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(54) **Title:** AMINO ACID SEQUENCES DIRECTED AGAINST IL-6R AND POLYPEPTIDES COMPRISING THE SAME FOR THE TREATMENT OF DISEASES AND DISORDERS ASSOCIATED WITH IL-6-MEDIATED SIGNALLING

(57) **Abstract:** The invention relates to amino acid sequences that are directed against and/or that can specifically bind to IL-6 receptor, compounds or constructs that comprise said amino acid sequence, nucleic acids that encode said amino acid sequences, compounds or constructs, pharmaceutical compositions comprising said amino acid sequences, compounds or constructs as well as methods for the prevention and/or treatment of diseases and disorders associated with IL-6 receptor.

Amino acid sequences directed against IL-6R and polypeptides comprising the same for the treatment of diseases and disorders associated with IL-6-mediated signalling

5 The present invention relates to amino acid sequences that are directed against (as defined herein) Interleukin-6 Receptor (IL-6R), as well as to compounds or constructs, and in particular proteins and polypeptides, that comprise or essentially consist of one or more such amino acid sequences (also referred to herein as "*amino acid sequences of the invention*", "*compounds of the invention*", and "*polypeptides of the invention*", respectively).

10 The invention also relates to nucleic acids encoding such amino acid sequences and polypeptides (also referred to herein as "*nucleic acids of the invention*" or "*nucleotide sequences of the invention*"); to methods for preparing such amino acid sequences and polypeptides; to host cells expressing or capable of expressing such amino acid sequences or polypeptides; to compositions, and in particular to pharmaceutical compositions, that 15 comprise such amino acid sequences, polypeptides, nucleic acids and/or host cells; and to uses of such amino acid sequences or polypeptides, nucleic acids, host cells and/or compositions, in particular for prophylactic, therapeutic or diagnostic purposes, such as the prophylactic, therapeutic or diagnostic purposes mentioned herein.

20 Other aspects, embodiments, advantages and applications of the invention will become clear from the further description herein.

The interaction of IL-6, a protein originally identified as a B cell differentiation factor (Hirano et al., 1985; EP0257406), with IL-6R (Yamasaki et al., 1988; EP0325474) results in the formation of the IL-6/IL-6R complex. This complex binds to gp130 (Taga et al., 1989; EP0411946), a membrane protein on a target cell, which transmits various physiological 25 actions of IL-6. IL-6 is currently known to be involved in - amongst others - the regulation of the immune response, hematopoiesis, the acute phase response, bone metabolism, angiogenesis, and inflammation. Deregulation of IL-6 production is implicated in the pathology of several autoimmune and chronic inflammatory proliferative disease processes (Ishihara and Hirano, 2002). As a consequence, inhibitors of IL-6 induced signaling have 30 attracted much attention in the past (Hirano et al., 1990). Polypeptides specifically binding to IL-6 (Klein et al., 1991; EP0312996), IL-6R (EP0409607) or gp130 (Saito et al., 1993; EP0572118) proved to exhibit an efficient inhibitory effect on IL-6 functioning.

IL-6 overproduction and signalling (and in particular so-called trans-signalling) are involved in various diseases and disorders, such as sepsis (Starnes et al., 1999) and various forms of cancer such as multiple myeloma disease (MM), renal cell carcinoma (RCC), plasma cell leukaemia (Klein et al., 1991), lymphoma, B-lymphoproliferative disorder (BLPD) and 5 prostate cancer. Non-limiting examples of other diseases caused by excessive IL-6 production or signalling include bone resorption (osteoporosis) (Roodman et al., 1992; Jilka et al., 1992), cachexia (Strassman et al., 1992), psoriasis, mesangial proliferative glomerulonephritis, Kaposi's sarcoma, AIDS-related lymphoma (Emilie et al., 1994), inflammatory diseases and 10 disorder such as rheumatoid arthritis, systemic onset juvenile idiopathic arthritis, hypergammaglobulinemia (Grau et al., 1990); Crohn's disease, ulcerative colitis, systemic lupus erythematosus (SLE), multiple sclerosis, Castleman's disease, IgM gammopathy, cardiac myxoma, asthma (in particular allergic asthma) and autoimmune insulin-dependent diabetes mellitus (Campbell et al., 1991). Other IL-6 related disorders will be clear to the skilled person.

15 As can for example be seen from the references above, the prior art describes antibodies and antibody fragments directed against human IL-6, against human IL-6R and against human gp130 protein for the prevention and treatment of IL-6 relates disorders. Examples are Tocilizumab (see Woo P, et al. *Arthritis Res Ther.* (2005) 7: 1281-8, Nishimoto N et al. *Blood.* (2005) 106: 2627-32, Ito H et al. *Gastroenterology.* (2004) 126: 989-96, Choy 20 EH et al. *Arthritis Rheum.* (2002) 46: 3143-50.), BE8 (see Bataille R et al. *Blood* (1995) 86:685-91, Emilie D et al. *Blood* (1994) 84:2472-9, Beck JT et al. *N Engl J Med.* (1994) 330:602-5, Wendling D et al. *J Rheumatol.* (1993) 20:259-62.) and CNTO-328 of Centocor (see *Journal of Clinical Oncology*, (2004) 22/14S: 2560; *Journal of Clinical Oncology*, (2004) 22/14S: 2608; *Int J Cancer* (2004) 111:592-5). Another active principle known in the art for 25 the prevention and treatment of IL-6 related disorders is an Fc fusion of soluble gp130 (see Becker C et al. *Immunity.* (2004) 21: 491-501, Doganci A et al. *J Clin Invest.* (2005) 115:313-25, Nowell MA et al. *J Immunol.* (2003) 171: 3202-9., Atreya R et al. *Nat Med.* (2000) 6:583-8.)

30 The amino acid sequences, polypeptides and compositions of the present invention can generally be used to modulate, and in particular inhibit and/or prevent, binding of IL-6R to IL-6 and/or binding of the IL-6/IL-6R complex to gp130 and thus to modulate, and in particular inhibit or prevent, the signalling that is mediated by IL-6R, IL-6, IL6/IL-6R complex or gp130 to modulate the biological pathways in which IL-6R, IL-6, the IL6/IL-6R

complex and/or gp130 are involved, and/or to modulate the biological mechanisms, responses and effects associated with such signalling or these pathways.

In the context of the present invention "*modulating the interaction between IL-6/IL-6R complex and gp130*" can for example mean:

5 - binding to IL-6R (i.e. as such or as present in the IL-6/IL-6R complex) in such a way that the formation of the IL-6/IL-6R complex is inhibited or affected (e.g. fully or partially disrupted) in such a way that the binding of the complex to - e.g. its affinity for - gp130 is reduced (or reversely, that the binding of gp 130 to - e.g. its affinity for – the complex is reduced), so that the signaling induced/mediated by the binding of the

10 complex to gp130 is modulated (e.g. reduced);

or

- binding to IL-6R (i.e. as such or as present in the IL-6/IL-6R complex) in such a way that the formation of the IL-6/IL-6R complex essentially is not affected but that the binding of said complex to gp130 is modulated (e.g. inhibited), so that the signalling induced/mediated by the binding of the complex to gp130 is modulated (e.g. reduced);

15 both compared to the formation of the complex and its binding to gp130 without the presence of the amino acid sequence or Nanobody of the invention.

Accordingly, in one specific, but non-limiting aspect, the invention provides polypeptides and compositions that are, and/or that can be used as, an antagonist of IL-6, of 20 IL-6R, of IL-6- or IL-6R-mediated signalling, and/or of the biological pathways mechanisms, responses and/or effects in which IL-6, IL-6R and/or IL-6- or IL-6R mediated signalling are involved.

As such, the amino acid sequences, polypeptides and compositions of the invention may for example bind an epitope that lies in, forms part of, or overlaps with (i.e. in the 25 primary or tertiary structure) or is in close proximity to (i.e. in the primary or tertiary structure) to the IL-6 binding site on IL-6R (for example, competitively with IL-6); bind an epitope that lies in, forms part of, or overlaps with (i.e. in the primary or tertiary structure) or is in close proximity to (i.e. in the primary or tertiary structure) the gp130 binding site on IL-6R (for example, competitively with gp130); and/or bind an epitope that lies in, forms part of, 30 or overlaps with (i.e. in the primary or tertiary structure) or is in close proximity to (i.e. in the primary or tertiary structure) the gp 130 binding site on the complex (for example, competitively with gp130). Preferably, any such epitope is an extracellular epitope. Some

specific epitopes to which the amino acid sequences, Nanobodies and polypeptides of the invention may preferably bind will become clear from the further description herein.

As such, the amino acid sequences, polypeptides and compositions of the present invention can be used for the prevention and treatment of diseases and disorders associated with IL-6R, IL-6 and/or with the IL-6/IL-6R complex (optionally in further complex with gp130), and/or with the signaling pathway(s) and/or the biological functions and responses in which IL-6 and/or the IL-6/IL-6R complex (optionally in further complex with gp130) are involved, and in particular for the prevention and treatment of diseases and disorders associated with IL-6R, IL-6 and/or with the IL-6/IL-6R complex (optionally in further complex with gp130), and/or with the signaling pathway(s) and/or the biological functions and responses in which IL-6R, IL-6 and/or with the IL-6/IL-6R complex (optionally in further complex with gp130) are involved, which are characterized by excessive and/or unwanted signalling mediated by IL-6R or by the pathway(s) in which IL-6R is involved. Examples of such diseases and disorders associated with IL-6R, IL-6 and/or with the IL-6/IL-6R complex, and/or with the signaling pathway(s) and/or the biological functions and responses in which IL-6 and/or the IL-6/IL-6R complex are involved, will be clear to the skilled person based on the disclosure herein, and for example include the following diseases and disorders: sepsis (Starnes et al., 1999) and various forms of cancer such as multiple myeloma disease (MM), renal cell carcinoma (RCC), plasma cell leukaemia (Klein et al., 1991), lymphoma, B-lymphoproliferative disorder (BLPD) and prostate cancer. Non-limiting examples of other diseases caused by excessive IL-6 production or signalling include bone resorption (osteoporosis) (Roodman et al., 1992; Jilka et al., 1992), cachexia (Strassman et al., 1992), psoriasis, mesangial proliferative glomerulonephritis, Kaposi's sarcoma, AIDS-related lymphoma (Emilie et al., 1994), inflammatory diseases and disorder such as rheumatoid arthritis, systemic onset juvenile idiopathic arthritis, hypergammaglobulinemia (Grau et al., 1990); Crohn's disease, ulcerative colitis, systemic lupus erythematosus (SLE), multiple sclerosis, Castleman's disease, IgM gammopathy, cardiac myxoma, asthma (in particular allergic asthma) and autoimmune insulin-dependent diabetes mellitus (Campbell et al., 1991). Other IL-6R, IL-6 and/or IL-6/IL-6R complex related disorders will be clear to the skilled person. Such diseases and disorders are also generally referred to herein as "*IL-6R related disorders*".

Thus, without being limited thereto, the amino acid sequences and polypeptides of the invention can for example be used to prevent and/or to treat all diseases and disorders that are

currently being prevented or treated with active principles that can modulate IL-6R-mediated signalling, such as those mentioned in the prior art cited above. It is also envisaged that the polypeptides of the invention can be used to prevent and/or to treat all diseases and disorders for which treatment with such active principles is currently being developed, has been

5 proposed, or will be proposed or developed in future. In addition, it is envisaged that, because of their favourable properties as further described herein, the polypeptides of the present invention may be used for the prevention and treatment of other diseases and disorders than those for which these known active principles are being used or will be proposed or developed; and/or that the polypeptides of the present invention may provide new methods 10 and regimens for treating the diseases and disorders described herein.

Other applications and uses of the amino acid sequences and polypeptides of the invention will become clear to the skilled person from the further disclosure herein.

Generally, it is an object of the invention to provide pharmacologically active agents, as well as compositions comprising the same, that can be used in the diagnosis, prevention 15 and/or treatment of one or more IL-6R related disorders (as defined herein); and to provide methods for the diagnosis, prevention and/or treatment of such diseases and disorders that involve the administration and/or use of such agents and compositions.

In particular, it is an object of the invention to provide such pharmacologically active agents, compositions and/or methods that have certain advantages compared to the agents, 20 compositions and/or methods that are currently used and/or known in the art. These advantages will become clear from the further description below.

More in particular, it is an object of the invention to provide therapeutic proteins that can be used as pharmacologically active agents, as well as compositions comprising the same, for the diagnosis, prevention and/or treatment of one or more IL-6R related disorders (as 25 defined herein); and to provide methods for the diagnosis, prevention and/or treatment of such diseases and disorders that involve the administration and/or the use of such therapeutic proteins and compositions.

Accordingly, it is a specific object of the present invention to provide amino acid sequences and polypeptides that are directed against (as defined herein) IL-6R, in particular 30 against IL-6R from a warm-blooded animal, more in particular against IL-6R from a mammal, and especially against human IL-6R; and to provide proteins and polypeptides comprising or essentially consisting of at least one such amino acid sequence.

In particular, it is a specific object of the present invention to provide such amino acid sequences and such proteins and/or polypeptides that are suitable for prophylactic, therapeutic and/or diagnostic use in a warm-blooded animal, and in particular in a mammal, and more in particular in a human being.

5 More in particular, it is a specific object of the present invention to provide such amino acid sequences and such proteins and/or polypeptides that can be used for the prevention, treatment, alleviation and/or diagnosis of one or more IL-6R related disorders (as defined herein) in a warm-blooded animal, in particular in a mammal, and more in particular in a human being.

10 It is also a specific object of the invention to provide such amino acid sequences and such proteins and/or polypeptides that can be used in the preparation of pharmaceutical or veterinary compositions for the prevention and/or treatment of one or more IL-6R related disorders (as defined herein) in a warm-blooded animal, in particular in a mammal, and more in particular in a human being.

15 In the invention, generally, these objects are achieved by the use of the amino acid sequences, proteins, polypeptides and compositions that are described herein.

In general, the invention provides amino acid sequences and polypeptides that are directed against (as defined herein) and/or can specifically bind (as defined herein) to IL-6R; as well as compounds and constructs, and in particular proteins and polypeptides, that 20 comprise at least one such amino acid sequence.

More in particular, the invention provides amino acid sequences that can bind to IL-6R with an affinity (suitably measured and/or expressed as a K_D -value (actual or apparent), a K_A -value (actual or apparent), a k_{on} -rate and/or a k_{off} -rate, or alternatively as an IC_{50} value, as further described herein) that is as defined herein; as well as compounds and constructs, and in particular proteins and polypeptides, that comprise at least one such amino acid sequence.

25 In particular, amino acid sequences and polypeptides of the invention are preferably such that they:

- bind to IL-6R with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter or less, and preferably 10^{-7} to 10^{-12} moles/liter or less and more preferably 10^{-8} to 10^{-12} moles/liter (i.e. with an association constant (K_A) of 10^5 to 10^{12} liter/ moles or more, and preferably 10^7 to 10^{12} liter/moles or more and more preferably 10^8 to 10^{12} liter/moles);

30 and/or such that they:

- bind to IL-6R with a k_{on} -rate of between $10^2 \text{ M}^{-1} \text{s}^{-1}$ to about $10^7 \text{ M}^{-1} \text{s}^{-1}$, preferably between $10^3 \text{ M}^{-1} \text{s}^{-1}$ and $10^7 \text{ M}^{-1} \text{s}^{-1}$, more preferably between $10^4 \text{ M}^{-1} \text{s}^{-1}$ and $10^7 \text{ M}^{-1} \text{s}^{-1}$, such as between $10^5 \text{ M}^{-1} \text{s}^{-1}$ and $10^7 \text{ M}^{-1} \text{s}^{-1}$;

and/or such that they:

5

- bind to IL-6R with a k_{off} rate between 1 s^{-1} ($t_{1/2}=0.69 \text{ s}$) and 10^{-6} s^{-1} (providing a near irreversible complex with a $t_{1/2}$ of multiple days), preferably between 10^{-2} s^{-1} and 10^{-6} s^{-1} , more preferably between 10^{-3} s^{-1} and 10^{-6} s^{-1} , such as between 10^{-4} s^{-1} and 10^{-6} s^{-1} .

Preferably, a monovalent amino acid sequence of the invention (or a polypeptide that contains only one amino acid sequence of the invention) is preferably such that it will bind to 10 IL-6R with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM.

Some preferred IC50 values for binding of the amino acid sequences or polypeptides of the invention to IL-6R will become clear from the further description and examples herein.

For binding to IL-6R, an amino acid sequence of the invention will usually contain 15 within its amino acid sequence one or more amino acid residues or one or more stretches of amino acid residues (i.e. with each “stretch” comprising two or more amino acid residues that are adjacent to each other or in close proximity to each other, i.e. in the primary or tertiary structure of the amino acid sequence) via which the amino acid sequence of the invention can bind to IL-6R, which amino acid residues or stretches of amino acid residues thus form the 20 “site” for binding to IL-6R (also referred to herein as the “*antigen binding site*”).

The amino acid sequences provided by the invention are preferably in essentially 25 isolated form (as defined herein), or form part of a protein or polypeptide of the invention (as defined herein), which may comprise or essentially consist of one or more amino acid sequences of the invention and which may optionally further comprise one or more further amino acid sequences (all optionally linked via one or more suitable linkers). For example, and without limitation, the one or more amino acid sequences of the invention may be used as 30 a binding unit in such a protein or polypeptide, which may optionally contain one or more further amino acid sequences that can serve as a binding unit (i.e. against one or more other targets than IL-6R), so as to provide a monovalent, multivalent or multispecific polypeptide of the invention, respectively, all as described herein. Such a protein or polypeptide may also be in essentially isolated form (as defined herein).

Generally, when an amino acid sequence of the invention (or a compound, construct or polypeptide comprising the same) is intended for administration to a subject (for example for

therapeutic and/or diagnostic purposes as described herein), it is preferably either an amino acid sequence that does not occur naturally in said subject; or, when it does occur naturally in said subject, in essentially isolated form (as defined herein).

It will also be clear to the skilled person that for pharmaceutical use, the amino acid

5 sequences of the invention (as well as compounds, constructs and polypeptides comprising the same) are preferably directed against human IL-6R; whereas for veterinary purposes, the amino acid sequences and polypeptides of the invention are preferably directed against IL-6R from the species to be treated, or at least cross-reactive with IL-6R from the species to be treated.

10 Furthermore, an amino acid sequence of the invention (or compound, construct or polypeptide comprising one or more such amino acid sequences) may optionally, and in addition to the at least one binding site for binding against IL-6R, contain one or more further binding sites for binding against other antigens, proteins or targets.

15 The efficacy of the amino acid sequences and polypeptides of the invention, and of compositions comprising the same, can be tested using any suitable in vitro assay, cell-based assay, in vivo assay and/or animal model known per se, or any combination thereof, depending on the specific disease or disorder involved. Suitable assays and animal models will be clear to the skilled person, and for example include proliferation assays using IL6-dependent cell lines including B9, XG1 and 7TD1, collagen induced arthritis model, 20 transplant model of synovial tissue in SCID mice, xenograft models of various human cancers, including lymphoma, myeloma, prostate cancer and renal cell carcinoma, IBD models including TNBS, DSS and IL10 knockout models, as well as the assays and animal models used in the experimental part below and in the prior art cited herein (Peake et al., Rheumatology 2006; 45(12):1485-9; Wahid et al.; Clin Exp Immunol. 2000, 122:133-142; 25 Matsuno et al., Arthritis and rheumatism, 1998, 41: 2014-2021).

Also, according to the invention, amino acid sequences and polypeptides that are directed against IL-6R from a first species of warm-blooded animal may or may not show cross-reactivity with IL-6R from one or more other species of warm-blooded animal, by which is meant that these amino acid sequences are also "directed against" (as defined herein) and/or are capable of specific binding to (as defined herein) IL-6R from said warm-blooded animal. For example, amino acid sequences and polypeptides directed against human IL-6R may or may not show cross reactivity with IL-6R from one or more other species of primates (such as, without limitation, monkeys from the genus *Macaca* (such as, and in particular,

cynomologus monkeys (*Macaca fascicularis*) and/or rhesus monkeys (*Macaca mulatta*) and baboon (*Papio ursinus*)) and/or with IL-6R from one or more species of animals that are often used in animal models for diseases (for example mouse, rat, rabbit, pig or dog), and in particular in animal models for diseases and disorders associated with IL-6R (such as the

5 species and animal models mentioned herein). In this respect, it will be clear to the skilled person that such cross-reactivity, when present, may have advantages from a drug development point of view, since it allows the amino acid sequences and polypeptides against human IL-6R to be tested in such disease models. In a preferred but non-limiting aspect, the amino acid sequences of the invention (as well as compounds, constructs and polypeptides 10 comprising the same) may be cross-reactive with the amino acid sequence for IL-6R from *Macaca fascicularis* that is given in SEQ ID NO: 633. For this sequence and the corresponding cDNA sequence, reference is also made to the non-prepublished US provisional application filed by Ablynx N.V. on July 19, 2007 entitled "*Receptor for interleukin-6 (IL-6) from Macaca fascicularis*"; see SEQ ID NO: 3 and Figure 1B for the 15 cDNA sequence and SEQ ID NO: 4 and Figure 3B for the amino acid sequence.

More generally, amino acid sequences and polypeptides of the invention that are cross-reactive with IL-6R from multiple species of mammal will usually be advantageous for use in veterinary applications, since it will allow the same amino acid sequence or polypeptide to be used across multiple species. Thus, it is also encompassed within the scope of the invention 20 that amino acid sequences and polypeptides directed against IL-6R from one species of animal (such as amino acid sequences and polypeptides against human IL-6R) can be used in the treatment of another species of animal, as long as the use of the amino acid sequences and/or polypeptides provide the desired effects in the species to be treated.

The present invention is in its broadest sense also not particularly limited to or defined 25 by a specific antigenic determinant, epitope, part, domain, subunit or confirmation (where applicable) of IL-6R against which the amino acid sequences and polypeptides of the invention are directed. However, it is generally assumed and preferred that the amino acid sequences and polypeptides of the invention are preferably directed against any epitope of the IL-6 receptor involved in the interaction of the IL-6 receptor with IL-6.

30 Such epitopes or interaction sites have been described in detail in Boulanger et al. (2003) (Science 300, 2101-2104) and reference is specifically made to figure 2 in cited reference. More preferably, the amino acid sequences and polypeptides of the present invention are directed against an extracellular domain of the IL-6 receptor. Still more

preferably, the amino acid sequences and polypeptides of the present invention are directed against the extracellular D3 domain of the IL-6 receptor. Still more preferably, the amino acid sequences and polypeptides of the present invention interact with one or more of the 18 contact residues as described in Boulanger et al. 2003 (Science 300, 2101-2104) present in the 5 extracellular D3 domain of the IL-6 receptor that contribute to the interaction of the IL-6 receptor with IL-6. Most preferably, the amino acid sequences and polypeptides of the present invention interact with amino acid residues Phe229 and Phe279 present in the extracellular D3 domain of the IL-6 receptor.

Thus, in one preferred, but non-limiting aspect, the amino acid sequences and 10 polypeptides of the invention are directed against any epitope of the IL-6 receptor involved in the interaction of the IL-6 receptor with IL-6, and are as further defined herein.

Alternatively, the amino acid sequences and polypeptides of the invention are directed against any epitope of the IL-6 receptor involved in the interaction of the IL-6 receptor with gp130. Such epitopes or interaction sites have been described in detail in Boulanger et al. 15 (2003) (Science 300, 2101-2104) and reference is specifically made to figure 2 in cited reference.

In this context, according to a non-limiting aspect, amino acid sequences and 20 polypeptides of the invention are preferably such that they can compete for binding to the IL-6 receptor with the commercially available human-mouse reconstituted chimeric monoclonal anti-IL6R antibody Tocilizumab (MRA) (Chugai/Roche) or an antigen binding fragment thereof (see for example WO 92/19759 and corresponding European patent EP 0628639, as well as Shinkura et al., 1998, Anticancer Research 18, 1217-1222), for example in the assay 25 described in Example 29; and/or such that they can bind to the same epitope or binding site on IL-6R as Tocilizumab, or to an epitope close to said binding site and/or overlapping with said binding site.

Also, according to a non-limiting aspect, amino acid sequences and polypeptides of 30 the invention are preferably such that they can compete for binding to the IL-6 receptor with the reference IgG and/or reference Fab according to patent EP 0628639; and/or such that they can bind to the same epitope or binding site on IL-6R as said reference IgG or reference Fab, or to an epitope close to said binding site and/or overlapping with said binding site. For the preparation and sequence of said reference IgG and reference Fab, reference is made to Reference Example 1 below, as well as to SEQ ID NO's: 629 to 632.

Thus, generally and without limitation, amino acid sequences and polypeptides of the invention may be directed against any epitope of the IL-6 receptor involved in the interaction of the IL-6 receptor with IL-6 and/or gp130.

The amino acid sequences and polypeptides of the invention are also preferably (but without limitation) such that they effect a decrease (i.e. by at least 1 percent such as by at least 10 percent or more) in the levels of C-reactive protein (CRP) in a mammal (such as a human subject or in a suitable animal model for inflammation such as the cynomolgus monkey model used in the experimental part below) when they are administered to said mammal in a therapeutically relevant amount.

It is also within the scope of the invention that, where applicable, an amino acid sequence or polypeptide of the invention can bind to two or more antigenic determinants, epitopes, parts, domains, subunits or confirmations of IL-6R. In such a case, the antigenic determinants, epitopes, parts, domains or subunits of IL-6R to which the amino acid sequences and/or polypeptides of the invention bind may be essentially the same (for example, if IL-6R contains repeated structural motifs or occurs in a multimeric form) or may be different (and in the latter case, the amino acid sequences and polypeptides of the invention may bind to such different antigenic determinants, epitopes, parts, domains, subunits of IL-6R with an affinity and/or specificity which may be the same or different). Also, for example, when IL-6R exists in an activated conformation and in an inactive conformation, the amino acid sequences and polypeptides of the invention may bind to either one of these confirmation, or may bind to both these confirmations (i.e. with an affinity and/or specificity which may be the same or different). Also, for example, the amino acid sequences and polypeptides of the invention may bind to a conformation of IL-6R in which it is bound to a pertinent ligand, may bind to a conformation of IL-6R in which it is not bound to a pertinent ligand, or may bind to both such conformations (again with an affinity and/or specificity which may be the same or different).

It is also expected that the amino acid sequences and polypeptides of the invention will generally bind to all naturally occurring or synthetic analogs, variants, mutants, alleles, parts and fragments of IL-6R; or at least to those analogs, variants, mutants, alleles, parts and fragments of IL-6R that contain one or more antigenic determinants or epitopes that are essentially the same as the antigenic determinant(s) or epitope(s) to which the amino acid sequences and polypeptides of the invention bind in IL-6R (e.g. in wild-type IL-6R). Again, in such a case, the amino acid sequences and polypeptides of the invention may bind to such

analogs, variants, mutants, alleles, parts and fragments with an affinity and/or specificity that are the same as, or that are different from (i.e. higher than or lower than), the affinity and specificity with which the amino acid sequences of the invention bind to (wild-type) IL-6R. It is also included within the scope of the invention that the amino acid sequences and

5 polypeptides of the invention bind to some analogs, variants, mutants, alleles, parts and fragments of IL-6R, but not to others.

When IL-6R exists in a monomeric form and in one or more multimeric forms, it is within the scope of the invention that the amino acid sequences and polypeptides of the invention only bind to IL-6R in monomeric form, only bind to IL-6R in multimeric form, or

10 bind to both the monomeric and the multimeric form. Again, in such a case, the amino acid sequences and polypeptides of the invention may bind to the monomeric form with an affinity and/or specificity that are the same as, or that are different from (i.e. higher than or lower than), the affinity and specificity with which the amino acid sequences of the invention bind to the multimeric form.

15 Also, when IL-6R can associate with other proteins or polypeptides to form protein complexes (e.g. with multiple subunits), it is within the scope of the invention that the amino acid sequences and polypeptides of the invention bind to IL-6R in its non-associated state, bind to IL-6R in its associated state, or bind to both. In all these cases, the amino acid sequences and polypeptides of the invention may bind to such multimers or associated protein

20 complexes with an affinity and/or specificity that may be the same as or different from (i.e. higher than or lower than) the affinity and/or specificity with which the amino acid sequences and polypeptides of the invention bind to IL-6R in its monomeric and non-associated state.

Also, as will be clear to the skilled person, proteins or polypeptides that contain two or more amino acid sequences directed against IL-6R may bind with higher avidity to IL-6R

25 than the corresponding monomeric amino acid sequence(s). For example, and without limitation, proteins or polypeptides that contain two or more amino acid sequences directed against different epitopes of IL-6R may (and usually will) bind with higher avidity than each of the different monomers, and proteins or polypeptides that contain two or more amino acid sequences directed against IL-6R may (and usually will) bind also with higher avidity to a

30 multimer of IL-6R.

Generally, amino acid sequences and polypeptides of the invention will at least bind to those forms of IL-6R (including monomeric, multimeric and associated forms) that are the

most relevant from a biological and/or therapeutic point of view, as will be clear to the skilled person.

It is also within the scope of the invention to use parts, fragments, analogs, mutants, variants, alleles and/or derivatives of the amino acid sequences and polypeptides of the invention, and/or to use proteins or polypeptides comprising or essentially consisting of one or more of such parts, fragments, analogs, mutants, variants, alleles and/or derivatives, as long as these are suitable for the uses envisaged herein. Such parts, fragments, analogs, mutants, variants, alleles and/or derivatives will usually contain (at least part of) a functional antigen-binding site for binding against IL-6R; and more preferably will be capable of specific binding to IL-6R, and even more preferably capable of binding to IL-6R with an affinity (suitably measured and/or expressed as a K_D -value (actual or apparent), a K_A -value (actual or apparent), a k_{on} -rate and/or a k_{off} -rate, or alternatively as an IC_{50} value, as further described herein) that is as defined herein. Some non-limiting examples of such parts, fragments, analogs, mutants, variants, alleles, derivatives, proteins and/or polypeptides will become clear from the further description herein. Additional fragments or polypeptides of the invention may also be provided by suitably combining (i.e. by linking or genetic fusion) one or more (smaller) parts or fragments as described herein.

In one specific, but non-limiting aspect of the invention, which will be further described herein, such analogs, mutants, variants, alleles, derivatives have an increased half-life in serum (as further described herein) compared to the amino acid sequence from which they have been derived. For example, an amino acid sequence of the invention may be linked (chemically or otherwise) to one or more groups or moieties that extend the half-life (such as PEG), so as to provide a derivative of an amino acid sequence of the invention with increased half-life.

In one specific, but non-limiting aspect, the amino acid sequence of the invention may be an amino acid sequence that comprises an immunoglobulin fold or may be an amino acid sequence that, under suitable conditions (such as physiological conditions) is capable of forming an immunoglobulin fold (i.e. by folding). Reference is inter alia made to the review by Halaby et al., J. (1999) Protein Eng. 12, 563-71. Preferably, when properly folded so as to form an immunoglobulin fold, such an amino acid sequence is capable of specific binding (as defined herein) to IL-6R; and more preferably capable of binding to IL-6R with an affinity (suitably measured and/or expressed as a K_D -value (actual or apparent), a K_A -value (actual or apparent), a k_{on} -rate and/or a k_{off} -rate, or alternatively as an IC_{50} value, as further described

herein) that is as defined herein. Also, parts, fragments, analogs, mutants, variants, alleles and/or derivatives of such amino acid sequences are preferably such that they comprise an immunoglobulin fold or are capable for forming, under suitable conditions, an immunoglobulin fold.

5 In particular, but without limitation, the amino acid sequences of the invention may be amino acid sequences that essentially consist of 4 framework regions (FR1 to FR4 respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively); or any suitable fragment of such an amino acid sequence (which will then usually contain at least some of the amino acid residues that form at least one of the CDR's, as further described 10 herein).

The amino acid sequences of the invention may in particular be an immunoglobulin sequence or a suitable fragment thereof, and more in particular be an immunoglobulin variable domain sequence or a suitable fragment thereof, such as light chain variable domain sequence (e.g. a V_L -sequence) or a suitable fragment thereof; or a heavy chain variable 15 domain sequence (e.g. a V_H -sequence) or a suitable fragment thereof. When the amino acid sequence of the invention is a heavy chain variable domain sequence, it may be a heavy chain variable domain sequence that is derived from a conventional four-chain antibody (such as, without limitation, a V_H sequence that is derived from a human antibody) or be a so-called V_{HH} -sequence (as defined herein) that is derived from a so-called "heavy chain antibody" (as 20 defined herein).

However, it should be noted that the invention is not limited as to the origin of the amino acid sequence of the invention (or of the nucleotide sequence of the invention used to express it), nor as to the way that the amino acid sequence or nucleotide sequence of the invention is (or has been) generated or obtained. Thus, the amino acid sequences of the 25 invention may be naturally occurring amino acid sequences (from any suitable species) or synthetic or semi-synthetic amino acid sequences. In a specific but non-limiting aspect of the invention, the amino acid sequence is a naturally occurring immunoglobulin sequence (from any suitable species) or a synthetic or semi-synthetic immunoglobulin sequence, including but not limited to "humanized" (as defined herein) immunoglobulin sequences (such as partially 30 or fully humanized mouse or rabbit immunoglobulin sequences, and in particular partially or fully humanized V_{HH} sequences or Nanobodies), "camelized" (as defined herein) immunoglobulin sequences, as well as immunoglobulin sequences that have been obtained by techniques such as affinity maturation (for example, starting from synthetic, random or

naturally occurring immunoglobulin sequences), CDR grafting, veneering, combining fragments derived from different immunoglobulin sequences, PCR assembly using overlapping primers, and similar techniques for engineering immunoglobulin sequences well known to the skilled person; or any suitable combination of any of the foregoing. Reference is 5 for example made to the standard handbooks, as well as to the further description and prior art mentioned herein.

Similarly, the nucleotide sequences of the invention may be naturally occurring nucleotide sequences or synthetic or semi-synthetic sequences, and may for example be sequences that are isolated by PCR from a suitable naturally occurring template (e.g. DNA or 10 RNA isolated from a cell), nucleotide sequences that have been isolated from a library (and in particular, an expression library), nucleotide sequences that have been prepared by introducing mutations into a naturally occurring nucleotide sequence (using any suitable technique known per se, such as mismatch PCR), nucleotide sequence that have been prepared by PCR using overlapping primers, or nucleotide sequences that have been prepared 15 using techniques for DNA synthesis known per se.

The amino acid sequence of the invention may in particular be a domain antibody (or an amino acid sequence that is suitable for use as a domain antibody), a single domain antibody (or an amino acid sequence that is suitable for use as a single domain antibody), a "dAb" (or an amino acid sequence that is suitable for use as a dAb) or a Nanobody® (as 20 defined herein, and including but not limited to a V_{HH} sequence); other single variable domains, or any suitable fragment of any one thereof. For a general description of (single) domain antibodies, reference is also made to the prior art cited above, as well as to EP 0 368 684. For the term "dAb's", reference is for example made to Ward et al. (Nature 1989 Oct 12; 341 (6242): 544-6), to Holt et al., Trends Biotechnol., 2003, 21(11):484-490; as well as to for 25 example WO 06/030220, WO 06/003388 and other published patent applications of Domantis Ltd.. It should also be noted that, although less preferred in the context of the present invention because they are not of mammalian origin, single domain antibodies or single variable domains can be derived from certain species of shark (for example, the so-called "IgNAR domains", see for example WO 05/18629).

30 In particular, the amino acid sequence of the invention may be a Nanobody® (as defined herein) or a suitable fragment thereof. [Note: Nanobody®, Nanobodies® and Nanoclone® are registered trademarks of Ablynx N.V.] Such Nanobodies directed against IL-6R will also be referred to herein as "Nanobodies of the invention".

For a general description of Nanobodies, reference is made to the further description below, as well as to the prior art cited herein. In this respect, it should however be noted that this description and the prior art mainly described Nanobodies of the so-called “V_H3 class” (i.e. Nanobodies with a high degree of sequence homology to human germline sequences of the V_H3 class such as DP-47, DP-51 or DP-29), which Nanobodies form a preferred aspect of this invention. It should however be noted that the invention in its broadest sense generally covers any type of Nanobody directed against IL-6R, and for example also covers the Nanobodies belonging to the so-called “V_H4 class” (i.e. Nanobodies with a high degree of sequence homology to human germline sequences of the V_H4 class such as DP-78), as for example described in the US provisional application 60/792,279 by Ablynx N.V. entitled “DP-78-like Nanobodies” filed on April 14, 2006.

Generally, Nanobodies (in particular V_{HH} sequences and partially humanized Nanobodies) can in particular be characterized by the presence of one or more “*Hallmark residues*” (as described herein) in one or more of the framework sequences (again as further described herein).

Thus, generally, a Nanobody can be defined as an amino acid sequence with the (general) structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

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in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which one or more of the Hallmark residues are as further defined herein.

In particular, a Nanobody can be an amino acid sequence with the (general) structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which the framework sequences are as further defined herein.

Thus, the invention also relates to such Nanobodies that can bind to (as defined herein) and/or are directed against IL-6R, to suitable fragments thereof, as well as to polypeptides that comprise or essentially consist of one or more of such Nanobodies and/or suitable fragments.

SEQ ID NO's 399 to 471 give the amino acid sequences of a number of V_{HH} sequences that have been raised against IL-6R.

Accordingly, some particularly preferred Nanobodies of the invention are Nanobodies which can bind (as further defined herein) to and/or are directed against to IL-6R and which:

- 5 a) have 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 399 to 471, in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences are disregarded. In this respect, reference is also made to Table A-1, which lists the framework 1 sequences (SEQ ID NO's: 42 to 92), framework 2 sequences (SEQ ID NO's: 144 to 194),
10 framework 3 sequences (SEQ ID NO's: 246 to 296) and framework 4 sequences (SEQ ID NO's: 348 to 398) of the Nanobodies of SEQ ID NO's: 399 to 471 (with respect to the amino acid residues at positions 1 to 4 and 27 to 30 of the framework 1 sequences, reference is also made to the comments made below. Thus, for determining the degree of amino acid identity, these residues are preferably disregarded);
15 and in which:
 - b) preferably one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table A-3 below (it being understood that V_{HH} sequences will contain one or more Hallmark residues; and that partially humanized Nanobodies will usually, and preferably, [still] contain one or more Hallmark residues [although it is also within the scope of the invention to provide - where suitable in accordance with the invention - partially humanized Nanobodies in which all Hallmark residues, but not one or more of the other amino acid residues, have been humanized]; and that in fully humanized Nanobodies, where suitable in accordance with the invention, all amino acid residues at the positions of the Hallmark residues will be amino acid residues that occur in a human V_{H3} sequence. As will be clear to the skilled person based on the disclosure herein that such V_{HH} sequences, such partially humanized Nanobodies with at least one Hallmark residue, such partially humanized Nanobodies without Hallmark residues and such fully humanized Nanobodies all form aspects of this invention).
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In these Nanobodies, the CDR sequences are generally as further defined herein.

Again, such Nanobodies may be derived in any suitable manner and from any suitable source, and may for example be naturally occurring V_{HH} sequences (i.e. from a suitable species of Camelid) or synthetic or semi-synthetic amino acid sequences, including but not

limited to “humanized” (as defined herein) Nanobodies, “camelized” (as defined herein) immunoglobulin sequences (and in particular camelized heavy chain variable domain sequences), as well as Nanobodies that have been obtained by techniques such as affinity maturation (for example, starting from synthetic, random or naturally occurring

5 immunoglobulin sequences), CDR grafting, veneering, combining fragments derived from different immunoglobulin sequences, PCR assembly using overlapping primers, and similar techniques for engineering immunoglobulin sequences well known to the skilled person; or any suitable combination of any of the foregoing as further described herein. Also, when a Nanobody comprises a V_{HH} sequence, said Nanobody may be suitably humanized, as further
10 described herein, so as to provide one or more further (partially or fully) humanized Nanobodies of the invention. Similarly, when a Nanobody comprises a synthetic or semi-synthetic sequence (such as a partially humanized sequence), said Nanobody may optionally be further suitably humanized, again as described herein, again so as to provide one or more further (partially or fully) humanized Nanobodies of the invention.

15 In particular, humanized Nanobodies may be amino acid sequences that are as generally defined for Nanobodies in the previous paragraphs, but in which at least one amino acid residue is present (and in particular, in at least one of the framework residues) that is and/or that corresponds to a humanizing substitution (as defined herein). Some preferred, but non-limiting humanizing substitutions (and suitable combinations thereof) will become clear
20 to the skilled person based on the disclosure herein. In addition, or alternatively, other potentially useful humanizing substitutions can be ascertained by comparing the sequence of the framework regions of a naturally occurring V_{HH} sequence with the corresponding framework sequence of one or more closely related human V_H sequences, after which one or more of the potentially useful humanizing substitutions (or combinations thereof) thus
25 determined can be introduced into said V_{HH} sequence (in any manner known per se, as further described herein) and the resulting humanized V_{HH} sequences can be tested for affinity for the target, for stability, for ease and level of expression, and/or for other desired properties. In this way, by means of a limited degree of trial and error, other suitable humanizing substitutions (or suitable combinations thereof) can be determined by the skilled person based on the
30 disclosure herein. Also, based on the foregoing, (the framework regions of) a Nanobody may be partially humanized or fully humanized.

Some particularly preferred humanized Nanobodies of the invention are humanized variants of the Nanobodies of SEQ ID NO's: 399 to 471.

Thus, some other preferred Nanobodies of the invention are Nanobodies which can bind (as further defined herein) to IL-6R and which:

- a) are a humanized variant of one of the amino acid sequences of SEQ ID NO's: 399 to 471; and/or
- 5 b) have 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 399 to 471, in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences are disregarded;

and in which:

- c) preferably one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 10 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table A-3 below

Some preferred, but non-limiting examples of humanized Nanobodies of the Invention are given in SEQ ID NO's: 609 to 628.

According to another specific aspect of the invention, the invention provides a number 15 of stretches of amino acid residues (i.e. small peptides) that are particularly suited for binding to IL-6R. These stretches of amino acid residues may be present in, and/or may be incorporated into, an amino acid sequence of the invention, in particular in such a way that they form (part of) the antigen binding site of an amino acid sequence of the invention. As these stretches of amino acid residues were first generated as CDR sequences of heavy chain antibodies or V_{HH} 20 sequences that were raised against IL-6R (or may be based on and/or derived from such CDR sequences, as further described herein), they will also generally be referred to herein as “CDR sequences” (i.e. as CDR1 sequences, CDR2 sequences and CDR3 sequences, respectively). It should however be noted that the invention in its broadest sense is not limited to a specific 25 structural role or function that these stretches of amino acid residues may have in an amino acid sequence of the invention, as long as these stretches of amino acid residues allow the amino acid sequence of the invention to bind to IL-6R. Thus, generally, the invention in its broadest sense comprises any amino acid sequence that is capable of binding to IL-6R and that comprises one or more CDR sequences as described herein and, and in particular a suitable combination of two or more such CDR sequences, that are suitably linked to each 30 other via one or more further amino acid sequences, such that the entire amino acid sequence forms a binding domain and/or binding unit that is capable of binding to IL-6R. It should however also be noted that the presence of only one such CDR sequence in an amino acid sequence of the invention may by itself already be sufficient to provide an amino acid

sequence of the invention that is capable of binding to IL-6R; reference is for example again made to the so-called “Expedite fragments” described in WO 03/050531.

Thus, in another specific, but non-limiting aspect, the amino acid sequence of the invention may be an amino acid sequence that comprises at least one amino acid sequence that is chosen from the group consisting of the CDR1 sequences, CDR2 sequences and CDR3 sequences that are described herein (or any suitable combination thereof). In particular, an amino acid sequence of the invention may be an amino acid sequence that comprises at least one antigen binding site, wherein said antigen binding site comprises at least one amino acid sequence that is chosen from the group consisting of the CDR1 sequences, CDR2 sequences and CDR3 sequences that are described herein (or any suitable combination thereof).

Generally, in this aspect of the invention, the amino acid sequence of the invention may be any amino acid sequence that comprises at least one stretch of amino acid residues, in which said stretch of amino acid residues has an amino acid sequence that corresponds to the sequence of at least one of the CDR sequences described herein. Such an amino acid sequence may or may not comprise an immunoglobulin fold. For example, and without limitation, such an amino acid sequence may be a suitable fragment of an immunoglobulin sequence that comprises at least one such CDR sequence, but that is not large enough to form a (complete) immunoglobulin fold (reference is for example again made to the “Expedite fragments” described in WO 03/050531). Alternatively, such an amino acid sequence may be a suitable “protein scaffold” that comprises least one stretch of amino acid residues that corresponds to such a CDR sequence (i.e. as part of its antigen binding site). Suitable scaffolds for presenting amino acid sequences will be clear to the skilled person, and for example comprise, without limitation, to binding scaffolds based on or derived from immunoglobulins (i.e. other than the immunoglobulin sequences already described herein), protein scaffolds derived from protein A domains (such as Affibodies™), tendamistat, fibronectin, lipocalin, CTLA-4, T-cell receptors, designed ankyrin repeats, avimers and PDZ domains (Binz et al, Nat. Biotech 2005, Vol 23:1257), and binding moieties based on DNA or RNA including but not limited to DNA or RNA aptamers (Ulrich et al. Comb Chem High Throughput Screen 2006 9(8):619-32).

Again, any amino acid sequence of the invention that comprises one or more of these CDR sequences is preferably such that it can specifically bind (as defined herein) to IL-6R, and more in particular such that it can bind to IL-6R with an affinity (suitably measured and/or expressed as a K_D -value (actual or apparent), a K_A -value (actual or apparent), a k_{on} -rate

and/or a k_{off} -rate, or alternatively as an IC_{50} value, as further described herein), that is as defined herein.

More in particular, the amino acid sequences according to this aspect of the invention may be any amino acid sequence that comprises at least one antigen binding site, wherein said antigen binding site comprises at least two amino acid sequences that are chosen from the group consisting of the CDR1 sequences described herein, the CDR2 sequences described herein and the CDR3 sequences described herein, such that (i) when the first amino acid sequence is chosen from the CDR1 sequences described herein, the second amino acid sequence is chosen from the CDR2 sequences described herein or the CDR3 sequences described herein; (ii) when the first amino acid sequence is chosen from the CDR2 sequences described herein, the second amino acid sequence is chosen from the CDR1 sequences described herein or the CDR3 sequences described herein; or (iii) when the first amino acid sequence is chosen from the CDR3 sequences described herein, the second amino acid sequence is chosen from the CDR1 sequences described herein or the CDR3 sequences described herein.

Even more in particular, the amino acid sequences of the invention may be amino acid sequences that comprise at least one antigen binding site, wherein said antigen binding site comprises at least three amino acid sequences that are chosen from the group consisting of the CDR1 sequences described herein, the CDR2 sequences described herein and the CDR3 sequences described herein, such that the first amino acid sequence is chosen from the CDR1 sequences described herein, the second amino acid sequence is chosen from the CDR2 sequences described herein, and the third amino acid sequence is chosen from the CDR3 sequences described herein. Preferred combinations of CDR1, CDR2 and CDR3 sequences will become clear from the further description herein. As will be clear to the skilled person, such an amino acid sequence is preferably an immunoglobulin sequence (as further described herein), but it may for example also be any other amino acid sequence that comprises a suitable scaffold for presenting said CDR sequences.

Thus, in one specific, but non-limiting aspect, the invention relates to an amino acid sequence directed against IL-6R, that comprises one or more stretches of amino acid residues chosen from the group consisting of:

- a) the amino acid sequences of SEQ ID NO's: 93 to 143;
- b) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 93 to 143;

- c) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 93 to 143;
- d) the amino acid sequences of SEQ ID NO's: 195 to 245;
- e) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: SEQ ID NO's: 195 to 245;
- 5 f) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 195 to 245;
- g) the amino acid sequences of SEQ ID NO's: 297 to 347;
- h) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 297 to 347;
- 10 i) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 297 to 347;
- or any suitable combination thereof.

When an amino acid sequence of the invention contains one or more amino acid

15 sequences according to b) and/or c):

- i) any amino acid substitution in such an amino acid sequence according to b) and/or c) is preferably, and compared to the corresponding amino acid sequence according to a), a conservative amino acid substitution, (as defined herein);

and/or

20 ii) the amino acid sequence according to b) and/or c) preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the corresponding amino acid sequence according to a);

and/or

- iii) the amino acid sequence according to b) and/or c) may be an amino acid sequence that is derived from an amino acid sequence according to a) by means of affinity maturation using one or more techniques of affinity maturation known per se.

25 Similarly, when an amino acid sequence of the invention contains one or more amino acid sequences according to e) and/or f):

- i) any amino acid substitution in such an amino acid sequence according to e) and/or f) is preferably, and compared to the corresponding amino acid sequence according to d), a conservative amino acid substitution, (as defined herein);

and/or

ii) the amino acid sequence according to e) and/or f) preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the corresponding amino acid sequence according to d);

and/or

5 iii) the amino acid sequence according to e) and/or f) may be an amino acid sequence that is derived from an amino acid sequence according to d) by means of affinity maturation using one or more techniques of affinity maturation known per se.

Also, similarly, when an amino acid sequence of the invention contains one or more amino acid sequences according to h) and/or i):

10 i) any amino acid substitution in such an amino acid sequence according to h) and/or i) is preferably, and compared to the corresponding amino acid sequence according to g), a conservative amino acid substitution, (as defined herein);

and/or

15 ii) the amino acid sequence according to h) and/or i) preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the corresponding amino acid sequence according to g);

and/or

20 iii) the amino acid sequence according to h) and/or i) may be an amino acid sequence that is derived from an amino acid sequence according to g) by means of affinity maturation using one or more techniques of affinity maturation known per se.

It should be understood that the last preceding paragraphs also generally apply to any amino acid sequences of the invention that comprise one or more amino acid sequences according to b), c), e), f), h) or i), respectively.

25 In this specific aspect, the amino acid sequence preferably comprises one or more stretches of amino acid residues chosen from the group consisting of:

i) the amino acid sequences of SEQ ID NO's: 93 to 143;
ii) the amino acid sequences of SEQ ID NO's: 195 to 245; and
iii) the amino acid sequences of SEQ ID NO's: 297 to 347;

or any suitable combination thereof.

30 Also, preferably, in such an amino acid sequence, at least one of said stretches of amino acid residues forms part of the antigen binding site for binding against IL-6R.

In a more specific, but again non-limiting aspect, the invention relates to an amino acid sequence directed against IL-6R, that comprises two or more stretches of amino acid residues chosen from the group consisting of:

- a) the amino acid sequences of SEQ ID NO's: 93 to 143;
- 5 b) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 93 to 143;
- c) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 93 to 143;
- d) the amino acid sequences of SEQ ID NO's: 195 to 245;
- 10 e) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 195 to 245;
- f) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 195 to 245;
- 15 g) the amino acid sequences of SEQ ID NO's: 297 to 347;
- h) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 297 to 347;
- i) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 297 to 347;

such that (i) when the first stretch of amino acid residues corresponds to one of the amino acid sequences according to a), b) or c), the second stretch of amino acid residues corresponds to one of the amino acid sequences according to d), e), f), g), h) or i); (ii) when the first stretch of amino acid residues corresponds to one of the amino acid sequences according to d), e) or f), the second stretch of amino acid residues corresponds to one of the amino acid sequences according to a), b), c), g), h) or i); or (iii) when the first stretch of amino acid residues corresponds to one of the amino acid sequences according to g), h) or i), the second stretch of amino acid residues corresponds to one of the amino acid sequences according to a), b), c), d), e) or f).

In this specific aspect, the amino acid sequence preferably comprises two or more stretches of amino acid residues chosen from the group consisting of:

- 30 i) the amino acid sequences of SEQ ID NO's: 93 to 143;
- ii) the amino acid sequences of SEQ ID NO's: 195 to 245; and
- iii) the amino acid sequences of SEQ ID NO's: 297 to 347;

such that, (i) when the first stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 93 to 143, the second stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 195 to 245 or of SEQ ID NO's: 297 to 347; (ii) when the first stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 195 to 245, the second stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 93 to 143 or of SEQ ID NO's: 297 to 347; or (iii) when the first stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 297 to 347, the second stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 93 to 143 or of SEQ ID NO's: 195 to 245.

Also, in such an amino acid sequence, the at least two stretches of amino acid residues again preferably form part of the antigen binding site for binding against IL-6R.

In an even more specific, but non-limiting aspect, the invention relates to an amino acid sequence directed against IL-6R, that comprises three or more stretches of amino acid residues, in which the first stretch of amino acid residues is chosen from the group consisting of:

- a) the amino acid sequences of SEQ ID NO's: 93 to 143;
- b) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 93 to 143;
- 20 c) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 93 to 143;

the second stretch of amino acid residues is chosen from the group consisting of:

- d) the amino acid sequences of SEQ ID NO's: 195 to 245;
- e) amino acid sequences that have at least 80% amino acid identity with at least one of the 25 amino acid sequences of SEQ ID NO's: 195 to 245;
- f) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 195 to 245;

and the third stretch of amino acid residues is chosen from the group consisting of:

- g) the amino acid sequences of SEQ ID NO's: 297 to 347;
- 30 h) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 297 to 347;
- i) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 297 to 347.

Preferably, in this specific aspect, the first stretch of amino acid residues is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 93 to 143; the second stretch of amino acid residues is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 195 to 245; and the third stretch of amino acid residues is chosen 5 from the group consisting of the amino acid sequences of SEQ ID NO's: 297 to 347.

Again, preferably, in such an amino acid sequence, the at least three stretches of amino acid residues forms part of the antigen binding site for binding against IL-6R.

Preferred combinations of such stretches of amino acid sequences will become clear from the further disclosure herein.

10 Preferably, in such amino acid sequences the CDR sequences have at least 70% amino acid identity, preferably at least 80% amino acid identity, more preferably at least 90% amino acid identity, such as 95% amino acid identity or more or even essentially 100% amino acid identity with the CDR sequences of at least one of the amino acid sequences of SEQ ID NO's 399 to 471

15 Also, such amino acid sequences are preferably such that they can specifically bind (as defined herein) to IL-6R; and more in particular bind to IL-6R with an affinity (suitably measured and/or expressed as a K_D -value (actual or apparent), a K_A -value (actual or apparent), a k_{on} -rate and/or a k_{off} -rate, or alternatively as an IC_{50} value, as further described herein) that is as defined herein.

20 When the amino acid sequence of the invention essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), the amino acid sequence of the invention is preferably such that:

- CDR1 is chosen from the group consisting of:
 - a) the amino acid sequences of SEQ ID NO's: 93 to 143;
 - 25 b) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 93 to 143;
 - c) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 93 to 143;

and/or

- 30 - CDR2 is chosen from the group consisting of:
 - d) the amino acid sequences of SEQ ID NO's: 195 to 245;
 - e) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 195 to 245;

f) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 195 to 245;

and/or

- CDR3 is chosen from the group consisting of:

5 g) the amino acid sequences of SEQ ID NO's: 297 to 347;

h) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 297 to 347;

i) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 297 to 347.

10 In particular, such an amino acid sequence of the invention may be such that CDR1 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 93 to 143; and/or CDR2 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 195 to 245; and/or CDR3 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 297 to 347.

15 In particular, when the amino acid sequence of the invention essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), the amino acid sequence of the invention is preferably such that:

- CDR1 is chosen from the group consisting of:

20 a) the amino acid sequences of SEQ ID NO's: 93 to 143;

b) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 93 to 143;

c) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 93 to 143;

25 and

- CDR2 is chosen from the group consisting of:

d) the amino acid sequences of SEQ ID NO's: 195 to 245;

e) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 195 to 245;

30 f) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 195 to 245;

and

- CDR3 is chosen from the group consisting of:

- g) the amino acid sequences of SEQ ID NO's: 297 to 347;
- h) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 297 to 347;
- i) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 297 to 347; or any suitable fragment of such an amino acid sequence

5 In particular, such an amino acid sequence of the invention may be such that CDR1 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 93 to 143; and CDR2 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 10 195 to 245; and CDR3 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 297 to 347.

Again, preferred combinations of CDR sequences will become clear from the further description herein.

15 Also, such amino acid sequences are preferably such that they can specifically bind (as defined herein) to IL-6R; and more in particular bind to IL-6R with an affinity (suitably measured and/or expressed as a K_D -value (actual or apparent), a K_A -value (actual or apparent), a k_{on} -rate and/or a k_{off} -rate, or alternatively as an IC_{50} value, as further described herein) that is as defined herein.

20 In one preferred, but non-limiting aspect, the invention relates to an amino acid sequence that essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which the CDR sequences of said amino acid sequence have at least 70% amino acid identity, preferably at least 80% amino acid identity, more preferably at least 90% amino acid identity, such as 95% amino acid identity or more or even essentially 100% amino acid identity with the CDR 25 sequences of at least one of the amino acid sequences of SEQ ID NO's: 399 to 471. This degree of amino acid identity can for example be determined by determining the degree of amino acid identity (in a manner described herein) between said amino acid residue and one or more of the sequences of SEQ ID NO's: 399 to 471, in which the amino acid residues that form the framework regions are disregarded. Such amino acid sequences of the invention can 30 be as further described herein.

In such an amino acid sequence of the invention, the framework sequences may be any suitable framework sequences, and examples of suitable framework sequences will be clear to

the skilled person, for example on the basis the standard handbooks and the further disclosure and prior art mentioned herein.

The framework sequences are preferably (a suitable combination of) immunoglobulin framework sequences or framework sequences that have been derived from immunoglobulin framework sequences (for example, by humanization or camelization). For example, the framework sequences may be framework sequences derived from a light chain variable domain (e.g. a V_L -sequence) and/or from a heavy chain variable domain (e.g. a V_H -sequence). In one particularly preferred aspect, the framework sequences are either framework sequences that have been derived from a V_{HH} -sequence (in which said framework sequences may 5 optionally have been partially or fully humanized) or are conventional V_H sequences that have 10 been camelized (as defined herein).

The framework sequences are preferably such that the amino acid sequence of the invention is a domain antibody (or an amino acid sequence that is suitable for use as a domain antibody); is a single domain antibody (or an amino acid sequence that is suitable for use as a 15 single domain antibody); is a "dAb" (or an amino acid sequence that is suitable for use as a dAb); or is a Nanobody® (including but not limited to V_{HH} sequence). Again, suitable framework sequences will be clear to the skilled person, for example on the basis the standard handbooks and the further disclosure and prior art mentioned herein.

In particular, the framework sequences present in the amino acid sequences of the 20 invention may contain one or more of Hallmark residues (as defined herein), such that the amino acid sequence of the invention is a Nanobody®. Some preferred, but non-limiting examples of (suitable combinations of) such framework sequences will become clear from the further disclosure herein.

Again, as generally described herein for the amino acid sequences of the invention, it 25 is also possible to use suitable fragments (or combinations of fragments) of any of the foregoing, such as fragments that contain one or more CDR sequences, suitably flanked by and/or linked via one or more framework sequences (for example, in the same order as these CDR's and framework sequences may occur in the full-sized immunoglobulin sequence from which the fragment has been derived). Such fragments may also again be such that they 30 comprise or can form an immunoglobulin fold, or alternatively be such that they do not comprise or cannot form an immunoglobulin fold.

In one specific aspect, such a fragment comprises a single CDR sequence as described herein (and in particular a CDR3 sequence), that is flanked on each side by (part of) a

framework sequence (and in particular, part of the framework sequence(s) that, in the immunoglobulin sequence from which the fragment is derived, are adjacent to said CDR sequence. For example, a CDR3 sequence may be preceded by (part of) a FR3 sequence and followed by (part of) a FR4 sequence). Such a fragment may also contain a disulphide bridge,

5 and in particular a disulphide bridge that links the two framework regions that precede and follow the CDR sequence, respectively (for the purpose of forming such a disulphide bridge, cysteine residues that naturally occur in said framework regions may be used, or alternatively cysteine residues may be synthetically added to or introduced into said framework regions). For a further description of these “Expedite fragments”, reference is again made to WO

10 03/050531).

Table A-1: Preferred combinations of CDR and framework sequences.

Clone	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
PMP40C9(t)	42	EVQLVESGGGLVQPG GSLRLSCAASGFLSLD	93	YYAIG	144	WFRQAPGK EREGVS	195	CMDSSAGTT STYYSDSVK G	246	RFTISRDDAKN TVYLQMNSLKP EDTAVYYCAA	297	DGHLNWGQ RYVPCSQIS WRGWNDY	348	WGQGTQ VTVSS
PMP34F8(t)	43	EVQLVESGGGLVQPG GSLRLSCAASGFLSLD	94	YYAIG	145	WFRQAPGK EREGVS	196	CMDSSDGTT NTYYSDSVK G	247	RFTISRDDAKN TVYLQMNSLKP EDTASYYCAA	298	DGHLNWGQ PYVPCSQIS WRGWNDY	349	WGQGTQ VTVSS
PMP34E9(t)	44	EVQLVESGGGLVQPG GSLRLSCAASGFTLD	95	YYAIG	146	WFRQAPGK EREGVS	197	CISSDGSTY YADSVKG	248	RFTISRDDAKN TVYLQMNSLKP EDTAAYYCAT	299	DRSVYYCSG DAPEEYY	350	WGQGTQ VTVSS
PMP34D2(t)	45	EVQLVESGGGLVQPG GSLRLSCAASGFTLD	96	YFAIG	147	WFRQAPGK ERERVS	198	CISSDGSTY YADSVKG	249	RFTISRDDAKN TVYLQMNSLKP EDTAVYYCAT	300	DRSVYYCSG GAPEEYY	351	WGQGTQ VTVSS
PMP34C3(t)	46	EVQLVESGGGLVQPG GSLRLSCV/ASGFLSLD	97	YYVIG	148	WFRQAPGK EREGVS	199	CISSDGSTY YADSVKG	250	RFTISRDDAKN TVYLQMNSLKP EDTAVYYCAA	301	DLRLTPEFC VDSAPYDY	352	WGRGTQ VTVSS
PMP34A5(t)	47	EVQLVESGGGLVQPG GSLRLSCAASGFTLG	98	YFAIG	149	WFRQAPGK EREGVS	200	CISSDGSTY YADSVKG	251	RFTVSRDNAK NTVYLQMNLSK PEDTAVYYCAT	302	DRSVYYCSG GAPEEYY	353	WGQGTQ VTVSS
PMP33G3(t)	48	EVQLVESGGGLVQ PGGSRLSCAASG FTLG	99	YFAIG	150	WFRQAPG KEREVS	201	CISSDGSA YYADSVKG	252	RFTVSRDNA KNTVYLQMN SLKPEDTAVY YCAT	303	DRSVYYCS GGAPEEYY	354	WGQGT QVTVSS
PMP33C10(t)	49	EVQLVESGGGLVQ PGGSRLSCAASG FTFD								RFTISRDDAKN NTVYLQMN LKPEDTAVYY CVK		GSTAIVGV PPYPDEY DY	355	WGQGT QVTVSS

Table A-1 (continued):

CLONE	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
PMP33A2(t)	50	EVQLVESGGGLVQPG GSLRLSCAASGFSLD	101	YYAIG	152	WFRQAPG KEREVGS	203	CMDSSGGTT STYSDSVKG	254	RFTISRDDAKN TVYLQMNSLKP EDTAVYYCAA	305	DGHLNWQGQ RYVPCSQIS WRGWNDY	356	WGQGT QVTVSS
PMP32H5(t)	51	EVQLVESGGGLVQPG GSLRLSCAASGFTFG	102	SYDMS	153	WVRQAPG KGPEWVS	204	AINSGGGST YYADSVKG	255	RFTISRDNAKN TLYLQMNSLKP EDTAVYYCAT	306	DWRYSDYDL PLPPPGDY	357	WGQGT QVTVSS
PMP32F10(t)	52	EVQLVESGGGLVQAG GSLRLSCAASGFTFD	103	DYAIG	154	WFRQAPG KEREGIS	205	CISSDGST YYADSVKG	256	RFTISSDNAKN TVYLQMNSLKP EDTAVYYCAA	307	EPPDSSWIL DGSPEFFKF	358	WGQGT QVTVSS
PMP31F4(t)	53	EVQLVESGGGLVQPG GSLRLSCAASGFTFG	104	SYDMS	155	WVRQAPG KGPEWVS	206	AINSGGGST YYADSVKG	257	RFTISRDNAKN TLYLQMNSLKP EDTAVYYCAT	308	DWRYSDYDL PLPPPGDY	359	WGQGT QVTVSS
PMP31D2(t)	54	EVQLVESGGGLVQAG GSLRLSCAASGFTFD	105	DYAIG	156	WFRQAPG KEREVGS	207	GISSDGNT YYADSVKG	258	RFTISSDNAKN TVYLQMNSLKP EDTAVYYCAA	309	EPPDSNWYL DGSPEFFKF	360	WGQGT QVTVSS
PMP31C8(t)	55	EVQLVESGGGLVQPG GSLRLSCAASGFTFG	106	SYDMS	157	WVRQAPG KGPEWVS	208	AINSGGGST YYADSVKG	259	RFTISRDNAKN TLYLQMNSLKP EDTAVYYCAT	310	DWRYSDYDL PLPPPGDY	361	WGQGT QVTVSS
PMP31C5(t)	56	EVQLVESGGGLVQAG GSLRLSCAASGFTFD	107	DYAIG	158	WFRQAPG KEREVGS	209	CISSDGST YYADSVKG	260	RFTISSDNAKN TVYLLMNSLKP EDTAVYYCAA	311	EPPDSMWSL DGSPEFFKF	362	WGQGT QVTVSS
PMP31B4(t)	57	EVQLVESGGGLVQPG GSLRLSCAASGFTFG	108	SYDMS	159	WVRQAPG KGPEWVS	210	AINSGGGST YYADSVKG	261	RFTISRDNAKN TLYLQMNSLKP EDTAVYYCAT	312	DWRYSDYDL PLPPPGDY	363	WGQGT QVTVSS

Table A-1 (continued):

CLONE	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
PMP31B11(t)	58	EVQLVESGGGLVQPG GSLRLSCAASGFTFG	109	SYDMS	160	WVRQAPG KGPEWVS	211	AINSGGGST YYADSVKG	262	RFTISRDNAKN TLYQMNLSLKP EDTAVYYCAT	313	DWRYSDYDL PLPPPGDY	364	WGQGTQ VTVSS
PMP30G11(t)	59	EVQLVESGGGLVQPG GSLRLSCVASGFTSLD	110	YYVIG	161	WFRQAPG KEREVGS	212	CISSDGSTY YADSVKG	263	RFTISRDNAKN TVLQMNLSLKP EDTAVYYCAA	314	DLLRTPEFC VDSAPYDY	365	WGQGTQ VTVSS
PMP30B6(t)	60	EVQLVESGGGLVQPG GSLRLSCAASGFTLD	111	YYVIG	162	WFRQAPG KEREAVA	213	CISSDRSTY YADSVKG	264	RFTISRDNAKN TGQLQMNLSK PEDTAVYYCAA	315	DLLRTPEFC SDSAPYDY	366	WGQGTQ VTVSS
PMP30B1(t)	61	EVQLVESGGGLVQPG GSLRLSCAASGFTLD	112	YYAIG	163	WWRQAPG KGREGVS	214	CISGDDGST NYADSVKG	265	RFTISRDNAKN TVLQMNLSLKP EDTAVYYCAT	316	DRSVYYCSG GAPEEYY	367	WGQGTQ VTVSS
PMP30A2(t)	62	EVQLVESGGGLVQPG GSLRLSCAASGFTLD	113	YYVIG	164	WFRQAPG KEREVGS	215	CIGSSDDST YYADSVKG	266	RFTISRDNAKN TVLQMNLSLKP EDTAVYYCAA	317	DLLRTPEFC DSAPYDY	368	WGQGTQ VTVSS
PMP30A10(t)	63	EVQLVESGGGLVQPG GSLRLSCAASGFTFD	114	DYGMS	165	WVRQAPG KGLEWVS	216	AISWNGGST YYTESMKG	267	RFTISRDNAKN TVLQMNLSLKP EDTAVYYCVK	318	GSTAIVGVPP TYPDEYDY	369	WGQGTQ VTVSS
PMP28H6(t)	64	EVQLVESGGGLVQPG GSLRLSCAASGFTFG	115	SYDMS	166	WVRQAPG KGPEWVS	217	AINSGGGST YYADSVKG	268	RFTISRDNAKN TLYQMNLSLKP EDTAVYYCAT	319	DWRYSDYDL PLPPPGDY	370	WGQGTQ VTVSS
PMP28F7(t)	65	EVQLVESGGGLVQPG GSLRLSCAASGFTFG	116	SYDMS	167	WVRQAPG KGPEWVS	218	AINSGGGST YYADSVKG	269	RFTISRDNAKN TLYQMNLSLKP EDTAVYYCAT	320	DWRYSDYDL PLPPPGDY	371	WGQGTQ VTVSS
PMP28D4(t)	66	EVQLVESGGGLVQPG GSLRLSCAASGFTFG	117	SYDMS	168	WVRQAPG KGPEWVS	219	AINSGGGST YYADSVKG	270	RFTISRDNAKN TLYQMNLSLKP EDTAVYYCAT	321	DWRYSDYDL PLPPPGDY	372	WGQGTQ VTVSS

Table A-1 (continued):

CLONE	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
PMP28C7(t)	67	EVQLVESGGGLVQPG GSLRLSCAASGFTFG	118	SYDMS	169	WVRQAPG KGPEWVS	220	AINSGGDNTY YADSVKG	271	RFTISRDNNAKNT LYLQMNSLKPED TAVYYCAT	322	DWRYSDYDLP LPPPGDY	373	WGQGTQ VTVSS
PMP28B1(t)	68	EVQLVESGGGLVQPG GSLRLSCAASGFTLN	119	YYAIG	170	WFRQAPG KEREVGS	221	CISSDGSTY YADSVKG	272	RFTISRDNNAKNT FYLQMNSLKPED TAVYYCAA	323	EGLGDSDFPC GAAWYNDY	374	WGQGTQ VTVSS
PMP28A2(t)	69	EVQLVESGGGLVQPG GSLRLSCAASGFTFG	120	SYDMS	171	WVRQAPG KGPEWVS	222	AINSGGGSTY YADSVKG	273	RFTISRDNNAKNT LYLQMNSLKPED TAVYYCAT	324	DWRYSDYDLP LPPPGDY	375	WGQGTQ VTVSS
PMP40H5	70	EVQLVESGGGLVQPG GSLRLSCAASGFSLD	121	YYAIG	172	WFRQAPG KEREVGS	223	CMDSSGGTT STYYSDSVKG	274	RFTISRDNNAKNT VYLQMNSLKPED TAVYYCAA	325	DGHLNWGQR YVPCSQISWR GWNDY	376	WGQGTQ VTVSS
PMP35H4	71	EVQLVESGGGLVQPG GSLRLSCAASGFTFD	122	DYGMS	173	WVRQAPG RATEWVS	224	AISWNGNNTY YTESMKG	275	RFTISRDNNAKNT VYLQMNSLKPED TAVYYCVK	326	GSTAIVGVPP YPDEYDY	377	WGQGTQ VTVSS
PMP35F4	72	EVQLVESGGGLVQAG GSLRLSCAASGRTFS	123	SYDMG	174	WYRQAPG KEREVFA	225	IIIWNSSTYYA DSVKG	276	RFTISRDNNAKNT VYLQMNSLKPED TAIYYCNA	327	QYGLGYAEDY	378	WGQGTQ VTVSS
PMP35E11	73	EVQLVESGGGLVQAG GSLRLSCAASGRTFD	124	DYAIG	175	WFRQAPG KEHEGV	226	CISSDGSTY YADSVKG	277	RFTISRDNNAKNT VYLQMNSLKPED TAVYYCAA	328	ERDVPARSLC GSYYWYDY	379	RQGQTQ VTVSS
PMP35C10	74	EVQLVESGGGLVQAG GSLRLSCAASGRTFS	125	SYDMG	176	WYRQAPG KEREVFA	227	VIHWSSGGSTY YADPVKG	278	RFTISRDNNAKNT VYLQMNSLKPED TAIYYCNA	329	FLPGPEGFH Y	380	WGQGTQ VTVSS
PMP34G9	75	EVQLVESGGGLVQAG GSLRLSCAASGRTSS	126	SYDMT	177	WYRQVPG KEREVFA	228	VIWSGGSTY YADSVKG	279	RFTISRDNNAKNT VYLQMNSLKPED TAIYYCNA	330	YTGGDDY	381	WGQGTQ VTVSS

Table A-1 (continued):

CLONE	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
PMP34G3	76	EVQLVESGGGLVQPG GSLRLSCAASGFTLD	127	YYAIG	178	WFRQAPG KEREV/S	229	CISSSDGSTY YADSV/KG	280	RFTISRDNAKN TVYLQMNSLKP EDTAAYYCAT	331	DRSVYYCSG GAPEEYY	382	WGQGTQ VTVSS
PMP34E10	77	EVQLVESGGGLVQAG GSLRLSCAASGRTFS	128	SYAMG	179	WGRQAPG KEREV/A	230	TISWSGGST YYADSV/KG	281	RFTISRDNAKN TVYLQMNSLKP EDTAVYYCAA	332	DLAEFKYSD YADY	383	WGQGTQ VTVSS
PMP34C11	78	EVQLVESGGGLVQPG GSLRLSCAAGFTLD	129	YSAIG	180	WFRQAPG KEREMFS	231	CISGSDGST WYADSV/AG	282	RFTISFDNAKN TVYLQMNSLKP EDTGlyCAV	333	TGGVRGPCA YEYEV	384	WGQGTQ VTVSS
PMP34A12	79	EVQLVESGGGLVQPG GSLRLSCVASGFSLD	130	YYVIG	181	WFRQAPG KEREV/S	232	CISSSDGSTY YADSV/KG	283	RFTISRDNAKN TVYLQMNSLKP EDTAVYYCAA	334	DLLRTEF/C VDSAPYDY	385	WGQGTQ VTVSS
PMP33A3	80	EVQLVESGGGLVQPG GSLRLSCAASGFTLD	131	YGAIG	182	WFRQAPG KEREV/S	233	CISSSTGSTY YADSV/KG	284	RFTISRDNGKN TVYLQMNSLKP EDTAVYYCAA	335	DKMW/SPCL VAAANEEALF EYDY	386	WGQGTQ VTVSS
PMP32E2	81	EVQLVESGGGLVQAG GSLRLSCAASGNID	132	DNTMGWT	183	WNRQPPG KQRELVA	234	IIATDGSTNY ADSV/KG	285	RFTISRDNAKN TVYLQMNSLKP EDTAVYYCNL	336	FSLRLGRDY	387	WGQGTQ VTVSS
PMP32E10	82	EVQLVESGGGLVQPG GSLRLSCAASGFTFG	133	SYDMS	184	WVRQAPG KGPEW/S	235	AINSGGGST YYADSV/KG	286	RFTISRDNAKN TLYLQMNSLKP EDTAVYYCAT	337	DW/RYSDYDL PLPPGDY	388	WGQGTQ VTVSS
PMP32C9	83	EVQLVESGGGLVQAG GSLRLSCAASGFTFD	134	DYDIG	185	WFRQAPG KEREV/S	236	GISSSDGNT YYADSV/KG	287	RFTISSDNAKN TVYLQMNSLKP EDTAVYYCAA	338	EPPDSSWYL DGSPEFFKY	389	WGQGTQ VTVSS
PMP31A4	84	EVQLVESGGGLVQAG GSLRLSCAASGSIFK	135	VNAMG	186	WYRQAPG KQRELVA	237	GISSGGSTNY ADSV/KG	288	RLTISRDNAKN TVYLQMNSLKP EDTAVYYCSF	339	VTTNSDYDL GRDY	390	WGQGTQ VTVSS

Table A-1 (continued):

CLONE	ID	FR1	ID	CDR1	ID	FR2	ID	CDR2	ID	FR3	ID	CDR3	ID	FR4
PMP30C11	85	EVQLVESGGGLVQAG GSRLSCAASGRTFS	136	SYDMG	187	WYRQAPG KEREFGVA	238	VISRSGSSTY YADSVKG	289	RFTISRDNAKNT VYLQMNLSLKPED TAIYYCKA	340	EVVAGD YDY	391	WGQGTQV TVSS
PMP28G3	86	EVQLVESGGGLVQAG GSRLSCSCTASGNIFS	137	TETMG	188	WYRQPPG KQRDVV	239	ATITHGGTTN YADSVKG	290	RFTISRDNRKNT VYLQMNLSLKPED GVYYCNA	341	RSSWYS PEY	392	WGQGTQV TVSS
PMP28E11	87	EVQLVESGGGFVQAG GSRLSCIASGDNFS	138	INRMG	189	WYRQALG KQRELVA	240	IITNHGSTNY ADAVKG	291	RFTISRDYAKNTV YLQMMNGLKPDFT AVYYCNA	342	YISEVGT WRDDY	393	WGQGTQV TVSS
059B.IL6R.cl5.7(t)	88	EVQLVESGGGLVQAG GSRLSCAASGRTFS	139	GADAG	190	WNRQTPG KEREFGVA	241	AINWSGNST YYADSVKG	292	RFTISRDNAKNT VYLQMNLSLKPED TAIYYCHA	343	FRDDYYS	394	EGKGTLVT VSS
059A.IL6Rcl4(t)	89	EVQLVESGGGLVQAG GSRLSCAASGRTLS	140	SYDMG	191	WYRQGPG KEREFGVA	242	AISWSSGGT DYVDSVKG	293	RFTISRDYAKNT YLQMNLSLKPED TAIYYCNA	344	LGTTDSD YEGETY	395	WGQGTQV TVSS
059A.IL6Rcl3(t)	90	EVQLVESGGGLVQPG GSRLSCAASGFTLD	141	SYAIG	192	WFRQAPG KEPEGVS	243	CISTSDGSTY YADSVKG	294	RFTISRDNAKNT VYLQMNLSLKPED TAIYYCTA	345	DGGPHA PLTVQD MCVMAIA DY	396	WGQGTQV TVSS
059A.IL6Rcl2(t)	91	EVQLVESGGGLVQAG GSRLSCAASGRTFS	142	NIAMG	193	WIREAPGK EREFVVA	244	ALTWSGGST YYADSVKG	295	RFTISRDYAKNTV YLQMNKLKPEDT AVYYCVA	346	DEEIHILV SISIADF	397	WGQGTQV TVSS
059A.IL6Rcl1(t)	92	EVQLVESGGGLVQAG GSRLSCAASGLTDD	143	DFAIG	194	WFRQAPG KEPEGVS	245	CISSSDGSTY YADSVKG	296	RFTISRDNAKNTV YLQMNLSLKPEDT AVYFCTA	347	LFDRCGS TWYYGM DY	398	WGKGTLV TVSS

In another aspect, the invention relates to a compound or construct, and in particular a protein or polypeptide (also referred to herein as a “*compound of the invention*” or “*polypeptide of the invention*”, respectively) that comprises or essentially consists of one or more amino acid sequences of the invention (or suitable fragments thereof), and optionally 5 further comprises one or more other groups, residues, moieties or binding units. As will become clear to the skilled person from the further disclosure herein, such further groups, residues, moieties, binding units or amino acid sequences may or may not provide further functionality to the amino acid sequence of the invention (and/or to the compound or construct in which it is present) and may or may not modify the properties of the amino acid 10 sequence of the invention.

For example, such further groups, residues, moieties or binding units may be one or more additional amino acid sequences, such that the compound or construct is a (fusion) protein or (fusion) polypeptide. In a preferred but non-limiting aspect, said one or more other groups, residues, moieties or binding units are immunoglobulin sequences. Even more 15 preferably, said one or more other groups, residues, moieties or binding units are chosen from the group consisting of domain antibodies, amino acid sequences that are suitable for use as a domain antibody, single domain antibodies, amino acid sequences that are suitable for use as a single domain antibody, “dAb”’s, amino acid sequences that are suitable for use as a dAb, or Nanobodies®.

20 Alternatively, such groups, residues, moieties or binding units may for example be chemical groups, residues, moieties, which may or may not by themselves be biologically and/or pharmacologically active. For example, and without limitation, such groups may be linked to the one or more amino acid sequences of the invention so as to provide a “derivative” of an amino acid sequence or polypeptide of the invention, as further described 25 herein.

Also within the scope of the present invention are compounds or constructs, that comprises or essentially consists of one or more derivates as described herein, and optionally further comprises one or more other groups, residues, moieties or binding units, optionally linked via one or more linkers. Preferably, said one or more other groups, residues, moieties 30 or binding units are amino acid sequences.

In the compounds or constructs described above, the one or more amino acid sequences of the invention and the one or more groups, residues, moieties or binding units may be linked directly to each other and/or via one or more suitable linkers or spacers. For

example, when the one or more groups, residues, moieties or binding units are amino acid sequences, the linkers may also be amino acid sequences, so that the resulting compound or construct is a fusion (protein) or fusion (polypeptide).

The compounds or polypeptides of the invention can generally be prepared by a

5 method which comprises at least one step of suitably linking the one or more amino acid sequences of the invention to the one or more further groups, residues, moieties or binding units, optionally via the one or more suitable linkers, so as to provide the compound or polypeptide of the invention. Polypeptides of the invention can also be prepared by a method which generally comprises at least the steps of providing a nucleic acid that encodes a

10 polypeptide of the invention, expressing said nucleic acid in a suitable manner, and recovering the expressed polypeptide of the invention. Such methods can be performed in a manner known per se, which will be clear to the skilled person, for example on the basis of the methods and techniques further described herein.

The process of designing/selecting and/or preparing a compound or polypeptide of the

15 invention, starting from an amino acid sequence of the invention, is also referred to herein as “formatting” said amino acid sequence of the invention; and an amino acid of the invention that is made part of a compound or polypeptide of the invention is said to be “formatted” or to be “*in the format of*” said compound or polypeptide of the invention. Examples of ways in which an amino acid sequence of the invention can be formatted and examples of such

20 formats will be clear to the skilled person based on the disclosure herein; and such formatted amino acid sequences form a further aspect of the invention.

In one specific aspect of the invention, a compound of the invention or a polypeptide of the invention may have an increased half-life, compared to the corresponding amino acid sequence of the invention. Some preferred, but non-limiting examples of such compounds and

25 polypeptides will become clear to the skilled person based on the further disclosure herein, and for example comprise amino acid sequences or polypeptides of the invention that have been chemically modified to increase the half-life thereof (for example, by means of pegylation); amino acid sequences of the invention that comprise at least one additional binding site for binding to a serum protein (such as serum albumin); or polypeptides of the

30 invention that comprise at least one amino acid sequence of the invention that is linked to at least one moiety (and in particular at least one amino acid sequence) that increases the half-life of the amino acid sequence of the invention. Examples of polypeptides of the invention that comprise such half-life extending moieties or amino acid sequences will become clear to

the skilled person based on the further disclosure herein; and for example include, without limitation, polypeptides in which the one or more amino acid sequences of the invention are suitable linked to one or more serum proteins or fragments thereof (such as (human) serum albumin or suitable fragments thereof) or to one or more binding units that can bind to serum 5 proteins (such as, for example, domain antibodies, amino acid sequences that are suitable for use as a domain antibody, single domain antibodies, amino acid sequences that are suitable for use as a single domain antibody, “dAb”’s, amino acid sequences that are suitable for use as a dAb, or Nanobodies can bind to serum proteins such as serum albumin (such as human serum albumin), serum immunoglobulins such as IgG, or transferrine; reference is made to the 10 further description and references mentioned herein); polypeptides in which an amino acid sequence of the invention is linked to an Fc portion (such as a human Fc) or a suitable part or fragment thereof; or polypeptides in which the one or more amino acid sequences of the invention are suitable linked to one or more small proteins or peptides that can bind to serum proteins (such as, without limitation, the proteins and peptides described in WO 91/01743, 15 WO 01/45746, WO 02/076489).

Generally, the compounds or polypeptides of the invention with increased half-life preferably have a half-life that is at least 1.5 times, preferably at least 2 times, such as at least 5 times, for example at least 10 times or more than 20 times, greater than the half-life of the corresponding amino acid sequence of the invention per se. For example, the compounds or 20 polypeptides of the invention with increased half-life may have a half-life that is increased with more than 1 hours, preferably more than 2 hours, more preferably more than 6 hours, such as more than 12 hours, or even more than 24, 48 or 72 hours, compared to the corresponding amino acid sequence of the invention per se.

In a preferred, but non-limiting aspect of the invention, such compounds or 25 polypeptides of the invention have a serum half-life that is increased with more than 1 hour, preferably more than 2 hours, more preferably more than 6 hours, such as more than 12 hours, or even more than 24, 48 or 72 hours, compared to the corresponding amino acid sequence of the invention per se.

In another preferred, but non-limiting aspect of the invention, such compounds or 30 polypeptides of the invention exhibit a serum half-life in human of at least about 12 hours, preferably at least 24 hours, more preferably at least 48 hours, even more preferably at least 72 hours or more. For example, compounds or polypeptides of the invention may have a half-life of at least 5 days (such as about 5 to 10 days), preferably at least 9 days (such as about 9

to 14 days), more preferably at least about 10 days (such as about 10 to 15 days), or at least about 11 days (such as about 11 to 16 days), more preferably at least about 12 days (such as about 12 to 18 days or more), or more than 14 days (such as about 14 to 19 days).

In another aspect, the invention relates to a nucleic acid that encodes an amino acid sequence of the invention or a polypeptide of the invention (or a suitable fragment thereof). Such a nucleic acid will also be referred to herein as a "*nucleic acid of the invention*" and may for example be in the form of a genetic construct, as further described herein.

In another aspect, the invention relates to a host or host cell that expresses (or that under suitable circumstances is capable of expressing) an amino acid sequence of the invention and/or a polypeptide of the invention; and/or that contains a nucleic acid of the invention. Some preferred but non-limiting examples of such hosts or host cells will become clear from the further description herein.

The invention further relates to a product or composition containing or comprising at least one amino acid sequence of the invention (or a suitable fragment thereof), at least one polypeptide of the invention and/or at least one nucleic acid of the invention, and optionally one or more further components of such compositions known per se, i.e. depending on the intended use of the composition. Such a product or composition may for example be a pharmaceutical composition (as described herein), a veterinary composition or a product or composition for diagnostic use (as also described herein). Some preferred but non-limiting examples of such products or compositions will become clear from the further description herein.

In another specific, but non-limiting aspect, the amino acid sequences and polypeptides described herein are such that they (a) specifically bind (as defined herein) to the IL-6 receptor; and (b) are capable of downregulating the IL-6 receptor and/or are capable of inhibiting, decreasing or downregulating the signalling of the IL-6 receptor and/or the pathway(s), mechanism(s) or signalling in which the IL-6 or IL-6R is involved. As will be clear to the skilled person, such an amino acid sequence or polypeptide can generally be used as an antagonist of IL-6, of the IL-6 receptor and/or of the biological pathways, mechanisms or effects in which IL-6, IL-6R and/or IL-6/IL-6R mediated signalling is involved. Any such decrease or downregulation (which can be at least 1%, such as at least 5%, or more than 10%, or up to 50% or 100% or more in a relevant parameter, compared to the same parameter under conditions in which the amino acid sequence or polypeptide is not bound to the IL-6

receptor), may be measured in any suitable manner known per se, for example using one of the assays used in the Experimental Part and/or mentioned herein.

For example, such antagonistic amino acid sequences and polypeptides may be competitive of non-competitive inhibitors of the binding of IL-6 to IL-6R.

5 More in particular, and in addition to (a) and (b) above, and optionally in addition to (d) and/or (e) below, such antagonistic amino acid sequences and polypeptides may bind to IL-6R in such a way that (c) binding of IL-6 to IL-6R is blocked, inhibited or reduced; compared to the binding of IL-6 to its receptor without the presence of the amino acid sequence or Nanobody of the invention.

10 For example, and without limitation, such antagonistic amino acid sequences and polypeptides may bind to or close to the IL-6 om IL-6R.

Also, in addition to (a) and (b) above, and optionally in addition to (c) above or (e) below, such antagonistic amino acid sequences and polypeptides may bind to IL-6R (i.e. as such or as present in the IL-6/IL-6R complex) in such a way that (d) the formation of the IL-15 6/IL-6R complex is inhibited or affected (e.g. fully or partially disrupted) in such a way that the binding of the complex to - e.g. its affinity for - gp130 is reduced (or reversely, that the binding of gp 130 to - e.g. its affinity for – the complex is reduced), so that the signalling induced/mediated by the binding of the complex to gp130 is modulated (e.g. reduced); compared to the formation of the complex and its binding to gp130 without the presence of 20 the amino acid sequence or Nanobody of the invention.

Also, in addition to (a) and (b) above, and optionally in addition to (c) or (d) above, such antagonistic amino acid sequences and polypeptides may bind to IL-6R (i.e. as such or as present in the IL-6/IL-6R complex) in such a way that (e) binding to IL-6R (i.e. as such or as present in the IL-6/IL-6R complex) in such a way that the formation of the IL-6/IL-6R 25 complex essentially is not affected but that the binding of said complex to gp130 is modulated (e.g. inhibited), so that the signalling induced/mediated by the binding of the complex to gp130 is modulated (e.g. reduced); compared to the formation of the complex and its binding to gp130 without the presence of the amino acid sequence or Nanobody of the invention.

Alternatively, such antagonistic amino acid sequences and polypeptides may bind to 30 another epitope, site, domain or region on the IL-6 receptor (e.g. allosteric binding) such that the IL-6 receptor becomes less sensitive for binding of IL-6 (and/or that the signalling of the IL-6 receptor upon binding of IL-6 is reduced).

It is also possible that such antagonistic amino acid sequences and polypeptides may bind to another epitope, site, domain or region on the IL-6 receptor such that the ligand-mediated dimerization of the growth factor receptor is prevented, reduced or inhibited.

Accordingly, in the context of the present invention, “modulating” or “to modulate” generally means exercising an agonistic or antagonistic effect, respectively, with respect to IL-6, IL-6R and/or the biological pathways, responses, signalling, mechanisms or effects in which IL-6 and/or IL-6R is involved. In particular, “modulating” or “to modulate” may mean either an such an agonistic or antagonistic effect (i.e. a full or partial agonistic or antagonistic effect, respectively), as measured using a suitable in vitro, cellular or in vivo assay (such as those mentioned herein), that leads to a change in a relevant parameter by at least 1%, preferably at least 5%, such as at least 10% or at least 25%, for example by at least 50%, at least 60%, at least 70%, at least 80%, or 90% or more, compared to same parameter in the same assay under the same conditions but without the presence of the amino acid sequence, Nanobody or polypeptide of the invention.

15 The invention further relates to methods for preparing or generating the amino acid sequences, polypeptides, nucleic acids, host cells, products and compositions described herein. Some preferred but non-limiting examples of such methods will become clear from the further description herein.

Generally, these methods may comprise the steps of:

20 a) providing a set, collection or library of amino acid sequences; and
b) screening said set, collection or library of amino acid sequences for amino acid sequences that can bind to and/or have affinity for IL-6R;
and
c) isolating the amino acid sequence(s) that can bind to and/or have affinity for IL-6R.

25 In such a method, the set, collection or library of amino acid sequences may be any suitable set, collection or library of amino acid sequences. For example, the set, collection or library of amino acid sequences may be a set, collection or library of immunoglobulin sequences (as described herein), such as a naïve set, collection or library of immunoglobulin sequences; a synthetic or semi-synthetic set, collection or library of immunoglobulin sequences; and/or a set, collection or library of immunoglobulin sequences that have been subjected to affinity maturation.

30 Also, in such a method, the set, collection or library of amino acid sequences may be a set, collection or library of heavy chain variable domains (such as V_H domains or V_{HH}

domains) or of light chain variable domains. For example, the set, collection or library of amino acid sequences may be a set, collection or library of domain antibodies or single domain antibodies, or may be a set, collection or library of amino acid sequences that are capable of functioning as a domain antibody or single domain antibody.

5 In a preferred aspect of this method, the set, collection or library of amino acid sequences may be an immune set, collection or library of immunoglobulin sequences, for example derived from a mammal that has been suitably immunized with IL-6R or with a suitable antigenic determinant based thereon or derived therefrom, such as an antigenic part, fragment, region, domain, loop or other epitope thereof. In one particular aspect, said 10 antigenic determinant may be an extracellular part, region, domain, loop or other extracellular epitope(s).

In the above methods, the set, collection or library of amino acid sequences may be displayed on a phage, phagemid, ribosome or suitable micro-organism (such as yeast), such as to facilitate screening. Suitable methods, techniques and host organisms for displaying and 15 screening (a set, collection or library of) amino acid sequences will be clear to the person skilled in the art, for example on the basis of the further disclosure herein. Reference is also made to the review by Hoogenboom in *Nature Biotechnology*, 23, 9, 1105-1116 (2005).

In another aspect, the method for generating amino acid sequences comprises at least the steps of:

20 a) providing a collection or sample of cells expressing amino acid sequences;

b) screening said collection or sample of cells for cells that express an amino acid sequence that can bind to and/or have affinity for IL-6R;

and

c) either (i) isolating said amino acid sequence; or (ii) isolating from said cell a nucleic acid sequence that encodes said amino acid sequence, followed by expressing said 25 amino acid sequence.

For example, when the desired amino acid sequence is an immunoglobulin sequence, the collection or sample of cells may for example be a collection or sample of B-cells. Also, in this method, the sample of cells may be derived from a mammal that has been suitably 30 immunized with IL-6R or with a suitable antigenic determinant based thereon or derived therefrom, such as an antigenic part, fragment, region, domain, loop or other epitope thereof. In one particular aspect, said antigenic determinant may be an extracellular part, region, domain, loop or other extracellular epitope(s).

The above method may be performed in any suitable manner, as will be clear to the skilled person. Reference is for example made to EP 0 542 810, WO 05/19824, WO 04/051268 and WO 04/106377. The screening of step b) is preferably performed using a flow cytometry technique such as FACS. For this, reference is for example made to Lieby et al., 5 Blood, Vol. 97, No. 12, 3820.

In another aspect, the method for generating an amino acid sequence directed against IL-6R may comprise at least the steps of:

- a) providing a set, collection or library of nucleic acid sequences encoding amino acid sequences;
- 10 b) screening said set, collection or library of nucleic acid sequences for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for IL-6R;

and

- c) isolating said nucleic acid sequence, followed by expressing said amino acid sequence.

15 In such a method, the set, collection or library of nucleic acid sequences encoding amino acid sequences may for example be a set, collection or library of nucleic acid sequences encoding a naïve set, collection or library of immunoglobulin sequences; a set, collection or library of nucleic acid sequences encoding a synthetic or semi-synthetic set, collection or library of immunoglobulin sequences; and/or a set, collection or library of 20 nucleic acid sequences encoding a set, collection or library of immunoglobulin sequences that have been subjected to affinity maturation.

Also, in such a method, the set, collection or library of nucleic acid sequences may encode a set, collection or library of heavy chain variable domains (such as V_H domains or V_{HH} domains) or of light chain variable domains. For example, the set, collection or library of 25 nucleic acid sequences may encode a set, collection or library of domain antibodies or single domain antibodies, or a set, collection or library of amino acid sequences that are capable of functioning as a domain antibody or single domain antibody.

In a preferred aspect of this method, the set, collection or library of amino acid sequences may be an immune set, collection or library of nucleic acid sequences, for example 30 derived from a mammal that has been suitably immunized with IL-6R or with a suitable antigenic determinant based thereon or derived therefrom, such as an antigenic part, fragment, region, domain, loop or other epitope thereof. In one particular aspect, said antigenic

determinant may be an extracellular part, region, domain, loop or other extracellular epitope(s).

The set, collection or library of nucleic acid sequences may for example encode an immune set, collection or library of heavy chain variable domains or of light chain variable domains. In one specific aspect, the set, collection or library of nucleotide sequences may encode a set, collection or library of V_{HH} sequences.

In the above methods, the set, collection or library of nucleotide sequences sequences may be displayed on a phage, phagemid, ribosome or suitable micro-organism (such as yeast), such as to facilitate screening. Suitable methods, techniques and host organisms for displaying and screening (a set, collection or library of) nucleotide sequences encoding amino acid sequences will be clear to the person skilled in the art, for example on the basis of the further disclosure herein. Reference is also made to the review by Hoogenboom in *Nature Biotechnology*, 23, 9, 1105-1116 (2005).

