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(54) Title: ANTIGEN-BINDING PROTEINS

(57) **Abrégé/Abstract:**

The invention relates to linkers suitable for use in antigen-binding proteins, certain antigen binding proteins and methods of making such proteins and uses thereof.



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(57) Abstract: The invention relates to linkers suitable for use in antigen-binding proteins, certain antigen binding proteins and methods of making such proteins and uses thereof.



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Antigen-binding proteins

Background

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Antibodies are well known for use in therapeutic applications.

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Antibodies are heteromultimeric glycoproteins comprising at least two heavy and two light chains. Aside from IgM, intact antibodies are usually heterotetrameric glycoproteins of approximately 150Kda, composed of two identical light (L) chains and two identical heavy (H) chains. Typically, each light chain is linked to a heavy chain by one covalent disulfide bond while the number of disulfide linkages between the heavy chains of different immunoglobulin isotypes varies. Each heavy and light chain also has intrachain disulfide bridges. Each heavy chain has at one end a variable domain (VH) followed by a number of constant regions. Each light chain has a variable domain (VL) and a constant region at its other end; the constant region of the light chain is aligned with the first constant region of the heavy chain and the light chain variable domain is aligned with the variable domain of the heavy chain. The light chains of antibodies from most vertebrate species can be assigned to one of two types called Kappa and Lambda based on the amino acid sequence of the constant region. Depending on the amino acid sequence of the constant region of their heavy chains, human antibodies can be assigned to five different classes, IgA, IgD, IgE, IgG and IgM. IgG and IgA can be further subdivided into subclasses, IgG1, IgG2, IgG3 and IgG4; and IgA1 and IgA2. Species variants exist with mouse and rat having at least IgG2a, IgG2b. The variable domain of the antibody confers binding specificity upon the antibody with certain regions displaying particular variability called complementarity determining regions (CDRs). The more conserved portions of the variable region are called Framework regions (FR). The variable domains of intact heavy and light chains each comprise four FR connected by three CDRs. The CDRs in each chain are held together in close proximity by the FR regions and with the CDRs from the other chain contribute to the formation of the antigen binding site of antibodies. The constant regions are not directly involved in the binding of the antibody to the antigen but exhibit various effector functions such as participation in antibody dependent cell-mediated cytotoxicity (ADCC), phagocytosis via binding to Fcγ receptor, half-life/clearance rate via neonatal Fc receptor (FcRn) and complement dependent cytotoxicity via the C1q component of the complement cascade.

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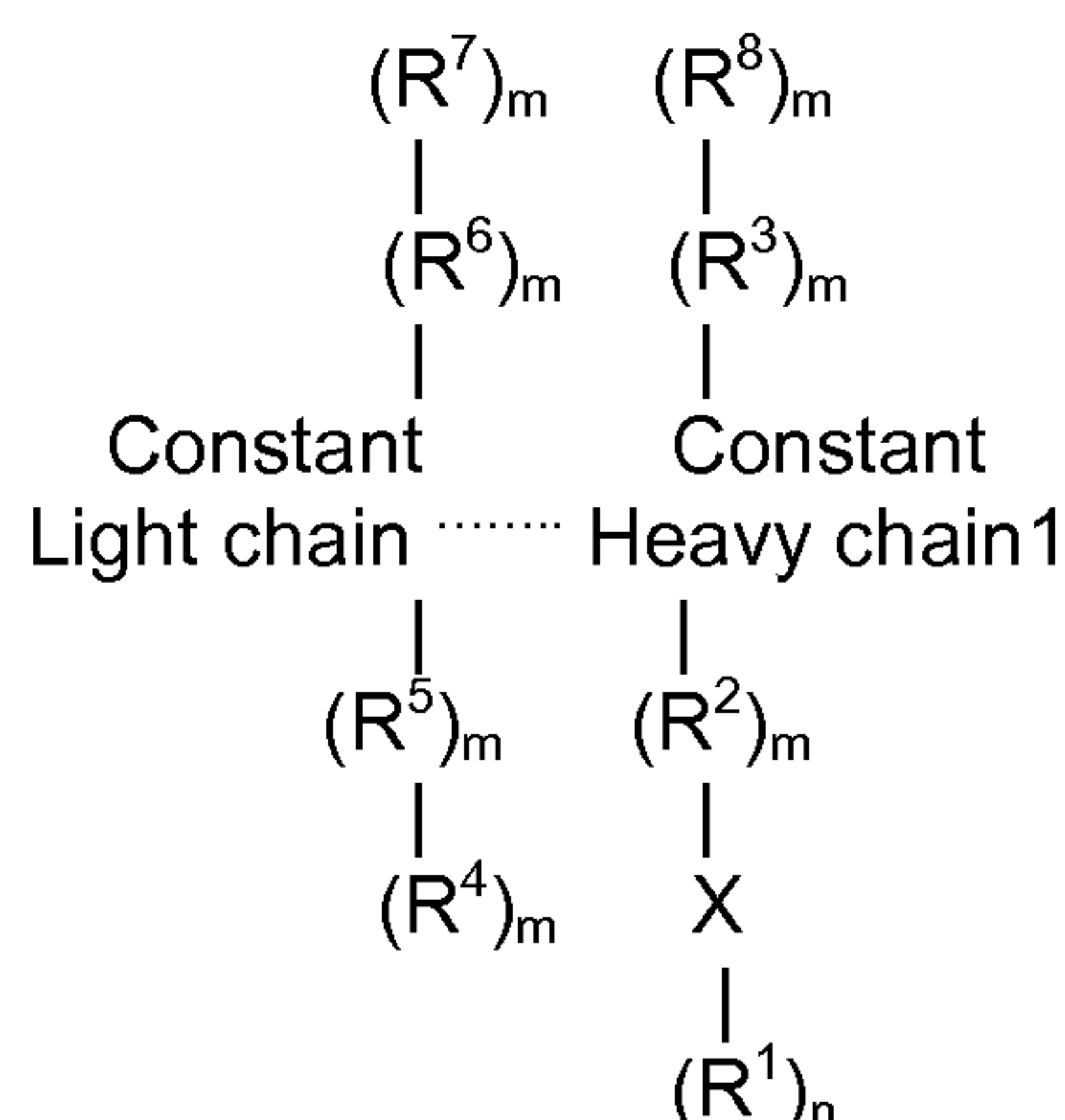
The nature of the structure of an IgG antibody is such that there are two antigen-binding sites, both of which are specific for the same epitope. They are therefore, monospecific.

A bispecific antibody is an antibody having binding specificities for at least two different epitopes. Methods of making such antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the coexpression of two immunoglobulin H chain-L chain pairs, where the two H chains have different binding specificities see Millstein *et al*, Nature 305 537-539 (1983), WO93/08829 and Traunecker *et al* EMBO, 10, 1991, 3655-3659. Because of the random assortment of H and L chains, a potential mixture of ten different antibody structures are produced of which only one has the desired binding specificity. An alternative approach involves fusing the variable domains with the desired binding specificities to heavy chain constant region comprising at least part of the hinge region, CH2 and CH3 regions. It is preferred to have the CH1 region containing the site necessary for light chain binding present in at least one of the fusions. DNA encoding these fusions, and if desired the L chain are inserted into separate expression vectors and are then cotransfected into a suitable host organism. It is possible though to insert the coding sequences for two or all three chains into one expression vector. In one approach, a bispecific antibody is composed of a H chain with a first binding specificity in one arm and a H-L chain pair, providing a second binding specificity in the other arm, see WO94/04690. Also see Suresh *et al* Methods in Enzymology 121, 210, 1986. Other approaches include antibody molecules which comprise single domain binding sites which is set out in WO2007/095338.

Summary of invention

The present invention relates to an antigen-binding protein comprising a protein scaffold which is linked to one or more epitope-binding domains wherein the antigen-binding protein has at least two antigen binding sites at least one of which is from an epitope binding domain and at least one of which is from a paired VH/VL domain.

The invention further relates to antigen-binding proteins comprising at least one homodimer comprising two or more structures of formula I:



(I)

wherein

5 X represents a constant antibody region comprising constant heavy domain 2 and constant heavy domain 3;

R^1 , R^4 , R^7 and R^8 represent a domain independently selected from an epitope-binding domain;

10 R^2 represents a domain selected from the group consisting of constant heavy chain 1, and an epitope-binding domain;

R^3 represents a domain selected from the group consisting of a paired VH and an epitope-binding domain;

15 R^5 represents a domain selected from the group consisting of constant light chain, and an epitope-binding domain;

20 R^6 represents a domain selected from the group consisting of a paired VL and an epitope-binding domain;

n represents an integer independently selected from: 0, 1, 2, 3 and 4;
m represents an integer independently selected from: 0 and 1,

25 wherein the Constant Heavy chain 1 and the Constant Light chain domains are associated;

wherein at least one epitope binding domain is present;

30 wherein when R^3 represents a paired VH domain, R^6 represents a paired VL domain, so that the two domains are together capable of binding antigen;

and wherein the epitope binding domains are linked to the rest of the molecule by the linkers of the present invention.

35 The invention relates to IgG based structures which comprise monoclonal antibodies, or fragments linked to one or more domain antibodies, and to methods of making such proteins and uses thereof, particularly uses in therapy.

40 The invention also provides a polynucleotide sequence encoding a heavy chain of any of the antigen binding proteins described herein, and a polynucleotide encoding a light chain of any of the antigen binding proteins described herein. Such polynucleotides represent the coding sequence which corresponds to the equivalent

polypeptide sequences, however it will be understood that such polynucleotide sequences could be cloned into an expression vector along with a start codon, an appropriate signal sequence and a stop codon.

5 The invention also provides a recombinant transformed or transfected host cell comprising one or more polynucleotides encoding a heavy chain and a light chain of any of the antigen binding proteins described herein.

10 The invention further provides a method for the production of any of the antigen binding proteins described herein which method comprises the step of culturing a host cell comprising a first and second vector, said first vector comprising a polynucleotide encoding a heavy chain of any of the antigen binding proteins described herein and said second vector comprising a polynucleotide encoding a light chain of any of the antigen binding proteins described herein, in a serum- free culture media.

15 The invention further provides a pharmaceutical composition comprising an antigen binding protein as described herein a pharmaceutically acceptable carrier.

20 The invention also provides a mAbdAb comprising the heavy chain sequence set out in SEQ ID NO:85 and the light chain sequence set out in SEQ ID NO: 3, or the heavy chain sequence set out in SEQ ID NO:86 and the light chain sequence set out in SEQ ID NO: 3, or the heavy chain sequence set out in SEQ ID NO 2.and the light chain sequence set out in SEQ ID NO: 87, or the heavy chain sequence set out in SEQ ID NO:2.and the light chain sequence set out in SEQ ID NO: 88, or the heavy chain sequence set out in SEQ ID NO:85.and the light chain sequence set out in SEQ ID NO: 87, or the heavy chain sequence set out in SEQ ID NO:85.and the light chain sequence set out in SEQ ID NO: 88,or the heavy chain sequence set out in SEQ ID NO:86.and the light chain sequence set out in SEQ ID NO: 87, or the heavy chain sequence set out in SEQ ID NO:86.and the light chain sequence set out in
30 SEQ ID NO: 88.

Definitions

The term 'Protein Scaffold' as used herein includes but is not limited to an immunoglobulin (Ig) scaffold, for example an IgG scaffold, which may be a four chain or two chain antibody, or which may comprise only the Fc region of an antibody, or which may comprise one or more constant regions from an antibody, which constant regions may be of human or primate origin, or which may be an artificial chimera of human and primate constant regions. Such protein scaffolds may comprise antigen-binding sites in addition to the one or more constant regions, for example where the protein scaffold comprises a full IgG. Such protein scaffolds will be capable of being linked to other protein domains, for example protein domains which have antigen-binding sites, for example epitope-binding domains or ScFv domains.

A "domain" is a folded protein structure which has tertiary structure independent of the rest of the protein. Generally, domains are responsible for discrete functional properties of proteins and in many cases may be added, removed or transferred to other proteins without loss of function of the remainder of the protein and/or of the domain. A "single antibody variable domain" is a folded polypeptide domain comprising sequences characteristic of antibody variable domains. It therefore includes complete antibody variable domains and modified variable domains, for example, in which one or more loops have been replaced by sequences which are not characteristic of antibody variable domains, or antibody variable domains which have been truncated or comprise N- or C-terminal extensions, as well as folded fragments of variable domains which retain at least the binding activity and specificity of the full-length domain.

The phrase "immunoglobulin single variable domain" refers to an antibody variable domain (V_H , V_{HH} , V_L) that specifically binds an antigen or epitope independently of a different V region or domain. An immunoglobulin single variable domain can be present in a format (e.g., homo- or hetero-multimer) with other, different variable regions or variable domains where the other regions or domains are not required for antigen binding by the single immunoglobulin variable domain (i.e., where the immunoglobulin single variable domain binds antigen independently of the additional variable domains). A "domain antibody" or "dAb" is the same as an "immunoglobulin single variable domain" which is capable of binding to an antigen as the term is used herein. An immunoglobulin single variable domain may be a human antibody variable domain, but also includes single antibody variable domains from other species such as rodent (for example, as disclosed in WO 00/29004), nurse shark and *Camelid* V_{HH} dAbs. Camelid V_{HH} are immunoglobulin single variable domain polypeptides that are derived from species including camel, llama, alpaca, dromedary, and guanaco, which produce heavy chain antibodies naturally devoid of light chains. Such V_{HH} domains may be humanised according to standard techniques available in the art, and such domains are still considered to be "domain antibodies"

according to the invention. As used herein "V_H includes camelid V_{HH} domains. NARV are another type of immunoglobulin single variable domain which were identified in cartilaginous fish including the nurse shark. These domains are also known as Novel Antigen Receptor variable region (commonly abbreviated to V(NAR) or NARV). For
 5 further details see Mol. Immunol. **44**, 656-665 (2006) and US20050043519A.

The term "Epitope-binding domain" refers to a domain that specifically binds an antigen or epitope independently of a different V region or domain, this may be an immunoglobulin single variable domain for example a human, camelid (nanobody) or
 10 shark immunoglobulin single variable domain or it may be a non-immunoglobulin domain, for example a domain which is a derivative of a scaffold selected from the group consisting of CTLA-4 (Evibody); lipocalin; Protein A derived molecules such as Z-domain of Protein A (Affibody, SpA), A-domain (Avimer/Maxibody); Heat shock proteins such as GroEI and GroES; transferrin (trans-body); ankyrin repeat protein
 15 (DARPin); peptide aptamer; C-type lectin domain (Tetranectin); human γ -crystallin and human ubiquitin (affilins); PDZ domains; scorpion toxinkunitz type domains of human protease inhibitors; and fibronectin (adnectin); which has been subjected to protein engineering in order to obtain binding to a ligand other than the natural ligand.

20 CTLA-4 (Cytotoxic T Lymphocyte-associated Antigen 4) is a CD28-family receptor expressed on mainly CD4⁺ T-cells. Its extracellular domain has a variable domain-like Ig fold. Loops corresponding to CDRs of antibodies can be substituted with heterologous sequence to confer different binding properties. CTLA-4 molecules engineered to have different binding specificities are also known as Evibodies. For
 25 further details see Journal of Immunological Methods **248 (1-2)**, 31-45 (2001)

Lipocalins are a family of extracellular proteins which transport small hydrophobic molecules such as steroids, bilins, retinoids and lipids. They have a rigid β -sheet secondary structure with a number of loops at the open end of the conical structure
 30 which can be engineered to bind to different target antigens. Anticalins are between 160-180 amino acids in size, and are derived from lipocalins. For further details see Biochim Biophys Acta **1482**: 337-350 (2000), US7250297B1 and US20070224633

An affibody is a scaffold derived from Protein A of *Staphylococcus aureus* which can
 35 be engineered to bind to antigen. The domain consists of a three-helical bundle of approximately 58 amino acids. Libraries have been generated by randomisation of surface residues. For further details see Protein Eng. Des. Sel. **17**, 455-462 (2004) and EP1641818A1

Avimers are multidomain proteins derived from the A-domain scaffold family. The native domains of approximately 35 amino acids adopt a defined disulphide bonded structure. Diversity is generated by shuffling of the natural variation exhibited by the family of A-domains. For further details see Nature Biotechnology **23(12)**, 1556 -
5 1561 (2005) and Expert Opinion on Investigational Drugs **16(6)**, 909-917 (June 2007)

A transferrin is a monomeric serum transport glycoprotein. Transferrins can be engineered to bind different target antigens by insertion of peptide sequences in a permissive surface loop. Examples of engineered transferrin scaffolds include the
10 Trans-body. For further details see J. Biol. Chem **274**, 24066-24073 (1999).

Designed Ankyrin Repeat Proteins (DARPs) are derived from Ankyrin which is a family of proteins that mediate attachment of integral membrane proteins to the cytoskeleton. A single ankyrin repeat is a 33 residue motif consisting of two α -helices
15 and a β -turn. They can be engineered to bind different target antigens by randomising residues in the first α -helix and a β -turn of each repeat. Their binding interface can be increased by increasing the number of modules (a method of affinity maturation). For further details see J. Mol. Biol. **332**, 489-503 (2003), PNAS **100(4)**, 1700-1705 (2003) and J. Mol. Biol. 369, 1015-1028 (2007) and US20040132028A1.

20

Fibronectin is a scaffold which can be engineered to bind to antigen. Adnectins consists of a backbone of the natural amino acid sequence of the 10th domain of the 15 repeating units of human fibronectin type III (FN3). Three loops at one end of the β -sandwich can be engineered to enable an Adnectin to specifically recognize a
25 therapeutic target of interest. For further details see Protein Eng. Des. Sel. **18**, 435-444 (2005), US20080139791, WO2005056764 and US6818418B1.

Peptide aptamers are combinatorial recognition molecules that consist of a constant scaffold protein, typically thioredoxin (TrxA) which contains a constrained variable
30 peptide loop inserted at the active site. For further details see Expert Opin. Biol. Ther. **5**, 783-797 (2005).

Microbodies are derived from naturally occurring microproteins of 25-50 amino acids in length which contain 3-4 cysteine bridges – examples of microproteins include
35 KalataB1 and conotoxin and knottins. The microproteins have a loop which can be

engineered to include upto 25 amino acids without affecting the overall fold of the microprotein. For further details of engineered knottin domains, see WO2008098796.

Other epitope binding domains include proteins which have been used as a scaffold
5 to engineer different target antigen binding properties include human γ -crystallin and human ubiquitin (affilins), kunitz type domains of human protease inhibitors, PDZ-domains of the Ras-binding protein AF-6, scorpion toxins (charybdotoxin), C-type lectin domain (tetranectins) are reviewed in Chapter 7 – Non-Antibody Scaffolds from Handbook of Therapeutic Antibodies (2007, edited by Stefan Dubel) and Protein
10 Science **15**:14-27 (2006). Epitope binding domains of the present invention could be derived from any of these alternative protein domains.

As used herein, the terms “paired VH domain”, “paired VL domain”, and “paired VH/VL domains” refer to antibody variable domains which specifically bind antigen
15 only when paired with their partner variable domain. There is always one VH and one VL in any pairing, and the term “paired VH domain” refers to the VH partner, the term “paired VL domain” refers to the VL partner, and the term “paired VH/VL domains” refers to the two domains together.

20 In one embodiment of the invention the antigen binding site binds to antigen with a Kd of at least 1mM, for example a Kd of 10nM, 1nM, 500pM, 200pM, 100pM, to each antigen as measured by BiacoreTM, such as the BiacoreTM method as described in Example 7.

25 As used herein, the term “antigen binding site” refers to a site on a protein which is capable of specifically binding to antigen, this may be a single domain, for example an epitope-binding domain, or it may be paired VH/VL domains as can be found on a standard antibody. In some aspects of the invention single-chain Fv (ScFv) domains can provide antigen-binding sites.

30 The terms “mAb/dAb” and dAb/mAb” are used herein to refer to antigen-binding proteins of the present invention. The two terms can be used interchangeably, and are intended to have the same meaning as used herein.

35 The term “constant heavy chain 1” is used herein to refer to the CH1 domain of an immunoglobulin heavy chain.

The term “constant light chain” is used herein to refer to the constant domain of an immunoglobulin light chain.

40

Detailed description of Invention

5 The present invention relates to an antigen-binding protein comprising a protein scaffold which is linked to one or more epitope-binding domains wherein the antigen-binding protein has at least two antigen binding sites at least one of which is from an epitope binding domain and at least one of which is from a paired VH/VL domain.

10 Such antigen-binding proteins comprise a protein scaffold, for example an Ig scaffold such as IgG, for example a monoclonal antibody, which is linked to one or more epitope-binding domains, for example a domain antibody, wherein the binding protein has at least two antigen binding sites, at least one of which is from an epitope binding domain, and to methods of producing and uses thereof, particularly uses in therapy.

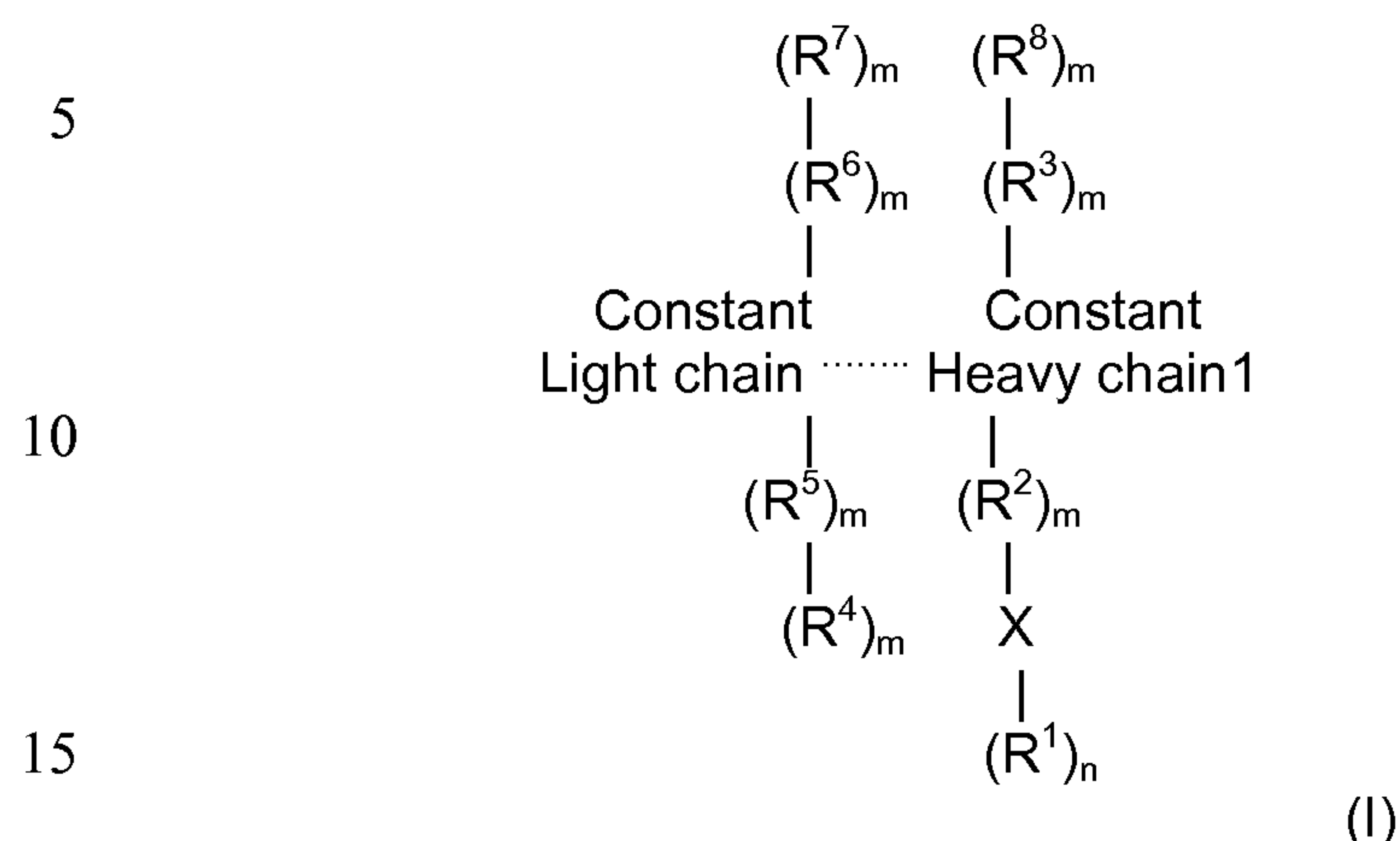
15 The antigen-binding proteins of the present invention are also referred to as mAbdAbs or dAbmAbs.

20 In one embodiment the protein scaffold of the antigen-binding protein of the present invention is an Ig scaffold, for example an IgG scaffold or IgA scaffold. The IgG scaffold may comprise all the domains of an antibody (i.e. CH1, CH2, CH3, VH, VL). The antigen-binding protein of the present invention may comprise an IgG scaffold selected from IgG1, IgG2, IgG3, IgG4 or IgG4PE.

25 The antigen-binding protein of the present invention has at least two antigen binding sites, for examples it has two binding sites, for example where the first binding site has specificity for a first epitope on an antigen and the second binding site has specificity for a second epitope on the same antigen. In a further embodiment there are 4 antigen binding sites, or 6 antigen binding sites, or 8 antigen binding sites, or 10 or more antigen-binding sites. In one embodiment the antigen binding protein has
30 specificity for more than one antigen, for example two antigens, or for three antigens, or for four antigens.

In another aspect the invention relates to an antigen-binding protein comprising at least one homodimer comprising two or more structures of formula I:

35



wherein

20 X represents a constant antibody region comprising constant heavy domain 2 and constant heavy domain 3;

R^1 , R^4 , R^7 and R^8 represent a domain independently selected from an epitope-binding domain;

25 R^2 represents a domain selected from the group consisting of constant heavy chain 1, and an epitope-binding domain;

30 R^3 represents a domain selected from the group consisting of a paired VH and an epitope-binding domain;

R^5 represents a domain selected from the group consisting of constant light chain, and an epitope-binding domain;

35 R^6 represents a domain selected from the group consisting of a paired VL and an epitope-binding domain;

n represents an integer independently selected from: 0, 1, 2, 3 and 4;
 m represents an integer independently selected from: 0 and 1,

40 wherein the Constant Heavy chain 1 and the Constant Light chain domains are associated;

wherein at least one epitope binding domain is present;

45 and when R^3 represents a paired VH domain, R^6 represents a paired VL domain, so that the two domains are together capable of binding antigen.

In one embodiment R^6 represents a paired VL and R^3 represents a paired VH.

5 In a further embodiment either one or both of R^7 and R^8 represent an epitope binding domain.

In yet a further embodiment either one or both of R^1 and R^4 represent an epitope binding domain.

10 In one embodiment R^4 is present.

In one embodiment R^1 , R^7 and R^8 represent an epitope binding domain.

15 In one embodiment R^1 , R^7 and R^8 , and R^4 represent an epitope binding domain.

In one embodiment $(R^1)_n$, $(R^2)_m$, $(R^4)_m$ and $(R^5)_m = 0$, i.e. are not present, R^3 is a paired VH domain, R^6 is a paired VL domain, R^8 is a VH dAb, and R^7 is a VL dAb.

20 In another embodiment $(R^1)_n$, $(R^2)_m$, $(R^4)_m$ and $(R^5)_m$ are 0, i.e. are not present, R^3 is a paired VH domain, R^6 is a paired VL domain, R^8 is a VH dAb, and $(R^7)_m = 0$ i.e. not present.

25 In another embodiment $(R^2)_m$ and $(R^5)_m$ are 0, i.e. are not present, R^1 is a dAb, R^4 is a dAb, R^3 is a paired VH domain, R^6 is a paired VL domain, $(R^8)_m$ and $(R^7)_m = 0$ i.e. not present.

In one embodiment of the present invention the epitope binding domain is an immunoglobulin single variable domain.

30 It will be understood that any of the antigen-binding proteins described herein will be capable of neutralising one or more antigens.

35 The term "neutralises" and grammatical variations thereof as used throughout the present specification in relation to antigen binding proteins of the invention means that a biological activity of the target is reduced, either totally or partially, in the presence of the antigen binding proteins of the present invention in comparison to the activity of the target in the absence of such antigen binding proteins. Neutralisation may be due to but not limited to one or more of blocking ligand binding, preventing the ligand activating the receptor, down regulating the receptor or affecting effector
40 functionality.

Levels of neutralisation can be measured in several ways, for example by use of any of the assays as set out in the examples and methods below, for example in an

assay which measures inhibition of ligand binding to receptor. The neutralisation of ligand in such assays can be measured by assessing the decreased binding between the ligand and its receptor in the presence of neutralising antigen binding protein.

5 Levels of neutralisation can also be measured, for example in a TF1 assay which may be carried out for example as described in Example 4.3 or 4.4. The neutralisation of IL-4, IL-13 or both of these cytokines in this assay is measured by assessing the inhibition of TF1 cell proliferation in the presence of neutralising antigen binding protein.

10

Other methods of assessing neutralisation, for example, by assessing the decreased binding between the ligand and its receptor in the presence of neutralising antigen binding protein are known in the art, and include, for example ELISA ligand receptor inhibition assays or Biacore™ assays.

15

In an alternative aspect of the present invention there is provided antigen binding proteins which have at least substantially equivalent neutralising activity to the antibodies exemplified herein, for example antigen binding proteins which retain the neutralising activity of BPC2201, BPC2202, BPC2203, BPC2209, BPC2210, 20 BPC2204 to BPC2208 and BPC2220 in the IL-13/4 binding assay as set out in Example 2.

The antigen binding proteins of the invention include those which have specificity for IL-13, for example which comprise an epitope-binding domain which is capable of 25 binding to IL-13, or which comprise a paired VH/VL which binds to IL-13. The antigen binding protein may comprise an antibody which is capable of binding to IL-13. The antigen binding protein may comprise an immunoglobulin single variable domain which is capable of binding to IL-13.

In one embodiment the antigen-binding protein of the present invention has 30 specificity for more than one antigen, for example where it is capable of binding two or more antigens selected from IL-13, IL-5, and IL-4, for example where it is capable of binding IL-13 and IL-4, or where it is capable of binding IL-13 and IL-5, or where it is capable of binding IL-5 and IL-4.

In one embodiment the antigen-binding protein of the present invention has 35 specificity for more than one antigen, for example where it is capable of binding two or more antigens selected from IL-13, IL-5, and IL-4, for example where it is capable of binding IL-13 and IL-4 simultaneously, or where it is capable of binding IL-13 and IL-5 simultaneously, or where it is capable of binding IL-5 and IL-4 simultaneously.

40 It will be understood that any of the antigen-binding proteins described herein may be capable of binding two or more antigens simultaneously, for example, as determined by stoichiometry analysis by using a suitable assay such as that described in Example 3.

Examples of antigen-binding proteins of the invention include IL-13 antibodies which have an epitope binding domain with a specificity for IL-4, for example an anti-IL-4 immunoglobulin single variable domain, attached to the c-terminus or the n-terminus of the heavy chain or the c-terminus or n-terminus of the light chain. Antigen binding proteins of the present invention include IL-13 antibodies with an IL-4 epitope binding domain attached to the n-terminus of the heavy chain. Antigen binding proteins of the present invention include IL-13 antibodies with an IL-4 epitope binding domain attached to the n-terminus of the light chain. Antigen binding proteins of the present invention include IL-13 antibodies with an IL-4 epitope binding domain attached to the c-terminus of the heavy chain. Antigen binding proteins of the present invention include IL-13 antibodies with an IL-4 epitope binding domain attached to the c-terminus of the light chain. Such antigen-binding proteins may also have one or more further epitope binding domains with the same or different antigen-specificity attached to the c-terminus and/or the n-terminus of the heavy chain and/ or the c-terminus and/or n-terminus of the light chain. IL-13 antibodies of use in the present invention include those comprising a heavy chain sequences as set out in SEQ ID NO: 28 and a light chain sequence as set out in SEQ ID NO: 27.

Examples of such antigen-binding proteins include IL-4 antibodies which have an epitope binding domain with a specificity for IL-13, for example an anti-IL-13 immunoglobulin single variable domain, attached to the c-terminus or the n-terminus of the heavy chain or the c-terminus or n-terminus of the light chain.

Antigen binding proteins of the present invention include IL-4 antibodies with an IL-13 epitope binding domain attached to the n-terminus of the heavy chain. Antigen binding proteins of the present invention include IL-4 antibodies with an IL-13 epitope binding domain attached to the n-terminus of the light chain. Antigen binding proteins of the present invention include IL-4 antibodies with an IL-13 epitope binding domain attached to the c-terminus of the heavy chain. Antigen binding proteins of the present invention include IL-4 antibodies with an IL-13 epitope binding domain attached to the c-terminus of the light chain. Such antigen-binding proteins may also have one or more further epitope binding domains with the same or different antigen-specificity attached to the c-terminus and/or the n-terminus of the heavy chain and/ or the c-terminus and/or n-terminus of the light chain.

IL-4 antibodies of use in the present invention include those comprising a heavy chain sequences as set out in SEQ ID NO:2 and a light chain sequence as set out in SEQ ID NO:3.

Examples of such antigen-binding proteins include IL-13 antibodies which have an epitope binding domain with a specificity for IL-5, for example an anti-IL-5 immunoglobulin single variable domain, attached to the c-terminus or the n-terminus of the heavy chain or the c-terminus or n-terminus of the light chain.. Antigen binding proteins of the present invention include IL-13 antibodies with an IL-5 epitope binding domain attached to the n-terminus of the heavy chain. Antigen binding proteins of the

present invention include IL-13 antibodies with an IL-5 epitope binding domain attached to the n-terminus of the light chain. Antigen binding proteins of the present invention include IL-13 antibodies with an IL-5 epitope binding domain attached to the c-terminus of the heavy chain. Antigen binding proteins of the present invention include IL-13 antibodies with an IL-5 epitope binding domain attached to the c-terminus of the light chain. Such antigen-binding proteins may also have one or more further epitope binding domains with the same or different antigen-specificity attached to the c-terminus and/or the n-terminus of the heavy chain and/ or the c-terminus and/or n-terminus of the light chain.

Examples of such antigen-binding proteins include IL-5 antibodies which have an epitope binding domain with a specificity for IL-13, for example an anti-IL-13 immunoglobulin single variable domain, attached to the c-terminus or the n-terminus of the heavy chain or the c-terminus or n-terminus of the light chain.

Antigen binding proteins of the present invention include IL-5 antibodies with an IL-13 epitope binding domain attached to the n-terminus of the heavy chain. Antigen binding proteins of the present invention include IL-5 antibodies with an IL-13 epitope binding domain attached to the n-terminus of the light chain. Antigen binding proteins of the present invention include IL-5 antibodies with an IL-13 epitope binding domain attached to the c-terminus of the heavy chain. Antigen binding proteins of the present invention include IL-5 antibodies with an IL-13 epitope binding domain attached to the c-terminus of the light chain. Such antigen-binding proteins may also have one or more further epitope binding domains with the same or different antigen-specificity attached to the c-terminus and/or the n-terminus of the heavy chain and/ or the c-terminus and/or n-terminus of the light chain. IL-5 antibodies of use in the present invention include those comprising a heavy chain sequences as set out in SEQ ID NO: 91 and a light chain sequence as set out in SEQ ID NO: 92

Examples of such antigen-binding proteins include IL-4 antibodies which have an epitope binding domain with a specificity for IL-5, for example an anti-IL-5 immunoglobulin single variable domain, attached to the c-terminus or the n-terminus of the heavy chain or the c-terminus or n-terminus of the light chain.. Antigen binding proteins of the present invention include IL-4 antibodies with an IL-5 epitope binding domain attached to the n-terminus of the heavy chain. Antigen binding proteins of the present invention include IL-4 antibodies with an IL-5 epitope binding domain attached to the n-terminus of the light chain. Antigen binding proteins of the present invention include IL-4 antibodies with an IL-5 epitope binding domain attached to the c-terminus of the heavy chain. Antigen binding proteins of the present invention include IL-4 antibodies with an IL-5 epitope binding domain attached to the c-terminus of the light chain. Such antigen-binding proteins may also have one or more further epitope binding domains with the same or different antigen-specificity attached to the c-terminus and/or the n-terminus of the heavy chain and/ or the c-terminus and/or n-terminus of the light chain.

Examples of such antigen-binding proteins include IL-5 antibodies which have an epitope binding domain with a specificity for IL-4, for example an anti-IL-4 immunoglobulin single variable domain, attached to the c-terminus or the n-terminus of the heavy chain or the c-terminus or n-terminus of the light chain.

Antigen binding proteins of the present invention include IL-5 antibodies with an IL-4 epitope binding domain attached to the n-terminus of the heavy chain. Antigen binding proteins of the present invention include IL-5 antibodies with an IL-4 epitope binding domain attached to the n-terminus of the light chain. Antigen binding proteins of the present invention include IL-5 antibodies with an IL-4 epitope binding domain attached to the c-terminus of the heavy chain. Antigen binding proteins of the present invention include IL-5 antibodies with an IL-4 epitope binding domain attached to the c-terminus of the light chain. Such antigen-binding proteins may also have one or more further epitope binding domains with the same or different antigen-specificity attached to the c-terminus and/or the n-terminus of the heavy chain and/or the c-terminus and/or n-terminus of the light chain.

The invention also provides a trispecific binding protein which is capable of binding to IL-4, IL-13 and IL-5.

Examples of such antigen-binding proteins include IL-5 antibodies which have an epitope binding domain with a specificity for IL-4, for example an anti-IL-4 immunoglobulin single variable domain, attached to the c-terminus or the n-terminus of the heavy chain or the c-terminus or n-terminus of the light chain and an epitope binding domain with a specificity for IL-13, for example an anti-IL-13 immunoglobulin single variable domain, attached to the c-terminus or the n-terminus of the heavy chain or the c-terminus or n-terminus of the light chain.

Antigen binding proteins of the present invention include IL-5 antibodies with an IL-4 epitope binding domain attached to the n-terminus of the heavy chain and an IL-13 epitope binding domain attached to the n-terminus of the light chain.

Antigen binding proteins of the present invention include IL-5 antibodies with an IL-4 epitope binding domain attached to the n-terminus of the heavy chain and an IL-13 epitope binding domain attached to the c-terminus of the light chain.

Antigen binding proteins of the present invention include IL-5 antibodies with an IL-4 epitope binding domain attached to the n-terminus of the heavy chain and an IL-13 epitope binding domain attached to the c-terminus of the heavy chain.

Antigen binding proteins of the present invention include IL-5 antibodies with an IL-4 epitope binding domain attached to the n-terminus of the light chain and an IL-13 epitope binding domain attached to the c-terminus of the light chain.

Antigen binding proteins of the present invention include IL-5 antibodies with an IL-4 epitope binding domain attached to the n-terminus of the light chain and an IL-13 epitope binding domain attached to the c-terminus of the heavy chain.

Antigen binding proteins of the present invention include IL-5 antibodies with an IL-4 epitope binding domain attached to the n-terminus of the light chain and an IL-13 epitope binding domain attached to the n-terminus of the heavy chain.

Antigen binding proteins of the present invention include IL-5 antibodies with an IL-4 epitope binding domain attached to the c-terminus of the heavy chain and an IL-13 epitope binding domain attached to the c-terminus of the light chain.

Antigen binding proteins of the present invention include IL-5 antibodies with an IL-4 epitope binding domain attached to the c-terminus of the heavy chain and an IL-13 epitope binding domain attached to the n-terminus of the light chain.

Antigen binding proteins of the present invention include IL-5 antibodies with an IL-4 epitope binding domain attached to the c-terminus of the heavy chain and an IL-13 epitope binding domain attached to the n-terminus of the heavy chain.

Antigen binding proteins of the present invention include IL-5 antibodies with an IL-4 epitope binding domain attached to the c-terminus of the light chain and an IL-13 epitope binding domain attached to the c-terminus of the heavy chain.

Antigen binding proteins of the present invention include IL-5 antibodies with an IL-4 epitope binding domain attached to the c-terminus of the light chain and an IL-13 epitope binding domain attached to the n-terminus of the heavy chain.

Antigen binding proteins of the present invention include IL-5 antibodies with an IL-4 epitope binding domain attached to the c-terminus of the light chain and an IL-13 epitope binding domain attached to the n-terminus of the light chain.

Such antigen-binding proteins may also have one or more further epitope binding domains with the same or different antigen-specificity attached to the c-terminus and/or the n-terminus of the heavy chain and/ or the c-terminus and/or n-terminus of the light chain.

The antigen binding proteins of the invention include those which have specificity for IL-18, for example which comprises an epitope-binding domain which is capable of binding to IL-18, or which comprises a paired VH/VL which binds to IL-18.

The antigen binding protein may comprise an antibody which is capable of binding to IL-18. The antigen binding protein may comprise an immunoglobulin single variable domain which is capable of binding to IL-18.

The invention also provides a trispecific binding protein which is capable of binding to IL-4, IL-13 and IL-18.

Examples of such antigen-binding proteins include IL-18 antibodies which have an epitope binding domain with a specificity for IL-4, for example an anti-IL-4 immunoglobulin single variable domain, attached to the c-terminus or the n-terminus of the heavy chain or the c-terminus or n-terminus of the light chain and an epitope

binding domain with a specificity for IL-13, for example an anti-IL-13 immunoglobulin single variable domain, attached to the c-terminus or the n-terminus of the heavy chain or the c-terminus or n-terminus of the light chain.

