HEAVY CHAIN VARIABLE DOMAINS THAT BIND TO FREE LIGHT CHAINS

FIELD OF THE INVENTION

The present invention relates to antibodies binding to, or pairing with, free light chains, such as free kappa light chains and/or (kappa) amyloidogenic light chains, and formulations comprising the same. The invention further relates to the use of the anti-light chain antibodies and formulations in therapy and/or diagnosis.

BACKGROUND OF THE INVENTION

Light chain (AL) amyloidosis is the most common form of systemic amyloid disease, affecting approximately 1 in 100,000 people per year (Gertz, 2018). AL amyloidosis is classified as a plasma-cell (PC) dyscrasia, in which a mutant PC clone expand abnormally, resulting in the synthesis of large concentrations of free light chains (FLCs) (Fotiou et al., 2020). In standard conditions, light chains (LCs) and heavy chains (HCs) are produced at nearly equivalent ratios within plasma cells, before being incorporated into antibodies. However, in AL amyloidosis, the abnormal large excess of FLCs that are produced as well as the presence of destabilising mutations, triggers the aggregation of FLCs into amyloid deposits (also named fibrils), which are systemically deposited in almost every organ (e.g. heart, kidneys, skin, liver and nerves). Such amyloid fibrils deposition in organs leads to their dysfunction. For instance more than 3/4 AL amyloidosis patients have such deposits in the heart, leading to the development of rapidly progressive form of cardiomyopathy, with rather poor prognosis (Merlini et al., 2013). In another example, representing about 85% of cases, AL amyloidosis is the most common form of amyloidogenesis in the kidney (Gurung and Tingting, 2022),

No cure currently exists for AL amyloidosis. Unfortunately, patients diagnosed with this disease have generally a very poor prognosis, with a typical survival time from 6 months to 3 years (Weiss et al., 2016). Current treatments focus on the management of symptoms and/or aim to limit further the production of FLCs. The latter type of treatments therefore focuses on depletion of the plasma cell clones, which is typically poorly tolerated, in particular for patients with severe cardiac deposits. Although there are no therapeutics available on the market aiming at clearing the amyloid deposits, this area is currently being targeted, including via passive immunotherapy. The mAb anselamimab for instance, currently in phase III clinical trial for primary systemic amyloidosis, targets amyloid fibrils, of λ or κ origin, for removal (Edwards et al., 2021). The antibody birtamimab, also in phase III clinical trial for Mayo Stage IV AL amyloidosis, appears to show some benefit for AL amyloidosis patients with advanced cardiac involvement (Gertz et al., 2022). However, it is noted that birtamimab does not target the fibrils themselves, but rather the serum amyloid protein (SAP), which colocalises with amyloid deposits. Broggini et al, 2023 have recently identified llama-derived nanobodies that bind and stabilise H3, a lambda amyloidogenic LC.

Therefore, there remains a need to provide antibodies, such as VH chains or nanobodies, which specifically target the free light chains, such as free kappa light chains and/or (kappa) amyloidogenic light chains, stabilise them and as such prevent and/or inhibit the formation of (amyloid) fibrils. Such antibodies would be very useful in diagnosis and/or therapy, including for prevention and therapeutic intervention in inflammatory diseases or disorders, especially those involving altered profiles of FLCs.

SUMMARY OF THE INVENTION

The present invention addresses the above-identified need by providing new antibodies binding to, or pairing with, free light chains, such as free kappa light chains and/or (kappa) amyloidogenic light chains. These antibodies can be useful in diagnosis or therapy, notably in the treatment of disorders involving free (kappa)(amyloidogenic) light chains-based fibrils. These antibodies have notably high specificity for the free light chains, in particular free kappa light chains, and prevent and/or inhibit the formation of light chains dimers and resulting light chains fibrils.

In a first aspect, the present invention provides an antibody that specifically binds to, or pairs with, free light chains, wherein said antibody comprises a heavy chain variable region comprising: i) a CDR-H1 consisting of SEQ ID NO: 1, 4, 7, 10 or 13, a CDR-H2 consisting of SEQ ID NO: 2, 5, 8, 11 or 14 and a CDR-H3 consisting of SEQ ID NO: 3, 6, 9, 12 or 15; or ii) CDR-H1, CDR-H2 and CDR-H3 sequences that have at least 70%, 80%, 90%, 91%, 92%, 93%, 94 %, 95%, 96%, 97%, 98%, or 99% identity or similarity to any of the sequences listed in i. Preferably, the antibody is selected from the group consisting of: i) an antibody moiety comprising a VH domain but no VL domain or ii) a nanobody.

In a second aspect, the present invention provides an antibody that specifically binds to, or pairs with, free light chains, wherein said antibody comprises: i. CDR-H1 consisting of SEQ ID NO: 1; a CDR-H2 consisting of SEQ ID NO: 2; and a CDR-H3 consisting of SEQ ID NO: 3; ii. A C CDR-H1 consisting of SEQ ID NO: 4; a CDR-H2 consisting of SEQ ID NO: 5; and a CDR-H3 consisting of SEQ ID NO: 6; iii. A CDR-H1 consisting of SEQ ID NO: 7; a CDR-H2 consisting of SEQ ID NO: 8; and a CDR-H3 consisting of SEQ ID NO: 9; iv. A CDR-H1 consisting of SEQ ID NO: 10; a CDR-H2 consisting of SEQ ID NO: 11; and a CDR-H3 consisting of SEQ ID NO: 12; v. a CDR-H1 consisting of SEQ ID NO: 13; a CDR-H2 consisting of SEQ ID NO: 14; and a CDR-H3 consisting of SEQ ID NO: 15; or vi. CDR-H1, CDR-H2 and CDR-H3 sequences having at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity or similarity to any of the sequences listed in any one of i. to v.

In a third aspect, the present invention provides an antibody that specifically binds to, or pairs with, free light chains, wherein the antibody has a heavy chain variable region comprising any one of SEQ ID NO: 16 to 26 or a sequence that has at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereof.

In further aspects of the invention are covered isolated polynucleotides encoding the antibody, cloning or expression vectors, host cells, processes for the production of the antibody, pharmaceutical compositions comprising the antibody, and their use in therapy and/or diagnosis.

DETAILED DESCRIPTION OF THE INVENTION

The present disclosure will now be described with respect to particular non-limiting aspects and embodiments thereof and with reference to certain figures and examples.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in art to which the subject matter herein belongs. As used in the specification and claims, the following definitions are supplied to facilitate the understanding of the present invention.

- The term “and/or” used in a phrase such as “A and/or B” herein is intended to include “A and B”, “A or B”, “A”, and “B”.

- The forms “a”, “an”, and “the” include both single and plural referents unless the content clearly dictates otherwise. Thus, for example, reference to “an antibody” includes both “an antibody” and “antibodies”.

- The term “comprising” does not exclude other elements. For the purpose of the present disclosure, the term “consisting of’ is considered to be a preferred embodiment of the term “comprising of’.

- The term “free light chain”, as used herein, refers to an immunoglobulin light chain that is free from a partner immunoglobulin heavy chain. In the context of the invention as a whole, "free light chain" does not exclude the possibility of monomers, dimers, trimers or other oligomeric /polymeric forms of light chains. It will be understood that “free light chain” and “light chain” will be used interchangeably herein, as the invention as a whole is in the context of formation of a light chain amyloid fibril and the aim to prevent it with the antibody of the invention.

- The term “amyloidogenic light chain” refers to an immunoglobulin light chain polypeptide involved in amyloidogenesis.

- the terms “SMA”, “LEN” and “REC”, as used herein, refer to three of the most investigated light chains proteins, in the frame of understanding AL amyloidosis. LEN and REC have been isolated from the urine of patients with multiple myeloma and AL amyloidosis respectively, they are both 114 amino acid residues long and belong to the κ4 sub-type. LEN is a non-amyloidogenic immunoglobulin light chain protein (i.e. it does not form amyloid fibrils), which differs from the germline κ4 sequence at a single position (See Figure 9; see SEQ ID NO: 45)(Solomon, 1985). SMA is an amyloidogenic immunoglobulin light chain protein, also containing 114 amino acid residues and belonging to the κ4 sub-type. It has been isolated from lymph node-derived amyloid plaques of a patient suffering from AL amyloidosis (Pras et al. 1968). SMA contains a total of eight aa mutations (See Figure 9; see SEQ ID NO: 46) relative to the germline κ4 sequence. REC is

also an amyloidogenic protein which differs from the germline κ4 sequence at 14 positions (See Figure 9; see SEQ ID NO:47)(Stevens et al., 1995).