The invention also relates to amino acid sequences that are obtained by the above methods, or alternatively by a method that comprises the one of the above methods and in addition at least the steps of determining the nucleotide sequence or amino acid sequence of said immunoglobulin sequence; and of expressing or synthesizing said amino acid sequence in a manner known per se, such as by expression in a suitable host cell or host organism or by chemical synthesis.

Also, following the steps above, one or more amino acid sequences of the invention may be suitably humanized (or alternatively camelized); and/or the amino acid sequence(s) thus obtained may be linked to each other or to one or more other suitable amino acid sequences (optionally via one or more suitable linkers) so as to provide a polypeptide of the invention. Also, a nucleic acid sequence encoding an amino acid sequence of the invention may be suitably humanized (or alternatively camelized) and suitably expressed; and/or one or more nucleic acid sequences encoding an amino acid sequence of the invention may be linked to each other or to one or more nucleic acid sequences that encode other suitable amino acid sequences (optionally via nucleotide sequences that encode one or more suitable linkers), after which the nucleotide sequence thus obtained may be suitably expressed so as to provide a polypeptide of the invention.

The invention further relates to applications and uses of the amino acid sequences, polypeptides, nucleic acids, host cells, products and compositions described herein, as well as to methods for the prevention and/or treatment for diseases and disorders associated with IL-

6R. Some preferred but non-limiting applications and uses will become clear from the further description herein.

Other aspects, embodiments, advantages and applications of the invention will also become clear from the further description herein, in which the invention will be described and 5 discussed in more detail with reference to the Nanobodies of the invention and of polypeptides of the invention comprising the same, which form some of the preferred aspects of the invention.

As will become clear from the further description herein, Nanobodies generally offer certain advantages (outlined herein) compared to "dAb's" or similar (single) domain 10 antibodies or immunoglobulin sequences, which advantages are also provided by the Nanobodies of the invention. However, it will be clear to the skilled person that the more general aspects of the teaching below can also be applied (either directly or analogously) to other amino acid sequences of the invention.

15 Detailed description of the invention.

The above and other aspects, embodiments and advantages of the invention will become clear from the further description hereinbelow, in which:

a) Unless indicated or defined otherwise, all terms used have their usual meaning in the art, which will be clear to the skilled person. Reference is for example made to the 20 standard handbooks, such as Sambrook et al, "Molecular Cloning: A Laboratory Manual" (2nd.Ed.), Vols. 1-3, Cold Spring Harbor Laboratory Press (1989); F. Ausubel et al, eds., "Current protocols in molecular biology", Green Publishing and Wiley Interscience, New York (1987); Lewin, "Genes II", John Wiley & Sons, New York, N.Y., (1985); Old et al., "Principles of Gene Manipulation: An Introduction to Genetic 25 Engineering", 2nd edition, University of California Press, Berkeley, CA (1981); Roitt et al., "Immunology" (6th. Ed.), Mosby/Elsevier, Edinburgh (2001); Roitt et al., Roitt's Essential Immunology, 10th Ed. Blackwell Publishing, UK (2001); and Janeway et al., "Immunobiology" (6th Ed.), Garland Science Publishing/Churchill Livingstone, New York (2005), as well as to the general background art cited herein;

30 b) Unless indicated otherwise, the term "immunoglobulin sequence" - whether used herein to refer to a heavy chain antibody or to a conventional 4-chain antibody - is used as a general term to include both the full-size antibody, the individual chains thereof, as well as all parts, domains or fragments thereof (including but not limited to antigen-binding

domains or fragments such as V_{HH} domains or V_H/V_L domains, respectively). In addition, the term “sequence” as used herein (for example in terms like “immunoglobulin sequence”, “antibody sequence”, “variable domain sequence”, “ V_{HH} sequence” or “protein sequence”), should generally be understood to include both the relevant amino acid sequence as well as nucleic acid sequences or nucleotide sequences encoding the same, unless the context requires a more limited interpretation;

5

- 10 c) Unless indicated otherwise, all methods, steps, techniques and manipulations that are not specifically described in detail can be performed and have been performed in a manner known *per se*, as will be clear to the skilled person. Reference is for example again made to the standard handbooks and the general background art mentioned herein and to the further references cited therein;
- d) Amino acid residues will be indicated according to the standard three-letter or one-letter amino acid code, as mentioned in Table A-2;

Table A-2: one-letter and three-letter amino acid code

Nonpolar, uncharged (at pH 6.0 – 7.0) ⁽³⁾	Alanine	Ala	A
	Valine	Val	V
	Leucine	Leu	L
	Isoleucine	Ile	I
	Phenylalanine	Phe	F
	Methionine ⁽¹⁾	Met	M
	Tryptophan	Trp	W
	Proline	Pro	P
Polar, uncharged (at pH 6.0-7.0)	Glycine ⁽²⁾	Gly	G
	Serine	Ser	S
	Threonine	Thr	T
	Cysteine	Cys	C
	Asparagine	Asn	N
	Glutamine	Gln	Q
	Tyrosine	Tyr	Y
Polar, charged (at pH 6.0-7.0)	Lysine	Lys	K
	Arginine	Arg	R
	Histidine ⁽⁴⁾	His	H
	Aspartate	Asp	D
	Glutamate	Glu	E
<u>Notes:</u>			
a) Sometimes also considered to be a polar uncharged amino acid.			
b) Sometimes also considered to be a nonpolar uncharged amino acid.			
c) As will be clear to the skilled person, the fact that an amino acid residue is referred to in this Table as being either charged or uncharged at pH 6.0 to 7.0 does not reflect in any way on the charge said amino acid residue may have at a pH lower than 6.0 and/or at a pH higher than 7.0; the amino acid residues mentioned in the Table can be either charged and/or uncharged at such a higher or lower pH, as will be clear to the skilled person.			
d) As is known in the art, the charge of a His residue is greatly dependant upon even small shifts in pH, but a His residue can generally be considered essentially uncharged at a pH of about 6.5.			

5 e) For the purposes of comparing two or more nucleotide sequences, the percentage of “sequence identity” between a first nucleotide sequence and a second nucleotide sequence may be calculated by dividing [*the number of nucleotides in the first nucleotide sequence that are identical to the nucleotides at the corresponding positions in the second nucleotide sequence*] by [*the total number of nucleotides in the first nucleotide sequence*] and multiplying by [100%], in which each deletion, insertion, substitution or addition of a nucleotide in the second nucleotide sequence - compared to the first nucleotide sequence - is considered as a difference at a single nucleotide (position).

10 Alternatively, the degree of sequence identity between two or more nucleotide sequences may be calculated using a known computer algorithm for sequence alignment such as NCBI Blast v2.0, using standard settings.

15 Some other techniques, computer algorithms and settings for determining the degree of sequence identity are for example described in WO 04/037999, EP 0 967 284, EP 1 085 089, WO 00/55318, WO 00/78972, WO 98/49185 and GB 2 357 768-A.

20 Usually, for the purpose of determining the percentage of “sequence identity” between two nucleotide sequences in accordance with the calculation method outlined hereinabove, the nucleotide sequence with the greatest number of nucleotides will be taken as the “first” nucleotide sequence, and the other nucleotide sequence will be taken as the “second” nucleotide sequence;

25 f) For the purposes of comparing two or more amino acid sequences, the percentage of “sequence identity” between a first amino acid sequence and a second amino acid sequence (as referred to herein as “*amino acid identity*”) may be calculated by dividing [*the number of amino acid residues in the first amino acid sequence that are identical to the amino acid residues at the corresponding positions in the second amino acid sequence*] by [*the total number of amino acid residues in the first amino acid sequence*] and multiplying by [100%], in which each deletion, insertion, substitution or addition of an amino acid residue in the second amino acid sequence - compared to the first amino acid sequence - is considered as a difference at a single amino acid residue (position), i.e. as an “amino acid difference” as defined herein.

Alternatively, the degree of sequence identity between two amino acid sequences may be calculated using a known computer algorithm, such as those mentioned above for determining the degree of sequence identity for nucleotide sequences, again using standard settings.

5 Usually, for the purpose of determining the percentage of "sequence identity" between two amino acid sequences in accordance with the calculation method outlined hereinabove, the amino acid sequence with the greatest number of amino acid residues will be taken as the "first" amino acid sequence, and the other amino acid sequence will be taken as the "second" amino acid sequence.

10 Also, in determining the degree of sequence identity between two amino acid sequences, the skilled person may take into account so-called "conservative" amino acid substitutions, which can generally be described as amino acid substitutions in which an amino acid residue is replaced with another amino acid residue of similar chemical structure and which has little or essentially no influence on the function, activity or other biological properties of the polypeptide. Such conservative amino acid substitutions are well known in the art, for example from WO 04/037999, GB-A-3 357 15 768, WO 98/49185, WO 00/46383 and WO 01/09300; and (preferred) types and/or combinations of such substitutions may be selected on the basis of the pertinent teachings from WO 04/037999 as well as WO 98/49185 and from the further references 20 cited therein.

Such conservative substitutions preferably are substitutions in which one amino acid within the following groups (a) – (e) is substituted by another amino acid residue within the same group: (a) small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr, Pro and Gly; (b) polar, negatively charged residues and their (uncharged) amides: Asp, Asn, Glu and Gln; (c) polar, positively charged residues: His, Arg and Lys; (d) large 25 aliphatic, nonpolar residues: Met, Leu, Ile, Val and Cys; and (e) aromatic residues: Phe, Tyr and Trp.

Particularly preferred conservative substitutions are as follows: Ala into Gly or into Ser; Arg into Lys; Asn into Gln or into His; Asp into Glu; Cys into Ser; Gln into Asn; Glu 30 into Asp; Gly into Ala or into Pro; His into Asn or into Gln; Ile into Leu or into Val; Leu into Ile or into Val; Lys into Arg, into Gln or into Glu; Met into Leu, into Tyr or into Ile; Phe into Met, into Leu or into Tyr; Ser into Thr; Thr into Ser; Trp into Tyr; Tyr into Trp; and/or Phe into Val, into Ile or into Leu.

Any amino acid substitutions applied to the polypeptides described herein may also be based on the analysis of the frequencies of amino acid variations between homologous proteins of different species developed by Schulz et al., *Principles of Protein Structure*, Springer-Verlag, 1978, on the analyses of structure forming potentials developed by 5 Chou and Fasman, *Biochemistry* 13: 211, 1974 and *Adv. Enzymol.*, 47: 45-149, 1978, and on the analysis of hydrophobicity patterns in proteins developed by Eisenberg et al., *Proc. Nad. Acad. Sci. USA* 81: 140-144, 1984; Kyte & Doolittle; *J Molec. Biol.* 157: 10 105-132, 1981, and Goldman et al., *Ann. Rev. Biophys. Chem.* 15: 321-353, 1986, all incorporated herein in their entirety by reference. Information on the primary, secondary and tertiary structure of Nanobodies is given in the description herein and in the general background art cited above. Also, for this purpose, the crystal structure of a V_{HH} domain from a llama is for example given by Desmyter et al., *Nature Structural Biology*, Vol. 3, 9, 803 (1996); Spinelli et al., *Natural Structural Biology* (1996); 3, 752-757; and 15 Decanniere et al., *Structure*, Vol. 7, 4, 361 (1999). Further information about some of the amino acid residues that in conventional V_H domains form the V_H/V_L interface and potential camelizing substitutions on these positions can be found in the prior art cited above.

- 20 g) Amino acid sequences and nucleic acid sequences are said to be “exactly the same” if they have 100% sequence identity (as defined herein) over their entire length;
- h) When comparing two amino acid sequences, the term “*amino acid difference*” refers to an insertion, deletion or substitution of a single amino acid residue on a position of the first sequence, compared to the second sequence; it being understood that two amino acid sequences can contain one, two or more such amino acid differences;
- i) When a nucleotide sequence or amino acid sequence is said to “comprise” another 25 nucleotide sequence or amino acid sequence, respectively, or to “essentially consist of” another nucleotide sequence or amino acid sequence, this may mean that the latter nucleotide sequence or amino acid sequence has been incorporated into the firstmentioned nucleotide sequence or amino acid sequence, respectively, but more usually this generally means that the firstmentioned nucleotide sequence or amino acid sequence comprises within its sequence a stretch of nucleotides or amino acid residues, respectively, that has the same nucleotide sequence or amino acid sequence, respectively, as the latter sequence, irrespective of how the firstmentioned sequence has 30 actually been generated or obtained (which may for example be by any suitable method

described herein). By means of a non-limiting example, when a Nanobody of the invention is said to comprise a CDR sequence, this may mean that said CDR sequence has been incorporated into the Nanobody of the invention, but more usually this generally means that the Nanobody of the invention contains within its sequence a stretch of amino acid residues with the same amino acid sequence as said CDR sequence, irrespective of how said Nanobody of the invention has been generated or obtained. It should also be noted that when the latter amino acid sequence has a specific biological or structural function, it preferably has essentially the same, a similar or an equivalent biological or structural function in the firstmentioned amino acid sequence (in other words, the firstmentioned amino acid sequence is preferably such that the latter sequence is capable of performing essentially the same, a similar or an equivalent biological or structural function). For example, when a Nanobody of the invention is said to comprise a CDR sequence or framework sequence, respectively, the CDR sequence and framework are preferably capable, in said Nanobody, of functioning as a CDR sequence or framework sequence, respectively. Also, when a nucleotide sequence is said to comprise another nucleotide sequence, the firstmentioned nucleotide sequence is preferably such that, when it is expressed into an expression product (e.g. a polypeptide), the amino acid sequence encoded by the latter nucleotide sequence forms part of said expression product (in other words, that the latter nucleotide sequence is in the same reading frame as the firstmentioned, larger nucleotide sequence).

10 j) A nucleic acid sequence or amino acid sequence is considered to be “*(in) essentially isolated (form)*” - for example, compared to its native biological source and/or the reaction medium or cultivation medium from which it has been obtained - when it has been separated from at least one other component with which it is usually associated in said source or medium, such as another nucleic acid, another protein/polypeptide, another biological component or macromolecule or at least one contaminant, impurity or minor component. In particular, a nucleic acid sequence or amino acid sequence is considered “essentially isolated” when it has been purified at least 2-fold, in particular at least 10-fold, more in particular at least 100-fold, and up to 1000-fold or more. A nucleic acid sequence or amino acid sequence that is “in essentially isolated form” is preferably essentially homogeneous, as determined using a suitable technique, such as a suitable chromatographical technique, such as polyacrylamide-gel electrophoresis;

5 k) The term “domain” as used herein generally refers to a globular region of an antibody chain, and in particular to a globular region of a heavy chain antibody, or to a polypeptide that essentially consists of such a globular region. Usually, such a domain will comprise peptide loops (for example 3 or 4 peptide loops) stabilized, for example, as a sheet or by disulfide bonds.

10 l) The term ‘antigenic determinant’ refers to the epitope on the antigen recognized by the antigen-binding molecule (such as a Nanobody or a polypeptide of the invention) and more in particular by the antigen-binding site of said molecule. The terms “antigenic determinant” and “epitope” may also be used interchangeably herein.

15 m) An amino acid sequence (such as a Nanobody, an antibody, a polypeptide of the invention, or generally an antigen binding protein or polypeptide or a fragment thereof) that can (specifically) bind to, that has affinity for and/or that has specificity for a specific antigenic determinant, epitope, antigen or protein (or for at least one part, fragment or epitope thereof) is said to be “against” or “directed against” said antigenic determinant, epitope, antigen or protein. In particular, an amino acid sequence that “against” or “directed against” an antigenic determinant, epitope, antigen or protein (or for at least one part, fragment or epitope thereof) and/or that can specifically bind to, that has affinity for and/or that has specificity for a specific antigenic determinant, epitope, antigen or protein (or for at least one part, fragment or epitope thereof) is defined herein as an amino acid sequence that can bind to said antigenic determinant, epitope, antigen or protein (or for at least one part, fragment or epitope thereof) with a dissociation constant (K_D) of 10^5 to 10^{-12} moles/liter or less, and preferably 10^{-7} to 10^{-12} moles/liter or less and more preferably 10^{-8} to 10^{-12} moles/liter (i.e. with an association constant (K_A) of 10^5 to 10^{12} liter/ moles or more, and preferably 10^7 to 10^{12} liter/moles or more and more preferably 10^8 to 10^{12} liter/moles).

20 n) The term “*specificity*” refers to the number of different types of antigens or antigenic determinants to which a particular antigen-binding molecule or antigen-binding protein (such as a Nanobody or a polypeptide of the invention) molecule can bind. The specificity of an antigen-binding protein can be determined based on affinity and/or avidity. The affinity, represented by the equilibrium constant for the dissociation of an antigen with an antigen-binding protein (K_D), is a measure for the binding strength between an antigenic determinant and an antigen-binding site on the antigen-binding protein: the lesser the value of the K_D , the stronger the binding strength between an

antigenic determinant and the antigen-binding molecule (alternatively, the affinity can also be expressed as the affinity constant (K_A), which is $1/K_D$). As will be clear to the skilled person (for example on the basis of the further disclosure herein), affinity can be determined in a manner known per se, depending on the specific antigen of interest.

5 Avidity is the measure of the strength of binding between an antigen-binding molecule (such as a Nanobody or polypeptide of the invention) and the pertinent antigen. Avidity is related to both the affinity between an antigenic determinant and its antigen binding site on the antigen-binding molecule and the number of pertinent binding sites present on the antigen-binding molecule. Typically, antigen-binding proteins (such as the amino acid sequences, Nanobodies and/or polypeptides of the invention) will bind to their antigen with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter or less, and preferably 10^{-7} to 10^{-12} moles/liter or less and more preferably 10^{-8} to 10^{-12} moles/liter (i.e. with an association constant (K_A) of 10^5 to 10^{12} liter/ moles or more, and preferably 10^7 to 10^{12} liter/moles or more and more preferably 10^8 to 10^{12} liter/moles). Any K_D value greater than 10^4 mol/liter (or any K_A value lower than $10^4 M^{-1}$) liters/mol is generally considered to indicate non-specific binding. Preferably, a monovalent immunoglobulin sequence of the invention will bind to the desired serum protein with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM. Specific binding of an antigen-binding protein to an antigen or antigenic determinant can be determined in any suitable manner known per se, including, for example, Scatchard analysis and/or competitive binding assays, such as radioimmunoassays (RIA), enzyme immunoassays (EIA) and sandwich competition assays, and the different variants thereof known per se in the art; as well as the other techniques mentioned herein.

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The dissociation constant may be the actual or apparent dissociation constant, as will be clear to the skilled person. Methods for determining the dissociation constant will be clear to the skilled person, and for example include the techniques mentioned herein. In this respect, it will also be clear that it may not be possible to measure dissociation constants of more than 10^{-4} moles/liter or 10^{-3} moles/liter (e.g. of 10^{-2} moles/liter). Optionally, as will also be clear to the skilled person, the (actual or apparent) dissociation constant may be calculated on the basis of the (actual or apparent) association constant (K_A), by means of the relationship [$K_D = 1/K_A$].

The affinity denotes the strength or stability of a molecular interaction. The affinity is commonly given as by the K_D , or dissociation constant, which has units of mol/liter (or M). The affinity can also be expressed as an association constant, K_A , which equals $1/K_D$ and has units of $(\text{mol/liter})^{-1}$ (or M^{-1}). In the present specification, the stability of the interaction between two molecules (such as an amino acid sequence, Nanobody or polypeptide of the invention and its intended target) will mainly be expressed in terms of the K_D value of their interaction; it being clear to the skilled person that in view of the relation $K_A = 1/K_D$, specifying the strength of molecular interaction by its K_D value can also be used to calculate the corresponding K_A value. The K_D -value characterizes the strength of a molecular interaction also in a thermodynamic sense as it is related to the free energy (DG) of binding by the well known relation $DG = RT \cdot \ln(K_D)$ (equivalently $DG = -RT \cdot \ln(K_A)$), where R equals the gas constant, T equals the absolute temperature and ln denotes the natural logarithm.

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The K_D for biological interactions which are considered meaningful (e.g. specific) are typically in the range of $10^{-10} M$ (0.1 nM) to $10^{-5} M$ (10000nM). The stronger an interaction is, the lower is its K_D .

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The K_D can also be expressed as the ratio of the dissociation rate constant of a complex, denoted as k_{off} , to the rate of its association, denoted k_{on} (so that $K_D = k_{\text{off}}/k_{\text{on}}$ and $K_A = k_{\text{on}}/k_{\text{off}}$). The off-rate k_{off} has units s^{-1} (where s is the SI unit notation of second). The on-rate k_{on} has units $M^{-1}s^{-1}$. The on-rate may vary between $10^2 M^{-1}s^{-1}$ to about $10^7 M^{-1}s^{-1}$, approaching the diffusion-limited association rate constant for bimolecular interactions. The off-rate is related to the half-life of a given molecular interaction by the relation $t_{1/2} = \ln(2)/k_{\text{off}}$. The off-rate may vary between $10^{-6} s^{-1}$ (near irreversible complex with a $t_{1/2}$ of multiple days) to $1 s^{-1}$ ($t_{1/2} = 0.69 s$).

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The affinity of a molecular interaction between two molecules can be measured via different techniques known per se, such as the well known surface plasmon resonance (SPR) biosensor technique (see for example Ober *et al.*, *Intern. Immunology*, 13, 1551-1559, 2001) where one molecule is immobilized on the biosensor chip and the other molecule is passed over the immobilized molecule under flow conditions yielding k_{on} , k_{off} measurements and hence K_D (or K_A) values. This can for example be performed using the well-known BIACORE instruments.

It will also be clear to the skilled person that the measured K_D may correspond to the apparent K_D if the measuring process somehow influences the intrinsic binding affinity

of the implied molecules for example by artifacts related to the coating on the biosensor of one molecule. Also, an apparent K_D may be measured if one molecule contains more than one recognition sites for the other molecule. In such situation the measured affinity may be affected by the avidity of the interaction by the two molecules.

5 Another approach that may be used to assess affinity is the 2-step ELISA (Enzyme-Linked Immunosorbent Assay) procedure of Friguet *et al.* (J. Immunol. Methods, 77, 305-19, 1985). This method establishes a solution phase binding equilibrium measurement and avoids possible artifacts relating to adsorption of one of the molecules on a support such as plastic.

10 However, the accurate measurement of K_D may be quite labor-intensive and as consequence, often apparent K_D values are determined to assess the binding strength of two molecules. It should be noted that as long all measurements are made in a consistent way (e.g. keeping the assay conditions unchanged) apparent K_D measurements can be used as an approximation of the true K_D and hence in the present document K_D and apparent K_D should be treated with equal importance or relevance.

15 Finally, it should be noted that in many situations the experienced scientist may judge it to be convenient to determine the binding affinity relative to some reference molecule. For example, to assess the binding strength between molecules A and B, one may e.g. use a reference molecule C that is known to bind to B and that is suitably labeled with a 20 fluorophore or chromophore group or other chemical moiety, such as biotin for easy detection in an ELISA or FACS (Fluorescent activated cell sorting) or other format (the fluorophore for fluorescence detection, the chromophore for light absorption detection, the biotin for streptavidin-mediated ELISA detection). Typically, the reference molecule C is kept at a fixed concentration and the concentration of A is varied for a 25 given concentration or amount of B. As a result an IC_{50} value is obtained corresponding to the concentration of A at which the signal measured for C in absence of A is halved. Provided $K_{D\text{ ref}}$, the K_D of the reference molecule, is known, as well as the total concentration c_{ref} of the reference molecule, the apparent K_D for the interaction A-B can be obtained from following formula: $K_D = IC_{50}/(1+c_{\text{ref}}/K_{D\text{ ref}})$. Note that if $c_{\text{ref}} \ll K_{D\text{ ref}}$, $K_D \approx IC_{50}$. Provided the measurement of the IC_{50} is performed in a consistent way (e.g. keeping c_{ref} fixed) for the binders that are compared, the strength or stability of a 30 molecular interaction can be assessed by the IC_{50} and this measurement is judged as equivalent to K_D or to apparent K_D throughout this text.

o) The half-life of an amino acid sequence, compound or polypeptide of the invention can generally be defined as the time taken for the serum concentration of the amino acid sequence, compound or polypeptide to be reduced by 50%, *in vivo*, for example due to degradation of the sequence or compound and/or clearance or sequestration of the sequence or compound by natural mechanisms. The *in vivo* half-life of an amino acid sequence, compound or polypeptide of the invention can be determined in any manner known *per se*, such as by pharmacokinetic analysis. Suitable techniques will be clear to the person skilled in the art, and may for example generally involve the steps of suitably administering to a warm-blooded animal (i.e. to a human or to another suitable mammal, such as a mouse, rabbit, rat, pig, dog or a primate, for example monkeys from the genus *Macaca* (such as, and in particular, cynomologus monkeys (*Macaca fascicularis*) and/or rhesus monkeys (*Macaca mulatta*)) and baboon (*Papio ursinus*)) a suitable dose of the amino acid sequence, compound or polypeptide of the invention; collecting blood samples or other samples from said animal; determining the level or concentration of the amino acid sequence, compound or polypeptide of the invention in said blood sample; and calculating, from (a plot of) the data thus obtained, the time until the level or concentration of the amino acid sequence, compound or polypeptide of the invention has been reduced by 50% compared to the initial level upon dosing. Reference is for example made to the Experimental Part below, as well as to the standard handbooks, such as Kenneth, A et al: Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists and Peters et al, Pharmacokinetic analysis: A Practical Approach (1996). Reference is also made to "Pharmacokinetics", M Gibaldi & D Perron, published by Marcel Dekker, 2nd Rev. edition (1982). As will also be clear to the skilled person (see for example pages 6 and 7 of WO 04/003019 and in the further references cited therein), the half-life can be expressed using parameters such as the t_{1/2}-alpha, t_{1/2}-beta and the area under the curve (AUC). In the present specification, an "increase in half-life" refers to an increase in any one of these parameters, such as any two of these parameters, or essentially all three these parameters. As used herein "increase in half-life" or "increased half-life" in particular refers to an increase in the t_{1/2}-beta, either with or without an increase in the t_{1/2}-alpha and/or the AUC or both.

p) As also further described herein, the total number of amino acid residues in a Nanobody can be in the region of 110-120, is preferably 112-115, and is most preferably 113. It

should however be noted that parts, fragments, analogs or derivatives (as further described herein) of a Nanobody are not particularly limited as to their length and/or size, as long as such parts, fragments, analogs or derivatives meet the further requirements outlined herein and are also preferably suitable for the purposes described herein;

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q) The amino acid residues of a Nanobody are numbered according to the general numbering for V_H domains given by Kabat et al. ("Sequence of proteins of immunological interest", US Public Health Services, NIH Bethesda, MD, Publication No. 91), as applied to V_{HH} domains from Camelids in the article of Riechmann and Muylldermans, J. Immunol. Methods 2000 Jun 23; 240 (1-2):185-195 (see for example Figure 2 of said article or referred to herein. According to this numbering, FR1 of a Nanobody comprises the amino acid residues at positions 1-30, CDR1 of a Nanobody comprises the amino acid residues at positions 31-35, FR2 of a Nanobody comprises the amino acids at positions 36-49, CDR2 of a Nanobody comprises the amino acid residues at positions 50-65, FR3 of a Nanobody comprises the amino acid residues at positions 66-94, CDR3 of a Nanobody comprises the amino acid residues at positions 95-102, and FR4 of a Nanobody comprises the amino acid residues at positions 103-113. [In this respect, it should be noted that - as is well known in the art for V_H domains and for V_{HH} domains - the total number of amino acid residues in each of the CDR's may vary and may not correspond to the total number of amino acid residues indicated by the Kabat numbering (that is, one or more positions according to the Kabat numbering may not be occupied in the actual sequence, or the actual sequence may contain more amino acid residues than the number allowed for by the Kabat numbering). This means that, generally, the numbering according to Kabat may or may not correspond to the actual numbering of the amino acid residues in the actual sequence. Generally, however, it can be said that, according to the numbering of Kabat and irrespective of the number of amino acid residues in the CDR's, position 1 according to the Kabat numbering corresponds to the start of FR1 and vice versa, position 36 according to the Kabat numbering corresponds to the start of FR2 and vice versa, position 66 according to the Kabat numbering corresponds to the start of FR3 and vice versa, and position 103 according to the Kabat numbering corresponds to the start of FR4 and vice versa.]. Alternative methods for numbering the amino acid residues of V_H domains, which methods can also be applied in an analogous manner to V_{HH} domains from Camelids

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and to Nanobodies, are the method described by Chothia et al. (Nature 342, 877-883 (1989)), the so-called “AbM definition” and the so-called “contact definition”.

However, in the present description, claims and figures, the numbering according to Kabat as applied to V_{HH} domains by Riechmann and Muyldermans will be followed, unless indicated otherwise; and

5 r) The Figures, Sequence Listing and the Experimental Part/Examples are only given to further illustrate the invention and should not be interpreted or construed as limiting the scope of the invention and/or of the appended claims in any way, unless explicitly indicated otherwise herein.

10 For a general description of heavy chain antibodies and the variable domains thereof, reference is inter alia made to the prior art cited herein, to the review article by Muyldermans in Reviews in Molecular Biotechnology 74(2001), 277-302; as well as to the following patent applications, which are mentioned as general background art: WO 94/04678, WO 95/04079 and WO 96/34103 of the Vrije Universiteit Brussel; WO 94/25591, WO 99/37681, WO 15 00/40968, WO 00/43507, WO 00/65057, WO 01/40310, WO 01/44301, EP 1134231 and WO 02/48193 of Unilever; WO 97/49805, WO 01/21817, WO 03/035694, WO 03/054016 and WO 03/055527 of the Vlaams Instituut voor Biotechnologie (VIB); WO 03/050531 of Algonomics N.V. and Ablynx N.V.; WO 01/90190 by the National Research Council of Canada; WO 03/025020 (= EP 1 433 793) by the Institute of Antibodies; as well as WO 20 04/041867, WO 04/041862, WO 04/041865, WO 04/041863, WO 04/062551, WO 05/044858, WO 06/40153, WO 06/079372, WO 06/122786, WO 06/122787 and WO 06/122825, by Ablynx N.V. and the further published patent applications by Ablynx N.V. Reference is also made to the further prior art mentioned in these applications, and in particular to the list of references mentioned on pages 41-43 of the International application 25 WO 06/040153, which list and references are incorporated herein by reference.

In accordance with the terminology used in the above references, the variable domains present in naturally occurring heavy chain antibodies will also be referred to as “ V_{HH} domains”, in order to distinguish them from the heavy chain variable domains that are present in conventional 4-chain antibodies (which will be referred to hereinbelow as “ V_H domains”) and from the light chain variable domains that are present in conventional 4-chain antibodies (which will be referred to hereinbelow as “ V_L domains”).

30 As mentioned in the prior art referred to above, V_{HH} domains have a number of unique structural characteristics and functional properties which make isolated V_{HH} domains (as well

as Nanobodies based thereon, which share these structural characteristics and functional properties with the naturally occurring V_{HH} domains) and proteins containing the same highly advantageous for use as functional antigen-binding domains or proteins. In particular, and without being limited thereto, V_{HH} domains (which have been “designed” by nature to

5 functionally bind to an antigen without the presence of, and without any interaction with, a light chain variable domain) and Nanobodies can function as a single, relatively small, functional antigen-binding structural unit, domain or protein. This distinguishes the V_{HH} domains from the V_H and V_L domains of conventional 4-chain antibodies, which by themselves are generally not suited for practical application as single antigen-binding proteins
10 or domains, but need to be combined in some form or another to provide a functional antigen-binding unit (as in for example conventional antibody fragments such as Fab fragments; in ScFv's fragments, which consist of a V_H domain covalently linked to a V_L domain).

Because of these unique properties, the use of V_{HH} domains and Nanobodies as single antigen-binding proteins or as antigen-binding domains (i.e. as part of a larger protein or

15 polypeptide) offers a number of significant advantages over the use of conventional V_H and V_L domains, scFv's or conventional antibody fragments (such as Fab- or $F(ab')_2$ -fragments):

- only a single domain is required to bind an antigen with high affinity and with high selectivity, so that there is no need to have two separate domains present, nor to assure that these two domains are present in the right spacial conformation and configuration
20 (i.e. through the use of especially designed linkers, as with scFv's);
- V_{HH} domains and Nanobodies can be expressed from a single gene and require no post-translational folding or modifications;
- V_{HH} domains and Nanobodies can easily be engineered into multivalent and multispecific formats (as further discussed herein);
- V_{HH} domains and Nanobodies are highly soluble and do not have a tendency to aggregate (as with the mouse-derived antigen-binding domains described by Ward et al., Nature, Vol. 341, 1989, p. 544);
- V_{HH} domains and Nanobodies are highly stable to heat, pH, proteases and other denaturing agents or conditions (see for example Ewert et al, *supra*);
- 30 - V_{HH} domains and Nanobodies are easy and relatively cheap to prepare, even on a scale required for production. For example, V_{HH} domains, Nanobodies and proteins/polypeptides containing the same can be produced using microbial

fermentation (e.g. as further described below) and do not require the use of mammalian expression systems, as with for example conventional antibody fragments;

- V_{HH} domains and Nanobodies are relatively small (approximately 15 kDa, or 10 times smaller than a conventional IgG) compared to conventional 4-chain antibodies and antigen-binding fragments thereof, and therefore show high(er) penetration into tissues (including but not limited to solid tumors and other dense tissues) than such conventional 4-chain antibodies and antigen-binding fragments thereof;
- V_{HH} domains and Nanobodies can show so-called cavity-binding properties (inter alia due to their extended CDR3 loop, compared to conventional V_H domains) and can therefore also access targets and epitopes not accessible to conventional 4-chain antibodies and antigen-binding fragments thereof. For example, it has been shown that V_{HH} domains and Nanobodies can inhibit enzymes (see for example WO 97/49805; Transue et al., (1998), *supra*; Lauwereys et al., (1998), *supra*).

As mentioned above, the invention generally relates to Nanobodies directed against

15 the IL-6 receptor, as well as to polypeptides comprising or essentially consisting of one or more of such Nanobodies, that can be used for the prophylactic, therapeutic and/or diagnostic purposes described herein.

As also further described herein, the invention further relates to nucleic acids encoding such Nanobodies and polypeptides, to methods for preparing such Nanobodies and polypeptides, to host cells expressing or capable of expressing such Nanobodies or polypeptides, to compositions comprising such Nanobodies, polypeptides, nucleic acids or host cells, and to uses of such Nanobodies, polypeptides, nucleic acids, host cells or compositions.

Generally, it should be noted that the term Nanobody as used herein in its broadest sense is not limited to a specific biological source or to a specific method of preparation. For example, as will be discussed in more detail below, the Nanobodies of the invention can generally be obtained: (1) by isolating the V_{HH} domain of a naturally occurring heavy chain antibody; (2) by expression of a nucleotide sequence encoding a naturally occurring V_{HH} domain; (3) by “humanization” (as described herein) of a naturally occurring V_{HH} domain or 30 by expression of a nucleic acid encoding a such humanized V_{HH} domain; (4) by “camelization” (as described herein) of a naturally occurring V_H domain from any animal species, and in particular a from species of mammal, such as from a human being, or by expression of a nucleic acid encoding such a camelized V_H domain; (5) by “camelisation” of a

“domain antibody” or “Dab” as described by Ward et al (supra), or by expression of a nucleic acid encoding such a camelized V_H domain; (6) by using synthetic or semi-synthetic techniques for preparing proteins, polypeptides or other amino acid sequences known per se; (7) by preparing a nucleic acid encoding a Nanobody using techniques for nucleic acid

5 synthesis known per se, followed by expression of the nucleic acid thus obtained; and/or (8) by any combination of one or more of the foregoing. Suitable methods and techniques for performing the foregoing will be clear to the skilled person based on the disclosure herein and for example include the methods and techniques described in more detail herein.

One preferred class of Nanobodies corresponds to the V_{HH} domains of naturally 10 occurring heavy chain antibodies directed against the IL-6 receptor. As further described herein, such V_{HH} sequences can generally be generated or obtained by suitably immunizing a species of Camelid with the IL-6 receptor (i.e. so as to raise an immune response and/or heavy chain antibodies directed against the IL-6 receptor), by obtaining a suitable biological sample from said Camelid (such as a blood sample, serum sample or sample of B-cells), and by 15 generating V_{HH} sequences directed against the IL-6 receptor, starting from said sample, using any suitable technique known per se. Such techniques will be clear to the skilled person and/or are further described herein.

Alternatively, such naturally occurring V_{HH} domains against the IL-6 receptor, can be 20 obtained from naïve libraries of Camelid V_{HH} sequences, for example by screening such a library using the IL-6 receptor, or at least one part, fragment, antigenic determinant or epitope thereof using one or more screening techniques known per se. Such libraries and techniques are for example described in WO 99/37681, WO 01/90190, WO 03/025020 and WO 03/035694. Alternatively, improved synthetic or semi-synthetic libraries derived from 25 naïve V_{HH} libraries may be used, such as V_{HH} libraries obtained from naïve V_{HH} libraries by techniques such as random mutagenesis and/or CDR shuffling, as for example described in WO 00/43507.

Yet another technique for obtaining V_{HH} sequences directed against the IL-6 receptor, involves suitably immunizing a transgenic mammal that is capable of expressing heavy chain 30 antibodies (i.e. so as to raise an immune response and/or heavy chain antibodies directed against the IL-6 receptor), obtaining a suitable biological sample from said transgenic mammal (such as a blood sample, serum sample or sample of B-cells), and then generating V_{HH} sequences directed against The IL-6 receptor, starting from said sample, using any suitable technique known per se. For example, for this purpose, the heavy chain antibody-

expressing mice and the further methods and techniques described in WO 02/085945, WO 04/049794, WO 06/008548 and Janssens et al., Proc. Natl. Acad. Sci. USA. 2006 Oct 10;103(41):15130-5 can be used.

A particularly preferred class of Nanobodies of the invention comprises Nanobodies
5 with an amino acid sequence that corresponds to the amino acid sequence of a naturally
occurring V_{HH} domain, but that has been “humanized”, i.e. by replacing one or more amino
acid residues in the amino acid sequence of said naturally occurring V_{HH} sequence (and in
particular in the framework sequences) by one or more of the amino acid residues that occur
at the corresponding position(s) in a V_H domain from a conventional 4-chain antibody from a
10 human being (e.g. indicated above). This can be performed in a manner known per se, which
will be clear to the skilled person, for example on the basis of the further description herein
and the prior art on humanization referred to herein. Again, it should be noted that such
humanized Nanobodies of the invention can be obtained in any suitable manner known per se
(i.e. as indicated under points (1) – (8) above) and thus are not strictly limited to polypeptides
15 that have been obtained using a polypeptide that comprises a naturally occurring V_{HH} domain
as a starting material.

Another particularly preferred class of Nanobodies of the invention comprises
Nanobodies with an amino acid sequence that corresponds to the amino acid sequence of a
naturally occurring V_H domain, but that has been “camelized”, i.e. by replacing one or more
20 amino acid residues in the amino acid sequence of a naturally occurring V_H domain from a
conventional 4-chain antibody by one or more of the amino acid residues that occur at the
corresponding position(s) in a V_{HH} domain of a heavy chain antibody. This can be performed
in a manner known per se, which will be clear to the skilled person, for example on the basis
of the further description herein. Such “camelizing” substitutions are preferably inserted at
25 amino acid positions that form and/or are present at the V_H - V_L interface, and/or at the so-
called Camelidae hallmark residues, as defined herein (see for example WO 94/04678 and
Davies and Riechmann (1994 and 1996), *supra*). Preferably, the V_H sequence that is used as a
starting material or starting point for generating or designing the camelized Nanobody is
preferably a V_H sequence from a mammal, more preferably the V_H sequence of a human
30 being, such as a V_H3 sequence. However, it should be noted that such camelized Nanobodies
of the invention can be obtained in any suitable manner known per se (i.e. as indicated under
points (1) – (8) above) and thus are not strictly limited to polypeptides that have been

obtained using a polypeptide that comprises a naturally occurring V_H domain as a starting material.

For example, again as further described herein, both “humanization” and “camelization” can be performed by providing a nucleotide sequence that encodes a naturally occurring V_{HH} domain or V_H domain, respectively, and then changing, in a manner known per se, one or more codons in said nucleotide sequence in such a way that the new nucleotide sequence encodes a “humanized” or “camelized” Nanobody of the invention, respectively. This nucleic acid can then be expressed in a manner known per se, so as to provide the desired Nanobody of the invention. Alternatively, based on the amino acid sequence of a naturally occurring V_{HH} domain or V_H domain, respectively, the amino acid sequence of the desired humanized or camelized Nanobody of the invention, respectively, can be designed and then synthesized *de novo* using techniques for peptide synthesis known per se. Also, based on the amino acid sequence or nucleotide sequence of a naturally occurring V_{HH} domain or V_H domain, respectively, a nucleotide sequence encoding the desired humanized or camelized Nanobody of the invention, respectively, can be designed and then synthesized *de novo* using techniques for nucleic acid synthesis known per se, after which the nucleic acid thus obtained can be expressed in a manner known per se, so as to provide the desired Nanobody of the invention.

Other suitable methods and techniques for obtaining the Nanobodies of the invention and/or nucleic acids encoding the same, starting from naturally occurring V_H sequences or preferably V_{HH} sequences, will be clear from the skilled person, and may for example comprise combining one or more parts of one or more naturally occurring V_H sequences (such as one or more FR sequences and/or CDR sequences), one or more parts of one or more naturally occurring V_{HH} sequences (such as one or more FR sequences or CDR sequences), and/or one or more synthetic or semi-synthetic sequences, in a suitable manner, so as to provide a Nanobody of the invention or a nucleotide sequence or nucleic acid encoding the same.

Optionally, a Nanobody of the invention may also, and in addition to the at least one binding site for binding against IL-6R, contain one or more further binding sites for binding against other antigens, proteins or targets. For methods and positions for introducing such second binding sites, reference is for example made to Keck and Huston, *Biophysical Journal*, 71, October 1996, 2002-2011; EP 0 640 130; WO 06/07260 and the US provisional

application by Ablynx N.V. entitled "*Immunoglobulin domains with multiple binding sites*" filed on November 27, 2006.

According to one preferred, but non-limiting aspect of the invention, a Nanobody in its broadest sense can be generally defined as a polypeptide comprising:

5 a) an amino acid sequence that is comprised of four framework regions/sequences interrupted by three complementarity determining regions/sequences, in which the amino acid residue at position 108 according to the Kabat numbering is Q;
and/or:
b) an amino acid sequence that is comprised of four framework regions/sequences
10 interrupted by three complementarity determining regions/sequences, in which the amino acid residue at position 45 according to the Kabat numbering is a charged amino acid (as defined herein) or a cysteine residue, and position 44 is preferably an E;
and/or:
c) an amino acid sequence that is comprised of four framework regions/sequences
15 interrupted by three complementarity determining regions/sequences, in which the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of P, R and S, and is in particular chosen from the group consisting of R and S.

Thus, in a first preferred, but non-limiting aspect, a Nanobody of the invention may
20 have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which
25 CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which
a) the amino acid residue at position 108 according to the Kabat numbering is Q;
and/or in which:
b) the amino acid residue at position 45 according to the Kabat numbering is a charged
30 amino acid or a cysteine and the amino acid residue at position 44 according to the Kabat numbering is preferably E;
and/or in which:

c) the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of P, R and S, and is in particular chosen from the group consisting of R and S;

and in which:

5 d) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred embodiments herein, and are more preferably as defined according to one of the more preferred embodiments herein.

In particular, a Nanobody in its broadest sense can be generally defined as a polypeptide comprising:

10 a) an amino acid sequence that is comprised of four framework regions/sequences interrupted by three complementarity determining regions/sequences, in which the amino acid residue at position 108 according to the Kabat numbering is Q;

and/or:

b) an amino acid sequence that is comprised of four framework regions/sequences interrupted by three complementarity determining regions/sequences, in which the amino acid residue at position 44 according to the Kabat numbering is E and in which the amino acid residue at position 45 according to the Kabat numbering is an R;

and/or:

c) an amino acid sequence that is comprised of four framework regions/sequences interrupted by three complementarity determining regions/sequences, in which the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of P, R and S, and is in particular chosen from the group consisting of R and S.

Thus, according to a preferred, but non-limiting aspect, a Nanobody of the invention

25 may have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which

30 CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which

a) the amino acid residue at position 108 according to the Kabat numbering is Q;

and/or in which:

b) the amino acid residue at position 44 according to the Kabat numbering is E and in which the amino acid residue at position 45 according to the Kabat numbering is an R; and/or in which:

5 c) the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of P, R and S, and is in particular chosen from the group consisting of R and S;

10 and in which:

d) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred embodiments herein, and are more preferably as defined according to one of the more preferred embodiments herein.

In particular, a Nanobody against the IL-6 receptor, according to the invention may have the structure:

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

15 in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which

20 a) the amino acid residue at position 108 according to the Kabat numbering is Q; and/or in which:

b) the amino acid residue at position 44 according to the Kabat numbering is E and in which the amino acid residue at position 45 according to the Kabat numbering is an R; and/or in which:

25 c) the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of P, R and S, and is in particular chosen from the group consisting of R and S;

30 and in which:

d) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred embodiments herein, and are more preferably as defined according to one of the more preferred embodiments herein.

In particular, according to one preferred, but non-limiting aspect of the invention, a Nanobody can generally be defined as a polypeptide comprising an amino acid sequence that

is comprised of four framework regions/sequences interrupted by three complementarity determining regions/sequences, in which;

- a-1) the amino acid residue at position 44 according to the Kabat numbering is chosen from the group consisting of A, G, E, D, G, Q, R, S, L; and is preferably chosen from the group consisting of G, E or Q; and
- 5 a-2) the amino acid residue at position 45 according to the Kabat numbering is chosen from the group consisting of L, R or C; and is preferably chosen from the group consisting of L or R; and
- a-3) the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of W, R or S; and is preferably W or R, and is most preferably W;
- 10 a-4) the amino acid residue at position 108 according to the Kabat numbering is Q;
or in which:
 - b-1) the amino acid residue at position 44 according to the Kabat numbering is chosen from the group consisting of E and Q; and
 - 15 b-2) the amino acid residue at position 45 according to the Kabat numbering is R; and
 - b-3) the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of W, R and S; and is preferably W;
 - b-4) the amino acid residue at position 108 according to the Kabat numbering is chosen from the group consisting of Q and L; and is preferably Q;
- 20 or in which:
 - c-1) the amino acid residue at position 44 according to the Kabat numbering is chosen from the group consisting of A, G, E, D, Q, R, S and L; and is preferably chosen from the group consisting of G, E and Q; and
 - c-2) the amino acid residue at position 45 according to the Kabat numbering is chosen from the group consisting of L, R and C; and is preferably chosen from the group consisting of L and R; and
 - 25 c-3) the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of P, R and S; and is in particular chosen from the group consisting of R and S; and
 - c-4) the amino acid residue at position 108 according to the Kabat numbering is chosen from the group consisting of Q and L; is preferably Q;
30 and in which

d) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred embodiments herein, and are more preferably as defined according to one of the more preferred embodiments herein.

Thus, in another preferred, but non-limiting aspect, a Nanobody of the invention may

5 have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which

10 CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which:

a-1) the amino acid residue at position 44 according to the Kabat numbering is chosen from the group consisting of A, G, E, D, G, Q, R, S, L; and is preferably chosen from the group consisting of G, E or Q;

15 and in which:

a-2) the amino acid residue at position 45 according to the Kabat numbering is chosen from the group consisting of L, R or C; and is preferably chosen from the group consisting of L or R;

and in which:

20 a-3) the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of W, R or S; and is preferably W or R, and is most preferably W; and in which

a-4) the amino acid residue at position 108 according to the Kabat numbering is Q;

and in which:

25 d) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred embodiments herein, and are more preferably as defined according to one of the more preferred embodiments herein.

In another preferred, but non-limiting aspect, a Nanobody of the invention may have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which:

b-1) the amino acid residue at position 44 according to the Kabat numbering is chosen from
5 the group consisting of E and Q;

and in which:

b-2) the amino acid residue at position 45 according to the Kabat numbering is R;

and in which:

b-3) the amino acid residue at position 103 according to the Kabat numbering is chosen from
10 the group consisting of W, R and S; and is preferably W;

and in which:

b-4) the amino acid residue at position 108 according to the Kabat numbering is chosen from
the group consisting of Q and L; and is preferably Q;

and in which:

15 d) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according
to one of the preferred embodiments herein, and are more preferably as defined
according to one of the more preferred embodiments herein.

In another preferred, but non-limiting aspect, a Nanobody of the invention may have
the structure

20

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which
CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in
25 which:

c-1) the amino acid residue at position 44 according to the Kabat numbering is chosen from
the group consisting of A, G, E, D, Q, R, S and L; and is preferably chosen from the
group consisting of G, E and Q;

and in which:

30 c-2) the amino acid residue at position 45 according to the Kabat numbering is chosen from
the group consisting of L, R and C; and is preferably chosen from the group consisting
of L and R;

and in which:

c-3) the amino acid residue at position 103 according to the Kabat numbering is chosen from the group consisting of P, R and S; and is in particular chosen from the group consisting of R and S;

and in which:

5 c-4) the amino acid residue at position 108 according to the Kabat numbering is chosen from the group consisting of Q and L; is preferably Q;

and in which:

d) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred embodiments herein, and are more preferably as defined according to one of the more preferred embodiments herein.

10 Two particularly preferred, but non-limiting groups of the Nanobodies of the invention are those according to a) above; according to (a-1) to (a-5) above; according to b) above; according to (b-1) to (b-4) above; according to (c) above; and/or according to (c-1) to (c-4) above, in which;

15 a) the amino acid residues at positions 44-47 according to the Kabat numbering form the sequence GLEW (or a GLEW-like sequence as defined herein) and the amino acid residue at position 108 is Q;

or in which:

20 b) the amino acid residues at positions 43-46 according to the Kabat numbering form the sequence KERE or KQRE (or a KERE-like sequence) and the amino acid residue at position 108 is Q or L, and is preferably Q.

Thus, in another preferred, but non-limiting aspect, a Nanobody of the invention may have the structure

25 FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which:

30 a) the amino acid residues at positions 44-47 according to the Kabat numbering form the sequence GLEW (or a GLEW-like sequence as defined herein) and the amino acid residue at position 108 is Q;

and in which:

b) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred embodiments herein, and are more preferably as defined according to one of the more preferred embodiments herein.

In another preferred, but non-limiting aspect, a Nanobody of the invention may have

5 the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which
10 CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which:

a) the amino acid residues at positions 43-46 according to the Kabat numbering form the sequence KERE or KQRE (or a KERE-like sequence) and the amino acid residue at position 108 is Q or L, and is preferably Q;

15 and in which:

b) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred embodiments herein, and are more preferably as defined according to one of the more preferred embodiments herein.

In the Nanobodies of the invention in which the amino acid residues at positions 43-46
20 according to the Kabat numbering form the sequence KERE or KQRE, the amino acid residue at position 37 is most preferably F. In the Nanobodies of the invention in which the amino acid residues at positions 44-47 according to the Kabat numbering form the sequence GLEW, the amino acid residue at position 37 is chosen from the group consisting of Y, H, I, L, V or F, and is most preferably V.

25 Thus, without being limited hereto in any way, on the basis of the amino acid residues present on the positions mentioned above, the Nanobodies of the invention can generally be classified on the basis of the following three groups:

a) The “GLEW-group”: Nanobodies with the amino acid sequence GLEW at positions 44-47 according to the Kabat numbering and Q at position 108 according to the Kabat
30 numbering. As further described herein, Nanobodies within this group usually have a V at position 37, and can have a W, P, R or S at position 103, and preferably have a W at position 103. The GLEW group also comprises some GLEW-like sequences such as those mentioned in Table A-3 below;

5 b) The “*KERE-group*”: Nanobodies with the amino acid sequence KERE or KQRE (or another KERE-like sequence) at positions 43-46 according to the Kabat numbering and Q or L at position 108 according to the Kabat numbering. As further described herein, Nanobodies within this group usually have a F at position 37, an L or F at position 47; and can have a W, P, R or S at position 103, and preferably have a W at position 103;

10 c) The “*103 P, R, S-group*”: Nanobodies with a P, R or S at position 103. These Nanobodies can have either the amino acid sequence GLEW at positions 44-47 according to the Kabat numbering or the amino acid sequence KERE or KQRE at positions 43-46 according to the Kabat numbering, the latter most preferably in combination with an F at position 37 and an L or an F at position 47 (as defined for the KERE-group); and can have Q or L at position 108 according to the Kabat numbering, and preferably have Q.

15 Also, where appropriate, Nanobodies may belong to (i.e. have characteristics of) two or more of these classes. For example, one specifically preferred group of Nanobodies has GLEW or a GLEW-like sequence at positions 44-47; P,R or S (and in particular R) at position 103; and Q at position 108 (which may be humanized to 108L).

20 Thus, in another preferred, but non-limiting aspect, a Nanobody of the invention may be a Nanobody belonging to the GLEW-group (as defined herein), and in which CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred embodiments herein, and are more preferably as defined according to one of the more preferred embodiments herein.

25 In another preferred, but non-limiting aspect, a Nanobody of the invention may be a Nanobody belonging to the KERE-group (as defined herein), and CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred embodiments herein, and are more preferably as defined according to one of the more preferred embodiments herein.

30 Thus, in another preferred, but non-limiting aspect, a Nanobody of the invention may be a Nanobody belonging to the 103 P, R, S-group (as defined herein), and in which CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred embodiments herein, and are more preferably as defined according to one of the more preferred embodiments herein.

Also, more generally and in addition to the 108Q, 43E/44R and 103P,R,S residues mentioned above, the Nanobodies of the invention can contain, at one or more positions that

in a conventional V_H domain would form (part of) the V_H/V_L interface, one or more amino acid residues that are more highly charged than the amino acid residues that naturally occur at the same position(s) in the corresponding naturally occurring V_H sequence, and in particular one or more charged amino acid residues (as mentioned in Table A-2). Such substitutions

5 include, but are not limited to, the GLEW-like sequences mentioned in Table A-3 below; as well as the substitutions that are described in the International Application WO 00/29004 for so-called “microbodies”, e.g. so as to obtain a Nanobody with Q at position 108 in combination with KLEW at positions 44-47. Other possible substitutions at these positions will be clear to the skilled person based upon the disclosure herein.

10 In one embodiment of the Nanobodies of the invention, the amino acid residue at position 83 is chosen from the group consisting of L, M, S, V and W; and is preferably L.

Also, in one embodiment of the Nanobodies of the invention, the amino acid residue at position 83 is chosen from the group consisting of R, K, N, E, G, I, T and Q; and is most preferably either K or E (for Nanobodies corresponding to naturally occurring V_{HH} domains) 15 or R (for “humanized” Nanobodies, as described herein). The amino acid residue at position 84 is chosen from the group consisting of P, A, R, S, D T, and V in one embodiment, and is most preferably P (for Nanobodies corresponding to naturally occurring V_{HH} domains) or R (for “humanized” Nanobodies, as described herein).

Furthermore, in one embodiment of the Nanobodies of the invention, the amino acid 20 residue at position 104 is chosen from the group consisting of G and D; and is most preferably G.

Collectively, the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108, which in the Nanobodies are as mentioned above, will also be referred to herein as the “Hallmark Residues”. The Hallmark Residues and the amino acid residues at the 25 corresponding positions of the most closely related human V_H domain, V_H3 , are summarized in Table A-3.

Some especially preferred but non-limiting combinations of these Hallmark Residues as occur in naturally occurring V_{HH} domains are mentioned in Table A-4. For comparison, the corresponding amino acid residues of the human V_H3 called DP-47 have been indicated in 30 italics.

Table A-3: Hallmark Residues in Nanobodies

Position	Human V _{H3}	Hallmark Residues
11	L, V; predominantly L	L, M, S, V, W; preferably L
37	V, I, F; usually V	F ⁽¹⁾ , Y, H, I, L or V, preferably F ⁽¹⁾ or Y
44 ⁽⁸⁾	G	G ⁽²⁾ , E ⁽³⁾ , A, D, Q, R, S, L; preferably G ⁽²⁾ , E ⁽³⁾ or Q; most preferably G ⁽²⁾ or E ⁽³⁾
45 ⁽⁸⁾	L	L ⁽²⁾ , R ⁽³⁾ , C, I, L, P, Q, V; preferably L ⁽²⁾ or R ⁽³⁾
47 ⁽⁸⁾	W, Y	W ⁽²⁾ , L ⁽¹⁾ or F ⁽¹⁾ , A, G, I, M, R, S, V or Y; preferably W ⁽²⁾ , L ⁽¹⁾ , F ⁽¹⁾ or R
83	R or K; usually R	R, K ⁽⁵⁾ , N, E ⁽⁵⁾ , G, I, M, Q or T; preferably K or R; most preferably K
84	A, T, D; predominantly A	P ⁽⁵⁾ , A, L, R, S, T, D, V; preferably P
103	W	W ⁽⁴⁾ , P ⁽⁶⁾ , R ⁽⁶⁾ , S; preferably W
104	G	G or D; preferably G
108	L, M or T; predominantly L	Q, L ⁽⁷⁾ or R; preferably Q or L ⁽⁷⁾

Notes:

- a) In particular, but not exclusively, in combination with KERE or KQRE at positions 43-46.
- b) Usually as GLEW at positions 44-47.
- c) Usually as KERE or KQRE at positions 43-46, e.g. as KEREL, KEREF, KQREL, KQREF or KEREG at positions 43-47. Alternatively, also sequences such as TERE (for example TEREL), KECE (for example KECEL or KECER), RERE (for example REREG), QERE (for example QEREG), KGRE (for example KGREG), KDRE (for example KDREV) are possible. Some other possible, but less preferred sequences include for example DECKL and NVCEL.
- d) With both GLEW at positions 44-47 and KERE or KQRE at positions 43-46.
- e) Often as KP or EP at positions 83-84 of naturally occurring V_{HH} domains.
- f) In particular, but not exclusively, in combination with GLEW at positions 44-47.
- g) With the proviso that when positions 44-47 are GLEW, position 108 in (non-humanized) VHH sequences that also contain a W at 103.
- h) The GLEW group also contains GLEW-like sequences at positions 44-47, such as for example GVEW, EPEW, GLER, DQEWE, DLEW, GIEW, ELEW, GPEW, EWLP, GPER, GLER and ELEW.

Table A-4: Some preferred but non-limiting combinations of Hallmark Residues in naturally occurring Nanobodies.

For humanization of these combinations, reference is made to the specification.

	11	37	44	45	47	83	84	103	104	108
<i>DP-47</i> (<i>human</i>)	<i>M</i>	<i>V</i>	<i>G</i>	<i>L</i>	<i>W</i>	<i>R</i>	<i>A</i>	<i>W</i>	<i>G</i>	<i>L</i>
“KERE” group	<i>L</i>	<i>F</i>	<i>E</i>	<i>R</i>	<i>L</i>	<i>K</i>	<i>P</i>	<i>W</i>	<i>G</i>	<i>Q</i>
	<i>L</i>	<i>F</i>	<i>E</i>	<i>R</i>	<i>F</i>	<i>E</i>	<i>P</i>	<i>W</i>	<i>G</i>	<i>Q</i>
	<i>L</i>	<i>F</i>	<i>E</i>	<i>R</i>	<i>F</i>	<i>K</i>	<i>P</i>	<i>W</i>	<i>G</i>	<i>Q</i>
	<i>L</i>	<i>Y</i>	<i>Q</i>	<i>R</i>	<i>L</i>	<i>K</i>	<i>P</i>	<i>W</i>	<i>G</i>	<i>Q</i>
	<i>L</i>	<i>F</i>	<i>L</i>	<i>R</i>	<i>V</i>	<i>K</i>	<i>P</i>	<i>Q</i>	<i>G</i>	<i>Q</i>
	<i>L</i>	<i>F</i>	<i>Q</i>	<i>R</i>	<i>L</i>	<i>K</i>	<i>P</i>	<i>W</i>	<i>G</i>	<i>Q</i>
	<i>L</i>	<i>F</i>	<i>E</i>	<i>R</i>	<i>F</i>	<i>K</i>	<i>P</i>	<i>W</i>	<i>G</i>	<i>Q</i>
“GLEW” group	<i>L</i>	<i>V</i>	<i>G</i>	<i>L</i>	<i>W</i>	<i>K</i>	<i>S</i>	<i>W</i>	<i>G</i>	<i>Q</i>
	<i>M</i>	<i>V</i>	<i>G</i>	<i>L</i>	<i>W</i>	<i>K</i>	<i>P</i>	<i>R</i>	<i>G</i>	<i>Q</i>

In the Nanobodies, each amino acid residue at any other position than the Hallmark Residues can be any amino acid residue that naturally occurs at the corresponding position (according to the Kabat numbering) of a naturally occurring V_{HH} domain.

Such amino acid residues will be clear to the skilled person. Tables A-5 – A-8 mention some non-limiting residues that can be present at each position (according to the Kabat numbering) of the FR1, FR2, FR3 and FR4 of naturally occurring V_{HH} domains. For each position, the amino acid residue that most frequently occurs at each position of a naturally occurring V_{HH} domain (and which is the most preferred amino acid residue for said position in a Nanobody) is indicated in bold; and other preferred amino acid residues for each position have been underlined (note: the number of amino acid residues that are found at positions 26-30 of naturally occurring V_{HH} domains supports the hypothesis underlying the numbering by Chothia (supra) that the residues at these positions already form part of CDR1.)

In Tables A-5 – A-8, some of the non-limiting residues that can be present at each position of a human V_{H3} domain have also been mentioned. Again, for each position, the amino acid residue that most frequently occurs at each position of a naturally occurring human V_{H3} domain is indicated in bold; and other preferred amino acid residues have been underlined.

For reference only, Tables A-5 to A-8 also contain data on the V_{HH} entropy (“ V_{HH} Ent.”) and V_{HH} variability (“ V_{HH} Var.”) at each amino acid position for a representative sample of 1118 V_{HH} sequences (data kindly provided by David Lutje Hulsing and Prof. Theo Verrrips of Utrecht University). The values for the V_{HH} entropy and the V_{HH} variability provide a measure for the variability and degree of conservation of amino acid residues between the 1118 V_{HH} sequences analyzed: low values (i.e. <1, such as < 0.5) indicate that an amino acid residue is highly conserved between the V_{HH} sequences (i.e. little variability). For example, the G at position 8 and the G at position 9 have values for the V_{HH} entropy of 0.1 and 0 respectively, indicating that these residues are highly conserved and have little variability (and in case of position 9 is G in all 1118 sequences analysed), whereas for residues that form part of the CDR's generally values of 1.5 or more are found (data not shown). Note that (1) the amino acid residues listed in the second column of Tables A-5 – A-8 are based on a bigger sample than the 1118 V_{HH} sequences that were analysed for determining the V_{HH} entropy and V_{HH} variability referred to in the last two columns; and (2) the data represented below support the hypothesis that the amino acid residues at positions 27-30 and maybe even also at positions 93 and 94 already form part of the CDR's (although the invention is not limited to any specific hypothesis or explanation, and as mentioned above,

herein the numbering according to Kabat is used). For a general explanation of sequence entropy, sequence variability and the methodology for determining the same, see Oliveira et al., PROTEINS: Structure, Function and Genetics, 52: 544-552 (2003).

Table A-5: Non-limiting examples of amino acid residues in FR1 (for the footnotes, see the footnotes to Table A-3)

Position	Amino acid residue(s):		V _{HH} Ent.	V _{HH} Var.
	Human V _{H3}	Camelid V _{HH} 's		
1	E, Q	Q, A, E	-	-
2	V	V	0.2	1
3	Q	Q, K	0.3	2
4	L	L	0.1	1
5	V, L	Q, E, L, V	0.8	3
6	E	E, D, Q, A	0.8	4
7	S, T	S, F	0.3	2
8	G, R	G	0.1	1
9	G	G	0	1
10	G, V	G, D, R	0.3	2
11	Hallmark residue: L, M, S, V, W; preferably L			0.8
12	V, I	V, A	0.2	2
13	Q, K, R	Q, E, K, P, R	0.4	4
14	P	A, Q, A, G, P, S, T, V	1	5
15	G	G	0	1
16	G, R	G, A, E, D	0.4	3
17	S	S, E	0.5	2
18	L	L, V	0.1	1
19	R, K	R, K, L, N, S, T	0.6	4
20	L	L, E, I, V	0.5	4
21	S	S, A, F, T	0.2	3
22	C	C	0	1
23	A, T	A, D, E, P, S, T, V	1.3	5
24	A	A, I, L, S, T, V	1	6

Table A-5: Non-limiting examples of amino acid residues in FR1 (continued)

Position	Amino acid residue(s):		V _{HH} Ent.	V _{HH} Var.
	Human V _{H3}	Camelid V _{HH} 's		
25	S	S, A, F, P, T	0.5	5
26	G	G, A, D, E, R, S, T, V	0.7	7
27	F	S, F, R, L, P, G, N,	2.3	13
28	T	N, T, E, D, S, I, R, A, G, R, F, Y	1.7	11
29	F, <u>V</u>	F, L, D, S, I, G, V, A	1.9	11
30	S, <u>D</u>, G	N, S, E, G, A, D, M, T	1.8	11

Table A-6: Non-limiting examples of amino acid residues in FR2 (for the footnotes, see the footnotes to Table A-3)

Position	Amino acid residue(s):		V _{HH} Ent.	V _{HH} Var.
	Human V _{H3}	Camelid V _{HH} 's		
36	W	W	0.1	1
37	Hallmark residue: F ⁽¹⁾ , H, I, L, Y or V, preferably F ⁽¹⁾ or Y		1.1	6
38	R	R	0.2	1
39	Q	Q, H, P, R	0.3	2
40	A	A, F, G, L, P, T, V	0.9	7
41	P, S, T	P, A, L, S	0.4	3
42	G	G, E	0.2	2
43	K	K, D, E, N, Q, R, T, V	0.7	6
44	Hallmark residue: G ⁽²⁾ , E ⁽³⁾ , A, D, Q, R, S, L; preferably G ⁽²⁾ , E ⁽³⁾ or Q; most preferably G ⁽²⁾ or E ⁽³⁾ .		1.3	5
45	Hallmark residue: L ⁽²⁾ , R ⁽³⁾ , C, I, L, P, Q, V; preferably L ⁽²⁾ or R ⁽³⁾		0.6	4
46	E, V	E, D, K, Q, V	0.4	2
47	Hallmark residue: W ⁽²⁾ , L ⁽¹⁾ or F ⁽¹⁾ , A, G, I, M, R, S, V or Y; preferably W ⁽²⁾ , L ⁽¹⁾ , F ⁽¹⁾ or R		1.9	9
48	V	V, I, L	0.4	3
49	S, <u>A</u>, <u>G</u>	A, <u>S</u>, G, T, V	0.8	3

Table A-7: Non-limiting examples of amino acid residues in FR3 (for the footnotes, see the footnotes to Table A-3)

Position	Amino acid residue(s):		V _{HH} Ent.	V _{HH} Var.
	Human V _{H3}	Camelid V _{HH} 's		
66	R	R	0.1	1
67	F	F, L, V	0.1	1
68	T	T, A, N, S	0.5	4
69	I	I, L, M, V	0.4	4
70	S	S, A, F, T	0.3	4
71	R	R, G, H, I, L, K, Q, S, T, W	1.2	8
72	D, E	D, E, G, N, V	0.5	4
73	N, D, G	N, A, D, F, I, K, L, R, S, T, V, Y	1.2	9
74	A, S	A, D, G, N, P, S, T, V	1	7
75	K	K, A, E, K, L, N, Q, R	0.9	6
76	N, S	N, D, K, R, S, T, Y	0.9	6
77	S, T, I	T, A, E, I, M, P, S	0.8	5
78	L, A	V, L, A, F, G, I, M	1.2	5
79	Y, H	Y, A, D, F, H, N, S, T	1	7
80	L	L, F, V	0.1	1
81	Q	Q, E, I, L, R, T	0.6	5
82	M	M, I, L, V	0.2	2
82a	N, G	N, D, G, H, S, T	0.8	4
82b	S	S, N, D, G, R, T	1	6
82c	L	L, P, V	0.1	2
83	Hallmark residue: R, K ⁽⁵⁾ , N, E ⁽⁵⁾ , G, I, M, Q or T; preferably K or R; most preferably K		0.9	7
84	Hallmark residue: P ⁽⁵⁾ , A, D, L, R, S, T, V; preferably P		0.7	6
85	E, G	E, D, G, Q	0.5	3
86	D	D	0	1
87	T, M	T, A, S	0.2	3

Table A-7: Non-limiting examples of amino acid residues in FR3 (continued)

Position	Amino acid residue(s):		V _{HH} Ent.	V _{HH} Var.
	Human V _{H3}	Camelid V _{HH} 's		
88	A	A, <u>G</u>, S	0.3	2
89	V, L	V, A, D, I, L, M, N, R, T	1.4	6
90	Y	Y, F	0	1
91	Y, H	Y, D, F, H, L, S, T, V	0.6	4
92	C	C	0	1
93	A, K, T	A, <u>N</u>, G, H, K, N, R, S, T, V, Y	1.4	10
94	K, R, T	A, <u>V</u>, C, F, G, I, K, L, R, S or T	1.6	9

Table A-8: Non-limiting examples of amino acid residues in FR4 (for the footnotes, see the footnotes to Table A-3)

Position	Amino acid residue(s):		V _{HH} Ent.	V _{HH} Var.
	Human V _{H3}	Camelid V _{HH} 's		
103	Hallmark residue: W ⁽⁴⁾ , P ⁽⁶⁾ , R ⁽⁶⁾ , S; preferably W		0.4	2
104	Hallmark residue: G or D; preferably G		0.1	1
105	Q, R	Q, E, K, P, R	0.6	4
106	G	G	0.1	1
107	T	T, A, I	0.3	2
108	Hallmark residue: Q, L ⁽⁷⁾ or R; preferably Q or L ⁽⁷⁾		0.4	3
109	V	V	0.1	1
110	T	T, I, A	0.2	1
111	V	V, A, I	0.3	2
112	S	S, F	0.3	1
113	S	S, A, L, P, T	0.4	3

Thus, in another preferred, but not limiting aspect, a Nanobody of the invention can have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which:

a) the Hallmark residues are as defined herein;

10 and in which:

b) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred embodiments herein, and are more preferably as defined according to one of the more preferred embodiments herein.

In another preferred, but not limiting aspect, a Nanobody of the invention can have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which 20 CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which:

and in which

a) FR1 is chosen from the group consisting of the amino acid sequence:

[1] QVQLQESGGGXVQAGGSLRLSCAASG [26] [SEQ ID NO: 1]

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with the above amino acid sequence; in which

30 i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-5; and/or

ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 “amino acid difference(s)” (as defined herein) with one of the above amino acid sequences, in which:

i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-5; and/or

10 ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and in which:

b) FR2 is chosen from the group consisting of the amino acid sequence:

[36] WXRQAPGKXXEXVA [49]

[SEQ ID NO: 2]

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with the above amino acid sequence; in which

20 i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-6; and/or

ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 “amino acid difference(s)” (as defined herein) with one of the above amino acid sequences, in which:

30 i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-6; and/or

- ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and in which:

- c) FR3 is chosen from the group consisting of the amino acid sequence:

[66] RFTISRDNAKNTVYLQMNSLXXEDTAVYYCAA [94] [SEQ ID NO: 3]

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with the above amino acid sequence; in which

- i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-7; and/or
- ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 “amino acid difference(s)” (as defined herein) with one of the above amino acid sequences, in which:

- i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-7; and/or
- ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and in which:

- d) FR4 is chosen from the group consisting of the amino acid sequence:

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with the above amino acid sequence; in which

- i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-8; and/or
- ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

10 and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 “amino acid difference(s)” (as defined herein) with one of the above amino acid sequences, in which:

- i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-8; and/or
- ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s);

and in which:

20 e) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred embodiments herein, and are more preferably as defined according to one of the more preferred embodiments herein;

in which the Hallmark Residues are indicated by “X” and are as defined hereinabove and in which the numbers between brackets refer to the amino acid positions according to the Kabat numbering.

In another preferred, but not limiting aspect, a Nanobody of the invention can have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

30

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which:

- a) FR1 is chosen from the group consisting of the amino acid sequence:

[1] QVQLQESGGGLVQAGGSLRLSCAASG [26]

[SEQ ID NO: 5]

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with the above amino acid sequence; in which

- i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-5; and/or
- ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and
- iii) the Hallmark residue at position is as indicated in the sequence above; and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 “amino acid difference(s)” (as defined herein) with one of the above amino acid sequences, in which:
 - i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-5; and/or
 - ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and
 - iii) the Hallmark residue at position is as indicated in the sequence above;

and in which:

- b) FR2 is chosen from the group consisting of the amino acid sequences:

[36] WFRQAPGKERELVA [49]	[SEQ ID NO: 6]
[36] WFRQAPGKEREVVA [49]	[SEQ ID NO: 7]
[36] WFRQAPGKEREWA [49]	[SEQ ID NO: 8]
[36] WFRQAPGKQRELVA [49]	[SEQ ID NO: 9]
[36] WFRQAPGKQREFVA [49]	[SEQ ID NO: 10]
30 [36] WYRQAPGKGLEWA [49]	[SEQ ID NO: 11]

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of the above amino acid sequences; in which

- i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-6; and/or
- ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and
- iii) the Hallmark residues at positions 37, 44, 45 and 47 are as indicated in each of the sequences above;

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with one of the above amino acid sequences, in which:

- i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-6; and/or
- ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and
- iii) the Hallmark residues at positions 37, 44, 45 and 47 are as indicated in each of the sequences above;

20 and in which:

- c) FR3 is chosen from the group consisting of the amino acid sequence:

[66] RFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA [94] [SEQ ID NO: 12]

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with the above amino acid sequence; in which

- i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-7; and/or
- ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and

iii) the Hallmark residues at positions 83 and 84 are as indicated in each of the sequences above;

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 “amino acid difference(s)” (as defined herein) with one of the above amino acid sequences, in which:

- i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-7; and/or
- ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and
- iii) the Hallmark residues at positions 83 and 84 are as indicated in each of the sequences above;

and in which:

d) FR4 is chosen from the group consisting of the amino acid sequences:

[103] WGQGTQVTVSS [113]

[SEQ ID NO: 13]

[103] WGQGTLVTVSS [113]

[SEQ ID NO: 14]

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of the above amino acid sequence; in which

- i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-8; and/or
- ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and
- iii) the Hallmark residues at positions 103, 104 and 108 are as indicated in each of the sequences above;

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 “amino acid difference(s)” (as defined herein) with one of the above amino acid sequences, in which:

- i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-8; and/or
- ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and
- iii) the Hallmark residues at positions 103, 104 and 108 are as indicated in each of the sequences above;

and in which:

10 e) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred embodiments herein, and are more preferably as defined according to one of the more preferred embodiments herein.

In another preferred, but not limiting aspect, a Nanobody of the invention can have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which:

20 and in which

a) FR1 is chosen from the group consisting of the amino acid sequence:

[1] QVQLQESGGGLVQAGGSLRLSCAASG [26] [SEQ ID NO: 5]

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 “amino acid difference(s)” (as defined herein) with one of the above amino acid sequences, in which:

- i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-5; and/or
- ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and
- iii) the Hallmark residue at position is as indicated in the sequence above;

30

and in which:

b) FR2 is chosen from the group consisting of the amino acid sequences:

[36] WFRQAPGKERELVA [49]	[SEQ ID NO: 6]
[36] WFRQAPGKEREVFA [49]	[SEQ ID NO: 7]
[36] WFRQAPGKEREWA [49]	[SEQ ID NO: 8]
[36] WFRQAPGKQRELVA [49]	[SEQ ID NO: 9]
[36] WFRQAPGKQREFVA [49]	[SEQ ID NO: 10]

10 and/or from the group consisting of amino acid sequences that have 2 or only 1 “amino acid difference(s)” (as defined herein) with one of the above amino acid sequences, in which:

- i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-6; and/or
- ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and
- iii) the Hallmark residues at positions 37, 44, 45 and 47 are as indicated in each of the sequences above;

20 and in which:

c) FR3 is chosen from the group consisting of the amino acid sequence:

[66] RFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA [94] [SEQ ID NO: 12]

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 “amino acid difference(s)” (as defined herein) with one of the above amino acid sequences, in which:

- i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-7; and/or
- ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and

iii) the Hallmark residues at positions 83 and 84 are as indicated in each of the sequences above;

and in which:

d) FR4 is chosen from the group consisting of the amino acid sequences:

[103] WGQGTQVTVSS [113] [SEQ ID NO: 13]

[103] WGQGTLVTVSS [113] [SEQ ID NO: 14]

and/or from the group consisting of amino acid sequences that have 3, 2 or only 10 1 “amino acid difference(s)” (as defined herein) with one of the above amino acid sequences, in which:

- i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-8; and/or
- ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and
- iii) the Hallmark residues at positions 103, 104 and 108 are as indicated in each of the sequences above;

20 and in which:

- e) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred embodiments herein, and are more preferably as defined according to one of the more preferred embodiments herein.

In another preferred, but not limiting aspect, a Nanobody of the invention can have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

30 in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which:

a) FR1 is chosen from the group consisting of the amino acid sequence:

[1] QVQLQESGGGLVQAGGSLRLSCAASG [26] [SEQ ID NO: 5]

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 “amino acid difference(s)” (as defined herein) with one of the above amino acid sequences, in which:

- i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-5; and/or
- ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and
- iii) the Hallmark residue at position is as indicated in the sequence above;

and in which:

b) FR2 is chosen from the group consisting of the amino acid sequence:

[36] WYRQAPGKGLEWA [49]

[SEQ ID NO: 11]

and/or from the group consisting of amino acid sequences that have 2 or only 1 “amino acid difference(s)” (as defined herein) with one of the above amino acid sequences, in which:

- i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-6; and/or
- ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and
- iii) the Hallmark residues at positions 37, 44, 45 and 47 are as indicated in each of the sequences above;

and in which:

c) FR3 is chosen from the group consisting of the amino acid sequence:

[66] RFTISRDNAKNTVYLQMNSLKPEDTAVYYCAA [94] [SEQ ID NO: 12]

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 “amino acid difference(s)” (as defined herein) with one of the above amino acid sequences, in which:

- i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-7; and/or
- ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and
- 10 iii) the Hallmark residues at positions 83 and 84 are as indicated in each of the sequences above;

and in which:

- d) FR4 is chosen from the group consisting of the amino acid sequence:

[103] WGQGTQVTVSS [113]

[SEQ ID NO: 13]

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 “amino acid difference(s)” (as defined herein) with one of the above amino acid sequences, in which:

- i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-8; and/or
- 20 ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s); and
- iii) the Hallmark residues at positions 103, 104 and 108 are as indicated in each of the sequences above;

and in which:

- e) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred embodiments herein, and are more preferably as defined according to one of the more preferred embodiments herein.

Some other framework sequences that can be present in the Nanobodies of the invention can be found in the European patent EP 656 946 mentioned above (see for example also the granted US equivalent 5,759,808),

In another preferred, but not limiting aspect, a Nanobody of the invention can have the structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which:

and in which

10 a) FR1 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 42-92, or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with at least one of said FR1 sequences; in which

- i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-5; and/or
- ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to said FR1 sequence; and
- iii) the Hallmark residue at position is as indicated in said FR1 sequence; and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with at least one of said FR1 sequences, in which:

 - i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-5; and/or
 - ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to said FR1 sequence; and
 - iii) the Hallmark residue at position is as indicated in said FR1 sequence;

20 and in which:

b) FR2 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 144-194, or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least

99% sequence identity (as defined herein) with at least one of said FR2 sequences; in which

- i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-6; and/or
- ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to said FR2 sequence; and
- iii) the Hallmark residues at positions 37, 44, 45 and 47 are as indicated in said FR2 sequence;

10 and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 “amino acid difference(s)” (as defined herein) with at least one of said FR2 sequences, in which:

- i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-6; and/or
- ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to said FR2 sequence; and
- iii) the Hallmark residues at positions 37, 44, 45 and 47 are as indicated in said FR2 sequence;

20 and in which:

- c) FR3 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 246-296, or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with at least one of said FR3 sequences; in which
 - i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-7; and/or
 - ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to said FR3 sequence; and
 - iii) the Hallmark residues at positions 83 and 84 are as indicated in said FR3 sequence;

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 “amino acid difference(s)” (as defined herein) with at least one of said FR3 sequences, in which:

- i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-7; and/or
- ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to said FR3 sequence; and
- iii) the Hallmark residues at positions 83 and 84 are as indicated in said FR3 sequence;

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and in which:

- d) FR4 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 348-398, or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with at least one of said FR4 sequences; in which

- i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-8; and/or
- ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to said FR4 sequence; and
- iii) the Hallmark residues at positions 103, 104 and 108 are as indicated in said FR3 sequence;

20

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 “amino acid difference(s)” (as defined herein) with at least one of said FR4 sequences, in which:

- i) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Table A-8; and/or
- ii) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to said FR4 sequence; and
- iii) the Hallmark residues at positions 103, 104 and 108 are as indicated in said FR4 sequence;

30

and in which:

e) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred embodiments herein, and are more preferably as defined according to one of the more preferred embodiments herein.

Some particularly preferred Nanobodies of the invention can be chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 399-449, or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with at least one of said amino acid sequences; in which

- a) the Hallmark residues can be as indicated in Table A-3 above;
- 10 b) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Tables A-5 – A-8; and/or
- c) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequence(s).

Some even more particularly preferred Nanobodies of the invention can be chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 399-449, or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with at least one of said amino acid sequences; in which

- 20 a) the Hallmark residues are as indicated in the pertinent sequence from SEQ ID NO's: 399-449;
- b) any amino acid substitution at any position other than a Hallmark position is preferably either a conservative amino acid substitution (as defined herein) and/or an amino acid substitution as defined in Tables A-5 – A-8; and/or
- c) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the pertinent sequence chosen from SEQ ID NO's: 399-449.

Some of the most preferred Nanobodies of the invention against the IL-6 receptor can be chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 399-449.

30 Preferably, the CDR sequences and FR sequences in the Nanobodies of the invention are such that the Nanobody of the invention binds to the IL-6 receptor, with an affinity (suitably measured and/or expressed as a K_D -value (actual or apparent), a K_A -value (actual or apparent), a k_{on} -rate and/or a k_{off} -rate, or alternatively as an IC_{50} value, as further described

herein) that is as defined herein; as well as compounds and constructs, and in particular proteins and polypeptides, that comprise at least one such amino acid sequence.

In particular, amino acid sequences and polypeptides of the invention are preferably such that they:

- bind to IL-6R with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter or less, and preferably 10^{-7} to 10^{-12} moles/liter or less and more preferably 10^{-8} to 10^{-12} moles/liter (i.e. with an association constant (K_A) of 10^5 to 10^{12} liter/ moles or more, and preferably 10^7 to 10^{12} liter/moles or more and more preferably 10^8 to 10^{12} liter/moles);

and/or such that they:

10 - bind to IL-6R with a k_{on} -rate of between $10^2 \text{ M}^{-1} \text{ s}^{-1}$ to about $10^7 \text{ M}^{-1} \text{ s}^{-1}$, preferably between $10^3 \text{ M}^{-1} \text{ s}^{-1}$ and $10^7 \text{ M}^{-1} \text{ s}^{-1}$, more preferably between $10^4 \text{ M}^{-1} \text{ s}^{-1}$ and $10^7 \text{ M}^{-1} \text{ s}^{-1}$, such as between $10^5 \text{ M}^{-1} \text{ s}^{-1}$ and $10^7 \text{ M}^{-1} \text{ s}^{-1}$;

and/or such that they:

- bind to IL-6R with a k_{off} rate between 1 s^{-1} ($t_{1/2}=0.69 \text{ s}$) and 10^{-6} s^{-1} (providing a near irreversible complex with a $t_{1/2}$ of multiple days), preferably between 10^{-2} s^{-1} and 10^{-6} s^{-1} , more preferably between 10^{-3} s^{-1} and 10^{-6} s^{-1} , such as between 10^{-4} s^{-1} and 10^{-6} s^{-1} .

Preferably, a monovalent amino acid sequence of the invention (or a polypeptide that contains only one amino acid sequence of the invention) is preferably such that it will bind to IL-6R with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM.

Some preferred IC_{50} values for binding of the amino acid sequences or polypeptides of the invention to IL-6R will become clear from the further description and examples herein.

According to one non-limiting aspect of the invention, a Nanobody may be as defined herein, but with the proviso that it has at least “one amino acid difference” (as defined herein) in at least one of the framework regions compared to the corresponding framework region of a naturally occurring human V_H domain, and in particular compared to the corresponding framework region of DP-47. More specifically, according to one non-limiting aspect of the invention, a Nanobody may be as defined herein, but with the proviso that it has at least “one amino acid difference” (as defined herein) at at least one of the Hallmark residues (including those at positions 108, 103 and/or 45) compared to the corresponding framework region of a naturally occurring human V_H domain, and in particular compared to the corresponding framework region of DP-47. Usually, a Nanobody will have at least one such amino acid difference with a naturally occurring V_H domain in at least one of FR2 and/or FR4, and in

particular at at least one of the Hallmark residues in FR2 and/or FR4 (again, (including those at positions 108, 103 and/or 45).

Also, a humanized Nanobody of the invention may be as defined herein, but with the proviso that it has at least “one amino acid difference” (as defined herein) in at least one of the framework regions compared to the corresponding framework region of a naturally occurring VHH domain. More specifically, according to one non-limiting aspect of the invention, a humanized Nanobody may be as defined herein, but with the proviso that it has at least “one amino acid difference” (as defined herein) at at least one of the Hallmark residues (including those at positions 108, 103 and/or 45) compared to the corresponding framework region of a naturally occurring VHH domain. Usually, a humanized Nanobody will have at least one such amino acid difference with a naturally occurring VHH domain in at least one of FR2 and/or FR4, and in particular at at least one of the Hallmark residues in FR2 and/or FR4 (again, including those at positions 108, 103 and/or 45).

As will be clear from the disclosure herein, it is also within the scope of the invention to use natural or synthetic analogs, mutants, variants, alleles, homologs and orthologs (herein collectively referred to as “*analogs*”) of the Nanobodies of the invention as defined herein, and in particular analogs of the Nanobodies of SEQ ID NO’s: 399-449. Thus, according to one embodiment of the invention, the term “Nanobody of the invention” in its broadest sense also covers such analogs.

Generally, in such analogs, one or more amino acid residues may have been replaced, deleted and/or added, compared to the Nanobodies of the invention as defined herein. Such substitutions, insertions or deletions may be made in one or more of the framework regions and/or in one or more of the CDR’s. When such substitutions, insertions or deletions are made in one or more of the framework regions, they may be made at one or more of the Hallmark residues and/or at one or more of the other positions in the framework residues, although substitutions, insertions or deletions at the Hallmark residues are generally less preferred (unless these are suitable humanizing substitutions as described herein).

By means of non-limiting examples, a substitution may for example be a conservative substitution (as described herein) and/or an amino acid residue may be replaced by another amino acid residue that naturally occurs at the same position in another V_{HH} domain (see Tables A-5 – A-8 for some non-limiting examples of such substitutions), although the invention is generally not limited thereto. Thus, any one or more substitutions, deletions or insertions, or any combination thereof, that either improve the properties of the Nanobody of the invention or that at least do not detract too much from the desired properties or from the

balance or combination of desired properties of the Nanobody of the invention (i.e. to the extent that the Nanobody is no longer suited for its intended use) are included within the scope of the invention. A skilled person will generally be able to determine and select suitable substitutions, deletions or insertions, or suitable combinations of thereof, based on the disclosure herein and optionally after a limited degree of routine experimentation, which may for example involve introducing a limited number of possible substitutions and determining their influence on the properties of the Nanobodies thus obtained.

For example, and depending on the host organism used to express the Nanobody or polypeptide of the invention, such deletions and/or substitutions may be designed in such a way that one or more sites for post-translational modification (such as one or more glycosylation sites) are removed, as will be within the ability of the person skilled in the art. Alternatively, substitutions or insertions may be designed so as to introduce one or more sites for attachment of functional groups (as described herein), for example to allow site-specific pegylation (again as described herein).

As can be seen from the data on the V_{HH} entropy and V_{HH} variability given in Tables A-5 – A-8 above, some amino acid residues in the framework regions are more conserved than others. Generally, although the invention in its broadest sense is not limited thereto, any substitutions, deletions or insertions are preferably made at positions that are less conserved. Also, generally, amino acid substitutions are preferred over amino acid deletions or insertions.

The analogs are preferably such that they can bind to the IL-6 receptor, with affinity (suitably measured and/or expressed as a K_D -value (actual or apparent), a K_A -value (actual or apparent), a k_{on} -rate and/or a k_{off} -rate, or alternatively as an IC_{50} value, as further described herein) that is as defined herein; as well as compounds and constructs, and in particular proteins and polypeptides, that comprise at least one such amino acid sequence.

In particular, amino acid sequences and polypeptides of the invention are preferably such that they:

- bind to IL-6R with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter or less, and preferably 10^{-7} to 10^{-12} moles/liter or less and more preferably 10^{-8} to 10^{-12} moles/liter (i.e. with an association constant (K_A) of 10^5 to 10^{12} liter/ moles or more, and preferably 10^7 to 10^{12} liter/moles or more and more preferably 10^8 to 10^{12} liter/moles);

and/or such that they:

- bind to IL-6R with a k_{on} -rate of between $10^2 \text{ M}^{-1} \text{s}^{-1}$ to about $10^7 \text{ M}^{-1} \text{s}^{-1}$, preferably between $10^3 \text{ M}^{-1} \text{s}^{-1}$ and $10^7 \text{ M}^{-1} \text{s}^{-1}$, more preferably between $10^4 \text{ M}^{-1} \text{s}^{-1}$ and $10^7 \text{ M}^{-1} \text{s}^{-1}$, such as between $10^5 \text{ M}^{-1} \text{s}^{-1}$ and $10^7 \text{ M}^{-1} \text{s}^{-1}$;

and/or such that they:

- bind to IL-6R with a k_{off} rate between 1s^{-1} ($t_{1/2}=0.69\text{ s}$) and 10^{-6}s^{-1} (providing a near irreversible complex with a $t_{1/2}$ of multiple days), preferably between 10^{-2}s^{-1} and 10^{-6}s^{-1} , more preferably between 10^{-3}s^{-1} and 10^{-6}s^{-1} , such as between 10^{-4}s^{-1} and 10^{-6}s^{-1} .

Preferably, a monovalent amino acid sequence of the invention (or a polypeptide that contains only one amino acid sequence of the invention) is preferably such that it will bind to IL-6R with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM.

Some preferred IC50 values for binding of the amino acid sequences or polypeptides 10 of the invention to IL-6R will become clear from the further description and examples herein.

The analogs are preferably also such that they retain the favourable properties the Nanobodies, as described herein.

Also, according to one preferred embodiment, the analogs have a degree of sequence identity of at least 70%, preferably at least 80%, more preferably at least 90%, such as at least 95% or 99% or more; and/or preferably have at most 20, preferably at most 10, even more preferably at most 5, such as 4, 3, 2 or only 1 amino acid difference (as defined herein), with one of the Nanobodies of SEQ ID NOS 399-449.

Also, the framework sequences and CDR's of the analogs are preferably such that they are in accordance with the preferred embodiments defined herein. More generally, as 20 described herein, the analogs will have (a) a Q at position 108; and/or (b) a charged amino acid or a cysteine residue at position 45 and preferably an E at position 44, and more preferably E at position 44 and R at position 45; and/or (c) P, R or S at position 103.

One preferred class of analogs of the Nanobodies of the invention comprise Nanobodies that have been humanized (i.e. compared to the sequence of a naturally occurring Nanobody of the invention). As mentioned in the background art cited herein, such humanization generally involves replacing one or more amino acid residues in the sequence of a naturally occurring V_{HH} with the amino acid residues that occur at the same position in a human V_H domain, such as a human V_H3 domain. Examples of possible humanizing substitutions or combinations of humanizing substitutions will be clear to the skilled person, 30 for example from the Tables herein, from the possible humanizing substitutions mentioned in the background art cited herein, and/or from a comparision between the sequence of a Nanobody and the sequence of a naturally occurring human V_H domain.

The humanizing substitutions should be chosen such that the resulting humanized Nanobodies still retain the favourable properties of Nanobodies as defined herein, and more

preferably such that they are as described for analogs in the preceding paragraphs. A skilled person will generally be able to determine and select suitable humanizing substitutions or suitable combinations of humanizing substitutions, based on the disclosure herein and optionally after a limited degree of routine experimentation, which may for example involve introducing a limited number of possible humanizing substitutions and determining their influence on the properties of the Nanobodies thus obtained.

Generally, as a result of humanization, the Nanobodies of the invention may become more “human-like”, while still retaining the favorable properties of the Nanobodies of the invention as described herein. As a result, such humanized Nanobodies may have several advantages, such as a reduced immunogenicity, compared to the corresponding naturally occurring V_{HH} domains. Again, based on the disclosure herein and optionally after a limited degree of routine experimentation, the skilled person will be able to select humanizing substitutions or suitable combinations of humanizing substitutions which optimize or achieve a desired or suitable balance between the favourable properties provided by the humanizing substitutions on the one hand and the favourable properties of naturally occurring V_{HH} domains on the other hand.

The Nanobodies of the invention may be suitably humanized at any framework residue(s), such as at one or more Hallmark residues (as defined herein) or at one or more other framework residues (i.e. non-Hallmark residues) or any suitable combination thereof.

20 One preferred humanizing substitution for Nanobodies of the “P,R,S-103 group” or the “KERE group” is Q108 into L108. Nanobodies of the “GLEW class” may also be humanized by a Q108 into L108 substitution, provided at least one of the other Hallmark residues contains a camelid (camelizing) substitution (as defined herein). For example, as mentioned above, one particularly preferred class of humanized Nanobodies has GLEW or a GLEW-like sequence at positions 44-47; P, R or S (and in particular R) at position 103, and an L at position 108.

30 The humanized and other analogs, and nucleic acid sequences encoding the same, can be provided in any manner known per se. For example, the analogs can be obtained by providing a nucleic acid that encodes a naturally occurring V_{HH} domain, changing the codons for the one or more amino acid residues that are to be substituted into the codons for the corresponding desired amino acid residues (e.g. by site-directed mutagenesis or by PCR using suitable mismatch primers), expressing the nucleic acid/nucleotide sequence thus obtained in a suitable host or expression system; and optionally isolating and/or purifying the analog thus obtained to provide said analog in essentially isolated form (e.g. as further described herein).

This can generally be performed using methods and techniques known per se, which will be clear to the skilled person, for example from the handbooks and references cited herein, the background art cited herein and/or from the further description herein. Alternatively, a nucleic acid encoding the desired analog can be synthesized in a manner known per se (for example using an automated apparatus for synthesizing nucleic acid sequences with a predefined amino acid sequence) and can then be expressed as described herein. Yet another technique may involve combining one or more naturally occurring and/or synthetic nucleic acid sequences each encoding a part of the desired analog, and then expressing the combined nucleic acid sequence as described herein. Also, the analogs can be provided using chemical synthesis of the pertinent amino acid sequence using techniques for peptide synthesis known per se, such as those mentioned herein.

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In this respect, it will be also be clear to the skilled person that the Nanobodies of the invention (including their analogs) can be designed and/or prepared starting from human V_H sequences (i.e. amino acid sequences or the corresponding nucleotide sequences), such as for example from human V_H 3 sequences such as DP-47, DP-51 or DP-29, i.e. by introducing one or more camelizing substitutions (i.e. changing one or more amino acid residues in the amino acid sequence of said human V_H domain into the amino acid residues that occur at the corresponding position in a V_{HH} domain), so as to provide the sequence of a Nanobody of the invention and/or so as to confer the favourable properties of a Nanobody to the sequence thus obtained. Again, this can generally be performed using the various methods and techniques referred to in the previous paragraph, using an amino acid sequence and/or nucleotide sequence for a human V_H domain as a starting point.