5 Antigen binding proteins of the present invention include IL-18 antibodies with an IL-4 epitope binding domain attached to the n-terminus of the heavy chain and an IL-13 epitope binding domain attached to the n-terminus of the light chain.

Antigen binding proteins of the present invention include IL-18 antibodies with an IL-4 epitope binding domain attached to the n-terminus of the heavy chain and an IL-13 epitope binding domain attached to the c-terminus of the light chain.

10 Antigen binding proteins of the present invention include IL-18 antibodies with an IL-4 epitope binding domain attached to the n-terminus of the heavy chain and an IL-13 epitope binding domain attached to the c-terminus of the heavy chain.

15 Antigen binding proteins of the present invention include IL-18 antibodies with an IL-4 epitope binding domain attached to the n-terminus of the light chain and an IL-13 epitope binding domain attached to the c-terminus of the light chain.

Antigen binding proteins of the present invention include IL-18 antibodies with an IL-4 epitope binding domain attached to the n-terminus of the light chain and an IL-13 epitope binding domain attached to the c-terminus of the heavy chain.

20 Antigen binding proteins of the present invention include IL-18 antibodies with an IL-4 epitope binding domain attached to the n-terminus of the light chain and an IL-13 epitope binding domain attached to the n-terminus of the heavy chain.

25 Antigen binding proteins of the present invention include IL-18 antibodies with an IL-4 epitope binding domain attached to the c-terminus of the heavy chain and an IL-13 epitope binding domain attached to the c-terminus of the light chain.

Antigen binding proteins of the present invention include IL-18 antibodies with an IL-4 epitope binding domain attached to the c-terminus of the heavy chain and an IL-13 epitope binding domain attached to the n-terminus of the light chain.

30 Antigen binding proteins of the present invention include IL-18 antibodies with an IL-4 epitope binding domain attached to the c-terminus of the heavy chain and an IL-13 epitope binding domain attached to the n-terminus of the heavy chain.

35 Antigen binding proteins of the present invention include IL-18 antibodies with an IL-4 epitope binding domain attached to the c-terminus of the light chain and an IL-13 epitope binding domain attached to the c-terminus of the heavy chain.

Antigen binding proteins of the present invention include IL-18 antibodies with an IL-4 epitope binding domain attached to the c-terminus of the light chain and an IL-13 epitope binding domain attached to the n-terminus of the heavy chain.

40 Antigen binding proteins of the present invention include IL-18 antibodies with an IL-4 epitope binding domain attached to the c-terminus of the light chain and an IL-13 epitope binding domain attached to the n-terminus of the light chain.

Such antigen-binding proteins may also have one or more further epitope binding domains with the same or different antigen-specificity attached to the c-terminus and/or the n-terminus of the heavy chain and/ or the c-terminus and/or n-terminus of the light chain.

5

The antigen binding proteins of the invention include those which have specificity for TNF α , for example which comprises an epitope-binding domain which is capable of binding to TNF α , or which comprises a paired VH/VL which binds to TNF α .

10 The antigen binding protein may comprise an antibody which is capable of binding to TNF α . The antigen binding protein may comprise an immunoglobulin single variable domain which is capable of binding to TNF α .

In one embodiment the antigen-binding protein of the present invention has specificity for more than one antigen, for example where it is capable of binding two or more antigens selected from TNF α , EGFR and VEGF, for example where it is
15 capable of binding TNF α and EGFR, or where it is capable of binding TNF α and VEGF, or where it is capable of binding EGFR and VEGF. Examples of such antigen-binding proteins include TNF α antibodies which have an epitope binding domain with a specificity for EGFR, for example an anti-EGFR immunoglobulin single variable domain, attached to the c-terminus or the n-terminus of the heavy chain or the c-terminus or n-terminus of the light chain.
20

Antigen binding proteins of the present invention include TNF α antibodies with an EGFR epitope binding domain attached to the n-terminus of the heavy chain. Antigen binding proteins of the present invention include TNF α antibodies with an EGFR
25 epitope binding domain attached to the n-terminus of the light chain. Antigen binding proteins of the present invention include TNF α antibodies with an EGFR epitope binding domain attached to the c-terminus of the heavy chain. Antigen binding proteins of the present invention include TNF α antibodies with an EGFR epitope binding domain attached to the c-terminus of the light chain. Such antigen-binding
30 proteins may also have one or more further epitope binding domains with the same or different antigen-specificity attached to the c-terminus and/or the n-terminus of the heavy chain and/ or the c-terminus and/or n-terminus of the light chain.

Antigen binding proteins of the present invention include EGFR antibodies with an
35 TNF α epitope binding domain attached to the n-terminus of the heavy chain. Antigen binding proteins of the present invention include EGFR antibodies with an TNF α epitope binding domain attached to the n-terminus of the light chain. Antigen binding proteins of the present invention include EGFR antibodies with an TNF α epitope binding domain attached to the c-terminus of the heavy chain. Antigen binding
40 proteins of the present invention include EGFR antibodies with an TNF α epitope binding domain attached to the c-terminus of the light chain. Such antigen-binding proteins may also have one or more further epitope binding domains with the same

or different antigen-specificity attached to the c-terminus and/or the n-terminus of the heavy chain and/ or the c-terminus and/or n-terminus of the light chain.

5 Examples of such antigen-binding proteins include TNF α antibodies which have an epitope binding domain with a specificity for VEGF, for example an anti-VEGF immunoglobulin single variable domain, attached to the c-terminus or the n-terminus of the heavy chain or the c-terminus or n-terminus of the light chain.

10 The antigen-binding protein of the present invention may have specificity for more than one antigen, for example where it is capable of binding TNF α , and one or both antigens selected from IL-4 and IL-13, for example where it is capable of binding TNF α and IL-4, or where it is capable of binding TNF α and IL-13, or where it is capable of binding TNF α and IL-13 and IL-4. Examples of such antigen-binding proteins include IL-13 antibodies which have an epitope binding domain with a
15 specificity for TNF α , for example an anti- TNF α adnectin, attached to the c-terminus or the n-terminus of the heavy chain or the c-terminus or n-terminus of the light chain. Other examples of such antigen-binding proteins include IL-4 antibodies which have an epitope binding domain with a specificity for TNF α , for example an anti- TNF α adnectin, attached to the c-terminus or the n-terminus of the heavy chain or the c-terminus or n-terminus of the light chain.
20

Antigen binding proteins of the present invention include TNF α antibodies with an VEGF epitope binding domain attached to the n-terminus of the heavy chain. Antigen binding proteins of the present invention include TNF α antibodies with an VEGF
25 epitope binding domain attached to the n-terminus of the light chain. Antigen binding proteins of the present invention include TNF α antibodies with an VEGF epitope binding domain attached to the c-terminus of the heavy chain. Antigen binding proteins of the present invention include TNF α antibodies with an VEGF epitope binding domain attached to the c-terminus of the light chain. Such antigen-binding
30 proteins may also have one or more further epitope binding domains with the same or different antigen-specificity attached to the c-terminus and/or the n-terminus of the heavy chain and/ or the c-terminus and/or n-terminus of the light chain.

Antigen binding proteins of the present invention include VEGF antibodies with an
35 TNF α epitope binding domain attached to the n-terminus of the heavy chain. Antigen binding proteins of the present invention include VEGF antibodies with an TNF α epitope binding domain attached to the n-terminus of the light chain. Antigen binding proteins of the present invention include VEGF antibodies with an TNF α epitope binding domain attached to the c-terminus of the heavy chain. Antigen binding
40 proteins of the present invention include VEGF antibodies with an TNF α epitope binding domain attached to the c-terminus of the light chain. Such antigen-binding proteins may also have one or more further epitope binding domains with the same

or different antigen-specificity attached to the c-terminus and/or the n-terminus of the heavy chain and/ or the c-terminus and/or n-terminus of the light chain.

5 The antigen binding proteins of the invention include those which have specificity for CD-20, for example which comprises an epitope-binding domain which is capable of binding to CD-20, or which comprises a paired VH/VL which binds to CD-20.

The antigen binding protein may comprise an antibody which is capable of binding to CD-20. The antigen binding protein may comprise an immunoglobulin single variable domain which is capable of binding to CD-20.

10 Such antigen-binding proteins may also have one or more further epitope binding domains with the same or different antigen-specificity attached to the c-terminus and/or the n-terminus of the heavy chain and/ or the c-terminus and/or n-terminus of the light chain.

15 The antigen binding proteins of the invention include those which have specificity for IL1R1, for example which comprise an epitope-binding domain which is capable of binding to IL1R1, or which comprises a paired VH/VL which binds to IL1R1.

The antigen binding protein may comprise an antibody which is capable of binding to IL1R1. The antigen binding protein may comprise an immunoglobulin single variable domain which is capable of binding to IL1R1.

20 In one embodiment the antigen-binding protein of the present invention has specificity for more than one antigen, for example where it is capable of binding IL1R1 and a second antigen, for example where it is capable of binding IL1R1 and VEGF. Examples of such antigen-binding proteins include IL1R1 antibodies which have an epitope binding domain with a specificity for VEGF, for example an anti-VEGF immunoglobulin single variable domain, attached to the c-terminus or the n-terminus of the heavy chain or the c-terminus or n-terminus of the light chain.

30 Antigen binding proteins of the present invention include IL1R1 antibodies with an VEGF epitope binding domain attached to the n-terminus of the heavy chain. Antigen binding proteins of the present invention include IL1R1 antibodies with an VEGF epitope binding domain attached to the n-terminus of the light chain. Antigen binding proteins of the present invention include IL1R1 antibodies with an VEGF epitope binding domain attached to the c-terminus of the heavy chain. Antigen binding proteins of the present invention include IL1R1 antibodies with an VEGF epitope binding domain attached to the c-terminus of the light chain. Such antigen-binding proteins may also have one or more further epitope binding domains with the same or different antigen-specificity attached to the c-terminus and/or the n-terminus of the heavy chain and/ or the c-terminus and/or n-terminus of the light chain.

40 Antigen binding proteins of the present invention include VEGF antibodies with an IL1R1 epitope binding domain attached to the n-terminus of the heavy chain. Antigen binding proteins of the present invention include VEGF antibodies with an IL1R1

epitope binding domain attached to the n-terminus of the light chain. Antigen binding proteins of the present invention include VEGF antibodies with an IL1R1 epitope binding domain attached to the c-terminus of the heavy chain. Antigen binding proteins of the present invention include VEGF antibodies with an IL1R1 epitope binding domain attached to the c-terminus of the light chain. Such antigen-binding proteins may also have one or more further epitope binding domains with the same or different antigen-specificity attached to the c-terminus and/or the n-terminus of the heavy chain and/ or the c-terminus and/or n-terminus of the light chain.

- 10 The antigen binding proteins of the invention include those which have specificity for EGFR, for example which comprises an epitope-binding domain which is capable of binding to EGFR, or which comprises a paired VH/VL which binds to EGFR. The antigen binding protein may comprise an antibody which is capable of binding to EGFR. The antigen binding protein may comprise an immunoglobulin single variable domain which is capable of binding to EGFR.

In one embodiment the antigen-binding protein of the present invention has specificity for more than one antigen, for example where it is capable of binding two or more antigens selected from EGFR, IGF-1R, VEGFR2 and VEGF, for example where it is capable of binding EGFR and IGF-1R, or where it is capable of binding EGFR and VEGF, or where it is capable of binding VEGF and IGF-1R, or where it is capable of binding EGFR and VEGFR2, or where it is capable of binding IGF-1R and VEGFR2, or where it is capable of binding VEGF and VEGFR2, or where it is capable of binding EGFR, IGF-1R and VEGFR2, or where it is capable of binding VEGF, IGF-1R and VEGFR2, or where it is capable of binding EGFR, VEGF and VEGFR2, or where it is capable of binding EGFR, VEGF and IGF1R. Examples of such antigen-binding proteins include EGFR antibodies which have an epitope binding domain with a specificity for VEGFR2, for example an anti-VEGFR2 adnectin, attached to the c-terminus or the n-terminus of the heavy chain or the c-terminus or n-terminus of the light chain.

Examples of such antigen-binding proteins include EGFR antibodies which have an epitope binding domain with a specificity for VEGF, for example an anti-VEGF immunoglobulin single variable domain, attached to the c-terminus or the n-terminus of the heavy chain or the c-terminus or n-terminus of the light chain.

35 Examples of such antigen-binding proteins include VEGF antibodies which have an epitope binding domain with a specificity for EGFR, for example an anti-EGFR immunoglobulin single variable domain, attached to the c-terminus or the n-terminus of the heavy chain or the c-terminus or n-terminus of the light chain.

Examples of such antigen-binding proteins include IGF-1R antibodies which have an epitope binding domain with a specificity for VEGF, for example an anti-VEGF lipocalin, attached to the c-terminus or the n-terminus of the heavy chain or the c-terminus or n-terminus of the light chain.

Examples of such antigen-binding proteins include IGF-1R antibodies which have an epitope binding domain with a specificity for VEGFR2, for example an anti-VEGFR2 adnectin, attached to the c-terminus or the n-terminus of the heavy chain or the c-terminus or n-terminus of the light chain.

5

The antigen binding proteins of the invention include those which have specificity for IL-23, for example which comprises an epitope-binding domain which is capable of binding to IL-23, or which comprises a paired VH/VL which binds to IL-23.

10 The antigen binding protein may comprise an antibody which is capable of binding to IL-23. The antigen binding protein may comprise an immunoglobulin single variable domain which is capable of binding to IL-23.

In one embodiment the antigen-binding protein of the present invention has specificity for more than one antigen, for example where it is capable of binding two or more antigens selected from TH17 type cytokines, for example. IL-17, IL-22, or IL-21, for example where it is capable of binding IL-23 and IL-17, or where it is capable of binding IL-23 and IL-21, or where it is capable of binding IL-23 and IL-22.

15 Examples of such antigen-binding proteins include IL-23 antibodies which have an epitope binding domain with a specificity for IL-17, for example an anti-IL-17 immunoglobulin single variable domain, attached to the c-terminus or the n-terminus of the heavy chain or the c-terminus or n-terminus of the light chain.

The antigen binding proteins of the invention include those which have specificity for PDGFR α , for example which comprises an epitope-binding domain which is capable of binding to PDGFR α , or which comprises a paired VH/VL which binds to PDGFR α .

25 The antigen binding protein may comprise an antibody which is capable of binding to PDGFR α . The antigen binding protein may comprise an immunoglobulin single variable domain which is capable of binding to PDGFR α .

The antigen binding proteins of the invention include those which have specificity for FGFR1, for example which comprises an epitope-binding domain which is capable of binding to FGFR1, or which comprises a paired VH/VL which binds to FGFR1. The antigen binding protein may comprise an antibody which is capable of binding to FGFR1. The antigen binding protein may comprise an immunoglobulin single variable domain which is capable of binding to FGFR1.

30 The antigen binding proteins of the invention include those which have specificity for FGFR3, for example which comprises an epitope-binding domain which is capable of binding to FGFR3, or which comprises a paired VH/VL which binds to FGFR3. The antigen binding protein may comprise an antibody which is capable of binding to FGFR3. The antigen binding protein may comprise an immunoglobulin single variable domain which is capable of binding to FGFR3.

40 The antigen binding proteins of the invention include those which have specificity for VEGFR2, for example which comprises an epitope-binding domain which is capable of binding to VEGFR2, or which comprises a paired VH/VL which binds to VEGFR2.

The antigen binding protein may comprise an antibody which is capable of binding to VEGFR2. The antigen binding protein may comprise an immunoglobulin single variable domain which is capable of binding to VEGFR2.

5 The antigen binding proteins of the invention include those which have specificity for VEGFR3, for example which comprises an epitope-binding domain which is capable of binding to VEGFR3, or which comprises a paired VH/VL which binds to VEGFR3. The antigen binding protein may comprise an antibody which is capable of binding to VEGFR3. The antigen binding protein may comprise an immunoglobulin single variable domain which is capable of binding to VEGFR3.

10 The antigen binding proteins of the invention include those which have specificity for VE cadherin, for example which comprises an epitope-binding domain which is capable of binding to VE cadherin, or which comprises a paired VH/VL which binds to VE cadherin.

15 The antigen binding protein may comprise an antibody which is capable of binding to VE cadherin. The antigen binding protein may comprise an immunoglobulin single variable domain which is capable of binding to VE cadherin.

The antigen binding proteins of the invention include those which have specificity for neuropilin, for example which comprises an epitope-binding domain which is capable of binding to neuropilin, or which comprises a paired VH/VL which binds to neuropilin.

20 The antigen binding protein may comprise an antibody which is capable of binding to neuropilin. The antigen binding protein may comprise an immunoglobulin single variable domain which is capable of binding to neuropilin.

The antigen binding proteins of the invention include those which have specificity for Flt-3, for example which comprises an epitope-binding domain which is capable of binding to Flt-3, or which comprises a paired VH/VL which binds to Flt-3.

25

The antigen binding protein may comprise an antibody which is capable of binding to Flt-3. The antigen binding protein may comprise an immunoglobulin single variable domain which is capable of binding to Flt-3.

30 The antigen binding proteins of the invention include those which have specificity for ron, for example which comprises an epitope-binding domain which is capable of binding ron, or which comprises a paired VH/VL which binds to ron.

The antigen binding protein may comprise an antibody which is capable of binding to ron. The antigen binding protein may comprise an immunoglobulin single variable domain which is capable of binding to ron.

35 The antigen binding proteins of the invention include those which have specificity for Trp-1, for example which comprises an epitope-binding domain which is capable of binding Trp-1, or which comprises a paired VH/VL which binds to Trp-1.

40 The antigen binding protein may comprise an antibody which is capable of binding to Trp-1. The antigen binding protein may comprise an immunoglobulin single variable domain which is capable of binding to Trp-1.

In one embodiment the antigen-binding protein of the present invention has specificity for more than one antigen, for example where it is capable of binding two

or more antigens which are implicated in cancer, for example where it is capable of binding two or more antigens selected from PDGFR α , FGFR1, FGFR3, VEGFR2, VEGFR3, IGF1R, EGFR and VEGF, VE cadherin, neuropilin, Flt-3, ron, Trp-1, CD-20 for example where it is capable of binding PDGFR α and FGFR1, or where it is
 5 capable of binding PDGFR α and VEGF, or where it is capable of binding PDGFR α and FGFR3, or where it is capable of binding PDGFR α and VEGFR2, or where it is capable of binding PDGFR α and VEGFR3, or where it is capable of binding PDGFR α and IGF1R, or where it is capable of binding PDGFR α and EGFR, or where it is capable of binding PDGFR α and VEGF, or where it is capable of binding PDGFR α
 10 and VE cadherin, or where it is capable of binding PDGFR α and neuropilin, or where it is capable of binding PDGFR α and Flt-3, or where it is capable of binding PDGFR α and ron, or where it is capable of binding PDGFR α and Trp1, or where it is capable of binding PDGFR α and CD-20, or where it is capable of binding FGFR1 and FGFR3, or where it is capable of binding FGFR1 and VEGFR2, or where it is capable of
 15 binding FGFR1 and VEGFR3, or where it is capable of binding FGFR1 and IGF1R, or where it is capable of binding FGFR1 and EGFR, or where it is capable of binding FGFR1 and VEGF, or where it is capable of binding FGFR1 and VE cadherin, or where it is capable of binding FGFR1 and neuropilin, or where it is capable of binding FGFR1 and Flt-3, or where it is capable of binding FGFR1 and ron, or where it is capable of binding FGFR1 and Trp-1, or where it is capable of binding FGFR1 and CD-20, or where it is capable of binding FGFR3 and VEGFR2, or where it is capable of binding FGFR3 and VEGFR3, or where it is capable of binding FGFR3 and IGF1R, or where it is capable of binding FGFR3 and EGFR, or where it is capable of binding FGFR3 and VEGF, or where it is capable of binding FGFR3 and VE cadherin, or
 20 where it is capable of binding FGFR3 and neuropilin, or where it is capable of binding FGFR3 and Flt-3, or where it is capable of binding FGFR3 and ron, or where it is capable of binding FGFR3 and Trp-1, or where it is capable of binding FGFR3 and CD-20, or where it is capable of binding VEGFR2 and VEGFR3, or or where it is capable of binding VEGFR2 and IGF1R, or where it is capable of binding VEGFR2 and EGFR, or where it is capable of binding VEGFR2 and VEGF, or where it is capable of binding VEGFR2 and VE cadherin, or where it is capable of binding VEGFR2 and neuropilin, or where it is capable of binding VEGFR2 and Flt-3, or where it is capable of binding VEGFR2 and ron, or where it is capable of binding VEGFR2 and Trp-1, or where it is capable of binding VEGFR2 and CD-20, or where
 25 it is capable of binding VEGFR3 and IGF-1R, or where it is capable of binding VEGFR3 and EGFR, or where it is capable of binding VEGFR3 and VEGF, or where it is capable of binding VEGFR3 and VE cadherin, or where it is capable of binding VEGFR3 and neuropilin, or where it is capable of binding VEGFR3 and Flt-3, or where it is capable of binding VEGFR3 and Trp-1, or where it is capable of binding VEGFR3 and CD-20, or where
 30 it is capable of binding IGF1R and EGFR, or where it is capable of binding IGF1R and VEGF, or where it is capable of binding IGF1R and VE cadherin, or where it is capable of binding IGF1R and neuropilin, or where it is capable of binding IGF1R and Flt-3, or where it is capable of binding IGF1R and ron,

or where it is capable of binding IGF1R and Trp-1, or where it is capable of binding IGF1R and CD-20, or where it is capable of binding EGFR and VEGF, or where it is capable of binding EGFR and VE cadherin, or where it is capable of binding EGFR and neuropilin, or where it is capable of binding EGFR and Flt-3, or where it is capable of binding EGFR and ron, or where it is capable of binding EGFR and Trp-1, or where it is capable of binding EGFR and CD-20, or where it is capable of binding VEGF and VE cadherin, or where it is capable of binding VEGF and neuropilin, or where it is capable of binding VEGF and Flt-3, or where it is capable of binding VEGF and ron, or where it is capable of binding VEGF and Trp-1, or where it is capable of binding VEGF and CD-20, or where it is capable of binding VE cadherin and neuropilin, or where it is capable of binding VE cadherin and Flt-3, or where it is capable of binding VE cadherin and ron, or where it is capable of binding VE cadherin and Trp-1, or where it is capable of binding VE cadherin and CD-20, or where it is capable of binding neuropilin and Flt-3, or where it is capable of binding neuropilin and ron, or where it is capable of binding neuropilin and Trp-1, or where it is capable of binding neuropilin and CD-20, or where it is capable of binding Flt-3 and ron, or where it is capable of binding Flt-3 and Trp-1, or where it is capable of binding Flt-3 and CD-20, or where it is capable of binding ron and Trp-1, or where it is capable of binding ron and CD-20, and or where it is capable of binding Trp-1 and CD-20.

Such antigen-binding proteins may also have one or more further epitope binding domains with the same or different antigen-specificity attached to the c-terminus and/or the n-terminus of the heavy chain and/ or the c-terminus and/or n-terminus of the light chain.

The antigen binding proteins of the invention include those which have specificity for beta-amyloid, for example which comprise an epitope-binding domain which is capable of binding to beta-amyloid, or which comprises a paired VH/VL which binds to beta-amyloid.

The antigen binding protein may comprise an antibody which is capable of binding to beta-amyloid. The antigen binding protein may comprise an immunoglobulin single variable domain which is capable of binding to beta-amyloid.

The antigen binding proteins of the invention include those which have specificity for CD-3, for example which comprise an epitope-binding domain which is capable of binding to CD-3, or which comprises a paired VH/VL which binds to CD-3.

The antigen binding protein may comprise an antibody which is capable of binding to CD-3. The antigen binding protein may comprise an immunoglobulin single variable domain which is capable of binding to CD-3.

The antigen binding proteins of the invention include those which have specificity for gpIIIb/IIa, for example which comprise an epitope-binding domain which is capable of binding to gpIIIb/IIa, or which comprises a paired VH/VL which binds to gpIIIb/IIa.

The antigen binding protein may comprise an antibody which is capable of binding to gpIIIb/IIa. The antigen binding protein may comprise an immunoglobulin single variable domain which is capable of binding to gpIIIb/IIa.

The antigen binding proteins of the invention include those which have specificity for TGFbeta, for example which comprise an epitope-binding domain which is capable of binding to TGFbeta, or which comprises a paired VH/VL which binds to TGFbeta.

5 The antigen binding protein may comprise an antibody which is capable of binding to TGFbeta. The antigen binding protein may comprise an immunoglobulin single variable domain which is capable of binding to TGFbeta.

10 In one embodiment of the present invention there is provided an antigen binding protein according to the invention described herein and comprising a constant region such that the antibody has reduced ADCC and/or complement activation or effector functionality. In one such embodiment the heavy chain constant region may comprise a naturally disabled constant region of IgG2 or IgG4 isotype or a mutated IgG1 constant region. Examples of suitable modifications are described in EP0307434. One example comprises the substitutions of alanine residues at positions 235 and
15 237 (EU index numbering).

In one embodiment the antigen-binding proteins of the present invention will retain Fc functionality for example will be capable of one or both of ADCC and CDC activity. Such antigen-binding proteins may comprise an epitope-binding domain located on
20 the light chain, for example on the c-terminus of the light chain.

The invention also provides a method of maintaining ADCC and CDC function of antigen-binding proteins by positioning of the epitope binding domain on the light chain of the antibody in particular, by positioning the epitope binding domain on the
25 c-terminus of the light chain. Such ADCC and CDC function can be measured by any suitable assay which will be known to the person skilled in the art.

The invention also provides a method of reducing CDC function of antigen-binding proteins by positioning of the epitope binding domain on the heavy chain of the
30 antibody, in particular, by positioning the epitope binding domain on the c-terminus of the heavy chain. Such CDC function can be measured by any suitable assay, which will be known to the person skilled in the art.

35 In a further embodiment the antigen-binding protein of the present invention is capable of binding two or more antigens selected from VEGF, IGF-1R and EGFR, for example it is capable of binding EGFR and VEGF, or EGFR and IGF1R, or IGF1R and VEGF, or for example it is capable of binding to TNF and IL1-R. In embodiments of the invention which comprise an IGF-1R binding site, the IGF-1R binding site of the antigen-binding protein of the invention may comprise a paired VH/VL domain in
40 the protein scaffold.

In one embodiment, the antigen binding proteins comprise an epitope-binding domain which is an immunoglobulin single variable domain for example the epitope

binding domain may be a human VH or human VL, or a camelid V_{HH} (nanobody) or a shark dAb (NARV).

In one embodiment the antigen binding proteins comprise an epitope-binding domain which is a derivative of a non-immunoglobulin scaffold, for example a non-

5 immunoglobulin domain selected from the group consisting of CTLA-4 (Evibody); lipocalin; Protein A derived molecules such as Z-domain of Protein A (Affibody, SpA), A-domain (Avimer/Maxibody); Heat shock proteins such as GroEl and GroES; transferrin (trans-body); ankyrin repeat protein (DARPin); peptide aptamer; C-type lectin domain (Tetranectin); human γ -crystallin and human ubiquitin
10 (affilins); PDZ domains; scorpion toxin/kunitz type domains of human protease inhibitors; and fibronectin (adnectin); which has been subjected to protein engineering in order to obtain binding to a ligand other than the natural ligand.

The antigen binding proteins of the present invention may comprise a protein scaffold
15 attached to an epitope binding domain which is an adnectin, for example an IgG scaffold with an adnectin attached to the c-terminus of the heavy chain, or it may comprise a protein scaffold attached to an adnectin, for example an IgG scaffold with an adnectin attached to the n-terminus of the heavy chain, or it may comprise a protein scaffold attached to an adnectin, for example an IgG scaffold with an adnectin
20 attached to the c-terminus of the light chain, or it may comprise a protein scaffold attached to an adnectin, for example an IgG scaffold with an adnectin attached to the n-terminus of the light chain.

In other embodiments it may comprise a protein scaffold, for example an IgG scaffold, attached to an epitope binding domain which is CTLA-4, for example an IgG
25 scaffold with CTLA-4 attached to the n-terminus of the heavy chain, or it may comprise for example an IgG scaffold with CTLA-4 attached to the c-terminus of the heavy chain, or it may comprise for example an IgG scaffold with CTLA-4 attached to the n-terminus of the light chain, or it may comprise an IgG scaffold with CTLA-4 attached to the c-terminus of the light chain.

30 In other embodiments it may comprise a protein scaffold, for example an IgG scaffold, attached to an epitope binding domain which is a lipocalin, for example an IgG scaffold with a lipocalin attached to the n-terminus of the heavy chain, or it may comprise for example an IgG scaffold with a lipocalin attached to the c-terminus of the heavy chain, or it may comprise for example an IgG scaffold with a lipocalin
35 attached to the n-terminus of the light chain, or it may comprise an IgG scaffold with a lipocalin attached to the c-terminus of the light chain.

In other embodiments it may comprise a protein scaffold, for example an IgG scaffold, attached to an epitope binding domain which is an SpA, for example an IgG scaffold with an SpA attached to the n-terminus of the heavy chain, or it may
40 comprise for example an IgG scaffold with an SpA attached to the c-terminus of the heavy chain, or it may comprise for example an IgG scaffold with an SpA attached to the n-terminus of the light chain, or it may comprise an IgG scaffold with an SpA attached to the c-terminus of the light chain.

In other embodiments it may comprise a protein scaffold, for example an IgG scaffold, attached to an epitope binding domain which is an affibody, for example an IgG scaffold with an affibody attached to the n-terminus of the heavy chain, or it may comprise for example an IgG scaffold with an affibody attached to the c-terminus of the heavy chain, or it may comprise for example an IgG scaffold with an affibody attached to the n-terminus of the light chain, or it may comprise an IgG scaffold with an affibody attached to the c-terminus of the light chain.

In other embodiments it may comprise a protein scaffold, for example an IgG scaffold, attached to an epitope binding domain which is an affimer, for example an IgG scaffold with an affimer attached to the n-terminus of the heavy chain, or it may comprise for example an IgG scaffold with an affimer attached to the c-terminus of the heavy chain, or it may comprise for example an IgG scaffold with an affimer attached to the n-terminus of the light chain, or it may comprise an IgG scaffold with an affimer attached to the c-terminus of the light chain.

In other embodiments it may comprise a protein scaffold, for example an IgG scaffold, attached to an epitope binding domain which is a GroEI, for example an IgG scaffold with a GroEI attached to the n-terminus of the heavy chain, or it may comprise for example an IgG scaffold with a GroEI attached to the c-terminus of the heavy chain, or it may comprise for example an IgG scaffold with a GroEI attached to the n-terminus of the light chain, or it may comprise an IgG scaffold with a GroEI attached to the c-terminus of the light chain.

In other embodiments it may comprise a protein scaffold, for example an IgG scaffold, attached to an epitope binding domain which is a transferrin, for example an IgG scaffold with a transferrin attached to the n-terminus of the heavy chain, or it may comprise for example an IgG scaffold with a transferrin attached to the c-terminus of the heavy chain, or it may comprise for example an IgG scaffold with a transferrin attached to the n-terminus of the light chain, or it may comprise an IgG scaffold with a transferrin attached to the c-terminus of the light chain.

In other embodiments it may comprise a protein scaffold, for example an IgG scaffold, attached to an epitope binding domain which is a GroES, for example an IgG scaffold with a GroES attached to the n-terminus of the heavy chain, or it may comprise for example an IgG scaffold with a GroES attached to the c-terminus of the heavy chain, or it may comprise for example an IgG scaffold with a GroES attached to the n-terminus of the light chain, or it may comprise an IgG scaffold with a GroES attached to the c-terminus of the light chain.

In other embodiments it may comprise a protein scaffold, for example an IgG scaffold, attached to an epitope binding domain which is a DARPin, for example an IgG scaffold with a DARPin attached to the n-terminus of the heavy chain, or it may comprise for example an IgG scaffold with a DARPin attached to the c-terminus of the heavy chain, or it may comprise for example an IgG scaffold with a DARPin attached to the n-terminus of the light chain, or it may comprise an IgG scaffold with a DARPin attached to the c-terminus of the light chain.

In other embodiments it may comprise a protein scaffold, for example an IgG scaffold, attached to an epitope binding domain which is a peptide aptamer, for example an IgG scaffold with a peptide aptamer attached to the n-terminus of the heavy chain, or it may comprise for example an IgG scaffold with a peptide aptamer attached to the c-terminus of the heavy chain, or it may comprise for example an IgG scaffold with a peptide aptamer attached to the n-terminus of the light chain, or it may comprise an IgG scaffold with a peptide aptamer attached to the c-terminus of the light chain.

10 In one embodiment of the present invention there are four epitope binding domains, for example four domain antibodies, two of the epitope binding domains may have specificity for the same antigen, or all of the epitope binding domains present in the antigen-binding protein may have specificity for the same antigen.

15 Protein scaffolds of the present invention are linked to epitope-binding domains by the use of linkers. Examples of suitable linkers include amino acid sequences which may be from 1 amino acid to 150 amino acids in length, or from 1 amino acid to 140 amino acids, for example, from 1 amino acid to 130 amino acids, or from 1 to 120 amino acids, or from 1 to 80 amino acids, or from 1 to 50 amino acids, or from 1 to 20 amino acids, or from 1 to 10 amino acids, or from 5 to 18 amino acids. Such
20 sequences may have their own tertiary structure, for example, a linker of the present invention may comprise a single variable domain. The size of a linker in one embodiment is equivalent to a single variable domain. Suitable linkers may be of a size from 1 to 20 angstroms, for example less than 15 angstroms, or less than 10
25 angstroms, or less than 5 angstroms.

In one embodiment of the present invention at least one of the epitope binding domains is directly attached to the Ig scaffold with a linker comprising any one of those set out in SEQ ID NO: 51-78, 112-114, or multiples of such linkers.

30 In another embodiment at least one of the epitope binding domains is directly attached to the Ig scaffold with a linker comprising any one of those set out in SEQ ID NO: 53-60, SEQ ID NO: 62-72, SEQ ID NO: 74, SEQ ID NO: 76-78, SEQ ID NO: 112-114, or multiples of such linkers.

35 Linkers of use in the antigen-binding proteins of the present invention may comprise alone or in addition to other linkers, one or more sets of GS residues, for example 'GSPAS' or 'PASGS' or 'GSPASGS'.

40 In one embodiment the epitope binding domain is linked to the Ig scaffold by the linker '(PAS)_n(GS)_m'. In another embodiment the epitope binding domain is linked to the Ig scaffold by the linker '(GGGGS)_p(GS)_m'. In another embodiment the epitope binding domain is linked to the Ig scaffold by the linker '(TVAAPS)_p(GS)_m'. In another

embodiment the epitope binding domain is linked to the Ig scaffold by the linker ' $(GS)_m(TVAAPSGS)_p$ '. In another embodiment the epitope binding domain is linked to the Ig scaffold by the linker ' $(PAVPPP)_n(GS)_m$ '. In another embodiment the epitope binding domain is linked to the Ig scaffold by the linker ' $(TVSDVP)_n(GS)_m$ '. In another embodiment the epitope binding domain is linked to the Ig scaffold by the linker ' $(TGLDSP)_n(GS)_m$ '. In all such embodiments, $n = 1-10$, and $m = 0-4$, and $p=2-10$.

Examples of such linkers include $(PAS)_n(GS)_m$ wherein $n=1$ and $m=1$ (SEQ ID NO: 58), $(PAS)_n(GS)_m$ wherein $n=2$ and $m=1$ (SEQ ID NO: 59), $(PAS)_n(GS)_m$ wherein $n=3$ and $m=1$ (SEQ ID NO:60), $(PAS)_n(GS)_m$ wherein $n=4$ and $m=1$, $(PAS)_n(GS)_m$ wherein $n=2$ and $m=0$, $(PAS)_n(GS)_m$ wherein $n=3$ and $m=0$, $(PAS)_n(GS)_m$ wherein $n=4$ and $m=0$.

Examples of such linkers include $(GGGGS)_p(GS)_m$ wherein $p=2$ and $m=0$ (SEQ ID NO: 62), $(GGGGS)_p(GS)_m$ wherein $p=3$ and $m=0$ (SEQ ID NO:63), $(GGGGS)_p(GS)_m$ wherein $p=4$ and $m=0$.

Examples of such linkers include $(TVAAPS)_p(GS)_m$ wherein $p=2$ and $m=1$ (SEQ ID NO: 112), $(TVAAPS)_p(GS)_m$ wherein $p=3$ and $m=1$ (SEQ ID NO:113), $(TVAAPS)_p(GS)_m$ wherein $p=4$ and $m=1$ (SEQ ID NO:114), $(TVAAPS)_p(GS)_m$ wherein $p=2$ and $m=0$, $(TVAAPS)_p(GS)_m$ wherein $p=3$ and $m=0$, $(TVAAPS)_p(GS)_m$ wherein $p=4$ and $m=0$.

Examples of such linkers include $(GS)_m(TVAAPSGS)_p$ wherein $p=2$ and $m=1$ (SEQ ID NO:53), $(GS)_m(TVAAPSGS)_p$ wherein $p=3$ and $m=1$ (SEQ ID NO:54), or $(GS)_m(TVAAPSGS)_p$ wherein $p=4$ and $m=1$ (SEQ ID NO:55), $(GS)_m(TVAAPSGS)_p$ wherein $p=5$ and $m=1$ (SEQ ID NO:56), $(GS)_m(TVAAPSGS)_p$ wherein $p=6$ and $m=1$ (SEQ ID NO:57).

Examples of such linkers include $(PAVPPP)_n(GS)_m$ wherein $n=1$ and $m=1$ (SEQ ID NO: 64), $(PAVPPP)_n(GS)_m$ wherein $n=2$ and $m=1$ (SEQ ID NO: 65), $(PAVPPP)_n(GS)_m$ wherein $n=3$ and $m=1$ (SEQ ID NO:66), $(PAVPPP)_n(GS)_m$ wherein $n=4$ and $m=1$, $(PAVPPP)_n(GS)_m$ wherein $n=2$ and $m=0$, $(PAVPPP)_n(GS)_m$ wherein $n=3$ and $m=0$, $(PAVPPP)_n(GS)_m$ wherein $n=4$ and $m=0$.

Examples of such linkers include $(TVSDVP)_n(GS)_m$ wherein $n=1$ and $m=1$ (SEQ ID NO: 67), $(TVSDVP)_n(GS)_m$ wherein $n=2$ and $m=1$ (SEQ ID NO: 68), $(TVSDVP)_n(GS)_m$ wherein $n=3$ and $m=1$ (SEQ ID NO:69), $(TVSDVP)_n(GS)_m$ wherein $n=4$ and $m=1$, $(TVSDVP)_n(GS)_m$ wherein $n=2$ and $m=0$, $(TVSDVP)_n(GS)_m$ wherein $n=3$ and $m=0$, $(TVSDVP)_n(GS)_m$ wherein $n=4$ and $m=0$.

Examples of such linkers include $(TGLDSP)_n(GS)_m$ wherein $n=1$ and $m=1$ (SEQ ID NO: 70), $(TGLDSP)_n(GS)_m$ wherein $n=2$ and $m=1$ (SEQ ID NO: 71), $(TGLDSP)_n(GS)_m$

wherein $n=3$ and $m=1$ (SEQ ID NO:72), $(\text{TGLDSP})_n(\text{GS})_m$ wherein $n=4$ and $m=1$, $(\text{TGLDSP})_n(\text{GS})_m$ wherein $n=2$ and $m=0$, $(\text{TGLDSP})_n(\text{GS})_m$ wherein $n=3$ and $m=0$, $(\text{TGLDSP})_n(\text{GS})_m$ wherein $n=4$ and $m=0$.

- 5 In one embodiment, the antigen-binding protein of the present invention comprises at least one epitope binding domain which is capable of binding human serum albumin.

In one embodiment, there are at least 3 antigen binding sites, for example there are 4, or 5 or 6 or 8 or 10 antigen binding sites and the antigen binding protein is capable
10 of binding at least 3 or 4 or 5 or 6 or 8 or 10 antigens, for example it is capable of binding 3 or 4 or 5 or 6 or 8 or 10 antigens simultaneously.

The invention also provides a mAbdAb comprising the heavy chain sequence set out in SEQ ID NO:85 and the light chain sequence set out in SEQ ID NO: 3, or the heavy
15 chain sequence set out in SEQ ID NO:86 and the light chain sequence set out in SEQ ID NO: 3, or the heavy chain sequence set out in SEQ ID NO 2.and the light chain sequence set out in SEQ ID NO: 87, or the heavy chain sequence set out in SEQ ID NO:2.and the light chain sequence set out in SEQ ID NO: 88, or the heavy chain sequence set out in SEQ ID NO:85.and the light chain sequence set out in
20 SEQ ID NO: 87, or the heavy chain sequence set out in SEQ ID NO:85.and the light chain sequence set out in SEQ ID NO: 88,or the heavy chain sequence set out in SEQ ID NO:86.and the light chain sequence set out in SEQ ID NO: 87, or the heavy chain sequence set out in SEQ ID NO:86.and the light chain sequence set out in SEQ ID NO: 88. Any of the linkers used in SEQ ID NO: 85-88 could be replaced with
25 any of the other linkers or combinations of linkers described herein, in particular the 'GS' portion of the linker could be removed.

