD.WT、AFD.v8和AFD.v14)。对于照片(图8)中的溶液，AFD.v14的浓度为344mg/mL，然后其被进一步浓缩至372mg/mL。对于图8中的溶液，AFD.v8的浓度为269mg/ml。对于图8中的溶液，aFD.WT的浓度为227mg/mL。在离心后，在aFD.WT溶液的情况下观察到沉淀，但是对于SIESD(AFD.v8)和SIESD.N103S(AFD.v14)的溶液则没有观察到沉淀。蛋白浓度数据(表5)指示，在PBS中，aFD.WT仅能够被浓缩至227mg/mL，之后则观察到沉淀，而SIESD(AFD.v8)(≥269mg/mL)和SIESD.N103S(AFD.v14)(≥372mg/mL)的溶解性极限更高。因为SIESD(AFD.v8)在269mg/mL没有观察到沉淀，并且没有进行进一步浓缩溶液的尝试，所以这是该变体在PBS中的溶解性的下限。类似地，SIESD.N103S(AFD.v14)在PBS中的溶解性的下限是372mg/mL。在PBS中的SIESD(AFD.v8)的269mg/mL溶液在2-8℃孵育4周后保持清澈。类似地，在PBS中的SIESD.N103S(AFD.v14)的372mg/mL溶液在2-8℃孵育3周后浊度没有任何明显增加。在该浓度，在使用分子排阻色谱法(SEC)测量时，聚集体%的变化很小(图9)，在2-8℃在3周内，从0.9%增加至2.1%(3周孵育前的SEC数据(0.9%聚集体)显示在图9中；3周孵育后的SEC数据是未显示的数据)。

[0365] 表5.AFD.Ab变体的溶解性(在PBS(pH 7.3)中)

[0366]

AFD.Ab变体#	等电点(pI)*	离心后的浓度(mg/ml)
aFD.WT	7.1	227
SIESD(AFD.v8)	7.3	269
SIESD.N103S(AFD.v14)	7.4	372

[0367] *pI值通过成像毛细管等电聚焦(icIEF)确定

[0368] 还比较了变体SIESD(AFD.v8)和SIESD.N103S(AFD.v14)在可以代表用于经由玻璃体内注射施用的药物的制剂的具有pH 5.5(20mM HCl pH 5.5)和不同NaCl浓度的缓冲液中的溶解性。制备蛋白浓度为约100mg/mL的溶液并且将其对测试缓冲液透析。然后使用Amicon Centriprep YM-10离心过滤单元将这些溶液浓缩。表6中列出了使溶液在环境温度保持在视觉上清澈的获得的浓度。SIESD(AFD.v8)在pH 5.5和低NaCl浓度具有达到314mg/mL的高溶解性。在添加100mM NaCl的情况下,SIESD.N103S(AFD.v14)的达到278mg/mL的高浓度也是可实现的。

[0369] 表6.AFD.Ab变体在pH 5.5(20mM His-HCl pH 5.5)和不同NaCl浓度下的溶解性

[0370]

NaCl浓度,mM	SIESD(AFD.v8)mg/mL	SIESD.N103S(AFD.v14)mg/mL
0	314	NT
50	290	200
100	NT	278

[0371] NT=未测

[0372] 虽然与aFD.WT相比,SIESD.N103S(AFD.v14)具有两个(2)带较少负电的残基,但电荷的这些变化并未导致pI的明显变化(表5),如通过成像毛细管等电聚焦测量的(iCIEF)(Salas-Solano等,J.Sep Sci,35(22):3124(2012))。预期蛋白质在接近pI的pH具有最小的溶解性(Green,A.A.,J.Biol.Chem.,93:517-542(1931))。对于SIESD.N103S(AFD.v14),在PBS(pH 7.3)中的增加的溶解性与pI的改变不相关。更确切地,LC-CDR-L1中Asp到Ser的氨基酸变化(VL-D28S和D31S)表现为改变分子表面的电荷分布。

[0373] b.异构化和脱酰胺

[0374] 为了模拟变体对在长效递送系统中可能发现的多种条件的暴露,在37℃在变化的pH和盐条件下对抗体进行应激达数周。具体地,在以下五种不同的制剂中对抗体进行评估:

[0375] 制剂1:10mg/mL,10mM磷酸缓冲液,pH 2.5,

[0376] 制剂2:10mg/mL,10mM组氨酸HCl,pH 5.5,

[0377] 制剂3:10mg/mL,10mM磷酸缓冲液,pH 7.4(“低盐”),

[0378] 制剂4:10mg/mL,pH 7.4PBS(“高盐”;10mM磷酸,137mM NaCl);和

[0379] 制剂5:100mg/mL,pH 7.4PBS

[0380] 所有溶液都具有0.02%PS20,在37℃孵育并且每2周取样一次。评估低盐条件(pH 2.5、5.5和7.4)液体制剂中化学分解的作用。将PBS用作人玻璃体的pH和离子强度的模拟物。此外,将10mM磷酸盐,pH 7.4与PBS条件相比将揭示对化学和物理稳定性的离子强度作用。在孵育期间有规律地对PBS样品进行缓冲液交换以模仿玻璃体的交换。在所有5种条件下评估野生型AFD(“WT”或“aFD.WT”)和aVEGF。在除4号以外的所有制剂中测试D30E

(AFD.v2)和TM(AFD.v6)变体。在制剂2和5中测试SIESD(AFD.v8)和SIESD.N103S(AFD.v14)。

[0381] 被量化的化学分解是脱酰胺和异构化的脱水步骤,脱酰胺被表征为形成酸性峰,而异构化的脱水步骤被表征为形成长时间存在的长效琥珀酰亚胺(Asu)中间体,其作为碱性峰被检测到。使用阳离子交换色谱法(Dionex ProPac SAX-10columns)(IEC)来量化脱酰胺的Asn物质和脱水的Asp物质在不同制剂中的抗体样品内的出现。

[0382] 对于所有测试的条件,在受试抗体中,aFD.WT显示主峰损失和碱性峰增加的最大速率。这在pH 5.5最明显,aVEGF、D30E(AFD.v2)、TM(AFD.v6)、SIESD(AFD.v8)和SIESD.N103S(AFD.v14)都显示比aFD.WT明显低的主峰损失速率,参见图3A。SIESD.N103S(AFD.v14)显示最慢的主峰损失速率,所述速率在pH 5.5与aVEGF相似(图3A)并且在PBS中甚至比aVEGF更慢(图3B)。如图4B中所示(100mg/ml的Fab,在PBS(pH 7.3)中),D30E(AFD.v2)显示的碱性峰形成的速率为aFD.WT的约一半,而TM(AFD.v6)、SIESD(AFD.v8)和SIESD.N103S(AFD.14)显示可忽略的碱性峰形成。相反,如图4B中所示(100mg/ml的Fab,在PBS(pH 7.3)中),对于PBS条件,aFD.WT和D30E(AFD.v2)的酸性峰产生的速率是相当的并且是对于TM(AFD.v6)和SIESD(AFD.v8)所确定的约2倍慢。PBS中SIESD.N103S(AFD.v14)的酸性峰形成基本上可忽略。

[0383] c.聚集

[0384] 利用尺寸排阻色谱法(SEC)来量化受试抗体的聚集体和单体的形成。使用的柱子是TSK-GEL Super SW2000(Tosoh Biosci.)。基于生产商的说明来使用材料和条件(www.tskgel.com)。

[0385] 在PBS中配制的100mg/ml的Fab的受试抗体的基于SEC数据的在37℃随时间的单体%显示在图5中。aFD.WT显示单体峰级分每月下降3-4%。与aFD.WT相比负电荷无变化的AFD.v2(D30E)显示相似的单体损失速率。AFD.v6、AFD.v8和AFD.v14显示下降的单体损失速率。第零天时单体含量的差异反映纯化的制剂在均一性方面的微小变化。在pH 5.5和7.4(无盐)在10mg/ml蛋白浓度下,D30E(AFD.v2)和TM(AFD.v6)的聚集速率是可比的。在pH 7.4添加盐不影响AFD.Ab变体的聚集速率但是其使aVEGF的聚集速率加倍。聚集依赖于蛋白浓度,因为在PBS中将浓度从10mg/mL增加至100mg/mL使得所有受试样品的聚集速率增加(表7)。在10mg/mL浓度在10mM磷酸缓冲液pH 7.4且无NaCl中,以及在10mg/mL浓度在PBS中的聚集是最小的(表7)。在37℃,在100mg/mL浓度在PBS中的aFD.WT和D30E(AFD.v2)的单体损失(在40天内分别为5.8%和7.3%)远大于在100mg/mL在PBS中的aVEGF、TM(AFD.v6)、SIESD(AFD.v8)和SIESD.N103S(AFD.v14)的单体损失(40天内分别为1.8%、1.5%、0.7%和1.5%)。这些数据暗示AFD.v6、AFD.v8和AFD.v14具有比aFD.WT和AFD.v2更少的聚集并且可以更适合作为治疗剂,因为其可以较少地倾向于体内免疫原性。

[0386] 表7.在40天通过SEC确定的盐和蛋白浓度对AFD.Ab变体和aVEGF的聚集的作用

[0387]

制剂	40天后单体%的减小					
条件	aFD.WT	D30E (AFD.v2)	TM (AFD.v6)	SIESD (AFD.v8)	SIESD.N103S (AFD.v14)	aVEGF
10 mg/mL，在10 mM 磷酸钠 pH 7.4 中	1.6	2.1	.9			.36
10 mg/mL，在PBS 中	1.5		.8			.63
100 mg/mL，在PBS 中	5.8	7.3	1.5	0.7	1.5	1.78

[0388] 为检测作为pH的函数形成的碎片，进行毛细管电泳十二烷基硫酸钠(CE-SDS)，使用Beckman PA800System，利用无涂层的内径为50μm的熔融二氧化硅毛细管(Polymicro Technologies,Inc)。通过Beckman Coulter NXp Liquid Handling Robot利用相当于Q12695的自动化来制备样品。以5kV的电压用15秒将样品注射到毛细管中，然后以15kV的电压固定30分钟。所有样品都在环境温度下运行。所有受试抗体的电泳图都与aFD.WT的电泳图类似。仅在pH 2.5观察到明显的碎片。在任何条件下都没有观察到更高分子量的物质，指示形成的任何聚集体都是可用SDS分解的并且不是共价相连的。

[0389] 以上稳定性结果显示：抗-因子D的三重(TM(AFD.v6))和四重(SIESD(AFD.v8))突变体变体的化学稳定性与aFD.WT或D30E(AFD.v2)相比明显提高。在该系列中，SIESD.N103S(AFD.14)在pH 5.5和在PBS中具有最高的化学稳定性，类似于aVEGF的稳定性。异构化和脱酰胺位点都被去除并且在中性pH的溶解性提高，同时保持了fD结合亲和力。基于以上发现，本文中描述的所选的抗-因子D变体，尤其是SIESD(AFD.v8)和SIESD.N103S(AFD.v14)变体，适用于高浓度制剂和经由例如端口递送系统(PDS)设备的长效递送两者。例如，在生理条件的pH(～7.3)和离子强度(～150mM NaCl)下，利用耐久性的、可再填充的设备如端口递送系统的长效递送可能需要高浓度制剂和低的聚集倾向。

[0390] HVR序列列表(变体中的置换是加下划线的)

[0391]

SEQ ID NO:	描述	序列
5	WT 的 HVR-L1	ITSTDIDDDMN
6	WT/TM (AFD.v6)/TM.92E (AFD.v7)/SIESD (AFD.v8)/SIESD.N103S (AFD.v14)的 HVR-L2	GGNTLRP
7	WT/TM (AFD.v6)/SIESD (AFD.v8)/SIESD.N103S (AFD.v14)的 HVR-L3	LQSDSLPYT
8	WT/TM (AFD.v6)/TM.92E (AFD.v7)/SIESD (AFD.v8)/SIESD.N103S (AFD.v14)的 HVR-H1	GYTFTNYGMN
9	WT 的 HVR-H2	WINTYTGETTYADDFKG
10	WT/TM (AFD.v6)/TM.92E (AFD.v7)/SIESD (AFD.v8)的 HVR-H3	EGGVNN
11	TM (AFD.v6)/TM.D92E (AFD.v7)的 HVR-L1	ITSTDIESDMN
12	TM (AFD.v6)/TM.92E (AFD.v7)/SIESD (AFD.v8)/SIESD.N103S (AFD.v14)的 HVR-H2	WINTYTGETTYAEDFKG
13	TM.D92E (AFD.v7)的 HVR-L3	LQSESLPYT
14	SIESD (AFD.v8)/SIESD.N103S	ITSTSIESDMN

[0392]

SEQ ID NO:	描述	序列
	(AFD.v14)的 HVR-L1	
15	SIESD.N103S (AFD.v14)的 HVR-H3	EGGVSN

[0393] 实施例4:SIESD(AFD.v8)的高浓度制剂的稳定性

[0394] 基于以上稳定性研究,通过进行热(37℃)应力测试来评价AFD.v8对于低离子强度、pH 5.5缓冲液中的高浓度制剂的适用性。制备约100mg/mL AFD.v8的溶液,并且相对于pH 5.5的20mM组氨酸盐酸盐(His-HCl)透析,然后使用Amicon YM-10离心过滤器单元浓缩。使用280nm处的吸光度测量确定从过滤器单元移出后的蛋白质浓度为272mg/mL。使用灭菌的Spin-X(Costar)离心管过滤器通过0.22μm乙酸纤维素滤膜过滤100μL的等分试样。将包含在具有咬封盖的eppendorf管中的过滤后的溶液用石蜡膜密封并置于保持于37℃的温室中。在0、1、2、4和8个月的预定时间,取出试管,通过加入900μL储存缓冲液(10mM His-HCl,pH 5.5,10%海藻糖,0.01%聚山梨醇酯20)稀释10倍,并且冻存于-70℃直至可以进行分析。对于样品,通过离子交换色谱法(IEC)分析电荷变体的产生,通过尺寸排阻色谱法(SEC)分析聚集体的存在,通过表面等离子共振(SPR)测量分析抗原结合能力的保留,并且通过肽作图分析可变结构域中的具体化学变化。如实施例3(第00292段)中所述进行IEC,如实施例3(第00294段)中所述进行SEC。如下所述进行SPR和肽作图。

[0395] a.肽作图

[0396] 在1.5mL eppendorf管中用RCM缓冲液(6M盐酸胍,360mM Tris,2mM EDTA,pH8.6)将AFD.v8样品稀释至1mg/mL。通过加入1M二硫苏糖醇(DTT)至20mM的最终浓度,然后在37℃孵育1小时来引发还原反应。还原后,通过加入1M碘乙酸(IAA)至50mM的最终浓度并在室温下避光孵育15分钟来进行烷基化反应。使用G-25Minitrap柱,将还原和烷基化的样品缓冲液交换到消化缓冲液(25mM Tris,2.0M $CaCl_2$,pH 8.2)中。以1:40的胰蛋白酶与蛋白质的比率(按质量计)加入胰蛋白酶,通过在37℃温育4小时使其消化完全。

[0397] LC/MS-MS

[0398] 连接到Thermo Orbitrap Q Exactive质谱仪的Waters H-Class Acquity用LC/MS-MS分析。将10μg的胰蛋白酶消化的样品加载到Waters Acquity UPLC CSH柱上并使用以下LC条件运行:

[0399] 流动相A-H_2O中的0.1%FA

[0400] 流动相B-ACN中的0.1%FA

[0401] 柱温:77C

[0402] 流动速率:0.2mL/min

[0403]

时间(分钟)	%B
0	1.0

2	1.0
7	13.0
42	35.0
44.1	95.0
46.0	95.0
46.1	1.0
64	1.0

[0404] 对于质谱分析，使用FTMS(傅里叶变换MS，Orbitrap)以35K分辨率检测完整的MS1扫描。在全部MS1扫描中检测到的前8个离子(动态排除关闭)被选择用于在使用FTMS检测的数据依赖性MS2扫描中的HCD片段化。使用Thermo Scientific的XCalibur软件进行下游数据分析，包括提取离子色谱和定量天然和翻译后修饰的肽。

[0405] b.通过SPR测量结合能力

[0406] 通过SPR测量来评价结合固定的人因子D的功能活性。将S系列CM5传感器芯片对接到Biacore®T200仪器[1](GE Healthcare)中，用1X运行缓冲液引发，并且遵循制造商提供的方案用70％甘油进行标准化。传感器芯片表面使用具有制造商提供的材料和建议的方案的胺偶联试剂盒激活以用于抗原的胺偶联。在传感器芯片的流动池上，人因子D(fD)通过注射用10mM乙酸钠(pH 5)稀释fD(2.4mg/mL)制备的含有100μg/mL fD的溶液进行共价固定。流速为10μL/分钟，并且使用70μL的注射体积。这在fD的约5000共振单位(RU)的多次实验中产生了典型的偶联密度。通过注射70μL 1M乙醇胺封闭未反应的胺偶联位点。

[0407] 使用Biacore®T200评价软件的校准依赖性浓度分析程序确定AFD的抗原结合活性浓度。通过以下方法制备未受应力的AFD的标准曲线：将标准物重量稀释至5μg/mL，然后连续2倍稀释以产生2.5、1.25、0.625、0.313、0.156和0.078μg/mL的样品。通过重量稀释制备测试样品以获得约0.5、1.0或1.5μg/mL的蛋白质浓度。使用1X运行缓冲液(10mM HEPES pH 7.4，150mM NaCl，0.01％PS20)制备所有样品(200μL体积)。将60μL等分试样以10μL/分钟的流速注射到特异性抗原表面上，传感器芯片保持在25℃并且用1X运行缓冲液引发。在样品注射接近结束时由SPR信号确定与特异性抗原结合的抗体。在每个结合周期结束时通过注射30μL的10mM Gly-HCl pH 2.1来洗脱结合的抗体，以引起抗体-抗原复合物的解离。将起始材料的标准曲线用于确定SPR信号与抗体浓度之间的关系，使用四参数函数来分析数据。基于观察到的SPR信号，将由标准曲线计算的参数用于计算测试样品的抗原结合浓度。通过吸光度测量确定的该浓度与蛋白质浓度的比率给出了结合分数或百分比。

[0408] c.结果

[0409] 该热应力测试的结果表明AFD.v8是高浓度制剂中的稳定分子。在37℃下4个月后还保持了大于80％的抗原结合能力(图10A)。在4个月时发生非常少的聚集体形成，并且蛋白质为接近100％单体(通过SEC)(图10B)。酸性物质(图10B，％酸性-IEC)增加至约15％并且碱性物质增加至约20％(图10B，％碱性-IEC)表示4个月时发生的一些化学变化。在37℃下8个月后，酸性和碱性物质又有所增加，单体含量降低，并且因子D结合能力降低。肽作图表明酸性物质主要来源于CDR-H3Asn-103(根据Kabat编号的Asn-101)的脱酰胺作用(图10B，CDR-H3N101％脱酰胺作用)，而碱性变体是由N端处的焦谷氨酸形成(图10B，HC-E1％琥珀酰亚胺)和CDR-H3残基Glu-99(根据Kabat编号的Glu-95)(图10B，CDR-H3E97％异构化)的

异构化所促成的。由于Asn-103和Glu-99(根据Kabat编号,分别为Asn-101和Glu-95)与lamaplizumab和因子D的共晶体结构中的因子D接触(Katschke KJ,Jr.,Wu P,Ganesan R,Kelley RF,Mathieu MA,Hass PE,Murray J,Kirchhofer D,Weismann C,van Lookeren Campange M,"Inhibiting alternative pathway complement activation by targeting the factor D exosite",J.Biol.Chem.(2012)287:12886-92),而重链的N-末端并非如此,所以Asn-103脱酰胺和Glu-99异构化以及单体含量降低可能直接导致在8个月时因子D结合的损失。尽管如此,由于这些化学和物理变化的速率缓慢,再加上预期在较低温度下速率进一步降低,储存于2-8℃或冷冻于-20℃的AFD.v8的高浓度液体制剂似乎将给出可接受的货架期。

[0410] 实施例5:AFD.v8/v14的兔pK

[0411] 在兔中进行AFD.v8和AFD.v14的体内pK研究。由单剂量实验确定pK参数,因为在重复用药后或在通过持续递送制剂增加暴露时,人源化抗体在兔中有免疫原性。

[0412] 动物的护理遵循Genentech Institutional动物护理和使用委员会指南。将天然新西兰白(NZW)兔(41只雄性动物;3.1kg至4.1kg并且在用药时为约4月龄)分配到各剂量组并且利用在Charles River Laboratories的测试项目进行用药。

[0413] 经由单次双侧玻璃体内注射将SIESD(AFD.v8)、SIESD.N103S(AFD.v14)或雷珠单抗施用至兔并且进行观察达27天。在治疗前一天,紧接着注射后,将局部抗生素(托普霉素眼膏)两次施用于双眼,并且在注射后一天施用两次,在第1天和第2天送去验尸的动物除外。在用药前,将散瞳剂液滴(1%托吡卡胺)施用于每只眼以进行充分扩瞳。在所述过程之前或期间用异氟醚/氧气使动物镇静。还在注射前将爱尔卡因(0.5%)施用于每只眼。用稀释在无菌水中的苯扎氯铵(ZephiranTM)冲洗结膜,U.S.P.至1:10,000(v/v)。

[0414] 在快要用药前在层流柜中充填注射器。通过单次30μL玻璃体内注射(0.3mg剂量)将Fab施用于所有动物的双眼。由委员会认证的兽医眼科医生利用消毒的带有30规格x 1/2"针头的100μL Hamilton Luer Lock注射器来施用剂量。为了模拟临床用药,在下前方象限,即对于左眼和右眼,分别在5点钟和7点钟位置(当面向动物时),给眼睛用药。在处理后立即通过裂隙灯活组织显微镜检查和/或间接的检眼镜检查对眼睛进行检查。

[0415] 在通过静脉内注射戊巴比妥钠进行麻醉后,通过切割腋动脉或股动脉对所有动物进行放血。收集房水、玻璃体液和视网膜组织,在液氮中速冻并且存储在-80℃。通过在50mM Tris-HCl pH 8.0,1M NaCl中的匀浆化提取视网膜中的抗体Fab。通过以下描述的GRIP ELISA确定测试品的玻璃体和视网膜浓度。低于LLOQ的值不被用于药物动力学分析或用于绘图或概述。通过非区室分析利用标称时间和剂量来确定药物动力学参数(Phoenix WinNonlin,Pharsight Corp,Mountain View,CA)。

[0416] 在通用免疫球蛋白药物动力学(GRIP)ELISA中进行对SIESD(AFD.v8)、SIESD.N103S(AFD.v14)和雷珠单抗的分析,本文所述除外。将绵羊抗-人-IgG(The Binding Site;San Diego,CA)稀释至1000ng/mL,在0.5M碳酸盐/碳酸氢盐,pH 9.6中,并且在4℃过夜孵育期间涂覆在384孔ELISA平板(Nunc;Neptune,NJ)上。将平板用PBS加上0.05%Tween-20洗涤并且在与PBS加上0.5%牛血清白蛋白(BSA)一起孵育1至2小时期间进行封闭。该温育和所有后续温育都在温和的搅拌下在室温进行。通过在测定缓冲液(PBS,0.5%BSA,15ppm Proclin,0.05%Tween 20,0.25%CHAPS,5mM EDTA,0.35M NaCl,(pH 7.4))中40-

0.625ng/mL连续稀释AFD.v8、AFD.v14或雷珠单抗来制备标准曲线。兔玻璃体或视网膜匀浆样品分别在测定缓冲液中进行最小1:100或1:50稀释。然后将稀释的标准物、对照及样品在清洗过的平板上孵育1-2小时。在洗涤步骤后，在利用在测定稀释液(PBS+0.5％BSA+0.05％Tween 20+10ppm Proclin)中的稀释至83.3ng/mL的缀合了HRP的绵羊抗-人IgG mAb(Bethyl Laboratories Inc;Montgomery,TX)的1.5小时孵育期间检测与平板结合的AFD.v8、AFD.v14或雷珠单抗。在最后洗涤后，添加四甲基联苯胺过氧化物酶底物(Moss,Inc.,Pasadena,MD)，显色10-15分钟，并且用1M磷酸终止反应。使用酶标仪(Multiscan Ascent,Thermo Fischer;Waltham,MA)在450nm对平板进行读数，以620nm作为参比。使用内部的基于Excel的软件，由相应的标准曲线的四参数拟合计算AFD.v8、AFD.v14或雷珠单抗的浓度。考虑到玻璃体或视网膜匀浆中的最小稀释，AFD.v8、AFD.v14或雷珠单抗在兔玻璃体或视网膜匀浆中的最小可量化浓度分别为62.5ng/mL或31.25ng/mL。

[0417] 玻璃体内注射0.3mg SIESD(AFD.v8)、SIESD.N103S(AFD.v14)或比较剂量的雷珠单抗(抗-VEGF)观察到的时间依赖性浓度曲线显示在图11中。

[0418] 使用非区室模型的玻璃体数据分析指示SIESD(AFD.v8)和SIESD.N103S(AFD.v14)两者都具有与雷珠单抗非常相似的清除性质。在三眼室：玻璃体液、房水和视网膜中，三种蛋白质全部给出非常相似的暴露，如AUC参数反映的。计算的雷珠单抗的PK参数与之前在兔子中的研究的结果一致(Gaudrealt等,Retina,27:1260-6(2007))。SIESD(AFD.v8)和SIESD.N103S(AFD.v14)都显示不依赖于靶标的眼清除性质，这使得这些分子适合开发。

[0419] 实施例6:AFD.v8/v14的粘度

[0420] 因为低粘度对于玻璃体内给药是重要的，因此在不同蛋白浓度在pH5.5，低盐缓冲液中测量SIESD(AFD.v8)和SIESD.N103S(AFD.v14)的粘度。使用1000s^{-1}的剪切速率在恒温于25℃的TA Instruments锥板流变仪上进行粘度测量。

[0421] aFD.WT、SIESD(AFD.v8)和SIESD.N103S(AFD.v14)给出类似的依赖于蛋白浓度的粘度曲线，其中即使在超过200mg/mL的浓度，粘度对于玻璃体内注射来说也是可接受的(<30cP)(图12)。

[0422] 实施例7.进一步修饰抗-因子D抗体变体用于聚合物缀合物

[0423] 以上实施例中所述的aFD.WT和变体是Fab片段。虽然其轻链和重链的可变结构域(VL和VH)序列不同，如图1B中所示，但其恒定结构域CL和CH1保持相同。特别地，如图1A(SEQ ID NO:2)、图1C(SEQ ID NO:27)和图1D(SEQ ID NO:29)中所示，重链的CH1结构域终止于苏氨酸残基。为了制备用于聚合物缀合如PEG化的AFD.Ab变体，通过添加自Fab'对应物的铰链区起的第一个半胱氨酸残基将Fab片段的重链进一步修饰(例如，对于AFD.v8为Cys-修饰的HC(Fab-C)(SEQ ID NO:30)，对于AFD.v14为Cys-修饰的HC(Fab-C)(SEQ ID NO:32))，使得添加的半胱氨酸充当PEG聚合物的连接位点。所得的片段可以因此与多臂PEG的一个臂缀合。还通过添加自Fab'对应物的铰链区起的头四个残基，即Cys-Pro-Pro-Cys(SEQ ID NO:21)来修饰Fab片段的重链(例如对于AFD.v8为Cys-Pro-Pro-Cys-修饰的HC(SEQ ID NO:31)，对于AFD.v14为Cys-Pro-Pro-Cys-修饰的HC(SEQ ID NO:33))，使得添加的两个Cys都充当PEG的连接位点，导致修饰的AFD.Ab片段能够连接两个PEG分子。

[0424] 遵循随试剂盒提供的方案，使用QuikChangeII®(Agilent)诱变试剂盒制备Cys-修饰的和Cys-Pro-Pro-Cys-修饰的变体。合成载有所需的密码子改变的寡核苷酸引物。通

过DNA测序鉴定和确定具有设计的改变的质粒。对于小规模表达，将DNA转化到大肠杆菌菌株64B4中。将单菌落挑取到含有50μg/mL羧苄青霉素(carbenecillin)(培养基制备代码A3232)的5mL LB培养基(培养基制备编码A2008)中，并且在14mL培养管中在以200RPM进行振荡的情况下在Innova培养箱中在37℃培养过夜。将这些培养物用于接种在1L带挡板的摇瓶中的250mL的完全豆粕(soy crap)培养基(培养基制备编码A4564)(50μg/mL羧苄青霉素)中。将培养物在30℃在以200RPM进行振荡的情况下培养过夜，然后通过离心收集。如实施例1所描述的，将细胞沉淀用PopCulture介质(Invitrogen)裂解，并且纯化Fab。对于较大规模的Fab-C制备，将来自10L转化细胞发酵的细胞糊悬浮在提取缓冲液中并且使用微型流化仪将其匀浆化，并且如实施例8所描述的对Fab-C进行纯化。

[0425] 实施例8:AFD.v14缀合物的制备

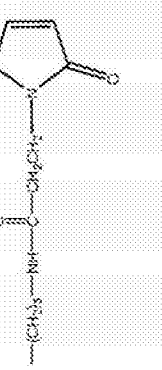
[0426] 将实施例7中制备的含有Cys修饰的HC(SEQ ID NO:32)的AFD.v14变体(本文中称为“Cys修饰的AFD.v14变体”或“AFD.v14.C”)与具有不同核心结构的市售马来酰亚胺官能化的多臂PEG缀合。

[0427] a.马来酰亚胺官能化的多臂PEG

[0428] 将下表8中详述的马来酰亚胺官能化的多臂PEG用于缀合反应中：

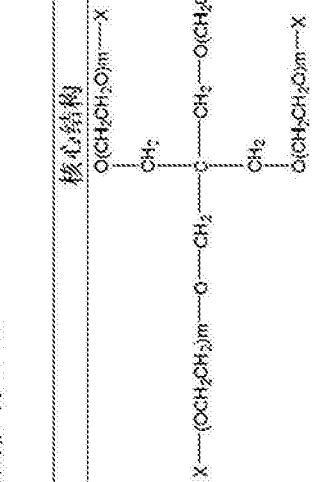
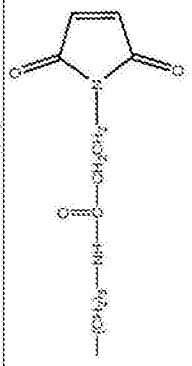
[0429]

表 8. 马来酰亚胺官能化的多臂 PEG

PEG	供应商	核心结构	官能团(X)	*多分散性	平均 MW
8ARM (TP)-PEG-MAL	JenKem Technology, USA	三季戊四醇(TP)		~1.04	40,000
8ARM-PEG-MAL	JenKem Technology, USA	六甘油(HG)		~1.08	40,000
Sunbright® HGEO-400MA	NOF America Corp.	六甘油(HGEO)		~1.33	40,000
Sunbright® DX-400MA	NOF America Corp.	丁二醇		~1.01	40,000

[0430]

表 8. 马来酰亚胺官能化的多臂 PEG

PEG	供应商	核心结构	官能团(X)	*多分散性	平均 MW
Sunbright® PTE-400MA	NOF America Corp.			--	40,000

*如供应商提供的。注意，这是指 PEG 链长度。

[0431] 使用MALDI对8ARM(TP)-PEG-MAL(JenKem Technology,USA)和Sunbright®HGEO-400MA(NOF America,Corp.)进行分析,以比较含有TP核心或HGEO核心的PEG八聚体的均匀

性。结果显示在图13A和13B中。

[0432] 从图13A和13B可以看出，含有TP核心的8ARM(TP)-PEG-MAL比含有HGEO核心的Sunbright®HGEO-400MA更均匀。

[0433] b.Cys修饰的AFD.v14变体与马来酰亚胺官能化的多臂PEG的缀合

[0434] 使用Gamma Plus树脂捕获实施例7中制备的Cys修饰的AFD.v14变体，用6.5mM GSH pH 8.5洗涤5个柱体积以将c-末端半胱氨酸去封闭并破坏Fab-C二聚体形成，接着洗脱到0.1M乙酸(pH 2.9)中。使用SP Sepharose高效强阳离子交换树脂(来自GE Healthcare)，在25mM乙酸钠(pH 5.0)中，用0.05％Triton X-100+0.05％Triton X-114将Cys修饰的AFD.v14单体进一步分离19小时以去除内毒素。在20个柱体积内，使用0-20％25mM乙酸钠(pH 5.0)+1M NaCl之间的梯度进行洗脱。然后通过使用1M HEPES pH 7.2滴定至pH 6.5来制备具有去封闭的c-末端半胱氨酸的单体Fab-C以用于PEG化。然后，将Cys修饰的AFD.v14Fab-C以约5mg/mL的浓度在25mM乙酸钠(pH 6.5)、150mM NaCl、4mM EDTA中与马来酰亚胺功能化的多臂PEG缀合。Cys修饰的AFD.v14变体未被进一步浓缩以使由于Fab-C二聚化引起的半胱氨酸反应性损失最小化。在平衡至室温后，将马来酰亚胺功能化的多臂PEG重悬于25mM乙酸钠(pH5.0)中至10mg/mL的浓度。将pH保持在低于pH 6以避免马来酰亚胺开环。一旦PEG溶解，将其以0.1125:1的PEG与Fab-C的摩尔比添加至Fab-C池中。然后将混合物在温和的震荡下在室温过夜。第二天，通过SEC-MALS检查缀合效率(#Fab/PEG)。

[0435] 实施例9:AFD.v14缀合物的纯化和表征

[0436] 纯化实施例8中制备的缀合物并使用SEC-MALS分析以确认PEG化并确定不同PEG核心结构的缀合效率。除非另有指明，使用在等度条件下使用磷酸盐缓冲盐水(PBS)(pH 7.2)、150mM NaCl以0.8mL/分钟运行的300x 8mm Shodex OH pak SB-804HQ，通过尺寸排阻色谱(SEC)确定缀合效率。摩尔质量使用Wyatt Technology的直列静态多角度激光散射(MALS)确定。将光子相关光谱用于确定流体动力学半径(RH)，使用准弹性光散射(QELS)，即具有99.0°处检测的单光子计数模块(也属于Wyatt Technology)。使用Wyatt所有的Astra软件处理原始数据，其中使用利妥昔单抗标准品设定摩尔质量和RH常数。

[0437] a.Cys修饰的AFD.v14-8ARM(TP)-PEG-MAL缀合物

[0438] 在20mM His-乙酸盐(pH 5.5)、50mM NaCl(等度梯度)中，在Sephacryl S-300HR(GE Healthcare)柱上使用SEC纯化实施例8中制备的Cys修饰的AFD.v14-8ARM(TP)-PEG-MAL缀合物(含有TP核心结构)(下文中称为“AFD.v14TP缀合物”或“AFD.v14.C+TP八聚体”)。使用Wyatt Technology的直列静态多角度激光散射(MALS)和Shodex OH pak SB-804，确定摩尔质量和缀合效率(图14C)。使用Wyatt所有的Astra软件分析原始数据，其中使用利妥昔单抗标准品设定摩尔质量常数。摩尔质量用于估计与每个PEG连接的AFD.v14变体的平均数量。结果显示于图14A、14B和14C以及表9中。

[0439] 表9

[0440]

级分#	摩尔质量 (g/mol)	估计的 Fab 化*
B2	502,000	agg
B3	470,200	n/d
B4	453,200	n/d
B5	444,300	8 个 Fab/PEG
B6	430,400	8 个 Fab/PEG
B7	410,900	8 个 Fab/PEG
C1	388,100	7 个 Fab/PEG
C2	349,100	6-7 个 Fab/PEG

[0441] agg＝聚集物;n/d＝未确定

[0442] *B5-B7视为MALS测量中基于误差%的8个Fab/PEG。

[0443] 从表9中可以看出,Cys修饰的AFD.v14变体(AFD.v14.C)与具有TP核心的多臂PEG八聚体的缀合产生了包含8个Fab/PEG的缀合物,这表明使用包含TP核心的PEG八聚体可以实现良好的缀合效率(例如,8个Fab/PEG从缀合的约45%回收)。

[0444] b.Cys修饰的AFD.v14-8ARM-PEG-MAL缀合物

[0445] 在20mM His-乙酸盐(pH 5.5)、50mM NaCl(等度梯度)中,在Sephacryl S-300HR(GE Healthcare)柱上使用SEC纯化实施例8中制备的Cys修饰的AFD.v14-8ARM-PEG-MAL缀合物(含有HG核心结构(JenKem))(下文中称为“AFD.v14HG缀合物”或“AFD.v14.C+HG八聚体”)。使用Tosoh G3000PW柱和Wyatt Technology的直列静电MALS确定摩尔质量和缀合效率。将光子相关光谱用于确定流体动力学半径(RH),使用准弹性光散射(QELS),即具有99°处检测的单光子计数模块(也属于Wyatt Technology)。使用Wyatt所有的Astra软件分析原始数据,其中使用利妥昔单抗标准品设定摩尔质量和RH常数。摩尔质量用于估计与每个PEG连接的AFD.v14变体的数量。结果显示于图15A、15B和15C以及表10中。

[0446] 表10

[0447]

级分#	Mw(kDa)	估计的Fab化	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个Fab/PEG	12.3(±3.7%)
F1	409.2(±0.2%)	7-8个Fab/PEG	10.8(±3.7%)
F4	342.2(±0.1%)	6-7个Fab/PEG	9.7(±2.9%)
F6	325.5(±0.2%)	6个Fab/PEG	9.5(±0.3%)
G2	302.4(±0.2%)	5-6个Fab/PEG	9.3(±3.1%)

[0448] agg＝聚集物;n/d＝未确定

[0449] 从表10中可以看出,Cys修饰的AFD.v14变体(AFD.v14.C)与包含HG核心的PEG八聚体的缀合产生了包含8个Fab/PEG的缀合物。然而,包含8个Fab/PEG的缀合物的回收率(约20%回收)约为使用具有TP核心的缀合物时包含8个Fab/PEG的缀合物的量的一半。相较于与TP核心的缀合所观察到的,与HG核心的缀合还产生了更多的包含5-7个Fab/PEG的缀合物,以及显著更多的聚集体。

[0450] 为了改善Fab化的估计和RH测量,将含有S-300纯化后获得的级分的产物汇集并备选地在PBS(pH 7.4)中在10/300Sephacryl S-400HR(GE Healthcare)柱上使用SEC-MALS进行分析,其以0.25mL/分钟运行。如上所描述的确定摩尔质量和RH。SEC和MALS结果显示在图16A和16B中。

[0451] 使用8ARM-PEG-MAL(HG核心)制备并使用Sephacryl S-400HR分析的缀合物具有12.2nm(±4.5%)的平均RH,340.3kDa(±8.9%)的平均摩尔质量,和6.4个Fab/PEG的平均值。

[0452] b.Cys修饰的AFD.v14-HGEO-400MA缀合物

[0453] 在20mM His-乙酸盐(pH 5.5)、50mM NaCl(等度梯度)中,在Sephacryl S-300HR(GE Healthcare)柱上使用SEC纯化实施例8中制备的Cys修饰的AFD.v14-HGEO-400MA缀合物(含有Sunbright®HGEO-400MA PEG)(下文中称为“AFD.v14HGEO缀合物”或“AFD.v14.C+HGEO八聚体”)。如上所描述的,使用Sephacryl S-400HR确定摩尔质量、缀合效率和RH(在PBS pH7.4中以0.25mL/分钟运行)。

[0454] 结果显示在图17A和17B中。使用Sunbright®HGEO-400MA PEG(HGEO核心)制备的缀合物具有15.2nm(±4.5%)的平均RH,423.8kDa(±10.6%)的平均摩尔质量,和8.2个Fab/PEG的平均值。

[0455] 在Sephacryl S-300HR柱上使用SEC纯化AFD.v14HGEO缀合物之后,备选地使用Tosoh G3000PW柱和Wyatt Technology的直列静态MALS确定缀合效率和摩尔质量(如上所描述的)。摩尔质量用于估计与每个PEG连接的AFD.v14变体的数量。来自该分析的结果显示于图18A和18B以及表11中。

[0456] 表11

[0457]

级分#	摩尔质量(g/mol)	估计的Fab化
B1	2,145,000(±0.8%)	agg
B2	665,800(±0.7%)	agg
B3	426,400(±0.8%)	8个Fab/PEG
B4	296,400(±0.8%)	6个Fab/PEG
B5	246,200(±0.8%)	5个Fab/PEG

B6	215,000(±0.8%)	n/d

[0458] agg＝聚集物;n/d＝未确定

[0459] 从表11中可以看出,Cys修饰的AFD.v14变体(AFD.v14.C)与包含HGEO核心的PEG八聚体的缀合产生了包含8个Fab/PEG的缀合物。相较于与TP核心的缀合所观察到的,与HGEO核心的缀合还产生了更多的包含5-6个Fab/PEG的缀合物。最后,相比于TP核心,与HGEO核心的缀合导致更多的聚集体以及更低的缀合效率。

[0460] 实施例10:AFD.v14缀合物的富集

[0461] 增加玻璃体内制剂中Fab浓度而不显著增加制剂粘度的一种方法是增加制剂中高度Fab化的缀合物的百分比。在该实例中,将阳离子交换色谱用于富集高度Fab化的缀合物。

[0462] 合并来自实施例9a中描述的AFD.v14.C+TP八聚体的SEC纯化的级分B4-B7(8个Fab/PEG的估计Fab化),并且在25mM乙酸钠pH 5.0中使用SP Sepharose高效强阳离子交换树脂(GE Healthcare)进行阳离子交换色谱(CEX),其中使用0.05%Triton X-100+0.05%Triton X-114洗涤19小时以去除内毒素,然后在50个柱体积(CV)内使用25mM乙酸钠pH 5.0加上1M NaCl在10-20%之间梯度洗脱。如上所描述的,使用Shodex OH pak SB-804HQ,使用SEC-MALS+QELS分析级分。结果显示在图19A、19B、19C和表12中。

[0463] 表12

[0464]

