



(86) Date de dépôt PCT/PCT Filing Date: 2007/12/19
(87) Date publication PCT/PCT Publication Date: 2008/06/26
(85) Entrée phase nationale/National Entry: 2009/06/18
(86) N° demande PCT/PCT Application No.: EP 2007/064243
(87) N° publication PCT/PCT Publication No.: 2008/074839
(30) Priorité/Priority: 2006/12/19 (US60/875,860)

(51) Cl.Int./Int.Cl. *C07K 16/28* (2006.01),
A61K 39/395 (2006.01), *C12N 15/13* (2006.01)
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(54) Titre : SEQUENCES D'ACIDES AMINES DIRIGÉES CONTRE LES GPCR ET POLYPEPTIDES LES CONTENANT
POUR LE TRAITEMENT DE MALADIES ET DE TROUBLES LIÉS AU GPCR
(54) Title: AMINO ACID SEQUENCES DIRECTED AGAINST GPCRS AND POLYPEPTIDES COMPRISING THE SAME
FOR THE TREATMENT OF GPCR-RELATED DISEASES AND DISORDERS

(57) **Abrégé/Abstract:**

The present invention relates to amino acid sequences that are directed G-protein coupled receptors (GPCRs), 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. The invention also relates to nucleic acids encoding such amino acid sequences and polypeptides 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 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.



(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
26 June 2008 (26.06.2008)

PCT

(10) International Publication Number
WO 2008/074839 A3

(51) International Patent Classification:

C07K 16/28 (2006.01) **C12N 15/13** (2006.01)
A61K 39/395 (2006.01)

(21) International Application Number:

PCT/EP2007/064243

(22) International Filing Date:

19 December 2007 (19.12.2007)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

60/875,860 19 December 2006 (19.12.2006) US

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(81) **Designated States** (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- *with international search report*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*
- *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

(88) Date of publication of the international search report:

20 November 2008

(54) **Title**: AMINO ACID SEQUENCES DIRECTED AGAINST GPCRS AND POLYPEPTIDES COMPRISING THE SAME FOR THE TREATMENT OF GPCR-RELATED DISEASES AND DISORDERS

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WO 2008/074839 A3

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AMINO ACID SEQUENCES DIRECTED AGAINST GPCRs AND POLYPEPTIDES
COMPRISING THE SAME FOR THE TREATMENT OF GPCR-RELATED
DISEASES AND DISORDERS

The present invention relates to amino acid sequences that are directed against (as defined herein) G-protein coupled receptors (GPCRs), 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 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.

GPCRs are a well-known class of receptors. Reference is for example made to the following reviews: Surgand et al., Proteins 62:509-538 (2006); Vassilatis et al., Proc Natl Acad Sci U S A 100:4903-4908 (2003) and Pierce et al., Nat Rev Mol Cell Biol 3:639-650 (2002); as well as to for example: George et al., Nat Rev Drug Discov 1:808-820 (2002); Kenakin, Trends Pharmacol Sci 25:186-192 (2002); Rios et al., Pharmacol Ther 92:71-87 (2001); Jacoby et al., ChemMedChem 2006, 1, 760-782; and Schlyer and Horuk, Drug Discovery Today, 11, 11/12. June 2006, 481; and also for example to Rosenkilde, Oncogene (2001), 20, 1582-1593 and Sadee et al., AAPS PharmSci 2001; 3; 1-16; as well as to the further references cited therein.

30 G-protein-coupled receptors (GPCRs) are the largest class of cell-surface receptors (more than 1000 genes are present in the human genome). They can be activated by a diverse array of stimuli, e.g. hormones, peptides, amino acids, photons of light, and these receptors play a large role in the central nervous system and in the periphery. GPCRs are proteins with 7 transmembrane domains with highly conserved domains.

As half of all known drugs work through G-protein coupled receptors, it is commercially very attractive to select Nanobodies against this protein family. It was estimated that in the year 2000 half of all modern drugs and almost one-quarter of the top 200 best-selling drugs are directed against or modulate GPCR targets (approximately 30 in total). However, due to their architecture of 7 membrane-spanning helices and their strong tendency to aggregate, it's a very challenging target class.

GPCRs can be grouped on the basis of sequence homology into several distinct families. Although all GPCRs have a similar architecture of seven membrane-spanning α -helices, the different families within this receptor class show no sequence homology to one another, thus suggesting that the similarity of their transmembrane domain structure might define common functional requirements. Depending on the size of the extracellular domain three families are discriminated (Fig.1).

- Members of Family 1 (also called family A or rhodopsin-like family; panel a of Fig. 1) only have small extracellular loops and the interaction of the ligands occurs with residues within the transmembrane cleft. This is by far the largest group (>90% of the GPCRs) and contains receptors for odorants, small molecules such as catecholamines and amines, (neuro)peptides and glycoprotein hormones. Rhodopsin, which belongs to this family, is the only GPCR for which the structure has been solved.
- Family 2 or family B GPCRs (Fig. 1, panel b) are characterized by a relatively long amino terminal extracellular domain involved in ligand-binding. Little is known about the orientation of the transmembrane domains, but it is probably quite different from that of rhodopsin. Ligands for these GPCRs are hormones, such as glucagon, gonadotropin-releasing hormone and parathyroid hormone.
- Family 3 members (Fig. 1, panel c) also have a large extracellular domain, which functions like a "Venus fly trap" since it can open and close with the agonist bound inside. Family members are the metabotropic glutamate, the Ca^{2+} -sensing and the γ -aminobutyric acid (GABA)_B receptors.

Traditionally small molecules are used for development of drugs directed against GPCRs, not only because pharmaceutical companies have historical reasons to work with these, but more importantly because of the structural constraints of Family 1 GPCRs, which have the ligand binding site within the transmembrane cleft (Nat Rev Drug Discov. (2004) The state of GPCR research in 2004. Nature Reviews Drug Discovery GPCR Questionnaire Participants 3(7):575, 577-626). For this reason it proved to be difficult or impossible to generate monoclonal antibodies against this target class. The amino acid sequences of the

invention (and in particular Nanobodies of the invention) can solve this particular problem by means of their intrinsic property of binding via extended CDR loops into cavities (as further described herein).

Some non-limiting examples of therapeutically relevant GPCRs are for example the following, which are all targets of known drugs that have either been approved or are in clinical development. The text between brackets indicates the desired action of an amino acid sequence, a Nanobody or a polypeptide of the invention (i.e. as agonist or antagonist):

Class A GPCRs

- 10 - Muscarinic M1 receptor (agonist)
- Adrenoceptor (agonist);
- Histamine receptor (agonist);
- 5-HT GPCR (agonist);
- Cannabinoid receptor (agonist);
- Class A hormone protein GPCR (agonist);
- Chemokine (agonist);
- Galanin (agonist);
- Melanocortin (agonist);
- Neuropeptide Y receptor (agonist);
- 20 - Neurotensin receptor (agonist);
- Opioid (agonist);
- Somatostatin (agonist);
- Vasopressin like receptor (agonist);
- Prostanoid receptor (agonist)

Class B GPCRs

- ACTH releasing factor receptor (modulator);

Class C GPCRs

- 30 - GABA B receptor (agonist);
- Metabotropic glutamate receptor (agonist);

Some other non-limiting examples of therapeutically relevant GPCRs are mentioned in Table C. A more extensive list of human GPCRs is given in Table D.

The polypeptides and compositions of the present invention can generally be used to modulate (as defined herein), and in particular inhibit and/or prevent, GPCRs mediated signalling and/or to modulate the biological pathways in which GPCRs are involved, and/or to modulate the biological mechanisms, responses and effects associated with such signalling or these pathways.

As such, the polypeptides and compositions of the present invention can be used for the prevention and treatment (as defined herein) of GPCR-related diseases and disorders. Generally, "GPCR-related diseases and disorders" can be defined as diseases and disorders that can be prevented and/or treated, respectively, by suitably administering to a subject in
10 need thereof (i.e. having the disease or disorder or at least one symptom thereof and/or at risk of attracting or developing the disease or disorder) of either a polypeptide or composition of the invention (and in particular, of a pharmaceutically active amount thereof) and/or of a known active principle active against GPCRs or a biological pathway or mechanism in which GPCRs is involved (and in particular, of a pharmaceutically active amount thereof). Examples of such GPCR-related diseases and disorders will be clear to the skilled person based on the disclosure herein.

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 GPCRs-
20 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 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 and regimens for treating the diseases and disorders described herein.

Other applications and uses of the amino acid sequences and polypeptides of the
30 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 and/or treatment of GPCR-related diseases and disorders and of the further diseases and disorders mentioned 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, 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 GPCR-related diseases and disorders and of the further diseases and disorders mentioned 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 that are directed against (as defined herein) GPCRs, in particular against GPCRs from a warm-blooded animal, more in particular against GPCRs from a mammal, and especially against human GPCRs; 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.

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 diseases, disorders or conditions associated with GPCRs and/or mediated by GPCRs (such as the diseases, disorders and conditions mentioned herein) in a warm-blooded animal, in particular in a mammal, and more in particular in a human being.

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 diseases, disorders or conditions associated with and/or mediated by GPCRs (such as the diseases, disorders and conditions mentioned herein) in a warm-blooded animal, in particular in a mammal, and more in particular in a human being.

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 that are directed against (as defined herein) and/or can specifically bind (as defined herein) to GPCRs; as well as compounds and constructs, and in particular proteins and polypeptides, that comprise at least one such amino acid sequence.

More in particular, the invention provides amino acid sequences that can bind to GPCRs 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 GPCRs 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 GPCRs 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 GPCRs 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 GPCRs 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 GPCRs will become clear from the further description and examples herein.

For binding to GPCRs, an amino acid sequence of the invention will usually contain 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 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 GPCRs, which amino acid residues or stretches of amino acid residues thus form the “site” for binding to GPCRs (also referred to herein as the “*antigen binding site*”).

10 The amino acid sequences provided by the invention are preferably in essentially 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 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 GPCRs), 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).

20 The amino acid sequences and polypeptides of the invention as such preferably essentially consist of a single amino acid chain that is not linked via disulphide bridges to any other amino acid sequence or chain (but that may or may not contain one or more intramolecular disulphide bridges. For example, it is known that Nanobodies – as described herein - may sometimes contain a disulphide bridge between CDR3 and CDR1 or FR2). However, it should be noted that one or more amino acid sequences of the invention may be linked to each other and/or to other amino acid sequences (e.g. via disulphide bridges) to provide peptide constructs that may also be useful in the invention (for example Fab’ fragments, F(ab’)₂ fragments, ScFv constructs, “diabodies” and other multispecific constructs. Reference is for example made to the review by Holliger and Hudson, Nat Biotechnol. 2005 Sep;23(9):1126-36).

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

Furthermore, an amino acid sequence of the invention may optionally, and in addition to the at least one binding site for binding against GPCRs, contain one or more further binding sites for binding against other antigens, proteins or targets.

10 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 the expression of the receptors in *Xenopus* oocytes, after which the coupling of many GPCRs to ion channels allows the activation or inhibition of these GPCRs to be monitored in oocytes via voltage clamping techniques. Heterologous GPCRs can be functionally expressed in the oocyte by injecting exogenous, GPCR-encoding mRNA into the oocyte and then allowing 20 the oocyte's endogenous cellular machinery to translate and insert the receptors into the plasma

20 membrane. (See, e.g., Houamed et al., *Science* 252:1318-21, 1991; Dahmen et al., *J. Neurochem.* 58:1176-79, 1992.) Following functional expression of receptors, the ability of ligands to induce transmembrane conductance changes can be observed via a two-electrode voltage clamp system (Dahmen et al., *supra*), which can detect either a depolarization or hyperpolarization of the membrane potential.

Other techniques for screening GPCRs will be clear to the skilled person, for example from the handbooks, reviews and prior art cited herein. These include for example the radioligand binding assays, as for example used in Lundstrom et al., *J Struct Funct Genomics*. 2006 Nov 22; [Epub ahead of print] and as described in Andre' et al., *Protein Sci* 5:1115 (2006); Hassaine et al., (2006) *Prot Purif Expr* 45:343; Nicholson et al. *J Pharmacol Exp*

30 *Ther.* 2006 May;317(2):771-7. [Epub 2006 Jan 25]. and Vilardaga et al., *J Biol Chem.* 2001 Sep 7;276(36):33435-43. Epub 2001 May 31, for example using membrane preparations that can be made as described in Hovius et al., (1998) *J Neurochem* 70:824. Some HTS techniques for screening GPCRs are mentioned in Table 4 of the review by Jacoby et al.,

Also, according to the invention, amino acid sequences and polypeptides that are directed against GPCRs from a first species of warm-blooded animal may or may not show cross-reactivity with GPCRs from one or more other species of warm-blooded animal. For example, amino acid sequences and polypeptides directed against human GPCRs may or may not show cross reactivity with GPCRs from one or more other species of primates (such as, without limitation, monkeys from the genus *Macaca* (such as, and in particular, cynomolgus monkeys (*Macaca fascicularis*) and/or rhesus monkeys (*Macaca mulatta*)) and baboon (*Papio ursinus*)) and/or with GPCRs 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
10 animal models for diseases and disorders associated with GPCRs (such as the 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 GPCRs to be tested in such disease models.

More generally, amino acid sequences and polypeptides of the invention that are cross-reactive with GPCRs 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 that amino acid sequences and polypeptides directed against GPCRs from
20 one species of animal (such as amino acid sequences and polypeptides against human GPCRs) 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 by a specific antigenic determinant, epitope, part, domain, subunit or conformation (where applicable) of GPCRs against which the amino acid sequences and polypeptides of the invention are directed. For example, the amino acid sequences and polypeptides may or may not be directed against an “interaction site” (as defined herein). For example, amino acid sequences of the invention may be raised by suitably immunizing a mammal (such as a
30 Camelid) with GPCR that has been expressed in a suitable expression system or that has been isolated from a suitable cell or cell fraction. In particular, amino acid sequences of the invention may be raised against GPCRs (or suitable parts or fragments thereof) that have been refolded (for example using the techniques described in the review by Kiefer, Biochim. Biophys. Acta, 1610 (2003), 57-62), and amino acid sequences, Nanobodies and polypeptides

that are directed against and/or that have been raised against a refolded GPCR form a further aspect of the invention.

The amino acid sequences and polypeptides of the invention may generally be directed against any desired GPCR, and may in particular be directed against a GPCR that has at least one extracellular loop or domain. Examples of such GPCRs will be clear to the skilled person based on the prior art cited herein

According to a specific aspect of the invention, an amino acid sequence or polypeptide of the invention may be directed against (as defined herein) a GPCR that is expressed on the surface of a cell and/or against at least one extracellular region, domain, loop or other
10 extracellular epitope of a GPCR. For example, such amino acid sequences may be raised by suitably immunizing a mammal (such as a Camelid) with a cell or cell fraction that has a GPCR or its surface. For example, the mammal (and in particular, Camelid) may be suitably immunized with whole cells that express (and preferably overexpress) the desired GPCR, such as with cells of a cell line that (over)expresses the desired GPCR.

In particular, according to this aspect, an amino acid sequence or polypeptide of the invention is directed against (as defined herein) at least one extracellular region, domain, loop or other extracellular epitope of a GPCR; and is preferably further such that said amino acid sequence or polypeptide of the invention is capable of modulating (as defined herein) said GPCR. More in particular, according to this aspect, an amino acid sequence or polypeptide of
20 the invention is directed against (as defined herein) at least one extracellular region, domain, loop or other extracellular epitope of a GPCR; and is preferably further such that said amino acid sequence or polypeptide of the invention is capable of (fully or partially) blocking said GPCR.

According to this aspect of the invention, the amino acid sequence or polypeptide of the invention may be directed against any suitable extracellular part, region, domain, loop or other extracellular epitope of a GPCR, but is preferably directed against one of the extracellular parts of the transmembrane domains or more preferably against one of the extracellular loops that link the transmembrane domains.

The amino acid sequence of such suitable extracellular parts, regions, domains, loops
30 or epitopes may be derived by Kyte-Doolittle analysis of the amino acid sequence of the pertinent GPCR; by aligning GPCRs belonging to the same (sub)families and identifying the various transmembrane domains and extracellular parts, regions, domain or loops; by TMAP-analysis; or by any suitable combination thereof. The invention also relates to amino acid sequences and (as further defined herein) that are directed against and/or have been raised

against such extracellular parts, regions, domains, loops or epitopes (and/or that are directed against and/or have been raised against suitable parts or fragments of such extracellular parts, regions, domains, loops or epitopes and/or against synthetic or semi-synthetic peptides that are derived from or based on such extracellular parts, regions, domains, loops or epitopes).

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

- bind to an extracellular part, region, domain, loop or other extracellular epitope of a GPCR (as described herein) or against a peptide derived therefrom 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 an extracellular part, region, domain, loop or other extracellular epitope of a GPCR (as described herein) or against a peptide derived therefrom 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 an extracellular part, region, domain, loop or other extracellular epitope of a GPCR (as described herein) or against a peptide derived therefrom 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 bind to an extracellular part, region, domain, loop or other extracellular epitope of a GPCR (as described herein) with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM.

- Also, according to this aspect, any multivalent or multispecific (as defined herein) polypeptides of the invention may also be suitably directed against two or more different extracellular parts, regions, domains, loops or other extracellular epitopes on the same antigen, for example against two different extracellular loops, against two different extracellular parts of the transmembrane domains or against one extracellular loops and one

extracellular loop. Such multivalent or multispecific polypeptides of the invention may also have (or be engineered and/or selected for) increased avidity and/or improved selectivity for the desired GPCR, and/or for any other desired property or combination of desired properties that may be obtained by the use of such multivalent or multispecific polypeptides.

Generally, it is expected that amino acid sequences and polypeptides of the invention that are directed against an extracellular loop or domain of a GPCR (or against a small peptide derived therefrom or based thereon), and/or that have been screened against, selected using and/or raised against an extracellular loop or domain of a GPCR (or against a small peptide derived therefrom or based thereon) will also be able to bind (and in particular, to specifically
10 bind, as defined herein) to such an extracellular loop or domain (or peptide derived therefrom) that forms part of a GPCR (or at least one subunit thereof) that is present on the surface of a cell. Thus, such (peptides derived from) an extracellular loop or domain may find particular use in methods for generating amino acid sequences and polypeptides of the invention (as defined herein); and such methods and uses form further aspects of the invention; as do amino acid sequences, Nanobodies and polypeptides of the invention that are directed against or raised against such an extracellular loop, domain or peptide derived therefrom.

For example, such a method may comprises one of the following steps or a suitable combination of both of the following steps:

- a) a step of suitably immunizing a Camelid with a suitable antigen that comprises the
20 desired extracellular part, region, domain, loop or other extracellular epitope(s), or with a suitable peptide derived therefrom or based thereon, such that an immune response against the desired extracellular part, region, domain, loop or other extracellular epitope(s) is raised. The antigen may be any suitable antigen that is capable of raising an immune response against the desired extracellular part, region, domain, loop or other extracellular epitope(s); such as, for example and without limitation, whole cells that have the desired extracellular part, region, domain, loop or other extracellular epitope(s) on their surface, cell wall fragments thereof or any other suitable preparation derived from such cells, vesicles that have the desired extracellular part, region, domain, loop or other extracellular epitope(s) on their surface, a subunit or fragment of a subunit of a
30 GPCR that comprises the desired extracellular part, region, domain, loop or other extracellular epitope(s), or a synthetic or semi-synthetic peptide that comprises and/or is based on (the amino acid sequence of) the desired extracellular part, region, domain, loop or other extracellular epitope(s);
and/or

b) a step of screening for affinity and/or binding for the desired extracellular part, region, domain, loop or other extracellular epitope(s). This may for example be performed by screening a set, collection or library of cells that express heavy chain antibodies on their surface (e.g. B-cells obtained from a suitably immunized Camelid), by screening of a (naïve or immune) library of VHH sequences or Nanobody sequences, or by screening of a (naïve or immune) library of nucleic acid sequences that encode VHH sequences or Nanobody sequences; which may all be performed in a manner known per se, for which reference is made to the further disclosure and prior art mentioned herein;

and which method may optionally further comprise one or more other suitable steps known per se, such as, for example and without limitation, a step of affinity maturation, a step of expressing the desired amino acid sequence, a step of screening for binding and/or for activity against the desired antigen (in this case, the GPCR), a step of determining the desired amino acid sequence or nucleotide sequence, a step of introducing one or more humanizing substitutions (e.g. as further described herein), a step of formatting in a suitable multivalent and/or multispecific format, a step of screening for the desired biological and/or physiological properties (i.e. using a suitable assay, such as those described herein); and/or any suitable combination of one or more of such steps, in any suitable order.

Such methods and the amino acid sequences obtained via such methods, as well as proteins and polypeptides comprising or essentially consisting of the same, form further aspects of this invention.

The amino acid sequences, Nanobodies and polypeptides of the invention may also bind to the GPCR at the same site as the endogenous agonist (i.e. at an orthosteric site), so as to activate or increase receptor signalling; or alternatively so as to decrease or inhibit receptor signalling.

The amino acid sequences, Nanobodies and polypeptides of the invention may also bind to the GPCR in such a way that they block of the constitutive activity of the GPCR.

The amino acid sequences, Nanobodies and polypeptides of the invention may also bind to the GPCR in such a way that they mediate allosteric modulation (e.g. bind to the GPCR at an allosteric site). In this way, the amino acid sequences, Nanobodies and polypeptides of the invention may modulation of the receptor function by binding to different regions in the receptor (e.g. at allosteric sites). Reference is for example made to George et al., Nat Rev Drug Discov 1:808-820 (2002); Kenakin, Trends Pharmacol Sci 25:186-192 (2002) and Rios et al., Pharmacol Ther 92:71-87 (2001)).

The amino acid sequences, Nanobodies and polypeptides of the invention may also bind to the GPCR in such a way that they inhibit or enhance the assembly of GPCR functional homodimers or heterodimers.

The amino acid sequences, Nanobodies and polypeptides of the invention may also bind to the GPCR in such a way that they prolong the duration of the GPCR-mediated signalling.

The amino acid sequences, Nanobodies and polypeptides of the invention may also enhance receptor signalling by increasing receptor-ligand affinity.

Polypeptides of the invention that are directed against a GPCR and its ligand may also
10 provide for enhanced binding of the ligand to the GPCR by cross-linking the ligand to the orthosteric site; and/or stabilize of the binding of the ligand to the orthosteric site. Thus, a further aspect of the invention relates to a multispecific polypeptide of the invention (as defined herein) that comprises at least one amino acid sequence of the invention (such as a Nanobody) against a GPCR proteinase and at least one binding unit directed against its natural ligand. Such multispecific proteins may further be as described herein.

Also, as will be clear from the further disclosure herein, and depending on the GPCR against which they are directed and their desired (therapeutic) effect, the amino acid sequences, Nanobodies and polypeptides of the invention may act as (full or partial) agonists, (full or partial, and competitive or non-competitive) antagonists or as inverse agonists of the
20 GPCR (and/or of the ligand of the GPCR) and/or of the biological function, pathway, mechanism, effect, signalling or response associated therewith. They may do so in an irreversible but preferably reversible manner.

In a preferred embodiment, the amino acid sequence or polypeptide of the invention is a “monoclonal” amino acid sequence or polypeptide, by which is meant that at least each of the one or more amino acid sequences directed against the GPCR that are present in said protein or polypeptide (and preferably all of the immunoglobulin sequences that are present in said protein or polypeptide) are “monoclonal” as commonly understood by the skilled person. In this respect, it should however be noted that, as further described herein, the present invention explicitly covers multivalent or multispecific proteins that comprise two or more
30 immunoglobulin sequences (and in particular monoclonal immunoglobulin sequences) that are directed against different parts, regions, domains, loops or epitopes of the same GPCR, and in particular against different extracellular parts, regions, domains, loops or epitopes of the same GPCR.

As further described herein, a polypeptide of the invention may contain two or more amino acid sequences of the invention that are directed against a GPCR. Generally, such polypeptides will bind to a GPCR with increased avidity compared to a single amino acid sequence of the invention. Such a polypeptide may for example comprise two amino acid sequences of the invention that are directed against the same antigenic determinant, epitope, part, domain, subunit or confirmation (where applicable) of a GPCR (which may or may not be an interaction site); or comprise at least one “first” amino acid sequence of the invention that is directed against a first same antigenic determinant, epitope, part, domain, subunit or confirmation (where applicable) of a GPCR (which may or may not be an interaction site);
10 and at least one “second” amino acid sequence of the invention that is directed against a second antigenic determinant, epitope, part, domain, subunit or confirmation (where applicable) different from the first (and which again may or may not be an interaction site). Preferably, in such “biparatopic” polypeptides of the invention, at least one amino acid sequence of the invention is directed against an interaction site (as defined herein), although the invention in its broadest sense is not limited thereto.

For example, when a polypeptide of the invention is a biparatopic polypeptide of the invention, it may contain at least one amino acid sequence of the invention (such as a Nanobody) that is directed against a first extracellular part, region, domain, loop or other extracellular epitope of a GPCR and at least one amino acid sequence of the invention (such
20 as a Nanobody) that is directed against a second extracellular part, region, domain, loop or other extracellular epitope of a GPCR different from said first extracellular part, region, domain, loop or other extracellular epitope.

Also, when the target is part of a binding pair (for example, a receptor-ligand binding pair), the amino acid sequences and polypeptides may be such that they compete with the cognate binding partner (e.g. the ligand, receptor or other binding partner, as applicable) for binding to the target, and/or such that they (fully or partially) neutralize binding of the binding partner to the target.

It is also within the scope of the invention that, where applicable, an amino acid sequence of the invention can bind to two or more antigenic determinants, epitopes, parts,
30 domains, subunits or confirmations of GPCRs. In such a case, the antigenic determinants, epitopes, parts, domains or subunits of GPCRs to which the amino acid sequences and/or polypeptides of the invention bind may be essentially the same (for example, if GPCRs 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 GPCRs with an affinity and/or specificity which may be the same or different). Also, for example, when GPCRs 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 GPCRs in which it is bound to a pertinent ligand, may bind to a conformation of GPCRs in which it 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).

10 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 GPCRs; or at least to those analogs, variants, mutants, alleles, parts and fragments of GPCRs 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 GPCRs (e.g. in wild-type GPCRs). 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-
20 type) GPCRs. It is also included within the scope of the invention that the amino acid sequences and polypeptides of the invention bind to some analogs, variants, mutants, alleles, parts and fragments of GPCRs, but not to others.

When GPCRs 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 GPCRs in monomeric form, only bind to GPCRs in multimeric form, or 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
30 to the multimeric form.

Also, when GPCRs 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 GPCRs in its non-associated state, bind to GPCRs 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 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 GPCRs 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 GPCRs may bind with higher avidity to GPCRs 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 GPCRs may (and usually will) bind with higher avidity than each
10 of the different monomers, and proteins or polypeptides that contain two or more amino acid sequences directed against GPCRs may (and usually will) bind also with higher avidity to a multimer of GPCRs.

Generally, amino acid sequences and polypeptides of the invention will at least bind to those forms of GPCRs (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
20 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 GPCRs; and more preferably will be capable of specific binding to GPCRs, and even more preferably capable of binding to GPCRs 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
30 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 GPCRs; and more preferably capable of binding to GPCRs 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.

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 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 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 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 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 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 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 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 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 NanobodyTM (as 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 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).

10 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 GPCRs 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 GPCRs, and for example also covers the
20 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 (see also PCT/EP2007/003259)..

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

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

10 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.

More 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:

20 i) 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;

and in which:

ii) said amino acid sequence has at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1 to 22, in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences (indicated with X in the sequences of SEQ ID NO's: 1 to 22) are disregarded.

In these Nanobodies, the CDR sequences are generally as further defined herein.

30 Thus, the invention also relates to such Nanobodies that can bind to (as defined herein) and/or are directed against GPCRs, 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 413 to 453 and 517 to 525 give the amino acid sequences of a number of V_{HH} sequences that have been raised against three representative GPCRs, and in particular against the Cannabinoid Receptor 1 (CB1R, see for example Pacher et al, Pharmacol Rev

58:389-462); the parathyroid hormone receptor (PTH1R, see for example Gensure et al, Biochem Biophys Res Commun 328:666-678); and melanocortin 4 receptor (MC4R), which are given in SEQ ID NO's 413 to 430, 517 and 518; SEQ ID NO'S 431 to 453; and SEQ ID NO's: 519 to 525, respectively.

In particular, the invention in some specific aspects provides:

- 10 — amino acid sequences that are directed against (as defined herein) CB1R and that have at least 80%, preferably at least 85%, such as 90% or 95% or more sequence identity with at least one of the amino acid sequences of SEQ ID NO's:413 to 453 and/or 517 to 525. These amino acid sequences may further be such that they neutralize binding of the cognate ligand to CB1R; and/or compete with the cognate ligand for binding to CB1R; and/or are directed against an interaction site (as defined herein) on CB1R (such as the ligand binding site);
- amino acid sequences that cross-block (as defined herein) the binding of at least one of the amino acid sequences of SEQ ID NO's: 413 to 453 and/or 517 to 525 to CB1R and/or that compete with at least one of the amino acid sequences of SEQ ID NO's: 413 to 453 and/or 517 to 525 for binding to CB1R. Again, these amino acid sequences may further be such that they neutralize binding of the cognate ligand to CB1R; and/or compete with the cognate ligand for binding to CB1R; and/or are directed against an interaction site (as defined herein) on CB1R (such as the ligand binding site);
- 20 — amino acid sequences that are directed against (as defined herein) PTHR1 and that have at least 80%, preferably at least 85%, such as 90% or 95% or more sequence identity with at least one of the amino acid sequences of SEQ ID NO's: 431 to 453 . These amino acid sequences may further be such that they neutralize binding of the cognate ligand to PTHR1; and/or compete with the cognate ligand (e.g. with parathyroid hormone (PTH) and/or with parathyroid hormone-like hormone (PTH1H)) for binding to PTHR1; and/or are directed against an interaction site (as defined herein) on PTHR1 (such as the ligand binding site);
- amino acid sequences that cross-block (as defined herein) the binding of at least one of the amino acid sequences of SEQ ID NO's: 431 to 453 to PTHR1 and/or that compete with at least one of the amino acid sequences of SEQ ID NO's: 431 to 453 for binding to PTHR1. Again, these amino acid sequences may further be such that they neutralize binding of the cognate ligand to PTHR1; and/or compete with the cognate ligand for
- 30

binding to PTHR1; and/or are directed against an interaction site (as defined herein) on PTHR1 (such as the ligand binding site);

- amino acid sequences that are directed against (as defined herein) MC4R and that have at least 80%, preferably at least 85%, such as 90% or 95% or more sequence identity with at least one of the amino acid sequences of SEQ ID NO's: 519 to 525. These amino acid sequences may further be such that they neutralize binding of the cognate ligand to MC4R; and/or compete with the cognate ligand (e.g. with at least one melanocortin) for binding to MC4R; and/or are directed against an interaction site (as defined herein) on MC4R (such as the ligand binding site);
- 10 – amino acid sequences that cross-block (as defined herein) the binding of at least one of the amino acid sequences of SEQ ID NO's: 519 to 525 to MC4R and/or that compete with at least one of the amino acid sequences of SEQ ID NO's: 519 to 525 for binding to MC4R. Again, these amino acid sequences may further be such that they neutralize binding of the cognate ligand to MC4R; and/or compete with the cognate ligand for binding to MC4R; and/or are directed against an interaction site (as defined herein) on MC4R (such as the ligand binding site);

which amino acid sequences may be as further described herein (and may for example be Nanobodies); as well as polypeptides of the invention that comprise one or more of such amino acid sequences (which may be as further described herein, and may for example be
 20 bispecific and/or biparatopic polypeptides as described herein), and nucleic acid sequences that encode such amino acid sequences and polypeptides. Such amino acid sequences and polypeptides do not include any naturally occurring ligands.

In one embodiment, the amino acid sequences and peptides of the invention (which may be as further described herein) are directed against a receptor (i.e. a GPCR) from the family of cannabinoid receptor family

It is expected that amino acid sequences, Nanobodies and polypeptides of the invention of the invention directed against CB1R (and in particular antagonists), as well as compositions comprising the same, may find particular use in the prevention and treatment of for example cachexia and anorexia; pain and inflammation; mental disorders such as
 30 schizophrenia; anxiety and depression; drug abuse disorders and insomnia; Syndrome X; nicotine dependence; obesity; psychosis; atherosclerosis; hypotension; lipid metabolism disorders; metabolic disorders; non-insulin dependent diabetes; as an appetite suppressant. Antagonists of CB1R may also find use as antiarteriosclerotic agents; antipsychotic agents; hypoglycemic agents; and antihyperlipidemic agents.

In one embodiment, the amino acid sequences and peptides of the invention (which may be as further described herein) are directed against a receptor (i.e. a GPCR) from the family of parathyroid hormone receptors (for examples, receptors for parathyroid hormone (PTH) and/or parathyroid hormone-like hormone (PTHLH)).

It is expected that amino acid sequences, Nanobodies and polypeptides of the invention of the invention directed against PTHR1, as well as compositions comprising the same, may find particular use in the prevention and treatment of osteoporosis and disorders of bone metabolism (agonist); hyperparathyroidism (antagonist) and Jansen's chondrodysplasia (inverse agonist). Antagonists of PTHR1 may also find use as agents for the prevention or
10 treatment of hypercalcemia; bone metastases and cachexia.

In one embodiment, the amino acid sequences and polypeptides of the invention (which may be as further described herein) are directed against a receptor from the family of melanocortin receptors, such as MC1R, MC2R, MC3R, MC4R and MC4R. It is expected that amino acid sequences, Nanobodies and polypeptides of the invention of the invention directed against such receptors, and in particular against MC4R, as well as compositions comprising the same, may find particular use in the prevention and treatment of diseases and disorders associated with melanocortin receptors (e.g. with insufficient, undesired or abnormal signalling mediated by melanocortin receptors), such as diseases and disorders that are caused by and/or associated with aberrations in physiological processes such as energy homeostasis
20 (for example, cachexia and/or obesity), immunity, inflammation, sexual function, pigmentation and neurite outgrowth. Reference is inter alia made to the review by Bednarek and Fong, Expert Opin. Ther. Patents (2004) 14(3): 327-336, which also lists a number of synthetic ligands for receptors belonging to the family of melanocortin receptors, and it is expected that the amino acid sequences and polypeptides of the invention may be used for all diseases and disorders that are/can be treated with such ligands and/or for which said ligands are being developed. Reference is also made to the review by Getting, S. J. 2006, Pharmacol Ther 111:1-15.

Accordingly, some particularly preferred Nanobodies of the invention are Nanobodies which can bind (as further defined herein) to and/or are directed against to GPCRs and which:
30 i) have at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 413 to 453 and 517 to 525, 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: 126 to 166 and 454 to 462), framework 2

sequences (SEQ ID NO's: 208 to 248 and 472 to 480), framework 3 sequences (SEQ ID NO's: 290 to 330 and 490 to 498) and framework 4 sequences (SEQ ID NO's: 372 to 412 and 508 to 516) of the Nanobodies of SEQ ID NO's: 413 to 453 and 517 to 525 (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);

and in which:

- ii) 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.

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 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 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.

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

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 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
10 (or suitable combinations thereof) can be determined by the skilled person based on the 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: 413 to 453 and/or 517 to 525.

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

- i) are a humanized variant of one of the amino acid sequences of SEQ ID NO's: 413 to 453 and/or 517 to 525; and/or
- ii) have at least 80% amino acid identity with at least one of the amino acid sequences of
20 SEQ ID NO's: 413 to 453 and/or 517 to 525, 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:

- i) 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.

According to another specific aspect of the invention, the invention provides a number of stretches of amino acid residues (i.e. small peptides) that are particularly suited for binding to GPCRs. These stretches of amino acid residues may be present in, and/or may be
30 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} sequences that were raised against GPCRs (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 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 GPCRs. Thus, generally, the invention in its broadest sense comprises any amino acid sequence that is capable of binding to GPCRs and that comprises one or more CDR sequences as described herein, and in particular a suitable combination of two or more such CDR sequences, that are suitably linked to each other via one or more further amino acid sequences, such that the entire amino acid

10 sequence forms a binding domain and/or binding unit that is capable of binding to GPCRs. 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 GPCRs; 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

20 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)

30 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 GPCRs, and more in particular such that it can bind to GPCRs 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 GPCRs, 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: 167 to 207 and/or 463 to 471;
- 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: 167 to 207 and/or 463 to 471;
- 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: 167 to 207 and/or 463 to 471;
- d) the amino acid sequences of SEQ ID NO's: 249 to 289 and/or 481 to 489;
- 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: 249 to 289 and/or 481 to 489;
- 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: 249 to 289 and/or 481 to 489;
- g) the amino acid sequences of SEQ ID NO's: 331 to 371 and/or 499 to 507;
- 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: 331 to 371 and/or 499 to 507;
- 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: 331 to 371 and/or 499 to 507;

or any suitable combination thereof.

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

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

10 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

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):

20 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

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

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.

30 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.

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: 167 to 207 and/or 463 to 471;
 - ii) the amino acid sequences of SEQ ID NO's: 249 to 289 and/or 481 to 489; and
 - iii) the amino acid sequences of SEQ ID NO's: 331 to 371 and/or 499 to 507;
- or any suitable combination thereof.

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 GPCRs.

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

- 10 a) the amino acid sequences of SEQ ID NO's: 167 to 207 and/or 463 to 471;
- 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: 167 to 207 and/or 463 to 471;
- 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: 167 to 207 and/or 463 to 471;
- d) the amino acid sequences of SEQ ID NO's: 249 to 289 and/or 481 to 489;
- 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: 249 to 289 and/or 481 to 489;
- 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: 249 to 289 and/or 481 to 489;
- 20 g) the amino acid sequences of SEQ ID NO's: 331 to 371 and/or 499 to 507;
- 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: 331 to 371 and/or 499 to 507;
- 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: 331 to 371 and/or 499 to 507;

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:

- i) the amino acid sequences of SEQ ID NO's: 167 to 207 and/or 463 to 471;
- ii) the amino acid sequences of SEQ ID NO's: 249 to 289 and/or 481 to 489; and
- iii) the amino acid sequences of SEQ ID NO's: 331 to 371 and/or 499 to 507;

such that, (i) when the first stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 167 to 207 and/or 463 to 471, the second stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 249 to 289 and/or 481 to 489 or of SEQ ID NO's: 331 to 371 and/or 499 to 507; (ii) when the first stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 249 to 289 and/or 481 to 489, the second stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 167 to 207 and/or 463 to 471 or of SEQ ID NO's: 331 to 371 and/or 499 to 507; or (iii) when the first stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 331 to 371 and/or 499 to 507, the second stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 167 to 207 and/or 463 to 471 or of SEQ ID NO's: 249 to 289 and/or 481 to 489.

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 GPCRs.

In an even more specific, but non-limiting aspect, the invention relates to an amino acid sequence directed against GPCRs, 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: 167 to 207 and/or 463 to 471;
- 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: 167 to 207 and/or 463 to 471;
- 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: 167 to 207 and/or 463 to 471;

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

- d) the amino acid sequences of SEQ ID NO's: 249 to 289 and/or 481 to 489;
- 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: 249 to 289 and/or 481 to 489;
- 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: 249 to 289 and/or 481 to 489;

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: 331 to 371 and/or 499 to 507;
- 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: 331 to 371 and/or 499 to 507;
- 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: 331 to 371 and/or 499 to 507.

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: 167 to 207 and/or 463 to 471; the second stretch of amino acid residues is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 249 to 289 and/or 481 to 489; and the third stretch
10 of amino acid residues is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 331 to 371 and/or 499 to 507.

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 GPCRs.

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

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:
20 413 to 453 and 517 to 525. 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 sequence and one or more of the sequences of SEQ ID NO's: 413 to 453 and 517 to 525, in which the amino acid residues that form the framework regions are disregarded. Also, such amino acid sequences of the invention can be as further described herein.

Also, such amino acid sequences are preferably such that they can specifically bind (as defined herein) to GPCRs; and more in particular bind to GPCRs 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.

30 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: 167 to 207 and/or 463 to 471;

- 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: 167 to 207 and/or 463 to 471;
- 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: 167 to 207 and/or 463 to 471;

and/or

- CDR2 is chosen from the group consisting of:

- d) the amino acid sequences of SEQ ID NO's: 249 to 289 and/or 481 to 489;
- 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: 249 to 289 and/or 481 to 489;

- 10 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: 249 to 289 and/or 481 to 489;

and/or

- CDR3 is chosen from the group consisting of:

- g) the amino acid sequences of SEQ ID NO's: 331 to 371 and/or 499 to 507;
- 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: 331 to 371 and/or 499 to 507;
- 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: 331 to 371 and/or 499 to 507.

20 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: 167 to 207 and/or 463 to 471; and/or CDR2 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 249 to 289 and/or 481 to 489; and/or CDR3 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 331 to 371 and/or 499 to 507.

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:

- a) the amino acid sequences of SEQ ID NO's: 167 to 207 and/or 463 to 471;
- 30 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: 167 to 207 and/or 463 to 471;
- 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: 167 to 207 and/or 463 to 471;

and

- CDR2 is chosen from the group consisting of:
 - d) the amino acid sequences of SEQ ID NO's: 249 to 289 and/or 481 to 489;
 - 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: 249 to 289 and/or 481 to 489;
 - 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: 249 to 289 and/or 481 to 489;

and

- CDR3 is chosen from the group consisting of:
 - g) the amino acid sequences of SEQ ID NO's: 331 to 371 and/or 499 to 507;
 - 10 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: 331 to 371 and/or 499 to 507;
 - 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: 331 to 371 and/or 499 to 507; or any suitable fragment of such an amino acid sequence

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: 167 to 207 and/or 463 to 471; and CDR2 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 249 to 289 and/or 481 to 489; and CDR3 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 331 to 371 and/or 499 to 507.

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

Also, such amino acid sequences are preferably such that they can specifically bind (as defined herein) to GPCRs; and more in particular bind to GPCRs 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.

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 sequences of at least one of the amino acid sequences of SEQ ID NO's: 413 to 453 and 517 to 525. 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 sequence and one or more of the sequences of SEQ ID NO's: 413 to 453 and 517 to 525, in which the amino acid residues that form the framework regions are disregarded. Such amino acid sequences of the invention can be as further described herein.

In an even more specific, but non-limiting aspect, the invention relates to an amino acid sequence directed against CB1R (such as a Nanobody of the invention, as further described herein), 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: 167 to 184, 463 and/or 464 ;
 - 10 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: 167 to 184, 463 and/or 464 ;
 - 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: 167 to 184, 463 and/or 464 ;
 - d) the amino acid sequences of SEQ ID NO's: 249 to 266, 481 and/or 482;
 - 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: 249 to 266, 481 and/or 482;
 - 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: 249 to 266, 481 and/or 482;
 - g) the amino acid sequences of SEQ ID NO's: 331 to 348, 499 and/or 500;
 - 20 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: 331 to 348, 499 and/or 500;
 - 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: 331 to 348, 499 and/or 500;
- or any suitable combination thereof.

When an amino acid sequence of the invention contains one or more amino acid 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);
 - 30 and/or
 - 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.

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

10 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

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):

20 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

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

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.

30 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.

In this specific aspect, the amino acid sequence preferably 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: 167 to 184, 463 and/or 464 ;
 - b) the amino acid sequences of SEQ ID NO's: 249 to 266, 481 and/or 482; and
 - c) the amino acid sequences of SEQ ID NO's: 331 to 348, 499 and/or 500;
- or any suitable combination thereof.

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 CB1R.

