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Feng et al.

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(54) **TETRA-SPECIFIC, OCTAMERIC BINDING AGENTS AND ANTIBODIES AGAINST *CLOSTRIDIUM DIFFICILE* TOXIN A AND TOXIN B FOR TREATMENT OF *C. DIFFICILE* INFECTION**

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CPC ***C07K 16/1282*** (2013.01); ***A61P 31/04*** (2018.01); *A61K 2039/505* (2013.01); *C07K 2317/24* (2013.01); *C07K 2317/30* (2013.01); *C07K 2317/31* (2013.01); *C07K 2317/35* (2013.01); *C07K 2317/52* (2013.01); *C07K 2317/524* (2013.01); *C07K 2317/526* (2013.01); *C07K 2317/569* (2013.01); *C07K 2317/60* (2013.01); *C07K 2317/76* (2013.01); *C07K 2317/92* (2013.01); *C07K 2317/94* (2013.01); *C07K 2319/00* (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

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Primary Examiner — Oluwatosin A Ogunbiyi
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(57) **ABSTRACT**

Novel, antibody-based binding agents derived from human and camelid immunoglobulins are described. These binding agents recognize and bind with specificity to *Clostridium difficile* toxin A and/or toxin B and in some cases exhibit toxin neutralizing activity. These binding agents can be used to treat or prevent primary and recurrent CDI. The binding agents include camelid V_HH peptide monomers, linked groups of V_HH peptide monomers, V_HH peptide monomers joined to antibody Fc domains, and V_HH peptide monomers joined to IgG antibodies.

12 Claims, 14 Drawing Sheets

Specification includes a Sequence Listing.

Figure 1

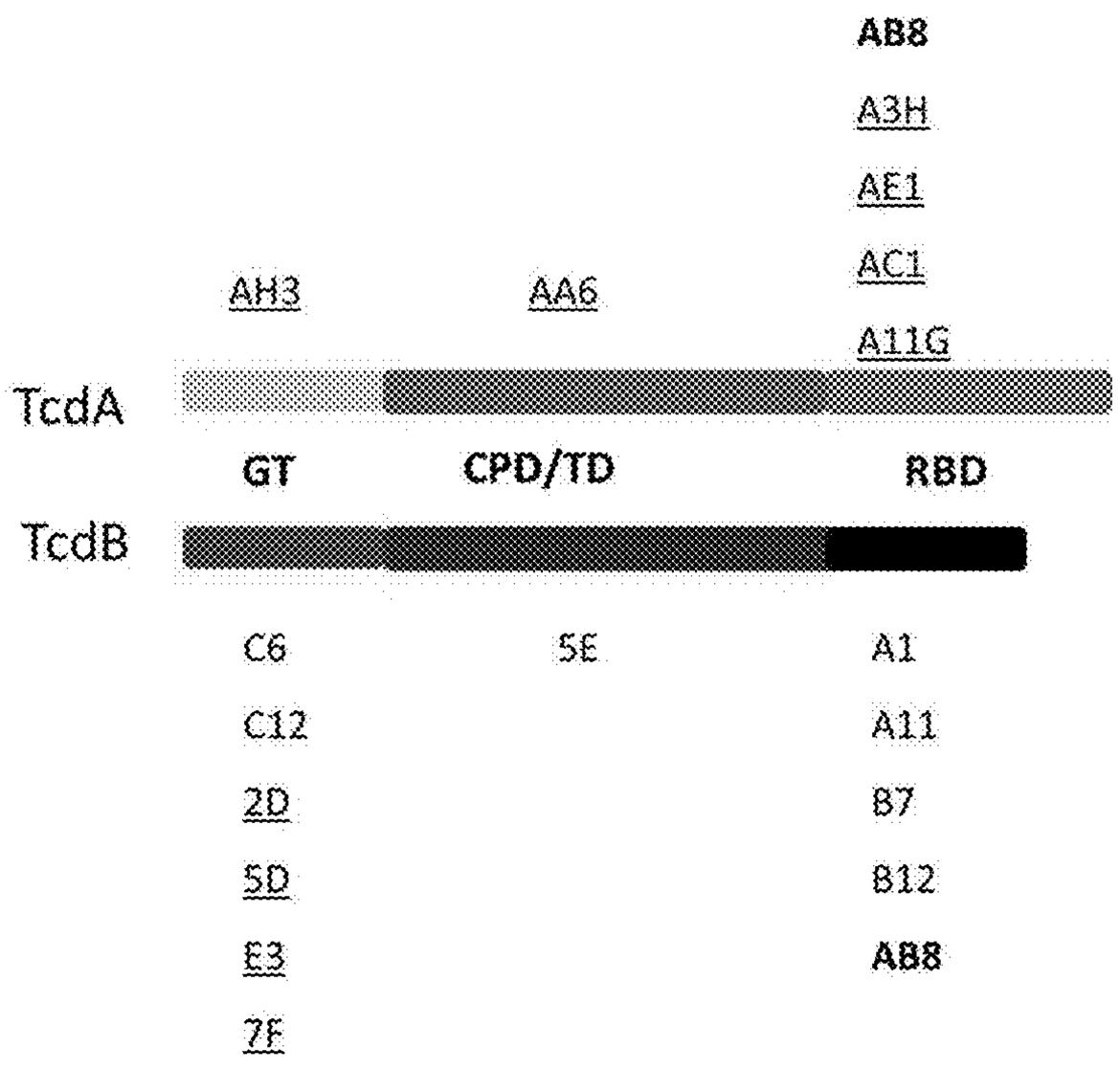

AB8
A3H
AE1
AC1
A11G
AH3
AA6
TcdA
GT
CPD/TD
RBD
TcdB
C6
5E
A1
C12
A11
2D
B7
5D
B12
E3
A88
7F

Figure 2

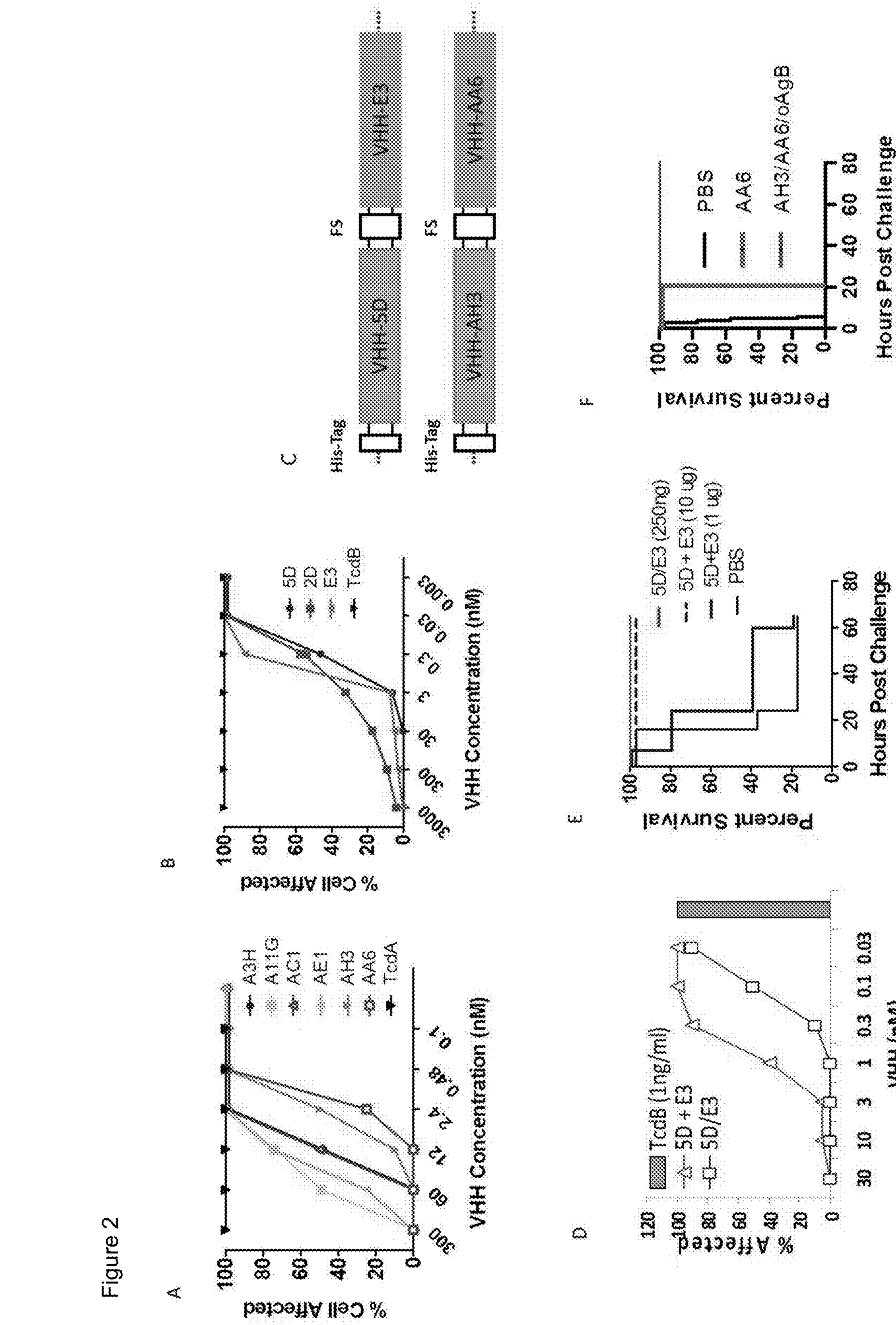

A
% Cell Affected
VHH Concentration (nM)
A3H
A11G
AC1
AE1
AH3
AA6
TcdA
B
% Cell Affected
VHH Concentration (nM)
5D
2D
E3
TcdB
C
His-Tag
VHH-5D
VHH-AH3
His-Tag
VHH-E3
VHH-AA6
FS
D
TcdB (1ng/ml)
5D + E3
5D/E3
% Affected
VHH (nM)
E
Percent Survival
Hours Post Challenge
5D/E3 (250mg)
5D + E3 (10 ug)
5D+E3 (1 ug)
PBS
F
Percent Survival
Hours Post Challenge
PBS
AA6
AH3/AA6/oAgB

Figure 3

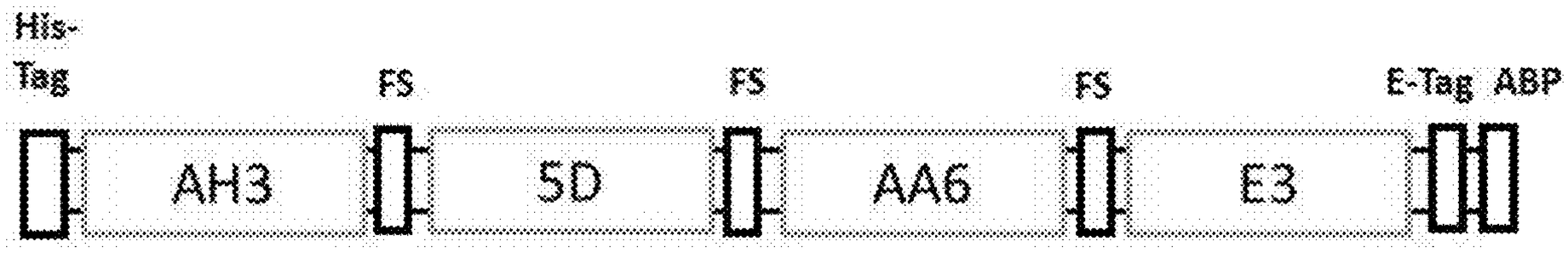


Figure 4A

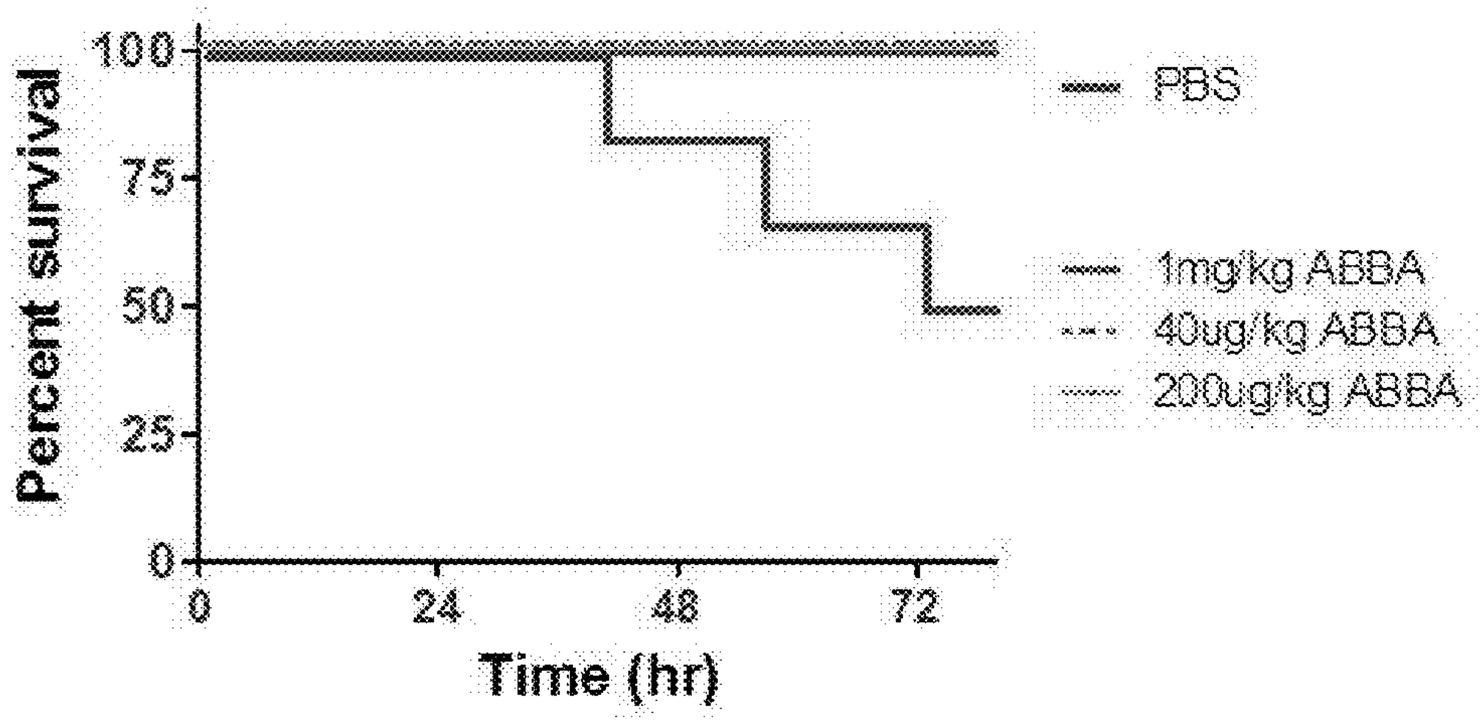


Figure 4B

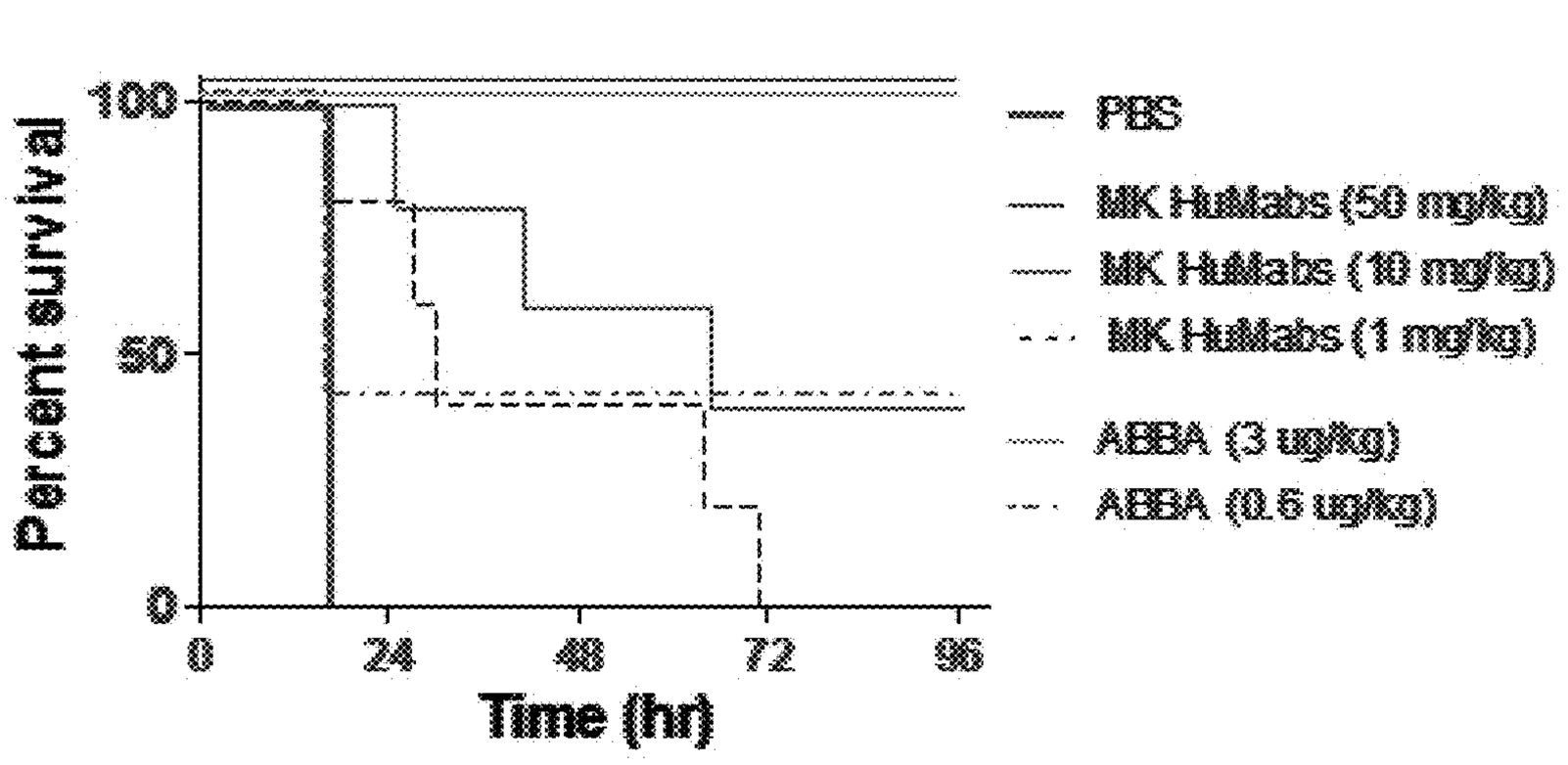


Figure 5

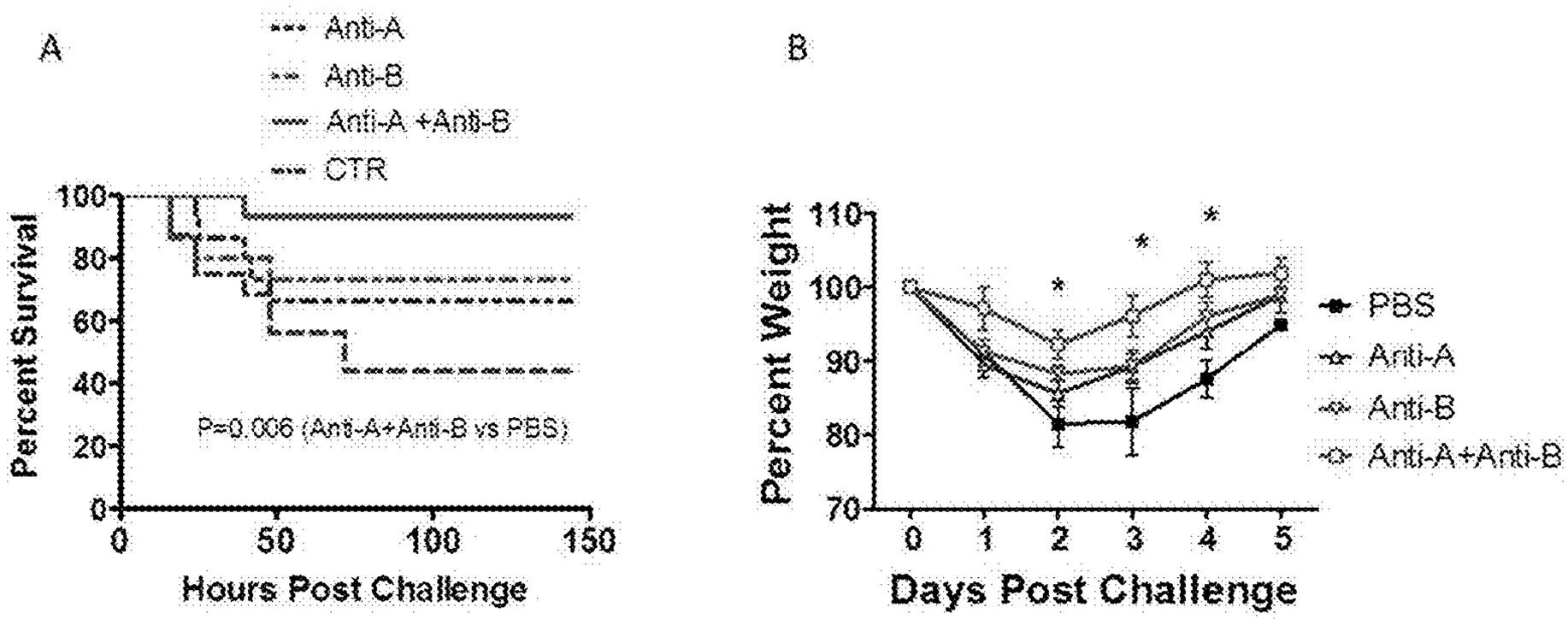


Figure 6

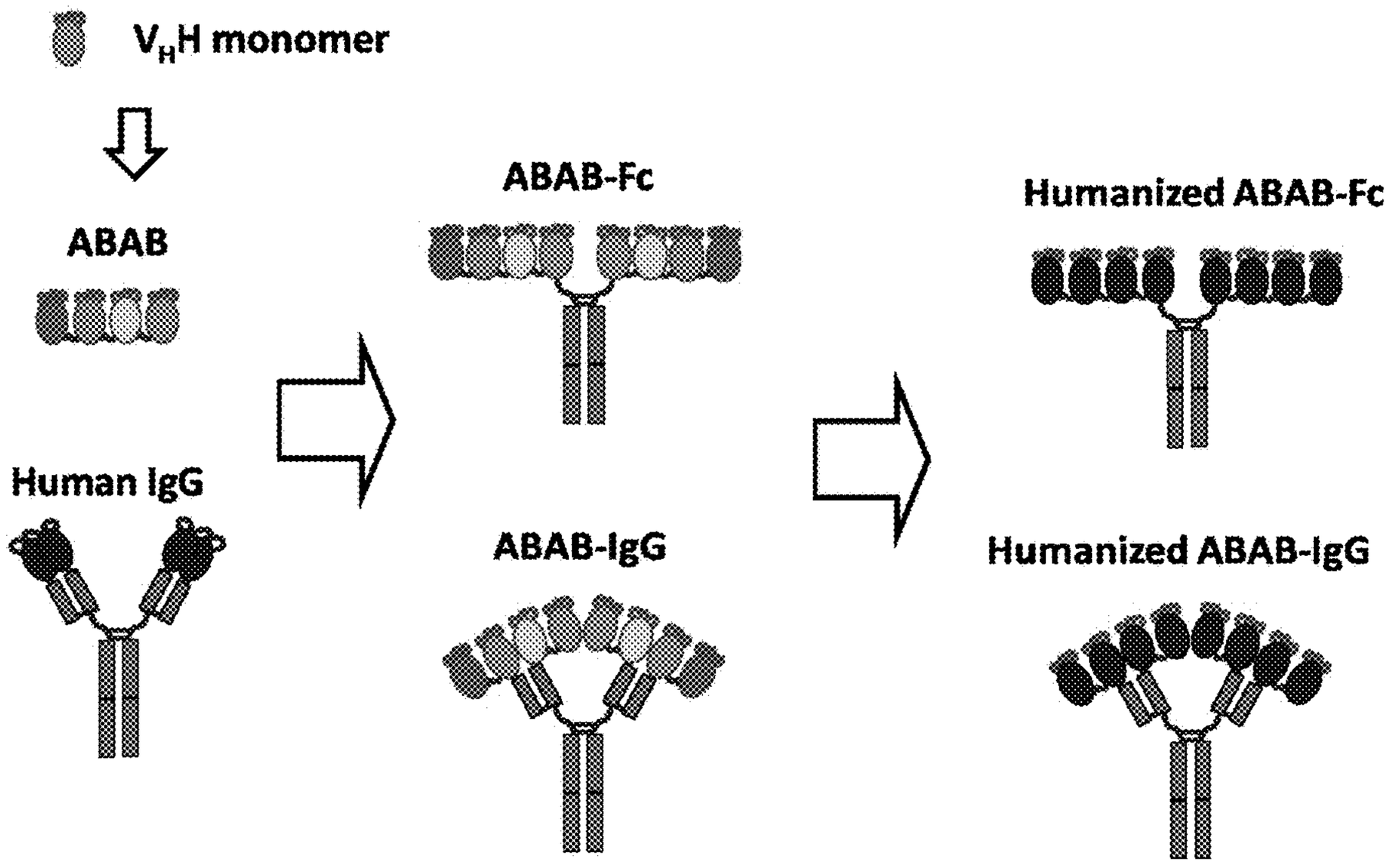


Figure 7

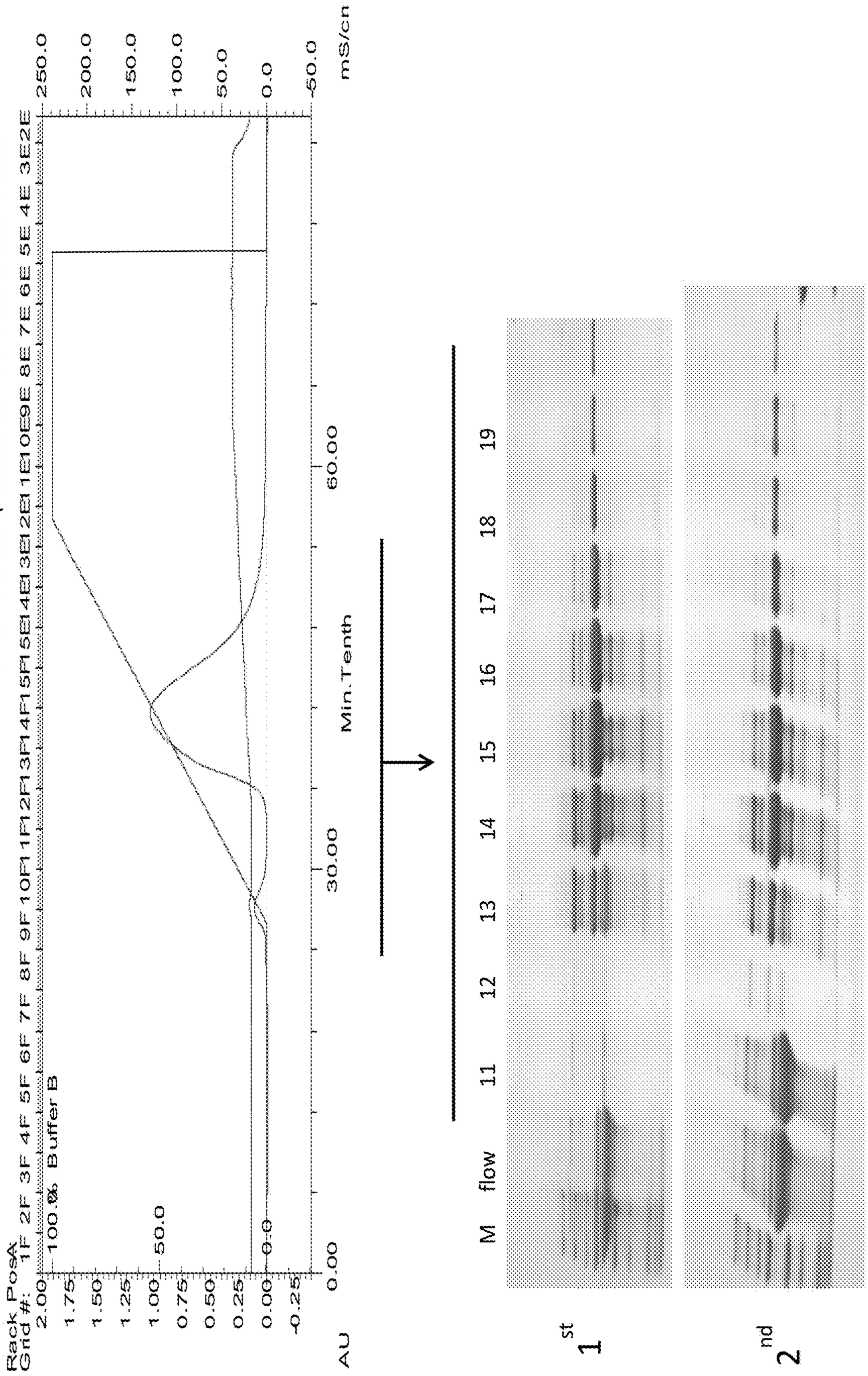

Fractions Fc-ABBA purification from 2 liter culture medium
mS/cm
250.0
200.0
150.0
100.0
50.0
0.0
-50.0
Min.Tenth
30.00
60.00
Rack PosA
Grid #: 1F 2F 3F 4F 5F 6F 7F 8F 9F 10F1 1F1 2F1 3F1 4F1 5F1 5E1 4E1 3E1 2E1 1E1 0E9E 8E 7E 6E 5E 4E 3E2E
A
B
100.0% Buffer B
50.0
0.0
AU
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-0.25
0.00
M flow 11 12 13 14 15 16 17 18 19
1st
2nd