级分#	摩尔质量(g/mol)	估计的Fab化	GEL泳道#
3B11	335,000	6个Fab/PEG	1,8
3E12	367,100	7个Fab/PEG	2,9
4A7	414,200	8个Fab/PEG	3,10
4C5	430,000	8个Fab/PEG	4,11
4F3	483,900	n/d	5,12
4H9	567,400	n/d	6,13

[0465] 汇集在SP Sepharose树脂上由CEX获得的含有缀合物的级分,并且使用在等度条件下使用磷酸盐缓冲盐水(PBS)(pH 7.4)、150mM NaCl以0.8mL/分钟运行的300x 8mm Shodex OH pak SB-804HQ进行分析。如上所描述的确定摩尔质量和RH。MALS结果显示于图20中。

[0466] 富集后,获得了使用8ARM(TP)-PEG-MAL(TP核心)制备的缀合物,其具有10.5nm(±2.5%)的平均RH,407.1kDa(±0.2%)的平均摩尔质量,和7.8个Fab/PEG的平均值。

[0467] 汇集上述的TP缀合物的阳离子交换色谱纯化后获得的含有缀合物的级分(CEX池),并且与以下级分进行比较:使用Sephacryl S-300HR(GE Healthcare)(参见实施例9a)的SEC后获得的汇集级分(S300池),和在20mM His-乙酸盐、pH 5.5、50mM NaCl(等度梯度)中使用Sephacryl S-400HR(GE Healthcare)的SEC后获得的汇集级分(数据未显示)(S400池)。将汇集的组分进行毛细管SDS凝胶电泳(CE-SDS),并且结果显示于图21A和21B中。

[0468] 从图21A和21B中可以看出,S-300和S-400树脂上的纯化得到了类似的结果。然而,使用CEX富集缀合物显著地富集了包含8个Fab/PEG的缀合物的量,而去除了较低和较高分子量污染物。

[0469] 实施例11:PEG核心的比较

[0470] 使用SEC-MALS比较实施例8中制备的缀合物的性质，包括8ARM-(TP)-PEG-MAL(含有TP核心结构)、8ARM-PEG-MAL(含有HG核心结构(JenKem))、或Sunbright®-DX-400MA PEG(含有丁二醇核心结构，本文中称为“AFD.v14Dx缀合物”或“AFD.v14.C+DX八聚体”)。在20mM His-乙酸盐(pH 5.5)、50mM NaCl(等度梯度)中，在Sephacryl S-300HR(GE Healthcare)柱上使用SEC纯化HG和DX缀合物。对于TP缀合物，使用如实施例9a所述在Sephacryl S-300HR上纯化后获得的汇集级分(“CEX负载”)和如实施例10所述在CEX富集后获得的汇集级分(“TP最终”)。使用Wyatt Technology的MALS以及在等度条件下使用磷酸盐缓冲盐水(PBS)(pH 7.2)、150mM NaCl以0.8mL/分钟运行的300x 8mm Shodex OH pak SB-804Hq来确定摩尔质量和缀合效率。如上所述确定RH。结果显示在图22A、22B和表13中。

[0471] 表13

[0472]

缀合物	PEG 核心结构	M_W (kDa)	多分散性 (Mw/Mn)	R_H (nm)	凝胶泳道#
AFD.v14.C + TP 八聚体*	三季戊四醇(TP)	407.1	1.004	10.5	6, 12
AFD.v14.C + HG 八聚体	六聚甘油(HG, JenKem)	539.1	1.289	12.8	4, 10
AFD.v14.C + DX 八聚体	丁二醇(DX)	355.6	1.005	9.2	3, 9

[0473] *数据是对于最终的TP。凝胶泳道5和11(图22A)是CEX负载。

[0474] 使用本领域已知的方法确定多分散性，并且特别地使用可商购自Wyatt Technology的Astra软件来确定多分散性。

[0475] 可从这些结果观察到，虽然AFD.v14DX缀合物具有低的多分散性，但是其无法提供与AFD.v14.C+TP八聚体一样高的缀合效率。

[0476] 实施例12:AFD.v14缀合物的粘度

[0477] 由于低粘度对于玻璃体内施用是重要的，所以在pH 7.4磷酸盐缓冲盐水(PBS)溶液中，在不同的蛋白浓度下测量实施例8中制备的、与PEG八聚体(8ARM(HG)-PEG-MAL，来自JenKem Technology,USA;AFD.v14.C+HG八聚体)或PEG四聚体(Sunbright®PTE-400MA，来自NOF America Corp.)缀合的Cys-修饰的AFD.v14变体(AFD.v14.C)的粘度。使用1000s^{-1}的剪切速率在恒温于40℃的TA Instruments锥板流变仪上进行粘度测量。结果显示在图23中。

[0478] 从图23中可以看出，在相当的粘度下，相比于缀合至四聚体，AFD.v14变体与HGEO八聚体的缀合允许更高的蛋白浓度。

[0479] 将不同蛋白浓度下的AFD.v14HGEO缀合物(AFD.v14.C+HGEO八聚体)粘度与AFD.v14.C+TP八聚体的粘度进行比较。在20mM His-Ace、50mM NaCl制剂中，在pH 6.5下，在不同蛋白浓度下测量粘度。使用1000s^{-1}的剪切速率在恒温于20℃的TAInstruments锥板流

变仪上进行粘度测量。结果显示在图24中。

[0480] 从图24中可以看出，在相当的蛋白浓度下，AFD.v14.C+TP八聚体具有比AFD.v14.C+HGEO八聚体更低的粘度。

[0481] 实施例13:AFD.v14缀合物的热稳定性

[0482] 为了模拟AFD.v14缀合物对可能在长效递送系统中发现的条件的暴露，将AFD.v14.C+TP八聚体(实施例8中制备)的样品为在37℃在两种不同的pH和盐条件下应激数周。特别地，缀合物在以下制剂中进行评价：

[0483] 制剂1:10mg/mL，PBS;和，

[0484] 制剂2:10mg/mL，20mM组氨酸HCl，50mM NaCl，pH 6.5。

[0485] 将PBS用作人玻璃体的pH和离子强度的模拟物。将在PBS或20mM His-乙酸盐pH 6.5、50mM NaCl中以10mg/mL配制的AFD.v14-TP缀合物溶液的等分试样(100μL)通过使用0.22μmCostar®Spin-X离心管(Corning)的离心过滤进行无菌过滤，然后在37℃温育0、2、4或8周(分别为(T0、T2w、T4w或T8w)。温育通过在-70℃冷冻而终止。在解冻后，如上所描述的，使用Shodex OH pak SB-804HQ通过SEC-MALS分析样品，通过CE-SDS和biacore来评价fD结合能力，如下所描述的。图25A和25B中显示了通过CE-SDS确定的缀合物的相对峰面积作为孵育时间的函数，其表明37℃下缀合物的1％/周的减少。通过SEC-MALS观察到缀合物中相似的变化，其中游离Fab和二聚体种类增加(图26)。确定测量中不存在大于标准误差的结合能力的变化(±10％)，用于在37℃温育缀合物(图28)。即使在磷酸盐缓冲盐水(PBS)中在37℃达8周后且在pH 6.5下4周后，结合能力仍保持稳定。

[0486] a.CE-SDS分析

[0487] 材料和试剂:在使用前从-70℃解冻AFD.v14.C+Tp八聚体样品。氰化钾(KCN)和3-(2-呋喃甲酰基)喹啉-2-甲醛(FQ)试剂购自Molecular Probes(Eugene,OR,USA)。磷酸二氢钠和磷酸氢二钠、二甲亚砜(DMSO)和二硫苏糖醇(DTT)以及N-乙基马来酰亚胺购自Sigma-Aldrich(St.Louis,MO,USA)。十二烷基磺酸钠(SDS)、0.1M氢氧化钠(NaOH)和0.1M盐酸(HCl)试剂购自J.T.。可替换的筛选凝胶购自Beckman Coulter,Inc.(Fullerton,CA,USA)。

[0488] 溶液:使用来自Millipore纯化系统(Billerica,MA,USA)的去离子的18.2MΩ水制备水溶液。0.1M磷酸钠、pH 6.7反应缓冲液和4％SDS的溶液通过0.2μm膜滤器(Millipore,Bedford,MA,USA)过滤并在施用前稀释。在二甲亚砜(DMSO)中制备20mM荧光FQ的储备溶液并且避光储存于-20℃。在使用前解冻等分试样并用水稀释。

[0489] FQ标记程序:使用NAP-5凝胶过滤柱(GE Healthcare,Piscataway,NJ,USA)将AFD.v14.C+TP八聚体(300μg)的溶液换到0.5mL磷酸钠反应缓冲液中以去除可能竞争的制剂成分。将250μL的脱盐缀合物的等分试样与30μL的溶于4％SDS中的150mM N-乙基马来酰亚胺混合，并且在70℃温育5分钟以控制变性条件下的二硫键重排(参见，例如，Michels,D.A.,Brady,L.J.,Guo,A.,Balland,A.,Anal Chem 2007,79,5963-5971)。将十微升的每份2.5mM FQ和30mM KCN试剂添加到SDS-AFD.v14溶液中，并且将最终溶液在50℃温育10分钟，然后用1％SDS稀释三倍以猝灭反应。为了减少分析，将稀释的样品的等分试样与50mM DTT在70℃温育10分钟。

[0490] CE-SDS分析:使用包裹在40℃热控制药筒中的50μm ID的31.2cm(21cm有效长度)熔融二氧化硅毛细管(Polymicro technologies,Phoenix,AZ,USA)进行AFD.v14.C+TP八聚

体样品的分离。全自动Beckman PA800+系统(Beckman Coulter,Brea,CA,USA)配备有LIF检测器,并且使用32Karat 9.1版以控制仪器。LIF检测器使用在488nm处激发的3.5mW氩离子激光器;通过600±20nm带通滤波器(Edmund Optics,Barrington,NJ,USA)收集发射。电压以负模式(反极性)施加。将样品溶液在5kV下电动引入25s并在17kV下分离。在运行之间,毛细管分别用0.1M NaOH、0.1M HCl和Beckman凝胶缓冲液洗涤5分钟、1分钟、1分钟和10分钟。(参见,例如,Michels等人,Anal Chem 2007,79,5963-5971;Michels等人,Electrophoresis 2012,33,815-826)。

[0491] b.结合能力

[0492] 以下材料购自GE Healthcare:Series S CM5传感器芯片(目录号BR-1005-30);10X Biacore®运行缓冲液(目录号BR-1006-71):0.1M Hepes pH 7.4,1.5M NaCl,0.5% Polysorbate®20;再生溶液(目录号BR-1003-55):10mM Gly-HCl pH 2.1;和胺偶联试剂盒(目录号BR-1000-50)。将S系列CM5传感器芯片对接到Biacore®T200仪器(GE Healthcare)中,用1X运行缓冲液引发,并且遵循制造商提供的方案用70%甘油进行标准化。传感器芯片表面使用具有制造商提供的材料和建议的方案的胺偶联试剂盒激活以用于抗原的胺偶联。人因子D(fD)通过注射用10mM乙酸钠(pH 5)稀释fD(PUR#20491,2.4mg/mL)制备的含有100μg/mL抗原的溶液进行共价固定。流速为10μL/分钟,并且使用70μL的注射体积。这在fD的约5000共振单位(RU)的多次实验中产生了典型的偶联密度。通过注射70μL 1M乙醇胺封闭未反应的胺偶联位点。使用Biacore®T200评价软件的校准依赖性浓度分析程序确定抗体Fab的抗原结合活性浓度。通过以下方法制备AFD.v14.C+TP八聚体的标准曲线:将储备溶液重量稀释至5μg/mL,然后连续2倍稀释以产生2.5、1.25、0.625、0.313、0.156和0.078μg/mL的样品。通过重量稀释制备测试样品以获得约0.5、1.0或1.5μg/mL的蛋白质浓度。所有样品(200μL体积)使用1X运行缓冲液制备。将60μL等分试样以10μL/分钟的流速注射到特异性抗原表面上,传感器芯片保持在25℃并且用1X运行缓冲液引发。在样品注射接近结束时由SPR信号确定与特异性抗原结合的抗体。在每个结合周期结束时通过注射30μL的10mM Gly-HCl pH 2.1来洗脱结合的抗体,以引起抗体-抗原复合物的解离。将AFD.v14.C+TP八聚体的标准曲线用于确定SPR信号与抗体浓度之间的关系,使用四参数函数来分析数据。基于观察到的SPR信号,将由标准曲线计算的参数用于计算测试样品的抗原结合浓度。通过吸光度测量确定的该浓度与蛋白质浓度的比率给出了结合分数或百分比。

[0493] 实施例14:AFD.v14缀合物的食蟹猴PK

[0494] 在食蟹猴中进行实施例8中制备的并如实施例9a和10所述纯化的AFD.v14.C+TP八聚体的体内pK研究。PK参数由单剂量实验确定。使用未缀合的、未修饰的AFD.v14(SIESD.N103S)作为对照。动物的护理遵循Genentech Institutional动物护理和使用委员会指南。

[0495] a.研究参数

[0496] 将食蟹猴(28只雄性动物;2kg至4kg,并且在给药时年龄为大约2-7岁)分配至四个给药组中的一个。通过30号针(100μl剂量体积),第1组(对照)动物(4只动物)接受5mg/眼(10mg/动物)的AFD.v14的单次双侧玻璃体内剂量。通过30号针(2次注射,每眼中50μl;100μl总剂量体积),第2组和第3组动物(每组10只动物)分别接受基于Fab重量的1或4mg/眼(2或8mg/动物)的AFD.v14.C+TP八聚体的双侧玻璃体内剂量。在注射前,使动物镇静(10mg/kg盐

酸氯胺酮,0.5mg/kg地西泮)并用局部用丙美卡因处理。然后将AFD.v14或AFD.v14.C+TP八聚体通过巩膜和角膜缘后4mm处的平坦部施用,其中使针指向晶状体后方进入中玻璃体。第4组动物(4只动物)以0.4mg/动物接受AFD.v14.C+TP八聚体的单次IV大剂量(1mL)。对于IV施用,将AFD.v14.C+TP八聚体配制为10mM琥珀酸钠、10%海藻糖和0.05%吐温-20(pH 5.0)。

[0497] 从第1、2和3组收集眼组织。在给药后的以下时间:第1组-第1(24小时)、2、4和8天;第2组和第3组-第1(24小时)、4、8、12和20天,将来自第1组的一只动物(2只眼睛)和各自来自第2组和第3组的两只动物(4只眼睛)安乐死。安乐死后,将两只眼睛摘除,并且从两只眼睛中收集玻璃体液、房水和视网膜组织。在快速冷冻眼睛几天后使用滤纸收集完整的视网膜层。确定AFD.v14和AFD.v14.C+TP八聚体在玻璃体液和房水和视网膜组织中的浓度。

[0498] 通过股静脉或头静脉收集所有血液样品(约1mL)。在IVT或IV给药后的以下时间抽取样品:第1组-1小时、6小时和第1(24小时)、2、3、4、5和7天;第2组和第3组-1小时、6小时和第1(24小时)、2、4、6、8、12和20天;第4组-1小时、6小时和第1(24小时)、2、4、7、11、14、17、21、24和28天。在血液收集的一小时内,将样品在室温凝结,并且通过离心分离血清并在-60℃至-80℃储存。

[0499] 研究方案的细节在表14中列出。

[0500] 表14.食蟹猴pK研究参数

[0501]

组	剂量	途径	动物数量	眼部时间点(天)	血清时间点
1	5 mg/眼	IVT(双侧)	4	1、2、4、8	1和6小时;1、2、3、4、5、7天
2	1 mg/眼	IVT(双侧)	10	1、4、8、12、20	6小时;1、2、4、5、8、12、20天
3	4 mg/眼	IVT(双侧)	10	1、4、8、12、20	6小时;1、2、4、6、8、12、20天
4	0.4 mg/动物	IV	4	n/a	6小时;1、2、4、7、11、14、17、21、24、28天

[0502] b.AFD.v14和AFD.v14.C+TP八聚体的药代动力学测定

[0503] 将Gyrolab XP测定用于定量食蟹猴血清、玻璃体液、房水和视网膜匀浆中的AFD.v14和AFD.v14.C+TP八聚体。将样品在样品缓冲液(磷酸盐缓冲盐水(PBS),0.5%牛血清白蛋白(BSA),15ppm Proclin(Sigma-Aldrich),0.05%吐温20,0.25%CHAPS,50μg/mL muIgG(Equitech Bio,目录号SLM66),5mM EDTA(pH 7.4))中以1:4-1:3000稀释。通过在样品缓冲液中连续稀释AFD.v14或AFD.v14.C+TP八聚体(从2.06-1500ng/mL)制备AFD.v14和AFD.v14.C+TP八聚体的标准曲线。以PBS/0.01%吐温20/0.02%NaN3中的100μg/mL的生物素缀合的山羊抗-人IgG(HC+LC,Bethyl,目录号A80-319B)和Rexxip F(Gyrolab)中的25nM的Alexa-抗-CDR(克隆234,Genentech)应用捕获和检测试剂测定在Gyrolab Bioaffy200CD上进行,并且洗涤步骤使用PBS/0.01%吐温20/0.02%NaN_3,接着使用Gyros pH 11洗涤缓冲液。如制造商所描述的以1%PMT设置运行仪器和分析数据。AFD.v14和AFD.v14.C+TP八聚体缀合物的浓度由其标准曲线的五参数拟合确定。食蟹猴血清、玻璃体液、房水和视网膜匀浆中的AFD.v14和AFD.v14.C+TP八聚体缀合物的最小可定量浓度为8.24ng/mL(0.16nM)。

[0504] 玻璃体液、房水和视网膜pK结果显示于图29A(玻璃体)和29B(玻璃体,归一化的)、图30A(房水)和30B(房水、归一化的)、和图31A(视网膜)和31B(视网膜,归一化的)和下表15-17中。

[0505] 表15.AFD.v14对照(第1组)和AFD.v14.C+TP八聚体(第2组和第3组)的玻璃体PK

[0506]

组	剂量 (μg/眼)	$T_{1/2}$ (天)	AUC (天*μg/mL)	AUC/剂量 (天*μg/mL/mg 剂量)	$T_{1/2}$ ext*	Vss (mL)	Cl (mL/天)
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

[0507] *相比于对照的增加

[0508] 表16.AFD.v14.C+TP八聚体的房水PK

[0509]

组	剂量(μg/眼)	$T_{1/2}$(天)	AUC_{last}(天*μg/mL)	Vz(mL)	Cl/F(mL/天)
2	1000	3	434	12	2.73
3	4000	5.2	1430	20	2.58

[0510] 表17.AFD.v14.C+TP八聚体的视网膜PK

[0511]

组	剂量(μg/眼)	$T_{1/2}$(天)	AUC_{last}(天*μg/mL)	Vz(mL)	Cl/F(mL/天)
2	1000	3.6	31	196	38
3	4000	5.9	98	309	36

[0512] 从表15可以看出,第2组(3.5天)和第3组(5天)的玻璃体终末半衰期比未缀合的AFD.v14对照(第1组)都要更长,并且比未缀合的lampalizumab和雷珠单抗Fab的平均半衰期(约2.34天)更长。缀合的AFD.v14第2组和第3组的平均AUC/mg-剂量(约2040)高于未缀合的lampalizumab Fab的平均AUC/mg-剂量(约1733)。基于玻璃体终末半衰期,4.0mg/眼剂量

比1.0mg/眼剂量清除得更慢。从表16和17以及图30和31可以看出，与未缀合的Fab相比，第2组和第3组(缀合的AFD.v14)在房水和视网膜中也观察到更长的终末半衰期。

[0513] 第1-3组的血清pK结果在图32A和32B(归一化)中显示，并且第4组的血清pK结果在图32C中显示。

[0514] 从图32A和32B中可以看出，第2组和第3组(AFD.v14.C+TP八聚体)的血清pK曲线彼此平行(图32A)，并且在剂量归一化后重叠(图32B)。第2组和第3组的血清AUC与剂量成比例，直到最后测量的时间点。

[0515] 第4组(AFD.v14.C+TP八聚体；IV剂量)的终末半衰期为7.5天，并且清除率为15.8mL/天(5.64mL/kg/天(第4组猴子的平均重量是2.8kg))。在第21、24和28天测量时，4只第4组猴中的3只的血清浓度降至检测限以下。

[0516] c.食蟹猴血清中因子D的药效学测定

[0517] 将夹心ELISA用于定量食蟹猴血清、玻璃体液、房水和视网膜匀浆中的因子D(fD)。在包被缓冲液(0.05M碳酸钠，pH 9.6)中将小鼠抗-人因子D克隆4676(Genentech)稀释至1μg/mL，并且在384孔Maxisorp平板(Thermo Scientific，目录号464718)上在4℃温育过夜。将平板用PBS加0.05％吐温20洗涤，并且在用PBS加0.5％牛血清白蛋白(BSA)温育2小时期间进行封闭。该温育和所有后续温育都在温和的搅拌下在室温进行。通过在样品缓冲液(补充有500ng/mL的AFD.v14治疗剂和50μg/mL小鼠IgG的测定缓冲液)中连续稀释fD(从0.04至5ng/mL)来制备食蟹猴fD标准曲线。血清样品和对照在样品缓冲液中稀释至最低1:100。在样品缓冲液中将玻璃体液、房水和视网膜匀浆样品和对照稀释至最小1:10。然后将稀释的标准品、对照和样品在平板上温育2小时，并且使用对于AFD.Ab的生物素缀合的小鼠抗-CDR mAb(克隆242，1μg/mL)检测与平板结合的fD/AFD.Ab复合物达一小时，接着还用高灵敏度SA-HRP(3ng/mL，Pierce目录号21130)检测一小时。最终的洗涤之后，添加四甲基联苯胺(Moss，目录号TMBE-1000)并显色10-15分钟，并且用1M磷酸终止反应。使用酶标仪，采用620nm参比在450nm处读板。由标准曲线的四参数拟合确定fD的浓度。食蟹猴血清中的最小可定量浓度为3.9ng/mL(0.16nM)。食蟹猴玻璃体液、房水和视网膜匀浆中的最小可定量浓度为0.39ng/ml(0.016nM)。

[0518] 第2、3和4组的平均血清fD和AFD.v14.C+TP八聚体浓度在图33A中显示。从图33A中可以看出，在测试的所有时间点，血清fD浓度高于AFD.Ab浓度。这些结果表明所有组中均维持系统性AP补体活性。

[0519] 第2和3组的平均眼部fD和AFD.v14.C+TP八聚体浓度在图33B中显示。从图33B中可以看出，在测试的所有时间点，玻璃体液、房水和视网膜匀浆中的AFD.Ab浓度超过了fD浓度。

[0520] 实施例15：抗-因子D抗体变体和缀合物对抑制因子D的效力

[0521] 在因子D依赖性因子B激活的时间分辨荧光能量转移(TR-FRET)测定中，确定包含Cys-修饰的Fab变体的AFD.Ab变体或缀合物对因子D的抑制效力。

[0522] 根据实施例8所述的程序，将Cys-修饰的AFD.v14变体(AFD.v14.C)和含有实施例7中制备的Cys-修饰的HC(SEQ ID NO:30)的AFD.v8变体(“Cys-修饰的AFD.v8变体”或“AFD.v8.C”)各自与马来酰亚胺功能化的多臂PEG四聚体(Sunbright® PTE-400MA，来自NOF America Corp.)缀合，以形成缀合物(下文中分别称为“AFD.v14四聚体”或“AFD.v8四聚

体”)。

[0523] 以4x浓度在酶促反应缓冲液(ERB;75mM NaCl,1mM MgCl2,25mM Tris,0.005%聚山梨醇酯20,pH 7.3)中制备AFD.Ab Fab变体、缀合物或Fab对照的稀释物,并且与0.5nM或0.2nM因子D(分别为125pM或50pM)(fD,Complement Technology;Tyler,TX)或ERB(无酶对照)以相等体积进行组合。将雷珠单抗(抗-VEGF)用作阴性对照。将因子D/AFD.Ab或因子D/缀合物混合物(7μl/孔)添加到364孔Proxiplate F plus黑色平板(Perkin Elmer Health Sciences;Waltham,MA)中,随后添加7μl/孔的底物。底物由7μg/mL(40nM)的C3b(Complement Technology)和1μg/mL(15nM)的因子B(Complement Technology)组成。将AFD.Ab Fab或缀合物、酶、辅因子和底物在温和的搅拌下在室温温育45分钟。用7μl/孔的检测试剂鸡尾酒混合物终止反应,所述混合物由8nM的生物素化的抗-因子Bb(2F12,GNE PRO282909)、4nM的铕缀合的抗-因子Ba(1C3的定制缀合,Life Technologies的GNE PRO282908;Madison,WI)和25nM的链霉抗生物素蛋白-Alexa 647组成。将平板在黑暗中在室温温育30分钟。通过在337nm处激发并检测620nm处的铕发射和665nm处的Alexa荧光发射,用PHERAstar FS酶标仪(BMG LabTech;Cary,NC)检测时间分辨荧光能量转移。通过使用四参数拟合模型(KaleidaGraph Synergy Software;Reading,PA)的非线性回归分析确定引起半最大抑制(IC50)的AFD.Ab或缀合物浓度。

[0524] TR-FRET测定的抑制曲线显示在图34A中(表18)。Lampalizumab抑制因子D依赖性fB激活的IC50为24pM,并且IC50的标准误差为±25%。针对AFD.v8和AFD.v14的IC50与针对lampalizumab所测量的IC50相当。参见图34A(表18)。缀合的Cys-修饰的AFD.Ab格式(AFD.v8四聚体和AFD.v14四聚体)相比于未缀合的Fab的IC50差异很可能是由处理更粘稠的PEG化分子的难度所造成的(图34A,表18)。

[0525] 表18.因子D依赖性因子B激活的IC50(50pM fD)

[0526]

分子	平均 IC50 fB 激活 (pM)
AFD.v8	27.65
AFD.v14	34.03
AFD.v8 四聚体	11.03
AFD.v14 四聚体	14.77
雷珠单抗	n/a
Lampalizumab	24.38

[0527] 在添加125pM fD的情况下,在使用上述程序的因子D依赖性因子B激活的TR-FRET测定中,还确定了AFD.v14.C+TP八聚体抑制因子D的效力。AFD.v14.C+TP八聚体(AFD.v14.C+TP八聚体)的IC50与AFD.v14、Cys-修饰的AFD.v14(“AFD.v14.C”)和lampalizumab相当。将雷珠单抗用作阴性对照。结果显示在图34B和表19中。

[0528] 表19.因子D依赖性因子B激活的IC50(125pM fD)

[0529]

分子	平均 IC50 fB 激活 (pM)
Lampalizumab	72
AFD.v14	87
AFD.v14 TP 八聚体(S200 池)*	104
AFD.v14 TP 八聚体(CEX 池)^	77
AFD.v14.C	72
雷珠单抗	n/a

[0530] *在Sephacryl S-200HR(GE Healthcare)柱上使用SEC纯化后获得的。

[0531] ^在CEX富集(实施例10)后获得的。

[0532] AFD.v14TP八聚体(S300池和CEX池)的IC50是有效的,并且是与针对未缀合的Fab(lampalizumab,AFD.v14,AFD.v14.C)所测量的相当。使用阳离子交换色谱富集AFD.v14TP八聚体导致更有效的产物。

[0533] 实施例16:抗-因子D抗体变体和缀合物对系统性补体旁路活性的影响

[0534] 先前已经显示了Lampalizumab瞬时抑制食蟹猴中的系统性补体功能(参见Loyet等人,J.Pharmacol.Exp.Ther.,2014,Vol.351,pp.527-537)。在当前实例中,在食蟹猴中评价玻璃体内施用抗-因子D抗体变体或AFD.Ab缀合物对系统性补体旁路途径(AP)活性的影响。

[0535] a.食蟹猴中的药代动力学/药效学研究

[0536] AFD.Ab变体和缀合物通过单剂量IVT或静脉内注射施用至中国来源的雄性食蟹猴(M.fascicularis)以评价分子的药代动力学(PK)和药效学(PD)。这些研究在Covance实验室(Madison,WI)进行。所有程序均遵从美国农业部动物福利法条例(9CFR 3)、实验动物护理和使用指南以及实验室动物福利办公室的规定进行。

[0537] 进行了四项研究。在第一项(对照)研究中(研究1,n=10),将lampalizumab施用至两只眼睛,以两个50μL IVT剂量,间隔15分钟。这些动物接受10mg/眼,总计20mg/动物。在给药前(第-2天)和给药后以下时间点收集血液:45分钟,以及2、6、10、24、34、48、96、120、154、192、288和384小时。在24、48、120、192和384小时收集血液后,将每组两只动物从研究中移出,并且进行安乐死以收集眼基质。lampalizumab对照研究先前已在Loyet等人,J.Pharmacol.Exp.Ther.,2014,351:527-537中描述。

[0538] 在研究2(n=3)中,将AFD.v14施用至两只眼睛,以两个50μL IVT剂量,间隔15分钟。这些动物接受25mg/眼,总计50mg/动物。在给药前(第-1和-3天)和给药后以下时间点收集血液:30分钟,以及2、8、24、48和96小时。

[0539] 在研究3(n=10)中,将AFD.v14.C+Tp八聚体施用至两只眼睛,以两个50μL IVT剂量,间隔15分钟,以提供3.9mg/眼的AFD.v14,总计7.8mg/动物的AFD.v14。在给药前(第-1和-2周)和给药后以下时间点收集血液:1、6、24、48、72、96、144、192、288和480小时。将每个

时间点(在24、96、192、288和480小时)的每组两只动物从研究中移出,并且进行安乐死以收集眼基质。

[0540] 在研究4中,将AFD.v14.C+HG八聚体以两个50μL IVT剂量施用至两只眼睛,间隔15分钟,以提供7.1mg/眼的AFD.v14(n=2)或11.8mg/眼的AFD.v14(n=1),总计14.2mg/动物的AFD.v14或23.6mg/动物的AFD.v14。在给药前(第-7和-1天)和给药后以下时间点收集血液:1、6、24、96和168小时。

[0541] 对于所有研究,通过股静脉从每只动物收集给药前和给药后血清样品以用于PK和PD分析。在每个时间点,将全血收集到血清分离管中,允许在环境温度凝结至少20分钟,然后在设置为2℃-8℃的温度范围的冷冻离心机中离心。在离心20分钟内收集血清并储存在-60℃和-80℃之间直至分析。

[0542] b.总AFD.v14/缀合物分析

[0543] 将Gyrolab XP测定用于定量食蟹猴血清中的AFD.v14,AFD.v14.C+TP八聚体和AFD.v14.C+HG八聚体。将样品在样品缓冲液(磷酸盐缓冲盐水(PBS),0.5%牛血清白蛋白(BSA),15ppm Proclin(Sigma-Aldrich),0.05%吐温20,0.25%CHAPS,50μg/mL muIgG(Equitech Bio,目录号SLM66),5mM EDTA(pH 7.4))中以1:4-1:3000稀释。通过在样品缓冲液中连续稀释AFD.v14、AFD.v14.C+TP八聚体或AFD.v14.C+HG八聚体(从2.06-1500ng/mL)制备AFD.v14和AFD.v14TP和HG缀合物标准曲线。以PBS/0.01%吐温20/0.02%NaN3中的100μg/mL的生物素缀合的山羊抗-人IgG(HC+LC,Bethyl,目录号A80-319B)和Rexxip F(Gyrolab)中的25nM的Alexa-抗-CDR(克隆234,Genentech)应用捕获和检测试剂测定在Gyrolab Bioaffy 200CD上进行,并且洗涤步骤使用PBS/0.01%吐温20/0.02%NaN_3,接着使用Gyros pH 11洗涤缓冲液。如制造商所描述的以1%PMT设置运行仪器和分析数据。AFD.v14、AFD.v14.C+TP八聚体和AFD.v14.C+HG八聚体的浓度由其标准曲线的五参数拟合确定。对于食蟹猴血清中的AFD.v14、AFD.v14.C+TP八聚体和AFD.v14.C+HG八聚体,最小可定量浓度为8.24ng/mL(0.16nM)。

[0544] c.食蟹猴血清中因子D的药效学测定

[0545] 使用夹心ELISA来定量食蟹猴血清中的因子D(fD)。在包被缓冲液(0.05M碳酸钠,pH 9.6)中将小鼠抗-人因子D克隆4676(Genentech)稀释至1μg/mL,并且在384孔Maxisorp平板(Thermo Scientific,目录号464718)上在4℃温育过夜。将平板用PBS加0.05%吐温20洗涤,并且在用PBS加0.5%牛血清白蛋白(BSA)温育2小时期间进行封闭。该温育和所有后续温育都在温和的搅拌下在室温进行。通过在样品缓冲液(补充有500ng/mL的AFD.v14治疗剂和50μg/mL小鼠IgG的测定缓冲液)中连续稀释fD(从0.04至5ng/mL)来制备食蟹猴fD标准曲线。血清样品和对照在样品缓冲液中稀释至最低1:100。然后将稀释的标准品、对照和样品在平板上温育2小时,并且使用对于AFD.Ab的生物素缀合的小鼠抗-CDR mAb(克隆242,1μg/mL)检测与平板结合的fD/AFD.Ab复合物达一小时,接着还用高灵敏度SA-HRP(3ng/mL,Pierce目录号21130)检测一小时。最终的洗涤之后,添加四甲基联苯胺(Moss,目录号TMBE-1000)并显色10-15分钟,并且用1M磷酸终止反应。使用酶标仪,采用620nm参比在450nm处读板。由标准曲线的四参数拟合确定fD的浓度。食蟹猴血清中的最小可定量浓度为3.9ng/mL(0.16nM)。

[0546] d.AP溶血测定

[0547] 在溶血测定中评价AFD.v14和AFD.v14.C+TP八聚体抑制AP活性的能力，其中将血清(人或猴)与兔红细胞组合，如Pangburn(Methods Enzymol,1988,162:639-653)和Katschke等人(J.Biol.Chem.,2009,284:10473-10479)所设计和描述的。为了确保补体激活不是通过经典补体途径(CP)发生的，使用C1q耗尽的人血清(Complement Technologies,Tyler,TX)，并且缓冲液包含EGTA以螯合钙(即CP活性所必需的阳离子)。

[0548] 将C1q耗尽的人血清用于激活AP。存在于10％C1q耗尽的人血清中的fD浓度在孔中为9.6nM，该值与先前报道的血清中的fD水平一致(Barnum等人，J.Immunol.Methods,1984,67:303-309;Loyet等人，Invest.Ophthalmol.Vis.Sci.,2012,53:6628-6637)。

[0549] e.AFD.v14.C+HG八聚体处理的食蟹猴血清中系统性AP活性抑制的确定

[0550] 为了评价用AFD.v14.C+HG八聚体或AFD.v14.C+TP八聚体给药后系统性AP活性的任何潜在抑制的时程和剂量依赖性，进行基于平板的WIESLAB补体系统AP ELISA(来自该测定的数据在图35中称为“％AP补体活性”)或与上述体外AP溶血测定类似的离体测定(来自该测定的数据在图35中称为“％相对溶血”)。然而，在该测定中，代替将外源性AFD.v14.C+HG八聚体或AFD.v14.C+TP八聚体的稀释曲线添加到血清样品中，将样品本身连续地稀释，其具有由注射剂量的AFD.v14.C+HG八聚体或AFD.v14.C+TP八聚体引起的溶血活性的任何抑制。

[0551] 如以上所描述的，制备红细胞并进行测定，用于具有以下修改的AP溶血测定。为了确定对应于最大裂解的吸光度，用无菌水(80μl/孔)制备全裂解对照，同时将GVB添加到所有其他孔(50μl)中。将食蟹猴血清样品在六个点上以1:1.5连续稀释，并且与阴性对照(仅缓冲液)一起添加到96孔U底聚丙烯板(30μl/孔)中。总裂解对照表示最大(100％)溶血。一式三份地收集数据点，并且将平均最大溶血百分比相对测定中最终血清稀释度的倒数作图。通过使用四参数拟合模型的非线性回归分析，确定50％最大溶血(AH50)值(定义为50％最大溶血)。对于没有达到饱和的那些曲线，使用曲线拟合估算AH50，其中将上渐近线固定于100％。将每个个体时间点的相对溶血百分比计算为[(个体时间点的给药后AH50)/(给药前AH50)]×100。来自每个个体正常食蟹猴的血清的AH50值可以从AH50值的整体平均值变化多达2倍。因此，将来自每个研究动物的给药前和给药后样品在相同的测定板上运行，以确保将AP活性的给药后变化与个体动物的基线补体活性直接进行比较。

[0552] f.结果

[0553] 相比于总fD和治疗活性的相对溶血百分比显示于图35A(lampalizumab,10mg/眼)、图35B(AFD.v14,25mg/眼)和图35C(AFD.v14.C+TP八聚体,3.9mg/眼)中。lampalizumab数据(图35A)是在IVT施用10mg/眼的lampalizumab之后获得的比较数据，如Loyet等人，J.Pharmacol.Exp.Ther.,2014,351:527-537)中所描述的。从图35B可以看出，施用25mg/眼的AFD.v14瞬时抑制系统性AP活性，其中活性在施用后24小时恢复至基线，类似于先前对lampalizumab所观察到的结果(图35A)。相比之下，在施用3.9mg/眼的AFD.v14.C+TP八聚体后未观察到系统性AP抑制(图35C)。不希望受任何特定理论束缚，据信与Fab(例如，lampalizumab和AFD.v14)相比，用缀合物获得的较慢的从眼睛的清除率允许fD在较早的时间点使AFD.Ab饱和，防止系统性补体抑制。

[0554] 相对于总fD和总缀合物的相对AP补体活性百分比显示于图35D(AFD.v14.C+HG八聚体,7.1mg/眼)和图35E(AFD.v14.C+HG八聚体,11.8mg/眼)中。从这些图中可以看出，对于

IVT剂量高达11.8mg/眼的AFD.v14.C+HG八聚体，观察到可忽略的系统性补体抑制。由于从眼睛清除较慢，所以缀合物浓度保持低于fD的摩尔浓度，特别是在早于10小时的时间点。这与类似的AFD.Ab Fab眼部给药浓度相反，其中在这些早期时间点，摩尔浓度超过摩尔fD浓度并导致系统性AP抑制。

[0555] 本领域技术人员将认识到，或者能够仅使用常规实验确定，本文所述发明的具体实施方案的许多等价物。所述等价物旨在涵盖于以下权利要求中。

[0556] 尽管为了清楚理解的目的已经通过说明和实施例的方式较详细地描述了前述发明，但是描述和实施例不应被解释为限制本发明的范围。本文引用的所有专利和科学文献的公开内容明确地通过引用整体并入。

[0557] 上述书面说明书被认为足以使得本领域技术人员能够实施本发明。本发明的范围不受保藏的构建体的限制，因为保藏的实施方案意在作为本发明的某些方面的简单说明，并且功能相当的任何构建体都在本发明的范围内。实际上，根据以上描述，除本文中显示和描述的那些修改之外的本发明的各种修改对本领域技术人员将是明显的，并且落入所附权利要求书的范围内。

序列表

<110> 健泰科生物技术公司
豪夫迈·罗氏有限公司

<120> 抗-因子D抗体变体缀合物及其用途

<130> P33044-WO

<150> US 62/249,020
<151> 2015-10-30

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<151> 2015-11-04

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[0001]

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Ser Gly Gly Asn Thr Leu Arg Pro Gly Val Pro Ser Arg Phe Ser Gly
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Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
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Glu Asp Val Ala Thr Tyr Tyr Cys Leu Gln Ser Asp Ser Leu Pro Tyr
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Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
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Ser Gly Gly Asn Thr Leu Arg Pro Gly Val Pro Ser Arg Phe Ser Gly
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Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
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Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
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35 40 45

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Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
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35 40 45

Ser Gly Gly Asn Thr Leu Arg Pro Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
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Glu Asp Val Ala Thr Tyr Tyr Cys Leu Gln Ser Asp Ser Leu Pro Tyr
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Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
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Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
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Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

[0013]

Phe Asn Arg Gly Glu Cys
210

<210> 27
<211> 223
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 27

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe

50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

[0014] Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr
210 215 220

<210> 28
<211> 214
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 28

Asp Ile Gln Val Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ile Thr Ser Thr Ser Ile Glu Ser Asp
20 25 30

Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Leu Ile
35 40 45

Ser Gly Gly Asn Thr Leu Arg Pro Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Val Ala Thr Tyr Tyr Cys Leu Gln Ser Asp Ser Leu Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

[0015]

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> 29
<211> 223
<212> PRT

〈213〉 人工序列

〈220〉
〈223〉 合成多肽

〈400〉 29

[0016]

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Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1               5                  10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
            20                  25                  30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
    50                  55                  60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Glu Arg Glu Gly Gly Val Ser Asn Trp Gly Gln Gly Thr Leu Val Thr
            100                 105                 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
        115                 120                 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
    130                 135                 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145                 150                 155                 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
                165                 170                 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
            180                 185                 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
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195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr
210 215 220

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<400> 30

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

[0017]

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys
210 215 220

<210> 31
<211> 227
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 31

[0018]

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro

115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys
210 215 220

[0019] Pro Pro Cys
225

<210> 32
<211> 224
<212> PRT
<213> 人工序列

<220>
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<400> 32

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Ser Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

[0020]

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys
210 215 220

<210> 33
<211> 227
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 33

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr

20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Ser Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

[0021] Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys
210 215 220

Pro Pro Cys
225

<210> 34

<211> 223
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 34

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

[0022]

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Leu
210 215 220

<210> 35
<211> 222
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<220>
<223> 合成多肽

<400> 35

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

[0023]

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala

145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
210 215 220

<210> 36
<211> 221
<212> PRT
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<220>
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[0024] <400> 36

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
210 215 220

[0025]

<210> 37
<211> 220
<212> PRT
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<220>
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<400> 37

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr

65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

[0026] Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220

<210> 38
<211> 219
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 38

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

[0027]

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
210 215

<210> 39
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<213> 人工序列

<220>
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<400> 39

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

[0028] Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys

210 215 220

<210> 40
<211> 227
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 40

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp Asp Phe
50 55 60

[0029]