In a more specific, but again non-limiting aspect, the invention relates to an amino acid sequence directed against CB1R, that comprises two or more stretches of amino acid residues chosen from the group consisting of:

- 10 a) the amino acid sequences of SEQ ID NO's: 167 to 184, 463 and/or 464 ;
- 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: 167 to 184, 463 and/or 464 ;
- 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: 167 to 184, 463 and/or 464 ;
- d) the amino acid sequences of SEQ ID NO's: 249 to 266, 481 and/or 482;
- 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: 249 to 266, 481 and/or 482;
- 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: 249 to 266, 481 and/or 482;
- 20 g) the amino acid sequences of SEQ ID NO's: 331 to 348, 499 and/or 500;
- 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: 331 to 348, 499 and/or 500;
- 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: 331 to 348, 499 and/or 500;

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:

- a) the amino acid sequences of SEQ ID NO's: 167 to 184, 463 and/or 464 ;
- b) the amino acid sequences of SEQ ID NO's: 249 to 266, 481 and/or 482; and
- c) the amino acid sequences of SEQ ID NO's: 331 to 348, 499 and/or 500;

such that, (i) when the first stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 167 to 184, 463 and/or 464 , the second stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 249 to 266, 481 and/or 482 or of SEQ ID NO's: 331 to 348, 499 and/or 500; (ii) when the first stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 249 to 266, 481 and/or 482, the second stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 167 to 184, 463 and/or 464 or of SEQ ID NO's: 331 to 348, 499 and/or 500; or (iii) when the first stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 331 to 348, 499 and/or 500, the second stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 167 to 184, 463 and/or 464 or of SEQ ID NO's: 249 to 266, 481 and/or 482.

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 CB1R.

In an even more specific, but non-limiting aspect, the invention relates to an amino acid sequence directed against CB1R, 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: 167 to 184, 463 and/or 464 ;
- 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: 167 to 184, 463 and/or 464 ;
- 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: 167 to 184, 463 and/or 464 ;

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

- d) the amino acid sequences of SEQ ID NO's: 249 to 266, 481 and/or 482;
- 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: 249 to 266, 481 and/or 482;
- 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: 249 to 266, 481 and/or 482;

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: 331 to 348, 499 and/or 500;
- 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: 331 to 348, 499 and/or 500;
- 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: 331 to 348, 499 and/or 500.

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: 167 to 184, 463 and/or 464 ; the second stretch of amino acid residues is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 249 to 266, 481 and/or 482; and the third stretch
 10 of amino acid residues is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 331 to 348, 499 and/or 500.

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 CB1R.

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

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:
 20 413 to 430, 517 and 518 . 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 sequence and one or more of the sequences of SEQ ID NO's: 413 to 430, 517 and 518 , in which the amino acid residues that form the framework regions are disregarded. Also, such amino acid sequences of the invention can be as further described herein.

Also, such amino acid sequences are preferably such that they can specifically bind (as defined herein) to CB1R; and more in particular bind to CB1R 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.

30 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: 167 to 184, 463 and/or 464 ;

- 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: 167 to 184, 463 and/or 464 ;
- 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: 167 to 184, 463 and/or 464 ;

and/or

- CDR2 is chosen from the group consisting of:

- d) the amino acid sequences of SEQ ID NO's: 249 to 266, 481 and/or 482;
- 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: 249 to 266, 481 and/or 482;

- 10 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: 249 to 266, 481 and/or 482;

and/or

- CDR3 is chosen from the group consisting of:

- g) the amino acid sequences of SEQ ID NO's: 331 to 348, 499 and/or 500;
- 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: 331 to 348, 499 and/or 500;
- 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: 331 to 348, 499 and/or 500.

20 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: 167 to 184, 463 and/or 464 ; and/or CDR2 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 249 to 266, 481 and/or 482; and/or CDR3 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 331 to 348, 499 and/or 500.

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:

- a) the amino acid sequences of SEQ ID NO's: 167 to 184, 463 and/or 464 ;
- 30 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: 167 to 184, 463 and/or 464 ;
- 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: 167 to 184, 463 and/or 464 ;

and

- CDR2 is chosen from the group consisting of:
 - d) the amino acid sequences of SEQ ID NO's: 249 to 266, 481 and/or 482;
 - 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: 249 to 266, 481 and/or 482;
 - 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: 249 to 266, 481 and/or 482;

and

- CDR3 is chosen from the group consisting of:
 - g) the amino acid sequences of SEQ ID NO's: 331 to 348, 499 and/or 500;
 - 10 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: 331 to 348, 499 and/or 500;
 - 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: 331 to 348, 499 and/or 500; or any suitable fragment of such an amino acid sequence

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: 167 to 184, 463 and/or 464 ; and CDR2 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 249 to 266, 481 and/or 482; and CDR3 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 331 to 348, 499 and/or 500.

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

Also, such amino acid sequences are preferably such that they can specifically bind (as defined herein) to CB1R; and more in particular bind to CB1R 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.

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 sequences of at least one of the amino acid sequences of SEQ ID NO's: 413 to 430, 517 and 518 . 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 sequence and one or more of the sequences of SEQ ID NO's: 413 to 430, 517 and 518, in which the amino acid residues that form the framework regions are disregarded. Such amino acid sequences of the invention can be as further described herein.

In another specific, but non-limiting aspect, the invention relates to an amino acid sequence directed against PTHR1 (such as a Nanobody of the invention, as further described herein), 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: 185 to 207;
- 10 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: 185 to 207;
- 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: 185 to 207;
- d) the amino acid sequences of SEQ ID NO's: 267 to 289;
- 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: 267 to 289;
- 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: 267 to 289;
- g) the amino acid sequences of SEQ ID NO's: 349 to 371;
- 20 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: 349 to 371;
- 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: 349 to 371;

or any suitable combination thereof.

When an amino acid sequence of the invention contains one or more amino acid 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);
- 30 and/or
- 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.

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

10 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

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):

20 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

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

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.

30 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.

In this specific aspect, the amino acid sequence preferably 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: 185 to 207;
 - b) the amino acid sequences of SEQ ID NO's: 267 to 289; and
 - c) the amino acid sequences of SEQ ID NO's: 349 to 371;
- or any suitable combination thereof.

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 PTHR1.

In a more specific, but again non-limiting aspect, the invention relates to an amino acid sequence directed against PTHR1, that comprises two or more stretches of amino acid residues chosen from the group consisting of:

- 10 a) the amino acid sequences of SEQ ID NO's: 185 to 207;
- 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: 185 to 207;
- 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: 185 to 207;
- d) the amino acid sequences of SEQ ID NO's: 267 to 289;
- 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: 267 to 289;
- 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: 267 to 289;
- 20 g) the amino acid sequences of SEQ ID NO's: 349 to 371;
- 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: 349 to 371;
- 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: 349 to 371;

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:

- a) the amino acid sequences of SEQ ID NO's: 185 to 207;
- b) the amino acid sequences of SEQ ID NO's: 267 to 289; and
- c) the amino acid sequences of SEQ ID NO's: 349 to 371;

such that, (i) when the first stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 185 to 207, the second stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 267 to 289 or of SEQ ID NO's: 349 to 371; (ii) when the first stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 267 to 289, the second stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 185 to 207 or of SEQ ID NO's: 349 to 371; or (iii) when the first stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 349 to 371, the second stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 185 to 207 or of SEQ ID NO's: 267 to 289.

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 PTHR1.

In an even more specific, but non-limiting aspect, the invention relates to an amino acid sequence directed against PTHR1, 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: 185 to 207;
- 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: 185 to 207;
- 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: 185 to 207;

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

- d) the amino acid sequences of SEQ ID NO's: 267 to 289;
- 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: 267 to 289;
- 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: 267 to 289;

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: 349 to 371;

- 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: 349 to 371;
- 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: 349 to 371.

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: 185 to 207; the second stretch of amino acid residues is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 267 to 289; and the third stretch of amino acid residues is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 349 to 371.

10 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 PTHR1.

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

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: 431 to 453. 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
20 sequence and one or more of the sequences of SEQ ID NO's: 431 to 453, in which the amino acid residues that form the framework regions are disregarded. Also, such amino acid sequences of the invention can be as further described herein.

Also, such amino acid sequences are preferably such that they can specifically bind (as defined herein) to CB1R; and more in particular bind to CB1R 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.

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
30 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: 185 to 207;
 - 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: 185 to 207;

- 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: 185 to 207;

and/or

- CDR2 is chosen from the group consisting of:

- d) the amino acid sequences of SEQ ID NO's: 267 to 289;
- 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: 267 to 289;
- 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: 267 to 289;

10 and/or

- CDR3 is chosen from the group consisting of:

- g) the amino acid sequences of SEQ ID NO's: 349 to 371;
- 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: 349 to 371;
- 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: 349 to 371.

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: 185 to 207; and/or CDR2 is chosen from the group consisting of the amino acid sequences of SEQ ID
20 NO's: 267 to 289; and/or CDR3 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 349 to 371.

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:

- a) the amino acid sequences of SEQ ID NO's: 185 to 207;
- 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: 185 to 207;
- 30 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: 185 to 207;

and

- CDR2 is chosen from the group consisting of:

- d) the amino acid sequences of SEQ ID NO's: 267 to 289;

- 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: 267 to 289;
- 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: 267 to 289;

and

- CDR3 is chosen from the group consisting of:

- g) the amino acid sequences of SEQ ID NO's: 349 to 371;
- 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: 349 to 371;
- 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: 349 to 371; or any suitable fragment of such an amino acid sequence

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: 185 to 207; and CDR2 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 267 to 289; and CDR3 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 349 to 371.

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

- 20 Also, such amino acid sequences are preferably such that they can specifically bind (as defined herein) to PTHR1; and more in particular bind to PTHR1 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.

- 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 sequences of at least one of the amino acid sequences of SEQ ID NO's: 431 to 453. 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 sequence and one or more of the sequences of SEQ ID NO's: 431 to 453, in which the amino acid residues that
- 30

form the framework regions are disregarded. Such amino acid sequences of the invention can be as further described herein.

In another specific, but non-limiting aspect, the invention relates to an amino acid sequence directed against MC4R (such as a Nanobody of the invention, as further described herein), 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: 465 to 471;
- 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: 465 to 471;
- 10 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: 465 to 471;
- d) the amino acid sequences of SEQ ID NO's: 483 to 489;
- 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: 483 to 489;
- 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: 483 to 489;
- g) the amino acid sequences of SEQ ID NO's: 501 to 507;
- 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: 501 to 507;
- 20 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: 501 to 507;

or any suitable combination thereof.

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

- 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);
- 30

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.

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

10 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

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):

20 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

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

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.

30 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.

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: 465 to 471;
 - ii) the amino acid sequences of SEQ ID NO's: 483 to 489; and
 - iii) the amino acid sequences of SEQ ID NO's: 501 to 507;
- or any suitable combination thereof.

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 MC4R.

In a more specific, but again non-limiting aspect, the invention relates to an amino acid sequence directed against MC4R, that comprises two or more stretches of amino acid residues chosen from the group consisting of:

- 10 a) the amino acid sequences of SEQ ID NO's: 465 to 471;
- 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: 465 to 471;
- 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: 465 to 471;
- d) the amino acid sequences of SEQ ID NO's: 483 to 489;
- 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: 483 to 489;
- 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: 483 to 489;
- 20 g) the amino acid sequences of SEQ ID NO's: 501 to 507;
- 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: 501 to 507;
- 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: 501 to 507;

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:

- i) the amino acid sequences of SEQ ID NO's: 465 to 471;
- ii) the amino acid sequences of SEQ ID NO's: 483 to 489; and
- iii) the amino acid sequences of SEQ ID NO's: 501 to 507;

such that, (i) when the first stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 465 to 471, the second stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 483 to 489 or of SEQ ID NO's: 501 to 507; (ii) when the first stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 483 to 489, the second stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 465 to 471 or of SEQ ID NO's: 501 to 507; or (iii) when the first stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 501 to 507, the second stretch of amino acid residues corresponds to one of the amino acid sequences of SEQ ID NO's: 465 to 471 or of SEQ ID NO's: 483 to 489.

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 MC4R.

In an even more specific, but non-limiting aspect, the invention relates to an amino acid sequence directed against MC4R, 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: 465 to 471;
- 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: 465 to 471;
- 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: 465 to 471;

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

- d) the amino acid sequences of SEQ ID NO's: 483 to 489;
- 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: 483 to 489;
- 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: 483 to 489;

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: 501 to 507;

- 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: 501 to 507;
- 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: 501 to 507.

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: 465 to 471; the second stretch of amino acid residues is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 483 to 489; and the third stretch of amino acid residues is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 501 to 507.

10 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 MC4R.

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

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: 519 to 525. 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
20 sequence and one or more of the sequences of SEQ ID NO's: 519 to 525, in which the amino acid residues that form the framework regions are disregarded. Also, such amino acid sequences of the invention can be as further described herein.

Also, such amino acid sequences are preferably such that they can specifically bind (as defined herein) to CB1R; and more in particular bind to CB1R 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.

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
30 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: 465 to 471;
 - 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: 465 to 471;

- 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: 465 to 471;

and/or

- CDR2 is chosen from the group consisting of:

- d) the amino acid sequences of SEQ ID NO's: 483 to 489;
- 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: 483 to 489;
- 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: 483 to 489;

10 and/or

- CDR3 is chosen from the group consisting of:

- g) the amino acid sequences of SEQ ID NO's: 501 to 507;
- 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: 501 to 507;
- 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: 501 to 507.

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: 465 to 471; and/or CDR2 is chosen from the group consisting of the amino acid sequences of SEQ ID
20 NO's: 483 to 489; and/or CDR3 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 501 to 507.

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:

- a) the amino acid sequences of SEQ ID NO's: 465 to 471;
- 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: 465 to 471;
- 30 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: 465 to 471;

and

- CDR2 is chosen from the group consisting of:

- d) the amino acid sequences of SEQ ID NO's: 483 to 489;

- 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: 483 to 489;
- 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: 483 to 489;

and

- CDR3 is chosen from the group consisting of:

- g) the amino acid sequences of SEQ ID NO's: 501 to 507;
- 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: 501 to 507;
- 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: 501 to 507; or any suitable fragment of such an amino acid sequence

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: 465 to 471; and CDR2 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 483 to 489; and CDR3 is chosen from the group consisting of the amino acid sequences of SEQ ID NO's: 501 to 507.

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

- 20 Also, such amino acid sequences are preferably such that they can specifically bind (as defined herein) to MC4R; and more in particular bind to MC4R 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.

- 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 sequences of at least one of the amino acid sequences of SEQ ID NO's: 519 to 525. 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 sequence and one or more of the sequences of SEQ ID NO's: 519 to 525, in which the amino acid residues that
- 30

form the framework regions are disregarded. Such amino acid sequences of the invention can be as further described herein.

In all of the above amino acid sequences 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
10 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 optionally have been partially or fully humanized) or are conventional V_H sequences that have 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 single domain antibody); is a "dAb" (or an amino acid sequence that is suitable for use as a
20 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 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 is also possible to use suitable fragments (or combinations of fragments) of any of the
30 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

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, and in particular a disulphide bridge that links the two framework regions that precede and
10 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 03/050531, as well as to as well as to the US provisional application of Ablynx N.V. entitled "*Peptides capable of binding to serum proteins*" of Ablynx N.V. (inventors: Revets, Hilde Adi Pierrette; Kolkman, Joost Alexander; and Hoogenboom, Hendricus Renerus Jacobus Mattheus) filed on December 5, 2006 (see also PCT/EP2007/063348).

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
20 "*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 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 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)
30 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 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.

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 herein.

Also within the scope of the present invention are compounds or constructs, that
10 comprises or essentially consists of one or more derivatives 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 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).

20 As will be clear from the further description above and herein, this means that the amino acid sequences of the invention can be used as "building blocks" to form polypeptides of the invention, i.e. by suitably combining them with other groups, residues, moieties or binding units, in order to form compounds or constructs as described herein (such as, without limitations, the biparatopic, bi/multivalent and bi/multispecific polypeptides of the invention described herein) which combine within one molecule one or more desired properties or biological functions.

The compounds or polypeptides of the invention can generally be prepared by a 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
30 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 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 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
10 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 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
20 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 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
30 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 proteins such as serum albumin (such as human serum albumin), serum immunoglobulins such as IgG, or transferrin; reference is made to the 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, WO 01/45746, WO 02/076489 and to the US provisional application of Ablynx N.V. entitled "*Peptides capable of binding to serum proteins*" of Ablynx N.V. filed on December 5, 2006. (see also PCT/EP2007/063348).

10 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 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 polypeptides of the invention have 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 of the invention per se.

20 In another preferred, but non-limiting aspect of the invention, such compounds 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 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).
30 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, at least one polypeptide of the invention (or a suitable fragment thereof) 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
10 examples of such products or compositions will become clear from the further description herein.

The invention also relates to the use of an amino acid sequence, Nanobody or polypeptide of the invention, or of a composition comprising the same, in (methods or compositions for) modulating a GPCR, either in vitro (e.g. in an in vitro or cellular assay) or in vivo (e.g. in an a single cell or in a multicellular organism, and in particular in a mammal, and more in particular in a human being, such as in a human being that is at risk of or suffers from a GPCR related disease or disorder).

The invention also relates to methods for modulating a GPCR, either in vitro (e.g. in an in vitro or cellular assay) or in vivo (e.g. in an a single cell or multicellular organism, and
20 in particular in a mammal, and more in particular in a human being, such as in a human being that is at risk of or suffers from a GPCR related disease or disorder), which method comprises at least the step of contacting a GPCR with at least one amino acid sequence, Nanobody or polypeptide of the invention, or with a composition comprising the same, in a manner and in an amount suitable to modulate a GPCR, with at least one amino acid sequence, Nanobody or polypeptide of the invention.

The invention also relates to the use of an one amino acid sequence, Nanobody or polypeptide of the invention in the preparation of a composition (such as, without limitation, a pharmaceutical composition or preparation as further described herein) for modulating (as defined herein) a GPCR, either in vitro (e.g. in an in vitro or cellular assay) or in vivo (e.g. in
30 an a single cell or multicellular organism, and in particular in a mammal, and more in particular in a human being, such as in a human being that is at risk of or suffers from a GPCR related disease or disorder).

In the context of the present specification, “modulating” may be as generally defined herein, but may for also involve allosteric modulation (see for example

George et al., Nat Rev Drug Discov 1:808-820 (2002); Kenakin, Trends Pharmacol Sci 25:186-192 (2002) and Rios et al., Pharmacol Ther 92:71-87 (2001)) and/or reducing or inhibiting the binding of a GPCR to one of its substrates or ligands and/or competing with a natural ligand, substrate for binding to a GPCR. Modulating may also involve activating a GPCR or the mechanism or pathway in which it is involved. Modulating may be reversible or irreversible, but for pharmaceutical and pharmacological purposes will usually be in a reversible manner.

10 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:

- 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 GPCRs;
- and
- c) isolating the amino acid sequence(s) that can bind to and/or have affinity for GPCRs.

20 In particular, in step b) of such a method, the set, collection or library may be screened for amino acid sequences that can bind to and/or have affinity for GPCRs that are expressed on the surface of a suitable cell; for amino acid sequences that can bind to and/or have affinity for an extracellular part, region, domain, loop or other extracellular epitope of a GPCR (as described herein); and/or for amino acid sequences that can bind to and/or have affinity for a peptide that has been derived from or based on the amino acid sequence of an extracellular part, region, domain, loop or other extracellular epitope of a GPCR. This can be performed using methods and techniques known per se, for example those mentioned herein.

30 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.

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.

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 GPCRs 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), or a suitable peptide derived therefrom. Alternatively, as mentioned herein, 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 a refolded GPCR or with a cell, or cell fraction or preparation derived from a cell that has a GPCR on its surface.

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 screening (a set, collection or library of) amino acid sequences will be clear to the person
20 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:

- 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 GPCRs;
- 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
30 amino acid sequence.

In particular, in step b) of such a method, the set, collection or library may be screened for cells that express amino acid sequences that can bind to and/or have affinity for GPCRs that are expressed on the surface of a suitable cell; for cells that express amino acid sequences that can bind to and/or have affinity for an extracellular part, region, domain, loop or other

extracellular epitope of a GPCR (as described herein); and/or for cells that express amino acid sequences that can bind to and/or have affinity for a peptide that has been derived from or based on the amino acid sequence of an extracellular part, region, domain, loop or other extracellular epitope of a GPCR. This can be performed using methods and techniques known per se, for example those mentioned herein.

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 immunized with GPCRs 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), or a suitable peptide derived therefrom.. Alternatively, as mentioned herein, the sample of cells may be derived from a mammal that has been suitably immunized with a refolded GPCR or with a cell, or cell fraction or preparation derived from a cell that has a GPCR on its surface.

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., Blood, Vol. 97, No. 12, 3820 (2001).

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

- a) providing a set, collection or library of nucleic acid sequences encoding amino acid sequences;
- 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 GPCRs;

and

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

In particular, in step b) of such a method, the set, collection or library may be screened for nucleotide sequences that encode amino acid sequences that can bind to and/or have affinity for GPCRs that are expressed on the surface of a suitable cell; for nucleotide sequences that encode amino acid sequences that can bind to and/or have affinity for an extracellular part, region, domain, loop or other extracellular epitope of a GPCR (as described

herein); and/or for nucleotide sequences that encode amino acid sequences that can bind to and/or have affinity for a peptide that has been derived from or based on the amino acid sequence of an extracellular part, region, domain, loop or other extracellular epitope of a GPCR. This can be performed using methods and techniques known per se, for example those mentioned herein.

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 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 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 derived from a mammal that has been suitably immunized with GPCRs 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), or a suitable peptide derived therefrom. Alternatively, as mentioned herein, the set, collection or library of nucleic acid sequences may be an immune set, collection or library derived from a mammal that has been suitably immunized with a refolded GPCR or with a cell, or cell fraction or preparation derived from a cell that has a GPCR on its surface.

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 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
10 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
20 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 GPCRs. Some preferred but non-limiting applications and uses will become clear from the further description herein. For example, as mentioned herein, it is expected that amino acid sequences, Nanobodies and polypeptides of the invention that are directed against olfactory GPCRs can find use as artificial flavourings or even perfumes. The amino acid sequences,
30 Nanobodies and polypeptides of the invention may also find use as markers for detecting cells that express the GPCRs against which they are directed, for example in vitro (e.g. using Western blot, immunoprecipitation or immunofluorescence techniques) or in vivo (e.g. using suitable imaging techniques). The amino acid sequences, Nanobodies and polypeptides of the

invention may also find use in affinity purification techniques for (cells expressing) the GPCRs against which they are directed.

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 discussed in more detail with reference to the Nanobodies of the invention and 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.

Detailed description of the invention

G protein-coupled receptors (GPCRs), also known as seven transmembrane receptors, 7TM receptors, heptahelical receptors, and G protein linked receptors (GPLR), are a protein family of transmembrane receptors that transduce an extracellular signal (ligand binding) into an intracellular signal (G protein activation). GPCRs are integral membrane proteins that
20 possess seven membrane-spanning domains or transmembrane helices. The extracellular parts of the receptor can be glycosylated. These extracellular loops also contain two highly conserved cysteine residues which build disulfide bonds to stabilize the receptor structure.

The GPCRs form the largest and most diverse group of transmembrane proteins involved in signal transduction (Howard et al., Trends Pharmacol. Sci. 22:132-40, 2001). GPCRs are involved in various cellular and biological functions, such as stimulus-response pathways (from intercellular communication to physiological senses), including, for example, embryogenesis, neurotransmitter release, neurosensation (e.g., 15 chemosensory functions such as taste and smell) (Mombaerts, Science 286:707-711, 1999), neuronal axon pathfinding (Mombaerts et al., Cell 87:675, 1996; Mombaerts et al., Cold Spring Harbor Symp. Quant.
30 Biol. 56:135, 1996), leukocyte targeting to sites of inflammation (Tager et al., J. Exp. Med., 192:439-46, 2000), and cell survival, proliferation, and differentiation. (Ryan et al., J. Biol Chem. 273:13613-24, 1998).

The complexity of the GPCR repertoire surpasses that of the immunoglobulin and T cell receptor genes combined, with members of the GPCR superfamily estimated at as many

as 2,000, or more than 1.5% of the human genome. Further, members of the GPCR superfamily are the direct or indirect target of more than 50% of the current pharmaceutical drugs used clinically in humans.

The diversity of functions is matched by the wide range of ligands recognized by members of the family, from photons (rhodopsin, the archetypal GPCR) to small molecules (in the case of the histamine receptors) to proteins (for example, chemokine receptors). For an overview of the human GPCR family and ligands of human GPCRs reference is made to Figure 1 in the US application 2002/0106739.

GPCRs can be grouped into 4 classes based on structural homology and functional similarity:

- 10 Class A (rhodopsin-like), Class B (secretin-like), Class C (metabotropic/ pheromone), and Class D (Fungal pheromone), of which Class A receptors, Class B receptors, and receptors with virtually non-existent carboxyl-terminal tails form the major classes. GPCRs can be classified accordingly based on their interactions with an affinity for rat,8-arrestin-2 in HEK-293 cells and may be predicted based on the amino acid residues in their carboxyl- terminal tail and the length of their carboxyl-terminal tail. A Class B receptor is a GPCR that has one or more sites of phosphorylation (e.g., clusters of phosphorylation sites) properly positioned in its carboxyl- terminal tail such that it does recruit rat 8-arrestin-2 to endosomes in HEK-293 cells under conditions as described in U.S. Patent No 5,891,646, Oakley, et al., Journal of Biological Chemistry, Vol 275, No. 22, pp 17201-17210, June 2, 2000, and Oakley et al.,
- 20 Journal of Biological Chemistry, Vol. 276, No. 22, pp 19452-19460, 2001. A Class A receptor is a GPCR that does not have one or more sites of phosphorylation (e.g., clusters of phosphorylation sites) properly positioned in its carboxyl-terminal tail such that it does not recruit rat p-arrestin-2 to endosomes in HEK- 293 cells under conditions as described above for Class B receptors. Receptors with virtually non existent carboxyl-terminal tails include, for example, olfactory and taste receptors.

Some examples of the biological and physiological roles of GPCRs include:

- the visual sense: the opsins use a photoisomerization reaction to translate electromagnetic radiation into cellular signals. Rhodopsin, for example, uses the conversion of 11-cis-retinal to all-trans-retinal for this purpose.
- 30 - the sense of smell: receptors of the olfactory epithelium bind odorants (olfactory receptors) and pheromones (vomeronasal receptors)
- behavioral and mood regulation: receptors in the mammalian brain bind several different neurotransmitters, including serotonin, dopamine, GABA and glutamate.

- regulation of immune system activity and inflammation: chemokine receptors bind ligands that mediate intercellular communication between cells of the immune system; receptors such as histamine receptors bind inflammatory mediators and engage target cell types in the inflammatory response
- autonomic nervous system transmission: both the sympathetic and parasympathetic nervous systems are regulated by GPCR pathways. These systems are responsible for control of many automatic functions of the body such as blood pressure, heart rate and digestive processes.

Generally, for GPCRs reference is made to the standard handbooks, such as the G
 10 Protein Coupled Receptors Handbook, L. Devi (Ed.), Humana Press, 2005, as well as to the available databases, such as GPCRDB (see for example <http://www.gpcr.org/7tm/htmls/entries.html>).

Thus, generally, as used herein, the term "G-protein coupled receptor" (or "GPCR") refers to a receptor that, when expressed by a cell, associates with a G-protein (e.g., a protein composed of α , β and γ subunits and which hydrolyzes GTP). Preferably, the GPCR is a "seven transmembrane segment receptor" (or "7 TMS receptor"), which refers to a protein that structurally comprises seven hydrophobic transmembrane spanning regions.

Some non-limiting examples of GPCRs include, but are not limited to:

- GPCRs that are known targets for pharmaceuticals (either small molecules or
 20 biologicals) that are currently on the market or in clinical development (for example, those mentioned herein);
- the luteinizing hormone releasing hormone (LHRH) (also known as gonadotropin releasing hormone, GnRH) receptor, the M1 muscarinic receptor and the D2-adrenergic receptor;
- opioid receptors, endothelin receptors, angiotensin receptors, neuropeptide Y receptors and serotonin K receptors;
- GPCRs that couple to (i.e., associates with) a Gq/11 G-protein, such as LHRH (=GnRH), acetylcholine (m1, 3 and 5 subtypes), M1 muscarinic, adenosine 1, CC-
 30 adrenergic (α 1A, α 1B and α 1C subtypes), angiotensin (AT1A subtype), bombesin (BB1 and BB2 subtypes), bradykinin (B2 subtype), C5a, cholecystokinin (CCKa and CCKb subtypes), endothelin (E1 and E2 subtypes), glutamate (mGlu1, 5 subtypes), 5HT (2A, 2B and 2C subtypes), histamine (H1 subtype), neurotensin, neurokinin (NK1, 2, 3 subtypes), oxytocin, thyrotropin releasing hormone (TRH), thyroid stimulating hormone (TSH), thromboxane A2 and vasopressin (V1a subtypes);

- GPCRs that couple to a Gs G-protein, such as the following receptors: P2-adrenergic, cardiac P- adrenergic, histamine (H2 subtype), thyrotropin, growth hormone releasing factor, adrenocorticotrophic hormone (ACTH), 5HT4, follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), GLP- 1, glucagon, dopamine5 (D5), doparninel (DI), calcitonin, adenosine2p (A2p), vasopressin2, vasoactive intestinal polypeptide and parathyroid hormone;
- GPCRs that couple to a Gi G-protein, such as the following receptors: 5HT (I A, I B, I D and I F subtypes), mGlutamineR (2, 3 subtypes), dopamine4 (D4), dopamine-2 (D2) cannabinoid, adenosine3 (A3), somatostatin (4, 3 subtypes), t-opioid, 6- opioid, K-
10 Opioid, neuropeptide Y (1, 2 subtypes);
- The GPCRs mentioned in US 2002/0106739;
- The GPCRs listed in Table 1 of Lundstrom et al., J. Struct. Funct.Genomics, 2006 Nov 22; [Epub ahead of print]
- GPCRs that are so-called "orphan" receptors, i.e. a GPCR that is structurally similar to other GPCRs but for which the natural ligand is not yet known;
- The GPCRs mentioned in Table C;
- The GPCRs mentioned in Table D.

Other GPCRs will be clear to the skilled person, for example from the standard handbooks, such as the G Protein Coupled Receptors Handbook, L. Devi (Ed.), Humana
20 Press, 2005; as well as from the standard databases, such as GPCRDB (see for example <http://www.gpcr.org/7tm/htmls/entries.html>).

In the present description, examples and claims:

- 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 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
30 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;

- 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”, “VHH sequence” or “protein sequence”), should generally be understood to include both the relevant amino acid sequence as well as nucleic acids or nucleotide sequences encoding the same, unless the context requires a more limited interpretation. Also, the term “nucleotide sequence” as used herein also encompasses a nucleic acid molecule with said nucleotide sequence, so that the terms “nucleotide sequence” and “nucleic acid” should be considered equivalent and are used interchangeably herein;
- 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; as well as to for example the following reviews Presta, *Adv. Drug Deliv. Rev.* 2006, 58 (5-6): 640-56; Levin and Weiss, *Mol. Biosyst.* 2006, 2(1): 49-57; Irving et al., *J. Immunol. Methods*, 2001, 248(1-2), 31-45; Schmitz et al., *Placenta*, 2000, 21 Suppl. A, S106-12, Gonzales et al., *Tumour Biol.*, 2005, 26(1), 31-43, which describe techniques for protein engineering, such as affinity maturation and other techniques for improving the specificity and other desired properties of proteins such as immunoglobulins.
- 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

Notes:

- (1) Sometimes also considered to be a polar uncharged amino acid.
- (2) Sometimes also considered to be a nonpolar uncharged amino acid.
- (3) 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.
- (4) 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.

- 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.

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.

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
20 as the “second” nucleotide sequence;

- 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 (also 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
30 (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.

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.

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-3 357 768, 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 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 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 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 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. Nat. Acad Sci. USA 81: 140-144, 1984; Kyte & Doolittle; J Molec. Biol. 157: 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., Nature Structural Biology (1996); 3, 752-757; and 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.

- 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 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 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).

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;

k) The term “*domain*” as used herein generally refers to a globular region of an amino acid sequence (such as 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. The term “*binding domain*” refers to such a domain that is directed against an antigenic determinant (as defined herein);

- 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.
- 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.
- 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. 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 antigen 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.

10 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
20 $1/K_D$ 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 (ΔG) of binding by the well known relation $\Delta G = RT \cdot \ln(K_D)$ (equivalently $\Delta G = -RT \cdot \ln(K_A)$), where R equals the gas constant, T equals the absolute temperature and ln denotes the natural logarithm.

30 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 (10000 nM). The stronger an interaction is, the lower is its K_D .

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 $\text{M}^{-1}\text{s}^{-1}$. The on-rate may vary between $10^2 \text{ M}^{-1}\text{s}^{-1}$ to about $10^7 \text{ M}^{-1}\text{s}^{-1}$, 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} ($t_{1/2} = 0.69 \text{ s}$).

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 artefacts 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.

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 artefacts relating to adsorption of one of the molecules on a support such as plastic.

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.

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 labelled with a

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 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 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, cynomolgus 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) In the context of the present invention, "modulating" or "to modulate" generally means either reducing or inhibiting the activity of, or alternatively increasing the activity of, a target or antigen, as measured using a suitable in vitro, cellular or in vivo assay. In particular, "modulating" or "to modulate" may mean either reducing or inhibiting the activity of, or alternatively increasing a (relevant or intended) biological activity of, a target or antigen, as measured using a suitable in vitro, cellular or in vivo assay (which will usually depend on the target or antigen involved), 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 activity of the target or antigen in the same assay under the same conditions but without the presence of the construct of the invention.

As will be clear to the skilled person, "modulating" may also involve effecting a change (which may either be an increase or a decrease) in affinity, avidity, specificity and/or selectivity of a target or antigen for one or more of its ligands, binding partners, partners for association into a homomultimeric or heteromultimeric form, or substrates; and/or effecting a change (which may either be an increase or a decrease) in the sensitivity of the target or antigen for one or more conditions in the medium or surroundings in which the target or antigen is present (such as pH, ion strength, the presence of co-factors, etc.), compared to the same conditions but without the presence of the construct of the invention. As will be clear to the skilled person, this may again be determined in any suitable manner and/or using any suitable assay known per se, depending on the target or antigen involved.

“Modulating” may also mean effecting a change (i.e. an activity as an agonist, as an antagonist or as a reverse agonist, respectively, depending on the target or antigen and the desired biological or physiological effect) with respect to one or more biological or physiological mechanisms, effects, responses, functions, pathways or activities in which the target or antigen (or in which its substrate(s), ligand(s) or pathway(s) are involved, such as its signalling pathway or metabolic pathway and their associated biological or physiological effects) is involved. Again, as will be clear to the skilled person, such an action as an agonist or an antagonist may be determined in any suitable manner and/or using any suitable (in vitro and usually cellular or in assay) assay known per se,

10 depending on the target or antigen involved. In particular, an action as an agonist or antagonist may be such that an intended biological or physiological activity is increased or decreased, respectively, 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 the biological or physiological activity in the same assay under the same conditions but without the presence of the construct of the invention. Modulating may for example also involve allosteric modulation of the target or antigen; and/or reducing or inhibiting the binding of the target or antigen to one of its substrates or ligands and/or competing with a natural ligand, substrate for binding to the target or antigen. Modulating may also involve activating the target or antigen or the mechanism
20 or pathway in which it is involved. Modulating may for example also involve effecting a change in respect of the folding or confirmation of the target or antigen, or in respect of the ability of the target or antigen to fold, to change its confirmation (for example, upon binding of a ligand), to associate with other (sub)units, or to disassociate. Modulating may for example also involve effecting a change in the ability of the target or antigen to transport other compounds or to serve as a channel for other compounds (such as ions).

Modulating may be reversible or irreversible, but for pharmaceutical and pharmacological purposes will usually be in a reversible manner.

q) In respect of a target or antigen, the term “interaction site” on the target or antigen
30 means a site, epitope, antigenic determinant, part, domain or stretch of amino acid residues on the target or antigen that is a site for binding to a ligand, receptor or other binding partner, a catalytic site, a cleavage site, a site for allosteric interaction, a site involved in multimerisation (such as homomerization or heterodimerization) of the target or antigen; or any other site, epitope, antigenic determinant, part, domain or

stretch of amino acid residues on the target or antigen that is involved in a biological action or mechanism of the target or antigen. More generally, an “interaction site” can be any site, epitope, antigenic determinant, part, domain or stretch of amino acid residues on the target or antigen to which an amino acid sequence or polypeptide of the invention can bind such that the target or antigen (and/or any pathway, interaction, signalling, biological mechanism or biological effect in which the target or antigen is involved) is modulated (as defined herein).

- 10 r) An amino acid sequence or polypeptide is said to be “*specific for*” a first target or antigen compared to a second target or antigen when it binds to the first antigen with an affinity (as described above, and suitably expressed as a K_D value, K_A value, K_{off} rate and/or K_{on} rate) that is at least 10 times, such as at least 100 times, and preferably at least 1000 times, and up to 10,000 times or more better than the affinity with which said amino acid sequence or polypeptide binds to the second target or polypeptide. For example, the first antigen may bind to the target or antigen with a K_D value that is at least 10 times less, such as at least 100 times less, and preferably at least 1000 times less, such as 10,000 times less or even less than that, than the K_D with which said amino acid sequence or polypeptide binds to the second target or polypeptide. Preferably, when an amino acid sequence or polypeptide is “specific for” a first target or antigen compared to a second target or antigen, it is directed against (as defined herein) said first target or antigen, but not directed against said second target or antigen.

- 20 s) The terms “cross-block”, “cross-blocked” and “cross-blocking” are used interchangeably herein to mean the ability of an amino acid sequence or other binding agents (such as a polypeptide of the invention) to interfere with the binding of other amino acid sequences or binding agents of the invention to a given target. The extent to which an amino acid sequence or other binding agents of the invention is able to interfere with the binding of another to [target], and therefore whether it can be said to cross-block according to the invention, can be determined using competition binding assays. One particularly suitable quantitative assay uses a Biacore machine which can measure the extent of interactions using surface plasmon resonance technology. Another
- 30 suitable quantitative cross-blocking assay uses an ELISA-based approach to measure competition between amino acid sequence or another binding agents in terms of their binding to the target.

The following generally describes a suitable Biacore assay for determining whether an amino acid sequence or other binding agent cross-blocks or is capable of cross-blocking

according to the invention. It will be appreciated that the assay can be used with any of the amino acid sequence or other binding agents described herein. The Biacore machine (for example the Biacore 3000) is operated in line with the manufacturer's recommendations. Thus in one cross-blocking assay, the target protein is coupled to a CM5 Biacore chip using standard amine coupling chemistry to generate a surface that is coated with the target. Typically 200- 800 resonance units of the target would be coupled to the chip (an amount that gives easily measurable levels of binding but that is readily saturable by the concentrations of test reagent being used). Two test amino acid sequences (termed A* and B*) to be assessed for their ability to cross- block each other are mixed at a one to one molar ratio of binding sites in a suitable buffer to create the test mixture. When calculating the concentrations on a binding site basis the molecular weight of an amino acid sequence is assumed to be the total molecular weight of the amino acid sequence divided by the number of target binding sites on that amino acid sequence. The concentration of each amino acid sequence in the test mix should be high enough to readily saturate the binding sites for that amino acid sequence on the target molecules captured on the Biacore chip. The amino acid sequences in the mixture are at the same molar concentration (on a binding basis) and that concentration would typically be between 1.00 and 1.5 micromolar (on a binding site basis). Separate solutions containing A* alone and B* alone are also prepared. A* and B* in these solutions should be in the same buffer and at the same concentration as in the test mix. The test mixture is passed over the target-coated Biacore chip and the total amount of binding recorded. The chip is then treated in such a way as to remove the bound amino acid sequences without damaging the chip-bound target. Typically this is done by treating the chip with 30 mM HCl for 60 seconds. The solution of A* alone is then passed over the target-coated surface and the amount of binding recorded. The chip is again treated to remove all of the bound amino acid sequences without damaging the chip-bound target. The solution of B* alone is then passed over the target-coated surface and the amount of binding recorded. The maximum theoretical binding of the mixture of A* and B* is next calculated, and is the sum of the binding of each amino acid sequence when passed over the target surface alone. If the actual recorded binding of the mixture is less than this theoretical maximum then the two amino acid sequences are cross-blocking each other. Thus, in general, a cross-blocking amino acid sequence or other binding agent according to the invention is one which will bind to the target in the above Biacore cross-blocking assay such that during the assay and in the presence of

a second amino acid sequence or other binding agent of the invention the recorded binding is between 80% and 0.1% (e.g. 80% to 4%) of the maximum theoretical binding, specifically between 75% and 0.1% (e.g. 75% to 4%) of the maximum theoretical binding, and more specifically between 70% and 0.1% (e.g. 70% to 4%) of maximum theoretical binding (as just defined above) of the two amino acid sequences or binding agents in combination. The Biacore assay described above is a primary assay used to determine if amino acid sequences or other binding agents cross-block each other according to the invention. On rare occasions particular amino acid sequences or other binding agents may not bind to target coupled via amine chemistry to a CM5

10 Biacore chip (this usually occurs when the relevant binding site on target is masked or destroyed by the coupling to the chip). In such cases cross-blocking can be determined using a tagged version of the target, for example a N-terminal His-tagged version (R & D Systems, Minneapolis, MN, USA; 2005 cat# 1406-ST-025). In this particular format, an anti-His amino acid sequence would be coupled to the Biacore chip and then the His-tagged target would be passed over the surface of the chip and captured by the anti-His amino acid sequence. The cross blocking analysis would be carried out essentially as described above, except that after each chip regeneration cycle, new His-tagged target would be loaded back onto the anti-His amino acid sequence coated surface. In addition to the example given using N-terminal His-tagged [target], C-terminal His-tagged target

20 could alternatively be used. Furthermore, various other tags and tag binding protein combinations that are known in the art could be used for such a cross-blocking analysis (e.g. HA tag with anti-HA antibodies; FLAG tag with anti-FLAG antibodies; biotin tag with streptavidin).

The following generally describes an ELISA assay for determining whether an amino acid sequence or other binding agent directed against a target cross-blocks or is capable of cross-blocking as defined herein. It will be appreciated that the assay can be used with any of the amino acid sequences (or other binding agents such as polypeptides of the invention) described herein. The general principal of the assay is to have an amino acid sequence or binding agent that is directed against the target coated onto the wells of

30 an ELISA plate. An excess amount of a second, potentially cross-blocking, anti-target amino acid sequence is added in solution (i.e. not bound to the ELISA plate). A limited amount of the target is then added to the wells. The coated amino acid sequence and the amino acid sequence in solution compete for binding of the limited number of target molecules. The plate is washed to remove excess target that has not been bound by the

coated amino acid sequence and to also remove the second, solution phase amino acid sequence as well as any complexes formed between the second, solution phase amino acid sequence and target. The amount of bound target is then measured using a reagent that is appropriate to detect the target. An amino acid sequence in solution that is able to cross-block the coated amino acid sequence will be able to cause a decrease in the number of target molecules that the coated amino acid sequence can bind relative to the number of target molecules that the coated amino acid sequence can bind in the absence of the second, solution phase, amino acid sequence. In the instance where the first amino acid sequence, e.g. an Ab-X, is chosen to be the immobilized amino acid sequence, it is coated onto the wells of the ELISA plate, after which the plates are blocked with a suitable blocking solution to minimize non-specific binding of reagents that are subsequently added. An excess amount of the second amino acid sequence, i.e. Ab-Y, is then added to the ELISA plate such that the moles of Ab-Y [target] binding sites per well are at least 10 fold higher than the moles of Ab-X [target] binding sites that were used, per well, during the coating of the ELISA plate. [target] is then added such that the moles of [target] added per well are at least 25-fold lower than the moles of Ab-X [target] binding sites that were used for coating each well. Following a suitable incubation period the ELISA plate is washed and a reagent for detecting the target is added to measure the amount of target specifically bound by the coated anti-[target] amino acid sequence (in this case Ab-X). The background signal for the assay is defined as the signal obtained in wells with the coated amino acid sequence (in this case Ab-X), second solution phase amino acid sequence (in this case Ab-Y), [target] buffer only (i.e. no target) and target detection reagents. The positive control signal for the assay is defined as the signal obtained in wells with the coated amino acid sequence (in this case Ab-X), second solution phase amino acid sequence buffer only (i.e. no second solution phase amino acid sequence), target and target detection reagents. The ELISA assay may be run in such a manner so as to have the positive control signal be at least 6 times the background signal. To avoid any artefacts (e.g. significantly different affinities between Ab-X and Ab-Y for [target]) resulting from the choice of which amino acid sequence to use as the coating amino acid sequence and which to use as the second (competitor) amino acid sequence, the cross-blocking assay may to be run in two formats: 1) format 1 is where Ab-X is the amino acid sequence that is coated onto the ELISA plate and Ab-Y is the competitor amino acid sequence that is in solution and 2) format 2 is where Ab-Y is the amino acid sequence that is coated onto the ELISA plate and Ab-X is the

competitor amino acid sequence that is in solution. Ab-X and Ab-Y are defined as cross-blocking if, either in format 1 or in format 2, the solution phase anti-target amino acid sequence is able to cause a reduction of between 60% and 100%, specifically between 70% and 100%, and more specifically between 80% and 100%, of the target detection signal {i.e. the amount of target bound by the coated amino acid sequence) as compared to the target detection signal obtained in the absence of the solution phase anti- target amino acid sequence (i.e. the positive control wells).