20

Some preferred, but non-limiting camelizing substitutions can be derived from Tables A-5 – A-8. It will also be clear that camelizing substitutions at one or more of the Hallmark residues will generally have a greater influence on the desired properties than substitutions at one or more of the other amino acid positions, although both and any suitable combination thereof are included within the scope of the invention. For example, it is possible to introduce one or more camelizing substitutions that already confer at least some the desired properties, and then to introduce further camelizing substitutions that either further improve said properties and/or confer additional favourable properties. Again, the skilled person will generally be able to determine and select suitable camelizing substitutions or suitable combinations of camelizing substitutions, based on the disclosure herein and optionally after a limited degree of routine experimentation, which may for example involve introducing a

30

limited number of possible camelizing substitutions and determining whether the favourable properties of Nanobodies are obtained or improved (i.e. compared to the original V_H domain).

Generally, however, such camelizing substitutions are preferably such that the resulting an amino acid sequence at least contains (a) a Q at position 108; and/or (b) a charged amino acid or a cysteine residue at position 45 and preferably also an E at position 44, and more preferably E at position 44 and R at position 45; and/or (c) P, R or S at position 103; and optionally one or more further camelizing substitutions. More preferably, the camelizing substitutions are such that they result in a Nanobody of the invention and/or in an analog thereof (as defined herein), such as in a humanized analog and/or preferably in an analog that is as defined in the preceding paragraphs.

As will also be clear from the disclosure herein, it is also within the scope of the invention to use parts or fragments, or combinations of two or more parts or fragments, of the Nanobodies of the invention as defined herein, and in particular parts or fragments of the Nanobodies of SEQ ID NO's 399-449. Thus, according to one embodiment of the invention, the term "Nanobody of the invention" in its broadest sense also covers such parts or fragments.

Generally, such parts or fragments of the Nanobodies of the invention (including analogs thereof) have amino acid sequences in which, compared to the amino acid sequence of the corresponding full length Nanobody of the invention (or analog thereof), one or more of the amino acid residues at the N-terminal end, one or more amino acid residues at the C-terminal end, one or more contiguous internal amino acid residues, or any combination thereof, have been deleted and/or removed.

The parts or fragments are preferably such that they can bind to the IL-6 receptor, with an affinity (suitably measured and/or expressed as a K_D-value (actual or apparent), a K_A-value (actual or apparent), a k_{on}-rate and/or a k_{off}-rate, or alternatively as an IC₅₀ value, as further described herein) that is as defined herein; as well as compounds and constructs, and in particular proteins and polypeptides, that comprise at least one such amino acid sequence.

In particular, amino acid sequences and polypeptides of the invention are preferably such that they:

30 - bind to IL-6R with a dissociation constant (K_D) of 10⁻⁵ to 10⁻¹² moles/liter or less, and preferably 10⁻⁷ to 10⁻¹² moles/liter or less and more preferably 10⁻⁸ to 10⁻¹² moles/liter (i.e. with an association constant (K_A) of 10⁵ to 10¹² liter/ moles or more, and preferably 10⁷ to 10¹² liter/moles or more and more preferably 10⁸ to 10¹² liter/moles);

and/or such that they:

- bind to IL-6R with a k_{on} -rate of between $10^2 \text{ M}^{-1}\text{s}^{-1}$ to about $10^7 \text{ M}^{-1}\text{s}^{-1}$, preferably between $10^3 \text{ M}^{-1}\text{s}^{-1}$ and $10^7 \text{ M}^{-1}\text{s}^{-1}$, more preferably between $10^4 \text{ M}^{-1}\text{s}^{-1}$ and $10^7 \text{ M}^{-1}\text{s}^{-1}$, such as between $10^5 \text{ M}^{-1}\text{s}^{-1}$ and $10^7 \text{ M}^{-1}\text{s}^{-1}$;

and/or such that they:

- bind to IL-6R with a k_{off} rate between 1s^{-1} ($t_{1/2}=0.69 \text{ s}$) and 10^{-6} s^{-1} (providing a near irreversible complex with a $t_{1/2}$ of multiple days), preferably between 10^{-2} s^{-1} and 10^{-6} s^{-1} , more preferably between 10^{-3} s^{-1} and 10^{-4} s^{-1} , such as between 10^{-4} s^{-1} and 10^{-6} s^{-1} .

Preferably, a monovalent amino acid sequence of the invention (or a polypeptide that contains only one amino acid sequence of the invention) is preferably such that it will bind to IL-6R with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM.

Some preferred IC50 values for binding of the amino acid sequences or polypeptides of the invention to IL-6R will become clear from the further description and examples herein.

The affinity of the analog against the IL-6 receptor, can be determined in a manner known per se, for example using the assay described herein.

Any part or fragment is preferably such that it comprises at least 10 contiguous amino acid residues, preferably at least 20 contiguous amino acid residues, more preferably at least 30 contiguous amino acid residues, such as at least 40 contiguous amino acid residues, of the amino acid sequence of the corresponding full length Nanobody of the invention.

Also, any part or fragment is such preferably that it comprises at least one of CDR1, CDR2 and/or CDR3 or at least part thereof (and in particular at least CDR3 or at least part thereof). More preferably, any part or fragment is such that it comprises at least one of the CDR's (and preferably at least CDR3 or part thereof) and at least one other CDR (i.e. CDR1 or CDR2) or at least part thereof, preferably connected by suitable framework sequence(s) or at least part thereof. More preferably, any part or fragment is such that it comprises at least one of the CDR's (and preferably at least CDR3 or part thereof) and at least part of the two remaining CDR's, again preferably connected by suitable framework sequence(s) or at least part thereof.

According to another particularly preferred, but non-limiting embodiment, such a part or fragment comprises at least CDR3, such as FR3, CDR3 and FR4 of the corresponding full length Nanobody of the invention, i.e. as for example described in the International application WO 03/050531 (Lasters et al.).

As already mentioned above, it is also possible to combine two or more of such parts or fragments (i.e. from the same or different Nanobodies of the invention), i.e. to provide an

analog (as defined herein) and/or to provide further parts or fragments (as defined herein) of a Nanobody of the invention. It is for example also possible to combine one or more parts or fragments of a Nanobody of the invention with one or more parts or fragments of a human V_H domain.

According to one preferred embodiment, the parts or fragments have a degree of sequence identity of at least 50%, preferably at least 60%, more preferably at least 70%, even more preferably at least 80%, such as at least 90%, 95% or 99% or more with one of the Nanobodies of SEQ ID NOS: 399-449.

10 The parts and fragments, and nucleic acid sequences encoding the same, can be provided and optionally combined in any manner known per se. For example, such parts or fragments can be obtained by inserting a stop codon in a nucleic acid that encodes a full-sized Nanobody of the invention, and then expressing the nucleic acid thus obtained in a manner known per se (e.g. as described herein). Alternatively, nucleic acids encoding such parts or fragments can be obtained by suitably restricting a nucleic acid that encodes a full-sized Nanobody of the invention or by synthesizing such a nucleic acid in a manner known per se. Parts or fragments may also be provided using techniques for peptide synthesis known per se.

20 The invention in its broadest sense also comprises derivatives of the Nanobodies of the invention. Such derivatives can generally be obtained by modification, and in particular by chemical and/or biological (e.g enzymatical) modification, of the Nanobodies of the invention and/or of one or more of the amino acid residues that form the Nanobodies of the invention.

Examples of such modifications, as well as examples of amino acid residues within the Nanobody sequence that can be modified in such a manner (i.e. either on the protein backbone but preferably on a side chain), methods and techniques that can be used to introduce such modifications and the potential uses and advantages of such modifications will be clear to the skilled person.

30 For example, such a modification may involve the introduction (e.g. by covalent linking or in an other suitable manner) of one or more functional groups, residues or moieties into or onto the Nanobody of the invention, and in particular of one or more functional groups, residues or moieties that confer one or more desired properties or functionalities to the Nanobody of the invention. Example of such functional groups will be clear to the skilled person.

For example, such modification may comprise the introduction (e.g. by covalent binding or in any other suitable manner) of one or more functional groups that increase the half-life, the solubility and/or the absorption of the Nanobody of the invention, that reduce

the immunogenicity and/or the toxicity of the Nanobody of the invention, that eliminate or attenuate any undesirable side effects of the Nanobody of the invention, and/or that confer other advantageous properties to and/or reduce the undesired properties of the Nanobodies and/or polypeptides of the invention; or any combination of two or more of the foregoing. Examples of such functional groups and of techniques for introducing them will be clear to the skilled person, and can generally comprise all functional groups and techniques mentioned in the general background art cited hereinabove as well as the functional groups and techniques known per se for the modification of pharmaceutical proteins, and in particular for the modification of antibodies or antibody fragments (including ScFv's and single domain antibodies), for which reference is for example made to Remington's Pharmaceutical Sciences, 16th ed., Mack Publishing Co., Easton, PA (1980). Such functional groups may for example be linked directly (for example covalently) to a Nanobody of the invention, or optionally via a suitable linker or spacer, as will again be clear to the skilled person.

One of the most widely used techniques for increasing the half-life and/or reducing the immunogenicity of pharmaceutical proteins comprises attachment of a suitable pharmacologically acceptable polymer, such as poly(ethyleneglycol) (PEG) or derivatives thereof (such as methoxypoly(ethyleneglycol) or mPEG). Generally, any suitable form of pegylation can be used, such as the pegylation used in the art for antibodies and antibody fragments (including but not limited to (single) domain antibodies and ScFv's); reference is made to for example Chapman, Nat. Biotechnol., 54, 531-545 (2002); by Veronese and Harris, Adv. Drug Deliv. Rev. 54, 453-456 (2003), by Harris and Chess, Nat. Rev. Drug. Discov., 2, (2003) and in WO 04/060965. Various reagents for pegylation of proteins are also commercially available, for example from Nektar Therapeutics, USA.

Preferably, site-directed pegylation is used, in particular via a cysteine-residue (see for example Yang et al., Protein Engineering, 16, 10, 761-770 (2003). For example, for this purpose, PEG may be attached to a cysteine residue that naturally occurs in a Nanobody of the invention, a Nanobody of the invention may be modified so as to suitably introduce one or more cysteine residues for attachment of PEG, or an amino acid sequence comprising one or more cysteine residues for attachment of PEG may be fused to the N- and/or C-terminus of a Nanobody of the invention, all using techniques of protein engineering known per se to the skilled person.

Preferably, for the Nanobodies and proteins of the invention, a PEG is used with a molecular weight of more than 5000, such as more than 10,000 and less than 200,000, such as less than 100,000; for example in the range of 20,000-80,000.

Another, usually less preferred modification comprises N-linked or O-linked glycosylation, usually as part of co-translational and/or post-translational modification, depending on the host cell used for expressing the Nanobody or polypeptide of the invention.

Yet another modification may comprise the introduction of one or more detectable labels or other signal-generating groups or moieties, depending on the intended use of the labelled Nanobody. Suitable labels and techniques for attaching, using and detecting them will be clear to the skilled person, and for example include, but are not limited to, fluorescent labels (such as fluorescein, isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde, and fluorescamine and fluorescent metals such as ¹⁵²Eu or others metals from the lanthanide series), phosphorescent labels, chemiluminescent labels or bioluminescent labels (such as luminal, isoluminol, theromatic acridinium ester, imidazole, acridinium salts, oxalate ester, dioxetane or GFP and its analogs), radio-isotopes (such as ³H, ¹²⁵I, ³²P, ³⁵S, ¹⁴C, ⁵¹Cr, ³⁶Cl, ⁵⁷Co, ⁵⁸Co, ⁵⁹Fe, and ⁷⁵Se), metals, metals chelates or metallic cations (for example metallic cations such as ^{99m}Tc, ¹²³I, ¹¹¹In, ¹³¹I, ⁹⁷Ru, ⁶⁷Cu, ⁶⁷Ga, and ⁶⁸Ga or other metals or metallic cations that are particularly suited for use in *in vivo*, *in vitro* or *in situ* diagnosis and imaging, such as (¹⁵⁷Gd, ⁵⁵Mn, ¹⁶²Dy, ⁵²Cr, and ⁵⁶Fe), as well as chromophores and enzymes (such as malate dehydrogenase, staphylococcal nuclease, delta-V-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, biotinavidin peroxidase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, β-galactosidase, ribonuclease, urease, catalase, glucose-VI-phosphate dehydrogenase, glucoamylase and acetylcholine esterase). Other suitable labels will be clear to the skilled person, and for example include moieties that can be detected using NMR or ESR spectroscopy.

Such labelled Nanobodies and polypeptides of the invention may for example be used for *in vitro*, *in vivo* or *in situ* assays (including immunoassays known per se such as ELISA, RIA, EIA and other “sandwich assays”, etc.) as well as *in vivo* diagnostic and imaging purposes, depending on the choice of the specific label.

As will be clear to the skilled person, another modification may involve the introduction of a chelating group, for example to chelate one of the metals or metallic cations referred to above. Suitable chelating groups for example include, without limitation, diethylenetriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

Yet another modification may comprise the introduction of a functional group that is one part of a specific binding pair, such as the biotin-(strept)avidin binding pair. Such a functional group may be used to link the Nanobody of the invention to another protein,

polypeptide or chemical compound that is bound to the other half of the binding pair, i.e. through formation of the binding pair. For example, a Nanobody of the invention may be conjugated to biotin, and linked to another protein, polypeptide, compound or carrier conjugated to avidin or streptavidin. For example, such a conjugated Nanobody may be used as a reporter, for example in a diagnostic system where a detectable signal-producing agent is conjugated to avidin or streptavidin. Such binding pairs may for example also be used to bind the Nanobody of the invention to a carrier, including carriers suitable for pharmaceutical purposes. One non-limiting example are the liposomal formulations described by Cao and Suresh, *Journal of Drug Targetting*, 8, 4, 257 (2000). Such binding pairs may also be used to link a therapeutically active agent to the Nanobody of the invention.

10 For some applications, in particular for those applications in which it is intended to kill a cell that expresses the target against which the Nanobodies of the invention are directed (e.g. in the treatment of cancer), or to reduce or slow the growth and/or proliferation such a cell, the Nanobodies of the invention may also be linked to a toxin or to a toxic residue or moiety. Examples of toxic moieties, compounds or residues which can be linked to a Nanobody of the invention to provide – for example – a cytotoxic compound will be clear to the skilled person and can for example be found in the prior art cited above and/or in the further description herein. One example is the so-called ADEPT™ technology WO 03/055527.

20 Other potential chemical and enzymatical modifications will be clear to the skilled person. Such modifications may also be introduced for research purposes (e.g. to study function-activity relationships). Reference is for example made to Lundblad and Bradshaw, *Biotechnol. Appl. Biochem.*, 26, 143-151 (1997).

Preferably, the derivatives are such that they bind to the IL-6 receptor, with an affinity (suitably measured and/or expressed as a K_D -value (actual or apparent), a K_A -value (actual or apparent), a k_{on} -rate and/or a k_{off} -rate, or alternatively as an IC_{50} value, as further described herein) that is as defined herein; as well as compounds and constructs, and in particular proteins and polypeptides, that comprise at least one such amino acid sequence.

In particular, amino acid sequences and polypeptides of the invention are preferably such that they:

30 - bind to IL-6R with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter or less, and preferably 10^{-7} to 10^{-12} moles/liter or less and more preferably 10^{-8} to 10^{-12} moles/liter (i.e. with an association constant (K_A) of 10^5 to 10^{12} liter/ moles or more, and preferably 10^7 to 10^{12} liter/moles or more and more preferably 10^8 to 10^{12} liter/moles); and/or such that they:

- bind to IL-6R with a k_{on} -rate of between $10^2 \text{ M}^{-1}\text{s}^{-1}$ to about $10^7 \text{ M}^{-1}\text{s}^{-1}$, preferably between $10^3 \text{ M}^{-1}\text{s}^{-1}$ and $10^7 \text{ M}^{-1}\text{s}^{-1}$, more preferably between $10^4 \text{ M}^{-1}\text{s}^{-1}$ and $10^7 \text{ M}^{-1}\text{s}^{-1}$, such as between $10^5 \text{ M}^{-1}\text{s}^{-1}$ and $10^7 \text{ M}^{-1}\text{s}^{-1}$;

and/or such that they:

- bind to IL-6R with a k_{off} rate between 1s^{-1} ($t_{1/2}=0.69 \text{ s}$) and 10^{-6} s^{-1} (providing a near irreversible complex with a $t_{1/2}$ of multiple days), preferably between 10^{-2} s^{-1} and 10^{-6} s^{-1} , more preferably between 10^{-3} s^{-1} and 10^{-6} s^{-1} , such as between 10^{-4} s^{-1} and 10^{-6} s^{-1} .

Preferably, a monovalent amino acid sequence of the invention (or a polypeptide that contains only one amino acid sequence of the invention) is preferably such that it will bind to 10 IL-6R with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM.

Some preferred IC50 values for binding of the amino acid sequences or polypeptides of the invention to IL-6R will become clear from the further description and examples herein.

The affinity of a derivative of a Nanobody of the invention against the IL-6 receptor, can be determined in a manner known per se, for example using the assay described herein.

As mentioned above, the invention also relates to proteins or polypeptides that essentially consist of or comprise at least one Nanobody of the invention. By “essentially consist of” is meant that the amino acid sequence of the polypeptide of the invention either is exactly the same as the amino acid sequence of a Nanobody of the invention or corresponds to 20 the amino acid sequence of a Nanobody of the invention which has a limited number of amino acid residues, such as 1-20 amino acid residues, for example 1-10 amino acid residues and preferably 1-6 amino acid residues, such as 1, 2, 3, 4, 5 or 6 amino acid residues, added at the amino terminal end, at the carboxy terminal end, or at both the amino terminal end and the carboxy terminal end of the amino acid sequence of the Nanobody.

Said amino acid residues may or may not change, alter or otherwise influence the (biological) properties of the Nanobody and may or may not add further functionality to the Nanobody. For example, such amino acid residues:

- a) can comprise an N-terminal Met residue, for example as result of expression in a heterologous host cell or host organism.
- 30 b) may form a signal sequence or leader sequence that directs secretion of the Nanobody from a host cell upon synthesis. Suitable secretory leader peptides will be clear to the skilled person, and may be as further described herein. Usually, such a leader sequence will be linked to the N-terminus of the Nanobody, although the invention in its broadest sense is not limited thereto;

c) may form a sequence or signal that allows the Nanobody to be directed towards and/or to penetrate or enter into specific organs, tissues, cells, or parts or compartments of cells, and/or that allows the Nanobody to penetrate or cross a biological barrier such as a cell membrane, a cell layer such as a layer of epithelial cells, a tumor including solid tumors, or the blood-brain-barrier. Examples of such amino acid sequences will be clear to the skilled person. Some non-limiting examples are the small peptide vectors (“Pep-trans vectors”) described in WO 03/026700 and in Temsamani et al., Expert Opin. Biol. Ther., 1, 773 (2001); Temsamani and Vidal, Drug Discov. Today, 9, 1012 (004) and Rousselle, J. Pharmacol. Exp. Ther., 296, 124-131 (2001), and the membrane translocator sequence described by Zhao et al., Apoptosis, 8, 631-637 (2003). C-terminal and N-terminal amino acid sequences for intracellular targeting of antibody fragments are for example described by Cardinale et al., Methods, 34, 171 (2004). Other suitable techniques for intracellular targeting involve the expression and/or use of so-called “intrabodies” comprising a Nanobody of the invention, as mentioned below;

10 d) may form a “tag”, for example an amino acid sequence or residue that allows or facilitates the purification of the Nanobody, for example using affinity techniques directed against said sequence or residue. Thereafter, said sequence or residue may be removed (e.g. by chemical or enzymatical cleavage) to provide the Nanobody sequence (for this purpose, the tag may optionally be linked to the Nanobody sequence via a cleavable linker sequence or contain a cleavable motif). Some preferred, but non-limiting examples of such residues are multiple histidine residues, glutatione residues and a myc-tag such as AAAEQKLISEEDLNGAA [SEQ ID NO:31];

20 e) may be one or more amino acid residues that have been functionalized and/or that can serve as a site for attachment of functional groups. Suitable amino acid residues and functional groups will be clear to the skilled person and include, but are not limited to, the amino acid residues and functional groups mentioned herein for the derivatives of the Nanobodies of the invention.

According to another embodiment, a polypeptide of the invention comprises a Nanobody of the invention, which is fused at its amino terminal end, at its carboxy terminal end, or both at its amino terminal end and at its carboxy terminal end to at least one further amino acid sequence, i.e. so as to provide a fusion protein comprising said Nanobody of the invention and the one or more further amino acid sequences. Such a fusion will also be referred to herein as a “Nanobody fusion”.

The one or more further amino acid sequence may be any suitable and/or desired amino acid sequences. The further amino acid sequences may or may not change, alter or otherwise influence the (biological) properties of the Nanobody, and may or may not add further functionality to the Nanobody or the polypeptide of the invention. Preferably, the further amino acid sequence is such that it confers one or more desired properties or functionalities to the Nanobody or the polypeptide of the invention.

Example of such amino acid sequences will be clear to the skilled person, and may generally comprise all amino acid sequences that are used in peptide fusions based on conventional antibodies and fragments thereof (including but not limited to ScFv's and single domain antibodies). Reference is for example made to the review by Holliger and Hudson, *Nature Biotechnology*, 23, 9, 1126-1136 (2005),

For example, such an amino acid sequence may be an amino acid sequence that increases the half-life, the solubility, or the absorption, reduces the immunogenicity or the toxicity, eliminates or attenuates undesirable side effects, and/or confers other advantageous properties to and/or reduces the undesired properties of the polypeptides of the invention, compared to the Nanobody of the invention *per se*. Some non-limiting examples of such amino acid sequences are serum proteins, such as human serum albumin (see for example WO 00/27435) or haptic molecules (for example haptens that are recognized by circulating antibodies, see for example WO 98/22141).

The further amino acid sequence may also provide a second binding site, which binding site may be directed against any desired protein, polypeptide, antigen, antigenic determinant or epitope (including but not limited to the same protein, polypeptide, antigen, antigenic determinant or epitope against which the Nanobody of the invention is directed, or a different protein, polypeptide, antigen, antigenic determinant or epitope). For example, the further amino acid sequence may provide a second binding site that is directed against a serum protein (such as, for example, human serum albumin or another serum protein such as IgG), so as to provide increased half-life in serum. Reference is for example made to EP 0 368 684, WO 91/01743, WO 01/45746 and WO 04/003019 (in which various serum proteins are mentioned), the International application by Ablynx N.V. entitled "Nanobodies against amyloid-beta and polypeptides comprising the same for the treatment of degenerative neural diseases such as Alzheimer's disease" (in which various other proteins are mentioned), as well as to Harmsen et al., *Vaccine*, 23 (41); 4926-42, 2005.

According to another embodiment, the one or more further amino acid sequences may comprise one or more parts, fragments or domains of conventional 4-chain antibodies (and in

particular human antibodies) and/or of heavy chain antibodies. For example, although usually less preferred, a Nanobody of the invention may be linked to a conventional (preferably human) V_H or V_L domain domain or to a natural or synthetic analog of a V_H or V_L domain, again optionally via a linker sequence (including but not limited to other (single) domain antibodies, such as the dAb's described by Ward et al.).

In one specific aspect of the invention, a Nanobody of the invention or a compound, construct or polypeptide of the invention comprising at least one Nanobody of the invention may have an increased half-life, compared to the corresponding amino acid sequence of the invention. Some preferred, but non-limiting examples of such Nanobodies, compounds and polypeptides will become clear to the skilled person based on the further disclosure herein, and for example comprise Nanobodies sequences or polypeptides of the invention that have been chemically modified to increase the half-life thereof (for example, by means of pegylation); amino acid sequences of the invention that comprise at least one additional binding site for binding to a serum protein (such as serum albumin. Reference is for example made to the US provisional application by Ablynx N.V. entitled "*Immunoglobulin domains with multiple binding sites*" filed on November 27, 2006); or polypeptides of the invention that comprise at least one Nanobody of the invention that is linked to at least one moiety (and in particular at least one amino acid sequence) that increases the half-life of the Nanobody of the invention. Examples of polypeptides of the invention that comprise such half-life extending moieties or amino acid sequences will become clear to the skilled person based on the further disclosure herein; and for example include, without limitation, polypeptides in which the one or more Nanobodies of the invention are suitable linked to one or more serum proteins or fragments thereof (such as serum albumin or suitable fragments thereof) or to one or more binding units that can bind to serum proteins (such as, for example, Nanobodies or (single) domain antibodies that can bind to serum proteins such as serum albumin, serum immunoglobulins such as IgG, or transferrine); polypeptides in which a Nanobody of the invention is linked to an Fc portion (such as a human Fc) or a suitable part or fragment thereof; or polypeptides in which the one or more Nanobodies of the invention are suitable linked to one or more small proteins or peptides that can bind to serum proteins (such as, without limitation, the proteins and peptides described in WO 91/01743, WO 01/45746, WO 02/076489).

Generally, the Nanobodies of the invention (or compounds, constructs or polypeptides comprising the same) with increased half-life preferably have a half-life that is at least 1.5 times, preferably at least 2 times, such as at least 5 times, for example at least 10 times or

more than 20 times, greater than the half-life of the corresponding amino acid sequence of the invention per se. For example, the Nanobodies, compounds, constructs or polypeptides of the invention with increased half-life may have a half-life that is increased with more than 1 hours, preferably more than 2 hours, more preferably more than 6 hours, such as more than 12 hours, or even more than 24, 48 or 72 hours, compared to the corresponding amino acid sequence of the invention per se.

In a preferred, but non-limiting aspect of the invention, such Nanobodies, compound, constructs or polypeptides of the invention exhibit a serum half-life in human of at least about 12 hours, preferably at least 24 hours, more preferably at least 48 hours, even more preferably 10 at least 72 hours or more. For example, compounds or polypeptides of the invention may have a half-life of at least 5 days (such as about 5 to 10 days), at preferably at least 9 days (such as about 9 to 14 days), more preferably at least about 10 days (such as about 10 to 15 days), or at least about 11 days (such as about 11 to 16 days), more preferably at least about 12 days (such as about 12 to 18 days or more), or more than 14 days (such as about 14 to 19 days).

In another one aspect of the invention, a polypeptide of the invention comprises one or more (such as two or preferably one) Nanobodies of the invention linked (optionally via one or more suitable linker sequences) to one or more (such as two and preferably one) amino acid sequences that allow the resulting polypeptide of the invention to cross the blood brain barrier. In particular, said one or more amino acid sequences that allow the resulting 20 polypeptides of the invention to cross the blood brain barrier may be one or more (such as two and preferably one) Nanobodies, such as the Nanobodies described in WO 02/057445, of which FC44 (SEQ ID NO: 189 of WO 06/040153) and FC5 (SEQ ID NO: 190 of WO 06/040154) are preferred examples.

In particular, it has been described in the art that linking fragments of immunoglobulins (such as V_H domains) to serum albumin or to fragments thereof can be used to increase the half-life. Reference is made to WO 00/27435 and WO 01/077137). According to the invention, the Nanobody of the invention is preferably either directly linked to serum albumin (or to a suitable fragment thereof) or via a suitable linker, and in particular via a suitable peptide linked so that the polypeptide of the invention can be expressed as a 30 genetic fusion (protein). According to one specific aspect, the Nanobody of the invention may be linked to a fragment of serum albumin that at least comprises the domain III of serum albumin or part thereof. Reference is for example made to the US provisional application 60/788,256 of Ablynx N.V. entitled "*Albumin derived amino acid sequence, use thereof for*

increasing the half-life of therapeutic proteins and of other therapeutic proteins and entities, and constructs comprising the same" filed on March 31, 2006.

Some preferred, but non-limiting, examples of fusions of anti-IL6R Nanobodies to human serum albumin are given in SEQ ID NO's: 603-608.

Alternatively, the further amino acid sequence may provide a second binding site or binding unit that is directed against a serum protein (such as, for example, human serum albumin or another serum protein such as IgG), so as to provide increased half-life in serum. Such amino acid sequences for example include the Nanobodies described below, as well as the small peptides and binding proteins described in WO 91/01743, WO 01/45746 and WO 10 02/076489 and the dAb's described in WO 03/002609 and WO 04/003019. Reference is also made to Harmsen et al., Vaccine, 23 (41); 4926-42, as well as to EP 0 368 684, as well as to the following the US provisional applications 60/843,349, 60/850,774, 60/850,775 by Ablynx N.V. mentioned herein.

Such amino acid sequences may in particular be directed against serum albumin (and more in particular human serum albumin) and/or against IgG (and more in particular human IgG). For example, such amino acid sequences may be amino acid sequences that are directed against (human) serum albumin and that amino acid sequences that can bind to amino acid residues on (human) serum albumin that are not involved in binding of serum albumin to FcRn (see for example WO 06/0122787) and/or amino acid sequences that are capable of binding to amino acid residues on serum albumin that do not form part of domain III of serum albumin (see again see for example WO 06/0122787); amino acid sequences that have or can provide an increased half-life (see for example the US provisional application 60/843,349 by Ablynx N.V. entitled "*Serum albumin binding proteins with long half-lives*" filed on September 8, 2006); amino acid sequences against human serum albumin that are cross-reactive with serum albumin from at least one species of mammal, and in particular with at least one species of primate (such as, without limitation, monkeys from the genus *Macaca* (such as, and in particular, cynomologus monkeys (*Macaca fascicularis*) and/or rhesus monkeys (*Macaca mulatta*)) and baboon (*Papio ursinus*), reference is again made to the US provisional application 60/843,349); amino acid sequences that can bind to serum albumin in a pH independent manner (see for example the US provisional application 60/850,774 by Ablynx N.V. entitled "*Amino acid sequences that bind to serum proteins in a manner that is essentially independent of the pH, compounds comprising the same, and uses thereof*", filed on October 11, 2006) and/or amino acid sequences that are conditional binders (see for example the US provisional application 60/850,775 by Ablynx N.V. entitled "*Amino acid*

sequences that bind to a desired molecule in a conditional manner”, filed on October 11, 2006).

The at least one Nanobody may also be linked to one or more (preferably human) CH₁, CH₂ and/or CH₃ domains, optionally via a linker sequence. For instance, a Nanobody linked to a suitable CH₁ domain could for example be used - together with suitable light chains - to generate antibody fragments/structures analogous to conventional Fab fragments or F(ab')2 fragments, but in which one or (in case of an F(ab')2 fragment) one or both of the conventional V_H domains have been replaced by a Nanobody of the invention. Also, two Nanobodies could be linked to a CH3 domain (optionally via a linker) to provide a construct with increased half-life in vivo.

According to one specific embodiment of a polypeptide of the invention, one or more Nanobodies of the invention may be linked to one or more antibody parts, fragments or domains that confer one or more effector functions to the polypeptide of the invention and/or may confer the ability to bind to one or more Fc receptors. For example, for this purpose, and without being limited thereto, the one or more further amino acid sequences may comprise one or more CH₂ and/or CH₃ domains of an antibody, such as from a heavy chain antibody (as described herein) and more preferably from a conventional human 4-chain antibody; and/or may form (part of) and Fc region, for example from IgG, from IgE or from another human Ig. For example, WO 94/04678 describes heavy chain antibodies comprising a Camelid V_{HH} domain or a humanized derivative thereof (i.e. a Nanobody), in which the Camelidae CH₂ and/or CH₃ domain have been replaced by human CH₂ and CH₃ domains, so as to provide an immunoglobulin that consists of 2 heavy chains each comprising a Nanobody and human CH2 and CH3 domains (but no CH1 domain), which immunoglobulin has the effector function provided by the CH2 and CH3 domains and which immunoglobulin can function without the presence of any light chains. Other amino acid sequences that can be suitably linked to the Nanobodies of the invention so as to provide an effector function will be clear to the skilled person, and may be chosen on the basis of the desired effector function(s).

Reference is for example made to WO 04/058820, WO 99/42077 and WO 05/017148, as well as the review by Holliger and Hudson, *supra*. Coupling of a Nanobody of the invention to an Fc portion may also lead to an increased half-life, compared to the corresponding Nanobody of the invention. For some applications, the use of an Fc portion and/or of constant domains (i.e. CH₂ and/or CH₃ domains) that confer increased half-life without any biologically significant effector function may also be suitable or even preferred. Other suitable constructs comprising one or more Nanobodies and one or more constant domains with increased half-

life in vivo will be clear to the skilled person, and may for example comprise two Nanobodies linked to a CH3 domain, optionally via a linker sequence. Generally, any fusion protein or derivatives with increased half-life will preferably have a molecular weight of more than 50 kD, the cut-off value for renal absorption.

The further amino acid sequences may also form a signal sequence or leader sequence that directs secretion of the Nanobody or the polypeptide of the invention from a host cell upon synthesis (for example to provide a pre-, pro- or prepro- form of the polypeptide of the invention, depending on the host cell used to express the polypeptide of the invention).

The further amino acid sequence may also form a sequence or signal that allows the 10 Nanobody or polypeptide of the invention to be directed towards and/or to penetrate or enter into specific organs, tissues, cells, or parts or compartments of cells, and/or that allows the Nanobody or polypeptide of the invention to penetrate or cross a biological barrier such as a cell membrane, a cell layer such as a layer of epithelial cells, a tumor including solid tumors, or the blood-brain-barrier. Suitable examples of such amino acid sequences will be clear to the skilled person, and for example include, but are not limited to, the “Peptrans” vectors mentioned above, the sequences described by Cardinale et al. and the amino acid sequences and antibody fragments known per se that can be used to express or produce the Nanobodies and polypeptides of the invention as so-called “intrabodies”, for example as described in WO 94/02610, WO 95/22618, US-A-7004940, WO 03/014960, WO 99/07414; WO 05/01690; EP 20 1 512 696; and in Cattaneo, A. & Biocca, S. (1997) Intracellular Antibodies: Development and Applications. Landes and Springer-Verlag; and in Kontermann, Methods 34, (2004), 163-170, and the further references described therein.

For some applications, in particular for those applications in which it is intended to kill a cell that expresses the target against which the Nanobodies of the invention are directed (e.g. in the treatment of cancer), or to reduce or slow the growth and/or proliferation of such a cell, the Nanobodies of the invention may also be linked to a (cyto)toxic protein or polypeptide. Examples of such toxic proteins and polypeptides which can be linked to a Nanobody of the invention to provide – for example – a cytotoxic polypeptide of the invention will be clear to the skilled person and can for example be found in the prior art cited above and/or in the 30 further description herein. One example is the so-called ADEPT™ technology described in WO 03/055527.

According to one preferred, but non-limiting embodiment, said one or more further amino acid sequences comprise at least one further Nanobody, so as to provide a polypeptide of the invention that comprises at least two, such as three, four, five or more Nanobodies, in

which said Nanobodies may optionally be linked via one or more linker sequences (as defined herein). Polypeptides of the invention that comprise two or more Nanobodies, of which at least one is a Nanobody of the invention, will also be referred to herein as “multivalent” polypeptides of the invention, and the Nanobodies present in such polypeptides will also be referred to herein as being in a “multivalent format”. For example a “bivalent” polypeptide of the invention comprises two Nanobodies, optionally linked via a linker sequence, whereas a “trivalent” polypeptide of the invention comprises three Nanobodies, optionally linked via two linker sequences; etc.; in which at least one of the Nanobodies present in the polypeptide, and up to all of the Nanobodies present in the polypeptide, is/are a Nanobody of the invention.

10 In a multivalent polypeptide of the invention, the two or more Nanobodies may be the same or different, and may be directed against the same antigen or antigenic determinant (for example against the same part(s) or epitope(s) or against different parts or epitopes) or may alternatively be directed against different antigens or antigenic determinants; or any suitable combination thereof. For example, a bivalent polypeptide of the invention may comprise (a) two identical Nanobodies; (b) a first Nanobody directed against a first antigenic determinant of a protein or antigen and a second Nanobody directed against the same antigenic determinant of said protein or antigen which is different from the first Nanobody; (c) a first Nanobody directed against a first antigenic determinant of a protein or antigen and a second Nanobody directed against another antigenic determinant of said protein or antigen; or (d) a first Nanobody directed against a first protein or antigen and a second Nanobody directed against a second protein or antigen (i.e. different from said first antigen). Similarly, a trivalent polypeptide of the invention may, for example and without being limited thereto, comprise (a) three identical Nanobodies; (b) two identical Nanobodies against a first antigenic determinant of an antigen and a third Nanobody directed against a different antigenic determinant of the same antigen; (c) two identical Nanobodies against a first antigenic determinant of an antigen and a third Nanobody directed against a second antigen different from said first antigen; (d) a first Nanobody directed against a first antigenic determinant of a first antigen, a second Nanobody directed against a second antigenic determinant of said first antigen and a third Nanobody directed against a second antigen different from said first antigen; or (e) a first Nanobody directed against a first antigen, a second Nanobody directed against a second antigen different from said first antigen, and a third Nanobody directed against a third antigen different from said first and second antigen.

Polypeptides of the invention that contain at least two Nanobodies, in which at least one Nanobody is directed against a first antigen (i.e. against the IL-6 receptor) and at least one

Nanobody is directed against a second antigen (i.e. different from the IL-6 receptor), will also be referred to as “multispecific” polypeptides of the invention, and the Nanobodies present in such polypeptides will also be referred to herein as being in a “multispecific format”. Thus, for example, a “bispecific” polypeptide of the invention is a polypeptide that comprises at least one Nanobody directed against a first antigen (i.e. the IL-6 receptor) and at least one further Nanobody directed against a second antigen (i.e. different from the IL-6 receptor), whereas a “trispecific” polypeptide of the invention is a polypeptide that comprises at least one Nanobody directed against a first antigen (i.e. the IL-6 receptor), at least one further Nanobody directed against a second antigen (i.e. different from the IL-6 receptor) and at least 10 one further Nanobody directed against a third antigen (i.e. different from both the IL-6 receptor, and the second antigen); etc.

Accordingly, in its simplest form, a bispecific polypeptide of the invention is a bivalent polypeptide of the invention (as defined herein), comprising a first Nanobody directed against the IL-6 receptor, and a second Nanobody directed against a second antigen, in which said first and second Nanobody may optionally be linked via a linker sequence (as defined herein); whereas a trispecific polypeptide of the invention in its simplest form is a trivalent polypeptide of the invention (as defined herein), comprising a first Nanobody directed against the IL-6 receptor, a second Nanobody directed against a second antigen and a third Nanobody directed against a third antigen, in which said first, second and third 20 Nanobody may optionally be linked via one or more, and in particular one and more in particular two, linker sequences.

Some preferred, but non-limiting examples of bivalent bispecific polypeptides of the invention are given in SEQ ID NO's: 450-471 and 559-602. Some preferred, but non-limiting examples of trivalent bispecific polypeptides of the invention are given in SEQ ID NO's: 478-558.

However, as will be clear from the description hereinabove, the invention is not limited thereto, in the sense that a multispecific polypeptide of the invention may comprise at least one Nanobody against the IL-6 receptor, and any number of Nanobodies directed against one or more antigens different from the IL-6 receptor.

30 Furthermore, although it is encompassed within the scope of the invention that the specific order or arrangement of the various Nanobodies in the polypeptides of the invention may have some influence on the properties of the final polypeptide of the invention (including but not limited to the affinity, specificity or avidity for the IL-6 receptor, or against the one or more other antigens), said order or arrangement is usually not critical and may be suitably

chosen by the skilled person, optionally after some limited routine experiments based on the disclosure herein. Thus, when reference is made to a specific multivalent or multispecific polypeptide of the invention, it should be noted that this encompasses any order or arrangements of the relevant Nanobodies, unless explicitly indicated otherwise.

Finally, it is also within the scope of the invention that the polypeptides of the invention contain two or more Nanobodies and one or more further amino acid sequences (as mentioned herein).

For multivalent and multispecific polypeptides containing one or more V_{HH} domains and their preparation, reference is also made to Conrath et al., J. Biol. Chem., Vol. 276, 10.

10 7346-7350, 2001; Muyldermans, Reviews in Molecular Biotechnology 74 (2001), 277-302; as well as to for example WO 96/34103 and WO 99/23221. Some other examples of some specific multispecific and/or multivalent polypeptides of the invention can be found in the applications by Ablynx N.V. referred to herein.

One preferred, but non-limiting example of a multispecific polypeptide of the invention comprises at least one Nanobody of the invention and at least one Nanobody that provides for an increased half-life. Some preferred, but non-limiting examples of such Nanobodies include Nanobodies directed against serum proteins, such as human serum albumin, thyroxine-binding protein, (human) transferrin, fibrinogen, an immunoglobulin such as IgG, IgE or IgM, or one of the other serum proteins listed in WO 04/003019.

20 For example, for experiments in mice, Nanobodies against mouse serum albumin (MSA) can be used, whereas for pharmaceutical use, Nanobodies against human serum albumin can be used.

Another embodiment of the present invention is a polypeptide construct as described above wherein said at least one (human) serum protein is any of (human) serum albumin, (human) serum immunoglobulins, (human) thyroxine-binding protein, (human) transferrin, (human) fibrinogen, etc.

According to a specific, but non-limiting aspect of the invention, the polypeptides of the invention contain, besides the one or more Nanobodies of the invention, at least one Nanobody against human serum albumin. Although these Nanobodies against human serum 30 albumin may be as generally described in the applications by Ablynx N.V. cited above (see for example W04/062551), according to a particularly preferred, but non-limiting embodiment, said Nanobody against human serum albumin consists of 4 framework regions (FR1 to FR4 respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively), in which:

i) CDR1 is an amino acid sequence chosen from the group consisting of:

SFGMS	[SEQ ID NO: 15]
LNLMG	[SEQ ID NO: 16]
INLLG	[SEQ ID NO: 17]
NYWMY;	[SEQ ID NO: 18]

and/or from the group consisting of amino acid sequences that have 2 or only 1 “amino acid difference(s)” (as defined herein) with one of the above amino acid sequences, in which:

(1) any amino acid substitution is preferably a conservative amino acid substitution
10 (as defined herein); and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequences; and in which:

ii) CDR2 is an amino acid sequence chosen from the group consisting of:

SISGSGSDTLYADSVKG	[SEQ ID NO: 19]
TITVGDSTNYADSVKG	[SEQ ID NO: 20]
TITVGDSTS YADSVKG	[SEQ ID NO: 21]
SINGRGDDTRYADSVKG	[SEQ ID NO: 22]
AISADSSTKNYADSVKG	[SEQ ID NO: 23]
AISADSSDKRYADSVKG	[SEQ ID NO: 24]
RISTGGGYSYYADSVKG	[SEQ ID NO: 25]

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of the above amino acid sequences; in which

(1) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequences;

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1

30 “amino acid difference(s)” (as defined herein) with one of the above amino acid sequences, in which:

(1) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequences; and in which:

iii) CDR3 is an amino acid sequence chosen from the group consisting of:

DREAQVDTLDFDY

[SEQ ID NO: 26]

or from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with one of the above amino acid sequences; in which

(1) any amino acid substitution is preferably a conservative amino acid substitution

10 (as defined herein); and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequences;

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 “amino acid difference(s)” (as defined herein) with one of the above amino acid sequences, in which:

(1) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

(2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequences;

20 or from the group consisting of:

GGSLSR

[SEQ ID NO: 27]

RRTWHSEL

[SEQ ID NO: 28]

GRSVSRS

[SEQ ID NO: 29]

GRGSP

[SEQ ID NO: 30]

and/or from the group consisting of amino acid sequences that have 3, 2 or only 1 “amino acid difference(s)” (as defined herein) with one of the above amino acid sequences, in which:

(1) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or

30 (2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequences.

In another aspect, the invention relates to a Nanobody against human serum albumin, which consist of 4 framework regions (FR1 to FR4 respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively), which is chosen from

the group consisting of Nanobodies with the one of the following combinations of CDR1, CDR2 and CDR3, respectively:

- CDR1: SFGMS; CDR2: SISGSGSDTLYADSVKG; CDR3: GGSLSR;
- CDR1: LNLMG; CDR2: TITVGDSTNYADSVKG; CDR3: RRTWHSEL;
- CDR1: INLLG; CDR2: TITVGDSTSYADSVKG; CDR3: RRTWHSEL;
- CDR1: SFGMS; CDR2: SINGRGDDTRYADSVKG; CDR3: GRSVSR;
- CDR1: SFGMS; CDR2: AISADSSDKRYADSVKG; CDR3: GRGSP;
- CDR1: SFGMS; CDR2: AISADSSDKRYADSVKG; CDR3: GRGSP;
- CDR1: NYWMY; CDR2: RISTGGGYSYYADSVKG; CDR3:

10 DREAQVDTLDFDY.

In the Nanobodies of the invention that comprise the combinations of CDR's mentioned above, each CDR can be replaced by a CDR chosen from the group consisting of amino acid sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with the mentioned CDR's; in which

- (1) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or
- (2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequences;

20 and/or chosen from the group consisting of amino acid sequences that have 3, 2 or only 1 (as indicated in the preceding paragraph) "amino acid difference(s)" (as defined herein) with the mentioned CDR(s) one of the above amino acid sequences, in which:

- (1) any amino acid substitution is preferably a conservative amino acid substitution (as defined herein); and/or
- (2) said amino acid sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the above amino acid sequences.

However, of the Nanobodies of the invention that comprise the combinations of CDR's mentioned above, Nanobodies comprising one or more of the CDR's listed above are particularly preferred; Nanobodies comprising two or more of the CDR's listed above are 30 more particularly preferred; and Nanobodies comprising three of the CDR's listed above are most particularly preferred.

In these Nanobodies against human serum albumin, the Framework regions FR1 to FR4 are preferably as defined hereinabove for the Nanobodies of the invention.

Some preferred, but non-limiting examples of Nanobodies directed against human serum albumin that can be used in the polypeptides of the invention are listed in Table A-9 below. ALB-8 is a humanized version of ALB-1.

Table A-9: Preferred, but non-limiting examples of albumin-binding Nanobodies

<Name, SEQ ID #; PRT (protein); ->	
Sequence	
<PMP 6A6 (ALB-1), SEQ ID NO:32 ;PRT;->	AVQLVESGGGLVQPGNSLRLSCAASGFTFRSGMSWVRQAPGKEPEWVSSISGSGSDTLYADSVKG RFTISRDNAKTTLYLQMNSLKPEDTAVYYCTIGGSLSRSSQGTQVTVSS
<ALB-8 (humanized ALB-1), SEQ ID NO:33 ;PRT;->	EVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKG RFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTQVTVSS
<PMP 6A8 (ALB-2), SEQ ID NO:34 ;PRT;->	AVQLVESGGGLVQGGGSLRLACAASERIFDLNLMGWYRQGPGNERELVATCITVGDSTNYADSVKG RFTISMDYTKQTQVYLMNSLRPEDTGLYYCKIRRTWHSELWGQGTQVTVSS

Some preferred, but non-limiting examples of polypeptides of the invention that comprise at least one Nanobody against IL-6R and at least one Nanobody that provides for 10 increased half-life are given in SEQ ID NO's 478 to 602.

Generally, any derivatives and/or polypeptides of the invention with increased half-life (for example pegylated Nanobodies or polypeptides of the invention, multispecific Nanobodies directed against the IL-6 receptor and (human) serum albumin, or Nanobodies fused to an Fc portion, all as described herein) have a half-life that is at least 1.5 times, preferably at least 2 times, such as at least 5 times, for example at least 10 times or more than 20 times, the half-life of the corresponding Nanobody of the invention.

Also, any derivatives or polypeptides of the invention with an increase half-life preferably have a half-life of more than 1 hour, preferably more than 2 hours, more preferably of more than 6 hours, such as of more than 12 hours, and for example of about one day, two days, one week, two weeks or three weeks, and preferably no more than 2 months, although the latter may be less critical. 20

Half-life can generally be defined as the time taken for the serum concentration of the polypeptide to be reduced by 50%, *in vivo*, for example due to degradation of the ligand and/or clearance or sequestration of the ligand by natural mechanisms. Methods for pharmacokinetic analysis and determination of half-life are familiar to those skilled in the art. Details may be

found in Kenneth, A et al: Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists and in Peters et al, Pharmacokinetic analysis: A Practical Approach (1996). Reference is also made to "Pharmacokinetics", M Gibaldi & D Perron, published by Marcel Dekker, 2 nd Rev. ex edition (1982).

According to one aspect of the invention the polypeptides are capable of binding to one or more molecules which can increase the half-life of the polypeptide in vivo.

The polypeptides of the invention are stabilised in vivo and their half-life increased by binding to molecules which resist degradation and/or clearance or sequestration. Typically, such molecules are naturally occurring proteins which themselves have a long half-life in vivo.

Another preferred, but non-limiting example of a multispecific polypeptide of the invention comprises at least one Nanobody of the invention and at least one Nanobody that directs the polypeptide of the invention towards, and/or that allows the polypeptide of the invention to penetrate or to enter into specific organs, tissues, cells, or parts or compartments of cells, and/or that allows the Nanobody to penetrate or cross a biological barrier such as a cell membrane, a cell layer such as a layer of epithelial cells, a tumor including solid tumors, or the blood-brain-barrier. Examples of such Nanobodies include Nanobodies that are directed towards specific cell-surface proteins, markers or epitopes of the desired organ, tissue or cell (for example cell-surface markers associated with tumor cells), and the single-domain brain targeting antibody fragments described in WO 02/057445, of which FC44 (SEQ ID NO 35) and FC5 (SEQ ID NO: 36) are preferred examples.

Table A-10: Sequence listing of FC44 and FC5

<Name, SEQ ID #; PRT (protein); ->
Sequence
< FC44, SEQ ID NO:35 ;PRT;->
EVQLQASGGGLVQAGGSLRLSCASVRTFSIYAMGWFRQAPGKEREVFAGINRSGDVTKYADFVKGRFSISRDNAKNMVYLQMNSLKPEDTALYYCAATWAYDTVGALTSGYNFWGQGTQVTVSS
< FC5, SEQ ID NO:36 ;PRT;->
EVQLQASGGGLVQAGGSLRLSCAASGFKITHYTMGWFRQAPGKEREVFVSRTWGGDNTFYSNSVKG RFTISRDNAKNTVYLQMNSLKPEDTADYYCAAGSTSTATPLRVDYWGKGTQVTVSS

In the polypeptides of the invention, the one or more Nanobodies and the one or more polypeptides may be directly linked to each other (as for example described in WO 99/23221)

and/or may be linked to each other via one or more suitable spacers or linkers, or any combination thereof.

Suitable spacers or linkers for use in multivalent and multispecific polypeptides will be clear to the skilled person, and may generally be any linker or spacer used in the art to link amino acid sequences. Preferably, said linker or spacer is suitable for use in constructing proteins or polypeptides that are intended for pharmaceutical use.

Some particularly preferred spacers include the spacers and linkers that are used in the art to link antibody fragments or antibody domains. These include the linkers mentioned in the general background art cited above, as well as for example linkers that are used in the art to construct diabodies or ScFv fragments (in this respect, however, it should be noted that, whereas in diabodies and in ScFv fragments, the linker sequence used should have a length, a degree of flexibility and other properties that allow the pertinent V_H and V_L domains to come together to form the complete antigen-binding site, there is no particular limitation on the length or the flexibility of the linker used in the polypeptide of the invention, since each Nanobody by itself forms a complete antigen-binding site).

For example, a linker may be a suitable amino acid sequence, and in particular amino acid sequences of between 1 and 50, preferably between 1 and 30, such as between 1 and 10 amino acid residues. Some preferred examples of such amino acid sequences include gly-ser linkers, for example of the type $(\text{gly}_x\text{ser}_y)_z$, such as (for example $(\text{gly}_4\text{ser})_3$ or $(\text{gly}_3\text{ser}_2)_3$, as described in WO 99/42077, hinge-like regions such as the hinge regions of naturally occurring heavy chain antibodies or similar sequences (such as described in WO 94/04678).

Some other particularly preferred linkers are poly-alanine (such as AAA), as well as the linkers mentioned in Table A-11, of which AAA, GS-7 and GS-9 are particularly preferred.

Table A-11: Sequence listing of linkers

< GS7, SEQ ID NO:40 ;PRT; ->

SGGSGGS

< Llama upper long hinge region, SEQ ID NO:41 ;PRT; ->

EPKTPKPQPA

Other suitable linkers generally comprise organic compounds or polymers, in particular those suitable for use in proteins for pharmaceutical use. For instance, poly(ethyleneglycol) moieties have been used to link antibody domains, see for example WO 04/081026.

It is encompassed within the scope of the invention that the length, the degree of flexibility and/or other properties of the linker(s) used (although not critical, as it usually is for linkers used in ScFv fragments) may have some influence on the properties of the final 10 polypeptide of the invention, including but not limited to the affinity, specificity or avidity for The IL-6 receptor, or for one or more of the other antigens. Based on the disclosure herein, the skilled person will be able to determine the optimal linker(s) for use in a specific polypeptide of the invention, optionally after some limited routine experiments.

For example, in multivalent polypeptides of the invention that comprise Nanobodies directed against a multimeric antigen (such as a multimeric receptor or other protein), the length and flexibility of the linker are preferably such that it allows each Nanobody of the invention present in the polypeptide to bind to the antigenic determinant on each of the subunits of the multimer. Similarly, in a multispecific polypeptide of the invention that comprises Nanobodies directed against two or more different antigenic determinants on the 20 same antigen (for example against different epitopes of an antigen and/or against different subunits of a multimeric receptor, channel or protein), the length and flexibility of the linker are preferably such that it allows each Nanobody to bind to its intended antigenic determinant. Again, based on the disclosure herein, the skilled person will be able to determine the optimal linker(s) for use in a specific polypeptide of the invention, optionally after some limited routine experiments.

It is also within the scope of the invention that the linker(s) used confer one or more other favourable properties or functionality to the polypeptides of the invention, and/or provide one or more sites for the formation of derivatives and/or for the attachment of functional groups (e.g. as described herein for the derivatives of the Nanobodies of the 30 invention). For example, linkers containing one or more charged amino acid residues (see

Table A-2 above) can provide improved hydrophilic properties, whereas linkers that form or contain small epitopes or tags can be used for the purposes of detection, identification and/or purification. Again, based on the disclosure herein, the skilled person will be able to determine the optimal linkers for use in a specific polypeptide of the invention, optionally after some limited routine experiments.

Finally, when two or more linkers are used in the polypeptides of the invention, these linkers may be the same or different. Again, based on the disclosure herein, the skilled person will be able to determine the optimal linkers for use in a specific polypeptide of the invention, optionally after some limited routine experiments.

10 Usually, for easy of expression and production, a polypeptide of the invention will be a linear polypeptide. However, the invention in its broadest sense is not limited thereto. For example, when a polypeptide of the invention comprises three or more Nanobodies, it is possible to link them by use of a linker with three or more “arms”, which each “arm” being linked to a Nanobody, so as to provide a “star-shaped” construct. It is also possible, although usually less preferred, to use circular constructs.

The invention also comprises derivatives of the polypeptides of the invention, which may be essentially analogous to the derivatives of the Nanobodies of the invention, i.e. as described herein.

20 The invention also comprises proteins or polypeptides that “essentially consist” of a polypeptide of the invention (in which the wording “essentially consist of” has essentially the same meaning as indicated hereinabove).

According to one embodiment of the invention, the polypeptide of the invention is in essentially isolated from, as defined herein.

30 The amino acid sequences, Nanobodies, polypeptides and nucleic acids of the invention can be prepared in a manner known per se, as will be clear to the skilled person from the further description herein. For example, the Nanobodies and polypeptides of the invention can be prepared in any manner known per se for the preparation of antibodies and in particular for the preparation of antibody fragments (including but not limited to (single) domain antibodies and ScFv fragments). Some preferred, but non-limiting methods for preparing the amino acid sequences, Nanobodies, polypeptides and nucleic acids include the methods and techniques described herein.

As will be clear to the skilled person, one particularly useful method for preparing an amino acid sequence, Nanobody and/or a polypeptide of the invention generally comprises the steps of:

- the expression, in a suitable host cell or host organism (also referred to herein as a “host of the invention”) or in another suitable expression system of a nucleic acid that encodes said amino acid sequence, Nanobody or polypeptide of the invention (also referred to herein as a “nucleic acid of the invention”), optionally followed by:
- isolating and/or purifying the amino acid sequence, Nanobody or polypeptide of the invention thus obtained.

In particular, such a method may comprise the steps of:

- cultivating and/or maintaining a host of the invention under conditions that are such that said host of the invention expresses and/or produces at least one amino acid sequence, Nanobody and/or polypeptide of the invention; optionally followed by:
- isolating and/or purifying the amino acid sequence, Nanobody or polypeptide of the invention thus obtained.

A nucleic acid of the invention can be in the form of single or double stranded DNA or RNA, and is preferably in the form of double stranded DNA. For example, the nucleotide sequences of the invention may be genomic DNA, cDNA or synthetic DNA (such as DNA with a codon usage that has been specifically adapted for expression in the intended host cell or host organism).

According to one embodiment of the invention, the nucleic acid of the invention is in essentially isolated from, as defined herein.

20 The nucleic acid of the invention may also be in the form of, be present in and/or be part of a vector, such as for example a plasmid, cosmid or YAC, which again may be in essentially isolated form.

The nucleic acids of the invention can be prepared or obtained in a manner known per se, based on the information on the amino acid sequences for the polypeptides of the invention given herein, and/or can be isolated from a suitable natural source. To provide analogs, nucleotide sequences encoding naturally occurring V_{HH} domains can for example be subjected to site-directed mutagenesis, so as to provide a nucleic acid of the invention encoding said analog. Also, as will be clear to the skilled person, to prepare a nucleic acid of the invention, also several nucleotide sequences, such as at least one nucleotide sequence 30 encoding a Nanobody and for example nucleic acids encoding one or more linkers can be linked together in a suitable manner.

Techniques for generating the nucleic acids of the invention will be clear to the skilled person and may for instance include, but are not limited to, automated DNA synthesis; site-directed mutagenesis; combining two or more naturally occurring and/or synthetic sequences

(or two or more parts thereof), introduction of mutations that lead to the expression of a truncated expression product; introduction of one or more restriction sites (e.g. to create cassettes and/or regions that may easily be digested and/or ligated using suitable restriction enzymes), and/or the introduction of mutations by means of a PCR reaction using one or more “mismatched” primers, using for example a sequence of a naturally occurring GPCR ???? as a template. These and other techniques will be clear to the skilled person, and reference is again made to the standard handbooks, such as Sambrook et al. and Ausubel et al., mentioned above, as well as the Examples below.

10 The nucleic acid of the invention may also be in the form of, be present in and/or be part of a genetic construct, as will be clear to the person skilled in the art. Such genetic constructs generally comprise at least one nucleic acid of the invention that is optionally linked to one or more elements of genetic constructs known per se, such as for example one or more suitable regulatory elements (such as a suitable promoter(s), enhancer(s), terminator(s), etc.) and the further elements of genetic constructs referred to herein. Such genetic constructs comprising at least one nucleic acid of the invention will also be referred to herein as “genetic constructs of the invention”.

20 The genetic constructs of the invention may be DNA or RNA, and are preferably double-stranded DNA. The genetic constructs of the invention may also be in a form suitable for transformation of the intended host cell or host organism, in a form suitable for integration into the genomic DNA of the intended host cell or in a form suitable for independent replication, maintenance and/or inheritance in the intended host organism. For instance, the genetic constructs of the invention may be in the form of a vector, such as for example a plasmid, cosmid, YAC, a viral vector or transposon. In particular, the vector may be an expression vector, i.e. a vector that can provide for expression in vitro and/or in vivo (e.g. in a suitable host cell, host organism and/or expression system).

In a preferred but non-limiting embodiment, a genetic construct of the invention comprises

- a) at least one nucleic acid of the invention; operably connected to
- b) one or more regulatory elements, such as a promoter and optionally a suitable terminator;

30 and optionally also

- c) one or more further elements of genetic constructs known per se;
in which the terms “regulatory element”, “promoter”, “terminator” and “operably connected” have their usual meaning in the art (as further described herein); and in which said

“further elements” present in the genetic constructs may for example be 3’- or 5’-UTR sequences, leader sequences, selection markers, expression markers/reporter genes, and/or elements that may facilitate or increase (the efficiency of) transformation or integration. These and other suitable elements for such genetic constructs will be clear to the skilled person, and may for instance depend upon the type of construct used, the intended host cell or host organism; the manner in which the nucleotide sequences of the invention of interest are to be expressed (e.g. via constitutive, transient or inducible expression); and/or the transformation technique to be used. For example, regulatory sequences, promoters and terminators known per se for the expression and production of antibodies and antibody fragments (including but not limited to (single) domain antibodies and ScFv fragments) may be used in an essentially analogous manner.

10 Preferably, in the genetic constructs of the invention, said at least one nucleic acid of the invention and said regulatory elements, and optionally said one or more further elements, are “operably linked” to each other, by which is generally meant that they are in a functional relationship with each other. For instance, a promoter is considered “operably linked” to a coding sequence if said promoter is able to initiate or otherwise control/regulate the transcription and/or the expression of a coding sequence (in which said coding sequence should be understood as being “under the control of” said promoter). Generally, when two nucleotide sequences are operably linked, they will be in the same orientation and usually also 20 in the same reading frame. They will usually also be essentially contiguous, although this may also not be required.

Preferably, the regulatory and further elements of the genetic constructs of the invention are such that they are capable of providing their intended biological function in the intended host cell or host organism.

For instance, a promoter, enhancer or terminator should be “operable” in the intended host cell or host organism, by which is meant that (for example) said promoter should be capable of initiating or otherwise controlling/regulating the transcription and/or the expression of a nucleotide sequence - e.g. a coding sequence - to which it is operably linked (as defined herein).

30 Some particularly preferred promoters include, but are not limited to, promoters known per se for the expression in the host cells mentioned herein; and in particular promoters for the expression in the bacterial cells, such as those mentioned herein and/or those used in the Examples.

A selection marker should be such that it allows - i.e. under appropriate selection conditions - host cells and/or host organisms that have been (successfully) transformed with the nucleotide sequence of the invention to be distinguished from host cells/organisms that have not been (successfully) transformed. Some preferred, but non-limiting examples of such markers are genes that provide resistance against antibiotics (such as kanamycin or ampicillin), genes that provide for temperature resistance, or genes that allow the host cell or host organism to be maintained in the absence of certain factors, compounds and/or (food) components in the medium that are essential for survival of the non-transformed cells or organisms.

10 A leader sequence should be such that - in the intended host cell or host organism - it allows for the desired post-translational modifications and/or such that it directs the transcribed mRNA to a desired part or organelle of a cell. A leader sequence may also allow for secretion of the expression product from said cell. As such, the leader sequence may be any pro-, pre-, or prepro-sequence operable in the host cell or host organism. Leader sequences may not be required for expression in a bacterial cell. For example, leader sequences known per se for the expression and production of antibodies and antibody fragments (including but not limited to single domain antibodies and ScFv fragments) may be used in an essentially analogous manner.

20 An expression marker or reporter gene should be such that - in the host cell or host organism - it allows for detection of the expression of (a gene or nucleotide sequence present on) the genetic construct. An expression marker may optionally also allow for the localisation of the expressed product, e.g. in a specific part or organelle of a cell and/or in (a) specific cell(s), tissue(s), organ(s) or part(s) of a multicellular organism. Such reporter genes may also be expressed as a protein fusion with the amino acid sequence of the invention. Some preferred, but non-limiting examples include fluorescent proteins such as GFP.

30 Some preferred, but non-limiting examples of suitable promoters, terminator and further elements include those that can be used for the expression in the host cells mentioned herein; and in particular those that are suitable for expression in bacterial cells, such as those mentioned herein and/or those used in the Examples below. For some (further) non-limiting examples of the promoters, selection markers, leader sequences, expression markers and further elements that may be present/used in the genetic constructs of the invention - such as terminators, transcriptional and/or translational enhancers and/or integration factors - reference is made to the general handbooks such as Sambrook et al. and Ausubel et al. mentioned above, as well as to the examples that are given in WO 95/07463, WO 96/23810,

WO 95/07463, WO 95/21191, WO 97/11094, WO 97/42320, WO 98/06737, WO 98/21355, US-A-7,207,410, US-A- 5,693,492 and EP 1 085 089. Other examples will be clear to the skilled person. Reference is also made to the general background art cited above and the further references cited herein.

The genetic constructs of the invention may generally be provided by suitably linking the nucleotide sequence(s) of the invention to the one or more further elements described above, for example using the techniques described in the general handbooks such as Sambrook et al. and Ausubel et al., mentioned above.

Often, the genetic constructs of the invention will be obtained by inserting a nucleotide sequence of the invention in a suitable (expression) vector known per se. Some preferred, but non-limiting examples of suitable expression vectors are those used in the Examples below, as well as those mentioned herein.

The nucleic acids of the invention and/or the genetic constructs of the invention may be used to transform a host cell or host organism, i.e. for expression and/or production of the amino acid sequence, Nanobody or polypeptide of the invention. Suitable hosts or host cells will be clear to the skilled person, and may for example be any suitable fungal, prokaryotic or eukaryotic cell or cell line or any suitable fungal, prokaryotic or eukaryotic organism, for example:

- a bacterial strain, including but not limited to gram-negative strains such as strains of *Escherichia coli*; of *Proteus*, for example of *Proteus mirabilis*; of *Pseudomonas*, for example of *Pseudomonas fluorescens*; and gram-positive strains such as strains of *Bacillus*, for example of *Bacillus subtilis* or of *Bacillus brevis*; of *Streptomyces*, for example of *Streptomyces lividans*; of *Staphylococcus*, for example of *Staphylococcus carnosus*; and of *Lactococcus*, for example of *Lactococcus lactis*;
- a fungal cell, including but not limited to cells from species of *Trichoderma*, for example from *Trichoderma reesei*; of *Neurospora*, for example from *Neurospora crassa*; of *Sordaria*, for example from *Sordaria macrospora*; of *Aspergillus*, for example from *Aspergillus niger* or from *Aspergillus sojae*; or from other filamentous fungi;
- a yeast cell, including but not limited to cells from species of *Saccharomyces*, for example of *Saccharomyces cerevisiae*; of *Schizosaccharomyces*, for example of *Schizosaccharomyces pombe*; of *Pichia*, for example of *Pichia pastoris* or of *Pichia methanolica*; of *Hansenula*, for example of *Hansenula polymorpha*; of *Kluyveromyces*,

for example of *Kluyveromyces lactis*; of *Arxula*, for example of *Arxula adeninivorans*; of *Yarrowia*, for example of *Yarrowia lipolytica*;

- an amphibian cell or cell line, such as *Xenopus oocytes*;
- an insect-derived cell or cell line, such as cells/cell lines derived from lepidoptera, including but not limited to *Spodoptera* SF9 and Sf21 cells or cells/cell lines derived from *Drosophila*, such as Schneider and Kc cells;
- a plant or plant cell, for example in tobacco plants; and/or
- a mammalian cell or cell line, for example a cell or cell line derived from a human, a cell or a cell line from mammals including but not limited to CHO-cells, BHK-cells (for example BHK-21 cells) and human cells or cell lines such as HeLa, COS (for example COS-7) and PER.C6 cells;

10 as well as all other hosts or host cells known per se for the expression and production of antibodies and antibody fragments (including but not limited to (single) domain antibodies and ScFv fragments), which will be clear to the skilled person. Reference is also made to the general background art cited hereinabove, as well as to for example WO 94/29457; WO 96/34103; WO 99/42077; Frenken et al., (1998), *supra*; Riechmann and Muyldermans, (1999), *supra*; van der Linden, (2000), *supra*; Thomassen et al., (2002), *supra*; Joosten et al., (2003), *supra*; Joosten et al., (2005), *supra*; and the further references cited herein.

20 The amino acid sequences, Nanobodies and polypeptides of the invention can also be introduced and expressed in one or more cells, tissues or organs of a multicellular organism, for example for prophylactic and/or therapeutic purposes (e.g. as a gene therapy). For this purpose, the nucleotide sequences of the invention may be introduced into the cells or tissues in any suitable way, for example as such (e.g. using liposomes) or after they have been inserted into a suitable gene therapy vector (for example derived from retroviruses such as adenovirus, or parvoviruses such as adeno-associated virus). As will also be clear to the skilled person, such gene therapy may be performed *in vivo* and/or *in situ* in the body of a patient by administering a nucleic acid of the invention or a suitable gene therapy vector encoding the same to the patient or to specific cells or a specific tissue or organ of the patient; or suitable cells (often taken from the body of the patient to be treated, such as explanted 30 lymphocytes, bone marrow aspirates or tissue biopsies) may be treated *in vitro* with a nucleotide sequence of the invention and then be suitably (re-)introduced into the body of the patient. All this can be performed using gene therapy vectors, techniques and delivery systems which are well known to the skilled person, and for example described in Culver, K. W., "Gene Therapy", 1994, p. xii, Mary Ann Liebert, Inc., Publishers, New York, N.Y.);

Giordano, Nature F Medicine 2 (1996), 534-539; Schaper, Circ. Res. 79 (1996), 911-919; Anderson, Science 256 (1992), 808-813; Verma, Nature 389 (1994), 239; Isner, Lancet 348 (1996), 370-374; Muhlhauser, Circ. Res. 77 (1995), 1077-1086; Onodera, Blood 91; (1998), 30-36; Verma, Gene Ther. 5 (1998), 692-699; Nabel, Ann. N.Y. Acad. Sci. : 811 (1997), 289-292; Verzeletti, Hum. Gene Ther. 9 (1998), 2243-51; Wang, Nature Medicine 2 (1996), 714-716; WO 94/29469; WO 97/00957, US 5,580,859; US 5,5895466; or Schaper, Current Opinion in Biotechnology 7 (1996), 635-640. For example, in situ expression of ScFv fragments (Afanasieva et al., Gene Ther., 10, 1850-1859 (2003)) and of diabodies (Blanco et al., J. Immunol, 171, 1070-1077 (2003)) has been described in the art.

10 For expression of the Nanobodies in a cell, they may also be expressed as so-called "intrabodies", as for example described in WO 94/02610, WO 95/22618 and US-A-7004940; WO 03/014960; in Cattaneo, A. & Biocca, S. (1997) Intracellular Antibodies: Development and Applications. Landes and Springer-Verlag; and in Kontermann, Methods 34, (2004), 163-170.

20 The amino acid sequences, Nanobodies and polypeptides of the invention can for example also be produced in the milk of transgenic mammals, for example in the milk of rabbits, cows, goats or sheep (see for example US-A-6,741,957, US-A-6,304,489 and US-A-6,849,992 for general techniques for introducing transgenes into mammals), in plants or parts of plants including but not limited to their leaves, flowers, fruits, seed, roots or tubers (for example in tobacco, maize, soybean or alfalfa) or in for example pupae of the silkworm *Bombyx mori*.

Furthermore, the amino acid sequences, Nanobodies and polypeptides of the invention can also be expressed and/or produced in cell-free expression systems, and suitable examples of such systems will be clear to the skilled person. Some preferred, but non-limiting examples include expression in the wheat germ system; in rabbit reticulocyte lysates; or in the *E. coli* Zubay system.

30 As mentioned above, one of the advantages of the use of Nanobodies is that the polypeptides based thereon can be prepared through expression in a suitable bacterial system, and suitable bacterial expression systems, vectors, host cells, regulatory elements, etc., will be clear to the skilled person, for example from the references cited above. It should however be noted that the invention in its broadest sense is not limited to expression in bacterial systems.

Preferably, in the invention, an (in vivo or in vitro) expression system, such as a bacterial expression system, is used that provides the polypeptides of the invention in a form that is suitable for pharmaceutical use, and such expression systems will again be clear to the

skilled person. As also will be clear to the skilled person, polypeptides of the invention suitable for pharmaceutical use can be prepared using techniques for peptide synthesis.

For production on industrial scale, preferred heterologous hosts for the (industrial) production of Nanobodies or Nanobody-containing protein therapeutics include strains of *E. coli*, *Pichia pastoris*, *S. cerevisiae* that are suitable for large scale expression/production/fermentation, and in particular for large scale pharmaceutical expression/production/fermentation. Suitable examples of such strains will be clear to the skilled person. Such strains and production/expression systems are also made available by companies such as Biovitrum (Uppsala, Sweden).

10 Alternatively, mammalian cell lines, in particular Chinese hamster ovary (CHO) cells, can be used for large scale expression/production/fermentation, and in particular for large scale pharmaceutical expression/production/fermentation. Again, such expression/production systems are also made available by some of the companies mentioned above.

The choice of the specific expression system would depend in part on the requirement for certain post-translational modifications, more specifically glycosylation. The production of a Nanobody-containing recombinant protein for which glycosylation is desired or required would necessitate the use of mammalian expression hosts that have the ability to glycosylate the expressed protein. In this respect, it will be clear to the skilled person that the glycosylation pattern obtained (i.e. the kind, number and position of residues attached) will 20 depend on the cell or cell line that is used for the expression. Preferably, either a human cell or cell line is used (i.e. leading to a protein that essentially has a human glycosylation pattern) or another mammalian cell line is used that can provide a glycosylation pattern that is essentially and/or functionally the same as human glycosylation or at least mimics human glycosylation. Generally, prokaryotic hosts such as *E. coli* do not have the ability to glycosylate proteins, and the use of lower eukaryotes such as yeast usually leads to a glycosylation pattern that differs from human glycosylation. Nevertheless, it should be understood that all the foregoing host cells and expression systems can be used in the invention, depending on the desired amino acid sequence, Nanobody or polypeptide to be obtained.

30 Thus, according to one non-limiting embodiment of the invention, the amino acid sequence, Nanobody or polypeptide of the invention is glycosylated. According to another non-limiting embodiment of the invention, the amino acid sequence, Nanobody or polypeptide of the invention is non-glycosylated.

According to one preferred, but non-limiting embodiment of the invention, the amino acid sequence, Nanobody or polypeptide of the invention is produced in a bacterial cell, in particular a bacterial cell suitable for large scale pharmaceutical production, such as cells of the strains mentioned above.

According to another preferred, but non-limiting embodiment of the invention, the amino acid sequence, Nanobody or polypeptide of the invention is produced in a yeast cell, in particular a yeast cell suitable for large scale pharmaceutical production, such as cells of the species mentioned above.

According to yet another preferred, but non-limiting embodiment of the invention, the 10 amino acid sequence, Nanobody or polypeptide of the invention is produced in a mammalian cell, in particular in a human cell or in a cell of a human cell line, and more in particular in a human cell or in a cell of a human cell line that is suitable for large scale pharmaceutical production, such as the cell lines mentioned hereinabove.

When expression in a host cell is used to produce the amino acid sequences, Nanobodies and the polypeptides of the invention, the amino acid sequences Nanobodies and polypeptides of the invention can be produced either intracellularly (e.g. in the cytosol, in the periplasma or in inclusion bodies) and then isolated from the host cells and optionally further purified; or can be produced extracellularly (e.g. in the medium in which the host cells are cultured) and then isolated from the culture medium and optionally further purified. When 20 eukaryotic host cells are used, extracellular production is usually preferred since this considerably facilitates the further isolation and downstream processing of the Nanobodies and proteins obtained. Bacterial cells such as the strains of *E. coli* mentioned above normally do not secrete proteins extracellularly, except for a few classes of proteins such as toxins and hemolysin, and secretory production in *E. coli* refers to the translocation of proteins across the inner membrane to the periplasmic space. Periplasmic production provides several advantages over cytosolic production. For example, the N-terminal amino acid sequence of the secreted product can be identical to the natural gene product after cleavage of the secretion signal sequence by a specific signal peptidase. Also, there appears to be much less protease activity in the periplasm than in the cytoplasm. In addition, protein purification is simpler due to 30 fewer contaminating proteins in the periplasm. Another advantage is that correct disulfide bonds may form because the periplasm provides a more oxidative environment than the cytoplasm. Proteins overexpressed in *E. coli* are often found in insoluble aggregates, so-called inclusion bodies. These inclusion bodies may be located in the cytosol or in the periplasm; the recovery of biologically active proteins from these inclusion bodies requires a

denaturation/refolding process. Many recombinant proteins, including therapeutic proteins, are recovered from inclusion bodies. Alternatively, as will be clear to the skilled person, recombinant strains of bacteria that have been genetically modified so as to secrete a desired protein, and in particular a amino acid sequence, Nanobody or a polypeptide of the invention, can be used.

Thus, according to one non-limiting embodiment of the invention, the amino acid sequence, Nanobody or polypeptide of the invention is an amino acid sequence, Nanobody or polypeptide that has been produced intracellularly and that has been isolated from the host cell, and in particular from a bacterial cell or from an inclusion body in a bacterial cell.

10 According to another non-limiting embodiment of the invention, the amino acid sequence, Nanobody or polypeptide of the invention is an amino acid sequence, Nanobody or polypeptide that has been produced extracellularly, and that has been isolated from the medium in which the host cell is cultivated.

Some preferred, but non-limiting promoters for use with these host cells include,

- for expression in *E. coli*: lac promoter (and derivatives thereof such as the lacUV5 promoter); arabinose promoter; left- (PL) and rightward (PR) promoter of phage lambda; promoter of the trp operon; hybrid lac/trp promoters (tac and trc); T7-promoter (more specifically that of T7-phage gene 10) and other T-phage promoters; promoter of the Tn10 tetracycline resistance gene; engineered variants of the above promoters that include one or more copies of an extraneous regulatory operator sequence;
- for expression in *S. cerevisiae*: constitutive: ADH1 (alcohol dehydrogenase 1), ENO (enolase), CYC1 (cytochrome c iso-1), GAPDH (glyceraldehydes-3-phosphate dehydrogenase), PGK1 (phosphoglycerate kinase), PYK1 (pyruvate kinase); regulated: GAL1,10,7 (galactose metabolic enzymes), ADH2 (alcohol dehydrogenase 2), PHO5 (acid phosphatase), CUP1 (copper metallothionein); heterologous: CaMV (cauliflower mosaic virus 35S promoter);
- for expression in *Pichia pastoris*: the AOX1 promoter (alcohol oxidase I);
- for expression in mammalian cells: human cytomegalovirus (hCMV) immediate early enhancer/promoter; human cytomegalovirus (hCMV) immediate early promoter variant that contains two tetracycline operator sequences such that the promoter can be regulated by the Tet repressor; Herpes Simplex Virus thymidine kinase (TK) promoter; Rous Sarcoma Virus long terminal repeat (RSV LTR) enhancer/promoter; elongation factor 1 α (hEF-1 α) promoter from human, chimpanzee, mouse or rat; the SV40 early promoter; HIV-1 long terminal repeat promoter; β -actin promoter;

Some preferred, but non-limiting vectors for use with these host cells include:

- vectors for expression in mammalian cells: pMAMneo (Clontech), pcDNA3 (Invitrogen), pMC1neo (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593), pBPV-1 (8-2) (ATCC 37110), pdBPV-MMTneo (342-12) (ATCC 37224), pRSVgpt (ATCC37199), pRSVneo (ATCC37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460) and 1ZD35 (ATCC 37565), as well as viral-based expression systems, such as those based on adenovirus;
- vectors for expression in bacterial cells: pET vectors (Novagen) and pQE vectors (Qiagen);
- 10 - vectors for expression in yeast or other fungal cells: pYES2 (Invitrogen) and Pichia expression vectors (Invitrogen);
- vectors for expression in insect cells: pBlueBacII (Invitrogen) and other baculovirus vectors
- vectors for expression in plants or plant cells: for example vectors based on cauliflower mosaic virus or tobacco mosaic virus, suitable strains of Agrobacterium, or Ti-plasmid based vectors.

Some preferred, but non-limiting secretory sequences for use with these host cells include:

- for use in bacterial cells such as E. coli: PelB, Bla, OmpA, OmpC, OmpF, OmpT, StII, PhoA, PhoE, MalE, Lpp, LamB, and the like; TAT signal peptide, hemolysin C-terminal secretion signal;
- for use in yeast: α -mating factor prepro-sequence, phosphatase (pho1), invertase (Suc), etc.;
- for use in mammalian cells: indigenous signal in case the target protein is of eukaryotic origin; murine Ig κ -chain V-J2-C signal peptide; etc.

Suitable techniques for transforming a host or host cell of the invention will be clear to the skilled person and may depend on the intended host cell/host organism and the genetic construct to be used. Reference is again made to the handbooks and patent applications mentioned above.

30 After transformation, a step for detecting and selecting those host cells or host organisms that have been successfully transformed with the nucleotide sequence/genetic construct of the invention may be performed. This may for instance be a selection step based on a selectable marker present in the genetic construct of the invention or a step involving the detection of the amino acid sequence of the invention, e.g. using specific antibodies.

The transformed host cell (which may be in the form or a stable cell line) or host organisms (which may be in the form of a stable mutant line or strain) form further aspects of the present invention.

Preferably, these host cells or host organisms are such that they express, or are (at least) capable of expressing (e.g. under suitable conditions), an amino acid sequence, Nanobody or polypeptide of the invention (and in case of a host organism: in at least one cell, part, tissue or organ thereof). The invention also includes further generations, progeny and/or offspring of the host cell or host organism of the invention, that may for instance be obtained by cell division or by sexual or asexual reproduction.

10 To produce/obtain expression of the amino acid sequences of the invention, the transformed host cell or transformed host organism may generally be kept, maintained and/or cultured under conditions such that the (desired) amino acid sequence, Nanobody or polypeptide of the invention is expressed/produced. Suitable conditions will be clear to the skilled person and will usually depend upon the host cell/host organism used, as well as on the regulatory elements that control the expression of the (relevant) nucleotide sequence of the invention. Again, reference is made to the handbooks and patent applications mentioned above in the paragraphs on the genetic constructs of the invention.

20 Generally, suitable conditions may include the use of a suitable medium, the presence of a suitable source of food and/or suitable nutrients, the use of a suitable temperature, and optionally the presence of a suitable inducing factor or compound (e.g. when the nucleotide sequences of the invention are under the control of an inducible promoter); all of which may be selected by the skilled person. Again, under such conditions, the amino acid sequences of the invention may be expressed in a constitutive manner, in a transient manner, or only when suitably induced.

It will also be clear to the skilled person that the amino acid sequence, Nanobody or polypeptide of the invention may (first) be generated in an immature form (as mentioned above), which may then be subjected to post-translational modification, depending on the host cell/host organism used. Also, the amino acid sequence, Nanobody or polypeptide of the invention may be glycosylated, again depending on the host cell/host organism used.

30 The amino acid sequence, Nanobody or polypeptide of the invention may then be isolated from the host cell/host organism and/or from the medium in which said host cell or host organism was cultivated, using protein isolation and/or purification techniques known per se, such as (preparative) chromatography and/or electrophoresis techniques, differential precipitation techniques, affinity techniques (e.g. using a specific, cleavable amino acid

sequence fused with the amino acid sequence, Nanobody or polypeptide of the invention) and/or preparative immunological techniques (i.e. using antibodies against the amino acid sequence to be isolated).

Generally, for pharmaceutical use, the polypeptides of the invention may be formulated as a pharmaceutical preparation or compositions comprising at least one polypeptide of the invention and at least one pharmaceutically acceptable carrier, diluent or excipient and/or adjuvant, and optionally one or more further pharmaceutically active polypeptides and/or compounds. By means of non-limiting examples, such a formulation may be in a form suitable for oral administration, for parenteral administration (such as by 10 intravenous, intramuscular or subcutaneous injection or intravenous infusion), for topical administration, for administration by inhalation, by a skin patch, by an implant, by a suppository, etc.. Such suitable administration forms - which may be solid, semi-solid or liquid, depending on the manner of administration - as well as methods and carriers for use in the preparation thereof, will be clear to the skilled person, and are further described herein.

Thus, in a further aspect, the invention relates to a pharmaceutical composition that contains at least one amino acid of the invention, at least one Nanobody of the invention or at least one polypeptide of the invention and at least one suitable carrier, diluent or excipient (i.e. suitable for pharmaceutical use), and optionally one or more further active substances.

Generally, the amino acid sequences, Nanobodies and polypeptides of the invention 20 can be formulated and administered in any suitable manner known per se, for which reference is for example made to the general background art cited above (and in particular to WO 04/041862, WO 04/041863, WO 04/041865 and WO 04/041867) as well as to the standard handbooks, such as Remington's Pharmaceutical Sciences, 18th Ed., Mack Publishing Company, USA (1990) or Remington, the Science and Practice of Pharmacy, 21th Edition, Lippincott Williams and Wilkins (2005).

For example, the amino acid sequences, Nanobodies and polypeptides of the invention may be formulated and administered in any manner known per se for conventional antibodies and antibody fragments (including ScFv's and diabodies) and other pharmaceutically active proteins. Such formulations and methods for preparing the same will be clear to the skilled 30 person, and for example include preparations suitable for parenteral administration (for example intravenous, intraperitoneal, subcutaneous, intramuscular, intraluminal, intra-arterial or intrathecal administration) or for topical (i.e. transdermal or intradermal) administration.

Preparations for parenteral administration may for example be sterile solutions, suspensions, dispersions or emulsions that are suitable for infusion or injection. Suitable

carriers or diluents for such preparations for example include, without limitation, sterile water and aqueous buffers and solutions such as physiological phosphate-buffered saline, Ringer's solutions, dextrose solution, and Hank's solution; water oils; glycerol; ethanol; glycols such as propylene glycol or as well as mineral oils, animal oils and vegetable oils, for example peanut oil, soybean oil, as well as suitable mixtures thereof. Usually, aqueous solutions or suspensions will be preferred.

The amino acid sequences, Nanobodies and polypeptides of the invention can also be administered using gene therapy methods of delivery. See, *e.g.*, U.S. Patent No. 5,399,346, which is incorporated by reference in its entirety. Using a gene therapy method of delivery, 10 primary cells transfected with the gene encoding an amino acid sequence, Nanobody or polypeptide of the invention can additionally be transfected with tissue specific promoters to target specific organs, tissue, grafts, tumors, or cells and can additionally be transfected with signal and stabilization sequences for subcellularly localized expression.

Thus, the amino acid sequences, Nanobodies and polypeptides of the invention may be systemically administered, *e.g.*, orally, in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an assimilable edible carrier. They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the amino acid sequences, Nanobodies and polypeptides of the invention may be combined with one or more 20 excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of the amino acid sequence, Nanobody or polypeptide of the invention. Their percentage in the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of a given unit dosage form. The amount of the amino acid sequence, Nanobody or polypeptide of the invention in such therapeutically useful compositions is such that an effective dosage level will be obtained.

The tablets, troches, pills, capsules, and the like may also contain the following: binders such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a 30 lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring may be added. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form

of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like. A syrup or elixir may contain the amino acid sequences, Nanobodies and polypeptides of the invention, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the amino acid sequences, Nanobodies and polypeptides of the invention may be incorporated into sustained-release preparations and devices.

Preparations and formulations for oral administration may also be provided with an 10 enteric coating that will allow the constructs of the invention to resist the gastric environment and pass into the intestines. More generally, preparations and formulations for oral administration may be suitably formulated for delivery into any desired part of the gastrointestinal tract. In addition, suitable suppositories may be used for delivery into the gastrointestinal tract.

The amino acid sequences, Nanobodies and polypeptides of the invention may also be administered intravenously or intraperitoneally by infusion or injection. Solutions of the amino acid sequences, Nanobodies and polypeptides of the invention or their salts can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils.

20 Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. In all cases, the ultimate dosage form must be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper 30 fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or

sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the amino acid sequences, Nanobodies and polypeptides of the invention in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

For topical administration, the amino acid sequences, Nanobodies and polypeptides of the invention may be applied in pure form, i.e., when they are liquids. However, it will generally be desirable to administer them to the skin as compositions or formulations, in combination with a dermatologically acceptable carrier, which may be a solid or a liquid.

Useful solid carriers include finely divided solids such as talc, clay, microcrystalline cellulose, silica, alumina and the like. Useful liquid carriers include water, hydroxyalkyls or glycols or water-alcohol/glycol blends, in which the amino acid sequences, Nanobodies and polypeptides of the invention can be dissolved or dispersed at effective levels, optionally with the aid of non-toxic surfactants. Adjuvants such as fragrances and additional antimicrobial agents can be added to optimize the properties for a given use. The resultant liquid compositions can be applied from absorbent pads, used to impregnate bandages and other dressings, or sprayed onto the affected area using pump-type or aerosol sprayers.

Thickeners such as synthetic polymers, fatty acids, fatty acid salts and esters, fatty alcohols, modified celluloses or modified mineral materials can also be employed with liquid carriers to form spreadable pastes, gels, ointments, soaps, and the like, for application directly to the skin of the user.

Examples of useful dermatological compositions which can be used to deliver the amino acid sequences, Nanobodies and polypeptides of the invention to the skin are known to the art; for example, see Jacquet et al. (U.S. Pat. No. 4,608,392), Geria (U.S. Pat. No. 4,992,478), Smith et al. (U.S. Pat. No. 4,559,157) and Wortzman (U.S. Pat. No. 4,820,508).

Useful dosages of the amino acid sequences, Nanobodies and polypeptides of the invention can be determined by comparing their *in vitro* activity, and *in vivo* activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art; for example, see U.S. Pat. No. 4,938,949.

Generally, the concentration of the amino acid sequence, Nanobodies and polypeptides of the invention in a liquid composition, such as a lotion, will be from about 0.1-25 wt-%, preferably from about 0.5-10 wt-%. The concentration in a semi-solid or solid composition such as a gel or a powder will be about 0.1-5 wt-%, preferably about 0.5-2.5 wt-%.

The amount of the amino acid sequences, Nanobodies and polypeptides of the invention required for use in treatment will vary not only with the particular amino acid sequence, Nanobody or polypeptide selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or clinician. Also the dosage of the amino acid sequences, Nanobodies and polypeptides of the invention varies depending on the target cell, tumor, tissue, graft, or organ.

The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, *e.g.*, into a number of discrete loosely spaced administrations; such as multiple inhalations from an insufflator or by application of a plurality of drops into the eye.

An administration regimen could include long-term, daily treatment. By "long-term" is meant at least two weeks and preferably, several weeks, months, or years of duration. Necessary modifications in this dosage range may be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein. See Remington's Pharmaceutical Sciences (Martin, E.W., ed. 4), Mack Publishing Co., Easton, PA. The dosage can also be adjusted by the individual physician in the event of any complication.

In another aspect, the invention relates to a method for the prevention and/or treatment of at least one IL-6R related disorders, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of an amino acid sequence of the invention, of a polypeptide of the invention, and/or of a pharmaceutical composition comprising the same.

In the context of the present invention, the term "prevention and/or treatment" not only comprises preventing and/or treating the disease, but also generally comprises preventing the onset of the disease, slowing or reversing the progress of disease, preventing or slowing the onset of one or more symptoms associated with the disease, reducing and/or alleviating one or more symptoms associated with the disease, reducing the severity and/or the duration of the disease and/or of any symptoms associated therewith and/or preventing a further increase in the severity of the disease and/or of any symptoms associated therewith, preventing, reducing

or reversing any physiological damage caused by the disease, and generally any pharmacological action that is beneficial to the patient being treated.

The subject to be treated may be any warm-blooded animal, but is in particular a mammal, and more in particular a human being. As will be clear to the skilled person, the subject to be treated will in particular be a person suffering from, or at risk of, the diseases and disorders mentioned herein.

The invention relates to a method for the prevention and/or treatment of at least one disease or disorder that is associated with IL-6R, with its biological or pharmacological activity, and/or with the biological pathways or signalling in which IL-6R is involved, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of an amino acid sequence of the invention, of a Nanobody of the invention, of a polypeptide of the invention, and/or of a pharmaceutical composition comprising the same. In particular, the invention relates to a method for the prevention and/or treatment of at least one disease or disorder that can be treated by modulating IL-6R, its biological or pharmacological activity, and/or the biological pathways or signalling in which IL-6R is involved, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of an amino acid sequence of the invention, of a Nanobody of the invention, of a polypeptide of the invention, and/or of a pharmaceutical composition comprising the same. In particular, said pharmaceutically effective amount may be an amount that is sufficient to modulate IL-6R, its biological or pharmacological activity, and/or the biological pathways or signalling in which IL-6R is involved

The invention also relates to a method for the prevention and/or treatment of at least one disease or disorder that can be prevented and/or treated by administering of an amino acid sequence of the invention or polypeptide of the invention to a patient, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of an amino acid sequence of the invention, of a polypeptide of the invention, and/or of a pharmaceutical composition comprising the same.

More in particular, the invention relates to a method for the prevention and/or treatment of at least one disease or disorder chosen from the group consisting of the diseases and disorders listed herein, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of an amino acid sequence of the invention, of an amino acid sequence of the invention, of a polypeptide of the invention, and/or of a pharmaceutical composition comprising the same.

In another embodiment, the invention relates to a method for immunotherapy, and in particular for passive immunotherapy, which method comprises administering, to a subject suffering from or at risk of the diseases and disorders mentioned herein, a pharmaceutically active amount of an amino acid sequence of the invention, of an amino acid sequence of the invention, of a polypeptide of the invention, and/or of a pharmaceutical composition comprising the same.

In the above methods, the amino acid sequences, Nanobodies and/or polypeptides of the invention and/or the compositions comprising the same can be administered in any suitable manner, depending on the specific pharmaceutical formulation or composition to be used. Thus, the amino acid sequences, Nanobodies and/or polypeptides of the invention and/or the compositions comprising the same can for example be administered orally, intraperitoneally (e.g. intravenously, subcutaneously, intramuscularly, or via any other route of administration that circumvents the gastrointestinal tract), intranasally, transdermally, topically, by means of a suppository, by inhalation, again depending on the specific pharmaceutical formulation or composition to be used. The clinician will be able to select a suitable route of administration and a suitable pharmaceutical formulation or composition to be used in such administration, depending on the disease or disorder to be prevented or treated and other factors well known to the clinician.

The amino acid sequences, Nanobodies and/or polypeptides of the invention and/or the compositions comprising the same are administered according to a regime of treatment that is suitable for preventing and/or treating the disease or disorder to be prevented or treated. The clinician will generally be able to determine a suitable treatment regimen, depending on factors such as the disease or disorder to be prevented or treated, the severity of the disease to be treated and/or the severity of the symptoms thereof, the specific amino acid sequences, Nanobody or polypeptide of the invention to be used, the specific route of administration and pharmaceutical formulation or composition to be used, the age, gender, weight, diet, general condition of the patient, and similar factors well known to the clinician.

Generally, the treatment regimen will comprise the administration of one or more amino acid sequences, Nanobodies and/or polypeptides of the invention, or of one or more compositions comprising the same, in one or more pharmaceutically effective amounts or doses. The specific amount(s) or doses to administered can be determined by the clinician, again based on the factors cited above.

Generally, for the prevention and/or treatment of the diseases and disorders mentioned herein and depending on the specific disease or disorder to be treated, the potency of the

specific amino acid sequence, Nanobody and polypeptide of the invention to be used, the specific route of administration and the specific pharmaceutical formulation or composition used, the amino acid sequences, Nanobodies and polypeptides of the invention will generally be administered in an amount between 1 gram and 0.01 microgram per kg body weight per day, preferably between 0.1 gram and 0.1 microgram per kg body weight per day, such as about 1, 10, 100 or 1000 microgram per kg body weight per day, either continuously (e.g. by infusion), as a single daily dose or as multiple divided doses during the day. The clinician will generally be able to determine a suitable daily dose, depending on the factors mentioned herein. It will also be clear that in specific cases, the clinician may choose to deviate from 10 these amounts, for example on the basis of the factors cited above and his expert judgment. Generally, some guidance on the amounts to be administered can be obtained from the amounts usually administered for comparable conventional antibodies or antibody fragments against the same target administered via essentially the same route, taking into account however differences in affinity/avidity, efficacy, biodistribution, half-life and similar factors well known to the skilled person.

Usually, in the above method, a single amino acid sequence, Nanobody or polypeptide of the invention will be used. It is however within the scope of the invention to use two or more amino acid sequences, Nanobodies and/or polypeptides of the invention in combination.

20 The amino acid sequences, Nanobodies and polypeptides of the invention may also be used in combination with one or more further pharmaceutically active compounds or principles, i.e. as a combined treatment regimen, which may or may not lead to a synergistic effect. Again, the clinician will be able to select such further compounds or principles, as well as a suitable combined treatment regimen, based on the factors cited above and his expert judgement.

In particular, the amino acid sequences, Nanobodies and polypeptides of the invention may be used in combination with other pharmaceutically active compounds or principles that are or can be used for the prevention and/or treatment of the diseases and disorders cited herein, as a result of which a synergistic effect may or may not be obtained. Examples of such compounds and principles, as well as routes, methods and pharmaceutical formulations or 30 compositions for administering them will be clear to the clinician.