- The term “antibody” refers to whole antibodies and functionally active fragments thereof (i.e., molecules that contain an antigen binding domain that specifically binds an antigen, also termed antigen-binding fragments). Features described herein with respect to antibodies also apply to antibody fragments unless context dictates otherwise. Whole antibodies, also known as “immunoglobulins (Ig)” generally relate to intact or full-length antibodies i.e. comprising the elements of two heavy chains and two light chains, inter-connected by disulphide bonds, which assemble to define a characteristic Y-shaped three-dimensional structure. Classical natural whole antibodies are monospecific in that they bind one antigen type, and bivalent in that they have two independent antigen binding domains.

- The terms “intact antibody”, “full-length antibody” and “whole antibody” are used interchangeably to refer to a monospecific bivalent antibody having a structure similar to a native antibody structure, including an Fc region as defined herein. In whole antibodies, each light chain comprises a light chain variable region (abbreviated herein as VL) and a light chain constant region (CL). Light chains can be kappa (κ) or lambda (λ). The typical kappa-to-lambda ratio in the normal human is between 0.26 and 1.65 (Rajkumar et al., 2005). Each heavy chain comprises a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region (CH) constituted of three constant domains CH1, CH2 and CH3, or four constant domains CH1, CH2, CH3 and CH4, depending on the Ig class. The “class” of an Ig or antibody refers to the type of constant region and includes IgA, IgD, IgE, IgG and IgM and several of them can be further divided into subclasses, e.g. IgG1, IgG2, IgG3, IgG4. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.,* effector cells) and the first component (Clq) of the classical complement system. The VH and VL regions of the antibody according to the present invention can be further subdivided into regions of hypervariability (or “hypervariable regions”, or HVR) determining the recognition of the antigen, termed complementarity determining regions (CDR), interspersed with regions that are more structurally conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The CDRs and the FR together form a variable region. By convention, the CDRs in the heavy chain variable region of an antibody or antigen-binding fragment thereof are referred as CDR-H1, CDR-H2 and CDR-H3 and in the light chain variable regions as CDR-L1, CDR-L2 and CDR-L3. They are numbered sequentially in the direction from the N-terminus to the C-terminus of each chain.

CDRs are conventionally numbered according to a system devised by Kabat *et al.* This system is set forth in Kabat *et al.*, 1991. This numbering system is used in the present specification except where otherwise indicated.

The CDRs of the heavy chain variable domain typically comprise residues 31-35 (CDR-H1), residues 50-65 (CDR-H2) and residues 95-102 (CDR-H3) according to the Kabat numbering system. However, according to Chothia (1987), the loop equivalent to CDR-H1 extends from residue 26 to residue 32. Thus, unless indicated otherwise 'CDR-H1' as employed herein is intended to refer to residues 26 to 35, as described by a combination of the Kabat numbering system and Chothia's topological loop definition. The CDRs of the light chain variable domain are typically located at residues 24-34 (CDR-L1), residues 50-56 (CDR-L2) and residues 89-97 (CDR-L3) according to the Kabat numbering system. In addition to the CDR loops, a fourth loop exists between CDR-2 (CDR-L2 or CDR-H2) and CDR-3 (CDR-L3 or CDR-H3) which is formed by framework 3 (FR3). The Kabat numbering system defines framework 3 as positions 66-94 in a heavy chain and positions 57-88 in a light chain.

- The terms "constant domain(s)" or "constant region", as used herein, are used interchangeably to refer to the domain(s) of an antibody which is outside the variable regions. The constant domains are identical in all antibodies of the same isotype but are different from one isotype to another. Typically, the constant region of a heavy chain is formed, from N to C terminal, by CH1-hinge - CH2-CH3-optionally CH4, comprising three or four constant domains.

The constant region domains of the antibody molecule of the present invention, if present, may be selected having regard to the proposed function of the antibody molecule, and in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgD, IgE, IgG or IgM domains. In particular, human IgG constant region domains may be used, especially of the IgG1 and IgG3 isotypes when the antibody molecule is intended for therapeutic uses and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the antibody molecule is intended for therapeutic purposes and antibody effector functions are not required. It will be appreciated that sequence variants of these constant region domains may also be used. For example, IgG4 molecules in which the serine at position 241 (numbered according to the Kabat numbering system) has been changed to proline as described in Angal *et al.* (Angal *et al.*, 1993).

- "Fc", "Fc fragment", and "Fc region" are used interchangeably to refer to the C-terminal region of an antibody comprising the constant region of an antibody excluding the first constant region immunoglobulin domain. Thus, Fc refers to the last two constant domains, C_{H2} and C_{H3}, of IgA, IgD, and IgG, or the last three constant domains of IgE and IgM, and the flexible hinge N-terminal to these domains. The human IgG1 heavy chain Fc region is defined herein to comprise residues C226 to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat. In the context of human IgG1, the lower hinge refers to positions 226-236, the CH2 domain refers to positions 237-340 and the CH3 domain refers to positions 341-447 according to the EU index as in Kabat. The corresponding Fc region of other immunoglobulins can be identified by sequence alignments. In the context of the present disclosure, when present, the constant region or Fc region

may be natural, as defined above, or else may be modified in various ways, provided that it comprises a functional FcR binding domain, and preferably a functional FcRn binding domain.

- The term “antibody” encompasses monovalent antibodies, i.e. antibodies comprising only one antigen binding domain (e.g. one-armed antibodies comprising a full-length heavy chain and a full-length light chain interconnected, also termed “half-antibody”), and multivalent antibodies, or antibodies comprising more than one antigen binding domain.

- The term “antibody “ according to the invention also encompasses antigen-binding fragments of antibodies. Antigen-binding fragments of antibodies include single chain antibodies (e.g. scFv and dsscfv), Fab, Fab’, $F(ab')_2$, Fv, single domain antibodies, also called nanobodies (e.g. VH or VL, or VHH or VNAR).

- The term “Fab fragment” as used herein refers to an antibody fragment comprising a light chain fragment comprising a VL (variable light) domain and a constant domain of a light chain (CL), and a VH (variable heavy) domain and a first constant domain (CH1) of a heavy chain. A typical “Fab’ fragment” comprises a heavy and a light chain pair in which the heavy chain comprises a variable region VH, a constant domain CH1 and a natural or modified hinge region and the light chain comprises a variable region VL and a constant domain CL. Dimers of a Fab’ according to the present disclosure create a $F(ab')_2$ where, for example, dimerization may be through the hinge.

- The term “single domain antibody” as used herein refers to an antibody fragment consisting of a single monomeric variable antibody domain. Examples of single domain antibodies include VH, VL, VHH or VNAR.

- The term “Fv” refers to two variable domains, for example co-operative variable domains, such as a cognate pair or affinity matured variable domains, i.e. a VH and VL pair.

- The terms “Single chain variable fragment” or “scFv” as employed herein refer to a single chain variable fragment which is stabilised by a peptide linker between the VH and VL variable domains.

- The terms “multispecific” or “multi-specific antibody” as employed herein refer to an antibody as described herein which has at least two binding domains, i.e. two or more binding domains, for example two or three binding domains, wherein the at least two binding domains independently bind two different antigens or two different epitopes on the same antigen. Multi-specific antibodies are generally monovalent for each specificity (antigen). Multi-specific antibodies described herein encompass monovalent and multivalent, e.g. bivalent, trivalent, tetravalent multi-specific antibodies.

- The term “antigen binding domain” as employed herein refers to a portion of the antibody, which comprises a part or the whole of one or more variable domains, for example a part or the whole of a pair of variable domains VH and VL, that interacts specifically with the target antigen. A binding domain may comprise a single domain antibody. In one embodiment, each binding domain is monovalent. Preferably each binding domain comprises no more than one VH and one VL.

