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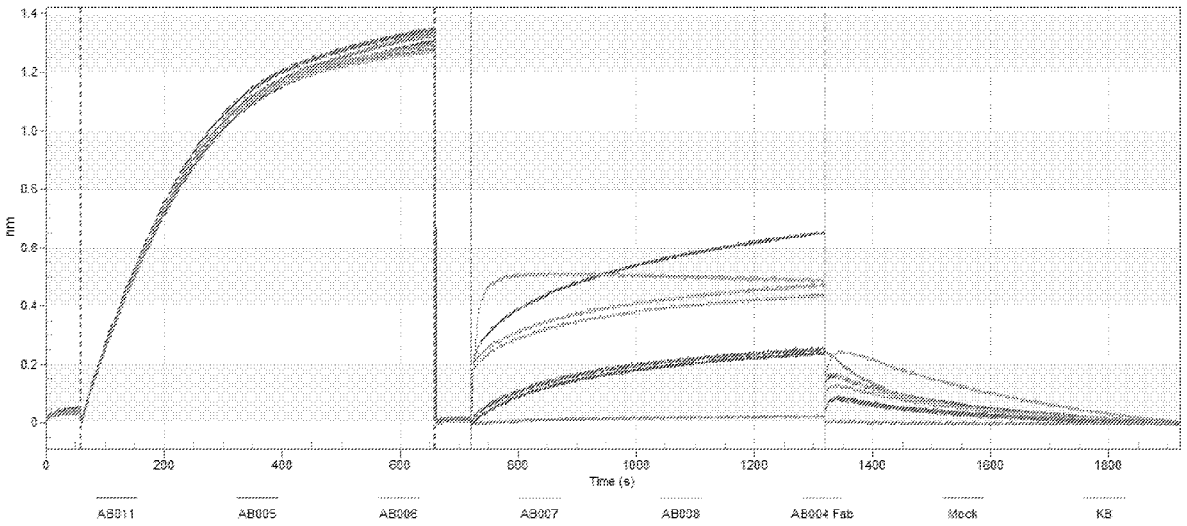

FIG. 1

(57) **Abstract:** Described herein are isolated monovalent antibodies or antibody fragments thereof that bind human properdin. Such antibodies are useful in methods of treatment for diseases mediated by alternative complement pathway dysregulation.

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MONOVALENT ANTI-PROPERDIN ANTIBODIES AND ANTIBODY FRAGMENTS

BACKGROUND

The complement system plays a central role in the clearance of immune complexes and in immune responses to infectious agents, foreign antigens, virus-infected cells and tumor cells. Complement activation occurs primarily by three pathways: the classical pathway, the lectin pathway and the alternative pathway. Uncontrolled activation or insufficient regulation of the alternative complement pathway can lead to systemic inflammation, cellular injury and tissue damage. The alternative complement pathway has been implicated in the pathogenesis of a growing number of diverse diseases. Properdin positively regulates alternative complement pathway activation by binding and stabilizing the C3 and C5 convertase complexes (C3bBb and C3bnBb). Inhibition or modulation of properdin activity is an important therapeutic strategy to mitigate symptoms and slow progression of diseases associated with alternative complement pathway dysregulation. There continues to be an unmet need for effectively regulating properdin activity.

SUMMARY

Described herein are isolated monovalent antibodies and antibody fragments thereof that specifically or substantially specifically bind properdin and selectively block alternative complement pathway activation. By inhibiting the functional activity of properdin, the monovalent antibody described herein inhibits alternative complement pathway-induced assembly of the membrane attack complex. In addition, selective binding of a single properdin molecule with a monovalent antibody can reduce undesirable immune complexes, resulting from aggregation. Thus, the selective targeting of a single properdin monomer or multimer can, in turn, improve clinical benefits for patients with disease mediated by alternative complement pathway dysregulation.

In one embodiment, the disclosure is directed to an isolated monovalent antibody or antibody fragment thereof, where the antibody or antibody fragment thereof binds human properdin. In a particular embodiment, the antibody or fragment is a camelid antibody. In a particular embodiment, the antibody or fragment is a single-domain antibody. In a particular embodiment, the antibody or fragment binds to TSR0 and/or TSR1 of human properdin. In a particular embodiment, the antibody or fragment binds an epitope within the amino acid sequence LCQPCRSPRWSLWSTWAPCSVTCSEGSQLRYRRCVGWNGQ (SEQ ID NO: 8). In a particular embodiment, the antibody or fragment binds to mouse properdin with an affinity of less than 50 nM. In a particular embodiment, the antibody or fragment comprises at least one or all three CDRs selected from: a) a CDR-H1 including the amino acid sequence GRIFEVNMMA (SEQ ID NO: 9); b) a CDR-H2 including the amino acid sequence RVGTTX$_1$YADSVKG (SEQ ID NO: 10), where X$_1$ is a polar or a nonpolar amino acid; and c) a CDR-H3 including the amino acid sequence LQYX$_2$RYGGAEY (SEQ ID NO: 11), where X$_2$ is a polar amino acid. In a particular embodiment, CDR-H2 includes the amino acid sequence RVGTTVYADSVKG (SEQ ID NO: 12). In a particular embodiment, CDR-H3 includes the amino acid sequence LQYDRYGGAEY (SEQ ID NO: 12). In a particular embodiment, CDR-H2 includes the amino acid sequence RVGTTTYADSVKG (SEQ ID NO: 15). In a particular embodiment, CDR-H3 has the amino acid sequence LQYSRYGGAEY (SEQ ID NO: 14). In a particular embodiment, CDR-H3 has the amino acid sequence LQYDRYGGAEY (SEQ ID NO: 13). In a

particular embodiment, CDR-H3 has the amino acid sequence LQYDRYGGAEY (SEQ ID NO: 13). In a particular embodiment, CDR-H3 has the amino acid sequence LQYSRYGGAEY (SEQ ID NO: 14). In a particular embodiment, the antibody or fragment includes 3 CDRs with the following sequences: a) a CDR-H1 having the amino acid sequence GRISSIIHMA (SEQ ID NO: 16); b) a CDR-H2 having the amino acid sequence RVGTTVYADSVKG (SEQ ID NO: 12); and c) a CDR-H3 having the amino acid sequence LQYEKHGGADY (SEQ ID NO: 17). In a particular embodiment, the antibody includes 6 CDRs with the following sequences: a) a CDR-H1 having the amino acid sequence GYIFTNYPIH (SEQ ID NO: 18); b) a CDR-H2 having the amino acid sequence FIDPGGGYDEPDERFRD (SEQ ID NO: 19); c) a CDR-H3 having the amino acid sequence RGGGYYLDY (SEQ ID NO: 20); d) a CDR-L1 having the amino acid sequence RASQDISFFLN (SEQ ID NO: 21); e) a CDR-L2 having the amino acid sequence YTSRYHS (SEQ ID NO: 22); and f) a CDR-L3 having the amino acid sequence QHGNTLPWT (SEQ ID NO: 23). In a particular embodiment, the antibody includes 6 CDRs with the following sequences: a) a CDR-H1 having the amino acid sequence GFSLTTYGVH (SEQ ID NO: 24); b) a CDR-H2 having the amino acid sequence VIWSGGDTDYNASFIS (SEQ ID NO: 25); c) a CDR-H3 having the amino acid sequence NKDYYTNYDFTMDY (SEQ ID NO: 26); d) a CDR-L1 having the amino acid sequence KSSQSVLYSSNQKNFLA (SEQ ID NO: 27); e) a CDR-L2 having the amino acid sequence WASTRES (SEQ ID NO: 28); and f) a CDR-L3 having the amino acid sequence HQYLSSYT (SEQ ID NO: 29). In a particular embodiment, the antibody includes 6 CDRs with the following sequences: a) a CDR-H1 having the amino acid sequence GYTFIDYWIE (SEQ ID NO: 30); b) a CDR-H2 having the amino acid sequence EIFPGSGTINHNEKFKD (SEQ ID NO: 31); c) a CDR-H3 having the amino acid sequence EGLDY (SEQ ID NO: 32); d) a CDR-L1 having the amino acid sequence SASSSVSYIY (SEQ ID NO: 33); e) a CDR-L2 having the amino acid sequence DTSTLAS (SEQ ID NO: 34); and f) a CDR-L3 having the amino acid sequence QQWSRNPFT (SEQ ID NO: 35). In a particular embodiment, the antibody includes 6 CDRs with the following sequences: a) a CDR-H1 having the amino acid sequence GFSLTSYGVH (SEQ ID NO: 36); b) a CDR-H2 having the amino acid sequence VIWSGGSTDYNAAFIS (SEQ ID NO: 37); c) a CDR-H3 having the amino acid sequence NKDFYSNYDYTMDY (SEQ ID NO: 38); d) a CDR-L1 having the amino acid sequence KSSQSVLYSSNQKNFLA (SEQ ID NO: 27); e) a CDR-L2 having the amino acid sequence WASTRES (SEQ ID NO: 28); and f) a CDR-L3 having the amino acid sequence HQYLSSYT (SEQ ID NO: 29). In a particular embodiment, the antibody includes 6 CDRs with the following sequences: a) a CDR-H1 having the amino acid sequence GYTXTAYGIN (SEQ ID NO: 39); b) a CDR-H2 having the amino acid sequence YIYIGNGYTDYNEKFKG (SEQ ID NO: 40); c) a CDR-H3 having the amino acid sequence SGWDEDYAMDF (SEQ ID NO: 41); d) a CDR-L1 having the amino acid sequence RASENIYSYLA (SEQ ID NO: 42); e) a CDR-L2 having the amino acid sequence HAKTLAE (SEQ ID NO: 43); and f) a CDR-L3 having the amino acid sequence QHHYGPPPT (SEQ ID NO: 44). In a particular embodiment, the antibody or fragment inhibits an activity of human properdin.

In one embodiment, the disclosure is directed to use of an isolated monovalent antibody or antibody fragment thereof that binds human properdin in a method of treating a disease mediated by alternative complement pathway dysregulation or in the manufacture of a medicament for treating a disease mediated by alternative complement pathway dysregulation.

In one embodiment, the disclosure is directed to a method of treating a disease mediated by alternative complement pathway dysregulation. The methods includes administering an effective amount

of the antibody of an isolated monovalent antibody or antibody fragment thereof, where the antibody or antibody fragment binds human properdin to a patient in need thereof. In a particular embodiment, the disease is autoimmune thrombotic thrombocytopenic purpura (TTP), hemolytic uremic syndrome (HUS), atypical hemolytic uremic syndrome (aHUS), paroxysmal nocturnal hemoglobinuria (PNH), IgA nephropathy (Berger's disease), asthma (e.g., severe asthma), C3 glomerulopathy (C3G), Gaucher disease, Hidradentitis suppurativa, Behcet's disease, severe burn, early sepsis, dermatomyositis, pneumococcal meningitis, Alzheimer's disease, cancer metastasis, acute respiratory distress syndrome (ARDS), acute lung injury (ACI), transfusion-related lung injury (TRALI), hemodialysis induced thrombosis, epidermolysis bullosa acquisita (EBA), uveitis, Parkinson's disease, primary biliary atresia, antineutrophil cytoplasmic antibodies (ANCA) vasculitis, retinal degeneration, broad thrombotic microangiopathy (TMA), broad TMA (APS), hematopoietic stem cell therapy (HSCT) TMA, age-related macular degeneration (AMD), pre-eclampsia, hemolysis, elevated liver enzymes, and low platelet (HELLP) syndrome, multiple sclerosis, antiphospholipid syndrome (APS), relapsing polychondritis, ischemic injury, stroke, graft versus host disease (GvHD), chronic obstructive pulmonary disease (COPD), emphysema, atherosclerosis, acute coronary syndrome, hemorrhagic shock, rheumatoid arthritis, dialysis (cardiovascular risk), cardiovascular disease, placental malaria, antiphospholipid syndrome (APS) pregnancy loss, membranoproliferative (MP) glomerulonephritis, membranous nephritism, encephalitis, brain injury, N-methyl-D-aspartate (NMDA) receptor antibody encephalitis, malaria hemolytic crisis, abdominal aortic aneurysm (AAA), or thoracoabdominal aortic aneurysm (TAA).

In one embodiment, the disclosure is directed to a method of inhibiting alternative complement pathway membrane attack complex assembly. The method includes administering an effective amount of an antibody, antibody derivative or fragment thereof to a patient in need thereof. In a particular embodiment, the method inhibits alternative complement pathway dependent hemolysis.

In some embodiments, the antibody or fragment thereof includes the sequence of:

EVQLVESGGGLVKPGGSLRLSCAASGRPVSNYAAAWFRQAPGKEREFVSAINWQKTATYADSVKGRFTI SRDNAKNSLYLQMNSLRAEDTAVYYCAAVFRVVAPKTQYDYDYWGQGTLVTVSSGGGGSGGGGSGG GGSLEVQLVESGGGLVQAGGSLRLSCAASGRISSIIHMAWYRQAPGKQRELVAEISRVGTTVYADSVKG RFTISRDDAKNTVTLQMNSLKPEDTAVYYCNALQYEKHGGADYWGQGTQVTVSS (SEQ ID NO: 45).

In some embodiments, the antibody or fragment thereof includes the sequence of:

EVQLVESGGGLVKPGGSLRLSCAASGRPVSNYAAAWFRQAPGKEREFVSAINWQKTATYADSVKGRFTI SRDNAKNSLYLQMNSLRAEDTAVYYCAAVFRVVAPKTQYDYDYWGQGTLVTVSSGGGGSGGGGSGG GGSEVQLLESGGGLVQPGGSLRLSCAASGRISSIIHMAWFRQAPGKERELVSEISRVGTTVYADSVKGRF TISRDNSKNTLYLQMNSLKPEDTAVYYCNALQYEKHGGADYWGQGTLVTVSS (SEQ ID NO: 46).

In some embodiments, the antibody or fragment thereof includes the sequence of:

EVQLVESGGGLVKPGGSLRLSCAASGRPVSNYAAAWFRQAPGKEREFVSAINWQKTATYADSVKGRFTI SRDNAKNSLYLQMNSLRAEDTAVYYCAAVFRVVAPKTQYDYDYWGQGTLVTVSSGGGGDGGGGDGG GGEVQLVESGGGLVQAGGSLRLSCAASGRISSIIHMAWYRQAPGKQRELVAEISRVGTTVYADSVKGRF TISRDDAKNTVTLQMNSLKPEDTAVYYCNALQYEKHGGADYWGQGTQVTVSS (SEQ ID NO: 47).

In some embodiments, the antibody or fragment thereof includes the sequence of:

EVQLVESGGGLVKPGGSLRLSCAASGRPVSNYAAAWFRQAPGKEREFVSAINWQKTATYADSVKGRFTI SRDNAKNSLYLQMNSLRAEDTAVYYCAAVFRVVAPKTQYDYDYWGQGTLVTVSSGGGGEGGGGEGG

GGEVQLVESGGGLVQAGGSLRLSCAASGRISSIIHMAWYRQAPGKQRELVAEISRVGTTVYADSVKGRF
TISRDDAKNTVTLQMNSLKPEDTAVYYCNALQYEKHGGADYWGQGTQVTVSS (SEQ ID NO: 48).

In some embodiments, the antibody or fragment thereof includes the sequence of:

EVQLVESGGGLVKPGGSLRLSCAASGRPVSNYAAAWFRQAPGKEREFVSAINWQKTATYADSVKGRFTI
SRDNAKNSLYLQMNSLRAEDTAVYYCAAVFRVVAPKTQYDYDYWGQGTLVTVSSGGGGSGGGGSGG
GGSEVQLVESGGGLVQPGGSLRLSCAASGRISSIIHMAWVRQAPGKQRELVSEISRVGTTVYADSVKGR
FTISRDNSKNTLYLQMNSLRAEDTAVYYCNALQYEKHGGADYWGQGTLVTVSS (SEQ ID NO: 49).

In some embodiments, the antibody or fragment thereof includes the sequence of:

EVQLVESGGGLVKPGGSLRLSCAASGRPVSNYAAAWFRQAPGKEREFVSAINWQKTATYADSVKGRFTI
SRDNAKNSLYLQMNSLRAEDTAVYYCAAVFRVVAPKTQYDYDYWGQGTLVTVSSGGGGDGGGGDGG
GGEVQLLESGGGLVQPGGSLRLSCAASGRISSIIHMAWFRQAPGKERELVSEISRVGTTVYADSVKGRFT
ISRDNSKNTLYLQMNSLKPEDTAVYYCNALQYEKHGGADYWGQGTLVTVSS (SEQ ID NO: 50).

In some embodiments, the antibody or fragment thereof includes the sequence of:

EVQLVESGGGLVKPGGSLRLSCAASGRPVSNYAAAWFRQAPGKEREFVSAINWQKTATYADSVKGRFTI
SRDNAKNSLYLQMNSLRAEDTAVYYCAAVFRVVAPKTQYDYDYWGQGTLVTVSSGGGGEGGGGEGG
GGEVQLLESGGGLVQPGGSLRLSCAASGRISSIIHMAWFRQAPGKERELVSEISRVGTTVYADSVKGRFT
ISRDNSKNTLYLQMNSLKPEDTAVYYCNALQYEKHGGADYWGQGTLVTVSS (SEQ ID NO: 51).

In some embodiments, the antibody or fragment thereof includes the sequence of:

EVQLVESGGGLVKPGGSLRLSCAASGRPVSNYAAAWFRQAPGKEREFVSAINWQKTATYADSVKGRFTI
SRDNAKNSLYLQMNSLRAEDTAVYYCAAVFRVVAPKTQYDYDYWGQGTLVTVSSGGGGDGGGGDGG
GGEVQLVESGGGLVQPGGSLRLSCAASGRISSIIHMAWVRQAPGKQRELVSEISRVGTTVYADSVKGRF
TISRDNSKNTLYLQMNSLRAEDTAVYYCNALQYEKHGGADYWGQGTLVTVSS (SEQ ID NO: 52).

In some embodiments, the antibody or fragment thereof includes the sequence of:

LEVQLVESGGGLVQAGGSLRLSCAASGRISSIIHMAWYRQAPGKQRELVAEISRVGTTVYADSVKGRFTI
SRDDAKNTVTLQMNSLKPEDTAVYYCNALQYEKHGGADYWGQGTQVTVSSRKCCVECPPCPAPPVAG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSL
SLGK (SEQ ID NO: 53).

In some embodiments, the antibody or fragment thereof is an LVP058 anti-properdin monovalent antibody VHH linked to an hG1 without a C1q binding domain and includes the sequence of:

LEVQLVESGGGLVQAGGSLRLSCAASGRISSIIHMAWYRQAPGKQRELVAEISRVGTTVYADSVKGRFTI
SRDDAKNTVTLQMNSLKPEDTAVYYCNALQYEKHGGADYWGQGTQVTVSSPKSCDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV
SVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK (SEQ ID NO: 54).

In some embodiments, the antibody or fragment thereof is an LVP058 anti-properdin monovalent antibody V_{HH} linked to an anti-albumin V_{HH} by a $(G4S)_3$ linker and includes includes the sequence of:

LEVQLVESGGGLVQAGGSLRLSCAASGRISSIIHMAWYRQAPGKQRELVAEISRVGTTVYADSVKGRFTI
SRDDAKNTVTLQMNSLKPEDTAVYYCNALQYEKHGGADYWGQGTQVTVSSGGGGSGGGGSGGGGSE

VQLVESGGGLVKPGGSLRLSCAASGRPVSNYAAAWFRQAPGKEREFVSAINWQKTATYADSVKGRFTIS RDNAKNSLYLQMNSLRAEDTAVYYCAAVFRVVAPKTQYDYDYWGQGTLVTVSS (SEQ ID NO: 55).