Figure 8

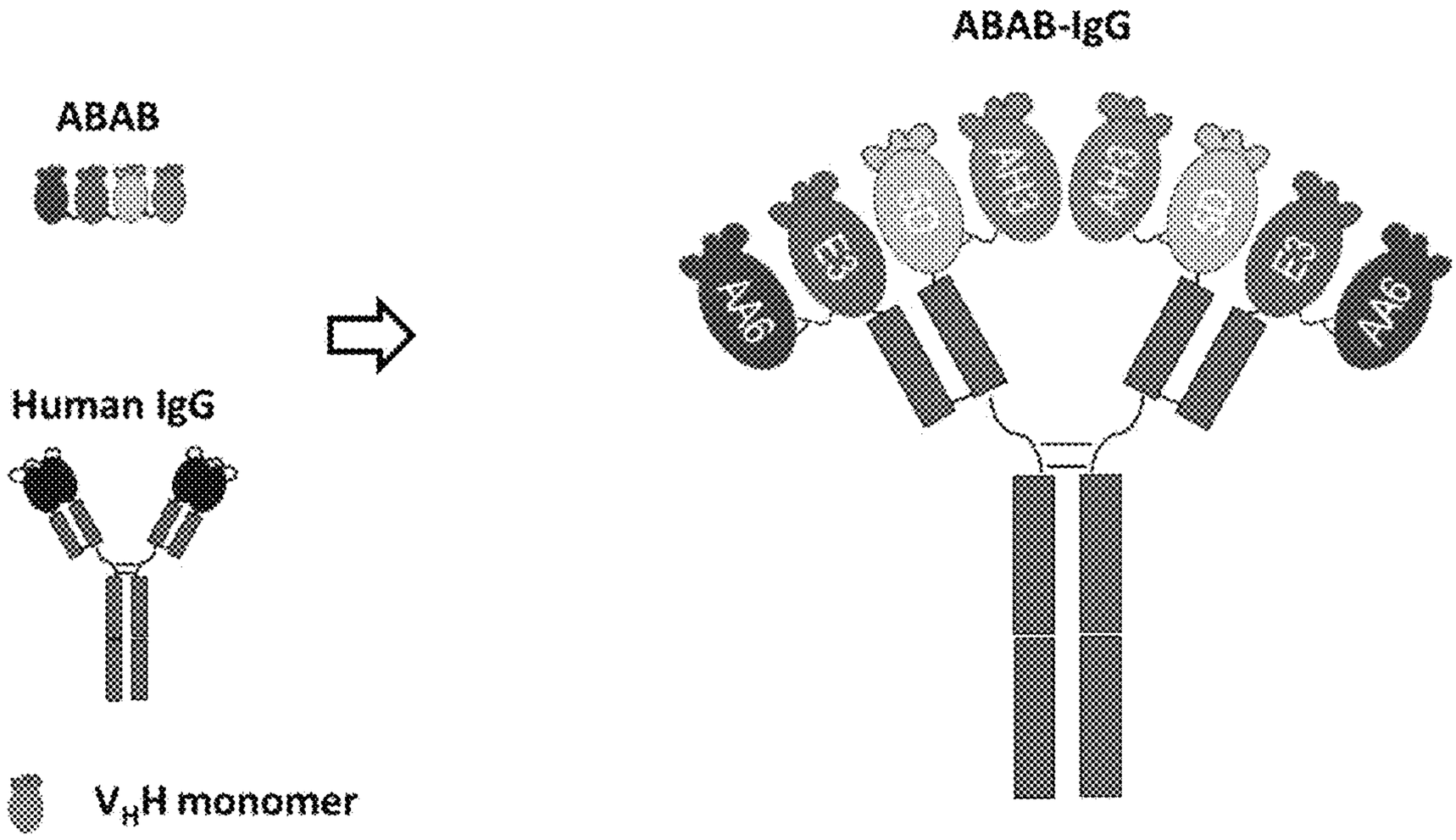

ABAB
Human IgG
V_HH monomer
CDRs
ABAB-IgG
Current product
IgG-ABAB half life: 5-7 days;
ABBA half life: 7.8h

Figure 9

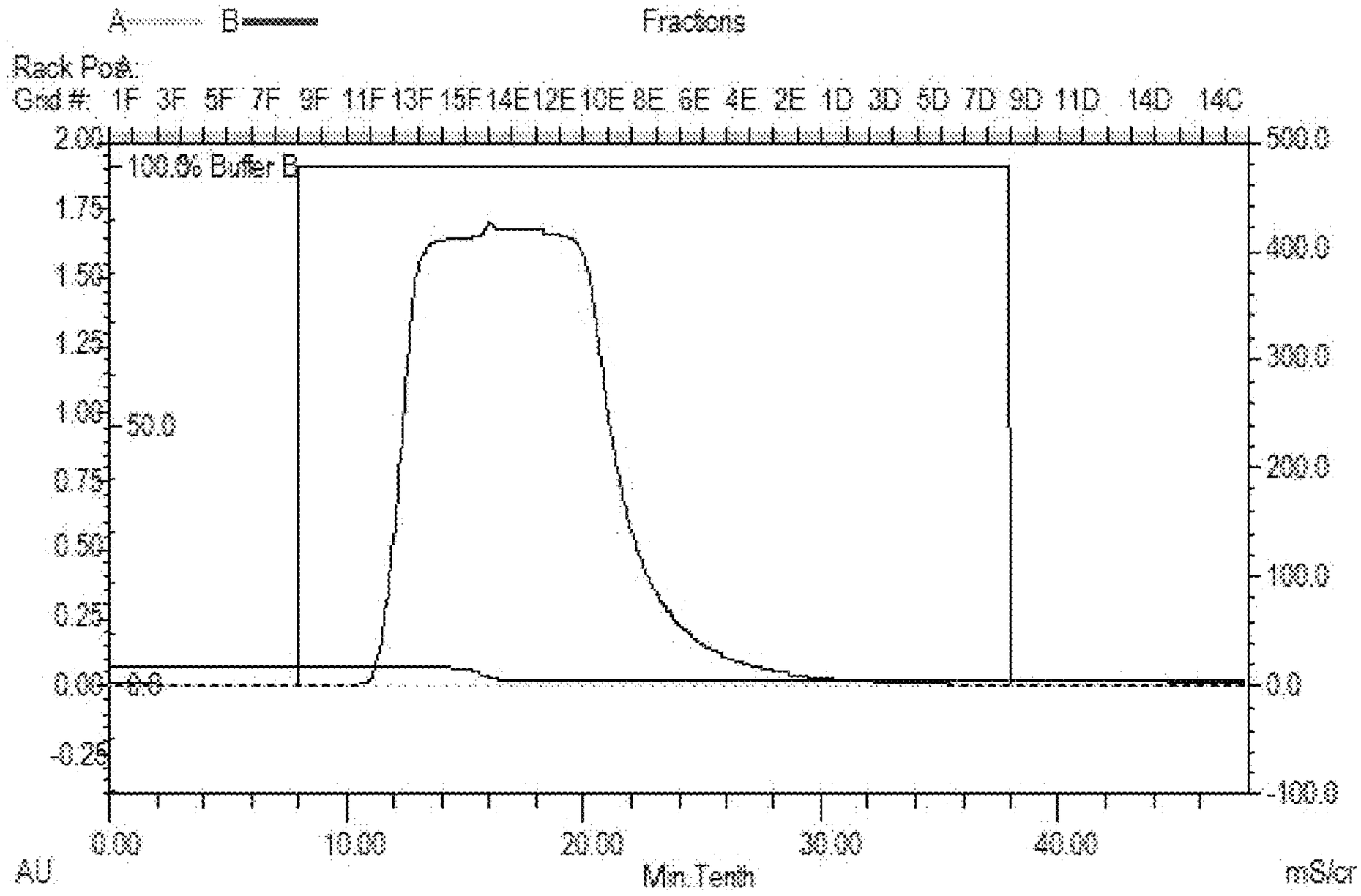

A
B
Fractions
Rack Pos:
Grid #: 1F 3F 5F 7F 9F 11F 13F 15F 14E 12E 10E 8E 6E 4E 2E 1D 3D 5D 7D 9D 11D 14D 14C
100.0% Buffer B
50.0
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-0.25
500.0
400.0
300.0
200.0
100.0
0.0
-100.0
0.00
10.00
20.00
30.00
40.00
AU
Min.Tenth
mS/cr

Figure 10

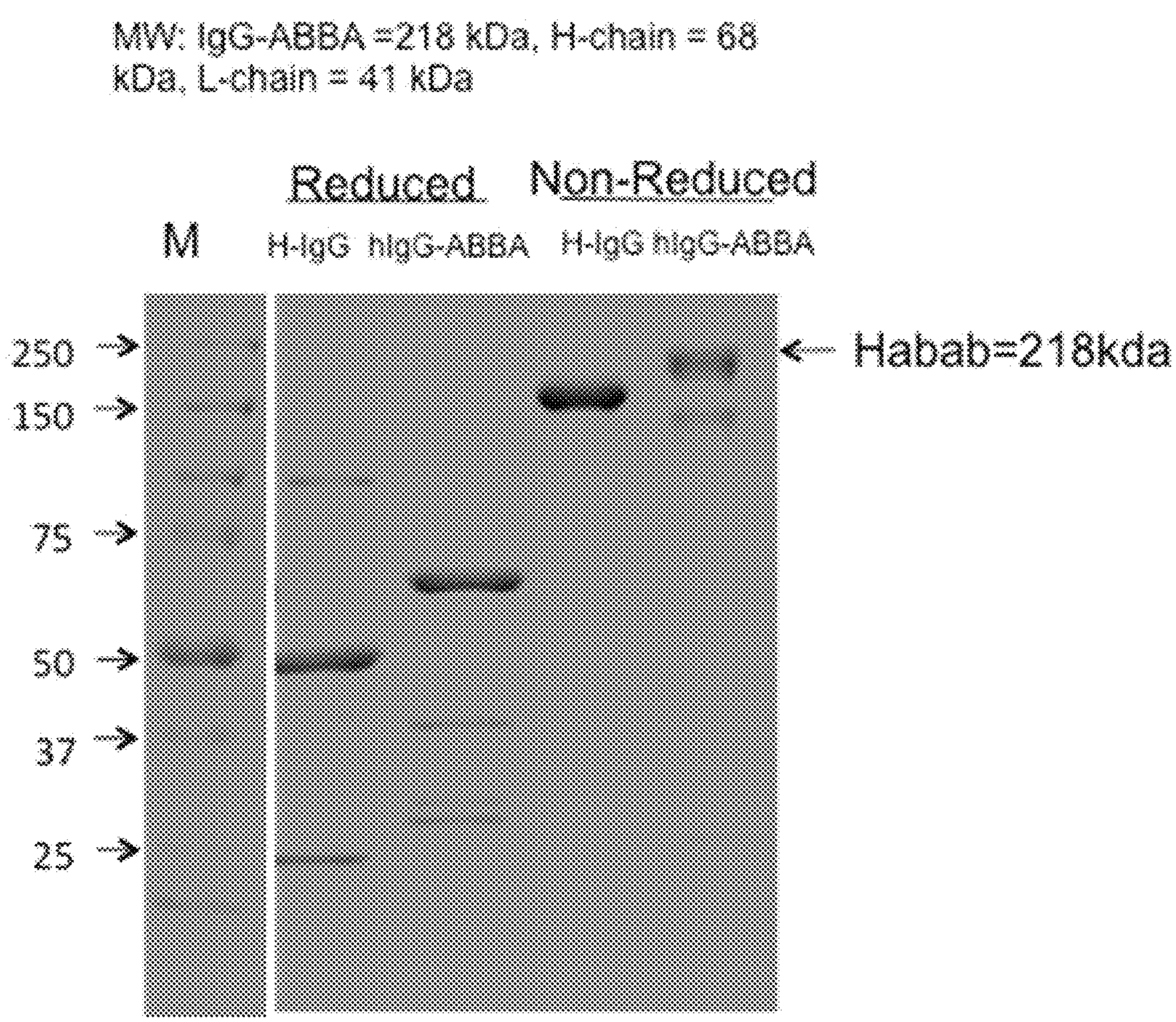

MW: IgG-ABBA =218 kDa, H-chain = 68 kDa, L-chain = 41 kDa
Reduced
Non-Reduced
M
H-IgG
hIgG-ABBA
H-IgG
hIgG-ABBA
250
150
75
50
37
25
Hababb=218kda