- The term “chimeric” antibody refers to an antibody in which the variable domain (or at least a portion thereof) of the heavy and/or light chain is derived from a particular source or species, for

example a mouse, rat, rabbit or similar while the remainder of the heavy and/or light chain (i.e. the constant region) is derived from another species such as a human. Chimeric antibodies are composed of elements derived from two different species such that the element retains the characteristics of the species from which it is derived. A subcategory of “chimeric antibodies” is “humanized antibodies”. Humanised antibodies (which include CDR-grafted antibodies) are antibody molecules having one or more complementarity determining regions (CDRs) from a non-human species and a framework region from a human immunoglobulin molecule. It will be appreciated that it may only be necessary to transfer the specificity determining residues of the CDRs rather than the entire CDR. Humanised antibodies may optionally further comprise one or more framework residues derived from the non-human species from which the CDRs were derived.

- The term “fully human” antibodies refers to antibodies in which the variable regions and the constant regions (where present) of both the heavy and the light chains are all of human origin, or substantially identical to sequences of human origin, but not necessarily from the same antibody. Examples of fully human antibodies may include antibodies produced, for example by the phage display methods described above and antibodies produced by mice in which the murine immunoglobulin variable and optionally the constant region genes have been replaced by their human counterparts.

- Within the present invention, the term “epitope” is used interchangeably for both conformational and linear epitopes. A conformational epitope is composed of discontinued sections of the antigen’s amino acid primary sequence and a linear epitope is formed by a sequence formed by continuous amino acids.

- The term “isolated” antibody refers to an antibody which has been separated (e.g. by purification means) from a component of its natural environment.

- The term “isolated” polynucleotide means that the polynucleotide exists in a physical milieu distinct from that in which it may occur in nature.

- The term “K_D” as used herein refers to the equilibrium dissociation constant which is obtained from the ratio of K_d to K_a (i.e. K_d/K_a) and is expressed as a molar concentration (M). K_d and K_a refers to the dissociation rate and association rate, respectively, of a particular antigen-antibody (or antigen-binding fragment thereof) interaction. K_D values for antibodies can be determined using methods well established in the art, such as(but not limited to) those used in the example section.

- The term “blocking”, “blocks” and the like, in the context of antibodies describe an antibody that is capable of inhibiting or attenuating at least one of the biological activities of its target (here, light chains or amyloidogenic light chains). Alternatively, the term “neutralizing” or neutralizes” can be used.

- The term “specifically binds” refers to an antibody which binds with preferential or high affinity to the protein of interest (e.g. light chains or amyloidogenic light chains) but does not substantially bind to other proteins. In other words, the antibody binds to the protein of interest with no significant cross-reactivity to any other molecule. The specificity of an antibody may be further studied by

determining whether or not the antibody binds to other related proteins as discussed above or whether it discriminates between them.

Binding of antibody and antigen is mediated by the interaction of amino acids at the paratope-epitope interface of an antibody-antigen complex. Conventional immunoglobulin antigen binding sites are formed of heavy and light chain variable regions; binding to the epitope is mainly formed by the three hypervariable regions termed complementarity-determining regions (CDRs) within each chain. The number of CDR loop residues directly involved in binding to the target will vary from antibody to antibody and from epitope to epitope. Recombinant DNA technology and library-based engineering has allowed optimisation of antibody paratopes directly to modulate binding affinity, specificity and introduce new functions such as bispecificity. The third hypervariable loops, both CDR-L3 (light chain) and more commonly, owing to its greater diversity during repertoire development, CDR-H3 (heavy chain), are the most critical hypervariable loops determining antigen binding specificity and affinity, and are located at the middle of the paratope region upon the antibody and typically involved in conveying exquisite binding to target. Importantly within conventional two-chain antibodies, the heavy-and light chain packing regions are excluded from solvent and though potentially critical for antibody folding, are not directly involved in binding to target antigen.

Within the camelid repertoire of single domain antibodies, the heavy chain is expressed autonomously, independent of the light chain, owing to differential RNA splicing 3'- of the V(H)H meaning that the IgG1-containing CH1 is skipped, and the variable heavy chain is instead expressed as a fusion to the alternative IgG2/3 serotype immunoglobulin. The paratope of the V(H)H repertoire can adopt conformations distinct from those of conventional antibodies owing to the lack of light chain packing at the light-heavy chain interface. In particular, the exposed CDR-3 is conformationally freer to explore distinct paratope shapes compared to conventional antibodies. Paratope residues directly interacting with epitope can be present within CDRs 1-3, but also within frameworks, most notably in framework 2 (the interface which directly packs or pair with the light chain).

- As used herein, the terms "treatment", "treating" and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment" thus covers any treatment of a disease in a mammal, particularly in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; and (c) relieving the disease, i.e., causing regression of the disease.

- The term "therapeutically effective amount" refers to the amount of an active ingredient (such as the antibodies according to the invention) that, when administered to a mammal or other subject for treating a disease, is sufficient to produce such treatment for the disease.

- The term “biological sample” encompasses a variety of sample types obtained from an individual and can be used in a diagnostic or monitoring assay. The definition encompasses cerebrospinal fluid, blood such as plasma and serum, and other liquid samples of biological origin such as urine and saliva, solid tissue samples such as a biopsy specimen or tissue cultures or cells derived therefrom and the progeny thereof. The definition also includes samples that have been manipulated in any way after their procurement, such as by treatment with reagents, solubilization, or enrichment for certain components, such as polynucleotides

As it will now be described in more detail, the present invention is based on the identification, using phage-display screening methods, of specific molecules comprising or consisting of variable heavy (VH) domains, such as C03 or C06, targeted towards at least three well-known forms of FLCs, i.e. SMA, LEN and REC. Using gel filtration, differential scanning fluorimetry, circular dichroism and surface plasmon resonance, the VHs were characterised to bind and kinetically stabilise FLCs. These molecules (such as C03 and C06) were able not only to bind, or pairs, and stabilise the (Free) LCs but were also, and more importantly, able to inhibit LC dimers formation and thus fibril formation of LCs, whereby even a 10:1 LC:VH concentration was able to reduce aggregation to baseline levels. Atomic resolution X-ray crystallographic data of a LC:VH complex revealed binding in a 1:1 ratio, mimicking both the dimeric antigen binding site of the native Ig molecule and the native LC homodimeric structure. The inventors were able to identify binders for specific types of LC as well as pan-LC binders. Such finding is important from a therapeutic viewpoint, for instance providing options for the physicians. Indeed, in one hand it may be beneficial to use pan-LC binders to treat any type of diseases/disorders involving LCs dimers and/or formation of LCs fibrils. In another hand it may also be beneficial to be able to specifically target a specific form of LC for a given patient.

The present invention also provides evidence that the anti-light chain (LC) antibodies of the invention, preferably the VH-based antibodies of the invention, are significantly interacting (or pairing) with the LC through their framework 2 region, mimicking the dimeric antigen binding sites of the native immunoglobulin molecule, instead of the classical “epitope binding“ of an antibody to its target.

The main object of the present invention is an antibody that specifically binds to, or pairs with, free light chains, such as free kappa light chains and/or (kappa) amyloidogenic light chains, wherein the antibody comprises a heavy chain variable region comprising:

i. a CDR-H1 consisting of SEQ ID NO: 1, 4, 7, 10 or 13, a CDR-H2 consisting of SEQ ID NO: 2, 5, 8, 11 or 14 and a CDR-H3 consisting of SEQ ID NO: 3, 6, 9, 12 or 15; or
ii. CDR-H1, CDR-H2 and CDR-H3 sequences that have at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity or similarity to any of the sequences listed in i.

As non-limiting examples, the antibody according to the invention can be: a) an antibody which comprises a CDR-H1 consisting of SEQ ID NO: 1; a CDR-H2 consisting of SEQ ID NO: 2; and a CDR-H3 consisting of SEQ ID NO: 3 (e.g. such as in antibody “C03”), b) an antibody which comprises a CDR-H1 consisting of SEQ ID NO: 4; a CDR-H2 consisting of SEQ ID NO: 5; and a CDR-H3 consisting of SEQ ID NO: 6 (e.g. such as in antibody “C06”), c) an antibody which comprises a CDR-H1 consisting of SEQ ID NO: 7; a CDR-H2 consisting of SEQ ID NO: 8; and a CDR-H3 consisting of SEQ ID NO: 9 (e.g. such as in antibody “A03”), d) an antibody which comprises a CDR-H1 consisting of SEQ ID NO: 10; a CDR-H2 consisting of SEQ ID NO: 11; and a CDR-H3 consisting of SEQ ID NO: 12 (e.g. such as in antibody “D07”), e) an antibody which comprises a CDR-H1 consisting of SEQ ID NO: 13; a CDR-H2 consisting of SEQ ID NO: 14; and a CDR-H3 consisting of SEQ ID NO: 15 (e.g. such as in antibody “D09”), or f) an antibody which comprises CDR-H1, CDR-H2 and CDR-H3 sequences having at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity or similarity to any of the sequences listed in any one of a) to e).