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys
210 215 220

Pro Pro Cys
225

〈210〉 41
〈211〉 227
〈212〉 PRT
〈213〉 人工序列

〈220〉
〈223〉 合成多肽

〈400〉 41

[0030]

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro

115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys
210 215 220

[0031] Pro Pro Ser
225

<210> 42
<211> 227
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<213> 人工序列

<220>
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<400> 42

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

[0032]

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Ser
210 215 220

Pro Pro Cys
225

<210> 43
<211> 227
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<213> 人工序列

<220>
<223> 合成多肽

<400> 43

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala

1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

[0033] Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Ala
210 215 220

Pro Pro Cys

225

<210> 44
<211> 227
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 44

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp Asp Phe
50 55 60

[0034]

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Ser
210 215 220

Gly Gly Cys
225

<210> 45
<211> 223
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 45

[0035]
Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro

115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Tyr Gly Pro Pro Cys
210 215 220

[0036] <210> 46
<211> 216
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<220>
<223> 合成多肽

<400> 46

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly
180 185 190

[0037]

Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Thr Val Glu Arg Lys
210 215

<210> 47
<211> 217
<212> PRT
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<220>
<223> 合成多肽

<400> 47

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met

35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

[0038] Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly
180 185 190

Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Thr Val Glu Arg Lys Cys
210 215

〈210〉 48
〈211〉 220
〈212〉 PRT
〈213〉 人工序列

〈220〉
〈223〉 合成多肽

〈400〉 48

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

[0039]

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro
210 215 220

〈210〉 49
〈211〉 219
〈212〉 PRT
〈213〉 人工序列

〈220〉
〈223〉 合成多肽

〈400〉 49

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp Asp Phe
50 55 60

[0040] Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly

180 185 190

Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro
210 215

<210> 50
<211> 218
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 50

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

[0041]

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Arg Val Glu Ser Lys Tyr Gly
210 215

〈210〉 51
〈211〉 217
〈212〉 PRT
〈213〉 人工序列

〈220〉
〈223〉 合成多肽

[0042]

〈400〉 51

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr

100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys
195 200 205

[0043] Val Asp Lys Arg Val Glu Ser Lys Tyr
210 215

<210> 52
<211> 216
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 52

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

[0044]

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Arg Val Glu Ser Lys
210 215

<210> 53
<211> 221
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 53

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr

20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

[0045] Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys
210 215 220

<210> 54
<211> 108
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 54

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

[0046]

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr
100 105

<210> 55
<211> 108
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 55

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser

50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Leu
100 105

<210> 56
<211> 109
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 56

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

[0047]

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys
100 105

<210> 57
<211> 112
<212> PRT

〈213〉 人工序列

〈220〉
〈223〉 合成多肽

〈400〉 57

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

[0048] Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
100 105 110

〈210〉 58
〈211〉 112
〈212〉 PRT
〈213〉 人工序列

〈220〉
〈223〉 合成多肽

〈400〉 58

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Ser
100 105 110

<210> 59
<211> 112
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 59

[0049]

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Ser Pro Pro Cys
100 105 110

<210> 60

<211> 112
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 60

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

[0050]

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Ala Pro Pro Cys
100 105 110

<210> 61
<211> 112
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 61

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser

35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Ser Gly Gly Cys
100 105 110

<210> 62
<211> 108
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

[0051] <400> 62

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val Glu Pro Lys Ser Cys Tyr Gly Pro Pro Cys
100 105

<210> 63
<211> 107
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 63

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

[0052]

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
100 105

<210> 64
<211> 106
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 64

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
100 105

<210> 65
<211> 105
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

[0053]

<400> 65

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys

100 105

<210> 66
<211> 104
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 66

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

[0054]

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val Glu Pro Lys Ser Cys Asp
100

<210> 67
<211> 101
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 67

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1 5 10 15

Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr

20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr
65 70 75 80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Thr Val Glu Arg Lys
100

〈210〉 68
〈211〉 102
〈212〉 PRT
〈213〉 人工序列

[0055]

〈220〉
〈223〉 合成多肽

〈400〉 68

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1 5 10 15

Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln Thr
65 70 75 80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Thr Val Glu Arg Lys Cys
100

<210> 69
<211> 105
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 69

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1 5 10 15

Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

[0056]

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr
65 70 75 80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Arg Val Glu Ser Lys Tyr Gly Pro Pro
100 105

<210> 70
<211> 104
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 70

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1 5 10 15

Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr
65 70 75 80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Arg Val Glu Ser Lys Tyr Gly Pro
100

〈210〉 71
〈211〉 103
〈212〉 PRT
[0057] 〈213〉 人工序列

〈220〉
〈223〉 合成多肽

〈400〉 71

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1 5 10 15

Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr
65 70 75 80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys

85 90 95

Arg Val Glu Ser Lys Tyr Gly
100

<210> 72
<211> 102
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 72

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1 5 10 15

Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

[0058]

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr
65 70 75 80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Arg Val Glu Ser Lys Tyr
100

<210> 73
<211> 101
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 73

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg

1 5 10 15

Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr
65 70 75 80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Arg Val Glu Ser Lys
100

[0059] <210> 74
<211> 106
<212> PRT
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<220>
<223> 合成多肽

<400> 74

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
1 5 10 15

Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr
65 70 75 80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys
100 105

<210> 75
<211> 227
<212> PRT
<213> 人工序列

<220>
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<400> 75

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
[0060] 35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala

145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Ser
210 215 220

Pro Pro Cys
225

[0061]

<210> 76
<211> 227
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 76

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

[0062]

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys
210 215 220

Pro Pro Ser
225

<210> 77
<211> 227
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 77

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met

35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

[0063] Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Ala
210 215 220

Pro Pro Cys
225

<210> 78
<211> 227
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 78

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

[0064]

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Ser
210 215 220

Gly Gly Cys
225

<210> 79
<211> 223
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 79

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

[0065] Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala

145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Tyr Gly Pro Pro Cys
210 215 220

<210> 80
<211> 223
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

[0066] <400> 80

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Leu
210 215 220

[0067]

<210> 81
<211> 222
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 81

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr

65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

[0068] Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
210 215 220

<210> 82
<211> 221
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 82

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

[0069]

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
210 215 220

<210> 83
<211> 220
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 83

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
[0070] 100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys

210 215 220

<210> 84
<211> 219
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 84

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

[0071]

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
210 215

<210> 85
<211> 216
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 85

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

[0072]

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val

130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly
180 185 190

Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Thr Val Glu Arg Lys
210 215

<210> 86
<211> 217
<212> PRT
<213> 人工序列

[0073]

<220>
<223> 合成多肽

<400> 86

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly
180 185 190

Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys
195 200 205

[0074]

Val Asp Lys Thr Val Glu Arg Lys Cys
210 215

<210> 87
<211> 220
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 87

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe

50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

[0075] Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro
210 215 220

<210> 88
<211> 219
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 88

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

[0076]

Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro
210 215

<210> 89
<211> 218
<212> PRT

〈213〉 人工序列

〈220〉
〈223〉 合成多肽

〈400〉 89

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

[0077] Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys

195 200 205

Val Asp Lys Arg Val Glu Ser Lys Tyr Gly
210 215

<210> 90
<211> 217
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 90

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

[0078]

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Arg Val Glu Ser Lys Tyr
210 215

<210> 91
<211> 216
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 91

[0079]

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro

115 120 125

Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Arg Val Glu Ser Lys
210 215

[0080] <210> 92
<211> 221
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 92

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

[0081]

Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys
210 215 220

<210> 93
<211> 227
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 93

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met

35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Ser Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

[0082] Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Ser
210 215 220

Pro Pro Cys
225

<210> 94
<211> 227
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 94

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

[0083]

Glu Arg Glu Gly Gly Val Ser Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys
210 215 220

Pro Pro Ser
225

<210> 95
<211> 227
<212> PRT
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<220>
<223> 合成多肽

<400> 95

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

[0084] Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Ser Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala

145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Ala
210 215 220

Pro Pro Cys
225

<210> 96
<211> 227
<212> PRT
<213> 人工序列

[0085]

<220>
<223> 合成多肽

<400> 96

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Ser Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

[0086]

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Ser
210 215 220

Gly Gly Cys
225

<210> 97
<211> 223
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 97

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met

35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Ser Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

[0087] Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Tyr Gly Pro Pro Cys
210 215 220

<210> 98
<211> 223
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 98

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Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1               5                  10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
            20                  25                  30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
    50                  55                  60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Glu Arg Glu Gly Gly Val Ser Asn Trp Gly Gln Gly Thr Leu Val Thr
            100                 105                 110
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[0088]

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Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
        115                 120                 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
    130                 135                 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145                 150                 155                 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
                165                 170                 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
            180                 185                 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
        195                 200                 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Leu
    210                 215                 220
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<210> 99
<211> 222
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 99

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

[0089] Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Ser Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly

180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
210 215 220

<210> 100
<211> 221
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 100

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

[0090]

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Ser Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
210 215 220

<210> 101
<211> 220
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

[0091]

<400> 101

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Ser Asn Trp Gly Gln Gly Thr Leu Val Thr

100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

[0092] Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220

<210> 102
<211> 219
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 102

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Ser Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

[0093]

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
210 215

<210> 103
<211> 216
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 103

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr

20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Ser Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

[0094] Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly
180 185 190

Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Thr Val Glu Arg Lys
210 215

<210> 104
<211> 217
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 104

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

[0095]

Glu Arg Glu Gly Gly Val Ser Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly
180 185 190

Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Thr Val Glu Arg Lys Cys
210 215

<210> 105
<211> 220
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 105

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

[0096] Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Ser Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly

165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro
210 215 220

<210> 106
<211> 219
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 106

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

[0097]

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Ser Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro
210 215

〈210〉 107
〈211〉 218
〈212〉 PRT
[0098] 〈213〉 人工序列

〈220〉
〈223〉 合成多肽

〈400〉 107

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys

85 90 95

Glu Arg Glu Gly Gly Val Ser Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

[0099] Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Arg Val Glu Ser Lys Tyr Gly
210 215

<210> 108
<211> 217
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 108

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Ser Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

[0100]

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Arg Val Glu Ser Lys Tyr
210 215

<210> 109
<211> 216
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 109

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala

1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Ser Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

[0101] Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Arg Val Glu Ser Lys
210 215

<210> 110

<211> 221
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<400> 110

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

[0102]

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Ser Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys
210 215 220

<210> 111
<211> 4
<212> PRT
<213> 人工序列

<220>
<223> 合成肽

<400> 111

Ser Pro Pro Cys
1

<210> 112
<211> 4
<212> PRT
<213> 人工序列

[0103]

<220>
<223> 合成肽

<400> 112

Cys Pro Pro Ser
1

<210> 113
<211> 4
<212> PRT
<213> 人工序列

<220>
<223> 合成肽

<400> 113

Ala Pro Pro Cys
1

<210> 114
<211> 4
<212> PRT
<213> 人工序列

<220>
<223> 合成肽

<400> 114

Ser Gly Gly Cys
1

<210> 115
<211> 223
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<220>
<221> MOD_RES
<222> (223)..(223)
<223> Xaa可以是除Thr以外的任何氨基酸

<400> 115

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

[0104]

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Asp Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Xaa
210 215 220

[0105]

<210> 116
<211> 108
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<220>
<221> MOD_RES
<222> (108)..(108)
<223> Xaa可以是除Thr以外的任何氨基酸

<400> 116

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Xaa
100 105

<210> 117
<211> 223
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<220>
<221> MOD_RES
<222> (223)..(223)
<223> Xaa可以是除Thr以外的任何氨基酸

[0106] <400> 117

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Asn Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Xaa
210 215 220

[0107]

<210> 118
<211> 223
<212> PRT
<213> 人工序列

<220>
<223> 合成多肽

<220>
<221> MOD_RES
<222> (223)..(223)
<223> Xaa可以是除Thr以外的任何氨基酸

<400> 118

Glu Val Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Thr Thr Tyr Ala Glu Asp Phe
50 55 60

Lys Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Glu Arg Glu Gly Gly Val Ser Asn Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
115 120 125

[0108] Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
130 135 140

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
145 150 155 160

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
165 170 175

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
180 185 190

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
195 200 205

Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Xaa
210 215 220

WT 轻链

```
DIQVTQSPSS LSASVGDRVT ITCITSTDID DDMNWYQQKP GKVPKLLISG GNTLRPGVPS  60
RFSGSGSGTD FTLTISSLQP EDVATYYCLQ SDSLPYTFGQ GTKVEIKRTV AAPSVFIFPP 120
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT 180
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC  (SEQ ID NO:1)              214
```

WT 重链

```
EVQLVQSGPE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW INTYTGETTY  60
ADDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCEREG GVNNWGQGTL VTVSSASTKG 120
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL 180
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT  (SEQ ID NO:2)     223
```

图1A

VL结构域

WT	DIQVTQSPSSLSASVGDRVTITCITSTDIDDDMNWYQQKPGKVPKLLISGGNTLRPGVPSRFSGSGSGTDFTLTISSLQP	
TM	DIQVTQSPSSLSASVGDRVTITCITSTDIESDMNWYQQKPGKVPKLLISGGNTLRPGVPSRFSGSGSGTDFTLTISSLQP	
TM.D92E	DIQVTQSPSSLSASVGDRVTITCITSTDIESDMNWYQQKPGKVPKLLISGGNTLRPGVPSRFSGSGSGTDFTLTISSLQP	
SIESD	DIQVTQSPSSLSASVGDRVTITCITSTSIESDMNWYQQKPGKVPKLLISGGNTLRPGVPSRFSGSGSGTDFTLTISSLQP	
SIESD.N103S	DIQVTQSPSSLSASVGDRVTITCITSTSIESDMNWYQQKPGKVPKLLISGGNTLRPGVPSRFSGSGSGTDFTLTISSLQP	
WT	EDVATYYCLQSDSLPYTFGQGTKVEIK	(SEQ ID NO:3)
TM	EDVATYYCLQSDSLPYTFGQGTKVEIK	(SEQ ID NO:16)
TM.D92E	EDVATYYCLQSESLPYTFGQGTKVEIK	(SEQ ID NO:18)
SIESD	EDVATYYCLQSDSLPYTFGQGTKVEIK	(SEQ ID NO:19)
SIESD.N103S	EDVATYYCLQSDSLPYTFGQGTKVEIK	(SEQ ID NO:19)

VH结构域

WT	EVQLVQSGPELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTGETTYADDFKGRFVFSLDTSVSTAY	
TM	EVQLVQSGPELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTGETTYAEDFKGRFVFSLDTSVSTAY	
TM.D92E	EVQLVQSGPELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTGETTYAEDFKGRFVFSLDTSVSTAY	
SIESD	EVQLVQSGPELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTGETTYAEDFKGRFVFSLDTSVSTAY	
SIESD.N103S	EVQLVQSGPELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTGETTYAEDFKGRFVFSLDTSVSTAY	
WT	LQISSLKAEDTAVYYCEREGGVNNWGQGTLVTVSS	(SEQ ID NO:4)
TM	LQISSLKAEDTAVYYCEREGGVNNWGQGTLVTVSS	(SEQ ID NO:17)
TM.D92E	LQISSLKAEDTAVYYCEREGGVNNWGQGTLVTVSS	(SEQ ID NO:17)
SIESD	LQISSLKAEDTAVYYCEREGGVNNWGQGTLVTVSS	(SEQ ID NO:17)
SIESD.N103S	LQISSLKAEDTAVYYCEREGGVSNWGQGTLVTVSS	(SEQ ID NO:20)

图1B

SIESD (AFD.v8)轻链

```
DIQVTQSPSS LSASVGDRVT ITCIISISIE SDMNWYQQKP GKVPKLLISG GNTLRPGVPS  60
RFSGSGSGTD FTLTISSLQP EDVATYYCLQ SDSLPYTFGQ GTKVEIKRTV AAPSVFIFPP 120
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT 180
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC  (SEQ ID NO:26)             214
```

SIESD (AFD.v8)重链

```
EVQLVQSGPE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW INTYTGETTY  60
AEDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCEREG GVNNWGQGTL VTVSSASTKG 120
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL 180
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT   (SEQ ID NO:27)  223
```

Cys-修饰的SIESD (AFD.v8)重链

```
EVQLVQSGPE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW INTYTGETTY  60
AEDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCEREG GVNNWGQGTL VTVSSASTKG 120
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL 180
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTC    (SEQ ID NO:30)  223
```

Cys-Pro-Pro-Cys-修饰的SIESD (AFD.v8)重链

```
EVQLVQSGPE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW INTYTGETTY  60
AEDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCEREG GVNNWGQGTL VTVSSASTKG 120
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL 180
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTCPPC (SEQ ID NO:31) 223
```

图1C

SIESD.N103S (AFD.v14) 轻链

```
DIQVTQSPSS LSASVGDRVT ITCITSISIE SDMNWYQQKP GKVPKLLISG GNTLRPGVPS  60
RFSGSGSGTD FTLTISSLQP EDVATYYCLQ SDSLPYTFGQ GTKVEIKRTV AAPSVFIFPP 120
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT 180
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC  (SEQ ID NO:28)             214
```

SIESD.N103S (AFD.v14) 重链

```
EVQLVQSGPE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW INTYTGETTY  60
AEDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCEREG GVSNWGQGTL VTVSSASTKG 120
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL 180
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT   (SEQ ID NO:29)  223
```

Cys-修饰的 SIESD.N103S (AFD.v14) 重链

```
EVQLVQSGPE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW INTYTGETTY  60
AEDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCEREG GVSNWGQGTL VTVSSASTKG 120
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL 180
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTC    (SEQ ID NO:32)  223
```

Cys-Pro-Pro-Cys-修饰的 SIESD.N103S (AFD.v14) 重链

```
EVQLVQSGPE LKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW INTYTGETTY  60
AEDFKGRFVF SLDTSVSTAY LQISSLKAED TAVYYCEREG GVSNWGQGTL VTVSSASTKG 120
PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL 180
SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTCPPC (SEQ ID NO:33) 223
```

图1D

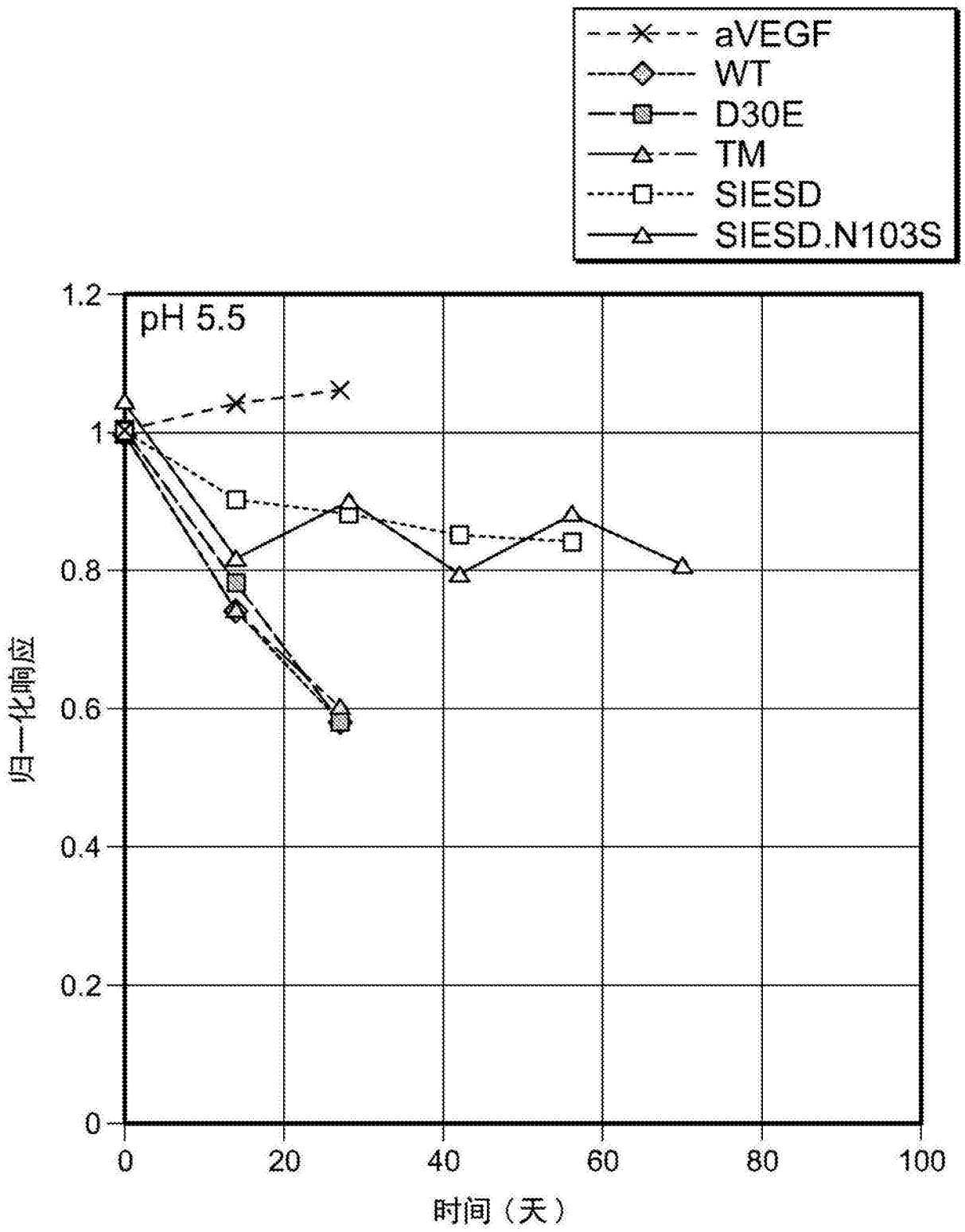

aVEGF
WT
D30E
TM
SIESD
SIESD.N103S
pH 5.5
1.2
1
0.8
0.6
0.4
0.2
0
0
20
40
60
80
100
归一化响应
时间（天）

图2A

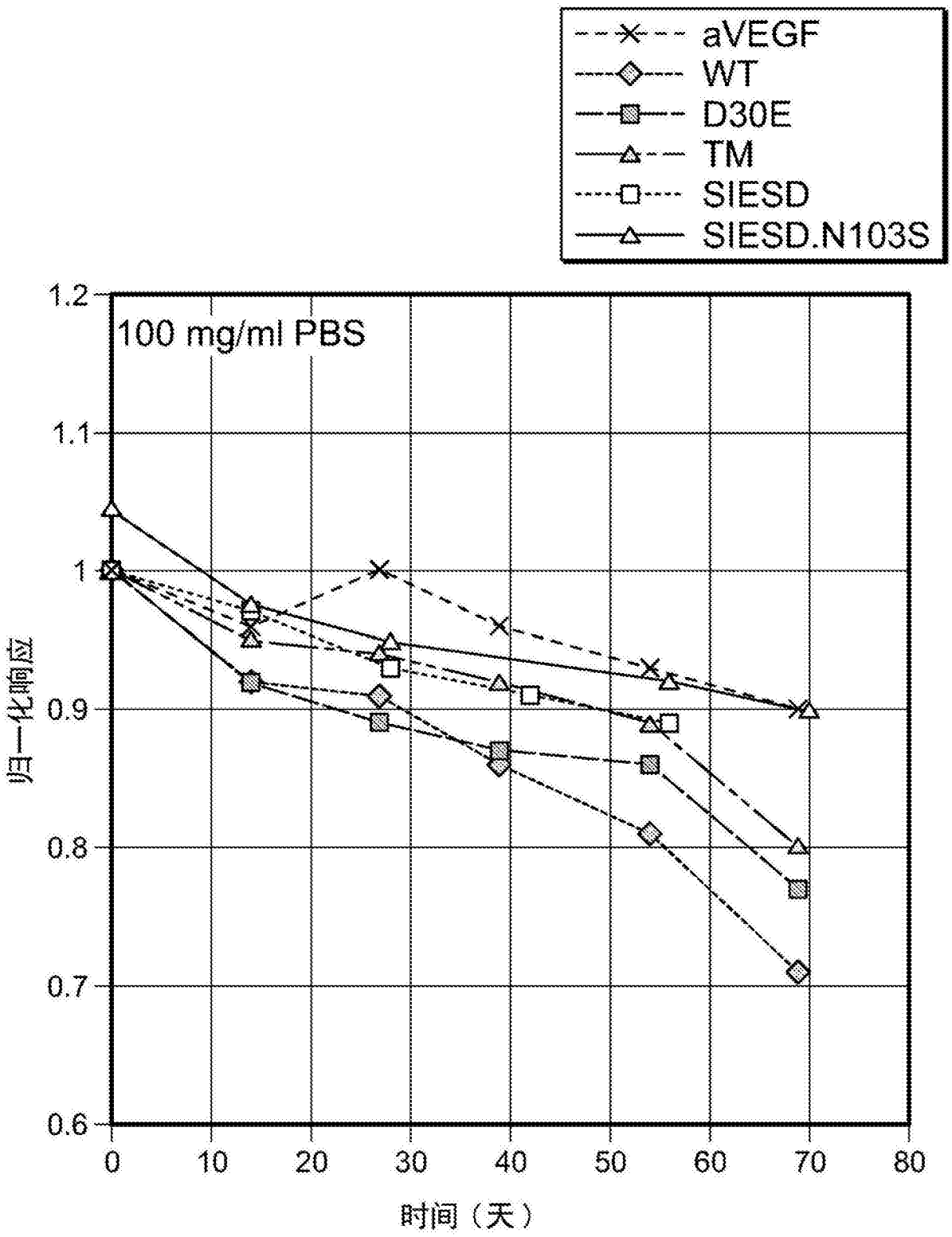

aVEGF
WT
D30E
TM
SIESD
SIESD.N103S
100 mg/ml PBS
1.2
1.1
1
0.9
0.8
0.7
0.6
归一化响应
0
10
20
30
40
50
60
70
80
时间（天）

图2B

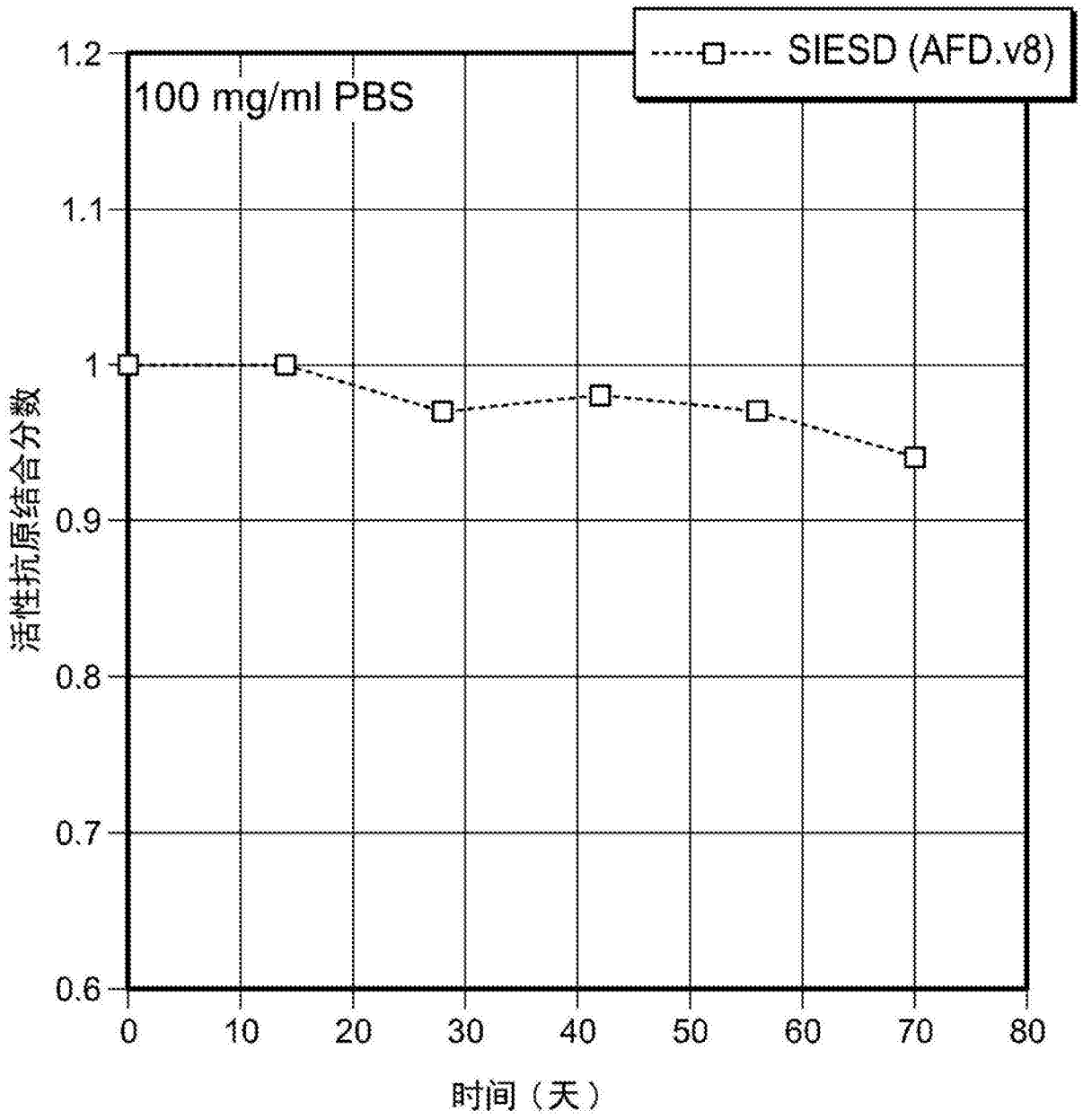

SIESD (AFD.v8)
100 mg/ml PBS
活性抗原结合分数
1.2
1.1
1
0.9
0.8
0.7
0.6
0
10
20
30
40
50
60
70
80
时间（天）

图2C

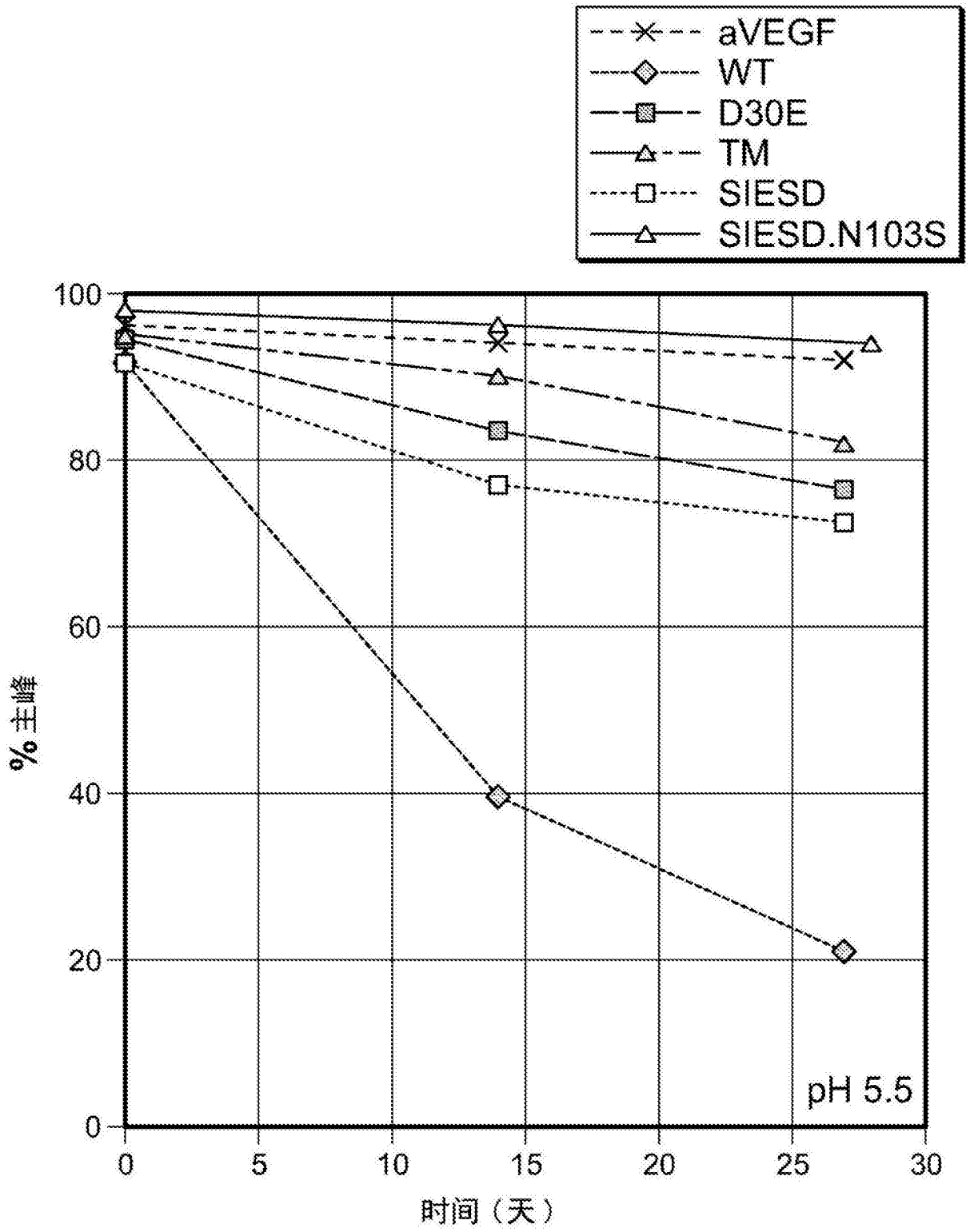

aVEGF
WT
D30E
TM
SIESD
SIESD.N103S
100
80
60
40
20
0
% 主峰
0
5
10
15
20
25
30
时间（天）
pH 5.5

图3A

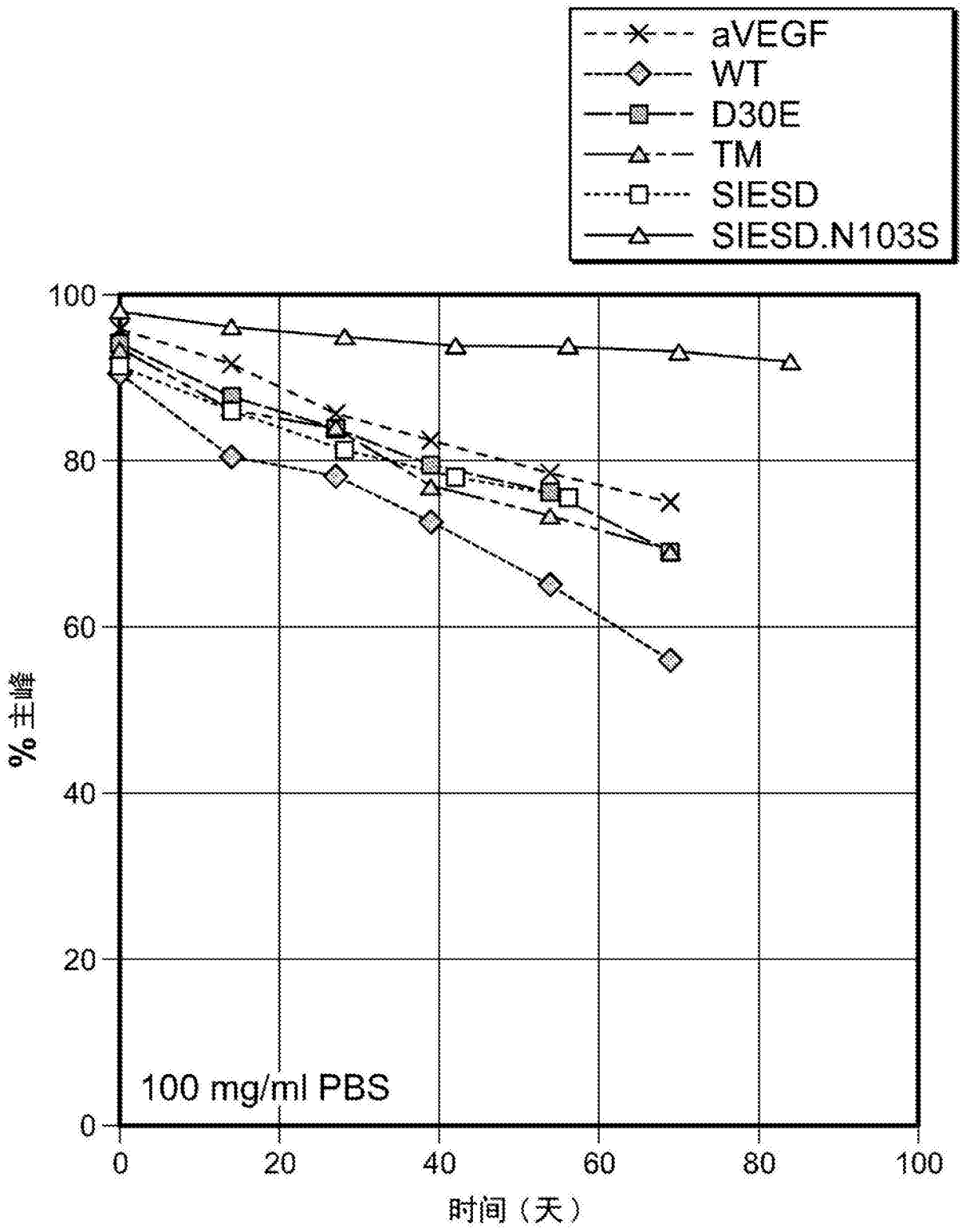

aVEGF
WT
D30E
TM
SIESD
SIESD.N103S
100
80
60
40
20
0
% 主峰
100 mg/ml PBS
0
20
40
60
80
100
时间（天）

图3B

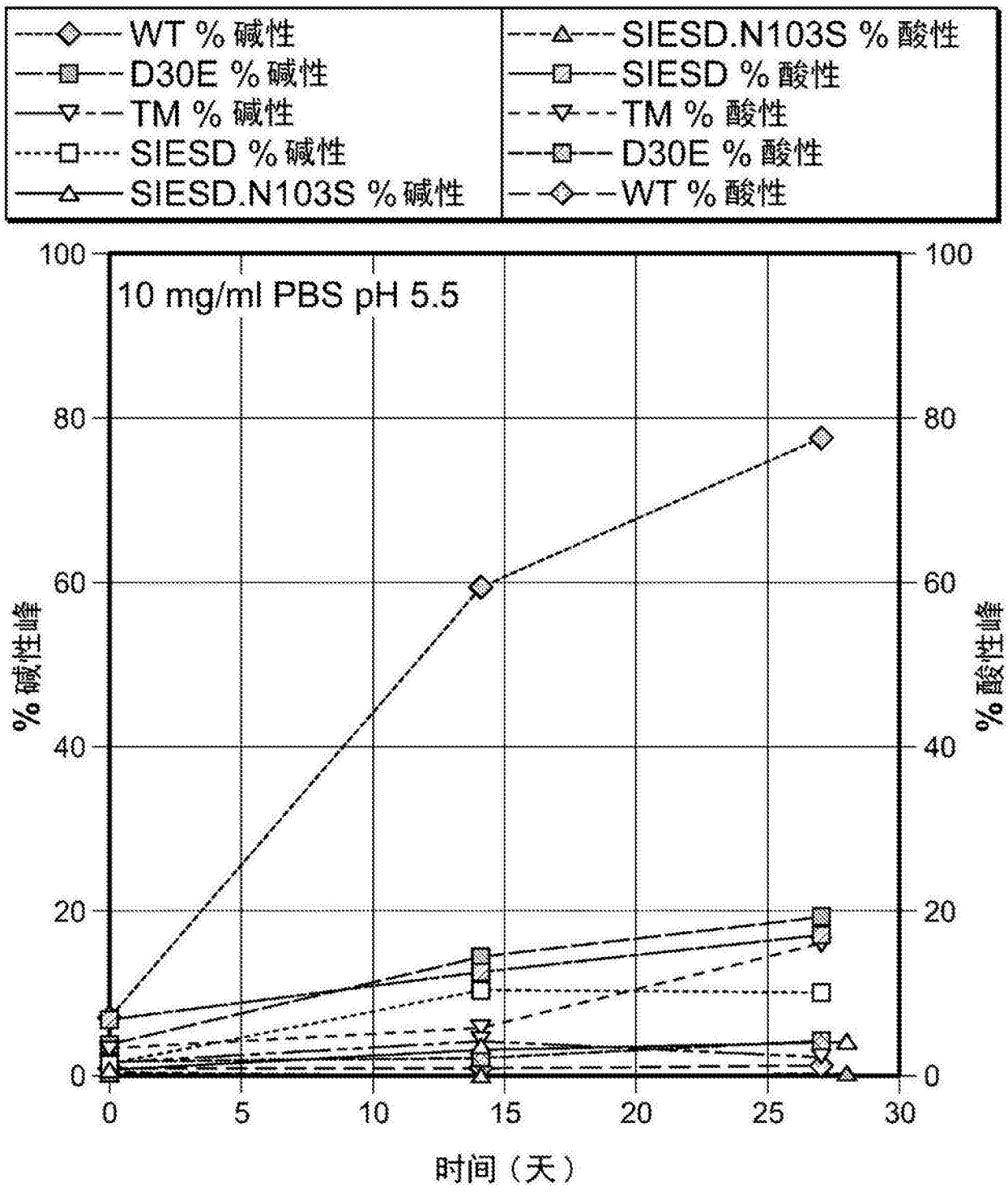

WT % 碱性
D30E % 碱性
TM % 碱性
SIESD % 碱性
SIESD.N103S % 碱性
SIESD.N103S % 酸性
SIESD % 酸性
TM % 酸性
D30E % 酸性
WT % 酸性
10 mg/ml PBS pH 5.5
% 碱性峰
% 酸性峰
时间（天）
0
20
40
60
80
100
0
5
10
15
20
25
30