When two or more substances or principles are to be used as part of a combined treatment regimen, they can be administered via the same route of administration or via different routes of administration, at essentially the same time or at different times (e.g. essentially simultaneously, consecutively, or according to an alternating regime). When the

substances or principles are to be administered simultaneously via the same route of administration, they may be administered as different pharmaceutical formulations or compositions or part of a combined pharmaceutical formulation or composition, as will be clear to the skilled person.

Also, when two or more active substances or principles are to be used as part of a combined treatment regimen, each of the substances or principles may be administered in the same amount and according to the same regimen as used when the compound or principle is used on its own, and such combined use may or may not lead to a synergistic effect. However, when the combined use of the two or more active substances or principles leads to a synergistic effect, it may also be possible to reduce the amount of one, more or all of the substances or principles to be administered, while still achieving the desired therapeutic action. This may for example be useful for avoiding, limiting or reducing any unwanted side-effects that are associated with the use of one or more of the substances or principles when they are used in their usual amounts, while still obtaining the desired pharmaceutical or therapeutic effect.

The effectiveness of the treatment regimen used according to the invention may be determined and/or followed in any manner known per se for the disease or disorder involved, as will be clear to the clinician. The clinician will also be able, where appropriate and on a case-by-case basis, to change or modify a particular treatment regimen, so as to achieve the desired therapeutic effect, to avoid, limit or reduce unwanted side-effects, and/or to achieve an appropriate balance between achieving the desired therapeutic effect on the one hand and avoiding, limiting or reducing undesired side effects on the other hand.

Generally, the treatment regimen will be followed until the desired therapeutic effect is achieved and/or for as long as the desired therapeutic effect is to be maintained. Again, this can be determined by the clinician.

In another aspect, the invention relates to the use of an amino acid sequence, Nanobody or polypeptide of the invention in the preparation of a pharmaceutical composition for prevention and/or treatment of at least one IL-6R related disorders.

The subject to be treated may be any warm-blooded animal, but is in particular a mammal, and more in particular a human being. As will be clear to the skilled person, the subject to be treated will in particular be a person suffering from, or at risk of, the diseases and disorders mentioned herein.

The invention also relates to the use of an amino acid sequence, Nanobody or polypeptide of the invention in the preparation of a pharmaceutical composition for the

prevention and/or treatment of at least one disease or disorder that can be prevented and/or treated by administering an amino acid sequence, Nanobody or polypeptide of the invention to a patient.

More in particular, the invention relates to the use of an amino acid sequence, Nanobody or polypeptide of the invention in the preparation of a pharmaceutical composition for the prevention and/or treatment of IL-6R related disorders, and in particular for the prevention and treatment of one or more of the diseases and disorders listed herein.

Again, in such a pharmaceutical composition, the one or more amino acid sequences, Nanobodies or polypeptides of the invention may also be suitably combined with one or more other active principles, such as those mentioned herein.

Finally, although the use of the Nanobodies of the invention (as defined herein) and of the polypeptides of the invention is much preferred, it will be clear that on the basis of the description herein, the skilled person will also be able to design and/or generate, in an analogous manner, other (single) domain antibodies against the IL-6 receptor, as well as polypeptides comprising such (single) domain antibodies (in which the terms "domain antibody" and "single domain antibody" have their usual meaning in the art).

Thus, one further aspect of the invention relates to domain antibodies or single domain antibodies against the IL-6 receptor, and to polypeptides that comprise at least one such (single) domain antibody and/or that essentially consist of such a (single) domain antibody.

In particular, such a (single) domain antibody against the IL-6 receptor may comprise 3 CDR's, in which said CDR's are as defined above for the Nanobodies of the invention. For example, such (single) domain antibodies may be the single domain antibodies known as "dAb's", which are for example as described by Ward et al, *supra*, but which have CDR's that are as defined above for the Nanobodies of the invention. However, as mentioned above, the use of such "dAb's" will usually have several disadvantages compared to the use of the corresponding Nanobodies of the invention. Thus, any (single) domain antibodies against the IL-6 receptor according to this aspect of the invention will preferably have framework regions that provide these (single) domain antibodies against the IL-6 receptor with properties that make them substantially equivalent to the Nanobodies of the invention.

Thus, in its broadest sense, the invention relates to an amino acid sequence that essentially consists of four framework regions (FR1 to FR4, respectively) and three complimentarity determining regions (CDR1 to CDR3, respectively), and that is directed against (as defined herein) the IL-6 receptor. Such an amino acid sequence preferably contains between 80 and 200 amino acid residues, such as between 90 and 150 amino acid

residues, such as about 100-130 amino acid residues (although suitable fragments of such an amino acid sequence - i.e. essentially as described herein for the Nanobodies of the invention or equivalent thereto - may also be used), and is preferably such that it forms an immunoglobulin fold or such that, under suitable conditions, it is capable of forming an immunoglobulin fold (i.e. by suitable folding). The amino acid sequence is preferably chosen from Nanobodies, domain antibodies, single domain antibodies or “dAb’s”, and is most preferably a Nanobody as defined herein. The CDR’s may be any suitable CDR’s (for which reference is made to the disclosure herein), but are preferably as defined herein.

In further aspects, the invention also relates to proteins and polypeptides that comprise 10 at least one such amino acid sequence; to nucleic acids encoding such amino acid sequences, proteins and polypeptides; to methods for preparing such amino acid sequences, proteins and polypeptides; to host cells expressing or capable of expressing such amino acid sequences, proteins or polypeptides; to compositions, and in particular to pharmaceutical compositions, that comprise such amino acid sequences, proteins, polypeptides, nucleic acids and/or host cells; and to uses of such amino acid sequences, proteins, polypeptides, nucleic acids, host cells and/or compositions, in particular for prophylactic, therapeutic or diagnostic purposes, such as the prophylactic, therapeutic or diagnostic purposes mentioned herein. All these aspects will be clear to the skilled person based on the disclosure herein, and may be essentially the same or equivalent to the embodiments described herein for the Nanobodies of 20 the invention.

Such an amino acid sequence preferably contains between 80 and 200 amino acid residues, such as between 90 and 150 amino acid residues, such as about 100-130 amino acid residues, although suitable fragments of such an amino acid sequence (i.e. essentially as described herein for the Nanobodies of the invention or equivalent thereto) may also be used.

Furthermore, it will also be clear to the skilled person that it may be possible to “graft” one or more of the CDR’s mentioned above for the Nanobodies of the invention onto other “scaffolds”, including but not limited to human scaffolds or non-immunoglobulin scaffolds. Suitable scaffolds and techniques for such CDR grafting will be clear to the skilled person and are well known in the art, see for example US-A-7,180,370, WO 01/27160, EP 0 605 522, 30 EP 0 460 167, US-A-7,054,297, Nicaise et al., Protein Science (2004), 13:1882-1891; Ewert et al., Methods, 2004 Oct; 34(2):184-199; Kettleborough et al., Protein Eng. 1991 Oct; 4(7): 773-783; O’Brien and Jones, Methods Mol. Biol. 2003; 207: 81-100; and Skerra, J. Mol. Recognit. 2000; 13: 167-187, and Saerens et al., J. Mol. Biol. 2005 Sep 23;352(3):597-607, and the further references cited therein. For example, techniques known per se for grafting

mouse or rat CDR's onto human frameworks and scaffolds can be used in an analogous manner to provide chimeric proteins comprising one or more of the CDR's of the Nanobodies of the invention and one or more human framework regions or sequences.

Thus, in another embodiment, the invention comprises a chimeric polypeptide comprising at least one CDR sequence chosen from the group consisting of CDR1 sequences, CDR2 sequences and CDR3 sequences mentioned herein for the Nanobodies of the invention. Preferably, such a chimeric polypeptide comprises at least one CDR sequence chosen from the group consisting of the CDR3 sequences mentioned herein for the Nanobodies of the invention, and optionally also at least one CDR sequence chosen from the group consisting of the CDR1 sequences and CDR2 sequences mentioned herein for the Nanobodies of the invention. For example, such a chimeric polypeptide may comprise one CDR sequence chosen from the group consisting of the CDR3 sequences mentioned herein for the Nanobodies of the invention, one CDR sequence chosen from the group consisting of the CDR1 sequences mentioned herein for the Nanobodies of the invention and one CDR sequence chosen from the group consisting of the CDR1 sequences and CDR2 sequences mentioned herein for the Nanobodies of the invention. The combinations of CDR's that are mentioned herein as being preferred for the Nanobodies of the invention will usually also be preferred for these chimeric polypeptides.

In said chimeric polypeptides, the CDR's may be linked to further amino acid sequences and/or may be linked to each other via amino acid sequences, in which said amino acid sequences are preferably framework sequences or are amino acid sequences that act as framework sequences, or together form a scaffold for presenting the CDR's. Reference is again made to the prior art mentioned in the last paragraph. According to one preferred embodiment, the amino acid sequences are human framework sequences, for example V_H 3 framework sequences. However, non-human, synthetic, semi-synthetic or non-immunoglobulin framework sequences may also be used. Preferably, the framework sequences used are such that (1) the chimeric polypeptide is capable of binding the IL-6 receptor, i.e. with an affinity that is at least 1%, preferably at least 5%, more preferably at least 10%, such as at least 25% and up to 50% or 90% or more of the affinity of the corresponding Nanobody of the invention; (2) the chimeric polypeptide is suitable for pharmaceutical use; and (3) the chimeric polypeptide is preferably essentially non-immunogenic under the intended conditions for pharmaceutical use (i.e. indication, mode of administration, dosis and treatment regimen) thereof (which may be essentially analogous to the conditions described herein for the use of the Nanobodies of the invention).

According to one non-limiting embodiment, the chimeric polypeptide comprises at least two CDR sequences (as mentioned above) linked via at least one framework sequence, in which preferably at least one of the two CDR sequences is a CDR3 sequence, with the other CDR sequence being a CDR1 or CDR2 sequence. According to a preferred, but non-limiting embodiment, the chimeric polypeptide comprises at least two CDR sequences (as mentioned above) linked at least two framework sequences, in which preferably at least one of the three CDR sequences is a CDR3 sequence, with the other two CDR sequences being CDR1 or CDR2 sequences, and preferably being one CDR1 sequence and one CDR2 sequence. According to one specifically preferred, but non-limiting embodiment, the chimeric 10 polypeptides have the structure FR1' - CDR1 - FR2' - CDR2 - FR3' - CDR3 - FR4', in which CDR1, CDR2 and CDR3 are as defined herein for the CDR's of the Nanobodies of the invention, and FR1', FR2', FR3' and FR4' are framework sequences. FR1', FR2', FR3' and FR4' may in particular be Framework 1, Framework 2, Framework 3 and Framework 4 sequences, respectively, of a human antibody (such as V_H3 sequences) and/or parts or fragments of such Framework sequences. It is also possible to use parts or fragments of a chimeric polypeptide with the structure FR1' - CDR1 - FR2' - CDR2 - FR3' - CDR3 - FR4. Preferably, such parts or fragments are such that they meet the criteria set out in the preceding paragraph.

The invention also relates to proteins and polypeptides comprising and/or essentially 20 consisting of such chimeric polypeptides, to nucleic acids encoding such proteins or polypeptides; to methods for preparing such proteins and polypeptides; to host cells expressing or capable of expressing such proteins or polypeptides; to compositions, and in particular to pharmaceutical compositions, that comprise such proteins or polypeptides, nucleic acids or host cells; and to uses of such proteins or polypeptides, such nucleic acids, such host cells and/or such compositions, in particular for prophylactic, therapeutic or diagnostic purposes, such as the prophylactic, therapeutic or diagnostic purposes mentioned herein. For example, such proteins, polypeptides, nucleic acids, methods, host cells, compositions and uses may be analogous to the proteins, polypeptides, nucleic acids, methods, host cells, compositions and use described herein for the Nanobodies of the 30 invention.

It should also be noted that, when the Nanobodies of the inventions contain one or more other CDR sequences than the preferred CDR sequences mentioned above, these CDR sequences can be obtained in any manner known per se, for example from Nanobodies (preferred), V_H domains from conventional antibodies (and in particular from human

antibodies), heavy chain antibodies, conventional 4-chain antibodies (such as conventional human 4-chain antibodies) or other immunoglobulin sequences directed against the IL-6 receptor. Such immunoglobulin sequences directed against the IL-6 receptor can be generated in any manner known per se, as will be clear to the skilled person, i.e. by immunization with the IL-6 receptor or by screening a suitable library of immunoglobulin sequences with the IL-6 receptor, or any suitable combination thereof. Optionally, this may be followed by techniques such as random or site-directed mutagenesis and/or other techniques for affinity maturation known per se. Suitable techniques for generating such immunoglobulin sequences will be clear to the skilled person, and for example include the screening techniques reviewed 10 by Hoogenboom, *Nature Biotechnology*, 23, 9, 1105-1116 (2005). Other techniques for generating immunoglobulins against a specified target include for example the Nanoclone® technology (as for example described in the published US patent application 2006-0211088), so-called SLAM technology (as for example described in the European patent application 0 542 810), the use of transgenic mice expressing human immunoglobulins or the well-known hybridoma techniques (see for example Larrick et al, *Biotechnology*, Vol.7, 1989, p. 934). All these techniques can be used to generate immunoglobulins against the IL-6 receptor, and the CDR's of such immunoglobulins can be used in the Nanobodies of the invention, i.e. as outlined above. For example, the sequence of such a CDR can be determined, synthesized and/or isolated, and inserted into the sequence of a Nanobody of the invention (e.g. so as to 20 replace the corresponding native CDR), all using techniques known per se such as those described herein, or Nanobodies of the invention containing such CDR's (or nucleic acids encoding the same) can be synthesized de novo, again using the techniques mentioned herein.

Further uses of the amino acid sequences, Nanobodies, polypeptides, nucleic acids, genetic constructs and hosts and host cells of the invention will be clear to the skilled person based on the disclosure herein. For example, and without limitation, the amino acid sequences of the invention can be linked to a suitable carrier or solid support so as to provide a medium than can be used in a manner known per se to purify IL-6R from compositions and preparations comprising the same. Derivatives of the amino acid sequences of the invention that comprise a suitable detectable label can also be used as markers to determine 30 (qualitatively or quantitatively) the presence of IL-6R in a composition or preparation or as a marker to selectively detect the presence of IL-6R on the surface of a cell or tissue (for example, in combination with suitable cell sorting techniques).

The invention will now be further described by means of the following non-limiting examples and figures, in which the Figures show:

- **Fig. 1** Analysis of immune response in llamas 81 and 82 by ELISA.
- **Fig. 2** FACS analysis of immune response in llamas 081 and 082.
- **Fig. 3** Schematic representation of Alphascreen assays used to identify Nanobodies against the IL6 binding site on IL6R.
- **Fig. 4** SDS-PAGE of purified monovalent anti IL6R Nanobodies. M=molecular weight markers. The identity of the Nanobodies is shown on top of the gel.
- **Fig. 5** Protein sequences of anti-IL6R Nanobodies.
- 10 - **Fig. 6** Protein sequences of a selected subset of inhibitory anti-IL6R Nanobodies.
- **Fig. 7** Binding of monovalent inhibitory anti IL6R Nanobodies to IL6-R on U266 cells. Fluorescence intensity is blotted on the X-axis, the number of events on the Y-axis. Purified anti-IL6-R antibody BR-6 was included.
- **Fig. 8, 9 and 10** Antagonistic activity of 14 monovalent anti IL6R Nanobodies in alphascreen for inhibition of the IL6/IL6-R interaction.
- **Fig. 11** Antagonistic activity of 14 monovalent anti IL6R Nanobodies in cell-based assay (XG-1). BR6, BN12 and the Reference-Fab were included as a reference.
- **Fig. 12:** Competitive binding of monovalent anti IL6R Nanobodies to sIL6-R in human plasma
- 20 - **Fig. 13** Competitive binding of monovalent anti IL6R Nanobodies to sIL6-R in cynomolgus plasma
- **Fig. 14** SDS-PAGE of purified bispecific anti IL6R/anti SA Nanobodies. M=molecular weight markers. The identity of the Nanobodies is shown on top of the gel.
- **Fig. 15** Binding of bispecific anti IL6R/anti SA Nanobodies to IL6-R on U266 cells. Fluorescence intensity is blotted on the X-axis, the number of events on the Y-axis. Purified anti-IL6-R antibody BR-6 was included.
- **Fig. 16 and 17** Antagonistic activity of bispecific anti IL6R/anti SA Nanobodies in Alphascreen for inhibition of the IL6/IL6-R interaction.
- **Fig. 18, 19 and 20** Antagonistic activity of bispecific anti IL6R/anti SA Nanobodies in cell-based assay (XG-1). BR6, BN12 and the Reference-Fab were included as a reference.
- 30 - **Fig. 21 and 22** Antagonistic activity of bispecific anti IL6R/anti SA Nanobodies in the presence of 1 mg/mL human serum albumin in cell-based assay (XG-1). BR6, BN12 and the Reference-Fab were included as a reference.

- **Fig. 23** SDS-PAGE of purified trivalent anti IL6R/anti SA Nanobodies. M=molecular weight markers. The identity of the Nanobodies is shown on top of the gel.
- **Fig. 24** Binding of trivalent anti IL6R/anti SA Nanobodies to IL6-R in Biacore.
- **Fig. 25** Binding of trivalent anti IL6R/anti SA Nanobodies to IL6-R on U266 cells. Fluorescence intensity is blotted on the X-axis, the number of events on the Y-axis. Purified anti-IL6-R antibody BR-6 was included.
- **Fig. 26** Antagonistic activity of trivalent anti IL6R/anti SA Nanobodies in the absence and in the presence of 1 mg/mL human serum albumin in cell-based assay (XG-1).
- **Fig. 27** Sequences of different humanized variants of IL6R03, IL6R04 and IL6R13.
- 10 - **Fig. 28, 29, 30 and 31** Antagonistic activity of humanized variants of IL6R03, IL6R04 and IL6R13 anti IL6R Nanobodies in Alphascreen for inhibition of the IL6/IL6-R interaction.
- **Fig. 32 and 33** Concentration of humanized variants of IL6R03, IL6R04 and IL6R13 anti IL6R Nanobodies after incubation at different temperatures.
- **Fig. 34** Antagonistic activity in cell-based assay (XG-1) of humanized Nanobodies.
- **Fig. 35** Flow cytometric analysis of IMAC-purified Nanobodies from selected clones. IMAC-purified Nanobodies IL6R-24, IL6R-44, IL6R-202 and IL6R-203 were added to cyno IL6R-positive CHO cells. Detection was performed using a PE-labeled rabbit polyclonal anti-Nanobody serum (R23). Nanobodies binding to cells was measured by an increase in fluorescence intensity as compared to cells that were incubated with FACS buffer (PBS + 10% FBS) followed by PE-labeled R23 antiserum. Fluorescence intensity is blotted on the X-axis, the number of events on the Y-axis.
- 20 - **Fig. 36 and 37** Binding inhibition curve for ALEXA⁶⁴⁷-labeled hIL-6 (625 pM final) competed with unlabeled hIL-6, Nanobody IL6R-202 and Nanobody IL6R-203. The average fluorescence and standard deviation is plotted against the concentration of unlabeled competitor. IC₅₀ values were calculated in graph pad Prism.
- **Fig. 38** Flow cytometric analysis of purified IL6R04-HSA and HSA-IL6R04 fusion proteins and IL6R04. Purified Nanobody constructs were added to U266 cells. Detection was performed using a PE-labeled rabbit polyclonal anti-Nanobody serum (R23). Nanobody-HSA fusion protein and Nanobody binding to cells was measured by an increase in fluorescence intensity as compared to cells that were incubated with FACS buffer (PBS + 10% FBS) followed by PE-labeled R23 antiserum. Fluorescence intensity is blotted on the X-axis, the number of events on the Y-axis. Purified anti-IL6R antibodies BR6 and BN12 were included as positive control.

- **Fig. 39** Proliferative response of the TF1 cell line to Nanobody-HSA formats. 1.25×10^4 cells were seeded in triplicate in the presence of 200 pg/ml IL-6 and the indicated dilutions of Nanobody-HSA formats or control antibodies BR-6 or BN-12. The cells were pulsed with 0.5 μ Ci of [³H]thymidine for the last 6 h of a 72-h culture period, after which the incorporated radioactivity was determined.
- **Fig. 40** PK analysis of Nanobody IL-6R04-HSA in Balb/c mice. The mean half-life of Nanobody IL-6R04-HSA is 1.10 days.
- **Fig. 41** Mean observed (\pm SD) plasma concentration-time profiles after intravenous administration of 2.00 mg/kg of (A, B) IL6R04-HSA, (C,D) IL6R202 and (E,F) IL6R203.
- **Fig. 42** The level of CRP in cynomolgus monkey for the evaluation of in vivo efficacy of anti-IL6R Nanobodies
- **Fig. 43, 44 and 45** Analysis of epitope specificity of a panel of Nanobodies in comparison with the Reference-Fab and Reference IgG (see Reference Example 1 and SEQ ID NOs: 629 to 632) using ELISA
- **Fig. 46** Evaluation of specificity of a panel of Nanobodies in comparison with the Reference-Fab (see example 29, first paragraph) using Biacore
- **Fig. 47, 48, 49 and 50** Potency of anti IL6R Nanobodies to inhibit the binding of human IL6 to human, rhesus and cynomolgus monkey soluble IL6R present in plasma

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EXAMPLES

Experimental part

Interleukin-6 (IL6) is a pleiotropic cytokine involved in many physiological processes including regulation of inflammation, immune responses and hematopoiesis. IL6 exerts its biological activities through 2 membrane molecules, a ligand binding 80kDa chain (IL6-R) and a non-ligand-binding signal transducer gp130. Formation of the IL6-IL6-R-gp130 signaling complex occurs sequentially: first IL6 binds to IL6-R via interaction site I (Kd: ~ 10 nM). Next step is binding of this complex to gp130 via interaction sites II and III (Kd: 0.8 nM). Interaction sites II and III are composite sites comprising residues of both IL6 and IL6-R. IL6 and IL6-R alone have no detectable affinity for gp130. The exact stoichiometry and composition of the IL6-IL6-R-gp130 complex is still under debate. The crystal structure of IL6-IL6-R-gp130 complex has been solved (Boulanger (2003) *Science* 300, 2101-2104) and

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suggests a 2:2:2 stoichiometry. Besides the membrane-bound IL6-R, a soluble form (sIL6-R) can be generated by proteolytic cleavage (TACE/ADAM17) or alternative splicing. The complex of IL6 and sIL6-R can also bind to gp130. Interestingly, this also happens in cells which do not express endogenous IL6-R. Consequently, cells which release the sIL6-R protein render cells which only express gp130 responsive towards the cytokine IL6. This mechanism has been termed trans-signaling.

Overexpression of IL6 has been implicated in the pathogenesis of several clinical disorders including chronic autoimmune diseases, multiple myeloma, Castleman's disease, post-menopausal osteoporosis and renal cell carcinoma. To date, a number of inhibitors of the IL6 signaling pathway have been developed including CNTO-328 (anti-IL6 MAb, Centocor), Tocilizumab (anti-IL6-R MAb, Chugai/Roche) and C326 (anti-IL6 avimer, Avidia). CNTO-328 and Tocilizumab are currently in clinical trials for MM, RCC, RA, soJIA, CD and SLE. Tocilizumab is available on the Japanese market since 2005 for treatment of Castleman's disease (Actemra).

Materials

- Human IL6 was obtained from Diaclone as a recombinant protein produced in E. coli.
- Human bio-IL6 was obtained from Diaclone as human IL6 biotinylated by PE (6 biotins/molecule).
- Human soluble IL6-R was obtained from Peprotech as a recombinant protein produced in HEK293 cells and from R&D Systems as a recombinant protein produced in *Sf*21 cells.
- MAb BR-6 is a neutralizing anti-IL6-R monoclonal antibody obtained from Diaclone.
- MAb BN-12 is a non-neutralizing anti-IL6-R monoclonal antibody obtained from Diaclone.
- MAb M182 is a biotinylated anti-IL6-R monoclonal antibody obtained from BD Biosciences.
- Llama IgG (h&I) antibody HRP (horse radish peroxidase) conjugated is a polyclonal antibody against llama IgG raised in goat obtained from Bethyl Labs.
- The Reference Fab and Reference IgG were generated as described in the Reference Example below.

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Example 1: Immunizations

Two llamas (081 and 082) were immunized with human IL6-R (Peprotech) according to the scheme outlined in Table C-1.

Table C-1 Immunization protocol

Day	Llama 081	Llama 082	Tissue collection
0	100 µg	100 µg	10 ml pre-immune blood
7	100 µg	100 µg	-
14	50 µg	50 µg	-
21	50 µg	50 µg	-
28	50 µg	50 µg	10 ml immune blood
35	50 µg	50 µg	-
39			150 ml immune blood PBL1 lymph node bow biopsy
43			150 ml immune blood PBL2
52	50 µg	50 µg	
59			100 ml immune blood NC1

After completion of the protocol the immune response in each animal was analyzed by ELISA. To this end, biotinylated IL6-R (2 µg/ml) was captured in a neutravidin coated microtiter plate. Serial dilutions of serum samples collected at days 0, 28, 39 and 43 were added (starting dilution: 1/500) and bound llama IgG was detected by addition of goat anti-llama IgG HRP labeled. TMB was used as a substrate. Results are shown in Figure 1.

Immune responses were also analyzed by FACS: serial dilutions (starting dilution: 1/100) of serum samples collected at days 0, 28 and 39 were incubated with U266B1 cells (human myeloma). Bound llama IgG was detected by goat anti-llama IgG FITC labeled. Results are shown in Figure 2.

Example 2: Library construction

RNA extracted from peripheral blood lymphocytes and lymph node obtained from llama 081 and 082 was used as starting material for RT-PCR to amplify Nanobody encoding gene fragments. These fragments were cloned into phagemid vector pAX50. Phage was prepared according to standard methods (see for example the prior art and applications filed by Ablynx N.V. cited herein) and stored after filter sterilization at 4°C for further use. The characteristics of the constructed libraries are shown in Table C-2.

Table C-2 Size and percentages of inserts of constructed libraries

Library size	% insert
Llama 81	6×10^7
Llama 82	5×10^7

Example 3: Selections

Selections were carried out with the above libraries using various conditions as summarized in Table C-3.

Table C-3 Experimental conditions used in different selection strategies

Method	Immobilization/ capture	Antigen	Concentration/ amount	Elution
Magnetic beads	Streptavidin	bio-IL6-R	0, 1, 10, 100 ng	Trypsin
Solution	Streptavidin beads	bio-IL6-R	0, 0.01, 0.1, 1 nM	Trypsin
Plate	BN-12	IL6-R (Peptech)	0, 1, 10, 100 nM	Trypsin
Plate	BN-12	IL6-R (R&D)	0, 1, 10, 100 nM	Trypsin

Only a single round of selection was performed for all conditions. Each selection output was analyzed for enrichment factor (# phage present in eluate relative to control), diversity (HinfI profiling) and percentage of IL6-R positive clones (ELISA). Based on these parameters the best selections were chosen for further analysis. To this end, the output from each selection was recloned as a pool into the expression vector pAX51. Colonies were picked and grown in 96 deep well plates (1 ml volume) and induced by adding IPTG for

10 Nanobody expression. Periplasmic extracts (volume: ~ 80 µl) were prepared according to standard methods (see for example the prior art and applications filed by Ablynx N.V. cited herein).

Example 4: Screening

Periplasmic extracts were analyzed first for their ability to inhibit the IL6-IL6-R interaction. To this end, 2 independent Alphascreen assays were set up which are depicted schematically in Figure 3. In assay 1, the periplasmic extracts were incubated with biotinylated IL6 (3 nM), soluble IL6 receptor (1 nM), streptavidin coated donor beads and MAAb BN-12 coated acceptor beads (20 µg/ml). Nanobodies positive in this assay could either 20 inhibit the IL6-IL6-R interaction or IL6-R-MAAb BN-12 interaction. To discriminate between these 2 possibilities a second assay was set up (Assay 2). In this assay the periplasmic extract were incubated with bio-IL6-R (0.3 nM), streptavidin coated donor beads and MAAb BN-12 coated acceptor beads (10 µg/ml). Nanobodies positive in assay 1 but negative in assay 2 were

considered as IL6-IL6-R inhibitors. Periplasmic extracts were diluted 25-fold in both assays which corresponds roughly to a final concentration of 40 nM.

This resulted in two different subclasses of anti-IL6-R Nanobodies; i.e.

a) Nanobodies against IL6-R that were capable of modulating (e.g. partially or fully reducing or preventing) binding of IL6 to IL6-R. In the present example, these were obtained in selections where IL6-R was immobilized on MAb BN-12 (although other methods of obtaining such Nanobodies will be clear to the skilled person).

b) Nanobodies against IL6-R that were capable of modulating (e.g. partially or fully reducing or preventing) binding of IL6-R to MAb BN-12. In the present example, these were obtained in alternative selection strategies where IL6-R was not immobilized on MAb BN-12 (although other methods of obtaining such Nanobodies will be clear to the skilled person).

A statistical overview of the screening effort is shown in Table C-4. Nanobodies showing the strongest inhibition were selected for further characterization.

Table C-4 Screening for Nanobodies that inhibit the IL6/IL6-R interaction

Assay	# clones screened	# inhibitors (%)	# clones sequenced	# unique sequences
IL6-IL6-R	1536	72 (4.7%)	46	14

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Example 5: Nanobody expression and purification

Selected Nanobodies were expressed in *E.coli* as c-myc, His6-tagged proteins in a culture volume of 50 mL. Expression was induced by addition of 1 mM IPTG and allowed to continue for 4h at 37°C. After spinning the cell cultures, periplasmic extracts were prepared by freeze-thawing the pellets. These extracts were used as starting material for immobilized metal affinity chromatography (IMAC). Nanobodies were eluted from the column with 150 mM imidazole and subsequently dialyzed against PBS. Total yield and yield per liter of cell culture are listed in Table C-5.

SDS-PAGE of purified Nanobodies is shown in Figure 4.

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Table C-5 Expression yields of anti-IL6-R Nanobodies in *E. coli*

Nanobody ID	yield (mg)	yield (mg/l)	Nanobody ID	yield (mg)	yield (mg/l)
PMP40H5	0.14	0.6	PMP34G9	0.09	1.8
PMP35E11	0.65	2.6	PMP31A4	1.06	4.2
PMP32C9	0.33	6.5	PMP32E2	1.57	6.3
PMP35H4	0.49	9.8	PMP33A3	0.33	1.3
PMP32E10	0.78	3.1	PMP34A12	0.57	2.3
PMP30C11	0.63	2.5	PMP28E11	0.08	1.6
PMP35C10	0.53	2.1	PMP35F4	0.24	1.0

Example 6: Characterization of monovalent Nanobodies

For simplicity, monovalent clones were renamed. An overview is given in Table C-6 below.

Table C-6 Overview of nomenclature of monovalent anti-IL6R Nanobodies

ID	Original name
IL6R01	PMP28E11
IL6R02	PMP30C11
IL6R03	PMP31A4
IL6R04	PMP32C9
IL6R05	PMP32E10
IL6R06	PMP32E2
IL6R07	PMP33A3
IL6R08	PMP34A12
IL6R09	PMP34G9
IL6R10	PMP35C10
IL6R11	PMP35E11
IL6R12	PMP35F4
IL6R13	PMP35H4
IL6R14	PMP40H5

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a) *Binding to IL6-R in Biacore*

Nanobodies showing the strongest inhibition were selected for off-rate analysis on Biacore and DNA sequencing (Figure 5 and Table C-7).

A subset of 14 inhibitory Nanobodies and 3 control Nanobodies (which do not inhibit the interaction between IL6 and IL6-R) were selected for further analysis in cell based assays. Figure 6 shows the protein sequences of this selected subset of Nanobodies.

Affinity constants (Kd) of these 14 individual inhibitory Nanobodies were determined by surface plasmon resonance on a Biacore 3000 instrument. In brief, IL6-R is amine-coupled to a CM5 sensor chip at a density of 800-1000 RU. Remaining reactive groups are inactivated. Nanobody binding is assessed at various concentrations ranging from 0.5 to 50 nM. Each sample is injected for 4 min at a flow rate of 45 μ l/min to allow for binding to chip-bound antigen. Next, binding buffer without Nanobody is sent over the chip at the same flow rate to allow for dissociation of bound Nanobody. After 10 min, remaining bound analyte is removed by injecting regeneration solution (Glycine/HCl pH1.5). Binding curves obtained at different concentrations of Nanobody are used to calculate Kd values. In Table 10 C-8, an overview of k_d/k_{off} , k_a , and K_d values for the selected subset of 14 Nanobodies is shown.

Table C-7 Off rates (obtained from Biacore-analysis) of anti-IL6-R Nanobodies

Clone ID	k_{off} (s ⁻¹)	clone ID	k_{off} (s ⁻¹)
PMP 35 A3	1,18E-03	PMP 33 B11	1,42E-03
PMP 35 C6	9,32E-04	PMP 33 D1	9,34E-04
PMP 35 D10	3,26E-04	PMP 33 H10	6,69E-04
PMP 35 G9	1,86E-03	PMP 33 H7	1,49E-03
PMP 40 F12	4,39E-04	PMP 35 C10	5,09E-04
PMP 31 C5	1,11E-04	PMP 35 E2	7,54E-04
PMP 31 D2	2,81E-04	PMP 35 E11	4,17E-04
PMP 32 C9	1,50E-04	PMP 35 G11	2,05E-04
PMP 32 F10	5,80E-04	PMP 30 A10	2,68E-03
PMP 28 A2	1,24E-03	PMP 33 C10	3,08E-03
PMP 28 C7	1,31E-03	PMP 35 H4	1,78E-04
PMP 28 D4	1,85E-03	PMP 30 D4	3,24E-04
PMP 28 F7	1,26E-03	PMP 30 H1	2,83E-04
PMP 28 H6	2,47E-03	PMP 33 A2	5,00E-04
PMP 31 B11	1,24E-03	PMP 34 F8	1,42E-04
PMP 31 B4	1,28E-03	PMP 35 B4	3,03E-04
PMP 31 C8	1,25E-03	PMP 40 C9	1/3,6E-04
PMP 31 F4	1,23E-03	PMP 40 H5	1,14E-04
PMP 32 E10	1,27E-03	PMP 30 B9	1,56E-04
PMP 32 H5	1,28E-03	PMP 30 F1	1,01E-03
PMP 32 D12	2,70E-03	PMP 34 B4	9,53E-04
PMP 30 A2	2,57E-03	PMP 34 F10	1,63E-03
PMP 30 B6	8,42E-04	PMP 40 A2	5,69E-04
PMP 30 G11	1,64E-03	PMP 28 G3	1,93E-03
PMP 34 A12	To be determined	PMP 30 C11	2,94E-03
PMP 34 C3	4,35E-04	PMP 31 A4	1,60E-03

Table C-7 (Continued)

PMP 35 H7	1,48E-03	PMP 34 C11	3,67E-03
PMP 33 G3	1,19E-03	PMP 34 E10	2,00E-03
PMP 34 A5	1,68E-03	PMP 34 G9	1,39E-03
PMP 34 D2	3,31E-04	PMP 35 F4	8,96E-04
PMP 34 E9	5,03E-04	PMP 28 B1	1,34E-03
PMP 34 G3	1,40E-04	PMP 28 E11	To be determined
PMP 30 B1	1,00E-03	PMP 32 E2	8,86E-04
PMP 28 G1	3,17E-03	PMP 33 A3	2,42E-04
PMP 30 B3	2,96E-04	PMP 28 B2	5,39E-03
PMP 30 B7	6,66E-04	PMP 28 D1	1,45E-02

Table C-8 Overview of k_d/k_{off} , k_a -, and K_d -values for a selected subset of 14 inhibitory anti-IL6-R Nanobodies.

Nanobody ID	k_d/k_{off} (s⁻¹)	k_a (1/Ms)	K_d (nM)
IL6R01	1.10 E-04	2.62 E+05	0.418
IL6R02	2.94 E-03	8.40 E+05	5.90
	4.95 E-03		
IL6R03	1.47 E-03	4.84 E+05	3.03
	1.60 E-03		
IL6R04	9.42 E-05	3.65 E+05	0.26
	1.50 E-04		
IL6R05	1.41 E-03	1.44 E+05	9.79
	1.27 E-03		
IL6R06	8.86 E-04	1.07 E+06	7.10
	7.57 E-03		
IL6R07	2.42 E-04		
IL6R08	1.97 E-03	1.94 E+05	10.2
IL6R09	1.29 E-03	6.41 E+05	2.01
	1.30 E-03	1.11 E+06	1.17
IL6R10	1.39 E-03		
	5.26 E-04	4.14 E+05	1.27
IL6R11	5.09 E-04		
	3.40 E-04	3.91 E+05	0.87
IL6R12	3.96 E-04	2.15 E+05	1.85
	4.17 E-04		
IL6R13	1.16 E-03	6.78 E+05	1.71
	8.96 E-04		
IL6R14	1.21 E-04	2.31 E+05	0.53
	1.09 E-04	1.37 E+05	0.79
Reference Fab	1.78 E-04		
	1.00 E-04	4.02 E+05	0.25
	1.14 E-04		
	6.60 E-04	7.68 E+05	0.86

b) Binding to IL6-R on U266 cells

Binding to membrane-bound IL6R expressed on U266 cells was analyzed in FACS. Flow cytometric analysis was performed of IMAC-purified Nanobodies from selected clones (IL6R04, IL6R09, IL6R11, IL6R13, IL6R14). IMAC-purified Nanobodies were added to IL6-R positive U266 cells. Detection was performed by a monoclonal anti-myc antibody followed by a PE-labeled polyclonal anti-mouse antibody (Jackson ImmunoResearch Laboratories 115-115-164 Lot 69854). Nanobodies binding to cells was measured by an increase in fluorescence intensity as compared to cells that were incubated with FACS buffer (PBS + 10% FBS) followed by monoclonal anti-myc antibody and/or PE-labeled polyclonal anti-mouse antibody. Results are shown in Figure 7. Fluorescence intensity is blotted on the X-axis, the number of events on the Y-axis. Purified anti-IL6-R antibody BR-6 was included.

c) Epitope mapping

Nanobodies were analyzed for competition with Tocilizumab-Fab. The 14 purified Nanobodies were tested in Alphascreen for inhibition of the Reference-Fab/IL6R interaction. A fixed concentration of purified proteins (100 nM) was added to biotinylated IL6-R (1 nM) and incubated for 15 min. Subsequently Reference-Fab-coated acceptor beads were added and this mixture was incubated for 1 hour. Finally streptavidin donor beads were added and after 1 hour incubator the plate was read on the Envision microplate reader. BR-6, BN12 and the Reference-Fab were included as reference. Results, shown in Table C-9, are expressed as the % binding retained in competition with Reference-Fab. The lower the number, the higher the competition, the higher the overlap in epitope.

Table C-9 Inhibition of the Reference-Fab/IL6R interaction by 14 selected inhibitory anti-IL6-R Nanobodies.

Nanobody ID	% binding retained in competition with Reference-Fab
IL6R01	49
IL6R02	86
IL6R03	5
IL6R04	50
IL6R05	64
IL6R06	36
IL6R07	80
IL6R08	99
IL6R09	62
IL6R10	102
IL6R11	40
IL6R12	103
IL6R13	25
IL6R14	96

d) Competition assays/ Antagonistic activity in Alphascreen

The 14 purified Nanobodies were tested in Alphascreen for inhibition of the IL6/IL6-R interaction. Serial dilutions of purified proteins (concentration range: 500 nM - 10 pM) were added to IL6-R (0.3 nM) and incubated for 15 min. Subsequently 3 nM bio-IL6 and BN12-coated acceptor beads were added and this mixture was incubated for 1 hour. Finally streptavidin donor beads were added and after 1 hour incubator the plate was read on the Envision microplate reader. BR-6 and the Reference-Fab were included as reference. Results are shown in Figures 8, 9 and 10. Dose-response curves were observed for all 14 Nanobodies with IC₅₀-values ranging from 48 pM to 1.7 nM. As can be seen from Figures 8, 9 and 10, the invention provides Nanobodies that, under the conditions of this assay, exhibit essentially full (i.e. >90%; preferably >95%) inhibition of the IL6-IL6-R interaction; or alternatively Nanobodies that, under the conditions of the assay, provide partial inhibition (i.e. between 25% and 90%; preferably between 40% and 75%) of the IL6-IL6-R interaction (e.g. IL6-R07).

e) Antagonistic activity in cell-based assay (XG-1)

All purified Nanobodies were tested in the XG1 assay. XG1 is an IL6-dependent human myeloma cell line. Half-maximal proliferation is achieved at ~20 pg/ml of IL6. Assays were essentially performed as described by Zhang et al. (Blood 83: 3654-3663). BR6, BN12 and the Reference-Fab were included as a reference. Results are outlined in Figure 11. IC50 values ranged from 50 nM to 90 pM and are presented in Table C-10.

Table C-10 IC50 values of 14 selected inhibitory anti-IL6-R Nanobodies measured in XG-1 cell based assay

ID	IC50 (nM)
IL6R02	31.04
IL6R03	16.16
IL6R04	0.089
IL6R05	7.295
IL6R06	42.05
IL6R07	50.47
IL6R08	36.6
IL6R09	2.745
IL6R10	2.546
IL6R11	5.366
IL6R12	2.753
IL6R13	1.395
IL6R14	0.603
Reference-Fab	5.985
BN12	0.2721
BR6	0.064

f) Potency of monovalent wild type Nanobodies in cell-based assay (TF-1)

The TF-1 cell (ECACC) line was maintained between 2-9x100,000 cells/mL using RPMI 1640 supplemented with 2 mM Glutamine, 1% Sodium pyruvate, 3 ng/mL Human GM-CSF (eBiosciences) and 10% Foetal Bovine serum (Gibco). Cells were subcultured 3

times a week and were maintained at 37% and a 5% CO₂ atmosphere. The same batch of GM-CSF (Lot E019991) and of Foetal Bovine Serum (lot no 41Q4556K) was used.

The cell-based assay was performed similarly as described in de Hon, F. D., Ehlers, M., Rose-John, S., Ebeling, S. B., Bos, H. K., Aarden, L. A., and Brakenhoff, J. P. (1994) J Exp Med 180, 2395-2400. Cell suspensions were centrifuged for 5 min at 200g and the supernatant was removed. Cells were resuspended in RPMI 1640 supplemented with 2 mM Glutamine, 1% Sodium pyruvate and 10% Foetal Bovine serum, were seeded at a density of 12500 cells/well in a 96-well plate and incubated for 72h with different dilutions of Nanobodies® and a constant amount of 500 pg/mL IL-6. The 96-well plates were incubated 10 in a humid chamber. Every sample was analysed in triplicate. The total volume/well was 200 µL. During the last 6h of the incubation, cells were pulse-labeled with 0.2 µCi/well of ³H-thymidine (GE Healthcare) in a total volume of 20 µL. Cells were harvested with a semiautomatic cell harvester (Filtermate harvester, PerkinElmer) and the ³H-thymidine incorporation was measured using a Topcount NXT counter (PerkinElmer). Results are expressed as average counts per minute (cpm) per well. IC 50 values are summarised in Table C-11.

Table C-11 IC50 values obtained in TF-1 assay of monovalent wild type Nanobodies

IC50 (nM)	
TF-1	
IL6R04	0,545
IL6R14	1,751
IL6R11	6,623
IL6R12	6,915
IL6R13	7,197
IL6R10	9,147
IL6R09	10,01
IL6R05	19,94
IL6R03	28,05
IL6R02	29,66
IL6R07	35
IL6R08	42,88
IL6R06	77,23

g) Binding to sIL6-R in human plasma

Soluble human IL6-R is present in plasma in the range of 80 ng/ml-400 ng/ml (Jones et al., 2001, FASEB Journal 15:43-58). To analyze whether Nanobodies bind to naturally

occurring sIL6R, binding of monovalent nanobodies to U266 cells was performed in presence of human plasma. Competitive binding could be demonstrated indicating that soluble IL6-R is bound by the Nanobodies analyzed. Inhibition of binding of monovalent IMAC-purified Nanobodies from selected clones (IL6R03, IL6R04, IL6R13) to U266 cells in the presence of human plasma was measured. IMAC-purified Nanobodies were added to IL6-R positive U266 cells in the presence of human plasma. Detection was performed by a monoclonal anti-myc antibody followed by a PE-labeled polyclonal anti-mouse antibody. Inhibition of binding of Nanobodies to cells in the presence of human plasma was measured by a decrease in fluorescence intensity as compared to the fluorescence intensity of Nanobodies binding to cells in the absence of human plasma. Results are shown in Figure 12. Fluorescence intensity is blotted on the X-axis, the number of events on the Y-axis. Purified anti-IL6-R antibody BR-6 was included. U266 cells incubated with monoclonal anti-myc antibody followed by a PE-labeled polyclonal anti-mouse antibody in the presence/absence of human plasma served as background staining control.

10 *h) Binding to sIL6-R in cynomolgus plasma*

Soluble human IL6-R is present in plasma in the range of 80 ng/ml-400 ng/ml (Jones et al., 2001, FASEB Journal 15:43-58). To analyze whether Nanobodies bind to naturally occurring sIL6R, binding of monovalent nanobodies to U266 cells was performed in presence 20 of cynomolgus plasma. Competitive binding could be demonstrated indicating that soluble IL6-R is bound by the Nanobodies analyzed. Inhibition of binding of monovalent IMAC-purified Nanobodies from selected clones (IL6R04, IL6R13) to U266 cells in the presence of cynomolgus plasma. IMAC-purified Nanobodies were added to IL6-R positive U266 cells in the presence of cynomolgus plasma was measured. Detection was performed by a monoclonal anti-myc antibody followed by a PE-labeled polyclonal anti-mouse antibody. Inhibition of binding of Nanobodies to cells in the presence of cynomolgus plasma was measured by a decrease in fluorescence intensity as compared to the fluorescence intensity of Nanobodies binding to cells in the absence of cynomolgus plasma. Results are shown in Figure 13. Fluorescence intensity is blotted on the X-axis, the number of events on the Y-axis. Purified 30 anti-IL6-R antibody BR-6 was included. U266 cells incubated with monoclonal anti-myc antibody followed by a PE-labeled polyclonal anti-mouse antibody in the presence/absence of cynomolgus plasma served as background staining control.

i) Cross-reactivity to mouse IL6-R

Binding to mouse IL6-R (R&D Systems, cat#1830-SR/CF) was analyzed in ELISA. A maxisorp 96-well plate was coated with mouse IL6-R (1 µg/ml), blocked and incubated with a dilution series of Nanobodies (500 nM-0.16 nM). Bound Nanobodies were detected using anti-Myc and anti-mouse horse raddish peroxidase using TMB substrate. No binding could be observed.

Example 7: Construction and expression of bi-specific anti-IL6-R Nanobodies

All 14 selected Nanobodies were also expressed as bispecifics consisting of a C-terminal anti-HSA Nanobody (ALB-1), a 9 amino acid Gly/Ser linker and an N-terminal anti-IL6-R Nanobody. These constructs were expressed in *E.coli* as c-myc, His6-tagged proteins and subsequently purified from the culture medium by immobilized metal affinity chromatography (IMAC) and size exclusion chromatography (SEC). Total yield and yield per liter of cell culture are listed in Table C-12. SDS-PAGE of purified Nanobodies is shown in Figure 14.

Table C-12 Expression yields of bi-specific anti-IL6-R Nanobodies in *E. coli*

Nanobody ID	yield (mg)	yield (mg/L)
PMP28E11-9GS –ALB-1	tbd	
PMP30C11-9GS –ALB-1	1,06	4,22
PMP31A4-9GS –ALB-1	tbd	
PMP32C9-9GS –ALB-1	0,50	1,98
PMP32E2-9GS –ALB-1	1,05	4,19
PMP32E10-9GS –ALB-1	1,25	5,00
PMP33A3-9GS –ALB-1	tbd	
PMP34A12-9GS –ALB-1	2,11	8,44
PMP34G9-9GS –ALB-1	3,30	13,2
PMP35C10-9GS –ALB-1	0,93	3,74
PMP35E11-9GS –ALB-1	1,82	7,28
PMP35F4-9GS –ALB-1	tbd	
PMP35H4-9GS –ALB-1	1,88	7,52
PMP40H5-9GS-ALB-1	tbd	

Example 8: Characterization of bi-specific anti-IL6-R Nanobodies

For simplicity, bispecific clones were renamed. An overview is given in Table C-13 below.

Table C-13 Overview of nomenclature of bispecific anti-IL6R Nanobodies

ID	Formatted Nanobody
IL6R21	Bispecific PMP28E11-9AA GlySer-ALB-1
IL6R22	Bispecific PMP30C11-9AA GlySer- ALB-1
IL6R23	Bispecific PMP31A4-9AA GlySer- ALB-1
IL6R24	Bispecific PMP32C9-9AA GlySer- ALB-1
IL6R25	Bispecific PMP32E10-9AA GlySer- ALB-1
IL6R26	Bispecific PMP32E2-9AA GlySer- ALB-1
IL6R27	Bispecific PMP33A3-9AA GlySer- ALB-1
IL6R28	Bispecific PMP34A12-9AA GlySer- ALB-1
IL6R29	Bispecific PMP34G9-9AA GlySer- ALB-1
IL6R30	Bispecific PMP35C10-9AA GlySer- ALB-1
IL6R31	Bispecific PMP35E11-9AA GlySer- ALB-1
IL6R32	Bispecific PMP35F4-9AA GlySer- ALB-1
IL6R33	Bispecific PMP35H4-9AA GlySer- ALB-1
IL6R34	Bispecific PMP40H5-9AA GlySer- ALB-1

10 a) *Binding to IL6-R in Biacore*

Affinity constants (Kd) of the 14 bispecific (anti-IL6R/anti-HSA) Nanobodies (table C-14) were determined by surface plasmon resonance on a Biacore 3000 instrument. In brief, IL6-R is amine-coupled to a CM5 sensor chip at a density of 800-1000 RU. Remaining reactive groups are inactivated. Nanobody binding is assessed at various concentrations ranging from 0.5 to 50 nM. Each sample is injected for 4 min at a flow rate of 45 µl/min to allow for binding to chip-bound antigen. Next, binding buffer without Nanobody is sent over the chip at the same flow rate to allow for dissociation of bound Nanobody. After 10 min, remaining bound analyte is removed by injecting regeneration solution (Glycine/HCl pH1.5). Binding curves obtained at different concentrations of Nanobody are used to calculate Kd values. In Table C-14 an overview of k_d/k_{off}, k_a, and K_d values for the 14 bispecific Nanobodies is shown.

Table C-14 Overview of k_d/k_{off} , k_a -, and K_d -values for binding of 14 bispecific (anti-IL6-R/anti-HSA) Nanobodies to IL6-R

Nanobody ID	$k_d/k_{off} (s^{-1})$	$k_a (1/Ms)$	$K_d (nM)$
IL6R22	5.65 E-03	3.33 E+05	16.9
IL6R23	1.46 E-03	3.19 E+05	4.59
IL6R24	1.10 E-04	3.68 E+05	0.3
IL6R25	1.21 E-03	1.18 E+05	10.3
IL6R26	6.90 E-03	4.46 E+05	15.5
IL6R28	5.26 E-04	2.41 E+05	2.18
IL6R29	1.49 E-03	7.13 E+05	2.1
IL6R30	1.20 E-03	1.60 E+05	7.52
IL6R31	3.77 E-04	1.62 E+05	2.32
IL6R32	1.26 E-03	1.01 E+06	1.3
IL6R33	1.25 E-04	1.13 E+05	1.11
IL6R34	1.08 E-04	2.58 E+05	0.417

b) Binding to human and mouse serum albumin in Biacore

Binding of Nanobodies® to serum albumin was characterized by surface plasmon resonance in a Biacore 3000 instrument, and an equilibrium constant, K_D , was determined. In brief, serum albumin from different species was covalently bound to CM5 sensor chips surface via amine coupling until an increase of 500 response units was reached. Remaining reactive groups were inactivated. Nanobody® binding was assessed using a series of different concentrations. Each Nanobody® at each concentration was injected for 4 minutes at a flow rate of 45 μ l/min to allow for binding to chip-bound antigen. Next, binding buffer without Nanobody® was sent over the chip at the same flow rate to allow dissociation of bound Nanobody®. After 15 min, remaining bound analyte was removed by injecting regeneration solution (50 mM NaOH).

From the sensorgrams obtained for the different concentrations of each analyte, K_D values were calculated via kinetic data analysis. Results are presented in Table C-15 below.

c) *Binding to IL6-R on U266 cells*

Flow cytometric analysis of bispecific IMAC-purified Nanobodies from selected clones (IL6R23, IL6R24, IL6R29, IL6R33) was done. IMAC-purified Nanobodies were added to IL6-R positive U266 cells. Detection was performed by a monoclonal anti-myc antibody followed by a PE-labeled polyclonal anti-mouse antibody. Nanobodies binding to cells was measured by an increase in fluorescence intensity as compared to cells that were incubated with FACS buffer (PBS + 10% FBS) followed by monoclonal anti-myc antibody and/or PE-labeled polyclonal anti-mouse antibody. Results are shown in Figure 15. Fluorescence intensity is plotted on the X-axis, the number of events on the Y-axis. Purified 10 anti-IL6-R antibody BR-6 was included.

Table C-15 Overview of k_d/k_{off} , k_a , and K_d -values for binding of 14 bispecific (anti-IL6-R/anti-HSA) Nanobodies® to human, mouse, cyno, rhesus and baboon serum albumin

Nanobody ID	Human SA			Mouse SA			Cyno SA			Rhesus SA			Baboon SA			
	k_d/k_{off} (s ⁻¹)	k_a (1/Ms)	K_d (nM)	k_d/k_{off} (s ⁻¹)	k_a (1/Ms)	K_d (nM)	k_d/k_{off} (s ⁻¹)	k_a (1/Ms)	K_d (nM)	k_d/k_{off} (s ⁻¹)	k_a (1/Ms)	K_d (nM)	k_d/k_{off} (s ⁻¹)	k_a (1/Ms)	K_d (nM)	
IL6R22	3.27 E-03	2.94 E+05	11.1	5.08 E-02	4.72 E+05	108										
IL6R23	7.05 E-03	4.58 E+05	15.4	5.50 E-02	2.00 E+05	275	2.77 E-03	1.00 E+05	27.6	2.67 E-03	1.12 E+05	23.8	7.47 E-03	3.22 E+05	23.2	
	3.08 E-03	1.92 E+05	16													
IL6R24	3.31 E-03	2.21 E+05	15	4.95 E-02	4.06 E+05	122	3.34 E-03	1.18 E+05	28.3	3.53 E-03	1.25 E+05	28.3	4.98 E-03	1.24 E+05	40.3	
IL6R25	3.17 E-03	2.29 E+05	13.9	4.74 E-02	3.89 E+05	122										
IL6R26	3.13 E-03	3.33 E+05	9.39	4.38 E-02	6.01 E+05	73										
IL6R28	3.78 E-03	3.56 E+05	10.6	4.78 E-02	2.65 E+05	180										
IL6R29	3.13 E-03	2.91 E+05	10.8	4.58 E-02	5.52 E+05	83	3.07 E-03	1.62 E+05	19	3.29 E-03	1.60 E+05	20.6	4.53 E-03	1.69 E+05	26.8	

Table C-15 Overview of k_d/k_{off} , k_a -, and K_d -values for binding of 14 bispecific (anti-IL6-R/anti-HSA) Nanobodies® to human, mouse, cyno, rhesus and baboon serum albumin (continued)

d) Epitope mapping

Bispecific Nanobodies were analyzed for competition with Reference-Fab. The 14 purified bispecific Nanobodies were tested in Alphascreen for inhibition of the Reference-Fab/IL6R interaction. A fixed concentration of purified proteins (100 nM) was added to biotinylated IL6-R (1 nM) and incubated for 15 min. Subsequently Reference-Fab-coated acceptor beads were added and this mixture was incubated for 1 hour. Finally streptavidin donor beads were added and after 1 hour incubator the plate was read on the Envision microplate reader. BR-6, BN12 and the Reference-Fab were included as reference. Results, shown in Table C-16, are expressed as the % binding retained in competition with Reference-Fab. The lower the number, the higher the competition, the higher the overlap in epitope.

Table C-16 Inhibition of the Reference-Fab/IL6R interaction by 14 bispecific (anti-IL6R/anti-HSA) Nanobodies

Nanobody ID	% binding retained in competition with Reference-Fab
IL6R22	71
IL6R23	4
IL6R24	35
IL6R25	44
IL6R26	25
IL6R28	82
IL6R29	37
IL6R30	78
IL6R31	37
IL6R32	58
IL6R33	17
IL6R34	31

e) *Antagonistic activity in Alphascreen*

The 14 purified bispecific Nanobodies were tested in Alphascreen for inhibition of the IL6/IL6R interaction. Serial dilutions of purified proteins (concentration range: 500 nM - 10 pM) were added to IL6-R (0.3 nM) and incubated for 15 min. Subsequently 3 nM bio-IL6 and BN12-coated acceptor beads were added and this mixture was incubated for 1 hour. Finally streptavidin donor beads were added and after 1 hour incubator the plate was read on the Envision microplate reader. BR-6 and the Reference-Fab fragment were included as reference. Dose-response curves, shown in Figures 16 and 17, were observed for Nanobodies with IC₅₀-values ranging from 55 pM to 1.7 nM (Table C-17).

Table C-17 IC50 values of 14 bispecific (anti-IL6-R/anti-HSA) Nanobodies

ID	IC50 (M)
IL6R21	
IL6R22	5.594 E-10
IL6R23	
IL6R24	1.451 E-10
IL6R25	6.429 E-10
IL6R26	1.666 E-09
IL6R27	
IL6R28	3.259 E-10
IL6R29	1.234 E-10
IL6R30	3.431 E-10
IL6R31	1.309 E-10
IL6R32	2.678 E-10
IL6R33	1.386 E-10
IL6R34	1.455 E-10

f) *Antagonistic activity in cell-based assay (XG-1)*

All 14 bispecific Nanobodies were tested in the XG1 assay. XG1 is an IL6-dependent human myeloma cell line. Half-maximal proliferation is achieved at ~20 pg/ml of IL6. Assays were essentially performed as described by Zhang et al. (**Blood** 83: 3654-3663). BR6, BN12 and the Reference-Fab were included as a reference. Results are outlined in Figures 18, 19 and 20. IC50 values ranged from 50 nM to 90 pM and are presented in Table C-18.

Table C-18 IC50 values of 14 bispecific (anti-IL6-R/anti-HSA) Nanobodies measured in XG-1 cell based assay

ID	IC50 (nM)
IL6R21	
IL6R22	50.17
IL6R23	13.97
IL6R24	0.1542
IL6R25	8.363
IL6R26	65.33
IL6R27	
IL6R28	4.364
IL6R29	3.022
IL6R30	27.16
IL6R31	4.566
IL6R32	1.61
IL6R33	1.574
IL6R34	0.339

Nanobodies were also analyzed in the presence of 1 mg/mL human serum albumin. IC50 values range from 17 nM to 100 pM for monovalent Nanobodies and from 37 nM to 670 pM for bispecific Nanobodies. Results are shown in Figures 21 and 22 and Table C-19.

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Table C-19 IC50 values of monovalent (anti-IL6R) and bispecific (anti-IL6-R/anti-HSA) Nanobodies measured in XG-1 cell based assay

ID	IC50 (nM)
IL6R03	17.54
IL6R04	0.099
IL6R09	2.985
IL6R13	1.321
IL6R14	0.751
IL6R23	37.26
IL6R24	0.667
IL6R29	8.691
IL6R33	8.071
IL6R34	1.356

Example 9: Construction, expression and purification of trivalent anti-IL6-R Nanobodies

Nanobodies were also expressed as trivalent bispecifics consisting of an N-terminal anti-IL6R Nanobody, a C-terminal anti-IL6R Nanobody and an anti-HSA Nanobody (ALB-1) in the middle, connecting the different building blocks with a 9 amino acid Gly/Ser linker (SEQ ID NO's 478 to 492). These constructs were expressed in *E.coli* as c-myc, His6-tagged proteins and subsequently purified from the culture medium by immobilized metal affinity chromatography (IMAC) and size exclusion chromatography (SEC). SDS-PAGE of purified Nanobodies is shown in Figure 23.

Example 10: Characterization of trivalent anti-IL6-R Nanobodies

a) Binding to IL6R on Biacore

Affinity constants (Kd) of the 3 trivalent Nanobodies were determined by surface plasmon resonance on a Biacore 3000 instrument. In brief, IL6-R is amine-coupled to a CM5 sensor chip at a density of 800-1000 RU. Remaining reactive groups are inactivated. Nanobody binding is assessed at various concentrations ranging from 0.5 to 50 nM. Each sample is injected for 4 min at a flow rate of 45 μ l/min to allow for binding to chip-bound

antigen. Next, binding buffer without Nanobody is sent over the chip at the same flow rate to allow for dissociation of bound Nanobody. After 10 min, remaining bound analyte is removed by injecting regeneration solution (Glycine/HCl pH1.5). Avid binding could be demonstrated for the trivalent Nanobody IL6R49 (SEQ ID NO 480) as compared to the corresponding monovalent Nanobody IL6R09 or the bispecific Nanobody IL6R29 (Figure 24).

b) Binding to IL6R on U266 cells

Flow cytometric analysis of bispecific trivalent IMAC-purified Nanobodies from selected clones (IL6R44 (SEQ ID NO 479), IL6R53 (SEQ ID NO 481)). IMAC-purified Nanobodies were added to IL6-R positive U266 cells. Detection was performed by a monoclonal anti-myc antibody followed by a PE-labeled polyclonal anti-mouse antibody. Nanobodies binding to cells was measured by an increase in fluorescence intensity as compared to cells that were incubated with FACS buffer (PBS + 10% FBS) followed by monoclonal anti-myc antibody and/or PE-labeled polyclonal anti-mouse antibody. Results are shown in Figure 25. Fluorescence intensity is blotted on the X-axis, the number of events on the Y-axis. Purified anti-IL6-R antibody BR-6 was included.

c) Antagonistic activity in cell based assay (XG-1)

Purified trivalent Nanobodies IL6R44, IL6R49 and IL6R53 (SEQ ID NO's 479, 480 and 481) were tested in the XG1 assay. XG1 is an IL6-dependent human myeloma cell line. Half-maximal proliferation is achieved at ~20 pg/ml of IL6. Assays were essentially performed as described by Zhang et al. (**Blood** 83: 3654-3663). BR6, BN12 and the Reference-Fab were included as a reference. Results are outlined in Figure 26.

Example 11: Binding to human, cynomolgus monkey and mouse serum albumin in Biacore of wild type trivalent Nanobody® constructs.

Binding of Nanobodies® to serum albumin was characterized by surface plasmon resonance in a Biacore 3000 instrument, and an equilibrium constant, K_D , was determined. In brief, serum albumin from different species was covalently bound to CM5 sensor chips surface via amine coupling until an increase of 500 response units was reached. Remaining reactive groups were inactivated. Nanobody® binding was assessed using a series of different

concentrations. Each Nanobody® at each concentration was injected for 4 minutes at a flow rate of 45 μ l/min to allow for binding to chip-bound antigen. Next, binding buffer without Nanobody® was sent over the chip at the same flow rate to allow dissociation of bound Nanobody®. After 15 min, remaining bound analyte was removed by injecting regeneration solution (50 mM NaOH).

From the sensorgrams obtained for the different concentrations of each analyte, K_D values were calculated via kinetic data analysis. Results are presented in Table C-20 below.

Table C-20 K_D values of trivalent bispecific Nanobodies to serum albumin from different species

Nanobody ID	Human SA	Mouse SA	Cyno SA
IL6R44	51.4 nM	993 nM	43 nM
IL6R53	35 nM	497 nM	Not determined

Example 12: Humanization

DNA fragments encoding humanized versions of Nanobodies® IL6R03, IL6R04 and IL6R13 were assembled from oligonucleotides using a PCR overlap extension method (Stemmer et al., 1995). The sequences of different variants are shown in Figure 27.

a) Antagonistic activity in Alpha screen

Humanized clones of IL6R03 (IL6R61, IL6R62, IL6R63, IL6R64 and IL6R65; SEQ ID NOS : 609 to 613), IL6R04 (IL6R71, IL6R72, IL6R73, IL6R74 and IL6R75; SEQ ID NOS : 614 to 618) and IL6R13 (IL6R81, IL6R82, IL6R83, IL6R84 and IL6R88; SEQ ID NOS : 619, 620, 621, 622 and 626, respectively) were tested in Alphascreen for inhibition of the IL6/IL6R interaction. Serial dilutions of purified proteins (concentration range: 500 nM - 10 pM) were added to IL6-R (0.3 nM) and incubated for 15 min. Subsequently 3 nM bio-IL6 and BN12-coated acceptor beads were added and this mixture was incubated for 1 hour. Finally streptavidin donor beads were added and after 1 hour incubator the plate was read on the Envision microplate reader. BR-6 and the Reference-Fab fragment were included as reference. Dose-response curves are shown in Figures 28, 29, 30 and 31. No significant

differences between the final different humanized clones (IL6R65, IL6R75 and IL6R88) and their corresponding non-humanized versions (IL6R03, IL6R04 and IL6R13, respectively) were observed.

b) Temperature stability tests

Temperature stability tests were performed for humanized clones of IL6R03 (IL6R65), IL6R04 (IL6R75) and IL6R13 (IL6R81, IL6R82, IL6R83 and IL6R84). Samples were diluted at 200 µg/ml and divided in 5*2 aliquots containing 60 µl. The different vials were incubated each at a given temperature ranging from 37°C to 90°C (37, 50, 70 and 90°C) for a period of 1 hr. (lid temperature: 105°C) (control was stored at 4°C). Thereafter, the samples were held at 25°C for 2hrs (ramping rate: 0,05) and stored over night at 4°C. Precipitates were removed by centrifugation for 30 min at 14.000rpm. Supernatant was carefully removed and further analysed. OD at 280nm was measured and the concentration was calculated based on the extinction coefficients. Results are shown in Figures 32 and 33.

c) Binding to IL6-R in Biacore

Humanized clones of IL6R03 (IL6R61, IL6R62, IL6R63, IL6R64 and IL6R65), IL6R04 (IL6R71, IL6R72, IL6R73, IL6R74 and IL6R75) and IL6R13 (IL6R81, IL6R82, IL6R83, IL6R84 and IL6R88) were selected for off-rate analysis on Biacore and DNA sequencing (Table C-21 and Figure 27).

Affinity constants (Kd) of these Nanobodies were determined by surface plasmon resonance on a Biacore 3000 instrument. In brief, IL6-R is amine-coupled to a CM5 sensor chip at a density of 800-1000 RU. Remaining reactive groups are inactivated. Nanobody binding was assessed at various concentrations ranging from 0.5 to 50 nM. Each sample was injected for 4 min at a flow rate of 45 µl/min to allow for binding to chip-bound antigen. Next, binding buffer without Nanobody was sent over the chip at the same flow rate to allow for dissociation of bound Nanobody. After 10 min, remaining bound analyte was removed by injecting regeneration solution (Glycine/HCl pH1.5). Binding curves obtained at different concentrations of Nanobody were used to calculate Kd values. In Table C-21, an overview of k_d, k_a, and K_d values for the humanized Nanobodies is shown.

Table C-21: Overview of K_D , k_a and k_d values for binding of humanized anti-IL-6 receptor Nanobodies® to the human IL-6 receptor.

		IL6R
IL6R61	KD (nM) ka (1/Ms) kd (1/s)	2 8,50E+05 1,70E-03
IL6R62	KD (nM) ka (1/Ms) kd (1/s)	2,22 9,29E+05 2,07E-03
IL6R63	KD (nM) ka (1/Ms) kd (1/s)	3,69 9,90E+05 3,65E-03
IL6R64	KD (nM) ka (1/Ms) kd (1/s)	1,00E-03
IL6R65	KD (nM) ka (1/Ms) kd (1/s)	4 6,00E+05 2,35E-03
IL6R71	KD (nM) ka (1/Ms) kd (1/s)	0,22 7,03E+05 1,53E-04
IL6R72	KD (nM) ka (1/Ms) kd (1/s)	0,33 5,43E+05 1,80E-04
IL6R73	KD (nM) ka (1/Ms) kd (1/s)	0,33 6,98E+05 2,33E-04
IL6R74	KD (nM) ka (1/Ms) kd (1/s)	0,16 7,67E+05 1,22E-04
IL6R75	KD (nM) ka (1/Ms) kd (1/s)	<0,1 1,00E+06 <1E-04
IL6R81	KD (nM)	0,4

	ka (1/Ms) kd (1/s)	3,20E+05 1,28E-04
IL6R82	KD (nM) ka (1/Ms) kd (1/s)	5,07 6,19E+05 3,14E-03
IL6R83	KD (nM) ka (1/Ms) kd (1/s)	0,34 3,50E+05 1,20E-04
IL6R84	KD (nM) ka (1/Ms) kd (1/s)	5,36 7,62E+05 4,09E-03
IL6R85	KD (nM) ka (1/Ms) kd (1/s)	2,06E-03
IL6R86	KD (nM) ka (1/Ms) kd (1/s)	1,70E-03
IL6R87	KD (nM) ka (1/Ms) kd (1/s)	1,05E-04
IL6R88	KD (nM) ka (1/Ms) kd (1/s)	0,9 2,30E+05 2,13E-04 3,10E-04
IL6R89	KD (nM) ka (1/Ms) kd (1/s)	1,90E-04
IL6R90	KD (nM) ka (1/Ms) kd (1/s)	1,92E-03

Example 13: Antagonistic activity in cell-based assay (XG-1) of humanized Nanobodies

Humanized Nanobodies were analysed in the XG-1 assay. XG-1 is an IL6-dependent human myeloma cell line. Half-maximal proliferation is achieved using ~20 pg/ml IL6. Assays were performed as described by Zhang et al. (Blood 83: 3654-3663). BR6, BN12 were included as a reference. Results are shown in Figure 34.

Example 14: Construction of bivalent and trivalent humanized nanobodies

Nanobodies were constructed to bivalent (SEQ ID NO's 559 to 602) and trivalent (SEQ ID NO's 493 to 558) constructs using the humanized anti-IL6R building blocks and humanized anti-serum albumin building blocks. A 9-amino acid GlySer linker was used to link the different building blocks. These constructs were expressed in *E. coli* and purified using ProtA followed by size exclusion chromatography (SEC).

Example 15: Binding to IL6-R in Biacore of humanized bivalent and trivalent Nanobodies

Affinity constants (K_d) of the bispecific bivalent and trivalent humanized Nanobodies were determined by surface plasmon resonance on a Biacore 3000 instrument. In brief, IL6-R was amine-coupled to a CM5 sensor chip at a density of 800-1000 RU. Remaining reactive groups were inactivated. Nanobody binding was assessed at various concentrations ranging from 0.5 to 50 nM. Each sample was injected for 4 min at a flow rate of 45 μ l/min to allow for binding to chip-bound antigen. Next, binding buffer without Nanobody was sent over the chip at the same flow rate to allow for dissociation of bound Nanobody. After 10 min, remaining bound analyte was removed by injecting regeneration solution (Glycine/HCl pH1.5). Binding curves obtained at different concentrations of Nanobody were used to calculate K_d values. In Table C-22 an overview of k_d/k_{off} , k_a , and K_d values for the bispecific Nanobodies is shown.

Table C-22 Overview of k_d/k_{off} , k_a and K_d -values for binding of bispecific humanized bivalent and trivalent Nanobodies to IL6-R

Nanobody ID	k_d/k_{off} (s^{-1})	k_a (1/Ms)	K_d (nM)
IL6R202	5.7 – 4.5 E+05	1.4 – 1.45 E-04	0.25- 0.32
IL6R203	3.8 E+05	~1.5 E-05	~ 0.040

Example 16: Binding to serum albumin from different species of humanized bivalent and trivalent Nanobodies

Binding of Nanobodies® to serum albumin was characterized by surface plasmon resonance in a Biacore 3000 instrument, and an equilibrium constant, K_D , was determined. In brief, serum albumin from different species was covalently bound to CM5 sensor chips surface via amine coupling until an increase of 500 response units was reached. Remaining reactive groups were inactivated. Nanobody® binding was assessed using a series of different concentrations. Each Nanobody® at each concentration was injected for 4 minutes at a flow rate of 45 μ l/min to allow for binding to chip-bound antigen. Next, binding buffer without Nanobody® was sent over the chip at the same flow rate to allow dissociation of bound Nanobody®. After 15 min, remaining bound analyte was removed by injecting regeneration solution (50 mM NaOH).

From the sensorgrams obtained for the different concentrations of each analyte, K_D values were calculated via kinetic data analysis. Results are presented in Table C-23 below.

Table C-23 Overview of k_d/k_{off} , k_a and K_d -values for binding of humanized bivalent and trivalent Nanobodies to serum albumin from different species

Cyno serum albumin

Nanobody ID	$k_d/k_{off} (s^{-1})$	$k_a (1/Ms)$	$K_d (nM)$
IL6R202	1.19 E+05	5.20 E-03	43.6
IL6R203	8.06 E+04	4.36 E-03	54.1

Mouse serum albumin

Nanobody ID	$K_d (nM)$
IL6R202	493
IL6R203	1009

Human serum albumin

Nanobody ID	$k_d/k_{off} (s^{-1})$	$k_a (1/Ms)$	$K_d (nM)$
IL6R202	1.07 E+5	5.56 E-03	52.1
IL6R203	6.47 E+04	4.53 E-03	70

Example 17: Antagonistic activity in cell-based assay (TF-1)

The TF-1 cell (ECACC) line was maintained between 2-9x100,000 cells/mL using RPMI 1640 supplemented with 2 mM Glutamine, 1% Sodium pyruvate, 3 ng/mL Human GM-CSF (eBiosciences) and 10% Foetal Bovine serum (Gibco). Cells were subcultured 3 times a week and were maintained at 37% and a 5% CO₂ atmosphere. The same batch of GM-CSF (Lot E019991) and of Foetal Bovine Serum (lot no 41Q4556K) was used.

The cell-based assay was performed similarly as described in de Hon, F. D., Ehlers, M., Rose-John, S., Ebeling, S. B., Bos, H. K., Aarden, L. A., and Brakenhoff, J. P. (1994) J Exp Med 180, 2395-2400. Cell suspensions were centrifuged for 5 min at 200g and the supernatant was removed. Cells were resuspended in RPMI 1640 supplemented with 2 mM Glutamine, 1% Sodium pyruvate and 10% Foetal Bovine serum, were seeded at a density of 12500 cells/well in a 96-well plate and incubated for 72h with different dilutions of Nanobodies® and a constant amount of 500 pg/mL IL-6. The 96-well plates were incubated in a humid chamber. Every sample was analysed in triplicate. The total volume/well was 200 µL. During the last 6h of the incubation, cells were pulse-labeled with 0.2 µCi/well of ³H-thymidine (GE Healthcare) in a total volume of 20 µL. Cells were harvested with a semiautomatic cell harvester (Filtermate harvester, PerkinElmer) and the ³H-thymidine incorporation was measured using a Topcount NXT counter (PerkinElmer). Results are expressed as average counts per minute (cpm) per well. IC 50 values are summarised in Table C-24.

Table C-24 IC50 values obtained in TF-1 assay of humanized bivalent and trivalent Nanobodies

IC50 value (nM)		
Nanobody	IC50 (nM)	Stand. Dev.
IL6R202	0.180	0.061
IL6R203	0.056	0.019

Example 18: Antagonistic activity in cell-based TF-1 assay in presence of serum albumin

To test the potential of the bivalent and trivalent Nanobodies to block the IL-6/IL6R interaction, a proliferation assay was performed using the IL-6 sensitive TF1 erythroleukemia cell line. Briefly, serial dilutions of the Nanobodies (pre-incubated with 1% serum albumin)

were added to 1.25×10^4 TF-1 cells in triplicate in the presence of a fixed concentration of IL-6 (200 pg/ml). After 72h incubation, cells were pulsed with 0.5 μ Ci of [3 H]thymidine and incubated for an additional 6h prior to freezing at -80°C. Cells were subsequently thawed and embedded on glass fiber membranes using a cell harvester (Perkin Elmer Life Sciences, Wellesley, MA, USA). After several washings with water, filters were air-dried and counted using a γ -counter (Perkin Elmer Life Sciences). The monoclonal antibody BR-6 known to neutralize the IL6/IL6R interaction was used as positive control. The non-neutralizing monoclonal antibody BN-12 was used as negative control. Results are summarized in the table C-25.

Table C-25 IC50 values of inhibition of IL-6/IL6R interaction by bivalent and trivalent anti-IL6R Nanobodies

Compound	IC50 (w/o HSA)	IC50 (+ 1% HSA)
IL6R24	0.305 ± 0.073 (n=4)	0.870 ± 0.326 (n=4)
IL6R44	0.122 ± 0.132 (n=4)	0.144 ± 0.052 (n=4)
IL6R202	0.190 ± 0.056 (n=2)	0.6 ± 0.141 (n=2)
IL6R203	ND	0.092 (n=1)
BR6	0.042 ± 0.014 (n=2)	0.088 ± 0.036 (n=3)

Example 19: Binding of anti-hIL-6R Nanobodies to cynomolgus monkey IL-6R positive cells.

To verify whether the anti-human IL-6R Nanobodies are able to recognize cynomolgus IL-6R, binding to CHO-K1 cells that are stably transfected with cynomolgus IL-6R was assessed by flow cytometry.

Cell binding assays were carried out by initially incubating 200,000 cells with purified Nanobodies. After incubation, the cells were washed with FACS buffer. Cells were subsequently incubated with phycoerythrin labeled rabbit anti-VHH antiserum. To omit signals arising from dead cells a TOPRO-3 (Invitrogen) staining was carried out. Cells were finally analyzed on a BD FACSArray Bioanalyzer System (BD Biosciences).

Figure 35 depicts binding of Nanobody constructs to cyno IL-6R transfected CHO-K1 cells as measured by flow cytometric analysis. It can be seen that the constructs IL6R-24, IL6R-44, IL6R-202 and IL6R-203 show clearly discernable shifts in fluorescence intensity as compared to the fluorescence intensity for cells incubated only with FACS buffer alone in the absence of any construct but with all appropriate detection agents as used for the detection of Nanobody constructs.

Example 20: Anti-human IL-6R Nanobodies compete with human IL-6 for binding to cynomolgus IL-6R

To evaluate the capacity of expressed Nanobodies to block the binding of human IL-6 to cynomolgus IL-6R, a human IL-6 competitive homogeneous cell-based assay was performed. The FMAT 8200 HTS system (Applied Biosystems) assay was performed as follows: CHO-K1 cells stably expressing cynomolgus IL-6R were detached using 0,25% (vol/vol) trypsin and suspended in RPMI supplemented with 10% fetal calf serum (FCS), glutamine, and penicillin-streptomycin. Aliquots of 60 µl (4x10³ cells) were plated into FMAT system 384-well plates (PE Biosystems, CA) and allowed to adhere for 24h. After overnight adherence, culture supernatant was removed by gently tapping the plate. To initiate the competitive screen, 20 µl ALEXA⁶⁴⁷-labeled human IL-6 (625 pM final concentration) and 20 µl Nanobody or cold h-IL6 dilution were added to the cell-containing FMAT system 384-well plates (PE Biosystems, CA). The plates were scanned after 10 hours of incubation. A well was considered positive if it had a count of over 50 events. Figures 36 and 37 depict the results of experiments measuring the capacity of anti hIL-6R Nanobodies to block binding of hIL-6 to cynomolgus IL-6R as measured by competitive FMAT. It can be seen that the constructs IL6R-202 and IL6R-203 clearly compete with the binding of hIL-6 to cynomolgus IL-6R. The positive control cold hIL-6 shows, as expected, clearly discernable competition with ALEXA⁶⁴⁷-labeled hIL-6.

Example 21: Construction of anti-IL-6R Nanobody - human serum albumin fusion proteins

HSA was constructed by gene assembly and cloned into pPICZ α A as XhoI/NotI fragment.

For the generation of a NB04-HSA fusion protein, NB04 was amplified by PCR using primer pair: 5'-GGGTATCTCTCGAGAAAAGAGAGAGCTGAAGCTGAGGTGCAG CTGGTGGAGTCTGGG-3' and 5'-TCTCTTCTCCTAGGTCTTGAATCTGTGGGC GACTTCAGAT TTATGAGCATCTGAGGAGACGGTGACCTG-3'.

The resulting PCR product was cloned into PCR4TOPO and used to clone IL6R04 N-terminally of HSA. Hereto, pPICZ α A HSA and TOPO NB04-N were digested with XhoI and AvrII and ligated to each other.

To connect NB04 to the C-terminus of HSA interspaced with a linker, the following nested-PCR was performed: NB04 was first amplified by PCR using primer pair:

5'-GTGGATCCGGAGGCAGTGGAGGTTCTGGTGGGTCAAGGAGGTGAG
GTGCAGCTGGTGGAGTCTGGG -3' and 5'-TAGAAAGCTGGCGGCCGCTTA
TTATGAGGAGACGGTGACCTG -3'.

A second PCR was performed on the obtained product to insert a GlySer linker by use of primer pair 5'- TTGGTTGCGGCCAGTCAGGCCGCACTTGGTTGGGTGGAT
CCGGAGGCAGTG-3' and 5'-TAGAAAGCTGGCGGCCGCTTATTATGAGGA
GACGGTGACCTG-3'.

The resulting PCR product was cloned into PCR4TOPO and used to clone NB04 at the C-terminus of HSA. Hereto, pPICZ α A HSA and TOPO NB04-C were digested with NotI and SfiI and ligated to each other.

Example 22: Binding of IL6R04-HSA and HSA-IL6R04 to IL-6R-positive cell lines in flow cytometric analysis

The test for binding to the IL-6R positive human multiple myeloma cell line U266 was carried out with purified IL6R04-HSA (SEQ ID NO 604) , HSA-IL6R04 (SEQ ID NO 603) and IL6R04 (SEQ ID NO 440) using the commercially available murine anti-human IL6R antibodies BR6 and BN12 as positive controls.

Cell binding assays were carried out by initially incubating 200,000 cells with 5 nM purified Nanobody-HSA fusion proteins. After incubation, the cells were washed with FACS buffer. Cells were subsequently incubated with phycoerythrin labeled rabbit anti-VHH antiserum. To omit signals arising from dead cells a TOPRO-3 (Invitrogen) staining was carried out. Cells were finally analyzed on a BD FACSArray Bioanalyzer System (BD Biosciences).

Figure 38 depicts binding of the Nanobody-HSA fusion constructs to U266 cells as measured by flow cytometric analysis. The positive controls BR6 and BN12 show, as expected, a clearly discernable shift in fluorescence intensity as compared to the respective control (anti-IgG^{PE}). The constructs IL6R04-HSA and HSA-IL6R04 tested show binding to the IL6R-positive U266 cells as indicated by the shifts in fluorescence intensity as compared to the fluorescence intensity for cells incubated only with FACS buffer in the absence of any construct but with all appropriate detection agents as used for the detection of the Nanobody

constructs. No obvious difference in binding to U266 cells was observed between IL6R04-HSA, HSA-IL6R04 and the monovalent IL6R04 Nanobody.

Example 23: Screening of kinetic off-rate constants via surface plasmon resonance (BIAcore).

Affinity constants (Kd) of the anti-IL6R Nanobody-HSA constructs were determined by surface plasmon resonance on a Biacore 3000 instrument. In brief, IL6-R is amine-coupled to a CM5 sensor chip at a density of 800-1000 RU. Remaining reactive groups are inactivated. Nanobody binding is assessed at various concentrations ranging from 0.5 to 50 nM. Each sample is injected for 4 min at a flow rate of 45 µl/min to allow for binding to chip-bound antigen. Next, binding buffer without Nanobody is sent over the chip at the same flow rate to allow for dissociation of bound Nanobody. After 10 min, remaining bound analyte is removed by injecting regeneration solution (Glycine/HCl pH1.5). Binding curves obtained at different concentrations of Nanobody are used to calculate Kd values. The binding characteristics are summarized in Table C-26.

Table C-26 Surface plasmon resonance measurements of the interaction between hIL-6R and anti-IL6R Nanobody-HSA constructs

	Ka (1/Ms)	Kd (1/s)	KD (nM)
IL6R04	5.36E+05	1.12E-04	0.20
HSA-IL6R04	2.52E+05	5.62E-05	0.22
IL6R04-HSA	1.89E+05	5.60E-05	0.30

Example 24: Anti-IL6R Nanobody-albumin fusion can block hIL-6 induced proliferation of the erythroleukemia cell line TF1.

To test the potential of the anti-IL6R Nanobody-albumin fusions to block the IL-6/IL6R interaction, a proliferation assay was performed using the IL-6 sensitive TF1 erythroleukemia cell line. Briefly, serial dilutions of the anti-IL6R Nanobody-albumin fusions were added to 1.25×10^4 TF-1 cells in triplicate in the presence of a fixed concentration of IL-6 (200 pg/ml). After 72h incubation, cells were pulsed with 0.5 µCi of [³H]thymidine and incubated for an additional 6h prior to freezing at -80°C. Cells were

subsequently thawed and embedded on glass fiber membranes using a cell harvester (Perkin Elmer Life Sciences, Wellesley, MA, USA). After several washings with water, filters were air-dried and counted using a γ -counter (Perkin Elmer Life Sciences). The monoclonal antibody BR-6 known to neutralize the IL6/IL6R interaction was used as positive control. The non-neutralizing monoclonal antibody BN-12 was used as negative control.

As shown in Figure 39, both IL6R04-HSA and HSA-IL6R04 can efficiently block the IL-6 dependent proliferation of TF1 cells. As expected, antibody BR-6 efficiently blocked IL-6 dependent proliferation of TF1 cells whereas BN-12 had no effect.

Example 25: Generation of multivalent Nanobody-albumin fusion protein formats

To potentially increase the biological effect of Nanobody-albumin molecules, bivalent fusions are generated whereby 2 Nanobodies are fused at the N-terminal side of serum albumin. The Nanobodies are fused head-to-tail using an amino acid linker peptide consisting of different lengths.

Here we describe the construction and characterization of bivalent Nanobody-albumin fusion proteins consisting of two identical anti-IL6-R Nanobodies separated by a 3 Ala, 9 (GS) or 20 (GS) amino acid linker peptide. DNA segments encoding Nanobody IL6R-201 were head-to-tail genetically linked and fused to human serum albumin at its N-terminal side, resulting in constructs IL6R201-HSA (SEQ ID NO 605), IL6R201-AAA-IL6R201-HSA (SEQ ID NO 608), IL6R201-9GS-IL6R201-HSA (SEQ ID NO 606), IL6R201-20GS-IL6R201-HSA (SEQ ID NO 607). All Nanobody-albumin fusion proteins were expressed in *Pichia pastoris* in shake flasks or in a fermentor. Production levels of >50 mg/l were obtained.

Example 26: Fusion of Nanobody to albumin increases its serum half-life

The pharmacokinetic study of a HSA fusion Nanobody was conducted in Balbc mice. The mice are preferably 8 up to 12 weeks old. We tested the pharmacokinetic profile from Nanobody, IL6R04-HSA and Nanobody HSA-IL6R04. The Nanobody was injected in three mice. The mice were administrated with 200 μ g/200 μ l via an intravenous infusion. Blood samples were taken at 15', 1h, 2h, 4h, 8h, 24h, day 2, day 3, day 4, day 5, day 8 and day 15 after the start of infusion. 100 μ l whole blood was withdrawn per sampling and the serum was isolated after 1hr incubation at 37°C. The plasma samples are stored by -20°C.

Serum samples were tested for the determination of plasma levels of half-life extension Nanobodies.

Micro titer plates (Maxisorb) are coated with 0.5 µg/ml sIL-6R (Prepotech) in PBS at 100 µl/well overnight at 4°C. The plates are washed with PBS containing 0.1%Tween20 and blocked for 2 hours at RT with PBS containing 1% casein. Plasma samples are then diluted in a non-coated plate and incubated for 30 minutes at room temperature. 100µl of all the dilutions is then transferred to the coated plate and allowed to bind for 1 hour at room temperature. After 1 hour, the plate is washed and 100 ul of 1/2000 dilution of biotinylated goat anti-human albumin in PBS containing 1% casein is added per well. After further incubation for half an hour at room temperature, wells are washed and 100µl of 1/2000 dilution of streptavidin conjugated with horse radish peroxidase in PBS containing 1% casein. This enzyme catalyzes a chemical reaction with the substrate slow TMB, which results in a colorimetric change. After stopping this reaction with HCl, the intensity of the color can be measured by a spectrophotometer, which determines the optical density of the reaction product, using a 450 nm wavelength of light.

The half-life extension Nanobody concentration in the plasma samples is determined towards a standard curve. The concentration determination is determined with the sigmoidal dose-response curve with variable slope.

Figure 40 shows that the albumin fusion prolongs the half-life of a monovalent Nanobody targeting IL6R (IL6R-HSA) and has been determined to be 1.10 days.

The half-life for HSA-IL6R04 has been determined to be 1.0 days.

As a non-formatted Nanobody is cleared within the first hour, the increase in half-life by fusing with human albumin is more than 25 fold.

Example 27: Pharmacokinetic profile in cynomolgus monkey

A pharmacokinetic study of IL6R202, IL6R203 and IL6R04-HSA was conducted in cynomolgus monkeys. The Nanobody was administered intravenously by bolus injection (1.0 ml/kg, approximately 30 sec) in the vena cephalica of the left or right arm to obtain a dose of 2.0 mg/kg. Table C-27 summarizes the dosing regimen for all monkeys.

Table C-27: Dosing regimen of all animals

Group	Test item	Route	Animal	Animal ID	Dose Volume (ml/kg)	Dose (mg/kg)
1	IL6R04-HSA	Iv (bolus)	Cynomolgus Monkey	1m	1.0	2.0
			Cynomolgus Monkey	2 f	1.0	2.0
2	IL6R202	Iv (bolus)	Cynomolgus Monkey	3 m	1.0	2.0
			Cynomolgus Monkey	4 f	1.0	2.0
3	IL6R203	Iv (bolus)	Cynomolgus Monkey	5 m	1.0	2.0
			Cynomolgus Monkey	6 f	1.0	2.0

The anti-IL6R Nanobody concentration in the plasma samples was determined as follows:

a) Immunodetection of IL6R04-HSA in plasma samples

Maxisorb micro titer plates (Nunc, Article No. 430341) were coated overnight at 4°C with 100 µl of a 0.5 µg/ml solution of sIL6R (Peprotech, Article No. 20006R) in DPBS (pH 7.4). After coating, the plates were washed three times (PBS containing 0.1% Tween20) and blocked for 2 hours at room temperature (RT) with PBS containing 1% casein (250 µl/well). Plasma samples and serial dilutions of Nanobody-standards (spiked in 100% pooled blank cynomolgus plasma) were diluted in PBS (in a separate non-coated plate) to obtain the desired concentration/dilution in a final sample matrix consisting of 10% pooled cynomolgus

plasma in PBS. All pre-dilutions were incubated for 30 minutes at RT in the non-coated plate. After the blocking step, plates were washed three times (PBS containing 0.1% Tween20), and an aliquot of each sample dilution (100 µl) was transferred to the coated plates and allowed to bind for 1 hour at RT. After sample incubation, the plates were washed three times (PBS containing 0.1% Tween20) and incubated with 100 µl of 1/2000 dilution (in PBS containing 0.1% casein) of polyclonal goat anti-human serum albumin (Nordic Immunology, Article No. 5184). After 1 hour at RT, the plates were washed three times (PBS containing 0.1% Tween20) and incubated with 100 µl of a 1/2000 dilution (in PBS containing 1% casein) of streptavidine conjugated with horseradish peroxidase (DaktoCytomation, Article No. P0397). After 30 minutes, plates were washed three times (PBS containing 0.1% Tween20) and incubated with 100 µl of slow TMB (Pierce, Article No. 34024). After 20 minutes, the reaction was stopped with 100 µl HCl (1N). The absorbance of each well was measured at 450 nm (Tecan Sunrise spectrophotometer), and corrected for absorbance at 620 nm. This assay measures free and sIL6R-bound IL6R04-HSA Nanobodies. Concentration in each plasma sample was determined based on a sigmoidal standard curve with variable slope of the respective Nanobody. The LLOQ and ULOQ of IL6R04-HSA were 2.00 ng/ml and 100 ng/ml, respectively. Each individual plasma sample was analyzed in three independent assays and an average plasma concentration was calculated for pharmacokinetic data analysis.

b) Immunodetection of IL6R202 and IL6R203 in plasma samples

Maxisorb micro titer plates (Nunc, Article No. 430341) were coated overnight at 4°C with 100 µl of a 5 µg/ml solution of 12B2-GS9-12B2 (B2#1302nr4.3.9) in bicarbonate buffer (50 mM, pH 9.6). After coating, the plates were washed three times with PBS containing 0.1% Tween20 and blocked for 2 hours at room temperature (RT) with PBS containing 1% casein (250 µl/well). Plasma samples and serial dilutions of Nanobody-standards (spiked in 100% pooled blank cynomolgus plasma) were diluted in PBS in a separate non-coated plate (Nunc, Article No. 249944) to obtain the desired concentration/dilution in a final sample matrix consisting of 10% pooled cynomolgus plasma in PBS. All pre-dilutions were incubated for 30 minutes at RT in the non-coated plate. After the blocking step, the coated plates were washed three times (PBS containing 0.1% Tween20), and an aliquot of each sample dilution (100 µl) was transferred to the coated plates and allowed to bind for 1 hour at

RT. After sample incubation, the plates were washed three times (PBS containing 0.1% Tween20) and incubated for 1 hour at RT with 100 µl of a 100 ng/ml solution of sIL6R in PBS (Peprotech, Article No. 20006R). After 1 hour at RT, the plates were washed three times (PBS containing 0.1% Tween20) and incubated with 100 µl of a 250 ng/ml solution of a biotinylated polyclonal anti-IL6R antibody in PBS containing 1% casein (R&D systems, Article No. BAF227). After incubation for 30 minutes (RT), plates were washed three times (PBS containing 0.1% Tween20) and incubated for 30 minutes (RT) with 100 µl of a 1/5000 dilution (in PBS containing 1% casein) of streptavidine conjugated with horseradish peroxidase (DaktoCytomation, Article No. P0397). After 30 minutes, plates were washed three times (PBS containing 0.1% Tween20) and incubated with 100 µl of slow TMB (Pierce, Article No. 34024). After 20 minutes, the reaction was stopped with 100 µl HCl (1N). The absorbance of each well was measured at 450 nm (Tecan Sunrise spectrophotometer), and corrected for absorbance at 620 nm. This assay measures free Nanobody as well as Nanobodies bound to sIL6R and/or cynomolgus serum albumin. Concentration in each plasma sample was determined based on a sigmoidal standard curve with variable slope of the respective Nanobody. The LLOQ and ULOQ of IL6R202 were 7.00 ng/ml and 300 ng/ml, respectively. The LLOQ and ULOQ of IL6R203 were 2.00 ng/ml and 70.0 ng/ml, respectively.

Each individual plasma sample was analyzed in two independent assays and an average plasma concentration was calculated for pharmacokinetic data analysis.

Mean observed plasma concentration-time profiles (\pm SD) after intravenous administration of IL6R04-HSA (2.0 mg/kg), IL6R202 or IL6R203 are shown in Figure 41.

Basic pharmacokinetic parameters of IL6R04-HSA, IL6R202 and IL6R203 after a single intravenous administration at 2.00 mg/kg in the male and female cynomolgus monkey are listed in Tables C-28, C-29 and C-30, respectively. All parameters were calculated with two-compartmental modeling, with elimination from the central compartment.

Table C-28: Basic pharmacokinetic parameters¹ of IL6R04-HSA after a single intravenous administration at 2.00 mg/kg in the male and female Cynomolgus Monkey.