The anti-light chain antibodies according to the invention (i.e. comprising any one of the above combinations of CDR sequences) are particularly inventive because they provide for an antibody with high affinity for free light chains, such as the kappa light chains and/or kappa amyloidogenic light chains. As an example, without any limitations, such (free) light chains can be LEN, SMA and/or REC, and/or such amyloidogenic light chains can be SMA and/or REC.

In one embodiment of the present invention, the anti-light chains antibody further comprises framework regions (FRs) comprising (or consisting of):

i. A FR1 comprising SEQ ID NO: 27, 31, 36, 38 or 42 or a sequence having at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereof ;
ii. A FR2 comprising SEQ ID NO: 28, 32, 33 or 43 or a sequence having at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereof ;
iii. A FR3 comprising SEQ ID NO: 29, 34, 37, 39, 40 or 44 or a sequence having at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereof ; and/or
iv. A FR4 comprising SEQ ID NO: 30, 35 or 41 or a sequence that has at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereof.

In another embodiment, the present invention provides an antibody that specifically binds to, or pairs with, free light chains, such as free kappa light chains and/or (kappa) amyloidogenic light chains, wherein the antibody has a heavy chain variable region comprising any one of SEQ ID NO: 16 to 20, or a sequence that has at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereof.

Also covered by the invention is an anti-light chains (alternatively anti-free light chains), such as an anti-kappa light chains and/or an anti-(kappa) amyloidogenic light chains, antibody comprising a heavy chain variable region comprising a sequence having at least 70%, 80%, 90%, 91%, 92%,

93%, 94%, 95%, 96%, 97%, 98%, or 99% identity or similarity to the sequence given in any one of SEQ ID NO: 21 to 26.

In another embodiment, the present invention provides an antibody that specifically binds to, or pairs with, free light chains, such as kappa light chains and/or (kappa) amyloidogenic light chains, wherein the antibody further comprises an Fc domain. Non-limiting examples of Fc domains comprise any one of SEQ ID Nos: 48-52, or a sequence that has at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereof. The heavy chain variable region can be linked as such to the Fc domains or can include a linker (alternatively named a hinge).

The antibody according to the invention as a whole is preferably selected from the group consisting of i) an antibody moiety comprising a VH domain but no VL domain, and ii) a nanobody (that by definition are not associated to any light chains). In other words, the antibody according to the invention as a whole preferably consists of only one heavy chain variable domain and active fragments thereof or on only one full heavy chain and active fragments thereof. The antibody herein described can be chimeric or humanized. Should the antibodies be humanised, suitable framework regions for the heavy chain of the humanized antibody according to the present invention can be derived from human germlines. Standard humanisation methods can be used, such as those described by Adair (e.g. WO9109967).

Although they are preferably in a VH only format or nanobody format, alternately the VHs according to the invention can be associated with VL comprising CDRs that specifically bind to free light chains, such as kappa light chains and/or (kappa) amyloidogenic light chains. In such a case, the resulting antibodies would likely bind to the targeted free light chains, such as kappa light chains and/or (kappa) amyloidogenic light chains, as regular antibodies, i.e. via their CDRs, instead of pairing between VHs and VLs. In another alternative they can be associated with VL comprising CDRs that specifically bind to, or pair with, one or more other targets in order to obtain a bispecific or multispecific antibody.

The anti-light chains antibody according to the invention, as a whole neutralises/inhibits the formation of light chains fibrils. In particular, they mainly bind to (or pair with) kappa light chains and/or kappa amyloidogenic light chains, such binding between the antibody according to the invention and the light chains preventing/neutralising/inhibiting the formation of light chains fibrils. In some non-limiting examples, the antibodies of the invention can bind LEN, SMA and/or REC light chains.

The anti-light chains antibody according to the invention as a whole has preferably an equilibrium dissociation constant (KD) of 1µM or less than 1µM for at least one light chain(s), such as kappa light chain(s) or kappa amyloidogenic light chain(s), such as a dissociation constant (K_D) of 900nM or less than 900nM, 800nM or less than 800nM, 700nM or less than 700nM, 600nM or less than 600nM, 500nM or less than 500nM, 100nM or less than 100nM, 50 nM or less than 50nM, 40nM or less than 40nM, 30nM or less than 30nM, 20nM or less than 20nM, as measured in an assay in which the anti-light chains antibody is the immobilised moiety (an example of such an assay is

provided in Example 1). In certain embodiments, the kD is as low as 16nM or below, when using such an assay. The KD can be measured/determined by any standard methods. For instance, the constant of dissociation can be determined by surface plasmon resonance (SPR) at a temperature of 25°C, between an antibody of the invention and light chains, such as kappa light chains and/or (kappa) amyloidogenic light chains.

In one embodiment of the invention, herein provided is an antibody that cross-competes for binding to, or pairing with, light chains, such as kappa light chains and/or (kappa) amyloidogenic light chains, with an antibody comprises heavy chain variable region comprising:

i. a CDR-H1 consisting of SEQ ID NO: 1, 4, 7, 10 or 13, a CDR-H2 consisting of SEQ ID NO: 2, 5, 8, 11 or 14 and a CDR-H3 consisting of SEQ ID NO: 3, 6, 9, 12 or 15;
ii. a CDR-H1 consisting of SEQ ID NO: 1; a CDR-H2 consisting of SEQ ID NO: 2; and a CDR-H3 consisting of SEQ ID NO: 3;
iii. a CDR-H1 consisting of SEQ ID NO: 4; a CDR-H2 consisting of SEQ ID NO: 5; and a CDR-H3 consisting of SEQ ID NO: 6;
iv. a CDR-H1 consisting of SEQ ID NO: 7; a CDR-H2 consisting of SEQ ID NO: 8; and a CDR-H3 consisting of SEQ ID NO: 9;
v. a CDR-H1 consisting of SEQ ID NO: 10; a CDR-H2 consisting of SEQ ID NO: 11; and a CDR-H3 consisting of SEQ ID NO: 12;
vi. a CDR-H1 consisting of SEQ ID NO: 13; a CDR-H2 consisting of SEQ ID NO: 14; and a CDR-H3 consisting of SEQ ID NO: 15; or
vii. a sequence according to any one of SEQ ID NO: 16-26;
viii. CDR-H1, CDR-H2 and CDR-H3 that have at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity or similarity to any of the sequences listed in any one of i. to vi.; or
ix. a sequence that has at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity or similarity to any of the sequences listed in any one of vii.

To determine if an antibody competes for binding with a reference antibody, the above-described binding methodology is performed in two different experimental setups. In a first setup, the reference antibody is allowed to bind to the antigen under saturating conditions followed by assessment of binding of the test antibody to the antigen. In a second setup, the test antibody is allowed to bind to the antigen under saturating conditions followed by assessment of binding of the reference antibody to the protein/peptide. If, in both experimental setups, only the first (saturating) antibody is capable of binding to the protein/peptide, then it is concluded that the test antibody and the reference antibody compete for binding to the antigen. As will be appreciated by the skilled person, an antibody that competes for binding with a reference antibody may not necessarily bind to the identical epitope as the reference antibody, but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope or cause a conformational change leading to the lack of binding.

Two antibodies bind to the same or overlapping epitope/pairing site if each competitively inhibits (blocks) binding/pairing of the other to the antigen. Alternatively, two antibodies have the same epitope/pairing site if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies have overlapping epitopes/pairing sites if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

Additional routine experimentation (e.g., peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antibody is in fact due to binding to the same part of the antigen as the reference antibody or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA, SPR, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art.