图4A

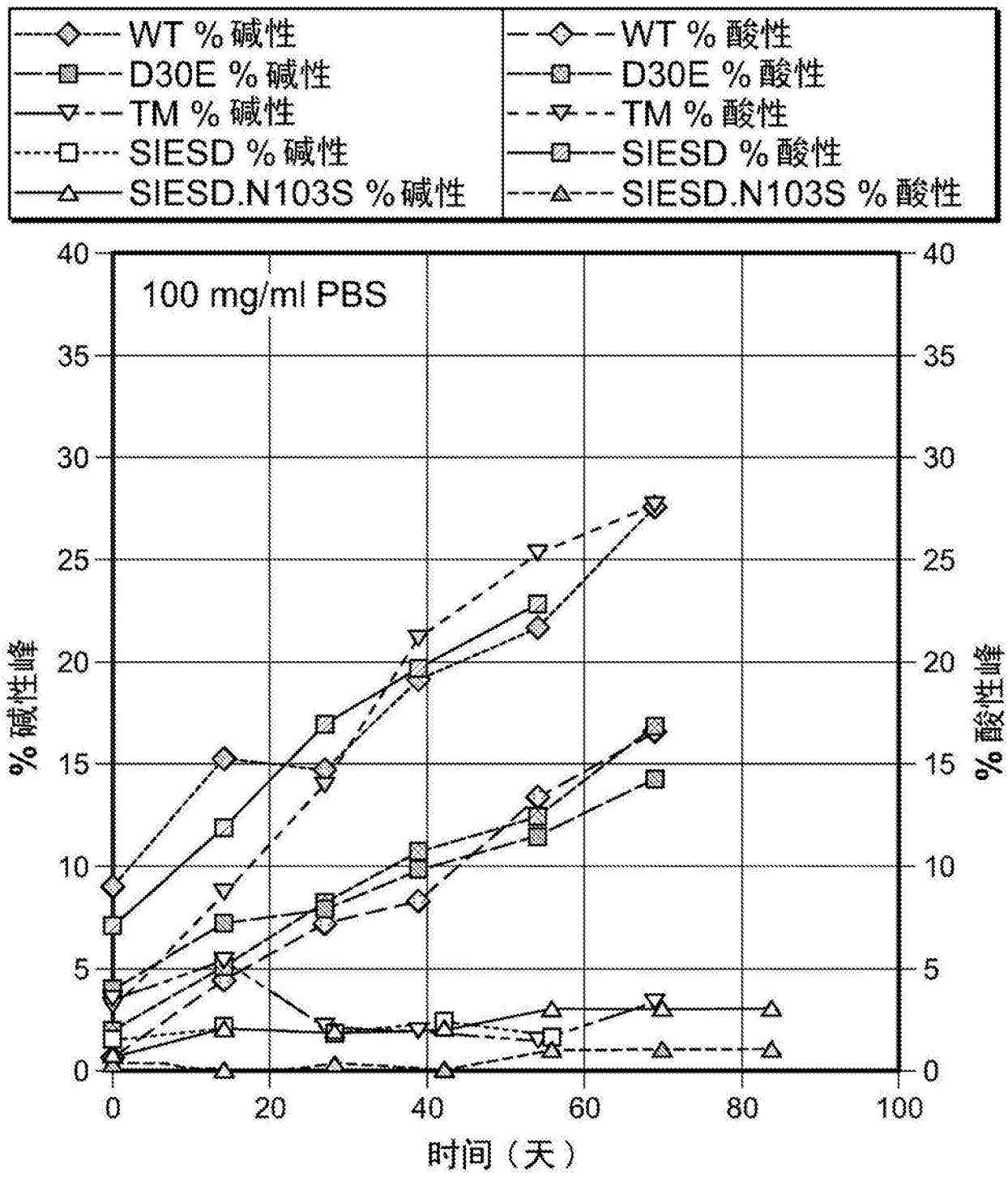

WT % 碱性
D30E % 碱性
TM % 碱性
SIESD % 碱性
SIESD.N103S % 碱性
WT % 酸性
D30E % 酸性
TM % 酸性
SIESD % 酸性
SIESD.N103S % 酸性
100 mg/ml PBS
% 碱性峰
% 酸性峰
时间（天）
0
5
10
15
20
25
30
35
40
0
20
40
60
80
100

图4B

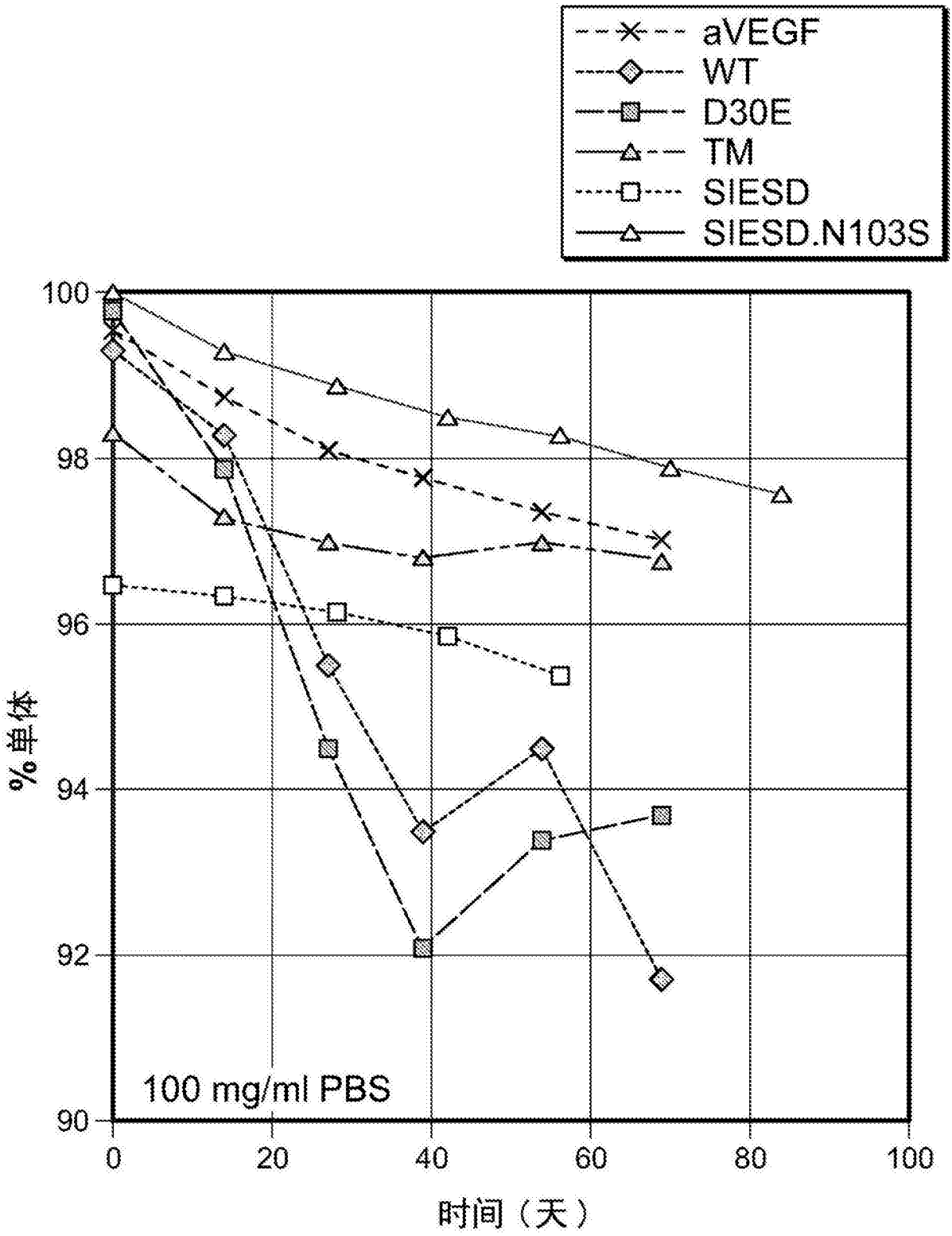

aVEGF
WT
D30E
TM
SIESD
SIESD.N103S
100
98
96
94
92
90
% 单体
100 mg/ml PBS
0
20
40
60
80
100
时间（天）

图5

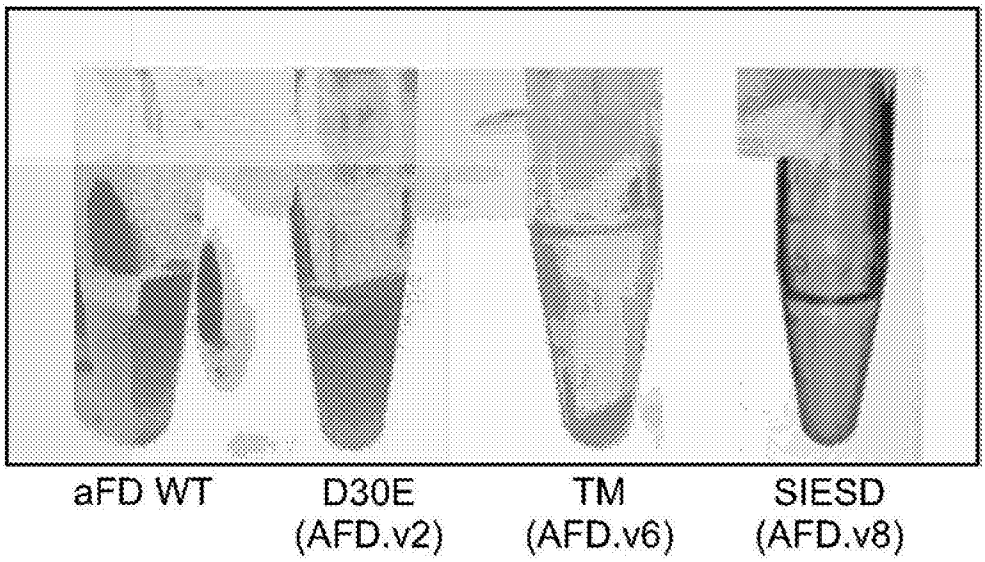


图6

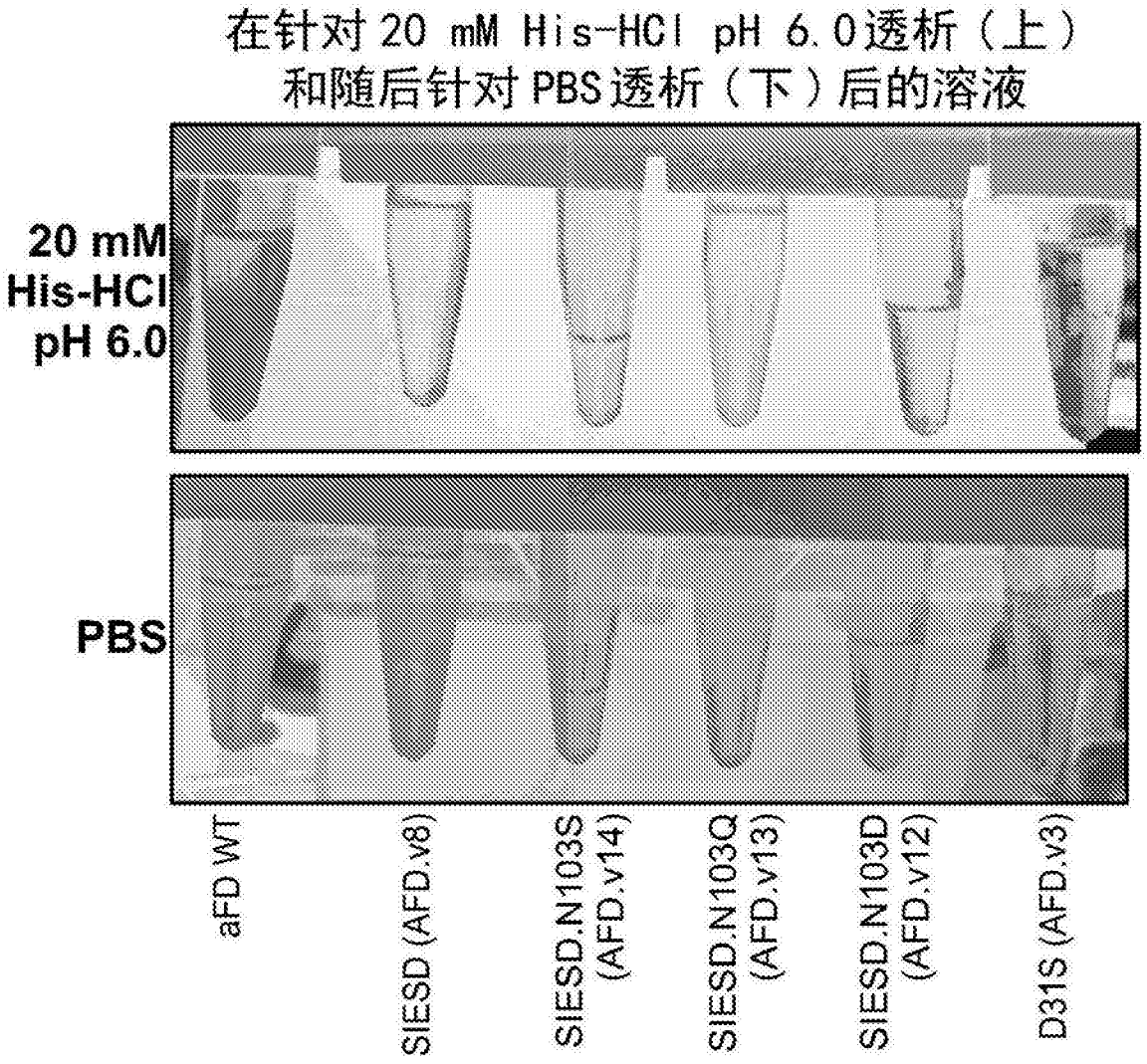

在针对 20 mM His-HCl pH 6.0透析（上）
和随后针对 PBS透析（下）后的溶液
20 mM
His-HCl
pH 6.0
PBS
aFD WT
SIESD (AFD.v8)
SIESD.N103S
(AFD.v14)
SIESD.N103Q
(AFD.v13)
SIESD.N103D
(AFD.v12)
D31S (AFD.v3)

图7

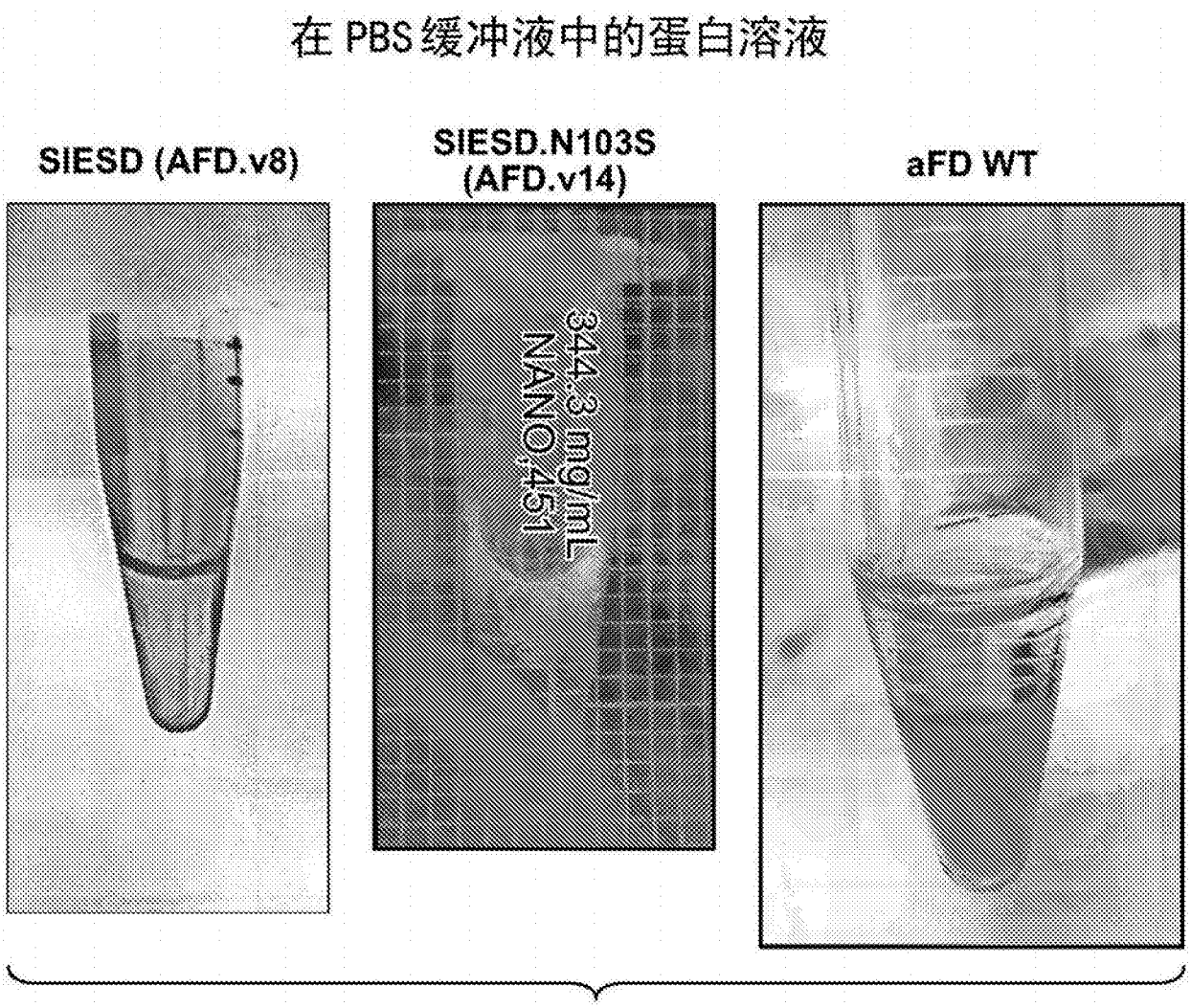
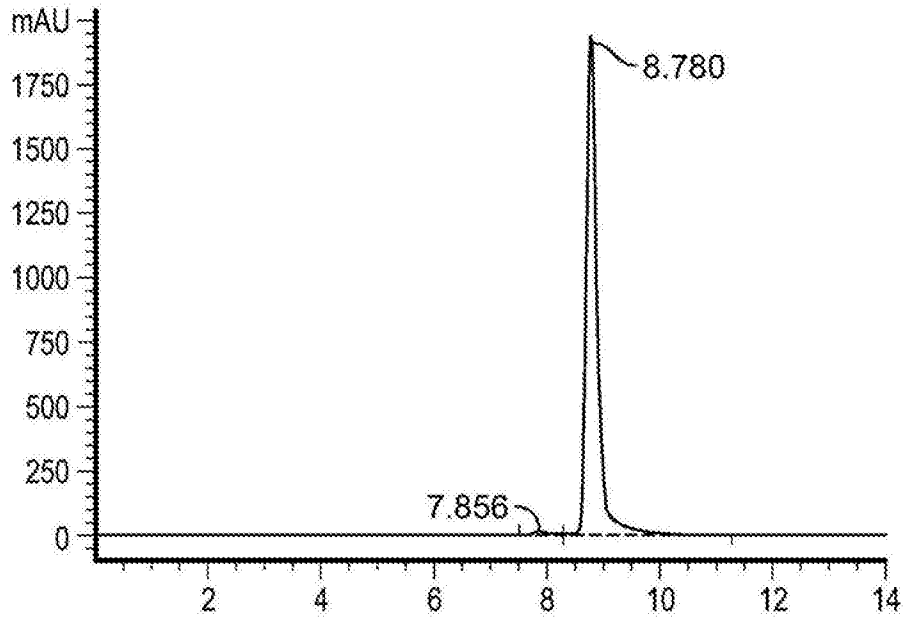

在PBS缓冲液中的蛋白溶液
SIESD (AFD.v8)
SIESD.N103S (AFD.v14)
aFD WT
344.3 mg/mL
NANO;451

图8

在 PBS 中 372 mg/mL SIESD.N103S (AFD.v14) 的 SEC 性质
DAD1 A, Sig=280, 2 Ref=关



面积百分比报告

分类 : 信号
倍增器 : 1.0000
稀释 : 1.0000
关于 ISTD 使用倍增器 & 稀释

信号 1: DAD1 A, Sig=280, 2 Ref= 关

峰 #	保留时间 [min]	类型	宽度 [min]	面积 [mAU*s]	高度 [mAU]	面积 %
1	7.856	VV	0.2537	241.13916	12.69158	0.94195
2	8.780	VB	0.1986	2.53587e4	1939.25708	99.05805
总共 :				2.55999e4	1951.94866	

图9

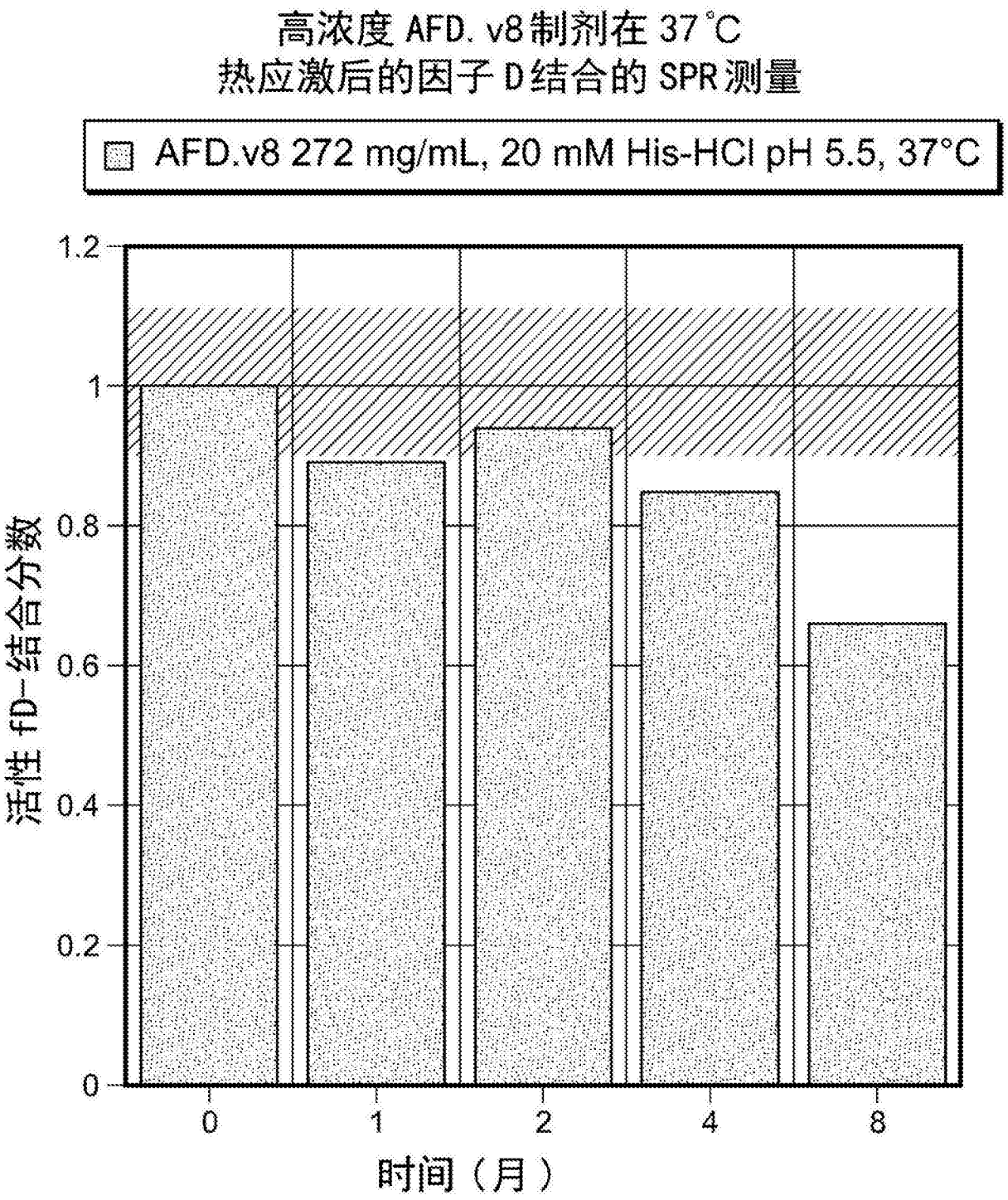

高浓度 AFD. v8制剂在 37°C
热应激后的因子 D结合的 SPR测量
AFD.v8 272 mg/mL, 20 mM His-HCl pH 5.5, 37°C
活性 fD-结合分数
1.2
1
0.8
0.6
0.4
0.2
0
0
1
2
4
8
时间（月）

图10A

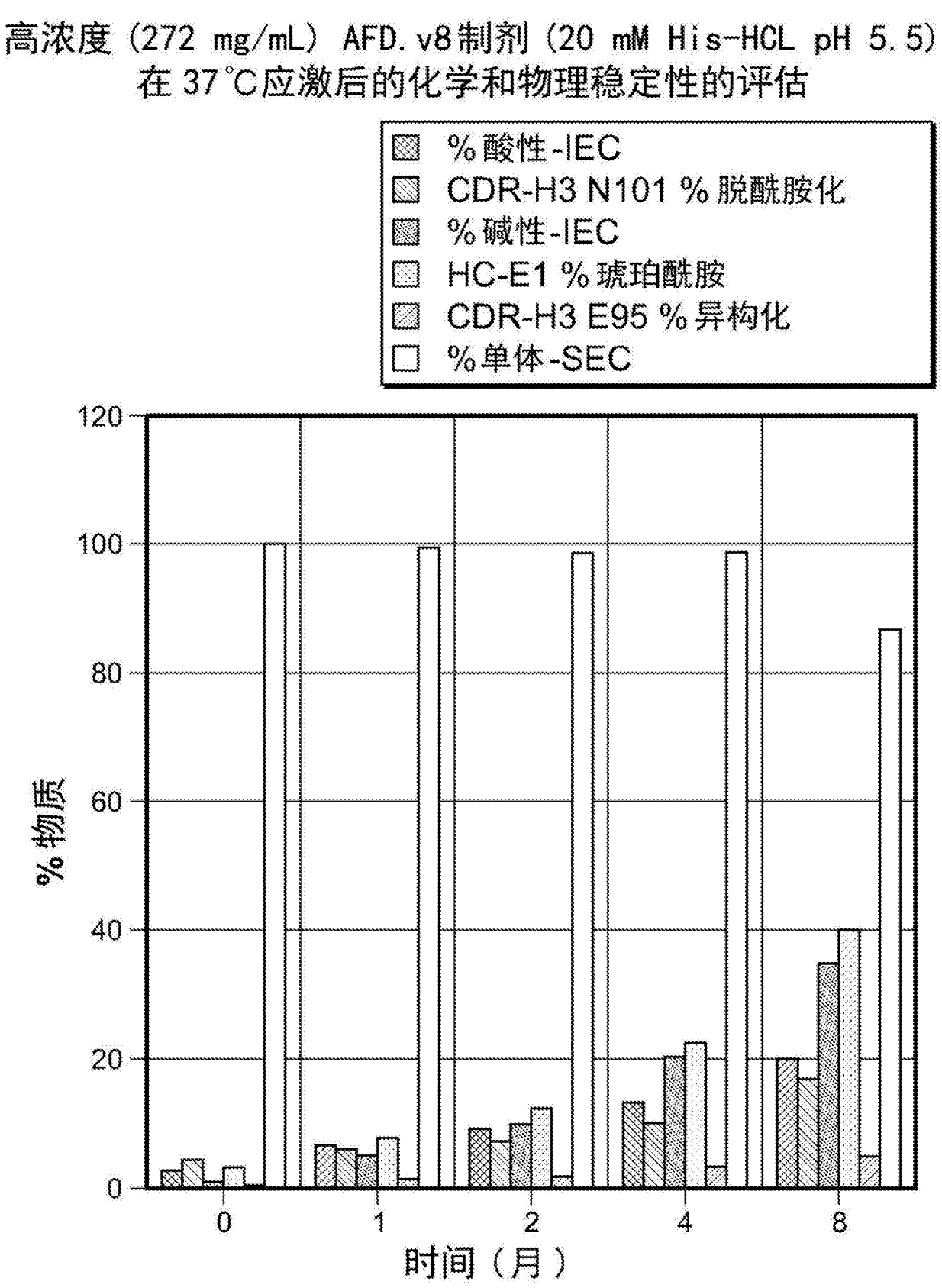
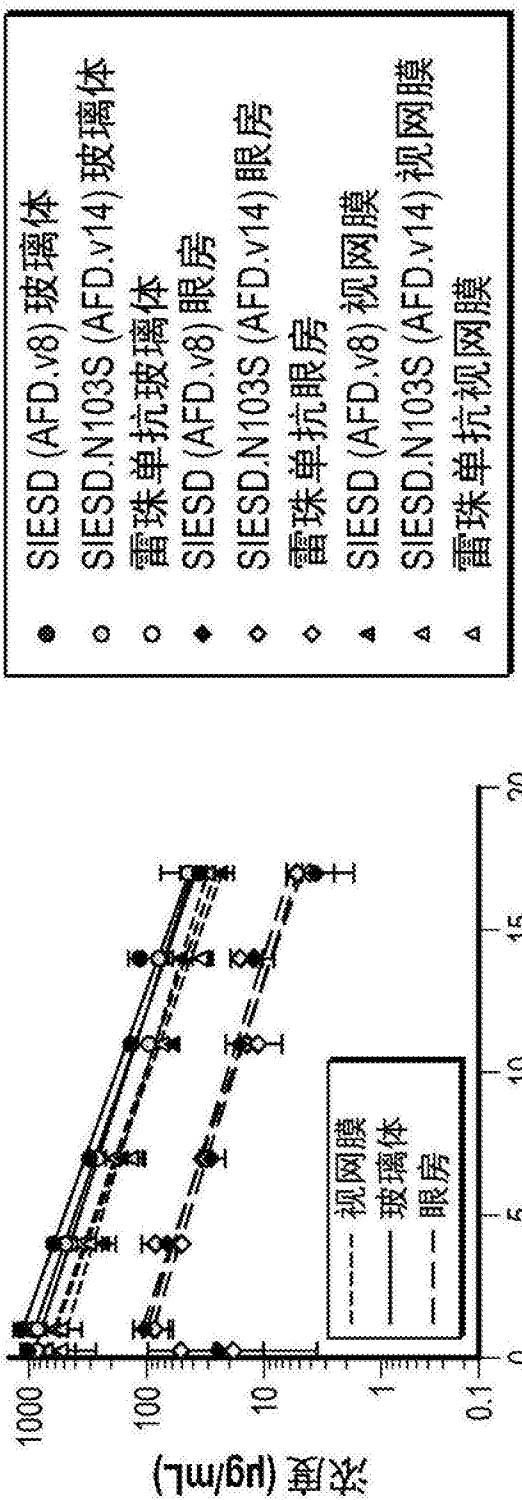

高浓度（272 mg/mL）AFD.v8制剂（20 mM His-HCL pH 5.5）
在37℃应激后的化学和物理稳定性的评估
%酸性-IEC
CDR-H3 N101 %脱酰胺化
%碱性-IEC
HC-E1 %琥珀酰胺
CDR-H3 E95 %异构化
%单体-SEC
120
100
80
60
40
20
0
% 物质
0
1
2
4
8
时间（月）

图10B



	玻璃体（液体）				眼房		视网膜	
分子*	半衰期（天）	$AUC_{全}$（天*μg/mL）	CL（mL/天）	V_{ss}（mL）	半衰期（天）	$AUC_{全}$（天*μg/mL）	半衰期（天）	$AUC_{全}$（天*μg/mL）
SIESD (AFD.v8)	3.4	4460	0.22	1.0	3.5	411	3.4	2489
SIESD.N103S (AFD.v14)	3.7	3450	0.28	1.4	4.0	432	4.0	2199
雷珠单抗	3.5	3600	0.27	1.3	3.9	353	3.5	2453

*1 mg/眼剂量，研究 14-0995

图 11

图11

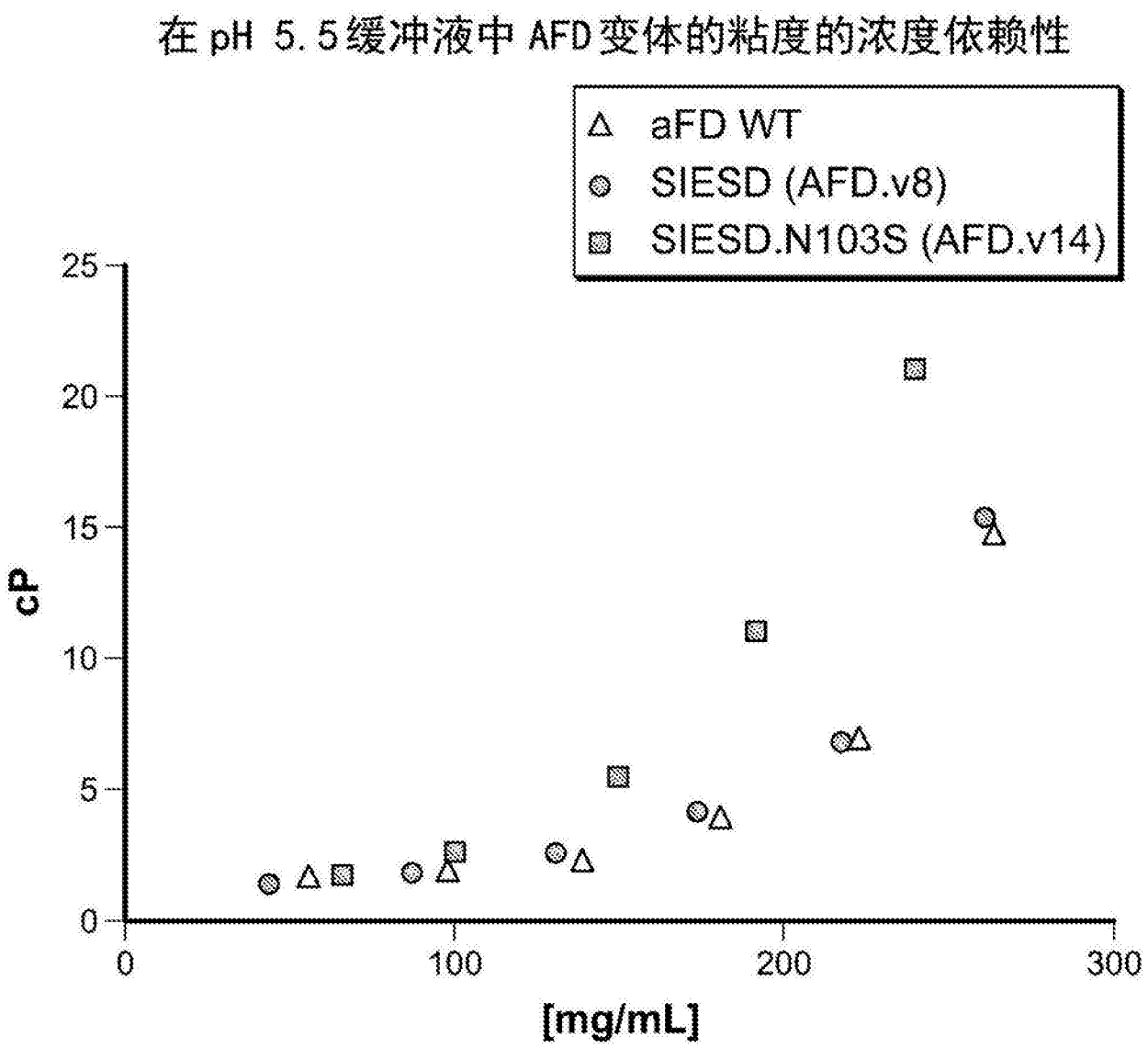

在 pH 5.5缓冲液中 AFD变体的粘度的浓度依赖性
aFD WT
SIESD (AFD.v8)
SIESD.N103S (AFD.v14)
cP
25
20
15
10
5
0
0
100
200
300
[mg/mL]

图12

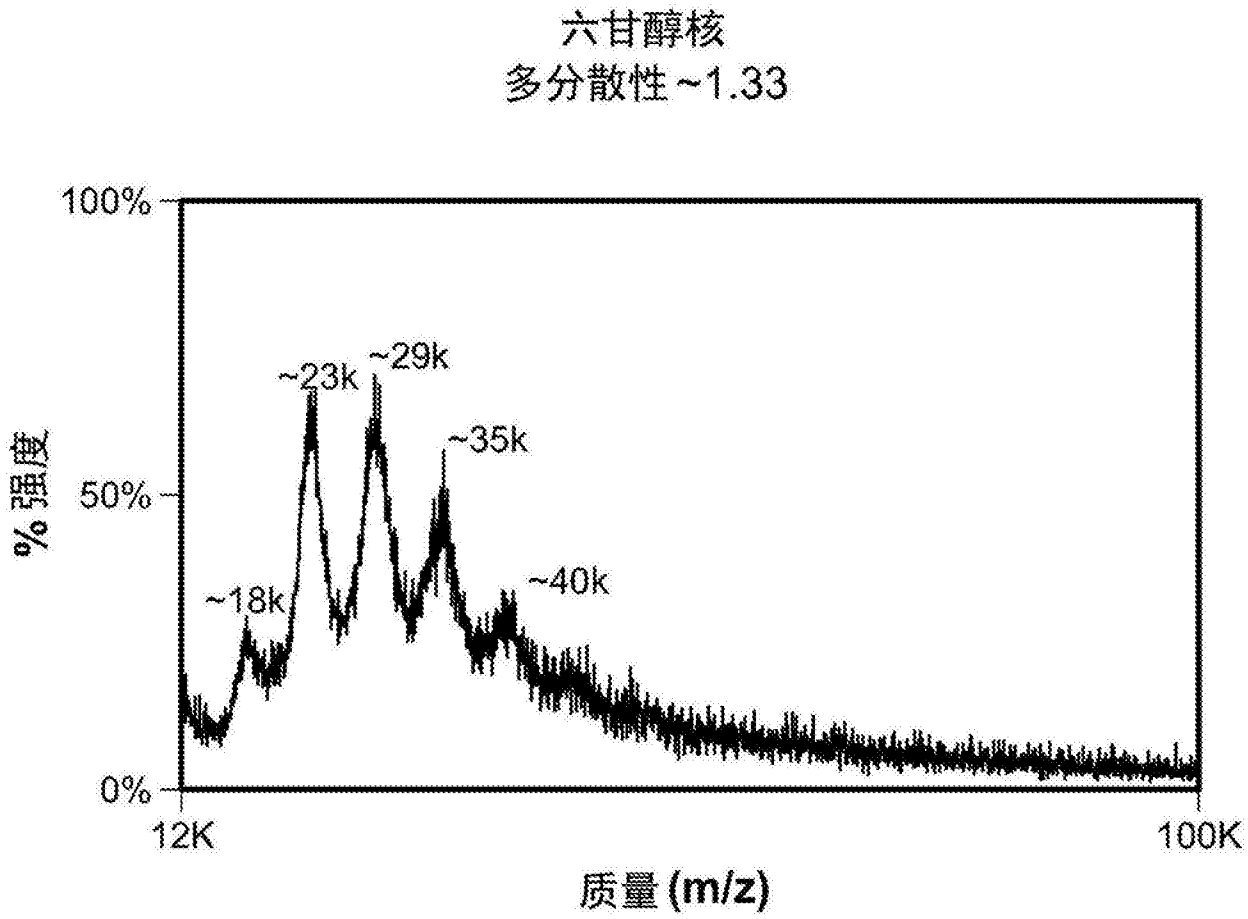


图13A

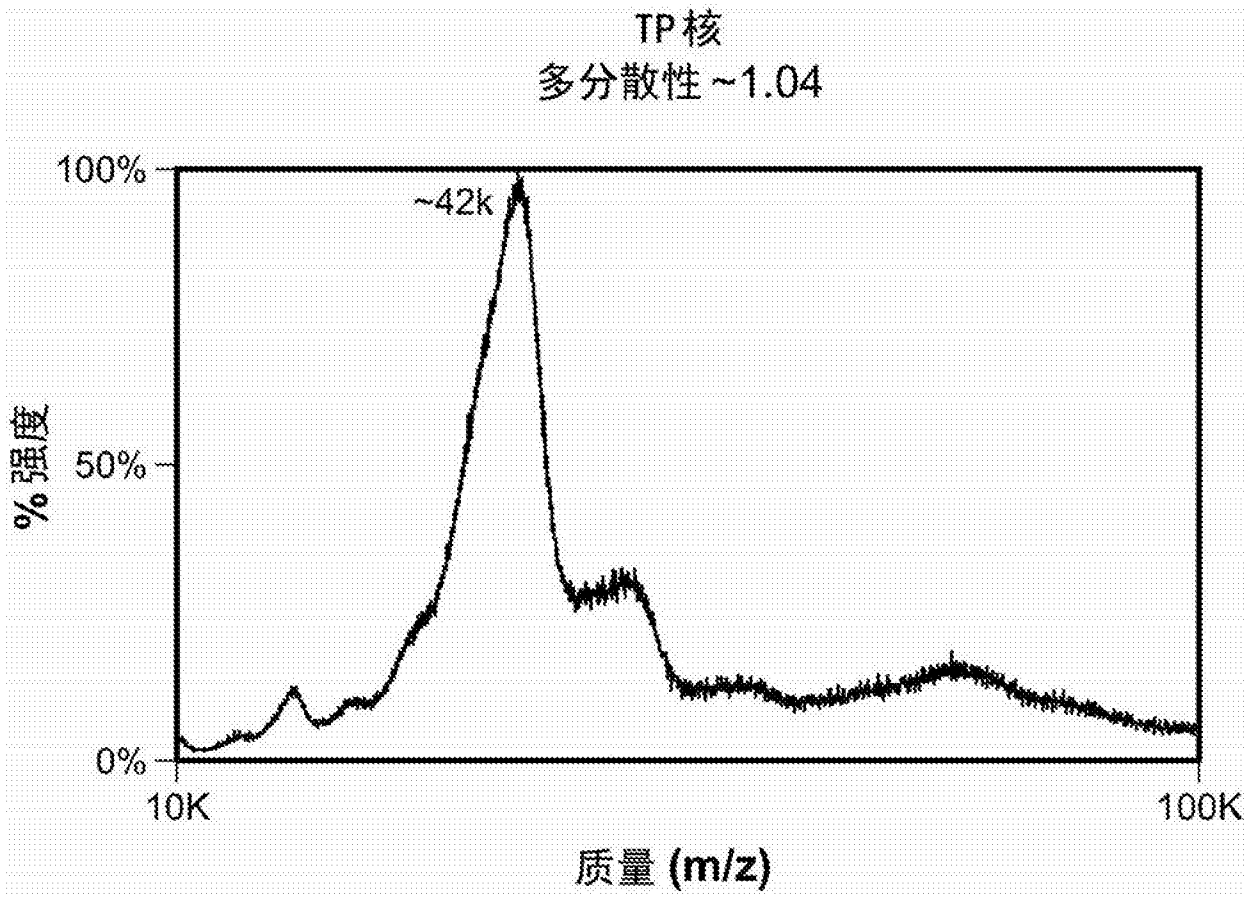


图13B

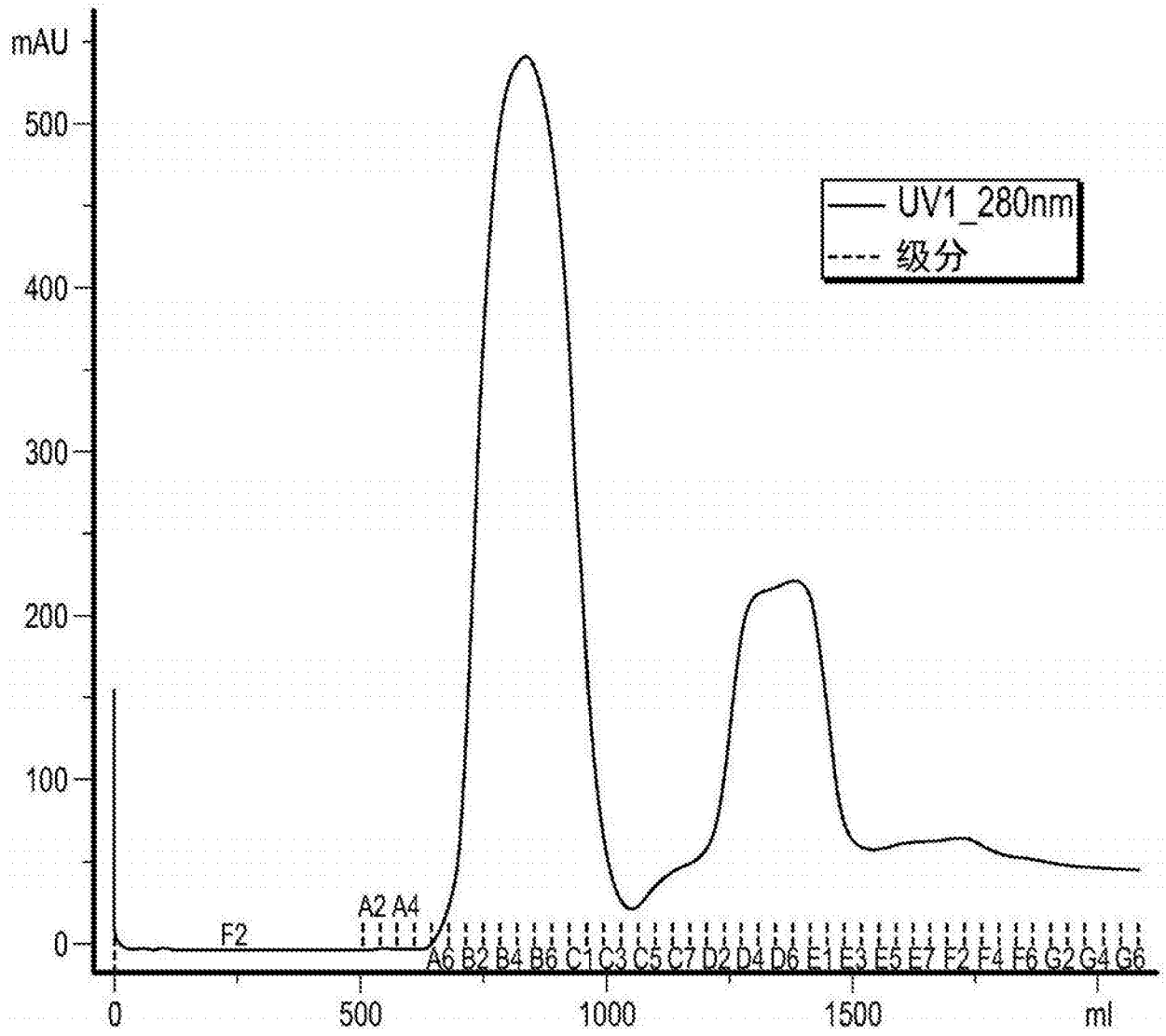

mAU
500
400
300
200
100
0
UV1_280nm
级分
A2 A4
F2
A6 B2 B4 B6 C1 C3 C5 C7 D2 D4 D6 E1 E3 E5 E7 F2 F4 F6 G2 G4 G6
0
500
1000
1500
ml