Definitions

As used herein, the term "monovalent antibody or antibody fragment thereof" refers to an antibody or antigen binding fragment thereof comprising a single binding domain, *e.g.*, V_H or V_{HH}, for an antigen, *e.g.*, a single properdin molecule. In one embodiment, the bound antigen molecule is part of a multimer, *e.g.*, a trimer or higher order multimer of properdin monomers. Antibodies generally, including monovalent antibodies or antibody fragments thereof, bind with a high degree of specificity to a particular antigen.

As used herein, the term "single domain antibody" defines molecules where the antigen binding site is present on, and formed by, a single immunoglobulin domain. Generally, the antigen binding site of an immunoglobulin single variable domain is formed by no more than three CDRs. The single variable domain may, for example, include a light chain variable domain sequence (a V_L sequence) or a suitable fragment thereof; or a heavy chain variable domain sequence (*e.g.*, a V_H sequence or V_{HH} sequence) or a suitable fragment thereof; as long as it is capable of forming a single antigen binding unit (*i.e.*, a functional antigen binding unit that essentially is the single variable domain, such that the single antigen binding domain does not need to interact with another variable domain to form a functional antigen binding unit).

As used herein, the term "camelid antibody" refers to an antibody derived from a camelid species, for example, in a camel, dromedary, llama, alpaca or guanaco. Camelid antibodies differ from those of most other mammals in that they lack a light chain, and thus include only heavy chains with complete and diverse antigen binding capabilities (Hamers-Casterman, C. *et al.*, *Nature*, 363:446-8, 1993).

As used herein, the term "V_{HH}" refers to a single heavy chain variable domain antibody devoid of light chains. V_{HH} chains, for example, can be of the type that can be found in *Camelidae* or cartilaginous fish that are naturally devoid of light chains or to a synthetic and non-immunized V_{HH} that can be constructed accordingly. Each heavy chain includes a variable region encoded by V-, D- and J-exons. A V_{HH} may be a natural V_{HH} antibody, *e.g.*, a camelid antibody, or a recombinant protein including a heavy chain variable domain.

As used herein, the term an "isolated antibody" refers to an antibody that is substantially free of other antibodies having different antigenic specificities (*e.g.*, an isolated antibody that binds to properdin is substantially free of contaminants, *e.g.*, antibodies that do not bind to properdin). In addition, an "isolated" antibody is one that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that could interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes.

As used herein, the term "specific binding" of an antibody or fragment thereof, polypeptide, or peptidomimetic is binding to a target molecule that is measurably different from binding to molecules that are not target molecules. As used herein, specific binding refers to a greater than 95% preference for binding a particular antigen versus background ("non-specific") binding. "Substantially specific" binding refers to a greater than about 80% preference for binding a particular antigen versus background. Binding can be measured using a variety of methods including, but not limited to, Western blot,

immunoblot, enzyme-linked immunosorbant assay ("ELISA"), radioimmunoassay ("RIA"), immunoprecipitation, surface plasmon resonance, bio-layer interferometry, chemiluminescence, fluorescent polarization, phosphorescence, immunohistochemical analysis, matrix-assisted laser desorption/ionization time-of-flight ("MALDI-TOF") mass spectrometry, microcytometry, microarray, microscopy, fluorescence activated cell sorting ("FACS") and flow cytometry.

As used herein, the term "human properdin" refers to a 469 amino acid soluble glycoprotein found in plasma that has seven thrombospondin type I repeats (TSR) with the N-terminal domain,TSR0, being a truncated domain. Human properdin, a 53 kDa protein, includes a signal peptide (amino acids 1-28), and six, non-identical TSR repeats about 60 amino acids each, as follows: amino acids 80-134 (TSR1), amino acids 139-191 (TSR2), amino acids 196-255 (TSR3), amino acids 260-313 (TSR4), amino acids 318-377 (TSR5), and amino acids 382-462 (TSR6). Properdin is formed by oligomerization of a rod-like monomer into cyclic dimers, trimers, and tetramers. The amino acid sequence of human properdin is found in the GenBank database under the following accession numbers: for human properdin, see, *e.g.*, GenBank Accession Nos. AAA36489, NP_002612, AAH15756, AAP43692, S29126 and CAA40914. Properdin is a positive regulator of the alternative complement activation cascade. Known binding ligands for properdin include C3b, C3bB and C3bBb (Blatt, A. *et al.*, *Immunol. Rev.*, 274:172-90, 2016).

As used herein, the term "mouse properdin" refers to a 457 amino acid soluble glycoprotein found in plasma that has seven TSRs with the N-terminal domain, TSR0, being truncated. Mouse properdin, a 50 kDa protein, includes a signal peptide (amino acids 1-24), and six, non-identical TSRs of about 60 amino acids each, as follows: amino acids 73-130 (TSR1), amino acids 132-187 (TSR2), amino acids 189-251 (TSR3), amino acids 253-309 (TSR4), amino acids 311-372 (TSR5), and amino acids 374-457 (TSR6). Mouse properdin is formed by oligomerization of a rod-like monomer into cyclic dimers, trimers, and tetramers. The amino acid sequence of mouse properdin is found, for example, in the GenBank database (GenBank Accession Nos. P11680 and S05478).

As used herein, the term "TSR0 domain" refers to the truncated domain of properdin that precedes the TSR1 domain of properdin. For example, the TSR0 domain of human properdin includes amino acids 28-76.

As used herein, the term "TSR1 domain" refers to the domain of properdin adjacent to the TSR0 domain of properdin. For example, the TSR0 domain of human properdin includes amino acids 77-134.

As used herein, the term "an activity of properdin" refers to the biological activity of properdin including, but not limited to, binding interactions that lead to the stability of the C3/C5 convertase. Properdin binds most avidly to C3b,Bb- the alternative pathway C3/C5 convertase, but also binds to C3b; C3b,B and C3b,Bb. One function is to stabilize the C3b,Bb complex allowing increased alternative pathway activation (Pangburn, M., *Methods Enzymol.*, 162:639-53, 1988; Nolan, K. & Reid, K., *Methods Enzymol.*, 223:35-46, 1993). Properdin enhances formation of the alternative pathway C3 convertase by increasing binding of factor B to P,C3b complexes. Thus, properdin is an accelerator (positive regulator) of complement activation. Properdin also has been implicated in initiating activation of the alternative pathway by binding to the target surface and initiating C3/C5 convertase formation (Kemper C. & Hourcade, D., *Mol. Immunol.*, 45:4048-56, 2008).

As used herein, the term "alternative complement pathway" refers to one of three pathways of complement activation (the others being the classical pathway and the lectin pathway). The alternative

complement pathway is typically activated by bacteria, parasites, viruses or fungi, although IgA Abs and certain IgL chains have also been reported to activate this pathway.

As used herein, the term "alternative complement pathway dysregulation" refers to any aberration in the ability of the alternative complement pathway to provide host defense against pathogens and clear immune complexes and damaged cells and for immunoregulation. Alternative complement pathway dysregulation can occur both in fluid phase as well as at cell surface and can lead to excessive complement activation or insufficient regulation, both causing tissue injury.

As used herein, the term "a disease mediated by alternative complement pathway dysregulation" refers to an interruption, cessation or disorder of body functions, systems or organs caused by alternative complement pathway dysregulation. Such diseases would benefit from treatment with a composition or formulation described herein. In some embodiments, the disease is caused by any aberration in the ability of the alternative complement pathway to provide host defense against pathogens and clear immune complexes and damaged cells, and for immunoregulation. Also encompassed herein are diseases, directly or indirectly, mediated by dysregulation of one or more components of the alternative complement pathway, or a product generated by the alternative complement pathway.

As used herein, the term "alternative complement pathway-dependent membrane attack complex assembly" refers to a terminal complex formed as a result of alternative complement pathway activation and includes complement components C5, C6, C7, C8 and C9. Assembly of the membrane attack complex (MAC) leads to cell lysis.

As used herein, the term "alternative complement pathway dependent hemolysis" refers to the lysis of red blood cells mediated by increased alternative complement pathway-dependent MAC assembly and/or deposition on red blood cells.

As used herein, the term "linker" refers to a linkage between two elements, e.g., protein domains. A linker can be a covalent bond or a spacer. The term "bond" refers to a chemical bond, e.g., an amide bond or a disulfide bond, or any kind of bond created from a chemical reaction, e.g., chemical conjugation. A linker may refer to a moiety (e.g., a polyethylene glycol (PEG) polymer) or an amino acid sequence (e.g., a 3-200 amino acid, 3-150 amino acid, or 3-100 amino acid sequence) occurring between two polypeptides or polypeptide domains to provide space and/or flexibility between the two polypeptides or polypeptide domains. An amino acid spacer may be part of the primary sequence of a polypeptide (e.g., joined to the spaced polypeptides or polypeptide domains via the polypeptide backbone). A linker may comprise one or more glycine and serine residues.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts bio-layer interferometry data obtained using an Octet™ biosensor with a model system in which selected anti-properdin antibodies specifically bind human properdin. The graph shows equilibrium dissociation over time.

FIG. 2 depicts bio-layer interferometry data obtained using an Octet™ biosensor with a model system in which selected anti-properdin antibodies showed weak or no binding to mouse properdin. The graph shows equilibrium dissociation over time.

FIG. 3 depicts bio-layer interferometry data obtained using an Octet™ biosensor with a model system in which selected anti-properdin antibodies showed specific, but weak binding to cynomolgus properdin. The graph shows equilibrium dissociation over time.

FIG. 4 shows that selected anti-properdin antibodies inhibit activity of human properdin in an alternative complement pathway hemolysis assay.

FIG. 5A to FIG. 5C show the characterization of selected anti-properdin antibodies by mass spectrometry.

FIG. 6A and FIG. 6B show the binding affinity of selected anti-properdin antibodies to biotinylated properdin using a properdin capture method.

FIG. 7 shows the binding affinity of selected anti-properdin bispecific antibodies to biotinylated properdin using a properdin capture method.

FIG. 8A and FIG. 8B show selected anti-properdin bispecific antibodies inhibit activity of human and cynomolgus properdin in an alternative complement pathway hemolysis assay. An anti-properdin antibody was used as the control.

FIG. 9A and FIG. 9B show selected anti-properdin bispecific antibodies inhibit activity of human and cynomolgus properdin in an alternative complement pathway hemolysis assay.

FIG. 10A and FIG. 10B show the binding affinity of selected anti-properdin bispecific antibodies to biotinylated properdin using a properdin capture method.

FIG. 11A and FIG. 11B show the binding affinity of selected anti-properdin bispecific antibodies to biotinylated properdin using a properdin capture method.

FIG. 12A and FIG. 12B show the binding affinity of selected anti-properdin bispecific antibodies to biotinylated properdin using a properdin capture method.

DETAILED DESCRIPTION

Properdin is a positive regulator of the alternative complement pathway. Described herein are novel monovalent antibodies that bind to a single properdin molecule and are useful for treating diseases mediated by dysregulation of the alternative complement pathway. Described herein is the discovery that immune complexes resulting from bivalent antibodies binding more than one properdin multimer exhibit toxicity as therapeutic agents for inhibiting aberrant activation of the alternative complement pathway. Monovalent antibodies described herein have a 1:1 binding ratio to properdin and, by design, cannot form antibody/properdin aggregates containing more than one properdin multimer, providing an advantage over bivalent and multivalent antibodies.

The sections that follow provide a description of monovalent antibodies or antibody fragments that can be administered to a patient with disease mediated by alternative complement pathway dysregulation.

Anti-properdin antibodies

Described herein are monovalent anti-properdin antibodies, antibody derivatives (*e.g.*, engineered antibodies, humaneered antibodies, chimeric antibodies, substituted antibodies, humanized antibodies *etc.*) and antibody fragments thereof that inhibit properdin, a positive regulator of the alternate pathway of complement, and subsequently destabilize the C3- and C5-convertase enzyme complexes.

The antibodies described herein can inhibit, for example, properdin binding to C3b, C3Bb, and C3bBb. Inhibition of properdin leads to reduced alternative pathway complement activation, indicating a therapeutic benefit for patients afflicted with a disease of alternative pathway dysregulation wherein the alternative pathway is hyper-activated.

Anti-properdin antibodies described herein can be produced by using full-length properdin, properdin polypeptides, and/or using antigenic properdin epitope-bearing peptides, for example, a fragment of the properdin polypeptide. Properdin peptides and polypeptides can be isolated and used to generate antibodies as natural polypeptides, recombinant or synthetic recombinant polypeptides. All antigens useful for producing anti-properdin antibodies can be used to generate monovalent antibodies. Suitable monovalent antibody formats, and methods for producing them, are known in the art (*e.g.*, WO 2007/048037 and WO 2007/059782, the entire contents of which are incorporated herein by reference).

The anti-properdin antibody may be a monoclonal antibody or derived from a monoclonal antibody. Suitable monoclonal antibodies to selected antigens may be prepared by known techniques ("Monoclonal Antibodies: A manual of techniques," Zola (CRC Press, 1988); "Monoclonal Hybridoma Antibodies: Techniques and Applications," Hurrell (CRC Press, 1982), the entire contents of which are incorporated herein by reference).

In other embodiments, the antibody may be a single-domain antibody, such as a V_{HH}. Such antibodies exist naturally in camelids and sharks (Saerens, D. *et al.*, *Curr. Opin. Pharmacol.*, 8:600-8, 2008). Camelid antibodies are described in, for example, U.S. Pat. Nos.5,759,808; 5,800,988; 5,840,526; 5,874,541; 6,005,079; and 6,015,695, the entire contents of each of which are incorporated herein by reference. The cloned and isolated V_{HH} domain is a stable polypeptide that features the full antigen-binding capacity of the original heavy-chain antibody. V_{HH} domains, with their unique structural and functional properties, combine the advantages of conventional antibodies (high target specificity, high target affinity and low inherent toxicity) with important features of small molecule drugs (the ability to inhibit enzymes and access receptor clefts). Furthermore, they are stable, have the potential to be administered by means other than injection, are easier to manufacture, and can be humanized (U.S. Pat. No. 5,840,526; U.S. Pat. No. 5,874,541; U.S. Pat. No. 6,005,079, U.S. Pat. No. 6,765,087; EP 1589107; WO 97/34103; WO 97/49805; U.S. Pat. No. 5,800,988; U.S. Pat. No. 5,874,541 and U.S. Pat. No. 6,015,695, the entire contents of each of which are incorporated herein by reference).

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVQLVESGGGLVQAGGSLRLSCAASGRTFSSYAMGWFRQAPGKEREFVAAIGWNGEGIYYAD SVKGRFTISRDNAKNTGYLQMNSLKPEDTAVYYCAADSEGVVPGFPIAYWGQGTQVTVSG (SEQ ID NO: 71)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVQLVESGGGLVQPGGSLRLSCAASGFPLNSYAIGWFRQAPGKEREGVSCISVSDDSTYYTDS VKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAVDSAPLYGDYVCKPLENEYDFWGQGTQVTVSG (SEQ ID NO: 72).

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVQLVESGGGLVQAGGSLXLSCAASGSDRRINGMGWYRHPPGKQRELVAAITSGGSTNYADS VKGRFTISTNNANNMMYLQMNSLKPEDTAVYYCAIDEFGTGWLDYCGQGTQVTVSG (SEQ ID NO: 73).

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVQLVESGGGLVQPGGSLRLSCAASGRPFSSYAMGWFRQAPGKEREIVAGLSWSGGNVYYAD SVKGRFTISRDNAKNTGDLQMNSLKPEDTAVYYCAIGPKLTTGPTAYRYWGQGTQVTVSS (SEQ ID NO: 74)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVQLVESGGGLVQPGGSLRLSCATSGGTFSSYAMGWFRQAPGKEREFVAAITWNGSNRYYAD SVKGRFTISRDNAKSTVYLQMNSLKPEDTAVYYCAAEHSTRYSGFYYYTRGETYHYWGQGTQVTVSG (SEQ ID NO: 75)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVQLVESGGGLVQAGGSLRLSCAASGRTFSTLGMGWFRQAPGKERQFVAAINWSGSSTYYAN SVKGRFTISRDNAQSTMYLQMNSLKPEDTAVYYCAADLDSRYSAYYYYSDESQYDYWGQGTLVTVSG (SEQ ID NO: 76)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVQVVESGGGLVQPGGSLSLSCAASGRTFSSYAMGWFRQAPGKEREFVAAITWDGANIYYAD SVKGRFTLSRDNAENTVWLQLNSLKPEDTAVYYCAAAESGRYSGRDYYSAPGVYLYWGQGTLVTVSG (SEQ ID NO: 77)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVQLVESGGGLVQAGGSLRLSCAASGSIFDINAMGWYRQAPGKQRELVADITSSGSTNYADSV KGRFTISRDNAKNTVYLQMNSLKPEDTAVYTCAAESIRESQNRHQLGYMGPLYDYWGQGTQVTVSG (SEQ ID NO: 78)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVQLIESGGGLVQAGDSLRLSCAASEGTFSRFAMGWFRQAPGKEREFVAAINWSGGITYYADS VKGRFTISRDNAKNTVYLQMNSLKPEDTADYYCAAETTTRYSGYYYYEDNKSYDYWGQGTLVTVSG (SEQ ID NO: 79)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVQVVESGGGLRQTGGSLRLSCTASGRIFEVNMMAWYRQAPGKQRELVAEISRVGTTVYADSV KGRFTISRDSAKNTVTLQMNSLKSEDTAVYYCNALQYDRYGGAEYWGQGTQVTVSS (SEQ ID NO: 58)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVLLEESGGGLERTGGSLRLSCAASGSIFSVNSMTWYRQAPGKRREFLGTITEEGRTNYADSVK GRFTISRDNAKNTMYLQMNSLKPEDTAVYYCYANLISSEDRTFGVWGQGTQVTVSS (SEQ ID NO: 80)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVHLVESGGGLVQAGGSLRLSCTASGGTVGDYAVGWFRQAPGKERELIGVVSRLGARTGYAD SVLGRFTISRDDVKNTVFLQMDSVKPEDTAVYYCAARRDYSFEVVPYDYWGQGTQVTVSG (SEQ ID NO: 81)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVQMVESGGGLVQAGGSLRLSCAASGLTNRIRIMGWYRQAPGKLRELVATITNDGSTHYADSV KGRFTISTDNAKNTVFLQMNSLKPEDTAVYICNVGENWGPAYWGQGTQVTVSG (SEQ ID NO: 82)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVQLVESGGGLVQPGGSLRLSCAASGFPLNSYAIGWFRQAPGKEREGVSCISVSDDSTYYTDS VKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAVDSAPLYGDYVCKPLENEYDFWGQGTQVTVSG (SEQ ID NO: 72)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVRLTESGGGLVQYGTNLTLTCVASGLISTRNKMGWFRRRSGGQREFVASSTVLSDDVIQDDIA ETVKGRFAVARNDYKNILYLQMTAVKPEDTGFYWCASGTSLFGASRREDDFNAWGVGTQVTVSA (SEQ ID NO: 83)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVQLAESGGGLVQAGDSLKLSCTASGRIFEVNMMAWYRQAPGKDRELVAEISRVGTTTYADSV KGRFTISRDSAKNTVTLQMNSLKSEDTAVYYCNALQYSRYGGAEYWGQGTQVTVSG (SEQ ID NO: 59)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

EVQLVESGGGLVQPGGSLRLSCAASGFTFGSADMSWVRQAPGKGPEWVSAINSNGGSTYYAA SVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCAQGNWYTEEYHYWGQGTLVTVSG (SEQ ID NO: 84)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVRLVESGGGLVQAGDSLRLSCAASGRTLSSYAMGWFRQAPGKEREFVAATTWRDTSTYYAD SVKGRFTISRDNAKNTVYLQMNSLKPEDTAAYYCAAEEPSKYSGRDYYMMGDSYDYWGQGTQVTVSS (SEQ ID NO: 85)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