The epitope/pairing site can be identified by any suitable epitope mapping method known in the art in combination with any one of the antibodies provided by the present invention. Examples of such methods include screening peptides of varying lengths derived from full length light chains (such as kappa light chains and/or (kappa) amyloidogenic light chains) for binding to, pairing with, the antibody or fragment thereof of the present invention and identifying the smallest fragment that can specifically bind to, or pair with, the antibody containing the sequence of the epitope recognized by the antibody. Light chain peptides may be produced synthetically or by proteolytic digestion of the light chains. Peptides that bind the antibody can be identified by, for example, mass spectrometric analysis. Methodologies such as X-ray crystallography, Nuclear magnetic resonance (NMR) spectroscopy, Hydrogen deuterium exchange mass spectrometry (HDX-MS) or yet cryo-EM can be used to identify the epitope bound by an antibody. Typically, when the epitope determination is performed by X-ray crystallography, amino acid residues of the antigen within 4Å from CDRs are considered to be amino acid residues part of the epitope. Once identified, the epitope may serve for preparing fragments which bind an antibody of the present invention and, if required, used as an immunogen to obtain additional antibodies which bind the same epitope.

It will also be understood by one skilled in the art that antibodies may undergo a variety of posttranslational modifications. The type and extent of these modifications often depends on the host cell line used to express the antibody as well as the culture conditions. Such modifications may include variations in glycosylation, methionine oxidation, diketopiperazine formation, aspartate isomerization and asparagine deamidation. A frequent modification is the loss of a carboxy-terminal basic residue (such as lysine or arginine) due to the action of carboxypeptidases. Accordingly, the C-terminal lysine of the antibody heavy chain may be absent. In one embodiment, a C-terminal amino acid from the antibody is cleaved during post-translation modifications. In another embodiment, an N-terminal amino acid from the antibody is cleaved during post-translation modifications. In certain further embodiments, antibody variants having one or more amino acid substitutions, insertions, and/or deletions are provided. Sites of interest for substitutional

mutagenesis include the CDRs and framework regions. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, e.g., retained/improved antigen binding and/or decreased immunogenicity.

In certain embodiments, amino acid sequence variants of the antibodies described herein are contemplated. For example, it may be desirable to improve the binding/pairing affinity and/or other biological properties of the antibody. Amino acid sequence variants of the anti-light chains antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the protein, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences (such as in one or more CDRs and/or framework sequences in the VH domain) of the anti-light chains antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics.

In certain embodiments of the variant VH sequences provided herein, each CDR (or HVR) either is unaltered, or contains no more than one, two or three amino acid substitutions.

It will be appreciated that one or more amino acid substitutions, additions and/or deletions may be made to the CDRs provided by the present invention without significantly altering the ability of the antibody to bind to, or pair with, light chains, such as kappa light chains and/or (kappa) amyloidogenic light chains, and to neutralize the formation of fibrils. The effect of any amino acid substitutions, additions and/or deletions can be readily tested by one skilled in the art, for example by using the methods described herein, particularly those illustrated in the Examples, to determine light chains binding and inhibition of the formation of fibrils.

Consequently, in certain embodiments of the variant VH sequences, each CDR either contains no more than one, two or three amino acid substitutions, wherein such amino-acid substitutions are conservative, and wherein the antibody retains its binding properties to light chains, such as kappa light chains and/or (kappa) amyloidogenic light chains.

Therefore, herein provided is an antibody variant that specifically binds to, or pairs with, free light chains, such as free kappa light chains and/or (kappa) amyloidogenic light chains, wherein said antibody variant comprises a heavy chain variable region comprising CDR-H1, CDR-H2 and CDR-H3 sequences that have at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity or similarity to any of the sequences listed in any one of the following i. to vi.: i) CDR-H1 consisting of SEQ ID NO: 1, 4, 7, 10 or 13, a CDR-H2 consisting of SEQ ID NO: 2, 5, 8, 11 or 14 and a CDR-H3 consisting of SEQ ID NO: 3, 6, 9, 12 or 15, ii) CDR-H1 consisting of SEQ ID NO: 1; a CDR-H2 consisting of SEQ ID NO: 2; and a CDR-H3 consisting of SEQ ID NO: 3; iii) a CDR-H1 consisting of SEQ ID NO: 4; a CDR-H2 consisting of SEQ ID NO: 5; and a CDR-H3 consisting of SEQ ID NO: 6; iv) a CDR-H1 consisting of SEQ ID NO: 7; a CDR-H2 consisting of SEQ ID NO: 8; and a CDR-H3 consisting of SEQ ID NO: 9; v) a CDR-H1 consisting of SEQ ID NO: 10; a CDR-H2 consisting of SEQ ID NO: 11; and a CDR-H3 consisting of SEQ ID NO: 12; vi) a

CDR-H1 consisting of SEQ ID NO: 13; a CDR-H2 consisting of SEQ ID NO: 14; and a CDR-H3 consisting of SEQ ID NO: 15.

Also provided is an antibody variant that further comprises framework regions (FRs) comprising or consisting of FR1, FR2, FR3 and FR4 sequences that have at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity or similarity to any of the sequences listed in any one of the following i. to iv.: i. a FR1 comprising SEQ ID NO: 27, 31, 36, 38 or 42; ii. a FR2 comprising SEQ ID NO: 28, 32, 33 or 43; iii. a FR3 comprising SEQ ID NO: 29, 34, 37, 39, 40 or 44; and/or iv. a FR4 comprising SEQ ID NO: 30, 35 or 41.

As examples of antibody variants according to the present invention (i.e. comprising CDRs and FRs as described herein), such antibodies can comprise a heavy chain variable region comprising a sequence having at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity or similarity to the sequence given in any one of SEQ ID NO: 16 to 20.

Also covered by the invention is an anti-light chains, such as an anti-kappa light chains and/or an anti-(kappa) amyloidogenic light chains, antibody variant comprising a heavy chain variable region comprising a sequence having at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity or similarity to the sequence given in any one of SEQ ID NO: 21 to 26.

In a further alternative embodiment, an anti-light chains antibody variant, such as an anti-kappa light chains and/or an anti-(kappa) amyloidogenic light chains antibody variant, of the present invention comprises a Fc domain comprising a sequence having at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity or similarity to the sequence given in any one of SEQ ID NO: 48-52.

The anti-light chains antibody variants provided herein by the invention retain the advantageous properties of the parental antibody (i.e. unmodified antibody), i.e. the functional properties herein described. In one example, an anti-light chains antibody variant provided by the invention has a dissociation constant (K_D) of 1μM or less than 1μM for at least one light chain(s), such as kappa light chain(s) or kappa amyloidogenic light chain(s), such as a dissociation constant (K_D) of 900nM or less than 900nM, 800nM or less than 800nM, 700nM or less than 700nM, 600nM or less than 600nM, 500nM or less than 500nM, 100nM or less than 100nM, 50 nM or less than 50nM, 40nM or less than 40nM, 30nM or less than 30nM, 20nM or less than 20nM, as measured in an assay in which the anti-light chains antibody is the immobilised moiety (an example of such an assay is provided in Example 1). In certain embodiments, the K_D is as low as 16nM or below, using such an assay. The K_D can be measured/determined by any standard methods. For instance, the constant of dissociation can be determined by SPR at a temperature of 25°C, between an antibody of the invention and light chains, such as kappa and/or amyloidogenic light chains.

Degrees of identity and similarity between sequences can be readily calculated. The “% sequence identity” (or “% sequence similarity”) is calculated by: (1) comparing two optimally aligned sequences over a window of comparison (e.g., the length of the longer sequence, the length of the shorter sequence, a specified window, etc.), (2) determining the number of positions containing

identical (or similar) amino-acids (e.g., identical amino acids occurs in both sequences, similar amino acid occurs in both sequences) to yield the number of matched positions, (3) dividing the number of matched positions by the total number of positions in the comparison window (e.g., the length of the longer sequence, the length of the shorter sequence, a specified window), and (4) multiplying the result by 100 to obtain the % sequence identity or percent sequence similarity.

Methods of alignment of sequences for comparison are well-known in the art. Preferred examples of algorithms that are suitable for determining percent sequence identity and sequence similarity include the BLAST and BLAST 2.0 algorithms. Polypeptide sequences also can be compared using FASTA using default or recommended parameters. FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences.

In certain embodiments, substitutions, insertions, or deletions may occur within one or more CDR so long as such alterations do not substantially reduce the ability of the antibody to bind the target. For example, conservative alterations that do not substantially reduce binding affinity may be made in CDRs. Such alterations may be made outside of antigen contacting residues in the CDRs.

Conservative substitutions are shown in Table 1 together with more substantial “exemplary substitutions”.