	Monkey 1m	Monkey 2f ²	Mean		SD	CV(%)
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$C_{(0)}$ ($\mu\text{g}/\text{ml}$)	50.6	49.0	49.8	\pm	1.13	2.27
V_{ss} (mL/kg)	79.1	77.2	78.2	\pm	1.34	1.72
V_z (mL/kg)	84.4	80.8	82.6	\pm	2.55	3.08
V_c (mL/kg)	39.6	40.8	40.2	\pm	0.849	2.11
V_t (mL/kg)	39.5	36.4	38.0	\pm	2.19	5.78
CL ($\text{mL}/\text{day}/\text{kg}$)	10.3	10.5	10.4	\pm	0.141	1.36
CL _d ($\text{mL}/\text{day}/\text{kg}$)	41.6	55.7	48.7	\pm	9.97	20.5
$t_{1/2\alpha}$ (day)	0.309	0.229	0.269	\pm	0.0566	21.0
$t_{1/2\beta}$ (day)	5.69	5.33	5.51	\pm	0.255	4.62
MRT (day)	7.70	7.37	7.54		0.233	3.10
AUC _{inf} ($\mu\text{g}\cdot\text{day}/\text{ml}$)	194	191	193	\pm	2.12	1.10
AUC _{inf} /D ($\text{kg}\cdot\text{day}/\text{ml}$)	0.0970	0.0955	0.0963		0.00106	1.10

¹ All parameters were calculated with two-compartmental modeling

² Estimate of PK parameters should be interpreted with caution because the method of calculation ignores the immunological clearance due to neutralizing antibodies.

Table C-29: Basic pharmacokinetic parameters¹ of IL6R202 after a single intravenous administration at 2.00 mg/kg in the male and female Cynomolgus Monkey.

	Monkey 3m	Monkey 4f	Mean		SD	CV(%)
$C_{(0)}$ ($\mu\text{g}/\text{ml}$)	57.6	56.5	57.1	\pm	0.778	1.36
V_{ss} (mL/kg)	65.1	60.6	62.9	\pm	3.18	5.06
V_z (mL/kg)	70.0	64.6	67.3	\pm	3.82	5.67
V_c (mL/kg)	34.7	35.4	35.1	\pm	0.495	1.41
V_t (mL/kg)	30.4	25.2	27.8	\pm	3.68	13.2
CL ($\text{mL}/\text{day}/\text{kg}$)	6.97	6.74	6.86	\pm	0.163	2.37
CL _d ($\text{mL}/\text{day}/\text{kg}$)	22.1	19.1	20.6	\pm	2.12	10.3
$t_{1/2\alpha}$ (day)	0.474	0.500	0.487	\pm	0.0184	3.78
$t_{1/2\beta}$ (day)	6.96	6.64	6.80	\pm	0.226	3.33

Table C-30: Basic pharmacokinetic parameters¹ of IL6R203 after a single intravenous administration at 2.00 mg/kg in the male and female Cynomolgus Monkey.

	Monkey 5m	Monkey 6f	Mean		SD	CV(%)
C ₍₀₎ (µg/ml)	67.1	87.8	77.5	±	14.6	18.9
V _{ss} (mL/kg)	41.5	34.1	37.8	±	5.23	13.8
V _z (mL/kg)	42.5	35.2	38.9	±	5.16	13.3
V _c (mL/kg)	29.8	22.8	26.3	±	4.95	18.8
V _t (mL/kg)	11.7	11.3	11.5	±	0.283	2.46
CL (mL/day/kg)	6.98	7.11	7.05	±	0.0919	1.30
CL _d (mL/day/kg)	22.7	27.2	25.0	±	3.18	12.8
t _{1/2α} (day)	0.250	0.187	0.219	±	0.0445	20.4
t _{1/2β} (day)	4.22	3.43	3.83	±	0.559	14.6
MRT (day)	5.94	4.80	5.37		0.806	15.0
AUC _{inf} (µg•day/ml)	286	281	284	±	3.54	1.25
AUC _{inf} /D (kg•day/ml)	0.143	0.141	0.142		0.00141	1.00

¹ All parameters were calculated with two-compartmental modeling

Example 28: Efficacy in cynomolgus monkey

In human and non-human primates, human IL-6 (hIL-6) has been reported to induce acute phase protein synthesis (Gauldi et al, 1987; Asano et al., 1990). Acute phase proteins are defined as a set of plasma proteins, like C-reactive protein (CRP), serum amyloid A, haptoglobin, fibrinogen, albumin, transferrin and C3, increasing or decreasing concentrations by at least 25% in inflammatory disorders, mainly due to changes in their production by hepatocytes (Gabay & Kushner, 1999). Patterns of cytokine production and acute phase response differ in different inflammatory conditions. Therefore, acute phase changes reflect the presence and intensity of inflammation, making them diagnostically relevant.

In this study, the ability of IL6R202 and IL6R203 to block *in vivo* IL-6 induced responses was examined in primates.

Adult cynomolgus monkeys (*Macaca fascicularis*), aged between 3 and 4 years and weighing below 3 kg were housed individually in an environment controlled room, at room temperature. The body weight of all individuals was recorded at first administration and weekly from there on. The animals were numbered and coupled in groups of 2 individuals (one male and one female) with approximately equal mean body weight.

The lyophilized hIL-6 (Gentaur) was dissolved in 0.5 M acetic acid to a final concentration of 100 µg/mL. One vial containing 500 µg IL-6 was dissolved in 5 mL 0.5 M acetic acid. First, 3 mL of 0.5 M acetic acid was added to the vial and the solution was swirled until all lyophilisate was dissolved. The solution was transferred to a sterile polystyrene tube. The vial was rinsed with 2 mL of 0.5 M acetic acid and this solution was transferred to the polystyrene tube as well, with subsequent gentle mixing of the solution.

Human IL-6 was administered via an s.c. bolus injection of 1 mL/kg b.w. in 1% autologues Cynomolgus monkey heat inactivated serum in the dorsal region.

8 groups (group 4 to 11, Table C-31) of animals received a single i.v. injection of IL6R202 or IL6R203, in one of four different dosages; namely 0.6, 2, 6 or 20 mg/kg b.w. in D-PBS. The Nanobodies® were administered via an i.v. bolus injection of 1 mL/kg b.w. in D-PBS into the vena cephalica of the left or right arm. Subsequently after 1 hour, all animals were injected with the first of 7 daily injections s.c. of hIL-6 (5 µg/kg b.w.). Two individuals of one group (group 12, 23m and 24f, Table C-31) received no Nanobody® preadministration and served as a negative control.

Blood samples were collected prior to injection on test day 0 and from there on daily from test day 1 to test day 14 and on day 21.

The inflammation parameter CRP (serum C-reactive protein levels) is one of the most important parameters for evaluating efficacy with respect to acute phase response in this animal model (animal model that was used in the current study is described in detail in Asano et al. (1990) Blood 8, 1602-1605). Furthermore, CRP is also one of the most important non-subjective parameters used in evaluating efficacy of anti-inflammatory response compounds in human subjects as part of clinical trials, which is well documented in the following references: Choy et al. (2002) Arthritis and Rheumatism 46 (12) 3143-50; Nishimoto et al. (2004) Arthritis and Rheumatism 50 (6) 1761-69; Maini et al. (2006) Arthritis and

Rheumatism 54 (9) 2817-29. An increase in the levels of CRP is one of the most relevant indications of inflammation.

In the current animal model, the CRP levels and a number of other parameters were measured (results for the latter not shown); the inflammation parameter ESR (erythrocyte sedimentation rate) could not be determined in a quantitative manner (i.e. to provide a dose-response curve) using this model.

Table C-31 Chronologic schedule of hIL-6 and test item administration

Animal group	Animal number		Nanobody®			hIL6	
	Male	Female	Test item	Admin (day)	dose	Admin (day)	dose
4	7	8	IL6R202	1	0.6 mg/kg b.w.	1, 2, 3, 4, 5, 6, 7	5 µg/kg b.w.
5	9	10	IL6R202	1	2 mg/kg b.w.	1, 2, 3, 4, 5, 6, 7	5 µg/kg b.w.
6	11	12	IL6R202	1	6 mg/kg b.w.	1, 2, 3, 4, 5, 6, 7	5 µg/kg b.w.
7	13	14	IL6R202	1	20 mg/kg b.w.	1, 2, 3, 4, 5, 6, 7	5 µg/kg b.w.
8	15	16	IL6R203	1	0.6 mg/kg b.w.	1, 2, 3, 4, 5, 6, 7	5 µg/kg b.w.
9	17	18	IL6R203	1	2 mg/kg b.w.	1, 2, 3, 4, 5, 6, 7	5 µg/kg b.w.
10	19	20	IL6R203	1	6 mg/kg b.w.	1, 2, 3, 4, 5, 6, 7	5 µg/kg b.w.
11	21	22	IL6R203	1	20 mg/kg b.w.	1, 2, 3, 4, 5, 6, 7	5 µg/kg b.w.
12	23	24	-	-	-	1, 2, 3, 4, 5, 6, 7	5 µg/kg b.w.

Data on the level of CRP are presented in Figure 42. At test day 2, the serum concentration of CRP in all groups, control and test, reached a maximum of up to 13 times the base level. A clear correlation between the administered dosage of either Nanobody® and the level of increase in CRP was observed.

Example 29: Competition ELISA with Reference IgG.

To analyze epitope specificity of a panel of Nanobodies in comparison with the Reference IgG, an ELISA experiment was designed where the Reference IgG MRA was coated, and a dilution series of Nanobodies pre-incubated with a constant amount of IL6R was subsequently transferred to the wells.

Detection was performed using biotinylated anti-IL6R Mab M182 (Pharmingen) and streptavidin-HRP. Data indicate that only for Reference Fab, IL6R-03, IL6R65, IL6R13 and IL6R88 a competitive binding to the Reference IgG was observed.

Data are presented in Figures 43, 44 and 45.

Example 30: Biacore Reference-Fab.

To analyze epitope specificity of a panel of Nanobodies in comparison with the Reference-Fab, a Biacore experiment was designed where BN12, a non-neutralizing anti-human IL6R Mab (Diaclone) was coupled to the chip in Sodium acetate pH=4. Next, 100 nM IL-6R was injected on the chip. Then, 100 nM of an anti-IL-6R nanobody was injected. Finally, a mixture of 100 nM Reference-Fab and 100 nM Nanobody was injected.

Results (Figure 46) showed that, for all Nanobodies tested, the Reference-Fab was still binding to IL-6R when IL-6R was already bound by the Nanobodies. This indicated that the anti-IL-6R Nanobodies possibly recognized a different epitope on IL-6R. Only for Nanobodies IL6R-03, IL6R65, IL6R06, IL6R13 and IL6R88 a reduced binding was observed.

Example 31: Potency to inhibit binding of human IL6 to human, rhesus and cynomolgus monkey soluble IL6R from plasma

Potency to inhibit the binding of human IL6 to human, rhesus and cynomolgus monkey soluble IL6R present in plasma was analyzed for a panel of Nanobodies. A non-neutralizing anti-IL6R Mab BN12 (Diaclone) was coated on a microtiter well plate. Subsequently, plasma from human, rhesus or cynomolgus monkey pre-incubated with human IL6 and a dilution serie of Nanobody was applied to the plate. Detection was performed using anti-IL6-biotine followed by streptavidin-HRP reagent. Data are presented in Figures 47, 48, 49 and 50.

Reference Example 1: preparation of reference Fab and IgG according to EP 0 628 639 (= WO 92/19759).

Two representative anti-human IL-6R immunoglobulins according to EP 0 628 639 (a Fab fragment and a full-sized IgG) were generated and used as reference compounds herein.

The Fab fragment and full-sized IgG were constructed based on the L-chain called “RV_{La}” (see EP 0 628 639 B1, Table 2, version (a)) and the H-chain called “RV_{Hf}” (see EP 0 628 639 B1, Table 3, version (f)). These particular L-chain and H-chain were chosen for the purposes of constructing the reference compounds because, according to EP 0 268 639 B1 (see for example paragraph [0074]), a reshaped human antibody comprising said L-chain and said H-chain exhibited an ability to bind to human IL-6R at the same level as PM1, a mouse monoclonal antibody against human IL-6R (see again EP 0 628 639 B1, paragraph [009] and the further references cited therein).

The full-length reference IgG consisted of the amino acid sequences of SEQ ID NO: 629 (heavy chain) and SEQ ID NO: 630 (light chain). The Fab fragment consisted of the amino acid sequences of SEQ ID NO: 631 (heavy chain regions V_Lb and V_Hf fused to the CH1 region of human IgG1) and SEQ ID NO: 632 (reshaped human PM-1 variable light chain fused to human C_{kappa}).

Encoding DNA fragments were generated by assembly PCR using partially overlapping oligonucleotides. PCR products were cloned into a single, bi-cistronic vector which enables expression of functional, disulphide-linked Fab fragments in the periplasm of *E. coli*. Full-length IgG was produced in CHO cells transfected with 2 expression vectors containing the genes for the light and heavy chains. The gene encoding the heavy chain was

created by fusing $V_H f$ to the constant region of human IgG1. The light chain was as described in EP 0 268 639.

Table B-1: Nanobodies and polypeptides against the IL-6 receptor.

“(t)” refers to a translated protein. Leader sequences and N-terminal sequences are given as SEQ ID NO’s: 472-477.

Nanobodies against the IL-6 receptor	
<	> PMP40C9(t) , SEQ ID NO: 399 ;PRT;-> EVQLVESGGGLVQPGGSLRLSCAASGFSLDYYAIGWFRQAPGKEREGVSCM DSSAGTTSTYYSDSVKGRFTISRDDAKNTVYLQMNSLKPEDTAVYYCAADGHLNW GQRYVPCSQISWRGWNDYWGQGTQTVSS
<	> PMP34F8(t), SEQ ID NO: 400 ;PRT;-> EVQLVESGGGLVQPGGSLRLSCAASGFSLDYYAIGWFRQAPGKEREGVSCM DSSDGTTNTYYSDSVKGRFTISRDDAKNTVYLQMNSLKPEDTASYYCAADGHLNW GQPYVPCSQISWRGWNDYWGQGTQTVSS
<	> PMP34E9(t), SEQ ID NO: 401 ;PRT;-> EVQLVESGGGLVQPGGSLRLSCAASGFTLDYYAIGWFRQAPGKEREGVSCISS SDGSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAAYYCATDRSVYYCSGDA PEEYYWGQGTQTVSS
<	> PMP34D2(t) , SEQ ID NO: 402 ;PRT;-> EVQLVESGGGLVQPGGSLRLSCAASGFTLDYFAIGWFRQAPGKERERVSCISS SDGSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCATDRSVYYCSGGA PEEYYWGQGTQTVSS
<	> PMP34C3(t) , SEQ ID NO: 403 ;PRT;-> EVQLVESGGGLVQPGGSLRLSCVASGFSLDYYVIGWFRQAPGKEREGVSCISS SDGSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAADLLRTPEFCVD SAPYDYWGRGTQTVSS
<	> PMP34A5(t) , SEQ ID NO: 404 ;PRT;-> EVQLVESGGGLVQPGGSLRLSCAASGFTLGYFAIGWFRQAPGKEREGVSCISS SDGSTYYADSVKGRFTVSRDNAKNTVYLQMNSLKPEDTAVYYCATDRSVYYCSGG APEEYYWGQGTQTVSS
<	> PMP33G3(t) , SEQ ID NO: 405 ;PRT;-> EVQLVESGGGLVQPGGSLRLSCAASGFTLGYFAIGWFRQAPGKEREGVSCISS

SDGSAYYADSVKGRFTVSRDNAKNTVYLQMNSLKPEDTAVYYCATDRSVYYCSGG
APEEYYWGQGTQTVSS

< > PMP33C10(t) , SEQ ID NO: 406 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMWSVRQAPGKGLEWVSAI
SWNGGSTYYTESMKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCVKGSTAIVGVP
PTYPDEYDYWGQGTQTVSS

< > PMP33A2(t) , SEQ ID NO: 407 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAASGFSLDYYAIGWFRQAPGKEREGVSCM
DSSGGTTSTYYSDSVKGRFTISRDDAKNTVYLQMNSLKPEDTAVYYCAADGHLNW
GQRYVPCSQISWRGWNDYWGQGTQTVSS

< > PMP32H5(t) , SEQ ID NO: 408 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAPGKGPEWVSAI
NSGGGSTYYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCATDWRYSDYD
LPLPPPGDYWGQGTQTVSS

< > PMP32F10(t) , SEQ ID NO: 409 ;PRT;->
EVQLVESGGGLVQAGGSLRLSCAASGFTFDDYAIGWFRQAPGKEREGISCISS
SDGSTYYADSVKGRFTISSLNAKNTVYLQMNSLKPEDTAVYYCAAEPPDSSWILDGS
PEFFKFWGQGTQTVSS

< > PMP31F4(t), SEQ ID NO: 410 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAPGKGPEWVSAI
NSGGGSTYYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCATDWRYSDYD
LPLPPPGDYWGQGTQTVSS

< > PMP31D2(t) , SEQ ID NO: 411 ;PRT;->
EVQLVESGGGLVQAGGSLRLSCAASGFTFDDYAIGWFRQAPGKEREGVSGIS
SSDGNTYYADSVKGRFTISSLNAKNTVYLQMNSLKPEDTAVYYCAAEPPDSNWYLD
GSPEFFKFWGQGTQTVSS

< > PMP31C8(t) , SEQ ID NO: 412 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAPGKGPEWVSAI
NSGGGSTYYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCATDWRYSDYD
LPLPPPGDYWGQGTQTVSS

< > PMP31C5(t) , SEQ ID NO: 413 ;PRT;->
EVQLVESGGGLVQAGGSLRLSCAASGFTFDDYAIGWFRQAPGKEREGVSCIS
SSDGSTYYADSVKGRFTISSDNAKNTVYLLMNSLKPEDTAVYYCAEPPDSMWSDL
GSPEFFKFWGQGTQTVSS

< > PMP31B4(t) , SEQ ID NO: 414 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAPGKGPEWVSAI
NSGGGSTYYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCATDWRYSDYD
LPLPPPGDYWGQGTQTVSS

< > PMP31B11(t) , SEQ ID NO: 415 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAPGKGPEWVSAI
NSGGGSTYYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCATDWRYSDYD
LPLPPPGDYWGQGTQTVSS

< > PMP30G11(t) , SEQ ID NO: 416 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCVASGFSLDYYVIGWFRQAPGKEREGVSCISS
SDGSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAADLLRTPEFCVD
SAPYDYWGQGTQTVSS

< > PMP30B6(t) , SEQ ID NO: 417 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAASGFTLDYYVIGWFRQAPGKEREAVACIS
SSDRSTYYADSVKGRFTISRDNAKNTGYLQMNSLKPEDTAVYYCAADLLRTPEFCSD
SAPYDYWGQGTQTVSS

< > PMP30B1(t) , SEQ ID NO: 418 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAASGFTLDYYAIGWWRQAPGKGREGVSCIS
SGDGSTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCATDRSVYYCSGG
APEEYYWGQGTQTVSS

< > PMP30A2(t) , SEQ ID NO: 419 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAASGFTLDYYVIGWFRQAPGKEREGVSCIG
SSDDSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAADLLRTPEFCT
DSAPYDYWGQGTQTVSS

< > PMP30A10(t) , SEQ ID NO: 420 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMWSVRQAPGKGLEWVSAI

SWNGGSTYYTESMKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCVKGSTAIVGVP
PTYPDEYDYWGQGTQTVSS

< > PMP28H6(t) , SEQ ID NO: 421 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAPGKGPEWVSAI
NSGGGSTYYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCATDWRYSDYD
LPLPPPGDYWGQGTQTVSS

< > PMP28F7(t), SEQ ID NO: 422 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAPGKGPEWVSAI
NSGGGSTYYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCATDWRYSDYD
LPLPPPGDYWGQGTQTVSS

< > PMP28D4(t) , SEQ ID NO: 423 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAPGKGPEWVSAI
NSGGGSTYYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCATDWRYSDYD
LPLPPPGDYWGQGTQTVSS

< > PMP28C7(t) , SEQ ID NO: 424 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAPGKGPEWVSAI
NSGGDNTYYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCATDWRYSDYD
LPLPPPGDYWGQGTQTVSS

< > PMP28B1(t) , SEQ ID NO: 425 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAASGFTLNYYAIGWFRQAPGKEREGVSCISS
SDGSTYYADSVKGRFTISRDNAKNTFYLQMNSLKPEDTAVYYCAAEGLGDSDSPCG
AAWYNDYWGQGTQTVSS

< > PMP28A2(t) , SEQ ID NO: 426 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAPGKGPEWVSAI
NSGGGSTYYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCATDWRYSDYD
LPLPPPGDYWGQGTQTVSS

< > PMP40H5 , SEQ ID NO: 427 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAASGFSLDYYAIGWFRQAPGKEREGVSCM
DSSSGTTSTYYSDSVKGRFTISRDDAKNTVYLQMNSLKPEDTAVYYCAADGHLNWG
QRVPCSQISWRGWNDYWGQGTQTVSS

< >PMP35H4 , SEQ ID NO: 428 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMWSVRQAPGRATEWVSAI
SWNGNNNTYYTESMKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCVKGSTAIVGV
PPTYPDEYDYWGQGTQTVSS

< >PMP35F4 , SEQ ID NO: 429 ;PRT;->
EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYDMGWYRQAPGKEREFAIIT
WNSSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAIYYCNAQYGLGYAEDY
WGQGTQTVSS

< >PMP35E11 , SEQ ID NO: 430 ;PRT;->
EVQLVESGGGLVQAGGSLRLSCAASGFTFDDYAIGWFRQAPGKEHEGVSCIS
SSDGSTYYADSVKGRFTISSDNAKNTVYLQMNSLKPEDTAVYYCAAERDVPARSLC
GSYYWYDYRGQGTQTVSS

< >PMP35C10 , SEQ ID NO: 431 ;PRT;->
EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYDMGWYRQAPGKEREFAVI
HWSSGSTYYADPVKGRFTISRDNAKNTVYLQMNSLKPEDTAIYYCNAFLPGPEGFH
DYWGQGTQTVSS

< >PMP34G9 , SEQ ID NO: 432 ;PRT;->
EVQLVESGGGLVQAGGSLRLSCAASGRTSSSYDMTWYRQVPGKEREFAVIS
WSGGSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAIYYCNAYTGGDDYW
QQGTQTVSS

< >PMP34G3 , SEQ ID NO: 433 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAASGFTLDYYAIGWFRQAPGKERERVSCISS
SDGSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAAYYCATDRSVYYCSGGA
PEEYYWGQGTQTVSS

< >PMP34E10 , SEQ ID NO: 434 ;PRT;->
EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYAMGWGRQAPGKEREFAVIS
WSGGSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAADLAEFKYS
YADYWGQGTQTVSS

< >PMP34C11 , SEQ ID NO: 435 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAAAGFTLDYSAIGWFRQAPGKEREMFSCIS

GSDGSTWYADSVAGRFTISFDNAKNTVYLQMNSLKPEDTGLYICAVTGGVRGPCAY EYEYWGQGTQTVSS
< >PMP34A12 , SEQ ID NO: 436 ;PRT;-> EVQLVESGGGLVQPGGSLRLSCVASGFSLDYYVIGWFRQAPGKEREGVSCISS SDGSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAADLLRTPEFCVD SAPYDYWGQGTQTVSS
< >PMP33A3 , SEQ ID NO: 437 ;PRT;-> EVQLVESGGGLVQPGGSLRLSCAASGFTLDYGAIGWFRQAPGKEREGVSCISS STGSTYYADSVKGRFTISRDNGKNTVYLQMNSLKPEDTAVYYCAADKMWSPCLVA ANEEALFEYDYWGQGTQTVSS
< >PMP32E2 , SEQ ID NO: 438 ;PRT;-> EVQLVESGGGLVQAGGSLRLSCAASGNIFDDNTMGWTWNRQPPGKQRELVA IIATDGSTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNLFSLRLGRDY WGQGTQTVSS
< >PMP32E10 , SEQ ID NO: 439 ;PRT;-> EVQLVESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAPGKGPEWVSAI NSGGGSTYYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCATDWRYSDYD LPLPPPGDYWGQGTQTVSS
< >PMP32C9 , SEQ ID NO: 440 ;PRT;-> EVQLVESGGGLVQAGGSLRLSCAASGFTFDDYDIGWFRQAPGKEREGVSGIS SSDGNTYYADSVKGRFTISSDNAKNTVYLQMNSLKPEDTAVYYCAAEPPDSSWYLD GSPEFFKYWGQGTQTVSS
< >PMP31A4 , SEQ ID NO: 441 ;PRT;-> EVQLVESGGGLVQAGGSLRLSCAASGSIFKVNAMEGWYRQAPGKQRELVAGII SGGSTNYADSVKGRLTISRDNAKNTVYLQMNSLKPEDTAVYYCSFVTTNSDYDLGR DYWGQGTQTVSS
< >PMP30C11 , SEQ ID NO: 442 ;PRT;-> EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYDMGWYRQAPGKEREFVAI SRSGSSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAIYYCKAEVVAGDYDY WGQGTQTVSS

< >PMP28G3 , SEQ ID NO: 443 ;PRT;->
EVQLVESGGGLVQAGGSLRLSCTASGNIFSTETMGWYRQPPGKQRDVVATIT HGGTTNYADSVKGRFTISRDNRKNTVYLQMNSLKPEDTGYYYCNARSSWYSPEYW GQGTQTVSS
< >PMP28E11 , SEQ ID NO: 444 ;PRT;->
EVQLVESGGGFVQAGGSLRLSCIASGDNFSINRMGWYRQALGKQRELVAIIT NHGSTNYADAVKGRFTISRDYAKNTVYLQMNGLPDDTAVYYCNAIYSEVGTWRD DYWGQQIQTVSS
< > 059B.IL6-R.cl5.7(t), SEQ ID NO: 445 ;PRT;->
EVQLVESGGGLVQAGGSLRLSCAASGRTFSGADAGWNRQTPGKEREFAAI NWSGNSTYYADSVKGRFTVSRDNAKNTVYLQMNSLKPEDTAVYYCHAFRDDYYSE GKGTLTVSS
< > 059A.IL6-Rcl4(t) , SEQ ID NO: 446 ;PRT;->
EVQLVESGGGLVQAGGSLRLSCAASGRTLSSYDMGWYRQGPGKEREFAAI SWSGGGTDYVDSVKGRFTISRDTAKNTMYLQMNSLKPEDTAIYYCNALGTTDSDYE GELYWGQGTQTVSS
< > 059A.IL6-Rcl3(t) , SEQ ID NO: 447 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAASGFTLDSYAIGWFRQAPGKEPEGVSCIST SDGSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCTADGGPHAPLTVQ DMCVMAIADYWGQGTQTVSS
< > 059A.IL6-Rcl2(t) , SEQ ID NO: 448 ;PRT;->
EVQLVESGGGLVQAGGSLRLSCAASGRTFSNIAMGWIREAPGKEREFAALT WSGGSTYYADSVKGRFTISRDSAKNTVYLQMNLKPEDTAVYYCVADEEIHLSISI ADFWGQGTQTVSS
< > 059A.IL6-Rcl1(t) , SEQ ID NO: 449 ;PRT;->
EVQLVESGGGLVQAGGSLRLSCAASGLTDDFAIGWFRQAPGKEPEGVSCISS SDGSTYYADSVKGRFTISSLNAKNTVYLQMNSLKPEDTAVYFCTALFDRCGSTWYY GMDYWGKGTLTVSS
Bispecific polypeptides against the IL6 receptor
< >063.IL-6RPMP35C10cl8 , SEQ ID NO: 450 ;PRT;->
EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYDMGWYRQAPGKEREFAVI

HWSSGSTYYADPVKGRFTISRDNAKNTVYLQMNSLKPEDTAIYYCNAFLPGPEGFH
DYWGQGTQTVSSGGGGGGSEVQLVESGGGLVQPGNSRLSCAASGFTFRSGM
SWVRQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDT
AVYYCTIGGSLSRSSQGTQTVSS

< >063.IL-6RPMP34G9cl2 , SEQ ID NO: 451 ;PRT;->
EVQLVESGGGLVQAGGSLRLSCAASGRTSSSYDMTWYRQVPGKEREFAVIS
WSGGSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAIYYCNAYTGGDDYW
GQGTQTVSSGGGGGGSEVQLVESGGGLVQPGNSRLSCAASGFTFRSGMSWV
RQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTAVY
YCTIGGSLSRSSQGTQTVSS

< >063.IL-6RPMP34G3cl4-5 , SEQ ID NO: 452 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAASGFTLDYYAIGWFRQAPGKERERVSCISS
SDGSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAAYYCATDRSVYYCSGGA
PEEYYWGQGTQTVSSGGGGGGSEVQLVESGGGLVQPGNSRLSCAASGFTFRS
FGMSWVRQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKP
EDTAVYYCTIGGSLSRSSQGTQTVSS

< >063.IL-6RPMP34A12cl2 , SEQ ID NO: 453 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCVASGFSLDYYVIGWFRQAPGKEREGVSCISS
SDGSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAADLLRTPEFCVD
SAPYDYWGQGTQTVSSGGGGGGSEVQLVESGGGLVQPGNSRLSCAASGFTFR
SFGMSWVRQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKP
PEDTAVYYCTIGGSLSRSSQGTQTVSS

< >063.IL-6RPMP31A4cl4 , SEQ ID NO: 454 ;PRT;->
EVQLVESGGGLVQAGGSLRLSCAASGSIFKVNAMEGWYRQAPGKQRELVAGII
SGGSTNYADSVKGRLTISRDNAKNTVYLQMNSLKPEDTAVYYCSFTTNSDYDLGR
DYWGQGTQTVSSGGGGGGSEVQLVESGGGLVQPGNSRLSCAASGFTFRSGM
SWVRQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDT
AVYYCTIGGSLSRSSQGTQTVSS

< >063.IL-6R.PMP40H5cl4 , SEQ ID NO: 455 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAASGFSLDYYAIGWFRQAPGKEREGVSCM
DSSSGTTSTYYSDSVKGRFTISRDDAKNTVYLQMNSLKPEDTAVYYCAADGHLNWG
QRVPCSQISWRGWNDYWGQGTQTVSSGGGGGGSEVQLVESGGGLVQPGNSL

RLSCAASGFTFRSGMSWVRQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISRDNA
KTTLYLQMNSLKPEDTAVYYCTIGGSLSRSSQGTQTVSS

< >063.IL-6R.PMP35F4cl3 , SEQ ID NO: 456 ;PRT;->
EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYDMGWYRQAPGKEREVVAIIT
WNSSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAIYYCNAQYGLGYAEDY
WGQGTQTVSSGGGGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFRSGMSW
VRQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTAV
YYCTIGGSLSRSSQGTQTVSS

< >063.IL-6R.PMP35E11cl1 , SEQ ID NO: 457 ;PRT;->
EVQLVESGGGLVQAGGSLRLSCAASGFTFDDYAIGWFRQAPGKEHEGVSCIS
SSDGSTYYADSVKGRFTISSDNAKNTVYLQMNSLKPEDTAVYYCAAERDVPARSLC
GSYYWYDYZRGQGTQTVSSGGGGGGSEVQLVESGGGLVQPGNSLRLSCAASGF
TFRSGMSWVRQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQM
SLKPEDTAVYYCTIGGSLSRSSQGTQTVSS

< >063.IL-6R.PMP34E10cl3 , SEQ ID NO: 458 ;PRT;->
EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYAMGWGRQAPGKEREVATIS
WSGGSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCAADLAEFKYSD
YADYWQGTQTVSSGGGGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFRSF
GMSWVRQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKP
EDTAVYYCTIGGSLSRSSQGTQTVSS

< >063.IL-6R.PMP34C11cl3 , SEQ ID NO: 459 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAAAGFTLDYSAIGWFRQAPGKEREMFSCIS
GSDGSTWYADSVAGRFTISFDNAKNTVYLQMNSLKPEDTGLYICAVTGGVRGPCAY
EYEYWGQGTQTVSSGGGGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFRSF
GMSWVRQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKP
EDTAVYYCTIGGSLSRSSQGTQTVSS

< >063.IL-6R.PMP33A3cl1 , SEQ ID NO: 460 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAASGFTLDYGAIGWFRQAPGKEREGVSCISS
STGSTYYADSVKGRFTISRDNGKNTVYLQMNSLKPEDTAVYYCAADKMWSPCLVA
ANEEALFEYDYWGQGTQTVSSGGGGGGSEVQLVESGGGLVQPGNSLRLSCAAS
GFTFRSGMSWVRQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQ
MNSLKPEDTAVYYCTIGGSLSRSSQGTQTVSS

< >063.IL-6R.PMP32E2cl4 , SEQ ID NO: 461 ;PRT;->
EVQLVESGGGLVQAGGSLRLSCAASGNIFDDNTMGWTWNRQPPGKQRELVA
IIATDGSTNYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCNLFSLRLGRDY
WGQGTQVTVSSGGGGGGSEVQLVESGGGLVQPGNSRLSCAASGFTFRSGMSW
VRQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTAV
YYCTIGGSLSRSSQGTQTVSS

< >063.IL-6R.PMP32E10cl1 , SEQ ID NO: 462 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAASGFTFGSYDMSWVRQAPGKGPEWVSAI
NSGGGSTYYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCATDWRYSDYD
LPLPPPGDYWGQGTQVTVSSGGGGGGSEVQLVESGGGLVQPGNSRLSCAASGF
TFRSGMSWVRQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQM
SLKPEDTAVYYCTIGGSLSRSSQGTQTVSS

< >063.IL-6R.PMP32C9cl2 , SEQ ID NO: 463 ;PRT;->
EVQLVESGGGLVQAGGSLRLSCAASGFTFDDYDIGWFRQAPGKEREGVSGIS
SSDGNTYYADSVKGRFTISSDNAKNTVYLQMNSLKPEDTAVYYCAAEPDSSWYLD
GSPEFFKYWGQGTQVTVSSGGGGGGSEVQLVESGGGLVQPGNSRLSCAASGFT
FRSGMSWVRQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNS
LKPEDTAVYYCTIGGSLSRSSQGTQTVSS

< >063.IL-6R.PMP30C11cl2 , SEQ ID NO: 464 ;PRT;->
EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYDMGWYRQAPGKEREFVAI
SRSGSSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAIYYCKAEVVAGDYDY
WGQGTQVTVSSGGGGGGSEVQLVESGGGLVQPGNSRLSCAASGFTFRSGMSW
VRQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTAV
YYCTIGGSLSRSSQGTQTVSS

< >063.IL-6R.PMP28G3cl3 , SEQ ID NO: 465 ;PRT;->
EVQLVESGGGLVQAGGSLRLSCTASGNIFSTETMGWYRQPPGKQRDVVATIT
HGGTTNYADSVKGRFTISRDNRKNTVYLQMNSLKPEDTGVYYCNARSSWYSPEYW
GQGTQVTVSSGGGGGGSEVQLVESGGGLVQPGNSRLSCAASGFTFRSGMSWV
RQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTAVY
YCTIGGSLSRSSQGTQTVSS

< >063.IL-6R.PMP28E11cl4 , SEQ ID NO: 466 ;PRT;->
EVQLVESGGGFVQAGGSLRLSCIASGDNFSINRMGWYRQALGKQRELVAIIT

NHGSTNYADAVKGRFTISRDYAKNTVYLQMNGLPDDTAVYYCNAYISEVGTWRD
DYWGQQIQTVSSGGGGGGSEVQLVESGGGLVQPGNRLSCAASGFTFRSGM
SWVRQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDT
AVYYCTIGGSLSRSSQGTQTVVSS

< > 059B.IL6-R.c15.7(t), SEQ ID NO: 467 ;PRT;->
EVQLVESGGGLVQAGGSLRLSCAASGRTFSGADAGWNRQTPGKEREVAAI
NWSGNSTYYADSVKGRFTVSRDNAKNTVYLQMNSLKPEDTAVYYCHAFRDDYYSE
GKGTLTVSSGGGGGGSEVQLVESGGGLVQPGNRLSCAASGFTFRSGMSWV
RQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTAVY
YCTIGGSLSRSSQGTQTVVSS

< > 059A.IL6-Rc14(t) , SEQ ID NO: 468 ;PRT;->
EVQLVESGGGLVQAGGSLRLSCAASGRTLSSYDMGWYRQGPGKEREVAAI
SWSGGGTDYVDSVKGRFTISRDTAKNTMYLQMNSLKPEDTAIYYCNALGTTDSDYE
GELYWGQQGTQTVTSSGGGGGGSEVQLVESGGGLVQPGNRLSCAASGFTFSSF
GMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRP
EDTAVYYCTIGGSLSRSSQGTQTVVSS

< > 059A.IL6-Rc13(t) , SEQ ID NO: 469 ;PRT;->
EVQLVESGGGLVQPGGSLRLSCAASGFTLDSYAIGWFRQAPGKEPEGVSCIST
SDGSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCTADGGPHAPLTQ
DMCVMAIADYWGQGTQTVTSSGGGGGGSEVQLVESGGGLVQPGNRLSCAAS
GFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQ
MNSLRPEDTAVYYCTIGGSLSRSSQGTQTVVSS

< > 059A.IL6-Rc12(t) , SEQ ID NO: 470 ;PRT;->
EVQLVESGGGLVQAGGSLRLSCAASGRTFSNIAMGWIREAPGKEREVAAALT
WSGGSTYYADSVKGRFTISRDSAKNTVYLQMNLKPEDTAVYYCVADEEHLIVSISI
ADFWGQGTQTVTSSGGGGGGSEVQLVESGGGLVQPGNRLSCAASGFTFSSFG
MSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRP
DTAVYYCTIGGSLSRSSQGTQTVVSS

< > 059A.IL6-Rc11(t) , SEQ ID NO: 471 ;PRT;->
EVQLVESGGGLVQAGGSLRLSCAASGLTDDFAIGWFRQAPGKEPEGVSCISS
SDGSTYYADSVKGRFTIISDNAKNTVYLQMNSLKPEDTAVYFCTALFDRCGSTWYY
GMDYWGKGTLTVTSSGGGGGGSEVQLVESGGGLVQPGNRLSCAASGFTFSSF

GMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRP
EDTAVYYCTIGGSLSRSSQGTQTVSS

Leader sequences and N-terminal sequences

< > llama leader 1 , SEQ ID NO: 472 ;PRT;->

VKKLLFAIPLVVVFYAAQPAMA

< > llama leader 2 , SEQ ID NO: 473 ;PRT;->

VKKLLFAIPLVVVFYAAQPAIA

< > llama leader 3 , SEQ ID NO: 474 ;PRT;-> FELASVAQA

< > leader sequence , SEQ ID NO: 475 ;PRT;->

MKKTAIAIAVALAGLATVAQA

< > leader sequence , SEQ ID NO: 476 ;PRT;->

MKKTAIAFAVALAGLATVAQA

< > N-terminal sequence , SEQ ID NO: 477 ;PRT;->

AAAEQKLISEEDLNGAAHHHHH**

Trivalent bispecific polypeptides directed against the IL-6 receptor and human serum albumin

< IL6R43 , SEQ ID NO: 478 ;PRT;-> "

EVQLVESGGGLVQAGGSLRLSCAASGSIFKVNAMGWYRQAPGKQRELVAGIISGGSTNYADSVKGRLTISRDNAKNTVYLQMNSLKPEDTAVYYCSFVTTNSDYDLGRDYWGQGTQTVSSGGGGSGGSAVQLVESGGGLVQPGNRLSCAASGFTFRSFGMSWVRQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTAVYYCTIGGSLSRSSQGTQTVSSGGGGSGGSEVQLVESGGGLVQAGGSLRLSCAASGSIFKVNAMGWYRQAPGKQRELVAGIISGGSTNYADSVKGRLTISRDNAKNTVYLQMNSLKPEDTAVYYCSFVTTNSDYDLGRDYWGQGTQTVSS

< IL6R44 , SEQ ID NO: 479 ;PRT;-> "

EVQLVESGGGLVQAGGSLRLSCAASGFTFDDYDIGWFRQAPGKEREGVSGISSSDGNTYYADSVKGRFTIISDNAKNTVYLQMNSLKPEDTAVYYCAAEPDSSWYLDGSPEFFKYWGQGTQTVSSGGGGSGGSAVQLVESGGGLVQPGNRLSCAASGFT

FRSGMSWVRQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNS
LKPEDTAVYYCTIGGSLRSSQGTQTVSSGGGGGGSEVQLVESGGGLVQAGGSL
RLSCAASGFTFDDYDIGWFRQAPGKEREVGSISSDGNTYYADSVKGRFTISSDNA
KNTVYLQMNSLKPEDTAVYYCAAEPPDSSWYLDGSPEFFKYWGQGTQTVSS

< IL6R49 , SEQ ID NO: 480 ";PRT;->"
EVQLVESGGGLVQAGGSLRLSCAASGRTSSYDMTWYRQVPGKEREFAVIS
WSGGSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAIYYCNAYTGGGDDYW
GQGTQTVSSGGGGGGSAVQLVESGGGLVQPGNSLRLSCAASGFTFRSGMSWV
RQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTAVY
YCTIGGSLRSSQGTQTVSSGGGGGGSEVQLVESGGGLVQAGGSLRLSCAASGR
TSSSYDMTWYRQVPGKEREFAVISWSGGSTYYADSVKGRFTISRDNAKNTVYLQM
NSLKPEDTAIYYCNAYTGGGDDYWQGTQTVSS

< IL6R53 , SEQ ID NO: 481 ";PRT;->"
EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGRATEWVSAI
SWNGNNTYYTESMKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCVKGSTAIVGV
PPTYPDEYDYWGQGTQTVSSGGGGGGSAVQLVESGGGLVQPGNSLRLSCAASG
FTFRSGMSWVRQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQM
NSLKPEDTAVYYCTIGGSLRSSQGTQTVSSGGGGGGSEVQLVESGGGLVQPGG
SLRLSCAASGFTFDDYGMSWVRQAPGRATEWVSAISWNGNNTYYTESMKGRFTISR
DNAKNTVYLQMNSLKPEDTAVYYCVKGSTAIVGVPPYPDEYDYWGQGTQTVSS

< IL6R54 , SEQ ID NO: 482 ";PRT;->"
EVQLVESGGGLVQPGGSLRLSCAASGFSLDYYAIGWFRQAPGKEREVGSCM
DSSSGTTSTYYSDSVKGRFTISRDDAKNTVYLQMNSLKPEDTAVYYCAADGHLNWG
QRYVPCSQISWRGWNDYWGQGTQTVSSGGGGGGSAVQLVESGGGLVQPGNSL
RLSCAASGFTFRSGMSWVRQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISRDNA
KTTLYLQMNSLKPEDTAVYYCTIGGSLRSSQGTQTVSSGGGGGGSEVQLVESG
GLVQPGGSLRLSCAASGFSLDYYAIGWFRQAPGKEREVGSCMDSSGTTSTYYSDS
VKGRFTISRDDAKNTVYLQMNSLKPEDTAVYYCAADGHLNWGQRYVPCSQISWRG
WNDYWGQGTQTVSS

< , SEQ ID NO: 483 ";PRT;->"
AVQLVESGGGLVQPGNSLRLSCAASGFTFRSGMSWVRQAPGKEPEWVSSIS
GSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTAVYYCTIGGSLRSSQGT

QVTVSSGGGSGGGSEVQLVESGGLVQAGGSLRLSCAASGSIFKVNAMGWYRQA
PGKQRELVAGIISGGSTNYADSVKGRLTISRDNAKNTVYLMQNSLKPEDTAVYYCSF
VTTNSDYDLGRDYWGQGTQVTVSSGGGSGGGSEVQLVESGGLVQAGGSLRLSC
AASGSIFKVNAMGWYRQAPGKQRELVAGIISGGSTNYADSVKGRLTISRDNAKNTV
YLMQNSLKPEDTAVYYCSFVTTNSDYDLGRDYWGQGTQVTVSS

< , SEQ ID NO: 484 ";PRT;-> "

AVQLVESGGLVQPGNSLRLSCAASGFTFRSGMSWVRQAPGKEPEWVSSIS
GSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTAVYYCTIGGSLSRSSQGT
QVTVSSGGGSGGGSEVQLVESGGLVQAGGSLRLSCAASGFTFDDYDIGWFRQAP
GKEREGVSGISSLGDGNTYYADSVKGRFTISSDNAKNTVYLMQNSLKPEDTAVYYCA
AEPPDSSWYLDGSPEFFKYWGQGTQVTVSSGGGSGGGSEVQLVESGGLVQAGGS
LRLSCAASGFTFDDYDIGWFRQAPGKEREGVSGISSLGDGNTYYADSVKGRFTISSDNA
KNTVYLMQNSLKPEDTAVYYCAAEPPDSSWYLDGSPEFFKYWGQGTQVTVSS

< , SEQ ID NO: 485 ";PRT;-> "

AVQLVESGGLVQPGNSLRLSCAASGFTFRSGMSWVRQAPGKEPEWVSSIS
GSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTAVYYCTIGGSLSRSSQGT
QVTVSSGGGSGGGSEVQLVESGGLVQAGGSLRLSCAASGRTSSYDMTWYRQV
PGKEREFVAVISWSGGSTYYADSVKGRFTISRDNAKNTVYLMQNSLKPEDTAIYYCN
AYTGGDDYWGQGTQVTVSSGGGSGGGSEVQLVESGGLVQAGGSLRLSCAASG
RTSSYDMTWYRQVPGKEREFVAVISWSGGSTYYADSVKGRFTISRDNAKNTVYLM
MNSLKPEDTAIYYCNAYTGGDDYWGQGTQVTVSS

< , SEQ ID NO: 486 ";PRT;-> "

AVQLVESGGLVQPGNSLRLSCAASGFTFRSGMSWVRQAPGKEPEWVSSIS
GSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTAVYYCTIGGSLSRSSQGT
QVTVSSGGGSGGGSEVQLVESGGLVQPGGSLRLSCAASGFTFDDYGMWSVRQA
PGRATEWVSAISWNGNNTYYTESMKGRFTISRDNAKNTVYLMQNSLKPEDTAVYY
CVKGSTAIVGPPTYPDEYDYWGQGTQVTVSSGGGSGGGSEVQLVESGGLVQPG
GSLRLSCAASGFTFDDYGMWSVRQAPGRATEWVSAISWNGNNTYYTESMKGRFTIS
RDNAKNTVYLMQNSLKPEDTAVYYCVKGSTAIVGPPTYPDEYDYWGQGTQVTVS
S

< , SEQ ID NO: 487 ";PRT;-> "

AVQLVESGGLVQPGNSLRLSCAASGFTFRSGMSWVRQAPGKEPEWVSSIS

GSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTAVYYCTIGGSLSRSSQGT
QVTVSSGGGGSGGGSEVQLVESGGLVQPGGSLRLSCAASGFSLDYYAIGWFRQAP
GKEREGVSCMDSSGTTSTYYSDSVKGRFTISRDDAKNTVYLQMNSLKPEDTAVYY
CAADGHLNWGQRYVPCSQISWRGWNDYWGQGTQVTVSSGGGGSGGGSEVQLVES
GGGLVQPGGSLRLSCAASGFSLDYYAIGWFRQAPGKEREGVSCMDSSGTTSTYYSD
SVKGRFTISRDDAKNTVYLQMNSLKPEDTAVYYCAADGHLNWGQRYVPCSQISWR
GWNDYWGQGTQVTVSS

< , SEQ ID NO: 488 ";PRT;-> "

EVQLVESGGLVQAGGSLRLSCAASGSIFKVNAMEWYRQAPGKQRELVAGII
SGGSTNYADSVKGRLTISRDNAKNTVYLQMNSLKPEDTAVYYCSFVTTNSDYDLGR
DYWGQGTQVTVSSGGGGSGGGSEVQLVESGGLVQAGGSLRLSCAASGSIFKVNA
MGWYRQAPGKQRELVAGIISGGSTNYADSVKGRLTISRDNAKNTVYLQMNSLKPED
TAVYYCSFVTTNSDYDLGRDYWGQGTQVTVSSGGGGSGGGSAVQLVESGGLVQP
GNSLRLSCAASGFTFRSGMSWVRQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISR
DNAKTTLYLQMNSLKPEDTAVYYCTIGGSLSRSSQGTQVTVSS

< , SEQ ID NO: 489 ";PRT;-> "

EVQLVESGGLVQAGGSLRLSCAASGFTFDDYDIGWFRQAPGKEREGVSGIS
SSDGNTYYADSVKGRFTIISDNAKNTVYLQMNSLKPEDTAVYYCAAEPPDSSWYLD
GSPEFFKYWGQGTQVTVSSGGGGSGGGSEVQLVESGGLVQAGGSLRLSCAASGFT
FDDYDIGWFRQAPGKEREGVSGIISSDGNTYYADSVKGRFTIISDNAKNTVYLQMNS
LKPEDTAVYYCAAEPPDSSWYLDGSPEFFKYWGQGTQVTVSSGGGGSGGGSAVQL
VESGGGLVQPGNSLRLSCAASGFTFRSGMSWVRQAPGKEPEWVSSISGSGSDTLYA
DSVKGRFTISRDNAKTTLYLQMNSLKPEDTAVYYCTIGGSLSRSSQGTQVTVSS

< , SEQ ID NO: 490 ";PRT;-> "

EVQLVESGGLVQAGGSLRLSCAASGRTSSYDMTWYRQVPGKEREFVAVIS
WSGGSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAIYYCAYTGGDDYW
GQGTQVTVSSGGGGSGGGSEVQLVESGGLVQAGGSLRLSCAASGRTSSYDMTW
YRQVPGKEREFVAVISWSGGSTYYADSVKGRFTISRDNAKNTVYLQMNSLKPEDTAI
YYCAYTGGDDYWGQGTQVTVSSGGGGSGGGSAVQLVESGGLVQPGNSLRLSC
AASGFTFRSGMSWVRQAPGKEPEWVSSISGSGSDTLYADSVKGRFTISRDNAKTT
YLQMNSLKPEDTAVYYCTIGGSLSRSSQGTQVTVSS

< , SEQ ID NO: 491 ";PRT;->"

EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGRATEWVSAI
SWNGNNNTYYTESMKGRFTISRDNAKNTVYLQMNSLKPEDTAVYYCVKGSTAIVGV
PPTYPDEYDYWGQGTQTVSSGGGGSGGGSEVQLVESGGGLVQPGGSLRLSCAASG
FTFDDYGMSWVRQAPGRATEWVSAISWNGNNNTYYTESMKGRFTISRDNAKNTVYL
QMNSLKPEDTAVYYCVKGSTAIVGPPTYPDEYDYWGQGTQTVSSGGGGSGGG
AVQLVESGGGLVQPGNSLRLSCAASGFTFRSGMSWVRQAPGKEPEWVSSISGSGSD
TLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTAVYYCTIGGSLSRSSQGTQTVS
S

< , SEQ ID NO: 492 ";PRT;->"

EVQLVESGGGLVQPGGSLRLSCAASGFSLDYYAIGWFRQAPGKEREJVSCM
DSSSGTTSTYYSDSVKGRFTISRDDAKNTVYLQMNSLKPEDTAVYYCAADGHLNWG
QRYVPCSQISWRGWNDYWGQGTQTVSSGGGGSGGGSEVQLVESGGGLVQPGGSL
RLSCAASGFSLDYYAIGWFRQAPGKEREJVSCMDSSGTTSTYYSDSVKGRFTISRD
DAKNTVYLQMNSLKPEDTAVYYCAADGHLNWGQRYVPCSQISWRGWNDYWGQG
TQTVSSGGGGSGGSAVQLVESGGGLVQPGNSLRLSCAASGFTFRSGMSWVRQA
PGKEPEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLKPEDTAVYYCTI
GGSLSRSSQGTQTVSS

Humanized trivalent bispecific polypeptides directed against the IL-6 receptor and human serum albumin

< , SEQ ID NO: 493 ";PRT;->"

EVQLVESGGGLVQPGGSLRLSCAASGSIFKVNAMEWYRQAPGKGRELVAGII
SGGSTNYADSVKGRLTISRDNAKNTVYLQMNSLRPEDTAVYYCSFVTTNSDYDLGR
DYWGQGTLTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTSSFGM
SWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDT
AVYYCTIGGSLSRSSQGTLTVSSGGGGSGGGSEVQLVESGGGLVQPGGSLRLSCAA
SGSIFKVNAMEWYRQAPGKGRELVAGIISGGSTNYADSVKGRLTISRDNAKNTVYL
QMNSLRPEDTAVYYCSFVTTNSDYDLGRDYWGQGTLTVSS

< , SEQ ID NO: 494 ";PRT;->"

EVQLVESGGGLVQPGGSLRLSCAASGSIFKVNAMEWYRQAPGKGRELVAGII
SGGSTNYADSVKGRFTISRDNAKNTVYLQMNSLRPEDTAVYYCSFVTTNSDYDLGR
DYWGQGTLTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTSSFGM

SWVRQAPGKGLEWVSSISGSQDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDT
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< , SEQ ID NO: 495 ";PRT;-> "

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< , SEQ ID NO: 496 ";PRT;-> "

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< IL6R67 , SEQ ID NO: 497 ";PRT;-> "

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SGSIFKVNAMEWYRQAPGKGRELVAGIISGGSTNYADSVKGRFTISRDNAKNTLYLQ
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< , SEQ ID NO: 498 ";PRT;-> "

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SSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSL
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LSCAASGFTFDDYDIGWFRQAPGKGREGVSGISSLGNTYYADSVKGRFTISSLNAK
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< , SEQ ID NO: 499 " ;PRT; -> "

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SFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSL
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< , SEQ ID NO: 500 ";PRT;-> "

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< , SEQ ID NO: 501 ";PRT;->"

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RPEDTAVYYCTIGGSLRSSQGTLTVSSGGGGSGGSEVQLVESGGGLVQPGGSLR
LSCAASGFTFDDYDIGWFRQAPGKGREGVSGISSLGDNTYYADSVKGRFTISSLNAK
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< IL6R203 , SEQ ID NO: 502 ";PRT;->"

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< , SEQ ID NO: 503 ";PRT;-> "

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< , SEQ ID NO: 504 ";PRT;-> "

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< , SEQ ID NO: 505 ";PRT;-> "

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< , SEQ ID NO: 506 ";PRT;-> "

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< , SEQ ID NO: 507 ";PRT;-> "

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SLRPEDTAVYYCTIGGSLRSSQGTLTVSSGGGSGGGSEVQLVESGGGLVQPGGS
LRLSCAASGFTFDDYGMSWVRQAPGRGLEWVSAISWNGNNTYYTESMKGRFTISRD
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< , SEQ ID NO: 508 ";PRT;-> "

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PTYPDEYDYWGQGTLTVSSGGGSGGGSEVQLVESGGGLVQPGNSRLSCAASGF
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SLRPEDTAVYYCTIGGSLRSSQGTLTVSSGGGSGGGSEVQLVESGGGLVQPGGS
LRLSCAASGFTFDDYGMSWVRQAPGRALEWVSAISWNGNNTYYTESMKGRFTISRD
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< , SEQ ID NO: 509 ";PRT;-> "

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SLRPEDTAVYYCTIGGSLRSSQGTLTVSSGGGSGGGSEVQLVESGGGLVQPGGS
LRLSCAASGFTFDDYGMSWVRQAPGKATEWVSAISWNGNNTYYTESMKGRFTISRD
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< IL6R92 , SEQ ID NO: 510 ";PRT;-> "

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PTYPDEYDYWGQGTLTVSSGGGSGGGSEVQLVESGGGLVQPGNSRLSCAASGF

TFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMN
SLRPEDTAVYYCTIGGSLRSSQGTLTVSSGGGSGGGSEVQLVESGGGLVQPGGS
LRLSCAASGFTFDDYGMSWVRQAPGKGTEWVSAISWNGNNTYYTESMKGRFTISRD
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< , SEQ ID NO: 511 ";PRT;-> "

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< , SEQ ID NO: 512 ";PRT;-> "

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< , SEQ ID NO: 513 ";PRT;-> "

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< , SEQ ID NO: 514 ";PRT;-> "

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< , SEQ ID NO: 515 ";PRT;-> "

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< , SEQ ID NO: 516 ";PRT;-> "

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< , SEQ ID NO: 517 ";PRT;-> "

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< , SEQ ID NO: 518 ";PRT;-> "

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< , SEQ ID NO: 519 ";PRT;-> "

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< , SEQ ID NO: 520 ";PRT;-> "

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< , SEQ ID NO: 521 ";PRT;-> "

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< , SEQ ID NO: 522 ";PRT;-> "

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< , SEQ ID NO: 523 ";PRT;-> "

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< , SEQ ID NO: 524 ";PRT;-> "

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< , SEQ ID NO: 525 ";PRT;-> "

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MNSLRPEDTAVYYCVKGSTAIVGPPTYPDEYDYWGQGTLTVSSGGGGSGGGSEV
QLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLY
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< , SEQ ID NO: 526 ";PRT;-> "

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QLVESGGGLVQPGNSLRLSCAASGFTSSFGMSWVRQAPGKGLEWVSSISGSGSDL
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< , SEQ ID NO: 527 ";PRT;-> "

EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMWSVRQAPGRATEWVSAI
SWNGNNTYYTESMKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCVKGSTAIVGP
PTYPDEYDYWGQGTLTVSSGGGGSGGSEVQLVESGGGLVQPGGSLRLSCAASGF
TFDDYGMWSVRQAPGRATEWVSAISWNGNNTYYTESMKGRFTISRDNAKNTLYLQ
MNSLRPEDTAVYYCVKGSTAIVGPPTYPDEYDYWGQGTLTVSSGGGGSGGSEV
QLVESGGGLVQPGNSLRLSCAASGFTSSFGMSWVRQAPGKGLEWVSSISGSGSDL
YADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLTVSS

< , SEQ ID NO: 528 ";PRT;-> "

EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMWSVRQAPGKGLEWVSAI
SWNGNNTYYTESMKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCVKGSTAIVGP
PTYPDEYDYWGQGTLTVSSGGGGSGGSEVQLVESGGGLVQPGGSLRLSCAASGF
TFDDYGMWSVRQAPGKGLEWVSAISWNGNNTYYTESMKGRFTISRDNAKNTLYLQ
MNSLRPEDTAVYYCVKGSTAIVGPPTYPDEYDYWGQGTLTVSSGGGGSGGSEV
QLVESGGGLVQPGNSLRLSCAASGFTSSFGMSWVRQAPGKGLEWVSSISGSGSDL
YADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLTVSS

< , SEQ ID NO: 529 ";PRT;-> "

EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMWSVRQAPGRGLEWVSAI
SWNGNNTYYTESMKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCVKGSTAIVGP
PTYPDEYDYWGQGTLTVSSGGGGSGGSEVQLVESGGGLVQPGGSLRLSCAASGF
TFDDYGMWSVRQAPGRGLEWVSAISWNGNNTYYTESMKGRFTISRDNAKNTLYLQ
MNSLRPEDTAVYYCVKGSTAIVGPPTYPDEYDYWGQGTLTVSSGGGGSGGSEV
QLVESGGGLVQPGNSLRLSCAASGFTSSFGMSWVRQAPGKGLEWVSSISGSGSDL
YADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLTVSS

< , SEQ ID NO: 530 ";PRT;-> "

EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMWSVRQAPGRALEWVSAI
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PTYPDEYDYWGQGTLTVSSGGGGSGGSEVQLVESGGGLVQPGGSLRLSCAASGF

TFDDYGMWSVRQAPGRALEWVSAISWNGNNTYYTESMKGRFTISRDNAKNTLYLQ
MNSLRPEDTAVYYCVKGSTAIVGPPTYPDEYDYWGQGTLTVSSGGGGSGGSEV
QLVESGGGLVQPGNSLRLSCAASGFTSSFGMSWVRQAPGKGLEWVSSISGSGSDL
YADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLTVSS

< , SEQ ID NO: 531 ";PRT;-> "

EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMWSVRQAPGKATEWVSAI
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PTYPDEYDYWGQGTLTVSSGGGGSGGSEVQLVESGGGLVQPGGSLRLSCAASGF
TFDDYGMWSVRQAPGKATEWVSAISWNGNNTYYTESMKGRFTISRDNAKNTLYLQ
MNSLRPEDTAVYYCVKGSTAIVGPPTYPDEYDYWGQGTLTVSSGGGGSGGSEV
QLVESGGGLVQPGNSLRLSCAASGFTSSFGMSWVRQAPGKGLEWVSSISGSGSDL
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< , SEQ ID NO: 532 ";PRT;-> "

EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMWSVRQAPGKGTEWVSAI
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PTYPDEYDYWGQGTLTVSSGGGGSGGSEVQLVESGGGLVQPGGSLRLSCAASGF
TFDDYGMWSVRQAPGKGTEWVSAISWNGNNTYYTESMKGRFTISRDNAKNTLYLQ
MNSLRPEDTAVYYCVKGSTAIVGPPTYPDEYDYWGQGTLTVSSGGGGSGGSEV
QLVESGGGLVQPGNSLRLSCAASGFTSSFGMSWVRQAPGKGLEWVSSISGSGSDL
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< , SEQ ID NO: 533 ";PRT;-> "

EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMWSVRQAPGRGTEWVSAI
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PTYPDEYDYWGQGTLTVSSGGGGSGGSEVQLVESGGGLVQPGGSLRLSCAASGF
TFDDYGMWSVRQAPGRGTEWVSAISWNGNNTYYTESMKGRFTISRDNAKNTLYLQ
MNSLRPEDTAVYYCVKGSTAIVGPPTYPDEYDYWGQGTLTVSSGGGGSGGSEV
QLVESGGGLVQPGNSLRLSCAASGFTSSFGMSWVRQAPGKGLEWVSSISGSGSDL
YADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLTVSS

< , SEQ ID NO: 534 ";PRT;-> "

EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMWSVRQAPGKALEWVSAI
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PTYPDEYDYWGQGTLTVSSGGGGSGGSEVQLVESGGGLVQPGGSLRLSCAASGF

TFDDYGMSWVRQAPGKALEWVSAISWNGNNTYYTESMKGRFTISRDNAKNTLYLQ
MNSLRPEDTAVYYCVKGSTAIVGPPTYPDEYDYWGQGTLTVSSGGGGSGGSEV
QLVESGGGLVQPGNSLRLSCAASGFTSSFGMSWVRQAPGKGLEWVSSISGSGSDL
YADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLTVSS

< , SEQ ID NO: 535 ";PRT;-> "

EVQLVESGGGLVQPGNSLRLSCAASGFTFSDYGMWSWVRQAPGKGTEWVSAI
SWNGNNTYYTESMKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCVKGSTAIVGP
PTYPDEYDYWGQGTLTVSSGGGGSGGSEVQLVESGGGLVQPGNSLRLSCAASGF
TFSDYGMWSWVRQAPGKGTEWVSAISWNGNNTYYTESMKGRFTISRDNAKNTLYLQ
MNSLRPEDTAVYYCVKGSTAIVGPPTYPDEYDYWGQGTLTVSSGGGGSGGSEV
QLVESGGGLVQPGNSLRLSCAASGFTSSFGMSWVRQAPGKGLEWVSSISGSGSDL
YADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLTVSS

< , SEQ ID NO: 536 ";PRT;-> "

EVQLVESGGGLVQPGNSLRLSCAASGFTFSDYGMWSWVRQAPGKGTEWVSAI
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PTYPDEYDYWGQGTLTVSSGGGGSGGSEVQLVESGGGLVQPGNSLRLSCAASGF
TFSDYGMWSWVRQAPGKGTEWVSAISWNGNNTYYTESMKGRFTISRDNAKNTLYLQ
MNSLRPEDTAVYYCAKGSTAIVGPPTYPDEYDYWGQGTLTVSSGGGGSGGSEV
QLVESGGGLVQPGNSLRLSCAASGFTSSFGMSWVRQAPGKGLEWVSSISGSGSDL
YADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLTVSS

< , SEQ ID NO: 537 ";PRT;-> "

EVQLVESGGGLVQPGNSLRLSCAASGFTSSFGMSWVRQAPGKGLEWVSSIS
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LTVSSGGGGSGGSEVQLVESGGGLVQPGNSLRLSCAASGSIFKVNAMEWYRQAP
GKGRELVAGIISGGSTNYADSVKGRLTISRDNAKNTVYLVQMNSLRPEDTAVYYCSFV
TTNSDYDLGRDYWGQGTLTVSSGGGGSGGSEVQLVESGGGLVQPGNSLRLSCA
ASGSIFKVNAMEWYRQAPGKGRELVAGIISGGSTNYADSVKGRLTISRDNAKNTVY
LQMNSLRPEDTAVYYCSFVTTNSDYDLGRDYWGQGTLTVSS

< , SEQ ID NO: 538 ";PRT;-> "

EVQLVESGGGLVQPGNSLRLSCAASGFTSSFGMSWVRQAPGKGLEWVSSIS
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GKGRELVAGIISSGGSTNYADSVKGRFTISRDNAKNTVYLQMNSLRPEDTAVYYCSFV
TTNSDYDLGRDYWGQGTLTVSSGGGGGGSEVQLVESGGGLVQPGGSLRLSCA
ASGSIFKVNAMEWYRQAPGKGRELVAGIISSGGSTNYADSVKGRFTISRDNAKNTVY
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< , SEQ ID NO: 539 ";PRT;->"
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GKGRELVAGIISSGGSTNYADSVKGRLTISRDNAKNTLYLQMNSLRPEDTAVYYCSFV
TTNSDYDLGRDYWGQGTLTVSSGGGGGGSEVQLVESGGGLVQPGGSLRLSCA
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< , SEQ ID NO: 540 ";PRT;->"
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GKGRELVAGIISSGGSTNYADSVKGRLTISRDNAKNTVYLQMNSLRPEDTAVYYCAF
VTTNSDYDLGRDYWGQGTLTVSSGGGGGGSEVQLVESGGGLVQPGGSLRLSC
AASGSIFKVNAMEWYRQAPGKGRELVAGIISSGGSTNYADSVKGRLTISRDNAKNTV
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< , SEQ ID NO: 541 ";PRT;->"
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GSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGT
LTVSSGGGGGGSEVQLVESGGGLVQPGGSLRLSCAASGSIFKVNAMEWYRQAP
GKGRELVAGIISSGGSTNYADSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAFV
TTNSDYDLGRDYWGQGTLTVSSGGGGGGSEVQLVESGGGLVQPGGSLRLSCA
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< , SEQ ID NO: 542 ";PRT;->"
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GSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGT
LTVSSGGGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFDDYDIGWFRQAP

GKGREGVSGISSLGDGNTYYADSVKGRFTISSLNAKNTVYLQMNSLRPEDTAVYYCA
AEPPDSSWYLDGSPEFFKYWGQGTLTVSSGGGGSGGSEVQLVESGGGLVQPGGS
LRLSCAASGFTFDDYDIGWFRQAPGKGREGVSGISSLGDGNTYYADSVKGRFTISSLNA
AKNTVYLQMNSLRPEDTAVYYCAAEPPDSSWYLDGSPEFFKYWGQGTLTVSS

< , SEQ ID NO: 543 ";PRT;-> "

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LTVSSGGGGSGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFSDYDIGWFRQAPG
KGREGVSGISSLGDGNTYYADSVKGRFTISSLNAKNTVYLQMNSLRPEDTAVYYCAA
EPPDSSWYLDGSPEFFKYWGQGTLTVSSGGGGSGGSEVQLVESGGGLVQPGGSL
LRLSCAASGFTFSDYDIGWFRQAPGKGREGVSGISSLGDGNTYYADSVKGRFTISSLNA
AKNTVYLQMNSLRPEDTAVYYCAAEPPDSSWYLDGSPEFFKYWGQGTLTVSS

< , SEQ ID NO: 544 ";PRT;-> "

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LTVSSGGGGSGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFDDYDIGWFRQAP
KGREGVSGISSLGDGNTYYADSVKGRFTISSLNAKNTVYLQMNSLRPEDTAVYYCA
AEPPDSSWYLDGSPEFFKYWGQGTLTVSSGGGGSGGSEVQLVESGGGLVQPGGS
LRLSCAASGFTFDDYDIGWFRQAPGKGREGVSGISSLGDGNTYYADSVKGRFTISSLNA
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< , SEQ ID NO: 545 ";PRT;-> "

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LTVSSGGGGSGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFDDYDIGWFRQAP
KGREGVSGISSLGDGNTYYADSVKGRFTISSLNAKNTLYLQMNSLRPEDTAVYYCA
AEPPDSSWYLDGSPEFFKYWGQGTLTVSSGGGGSGGSEVQLVESGGGLVQPGGS
LRLSCAASGFTFDDYDIGWFRQAPGKGREGVSGISSLGDGNTYYADSVKGRFTISSLNA
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< , SEQ ID NO: 546 ";PRT;-> "

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KGREGVSGISSLGDGNTYYADSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAA
EPPDSSWYLDGSPEFFKYWGQGTLTVSSGGGGGGSEVQLVESGGGLVQPGGSL
RLSCAASGFTFSDYDIGWFRQAPGKGREGVSGISSLGDGNTYYADSVKGRFTISRDNA
KNTLYLQMNSLRPEDTAVYYCAAEPDSSWYLDGSPEFFKYWGQGTLTVSS

< , SEQ ID NO: 547 ";PRT;-> "

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LTVSSGGGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAP
GRATEWVSAISWNGNNTYYTESMKGRFTISRDNAKNTVYLQMNSLRPEDTAVYYC
VKGSTAIVGPPTYPDEYDYWGQGTLTVSSGGGGGGSEVQLVESGGGLVQPGG
SLRLSCAASGFTFDDYGMSWVRQAPGRATEWVSAISWNGNNTYYTESMKGRFTISR
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< , SEQ ID NO: 548 ";PRT;-> "

EVQLVESGGGLVQPGNSRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSIS
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LTVSSGGGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAP
GKGLEWVSAISWNGNNTYYTESMKGRFTISRDNAKNTVYLQMNSLRPEDTAVYYC
VKGSTAIVGPPTYPDEYDYWGQGTLTVSSGGGGGGSEVQLVESGGGLVQPGG
SLRLSCAASGFTFDDYGMSWVRQAPGKGLEWVSAISWNGNNTYYTESMKGRFTISR
DNAKNTVYLQMNSLRPEDTAVYYCVKGSTAIVGPPTYPDEYDYWGQGTLTVSS

< , SEQ ID NO: 549 ";PRT;-> "

EVQLVESGGGLVQPGNSRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSIS
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LTVSSGGGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAP
GRATEWVSAISWNGNNTYYTESMKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYC
VKGSTAIVGPPTYPDEYDYWGQGTLTVSSGGGGGGSEVQLVESGGGLVQPGG
SLRLSCAASGFTFDDYGMSWVRQAPGRATEWVSAISWNGNNTYYTESMKGRFTISR
DNAKNTLYLQMNSLRPEDTAVYYCVKGSTAIVGPPTYPDEYDYWGQGTLTVSS

< , SEQ ID NO: 550 ";PRT;-> "

EVQLVESGGGLVQPGNSRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSIS
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LTVSSGGGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAP

GKGLEWVSAISWGNNTYYTESMKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYC
VKGSTAIVGPPTYPDEYDYWGQGTLTVSSGGGGGGSEVQLVESGGGLVQPGG
SLRLSCAASGFTFDDYGMWSVRQAPGKGLEWVSAISWGNNTYYTESMKGRFTISR
DNAKNTLYLQMNSLRPEDTAVYYCVKGSTAIVGPPTYPDEYDYWGQGTLTVSS

< , SEQ ID NO: 551 ";PRT;-> "

EVQLVESGGGLVQPGNSRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSIS
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LTVSSGGGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMWSVRQAP
GRGLEWVSAISWGNNTYYTESMKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYC
VKGSTAIVGPPTYPDEYDYWGQGTLTVSSGGGGGGSEVQLVESGGGLVQPGG
SLRLSCAASGFTFDDYGMWSVRQAPGRGLEWVSAISWGNNTYYTESMKGRFTISR
DNAKNTLYLQMNSLRPEDTAVYYCVKGSTAIVGPPTYPDEYDYWGQGTLTVSS

< , SEQ ID NO: 552 ";PRT;-> "

EVQLVESGGGLVQPGNSRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSIS
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LTVSSGGGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMWSVRQAP
GRALEWVSAISWGNNTYYTESMKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYC
VKGSTAIVGPPTYPDEYDYWGQGTLTVSSGGGGGGSEVQLVESGGGLVQPGG
SLRLSCAASGFTFDDYGMWSVRQAPGRALEWVSAISWGNNTYYTESMKGRFTISR
DNAKNTLYLQMNSLRPEDTAVYYCVKGSTAIVGPPTYPDEYDYWGQGTLTVSS

< , SEQ ID NO: 553 ";PRT;-> "

EVQLVESGGGLVQPGNSRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSIS
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LTVSSGGGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMWSVRQAP
GKATEWVSAISWGNNTYYTESMKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYC
VKGSTAIVGPPTYPDEYDYWGQGTLTVSSGGGGGGSEVQLVESGGGLVQPGG
SLRLSCAASGFTFDDYGMWSVRQAPGKATEWVSAISWGNNTYYTESMKGRFTISR
DNAKNTLYLQMNSLRPEDTAVYYCVKGSTAIVGPPTYPDEYDYWGQGTLTVSS

< , SEQ ID NO: 554 ";PRT;-> "

EVQLVESGGGLVQPGNSRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSIS
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LTVSSGGGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMWSVRQAP

GKGTEWVSAISWGNNTYYTESMKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYC
VKGSTAIVGPPTYPDEYDYWGQGTLTVSSGGGGGGSEVQLVESGGGLVQPGG
SLRLSCAASGFTFDDYGMWSVRQAPGKTEWVSAISWGNNTYYTESMKGRFTISR
DNAKNTLYLQMNSLRPEDTAVYYCVKGSTAIVGPPTYPDEYDYWGQGTLTVSS

< , SEQ ID NO: 555 ";PRT;-> "

EVQLVESGGGLVQPGNSRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSIS
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LTVSSGGGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMWSVRQAP
GRGTEWVSAISWGNNTYYTESMKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYC
VKGSTAIVGPPTYPDEYDYWGQGTLTVSSGGGGGGSEVQLVESGGGLVQPGG
SLRLSCAASGFTFDDYGMWSVRQAPGRGTEWVSAISWGNNTYYTESMKGRFTISR
DNAKNTLYLQMNSLRPEDTAVYYCVKGSTAIVGPPTYPDEYDYWGQGTLTVSS

< , SEQ ID NO: 556 ";PRT;-> "

EVQLVESGGGLVQPGNSRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSIS
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LTVSSGGGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMWSVRQAP
GKALEWVSAISWGNNTYYTESMKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYC
VKGSTAIVGPPTYPDEYDYWGQGTLTVSSGGGGGGSEVQLVESGGGLVQPGG
SLRLSCAASGFTFDDYGMWSVRQAPGKALEWVSAISWGNNTYYTESMKGRFTISR
DNAKNTLYLQMNSLRPEDTAVYYCVKGSTAIVGPPTYPDEYDYWGQGTLTVSS

< , SEQ ID NO: 557 ";PRT;-> "

EVQLVESGGGLVQPGNSRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSIS
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LTVSSGGGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFSDYGMWSVRQAP
GKGTEWVSAISWGNNTYYTESMKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYC
VKGSTAIVGPPTYPDEYDYWGQGTLTVSSGGGGGGSEVQLVESGGGLVQPGG
SLRLSCAASGFTFSDYGMWSVRQAPGKTEWVSAISWGNNTYYTESMKGRFTISR
DNAKNTLYLQMNSLRPEDTAVYYCVKGSTAIVGPPTYPDEYDYWGQGTLTVSS

< , SEQ ID NO: 558 ";PRT;-> "

EVQLVESGGGLVQPGNSRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSIS
GSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGT
LTVSSGGGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFSDYGMWSVRQAP

GKGTEWVSAISWNGNNTYYTESMKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYC
AKGSTAIVGPPTYPDEYDYWGQGTLTVSSGGGGGGSEVQLVESGGGLVQPGG
SLRLSCAASGFTFSDYGMWSVRQAPGKGTWVSAISWNGNNTYYTESMKGRFTISR
DNAKNTLYLQMNSLRPEDTAVYYCAKGSTAIVGPPTYPDEYDYWGQGTLTVSS

Humanized bispecific polypeptides against the IL6 receptor and human serum albumin

< , SEQ ID NO: 559 ";PRT;-> "

EVQLVESGGGLVQPGGSLRLSCAASGSIFKVNAMEWYRQAPGKGRELVAGII
SGGSTNYADSVKGRLTISRDNAKNTVYLQMNSLRPEDTAVYYCSFVTTNSDYDLGR
DYWGQGTLTVSSGGGGGGSEVQLVESGGGLVQPGNSRLSCAASGFTSSFGM
SWVRQAPGKGLEWVSSISGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDT
AVYYCTIGGSLRSSQGTLTVSS

< , SEQ ID NO: 560 ";PRT;-> "

EVQLVESGGGLVQPGGSLRLSCAASGSIFKVNAMEWYRQAPGKGRELVAGII
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DYWGQGTLTVSSGGGGGGSEVQLVESGGGLVQPGNSRLSCAASGFTSSFGM
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< , SEQ ID NO: 561 ";PRT;-> "

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< , SEQ ID NO: 562 ";PRT;-> "

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AVYYCTIGGSLRSSQGTLTVSS

< IL6R66 , SEQ ID NO: 563 ";PRT;->"
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< , SEQ ID NO: 564 ";PRT;->"
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GSPEFFKYWGQQGTLTVSSGGGGSGGGSEVQLVESGGGLVQPGNSRLSCAASGFTF
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< , SEQ ID NO: 565 ";PRT;->"
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< , SEQ ID NO: 566 ";PRT;->"
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< , SEQ ID NO: 567 ";PRT;->"
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GSPEFFKYWGQQGTLTVSSGGGGSGGGSEVQLVESGGGLVQPGNSRLSCAASGFTF
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< IL6R202 , SEQ ID NO: 568 ";PRT;->"
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< , SEQ ID NO: 569 ";PRT;->"
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< , SEQ ID NO: 570 ";PRT;->"
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< , SEQ ID NO: 571 ";PRT;->"
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PTYPDEYDYWGQGTLTVSSGGGGGGSEVQLVESGGGLVQPGNSRLSCAASGF
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< , SEQ ID NO: 572 ";PRT;->"
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PTYPDEYDYWGQGTLTVSSGGGGGGSEVQLVESGGGLVQPGNSRLSCAASGF
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SLRPEDTAVYYCTIGGSLRSSQGTLTVSS

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< , SEQ ID NO: 574 ";PRT;-> "

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PTYPDEYDYWGQGTLTVSSGGGSGGGSEVQLVESGGGLVQPGNSRLSCAASGF
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< , SEQ ID NO: 575 ";PRT;-> "

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PTYPDEYDYWGQGTLTVSSGGGSGGGSEVQLVESGGGLVQPGNSRLSCAASGF
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< IL6R91 , SEQ ID NO: 576 ";PRT;-> "

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PTYPDEYDYWGQGTLTVSSGGGSGGGSEVQLVESGGGLVQPGNSRLSCAASGF
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< , SEQ ID NO: 577 ";PRT;-> "

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PTYPDEYDYWGQGTLTVSSGGGSGGGSEVQLVESGGGLVQPGNSRLSCAASGF
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< , SEQ ID NO: 578 ";PRT;-> "

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PTYPDEYDYWGQGTLTVSSGGGSGGGSEVQLVESGGGLVQPGNSRLSCAASGF
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< , SEQ ID NO: 579 ";PRT;->"
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PTYPDEYDYWGQGTLTVSSGGGSGGGSEVQLVESGGGLVQPGNSRLSCAASGF
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< , SEQ ID NO: 580 ";PRT;->"
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PTYPDEYDYWGQGTLTVSSGGGSGGGSEVQLVESGGGLVQPGNSRLSCAASGF
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< , SEQ ID NO: 581 ";PRT;->"
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< , SEQ ID NO: 582 ";PRT;->"
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LTVSSGGGSGGGSEVQLVESGGGLVQPGGSLRLSCAASGSIFKVNAMEWYRQAP
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TTNSDYDLGRDYWGQGTLTVSS

< , SEQ ID NO: 583 ";PRT;->"
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LTVSSGGGSGGGSEVQLVESGGGLVQPGGSLRLSCAASGSIFKVNAMEWYRQAP
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< , SEQ ID NO: 584 ";PRT;->"
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< , SEQ ID NO: 585 ";PRT;->"
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LTVSSGGGGGGSEVQLVESGGLVQPGGSLRLSCAASGSIFKVNAMGWYRQAP
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TTNSDYDLGRDYWGQGTLTVSS

< , SEQ ID NO: 586 ";PRT;->"
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LTVSSGGGGGGSEVQLVESGGLVQPGGSLRLSCAASGFTFDDYDIGWFRQAP
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< , SEQ ID NO: 587 ";PRT;->"
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LTVSSGGGGGGSEVQLVESGGLVQPGGSLRLSCAASGFTFSDYDIGWFRQAPG
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< , SEQ ID NO: 588 ";PRT;->"
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LTVSSGGGGGGSEVQLVESGGLVQPGGSLRLSCAASGFTFDDYDIGWFRQAP
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< , SEQ ID NO: 589 ";PRT;->"
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LTVSSGGGGGGSEVQLVESGGLVQPGGSLRLSCAASGFTFDDYDIGWFRQAP

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< , SEQ ID NO: 590 ";PRT;-> "

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< , SEQ ID NO: 591 ";PRT;-> "

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LTVSSGGGGSGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAP
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< , SEQ ID NO: 592 ";PRT;-> "

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LTVSSGGGGSGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAP
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< , SEQ ID NO: 593 ";PRT;-> "

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< , SEQ ID NO: 594 ";PRT;-> "

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< , SEQ ID NO: 595 ";PRT;->"
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LTVSSGGGSGGGSEVQLVESGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAP
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VKGSTAIVGPPTYPDEYDYWGQQGTLTVSS

< , SEQ ID NO: 596 ";PRT;->"
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LTVSSGGGSGGGSEVQLVESGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAP
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VKGSTAIVGPPTYPDEYDYWGQQGTLTVSS

< , SEQ ID NO: 597 ";PRT;->"
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LTVSSGGGSGGGSEVQLVESGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAP
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< , SEQ ID NO: 598 ";PRT;->"
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VKGSTAIVGPPTYPDEYDYWGQQGTLTVSS

< , SEQ ID NO: 599 ";PRT;->"
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LTVSSGGGSGGGSEVQLVESGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAP
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VKGSTAIVGPPTYPDEYDYWGQQGTLTVSS

< , SEQ ID NO: 600 ";PRT;->"
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GSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGT
LTVSSGGGGGGSEVQLVESGGLVQPGGSLRLSCAASGFTFDDYGMWSVRQAP
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VKGSTAIVGPPTYPDEYDYWGQGTLTVSS

< , SEQ ID NO: 601 ";PRT;->"
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GSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGT
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< , SEQ ID NO: 602 ";PRT;->"
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LTVSSGGGGGGSEVQLVESGGLVQPGGSLRLSCAASGFTFSDYGMWSVRQAP
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Anti-IL-6 receptor Nanobody-human serum albumin fusion proteins

< HSA-(GGS)4GG-IL6R04 , SEQ ID NO: 603 ";PRT;->"
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CVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPRNECFLQHKDD
NPNLPLRVRPEVDVMCTAFHDNEETFLKKLYEIARRHPFYAPELLFFAKRYKAAF
TECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLS
QRFPKAFAEVSKLVTDLTVHTECCHGDLLECADDRADLAKYICENQDSISSKLKE
CCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYE
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PCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESVNRRPCFSALEVDETYVPKEFN
AETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCC
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GSLRLSCAASGFTFDDYDIGWFRQAPGKEREGVSGISSLGNTYYADSVKGRFTISSL
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< IL6R04-HSA , SEQ ID NO: 604 ";PRT;-> "

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GSPEFFKYWGQGTQTVSSDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFE
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KQEPERNECFLQHKDDNPNLPRLRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYF
YAPELLFFAKRYKAAFTECQCQAADKAACLLPKLDELRDEGKASSAKQRLKASLQK
FGERAFKAWAVARLSQRFPKAFAEVSKLVTDLTKVHTECCHGDLLECADDRADLA
KYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNY
AEAKDVFGLMFLYEYARRHPDYSVVLLRLAKTYETTLEKCCAAADPHECYAKVF
DEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKVPQVSTPTLVEVSRLNG
KVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPC
FSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLK
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< IL6R201-HSA , SEQ ID NO: 605 ";PRT;-> "

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYDIGWFRQAPGKGREGVSGISSL
SDGNTYYADSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAEPDSSWYLDG
SPEFFKYWGQGTLTVSSDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDH
VKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCA
KQEPERNECFLQHKDDNPNLPRLRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYA
PELLFFAKRYKAAFTECQCQAADKAACLLPKLDELRDEGKASSAKQRLKASLQKFG
ERAFAKAWAVARLSQRFPKAFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAK
YICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYA
EAKDVFGLMFLYEYARRHPDYSVVLLRLAKTYETTLEKCCAAADPHECYAKVFDE
FKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKVPQVSTPTLVEVSRLNGKV
GSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFS
ALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKA
VMDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGL

< IL6R201-9GS-IL6R201-HSA , SEQ ID NO: 606 ";PRT;-> "

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYDIGWFRQAPGKGREGVSGISSL

SDGNTYYADSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAEPDSSWYLDG
SPEFFKYWGQGTLTVSSGGGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFTFS
DYDIGWFRQAPGKGREGVSGISSLGNTYYADSVKGRFTISRDNAKNTLYLQMNSL
RPEDTAVYYCAAEPDSSWYLDGSPEFFKYWGQGTLTVSSDAHKSEVAHRFKDLG
EENFKALVLIAFAQYLQQCPFEDHVKLNEVTEFAKTCVADESAENCDKSLHTLFGD
KLCTVATLRETYGEMADCCAKQEPECFLQHKDDNPNLPRLVRPEVDVMCTAF
HDNEETFLKKLYEIARRHPYFYAPELFFAKRYKAAFTECCQAADKAACLLPKLDE
LRDEGKASSAKQRLKCASLQKFGERAFAVARLSQRFPKAFAEVSKLVTDLTK
VHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDE
MPADLPSLAADFVESKDVKNYAEAKDVFLGMFLYEYARRHPDYSVVLLRLAKT
YETTLEKCCAAADPHECYAKVDFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLV
RYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHE
KTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQI
KKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCKADDKETCFAEEGKKLVA
ASQAALGL

< IL6R201-20GS-IL6R201-HSA , SEQ ID NO: 607 ";PRT;->"
EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYDIGWFRQAPGKGREGVSGISSL
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RLSCAASGFTFSDYDIGWFRQAPGKGREGVSGISSLGNTYYADSVKGRFTISRDNA
KNTLYLQMNSLRPEDTAVYYCAAEPDSSWYLDGSPEFFKYWGQGTLTVSSDAH
KSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLNEVTEFAKTCVADESA
ENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPECFLQHKDDNPNLPRL
VRPEVDVMCTAFHDNEETFLKKLYEIARRHPYFYAPELFFAKRYKAAFTECCQA
ADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFAVARLSQRFPKAFAE
EVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLL
EKSHCIAEVENDEMPADLPSLAADFVESKDVKNYAEAKDVFLGMFLYEYARRHPD
YSVVLLRLAKTYETTLEKCCAAADPHECYAKVDFKPLVEEPQNLIKQNCELFEQ
LGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDY
SVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFH
ADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCKADDKET
CFAEEGKKLV

< IL6R201-AAA-IL6R201-HSA , SEQ ID NO: 608 ";PRT;->"
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SPEFFKYWGQGTLTVSSAAAEVQLVESGGGLVQPGGSLRLSCAASGFTFSDYDIGW
FRQAPGKGREGVSGISSLGDGNTYYADSVKGRFTISRDNAKNTLYLQMNSLRPEDTAV
YYCAAEPPDSSWYLDGSPEFFKYWGQGTLTVSSDAHKSEVAHRFKDLGEENFKAL
VLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVA
TLRETYGEMADCCAKQEPECNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETF
LKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGK
ASSAKQRLKCASLQKFGERAFAKAWAVARLSQRFPKAFAEVSKLVTDLTKVHTECC
HGDLLCADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLPS
LAADFVESKDVCKNYAEAKDVFGLMFLYEYARRHPDYSVVLRLAKTYETTLEKC
CAAADPHECYAKVDFEKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVP
QVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDR
VTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICLSEKERQIKKQTALV
ELVHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQAALGL

< IL6R61 , SEQ ID NO: 609 ";PRT;->"
EVQLVESGGGLVQPGGSLRLSCAASGSIFKVNAMGWYRQAPGKGRELVAGII
SGGSTNYADSVKGRLTISRDNAKNTVYLQMNSLRPEDTAVYYCSFVTTNSDYDLGR
DYWGQGTLTVSS

< IL6R62 , SEQ ID NO: 610 ";PRT;->"
EVQLVESGGGLVQPGGSLRLSCAASGSIFKVNAMGWYRQAPGKGRELVAGII
SGGSTNYADSVKGRFTISRDNAKNTVYLQMNSLRPEDTAVYYCSFVTTNSDYDLGR
DYWGQGTLTVSS

< IL6R63 , SEQ ID NO: 611 ";PRT;->"
EVQLVESGGGLVQPGGSLRLSCAASGSIFKVNAMGWYRQAPGKGRELVAGII
SGGSTNYADSVKGRLTISRDNAKNTLYLQMNSLRPEDTAVYYCSFVTTNSDYDLGR
DYWGQGTLTVSS

< IL6R64 , SEQ ID NO: 612 ";PRT;->"
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SGGSTNYADSVKGRLTISRDNAKNTVYLQMNSLRPEDTAVYYCAFVTTNSDYDLGR
DYWGQGTLTVSS

< IL6R65 , SEQ ID NO: 613 ";PRT;->"	EVQLVESGGGLVQPGGSLRLSCAASGSIFKVNAMGWYRQAPGKGRELVAGIISGGSTNYADSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAFVTTNSDYDLGRDYWGQGTLTVSS
< IL6R71 , SEQ ID NO: 614 ";PRT;->"	EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYDIGWFRQAPGKGREGVSGISSSDGNTYYADSVKGRFTIISDNAKNTVYLQMNSLRPEDTAVYYCAAEPPDSSWYLDGSPEFFKYWGQGTLTVSS
< IL6R72 , SEQ ID NO: 615 ";PRT;->"	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYDIGWFRQAPGKGREGVSGISSDGNTYYADSVKGRFTIISDNAKNTVYLQMNSLRPEDTAVYYCAAEPPDSSWYLDGSPEFFKYWGQGTLTVSS
< IL6R73 , SEQ ID NO: 616 ";PRT;->"	EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYDIGWFRQAPGKGREGVSGISSSDGNTYYADSVKGRFTISRDNAKNTVYLQMNSLRPEDTAVYYCAAEPPDSSWYLDGSPEFFKYWGQGTLTVSS
< IL6R74 , SEQ ID NO: 617 ";PRT;->"	EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYDIGWFRQAPGKGREGVSGISSSDGNTYYADSVKGRFTIISDNAKNTLYLQMNSLRPEDTAVYYCAAEPPDSSWYLDGSPEFFKYWGQGTLTVSS
< IL6R75 , SEQ ID NO: 618 ";PRT;->"	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYDIGWFRQAPGKGREGVSGISSDGNTYYADSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAEPPDSSWYLDGSPEFFKYWGQGTLTVSS
< IL6R81 , SEQ ID NO: 619 ";PRT;->"	EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGRATEWVSAISWNGNNNTYYTESMKGRFTISRDNAKNTVYLQMNSLRPEDTAVYYCVKGSTAIVGVPPTYPDEYDYWGQGTLTVSS
< IL6R82 , SEQ ID NO: 620 ";PRT;->"	EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWVSAI

SWNGNNNTYYTESMKGRFTISRDNAKNTVYLQMNSLRPEDTAVYYCVKGSTAIVGVP PTYPDEYDYWGQGTLTVSS
< IL6R83 , SEQ ID NO: 621 ";PRT;->" EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGRATEWVSAI SWNGNNNTYYTESMKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCVKGSTAIVGVP PTYPDEYDYWGQGTLTVSS
< IL6R84 , SEQ ID NO: 622 ";PRT;->" EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWVSAI SWNGNNNTYYTESMKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCVKGSTAIVGVP PTYPDEYDYWGQGTLTVSS
< IL6R85 , SEQ ID NO: 623 ";PRT;->" EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGRGLEWVSAI SWNGNNNTYYTESMKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCVKGSTAIVGVP PTYPDEYDYWGQGTLTVSS
< IL6R86 , SEQ ID NO: 624 ";PRT;->" EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGRALEWVSAI SWNGNNNTYYTESMKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCVKGSTAIVGVP PTYPDEYDYWGQGTLTVSS
< IL6R87 , SEQ ID NO: 625 ";PRT;->" EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGKATEWVSAI SWNGNNNTYYTESMKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCVKGSTAIVGVP PTYPDEYDYWGQGTLTVSS
< IL6R88 , SEQ ID NO: 626 ";PRT;->" EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGTEWVSAI SWNGNNNTYYTESMKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCVKGSTAIVGVP PTYPDEYDYWGQGTLTVSS
< IL6R89 , SEQ ID NO: 627 ";PRT;->" EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGRGTEWVSAI SWNGNNNTYYTESMKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCVKGSTAIVGVP PTYPDEYDYWGQGTLTVSS

< IL6R90 , SEQ ID NO: 628 ";PRT;->"
EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYGMSWVRQAPGKALEWVSAI
SWNGNNNTYYTESMKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCVKGSTAIVGVP
PTYPDEYDYWGQGTLTVSS

< REFERENCE IGG HEAVY CHAIN , SEQ ID NO: 629 ";PRT;->"
QVQLQESGPGLVRPSQTLSTLTCTVSGYSITSDHAWSWVRQPPGRGLEWIGYIS
YSGITTYNPSLKSRTMLRDTSKNQFSLRLSSVTAADTAVYYCARSLARTTAMDYW
GQGSLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTWSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI
SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

< REFERENCE IGG LIGHT CHAIN , SEQ ID NO: 630 ";PRT;->"
DIQMTQSPSSLSASVGDRVITCRASQDISSYLNWYQQKPGKAPKLLIYYTSR
LHSGVPSRFSGSQGTDFFTISSLQPEDIATYYCQQGNTLPYTFGQGTKVEIKRTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK
DSTYSLSSTTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

< REFERENCE FAB HEAVY CHAIN , SEQ ID NO: 631 ";PRT;->"
QVQLQESGPGLVRPSQTLSTLTCTVSGYSITSDHAWSWVRQPPGRGLEWIGYIS
YSGITTYNPSLKSRTMLRDTSKNQFSLRLSSVTAADTAVYYCARSLARTTAMDYW
GQGSLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTWSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC

< REFERENCE FAB LIGHT CHAIN , SEQ ID NO: 632 ";PRT;->"
DIQMTQSPSSLSASVGDRVITCRASQDISSYLNWYQQKPGKAPKLLIYYTSR
LHSGVPSRFSGSQGTDFFTISSLQPEDIATYYCQQGNTLPYTFGQGTKVEIKRTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK
DSTYSLSSTTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

< IL6R MACACA FASCICULARIS , SEQ ID NO: 633 ";PRT;->"
LAPGGCPAQEVARGVLTSLPGDSVLTCPGGEPEDNATVHWVLRKPAEGSHL
SRWAGVGRLLRSVQLHDSGNYSCYAGRPAATVHLLVDVPPEPQLSCFRKSPLS

NVVCEWGPRTSPPTKAVLLVRKFQNSPAEDFQEPCQYSQESQKFSCQLAVPEGDS
SFYIVSMCVASSVGSKLSKTQTFQGCGILQPDPPANITVTAVARNPRWLSVTWQDPH
SWNSSFYRLRFELRYRAERSKTFTWMVKDLQHHCVIHDAWSGLRHVVQLRAQEE
FGQGEWSEWSPEAMGTPWTESRSPPAENEVSTPTQAPTTNKDDDNILSGDSANATSL
PVQD

The term and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

All of the references described herein are incorporated by reference, in particular of the teaching that is referenced hereinabove.