EVQLVESGGGLVQPGGSLRLSCAASGFTFGSADMSWVRQAPGKGPEWVSAINSNGGSTYYAA SVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCAQGNWYTEEYHYWGQGTQVTVSG (SEQ ID NO: 86)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVQLVESGGGLVQAGGSLRLSCAASGRTFSNYAMAWFRQAPGKEREFVASISGSGDSRYYAD SVKGRFTISRDNAKNTVYLQTNSPKPEDTAVYYCAAVLPTRYSGFYYYSDGTQYHYWGQGTQVTVSS (SEQ ID NO: 87)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVNLVESGGGSVQAGGSLRLSCAASENINVINDMGWYRQAPGKQRELVAVITGHDNINYADSAT GRFTISTYTWNTENLQMNMLKPEDTAVYYCNADITYANGRFNDWGQGTQVTVSS (SEQ ID NO: 88)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVHLVESGGGLVQAGGSLRLSCAASGRTFSSYAMGWFRQPPGKEREFVAAITWSGSSIYYADS VKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAAEETSKYSGSYYYMMGDSYDYWGQGTQVTVSG (SEQ ID NO: 89)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVQLVESGGGLVQAGGSLRLSCAASGRTFSSYAMGWFRQAPGKEREFVAAVPWTYGSKYYAD SVKGRFTISRDDAKNTVYLQMNNLKPEDTAVYYCAADSSAGYYSGFDYYSAATPYDLWGQGTQVTVSG (SEQ ID NO: 90)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVQLVESGGGLVQPGGSLRLSCAASGSDYYAIGWFRQAPGKEREGVSCMSRTDGSTYYADSV KDRFTISRDYAKNTVYLQMNSLKPEDTAVYYCGLDRSYPTGGISCLFGDFGSWGQGTQVTVSG (SEQ ID NO: 91)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVQLVESGGGLVQAGGSLRLSCAASGRTFSSYNMGWFRQRHGNEREFVATISWSGRSTYYAD SVKGRFAISRDNANTTVYLQMNSLKPEDSAVYYCAASTRGWYGTQEDDYNFWGQGTQVTVSG (SEQ ID NO: 92)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVQLVESGGGLVQAGGSLRLSCAASGRISSIIHMAWYRQAPGKQRELVAEISRVGTTVYADSVK GRFTISRDDAKNTVTLQMNSLKPEDTAVYYCNALQYEKHGGADYWGQGTQVTVSG (SEQ ID NO: 60)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVQLVESGGGLVQAGGSLRLSCAASGGTFSSYSMGWFRQAPGKEREFVAAITWNGVSTYYAD SVKGRFTISRDNAKNTVYLQMNSLKPTDTAVYYCAAEITTRYSGFYYYEDNKSYDYWGQGTQVTVSS (SEQ ID NO: 93)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVQLVESGGGLRQTGESLRLSCTASGRIFEVNMMAWYRQAPGKQRELVAEISRVGTTTYADSV KGRFTISRDSAKNTVTLQMNSLKSEDTAVYYCNALQYDRYGGAEYWGQGTQVTVSG (SEQ ID NO: 61)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

EVQLVESGGGLVQPGGSLRLSCAASGFTLDYYAIGWFRQAPGKEREGVSCISRTDGSTYYADS VKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAVDDSYPTGGISCLFGHFGSWGQGTQVTVSS (SEQ ID NO: 94)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVQLVESGGGLVQAGDSLRLSCAASGFTFSSYAMGWFRQAPGKEREFVAAITWSGVSTYYAD SVKGRFTISRDNAKNRVYLQMNSLKPEDTAVYSCAADGSGRYSGMEYYNRDWVYDYWGQGTQVTVSS (SEQ ID NO: 95)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVHMVESGGGLVQAGGSLRFSCAASGNIFTISTLDWYRQAPGEQRELVATLTPDGITDYAGSVK GRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNAWRYSDDYRGRVDYWGQGTQVTVSG (SEQ ID NO: 96)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVQLIESGGGLVQEGASLRLSCAGSGPMFSRLAVGWFRQAPGKEREFVAVINWSGSADFYTNS VKGRFTISRDNAKNTVYLEMNTLKPEDSAVYYCAADQNPLTLRTGVRDVGRQWGQGTEVTVSS (SEQ ID NO: 97)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVQLVESGGGLVQAGGSLRLSCAASGRTFSSYAMGWFRQAPGKEREFVAAITWRGASTYYAD PVKGRFTISRDNAKNTVYLQMSSLKPEDTAVYYCAAEEPSYYSGSYYYMMGDSYNYWGQGTQVTVSG (SEQ ID NO: 98)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence as its heavy chain variable domain:

QVQLVESGGGLVQAGGSLRLSCTASGRTFSNYAMGWFRQAPGKEREFLAAISRSGESTNYATF VKGRFTIARDNAKNTVSLQMNSLKPEDTAVYFCAAKVAVLVSTTYNSQYDYWGQGTQVTVSS (SEQ ID NO: 99).

Anti-properdin antibodies, antibody derivatives and fragments thereof disclosed herein include those that have one or more, or all, of the following CDRs:

a. a CDR-H1 having the amino acid sequence GRIFEVNMMA (SEQ ID NO: 9);

b. a CDR-H2 having the amino acid sequence RVGTTVYADSVKG (SEQ ID NO: 12);

c. a CDR-H3 having the amino acid sequence LQYDRYGGAEY (SEQ ID NO: 13).

Additional anti-properdin antibodies, antibody derivatives and fragments thereof disclosed herein include those that have one or more, or all, of the following CDRs:

a. a CDR-H1 having the amino acid sequence GRIFEVNMMA (SEQ ID NO: 9);

b. a CDR-H2 having the amino acid sequence RVGTTTYADSVKG (SEQ ID NO: 15); and

c. a CDR-H3 having the amino acid sequence LQYSRYGGAEY (SEQ ID NO: 14).

Additional anti-properdin antibodies, antibody derivatives and fragments thereof disclosed herein include those that have one or more, or all, of the following CDRs:

a. a CDR-H1 having the amino acid sequence GRIFEVNMMA (SEQ ID NO: 9);

b. a CDR-H2 having the amino acid sequence RVGTTTYADSVKG (SEQ ID NO: 15); and

c. a CDR-H3 having the amino acid sequence LQYDRYGGAEY (SEQ ID NO: 13).

Additional anti-properdin antibodies, antibody derivatives and fragments thereof disclosed herein include those that have one or more, or all, of the following CDRs:

a. a CDR-H1 having the amino acid sequence GRISSIIHMA (SEQ ID NO: 16);

b. a CDR-H2 having the amino acid sequence RVGTTVYADSVKG (SEQ ID NO: 12); and

c. a CDR-H3 having the amino acid sequence LQYEKHGGADY (SEQ ID NO: 17).

Humanized camelid V_{HH} polypeptides are taught, for example in W004/041862, the teachings of which are incorporated herein in their entirety. It will be understood by one of skill in the art that naturally occurring camelid antibody single variable domain polypeptides can be modified (*e.g.*, amino acid. substitutions at positions 45 and 103 (W004/041862)) to generate humanized camelid V_{HH} polypeptides. Also included herein are antibody single variable domain polypeptides that are nurse shark V_{HH} (Greenberg, A. et al., Nature, 374:168-73, 1995; U.S. Patent Publication No. 20050043519).

Additional anti-properdin antibodies, antibody derivatives and fragments thereof disclosed herein include those that have one or more, or all (*e.g.*, to create a scFv or dAb), of the following CDRs:

a) a CDR-H1 having the amino acid sequence GYIFTNYPIH (SEQ ID NO: 18);

b) a CDR-H2 having the amino acid sequence FIDPGGGYDEPDERFRD (SEQ ID NO: 19);

c) a CDR-H3 having the amino acid sequence RGGGYYLDY (SEQ ID NO: 20);

d) a CDR-L1 having the amino acid sequence RASQDISFFLN (SEQ ID NO: 21);

e) a CDR-L2 having the amino acid sequence YTSRYHS (SEQ ID NO: 22); and

f) a CDR-L3 having the amino acid sequence QHGNTLPWT (SEQ ID NO: 23).

Additional anti-properdin antibodies, antibody derivatives and fragments thereof disclosed herein include those that have one or more, or all (*e.g.*, to create a scFv), of the following CDRs:

a) a CDR-H1 having the amino acid sequence GFSLTTYGVH (SEQ ID NO: 24);

b) a CDR-H2 having the amino acid sequence VIWSGGDTDYNASFIS (SEQ ID NO: 25);

c) a CDR-H3 having the amino acid sequence NKDYYTNYDFTMDY (SEQ ID NO: 26);

d) a CDR-L1 having the amino acid sequence KSSQSVLYSSNQKNFLA (SEQ ID NO: 27);

e) a CDR-L2 having the amino acid sequence WASTRES (SEQ ID NO: 28); and

f) a CDR-L3 having the amino acid sequence HQYLSSYT (SEQ ID NO: 29).

Additional anti-properdin antibodies, antibody derivatives and fragments thereof disclosed herein include those that have one or more, or all (*e.g.*, to create a scFv), of the following CDRs:

a) a CDR-H1 having the amino acid sequence GYTFIDYWIE (SEQ ID NO: 30);

b) a CDR-H2 having the amino acid sequence EIFPGSGTINHNEKFKD (SEQ ID NO: 31);

c) a CDR-H3 having the amino acid sequence EGLDY (SEQ ID NO: 32);

d) a CDR-L1 having the amino acid sequence SASSSVSYIY (SEQ ID NO: 33);

e) a CDR-L2 having the amino acid sequence DTSTLAS (SEQ ID NO: 34); and

f) a CDR-L3 having the amino acid sequence QQWSRNPFT (SEQ ID NO: 35).

Additional anti-properdin antibodies, antibody derivatives and fragments thereof disclosed herein include those that have one or more, or all (*e.g.*, to create a scFv), of the following CDRs:

a) a CDR-H1 having the amino acid sequence GFSLTSYGVH (SEQ ID NO: 36);

b) a CDR-H2 having the amino acid sequence VIWSGGSTDYNAAFIS (SEQ ID NO: 37);

c) a CDR-H3 having the amino acid sequence NKDFYSNYDYTMDY (SEQ ID NO: 38);

d) a CDR-L1 having the amino acid sequence KSSQSVLYSSNQKNFLA (SEQ ID NO: 27);

e) a CDR-L2 having the amino acid sequence WASTRES (SEQ ID NO: 28); and

f) a CDR-L3 having the amino acid sequence HQYLSSYT (SEQ ID NO: 29).

Additional anti-properdin antibodies, antibody derivatives and fragments thereof disclosed herein include those that have one or more, or all (*e.g.*, to create a scFv), of the following CDRs:

a) a CDR-H1 having the amino acid sequence GYTXTAYGIN (SEQ ID NO: 39);

b) a CDR-H2 having the amino acid sequence YIYIGNGYTDYNEKFKG (SEQ ID NO: 40);

c) a CDR-H3 having the amino acid sequence SGWDEDYAMDF (SEQ ID NO: 41);

d) a CDR-L1 having the amino acid sequence RASENIYSYLA (SEQ ID NO: 42);

e) a CDR-L2 having the amino acid sequence HAKTLAE (SEQ ID NO: 43); and

f) a CDR-L3 having the amino acid sequence QHHYGPPPT (SEQ ID NO: 44).

In some embodiments, the antibody or antibody fragment thereof includes the following sequence:

LEVQLVESGGGLVQAGGSLRLSCAASGRISSIIHMAWYRQAPGKQRELVAEISRVGTTVYADSV KGRFTISRDDAKNTVTLQMNSLKPEDTAVYYCNALQYEKHGGADYWGQGTQVTVSSRKCCVECPPCPA PPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQK SLSLSLGK (SEQ ID NO: 53)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence:

LEVQLVESGGGLVQAGGSLRLSCAASGRISSIIHMAWYRQAPGKQRELVAEISRVGTTVYADSV KGRFTISRDDAKNTVTLQMNSLKPEDTAVYYCNALQYEKHGGADYWGQGTQVTVSSPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK (SEQ ID NO: 54)

In some embodiments, the antibody or antibody fragment thereof includes the following light chain and heavy chain sequences:

DIQMTQSPSSLSASVGDRVTITCRASQDISFFLNWYQQKPGKAPKLLIYYTSRYHSGVPSRFSGS GSGTDFTLTISSLQPEDFATYYCQHGNTLPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSP VTKSFNRGEC (SEQ ID NO: 56); and

QVQLVQSGAEVKKPGASVKVSCKASGYIFTNYPIHWVRQAPGQGLEWMGFIDPGGGYDEPDE RFRDRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARRGGGYYLDYWGQGTLVTVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI

CNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 57)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence:

LEVQLVESGGGLVQAGGSLRLSCAASGRISSIIHMAWYRQAPGKQRELVAEISRVGTTVYADSV
KGRFTISRDDAKNTVTLQMNSLKPEDTAVYYCNALQYEKHGGADYWGQGTQVTVSSGGGGSGGGGSG
GGGSEVQLVESGGGLVKPGGSLRLSCAASGRPVSNYAAAWFRQAPGKEREFVSAINWQKTATYADSVK
GRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAAVFRVVAPKTQYDYDYWGQGTLVTVSS (SEQ ID NO: 55)

In some embodiments, the antibody or antibody fragment thereof includes the following sequence:

EVQLVESGGGLVKPGGSLRLSCAASGRPVSNYAAAWFRQAPGKEREFVSAINWQKTATYADSV
KGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAAVFRVVAPKTQYDYDYWGQGTLVTVSSGGGGSGGG
GSGGGGSLEVQLVESGGGLVQAGGSLRLSCAASGRISSIIHMAWYRQAPGKQRELVAEISRVGTTVYAD
SVKGRFTISRDDAKNTVTLQMNSLKPEDTAVYYCNALQYEKHGGADYWGQGTQVTVSS (SEQ ID NO: 45)

Anti-properdin antibody fragments and derivatives

Some naturally occurring antibodies include two antigen binding domains and are therefore divalent. A number of smaller antigen binding fragments of naturally occurring antibodies have been identified following protease digestion. These include, for example, the "Fab fragment" (V_L-C_L-C_H1-V_H), "Fab′ fragment" (a Fab with the heavy chain hinge region), and "F(ab′)$_2$ fragment" (a dimer of Fab′ fragments joined by the heavy chain hinge region). Recombinant methods have been used to generate such fragments and to generate even smaller antibody fragments, *e.g.*, those referred to as "single chain Fv" (variable fragment) or "scFv," consisting of V_L and V_H joined by a synthetic peptide linker (V_L-linker-V_H). Fab fragments, Fab′ fragments and scFv fragments are monovalent for antigen binding, as they each include only one antigen binding domain including one V_H/V_L dimer. Even smaller monovalent antibody fragments are the dAbs, which include only a single immunoglobulin variable domain, *e.g.*, V_H or V_L, that alone specifically binds antigen, *i.e.*, without the need for a complementary V_L or V_H domain, respectively. A dAb binds antigen independently of other V domains; however, a dAb can be present in a homo- or hetero-multimer with other V_H or V_L domains where the other domains are not required for antigen binding by the dAb, *i.e.*, where the dAb binds antigen independently of the additional V_H or V_L domains.

Linkers

In the present invention, a linker is used to describe a linkage or connection between polypeptides or protein domains and/or associated non-protein moieties. In some embodiments, a linker is a linkage or connection between at least two polypeptide constructs, e.g., such that the two polypeptide constructs are joined to each other in tandem series (e.g., a monovalent antibody linked to a second

polypeptide or monovalent antibody). A linker can attach the N-terminus or C-terminus of one antibody construct to the N-terminus or C-terminus of a second polypeptide construct.

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

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

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

In the present invention, a linker between two peptide constructs can be an amino acid linker including from 1-200 (e.g., 1-4, 1-10, 1-20, 1-30, 1-40, 2-10, 2-12, 2-16, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200) amino acids. Suitable peptide linkers are known in the art, and include, for example, peptide linkers containing flexible amino acid residues such as glycine and serine. In certain embodiments, a linker can contain single motifs or multiple different or repeating motifs, of GS, GGS, GGGGS (SEQ ID NO: 1), GGSG (SEQ ID NO: 2), or SGGG (SEQ ID NO: 3). Exemplary motifs have the sequence of $(G4S)_n$, $(G4D)_n$, $(G4E)_n$, $(G4A)_n$ where n = 1, 2, 3, 4, 5, or more, and combinations thereof. Other linkers include the sequences GGGGD (SEQ ID NO: 63), GGGGE (SEQ ID NO: 64), and GGGGA (SEQ ID NO: 100). Linkers can be designed by combining these various motifs. Such linkers include GGGGSGGGGSGGGGS (SEQ ID NO: 4), GGGGDGGGGDGGGG (SEQ ID NO: 5), GGGGEGGGGEGGGG (SEQ ID NO: 6), and GGGGAGGGGAGGGGS (SEQ ID NO: 101).

Bispecific Constructs

The invention also features bispecific constructs where two antigen binding polypeptides are linked (e.g., by a linker such as the linker of any one of SEQ ID NOs: 1-6, 63-64, and 100-101). Such bispecific constructs may include an anti-properdin binding polypeptide (e.g., a monovalent antibody) connected by a linker to a second polypeptide (e.g., a second monovalent antibody). The second polypeptide can enhance *in vivo* stability of the bispecific construct. In some embodiments, the second polypeptide is an albumin binding molecule, an albumin binding peptide, or an anti-albumin antibody (e.g.,

a monovalent antibody), or a modified form thereof. Albumin binding peptides are known in the art and are described, for example, in WO 2007/106120 (see Tables 1 to 9) and Dennis et al., 2002, J Biol. Chem. 277: 35035-35043, the disclosures of which are hereby incorporated by reference.

In some embodiments, the second polypeptide is a Fc domain that enhances *in vivo* stability of the construct.

Exemplary bispecific constructs are shown below in Example 5.

In some embodiments, a monovalent anti-properdin antibody is linked to a monovalent anti-albumin antibody. The monovalent anti-properdin antibody may be linked by its N-terminus or C-terminus to the N-terminus or C-terminus of the monovalent anti-albumin antibody.

The monovalent anti-properdin antibody may be linked by its N-terminus or C-terminus to the N-terminus or C-terminus of the monovalent anti-albumin antibody with a linker with the amino acid sequence of any one of SEQ ID NOs: 1-6, 63-64, and 100-101.

In some embodiments, a monovalent anti-properdin antibody including the amino acid sequence of SEQ ID NO: 58 is linked to a monovalent anti-albumin antibody. The monovalent anti-properdin antibody including the sequence of SEQ ID NO: 58 may be linked by its N-terminus or C-terminus to the N-terminus or C-terminus of the monovalent anti-albumin antibody with a linker including the amino acid sequence of any one of SEQ ID NOs: 1-6, 63-64, and 100-101.

In some embodiments, a monovalent anti-properdin antibody including the amino acid sequence of SEQ ID NO: 59 is linked to a monovalent anti-albumin antibody. The monovalent anti-properdin antibody including the sequence of SEQ ID NO: 59 may be linked by its N-terminus or C-terminus to the N-terminus or C-terminus of the monovalent anti-albumin antibody with a linker including the amino acid sequence of any one of SEQ ID NOs: 1-6, 63-64, and 100-101.

In some embodiments, a monovalent anti-properdin antibody including the amino acid sequence of SEQ ID NO: 60 is linked to a monovalent anti-albumin antibody. The monovalent anti-properdin antibody including the sequence of SEQ ID NO: 60 may be linked by its N-terminus or C-terminus to the N-terminus or C-terminus of the monovalent anti-albumin antibody with a linker including the amino acid sequence of any one of SEQ ID NOs: 1-6, 63-64, and 100-101.