Table 1: Examples of amino-acid substitutions

Original Residue	Exemplary Substitutions	Conservative Substitution
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys(C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe	Leu
Leu (L)	Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser

Original Residue	Exemplary Substitutions	Conservative Substitution
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala;	Leu

Substantial modifications in the biological properties of an antibody variant can be accomplished by selecting substitutions that differ significantly in their effect on maintaining the structure of the polypeptide backbone in the area of the substitution, the charge or hydrophobicity of the molecule at the target site, or the bulk of the side chain.

One type of substitutional variant involves substituting one or more CDR region residues of a parent antibody (humanized or human antibody). Generally, the resulting variant(s) selected for further study will have changes in certain biological properties (e.g., increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, e.g., using phage display -based affinity maturation techniques. Briefly, one or more CDR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g. binding affinity).

Alterations (e.g., substitutions) may be made in CDRs, e.g., to improve antibody affinity. Such alterations may be made in Hyper Variable Region (HVR) "hotspots," i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process, and/or residues that contact antigen, with the resulting variant VH being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been well described in the literature. In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. One of the methods that can be used for identification of residues or regions of an antibody that may be targeted for mutagenesis is alanine scanning mutagenesis. Alternatively, or additionally, an X-ray structure of an antigen-antibody complex can be used to identify contact points between the antibody and its antigen. Variants may be screened to determine whether they contain the desired properties.

Antibodies generated against light chains, such as kappa light chains and/or (kappa) amyloidogenic light chains, may be obtained after immunization of an animal by administering light chains or a portion thereof to an animal, preferably a non-human animal, using well-known and routine protocols. Many animals, such as rabbits, mice, rats, sheep, cows, llamas, camels or pigs may be immunized.

Monoclonal antibodies may be made by a variety of techniques, including but not limited to, the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing

transgenic animals containing all or a part of the human immunoglobulin loci. One non-limiting method for making antibodies is described in the example section.

Herein described is also a method of identifying an antibody according to the invention, said method comprising:

a) immunizing a non-human animal with a light chain immunogenic composition;
b) recovering B cells from said non-human mammal;
c) selecting the antibodies produced by said B cells that have at least one or more of the following properties:
    i. bind to, or pair with, free light chain with an affinity represented by a dissociation constant K_D 500nM or of less than 500nM; and/or
    ii. block, prevent or inhibit the formation of free light chain fibrils.

The present invention also provides an isolated polynucleotide encoding the antibodies according to the present invention. Therefore, herein provided is an isolated polynucleotide encoding an antibody comprises heavy chain variable region comprising:

i. a CDR-H1 consisting of SEQ ID NO: 1, 4, 7, 10 or 13, a CDR-H2 consisting of SEQ ID NO: 2, 5, 8, 11 or 14 and a CDR-H3 consisting of SEQ ID NO: 3, 6, 9, 12 or 15;
ii. a CDR-H1 consisting of SEQ ID NO: 1; a CDR-H2 consisting of SEQ ID NO: 2; and a CDR-H3 consisting of SEQ ID NO: 3;
iii. a CDR-H1 consisting of SEQ ID NO: 4; a CDR-H2 consisting of SEQ ID NO: 5; and a CDR-H3 consisting of SEQ ID NO: 6;
iv. a CDR-H1 consisting of SEQ ID NO: 7; a CDR-H2 consisting of SEQ ID NO: 8; and a CDR-H3 consisting of SEQ ID NO: 9;
v. a CDR-H1 consisting of SEQ ID NO: 10; a CDR-H2 consisting of SEQ ID NO: 11; and a CDR-H3 consisting of SEQ ID NO: 12;
vi. a CDR-H1 consisting of SEQ ID NO: 13; a CDR-H2 consisting of SEQ ID NO: 14; and a CDR-H3 consisting of SEQ ID NO: 15; or
vii. a sequence according to any one of SEQ ID NO: 16-26;
viii. CDR-H1, CDR-H2 and CDR-H3 that have at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity or similarity to any of the sequences listed in any one of i. to vi.; or
ix. a sequence that has at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity or similarity to any of the sequences listed in any one of vii.

The isolated polynucleotide according to the present invention may further comprise a sequence encoding a Fc domain, such as a Fc domain comprising any one of SEQ ID Nos: 48-52, or a sequence that has at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereof.

The isolated polynucleotide according to the present invention may comprise synthetic DNA, for instance produced by chemical processing, cDNA, genomic DNA or yet mRNA or any combination thereof.

The present invention also provides for a cloning or expression vector comprising one or more polynucleotides described herein. In one example, the cloning or expression vector according to the present invention comprises one or more isolated polynucleotides as described above.

Standard techniques of molecular biology may be used to prepare DNA sequences coding for the antibody of the present invention. Desired DNA sequences may be synthesized completely or in part using oligonucleotide synthesis techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate.

General methods by which the vectors may be constructed, transfection methods and culture methods are well known to those skilled in the art.

Also provided is a host cell comprising one or more isolated polynucleotide sequences according to the invention or one or more cloning or expression vectors comprising one or more isolated polynucleotide sequences encoding an antibody of the present invention. Any suitable host cell/vector system may be used for expression of the polynucleotide sequences encoding the antibody of the present invention. Bacterial, for example *E. coli*, and other microbial systems may be used. In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern. Further host cells that can be used are any eukaryotic cells, for example mammalian. Suitable mammalian host cells include Chinese Hamster Ovary (CHO) cell, human embryonic cell (HEK cell) or lymphoid cell (e.g., Y0, NS0, Sp20 cell). Suitable types of CHO cells according to the present invention may include CHO and CHO-K1 cells including dhfr- CHO cells, such as CHO-DG44 cells and CHO-DXB11 cells and which may be used with a DHFR selectable marker or CHOK1-SV cells which may be used with a glutamine synthetase selectable marker. Other cell types of use in expressing antibodies include lymphocytic cell lines, e.g., NS0 myeloma cells and SP2 cells, COS cells; or human embryonic cells such as HEK293, HEK293F, HEK293S or EK293T. The host cell may be stably transformed or transfected with the isolated polynucleotide sequences or the expression vectors according to the present invention.

The present invention also provides a process for the production of an antibody according to the present invention comprising culturing a host cell according to the present invention under conditions suitable for producing the antibody according to the invention and isolating the antibody.

The present invention also provides a process for the production of a pharmaceutical composition comprising an antibody according to the present invention comprising culturing a host cell according to the present invention under conditions suitable for producing the antibody according

to the invention, isolating the antibody, and formulating the antibody into a pharmaceutical composition.

The antibody of the invention preferably comprises only a heavy chain polypeptide, in which case only a heavy chain polypeptide coding sequence needs to be used to transfect the host cells (in only one vector). For production of antibodies comprising both heavy and light chains, the cell line may be transfected with two vectors, a first vector encoding a light chain polypeptide and a second vector encoding a heavy chain polypeptide. Alternatively, a single vector may be used, the vector including sequences encoding light chain and heavy chain polypeptides.

Thus, here is provided a process for culturing a host cell and expressing an antibody according to the invention, isolating the antibody and optionally purifying said antibody to provide an isolated antibody.

The present invention also provides a process for the production of an antibody according to the present invention comprising culturing a host cell containing a vector of the present invention under conditions suitable for leading to expression of protein from DNA encoding the antibody molecule of the present invention and isolating the antibody molecule.

In one embodiment there is provided a purified antibody, for example a humanized antibody, in particular an antibody according to the invention, in substantially purified form, in particular free or substantially free of endotoxin and/or host cell protein or DNA.