图14A

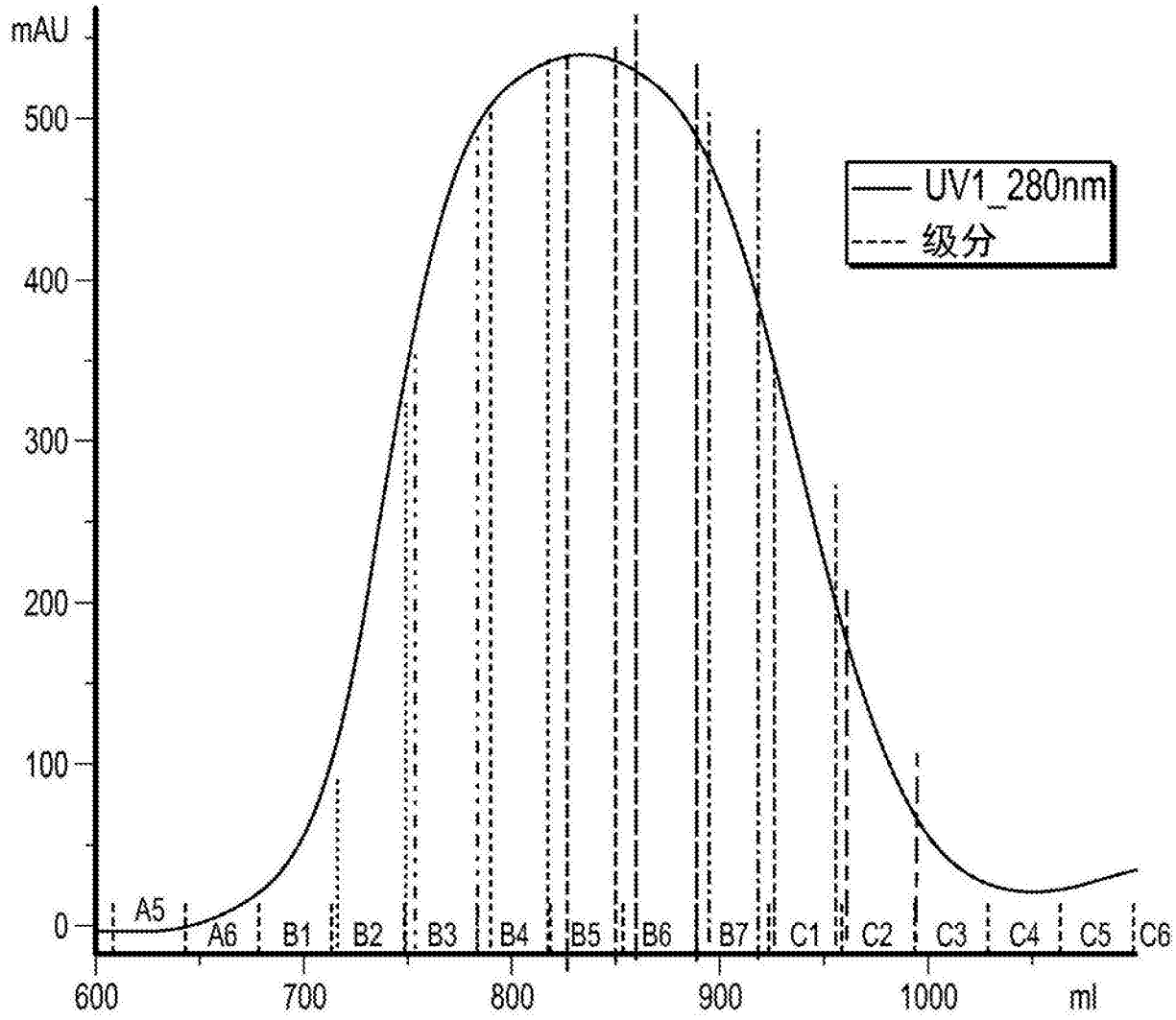

mAU
500
400
300
200
100
0
UV1_280nm
级分
A5
A6
B1
B2
B3
B4
B5
B6
B7
C1
C2
C3
C4
C5
C6
600
700
800
900
1000
ml

图14B

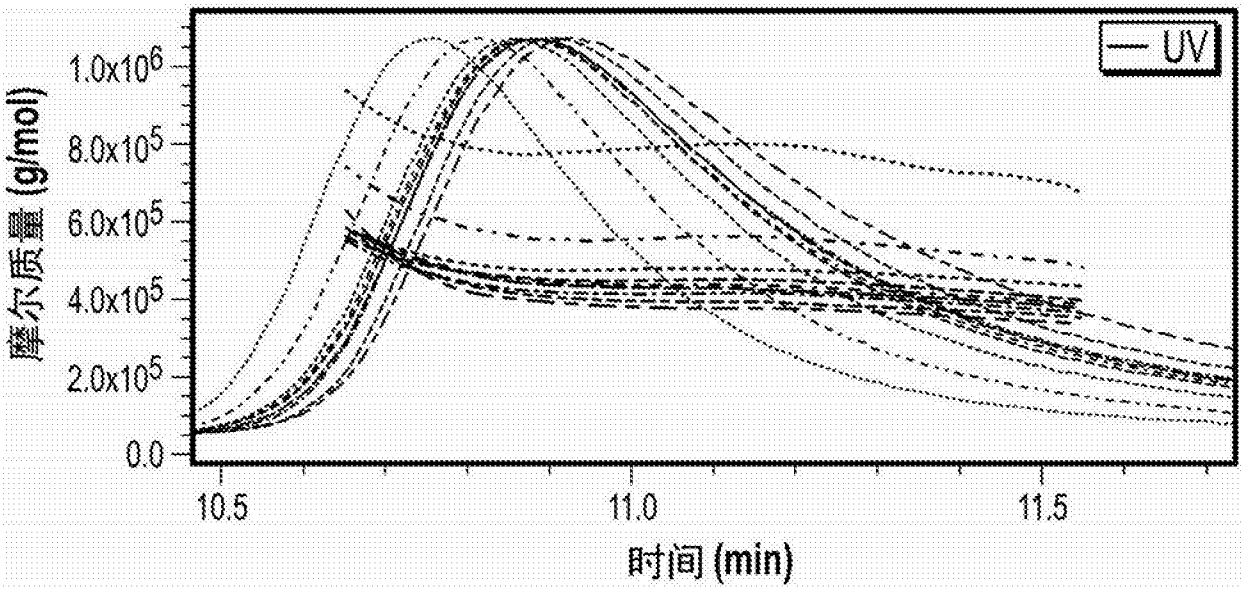

— UV
1.0x10^6
8.0x10^5
6.0x10^5
4.0x10^5
2.0x10^5
0.0
摩尔质量 (g/mol)
10.5
11.0
11.5
时间 (min)

图14C

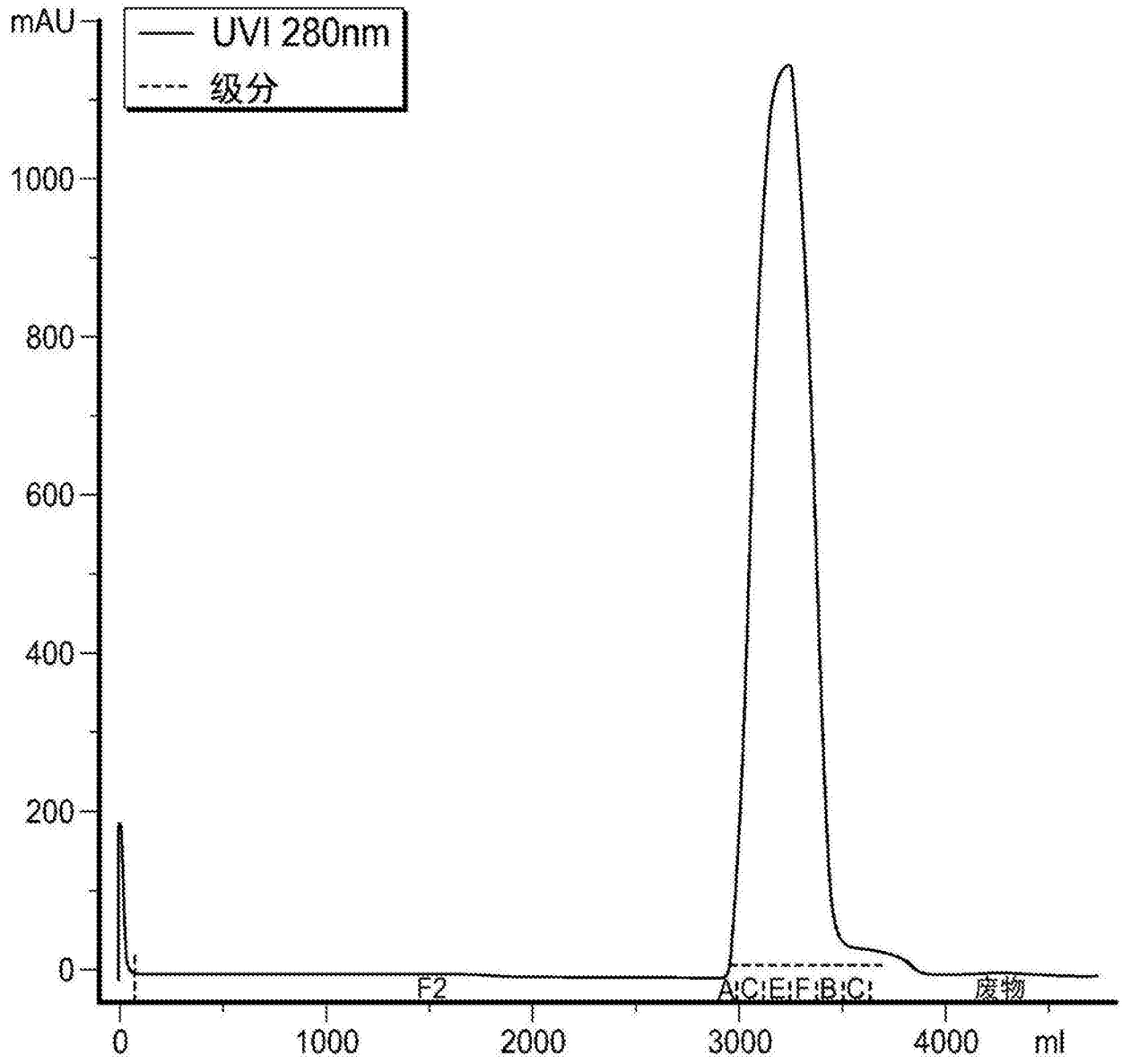

mAU
UVI 280nm
级分
1000
800
600
400
200
0
F2
A1
C1
E1
F1
B1
C1
废物
0
1000
2000
3000
4000
ml

图15A

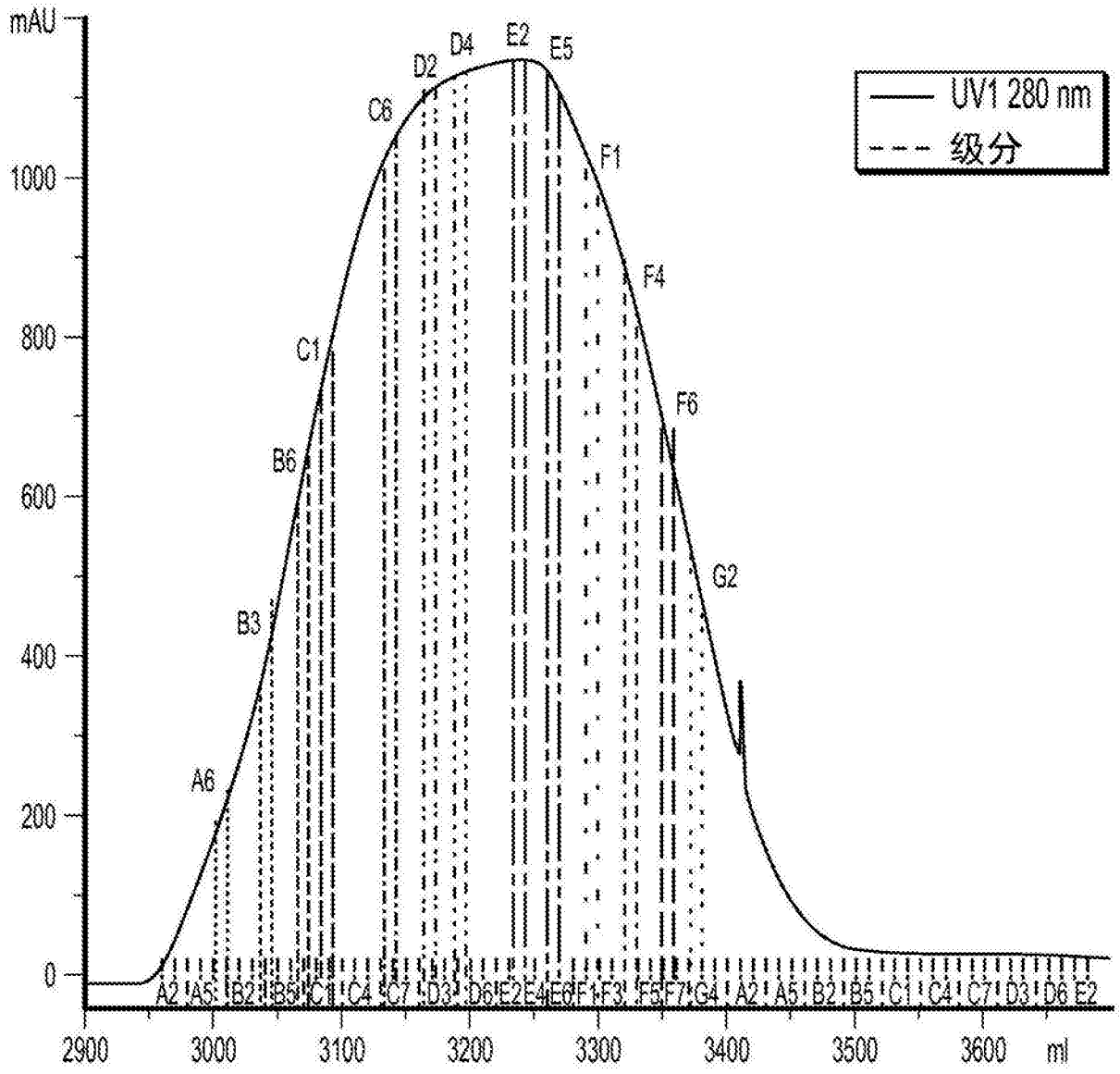

mAU
1000
800
600
400
200
0
UV1 280 nm
级分
A6
B3
B6
C1
C6
D2
D4
E2
E5
F1
F4
F6
G2
A2 A5 B2 B5 C1 C4 C7 D3 D6 E2 E4 E6 F1 F3 F5 F7 G4 A2 A5 B2 B5 C1 C4 C7 D3 D6 E2
2900
3000
3100
3200
3300
3400
3500
3600
ml

图15B

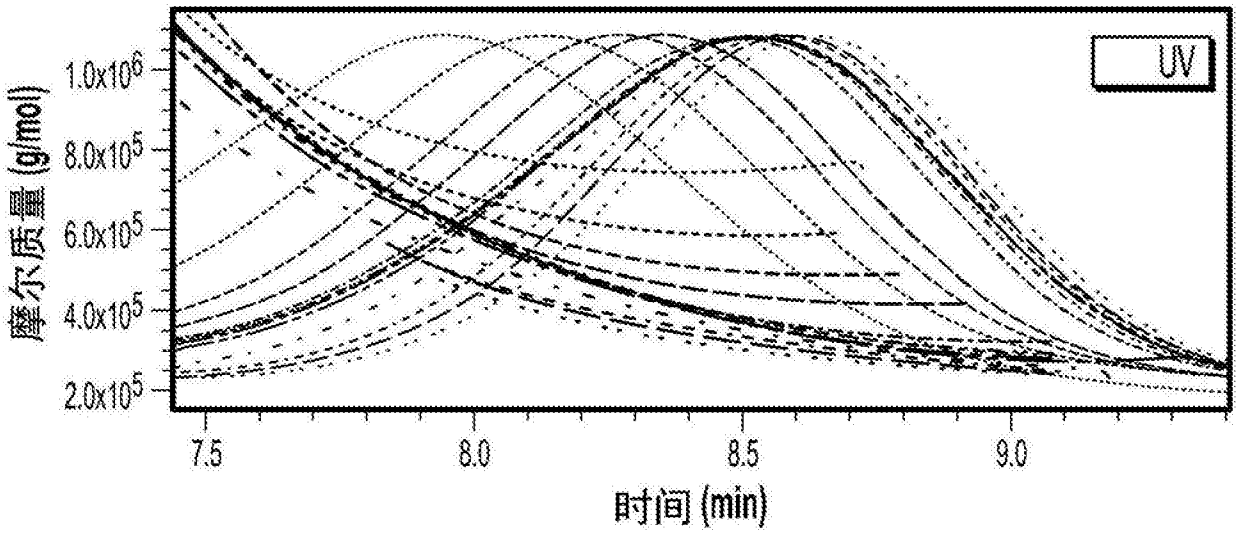

UV
摩尔质量 (g/mol)
1.0x10^6
8.0x10^5
6.0x10^5
4.0x10^5
2.0x10^5
7.5
8.0
8.5
9.0
时间 (min)

图15C

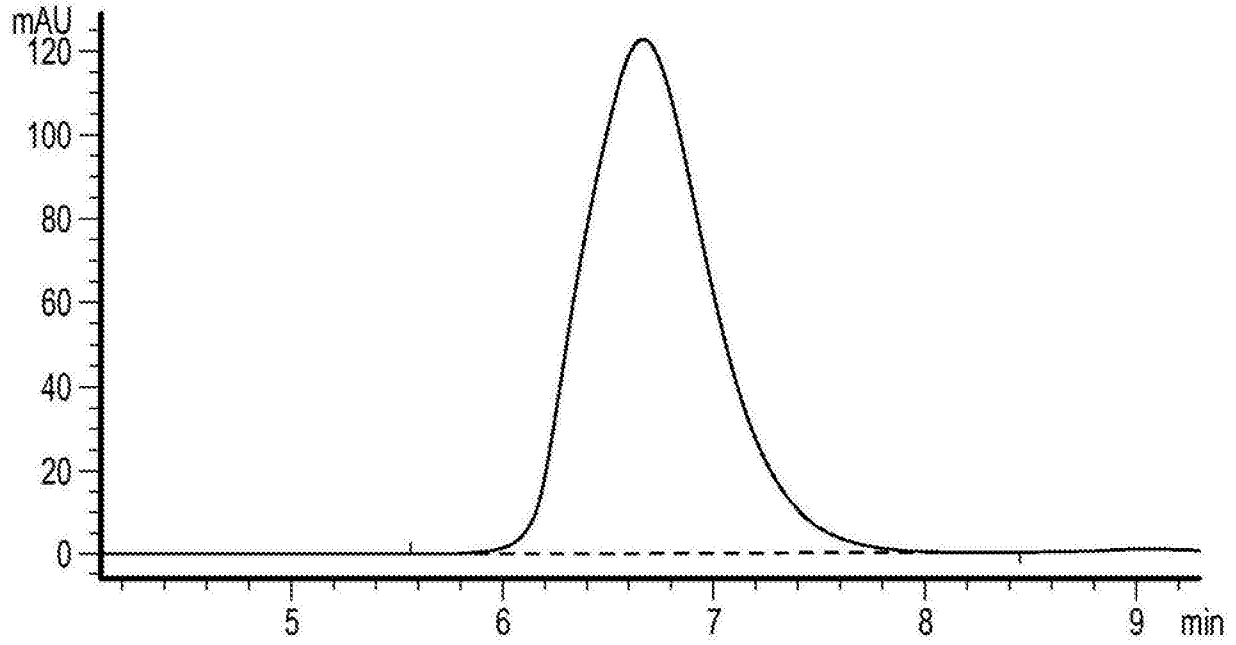

mAU
120
100
80
60
40
20
0
5
6
7
8
9
min

图16A

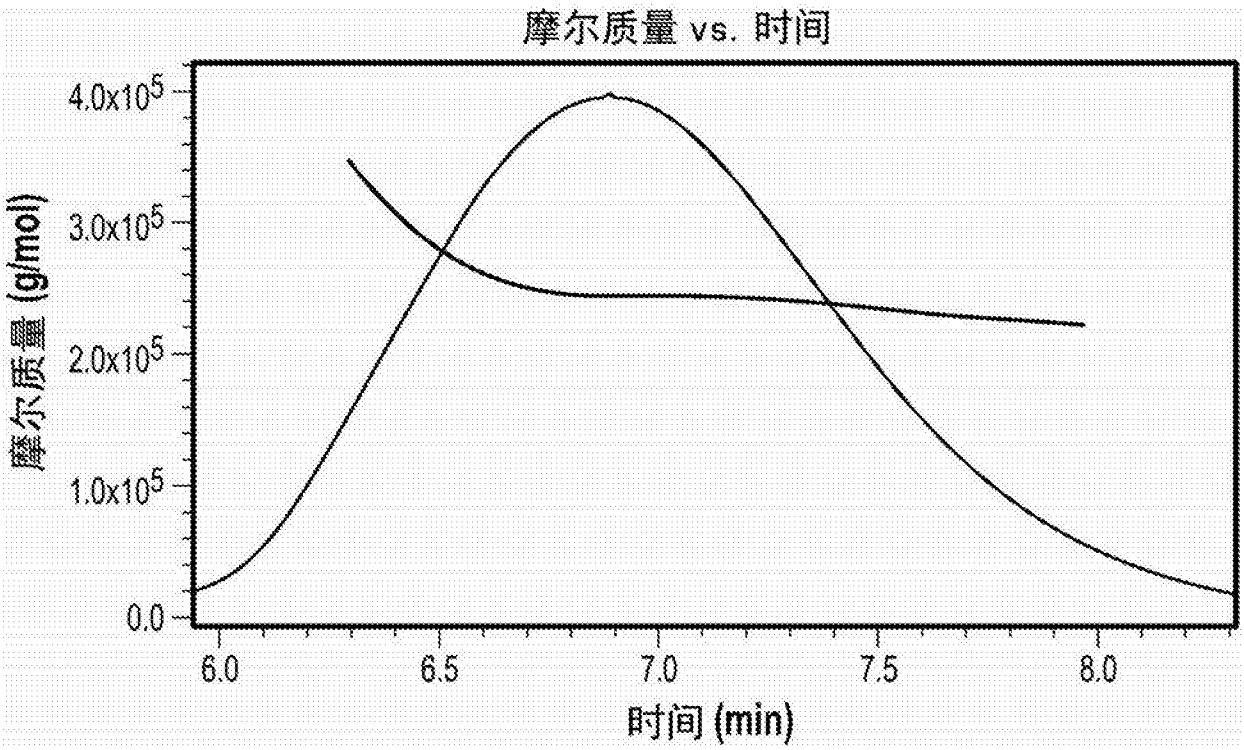

摩尔质量 vs. 时间
摩尔质量 (g/mol)
4.0x10^5
3.0x10^5
2.0x10^5
1.0x10^5
0.0
6.0
6.5
7.0
7.5
8.0
时间 (min)

图16B

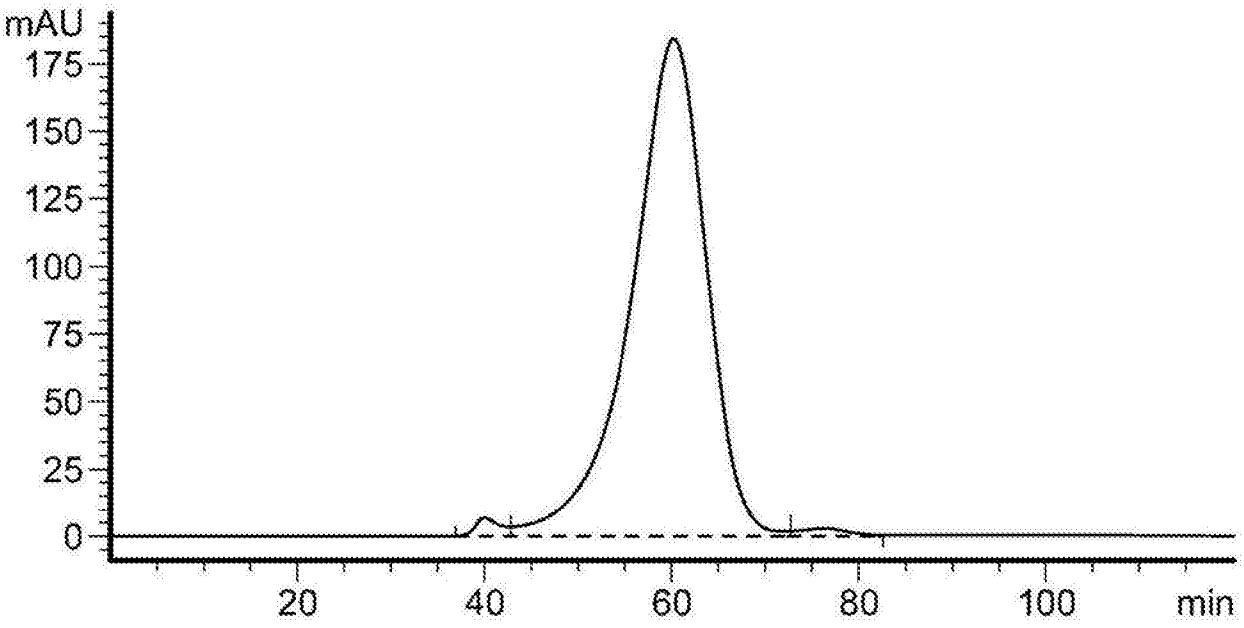

mAU
175
150
125
100
75
50
25
0
20
40
60
80
100
min

图17A

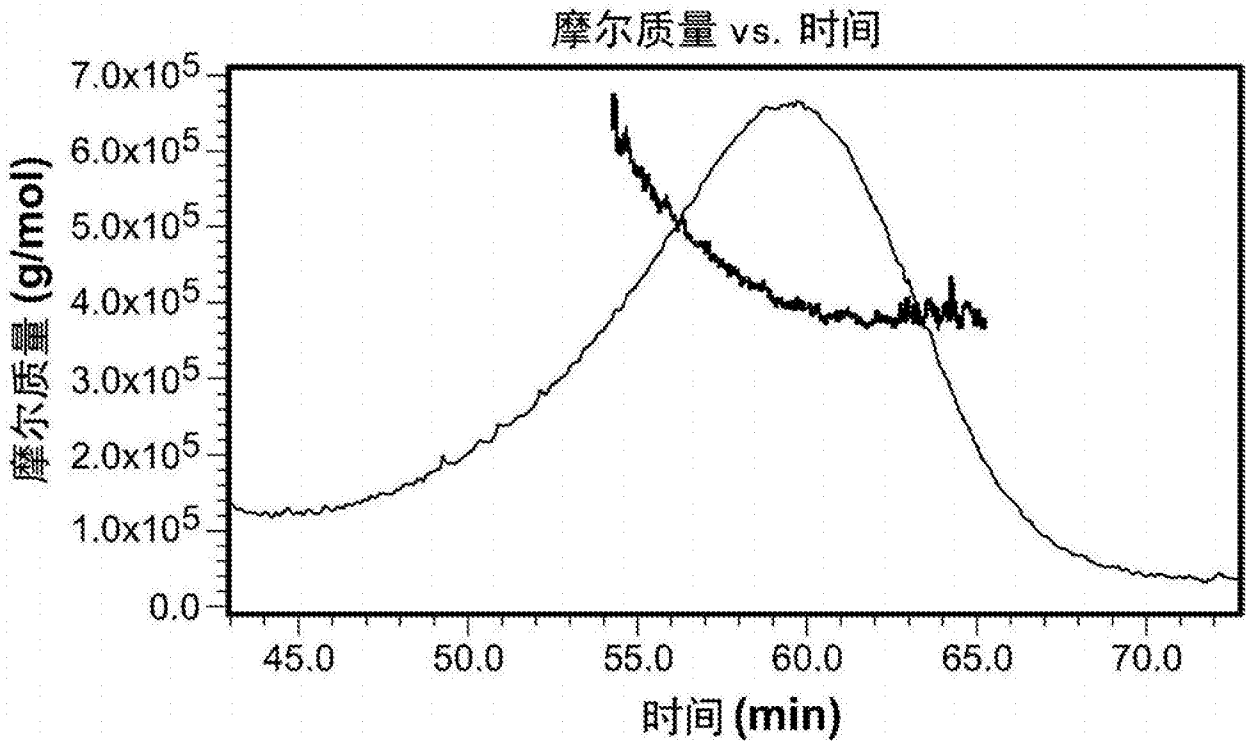

摩尔质量 vs. 时间
摩尔质量 (g/mol)
7.0x10^5
6.0x10^5
5.0x10^5
4.0x10^5
3.0x10^5
2.0x10^5
1.0x10^5
0.0
45.0
50.0
55.0
60.0
65.0
70.0
时间 (min)

图17B

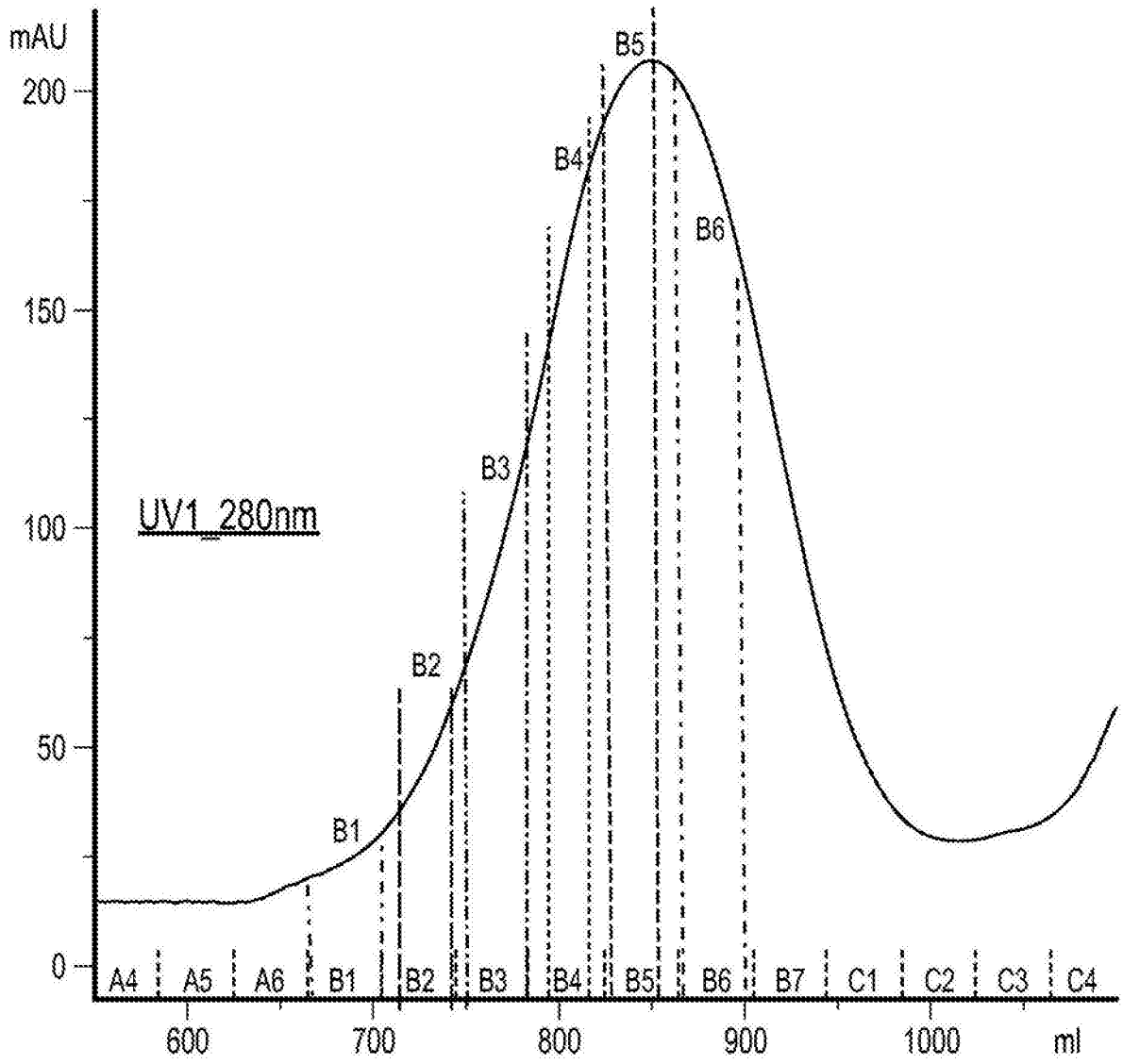

mAU
200
150
100
50
0
UV1_280nm
B1
B2
B3
B4
B5
B6
A4
A5
A6
B1
B2
B3
B4
B5
B6
B7
C1
C2
C3
C4
600
700
800
900
1000
ml

图18A

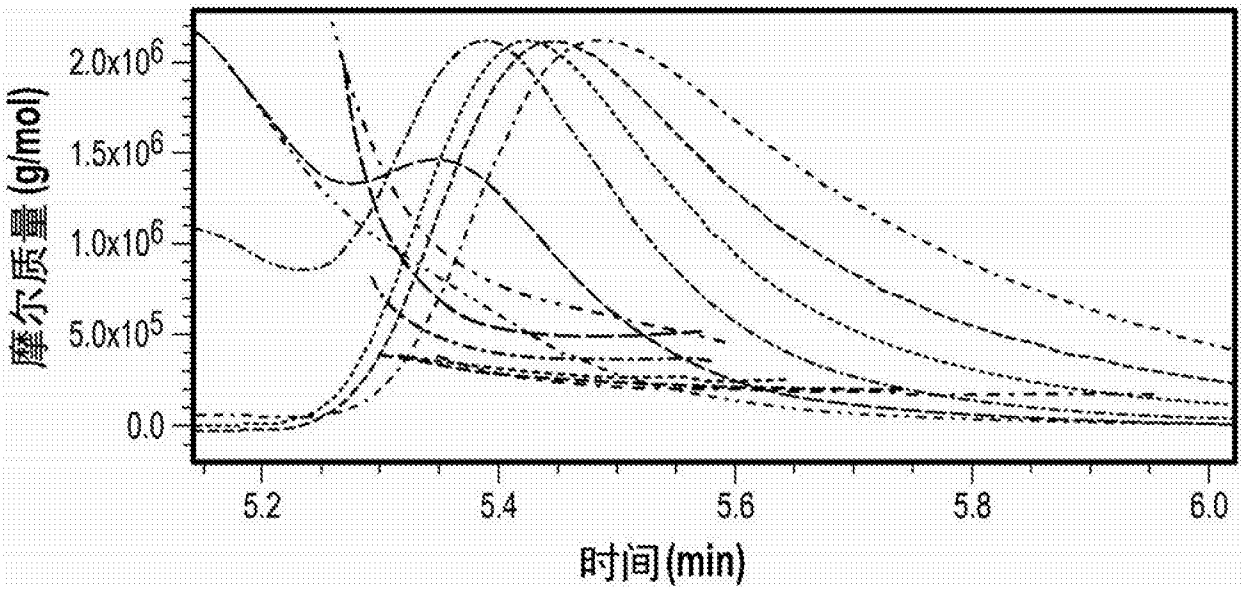


图18B

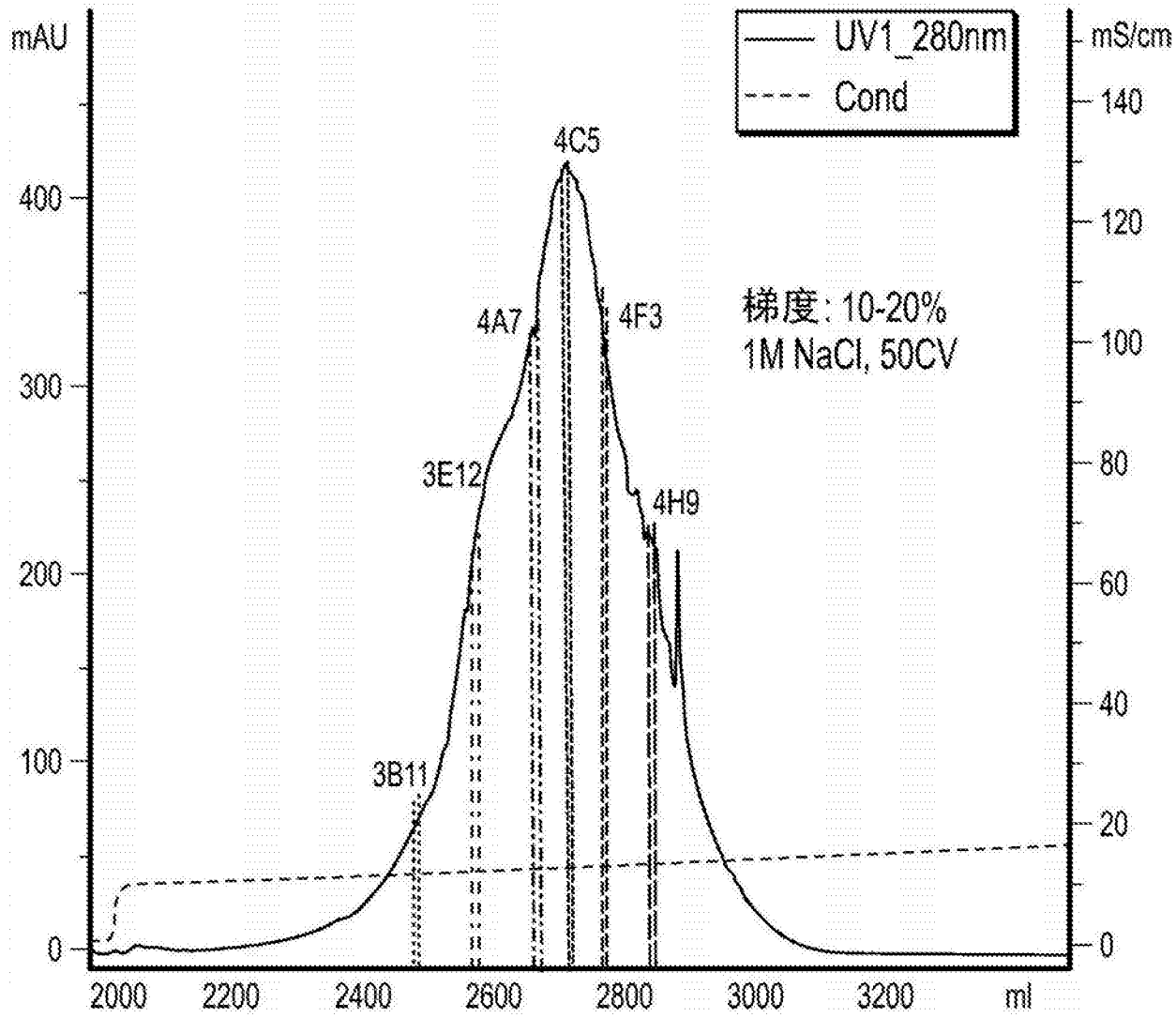

mAU
mS/cm
UV1_280nm
Cond
梯度: 10-20%
1M NaCl, 50CV
4C5
4A7
4F3
3E12
4H9
3B11
0
100
200
300
400
0
20
40
60
80
100
120
140
2000
2200
2400
2600
2800
3000
3200
ml