Figure 11A

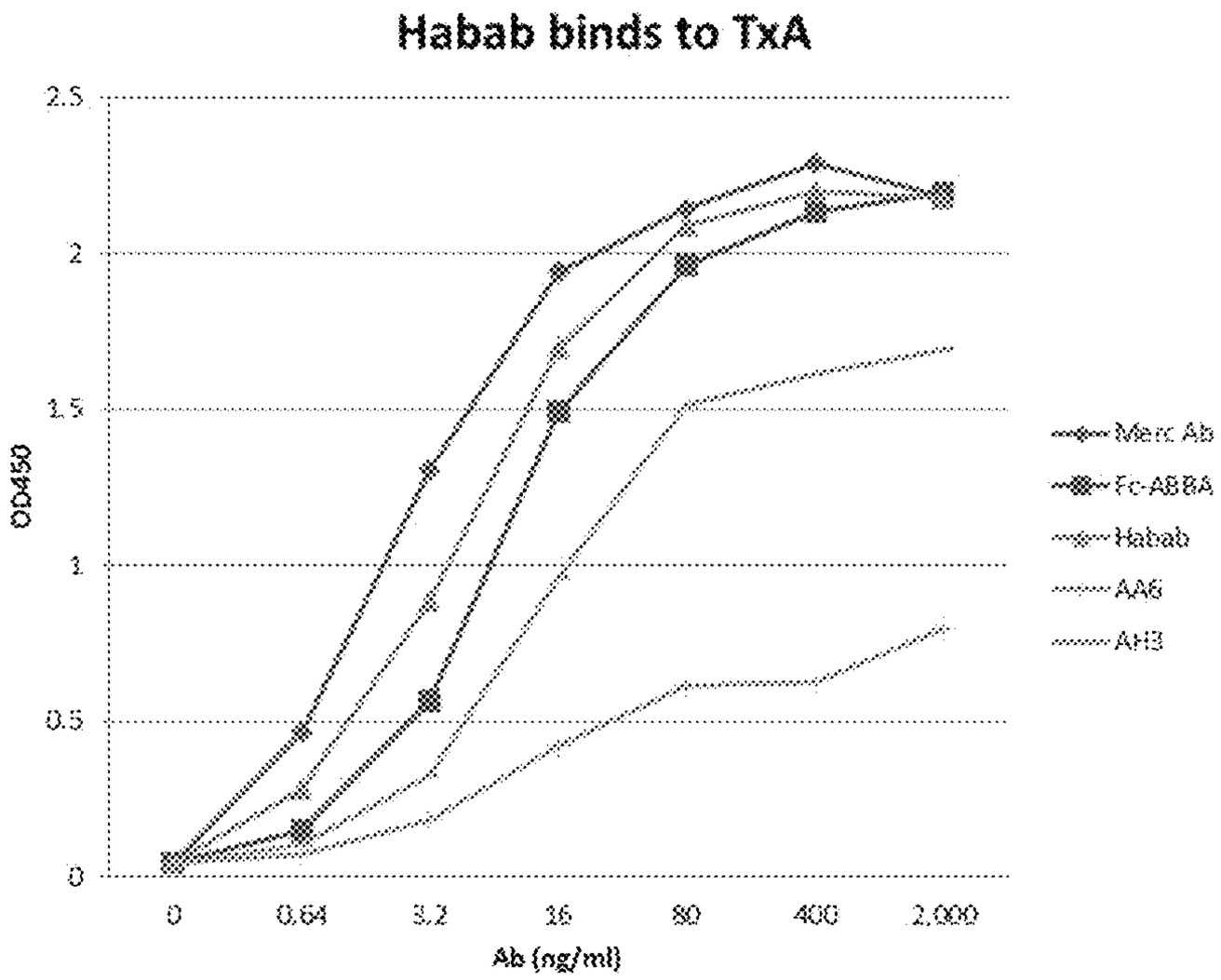


Figure 11B

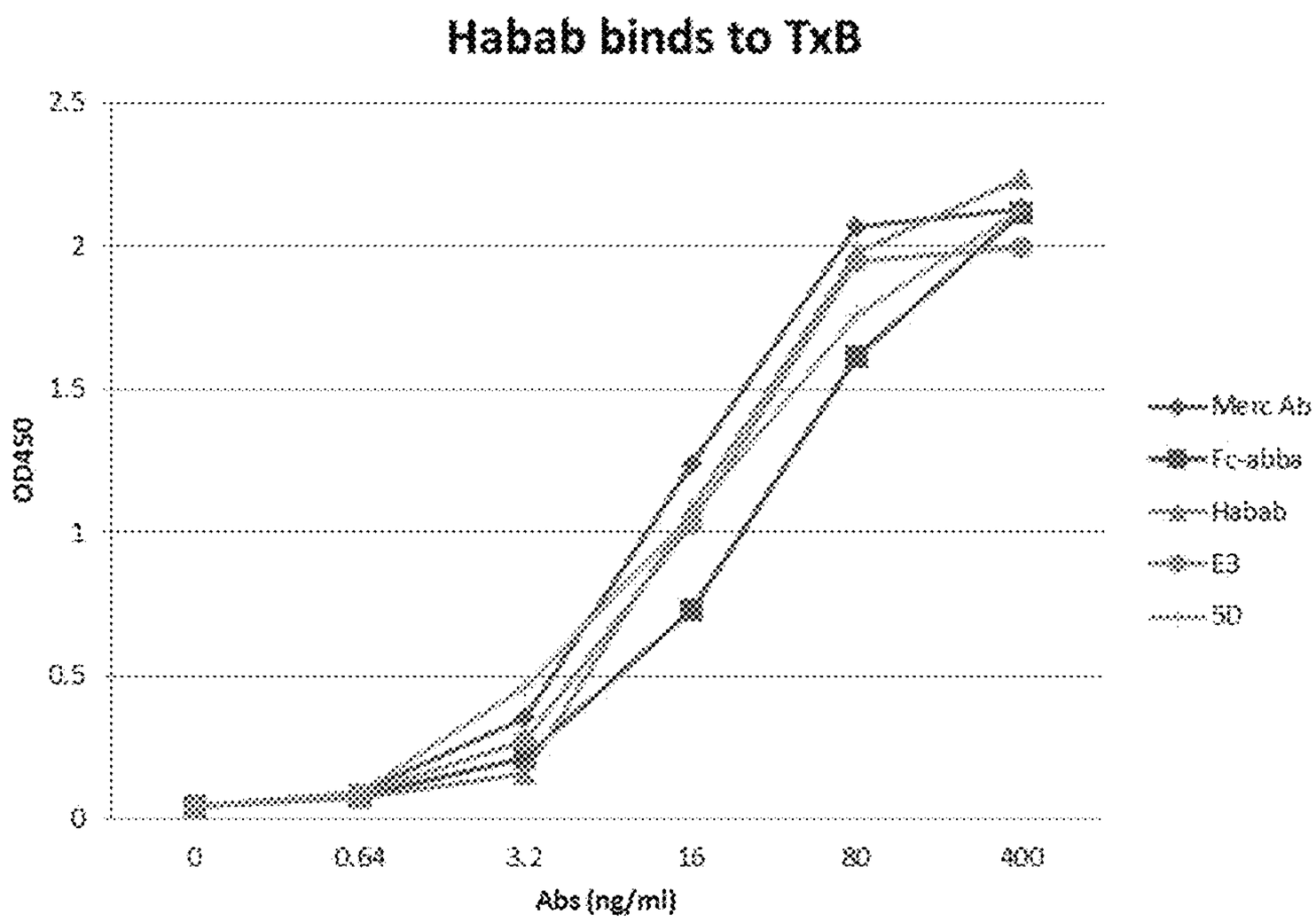


Figure 12A

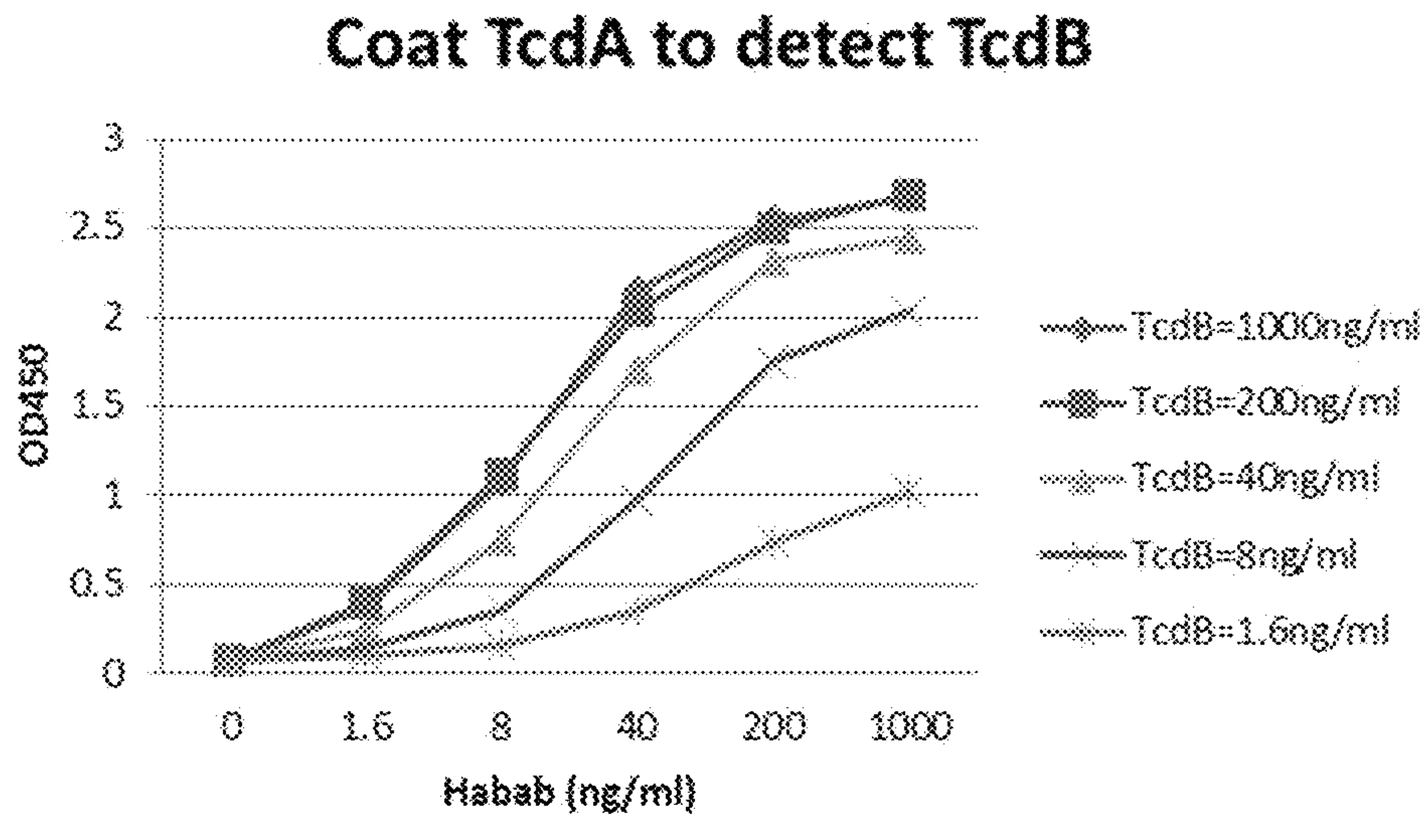


Figure 12B

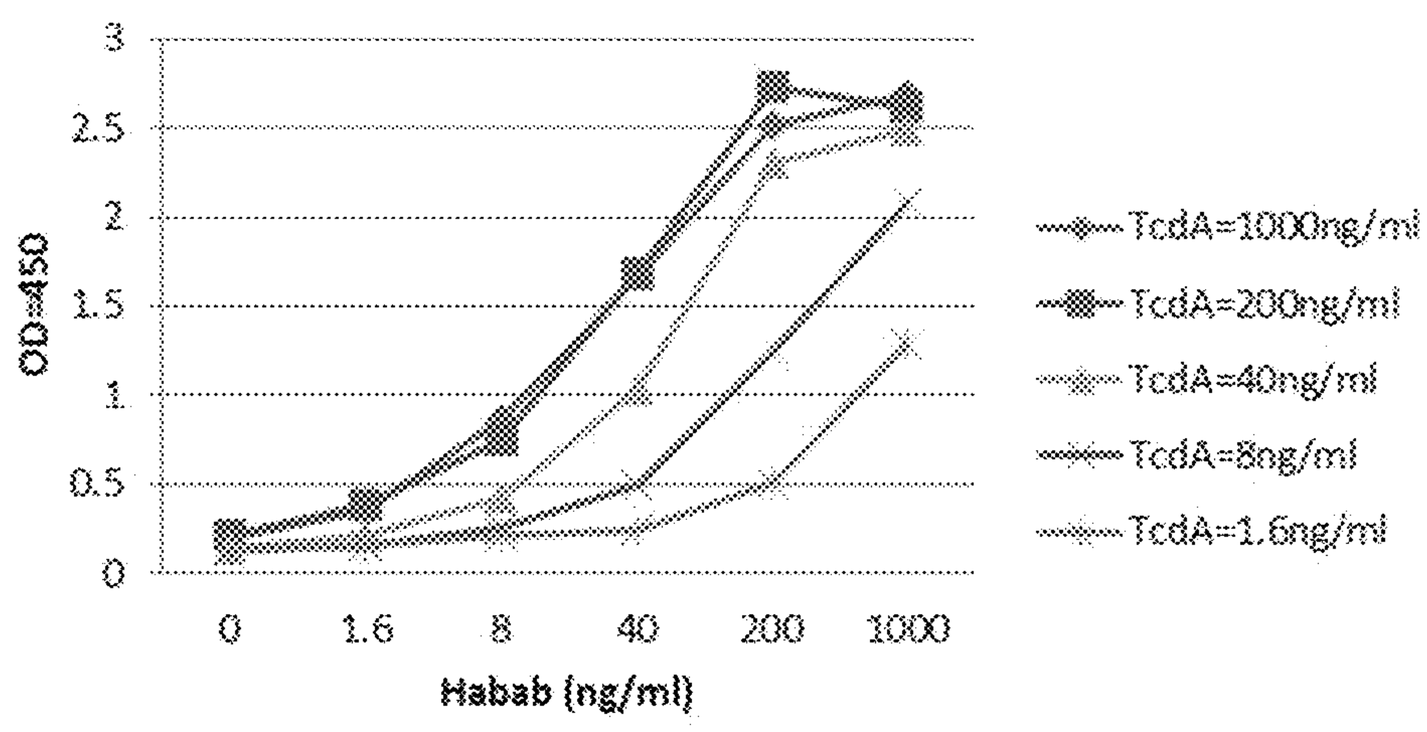


Figure 13A

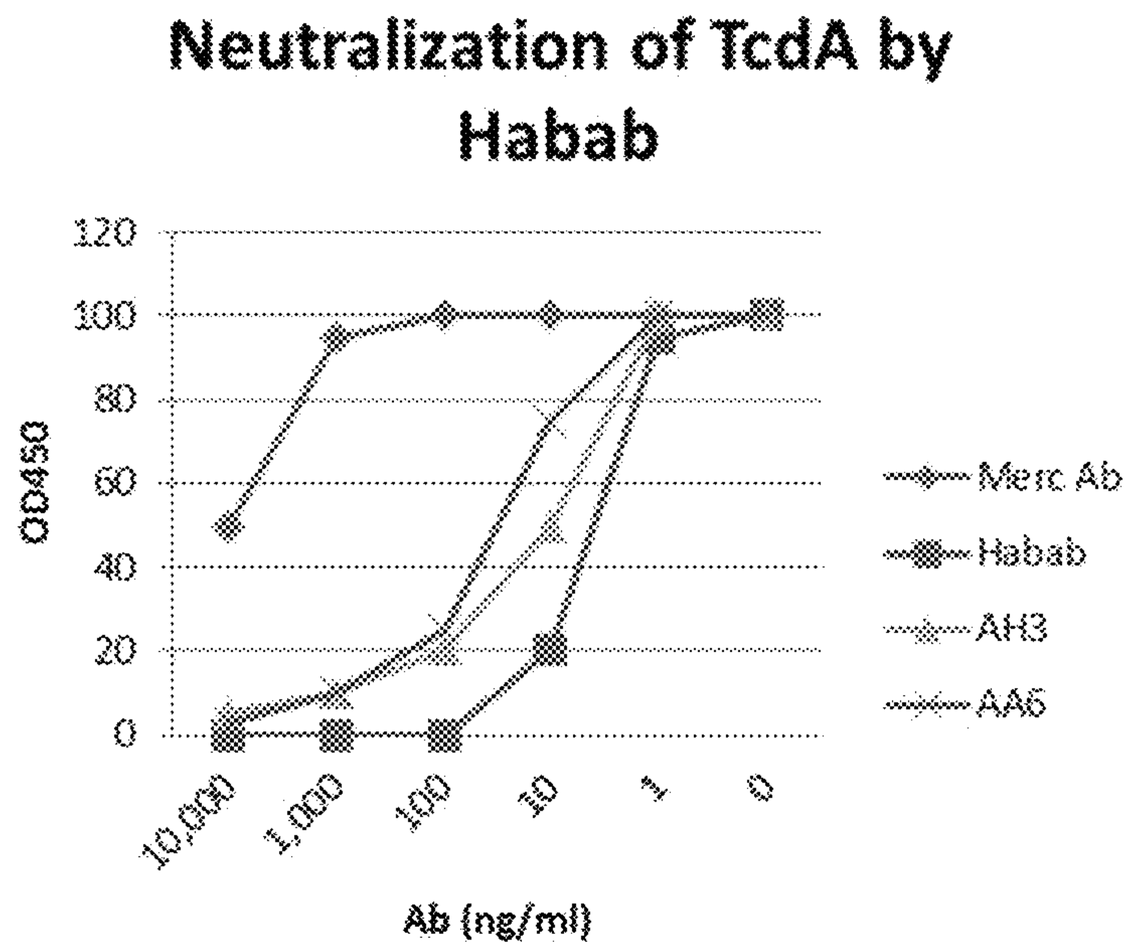


Figure 13B

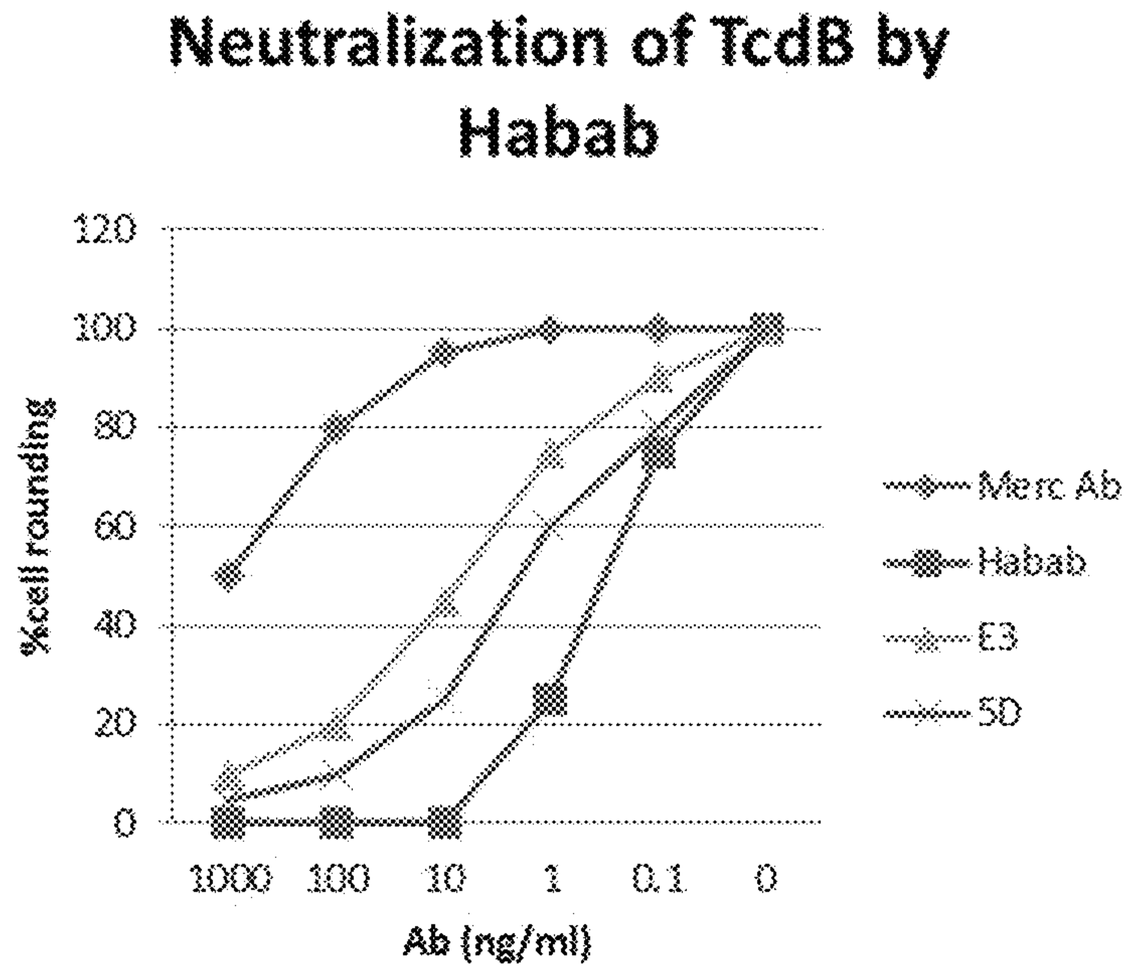
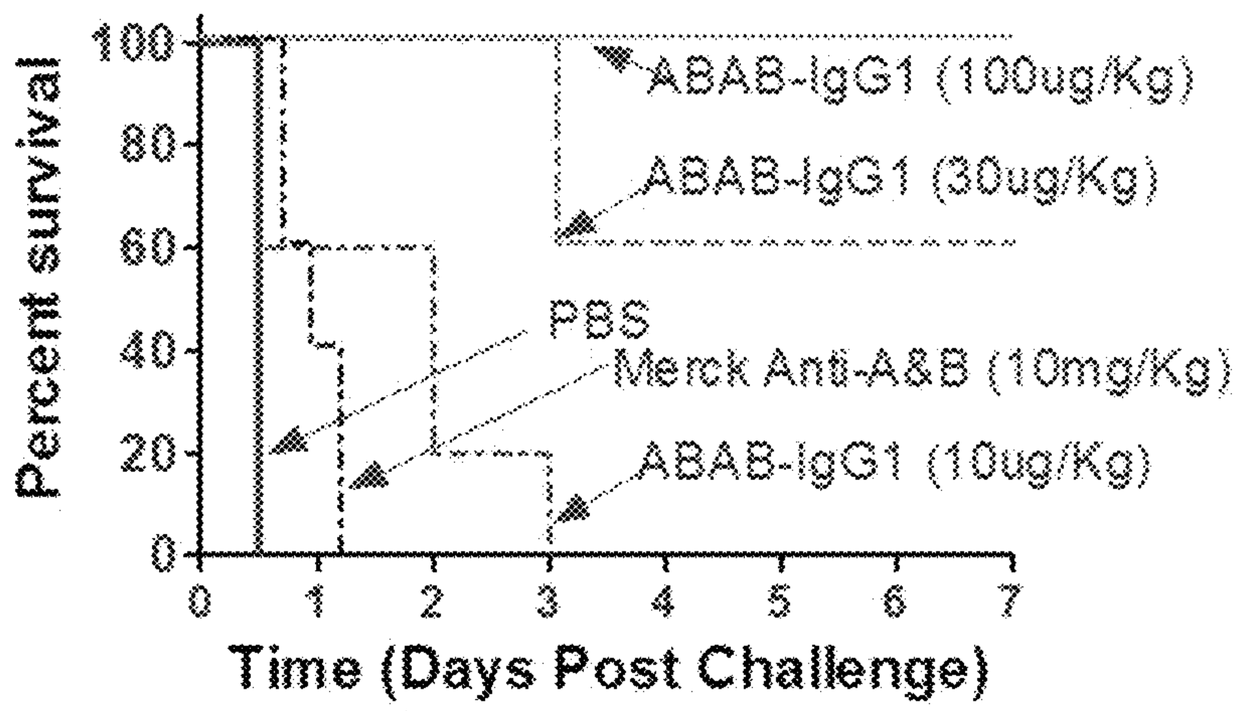


Figure 14


Percent survival
100
80
60
40
20
0
0
1
2
3
4
5
6
7
Time (Days Post Challenge)
ABAB-IgG1 (100ug/Kg)
ABAB-IgG1 (30ug/Kg)
PBS
Merck Anti-A&B (10mg/Kg)
ABAB-IgG1 (10ug/Kg)

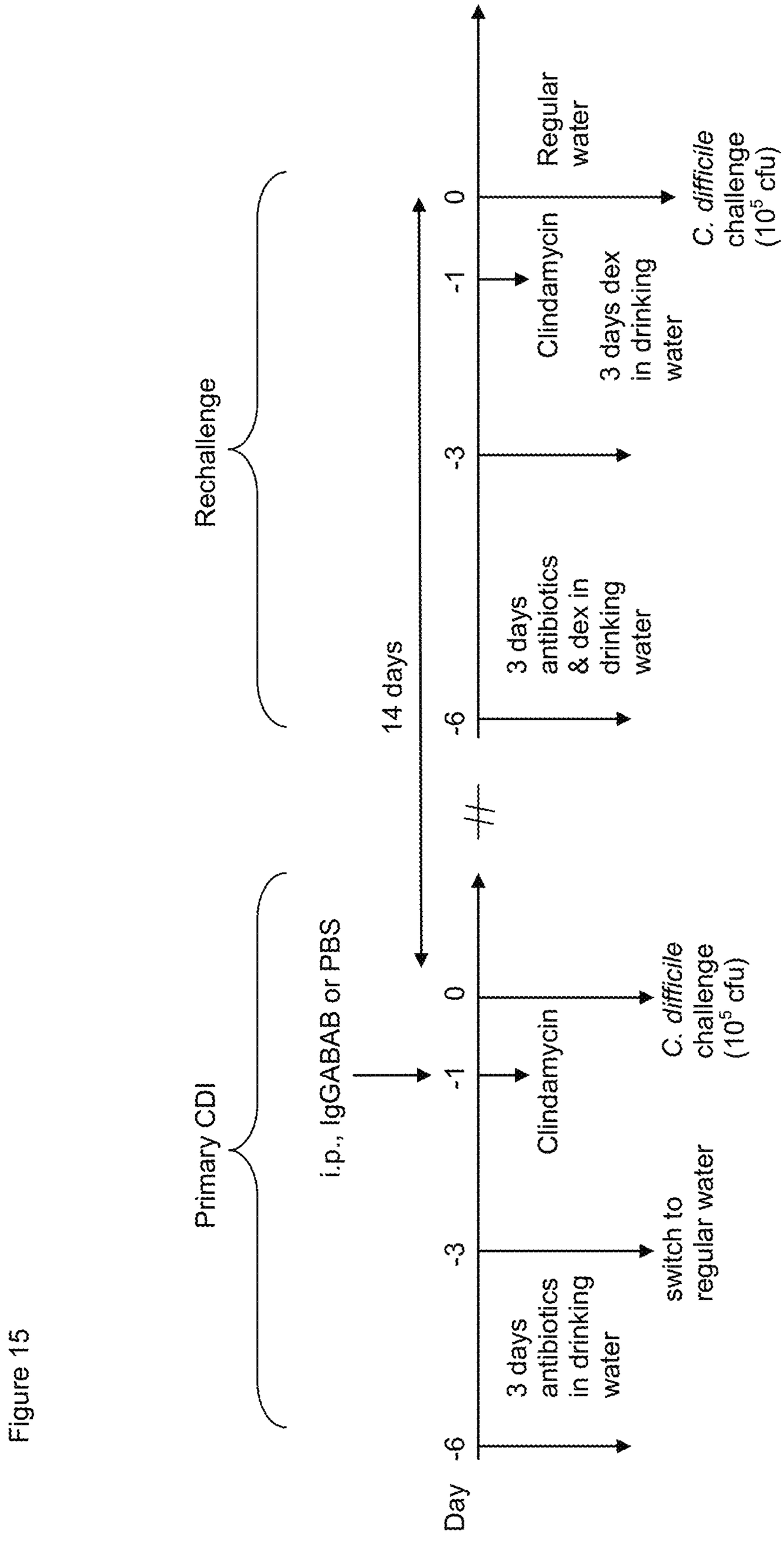

Day
-6
-3
-1
0
3 days antibiotics in drinking water
switch to regular water
Clindamycin
C. difficile challenge (10^5 cfu)
Primary CDI
i.p., IgGABAB or PBS
14 days
3 days antibiotics & dex in drinking water
3 days dex in drinking water
Regular water
Rechallenge

Figure 15

Figure 16

	Diarrhea			Weight Change			Survival
	occurrence	Day1 score	Day2 score	Overall	Day2	Day3	Day4	
200ug/kg		-	-	-	√	-	√	√
1mg/kg	√	-	√	-	√	√	-	√
5mg/kg	√	√	√	√	√	√	√	√

Figure 17

	Diarrhea			Weight Change			Survival
	occurrence	Day1 score	Day2 score	Overall	Day2	Day3	Day4	
200ug/kg	√	√	-	-	-	-	-	-
1mg/kg	-	-	-	-	-	-	√	-
5mg/kg	-	-	-	√	-	-	√	-

TETRA-SPECIFIC, OCTAMERIC BINDING AGENTS AND ANTIBODIES AGAINST *CLOSTRIDIUM DIFFICILE* TOXIN A AND TOXIN B FOR TREATMENT OF *C. DIFFICILE* INFECTION

STATEMENT OF FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

This invention was made with government support under Grant No. DK084509 and Grant No. AI109776 awarded by the National Institutes of Health. The government has certain rights in the invention.

SEQUENCE LISTING

A sequence listing in electronic (ASCII text file) format is filed with this application and incorporated herein by reference. The name of the ASCII text file is "2016_0045A_ST25"; the file was created on Feb. 5, 2016; the size of the file is 108 KB.

BACKGROUND

The bacterium *Clostridium difficile* is the most common cause of nosocomial antibiotic-associated diarrhea as well as the etiologic agent of pseudomembranous colitis. It is estimated that over 500,000 cases of *C. difficile*-associated disease (CDI) occur annually in the United States, with the annual mortality rate ranging from about 3-17%, depending on the strains.

Available options for treating CDI patients are limited and the recurrence rate is high (20-35% of patients). The risk of further episodes of CDI in recurrent patients can be more than 50% and a subset of patients will have multiple recurrences. Recurrent CDI can be caused by the same strain or different ones. With the emergence of hypervirulent and antibiotic-resistant strains, the incidence of mortality in patients with *C. difficile* infection is increasing rapidly.

Standard therapy includes antibiotic treatment (vancomycin and metronidazole), which is not fully effective and has a disruptive effect on gut microflora leading to multiple relapses. While other interventions have been tried (e.g., probiotics, toxin-absorbing polymers, and toxoid vaccines), neither prevention nor treatment strategies have kept up with the increased incidence and seriousness of this infection.

Newer immune-based therapies have been shown to be somewhat effective in clinical trials and include intravenous immunoglobulin (IVIG) against severe CDI and human monoclonal antibodies against recurrent CDI. Fidaxomicin, a narrow spectrum macrocyclic antibiotic, has shown an effect similar to oral vancomycin on CDI but was significantly better at lowering the relapse rate.

It is a frustrating condition that is difficult to treat and may affect patients for months or even years, causing tremendous morbidity and mortality. Accordingly, there is a need for new treatments for both primary and recurrent CDI and preventions for subjects at risk of developing CDI.

BRIEF SUMMARY OF INVENTION

C. difficile-associated disease is mainly caused by two large exotoxins, i.e., toxin A (TcdA) and toxin B (TcdB), produced by the bacteria. These toxins are structurally similar, 300-kDa single-chain proteins that exhibit similar modes of action on host cells. Both toxins target host Rho GTPases, leading to enzyme inactivation, followed by cytoskeleton disorganization and apoptosis. In intestinal epithelial cells, TcdA catalyzes glucosylation of the Rho GTPases, leading to reorganization of the actin cytoskeleton with accompanying morphological changes such as complete rounding of cells and destruction of the intestinal barrier function. The toxins can individually cause CDI in animals, and $TcdA^-$ $TcdB^-$ strains of the bacteria are avirulent.

Systemic and mucosal antibodies against the toxins confer protection against CDI. Because TcdA and TcdB are essential virulence factors for *C. difficile*, antibodies produced against both toxins can treat and protect against toxigenic *C. difficile* infection in animal models.

The present invention builds on existing knowledge regarding anti-TcdA and anti-TcdB antibodies for the treatment and prevention of CDI, and the symptoms of CDI. Provided herein are novel, antibody-based binding agents derived from human and camelid immunoglobulins. These binding agents recognize and bind with specificity to *C. difficile* TcdA and/or TcdB. Some of these binding agents exhibit toxin-neutralizing activity. These binding agents can be used to treat or prevent primary and recurrent CDI, as well as the symptoms of primary and recurrent CDI.

As discussed in detail below, camelid animals produce a class of functional immunoglobulins that lack light chains and are thus heavy chain-only antibodies (HCAbs). The V_H domain of HCAbs, called V_HH, is similar to the conventional human V_H domain but has unique sequence and structural characteristics. DNA encoding this domain can be readily cloned and expressed in microbes to yield soluble protein monomers that retain the antigen-binding properties of the parent HCAb. These V_HH peptide monomer binding agents are small (~15 kDa), easy to produce, and generally more stable than conventional antibody fragments. They can also be produced as fusion proteins with human antibodies, such as IgG, and fragments of human antibodies, such as Fc domains.

The binding agents of the present invention thus include simple V_HH peptide monomers and linked groups of V_HH peptide monomers (comprising 2, 3, 4, or more monomers), as well as more complex binding agents that comprise V_HH peptide monomers joined to antibody Fc domains, as well as V_HH peptide monomers joined to partial or full IgG antibodies.

In a first embodiment, the present invention is directed to binding agents comprising V_HH peptide monomers and linked groups of V_HH peptide monomers comprising two, three, four, or more monomers, each of which binds TcdA and/or TcdB, preferably with specificity. Thus, the invention encompasses V_HH peptide binding agents comprising at least one V_HH peptide monomer, wherein each V_HH peptide monomer has binding specificity for a unique epitope of *C. difficile* toxin A (TcdA) or toxin B (TcdB). In certain aspects, these binding agents comprise two, three, four, or more linked V_HH peptide monomers. The V_HH peptide monomers include, but are not limited to, the V_HH peptide monomers 5D (SEQ ID NO:1), E3 (SEQ ID NO:3), AA6 (SEQ ID NO:5), and AH3 (SEQ ID NO:7).

In aspects of this embodiment where two or more monomer are linked, the monomers may be linked by flexible peptide linkers, generally comprising between 10 and 20 amino acids. Suitable linkers include, but are not limited to, linker-1 (SEQ ID NO:9), linker-2 (SEQ ID NO:11), and linker-3 (SEQ ID NO:13).

In certain aspects of this embodiment, the binding agents bind to TcdA and/or TcdB with specificity. In certain aspects of this embodiment, the binding agents exhibit TcdA and/or TcdB neutralizing activity.

In a specific aspect of this embodiment, the binding agent comprises four linked V_HH peptide monomers where two of the monomers have binding specificity for epitopes of TcdA and two of the monomers have binding specificity for epitopes of TcdB. The epitopes of TcdA may be the same or different. The epitopes of TcdB may be the same or different.

In a specific aspect of this embodiment, the binding agent comprises the amino acid sequence set forth in SEQ ID NO:19 or a sequence variant thereof having at least 95% sequence identity thereto, and wherein the sequence variant retains TcdA and/or TcdB binding specificity, or the sequence variant retains toxin neutralizing activity, or both. In some instances, variant amino acids of the sequence variant are located in framework regions of the V_HH peptide monomers.

In a second embodiment, the invention is directed to binding agents comprising V_HH peptide monomers joined to IgG antibodies, where the binding agents bind TcdA and/or TcdB. In these IgG-based binding agents, the variable regions of the light and heavy chains of IgG antibodies are replaced by one, two, three, four or more of the V_HH peptide monomers.

In certain aspects of this embodiment, these binding agents comprise two, three, four, or more linked V_HH peptide monomers joined to the amino termini of IgG light and heavy chains in place of the variable regions. The V_HH peptide monomers include, but are not limited to, the V_HH peptide monomers 5D (SEQ ID NO:1), E3 (SEQ ID NO:3), AA6 (SEQ ID NO:5), and AH3 (SEQ ID NO:7).

In aspects of this embodiment where two or more monomer are linked, the monomers may be linked by flexible peptide linkers, generally comprising between 10 and 20 amino acids. Suitable linkers include, but are not limited to, linker-1 (SEQ ID NO:9), linker-2 (SEQ ID NO:11), and linker-3 (SEQ ID NO:13).

In a first sub-embodiment, the invention is directed to tetra-specific, octameric binding agents comprising an IgG antibody, two sets of linked first and second V_HH peptide monomers, and two sets of linked third and fourth V_HH peptide monomers, wherein the IgG antibody comprises two arms, each arm comprising a heavy chain lacking a variable region and a light chain lacking a variable region, and each chain having an amino terminus, wherein for each arm of the antibody, one set of linked first and second V_HH peptide monomers is joined to the amino terminus of the light chain, and one set of linked third and fourth V_HH peptide monomers is joined to the amino terminus of the heavy chain, and wherein the V_HH peptide monomers have binding specificity for an epitope of *Clostridium difficile* toxin A (TcdA) or toxin B (TcdB). This binding agent is termed "tetra-specific" as it recognizes four different toxin epitopes. It is termed "octameric" as it bears eight V_HH peptide monomers (two copies of the first monomer, two copies of the second monomer, two copies of the third monomer, and two copies of the fourth monomer).

In this sub-embodiment, the first, second, third and fourth V_HH peptide monomers each has binding specificity for a different epitope.

In certain aspects of this sub-embodiment, two of the V_HH peptide monomers have binding specificity for epitopes of TcdA and two of the V_HH peptide monomers have binding specificity for epitopes of TcdB.

In certain aspects of this sub-embodiment, the V_HH peptide monomers independently have binding specificity for an epitope in the glucosyltransferase domain, cysteine protease domain, translocation domain or receptor binding domain of TcdA or TcdB.

In a specific aspect of this sub-embodiment, the light (kappa) chain of the binding agent comprises the amino acid sequence set forth in SEQ ID NO:46 (AA6/E3 kappa) or a sequence variant having at least 95% sequence identity thereto, and the heavy chain of the binding agent comprises the amino acid sequence set forth in SEQ ID NO:44 (AH3/5D heavy) or a sequence variant having at least 95% sequence identity thereto. As this binding agent is an IgG-based binding agent, it will be clear to the skilled artisan that two heavy chain polypeptides and two light chain polypeptides, having the noted amino acid sequences, will assemble through disulfide bonding to provide the complete binding agent. The sequence variants retain TcdA and/or TcdB binding specificity, or the sequence variants retain toxin-neutralizing activity, or both. The variant amino acids of the sequence variants may be located in framework regions of the V_HH peptide monomers.

In a second sub-embodiment, the invention is directed to bi-specific or tetra-specific, tetrameric binding agents comprising an IgG antibody and first, second, third and fourth V_HH peptide monomers, wherein the IgG antibody comprises two arms, each arm comprising a heavy chain lacking a variable region and a light chain lacking a variable region, and each chain having an amino terminus, wherein for a first arm of the antibody, the first V_HH peptide monomer is joined to the amino terminus of the light chain, and the second V_HH peptide monomer is joined to the amino terminus of the heavy chain, wherein for a second arm of the antibody, the third V_HH peptide monomer is joined to the amino terminus of the light chain, and the fourth V_HH peptide monomer is joined to the amino terminus of the heavy chain, and wherein the V_HH peptide monomers have binding specificity for an epitope of *Clostridium difficile* toxin A (TcdA) or toxin B (TcdB). When the binding agent is "tetra-specific", it recognizes four different toxin epitopes; when "bi-specific" it recognizes two different toxin epitopes. The binding agents are "tetrameric" as they bear four V_HH peptide monomers (when bi-specific, the first and third monomer have the same sequence and bind the same epitope, and the second and fourth monomers have the same sequence and bind the same epitope; when tetra-specific, each of the monomers has a different sequence and binds a different epitope).

When the binding agent is bi-specific, the first and second monomers have binding specificity for different epitopes, the first and third monomers have identical amino acid sequences, and the second and fourth monomers have identical amino acid sequences. One of the V_HH peptide monomers may have binding specificity for an epitope of TcdA and one of the V_HH peptide monomers may have binding specificity for an epitope of TcdB.

When the binding agent is tetra-specific, each of the V_HH peptide monomers has binding specificity for a different epitope. Two of the V_HH peptide monomers may have binding specificity for epitopes of TcdA and two of the V_HH peptide monomers may have binding specificity for epitopes of TcdB.

In certain aspects of this sub-embodiment, each of the V_HH peptide monomers has binding specificity for epitopes of TcdA.

In certain aspects of this sub-embodiment, each of the V_HH peptide monomers has binding specificity for epitopes of TcdB.

In certain aspects of this sub-embodiment, the V_HH peptide monomers independently have binding specificity

for an epitope in the glucosyltransferase domain, cysteine protease domain, translocation domain or receptor binding domain of TcdA or TcdB.

In a specific aspect of this sub-embodiment, the light (kappa) chain of the binding agent comprises the amino acid sequence set forth in SEQ ID NO:40 (AA6 kappa) or a sequence variant having at least 95% sequence identity thereto, and the heavy chain of the binding agent comprises the amino acid sequence set forth in SEQ ID NO:36 (AH3 heavy) or a sequence variant having at least 95% sequence identity thereto. As this binding agent is an IgG-based binding agent, it will be clear to the skilled artisan that two heavy chain polypeptides and two light chain polypeptides, having the noted amino acid sequences, will assemble through disulfide bonding to provide the complete binding agent. The sequence variants retain TcdA and/or TcdB binding specificity, or the sequence variants retain toxin neutralizing activity, or both. The variant amino acids of the sequence variant may be located in framework regions of the V_HH peptide monomers.

In another specific aspect of this sub-embodiment, the light (kappa) chain of the binding agent comprises the amino acid sequence set forth in SEQ ID NO:42 (E3 kappa) or a sequence variant having at least 95% sequence identity thereto, and the heavy chain of the binding agent comprises the amino acid sequence set forth in SEQ ID NO:38 (5D heavy) or a sequence variant having at least 95% sequence identity thereto. As this binding agent is an IgG-based binding agent, it will be clear to the skilled artisan that two heavy chain polypeptides and two light chain polypeptides, having the noted amino acid sequences, will assemble through disulfide bonding to provide the complete binding agent. The sequence variants retain TcdA and/or TcdB binding specificity, or the sequence variants retain toxin neutralizing activity, or both. The variant amino acids of the sequence variants may be located in framework regions of the V_HH peptide monomers.

In certain aspects of this embodiment and the sub-embodiments, the binding agents bind to TcdA and/or TcdB with specificity. In certain aspects of this embodiment, the binding agents exhibit TcdA and/or TcdB neutralizing activity.

In a third embodiment, the invention is directed to binding agents comprising V_HH peptide monomers joined to antibody Fc domains, where the binding agents bind TcdA and/or TcdB. In these Fc domain-based binding agents, one, two, three, four or more of the V_HH peptide monomers are joined to the hinge, C_H2 and C_H3 regions of each arm of Fc domain of an antibody heavy chain. Thus, the peptide monomers replace the Fab regions of an antibody.

In certain aspects of this embodiment, these binding agents comprise two, three, four, or more linked V_HH peptide monomers joined to the amino termini of the arms of the Fc domains. The V_HH peptide monomers include, but are not limited to, the V_HH peptide monomers 5D (SEQ ID NO:1), E3 (SEQ ID NO:3), AA6 (SEQ ID NO:5) and AH3 (SEQ ID NO:7).

In aspects of this embodiment where two or more monomer are linked, the monomers may be linked by flexible peptide linkers, generally comprising between 10 and 20 amino acids. Suitable linkers include, but are not limited to, linker-1 (SEQ ID NO:9), linker-2 (SEQ ID NO:11), and linker-3 (SEQ ID NO:13).

In a first sub-embodiment, the invention is directed to tetra-specific, octameric binding agents comprising an antibody Fc domain and two sets of linked first, second, third and fourth V_HH peptide monomers, wherein the antibody Fc domain comprises two arms, each arm comprising hinge, C_H2 and C_H3 regions of an antibody heavy chain, and each arm having an amino terminus, wherein for each arm of the Fc domain, one set of linked first, second, third and fourth V_HH peptide monomers is joined to the amino terminus of the arm, and where the V_HH peptide monomers have binding specificity for an epitope of *Clostridium difficile* toxin A (TcdA) or toxin B (TcdB). This binding agent is termed "tetra-specific" as it recognizes four different toxin epitopes. It is termed "octameric" as it bears eight V_HH peptide monomers (two copies of the first monomer, two copies of the second monomer, two copies of the third monomer, and two copies of the fourth monomer).

In certain aspects of this sub-embodiment, the first, second, third and fourth V_HH peptide monomers each has binding specificity for a different epitope.

In certain aspects of this sub-embodiment, two of the V_HH peptide monomers have binding specificity for epitopes of TcdA and two of the V_HH peptide monomers have binding specificity for epitopes of TcdB.

In certain aspects of this sub-embodiment, the V_HH peptide monomers independently have binding specificity for an epitope in the glucosyltransferase domain, cysteine protease domain, translocation domain or receptor binding domain of TcdA or TcdB.