In one embodiment the present invention provides a method for improving the potency of an epitope binding domain when attached to a protein scaffold, which
30 epitope binding domain has reduced potency when attached directly to a protein scaffold without a linker, compared with its potency as a naked epitope binding domain, i.e.without being linked to any protein scaffold, comprising the step of adding a peptide linker between the protein scaffold and the eptiope binding domain wherein the peptide linker comprises at least 10 amino acids, or wherein the peptide linker is
35 between 10 amino acids and 50 amino acids, or between 10 amino acids and 40 amino acids or between 10 amino acids and 36 amino acids, or between 12 amino acids and 50 amino acids, or between 12 and 40 amino acids, or between 12 and 36 amino acids. In one embodiment the peptide linker comprises one or more repeats of the linker 'TVAAPS', for example $(\text{TVAAPS})_p(\text{GS})_m$ for example the linker is selected
40 from $(\text{GS})_m(\text{TVAAPSGS})_p$ where $m = 0-4$, and $p=2-10$, $(\text{PAS})_n(\text{GS})_m$ wherein $n = 2-10$, and $m = 0-4$, $(\text{GGGGS})_p(\text{GS})_m$ wherein $m = 0-4$ and $p=2-10$, $(\text{PAVPPP})_n(\text{GS})_m$ wherein $n = 2-10$, and $m = 0-4$, $(\text{TVSDVP})_n(\text{GS})_m$ wherein $n = 2-10$, and $m = 0-4$ and $(\text{TGLDSP})_n(\text{GS})_m$ wherein $n = 2-10$, and $m = 0-4$.

In one embodiment of this method the protein scaffold is an Ig scaffold. The Ig scaffold may comprise an Fc domain of an antibody. In one such embodiment of this method the protein scaffold is an antibody. In another such embodiment of this method the protein scaffold comprises an immunoglobulin heavy chain and an immunoglobulin light chain, wherein the heavy chain comprises CH1-CH2-CH3, and the light chain comprises CL. In yet another such embodiment of this method the protein scaffold is a soluble receptor, i.e. a receptor fused to the Fc domain of an antibody for example the soluble receptor may be selected from Abatacept (marketed as Orencia) which is a fusion protein composed of an immunoglobulin fused to the extracellular domain of CTLA-4, a molecule capable of binding B7; Etanercept (Enbrel) which is a soluble recombinant human p75 tumour necrosis factor TNF receptor (TNFR2) and human IgG1 Fc portion fusion protein produced in a mammalian cell expression system, which is being developed for use in treating rheumatoid arthritis (RA) and other inflammatory conditions; and Atacicept which is a recombinant fusion protein that comprises the receptor portion of the B lymphocyte TACI receptor, which binds to and is activated by the cytokines BlyS and APRIL. The soluble protein comprises the fusion of the extracellular domain of the TACI receptor with the Fc portion of human IgG1. The TACI receptor is a member of the TNF receptor family. Atacicept binds to excess BlyS and APRIL, preventing their binding to B-cells, thereby regulating B-cell maturity and antibody production. It is being developed for the treatment of autoimmune disease.

In one embodiment the epitope binding domain is an immunoglobulin single variable domain, for example a human dAb, or a camelid VHH single variable domain (nanobody). In another embodiment, the epitope binding domain is a non-Ig domain, for example the non-Ig domains described herein.

In one embodiment the method for improving potency of an epitope binding domain in the context of a fusion protein comprises the following steps:

- (i) Determine the original potency of the naked epitope binding domain,
- (ii) Attach binding domain to the protein scaffold for example, an antibody,
- (iii) Determine potency of epitope binding domain whilst attached directly to protein scaffold
- (iv) If potency is lower when attached to a protein scaffold than the original potency then add a peptide linker comprising at least 10 amino acids between the epitope binding domain and the protein scaffold.

In another embodiment the method for improving potency of a human dAb in the context of a fusion protein comprises the following steps:

- (i) Determine the original potency of naked human dAb
- (ii) Genetically fuse the human dAb to an antibody,
- (iii) Determine potency of epitope binding domain whilst attached directly to the protein scaffold

(iv) If potency is lower when attached to a protein scaffold than the original potency then add a peptide linker comprising at least 10 amino acids between the epitope binding domain and the protein scaffold.

5 The potency of the epitope binding domain can be measured by assays known in the art, for example by assessing the binding affinity i.e. the ability of the epitope binding domain to bind to antigen, which may be assessed by BIAcore™ assays such as those described in Example 7. Alternatively the potency of the epitope binding domain can be measured by a suitable cell-based neutralisation assay for example a
10 TF1 assay which may be carried out for example as described in Example 4.3 or 4.4. The neutralisation of IL-4, IL-13 or both of these cytokines in this assay is measured by assessing the inhibition of TF1 cell proliferation in the presence of neutralising antigen binding protein.

15 In one embodiment the method described herein is capable of repairing the potency of the epitope binding domain so that the potency when attached to a protein scaffold is within 50% of the original potency as measured by BIAcore or by a suitable cell-based neutralisation assay. In another embodiment the repaired potency is within 40% of the original potency, or within 30% of the original potency or within 20% of the
20 original potency, or within 20% of the original potency, or within 15% of the original potency, or within 10% of the original potency. In another embodiment the binding affinity when attached to a protein scaffold is substantially the same as that of the original binding affinity.

25 The invention also provides the antigen-binding proteins for use in medicine, for example for use in the manufacture of a medicament for treating cancer or inflammatory diseases such as asthma, rheumatoid arthritis, or osteoarthritis.

30 The invention provides a method of treating a patient suffering from cancer or inflammatory diseases such as asthma, rheumatoid arthritis, or osteoarthritis, comprising administering a therapeutic amount of an antigen-binding protein of the invention.

35 The antigen-binding proteins of the invention may be used for the treatment of cancer or inflammatory diseases such as asthma, rheumatoid arthritis, or osteoarthritis.

The antigen-binding proteins of the invention may have some effector function. For example if the protein scaffold contains an Fc region derived from an antibody with effector function, for example if the protein scaffold comprises CH2 and CH3 from
40 IgG1. Levels of effector function can be varied according to known techniques, for example by mutations in the CH2 domain, for example wherein the IgG1 CH2 domain has one or more mutations at positions selected from 239 and 332 and 330, for example the mutations are selected from S239D and I332E and A330L such that

the antibody has enhanced effector function, and/or for example altering the glycosylation profile of the antigen-binding protein of the invention such that there is a reduction in fucosylation of the Fc region.

- 5 Protein scaffolds of use in the present invention include full monoclonal antibody scaffolds comprising all the domains of an antibody, or protein scaffolds of the present invention may comprise a non-conventional antibody structure, such as a monovalent antibody. Such monovalent antibodies may comprise a paired heavy and light chain wherein the hinge region of the heavy chain is modified so that the heavy chain does not homodimerise, such as the monovalent antibody described in WO2007059782. Other monovalent antibodies may comprise a paired heavy and light chain which dimerises with a second heavy chain which is lacking a functional variable region and CH1 region, wherein the first and second heavy chains are modified so that they will form heterodimers rather than homodimers, resulting in a monovalent antibody with two heavy chains and one light chain such as the monovalent antibody described in WO2006015371. Such monovalent antibodies can provide the protein scaffold of the present invention to which epitope binding domains can be linked.
- 20 Epitope-binding domains of use in the present invention are domains that specifically bind an antigen or epitope independently of a different V region or domain, this may be a domain antibody or may be a domain which is a derivative of a scaffold selected from the group consisting of CTLA-4 (Evibody); lipocalin; Protein A derived molecules such as Z-domain of Protein A (Affibody, SpA), A-domain (Avimer/Maxibody); Heat shock proteins such as GroEI and GroES; transferrin (trans-body); ankyrin repeat protein (DARPin); peptide aptamer; C-type lectin domain (Tetranectin); human γ -crystallin and human ubiquitin (affilins); PDZ domains; scorpion toxin; kunitz type domains of human protease inhibitors; and fibronectin (adnectin); which has been subjected to protein engineering in order to obtain binding to a ligand other than the natural ligand. In one embodiment this may be an domain antibody or other suitable domains such as a domain selected from the group consisting of CTLA-4, lipocalin, SpA, an Affibody, an avimer, GroEI, transferrin, GroES and fibronectin. In one embodiment this may be selected from an immunoglobulin single variable domain, an Affibody, an ankyrin repeat protein (DARPin) and an adnectin. In another embodiment this may be selected from an Affibody, an ankyrin repeat protein (DARPin) and an adnectin. In another embodiment this may be a domain antibody, for example a domain antibody selected from a human, camelid or shark (NARV) domain antibody.
- 40 Epitope-binding domains can be linked to the protein scaffold at one or more positions. These positions include the C-terminus and the N-terminus of the protein scaffold, for example at the C-terminus of the heavy chain and/or the C-terminus of

the light chain of an IgG, or for example the N-terminus of the heavy chain and/or the N-terminus of the light chain of an IgG.

5 In one embodiment, a first epitope binding domain is linked to the protein scaffold and a second epitope binding domain is linked to the first epitope binding domain, for example where the protein scaffold is an IgG scaffold, a first epitope binding domain may be linked to the c-terminus of the heavy chain of the IgG scaffold, and that epitope binding domain can be linked at its c-terminus to a second epitope binding domain, or for example a first epitope binding domain may be linked to the c-terminus
10 of the light chain of the IgG scaffold, and that first epitope binding domain may be further linked at its c-terminus to a second epitope binding domain, or for example a first epitope binding domain may be linked to the n-terminus of the light chain of the IgG scaffold, and that first epitope binding domain may be further linked at its n-terminus to a second epitope binding domain, or for example a first epitope binding
15 domain may be linked to the n-terminus of the heavy chain of the IgG scaffold, and that first epitope binding domain may be further linked at its n-terminus to a second epitope binding domain.

20 When the epitope-binding domain is a domain antibody, some domain antibodies may be suited to particular positions within the scaffold.

Domain antibodies of use in the present invention can be linked at the C-terminal end of the heavy chain and/or the light chain of conventional IgGs. In addition some immunoglobulin single variable domains can be linked to the C-terminal ends of both
25 the heavy chain and the light chain of conventional antibodies.

In antigen binding proteins where the N-terminus of immunoglobulin single variable domains are fused to an antibody constant domain (either C_H3 or CL), a peptide linker may help the immunoglobulin single variable domain to bind to antigen. Indeed,
30 the N-terminal end of an immunoglobulin single variable domain is located closely to the complementarity-determining regions (CDRs) involved in antigen-binding activity. Thus a short peptide linker acts as a spacer between the epitope-binding, and the constant domain of the protein scaffold, which may allow the immunoglobulin single variable domain CDRs to more easily reach the antigen, which may therefore bind
35 with high affinity.

The surroundings in which immunoglobulin single variable domains are linked to the IgG will differ depending on which antibody chain they are fused to:
When fused at the C-terminal end of the antibody light chain of an IgG scaffold, each
40 immunoglobulin single variable domain is expected to be located in the vicinity of the antibody hinge and the Fc portion. It is likely that such immunoglobulin single variable domains will be located far apart from each other. In conventional antibodies, the angle between Fab fragments and the angle between each Fab fragment and the Fc

portion can vary quite significantly. It is likely that – with mAbdAbs – the angle between the Fab fragments will not be widely different, whilst some angular restrictions may be observed with the angle between each Fab fragment and the Fc portion.

- 5 When fused at the C-terminal end of the antibody heavy chain of an IgG scaffold, each immunoglobulin single variable domain is expected to be located in the vicinity of the C_H3 domains of the Fc portion. This is not expected to impact on the Fc binding properties to Fc receptors (e.g. FcγRI, II, III and FcRn) as these receptors engage with the C_H2 domains (for the FcγRI, II and III class of receptors) or with the
- 10 hinge between the C_H2 and C_H3 domains (e.g. FcRn receptor). Another feature of such antigen-binding proteins is that both immunoglobulin single variable domains are expected to be spatially close to each other and provided that flexibility is provided by provision of appropriate linkers, these immunoglobulin single variable domains may even form homodimeric species, hence propagating the ‘zipped’
- 15 quaternary structure of the Fc portion, which may enhance stability of the antigen binding protein.

Such structural considerations can aid in the choice of the most suitable position to link an epitope-binding domain, for example an immunoglobulin single variable

20 domain, on to a protein scaffold, for example an antibody.

The size of the antigen, its localization (in blood or on cell surface), its quaternary structure (monomeric or multimeric) can vary. Conventional antibodies are naturally designed to function as adaptor constructs due to the presence of the hinge region,

25 wherein the orientation of the two antigen-binding sites at the tip of the Fab fragments can vary widely and hence adapt to the molecular feature of the antigen and its surroundings. In contrast immunoglobulin single variable domains linked to an antibody or other protein scaffold, for example a protein scaffold which comprises an antibody with no hinge region, may have less structural flexibility either directly or

30 indirectly.

Understanding the solution state and mode of binding at the immunoglobulin single variable domain is also helpful. Evidence has accumulated that *in vitro* dAbs can predominantly exist in monomeric, homo-dimeric or multimeric forms in solution

35 (Reiter et al. (1999) J Mol Biol 290 p685-698; Ewert et al (2003) J Mol Biol 325, p531-553, Jespers et al (2004) J Mol Biol 337 p893-903; Jespers et al (2004) Nat Biotechnol 22 p1161-1165; Martin et al (1997) Protein Eng. 10 p607-614; Sepulveda et al (2003) J Mol Biol 333 p355-365). This is fairly reminiscent to multimerisation events observed *in vivo* with Ig domains such as Bence-Jones proteins (which are

40 dimers of immunoglobulin light chains (Epp et al (1975) Biochemistry 14 p4943-4952; Huan et al (1994) Biochemistry 33 p14848-14857; Huang et al (1997) Mol immunol 34 p1291-1301) and amyloid fibers (James et al. (2007) J Mol Biol. **367**:603-8).

For example, it may be desirable to link dAbs that tend to dimerise in solution to the C-terminal end of the Fc portion in preference to the C-terminal end of the light chain as linking to the C-terminal end of the Fc will allow those dAbs to dimerise in the context of the antigen-binding protein of the invention.

5

The antigen-binding proteins of the present invention may comprise antigen-binding sites specific for a single antigen, or may have antigen-binding sites specific for two or more antigens, or for two or more epitopes on a single antigen, or there may be antigen-binding sites each of which is specific for a different epitope on the same or different antigens.

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The antigen-binding sites can each have binding specificity for an antigen, such as human or animal proteins, including cytokines, growth factors, cytokine receptors, growth factor receptors, enzymes (e.g., proteases), co-factors for enzymes, DNA binding proteins, lipids and carbohydrates. Suitable targets, including cytokines, growth factors, cytokine receptors, growth factor receptors and other proteins include but are not limited to: ApoE, Apo-SAA, BDNF, Cardiotrophin-1, CEA, CD40, CD40 Ligand, CD56, CD38, CD138, EGF, EGF receptor, ENA-78, Eotaxin, Eotaxin-2, Exodus-2, FAP α , FGF-acidic, FGF-basic, fibroblast growth factor-10, FLT3 ligand, Fractalkine (CX3C), GDNF, G-CSF, GM-CSF, GF- β 1, human serum albumin, insulin, IFN- γ , IGF-I, IGF-II, IL-1 α , IL-1 β , IL-1 receptor, IL-1 receptor type 1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8 (72 a.a.), IL-8 (77 a.a.), IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-16, IL-17, IL-18 (IGIF), Inhibin α , Inhibin β , IP-10, keratinocyte growth factor-2 (KGF-2), KGF, Leptin, LIF, Lymphotoxin, Mullerian inhibitory substance, monocyte colony inhibitory factor, monocyte attractant protein, M-CSF, c-fms, v-fmsMDC (67 a.a.), MDC (69 a.a.), MCP-1 (MCAF), MCP-2, MCP-3, MCP-4, MDC (67 a.a.), MDC (69 a.a.), MIG, MIP-1 α , MIP-1 β , MIP-3 α , MIP-3 β , MIP-4, myeloid progenitor inhibitor factor-1 (MPIF-1), NAP-2, Neurturin, Nerve growth factor, β -NGF, NT-3, NT-4, Oncostatin M, PDGF-AA, PDGF-AB, PDGF-BB, PF-4, RANTES, SDF1 α , SDF1 β , SCF, SCGF, stem cell factor (SCF), TARC, TGF- α , TGF- β , TGF- β 2, TGF- β 3, tumour necrosis factor (TNF), TNF- α , TNF- β , TNF receptor I, TNF receptor II, TNIL-1, TPO, VEGF, VEGF A, VEGF B, VEGF C, VEGF D, VEGF receptor 1, VEGF receptor 2, VEGF receptor 3, GCP-2, GRO/MGSA, GRO- β , GRO- γ , HCC1, 1-309, HER 1, HER 2, HER 3, HER 4, serum albumin, vWF, amyloid proteins (e.g., amyloid alpha), MMP12, PDK1, IgE, and other targets disclosed herein. It will be appreciated that this list is by no means exhaustive.

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In some embodiments, the protease resistant peptide or polypeptide binds a target in pulmonary tissue, such as a target selected from the group consisting of TNFR1, IL-1, IL-1R, IL-4, IL-4R, IL-5, IL-6, IL-6R, IL-8, IL-8R, IL-9, IL-9R, IL-10, IL-12, IL-12R, IL-13, IL-13R α 1, IL-13R α 2, IL-15, IL-15R, IL-16, IL-17R, IL-17, IL-18, IL-18R, IL-23, IL-23R, IL-25, CD2, CD4, CD11a, CD23, CD25, CD27, CD28, CD30, CD40, CD40L, CD56, CD138, ALK5, EGFR, FcER1, TGF β , CCL2, CCL18, CEA, CR8, CTGF, CXCL12 (SDF-1), chymase, FGF, Furin, Endothelin-1, Eotaxins (e.g.,

Eotaxin, Eotaxin-2, Eotaxin-3), GM-CSF, ICAM-1, ICOS, IgE, IFN α , I-309, integrins, L-selectin, MIF, MIP4, MDC, MCP-1, MMPs, neutrophil elastase, osteopontin, OX-40, PARC, PD-1, RANTES, SCF, SDF-1, siglec8, TARC, TGF β , Thrombin, Tim-1, TNF, TRANCE, Tryptase, VEGF, VLA-4, VCAM, α 4 β 7, CCR2, CCR3, CCR4, CCR5, CCR7, CCR8, α v β 6, α v β 8, cMET, CD8, vWF, amyloid proteins (e.g., amyloid α), MMP12, PDK1, and IgE.

In particular, the antigen-binding proteins of the present invention may be useful in treating diseases associated with IL-13, IL-5 and IL-4, for example atopic dermatitis, allergic rhinitis, crohn's disease, COPD, fibrotic diseases or disorders such as idiopathic pulmonary fibrosis, progressive systemic sclerosis, hepatic fibrosis, hepatic granulomas, schistosomiasis, leishmaniasis, diseases of cell cycle regulation such as Hodgkins disease, B cell chronic lymphocytic leukaemia, for example the proteins may be useful in treating asthma.

Antigen-binding proteins of the present invention may be useful in treating diseases associated with growth factors such as IGF-1R, VEGF, and EGFR, for example cancer or rheumatoid arthritis, examples of types of cancer in which such therapies may be useful are breast cancer, prostate cancer, lung cancer and myeloma.

Antigen-binding proteins of the present invention may be useful in treating diseases associated with TNF, for example arthritis, for example rheumatoid arthritis or osteoarthritis.

Antigen-binding proteins of the present invention may be useful in treating diseases associated with IL1-R, for example arthritis, for example rheumatoid arthritis or osteoarthritis.

Antigen-binding proteins of the present invention may be useful in treating diseases associated with CD-20, for example autoimmune diseases such as psoriasis, inflammatory bowel disease, ulcerative colitis, crohns disease, rheumatoid arthritis, juvenile rheumatoid arthritis, systemic lupus erythematosus, neurodegenerative diseases, for example multiple sclerosis, neutrophil driven diseases, for example COPD, Wegeners vasculitis, cystic fibrosis, Sjogrens syndrome, chronic transplant rejection, type 1 diabetes graft versus host disease, asthma, allergic diseases atopic dermatitis, eczematous dermatitis, allergic rhinitis, autoimmune diseases other including thyroiditis, spondyloarthropathy, ankylosing spondylitis, uveitis, polychonritis or scleroderma, or cancer e.g. B- cell lymphomas or mature B cell neoplasm such as CLL or SLL.

Antigen-binding proteins of the present invention may be useful in treating diseases associated with IL-17 and IL-23, for example psoriasis, inflammatory bowel disease, ulcerative colitis, crohns disease, rheumatoid arthritis, juvenile rheumatoid arthritis,

systemic lupus erythematosus, neurodegenerative diseases, for example multiple sclerosis, neutrophil driven diseases, for example COPD, Wegeners vasculitis, cystic fibrosis, Sjogrens syndrome, chronic transplant rejection, type 1 diabetes graft versus host disease, asthma, allergic diseases atopic dermatitis, eczematous dermatitis, allergic rhinitis, autoimmune diseases other including thyroiditis, spondyloarthropathy, ankylosing spondylitis, uveitis, polychonritis or scleroderma.

The antigen binding proteins of the present invention may be produced by transfection of a host cell with an expression vector comprising the coding sequence for the antigen binding protein of the invention. An expression vector or recombinant plasmid is produced by placing these coding sequences for the antigen binding protein in operative association with conventional regulatory control sequences capable of controlling the replication and expression in, and/or secretion from, a host cell. Regulatory sequences include promoter sequences, e.g., CMV promoter, and signal sequences which can be derived from other known antibodies. Similarly, a second expression vector can be produced having a DNA sequence which encodes a complementary antigen binding protein light or heavy chain. In certain embodiments this second expression vector is identical to the first except insofar as the coding sequences and selectable markers are concerned, so to ensure as far as possible that each polypeptide chain is functionally expressed. Alternatively, the heavy and light chain coding sequences for the antigen binding protein may reside on a single vector, for example in two expression cassettes in the same vector. A selected host cell is co-transfected by conventional techniques with both the first and second vectors (or simply transfected by a single vector) to create the transfected host cell of the invention comprising both the recombinant or synthetic light and heavy chains. The transfected cell is then cultured by conventional techniques to produce the engineered antigen binding protein of the invention. The antigen binding protein which includes the association of both the recombinant heavy chain and/or light chain is screened from culture by appropriate assay, such as ELISA or RIA. Similar conventional techniques may be employed to construct other antigen binding proteins.

Suitable vectors for the cloning and subcloning steps employed in the methods and construction of the compositions of this invention may be selected by one of skill in the art. For example, the conventional pUC series of cloning vectors may be used. One vector, pUC19, is commercially available from supply houses, such as Amersham (Buckinghamshire, United Kingdom) or Pharmacia (Uppsala, Sweden). Additionally, any vector which is capable of replicating readily, has an abundance of cloning sites and selectable genes (e.g., antibiotic resistance), and is easily manipulated may be used for cloning. Thus, the selection of the cloning vector is not a limiting factor in this invention.

The expression vectors may also be characterized by genes suitable for amplifying expression of the heterologous DNA sequences, e.g., the mammalian dihydrofolate reductase gene (DHFR). Other vector sequences include a poly A signal sequence,

such as from bovine growth hormone (BGH) and the betaglobin promoter sequence (betaglopro). The expression vectors useful herein may be synthesized by techniques well known to those skilled in this art.

5 The components of such vectors, e.g. replicons, selection genes, enhancers, promoters, signal sequences and the like, may be obtained from commercial or natural sources or synthesized by known procedures for use in directing the expression and/or secretion of the product of the recombinant DNA in a selected host. Other appropriate expression vectors of which numerous types are known in the art for mammalian, bacterial, insect, yeast, and fungal expression may also be
10 selected for this purpose.

The present invention also encompasses a cell line transfected with a recombinant plasmid containing the coding sequences of the antigen binding proteins of the present invention. Host cells useful for the cloning and other manipulations of these cloning vectors are also conventional. However, cells from various strains of
15 E. coli may be used for replication of the cloning vectors and other steps in the construction of antigen binding proteins of this invention.

Suitable host cells or cell lines for the expression of the antigen binding proteins of the invention include mammalian cells such as NS0, Sp2/0, CHO (e.g. DG44), COS, HEK, a fibroblast cell (e.g., 3T3), and myeloma cells, for example it may be
20 expressed in a CHO or a myeloma cell. Human cells may be used, thus enabling the molecule to be modified with human glycosylation patterns. Alternatively, other eukaryotic cell lines may be employed. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Sambrook et al., cited
25 above.

Bacterial cells may prove useful as host cells suitable for the expression of the recombinant Fabs or other embodiments of the present invention (see, e.g., Plückthun, A., Immunol. Rev., 130:151-188 (1992)). However, due to the tendency of proteins expressed in bacterial cells to be in an unfolded or improperly folded form
30 or in a non-glycosylated form, any recombinant Fab produced in a bacterial cell would have to be screened for retention of antigen binding ability. If the molecule expressed by the bacterial cell was produced in a properly folded form, that bacterial cell would be a desirable host, or in alternative embodiments the molecule may express in the bacterial host and then be subsequently re-folded. For example,
35 various strains of E. coli used for expression are well-known as host cells in the field of biotechnology. Various strains of B. subtilis, Streptomyces, other bacilli and the like may also be employed in this method.

Where desired, strains of yeast cells known to those skilled in the art are also available as host cells, as well as insect cells, e.g. Drosophila and Lepidoptera and
40 viral expression systems. See, e.g. Miller et al., Genetic Engineering, 8:277-298, Plenum Press (1986) and references cited therein.

The general methods by which the vectors may be constructed, the transfection methods required to produce the host cells of the invention, and culture methods

necessary to produce the antigen binding protein of the invention from such host cell may all be conventional techniques. Typically, the culture method of the present invention is a serum-free culture method, usually by culturing cells serum-free in suspension. Likewise, once produced, the antigen binding proteins of the invention
5 may be purified from the cell culture contents according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like. Such techniques are within the skill of the art and do not limit this invention. For example, preparation of altered antibodies are described in WO 99/58679 and WO 96/16990.

10 Yet another method of expression of the antigen binding proteins may utilize expression in a transgenic animal, such as described in U. S. Patent No. 4,873,316. This relates to an expression system using the animal's casein promoter which when transgenically incorporated into a mammal permits the female to produce the desired recombinant protein in its milk.

15 In a further aspect of the invention there is provided a method of producing an antibody of the invention which method comprises the step of culturing a host cell transformed or transfected with a vector encoding the light and/or heavy chain of the antibody of the invention and recovering the antibody thereby produced.

In accordance with the present invention there is provided a method of producing an
20 antigen binding protein of the present invention which method comprises the steps of;

- (a) providing a first vector encoding a heavy chain of the antigen binding protein;
- (b) providing a second vector encoding a light chain of the antigen binding
25 protein;
- (c) transforming a mammalian host cell (e.g. CHO) with said first and second vectors;
- (d) culturing the host cell of step (c) under conditions conducive to the secretion of the antigen binding protein from said host cell into said culture
30 media;
- (e) recovering the secreted antigen binding protein of step (d).

Once expressed by the desired method, the antigen binding protein is then examined for in vitro activity by use of an appropriate assay. Presently conventional ELISA
35 assay formats are employed to assess qualitative and quantitative binding of the antigen binding protein to its target. Additionally, other in vitro assays may also be used to verify neutralizing efficacy prior to subsequent human clinical studies performed to evaluate the persistence of the antigen binding protein in the body despite the usual clearance mechanisms.

40 The dose and duration of treatment relates to the relative duration of the molecules of the present invention in the human circulation, and can be adjusted by one of skill in the art depending upon the condition being treated and the general health of the

patient. It is envisaged that repeated dosing (e.g. once a week or once every two weeks) over an extended time period (e.g. four to six months) maybe required to achieve maximal therapeutic efficacy.

5 The mode of administration of the therapeutic agent of the invention may be any suitable route which delivers the agent to the host. The antigen binding proteins, and pharmaceutical compositions of the invention are particularly useful for parenteral administration, i.e., subcutaneously (s.c.), intrathecally, intraperitoneally, intramuscularly (i.m.), intravenously (i.v.), or intranasally.

10 Therapeutic agents of the invention may be prepared as pharmaceutical compositions containing an effective amount of the antigen binding protein of the invention as an active ingredient in a pharmaceutically acceptable carrier. In the prophylactic agent of the invention, an aqueous suspension or solution containing the antigen binding protein, may be buffered at physiological pH, in a form ready for injection. The compositions for parenteral administration will commonly comprise a
15 solution of the antigen binding protein of the invention or a cocktail thereof dissolved in a pharmaceutically acceptable carrier, for example an aqueous carrier. A variety of aqueous carriers may be employed, e.g., 0.9% saline, 0.3% glycine, and the like. These solutions may be made sterile and generally free of particulate matter. These solutions may be sterilized by conventional, well known sterilization techniques (e.g.,
20 filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, etc. The concentration of the antigen binding protein of the invention in such pharmaceutical formulation can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and
25 will be selected primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

Thus, a pharmaceutical composition of the invention for intramuscular injection could be prepared to contain 1 mL sterile buffered water, and between about 1 ng to about 100 mg, e.g. about 50 ng to about 30 mg, or about 5 mg to about 25 mg, of an
30 antigen binding protein of the invention. Similarly, a pharmaceutical composition of the invention for intravenous infusion could be made up to contain about 250 ml of sterile Ringer's solution, and about 1 to about 30 or about 5 mg to about 25 mg of an antigen binding protein of the invention per ml of Ringer's solution. Actual methods for preparing parenterally administrable compositions are well known or will be
35 apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton,

Pennsylvania. For the preparation of intravenously administrable antigen binding protein formulations of the invention see Lasmar U and Parkins D "The formulation of Biopharmaceutical products", Pharma. Sci.Tech.today, page 129-137, Vol.3 (3rd April 2000), Wang, W "Instability, stabilisation and formulation of liquid protein pharmaceuticals", Int. J. Pharm 185 (1999) 129-188, Stability of Protein Pharmaceuticals Part A and B ed Ahern T.J., Manning M.C., New York, NY: Plenum Press (1992), Akers,M.J. "Excipient-Drug interactions in Parenteral Formulations", J.Pharm Sci 91 (2002) 2283-2300, Imamura, K et al "Effects of types of sugar on stabilization of Protein in the dried state", J Pharm Sci 92 (2003) 266-274,Izutsu, Kkojima, S. "Excipient crystallinity and its protein-structure-stabilizing effect during freeze-drying", J Pharm. Pharmacol, 54 (2002) 1033-1039, Johnson, R, "Mannitol-sucrose mixtures-versatile formulations for protein lyophilization", J. Pharm. Sci, 91 (2002) 914-922.

Ha,E Wang W, Wang Y.j. "Peroxide formation in polysorbate 80 and protein stability", J. Pharm Sci, 91, 2252-2264,(2002) the entire contents of which are incorporated herein by reference and to which the reader is specifically referred.

In one embodiment the therapeutic agent of the invention, when in a pharmaceutical preparation, is present in unit dose forms. The appropriate therapeutically effective dose will be determined readily by those of skill in the art. Suitable doses may be calculated for patients according to their weight, for example suitable doses may be in the range of 0.01 to 20mg/kg, for example 0.1 to 20mg/kg, for example 1 to 20mg/kg, for example 10 to 20mg/kg or for example 1 to 15mg/kg, for example 10 to 15mg/kg. To effectively treat conditions of use in the present invention in a human, suitable doses may be within the range of 0.01 to 1000 mg, for example 0.1 to 1000mg, for example 0.1 to 500mg, for example 500mg, for example 0.1 to 100mg, or 0.1 to 80mg, or 0.1 to 60mg, or 0.1 to 40mg, or for example 1 to 100mg, or 1 to 50mg, of an antigen binding protein of this invention, which may be administered parenterally, for example subcutaneously, intravenously or intramuscularly. Such dose may, if necessary, be repeated at appropriate time intervals selected as appropriate by a physician.

The antigen binding proteins described herein can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immunoglobulins and art-known lyophilization and reconstitution techniques can be employed.

There are several methods known in the art which can be used to find epitope-binding domains of use in the present invention.

The term "library" refers to a mixture of heterogeneous polypeptides or nucleic acids. The library is composed of members, each of which has a single polypeptide or nucleic acid sequence. To this extent, "library" is synonymous with "repertoire."

5 Sequence differences between library members are responsible for the diversity present in the library. The library may take the form of a simple mixture of polypeptides or nucleic acids, or may be in the form of organisms or cells, for example bacteria, viruses, animal or plant cells and the like, transformed with a library of nucleic acids. In one example, each individual organism or cell contains
10 only one or a limited number of library members. Advantageously, the nucleic acids are incorporated into expression vectors, in order to allow expression of the polypeptides encoded by the nucleic acids. In a one aspect, therefore, a library may take the form of a population of host organisms, each organism containing one or more copies of an expression vector containing a single member of the library in
15 nucleic acid form which can be expressed to produce its corresponding polypeptide member. Thus, the population of host organisms has the potential to encode a large repertoire of diverse polypeptides.

A "universal framework" is a single antibody framework sequence corresponding to the regions of an antibody conserved in sequence as defined by Kabat ("Sequences
20 of Proteins of Immunological Interest", US Department of Health and Human Services) or corresponding to the human germline immunoglobulin repertoire or structure as defined by Chothia and Lesk, (1987) J. Mol. Biol. 196:910-917. There may be a single framework, or a set of such frameworks, which has been found to permit the derivation of virtually any binding specificity though variation in the
25 hypervariable regions alone.

Amino acid and nucleotide sequence alignments and homology, similarity or identity, as defined herein are in one embodiment prepared and determined using the algorithm BLAST 2 Sequences, using default parameters (Tatusova, T. A. *et al.*,
30 *FEMS Microbiol Lett*, 174:187-188 (1999)).

The epitope binding domain(s) and antigen binding sites can each have binding specificity for a generic ligand or any desired target ligand, such as human or animal proteins, including cytokines, growth factors, cytokine receptors, growth factor receptors, enzymes (e.g., proteases), co-factors for enzymes, DNA binding proteins,
35 lipids and carbohydrates. Suitable targets, including cytokines, growth factors, cytokine receptors, growth factor receptors and other proteins include but are not limited to: ApoE, Apo-SAA, BDNF, Cardiotrophin-1, CEA, CD40, CD40 Ligand, CD56, CD38, CD138, EGF, EGF receptor, ENA-78, Eotaxin, Eotaxin-2, Exodus-2, FAP α , FGF-acidic, FGF-basic, fibroblast growth factor-10, FLT3 ligand, Fractalkine
40 (CX3C), GDNF, G-CSF, GM-CSF, GF- β 1, human serum albumin, insulin, IFN- γ , IGF-I, IGF-II, IL-1 α , IL-1 β , IL-1 receptor, IL-1 receptor type 1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8 (72 a.a.), IL-8 (77 a.a.), IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-16, IL-17, IL-18 (IGIF), Inhibin α , Inhibin β , IP-10, keratinocyte growth factor-2 (KGF-2), KGF,

Leptin, LIF, Lymphotactin, Mullerian inhibitory substance, monocyte colony inhibitory factor, monocyte attractant protein, M-CSF, c-fms, v-fmsMDC (67 a.a.), MDC (69 a.a.), MCP-1 (MCAF), MCP-2, MCP-3, MCP-4, MDC (67 a.a.), MDC (69 a.a.), MIG, MIP-1 α , MIP-1 β , MIP-3 α , MIP-3 β , MIP-4, myeloid progenitor inhibitor factor-1 (MPIF-1), NAP-2, Neurturin, Nerve growth factor, β -NGF, NT-3, NT-4, Oncostatin M, PDGF-AA, PDGF-AB, PDGF-BB, PF-4, RANTES, SDF1 α , SDF1 β , SCF, SCGF, stem cell factor (SCF), TARC, TGF- α , TGF- β , TGF- β 2, TGF- β 3, tumour necrosis factor (TNF), TNF- α , TNF- β , TNF receptor I, TNF receptor II, TNIL-1, TPO, VEGF, VEGF A, VEGF B, VEGF C, VEGF D, VEGF receptor 1, VEGF receptor 2, VEGF receptor 3, GCP-2, GRO/MGSA, GRO- β , GRO- γ , HCC1, 1-309, HER 1, HER 2, HER 3, HER 4, serum albumin, vWF, amyloid proteins (e.g., amyloid alpha), MMP12, PDK1, IgE, and other targets disclosed herein. It will be appreciated that this list is by no means exhaustive.

In some embodiments, binding is to a target in pulmonary tissue, such as a target selected from the group consisting of TNFR1, IL-1, IL-1R, IL-4, IL-4R, IL-5, IL-6, IL-6R, IL-8, IL-8R, IL-9, IL-9R, IL-10, IL-12 IL-12R, IL-13, IL-13R α 1, IL-13Ra2, IL-15, IL-15R, IL-16, IL-17R, IL-17, IL-18, IL-18R, IL-23 IL-23R, IL-25, CD2, CD4, CD11a, CD23, CD25, CD27, CD28, CD30, CD40, CD40L, CD56, CD138, ALK5, EGFR, FcER1, TGFb, CCL2, CCL18, CEA, CR8, CTGF, CXCL12 (SDF-1), chymase, FGF, Furin, Endothelin-1, Eotaxins (e.g., Eotaxin, Eotaxin-2, Eotaxin-3), GM-CSF, ICAM-1, ICOS, IgE, IFNa, I-309, integrins, L-selectin, MIF, MIP4, MDC, MCP-1, MMPs, neutrophil elastase, osteopontin, OX-40, PARC, PD-1, RANTES, SCF, SDF-1, siglec8, TARC, TGFb, Thrombin, Tim-1, TNF, TRANCE, Tryptase, VEGF, VLA-4, VCAM, α 4 β 7, CCR2, CCR3, CCR4, CCR5, CCR7, CCR8, alphavbeta6, alphavbeta8, cMET, CD8, vWF, amyloid proteins (e.g., amyloid alpha), MMP12, PDK1, and IgE.

When a display system (e.g., a display system that links coding function of a nucleic acid and functional characteristics of the peptide or polypeptide encoded by the nucleic acid) is used in the methods described herein, eg in the selection of a dAb or other epitope binding domain, it is frequently advantageous to amplify or increase the copy number of the nucleic acids that encode the selected peptides or polypeptides. This provides an efficient way of obtaining sufficient quantities of nucleic acids and/or peptides or polypeptides for additional rounds of selection, using the methods described herein or other suitable methods, or for preparing additional repertoires (e.g., affinity maturation repertoires). Thus, in some embodiments, the methods of selecting epitope binding domains comprises using a display system (e.g., that links coding function of a nucleic acid and functional characteristics of the peptide or polypeptide encoded by the nucleic acid, such as phage display) and further comprises amplifying or increasing the copy number of a nucleic acid that encodes a selected peptide or polypeptide. Nucleic acids can be amplified using any suitable methods, such as by phage amplification, cell growth or polymerase chain reaction.

In one example, the methods employ a display system that links the coding function of a nucleic acid and physical, chemical and/or functional characteristics of the polypeptide encoded by the nucleic acid. Such a display system can comprise a plurality of replicable genetic packages, such as bacteriophage or cells (bacteria).

5 The display system may comprise a library, such as a bacteriophage display library. Bacteriophage display is an example of a display system.

A number of suitable bacteriophage display systems (e.g., monovalent display and multivalent display systems) have been described. (See, e.g., Griffiths *et al.*, U.S. Patent No. 6,555,313 B1 (incorporated herein by reference); Johnson *et al.*, U.S.

10 Patent No. 5,733,743 (incorporated herein by reference); McCafferty *et al.*, U.S. Patent No. 5,969,108 (incorporated herein by reference); Mulligan-Kehoe, U.S. Patent No. 5,702,892 (Incorporated herein by reference); Winter, G. *et al.*, *Annu. Rev. Immunol.* 12:433-455 (1994); Soumillon, P. *et al.*, *Appl. Biochem. Biotechnol.* 47(2-3):175-189 (1994); Castagnoli, L. *et al.*, *Comb. Chem. High Throughput*
15 *Screen*, 4(2):121-133 (2001).) The peptides or polypeptides displayed in a bacteriophage display system can be displayed on any suitable bacteriophage, such as a filamentous phage (e.g., fd, M13, F1), a lytic phage (e.g., T4, T7, lambda), or an RNA phage (e.g., MS2), for example.

Generally, a library of phage that displays a repertoire of peptides or
20 phagepolypeptides, as fusion proteins with a suitable phage coat protein (e.g., fd pIII protein), is produced or provided. The fusion protein can display the peptides or polypeptides at the tip of the phage coat protein, or if desired at an internal position. For example, the displayed peptide or polypeptide can be present at a position that is amino-terminal to domain 1 of pIII. (Domain 1 of pIII is also referred to as N1.) The
25 displayed polypeptide can be directly fused to pIII (e.g., the N-terminus of domain 1 of pIII) or fused to pIII using a linker. If desired, the fusion can further comprise a tag (e.g., myc epitope, His tag). Libraries that comprise a repertoire of peptides or polypeptides that are displayed as fusion proteins with a phage coat protein, can be produced using any suitable methods, such as by introducing a library of phage
30 vectors or phagemid vectors encoding the displayed peptides or polypeptides into suitable host bacteria, and culturing the resulting bacteria to produce phage (e.g., using a suitable helper phage or complementing plasmid if desired). The library of phage can be recovered from the culture using any suitable method, such as precipitation and centrifugation.