- t) As 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;
- u) 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 Muyldermans, J. Immunol. Methods 2000 Jun 23; 240 (1-2): 185-195 (see for example Figure 2 of this publication); 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 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

- v) 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.

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 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 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 WO 06/040153, which list and references are incorporated herein by reference.

In accordance with the terminology used in the art (see 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”).

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 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 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 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 spatial conformation and configuration (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 “dAb’s” 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);
- 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., Proteins 1998 Sep 1; 32(4): 515-22; Lauwereys et al., EMBO J. 1998 Jul 1; 17(13): 3512-20).

In a specific and preferred aspect, the invention provides Nanobodies against GPCRs, and in particular Nanobodies against GPCRs from a warm-blooded animal, and more in particular Nanobodies against GPCRs from a mammal, and especially Nanobodies against human GPCRs; as well as proteins and/or polypeptides comprising at least one such Nanobody.

In particular, the invention provides Nanobodies against GPCRs, and proteins and/or polypeptides comprising the same, that have improved therapeutic and/or pharmacological properties and/or other advantageous properties (such as, for example, improved ease of preparation and/or reduced costs of goods), compared to conventional antibodies against GPCRs or fragments thereof, compared to constructs that could be based on such conventional antibodies or antibody fragments (such as Fab’ fragments, F(ab’)₂ fragments, ScFv constructs, “diabodies” and other multispecific constructs (see for example the review

by Holliger and Hudson, Nat Biotechnol. 2005 Sep;23(9):1126-36)), and also compared to the so-called “dAb’s” or similar (single) domain antibodies that may be derived from variable domains of conventional antibodies. These improved and advantageous properties will become clear from the further description herein, and for example include, without limitation, one or more of:

- increased affinity and/or avidity for GPCRs, either in a monovalent format, in a multivalent format (for example in a bivalent format) and/or in a multispecific format (for example one of the multispecific formats described hereinbelow);
 - better suitability for formatting in a multivalent format (for example in a bivalent format);
 - better suitability for formatting in a multispecific format (for example one of the multispecific formats described hereinbelow);
 - improved suitability or susceptibility for “humanizing” substitutions (as defined herein);
 - less immunogenicity, either in a monovalent format, in a multivalent format (for example in a bivalent format) and/or in a multispecific format (for example one of the multispecific formats described hereinbelow);
 - increased stability, either in a monovalent format, in a multivalent format (for example in a bivalent format) and/or in a multispecific format (for example one of the multispecific formats described hereinbelow);
 - increased specificity towards GPCRs, either in a monovalent format, in a multivalent format (for example in a bivalent format) and/or in a multispecific format (for example one of the multispecific formats described hereinbelow);
 - decreased or where desired increased cross-reactivity with GPCRs from different species;
- and/or
- one or more other improved properties desirable for pharmaceutical use (including prophylactic use and/or therapeutic use) and/or for diagnostic use (including but not limited to use for imaging purposes), either in a monovalent format, in a multivalent format (for example in a bivalent format) and/or in a multispecific format (for example one of the multispecific formats described hereinbelow).

As generally described herein for the amino acid sequences of the invention, the Nanobodies of the invention are preferably in essentially 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 Nanobodies 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 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 GPCRs), so as to provide a monovalent, multivalent or multispecific polypeptide of the invention, respectively, all as described herein. In particular, such a protein or polypeptide may comprise or essentially consist of one or more Nanobodies of the invention and optionally one or more (other) Nanobodies (i.e. directed against other targets than GPCRs), all optionally linked via one or more suitable linkers, so as to provide a monovalent, multivalent or multispecific Nanobody construct, respectively, as further described herein. Such proteins or polypeptides may also be in essentially isolated form (as defined herein).

In a Nanobody of the invention, the binding site for binding against GPCRs is preferably formed by the CDR sequences. Optionally, a Nanobody of the invention may also, and in addition to the at least one binding site for binding against GPCRs, 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 and WO 06/07260.

As generally described herein for the amino acid sequences of the invention, when a Nanobody of the invention (or a polypeptide of the invention comprising the same) is intended for administration to a subject (for example for therapeutic and/or diagnostic purposes as described herein), it is preferably directed against human GPCRs; whereas for veterinary purposes, it is preferably directed against GPCRs from the species to be treated. Also, as with the amino acid sequences of the invention, a Nanobody of the invention may or may not be cross-reactive (i.e. directed against GPCRs from two or more species of mammal, such as against human GPCRs and GPCRs from at least one of the species of mammal mentioned herein).

Also, again as generally described herein for the amino acid sequences of the invention, the Nanobodies of the invention may generally be directed against any antigenic determinant, epitope, part, domain, subunit or conformation (where applicable) of GPCRs. However, it is generally assumed and preferred that the Nanobodies of the invention (and polypeptides comprising the same) are directed against and/or have been raised against at

least one extracellular region, domain, loop or other extracellular epitope of a GPCR (or a suitable peptide derived therefrom).

As already described herein, the amino acid sequence and structure of a Nanobody can be considered - without however being limited thereto - to be comprised of four framework regions or "FR's" (or sometimes also referred to as "FW's"), which are referred to in the art and herein as "Framework region 1" or "FR1"; as "Framework region 2" or "FR2"; as "Framework region 3" or "FR3"; and as "Framework region 4" or "FR4", respectively; which framework regions are interrupted by three complementary determining regions or "CDR's", which are referred to in the art as "Complementarity Determining Region 1" or "CDR1"; as
 10 "Complementarity Determining Region 2" or "CDR2"; and as "Complementarity Determining Region 3" or "CDR3", respectively. Some preferred framework sequences and CDR's (and combinations thereof) that are present in the Nanobodies of the invention are as described herein. Other suitable CDR sequences can be obtained by the methods described herein.

According to a non-limiting but preferred aspect of the invention, (the CDR sequences present in) the Nanobodies of the invention are such that:

- the Nanobodies can bind to GPCRs 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
 20 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:

- the Nanobodies can bind to GPCRs 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:

- the Nanobodies can bind to GPCRs 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}
 30 and 10^{-6} s^{-1} .

Preferably, (the CDR sequences present in) the Nanobodies of the invention are such that: a monovalent Nanobody of the invention (or a polypeptide that contains only one Nanobody of the invention) is preferably such that it will bind to GPCRs with an affinity less

than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM.

The affinity of the Nanobody of the invention against GPCRs can be determined in a manner known per se, for example using the general techniques for measuring K_D , K_A , k_{off} or k_{on} mentioned herein, as well as some of the specific assays described herein.

Some preferred IC50 values for binding of the Nanobodies of the invention (and of polypeptides comprising the same) to GPCRs will become clear from the further description and examples herein.

10 In a preferred but non-limiting aspect, the invention relates to a Nanobody (as defined herein) against GPCRs, which 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: 167 to 207 and/or 463 to 471;
- 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: 167 to 207 and/or 463 to 471;
- 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: 167 to 207 and/or 463 to 471;

and/or

- CDR2 is chosen from the group consisting of:

- 20
- d) the amino acid sequences of SEQ ID NO's: 249 to 289 and/or 481 to 489;
 - 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: 249 to 289 and/or 481 to 489;
 - 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: 249 to 289 and/or 481 to 489;

and/or

- CDR3 is chosen from the group consisting of:

- g) the amino acid sequences of SEQ ID NO's: 331 to 371 and/or 499 to 507;
- 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: 331 to 371 and/or 499 to 507;
- 30 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: 331 to 371 and/or 499 to 507;

or any suitable fragment of such an amino acid sequence.

In particular, according to this preferred but non-limiting aspect, the invention relates to a Nanobody (as defined herein) against GPCRs, which 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: 167 to 207 and/or 463 to 471;
 - 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: 167 to 207 and/or 463 to 471;
 - 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: 167 to 207 and/or 463 to 471;

and

- 10 - CDR2 is chosen from the group consisting of:
 - d) the amino acid sequences of SEQ ID NO's: 249 to 289 and/or 481 to 489;
 - 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: 249 to 289 and/or 481 to 489;
 - 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: 249 to 289 and/or 481 to 489;

and

- CDR3 is chosen from the group consisting of:
 - g) the amino acid sequences of SEQ ID NO's: 331 to 371 and/or 499 to 507;
 - 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: 331 to 371 and/or 499 to 507;
 - 20 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: 331 to 371 and/or 499 to 507;

or any suitable fragment of such an amino acid sequences.

In a specific but non-limiting aspect, the invention relates to a Nanobody (as defined herein) against CB1R, which 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: 167 to 184, 463 and/or 464;
 - 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: 167 to 184, 463 and/or 464;
 - 30 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: 167 to 184, 463 and/or 464;

and/or

- CDR2 is chosen from the group consisting of:

- d) the amino acid sequences of SEQ ID NO's: 249 to 266 and/or 481 and/or 482;
- 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: 249 to 266 and/or 481 and/or 482;
- 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: 249 to 266 and/or 481 and/or 482;

and/or

- CDR3 is chosen from the group consisting of:

- g) the amino acid sequences of SEQ ID NO's: 331 to 348 and/or 499 and/or 500;
- 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: 331 to 348 and/or 499 and/or 500;
- 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: 331 to 348 and/or 499 and/or 500;

or any suitable fragment of such an amino acid sequence.

In particular, according to this preferred but non-limiting aspect, the invention relates to a Nanobody (as defined herein) against CB1R, which 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: 167 to 184, 463 and/or 464;
- 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: 167 to 184, 463 and/or 464;
- 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: 167 to 184, 463 and/or 464;

and

- CDR2 is chosen from the group consisting of:

- d) the amino acid sequences of SEQ ID NO's: 249 to 266 and/or 481 and/or 482;
- 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: 249 to 266 and/or 481 and/or 482;
- 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: 249 to 266 and/or 481 and/or 482;

and

- CDR3 is chosen from the group consisting of:

- g) the amino acid sequences of SEQ ID NO's: 331 to 348 and/or 499 and/or 500;

- 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: 331 to 348 and/or 499 and/or 500;
- 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: 331 to 348 and/or 499 and/or 500;

or any suitable fragment of such an amino acid sequences.

In a specific but non-limiting aspect, the invention relates to a Nanobody (as defined herein) against PTHR1, which 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:

- 10 a) the amino acid sequences of SEQ ID NO's: 185 to 207;
- 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: 185 to 207;
- 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: 185 to 207;

and/or

- CDR2 is chosen from the group consisting of:

- d) the amino acid sequences of SEQ ID NO's: 267 to 289;
- 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: 267 to 289;
- 20 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: 267 to 289;

and/or

- CDR3 is chosen from the group consisting of:

- g) the amino acid sequences of SEQ ID NO's: 349 to 371;
- 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: 349 to 371;
- 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: 349 to 371;

or any suitable fragment of such an amino acid sequence.

- 30 In particular, according to this preferred but non-limiting aspect, the invention relates to a Nanobody (as defined herein) against PTHR1, which 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: 185 to 207;
- 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: 185 to 207;
- 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: 185 to 207;

and

- CDR2 is chosen from the group consisting of:

- d) the amino acid sequences of SEQ ID NO's: 267 to 289;
- 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: 267 to 289;
- 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: 267 to 289;

and

- CDR3 is chosen from the group consisting of:

- g) the amino acid sequences of SEQ ID NO's: 349 to 371;
- 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: 349 to 371;
- 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: 349 to 371;

or any suitable fragment of such an amino acid sequences.

In a specific but non-limiting aspect, the invention relates to a Nanobody (as defined herein) against MC4R, which 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: 465 to 471;
- 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: 465 to 471;
- 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: 465 to 471;

and/or

- CDR2 is chosen from the group consisting of:

- d) the amino acid sequences of SEQ ID NO's: 483 to 489;
- 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: 483 to 489;

- 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: 483 to 489;

and/or

- CDR3 is chosen from the group consisting of:

- g) the amino acid sequences of SEQ ID NO's: 501 to 507;
- 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: 501 to 507;
- 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: 501 to 507;

10 or any suitable fragment of such an amino acid sequence.

In particular, according to this preferred but non-limiting aspect, the invention relates to a Nanobody (as defined herein) against MC4R, which 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: 465 to 471;
- 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: 465 to 471;
- 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: 465 to 471;

20

and

- CDR2 is chosen from the group consisting of:

- d) the amino acid sequences of SEQ ID NO's: 483 to 489;
- 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: 483 to 489;
- 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: 483 to 489;

and

- CDR3 is chosen from the group consisting of:

30

- g) the amino acid sequences of SEQ ID NO's: 501 to 507;
- 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: 501 to 507;
- 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: 501 to 507;

or any suitable fragment of such an amino acid sequences.

As generally mentioned herein for the amino acid sequences of the invention, when a Nanobody of the invention contains one or more CDR1 sequences according to b) and/or c) as defined in the above paragraphs:

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

and/or

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

and/or

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

Similarly, when a Nanobody of the invention contains one or more CDR2 sequences according to e) and/or f) as defined in the above paragraphs:

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

and/or

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

and/or

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

30 Also, similarly, when a Nanobody of the invention contains one or more CDR3 sequences according to h) and/or i) as defined in the above paragraphs:

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

and/or

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

and/or

iii) the CDR according to h) and/or i) may be a CDR that is derived from a CDR 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 three paragraphs generally apply to any Nanobody of the invention that comprises one or more CDR1 sequences, CDR2 sequences and/or CDR3 sequences according to b), c), e), f), h) or i), respectively, as defined in the above paragraphs.

Of the Nanobodies of the invention, Nanobodies comprising one or more of the CDR's explicitly listed above are particularly preferred; Nanobodies comprising two or more of the CDR's explicitly listed above are more particularly preferred; and Nanobodies comprising three of the CDR's explicitly listed above are most particularly preferred.

Some particularly preferred, but non-limiting combinations of CDR sequences, as well as preferred combinations of CDR sequences and framework sequences, are mentioned in Table A-1 below, which lists the CDR sequences and framework sequences that are present in a number of preferred (but non-limiting) Nanobodies of the invention. As will be clear to the skilled person, a combination of CDR1, CDR2 and CDR3 sequences that occur in the same clone (i.e. CDR1, CDR2 and CDR3 sequences that are mentioned on the same line in Table A-1) will usually be preferred (although the invention in its broadest sense is not limited thereto, and also comprises other suitable combinations of the CDR sequences mentioned in Table A-1). Also, a combination of CDR sequences and framework sequences that occur in the same clone (i.e. CDR sequences and framework sequences that are mentioned on the same line in Table A-1) will usually be preferred (although the invention in its broadest sense is not limited thereto, and also comprises other suitable combinations of the CDR sequences and framework sequences mentioned in Table A-1, as well as combinations of such CDR sequences and other suitable framework sequences, e.g. as further described herein).

Also, in the Nanobodies of the invention that comprise the combinations of CDR's mentioned in Table A-1, 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:

- i) any amino acid substitution in such a CDR is preferably, and compared to the corresponding CDR sequence mentioned in Table A-1, a conservative amino acid substitution (as defined herein);

and/or

- ii) any such CDR sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the corresponding CDR sequence mentioned in Table A-1;

and/or

- 10 iii) any such CDR sequence is a CDR that is derived by means of a technique for affinity maturation known per se, and in particular starting from the corresponding CDR sequence mentioned in Table A-1.

However, as will be clear to the skilled person, the (combinations of) CDR sequences, as well as (the combinations of) CDR sequences and framework sequences mentioned in Table A-1 will generally be preferred.

Table A-1 CDR's and framework sequences of Nanobodies against three representative GPCRs (CB1R, PTHR1 and MC4R)

Clones against CB1R	S E Q	FR1	S E Q	CDR1	S E Q	FR2	S E Q	CDR2	S E Q	FR3	S E Q	CDR3	S E Q	FR4
LGPM P5_A6	1	EVQLVESGGG	1		2	WYRQAPG	2	VITRDGITYADSV	2	RFTISRDNAKNTMYL	3	GLPTGRG	3	WGQGTQV
	2	LVQAGGSLRLA	6		0	KQRELLA	4	KG	9	QMNSLKPEDTAVYY	3	SHSDY	7	TVSS
	6	CAASGSIFS	7	FNAMG	8				0	CHT	1		2	
LGPM P5_A7	1	EVQLVESGGG	1		2	WFRQAPGK	2	TIRWSTSTYYAD	2	RFTISSDNTKNTVDL	3	RSVYSYE	3	WGQGTQV
	2	LVQAGGSLRLS	6		0	EREFVA	5	SVKG	9	RMNSLTPEDTAVYY	3	YNY	7	TVSS
	7	CAASGRTL	8	NYDMG	9		0		1	CAA	2		3	
LGPM P5_B6	1	EVQLVESGGG	1		2	WFRQAPGK	2	TIRWSTSETYYAD	2	RFTISKDNTKNTVAL	3	RSVYSYE	3	WGQGTQV
	2	LVQAGGSLRLS	6		1	EREFVA	5	SVKG	9	QMNSLTPEDTALYY	3	YNY	7	TVSS
	8	CAASGRTL	9	NYDMG	0		1		2	CAA	3		4	
LGPM P5_B9	1	EVQLVESGGG	1		2	WFRQAPGK	2	TVRWGTSSYYAD	2	RFTISSDNTKNTVAL	3	RSVYSYE	3	WGQGTQV
	2	LVQAGGSLRLS	7		1	EREFVA	5	SVKG	9	QMNRSLTPEDTGVYY	3	YTY	7	TVSS
	9	CAASGRTL	0	NHDMG	1		2		3	CAA	4		5	
LGPM P5_D1 2	1	EVQLVESGGG	1		2	WFRQAPGK	2	AIYGTGGELVYYE	2	RFTISRDNAKSTMYL	3	ELTVRSID	3	WGQGTQV
	3	LVQAGGSLRLS	7		1	EREFVA	5	NSVKG	9	QMNSLKPEDTGVYY	3	LRRPLEY	7	TVSS
	0	CAASRRFTS	1	SFVMA	2		4		4	CAV	5	DY	6	
LGPM P5_F9	1	EVQLVESGGG	1		2	WFRQAPGK	2	TIRWSTSETYYAD	2	RFTISSDNTKNTVDL	3	RSVYSYE	3	WGQGTQV
	3	LVQAGGSLRLS	7		1	EREFVA	5	SVKG	9	QMNSLTPEDTAVYY	3	YNY	7	TVSS
	1	CAASGRTL	2	NYDMG	3		4		5	CAA	6		7	
LGPM P5_G1 1	1	EVQLVKSGGG	1		2	WFRQAPGK	2	TIRWSTGETYYTD	2	RFTISKDNTKNTVDL	3	RSVYSYE	3	WGQGTQV
	3	LVQAGGSLRLS	7		1	EREFVA	5	SVKG	9	QMNSLTPEDTAVYY	3	YNY	7	TVSS
	2	CAASGRTL	3	NYDMG	4		5		6	CAS	7		8	
LGPM P6_B7	1	EVQLVESGGG	1		2	WFRQAPGK	2	TIRWSTSETYYAD	2	RFTISSDNAKNTVDL	3	RSVYSYD	3	WGQGTQV
	3	LVQAGGSLRLS	7		1	EREFVA	5	SVKG	9	QMNSLTPGDTGVYY	3	YNY	7	TVSS
	3	CAASGRTL	4	NYDMG	5		6		7	CAA	8		9	
LGPM P6_C7	1	EVQLVESGGG	1		2	WFRQAPGK	2	TIRWSTSSYYAD	2	RFTISSDNIKNTVDL	3	RSVYSYE	3	WGQGTQV
	3	LVQAGGSLRLS	7		1	EREFVA	5	PVKG	9	QMNSLTPEDTAVYY	3	YNY	8	TVSS
	4	CAASGRTL	5	NYDVG	6		7		8	CAA	9		0	
LGPM P6_C9	1	EVQLVESGGG	1		2	WFRQAPGK	2	TIRWSTSETYYAD	2	RFTISSNTKNTVDL	3	RSVYSYE	3	WGQGTQV
	3	LVQAGGSLRLS	7		1	EREFVA	5	SVKG	9	QMNSLTPEDTAVYY	4	YNY	8	TVSS
	5	CAASGRTL	6	NYDMG	7		8		9	CAA	0		1	
LGPM P6_D2	1	EVQLMESGGG	1		2	WFRQAPGK	2	TIRWSTSETYYAD	3	RFTISKDNTKNTVAL	3	RSVYSYE	3	WGQGTQV
	3	LVQAGGSLRLS	7		1	EREFVA	5	SVKG	0	QMNSLTPEDTALYY	4	YNY	8	TVSS
	6	CAASGRTL	7	NYDMG	8		9		0	CAA	1		2	

Table A-1 (continued):

Clones against CB1R	S E Q	FR1	S E Q	CDR1	S E Q	FR2	S E Q	CDR2	S E Q	FR3	S E Q	CDR3	S E Q	FR4
LGPM P6_D5	1 3 7	EVQLVESGGG LEQAGGSLRLS CAVSRSEIFE	1 7 8		2 1 9	WFRQAPGK QRELVA	2 6 0	RISSTGDTNYADS VKG	3 0 1	RFTISRDNAKNTVYL QMNSLKPEDTAVYY CHV	3 4 2	AGPYGSK FA	3 8 3	WGQGTQV TVSS
LGPM P6_D1 2	1 3 8	EVQLVESGGG LVQAGGSLRLS CAASGRTL	1 7 9		2 2 0	WFRQAPGK EREFVA	2 6 1	TMRWSTGSTYYA DSVKG	3 0 2	RFTISSDNTKNTVDL QMNSLTPEDTAVYY CAA	3 4 3	RSVYSYE YNY	3 8 4	WGQGTQV TVSS
LGPM P6_E1 1	1 3 9	EVQLVESGGG LVQAGGSLRLS CAASGRTL	1 8 0		2 2 1	WFRQAPGK EREFVA	2 6 2	TIRWSTSETYYAD SVKG	3 0 3	RFTISSDNTKNTVDL QMNSLTPEDTGVYY CAA	3 4 4	RSVYSYD YNY	3 8 5	WGQGTQV TVSS
LGPM P6_F1 2	1 4 0	EVQLVESGGG LVQAGGSLRLS CAASGRTL	1 8 1		2 2 2	WFRQAPGK EREFVA	2 6 3	TIRWGTTDTYYAD SVKG	3 0 4	RFTISSDNTKNTVHL QMNSLTPEDTGVYY CAA	3 4 5	RSVYSYE YTY	3 8 6	WGQGTQV TVSS
LGPM P6_H4	1 4 1	EVQLVESGGG LVQAGGSLRLA CAASGSIFS	1 8 2		2 2 3	WYRQAPG KQRELLA	2 6 4	VITRDGITKYADSV KG	3 0 5	RFTISRDNAKNTMYL QMNSLKPEDTAVYY CHT	3 4 6	GLPTGRG SHSDY	3 8 7	WGQGTQV TVSS
LGPM P6_H9	1 4 2	EVQLVESGGG LVQAGGSLRLS CAASGRTL	1 8 3		2 2 4	WFRQAPGK EREFVA	2 6 5	TIRWSSKSTYYAD SVKG	3 0 6	RFTISSDNTKNTVDL RMNSLTPEDTAVYY CAA	3 4 7	RSVYSYE YNY	3 8 8	WGQGTQV TVSS
LGPM P6_H1 2	1 4 3	EVQLVESGGG LVQAGGSLRLS CAASGRTL	1 8 4		2 2 5	WFRQAPGK EREFVA	2 6 6	TIRWSTRETYT SVKG	3 0 7	RFTISSDNTKNTVHL QMNSLTPEDTAVYY CAA	3 4 8	RSVYSYE YNY	3 8 9	WGQGTQV TVSS
6F12	4 5 4	EVQLVESGG GLVQAGGSL RLSCAASGR TLS	4 6 3		4 7 2	WFRQAPG KEREFVA	4 8 1	TMRWGTSSSTYY ADSVKG	4 9 0	RFTISSDNTKNTV YLMNSLTPEDTA VYYCAA	4 9 9	RSVYSY EYTY	5 0 8	WGQGTQ VTVSS
5D12	4 5 5	EVQLVESGG GLVQAGGSL RLSCTASERT FS	4 6 4		4 7 3	WFRQAPG KERVFA	4 8 2	VIDWSGGLAYYA DSVKG	4 9 1	RFTISRDNAKKT YLMNSLKPEDTA VYYCAA	4 9 0	RSVYRG STKPYE YDY	5 0 9	WGQGTQ VTVSS

Clones against PTHR1	S E Q	FR1	S E Q	CDR1	S E Q	FR2	S E Q	CDR2	S E Q	FR3	S E Q	CDR3	S E Q	FR4
PMPL G22- A2	1 4 4	EVQLVESGGG LVQAGGSLRLS CSSSGFTST	1 8 5		2 2 6	WFRQAPGK ARTGVS	2 6 7	IISRDGGSTWYAD SVKG	2 6 7	RFTISSDNAKNTVYL QMNSLKPEDTAVY CAA	3 4 9	QLRHNFG MGDYDY	3 9 0	WGQGTQV TVSS
PMPL G22- A3	1 4 5	EVQLVESGGG LVQAGGSLRLS CAASGNIFA	1 8 6		2 2 7	WYRQPPG KREFVA	2 6 8	HVSHDGDSDMYAV SVKG	2 6 8	RFAISRKDATNLYLQ MNSLKPEDTAIFCR	3 5 0	LLNIPTQG RMEG	3 9 1	WGQGTQV TVSS
PMPL G22- A9	1 4 6	EVQLVESGGG LVQPGGSLRLS CAASGFTLD	1 8 7		2 2 8	WFRQAPGK EREGVS	2 6 9	CSNKDGGSTAYSDS VKG	2 6 9	RFTISRDNKNTVYL EMNGLKPEDAAIYY CAT	3 5 1	KSGRYCL SHMDY	3 9 2	WGIGTLVT VSS
PMPL G22- B7	1 4 7	EVQLVESGGG LVQTGGSLRLS CAASGMTFR	1 8 8		2 2 9	WYRQAPG ERREVA	2 7 0	TITIDGKTNYAEPL KG	2 7 0	RFTISRDNKNTIYL QMNSLKPEDTAVY CNA	3 5 2	VFSRGPL TY	3 9 3	WGRGTQVT VSS
PMPL G22- B11	1 4 8	EVQLVESGGG LVQAGGSLRLS CAASRSRSS	1 8 9		2 3 0	WYRQAPG KQREFVA	2 7 1	GITLGGSTIYADSV KG	2 7 1	RFTISMDSARNTGY LQMNSLKPEDTAVY YCNA	3 5 3	ALDGYSG SS	3 9 4	WGQGTQV TVSS
PMPL G22- B12	1 4 9	EVQLVESGGG LVQAGGSLRLS CVASGNIFS	1 9 0		2 3 1	WYRQAPG KQREFVA	2 7 2	HMSHDGTTNYED SVKG	2 7 2	RFTISRENAKNTLYL HMNSLKPEDTAIYY CRL	3 5 4	LTIPTTGR AEGF	3 9 5	WGQGTQV TVSS
PMPL G22- C3	1 5 0	EVQLVESGGG LVQAGGSLRLS CAASRSRSS	1 9 1		2 3 2	WYRQAPG KQREFVA	2 7 3	GIPVGGSTVYADS VKG	2 7 3	RFTISMDSARNRGY LQMNSLKPEDTAVY YCNA	3 5 5	YLDGYSG SS	3 9 6	WGQGTQV TVSS
PMPL G23- A3	1 5 1	EVQLVESGGG LVQAGGSLRLS CAASGLTFS	1 9 2		2 3 3	WFRQAPGK EREWVA	2 7 4	SINWSGGSTYYED SVEG	2 7 4	RFTISRDNKNTVNL QMNSLKPEDTAVY CAA	3 5 6	KRGHYSR EYDY	3 9 7	WGQGTQV TVSS
PMPL G23- C1	1 5 2	KVQLVESGGG LVQAGGSLRLS CAASASIIS	1 9 3		2 3 4	WYRQAPG KRREFVA	2 7 5	GILSDGRELYVDS VKG	2 7 5	RFTVSRDNKNTVY LQMNSLKPEDTAVY YCNL	3 5 7	DIDYRDY	3 9 8	WGQGTQV TVSS
PMPL G23- E12	1 5 3	EVQLVESGGG LVQAGGSLRLS CAASASIIS	1 9 4		2 3 5	WYRQAPG RRREFVA	2 7 6	GILSDGRELYVDS VKG	2 7 6	RFTVSRDNKNTVY LQMNSLKPEDTAVY YCNL	3 5 8	DIDYRDY	3 9 9	WGQGTQV TVSS
PMPL G23- F2	1 5 4	KVQLVESGGG LVQAGGSLRLS CAASGSIFS	1 9 5		2 3 6	WYRQAPG KQREFVA	2 7 7	HIYSDGSINYGNSV KG	2 7 7	RFTISRDNKNTVDL QMNNLKPEDTAVY CRL	3 5 9	LDTARGV GY	4 0 0	WGQGTQV TVSS
PMPL G23- H9	1 5 5	EVQLVESGGG LVQAGGSLRLS CAASASIIS	1 9 6		2 3 7	WYRQAPG KRREFVA	2 7 8	GILSDGRELYVDS VKG	2 7 8	RFTVSRDNKNTVY LQMNSLKPEDTAVY YCNL	3 6 9	DIDYRDY	4 0 1	WGQGTQV TVSS

Table A-1 (continued):

Clones against PTHR1	S E Q	FR1	S E Q	CDR1	S E Q	FR2	S E Q	CDR2	S E Q	FR3	S E Q	CDR3	S E Q	FR4
PMPL G26- A2	1 5 6	EVQLVESGGG LVQAGGALRLS CTASGITFK	1 9 7		2 3 8	WYRQAPG KEREWVT	2 7 9	TITSEGTANSADSV KG	3 2 0	RFTISRDNAKNTVYL QMNSLKSEDTAVYY CNA	3 6 1	QFTLARH LVY	4 0 2	WGQGTQV TVSS
PMPL G26- A6	1 5 7	EVQLVESGGG LVQAGGSLRLS CAASRSRSS	1 9 8		2 3 9	WYRQAPG KQREFVG	2 8 0	GITLGGSTIYADSV KG	3 2 1	RFTISMDSARNTGY LQMNSLKPEDTAVY YCNA	3 6 2	ALDGYSG SS	4 0 3	WGQGTQV TVSS
PMPL G26- B6	1 5 8	EVQLVESGGG LVQAGGSLRLS CAASRSRSS	1 9 9		2 4 0	WYRQAPG KQREFVA	2 8 1	GITLGGSTIYADSV KG	3 2 2	RFTISMDSARNTGY LQMSSLKPEDTAVY YCNA	3 6 3	VLDGYSG SS	4 0 4	WGQGTQV TVSS
PMPL G26- C4	1 5 9	EVQLVESGGG LVQAGGSLRLS CAASGNIF	2 0 0		2 4 1	WYRQPPG KEREFVA	2 8 2	HVSHDGYSMYAV SVEG	3 2 3	RFTISRENATNLYLQ MNSLKPEDTAIFYCR	3 6 4	LLTIPTQG RMEGF	4 0 5	WGQGTQV TVSS
PMPL G26- C5	1 6 0	EVQLVESGGG LVQAGGSLRLS CAASGNIFS	2 0 1		2 4 2	WYRQAPG KQREFVA	2 8 3	HMSRDGTSNYED SVKG	3 2 4	RFTISRENAKNTLYL QMNSLKPEDTAIYY CRL	3 6 5	LTIPTTGR KEGF	4 0 6	WGQGTQV TVSS
PMPL G26- D12	1 6 1	EVQLVESGGG LVQAGGSLRLS CAASGSISN	2 0 2		2 4 3	WQRELVAI KFSGGT	2 8 4		3 2 5	RFTISRDNAKNTVYL QMNSLRPEDTAVYY CRL	3 6 6	FLPSLAM GY	4 0 7	WGQGTQV TVSS
PMPL G26- E4	1 6 2	EVQLVESGGG LVQAGGSLRLS CAASGNIF	2 0 3		2 4 4	WYRQPPG KEREFVA	2 8 5	HVSHDGDSDMYAV SVK	3 2 6	GRFTISRKDATNLYL QMNSLKPEDTAIFY CR	3 6 7	LLNIPTQG RMEGF	4 0 8	WGQGTQV TVSS
PMPL G26- E6	1 6 3	EVQLVESGGG LVQAGGSLRLS CAASGNIF	2 0 4		2 4 5	WYRQPPG KEREFVA	2 8 6	HVSDDDGDSDMYAV SVK	3 2 7	GRFTISRKNATNLYL QMNSLKPEDTAIFY CR	3 6 8	LLNIPTQG RMEGF	4 0 9	WGQGTQV TVSS
PMPL G26- F2	1 6 4	EVQLVESGGG LVQAGGSLRLS CVASGFSD	2 0 5		2 4 6	WFRQAPG QDREPLA	2 8 7	CITSGGTRNYPNS VMA	3 2 8	RFRIDRDNVSKTVYL QINTLKPEDTAVYYC ST	3 6 9	ELDRQGG SAHECRE YDT	4 1 0	WGQGTQV TVSS
PMPL G26- G11	1 6 5	EVQLVESGGG LVQAGGSLRLS CAASESIS	2 0 6		2 4 7	WYRQGP KLRELA	2 8 8	QITDLHKDYAES A KG	3 2 9	RFTISRDNAKNTVDL QMNSLKPEDTGVYY CAA	3 7 0	FRGIMRP DY	4 1 1	WGQGTQV TVSS
PMPL G26- H11	1 6 6	EVQLVESGGG LVQAGKSLSL CAASDSRFS	2 0 7		2 4 8	WYRQAPG KQREWVT	2 8 9	TLLSTGSPYADSV KG	3 3 0	RFTISRDKHAKNTVYL QMHSKLPDDTANYI CFA	3 7 1	RLTPTGT V	4 1 2	WGQGTQV TVSS

Table A-1 (continued):

Clones against MC4R	S E Q	FR1	S E Q	CDR1	S E Q	FR2	S E Q	CDR2	S E Q	FR3	S E Q	CDR3	S E Q	FR4		
60-11B		EVQLVESGG GLVQPGGSL RLSCAASGFT LD	4 6 5				4 7 4	WFRQAPG KEREGVS	4 8 3	CISSSSSDTTY ADSVRG	4 9 2	RFSISRDSAKNTV YLQMNSLKPEDTA VYYCAA	5 0 1	DRHCLG SDWDEY DY	5 1 0	WGQGTQ VTVSS
	59-8A	EVQLVESGG GLVHAGDSL RLSCAASGFT LD	4 6 6				4 7 5	WFRQAPG KAREGLS	4 8 4	CISAEDGSTYYA DSVKG	4 9 3	RFIISRDNAKNTVY LQMNSLKPEDTAV YYCAA	5 0 2	EDSCLS SDYDAY DG	5 1 1	WGQGTQ VTVSS
		58-4H	EVQLVESGG GLAQPGGSL RLSCVASGF SLA	4 6 7				4 7 6	WFRQAPG KVREGVS	4 8 5	CISSSDGSTYYA DSVKG	4 9 4	RFTISRDNNAKNAV YLQMSTLKPEDTA VYYCAA	5 0 3	ERSCLS SDYDSY DA	5 1 2
60-5E		EVQLVESGG GLVQPGGSL RLSCAASGFT LD	4 6 8				4 7 7	WFRQAPG KEREGVS	4 8 6	CISSSDGSTYYA DSVKG	4 9 5	RFTISRDNNAKNTV YLQMNSLKPEDTA VYYCAA	5 0 4	DLGYGS NCLEYD F	5 1 3	WGQGTQ VTVSS
	60-4A	EVQLVESGG GLVQAGGSL RLSCAASGFT FD	4 6 9				4 7 8	WFRQAPG KEREGVS	4 8 7	CISRSDGSTYYA PSVKG	4 9 6	RFTISSDNAKNTV YLQMNSLKPEDTA VYYCAV	5 0 5	DLEYGS NCYEYD S	5 1 4	WGQGTQ VTVSS
58-10H		EVQLVESGG GLVQPGGSM RLSCAASGS SLD	4 7 0				4 7 9	WFRQAPG KEREGVS	4 8 8	CISGSDNSTYYA DSVKG	4 9 7	RFSISRDIKNTVY LQMNGLKPEDTA VYYCAA	5 0 6	KFLGFR QRSPTW FSRSWS EGSDYQ Y	5 1 5	WGQGTQ VTVSS
	60-10F	EVQLVESGG GLVQPGGSL RLSCAASGFT LD	4 7 1				4 8 0	WFRQAPG KEREGVS	4 8 9	CITSSDGSTYYA DSVKG	4 9 8	RFTISRDNNAKNTV YLQINSLKPEDTA VYYCAA	5 0 7	DLGVGT QCDEYD Y	5 1 6	WGQGTQ VTVSS

Thus, in the Nanobodies of the invention, at least one of the CDR1, CDR2 and CDR3 sequences present is suitably chosen from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table A-1; or from the group of CDR1, CDR2 and CDR3 sequences, respectively, 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 the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table A-1; and/or from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, that have 3, 2 or only 1 “amino acid difference(s)” (as defined herein) with at least one of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table A-1.

10 In this context, by “suitably chosen” is meant that, as applicable, a CDR1 sequence is chosen from suitable CDR1 sequences (i.e. as defined herein), a CDR2 sequence is chosen from suitable CDR2 sequences (i.e. as defined herein), and a CDR3 sequence is chosen from suitable CDR3 sequence (i.e. as defined herein), respectively. More in particular, the CDR sequences are preferably chosen such that the Nanobodies of the invention bind to GPCRs 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.

In particular, in the Nanobodies of the invention, at least the CDR3 sequence present is suitably chosen from the group consisting of the CDR3 sequences listed in Table A-1 or from
20 the group of CDR3 sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the CDR3 sequences listed in Table A-1; and/or from the group consisting of the CDR3 sequences that have 3, 2 or only 1 amino acid difference(s) with at least one of the CDR3 sequences listed in Table A-1.

Preferably, in the Nanobodies of the invention, at least two of the CDR1, CDR2 and CDR3 sequences present are suitably chosen from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table A-1 or from the group consisting of CDR1, CDR2 and CDR3 sequences, respectively, that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at
30 least one of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table A-1; and/or from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, that have 3, 2 or only 1 “amino acid difference(s)” with at least one of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table A-1.

In particular, in the Nanobodies of the invention, at least the CDR3 sequence present is suitably chosen from the group consisting of the CDR3 sequences listed in Table A-1 or from the group of CDR3 sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the CDR3 sequences listed in Table A-1, respectively; and at least one of the CDR1 and CDR2 sequences present is suitably chosen from the group consisting of the CDR1 and CDR2 sequences, respectively, listed in Table A-1 or from the group of CDR1 and CDR2 sequences, respectively, that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the CDR1 and CDR2 sequences, respectively, listed in Table A-1; and/or from the group consisting of the CDR1 and CDR2 sequences, respectively, that have 3, 2 or only 1 amino acid difference(s) with at least one of the CDR1 and CDR2 sequences, respectively, listed in Table A-1.

Most preferably, in the Nanobodies of the invention, all three CDR1, CDR2 and CDR3 sequences present are suitably chosen from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table A-1 or from the group of CDR1, CDR2 and CDR3 sequences, respectively, that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table A-1; and/or from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, that have 3, 2 or only 1 amino acid difference(s) with at least one of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table A-1.

Even more preferably, in the Nanobodies of the invention, at least one of the CDR1, CDR2 and CDR3 sequences present is suitably chosen from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table A-1. Preferably, in this aspect, at least one or preferably both of the other two CDR sequences present are suitably chosen from CDR sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the corresponding CDR sequences, respectively, listed in Table A-1; and/or from the group consisting of the CDR sequences that have 3, 2 or only 1 amino acid difference(s) with at least one of the corresponding sequences, respectively, listed in Table A-1.

In particular, in the Nanobodies of the invention, at least the CDR3 sequence present is suitably chosen from the group consisting of the CDR3 listed in Table A-1. Preferably, in this aspect, at least one and preferably both of the CDR1 and CDR2 sequences present are suitably chosen from the groups of CDR1 and CDR2 sequences, respectively, that have at least 80%,

preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the CDR1 and CDR2 sequences, respectively, listed in Table A-1; and/or from the group consisting of the CDR1 and CDR2 sequences, respectively, that have 3, 2 or only 1 amino acid difference(s) with at least one of the CDR1 and CDR2 sequences, respectively, listed in Table A-1.

Even more preferably, in the Nanobodies of the invention, at least two of the CDR1, CDR2 and CDR3 sequences present are suitably chosen from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table A-1. Preferably, in this aspect, the remaining CDR sequence present is suitably chosen from the group of CDR
10 sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the corresponding CDR sequences listed in Table A-1; and/or from the group consisting of CDR sequences that have 3, 2 or only 1 amino acid difference(s) with at least one of the corresponding sequences listed in Table A-1.

In particular, in the Nanobodies of the invention, at least the CDR3 sequence is suitably chosen from the group consisting of the CDR3 sequences listed in Table A-1, and either the CDR1 sequence or the CDR2 sequence is suitably chosen from the group consisting of the CDR1 and CDR2 sequences, respectively, listed in Table A-1. Preferably, in this aspect, the remaining CDR sequence present is suitably chosen from the group of CDR
20 sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the corresponding CDR sequences listed in Table A-1; and/or from the group consisting of CDR sequences that have 3, 2 or only 1 amino acid difference(s) with the corresponding CDR sequences listed in Table A-1.

Even more preferably, in the Nanobodies of the invention, all three CDR1, CDR2 and CDR3 sequences present are suitably chosen from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table A-1.

Also, generally, the combinations of CDR's listed in Table A-1 (i.e. those mentioned on the same line in Table A-1) are preferred. Thus, it is generally preferred that, when a CDR
30 in a Nanobody of the invention is a CDR sequence mentioned in Table A-1 or is suitably chosen from the group of CDR sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with a CDR sequence listed in Table A-1; and/or from the group consisting of CDR sequences that have 3, 2 or only 1 amino acid difference(s) with a CDR sequence listed in Table A-1, that at

least one and preferably both of the other CDR's are suitably chosen from the CDR sequences that belong to the same combination in Table A-1 (i.e. mentioned on the same line in Table A-1) or are suitably chosen from the group of CDR sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the CDR sequence(s) belonging to the same combination and/or from the group consisting of CDR sequences that have 3, 2 or only 1 amino acid difference(s) with the CDR sequence(s) belonging to the same combination. The other preferences indicated in the above paragraphs also apply to the combinations of CDR's mentioned in Table A-1.

Thus, by means of non-limiting examples, a Nanobody of the invention can for example comprise a CDR1 sequence that has more than 80 % sequence identity with one of the CDR1 sequences mentioned in Table A-1, a CDR2 sequence that has 3, 2 or 1 amino acid difference with one of the CDR2 sequences mentioned in Table A-1 (but belonging to a different combination), and a CDR3 sequence.

Some preferred Nanobodies of the invention may for example comprise: (1) a CDR1 sequence that has more than 80 % sequence identity with one of the CDR1 sequences mentioned in Table A-1; a CDR2 sequence that has 3, 2 or 1 amino acid difference with one of the CDR2 sequences mentioned in Table A-1 (but belonging to a different combination); and a CDR3 sequence that has more than 80 % sequence identity with one of the CDR3 sequences mentioned in Table A-1 (but belonging to a different combination); or (2) a CDR1 sequence that has more than 80 % sequence identity with one of the CDR1 sequences mentioned in Table A-1; a CDR2 sequence, and one of the CDR3 sequences listed in Table A-1; or (3) a CDR1 sequence; a CDR2 sequence that has more than 80% sequence identity with one of the CDR2 sequence listed in Table A-1; and a CDR3 sequence that has 3, 2 or 1 amino acid differences with the CDR3 sequence mentioned in Table A-1 that belongs to the same combination as the CDR2 sequence.