In a specific aspect of this sub-embodiment, the binding agent comprises the amino acid sequence set forth in SEQ ID NO:22 (ABAB-Fc) or a sequence variant having at least 95% sequence identity thereto, where the sequence variant retains TcdA and/or TcdB binding specificity, or the sequence variant retains toxin neutralizing activity, or both. As this binding agent is an Fc domain-based binding agent, it will be clear to the skilled artisan that two identical polypeptides, having the noted amino acid sequence, serve as the arms of the binding agent and that the arms will assemble through disulfide bonding to provide the complete binding agent. The variant amino acids of the sequence variant may be located in framework regions of the V_HH peptide monomers.

In a second sub-embodiment, the invention is directed to bi-specific, tetrameric binding agents comprising an antibody Fc domain and two sets of linked first and second V_HH peptide monomers, wherein the antibody Fc domain comprises two arms, each arm comprising hinge, C_H2 and C_H3 regions of an antibody heavy chain, and each arm having an amino terminus, wherein for each arm of the Fc domain, one set of linked first and second V_HH peptide monomers is joined to the amino terminus of the arm, and where the V_HH peptide monomers have binding specificity for an epitope of *Clostridium difficile* toxin A (TcdA) or toxin B (TcdB). This binding agent is termed "bi-specific" as it recognizes two different toxin epitopes. It is termed "tetrameric" as it bears four V_HH peptide monomers (two copies of the first monomer, and two copies of the second monomer).

In certain aspects of this sub-embodiment, the first and second V_HH peptide monomers have binding specificity for the same or different epitopes.

In certain aspects of this sub-embodiment, the V_HH peptide monomers independently have binding specificity for an epitope in the glucosyltransferase domain, cysteine protease domain, translocation domain or receptor binding domain of TcdA or TcdB.

In a specific aspect of this sub-embodiment, the binding agent comprises the amino acid sequence set forth in SEQ ID NO:32 (AH3/5D-Fc) or a sequence variant having at least 95% sequence identity thereto, where the sequence variant retains TcdA and/or TcdB binding specificity, or the

sequence variant retains toxin neutralizing activity, or both. As this binding agent is an Fc domain-based binding agent, it will be clear to the skilled artisan that two identical polypeptides, having the noted amino acid sequence, serve as the arms of the binding agent and that the arms will assemble through disulfide bonding to provide the complete binding agent. The variant amino acids of the sequence variant may be located in framework regions of the V_HH peptide monomers.

In another specific aspect of this sub-embodiment, the binding agent comprises the amino acid sequence set forth in SEQ ID NO:34 (AA6/E3-Fc) or a sequence variant having at least 95% sequence identity thereto, where the sequence variant retains TcdA and/or TcdB binding specificity, or the sequence variant retains toxin neutralizing activity, or both. As this binding agent is an Fc domain-based binding agent, it will be clear to the skilled artisan that two identical polypeptides, having the noted amino acid sequence, serve as the arms of the binding agent and that the arms will assemble through disulfide bonding to provide the complete binding agent. The variant amino acids of the sequence variant may be located in framework regions of the V_HH peptide monomers.

In certain aspects of this embodiment and the sub-embodiments, the binding agents bind to TcdA and/or TcdB with specificity. In certain aspects of this embodiment, the binding agents exhibit TcdA and/or TcdB neutralizing activity.

The invention includes humanized variants of each the binding agents provided in the various embodiments and aspects defined herein. Likewise, the invention includes epitope binding fragments of each the binding agents provided in the various embodiments and aspects defined herein.

The invention includes pharmaceutical formulations comprising one or more of the binding agents defined herein and a pharmaceutically acceptable carrier or diluent.

The invention includes polynucleotides comprising nucleotide sequences encoding each the binding agents provided in the various embodiments and aspects defined herein, as well as complementary strands thereof. The invention also includes expression vectors comprising the polynucleotides, and host cells comprising the expression vectors. The invention further includes methods of producing the binding agents define herein, comprising culturing the host cells under conditions promoting expression of the binding agents encoded by the expression vectors, and recovering the binding agents from the cell cultures.

In a fourth embodiment, the invention is directed to methods of treating or preventing a disease symptom induced by *C. difficile* in a subject comprising administering a therapeutically-effective amount of one or more binding agents as defined herein to a subject having *C. difficile* infection or a risk of developing *C. difficile* infection.

In a fifth embodiment, the invention is directed to methods of neutralizing *C. difficile* toxin TcdA and/or TcdB in a subject infected by *C. difficile* comprising administering a therapeutically-effective amount of one or more binding agents as defined herein to a subject having *C. difficile* infection.

In a sixth embodiment, the invention is directed to methods of treating or preventing *C. difficile* infection in a subject comprising administering a therapeutically-effective amount of one or more of the binding agents as defined herein to a subject having *C. difficile* infection or a risk of developing *C. difficile* infection.

In certain aspects of the sixth embodiment, the method further comprises administering a therapeutically-effective amount of an antibiotic to the subject.

In certain aspects of the methods, the binding agent is in a pharmaceutical formulation comprising the binding agent and a pharmaceutically acceptable carrier or diluent.

In certain aspects of the methods, the therapeutically-effective amount of the binding agent is between 10 ug/kg and 100 mg/kg of the agent per body weight of the subject.

In certain aspects of the methods, the agent is administered to the subject orally, parenterally or rectally.

The foregoing has outlined rather broadly the features and technical advantages of the present invention in order that the detailed description of the invention that follows may be better understood. Additional features and advantages of the invention will be described herein, which form the subject of the claims of the invention. It should be appreciated by those skilled in the art that any conception and specific embodiment disclosed herein may be readily utilized as a basis for modifying or designing other structures for carrying out the same purposes of the present invention. It should also be realized by those skilled in the art that such equivalent constructions do not depart from the spirit and scope of the invention as set forth in the appended claims. The novel features which are believed to be characteristic of the invention, both as to its organization and method of operation, together with further objects and advantages will be better understood from the following description when considered in connection with the accompanying figures. It is to be expressly understood, however, that any description, figure, example, etc. is provided for the purpose of illustration and description only and is by no means intended to define the limits the invention.

BRIEF DESCRIPTION OF DRAWINGS

FIG. **1**. A diagram of *C. difficile* toxins TcdA and TcdB, showing the glucosyltransferase domains (GT), cysteine protease domains (CPD), translocation domains (TD) and receptor binding domains (RBD) of each toxin. V_HHs that recognize and bind the different toxin domains are shown. Those that are underlined are those that have toxin-neutralizing activity.

FIG. **2**. Monomeric or dimeric V_HHs possess potent neutralizing activity. V_HHs block cell rounding induced by TcdA (FIG. **2**A) or TcdB (FIG. **2**B) at nM concentrations. (FIG. **2**C) Diagram of two heterodimers against TcdA or TcdB. $His_{(6)}$ tag on N-terminus facilitates purification; a flexible spacer (FS) separate the two V_HHs. (FIG. **2**D) Dimer 5D/E3 increases its neutralizing activity at least 10-fold over a simple mix of the two V_HHs. Heterodimers fully protected mice from lethal ip challenge with TcdB (FIG. **2**E) or TcdA (FIG. **2**F).

FIG. **3**. Diagram of ABAB. His-tag and E-tag are epitope tags for purification and detection, respectively. FS: flexible linker; ABP: albumin binding peptide.

FIGS. **4**A-**4**B. ABAB is highly potent in protecting mice from *C. difficile* spore (FIG. **4**A) and toxin (FIG. **4**B) challenge. MK HuMabs: a mixture of Merck anti-TcdA and anti-TcdB human monoclonal antibodies that are undergoing clinical trials.

FIG. **5**. Anti-toxin sera against both toxins protect mice from CDI. Mice were i.p. injected with 50 ul alpaca anti-sera against TcdA ("Anti-A"), TcdB ("Anti-B"), TcdA+TcdB ("Anti-A+Anti-B") or with 100 ul presera or PBS ("CTR") for 4 hours before *C. difficile* spore (UK1 strain, 10^6 spores/mouse) inoculation. Mouse survival (FIG. **5**A; Anti-A+

Anti-B vs. PBS, p=0.006) and weight loss (FIG. **5**B) are illustrated (*, p<0.05 between Anti-A+Anti-B vs. control).

FIG. **6**. Illustration of strategies for making binding agents of the invention.

FIG. **7**. Fractionation and purification of ABAB-Fc ("Fc-ABBA") from cell cultures.

FIG. **8**. The diagram of the ABAB and ABAB-IgG molecules.

FIG. **9**. Fractionation of culture supernatant from HEK293 cells expressing ABAB-IgG1. The peak shows the UV OD reading of the eluted ABAB-IgG1 from Protein A beads.

FIG. **10**. SDS-PAGE of reduced and non-reduced electrophoresis of purified ABAB-IgG1 ("IgG-ABBA" and "Hababb").

FIGS. **11**A-**11**B. ELISA analysis of binding of ABAB-IgG to TcdA (FIG. **11**A) and TcdB (FIG. **11**B) as compared with the binding of the individual VHHs to the respective toxins.

FIGS. **12**A-**12**B. Sandwich ELISA analysis of simultaneous binding of the tetraspecific antibody IgG-ABAB to both TcdA and TcdB. FIG. **12**A shows serially diluted ABAB-IgG added to ELISA plates coated with TcdA (TxA), followed by TcdB (TxB). FIG. **12**B shows serially diluted ABAB-IgG added to ELISA plates coated with TcdB (TxB), followed by TcdA (TxA).

FIGS. **13**A-**13**B. ABAB-IgG neutralizing activities against TcdA (FIG. **13**A) and TcdB (FIG. **13**B).

FIG. **14**. Graph showing in vivo neutralizing activity of ABAB-IgG against *C. difficile* infection in mice versus Merck antibodies against TcdA and TcdB.

FIG. **15**. Design of studies on the effects of prophylactic ABAB-IgG against *C. difficile* infection.

FIG. **16**. Effect of ABAB-IgG against CDI: prophylactic treatment—Summary.

FIG. **17**. Effect of ABAB-IgG against CDI: Re-challenge—Summary.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found, for example, in Benjamin Lewin, Genes VII, published by Oxford University Press, 2000 (ISBN 019879276X); Kendrew et al. (eds.); The Encyclopedia of Molecular Biology, published by Blackwell Publishers, 1994 (ISBN 0632021829); and Robert A. Meyers (ed.), Molecular Biology and Biotechnology: a Comprehensive Desk Reference, published by Wiley, John & Sons, Inc., 1995 (ISBN 0471186341); and other similar technical references.

As used herein, "a" or "an" may mean one or more. As used herein when used in conjunction with the word "comprising," the words "a" or "an" may mean one or more than one. As used herein "another" may mean at least a second or more. Furthermore, unless otherwise required by context, singular terms include pluralities and plural terms include the singular.

As used herein, "about" refers to a numeric value, including, for example, whole numbers, fractions, and percentages, whether or not explicitly indicated. The term "about" generally refers to a range of numerical values (e.g., +/−5-10% of the recited value) that one of ordinary skill in the art would consider equivalent to the recited value (e.g., having the same function or result). In some instances, the term "about" may include numerical values that are rounded to the nearest significant figure.

II. The Present Invention

The primary effectors of CDI in animals are the *C. difficile* exotoxins TcdA and TcdB (toxin A and B). These toxins are structurally similar, 300-kDa single-chain proteins that exhibit similar modes of action on host cells. Both toxins target host Rho GTPases, leading to enzyme inactivation, followed by cytoskeleton disorganization and apoptosis. In intestinal epithelial cells, TcdA catalyzes glucosylation of the Rho GTPases, leading to reorganization of the actin cytoskeleton with accompanying morphological changes such as complete rounding of cells and destruction of the intestinal barrier function. The toxins can individually cause CDI in animals, and $TcdA^{-}$ $TcdB^{-}$ strains of the bacteria are avirulent.

Numerous independent studies have demonstrated that systemic and mucosal antibodies against the toxins confer protection against CDI. Because TcdA and TcdB are essential virulence factors for *C. difficile*, antibodies produced against both toxins can protect against toxigenic *C. difficile* infection in animal models. In humans, high serum levels of antitoxin antibodies are associated with reduced disease severity and incidence of relapse. Therefore, a preventative rationale for systemically and orally administered antitoxin antibodies exists. However, monoclonal antibodies targeting a single epitope are typically low affinity, and use of such antibodies runs the risk of inducing mutations within the epitopes of the toxins thereby creating additional strains. Thus, neutralizing antitoxins targeting multiple, key, and conserved toxin epitopes are highly desirable.

Camelid animals produce a class of functional immunoglobulins that lack light chains and are thus heavy chain-only antibodies (HCAbs). HCAbs bind to target antigens with binding properties equivalent to those achieved by conventional human IgG. The V_H region of HCAbs, called V_HH, is similar to conventional V_H domains but has unique sequence and structural characteristics. DNA encoding this domain can readily be cloned and expressed in microbes to yield soluble protein monomers retaining the antigen-binding properties of the parent HCAb. These V_HH peptide monomer binding agents are small (~15 kDa), easy to produce, and generally more stable than conventional antibody fragments. They can also be produced in concert with IgG antibodies and antibody Fc domains.

The present invention utilizes the advantageous characteristics of HCAbs in the production of binding agents that can be used in the treatment and prevention of CDI. V_HH peptide monomers were screened for TcdA and TcdB epitope recognition and binding. Those monomers that exhibited epitope binding and had toxin-neutralizing activity were linked to produce the binding agents of the invention. The binding agents include simple V_HH peptide monomers and linked groups of V_HH peptide monomers (comprising 2, 3, 4, or more monomers), as well as more complex binding agents that comprise V_HH peptide monomers joined to antibody Fc domains, as well as V_HH peptide monomers joined to IgG antibodies (see FIG. **6**).

V_HH Monomers & V_HH Heterodimers

The inventors established an efficient platform to screen V_HH monomers against specific domains of both *C. difficile* toxins. Using highly immunogenic atoxic holotoxins for immunization, and bioactive chimeric toxins (with normal domain functions) for screening, panels of V_HH monomers

binding to different domains of TcdA or TcdB were prepared. A majority of these V_HH monomers possessed potent neutralizing activity and their binding to specific domains was determined (FIG. **1**).

Several of the V_HH monomers bind to highly conserved TcdA/TcdB epitopes. For example, the E3 V_HH monomer binds to the Rho GTPase binding site and blocks glucosylation; the AH3 V_HH monomer binds to the GT domain of the toxin; the 7F V_HH monomer binds to cysteine protease cleavage sites and blocks GT domain cleavage and release. Some V_HH monomers have potent toxin neutralizing activity, capable of blocking toxin cytotoxic activity at nM concentrations (monomers underlined in FIG. **1**; see also FIGS. **2**A and **2**B). Table 1 references amino and nucleic acid sequences in the Sequence Listing for some of these V_HH peptide monomers, both wild-type and codon-optimized versions. While both the optimized and non-optimized versions can be used in the production of the various binding agents of the present invention, the codon-optimized versions are preferred for expression in mammalian cells.

The present invention includes each of the V_HH peptide monomers referenced in Table 1 as well as sequence variants thereof having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity over the entire length of the peptide sequence and retaining the toxin binding and/or neutralizing activity of the wild-type peptide. The present invention also includes polynucleotide sequences encoding each of the V_HH peptide monomers of Table 1 and the sequence variants thereof, as well as complementary strands thereof.

TABLE 1

Name	Codon Optimized?	Location of epitope	SEQ ID NO for Amino Acid Seq.	SEQ ID NO for Nucleic Acid Seq.
5D	Yes	TcdB glucosyltransferase domain	1	2
E3	Yes	TcdB glucosyltransferase domain	3	4
AA6	Yes	TcdA cysteine protease domain	5	6
AH3	Yes	TcdA glucosyltransferase domain	7	8
5D	No	TcdB glucosyltransferase domain	48	49
E3	No	TcdB glucosyltransferase domain	50	51
AA6	No	TcdA cysteine protease domain	52	53
AH3	No	TcdA glucosyltransferase domain	54	55

To enhance the binding activity of the peptide monomers, V_HH peptide homo- and hetero-dimer binding agents were created, where two V_HH peptide monomers are linked (FIG. **2**C). Homodimer binding agents comprise two identical monomers that bind identical epitopes on two different toxins. Heterodimer binding agents comprise two different monomers that bind two distinct epitopes of the same toxin or distinct epitopes on two different toxins. The V_HH heterodimers were found to possess substantially enhanced neutralizing activities compared with equimolar mixtures of the individual V_HH peptide monomers comprising the heterodimers (FIG. **2**D). Indeed, heterodimers 5D/E3 and AH3/AA6 were found to fully protect mice from lethal systemic TcdB or TcdA challenge respectively, whereas mixed 5D and E3, or AA6 alone were only partially protective (FIGS. **2**E and F).

The V_HH monomers in the homo- and hetero-dimers are linked using a short, flexible linker of between 10 and 20 amino acids. Suitable linkers include those provided in Table 2. Table 2 also includes codon-optimized versions of the three linkers. While both the optimized and non-optimized versions can be used in the production of the various binding agents of the present invention, the codon-optimized versions are preferred for expression in mammalian cells.

TABLE 2

Name	Codon Optimized?	SEQ ID NO for Amino Acid Seq.	SEQ ID NO for Nucleic Acid Seq.
Linker-1	Yes	9	10
Linker-2	Yes	11	12
Linker-3	Yes	13	14
Linker-1	No	56	57
Linker-2	No	58	59
Linker-3	No	60	61

It will be understood by the skilled artisan that minor changes can be made to the sequence of the flexible linker without departing from the properties of the peptide. Sequence variants of the flexible linker having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity over the entire length of the peptide sequence and retaining properties of the linker upon which they are based may thus be used.

The present invention includes V_HH peptide homodimer binding agents comprising pairs of any of the monomers listed in Table 1, linked by a flexible linker as defined above. The present invention also includes V_HH peptide heterodimer binding agents comprising any combination of two of the monomers listed in Table 1, linked by a flexible linker as defined above. Exemplary heterodimers are provided in Table 3.

TABLE 3

Name	SEQ ID NO for Amino Acid Seq.	SEQ ID NO for Nucleic Acid Seq.
AH3-5D	15	16
AA6-E3	17	18
5D-E3	62	63
AH3-AA6	64	65

The present invention also includes sequence variants of the V_HH peptide homo- and hetero-dimers having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity over the entire length of the protein sequence and retaining the toxin binding and/or neutralizing activity of the wild-type protein. The present invention further includes polynucleotide sequences encoding each the V_HH peptide homo-hetero-dimers and the sequence variants thereof, as well as complementary strands thereof.

The invention also includes V_HH peptide homo- and hetero-trimer binding agents where three monomers are linked using the flexible linkers defined above in Table 2. Any combination of the monomers of Table 1 may be used, including trimers comprising three copies of the same mono-

mer, trimers comprising two copies of one monomer and a single copy of another, and trimers comprising three different monomers. Sequence variants of the V_HH peptide homo- and hetero-trimers are included in the invention, having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity over the entire length of the protein sequence and retaining the toxin binding and/or neutralizing activity of the wild-type protein. The present invention further includes polynucleotide sequences encoding each the V_HH peptide homo-hetero-trimers and the sequence variants thereof, as well as complementary strands thereof.

ABAB

The success of the peptide monomers and heterodimers allowed the inventors to develop binding agents comprising four linked V_HH peptide monomers. This was a goal of the research as earlier work had shown that the most useful agents in the treatment and prevention of CDI would be single antibodies that can simultaneously neutralize both TcdA and TcdB as this would be necessary in order to convey full protection against most pathogenic *C. difficile* strains. By creating tetra-specific binding agents that recognize and bind two epitopes on each of the toxins, the binding and neutralizing activity of the proteins might be strengthened. Therefore, four domain (tetra-specific) V_HH binding agents were generated.

The tetra-specific, tetrameric binding agents can be prepared from any combination of the monomers of Table 1, where the monomers are linked using the flexible linkers of Table 2. These binding agents will range from those having four copies of the same monomer, to those having three copies of the same monomer, to those having two copies of the same monomer, to those having four unique monomers, and variations therein. Sequence variants of the tetramers are included in the invention, having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity over the entire length of the protein sequence and retaining the toxin binding and/or neutralizing activity of the wild-type protein. The present invention further includes polynucleotide sequences encoding each tetramer and the sequence variants thereof, as well as complementary strands thereof.

ABAB is a particular binding agent of the invention that comprises four linked V_HH monomers, each of which has binding specificity for a different epitope of TcdA or TcdB. ABAB (sometimes also termed "ABBA" herein and in the figures) is thus a tetra-specific, tetrameric binding agent that consists of four distinct neutralizing V_HH monomers, two against TcdA and two against TcdB. This structural feature allows ABAB to bind simultaneously to two distinct neutralizing epitopes on each toxin. As described below, affinity/avidity and neutralizing activity of ABAB is more than 3-logs higher than human monoclonal antibodies (HuMabs) currently undergoing clinical trials for treatment of CDI.

ABAB binding agent was prepared by linking V_HH monomers AH3, 5D, E3, and AA6 (Table 1) using flexible linkers (Table 2). This binding agent targets conserved, non-overlapping epitopes and has excellent toxin neutralizing activity. In the design of ABAB (FIG. **3**), V_HH peptide monomers AH3 and AA6 were separated by placing the 5D between them because AH3 and AA6 bind to GT and TD respectively (FIG. **1**), which are spatially distant to each other. This design allowed AH3 and AA6 to bind to TcdA simultaneously.

The complete amino acid sequence comprising ABAB is provided in SEQ ID NO:19; the nucleic acid sequence encoding the protein is provided in SEQ ID NO:20. The present invention thus includes the ABAB binding agent provided in SEQ ID NO:19, as well as sequence variants of the ABAB binding agent having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity over the entire length of the protein sequence and retaining the toxin binding and/or neutralizing activity of the wild-type protein. The present invention further includes polynucleotide sequences encoding the ABAB binding agent (e.g., SEQ ID NO:20) and the sequence variants thereof, as well as complementary strands thereof.

In one variation of the ABAB binding agent, a $His_{(6)}$-tag (HHHHHH; SEQ ID NO:66) was provided at the amino terminus of the protein to aid in purification and an E-tag (GAPVPYPDPLEPR; SEQ ID NO:67) was provided at the carboxy terminus of the protein to aid in detection (see FIG. **3**). Because V_HH monomers have a half-life of 2-3 hr, in another variation an albumin-binding peptide (ABP, DICLPRWGCLWD; SEQ ID NO:21) was placed at the carboxyl end of the construct to increase its serum half-life to 10 hr (see FIG. **3**).

These binding agents bind to TcdA and/or TcdB with specificity. In certain aspects of the invention, the binding agents exhibit TcdA and/or TcdB neutralizing activity.

For the sake of clarity it can be noted that as used herein, "mono-specific", "bi-specific", "tri-specific", "tetra-specific", etc., mean the particular binding agent binds to 1, 2, 3, 4, etc., different epitopes, respectively. As used herein, "monomeric", "dimeric", "trimeric", "tetrameric", etc., mean that the particular binding agent has 1, 2, 3, 4, etc., separate V_HH peptide monomers that bind to the epitopes, respectively. Thus, a mono-specific, dimeric binding agent would display two V_HH peptide monomers that bind to the same epitope (e.g., a homodimer), and a bi-specific, dimeric binding agent would have two V_HH peptide monomers that bind to two different epitopes (e.g., a heterodimer). A tetra-specific, octameric binding agent has eight V_HH peptide monomers that recognize four different epitopes.

V_HH-Fc

It is well known that chimeric Fc-fusion proteins have the potential of increasing the half-life of a protein in vivo. This strategy has been applied in several FDA approved drugs, such as Etanercept. A proof-of principle study has shown that single-chain antibodies can be correctly assembled and expressed by B cells of transgenic mice carrying a mini-Ig construct encoding a dromedary V_HH and the Fc domain of human IgG. Also, a chimeric anti-EGFR/EGFRvIII V_HH, EG2-Fc exhibited excellent tumor accumulation in vivo and has pharmacokinetic properties that could improve glioblastoma targeting.

The present invention includes binding agents comprising V_HH peptide monomers joined to antibody Fc domains (V_HH-Fc), where the binding agents bind TcdA and/or TcdB. In these Fc domain-based binding agents, one, two, three, four or more of the V_HH peptide monomers are joined to the hinge, C_H2 and C_H3 regions of the Fc domain of an antibody heavy chain. Thus, the peptide monomers replace the Fab regions of the antibody.

The V_HH peptide monomers may be any of those provided in Table 1 above and include 5D (SEQ ID NO:1), E3 (SEQ ID NO:3), AA6 (SEQ ID NO:5) and AH3 (SEQ ID NO:7) V_HH peptide monomers. Where two or more monomers are linked, the monomers may be linked by flexible peptide linkers, generally comprising between 10 and 20 amino acids. Suitable linkers include those linkers provided in Table 2, such as linker-1 (SEQ ID NO:9), linker-2 (SEQ ID NO:11), and linker-3 (SEQ ID NO:13).

While the V_HH-Fc will typically be composed of two identical chains that self-assemble intracellularly after pro-

duction, the invention also includes V_HH-Fc binding agents comprising two different Fc chains. In such circumstances, the sequence of the V_HH monomer(s) alone may differ between the two Fc chains, or the Fc chains themselves may differ in sequence, or both the V_HH monomer(s) and the Fc chains may differ in sequence.

One type of V_HH-Fc binding agent is an octameric binding agent comprising an antibody Fc domain and first, second, third and fourth V_HH peptide monomers, where the V_HH peptide monomers have binding specificity for an epitope of *Clostridium difficile* toxin A (TcdA) or toxin B (TcdB), where the first, second, third and fourth V_HH peptide monomers are linked together and joined to amino termini of both antibody Fc domains, and where the antibody Fc domain comprises the hinge, C_H2 and C_H3 regions of an antibody heavy chain. Because this binding agent has four V_HH peptide monomers, it can be mono-specific (where all of the monomers bind the same epitope), bi-specific (where the monomers bind two different epitopes), tri-specific (where the monomers bind three different epitopes), or tetra-specific (where the monomers bind four different epitopes).

A specific example of a tetra-specific V_HH-Fc binding agent is the ABAB-Fc binding agent, a tetra-specific, octameric binding agent comprising an antibody Fc domain and two sets of linked first, second, third and fourth V_HH peptide monomers, wherein the antibody Fc domain comprises two arms, each arm comprising hinge, C_H2 and C_H3 regions of an antibody heavy chain, and each arm having an amino terminus, wherein for each arm of the Fc domain, one set of linked first, second, third and fourth V_HH peptide monomers is joined to the amino terminus of the arm, and where the V_HH peptide monomers have binding specificity for an epitope of *Clostridium difficile* toxin A (TcdA) or toxin B (TcdB). This binding agent is termed "tetra-specific" as it recognizes four different toxin epitopes. It is termed "octameric" as it bears eight V_HH peptide monomers (two copies of the first monomer, two copies of the second monomer, two copies of the third monomer, and two copies of the fourth monomer). ABAB-Fc was found to exhibit specific binding and neutralizing activity.

ABAB-Fc binding agent was prepared by generating an expression vector encoding the V_HH peptide monomers AH3/5D/AA6/E3 (linked in the noted order) joined to a human IgG1 Fc domain. The V_HH peptide monomers were separated by flexible linkers of Table 2. The nucleic acid sequence encoding each chain is provided in SEQ ID NO:23. The amino acid sequence of each chain is provided in SEQ ID NO:22. Upon self-assembly of pairs of the chains after expression, the tetra-specific, octameric binding agent resulted. The invention includes the ABAB-Fc binding agent of SEQ ID NO:22 and sequence variants having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity over the entire length of the protein sequence and retaining the toxin binding and/or neutralizing activity of the wild-type protein. The present invention further includes polynucleotide sequences encoding these sequence variants and complementary strands thereof.

Mono-specific V_HH-Fc binding agents (AH3-Fc, 5D-Fc, E3-Fc, AA6-Fc) and bi-specific V_HH-Fc binding agents (e.g., AH3/5D-Fc and AA6/E3-Fc) were also made using this Fc-fusion system. With respect to mono-specific binding agents, single V_HH peptide monomers were joined to human IgG1 Fc domains. Upon expression and assembly, pairs of the chains resulted in mono-specific, dimeric binding agents (when the chains were identical) or bi-specific, dimeric binding agents (when the chains were different). With respect to bi-specific binding agents, two linked V_HH peptide monomers (V_HH homo- or hetero-dimers) were joined to human IgG1 Fc domains. Upon expression and assembly, pairs of the chains resulted in bi-specific, tetrameric binding agents (when the chains were identical) or tetra-specific, tetrameric binding agents (when the chains were different). Table 4 provides the sequences for some these binding agents.

TABLE 4

Name	SEQ ID NO for Amino Acid Seq.	SEQ ID NO for Nucleic Acid Seq.
5D-Fc	24	25
E3-Fc	26	27
AA6-Fc	28	29
AH3-Fc	30	31
AH3-5D-Fc	32	33
AA6-E3-Fc	34	35

Specific pairings with one monomer include: 5D-Fc+5D-Fc; E3-Fc+E3-Fc; AA6-Fc+AA6-Fc; AH3-Fc+AH3-Fc; 5D-Fc+E3-Fc; 5D-Fc+AA6-Fc; 5D-Fc+AH3-Fc; E3-Fc+AA6-Fc; E3-Fc+AH3-Fc; and AA6-Fc+AH3-Fc. Specific pairings with two monomers include: AH3-5D-Fc+AH3-5D-Fc; AA6-E3-Fc+AA6-E3-Fc; and AH3-5D-Fc+AA6-E3-Fc.