35 The display system can comprise a repertoire of peptides or polypeptides that contains any desired amount of diversity. For example, the repertoire can contain peptides or polypeptides that have amino acid sequences that correspond to naturally occurring polypeptides expressed by an organism, group of organisms, desired tissue or desired cell type, or can contain peptides or polypeptides that have
40 random or randomized amino acid sequences. If desired, the polypeptides can share a common core or scaffold. For example, all polypeptides in the repertoire or library can be based on a scaffold selected from protein A, protein L, protein G, a fibronectin domain, an anticalin, CTLA4, a desired enzyme (e.g., a polymerase, a cellulase), or a

polypeptide from the immunoglobulin superfamily, such as an antibody or antibody fragment (e.g., an antibody variable domain). The polypeptides in such a repertoire or library can comprise defined regions of random or randomized amino acid sequence and regions of common amino acid sequence. In certain embodiments, all or substantially all polypeptides in a repertoire are of a desired type, such as a desired enzyme (e.g., a polymerase) or a desired antigen-binding fragment of an antibody (e.g., human V_H or human V_L). In some embodiments, the polypeptide display system comprises a repertoire of polypeptides wherein each polypeptide comprises an antibody variable domain. For example, each polypeptide in the repertoire can contain a V_H, a V_L or an Fv (e.g., a single chain Fv). Amino acid sequence diversity can be introduced into any desired region of a peptide or polypeptide or scaffold using any suitable method. For example, amino acid sequence diversity can be introduced into a target region, such as a complementarity determining region of an antibody variable domain or a hydrophobic domain, by preparing a library of nucleic acids that encode the diversified polypeptides using any suitable mutagenesis methods (e.g., low fidelity PCR, oligonucleotide-mediated or site directed mutagenesis, diversification using NNK codons) or any other suitable method. If desired, a region of a polypeptide to be diversified can be randomized. The size of the polypeptides that make up the repertoire is largely a matter of choice and uniform polypeptide size is not required. The polypeptides in the repertoire may have at least tertiary structure (form at least one domain).

Selection/Isolation/Recovery

An epitope binding domain or population of domains can be selected, isolated and/or recovered from a repertoire or library (e.g., in a display system) using any suitable method. For example, a domain is selected or isolated based on a selectable characteristic (e.g., physical characteristic, chemical characteristic, functional characteristic). Suitable selectable functional characteristics include biological activities of the peptides or polypeptides in the repertoire, for example, binding to a generic ligand (e.g., a superantigen), binding to a target ligand (e.g., an antigen, an epitope, a substrate), binding to an antibody (e.g., through an epitope expressed on a peptide or polypeptide), and catalytic activity. (See, e.g., Tomlinson *et al.*, WO 99/20749; WO 01/57065; WO 99/58655.)

In some embodiments, the protease resistant peptide or polypeptide is selected and/or isolated from a library or repertoire of peptides or polypeptides in which substantially all domains share a common selectable feature. For example, the domain can be selected from a library or repertoire in which substantially all domains bind a common generic ligand, bind a common target ligand, bind (or are bound by) a common antibody, or possess a common catalytic activity. This type of selection is particularly useful for preparing a repertoire of domains that are based on a parental peptide or polypeptide that has a desired biological activity, for example, when performing affinity maturation of an immunoglobulin single variable domain.

Selection based on binding to a common generic ligand can yield a collection or population of domains that contain all or substantially all of the domains that were components of the original library or repertoire. For example, domains that bind a target ligand or a generic ligand, such as protein A, protein L or an antibody, can be selected, isolated and/or recovered by panning or using a suitable affinity matrix. Panning can be accomplished by adding a solution of ligand (*e.g.*, generic ligand, target ligand) to a suitable vessel (*e.g.*, tube, petri dish) and allowing the ligand to become deposited or coated onto the walls of the vessel. Excess ligand can be washed away and domains can be added to the vessel and the vessel maintained under conditions suitable for peptides or polypeptides to bind the immobilized ligand. Unbound domains can be washed away and bound domains can be recovered using any suitable method, such as scraping or lowering the pH, for example. Suitable ligand affinity matrices generally contain a solid support or bead (*e.g.*, agarose) to which a ligand is covalently or noncovalently attached. The affinity matrix can be combined with peptides or polypeptides (*e.g.*, a repertoire that has been incubated with protease) using a batch process, a column process or any other suitable process under conditions suitable for binding of domains to the ligand on the matrix. domains that do not bind the affinity matrix can be washed away and bound domains can be eluted and recovered using any suitable method, such as elution with a lower pH buffer, with a mild denaturing agent (*e.g.*, urea), or with a peptide or domain that competes for binding to the ligand. In one example, a biotinylated target ligand is combined with a repertoire under conditions suitable for domains in the repertoire to bind the target ligand. Bound domains are recovered using immobilized avidin or streptavidin (*e.g.*, on a bead).

In some embodiments, the generic or target ligand is an antibody or antigen binding fragment thereof. Antibodies or antigen binding fragments that bind structural features of peptides or polypeptides that are substantially conserved in the peptides or polypeptides of a library or repertoire are particularly useful as generic ligands. Antibodies and antigen binding fragments suitable for use as ligands for isolating, selecting and/or recovering protease resistant peptides or polypeptides can be monoclonal or polyclonal and can be prepared using any suitable method.

LIBRARIES/REPERTOIRES

Libraries that encode and/or contain protease epitope binding domains can be prepared or obtained using any suitable method. A library can be designed to encode domains based on a domain or scaffold of interest (*e.g.*, a domain selected from a library) or can be selected from another library using the methods described herein. For example, a library enriched in domains can be prepared using a suitable polypeptide display system.

Libraries that encode a repertoire of a desired type of domain can readily be produced using any suitable method. For example, a nucleic acid sequence that encodes a desired type of polypeptide (*e.g.*, an immunoglobulin variable domain) can be obtained and a collection of nucleic acids that each contain one or more mutations

can be prepared, for example by amplifying the nucleic acid using an error-prone polymerase chain reaction (PCR) system, by chemical mutagenesis (Deng *et al.*, *J. Biol. Chem.*, 269:9533 (1994)) or using bacterial mutator strains (Low *et al.*, *J. Mol. Biol.*, 260:359 (1996)).

- 5 In other embodiments, particular regions of the nucleic acid can be targeted for diversification. Methods for mutating selected positions are also well known in the art and include, for example, the use of mismatched oligonucleotides or degenerate oligonucleotides, with or without the use of PCR. For example, synthetic antibody libraries have been created by targeting mutations to the antigen binding loops.
- 10 Random or semi-random antibody H3 and L3 regions have been appended to germline immunoglobulin V gene segments to produce large libraries with unmutated framework regions (Hoogenboom and Winter (1992) *supra*; Nissim *et al.* (1994) *supra*; Griffiths *et al.* (1994) *supra*; DeKruif *et al.* (1995) *supra*). Such diversification has been extended to include some or all of the other antigen binding loops (Cramer *et al.* (1996) *Nature Med.*, 2:100; Riechmann *et al.* (1995) *Bio/Technology*, 13:475; Morphosys, WO 97/08320, *supra*). In other embodiments, particular regions of the nucleic acid can be targeted for diversification by, for example, a two-step PCR strategy employing the product of the first PCR as a "mega-primer." (See, *e.g.*, Landt, O. *et al.*, *Gene* 96:125-128 (1990).) Targeted diversification can also be
- 20 accomplished, for example, by SOE PCR. (See, *e.g.*, Horton, R.M. *et al.*, *Gene* 77:61-68 (1989).)

- Sequence diversity at selected positions can be achieved by altering the coding sequence which specifies the sequence of the polypeptide such that a number of possible amino acids (*e.g.*, all 20 or a subset thereof) can be incorporated at that
- 25 position. Using the IUPAC nomenclature, the most versatile codon is NNK, which encodes all amino acids as well as the TAG stop codon. The NNK codon may be used in order to introduce the required diversity. Other codons which achieve the same ends are also of use, including the NNN codon, which leads to the production of the additional stop codons TGA and TAA. Such a targeted approach can allow the
- 30 full sequence space in a target area to be explored.

- Some libraries comprise domains that are members of the immunoglobulin superfamily (*e.g.*, antibodies or portions thereof). For example the libraries can comprise domains that have a known main-chain conformation. (See, *e.g.*, Tomlinson *et al.*, WO 99/20749.) Libraries can be prepared in a suitable plasmid
- 35 or vector. As used herein, vector refers to a discrete element that is used to introduce heterologous DNA into cells for the expression and/or replication thereof. Any suitable vector can be used, including plasmids (*e.g.*, bacterial plasmids), viral or bacteriophage vectors, artificial chromosomes and episomal vectors. Such vectors may be used for simple cloning and mutagenesis, or an expression vector can be
- 40 used to drive expression of the library. Vectors and plasmids usually contain one or more cloning sites (*e.g.*, a polylinker), an origin of replication and at least one selectable marker gene. Expression vectors can further contain elements to drive transcription and translation of a polypeptide, such as an enhancer element,

promoter, transcription termination signal, signal sequences, and the like. These elements can be arranged in such a way as to be operably linked to a cloned insert encoding a polypeptide, such that the polypeptide is expressed and produced when such an expression vector is maintained under conditions suitable for expression

5 (e.g., in a suitable host cell).

Cloning and expression vectors generally contain nucleic acid sequences that enable the vector to replicate in one or more selected host cells. Typically in cloning vectors, this sequence is one that enables the vector to replicate independently of the host chromosomal DNA and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2 micron plasmid origin is suitable for yeast, and various viral origins (e.g. SV40, adenovirus) are useful for cloning vectors in mammalian cells. Generally, the origin of replication is not needed for mammalian expression vectors, unless these are used in mammalian cells able to replicate high levels of DNA, such as COS cells.

Cloning or expression vectors can contain a selection gene also referred to as selectable marker. Such marker genes encode a protein necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will therefore not survive in the culture medium. Typical selection genes encode proteins that confer resistance to antibiotics and other toxins, e.g. ampicillin, neomycin, methotrexate or tetracycline, complement auxotrophic deficiencies, or supply critical nutrients not available in the growth media.

25 Suitable expression vectors can contain a number of components, for example, an origin of replication, a selectable marker gene, one or more expression control elements, such as a transcription control element (e.g., promoter, enhancer, terminator) and/or one or more translation signals, a signal sequence or leader sequence, and the like. Expression control elements and a signal or leader sequence, if present, can be provided by the vector or other source. For example, the transcriptional and/or translational control sequences of a cloned nucleic acid encoding an antibody chain can be used to direct expression.

A promoter can be provided for expression in a desired host cell. Promoters can be constitutive or inducible. For example, a promoter can be operably linked to a nucleic acid encoding an antibody, antibody chain or portion thereof, such that it directs transcription of the nucleic acid. A variety of suitable promoters for procaryotic (e.g., the β -lactamase and lactose promoter systems, alkaline phosphatase, the tryptophan (trp) promoter system, lac, tac, T3, T7 promoters for *E. coli*) and eucaryotic (e.g., simian virus 40 early or late promoter, Rous sarcoma virus long terminal repeat promoter, cytomegalovirus promoter, adenovirus late promoter, EG-1a promoter) hosts are available.

40 In addition, expression vectors typically comprise a selectable marker for selection of host cells carrying the vector, and, in the case of a replicable expression vector, an

origin of replication. Genes encoding products which confer antibiotic or drug resistance are common selectable markers and may be used in procaryotic (e.g., β -lactamase gene (ampicillin resistance), *Tet* gene for tetracycline resistance) and eucaryotic cells (e.g., neomycin (G418 or geneticin), gpt (mycophenolic acid), ampicillin, or hygromycin resistance genes). Dihydrofolate reductase marker genes permit selection with methotrexate in a variety of hosts. Genes encoding the gene product of auxotrophic markers of the host (e.g., *LEU2*, *URA3*, *HIS3*) are often used as selectable markers in yeast. Use of viral (e.g., baculovirus) or phage vectors, and vectors which are capable of integrating into the genome of the host cell, such as retroviral vectors, are also contemplated.

Suitable expression vectors for expression in prokaryotic (e.g., bacterial cells such as *E. coli*) or mammalian cells include, for example, a pET vector (e.g., pET-12a, pET-36, pET-37, pET-39, pET-40, Novagen and others), a phage vector (e.g., pCANTAB 5 E, Pharmacia), pRIT2T (Protein A fusion vector, Pharmacia), pCDM8, pCDNA1.1/amp, pcDNA3.1, pRc/RSV, pEF-1 (Invitrogen, Carlsbad, CA), pCMV-SCRIPT, pFB, pSG5, pXT1 (Stratagene, La Jolla, CA), pCDEF3 (Goldman, L.A., *et al.*, *Biotechniques*, 21:1013-1015 (1996)), pSVSPORT (GibcoBRL, Rockville, MD), pEF-Bos (Mizushima, S., *et al.*, *Nucleic Acids Res.*, 18:5322 (1990)) and the like. Expression vectors which are suitable for use in various expression hosts, such as prokaryotic cells (*E. coli*), insect cells (*Drosophila* Schneider S2 cells, Sf9), yeast (*P. methanolica*, *P. pastoris*, *S. cerevisiae*) and mammalian cells (eg, COS cells) are available.

Some examples of vectors are expression vectors that enable the expression of a nucleotide sequence corresponding to a polypeptide library member. Thus, selection with generic and/or target ligands can be performed by separate propagation and expression of a single clone expressing the polypeptide library member. As described above, a particular selection display system is bacteriophage display. Thus, phage or phagemid vectors may be used, for example vectors may be phagemid vectors which have an *E. coli* origin of replication (for double stranded replication) and also a phage origin of replication (for production of single-stranded DNA). The manipulation and expression of such vectors is well known in the art (Hoogenboom and Winter (1992) *supra*; Nissim *et al.* (1994) *supra*). Briefly, the vector can contain a β -lactamase gene to confer selectivity on the phagemid and a lac promoter upstream of an expression cassette that can contain a suitable leader sequence, a multiple cloning site, one or more peptide tags, one or more TAG stop codons and the phage protein pIII. Thus, using various suppressor and non-suppressor strains of *E. coli* and with the addition of glucose, iso-propyl thio- β -D-galactoside (IPTG) or a helper phage, such as VCS M13, the vector is able to replicate as a plasmid with no expression, produce large quantities of the polypeptide library member only or product phage, some of which contain at least one copy of the polypeptide-pIII fusion on their surface.

Antibody variable domains may comprise a target ligand binding site and/or a generic ligand binding site. In certain embodiments, the generic ligand binding site is a

binding site for a superantigen, such as protein A, protein L or protein G. The variable domains can be based on any desired variable domain, for example a human V_H (e.g., V_H 1a, V_H 1b, V_H 2, V_H 3, V_H 4, V_H 5, V_H 6), a human V_λ (e.g., V_λI, V_λII, V_λIII, V_λIV, V_λV, V_λVI or V_κ1) or a human V_κ (e.g., V_κ2, V_κ3, V_κ4, V_κ5, V_κ6, V_κ7, V_κ8, V_κ9 or V_κ10).

A still further category of techniques involves the selection of repertoires in artificial compartments, which allow the linkage of a gene with its gene product. For example, a selection system in which nucleic acids encoding desirable gene products may be selected in microcapsules formed by water-in-oil emulsions is described in WO99/02671, WO00/40712 and Tawfik & Griffiths (1998) *Nature Biotechnol* **16**(7), 652-6. Genetic elements encoding a gene product having a desired activity are compartmentalised into microcapsules and then transcribed and/or translated to produce their respective gene products (RNA or protein) within the microcapsules. Genetic elements which produce gene product having desired activity are subsequently sorted. This approach selects gene products of interest by detecting the desired activity by a variety of means.

Characterisation of the epitope binding domains.

The binding of a domain to its specific antigen or epitope can be tested by methods which will be familiar to those skilled in the art and include ELISA. In one example, binding is tested using monoclonal phage ELISA.

Phage ELISA may be performed according to any suitable procedure: an exemplary protocol is set forth below.

Populations of phage produced at each round of selection can be screened for binding by ELISA to the selected antigen or epitope, to identify "polyclonal" phage antibodies. Phage from single infected bacterial colonies from these populations can then be screened by ELISA to identify "monoclonal" phage antibodies. It is also desirable to screen soluble antibody fragments for binding to antigen or epitope, and this can also be undertaken by ELISA using reagents, for example, against a C- or N-terminal tag (see for example Winter *et al.* (1994) *Ann. Rev. Immunology* 12, 433-55 and references cited therein).

The diversity of the selected phage monoclonal antibodies may also be assessed by gel electrophoresis of PCR products (Marks *et al.* 1991, *supra*; Nissim *et al.* 1994 *supra*), probing (Tomlinson *et al.*, 1992) *J. Mol. Biol.* 227, 776) or by sequencing of the vector DNA.

E. Structure of dAbs

In the case that the dAbs are selected from V-gene repertoires selected for instance using phage display technology as herein described, then these variable domains comprise a universal framework region, such that is they may be recognised by a specific generic ligand as herein defined. The use of universal frameworks, generic
 5 ligands and the like is described in WO99/20749.

Where V-gene repertoires are used variation in polypeptide sequence may be located within the structural loops of the variable domains. The polypeptide sequences of either variable domain may be altered by DNA shuffling or by mutation
 10 in order to enhance the interaction of each variable domain with its complementary pair. DNA shuffling is known in the art and taught, for example, by Stemmer, 1994, Nature 370: 389-391 and U.S. Patent No. 6,297,053, both of which are incorporated herein by reference. Other methods of mutagenesis are well known to those of skill in the art.

15

Scaffolds for use in Constructing dAbs

i. Selection of the main-chain conformation

The members of the immunoglobulin superfamily all share a similar fold for their
 20 polypeptide chain. For example, although antibodies are highly diverse in terms of their primary sequence, comparison of sequences and crystallographic structures has revealed that, contrary to expectation, five of the six antigen binding loops of antibodies (H1, H2, L1, L2, L3) adopt a limited number of main-chain conformations, or canonical structures (Chothia and Lesk (1987) *J. Mol. Biol.*, **196**: 901; Chothia *et al.* (1989) *Nature*, **342**: 877). Analysis of loop lengths and key residues has therefore
 25 enabled prediction of the main-chain conformations of H1, H2, L1, L2 and L3 found in the majority of human antibodies (Chothia *et al.* (1992) *J. Mol. Biol.*, **227**: 799; Tomlinson *et al.* (1995) *EMBO J.*, **14**: 4628; Williams *et al.* (1996) *J. Mol. Biol.*, **264**: 220). Although the H3 region is much more diverse in terms of sequence, length and
 30 structure (due to the use of D segments), it also forms a limited number of main-chain conformations for short loop lengths which depend on the length and the presence of particular residues, or types of residue, at key positions in the loop and the antibody framework (Martin *et al.* (1996) *J. Mol. Biol.*, **263**: 800; Shirai *et al.* (1996) *FEBS Letters*, **399**: 1).

35

The dAbs are advantageously assembled from libraries of domains, such as libraries of V_H domains and/or libraries of V_L domains. In one aspect, libraries of domains are designed in which certain loop lengths and key residues have been chosen to ensure that the main-chain conformation of the members is known. Advantageously, these
 40 are real conformations of immunoglobulin superfamily molecules found in nature, to minimise the chances that they are non-functional, as discussed above. Germline V gene segments serve as one suitable basic framework for constructing antibody or T-cell receptor libraries; other sequences are also of use. Variations may occur at a low

frequency, such that a small number of functional members may possess an altered main-chain conformation, which does not affect its function.

Canonical structure theory is also of use to assess the number of different main-chain conformations encoded by ligands, to predict the main-chain conformation based on ligand sequences and to choose residues for diversification which do not affect the canonical structure. It is known that, in the human V_k domain, the L1 loop can adopt one of four canonical structures, the L2 loop has a single canonical structure and that 90% of human V_k domains adopt one of four or five canonical structures for the L3 loop (Tomlinson *et al.* (1995) *supra*); thus, in the V_k domain alone, different canonical structures can combine to create a range of different main-chain conformations. Given that the V_λ domain encodes a different range of canonical structures for the L1, L2 and L3 loops and that V_k and V_λ domains can pair with any V_H domain which can encode several canonical structures for the H1 and H2 loops, the number of canonical structure combinations observed for these five loops is very large. This implies that the generation of diversity in the main-chain conformation may be essential for the production of a wide range of binding specificities. However, by constructing an antibody library based on a single known main-chain conformation it has been found, contrary to expectation, that diversity in the main-chain conformation is not required to generate sufficient diversity to target substantially all antigens. Even more surprisingly, the single main-chain conformation need not be a consensus structure - a single naturally occurring conformation can be used as the basis for an entire library. Thus, in a one particular aspect, the dAbs possess a single known main-chain conformation.

The single main-chain conformation that is chosen may be commonplace among molecules of the immunoglobulin superfamily type in question. A conformation is commonplace when a significant number of naturally occurring molecules are observed to adopt it. Accordingly, in one aspect, the natural occurrence of the different main-chain conformations for each binding loop of an immunoglobulin domain are considered separately and then a naturally occurring variable domain is chosen which possesses the desired combination of main-chain conformations for the different loops. If none is available, the nearest equivalent may be chosen. The desired combination of main-chain conformations for the different loops may be created by selecting germline gene segments which encode the desired main-chain conformations. In one example, the selected germline gene segments are frequently expressed in nature, and in particular they may be the most frequently expressed of all natural germline gene segments.

In designing libraries the incidence of the different main-chain conformations for each of the six antigen binding loops may be considered separately. For H1, H2, L1, L2 and L3, a given conformation that is adopted by between 20% and 100% of the antigen binding loops of naturally occurring molecules is chosen. Typically, its

observed incidence is above 35% (i.e. between 35% and 100%) and, ideally, above 50% or even above 65%. Since the vast majority of H3 loops do not have canonical structures, it is preferable to select a main-chain conformation which is commonplace among those loops which do display canonical structures. For each of the loops, the conformation which is observed most often in the natural repertoire is therefore selected. In human antibodies, the most popular canonical structures (CS) for each loop are as follows: H1 - CS 1 (79% of the expressed repertoire), H2 - CS 3 (46%), L1 - CS 2 of V_κ(39%), L2 - CS 1 (100%), L3 - CS 1 of V_κ(36%) (calculation assumes a κ:λ ratio of 70:30, Hood *et al.* (1967) *Cold Spring Harbor Symp. Quant. Biol.*, **48**: 133). For H3 loops that have canonical structures, a CDR3 length (Kabat *et al.* (1991) *Sequences of proteins of immunological interest*, U.S. Department of Health and Human Services) of seven residues with a salt-bridge from residue 94 to residue 101 appears to be the most common. There are at least 16 human antibody sequences in the EMBL data library with the required H3 length and key residues to form this conformation and at least two crystallographic structures in the protein data bank which can be used as a basis for antibody modelling (2cgr and 1tet). The most frequently expressed germline gene segments that this combination of canonical structures are the V_H segment 3-23 (DP-47), the J_H segment JH4b, the V_κ segment O2/O12 (DPK9) and the J_κ segment J_κ1. V_H segments DP45 and DP38 are also suitable. These segments can therefore be used in combination as a basis to construct a library with the desired single main-chain conformation.

Alternatively, instead of choosing the single main-chain conformation based on the natural occurrence of the different main-chain conformations for each of the binding loops in isolation, the natural occurrence of combinations of main-chain conformations is used as the basis for choosing the single main-chain conformation. In the case of antibodies, for example, the natural occurrence of canonical structure combinations for any two, three, four, five, or for all six of the antigen binding loops can be determined. Here, the chosen conformation may be commonplace in naturally occurring antibodies and may be observed most frequently in the natural repertoire. Thus, in human antibodies, for example, when natural combinations of the five antigen binding loops, H1, H2, L1, L2 and L3, are considered, the most frequent combination of canonical structures is determined and then combined with the most popular conformation for the H3 loop, as a basis for choosing the single main-chain conformation.

Diversification of the canonical sequence

Having selected several known main-chain conformations or a single known main-chain conformation, dAbs can be constructed by varying the binding site of the molecule in order to generate a repertoire with structural and/or functional diversity. This means that variants are generated such that they possess sufficient diversity in their structure and/or in their function so that they are capable of providing a range of activities.

The desired diversity is typically generated by varying the selected molecule at one or more positions. The positions to be changed can be chosen at random or they may be selected. The variation can then be achieved either by randomisation, during
 5 which the resident amino acid is replaced by any amino acid or analogue thereof, natural or synthetic, producing a very large number of variants or by replacing the resident amino acid with one or more of a defined subset of amino acids, producing a more limited number of variants.

10 Various methods have been reported for introducing such diversity. Error-prone PCR (Hawkins *et al.* (1992) *J. Mol. Biol.*, 226: 889), chemical mutagenesis (Deng *et al.* (1994) *J. Biol. Chem.*, **269**: 9533) or bacterial mutator strains (Low *et al.* (1996) *J. Mol. Biol.*, **260**: 359) can be used to introduce random mutations into the genes that encode the molecule. Methods for mutating selected positions are also well known in
 15 the art and include the use of mismatched oligonucleotides or degenerate oligonucleotides, with or without the use of PCR. For example, several synthetic antibody libraries have been created by targeting mutations to the antigen binding loops. The H3 region of a human tetanus toxoid-binding Fab has been randomised to create a range of new binding specificities (Barbas *et al.* (1992) *Proc. Natl. Acad. Sci. USA*, **89**: 4457). Random or semi-random H3 and L3 regions have been appended to
 20 germline V gene segments to produce large libraries with unmutated framework regions (Hoogenboom & Winter (1992) *J. Mol. Biol.*, **227**: 381; Barbas *et al.* (1992) *Proc. Natl. Acad. Sci. USA*, **89**: 4457; Nissim *et al.* (1994) *EMBO J.*, **13**: 692; Griffiths *et al.* (1994) *EMBO J.*, **13**: 3245; De Kruif *et al.* (1995) *J. Mol. Biol.*, **248**: 97). Such
 25 diversification has been extended to include some or all of the other antigen binding loops (Cramer *et al.* (1996) *Nature Med.*, **2**: 100; Riechmann *et al.* (1995) *Bio/Technology*, **13**: 475; Morphosys, WO97/08320, *supra*).

30 Since loop randomisation has the potential to create approximately more than 10^{15} structures for H3 alone and a similarly large number of variants for the other five loops, it is not feasible using current transformation technology or even by using cell free systems to produce a library representing all possible combinations. For example, in one of the largest libraries constructed to date, 6×10^{10} different
 35 antibodies, which is only a fraction of the potential diversity for a library of this design, were generated (Griffiths *et al.* (1994) *supra*).

In a one embodiment, only those residues which are directly involved in creating or modifying the desired function of the molecule are diversified. For many molecules, the function will be to bind a target and therefore diversity should be concentrated in
 40 the target binding site, while avoiding changing residues which are crucial to the overall packing of the molecule or to maintaining the chosen main-chain conformation.

In one aspect, libraries of dAbs are used in which only those residues in the antigen binding site are varied. These residues are extremely diverse in the human antibody repertoire and are known to make contacts in high-resolution antibody/antigen complexes. For example, in L2 it is known that positions 50 and 53 are diverse in naturally occurring antibodies and are observed to make contact with the antigen. In contrast, the conventional approach would have been to diversify all the residues in the corresponding Complementarity Determining Region (CDR1) as defined by Kabat *et al.* (1991, *supra*), some seven residues compared to the two diversified in the library.. This represents a significant improvement in terms of the functional diversity required to create a range of antigen binding specificities.

In nature, antibody diversity is the result of two processes: somatic recombination of germline V, D and J gene segments to create a naive primary repertoire (so called germline and junctional diversity) and somatic hypermutation of the resulting rearranged V genes. Analysis of human antibody sequences has shown that diversity in the primary repertoire is focused at the centre of the antigen binding site whereas somatic hypermutation spreads diversity to regions at the periphery of the antigen binding site that are highly conserved in the primary repertoire (see Tomlinson *et al.* (1996) *J. Mol. Biol.*, **256**: 813). This complementarity has probably evolved as an efficient strategy for searching sequence space and, although apparently unique to antibodies, it can easily be applied to other polypeptide repertoires. The residues which are varied are a subset of those that form the binding site for the target. Different (including overlapping) subsets of residues in the target binding site are diversified at different stages during selection, if desired.

In the case of an antibody repertoire, an initial 'naive' repertoire is created where some, but not all, of the residues in the antigen binding site are diversified. As used herein in this context, the term "naive" or "dummy" refers to antibody molecules that have no pre-determined target. These molecules resemble those which are encoded by the immunoglobulin genes of an individual who has not undergone immune diversification, as is the case with fetal and newborn individuals, whose immune systems have not yet been challenged by a wide variety of antigenic stimuli. This repertoire is then selected against a range of antigens or epitopes. If required, further diversity can then be introduced outside the region diversified in the initial repertoire. This matured repertoire can be selected for modified function, specificity or affinity.

It will be understood that the sequences described herein include sequences which are substantially identical, for example sequences which are at least 90% identical, for example which are at least 91%, or at least 92%, or at least 93%, or at least 94% or at least 95%, or at least 96%, or at least 97% or at least 98%, or at least 99% identical to the sequences described herein.

For nucleic acids, the term "substantial identity" indicates that two nucleic acids, or designated sequences thereof, when optimally aligned and compared, are identical,

with appropriate nucleotide insertions or deletions, in at least about 80% of the nucleotides, usually at least about 90% to 95%, or at least about 98% to 99.5% of the nucleotides. Alternatively, substantial identity exists when the segments will hybridize under selective hybridization conditions, to the complement of the strand.

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For nucleotide and amino acid sequences, the term "identical" indicates the degree of identity between two nucleic acid or amino acid sequences when optimally aligned and compared with appropriate insertions or deletions. Alternatively, substantial identity exists when the DNA segments will hybridize under selective hybridization conditions, to the complement of the strand.

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The percent identity between two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = # of identical positions/total # of positions times 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm, as described in the non-limiting examples below.

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The percent identity between two nucleotide sequences can be determined using the GAP program in the GCG software package, using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. The percent identity between two nucleotide or amino acid sequences can also be determined using the algorithm of E. Meyers and W. Miller (Comput. Appl. Biosci., 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (J. Mol. Biol. 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package, using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

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By way of example, a polynucleotide sequence of the present invention may be identical to the reference sequence, that is be 100% identical, or it may include up to a certain integer number of nucleotide alterations as compared to the reference sequence. Such alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. The number of nucleotide alterations is determined by multiplying the total number of nucleotides in the reference sequence by the numerical percent of the respective percent

identity(divided by 100) and subtracting that product from said total number of nucleotides in the reference sequence, or:

$$nn \leq xn - (xn \bullet y),$$

wherein nn is the number of nucleotide alterations, xn is the total number of nucleotides in the reference sequence, and y is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and wherein any non-integer product of xn and y is rounded down to the nearest integer prior to subtracting it from xn. Alterations of the polynucleotide sequence of the reference sequence may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

Similarly, in another example, a polypeptide sequence of the present invention may be identical to the reference sequence encoded by SEQ ID NO: 5, that is be 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the % identity is less than 100%. Such alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of amino acid alterations for a given % identity is determined by multiplying the total number of amino acids in the polypeptide sequence encoded by SEQ ID NO: 5 by the numerical percent of the respective percent identity (divided by 100) and then subtracting that product from said total number of amino acids in the polypeptide sequence encoded by SEQ ID NO: 5, or:

$$na \leq xa - (xa \bullet y),$$

wherein na is the number of amino acid alterations, xa is the total number of amino acids in the polypeptide sequence encoded by SEQ ID NO: 5, and y is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc., and wherein any non-integer product of xa and y is rounded down to the nearest integer prior to subtracting it from xa.

Examples**Example 1 - Design and Construction of bispecific antigen binding proteins with various linkers**

5 An anti-IL-13 dAb DNA sequence (DOM10-53-616, SEQ ID NO: 1) was modified by PCR to include a BamHI site at the 5' end and an EcoRI site at the 3' end in order to facilitate cloning. The PCR product was cloned into a mammalian expression vector encoding the heavy chain of an anti-IL4 antibody, allowing the anti IL-13 dAb to be fused onto the C-terminus of the heavy chain via a GS linker (SEQ ID NO: 4, heavy
10 chain of bispecific antibody BPC 2201).

Forward and reverse primers encoding the TVAAPS linker were designed with BglII and BamHI cohesive ends to facilitate cloning into the BamHI site of the vector described above. This method allowed the sequential introduction of TVAAPSGS
15 linker sequences between the C-terminus of the CH3 domain and the N-terminus of the DOM-10-53-616 anti-IL-13 domain antibody. The resulting expression vectors encode SEQ ID NO: 5 and SEQ ID NO: 6 (the heavy chains of BPC2202 and BPC2203 respectively).

20 The DNA sequence of an anti-IL-4 mAb heavy chain was modified at the 3' end by PCR in order to incorporate various linker sequences. The resulting PCR fragments were restricted with HindIII and BamHI and cloned into an expression vector encoding SEQ ID NO: 4 also restricted with HindIII and BamHI. The resulting mammalian expression vectors encode the heavy chain of the anti-IL-4 mAb fused to
25 DOM-10-53-616 with various linkers between the C-terminus of the CH3 domain and the N-terminus of the DOM10-53-616 anti IL-13 dAb. This method generated expression vectors encoding the heavy chains of BPC2204-2215 and BPC2217-2220 (SEQ ID NOs: 7-8 and 11-21 and 23-25).

30 Plasmids encoding heavy chains of BPC2201-2210 and BPC2220 (SEQ ID NO's 4-8 and 11-16) were used as base constructs to generate alternative constructs. In the first stage, the DNA sequence encoding the DOM-10-53-616 anti-IL-13 domain antibody (SEQ ID NO: 1) was replaced with a DNA sequence encoding the DOM-9-155-154 anti IL-4 dAb (SEQ ID NO: 26) by restriction cloning using BamHI and
35 EcoRI. In the second stage, the DNA sequence encoding the variable heavy domain of the anti-IL4 antibody was replaced with a DNA sequence encoding the variable domain of an anti-IL13 antibody by restriction cloning using HindIII and SpeI. The resulting expression vectors encode the heavy chain-domain antibody fusion proteins SEQ ID NOs: 29-33 and 36-41.

40 Plasmids encoding heavy chains of BPC2211-2214, 2218 and 2219 (SEQ ID NO's 17-20, 24 and 25), were used as base constructs to generate alternative constructs. In the first stage, the DNA sequence encoding the DOM-10-53-616 anti-IL-13 domain

antibody (SEQ ID NO:1) was replaced with a DNA sequence encoding the DOM-9-155-154 anti IL-4 dAb (SEQ ID NO: 26) by restriction cloning using BamHI and EcoRI. In the second stage, the DNA sequence encoding the variable heavy domain of the anti-IL4 antibody was replaced with a DNA sequence encoding the variable domain of an anti-IL13 antibody by restriction cloning using HindIII and SpeI. The resulting expression vectors encode the heavy chain-domain antibody fusion proteins SEQ ID NOs: 42-45, 49 and 50.

Alternative heavy chain-domain antibody fusion proteins containing different linkers between the CH3 domain of the heavy chain and the domain antibody were designed and are listed in SEQ ID NOs: 9, 10, 22, 34-35 and 42-50. An alternative anti-IL-4 domain antibody sequence is given in SEQ ID NO. 80-82. An alternative anti-IL-13 domain antibody sequence is given in SEQ ID NO: 83. Linkers are listed in SEQ ID NOs: 51-78 and 112-114.

Table 1: summary of the antibodies and bispecific antigen binding proteins that have been designed. All of these have been constructed and expressed except for those identified with * i.e. BPC2232, 2233, 2243, 2244, 2245, 2238, 2239 and 2240.

Identifier	SEQ ID NO: of Heavy Chain	SEQ ID NO: of light chain	Linker
anti-IL-4 mAb	3	2	None
anti-IL-13 mAb (also described as 586 or A1L1)	28	27	None
BPC 2201	4	2	GS
BPC 2202	5	2	GS(TVAAPSGS) ₁
BPC 2203	6	2	GS(TVAAPSGS) ₂
BPC 2209	7	2	GS(TVAAPSGS) ₃
BPC 2210	8	2	GS(TVAAPSGS) ₄
BPC 2232*	9	2	GS(TVAAPSGS) ₅
BPC 2233*	10	2	GS(TVAAPSGS) ₆
BPC 2208	11	2	(PAS) ₁ GS
BPC 2204	12	2	(PAS) ₂ GS
BPC 2205	13	2	(PAS) ₃ GS
BPC 2206	14	2	(G ₄ S) ₁
BPC 2207	15	2	(G ₄ S) ₂
BPC 2220	16	2	(G ₄ S) ₃
BPC 2211	17	2	(PAVPPP) ₁ GS
BPC 2212	18	2	(PAVPPP) ₂ GS
BPC 2213	19	2	(PAVPPP) ₃ GS
BPC 2214	20	2	(TVSDVP) ₁ GS

BPC 2215	21	2	(TVSDVP) ₂ GS
BPC 2243*	22	2	(TVSDVP) ₃ GS
BPC 2217	23	2	(TGLDSP) ₁ GS
BPC 2218	24	2	(TGLDSP) ₂ GS
BPC 2219	25	2	(TGLDSP) ₃ GS
BPC2221	29	27	GS
BPC2222	30	27	GS(TVAAPSGS) ₁
BPC2223	31	27	GS(TVAAPSGS) ₂
BPC2230	32	27	GS(TVAAPSGS) ₃
BPC2231	33	27	GS(TVAAPSGS) ₄
BPC2244*	34	27	GS(TVAAPSGS) ₅
BPC2245*	35	27	GS(TVAAPSGS) ₆
BPC2229	36	27	(PAS) ₁ GS
BPC2224	37	27	(PAS) ₂ GS
BPC2225	38	27	(PAS) ₃ GS
BPC2226	39	27	(G ₄ S) ₁
BPC2227	40	27	(G ₄ S) ₂
BPC2228	41	27	(G ₄ S) ₃
BPC 2234	42	27	(PAVPPP) ₁ GS
BPC 2235	43	27	(PAVPPP) ₂ GS
BPC 2236	44	27	(PAVPPP) ₃ GS
BPC 2237	45	27	(TVSDVP) ₁ GS
BPC 2238*	46	27	(TVSDVP) ₂ GS
BPC 2239*	47	27	(TVSDVP) ₃ GS
BPC 2240*	48	27	(TGLDSP) ₁ GS
BPC 2241	49	27	(TGLDSP) ₂ GS
BPC 2242	50	27	(TGLDSP) ₃ GS
BPC 2246	29	2	GS
BPC 2247	30	2	GS(TVAAPSGS) ₁
BPC 2248	31	2	GS(TVAAPSGS) ₂
BPC 2249	32	2	GS(TVAAPSGS) ₃
BPC 2250	33	2	GS(TVAAPSGS) ₄
BPC 2251	36	2	(PAS) ₁ GS
BPC 2252	37	2	(PAS) ₂ GS
BPC 2253	38	2	(PAS) ₃ GS
BPC 2254	39	2	(G ₄ S) ₁
BPC 2255	40	2	(G ₄ S) ₂
BPC 2256	41	2	(G ₄ S) ₃

Expression plasmids encoding heavy chain and corresponding light chain of the bispecific antigen binding proteins shown in Table 1 were transiently co-transfected

into HEK 293-6E cells using 293-fectin (Invitrogen, 12347019). A tryptone feed was added to each cell culture up to 24 hours after transfection and the cells were harvested after 3 to 7 days. In some instances the supernatant was used as the test article in binding assays and BIAcore. In other instances, the bispecific antigen binding protein was purified using a Protein A column before being run in binding assays.

The supernatants were quantified with Gyros or the Beckman Coulter IMAGE nephelometer.

10 **Example 2 - IL-4 and IL-13 Binding ELISA**

96-well high binding plates were coated with 5µg/ml of either IL-4 or IL-13 in bicarbonate water and stored overnight at 4°C. The plates were washed twice with Phosphate Buffered Saline (PBS) or Tris-buffered saline (TBS) containing 0.05% of Tween-20. 200µL of blocking solution (3% BSA in PBS buffer) was added in each well and the plate was incubated for at least 1hour at room temperature. Another wash step was then performed. The supernatants or purified antibodies (mAbs) were successively diluted across the plates in blocking solution. After 1 hour incubation, the plates were washed. Goat anti-human kappa light chain specific peroxidase conjugated antibody (Sigma, A7164) was diluted in blocking solution to 1µg/mL and 50µL was added to each well. The plates were incubated between one to one and a half hours. After another wash step, 50µl of OPD (σ-phenylenediamine dihydrochloride) SigmaFast substrate solution was added to each well and the reaction was stopped 15 minutes later by addition of 25µL of 3M sulphuric acid. Absorbance was read at 490nm using the VersaMax Tunable Microplate Reader (Molecular Devices) using a basic endpoint protocol.

Figure 1 and Figure 2 show the results of the binding of several separate assays of purified bispecific antigen binding proteins with GS and GS(TVAAPSGS)_{n=1-4} linker (BPC 2201, 2202, 2203, 2209 and BPC 2210) to human IL-4 and human IL-13 respectively as determined by ELISA. BPC1001 (a positive control bispecific antigen binding protein which binds IL-13 and IL-4) showed binding to IL-4 and IL-13. In contrast a negative control antibody (labelled hybrid which does not recognise either IL-4 or IL-13) showed no binding to human IL-4 or IL-13. In this experiment the bispecific antigen binding proteins all show similar binding activity to human IL-4 and human IL-13.