Some particularly preferred Nanobodies of the invention may for example comprise: (1) a CDR1 sequence that has more than 80 % sequence identity with one of the CDR1 sequences mentioned in Table A-1; a CDR2 sequence that has 3, 2 or 1 amino acid difference with the CDR2 sequence mentioned in Table A-1 that belongs to the same combination; and a CDR3 sequence that has more than 80 % sequence identity with the CDR3 sequence mentioned in Table A-1 that belongs to the same combination; (2) a CDR1 sequence; a CDR 2 listed in Table A-1 and a CDR3 sequence listed in Table A-1 (in which the CDR2 sequence and CDR3 sequence may belong to different combinations).

Some even more preferred Nanobodies of the invention may for example comprise:

(1) a CDR1 sequence that has more than 80 % sequence identity with one of the CDR1 sequences mentioned in Table A-1; the CDR2 sequence listed in Table A-1 that belongs to the same combination; and a CDR3 sequence mentioned in Table A-1 that belongs to a different combination; or (2) a CDR1 sequence mentioned in Table A-1; a CDR2 sequence that has 3, 2 or 1 amino acid differences with the CDR2 sequence mentioned in Table A-1 that belongs to the same combination; and a CDR3 sequence that has more than 80% sequence identity with the CDR3 sequence listed in Table A-1 that belongs to the same or a different combination.

10 Particularly preferred Nanobodies of the invention may for example comprise a CDR1 sequence mentioned in Table A-1, a CDR2 sequence that has more than 80 % sequence identity with the CDR2 sequence mentioned in Table A-1 that belongs to the same combination; and the CDR3 sequence mentioned in Table A-1 that belongs to the same combination.

In the most preferred Nanobodies of the invention, the CDR1, CDR2 and CDR3 sequences present are suitably chosen from one of the combinations of CDR1, CDR2 and CDR3 sequences, respectively, listed in Table A-1.

20 According to another preferred, but non-limiting aspect of the invention (a) CDR1 has a length of between 1 and 12 amino acid residues, and usually between 2 and 9 amino acid residues, such as 5, 6 or 7 amino acid residues; and/or (b) CDR2 has a length of between 13 and 24 amino acid residues, and usually between 15 and 21 amino acid residues, such as 16 and 17 amino acid residues; and/or (c) CDR3 has a length of between 2 and 35 amino acid residues, and usually between 3 and 30 amino acid residues, such as between 6 and 23 amino acid residues.

In another preferred, but non-limiting aspect, the invention relates to a Nanobody in which the CDR sequences (as defined herein) have more than 80%, preferably more than 90%, more preferably more than 95%, such as 99% or more sequence identity (as defined herein) with the CDR sequences of at least one of the amino acid sequences of SEQ ID NO's: 413 to 453 and 517 to 525.

30 Generally, Nanobodies with the above CDR sequences may be as further described herein, and preferably have framework sequences that are also as further described herein. Thus, for example and as mentioned herein, such Nanobodies may be naturally occurring Nanobodies (from any suitable species), naturally occurring V_{HH} sequences (i.e. from a suitable species of Camelid) or synthetic or semi-synthetic amino acid sequences or

Nanobodies, including but not limited to partially humanized Nanobodies or V_{HH} sequences, fully humanized Nanobodies or V_{HH} sequences, camelized heavy chain variable domain sequences, as well as Nanobodies that have been obtained by the techniques mentioned herein.

Thus, in one specific, but non-limiting aspect, the invention relates to a humanized Nanobody, which consists of 4 framework regions (FR1 to FR4 respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively), in which CDR1 to CDR3 are as defined herein and in which said humanized Nanobody comprises at least one humanizing substitution (as defined herein), and in particular at least one humanizing substitution in at least one of its framework sequences (as defined herein).

In another preferred, but non-limiting aspect, the invention relates to a Nanobody 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: 413 to 453 and 517 to 525. 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 Nanobody and one or more of the sequences of SEQ ID NO's: 413 to 453 and 517 to 525, in which the amino acid residues that form the framework regions are disregarded. Such Nanobodies can be as further described herein.

In another preferred, but non-limiting aspect, the invention relates to a Nanobody with an amino acid sequence that is chosen from the group consisting of SEQ ID NO's: 413 to 453 and 517 to 525 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: 413 to 453 and 517 to 525.

Another preferred, but non-limiting aspect of the invention relates to humanized variants of the Nanobodies of SEQ ID NO's: 413 to 453 and 517 to 525, that comprise, compared to the corresponding native V_{HH} sequence, at least one humanizing substitution (as defined herein), and in particular at least one humanizing substitution in at least one of its framework sequences (as defined herein).

The polypeptides of the invention comprise or essentially consist of at least one Nanobody of the invention.

It will be clear to the skilled person that the Nanobodies that are mentioned herein as “preferred” (or “more preferred”, “even more preferred”, etc.) are also preferred (or more preferred, or even more preferred, etc.) for use in the polypeptides described herein. Thus, polypeptides that comprise or essentially consist of one or more “preferred” Nanobodies of the invention will generally be preferred, and polypeptides that comprise or essentially consist of one or more “more preferred” Nanobodies of the invention will generally be more preferred, etc..

Generally, proteins or polypeptides that comprise or essentially consist of a single Nanobody (such as a single Nanobody of the invention) will be referred to herein as “monovalent” proteins or polypeptides or as “monovalent constructs”. Proteins and polypeptides that comprise or essentially consist of two or more Nanobodies (such as at least two Nanobodies of the invention or at least one Nanobody of the invention and at least one other Nanobody) will be referred to herein as “multivalent” proteins or polypeptides or as “multivalent constructs”, and these may provide certain advantages compared to the corresponding monovalent Nanobodies of the invention. Some non-limiting examples of such multivalent constructs will become clear from the further description herein.

According to one specific, but non-limiting aspect, a polypeptide of the invention comprises or essentially consists of at least two Nanobodies of the invention, such as two or three Nanobodies of the invention. As further described herein, such multivalent constructs can provide certain advantages compared to a protein or polypeptide comprising or essentially consisting of a single Nanobody of the invention, such as a much improved avidity for GPCRs. Such multivalent constructs will be clear to the skilled person based on the disclosure herein.

According to another specific, but non-limiting aspect, a polypeptide of the invention comprises or essentially consists of at least one Nanobody of the invention and at least one other binding unit (i.e. directed against another epitope, antigen, target, protein or polypeptide), which is preferably also a Nanobody. Such proteins or polypeptides are also referred to herein as “multispecific” proteins or polypeptides or as “multispecific constructs”, and these may provide certain advantages compared to the corresponding monovalent Nanobodies of the invention (as will become clear from the further discussion herein of some preferred, but-nonlimiting multispecific constructs). Such multispecific constructs will be clear to the skilled person based on the disclosure herein.

According to yet another specific, but non-limiting aspect, a polypeptide of the invention comprises or essentially consists of at least one Nanobody of the invention,

optionally one or more further Nanobodies, and at least one other amino acid sequence (such as a protein or polypeptide) that confers at least one desired property to the Nanobody of the invention and/or to the resulting fusion protein. Again, such fusion proteins may provide certain advantages compared to the corresponding monovalent Nanobodies of the invention. Some non-limiting examples of such amino acid sequences and of such fusion constructs will become clear from the further description herein.

It is also possible to combine two or more of the above aspects, for example to provide a trivalent bispecific construct comprising two Nanobodies of the invention and one other Nanobody, and optionally one or more other amino acid sequences. Further non-limiting
10 examples of such constructs, as well as some constructs that are particularly preferred within the context of the present invention, will become clear from the further description herein.

In the above constructs, the one or more Nanobodies and/or other amino acid sequences may be directly linked to each other and/or suitably linked to each other via one or more linker sequences. Some suitable but non-limiting examples of such linkers will become clear from the further description herein.

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
20 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, see for example EP 0 368 684 B1, page 4); 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
30 for example include, without limitation, polypeptides in which the one or more Nanobodies of the invention are suitably 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

transferrin); 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 and to the US provisional application of Ablynx N.V. entitled "*Peptides capable of binding to serum proteins*" of Ablynx N.V. filed on December 5, 2006 (see also PCT/EP/2007/063348).

Again, as will be clear to the skilled person, such Nanobodies, compounds, constructs or polypeptides may contain one or more additional groups, residues, moieties or binding units, such as one or more further amino acid sequences and in particular one or more additional Nanobodies (i.e. not directed against GPCRs), so as to provide a tri- of multispecific Nanobody construct.

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 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 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

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, polypeptides comprising one or more Nanobodies of the invention are preferably such that they:

- bind to GPCRs 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 GPCRs 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 GPCRs 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 polypeptide that contains only one amino acid sequence of the invention is preferably such that it will bind to GPCRs with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM. In this respect, it will be clear to the skilled person that a polypeptide that contains two or more Nanobodies of the invention may bind to GPCRs with an increased avidity, compared to a polypeptide that contains only one amino acid sequence of the invention.

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

Another aspect of this invention relates to a nucleic acid that encodes an amino acid sequence of the invention (such as a Nanobody of the invention) or a polypeptide of the invention comprising the same. Again, as generally described herein for the nucleic acids of the invention, such a nucleic acid may be in the form of a genetic construct, as defined herein.

In another aspect, the invention relates to host or host cell that expresses or that is capable of expressing an amino acid sequence (such as a Nanobody) of the invention and/or a polypeptide of the invention comprising the same; 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.

Another aspect of the invention relates to a product or composition containing or comprising at least one amino acid sequence of the invention, 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.

The invention further relates to methods for preparing or generating the amino acid sequences, compounds, constructs, 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.

The invention further relates to applications and uses of the amino acid sequences, compounds, constructs, 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 GPCRs. 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 hereinbelow.

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 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 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 occurring heavy chain antibodies directed against GPCRs. As further described herein, such V_{HH} sequences can generally be generated or obtained by suitably immunizing a species of Camelid with GPCRs (i.e. so as to raise an immune response and/or heavy chain antibodies
10 directed against GPCRs), by obtaining a suitable biological sample from said Camelid (such as a blood sample, serum sample or sample of B-cells), and by generating V_{HH} sequences directed against GPCRs, 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 GPCRs, can be obtained from naïve libraries of Camelid V_{HH} sequences, for example by screening such a library using GPCRs, 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 naïve V_{HH} libraries
20 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.

Thus, in another aspect, the invention relates to a method for generating Nanobodies, that are directed against GPCRs. In one aspect, said method at least comprises the steps of:

- a) providing a set, collection or library of Nanobody sequences; and
- b) screening said set, collection or library of Nanobody sequences for Nanobody sequences that can bind to and/or have affinity for GPCRs;

and

- c) isolating the amino acid sequence(s) that can bind to and/or have affinity for GPCRs.

In such a method, the set, collection or library of Nanobody sequences may be a naïve
30 set, collection or library of Nanobody sequences; a synthetic or semi-synthetic set, collection or library of Nanobody sequences; and/or a set, collection or library of Nanobody sequences that have been subjected to affinity maturation.

In a preferred aspect of this method, the set, collection or library of Nanobody sequences may be an immune set, collection or library of Nanobody sequences, and in

particular an immune set, collection or library of V_{HH} sequences, that have been derived from a species of Camelid that has been suitably immunized with GPCRs 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).

In the above methods, the set, collection or library of Nanobody or V_{HH} 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) Nanobody 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 WO 03/054016 and to the review by Hoogenboom in Nature Biotechnology, 23, 9, 1105-1116 (2005).

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

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

In the method according to this aspect, 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 Camelid that has been suitably immunized with GPCRs or 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., Blood, Vol. 97, No. 12, 3820. Particular reference is made to the so-called "Nanoclone™" technique described in International application WO 06/079372 by Ablynx N.V.

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

- a) providing a set, collection or library of nucleic acid sequences encoding heavy chain antibodies or Nanobody sequences;
 - b) screening said set, collection or library of nucleic acid sequences for nucleic acid sequences that encode a heavy chain antibody or a Nanobody sequence that can bind to and/or has affinity for GPCRs;
- and
- c) isolating said nucleic acid sequence, followed by expressing the V_{HH} sequence present in said heavy chain antibody or by expressing said Nanobody sequence, respectively.

In such a method, the set, collection or library of nucleic acid sequences encoding heavy chain antibodies or Nanobody sequences may for example be a set, collection or library of nucleic acid sequences encoding a naïve set, collection or library of heavy chain antibodies or V_{HH} sequences; a set, collection or library of nucleic acid sequences encoding a synthetic or semi-synthetic set, collection or library of Nanobody sequences; and/or a set, collection or library of nucleic acid sequences encoding a set, collection or library of Nanobody sequences that have been subjected to affinity maturation.

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 encoding heavy chain antibodies or V_{HH} sequences derived from a Camelid that has been suitably immunized with GPCRs 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).

In the above methods, the set, collection or library of nucleotide 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 WO 03/054016 and to the review by Hoogenboom in Nature Biotechnology, 23, 9, 1105-1116 (2005).

As will be clear to the skilled person, the screening step of the methods described herein can also be performed as a selection step. Accordingly the term “screening” as used in the present description can comprise selection, screening or any suitable combination of selection and/or screening techniques. Also, when a set, collection or library of sequences is used, it may contain any suitable number of sequences, such as 1, 2, 3 or about 5, 10, 50, 100, 500, 1000, 5000, 10^4 , 10^5 , 10^6 , 10^7 , 10^8 or more sequences.

10 Also, one or more or all of the sequences in the above set, collection or library of amino acid sequences may be obtained or defined by rational, or semi-empirical approaches such as computer modelling techniques or biostatics or datamining techniques.

Furthermore, such a set, collection or library can comprise one, two or more sequences that are variants from one another (e.g. with designed point mutations or with randomized positions), comprise multiple sequences derived from a diverse set of naturally diversified sequences (e.g. an immune library)), or any other source of diverse sequences (as described for example in Hoogenboom et al, Nat Biotechnol 23:1105, 2005 and Binz et al, Nat Biotechnol 2005, 23:1247). Such set, collection or library of sequences can be displayed on the surface of a phage particle, a ribosome, a bacterium, a yeast cell, a mammalian cell, and
20 linked to the nucleotide sequence encoding the amino acid sequence within these carriers. This makes such set, collection or library amenable to selection procedures to isolate the desired amino acid sequences of the invention. More generally, when a sequence is displayed on a suitable host or host cell, it is also possible (and customary) to first isolate from said host or host cell a nucleotide sequence that encodes the desired sequence, and then to obtain the desired sequence by suitably expressing said nucleotide sequence in a suitable host organism. Again, this can be performed in any suitable manner known per se, as will be clear to the skilled person.

Yet another technique for obtaining V_{HH} sequences or Nanobody sequences directed against GPCRs involves suitably immunizing a transgenic mammal that is capable of
30 expressing heavy chain antibodies (i.e. so as to raise an immune response and/or heavy chain antibodies directed against GPCRs), obtaining a suitable biological sample from said transgenic mammal that contains (nucleic acid sequences encoding) said V_{HH} sequences or Nanobody sequences (such as a blood sample, serum sample or sample of B-cells), and then generating V_{HH} sequences directed against GPCRs, starting from said sample, using any

suitable technique known per se (such as any of the methods described herein or a hybridoma technique). 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 and WO 06/008548 and Janssens et al., Proc. Natl. Acad. Sci .USA. 2006 Oct 10;103(41):15130-5 can be used. For example, such heavy chain antibody expressing mice can express heavy chain antibodies with any suitable (single) variable domain, such as (single) variable domains from natural sources (e.g. human (single) variable domains, Camelid (single) variable domains or shark (single) variable domains), as well as for example synthetic or semi-synthetic (single) variable domains.

10 The invention also relates to the V_{HH} sequences or Nanobody 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 V_{HH} sequence or Nanobody sequence; and of expressing or synthesizing said V_{HH} sequence or Nanobody sequence in a manner known per se, such as by expression in a suitable host cell or host organism or by chemical synthesis.

As mentioned herein, a 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_{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
20 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 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 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
30 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 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 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 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
10 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
20 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
30 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 (which may then be suitably expressed). Nucleotide sequences encoding framework sequences of V_{HH} sequences or Nanobodies will be clear to the skilled person based on the disclosure herein and/or the further prior art cited herein (and/or may alternatively be obtained by PCR starting from the nucleotide sequences obtained using the methods described herein) and may be suitably combined with nucleotide sequences that encode the desired CDR's (for example, by PCR assembly using overlapping primers), so as to provide a nucleic acid encoding a Nanobody of the invention.

10 As mentioned herein, Nanobodies may in particular be characterized by the presence of one or more "*Hallmark residues*" (as described herein) in one or more of the framework sequences.

Thus, 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:

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:

20 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 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 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.

30 Thus, in a first 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

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 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:

10 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:

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

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

20 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:

30 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 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

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:

10 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:

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

In particular, a Nanobody against GPCRs according to 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 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:

30 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:

- d) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred aspects herein, and are more preferably as defined according to one of the more preferred aspects 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;

- 10 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
- 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;
- a-4) the amino acid residue at position 108 according to the Kabat numbering is Q;
- or in which:
- 20 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
- 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;
- 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
- 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
- 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;

and in which

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

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

10 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-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 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

20 L or R;

and in which:

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:

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

30 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 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 the group consisting of W, R and S; and is preferably W;

10 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:

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

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
30 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:

- 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 aspects herein, and are more preferably as defined according to one of the more preferred aspects herein.

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-4) 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 either:

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

or in which:

- ii) the amino acid residues at positions 43-46 according to the Kabat numbering form the sequence KERE or KQRE (or a KERE-like sequence as described) 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

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:

- i) 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:

- ii) CDR1, CDR2 and CDR3 are as defined herein, and are preferably as defined according to one of the preferred aspects herein, and are more preferably as defined according to one of the more preferred aspects 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:

- i) 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;

and in which:

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

In the Nanobodies of the invention in which the amino acid residues at positions 43-46 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.

- 20 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:

- i) 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 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. More generally, and without limitation, Nanobodies belonging to the GLEW-group can be defined as Nanobodies with a G at position 44 and/or with a W at position 47, in which position 46 is usually E and in which preferably position 45 is not a charged amino acid residue and not cysteine;
- 30 ii) 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. More generally, and without limitation, Nanobodies belonging to the KERE-group can be defined as Nanobodies with a K, Q or R at position 44 (usually K) in which position 45 is a charged amino acid residue or cysteine, and position 47 is as further defined herein;

- iii) 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.

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 L).

More generally, it should be noted that the definitions referred to above describe and apply to Nanobodies in the form of a native (i.e. non-humanized) V_{HH} sequence, and that humanized variants of these Nanobodies may contain other amino acid residues than those indicated above (i.e. one or more humanizing substitutions as defined herein). For example, and without limitation, in some humanized Nanobodies of the GLEW-group or the 103 P, R, S-group, Q at position 108 may be humanized to 108L. As already mentioned herein, other humanizing substitutions (and suitable combinations thereof) will become clear 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 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 disclosure herein.

Also, based on the foregoing, (the framework regions of) a Nanobody may be partially humanized or fully humanized.

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 aspects herein, and are more preferably as defined according to one of the more preferred aspects herein.

10 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 aspects herein, and are more preferably as defined according to one of the more preferred aspects herein.

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 aspects herein, and are more preferably as defined according to one of the more preferred aspects herein.

20 Also, more generally and in addition to the 108Q, 43E/44R and 103 P,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 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.

30 In one aspect 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 aspect 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) 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 aspect, 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 aspect of the Nanobodies of the invention, the amino acid 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 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 italics.

Table A-3: Hallmark Residues in Nanobodies

Position	Human V _H 3	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:

- (1) In particular, but not exclusively, in combination with KERE or KQRE at positions 43-46.
- (2) Usually as GLEW at positions 44-47.
- (3) 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 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.
- (4) With both GLEW at positions 44-47 and KERE or KQRE at positions 43-46.
- (5) Often as KP or EP at positions 83-84 of naturally occurring V_{HH} domains.
- (6) In particular, but not exclusively, in combination with GLEW at positions 44-47.
- (7) With the proviso that when positions 44-47 are GLEW, position 108 is always Q in (non-humanized) V_{HH} sequences that also contain a W at 103.
- (8) The GLEW group also contains GLEW-like sequences at positions 44-47, such as for example GVEW, EPEW, GLER, DQEW, 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 (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	L	F	E	R	L	K	P	W	G	Q
	L	F	E	R	F	E	P	W	G	Q
	L	F	E	R	F	K	P	W	G	Q
	L	Y	Q	R	L	K	P	W	G	Q
	L	F	L	R	V	K	P	Q	G	Q
	L	F	Q	R	L	K	P	W	G	Q
	L	F	E	R	F	K	P	W	G	Q
“GLEW” group	L	V	G	L	W	K	S	W	G	Q
	M	V	G	L	W	K	P	R	G	Q

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 to 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-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 Verrips 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)

Pos.	Amino acid residue(s):		V _{HH}	V _{HH}
	<i>Human V_{H3}</i>	<i>Camelid V_{HH}'s</i>	Ent.	Var.
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	2
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)

Pos.	Amino acid residue(s):		V _{HH}	V _{HH}
	<i>Human V_{H3}</i>	<i>Camelid V_{HH}'s</i>	Ent.	Var.
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)

Pos.	Amino acid residue(s):		V _{HH}	V _{HH}
	<i>Human V_{H3}</i>	<i>Camelid V_{HH}'s</i>	Ent.	Var.
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)

Pos.	Amino acid residue(s):		V _{HH}	V _{HH}
	<i>Human V_H3</i>	<i>Camelid V_{HH}'s</i>	Ent.	Var.
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, <u>D</u>, 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	<u>S</u>, <u>T</u>, I	T, A, E, I, M, P, S	0.8	5
78	L, A	V, <u>L</u>, 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, <u>N</u>, D, G, R, T	1	6
82c	L	L, P, V	0.1	2
83	<u>Hallmark residue:</u> R, K ⁽⁵⁾ , N, E ⁽⁵⁾ , G, I, M, Q or T; preferably K or R; most preferably K		0.9	7
84	<u>Hallmark residue:</u> 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)

Pos.	Amino acid residue(s):		V _{HH}	V _{HH}
	<i>Human V_{H3}</i>	<i>Camelid V_{HH}'s</i>	Ent.	Var.
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)

Pos.	Amino acid residue(s):		V _{HH}	V _{HH}
	<i>Human V_{H3}</i>	<i>Camelid V_{HH}'s</i>	Ent.	Var.
103	<u>Hallmark residue:</u> W ⁽⁴⁾ , P ⁽⁶⁾ , R ⁽⁶⁾ , S; preferably W		0.4	2
104	<u>Hallmark residue:</u> G or D; preferably G		0.1	1
105	Q, <u>R</u>	Q, E, K, P, R	0.6	4
106	G	G	0.1	1
107	T	T, A, I	0.3	2
108	<u>Hallmark residue:</u> 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 be defined as 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:

- 10 i) 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;

and in which:

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

The above Nanobodies may for example be V_{HH} sequences or may be humanized Nanobodies. When the above Nanobody sequences are V_{HH} sequences, they may be suitably humanized, as further described herein. When the Nanobodies are partially humanized

20 Nanobodies, they may optionally be further suitably humanized, again as described herein.

In particular, a Nanobody of the invention 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:

- i) (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 (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 -
- 30

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);

and in which:

- 10 ii) said amino acid sequence has at least 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1 to 22, in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences (indicated with X in the sequences of SEQ ID NO's: 1 to 22) are disregarded;

and in which:

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

The above Nanobodies may for example be V_{HH} sequences or may be humanized
20 Nanobodies. When the above Nanobody sequences are V_{HH} sequences, they may be suitably humanized, as further described herein. When the Nanobodies are partially humanized Nanobodies, they may optionally be further suitably humanized, again as described herein.

Table A-9: Representative amino acid sequences for Nanobodies of the KERE, GLEW and P,R,S 103 group.
The CDR's are indicated with XXXX

KERE sequence no. 1	SEQ ID NO:1	EVQLVESGGGLVQPGGSLRLSCAASGIPFSXXXXXWFRQAPGKQRDSVAXXXXXXRFTI SRDNAKNTVYLQMNSLKPEDTAVYRCYFXXXXXWGQGTQVTVSS
KERE sequence no. 2	SEQ ID NO:2	QVKLEESGGGLVQAGGSLRLSCVSGSGRTFSTXXXXXWFRLAPGKEREFVAXXXXXXRFTI SRDTASNRGYLMNNLTPTEDTAVYCAAXXXXXXWGQGTQVTVSS
KERE sequence no. 3	SEQ ID NO:3	AVQLVDSGGGLVQAGDSLKLSCALTGGAFTXXXXXWFRQTTPGREREFVAXXXXXXRFTI SRDNAKMMVYLRMNSLIPEDAAVYSCAAXXXXXXWGQGTQVTVSS
KERE sequence no. 4	SEQ ID NO:4	QVQLVESGGGLVEAGGSLRLSCTASESPFRXXXXXWFRQTSGQEREFVAXXXXXXRFTI SRDDAKNTVWLHGSTLKPEDTAVYCAAXXXXXXWGQGTQVTVSS
KERE sequence no. 5	SEQ ID NO:5	AVQLVESGGGLVQGGGSLRLACAASERIFDXXXXXWYRQGPGNERELVAXXXXXXRFTI SMDYTKQTVYLMNSLRPEDTGLYCKIXXXXXXWGQGTQVTVSS
KERE sequence no. 6	SEQ ID NO:6	DVKFVESGGGLVQAGGSLRLSCVASGFNFDXXXXXWFRQAPGKEREEVAXXXXXXRFT ISSEKDKNVYLMNSLKPEDTALYICAGXXXXXWGRGTQVTVSS
KERE sequence no. 7	SEQ ID NO:7	QVRLAESGGGLVQSGGSLRLSCVASGSTYTXXXXXWYRQYPGKQRALVAXXXXXXRFT IARDSTKDTFCLQMNNLKPEDTAVYCYAXXXXXXWGQGTQVTVSS
KERE sequence no. 8	SEQ ID NO:8	EVQLVESGGGLVQAGGSLRLSCAASGFTSDXXXXXWFRQAPGKPREGVSXXXXXRFT ISTDNAKNTVHLLMNRVNAEDTALYCAVXXXXXWGRGTRVTVSS
KERE sequence no. 9	SEQ ID NO:9	QVQLVESGGGLVQPGGSLRLSCQASGDISTXXXXXWYRQVPGKLREFVAXXXXXXRFTI SGDNAKRAIYLMNNLKPDDTAVYCNRXXXXXWGQGTQVTVSP
KERE sequence no. 10	SEQ ID NO:10	QVPVVESGGGLVQAGDSLRLFCVAPSFSTSTXXXXXWFRQAPGKEREFVAXXXXXXRFTI SRNATKNTLTLRMDSLKPEDTAVYCAAXXXXXXWGQGTQVTVSS
KERE sequence no. 11	SEQ ID NO:11	EVQLVESGGGLVQAGDSLRLFCTVSGGTASXXXXXWFRQAPGKEREFVAXXXXXXRFTI ARENAGNMVYLMNNLKPDDTALYTCAAXXXXXXWGRGTQVTVSS

Table A-9 (continued):

KERE sequence no. 12	SEQ ID NO:12	AVQLVESGGDSVQPGDSQTLSCAASGRNTSXXXXXWFRQAPGKERVFLEXXXXXRFT ISRDSAKNMMYLMNNLKPQDTAVYYCAAXXXXXXWGQGTQTVSS
KERE sequence no. 13	SEQ ID NO:13	AVQLVESGGGLVQAGGSLRLSCVSGLTSSXXXXXWFRQTPWQERDFVAXXXXXRFT ISRDNKDTVLLEMNFKPEDTAIYYCAAXXXXXXWGQGTQTVSS
KERE sequence no. 14	SEQ ID NO:14	AVQLVESGGGLVQAGASRLSCATSTRTLDXXXXXWFRQAPGRDREFVAXXXXXRFT VSRDSAENTVALQMNSLKPEDTAVYYCAAXXXXXXWGQGTQTVSS
KERE sequence no. 15	SEQ ID NO:15	QVQLVESGGGLVQPGGSLRLSCTVSRLTAHXXXXXWFRQAPGKEREAVSXXXXRFTI SRDYAGNTAFLQMDSLKPEDTGVYYCATXXXXXWGQGTQTVSS
KERE sequence no. 16	SEQ ID NO:16	EVQLVESGGELVQAGGSLKLSCTASGRNFVXXXXXWFRRAPGKEREFVAXXXXXRFT VSRDNGKNTAYLRMNSLKPEDTADYYCAVXXXXXLGSGTQTVSS
GLEW sequence no. 1	SEQ ID NO:17	AVQLVESGGGLVQPGGSLRLSCAASGFTSXXXXXWVRQAPGKLVESXXXXRFT ISRDNAKNTLYLQMNSLKPEDTAVYVCVXXXXXGSGGTQTVSS
GLEW sequence no. 2	SEQ ID NO:18	EVQLVESGGGLVQPGGSLRLSCVCSGCTXXXXXWVRQAPGKAEEWVSXXXXRF KISRDNAKNTLYLQMNSLGPEDTAMYYCQRXXXXXRGQGTQTVSS
GLEW sequence no. 3	SEQ ID NO:19	EVQLVESGGGLALPGGSLTLCVFSGSTFSXXXXXWVRHTPGKAEEWVSXXXXRFTI SRDNAKNTLYLEMNSLPEDTAMYYCGRXXXXXRSKGIQTVSS
P,R,S 103 sequence no. 1	SEQ ID NO:20	AVQLVESGGGLVQAGGSLRLSCAASGRTFSXXXXXWFRQAPGKEREFVAXXXXXRFTI SRDNAKNTVYLQMNSLKPEDTAVYYCAAXXXXXXRGQGTQTVSS
P,R,S 103 sequence no. 2	SEQ ID NO:21	DVQLVESGGDLVQPGGSLRLSCAASGFSFDXXXXXWLRQTPGKLEWVGXXXXRFT ISRDNAKNMLYLHLNLLKSEDTAVYYCRRXXXXXLGQGTQTVSS
P,R,S 103 sequence no. 3	SEQ ID NO:22	EVQLVESGGGLVQPGGSLRLSCVCSGCTXXXXXWVRQAPGKAEEWVSXXXXRF KISRDNAKNTLYLQMNSLGPEDTAMYYCQRXXXXXRGQGTQTVSS

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

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

in which:

- i) 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 in which:

- ii) FR1 is an amino acid sequence that has at least 80% amino acid identity with at least one of the following amino acid sequences:

10

Table A-10: Representative FW1 sequences for Nanobodies of the KERE-group.

KERE FW1 sequence no. 1	SEQ ID NO:23	QVQRVESGGGLVQAGGSLRLSCAASGRTSS
KERE FW1 sequence no. 2	SEQ ID NO:24	QVQLVESGGGLVQTGDSLRLSCSASGRTFS
KERE FW1 sequence no. 3	SEQ ID NO:25	QVKLEESGGGLVQAGDSLRLSCAATGRAFG
KERE FW1 sequence no. 4	SEQ ID NO:26	AVQLVESGGGLVQPGESLGLSCVASGRDFV
KERE FW1 sequence no. 5	SEQ ID NO:27	EVQLVESGGGLVQAGGSLRLSCEVLGRTAG
KERE FW1 sequence no. 6	SEQ ID NO:28	QVQLVESGGGWVQPGGSLRLSCAASETILS
KERE FW1 sequence no. 7	SEQ ID NO:29	QVQLVESGGGTVPGGSLNLSCVASGNTFN
KERE FW1 sequence no. 8	SEQ ID NO:30	EVQLVESGGGLAQPGGSLQLSCSAPGFTLD
KERE FW1 sequence no. 9	SEQ ID NO:31	AQELEESGGGLVQAGGSLRLSCAASGRTFN

and in which:

- iii) FR2 is an amino acid sequence that has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table A-11: Representative FW2 sequences for Nanobodies of the KERE-group.

KERE FW2 sequence no. 1	SEQ ID NO:41	WFRQAPGKEREFVA
KERE FW2 sequence no. 2	SEQ ID NO:42	WFRQTPGREREFVA
KERE FW2 sequence no. 3	SEQ ID NO:43	WYRQAPGKQREMVA
KERE FW2 sequence no. 4	SEQ ID NO:44	WYRQGPGKQRELVA
KERE FW2 sequence no. 5	SEQ ID NO:45	WIRQAPGKEREGVS
KERE FW2 sequence no. 6	SEQ ID NO:46	WFREAPGKEREGIS
KERE FW2 sequence no. 7	SEQ ID NO:47	WYRQAPGKERDLVA
KERE FW2 sequence no. 8	SEQ ID NO:48	WFRQAPGKQREEVS
KERE FW2 sequence no. 9	SEQ ID NO:49	WFRQPPGKVREFVG

and in which:

- iv) FR3 is an amino acid sequence that has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table A-12: Representative FW3 sequences for Nanobodies of the KERE-group.

KERE FW3 sequence no. 1	SEQ ID NO:50	RFTISRDNKNTVYLMNSLKPEDTAVYRCYF
KERE FW3 sequence no. 2	SEQ ID NO:51	RFAISRDNKNTGYLMNSLEPEDTAVYYCAA
KERE FW3 sequence no. 3	SEQ ID NO:52	RFTVARNNAKNTVNLEMNSLKPEDTAVYYCAA
KERE FW3 sequence no. 4	SEQ ID NO:53	RFTISRDIKNTVDLLMNNLEPEDTAVYYCAA
KERE FW3 sequence no. 5	SEQ ID NO:54	RLTISRDNVDTMYLMNSLKPEDTAVYYCAA
KERE FW3 sequence no. 6	SEQ ID NO:55	RFTISRDNKNTVYLMQMDNVKPEDTAIYYCAA
KERE FW3 sequence no. 7	SEQ ID NO:56	RFTISKDSGKNTVYLMQMTSLKPEDTAVYYCAT
KERE FW3 sequence no. 8	SEQ ID NO:57	RFTISRDSAKNMMYLMNNLKPQDTAVYYCAA
KERE FW3 sequence no. 9	SEQ ID NO:58	RFTISRENDKSTVYLLQNSLKPEDTAVYYCAA
KERE FW3 sequence no. 10	SEQ ID NO:59	RFTISRDYAGNTAYLMNSLKPEDTGVYYCAT

10 and in which:

- v) FR4 is an amino acid sequence that has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table A-13: Representative FW4 sequences for Nanobodies of the KERE-group.

KERE FW4 sequence no. 1	SEQ ID NO:60	WGQGTQVTVSS
KERE FW4 sequence no. 2	SEQ ID NO:61	WGKGTLVTVSS
KERE FW4 sequence no. 3	SEQ ID NO:62	RGQGTRVTVSS
KERE FW4 sequence no. 4	SEQ ID NO:63	WGLGTQVTISS

and in which:

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

In the above Nanobodies, one or more of the further Hallmark residues are preferably as described herein (for example, when they are V_{HH} sequences or partially humanized Nanobodies).

Also, the above Nanobodies may for example be V_{HH} sequences or may be humanized Nanobodies. When the above Nanobody sequences are V_{HH} sequences, they may be suitably humanized, as further described herein. When the Nanobodies are partially humanized Nanobodies, they may optionally be further suitably humanized, again as described herein.

With regard to framework 1, it will be clear to the skilled person that, when an amino acid sequence as outlined above is generated by expression of a nucleotide sequence, the first four amino acid sequences (i.e. amino acid residues 1-4 according to the Kabat numbering) may often be determined by the primer(s) that have been used to generate said nucleic acid. Thus, for determining the degree of amino acid identity, the first four amino acid residues are preferably disregarded.

Also, with regard to framework 1, and although amino acid positions 27 to 30 are according to the Kabat numbering considered to be part of the framework regions (and not the CDR's), it has been found by analysis of a database of more than 1000 V_{HH} sequences that the positions 27 to 30 have a variability (expressed in terms of V_{HH} entropy and V_{HH} variability – see Tables A-5 to A-8) that is much greater than the variability on positions 1 to 26. Because of this, for determining the degree of amino acid identity, the amino acid residues at positions 27 to 30 are preferably also disregarded.

In view of this, a Nanobody of the KERE class may be an amino acid sequence that is comprised of four framework regions/sequences interrupted by three complementarity determining regions/sequences, in which:

- i) 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 in which:
- ii) FR1 is an amino acid sequence that, on positions 5 to 26 of the Kabat numbering, has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table A-14: Representative FW1 sequences (amino acid residues 5 to 26) for Nanobodies of the KERE-group.

KERE FW1 sequence no. 10	SEQ ID NO:32	VESGGGLVQPGGSLRLSCAASG
KERE FW1 sequence no. 11	SEQ ID NO:33	VDSGGGLVQAGDSLKLSCALTG
KERE FW1 sequence no. 12	SEQ ID NO:34	VDSGGGLVQAGDSLRLSCAASG
KERE FW1 sequence no. 13	SEQ ID NO:35	VDSGGGLVEAGGSLRLSCQVSE
KERE FW1 sequence no. 14	SEQ ID NO:36	QDSGGGSVQAGGSLKLSCAASG
KERE FW1 sequence no. 15	SEQ ID NO:37	VQSGGRLVQAGDSLRLSCAASE
KERE FW1 sequence no. 16	SEQ ID NO:38	VESGGTLVQSGDSLKLSCASST
KERE FW1 sequence no. 17	SEQ ID NO:39	MESGGDSVQSGGSLTLSCVASG
KERE FW1 sequence no. 18	SEQ ID NO:40	QASGGGLVQAGGSLRLSCSASV

10 and in which:

- iii) FR2, FR3 and FR4 are as mentioned herein for FR2, FR3 and FR4 of Nanobodies of the KERE-class;

and in which:

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

The above Nanobodies may for example be V_{HH} sequences or may be humanized Nanobodies. When the above Nanobody sequences are V_{HH} sequences, they may be suitably humanized, as further described herein. When the Nanobodies are partially humanized

20 Nanobodies, they may optionally be further suitably humanized, again as described herein.

A Nanobody of the GLEW class may be an amino acid sequence that is comprised of four framework regions/sequences interrupted by three complementarity determining regions/sequences, in which

- i) preferably, when the Nanobody of the GLEW-class is a non-humanized Nanobody, the amino acid residue in position 108 is Q;
- ii) FR1 is an amino acid sequence that has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table A-15: Representative FW1 sequences for Nanobodies of the GLEW-group.

GLEW FW1 sequence no. 1	SEQ ID NO:64	QVQLVESGGGLVQPGGSLRLSCAASGFTFS
GLEW FW1 sequence no. 2	SEQ ID NO:65	EVHLVESGGGLVRPGGSLRLSCAAFGFIFK
GLEW FW1 sequence no. 3	SEQ ID NO:66	QVKLEESGGGLAQPGGSLRLSCVASGFTFS
GLEW FW1 sequence no. 4	SEQ ID NO:67	EVQLVESGGGLVQPGGSLRLSCVVCVSSGCT
GLEW FW1 sequence no. 5	SEQ ID NO:68	EVQLVESGGGLALPGGSLTLSCVFSGSTFS

and in which:

- iii) FR2 is an amino acid sequence that has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table A-16: Representative FW2 sequences for Nanobodies of the GLEW-group.

GLEW FW2 sequence no. 1	SEQ ID NO:72	WVRQAPGKVLEWVS
GLEW FW2 sequence no. 2	SEQ ID NO:73	WVRRPPGKGLEWVS
GLEW FW2 sequence no. 3	SEQ ID NO:74	WVRQAPGMGLEWVS
GLEW FW2 sequence no. 4	SEQ ID NO:75	WVRQAPGKEPEWVS
GLEW FW2 sequence no. 5	SEQ ID NO:76	WVRQAPGKDQEWVS
GLEW FW2 sequence no. 6	SEQ ID NO:77	WVRQAPGKAEEWVS
GLEW FW2 sequence no. 7	SEQ ID NO:78	WVRQAPGKGLEWVA
GLEW FW2 sequence no. 8	SEQ ID NO:79	WVRQAPGRATEWVS

10 and in which:

- iv) FR3 is an amino acid sequence that has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table A-17: Representative FW3 sequences for Nanobodies of the GLEW-group.

GLEW FW3 sequence no. 1	SEQ ID NO:80	RFTISRDNKNTLYLQMNSLKPEDTAVYYCVK
GLEW FW3 sequence no. 2	SEQ ID NO:81	RFTISRDNARNTLYLQMDSLIPEDTALYYCAR
GLEW FW3 sequence no. 3	SEQ ID NO:82	RFTSSRDNAKSTLYLQMNDLKPEDTALYYCAR
GLEW FW3 sequence no. 4	SEQ ID NO:83	RFIISRDNKNTLYLQMNSLGPEDTAMYYCQR
GLEW FW3 sequence no. 5	SEQ ID NO:84	RFTASRDNAKNTLYLQMNSLKSEDATARYYYCAR
GLEW FW3 sequence no. 6	SEQ ID NO:85	RFTISRDNKNTLYLQMDDLQSEDATAMYYCGR

and in which:

- v) FR4 is an amino acid sequence that has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table A-18: Representative FW4 sequences for Nanobodies of the GLEW-group.

GLEW FW4 sequence no. 1	SEQ ID NO:86	GSQGTQVTVSS
GLEW FW4 sequence no. 2	SEQ ID NO:87	LRGGTQVTVSS
GLEW FW4 sequence no. 3	SEQ ID NO:88	RGQGTLVTVSS
GLEW FW4 sequence no. 4	SEQ ID NO:89	RSRGIQVTVSS
GLEW FW4 sequence no. 5	SEQ ID NO:90	WGKGTQVTVSS
GLEW FW4 sequence no. 6	SEQ ID NO:91	WGQGTQVTVSS

and in which:

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

In the above Nanobodies, one or more of the further Hallmark residues are preferably as described herein (for example, when they are V_{HH} sequences or partially humanized Nanobodies).

With regard to framework 1, it will again be clear to the skilled person that, for determining the degree of amino acid identity, the amino acid residues on positions 1 to 4 and 27 to 30 are preferably disregarded.

In view of this, a Nanobody of the GLEW class may be an amino acid sequence that is comprised of four framework regions/sequences interrupted by three complementarity determining regions/sequences, in which:

- i) preferably, when the Nanobody of the GLEW-class is a non-humanized Nanobody, the amino acid residue in position 108 is Q;

and in which:

- ii) FR1 is an amino acid sequence that, on positions 5 to 26 of the Kabat numbering, has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table A-19: Representative FW1 sequences (amino acid residues 5 to 26) for Nanobodies of the KERE-group.

GLEW FW1 sequence no. 6	SEQ ID NO:69	VESGGGLVQPGGSLRLSCAASG
GLEW FW1 sequence no. 7	SEQ ID NO:70	EESGGGLAQPGGSLRLSCVASG
GLEW FW1 sequence no. 8	SEQ ID NO:71	VESGGGLALPGGSLTLSCVFSG

and in which:

- iii) FR2, FR3 and FR4 are as mentioned herein for FR2, FR3 and FR4 of Nanobodies of the GLEW-class;

and in which:

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

The above Nanobodies may for example be V_{HH} sequences or may be humanized Nanobodies. When the above Nanobody sequences are V_{HH} sequences, they may be suitably humanized, as further described herein. When the Nanobodies are partially humanized Nanobodies, they may optionally be further suitably humanized, again as described herein. In the above Nanobodies, one or more of the further Hallmark residues are preferably as described herein (for example, when they are V_{HH} sequences or partially humanized Nanobodies).

A Nanobody of the P, R, S 103 class may be an amino acid sequence that is comprised of four framework regions/sequences interrupted by three complementarity determining regions/sequences, in which

- i) the amino acid residue at position 103 according to the Kabat numbering is different from W;

and in which:

- ii) preferably the amino acid residue at position 103 according to the Kabat numbering is P, R or S, and more preferably R;

and in which:

- iii) FR1 is an amino acid sequence that has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table A-20: Representative FW1 sequences for Nanobodies of the P,R,S 103-group.