The antibody according to the invention may be provided in a pharmaceutical composition or diagnostic composition. Therefore, the present invention also provides for a pharmaceutical composition, or diagnostic composition, comprising the antibody according to the present invention, or a polynucleotide encoding the antibody according to the present invention, in combination with one or more of acceptable carriers, excipients and/or diluents, such as pharmaceutically acceptable carriers, excipients and/or diluents. Preferably, the pharmaceutical composition, or diagnostic composition, comprises an antibody which specifically binds free light chains, such as free kappa light chains and/or (kappa) amyloidogenic light chains, or a polynucleotide encoding such antibody, and one or more pharmaceutically acceptable carriers, excipients and/or diluents, wherein said antibody comprises a heavy chain variable region comprising:

i. CDR-H1 consisting of SEQ ID NO: 1, 4, 7, 10 or 13, a CDR-H2 consisting of SEQ ID NO: 2, 5, 8, 11 or 14 and a CDR-H3 consisting of SEQ ID NO: 3, 6, 9, 12 or 15;
ii. CDR-H1 consisting of SEQ ID NO: 1; a CDR-H2 consisting of SEQ ID NO: 2; and a CDR-H3 consisting of SEQ ID NO: 3;
iii. a CDR-H1 consisting of SEQ ID NO: 4; a CDR-H2 consisting of SEQ ID NO: 5; and a CDR-H3 consisting of SEQ ID NO: 6;
iv. a CDR-H1 consisting of SEQ ID NO: 7; a CDR-H2 consisting of SEQ ID NO: 8; and a CDR-H3 consisting of SEQ ID NO: 9;

v. a CDR-H1 consisting of SEQ ID NO: 10; a CDR-H2 consisting of SEQ ID NO: 11; and a CDR-H3 consisting of SEQ ID NO: 12;

vi. a CDR-H1 consisting of SEQ ID NO: 13; a CDR-H2 consisting of SEQ ID NO: 14; and a CDR-H3 consisting of SEQ ID NO: 15; or

vii. a sequence according to any one of SEQ ID NO: 16-26;

viii. CDR-H1, CDR-H2 and CDR-H3 that have at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity or similarity to any of the sequences listed in any one of i. to vi.; or

ix. a sequence that has at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity or similarity to any of the sequences listed in any one of vii.

The pharmaceutical compositions or diagnostic compositions, according to the invention can be used respectively in therapy and/or diagnosis. The pharmaceutical composition may be administered suitably to a patient to identify the therapeutically effective amount required. For any antibody, the therapeutically effective amount can be estimated initially either in cell culture assays or in animal models, usually in rodents, rabbits, dogs, pigs or primates. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans. The precise therapeutically effective amount for a human subject will depend upon the severity of the disease state, the general health of the subject, the age, weight and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities and tolerance/response to therapy. Generally, a therapeutically effective amount will be from 0.01 mg/kg to 500 mg/kg, for example 0.1 mg/kg to 200 mg/kg, such as 100mg/kg. Pharmaceutical compositions may be conveniently presented in unit dose forms containing a predetermined amount of an active agent of the invention per dose.

The pharmaceutical compositions or diagnostic compositions, according to the invention preferably comprise not only the antibody according to the invention, or a polynucleotide encoding such antibody, but also one or more (pharmaceutically) acceptable carriers, excipients (such as buffer, stabilizer, suspending, preservative, and/or dispersing agents or other materials well known to those skilled in the art) and/or diluent. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. Examples of the carriers, excipients and/or diluents, and method for preparing pharmaceutical compositions can be found in Remington's Pharmaceutical Sciences, 20th Edition, 2000, pub. Lippincott, Williams & Wilkins.

Suitable forms for administration include forms suitable for parenteral administration, e.g. by injection or infusion, for example by bolus injection or continuous infusion, in intravenous, inhalable or sub-cutaneous form. Where the product is for injection or infusion, it may take the form of a suspension, solution or emulsion in an oily or aqueous vehicle. Alternatively, the antibody according to the invention may be in dry form, for reconstitution before use with an appropriate

sterile liquid. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared.

Once formulated, the compositions of the invention can be administered directly to the subject or can be administered after reconstitution depending on the form. As an alternative they can be used for diagnosing purpose.

Also provided herein is the use of an antibody according to the invention, or a polynucleotide encoding such antibody, for the manufacture of a medicament.

Preferably, the pharmaceutical composition according to the present invention is adapted for administration to primate, such as human or non-human subjects.

Also herein encompassed is a pharmaceutical comprising an antibody that specifically binds light chains, such as kappa light chains and/or (kappa) amyloidogenic light chains, or a polynucleotide encoding such antibody, and one or more pharmaceutically acceptable carriers, excipients and/or diluents, for use in therapy and/or diagnosis, wherein said antibody comprises a heavy chain variable region comprising :

i. a CDR-H1 consisting of SEQ ID NO: 1, 4, 7, 10 or 13, a CDR-H2 consisting of SEQ ID NO: 2, 5, 8, 11 or 14 and a CDR-H3 consisting of SEQ ID NO: 3, 6, 9, 12 or 15;
ii. a CDR-H1 consisting of SEQ ID NO: 1; a CDR-H2 consisting of SEQ ID NO: 2; and a CDR-H3 consisting of SEQ ID NO: 3;
iii. a CDR-H1 consisting of SEQ ID NO: 4; a CDR-H2 consisting of SEQ ID NO: 5; and a CDR-H3 consisting of SEQ ID NO: 6;
iv. a CDR-H1 consisting of SEQ ID NO: 7; a CDR-H2 consisting of SEQ ID NO: 8; and a CDR-H3 consisting of SEQ ID NO: 9;
v. a CDR-H1 consisting of SEQ ID NO: 10; a CDR-H2 consisting of SEQ ID NO: 11; and a CDR-H3 consisting of SEQ ID NO: 12;
vi. a CDR-H1 consisting of SEQ ID NO: 13; a CDR-H2 consisting of SEQ ID NO: 14; and a CDR-H3 consisting of SEQ ID NO: 15; or
vii. a sequence according to any one of SEQ ID NO: 16-26;
viii. CDR-H1, CDR-H2 and CDR-H3 that have at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity or similarity to any of the sequences listed in any one of i. to vi.; or
ix. a sequence that has at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity or similarity to any of the sequences listed in any one of vii.

Herein also encompassed is an antibody according to the invention, a polynucleotide according to the invention or a pharmaceutical composition according to the invention for use in therapy, in particular for use in the treatment of a disorder or condition as described herein. Such antibody (or polynucleotide or pharmaceutical composition) is administered in a therapeutically effective amount.

The present invention provides a method of treating a disorder or condition as described herein in a subject in need thereof, the method comprising administering to the subject an antibody, a polynucleotide or a pharmaceutical composition according to the present invention. Such antibody is administered in a therapeutically effective amount.

The present invention also provides the use of an antibody, a polynucleotide or a pharmaceutical composition of the invention for the manufacture of a medicament, in particular for use in the treatment of a disorder or condition as described herein.

In the context of the present invention as a whole the disorder or condition is characterized by over expression of light chains, aggregation of light chains and/or formation and accumulation of light chains fibrils. Preferably, the disorder or condition is selected from the group consisting of light chain (AL) amyloidosis, Light chain deposition disease (LCDD), light- and heavy-chain deposition disease (LHCDD), Crystal-storing histiocytosis, asthma, an inflammatory and autoimmune disease (such as rheumatoid arthritis, Sjogren's disease, idiopathic urticaria, multiple sclerosis, diabetes mellitus, systemic lupus erythematosus, lupus nephritis and inflammatory bowel disease) and some categories of cancers such as multiple myeloma (including lambda and/or kappa myeloma).

As mentioned above, the inventors were able to identify pan-LC binders and more specific LC binders. Such finding is important from a therapeutic viewpoint. Indeed, in one hand it may be beneficial to use pan-LC binders to treat all amyloid light chain patients, without the need for preliminary diagnosing the patient in need thereof. Alternatively, a combination of two or more specific LC binders could be an option to avoid the need for preliminary diagnosing the patient in need thereof. In another hand it may also be beneficial to be able to specifically target the form of LC for a given patient. In such a case, the patient would need to have their light chains sequenced and a high-affinity therapeutic could then be designed onto the VH scaffold (personal medicine).

Herein also encompassed is the use of an anti-light chains antibody (such as an anti-kappa light chains and/or an anti-(kappa) amyloidogenic light chains,) or a variable domain fragment thereof for use as diagnostically active agents or in diagnostic assays, for example for diagnosing of a disorder or condition as described herein. The diagnosis may preferably be performed on biological samples, such as human biological samples (e.g. urine, blood, plasma, serum, red blood cells, tissue, saliva, placental tissue, bone marrow, breast milk, bronchoalveolar lavage, faeces, pleural fluid, synovial fluid, and semen). Diagnostic testing may preferably be performed in vitro.