Bi-specific, tetrameric V_HH-Fc binding agents were produced comprising an antibody Fc domain and two sets of linked first and second V_HH peptide monomers, wherein the antibody Fc domain comprises two arms, each arm comprising hinge, C_H2 and C_H3 regions of an antibody heavy chain, and each arm having an amino terminus, wherein for each arm of the Fc domain, one set of linked first and second V_HH peptide monomers is joined to the amino terminus of the arm, and where the V_HH peptide monomers have binding specificity for an epitope of *Clostridium difficile* toxin A (TcdA) or toxin B (TcdB). This binding agent is termed "bi-specific" as it recognizes two different toxin epitopes. It is termed "tetrameric" as it bears four V_HH peptide monomers (two copies of the first monomer, and two copies of the second monomer). The first and second V_HH peptide monomers may have binding specificity for the same or different epitopes. The V_HH peptide monomers may independently have binding specificity for an epitope in the glucosyltransferase domain, cysteine protease domain, translocation domain or receptor binding domain of TcdA or TcdB.

A specific example of a bi-specific, tetrameric V_HH-Fc binding agent comprises the amino acid sequence set forth in SEQ ID NO:32 (AH3/5D-Fc). The invention also includes sequence variants thereof having at least 95% sequence identity, where the sequence variant retains toxin-neutralizing activity. The variant amino acids of the sequence variant may be located in framework regions of the V_HH peptide monomers.

A specific example of a bi-specific, tetrameric V_HH-Fc binding agent comprises the amino acid sequence set forth in SEQ ID NO:34 (AA6/E3-Fc). The invention also includes sequence variants thereof having at least 95% sequence identity, where the sequence variant retains toxin-neutralizing activity. The variant amino acids of the sequence variant may be located in framework regions of the V_HH peptide monomers.

The V_HH-Fc binding agents bind to TcdA and/or TcdB with specificity. In certain aspects of the invention, the binding agents exhibit TcdA and/or TcdB neutralizing activity.

V_HH-IgG

The present invention also includes binding agents comprising V_HH peptide monomers joined to more of an antibody that the Fc domain alone. V_HH-IgG binding agents comprise one, two, three, four or more of the V_HH peptide monomers are joined to the light (kappa or lambda) and heavy chains of an IgG antibody lacking the variable regions of the antibody. Thus, the peptide monomers replace the variable regions of the antibody.

The V_HH peptide monomers may be any of those provided in Table 1 above and include 5D (SEQ ID NO:1), E3 (SEQ ID NO:3), AA6 (SEQ ID NO:5) and AH3 (SEQ ID NO:7) V_HH peptide monomers. Where two or more monomers are linked, the monomers may be linked by flexible peptide linkers, generally comprising between 10 and 20 amino acids. Suitable linkers include those linkers provided in Table 2, such as linker-1 (SEQ ID NO:9), linker-2 (SEQ ID NO:11), and linker-3 (SEQ ID NO:13).

V_HH-IgG binding agents include octameric binding agents comprising an IgG antibody and first, second, third and fourth V_HH peptide monomers, wherein the V_HH peptide monomers have binding specificity for an epitope of *Clostridium difficile* toxin A (TcdA) or toxin B (TcdB), wherein first and second V_HH peptide monomers are linked together and joined to amino termini of both light chains of the antibody, wherein the light chains lack the antibody variable regions, and wherein third and fourth V_HH peptide monomers are linked together and joined to amino termini of both heavy chains of the antibody, wherein the heavy chains lack the antibody variable regions. Because this binding agent has four V_HH peptide monomers, it can be monospecific (where all of the monomers bind the same epitope), bi-specific (where the monomers bind two different epitopes), tri-specific (where the monomers bind three different epitopes), or tetra-specific (where the monomers bind four different epitopes).

A specific example of a tetra-specific V_HH-IgG binding agent is the ABAB-IgG binding agent, a tetra-specific, octameric binding agent comprising an IgG antibody, two sets of linked first and second V_HH peptide monomers, and two sets of linked third and fourth V_HH peptide monomers, wherein the IgG antibody comprises two arms, each arm comprising a heavy chain lacking a variable region and a light chain lacking a variable region, and each chain having an amino terminus, wherein for each arm of the antibody, one set of linked first and second V_HH peptide monomers is joined to the amino terminus of the light chain, and one set of linked third and fourth V_HH peptide monomers is joined to the amino terminus of the heavy chain, and wherein the V_HH peptide monomers have binding specificity for an epitope of *Clostridium difficile* toxin A (TcdA) or toxin B (TcdB). This binding agent is termed "tetra-specific" as it recognizes four different toxin epitopes. It is termed "octameric" as it bears eight V_HH peptide monomers (two copies of the first monomer, two copies of the second monomer, two copies of the third monomer, and two copies of the fourth monomer). In certain aspects, the first, second, third and fourth V_HH peptide monomers may each have binding specificity for a different epitope. In certain aspects, two of the V_HH peptide monomers may have binding specificity for epitopes of TcdA and two of the V_HH peptide monomers may have binding specificity for epitopes of TcdB. In certain aspects, the V_HH peptide monomers independently have binding specificity for an epitope in the glucosyltransferase domain, cysteine protease domain, translocation domain or receptor binding domain of TcdA or TcdB.

A specific example of a tetra-specific, octameric ABAB-IgG binding agent comprises a light (kappa) chain having the amino acid sequence set forth in SEQ ID NO:46 (AA6/E3 kappa) or a sequence variant having at least 95% sequence identity thereto, and a heavy chain having the amino acid sequence set forth in SEQ ID NO:44 (AH3/5D heavy) or a sequence variant having at least 95% sequence identity thereto. In this aspect, the sequence variants retain toxin-neutralizing activity. The variant amino acids of the sequence variant may be located in framework regions of the V_HH peptide monomers. This binding agent was produced by preparing two separate expression vectors, the first encoding the V_HH peptide monomers AH3/5D (linked in the noted order) joined to the human IgG1 antibody heavy chain lacking the variable region and the second encoding the V_HH peptide monomers AA6/E3 (linked in the noted order) joined to the human IgG1 antibody light (kappa) chain lacking the variable region. The nucleotide sequence encoding the AA6/E3-IgG1 light (kappa) chain is provided in SEQ ID NO:47. The nucleotide sequence encoding the AH3/5D-IgG1 heavy chain is provided in SEQ ID NO:45. The invention includes sequence variants of ABAB-IgG having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity over the entire length of the protein sequence and retaining the toxin binding and/or neutralizing activity of the wild-type protein. The present invention further includes polynucleotide sequences encoding these sequence variants and complementary strands thereof.

Bi-specific or tetra-specific, tetrameric IgG binding agents are included in the invention. Such binding agents comprise an IgG antibody and first, second, third and fourth V_HH peptide monomers, wherein the IgG antibody comprises two arms, each arm comprising a heavy chain lacking a variable region and a light chain lacking a variable region, and each chain having an amino terminus, wherein for a first arm of the antibody, the first V_HH peptide monomer is joined to the amino terminus of the light chain, and the second V_HH peptide monomer is joined to the amino terminus of the heavy chain, wherein for a second arm of the antibody, the third V_HH peptide monomer is joined to the amino terminus of the light chain, and the fourth V_HH peptide monomer is joined to the amino terminus of the heavy chain, and where the V_HH peptide monomers have binding specificity for an epitope of *Clostridium difficile* toxin A (TcdA) or toxin B (TcdB). When the binding agent is "tetra-specific", it recognizes four different toxin epitopes; when "bi-specific" it recognizes two different toxin epitopes. The binding agents "tetrameric" as they bear four V_HH peptide monomers (when bi-specific, the first and second monomer have the same sequence and bind the same epitope, and the third and fourth monomers have the same sequence and bind the same epitope; when tetra-specific, each of the monomers has a different sequence and binds a different epitope).

When the binding agent is bi-specific, the first and third monomers have binding specificity for different epitopes, the first and second monomers have identical amino acid sequences, and the third and fourth monomers have identical amino acid sequences. In certain aspects, one of the V_HH peptide monomers has binding specificity for an epitope of TcdA and one of the V_HH peptide monomers has binding specificity for an epitope of TcdB.

When the binding agent is tetra-specific, each of the V_HH peptide monomers has binding specificity for a different

epitope. In certain aspects, two of the V_HH peptide monomers have binding specificity for epitopes of TcdA and two of the V_HH peptide monomers have binding specificity for epitopes of TcdB.

In certain aspects, each of the V_HH peptide monomers has binding specificity for epitopes of TcdA. In other aspects, each of the V_HH peptide monomers has binding specificity for epitopes of TcdB.

In certain aspects, the V_HH peptide monomers independently have binding specificity for an epitope in the glucosyltransferase domain, cysteine protease domain, translocation domain or receptor binding domain of TcdA or TcdB.

A specific example of a bi-specific, tetrameric IgG binding agent comprises a light (kappa) chain having the amino acid sequence set forth in SEQ ID NO:40 (AA6 kappa) and a heavy chain having the amino acid sequence set forth in SEQ ID NO:36 (AH3 heavy). The invention also includes sequence variants thereof having at least 95% sequence identity, where the sequence variant retains toxin neutralizing activity. The variant amino acids of the sequence variant may be located in framework regions of the V_HH peptide monomers.

Another specific example of a bi-specific, tetrameric IgG binding agent comprises a light (kappa) chain having the amino acid sequence set forth in SEQ ID NO:42 (E3 kappa) and a heavy chain having the amino acid sequence set forth in SEQ ID NO:38 (5D heavy). The invention also includes sequence variants thereof having at least 95% sequence identity, where the sequence variant retains toxin neutralizing activity. The variant amino acids of the sequence variant may be located in framework regions of the V_HH peptide monomers.

Table 5 provides the sequences used to generate bi-specific V_HH-IgG binding agents. Other suitable pairings include (i) 5D-IgG1-heavy chain+AA6-light (kappa or lambda) chain, and (ii) AH3-IgG1-heavy chain+E3-light (kappa or lambda) chain.

TABLE 5

Name	SEQ ID NO for Amino Acid Seq.	SEQ ID NO for Nucleic Acid Seq.
AH3-IgG1-heavy chain	36	37
5D-IgG1-heavy chain	38	39
AA6-IgG1-kappa chain	40	41
E3-IgG1-kappa chain	42	43

However, the present invention includes IgG1 heavy chains joined to any of AH3, 5D, AA6 and E3, and IgG1 light (kappa or lambda) chains joined to any of AH3, 5D, AA6 and E3. Further, all possible combinations of the heavy and light (kappa or lambda) chains are encompassed herein.

Humanized Binding Agents

Due to their small size and the high degree of identity of their framework to the human V_H framework of family III, V_HH peptide monomers are expected to exhibit low immunogenicity when administered to humans. While the systemic application of small monovalent V_HH monomers seems to induce little, if any, neutralizing antibody responses, protein immunogenicity generally increases with size and complexity. Two major hurdles for repeated and/or long-term in vivo use of V_HH monomers are their likely short half-life and potential immunogenicity. To increase the valence and circulating half-life, V_HH monomers can be fused with human IgG and Fc domains as discussed herein. To address possible immunogenicity, the V_HH monomers can be humanized as needed without compromising their expression level, affinity, solubility, and stability. These strategies should result in good expression, stability, and solubility of humanized V_HH monomers (hV_HH monomers), while retaining the antigen specificity and affinity of the loop donor V_HH.

hV_HH monomers that gain highest identity to human V_H gene(s) and possess the highest binding/neutralizing activity are selected, after which they are transformed into the V_HH-Fc and V_HH-IgG constructs to generate fully humanized binding agents, such as fully humanized ABAB-IgG and ABAB-Fc binding agents. The protein sequences of these humanized binding agents can be essentially identical to that of a human antibody variant, despite the non-human origin of some of its CDR segments that are responsible for the ability of the antibody to bind to its target antigen. Therefore, this strategy decreases the chance for potential immunogenicity in vivo and thus increase their safety and half-life in vivo.

The binding agents of the present invention thus encompasses humanized versions of each of the binding agents defined herein, comprising hV_HH peptide monomers.

Antibody Fragments

The binding agents of the invention include epitope binding fragments of each of the V_HH-Fc and V_HH-IgG binding agents defined herein. Because the V_HH-Fc and V_HH-IgG binding agents are comparable in structure to human IgG antibodies, where the variable regions are replace by the V_HH monomers, terms for human antibody fragments are also applicable to the such binding agents. The fragments include, but are not limited to, Fab fragments, $F(ab')_2$ fragments, single chain Fv (scFv) antibodies, and fragments produced by an Fab expression library, as well as bi-specific antibody and triple-specific antibodies.

The V_HH-Fc and V_HH-IgG binding agents of the invention include fully human, humanized, and chimeric binding agents. The binding agents may be monoclonal or polyclonal. Further, the binding agents may be recombinant binding agents.

The binding agents may be produced in any species of animal, though preferably from a mammal such as a human, simian, mouse, rat, rabbit, guinea pig, horse, cow, sheep, goat, pig, dog or cat. For example, the binding agents can be human or humanized, or any binding agent preparation suitable for administration to a human.

Polynucleotide, Expression Vectors, Host Cells and Method of Making

The invention includes polynucleotides comprising nucleotide sequences encoding each the binding agents provided herein, as well as complementary strands thereof.

The invention also includes expression vectors comprising the polynucleotides, and host cells comprising the expression vectors. Suitable expression vectors include, e.g., pcDNA3.1 and pSec-His. Suitable host cells include, e.g., Chinese hamster ovary cells (CHO cells) and human embryonic kidney cells 293 (HEK 293 cells).

The invention further includes methods of producing the binding agents defined herein, comprising culturing the host cells under conditions promoting expression of the binding agents encoded by the expression vectors, and recovering the binding agents from the cell cultures.

Methods of Treatment and Prevention

The binding agents of the invention can be used in methods of treating or preventing a disease symptom induced by *C. difficile* in a subject. These methods generally

comprise administering a therapeutically-effective amount of one or more binding agents as defined herein to a subject having *C. difficile* infection or a risk of developing *C. difficile* infection.

The binding agents of the invention can also be used in of neutralizing *C. difficile* toxin TcdA and/or TcdB in a subject infected by *C. difficile*. These methods generally comprise administering a therapeutically-effective amount of one or more binding agents as defined herein to a subject having *C. difficile* infection.

The binding agents of the invention can further be used in methods of treating *C. difficile* infection in a subject. These methods generally comprise administering a therapeutically-effective amount of one or more of the binding agents as defined herein to a subject having *C. difficile* infection. These same methods can be used to treat CDI, as defined herein.

The binding agents can also be used in immunoprophylaxis in order to prevent immediate CDI threats. In addition, passive immunoprophylaxis can be used to prevent both immediate and longer-term CDI threats. Each approach has its own particular advantages and is suitable to target a particular high-risk population. These methods generally comprises administering a therapeutically-effective amount of one or more of the binding agent as defined herein to a subject a risk of developing *C. difficile* infection.

Each of the methods of the invention may include administration of the one or more binding agents in a pharmaceutical formulation comprising the binding agents and a pharmaceutically acceptable carrier or diluent.

As used herein, the terms "treat", "treating", and "treatment" have their ordinary and customary meanings, and include one or more of: blocking, ameliorating, or decreasing in severity and/or frequency a symptom of a *C. difficile* infection or a *C. difficile*-related disease in a subject; and/or partly or fully inhibiting the biological activity and/or promoting the immunologic clearance of *C. difficile* TcdA and/or TcdB in a subject infected with *C. difficile*; and/or growth, division, spread, or proliferation of *C. difficile* cells or a *C. difficile* infection in a subject. Treatment means blocking, ameliorating, decreasing, or inhibiting by about 1% to about 100% versus a subject in which the methods of the present invention have not been practiced. Preferably, the blocking, ameliorating, decreasing, or inhibiting is about 100%, 99%, 98%, 97%, 96%, 95%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5% or 1% versus a subject in which the methods of the present invention have not been practiced.

As used herein, the terms "prevent", "preventing" and "prevention" have their ordinary and customary meanings, and include one or more of, stopping, averting, avoiding, alleviating or blocking *C. difficile* from colonizing, developing or progressing in a subject; and/or partly or fully inhibiting the biological activity and/or toxic effects of TcdA and/or TcdB in a subject infected with *C. difficile*; and/or stopping, averting, avoiding, alleviating or blocking the growth, division, spread, or proliferation of bacterial cells or bacterial infection in a subject. Prevention means stopping by at least about 95% versus a subject to which the prevention has not been administered. Preferably, the stopping is about 100%, about 99%, about 98%, about 97%, about 96% or about 95%. The results of the prevention may continue for a period of days (such as 1, 2, 3, 4, 5, 6 or 7 days), weeks (such as 1, 2, 3 or 4 weeks) or months (such as 1, 2, 3, 4, 5, 6 or more months).

The method of treating and preventing provided herein can be supplemented by also administering a therapeutically-effective amount of an antibiotic to the subject. Preferably, the antibiotic will have antibacterial activity against *C. difficile*.

Pharmaceutical Formulations

While the binding agents may be administered directly to a subject, the methods of the present invention are preferably based on the administration of a pharmaceutical formulation comprising one or more binding agents and a pharmaceutically acceptable carrier or diluent. Thus, the invention includes pharmaceutical formulations comprising one or more of the binding agents defined herein and a pharmaceutically acceptable carrier or diluent.

Pharmaceutically acceptable carriers and diluents are commonly known and will vary depending on the particular binding agent being administered and the mode of administration. Examples of generally used carriers and diluents include, without limitation: saline, buffered saline, dextrose, water-for-injection, glycerol, ethanol, and combinations thereof, stabilizing agents, solubilizing agents and surfactants, buffers and preservatives, tonicity agents, bulking agents, and lubricating agents. The formulations comprising binding agents will typically have been prepared and cultured in the absence of any non-human components, such as animal serum (e.g., bovine serum albumin).

Pharmaceutical formulations comprising one or more binding agents may be administered to a subject using modes and techniques known to the skilled artisan. Characteristic of CDI disease may make it more amenable to treatment and prevention using colonic delivery of therapeutic agents, i.e., targeted delivery of binding agents to the lower GI tract, e.g., the large intestine or colon. Other modes of delivery include, but are not limited to, oral, anal, via intravenous injection or aerosol administration. Other modes include, without limitation, intradermal, subcutaneous (s.c., s.q., sub-Q, Hypo), intramuscular (i.m.), intraperitoneal (i.p.), intra-arterial, intramedulary, intracardiac, intra-articular (joint), intrasynovial (joint fluid area), intracranial, intraspinal, and intrathecal (spinal fluids).

Depending on the means of administration, the dosage may be administered all at once, such as with an oral formulation in a capsule or liquid, or slowly over a period of time, such as with an intramuscular or intravenous administration.

The amount of binding agents, alone or in a pharmaceutical formulation, administered to a subject is an amount effective for the treatment or prevention of infection. Thus, therapeutically effective amounts are administered to subjects when the methods of the present invention are practiced. In general, between about 1 ug/kg and about 1000 mg/kg of the binding agent per body weight of the subject is administered. Suitable ranges also include between about 50 ug/kg and about 500 mg/kg, and between about 10 ug/kg and about 100 mg/kg. However, the amount of binding agent administered to a subject will vary between wide limits, depending upon the location, source, extent and severity of the infection, the age and condition of the subject to be treated, etc. A physician will ultimately determine appropriate dosages to be used.

Administration frequencies of the binding agents and pharmaceutical formulations comprising the binding agents will vary depending on factors that include the location of the bacterial infection, the particulars of the infection to be treated or prevented, and the mode of administration. Each formulation may be independently administered 4, 3, 2 or once daily, every other day, every third day, every fourth

day, every fifth day, every sixth day, once weekly, every eight days, every nine days, every ten days, bi-weekly, monthly and bi-monthly.

The duration of treatment or prevention will be based on location and severity of the infection being treated or the relative risk of contracting the infection, and will be best determined by the attending physician. However, continuation of treatment is contemplated to last for a number of days, weeks, or months.

In each embodiment and aspect of the invention, the subject is a human, a non-human primate, bird, horse, cow, goat, sheep, a companion animal, such as a dog, cat or rodent, or other mammal. The subjects to which the methods of the present invention can be applied include subjects having an underlying disease or condition that makes them more susceptible to *C. difficile* infections.

The invention also provides a kit comprising one or more containers filled with one or more binding agents or pharmaceutical formulations comprising binding agents. The kit may also include instructions for use. Associated with the kit may further be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

IV. Examples

V_HH Monomer and Heterodimer Binding Agents

An efficient platform to screen single domain (monomeric), mono-specific V_HH peptide monomers against specific domains of toxins TcdA and TcdB was established. Using highly immunogenic atoxic holotoxins for immunization, and bioactive chimeric toxins (with normal domain functions) for screening, panels of V_HH monomers binding to different domains of TcdA or TcdB were prepared. A majority of these V_HH monomers possessed potent neutralizing activity and their binding to specific domains was determined (FIG. **1**). The atoxic holotoxins have point mutations at their enzymatic glucosyltransferase domains as described previously (Wang et al., 2012). The bioactive chimeric toxins were created by switching the functional domains between TcdA and TcdB, which was also described previously (Wang, et al., 2012).

Several of the V_HH monomers bind to highly conserved TcdA/TcdB epitopes. For example, V_HH E3 binds to the Rho GTPase binding site and blocks glucosylation; V_HH AH3 binds to the GT domain of the toxin; V_HH 7F binds to cysteine protease cleavage sites and blocks GT domain cleavage and release. Some V_HH monomers have potent neutralizing activity capable of blocking toxin cytotoxic activity at nM concentrations (See Table 1; FIGS. **2**A and B).

To enhance the binding activity, two domain (dimeric), bi-specific V_HH heterodimers were created (Table 3; FIG. **2**C), allowing a single protein to target two distinctive epitopes of the toxins. These bi-specific V_HH heterodimers possessed substantially enhanced neutralizing activities compared with equimolar mixtures of the same two V_HH monomers (FIG. **2**D). Heterodimers 5D/E3 and AH3/AA6 were found to fully protect mice from lethal systemic TcdB or TcdA challenge respectively, whereas mixed 5D and E3, or AA6 alone were only partially protective (FIGS. **2**E and F).

The V_HH monomers comprising the heterodimers were linked using a flexible linker selected from SEQ ID NOs: 9-13 (Table 2).

ABAB Binding Agent

A four domain (tetrameric), tetra-specific V_HH binding agent termed ABAB was generated by linking V_HH monomers AH3, 5D, E3, and AA6. This tetra-specific, tetrameric binding agent targets conserved, non-overlapping epitopes and it has excellent toxin neutralizing activity. In the design of ABAB (FIG. **3**), V_HH peptide monomers AH3 and AA6 were separated by placing the 5D monomers between them because AH3 and AA6 bind to GT and TD respectively (FIG. **1**), which are spatially distant to each other. This design allowed AH3 and AA6 to bind to TcdA simultaneously.

In the construction of the ABAB binding agent, flexible linkers were placed between the V_HH monomers (see FIG. **3**). The complete nucleic acid sequence encoding ABAB is provided in SEQ ID NO:20; the amino acid sequence of the protein is provided in SEQ ID NO:19.

In certain variants, a $His_{(6)}$-tag was provided at the amino terminus of the protein to aid in purification, an E-tag was provided at the carboxy terminus of the protein to aid in detection, and/or an albumin-binding peptide (ABP, DICLPRWGCLWD; SEQ ID NO:21) was placed at the carboxyl end of the construct to increase serum half-life of the protein (See FIG. **3**).

ABAB was found to exhibit substantial enhanced binding affinity (Table 6) and neutralizing activity (Table 7) over the individual monomers. In Table 7, Vero cells were exposed to 5 ng/ml of TcdA in the presence of serially diluted AA6, AH3, ABAB or Merck anti-TcdA HuMab (Lowy et al., 2010). The minimal doses of antibodies protecting cells from TcdA-induced cell rounding are shown.

TABLE 6

	V_HHs	K_{on} (Ms^{-1})	K_{off} (s^{-1})	K_D (nM)
TcdA	AH3	2.20×10^4	7.10×10^{-4}	32.0
	AA6	3.52×10^4	6.92×10^{-4}	19.7
	ABAB	6.96×10^5	1.21×10^{-6}	0.002
TcdB	5D	1.52×10^6	9.94×10^{-4}	0.65
	E3	2.95×10^6	9.4×10^{-5}	0.03
	ABAB	1.79×10^6	3.57×10^{-6}	0.002

TABLE 7

AA6	AH3	ABAB	Merck Anti-TcdA HuMab
8 nM	8 nM	0.25 nM	>10 nM

ABAB was also found to compete with all four individual V_HH peptide monomers in a competition ELISA and can simultaneously bind to both TcdA and TcdB as determined by sandwich ELISA. Furthermore, ABAB is broadly reactive, capable of neutralizing toxins from the 13 different *C. difficile* strains that represent most of the current epidemic strains.

Since ABAB shows high potency in binding to and neutralizing both toxins, its efficacy in treating fulminant CDI was evaluated. A single injection with as low as 40 μg/kg of ABAB one-day post *C. difficile* spore challenge reversed fulminant CDI in mice. None of the ABAB-treated mice died whereas 50% of control mice became moribund by 3 days post-infection (FIG. **4**, left panel). ABAB is 4-log more potent in preventing mortality after systemic challenge

with TcdA and TcdB than the Merck HuMabs (FIG. **4**, right panel) (Lowy et al., 2010). Thus, ABAB possesses extraordinary in vivo efficacy against *C. difficile* toxins and spore challenge.

Animal and human studies demonstrated that passively administered antitoxin antibodies provide protection against CDI. The initial studies here also showed that antitoxin polysera protected mice from primary CDI (FIG. **5**) and recurrent/relapse CDI. These findings and results from FIG. **4** supported the hypothesis and provided the rationale for development of a parenteral ABAB immunization strategy for preventing CDI. To achieve the goal of optimizing ABAB for systemic delivery, chimeric and humanized ABAB were generated as illustrated in FIG. **6**, i.e., V_HH-Fc and V_HH-IgG binding agents as well as the humanized proteins hV_HH-Fc and hV_HH-IgG, after which leading proteins were evaluated for in vivo neutralizing activity and protection in animal models. Details regarding the preparation and testing of the additional binding agents are provided in the following paragraphs.

ABAB-Fc

ABAB-Fc binding agent was prepared by generating an expression vector encoding the V_HH peptide monomers AH3/5D/AA6/E3 (linked in the noted order) joined to a human IgG1 Fc domain. The V_HH peptide monomers were separated by flexible linkers of Table 2. The nucleic acid sequence encoding the protein is provided in SEQ ID NO:23. ABAB-Fc was expressed and purified from stable transfected HEK293 cell line culture supernatant using protein A beads (FIG. **7**) under conditions permitting disulfide bond formation and bi-valent molecule production. The expression levels were about 20 mg/L of culture supernatant. ABAB-Fc is fully functional in binding and neutralizing both TcdA and TcdB. The amino acid sequence of ABAB-Fc is provided in SEQ ID NO:22.

Mono-specific V_HH-Fc binding agents (AH3-Fc, 5D-Fc, E3-Fc, AA6-Fc) and bi-specific V_HH-Fc binding agents (AH3/5D-Fc) and AA6/E3-Fc) were also made using this Fc-fusion system. Table 4 above provides the sequences for these additional binding agents.

ABAB-IgG

As illustrated in FIG. **6**, bi-specific V_HH-IgG (AH3/5D-IgG and E3/AA6-IgG) can be generated by fusing monomers with human IgG heavy and light (kappa or lambda) chains separately. Tetra-specific V_HH-IgG (ABAB-IgG) binding agents can be generated by fusing dimers with human IgG heavy and light chains separately. Co-transfecting the heavy and light chain constructs generates the AH3/5D-IgG, E3/AA6-IgG and ABAB-IgG chimeric proteins. The separation of two V_HHs into heavy and light chains likely improves the yield and stability of bi-specific and tetra-specific V_HH chimeric proteins. This allows determination of whether V_HH-human IgG chimeric antibody helps the stability and efficacy of ABAB in vivo. Similarly, further improvement of in vivo half-life of ABAB-IgG can also be tested in ABAB-IgG variants with enhanced binding affinity to FcRn receptor.

Bi-specific (AH3/5D-IgG1 and E3/AA6-IgG1) and tetra-specific (ABAB-IgG1) IgG1 binding agents were prepared by co-transfecting expression vectors encoding the heavy and light (kappa) chain of each binding agent. The V_HH peptide monomers were separated by flexible linkers of Table 2.

Bi-specific, tetrameric V_HH-IgG1 binding agents were produced by preparing two separate expression vectors, the first encoding a V_HH peptide monomer joined to the human IgG1 antibody heavy chain (CH1-Hinge-CH2-CH3) lacking the heavy chain variable region and the second encoding a V_HH peptide monomer joined to the human IgG1 antibody light (kappa) chain (CK) lacking the light chain variable region. These binding agents are bi-specific and tetrameric in that each light chain of the resulting binding agent is linked to a first V_HH monomer and each heavy chain of the resulting binding agent is linked to a second V_HH monomer. Table 5 above provides the sequences for these additional binding agents. Suitable pairings include (i) AH3-IgG1-heavy chain+AA6-light (kappa or lambda) chain, (ii) 5D-IgG1-heavy chain+E3-light (kappa or lambda) chain, (iii) 5D-IgG1-heavy chain+AA6-light (kappa or lambda) chain, and (iv) AH3-IgG1-heavy chain+E3-light (kappa or lambda) chain.