P,R,S 103 FW1 sequence no. 1	SEQ ID NO:92	AVQLVESGGGLVQAGGSLRLSCAASGRTFS
P,R,S 103 FW1 sequence no. 2	SEQ ID NO:93	QVQLQESGGGMVQPGGSLRLSCAASGFDFG
P,R,S 103 FW1 sequence no. 3	SEQ ID NO:94	EVHLVESGGGLVRPGGSLRLSCAAFGFIFK
P,R,S 103 FW1 sequence no. 4	SEQ ID NO:95	QVQLAESGGGLVQPGGSLKLSCAASRTIVS
P,R,S 103 FW1 sequence no. 5	SEQ ID NO:96	QEHLVESGGGLVDIGGSLRLSCAASERIFS
P,R,S 103 FW1 sequence no. 6	SEQ ID NO:97	QVKLEESGGGLAQPGGSLRLSCVASGFTFS
P,R,S 103 FW1 sequence no. 7	SEQ ID NO:98	EVQLVESGGGLVQPGGSLRLSCVCVSSGCT
P,R,S 103 FW1 sequence no. 8	SEQ ID NO:99	EVQLVESGGGLALPGGSLTLSCVFSGSTFS

and in which

- iv) FR2 is an amino acid sequence that has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table A-21: Representative FW2 sequences for Nanobodies of the P,R,S 103-group.

P,R,S 103 FW2 sequence no. 1	SEQ ID NO:102	WFRQAPGKEREFVA
P,R,S 103 FW2 sequence no. 2	SEQ ID NO:103	WVRQAPGKVLEWVS
P,R,S 103 FW2 sequence no. 3	SEQ ID NO:104	WVRRPPGKGLEWVS
P,R,S 103 FW2 sequence no. 4	SEQ ID NO:105	WIRQAPGKEREGVS
P,R,S 103 FW2 sequence no. 5	SEQ ID NO:106	WVRQYPGKEPEWVS
P,R,S 103 FW2 sequence no. 6	SEQ ID NO:107	WFRQPPGKEHEFVA
P,R,S 103 FW2 sequence no. 7	SEQ ID NO:108	WYRQAPGKRTELVA
P,R,S 103 FW2 sequence no. 8	SEQ ID NO:109	WLRQAPGQGLEWVS
P,R,S 103 FW2 sequence no. 9	SEQ ID NO:110	WLRQTPGKGLEWVG
P,R,S 103 FW2 sequence no. 10	SEQ ID NO:111	WVRQAPGKAEEFVS

and in which:

- 10 v) FR3 is an amino acid sequence that has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table A-22: Representative FW3 sequences for Nanobodies of the P,R,S 103-group.

P,R,S 103 FW3 sequence no. 1	SEQ ID NO:112	RFTISRDN AKNTVYLQMNSLKPEDTAVYYCAA
P,R,S 103 FW3 sequence no. 2	SEQ ID NO:113	RFTISRDN ARNTLYLQMDSLIPEDTALYYCAR
P,R,S 103 FW3 sequence no. 3	SEQ ID NO:114	RFTISRDN AKNEMYLQMNNLKTEDTGVYWCGA
P,R,S 103 FW3 sequence no. 4	SEQ ID NO:115	RFTISSDSNRNMIY LQMNNLKPEDTAVYYCAA
P,R,S 103 FW3 sequence no. 5	SEQ ID NO:116	RFTISRDN AKNMLYLHLNNLKSEDTAVYYCRR
P,R,S 103 FW3 sequence no. 6	SEQ ID NO:117	RFTISRDN AKKTVYLRNLNLPEDTAVYSCNL
P,R,S 103 FW3 sequence no. 7	SEQ ID NO:118	RFKISRDN AKKTLYLQMNSLGPEDTAMYYCQR
P,R,S 103 FW3 sequence no. 8	SEQ ID NO:119	RFTVSRDN GKNTAYLRMNSLKPEDTADYYCAV

and in which:

- vi) FR4 is an amino acid sequence that has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table A-23: Representative FW4 sequences for Nanobodies of the P,R,S 103-group.

P,R,S 103 FW4 sequence no. 1	SEQ ID NO:120	RGQGTQVT VSS
P,R,S 103 FW4 sequence no. 2	SEQ ID NO:121	LRGGTQVT VSS
P,R,S 103 FW4 sequence no. 3	SEQ ID NO:122	GNKGTLVT VSS
P,R,S 103 FW4 sequence no. 4	SEQ ID NO:123	SSPGTQVT VSS
P,R,S 103 FW4 sequence no. 5	SEQ ID NO:124	SSQGTLVT VSS
P,R,S 103 FW4 sequence no. 6	SEQ ID NO:125	RSRGIQVT VSS

and in which:

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

In the above Nanobodies, one or more of the further Hallmark residues are preferably as described herein (for example, when they are V_{HH} sequences or partially humanized Nanobodies).

With regard to framework 1, it will again be clear to the skilled person that, for determining the degree of amino acid identity, the amino acid residues on positions 1 to 4 and 27 to 30 are preferably disregarded.

In view of this, a Nanobody of the P,R,S 103 class may be an amino acid sequence that is comprised of four framework regions/sequences interrupted by three complementarity determining regions/sequences, in which:

- i) the amino acid residue at position 103 according to the Kabat numbering is different from W;

and in which:

- ii) preferably the amino acid residue at position 103 according to the Kabat numbering is P, R or S, and more preferably R;

and in which:

- 10 iii) FR1 is an amino acid sequence that, on positions 5 to 26 of the Kabat numbering, has at least 80% amino acid identity with at least one of the following amino acid sequences:

Table A-24: Representative FW1 sequences (amino acid residues 5 to 26) for Nanobodies of the P,R,S 103-group.

P,R,S 103 FW1 sequence no. 9	SEQ ID NO:100	VESGGGLVQAGGSLRLSCAASG
P,R,S 103 FW1 sequence no. 10	SEQ ID NO:101	AESGGGLVQPGGSLKLSCAASR

and in which:

- iv) FR2, FR3 and FR4 are as mentioned herein for FR2, FR3 and FR4 of Nanobodies of the P,R,S 103 class;

and in which:

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

The above Nanobodies may for example be V_{HH} sequences or may be humanized Nanobodies. When the above Nanobody sequences are V_{HH} sequences, they may be suitably humanized, as further described herein. When the Nanobodies are partially humanized Nanobodies, they may optionally be further suitably humanized, again as described herein.

In the above Nanobodies, one or more of the further Hallmark residues are preferably as described herein (for example, when they are V_{HH} sequences or partially humanized Nanobodies).

- 30 In another preferred, but non-limiting aspect, the invention relates to a Nanobody as described above, 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: 413 to 453 and 517 to 525. 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 Nanobody and one or more of the sequences of SEQ ID NO's: 413 to 453 and 517 to 525, in which the amino acid residues that form the framework regions are disregarded. Such Nanobodies can be as further described herein.

As already mentioned herein, another preferred but non-limiting aspect of the invention relates to a Nanobody with an amino acid sequence that is chosen from the group consisting of SEQ ID NO's: 413 to 453 and 517 to 525 or from the group consisting of 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: 413 to 453 and 517 to 525.

Also, in the above Nanobodies:

- i) any amino acid substitution (when it is not a humanizing substitution as defined herein) is preferably, and compared to the corresponding amino acid sequence of SEQ ID NO's: 413 to 453 and 517 to 525, a conservative amino acid substitution, (as defined herein);
- 20 and/or:
- ii) its amino acid sequence preferably contains either only amino acid substitutions, or otherwise preferably no more than 5, preferably no more than 3, and more preferably only 1 or 2 amino acid deletions or insertions, compared to the corresponding amino acid sequence of SEQ ID NO's: 413 to 453 and 517 to 525;
- and/or
- iii) the CDR's may be CDR's that are derived by means of affinity maturation, for example starting from the CDR's of the corresponding amino acid sequence of SEQ ID NO's: 413 to 453 and 517 to 525.

Preferably, the CDR sequences and FR sequences in the Nanobodies of the invention are such that the Nanobodies of the invention (and polypeptides of the invention comprising the same):

- bind to GPCRs 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 GPCRs 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 GPCRs 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, CDR sequences and FR sequences present in the Nanobodies of the invention are such that the Nanobodies of the invention will bind to GPCRs with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM.

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 V_{HH} 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 V_{HH} domain. Usually, a humanized Nanobody will have at least one such amino acid difference with a naturally occurring V_{HH} 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 413 to 453 and 517 to 525.

Thus, according to one aspect of the invention, the term “Nanobody of the invention” in its
10 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
20 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 to 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
30 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 to 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.

10 Also, generally, amino acid substitutions are preferred over amino acid deletions or insertions.

The analogs are preferably such that they can bind to GPCRs 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 for the Nanobodies of the invention.

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

Also, according to one preferred aspect, 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
20 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: 413 to 453 and 517 to 525.

Also, the framework sequences and CDR's of the analogs are preferably such that they are in accordance with the preferred aspects defined herein. More generally, as 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
30 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, for example from the Tables herein, from the possible humanizing substitutions mentioned in

the background art cited herein, and/or from a comparison 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
10 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}
20 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. 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
30 position 108.

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.

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_H3 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.

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 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 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: 413 to 453 and 517 to 525. Thus, according to one aspect 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 GPCRs 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 for the Nanobodies of the invention.

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.

10 According to another particularly preferred, but non-limiting aspect, 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.

20 According to one preferred aspect, 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 413 to 453 and 517 to 525.

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
30 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.

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.

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
10 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
20 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.

30 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 ^{152}Eu or others metals from the lanthanide series), phosphorescent labels, chemiluminescent labels or bioluminescent labels (such as luminal, isoluminol, thermotropic acridinium ester, imidazole, acridinium salts, oxalate ester, dioxetane or GFP and its analogs), radio-isotopes (such as ^3H , ^{125}I , ^{32}P , ^{35}S , ^{14}C , ^{51}Cr , ^{36}Cl , ^{57}Co , ^{58}Co , ^{59}Fe , and ^{75}Se), metals, metal chelates or metallic cations (for example metallic cations such as $^{99\text{m}}\text{Tc}$, ^{123}I , ^{111}In , ^{131}I , ^{97}Ru , ^{67}Cu , ^{67}Ga , and ^{68}Ga 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 (^{157}Gd , ^{55}Mn , ^{162}Dy , ^{52}Cr , and ^{56}Fe), 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, beta-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.

10 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
20 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 Targeting, 8, 4, 257 (2000). Such binding pairs may also be used to link a therapeutically active agent to the Nanobody of the invention.

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,
30 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 described in WO 03/055527.

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 GPCRs 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 for the Nanobodies of the invention.

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 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:

- can comprise an N-terminal Met residue, for example as result of expression in a heterologous host cell or host organism.
- 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;
- 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;

- 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, glutathione residues and a myc-tag (see for example SEQ ID NO:31 of WO 06/12282).
- 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 aspect, 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.

For example, 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).

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),

10 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 haptenic molecules (for example haptens that are recognized by circulating antibodies, see for example WO 98/22141).

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 for made to WO 00/27435 and WO 01/077137).

20 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 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 (see also PCT/EP2007/002817).

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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 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, 2005, as well as to EP 0 368 684, as well as to the following the US provisional applications 60/843,349 (see also PCT/EP2007/059475), 60/850,774 (see also PCT/EP2007/060849), 60/850,775 (see also PCT/EP2007/060850) by Ablynx N.V. mentioned herein US provisional application of Ablynx N.V. entitled "*Peptides capable of binding to serum proteins*" filed on December 5, 2006 (see also PCT/EP2007/063348)..

Such amino acid sequences may in particular be directed against serum albumin (and
10 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 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 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; see also PCT/EP2007/059475); amino acid sequences against human
20 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, cynomolgus 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 and PCT/EP2007/059475); 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; see also and PCT/EP2007/059475) and/or amino acid sequences that are
30 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; see also PCT/EP2007/060850).

According to another aspect, 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 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.).

The at least one Nanobody may also be linked to one or more (preferably human) C_H1 , C_H2 and/or C_H3 domains, optionally via a linker sequence. For instance, a Nanobody linked to a suitable C_H1 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 C_H3 domain (optionally via a linker) to provide a construct with increased half-life in vivo.

According to one specific aspect of a polypeptide of the invention, one or more Nanobodies of the invention may be linked (optionally via a suitable linker or hinge region) to one or more constant domains (for example, 2 or 3 constant domains that can be used as part of/to form an Fc portion), to an Fc portion and/or 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 C_H2 and/or C_H3 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) an Fc region, for example from IgG (e.g. from IgG1, IgG2, IgG3 or IgG4), from IgE or from another human Ig such as IgA, IgD or IgM. 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 C_H2 and/or C_H3 domain have been replaced by human C_H2 and C_H3 domains, so as to provide an immunoglobulin that consists of 2 heavy chains each comprising a Nanobody and human C_H2 and C_H3 domains (but no C_H1 domain), which immunoglobulin has the effector function provided by the C_H2 and C_H3 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, WO 02/056910 and WO 05/017148, as well

as the review by Holliger and Hudson, supra; and to the non-prepublished US provisional application by Ablynx N.V. entitled “*Constructs comprising single variable domains and an Fc portion derived from IgE*” which has a filing date of December 4, 2007. 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. C_H2 and/or C_H3 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
10 for example comprise two Nanobodies linked to a C_H3 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.

In another one specific, but non-limiting, aspect, in order to form a polypeptide of the invention, one or more amino acid sequences of the invention may be linked (optionally via a suitable linker or hinge region) to naturally occurring, synthetic or semisynthetic constant domains (or analogs, variants, mutants, parts or fragments thereof) that have a reduced (or essentially no) tendency to self-associate into dimers (i.e. compared to constant domains that naturally occur in conventional 4-chain antibodies). Such monomeric (i.e. not self-associating) Fc chain variants, or fragments thereof, will be clear to the skilled person. For
20 example, Helm et al., J Biol Chem 1996 271 7494, describe monomeric Fc ϵ chain variants that can be used in the polypeptide chains of the invention.

Also, such monomeric Fc chain variants are preferably such that they are still capable of binding to the complement or the relevant Fc receptor(s) (depending on the Fc portion from which they are derived), and/or such that they still have some or all of the effector functions of the Fc portion from which they are derived (or at a reduced level still suitable for the intended use). Alternatively, in such a polypeptide chain of the invention, the monomeric Fc chain may be used to confer increased half-life upon the polypeptide chain, in which case the monomeric Fc chain may also have no or essentially no effector functions.

Bivalent/multivalent, bispecific/multispecific or biparatopic/multiparatopic
30 polypeptides of the invention may also be linked to Fc portions, in order to provide polypeptide constructs of the type that is described in the non-prepublished US provisional application entitled “*immunoglobulin constructs*” filed on December 4, 2007.

The further amino acid sequence may also form a sequence or signal that allows the 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 10 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 20 further description herein. One example is the so-called ADEPT™ technology described in WO 03/055527.

According to one preferred, but non-limiting aspect, 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 30 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.

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 Nanobody 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 Nanobody 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 GPCRs,) and at least one Nanobody is directed against a second antigen (i.e. different from GPCRs,), 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. GPCRs,) and at least one further Nanobody directed against a second antigen (i.e. different from GPCRs,), whereas a “trispecific” polypeptide of the invention is a polypeptide that comprises at least one Nanobody directed against a first antigen (i.e. GPCRs,), at least one further Nanobody directed against a second antigen (i.e. different from GPCRs,) and at least one further

Nanobody directed against a third antigen (i.e. different from both GPCRs, 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 GPCRs, 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 GPCRs, a second Nanobody directed against a second antigen and a third Nanobody directed
10 against a third antigen, in which said first, second and third Nanobody may optionally be linked via one or more, and in particular one and more, in particular two, linker sequences.

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 GPCRs, and any number of Nanobodies directed against one or more antigens different from GPCRs.

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 GPCRs, or against the one or more
20 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.
30 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 polypeptide 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. Such Nanobodies may for example be Nanobodies that are directed against a serum protein, and in particular a human serum protein, such as human serum albumin, thyroxine-binding protein, (human) transferrin, fibrinogen, an immunoglobulin such as IgG, IgE or IgM, or against one of the serum proteins listed in WO 04/003019. Of these, Nanobodies that can bind to serum albumin (and in particular human serum albumin) or to IgG (and in particular human IgG, see for example Nanobody VH-1 described in the review by Muyldermans, *supra*) are particularly preferred (although for
10 example, for experiments in mice or primates, Nanobodies against or cross-reactive with mouse serum albumin (MSA) or serum albumin from said primate, respectively, can be used. However, for pharmaceutical use, Nanobodies against human serum albumin or human IgG will usually be preferred). Nanobodies that provide for increased half-life and that can be used in the polypeptides of the invention include the Nanobodies directed against serum albumin that are described in WO 04/041865, in WO 06/122787 and in the further patent applications by Ablynx N.V., such as those mentioned above.

For example, the some preferred Nanobodies that provide for increased half-life for use in the present invention include Nanobodies that can bind to amino acid residues on (human) serum albumin that are not involved in binding of serum albumin to FcRn (see for
20 example WO 06/0122787); Nanobodies that are capable of binding to amino acid residues on serum albumin that do not form part of domain III of serum albumin (see for example WO 06/0122787); Nanobodies that have or can provide an increased half-life (see for example the US provisional application 60/843,349 by Ablynx N.V mentioned herein; see also PCT/EP2007/059475); Nanobodies 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, cynomolgus monkeys (*Macaca fascicularis*) and/or rhesus monkeys (*Macaca mulatta*)) and baboon (*Papio ursinus*)) (see for example the US provisional application 60/843,349 by Ablynx N.V; see also PCT/EP2007/059475); Nanobodies that can bind to
30 serum albumin in a pH independent manner (see for example the US provisional application 60/850,774 by Ablynx N.V. mentioned herein) and/or Nanobodies that are conditional binders (see for example the US provisional application 60/850,775 by Ablynx N.V. ; see also PCT/EP2007/060850).

Some particularly preferred Nanobodies that provide for increased half-life and that can be used in the polypeptides of the invention include the Nanobodies ALB-1 to ALB-10 disclosed in WO 06/122787 (see Tables II and III) of which ALB-8 (SEQ ID NO: 62 in WO 06/122787) is particularly preferred.

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.

Generally, any polypeptides of the invention with increased half-life that contain one or more Nanobodies of the invention, and any derivatives of Nanobodies of the invention or
10 of such polypeptides that have an 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 Nanobody of the invention per se. For example, such a derivative or polypeptides 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 Nanobody of the invention per se.

In a preferred, but non-limiting aspect of the invention, such derivatives or polypeptides may 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
20 more. For example, such derivatives or polypeptides 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).

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
30 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 and WO 06/040153, of which FC44 (SEQ ID NO: 189 of WO 06/040153) and FC5 (SEQ ID NO: 190 of WO 06/040154) are preferred examples.

10 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
20 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 $(gly_x ser_y)_z$, such as (for example $(gly_4 ser)_3$ or $(gly_3 ser_2)_3$, as
30 described in WO 99/42077 and the GS30, GS15, GS9 and GS7 linkers described in the applications by Ablynx mentioned herein (see for example WO 06/040153 and WO 06/122825), as well as 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 GS30 (SEQ ID NO: 85 in WO 06/122825) and GS9 (SEQ ID NO: 84 in WO 06/122825).

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 polypeptide of the invention, including but not limited to the affinity, specificity or avidity for GPCRs, 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 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 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.

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.

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 aspect of the invention, the polypeptide of the invention is in essentially isolated from, as defined herein.

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:

- i) 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:

- ii) 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:

- i) 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:
- ii) 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 aspect of the invention, the nucleic acid of the invention is in essentially isolated form, as defined herein.

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 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 form of GPCRs 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.

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
10 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”.

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
20 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 aspect, a genetic construct of the invention comprises

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

and optionally also

- iii) one or more further elements of genetic constructs known per se;

30 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 in the same reading frame. They will usually also be essentially contiguous, although this may also not be required.

20 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).

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
30 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.

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
10 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.

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
20 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.

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.
30 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.

10 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*;
- 20 - 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*,
30 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;

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.

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 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 5,589,546; 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.

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.

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 *Bombix 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.

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 (i.e. GMP grade) 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).

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 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.

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

According to one preferred, but non-limiting aspect 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 aspect 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 aspect of the invention, the 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
10 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 eukaryotic host cells are used, extracellular production is usually preferred since this considerably facilitates the further isolation and downstream processing of the Nanobodies
20 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 fewer contaminating proteins in the periplasm. Another advantage is that correct disulfide
30 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 an amino acid sequence, Nanobody or a polypeptide of the invention, can be used.

Thus, according to one non-limiting aspect 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.

According to another non-limiting aspect of the invention, the amino acid sequence,

10 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
20 (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
30 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);
- 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.

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 of 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.

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
20 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, 21st 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.

10 Preparations and formulations for oral administration may also be provided with an 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
10 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
20 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.
30 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 sequences, 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 GPCR-related diseases and 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 Nanobody 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 GPCRs, with its biological or pharmacological activity, and/or with the biological pathways or signalling in which GPCRs 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 GPCRs, its biological or pharmacological activity, and/or the biological pathways or signalling in which GPCRs 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 GPCRs, its biological or pharmacological activity, and/or the biological pathways or signalling in which GPCRs is involved; and/or an amount that provides a level of the amino acid sequence of the invention, of a Nanobody of the invention, of a polypeptide of the invention in the circulation that is sufficient to modulate GPCRs, its biological or pharmacological activity, and/or the biological pathways or signalling in which GPCRs is involved.

The invention furthermore 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 an amino acid sequence of the invention, a Nanobody of the invention or a 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 Nanobody 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 a Nanobody of the invention, of a polypeptide of the invention, and/or of a pharmaceutical composition comprising the same.

In another aspect, 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
10 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 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
20 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 sequence,
30 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 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.

The Nanobodies, amino acid sequences 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 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
10 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
20 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
30 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 GPCR-related diseases and disorders; and/or for use in one or more of the methods of treatment mentioned herein.

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 GPCR-related diseases and 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 amino acid sequences and in particular (single) domain antibodies against GPCRs, as well as polypeptides comprising such (single) domain antibodies.

For example, 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 such (single) domain antibodies or other protein 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, 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; 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.

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 GPCRs. Such immunoglobulin sequences directed against GPCRs can be generated in any manner known per se, as will be clear to the skilled person, i.e. by immunization with GPCRs or by screening a suitable library of immunoglobulin sequences with GPCRs, 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 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 GPCRs, 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 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 GPCRs 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 (qualitatively or quantitatively) the presence of GPCRs in a composition or preparation or as a marker to selectively detect the presence of GPCRs 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 in Figure 1 (which schematically shows the structure of the three different classes of GPCRs) and Figure 2 (which is a graph showing the screening results obtained in Example 3):

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Example 1: Immunization and library construction:

Mouse CB1R Membranes were purchased from Bioxtal (Mundolsheim, France).

N-terminal biotinylated MC4R peptide was purchased from NeoMPS S.A. (Strasbourg, France). Sequence: biotinyl-KTSLHLWNRSSYRLHS (SP061354; lot. Nr. CK01160). The peptide sequence is located in the extracellular part of MC4R.

Two llamas (086 and 087) were immunized with 6 boosts of refolded GPCRs (PTHR, CB1R) according to standard protocols. The proteins were refolded using a standard protocol, based on the techniques described by Kiefer (Biochim. Biophys. Acta, 1610 (2003), 57-62). Blood was collected from these animals after 7 and 10 days after boost 6

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Two llamas (100 and 101) were immunized with 6 boosts of KLH-NT peptide (derived from MC4R) according to Ablynx protocols. Blood was collected from these animals after 7 and 10 days after boost 6

Phage display libraries were prepared according to a standard protocol. Peripheral blood mononuclear cells were prepared from blood samples using Ficoll-Hypaque according to the manufacturer's instructions. Total RNA was extracted from these cells and used as starting material for RT-PCR to amplify Nanobody encoding gene fragments. These fragments were cloned into phagemid vector pAX50. Phage suspensions were prepared according to standard methods and stored after filter sterilization at 4°C prior to selections.

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Example 2: GPCR Nanobody selections:

Phage libraries were used for two rounds of selections on either refolded CB1R or on refolded PTHR. A second round on CB1R membranes was also performed for the CB1R Nanobody selection. All antigens were solid-phase immobilized on Nunc Maxisorp ELISA plates at 10 microg/well, 2 microg/well, 0.4 microg/well and 0 microg/well (background

control) for the first round of selection. For the second round of selection either 2 microg/well, 0.4 microg/well refolded GPCR was used or 5 microg/well and 1 microg/well CB1R membranes were used in the MaxiSorp plates. Interacting phages were retrieved from both first and second round of selections using triethylamine elution.

Individual clones were isolated and were grown on 10 ml or 50 ml scale, and induced by adding IPTG for nanobody expression. Periplasmic extracts (volume: 1 to 5 ml) were prepared according to standard methods.

Two phage libraries were used for selections for two rounds on biotinylated MC4R peptide. The peptide was captured by neutravidin which was solid-phase immobilized on Nunc Maxisorp ELISA plates at 500 ng/well. The peptide was coated in the first round of selection at a concentration ranging from 100 to 0.01 μ M. For the second round of selection the peptide was used at a concentration ranging from 5 to 0.05 μ M. Interacting phages were retrieved from both first and second round of selections using triethylamine elution. Individual clones were isolated and were grown 1 ml deep well plates, and induced by adding IPTG for nanobody expression. Periplasmic extracts were prepared according to standard methods.

Phage libraries 100 and 101 were used for selections for two rounds on biotinylated MC4R peptide. The peptide was captured by neutravidin which was solid-phase immobilized on Nunc Maxisorp ELISA plates at 500 ng/well. The peptide was coated in the first round of selection at a concentration ranging from 100 to 0.01 μ M. For the second round of selection the peptide was used at a concentration ranging from 5 to 0.05 μ M. Interacting phages were retrieved from both first and second round of selections using triethylamine elution.

Individual clones were isolated and were grown 1 ml deep well plates, and induced by adding IPTG for nanobody expression. Periplasmic extracts were prepared according to standard methods.

Example 3: Screening

For the determination of the binding specificity to the GPCRs, the clones were tested in a phage ELISA binding assay setup. 500 ng/well refolded GPCR or GPCR membrane was solid-phase immobilized on Maxisorp microtiter plates (Nunc), and free binding sites were blocked using 4% Marvel Milk in PBS. 10 μ l of phage suspension of the different clones in 100 μ l 2% Marvel PBS was incubated with the immobilized antigen. After 1 h incubation, non-bound phage was removed by washing with PBS-Tween, followed by PBS. Next mouse-anti-M13-HRP antibody was incubated with the interacting phages. After 1 h incubation, non-

bound phage was removed by washing with PBS-Tween, followed by PBS. Anti-M13 antibody presence was detected using TMB substrate (Pierce). Binding specificity was determined based on OD values compared to a control GPCR. The results are schematically shown in Figure 2.

The amino acid sequences were tested for activity against CB1R, PTHR1 and MC4R, respectively, using a standard radioligand assay (see for example Andre' et al., Protein Sci 5:1115 (2006); Hassaine et al., (2006) Prot Purif Expr 45:343; Nicholson et al. J Pharmacol Exp Ther. 2006 May;317(2):771-7. [Epub 2006 Jan 25]. and Vilardaga et al., J Biol Chem. 2001 Sep 7;276(36):33435-43. Epub 2001 May 31), for example using membrane
 10 preparations that can be made as described in Hovius et al., (1998) J Neurochem 70:824).

Example 3: Binding data

Binding of 4 clones directed against CB1R and of 4 clones against PTHR was measured on Biacore. The results are given in Tables B-2 and B-3.

Table B-2: Binding of clones against CB1R

	vHH6B7	vHH6H4	vHH5G11	vHH5D12
CB1R	12nM	36nM	12nM	non calculable

20 Table B-3: binding of clones on PTHR

	vHH22A3	vHH26F2	vHH23A3	vHH26A6	PTH(1-34) (ligand) 0.2-16nM
PTHR +CaCl2	9nM	70nM	NT	NT	
PTHR - CaCl2	17nM	/	22nM	17nM	4-23nM
PTHR Fos12	NT	360nM	NT	NT	260uM

Table B-4 gives the amino acid sequences of a number of Nanobodies against some representative GPCRs.

Table B-4: Nanobodies against some representative GPCRs.

- SEQ ID NO's 413 to 430, 517 and 518 : Nanobodies against CB1R
- SEQ ID NO's 431 to 453: Nanobodies against PTHR1
- SEQ ID NO's 519 to 525: Nanobodies against MCR4

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LGMP5_A6 SEQ ID NO 413 EVQLVESGGGLVQAGGSLRLACAASGSIFSFNAMGWYRQAPGKQRELL AVITRDGITKYADSVKGRFTISRDNKNTMYLQMNSLKPEDTAVYYCHTGLPT GRGSHSDYWGQGTQVTVSS
LGMP5_A7 SEQ ID NO 414 EVQLVESGGGLVQAGGSLRLSCAASGRTLSNYDMGWFRQAPGKEREFV ATIRWSTSSTYYADSVKGRFTISSDNTKNTVDLRMNSLTPEDTAVYYCAARSV SYEYNYWGQGTQVTVSS
LGMP5_B6 SEQ ID NO 415 EVQLVESGGGLVQAGGSLRLSCAASGRTLTYNYDMGWFRQAPGKEREFV ATIRWSTSETYYADSVKGRFTISKDNTKNTVALQMNSLTPEDTALYYCAARSV YSYEYNYWGQGTQVTVSS
LGMP5_B9 SEQ ID NO 416 EVQLVESGGGLVQAGGSLRLSCAASGRTLNNHDMGWFRQAPGKEREFV ATVRWGTSSTYYADSVKGRFTISSDNTKNTVALQMNRLTPEDTGVIYYCAARSV YSYEYTYWGQGTQVTVSS
LGMP5_D12 SEQ ID NO 417 EVQLVESGGGLVQAGGSLRLSCAASRRTFSSFVMAWFRQAPGKEREFV AAIYGTGGELVYYENSVKGRFTISRDNKSTMYLQMNSLKPEDTGVIYYCAVEL TVRSIDLRRPLEYDYWGQGTQVTVSS
LGMP5_F9 SEQ ID NO 418 EVQLVESGGGLVQAGGSLRLSCAASGRTLNYDMGWFRQAPGKEREFV ATIRWSTSETYYADSVKGRFTISSDNTKNTVDLQMNSLTPEDTAVYYCAARSV YSYEYNYWGQGTQVTVSS
LGMP5_G11 SEQ ID NO 419 EVQLVKSGGGLVQAGGSLRLSCAASGRTLSNYDMGWFRQAPGKEREFV ATIRWSTGETYYTDSVKGRFTISKDNTKNTVDLQMNSLTPEDTAVYYCASRSV YSYEYNYWGQGTQVTVSS

Table B-4 (continued):

LGMP6_B7 SEQ ID NO 420 EVQLVESGGGLVQAGGSLRLSCAASGRTLRNYDMGWFRQAPGKEREFV ATIRWSTSETYYADSVKGRFTISSDNAKNTVDLQMNSLTPGDTGVYYCAARSV YSYDYNWYGQGTQVTVSS
LGMP6_C7 SEQ ID NO 421 EVQLVESGGGLVQAGGSLRLSCAASGRTLSNYDVGWFRQAPGKEREFV ATIRWSTSTYYADPVKGRFTISSDNIKNTVDLQMNSLTPEDTAVYYCAARSV SYEYNYWYGQGTQVTVSS
LGMP6_C9 SEQ ID NO 422 EVQLVESGGGLVQAGGSLRLSCAASGRTLSNYDMGWFRQAPGKEREFV ATIRWSTSETYYADSVKGRFTISSNTKNTVDLQMNSLTPEDTAVYYCAARSV SYEYNYWYGQGTQVTVSS
LGMP6_D2 SEQ ID NO 423 EVQLMESGGGLVQAGGSLRLSCAASGRTLNTNYDMGWFRQAPGKEREFV ATIRWSTSETYYADSVKGRFTISKDNTKNTVALQMNSLTPEDTALYYCAARSV YSYEYNYWYGQGTQVTVSS
LGMP6_D5 SEQ ID NO 424 EVQLVESGGGLEQAGGSLRLSCAVSRISFETNTMAWFRQAPGKQRELVA RISSTGDTNYADSVKGRFTISRDNKNTVYLQMNSLKPEDTAVYYCHVAGPYG SKFAWYGQGTQVTVSS
LGMP6_D12 SEQ ID NO 425 EVQLVESGGGLVQAGGSLRLSCAASGRTLSNHDVGWFRQAPGKEREFV ATMRWSTGSTYYADSVKGRFTISSDNTKNTVDLQMNSLTPEDTAVYYCAARSV YSYEYNYWYGQGTQVTVSS
LGMP6_E11 SEQ ID NO 426 EVQLVESGGGLVQAGGSLRLSCAASGRTLRNYDLGWFRQAPGKEREFV ATIRWSTSETYYADSVKGRFTISSDNAKNTVDLQMNSLTPEDTGVYYCAARSV YSYDYNWYGQGTQVTVSS
LGMP6_F12 SEQ ID NO 427 EVQLVESGGGLVQAGGSLRLSCAASGRTLTHDMGWFRQAPGKEREFV ATIRWGTTDTYYADSVKGRFTISSDNTKNTVHLQMNSLTPEDTGVYYCAARSV YSYEYTYWYGQGTQVTVSS
LGMP6_H4 SEQ ID NO 428 EVQLVESGGGLVQAGGSLRLACAASGSIFSFNAMGWYRQAPGKQRELL AVITRDGITKYADSVKGRFTISRDNKNTMYLQMNSLKPEDTAVYYCHTGLPT GRGSHSDYWGQGTQVTVSS
LGMP6_H9 SEQ ID NO 429 EVQLVESGGGLVQAGGSLRLSCAASGRTLNDHDVGWFRQAPGKEREFV ATIRWSSKSTYYADSVKGRFTISSDNTKNTVDLRMNSLTPEDTAVYYCAARSV YSYEYNYWYGQGTQVTVSS

Table B-4 (continued):

LGPM6_H12	SEQ ID NO 430
EVQLVESGGGLVQAGGSLRLSCAASGRTLSNYDMGWFRQAPGKEREFV ATIRWSTRETYTDSVKGRFTISSDNTKNTVHLQMNSLTPEDTAVYYCAARSV YSYEYNYWGQGTQVTVSS	
PMPLG22-A2	SEQ ID NO 431
EVQLVESGGGLVQAGGSLRLSCSSSGFTSTDYAIGWFRQAPGKARTGVSI ISRSDGSTWYADSVKGRFTISSDNAKNTVYLQMNSLKPEDTAVYYCAAQLRHN FGMGDYDYWGQGTQVTVSS	
PMPLG22-A3	SEQ ID NO 432
EVQLVESGGGLVQAGGSLRLSCAASGNIFANNIMGWYRQPPGKEREFVA HVSHDGDSMYAVSVKGRFAISRKDATNLYLQMNSLKPEDTAIYFCRLLNIPTQG RMEGFWGQGTQVTVSS	
PMPLG22-A9	SEQ ID NO 433
EVQLVESGGGLVQPGGSLRLSCAASGFTLDGYAIGWFRQAPGKEREGVS CSNKDGSTAYSDSVKGRFTISRDNKNTVYLEMNGLPEDAAIYYCATKSGRY CLSHMDYWGIGTLVTVSS	
PMPLG22-B7	SEQ ID NO 434
EVQLVESGGGLVQTGGSLRLSCAASGMTFRLYALSWYRQAPGERREV ATITIDGKTNYAEPLKGRFTISRDNKNTIYLMNSLKPEDTAVYYCNAVFSRG PLTYWGRGTQVTVSS	
PMPLG22-B11	SEQ ID NO 435
EVQLVESGGGLVQAGGSLRLSCAASRSRSSFDDMGWYRQAPGKQREFV AGITLGGSTIYADSVKGRFTISMDSARNTGYLMNSLKPEDTAVYYCNAALDG YSGSSWGQGTQVTVSS	
PMPLG22-B12	SEQ ID NO 436
EVQLVESGGGLVQAGGSLRLSCVASGNIFSDNLMGWYRQAPGKQREFV AHMSHDGTTNYEDSVKGRFTISRENAKNTLYLHMNSLKPEDTAIYYCRLLTPT TGRAEGFWGQGTQVTVSS	
PMPLG22-C3	SEQ ID NO 437
EVQLVESGGGLVQAGGSLRLSCAASRSRSSFNDMGWYRQAPGKQREFV AGIPVGGSTVYADSVKGRFTISMDSARNRGYLMNSLKPEDTAVYYCNAYLDG YSGSSWGQGTQVTVSS	
PMPLG23-A3	SEQ ID NO 438
EVQLVESGGGLVQAGGSLRLSCAASGLTFSNYAMGWFRQAPGKEREW VASINWSGGSTYYEDSVEGRFTISRDNKNTVNLQMNSLKPEDTAVYYCAAKR GHYSREYDYWGQGTQVTVSS	
PMPLG23-C1	SEQ ID NO 439
KVQLVESGGGLVQAGGSLRLSCAASASISSAALGWYRQAPGKRREFVA GILSDGRELYVDSVKGRFTVSRDNKNTVYLQMNSLKPEDTAVYYCNLDIDYR DYWGQGTQVTVSS	

Table B-4 (continued):

PMPLG23-E12	SEQ ID NO 440
EVQLVESGGGLVQAGGSLRLSCAASASIISAAALGWYRQAPGRRREFVA GILSDGRELYVDSVKGRFTVSRDNAKNTVYLQMNSLKPEDTAVYYCNLDIDYR DYWGQGTQVTVSS	
PMPLG23-F2	SEQ ID NO 441
KVQLVESGGGLVQAGGSLRLSCAASGSIFSISLVGWYRQAPGKQREFVA HIYSDGSINYGNSVKGRFTISRDNKNTVDLQMNNLKPEDTAVYYCRLDITAR GVGYWGQGTQVTVSS	
PMPLG23-H9	SEQ ID NO 442
EVQLVESGGGLVQAGGSLRLSCAASASIISAAALGWYRQAPGKRREFVA GILSDGRELYVDSVKGRFTVSRDNAKNTVYLQMNSLKPEDTAVYYCNLDIDYR DYWGQGTQVTVSS	
PMPLG26-A2	SEQ ID NO 443
EVQLVESGGGLVQAGGALRLSCTASGITFKRYDMGWYRQAPGKEREW VTTITSEGTANSADSVKGRFTISRDNKNTVYLQMNSLKSEDTAVYYCNAQFTL ARHLVYWGQGTQVTVSS	
PMPLG26-A6	SEQ ID NO 444
EVQLVESGGGLVQAGGSLRLSCAASRSRSSFDDMGWYRQAPGKQREFV GGITLGGSTIYADSVKGRFTISMDSARNTGYLQMNSLKPEDTAVYYCNAALDG YSGSSWGQGTQVTVSS	
PMPLG26-B6	SEQ ID NO 445
EVQLVESGGGLVQAGGSLRLSCAASRSRSSFDDMGWYRQAPGKQREFV AGITLGGSTIYADSVKGRFTISMDSARNTGYLQMSSLKPEDTAVYYCNAVLDG YSGSSWGQGTQVTVSS	
PMPLG26-C4	SEQ ID NO 446
EVQLVESGGGLVQAGGSLRLSCAASGNIFANNIMGWYRQPPGKEREFVA HVSHDGYSMYAVSVEGRFTISRENATNLYLQMNSLKPEDTAIYFCRLLTIPPQG RMEGFWGQGTQVTVSS	
PMPLG26-C5	SEQ ID NO 447
EVQLVESGGGLVQAGGSLRLSCAASGNIFSDNIMGWYRQAPGKQREFV AHMSRDGTSNYEDSVKGRFTISRENAKNTLYLQMNSLKPEDTAIYYCRLLTIPT TGRKEGFWGQGTQVTVSS	
PMPLG26-D12	SEQ ID NO 448
EVQLVESGGGLVQAGGSLRLSCAASGSISNDNSMGWYRQAPGWQRELV AIKFSGGTTDIAESAAGRFTISRDNKNTVYLQMNSLRPEDTAVYYCRLFLPSLA MGYWGQGTQVTVSS	
PMPLG26-E4	SEQ ID NO 449
EVQLVESGGGLVQAGGSLRLSCAASGNIFANNIMGWYRQPPGKEREFVA HVSHDGDSMYAVSVKGRFTISRKDATNLYLQMNSLKPEDTAIYFCRLLNIPQTG RMEGFWGQGTQVTVSS	

Table B-4 (continued):

PMPLG26-E6 SEQ ID NO 450
EVQLVESGGGLVQAGGSLRLSCAASGNIFANNIMGWYRQPPGKEREFVA HVSDDGDSMYAVSVKGRFTISRKNATNLYLQMNSLKPEDTAIYFCRLLNIPQTQGR MEGFWGQGTQVTVSS
PMPLG26-F2 SEQ ID NO 451
EVQLVESGGGLVQAGGSLRLSCVASGFSFDDYAIGWFRQAPGQDREPLA CITSGGTRNYNPSVMARFRIDRDN SVKTVYLQINTLKPEDTAVYYCSTELDRQG GSAHECREYDTWGQGTQVTVSS
PMPLG26-G11 SEQ ID NO 452
EVQLVESGGGLVQAGGSLRLSCAASESISSEFNAMGWYRQGPGKLRELVA QITDLHKDYAESAKGRFTISRDN AKNTVDLQMNSLKPEDTGVYYCAA FRGIMR PDYWGQGTQVTVSS
PMPLG26-H11 SEQ ID NO 453
EVQLVESGGGLVQAGKSLSLSCAASDSRFSGRFNILNMGWYRQAPGKQ REWVTLLSTGSPYADSVKGRFTISR DHAKNTVYLQMHS LKPDDTANYICFAR LTPTGTVWGQGTQVTVSS
6F12 SEQ ID NO 517
EVQLVESGGGLVQAGGSLRLSCAASGRTLSDHDMGWFRQAPGKEREFV ATMRWGTSSSTYYADSVKGRFTISSDNTKNTVYLQMNSLTPEDTAVYYCAARSV YSYEYTYWGQGTQVTVSS
5D12 SEQ ID NO 518
EVQLVESGGGLVQAGGSLRLSCTASERTFSSYNMGWFRQAPGKERVFV AVIDWSGGLAYYADSVKGRFTISRDN AKKTVYLQMNSLKPEDTAVYYCAARS VYRGSTKPYEYDYWGQGTQVTVSS
60-11B SEQ ID NO 519
EVQLVESGGGLVQPGGSLRLSCAASGFTLDDYAVSWFRRAPGKEREGVS CISSSSSSDTYYADSVRGRFSISRDS AKNTVYLQMNSLKPEDTAVYYCAADRHC LGSDWDEYDYWGQGTQVTVSS
59-8A SEQ ID NO 520
EVQLVESGGGLVHAGDSLRLSCAASGFTLDDYAVSWFRQAPGKAREGL SCISAEDGSTYYADSVKGRFIISRDN AKNTVYLQMNSLKPEDTAVYYCAAEDSC LSSDYDAYDGDWGQGTQVTVSS
58-4H SEQ ID NO 521
EVQLVESGGGLAQPGGSLRLSCVASGFS LADY AISWFRQAPGKVREGVS CISSSDGSTYYADSVKGRFTISRDN AKNAVY LQMSTLKPEDTAVYYCAAERSCL SSDYDSYDAWGQGTQVTVSS
60-5E SEQ ID NO 522
EVQLVESGGGLVQPGGSLRLSCAASGFTLDDY AIGWFRQAPGKEREGVS CISSSDGSTYYADSVKGRFTISRDN AKNTVYLQMNSLKPEDTAVYYCAADLGY GSNCLEYDFWGQGTQVTVSS

Table B-4 (continued):

60-4A	SEQ ID NO 523	EVQLVESGGGLVQAGGSLRLSCAASGFTFDEYAIGWFRQAPGKEREGVS CISRSDGSTYYAPSVKGRFTISSDNAKNTVYLMNSLKPEDTAVYYCAVDLEYG SNCYEYDSWGQGTQVTVSS
58-10H	SEQ ID NO 524	EVQLVESGGGLVQPGGSMRLSCAASGSSLDAYGLGWFRQAPGKEREGV SCISGSDNSTYYADSVKGRFSISRDIKNTVYLMNGLKPEDTAVYYCAAKFLG FRQRSPTWFSRSWSEGSDYQYWGQGTQVTVSS
60-10E	SEQ ID NO 525	EVQLVESGGGLVQPGGSLRLSCAASGFTLDDYAIGWFRQAPGKEREGVS CITSSDGSTYYADSVKGRFTISRDNKNTVYMQNSLKPEDTAVYYCAADLGVG TQCDEYDYWGQGTQVTVSS

Table C: non-limiting list of some therapeutically relevant GPCRs (and desired action of an amino acid sequence, a Nanobody or a polypeptide of the invention).