In some embodiments, a monovalent anti-properdin antibody including the amino acid sequence of SEQ ID NO: 61 is linked to a monovalent anti-albumin antibody. The monovalent anti-properdin antibody including the sequence of SEQ ID NO: 61 may be linked by its N-terminus or C-terminus to the N-terminus or C-terminus of the monovalent anti-albumin antibody with a linker including the amino acid sequence of any one of SEQ ID NOs: 1-6, 63-64, and 100-101.

In some embodiments, a monovalent anti-properdin antibody including the amino acid sequence of SEQ ID NO: 60 is linked at its N-terminus to a monovalent anti-albumin antibody with a linker including the sequence of SEQ ID NO: 4.

In some embodiments, a monovalent anti-properdin antibody including the amino acid sequence of SEQ ID NO: 60 is linked at its N-terminus to a monovalent anti-albumin antibody with a linker including the sequence of SEQ ID NO: 5.

In some embodiments, a monovalent anti-properdin antibody including the amino acid sequence of SEQ ID NO: 60 is linked at its N-terminus to a monovalent anti-albumin antibody with a linker including the sequence of SEQ ID NO: 6.

In some embodiments, the bispecific construct includes the amino acid sequence of any one of SEQ ID NOs: 45-55, and 62.

In some embodiments, a monovalent anti-properdin antibody is linked at its N-terminus to a monovalent anti-albumin antibody with a linker including the sequence of SEQ ID NO: 1.

In some embodiments, a monovalent anti-properdin antibody is linked at its N-terminus to a monovalent anti-albumin antibody with a linker including the sequence of SEQ ID NO: 2.

In some embodiments, a monovalent anti-properdin antibody is linked at its N-terminus to a monovalent anti-albumin antibody with a linker including the sequence of SEQ ID NO: 3.

In some embodiments, a monovalent anti-properdin antibody is linked at its N-terminus to a monovalent anti-albumin antibody with a linker including the sequence of SEQ ID NO: 4.

In some embodiments, a monovalent anti-properdin antibody is linked at its N-terminus to a monovalent anti-albumin antibody with a linker including the sequence of SEQ ID NO: 5.

In some embodiments, a monovalent anti-properdin antibody is linked at its N-terminus to a monovalent anti-albumin antibody with a linker including the sequence of SEQ ID NO: 6.

In some embodiments, a monovalent anti-properdin antibody is linked at its N-terminus to a monovalent anti-albumin antibody with a linker including the sequence of SEQ ID NO: 63.

In some embodiments, a monovalent anti-properdin antibody is linked at its N-terminus to a monovalent anti-albumin antibody with a linker including the sequence of SEQ ID NO: 64.

In some embodiments, a monovalent anti-properdin antibody is linked at its C-terminus to a monovalent anti-albumin antibody with a linker including the sequence of SEQ ID NO: 1.

In some embodiments, a monovalent anti-properdin antibody is linked at its C-terminus to a monovalent anti-albumin antibody with a linker including the sequence of SEQ ID NO: 2.

In some embodiments, a monovalent anti-properdin antibody is linked at its C-terminus to a monovalent anti-albumin antibody with a linker including the sequence of SEQ ID NO: 3.

In some embodiments, a monovalent anti-properdin antibody is linked at its C-terminus to a monovalent anti-albumin antibody with a linker including the sequence of SEQ ID NO: 4.

In some embodiments, a monovalent anti-properdin antibody is linked at its C-terminus to a monovalent anti-albumin antibody with a linker including the sequence of SEQ ID NO: 5.

In some embodiments, a monovalent anti-properdin antibody is linked at its C-terminus to a monovalent anti-albumin antibody with a linker including the sequence of SEQ ID NO: 6.

In some embodiments, a monovalent anti-properdin antibody is linked at its C-terminus to a monovalent anti-albumin antibody with a linker including the sequence of SEQ ID NO: 63.

In some embodiments, a monovalent anti-properdin antibody is linked at its C-terminus to a monovalent anti-albumin antibody with a linker including the sequence of SEQ ID NO: 64.

Generation of single domain antibodies

In one embodiment, compositions and methods use a single domain antibody that is a heavy chain variable domain (V_H, *e.g.*, V_{HH}) or a light chain domain (V_L). Thus, one means of generating monovalent single domain antibodies specific for properdin is to amplify and express the V_H and V_L regions of the heavy chain and light chain gene sequences isolated, for example, from a hybridoma (*e.g.*, a mouse hybridoma) that expresses anti-properdin monoclonal antibody. The boundaries of V_H and V_L

domains are set out, for example, by Kabat *et al.* (Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, MD, 1991). The information regarding the boundaries of the V_H and V_L domains of heavy and light chain genes is used to design PCR primers that amplify the V domain from a heavy or light chain coding sequence encoding an antibody known to bind properdin. The amplified V domains are inserted into a suitable expression vector, *e.g.*, pHEN-1 (Hoogenboom, H. *et al.*, *Nucleic Acids Res.*, 19:4133-7, 1991) and expressed, for example, as a fusion of the V_H and V_L in a scFv or other suitable monovalent format. The resulting polypeptide can then be screened for high affinity monovalent binding to properdin. Screening for binding can be performed by methods known in the art. Single domain antibodies can be generated using methods known in the art (WO2005118642; Ward, E. *et al.*, *Nature*, 341:544-6, 1989; Holt, L. *et al.*, *Trends Biotechnol.*, 21:484-90, 2003). Each light chain domain may be either of the kappa or lambda subgroup. Methods for isolating V_H and V_L domains have been described in the art (EP0368684).

In one embodiment, the single domain antibody is obtained from a human, humanized rodent, camelid or shark. Any such single domain antibody can be optionally humanized. Humanization of camelid single domain antibodies requires the introduction and mutagenesis of a limited number of amino acids in a single polypeptide chain. This is in contrast to humanization of scFv, Fab, (Fab')2 and IgG, which requires the introduction of amino acid changes in two chains, the light and the heavy chain and the preservation of the assembly of both chains. In some embodiments, the single domain antibody includes V_{HH} domains. In some embodiments, the V_{HH} domains correspond to the V_{HH} domains of naturally occurring heavy chain antibodies directed against properdin. Such V_{HH} sequences can be generated, for example, by suitably immunizing a species of camelid with properdin (*i.e.*, so as to raise an immune response and/or heavy chain antibodies directed against properdin), by obtaining a suitable biological sample from said camelid (such as a blood sample, serum sample or sample of B-cells), and by generating V_{HH} sequences directed against properdin, starting from said sample, using any suitable technique known in the art (*e.g.*, the gene encoding the single domain antibody may be cloned by single cell PCR, or the B-cell(s) encoding the single domain antibody may be immortalized by EBV transformation, or by fusion to an immortal cell line).

Alternatively, such naturally occurring V_{HH} domains against properdin, can be obtained from naive libraries of camelid V_{HH} sequences, for example by screening such a library using properdin, or at least one part, fragment, antigenic determinant or epitope thereof using one or more screening techniques known in the art (WO 99/37681, WO 01/90190, WO 03/025020 and WO 03/035694). Alternatively, improved synthetic or semi-synthetic libraries derived from naive V_{HH} libraries may be used, such as V_{HH} libraries obtained from naive V_{HH} libraries by techniques such as random mutagenesis and/or CDR shuffling (WO 00/43507). In a certain embodiment, a V_{HH} library is constructed and expressed on phages after infection with helper phages. After several rounds of bio-panning, single domain antibodies against human properdin can be isolated and efficiently expressed.

A library of fusion proteins including V_{HH} or V_{HH} fragments can be displayed on a phage, phagemid, ribosome or suitable microorganism (such as yeast), to facilitate screening. Suitable methods, techniques and host organisms for displaying and screening (a set, collection or library of) fusion proteins including V_{HH} or V_{HH} fragments are known in the art (WO 03/054016; Hoogenboom, H., *Nat. Biotechnol.*, 23:1105-16, 2005).

In an additional embodiment, the method for generating fusion proteins including V_{HH} or V_{HH} fragment sequences includes at least the steps of: a) providing a collection or sample of cells derived from a species of camelid that express immunoglobulin sequences; b) screening the collection or sample of cells for (i) cells that express an immunoglobulin sequence that can bind to and/or have affinity for properdin; and (ii) cells that express heavy chain antibodies, in which substeps (i) and (ii) can be performed essentially as a single screening step or in any suitable order as two separate screening steps, to provide at least one cell that expresses a heavy chain antibody that can bind to and/or has affinity for properdin; and c) either (i) isolating from the cell the V_{HH} sequence present in the heavy chain antibody; or (ii) isolating from the cell a nucleic acid sequence that encodes the V_{HH} sequence present in the heavy chain antibody, followed by expressing the V_{HH} domain.

The method for generating an amino acid sequence directed against properdin can include at least the steps of: a) providing a set, collection or library of nucleic acid sequences encoding heavy chain antibodies or V_{HH} sequences ; b) screening the set, collection or library of nucleic acid sequences for nucleic acid sequences that encode a heavy chain antibody or a fusion protein including the V_{HH} sequence that can bind to and/or has affinity for properdin; and c) isolating the nucleic acid sequence, followed by expressing the V_{HH} sequence present in the heavy chain antibody or by expressing the fusion protein including the V_{HH} sequence, respectively.

Other suitable methods and techniques for obtaining the single domain antibodies and/or nucleic acids encoding the same, starting from naturally occurring V_H sequences or V_{HH} sequences may, for example, include combining one or more parts of one or more naturally occurring V_{HH} sequences (such as one or more framework region (FR) sequences and/or CDR sequences), one or more parts of one or more naturally occurring V_{HH} sequences (such as one or more framework region sequences or CDR sequences), and/or one or more synthetic or semi-synthetic sequences, in a suitable manner, so as to provide a monovalent anti-properdin single domain antibody or a nucleotide sequence or nucleic acid encoding the same. Nucleotide sequences encoding framework sequences of V_{HH} or single domain antibodies are known in the art and may alternatively be obtained polymerase chain reaction (PCR) starting from the nucleotide sequences obtained using the methods described herein. Such compositions can be suitably combined with nucleotide sequences that encode the desired CDRs (for example, by PCR assembly using overlapping primers), to provide a single domain antibody, or antibody fragment fused with a regulator of the alternative complement pathway or fragment thereof.

Generation of antibody fragments

Antibody fragments that recognize the same epitope as a parent antibody can be generated by known techniques. For example, antibody fragments can be prepared by proteolytic hydrolysis of an antibody or by expression in *E. coli* of the DNA coding for the fragment. The antibody fragments are antigen binding portions of an antibody, such as Fab, $F(ab')_2$, and scFV can be obtained by pepsin or papain digestion of whole antibodies by conventional methods or by genetic engineering techniques.

An antibody fragment can be produced by enzymatic cleavage of antibodies with pepsin to provide a 100 kDa fragment denoted $F(ab')_2$. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 50 kDa Fab' monovalent fragments. Alternatively, an enzymatic cleavage using

papain produces two monovalent Fab fragments and an Fc fragment directly (US Pat. Nos: 4,036,945 and 4,331,647; Nisonoff, A. *et al.*, *Arch. Biochem. Biophys.*, 89:230-44, 1960; Porter, R., *Biochem. J.*, 73:119-26, 1959; Edelman et al., in Methods in Enzymology Vol. I, page 422 (Academic Press 1967), and Coligan el al., Current Protocols in Immunology, Vol. 1, pages 2.8.1-2.8.10 and 2.10.-2.10.4 (John Wiley & Sons 1991).

Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody producing cells (Larrick, J & Fry, K. *METHODS- a companion to Methods in Enzymology Volume: New Techniques in Antibody Generation*, 2:106-110, 1991); Courtenay-Luck, "Genetic Manipulation of Monoclonal Antibodies," in Monoclonal Antibodies: Production, Engineering and Clinical Application, Ritter *et al.* (eds.), pages 166-179 (Cambridge University Press 1995); and Ward *et al.*, "Genetic Manipulation and Expression of Antibodies," in Monoclonal Antibodies: Principles And Applications, Birch *et al.*, (eds.), pages 137-185 (Wiley-Liss, Inc. 1995)).

Other antibody fragments, for example single domain antibody fragments, are known in the art and may be used in the claimed constructs (Muyldermans, S. *et al.*, *Trends Biochem. Sci.*, 26:230-5, 2001; Yau, K. *et al.*, *J. Immunol. Methods*, 281:161-75, 2003; Maass, D. *et al.*, *J. Immunol. Methods*, 324:13-25, 2007). The V_{HH} may have potent antigen binding capacity and can interact with novel epitopes that are inaccessible to conventional V_H-V_L pairs. Camelidae may be immunized with known antigens, such as properdin, and V_{HH}s can be isolated that bind to and neutralize the target antigen.

Screening monovalent antibodies for antigen binding

Library screening methods can be used to identify monovalent properdin-specific binding antibodies or fragments. Phage display technology provides an approach for the selection of antibodies that bind a desired target (*e.g.*, human properdin) from among large, diverse repertoires of antibodies (Smith, G., *Science*, 228:1315-7, 1985; Scott, J. & Smith, G., *Science*, 249:386-90, 1990; McCafferty, J. *et al.*, *Nature*, 348:552-4, 1990). These phage-antibody libraries can be grouped into two categories: natural libraries that use rearranged V genes harvested from human B-cells (Marks, J. *et al.*, *J. Mol. Biol.*, 222:581-97, 1991; Vaughan, T. *et al.*, *Nat. Biotechnol.*, 14:309-14, 1996) or synthetic libraries whereby germline V gene segments or other antibody polypeptide coding sequences are 'rearranged' *in vitro* (Hoogenboom, H. & Winter, G., *J. Mol. Biol.*, 227:381-8, 1992; Nissim, A. *et al.*, *EMBO J.*, 13:692-8, 1994; Griffiths, A. *et al.*, *EMBO J.*, 13:3245-60, 1994; de Kruif, J. *et al.*, *J. Mol. Biol.*, 248:97-105, 1995) or where synthetic CDRs are incorporated into a single rearranged V gene (Barbas, C. *et al.*, *Proc. Natl. Acad. Sci. USA*, 89:4457-61, 1992). Methods involving genetic display packages (*e.g.*, phage display, polysome display) are suited for the selection of monovalent properdin-specific antibody constructs because they generally express only monovalent fragments, rather than whole, divalent antibodies, on

the display packages. Methods for the preparation of phage display libraries displaying various antibody fragments are described in the preceding references and, for example, in U.S. Pat. No. 6,696,245, which is incorporated herein by reference in its entirety.

Following expression of a repertoire of single domain antibodies on the surface of phage, selection is performed by contacting the phage repertoire with immobilized target antigen (*e.g.*, properdin), washing to remove unbound phage, and propagation of the bound phage, the whole process frequently referred to as "panning." This process is applicable to the screening of monovalent single domain antibodies and antibody fragments that can be expressed on a display library (*e.g.*, scFv, Fab, (Fab')2, and V_{HH}; Harrison, J. *et al.*, *Meth. Enzymol.*, 267:83-109, 1996). Alternatively, phages are pre-selected for the expression of properly folded member variants by panning against an immobilized generic ligand (*e.g.*, protein A or protein L) that is only bound by folded members (WO 99/20749). This has the advantage of reducing the proportion of non-functional members, thereby increasing the proportion of members likely to bind a target antigen. The screening of phage antibody libraries is generally described, for example, by.

Screening is commonly performed using purified antigen immobilized on a solid support, for example, plastic tubes or wells, or on a chromatography matrix, for example Sepharose™ (Pharmacia). Screening or selection can also be performed on complex antigens, such as the surface of cells (Marks, J. *et al.*, *Biotechnology (NY)*, 11:1145-9, 1993; de Kruif, J. *et al.*, *Proc. Natl. Acad. Sci. USA*, 92:3938-42, 1995). Another alternative involves selection by binding biotinylated antigen in solution, followed by capture on streptavidin-coated beads. V_{HH} coding sequences are known in the art and may be used to construct camelid V_{HH} phage display libraries, which can be used for antibody fragment isolation by bio-panning techniques known in the art.

Expression of anti-properdin antibodies

The manipulation of nucleic acids can be carried out in recombinant vectors. As used herein, "vector" refers to a discrete element that is used to introduce heterologous DNA into cells for the expression and/or replication thereof. Methods to select or construct and, subsequently, use such vectors are known to one of skill in the art. Numerous vectors are publicly available, including bacterial plasmids, bacteriophage, artificial chromosomes and episomal vectors. Such vectors may be used for simple cloning and mutagenesis. A vector is selected to accommodate a polypeptide coding sequence of a desired size. A suitable host cell is transformed with the vector after *in vitro* cloning manipulations. Each vector contains various functional components, which generally include a cloning (or "polylinker") site and an origin of replication. An expression vector can further comprise one or more of the following: enhancer element, promoter, transcription termination and signal sequences- each positioned in the vicinity of the cloning site such that they are operatively linked to the gene encoding the polypeptide.

Both cloning and expression vectors generally contain nucleic acid sequences that enable the vector to replicate in one or more selected host cells. Typically cloning vectors comprise sequence elements that enable the vector to replicate independently of the host chromosomal DNA and includes origins of replication or autonomously replicating sequences. Such sequences are known for a variety of bacteria, yeast and viruses.

For screened libraries described herein, the vectors can be expression vectors that enable the expression of a polypeptide library member. Selection is performed by separate propagation and expression of a single clone expressing the polypeptide library member or by use of any selection display system. For bacteriophage display, phage or phagemid vectors can be used. Phagemid vectors have an *E. coli* origin of replication (for double stranded replication) and also a phage origin of replication (for production of single-stranded DNA).

Purification and concentration of monovalent antibodies

Monovalent antibodies secreted into the periplasmic space or into the medium of bacteria are harvested and purified according to known methods (Skerra, A. & Plückthun, A., Science, 240:1038-41, 1988; and Breitling, F. et al. (Gene, 104:147-53, 1991) describe the harvest of antibody polypeptides from the periplasm; Better, M. et al. (Science, 240:1041-3, 1988) describe harvest from the culture supernatant). For some antibody polypeptides, purification can also be achieved by binding to generic ligands, such as protein A or Protein L. Alternatively, the variable domains can be expressed with a peptide tag, *e.g.*, the Myc, HA or 6×His tags, which facilitates purification by affinity chromatography. If necessary, monovalent anti-properdin antibodies are concentrated by any of several methods well known in the art, including, for example, ultrafiltration, diafiltration and tangential flow filtration. The process of ultrafiltration uses semi-permeable membranes and pressure to separate molecular species on the basis of size and shape. The pressure is provided by gas pressure or by centrifugation. By selection of a molecular weight cutoff smaller than the target antibody (usually ⅓ to ⅙ the molecular weight of the target polypeptide, the anti-properdin antibody is retained when solvent and smaller solutes pass through the membrane.

Pharmaceutical compositions, dosage and administration

The antibodies described herein can be incorporated into pharmaceutical compositions suitable for administration to a subject. Typically, the pharmaceutical composition includes a monovalent anti-properdin antibody, antibody derivative or fragment thereof and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. The term "pharmaceutically acceptable carrier" excludes tissue culture medium including bovine or horse serum. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol or sodium chloride in the composition. Pharmaceutically acceptable substances include minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody.

The compositions as described herein may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (*e.g.*, injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The final form depends on the intended mode of administration and therapeutic application. Typical compositions are in the form

of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans with other antibodies. The composition(s) can delivered by, for example, parenteral injection (*e.g.*, intravenous, subcutaneous, intraperitoneal, intramuscular).

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the monovalent anti-properdin antagonist in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filter sterilization. Generally, dispersions are prepared by incorporating the monovalent anti-properdin antagonist into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants.

The antibodies described herein can be administered by a variety of methods known in the art, although for many therapeutic applications, the preferred route/mode of administration is intravenous injection or infusion. The polypeptide can also be administered by intramuscular or subcutaneous injection.