Tetra-specific, octameric ABAB-IgG binding agents were prepared. These binding agents are tetra-specific and octameric in that each light (kappa or lambda) chain of the resulting binding agent is joined to two (a first and second) linked V_HH monomers and each heavy chain of the resulting binding agent is joined to a two (a third and fourth) linked V_HH monomer, where the first, second, third and fourth monomers binds to a different epitope.

A particular tetra-specific, octameric ABAB-IgG (FIG. **8**) binding agent was produced by preparing two separate expression vectors, the first encoding the V_HH peptide monomers AH3/5D (linked in the noted order) joined to the human IgG1 antibody heavy chain (CH1-Hinge-CH2-CH3) lacking the heavy chain variable region and the second encoding the V_HH peptide monomers AA6/E3 (linked in the noted order) joined to the human IgG1 antibody light (kappa) chain (CK) lacking the light chain variable region. The nucleotide sequence encoding the AH3/5D-IgG1 heavy chain is provided in SEQ ID NO:45; the amino acid sequence is provided in SEQ ID NO:44. The nucleotide sequence encoding the AA6/E3-IgG1 kappa chain is provided in SEQ ID NO:47; the amino acid sequence is provided in SEQ ID NO:46.

The bi-specific (AH3/5D-IgG1 and E3/AA6-IgG1) and tetra-specific (ABAB-IgG1) IgG1 binding agents were expressed and purified from stable transfected HEK293 cell line culture supernatant using protein A beads (see FIG. **9** for ABAB-IgG1) under conditions permitting disulfide bond formation and bi-valent molecule production. SDS-PAGE shows more than 90% purity of the purified ABAB-IgG1 with total molecular weight (light and heavy chains together) around 218 KDa on non-reduced gel (FIG. **10**). The molecular weight of heavy chain is 68 KDa and light chain is 41 KDa showed on reduced gel.

The binding of ABAB-IgG1 to TcdA and TcdB was determined. FIGS. **11**A-**11**B illustrate the comparison of binding ABAB-IgG1 to both toxins with the individual components (AH3, AA6, E3, and 5D). FIG. **11**A shows the results of experiments where plates were coated with 1 ug/ml TcdA (TxA). Serially diluted ABAB-IgG was added in concentrations of 0, 0.64, 3.2, 16, 80, 400 and 2,000 ng/ml. The plates were washed and Merck Ab (anti-TcdA), Fc-ABBA (ABAB-Fc), Habab (ABAB-IgG), and V_HH anti-TcdB monomers AA6 and AH3 were added in the indicated amounts (ng/ml). Appropriate labeled antibodies were used for detection. FIG. **11**B shows the results of experiments where plates were coated with 1 ug/ml TcdB (TxB). Serially diluted ABAB-IgG was added in concentrations of 0, 0.64, 3.2, 16, 80 and 400 ng/ml. The plates were washed and Merck Ab (Anti-TcdB), Fc-abba (ABAB-Fc), Habab (ABAB-IgG), and V_HH anti-TcdB monomers E3 and 5D were added in the indicated amounts (ng/ml). Appropriate labeled antibodies were used for detection.

As expected, the tetra-specific antibody can bind to TcdA and TcdB simultaneously as determined by sandwich ELISA (FIGS. **12**A-**12**B). In a first set of experiments, plates were coated with 1 ug/ml TcdA (TxA). Serially diluted ABAB-IgG (Hababb) was added in concentrations of 0, 1.6, 8, 40, 200 and 1000 ng/ml. The plates were washed and the following amounts of TcdB were added: 1.6, 8, 40, 200, and 1000 ng/ml. Mouse anti-TxB antibodies (500×) and goat anti-mouse-IgG-HRP (3000×) antibodies were used for detection. The results provided in FIG. **12**A show that TxB is detected by coating TxA, suggesting IgG-ABAB binds to TxA/B simultaneously. In a second set of experiments, plates were coated with 1 ug/ml TcdB (TxB). Serially diluted ABAB-IgG (Habab) was added in concentrations of 0, 1.6, 8, 40, 200 and 1000 ng/ml. The plates were washed and the following amounts of TcdA were added: 1.6, 8, 40, 200, and 1000 ng/ml. Mouse anti-TxA antibodies (500×) and goat anti-mouse-IgG-HRP (3000×) antibodies were used for detection. The results provided in FIG. **12**B show that TxA is detected by coating TxB, again suggesting IgG-ABAB binds to TxA/B simultaneously.

The neutralizing activities of ABAB-IgG1 against cytopathic effects of the toxins on cultured cells were also examined. TcdA (100 ng/ml, FIG. **13**A) was mixed with serially diluted Merck anti-TcdA human monoclonal antibody, ABAB-IgG1 (Hababa), and V_HH anti-TcdA monomers AA6 and AH3 before adding to Vero cell monolayers in 100 ul culture medium and incubated at 37° C. for 24 hours. The results provided in FIG. **13**A show that ABAB-IgG1 is at least 1000-fold more potent than Merck antibodies in neutralizing TcdA. In similar experiments, TcdB (10 pg/ml, FIG. **13**B) was mixed with serially diluted Merck anti-TcdB human monoclonal antibody, ABAB-IgG1 (Hababa), and V_HH anti-TcdB monomers E3 and 5D before adding to Vero cell monolayers in 100 ul culture medium and incubated at 37° C. for 24 hours. The results provided in FIG. **13**B show that ABAB-IgG1 is at least 1000-fold more potent than Merck antibodies in neutralizing TcdB.

The in vivo neutralizing activities of ABAB-IgG1 were studied in a mouse model of CDI, the results of which are shown in FIG. **14**. Mice were challenged with lethal dose of a mixed TcdA and TcdB (25 ng each toxin per mouse) and 4 hour later, ABAB-IgG (10, 30 or 100 ug/kg), a mixture of Merck anti-toxin A and anti-toxin B antibodies (10 mg/kg) or PBS was administered to the mice. The results demonstrate that the neutralizing activity of ABAB-IgG was much greater than the Merck antibody, and at lower concentrations.

Animal Testing of ABAB-IgG

The ABAB-IgG1 binding agent was tested in both prophylactic treatment and re-challenge survival assays. FIG. **15** provides the experimental design of both studies. 6-8 week old female C57 mice were used, and the conditions included PBS: 10 ml/kg, i.p., n=14; ABAB-IgG: 200 ug/kg, i.p., n=10; ABAB-IgG: 1 mg/kg, i.p., n=10; ABAB-IgG: 5 mg/kg, i.p., n=10.

The table in FIG. **16** provides a summary of the results seen with prophylactic treatment of mice against *C. difficile* spores. ABAB-IgG or PBS was administered one day prior to administrating of *C. difficile* spores. As can be seen, ABAB-IgG showed dose-related prophylactic protection against CDI, where 5 mg/kg showed complete protection on all the parameters examined and 200 ug/kg was found to be more potent than 200 ug/kg of bi-specific V_HH fusion antibody ABA (Yang et al., 2014).

The table in FIG. **17** provides a summary of the results seen with re-challenge of mice against *C. difficile* spores. ABAB-IgG or PBS was administered 15 days prior to administrating of *C. difficile* spores. As can be seen, one dose of ABAB-IgG showed some protection against the CDI caused by re-challenge of spores, but the protection was much less efficient compared to that during the primary challenge. This may be due to the drop of the antibody level with time and the generation of antibody in the PBS group following primary challenge.

Expression, Purification and Evaluation of Binding Agents

A variety of selection criteria is used to select the binding agents generated in the experiments described in the approaches herein. First, each of the constructs defined herein can be used in transient transfections of 293T cells for making small-scale recombinant proteins by Protein A affinity chromatography. The production yield of each construct can be determined by quantitative ELISA. Second, binding activity of recombinant proteins can be screened using ELISA and surface plasmon resonance (SPR) to select constructs that preserve their original binding activities against the toxins. Third, the proteins are evaluated for neutralizing activity in in vitro assays (FIG. **2**).

Accumulating observations indicate that polyreactivity and/or autoreactivity of in vivo recombinant binding agents are potential issues related to their in vivo safety and half-life. The application of the selected ABAB binding agents as a systemic binding agent for preventing primary acute CDI likely requires that the chimeric and humanized ABAB proteins are limited in polyreactivity and/or autoreactivity. Progress in protein proteomics has made it possible to screen for polyreactivity and autoreactivity of recombinant antibodies in vitro, which is a great tool for surrogate therapeutic antibodies. Therefore, selected humanized binding agents with good yield, high binding affinity, and potent neutralizing activity can be further tested for potential polyreactivity and autoreactivity using the auto-antigen microarray test and ProtoArray protein microarrays (Invitrogen).

From the above in vitro assays, candidate ABAB-Fc and ABAB-IgG binding agents can be evaluated for their in vivo toxicity, serum half-life, and immunogenicity.

While the invention has been described with reference to certain particular embodiments thereof, those skilled in the art will appreciate that various modifications may be made without departing from the spirit and scope of the invention. The scope of the appended claims is not to be limited to the specific embodiments described.

REFERENCES

All patents and publications mentioned in this specification are indicative of the level of skill of those skilled in the art to which the invention pertains. Each cited patent and publication is incorporated herein by reference in its entirety. All of the following references have been cited in this application:

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Jachimska, B.; Wasilewska, M.; Adamczyk, Z., Characterization of globular protein solutions by dynamic light scattering, electrophoretic mobility, and viscosity measurements. *Langmuir* 24 (13), 6866-6872 (2008).

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Perdana, J.; Fox, M. B.; Schutyser, M. A. I.; Boom, R. M., Mimicking Spray Drying by Drying of Single Droplets Deposited on a Flat Surface. *Food Bioprocess Tech* 6 (4), 964-977 (2013).

Wang, H., et al. A chimeric toxin vaccine protects against primary and recurrent *Clostridium difficile* infection. *Infect Immun* 80, 2678-2688 (2012).

Yang, Z., et al. A novel multivalent, single-domain antibody targeting TcdA and TcdB prevents fulminant *Clostridium difficile* infection in mice. *J Infect Dis.* 210(6), 964-72 (2014).

SEQUENCE LISTING

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<223> OTHER INFORMATION: AH3-5D heterodimer

<400> SEQUENCE: 16

caggtacagc tggtggagac gggggggaggg ctggtacaac caggcgggtc actgaggctt 60

tcctgtgccg catctgggtt cacactggat tattcgtcca tagggtggtt tcggcaggct 120

cctggcaaag agcgtgaggg ggtctcatgt attagtagta gtggtgatag cacaaagtac 180

gccgattccg taaagggccg gtttacaacc tccagggata atgctaagaa caccgtatat 240

ctccagatga actctctgaa gcccgacgat acggccgtat attactgtgc ggctttcagg 300

-continued

gcgactatgt gcggcgtgtt ccctctgagc ccttacggca aggacgactg gggcaagggg 360

accctggtga ccgtatcctc aggcggtgga gggtctggtg ggggaggctc agggggtgga 420

ggcagccagg tgcaactggt tgaatctggg ggaggcttgg tacaacctgg gggatccctg 480

agactctctt gcgaggcctc cggattcacc ttggactact atggcatcgg ctggttccgc 540

cagcccccag ggaaggagcg ggaggccgtt tcatacatta gtgccagtgc ccggaccata 600

ctgtacgcag actctgtgaa gggacgcttt accatctcta gggacaatgc caaaaatgct 660

gtgtacctgc aaatgaacag cctcaagcgg gaggataccg cagtgtacta ctgcgcgaga 720

cggcgcttct ccgcttctag cgtgaataga tggctggccg acgactacga cgtgtgggga 780

cggggcacac aggtggctgt ctcgagc 807

<210> SEQ ID NO 17
<211> LENGTH: 247
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AA6-E3 heterodimer

<400> SEQUENCE: 17

Gln Leu Gln Leu Val Glu Thr Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Val Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Pro Glu Trp Ile
35 40 45

Ala Thr Ile Asn Thr Asp Gly Ser Thr Met Arg Asp Asp Ser Thr Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu
65 70 75 80

Gln Met Thr Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys Ala
85 90 95

Arg Gly Arg Val Ile Ser Ala Ser Ala Ile Arg Gly Ala Val Arg Gly
100 105 110

Pro Gly Thr Gln Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
115 120 125

Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Glu Ser Gly
130 135 140

Gly Gly Leu Val Gln Thr Gly Gly Ser Leu Arg Leu Ser Cys Ala Ser
145 150 155 160

Ser Gly Ser Ile Ala Gly Phe Glu Thr Val Thr Trp Ser Arg Gln Ala
165 170 175

Pro Gly Lys Ser Leu Gln Trp Val Ala Ser Met Thr Lys Thr Asn Asn
180 185 190

Glu Ile Tyr Ser Asp Ser Val Lys Gly Arg Phe Ile Ile Ser Arg Asp
195 200 205

Asn Ala Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu
210 215 220

Asp Thr Gly Val Tyr Phe Cys Lys Gly Pro Glu Leu Arg Gly Gln Gly
225 230 235 240

Ile Gln Val Thr Val Ser Ser
245

<210> SEQ ID NO 18
<211> LENGTH: 741

-continued

<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AA6-E3 heterodimer

<400> SEQUENCE: 18

caactgcagc tggtagagac aggggggcggc ttagttcagc ctggagggtc tctcagactg 60

tcatgcgctg cctctggctt taccttcagt gactacgtga tgacatgggt ccgccaagct 120

ccagggaagg ggcctgagtg gatcgctact attaatacag atggcagcac aatgcgggac 180

gactccacaa aggggcggtt caccatttcc agagacaacg ccaagaatac tctgtacctt 240

cagatgacca gtctgaaacc cgaggacact gctctgtact attgtgcaag aggccgggtg 300

atctctgctt ccgctatcag aggcgcagta aggggccctg gaacacaggt aaccgtttca 360

tccgggggag gcggttcagg cggtggggga tctggcgggg gtggatccca agttcagctg 420

gtcgaatccg ggggcggact ggtccagaca gggggctccc tgaggctctc ctgtgcatct 480

tccggaagca tcgccggctt cgagaccgtg acctggtctc gccaggctcc cgggaagtct 540

ctgcagtggg tcgcttccat gactaagact aacaacgaga tctactctga ctcagtgaaa 600

ggccgcttca tcatttctag agataacgct aaaaacacag tgtatctgca gatgaatagt 660

ctcaaacctg aagacacagg cgtgtatttc tgtaagggtc ctgagctgag gggccagggc 720

atccaggtaa cagtctcgag t 741

<210> SEQ ID NO 19
<211> LENGTH: 532
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ABAB binding agent

<400> SEQUENCE: 19

Gln Val Gln Leu Val Glu Thr Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Leu Asp Tyr Ser
20 25 30

Ser Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val
35 40 45

Ser Cys Ile Ser Ser Ser Gly Asp Ser Thr Lys Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Thr Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Phe Arg Ala Thr Met Cys Gly Val Phe Pro Leu Ser Pro Tyr
100 105 110

Gly Lys Asp Asp Trp Gly Lys Gly Thr Leu Val Thr Val Ser Ser Gly
115 120 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val
130 135 140

Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu
145 150 155 160

Arg Leu Ser Cys Glu Ala Ser Gly Phe Thr Leu Asp Tyr Tyr Gly Ile
165 170 175

Gly Trp Phe Arg Gln Pro Pro Gly Lys Glu Arg Glu Ala Val Ser Tyr
180 185 190

-continued

Ile Ser Ala Ser Ala Arg Thr Ile Leu Tyr Ala Asp Ser Val Lys Gly
195 200 205

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ala Val Tyr Leu Gln
210 215 220

Met Asn Ser Leu Lys Arg Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
225 230 235 240

Arg Arg Phe Ser Ala Ser Ser Val Asn Arg Trp Leu Ala Asp Asp Tyr
245 250 255

Asp Val Trp Gly Arg Gly Thr Gln Val Ala Val Ser Ser Gly Gly Gly
260 265 270

Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gln Leu Gln
275 280 285

Leu Val Glu Thr Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg
290 295 300

Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr Val Met Thr
305 310 315 320

Trp Val Arg Gln Ala Pro Gly Lys Gly Pro Glu Trp Ile Ala Thr Ile
325 330 335

Asn Thr Asp Gly Ser Thr Met Arg Asp Asp Ser Thr Lys Gly Arg Phe
340 345 350

Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln Met Thr
355 360 365

Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Arg Gly Arg
370 375 380

Val Ile Ser Ala Ser Ala Ile Arg Gly Ala Val Arg Gly Pro Gly Thr
385 390 395 400

Gln Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
405 410 415

Gly Gly Gly Gly Ser Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu
420 425 430

Val Gln Thr Gly Gly Ser Leu Arg Leu Ser Cys Ala Ser Ser Gly Ser
435 440 445

Ile Ala Gly Phe Glu Thr Val Thr Trp Ser Arg Gln Ala Pro Gly Lys
450 455 460

Ser Leu Gln Trp Val Ala Ser Met Thr Lys Thr Asn Asn Glu Ile Tyr
465 470 475 480

Ser Asp Ser Val Lys Gly Arg Phe Ile Ile Ser Arg Asp Asn Ala Lys
485 490 495

Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Gly
500 505 510

Val Tyr Phe Cys Lys Gly Pro Glu Leu Arg Gly Gln Gly Ile Gln Val
515 520 525

Thr Val Ser Ser
530

<210> SEQ ID NO 20
<211> LENGTH: 1596
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ABAB binding agent

<400> SEQUENCE: 20

caggtacagc tggtggagac gggggggaggg ctggtacaac caggcgggtc actgaggctt 60

tcctgtgccg catctgggtt cacactggat tattcgtcca tagggtggtt tcggcaggct 120

-continued

cctggcaaag agcgtgaggg ggtctcatgt attagtagta gtggtgatag cacaaagtac 180

gccgattccg taaagggccg gtttacaacc tccagggata atgctaagaa caccgtatat 240

ctccagatga actctctgaa gcccgacgat acggccgtat attactgtgc ggctttcagg 300

gcgactatgt gcggcgtgtt ccctctgagc ccttacggca aggacgactg gggcaagggg 360

accctggtga ccgtatcctc aggcggtgga gggtctggtg ggggaggctc agggggtgga 420

ggcagccagg tgcaactggt tgaatctggg ggaggcttgg tacaacctgg gggatccctg 480

agactctctt gcgaggcctc cggattcacc ttggactact atggcatcgg ctggttccgc 540

cagccccag ggaaggagcg ggaggccgtt tcatacatta gtgccagtgc ccggaccata 600

ctgtacgcag actctgtgaa gggacgcttt accatctcta gggacaatgc caaaaatgct 660

gtgtacctgc aaatgaacag cctcaagcgg gaggataccg cagtgtacta ctgcgcgaga 720

cggcgcttct ccgcttctag cgtgaataga tggctggccg acgactacga cgtgtgggga 780

cggggcacac aggtggctgt gtcttccggt ggcggaagcg gaggggggcag cgggggtggg 840

agcggtgggg gcagccaact gcagctggta gagacagggg gcggcttagt tcagcctgga 900

gggtctctca gactgtcatg cgctgcctct ggctttacct tcagtgacta cgtgatgaca 960

tgggtccgcc aagctccagg gaaggggcct gagtggatcg ctactattaa tacagatggc 1020

agcacaatgc gggacgactc cacaaagggg cggttcacca tttccagaga caacgccaag 1080

aatactctgt accttcagat gaccagtctg aaacccgagg acactgctct gtactattgt 1140

gcaagaggcc gggtgatctc tgcttccgct atcagaggcg cagtaagggg ccctggaaca 1200

caggtaaccg tttcatccgg gggaggcggt tcaggcggtg ggggatctgg cgggggtgga 1260

tcccaagttc agctggtcga atccgggggc ggactggtcc agacaggggg ctccctgagg 1320

ctctcctgtg catcttccgg aagcatcgcc ggcttcgaga ccgtgacctg gtctcgccag 1380

gctcccggga agtctctgca gtgggtcgct tccatgacta agactaacaa cgagatctac 1440

tctgactcag tgaaaggccg cttcatcatt tctagagata acgctaaaaa cacagtgtat 1500

ctgcagatga atagtctcaa acctgaagac acaggcgtgt atttctgtaa gggtcctgag 1560

ctgaggggcc agggcatcca ggtaacagtc tcgagt 1596

<210> SEQ ID NO 21
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Albumin-binding peptide

<400> SEQUENCE: 21

Asp Ile Cys Leu Pro Arg Trp Gly Cys Leu Trp Asp
1 5 10

<210> SEQ ID NO 22
<211> LENGTH: 761
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ABAB-Fc binding agent

<400> SEQUENCE: 22

Gln Val Gln Leu Val Glu Thr Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Leu Asp Tyr Ser
20 25 30

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```
Ser Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val
        35                  40                  45

Ser Cys Ile Ser Ser Ser Gly Asp Ser Thr Lys Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Thr Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Lys Pro Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Ala Phe Arg Ala Thr Met Cys Gly Val Phe Pro Leu Ser Pro Tyr
            100                 105                 110

Gly Lys Asp Asp Trp Gly Lys Gly Thr Leu Val Thr Val Ser Ser Gly
        115                 120                 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val
    130                 135                 140

Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu
145                 150                 155                 160

Arg Leu Ser Cys Glu Ala Ser Gly Phe Thr Leu Asp Tyr Tyr Gly Ile
                165                 170                 175

Gly Trp Phe Arg Gln Pro Pro Gly Lys Glu Arg Glu Ala Val Ser Tyr
            180                 185                 190

Ile Ser Ala Ser Ala Arg Thr Ile Leu Tyr Ala Asp Ser Val Lys Gly
        195                 200                 205

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ala Val Tyr Leu Gln
    210                 215                 220

Met Asn Ser Leu Lys Arg Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
225                 230                 235                 240

Arg Arg Phe Ser Ala Ser Ser Val Asn Arg Trp Leu Ala Asp Asp Tyr
                245                 250                 255

Asp Val Trp Gly Arg Gly Thr Gln Val Ala Val Ser Ser Gly Gly Gly
            260                 265                 270

Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gln Leu Gln
        275                 280                 285

Leu Val Glu Thr Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg
    290                 295                 300

Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr Val Met Thr
305                 310                 315                 320

Trp Val Arg Gln Ala Pro Gly Lys Gly Pro Glu Trp Ile Ala Thr Ile
                325                 330                 335

Asn Thr Asp Gly Ser Thr Met Arg Asp Asp Ser Thr Lys Gly Arg Phe
            340                 345                 350

Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln Met Thr
        355                 360                 365

Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Arg Gly Arg
    370                 375                 380

Val Ile Ser Ala Ser Ala Ile Arg Gly Ala Val Arg Gly Pro Gly Thr
385                 390                 395                 400

Gln Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
                405                 410                 415

Gly Gly Gly Gly Ser Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu
            420                 425                 430

Val Gln Thr Gly Gly Ser Leu Arg Leu Ser Cys Ala Ser Ser Gly Ser
        435                 440                 445
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```
Ile Ala Gly Phe Glu Thr Val Thr Trp Ser Arg Gln Ala Pro Gly Lys
    450                 455                 460

Ser Leu Gln Trp Val Ala Ser Met Thr Lys Thr Asn Asn Glu Ile Tyr
465                 470                 475                 480

Ser Asp Ser Val Lys Gly Arg Phe Ile Ile Ser Arg Asp Asn Ala Lys
                485                 490                 495

Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Gly
            500                 505                 510

Val Tyr Phe Cys Lys Gly Pro Glu Leu Arg Gly Gln Gly Ile Gln Val
        515                 520                 525

Thr Val Ser Ser Gly Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro
    530                 535                 540

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
545                 550                 555                 560

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
                565                 570                 575

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
            580                 585                 590

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
        595                 600                 605

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
    610                 615                 620

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
625                 630                 635                 640

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
                645                 650                 655

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met
            660                 665                 670

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
        675                 680                 685

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
    690                 695                 700

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
705                 710                 715                 720

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
                725                 730                 735

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
            740                 745                 750

Lys Ser Leu Ser Leu Ser Pro Gly Lys
        755                 760
```

<210> SEQ ID NO 23
<211> LENGTH: 2286
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ABAB-Fc binding agent

<400> SEQUENCE: 23

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caggtacagc tggtggagac gggggggaggg ctggtacaac caggcgggtc actgaggctt     60

tcctgtgccg catctgggtt cacactggat tattcgtcca tagggtggtt tcggcaggct    120

cctggcaaag agcgtgaggg ggtctcatgt attagtagta gtggtgatag cacaaagtac    180

gccgattccg taaagggccg gtttacaacc tccagggata atgctaagaa caccgtatat    240

ctccagatga actctctgaa gcccgacgat acggccgtat attactgtgc ggctttcagg    300
```

-continued

gcgactatgt gcggcgtgtt ccctctgagc ccttacggca aggacgactg gggcaagggg 360

accctggtga ccgtatcctc aggcggtgga gggtctggtg ggggaggctc agggggtgga 420

ggcagccagg tgcaactggt tgaatctggg ggaggcttgg tacaacctgg gggatccctg 480

agactctctt gcgaggcctc cggattcacc ttggactact atggcatcgg ctggttccgc 540

cagcccccag ggaaggagcg ggaggccgtt tcatacatta gtgccagtgc ccggaccata 600

ctgtacgcag actctgtgaa gggacgcttt accatctcta gggacaatgc caaaaatgct 660

gtgtacctgc aaatgaacag cctcaagcgg gaggataccg cagtgtacta ctgcgcgaga 720

cggcgcttct ccgcttctag cgtgaataga tggctggccg acgactacga cgtgtgggga 780

cggggcacac aggtggctgt gtcttccggt ggcggaagcg gaggggggcag cgggggtggg 840

agcggtgggg gcagccaact gcagctggta gagacagggg gcggcttagt tcagcctgga 900

gggtctctca gactgtcatg cgctgcctct ggctttacct tcagtgacta cgtgatgaca 960

tgggtccgcc aagctccagg gaaggggcct gagtggatcg ctactattaa tacagatggc 1020

agcacaatgc gggacgactc cacaaagggg cggttcacca tttccagaga caacgccaag 1080

aatactctgt accttcagat gaccagtctg aaacccgagg acactgctct gtactattgt 1140

gcaagaggcc gggtgatctc tgcttccgct atcagaggcg cagtaagggg ccctggaaca 1200

caggtaaccg tttcatccgg gggaggcggt tcaggcggtg ggggatctgg cgggggtgga 1260

tcccaagttc agctggtcga atccgggggc ggactggtcc agacaggggg ctccctgagg 1320

ctctcctgtg catcttccgg aagcatcgcc ggcttcgaga ccgtgacctg gtctcgccag 1380

gctcccggga agtctctgca gtgggtcgct tccatgacta agactaacaa cgagatctac 1440

tctgactcag tgaaaggccg cttcatcatt tctagagata acgctaaaaa cacagtgtat 1500

ctgcagatga atagtctcaa acctgaagac acaggcgtgt atttctgtaa gggtcctgag 1560

ctgaggggcc agggcatcca ggtaacagtc tcgagcggat ccgacaaaac tcacacatgc 1620

ccaccgtgcc cagcacctga actcctgggg ggaccgtcag tcttcctctt cccccaaaa 1680

cccaaggaca ccctcatgat ctcccggacc cctgaggtca catgcgtggt ggtggacgtg 1740

agccacgaag accctgaggt caagttcaac tggtacgtgg acggcgtgga ggtgcataat 1800

gccaagacaa agccgcggga ggagcagtac aacagcacgt accgtgtggt cagcgtcctc 1860

accgtcctgc accaggactg gctgaatggc aaggagtaca agtgcaaggt ctccaacaaa 1920

gccctcccag cccccatcga gaaaaccatc tccaaagcca aagggcagcc ccgagaacca 1980

caggtgtaca ccctgccccc atcccgggag gagatgacca agaaccaggt cagcctgacc 2040

tgcctggtca aaggcttcta tcccagcgac atcgccgtgg agtgggagag caatgggcag 2100

ccggagaaca actacaagac cacgcctccc gtgctggact ccgacggctc cttcttcctc 2160

tatagcaagc tcaccgtgga caagagcagg tggcagcagg ggaacgtctt ctcatgctcc 2220

gtgatgcatg aggctctgca caaccactac acgcagaaga gcctctccct gtctccgggt 2280

aaatga 2286

<210> SEQ ID NO 24
<211> LENGTH: 356
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 5D-Fc binding agent

<400> SEQUENCE: 24

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Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Glu Ala Ser Gly Phe Thr Leu Asp Tyr Tyr
            20                  25                  30

Gly Ile Gly Trp Phe Arg Gln Pro Pro Gly Lys Glu Arg Glu Ala Val
        35                  40                  45

Ser Tyr Ile Ser Ala Ser Ala Arg Thr Ile Leu Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ala Val Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Lys Arg Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Arg Arg Phe Ser Ala Ser Ser Val Asn Arg Trp Leu Ala Asp
            100                 105                 110

Asp Tyr Asp Val Trp Gly Arg Gly Thr Gln Val Ala Val Ser Ser Gly
        115                 120                 125

Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
    130                 135                 140

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
145                 150                 155                 160

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
                165                 170                 175

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
            180                 185                 190

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
        195                 200                 205

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
    210                 215                 220

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
225                 230                 235                 240