Class A GPCRs

- Acetylcholine receptor (agonist),
- Muscarinic receptor (agonist),
- Muscarinic M1 receptor (agonist),
- Muscarinic M2 receptor (agonist),
- Muscarinic M3 receptor (agonist),
- 10 - Muscarinic M4 receptor (agonist),
- Muscarinic M5 receptor (agonist)
- Muscarinic receptor (partial agonist)
- Adrenoceptor (agonist),
- Alpha adrenoceptor (agonist),
- Alpha 1 adrenoceptor (agonist),
- Alpha 1A adrenoceptor (agonist),
- Alpha 1B adrenoceptor (agonist)
- Alpha 1D adrenoceptor (agonist)
- Alpha 2 adrenoceptor (agonist),
- 20 - Alpha 2A adrenoceptor (agonist),
- Alpha 2B adrenoceptor (agonist),
- Alpha 2C adrenoceptor (agonist),
- Alpha 2 adrenoceptor (partial agonist)
- Alpha 3 adrenoceptor (agonist),
- Beta adrenoceptor (agonist),
- Beta 1 adrenoceptor (agonist),
- Beta 2 adrenoceptor (agonist),
- Beta 3 adrenoceptor (agonist),
- Dopamine receptor (agonist),
- 30 - Dopamine D5 receptor (agonist)
- Dopamine D1 receptor (agonist),
- Dopamine D2 receptor (agonist),
- Dopamine D3 receptor (agonist),
- Dopamine D4 receptor (agonist),

- Histamine receptor (agonist),
- Histamine H1 receptor (agonist),
- Histamine H2 receptor (agonist),
- Histamine H3 receptor (agonist),
- Histamine H4 receptor (agonist),
- 5-HT GPCR (agonist),
- 5-HT 1 (agonist),
- 5-HT 2 (agonist),
- 5-HT 4 (agonist),
- 10 - 5-HT 5a (agonist),
- 5-HT 5b (agonist)
- 5-HT 6 (agonist),
- 5-HT 7 (agonist),
- Trace amine-associated receptor (agonist),
- Trace amine-associated receptor-1 (agonist),
- Trace amine-associated receptor-2 (agonist)
- Trace amine-associated receptor-3 (agonist)
- Trace amine-associated receptor-4 (agonist)
- Trace amine-associated receptor-5 (agonist)
- 20 - Trace amine-associated receptor-6 (agonist)
- Trace amine-associated receptor-7 (agonist)
- Trace amine-associated receptor-8 (agonist)
- Trace amine-associated receptor-9 (agonist)
- Apelin receptor (agonist),
- Cannabinoid receptor (agonist),
- Cannabinoid CB1 receptor (agonist),
- Cannabinoid CB2 receptor (agonist),
- Lysosphingolipid receptor (agonist),
- Sphingosine-1-phosphate receptor-1 (agonist),
- 30 - Lysophosphatidate-1 receptor (agonist)
- Sphingosine-1-phosphate receptor-3 (agonist),
- Lysophosphatidate-2 receptor (agonist)
- Sphingosine-1-phosphate receptor-2 (agonist)
- Sphingosine-1-phosphate receptor-4 (agonist),

- Lysophosphatidate-3 receptor (agonist)
- Sphingosine-1-phosphate receptor-5 (agonist)
- Class A hormone protein GPCR (agonist),
- FSH (agonist),
- Luteinizing hormone receptor (agonist),
- TSH (agonist),
- Leukotriene (agonist),
- Leukotriene BLT receptor (agonist),
- Cysteinyl leukotriene receptor (agonist),
- 10 - Melatonin (agonist),
- Melatonin MT1 (agonist),
- Melatonin MT2 (agonist),
- Melatonin MT3 (agonist)
- Class A nucleotide like GPCR (agonist),
- Adenosine receptor (agonist),
- P2Y receptor (agonist),
- Class A orphan GPCR (agonist),
- Ghrelin (agonist),
- Class A peptide GPCR (agonist),
- 20 - Angiotensin receptor (agonist),
- Angiotensin I receptor (agonist),
- Angiotensin II receptor (agonist),
- Bombesin receptor (agonist),
- Bombesin BB1 receptor (agonist)
- Bombesin BB2 receptor (agonist)
- Bombesin bb3 receptor (agonist),
- Gastrin releasing peptide ligand,
- Neuromedin B ligand
- Neuromedin C ligand
- 30 - Bradykinin receptor (agonist),
- Bradykinin B1 receptor (agonist),
- Bradykinin B2 receptor (agonist),
- C3a receptor (agonist),
- C5a (agonist),

- CCK receptor (agonist),
- CCK 1 receptor (agonist),
- CCK 2 receptor (agonist),
- Gastrin (agonist),
- Chemokine (agonist),
- CC chemokine receptor (agonist),
- CCR1 chemokine (agonist),
- CCR2 chemokine (agonist),
- CCR3 chemokine (agonist),
- 10 - CCR4 chemokine (agonist),
- CCR5 chemokine (agonist),
- CCR6 chemokine (agonist),
- CCR7 chemokine (agonist)
- CCR8 chemokine (agonist),
- CCR9 chemokine (agonist)
- CCR10 chemokine (agonist),
- CCR11 chemokine (agonist)
- CX3C chemokine receptor (agonist),
- CX3CR1 chemokine (agonist),
- 20 - XCR1 chemokine (agonist)
- CXC chemokine receptor (agonist),
- CXCR1 chemokine (agonist)
- CXCR3 chemokine (agonist),
- CXCR4 chemokine (agonist),
- CXCR5 chemokine (agonist)
- Adrenomedullin receptor (agonist),
- Endothelin (agonist),
- Endothelin ET-A (agonist),
- Endothelin ET-B (agonist),
- 30 - Galanin (agonist),
- Galanin GAL1 (agonist),
- Galanin GAL2 (agonist),
- Galanin GAL3 (agonist)
- IL-9 (agonist),

- KiSS-1 receptor (agonist),
- Melanin concentrating hormone (agonist),
- MCH receptor-1 (agonist)
- MCH receptor-2 (agonist)
- Melanocortin (agonist),
- Melanocortin MC1 (agonist),
- ACTH receptor (agonist),
- Melanocortin MC3 (agonist),
- Melanocortin MC4 (agonist),
- 10 - Melanocortin MC5 (agonist),
- NK (agonist),
- NK1 (agonist),
- NK2 (agonist)
- NK3 (agonist), Drugs: 1
- Neuropeptide Y receptor (agonist),
- Neuropeptide Y1 receptor (agonist)
- Neuropeptide Y2 receptor (agonist),
- Neuropeptide Y4 receptor (agonist),
- Neuropeptide Y5 receptor (agonist),
- 20 - Neuropeptide Y6 receptor (agonist)
- Neurotensin receptor (agonist),
- Neurotensin NTS1 (agonist),
- Neurotensin NTS2 (agonist)
- Orexin & neuropeptide FF receptor (agonist),
- Orexin (agonist),
- Opioid (agonist),
- Delta opioid (agonist),
- Kappa opioid (agonist),
- Mu opioid (agonist),
- 30 - ORL1 receptor (agonist),
- Opioid (partial agonist)
- Sigma opioid (agonist),
- Orexin & neuropeptide FF receptor (agonist),
- Neuropeptide FF receptor (agonist),

- Neuropeptide FF1 receptor (agonist)
- Neuropeptide FF2 receptor (agonist),
- Orexin (agonist),
- Orexin-1 (agonist)
- Orexin-2 (agonist)
- Protease-activated receptor (agonist),
- Protease-activated receptor-1 (agonist),
- Protease-activated receptor-2 (agonist),
- Protease-activated receptor-3 (agonist)
- 10 - Protease-activated receptor-4 (agonist)
- Prokineticin receptor (agonist),
- Prokineticin receptor-1 (agonist),
- Prokineticin receptor-2 (agonist),
- Somatostatin (agonist),
- Somatostatin 1 (agonist),
- Somatostatin 2 (agonist),
- Somatostatin 3 (agonist),
- Somatostatin 4 (agonist),
- Somatostatin 5 (agonist),
- 20 - Urotensin II (agonist),
- Vasopressin like receptor (agonist),
- Oxytocin (agonist),
- Vasopressin (agonist),
- Vasopressin V1 (agonist),
- Vasopressin V2 (agonist),
- Prostanoid receptor (agonist),
- DP prostanoid (agonist),
- PGD2 (agonist),
- EP1 prostanoid (agonist),
- 30 - PGE2 (agonist),
- EP2 prostanoid (agonist),
- PGE2 (agonist),
- EP3 prostanoid (agonist),
- PGE2 (agonist),

- EP4 prostanoid (agonist),
- PGE2 (agonist),
- FP prostanoid (agonist),
- PGF2 alpha (agonist),
- IP prostanoid (agonist),
- Prostacyclin (agonist),
- Prostanoid receptor (partial agonist)
- TP prostanoid (agonist),
- Thromboxane A2 (agonist)
- 10 - Succinate receptor 1 (agonist)
- TRH (agonist),
- TRH1 (agonist)
- TRH2 (agonist)
- Vomeronasal type-1 receptor (agonist)
- Vomeronasal type-1 receptor-1 (agonist)
- Vomeronasal type-1 receptor-2 (agonist)
- Vomeronasal type-1 receptor-3 (agonist)
- Vomeronasal type-1 receptor-4 (agonist)
- Vomeronasal type-1 receptor-5 (agonist)
- 20 - Apelin receptor (modulator),
- Cannabinoid receptor (modulator),
- Chemokine receptor-like 1 (modulator),
- Lysosphingolipid receptor (modulator),
- Class A hormone protein GPCR (modulator),
- Leukotriene receptor (modulator),
- Melatonin receptor (modulator),
- Class A nucleotide like GPCR (modulator),
- Class A orphan GPCR (modulator),
- PAF receptor (modulator),
- 30 - Class A peptide GPCR (modulator),
- Prostanoid receptor (modulator),
- Succinate receptor 1 (modulator)
- TRH receptor (modulator),
- Vomeronasal type-1 receptor (modulator),

Class B GPCRs

- G-protein coupled receptor-3 (modulator),
- G-protein coupled receptor-3 (agonist)
- G-protein coupled receptor-3 (antagonist),
- G-protein coupled receptor-6 (modulator),
- G-protein coupled receptor-6 (agonist)
- G-protein coupled receptor-6 (antagonist),
- G-protein coupled receptor-12 (modulator),
- 10 - G-protein coupled receptor-12 (agonist)
- G-protein coupled receptor-12 (antagonist),
- G-protein coupled receptor-14 (modulator)
- G-protein coupled receptor-14 (agonist)
- G-protein coupled receptor-14 (antagonist)
- Class B GPCR (agonist),
- CRF-1 receptor (agonist)
- CRF-2 receptor (agonist),
- Calcitonin receptor (modulator),
- Calcitonin (agonist),
- 20 - Calcitonin (antagonist),
- ACTH releasing factor receptor (modulator),
- CRF-1 receptor (modulator),
- CRF-1 receptor (agonist)
- CRF-1 receptor (antagonist),
- CRF-2 receptor (modulator),
- CRF-2 receptor (agonist),
- CRF-2 receptor (antagonist),
- ACTH releasing factor (agonist),
- CRF-1 receptor (agonist)
- 30 - CRF-2 receptor (agonist),
- ACTH releasing factor (antagonist),
- CRF-1 receptor (antagonist),
- CRF-2 receptor (antagonist),
- Glucagon-like peptide receptor (modulator),

- Glucagon-like peptide 1 receptor (modulator),
- Glucagon-like peptide 2 receptor (modulator),
- Glucagon-like peptide (agonist),
- Glucagon-like peptide (antagonist),
- Glucagon receptor (modulator),
- Glucagon (agonist),
- Glucagon (antagonist),
- GHRH receptor (modulator),
- GHRH (agonist),
- 10 - Growth hormone releasing factor (antagonist),
- PACAP type I receptor (modulator),
- PACAP type I receptor (agonist),
- PACAP type I receptor (antagonist)
- PTH receptor (modulator),
- PTH-1 receptor (modulator)
- PTH-2 receptor (modulator)
- PTH (agonist),
- PTH (antagonist),
- Secretin receptor (modulator),
- 20 - Secretin (agonist),
- Secretin (antagonist)
- VIP receptor (modulator),
- VIP-1 receptor (modulator),
- VIP-2 receptor (modulator),
- VIP (agonist),
- VIP (antagonist),

Class C GPCRs

- Class C GPCR (modulator),
- 30 - Class C GPCR (agonist),
- GABA B receptor (agonist),
- Metabotropic glutamate receptor (agonist),
- Metabotropic glutamate receptor 1 (agonist),
- Metabotropic glutamate receptor 2 (agonist),

- Metabotropic glutamate receptor 3 (agonist),
- Metabotropic glutamate receptor 4 (agonist),
- Metabotropic glutamate receptor 5 (agonist),
- Metabotropic glutamate receptor 6 (agonist)
- Metabotropic glutamate receptor 7 (agonist)
- Metabotropic glutamate receptor 8 (agonist)

Table D: non-limiting list human GPCRs

10	5HT1A_HUMAN	(P08908)	HTR1A	Serotonin type 1	Homo sapiens (Human)
	5HT1B_HUMAN	(P28222)	HTR1B	Serotonin type 1	Homo sapiens (Human)
	5HT1D_HUMAN	(P28221)	HTR1D	Serotonin type 1	Homo sapiens (Human)
	5HT1E_HUMAN	(P28566)	HTR1E	Serotonin type 1	Homo sapiens (Human)
	5HT1F_HUMAN	(P30939)	HTR1F	Serotonin type 1	Homo sapiens (Human)
	5HT2A_HUMAN	(P28223)	HTR2A	Serotonin type 2	Homo sapiens (Human)
	5HT2B_HUMAN	(P41595)	HTR2B	Serotonin type 2	Homo sapiens (Human)
	5HT2C_HUMAN	(P28335)	HTR2C	Serotonin type 2	Homo sapiens (Human)
	5HT4R_HUMAN	(Q13639)	HTR4	Serotonin type 4	Homo sapiens (Human)
	5HT5A_HUMAN	(P47898)	HTR5A	Serotonin type 5	Homo sapiens (Human)
20	5HT6R_HUMAN	(P50406)	HTR6	Serotonin type 6	Homo sapiens (Human)
	5HT7R_HUMAN	(P34969)	HTR7	Serotonin type 7	Homo sapiens (Human)
	AA1R_HUMAN	(P30542)	ADORA1	Adenosine type 1	Homo sapiens (Human)
	AA2AR_HUMAN	(P29274)	ADORA2A	Adenosine type 2	Homo sapiens (Human)
	AA2BR_HUMAN	(P29275)	ADORA2B	Adenosine type 2	Homo sapiens (Human)
	AA3R_HUMAN	(P33765)	ADORA3	Adenosine type 3	Homo sapiens (Human)
	ACM1_HUMAN	(P11229)	CHRM1	Musc. acetylcholine Vertebrate type 1	Homo sapiens (Human)
	ACM2_HUMAN	(P08172)	CHRM2	Musc. acetylcholine Vertebrate type 2	Homo sapiens (Human)
	ACM3_HUMAN	(P20309)	CHRM3	Musc. acetylcholine Vertebrate type 3	Homo sapiens (Human)
	ACM4_HUMAN	(P08173)	CHRM4	Musc. acetylcholine Vertebrate type 4	Homo sapiens (Human)
30	ACM5_HUMAN	(P08912)	CHRM5	Musc. acetylcholine Vertebrate type 5	Homo sapiens (Human)
	ACTHR_HUMAN	(Q01718)	MC2R	Adrenocorticotropic hormone	Homo sapiens (Human)
	ADA1A_HUMAN	(P35348)	ADRA1A	Alpha Adrenoceptors type 1	Homo sapiens (Human)
	ADA1B_HUMAN	(P35368)	ADRA1B	Alpha Adrenoceptors type 1	Homo sapiens (Human)
	ADA1D_HUMAN	(P25100)	ADRA1D	Alpha Adrenoceptors type 1	Homo sapiens (Human)
	ADA2A_HUMAN	(P08913)	ADRA2A	Alpha Adrenoceptors type 2	Homo sapiens (Human)
	ADA2B_HUMAN	(P18089)	ADRA2B	Alpha Adrenoceptors type 2	Homo sapiens (Human)
	ADA2C_HUMAN	(P18825)	ADRA2C	Alpha Adrenoceptors type 2	Homo sapiens (Human)
	ADMR_HUMAN	(O15218)	ADMR	Adrenomedullin (G10D)	Homo sapiens (Human)
	ADRB1_HUMAN	(P08588)	ADRB1	Beta Adrenoceptors type 1	Homo sapiens (Human)
40	ADRB2_HUMAN	(P07550)	ADRB2	Beta Adrenoceptors type 2	Homo sapiens (Human)
	ADRB3_HUMAN	(P13945)	ADRB3	Beta Adrenoceptors type 3	Homo sapiens (Human)
	AGTR1_HUMAN	(P30556)	AGTR1	Angiotensin type 1	Homo sapiens (Human)
	AGTR2_HUMAN	(P50052)	AGTR2	Angiotensin type 2	Homo sapiens (Human)
	APJ_HUMAN	(P35414)	AGTRL1	APJ like	Homo sapiens (Human)
	BAI1_HUMAN	(O14514)	BAI1	Brain-specific angiogenesis inhibitor (BAI)	Homo sapiens (Human)
	BAI2_HUMAN	(O60241)	BAI2	Brain-specific angiogenesis inhibitor (BAI)	Homo sapiens (Human)
	BAI3_HUMAN	(O60242)	BAI3	Brain-specific angiogenesis inhibitor (BAI)	Homo sapiens (Human)
	BKRB1_HUMAN	(P46663)	BDKRB1	Bradykinin	Homo sapiens (Human)
	BKRB2_HUMAN	(P30411)	BDKRB2	Bradykinin	Homo sapiens (Human)
	BRS3_HUMAN	(P32247)	BRS3	Bombesin	Homo sapiens (Human)
	C3AR_HUMAN	(Q16581)	C3AR1	C5a anaphylatoxin	Homo sapiens (Human)
	C5ARL_HUMAN	(Q9P296)	GPR77	C5a anaphylatoxin	Homo sapiens (Human)
	C5AR_HUMAN	(P21730)	C5AR1	C5a anaphylatoxin	Homo sapiens (Human)
	CALCR_HUMAN	(P30988)	CALCR	Calcitonin	Homo sapiens (Human)
	CALRL_HUMAN	(Q16602)	CALCRL	Calcitonin	Homo sapiens (Human)
	CASR_HUMAN	(P41180)	CASR	Extracellular calcium-sensing	Homo sapiens (Human)

	CCBP2_HUMAN (O00590) CCBP2 C-C Chemokine type X Homo sapiens (Human)
	CCKAR_HUMAN (P32238) CCKAR CCK type A Homo sapiens (Human)
	CCR10_HUMAN (P46092) CCR10 C-C Chemokine type 10 Homo sapiens (Human)
	CCR1_HUMAN (P32246) CCR1 C-C Chemokine type 1 Homo sapiens (Human)
	CCR2_HUMAN (P41597) CCR2 C-C Chemokine type 2 Homo sapiens (Human)
	CCR3_HUMAN (P51677) CCR3 C-C Chemokine type 3 Homo sapiens (Human)
	CCR4_HUMAN (P51679) CCR4 C-C Chemokine type 4 Homo sapiens (Human)
	CCR5_HUMAN (P51681) CCR5 C-C Chemokine type 5 Homo sapiens (Human)
	CCR6_HUMAN (P51684) CCR6 C-C Chemokine type 6 Homo sapiens (Human)
10	CCR7_HUMAN (P32248) CCR7 C-C Chemokine type 7 Homo sapiens (Human)
	CCR8_HUMAN (P51685) CCR8 C-C Chemokine type 8 Homo sapiens (Human)
	CCR9_HUMAN (P51686) CCR9 C-C Chemokine type 9 Homo sapiens (Human)
	CCRL1_HUMAN (Q9NPB9) CCRL1 C-C Chemokine type 11 Homo sapiens (Human)
	CD97_HUMAN (P48960) CD97 EMR1 Homo sapiens (Human)
	CELR1_HUMAN (Q9NYQ6) CELSR1 Cadherin EGF LAG (CELSR) Homo sapiens (Human)
	CELR2_HUMAN (Q9HCU4) CELSR2 Cadherin EGF LAG (CELSR) Homo sapiens (Human)
	CELR3_HUMAN (Q9NYQ7) CELSR3 Cadherin EGF LAG (CELSR) Homo sapiens (Human)
	CLTR1_HUMAN (Q9Y271) CYSLTR1 Cysteinyl leukotriene Homo sapiens (Human)
	CLTR2_HUMAN (Q9NS75) CYSLTR2 Cysteinyl leukotriene Homo sapiens (Human)
20	CML1_HUMAN (Q99788) CMKLR1 Chemokine receptor-like 1 Homo sapiens (Human)
	CML2_HUMAN (Q99527) GPR30 Chemokine receptor-like 2 Homo sapiens (Human)
	CNR1_HUMAN (P21554) CNR1 Cannabinoid Homo sapiens (Human)
	CNR2_HUMAN (P34972) CNR2 Cannabinoid Homo sapiens (Human)
	CRFR1_HUMAN (P34998) CRHR1 Corticotropin releasing factor Homo sapiens (Human)
	CRFR2_HUMAN (Q13324) CRHR2 Corticotropin releasing factor Homo sapiens (Human)
	CX3C1_HUMAN (P49238) CX3CR1 C-X3-C Chemokine Homo sapiens (Human)
	CXCR1_HUMAN (P25024) IL8RA Interleukin-8 type A Homo sapiens (Human)
	CXCR3_HUMAN (P49682) CXCR3 C-X-C Chemokine type 3 Homo sapiens (Human)
	CXCR4_HUMAN (P61073) CXCR4 C-X-C Chemokine type 4 Homo sapiens (Human)
30	CXCR5_HUMAN (P32302) BLR1 C-X-C Chemokine type 5 Homo sapiens (Human)
	CXCR6_HUMAN (O00574) CXCR6 C-X-C Chemokine type 6 (Bonzo) Homo sapiens (Human)
	DRD1_HUMAN (P21728) DRD1 Dopamine Vertebrate type 1 Homo sapiens (Human)
	DRD2_HUMAN (P14416) DRD2 Dopamine Vertebrate type 2 Homo sapiens (Human)
	DRD3_HUMAN (P35462) DRD3 Dopamine Vertebrate type 3 Homo sapiens (Human)
	DRD4_HUMAN (P21917) DRD4 Dopamine Vertebrate type 4 Homo sapiens (Human)
	DRD5_HUMAN (P21918) DRD5 Dopamine Vertebrate type 1 Homo sapiens (Human)
	DUFFY_HUMAN (Q16570) DARC Duffy antigen Homo sapiens (Human)
	EBI2_HUMAN (P32249) EBI2 EBV-induced Homo sapiens (Human)
	EDG1_HUMAN (P21453) EDG1 Sphingosine 1-phosphate Edg-1 Homo sapiens (Human)
40	EDG2_HUMAN (Q92633) EDG2 Lysophosphatidic acid Edg-2 Homo sapiens (Human)
	EDG3_HUMAN (Q99500) EDG3 Sphingosine 1-phosphate Edg-3 Homo sapiens (Human)
	EDG4_HUMAN (Q9HBW0) EDG4 Lysophosphatidic acid Edg-4 Homo sapiens (Human)
	EDG5_HUMAN (O95136) EDG5 Sphingosine 1-phosphate Edg-5 Homo sapiens (Human)
	EDG6_HUMAN (O95977) EDG6 Sphingosine 1-phosphate Edg-6 Homo sapiens (Human)
	EDG7_HUMAN (Q9UBY5) EDG7 Lysophosphatidic acid Edg-7 Homo sapiens (Human)
	EDG8_HUMAN (Q9H228) EDG8 Sphingosine 1-phosphate Edg-8 Homo sapiens (Human)
	EDNRA_HUMAN (P25101) EDNRA Endothelin Homo sapiens (Human)
	EDNRB_HUMAN (P24530) EDNRB Endothelin Homo sapiens (Human)
	ELTD1_HUMAN (Q9HBW9) ELTD1 ETL receptors Homo sapiens (Human)
50	EMR1_HUMAN (Q14246) EMR1 EMR1 Homo sapiens (Human)

	EMR2_HUMAN (Q9UHX3)	EMR2	EMR1	Homo sapiens (Human)
	EMR3_HUMAN (Q9BY15)	EMR3	EMR1	Homo sapiens (Human)
	EMR4_HUMAN (Q86SQ3)	EMR4	fragments	Homo sapiens (Human)
	ETBR2_HUMAN (O60883)	GPR37L1	GPR37 / endothelin B-like	Homo sapiens (Human)
	FFAR1_HUMAN (O14842)	FFAR1	Free fatty acid receptor (GP40,GP41,GP43)	Homo sapiens (Human)
	FFAR2_HUMAN (O15552)	FFAR2	Free fatty acid receptor (GP40,GP41,GP43)	Homo sapiens (Human)
	FFAR3_HUMAN (O14843)	FFAR3	Free fatty acid receptor (GP40,GP41,GP43)	Homo sapiens (Human)
	FPR1_HUMAN (P21462)	FPR1	Fmet-leu-phe	Homo sapiens (Human)
	FPRL1_HUMAN (P25090)	FPRL1	Fmet-leu-phe	Homo sapiens (Human)
10	FPRL2_HUMAN (P25089)	FPRL2	Fmet-leu-phe	Homo sapiens (Human)
	FSHR_HUMAN (P23945)	FSHR	Follicle stimulating hormone	Homo sapiens (Human)
	FZD10_HUMAN (Q9ULW2)	FZD10	frizzled Group A (Fz 1&2&4&5&7-9)	Homo sapiens (Human)
	FZD1_HUMAN (Q9UP38)	FZD1	frizzled Group A (Fz 1&2&4&5&7-9)	Homo sapiens (Human)
	FZD2_HUMAN (Q14332)	FZD2	frizzled Group A (Fz 1&2&4&5&7-9)	Homo sapiens (Human)
	FZD3_HUMAN (Q9NPG1)	FZD3	frizzled Group B (Fz 3 & 6)	Homo sapiens (Human)
	FZD4_HUMAN (Q9ULV1)	FZD4	frizzled Group A (Fz 1&2&4&5&7-9)	Homo sapiens (Human)
	FZD5_HUMAN (Q13467)	FZD5	frizzled Group A (Fz 1&2&4&5&7-9)	Homo sapiens (Human)
	FZD6_HUMAN (O60353)	FZD6	frizzled Group B (Fz 3 & 6)	Homo sapiens (Human)
	FZD7_HUMAN (O75084)	FZD7	frizzled Group A (Fz 1&2&4&5&7-9)	Homo sapiens (Human)
20	FZD8_HUMAN (Q9H461)	FZD8	frizzled Group A (Fz 1&2&4&5&7-9)	Homo sapiens (Human)
	FZD9_HUMAN (O00144)	FZD9	frizzled Group A (Fz 1&2&4&5&7-9)	Homo sapiens (Human)
	G109A_HUMAN (Q8TDS4)	GPR109A	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	G109B_HUMAN (P49019)	GPR109B	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GABR1_HUMAN (Q9UBS5)	GABBR1	GABA-B subtype 1	Homo sapiens (Human)
	GABR2_HUMAN (O75899)	GABBR2	GABA-B subtype 2	Homo sapiens (Human)
	GALR1_HUMAN (P47211)	GALR1	Galanin	Homo sapiens (Human)
	GALR2_HUMAN (O43603)	GALR2	Galanin	Homo sapiens (Human)
	GALR3_HUMAN (O60755)	GALR3	Galanin	Homo sapiens (Human)
	GASR_HUMAN (P32239)	CCKBR	CCK type B	Homo sapiens (Human)
30	GHRHR_HUMAN (Q02643)	GHRHR	Growth hormone-releasing hormone	Homo sapiens (Human)
	GHSR_HUMAN (Q92847)	GHSR	Growth hormone secretagogue	Homo sapiens (Human)
	GIPR_HUMAN (P48546)	GIPR	Gastric inhibitory peptide	Homo sapiens (Human)
	GLP1R_HUMAN (P43220)	GLP1R	Glucagon	Homo sapiens (Human)
	GLP2R_HUMAN (O95838)	GLP2R	Glucagon	Homo sapiens (Human)
	GLR_HUMAN (P47871)	GCGR	Glucagon	Homo sapiens (Human)
	GNRHR_HUMAN (P30968)	GNRHR	Gonadotropin-releasing hormone type I	Homo sapiens (Human)
	GNRR2_HUMAN (Q96P88)	GNRHR2	Gonadotropin-releasing hormone type II	Homo sapiens (Human)
	GP101_HUMAN (Q96P66)	GPR101	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GP107_HUMAN (Q5VW38)	GPR107	Putative / unclassified other	Homo sapiens (Human)
40	GP110_HUMAN (Q5T601)	GPR110	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	GP111_HUMAN (Q8IZF7)	GPR111	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	GP112_HUMAN (Q8IZF6)	GPR112	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	GP113_HUMAN (Q8IZF5)	GPR113	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	GP114_HUMAN (Q8IZF4)	GPR114	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	GP115_HUMAN (Q8IZF3)	GPR115	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	GP116_HUMAN (Q8IZF2)	GPR116	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	GP119_HUMAN (Q8TDV5)	GPR119	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GP120_HUMAN (Q5NUL3)	GPR120	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GP123_HUMAN (Q86SQ6)	GPR123	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
50	GP124_HUMAN (Q96PE1)	GPR124	Putative / unclassified Class B GPCRs	Homo sapiens (Human)

10	GP125_HUMAN	(Q8IWK6)	GPR125	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	GP126_HUMAN	(Q86SQ4)	GPR126	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	GP128_HUMAN	(Q96K78)	GPR128	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	GP132_HUMAN	(Q9UNW8)	GPR132	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GP133_HUMAN	(Q6QNK2)	GPR133	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	GP135_HUMAN	(Q8IZ08)	GPR135	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GP139_HUMAN	(Q6DWJ6)	GPR139	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GP141_HUMAN	(Q7Z602)	GPR141	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GP142_HUMAN	(Q7Z601)	GPR142	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GP143_HUMAN	(P51810)	GPR143	Ocular albinism proteins	Homo sapiens (Human)
20	GP144_HUMAN	(Q7Z7M1)	GPR144	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	GP146_HUMAN	(Q96CH1)	GPR146	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GP148_HUMAN	(Q8TDV2)	GPR148	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GP149_HUMAN	(Q86SP6)	GPR149	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GP150_HUMAN	(Q8NGU9)	GPR150	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GP151_HUMAN	(Q8TDV0)	GPR151	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GP152_HUMAN	(Q8TDT2)	GPR152	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GP153_HUMAN	(Q6NV75)	GPR153	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GP154_HUMAN	(Q6W5P4)	GPR154	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GP155_HUMAN	(Q7Z3F1)	GPR155	Putative / unclassified other	Homo sapiens (Human)
30	GP156_HUMAN	(Q8NFN8)	GPR156	GABA-B like	Homo sapiens (Human)
	GP157_HUMAN	(Q5UAW9)	GPR157	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	GP158_HUMAN	(Q5T848)	GPR158	Putative / unclassified Class C GPCRs	Homo sapiens (Human)
	GP160_HUMAN	(Q9UJ42)	GPR160	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GP161_HUMAN	(Q8N6U8)	GPR161	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GP162_HUMAN	(Q16538)	GPR162	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GP171_HUMAN	(O14626)	GPR171	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GP173_HUMAN	(Q9NS66)	GPR173	SREB	Homo sapiens (Human)
	GP174_HUMAN	(Q9BXC1)	GPR174	Purinoreceptor P2RY5,8,9,10	GPR35,92,174 Homo sapiens (Human)
	GP175_HUMAN	(Q86W33)	GPR175	Putative / unclassified other	Homo sapiens (Human)
40	GP176_HUMAN	(Q14439)	GPR176	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GP179_HUMAN	(Q6PRD1)	GPR179	Putative / unclassified Class C GPCRs	Homo sapiens (Human)
	GPBAR_HUMAN	(Q8TDU6)	GPBAR1	G-protein coupled bile acid receptor	Homo sapiens (Human)
	GPC5B_HUMAN	(Q9NZH0)	GPRC5B	Orphan GPRC5	Homo sapiens (Human)
	GPC5C_HUMAN	(Q9NQ84)	GPRC5C	Orphan GPRC5	Homo sapiens (Human)
	GPC5D_HUMAN	(Q9NZD1)	GPRC5D	Orphan GPRC5	Homo sapiens (Human)
	GPC6A_HUMAN	(Q5T6X5)	GPRC6A	Orphan GPCR6	Homo sapiens (Human)
	GPR12_HUMAN	(P47775)	GPR12	GPR	Homo sapiens (Human)
	GPR15_HUMAN	(P49685)	GPR15	GPR	Homo sapiens (Human)
	GPR17_HUMAN	(Q13304)	GPR17	GPR	Homo sapiens (Human)
50	GPR18_HUMAN	(Q14330)	GPR18	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GPR19_HUMAN	(Q15760)	GPR19	GPR	Homo sapiens (Human)
	GPR1_HUMAN	(P46091)	GPR1	GPR	Homo sapiens (Human)
	GPR20_HUMAN	(Q99678)	GPR20	GPR	Homo sapiens (Human)
	GPR21_HUMAN	(Q99679)	GPR21	GPR	Homo sapiens (Human)
	GPR22_HUMAN	(Q99680)	GPR22	GPR	Homo sapiens (Human)
	GPR25_HUMAN	(O00155)	GPR25	GPR	Homo sapiens (Human)
	GPR26_HUMAN	(Q8NDV2)	GPR26	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GPR27_HUMAN	(Q9NS67)	GPR27	SREB	Homo sapiens (Human)

	GPR31_HUMAN	(O00270)	GPR31	GPR	Homo sapiens (Human)
	GPR32_HUMAN	(O75388)	GPR32	Chemokine receptor-like 1	Homo sapiens (Human)
	GPR33_HUMAN	(Q49SQ1)	GPR33	Chemokine receptor-like 1	Homo sapiens (Human)
	GPR34_HUMAN	(Q9UPC5)	GPR34	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GPR35_HUMAN	(Q9HC97)	GPR35	Purinoreceptor P2RY5,8,9,10 GPR35,92,174	Homo sapiens (Human)
	GPR37_HUMAN	(O15354)	GPR37	GPR37 / endothelin B-like	Homo sapiens (Human)
	GPR39_HUMAN	(O43194)	GPR39	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GPR3_HUMAN	(P46089)	GPR3	GPR	Homo sapiens (Human)
	GPR42_HUMAN	(O15529)	GPR42	Free fatty acid receptor (GP40,GP41,GP43)	Homo sapiens (Human)
10	GPR44_HUMAN	(Q9Y5Y4)	GPR44	Chemokine receptor-like 1	Homo sapiens (Human)
	GPR45_HUMAN	(Q9Y5Y3)	GPR45	GPR45 like	Homo sapiens (Human)
	GPR4_HUMAN	(P46093)	GPR4	GPR	Homo sapiens (Human)
	GPR52_HUMAN	(Q9Y2T5)	GPR52	GPR	Homo sapiens (Human)
	GPR55_HUMAN	(Q9Y2T6)	GPR55	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GPR56_HUMAN	(Q9Y653)	GPR56	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	GPR61_HUMAN	(Q9BZJ8)	GPR61	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GPR62_HUMAN	(Q9BZJ7)	GPR62	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GPR63_HUMAN	(Q9BZJ6)	GPR63	GPR45 like	Homo sapiens (Human)
	GPR64_HUMAN	(Q8IZP9)	GPR64	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
20	GPR6_HUMAN	(P46095)	GPR6	GPR	Homo sapiens (Human)
	GPR75_HUMAN	(O95800)	GPR75	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GPR78_HUMAN	(Q96P69)	GPR78	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GPR81_HUMAN	(Q9BXC0)	GPR81	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GPR82_HUMAN	(Q96P67)	GPR82	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GPR83_HUMAN	(Q9NYM4)	GPR83	Neuropeptide Y other	Homo sapiens (Human)
	GPR84_HUMAN	(Q9NQS5)	GPR84	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GPR85_HUMAN	(P60893)	GPR85	SREB	Homo sapiens (Human)
	GPR87_HUMAN	(Q9BY21)	GPR87	Purinoreceptor P2RY12-14 GPR87 (UDP-Glucose)	Homo sapiens (Human)
30	GPR88_HUMAN	(Q9GZN0)	GPR88	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	GPR92_HUMAN	(Q9H1C0)	GPR92	Purinoreceptor P2RY5,8,9,10 GPR35,92,174	Homo sapiens (Human)
	GPR97_HUMAN	(Q86Y34)	GPR97	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	GRPR_HUMAN	(P30550)	GRPR	Bombesin	Homo sapiens (Human)
	HRH1_HUMAN	(P35367)	HRH1	Histamine type 1	Homo sapiens (Human)
	HRH2_HUMAN	(P25021)	HRH2	Histamine type 2	Homo sapiens (Human)
	HRH3_HUMAN	(Q9Y5N1)	HRH3	Histamine type 3	Homo sapiens (Human)
	HRH4_HUMAN	(Q9H3N8)	HRH4	Histamine type 4	Homo sapiens (Human)
	KISSR_HUMAN	(Q969F8)	KISS1R	Kiss receptor (GPR54)	Homo sapiens (Human)
	LGR4_HUMAN	(Q9BXB1)	LGR4	LGR like (hormone receptors)	Homo sapiens (Human)
40	LGR5_HUMAN	(O75473)	LGR5	LGR like (hormone receptors)	Homo sapiens (Human)
	LGR6_HUMAN	(Q9HBX8)	LGR6	LGR like (hormone receptors)	Homo sapiens (Human)
	LGR8_HUMAN	(Q8WXD0)	LGR8	LGR like (hormone receptors)	Homo sapiens (Human)
	LPHN1_HUMAN	(O94910)	LPHN1	Latrophilin type 1	Homo sapiens (Human)
	LPHN2_HUMAN	(O95490)	LPHN2	Latrophilin type 2	Homo sapiens (Human)
	LPHN3_HUMAN	(Q9HAR2)	LPHN3	Latrophilin type 3	Homo sapiens (Human)
	LSHR_HUMAN	(P22888)	LHCGR	Lutropin-choriogonadotropic hormone	Homo sapiens (Human)
	LT4R1_HUMAN	(Q15722)	LTB4R	Leukotriene B4 receptor BLT1	Homo sapiens (Human)
	LT4R2_HUMAN	(Q9NPC1)	LTB4R2	Leukotriene B4 receptor BLT2	Homo sapiens (Human)
50	MAS1L_HUMAN	(P35410)	MAS1L	Mas proto-oncogene & Mas-related (MRGs)	Homo sapiens (Human)

	MASS1_HUMAN (Q8WXG9)	MASS1	Very large G-protein coupled receptor	Homo sapiens (Human)
	MAS_HUMAN (P04201)	MAS1	Mas proto-oncogene & Mas-related (MRGs)	Homo sapiens (Human)
	MC3R_HUMAN (P41968)	MC3R	Melanocortin hormone	Homo sapiens (Human)
	MC4R_HUMAN (P32245)	MC4R	Melanocortin hormone	Homo sapiens (Human)
	MC5R_HUMAN (P33032)	MC5R	Melanocortin hormone	Homo sapiens (Human)
	MCHR1_HUMAN (Q99705)	MCHR1	Melanin-concentrating hormone receptors	Homo sapiens (Human)
	MCHR2_HUMAN (Q969V1)	MCHR2	Melanin-concentrating hormone receptors	Homo sapiens (Human)
10	MGR1_HUMAN (Q13255)	GRM1	Metabotropic glutamate group I	Homo sapiens (Human)
	MGR2_HUMAN (Q14416)	GRM2	Metabotropic glutamate group II	Homo sapiens (Human)
	MGR3_HUMAN (Q14832)	GRM3	Metabotropic glutamate group II	Homo sapiens (Human)
	MGR4_HUMAN (Q14833)	GRM4	Metabotropic glutamate group III	Homo sapiens (Human)
	MGR5_HUMAN (P41594)	GRM5	Metabotropic glutamate group I	Homo sapiens (Human)
	MGR6_HUMAN (O15303)	GRM6	Metabotropic glutamate group III	Homo sapiens (Human)
	MGR7_HUMAN (Q14831)	GRM7	Metabotropic glutamate group III	Homo sapiens (Human)
	MGR8_HUMAN (O00222)	GRM8	Metabotropic glutamate group III	Homo sapiens (Human)
	MRGRD_HUMAN (Q8TDS7)	MRGPRD	Mas proto-oncogene & Mas-related (MRGs)	Homo sapiens (Human)
20	MRGRE_HUMAN (Q86SM8)	MRGPRE	fragments	Homo sapiens (Human)
	MRGRF_HUMAN (Q96AM1)	MRGPRF	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	MRGRG_HUMAN (Q86SM5)	MRGPRG	fragments	Homo sapiens (Human)
	MRGX1_HUMAN (Q96LB2)	MRGPRX1	Mas proto-oncogene & Mas-related (MRGs)	Homo sapiens (Human)
	MRGX2_HUMAN (Q96LB1)	MRGPRX2	Mas proto-oncogene & Mas-related (MRGs)	Homo sapiens (Human)
	MRGX3_HUMAN (Q96LB0)	MRGPRX3	Mas proto-oncogene & Mas-related (MRGs)	Homo sapiens (Human)
	MRGX4_HUMAN (Q96LA9)	MRGPRX4	Mas proto-oncogene & Mas-related (MRGs)	Homo sapiens (Human)
30	MSHR_HUMAN (Q01726)	MC1R	Melanocyte stimulating hormone	Homo sapiens (Human)
	MTLR_HUMAN (O43193)	MLNR	Growth hormone secretagogue like	Homo sapiens (Human)
	MTR1A_HUMAN (P48039)	MTNR1A	Melatonin	Homo sapiens (Human)
	MTR1B_HUMAN (P49286)	MTNR1B	Melatonin	Homo sapiens (Human)
	MTR1L_HUMAN (Q13585)	GPR50	Melatonin	Homo sapiens (Human)
	NK1R_HUMAN (P25103)	TACR1	Substance P (NK1)	Homo sapiens (Human)
	NK2R_HUMAN (P21452)	TACR2	Substance K (NK2)	Homo sapiens (Human)
	NK3R_HUMAN (P29371)	TACR3	Neuromedin K (NK3)	Homo sapiens (Human)
	NMBR_HUMAN (P28336)	NMBR	Bombesin	Homo sapiens (Human)
40	NMUR1_HUMAN (Q9HB89)	NMUR1	Neuromedin U	Homo sapiens (Human)
	NMUR2_HUMAN (Q9GZQ4)	NMUR2	Neuromedin U	Homo sapiens (Human)
	NPBW1_HUMAN (P48145)	NPBWR1	GPR	Homo sapiens (Human)
	NPBW2_HUMAN (P48146)	NPBWR2	GPR	Homo sapiens (Human)
	NPFF1_HUMAN (Q9GZQ6)	NPFFR1	Neuropeptide FF	Homo sapiens (Human)
	NPFF2_HUMAN (Q9Y5X5)	NPFFR2	Neuropeptide FF	Homo sapiens (Human)
	NPY1R_HUMAN (P25929)	NPY1R	Neuropeptide Y type 1	Homo sapiens (Human)
	NPY2R_HUMAN (P49146)	NPY2R	Neuropeptide Y type 2	Homo sapiens (Human)
	NPY4R_HUMAN (P50391)	PPYR1	Neuropeptide Y type 4	Homo sapiens (Human)
	NPY5R_HUMAN (Q15761)	NPY5R	Neuropeptide Y type 5	Homo sapiens (Human)
50	NTR1_HUMAN (P30989)	NTSR1	Neurotensin	Homo sapiens (Human)
	NTR2_HUMAN (O95665)	NTSR2	Neurotensin	Homo sapiens (Human)
	O00325_HUMAN (O00325)	PTGER3	Prostaglandin E2 subtype EP3	Homo sapiens (Human)