图19A

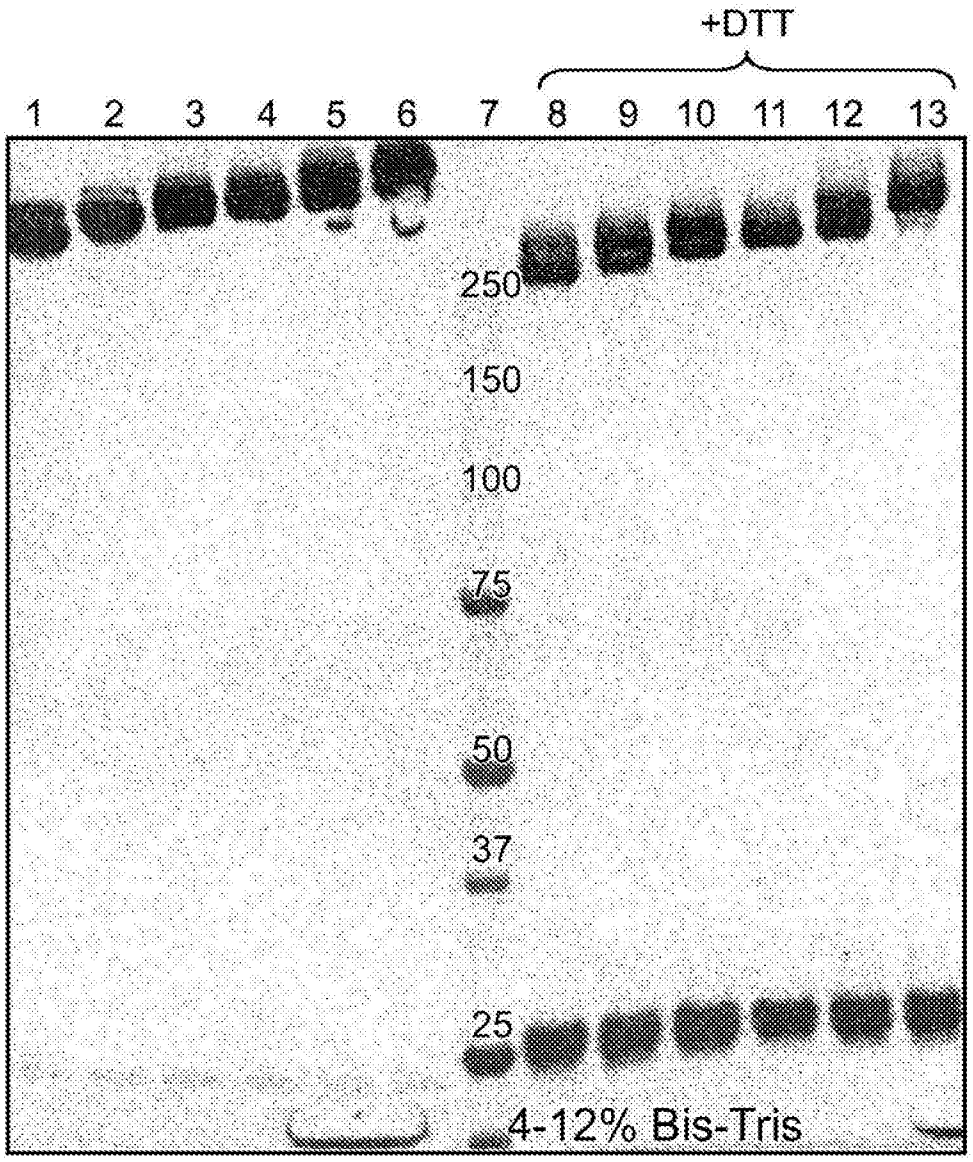


图19B

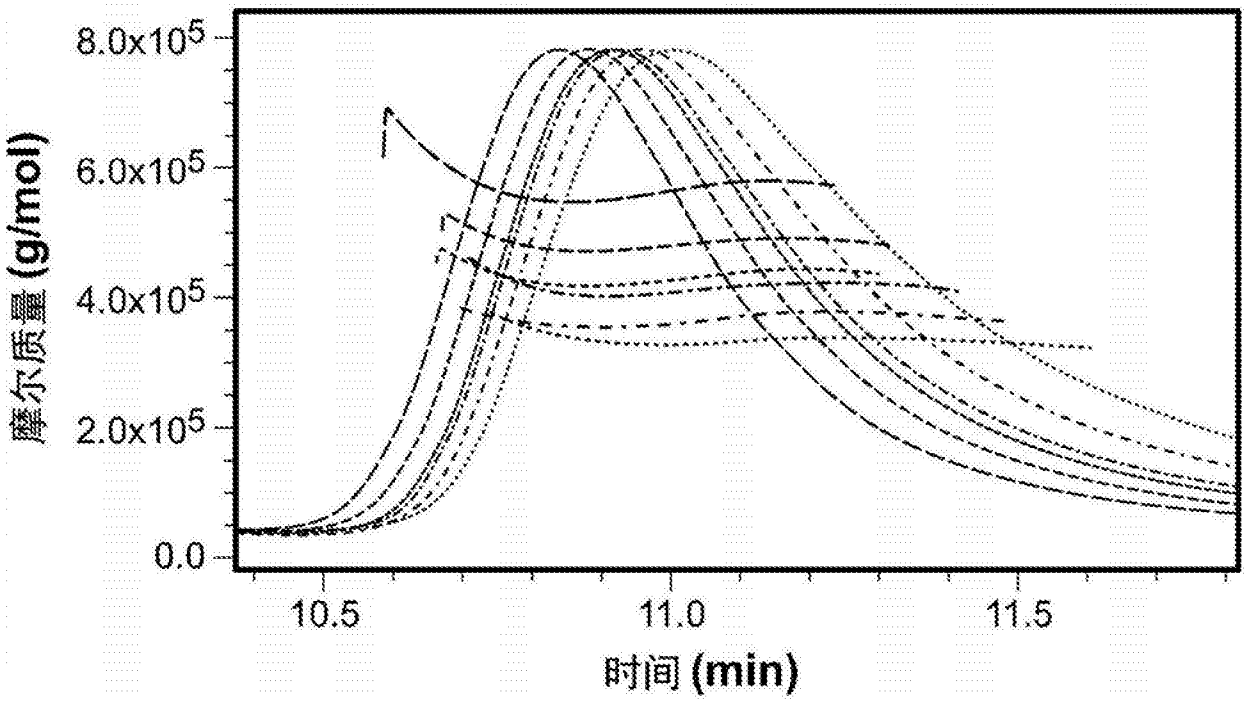

8.0x10^5
6.0x10^5
4.0x10^5
2.0x10^5
0.0
摩尔质量 (g/mol)
10.5
11.0
11.5
时间 (min)

图19C

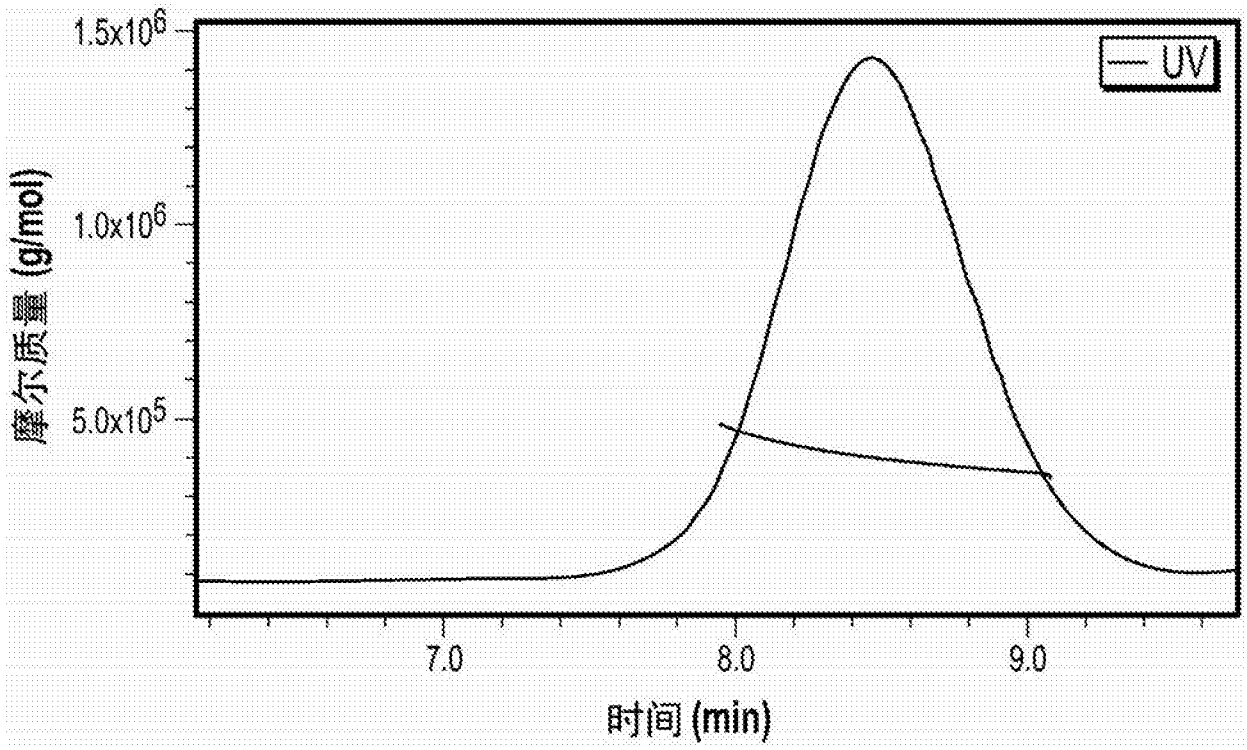

1.5x10^6
1.0x10^6
5.0x10^5
摩尔质量 (g/mol)
UV
7.0
8.0
9.0
时间 (min)

图20

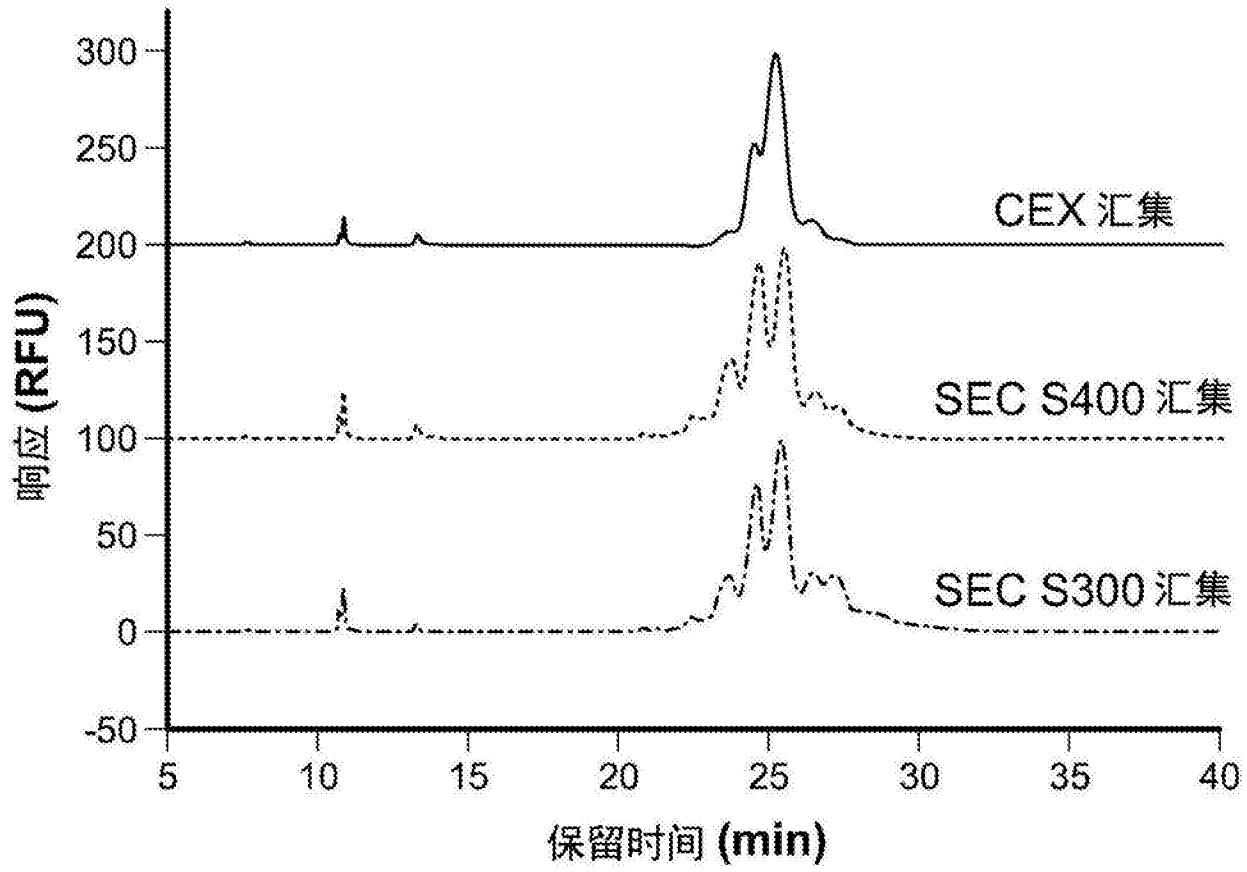

300
250
200
150
100
50
0
-50
响应 (RFU)
CEX 汇集
SEC S400 汇集
SEC S300 汇集
5
10
15
20
25
30
35
40
保留时间 (min)

图21A

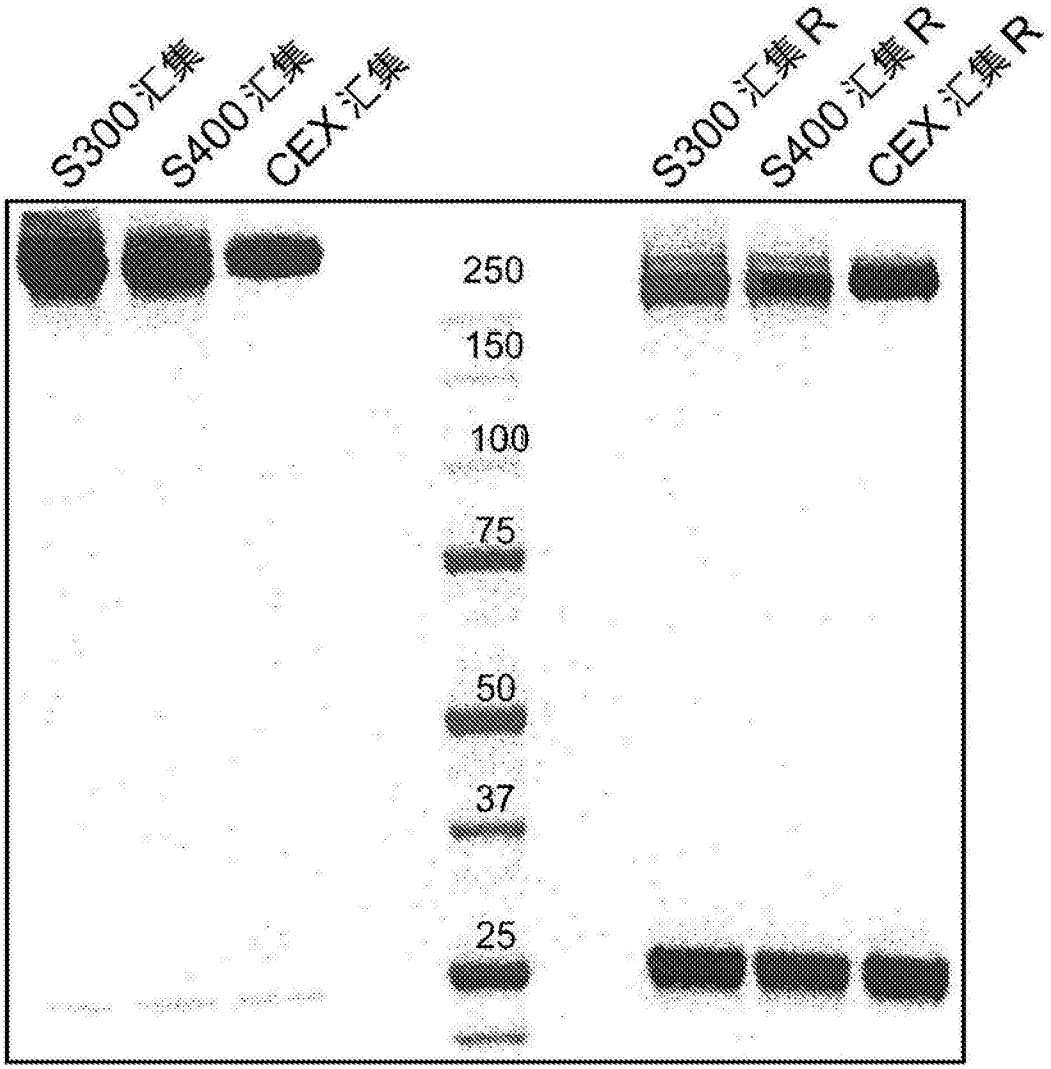

S300汇集
S400汇集
CEX汇集
S300汇集R
S400汇集R
CEX汇集R
250
150
100
75
50
37
25

图21B

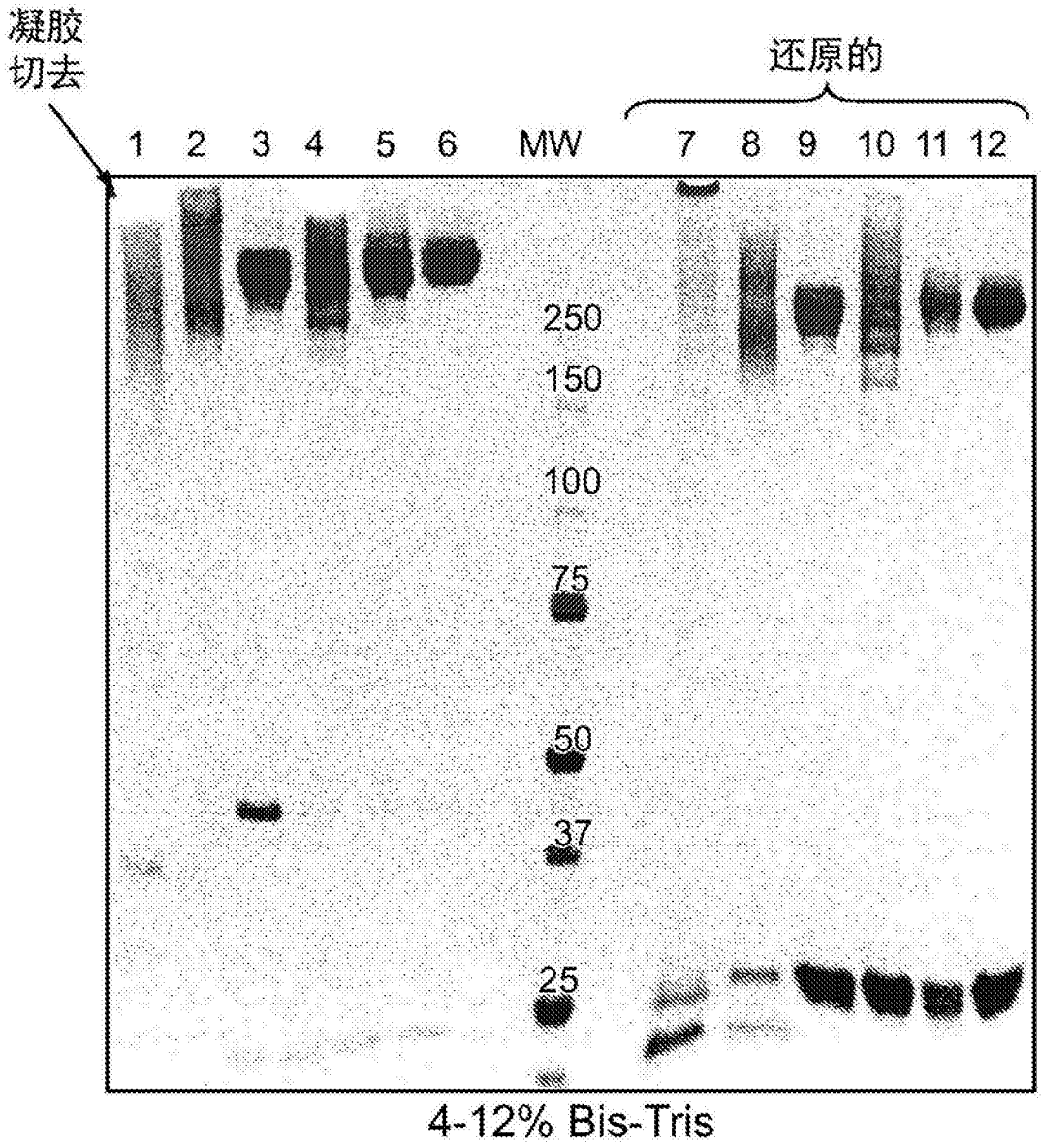

凝胶
切去
还原的
1 2 3 4 5 6 MW 7 8 9 10 11 12
250
150
100
75
50
37
25
4-12% Bis-Tris

图22A

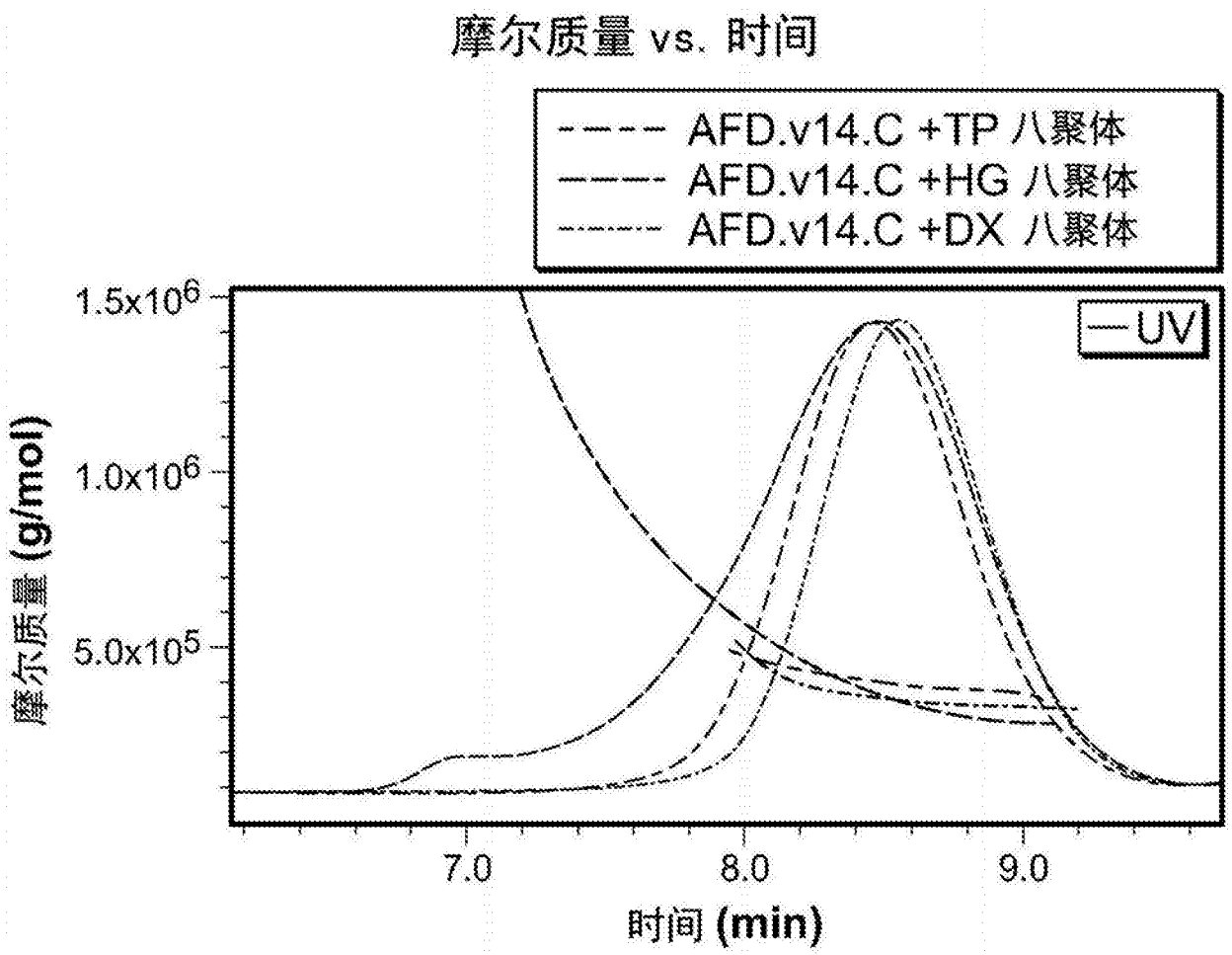

摩尔质量 vs. 时间
AFD.v14.C +TP 八聚体
AFD.v14.C +HG 八聚体
AFD.v14.C +DX 八聚体
UV
1.5x10^6
1.0x10^6
5.0x10^5
摩尔质量 (g/mol)
7.0
8.0
9.0
时间 (min)

图22B

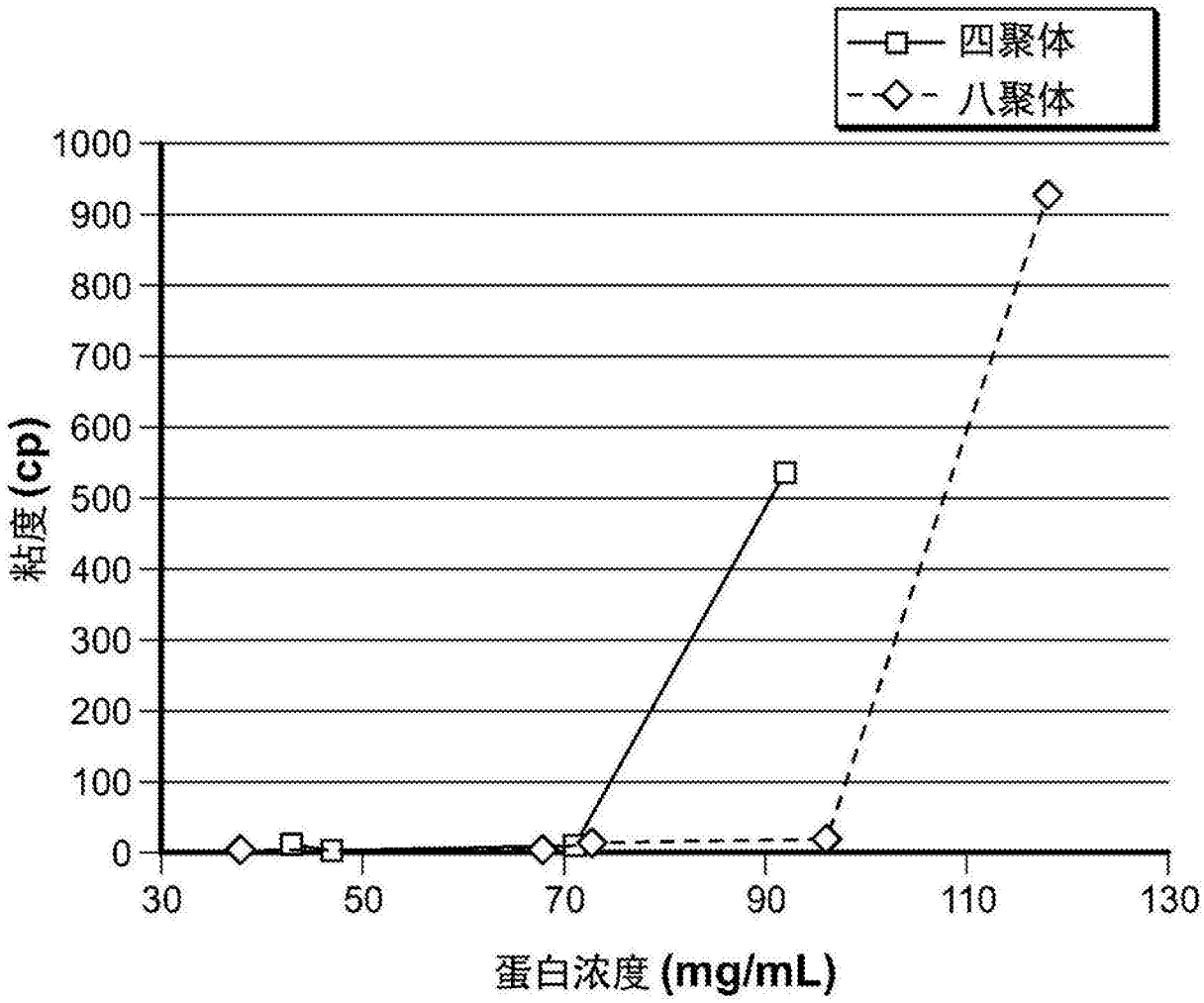

四聚体
八聚体
1000
900
800
700
600
500
400
300
200
100
0
粘度(cp)
30
50
70
90
110
130
蛋白浓度(mg/mL)

图23

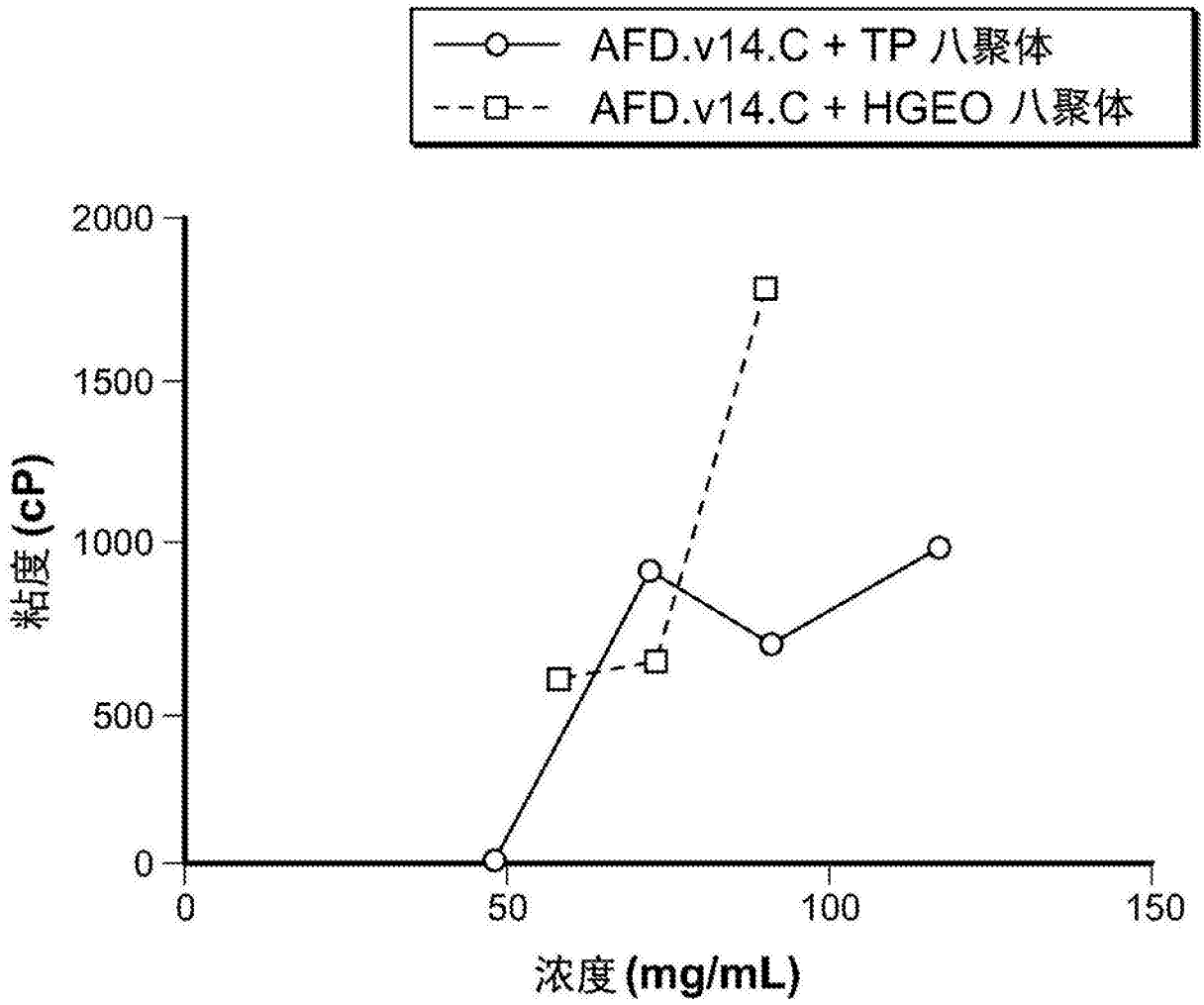

AFD.v14.C + TP 八聚体
AFD.v14.C + HGEO 八聚体
2000
1500
1000
500
0
粘度 (cP)
0
50
100
150
浓度 (mg/mL)

图24

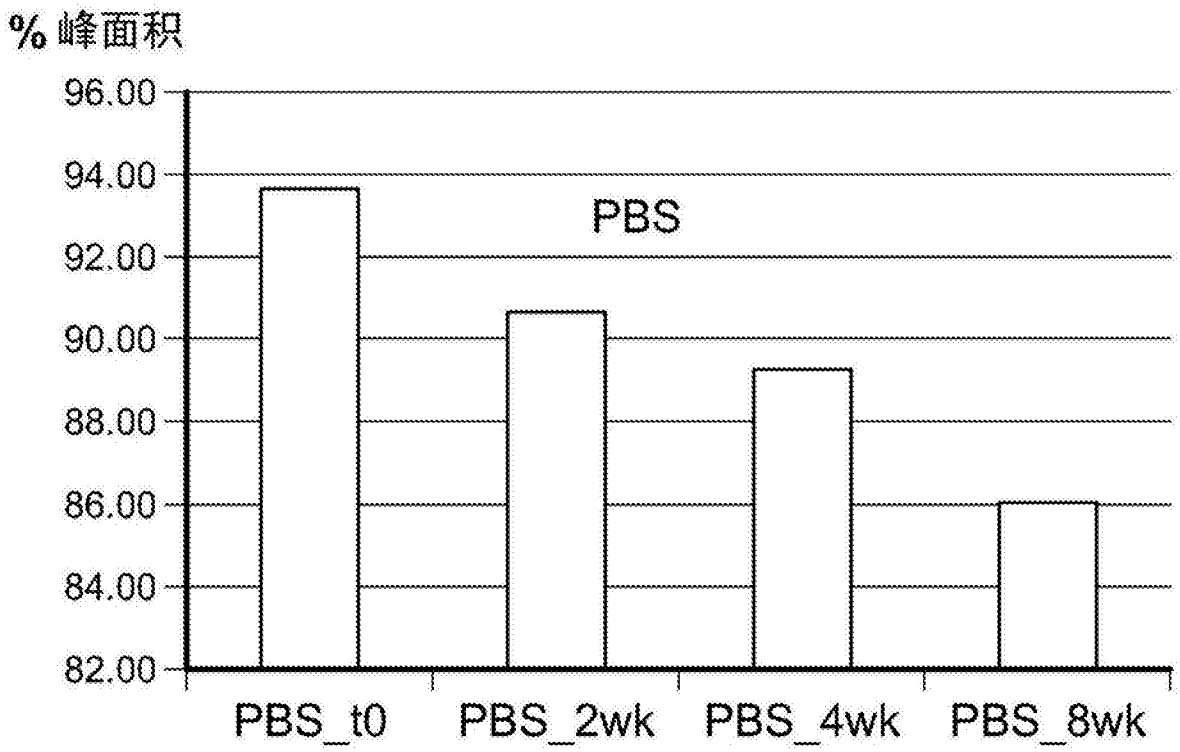

% 峰面积
96.00
94.00
92.00
90.00
88.00
86.00
84.00
82.00
PBS
PBS_t0
PBS_2wk
PBS_4wk
PBS_8wk

图25A

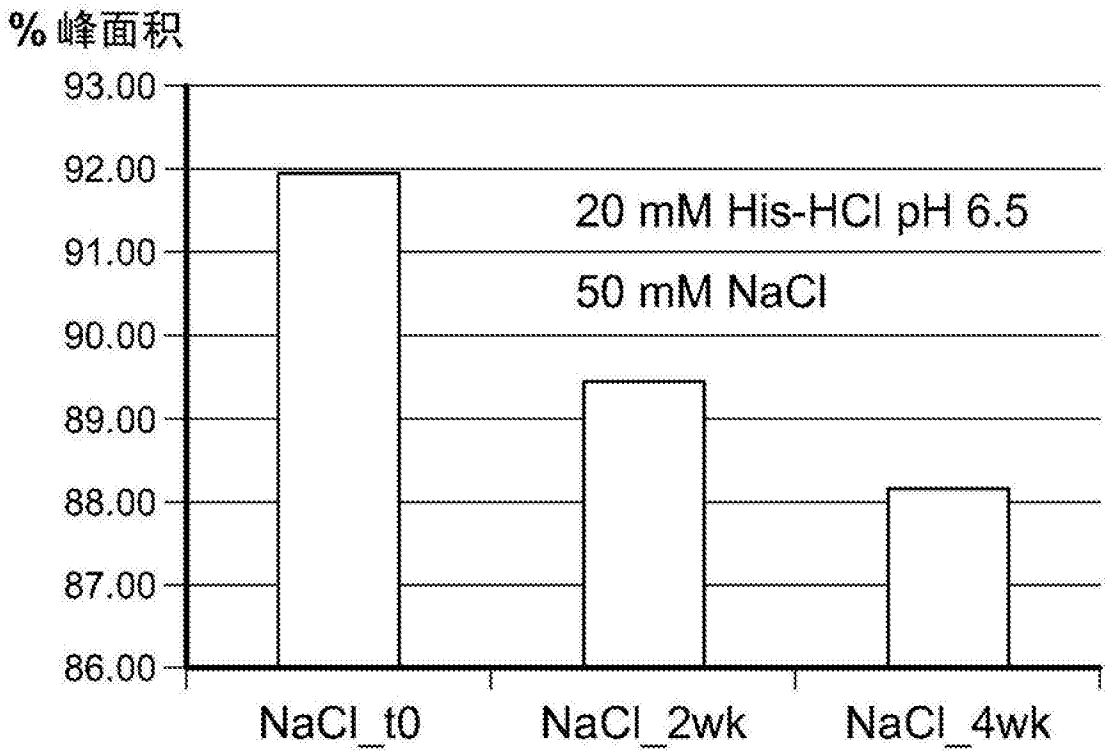
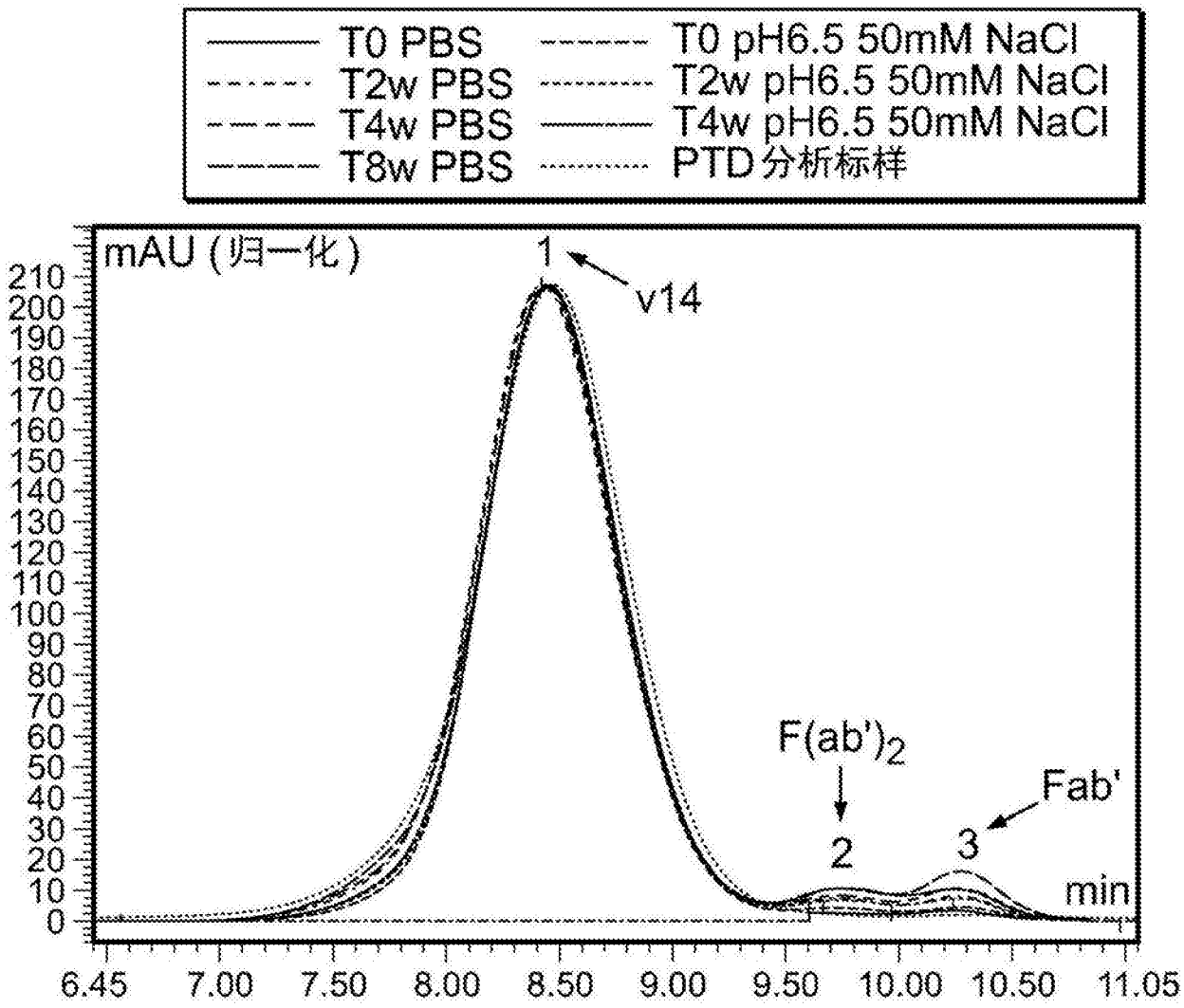