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
                245                 250                 255

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
            260                 265                 270

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
        275                 280                 285

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
    290                 295                 300

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
305                 310                 315                 320

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
                325                 330                 335

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
            340                 345                 350

Ser Pro Gly Lys
        355


<210> SEQ ID NO 25
<211> LENGTH: 1071
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 5D-Fc binding agent

<400> SEQUENCE: 25
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caggtgcaac tggttgaatc tgggggaggc ttggtacaac ctgggggatc cctgagactc 60

tcttgcgagg cctccggatt caccttggac tactatggca tcggctggtt ccgccagccc 120

ccagggaagg agcgggaggc cgtttcatac attagtgcca gtgcccggac catactgtac 180

gcagactctg tgaagggacg ctttaccatc tctagggaca atgccaaaaa tgctgtgtac 240

ctgcaaatga acagcctcaa gcgggaggat accgcagtgt actactgcgc gagacggcgc 300

ttctccgctt ctagcgtgaa tagatggctg gccgacgact acgacgtgtg gggacggggc 360

acacaggtgg ctgtctcgag cggatccgac aaaactcaca catgcccacc gtgcccagca 420

cctgaactcc tgggggggacc gtcagtcttc ctcttccccc caaaacccaa ggacaccctc 480

atgatctccc ggacccctga ggtcacatgc gtggtggtgg acgtgagcca cgaagaccct 540

gaggtcaagt tcaactggta cgtggacggc gtggaggtgc ataatgccaa gacaaagccg 600

cgggaggagc agtacaacag cacgtaccgt gtggtcagcg tcctcaccgt cctgcaccag 660

gactggctga atggcaagga gtacaagtgc aaggtctcca acaaagccct cccagccccc 720

atcgagaaaa ccatctccaa agccaaaggg cagccccgag aaccacaggt gtacaccctg 780

cccccatccc gggaggagat gaccaagaac caggtcagcc tgacctgcct ggtcaaaggc 840

ttctatccca gcgacatcgc cgtggagtgg gagagcaatg ggcagccgga gaacaactac 900

aagaccacgc ctcccgtgct ggactccgac ggctccttct tcctctatag caagctcacc 960

gtggacaaga gcaggtggca gcaggggaac gtcttctcat gctccgtgat gcatgaggct 1020

ctgcacaacc actacacgca gaagagcctc tccctgtctc cgggtaaatg a 1071

<210> SEQ ID NO 26
<211> LENGTH: 340
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E3-Fc binding agent

<400> SEQUENCE: 26

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Thr Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ser Ser Gly Ser Ile Ala Gly Phe Glu
20 25 30

Thr Val Thr Trp Ser Arg Gln Ala Pro Gly Lys Ser Leu Gln Trp Val
35 40 45

Ala Ser Met Thr Lys Thr Asn Asn Glu Ile Tyr Ser Asp Ser Val Lys
50 55 60

Gly Arg Phe Ile Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Gly Val Tyr Phe Cys Lys
85 90 95

Gly Pro Glu Leu Arg Gly Gln Gly Ile Gln Val Thr Val Ser Ser Gly
100 105 110

Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
115 120 125

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
130 135 140

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
145 150 155 160

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
165 170 175

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Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
            180                 185                 190

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
        195                 200                 205

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
    210                 215                 220

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
225                 230                 235                 240

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
                245                 250                 255

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
            260                 265                 270

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
        275                 280                 285

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
    290                 295                 300

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
305                 310                 315                 320

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
                325                 330                 335

Ser Pro Gly Lys
            340
```

<210> SEQ ID NO 27
<211> LENGTH: 1023
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E3-Fc binding agent

<400> SEQUENCE: 27

```
caagttcagc tggtcgaatc cgggggcgga ctggtccaga caggggctc cctgaggctc     60

tcctgtgcat cttccggaag catcgccggc ttcgagaccg tgacctggtc tcgccaggct    120

cccgggaagt ctctgcagtg ggtcgcttcc atgactaaga ctaacaacga gatctactct    180

gactcagtga aaggccgctt catcatttct agagataacg ctaaaaacac agtgtatctg    240

cagatgaata gtctcaaacc tgaagacaca ggcgtgtatt tctgtaaggg tcctgagctg    300

aggggccagg gcatccaggt aacagtctcg agcggatccg acaaaactca cacatgccca    360

ccgtgcccag cacctgaact cctgggggga ccgtcagtct tcctcttccc cccaaaaccc    420

aaggacaccc tcatgatctc ccggacccct gaggtcacat gcgtggtggt ggacgtgagc    480

cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc    540

aagacaaagc cgcgggagga gcagtacaac agcacgtacc gtgtggtcag cgtcctcacc    600

gtcctgcacc aggactggct gaatggcaag gagtacaagt gcaaggtctc caacaaagcc    660

ctcccagccc ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag    720

gtgtacaccc tgcccccatc ccgggaggag atgaccaaga accaggtcag cctgacctgc    780

ctggtcaaag gcttctatcc cagcgacatc gccgtggagt gggagagcaa tgggcagccg    840

gagaacaact acaagaccac gcctcccgtg ctggactccg acggctcctt cttcctctat    900

agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgctccgtg    960

atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccgggtaaa   1020

tga                                                                 1023
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<210> SEQ ID NO 28
<211> LENGTH: 350
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AA6-Fc binding agent

<400> SEQUENCE: 28

Gln Leu Gln Leu Val Glu Thr Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Val Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Pro Glu Trp Ile
35 40 45

Ala Thr Ile Asn Thr Asp Gly Ser Thr Met Arg Asp Asp Ser Thr Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu
65 70 75 80

Gln Met Thr Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys Ala
85 90 95

Arg Gly Arg Val Ile Ser Ala Ser Ala Ile Arg Gly Ala Val Arg Gly
100 105 110

Pro Gly Thr Gln Val Thr Val Ser Ser Gly Ser Asp Lys Thr His Thr
115 120 125

Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
130 135 140

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
145 150 155 160

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
165 170 175

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
180 185 190

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
195 200 205

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
210 215 220

Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
225 230 235 240

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
245 250 255

Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
260 265 270

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
275 280 285

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
290 295 300

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
305 310 315 320

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
325 330 335

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
340 345 350

<210> SEQ ID NO 29
<211> LENGTH: 1053

-continued

<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AA6-Fc binding agent

<400> SEQUENCE: 29

caactgcagc tggtagagac aggggcggc ttagttcagc ctggagggtc tctcagactg 60

tcatgcgctg cctctggctt taccttcagt gactacgtga tgacatgggt ccgccaagct 120

ccagggaagg ggcctgagtg gatcgctact attaatacag atggcagcac aatgcgggac 180

gactccacaa aggggcggtt caccatttcc agagacaacg ccaagaatac tctgtacctt 240

cagatgacca gtctgaaacc cgaggacact gctctgtact attgtgcaag aggccgggtg 300

atctctgctt ccgctatcag aggcgcagta aggggccctg gaacacaagt aactgtctcg 360

agcggatccg acaaaactca cacatgccca ccgtgcccag cacctgaact cctgggggga 420

ccgtcagtct tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggacccct 480

gaggtcacat gcgtggtggt ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg 540

tacgtggacg gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac 600

agcacgtacc gtgtggtcag cgtcctcacc gtcctgcacc aggactggct gaatggcaag 660

gagtacaagt gcaaggtctc caacaaagcc ctcccagccc ccatcgagaa aaccatctcc 720

aaagccaaag ggcagccccg agaaccacag gtgtacaccc tgcccccatc ccgggaggag 780

atgaccaaga accaggtcag cctgacctgc ctggtcaaag gcttctatcc cagcgacatc 840

gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg 900

ctggactccg acggctcctt cttcctctat agcaagctca ccgtggacaa gagcaggtgg 960

cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacacg 1020

cagaagagcc tctccctgtc tccgggtaaa tga 1053

<210> SEQ ID NO 30
<211> LENGTH: 356
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AH3-Fc binding agent

<400> SEQUENCE: 30

Gln Val Gln Leu Val Glu Thr Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Leu Asp Tyr Ser
20 25 30

Ser Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val
35 40 45

Ser Cys Ile Ser Ser Ser Gly Asp Ser Thr Lys Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Thr Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Phe Arg Ala Thr Met Cys Gly Val Phe Pro Leu Ser Pro Tyr
100 105 110

Gly Lys Asp Asp Trp Gly Lys Gly Thr Leu Val Thr Val Ser Ser Gly
115 120 125

Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
130 135 140

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Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
145                 150                 155                 160

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
                165                 170                 175

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
            180                 185                 190

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
        195                 200                 205

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
    210                 215                 220

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
225                 230                 235                 240

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
                245                 250                 255

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
            260                 265                 270

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
        275                 280                 285

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
    290                 295                 300

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
305                 310                 315                 320

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
                325                 330                 335

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
            340                 345                 350

Ser Pro Gly Lys
        355


<210> SEQ ID NO 31
<211> LENGTH: 1071
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AH3-Fc binding agent

<400> SEQUENCE: 31

caggtacagc tggtggagac gggggggaggg ctggtacaac caggcgggtc actgaggctt     60

tcctgtgccg catctgggtt cacactggat tattcgtcca tagggtggtt tcggcaggct    120

cctggcaaag agcgtgaggg ggtctcatgt attagtagta gtggtgatag cacaaagtac    180

gccgattccg taaagggccg gtttacaacc tccagggata atgctaagaa caccgtatat    240

ctccagatga actctctgaa gcccgacgat acggccgtat attactgtgc ggctttcagg    300

gcgactatgt gcggcgtgtt ccctctgagc ccttacggca aggacgactg gggcaagggg    360

accctggtga ccgtctcgag cggatccgac aaaactcaca catgcccacc gtgcccagca    420

cctgaactcc tgggggggacc gtcagtcttc ctcttccccc caaaacccaa ggacaccctc    480

atgatctccc ggacccctga ggtcacatgc gtggtggtgg acgtgagcca cgaagaccct    540

gaggtcaagt tcaactggta cgtggacggc gtggaggtgc ataatgccaa gacaaagccg    600

cgggaggagc agtacaacag cacgtaccgt gtggtcagcg tcctcaccgt cctgcaccag    660

gactggctga atggcaagga gtacaagtgc aaggtctcca acaaagccct cccagccccc    720

atcgagaaaa ccatctccaa agccaaaggg cagccccgag aaccacaggt gtacaccctg    780
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cccccatccc gggaggagat gaccaagaac caggtcagcc tgacctgcct ggtcaaaggc 840

ttctatccca gcgacatcgc cgtggagtgg gagagcaatg ggcagccgga gaacaactac 900

aagaccacgc ctcccgtgct ggactccgac ggctccttct tcctctatag caagctcacc 960

gtggacaaga gcaggtggca gcaggggaac gtcttctcat gctccgtgat gcatgaggct 1020

ctgcacaacc actacacgca gaagagcctc tccctgtctc cgggtaaatg a 1071

<210> SEQ ID NO 32
<211> LENGTH: 498
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AH3-5D-Fc binding agent

<400> SEQUENCE: 32

Gln Val Gln Leu Val Glu Thr Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Leu Asp Tyr Ser
20 25 30

Ser Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val
35 40 45

Ser Cys Ile Ser Ser Ser Gly Asp Ser Thr Lys Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Thr Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Phe Arg Ala Thr Met Cys Gly Val Phe Pro Leu Ser Pro Tyr
100 105 110

Gly Lys Asp Asp Trp Gly Lys Gly Thr Leu Val Thr Val Ser Ser Gly
115 120 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val
130 135 140

Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu
145 150 155 160

Arg Leu Ser Cys Glu Ala Ser Gly Phe Thr Leu Asp Tyr Tyr Gly Ile
165 170 175

Gly Trp Phe Arg Gln Pro Pro Gly Lys Glu Arg Glu Ala Val Ser Tyr
180 185 190

Ile Ser Ala Ser Ala Arg Thr Ile Leu Tyr Ala Asp Ser Val Lys Gly
195 200 205

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ala Val Tyr Leu Gln
210 215 220

Met Asn Ser Leu Lys Arg Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
225 230 235 240

Arg Arg Phe Ser Ala Ser Ser Val Asn Arg Trp Leu Ala Asp Asp Tyr
245 250 255

Asp Val Trp Gly Arg Gly Thr Gln Val Ala Val Ser Ser Gly Ser Asp
260 265 270

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
275 280 285

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
290 295 300

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
305 310 315 320

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Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
325 330 335

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
340 345 350

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
355 360 365

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
370 375 380

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
385 390 395 400

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
405 410 415

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
420 425 430

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
435 440 445

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
450 455 460

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
465 470 475 480

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
485 490 495

Gly Lys

<210> SEQ ID NO 33
<211> LENGTH: 1497
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AH3-5D-Fc binding agent

<400> SEQUENCE: 33

caggtacagc tggtggagac gggggggaggg ctggtacaac caggcgggtc actgaggctt 60

tcctgtgccg catctgggtt cacactggat tattcgtcca tagggtggtt tcggcaggct 120

cctggcaaag agcgtgaggg ggtctcatgt attagtagta gtggtgatag cacaaagtac 180

gccgattccg taaagggccg gtttacaacc tccagggata atgctaagaa caccgtatat 240

ctccagatga actctctgaa gcccgacgat acggccgtat attactgtgc ggctttcagg 300

gcgactatgt gcggcgtgtt ccctctgagc ccttacggca aggacgactg gggcaagggg 360

accctggtga ccgtatcctc aggcggtgga gggtctggtg ggggaggctc agggggtgga 420

ggcagccagg tgcaactggt tgaatctggg ggaggcttgg tacaacctgg gggatccctg 480

agactctctt gcgaggcctc cggattcacc ttggactact atggcatcgg ctggttccgc 540

cagccccag ggaaggagcg ggaggccgtt tcatacatta gtgccagtgc ccggaccata 600

ctgtacgcag actctgtgaa gggacgcttt accatctcta gggacaatgc caaaaatgct 660

gtgtacctgc aaatgaacag cctcaagcgg gaggataccg cagtgtacta ctgcgcgaga 720

cggcgcttct ccgcttctag cgtgaataga tggctggccg acgactacga cgtgtgggga 780

cggggcacac aggtggctgt ctcgagcgga tccgacaaaa ctcacacatg cccaccgtgc 840

ccagcacctg aactcctggg gggaccgtca gtcttcctct tccccccaaa acccaaggac 900

accctcatga tctcccggac ccctgaggtc acatgcgtgg tggtggacgt gagccacgaa 960

gaccctgagg tcaagttcaa ctggtacgtg gacggcgtgg aggtgcataa tgccaagaca 1020

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aagccgcggg aggagcagta caacagcacg taccgtgtgg tcagcgtcct caccgtcctg 1080

caccaggact ggctgaatgg caaggagtac aagtgcaagg tctccaacaa agccctccca 1140

gcccccatcg agaaaaccat ctccaaagcc aaagggcagc cccgagaacc acaggtgtac 1200

accctgcccc catcccggga ggagatgacc aagaaccagg tcagcctgac ctgcctggtc 1260

aaaggcttct atcccagcga catcgccgtg gagtgggaga gcaatgggca gccggagaac 1320

aactacaaga ccacgcctcc cgtgctggac tccgacggct ccttcttcct ctatagcaag 1380

ctcaccgtgg acaagagcag gtggcagcag gggaacgtct tctcatgctc cgtgatgcat 1440

gaggctctgc acaaccacta cacgcagaag agcctctccc tgtctccggg taaatga 1497

<210> SEQ ID NO 34
<211> LENGTH: 476
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AA6-E3-Fc binding agent

<400> SEQUENCE: 34

Gln Leu Gln Leu Val Glu Thr Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Val Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Pro Glu Trp Ile
35 40 45

Ala Thr Ile Asn Thr Asp Gly Ser Thr Met Arg Asp Asp Ser Thr Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu
65 70 75 80

Gln Met Thr Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys Ala
85 90 95

Arg Gly Arg Val Ile Ser Ala Ser Ala Ile Arg Gly Ala Val Arg Gly
100 105 110

Pro Gly Thr Gln Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
115 120 125

Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Glu Ser Gly
130 135 140

Gly Gly Leu Val Gln Thr Gly Gly Ser Leu Arg Leu Ser Cys Ala Ser
145 150 155 160

Ser Gly Ser Ile Ala Gly Phe Glu Thr Val Thr Trp Ser Arg Gln Ala
165 170 175

Pro Gly Lys Ser Leu Gln Trp Val Ala Ser Met Thr Lys Thr Asn Asn
180 185 190

Glu Ile Tyr Ser Asp Ser Val Lys Gly Arg Phe Ile Ile Ser Arg Asp
195 200 205

Asn Ala Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu
210 215 220

Asp Thr Gly Val Tyr Phe Cys Lys Gly Pro Glu Leu Arg Gly Gln Gly
225 230 235 240

Ile Gln Val Thr Val Ser Ser Gly Ser Asp Lys Thr His Thr Cys Pro
245 250 255

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
260 265 270

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val

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        275                 280                 285

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
    290                 295                 300

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
305                 310                 315                 320

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
                325                 330                 335

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
            340                 345                 350

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
        355                 360                 365

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
    370                 375                 380

Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
385                 390                 395                 400

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
                405                 410                 415

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
            420                 425                 430

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
        435                 440                 445

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
    450                 455                 460

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
465                 470                 475


<210> SEQ ID NO 35
<211> LENGTH: 1431
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AA6-E3-Fc binding agent

<400> SEQUENCE: 35

caactgcagc tggtagagac aggggggcggc ttagttcagc ctggagggtc tctcagactg     60

tcatgcgctg cctctggctt taccttcagt gactacgtga tgacatgggt ccgccaagct    120

ccagggaagg ggcctgagtg gatcgctact attaatacag atggcagcac aatgcgggac    180

gactccacaa aggggcggtt caccatttcc agagacaacg ccaagaatac tctgtacctt    240

cagatgacca gtctgaaacc cgaggacact gctctgtact attgtgcaag aggccgggtg    300

atctctgctt ccgctatcag aggcgcagta aggggccctg gaacacaggt aaccgtttca    360

tccgggggag gcggttcagg cggtggggga tctggcgggg gtggatccca agttcagctg    420

gtcgaatccg ggggcggact ggtccagaca gggggctccc tgaggctctc ctgtgcatct    480

tccggaagca tcgccggctt cgagaccgtg acctggtctc gccaggctcc cgggaagtct    540

ctgcagtggg tcgcttccat gactaagact aacaacgaga tctactctga ctcagtgaaa    600

ggccgcttca tcatttctag agataacgct aaaaacacag tgtatctgca gatgaatagt    660

ctcaaacctg aagacacagg cgtgtatttc tgtaagggtc ctgagctgag gggccagggc    720

atccaggtaa cagtctcgag cggatccgac aaaactcaca catgcccacc gtgcccagca    780

cctgaactcc tgggggggacc gtcagtcttc ctcttccccc caaaacccaa ggacaccctc    840

atgatctccc ggacccctga ggtcacatgc gtggtggtgg acgtgagcca cgaagaccct    900

gaggtcaagt tcaactggta cgtggacggc gtggaggtgc ataatgccaa gacaaagccg    960
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cgggaggagc agtacaacag cacgtaccgt gtggtcagcg tcctcaccgt cctgcaccag 1020

gactggctga atggcaagga gtacaagtgc aaggtctcca acaaagccct cccagccccc 1080

atcgagaaaa ccatctccaa agccaaaggg cagccccgag aaccacaggt gtacaccctg 1140

cccccatccc gggaggagat gaccaagaac caggtcagcc tgacctgcct ggtcaaaggc 1200

ttctatccca gcgacatcgc cgtggagtgg gagagcaatg ggcagccgga gaacaactac 1260

aagaccacgc ctcccgtgct ggactccgac ggctccttct tcctctatag caagctcacc 1320

gtggacaaga gcaggtggca gcaggggaac gtcttctcat gctccgtgat gcatgaggct 1380

ctgcacaacc actacacgca gaagagcctc tccctgtctc cgggtaaatg a 1431

<210> SEQ ID NO 36
<211> LENGTH: 457
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AH3-IgG1-heavy chain

<400> SEQUENCE: 36

Gln Val Gln Leu Val Glu Thr Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Leu Asp Tyr Ser
20 25 30

Ser Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val
35 40 45

Ser Cys Ile Ser Ser Ser Gly Asp Ser Thr Lys Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Thr Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Phe Arg Ala Thr Met Cys Gly Val Phe Pro Leu Ser Pro Tyr
100 105 110

Gly Lys Asp Asp Trp Gly Lys Gly Thr Leu Val Thr Val Ser Ser Ala
115 120 125

Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser
130 135 140

Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe
145 150 155 160

Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly
165 170 175

Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu
180 185 190

Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr
195 200 205

Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg
210 215 220

Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro
225 230 235 240

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
245 250 255

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
260 265 270

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr

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        275                 280                 285

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
    290                 295                 300

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
305                 310                 315                 320

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
                325                 330                 335

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
            340                 345                 350

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met
        355                 360                 365

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
    370                 375                 380

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
385                 390                 395                 400

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
                405                 410                 415

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
            420                 425                 430

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
        435                 440                 445

Lys Ser Leu Ser Leu Ser Pro Gly Lys
    450                 455


<210> SEQ ID NO 37
<211> LENGTH: 1374
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AH3-IgG1-heavy chain

<400> SEQUENCE: 37

caggtacagc tggtggagac gggggggaggg ctggtacaac caggcgggtc actgaggctt     60

tcctgtgccg catctgggtt cacactggat tattcgtcca tagggtggtt tcggcaggct    120

cctggcaaag agcgtgaggg ggtctcatgt attagtagta gtggtgatag cacaaagtac    180

gccgattccg taaagggccg gtttacaacc tccagggata atgctaagaa caccgtatat    240

ctccagatga actctctgaa gcccgacgat acggccgtat attactgtgc ggctttcagg    300

gcgactatgt gcggcgtgtt ccctctgagc ccttacggca aggacgactg gggcaagggg    360

accctggtga ccgtctcgag tgcgtcgacc aagggcccat cggtcttccc gctagcaccc    420

tcctccaaga gcacctctgg gggcacagcg gccctgggct gcctggtcaa ggactacttc    480

cccgaacctg tgacggtctc gtggaactca ggcgccctga ccagcggcgt gcacaccttc    540

ccggctgtcc tacagtcctc aggactctac tccctcagca gcgtggtgac cgtgccctcc    600

agcagcttgg gcacccagac ctacatctgc aacgtgaatc acaagcccag caacaccaag    660

gtggacaaga gagttgagcc caaatcttgt gacaaaactc acacatgccc accgtgccca    720

gcacctgaac tcctgggggg accgtcagtc ttcctcttcc ccccaaaacc caaggacacc    780

ctcatgatct cccggacccc tgaggtcaca tgcgtggtgg tggacgtgag ccacgaagac    840

cctgaggtca agttcaactg gtacgtggac ggcgtggagg tgcataatgc caagacaaag    900

ccgcgggagg agcagtacaa cagcacgtac cgtgtggtca gcgtcctcac cgtcctgcac    960

caggactggc tgaatggcaa ggagtacaag tgcaaggtct ccaacaaagc cctcccagcc   1020
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cccatcgaga aaaccatctc caaagccaaa gggcagcccc gagaaccaca ggtgtacacc 1080

ctgcccccat cccgggagga gatgaccaag aaccaggtca gcctgacctg cctggtcaaa 1140

ggcttctatc ccagcgacat cgccgtggag tgggagagca atgggcagcc ggagaacaac 1200

tacaagacca cgcctcccgt gctggactcc gacggctcct tcttcctcta tagcaagctc 1260

accgtggaca agagcaggtg gcagcagggg aacgtcttct catgctccgt gatgcatgag 1320

gctctgcaca accactacac gcagaagagc ctctccctgt ccccgggtaa atga 1374

<210> SEQ ID NO 38
<211> LENGTH: 457
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 5D-IgG1-heavy chain

<400> SEQUENCE: 38

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Glu Ala Ser Gly Phe Thr Leu Asp Tyr Tyr
20 25 30

Gly Ile Gly Trp Phe Arg Gln Pro Pro Gly Lys Glu Arg Glu Ala Val
35 40 45

Ser Tyr Ile Ser Ala Ser Ala Arg Thr Ile Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ala Val Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Arg Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Arg Arg Phe Ser Ala Ser Ser Val Asn Arg Trp Leu Ala Asp
100 105 110

Asp Tyr Asp Val Trp Gly Arg Gly Thr Gln Val Ala Val Ser Ser Ala
115 120 125

Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser
130 135 140

Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe
145 150 155 160

Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly
165 170 175

Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu
180 185 190

Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr
195 200 205

Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg
210 215 220

Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro
225 230 235 240

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
245 250 255

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
260 265 270

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
275 280 285

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
290 295 300

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Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
305                 310                 315                 320

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
                325                 330                 335

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
            340                 345                 350

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met
        355                 360                 365

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
    370                 375                 380

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
385                 390                 395                 400

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
                405                 410                 415

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
            420                 425                 430

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
        435                 440                 445

Lys Ser Leu Ser Leu Ser Pro Gly Lys
    450                 455


<210> SEQ ID NO 39
<211> LENGTH: 1374
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 5D-IgG1-heavy chain

<400> SEQUENCE: 39

caggtgcaac tggttgaatc tgggggaggc ttggtacaac ctgggggatc cctgagactc     60

tcttgcgagg cctccggatt caccttggac tactatggca tcggctggtt ccgccagccc    120

ccagggaagg agcgggaggc cgtttcatac attagtgcca gtgcccggac catactgtac    180

gcagactctg tgaagggacg ctttaccatc tctagggaca atgccaaaaa tgctgtgtac    240

ctgcaaatga acagcctcaa gcgggaggat accgcagtgt actactgcgc gagacggcgc    300

ttctccgctt ctagcgtgaa tagatggctg gccgacgact acgacgtgtg gggacggggc    360

acacaggtgg ctgtctcgag cgcgtcgacc aagggcccat cggtcttccc gctagcaccc    420

tcctccaaga gcacctctgg gggcacagcg gccctgggct gcctggtcaa ggactacttc    480

cccgaacctg tgacggtctc gtggaactca ggcgccctga ccagcggcgt gcacaccttc    540

ccggctgtcc tacagtcctc aggactctac tccctcagca gcgtggtgac cgtgccctcc    600

agcagcttgg gcacccagac ctacatctgc aacgtgaatc acaagcccag caacaccaag    660

gtggacaaga gagttgagcc caaatcttgt gacaaaactc acacatgccc accgtgccca    720

gcacctgaac tcctgggggg accgtcagtc ttcctcttcc ccccaaaacc caaggacacc    780

ctcatgatct cccggacccc tgaggtcaca tgcgtggtgg tggacgtgag ccacgaagac    840

cctgaggtca agttcaactg gtacgtggac ggcgtggagg tgcataatgc caagacaaag    900

ccgcgggagg agcagtacaa cagcacgtac cgtgtggtca gcgtcctcac cgtcctgcac    960

caggactggc tgaatggcaa ggagtacaag tgcaaggtct ccaacaaagc cctcccagcc   1020

cccatcgaga aaaccatctc caaagccaaa gggcagcccc gagaaccaca ggtgtacacc   1080

ctgccccccat cccgggagga gatgaccaag aaccaggtca gcctgacctg cctggtcaaa   1140

ggcttctatc ccagcgacat cgccgtggag tgggagagca atgggcagcc ggagaacaac   1200
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tacaagacca cgcctcccgt gctggactcc gacggctcct tcttcctcta tagcaagctc 1260

accgtggaca agagcaggtg gcagcagggg aacgtcttct catgctccgt gatgcatgag 1320

gctctgcaca accactacac gcagaagagc ctctccctgt ccccgggtaa atga 1374

<210> SEQ ID NO 40
<211> LENGTH: 228
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AA6-IgG1-kappa chain

<400> SEQUENCE: 40

Gln Leu Gln Leu Val Glu Thr Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Val Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Pro Glu Trp Ile
35 40 45

Ala Thr Ile Asn Thr Asp Gly Ser Thr Met Arg Asp Asp Ser Thr Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu
65 70 75 80

Gln Met Thr Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys Ala
85 90 95

Arg Gly Arg Val Ile Ser Ala Ser Ala Ile Arg Gly Ala Val Arg Gly
100 105 110

Pro Gly Thr Gln Val Thr Val Ser Ser Arg Thr Val Ala Ala Pro Ser
115 120 125

Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala
130 135 140

Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val
145 150 155 160

Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser
165 170 175

Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr
180 185 190

Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys
195 200 205

Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn
210 215 220

Arg Gly Glu Cys
225

<210> SEQ ID NO 41
<211> LENGTH: 687
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AA6-IgG1-kappa chain

<400> SEQUENCE: 41

caactgcagc tggtagagac aggggcggc ttagttcagc ctggagggtc tctcagactg 60

tcatgcgctg cctctggctt taccttcagt gactacgtga tgacatgggt ccgccaagct 120

ccagggaagg ggcctgagtg gatcgctact attaatacag atggcagcac aatgcgggac 180

gactccacaa aggggcggtt caccatttcc agagacaacg ccaagaatac tctgtacctt 240

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cagatgacca gtctgaaacc cgaggacact gctctgtact attgtgcaag aggccgggtg 300

atctctgctt ccgctatcag aggcgcagta aggggccctg gaacacaagt aactgtctcg 360

agccgtacgg tggctgcacc atctgtcttc atcttcccgc catctgatga gcagttgaaa 420

tctggaactg cctctgttgt gtgcctgctg aataacttct atcccagaga ggccaaagta 480

cagtggaagg tggataacgc cctccaatcg ggtaactccc aggagagtgt cacagagcag 540

gacagcaagg acagcaccta cagcctcagc agcaccctga cgctgagcaa agcagactac 600

gagaaacaca aagtctacgc ctgcgaagtc acccatcagg gcctgagctc gcccgtcaca 660

aagagcttca acaggggaga gtgttga 687

<210> SEQ ID NO 42
<211> LENGTH: 218
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E3-IgG1-kappa chain