	O00421_HUMAN	(O00421)	ccr6	C-C Chemokine other	Homo sapiens (Human)
	O10A1_HUMAN	(O95223)	OR10A1	fragments	Homo sapiens (Human)
	O10A3_HUMAN	(P58181)	OR10A3	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10A4_HUMAN	(Q9H209)	OR10A4	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10A5_HUMAN	(Q9H207)	OR10A5	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10A6_HUMAN	(Q8NH74)	OR10A6	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10A7_HUMAN	(Q8NGE5)	OR10A7	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10AD_HUMAN	(Q8NGE0)	OR10AD1	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10AG_HUMAN	(Q8NH19)	OR10AG1	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
10	O10C1_HUMAN	(Q96KK4)	OR10C1	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10D4_HUMAN	(Q8NGN7)	OR10D4	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10G2_HUMAN	(Q8NGC3)	OR10G2	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10G3_HUMAN	(Q8NGC4)	OR10G3	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10G4_HUMAN	(Q8NGN3)	OR10G4	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10G6_HUMAN	(Q8NH81)	OR10G6	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10G7_HUMAN	(Q8NGN6)	OR10G7	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10G8_HUMAN	(Q8NGN5)	OR10G8	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10G9_HUMAN	(Q8NGN4)	OR10G9	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
20	O10H1_HUMAN	(Q9Y4A9)	OR10H1	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10H2_HUMAN	(O60403)	OR10H2	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10H3_HUMAN	(O60404)	OR10H3	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10H4_HUMAN	(Q8NGA5)	OR10H4	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10H5_HUMAN	(Q8NGA6)	OR10H5	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10J1_HUMAN	(P30954)	OR10J1	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10J3_HUMAN	(Q5JRS4)	OR10J3	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10J5_HUMAN	(Q8NHC4)	OR10J5	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10J6_HUMAN	(Q8NGY7)	OR10J6	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10K1_HUMAN	(Q8NGX5)	OR10K1	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10K2_HUMAN	(Q6IF99)	OR10K2	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
30	O10P1_HUMAN	(Q8NGE3)	OR10P1	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10Q1_HUMAN	(Q8NGQ4)	OR10Q1	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10R2_HUMAN	(Q8NGX6)	OR10R2	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10S1_HUMAN	(Q8NGN2)	OR10S1	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10T2_HUMAN	(Q8NGX3)	OR10T2	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10V1_HUMAN	(Q8NGI7)	OR10V1	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10W1_HUMAN	(Q8NGF6)	OR10W1	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10X1_HUMAN	(Q8NGY0)	OR10X1	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	O10Z1_HUMAN	(Q8NGY1)	OR10Z1	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
40	O11A1_HUMAN	(Q9GZK7)	OR11A1	Olfactory II fam 11 / MOR106,121-122	Homo sapiens (Human)
	O11G2_HUMAN	(Q8NGC1)	OR11G2	Olfactory II fam 11 / MOR106,121-122	Homo sapiens (Human)
	O11H1_HUMAN	(Q8NG94)	OR11H1	Olfactory II fam 11 / MOR106,121-122	Homo sapiens (Human)
	O11H4_HUMAN	(Q8NGC9)	OR11H4	Olfactory II fam 11 / MOR106,121-122	Homo sapiens (Human)
	O11H6_HUMAN	(Q8NGC7)	OR11H6	Olfactory II fam 11 / MOR106,121-122	Homo sapiens (Human)
	O11L1_HUMAN	(Q8NGX0)	OR11L1	Olfactory II fam 11 / MOR106,121-122	Homo sapiens (Human)
	O12D2_HUMAN	(P58182)	OR12D2	Olfactory II fam 12 / MOR250	Homo sapiens (Human)
	O12D3_HUMAN	(Q9UGF7)	OR12D3	Olfactory II fam 12 / MOR250	Homo sapiens (Human)
	O13A1_HUMAN	(Q8NGR1)	OR13A1	Olfactory II fam 13 / MOR253	Homo sapiens (Human)
	O13C2_HUMAN	(Q8NGS9)	OR13C2	Olfactory II fam 13 / MOR253	Homo sapiens (Human)
	O13C3_HUMAN	(Q8NGS6)	OR13C3	Olfactory II fam 13 / MOR253	Homo sapiens (Human)
50	O13C4_HUMAN	(Q8NGS5)	OR13C4	Olfactory II fam 13 / MOR253	Homo sapiens (Human)

	O13C5_HUMAN (Q8NGS8)	OR13C5	Olfactory II fam 13 / MOR253	Homo sapiens (Human)
	O13C8_HUMAN (Q8NGS7)	OR13C8	Olfactory II fam 13 / MOR253	Homo sapiens (Human)
	O13C9_HUMAN (Q8NGT0)	OR13C9	Olfactory II fam 13 / MOR253	Homo sapiens (Human)
	O13D1_HUMAN (Q8NGV5)	OR13D1	Olfactory II fam 13 / MOR253	Homo sapiens (Human)
	O13F1_HUMAN (Q8NGS4)	OR13F1	Olfactory II fam 13 / MOR253	Homo sapiens (Human)
	O13G1_HUMAN (Q8NGZ3)	OR13G1	Olfactory II fam 13 / MOR253	Homo sapiens (Human)
	O13H1_HUMAN (Q8NG92)	OR13H1	Olfactory II fam 13 / MOR253	Homo sapiens (Human)
	O13J1_HUMAN (Q8NGT2)	OR13J1	Olfactory II fam 13 / MOR253	Homo sapiens (Human)
	O14694_HUMAN (O14694)	CCR5	fragments	Homo sapiens (Human)
10	O2A12_HUMAN (Q8NGT7)	OR2A12	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	O2A14_HUMAN (Q96R47)	OR2A14	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	O2A42_HUMAN (Q8NGT9)	OR2A42	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	O2AE1_HUMAN (Q8NHA4)	OR2AE1	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	O2AG1_HUMAN (Q9H205)	OR2AG1	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
20	O2AJ1_HUMAN (Q8NGZ0)	OR2AJ1	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	O2AK2_HUMAN (Q8NG84)	OR2AK2	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	O2AP1_HUMAN (Q8NGE2)	OR2AP1	Olfactory II fam 6 / MOR103-105,107-119	Homo sapiens (Human)
	O2T10_HUMAN (Q8NGZ9)	OR2T10	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	O2T11_HUMAN (Q8NH01)	OR2T11	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
30	O2T12_HUMAN (Q8NG77)	OR2T12	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	O2T27_HUMAN (Q8NH04)	OR2T27	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	O2T29_HUMAN (Q8NH02)	OR2T29	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	O2T33_HUMAN (Q8NG76)	OR2T33	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	O2T34_HUMAN (Q8NGX1)	OR2T34	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
40	O2T35_HUMAN (Q8NGX2)	OR2T35	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	O43192_HUMAN (O43192)		Vasopressin type 2	Homo sapiens (Human)
	O43200_HUMAN (O43200)	TSHR	fragments	Homo sapiens (Human)
	O43624_HUMAN (O43624)	OLFR 42A	fragments	Homo sapiens (Human)
	O43625_HUMAN (O43625)	OLFR 42A	fragments	Homo sapiens (Human)
	O43626_HUMAN (O43626)	OLFR 42B	fragments	Homo sapiens (Human)
	O43627_HUMAN (O43627)	OR2H5P	fragments	Homo sapiens (Human)
	O43789_HUMAN (O43789)		fragments	Homo sapiens (Human)
	O43871_HUMAN (O43871)	OR16-36	fragments	Homo sapiens (Human)
50	O43872_HUMAN (O43872)	OR16-37	fragments	Homo sapiens (Human)
	O43873_HUMAN (O43873)	OR16-88	fragments	Homo sapiens (Human)
	O43874_HUMAN (O43874)	OR16-89	fragments	Homo sapiens (Human)

	O43875_HUMAN (O43875)	OR16-90	fragments	Homo sapiens (Human)
	O43876_HUMAN (O43876)	OR17-130	fragments	Homo sapiens (Human)
	O43878_HUMAN (O43878)	OR17-137	fragments	Homo sapiens (Human)
	O43879_HUMAN (O43879)	OR17-15	fragments	Homo sapiens (Human)
	O43880_HUMAN (O43880)	OR17-16	fragments	Homo sapiens (Human)
	O43886_HUMAN (O43886)	OR7-139	fragments	Homo sapiens (Human)
	O43887_HUMAN (O43887)	OR7-140	fragments	Homo sapiens (Human)
	O43898_HUMAN (O43898)	GPR45 like		Homo sapiens (Human)
10	O4A15_HUMAN (Q8NGL6)	OR4A15	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
	O4A16_HUMAN (Q8NH70)	OR4A16	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
	O4A47_HUMAN (Q6IF82)	OR4A47	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
	O4F15_HUMAN (Q8NGB8)	OR4F15	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
	O4F17_HUMAN (Q8NGA8)	OR4F17	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
	O4F29_HUMAN (Q6IEY1)	OR4F29	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
	O51A2_HUMAN (Q8NGJ7)	OR51A2	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O51A4_HUMAN (Q8NGJ6)	OR51A4	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O51A7_HUMAN (Q8NH64)	OR51A7	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O51B2_HUMAN (Q9Y5P1)	OR51B2	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
20	O51B4_HUMAN (Q9Y5P0)	OR51B4	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O51B5_HUMAN (Q9H339)	OR51B5	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O51B6_HUMAN (Q9H340)	OR51B6	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O51D1_HUMAN (Q8NGF3)	OR51D1	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O51E1_HUMAN (Q8TCB6)	OR51E1	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O51E2_HUMAN (Q9H255)	OR51E2	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O51F1_HUMAN (Q8NH61)	OR51F2	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O51G1_HUMAN (Q8NGK1)	OR51G1	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O51G2_HUMAN (Q8NGK0)	OR51G2	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O51H1_HUMAN (Q8NH63)	OR51H1	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
30	O51I1_HUMAN (Q9H343)	OR51I1	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O51I2_HUMAN (Q9H344)	OR51I2	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O51L1_HUMAN (Q8NGJ5)	OR51L1	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O51M1_HUMAN (Q9H341)	OR51M1	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O51Q1_HUMAN (Q8NH59)	OR51Q1	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O51S1_HUMAN (Q8NGJ8)	OR51S1	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O51T1_HUMAN (Q8NGJ9)	OR51T1	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O51V1_HUMAN (Q9H2C8)	OR51V1	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O52A1_HUMAN (Q9UKL2)	OR52A1	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O52A5_HUMAN (Q9H2C5)	OR52A5	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
40	O52B2_HUMAN (Q96RD2)	OR52B2	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O52B4_HUMAN (Q8NGK2)	OR52B4	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O52B6_HUMAN (Q8NGF0)	OR52B6	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O52D1_HUMAN (Q9H346)	OR52D1	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O52E1_HUMAN (Q8NGJ3)	OR52E1	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O52E2_HUMAN (Q8NGJ4)	OR52E2	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O52E4_HUMAN (Q8NGH9)	OR52E4	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O52E5_HUMAN (Q8NH55)	OR52E5	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O52E6_HUMAN (Q96RD3)	OR52E6	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O52E8_HUMAN (Q6IFG1)	OR52E8	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O52H1_HUMAN (Q8NGJ2)	OR52H1	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
50	O52I1_HUMAN (Q8NGK6)	OR52I1	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)

10	O52I2_HUMAN (Q8NH67)	OR52I2	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O52J3_HUMAN (Q8NH60)	OR52J3	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O52K1_HUMAN (Q8NGK4)	OR52K1	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O52K2_HUMAN (Q8NGK3)	OR52K2	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O52L1_HUMAN (Q8NGH7)	OR52L1	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O52L2_HUMAN (Q8NGH6)	OR52L2	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O52M1_HUMAN (Q8NGK5)	OR52M1	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O52N1_HUMAN (Q8NH53)	OR52N1	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O52N2_HUMAN (Q8NGI0)	OR52N2	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O52N4_HUMAN (Q8NGI2)	OR52N4	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
20	O52N5_HUMAN (Q8NH56)	OR52N5	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O52P1_HUMAN (Q8NH57)	OR52P1	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O52R1_HUMAN (Q8NGF1)	OR52R1	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O52W1_HUMAN (Q6IF63)	OR52W1	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O56A1_HUMAN (Q8NGH5)	OR56A1	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O56A3_HUMAN (Q8NH54)	OR56A3	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O56A4_HUMAN (Q8NGH8)	OR56A4	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O56B1_HUMAN (Q8NGI3)	OR56B1	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O56B2_HUMAN (Q8NGI1)	OR56B2P	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
	O56B4_HUMAN (Q8NH76)	OR56B4	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
30	O5AC2_HUMAN (Q9NZP5)	OR5AC2	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	O5AK2_HUMAN (Q8NH90)	OR5AK2	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	O5AK3_HUMAN (Q8NH89)	OR5AK3	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	O5AN1_HUMAN (Q8NGI8)	OR5AN1	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	O5AP2_HUMAN (Q8NGF4)	OR5AP2	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	O5AR1_HUMAN (Q8NGP9)	OR5AR1	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	O5AS1_HUMAN (Q8N127)	OR5AS1	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	O5AT1_HUMAN (Q8NHC5)	OR5AT1	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	O5AU1_HUMAN (Q8NGC0)	OR5AU1	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	O5AV1_HUMAN (Q8NHC6)	OR5AV1P	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
40	O5AY1_HUMAN (Q8NGZ2)	OR5AY1	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	O5BF1_HUMAN (Q8NHC7)	OR5BF1	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	O60411_HUMAN (O60411)	fragments	Homo sapiens (Human)	
	O75228_HUMAN (O75228)	TBXA2R	Thromboxane	Homo sapiens (Human)
	O75307_HUMAN (O75307)	CCRL2	C-C Chemokine other	Homo sapiens (Human)
	O75824_HUMAN (O75824)	CCKBR	fragments	Homo sapiens (Human)
	O95220_HUMAN (O95220)	OR5D3	fragments	Homo sapiens (Human)
	O95499_HUMAN (O95499)	olfr89	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	O95950_HUMAN (O95950)	fragments	Homo sapiens (Human)	
	OPN3_HUMAN (Q9H1Y3)	OPN3	Rhodopsin Other	Homo sapiens (Human)

10	OPN4_HUMAN	(Q9UHM6)	OPN4	Rhodopsin Other	Homo sapiens (Human)
	OPN5_HUMAN	(Q6U736)	OPN5	Rhodopsin Other	Homo sapiens (Human)
	OPRD_HUMAN	(P41143)	OPRD1	Opioid type D	Homo sapiens (Human)
	OPRK_HUMAN	(P41145)	OPRK1	Opioid type K	Homo sapiens (Human)
	OPRM_HUMAN	(P35372)	OPRM1	Opioid type M	Homo sapiens (Human)
	OPRX_HUMAN	(P41146)	OPRL1	Opioid type X	Homo sapiens (Human)
	OPSB_HUMAN	(P03999)	OPN1SW	Rhodopsin Vertebrate type 3	Homo sapiens (Human)
	OPSD_HUMAN	(P08100)	RHO	Rhodopsin Vertebrate type 1	Homo sapiens (Human)
	OPSG_HUMAN	(P04001)	OPN1MW	Rhodopsin Vertebrate type 2	Homo sapiens (Human)
	OPSR_HUMAN	(P04000)	OPN1LW	Rhodopsin Vertebrate type 2	Homo sapiens (Human)
20	OPSX_HUMAN	(O14718)	RRH	Rhodopsin Other	Homo sapiens (Human)
	OR1A1_HUMAN	(Q9P1Q5)	OR1A1	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	OR1A2_HUMAN	(Q9Y585)	OR1A2	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	OR1B1_HUMAN	(Q8NGR6)	OR1B1	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	OR1C1_HUMAN	(Q15619)	OR1C1	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	OR1D2_HUMAN	(P34982)	OR1D2	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	OR1D4_HUMAN	(P47884)	OR1D4	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	OR1D5_HUMAN	(P58170)	OR1D5	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	OR1E1_HUMAN	(P30953)	OR1E1	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	OR1E2_HUMAN	(P47887)	OR1E2	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
30	OR1F1_HUMAN	(O43749)	OR1F1	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	OR1F2_HUMAN	(Q96R84)	OR1F2	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	OR1FC_HUMAN	(Q8NHA8)	OR1F12P	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	OR1G1_HUMAN	(P47890)	OR1G1	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	OR1I1_HUMAN	(O60431)	OR1I1	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	OR1J1_HUMAN	(Q8NGS3)	OR1J1	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	OR1J2_HUMAN	(Q8NGS2)	OR1J2	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	OR1J4_HUMAN	(Q8NGS1)	OR1J4	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	OR1K1_HUMAN	(Q8NGR3)	OR1K1	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	OR1L1_HUMAN	(Q8NH94)	OR1L1	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
40	OR1L3_HUMAN	(Q8NH93)	OR1L3	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	OR1L4_HUMAN	(Q8NGR5)	OR1L4	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	OR1L6_HUMAN	(Q8NGR2)	OR1L6	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	OR1L8_HUMAN	(Q8NGR8)	OR1L8	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	OR1M1_HUMAN	(Q8NGA1)	OR1M1	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	OR1N1_HUMAN	(Q8NGS0)	OR1N1	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	OR1N2_HUMAN	(Q8NGR9)	OR1N2	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	OR1Q1_HUMAN	(Q15612)	OR1Q1	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	OR1S1_HUMAN	(Q8NH92)	OR1S1	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	OR1S2_HUMAN	(Q8NGQ3)	OR1S2	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	OR2A2_HUMAN	(Q6IF42)	OR2A2	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2A4_HUMAN	(O95047)	OR2A4	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2A5_HUMAN	(Q96R48)	OR2A5	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2A7_HUMAN	(Q96R45)	OR2A7	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2B2_HUMAN	(Q9GZK3)	OR2B2	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2B3_HUMAN	(O76000)	OR2B3	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2B6_HUMAN	(P58173)	OR2B6	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2B8_HUMAN	(P59922)	OR2B8	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)

10	OR2BB_HUMAN (Q5JQS5)	OR2B11	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2C1_HUMAN (O95371)	OR2C1	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2C3_HUMAN (Q8N628)	OR2C3	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2D2_HUMAN (Q9H210)	OR2D2	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2D3_HUMAN (Q8NGH3)	OR2D3	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2F1_HUMAN (Q13607)	OR2F1	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2F2_HUMAN (O95006)	OR2F2	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2G2_HUMAN (Q8NGZ5)	OR2G2	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2G3_HUMAN (Q8NGZ4)	OR2G3	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2G6_HUMAN (Q5TZ20)	OR2G6	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
20	OR2H1_HUMAN (Q9GZK4)	OR2H1	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2H2_HUMAN (O95918)	OR2H2	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2I1_HUMAN (Q8NGU4)	OR2I1P	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2J1_HUMAN (Q9GZK6)	OR2J1	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2J2_HUMAN (O76002)	OR2J2	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2J3_HUMAN (O76001)	OR2J3	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2K1_HUMAN (Q8NGT1)	OR2K2	Olfactory II fam 13 / MOR253	Homo sapiens (Human)
	OR2L2_HUMAN (Q8NH16)	OR2L2	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2L3_HUMAN (Q8NG85)	OR2L3	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2L5_HUMAN (Q8NG80)	OR2L5	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
30	OR2L8_HUMAN (Q8NGY9)	OR2L8	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2LD_HUMAN (Q8N349)	OR2L13	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2M2_HUMAN (Q96R28)	OR2M2	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2M3_HUMAN (Q8NG83)	OR2M3	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2M4_HUMAN (Q96R27)	OR2M4	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2M7_HUMAN (Q8NG81)	OR2M7	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2S1_HUMAN (Q9NQN1)	OR2S2	Olfactory II fam 13 / MOR253	Homo sapiens (Human)
	OR2T1_HUMAN (O43869)	OR2T1	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2T2_HUMAN (Q6IF00)	OR2T2	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2T3_HUMAN (Q8NH03)	OR2T3	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
40	OR2T4_HUMAN (Q8NH00)	OR2T4	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2T5_HUMAN (Q6IEZ7)	OR2T5	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2T6_HUMAN (Q8NHC8)	OR2T6	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2V2_HUMAN (Q96R30)	OR2V2	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2W1_HUMAN (Q9Y3N9)	OR2W1	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	OR2W3_HUMAN (Q7Z3T1)	OR2W3	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
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OR2Y1_HUMAN (Q8NGV0)	OR2Y1	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
OR2Z1_HUMAN (Q8NG97)	OR2Z1	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
OR3A1_HUMAN (P47881)	OR3A1	Olfactory II fam 3 / MOR255	Homo sapiens (Human)
OR3A2_HUMAN (P47893)	OR3A2	Olfactory II fam 3 / MOR255	Homo sapiens (Human)
OR3A3_HUMAN (P47888)	OR3A3	Olfactory II fam 3 / MOR255	Homo sapiens (Human)
OR3A4_HUMAN (P47883)	OR3A4	Olfactory II fam 3 / MOR255	Homo sapiens (Human)
OR4A4_HUMAN (Q8NGN8)	OR4A4	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4A5_HUMAN (Q8NH83)	OR4A5	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4B1_HUMAN (Q8NGF8)	OR4B1	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4C3_HUMAN (Q8NH37)	OR4C3	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4C5_HUMAN (Q8NGB2)	OR4C5	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4C6_HUMAN (Q8NH72)	OR4C6	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4CB_HUMAN (Q6IEV9)	OR4C11	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4CC_HUMAN (Q96R67)	OR4C12	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4CD_HUMAN (Q8NGP0)	OR4C13	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4CF_HUMAN (Q8NGM1)	OR4C15	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4CG_HUMAN (Q8NGL9)	OR4C16	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4D1_HUMAN (Q15615)	OR4D1	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4D2_HUMAN (P58180)	OR4D2	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4D5_HUMAN (Q8NGN0)	OR4D5	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4D6_HUMAN (Q8NGJ1)	OR4D6	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4D9_HUMAN (Q8NGE8)	OR4D9	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4DA_HUMAN (Q8NGI6)	OR4D10	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4DB_HUMAN (Q8NGI4)	OR4D11	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4E2_HUMAN (Q8NGC2)	OR4E2	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4F3_HUMAN (O95013)	OR4F3	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4F4_HUMAN (Q96R69)	OR4F4	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4F5_HUMAN (Q8NH21)	OR4F5	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4F6_HUMAN (Q8NGB9)	OR4F6	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4K1_HUMAN (Q8NGD4)	OR4K1	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4K2_HUMAN (Q8NGD2)	OR4K2	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4K3_HUMAN (Q96R72)	OR4K3	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4K5_HUMAN (Q8NGD3)	OR4K5	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4KD_HUMAN (Q8NH42)	OR4K13	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4KE_HUMAN (Q8NGD5)	OR4K14	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4KF_HUMAN (Q8NH41)	OR4K15	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4KH_HUMAN (Q8NGC6)	OR4K17	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4L1_HUMAN (Q8NH43)	OR4L1	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4M1_HUMAN (Q8NGD0)	OR4M1	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4M2_HUMAN (Q8NGB6)	OR4M2	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4N2_HUMAN (Q8NGD1)	OR4N2	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4N4_HUMAN (Q8N0Y3)	OR4N4	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4N5_HUMAN (Q8IXE1)	OR4N5	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4P4_HUMAN (Q8NGL7)	OR4P4	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4Q3_HUMAN (Q8NH05)	OR4Q3	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4S1_HUMAN (Q8NGB4)	OR4S1	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4S2_HUMAN (Q8NH73)	OR4S2	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4X1_HUMAN (Q8NH49)	OR4X1	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
OR4X2_HUMAN (Q8NGF9)	OR4X2	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)

10	OR5A1_HUMAN (Q8NGJ0)	OR5A1	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5A2_HUMAN (Q8NGI9)	OR5A2	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5B2_HUMAN (Q96R09)	OR5B2	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5B3_HUMAN (Q8NH48)	OR5B3	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5BC_HUMAN (Q96R08)	OR5B12	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5BH_HUMAN (Q8NGF7)	OR5B17	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5C1_HUMAN (Q8NGR4)	OR5C1	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5DD_HUMAN (Q8NGL4)	OR5D13	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5DE_HUMAN (Q8NGL3)	OR5D14	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5DG_HUMAN (Q8NGK9)	OR5D16	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
20	OR5DI_HUMAN (Q8NGL1)	OR5D18	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5F1_HUMAN (Q95221)	OR5F1	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5H2_HUMAN (Q8NGV7)	OR5H2	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5H6_HUMAN (Q8NGV6)	OR5H6	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5I1_HUMAN (Q13606)	OR5I1	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
30	OR5J2_HUMAN (Q8NH18)	OR5J2	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5K1_HUMAN (Q8NHB7)	OR5K1	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5K2_HUMAN (Q8NHB8)	OR5K2	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5L1_HUMAN (Q8NGL2)	OR5L1	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5L2_HUMAN (Q8NGL0)	OR5L2	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
40	OR5M1_HUMAN (Q8NGP8)	OR5M1	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5M3_HUMAN (Q8NGP4)	OR5M3	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5M8_HUMAN (Q8NGP6)	OR5M8	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5M9_HUMAN (Q8NGP3)	OR5M9	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5MA_HUMAN (Q6IEU7)	OR5M10	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
50	OR5MB_HUMAN (Q96RB7)	OR5M11	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5P2_HUMAN (Q8WZ92)	OR5P2	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5P3_HUMAN (Q8WZ94)	OR5P3	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5R1_HUMAN (Q8NH85)	OR5R1	Olfactory II fam 8 / MOR161-171	Homo sapiens (Human)
	OR5T1_HUMAN (Q8NG75)	OR5T1	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5T2_HUMAN (Q8NGG2)	OR5T2	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5T3_HUMAN (Q8NGG3)	OR5T3	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5U1_HUMAN (Q9UGF5)	OR5U1	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR5V1_HUMAN (Q9UGF6)	OR5V1	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)

10	OR5W2_HUMAN (Q8NH69)	OR5W2	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR6A2_HUMAN (O95222)	OR6A2	Olfactory II fam 6 / MOR103-105,107-119	Homo sapiens (Human)
	OR6B1_HUMAN (O95007)	OR6B1	Olfactory II fam 6 / MOR103-105,107-119	Homo sapiens (Human)
	OR6B2_HUMAN (Q6IFH4)	OR6B2	Olfactory II fam 6 / MOR103-105,107-119	Homo sapiens (Human)
	OR6B3_HUMAN (Q8NGW1)	OR6B3	Olfactory II fam 6 / MOR103-105,107-119	Homo sapiens (Human)
	OR6C1_HUMAN (Q96RD1)	OR6C1	Olfactory II fam 6 / MOR103-105,107-119	Homo sapiens (Human)
	OR6C2_HUMAN (Q9NZP2)	OR6C2	Olfactory II fam 6 / MOR103-105,107-119	Homo sapiens (Human)
	OR6C3_HUMAN (Q9NZP0)	OR6C3	Olfactory II fam 6 / MOR103-105,107-119	Homo sapiens (Human)
	OR6C4_HUMAN (Q8NGE1)	OR6C4	Olfactory II fam 6 / MOR103-105,107-119	Homo sapiens (Human)
	OR6F1_HUMAN (Q8NGZ6)	OR6F1	Olfactory II fam 6 / MOR103-105,107-119	Homo sapiens (Human)
20	OR6J1_HUMAN (Q8NGC5)	OR6J1	Olfactory II fam 6 / MOR103-105,107-119	Homo sapiens (Human)
	OR6K2_HUMAN (Q8NGY2)	OR6K2	Olfactory II fam 6 / MOR103-105,107-119	Homo sapiens (Human)
	OR6K3_HUMAN (Q8NGY3)	OR6K3	Olfactory II fam 6 / MOR103-105,107-119	Homo sapiens (Human)
	OR6K6_HUMAN (Q8NGW6)	OR6K6	Olfactory II fam 6 / MOR103-105,107-119	Homo sapiens (Human)
	OR6M1_HUMAN (Q8NGM8)	OR6M1	Olfactory II fam 6 / MOR103-105,107-119	Homo sapiens (Human)
	OR6N1_HUMAN (Q8NGY5)	OR6N1	Olfactory II fam 6 / MOR103-105,107-119	Homo sapiens (Human)
	OR6N2_HUMAN (Q8NGY6)	OR6N2	Olfactory II fam 6 / MOR103-105,107-119	Homo sapiens (Human)
	OR6P1_HUMAN (Q8NGX9)	OR6P1	Olfactory II fam 6 / MOR103-105,107-119	Homo sapiens (Human)
	OR6Q1_HUMAN (Q8NGQ2)	OR6Q1	Olfactory II fam 6 / MOR103-105,107-119	Homo sapiens (Human)
	OR6S1_HUMAN (Q8NH40)	OR6S1	Olfactory II fam 6 / MOR103-105,107-119	Homo sapiens (Human)
30	OR6T1_HUMAN (Q8NGN1)	OR6T1	Olfactory II fam 6 / MOR103-105,107-119	Homo sapiens (Human)
	OR6V1_HUMAN (Q8N148)	OR6V1	Olfactory II fam 6 / MOR103-105,107-119	Homo sapiens (Human)
	OR6X1_HUMAN (Q8NH79)	OR6X1	Olfactory II fam 6 / MOR103-105,107-119	Homo sapiens (Human)
	OR6Y1_HUMAN (Q8NGX8)	OR6Y1	Olfactory II fam 6 / MOR103-105,107-119	Homo sapiens (Human)
	OR7A2_HUMAN (Q8NGA2)	OR7A2	Olfactory II fam 7 / MOR139-155	Homo sapiens (Human)
	OR7A5_HUMAN (Q15622)	OR7A5	Olfactory II fam 7 / MOR139-155	Homo sapiens (Human)
	OR7AA_HUMAN (O76100)	OR7A10	Olfactory II fam 7 / MOR139-155	Homo sapiens (Human)
	OR7AH_HUMAN (O14581)	OR7A17	Olfactory II fam 7 / MOR139-155	Homo sapiens (Human)
	OR7C1_HUMAN (O76099)	OR7C1	Olfactory II fam 7 / MOR139-155	Homo sapiens (Human)
	OR7C2_HUMAN (O60412)	OR7C2	Olfactory II fam 7 / MOR139-155	Homo sapiens (Human)
40	OR7D2_HUMAN (Q96RA2)	OR7D2	Olfactory II fam 7 / MOR139-155	Homo sapiens (Human)
	OR7D4_HUMAN (Q8NG98)	OR7D4	Olfactory II fam 7 / MOR139-155	Homo sapiens (Human)
	OR7G1_HUMAN (Q8NGA0)	OR7G1	Olfactory II fam 7 / MOR139-155	Homo sapiens (Human)
	OR7G2_HUMAN (Q8NG99)	OR7G2	Olfactory II fam 7 / MOR139-155	Homo sapiens (Human)
	OR7G3_HUMAN (Q8NG95)	OR7G3	Olfactory II fam 7 / MOR139-155	Homo sapiens (Human)
	OR8A1_HUMAN (Q8NGG7)	OR8A1	Olfactory II fam 8 / MOR161-171	Homo sapiens (Human)
	OR8B2_HUMAN (Q96RD0)	OR8B2	Olfactory II fam 8 / MOR161-171	Homo sapiens (Human)
	OR8B3_HUMAN (Q8NGG8)	OR8B3	Olfactory II fam 8 / MOR161-171	Homo sapiens (Human)
	OR8B4_HUMAN (Q96RC9)	OR8B4	Olfactory II fam 8 / MOR161-171	Homo sapiens (Human)
50				

	OR8B8_HUMAN (Q15620)	OR8B8	Olfactory II fam 8 / MOR161-171	Homo sapiens (Human)
	OR8BC_HUMAN (Q8NGG6)	OR8B12	Olfactory II fam 8 / MOR161-171	Homo sapiens (Human)
	OR8D1_HUMAN (Q8WZ84)	OR8D1	Olfactory II fam 8 / MOR161-171	Homo sapiens (Human)
	OR8D2_HUMAN (Q9GZM6)	OR8D2	Olfactory II fam 8 / MOR161-171	Homo sapiens (Human)
	OR8D4_HUMAN (Q8NGM9)	OR8D4	Olfactory II fam 8 / MOR161-171	Homo sapiens (Human)
	OR8G1_HUMAN (Q15617)	OR8G1	Olfactory II fam 8 / MOR161-171	Homo sapiens (Human)
	OR8G2_HUMAN (Q15614)	OR8G2	Olfactory II fam 8 / MOR161-171	Homo sapiens (Human)
	OR8G5_HUMAN (Q8NG78)	OR8G5	Olfactory II fam 8 / MOR161-171	Homo sapiens (Human)
	OR8H1_HUMAN (Q8NGG4)	OR8H1	Olfactory II fam 8 / MOR161-171	Homo sapiens (Human)
10	OR8H2_HUMAN (Q8N162)	OR8H2	Olfactory II fam 8 / MOR161-171	Homo sapiens (Human)
	OR8H3_HUMAN (Q8N146)	OR8H3	Olfactory II fam 8 / MOR161-171	Homo sapiens (Human)
	OR8I2_HUMAN (Q8N0Y5)	OR8I2	Olfactory II fam 8 / MOR161-171	Homo sapiens (Human)
	OR8J1_HUMAN (Q8NGP2)	OR8J1	Olfactory II fam 8 / MOR161-171	Homo sapiens (Human)
	OR8J3_HUMAN (Q8NGG0)	OR8J3	Olfactory II fam 8 / MOR161-171	Homo sapiens (Human)
	OR8K1_HUMAN (Q8NGG5)	OR8K1	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	OR8K3_HUMAN (Q8NH51)	OR8K3	Olfactory II fam 8 / MOR161-171	Homo sapiens (Human)
	OR8K5_HUMAN (Q8NH50)	OR8K5	Olfactory II fam 8 / MOR161-171	Homo sapiens (Human)
20	OR8S1_HUMAN (Q8NH09)	OR8S1	Olfactory unclassified class II	Homo sapiens (Human)
	OR8U1_HUMAN (Q8NH10)	OR8U1	Olfactory II fam 8 / MOR161-171	Homo sapiens (Human)
	OR9A2_HUMAN (Q8NGT5)	OR9A2	Olfactory II fam 9 / MOR120	Homo sapiens (Human)
	OR9A4_HUMAN (Q8NGU2)	OR9A4	Olfactory II fam 9 / MOR120	Homo sapiens (Human)
	OR9G1_HUMAN (Q8NH87)	OR9G1	Olfactory II fam 9 / MOR120	Homo sapiens (Human)
	OR9G4_HUMAN (Q8NGQ1)	OR9G4	Olfactory II fam 9 / MOR120	Homo sapiens (Human)
	OR9G5_HUMAN (Q8NGQ0)	OR9G5	Olfactory II fam 9 / MOR120	Homo sapiens (Human)
	OR9I1_HUMAN (Q8NGQ6)	OR9I1	Olfactory II fam 9 / MOR120	Homo sapiens (Human)
	OR9K2_HUMAN (Q8NGE7)	OR9K2	Olfactory II fam 9 / MOR120	Homo sapiens (Human)
	OR9Q1_HUMAN (Q8NGQ5)	OR9Q1	Olfactory II fam 9 / MOR120	Homo sapiens (Human)
	OR9Q2_HUMAN (Q8NGE9)	OR9Q2	Olfactory II fam 9 / MOR120	Homo sapiens (Human)
30	OX1R_HUMAN (O43613)	HCRT1	Orexin	Homo sapiens (Human)
	OX2R_HUMAN (O43614)	HCRT2	Orexin	Homo sapiens (Human)
	OXER1_HUMAN (Q8TDS5)	OXER1	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	OXGR1_HUMAN (Q96P68)	OXGR1	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	OXYR_HUMAN (P30559)	OXYR	Oxytocin / mesotocin	Homo sapiens (Human)
	P2RY1_HUMAN (P47900)	P2RY1	Purinoreceptor P2RY1-4,6,11 GPR91	Homo sapiens (Human)
	P2RY2_HUMAN (P41231)	P2RY2	Purinoreceptor P2RY1-4,6,11 GPR91	Homo sapiens (Human)
	P2RY4_HUMAN (P51582)	P2RY4	Purinoreceptor P2RY1-4,6,11 GPR91	Homo sapiens (Human)
	P2RY5_HUMAN (P43657)	P2RY5	Purinoreceptor P2RY5,8,9,10 GPR35,92,174	Homo sapiens (Human)
	P2RY6_HUMAN (Q15077)	P2RY6	Purinoreceptor P2RY1-4,6,11 GPR91	Homo sapiens (Human)
40	P2RY8_HUMAN (Q86VZ1)	P2RY8	Purinoreceptor P2RY5,8,9,10 GPR35,92,174	Homo sapiens (Human)
	P2RY9_HUMAN (Q99677)	GPR23	Purinoreceptor P2RY5,8,9,10 GPR35,92,174	Homo sapiens (Human)
	P2Y10_HUMAN (O00398)	P2RY10	Purinoreceptor P2RY5,8,9,10 GPR35,92,174	Homo sapiens (Human)
	P2Y11_HUMAN (Q96G91)	P2RY11	Purinoreceptor P2RY1-4,6,11 GPR91	Homo sapiens (Human)
	P2Y12_HUMAN (Q9H244)	P2RY12	Purinoreceptor P2RY12-14 GPR87 (UDP-Glucose)	Homo sapiens (Human)
	P2Y13_HUMAN (Q9BPV8)	P2RY13	Purinoreceptor P2RY12-14 GPR87 (UDP-Glucose)	Homo sapiens (Human)
	P2Y14_HUMAN (Q15391)	P2RY14	Purinoreceptor P2RY12-14 GPR87 (UDP-Glucose)	Homo sapiens (Human)
50	P78470_HUMAN (P78470)	fragments		Homo sapiens (Human)

	P78471_HUMAN (P78471)	fragments	Homo sapiens (Human)
	PACR_HUMAN (P41586)	ADCYAP1R1 PACAP	Homo sapiens (Human)
	PAR1_HUMAN (P25116)	F2R Thrombin	Homo sapiens (Human)
	PAR2_HUMAN (P55085)	F2RL1 Proteinase-activated	Homo sapiens (Human)
	PAR3_HUMAN (O00254)	F2RL2 Proteinase-activated	Homo sapiens (Human)
	PAR4_HUMAN (Q96RI0)	F2RL3 Proteinase-activated	Homo sapiens (Human)
	PD2R_HUMAN (Q13258)	PTGDR Prostaglandin E2/D2 subtype EP2	Homo sapiens (Human)
	PE2R1_HUMAN (P34995)	PTGER1 Prostaglandin E2 subtype EP1	Homo sapiens (Human)
10	PE2R2_HUMAN (P43116)	PTGER2 Prostaglandin E2/D2 subtype EP2	Homo sapiens (Human)
	PE2R3_HUMAN (P43115)	PTGER3 Prostaglandin E2 subtype EP3	Homo sapiens (Human)
	PE2R4_HUMAN (P35408)	PTGER4 Prostaglandin E2 subtype EP4	Homo sapiens (Human)
	PF2R_HUMAN (P43088)	PTGFR Prostaglandin F2-alpha	Homo sapiens (Human)
	PI2R_HUMAN (P43119)	PTGIR Prostacyclin	Homo sapiens (Human)
	PKR1_HUMAN (Q8TCW9)	PROKR1 Prokineticin receptors	Homo sapiens (Human)
	PKR2_HUMAN (Q8NFI6)	PROKR2 Prokineticin receptors	Homo sapiens (Human)
	PRLHR_HUMAN (P49683)	PRLHR Prolactin-releasing peptide (GPR10)	Homo sapiens (Human)
	PSYR_HUMAN (Q8IYL9)	GPR65 Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	PTAFR_HUMAN (P25105)	PTAFR Platelet activating factor	Homo sapiens (Human)
20	PTHR1_HUMAN (Q03431)	PTHR1 Parathyroid hormone	Homo sapiens (Human)
	PTHR2_HUMAN (P49190)	PTHR2 Parathyroid hormone	Homo sapiens (Human)
	Q13027_HUMAN (Q13027)	fragments	Homo sapiens (Human)
	Q13167_HUMAN (Q13167)	DRD3 Dopamine Vertebrate type 3	Homo sapiens (Human)
	Q14968_HUMAN (Q14968)	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	Q15613_HUMAN (Q15613)	tpcr110 fragments	Homo sapiens (Human)
	Q15616_HUMAN (Q15616)	OR5E1P fragments	Homo sapiens (Human)
	Q15618_HUMAN (Q15618)	OR7E18P fragments	Homo sapiens (Human)
	Q16144_HUMAN (Q16144)	CCK type B	Homo sapiens (Human)
	Q16292_HUMAN (Q16292)	thrombin receptor fragments	Homo sapiens (Human)
	Q16303_HUMAN (Q16303)	dopamine D4 receptor fragments	Homo sapiens (Human)
30	Q16503_HUMAN (Q16503)	fragments	Homo sapiens (Human)
	Q2F3K1_HUMAN (Q2F3K1)	CASR fragments	Homo sapiens (Human)
	Q2HIZ3_HUMAN (Q2HIZ3)	OR10H3 Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	Q2I7G5_HUMAN (Q2I7G5)	EMR1 EMR1	Homo sapiens (Human)
	Q2I8G2_HUMAN (Q2I8G2)	ADRA2A Alpha Adrenoceptors type 2	Homo sapiens (Human)
	Q2KHP3_HUMAN (Q2KHP3)	PTGFR Prostaglandin F2-alpha	Homo sapiens (Human)
	Q2L7J7_HUMAN (Q2L7J7)	ADORA2B fragments	Homo sapiens (Human)
	Q2M1L3_HUMAN (Q2M1L3)	GPR133 Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	Q2M1M6_HUMAN (Q2M1M6)	OR10J1 fragments	Homo sapiens (Human)
	Q2M1M8_HUMAN (Q2M1M8)	OR10J1 Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
40	Q2M1T0_HUMAN (Q2M1T0)	CASR Extracellular calcium-sensing	Homo sapiens (Human)
	Q2M1U3_HUMAN (Q2M1U3)	PTHR1 Parathyroid hormone	Homo sapiens (Human)
	Q2M1V1_HUMAN (Q2M1V1)	TAAR5 Trace amine	Homo sapiens (Human)
	Q2M1V7_HUMAN (Q2M1V7)	MRGPRE Mas proto-oncogene & Mas-related (MRGs)	Homo sapiens (Human)
	Q2M1W5_HUMAN (Q2M1W5)	TAAR1 Trace amine	Homo sapiens (Human)
	Q2M1Y3_HUMAN (Q2M1Y3)	OR2H2 Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	Q2M215_HUMAN (Q2M215)	RXFP1 LGR like (hormone receptors)	Homo sapiens (Human)
	Q2M229_HUMAN (Q2M229)	GLP1R Glucagon	Homo sapiens (Human)
50	Q2M249_HUMAN (Q2M249)	RHO Rhodopsin Vertebrate type 1	Homo sapiens (Human)