As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the antibody may be prepared with a carrier that will protect the antibody against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Monovalent single domain antibodies are suited for formulation as extended release preparations due, in part, to their small size- the number of moles per dose can be significantly higher than the dosage of, for example, full sized antibodies. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters and polylactic acid. Prolonged absorption of injectable compositions can be attained by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin. Many methods for the preparation of such formulations are known to those skilled in the art (*e.g.*, Sustained and Controlled Release Drug Delivery Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978). Methods applicable to the controlled or extended release of antibodies such as the monovalent single domain antibodies disclosed herein are known (US Pat. Nos: 6,306,406 and 6,346,274; U.S. Patent Application Nos: US20020182254 and US20020051808, the entire teachings of each of which are incorporated herein by reference).

In certain embodiments, a monovalent anti-properdin antibody, antibody derivative or fragment thereof can be orally administered, for example, with an inert diluent or an assimilable edible carrier. To administer a composition described herein by a method other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with a material to prevent its inactivation.

Additional active compounds can also be incorporated into the compositions. In certain embodiments, a monovalent anti-properdin antibody, antibody derivative or fragment thereof is co-formulated with and/or co-administered with one or more additional therapeutic agents. For example, a monovalent anti-properdin antibody, antibody derivative or fragment thereof can be co-formulated and/or co-administered with one or more additional antibodies that bind other targets (*e.g.*, antibodies that bind regulators of the alternative complement pathway). Such combination therapies may utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies. Additionally, the compositions described herein can be co-formulated or co-administered with other therapeutic agents to ameliorate side effects of administering the compositions described herein (*e.g.*, therapeutic agents that minimize risk of infection in an immunocompromised environment, for example, anti-bacterial agents, anti-fungal agents and anti-viral agents).

The pharmaceutical compositions can include a "therapeutically effective amount" or a "prophylactically effective amount" of a monovalent anti-properdin antagonist (*e.g.*, an antibody or derivative or fragment thereof). A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the antibody can vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the monovalent anti-properdin antagonist to elicit a desired response in the individual. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. In some embodiments, a prophylactic dose is used in subjects prior to or at an earlier stage of disease where the prophylactically effective amount will be less than the therapeutically effective amount.

Dosage regimens may be adjusted to provide the optimum desired response (*e.g.*, a therapeutic or prophylactic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. It is to be noted that dosage values can vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the administering clinician.

A non-limiting range for a therapeutically or prophylactically effective amount of a monovalent anti-properdin antibody, antibody derivative or fragment thereof is 0.1-20 mg/kg, more preferably 1-10 mg/kg. It is to be noted that dosage values can vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the administering clinician.

The efficacy of treatment with a monovalent anti-properdin antibody, antibody derivative or fragment thereof as described herein is judged by the skilled clinician on the basis of improvement in one

or more symptoms or indicators of the disease state or disorder being treated. An improvement of at least 10% (increase or decrease, depending upon the indicator being measured) in one or more clinical indicators is considered "effective treatment," although greater improvements are preferred, such as 20%, 30%, 40%, 50%, 75%, 90%, or even 100%, or, depending upon the indicator being measured, more than 100% (*e.g.*, two-fold, three-fold, ten-fold, etc., up to and including attainment of a disease-free state).

Use of monovalent anti-properdin antibodies

The compositions described herein can be used in methods of treating a disease or disorder mediated by alternative complement pathway dysfunction in an individual in need of such treatment, the method including administering to the individual a therapeutically effective amount of a composition that includes a monovalent anti-properdin antibody, antibody derivative or fragment thereof, preferably a composition including a single human immunoglobulin variable domain that binds human properdin. In one embodiment, the monovalent anti-properdin antibodies, antibody derivatives or fragments thereof described herein are useful in treating diseases mediated by alternative complement pathway dysregulation by inhibiting the alternative complement pathway activation in a mammal (*e.g.*, a human). Such disorders include, without limitation, systemic lupus erythromatosus and lupus nephritis, rheumatoid arthritis, antiphospholipid (aPL) Ab syndrome, glomerulonephritis, paroxysmal nocturnal hemoglobinuria (PNH) syndrome, inflammation, organ transplantation, intestinal and renal I/R injury, asthma (e.g., severe asthma), atypical hemolytic-uremic syndrome (aHUS), spontaneous fetal loss, DDD, Macular degeneration, TTP, IgA nephropathy (Berger's disease), C3 glomerulopathy (C3G), Gaucher disease, Hidradentitis suppurativa, Behcet's disease, dermatomyositis, severe burn, early sepsis, pneumococcal meningitis, Alzheimer's disease, cancer metastasis, acute respiratory distress syndrome (ARDS), acute lung injury (ACI), transfusion-related lung injury (TRALI), hemodialysis induced thrombosis, epidermolysis bullosa acquisita (EBA), uveitis, Parkinson's disease, primary biliary atresia, antineutrophil cytoplasmic antibodies (ANCA) vasculitis, retinal degeneration, broad thrombotic microangiopathy (TMA), broad TMA (APS), hematopoietic stem cell therapy (HSCT) TMA, age-related macular degeneration (AMD), pre-eclampsia, hemolysis, elevated liver enzymes, and low platelet (HELLP) syndrome, multiple sclerosis, antiphospholipid syndrome (APS), relapsing polychondritis, ischemic injury, stroke, graft versus host disease (GvHD), chronic obstructive pulmonary disease (COPD), emphysema, atherosclerosis, acute coronary syndrome, hemorrhagic shock, dialysis (cardiovascular risk), cardiovascular disease, placental malaria, APS pregnancy loss, membranoproliferative (MP) glomerulonephritis, membranous nephritism, encephalitis, brain injury, NMDA receptor antibody encephalitis, malaria hemolytic crisis, abdominal aortic aneurysm (AAA), and thoracoabdominal aortic aneurysm (TAA).

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a disclosure and description of how the methods and compounds claimed herein are performed, made. They are intended to be purely exemplary and are not intended to limit the scope of the disclosure.

Example 1. Generation of V_{HH}-His In-Fusion cloning Vector

The pBNJ391 vector was digested with the restriction enzymes BstEII and EcoRI to remove the hinge and Fc. The vector was gel purified, which produced a 1000bp release product. Annealed oligos UDEC6629/6630 were cloned into the pBNJ391 vector with BstEII/EcoRI. The annealed oligos contained the following sequences:

UDEC 6629 forward primer: GTCACCGTGTCGAGCCATCATCACCATCATCACTGATGAG (SEQ ID NO: 65)

UDEC 6630 reverse primer: AATTCTCATCATTTGTCATCATCATCCTTATAGTCGCTCGACACG (SEQ ID NO: 66)

The final vector contained a BstEII-6×His-EcoRI site.

Next, the pNGH0320 vector was digested with XhoI/BstEII (producing a 13 bp release product) and column purified. Next, a V_{HH} phage clone template was used to PCR amplify an insert. Forward primer UDEC 6438 (GTCCACTCCCTCGAGGTGCAGCTGGTGGAGTCTGGG; SEQ ID NO: 67) and reverse primer UDEC 6442 (GCTCGACACGGTGACCTGGGTCCCCTGGCCCCA; SEQ ID NO: 68), were used. The PCR products were purified and subsequently used in an In-Fusion protocol for cloning.

The pBNJ391 vector was digested with BstEII/EcoRI (50 ng/μL). Both complementary oligonucleotides were re-suspended at the same molar concentration, using TE Buffer. Equal volumes of both complementary oligonucleotides (at equimolar concentration) were mixed in a 1.5 mL tube. The tube was placed in a standard heat block at 90-95ºC for 3-5 minutes. The tube was removed from the apparatus and allowed to cool to room temperature (or at least below 30ºC). The tube was stored on ice or at 4ºC until further use.

A mix of 1 μL insert DNA (from the above nucleotide used for ligation to pBNJ391), 2 μL of pBNJ391 (EcoRI/BstEII, 100 ng), 1 μL of 10× Ligase Buffer (NEB B0202S Lot: 1091410), 1 μL of T4 DNA Ligase (NEB M0202L Lot: 0671502), and 5 μL of water formed the ligation reaction. The ligation reaction was incubated for 30 minutes at room temperature. 1 μL of the ligation reaction was transformed into 30 μL of DH10 chemical competent cells (InVitrogen 18297 Lot# 1552241) and 750 μL of SOC (NEBB9020S Lot# 2971403) was added. The tube was shaken for 1 hour at 37ºC and 10 μL and 100 μL were plated on an LB-carb/glucose plate. Plates were incubated over the weekend at room temperature.

Colonies were picked for PCR for insertion of the 6×His into pNGH0320. Eight colonies were screened and pBNJ391 was used as a negative control. 300 μL of TB/Carb/Glucose culture was added to isolated colonies and grown at 37ºC. Forward primer UDEC5276 (CATAATAGCTGACAGACTAACAGACTG; SEQ ID NO: 69) and reverse primer UDEC1977 (CGAAACAAGCGCTCATGAGCCCGAAGT; SEQ ID NO: 70), were used. For a 20 μL PCR reaction, DNA from a single colony was added to 10 μL Go Taq Green PCR Mix, 0.2 μL Forward Primer (100 μM), 0.2 μL Reverse Primer (100uM), and 9.6 μL H_2O, totaling 20 μL. PCR conditions are as follows: 95ºC for 3 minutes, 95ºC for 20 seconds, 50ºC for 20 seconds, and 72ºC for 1 minute 15 seconds. The cycle was repeated 30 times, followed by incubation at 72ºC for 5 minutes, and 4ºC until further use. 5 μL of the PCR product was mixed with 15 μL of water and run on a 2% E-gel. Two clones matched the predicted size. Plasmid maxi prep was performed using the overnight cultures with the Promega maxi prep kit.

To clone anti-properdin V_{HH} antibodies in V_{HH}-His tag format using in-fusion ligation of V_{HH} into pNGH0320, PCR was used to generate the V_{HH} insert with UDEC 6438- Infusion Forward Primer and UDEC 6442- Infusion reverse Primer for amplification of V_H phagemids from the Llama anti-properdin library pLNJ with an XhoI site for cloning into pNGH0317 by infusion. For a 60 μL PCR reaction, 30 μL 2×phusion PCR mix (NEB M0531s Lot: 0211412), 1 μL of bacterial culture, 0.1 μL Forward Primer UDEC 6438 (100 μM), 0.1 μL Reverse Primer UDEC 6442 (100 μM), and 28.8 μL H_2O, totaling 20 μL. PCR conditions are as follows: 98ºC for 3 minutes, 98C for 10 seconds, 52C for 15 seconds, 72C for 30-60 seconds, followed by 72ºC for 5 minutes. The cycle was repeated 30 times and held at 4C until further use. 5 μL of the PCR product was mixed with 15 μL of water and were run on a 2% E-gel. All clones matched the predicted size. Clones were pooled in reactions of eight and column purified using the Promega Wizard® SV Gel and PCR Clean-Up System, according to manufacturer's instructions. Plasmid maxi prep was performed using the overnight cultures with the Promega maxi prep kit.

For ligation of the insert, 2 μL of 5× In-Fusion HD Enzyme Premix (Clontech 639650 Lot: 1501713A), 2.5 μL of Vector pNGH0320 (XhoI/BstEII) 39.1 ng/μL (100 ng), 1 μL of purified PCR fragment (10-200 ng), and 4.5 μL of water formed the ligation reaction. The ligation reaction was incubated for 15 minutes at 50ºC.

For transformation, Stellar™ competent cells (Clontech) were thawed in an ice bath just before use. After thawing, cells were mixed gently to ensure even distribution, and then 50 μL of competent cells were moved into a 14 mL round bottom tube (falcon tube). 1 μL (less than 5 ng of DNA) was added to the cells. The tube was placed on ice for 30 min. Next, the cells were heat shocked for exactly 45 sec at 42ºC. Tubes were then placed on ice for 1-2 min. SOC medium was added to bring the final volume to 500 μL (SOC medium was warmed to 37C before using). The tube was incubated while shaking (160-225 rpm) for 1 hr at 37ºC. 10 μL of the solution was then place on LB plate containing carbenicillin. The plate was incubated overnight at 37C.

A colony PCR screen was performed for insertion of the 24 V_{HH} colonies for each pool. A total of 48 clones were picked for each pool. Vector pNGH0320.1 was used as a positive control. Forward primer UDEC5276 and reverse primer UDEC1977 were used. For a 20 μL PCR reaction, DNA from a single colony was added to 10 μL Go Taq Green PCR Mix, 0.2 μL Forward Primer (100 μM), 0.2 μL Reverse Primer (100 μM), and 9.6 μL H_2O, totaling 20 μL. PCR conditions are as follows: 95ºC for 3 minutes, 95ºC for 20 seconds, 50ºC for 20 seconds, and 72ºC for 1 minute 15 seconds. Repeat for 30 cycles, followed by 72ºC for 5 minutes, and hold 4ºC until further use. 5 μL of the PCR products was mixed with 15 μL of water and were run on a 2% E-gel. Sequence analysis was performed on all 48 clones.

Preliminary screening of an immunization-biased llama V_{HH} phage display library resulted in identification of 192 V_{HH}s that were ELISA positive for binding properdin. 57 V_{HH}s were cloned and expressed with a 6× histidine tag. Of these, 34 V_{HH}s were Octet-positive for binding properdin. A summary is shown below in Table 1.

Table 1. Summary of screening assay

	Screening with hP		Cross-reactivity screening on mouse and human fP		Cross-reactivity screening on monkey and human fB	
	Standard Procedure	NGS	Standard Procedure	NGS	Standard Procedure	NGS
ELISA positive	193		233		284	
Unique sequences (>3 different a.a. in CDR-H3)	72	NA	134	NA	90	NA
Cloned and expressed (with 6xhistidine tag)	57	NA	NA	NA	NA	NA
Octet positive	34	NA	NA	NA	NA	NA
Hemolysis positive (by using the purified VHH)	4	NA	NA	NA	NA	NA

Four functional V_{HH}s were found to effectively inhibit alternative complement pathway-mediated hemolysis and are shown below in Table 2.

Table 2. Anti-properdin V_{HH} sequences

Clone ID	V_{HH} sequence
AB005	QVQVVESGGGLRQTGGSLRLSCTASGRIFEVNMMAWYRQAPGKQRELVAEISRVGTTVYA DSVKGRFTISRDSAKNTVTLQMNSLKSEDTAVYYCNALQYDRYGGAEYWGQGTQVTVSS (SEQ ID NO: 58)
AB006	QVQLAESGGGLVQAGDSLKLSCTASGRIFEVNMMAWYRQAPGKDRELVAEISRVGTTTYAD SVKGRFTISRDSAKNTVTLQMNSLKSEDTAVYYCNALQYSRYGGAEYWGQGTQVTVSG (SEQ ID NO: 59)
AB007	QVQLVESGGGLVQAGGSLRLSCAASGRISSIIHMAWYRQAPGKQRELVAEISRVGTTVYADS VKGRFTISRDDAKNTVTLQMNSLKPEDTAVYYCNALQYEKHGGADYWGQGTQVTVSG (SEQ ID NO: 60)
AB008	QVQLVESGGGLRQTGESLRLSCTASGRIFEVNMMAWYRQAPGKQRELVAEISRVGTTTYAD SVKGRFTISRDSAKNTVTLQMNSLKSEDTAVYYCNALQYDRYGGAEYWGQGTQVTVSG (SEQ ID NO: 61)

Example 2. Binding of anti-properdin V_{HH} antibodies to human properdin

FIG. 1 shows kinetic binding measurements can be performed on an Octet instrument (FortéBio Inc.). All washes, dilutions, and measurements are performed in Kinetic buffer (FortéBio cat 185032) with the plate shaking at 1000 rpm. Streptavidin Biosensors (Forte Bio Cat:18-5019 lot: 1405301) were equilibrated in Kinetic buffer for 10 min and then loaded with 50 nm of biotinylated human properdin. For the association phase, 10 μg/mL of selected anti-properdin antibody or kinetics buffer blank was added to the biosensors preloaded with biotinylated human properdin respectively. Results show binding of AB005, AB006, AB007 and AB008 to human properdin.

FIG. 2 shows kinetic binding measurements can be performed on an Octet instrument (FortéBio Inc). All washes, dilutions, and measurements are performed in Kinetic buffer (FortéBio cat 185032) with the plate shaking at 1000 rpm. Streptavidin Biosensors (Forte Bio Cat:18-5019 lot: 1405301) were equilibrated in Kinetic buffer for 10 min and then loaded with 50 nm of biotinylated mouse properdin. For

the association phase, 10 μg/mL of selected anti-properdin antibody or kinetics buffer blank was added to the biosensors preloaded with biotinylated human properdin respectively. Results show weak or no binding of AB005, AB006, AB007, and AB008.

FIG. 3 shows kinetic binding measurements can be performed on an Octet instrument (FortéBio Inc.). All washes, dilutions, and measurements are performed in Kinetic buffer (FortéBio cat 185032) with the plate shaking at 1000 rpm. Streptavidin Biosensors (Forte Bio Cat:18-5019 lot: 1405301) were equilibrated in Kinetic buffer for 10 min and then loaded with 50 nm of biotinylated cynomolgus properdin. For the association phase, 10 μg/mL of selected anti-properdin antibody or kinetics buffer blank was added to the biosensors preloaded with biotinylated human properdin respectively. Results show weak binding of AB005, AB006, AB007, and AB008 to cynomolgus properdin.

Example 3. Alternative complement hemolysis assay

FIG. 4 shows an alternative complement pathway-mediated hemolysis assay based on the formation of a terminal complement-complex on the surface of the rabbit red blood cell (rRBC). As a result of the formation of this complex, the rRBCs are lysed. Agents that inhibit the formation of complement complexes are expected to inhibit cellular lysis. Various anti-properdin antigen binding fragments were tested to evaluate the effect on cellular lysis mediated by alternative complement activation. An "assay plate" was prepared by diluting 40% normal human serum with Gelatin veronal buffer (GVB) supplemented with 10 mM EGTA and 10 mM $MgCl_2$ (*e.g.*, 1600 μL normal human serum into 2400 μLGVB supplemented with 10 mM EGTA and 10 mM $MgCl_2$). 50 μL of this solution was distributed into each well of the assay plate (polystyrene). Next, the dilution plate (polypropylene) was prepared by adding 50 μL/well of 2× mAbs (*e.g.*, anti-properdin Fab) in GVB supplemented with 10 mM EGTA and 10 mM $MgCl_2$ at a concentration ranging from 0-100 nM to appropriate wells. As a positive control rabbit red blood cells were incubated in distilled water (100% lysis of cells) and for the negative control the red blood cells were incubated in GVB with 10 mM EDTA and 10 mM $MgCl_2$, respectively (0% lysis of cells).

50 μL/well was transferred from the dilution plate to assay plate. The assay plate was left at room temperature while proceeding to the next step. 400 μL of rRBCs were washed 4 times, each with 1 mL of GVB supplemented with 10 mM EGTA and 10 mM $MgCl_2$. rRBCs were spun at 2600 rpm for 1 minute after each wash. After the final wash, rRBCs were resuspend to a volume of 400 μL by adding 300 μL GVB supplemented with 10 mM EGTA and 10 mM $MgCl_2$. 50 μL of washed rRBCs were resuspended to 1 mL with GVB supplemented with 10 mM EGTA and 10 mM $MgCl_2$. 30 μL of this dilute solution was added to 100 μL of the prepared sample in the assay plate, yielding 1.5×10^6 cells/well. The plate was incubated for 30 minutes at 37ºC. The plate was then centrifuged at 1000×g for 5 min and 85 μL of the supernatant was transferred to a flat bottom 96 well plate. Hemolysis was determined by measuring OD at 415 nm. A progressive decrease in light scatter (due to the lysis of intact cells) was measured at 415 nm as a function of concentration. For the calculation, the total inhibition was calculated at each concentration of the anti-properdin V_{HH} and the results were expressed as a percentage of unlisted controls.