Figure 3 and Figure 4 show the results of the binding of several separate assays of purified bispecific antigen binding proteins with (PAS)_{n=1-3}GS and (G₄S)_{n=1-3} linkers (BPC 2204 to BPC 2208 and BPC 2220) to human IL-4 and human IL-13 respectively as determined by ELISA. BPC1001 (a positive control bispecific antigen binding protein which binds IL-13 and IL-4) showed binding to IL-4 and IL-13. In contrast a negative control antibody (labelled hybrid which does not recognise either

IL-4 or IL-13) showed no binding to human IL-4 or IL-13. In this experiment the bispecific antigen binding proteins all show similar binding activity to human IL-4 and human IL-13.

Figure 5 and Figure 6 show the results of the binding of purified bispecific antigen binding proteins with (PAVPPP)_{n=1-3}GS, (TVSDVP)_{n=1-2}GS and (TGLDSP)_{n=1-3}GS linkers (BPC 2211 to BPC 2215 and BPC 2217 to BPC 2219) to human IL-4 and human IL-13 respectively as determined by ELISA. BPC1001 (a positive control bispecific antigen binding protein which binds IL-13 and IL-4) showed binding to IL-4 and IL-13. In contrast a negative control antibody (labelled hybrid which does not recognise either IL-4 or IL-13) showed no binding to human IL-4 or IL-13. In this experiment the bispecific antigen binding proteins all show similar binding activity to human IL-4 and human IL-13.

Figures 7 and 8 show the results of the binding of purified bispecific antigen binding proteins with GS and GS(TVAAPSGS)_{n=1-4}, (PAS)_{n=1-3}GS and (G₄S)_{n=1-3} linker (BPC 2221-2231) to human IL-4 and human IL-13 respectively as determined by ELISA. BPC1001 (a positive control bispecific antigen binding protein which binds IL-13 and IL-4) showed binding to IL-4 and IL-13. In contrast a negative control antibody (labelled hybrid which does not recognise either IL-4 or IL-13) showed no binding to human IL-4 or IL-13. In this experiment the bispecific antigen binding proteins all show similar binding activity to human IL-13. In comparison, a significant increase in the binding of the dAb portion to IL-4 was observed on increasing the linker size from the GS(TVAAPSGS)₁ linker to the GS(TVAAPSGS)₂₋₄ linkers.

Figures 9 and 10 show the results of the binding of purified bispecific antigen binding proteins with (PAVPPP)_{n=1-3}GS and TVSDVPGS and (TGLDSP)_{n=2-3}GS linkers (BPC 2234 to BPC 2237, BPC 2241 and BPC 2242) to human IL-4 and human IL-13 respectively as determined by ELISA. BPC1001 (a positive control bispecific antigen binding protein which binds IL-13 and IL-4) showed binding to IL-4 and IL-13. In contrast a negative control antibody (labelled hybrid which does not recognise either IL-4 or IL-13) showed no binding to human IL-4 or IL-13. In this experiment the bispecific antigen binding proteins all show similar binding activity to human IL-13. In comparison, a trend was observed in the human IL-4 binding ELISA: as linker length increased, the binding activity of the dAb portion of the bispecific antigen binding proteins to IL-4 improved.

Example 3 -Stoichiometry assessment of antigen binding proteins (using Biacore™)

This example is prophetic. It provides guidance for carrying out an additional assay in which the antigen binding proteins of the invention can be tested,

Anti-human IgG is immobilised onto a CM5 biosensor chip by primary amine coupling. Antigen binding proteins are captured onto this surface after which a single concentration of IL-13 or IL-4 is passed over, this concentration is enough to saturate the binding surface and the binding signal observed reached full R-max.

5 Stoichiometries are then calculated using the given formula:

$$\text{Stoich} = R_{\text{max}} * M_w (\text{ligand}) / M_w (\text{analyte}) * R (\text{ligand immobilised or captured})$$

10 Where the stoichiometries are calculated for more than one analyte binding at the same time, the different antigens are passed over sequentially at the saturating antigen concentration and the stoichiometries calculated as above. The work can be carried out on the Biacore 3000, at 25°C using HBS-EP running buffer.

15 **Example 4 –Construction and testing of antigen binding proteins comprising the CDRH3 variant anti-IL-13 mAb and a mutated dAb (BPC1085, BPC1086 & BPC1087)**

4.1 Construction and expression

20 Plasmids encoding heavy chains consisting of an anti-IL-13 mAb and an anti-IL-4 dAb were used as base constructs to generate alternative plasmid constructs. A two step cloning strategy was required. In step 1, the DNA sequence encoding the VH of the anti-IL13 mAb component of the H chain was replaced with the DNA sequence encoding the VH of another humanized anti-IL13 antibody (SEQ ID NO:93) by restriction cloning using HindIII and SpeI. In step 2, the codon encoding the leucine at Kabat position 89 in the anti-IL4 dAb (DOM9-155-154, SEQ ID NO: 26) component of the mAbdAb was mutated by site directed mutagenesis to glutamine. All of the resulting heavy chain DNA sequences generated are given in SEQ ID NOs: 98-100. Table 2 provides a list of the molecules constructed and expressed.

30 **Table 2 – Summary of the antibodies constructed and expressed**

Identifier	Alternative names	Linker	Molecule description	Protein SEQ ID NO:
BPC1085	829H-GS(TVAAPSGS) ₂ -154 (89Q) <u>or</u> 829H-GS(TVAAPSGS) ₂ -256	GS(TVAAPSGS) ₂	H chain: Anti-human IL-13 mAb heavy chain Y100B_V – GS(TVAAPSGS) ₂ -DOM9-155-256	98
			L chain: Anti-human IL-13 mAb light chain	27
BPC1086	829H-GS(TVAAPSGS) ₃ -154 (89Q) <u>or</u> 829H-GS(TVAAPSGS) ₃ -256	GS(TVAAPSGS) ₃	H chain: Anti-human IL-13 mAb heavy chain Y100B_V – GS(TVAAPSGS) ₃ -DOM9-155-256	99
			L chain: Anti-human IL-13 mAb light chain	27

BPC1087	829H- GS(TVAAPSGS) ₄ - 154 (89Q) <u>or</u> 829H- GS(TVAAPSGS) ₄ - 256	GS(TVAAPSGS) ₄	H chain: Anti-human IL-13 mAb heavy chain Y100B_V – GS(TVAAPSGS) ₄ -DOM9-155-256	100
			L chain: Anti-human IL-13 mAb light chain	27

Heavy and light chain expression plasmids encoding BPC1085, BPC1086 and BPC1087 mAbdAbs were co-transfected into HEK 2936E cells using 293fectin (Invitrogen, 12347019). A tryptone feed was added to each of the cell cultures after 24 hours and the cells were harvested after 72 hours. The antibodies were purified using a Protein A column before being tested in binding assays.

BPC1085, BPC1086 and BPC1087 mAbdAbs were purified using Protein A affinity. 1ml Protein A columns were used (GE Healthcare) on the AKTA Xpress system, columns were equilibrated in PBS (Gibco/Invitrogen) and the antibodies eluted using Pierce IgG elute. Eluted fractions were neutralised using 1M Tris (Hydroxymethyl) Aminomethane buffer (in general 5-10% v/v). Eluted antibody fractions were pooled and analysed for aggregation by size exclusion chromatography and quantified by reading at OD_{280nm} using a spectrophotometer.

These were compared to equivalent mAbdAbs (2222, 2223, 2230 and 2231) which are described in Table 3. These comprise:

- i) a dAb which is identical to that used in BPC1085, BPC1086 and BPC1087 except for position 89 which is 'L' in BPC2222, BPC2223, BPC2230 & BPC2231 and 'Q' in BPC1085, BPC1086 & BPC1087).
- ii) same linkers
- iii) an IL-13 mAb sequence which is identical to that used BPC1085, BPC1086 and BPC1087 except for position 100B which is 'Y' in BPC2222, BPC2223, BPC2230 & BPC2231 and 'V' in BPC1085, BPC1086 & BPC1087).

Table 3:

Identifier	mAb	Linker	dAb	Heavy chain	Light chain
BPC2222	A1L1	GS(TVAAPSGS) ₁	DOM9-155-154	30	27
BPC2223	A1L1	GS(TVAAPSGS) ₂	DOM9-155-154	31	27
BPC2230	A1L1	GS(TVAAPSGS) ₃	DOM9-155-154	32	27
BPC2231	A1L1	GS(TVAAPSGS) ₄	DOM9-155-154	33	27

BPC2222, 2223, 2230 and 2231 mAbdAbs were purified using Protein A affinity. 1ml Protein A columns were used (GE Healthcare) on the AKTA Xpress system, columns were equilibrated in PBS (Gibco/Invitrogen) and the antibodies eluted using Pierce IgG elute. Eluted fractions were neutralised using 1M Tris (Hydroxymethyl) Aminomethane buffer (in general 5-10% v/v). Eluted antibody fractions were pooled

and analysed for aggregation by size exclusion chromatography and quantified by reading at OD_{280nm} using a spectrophotometer.

5 BPC2222, 2223, 2230 and 2231 showed aggregation of between 30-40%, with the aggregated material eluting before 10 minutes. Compared to BPC2222, 2223, 2230 and 2231 the constructs BPC1085, 1086 and 1087 showed lower levels of aggregation as assessed by size exclusion chromatography.

10 4.2 IL-4 Binding ELISA

Purified BPC1085, BPC1086 and BPC1087 mAbdAbs were tested for binding to IL-4 in a direct binding ELISA according to the following method.

15 96-well high binding plates were coated with 5µg/ml human IL-4 (made at GSK) in NaHCO₃ and stored overnight at 4°C. The plates were washed twice with Tris-Buffered Saline with 0.05% of Tween-20 (TBST). 100µL of blocking solution (1% BSA in TBST buffer) was added in each well and the plates were incubated for at least one hour at room temperature. The purified mAbdAbs were successively diluted across the plates in blocking solution. After one hour incubation, the plates were
20 washed three times. Goat anti-human kappa light chain specific peroxidase conjugated antibody (Sigma A7164) was diluted in blocking solution to 1µg/mL and 50µL was added to each well. The plates were incubated for one hour. After another three washing steps, 50µl of OPD (o-phenylenediamine dihydrochloride) SigmaFast substrate solution was added to each well and the reaction was stopped after about 5
25 minutes by addition of 25µL of 3M sulphuric acid. Absorbance was read at 490nm using the VersaMax Tunable Microplate Reader (Molecular Devices) using a basic endpoint protocol.

The experiment was carried out using mAbdAbs directly from tissue culture supernatants except for the positive control (anti-IL-4 mAb) and the anti-IL13 negative
30 control mAb, which were purified material.

These data are shown in Figure 11. The results of the ELISA confirmed that these purified mAbdAbs bound to IL-4. The positive controls anti-IL-4 mAb and BPC2231 also showed binding to IL-4 whereas the negative control mAb (anti IL-13 mAb)
35 showed no binding to IL-4. This indicated in this ELISA the dAb potency increased when the linker length was increased from GS(TVAAPSGS) to GS(TVAAPSGS)₂₋₄.

4.3 Neutralization of IL-4 in a TF-1 cell proliferation bioassay

40 Purified BPC1085, BPC1086 and BPC1087 mAbdAbs were tested for neutralization of human IL-4 in a TF-1 cell bioassay.

TF-1 cells proliferate in response to a number of different cytokines including human IL-4. The proliferative response of these cells for IL-4 can therefore be used to

measure the bioactivity of IL-4 and subsequently an assay has been developed to determine the IL-4 neutralisation potency (inhibition of IL-4 bioactivity) of mAbdAbs. The assay was performed in sterile 96-well tissue culture plates under sterile conditions and all test wells were performed in duplicate. Approximately 2.2ng/ml recombinant E.Coli-expressed human IL-4 was pre-incubated with various dilutions of mAbdAbs (usually from 560nM titrated in 3-fold dilutions to 0.009nM) in a total volume of 120µl for 1 hour at 37°C. An antibody of irrelevant specificity was similarly titrated as a negative control (anti-IL13 mAb). 50µl of these samples were then added to 50µl of TF-1 cells (at a concentration of 2×10^5 cells per ml) in a sterile 96-well tissue culture plate. Thus the final 100µl assay volume contained various dilutions of mAbdAbs (at a final concentration of 270nM titrated in 3-fold dilutions to 0.005nM), recombinant E.Coli-expressed human IL-4 (at a final concentration of 1.1ng/ml) and TF-1 cells (at a final concentration of 1×10^5 cells per ml). The assay plate was incubated at 37°C for approximately 4 days in a humidified CO₂ incubator. The amount of cell proliferation was then determined using the 'CellTitre 96® Non-Radioactive Cell Proliferation Assay' from Promega (catalogue number G4100), as described in the manufacturers instructions. The absorbance of the samples in the 96-well plate was read in a plate reader at 570nm. These data were entered on an Excel spreadsheet, values for duplicate test wells were averaged and the average background value (no mAb-dAb and no IL-4 test wells) was subtracted.

The capacity of the mAbdAbs to neutralise recombinant E.Coli-expressed human IL-4 bioactivity was expressed as that concentration of the mAb-dAb required to neutralise the bioactivity of the defined amount of human IL-4 (1.1ng/ml) by 50% (= ND₅₀). The lower the concentration of the mAbdAb required, the more potent the neutralisation capacity. The ND₅₀ data provided herein (Table 4) were calculated using the Robosage function in Excel. These data are represented graphically in Figure 12.

An anti-IL-4 mAb and DOM9-155-154 (SEQ ID NO: 26) were included as positive controls for neutralization of human and cynomolgus IL-4 in the TF-1 cell bioassays. Additionally, a dAb with specificity for an irrelevant antigen (dummy dAb) was also included as a negative control for neutralization of human or cynomolgus IL-4 in the TF-1 cell bioassays.

These were repeated several times and Figure 12 shows the results for one of these experiments. ND₅₀ values were calculated from the dataset. The ND₅₀ value is the concentration of the mAbdAb or mAb or dAb, which is able to neutralise the bioactivity of IL-4 by 50%. The mean ND₅₀ value and the number of times tested (n) are shown in Table 4.

Table 4

Molecule	Mean ND ₅₀ value & standard deviation (nM)	Number of repeats
BPC1085	9.21	3
BPC1086	4.32	3

BPC1087	3.59	3
Anti-IL-4 mAb	1.95	2
DOM9-155-154	0.98	2
Negative control dAb	did not neutralise	2

These data confirm that purified BPC1085, BPC1086 and BPC1087 mAbdAbs, neutralized the bioactivity of human and cyno IL-4. Anti-IL-4 mAb and DOM9-155-154 also neutralised the bioactivity of human and cynomolgus IL-4, whereas the negative dAb (dummy dAb) showed no neutralization in the same bioassay. All three mAbdAbs show good potency, and there is a clear trend of increasing dAb potency with increasing linker length was apparent from the neutralisation assays, despite the more crude ELISA not picking up this difference in potency. A negative control anti-IL-4 mAb) showed no neutralization in the same bioassay.

4.4 Neutralization of human IL-13 in a TF-1 cell proliferation bioassay

Purified BPC1085, BPC1086 and BPC1087 mAbdAbs were tested for neutralization of human IL-13 in a TF-1 cell bioassay as described below.

TF-1 cells proliferate in response to a number of different cytokines including human IL-13. The proliferative response of these cells for IL-13 can therefore be used to measure the bioactivity of IL-13 and subsequently an assay has been developed to determine the IL-13 neutralisation potency (inhibition of IL-13 bioactivity) of mAbdAbs.

The assay was performed in sterile 96-well tissue culture plates under sterile conditions and all test wells were performed in duplicate. Approximately 14ng/ml recombinant E.Coli-expressed human IL-13 was pre-incubated with various dilutions of mAbdAbs (usually from 560nM titrated in 3-fold dilutions to 0.009nM) in a total volume of 120µl for 1 hour at 37°C. An antibody and dAb of irrelevant specificity was similarly titrated as negative controls (anti-IL-4 mAb & DOM9-155-154 respectively). 50µl of these samples were then added to 50µl of TF-1 cells (at a concentration of 2×10^5 cells per ml) in a sterile 96-well tissue culture plate. Thus the final 100µl assay volume contained various dilutions of mAbdAbs (at a final concentration of 270nM titrated in 3-fold dilutions to 0.005nM), recombinant E.Coli-expressed human IL-13 (at a final concentration of 7ng/ml) and TF-1 cells (at a final concentration of 1×10^5 cells per ml). The assay plate was incubated at 37°C for approximately 4 days in a humidified CO₂ incubator. The amount of cell proliferation was then determined using the 'CellTitre 96® Non-Radioactive Cell Proliferation Assay' from Promega (catalogue number G4100), as described in the manufacturers instructions. The absorbance of the samples in the 96-well plate was read in a plate reader at 570nm. These data were entered on an Excel spreadsheet, values for duplicate test wells were averaged and the average background value (no mAb-dAb and no IL-13 test wells) was subtracted.

The capacity of the mAbdAbs to neutralise recombinant E.Coli-expressed human IL-13 bioactivity was expressed as that concentration of the mAb-dAb required to neutralise the bioactivity of the defined amount of human IL-13 (7ng/ml) by 50% (=

ND₅₀). The lower the concentration of the mAbdAb required, the more potent the neutralisation capacity. The ND₅₀ data provided herein (Table 5) were calculated using the Robosage function in Excel. These data are represented graphically in Figure 13.

5

An anti IL-13 mAb was included as a positive control for neutralization of human IL-13 in the TF-1 cell bioassays. Additionally, an anti-IL-4 mAb was also included as a negative control.

10 Figure 13 shows the result of the TF-1 cell neutralization assay. ND₅₀ values were calculated from the dataset. The ND₅₀ value is the concentration of the mAbdAb or mAb or dAb, which is able to neutralise the bioactivity of IL-13 by 50%. The mean ND₅₀ value and the number of times tested are shown in Table 5.

15 **Table 5**

Molecule	Mean ND ₅₀ value & standard deviation (nM)	Number of repeats
BPC1085	0.88	1
BPC1086	1.01	1
BPC1087	1.14	1
Anti-IL-13 mAb	5.01	1
Anti-IL-4 mAb	did not neutralise	1

These data confirm that purified BPC1085, BPC1086 and BPC1087 mAbdAbs, neutralized the bioactivity of recombinant human and cyno IL-13. A negative control anti-IL-4 mAb showed no neutralization in the same bioassay.

20

Example 5 – mAbdAbs comprising the DOM10-53-616 dAb

5.1 Expression analysis of anti-IL13 mAb-anti-IL4 dAb

Heavy and light chain expression plasmids encoding for BPC2202, BPC2203 ,
25 BPC2209 and BPC2210 as described in Example 1 were co-transfected into HEK 2936E cells using 293fectin (Invitrogen, 12347019) and expressed at small scale to produce antibody molecules. Molecules were assessed directly from the tissue culture supernatant and were quantified using the Gyrolab workstation. BPC2202, BPC2203, BPC2209 and BPC2210 all expressed well.

30

We attempted to make equivalent mAbdAbs using an alternative anti-IL-13 dAb sequence (DOM10-176-535 - SEQ ID NO: 97). However on expression, these molecules were highly aggregated, so no there was not enough material to use for futher testing.

35

5.2 Stressor Studies of mAbdAbs comprising the DOM10-53-616

BPC2202, BPC2203 , BPC2209 , BPC2210 were placed in PBS or 50mM acetate buffer and incubated at 37°C for up to 14 days. They were then analysed for the

presence of a visual precipitate, soluble aggregate and maintenance of the concentration of the molecule in solution.

Subsequent analysis indicated that in both PBS and acetate solutions there was a reduction in protein concentration in solution for these molecules, this was thought to be due to adsorption of the protein onto the eppendorf tube walls.

BPC2210 also exhibited a reduction in protein concentration in the acetate buffer solution, but had a slight increase in protein concentration after two weeks incubation in the PBS solution (and this may have been due to bacterial contamination).

Example 6 - Design and Construction of anti-IL4/ Anti-TNF α mAb-dAb constructs with various linker lengths

An anti-TNF α dAb DNA sequence was generated by PCR using overlapping oligonucleotide primers. The PCR product was cloned into a mammalian expression vector containing the heavy chain of an anti-IL4 antibody, allowing the anti TNF α dAb to be fused onto the C-terminus of the heavy chain via a TVAAPSGS linker (SEQ ID NO. 103 and 104, DNA and protein sequences of the heavy chain of BPC2626). The heavy chain of BPC2626 was also used as base construct to clone different linkers (TVAAPSGSx2, TVAAPSGSx3, TVAAPSGSx4) and generate the heavy chains of BPC2651, BPC2652 and BPC2653 respectively (SEQ ID. NO: 106 and 107, 108 and 109, 110 and 111, DNA and protein sequences of BPC2651, BPC2652 and BPC2653 respectively).

Expression plasmids encoding the heavy and corresponding light chains of BPC2626, BPC2651, BPC2652 and BPC2653 were transiently co-transfected into HEK 293-6E cells using 293 fectin (Invitrogen, 12347019). A tryptone feed was added to each cell culture 24 hours after transfection, and the cells were harvested after 3 to 7 days. The mAbdAbs were purified using a Protein A column before being assessed for activity. The constructs made and expressed are set out in Table 6.

Table 6

ANTIBODY ID	DESCRIPTION	SEQ ID NO: Polynucleotide sequence	SEQ ID NO: Amino acid sequence
BPC2626	PascoH-TVAAPSGS-PEP1-5-19 Heavy Chain	104	103
	Light Chain	3	105
BPC2651	PascoH-GS(TVAAPSGS) ₂ - PEP1-5-19 Heavy Chain	106	107
	Light Chain	3	105
BPC2652	PascoH-GS(TVAAPSGS) ₃ - PEP1-5-19 Heavy Chain	108	109
	Light Chain	3	105

BPC2653	PascoH-GS(TVAAPSGS) ₄ - PEP1-5-19 Heavy Chain	110	111
	Light Chain	3	105

6.1 TNF α Binding ELISA

96-well high binding plates were coated with 1 μ g/ml of TNF α (R&D Systems, 210-TA-010/CF) in Phosphate-Buffered Saline and stored overnight at 4°C. The plates were washed three times with Tris-Buffered Saline containing 0.05% of Tween-20. 100 μ L of blocking solution (1% BSA in Tris-Buffered Saline containing 0.05% of Tween-20 buffer) was added in each well and the plate was incubated for at least 1 hour at room temperature. Another wash step was then performed. Purified bispecific antibodies and a negative control antibody were successively diluted across the plates in blocking solution. After 1 hour incubation, the plates were washed. Goat anti-human kappa light chain specific peroxidase conjugated antibody H23 (Sigma, A7164) was diluted in blocking solution to 1 μ g/mL and 50 μ L was added to each well. The plates were incubated for one hour. After another wash step, 50 μ L of OPD (o-phenylenediamine dihydrochloride) SigmaFast substrate solution was added to each well and the reaction was stopped 15 minutes later by addition of 25 μ L of 3M sulphuric acid. Absorbance was read at 490nm using the VersaMax Tunable Microplate Reader (Molecular Devices) using a basic endpoint protocol.

Figure 14 shows the results of the TNF α binding ELISA and confirms that BPC2626, BPC2651, BPC2652 and BPC2653 bound to TNF α with an increase in the binding activity correlated to increased linker lengths. The negative control antibody did not show binding to the target. A trend of increasing dAb potency with increasing linker length was observed.

6.2 - IL4 Binding ELISA

These constructs were tested for binding to IL-4 and because of a technical issue with the assay the only conclusion we can draw is that they all bound.

Example 7 - Binding affinity of mAbsdAbs with varying linker length (BPC2221-2231) for IL-13 and IL-4 as assessed by BIAcoreTM analysis

Method

Anti Human IgG (GE Healthcare/Biacore) was immobilised on a CM5 chip by primary amine coupling; this surface was used as a capture surface for the antibody molecules to be tested. Recombinant E.coli-expressed Human IL4 and human IL13 were used at 256, 64, 16, 4 and 1nM, with 0nM (i.e. buffer alone) used to double reference the binding curves. Regeneration the anti-human IgG surface was with an injection of 100mM Phosphoric acid followed by an injection of 3M MgCl₂. The assay was run at 25°C using HBS-EP as running buffer. The data was fitted to 1:1 model inherent to the Biacore T100 analysis software.

The results of binding to human IL13 are shown in Table 7 and the result of binding to human IL4 are shown in Table 8.

Table 7

Molecule name	ka (M/s)	kd (1/s)	KD (nM)
BPC2221	1.049E+6	3.441E-4	0.328
BPC2222	1.108E+6	3.543E-4	0.320
BPC2223	1.410E+6	4.012E-4	0.285
BPC2224	1.156E+6	3.053E-4	0.264
BPC2225	1.178E+6	3.336E-4	0.283
BPC2226	1.105E+6	2.997E-4	0.271
BPC2227	1.082E+6	3.261E-4	0.301
BPC2228	1.071E+6	3.364E-4	0.314
BPC2229	1.066E+6	3.307E-4	0.310
BPC2230	1.067E+6	3.316E-4	0.311
BPC2231	1.099E+6	3.454E-4	0.314

5

Table 8

Molecule name	ka (M/s)	kd (1/s)	KD (nM)
BPC2221	7.268E+4	3.444E-4	4.74
BPC2222	5.723E+4	4.774E-4	8.34
BPC2223	5.856E+6	8.860E-8	0.00002
BPC2224	2.414E+4	7.553E-4	31.3
BPC2225	4.811E+4	3.498E-5	0.727
BPC2226	4.212E+6	3.253E-6	0.0008
BPC2227	1.573E+5	2.552E-4	1.62
BPC2228	3.199E+5	1.646E-4	0.515
BPC2229	3.457E+4	2.150E-4	6.22
BPC2230	2.835E+4	3.576E-4	12.6
BPC2231*	5.342E+6	7.332E-6	0.001

* Impossible off-rate, unable to analyse, very tight binding

** Actual kinetic values likely to be worse than calculated due to poor fitting for

10 BPC2229

Overall run quality was poor for IL4 binding, the analysis was complicated by the fact that at highest concentrations there was non-specific binding to the chip surface

Example 8 - Binding affinity of mAbdabs comprising the 616 dAb for IL-13 and IL-4 as assessed by BIAcore™ analysis

5 mAbdAbs BPC2201-2215, BPC2217-2220 and BPC2243, each of which comprise the DOM10-53-616 anti-IL-13 domain antibody (SEQ ID NO:1) were tested by BIAcore. All mAbdAbs bound to both IL-13 and IL-4 but they bound so tightly that off rates were impossible to measure.

10 **Example 9 – Binding Affinity of mAbdAbs with varying linker length (BPC2211-2231 & BPC2234-2237 & BPC2241-2242) for IL-13 and IL-4 as assessed by BIAcore™ analysis**

Method

15 Anti Human IgG (GE Healthcare/Biacore) was immobilised on a CM5 chip by primary amine coupling; this surface was used as a capture surface for the antibody molecules to be tested. Recombinant E.coli-expressed human IL4 was used at 256, 64, 16, 4 and 1nM and Recombinant E.coli-expressed human IL13 used at 256nM only, with 0nM (i.e. buffer alone) used to double reference the binding curves of both
20 IL4 and IL13 binding. Regeneration of the anti-human IgG surface was with an injection of 100mM Phosphoric acid followed by an injection of 3M MgCl₂. The assay was run at 25°C using HBS-EP as running buffer. The data was fitted to 1:1 model inherent to the Biacore T100 analysis software.

25 The results of binding to human IL13 and human IL4 are shown in Table 9.

Table 9

Molecule name/ analyte	ka (M/s)	kd (1/s)	KD (nM)	Quality of data
BPC2221 / IL4	7.07E+04	5.92E-04	8.368	Ok
BPC2221 / IL13	1.29E+06	4.52E-04	0.350	Yes
BPC2222 / IL4	6.52E+04	1.29E-04	1.980	Ok
BPC2222 / IL13	2.13E+06	6.04E-04	0.284	Ok
BPC2223 / IL4	1.30E+07	1.07E-04	0.008	No
BPC2223 / IL13	1.38E+06	4.51E-04	0.327	Yes
BPC2224 / IL4	2.52E+04	1.55E-03	61.370	Ok
BPC2224 / IL13	1.43E+06	4.74E-04	0.332	Yes
BPC2225 / IL4	5.78E+04	1.11E-04	1.912	Ok
BPC2225 / IL13	1.40E+06	4.77E-04	0.341	Yes
BPC2226 / IL4	2.86E+04	1.44E-03	50.130	Ok
BPC2226 / IL13	1.37E+06	4.55E-04	0.333	Yes
BPC2227 / IL4	2.07E+05	3.05E-04	1.476	Ok

BPC2227 / IL13	1.30E+06	4.68E-04	0.360	Yes
BPC2228 / IL4	4.24E+05	2.28E-04	0.539	Ok
BPC2228 / IL13	1.40E+06	4.82E-04	0.344	Yes
BPC2229 / IL4	3.17E+04	8.28E-04	26.080	Ok
BPC2229 / IL13	1.36E+06	4.71E-04	0.347	Yes
BPC2230 / IL4	1.86E+07	1.92E-04	0.010	Ok
BPC2230 / IL13	1.34E+06	4.51E-04	0.336	Yes
BPC2231 / IL4	1.92E+07	2.54E-04	0.013	Ok
BPC2231 / IL13	1.34E+06	4.67E-04	0.348	Yes
BPC2234 / IL4	2.73E+05	8.49E-05	0.312	Ok
BPC2234 / IL13	1.45E+06	4.49E-04	0.310	Yes
BPC2235 / IL4	9.71E+05	9.32E-05	0.096	Ok
BPC2235 / IL13	1.43E+06	4.72E-04	0.331	Yes
BPC2236 / IL4	5.46E+06	9.23E-05	0.017	Ok
BPC2236 / IL13	1.40E+06	4.73E-04	0.339	Yes
BPC2237 / IL4	2.06E+05	2.80E-04	1.359	Ok
BPC2237 / IL13	1.36E+06	4.42E-04	0.326	Yes
BPC2241 / IL4	6.17E+05	2.40E-04	0.389	Ok
BPC2241 / IL13	1.43E+06	4.37E-04	0.306	Yes
BPC2242 / IL4	3.45E+06	2.17E-04	0.063	Ok
BPC2242 / IL13	1.43E+06	4.57E-04	0.321	Yes

Some non-specific binding was seen with the highest concentrations of recombinant E.coli-expressed human IL4, which affected the quality of the data

5 **Example 10 - Binding Affinity of mAbdAbs comprising the original IL-13 mAb CDRH3 with varying linker lengths (BPC2222, BPC2223 & BPC2230-2231) for IL-13 and IL-4 as assessed by BIAcore™ analysis**

Method

- 10 Protein A was immobilised on a C1 chip by primary amine coupling; this surface was used as a capture surface for the antibody molecules to be tested. Recombinant E.coli-expressed human IL13 was used at 256, 64, 16, 4, and 1nM, recombinant E.coli-expressed human IL4 was used at 64, 16, 4, 1 and 0.25nM, with 0nM (i.e. buffer alone) used to double reference the binding curves of both IL4 and IL13

binding. Regeneration the Protein A surface was with 100mM Phosphoric acid. The assay was run at 25°C using HBS-EP as running buffer. The data was fitted to 1:1 model inherent to the Biacore T100 analysis software.

- 5 The results of binding to human IL13 are shown in Table 10 and the result of binding to human IL4 are shown in Table 11.

Table 10

Molecule name	ka(M/s)	kd(1/s)	KD (nM)
BPC2222	1.31E+06	4.93E-04	0.376
BPC2223	1.32E+06	4.90E-04	0.372
BPC2230	1.31E+06	4.88E-04	0.373
BPC2231	1.30E+06	5.13E-04	0.394

10 Table 11

Molecule name	ka(M/s)	kd(1/s)	KD (nM)
BPC2222	1.06E+05	1.09E-04	1.027
BPC2223	8.59E+06	1.56E-04	0.018
BPC2230*	2.48E+07	2.48E-04	0.010
BPC2231*	4.03E+07	2.31E-04	0.006

*The on-rate for BPC2230 and 2231 are beyond the sensitivity of Biacore, but the fact that we cannot accurately analyse this data does indicate that the interaction with IL4 is likely to be of high affinity with a fast on-rate.

15

Example 11 – Binding Affinity of mAbdAbs comprising the original IL-13 mAb CDRH3 with varying linker lengths (BPC2222, BPC2231) & variant anti-IL-13 mAb CDRH3 with varying linker lengths (BPC1085-1087) for IL-13 and IL-4 as assessed by BIAcore™ analysis

20

Method

Protein A was immobilised on a CM5 chip by primary amine coupling; this surface was used as a capture surface for the antibody molecules to be tested. Recombinant E.coli-expressed human IL13 was used at 256nM only, Recombinant E.coli-expressed human IL4 was used at 64, 16, 4 and 1nM, with 0nM (i.e. buffer alone) used to double reference the binding curves for both IL4 and IL13 binding.

25

Regeneration the Protein A surface was with 50mM NaOH. The assay was run at 25°C using HBS-EP as running buffer. The data was fitted to 1:1 model inherent to the Biacore T100 analysis software.

30

The results of binding to human IL13 are shown in Table12, and the results of binding to human IL4 are shown in Table 13.

35 Table 12

Molecule name	ka(M/s)	kd(1/s)	KD (nM)
BPC2222	1.64E+05	5.15E-05	0.314
BPC2231	5.36E+08	1.16E-03	0.002
BPC1085	1.87E+07	8.97E-04	0.048
BPC1086	7.99E+07	1.64E-03	0.021
BPC1087	9.86E+07	1.79E-03	0.018

On-rate for BPC1086 and BPC1087 are beyond the sensitivity of Biacore, but the fact that we cannot accurately analyse this data does indicate that the interaction with IL4 is likely to be of high affinity with a fast on-rate.

5 Table 13

Molecule name	ka(M/s)	kd(1/s)	KD (nM)
BPC2222	1.44E+06	4.44E-04	0.308
BPC2231	1.56E+06	4.95E-04	0.316
BPC1085	1.20E+06	6.39E-05	0.053
BPC1086	1.28E+06	6.57E-05	0.051
BPC1087	1.13E+06	6.42E-05	0.057

Example 12 - Stressor Studies of mAbdAbs

- 10 A number of mAbdAbs were placed in PBS or 50mM acetate buffer and incubated at 37°C for up to 14 days. They were then analysed for presence of a visual precipitate, soluble aggregate and maintenance of the concentration of the molecule in solution.

15 The results indicate that the mAbdAbs comprising the mutated dAb (BPC1085, 1086, 1087) behaved similarly to the non-mutated dAb (BPC2222, 2223, 2230, 2231) both catagories of mAbdAb appeared to be stable in both PBS and acetate buffers over the two week incubation period at 37°C, as indicated by no change in the protein concentration in the solutions. In addition none or very little change was noted for the levels of aggregates in the solutions and no precipitation was observed.

20

Example 13 - Effect of linker length on Rat and Cyno PK

- 25 The pharmacokinetics of BPC1085, BPC1086, and BPC1087 were investigated in separate studies following IV administration to rats. The PK of BPC1085 was also investigated in cynomologus monkeys following IV administration.

The PK of all three molecules in rat and BPC1085 in monkey were found to be consistent with that of a standard mAb.

Example 14

5 A panel of mAbdabs to a different target with a number of differing length linkers were expressed. Uncharacterised supernatants were put through some ELISA binding experiments and these preliminary results indicate similar trends to those described herein across different linker lengths.

10 A second panel of mAbdabs to a different target with a number of differing length linkers were expressed. Uncharacterised supernatants were put through some ELISA binding experiments and these preliminary results indicate variability across different dAbs and linker lengths.

Brief Description of Figures

Figure 1: A graph showing binding of purified bispecific antigen binding proteins with
 5 GS and GS(TVAAPSGS)_{n=1-4} linker (BPC 2201, 2202, 2203, 2209 and BPC 2210) to human IL-4 as determined by ELISA.

Figure 2: A graph showing binding of purified bispecific antigen binding proteins with GS and GS(TVAAPSGS)_{n=1-4} linker (BPC 2201, 2202, 2203, 2209 and BPC 2210) to human IL-13 as determined by ELISA.

10 Figure 3: A graph showing binding of purified bispecific antigen binding proteins with (PAS)_{n=1-3}GS and (G₄S)_{n=1-3} linkers (BPC 2204 to BPC 2208 and BPC 2220) to human IL-4 as determined by ELISA.

Figure 4: A graph showing binding of purified bispecific antigen binding proteins with (PAS)_{n=1-3}GS and (G₄S)_{n=1-3} linkers (BPC 2204 to BPC 2208 and BPC 2220) to
 15 human IL-13 as determined by ELISA.

Figure 5: A graph showing binding of purified bispecific antigen binding proteins with (PAVPPP)_{n=1-3}GS, (TVSDVP)_{n=1-2}GS and (TGLDSP)_{n=1-3}GS linkers (BPC 2211 to BPC 2215 and BPC 2217 to BPC 2219) to human IL-4 as determined by ELISA.

Figure 6: A graph showing binding of purified bispecific antigen binding proteins with
 20 (PAVPPP)_{n=1-3}GS, (TVSDVP)_{n=1-2}GS and (TGLDSP)_{n=1-3}GS linkers (BPC 2211 to BPC 2215 and BPC 2217 to BPC 2219) to human IL-13 as determined by ELISA.

Figure 7: A graph showing binding of purified bispecific antigen binding proteins with GS and GS(TVAAPSGS)_{n=1-4}, (PAS)_{n=1-3}GS and (G₄S)_{n=1-3} linker (BPC 2221 to BPC 2231) to human IL-4 as determined by ELISA.

25 Figure 8: A graph showing binding of purified bispecific antigen binding proteins with GS and GS(TVAAPSGS)_{n=1-4}, (PAS)_{n=1-3}GS and (G₄S)_{n=1-3} linker (BPC 2221 to BPC 2231) to human IL-13 as determined by ELISA.

Figure 9: A graph showing binding of purified bispecific antigen binding proteins with (PAVPPP)_{n=1-3}GS and TVSDVPGS and (TGLDSP)_{n=2-3}GS linkers (BPC 2234 to BPC
 30 2237, BPC 2241 and BPC 2242) to human IL-4 as determined by ELISA.

Figure 10: A graph showing binding of purified bispecific antigen binding proteins with (PAVPPP)_{n=1-3}GS and TVSDVPGS and (TGLDSP)_{n=2-3}GS linkers (BPC 2234 to BPC 2237, BPC 2241 and BPC 2242) to human IL-13 as determined by ELISA.

5 Figure 11: A graph showing binding of purified mAbdAbs (BPC1085, BPC1086 and BPC1087) to human IL-4 as determined by ELISA. The IL-4 control mAb is labelled as 'pascolizumab'.

10 Figure 12: A graph showing neutralization of human IL-4 by purified mAbdAbs (BPC1085, BPC1086 and BPC1087) to human IL-4 in the TF-1 cell bioassay. The IL-4 control mAb is labelled as 'pascolizumab'.

15 Figure 13: A graph showing neutralization of human IL-13 by purified mAbdAbs (BPC1085, BPC1086 and BPC1087) to human IL-13 in the TF-1 cell bioassay. The IL-4 control mAb is labelled as 'pascolizumab'.

Figure 14: A graph showing the binding of BPC2651, BPC2652 and BPC2653 to TNF α as determined by ELISA.