CLAIMS

1. Amino acid sequence that is directed against and/or that can specifically bind to IL-6R.
2. Amino acid sequence according to claim 1, wherein said amino acid sequence binds to an epitope of IL-6R, such that the interaction between IL-6R and IL-6 is modulated.
3. Amino acid sequence according to claims 1 or 2, wherein said amino acid sequence competes with IL-6 for binding to said epitope of IL-6R.
4. Amino acid sequence according to claims 2 or 3, wherein said epitope is located in an extracellular domain of IL-6R.
5. Amino acid sequence according to claim 4, wherein said epitope is located in the extracellular D3 domain of IL-6R.
6. Amino acid sequence according to claims 4 or 5, wherein said epitope comprises at least a fragment of the 18 contact residue stretch of the extracellular D3 domain of IL-6R, which is involved in the interaction between IL-6 and IL-6R.
7. Amino acid sequence according to claims 4 to 6, wherein said epitope comprises amino acid residue Phe229 and/or amino acid residue Phe279 of the extracellular D3 domain of IL-6R.
8. Amino acid sequence according to claim 1, wherein said amino acid sequence binds to an epitope of IL-6R, such that the interaction between the IL-6/IL-6R complex and gp130 is modulated.
9. Amino acid sequence according to claims 1 or 8, wherein said amino acid sequence competes with gp130 for binding to said epitope of IL-6R.

10. Amino acid sequence according to any of the preceding claims, that is in essentially isolated form.

11. Amino acid sequence according to any of the preceding claims, for administration to a subject, wherein said amino acid sequence does not naturally occur in said subject.

12. Amino acid sequence according to any of the preceding claims, that can specifically bind to IL-6R with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter or less, and preferably 10^{-7} to 10^{-12} moles/liter or less and more preferably 10^{-8} to 10^{-12} moles/liter.

13. Amino acid sequence according to any of the preceding claims, that can specifically bind to IL-6R with a rate of association (k_{on} -rate) of between $10^2 \text{ M}^{-1} \text{ s}^{-1}$ to about $10^7 \text{ M}^{-1} \text{ s}^{-1}$, preferably between $10^3 \text{ M}^{-1} \text{ s}^{-1}$ and $10^7 \text{ M}^{-1} \text{ s}^{-1}$, more preferably between $10^4 \text{ M}^{-1} \text{ s}^{-1}$ and $10^7 \text{ M}^{-1} \text{ s}^{-1}$, such as between $10^5 \text{ M}^{-1} \text{ s}^{-1}$ and $10^7 \text{ M}^{-1} \text{ s}^{-1}$.

14. Amino acid sequence according to any of the preceding claims, that can specifically bind to IL-6R with a rate of dissociation (k_{off} rate) between 1 s^{-1} and 10^{-6} s^{-1} preferably between 10^{-2} s^{-1} and 10^{-6} s^{-1} , more preferably between 10^{-3} s^{-1} and 10^{-6} s^{-1} , such as between 10^{-4} s^{-1} and 10^{-6} s^{-1} .

15. Amino acid sequence according to any of the preceding claims, that can specifically bind to IL-6R with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM.

16. Amino acid sequence according to any of the preceding claims, that is a naturally occurring amino acid sequence (from any suitable species) or a synthetic or semi-synthetic amino acid sequence.

17. Amino acid sequence according to any of the preceding claims, that comprises an immunoglobulin fold or that under suitable conditions is capable of forming an immunoglobulin fold.

18. Amino acid sequence according to any of the preceding claims, that essentially consists of 4 framework regions (FR1 to FR4 respectively) and 3 complementarity determining regions (CDR1 to CDR3), respectively.

19. Amino acid sequence according to any of the preceding claims, that is an immunoglobulin sequence.

20. Amino acid sequence according to any of the preceding claims, that is a naturally occurring immunoglobulin sequence (from any suitable species) or a synthetic or semi-synthetic immunoglobulin sequence.

21. Amino acid sequence according to any of the preceding claims that is a humanized immunoglobulin sequence, a camelized immunoglobulin sequence or an immunoglobulin sequence that has been obtained by techniques such as affinity maturation.

22. Amino acid sequence according to any of the preceding claims, that essentially consists of a light chain variable domain sequence (e.g. a V_L -sequence); or of a heavy chain variable domain sequence (e.g. a V_H -sequence).

23. Amino acid sequence according to any of the preceding claims, that essentially consists of a of a heavy chain variable domain sequence that is derived from a conventional four-chain antibody or that essentially consist of a heavy chain variable domain sequence that is derived from heavy chain antibody.

24. Amino acid sequence according to any of the preceding claims, that essentially consists of a domain antibody (or an amino acid sequence that is suitable for use as a domain antibody), of a single domain antibody (or an amino acid sequence that is suitable for use as a single domain antibody), of a "dAb" (or an amino acid sequence that is suitable for use as a dAb) or of a Nanobody® (including but not limited to a V_{HH} sequence).

25. Amino acid sequence according to any of the preceding claims, that essentially consists of a Nanobody®.

26. Amino acid sequence according to any of the preceding claims, that essentially consists of a Nanobody® that

- a) has 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 399 to 471, in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences are disregarded; and in which:
 - b) preferably one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table A-3.

27. Amino acid sequence according to any of the preceding claims, that essentially consists of a humanized Nanobody®.

28. Amino acid sequence according to any of the preceding claims, that in addition to the at least one binding site for binding against IL-6R, contains one or more further binding sites for binding against other antigens, proteins or targets.

29. Amino acid sequence directed against IL-6R, that comprises one or more stretches of amino acid residues chosen from the group consisting of:

- a) the amino acid sequences of SEQ ID NO's: 93 to 143;
- b) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 93 to 143;
- c) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 93 to 143;
- d) the amino acid sequences of SEQ ID NO's: 195 to 245;
- e) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 195 to 245;
- f) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 195 to 245;
- g) the amino acid sequences of SEQ ID NO's: 297 to 347;

- h) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 297 to 347;
- i) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 297 to 347;
or any suitable combination thereof.

30. Amino acid sequence according to claim 29, in which at least one of said stretches of amino acid residues forms part of the antigen binding site for binding against IL-6R.

31. Amino acid sequence according to claim 29, that comprises two or more stretches of amino acid residues chosen from the group consisting of:

- a) the amino acid sequences of SEQ ID NO's: 93 to 143;
- b) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 93 to 143;
- c) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 93 to 143;
- d) the amino acid sequences of SEQ ID NO's: 195 to 245;
- e) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 195 to 245;
- f) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 195 to 245;
- g) the amino acid sequences of SEQ ID NO's: 297 to 347;
- h) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 297 to 347;
- i) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 297 to 347;

such that (i) when the first stretch of amino acid residues corresponds to one of the amino acid sequences according to a), b) or c), the second stretch of amino acid residues corresponds to one of the amino acid sequences according to d), e), f), g), h) or i); (ii) when the first stretch of amino acid residues corresponds to one of the amino acid sequences according to d), e) or f), the second stretch of amino acid residues corresponds to one of the

amino acid sequences according to a), b), c), g), h) or i); or (iii) when the first stretch of amino acid residues corresponds to one of the amino acid sequences according to g), h) or i), the second stretch of amino acid residues corresponds to one of the amino acid sequences according to a), b), c), d), e) or f).

32. Amino acid sequence according to claim 31, in which the at least two stretches of amino acid residues forms part of the antigen binding site for binding against IL-6R.

33. Amino acid sequence according to any of claims 29 or 32, that comprises three or more stretches of amino acid residues, in which the first stretch of amino acid residues is chosen from the group consisting of:

- a) the amino acid sequences of SEQ ID NO's: 93 to 143;
- b) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 93 to 143;
- c) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 93 to 143;

the second stretch of amino acid residues is chosen from the group consisting of:

- d) the amino acid sequences of SEQ ID NO's: 195 to 245;
- e) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 195 to 245;
- f) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 195 to 245;

and the third stretch of amino acid residues is chosen from the group consisting of:

- g) the amino acid sequences of SEQ ID NO's: 297 to 347;
- h) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 297 to 347;
- i) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 297 to 347.

34. Amino acid sequence according to claim 33, in which the at least three stretches of amino acid residues forms part of the antigen binding site for binding against IL-6R.

35. Amino acid sequence according to any of claims 29 to 34, in which the CDR sequences of said amino acid sequence have at least 70% amino acid identity, preferably at least 80% amino acid identity, more preferably at least 90% amino acid identity, such as 95% amino acid identity or more or even essentially 100% amino acid identity with the CDR sequences of at least one of the amino acid sequences of SEQ ID NO's: 399 to 471.

36. Amino acid sequence according to any of claims 29 to 35, that is in essentially isolated form.

37. Amino acid sequence according to any of claims 29 to 36, for administration to a subject, wherein said amino acid sequence does not naturally occur in said subject.

38. Amino acid sequence according to any of claims 29 to 37, that can specifically bind to IL-6R with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter or less, and preferably 10^{-7} to 10^{-12} moles/liter or less and more preferably 10^{-8} to 10^{-12} moles/liter.

39. Amino acid sequence according to any of claims 29 to 38, that can specifically bind to IL-6R with a rate of association (k_{on} -rate) of between $10^2 \text{ M}^{-1}\text{s}^{-1}$ to about $10^7 \text{ M}^{-1}\text{s}^{-1}$, preferably between $10^3 \text{ M}^{-1}\text{s}^{-1}$ and $10^7 \text{ M}^{-1}\text{s}^{-1}$, more preferably between $10^4 \text{ M}^{-1}\text{s}^{-1}$ and $10^7 \text{ M}^{-1}\text{s}^{-1}$, such as between $10^5 \text{ M}^{-1}\text{s}^{-1}$ and $10^7 \text{ M}^{-1}\text{s}^{-1}$.

40. Amino acid sequence according to any of claims 29 to 39, that can specifically bind to IL-6R with a rate of dissociation (k_{off} rate) between 1s^{-1} and 10^{-6} s^{-1} preferably between 10^{-2} s^{-1} and 10^{-6} s^{-1} , more preferably between 10^{-3} s^{-1} and 10^{-6} s^{-1} , such as between 10^{-4} s^{-1} and 10^{-6} s^{-1} .

41. Amino acid sequence according to any of claims 29 to 40, that can specifically bind to IL-6R with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM.

42. Amino acid sequence according to any of claims 29 to 41, that is a naturally occurring amino acid sequence (from any suitable species) or a synthetic or semi-synthetic amino acid sequence.

43. Amino acid sequence according to any of claims 29 to 42, that comprises an immunoglobulin fold or that under suitable conditions is capable of forming an immunoglobulin fold.

44. Amino acid sequence according to any of claims 29 to 43, that is an immunoglobulin sequence.

45. Amino acid sequence according to any of claims 29 to 44, that is a naturally occurring immunoglobulin sequence (from any suitable species) or a synthetic or semi-synthetic immunoglobulin sequence.

46. Amino acid sequence according to any of claims 29 to 45, that is a humanized immunoglobulin sequence, a camelized immunoglobulin sequence or an immunoglobulin sequence that has been obtained by techniques such as affinity maturation.

47. Amino acid sequence according to any of claims 29 to 46, that essentially consists of a light chain variable domain sequence (e.g. a V_L -sequence); or of a heavy chain variable domain sequence (e.g. a V_H -sequence)

48. Amino acid sequence according to any of claims 29 to 47, that essentially consists of a of a heavy chain variable domain sequence that is derived from a conventional four-chain antibody or that essentially consist of a heavy chain variable domain sequence that is derived from heavy chain antibody.

49. Amino acid sequence according to any of claims 29 to 48, that essentially consists of a domain antibody (or an amino acid sequence that is suitable for use as a domain antibody), of a single domain antibody (or an amino acid sequence that is suitable for use as a

single domain antibody), of a "dAb" (or an amino acid sequence that is suitable for use as a dAb) or of a Nanobody® (including but not limited to a V_{HH} sequence).

50. Amino acid sequence according to any of claims 29 to 49, that essentially consists of a Nanobody®.

51. Amino acid sequence according to any of claims 29 to 50, that essentially consists of a Nanobody® that

- a) has 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 399 to 471, in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences are disregarded; and in which:
- b) preferably one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table A-3.

52. Amino acid sequence according to any of claims 29 to 51, that essentially consists of a humanized Nanobody®.

53. Amino acid sequence according to any of claims 29 to 52, that in addition to the at least one binding site for binding formed by the CDR sequences, contain one or more further binding sites for binding against other antigens, proteins or targets.

54. Amino acid sequence that essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

- CDR1 is chosen from the group consisting of:
- a) the amino acid sequences of SEQ ID NO's: 93 to 143;
- b) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 93 to 143;
- c) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 93 to 143;

and/or

- CDR2 is chosen from the group consisting of:
 - d) the amino acid sequences of SEQ ID NO's: 195 to 245;
 - e) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 195 to 245;
 - f) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 195 to 245;

and/or

- CDR3 is chosen from the group consisting of:
 - g) the amino acid sequences of SEQ ID NO's: 297 to 347;
 - h) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 297 to 347;
 - i) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 297 to 347.

55. Amino acid sequence that essentially consists of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which:

- CDR1 is chosen from the group consisting of:
 - a) the amino acid sequences of SEQ ID NO's: 93 to 143;
 - b) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 93 to 143;
 - c) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 93 to 143;

and

- CDR2 is chosen from the group consisting of:
 - d) the amino acid sequences of SEQ ID NO's: 195 to 245;
 - e) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 195 to 245;
 - f) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 195 to 245;

and

- CDR3 is chosen from the group consisting of:
 - g) the amino acid sequences of SEQ ID NO's: 297 to 347;
 - h) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 297 to 347;
 - i) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 297 to 347.

56. Amino acid sequence according to any of claims 54 or 55, in which the CDR sequences of said amino acid sequence have at least 70% amino acid identity, preferably at least 80% amino acid identity, more preferably at least 90% amino acid identity, such as 95% amino acid identity or more or even essentially 100% amino acid identity with the CDR sequences of at least one of the amino acid sequences of SEQ ID NO's: 399 to 471.

57. Amino acid sequence according to any of claims 54 to 56, that is in essentially isolated form.

58. Amino acid sequence according to any of claims 54 to 57, for administration to a subject, wherein said amino acid sequence does not naturally occur in said subject.

59. Amino acid sequence according to any of claims 54 to 58, that can specifically bind to IL-6R with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter or less, and preferably 10^{-7} to 10^{-12} moles/liter or less and more preferably 10^{-8} to 10^{-12} moles/liter.

60. Amino acid sequence according to any of claims 54 to 59, that can specifically bind to IL-6R with a rate of association (k_{on} -rate) of between $10^2 \text{ M}^{-1}\text{s}^{-1}$ to about $10^7 \text{ M}^{-1}\text{s}^{-1}$, preferably between $10^3 \text{ M}^{-1}\text{s}^{-1}$ and $10^7 \text{ M}^{-1}\text{s}^{-1}$, more preferably between $10^4 \text{ M}^{-1}\text{s}^{-1}$ and $10^7 \text{ M}^{-1}\text{s}^{-1}$, such as between $10^5 \text{ M}^{-1}\text{s}^{-1}$ and $10^7 \text{ M}^{-1}\text{s}^{-1}$.

61. Amino acid sequence according to any of claims 54 to 60, that can specifically bind to IL-6R with a rate of dissociation (k_{off} rate) between 1s^{-1} and 10^{-6} s^{-1} preferably between 10^{-2} s^{-1} and 10^{-6} s^{-1} , more preferably between 10^{-3} s^{-1} and 10^{-6} s^{-1} , such as between 10^{-4} s^{-1} and 10^{-6} s^{-1} .

62. Amino acid sequence according to any of claims 54 to 61, that can specifically bind to IL-6R with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM.

63. Amino acid sequence according to any of claims 54 to 62, that is a naturally occurring amino acid sequence (from any suitable species) or a synthetic or semi-synthetic amino acid sequence.

64. Amino acid sequence according to any of claims 54 to 63, that comprises an immunoglobulin fold or that under suitable conditions is capable of forming an immunoglobulin fold.

65. Amino acid sequence according to any of claims 54 to 64, that is an immunoglobulin sequence.

66. Amino acid sequence according to any of claims 54 to 65, that is a naturally occurring immunoglobulin sequence (from any suitable species) or a synthetic or semi-synthetic immunoglobulin sequence.

67. Amino acid sequence according to any of claims 54 to 66, that is a humanized immunoglobulin sequence, a camelized immunoglobulin sequence or an immunoglobulin sequence that has been obtained by techniques such as affinity maturation.

68. Amino acid sequence according to any of claims 54 to 67, that essentially consists of a light chain variable domain sequence (e.g. a V_L -sequence); or of a heavy chain variable domain sequence (e.g. a V_H -sequence)

69. Amino acid sequence according to any of claims 54 to 68, that essentially consists of a of a heavy chain variable domain sequence that is derived from a conventional four-chain antibody or that essentially consist of a heavy chain variable domain sequence that is derived from heavy chain antibody.

70. Amino acid sequence according to any of claims 54 to 69, that essentially consists of a domain antibody (or an amino acid sequence that is suitable for use as a domain antibody), of a single domain antibody (or an amino acid sequence that is suitable for use as a single domain antibody), of a "dAb" (or an amino acid sequence that is suitable for use as a dAb) or of a Nanobody® (including but not limited to a V_{HH} sequence).

71. Amino acid sequence according to any of claims 54 to 70, that essentially consists of a Nanobody®.

72. Amino acid sequence according to any of claims 54 to 71, that essentially consists of a Nanobody® that

- a) has 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 399 to 471, in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences are disregarded; and in which:
- b) preferably one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table A-3.

73. Amino acid sequence according to any of claims 54 to 72, that essentially consists of a humanized Nanobody®.

74. Amino acid sequence according to claims 54 to 73, that in addition to the at least one binding site for binding formed by the CDR sequences, contain one or more further binding sites for binding against other antigens, proteins or targets.

75. Nanobody that is directed against and/or that can specifically bind to IL-6R.

76. Nanobody according to claim 75, wherein said Nanobody binds to an epitope of IL-6R, such that the interaction between IL-6R and IL-6 is modulated.

77. Nanobody according to claim 75 or 76, wherein said Nanobody competes with IL-6 for binding to said epitope of IL-6R.

78. Nanobody according to claim 76 or 77, wherein said epitope is located in an extracellular domain of IL-6R.

79. Nanobody according to claim 78, wherein said epitope is located in the extracellular D3 domain of IL-6R.

80. Nanobody according to claim 78 or 79, wherein said epitope comprises at least a fragment of the 18 contact residue stretch of the extracellular D3 domain of IL-6R, which is involved in the interaction between IL-6 and IL-6R.

81. Nanobody according to claim 78 to 80, wherein said epitope comprises amino acid residue Phe229 and/or amino acid residue Phe279 of the extracellular D3 domain of IL-6R.

82. Nanobody according to claim 75, wherein said Nanobody binds to an epitope of IL-6R, such that the interaction between the IL-6/IL-6R complex and gp130 is modulated.

83. Nanobody according to claim 75 or 82, wherein said Nanobody competes with gp130 for binding to said epitope of IL-6R.

84. Nanobody according to any of claims 75 to 83, that is in essentially isolated form.

85. Nanobody according to any of claims 75 or 84, that can specifically bind to IL-6R with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter or less, and preferably 10^{-7} to 10^{-12} moles/liter or less and more preferably 10^{-8} to 10^{-12} moles/liter.

86. Nanobody according to any of claims 75 to 85, that can specifically bind to IL-6R with a rate of association (k_{on} -rate) of between 10^2 $M^{-1}s^{-1}$ to about 10^7 $M^{-1}s^{-1}$, preferably

between $10^3 \text{ M}^{-1} \text{s}^{-1}$ and $10^7 \text{ M}^{-1} \text{s}^{-1}$, more preferably between $10^4 \text{ M}^{-1} \text{s}^{-1}$ and $10^7 \text{ M}^{-1} \text{s}^{-1}$, such as between $10^5 \text{ M}^{-1} \text{s}^{-1}$ and $10^7 \text{ M}^{-1} \text{s}^{-1}$.

87. Nanobody according to any of claims 75 to 86, that can specifically bind to IL-6R with a rate of dissociation (k_{off} rate) between 1 s^{-1} and 10^{-6} s^{-1} preferably between 10^{-2} s^{-1} and 10^{-6} s^{-1} , more preferably between 10^{-3} s^{-1} and 10^{-6} s^{-1} , such as between 10^{-4} s^{-1} and 10^{-6} s^{-1} .

88. Nanobody according to any of claims 75 to 87, that can specifically bind to IL-6R with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM.

89. Nanobody according to any of claims 75 to 88, that is a naturally occurring Nanobody (from any suitable species) or a synthetic or semi-synthetic Nanobody.

90. Nanobody according to any of claims 75 to 89 that is a V_{HH} sequence, a partially humanized V_{HH} sequence, a fully humanized V_{HH} sequence sequence, a camelized heavy chain variable domain or an Nanobody that has been obtained by techniques such as affinity maturation.

91. Nanobody according to any of claims 75 to 90, that

- a) has 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 399 to 471, in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences are disregarded; and in which:
- b) preferably one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table A-3.

92. Nanobody according to any of claims 75 to 91, in which:

- CDR1 is chosen from the group consisting of:
- a) the amino acid sequences of SEQ ID NO's: 93 to 143;

b) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 93 to 143;

c) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 93 to 143;

and/or

- CDR2 is chosen from the group consisting of:

d) the amino acid sequences of SEQ ID NO's: 195 to 245;

e) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 195 to 245;

f) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 195 to 245;

and/or

- CDR3 is chosen from the group consisting of:

g) the amino acid sequences of SEQ ID NO's: 297 to 347;

h) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 297 to 347;

i) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 297 to 347.

93. Nanobody according to any of claims 75 to 92, in which:

- CDR1 is chosen from the group consisting of:

a) the amino acid sequences of SEQ ID NO's: 93 to 143;

b) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 93 to 143;

c) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 93 to 143;

and

- CDR2 is chosen from the group consisting of:

d) the amino acid sequences of SEQ ID NO's: 195 to 245;

e) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 195 to 245;

- f) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 195 to 245;

and

- CDR3 is chosen from the group consisting of:
- g) the amino acid sequences of SEQ ID NO's: 297 to 347;
- h) amino acid sequences that have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 297 to 347;
- i) amino acid sequences that have 3, 2, or 1 amino acid difference with at least one of the amino acid sequences of SEQ ID NO's: 297 to 347.

94. Nanobody according to any of claims 75 to 93, in which the CDR sequences have at least 70% amino acid identity, preferably at least 80% amino acid identity, more preferably at least 90% amino acid identity, such as 95% amino acid identity or more or even essentially 100% amino acid identity with the CDR sequences of at least one of the amino acid sequences of SEQ ID NO's: 399 to 471.

95. Nanobody according to any of claims 75 to 94, which is a partially humanized Nanobody.

96. Nanobody according to any of claims 75 to 95, which is a fully humanized Nanobody.

97. Nanobody according to any of claims 75 to 96, that is chosen from the group consisting of SEQ ID NO's: 399 to 471 or from the group consisting of from amino acid sequences that have more than 80%, preferably more than 90%, more preferably more than 95%, such as 99% or more sequence identity (as defined herein) with at least one of the amino acid sequences of SEQ ID NO's: 399 to 471.

98. Nanobody according to any of claims 75 to 97, which is a humanized Nanobody.

99. Nanobody according to any of claims 75 to 98, that is chosen from the group consisting of SEQ ID NO's: 399 to 471.

100. Compound or construct, that comprises or essentially consists of one or more amino acid sequences according to any of claims 1 to 74 and/or one or more Nanobodies according to any of claims 75 to 99, and optionally further comprises one or more other groups, residues, moieties or binding units, optionally linked via one or more linkers.

101. Compound or construct according to claim 100, in which said one or more other groups, residues, moieties or binding units are amino acid sequences.

102. Compound or construct according to claims 100 or 101, in which said one or more linkers, if present, are one or more amino acid sequences.

103. Compound or construct according to any of claims 100 to 102, in which said one or more other groups, residues, moieties or binding units are immunoglobulin sequences.

104. Compound or construct according to any of claims 100 to 103, in which said one or more other groups, residues, moieties or binding units are chosen from the group consisting of domain antibodies, amino acid sequences that are suitable for use as a domain antibody, single domain antibodies, amino acid sequences that are suitable for use as a single domain antibody, "dAb"'s , amino acid sequences that are suitable for use as a dAb, or Nanobodies.

105. Compound or construct according to any of claims 100 to 104, in which said one or more amino acid sequences of the invention are immunoglobulin sequences.

106. Compound or construct according to any of claims 100 to 105, in which said one or more amino acid sequences of the invention are chosen from the group consisting of

domain antibodies, amino acid sequences that are suitable for use as a domain antibody, single domain antibodies, amino acid sequences that are suitable for use as a single domain antibody, "dAb"'s , amino acid sequences that are suitable for use as a dAb, or Nanobodies.

107. Compound or construct, that comprises or essentially consists of one or more Nanobodies according to any of claims 75 to 99 and in which said one or more other groups, residues, moieties or binding units are Nanobodies.

108. Compound or construct according to any of claims 100 to 107, which is a multivalent construct.

109. Compound or construct according to any of claims 100 to 108, which is a multispecific construct.

110. Compound or construct according to any of claims 100 to 109, which has an increased half-life, compared to the corresponding amino acid sequence according to any of claims 1 to 74 per se or Nanobody according to any of claims 75 to 99 per se, respectively.

111. Compound or construct according to any of claims 100 to 110, in which said one or more other groups, residues, moieties or binding units provide the compound or construct with increased half-life, compared to the corresponding amino acid sequence according to any of claims 1 to 74 per se or Nanobody according to any of claims 75 to 99 per se, respectively.

112. Compound or construct according to claim 111, in which said one or more other groups, residues, moieties or binding units that provide the compound or construct with increased half-life is chosen from the group consisting of serum proteins or fragments thereof, binding units that can bind to serum proteins, an Fc portion, and small proteins or peptides that can bind to serum proteins.

113. Compound or construct according to claims 111 or 112, in which said one or more other groups, residues, moieties or binding units that provide the compound or construct with increased half-life is chosen from the group consisting of human serum albumin or fragments thereof.

114. Compound or construct according to any of claims 111 or 112, in which said one or more other groups, residues, moieties or binding units that provide the compound or construct with increased half-life are chosen from the group consisting of binding units that can bind to serum albumin (such as human serum albumin) or a serum immunoglobulin (such as IgG).

115. Compound or construct according to any of claim 114, in which said one or more other groups, residues, moieties or binding units that provide the compound or construct with increased half-life are chosen from the group consisting of domain antibodies, amino acid sequences that are suitable for use as a domain antibody, single domain antibodies, amino acid sequences that are suitable for use as a single domain antibody, "dAb"'s, amino acid sequences that are suitable for use as a dAb, or Nanobodies that can bind to serum albumin (such as human serum albumin) or a serum immunoglobulin (such as IgG).

116. Compound or construct according to any of claim 115, in which said one or more other groups, residues, moieties or binding units that provide the compound or construct with increased half-life is Nanobody that can bind to serum albumin (such as human serum albumin) or a serum immunoglobulin (such as IgG).

117. Compound or construct according to any of claims 111 to 116, that has a serum half-life that is at least 1.5 times, preferably at least 2 times, such as at least 5 times, for example at least 10 times or more than 20 times, greater than the half-life of the

corresponding amino acid sequence according to any of claims 1 to 74 per se or Nanobody according to any of claims 75 to 99 per se, respectively.

118. Compound or construct according to any of claims 100 to 117, that has a serum half-life that is increased with more than 1 hours, preferably more than 2 hours, more preferably more than 6 hours, such as more than 12 hours, or even more than 24, 48 or 72 hours, compared to the corresponding amino acid sequence according to any of claims 1 to 74 per se or Nanobody according to any of claims 75 to 99 per se, respectively.

119. Compound or construct according to any of claims 100 to 118, that has a serum half-life in human of at least about 12 hours, preferably at least 24 hours, more preferably at least 48 hours, even more preferably at least 72 hours or more. For example, compounds or polypeptides of the invention may have a half-life of at least 5 days (such as about 5 to 10 days), at preferably at least 9 days (such as about 9 to 14 days), more preferably at least about 10 days (such as about 10 to 15 days), or at least about 11 days (such as about 11 to 16 days), more preferably at least about 12 days (such as about 12 to 18 days or more), or more than 14 days (such as about 14 to 19 days).

120. Monovalent construct, comprising or essentially consisting of one amino acid sequence according to any of claims 1 to 74 and/or one Nanobody according to any of claims 75 to 99.

121. Monovalent construct according to claim 120, in which said amino acid sequence of the invention is chosen from the group consisting of domain antibodies, amino acid sequences that are suitable for use as a domain antibody, single domain antibodies, amino acid sequences that are suitable for use as a single domain antibody, "dAb"'s, amino acid sequences that are suitable for use as a dAb, or Nanobodies.

122. Monovalent construct, comprising or essentially consisting of one Nanobody according to any of claims 75 to 99.

123. Nucleic acid or nucleotide sequence, that encodes an amino acid sequence according to any of claims 1 to 74, a Nanobody according to any of claims 75 to 99, a compound or construct according to any of claims 100 to 119, or a monovalent construct according to any of claims 120 to 122.

124. Nucleic acid or nucleotide sequence according to claim 123, that is in the form of a genetic construct.

125. Host or host cell that expresses, or that under suitable circumstances is capable of expressing, an amino acid sequence according to any of claims 1 to 74, a Nanobody according to any of claims 75 to 99, a compound or construct according to any of claims 100 to 119, or a monovalent construct according to any of claims 120 to 122; and/or that comprises a nucleic acid or nucleotide sequence according to claim 123 or 124.

126. Method for producing an amino acid sequence according to any of claims 1 to 74, a Nanobody according to any of claims 75 to 99, a compound or construct according to any of claims 100 to 119, or a monovalent construct according to any of claims 120 to 122, said method at least comprising the steps of:

- a) expressing, in a suitable host cell or host organism or in another suitable expression system, a nucleic acid or nucleotide sequence according to claim 123 or 124; optionally followed by:
- b) isolating and/or purifying an amino acid sequence according to any of claims 1 to 74, a Nanobody according to any of claims 75 to 99, a compound or construct according to any of claims 100 to 119, or a monovalent construct according to any of claims 120 to 122, thus obtained.

127. Method for producing an amino acid sequence according to any of claims 1 to 74, a Nanobody according to any of claims 75 to 99, a compound or construct according to

any of claims 100 to 119, or a monovalent construct according to any of claims 120 to 122, said method at least comprising the steps of:

- a) cultivating and/or maintaining a host or host cell according to claim 125 under conditions that are such that said host or host cell expresses and/or produces at least one amino acid sequence according to any of claims 1 to 74, Nanobody according to any of claims 75 to 99, compound or construct according to any of claims 100 to 119, or monovalent construct according to any of claims 120 to 122,

optionally followed by:

- b) isolating and/or purifying the amino acid sequence according to any of claims 1 to 74, Nanobody according to any of claims 75 to 99, compound or construct according to any of claims 100 to 119, or monovalent construct according to any of claims 120 to 122, thus obtained.

128. Composition, comprising at least one amino acid sequence according to any of claims 1 to 74, Nanobody according to any of claims 75 to 99, compound or construct according to any of claims 100 to 119, or monovalent construct according to any of claims 120 to 122, or nucleic acid or nucleotide sequence according to claims 123 or 124.

129. Composition according to claim 128, which is a pharmaceutical composition

130. Composition according to claims 128 or 129, which is a pharmaceutical composition, that further comprises at least one pharmaceutically acceptable carrier, diluent or excipient and/or adjuvant, and that optionally comprises one or more further pharmaceutically active polypeptides and/or compounds.

131. Method for the prevention and/or treatment of at least one of the diseases and disorders associated with IL-6R and/or with the IL-6/IL-6R complex and/or with the signalling pathways and/or the biological functions and responses in which IL-6 and/or the IL-6/IL-6R complex are involved, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of at least one amino acid sequence according to any of claims 1 to 74, a Nanobody according to any of claims 75 to 99, a compound or

construct according to any of claims 100 to 119, or a monovalent construct according to any of claims 120 to 122, or composition according to any of claims 128 to 130.

132. Method according to claim 131, wherein said diseases and disorders associated with IL-6R and/or with the IL-6/IL-6R complex and/or with the signalling pathways and/or the biological functions and responses in which IL-6 and/or the IL-6/IL-6R complex are involved are chosen from the group consisting of sepsis, various forms of cancer, bone resorption, osteoporosis, cachexia, psoriasis, mesangial proliferative glomerulonephritis, Kaposi's sarcoma, AIDS-related lymphoma, and inflammatory diseases.

133. Method according to claim 132, wherein said various forms of cancer are chosen from the group consisting of multiple myeloma disease (MM), renal cell carcinoma (RCC), plasma cell leukaemia, lymphoma, B-lymphoproliferative disorder (BLPD), and prostate cancer.

134. Method according to claim 132, wherein said inflammatory diseases are chosen from the group consisting of rheumatoid arthritis, systemic onset juvenile idiopathic arthritis, hypergammaglobulinemia, Crohn's disease, ulcerative colitis, systemic lupus erythematosus (SLE), multiple sclerosis, Castleman's disease, IgM gammopathy, cardiac myxoma, asthma, allergic asthma and autoimmune insulin-dependent diabetes mellitus.

135. Method for the prevention and/or treatment of at least one disease or disorder that is associated with IL-6R, with its biological or pharmacological activity, and/or with the biological pathways or signalling in which IL-6R is involved, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of at least one amino acid sequence according to any of claims 1 to 74, Nanobody according to any of claims 75 to 99, compound or construct according to any of claims 100 to 119, or monovalent construct according to any of claims 120 to 122, or composition according to any of claims 128 to 130.

136. Method for the prevention and/or treatment of at least one disease or disorder that can be prevented and/or treated by administering, to a subject in need thereof, an amino acid sequence according to any of claims 1 to 74, a Nanobody according to any of claims 75 to 99, a compound or construct according to any of claims 100 to 119, or a monovalent construct according to any of claims 120 to 122, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of at least one amino acid sequence according to any of claims 1 to 74, Nanobody according to any of claims 75 to 99, compound or construct according to any of claims 100 to 119, or monovalent construct according to any of claims 120 to 122, or composition according to any of claims 128 to 130.

137. Method for immunotherapy, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of at least one amino acid sequence according to any of claims 1 to 74, Nanobody according to any of claims 75 to 99, compound or construct according to any of claims 100 to 119, or monovalent construct according to any of claims 120 to 122, or composition according to any of claims 128 to 130.

138. Use of an amino acid sequence according to any of claims 1 to 74, Nanobody according to any of claims 75 to 99, compound or construct according to any of claims 100 to 119, or monovalent construct according to any of claims 120 to 122, in the preparation of a pharmaceutical composition for prevention and/or treatment of at least one of the diseases and disorders associated with IL-6R and/or with the IL-6/IL-6R complex and/or with the signalling pathways and/or the biological functions and responses in which IL-6 and/or the IL-6/IL-6R complex are involved; and/or for use in one or more of the methods according to claims 131 to 137.

139. Part or fragment of an amino acid sequence according to any of claims 1 to 74, or of a Nanobody according to any of claims 75 to 99.

140. Part or fragment according to claim 139, that can specifically bind to IL-6R.

141. Part or fragment according to claim 140, wherein said part or fragment binds to an epitope of IL-6R, such that the interaction between IL-6R and IL-6 is modulated.

142. Part or fragment according to claims 140 or 141, wherein said part or fragment competes with IL-6 for binding to said epitope of IL-6R.

143. Part or fragment according to claims 141 or 142, wherein said epitope is located in an extracellular domain of IL-6R.

144. Part or fragment according to claim 143, wherein said epitope is located in the extracellular D3 domain of IL-6R.

145. Part or fragment according to claims 143 or 144, wherein said epitope comprises at least a fragment of the 18 contact residue stretch of the extracellular D3 domain of IL-6R, which is involved in the interaction between IL-6 and IL-6R.

146. Part or fragment according to claims 143 to 145, wherein said epitope comprises amino acid residue Phe229 and/or amino acid residue Phe279 of the extracellular D3 domain of IL-6R.

147. Part or fragment according to claim 140, wherein said part or fragment binds to an epitope of IL-6R, such that the interaction between the IL-6/IL-6R complex and gp130 is modulated.

148. Part or fragment according to claims 140 or 147, wherein said part or fragment competes with gp130 for binding to said epitope of IL-6R.

149. Part of fragment according to any of claims 139 to 148, that can specifically bind to IL-6R with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter or less, and preferably 10^{-7} to 10^{-12} moles/liter or less and more preferably 10^{-8} to 10^{-12} moles/liter.

150. Part or fragment according to any of claims 139 to 149, that can specifically bind to IL-6R with a rate of association (k_{on} -rate) of between $10^2 \text{ M}^{-1}\text{s}^{-1}$ to about $10^7 \text{ M}^{-1}\text{s}^{-1}$, preferably between $10^3 \text{ M}^{-1}\text{s}^{-1}$ and $10^7 \text{ M}^{-1}\text{s}^{-1}$, more preferably between $10^4 \text{ M}^{-1}\text{s}^{-1}$ and $10^7 \text{ M}^{-1}\text{s}^{-1}$, such as between $10^5 \text{ M}^{-1}\text{s}^{-1}$ and $10^7 \text{ M}^{-1}\text{s}^{-1}$.

151. Part or fragment according to any of claims 139 to 150, that can specifically bind to IL-6R with a rate of dissociation (k_{off} rate) between 1s^{-1} and 10^{-6} s^{-1} preferably between 10^{-2} s^{-1} and 10^{-6} s^{-1} , more preferably between 10^{-3} s^{-1} and 10^{-6} s^{-1} , such as between 10^{-4} s^{-1} and 10^{-6} s^{-1} .

152. Compound or construct, that comprises or essentially consists of one or more parts or fragments according to any of claims 139 to 151, and optionally further comprises one or more other groups, residues, moieties or binding units, optionally linked via one or more linkers.

153. Compound or construct according to claim 152, in which said one or more other groups, residues, moieties or binding units are amino acid sequences.

154. Compound or construct according to claims 152 or 153, in which said one or more linkers, if present, are one or more amino acid sequences.

155. Nucleic acid or nucleotide sequence, that encodes a part or fragment according to any of claims 139 to 151 or a compound or construct according to any of claims 152 to 154.

156. Composition, comprising at least one part or fragment according to any of claims 139 to 151, compound or construct according to any of claims 152 to 154, or nucleic acid or nucleotide sequence according to claim 155.

157. Derivative of an amino acid sequence according to any of claims 1 to 74, or of a Nanobody according to any of claims 75 to 99.

158. Derivative according to claim 157, that can specifically bind to IL-6R.

159. Derivative according to claim 158, wherein said derivative binds to an epitope of IL-6R, such that the interaction between IL-6R and IL-6 is modulated.

160. Derivative according to claims 158 or 159, wherein said derivative competes with IL-6 for binding to said epitope of IL-6R.

161. Derivative according to claims 159 or 160, wherein said epitope is located in an extracellular domain of IL-6R.

162. Derivative according to claim 161, wherein said epitope is located in the extracellular D3 domain of IL-6R.

163. Derivative according to claims 161 or 162, wherein said epitope comprises at least a fragment of the 18 contact residue stretch of the extracellular D3 domain of IL-6R, which is involved in the interaction between IL-6 and IL-6R.

164. Derivative according to claims 161 to 163, wherein said epitope comprises amino acid residue Phe229 and/or amino acid residue Phe279 of the extracellular D3 domain of IL-6R.

165. Derivative according to claim 158, wherein said derivative binds to an epitope of IL-6R, such that the interaction between the IL-6/IL-6R complex and gp130 is modulated.

166. Derivative according to claims 158 or 165, wherein said derivative competes with gp130 for binding to said epitope of IL-6R.

167. Derivative according to any of claims 158 to 166, that can specifically bind to IL-6R with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter or less, and preferably 10^{-7} to 10^{-12} moles/liter or less and more preferably 10^{-8} to 10^{-12} moles/liter.

168. Derivative according to any of claims 158 to 167, that can specifically bind to IL-6R with a rate of association (k_{on} -rate) of between $10^2 \text{ M}^{-1} \text{s}^{-1}$ to about $10^7 \text{ M}^{-1} \text{s}^{-1}$, preferably between $10^3 \text{ M}^{-1} \text{s}^{-1}$ and $10^7 \text{ M}^{-1} \text{s}^{-1}$, more preferably between $10^4 \text{ M}^{-1} \text{s}^{-1}$ and $10^7 \text{ M}^{-1} \text{s}^{-1}$, such as between $10^5 \text{ M}^{-1} \text{s}^{-1}$ and $10^7 \text{ M}^{-1} \text{s}^{-1}$.

169. Derivative according to any of claims 158 to 168, that can specifically bind to IL-6R with a rate of dissociation (k_{off} rate) between 1 s^{-1} and 10^{-6} s^{-1} preferably between 10^{-2} s^{-1} and 10^{-6} s^{-1} , more preferably between 10^{-3} s^{-1} and 10^{-6} s^{-1} , such as between 10^{-4} s^{-1} and 10^{-6} s^{-1} .

170. Derivative of a compound or construct according to any of claims 108 to 127 or 152 to 154.

171. Derivative according to claim 170, that can specifically bind to IL-6R.

172. Derivative according to claim 171, wherein said derivative binds to an epitope of IL-6R, such that the interaction between IL-6R and IL-6 is modulated.

173. Derivative according to claims 171 or 172, wherein said derivative competes with IL-6 for binding to said epitope of IL-6R.

174. Derivative according to claims 172 or 173, wherein said epitope is located in an extracellular domain of IL-6R.

175. Derivative according to claim 174, wherein said epitope is located in the extracellular D3 domain of IL-6R.

176. Derivative according to claims 174 or 175, wherein said epitope comprises at least a fragment of the 18 contact residue stretch of the extracellular D3 domain of IL-6R, which is involved in the interaction between IL-6 and IL-6R.

177. Derivative according to claims 174 to 176, wherein said epitope comprises amino acid residue Phe229 and/or amino acid residue Phe279 of the extracellular D3 domain of IL-6R.

178. Derivative according to claim 171, wherein said derivative binds to an epitope of IL-6R, such that the interaction between the IL-6/IL-6R complex and gp130 is modulated.

179. Derivative according to claims 171 or 178, wherein said derivative competes with gp130 for binding to said epitope of IL-6R.

180. Derivative according to any of claims 171 to 179, that can specifically bind to IL-6R with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter or less, and preferably 10^{-7} to 10^{-12} moles/liter or less and more preferably 10^{-8} to 10^{-12} moles/liter.

181. Derivative according to any of claims 171 to 180, that can specifically bind to IL-6R with a rate of association (k_{on} -rate) of between $10^2 \text{ M}^{-1} \text{ s}^{-1}$ to about $10^7 \text{ M}^{-1} \text{ s}^{-1}$, preferably between $10^3 \text{ M}^{-1} \text{ s}^{-1}$ and $10^7 \text{ M}^{-1} \text{ s}^{-1}$, more preferably between $10^4 \text{ M}^{-1} \text{ s}^{-1}$ and $10^7 \text{ M}^{-1} \text{ s}^{-1}$, such as between $10^5 \text{ M}^{-1} \text{ s}^{-1}$ and $10^7 \text{ M}^{-1} \text{ s}^{-1}$.

182. Derivative according to any of claims 171 to 181, that can specifically bind to IL-6R with a rate of dissociation (k_{off} rate) between 1 s^{-1} and 10^{-6} s^{-1} preferably between 10^{-2} s^{-1} and 10^{-6} s^{-1} , more preferably between 10^{-3} s^{-1} and 10^{-6} s^{-1} , such as between 10^{-4} s^{-1} and 10^{-6} s^{-1} .

183. Derivative according to any of claims 157 to 182, that has a serum half-life that is at least 1.5 times, preferably at least 2 times, such as at least 5 times, for example at least 10 times or more than 20 times, greater than the half-life of the corresponding amino acid

sequence according to any of claims 1 to 74 per se or Nanobody according to any of claims 75 to 99 per se, respectively.

184. Derivative according to any of claims 157 to 183, that has a serum half-life that is increased with more than 1 hours, preferably more than 2 hours, more preferably more than 6 hours, such as more than 12 hours, or even more than 24, 48 or 72 hours, compared to the corresponding amino acid sequence according to any of claims 1 to 74 per se or Nanobody according to any of claims 75 to 99 per se, respectively.

185. Derivative according to any of claims 157 to 184, that has a serum half-life in human of at least about 12 hours, preferably at least 24 hours, more preferably at least 48 hours, even more preferably at least 72 hours or more. For example, compounds or polypeptides of the invention may have a half-life of at least 5 days (such as about 5 to 10 days), at preferably at least 9 days (such as about 9 to 14 days), more preferably at least about 10 days (such as about 10 to 15 days), or at least about 11 days (such as about 11 to 16 days), more preferably at least about 12 days (such as about 12 to 18 days or more), or more than 14 days (such as about 14 to 19 days).

186. Derivative according to any of claims 157 to 185, that is a pegylated derivative.

187. Compound or construct, that comprises or essentially consists of one or more derivatives according to any of claims 157 to 186, and optionally further comprises one or more other groups, residues, moieties or binding units, optionally linked via one or more linkers.

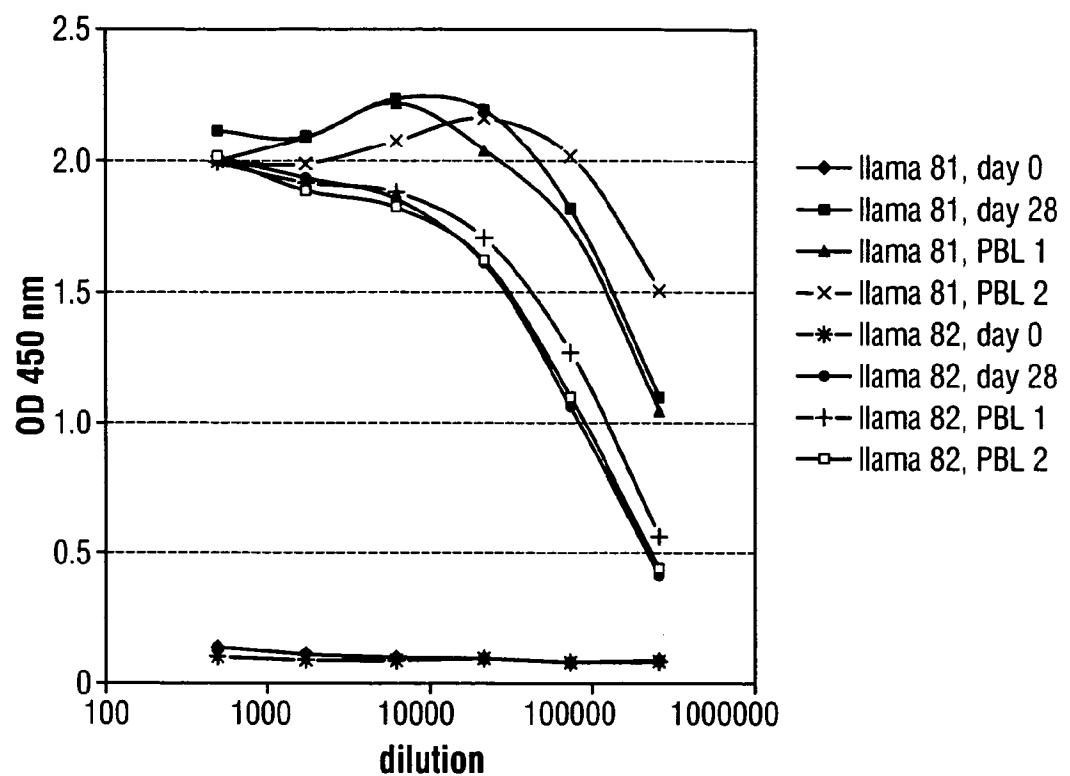
188. Compound or construct according to claim 187, in which said one or more other groups, residues, moieties or binding units are amino acid sequences.

189. Compound or construct according to claims 187 or 188, in which said one or more linkers, if present, are one or more amino acid sequences.

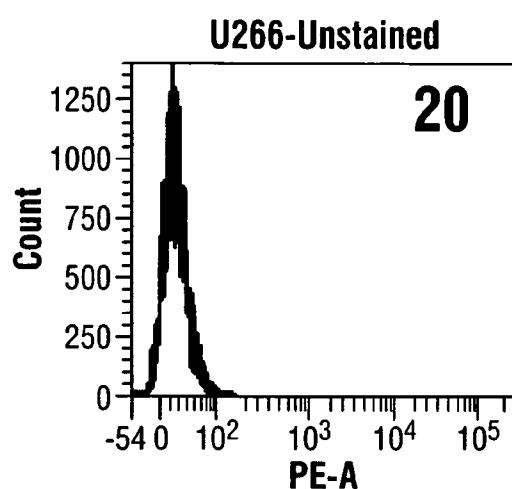
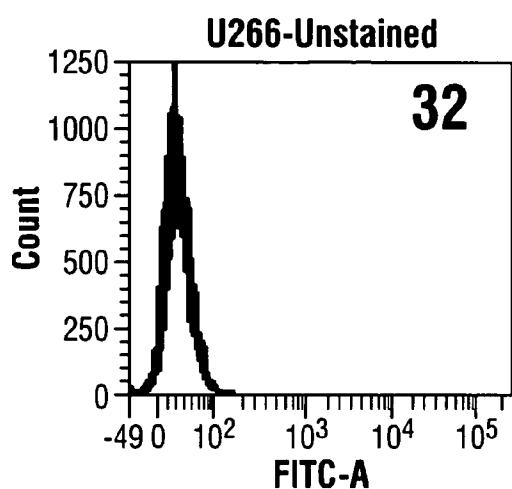
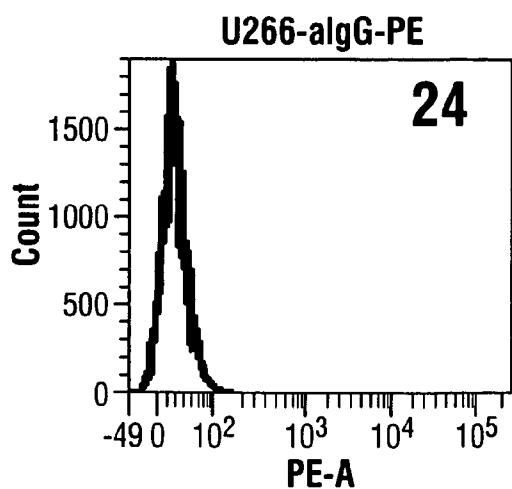
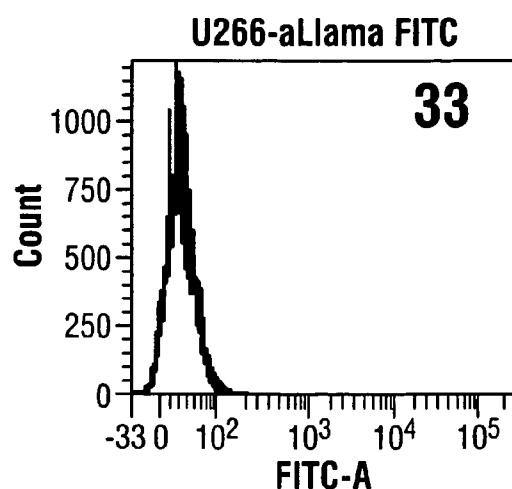
190. Nucleic acid or nucleotide sequence, that encodes (the amino acid sequence of) a derivative according to any of claims 157 to 186 or (the amino acid sequence of) a compound or construct according to any of claims 187 to 189.

191. Composition, comprising at least one derivative to any of claims 157 to 186, compound or construct according to any of claims 187 to 189, or nucleic acid or nucleotide sequence according to claim 190.

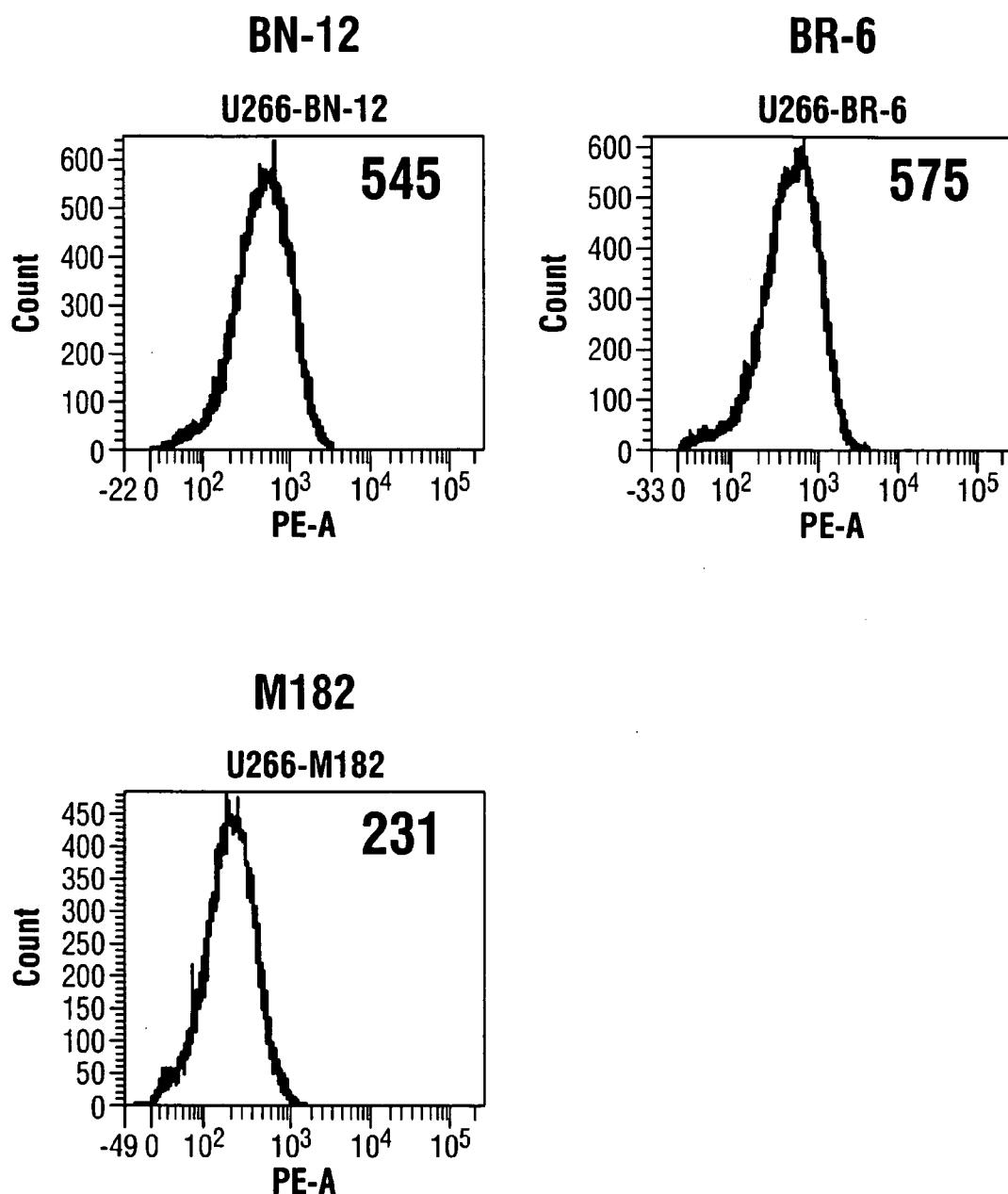
1 / 62

Fig. 1

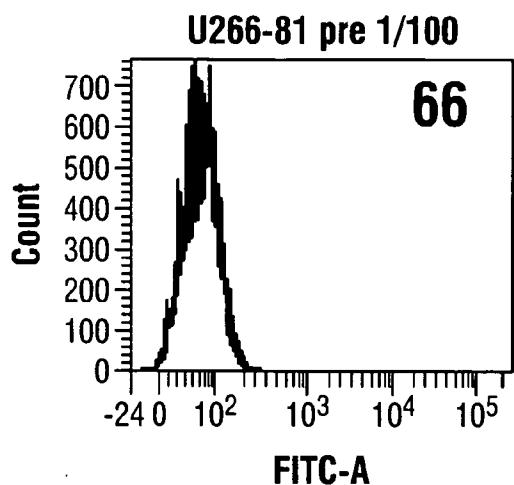
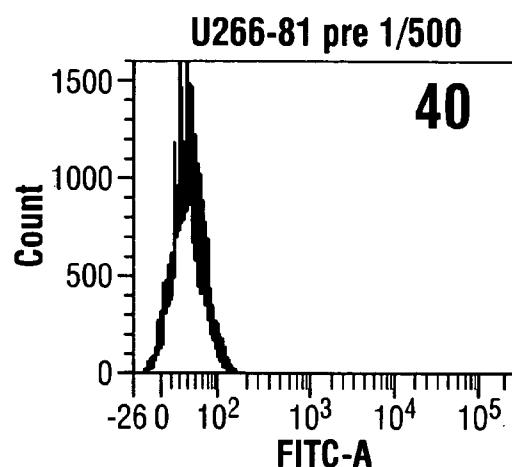
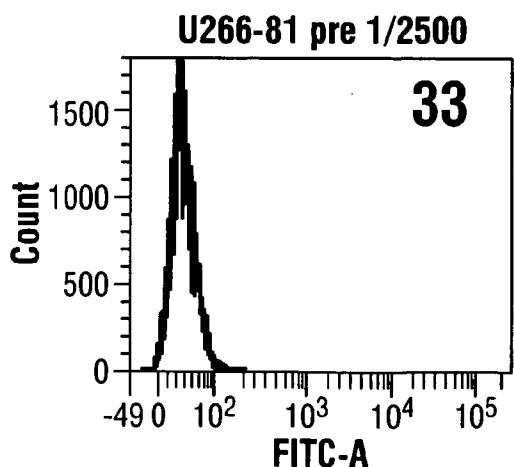
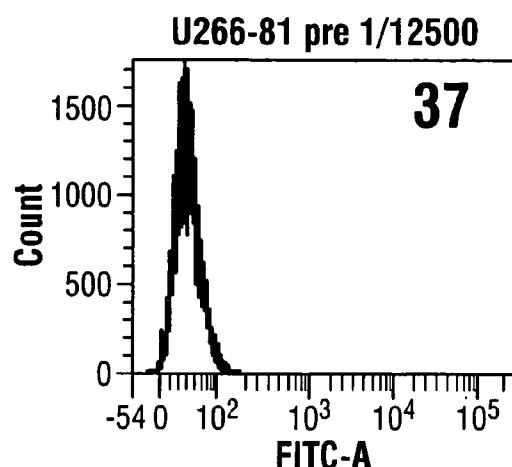
2 / 62

Fig. 2**U266B1****Unstained****a-mouse-IgG-PE****a-Llama-IgG-FITC**

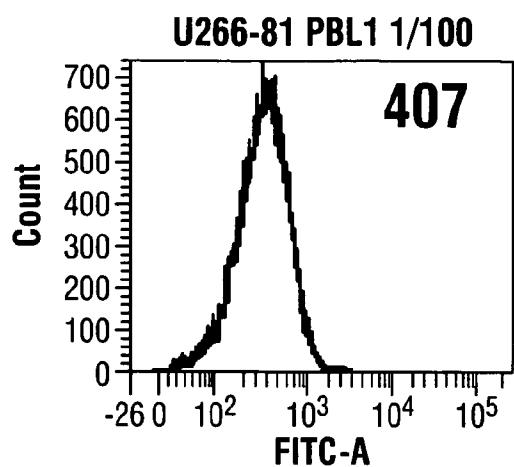
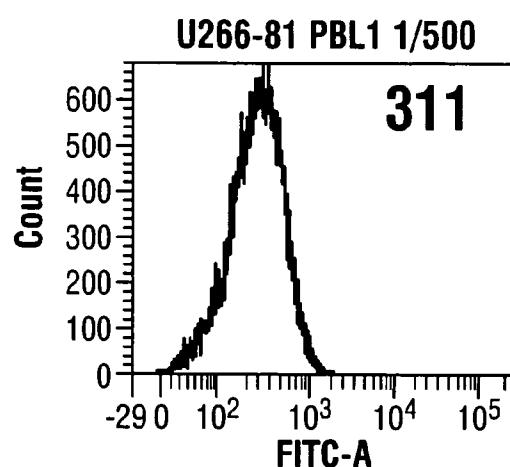
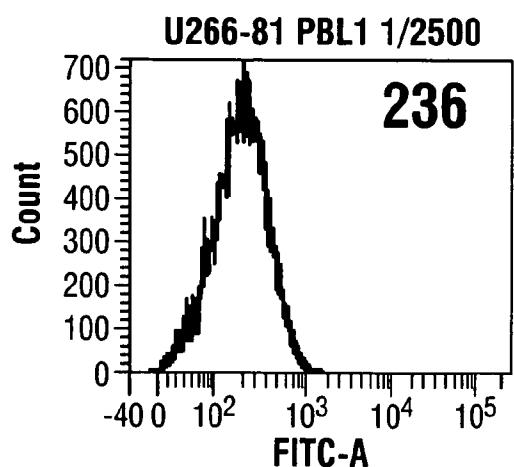
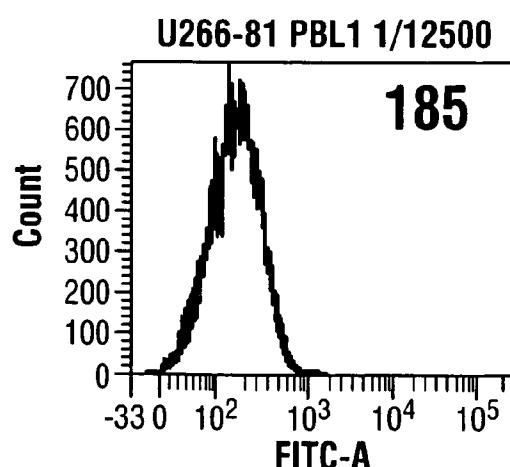
3 / 62

Fig. 2 (cont.)**U266B1**

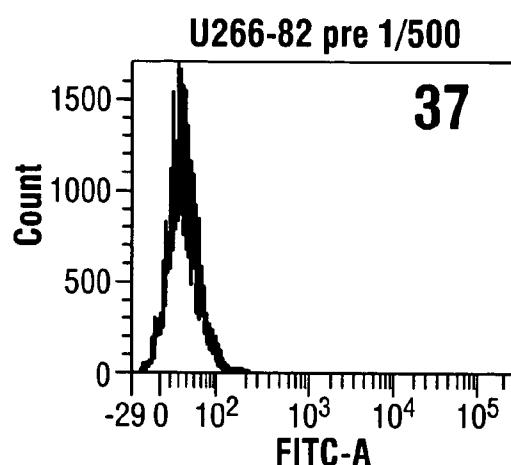
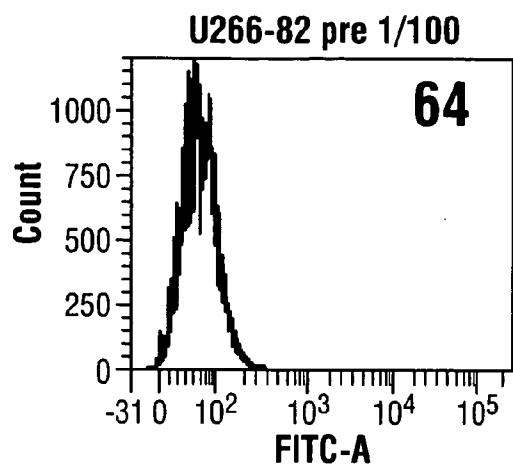
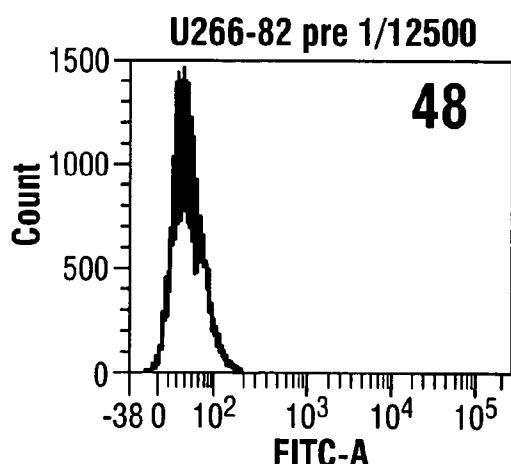
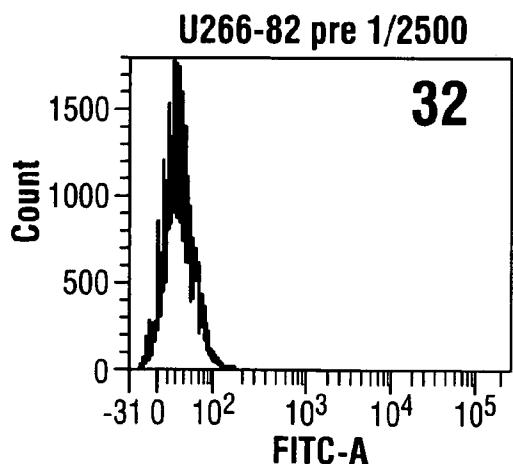
4 / 62

Fig. 2 (cont.)**U266B1****LI81 pre****1/100****1/500****1/2500****1/12500**

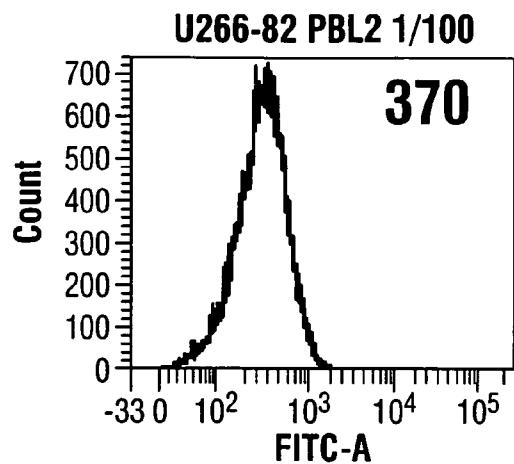
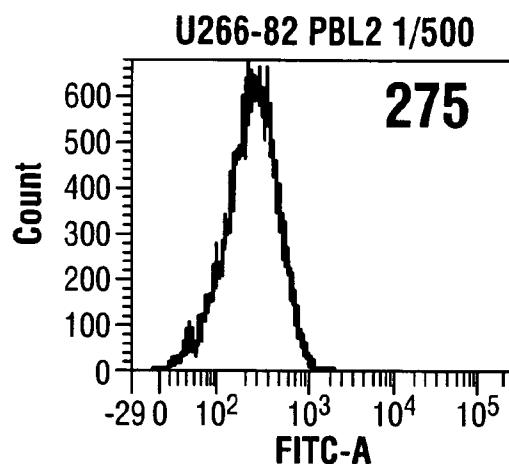
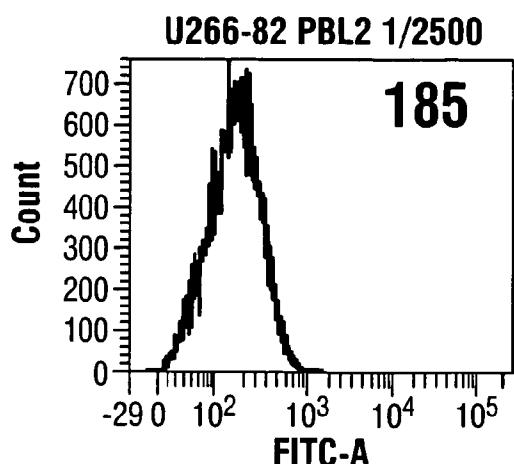
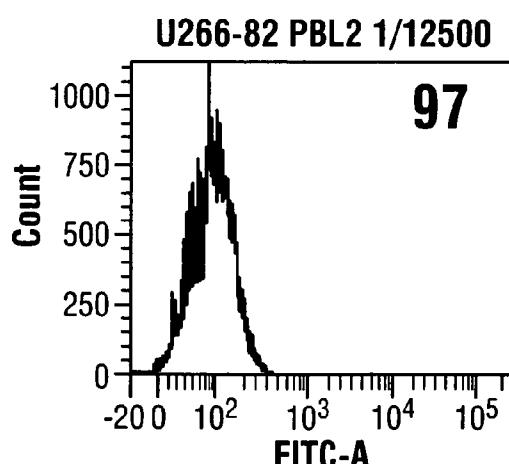
5 / 62

Fig. 2 (cont.)**U266B1****LI81 PBL1****1/100****1/500****1/2500****1/12500**

6 / 62

Fig. 2 (cont.)**U266B1****LI82 pre****1/100****1/500****1/2500****1/12500**

7 / 62

Fig. 2 (cont.)**U266B1****LI82 PBL2****1/100****1/500****1/2500****1/12500**

8 / 62

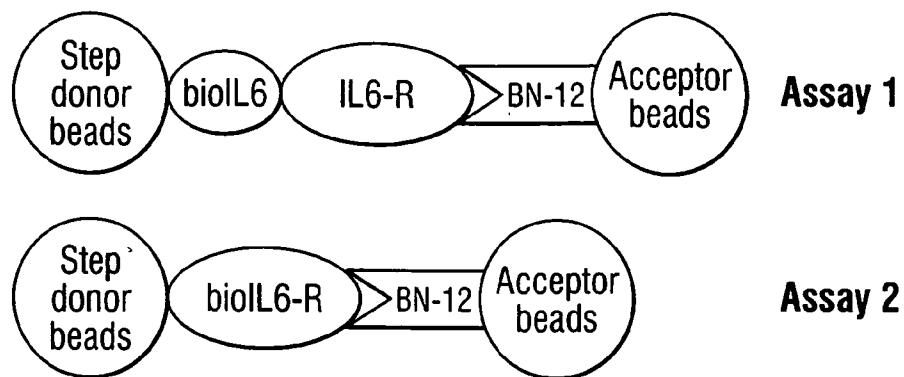
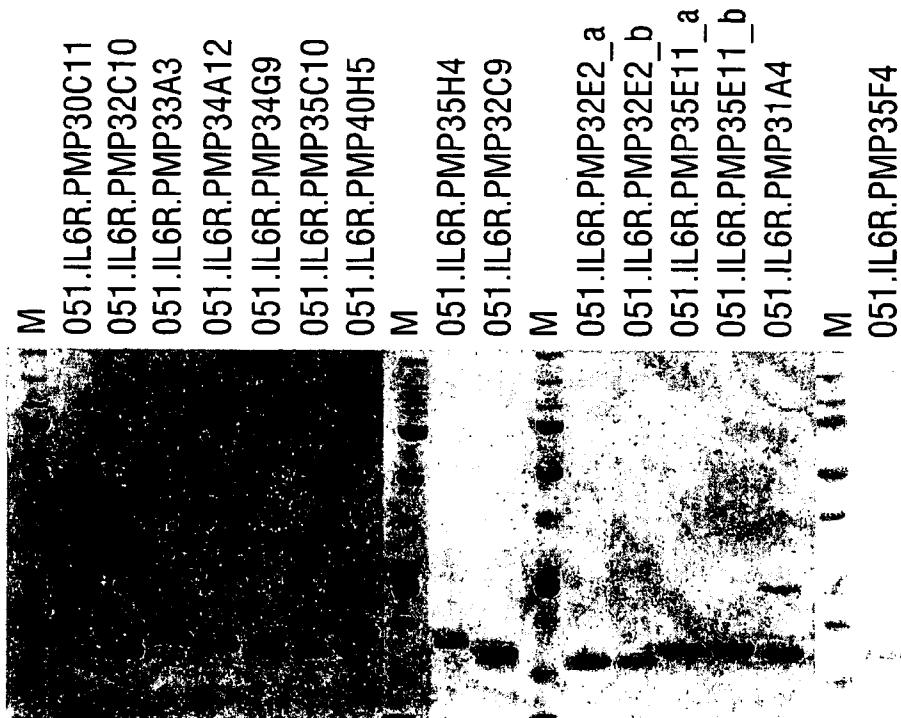
Fig. 3**Fig. 4**

Fig. 5

EVO¹VE²GG³EL⁴V⁵Q⁶EG⁷S⁸R⁹U¹⁰S¹¹C¹²A¹³S¹⁴G¹⁵F¹⁶T¹⁷G¹⁸S¹⁹W²⁰R²¹Q²²A²³P²⁴G²⁵K²⁶P²⁷E²⁸W²⁹S³⁰A³¹S³²G³³F³⁴T³⁵G³⁶S³⁷Y³⁸D³⁹S⁴⁰W⁴¹R⁴²Q⁴³A⁴⁴P⁴⁵G⁴⁶K⁴⁷P⁴⁸E⁴⁹W⁵⁰S⁵¹A⁵²S⁵³G⁵⁴F⁵⁵T⁵⁶G⁵⁷S⁵⁸Y⁵⁹D⁶⁰S⁶¹W⁶²R⁶³Q⁶⁴A⁶⁵P⁶⁶G⁶⁷K⁶⁸P⁶⁹E⁷⁰W⁷¹S⁷²A⁷³S⁷⁴G⁷⁵F⁷⁶T⁷⁷G⁷⁸S⁷⁹Y⁸⁰D⁸¹S⁸²W⁸³R⁸⁴Q⁸⁵A⁸⁶P⁸⁷G⁸⁸K⁸⁹P⁹⁰E⁹¹W⁹²S⁹³A⁹⁴S⁹⁵G⁹⁶F⁹⁷T⁹⁸G⁹⁹S¹⁰⁰Y¹⁰¹D¹⁰²S¹⁰³W¹⁰⁴R¹⁰⁵Q¹⁰⁶A¹⁰⁷P¹⁰⁸G¹⁰⁹K¹¹⁰P¹¹¹E¹¹²W¹¹³S¹¹⁴A¹¹⁵S¹¹⁶G¹¹⁷F¹¹⁸T¹¹⁹G¹²⁰S¹²¹Y¹²²D¹²³S¹²⁴W¹²⁵R¹²⁶Q¹²⁷A¹²⁸P¹²⁹G¹³⁰K¹³¹P¹³²E¹³³W¹³⁴S¹³⁵A¹³⁶S¹³⁷G¹³⁸F¹³⁹T¹⁴⁰G¹⁴¹S¹⁴²Y¹⁴³D¹⁴⁴S¹⁴⁵W¹⁴⁶R¹⁴⁷Q¹⁴⁸A¹⁴⁹P¹⁵⁰G¹⁵¹K¹⁵²P¹⁵³E¹⁵⁴W¹⁵⁵S¹⁵⁶A¹⁵⁷S¹⁵⁸G¹⁵⁹F¹⁶⁰T¹⁶¹G¹⁶²S¹⁶³Y¹⁶⁴D¹⁶⁵S¹⁶⁶W¹⁶⁷R¹⁶⁸Q¹⁶⁹A¹⁷⁰P¹⁷¹G¹⁷²K¹⁷³P¹⁷⁴E¹⁷⁵W¹⁷⁶S¹⁷⁷A¹⁷⁸S¹⁷⁹G¹⁸⁰F¹⁸¹T¹⁸²G¹⁸³S¹⁸⁴Y¹⁸⁵D¹⁸⁶S¹⁸⁷W¹⁸⁸R¹⁸⁹Q¹⁹⁰A¹⁹¹P¹⁹²G¹⁹³K¹⁹⁴P¹⁹⁵E¹⁹⁶W¹⁹⁷S¹⁹⁸A¹⁹⁹S²⁰⁰G²⁰¹F²⁰²T²⁰³G²⁰⁴S²⁰⁵Y²⁰⁶D²⁰⁷S²⁰⁸W²⁰⁹R²¹⁰Q²¹¹A²¹²P²¹³G²¹⁴K²¹⁵P²¹⁶E²¹⁷W²¹⁸S²¹⁹A²²⁰S²²¹G²²²F²²³T²²⁴G²²⁵S²²⁶Y²²⁷D²²⁸S²²⁹W²³⁰R²³¹Q²³²A²³³P²³⁴G²³⁵K²³⁶P²³⁷E²³⁸W²³⁹S²⁴⁰A²⁴¹S²⁴²G²⁴³F²⁴⁴T²⁴⁵G²⁴⁶S²⁴⁷Y²⁴⁸D²⁴⁹S²⁵⁰W²⁵¹R²⁵²Q²⁵³A²⁵⁴P²⁵⁵G²⁵⁶K²⁵⁷P²⁵⁸E²⁵⁹W²⁶⁰S²⁶¹A²⁶²S²⁶³G²⁶⁴F²⁶⁵T²⁶⁶G²⁶⁷S²⁶⁸Y²⁶⁹D²⁷⁰S²⁷¹W²⁷²R²⁷³Q²⁷⁴A²⁷⁵P²⁷⁶G²⁷⁷K²⁷⁸P²⁷⁹E²⁸⁰W²⁸¹S²⁸²A²⁸³S²⁸⁴G²⁸⁵F²⁸⁶T²⁸⁷G²⁸⁸S²⁸⁹Y²⁹⁰D²⁹¹S²⁹²W²⁹³R²⁹⁴Q²⁹⁵A²⁹⁶P²⁹⁷G²⁹⁸K²⁹⁹P³⁰⁰E³⁰¹W³⁰²S³⁰³A³⁰⁴S³⁰⁵G³⁰⁶F³⁰⁷T³⁰⁸G³⁰⁹S³¹⁰Y³¹¹D³¹²S³¹³W³¹⁴R³¹⁵Q³¹⁶A³¹⁷P³¹⁸G³¹⁹K³²⁰P³²¹E³²²W³²³S³²⁴A³²⁵S³²⁶G³²⁷F³²⁸T³²⁹G³³⁰S³³¹Y³³²D³³³S³³⁴W³³⁵R³³⁶Q³³⁷A³³⁸P³³⁹G³⁴⁰K³⁴¹P³⁴²E³⁴³W³⁴⁴S³⁴⁵A³⁴⁶S³⁴⁷G³⁴⁸F³⁴⁹T³⁵⁰G³⁵¹S³⁵²Y³⁵³D³⁵⁴S³⁵⁵W³⁵⁶R³⁵⁷Q³⁵⁸A³⁵⁹P³⁶⁰G³⁶¹K³⁶²P³⁶³E³⁶⁴W³⁶⁵S³⁶⁶A³⁶⁷S³⁶⁸G³⁶⁹F³⁷⁰T³⁷¹G³⁷²S³⁷³Y³⁷⁴D³⁷⁵S³⁷⁶W³⁷⁷R³⁷⁸Q³⁷⁹A³⁸⁰P³⁸¹G³⁸²K³⁸³P³⁸⁴E³⁸⁵W³⁸⁶S³⁸⁷A³⁸⁸S³⁸⁹G³⁹⁰F³⁹¹T³⁹²G³⁹³S³⁹⁴Y³⁹⁵D³⁹⁶S³⁹⁷W³⁹⁸R³⁹⁹Q⁴⁰⁰A⁴⁰¹P⁴⁰²G⁴⁰³K⁴⁰⁴P⁴⁰⁵E⁴⁰⁶W⁴⁰⁷S⁴⁰⁸A⁴⁰⁹S⁴¹⁰G⁴¹¹F⁴¹²T⁴¹³G⁴¹⁴S⁴¹⁵Y⁴¹⁶D⁴¹⁷S⁴¹⁸W⁴¹⁹R⁴²⁰Q⁴²¹A⁴²²P⁴²³G⁴²⁴K⁴²⁵P⁴²⁶E⁴²⁷W⁴²⁸S⁴²⁹A⁴³⁰S⁴³¹G⁴³²F⁴³³T⁴³⁴G⁴³⁵S⁴³⁶Y⁴³⁷D⁴³⁸S⁴³⁹W⁴⁴⁰R⁴⁴¹Q⁴⁴²A⁴⁴³P⁴⁴⁴G⁴⁴⁵K⁴⁴⁶P⁴⁴⁷E⁴⁴⁸W⁴⁴⁹S⁴⁵⁰A⁴⁵¹S⁴⁵²G⁴⁵³F⁴⁵⁴T⁴⁵⁵G⁴⁵⁶S⁴⁵⁷Y⁴⁵⁸D⁴⁵⁹S⁴⁶⁰W⁴⁶¹R⁴⁶²Q⁴⁶³A⁴⁶⁴P⁴⁶⁵G⁴⁶⁶K⁴⁶⁷P⁴⁶⁸E⁴⁶⁹W⁴⁷⁰S⁴⁷¹A⁴⁷²S⁴⁷³G⁴⁷⁴F⁴⁷⁵T⁴⁷⁶G⁴⁷⁷S⁴⁷⁸Y⁴⁷⁹D⁴⁸⁰S⁴⁸¹W⁴⁸²R⁴⁸³Q⁴⁸⁴A⁴⁸⁵P⁴⁸⁶G⁴⁸⁷K⁴⁸⁸P⁴⁸⁹E⁴⁹⁰W⁴⁹¹S⁴⁹²A⁴⁹³S⁴⁹⁴G⁴⁹⁵F⁴⁹⁶T⁴⁹⁷G⁴⁹⁸S⁴⁹⁹Y⁵⁰⁰D⁵⁰¹S⁵⁰²W⁵⁰³R⁵⁰⁴Q⁵⁰⁵A⁵⁰⁶P⁵⁰⁷G⁵⁰⁸K⁵⁰⁹P⁵¹⁰E⁵¹¹W⁵¹²S⁵¹³A⁵¹⁴S⁵¹⁵G⁵¹⁶F⁵¹⁷T⁵¹⁸G⁵¹⁹S⁵²⁰Y⁵²¹D⁵²²S⁵²³W⁵²⁴R⁵²⁵Q⁵²⁶A⁵²⁷P⁵²⁸G⁵²⁹K⁵³⁰P⁵³¹E⁵³²W⁵³³S⁵³⁴A⁵³⁵S⁵³⁶G⁵³⁷F⁵³⁸T⁵³⁹G⁵⁴⁰S⁵⁴¹Y⁵⁴²D⁵⁴³S⁵⁴⁴W⁵⁴⁵R⁵⁴⁶Q⁵⁴⁷A⁵⁴⁸P⁵⁴⁹G⁵⁵⁰K⁵⁵¹P⁵⁵²E⁵⁵³W⁵⁵⁴S⁵⁵⁵A⁵⁵⁶S⁵⁵⁷G⁵⁵⁸F⁵⁵⁹T⁵⁶⁰G⁵⁶¹S⁵⁶²Y⁵⁶³D⁵⁶⁴S⁵⁶⁵W⁵⁶⁶R⁵⁶⁷Q⁵⁶⁸A⁵⁶⁹P⁵⁷⁰G⁵⁷¹K⁵⁷²P⁵⁷³E⁵⁷⁴W⁵⁷⁵S⁵⁷⁶A⁵⁷⁷S⁵⁷⁸G⁵⁷⁹F⁵⁸⁰T⁵⁸¹G⁵⁸²S⁵⁸³Y⁵⁸⁴D⁵⁸⁵S⁵⁸⁶W⁵⁸⁷R⁵⁸⁸Q⁵⁸⁹A⁵⁹⁰P⁵⁹¹G⁵⁹²K⁵⁹³P⁵⁹⁴E⁵⁹⁵W⁵⁹⁶S⁵⁹⁷A⁵⁹⁸S⁵⁹⁹G⁶⁰⁰F⁶⁰¹T⁶⁰²G⁶⁰³S⁶⁰⁴Y⁶⁰⁵D⁶⁰⁶S⁶⁰⁷W⁶⁰⁸R⁶⁰⁹Q⁶¹⁰A⁶¹¹P⁶¹²G⁶¹³K⁶¹⁴P⁶¹⁵E⁶¹⁶W⁶¹⁷S⁶¹⁸A⁶¹⁹S⁶²⁰G⁶²¹F⁶²²T⁶²³G⁶²⁴S⁶²⁵Y⁶²⁶D⁶²⁷S⁶²⁸W⁶²⁹R⁶³⁰Q⁶³¹A⁶³²P⁶³³G⁶³⁴K⁶³⁵P⁶³⁶E⁶³⁷W⁶³⁸S⁶³⁹A⁶⁴⁰S⁶⁴¹G⁶⁴²F⁶⁴³T⁶⁴⁴G⁶⁴⁵S⁶⁴⁶Y⁶⁴⁷D⁶⁴⁸S⁶⁴⁹W⁶⁵⁰R⁶⁵¹Q⁶⁵²A⁶⁵³P⁶⁵⁴G⁶⁵⁵K⁶⁵⁶P⁶⁵⁷E⁶⁵⁸W⁶⁵⁹S⁶⁶⁰A⁶⁶¹S⁶⁶²G⁶⁶³F⁶⁶⁴T⁶⁶⁵G⁶⁶⁶S⁶⁶⁷Y⁶⁶⁸D⁶⁶⁹S⁶⁷⁰W⁶⁷¹R⁶⁷²Q⁶⁷³A⁶⁷⁴P⁶⁷⁵G⁶⁷⁶K⁶⁷⁷P⁶⁷⁸E⁶⁷⁹W⁶⁸⁰S⁶⁸¹A⁶⁸²S⁶⁸³G⁶⁸⁴F⁶⁸⁵T⁶⁸⁶G⁶⁸⁷S⁶⁸⁸Y⁶⁸⁹D⁶⁹⁰S⁶⁹¹W⁶⁹²R⁶⁹³Q⁶⁹⁴A⁶⁹⁵P⁶⁹⁶G⁶⁹⁷K⁶⁹⁸P⁶⁹⁹E⁷⁰⁰W⁷⁰¹S⁷⁰²A⁷⁰³S⁷⁰⁴G⁷⁰⁵F⁷⁰⁶T⁷⁰⁷G⁷⁰⁸S⁷⁰⁹Y⁷¹⁰D⁷¹¹S⁷¹²W⁷¹³R⁷¹⁴Q⁷¹⁵A⁷¹⁶P⁷¹⁷G⁷¹⁸K⁷¹⁹P⁷²⁰E⁷²¹W⁷²²S⁷²³A⁷²⁴S⁷²⁵G⁷²⁶F⁷²⁷T⁷²⁸G⁷²⁹S⁷³⁰Y⁷³¹D⁷³²S⁷³³W⁷³⁴R⁷³⁵Q⁷³⁶A⁷³⁷P⁷³⁸G⁷³⁹K⁷⁴⁰P⁷⁴¹E⁷⁴²W⁷⁴³S⁷⁴⁴A⁷⁴⁵S⁷⁴⁶G⁷⁴⁷F⁷⁴⁸T⁷⁴⁹G⁷⁵⁰S⁷⁵¹Y⁷⁵²D⁷⁵³S⁷⁵⁴W⁷⁵⁵R⁷⁵⁶Q⁷⁵⁷A⁷⁵⁸P⁷⁵⁹G⁷⁶⁰K⁷⁶¹P⁷⁶²E⁷⁶³W⁷⁶⁴S⁷⁶⁵A⁷⁶⁶S⁷⁶⁷G⁷⁶⁸F⁷⁶⁹T⁷⁷⁰G⁷⁷¹S⁷⁷²Y⁷⁷³D⁷⁷⁴S⁷⁷⁵W⁷⁷⁶R⁷⁷⁷Q⁷⁷⁸A⁷⁷⁹P⁷⁸⁰G⁷⁸¹K⁷⁸²P⁷⁸³E⁷⁸⁴W⁷⁸⁵S⁷⁸⁶A⁷⁸⁷S⁷⁸⁸G⁷⁸⁹F⁷⁹⁰T⁷⁹¹G⁷⁹²S⁷⁹³Y⁷⁹⁴D⁷⁹⁵S⁷⁹⁶W⁷⁹⁷R⁷⁹⁸Q⁷⁹⁹A⁸⁰⁰P⁸⁰¹G⁸⁰²K⁸⁰³P⁸⁰⁴E⁸⁰⁵W⁸⁰⁶S⁸⁰⁷A⁸⁰⁸S⁸⁰⁹G⁸¹⁰F⁸¹¹T⁸¹²G⁸¹³S⁸¹⁴Y⁸¹⁵D⁸¹⁶S⁸¹⁷W⁸¹⁸R⁸¹⁹Q⁸²⁰A⁸²¹P⁸²²G⁸²³K⁸²⁴P⁸²⁵E⁸²⁶W⁸²⁷S⁸²⁸A⁸²⁹S⁸³⁰G⁸³¹F⁸³²T⁸³³G⁸³⁴S⁸³⁵Y⁸³⁶D⁸³⁷S⁸³⁸W⁸³⁹R⁸⁴⁰Q⁸⁴¹A⁸⁴²P⁸⁴³G⁸⁴⁴K⁸⁴⁵P⁸⁴⁶E⁸⁴⁷W⁸⁴⁸S⁸⁴⁹A⁸⁵⁰S⁸⁵¹G⁸⁵²F⁸⁵³T⁸⁵⁴G⁸⁵⁵S⁸⁵⁶Y⁸⁵⁷D⁸⁵⁸S⁸⁵⁹W⁸⁶⁰R⁸⁶¹Q⁸⁶²A⁸⁶³P⁸⁶⁴G⁸⁶⁵K⁸⁶⁶P⁸⁶⁷E⁸⁶⁸W⁸⁶⁹S⁸⁷⁰A⁸⁷¹S⁸⁷²G⁸⁷³F⁸⁷⁴T⁸⁷⁵G⁸⁷⁶S⁸⁷⁷Y⁸⁷⁸D⁸⁷⁹S⁸⁸⁰W⁸⁸¹R⁸⁸²Q⁸⁸³A⁸⁸⁴P⁸⁸⁵G⁸⁸⁶K⁸⁸⁷P⁸⁸⁸E⁸⁸⁹W⁸⁹⁰S⁸⁹¹A⁸⁹²S⁸⁹³G⁸⁹⁴F⁸⁹⁵T⁸⁹⁶G⁸⁹⁷S⁸⁹⁸Y⁸⁹⁹D⁹⁰⁰S⁹⁰¹W⁹⁰²R⁹⁰³Q⁹⁰⁴A⁹⁰⁵P⁹⁰⁶G⁹⁰⁷K⁹⁰⁸P⁹⁰⁹E⁹¹⁰W⁹¹¹S⁹¹²A⁹¹³S⁹¹⁴G⁹¹⁵F⁹¹⁶T⁹¹⁷G⁹¹⁸S⁹¹⁹Y⁹²⁰D⁹²¹S⁹²²W⁹²³R⁹²⁴Q⁹²⁵A⁹²⁶P⁹²⁷G⁹²⁸K⁹²⁹P⁹³⁰E⁹³¹W⁹³²S⁹³³A⁹³⁴S⁹³⁵G⁹³⁶F⁹³⁷T⁹³⁸G⁹³⁹S⁹⁴⁰Y⁹⁴¹D⁹⁴²S⁹⁴³W⁹⁴⁴R⁹⁴⁵Q⁹⁴⁶A⁹⁴⁷P⁹⁴⁸G⁹⁴⁹K⁹⁵⁰P⁹⁵¹E⁹⁵²W⁹⁵³S⁹⁵⁴A⁹⁵⁵S⁹⁵⁶G⁹⁵⁷F⁹⁵⁸T⁹⁵⁹G⁹⁶⁰S⁹⁶¹Y⁹⁶²D⁹⁶³S⁹⁶⁴W⁹⁶⁵R⁹⁶⁶Q⁹⁶⁷A⁹⁶⁸P⁹⁶⁹G⁹⁷⁰K⁹⁷¹P⁹⁷²E⁹⁷³W⁹⁷⁴S⁹⁷⁵A⁹⁷⁶S⁹⁷⁷G⁹⁷⁸F⁹⁷⁹T⁹⁸⁰G⁹⁸¹S⁹⁸²Y⁹⁸³D⁹⁸⁴S⁹⁸⁵W⁹⁸⁶R⁹⁸⁷Q⁹⁸⁸A⁹⁸⁹P⁹⁹⁰G⁹⁹¹K⁹⁹²P⁹⁹³E⁹⁹⁴W⁹⁹⁵S⁹⁹⁶A⁹⁹⁷S⁹⁹⁸G⁹⁹⁹F⁹⁹⁹T⁹⁹⁹G⁹⁹⁹S⁹⁹⁹Y⁹⁹⁹D⁹⁹⁹S⁹⁹⁹W⁹⁹⁹R⁹⁹⁹Q⁹⁹⁹A⁹⁹⁹P⁹⁹⁹G⁹⁹⁹K⁹⁹⁹P⁹⁹⁹E⁹⁹⁹W⁹⁹⁹S⁹⁹⁹A⁹⁹⁹S⁹⁹⁹G⁹⁹⁹F⁹⁹⁹T⁹⁹⁹G⁹⁹⁹S⁹⁹⁹Y⁹⁹⁹D⁹⁹⁹S⁹⁹⁹W⁹⁹⁹R⁹⁹⁹Q⁹⁹⁹A⁹⁹⁹P⁹⁹⁹G⁹⁹⁹K⁹⁹⁹P⁹⁹⁹E⁹⁹⁹W⁹⁹⁹S⁹⁹⁹A⁹⁹⁹S⁹⁹⁹G⁹⁹⁹F⁹⁹⁹T⁹⁹⁹G⁹⁹⁹S⁹⁹⁹Y⁹⁹⁹D⁹⁹⁹S⁹⁹⁹W⁹⁹⁹R⁹⁹⁹Q⁹⁹⁹A⁹⁹⁹P⁹⁹⁹G⁹⁹⁹K⁹⁹⁹P⁹⁹⁹E⁹⁹⁹W⁹⁹⁹S⁹⁹⁹A⁹⁹⁹S⁹⁹⁹G⁹⁹⁹F⁹⁹⁹T⁹⁹⁹G⁹⁹⁹S⁹⁹⁹Y⁹⁹⁹D⁹⁹⁹S⁹⁹⁹W⁹⁹⁹R⁹⁹⁹Q⁹⁹⁹A⁹⁹⁹P⁹⁹⁹G⁹⁹⁹K⁹⁹⁹P⁹⁹⁹E⁹⁹⁹W⁹⁹⁹S⁹⁹⁹A⁹⁹⁹S⁹⁹⁹G⁹⁹⁹F⁹⁹⁹T⁹⁹⁹G⁹⁹⁹S⁹⁹⁹Y⁹⁹⁹D⁹⁹⁹S⁹⁹⁹W⁹⁹⁹R⁹⁹⁹Q⁹⁹⁹A⁹⁹⁹P⁹⁹⁹G⁹⁹⁹K⁹⁹⁹P⁹⁹⁹E⁹⁹⁹W⁹⁹⁹S⁹⁹⁹A⁹⁹⁹S⁹⁹⁹G⁹⁹⁹F⁹⁹⁹T⁹⁹⁹G⁹⁹⁹S⁹⁹⁹Y⁹⁹⁹D⁹⁹⁹S⁹⁹⁹W⁹⁹⁹R⁹⁹⁹Q⁹⁹⁹A⁹⁹⁹P⁹⁹⁹G⁹⁹⁹K⁹⁹⁹P⁹⁹⁹E⁹⁹⁹W⁹⁹⁹S⁹⁹⁹A⁹⁹⁹S⁹⁹⁹G⁹⁹⁹F⁹⁹⁹T⁹⁹⁹G⁹⁹⁹S⁹⁹⁹Y⁹⁹⁹D⁹⁹⁹S⁹⁹⁹W⁹⁹⁹R⁹⁹⁹Q⁹⁹⁹A⁹⁹⁹P⁹⁹⁹G⁹⁹⁹K⁹⁹⁹P⁹⁹⁹E⁹⁹⁹W⁹⁹⁹S⁹⁹⁹A⁹⁹⁹S⁹⁹⁹G⁹⁹⁹F⁹⁹⁹T⁹⁹⁹G⁹⁹⁹S⁹⁹⁹Y⁹⁹⁹D⁹⁹⁹S⁹⁹⁹W⁹⁹⁹R⁹⁹⁹Q⁹⁹⁹A⁹⁹⁹P⁹⁹⁹G⁹⁹⁹K⁹⁹⁹P⁹⁹⁹E⁹⁹⁹W⁹⁹⁹S⁹⁹⁹A⁹⁹⁹S⁹⁹⁹G⁹⁹⁹F⁹⁹⁹T⁹⁹⁹G⁹⁹⁹S⁹⁹⁹Y⁹⁹⁹D⁹⁹⁹S⁹⁹⁹W⁹⁹⁹R⁹⁹⁹Q⁹⁹⁹A⁹⁹⁹P⁹⁹⁹G⁹⁹⁹K⁹⁹⁹P⁹⁹⁹E⁹⁹⁹W⁹⁹⁹S⁹⁹⁹A⁹⁹⁹S⁹⁹⁹G⁹⁹⁹F⁹⁹⁹T⁹⁹⁹G⁹⁹⁹S⁹⁹⁹Y⁹⁹⁹D⁹⁹⁹S⁹⁹⁹W⁹⁹⁹R⁹⁹⁹Q⁹⁹⁹A⁹⁹⁹P⁹⁹⁹G⁹⁹⁹K⁹⁹⁹P⁹⁹⁹E⁹⁹⁹W⁹⁹⁹S⁹⁹⁹A⁹⁹⁹S<sup

Fig. 5 (cont.)