<400> SEQUENCE: 42

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Thr Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ser Ser Gly Ser Ile Ala Gly Phe Glu
20 25 30

Thr Val Thr Trp Ser Arg Gln Ala Pro Gly Lys Ser Leu Gln Trp Val
35 40 45

Ala Ser Met Thr Lys Thr Asn Asn Glu Ile Tyr Ser Asp Ser Val Lys
50 55 60

Gly Arg Phe Ile Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Gly Val Tyr Phe Cys Lys
85 90 95

Gly Pro Glu Leu Arg Gly Gln Gly Ile Gln Val Thr Val Ser Ser Arg
100 105 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
115 120 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
130 135 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145 150 155 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
165 170 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
180 185 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
195 200 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> SEQ ID NO 43
<211> LENGTH: 657
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E3-IgG1-kappa chain

<400> SEQUENCE: 43

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caagttcagc tggtcgaatc cgggggcgga ctggtccaga caggggctc cctgaggctc 60

tcctgtgcat cttccggaag catcgccggc ttcgagaccg tgacctggtc tcgccaggct 120

cccgggaagt ctctgcagtg ggtcgcttcc atgactaaga ctaacaacga gatctactct 180

gactcagtga aaggccgctt catcatttct agagataacg ctaaaaacac agtgtatctg 240

cagatgaata gtctcaaacc tgaagacaca ggcgtgtatt tctgtaaggg tcctgagctg 300

aggggccagg gcatccaggt aacagtctcg agccgtacgg tggctgcacc atctgtcttc 360

atcttcccgc catctgatga gcagttgaaa tctggaactg cctctgttgt gtgcctgctg 420

aataacttct atcccagaga ggccaaagta cagtggaagg tggataacgc cctccaatcg 480

ggtaactccc aggagagtgt cacagagcag gacagcaagg acagcaccta cagcctcagc 540

agcaccctga cgctgagcaa agcagactac gagaaacaca aagtctacgc ctgcgaagtc 600

acccatcagg gcctgagctc gcccgtcaca aagagcttca acaggggaga gtgttga 657

<210> SEQ ID NO 44
<211> LENGTH: 599
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AH3-5D-IgG1 heavy chain

<400> SEQUENCE: 44

Gln Val Gln Leu Val Glu Thr Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Leu Asp Tyr Ser
20 25 30

Ser Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val
35 40 45

Ser Cys Ile Ser Ser Ser Gly Asp Ser Thr Lys Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Thr Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Phe Arg Ala Thr Met Cys Gly Val Phe Pro Leu Ser Pro Tyr
100 105 110

Gly Lys Asp Asp Trp Gly Lys Gly Thr Leu Val Thr Val Ser Ser Gly
115 120 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val
130 135 140

Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu
145 150 155 160

Arg Leu Ser Cys Glu Ala Ser Gly Phe Thr Leu Asp Tyr Tyr Gly Ile
165 170 175

Gly Trp Phe Arg Gln Pro Pro Gly Lys Glu Arg Glu Ala Val Ser Tyr
180 185 190

Ile Ser Ala Ser Ala Arg Thr Ile Leu Tyr Ala Asp Ser Val Lys Gly
195 200 205

Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ala Val Tyr Leu Gln
210 215 220

Met Asn Ser Leu Lys Arg Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
225 230 235 240

Arg Arg Phe Ser Ala Ser Ser Val Asn Arg Trp Leu Ala Asp Asp Tyr
245 250 255

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Asp Val Trp Gly Arg Gly Thr Gln Val Ala Val Ser Ser Ala Ser Thr
            260                 265                 270

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
        275                 280                 285

Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
    290                 295                 300

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
305                 310                 315                 320

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
                325                 330                 335

Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
            340                 345                 350

Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu
        355                 360                 365

Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
    370                 375                 380

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
385                 390                 395                 400

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
                405                 410                 415

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
            420                 425                 430

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
        435                 440                 445

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
    450                 455                 460

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
465                 470                 475                 480

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
                485                 490                 495

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys
            500                 505                 510

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
        515                 520                 525

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
    530                 535                 540

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
545                 550                 555                 560

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
                565                 570                 575

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
            580                 585                 590

Leu Ser Leu Ser Pro Gly Lys
        595
```

<210> SEQ ID NO 45
<211> LENGTH: 1800
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AH3-5D-IgG1 heavy chain

<400> SEQUENCE: 45

caggtacagc tggtggagac gggggggaggg ctggtacaac caggcgggtc actgaggctt 60

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tcctgtgccg catctgggtt cacactggat tattcgtcca tagggtggtt tcggcaggct    120

cctggcaaag agcgtgaggg ggtctcatgt attagtagta gtggtgatag cacaaagtac    180

gccgattccg taaagggccg gtttacaacc tccagggata atgctaagaa caccgtatat    240

ctccagatga actctctgaa gcccgacgat acggccgtat attactgtgc ggctttcagg    300

gcgactatgt gcggcgtgtt ccctctgagc ccttacggca aggacgactg gggcaagggg    360

accctggtga ccgtatcctc aggcggtgga gggtctggtg ggggaggctc aggggtgga     420

ggcagccagg tgcaactggt tgaatctggg ggaggcttgg tacaacctgg gggatccctg    480

agactctctt gcgaggcctc cggattcacc ttggactact atggcatcgg ctggttccgc    540

cagcccccag ggaaggagcg ggaggccgtt tcatacatta gtgccagtgc ccggaccata    600

ctgtacgcag actctgtgaa gggacgcttt accatctcta gggacaatgc caaaaatgct    660

gtgtacctgc aaatgaacag cctcaagcgg gaggataccg cagtgtacta ctgcgcgaga    720

cggcgcttct ccgcttctag cgtgaataga tggctggccg acgactacga cgtgtgggga    780

cggggcacac aggtggctgt ctcgagcgcg tcgaccaagg gcccatcggt cttcccgcta    840

gcaccctcct ccaagagcac ctctgggggc acagcggccc tgggctgcct ggtcaaggac    900

tacttccccg aacctgtgac ggtctcgtgg aactcaggcg ccctgaccag cggcgtgcac    960

accttcccgg ctgtcctaca gtcctcagga ctctactccc tcagcagcgt ggtgaccgtg   1020

ccctccagca gcttgggcac ccagacctac atctgcaacg tgaatcacaa gcccagcaac   1080

accaaggtgg acaagagagt tgagcccaaa tcttgtgaca aaactcacac atgcccaccg   1140

tgcccagcac ctgaactcct gggggggaccg tcagtcttcc tcttcccccc aaaacccaag   1200

gacaccctca tgatctcccg gacccctgag gtcacatgcg tggtggtgga cgtgagccac   1260

gaagaccctg aggtcaagtt caactggtac gtggacggcg tggaggtgca taatgccaag   1320

acaaagccgc gggaggagca gtacaacagc acgtaccgtg tggtcagcgt cctcaccgtc   1380

ctgcaccagg actggctgaa tggcaaggag tacaagtgca aggtctccaa caaagccctc   1440

ccagccccca tcgagaaaac catctccaaa gccaaagggc agccccgaga accacaggtg   1500

tacaccctgc ccccatcccg ggaggagatg accaagaacc aggtcagcct gacctgcctg   1560

gtcaaaggct tctatcccag cgacatcgcc gtggagtggg agagcaatgg gcagccggag   1620

aacaactaca agaccacgcc tcccgtgctg gactccgacg gctccttctt cctctatagc   1680

aagctcaccg tggacaagag caggtggcag caggggaacg tcttctcatg ctccgtgatg   1740

catgaggctc tgcacaacca ctacacgcag aagagcctct ccctgtcccc gggtaaatga   1800
```

<210> SEQ ID NO 46
<211> LENGTH: 354
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AA6-E3-IgG1 light chain

<400> SEQUENCE: 46

```
Gln Leu Gln Leu Val Glu Thr Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
            20                  25                  30

Val Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Pro Glu Trp Ile
        35                  40                  45

Ala Thr Ile Asn Thr Asp Gly Ser Thr Met Arg Asp Asp Ser Thr Lys
    50                  55                  60
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Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu
65 70 75 80

Gln Met Thr Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys Ala
85 90 95

Arg Gly Arg Val Ile Ser Ala Ser Ala Ile Arg Gly Ala Val Arg Gly
100 105 110

Pro Gly Thr Gln Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
115 120 125

Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Glu Ser Gly
130 135 140

Gly Gly Leu Val Gln Thr Gly Gly Ser Leu Arg Leu Ser Cys Ala Ser
145 150 155 160

Ser Gly Ser Ile Ala Gly Phe Glu Thr Val Thr Trp Ser Arg Gln Ala
165 170 175

Pro Gly Lys Ser Leu Gln Trp Val Ala Ser Met Thr Lys Thr Asn Asn
180 185 190

Glu Ile Tyr Ser Asp Ser Val Lys Gly Arg Phe Ile Ile Ser Arg Asp
195 200 205

Asn Ala Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu
210 215 220

Asp Thr Gly Val Tyr Phe Cys Lys Gly Pro Glu Leu Arg Gly Gln Gly
225 230 235 240

Ile Gln Val Thr Val Ser Ser Arg Thr Val Ala Ala Pro Ser Val Phe
245 250 255

Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val
260 265 270

Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp
275 280 285

Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr
290 295 300

Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr
305 310 315 320

Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val
325 330 335

Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly
340 345 350

Glu Cys

<210> SEQ ID NO 47
<211> LENGTH: 1065
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AA6-E3-IgG1 light chain

<400> SEQUENCE: 47

caactgcagc tggtagagac aggggcggc ttagttcagc ctggagggtc tctcagactg 60

tcatgcgctg cctctggctt taccttcagt gactacgtga tgacatgggt ccgccaagct 120

ccagggaagg ggcctgagtg gatcgctact attaatacag atggcagcac aatgcgggac 180

gactccacaa aggggcggtt caccatttcc agagacaacg ccaagaatac tctgtacctt 240

cagatgacca gtctgaaacc cgaggacact gctctgtact attgtgcaag aggccgggtg 300

atctctgctt ccgctatcag aggcgcagta aggggccctg gaacacaggt aaccgtttca 360

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tccgggggag gcggttcagg cggtggggga tctggcgggg gtggatccca agttcagctg 420

gtcgaatccg ggggcggact ggtccagaca gggggctccc tgaggctctc ctgtgcatct 480

tccggaagca tcgccggctt cgagaccgtg acctggtctc gccaggctcc cgggaagtct 540

ctgcagtggg tcgcttccat gactaagact aacaacgaga tctactctga ctcagtgaaa 600

ggccgcttca tcatttctag agataacgct aaaaacacag tgtatctgca gatgaatagt 660

ctcaaacctg aagacacagg cgtgtatttc tgtaagggtc ctgagctgag gggccagggc 720

atccaggtaa cagtctcgag ccgtacggtg gctgcaccat ctgtcttcat cttcccgcca 780

tctgatgagc agttgaaatc tggaactgcc tctgttgtgt gcctgctgaa taacttctat 840

cccagagagg ccaaagtaca gtggaaggtg gataacgccc tccaatcggg taactcccag 900

gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg 960

ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc 1020

ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gttga 1065

<210> SEQ ID NO 48
<211> LENGTH: 127
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: VHH peptide monomer 5D

<400> SEQUENCE: 48

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Glu Ala Ser Gly Phe Thr Leu Asp Tyr Tyr
20 25 30

Gly Ile Gly Trp Phe Arg Gln Pro Pro Gly Lys Glu Arg Glu Ala Val
35 40 45

Ser Tyr Ile Ser Ala Ser Ala Arg Thr Ile Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ala Val Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Arg Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Arg Arg Phe Ser Ala Ser Ser Val Asn Arg Trp Leu Ala Asp
100 105 110

Asp Tyr Asp Val Trp Gly Arg Gly Thr Gln Val Ala Val Ser Ser
115 120 125

<210> SEQ ID NO 49
<211> LENGTH: 381
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: VHH peptide monomer 5D

<400> SEQUENCE: 49

caggtgcagc tcgtggagtc aggtggaggc ttggtgcagc ctgggggggtc tctgagactc 60

tcctgtgaag cctctggatt cactttggat tattatggta taggctggtt ccgccagccc 120

ccagggaagg agcgcgaggc ggtctcatat attagtgcca gtgcccgtac gatattgtat 180

gcagattccg tgaagggccg atttaccatc tccagagaca atgccaagaa cgcggtgtat 240

ctacaaatga acagcctgaa acgtgaggac acggctgtct attactgtgc gaggcggcga 300

ttctccgcgt ctagtgttaa tagatggctt gccgacgact atgacgtctg gggtcggggg 360

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acccaggtcg cggtgtcctc a 381

<210> SEQ ID NO 50
<211> LENGTH: 111
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: VHH peptide monomer E3

<400> SEQUENCE: 50

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Thr Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ser Ser Gly Ser Ile Ala Gly Phe Glu
20 25 30

Thr Val Thr Trp Ser Arg Gln Ala Pro Gly Lys Ser Leu Gln Trp Val
35 40 45

Ala Ser Met Thr Lys Thr Asn Asn Glu Ile Tyr Ser Asp Ser Val Lys
50 55 60

Gly Arg Phe Ile Ile Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Gly Val Tyr Phe Cys Lys
85 90 95

Gly Pro Glu Leu Arg Gly Gln Gly Ile Gln Val Thr Val Ser Ser
100 105 110

<210> SEQ ID NO 51
<211> LENGTH: 333
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: VHH peptide monomer E3

<400> SEQUENCE: 51

caggtgcagc tcgtggagtc gggcggaggc ttggtgcaga ctgggggtc tctgagactc 60

tcctgtgcat cctctggaag tatcgccggt ttcgaaaccg tgacctggtc ccgccaggct 120

cctggaaagt cgctccagtg ggtcgcatcg atgactaaaa ctaataacga gatctattca 180

gactccgtga agggccgatt catcatctcc agagacaacg ccaagaatac ggtgtatcta 240

caaatgaaca gcctgaaacc tgaggacaca ggcgtctatt tttgtaaagg tcctgagttg 300

aggggccagg ggatccaggt caccgtctcc tcg 333

<210> SEQ ID NO 52
<211> LENGTH: 121
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: VHH peptide monomer AA6

<400> SEQUENCE: 52

Gln Leu Gln Leu Val Glu Thr Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Val Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Pro Glu Trp Ile
35 40 45

Ala Thr Ile Asn Thr Asp Gly Ser Thr Met Arg Asp Asp Ser Thr Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu

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65 70 75 80

Gln Met Thr Ser Leu Lys Pro Glu Asp Thr Ala Leu Tyr Tyr Cys Ala
85 90 95

Arg Gly Arg Val Ile Ser Ala Ser Ala Ile Arg Gly Ala Val Arg Gly
100 105 110

Pro Gly Thr Gln Val Thr Val Ser Ser
115 120

<210> SEQ ID NO 53
<211> LENGTH: 363
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: VHH peptide monomer AA6

<400> SEQUENCE: 53

cagttgcagc tcgtggagac aggggaggc ttggtgcagc ctgggggtc tctgagactc 60

tcctgtgcag cctctggatt cacgttcagt gactacgtca tgacctgggt ccgccaggct 120

ccaggaaagg ggcccgaatg gatcgcaact attaatacgg acggtagcac gatgcgtgat 180

gactccacaa aaggccgatt caccatctcc agagacaacg ccaagaacac actgtatctg 240

caaatgacca gcctgaaacc ggaggacacg gccctgtatt actgtgcgag aggccgcgtg 300

atctccgcct ccgcgataag aggggcggtt aggggcccgg ggacccaggt caccgtctcc 360

tca 363

<210> SEQ ID NO 54
<211> LENGTH: 126
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: VHH peptide monomer AH3

<400> SEQUENCE: 54

Gln Val Gln Leu Val Glu Thr Gly Gly Leu Val Gln Pro Gly Gly Ser
1 5 10 15

Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Leu Asp Tyr Ser Ser
20 25 30

Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val Ser
35 40 45

Cys Ile Ser Ser Ser Gly Asp Ser Thr Lys Tyr Ala Asp Ser Val Lys
50 55 60

Gly Arg Phe Thr Thr Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Lys Pro Asp Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Ala Phe Arg Ala Thr Met Cys Gly Val Phe Pro Leu Ser Pro Tyr Gly
100 105 110

Lys Asp Asp Trp Gly Lys Gly Thr Leu Val Thr Val Ser Ser
115 120 125

<210> SEQ ID NO 55
<211> LENGTH: 378
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: VHH peptide monomer AH3

<400> SEQUENCE: 55

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caggtgcagc tcgtggagac gggggggcttg gtgcagcctg gggggtctct gagactctcc 60

tgtgcagcct ctggattcac tttggattat tcgtccatag gctggttccg ccaggcccca 120

gggaaggagc gtgaggggggt ctcatgtatt agtagtagtg gtgatagcac aaagtatgca 180

gactccgtga agggccgatt caccacctcc agagacaacg ccaagaacac ggtgtatctg 240

caaatgaaca gcctgaaacc tgacgacaca gccgtttatt actgtgcagc ttttagggcg 300

actatgtgcg gcgtgttccc ccttagcccc tacggcaagg acgactgggg caaagggacc 360

ctggtcaccg tctcctca 378

<210> SEQ ID NO 56
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Flexible linker 1

<400> SEQUENCE: 56

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15

<210> SEQ ID NO 57
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Flexible linker 1

<400> SEQUENCE: 57

ggcggtggtg gctctggtgg cggcggttcc ggtggcggtg gcagc 45

<210> SEQ ID NO 58
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Flexible linker 2

<400> SEQUENCE: 58

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15

<210> SEQ ID NO 59
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Flexible linker 2

<400> SEQUENCE: 59

ggtggaggcg gttcaggcgg aggtggctct ggcggtggcg gttcc 45

<210> SEQ ID NO 60
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Flexible linker 3

<400> SEQUENCE: 60

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15

<210> SEQ ID NO 61

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<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Flexible linker 3

<400> SEQUENCE: 61

ggcggtggtg gctctggtgg cggcggttcc ggtggcggtg gcagc 45

<210> SEQ ID NO 62
<211> LENGTH: 259
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 5D-E3 heterodimer

<400> SEQUENCE: 62

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Glu Ala Ser Gly Phe Thr Leu Asp Tyr Tyr
20 25 30

Gly Ile Gly Trp Phe Arg Gln Pro Pro Gly Lys Glu Arg Glu Ala Val
35 40 45

Ser Tyr Ile Ser Ala Ser Ala Arg Thr Ile Leu Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ala Val Tyr
65 70 75 80

Leu Gln Met Glu Thr Asn Ser Leu Lys Arg Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Ala Arg Arg Arg Phe Ser Ala Ser Ser Val Asn Arg Trp Leu
100 105 110

Ala Asp Asp Tyr Asp Val Trp Gly Arg Gly Thr Gln Val Ala Val Ser
115 120 125

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
130 135 140

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Thr Gly Gly
145 150 155 160

Ser Leu Arg Leu Ser Cys Ala Ser Ser Gly Ser Ile Ala Gly Phe Glu
165 170 175

Thr Val Thr Trp Ser Arg Gln Ala Pro Gly Lys Ser Leu Gln Trp Val
180 185 190

Ala Ser Met Glu Thr Thr Lys Thr Asn Asn Glu Ile Tyr Ser Asp Ser
195 200 205

Val Lys Gly Arg Phe Ile Ile Ser Arg Asp Asn Ala Lys Asn Thr Val
210 215 220

Tyr Leu Gln Met Glu Thr Asn Ser Leu Lys Pro Glu Asp Thr Gly Val
225 230 235 240

Tyr Phe Cys Lys Gly Pro Glu Leu Arg Gly Gln Gly Ile Gln Val Thr
245 250 255

Val Ser Ser

<210> SEQ ID NO 63
<211> LENGTH: 759
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 5D-E3 heterodimer

<400> SEQUENCE: 63

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caggtgcagc tcgtggagtc aggtggaggc ttggtgcagc ctgggggggtc tctgagactc 60

tcctgtgaag cctctggatt cactttggat tattatggta taggctggtt ccgccagccc 120

ccagggaagg agcgcgaggc ggtctcatat attagtgcca gtgcccgtac gatattgtat 180

gcagattccg tgaagggccg atttaccatc tccagagaca atgccaagaa cgcggtgtat 240

ctacaaatga acagcctgaa acgtgaggac acggctgtct attactgtgc gaggcggcga 300

ttctccgcgt ctagtgttaa tagatggctt gccgacgact atgacgtctg gggtcggggg 360

acccaggtcg cggtgtcctc aggcggtggt ggctctggtg gcggcggttc cggtggcggt 420

ggcagccagg tgcagctcgt ggagtcgggc ggaggcttgg tgcagactgg ggggtctctg 480

agactctcct gtgcatcctc tggaagtatc gccggtttcg aaaccgtgac ctggtcccgc 540

caggctcctg gaaagtcgct ccagtgggtc gcatcgatga ctaaaactaa taacgagatc 600

tattcagact ccgtgaaggg ccgattcatc atctccagag acaacgccaa gaatacggtg 660

tatctacaaa tgaacagcct gaaacctgag gacacaggcg tctatttttg taaaggtcct 720

gagttgaggg gccaggggat ccaggtcacc gtctcctcg 759

<210> SEQ ID NO 64
<211> LENGTH: 272
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AH3-AA6 heterodimer

<400> SEQUENCE: 64

Gln Val Gln Leu Val Glu Thr Gly Gly Leu Val Gln Pro Gly Gly Ser
1 5 10 15

Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Leu Asp Tyr Ser Ser
20 25 30

Ile Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val Ser
35 40 45

Cys Ile Ser Ser Ser Gly Asp Ser Thr Lys Tyr Ala Asp Ser Val Lys
50 55 60

Gly Arg Phe Thr Thr Ser Arg Asp Asn Ala Lys Asn Thr Val Tyr Leu
65 70 75 80

Gln Met Glu Thr Asn Ser Leu Lys Pro Asp Asp Thr Ala Val Tyr Tyr
85 90 95

Cys Ala Ala Phe Arg Ala Thr Met Glu Thr Cys Gly Val Phe Pro Leu
100 105 110

Ser Pro Tyr Gly Lys Asp Asp Trp Gly Lys Gly Thr Leu Val Thr Val
115 120 125

Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
130 135 140

Ser Gln Leu Gln Leu Val Glu Thr Gly Gly Gly Leu Val Gln Pro Gly
145 150 155 160

Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp
165 170 175

Tyr Val Met Glu Thr Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Pro
180 185 190

Glu Trp Ile Ala Thr Ile Asn Thr Asp Gly Ser Thr Met Glu Thr Arg
195 200 205

Asp Asp Ser Thr Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
210 215 220

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Asn Thr Leu Tyr Leu Gln Met Glu Thr Thr Ser Leu Lys Pro Glu Asp
225 230 235 240

Thr Ala Leu Tyr Tyr Cys Ala Arg Gly Arg Val Ile Ser Ala Ser Ala
245 250 255

Ile Arg Gly Ala Val Arg Gly Pro Gly Thr Gln Val Thr Val Ser Ser
260 265 270

<210> SEQ ID NO 65
<211> LENGTH: 786
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: AH3-AA6 heterodimer

<400> SEQUENCE: 65

caggtgcagc tcgtggagac gggggggcttg gtgcagcctg gggggtctct gagactctcc 60

tgtgcagcct ctggattcac tttggattat tcgtccatag gctggttccg ccaggcccca 120

gggaaggagc gtgaggggggt ctcatgtatt agtagtagtg gtgatagcac aaagtatgca 180

gactccgtga agggccgatt caccacctcc agagacaacg ccaagaacac ggtgtatctg 240

caaatgaaca gcctgaaacc tgacgacaca gccgtttatt actgtgcagc ttttagggcg 300

actatgtgcg gcgtgttccc ccttagcccc tacggcaagg acgactgggg caaagggacc 360

ctggtcaccg tctcctcagg cggtggtggc tctggtggcg gcggttccgg tggcggtggc 420

agccagttgc agctcgtgga gacaggggga ggcttggtgc agcctggggg gtctctgaga 480

ctctcctgtg cagcctctgg attcacgttc agtgactacg tcatgacctg ggtccgccag 540

gctccaggaa aggggcccga atggatcgca actattaata cggacggtag cacgatgcgt 600

gatgactcca caaaaggccg attcaccatc tccagagaca acgccaagaa cacactgtat 660

ctgcaaatga ccagcctgaa accggaggac acggccctgt attactgtgc gagaggccgc 720

gtgatctccg cctccgcgat aagaggggcg gttaggggcc cggggaccca ggtcaccgtc 780

tcctca 786

<210> SEQ ID NO 66
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: six-histidine tag

<400> SEQUENCE: 66

His His His His His His
1 5

<210> SEQ ID NO 67
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E-tag for protein purification

<400> SEQUENCE: 67

Gly Ala Pro Val Pro Tyr Pro Asp Pro Leu Glu Pro Arg
1 5 10

What is claimed is:

1. A tetra-specific, octameric binding agent comprising:

(a) an IgG antibody, two sets of linked first and second VHH peptide monomers, and two sets of linked third and fourth VHH peptide monomers,

wherein the IgG antibody comprises two arms, each arm comprising a heavy chain lacking a variable region and a light chain lacking a variable region, and each chain having an amino terminus,

wherein for each arm of the antibody, one set of linked first and second VHH peptide monomers is joined to the amino terminus of the light chain, and one set of linked third and fourth VHH peptide monomers is joined to the amino terminus of the heavy chain, and wherein the VHH peptide monomers have binding specificity for an epitope of *Clostridium difficile* toxin A (TcdA) or toxin B (TcdB); or

(b) an antibody Fc domain and two sets of linked first, second, third and fourth VHH peptide monomers,

wherein the antibody Fc domain comprises two arms, each arm comprising hinge, C_H2 and C_H3 regions of an antibody heavy chain, and each arm having an amino terminus,

wherein for each arm of the Fc domain, one set of linked first, second, third and fourth V_HH peptide monomers is joined to the amino terminus of the arm, and

wherein the V_HH peptide monomers have binding specificity for an epitope of *C. difficile* toxin A (TcdA) or toxin B (TcdB);

wherein for the binding agents of (a) and (b), the V_HH peptide monomers are (i) the 5D V_HH monomer (SEQ ID NO: 1), (ii) the E3 V_HH monomer (SEQ ID NO:3), (iii) the AA6 V_HH monomer (SEQ ID NO:5), and (iv) the AH3 V_HH monomer (SEQ ID NO:7);

wherein for the binding agent of (a), the light chain of the binding agent comprises the amino acid sequence set forth in SEQ ID NO:46, and wherein the heavy chain of the binding agent comprises the amino acid sequence set forth in SEQ ID NO:44; and

wherein for the binding agent of (b), the binding agent comprises the amino acid sequence set forth in SEQ ID NO:22.

2. The binding agent of claim **1**, wherein the first, second, third and fourth V_HH peptide monomers each has binding specificity for a different epitope.

3. The binding agent of claim **1**, wherein two of the V_HH peptide monomers have binding specificity for epitopes of TcdA and two of the V_HH peptide monomers have binding specificity for epitopes of TcdB.

4. The binding agent of claim **1**, wherein the V_HH peptide monomers independently have binding specificity for an epitope in the glucosyltransferase domain, cysteine protease domain, translocation domain or receptor binding domain of TcdA or TcdB.

5. The binding agent of claim **1**, wherein the binding agent is a binding agent of (a).

6. A method of producing a binding agent comprising culturing an isolated host cell comprising an expression vector comprising an isolated polynucleotide sequence comprising a nucleotide sequence encoding a binding agent of claim **1** under conditions promoting expression of the binding agent, and recovering the binding agent from the cell culture.

7. The binding agent of claim **1**, wherein the binding agent is a binding agent of (b).

8. A pharmaceutical formulation comprising a binding agent of any one of claims **1**, **5** and **7** and a pharmaceutically acceptable carrier or diluent.

9. A method of treating or preventing a disease symptom induced by *C. difficile* in a subject comprising administering a therapeutically-effective amount of one or more binding agent of any one of claims **1**, **5** and **7**, or pharmaceutical formulation comprising the one or more binding agent and a pharmaceutically acceptable carrier or diluent, to a subject having *C. difficile* infection or a risk of developing *C. difficile* infection.

10. A method of neutralizing *C. difficile* toxin TcdA and/or TcdB in a subject infected by *C. difficile* comprising administering a therapeutically-effective amount of one or more binding agent of any one of claims **1**, **5** and **7**, or pharmaceutical formulation comprising the one or more binding agent and a pharmaceutically acceptable carrier or diluent, to a subject having *C. difficile* infection.

11. A method of treating or preventing *C. difficile* disease in a subject comprising administering a therapeutically-effective amount of one or more binding agent of any one of claims **1**, **5** and **7**, or pharmaceutical formulation comprising the one or more binding agent and a pharmaceutically acceptable carrier or diluent, to a subject having *C. difficile* infection or a risk of developing *C. difficile* infection.

12. The method of claim **10**, where the neutralizing is partial or full neutralization.

* * * * *