Example 4. Binding Kinetics of monovalent anti-properdin V_{HH} antibodies to properdin

In Fig. 6, anti-properdin V_{HH} antibodies AB007 and AB008, respectively, were run at known concentrations over an immobilized sensor surface. Response level (RU) was plotted against time in the sensorgrams.

The binding affinities of anti-properdin V_{HH} antibodies were determined. The results are summarized in Table 3 below.

Table 3. Binding kinetics

Sample	k_a (1/Ms)	k_d (1/s)	K_D (M)	Chi^2
AB007	1.04e6	3.59e-4	3.44e-10	0.36
AB008	2.11e6	1.69e-3	8.03e-10	5.29

Sample	Antigen	k_a (1/Ms)	k_d (1/s)	K_D (M)	Chi^2	Comments
AB009	Human Properdin	1.69E+06	4.33E-05	2.55E-11	0.18	Good Fit
AB010	Human Properdin	1.09E+07	7.17E-05	6.59E-12	0.11	Good Fit

Example 5. Binding Kinetics of bispecific anti-properdin antibodies to properdin and alternative complement hemolysis assay

Bispecific constructs were created based on the anti-properdin constructs described above linked with a linker to an anti-albumin construct. The binding to properdin and alternative complement hemolysis was measured in similar assays as described above. The sequences of the constructs are shown in Table 4 below.

Table 4. anti-properdin construct sequences

Molecule	Description	AA Sequence
TPP-2225	anti-Albumin LVP058 $(G4S)_3$ Linker	EVQLVESGGGLVKPGGSLRLSCAASGRPVSNYAAAWFRQAPGKEREFV SAINWQKTATYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAAV FRVVAPKTQYDYDYWGQGTLVTVSSGGGGSGGGGSGGGGSLEVQLVE SGGGLVQAGGSLRLSCAASGRISSIIHMAWYRQAPGKQRELVAEISRVGT TVYADSVKGRFTISRDDAKNTVTLQMNSLKPEDTAVYYCNALQYEKHGG ADYWGQGTQVTVSS (SEQ ID NO: 45)
TPP-2951	Humanized anti-Albumin LVP058 $(G4S)_3$ Linker (7-backmutations)	EVQLVESGGGLVKPGGSLRLSCAASGRPVSNYAAAWFRQAPGKEREFV SAINWQKTATYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAAV FRVVAPKTQYDYDYWGQGTLVTVSSGGGGSGGGGSGGGGSEVQLLES GGGLVQPGGSLRLSCAASGRISSIIHMAWFRQAPGKERELVSEISRVGTT VYADSVKGRFTISRDNSKNTLYLQMNSLKPEDTAVYYCNALQYEKHGGA DYWGQGTLVTVSS (SEQ ID NO: 46)
TPP-3071	anti- Albumin LVP058 $(G4D)_2$ (G4) Linker	EVQLVESGGGLVKPGGSLRLSCAASGRPVSNYAAAWFRQAPGKEREFV SAINWQKTATYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAAV FRVVAPKTQYDYDYWGQGTLVTVSSGGGGDGGGGDGGGGEVQLVESG GGLVQAGGSLRLSCAASGRISSIIHMAWYRQAPGKQRELVAEISRVGTTV YADSVKGRFTISRDDAKNTVTLQMNSLKPEDTAVYYCNALQYEKHGGAD YWGQGTQVTVSS (SEQ ID NO: 47)
TPP-3072	anti- Albumin LVP058 $(G4E)_2$ (G4) Linker	EVQLVESGGGLVKPGGSLRLSCAASGRPVSNYAAAWFRQAPGKEREFV SAINWQKTATYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAAV FRVVAPKTQYDYDYWGQGTLVTVSSGGGGEGGGGEGGGGEVQLVESG GGLVQAGGSLRLSCAASGRISSIIHMAWYRQAPGKQRELVAEISRVGTTV YADSVKGRFTISRDDAKNTVTLQMNSLKPEDTAVYYCNALQYEKHGGAD YWGQGTQVTVSS (SEQ ID NO: 48)
TPP-3261	Humanized anti-Albumin LVP058 $(G4S)_3$ Linker (3-backmutations)	EVQLVESGGGLVKPGGSLRLSCAASGRPVSNYAAAWFRQAPGKEREFV SAINWQKTATYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAAV FRVVAPKTQYDYDYWGQGTLVTVSSGGGGSGGGGSGGGGSEVQLVES GGGLVQPGGSLRLSCAASGRISSIIHMAWVRQAPGKQRELVSEISRVGTT VYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCNALQYEKHGGA DYWGQGTLVTVSS (SEQ ID NO: 49)
TPP-3341	Humanized anti-Albumin LVP058 $(G4D)_2$ (G4) Linker (7-backmutations)	EVQLVESGGGLVKPGGSLRLSCAASGRPVSNYAAAWFRQAPGKEREFV SAINWQKTATYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAAV FRVVAPKTQYDYDYWGQGTLVTVSSGGGGDGGGGDGGGGEVQLLESG GGLVQPGGSLRLSCAASGRISSIIHMAWFRQAPGKERELVSEISRVGTTV YADSVKGRFTISRDNSKNTLYLQMNSLKPEDTAVYYCNALQYEKHGGAD YWGQGTLVTVSS (SEQ ID NO: 50)

Molecule	Description	AA Sequence
TPP-3342	Humanized anti-Albumin LVP058 $(G4E)_2$ (G4) Linker (7-backmutations)	EVQLVESGGGLVKPGGSLRLSCAASGRPVSNYAAAWFRQAPGKEREFV SAINWQKTATYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAAV FRVVAPKTQYDYDYWGQGTLVTVSSGGGGEGGGGEGGGGEVQLLESG GGLVQPGGSLRLSCAASGRISSIIHMAWFRQAPGKERELVSEISRVGTTV YADSVKGRFTISRDNSKNTLYLQMNSLKPEDTAVYYCNALQYEKHGGAD YWGQGTLVTVSS (SEQ ID NO: 51)
TPP-3343	Humanized anti-Albumin LVP058 $(G4D)_2$ (G4) Linker (3-backmutations)	EVQLVESGGGLVKPGGSLRLSCAASGRPVSNYAAAWFRQAPGKEREFV SAINWQKTATYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAAV FRVVAPKTQYDYDYWGQGTLVTVSSGGGGDGGGGDGGGGEVQLVESG GGLVQPGGSLRLSCAASGRISSIIHMAWVRQAPGKQRELVSEISRVGTTV YADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCNALQYEKHGGAD YWGQGTLVTVSS (SEQ ID NO: 52)
TPP-3344	Humanized anti-Albumin -LVP058 $(G4E)_2$ (G4) Linker (3-backmutations)	EVQLVESGGGLVKPGGSLRLSCAASGRPVSNYAAAWFRQAPGKEREFV SAINWQKTATYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAAV FRVVAPKTQYDYDYWGQGTLVTVSSGGGGEGGGGEGGGGEVQLVESG GGLVQPGGSLRLSCAASGRISSIIHMAWVRQAPGKQRELVSEISRVGTTV YADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCNALQYEKHGGAD YWGQGTLVTVSS (SEQ ID NO: 62)
TP-2221	LVP058_hG2-G4 - V_{HH} on silent human Fc	LEVQLVESGGGLVQAGGSLRLSCAASGRISSIIHMAWYRQAPGKQRELVA EISRVGTTVYADSVKGRFTISRDDAKNTVTLQMNSLKPEDTAVYYCNALQ YEKHGGADYWGQGTQVTVSSRKCCVECPPCPAPPVAGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFN STYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSL GK (SEQ ID NO: 53)
TP-2222	LVP058_ hG1_noC1q -V_{HH} on human Fc without C1q binding	LEVQLVESGGGLVQAGGSLRLSCAASGRISSIIHMAWYRQAPGKQRELVA EISRVGTTVYADSVKGRFTISRDDAKNTVTLQMNSLKPEDTAVYYCNALQ YEKHGGADYWGQGTQVTVSSPKSCDKTHTCPPCPAPELLGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS LSPGK (SEQ ID NO: 54)

Molecule	Description	AA Sequence
TPP-2224	LVP058 $(G4S)_3$ - anti-Alb - tandem V_{HH}	LEVQLVESGGGLVQAGGSLRLSCAASGRISSIIHMAWYRQAPGKQRELVA EISRVGTTVYADSVKGRFTISRDDAKNTVTLQMNSLKPEDTAVYYCNALQ YEKHGGADYWGQGTQVTVSSGGGGSGGGGSGGGGSEVQLVESGGGL VKPGGSLRLSCAASGRPVSNYAAAWFRQAPGKEREFVSAINWQKTATYA DSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAAVFRVVAPKTQYDY DYWGQGTLVTVSS (SEQ ID NO: 55)
TPP-2223	Anti-properdin control antibody without C1q binding domain	Light Chain Sequence: DIQMTQSPSSLSASVGDRVTITCRASQDISFFLNWYQQKPGKAPKLLIYYT SRYHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQHGNTLPWTFGQ GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC (SEQ ID NO: 56) Heavy Chain Sequence: QVQLVQSGAEVKKPGASVKVSCKASGYIFTNYPIHWVRQAPGQGLEWM GFIDPGGGYDEPDERFRDRVTMTRDTSTSTVYMELSSLRSEDTAVYYCA RRGGGYYLDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS LSPGK (SEQ ID NO: 57)

FIG. 7 shows kinetic binding measurements performed on an Octet instrument (FortéBio Inc.). All washes, dilutions, and measurements were performed in Kinetic buffer (FortéBio cat 185032) with the plate shaking at 1000 rpm. Streptavidin Biosensors (Forte Bio Cat:18-5019 lot: 1405301) were equilibrated in Kinetic buffer for 10 min and then loaded with 50 nm of biotinylated human properdin. For the association phase, 10 μg/mL of selected anti-properdin antibody or kinetics buffer blank was added to the biosensors preloaded with biotinylated human properdin, respectively. Results show binding of TPP-2225, TPP-2591, TPP-3071, TPP-3072, TPP-3261 to human properdin. The results show strong binding by all constructs to human properdin.

FIG. 8A-FIG. 8B show results of alternative complement pathway-mediated hemolysis assays based on the formation of a terminal complement-complex on the surface of the rabbit red blood cell (rRBC). As a result of the formation of this complex, the rRBCs are lysed. Agents that inhibit the formation of complement complexes are expected to inhibit cellular lysis. Various bispecific anti-properdin antigen binding constructs were tested to evaluate the effect on cellular lysis mediated by alternative complement activation. An “assay plate” was prepared by diluting 40% normal human serum

with Gelatin veronal buffer (GVB) supplemented with 10 mM EGTA and 10 mM $MgCl_2$ (*e.g.*, 1600 μL normal human serum into 2400 μLGVB supplemented with 10 mM EGTA and 10 mM $MgCl_2$). 50 μL of this solution was distributed into each well of the assay plate (polystyrene). Next, the dilution plate (polypropylene) was prepared by adding 50 μL/well of 2× mAbs (*e.g.*, anti-properdin Fab) in GVB supplemented with 10 mM EGTA and 10 mM $MgCl_2$ at a concentration ranging from 0-100 nM to appropriate wells. As a positive control rabbit red blood cells were incubated in distilled water (100% lysis of cells) and for the negative control the red blood cells were incubated in GVB with 10 mM EDTA and 10 mM $MgCl_2$, respectively (0% lysis of cells).

50 μL/well was transferred from the dilution plate to assay plate. The assay plate was left at room temperature while proceeding to the next step. 400 μL of rRBCs were washed 4 times, each with 1 mL of GVB supplemented with 10 mM EGTA and 10 mM $MgCl_2$. rRBCs were spun at 2600 rpm for 1 minute after each wash. After the final wash, rRBCs were resuspend to a volume of 400 μL by adding 300 μL GVB supplemented with 10 mM EGTA and 10 mM $MgCl_2$. 50 μL of washed rRBCs were resuspended to 1 mL with GVB supplemented with 10 mM EGTA and 10 mM $MgCl_2$. 30 μL of this dilute solution was added to 100 μL of the prepared sample in the assay plate, yielding 1.5×10^6 cells/well. The plate was incubated for 30 minutes at 37ºC. The plate was then centrifuged at 1000×g for 5 min and 85 μL of the supernatant was transferred to a flat bottom 96 well plate. Hemolysis was determined by measuring OD at 415 nm. A progressive decrease in light scatter (due to the lysis of intact cells) was measured at 415 nm as a function of concentration. For the calculation, the total inhibition was calculated at each concentration of the anti-properdin antibody construct and the results were expressed as a percentage of unlisted controls. FIG. 8A-FIG. 8B show hemolysis mediated by TPP-2221, TP-2222, TP-2223, TP-2224, and TP-2225 in human (FIG. 8A) and cynomolgus (FIG. 8B) serum. The control antibody is an anti-properdin antibody. FIG. 9A-FIG. 9B show hemolysis mediated by TPP-2225, TPP-2951, TPP-3261, TPP-3071, and TPP-3072 in human (FIG. 9A) and cynomolgus (FIG. 9B) serum.

FIG. 10A-FIG. 10B, FIG. 11A-FIG. 11B, and FIG. 12A-FIG. 12B show the binding kinetics of TPP-3261, TPP-2951, and TPP-2225 to human and cynomolgus properdin.

The binding affinities and IC50 values are shown for each construct in the following Tables 5-9.

Table 5. Binding kinetics of bispecific constructs

Molecule	**IC50 (nM)**	**Description**
Anti-properdin control	14.6-15.4	Anti-properdin
TPP-2221	7.1-8.4	LVP058_hG2-G4
TPP-2222	5.1-5.8	LVP058_ hG1_noC1q
TPP-2223	8.4-13.4	Anti-properdin hG1_noC1q
TPP-2224	13.9-15.8	LVP058-anti-Alb
TPP-2225	11.6-12.9	anti-Alb-LVP058

Table 6. Binding kinetics of bispecific constructs

Molecule	Affinity pH7.4 (nM)	Human IC50 (nM)	Cynomolgus IC50 (nM)	Description
TPP-2225	1.72E-10	20.04 to 64.49	11.19 to 12.3	Non-humanized (G4S)3 Linker
TPP-2951	3.01E-10	----	13.76 to 15.89	Humanized (7 back mutations)
TPP-3261	4.85E-10	28.82 to 30.96	14.8 to 23.83	Humanized (3 back mutations)
TPP-3071	-----	22.28 to 29.36	10.66 to 14.58	Non-humanized (G4D)2 G4 Linker
TPP-3072	-----	----	13.06 to 18.65	Non-humanized (G4E)2 G4 Linker

Table 7. Binding kinetics of bispecific constructs

Molecule	Human Properdin		Cynomolgus Properdin		Human Albumin		Cynomolgus Albumin	
	pH 7.4	pH 6.0	pH 7.4	pH 6.0	pH 7.4	pH 6.0	pH 7.4	pH 6.0
TPP-2225	1.72E-10	2.571E-9	1.979E-9	No fit	7.74e-10	6.46e-10	7.07e-9	2.30e-9
TPP-2951	3.01E-10		2.33E-9					
TPP-3261	4.85E-10		3.08E-9					

Table 8. Binding kinetics of bispecific constructs.

Molecule	Properdin Type	k_a (1/Ms)	k_d (1/s)	K_D (M)	Chi^2	Comments
TPP3261	Human	1.72e6	8.34e-4	4.85e-10	0.07	Good Fit
TPP3261	Cynomolgus	1.91e6	5.87e-3	3.08e-9	0.24	Good Fit
TPP2951	Human	1.78e6	5.74e-4	3.22e-10	0.06	Good Fit
TPP2951	Cynomolgus	1.82e6	4.26e-3	2.33e-9	0.17	Good Fit

Table 9. Binding kinetics of bispecific constructs.

Molecule	Properdin Type	k_a (1/Ms)	k_d (1/s)	K_D (M)	Chi^2	Comments
TPP2225	Human	2.03e6	3.49e-4	1.72e-10	0.04	Good Fit
TPP2951	Human	1.88e6	5.67e-4	3.01e-10	0.05	Good Fit

Other Embodiments

All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each independent publication or patent application was specifically and individually indicated to be incorporated by reference.

It will be understood that the compositions and methods described herein are capable of further modification(s), and this description is intended to include any variations, uses or adaptations following, in general, the principles disclosed herein- including such departures from the present disclosure that come within known or customary practice within the art that may be applied to the essential features hereinbefore set forth, and follows in the scope of the claims.

Reference to any prior art in the specification is not an acknowledgement or suggestion that this prior art forms part of the common general knowledge in any jurisdiction or that this prior art could reasonably be expected to be combined with any other piece of prior art by a skilled person in the art.

CLAIMS

1. An isolated monovalent antibody or antibody fragment thereof comprising a VHH domain that specifically binds human properdin, wherein the antibody or antibody fragment thereof comprises 3 CDRs with the following sequences:
   a) a CDR-H1 comprising the amino acid sequence GRISSIIHMA (SEQ ID NO: 16);
   b) a CDR-H2 comprising the amino acid sequence RVGTTVYADSVKG (SEQ ID NO: 12); and
   c) a CDR-H3 comprising the amino acid sequence LQYEKHGGADY (SEQ ID NO: 17).

2. A pharmaceutical composition comprising the isolated antibody or antibody fragment thereof of claim 1, as an active ingredient, and a pharmaceutically acceptable carrier.

3. The antibody or antibody fragment thereof of claim 1, wherein the antibody or antibody fragment thereof is linked to a second monovalent antibody or antibody fragment thereof by a poly-glycine linker, wherein the poly-glycine linker comprises a GGGGE (SEQ ID NO: 64) sequence.

4. The antibody or antibody fragment thereof of claim 3, wherein the second monovalent antibody or antibody fragment thereof specifically binds albumin.

5. The antibody or antibody fragment thereof of claim 3 or 4, wherein the second monovalent antibody or antibody fragment thereof is linked to the N-terminus of the antibody or antibody fragment thereof that binds human properdin.

6. An antibody construct comprising a VHH domain that specifically binds human properdin and a VHH domain that specifically binds albumin, wherein the antibody construct comprises six CDR sequences of SEQ ID NO: 51.

7. An isolated antibody construct comprising a VHH domain that specifically binds human properdin and a VHH domain that specifically binds albumin, wherein the isolated antibody construct comprises the sequence:
EVQLVESGGGLVKPGGSLRLSCAASGRPVSNYAAAWFRQAPGKEREFVSAINWQKTATYADSV
KGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAAVFRVVAPKTQYDYDYWGQGTLVTVSSGGG
GEGGGGEGGGGEVQLLESGGGLVQPGGSLRLSCAASGRISSIIHMAWFRQAPGKERELVSEIS
RVGTTVYADSVKGRFTISRDNSKNTLYLQMNSLKPEDTAVYYCNALQYEKHGGADYWGQGTLV
TVSS (SEQ ID NO: 51).