	Q2M2D2_HUMAN (Q2M2D2)	HTR5A	Serotonin type 5	Homo sapiens (Human)
	Q2M2E2_HUMAN (Q2M2E2)	GPR26	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	Q2M339_HUMAN (Q2M339)	TRHR	Thyrotropin-releasing hormone	Homo sapiens (Human)
	Q2M369_HUMAN (Q2M369)	GPR148	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	Q2M3C0_HUMAN (Q2M3C0)	PROKR2	Prokineticin receptors	Homo sapiens (Human)
	Q2M3E2_HUMAN (Q2M3E2)	VN1R4	Vomerolnasal receptors V1RL	Homo sapiens (Human)
	Q2M3F7_HUMAN (Q2M3F7)	GPR174	Purinoreceptor P2RY5,8,9,10 GPR35,92,174	Homo sapiens (Human)
10	Q2M3L0_HUMAN (Q2M3L0)	OR2S2	Olfactory II fam 13 / MOR253	Homo sapiens (Human)
	Q2M3M4_HUMAN (Q2M3M4)	OR13A1	Olfactory II fam 13 / MOR253	Homo sapiens (Human)
	Q2M3M5_HUMAN (Q2M3M5)	GCGR	Glucagon	Homo sapiens (Human)
	Q2M3T5_HUMAN (Q2M3T5)	OR2L2	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	Q2MZ38_HUMAN (Q2MZ38)	GNRHR2	fragments	Homo sapiens (Human)
	Q2NKN6_HUMAN (Q2NKN6)	BAI3	Brain-specific angiogenesis inhibitor (BAI)	Homo sapiens (Human)
	Q2NL85_HUMAN (Q2NL85)	GPRC5C	Orphan GPRC5	Homo sapiens (Human)
	Q2PNZ0_HUMAN (Q2PNZ0)	GPR115	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
20	Q2PNZ1_HUMAN (Q2PNZ1)	GPR111	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	Q2TBC9_HUMAN (Q2TBC9)	MAS1	Mas proto-oncogene & Mas-related (MRGs)	Homo sapiens (Human)
	Q2VPE4_HUMAN (Q2VPE4)		fragments	Homo sapiens (Human)
	Q2YD84_HUMAN (Q2YD84)		C-X-C Chemokine type 5	Homo sapiens (Human)
	Q2YD89_HUMAN (Q2YD89)		Prostacyclin	Homo sapiens (Human)
	Q2YDB9_HUMAN (Q2YDB9)		C-C Chemokine type 3	Homo sapiens (Human)
	Q2YEF8_HUMAN (Q2YEF8)		Interleukin-8 type A	Homo sapiens (Human)
	Q2YEG4_HUMAN (Q2YEG4)		Interleukin-8 type A	Homo sapiens (Human)
	Q2YEG5_HUMAN (Q2YEG5)		Interleukin-8 type A	Homo sapiens (Human)
30	Q2YEG7_HUMAN (Q2YEG7)		Interleukin-8 type A	Homo sapiens (Human)
	Q2YEG8_HUMAN (Q2YEG8)		Interleukin-8 type A	Homo sapiens (Human)
	Q32MN8_HUMAN (Q32MN8)	GALR2	Galanin	Homo sapiens (Human)
	Q32VQ0_HUMAN (Q32VQ0)	GPCRLTM7	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
	Q38L21_HUMAN (Q38L21)	CCR5	C-C Chemokine type 5	Homo sapiens (Human)
	Q3C1V7_HUMAN (Q3C1V7)		fragments	Homo sapiens (Human)
	Q3KNQ8_HUMAN (Q3KNQ8)	CCR8	C-C Chemokine type 8	Homo sapiens (Human)
	Q3KNR3_HUMAN (Q3KNR3)	CCR8	C-C Chemokine type 8	Homo sapiens (Human)
	Q3KNS9_HUMAN (Q3KNS9)	GPR22	GPR	Homo sapiens (Human)
	Q3KNV3_HUMAN (Q3KNV3)	GPRC5D	Orphan GPRC5	Homo sapiens (Human)
40	Q3KP37_HUMAN (Q3KP37)	CMKLR1	Chemokine receptor-like 1	Homo sapiens (Human)
	Q3KPF5_HUMAN (Q3KPF5)	P2RY5	Purinoreceptor P2RY5,8,9,10 GPR35,92,174	Homo sapiens (Human)
	Q3KRG8_HUMAN (Q3KRG8)	CEACAM1	fragments	Homo sapiens (Human)
	Q3KU23_HUMAN (Q3KU23)		LGR like (hormone receptors)	Homo sapiens (Human)
	Q3KU24_HUMAN (Q3KU24)		LGR like (hormone receptors)	Homo sapiens (Human)
	Q3KU25_HUMAN (Q3KU25)		LGR like (hormone receptors)	Homo sapiens (Human)
	Q3L3Q6_HUMAN (Q3L3Q6)	CCR5	C-C Chemokine type 5	Homo sapiens (Human)
	Q3MI45_HUMAN (Q3MI45)	MC2R	fragments	Homo sapiens (Human)
	Q3MIJ6_HUMAN (Q3MIJ6)	MC4R	Melanocortin hormone	Homo sapiens (Human)
	Q3MIL4_HUMAN (Q3MIL4)	GPR15	GPR	Homo sapiens (Human)
50	Q3MIS8_HUMAN (Q3MIS8)	OR5P2	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)

10	Q3MIV9_HUMAN (Q3MIV9)	GRM8	Metabotropic glutamate group III	Homo sapiens (Human)
	Q3MJ87_HUMAN (Q3MJ87)	PTGER4	Prostaglandin E2 subtype EP4	Homo sapiens (Human)
	Q3MJB1_HUMAN (Q3MJB1)	RXFP4	Somatostatin- and angiogenin-like peptide	Homo sapiens (Human)
	Q3MJC7_HUMAN (Q3MJC7)	OR6A2	Olfactory II fam 6 / MOR103-105,107-119	Homo sapiens (Human)
	Q3MJD3_HUMAN (Q3MJD3)	AVPR2	Vasopressin type 2	Homo sapiens (Human)
	Q3S2J4_HUMAN (Q3S2J4)	AVPR1	Vasopressin type 1	Homo sapiens (Human)
	Q3SAH0_HUMAN (Q3SAH0)	GPR34	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	Q3SAH2_HUMAN (Q3SAH2)	GPR34	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	Q3ZAR0_HUMAN (Q3ZAR0)	GPR50	Melatonin	Homo sapiens (Human)
	Q495D1_HUMAN (Q495D1)	OR5F1	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
20	Q495H1_HUMAN (Q495H1)	GPR120	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	Q495H7_HUMAN (Q495H7)	GPR119	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	Q499G4_HUMAN (Q499G4)	OPRK1	Opioid type K	Homo sapiens (Human)
	Q499H0_HUMAN (Q499H0)	GPR64	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	Q4G0I6_HUMAN (Q4G0I6)	HRH4	Histamine type 4	Homo sapiens (Human)
	Q4G0K7_HUMAN (Q4G0K7)	GPR116	fragments	Homo sapiens (Human)
	Q4G0Q6_HUMAN (Q4G0Q6)	MGC72080	fragments	Homo sapiens (Human)
	Q4KKW2_HUMAN (Q4KKW2)	PPYR1	Neuropeptide Y type 4	Homo sapiens (Human)
	Q4KN04_HUMAN (Q4KN04)	TAS2R8	Taste receptors T2R	Homo sapiens (Human)
	Q4KN27_HUMAN (Q4KN27)	MC3R	Melanocortin hormone	Homo sapiens (Human)
30	Q4KN29_HUMAN (Q4KN29)	TAS2R8	Taste receptors T2R	Homo sapiens (Human)
	Q4QRI5_HUMAN (Q4QRI5)	TACR2	Substance K (NK2)	Homo sapiens (Human)
	Q4QRI9_HUMAN (Q4QRI9)	HTR1F	fragments	Homo sapiens (Human)
	Q4QRJ0_HUMAN (Q4QRJ0)	DRD1	Dopamine Vertebrate type 1	Homo sapiens (Human)
	Q4QRJ1_HUMAN (Q4QRJ1)	CRHR1	Corticotropin releasing factor	Homo sapiens (Human)
	Q4QRJ3_HUMAN (Q4QRJ3)	FSHR	Follicle stimulating hormone	Homo sapiens (Human)
	Q4QRJ4_HUMAN (Q4QRJ4)	CRHR2	Corticotropin releasing factor	Homo sapiens (Human)
	Q4V749_HUMAN (Q4V749)	CCR10	C-C Chemokine type 10	Homo sapiens (Human)
	Q4V9L2_HUMAN (Q4V9L2)	MRGPRX1	Mas proto-oncogene & Mas-related (MRGs)	Homo sapiens (Human)
	Q4VAM0_HUMAN (Q4VAM0)	LGR5	LGR like (hormone receptors)	Homo sapiens (Human)
40	Q4VAM2_HUMAN (Q4VAM2)	LGR5	LGR like (hormone receptors)	Homo sapiens (Human)
	Q4VAT1_HUMAN (Q4VAT1)	GLP2R	fragments	Homo sapiens (Human)
	Q4VAT2_HUMAN (Q4VAT2)	GLP2R	fragments	Homo sapiens (Human)
	Q4VAT3_HUMAN (Q4VAT3)	GLP2R	Glucagon	Homo sapiens (Human)
	Q4VAT4_HUMAN (Q4VAT4)	GLP2R	fragments	Homo sapiens (Human)
	Q4VAV7_HUMAN (Q4VAV7)		Neuropeptide Y type 4	Homo sapiens (Human)
	Q4VAY7_HUMAN (Q4VAY7)	HTR1B	Serotonin type 1	Homo sapiens (Human)
	Q4VB06_HUMAN (Q4VB06)	OR3A1	Olfactory II fam 3 / MOR255	Homo sapiens (Human)
	Q4VBB0_HUMAN (Q4VBB0)	CCRL2	C-C Chemokine other	Homo sapiens (Human)
	Q4VBB4_HUMAN (Q4VBB4)	GPR68	GPR	Homo sapiens (Human)
50	Q4VBK6_HUMAN (Q4VBK6)	CHRM2	Musc. acetylcholine Vertebrate type 2	Homo sapiens (Human)
	Q4VBK7_HUMAN (Q4VBK7)	CHRM4	fragments	Homo sapiens (Human)
	Q4VBK8_HUMAN (Q4VBK8)	CNR2	fragments	Homo sapiens (Human)
	Q4VBL0_HUMAN (Q4VBL0)	NMBR	Bombesin	Homo sapiens (Human)
	Q4VBL2_HUMAN (Q4VBL2)	CCR2	C-C Chemokine type 2	Homo sapiens (Human)
	Q4VBL3_HUMAN (Q4VBL3)	GPR31	fragments	Homo sapiens (Human)
	Q4VBL6_HUMAN (Q4VBL6)	GPR52	GPR	Homo sapiens (Human)
	Q4VBL7_HUMAN (Q4VBL7)	GALR1	Galanin	Homo sapiens (Human)

	Q4VBL8_HUMAN (Q4VBL8)	TACR1	fragments	Homo sapiens (Human)
	Q4VBL9_HUMAN (Q4VBL9)	TACR3	fragments	Homo sapiens (Human)
	Q4VBM3_HUMAN (Q4VBM3)	CCR9	fragments	Homo sapiens (Human)
	Q4VBM7_HUMAN (Q4VBM7)	ADRA1A	Alpha Adrenoceptors type 1	Homo sapiens (Human)
	Q4VBN0_HUMAN (Q4VBN0)	GPR61	fragments	Homo sapiens (Human)
	Q4VBN1_HUMAN (Q4VBN1)	GPR81	fragments	Homo sapiens (Human)
	Q4VBN3_HUMAN (Q4VBN3)	GPR119	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	Q4VBN4_HUMAN (Q4VBN4)	CCRL1	fragments	Homo sapiens (Human)
	Q4VBN5_HUMAN (Q4VBN5)	GPR35	fragments	Homo sapiens (Human)
10	Q4VBN6_HUMAN (Q4VBN6)	F2RL2	fragments	Homo sapiens (Human)
	Q4VBN7_HUMAN (Q4VBN7)	P2RY10	Purinoreceptor P2RY5,8,9,10	GPR35,92,174 Homo sapiens (Human)
	Q4VBP0_HUMAN (Q4VBP0)	SSTR2	Somatostatin type 2	Homo sapiens (Human)
	Q4VBP1_HUMAN (Q4VBP1)	GIPR	fragments	Homo sapiens (Human)
	Q4VWM1_HUMAN (Q4VWM1)	OPRM1	Opioid type M	Homo sapiens (Human)
	Q4VWM2_HUMAN (Q4VWM2)	OPRM1	Opioid type M	Homo sapiens (Human)
	Q4VWM3_HUMAN (Q4VWM3)	OPRM1	Opioid type M	Homo sapiens (Human)
	Q4VWM4_HUMAN (Q4VWM4)	OPRM1	Opioid type M	Homo sapiens (Human)
	Q4VWM6_HUMAN (Q4VWM6)	OPRM1	Opioid type M	Homo sapiens (Human)
20	Q4VWX6_HUMAN (Q4VWX6)	OPRM	Opioid type M	Homo sapiens (Human)
	Q4W594_HUMAN (Q4W594)	ADRA2C	Alpha Adrenoceptors type 2	Homo sapiens (Human)
	Q4W5G7_HUMAN (Q4W5G7)	NPY2R	Neuropeptide Y type 2	Homo sapiens (Human)
	Q4ZFBV2_HUMAN (Q4ZFBV2)	GPR35	Purinoreceptor P2RY5,8,9,10	GPR35,92,174 Homo sapiens (Human)
	Q4ZIL0_HUMAN (Q4ZIL0)		fragments	Homo sapiens (Human)
	Q502U7_HUMAN (Q502U7)	GPR32	Chemokine receptor-like 1	Homo sapiens (Human)
	Q502U9_HUMAN (Q502U9)	GPR23	Purinoreceptor P2RY5,8,9,10	GPR35,92,174 Homo sapiens (Human)
	Q502V0_HUMAN (Q502V0)	XCR1	XC Chemokine	Homo sapiens (Human)
	Q502V1_HUMAN (Q502V1)	MC5R	Melanocortin hormone	Homo sapiens (Human)
30	Q502V2_HUMAN (Q502V2)	RXFP3	Somatostatin- and angiogenin-like peptide	Homo sapiens (Human)
	Q502V7_HUMAN (Q502V7)	TAS2R9	Taste receptors T2R	Homo sapiens (Human)
	Q502V9_HUMAN (Q502V9)	MAS1L	Mas proto-oncogene & Mas-related (MRGs)	Homo sapiens (Human)
	Q504X6_HUMAN (Q504X6)	MC2R	Adrenocorticotrophic hormone	Homo sapiens (Human)
	Q506J9_HUMAN (Q506J9)		Cannabinoid	Homo sapiens (Human)
	Q50KD4_HUMAN (Q50KD4)	Hosa(Biaka)-T2R55	fragments	Homo sapiens (Human)
	Q50KD6_HUMAN (Q50KD6)	Hosa(Adygei)-T2R55	fragments	Homo sapiens (Human)
	Q50KT0_HUMAN (Q50KT0)	Hosa(Biaka)-T2R9	fragments	Homo sapiens (Human)
	Q50KT1_HUMAN (Q50KT1)	Hosa(Japanese)-T2R9	fragments	Homo sapiens (Human)
40	Q50KU1_HUMAN (Q50KU1)	Hosa(Biaka)-T2R8	fragments	Homo sapiens (Human)
	Q50KU2_HUMAN (Q50KU2)	Hosa(Japanese)-T2R8	fragments	Homo sapiens (Human)
	Q50KV5_HUMAN (Q50KV5)	Hosa(Biaka)-T2R7	fragments	Homo sapiens (Human)
	Q50KV7_HUMAN (Q50KV7)	Hosa(Adygei)-T2R7	fragments	Homo sapiens (Human)
	Q52LG8_HUMAN (Q52LG8)	PTGER2	Prostaglandin E2/D2 subtype EP2	Homo sapiens (Human)
	Q52M04_HUMAN (Q52M04)	GIPR	Gastric inhibitory peptide	Homo sapiens (Human)
	Q52M68_HUMAN (Q52M68)	F2RL2	Proteinase-activated	Homo sapiens (Human)
	Q52R92_HUMAN (Q52R92)		fragments	Homo sapiens (Human)
	Q52R93_HUMAN (Q52R93)		fragments	Homo sapiens (Human)
	Q52R94_HUMAN (Q52R94)		fragments	Homo sapiens (Human)
50	Q53EM0_HUMAN (Q53EM0)		fragments	Homo sapiens (Human)

	Q53EZ5_HUMAN (Q53EZ5)	fragments	Homo sapiens (Human)
	Q53F99_HUMAN (Q53F99)	fragments	Homo sapiens (Human)
	Q53FA0_HUMAN (Q53FA0)	fragments	Homo sapiens (Human)
	Q53FA1_HUMAN (Q53FA1)	fragments	Homo sapiens (Human)
	Q53GA6_HUMAN (Q53GA6)	fragments	Homo sapiens (Human)
	Q53GM2_HUMAN (Q53GM2)	fragments	Homo sapiens (Human)
	Q53GP0_HUMAN (Q53GP0)	fragments	Homo sapiens (Human)
	Q53PC4_HUMAN (Q53PC4)	IL8RB Interleukin-8 type B	Homo sapiens (Human)
	Q53QQ5_HUMAN (Q53QQ5)	NTSR2 fragments	Homo sapiens (Human)
10	Q53QT9_HUMAN (Q53QT9)	GPR73 Prokineticin receptors	Homo sapiens (Human)
	Q53R18_HUMAN (Q53R18)	IL8RA Interleukin-8 type A	Homo sapiens (Human)
	Q53R22_HUMAN (Q53R22)	FZD5 frizzled Group A (Fz 1&2&4&5&7-9)	Homo sapiens (Human)
	Q53RU7_HUMAN (Q53RU7)	GPR39 fragments	Homo sapiens (Human)
	Q53RV4_HUMAN (Q53RV4)	tmp_locus_35 RDC1	Homo sapiens (Human)
	Q53S49_HUMAN (Q53S49)	LHCGR fragments	Homo sapiens (Human)
	Q53S59_HUMAN (Q53S59)	FZD7 frizzled Group A (Fz 1&2&4&5&7-9)	Homo sapiens (Human)
	Q53S69_HUMAN (Q53S69)	CXCR4 C-X-C Chemokine type 4	Homo sapiens (Human)
	Q53SF6_HUMAN (Q53SF6)	PTHR2 fragments	Homo sapiens (Human)
	Q53T00_HUMAN (Q53T00)	SCTR Secretin	Homo sapiens (Human)
20	Q53T35_HUMAN (Q53T35)	PTHR2 fragments	Homo sapiens (Human)
	Q53TA5_HUMAN (Q53TA5)	GPR113 Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	Q53TI1_HUMAN (Q53TI1)	HTR2B Serotonin type 2	Homo sapiens (Human)
	Q53TQ2_HUMAN (Q53TQ2)	TACR1 fragments	Homo sapiens (Human)
	Q53TR1_HUMAN (Q53TR1)	TACR1 fragments	Homo sapiens (Human)
	Q53TS5_HUMAN (Q53TS5)	CALCRL fragments	Homo sapiens (Human)
	Q53XJ8_HUMAN (Q53XJ8)	F2RL1 Proteinase-activated	Homo sapiens (Human)
	Q53XV0_HUMAN (Q53XV0)	Thrombin	Homo sapiens (Human)
	Q53XV5_HUMAN (Q53XV5)	Leukotriene B4 receptor BLT1	Homo sapiens (Human)
	Q53XZ3_HUMAN (Q53XZ3)	Musc. acetylcholine Vertebrate type 1	Homo sapiens (Human)
30	Q53Y09_HUMAN (Q53Y09)	Vasoactive intestinal polypeptide	Homo sapiens (Human)
	Q53YA1_HUMAN (Q53YA1)	CCBP2 C-C Chemokine type X	Homo sapiens (Human)
	Q53YJ4_HUMAN (Q53YJ4)	GALR3 Galanin	Homo sapiens (Human)
	Q53YY0_HUMAN (Q53YY0)	AGTR1 Angiotensin type 1	Homo sapiens (Human)
	Q53ZR7_HUMAN (Q53ZR7)	SSTR3 Somatostatin type 3	Homo sapiens (Human)
	Q541E0_HUMAN (Q541E0)	Somatostatin type 5	Homo sapiens (Human)
	Q546Q1_HUMAN (Q546Q1)	HTR4 Serotonin type 4	Homo sapiens (Human)
	Q548M6_HUMAN (Q548M6)	HRH3 Histamine type 3	Homo sapiens (Human)
	Q548Y0_HUMAN (Q548Y0)	HCRTR2 Orexin	Homo sapiens (Human)
	Q549E0_HUMAN (Q549E0)	CCR9 C-C Chemokine type 9	Homo sapiens (Human)
40	Q57Z87_HUMAN (Q57Z87)	NTSR2 fragments	Homo sapiens (Human)
	Q59EH9_HUMAN (Q59EH9)	fragments	Homo sapiens (Human)
	Q59ER8_HUMAN (Q59ER8)	fragments	Homo sapiens (Human)
	Q59ES7_HUMAN (Q59ES7)	fragments	Homo sapiens (Human)
	Q59FC0_HUMAN (Q59FC0)	fragments	Homo sapiens (Human)
	Q59FW2_HUMAN (Q59FW2)	fragments	Homo sapiens (Human)
	Q59G39_HUMAN (Q59G39)	fragments	Homo sapiens (Human)
	Q59G72_HUMAN (Q59G72)	fragments	Homo sapiens (Human)
	Q59G95_HUMAN (Q59G95)	fragments	Homo sapiens (Human)
	Q59GA2_HUMAN (Q59GA2)	fragments	Homo sapiens (Human)
50	Q59GB1_HUMAN (Q59GB1)	fragments	Homo sapiens (Human)

	Q59GE5_HUMAN (Q59GE5)	glutamate receptor homolog	fragments	Homo sapiens (Human)
	Q59GI0_HUMAN (Q59GI0)	fragments	Homo sapiens (Human)	
	Q59GL3_HUMAN (Q59GL3)	fragments	Homo sapiens (Human)	
	Q59GP3_HUMAN (Q59GP3)	fragments	Homo sapiens (Human)	
	Q59H16_HUMAN (Q59H16)	fragments	Homo sapiens (Human)	
	Q59HC2_HUMAN (Q59HC2)	fragments	Homo sapiens (Human)	
	Q59HG8_HUMAN (Q59HG8)	fragments	Homo sapiens (Human)	
	Q5CZ57_HUMAN (Q5CZ57)	EP3-I	Prostaglandin E2 subtype EP3	Homo sapiens (Human)
	Q5CZ59_HUMAN (Q5CZ59)	EP3e	Prostaglandin E2 subtype EP3	Homo sapiens (Human)
10	Q5CZ60_HUMAN (Q5CZ60)	EP3f	Prostaglandin E2 subtype EP3	Homo sapiens (Human)
	Q5CZ61_HUMAN (Q5CZ61)	EP3-VI	Prostaglandin E2 subtype EP3	Homo sapiens (Human)
	Q5CZ62_HUMAN (Q5CZ62)	EP3-V	Prostaglandin E2 subtype EP3	Homo sapiens (Human)
	Q5CZ63_HUMAN (Q5CZ63)	EP3-IV	Prostaglandin E2 subtype EP3	Homo sapiens (Human)
	Q5CZ64_HUMAN (Q5CZ64)	EP3-III	Prostaglandin E2 subtype EP3	Homo sapiens (Human)
	Q5EGP2_HUMAN (Q5EGP2)	GPR112	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	Q5EKM8_HUMAN (Q5EKM8)	CCR5	C-C Chemokine type 5	Homo sapiens (Human)
	Q5EKM9_HUMAN (Q5EKM9)	CCR5	C-C Chemokine type 5	Homo sapiens (Human)
	Q5EKN0_HUMAN (Q5EKN0)	CCR5	C-C Chemokine type 5	Homo sapiens (Human)
	Q5HYM4_HUMAN (Q5HYM4)	DKFZp686H1993	fragments	Homo sapiens (Human)
20	Q5HYQ4_HUMAN (Q5HYQ4)	GPR173	SREB	Homo sapiens (Human)
	Q5IFH6_HUMAN (Q5IFH6)	GPR24	Melanin-concentrating hormone receptors	Homo sapiens (Human)
	Q5IFI4_HUMAN (Q5IFI4)	GPR24	Melanin-concentrating hormone receptors	Homo sapiens (Human)
	Q5ISU3_HUMAN (Q5ISU3)	PPYR1	Neuropeptide Y type 4	Homo sapiens (Human)
	Q5JNZ1_HUMAN (Q5JNZ1)	DAQB-117O11.7-001	fragments	Homo sapiens (Human)
	Q5JPQ2_HUMAN (Q5JPQ2)	GPR64	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	Q5JPQ3_HUMAN (Q5JPQ3)	GPR64	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	Q5JPQ4_HUMAN (Q5JPQ4)	GPR64	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	Q5JPQ5_HUMAN (Q5JPQ5)	GPR64	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	Q5JPQ6_HUMAN (Q5JPQ6)	GPR64	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
30	Q5JQT0_HUMAN (Q5JQT0)	RP11-978I15.6	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	Q5JRH7_HUMAN (Q5JRH7)	CNR2	Cannabinoid	Homo sapiens (Human)
	Q5JSM8_HUMAN (Q5JSM8)	GPR101	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	Q5JU89_HUMAN (Q5JU89)	GPR112	fragments	Homo sapiens (Human)
	Q5JUH7_HUMAN (Q5JUH7)	EBI2	EBV-induced	Homo sapiens (Human)
	Q5JUH9_HUMAN (Q5JUH9)	GPR18	fragments	Homo sapiens (Human)
	Q5JVI7_HUMAN (Q5JVI7)	PTGFR	Prostaglandin F2-alpha	Homo sapiens (Human)
	Q5JVK3_HUMAN (Q5JVK3)	GPR112	fragments	Homo sapiens (Human)
	Q5JVL5_HUMAN (Q5JVL5)	CNR1	Cannabinoid	Homo sapiens (Human)
40	Q5KSY4_HUMAN (Q5KSY4)	CCR5	fragments	Homo sapiens (Human)
	Q5KU14_HUMAN (Q5KU14)	KPG_013	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	Q5KU17_HUMAN (Q5KU17)	KPG_011	Cysteinyl leukotriene	Homo sapiens (Human)
	Q5KU18_HUMAN (Q5KU18)	KPG_010	Purinoreceptor P2RY5,8,9,10 GPR35,92,174	Homo sapiens (Human)
	Q5KU19_HUMAN (Q5KU19)	KPG_009	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	Q5KU20_HUMAN (Q5KU20)	KPG_008	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	Q5KU21_HUMAN (Q5KU21)	KPG_007	Purinoreceptor P2RY5,8,9,10 GPR35,92,174	Homo sapiens (Human)
	Q5KU22_HUMAN (Q5KU22)	KPG_006	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
50	Q5KU27_HUMAN (Q5KU27)	KPG_005	Putative / unclassified Class A GPCRs	Homo sapiens (Human)

	Q5KU28_HUMAN (Q5KU28)	KPG_004	Leukotriene B4 receptor BLT2	Homo sapiens (Human)
	Q5KU34_HUMAN (Q5KU34)	KPG_003	ETL receptors	Homo sapiens (Human)
	Q5KU35_HUMAN (Q5KU35)	KPG_002	Purinoreceptor P2RY12-14 GPR87 (UDP-Glucose)	Homo sapiens (Human)
	Q5QIN9_HUMAN (Q5QIN9)	CCR5	C-C Chemokine type 5	Homo sapiens (Human)
	Q5QIP0_HUMAN (Q5QIP0)	CCR5	C-C Chemokine type 5	Homo sapiens (Human)
	Q5QIP1_HUMAN (Q5QIP1)	CCR5	C-C Chemokine type 5	Homo sapiens (Human)
	Q5RJ87_HUMAN (Q5RJ87)	DAQB-36F16.7-002	fragments	Homo sapiens (Human)
	Q5S4P5_HUMAN (Q5S4P5)	POGR	Olfactory I fam 51-52 /MOR1-42	Homo sapiens (Human)
10	Q5SQD8_HUMAN (Q5SQD8)	EDG3	Sphingosine 1-phosphate Edg-3	Homo sapiens (Human)
	Q5SQI9_HUMAN (Q5SQI9)	XXbac-BCX92J3.1-001	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	Q5ST16_HUMAN (Q5ST16)	DAQB-304F3.2-001	Olfactory II fam 11 / MOR106,121-122	Homo sapiens (Human)
	Q5ST27_HUMAN (Q5ST27)	XXbac-BCX147D4.2-001	Olfactory II fam 5 / MOR172-224,249,254	Homo sapiens (Human)
	Q5ST39_HUMAN (Q5ST39)	OR2J2	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	Q5STL4_HUMAN (Q5STL4)	GABBR1	fragments	Homo sapiens (Human)
	Q5STL7_HUMAN (Q5STL7)	GABBR1	fragments	Homo sapiens (Human)
20	Q5SUJ6_HUMAN (Q5SUJ6)	XXbac-BPG171B11.5-001	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	Q5SUJ7_HUMAN (Q5SUJ7)	XXbac-BPG171B11.3-001	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	Q5SUJ8_HUMAN (Q5SUJ8)	GABBR1	GABA-B subtype 1	Homo sapiens (Human)
	Q5SUJ9_HUMAN (Q5SUJ9)	GABBR1	GABA-B subtype 1	Homo sapiens (Human)
	Q5SUK1_HUMAN (Q5SUK1)	OR2H2	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	Q5SUL3_HUMAN (Q5SUL3)	GABBR1	GABA-B subtype 1	Homo sapiens (Human)
30	Q5SUN5_HUMAN (Q5SUN5)	MAS1L	Mas proto-oncogene & Mas-related (MRGs)	Homo sapiens (Human)
	Q5SUN6_HUMAN (Q5SUN6)	DAQB-12N14.4-001	Olfactory II fam 2 / MOR256-262,270-285	Homo sapiens (Human)
	Q5SUN7_HUMAN (Q5SUN7)	XXbac-BPG13B8.1-001	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	Q5SUN9_HUMAN (Q5SUN9)	XXbac-BPG13B8.6-001	Olfactory II fam 12 / MOR250	Homo sapiens (Human)
	Q5SWW2_HUMAN (Q5SWW2)	ELTD1	fragments	Homo sapiens (Human)
	Q5SWW3_HUMAN (Q5SWW3)	ELTD1	ETL receptors	Homo sapiens (Human)
	Q5SXP7_HUMAN (Q5SXP7)	RP11-294K24.1-001	GPR37 / endothelin B-like	Homo sapiens (Human)
40	Q5SY22_HUMAN (Q5SY22)	TAS1R1	fragments	Homo sapiens (Human)
	Q5SY23_HUMAN (Q5SY23)	TAS1R1	fragments	Homo sapiens (Human)
	Q5SY24_HUMAN (Q5SY24)	TAS1R1	fragments	Homo sapiens (Human)
	Q5T234_HUMAN (Q5T234)	GPR123	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	Q5T261_HUMAN (Q5T261)	EDG2	fragments	Homo sapiens (Human)
	Q5T2X9_HUMAN (Q5T2X9)	PPYR1	fragments	Homo sapiens (Human)
	Q5T2Y7_HUMAN (Q5T2Y7)	CELSR2	Cadherin EGF LAG (CELSR)	Homo sapiens (Human)
	Q5T5Y4_HUMAN (Q5T5Y4)	ADRB1	Beta Adrenoceptors type 1	Homo sapiens (Human)
	Q5T6D8_HUMAN (Q5T6D8)	GPR147	fragments	Homo sapiens (Human)
	Q5T6K0_HUMAN (Q5T6K0)	BAI2	Brain-specific angiogenesis inhibitor (BAI)	Homo sapiens (Human)
50	Q5T7Z3_HUMAN (Q5T7Z3)	RP11-64P14.4-001	Olfactory II fam 1 / MOR125-138,156	Homo sapiens (Human)
	Q5T8C0_HUMAN (Q5T8C0)	HTR2A	Serotonin type 2	Homo sapiens (Human)

	Q5T8P3_HUMAN (Q5T8P3)	GPR12	GPR	Homo sapiens (Human)
	Q5T9D2_HUMAN (Q5T9D2)	LPHN2	fragments	Homo sapiens (Human)
	Q5TBX0_HUMAN (Q5TBX0)	OPRM1	Opioid type M	Homo sapiens (Human)
	Q5TF06_HUMAN (Q5TF06)	RP3-365O12.1-001	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	Q5TGK2_HUMAN (Q5TGK2)	GPR161	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	Q5TGN7_HUMAN (Q5TGN7)	GPR126	Putative / unclassified Class B GPCRs	Homo sapiens (Human)
	Q5TGZ1_HUMAN (Q5TGZ1)	HTR6	Serotonin type 6	Homo sapiens (Human)
	Q5TH86_HUMAN (Q5TH86)	PTGER3	Prostaglandin E2 subtype EP3	Homo sapiens (Human)
10	Q5TH88_HUMAN (Q5TH88)	PTGER3	Prostaglandin E2 subtype EP3	Homo sapiens (Human)
	Q5U003_HUMAN (Q5U003)		C-C Chemokine type 1	Homo sapiens (Human)
	Q5U0H0_HUMAN (Q5U0H0)		Chemokine receptor-like 1	Homo sapiens (Human)
	Q5U5U4_HUMAN (Q5U5U4)	PTGER1	Prostaglandin E2 subtype EP1	Homo sapiens (Human)
	Q5VSV1_HUMAN (Q5VSV1)	RP11-180D21.2-001	Olfactory II fam 10 / MOR263-269	Homo sapiens (Human)
	Q5VT13_HUMAN (Q5VT13)	GPR82	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	Q5VT14_HUMAN (Q5VT14)	GPR34	Putative / unclassified Class A GPCRs	Homo sapiens (Human)
	Q5VT23_HUMAN (Q5VT23)	RP11-34P13.6-001	Olfactory II fam 4 / MOR225-248	Homo sapiens (Human)
20	Q5VTM0_HUMAN (Q5VTM0)	SLC31A2	Orexin	Homo sapiens (Human)
	Q5VTV7_HUMAN (Q5VTV7)	GPR145	Melanin-concentrating hormone receptors	Homo sapiens (Human)
	Q5VUF8_HUMAN (Q5VUF8)	HTR2C	Serotonin type 2	Homo sapiens (Human)
	Q5VUF9_HUMAN (Q5VUF9)	HTR2C	fragments	Homo sapiens (Human)
	Q5VUK8_HUMAN (Q5VUK8)	NMBR	Bombesin	Homo sapiens (Human)
	Q5VX01_HUMAN (Q5VX01)	HTR7	Serotonin type 7	Homo sapiens (Human)
	Q5VX02_HUMAN (Q5VX02)	HTR7	Serotonin type 7	Homo sapiens (Human)
	Q5VX03_HUMAN (Q5VX03)	HTR7	Serotonin type 7	Homo sapiens (Human)
	Q5VX04_HUMAN (Q5VX04)	HTR7	Serotonin type 7	Homo sapiens (Human)
30	Q5VX75_HUMAN (Q5VX75)	LPHN2	Latrophilin type 2	Homo sapiens (Human)
	Q5VX77_HUMAN (Q5VX77)	LPHN2	Latrophilin type 2	Homo sapiens (Human)
	Q5VX78_HUMAN (Q5VX78)	LPHN2	Latrophilin type 2	Homo sapiens (Human)
	Q5VX79_HUMAN (Q5VX79)	LPHN2	Latrophilin type 2	Homo sapiens (Human)
	Q5VX80_HUMAN (Q5VX80)	LPHN2	Latrophilin type 2	Homo sapiens (Human)
	Q5VX81_HUMAN (Q5VX81)	LPHN2	Latrophilin type 2	Homo sapiens (Human)
	Q5VX82_HUMAN (Q5VX82)	LPHN2	Latrophilin type 2	Homo sapiens (Human)
	Q5VX83_HUMAN (Q5VX83)	LPHN2	Latrophilin type 2	Homo sapiens (Human)
	Q5VXR2_HUMAN (Q5VXR2)	ADRA1D	Alpha Adrenoceptors type 1	Homo sapiens (Human)
	Q5VXY3_HUMAN (Q5VXY3)	CHRM3	fragments	Homo sapiens (Human)
40	Q5VY37_HUMAN (Q5VY37)	BAI3	Brain-specific angiogenesis inhibitor (BAI)	Homo sapiens (Human)
	Q5VZX0_HUMAN (Q5VZX0)	EDG2	Lysophosphatidic acid Edg-2	Homo sapiens (Human)
	Q5W0G9_HUMAN (Q5W0G9)	EDNRB	Endothelin	Homo sapiens (Human)
	Q5W0N7_HUMAN (Q5W0N7)	RP11-432E15.1	LGR like (hormone receptors)	Homo sapiens (Human)
	Q5Y190_HUMAN (Q5Y190)	RESDA1	Cadherin EGF LAG (CELSR)	Homo sapiens (Human)
	Q5ZGL8_HUMAN (Q5ZGL8)	HCTR-6	fragments	Homo sapiens (Human)
	Q5ZGX3_HUMAN (Q5ZGX3)	5HT1A	Serotonin type 1	Homo sapiens (Human)
	Q63ZY2_HUMAN (Q63ZY2)	GPR30	Chemokine receptor-like 2	Homo sapiens (Human)
	Q645Y1_HUMAN (Q645Y1)	TAS2R7	Taste receptors T2R	Homo sapiens (Human)
	Q659U6_HUMAN (Q659U6)	HCTR-5	fragments	Homo sapiens (Human)
50	Q66K38_HUMAN (Q66K38)	MC1R	Melanocyte stimulating hormone	Homo sapiens (Human)

DEMANDES OU BREVETS VOLUMINEUX

**LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVETS
COMPREND PLUS D'UN TOME.**

CECI EST LE TOME __1__ DE __2__

NOTE: Pour les tomes additionels, veuillez contacter le Bureau Canadien des Brevets.

JUMBO APPLICATIONS / PATENTS

**THIS SECTION OF THE APPLICATION / PATENT CONTAINS MORE
THAN ONE VOLUME.**

THIS IS VOLUME __1__ OF __2__

NOTE: For additional volumes please contact the Canadian Patent Office.

CLAIMS

1. Amino acid sequence that is directed against and/or that can specifically bind to a GPCR.
2. Amino acid sequence according to claim 1, that is in essentially isolated form.
3. Amino acid sequence according to claim 1 or 2, for administration to a subject, wherein said amino acid sequence does not naturally occur in said subject.
- 10 4. Amino acid sequence according to any of the preceding claims, that can specifically bind to a GPCR 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.
5. Amino acid sequence according to any of the preceding claims, that can specifically bind to a GPCR 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 $10^3 M^{-1}s^{-1}$ and $10^7 M^{-1}s^{-1}$, more preferably between $10^4 M^{-1}s^{-1}$ and $10^7 M^{-1}s^{-1}$, such as between $10^5 M^{-1}s^{-1}$ and $10^7 M^{-1}s^{-1}$.
- 20 6. Amino acid sequence according to any of the preceding claims, that can specifically bind to a GPCR 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 $10^{-6} s^{-1}$.
7. Amino acid sequence according to any of the preceding claims, that can specifically bind to a GPCR with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM.
- 30 8. 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.

9. 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.

10. 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).

11. Amino acid sequence according to any of the preceding claims, that is an immunoglobulin sequence.

12. 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.

13. 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.

14. 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).

15. Amino acid sequence according to any of the preceding claims, that essentially consists 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.

16. 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).

17. Amino acid sequence according to any of the preceding claims, that essentially consists of a Nanobody™.

18. Amino acid sequence according to any of the preceding claims, that essentially consists of a Nanobody™ that

- i) has 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 1 to 22, in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences are disregarded;

10 and in which:

- ii) 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.

19. Amino acid sequence according to any of the preceding claims, that essentially consists of a Nanobody™ that

- i) has 80% amino acid identity with at least one of the amino acid sequences of SEQ ID NO's: 868 to 973, in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences are disregarded;

20 and in which:

- ii) 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.

20. Amino acid sequence according to any of the preceding claims, that essentially consists of a humanized Nanobody™.

21. Amino acid sequence according to any of the preceding claims, that in addition to the at least one binding site for binding against a GPCR, contain one or more further
30 binding sites for binding against other antigens, proteins or targets.

22. Compound or construct, that comprises or essentially consists of one or more amino acid sequences according to any of claims 1 to 21, and optionally further comprises

one or more other groups, residues, moieties or binding units, optionally linked via one or more linkers.

23. Compound or construct according to claim 22, in which said one or more other groups, residues, moieties or binding units are amino acid sequences.

24. Compound or construct according to claim 23 in which said one or more linkers, if present, are one or more amino acid sequences.

10 25. Compound or construct according to any of claims 22 to 24, in which said one or more other groups, residues, moieties or binding units are immunoglobulin sequences.

26. Compound or construct according to any of claims 22 to 25, 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.

20 27. Compound or construct according to any of claims 22 to 26, in which said one or more amino acid sequences of the invention are immunoglobulin sequences.

28. Compound or construct according to any of claims 22 to 27, 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.

29. Compound or construct, that comprises or essentially consists of one or more Nanobodies according to any of claims 18 to 21 and in which said one or more other groups,
30 residues, moieties or binding units are Nanobodies.

30. Compound or construct according to any of claims 22 to 29, which is a multivalent construct.

31. Compound or construct according to any of claims 22 to 29, which is a multispecific construct.

32. Compound or construct according to any of claims 22 to 31, which has an increased half-life, compared to the corresponding amino acid sequence according to any of claims 1 to 21 per se.

33. Compound or construct according to claim 32, 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 21 per se.

34. Compound or construct according to claim 33, 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.

35. Compound or construct according to claim 33 or 34, 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.

36. Compound or construct according to claim 35, in which said one or more other groups, residues, moieties or binding units that provides 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).

37. Compound or construct according to claim 36, in which said one or more other groups, residues, moieties or binding units that provides 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).

38. Compound or construct according to claim 37, in which said one or more other groups, residues, moieties or binding units that provides the compound or construct with increased half-life is a Nanobody that can bind to serum albumin (such as human serum albumin) or a serum immunoglobulin (such as IgG).

39. Compound or construct according to any of claims 32 to 38, that has a serum
10 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 21 per se.

40. Compound or construct according to any of claims 32 to 39, 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 21 per se.

20 41. Compound or construct according to any of claims 32 to 40, 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, 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).

42. Monovalent construct, comprising or essentially consisting of one amino acid sequence according to any of claims 1 to 21.

30

43. Monovalent construct according to claim 42, 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.

44. Monovalent construct, comprising or essentially consisting of one Nanobody according to any of claims 1 to 21.

45. Nucleic acid or nucleotide sequence, that encodes an amino acid sequence according to any of claims 1 to 21, a compound or construct according to any of claims 23 to 41, or a monovalent construct according to any of claims 42 to 44.

10

46. Nucleic acid or nucleotide sequence according to claim 45, that is in the form of a genetic construct.

47. 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 21, a compound or construct according to any of claims 23 to 41, or a monovalent construct according to any of claims 42 to 44; and/or that comprises a nucleic acid or nucleotide sequence according to claim 45 or 46.

20

48. Method for producing an amino acid sequence according to any of claims 1 to 21, a compound or construct according to any of claims 23 to 41, or a monovalent construct according to any of claims 42 to 44, 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 45 or 46, or a genetic construct according to claim...

optionally followed by:

b) isolating and/or purifying the 1 to 21, the compound or construct according to any of claims 23 to 41, or the a monovalent construct according to any of claims 42 to 44 thus obtained

30

49. Method for producing an amino acid sequence according to any of claims 1 to 21, a compound or construct according to any of claims 23 to 41, or a monovalent construct according to any of claims 42 to 44, said method at least comprising the steps of:

- a) cultivating and/or maintaining a host or host cell according to claim 47 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 21, compound or construct according to any of claims 23 to 41, or monovalent construct according to any of claims 42 to 44; optionally followed by:
- b) isolating and/or purifying the amino acid sequence according to any of claims 1 to 21, the compound or construct according to any of claims 23 to 41, or the monovalent construct according to any of claims 42 to 44 thus obtained

10 50. Composition, comprising at least one amino acid sequence according to any of claims 1 to 21, compound or construct according to any of claims 22 to 41, monovalent construct according to any of claims 42 to 44, or nucleic acid or nucleotide sequence according to claim 45 or 46.

51. Composition according to claim 50, which is a pharmaceutical composition

52. Composition according to claim 50, 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
20 polypeptides and/or compounds.

53. Method for the prevention and/or treatment of at least one GPCR-related disease or disorder, 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 21, compound or construct according to any of claims 22 to 41, monovalent construct according to any of claims 42 to 44, or composition according to claim 51 or 52.

54. Method for the prevention and/or treatment of at least one disease or disorder that is associated with a GPCR, with its biological or pharmacological activity, and/or with
30 the biological pathways or signalling in which a GPCR 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 21, compound or construct according to any of claims 22 to 41, monovalent construct according to any of claims 42 to 44, or composition according to claim 51 or 52.

55. 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 21, compound or construct according to any of claims 22 to 41, monovalent construct according to any of claims 42 to 44, or composition according to claim 51 or 52, 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 21, compound or construct according to any of claims 22 to 41, monovalent construct according to any of claims 42 to 44, or composition according to claim 51 or 52.

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56. 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 21, compound or construct according to any of claims 22 to 41, monovalent construct according to any of claims 42 to 44, or composition according to claim 51 or 52.

20

57. Use of an amino acid sequence according to any of claims 1 to 21, compound or construct according to any of claims 22 to 41, monovalent construct according to any of claims 42 to 44 in the preparation of a pharmaceutical composition for prevention and/or treatment of at least one GPCR-related disease or disorder; and/or for use in one or more of the methods according to claims 53 to 56.

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