20

Table 14 – Sequence listing

Description	Sequence identifier (SEQ ID NO)	
	amino acid sequence	DNA sequence
DOM10-53-616 anti-IL-13 domain antibody	1	
anti-IL-4 mAb heavy chain	2	
anti-IL-4 mAb light chain	3	105
PascoH-GS-DOM10-53-616	4	
Anti-human IL-4 mAb heavy chain - GS(TVAAPSGS) ₁ -DOM10-53-616	5	
Anti-human IL-4 mAb heavy chain - GS(TVAAPSGS) ₂ -DOM10-53-616	6	
Anti-human IL-4 mAb heavy chain - GS(TVAAPSGS) ₃ -DOM10-53-616	7	
Anti-human IL-4 mAb heavy chain - GS(TVAAPSGS) ₄ -DOM10-53-616	8	
Anti-human IL-4 mAb heavy chain- GS(TVAAPSGS) ₅ -DOM10-53-616	9	
Anti-human IL-4 mAb heavy chain- GS(TVAAPSGS) ₆ -DOM10-53-616	10	
Anti-human IL-4 mAb heavy chain-(PAS) ₁ GS-DOM10-53-616	11	
Anti-human IL-4 mAb heavy chain-(PAS) ₂ GS-DOM10-53-616	12	
Anti-human IL-4 mAb heavy chain-(PAS) ₃ GS-DOM10-53-616	13	
Anti-human IL-4 mAb heavy chain-(G ₄ S) ₁ -DOM10-53-616	14	
Anti-human IL-4 mAb heavy chain-(G ₄ S) ₂ -DOM10-53-616	15	
Anti-human IL-4 mAb heavy chain-(G ₄ S) ₃ -DOM10-53-616	16	
Anti-human IL-4 mAb heavy chain-(PAVPPP) ₁ GS-DOM10-53-616	17	
Anti-human IL-4 mAb heavy chain-(PAVPPP) ₂ GS-DOM10-53-616	18	
Anti-human IL-4 mAb heavy chain-(PAVPPP) ₃ GS-DOM10-53-616	19	
Anti-human IL-4 mAb heavy chain-(TVSDVP) ₁ GS-DOM10-53-616	20	

Anti-human IL-4 mAb heavy chain-(TVSDVP) ₂ GS-DOM10-53-616	21	
Anti-human IL-4 mAb heavy chain-(TVSDVP) ₃ GS-DOM10-53-616	22	
Anti-human IL-4 mAb heavy chain-(TGLDSP) ₁ GS-DOM10-53-616	23	
Anti-human IL-4 mAb heavy chain-(TGLDSP) ₂ GS-DOM10-53-616	24	
Anti-human IL-4 mAb heavy chain-(TGLDSP) ₃ GS-DOM10-53-616	25	
DOM9-155-154 anti-IL-4 domain antibody	26	
Anti-IL-13 antibody (586) light chain	27	
Anti-IL-13 antibody (586) heavy chain	28	
Anti-IL-13 mAb heavy chain-GS-DOM9-155-154	29	
Anti-IL-13 mAb heavy chain-GS(TVAAPSGS) ₁ -DOM9-155-154	30	
Anti-IL-13 mAb heavy chain-GS(TVAAPSGS) ₂ -DOM9-155-154	31	
Anti-IL-13 mAb heavy chain-GS(TVAAPSGS) ₃ -DOM9-155-154	32	
Anti-IL-13 mAb heavy chain-GS(TVAAPSGS) ₄ -DOM9-155-154	33	
Anti-IL-13 mAb heavy chain-GS(TVAAPSGS) ₅ -DOM9-155-154	34	
Anti-IL-13 mAb heavy chain-GS(TVAAPSGS) ₆ -DOM9-155-154	35	
Anti-IL-13 mAb heavy chain-(PAS) ₁ GS-DOM9-155-154	36	
Anti-IL-13 mAb heavy chain-(PAS) ₂ GS-DOM9-155-154	37	
Anti-IL-13 mAb heavy chain-(PAS) ₃ GS-DOM9-155-154	38	
Anti-IL-13 mAb heavy chain-(G ₄ S) ₁ -DOM9-155-154	39	
Anti-IL-13 mAb heavy chain-(G ₄ S) ₂ -DOM9-155-154	40	
Anti-IL-13 mAb heavy chain-(G ₄ S) ₃ -DOM9-155-154	41	
Anti-IL-13 mAb heavy chain-(PAVPPP) ₁ GS-DOM9-155-154	42	
Anti-IL-13 mAb heavy chain-(PAVPPP) ₂ GS-DOM9-155-154	43	

Anti-IL-13 mAb heavy chain-(PAVPPP) ₃ GS-DOM9-155-154	44	
Anti-IL-13 mAb heavy chain-(TVSDVP) ₁ GS-DOM9-155-154	45	
Anti-IL-13 mAb heavy chain-(TVSDVP) ₂ GS-DOM9-155-154	46	
Anti-IL-13 mAb heavy chain-(TVSDVP) ₃ GS-DOM9-155-154	47	
Anti-IL-13 mAb heavy chain-(TGLDSP) ₁ GS-DOM9-155-154	48	
Anti-IL-13 mAb heavy chain-(TGLDSP) ₂ GS-DOM9-155-154	49	
Anti-IL-13 mAb heavy chain-(TGLDSP) ₃ GS-DOM9-155-154	50	
GS	51	
GS(TVAAPSGS) ₁	52	
GS(TVAAPSGS) ₂	53	
GS(TVAAPSGS) ₃	54	
GS(TVAAPSGS) ₄	55	
GS(TVAAPSGS) ₅	56	
GS(TVAAPSGS) ₆	57	
(PAS) ₁ GS	58	
(PAS) ₂ GS	59	
(PAS) ₃ GS	60	
(G ₄ S) ₁	61	
(G ₄ S) ₂	62	
(G ₄ S) ₃	63	
(PAVPPP) ₁ GS	64	
(PAVPPP) ₂ GS	65	
(PAVPPP) ₃ GS	66	
(TVSDVP) ₁ GS	67	
(TVSDVP) ₂ GS	68	
(TVSDVP) ₃ GS	69	
(TGLDSP) ₁ GS	70	
(TGLDSP) ₂ GS	71	
(TGLDSP) ₃ GS	72	
TVAAPSGS linker	73	
PAS linker	74	
TVAAPS linker	75	
PAVPPP linker	76	
TVSDVP linker	77	
TGLDSP linker	78	

586H-TVAAPSGS-154 Heavy chain	79	
DOM9-155-25	80	
DOM9-155-147	81	
DOM9-112-210	82	
DOM10-53-474	83	
Example signal peptide	84	
DOM10-53-616 dAb-GSTVAAPS-Anti-human IL-4 mAb heavy chain	85	
DOM10-53-616 dAb-GSASTKGPS-Anti-human IL-4 mAb heavy chain	86	
DOM10-53-616 dAb-GSTVAAPS-Anti-human IL-4 mAb light chain	87	
DOM10-53-616 dAb-GSASTKGPS-Anti-human IL-4 mAb light chain	88	
Anti-human IL-4 mAb light chain-TVAAPS-DOM10-53-616 dAb	89	
Anti-human IL-4 mAb heavy chain-TVAAPS-DOM10-53-616 dAb	90	
IL-5 mAb Heavy chain	91	
IL-5 mAb Light chain	92	
Anti IL13 humanised variant A1 Y100B Val (Heavy Chain)	93	
829H-(TVAAPS) ₂ GS-154 L89Q	94	
829H-(TVAAPS) ₃ GS-154 L89Q	95	
829H-(TVAAPS) ₄ GS-154 L89Q	96	
DOM10-176-535	97	
829H-GS(TVAAPSGS) ₂ -154 L89Q	98	
829H-GS(TVAAPSGS) ₃ -154 L89Q	99	
829H-GS(TVAAPSGS) ₄ -154 L89Q	100	
Anti-human IL-4 DOM9-155-256	101	
Anti-human IL-4 DOM9-155-256, CDRH3	102	
PascoH-TVAAPSGS-PEP1-5-19 Heavy Chain	104	103
PascoH-GS(TVAAPS) ₂ - PEP1-5-19 Heavy Chain	107	106
PascoH-GS(TVAAPS) ₃ - PEP1-5-19 Heavy Chain	109	108
PascoH-GS(TVAAPS) ₄ - PEP1-5-19 Heavy Chain	111	110

(TVAAPS) ₂ GS linker	112	
(TVAAPS) ₃ GS linker	113	
(TVAAPS) ₄ GS linker	114	

SEQ ID NO:1

5 GVQLLES GGGLVQPGGSLRLSCAASGFVFPWYDMGWVRQAPGKGLEWVSSIDWHGKITYYAD
SVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCATAEDEPGYDYWGQGTLVTVSS

SEQ ID NO:2

10 QVTLRESGPALVKPTQTLTLTCTFSGFSLSTSGMGVSWIRQPPGKGLEWLAHIYWDDDKRYN
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KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
15 ALHNHYTQKSLSLSPGK

SEQ ID NO:3

20 DIVLTQSPSSLSASVGDRVTITCKASQSV DYGDSYMNWYQQKPGKAPKLLIYAASNLESGI
PSRFGSGSGTDFTFTISS LQPEDIATYYCQQSNEDPPTFGQGTKVEIKRTVAAPSVFI FPP
SDEQLKSGTASV VCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLS
KADYEEKHKVYACEVTHQGLSSPVT KSFNRGEC

SEQ ID NO:4

25 QVTLRESGPALVKPTQTLTLTCTFSGFSLSTSGMGVSWIRQPPGKGLEWLAHIYWDDDKRYN
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KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
30 VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGK **GS**GVQLLES GGGLVQPGGSLRLSCAASGFVFPWYDMGWVRQAPGK
GLEWVSSIDWHGKITYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCATAEDEPGY
DYWGQGTLVTVSS

SEQ ID NO:5

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KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
40 PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
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45 TAEDEPGYDYWGQGTLVTVSS

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50 QVTLRESGPALVKPTQTLTLTCTFSGFSLSTSGMGVSWIRQPPGKGLEWLAHIYWDDDKRYN
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KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP

PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
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 VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
 ALHNHYTQKSLSLSPGK**GSTVAAPSGSTVAAPSGS**GVQLLES GGGGLVQPGGSLRLSCAASGF
 5 VFPWYDMGWVRQAPGKGLEWVSSIDWHGKITYYADSVKGRFTISRDN SKNTLYLQMNSLRAE
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 KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSL
 SSVVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
 PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
 TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
 15 VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHE
 ALHNHYTQKSLSLSPGK**GSTVAAPSGSTVAAPSGSTVAAPSGS**GVQLLES GGGGLVQPGGSLR
 LSCAASGFVFPWYDMGWVRQAPGKGLEWVSSIDWHGKITYYADSVKGRFTISRDN SKNTLYL
 QMNSLRAEDTAVYYCATAEDEPGYDYWGQGT LVT VSS

SEQ ID NO:8

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 KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSL
 SSVVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
 25 PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
 TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
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 VQPGGSLRLSCAASGFVFPWYDMGWVRQAPGKGLEWVSSIDWHGKITYYADSVKGRFTISRDN
 30 NSKNTLYLQMNSLRAEDTAVYYCATAEDEPGYDYWGQGT LVT VSS

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 35 KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSL
 SSVVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
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 LLES GGGGLVQPGGSLRLSCAASGFVFPWYDMGWVRQAPGKGLEWVSSIDWHGKITYYADSVK
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 KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSL
 SSVVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
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APSGSGVQLLES GGGGLVQPGGSLRLSCAASGFVFPWYDMGWVRQAPGKGLEWVSSIDWHGKI
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55

SEQ ID NO:11

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 KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
 5 SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
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 10 PGKGLEWVSSIDWHGKITYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCATAEDE
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SEQ ID NO:12

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 15 PSLKSRLTISKDTSRNQVVLMTNMDPVDATYYCARRETVFYWFYFDVWGRGTLVTVSSAST
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 SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
 PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
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 20 VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVCSVMHE
 ALHNHYTQKSLSLSPGK**PASPASGS**GVQLLES GGGGLVQPGGSLRLSCAASG FVFPWYDMGWV
 RQAPGKGLEWVSSIDWHGKITYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCATA
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SEQ ID NO:13

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 30 PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
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 35 ATAEDEPGYDYWGQGTLVTVSS

SEQ ID NO:14

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 40 KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
 SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
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 VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVCSVMHE
 45 ALHNHYTQKSLSLSPGK**GGGS**GVQLLES GGGGLVQPGGSLRLSCAASG FVFPWYDMGWVRQA
 PGKGLEWVSSIDWHGKITYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCATAEDE
 PGYDYWGQGTLVTVSS

SEQ ID NO:15

QVTLRESGPALVKPTQTLTLTCTFSGFSLSTSGMGVSWIRQPPGKGLEWLAHIYWDDDKRYN
 PSLKSRLTISKDTSRNQVVLMTNMDPVDATYYCARRETVFYWFYFDVWGRGTLVTVSSAST
 KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
 SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
 PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
 55 TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL

VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
 ALHNHYTQKSLSLSPGKGGGSGGGGSGVQLLESGGGLVQPGGSLRLSCAASGFVFPWYDMG
 WVRQAPGKGLEWVSSIDWHGKITYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCA
 TAEDEPGYDYWGQGT LVT VSS

5

SEQ ID NO:16

QVTLRESGPALVKPTQTLTLTCTFSGFSLSTSGMGVSWIRQPPGKGLEWLAHIYWDDDKRYN
 PSLKSRLTISKDTSRNQVVL TMTNMDPVD TATYYCARRET V FYWYFDVWGRGTLVT VSSAST
 KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSL
 10 SSVVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
 PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
 TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
 VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
 ALHNHYTQKSLSLSPGKGGGSGGGSGGGGSGVQLLESGGGLVQPGGSLRLSCAASGFVFP
 15 WYDMGWVRQAPGKGLEWVSSIDWHGKITYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTA
 VYYCATAEDEPGYDYWGQGT LVT VSS

SEQ ID NO:17

QVTLRESGPALVKPTQTLTLTCTFSGFSLSTSGMGVSWIRQPPGKGLEWLAHIYWDDDKRYN
 20 PSLKSRLTISKDTSRNQVVL TMTNMDPVD TATYYCARRET V FYWYFDVWGRGTLVT VSSAST
 KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSL
 SSVVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
 PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
 TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
 25 VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
 ALHNHYTQKSLSLSPGKPAVPPPGSGVQLLESGGGLVQPGGSLRLSCAASGFVFPWYDMGWV
 RQAPGKGLEWVSSIDWHGKITYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCATA
 EDEPGYDYWGQGT LVT VSS

SEQ ID NO:18

QVTLRESGPALVKPTQTLTLTCTFSGFSLSTSGMGVSWIRQPPGKGLEWLAHIYWDDDKRYN
 PSLKSRLTISKDTSRNQVVL TMTNMDPVD TATYYCARRET V FYWYFDVWGRGTLVT VSSAST
 KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSL
 SSVVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
 35 PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
 TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
 VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
 ALHNHYTQKSLSLSPGKPAVPPP PAVPPPGSGVQLLESGGGLVQPGGSLRLSCAASGFVFPW
 YDMGWVRQAPGKGLEWVSSIDWHGKITYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAV
 40 YYCATAEDEPGYDYWGQGT LVT VSS

SEQ ID NO:19

QVTLRESGPALVKPTQTLTLTCTFSGFSLSTSGMGVSWIRQPPGKGLEWLAHIYWDDDKRYN
 PSLKSRLTISKDTSRNQVVL TMTNMDPVD TATYYCARRET V FYWYFDVWGRGTLVT VSSAST
 45 KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSL
 SSVVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
 PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
 TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
 VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
 50 ALHNHYTQKSLSLSPGKPAVPPP PAVPPP PAVPPPGSGVQLLESGGGLVQPGGSLRLSCAAS
 GFVFPWYDMGWVRQAPGKGLEWVSSIDWHGKITYYADSVKGRFTISRDN SKNTLYLQMNSLR
 AEDTAVYYCATAEDEPGYDYWGQGT LVT VSS

SEQ ID NO:20

QVTLRESGPALVKPTQTLTLTCTFSGFSLSTSGMGVSWIRQPPGKGLEWLAHIYWDDDKRYN
 PSLKSRLTISKDTSRNQVVLMTNMDPVDATYYCARRETVFYWFYFDVWGRGTLVTVSSAST
 KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
 SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
 5 PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
 TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
 VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
 ALHNHYTQKSLSLSPGK**TVSDVPGS**GVQLLES GGGGLVQPGGSLRLSCAASGFVFPWYDMGWV
 RQAPGKGLEWVSSIDWHGKITYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCATA
 10 EDEPGYDYWGQGTTLVTVSS

SEQ ID NO:21

QVTLRESGPALVKPTQTLTLTCTFSGFSLSTSGMGVSWIRQPPGKGLEWLAHIYWDDDKRYN
 PSLKSRLTISKDTSRNQVVLMTNMDPVDATYYCARRETVFYWFYFDVWGRGTLVTVSSAST
 15 KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
 SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
 PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
 TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
 VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
 20 ALHNHYTQKSLSLSPGK**TVSDVPTVSDVPGS**GVQLLES GGGGLVQPGGSLRLSCAASGFVFPW
 YDMGWVRQAPGKGLEWVSSIDWHGKITYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAV
 YYCATAEDEPGYDYWGQGTTLVTVSS

SEQ ID NO:22

QVTLRESGPALVKPTQTLTLTCTFSGFSLSTSGMGVSWIRQPPGKGLEWLAHIYWDDDKRYN
 PSLKSRLTISKDTSRNQVVLMTNMDPVDATYYCARRETVFYWFYFDVWGRGTLVTVSSAST
 KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
 SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
 PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
 30 TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
 VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
 ALHNHYTQKSLSLSPGK**TVSDVPTVSDVPTVSDVPGS**GVQLLES GGGGLVQPGGSLRLSCAAS
 GFVFPWYDMGWVRQAPGKGLEWVSSIDWHGKITYYADSVKGRFTISRDN SKNTLYLQMNSLR
 AEDTAVYYCATAEDEPGYDYWGQGTTLVTVSS

35

SEQ ID NO:23

QVTLRESGPALVKPTQTLTLTCTFSGFSLSTSGMGVSWIRQPPGKGLEWLAHIYWDDDKRYN
 PSLKSRLTISKDTSRNQVVLMTNMDPVDATYYCARRETVFYWFYFDVWGRGTLVTVSSAST
 KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
 40 SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
 PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
 TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
 VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
 ALHNHYTQKSLSLSPGK**TGLDSPGS**GVQLLES GGGGLVQPGGSLRLSCAASGFVFPWYDMGWV
 45 RQAPGKGLEWVSSIDWHGKITYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCATA
 EDEPGYDYWGQGTTLVTVSS

SEQ ID NO:24

QVTLRESGPALVKPTQTLTLTCTFSGFSLSTSGMGVSWIRQPPGKGLEWLAHIYWDDDKRYN
 PSLKSRLTISKDTSRNQVVLMTNMDPVDATYYCARRETVFYWFYFDVWGRGTLVTVSSAST
 50 KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
 SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
 PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
 TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
 55 VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE

ALHNHYTQKSLSLSPGK**TGLDSPTGLDSPGS**GVQLLESGGGLVQPGGSLRLSCAASGFVFPW
YDMGWVRQAPGKGLEWVSSIDWHGKITYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAV
YYCATAEDEPGYDYWGQGT LVTVSS

5

SEQ ID NO:25

QVTLRSGPALVKPTQTLTLTCTFSGFSLSTSGMGVSWIRQPPGKGLEWLAHIYWDDDKRYN
PSLKSRLTISKDTSRNQVVLMTNMDPVDATYYCARRET VFYWYFDVWGRGTLVTVSSAST
KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
10 SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHE
ALHNHYTQKSLSLSPGK**TGLDSPTGLDSPTGLDSPGS**GVQLLESGGGLVQPGGSLRLSCAAS
15 GFVFPWYDMGWVRQAPGKGLEWVSSIDWHGKITYYADSVKGRFTISRDN SKNTLYLQMNSLR
AEDTAVYYCATAEDEPGYDYWGQGT LVTVSS

SEQ ID NO:26

DIQMTQSPSSLSASVGDRVTITCRASRPISDWLHWYQQKPGKAPKLLIAWASSLQGGVPSRF
20 SGSGSGTDFTLTITSSSLQPEDFATYYCLQEGWGPPTFGQG TKVEIKR

SEQ ID NO:27

DIVMTQSPSLPVTGPGEPAISCRSSQNIVHINGNTYLEWYLQKPGQSPRLLIYKISDRFSG
VPDRFSGSGSGTDFTLTKISRVEADDVGIYYCFQGSHVPWTFGQGTKLEIKRTVAAPSVFIFP
25 PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSTLT
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:28

QVQLVQSGAEVKKPGSSVKV SCKASGFYIKDTYMHWVRQAPGQGLEWMGTIDPANGNTKYVP
30 KFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARS IYDDYHYDDYYAMDYWGQGT LVTVS
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
35 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCS
VMHEALHNHYTQKSLSLSPGK

SEQ ID NO:29

QVQLVQSGAEVKKPGSSVKV SCKASGFYIKDTYMHWVRQAPGQGLEWMGTIDPANGNTKYVP
40 KFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARS IYDDYHYDDYYAMDYWGQGT LVTVS
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
45 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCS
VMHEALHNHYTQKSLSLSPGK**GS**DIQMTQSPSSLSASVGDRVTITCRASRPISDWLHWYQQK
PGKAPKLLIAWASSLQGGVPSRFSGSGSGTDFTLTITSSSLQPEDFATYYCLQEGWGPPTFGQG
TKVEIKR

SEQ ID NO:30

QVQLVQSGAEVKKPGSSVKV SCKASGFYIKDTYMHWVRQAPGQGLEWMGTIDPANGNTKYVP
KFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARS IYDDYHYDDYYAMDYWGQGT LVTVS
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
55 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV

VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
 VMHEALHNHYTQKSLSLSPGK**GSTVAAPSGS**DIQMTQSPSSLSASVGDRVTITCRASRPISD
 5 WLHWYQQKPGKAPKLLIAWASSLQGGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCLQEGW
 GPPTFGQGTKVEIKR

SEQ ID NO:31

QVQLVQSGAEVKKPGSSVKVCKASGFYIKDTYMHWRQAPGQGLEWMGTIDPANGNTKYVP
 10 KFQGRVTITADESTSTAYMELSSLRSEDYAVYYCARSYDDYHYDDYYAMDYWGQGLVTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
 LYSLSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
 15 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
 VMHEALHNHYTQKSLSLSPGK**GSTVAAPSGSTVAAPSGS**DIQMTQSPSSLSASVGDRVTITC
 RASRPISDWLHWYQQKPGKAPKLLIAWASSLQGGVPSRFSGSGSGTDFTLTISSLQPEDFAT
 YYCLQEGWGPPTFGQGTKVEIKR

SEQ ID NO:32

QVQLVQSGAEVKKPGSSVKVCKASGFYIKDTYMHWRQAPGQGLEWMGTIDPANGNTKYVP
 KFQGRVTITADESTSTAYMELSSLRSEDYAVYYCARSYDDYHYDDYYAMDYWGQGLVTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
 LYSLSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 25 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
 VMHEALHNHYTQKSLSLSPGK**GSTVAAPSGSTVAAPSGSTVAAPSGS**DIQMTQSPSSLSASV
 GDRVTITCRASRPISDWLHWYQQKPGKAPKLLIAWASSLQGGVPSRFSGSGSGTDFTLTISS
 30 LQPEDFATYYCLQEGWGPPTFGQGTKVEIKR

SEQ ID NO:33

QVQLVQSGAEVKKPGSSVKVCKASGFYIKDTYMHWRQAPGQGLEWMGTIDPANGNTKYVP
 KFQGRVTITADESTSTAYMELSSLRSEDYAVYYCARSYDDYHYDDYYAMDYWGQGLVTVS
 35 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
 LYSLSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
 40 VMHEALHNHYTQKSLSLSPGK**GSTVAAPSGSTVAAPSGSTVAAPSGSTVAAPSGS**DIQMTQS
 PSSLSASVGDRVTITCRASRPISDWLHWYQQKPGKAPKLLIAWASSLQGGVPSRFSGSGSGT
 DFTLTISSLQPEDFATYYCLQEGWGPPTFGQGTKVEIKR

SEQ ID NO:34

QVQLVQSGAEVKKPGSSVKVCKASGFYIKDTYMHWRQAPGQGLEWMGTIDPANGNTKYVP
 KFQGRVTITADESTSTAYMELSSLRSEDYAVYYCARSYDDYHYDDYYAMDYWGQGLVTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
 LYSLSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 50 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
 VMHEALHNHYTQKSLSLSPGK**GSTVAAPSGSTVAAPSGSTVAAPSGSTVAAPSGSTVAAPSG**
SDIQMTQSPSSLSASVGDRVTITCRASRPISDWLHWYQQKPGKAPKLLIAWASSLQGGVPSR
 FSGSGSGTDFTLTISSLQPEDFATYYCLQEGWGPPTFGQGTKVEIKR

55

SEQ ID NO:35

QVQLVQSGAEVKKPGSSVKVSCKASGFYIKDTYMHWVRQAPGQGLEWMGTIDPANGNTKYVP
 KFQGRVTITADESTSTAYMELSSLRSED TAVYYCARS IYDDYHYDDYYAMDYWGQGT LVTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSG
 5 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCS
 VMHEALHNHYTQKSLSLSPGK **GSTVAAPSGSTVAAPSGSTVAAPSGSTVAAPSGSTVAAPSG**
 10 **STVAAPSGS**DIQMTQSPSSLSASVGDRVTITCRASRPISDWLHWYQQKPGKAPKLLIAWASS
 LQGGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCLQEGWGPPTFGQGTKVEIKR

SEQ ID NO:36

QVQLVQSGAEVKKPGSSVKVSCKASGFYIKDTYMHWVRQAPGQGLEWMGTIDPANGNTKYVP
 15 KFQGRVTITADESTSTAYMELSSLRSED TAVYYCARS IYDDYHYDDYYAMDYWGQGT LVTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSG
 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
 20 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCS
 VMHEALHNHYTQKSLSLSPGK **PASGS**DIQMTQSPSSLSASVGDRVTITCRASRPISDWLHWY
 QQKPGKAPKLLIAWASSLQGGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCLQEGWGPPTF
 GQGTKVEIKR

SEQ ID NO:37

QVQLVQSGAEVKKPGSSVKVSCKASGFYIKDTYMHWVRQAPGQGLEWMGTIDPANGNTKYVP
 KFQGRVTITADESTSTAYMELSSLRSED TAVYYCARS IYDDYHYDDYYAMDYWGQGT LVTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSG
 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 30 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCS
 VMHEALHNHYTQKSLSLSPGK **PASPASGS**DIQMTQSPSSLSASVGDRVTITCRASRPISDWL
 HWYQQKPGKAPKLLIAWASSLQGGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCLQEGWGP
 35 PTFGQGTKVEIKR

SEQ ID NO:38

QVQLVQSGAEVKKPGSSVKVSCKASGFYIKDTYMHWVRQAPGQGLEWMGTIDPANGNTKYVP
 KFQGRVTITADESTSTAYMELSSLRSED TAVYYCARS IYDDYHYDDYYAMDYWGQGT LVTVS
 40 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSG
 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCS
 45 VMHEALHNHYTQKSLSLSPGK **PASPASPASGS**DIQMTQSPSSLSASVGDRVTITCRASRPIS
 DWLHWYQQKPGKAPKLLIAWASSLQGGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCLQEG
 WGPPTFGQGTKVEIKR

SEQ ID NO:39

QVQLVQSGAEVKKPGSSVKVSCKASGFYIKDTYMHWVRQAPGQGLEWMGTIDPANGNTKYVP
 KFQGRVTITADESTSTAYMELSSLRSED TAVYYCARS IYDDYHYDDYYAMDYWGQGT LVTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSG
 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 55 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS

LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
 VMHEALHNHYTQKSLSLSPGK**GGGGS**DIQMTQSPSSLSASVGDRVTITCRASRPISDWLHWY
 QQKPGKAPKLLIAWASSLQGGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCLQEGWGPPTF
 GQGTKVEIKR

5

SEQ ID NO:40

QVQLVQSGAEVKKPGSSVKVSCKASGFYIKDTYMHWVRQAPGQGLEWMGTIDPANGNTKYVP
 KFQGRVTITADESTSTAYMELSSLRSED TAVYYCARS IYDDYHYDDYYAMDYWGQGT LVTVS
 10 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
 15 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
 VMHEALHNHYTQKSLSLSPGK**GGGSGGGGS**DIQMTQSPSSLSASVGDRVTITCRASRPISD
 WLHWYQQKPGKAPKLLIAWASSLQGGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCLQEGW
 GPPTFGQGTKVEIKR

SEQ ID NO:41

QVQLVQSGAEVKKPGSSVKVSCKASGFYIKDTYMHWVRQAPGQGLEWMGTIDPANGNTKYVP
 KFQGRVTITADESTSTAYMELSSLRSED TAVYYCARS IYDDYHYDDYYAMDYWGQGT LVTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 25 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
 VMHEALHNHYTQKSLSLSPGK**GGGSGGGGS**DIQMTQSPSSLSASVGDRVTITCRAS
 RPISDWLHWYQQKPGKAPKLLIAWASSLQGGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
 LQEGWGPPTFGQGTKVEIKR

30

SEQ ID NO:42

QVQLVQSGAEVKKPGSSVKVSCKASGFYIKDTYMHWVRQAPGQGLEWMGTIDPANGNTKYVP
 KFQGRVTITADESTSTAYMELSSLRSED TAVYYCARS IYDDYHYDDYYAMDYWGQGT LVTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
 35 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
 VMHEALHNHYTQKSLSLSPGK**PAVPPPGS**DIQMTQSPSSLSASVGDRVTITCRASRPISDWL
 40 HWYQQKPGKAPKLLIAWASSLQGGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCLQEGWGP
 PTFGQGTKVEIKR

SEQ ID NO:43

QVQLVQSGAEVKKPGSSVKVSCKASGFYIKDTYMHWVRQAPGQGLEWMGTIDPANGNTKYVP
 KFQGRVTITADESTSTAYMELSSLRSED TAVYYCARS IYDDYHYDDYYAMDYWGQGT LVTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
 50 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
 VMHEALHNHYTQKSLSLSPGK**PAVPPPPAVPPPGS**DIQMTQSPSSLSASVGDRVTITCRASR
 PISDWLHWYQQKPGKAPKLLIAWASSLQGGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCL
 QEGWGPPTFGQGTKVEIKR

55

SEQ ID NO:44

QVQLVQSGAEVKKPGSSVKVSCKASGFYIKDTYMHWVRQAPGQGLEWMGTIDPANGNTKYVP
 KFQGRVTITADESTSTAYMELSSLRSED TAVYYCARS IYDDYHYDDYYAMDYWGQGT LVTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSG
 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 5 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCS
 VMHEALHNHYTQKSLSLSPGK **PAVPPPAVPPPAVPPPGS**DIQMTQSPSSLSASVGDRVTI
 TCRASRPISDWLHWYQQKPGKAPKLLIAWASSLQGGVPSRFSGSGSGTDFTLT ISSLQPEDF
 10 ATYYCLQEGWGPPTFGQGTKVEIKR

SEQ ID NO:45

QVQLVQSGAEVKKPGSSVKVSCKASGFYIKDTYMHWVRQAPGQGLEWMGTIDPANGNTKYVP
 KFQGRVTITADESTSTAYMELSSLRSED TAVYYCARS IYDDYHYDDYYAMDYWGQGT LVTVS
 15 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSG
 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCS
 20 VMHEALHNHYTQKSLSLSPGK **TVSDVPGS**DIQMTQSPSSLSASVGDRVTITCRASRPISDWL
 HWYQQKPGKAPKLLIAWASSLQGGVPSRFSGSGSGTDFTLT ISSLQPEDFATYYCLQEGWGP
 PTFGQGTKVEIKR

SEQ ID NO:46

QVQLVQSGAEVKKPGSSVKVSCKASGFYIKDTYMHWVRQAPGQGLEWMGTIDPANGNTKYVP
 KFQGRVTITADESTSTAYMELSSLRSED TAVYYCARS IYDDYHYDDYYAMDYWGQGT LVTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSG
 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 30 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCS
 VMHEALHNHYTQKSLSLSPGK **TVSDVPTVSDVPGS**DIQMTQSPSSLSASVGDRVTITCRASR
 PISDWLHWYQQKPGKAPKLLIAWASSLQGGVPSRFSGSGSGTDFTLT ISSLQPEDFATYYCL
 QEGWGPPTFGQGTKVEIKR

SEQ ID NO:47

QVQLVQSGAEVKKPGSSVKVSCKASGFYIKDTYMHWVRQAPGQGLEWMGTIDPANGNTKYVP
 KFQGRVTITADESTSTAYMELSSLRSED TAVYYCARS IYDDYHYDDYYAMDYWGQGT LVTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSG
 40 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCS
 VMHEALHNHYTQKSLSLSPGK **TVSDVPTVSDVPTVSDVPGS**DIQMTQSPSSLSASVGDRVTI
 45 TCRASRPISDWLHWYQQKPGKAPKLLIAWASSLQGGVPSRFSGSGSGTDFTLT ISSLQPEDF
 ATYYCLQEGWGPPTFGQGTKVEIKR

SEQ ID NO:48

QVQLVQSGAEVKKPGSSVKVSCKASGFYIKDTYMHWVRQAPGQGLEWMGTIDPANGNTKYVP
 KFQGRVTITADESTSTAYMELSSLRSED TAVYYCARS IYDDYHYDDYYAMDYWGQGT LVTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSG
 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
 55 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCS

VMHEALHNHYTQKSLSLSPGK**TGLDSPGS**DIQMTQSPSSLSASVGDRVITICRASRPISDWL
HWYQQKPGKAPKLLIAWASSLQGGVPSRFSGSGSGTDFTLTISLQPEDFATYYCLQEGWGP
PTFGQGTKVEIKR

5

SEQ ID NO:49

QVQLVQSGAEVKKPGSSVKVSCKASGFYIKDTYMHWVRQAPGQGLEWMGTIDPANGNTKYVP
KFQGRVTITADESTSTAYMELSSLRSED TAVYYCARS IYDDYHYDDYYAMDYWGQGT LVTVS
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSG
10 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPGK**TGLDSPTGLDSPGS**DIQMTQSPSSLSASVGDRVITICRASR
15 PISDWLHWYQQKPGKAPKLLIAWASSLQGGVPSRFSGSGSGTDFTLTISLQPEDFATYYCL
QEGWGPPTFGQGTKVEIKR

SEQ ID NO:50

QVQLVQSGAEVKKPGSSVKVSCKASGFYIKDTYMHWVRQAPGQGLEWMGTIDPANGNTKYVP
20 KFQGRVTITADESTSTAYMELSSLRSED TAVYYCARS IYDDYHYDDYYAMDYWGQGT LVTVS
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
25 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPGK**TGLDSPTGLDSPTGLDSPGS**DIQMTQSPSSLSASVGDRVIT
TCRASRPISDWLHWYQQKPGKAPKLLIAWASSLQGGVPSRFSGSGSGTDFTLTISLQPEDF
ATYYCLQEGWGPPTFGQGTKVEIKR

30 **SEQ ID NO:51**

GS

SEQ ID NO:52

GSTVAAPSGS

35

SEQ ID NO:53

GSTVAAPSGSTVAAPSGS

SEQ ID NO:54

40 GSTVAAPSGSTVAAPSGSTVAAPSGS

SEQ ID NO:55

GSTVAAPSGSTVAAPSGSTVAAPSGSTVAAPSGS

45 **SEQ ID NO:56**

GSTVAAPSGSTVAAPSGSTVAAPSGSTVAAPSGSTVAAPSGS

SEQ ID NO:57

GSTVAAPSGSTVAAPSGSTVAAPSGSTVAAPSGSTVAAPSGSTVAAPSGS

50

SEQ ID NO:58

PASGS

SEQ ID NO:59

55 PASPASGS

WO 2010/136483

PCT/EP2010/057231

SEQ ID NO:60
PASPASPASGS

5 **SEQ ID NO:61**
GGGGS

SEQ ID NO:62
GGGGSGGGGS

10 **SEQ ID NO:63**
GGGGSGGGGSGGGGS

SEQ ID NO:64
PAVPPPGS

15 **SEQ ID NO:65**
PAVPPPPAVPPPGS

SEQ ID NO:66
PAVPPPPAVPPPPAVPPPGS

20 **SEQ ID NO:67**
TVSDVPGS

25 **SEQ ID NO:68**
TVSDVPTVSDVPGS

SEQ ID NO:69
TVSDVPTVSDVPTVSDVPGS

30 **SEQ ID NO:70**
TGLDSPGS

SEQ ID NO:71
TGLDSPTGLDSPGS

35 **SEQ ID NO:72**
TGLDSPTGLDSPTGLDSPGS

40 **SEQ ID NO:73**
TVAAPSGS

SEQ ID NO:74
PAS

45 **SEQ ID NO:75**
TVAAPS

SEQ ID NO:76
PAVPPP

50 **SEQ ID NO:77**
TVSDVP

55

SEQ ID NO:78

TGLDSP

SEQ ID NO:79 = 586H-TVAAPS-154 (H chain)

5 QVQLVQSGAEVKKPGSSVKVSCKASGFYIKDTYMHWVRQAPGQGLEWMGTIDPANGNTKYVP
 KFQGRVTITADESTSTAYMELSSLRSED TAVYYCARS IYDDYHYDDYYAMDYWGQGT LVTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSG
 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 10 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
 VMHEALHNHYTQKSLSLSPGK **TVAAPS** GSDIQMTQSPSSLSASVGDRVTITCRASRPISDWL
 HWYQQKPGKAPKLLIAWASSLQGGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCLQEGWGP
 PTFGQGTKVEIKR
 15

SEQ ID NO: 80 = DOM9-155-25

DIQMTQSPSSLSASVGDRVTITCRASRPISDWLHWYQQKPGKAPKLLIAWASTLDSGVPSRF
 SGSGSGTDFTLTISSLQPEDFATYYCLQEGWGPPTFGQGTKVEIKR
 20

SEQ ID NO: 81 = DOM9-155-147

DIQMTQSPSSLSASVGDRVTITCRASRPISDWLHWYQQKPGKAPKLLIAWASSLYEGVPSRF
 SGSGSGTDFTLTISSLQPEDFATYYCLQEGWGPPTFGQGTKVEIKR

SEQ ID NO: 82 = DOM9-112-210

EVQLLES GGGLVQPGGSLRLSCAASGFTFRNFGMGWVRQAPGKGLEWVSWIISSGTETYYAD
 SVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCAKSLGRFDYWGQGT LVTVSS

SEQ ID NO: 83 = DOM10-53-474

30 GVQLLES GGGLVQPGGSLRLSCAASGFTFAWYDMGWVRQAPGKGLEWVSSIDWHGEVTTYAD
 SVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCATAEDEPGYDYWGQGT LVTVSS

SEQ ID NO: 84 (Example signal peptide sequence)

MGWSCIILFLVATATGVHS
 35

SEQ ID NO: 85 = 616-TVAAPS-Anti-human IL-4 mAb heavy chain

GVQLLES GGGLVQPGGSLRLSCAASGFVFPWYDMGWVRQAPGKGLEWVSSIDWHGKITYYAD
 SVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCATAEDEPGYDYWGQGT LVTVSSGSTVAA
 PSQVTLRESGPALVKPTQTLTLCTFSGFSLSTSGMGVSWIRQPPGKGLEWLAHIYWDDDKR
 40 YNPSLKSRLTISKDTSRNQVVLMTNMDPVD TATYYCARRET VFYWYFDVWGRGTLVTVSSA
 STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLY
 SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL
 FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS
 VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT
 45 CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM
 HEALHNHYTQKSLSLSPGK

SEQ ID NO: 86 = 616-ASTKG-Anti-human IL-4 mAb heavy chain

GVQLLES GGGLVQPGGSLRLSCAASGFVFPWYDMGWVRQAPGKGLEWVSSIDWHGKITYYAD

SVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCATAEDEPGYDYWGQGTLVTVSSGSASTK
GPSQVTLRESGPALVKPTQTLTLTCTFSGFSLSTSGMGVSWIRQPPGKGLEWLAHIYWDDDK
RYNPSLKSRLTISKDTSRNQVVLMTNMDPVDATYYCARRET VFYWYFDVWGRGTLVTVSS
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGL
5 YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF
LFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV
SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV
MHEALHNHYTQKSLSLSPGK

10 **SEQ ID NO: 87 = 616-GSTVAAPS-Anti-human IL-4 mAb Light chain**

GVQLLES GGGLVQPGGSLRLSCAASGFVFPWYDMGWVRQAPGKGLEWVSSIDWHGKITYYAD
SVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCATAEDEPGYDYWGQGTLVTVSSGSTVAA
PSDIVLTQSPSSLSASVGDRVITITCKASQSV DYGDSYMNWYQQKPGKAPKLLIYAASNLES
GIPSRFSGSGSGTDFTFTISSLQPED IATYYCQQSNEDPPTFGQGTKVEIKRTVAAPSVFIF
15 PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT
LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 88 = 616-GSASTKG- Anti-human IL-4 mAb Light chain

GVQLLES GGGLVQPGGSLRLSCAASGFVFPWYDMGWVRQAPGKGLEWVSSIDWHGKITYYAD
SVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCATAEDEPGYDYWGQGTLVTVSSGSASTK
20 GPSDIVLTQSPSSLSASVGDRVITITCKASQSV DYGDSYMNWYQQKPGKAPKLLIYAASNLE
SGIPSRFSGSGSGTDFTFTISSLQPED IATYYCQQSNEDPPTFGQGTKVEIKRTVAAPSVFI
FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL
TLISKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 89 = Anti-human IL-4 mAb Light chain-TVAAPS-616

DIVLTQSPSSLSASVGDRVITITCKASQSV DYGDSYMNWYQQKPGKAPKLLIYAASNLES
PSRFSGSGSGTDFTFTISSLQPED IATYYCQQSNEDPPTFGQGTKVEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
KADYEKHKVYACEVTHQGLSSPVTKSFNRGECTVAAPSGVQLLES GGGLVQPGGSLRLSCAA
SGFVFPWYDMGWVRQAPGKGLEWVSSIDWHGKITYYADSVKGRFTISRDN SKNTLYLQMNSL
30 RAEDTAVYYCATAEDEPGYDYWGQGTLVTVSS

SEQ ID NO: 90 = Anti-human IL-4 mAb heavy chain-TVAAPS-616 (Heavy chain)

QVTLRESGPALVKPTQTLTLTCTFSGFSLSTSGMGVSWIRQPPGKGLEWLAHIYWDDDKRYN
PSLKSRLTISKDTSRNQVVLMTNMDPVDATYYCARRET VFYWYFDVWGRGTLVTVSSAST
KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSL
35 SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
PKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPGK **TVAAPS** GVQLLES GGGLVQPGGSLRLSCAASGFVFPWYDMGWVRQ
40 APGKGLEWVSSIDWHGKITYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCATAED
EPGYDYWGQGTLVTVSS