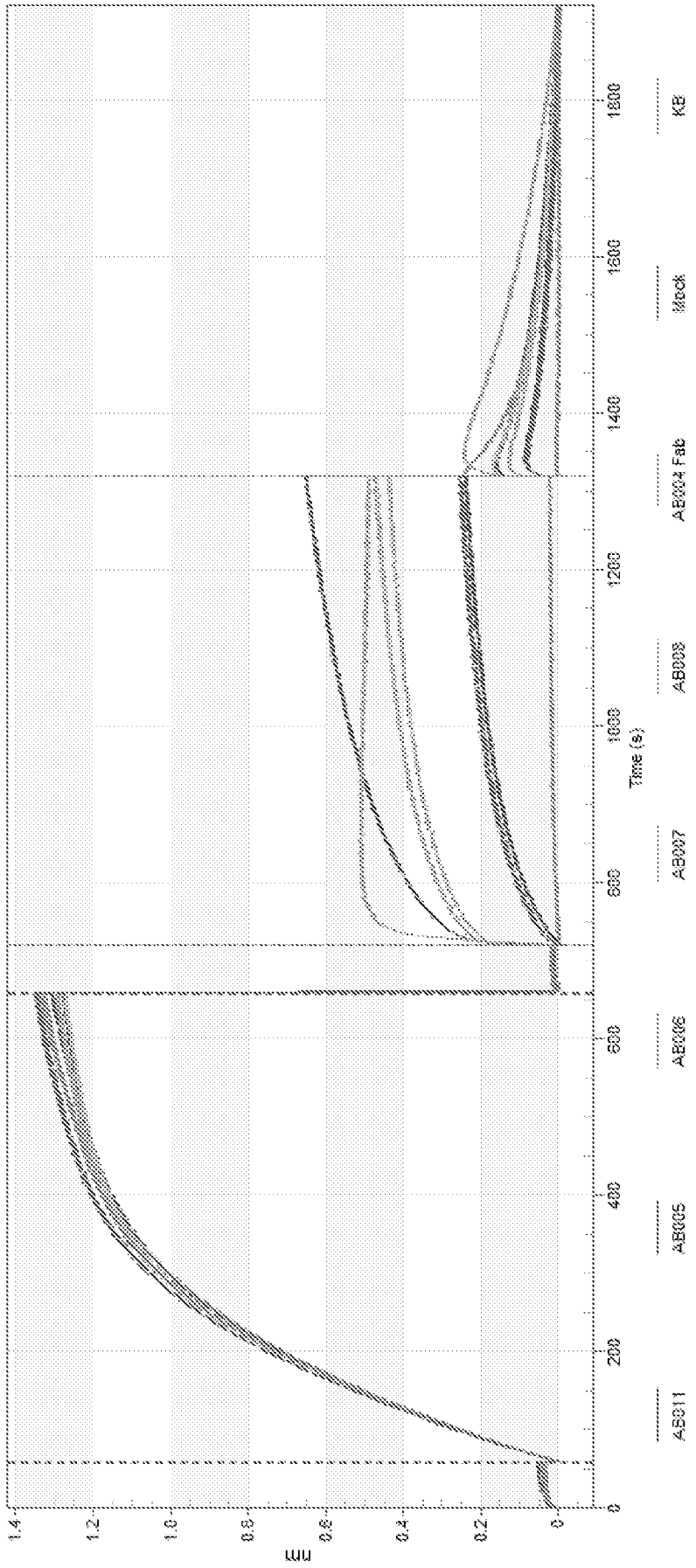

nm
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
0
200
400
600
800
1000
1200
1400
1600
1800
Time (s)
AB011
AB005
AB006
AB007
AB008
AB004 Fab
Mock
KB

FIG. 1

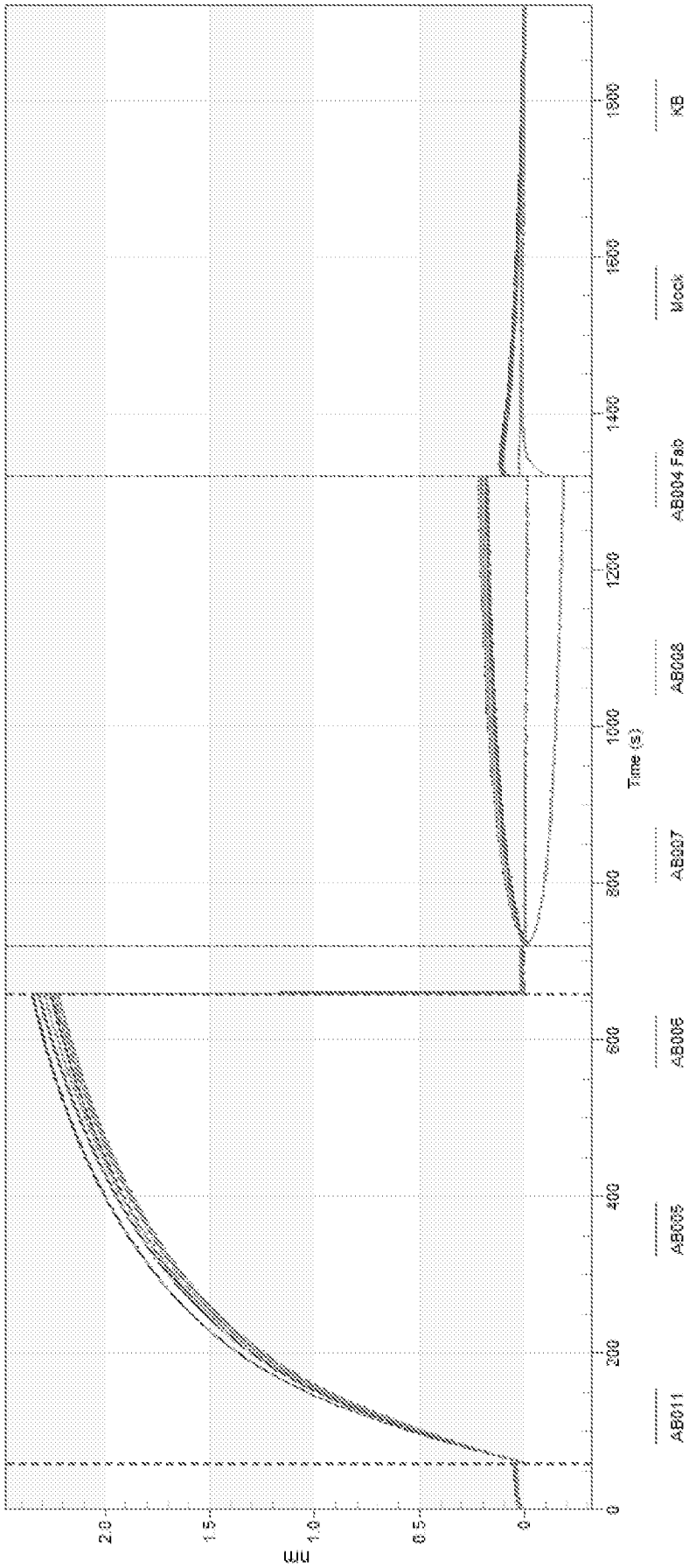

nm
2.0
1.5
1.0
0.5
0
0
200
400
600
800
1000
1200
1400
1600
1800
Time (s)
AB011
AB005
AB006
AB007
AB008
AB004 Fab
Mock
KB

FIG. 2

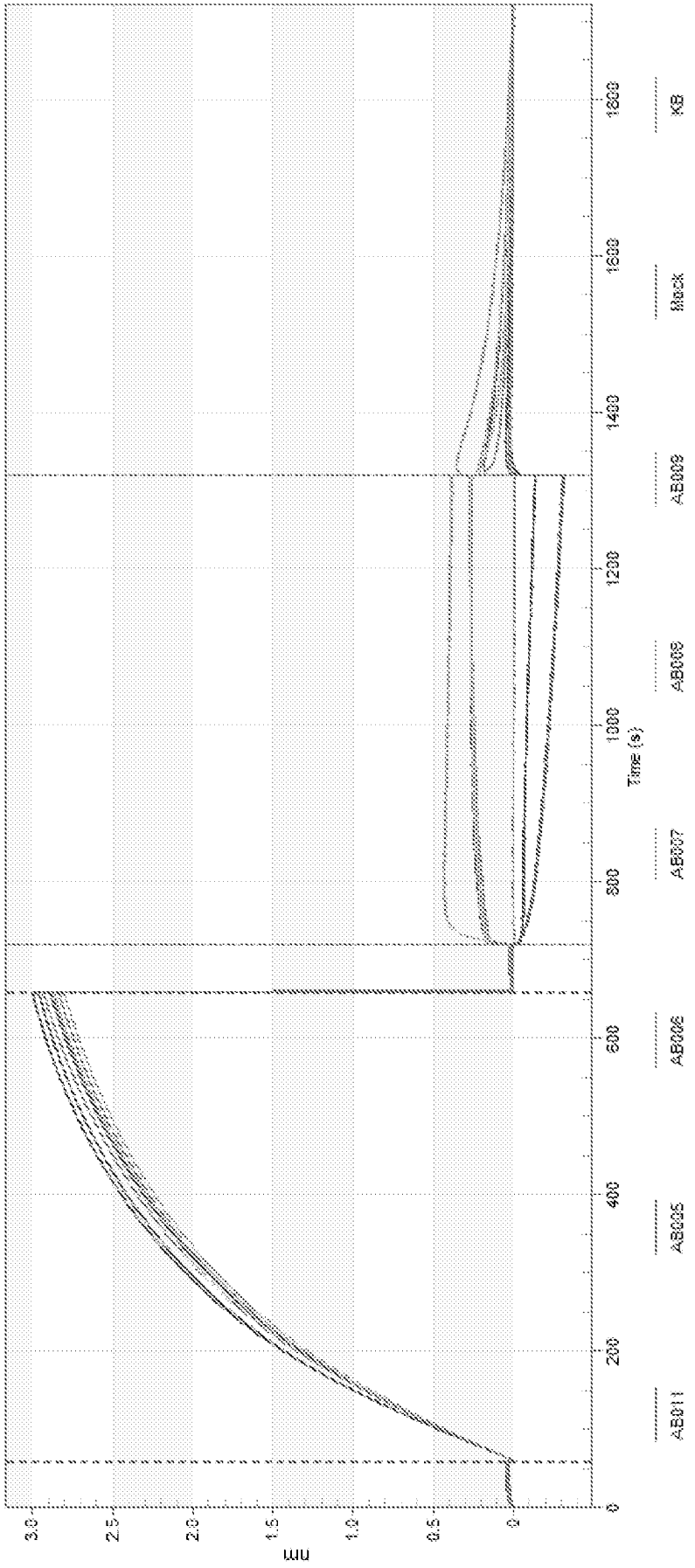

nm
3.0
2.5
2.0
1.5
1.0
0.5
0
0
200
400
600
800
1000
1200
1400
1600
1800
Time (s)
AB011
AB005
AB006
AB007
AB008
AB009
Mock
KB

FIG. 3

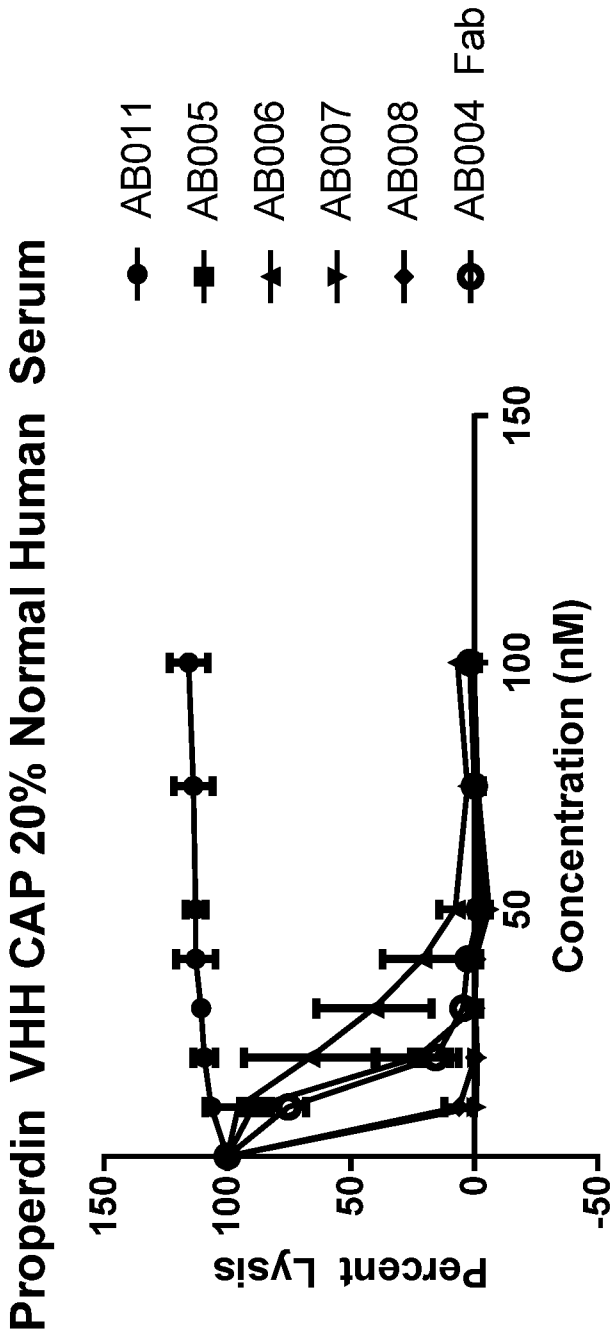

Properdin VHH CAP 20% Normal Human Serum
Percent Lysis
150
100
50
0
-50
50
100
150
Concentration (nM)
AB011
AB005
AB006
AB007
AB008
AB004 Fab

FIG. 4

FIG. 5C

Molecule	Theoretical	Experimental	
	MW (Da)	MW (Da)	ppm
AB007	13778.3	13777.0	-94.4
AB008	14068.6	14067.3	-92.4

FIG. 5A

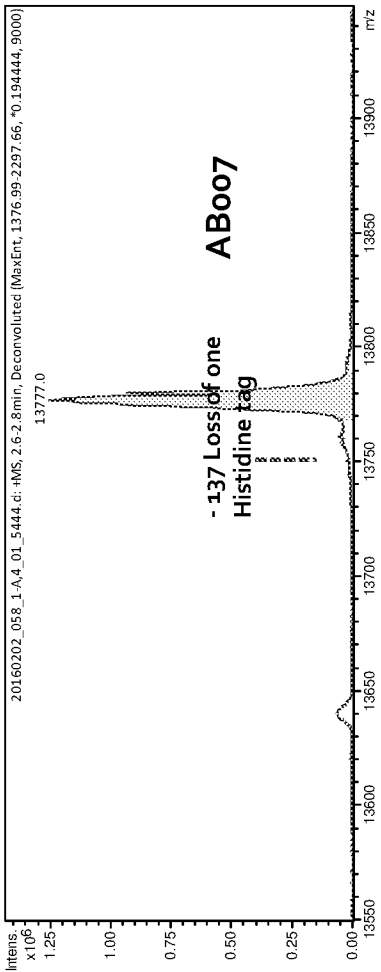


FIG. 5B

FIG. 6B

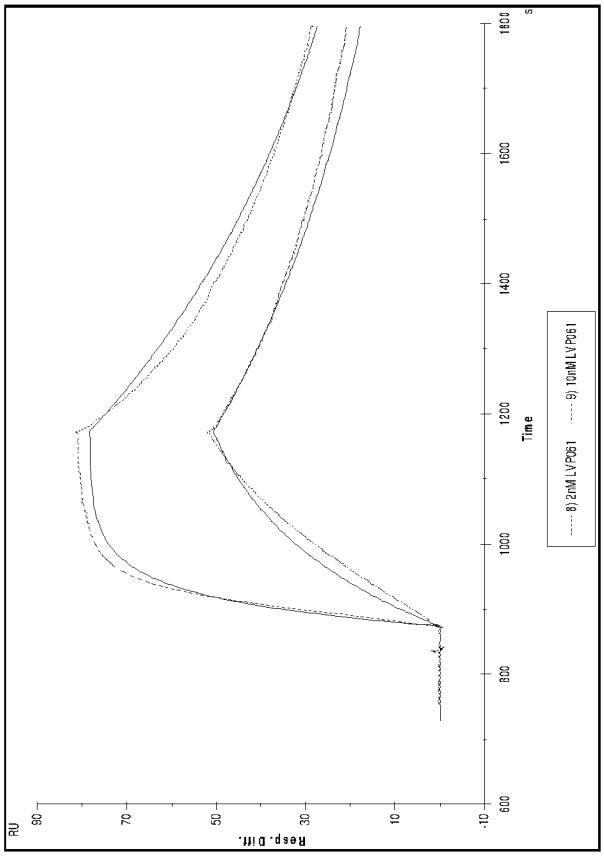
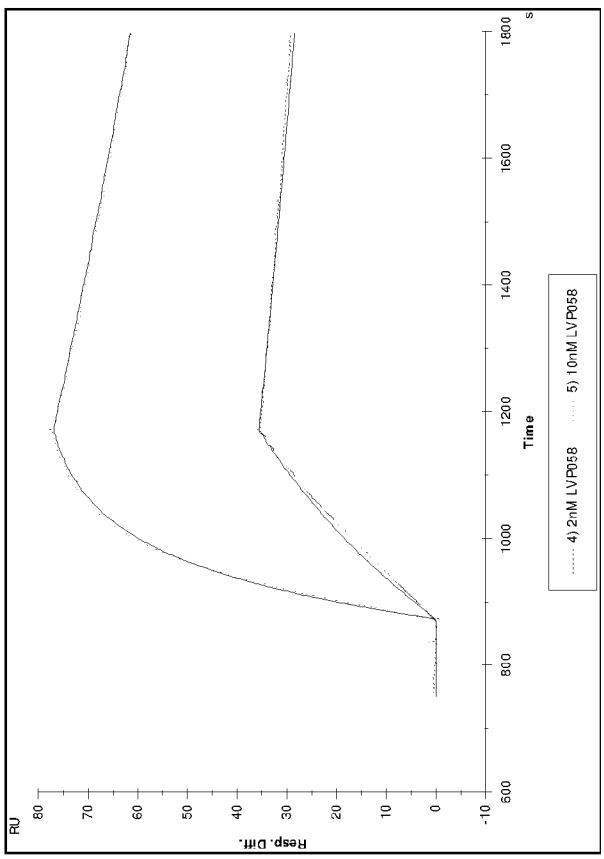

RU
Resp. Diff.
90
70
50
30
10
-10
600
800
1000
1200
1400
1600
1800
s
Time
8) 2nM LVP061
9) 10nM LVP061

FIG. 6A


RU
Resp. Diff.
80
70
60
50
40
30
20
10
0
-10
600
800
1000
1200
1400
1600
1800
s
Time
4) 2nM LVP058
5) 10nM LVP058

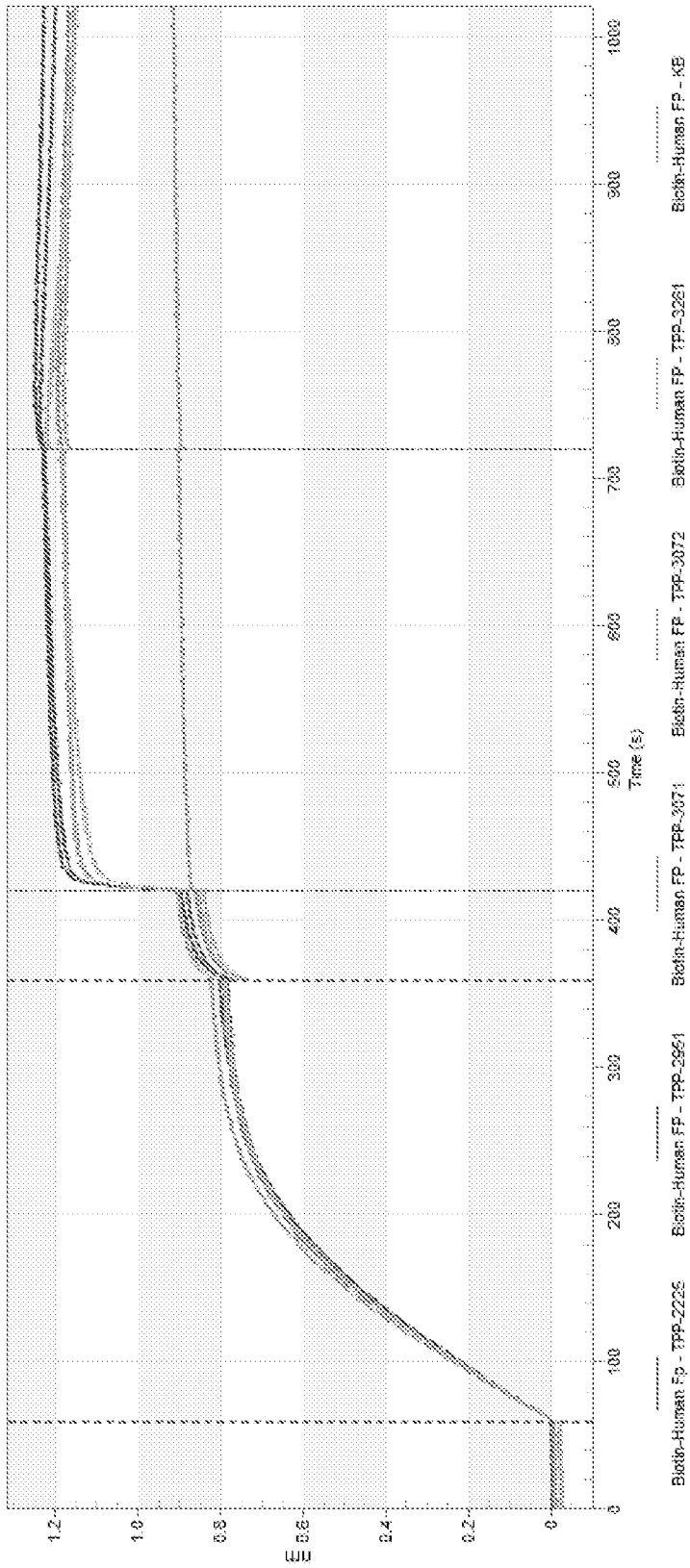

1.2
1.0
0.8
0.6
0.4
0.2
0
nm
0
100
200
300
400
500
600
700
800
900
1000
Time (s)
Biotin-Human Fp - TPP-2225
Biotin-Human FP - TPP-2641
Biotin-Human FP - TPP-3071
Biotin-Human FP - TPP-3372
Biotin-Human FP - TPP-3281
Biotin-Human FP - KB