PMP35H7	EVOLVETGGELVOPGGSLRUSCAASGFTLDYVIGMFRQAPGKEREVGSCISSSD--STYYADSVKGRTISRDNAKNTVYLOMNSIKPEDTAVYCAAD--LIRTPEFCDSDAP--Y-DWGGCTQVTVSS
PMP30A2	EVOLVETGGELVOPGGSLRUSCAASGFTLDYVIGMFRQAPGKEREVGSCISSSD--STYYADSVKGRTISRDNAKNTVYLOMNSIKPEDTAVYCAAD--LIRTPEFCDSDAP--Y-DWGGCTQVTVSS
PMP34C3	EVOLVETGGELVOPGGSLRUSCAASGFTLDYVIGMFRQAPGKEREVGSCISSSD--STYYADSVKGRTISRDNAKNTVYLOMNSIKPEDTAVYCAAD--LIRTPEFCDSDAP--Y-DWGGCTQVTVSS
PMP30G11	EVOLVETGGELVOPGGSLRUSCAASGFTLDYVIGMFRQAPGKEREVGSCISSSD--STYYADSVKGRTISRDNAKNTVYLOMNSIKPEDTAVYCAAD--LIRTPEFCDSDAP--Y-DWGGCTQVTVSS
PMP34A12	EVOLVETGGELVOPGGSLRUSCAASGFTLDYVIGMFRQAPGKEREVGSCISSSD--STYYADSVKGRTISRDNAKNTVYLOMNSIKPEDTAVYCAAD--LIRTPEFCDSDAP--Y-DWGGCTQVTVSS
PMP30B6	EVOLVETGGELVOPGGSLRUSCAASGFTLDYVIGMFRQAPGKEREVGSCISSSD--STYYADSVKGRTISRDNAKNTVYLOMNSIKPEDTAVYCAAD--LIRTPEFCDSDAP--Y-DWGGCTQVTVSS
PMP35E11	EVOLVETGGELVOPGGSLRUSCAASGFTLDYVIGMFRQAPGKEREVGSCISSSD--STYYADSVKGRTISRDNAKNTVYLOMNSIKPEDTAVYCAAEPPDS--RSLGGSY-WDYRGQCTQVTVSS
PMP35G11	EVOLVETGGELVOPGGSLRUSCAASGFTLDYVIGMFRQAPGKEREVGSCISSSD--STYYADSVKGRTISRDNAQNTVYLOMNSIKPEDTAVYCAAQDPVPA--RSLGGSY-WDYRGQCTQVTVSS
PMP32F10	EVOLVETGGELVOPGGSLRUSCAASGFTLDYVIGMFRQAPGKEREVGSCISSSD--STYYADSVKGRTISRDNAKNTVYLOMNSIKPEDTAVYCAAEPPDS--WILDGSE-FIKFWGCTQVTVSS
PMP31C5	EVOLVETGGELVOPGGSLRUSCAASGFTLDYVIGMFRQAPGKEREVGSCISSSD--STYYADSVKGRTISRDNAKNTVYLOMNSIKPEDTAVYCAAEPPDS--WILDGSE-FIKFWGCTQVTVSS
PMP31D2	EVOLVETGGELVOPGGSLRUSCAASGFTLDYVIGMFRQAPGKEREVGSCISSSD--NTYYADSVKGRTISRDNAKNTVYLOMNSIKPEDTAVYCAAEPPDS--WILDGSE-FIKFWGCTQVTVSS
PMP32G9	EVOLVETGGELVOPGGSLRUSCAASGFTLDYVIGMFRQAPGKEREVGSCISSSD--NTYYADSVKGRTISRDNAKNTVYLOMNSIKPEDTAVYCAAEPPDS--WILDGSE-FIKFWGCTQVTVSS
PMP30B1	EVOLVETGGELVOPGGSLRUSCAASGFTESSYDNGWYAPGKEREFTAVINSGS--STYYADSVKGRTISRDNAKNTVYLOMNSIKPEDTAVYCN--AQYG----IGYA-E-----DWGGCTQVTVSS
PMP33F4	EVOLVETGGELVOPGGSLRUSCAASGFTESSYDNGWYAPGKEREFTAVITN-S--STYYADSVKGRTISRDNAKNTVYLOMNSIKPEDTAVYCN--AQYG----IGYA-E-----DWGGCTQVTVSS
PMP31A4	EVOLVETGGELVOPGGSLRUSCAASGFTSYDNGWYAPGKEREFTAVIYAGI--SGG--STYYADSVKGRTISRDNAKNTVYLOMNSIKPEDTAVYCSFTTNSDILG--R-----DWGGCTQVTVSS
PMP33C10	EVOLVETGGELVOPGGSLRUSCAASGFTSYDNGWYAPGKEREFTAVIYSSG--STYYADPVGRTISRDNAKNTVYLOMNSIKPEDTAVYCN--AFLP--GPGEFH-----DWGGCTQVTVSS
PMP34G9	EVOLVETGGELVOPGGSLRUSCAASGFTSYDNGWYAPGKEREFTAVIYSGG--STYYADSVKGRTISRDNAKNTVYLOMNSIKPEDTAVYCN--AYTG--GCD-----DWGGCTQVTVSS
PMP30C11	EVOLVETGGELVOPGGSLRUSCAASGFTSYDNGWYAPGKEREFTAVIYCK--AEVW--AG--DI-----DWGGCTQVTVSS
PMP28E11	EVOLVETGGELVOPGGSLRUSCAASGFTSYDNGWYAPGKEREFTAVIYTAQTG--VPREYEYEWNGQCTQVTVSS
PMP32E2	EVOLVETGGELVOPGGSLRUSCAASGFTSYDNGWYAPGKEREFTAVIYCNLSLQGRD-----YWGCTQVTVSS
PMP33A3	EVOLVETGGELVOPGGSLRUSCAASGFTSYDNGWYAPGKEREFTAVIYCAADKNTVYLOMNSIKPEDTAVYCAAN--EALFEYDWGGCTQVTVSS

Fig. 6

		FR1	FR2	FR3	CDR3	FR4
Inhibitors						
PMP10H5	EVOLVESGGELVQAGGSIRLSCASGFLD	YAAIG	WTRQAPKERGVS CWDSSGGITSTYSDSFIG	RTTISRNARAKTVYLOMNSLKPEDAVYCAA	DGHINWGRVTPCSQLSTRGANDY	WGGGTQTVSS
PMP35E11	EVOLVESGGELVQAGGSIRLSCASGFLD	DIAIG	WTRQAPKERGVS CISSSDGTTYADSVKG	RTTISRNARAKTVYLOMNSLKPEDAVYCAA	ERDVPARSLOGSYWYDI	RGGCTQTVSS
PMP32C9	EVOLVESGGELVQAGGSIRLSCASGFLD	DIAIG	WTRQAPKERGVS GISSSDGTTYADSVKG	RTTISRNARAKTVYLOMNSLKPEDAVYCAA	EPDSSWLDGSPEFKY	WGGCTQTVSS
PMP35H4	EVOLVESGGELVQAGGSIRLSCASGFLD	DYAMS	WTRQAPKERAVS ALISNGNITYTTSMKG	RTTISRNARAKTVYLOMNSLKPEDAVYCAA	GSTAIVGPPPTPYEIDY	WGGCTQTVSS
PMP32E10	EVOLVESGGELVQAGGSIRLSCASGFLD	SYDMS	WTRQAPKGPWVS AINSGGGTTYADSVKG	RTTISRNARAKTVYLOMNSLKPEDAVYCAA	DWRSYDPLPPGDI	WGGCTQTVSS
PMP30C11	EVOLVESGGELVQAGGSIRLSCASGFLD	SYDMG	WTRQAPKERETVA VISRSGGTTYADSVKG	RTTISRNARAKTVYLOMNSLKPEDAVYCAA	EVVAGGYDY	WGGCTQTVSS
PMP55C10	EVOLVESGGELVQAGGSIRLSCASGFLD	SYDMG	WTRQAPKERETVA VHESSGTTYADSVKG	RTTISRNARAKTVYLOMNSLKPEDAVYCAA	ELPGPGFHDY	WGGCTQTVSS
PMP34G9	EVOLVESGGELVQAGGSIRLSCASGFLD	SYDET	WTRQAPKERETVA VISHSGGTTYADSVKG	RTTISRNARAKTVYLOMNSLKPEDAVYCAA	YTGGGDY	WGGCTQTVSS
PMP31A4	EVOLVESGGELVQAGGSIRLSCASGFLD	VNAMG	WTRQAPKORELVA GLISGGTTYADSVKG	RTTISRNARAKTVYLOMNSLKPEDAVYCAA	VTINSYDOLGRDY	WGGCTQTVSS
PMP32E2	EVOLVESGGELVQAGGSIRLSCASGFLD	DNTMAGT	WTRQPKORELVA LIATGTTYADSVKG	RTTISRNARAKTVYLOMNSLKPEDAVYCAA	FSLRGRDY	WGGCTQTVSS
PMP33A3	EVOLVESGGELVQAGGSIRLSCASGFLD	YGAIG	WTRQAPKERGVS CISSSTGTTYADSVKG	RTTISRNARAKTVYLOMNSLKPEDAVYCAA	DKWMSPLVIANEALFEDY	WGGCTQTVSS
PMP34A12	EVOLVESGGELVQAGGSIRLSCASGFLD	YYVIG	WTRQAPKERGVS CISSSDGTTYADSVKG	RTTISRNARAKTVYLOMNSLKPEDAVYCAA	DLIRPPEFCVDSAPIDY	WGGCTQTVSS
PMP28E11	EVOLVESGGELVQAGGSIRLSCASGFLD	YAAIG	WTRQAPKERETVS CISSSDGTTYADSVKG	RTTISRNARAKTVYLOMNSLKPEDAVYCAA	VHTTAQATGPORETEYEN	WGGCTQTVSS
PMP35F4	EVOLVESGGELVQAGGSIRLSCASGFLD	SYDMG	WTRQAPKERETVA ITTMISSTYADSVKG	RTTISRNARAKTVYLOMNSLKPEDAVYCAA	QYGLGQAEDY	WGGCTQTVSS
Non-inhibitors						
IL6R.c11	EVOLVESGGELVQAGGSIRLSCASGFLD	DEAIG	WTRQAPKERGVS CISSSDGTTYADSVKG	RTTISRNARAKTVYLOMNSLKPEDAVYCAA	LFDRGCGSTWYGMIDY	WGGCTQTVSS
IL6R.c13	EVOLVESGGELVQAGGSIRLSCASGFLD	SYAIG	WTRQAPKERGVS CISTSDGTTYADSVKG	RTTISRNARAKTVYLOMNSLKPEDAVYCAA	DGGRPLTVQDMCTMAYADY	WGGCTQTVSS
IL6R.c15	EVOLVESGGELVQAGGSIRLSCASGFLD	GADAG	WTRQPKEREETVA AINWSGNSTYADSVKG	RTTISRNARAKTVYLOMNSLKPEDAVYCAA	FRDYYIS	EGGGCTQTVSS

12 / 62

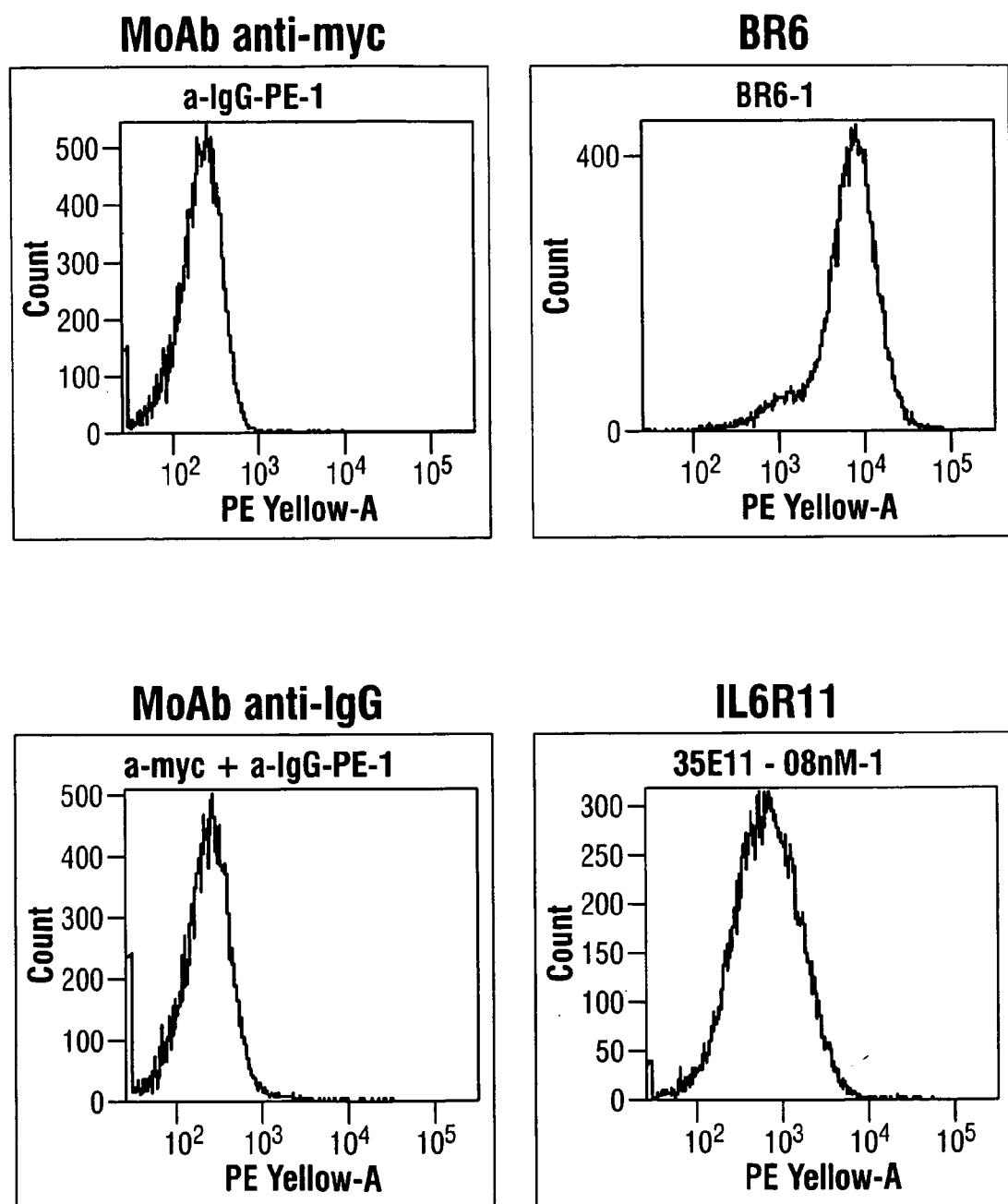
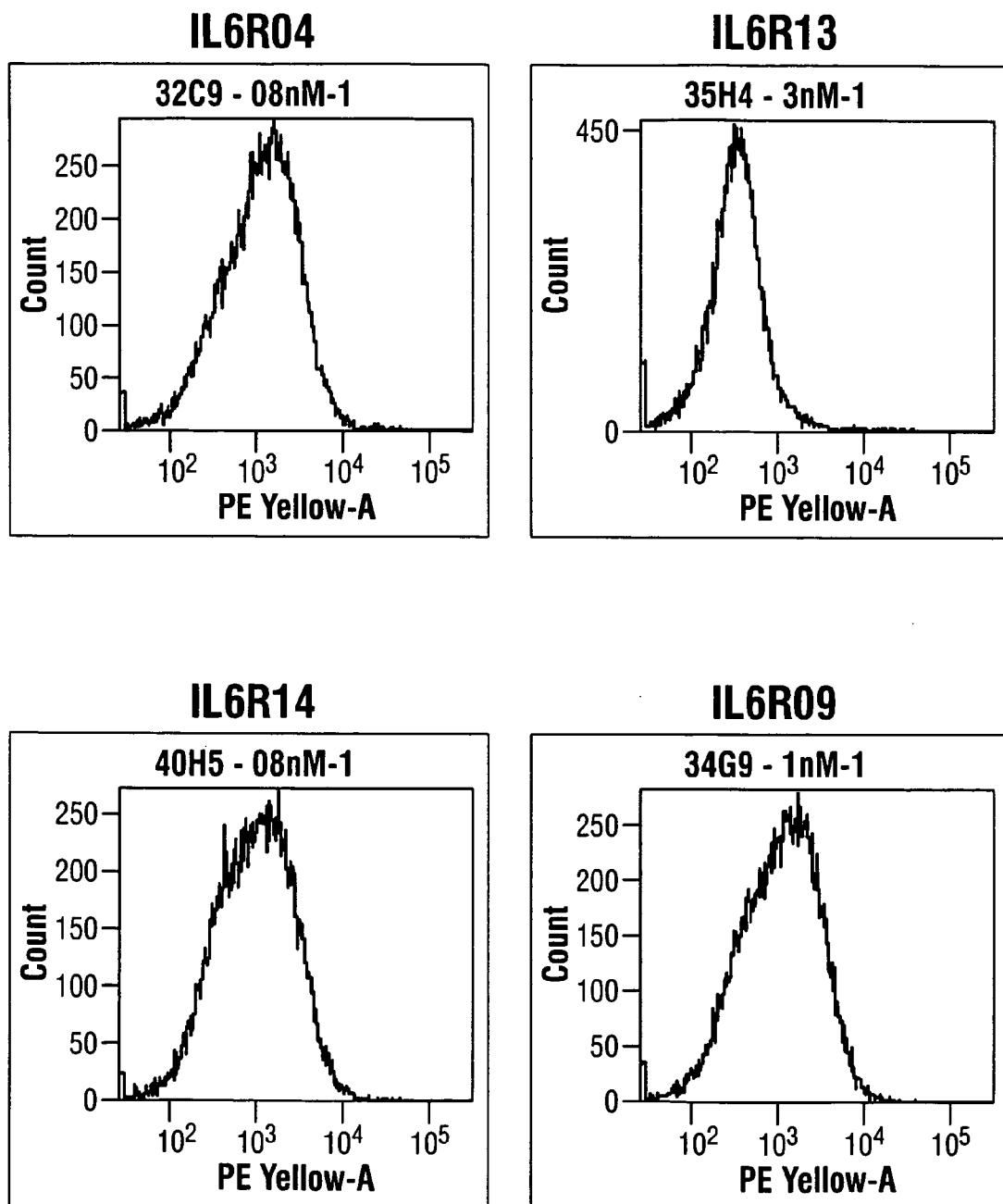
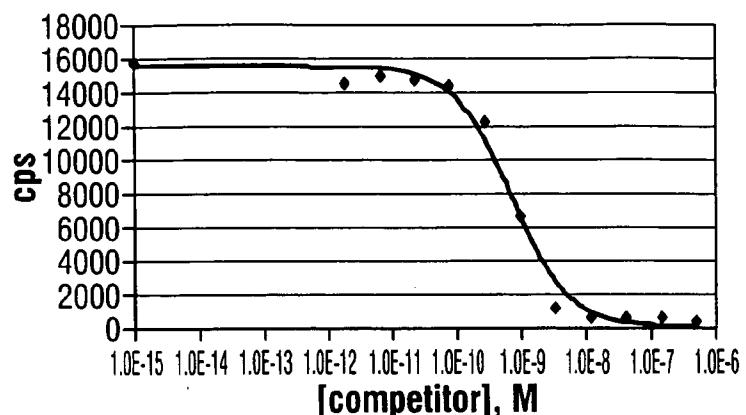
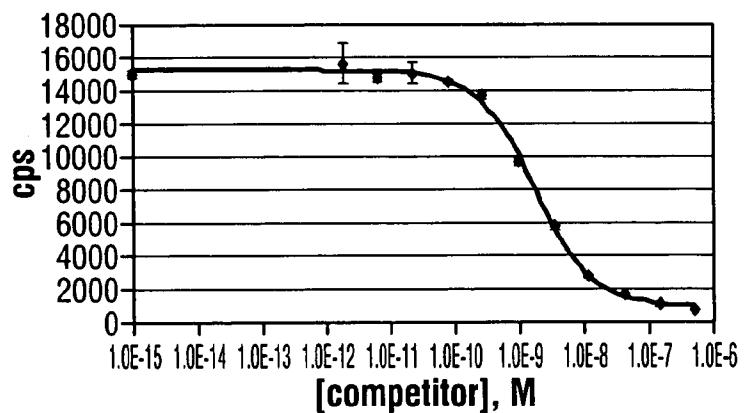
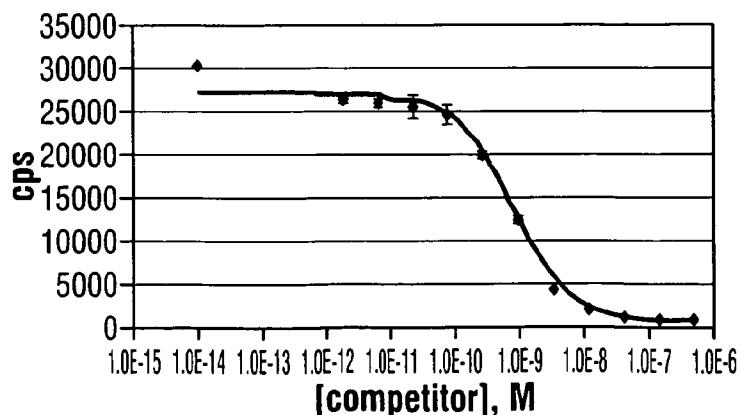
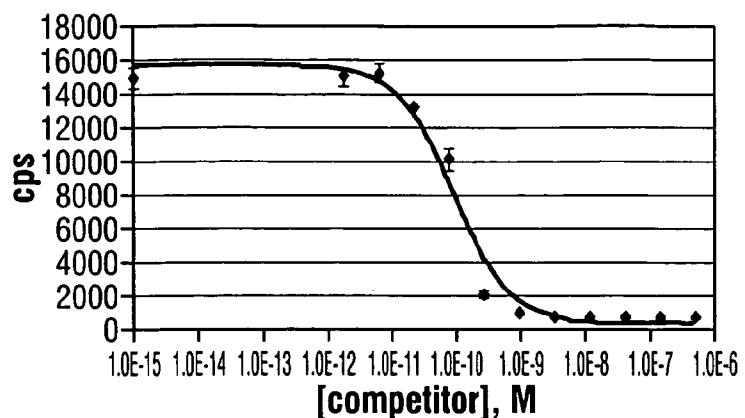
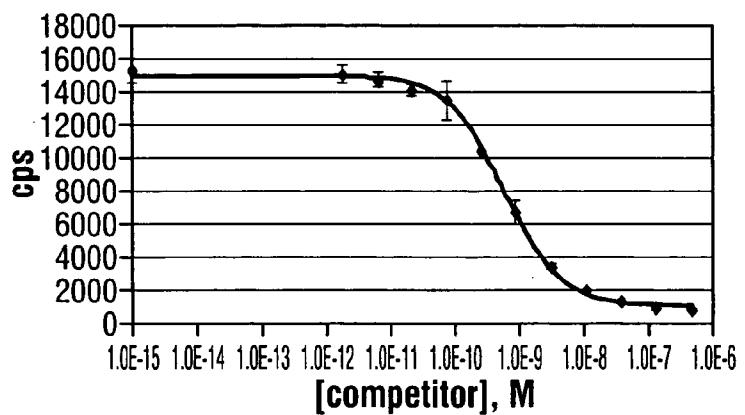
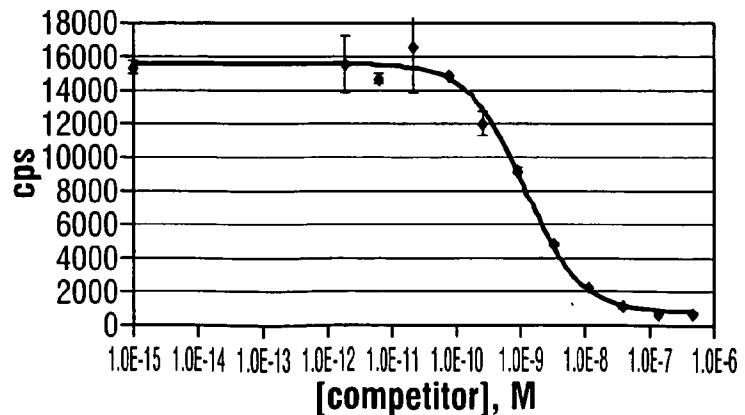
Fig. 7

Fig. 7 (cont.)

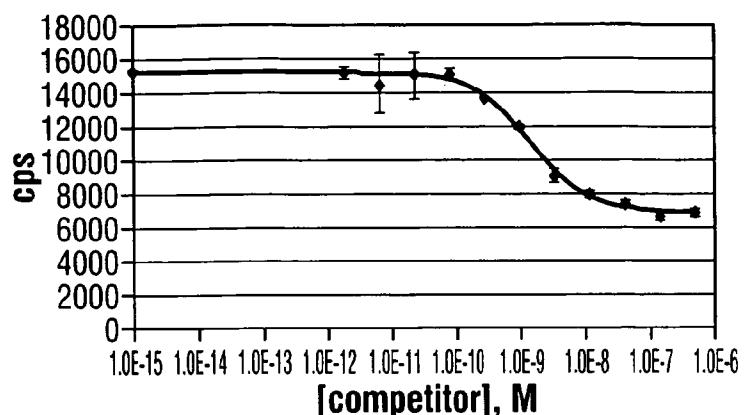
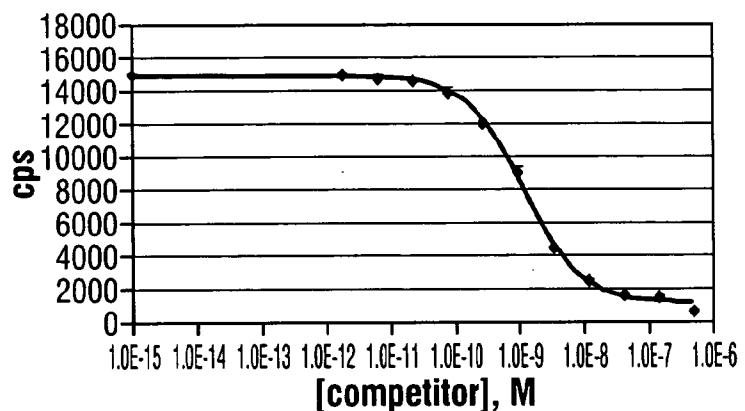
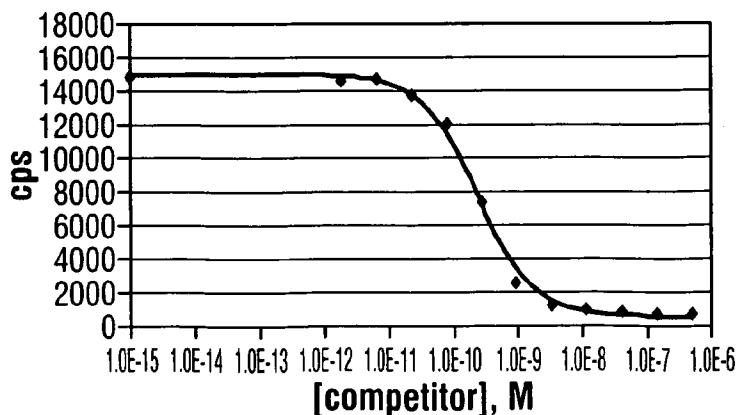
14 / 62

Fig. 8**IL6R01****IL6R02****IL6R03**

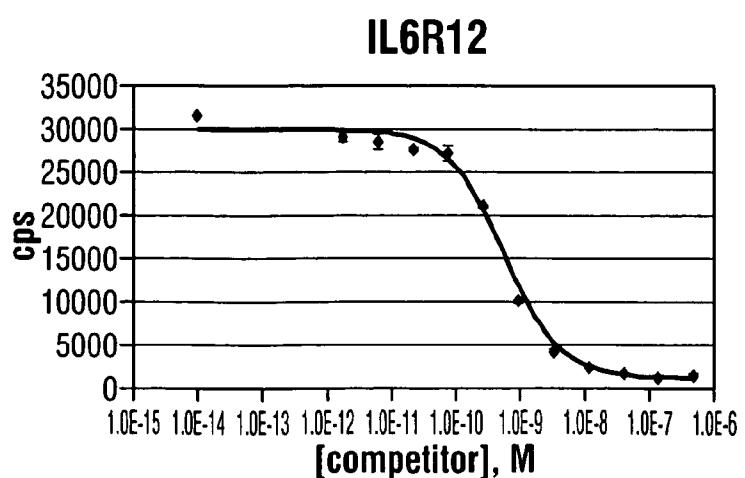
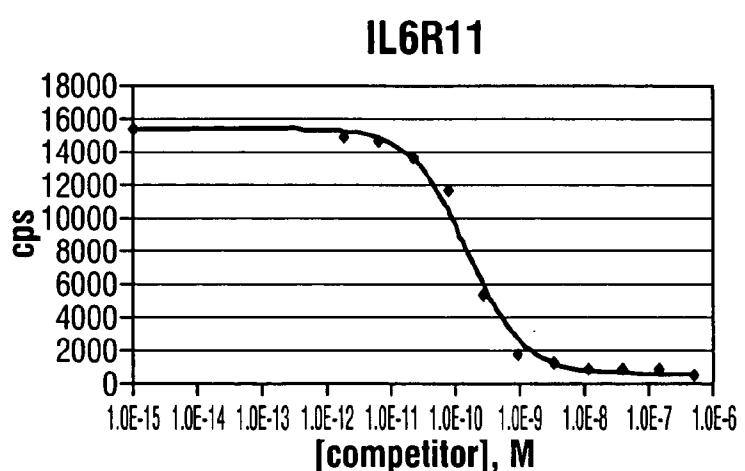
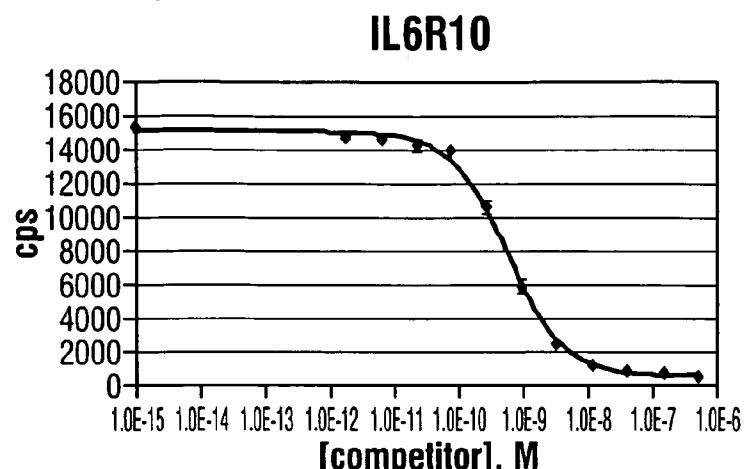
15 / 62

Fig. 8 (cont.)**IL6R04****IL6R05****IL6R06**

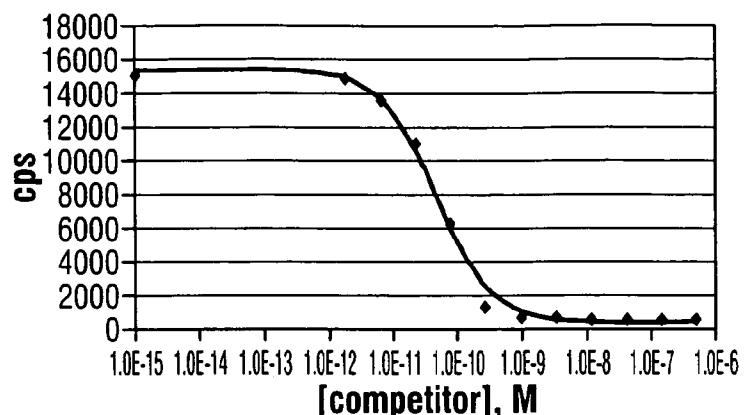
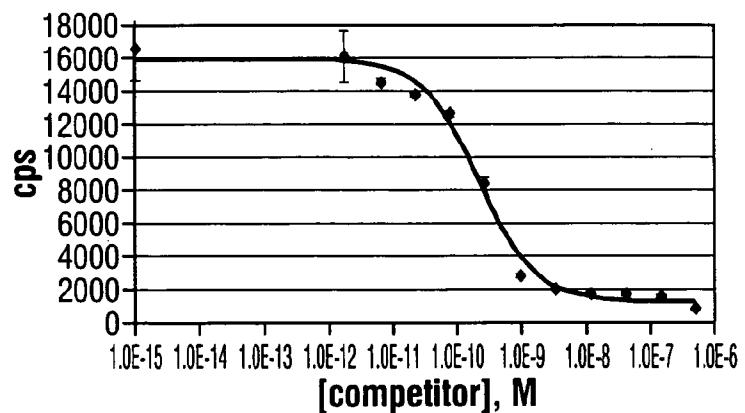
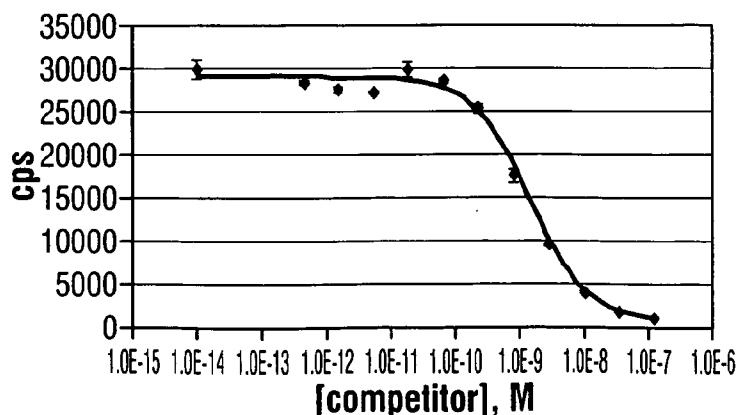
16 / 62

Fig. 9**IL6R07****IL6R08****IL6R09**

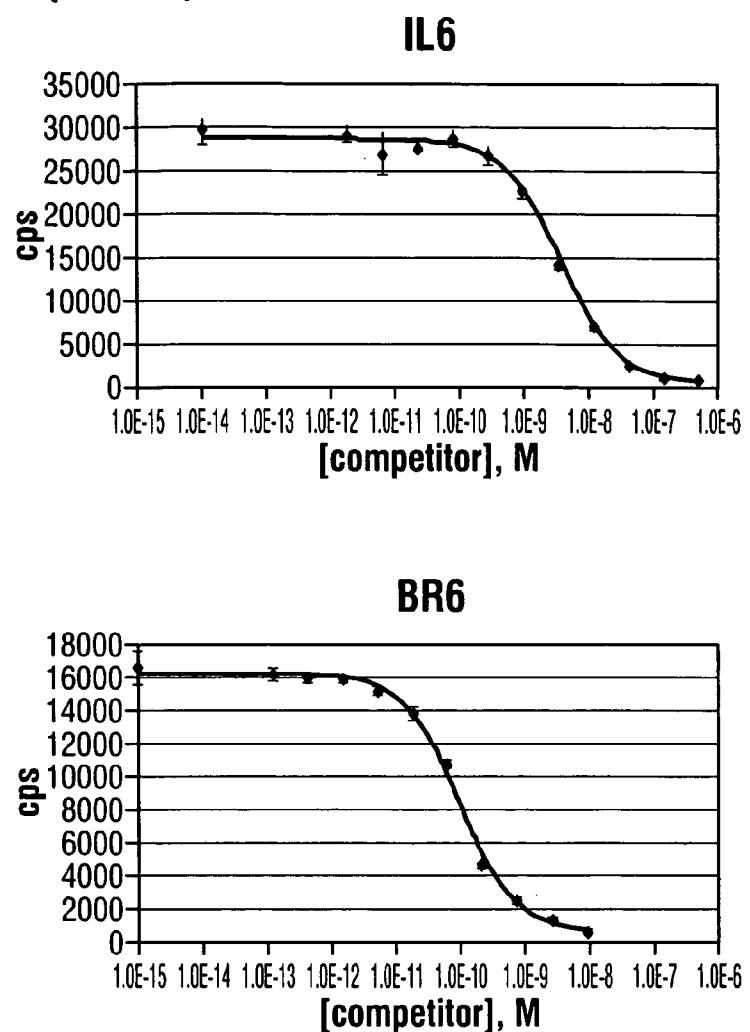
17 / 62

Fig. 9 (cont.)

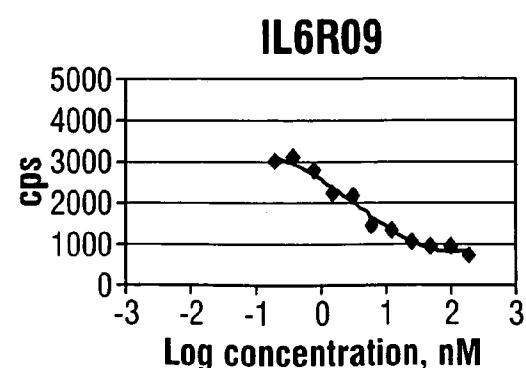
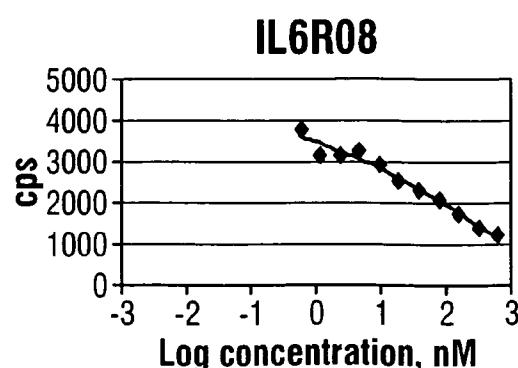
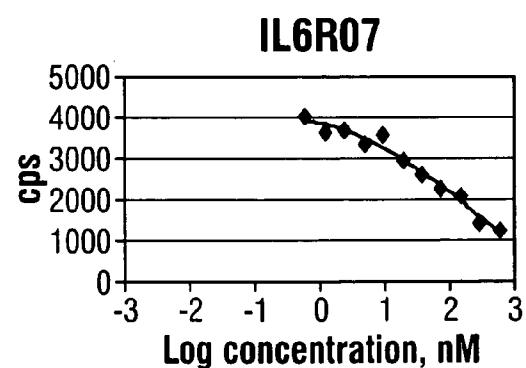
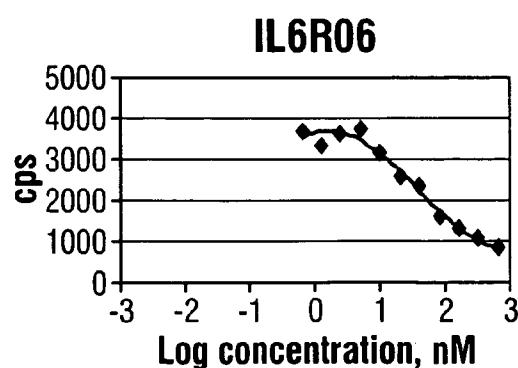
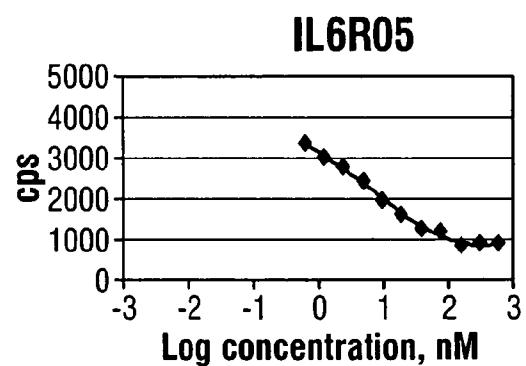
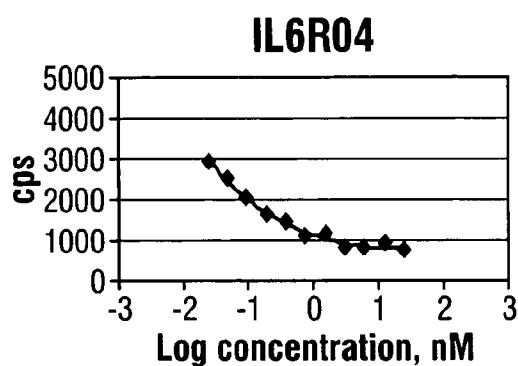
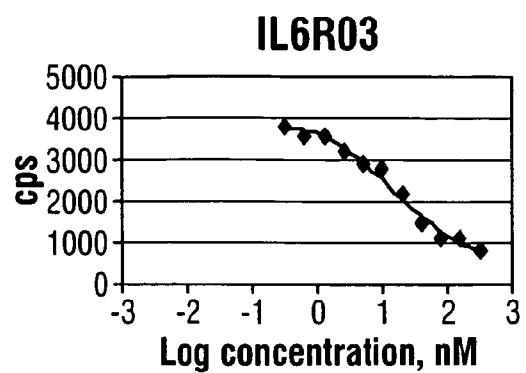
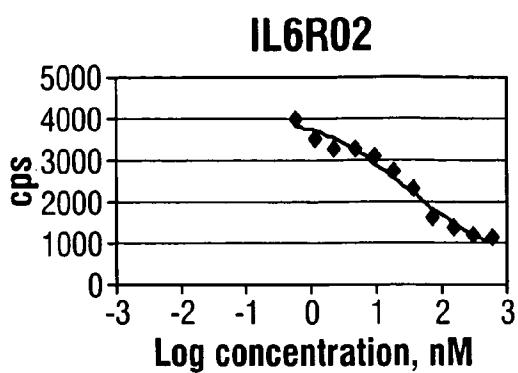
18 / 62

Fig. 10**IL6R13****IL6R14****Reference Fab**

19 / 62

Fig. 10 (cont.)

20 / 62

Fig. 11

21 / 62

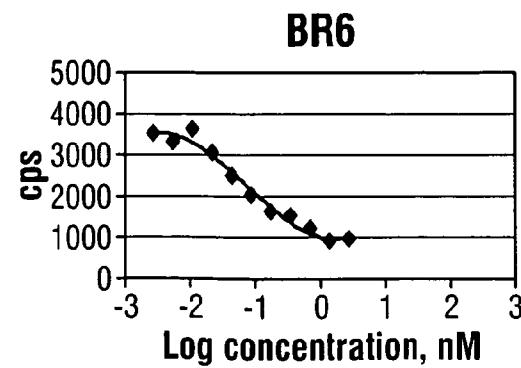
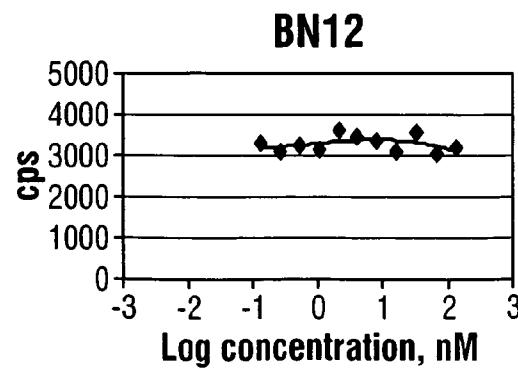
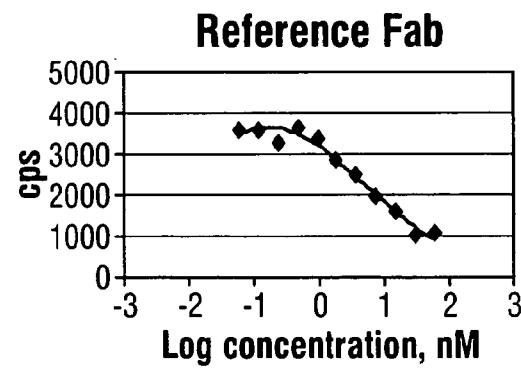
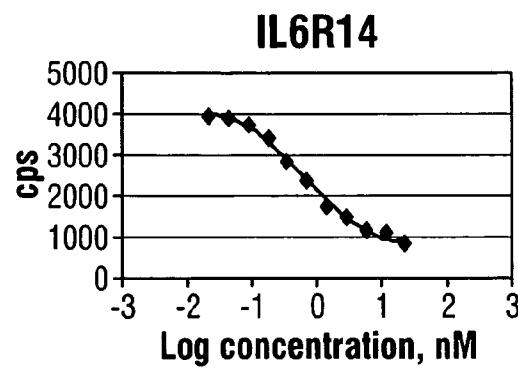
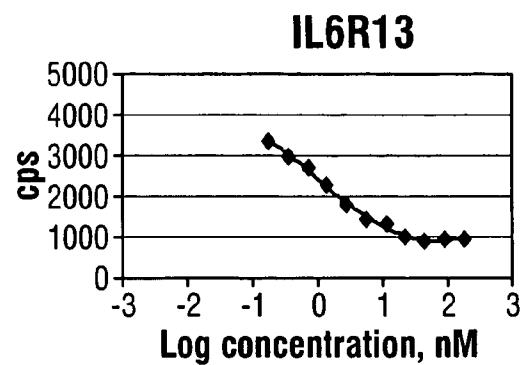
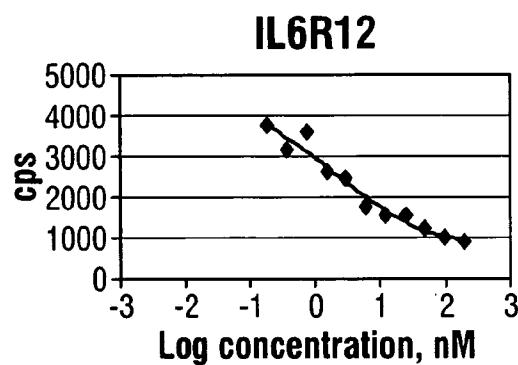
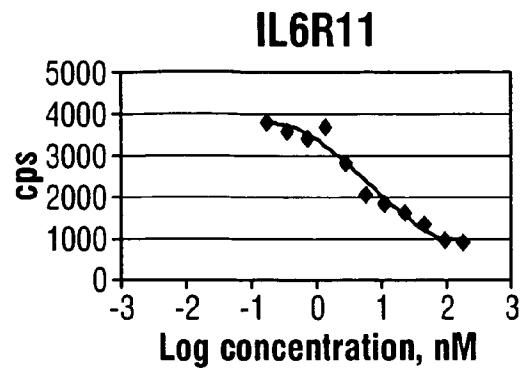
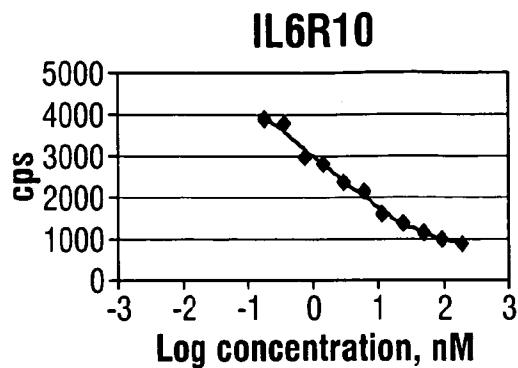
Fig. 11 (cont.)

Fig. 12

22 / 62

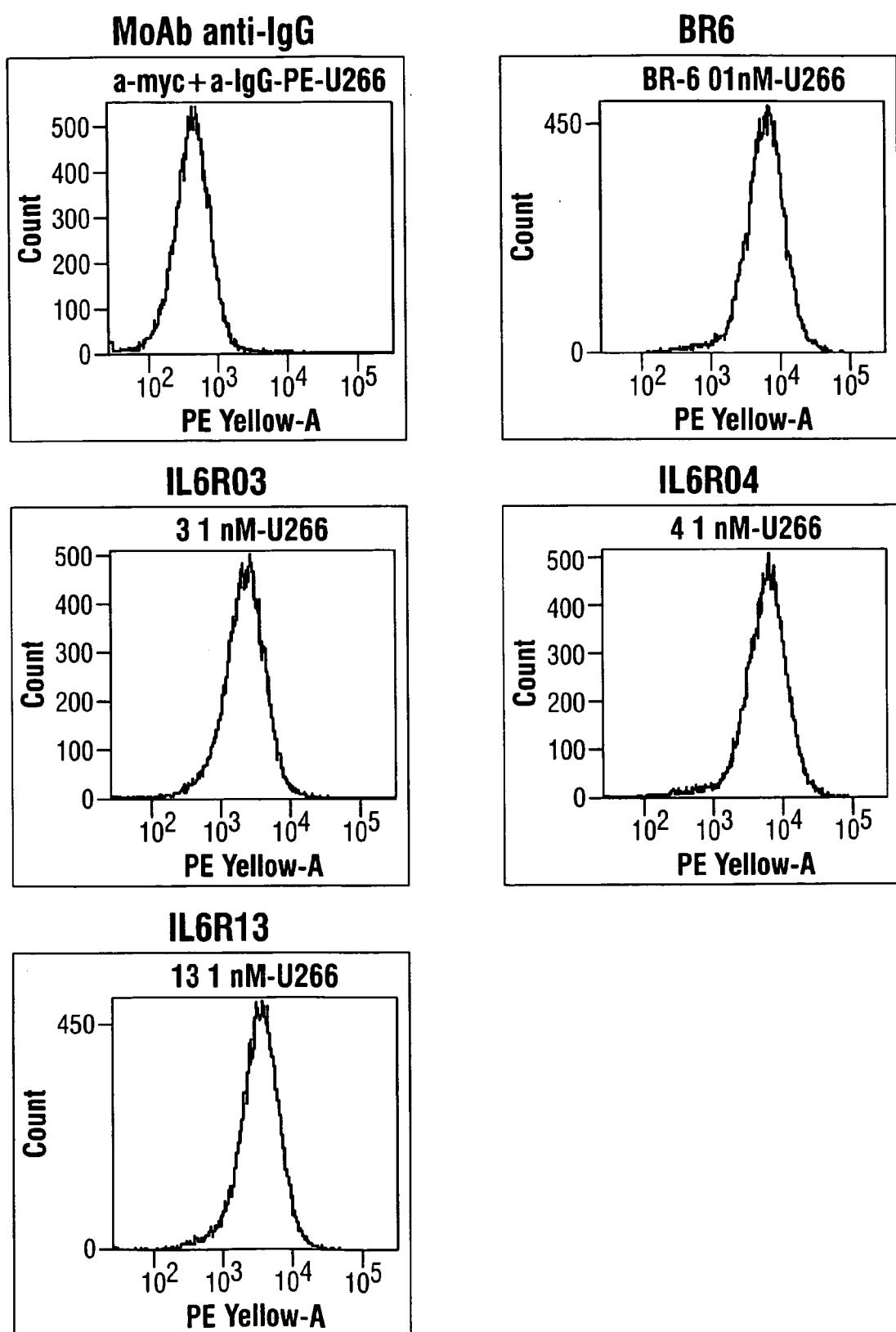
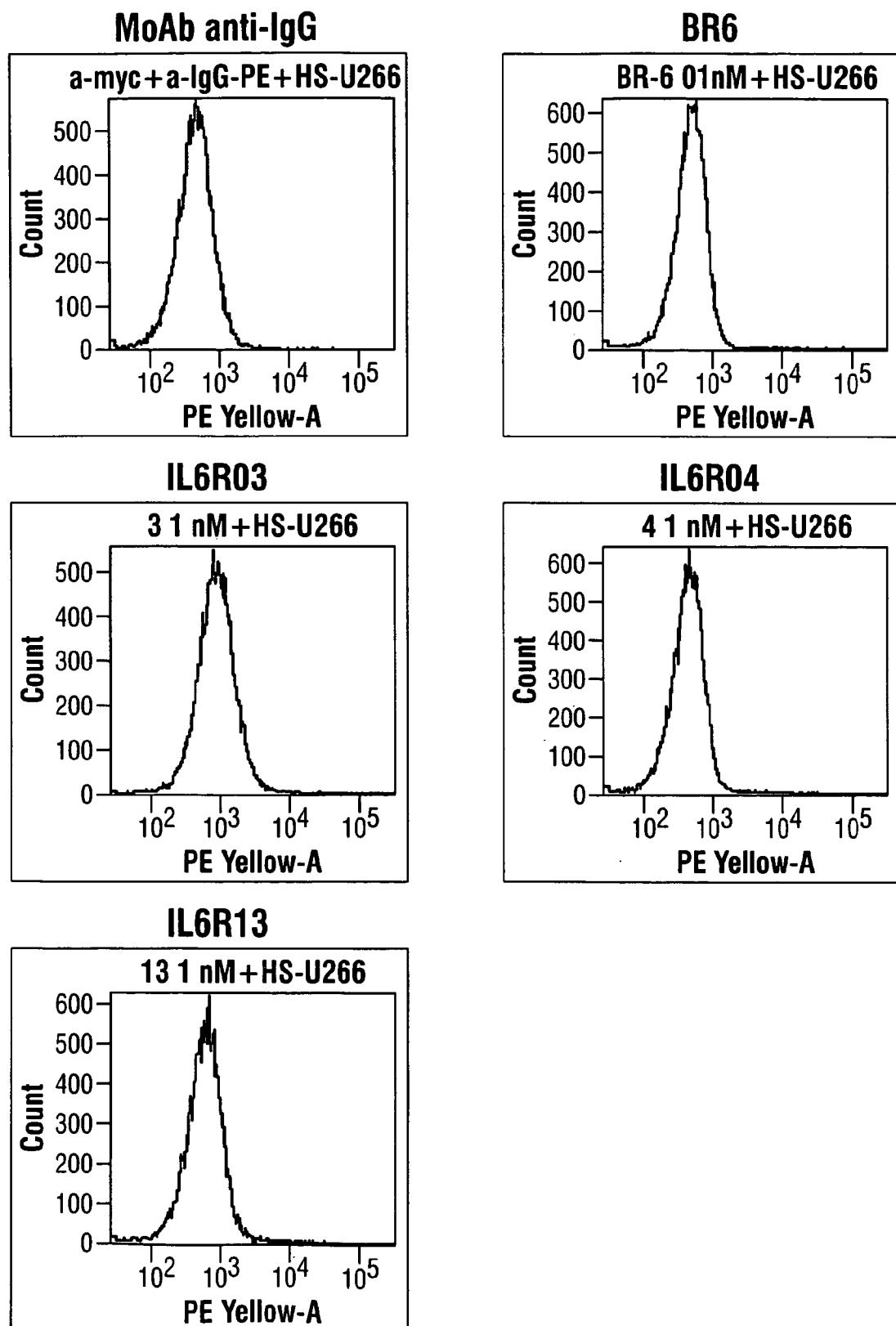
Without human plasma

Fig. 12 (cont.)

23 / 62

In the presence of human plasma

24 / 62

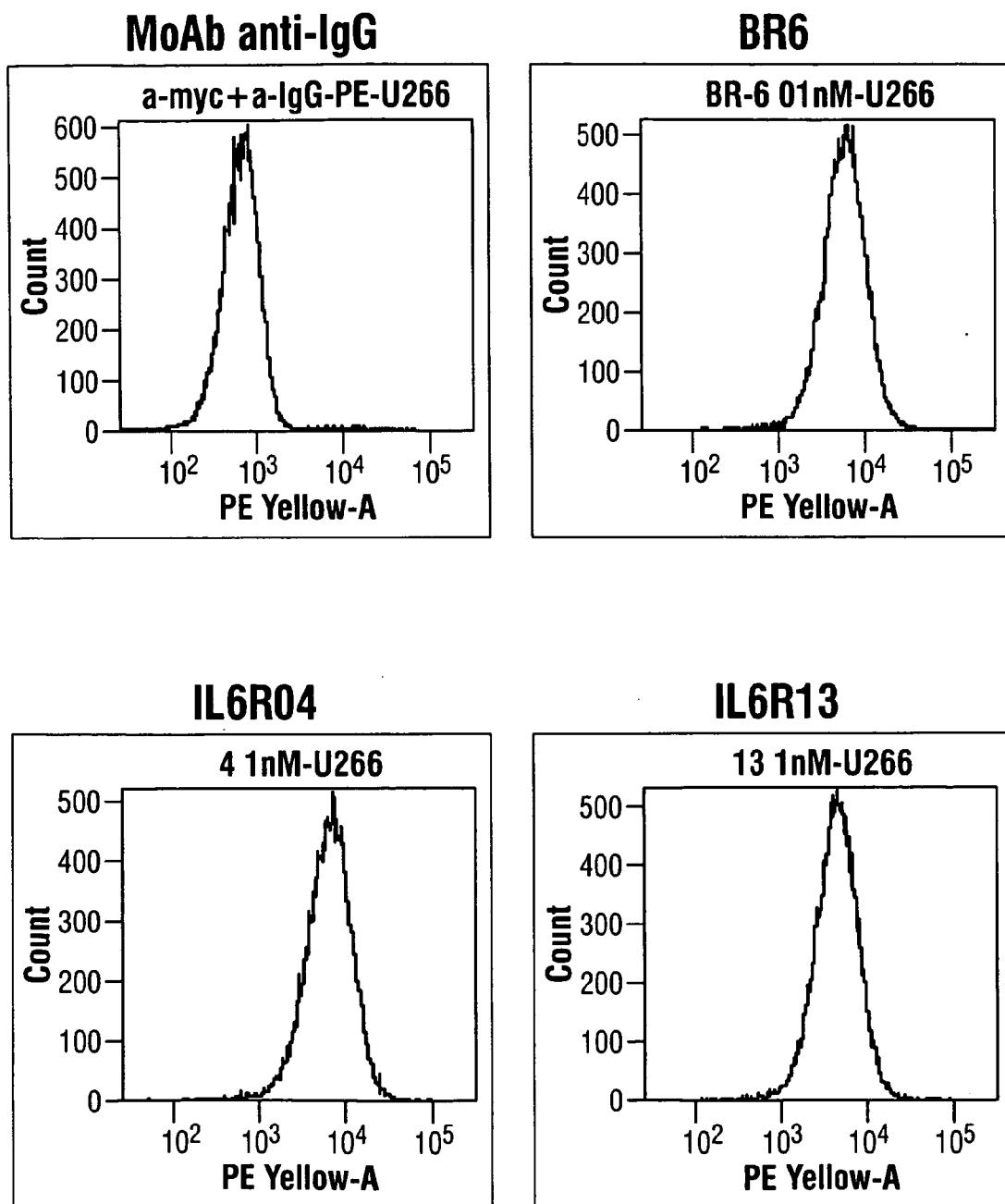
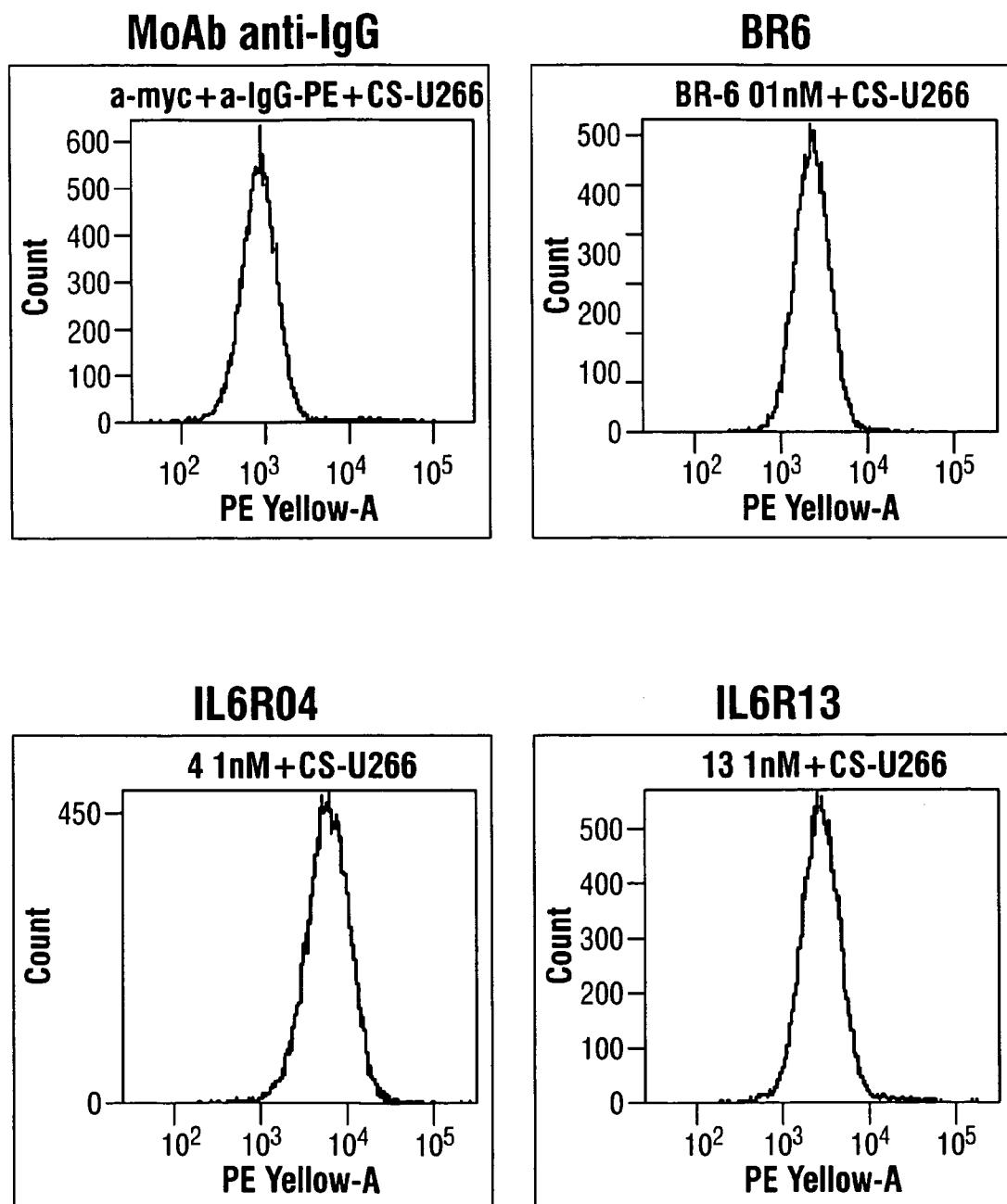
Fig. 13*Without cynomolgus plasma*

Fig. 13 (cont.)*In the presence of cynomolgus plasma*

26 / 62

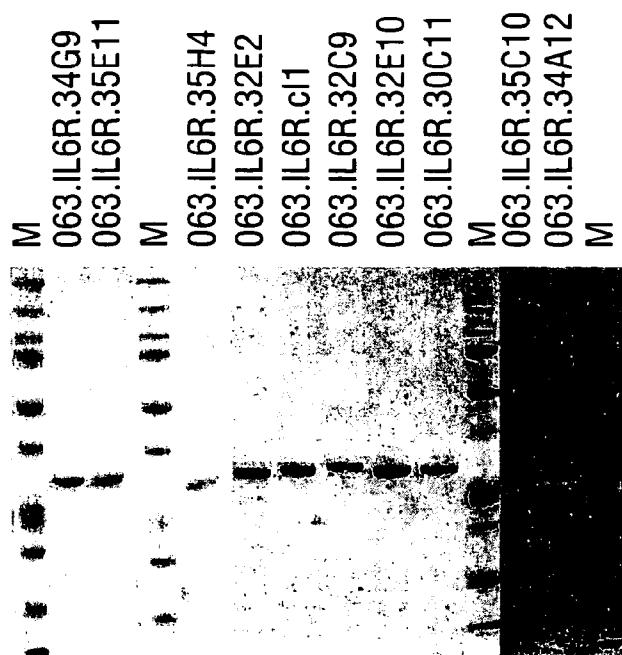
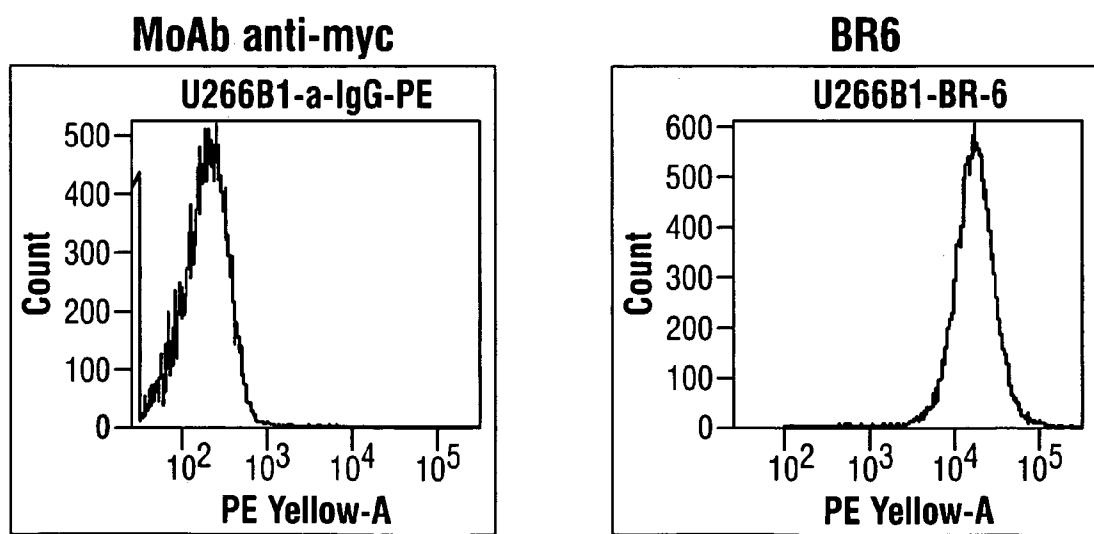
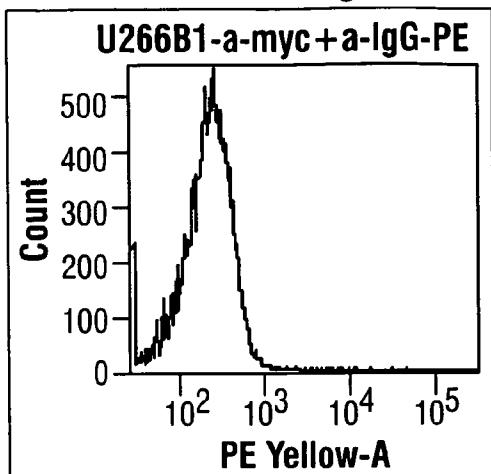
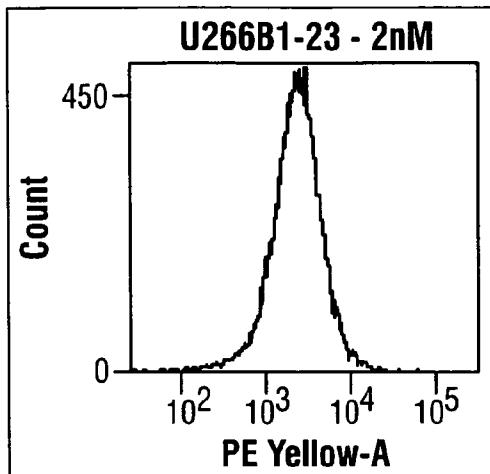
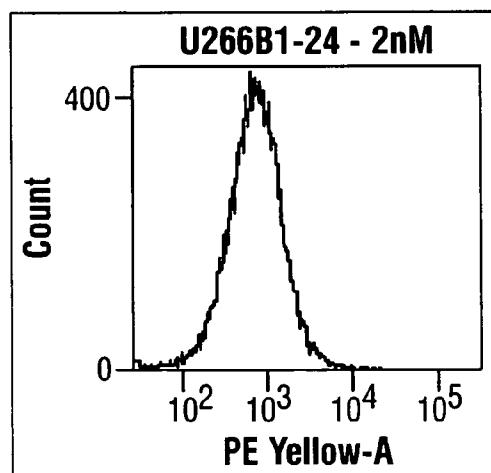
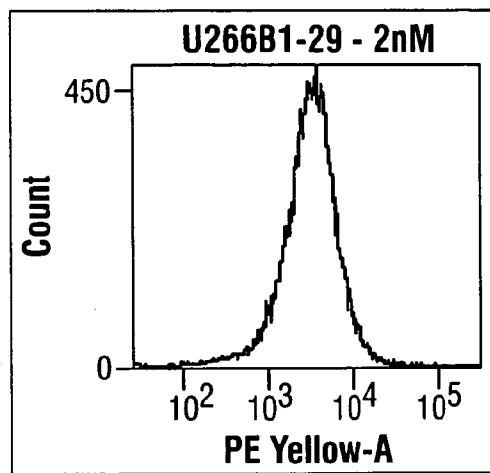
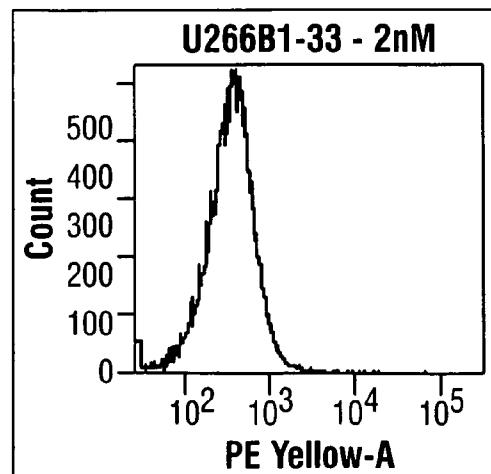
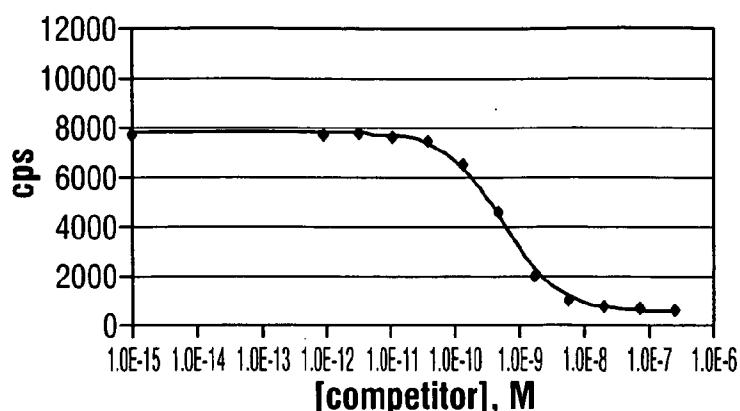
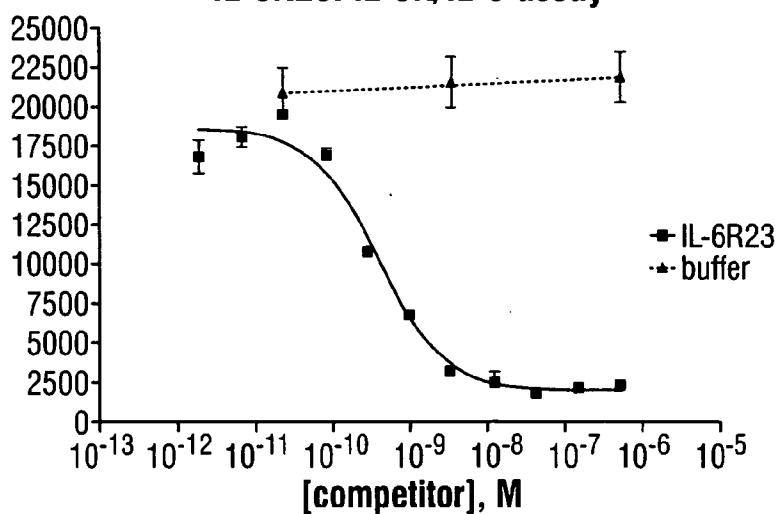
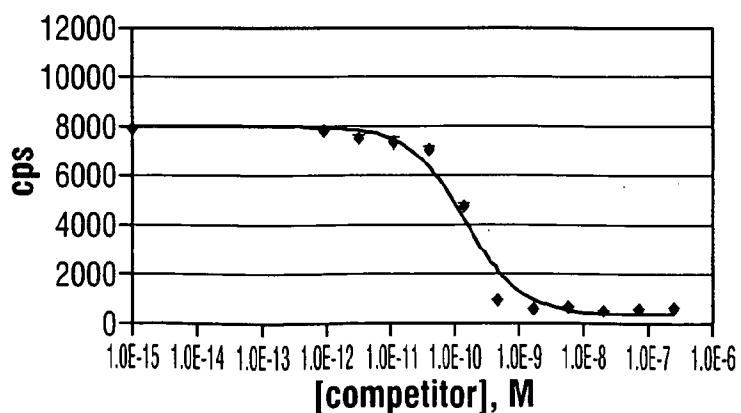
Fig. 14**Fig. 15**

Fig. 15 (cont.)

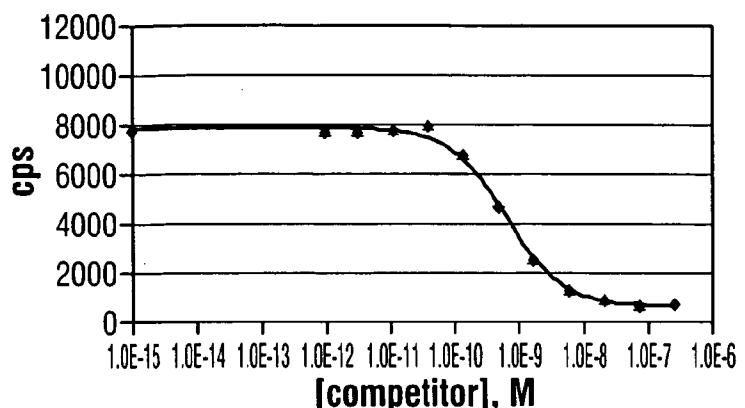
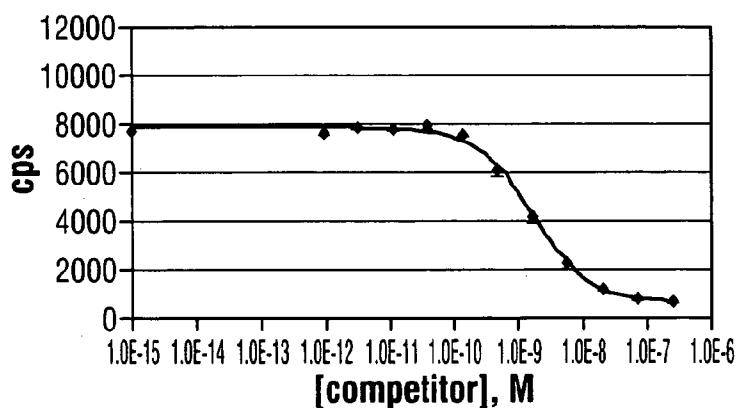
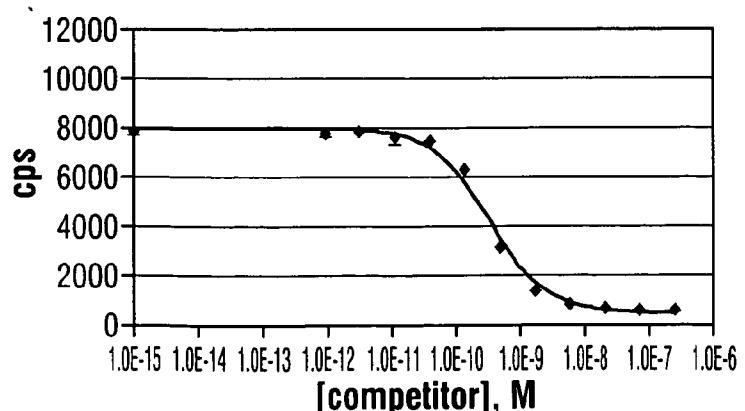
27 / 62

MoAb anti-IgG**IL6R23****IL6R24****IL6R29****IL6R33**

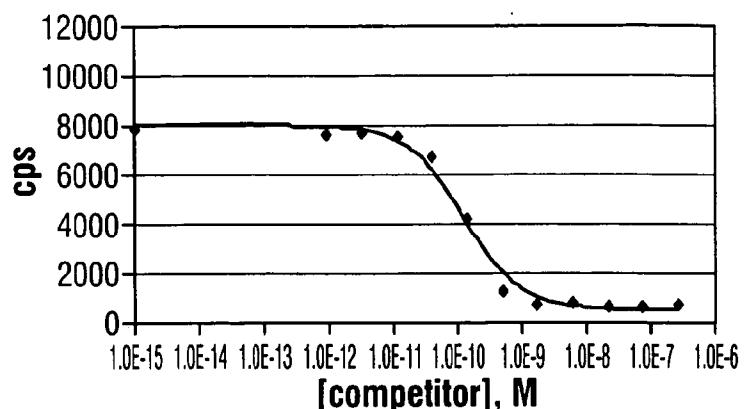
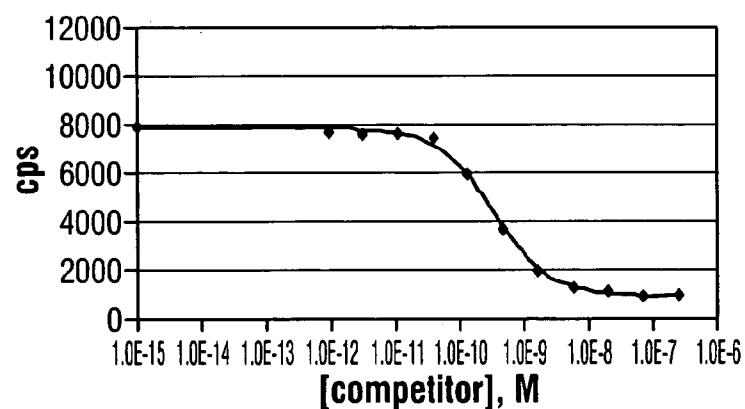
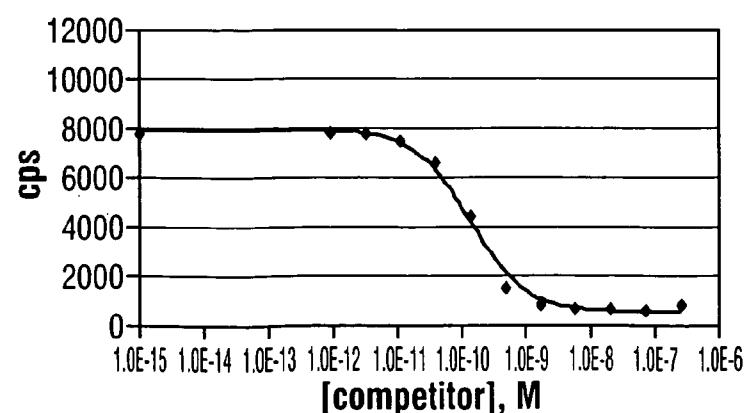
28 / 62

Fig. 16**IL6R22****IL-6R23: IL-6R/IL-6 assay****IL6R24**

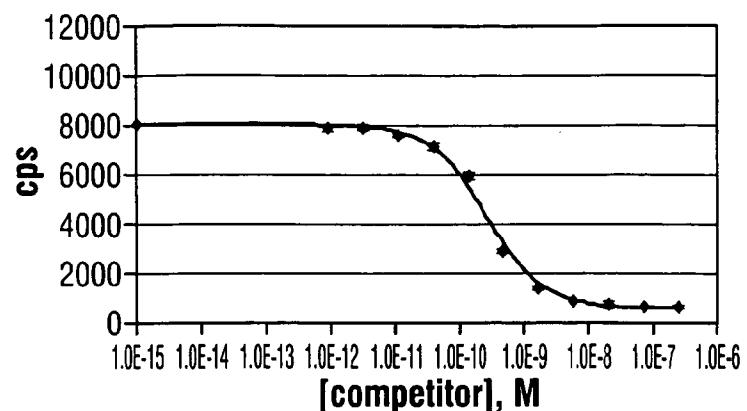
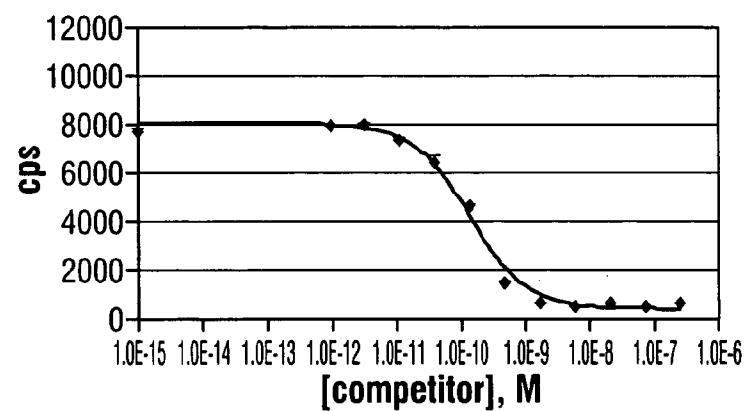
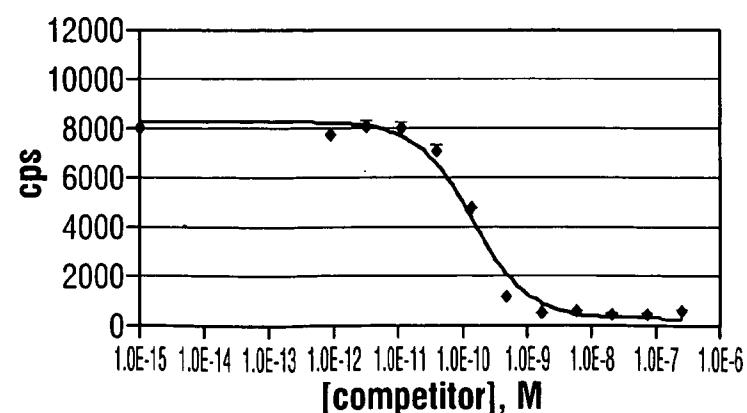
29 / 62

Fig. 16 (cont.)**IL6R25****IL6R26****IL6R28**

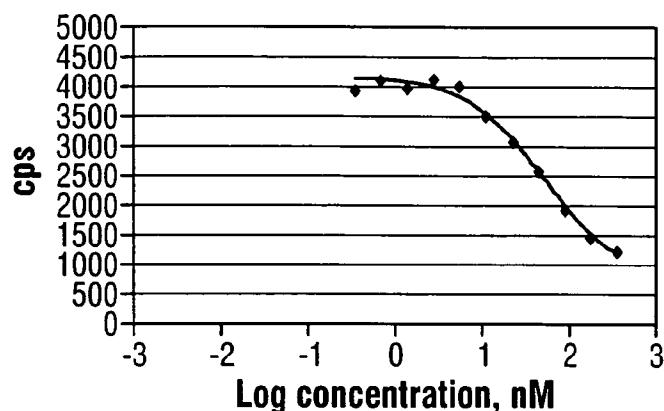
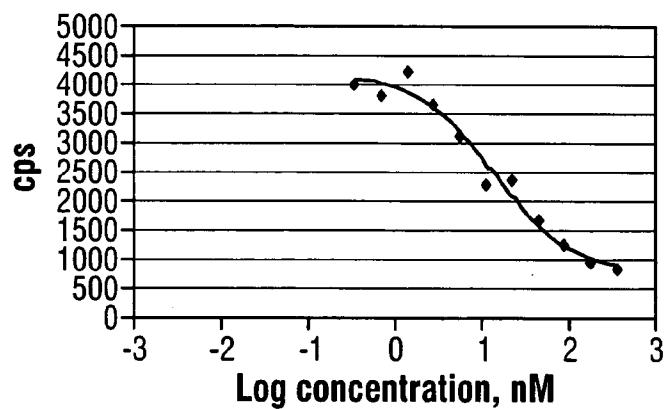
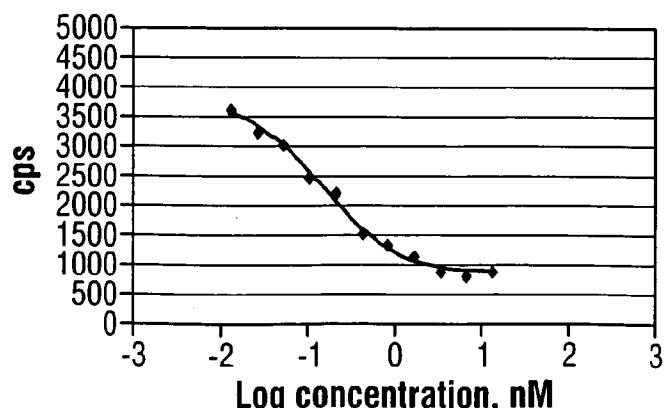
30 / 62

Fig. 17**IL6R29****IL6R30****IL6R31**

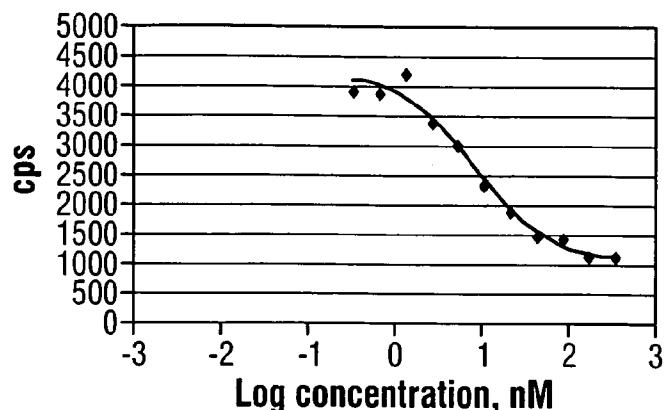
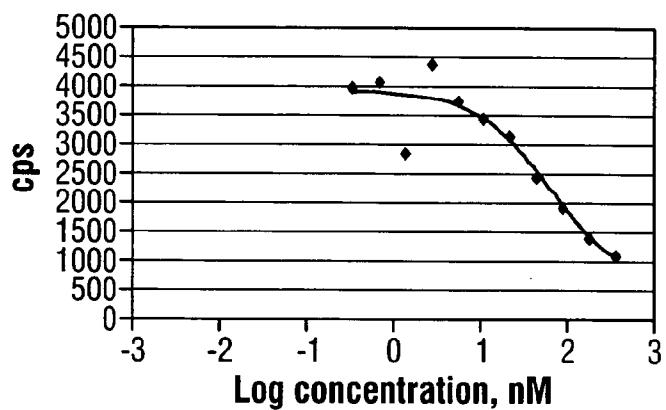
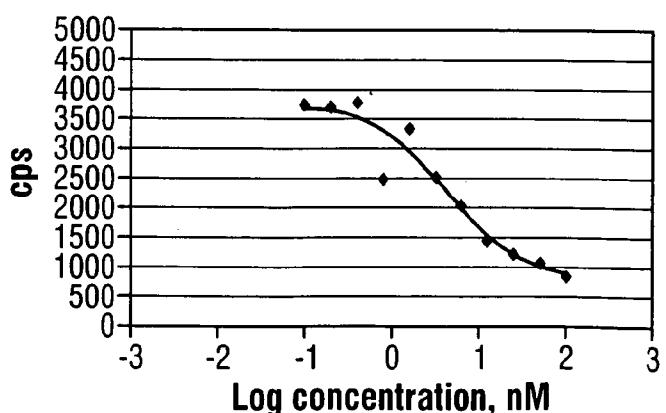
31 / 62

Fig. 17 (cont.)**IL6R32****IL6R33****IL6R34**

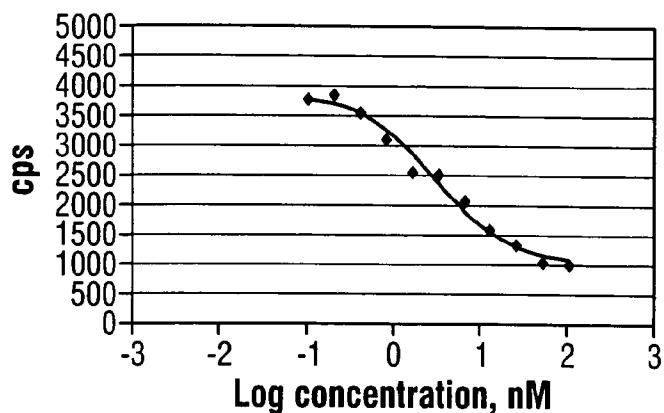
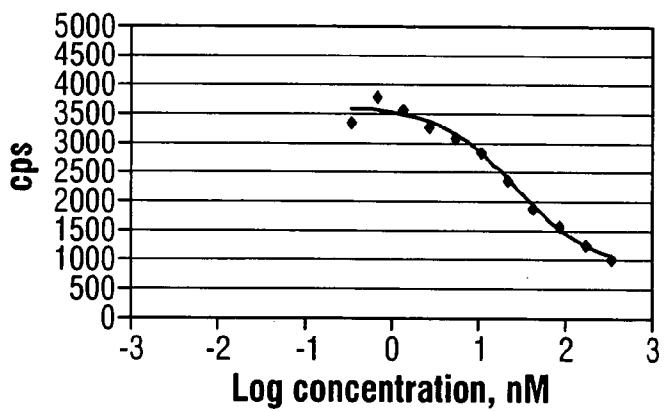
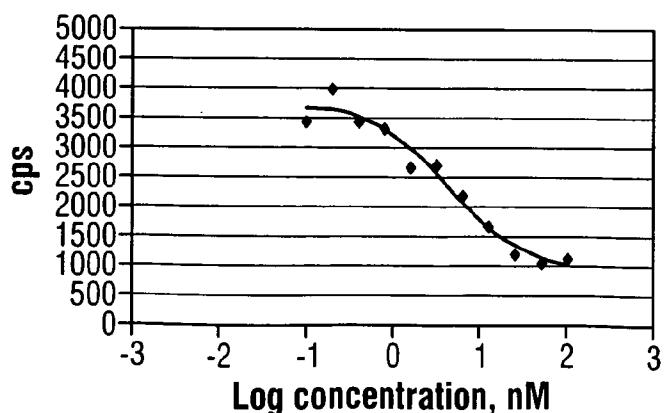
32 / 62

Fig. 18**IL6R22****IL6R23****IL6R24**

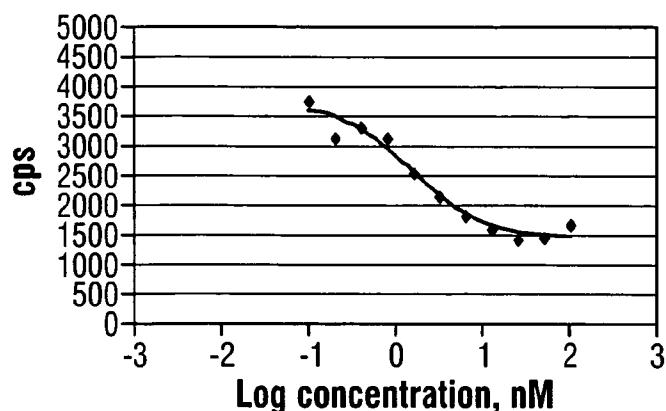
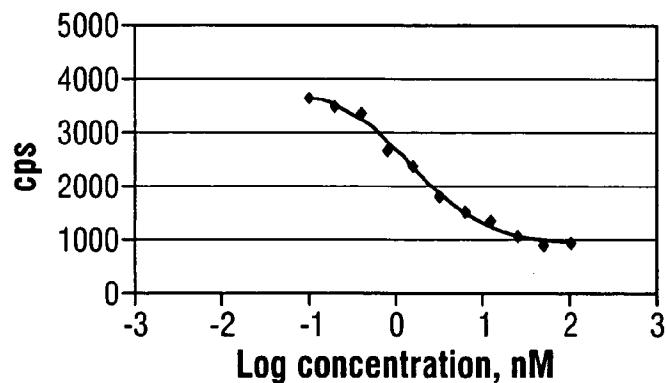
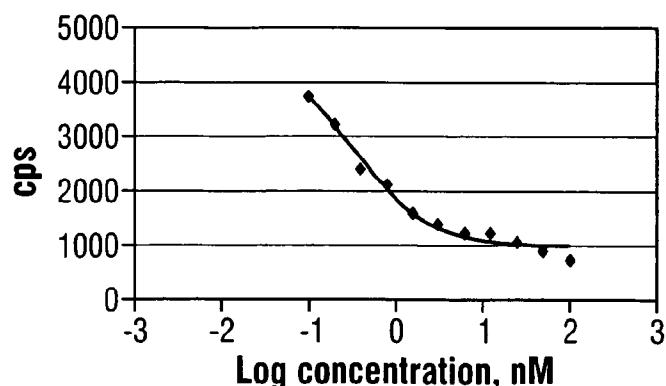
33 / 62

Fig. 18 (cont.)**IL6R25****IL6R26****IL6R28**

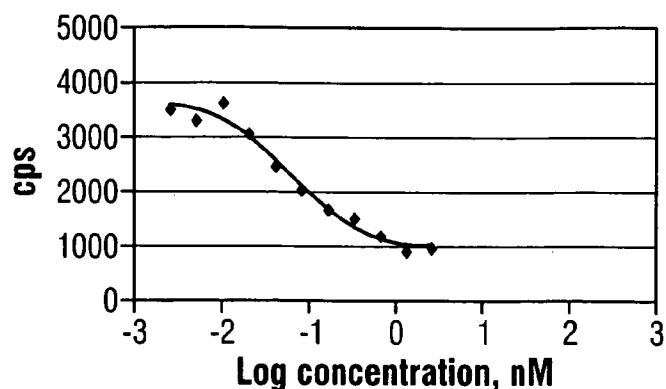
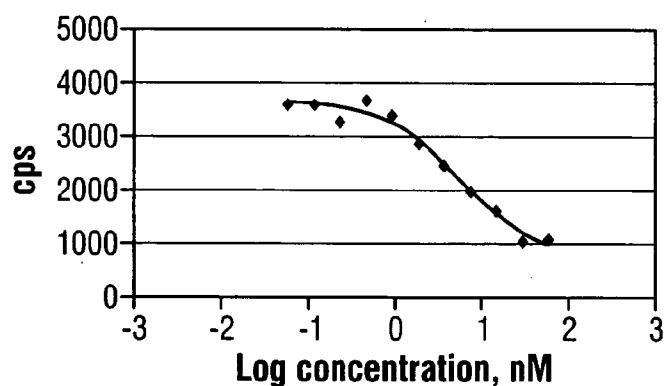
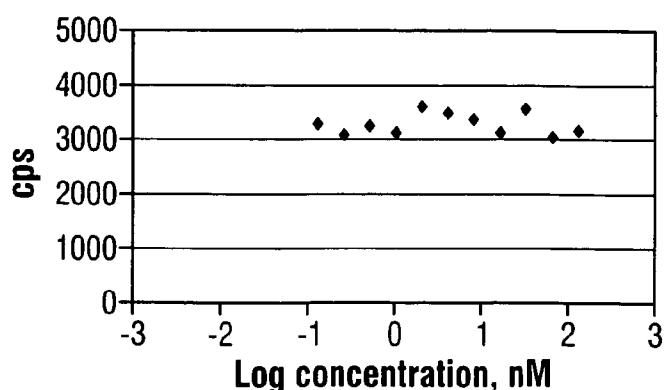
34 / 62

Fig. 19**IL6R29****IL6R30****IL6R31**

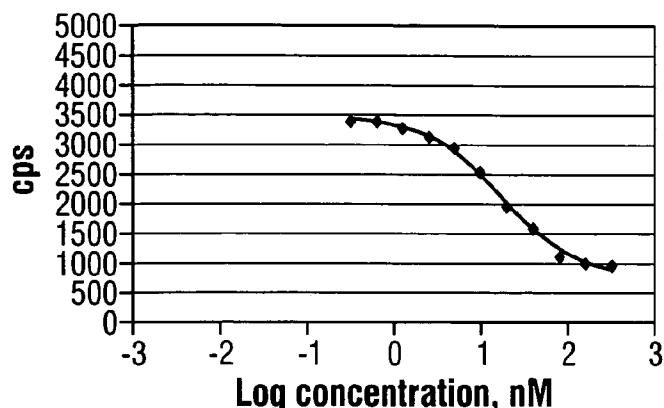
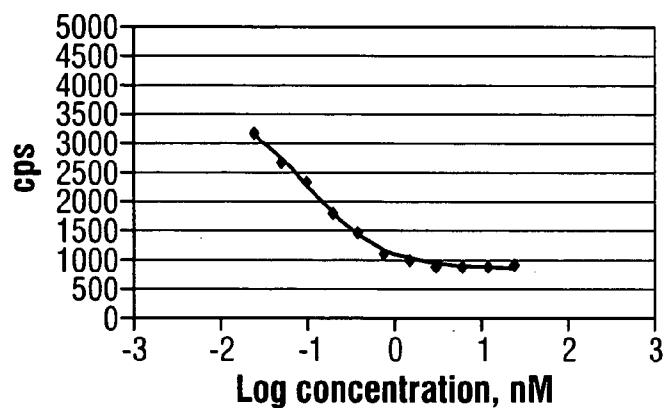
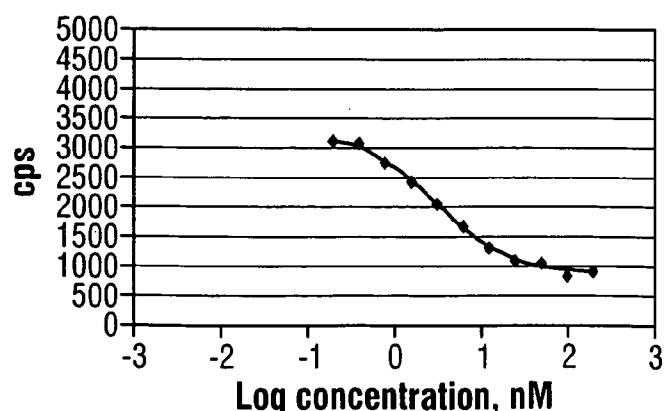
35 / 62