SEQ ID NO: 91 = Anti-IL-5 mAb Heavy chain

QVTLRESGPALVKPTQTLTLTCTVSGFSLTSYSVHWVRQPPGKGLEWLGVIWASGGTDYNSA
LMSRLSISKDTSRNQVVLMTNMDPVDATYYCARDPPSSLLRLDYWGRGTLVTVSSASTKG

PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
 VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPK
 PKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV
 LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
 5 GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEAL
 HNHYTQKSLSLSPGK

SEQ ID NO: 92 = Anti-IL-5 mAb Light chain

DIVMTQSPDSLAVSLGERATINCKSSQSLNLSGNQKNYLAWYQQKPGQPPKLLIYGASTRES
 10 GVPDRFSGSGSGTDFTLTISLQAEDVAVYYCQNVHSFPFTFGGGTKLEIKRTVAAPSVFIF
 PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT
 LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 93

15 QVQLVQSGAEVKKPGSSVKVCKASGFYIKDTYMHWVRQAPGQGLEWMGTIDPANGNTKYVP
 KFQGRVTITADESTSTAYMELSSLRSEDVAVYYCARSYDDYHVDDYYAMDYWGQGTLLVTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 FLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 20 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFS
 VMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 94 (829H-(TVAAPS)₂GS-154 L89Q)

25 QVQLVQSGAEVKKPGSSVKVCKASGFYIKDTYMHWVRQAPGQGLEWMGTIDPANGNTKYVP
 KFQGRVTITADESTSTAYMELSSLRSEDVAVYYCARSYDDYHVDDYYAMDYWGQGTLLVTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 FLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 30 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFS
 VMHEALHNHYTQKSLSLSPGKTVAAPSTVAAPSGSDIQMTQSPSSLSASVGDRVTITCRASR
 PISDWLHWYQQKPGKAPKLLIAWASSLQGGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQ
 QEGWGPPTFGQGTKVEIKR
 35

SEQ ID NO: 95**829H-(TVAAPS)₃GS-154 L89Q**

40 QVQLVQSGAEVKKPGSSVKVCKASGFYIKDTYMHWVRQAPGQGLEWMGTIDPANGNTKYVP
 KFQGRVTITADESTSTAYMELSSLRSEDVAVYYCARSYDDYHVDDYYAMDYWGQGTLLVTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 FLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
 45 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFS
 VMHEALHNHYTQKSLSLSPGKTVAAPSTVAAPSTVAAPSGSDIQMTQSPSSLSASVGDRVTI
 TCRASRPISDWLHWYQQKPGKAPKLLIAWASSLQGGVPSRFSGSGSGTDFTLTISLQPEDF
 ATYYCQQEGWGPPTFGQGTKVEIKR

50 SEQ ID NO: 96**829H-(TVAAPS)₄GS-154 L89Q**

QVQLVQSGAEVKKPGSSVKV SCKASGFYIKDTYMHWVRQAPGQGLEWMGTIDPANGNTKYVP
 KFQGRVTITADESTSTAYMELSSLRSED TAVYYCARS IYDDYHVDDYYAMDYWGQGT LVTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSG
 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 5 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCS
 VMHEALHNHYTQKSLSLSPGKTVAAPSTVAAPSTVAAPSTVAAPSGSDIQMTQSPSSLSASV
 GDRVTITCRASRPISDWLHWYQQKPGKAPKLLIAWASSLQGGVPSRFSGSGSGTDFTLT ISS
 10 LQPEDFATYYCQQEGWGPPTFGQGTKVEIKR

SEQ ID NO:97

DIQMTQSPSSLSASVGDRVTITCRASQWIGPYLNWYQQKPGKAPKPLIYMGYWAPSGVPSRF
 SGSGSGTDFTLT ISSLQPEDFATYYCQQDLYPPTFGQGTKVEIKR

15

SEQ ID NO: 98

QVQLVQSGAEVKKPGSSVKV SCKASGFYIKDTYMHWVRQAPGQGLEWMGTIDPANGNTKYVP
 KFQGRVTITADESTSTAYMELSSLRSED TAVYYCARS IYDDYHVDDYYAMDYWGQGT LVTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSG
 20 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCS
 VMHEALHNHYTQKSLSLSPGKGSTVAAPSGSTVAAPSGSDIQMTQSPSSLSASVGDRVTITC
 25 RASRPISDWLHWYQQKPGKAPKLLIAWASSLQGGVPSRFSGSGSGTDFTLT ISSLQPEDFAT
 YYCQQEGWGPPTFGQGTKVEIKR

SEQ ID NO: 99

QVQLVQSGAEVKKPGSSVKV SCKASGFYIKDTYMHWVRQAPGQGLEWMGTIDPANGNTKYVP
 KFQGRVTITADESTSTAYMELSSLRSED TAVYYCARS IYDDYHVDDYYAMDYWGQGT LVTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSG
 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 35 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCS
 VMHEALHNHYTQKSLSLSPGKGSTVAAPSGSTVAAPSGSTVAAPSGSDIQMTQSPSSLSASV
 GDRVTITCRASRPISDWLHWYQQKPGKAPKLLIAWASSLQGGVPSRFSGSGSGTDFTLT ISS
 LQPEDFATYYCQQEGWGPPTFGQGTKVEIKR

40

SEQ ID NO: 100

QVQLVQSGAEVKKPGSSVKV SCKASGFYIKDTYMHWVRQAPGQGLEWMGTIDPANGNTKYVP
 KFQGRVTITADESTSTAYMELSSLRSED TAVYYCARS IYDDYHVDDYYAMDYWGQGT LVTVS
 SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSG
 45 LYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
 FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
 LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCS
 VMHEALHNHYTQKSLSLSPGKGSTVAAPSGSTVAAPSGSTVAAPSGSTVAAPSGSDIQMTQS
 50 PSSLSASVGDRVTITCRASRPISDWLHWYQQKPGKAPKLLIAWASSLQGGVPSRFSGSGSGT
 DFTLT ISSLQPEDFATYYCQQEGWGPPTFGQGTKVEIKR

SEQ ID NO:101

DIQMTQSPSSLSASVGDRVTITCRASRPISDWLHWYQQKPGKAPKLLIAWASSLQGGVPSRF
 SGSGSGTDFTLT ISSLQPEDFATYYCQQEGWGPPTFGQGTKVEIKR

55

SEQ ID NO:102

QQEGWGPPT

5 SEQ ID NO: 103 – polynucleotide sequence of BPC2626 heavy chain

CAGGTGACCCTGAGGGAGAGCGGCCCCGCCCTGGTGAAGCCCACCCAGACCCTGACCCTGAC
 CTGCACCTTCAGCGGCTTTAGCCTCAGCACCTCCGGCATGGGCGTGAGCTGGATCAGGCAGC
 CACCCGGCAAAGGCCTGGAGTGGCTGGCCACATCTACTGGGACGACGACAAGAGGTACAAC
 CCCAGCCTGAAGAGCCGGCTGACCATCAGCAAGGATACCAGCAGGAACCAGGTGGTGCTGAC
 10 CATGACCAACATGGACCCCGTGGACACCGCTACCTACTACTGCGCCAGGAGGGAGACCGTCT
 TCTACTGGTACTTTCGACGTGTGGGGAAGGGGCACACTAGTGACCGTGTCCAGCGCCAGCACC
 AAGGGCCCCAGCGTGTTCCCCCTGGCCCCCAGCAGCAAGAGCACCAGCGGCGGCACAGCCGC
 CCTGGGCTGCCTGGTGAAGGACTACTTCCCCGAACCGGTGACCGTGTCTTGGAACAGCGGAG
 CCCTGACCAGCGGCGTGCACACCTTCCCCGCCGTGCTGCAGAGCAGCGGCCTGTACAGCCTG
 15 AGCAGCGTGGTGACCGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATCTGTAACGTGAA
 CCACAAGCCCAGCAACACCAAGGTGGACAAGAAGGTGGAGCCCCAAGAGCTGTGACAAGACCC
 ACACCTGCCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGAGGCCCCAGCGTGTTCTGTTCCTGTTCCCC
 CCAAGCCTAAGGACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGGA
 TGTGAGCCACGAGGACCCTGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACA
 20 ATGCCAAGACCAAGCCCAGGGAGGAGCAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTG
 ACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAGTGTAAGGTGTCCAACAAGGC
 CCTGCCTGCCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAGCCCCAGG
 TGTACACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTG
 GTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAA
 25 CAACTACAAGACCACCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACAGCAAGC
 TGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTTACGTGCTCCGTGATGCACGAG
 GCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGACCGTGGCCGC
 CCCCTCGGGATCCGACATCCAGATGACTCAGAGCCCCAGCAGCCTGAGCGCCAGCGTGGGCG
 ATAGGGTGACCATTACCTGCAGGGCCAGCCAGAGCATCGACAGCTACCTGCACTGGTACCAG
 30 CAGAAGCCCGGAAAGGCCCCCCAAGCTCCTGATCTACAGCGCCAGCGAGCTGCAGAGCGGCGT
 GCCTAGCAGGTTTTCTGGCAGCGGCAGCGGCACCGACTTCACCCTGACCATCTCCAGCCTGC
 AGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGTCTGTGGAGGCCCTTCACCTTCGGC
 CAGGGCACCAAGGTGGAGATCAAGAGG

SEQ ID NO: 104 – protein sequence of BPC2626 heavy chain

35 QVTLRESGPALVKPTQTLTLTCTFSGFSLSTSGMGVSWIRQPPGKGLEWLAHIYWDDDKRYN
 PSLKSRLTISKDTSRNQVVLMTNMDPVDATYYCARRETVFYWYFDVWGRGTLVTVSSAST
 KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
 SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
 PKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
 40 TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
 VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMHE
 ALHNHYTQKSLSLSPGKTVAAPSGSDIQMTQSPSSLSASVGDRVTITCRASQSIDSYLHWYQ
 QKPGKAPKLLIYSASELQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQVWVRPFTFG
 QGTKVEIKR

45

SEQ ID NO: 105 – polynucleotide sequence of light chain

GACATCGTGCTGACCCAGAGCCCCTCTTCCCTGAGCGCAAGCGTGGGCGATAGGGTGACCAT
 CACCTGCAAGGCCAGCCAGAGCGTGGACTACGACGGCGACAGCTACATGAACTGGTACCAGC
 AGAAGCCCGGCAAGGCCCCCAAACCTGCTGATCTACGCCGCCAGCAACCTCGAGTCAGGCATT
 5 CCCAGCAGGTTTAGCGGCAGCGGCAGCGGCACCGACTTCACCTTCACAATCAGCAGCCTGCA
 GCCCGAGGACATCGCCACCTACTACTGCCAGCAGAGCAACGAGGACCCTCCCACCTTCGGAC
 AGGGCACCAAGGTCGAGATCAAGCGTACGGTGGCCGCCCCCAGCGTGTTTCATCTTCCCCCCC
 AGCGATGAGCAGCTGAAGAGCGGCACCGCCAGCGTGGTGTGTCTGCTGAACAACTTCTACCC
 CCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAATGCCCTGCAGAGCGGCAACAGCCAGGAGA
 10 GCGTGACCGAGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCACCCCTGACCCTGAGC
 AAGGCCGACTACGAGAAGCACAAAGGTGTACGCCTGTGAGGTGACCCACCAGGGCCTGTCCAG
 CCCCCTGACCAAGAGCTTCAACCGGGGCGAGTGC

SEQ ID NO: 106 – polynucleotide sequence of BPC2651 heavy chain

CAGGTGACCCTGAGGGAGAGCGGCCCCGCCCTGGTGAAGCCCACCCAGACCCTGACCCTGAC
 CTGCACCTTCAGCGGCTTTAGCCTCAGCACCTCCGGCATGGGCGTGAGCTGGATCAGGCAGC
 CACCCGGCAAAGGCCTGGAGTGGCTGGCCACATCTACTGGGACGACGACAAGAGGTACAAC
 CCCAGCCTGAAGAGCCGGCTGACCATCAGCAAGGATACCAGCAGGAACCAGGTGGTGCTGAC
 CATGACCAACATGGACCCCGTGGACACCGCTACCTACTACTGCGCCAGGAGGGAGACCGTCT
 20 TCTACTGGTACTTCGACGTGTGGGGAAGGGGCACACTAGTGACCGTGTCCAGCGCCAGCACC
 AAGGGCCCCAGCGTGTTCCCCCTGGCCCCCAGCAGCAAGAGCACCAGCGGCGGCACAGCCGC
 CCTGGGCTGCCTGGTGAAGGACTACTTCCCCGAACCGGTGACCGTGTCTTGAACAGCGGAG
 CCCTGACCAGCGGCGTGCACACCTTCCCCGCCGTGCTGCAGAGCAGCGGCCTGTACAGCCTG
 AGCAGCGTGGTGACCGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATCTGTAACGTGAA
 25 CCACAAGCCCAGCAACACCAAGGTGGACAAGAAGGTGGAGCCCAAGAGCTGTGACAAGACCC
 ACACCTGCCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGAGGGCCCCAGCGTGTTCCCTGTTCCCC
 CCAAGCCTAAGGACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGA
 TGTGAGCCACGAGGACCCTGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACA
 ATGCCAAGACCAAGCCCAGGGAGGAGCAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTG
 30 ACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAGTGTAAGGTGTCCAACAAGGC
 CCTGCCTGCCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAGCCCCAGG
 TGTACACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTG
 GTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAA
 CAACTACAAGACCACCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACAGCAAGC
 35 TGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTTCAGCTGCTCCGTGATGCACGAG
 GCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGGGATCTACCGT
 GGCAGCACCATCCGGATCTACCGTAGCAGCACCATCCGGATCCGACATCCAGATGACTCAGA
 GCCCCAGCAGCCTGAGCGCCAGCGTGGGCGATAGGGTGACCATTACCTGCAGGGCCAGCCAG
 AGCATCGACAGCTACCTGCACTGGTACCAGCAGAAGCCCGGAAAGGCCCCCAAGCTCCTGAT
 40 CTACAGCGCCAGCGAGCTGCAGAGCGGCGTGCCTAGCAGGTTTTTCTGGCAGCGGCAGCGGCA
 CCGACTTCACCCTGACCATCTCCAGCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAG
 CAGGTCGTGTGGAGGCCCTTCACCTTCGGCCAGGGCACCAAGGTGGAGATCAAGAGG

SEQ ID NO: 107 – protein sequence of BPC2651 heavy chain

45 QVTLRESGPALVKPTQTLTLTCTFSGFSLSTSGMGVSWIRQPPGKGLEWLAHIYWDDDKRYN
 PSLKSRLTISKDTSRNQVVLMTNMDPVDATYYCARRET V FYWYFDVWGRGTLVTVSSAST

KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
 SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
 PKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
 TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
 5 VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQVMHE
 ALHNHYTQKSLSLSPGKGSTVAAPSGSTVAAPSGSDIQMTQSPSSLSASVGDRVTITCRASQ
 SIDSYLHWYQQKPGKAPKLLIYSASELQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQ
 QVWVRPFTFGQGTKVEIKR

10 SEQ ID NO: 108 – polynucleotide sequence of BPC2652 heavy chain

CAGGTGACCCTGAGGGAGAGCGGCCCCGCCCTGGTGAAGCCCACCCAGACCCTGACCCTGAC
 CTGCACCTTCAGCGGCTTTAGCCTCAGCACCTCCGGCATGGGCGTGAGCTGGATCAGGCAGC
 CACCCGGCAAAGGCCTGGAGTGGCTGGCCCACATCTACTGGGACGACGACAAGAGGTACAAC
 CCCAGCCTGAAGAGCCGGCTGACCATCAGCAAGGATACCAGCAGGAACCAGGTGGTGCTGAC
 15 CATGACCAACATGGACCCCGTGGACACCGCTACCTACTACTGCGCCAGGAGGGAGACCGTCT
 TCTACTGGTACTTCGACGTGTGGGGAAGGGGCACACTAGTGACCGTGTCCAGCGCCAGCACC
 AAGGGCCCCAGCGTGTTCCTTGGCCCCCAGCAGCAAGAGCACCAGCGGCGGCACAGCCGC
 CCTGGGCTGCCTGGTGAAGGACTACTTCCCCGAACCGGTGACCGTGTCTTGAACAGCGGAG
 CCCTGACCAGCGGCGTGCACACCTTCCCCGCCGTGCTGCAGAGCAGCGGCCCTGTACAGCCTG
 20 AGCAGCGTGGTGACCGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATCTGTAACGTGAA
 CCACAAGCCCAGCAACACCAAGGTGGACAAGAAGGTGGAGCCCAAGAGCTGTGACAAGACCC
 ACACCTGCCCCCTGCCCCTGCCCCCGAGCTGCTGGGAGGCCCCAGCGTGTTCCTGTTCCCC
 CCCAAGCCTAAGGACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGGA
 TGTGAGCCACGAGGACCCTGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACA
 25 ATGCCAAGACCAAGCCCAGGGAGGAGCAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTG
 ACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAGTGTAAAGTGTCCAACAAGGC
 CCTGCCTGCCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAGCCCCAGG
 TGTACACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTG
 GTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAA
 30 CAACTACAAGACCACCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACAGCAAGC
 TGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTTCAGCTGCTCCGTGATGCACGAG
 GCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGGGATCTACCGT
 GGCAGCACCATCAGGATCTACCGTGGCAGCACCATCAGGTTCAACAGTAGCTGCTCCTTCTG
 GATCCGACATCCAGATGACTCAGAGCCCCAGCAGCCTGAGCGCCAGCGTGGGCGATAGGGTG
 35 ACCATTACCTGCAGGGCCAGCCAGAGCATCGACAGCTACCTGCACTGGTACCAGCAGAAGCC
 CGGAAAGGCCCCCAAGCTCCTGATCTACAGCGCCAGCGAGCTGCAGAGCGGCGTGCCTAGCA
 GGTTTTCTGGCAGCGGCAGCGGCACCGACTTCACCCTGACCATCTCCAGCCTGCAGCCCGAG
 GACTTCGCCACCTACTACTGCCAGCAGGTCGTGTGGAGGCCCTTCACCTTCGGCCAGGGCAC
 CAAGGTGGAGATCAAGAGG

40

SEQ ID NO: 109 – protein sequence of BPC2652 heavy chain

QVTLRSGPALVKPTQTLTLTCTFSGFSLSTSGMGVSWIRQPPGKGLEWLAHIYWDDDKRYN
 PSLKSRLTISKDTSRNQVVLMTNMDPVDATYYCARRETVFYWFYFDVWGRGTLVTVSSAST
 45 KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
 SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
 PKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
 TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
 VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQVMHE
 50 ALHNHYTQKSLSLSPGKGSTVAAPSGSTVAAPSGSTVAAPSGSDIQMTQSPSSLSASVGDRV
 TITCRASQSIDSYLHWYQQKPGKAPKLLIYSASELQSGVPSRFSGSGSGTDFTLTISLQPE
 DFATYYCQQVWVRPFTFGQGTKVEIKR

SEQ ID NO: 110 – polynucleotide sequence of BPC2653 heavy chain

CAGGTGACCCTGAGGGAGAGCGGCCCCGCCCTGGTGAAGCCCACCCAGACCCTGACCCTGAC
 CTGCACCTTCAGCGGCTTTAGCCTCAGCACCTCCGGCATGGGCGTGAGCTGGATCAGGCAGC
 CACCCGGCAAAGGCCTGGAGTGGCTGGCCACATCTACTGGGACGACGACAAGAGGTACAAC
 5 CCCAGCCTGAAGAGCCGGCTGACCATCAGCAAGGATACCAGCAGGAACCAGGTGGTGCTGAC
 CATGACCAACATGGACCCCGTGGACACCGCTACCTACTACTGCGCCAGGAGGGAGACCGTCT
 TCTACTGGTACTTCGACGTGTGGGGAAGGGGCACACTAGTGACCGTGTCCAGCGCCAGCACC
 AAGGGCCCCAGCGTGTTCCCCCTGGCCCCCAGCAGCAAGAGCACCAGCGGCGGCACAGCCGC
 CCTGGGCTGCCTGGTGAAGGACTACTTCCCCGAACCGGTGACCGTGTCTTGGAACAGCGGAG
 10 CCCTGACCAGCGGCGTGCACACCTTCCCCGCCGTGCTGCAGAGCAGCGGCCTGTACAGCCTG
 AGCAGCGTGGTGACCGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATCTGTAACGTGAA
 CCACAAGCCCAGCAACACCAAGGTGGACAAGAAGGTGGAGCCCAAGAGCTGTGACAAGACCC
 ACACCTGCCCCCCCCCTGCCCTGCCCCCGAGCTGCTGGGAGGCCCCAGCGTGTTCTCTGTTCCCC
 CCCAAGCCTAAGGACACCCTGATGATCAGCAGAACCCCCGAGGTGACCTGTGTGGTGGTGA
 15 TGTGAGCCACGAGGACCCTGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACA
 ATGCCAAGACCAAGCCCAGGGAGGAGCAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTG
 ACCGTGCTGCACCAGGATTGGCTGAACGGCAAGGAGTACAAGTGTAAAGTGTCCAACAAGGC
 CCTGCCTGCCCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAGCCCCAGG
 TGTACACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTG
 20 GTGAAGGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAA
 CAACTACAAGACCACCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCCTGTACAGCAAGC
 TGACCGTGGACAAGAGCAGATGGCAGCAGGGCAACGTGTTTACGTGCTCCGTGATGCACGAG
 GCCCTGCACAATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAGGGATCTACCGT
 GGCAGCACCATCAGGATCTACCGTGGCAGCACCATCAGGTTCAACAGTAGCTGCTCCTTCTG
 25 GTTCAACAGTAGCTGCTCCTTCTGGATCCGACATCCAGATGACTCAGAGCCCCAGCAGCCTG
 AGCGCCAGCGTGGGCGATAGGGTGACCATTAACCTGCAGGGCCAGCCAGAGCATCGACAGCTA
 CCTGCACTGGTACCAGCAGAAGCCCGGAAAGGCCCCCAAGCTCCTGATCTACAGCGCCAGCG
 AGCTGCAGAGCGGCGTGCCTAGCAGGTTTTCTGGCAGCGGCAGCGGCACCGACTTCACCCTG
 ACCATCTCCAGCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGGTCGTGTGGAG
 30 GCCCTTCACCTTCGGCCAGGGCACCAAGGTGGAGATCAAGAGG

SEQ ID NO: 111– protein sequence of BPC2653 heavy chain

QVTLRESGPALVKPTQTLTLTCTFSGFSLSTSGMGVSWIRQPPGKGLEWLAHIYWDDDKRYN
 35 PSLKSRLTISKDTSRNQVVLMTNMDPVDATYYCARRETVFYWYFDVWGRGTLVTVSSAST
 KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
 SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
 PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
 TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
 40 VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHE
 ALHNHYTQKSLSLSPGKGSTVAAPSGSTVAAPSGSTVAAPSGSTVAAPSGSDIQMTQSPSSL
 SASVGDRVTITCRASQSIDSYLHWYQQKPGKAPKLLIYSASELQSGVPSRFSGSGSGTDFTL
 TISSLQPEDFATYYCQQVWRPFTFGQGTKVEIKR

45 **SEQ ID NO:112**
 TVAAPSTVAAPSGS

SEQ ID NO:113
 TVAAPSTVAAPSTVAAPSGS

50 **SEQ ID NO:114**
 TVAAPSTVAAPSTVAAPSTVAAPSGS

Claims

1. An antigen-binding protein comprising a protein scaffold which is linked to one or more epitope-binding domains wherein the antigen-binding protein has at least two antigen binding sites at least one of which is from an epitope binding domain and at least one of which is from a paired VH/VL domain wherein the epitope binding domain is linked to the protein scaffold by a peptide linker which comprises between 10 and 50 amino acids
2. An antigen-binding protein comprising a protein scaffold which is linked to one or more epitope-binding domains wherein the antigen-binding protein has at least two antigen binding sites at least one of which is from an epitope binding domain and at least one of which is from a paired VH/VL domain wherein the epitope binding domain is linked to the protein scaffold by a linker selected from those set out in SEQ ID NO: 53 to 60, SEQ ID NO:62-72, SEQ ID NO:74, SEQ ID NO:76-78, SEQ ID NO:112-114, or combinations or multiples of such linkers.
3. The antigen-binding protein according to claim 1 or 2 wherein the linker is selected from $(PAS)_n(GS)_m$, $(GGGGS)_p(GS)_m$, $(TVAAPS)_p(GS)_m$, $(GS)_m(TVAAPSGS)_p$, $(PAVPPP)_n(GS)_m$, $(TVSDVP)_n(GS)_m$, and $(TGLDSP)_n(GS)_m$, wherein $n = 1-10$, and $m = 0-4$, and $p=2-10$.
4. The antigen-binding protein according to claim 3 wherein the linker is selected from $(PAS)_n(GS)_m$ wherein $n = 1-10$, and $m = 0-4$.
5. The antigen-binding protein according to claim 4 wherein the linker is selected from $(TVAAPS)_p(GS)_m$ and $(GS)_m$ wherein $m = 0-10$, and $p=2-10$.
6. The antigen-binding protein according to any preceding claim wherein at least one epitope binding domain is an immunoglobulin single variable domain.
7. The antigen-binding protein according to claim 6 wherein the immunoglobulin single variable domain is a human dAb.
8. The antigen-binding protein according to claim 6 wherein the immunoglobulin single variable domain is a camelid dAb (nanobody) or a shark dAb (NARV).
9. The antigen-binding protein according to claim any one of claims 1 to 5 wherein at least one epitope binding domain is derived from a scaffold selected from CTLA-4 (Evibody); lipocalin; Protein A derived molecules such as Z-domain of Protein A (Affibody, SpA), A-domain (Avimer/Maxibody); Heat shock proteins such as GroEI and GroES; transferrin (trans-body); ankyrin repeat protein (DARPin); peptide aptamer; C-type lectin domain (Tetranectin); human γ -crystallin and human ubiquitin (affilins); PDZ domains; scorpion toxinkunitz type domains of human protease inhibitors; and fibronectin (adnectin).
10. The antigen-binding protein of any preceding claim wherein the antigen binding protein has specificity for more than one antigen.
11. The antigen-binding protein according to any preceding claim wherein the first binding site has specificity for a first epitope on an antigen and the second binding site has specificity for a second epitope on the same antigen.

12. The antigen-binding protein according to any preceding claim wherein the antigen-binding protein is capable of binding IL-13.
- 5 13. The antigen-binding protein according to any preceding claim wherein the antigen-binding protein is capable of binding two or more antigens selected from IL-13, IL-5, and IL-4.
- 10 14. The antigen-binding protein according to any preceding claim wherein the antigen-binding protein is capable of binding two or more antigens selected from VEGF, IGF-1R and EGFR,
- 15 15. The antigen-binding protein according to any preceding claim wherein the antigen-binding protein is capable of binding TNF α .
16. The antigen-binding protein according to claim 15 wherein the antigen-binding protein is capable of binding to TNF α and IL1-R.
- 20 17. The antigen-binding protein according to any preceding claim wherein the protein scaffold is an Ig scaffold.
18. The antigen-binding protein according to claim 17 wherein the Ig scaffold is an IgG scaffold.
- 25 19. The antigen-binding protein according to claim 18 wherein the IgG scaffold is selected from IgG1, IgG2, IgG3 and IgG4.
- 30 20. The antigen-binding protein according to any preceding claim wherein the protein scaffold comprises a monovalent antibody.
21. The antigen-binding protein according to any one of claims 1 to 19 wherein the IgG scaffold comprises all the domains of an antibody.
- 35 22. The antigen-binding protein according to any preceding claim which comprises four domain antibodies.
23. The antigen-binding protein according to any preceding claim wherein at least two of the antigen binding sites have specificity for the same antigen.
- 40 24. The antigen-binding protein according to any preceding claim wherein at least one of the epitope binding domains binds human serum albumin.
- 45 25. The antigen-binding protein according to any preceding claim comprising an epitope binding domain attached to the Ig scaffold at the N-terminus of the light chain.
- 50 26. The antigen-binding protein according to any one of claims 1 to 24 comprising an epitope binding domain attached to the Ig scaffold at the N-terminus of the heavy chain.
27. The antigen-binding protein according to any one of claims 1 to 24 comprising an epitope binding domain attached to the Ig scaffold at the C-terminus of the light chain.

28. The antigen-binding protein according to any one of claims 1 to 24 comprising an epitope binding domain attached to the Ig scaffold at the C-terminus of the heavy chain.
- 5 29. The antigen-binding protein according to any preceding claim for use in medicine.
- 10 30. The antigen-binding protein according to any preceding claim for the treatment of cancer or inflammatory diseases such as asthma, rheumatoid arthritis or osteoarthritis.
31. A polynucleotide sequence encoding a heavy chain of an antigen binding protein according to any one of claims 1 to 28.
- 15 32. A polynucleotide encoding a light chain of an antigen binding protein according to any one of claims 1 to 28.
- 20 33. A recombinant transformed or transfected host cell comprising one or more polynucleotide sequences encoding a heavy chain and a light chain of an antigen binding protein of any preceding claim.
- 25 34. A method for the production of an antigen binding protein according to claims 1 to 28 which method comprises the step of culturing a host cell of claim 33 and isolating the antigen binding protein.
- 30 35. A pharmaceutical composition comprising an antigen binding protein of any one of claims 1 to 28 and a pharmaceutically acceptable carrier.
- 35 36. A method for improving the potency of an epitope binding domain when attached to a protein scaffold, which epitope binding domain has reduced potency when attached directly to a protein scaffold without a linker, compared with its potency as a naked epitope binding domain, comprising the step of adding a peptide linker between the protein scaffold and the epitope binding domain wherein the peptide linker comprises at least 10 amino acids
37. A method according to claim 36 wherein the linker comprises between 10 amino acids and 50 amino acids.