% 峰面积
93.00
92.00
91.00
90.00
89.00
88.00
87.00
86.00
20 mM His-HCl pH 6.5
50 mM NaCl
NaCl_t0
NaCl_2wk
NaCl_4wk

图25B



	% 总共		
样品 ID	峰 1	峰 2	峰 3
T0 PBS	98.48	0.58	0.94
T2w PBS	95.82	2.02	2.16
T4w PBS	94.85	2.32	2.84
T8w PBS	93.00	2.77	4.23
T0 pH6.5, 50mM NaCl	97.53	1.21	1.26
T2w pH6.5, 50mM NaCl	95.99	2.00	2.02
T4w pH6.5, 50mM NaCl	93.94	3.22	2.84
PTD std	100.00	0.00	0.00

图26

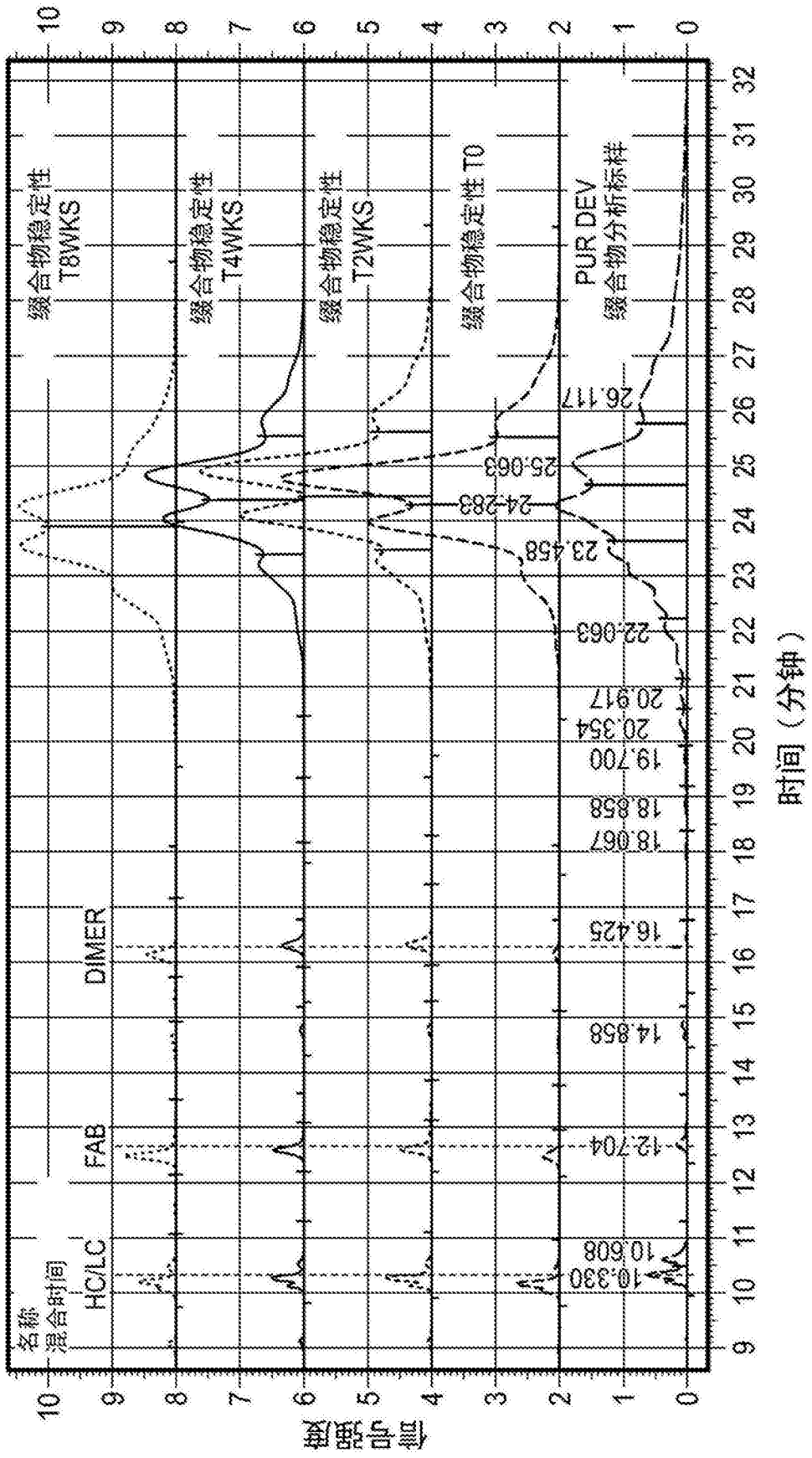

缀合物稳定性 T8WKS
缀合物稳定性 T4WKS
缀合物稳定性 T2WKS
缀合物稳定性 T0
PUR DEV
缀合物分析标样
DIMER
FAB
HC/LC
名称
混合时间
26.117
25.063
24.283
23.458
22.063
20.917
20.354
19.700
18.858
18.067
16.425
14.858
12.704
10.608
10.330
信号强度
时间（分钟）

图27

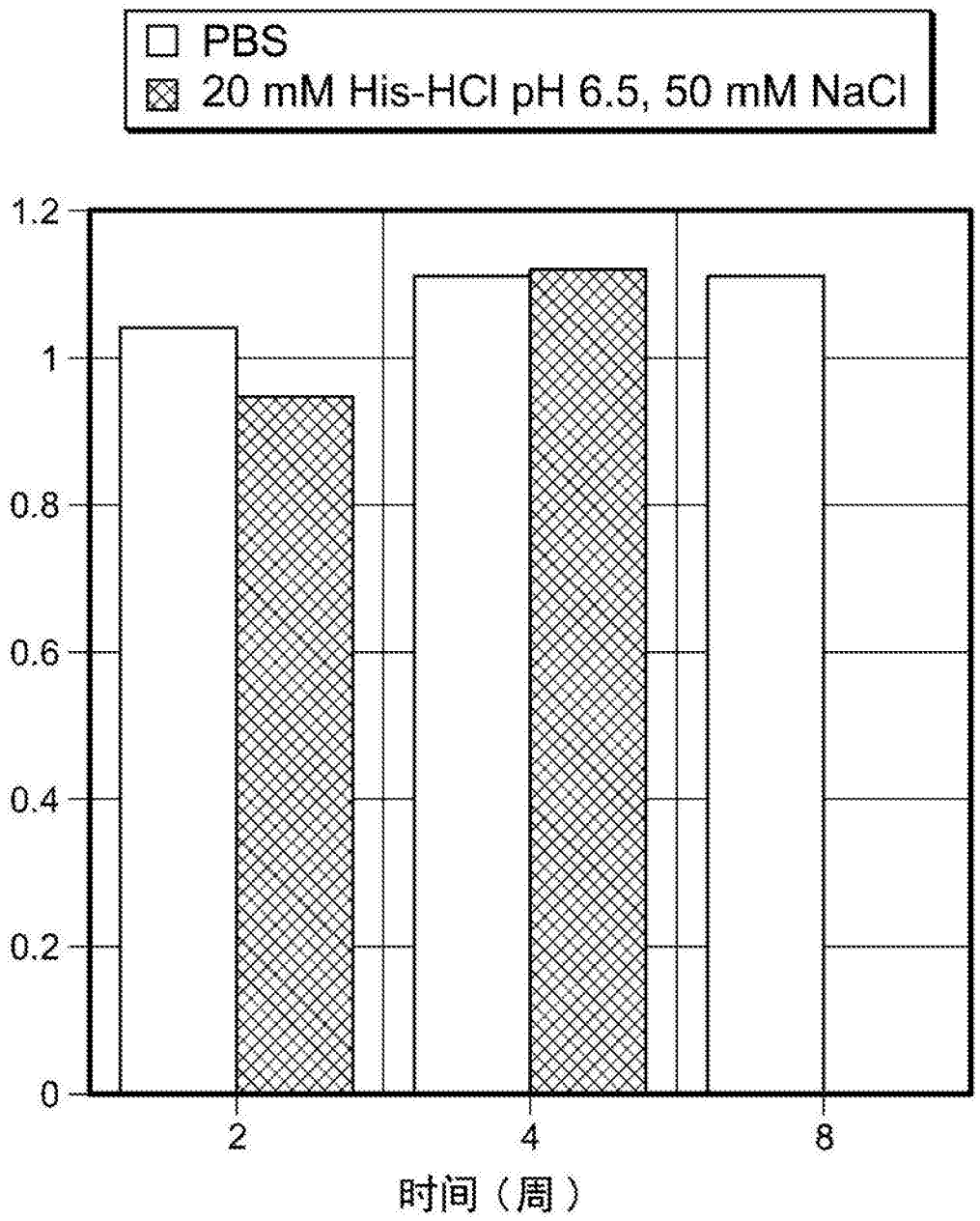


图28

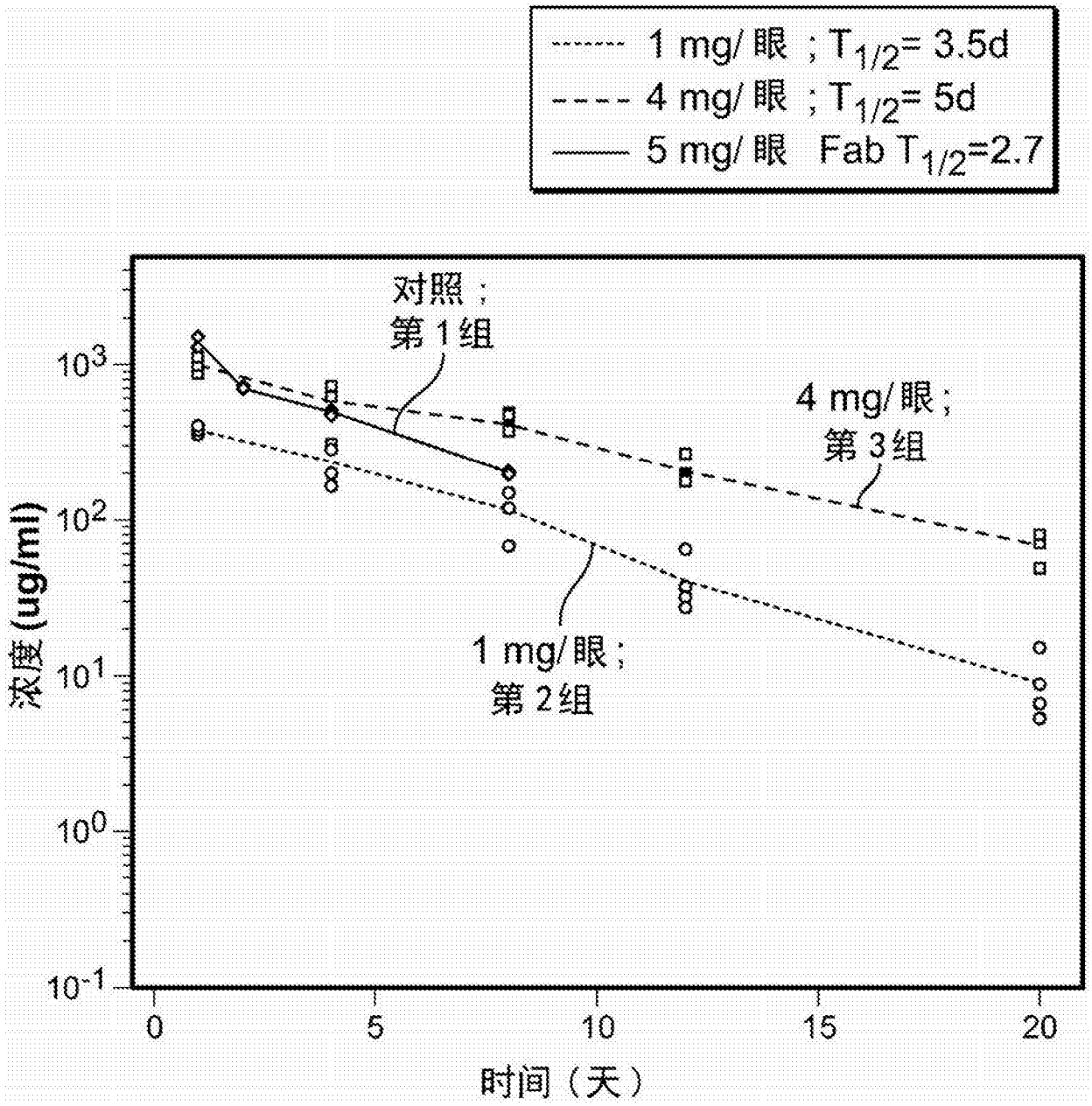

1 mg/眼 ; $T_{1/2}$= 3.5d
4 mg/眼 ; $T_{1/2}$= 5d
5 mg/眼 Fab $T_{1/2}$=2.7
对照；
第1组
4 mg/眼；
第3组
1 mg/眼；
第2组
浓度(ug/ml)
10^3
10^2
10^1
10^0
10^{-1}
0
5
10
15
20
时间（天）

图29A

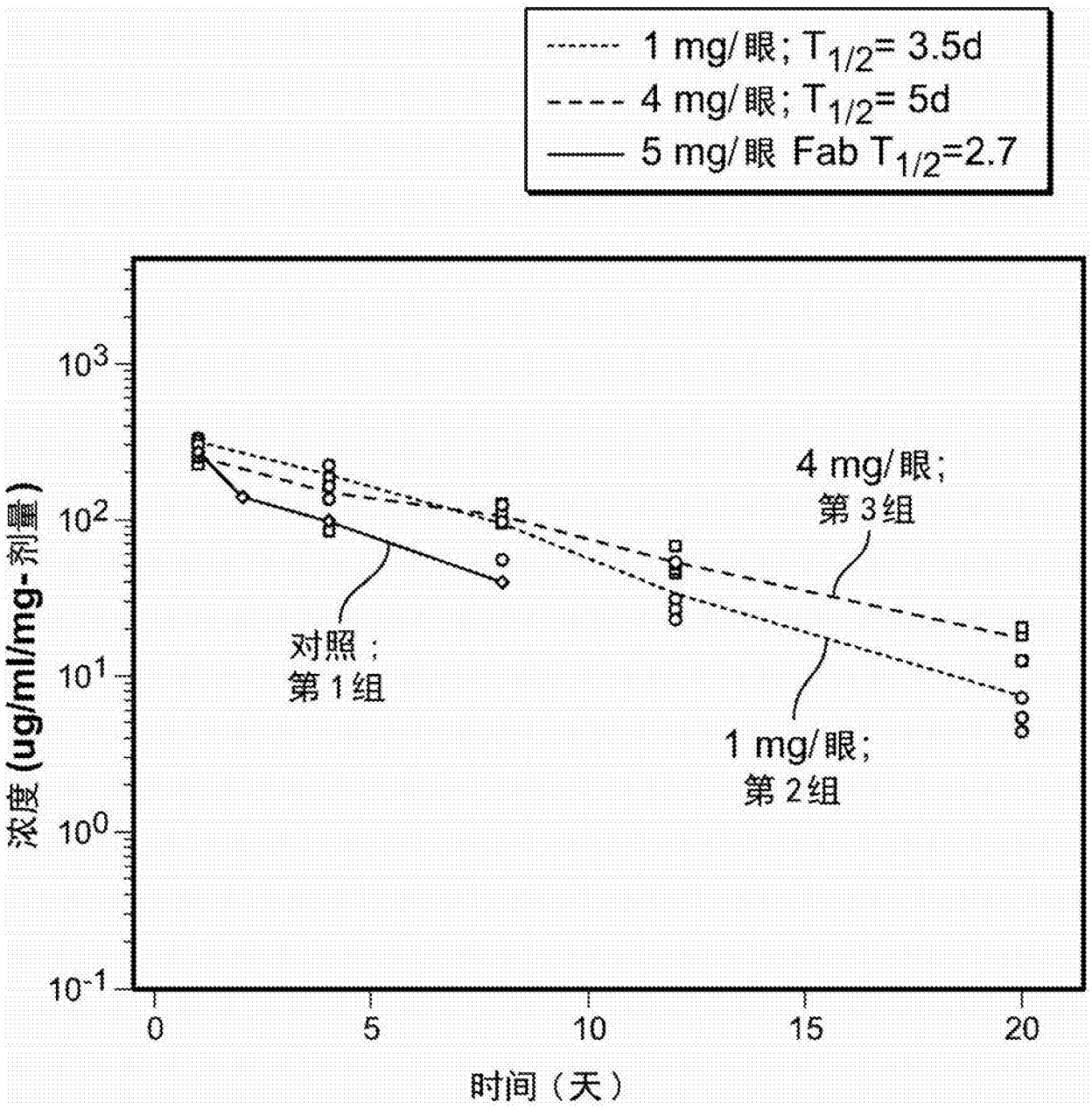

1 mg/眼; $T_{1/2}$= 3.5d
4 mg/眼; $T_{1/2}$= 5d
5 mg/眼 Fab $T_{1/2}$=2.7
浓度 (ug/ml/mg-剂量)
10^3
10^2
10^1
10^0
10^{-1}
0
5
10
15
20
时间（天）
4 mg/眼;
第 3 组
对照；
第 1 组
1 mg/眼;
第 2 组

图29B

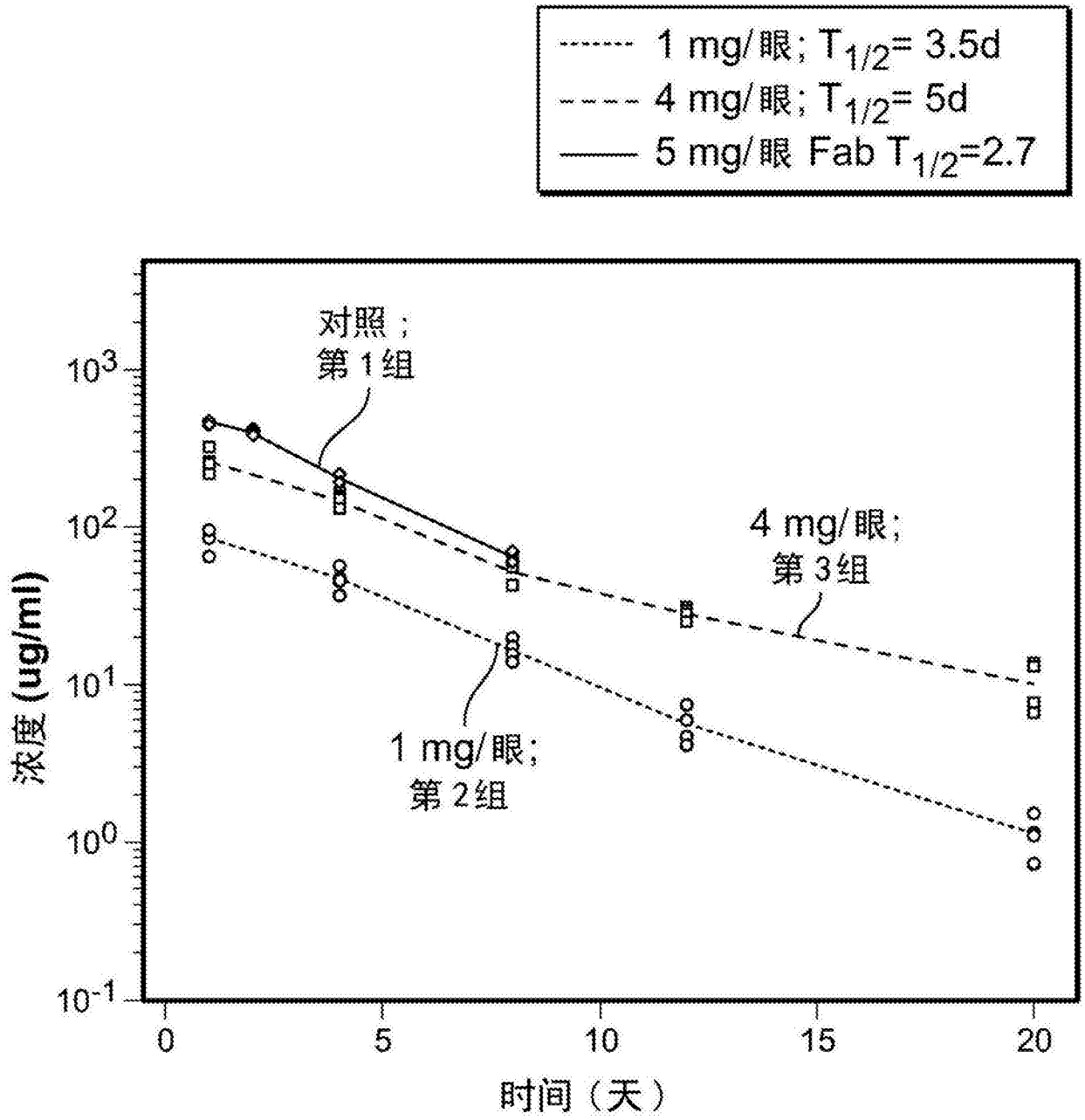

1 mg/眼; $T_{1/2}$= 3.5d
4 mg/眼; $T_{1/2}$= 5d
5 mg/眼 Fab $T_{1/2}$=2.7
对照；
第1组
4 mg/眼;
第3组
1 mg/眼;
第2组
浓度 (ug/ml)
10^3
10^2
10^1
10^0
10^{-1}
0
5
10
15
20
时间（天）

图30A

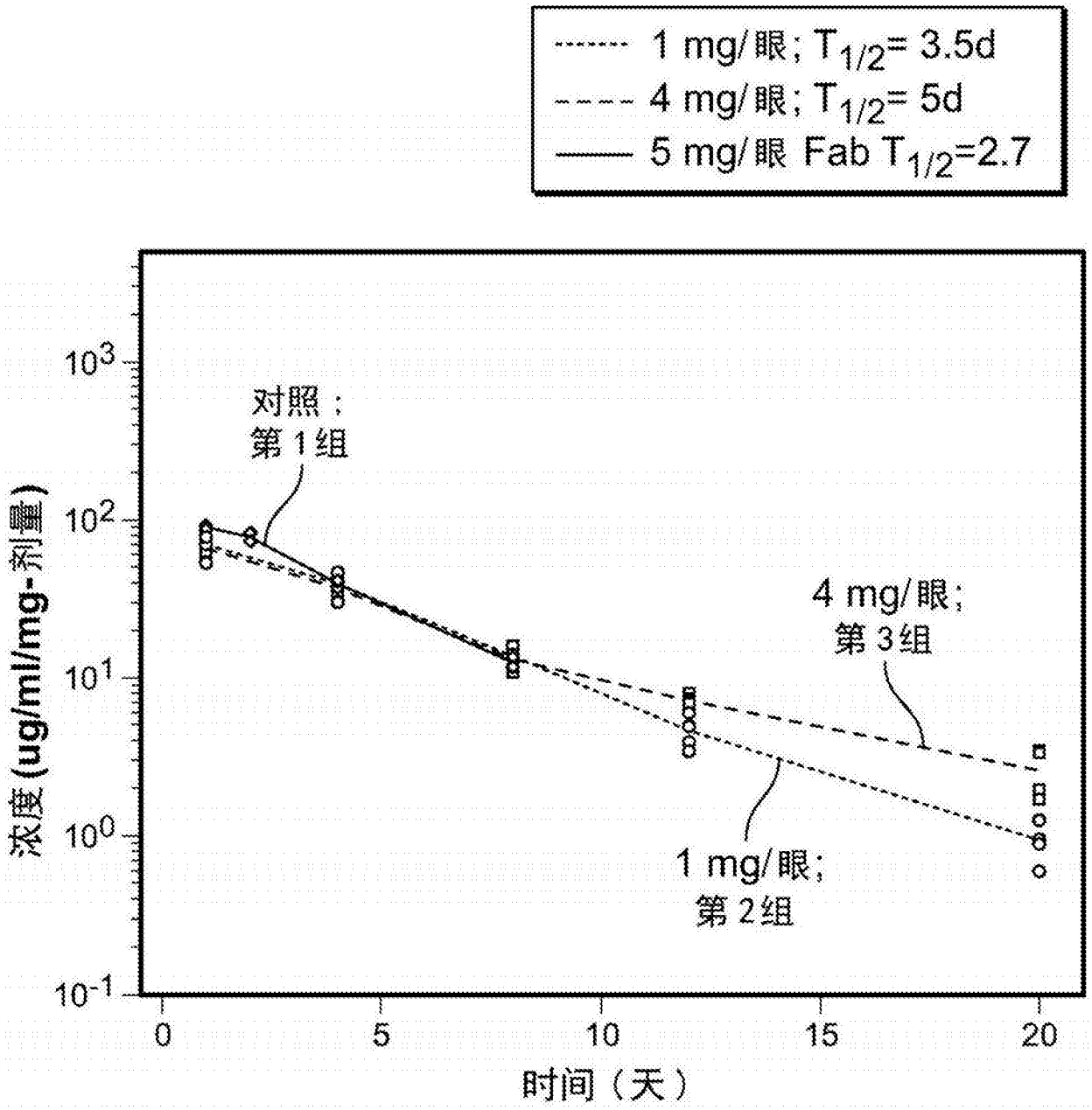

1 mg/眼; $T_{1/2}$= 3.5d
4 mg/眼; $T_{1/2}$= 5d
5 mg/眼 Fab $T_{1/2}$=2.7
浓度 (ug/ml/mg-剂量)
10^3
10^2
10^1
10^0
10^{-1}
对照：
第 1 组
4 mg/眼;
第 3 组
1 mg/眼;
第 2 组
0
5
10
15
20
时间（天）

图30B

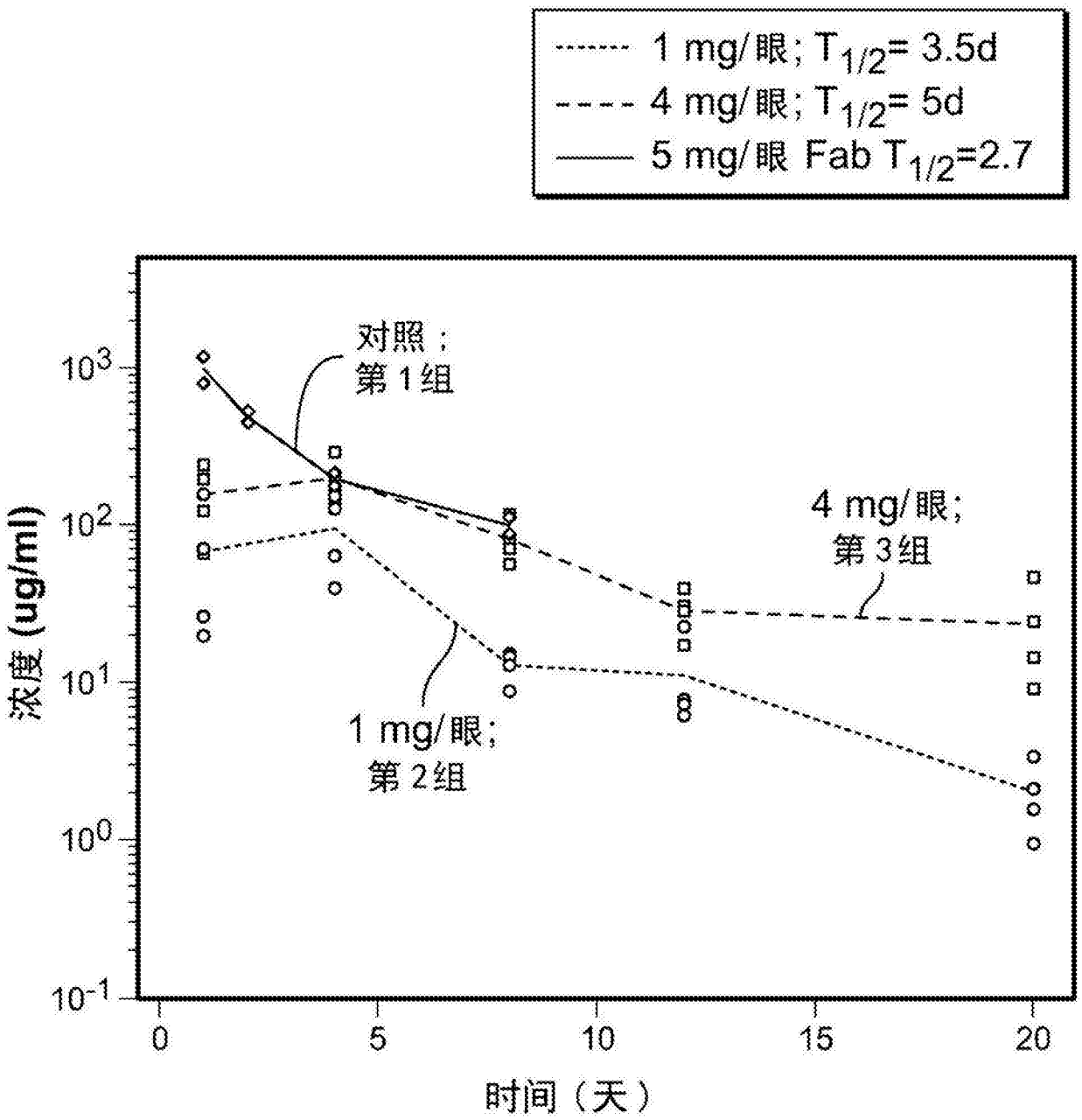

1 mg/眼; $T_{1/2}$= 3.5d
4 mg/眼; $T_{1/2}$= 5d
5 mg/眼 Fab $T_{1/2}$=2.7
对照；
第1组
4 mg/眼;
第3组
1 mg/眼;
第2组
浓度 (ug/ml)
10^3
10^2
10^1
10^0
10^{-1}
0
5
10
15
20
时间（天）

图31A

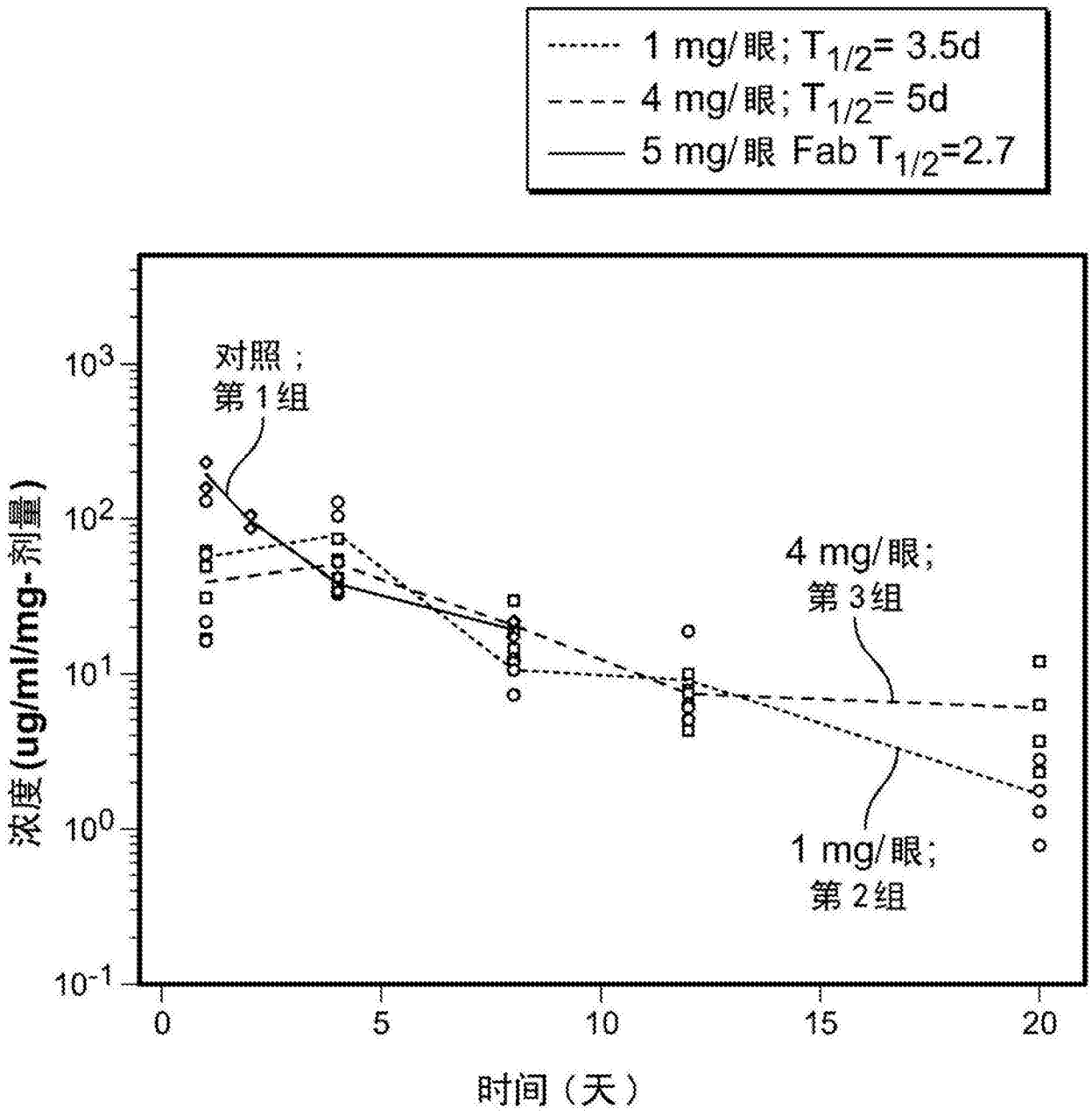

1 mg/眼; $T_{1/2}$= 3.5d
4 mg/眼; $T_{1/2}$= 5d
5 mg/眼 Fab $T_{1/2}$=2.7
对照；
第1组
4 mg/眼；
第3组
1 mg/眼；
第2组
浓度(ug/ml/mg-剂量)
10^3
10^2
10^1
10^0
10^{-1}
0
5
10
15
20
时间（天）

图31B

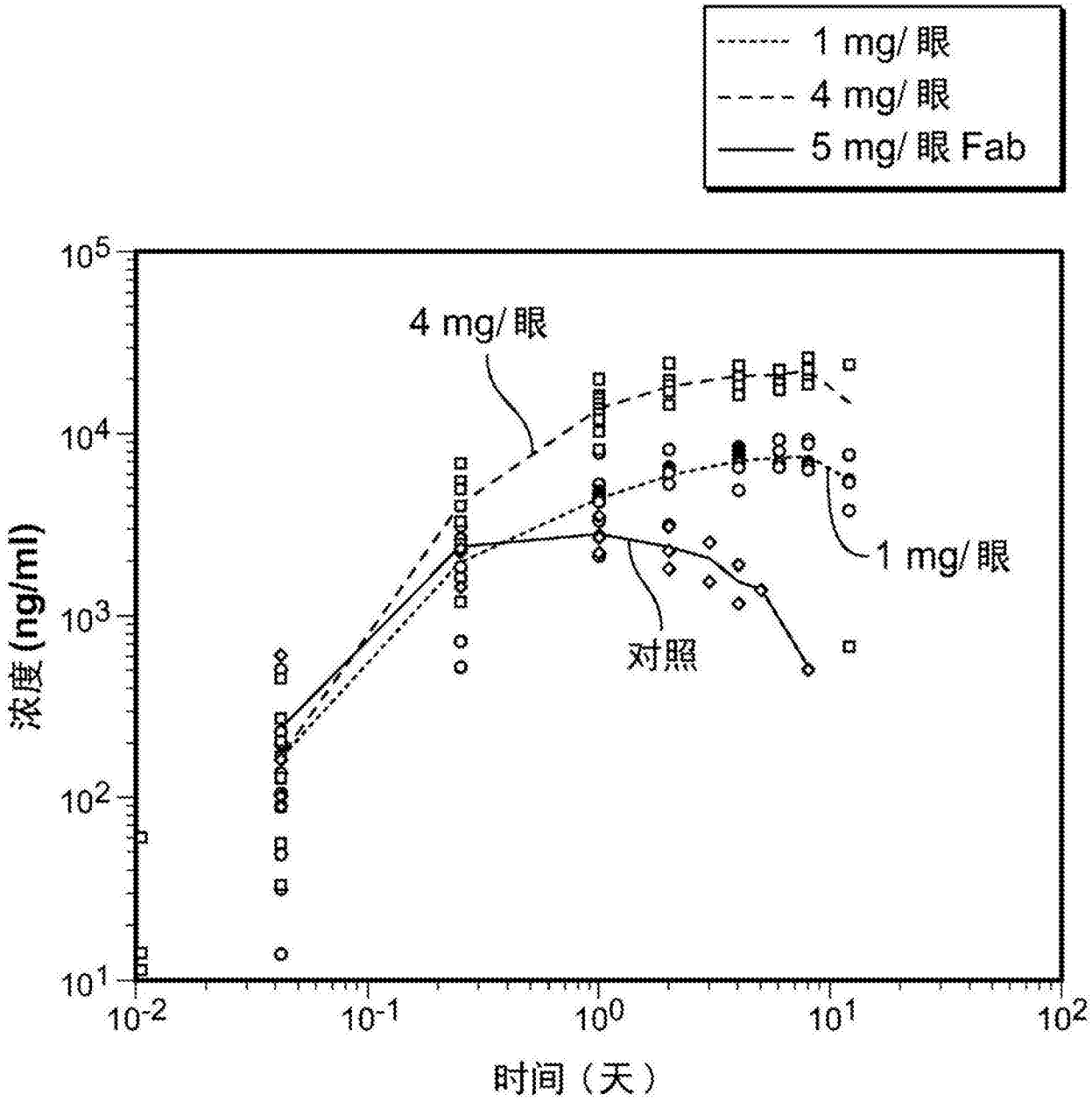

1 mg/眼
4 mg/眼
5 mg/眼 Fab
4 mg/眼
1 mg/眼
对照
浓度(ng/ml)
时间（天）
10^5
10^4
10^3
10^2
10^1
10^{-2}
10^{-1}
10^0
10^1
10^2