Fig. 19 (cont.)**IL6R32****IL6R33****IL6R34**

36 / 62

Fig. 20**BR6****Reference Fab****BN12**

37 / 62

Fig. 21**IL6R03****IL6R04****IL6R09**

38 / 62

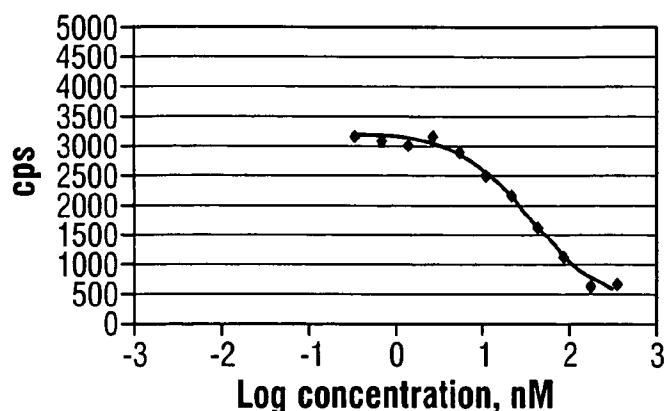
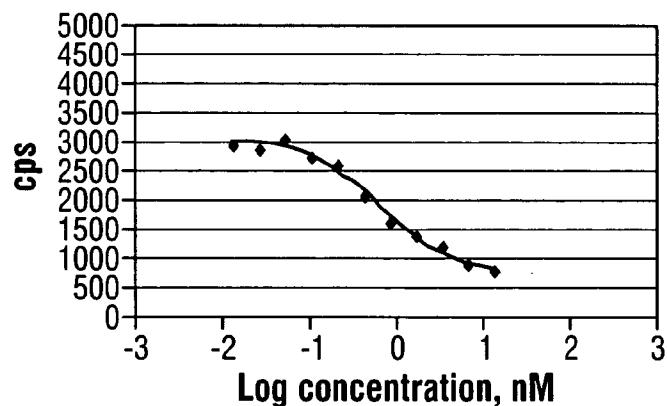
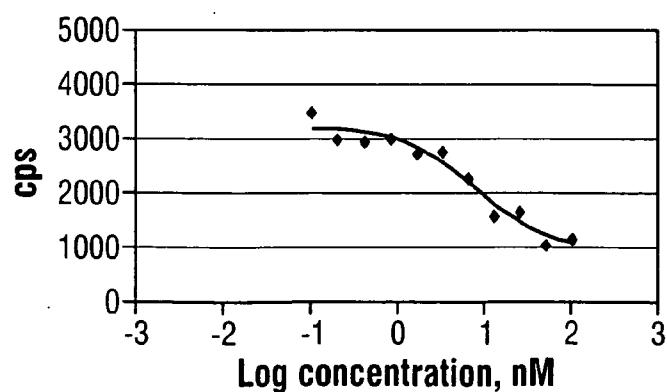
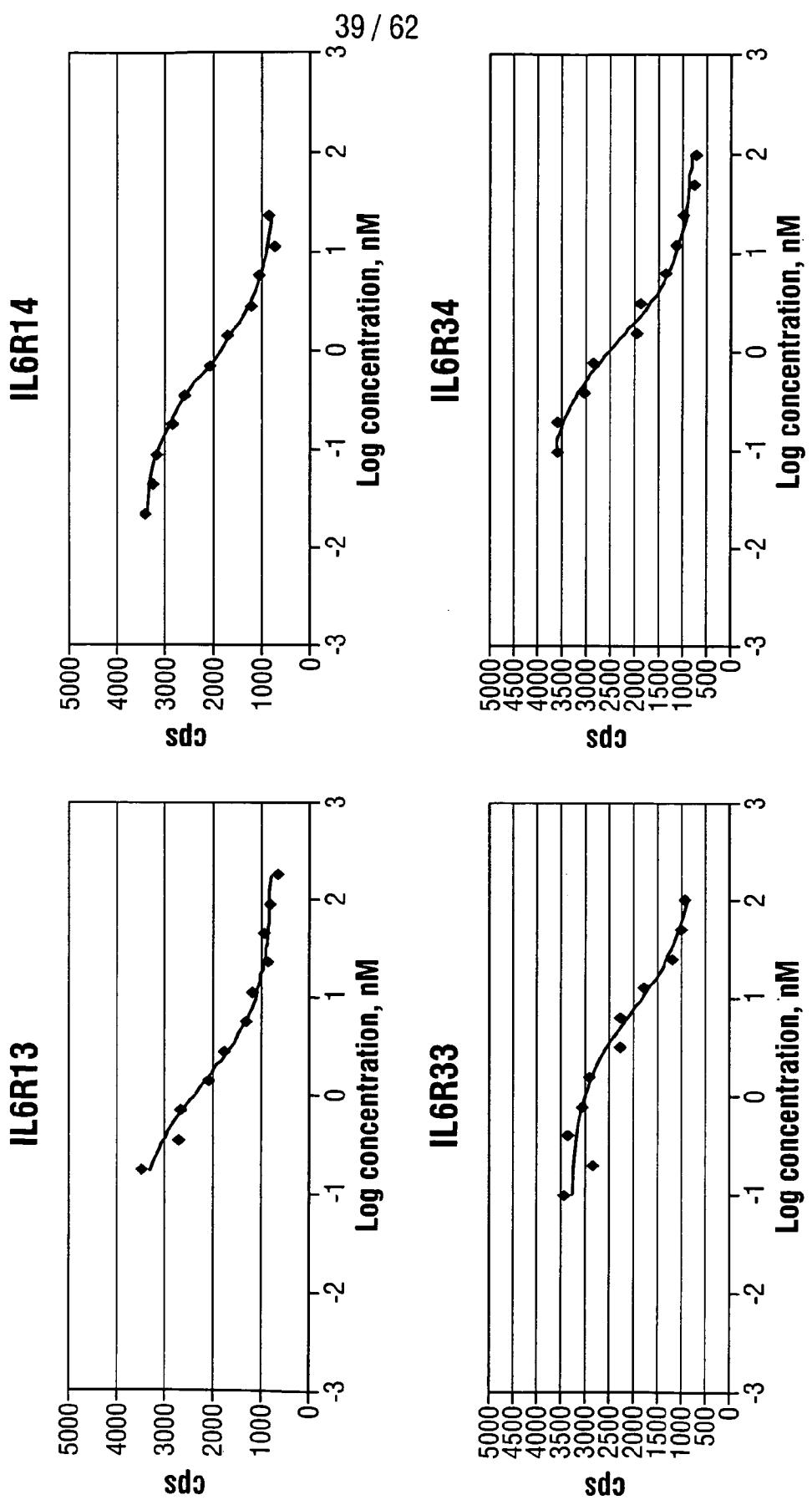
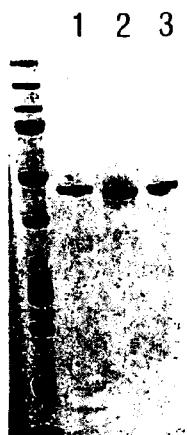
Fig. 21 (cont.)**IL6R23****IL6R24****IL6R29**

Fig. 22

40 / 62

Fig. 23

1=1 µg IL-6R 53
2=1 µg IL-6R 49
3=1 µg IL-6R 44

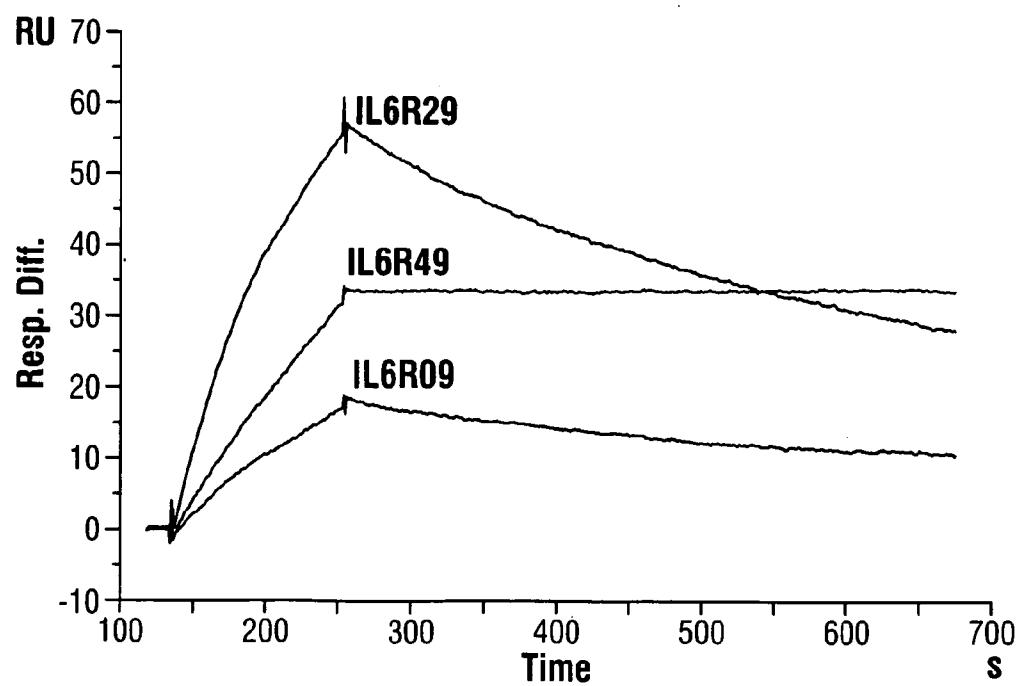
Fig. 24

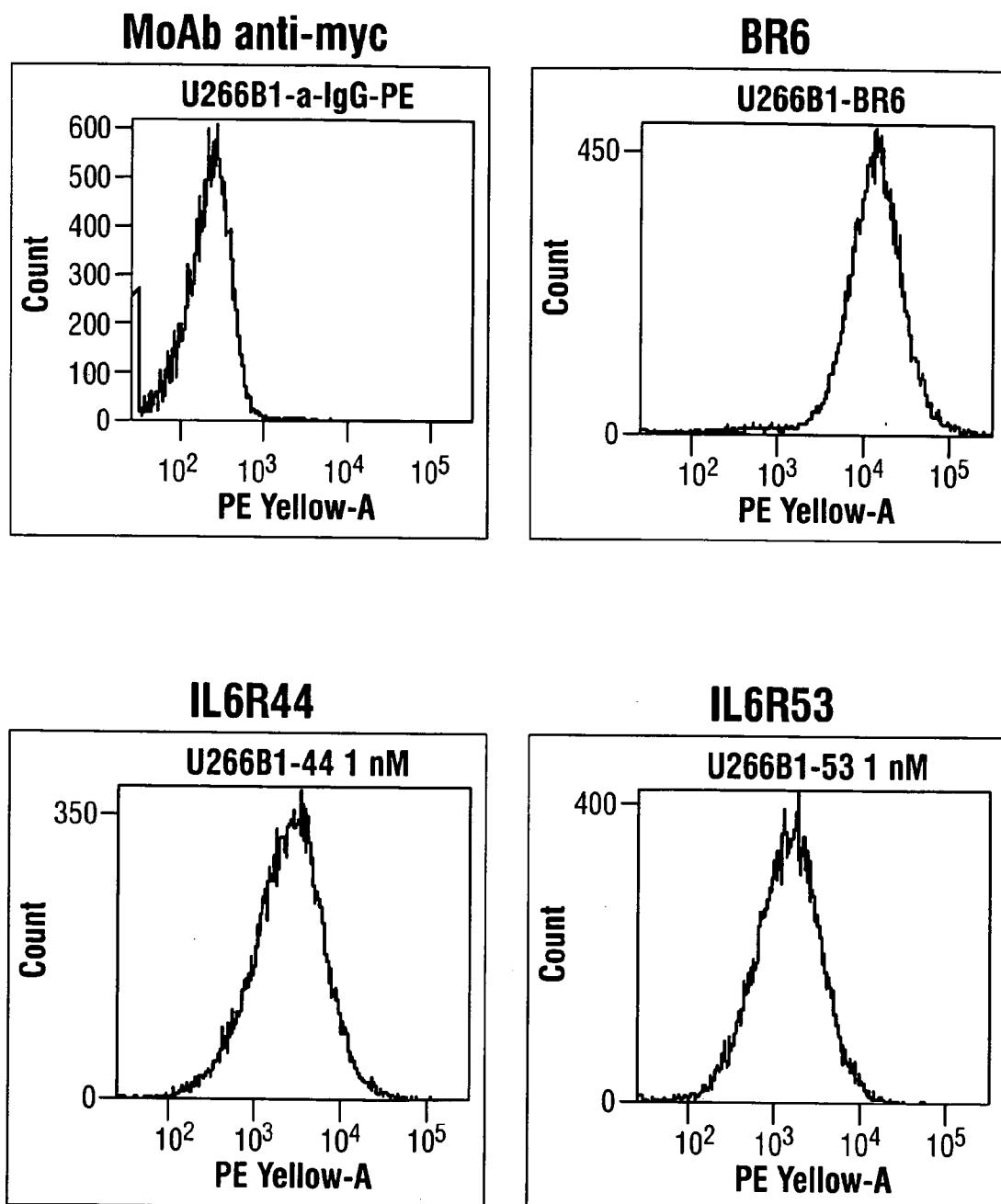
Fig. 25

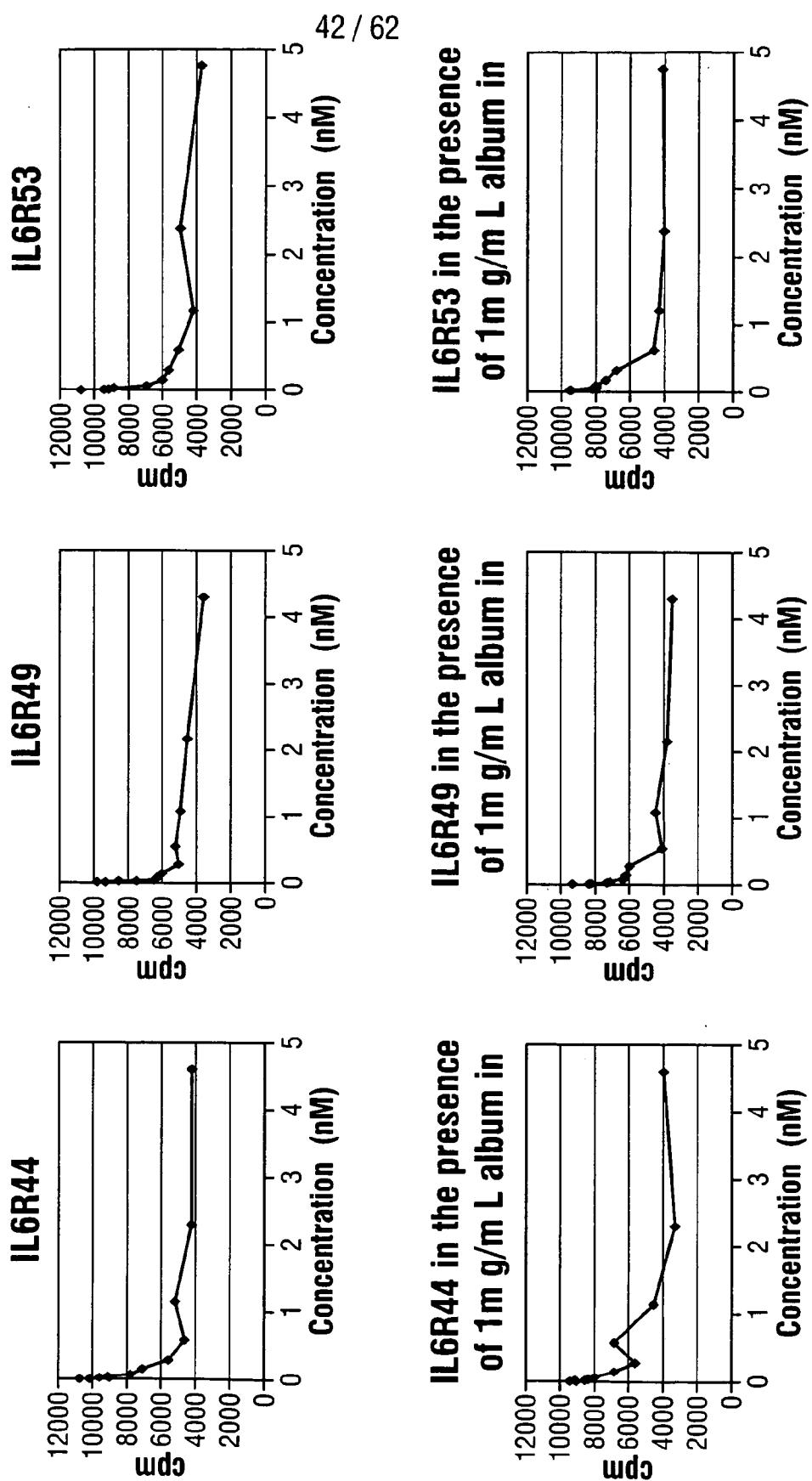
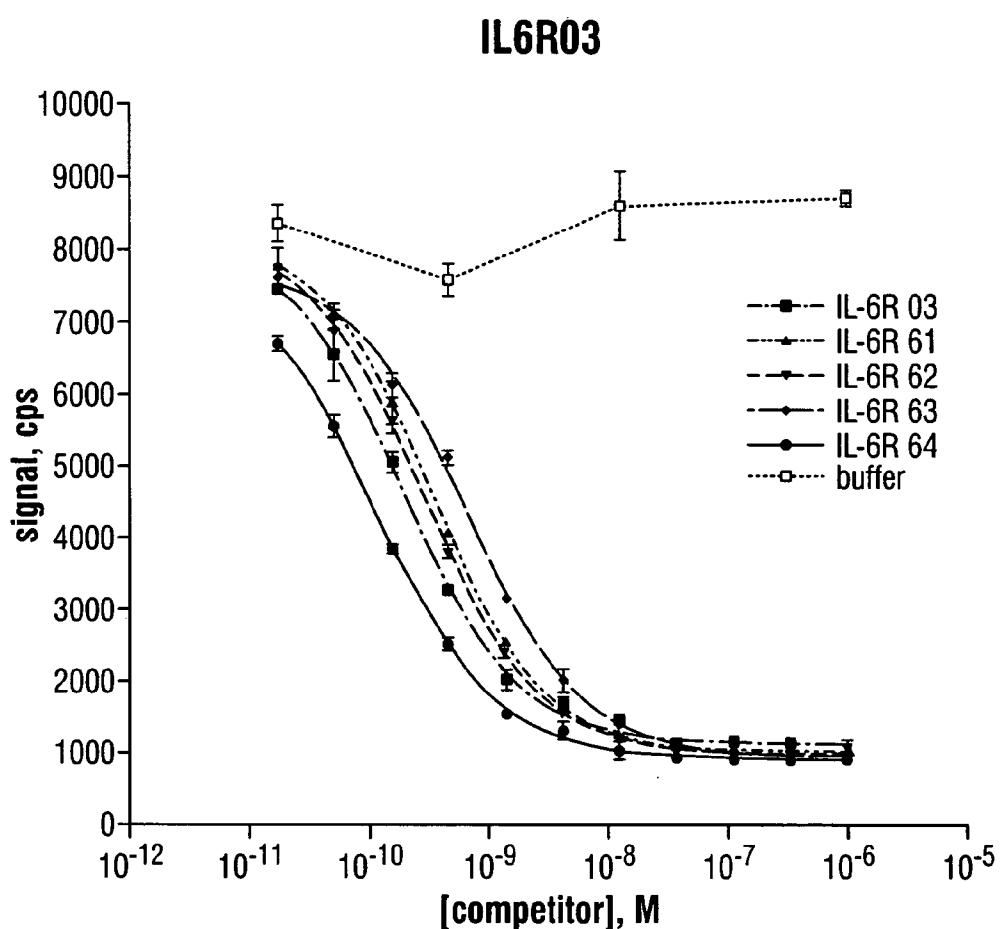
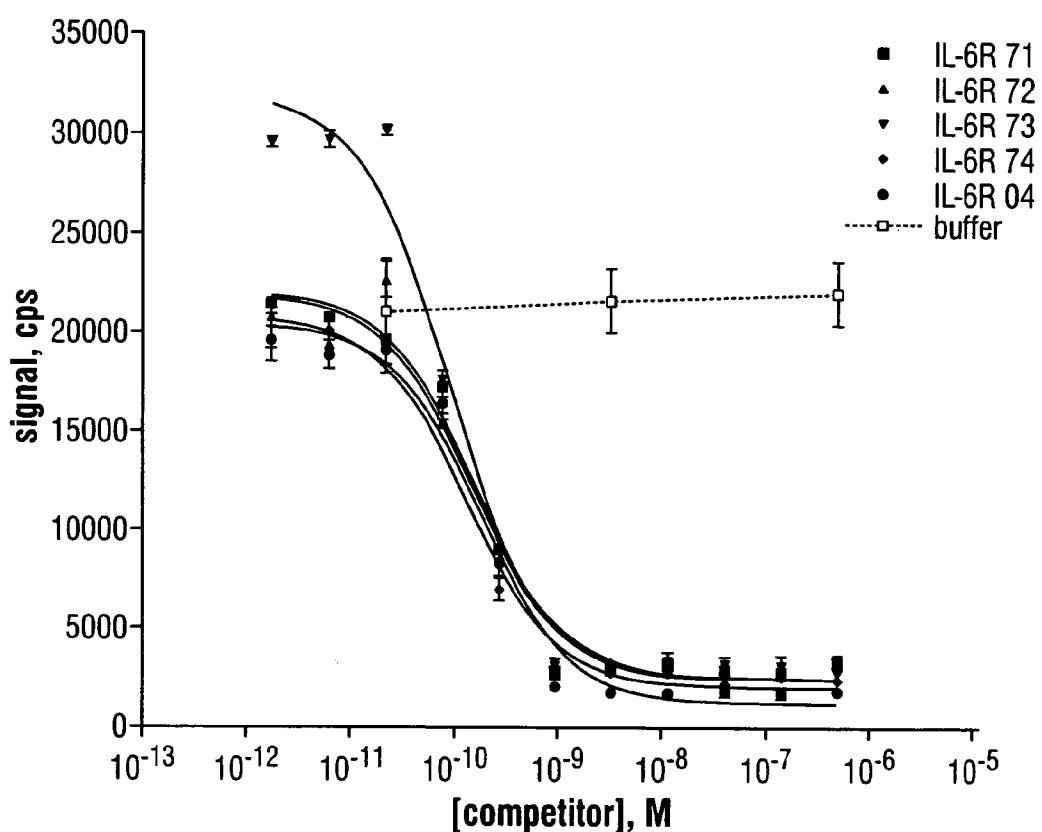
Fig. 26

Fig. 27

IL6R4	32C9 hum1	EVQVEGGGVWYQPESSRLSCAASGFTDDIGWFOAQGKRECEVSGISSSGCETTAAVGRTISSDIAKTVLQMSIRPDTAVYCAEPSSVYDGSPEEKYKGCTT
IL6R71	32C9 hum2	EVQVEGGGVWYQPESSRLSCAASGFTDDIGWFOAQGKRECEVSGISSSGCETTAAVGRTISSDIAKTVLQMSIRPDTAVYCAEPSSVYDGSPEEKYKGCTT
IL6R72	32C9 hum3	EVQVEGGGVWYQPESSRLSCAASGFTDDIGWFOAQGKRECEVSGISSSGCETTAAVGRTISSDIAKTVLQMSIRPDTAVYCAEPSSVYDGSPEEKYKGCTT
IL6R73	32C9 hum4	EVQVEGGGVWYQPESSRLSCAASGFTDDIGWFOAQGKRECEVSGISSSGCETTAAVGRTISSDIAKTVLQMSIRPDTAVYCAEPSSVYDGSPEEKYKGCTT
IL6R74		
IL6R75 PMP 32C9 hum5		
IL6R13	35H4 hum1	EVQVVEGGGVWYQPESSRLSCAASGFTDDIGWFOAQGKRECEVSGISSSGCETTAAVGRTISSDIAKTVLQMSIRPDTAVYCAEPSSVYDGSPEEKYKGCTT
IL6R81	35H4 hum2	EVQVVEGGGVWYQPESSRLSCAASGFTDDIGWFOAQGKRECEVSGISSSGCETTAAVGRTISSDIAKTVLQMSIRPDTAVYCAEPSSVYDGSPEEKYKGCTT
IL6R82	35H4 hum3	EVQVVEGGGVWYQPESSRLSCAASGFTDDIGWFOAQGKRECEVSGISSSGCETTAAVGRTISSDIAKTVLQMSIRPDTAVYCAEPSSVYDGSPEEKYKGCTT
IL6R83	35H4 hum3	EVQVVEGGGVWYQPESSRLSCAASGFTDDIGWFOAQGKRECEVSGISSSGCETTAAVGRTISSDIAKTVLQMSIRPDTAVYCAEPSSVYDGSPEEKYKGCTT
IL6R84	35H4 hum3	EVQVVEGGGVWYQPESSRLSCAASGFTDDIGWFOAQGKRECEVSGISSSGCETTAAVGRTISSDIAKTVLQMSIRPDTAVYCAEPSSVYDGSPEEKYKGCTT
IL6R03		
IL6R61	31A4 hum1	EVQVVEGGGVWYQPESSRLSCAASGFTTAKNAGTWRQPGREVALGTTGSGSTTAAVGRTISSDIAKTVLQMSIRPDTAVYCAEPSSVYDGSPEEKYKGCTT
IL6R62	31A4 hum2	EVQVVEGGGVWYQPESSRLSCAASGFTTAKNAGTWRQPGREVALGTTGSGSTTAAVGRTISSDIAKTVLQMSIRPDTAVYCAEPSSVYDGSPEEKYKGCTT
IL6R63	31A4 hum3	EVQVVEGGGVWYQPESSRLSCAASGFTTAKNAGTWRQPGREVALGTTGSGSTTAAVGRTISSDIAKTVLQMSIRPDTAVYCAEPSSVYDGSPEEKYKGCTT
IL6R64	31A4 hum4	EVQVVEGGGVWYQPESSRLSCAASGFTTAKNAGTWRQPGREVALGTTGSGSTTAAVGRTISSDIAKTVLQMSIRPDTAVYCAEPSSVYDGSPEEKYKGCTT
IL6R65 PMP 31A4 hum5		
IL6R13	35H4 hum5	EVQVVEGGGVWYQPESSRLSCAASGFTDDIGSWTRQPAQFLEWYSAISWGH--NYYTESMKGRTTSRQNAKTVLQMSIRPDTAVYCYKGSTAIVGPPPPDYEYDYGCTT
IL6R85	35H4 hum6	EVQVVEGGGVWYQPESSRLSCAASGFTDDIGSWTRQPAQFLEWYSAISWGH--NYYTESMKGRTTSRQNAKTVLQMSIRPDTAVYCYKGSTAIVGPPPPDYEYDYGCTT
IL6R86	35H4 hum7	EVQVVEGGGVWYQPESSRLSCAASGFTDDIGSWTRQPAQFLEWYSAISWGH--NYYTESMKGRTTSRQNAKTVLQMSIRPDTAVYCYKGSTAIVGPPPPDYEYDYGCTT
IL6R87	35H4 hum7	EVQVVEGGGVWYQPESSRLSCAASGFTDDIGSWTRQPAQFLEWYSAISWGH--NYYTESMKGRTTSRQNAKTVLQMSIRPDTAVYCYKGSTAIVGPPPPDYEYDYGCTT
IL6R88	35H4 hum8	EVQVVEGGGVWYQPESSRLSCAASGFTDDIGSWTRQPAQFLEWYSAISWGH--NYYTESMKGRTTSRQNAKTVLQMSIRPDTAVYCYKGSTAIVGPPPPDYEYDYGCTT
IL6R89	35H4 hum9	EVQVVEGGGVWYQPESSRLSCAASGFTDDIGSWTRQPAQFLEWYSAISWGH--NYYTESMKGRTTSRQNAKTVLQMSIRPDTAVYCYKGSTAIVGPPPPDYEYDYGCTT
IL6R90	35H4 hum10	EVQVVEGGGVWYQPESSRLSCAASGFTDDIGSWTRQPAQFLEWYSAISWGH--NYYTESMKGRTTSRQNAKTVLQMSIRPDTAVYCYKGSTAIVGPPPPDYEYDYGCTT

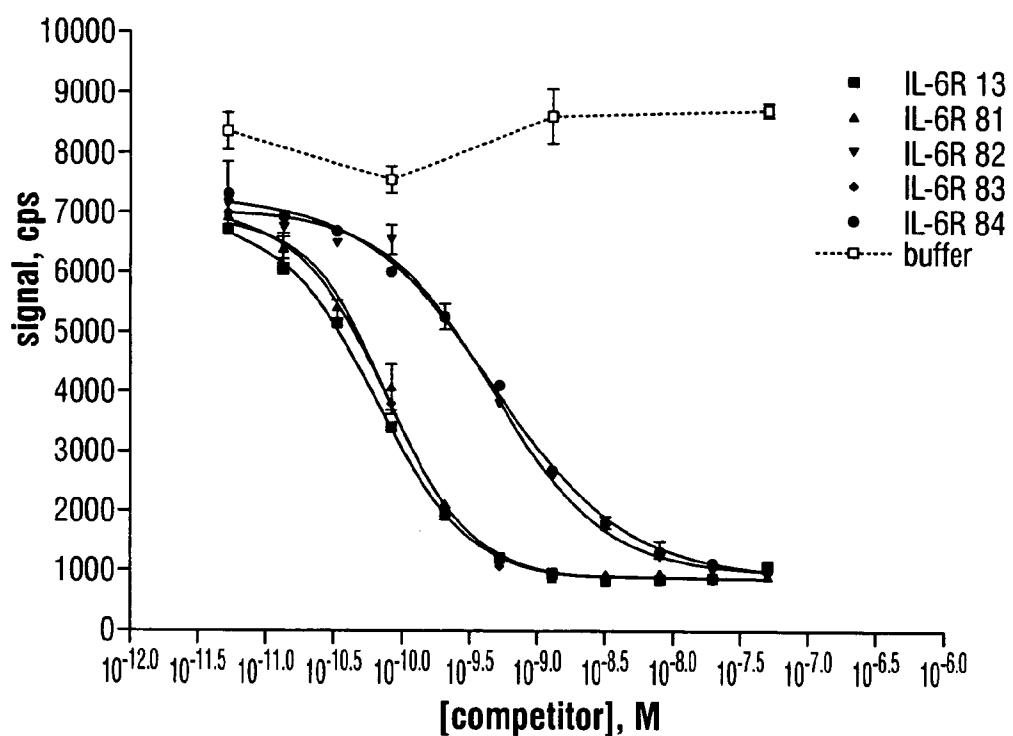
Fig. 28

	IL-6R 03	IL-6R 61	IL-6R 62	IL-6R 63	IL-6R 64
Sigmoidal dose-response (variable slope)					
Best-fit values					
BOTTOM	1112	1011	957.9	926.5	908.6
TOP	8238	8206	8255	7788	7903
LOGEC50	-9.729	-9.473	-9.552	-9.187	-9.967
HILLSLOPE	-0.8898	-0.9341	-0.8962	-0.8886	-0.8624
EC50	1.867e-010	3.362e-010	2.804e-010	6.496e-010	1.080e-010

Fig. 29**IL-6R04 humanized clones in teh IL-6/IL-6R assay**

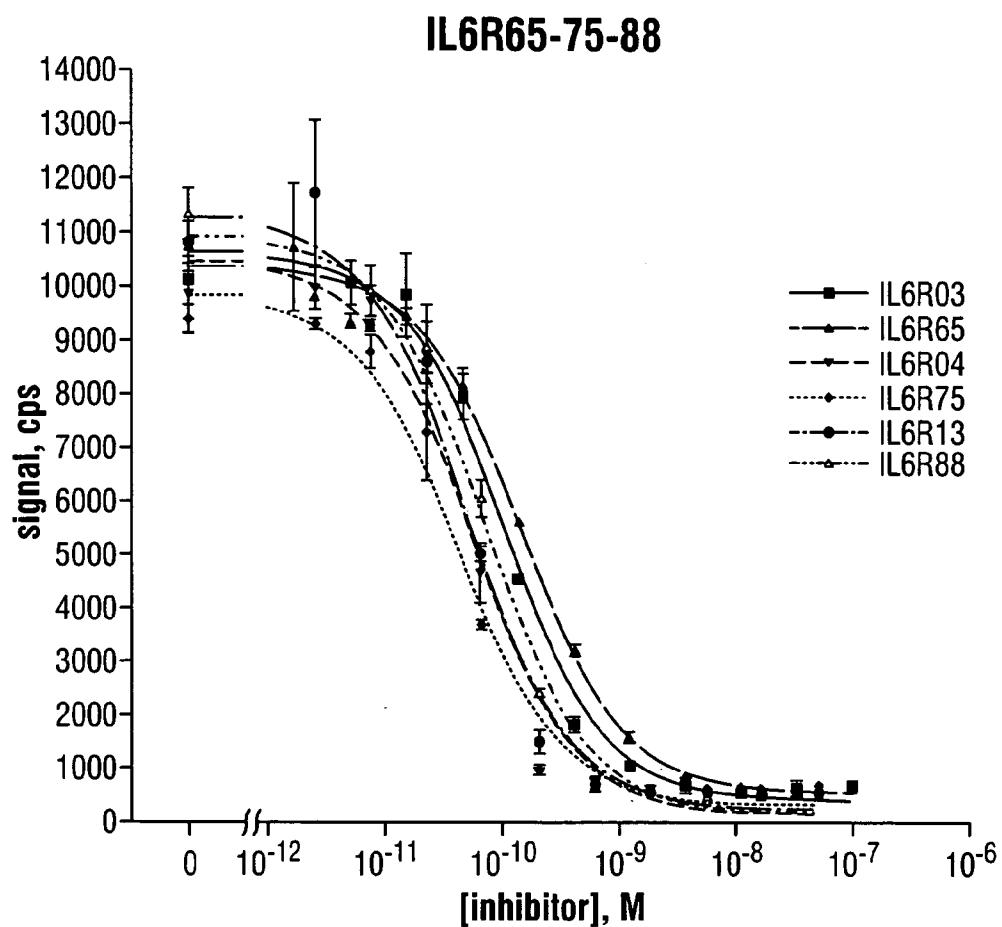
	IL-6R 71	IL-6R 72	IL-6R 73	IL-6R 74	IL-6R 04
Sigmoidal dose-response					
Best-fit values					
BOTTOM	2437	2456	2478	2030	1218
TOP	22034	22123	32032	20905	20499
LOGEC50	-9.816	-9.795	-10.04	-9.878	-9.744
EC50	1.527e-010	1.605e-010	9.165e-011	1.325e-010	1.802e-010

46 / 62

Fig. 30**IL6R13**

	IL-6R 13	IL-6R 81	IL-6R 82	IL-6R 83	IL-6R 84
Sigmoidal dose-response (variable slope)					
Best-fit values					
BOTTOM	859.7	865.8	925.7	860.9	901.0
TOP	6888	7027	7080	6955	7303
LOGEC50	-10.19	-10.13	-9.322	-10.11	-9.332
HILLSLOPE	-1.270	-1.369	-1.039	-1.442	-0.8822
EC50	6.437e-011	7.466e-011	4.764e-010	7.789e-011	4.654e-010

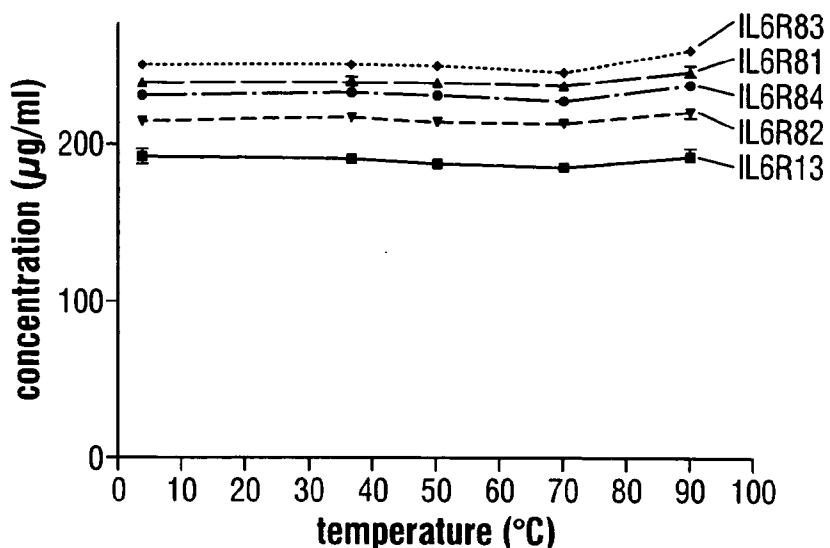
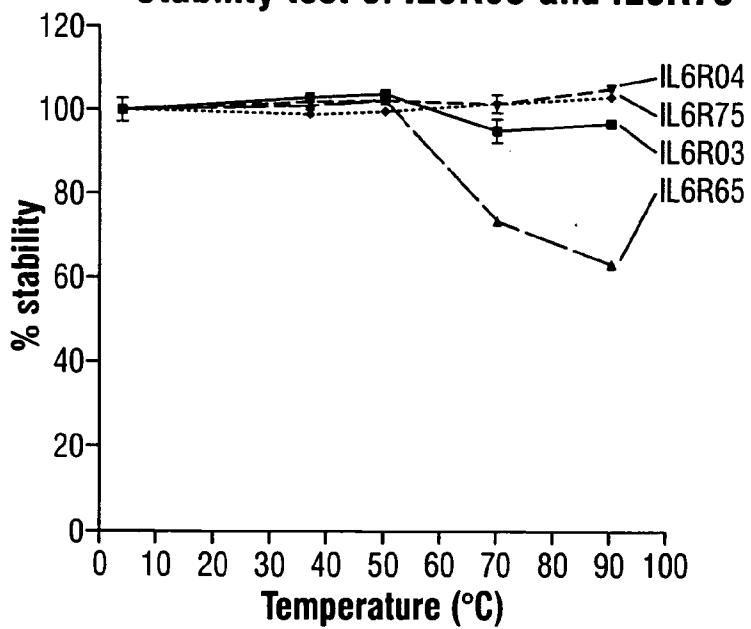
47 / 62

Fig. 31

	IL6R 03	IL6R 65	IL6R 04	IL6R 75	IL6R 13	IL6R 88
LOGEC50	-9.980	-9.833	-10.25	-10.36	-10.30	-10.14
EC50	1.047e-010	1.047e-010	5.680e-011	4.320e-011	5.052e-011	7.273e-011

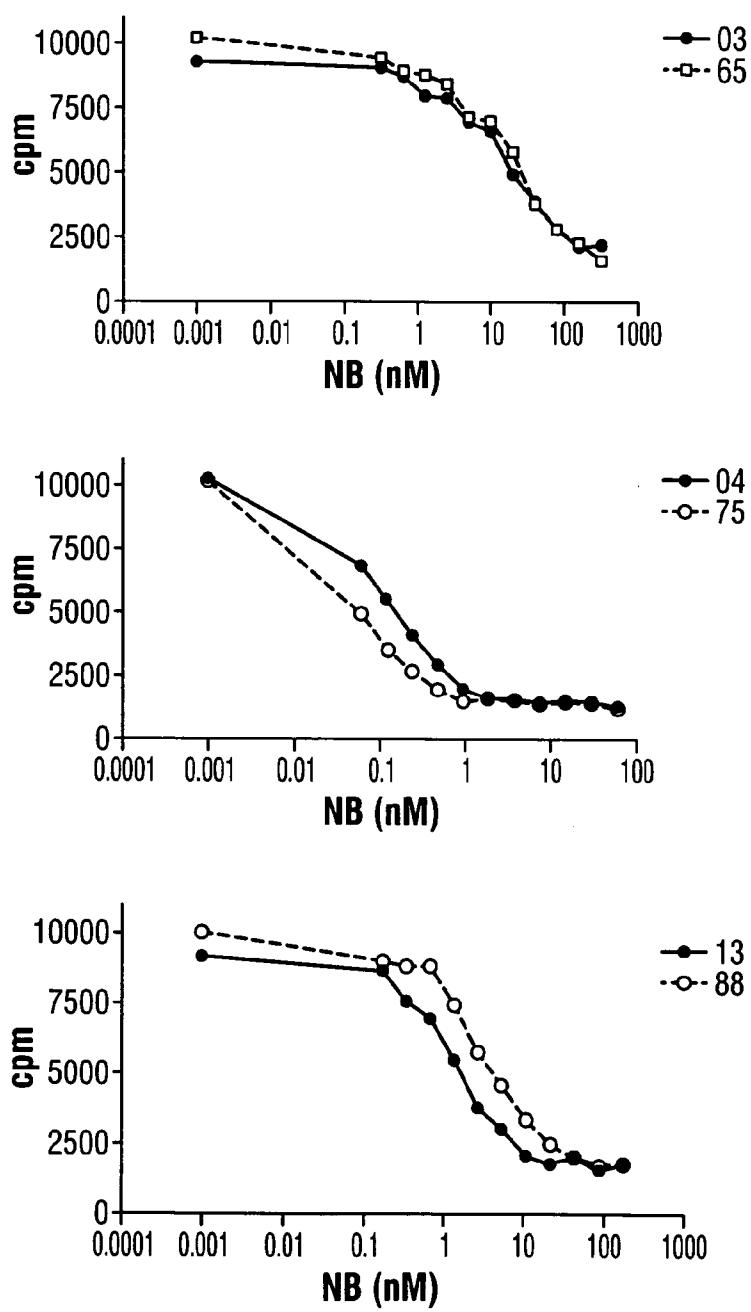
Fig. 32

48 / 62

Stability test of IL6R13+humanised versions**Fig. 33****Stability test of IL6R65 and IL6R75**

Temperature (°C)	IL6R03 (wt)		IL6R65 (hum)		IL6R04 (wt)		IL6R75 (hum)	
	Y1	Y2	Y1	Y2	Y1	Y2	Y1	Y2
4	102.510	97.490	100.160	99.840	100.310	99.690	100.410	99.590
37	102.510	103.350	100.470	101.100	102.460	101.540	99.310	99.030
50	102.680	103.770	102.350	101.720	102.150	101.540	99.030	100.410
70	97.490	92.470	73.240	73.870	103.380	99.380	100.410	101.800
90	96.650	97.070	62.910	63.220	105.540	104.620	103.730	101.800

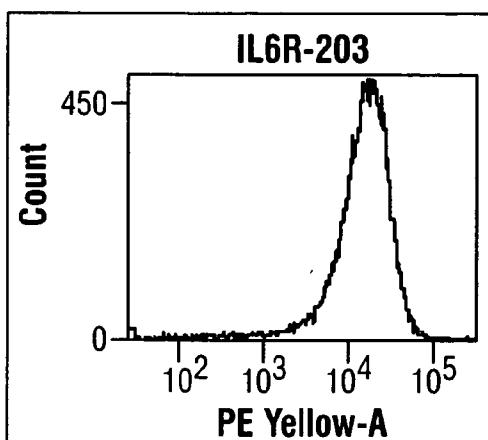
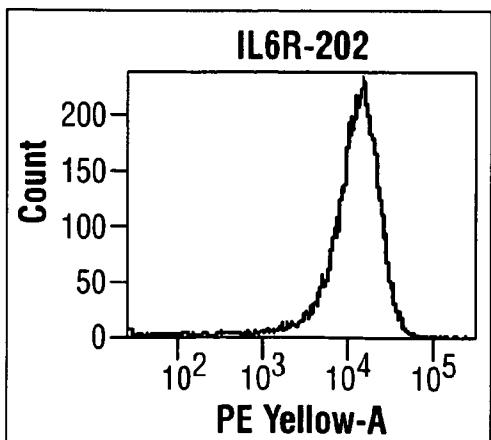
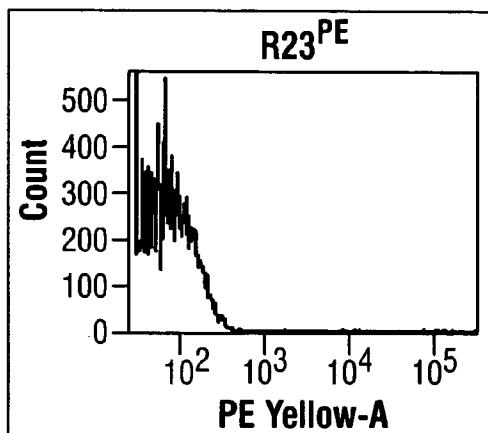
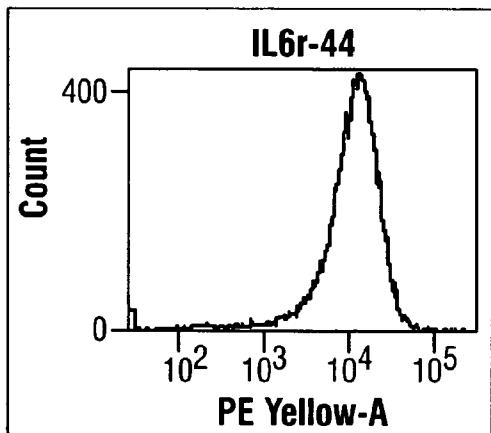
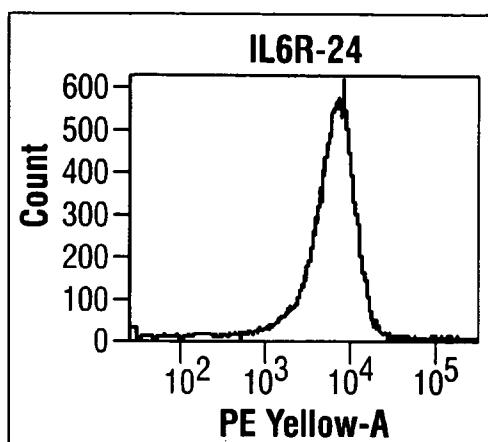
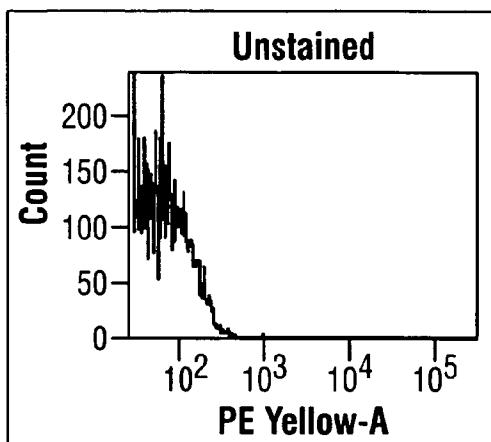
49 / 62

Fig. 34

IC 50 values			
WT	humanised		
3	17 nM	65	26 nM
4	0.1 nM	75	0.04 nM
13	1.4 nM	88	3.3 nM

Fig. 35

50 / 62



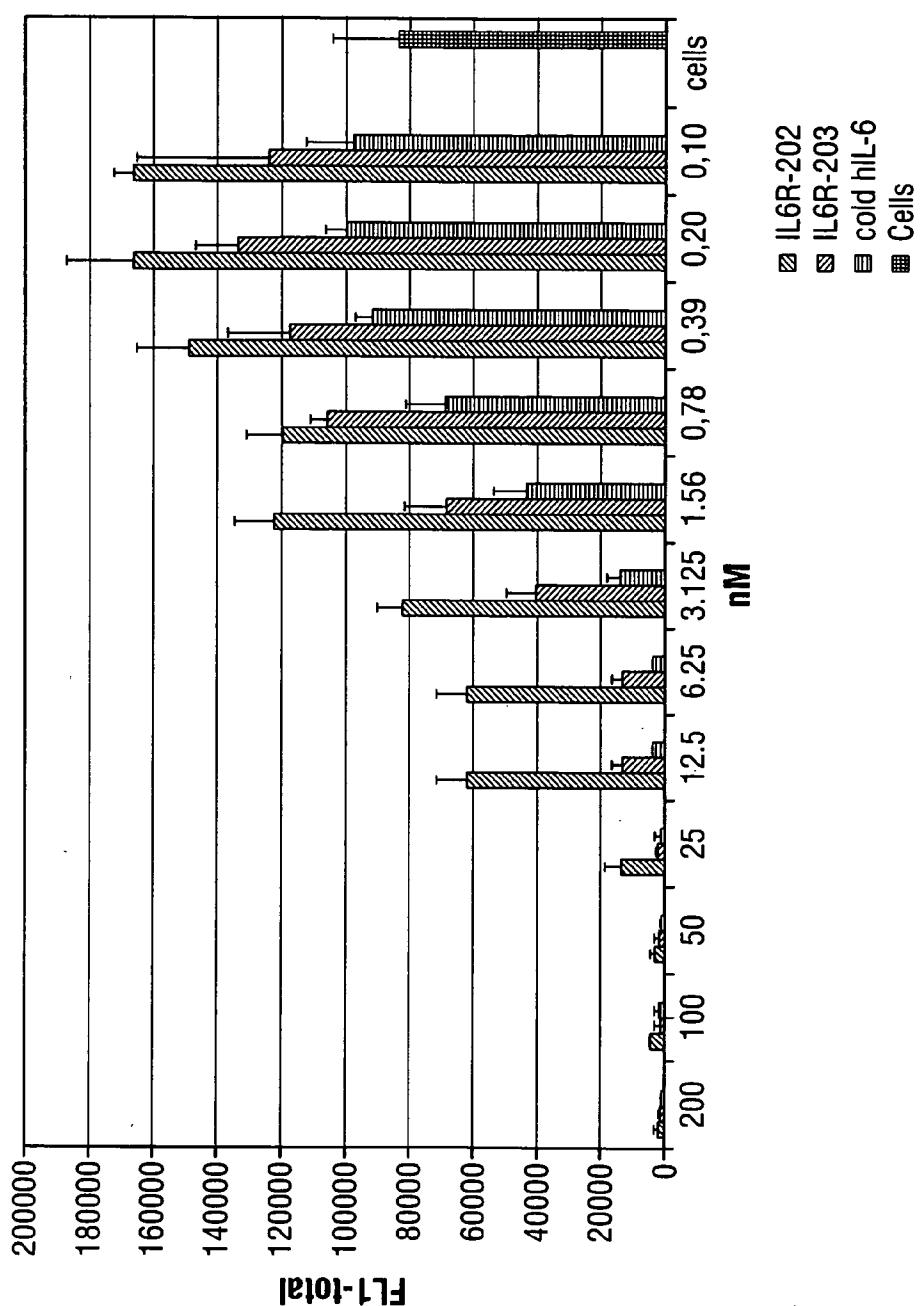


Fig. 36

52 / 62

Fig. 37

**Competition assay with IL-6 alexa 647
on CynoIL6R clone 4D5**

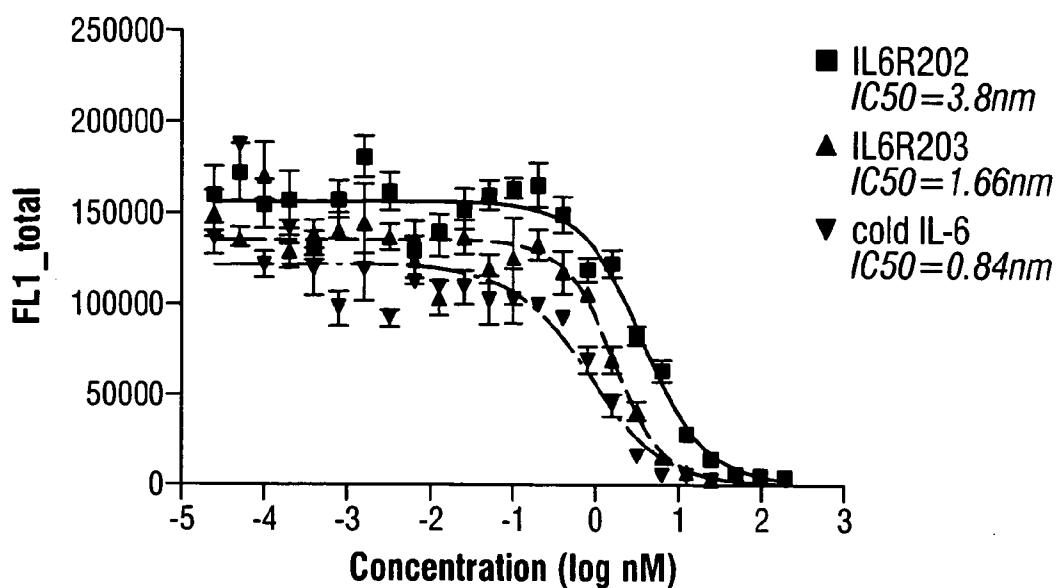
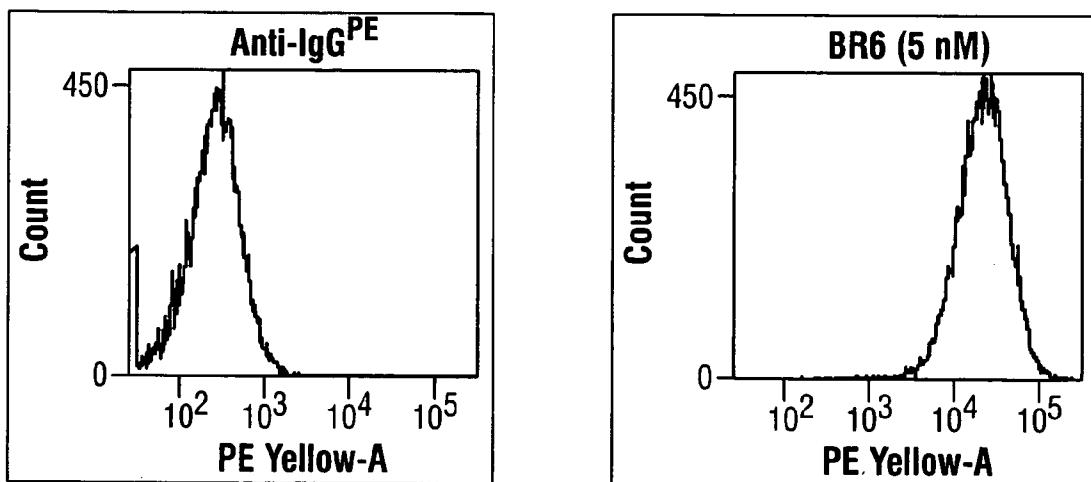
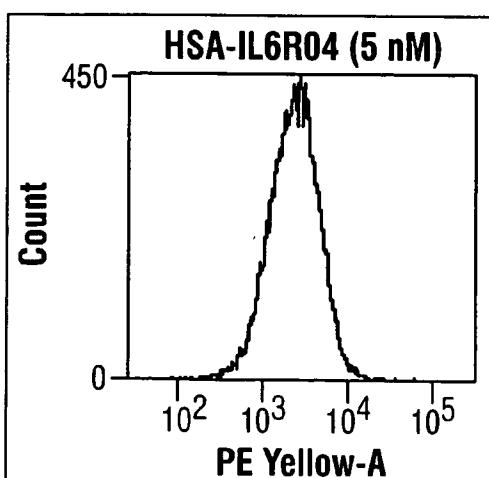
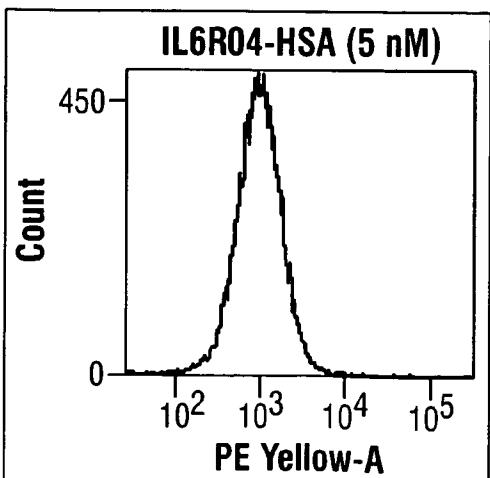
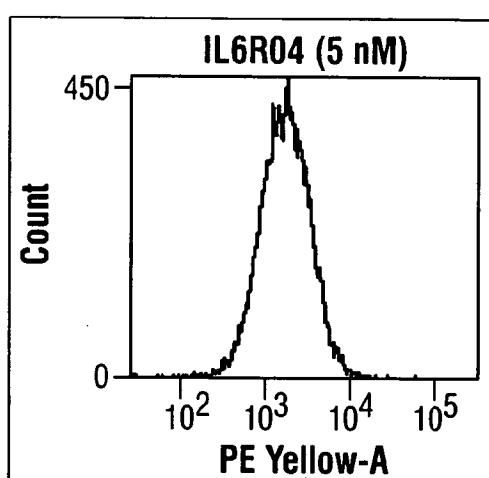
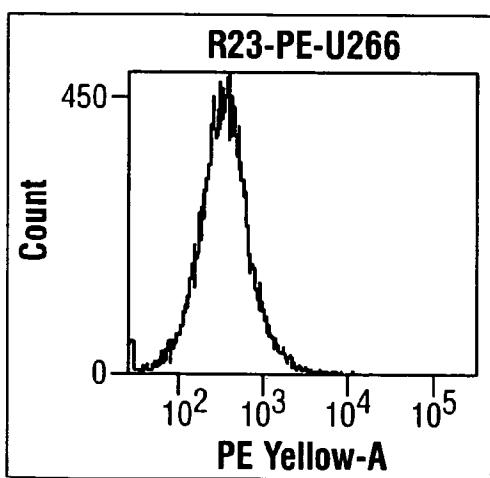
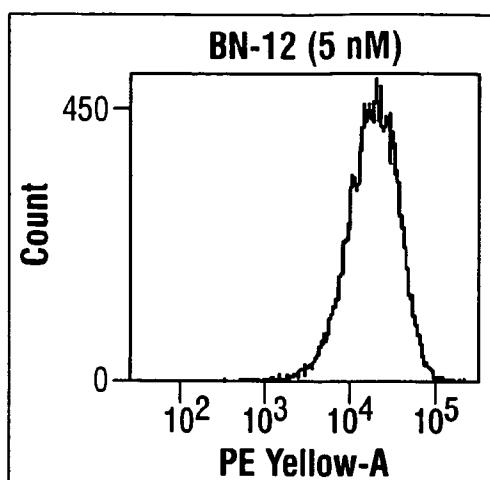
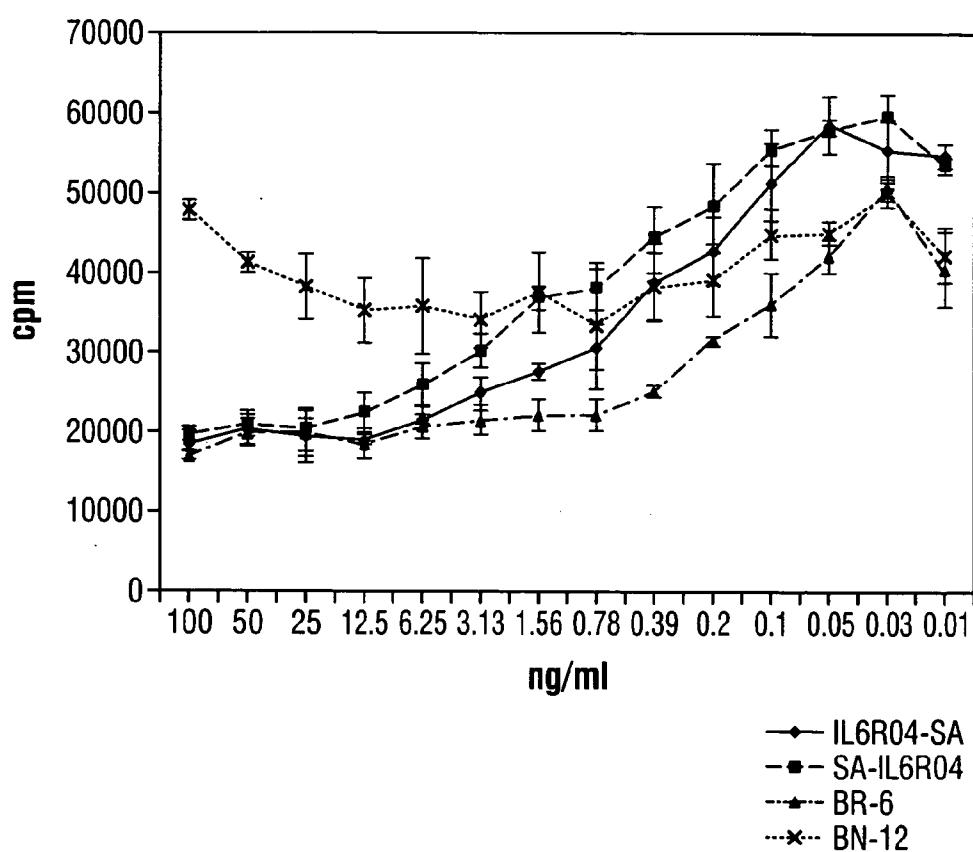
**Fig. 38**

Fig. 38 (cont.)

53 / 62



54 / 62

Fig. 39

55 / 62

Fig. 40

time after administration [d]	Nanobody concentration [$\mu\text{g/ml}$]		
	IL6R04-HSA-m1	IL6R04-HSA-m2	IL6R04-HSA-m3
0.01	230.361 \pm 27.291	153.093 \pm 9.864	267.718 \pm 13.272
0.04	181.800 \pm 22.200	116.827 \pm 4.651	193.174 \pm 37.839
0.08	138.344 \pm 21.009	91.874 \pm 7.251	200.765 \pm 28.966
0.17	95.549 \pm 10.658	103.977 \pm 10.059	79.150 \pm 14.135
0.33	107.148 \pm 30.335	56.946 \pm 8.855	120.554 \pm 18.909
1.00	60.861 \pm 29.498	35.076 \pm 0.565	63.953 \pm 21.415
2.00	22.024 \pm 4.140	15.653 \pm 0.897	23.096 \pm 1.437
3.00	11.160 \pm 0.235	7.889 \pm 1.890	10.842 \pm 0.901
5.00	6.283 \pm 0.166	4.191 \pm 0.501	5.957 \pm 0.257
8.00	0.560 \pm 0.008	0.371 \pm 0.037	0.496 \pm 0.037
14.00	<0.02	<0.02	<0.02
slope	-0.62	-0.62	-0.65
$t_{1/2}$	1.11	1.12	1.07
			days

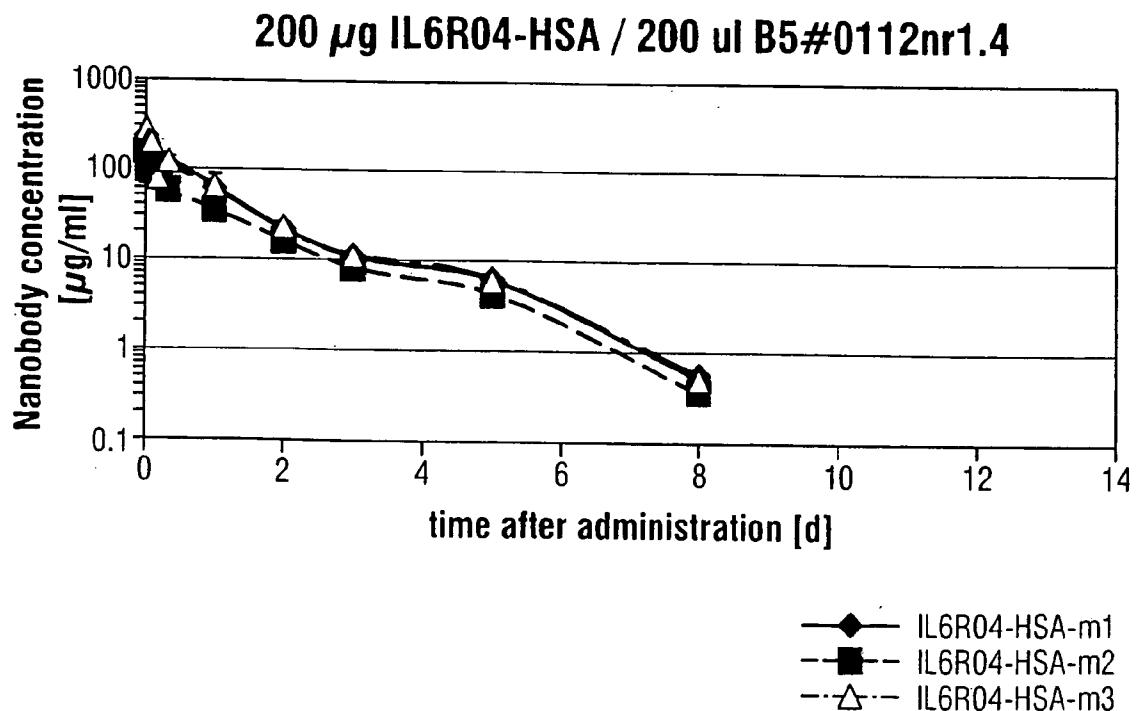
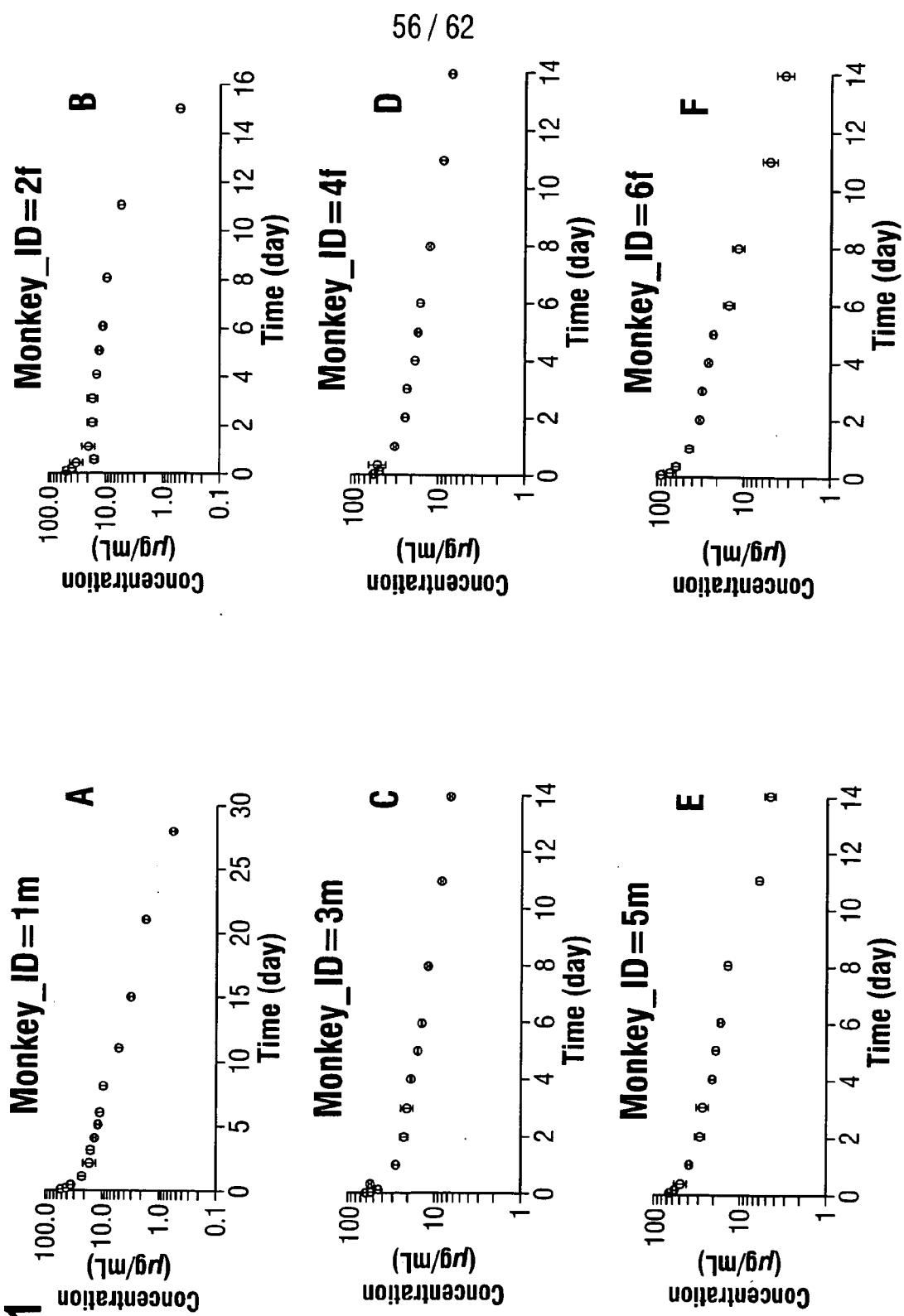
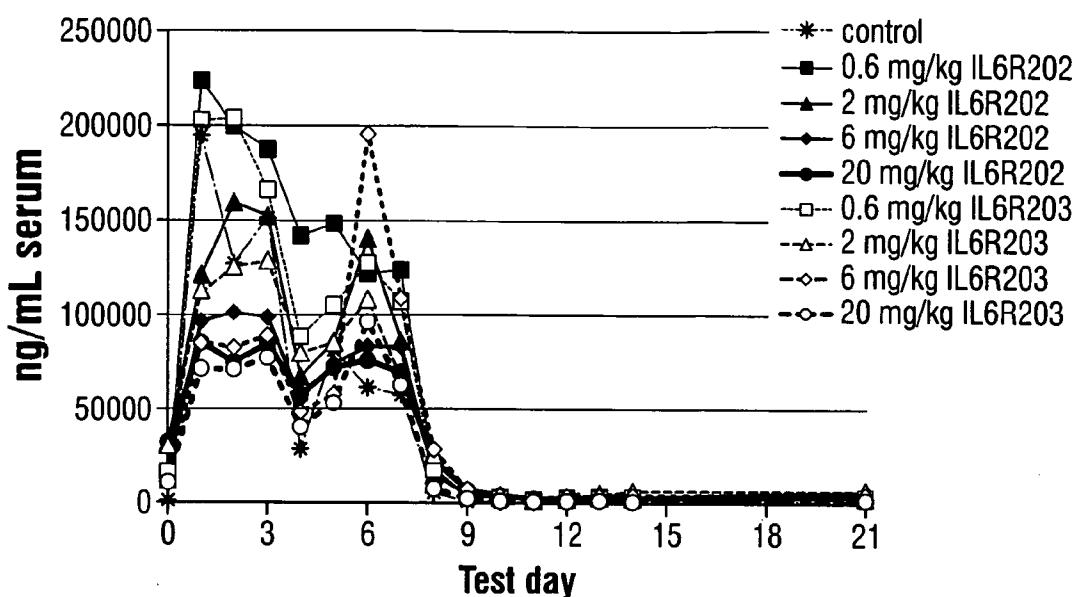
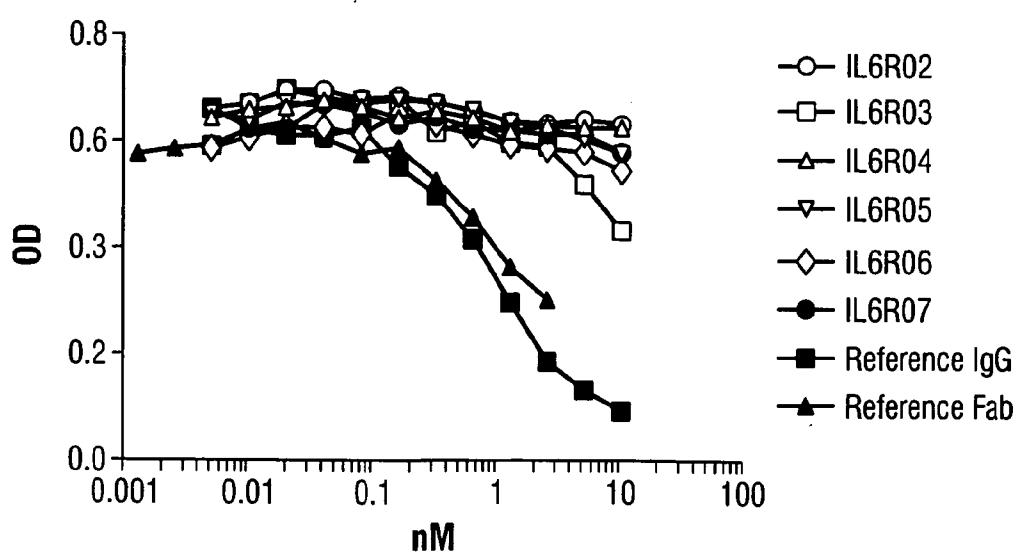
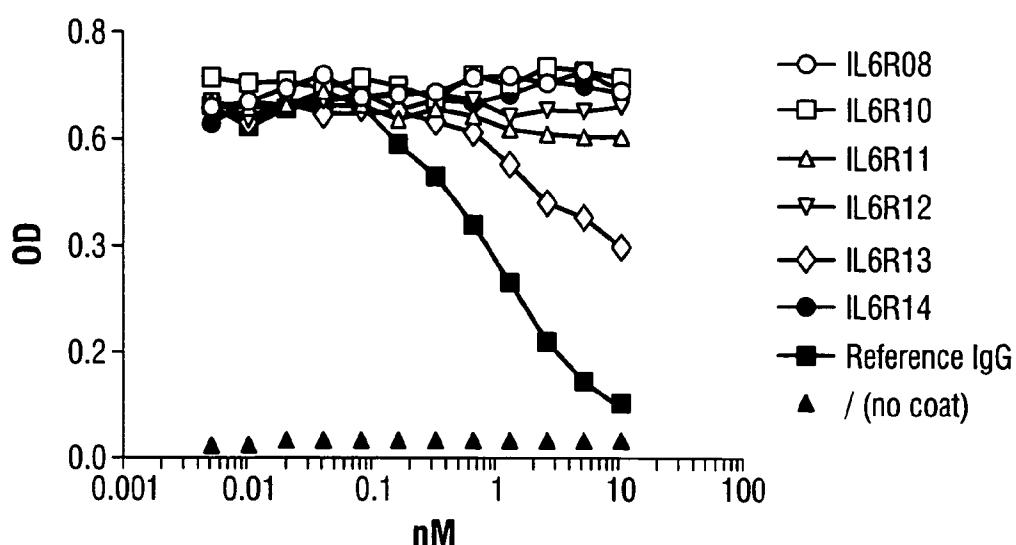
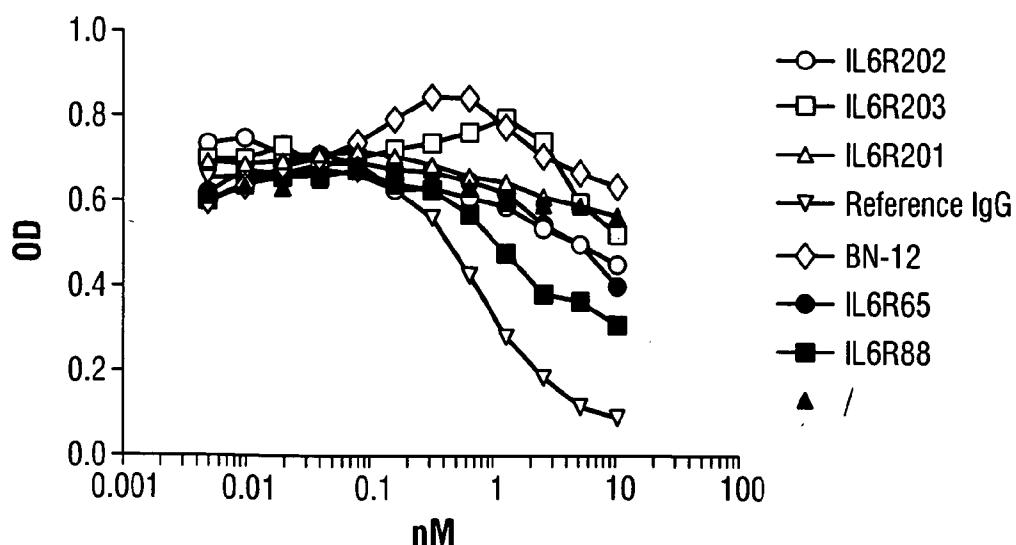


Fig. 41

57 / 62

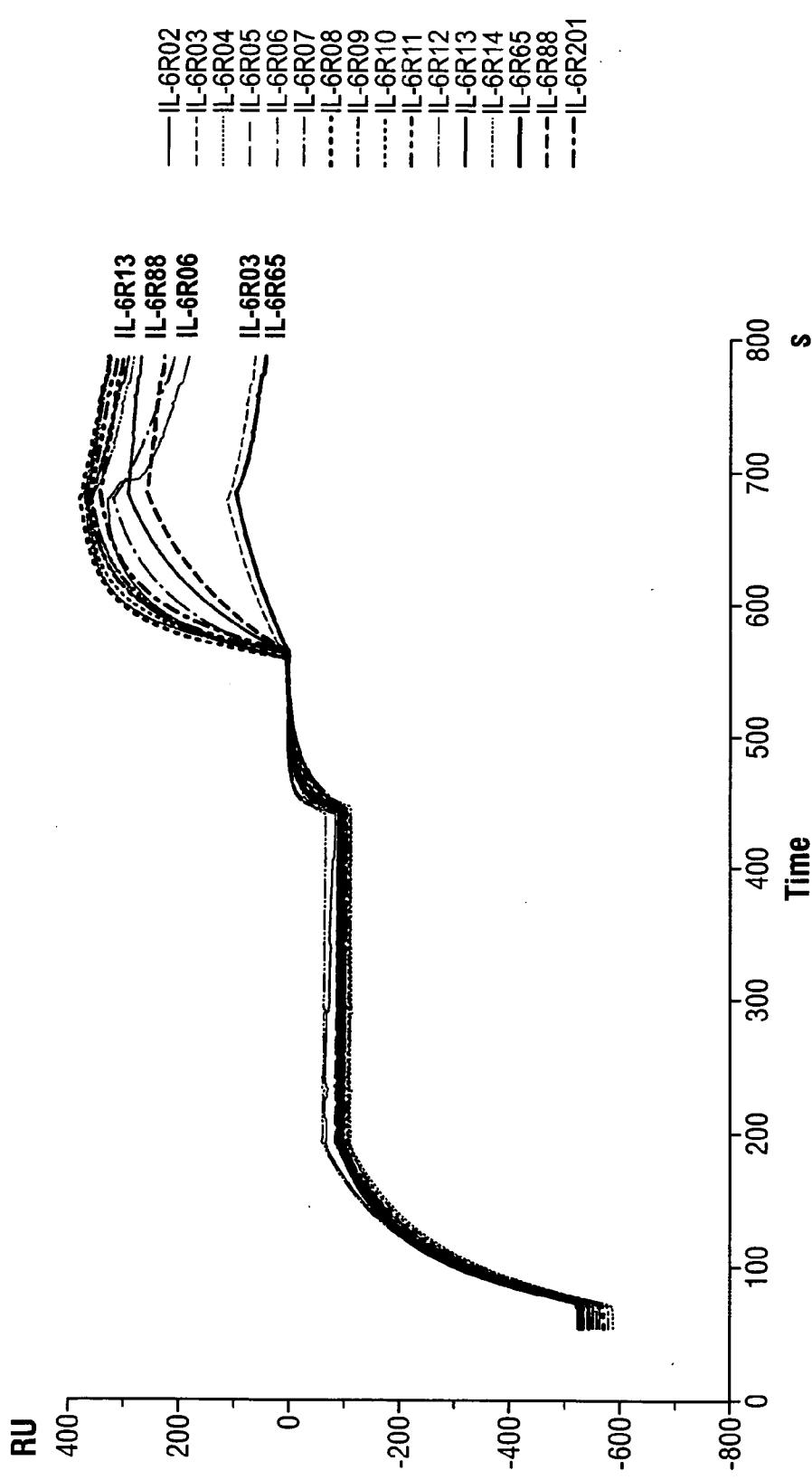
Fig. 42**CRP****Fig. 43****Epitope mapping**

58 / 62

Fig. 44**Epitope mapping****Fig. 45****Epitope mapping**

59 / 62

Fig. 46



60 / 62

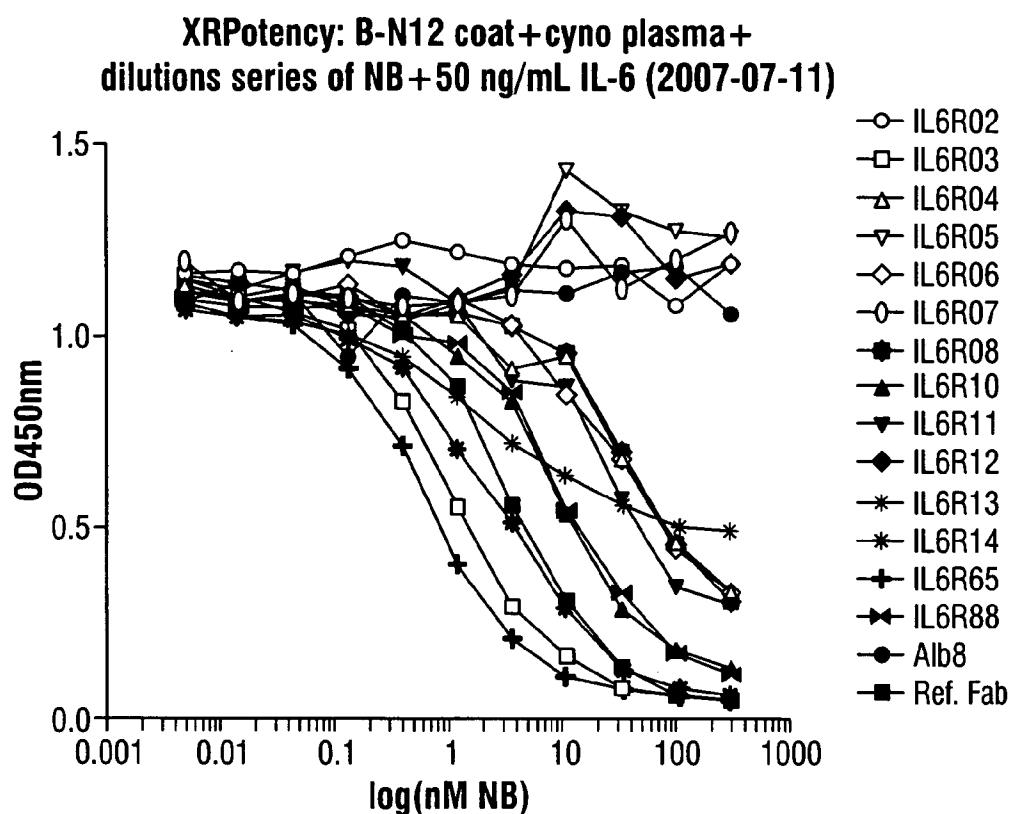
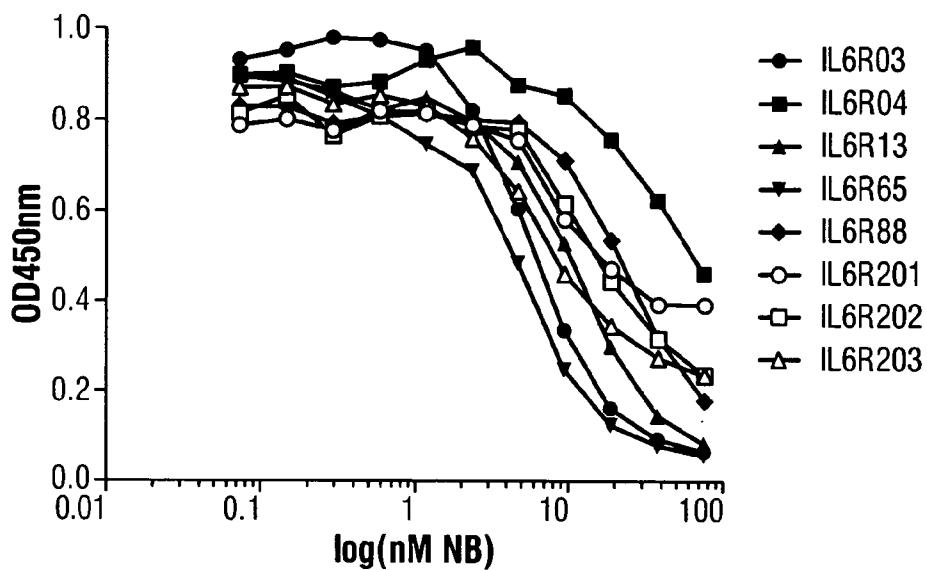
Fig. 47

Fig. 48

61 / 62

XRPotency: B-N12 coat + cyto plasma + dilutions series of NB + 50 ng/mL IL-6 (2007-07-12-CORR)



	IL6R03	IL6R04	IL6R13	IL6R65	IL6R88	IL6R201	IL6R202	IL6R203
EC50	5.885	36.94	11.66	4.910	23.27	9.360	14.32	6.857

Fig. 49

XRPotency - Proof of concept: B-N12 coat + rhesus plasma + dilutions series of NB + 40 ng/mL IL-6 (2007-07-18)

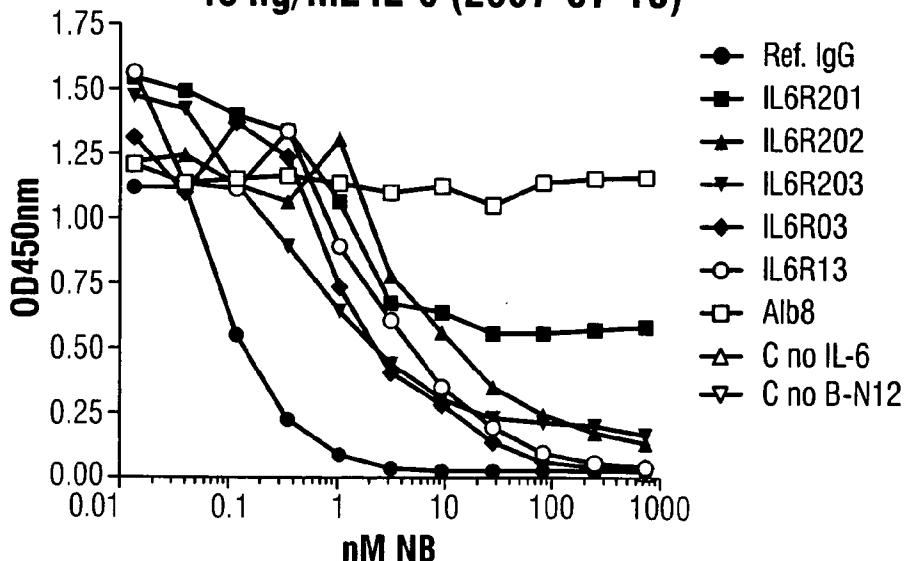
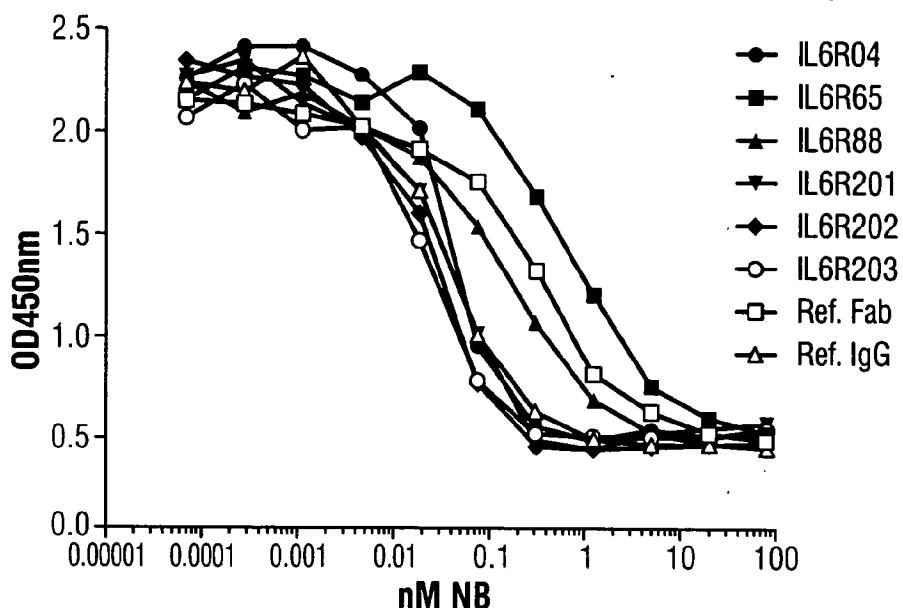


Fig. 50

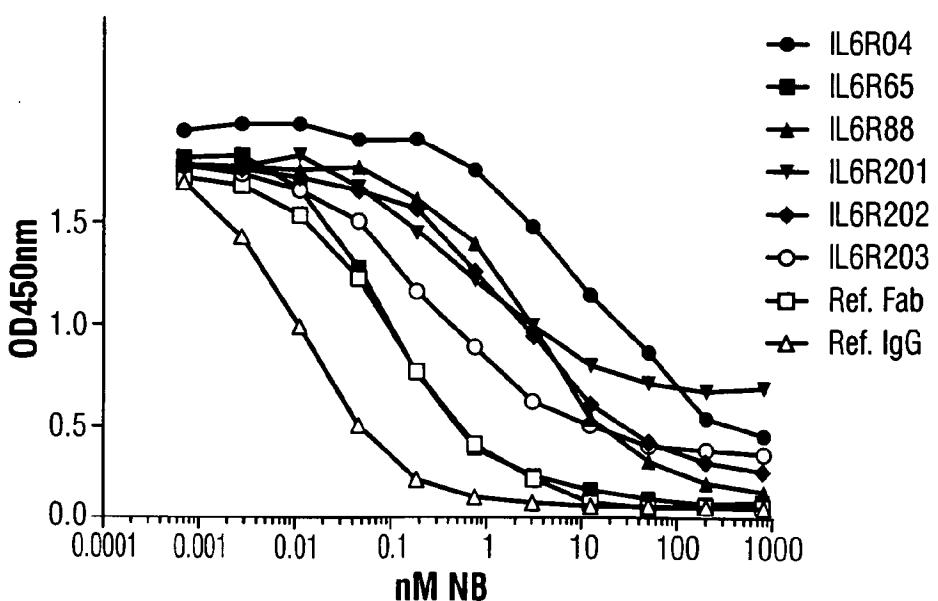
62 / 62

**XRPotency: B-N12 coat + human plasma + dilutions
series of NB + 50 ng/mL IL-6 (2007-07-13-CORR)**



	IL6R04	IL6R65	IL6R88	IL6R201	IL6R202	IL6R203	Ref. Fab	Ref. IgG
EC50	0.03938	0.7006	0.1433	0.03329	0.02441	0.02483	0.3024	0.03524

**XRPotency: B-N12 coat + cyno plasma + dilutions
series of NB + 50 ng/mL IL-6 (2007-07-13-CORR)**



	IL6R04	IL6R65	IL6R88	IL6R201	IL6R202	IL6R203	Ref. Fab	Ref. IgG
EC50	12.09	0.09343	3.320	0.6930	2.183	0.3041	0.1165	0.01209

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2007/058587

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, MEDLINE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>NISHIMOTO NORIHIRO ET AL: "Humanized anti-interleukin-6 receptor antibody treatment of multicentric Castleman disease" BLOOD, vol. 106, no. 8, October 2005 (2005-10), pages 2627-2632, XP002463362 ISSN: 0006-4971 the whole document</p> <p>-----</p> <p style="text-align: center;">-/-</p>	<p>1-4, 10, 11, 16, 17, 19, 100-103, 105, 123-138</p>
Y		<p>5-9, 12-15, 18, 20-99, 104, 106-122, 139-191</p>

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

2 January 2008

15/01/2008

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Authorized officer

Sommerfeld, Teresa

INTERNATIONAL SEARCH REPORT

 International application No
 PCT/EP2007/058587

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHOY E H S ET AL: "Therapeutic benefit of blocking interleukin-6 activity with an anti-interleukin-6 receptor monoclonal antibody in rheumatoid arthritis: a randomized, double-blind, placebo-controlled, dose-escalation trial" ARTHRITIS AND RHEUMATISM, LIPPINCOTT, PHILADELPHIA, US, vol. 46, no. 12, December 2002 (2002-12), pages 3143-3150, XP002298379 ISSN: 0004-3591 the whole document	1-4, 10, 11, 16, 17, 19, 100-103, 105, 123-132, 134-138
Y		5-9, 12-15, 18, 20-99, 104, 106-122, 133, 139-191
X	ITO HIROAKI ET AL: "A pilot randomized trial of a human anti-interleukin-6 receptor monoclonal antibody in active Crohn's disease" GASTROENTEROLOGY, ELSEVIER, PHILADELPHIA, PA, US, vol. 126, no. 4, April 2004 (2004-04), pages 989-996, XP002298383 ISSN: 0016-5085 the whole document	1-4, 10, 11, 16, 17, 19, 100-103, 105, 123-132, 134-138
Y		5-9, 12-15, 18, 20-99, 104, 106-122, 133, 139-191
X	EP 0 409 607 A (KISHIMOTO TADAMITSU [JP]) 23 January 1991 (1991-01-23)	1-4, 10, 11, 16, 17, 19, 100-103, 105, 128, 131
Y	the whole document	5-9, 12-15, 18, 20-99, 104, 106-122, 139-191
		----- -/-

INTERNATIONAL SEARCH REPORT

International application No PCT/EP2007/058587

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2006/079372 A (ABLYNX N V [BE]; HERMANS GUY [BE]; DE HAARD JOHANNES JOSEPH WILHE [NL]) 3 August 2006 (2006-08-03) the whole document -----	
A	REVETS H ET AL: "NANOBODIES AS NOVEL AGENTS FOR CANCER THERAPY" EXPERT OPINION ON BIOLOGICAL THERAPY, ASHLEY, LONDON, GB, vol. 5, no. 1, 2005, pages 111-124, XP009076361 ISSN: 1471-2598 the whole document -----	
A	HOLLIGER PHILIPP ET AL: "ENGINEERED ANTIBODY FRAGMENTS AND THE RISE OF SINGLE DOMAINS" NATURE BIOTECHNOLOGY, NATURE PUBLISHING GROUP, NEW YORK, NY, US, vol. 23, no. 9, September 2005 (2005-09), pages 1126-1136, XP008076746 ISSN: 1087-0156 the whole document -----	
E	WO 2007/104529 A (ABLYNX N V [BE]; KOLKMAN JOOST ALEXANDER [BE]; HERMANS GUY [BE]; HOOGE) 20 September 2007 (2007-09-20) the whole document -----	1-191

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2007/058587

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 131–137 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.
2. As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No PCT/EP2007/058587

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP 0409607	A 23-01-1991	AT 144713 T CA 2021594 A1 DE 69029015 D1 DE 69029015 T2 ES 2093017 T3 HK 49497 A JP 3139293 A SG 42954 A1		15-11-1996 21-01-1991 05-12-1996 22-01-1998 16-12-1996 25-04-1997 13-06-1991 17-10-1997
WO 2006079372	A 03-08-2006	AU 2005325801 A1 CA 2595682 A1 EP 1844073 A1 US 2006211088 A1		03-08-2006 03-08-2006 17-10-2007 21-09-2006
WO 2007104529	A 20-09-2007	NONE		