FIG. 7

FIG. 8A

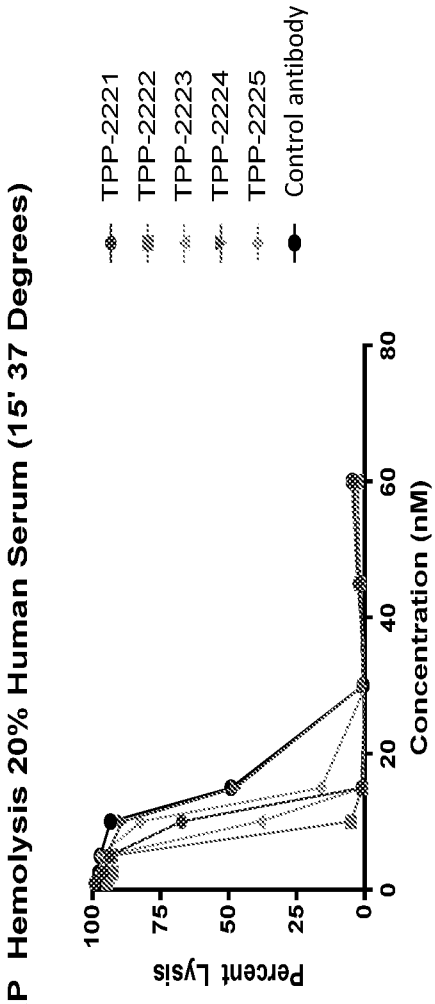

CAP Hemolysis 20% Human Serum (15' 37 Degrees)
Percent Lysis
100
75
50
25
0
0
20
40
60
80
Concentration (nM)
TPP-2221
TPP-2222
TPP-2223
TPP-2224
TPP-2225
Control antibody

FIG. 8B

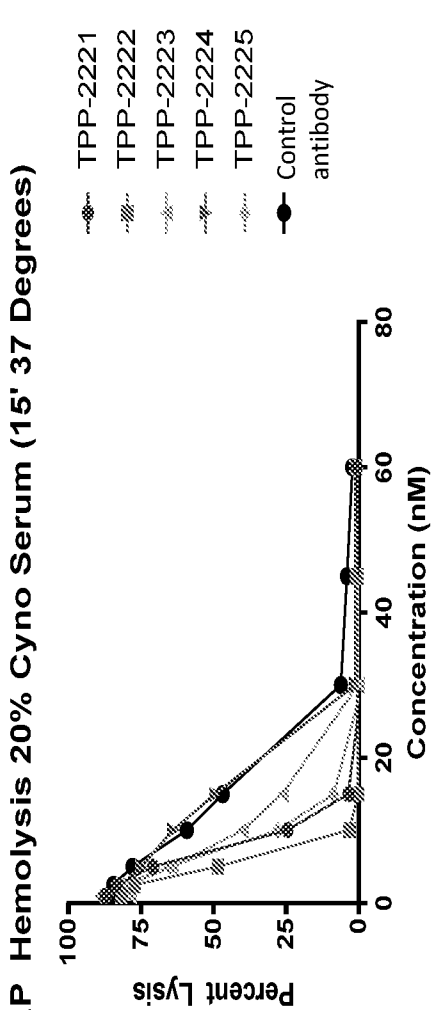

CAP Hemolysis 20% Cyno Serum (15' 37 Degrees)
Percent Lysis
100
75
50
25
0
0
20
40
60
80
Concentration (nM)
TPP-2221
TPP-2222
TPP-2223
TPP-2224
TPP-2225
Control antibody

FIG. 9A

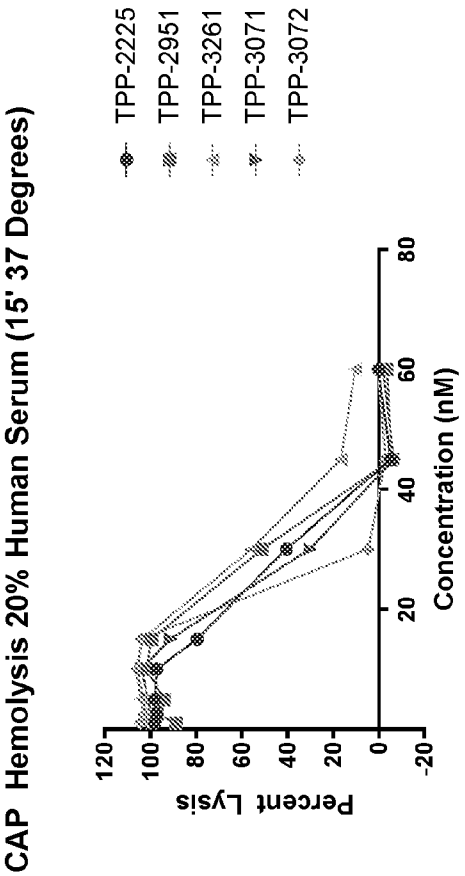
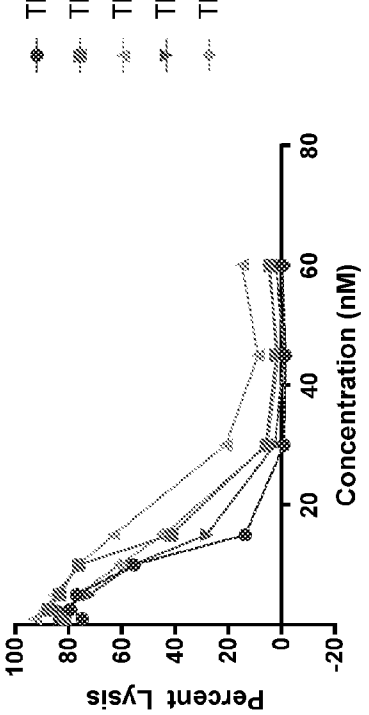

CAP Hemolysis 20% Human Serum (15' 37 Degrees)
Percent Lysis
120
100
80
60
40
20
0
-20
20
40
60
80
Concentration (nM)
TPP-2225
TPP-2951
TPP-3261
TPP-3071
TPP-3072

FIG. 9B


CAP Hemolysis 20% Cyno Serum (15' 37 Degrees)
Percent Lysis
100
80
60
40
20
0
-20
20
40
60
80
Concentration (nM)
TPP-2225
TPP-2951
TPP-3261
TPP-3071
TPP-3072

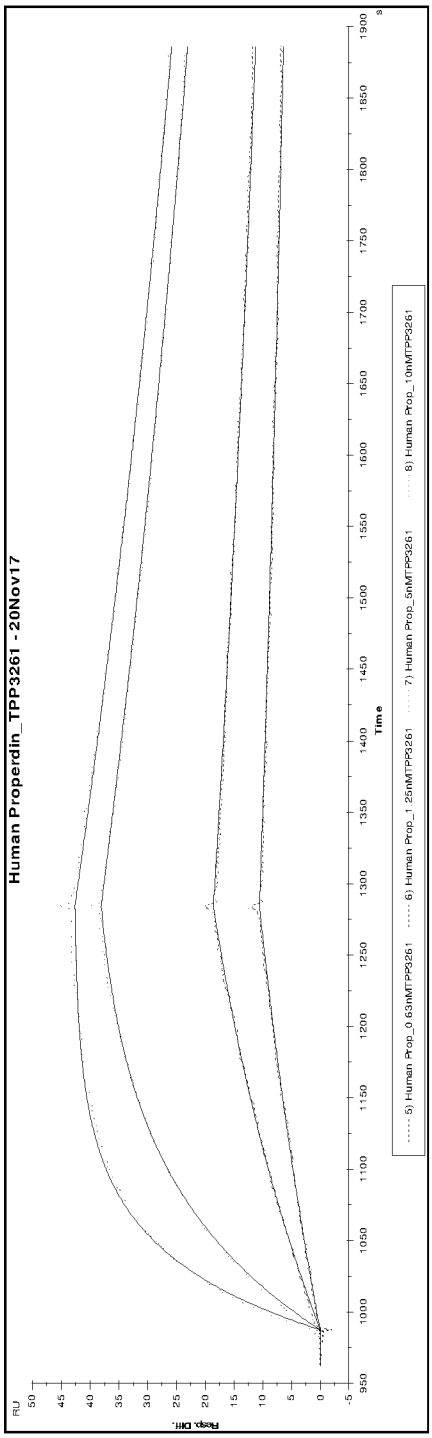


FIG. 10A

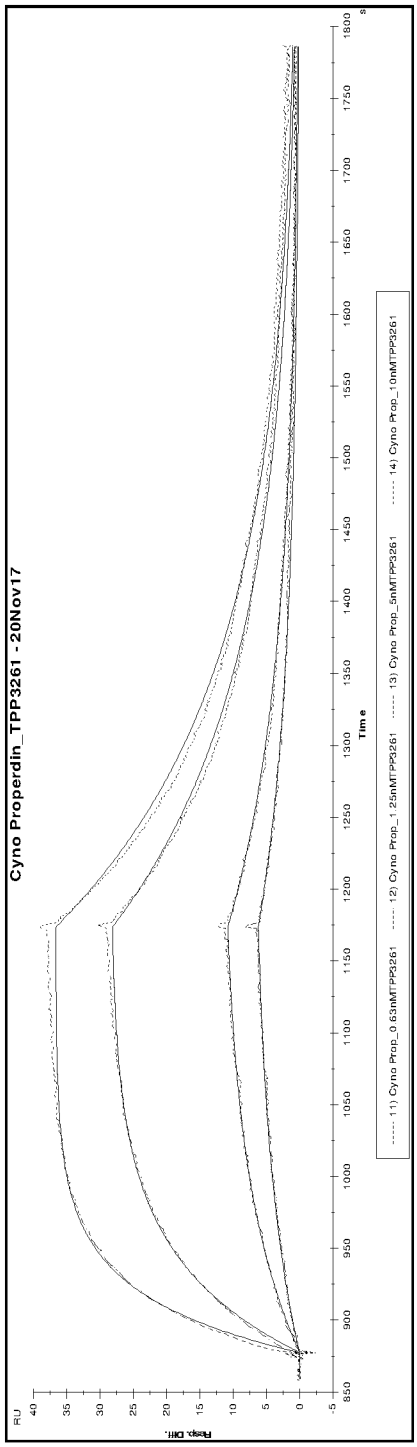


FIG. 10B

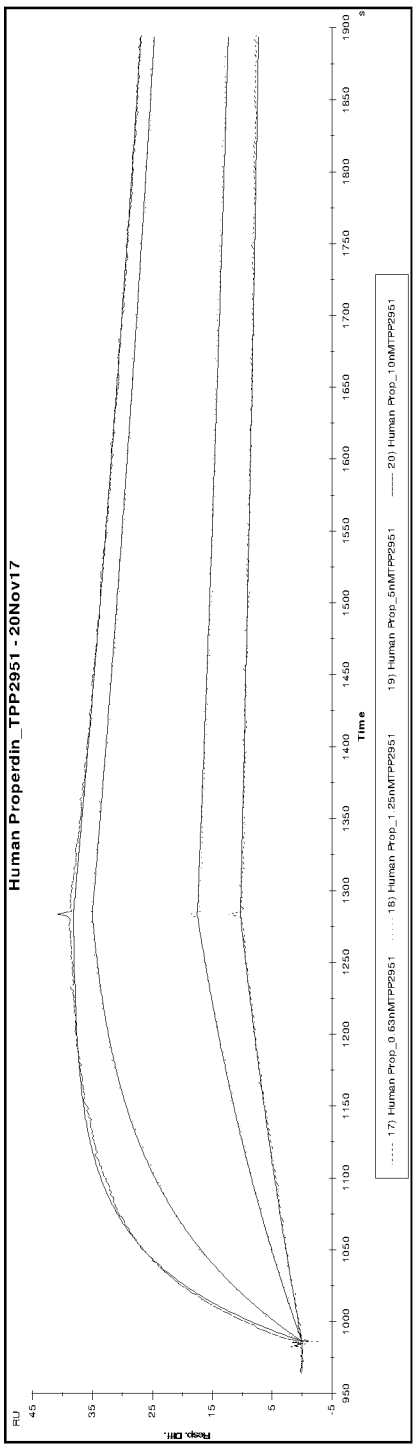


FIG. 11A

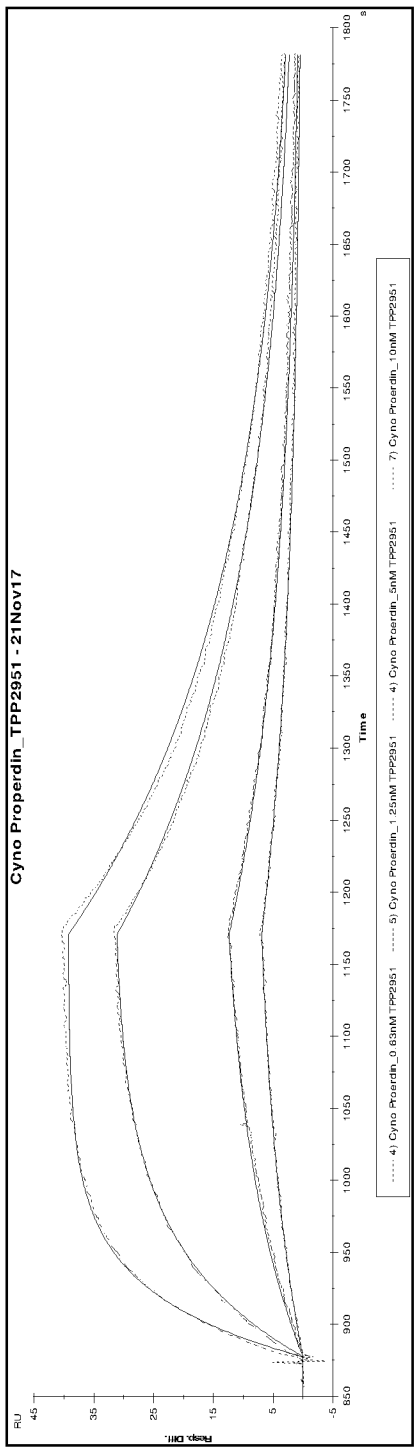


FIG. 11B

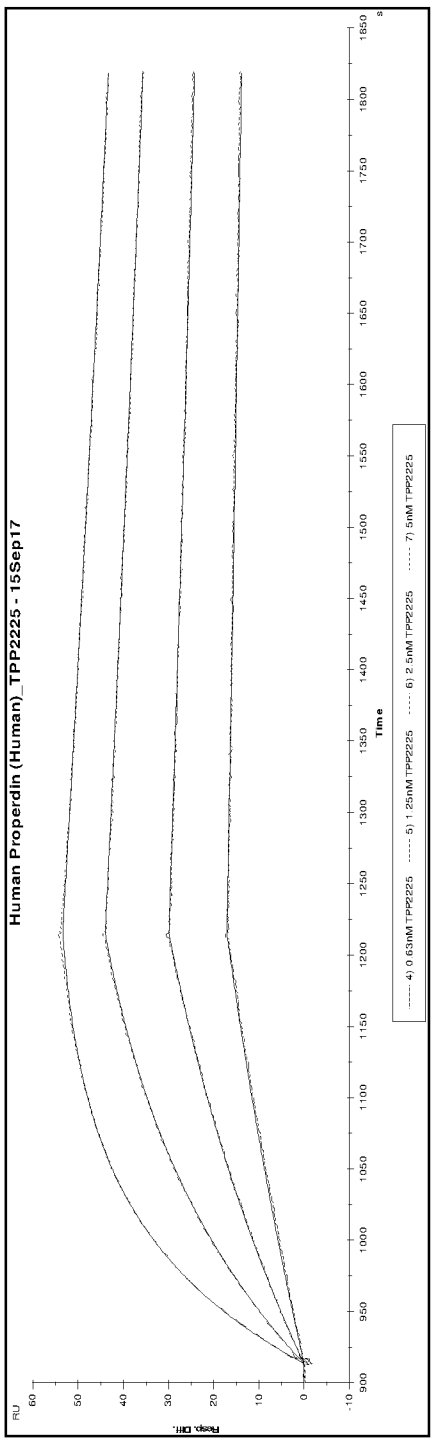


FIG. 12A

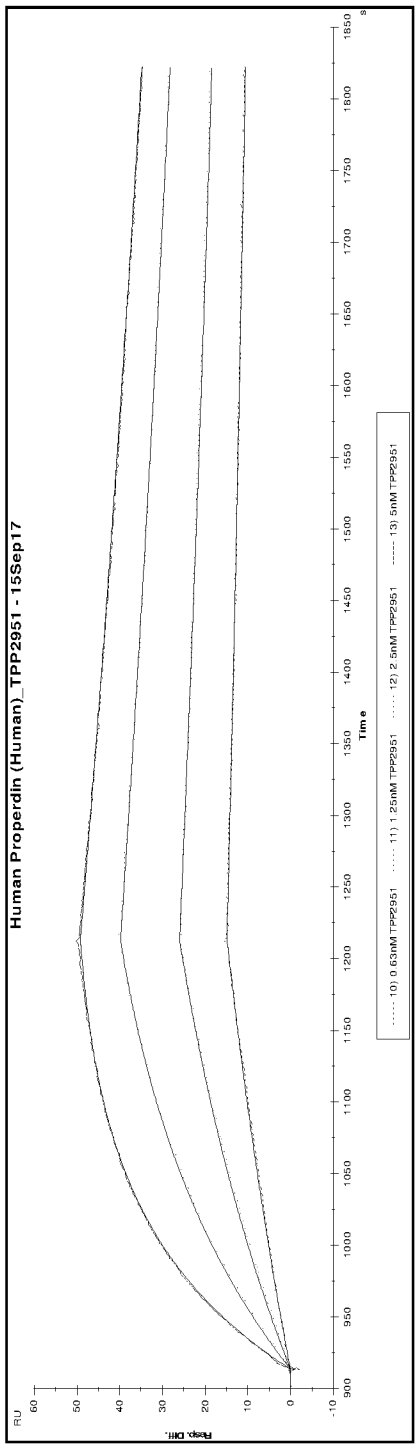


FIG. 12B