图32A

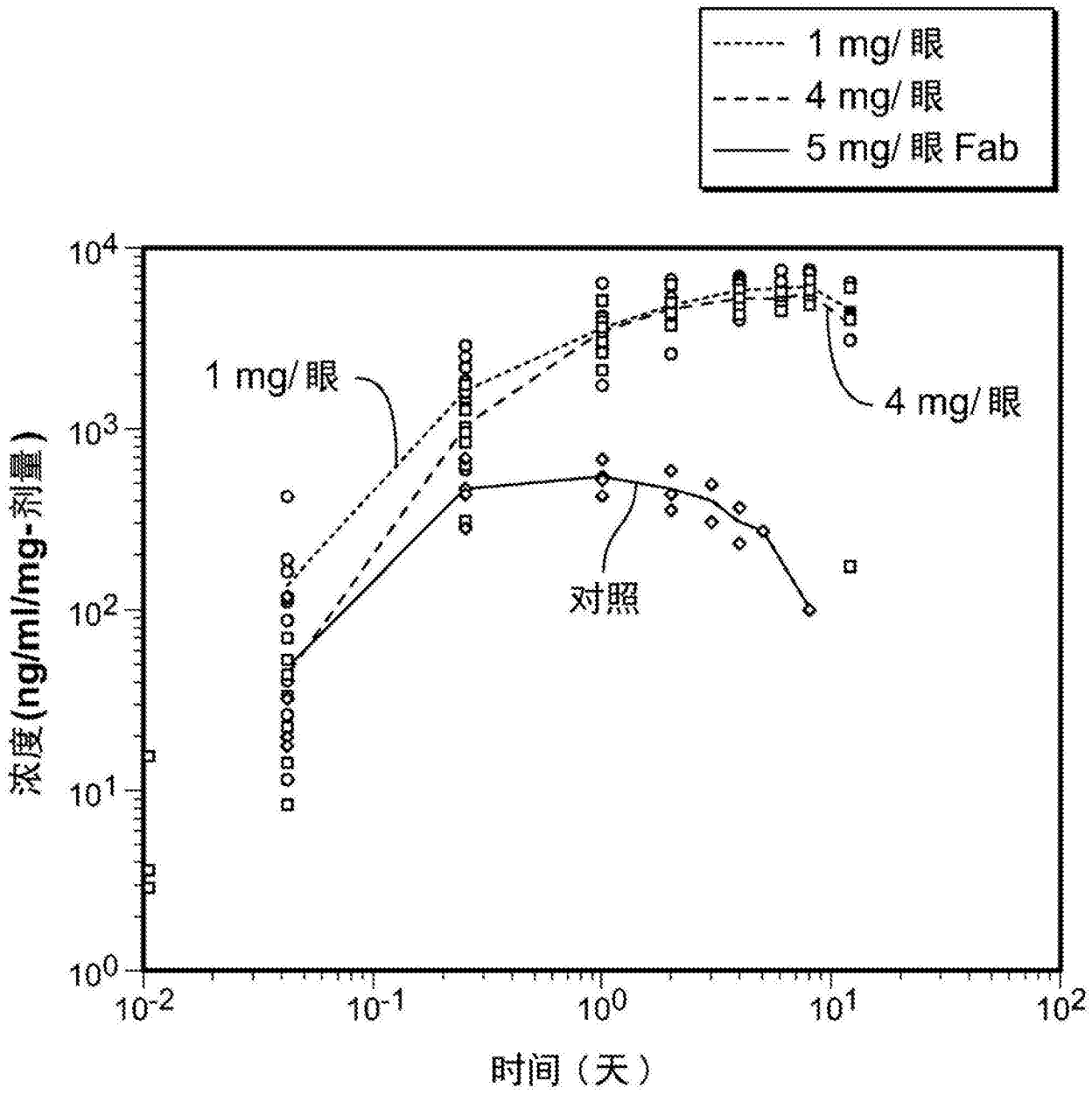


图32B

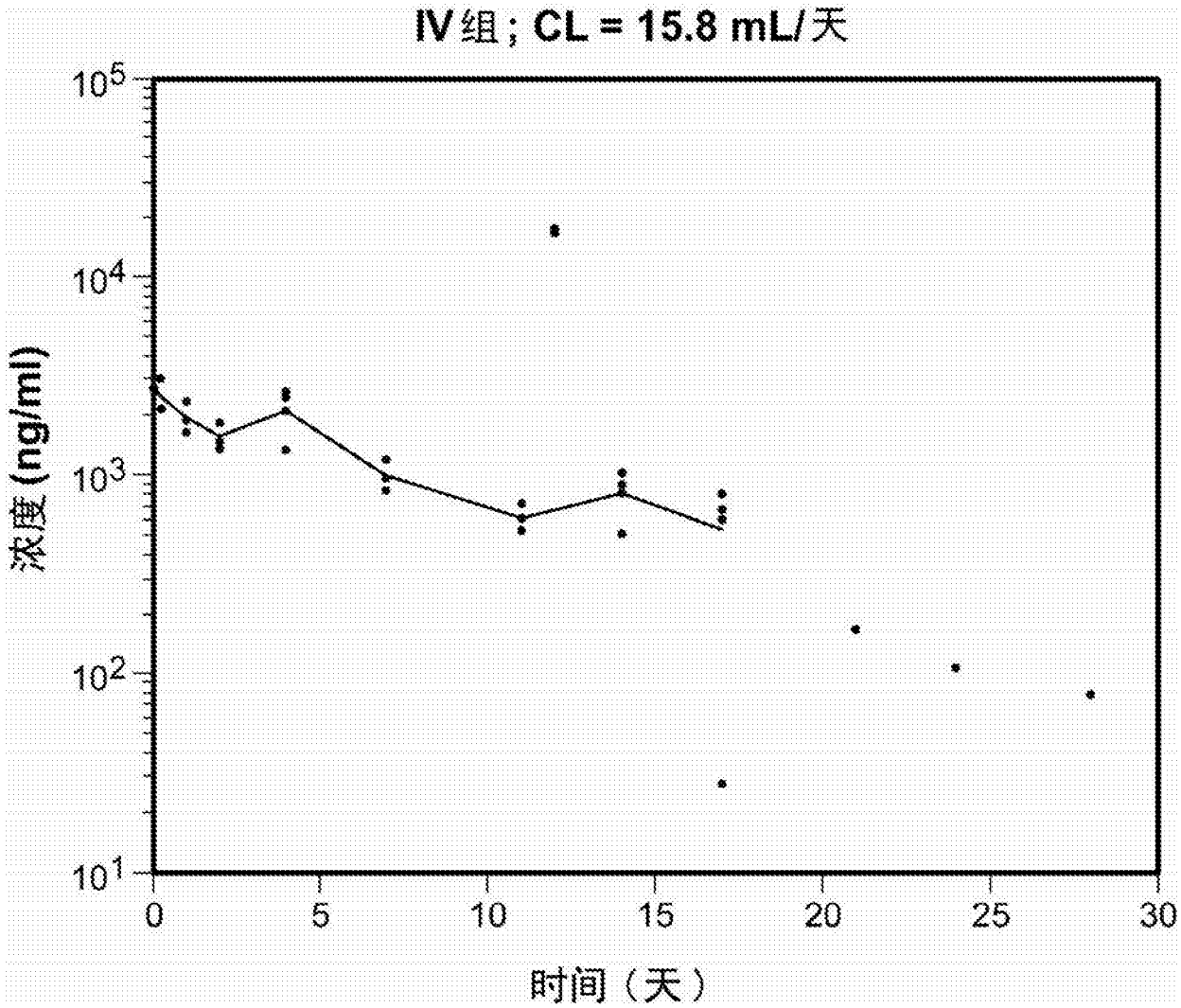


图32C

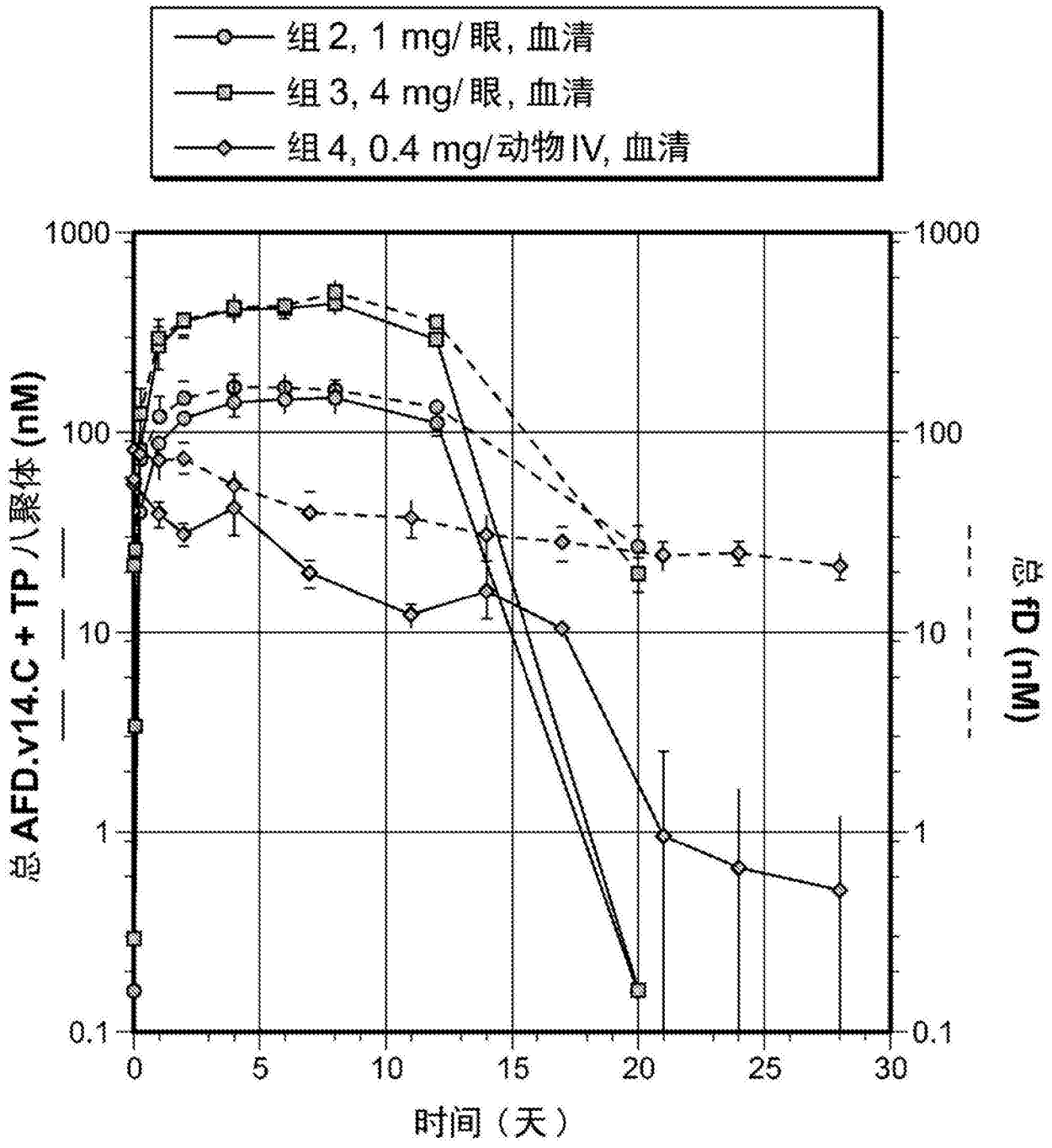

组2, 1 mg/眼, 血清
组3, 4 mg/眼, 血清
组4, 0.4 mg/动物IV, 血清
总 AFD.v14.C + TP 八聚体 (nM)
总 fD (nM)
1000
100
10
1
0.1
0
5
10
15
20
25
30
时间（天）

图33A

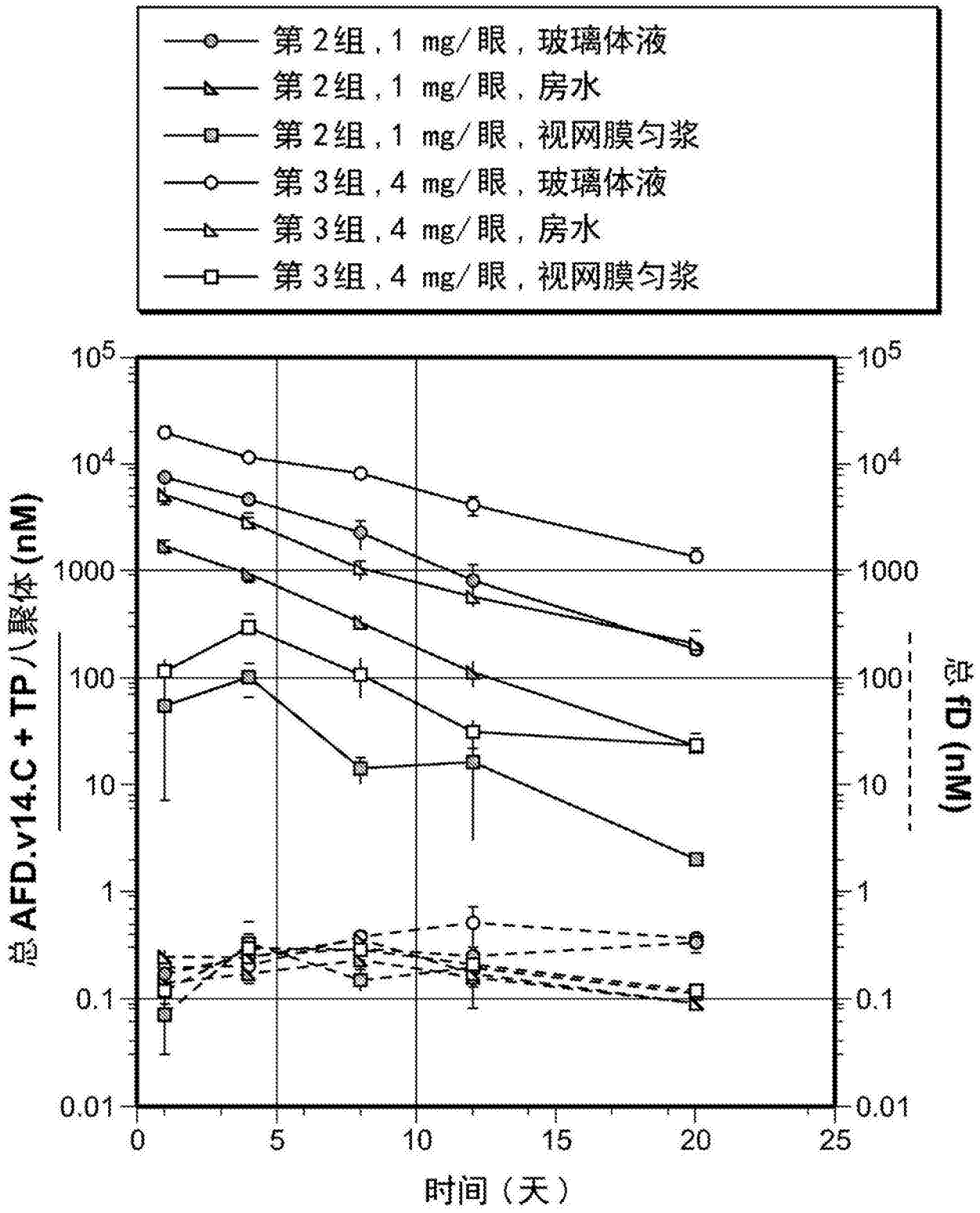

第2组，1 mg/眼，玻璃体液
第2组，1 mg/眼，房水
第2组，1 mg/眼，视网膜匀浆
第3组，4 mg/眼，玻璃体液
第3组，4 mg/眼，房水
第3组，4 mg/眼，视网膜匀浆
总 AFD.v14.C + TP八聚体 (nM)
游离 FD (nM)
时间（天）
0
5
10
15
20
25
0.01
0.1
1
10
100
1000
10^4
10^5

图33B

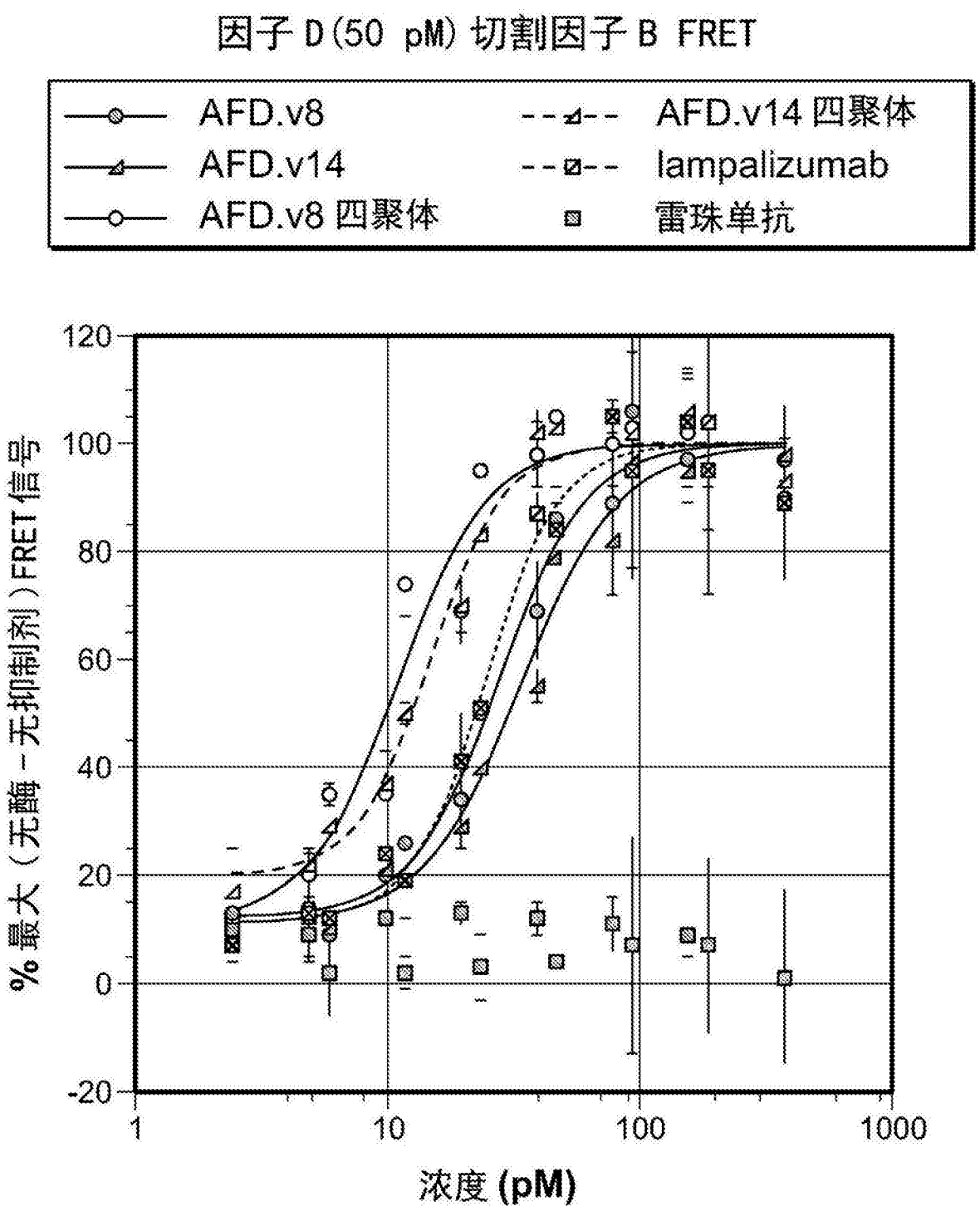

因子 D(50 pM) 切割因子 B FRET
AFD.v8
AFD.v14
AFD.v8 四聚体
AFD.v14 四聚体
lampalizumab
雷珠单抗
% 最大（无酶 - 无抑制剂）FRET 信号
120
100
80
60
40
20
0
-20
1
10
100
1000
浓度 (pM)

图34A

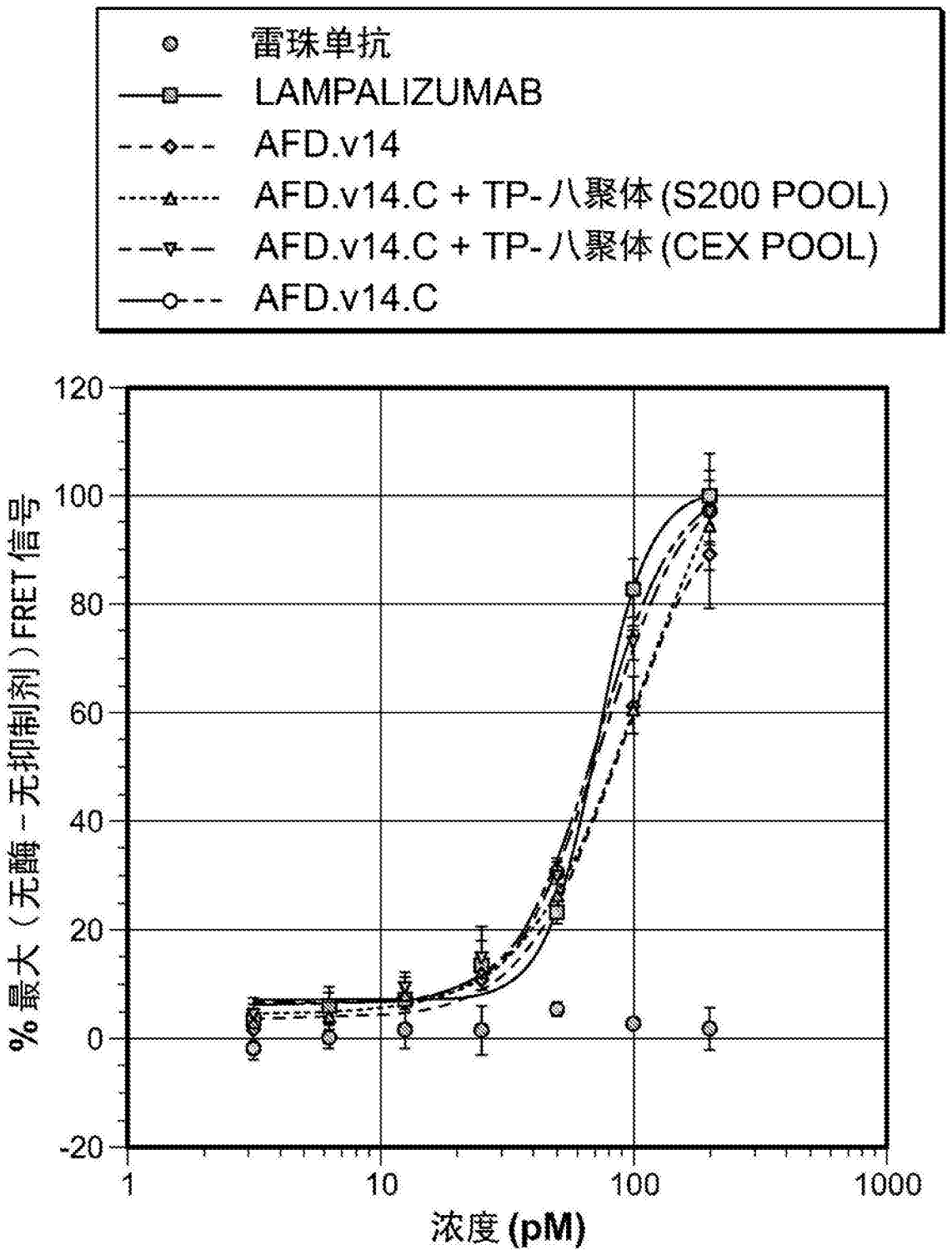

雷珠单抗
LAMPALIZUMAB
AFD.v14
AFD.v14.C + TP-八聚体 (S200 POOL)
AFD.v14.C + TP-八聚体 (CEX POOL)
AFD.v14.C
% 最大（无酶 - 无抑制剂）FRET 信号
120
100
80
60
40
20
0
-20
1
10
100
1000
浓度 (pM)

图34B

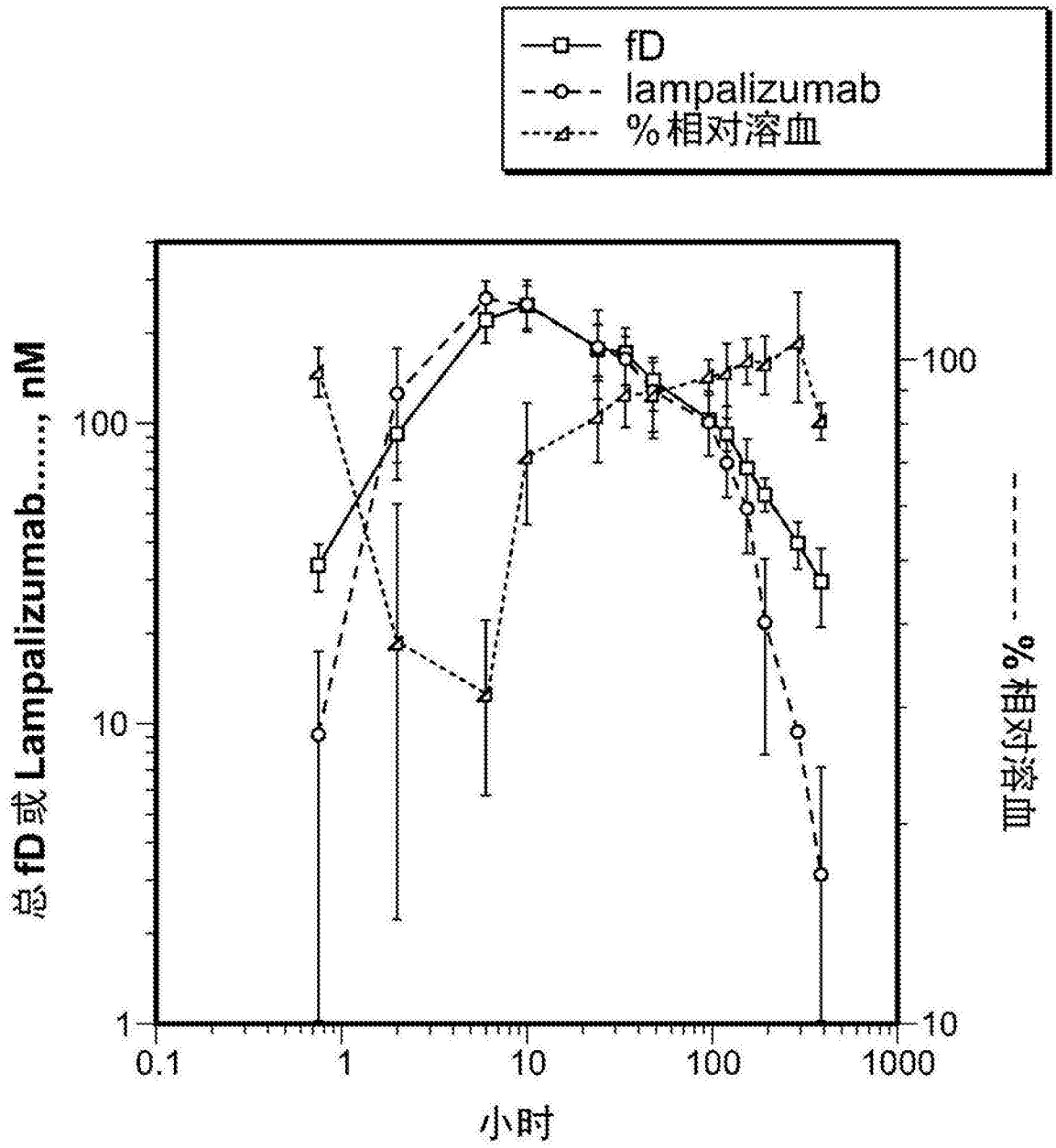

fD
lampalizumab
% 相对溶血
总 fD 或 Lampalizumab....., nM
100
10
1
0.1
1
10
100
1000
小时
100
10
% 相对溶血

图35A

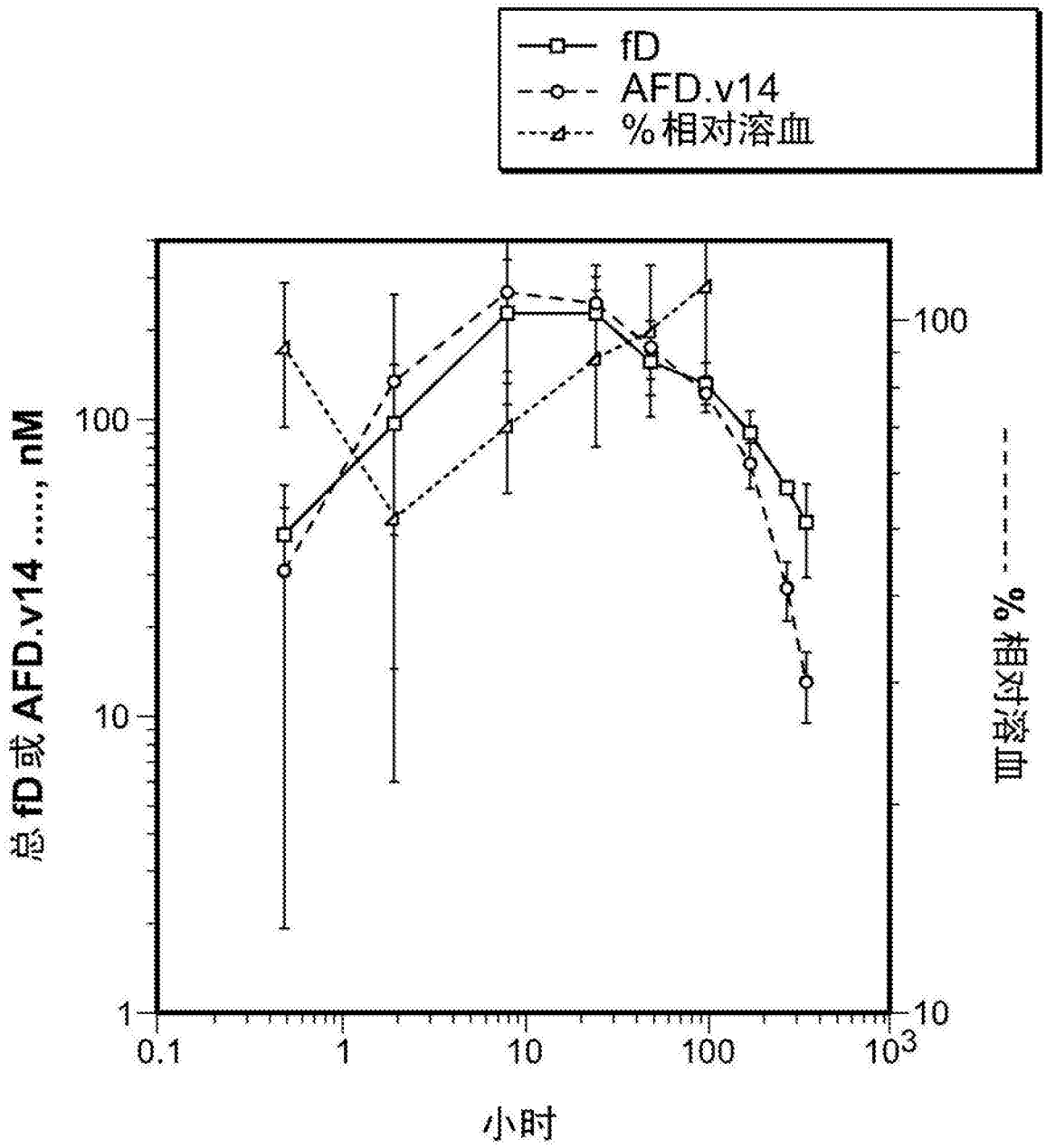

fD
AFD.v14
%相对溶血
总 fD或 AFD.v14 ....., nM
100
10
1
0.1
1
10
100
10^3
小时
100
10
% 相对溶血

图35B

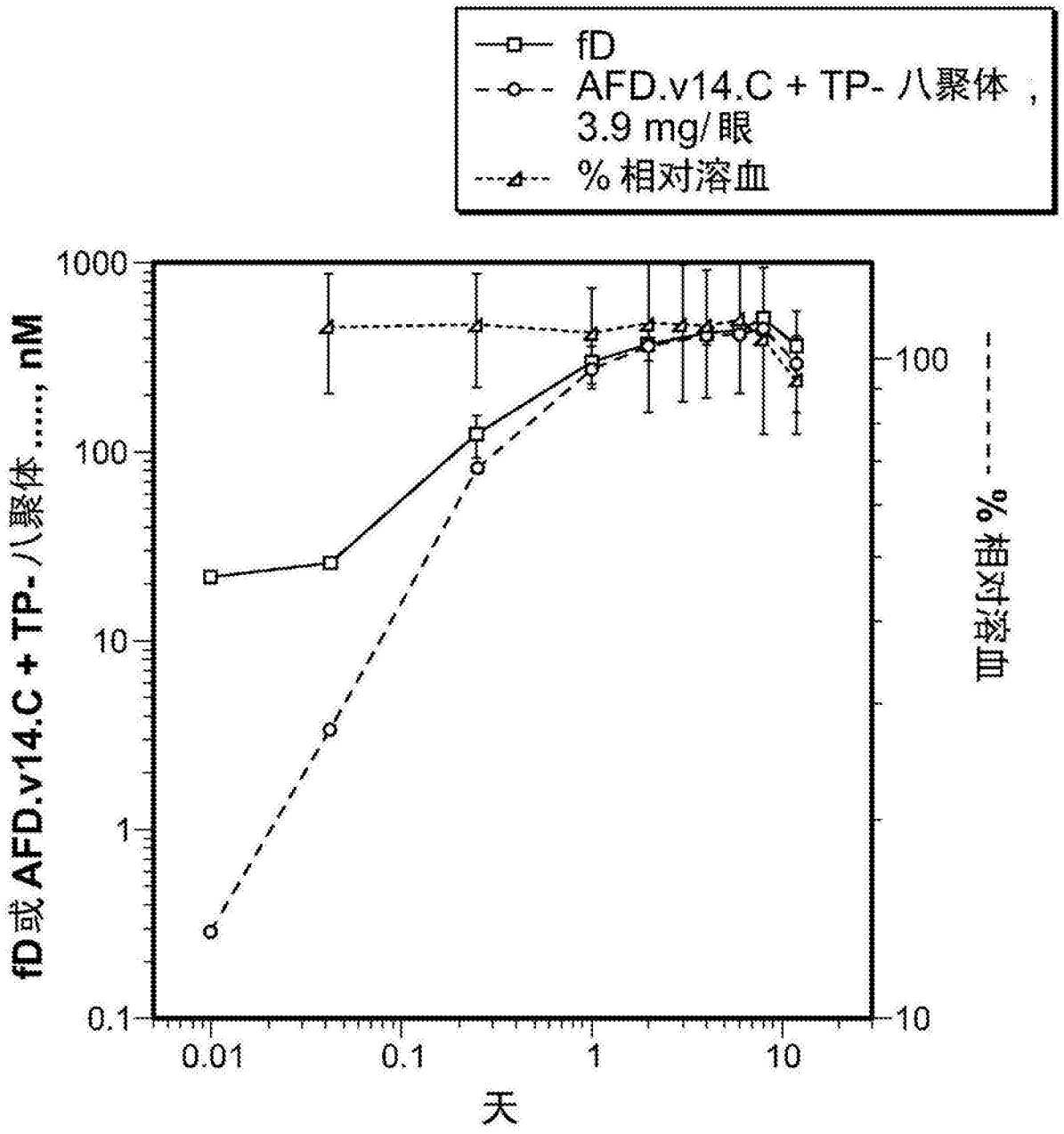

fD
AFD.v14.C + TP- 八聚体，3.9 mg/眼
% 相对溶血
1000
100
10
1
0.1
0.01
0.1
1
10
100
10
fD或AFD.v14.C + TP-八聚体...., nM
% 相对溶血
天

图35C

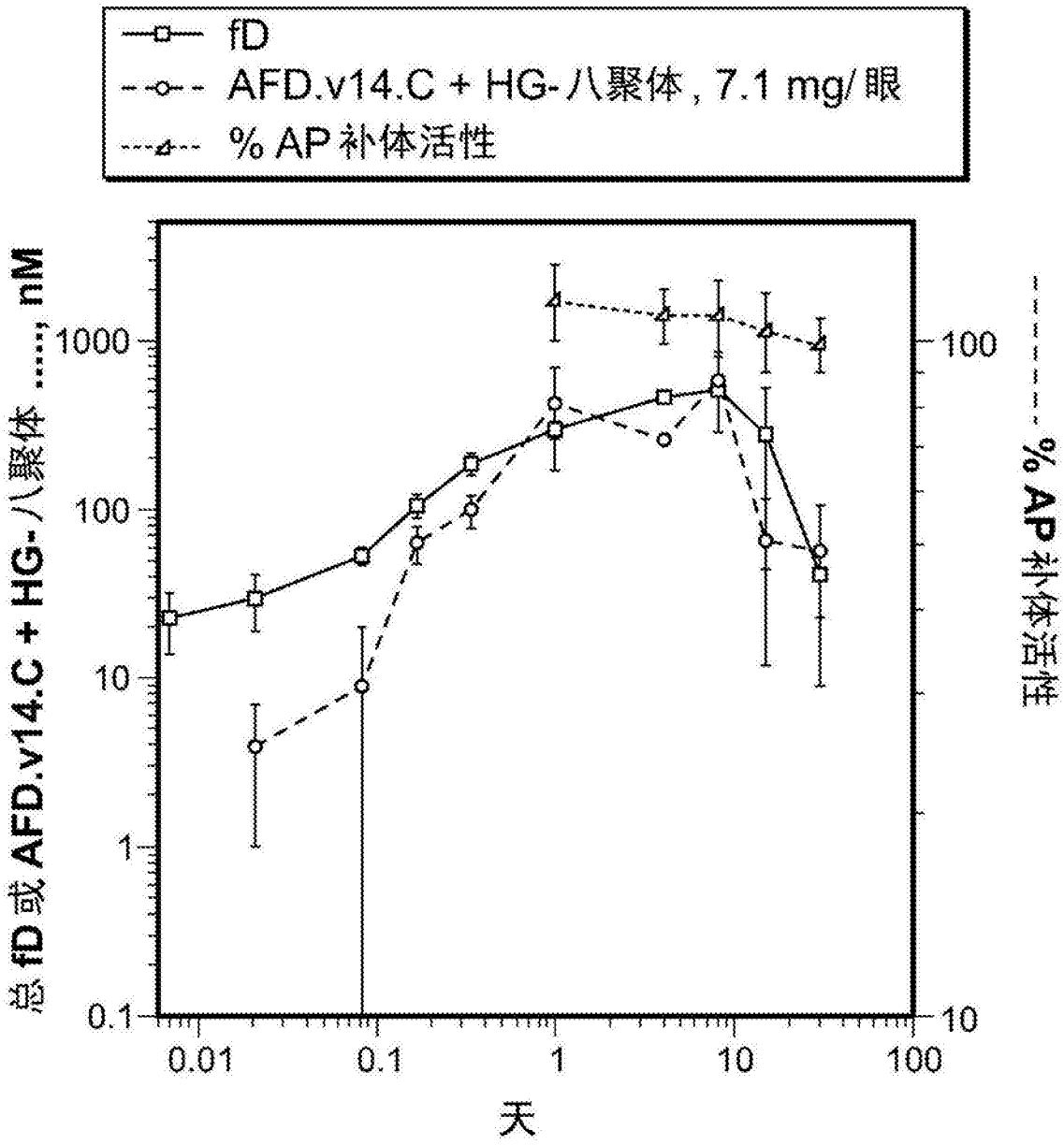

fD
AFD.v14.C + HG-八聚体, 7.1 mg/眼
% AP补体活性
总 fD或 AFD.v14.C + HG-八聚体 ....., nM
1000
100
10
1
0.1
0.01
0.1
1
10
100
天
100
10
% AP 补体活性

图35D

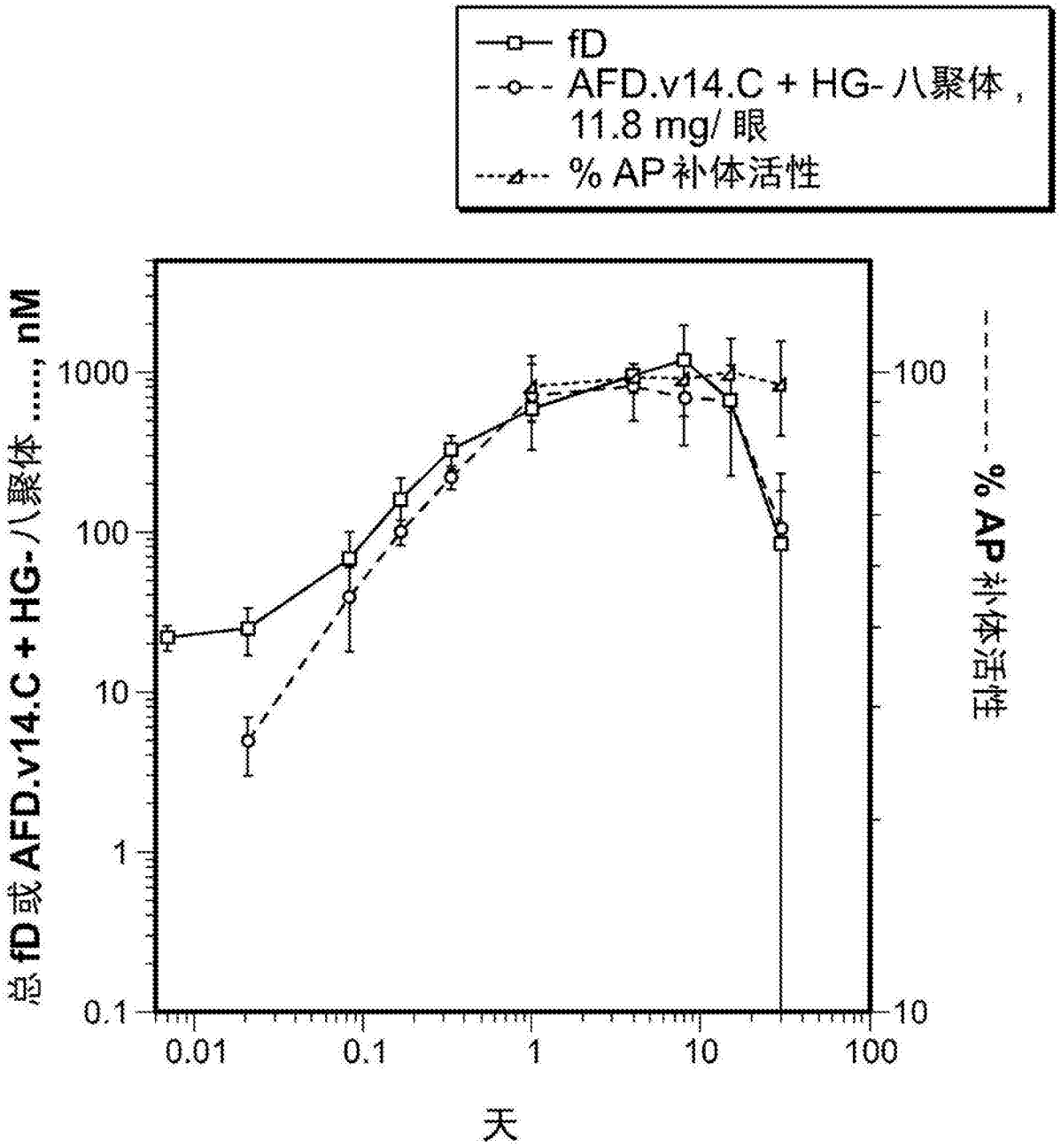

fD
AFD.v14.C + HG-八聚体，11.8 mg/ 眼
% AP 补体活性
总 fD 或 AFD.v14.C + HG-八聚体 ....., nM
1000
100
10
1
0.1
100
10
0.01
0.1
1
10
100
天
% AP 补体活性

图35E