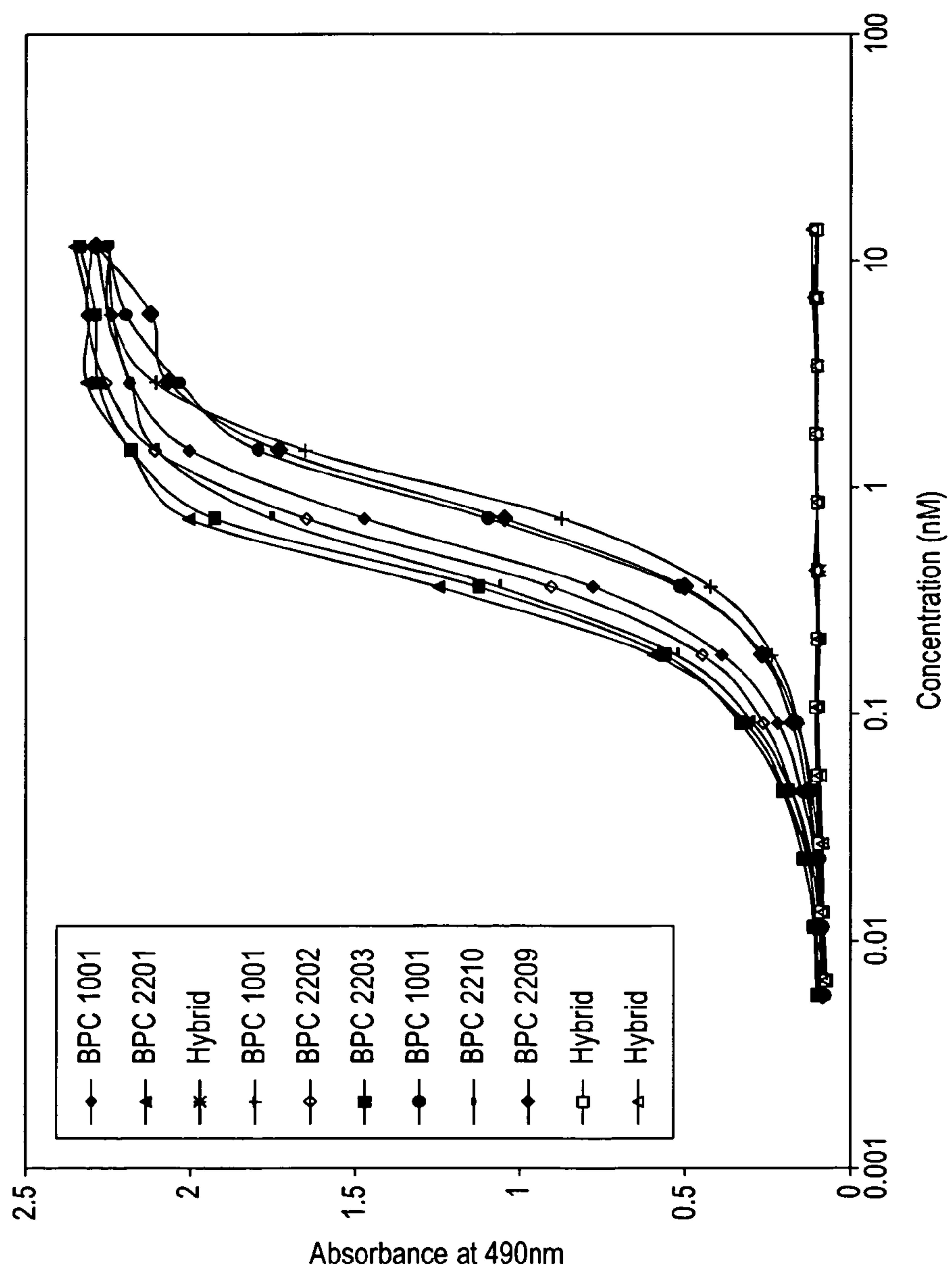


FIG. 1

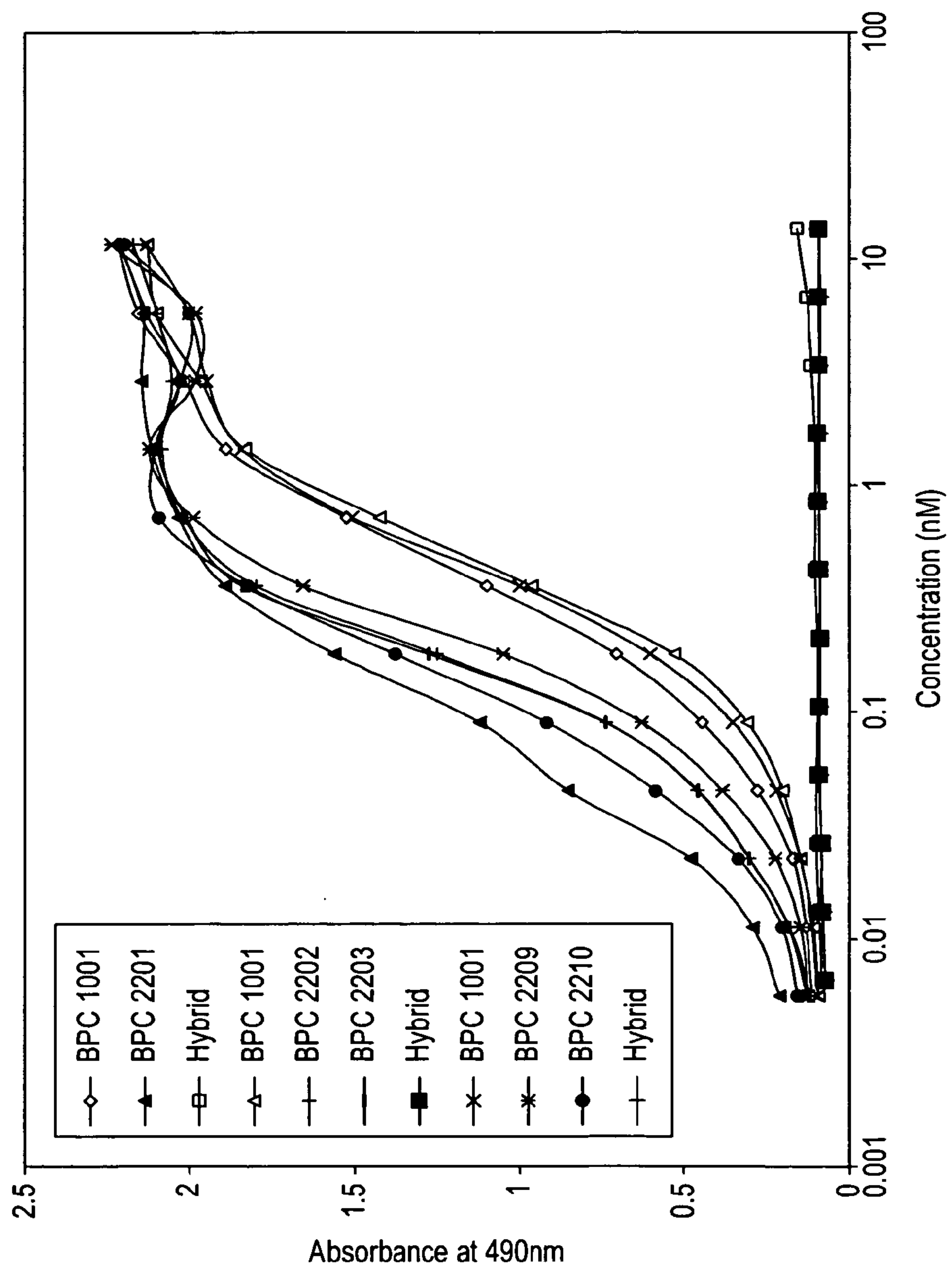


FIG. 2

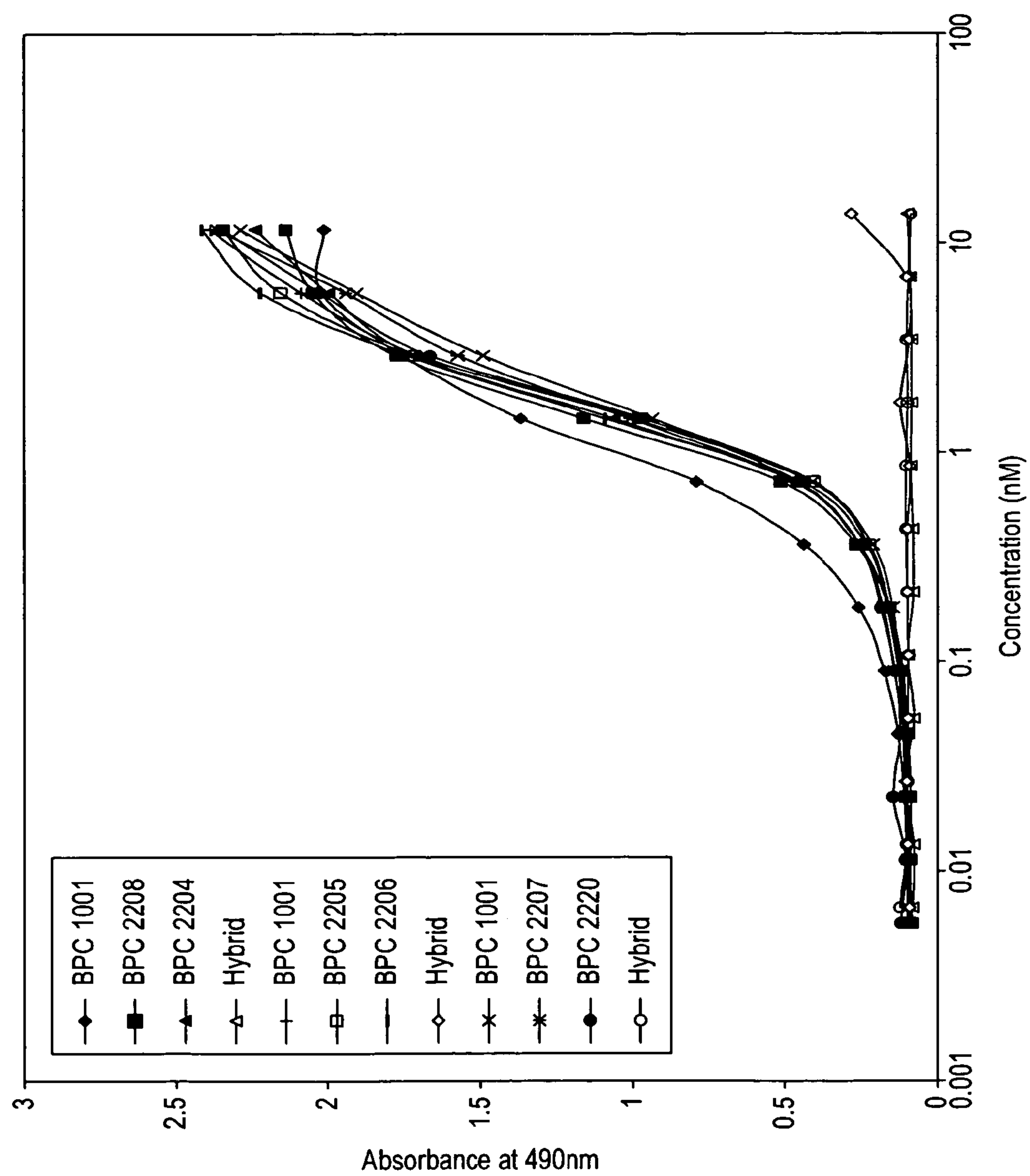


FIG. 3

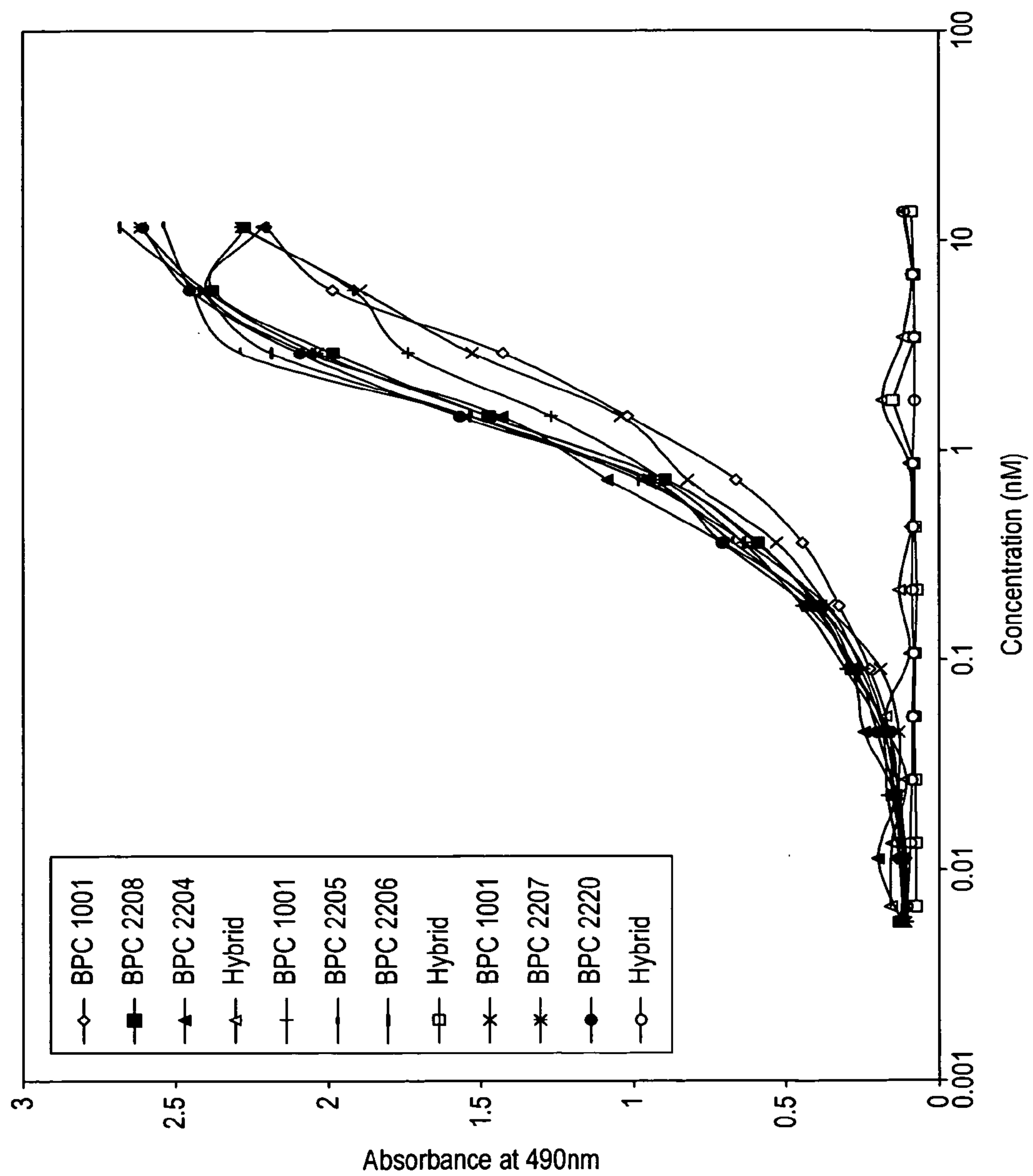


FIG. 4

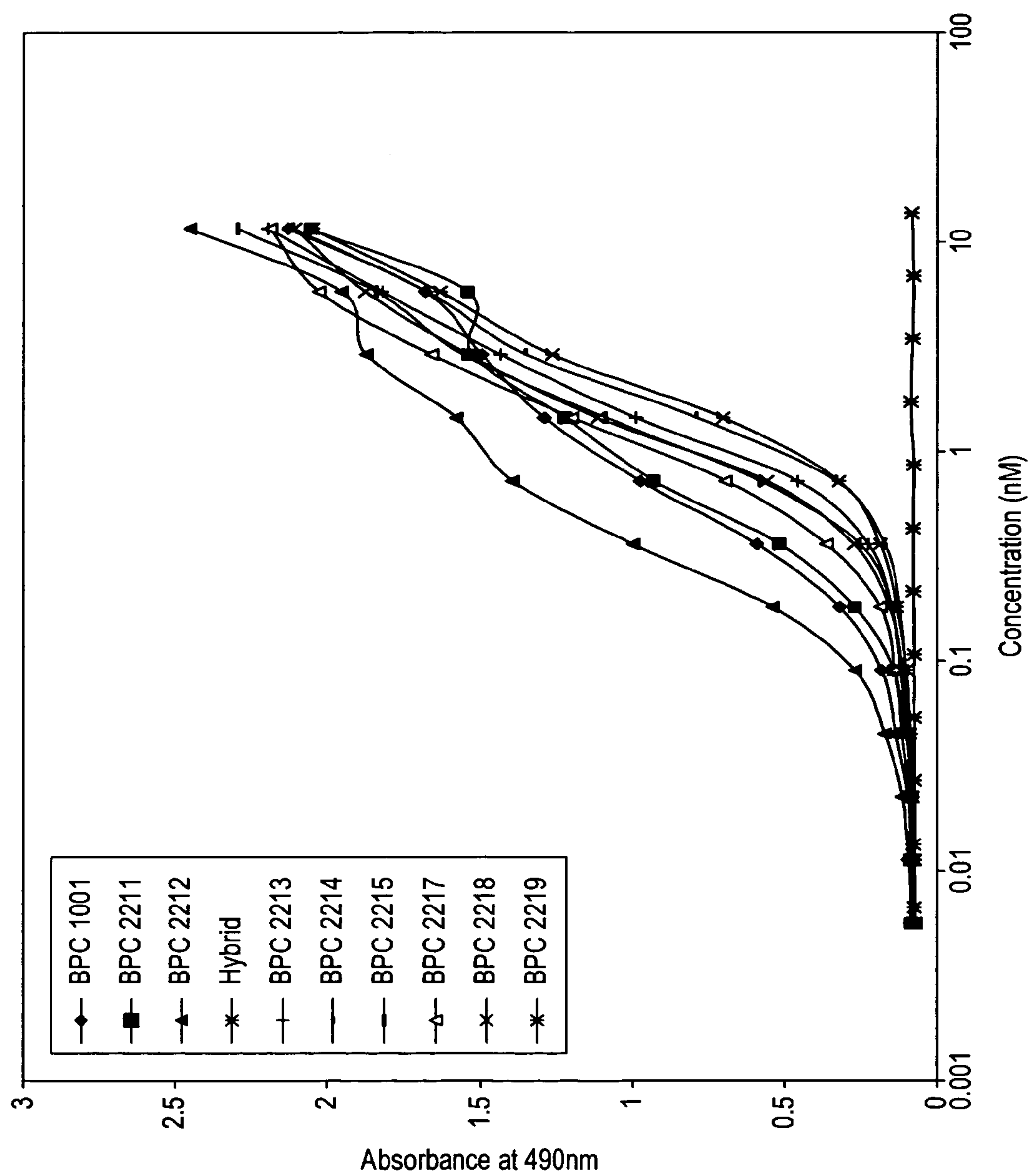


FIG. 5

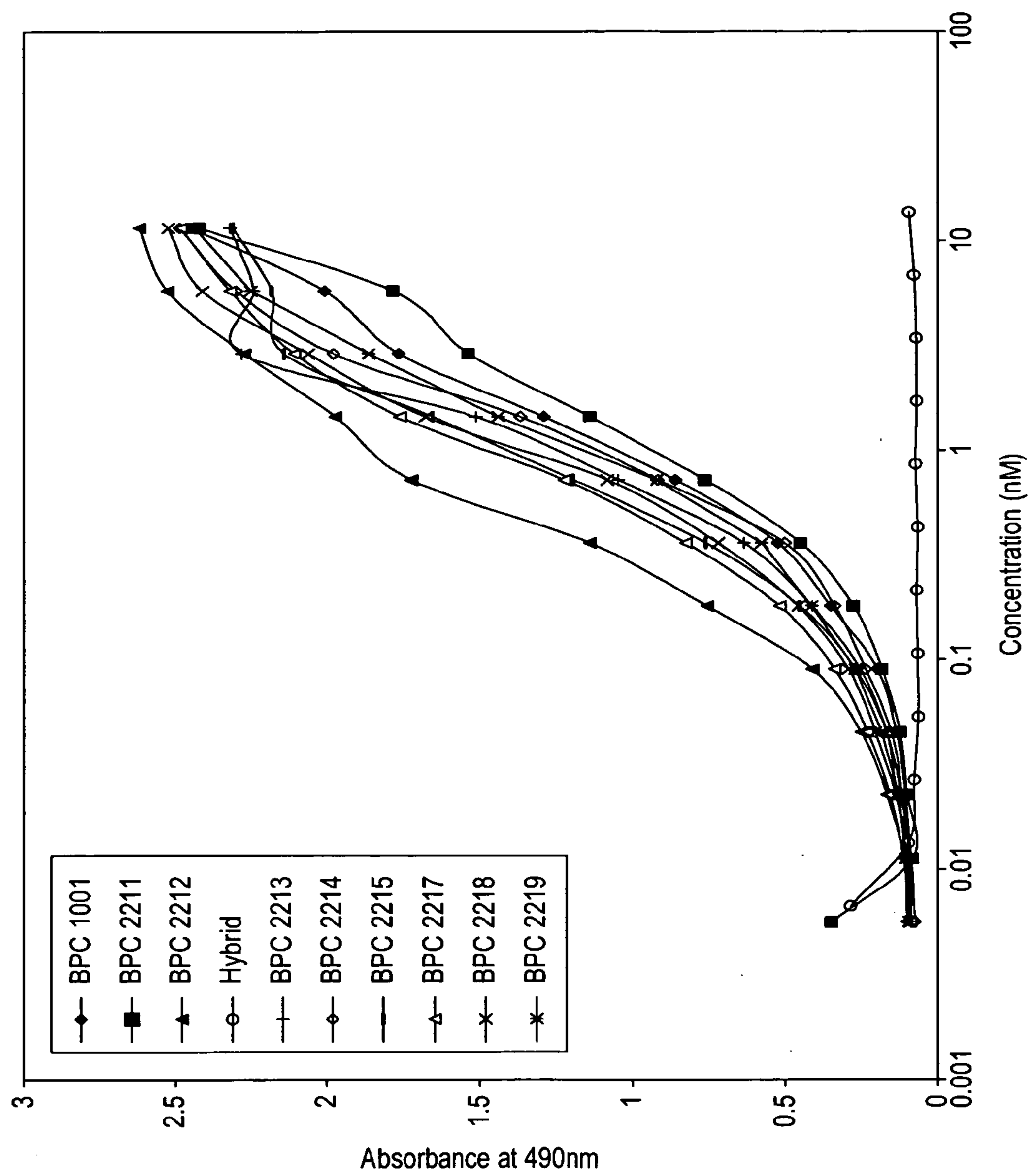


FIG. 6

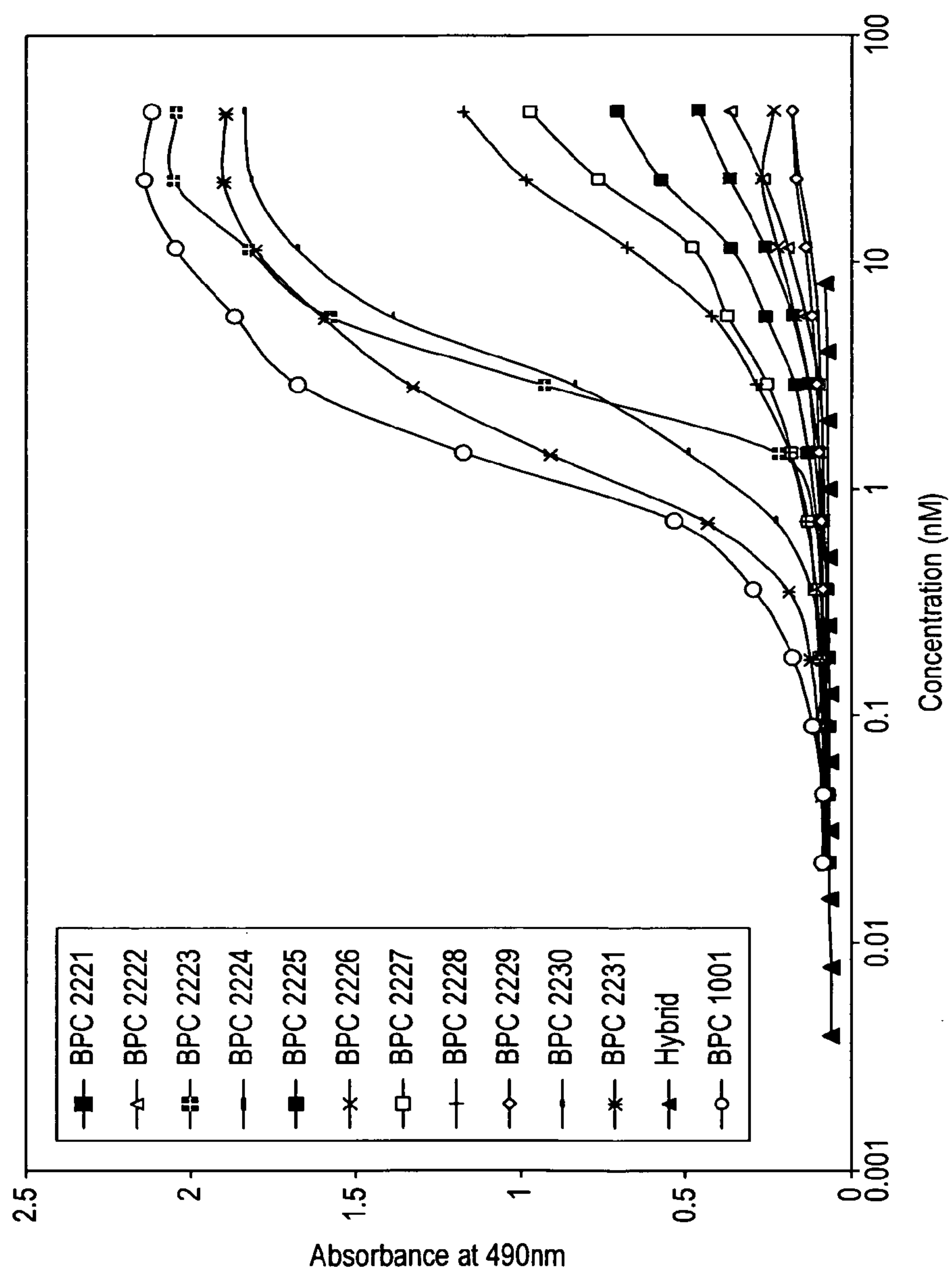


FIG. 7

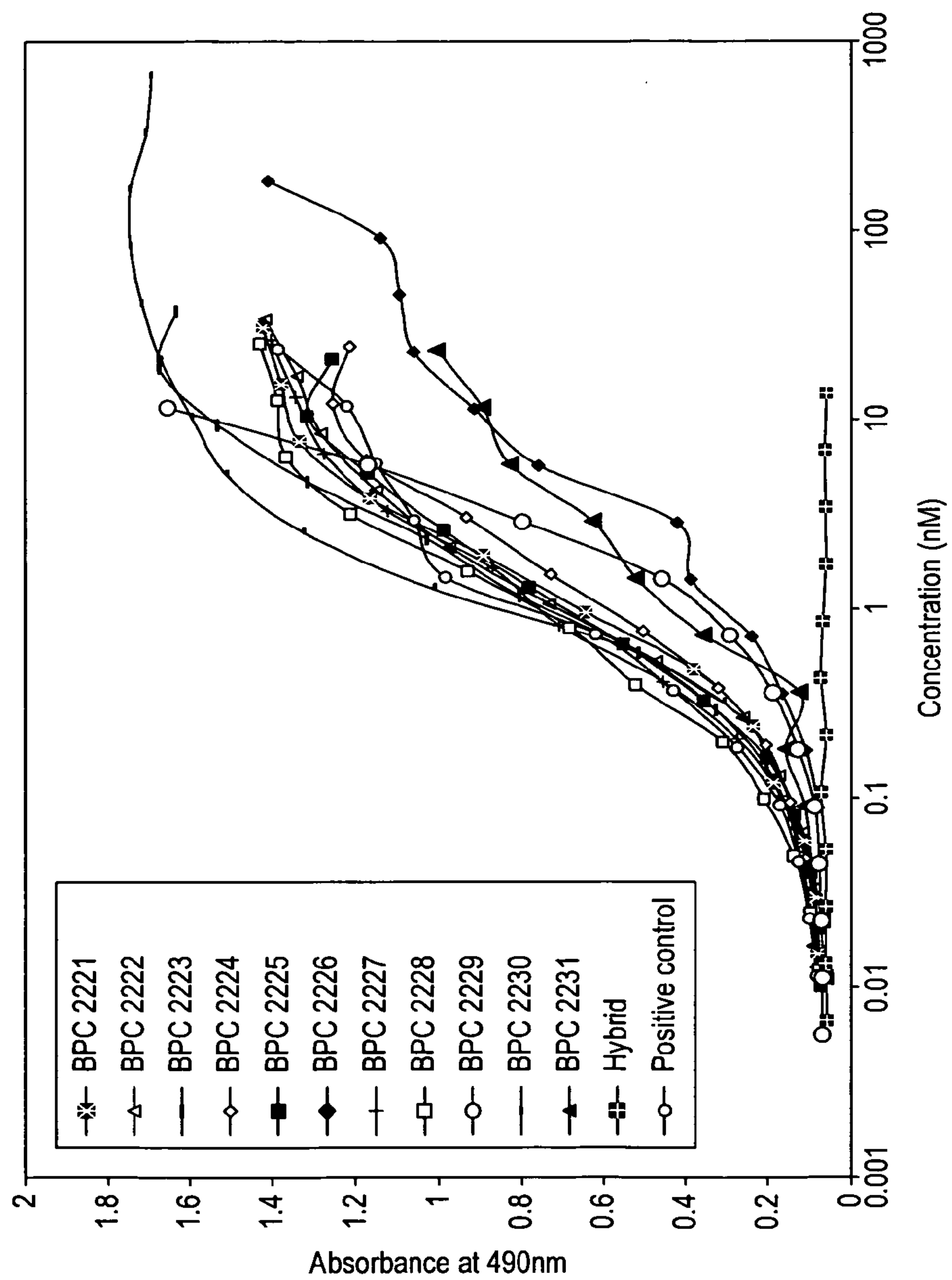


FIG. 8

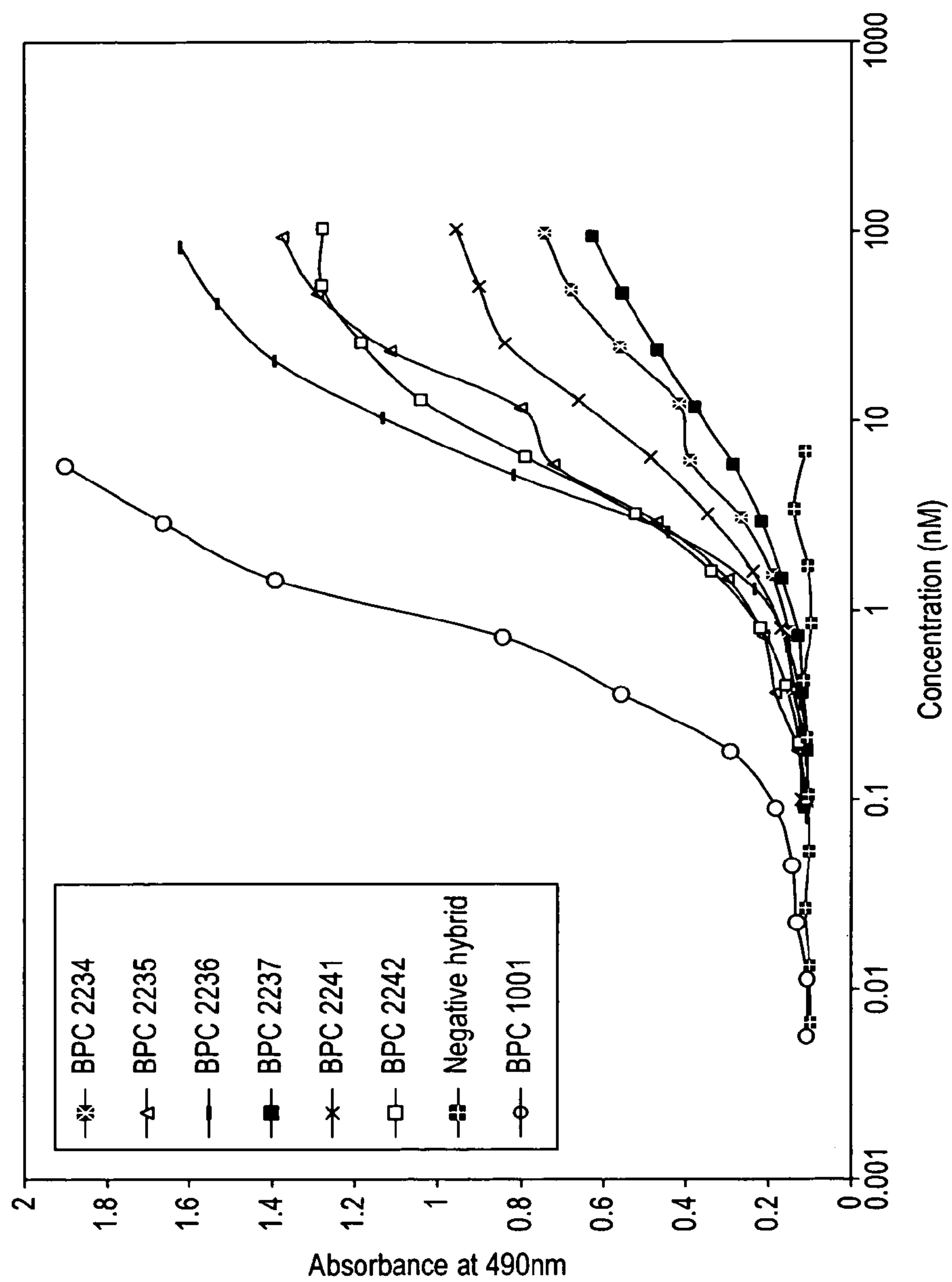


FIG. 9

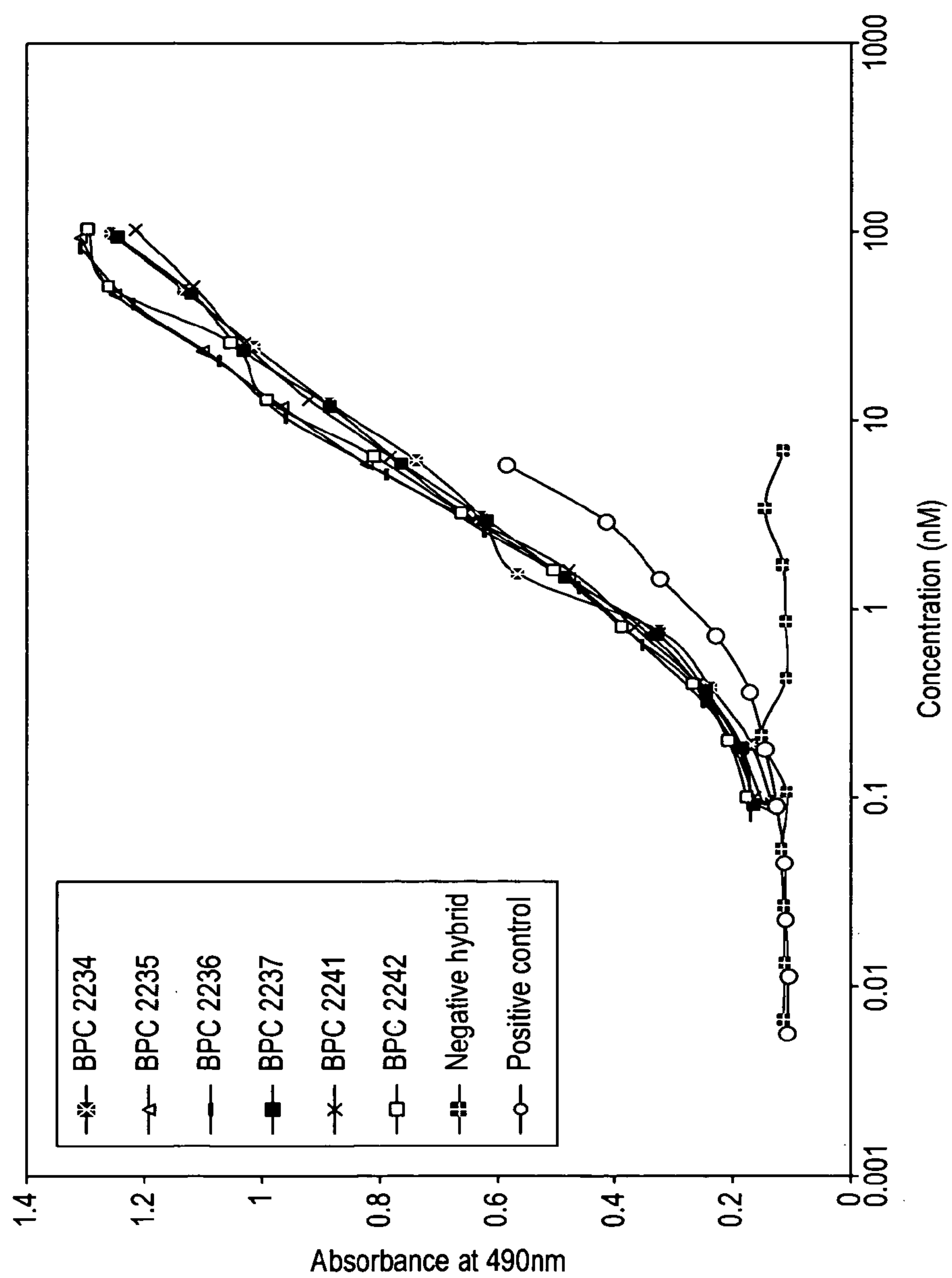


FIG. 10

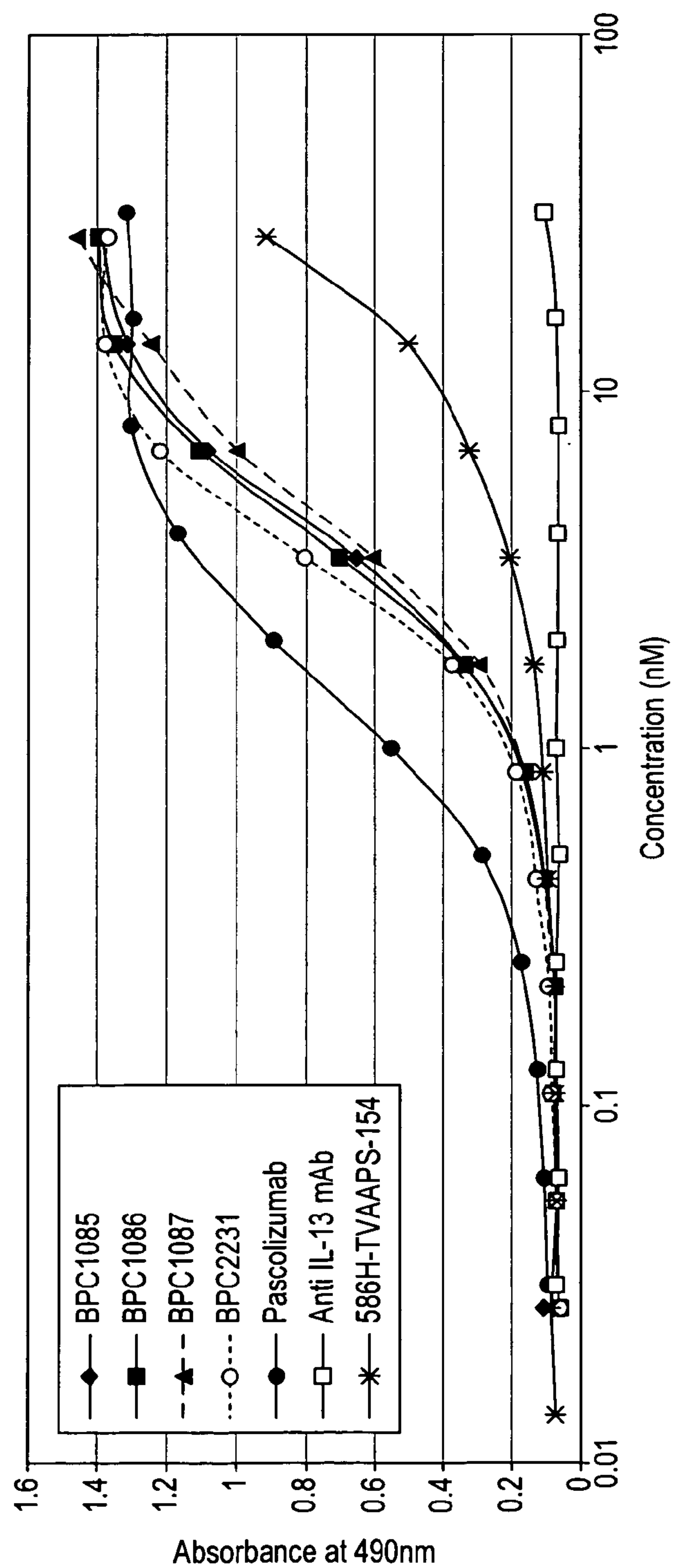


FIG. 11

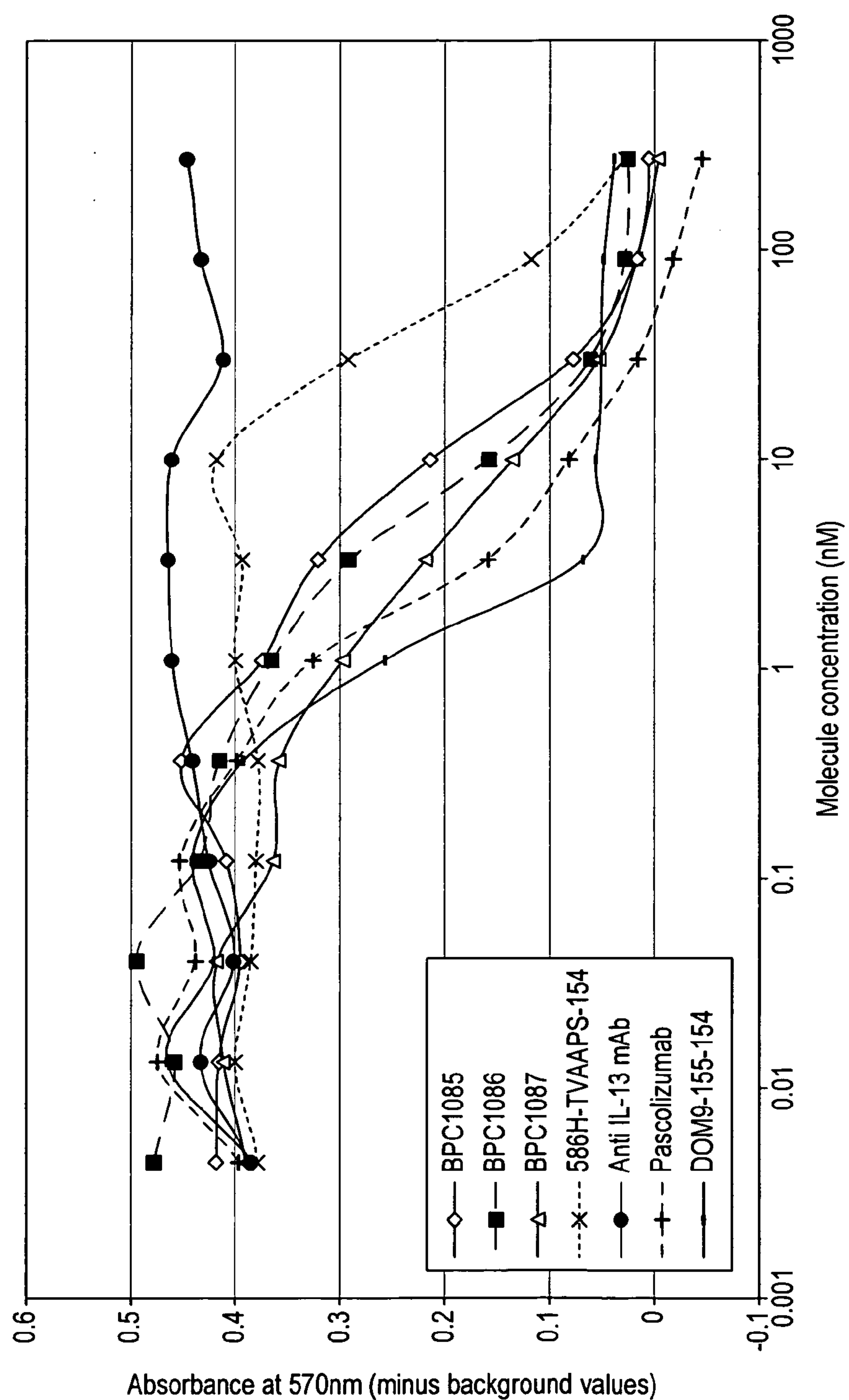


FIG. 12

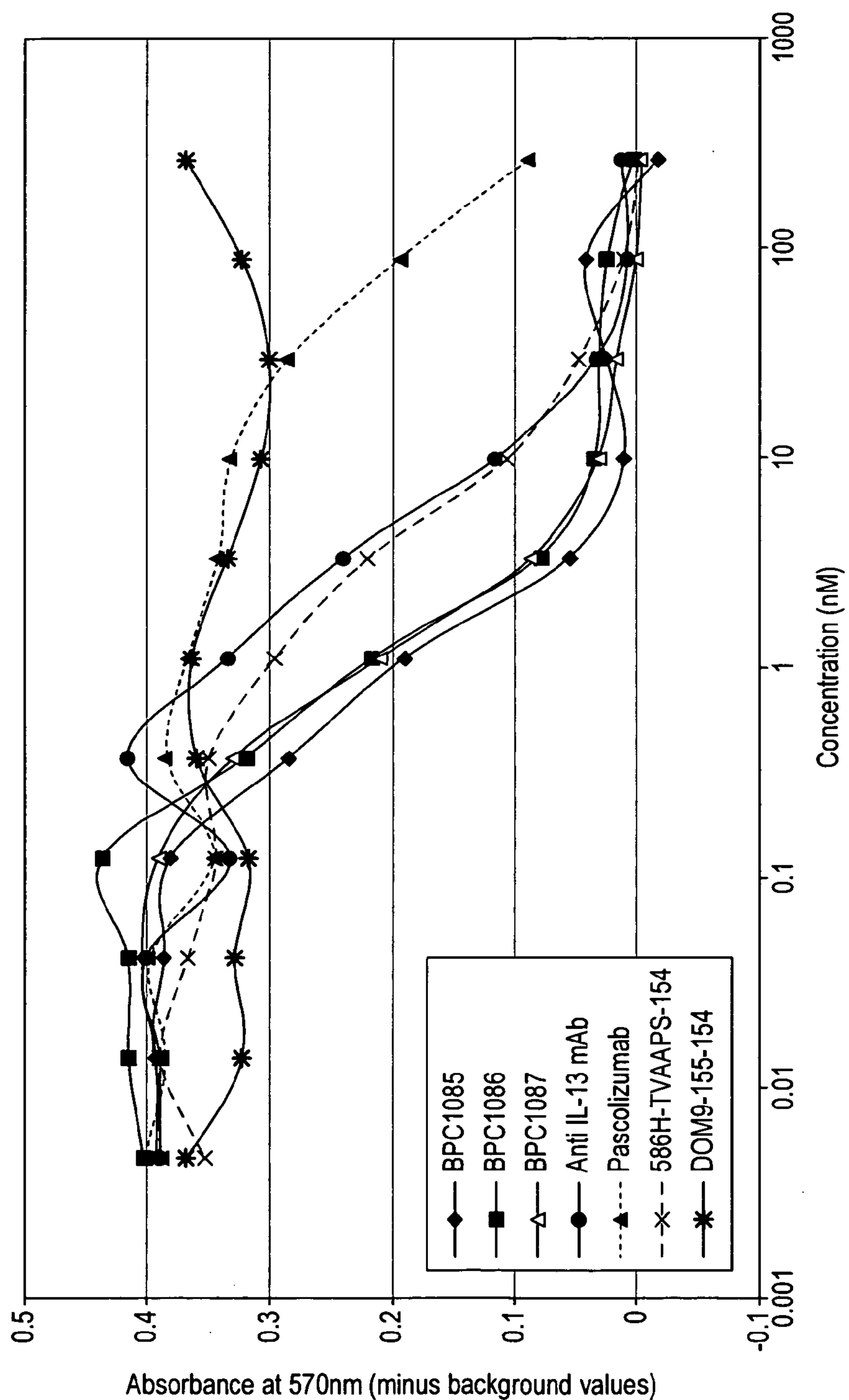


FIG. 13

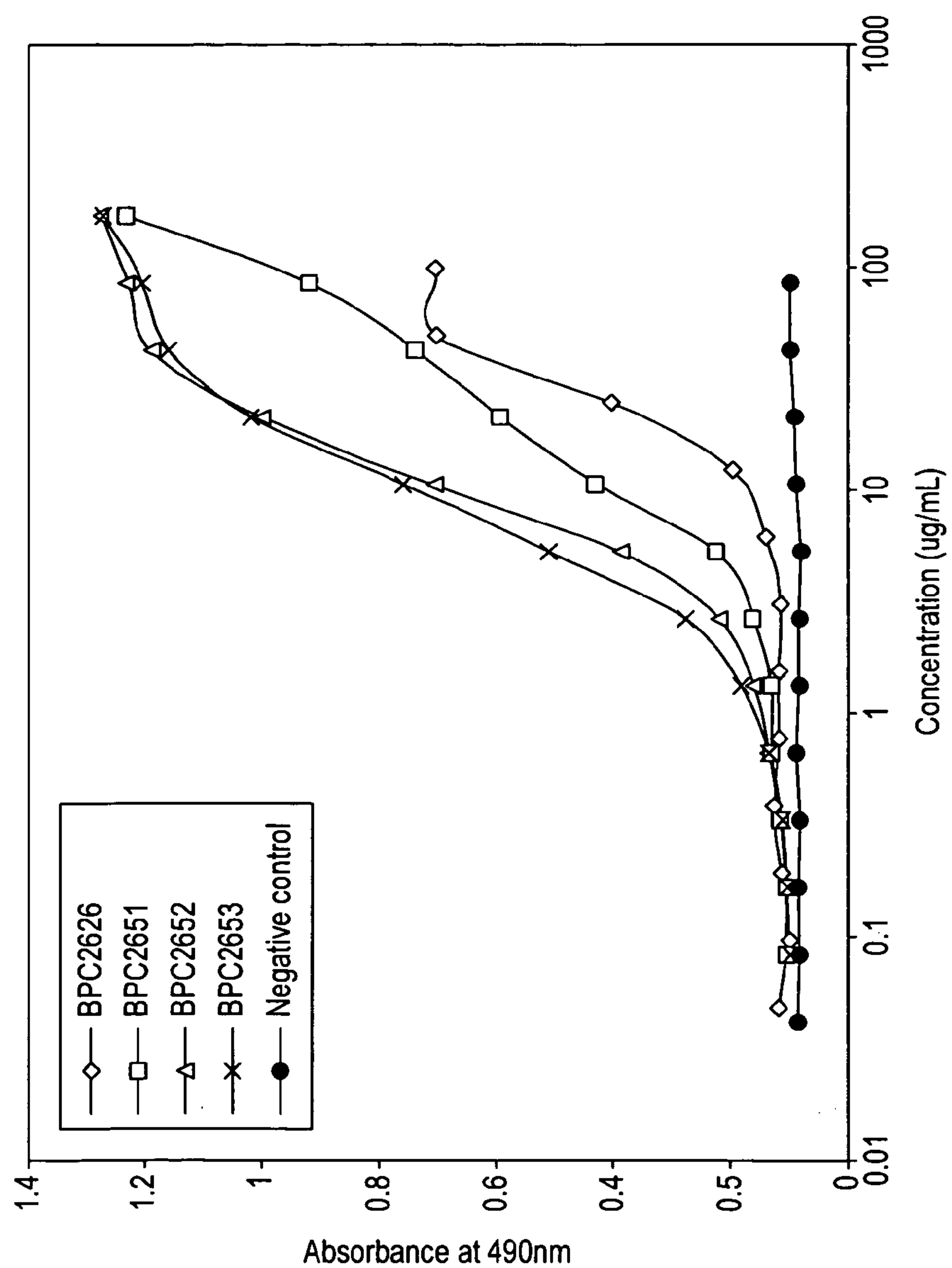


FIG. 14