As specified above, the anti-light chain (LC) antibodies of the invention, and more particularly the heavy chain domains of the invention, are significantly interacting (or pairing) with the FLC through their framework 2 regions, mimicking the dimeric antigen binding sites of the native immunoglobulin molecule, instead of the classical "epitope binding" of an antibody to its target. Therefore the term/notion of "anti-light chain (LC) antibody that specifically binds to free light chains" (and any equivalent thereof) in this document as a whole is interchangeable with "heavy chain domain that specifically interacts, or pairs, with free light chains". It follows that for instance:

- The present invention provides heavy chain domains that specifically interact, or pair, with free light chains, such as free kappa light chains and/or (kappa) amyloidogenic light chains, wherein the heavy chain domain comprises a heavy chain variable region comprising:
  i. a CDR-H1 consisting of SEQ ID NO: 1, 4, 7, 10 or 13, a CDR-H2 consisting of SEQ ID NO: 2, 5, 8, 11 or 14 and a CDR-H3 consisting of SEQ ID NO: 3, 6, 9, 12 or 15; or
  ii. a combination of CDR-H1, CDR-H2 and CDR-H3 sequences having at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity or similarity to any of the sequences listed in i.
- The heavy chain domains according to the invention can further comprise a heavy chain variable region comprising:
  i. CDR-H1 consisting of SEQ ID NO: 1; a CDR-H2 consisting of SEQ ID NO: 2; and a CDR-H3 consisting of SEQ ID NO: 3;
  ii. a CDR-H1 consisting of SEQ ID NO: 4; a CDR-H2 consisting of SEQ ID NO: 5; and a CDR-H3 consisting of SEQ ID NO: 6;
  iii. a CDR-H1 consisting of SEQ ID NO: 7; a CDR-H2 consisting of SEQ ID NO: 8; and a CDR-H3 consisting of SEQ ID NO: 9;
  iv. a CDR-H1 consisting of SEQ ID NO: 10; a CDR-H2 consisting of SEQ ID NO: 11; and a CDR-H3 consisting of SEQ ID NO: 12;
  v. a CDR-H1 consisting of SEQ ID NO: 13; a CDR-H2 consisting of SEQ ID NO: 14; and a CDR-H3 consisting of SEQ ID NO: 15; or
  vi. CDR-H1, CDR-H2 and CDR-H3 sequences having at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity or similarity to any of the sequences listed in any one of i. to v.
- The present invention provides heavy chain domains that specifically interact, or pair, with free light chains, such as kappa light chains and/or (kappa) amyloidogenic light chains, wherein the heavy chain domains have a heavy chain variable region comprising any one of SEQ ID NO: 16 to 26 or a sequence having at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereof.
- The heavy chain domains of the invention are selected from the group consisting of: i) a heavy chain domain comprising a VH domain but no VL domain or ii) a nanobody.
- The heavy chain domains of the invention are selected from the group consisting of chimeric heavy chain domains or humanized heavy chain domains.
- The heavy chain domains of the invention can further comprise a Fc domain selected from the group consisting of any one of SEQ ID NO: 48 to 52 or a sequence having at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereof.
- The heavy chain domains of the invention preferably neutralise or inhibit the formation of light chains dimers and/or light chains fibrils.

- The heavy chain domains of the invention preferably have an equilibrium dissociation constant (K_D) of less than 500 nM for light chains.
- Herein also provided are:
    - An Isolated polynucleotide encoding a heavy chain domain of the invention,
    - A cloning or expression vector comprising an isolated polynucleotide encoding a heavy chain domain of the invention,
    - A host cell comprising an isolated polynucleotide encoding a heavy chain domain of the invention or an expression vector comprising an isolated polynucleotide encoding a heavy chain domain of the invention.
    - A process for the production of a heavy chain domain of the invention, comprising culturing a host cell under suitable conditions for producing the heavy chain domain and isolating said heavy chain domain.
    - A pharmaceutical composition comprising a heavy chain domain of the invention, or a polynucleotide encoding said heavy chain domain, and one or more pharmaceutically acceptable carriers, excipients and/or diluents.
    - The heavy chain domains of the invention, the polynucleotide of the invention or the pharmaceutical compositions of the invention for use in therapy and/or diagnosis, such as in the treatment of a disorder or condition selected from the group consisting of light chain (AL) amyloidosis, Light chain deposition disease (LCDD), light- and heavy-chain deposition disease (LHCDD), Crystal-storing histiocytosis, asthma, an inflammatory and autoimmune disease (such as rheumatoid arthritis, Sjogren's disease, idiopathic urticaria, multiple sclerosis, diabetes mellitus, systemic lupus erythematosus, lupus nephritis and inflammatory bowel disease) and some categories of cancers such as multiple myeloma (including lambda myeloma and kappa myeloma).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Analytical gel filtration profiles for LEN (A), SMA (B) and REC (C) alone and in complex with C03.

Figure 2: Biacore SPR analysis of C03 binding to LEN (A), SMA (B) and REC (C). The light chains were injected over the captured C03 at varying concentrations. Example binding traces (shown in light grey) are given for each ligand and were fit with both a 1:1 binding model (i) and a heterogeneous ligand model (ii) (black line).

Figure 3: Thermal denaturation. Mean normalised fluorescence (%) measured by DSF for SMA (A), REC (C) and LEN (E) alone and in complex with C03 (i). Recorded Tm values were plotted with SEM error bars indicated (ii). * indicates a statistically significant increase in Tm of the LC:C03 complexes compared to the LCs alone (unpaired T-test $P<0.0001$). N=3 for each DSF experiment. Single transitions are observed for all proteins except LEN and the LEN:C03 complex. Here, two

transitions show a different cooperativity of unfolding, indicating the presence of two different conformations. Both transitions were analysed separately and plotted (E, iii). Thermal transition curves from Circular Dichroism (CD), at 202 nm, were plotted for the LCs between 30-70ºC with Boltzmann curves fit for SMA (B), REC (D) and LEN (F) alone and in complex with C03. Calculated Tm values are represented in the form of a bar chart (G) and given in Table 6.

Figure 4: Inhibition of SMA amyloid formation by C03 presence. A) 1:1 SMA:C03, B) 2:1 SMA:C03, C) 10:1 SMA:C03. D) Transmission electron microscope image of SMA (1mg/mL), E) Transmission electron microscope image of SMA (1mg/mL) at a higher resolution compared to D), F) 1:1 SMA:C03, G) 2:1 SMA:C03, H) 10:1 SMA:C03, I) C03 (1mg/mL) following incubation for 18 days. Scale bar 500nm.

Figure 5: Structural analysis of the SMA homodimer crystal structure. Magnified images of the A) hydrogen bond network involving the residues Gln44, Gln48 and Tyr93 for each halfmer (top one and bottom one) and B) the salt bridge interactions between His100 and Glu61 for each halfmer.

Figure 6: Structural analysis of the SMA:C03 heterodimer crystal structure. Magnified images of the A) hydrogen bond network involving SMA residues (Gln44, Tyr42, Tyr93 and Gln106) and C03 residues (Gln39, Lys43, Tyr95 and Tyr105) and B) the salt bridge interactions between His100(S)/Asp59(V) and Glu61(S)/Lys106(V).

Figure 7: Multiple sequence alignment of SMA and fifty randomly chosen (amyloidogenic) κ LC sequences, identified by an AL amyloidosis -base database search. Any sequences longer than 120 residues were cropped. Residues shown to be crucial for SMA:C03 binding are highlighted by the boxes.

Figure 8: Multiple sequence alignment of SMA and the fifty randomly chosen (amyloidogenic) λ LC sequences, identified by an AL amyloidosis -base database search. Any sequences longer than 120 residues were cropped. Residues shown to be crucial for SMA:C03 binding are highlighted by the boxes.

Figure 9: Structural overview and multiple sequence alignment of LEN, SMA and REC. The crystal structures of the canonical dimer LEN (left) and non-canonical dimer REC (right). LEN has both monomeric subunits orientated in the same direction, whereas the REC dimer has each subunit orientated 180º with respect to each other. There is no structural information available for SMA yet. The multiple sequence alignment of the germline κIV sequence (bottom) against LEN, SMA and REC is shown, with residues changes underlined. LEN differs from the germline by only one residue (N29S). SMA differs from LEN by a total of 8 residues and REC differs by 14 (highlighted across edges of the central triangle).

Figure 10: Full sequence alignment of the eleven hits chosen for further analysis. The conventional VH (FR2) and the three CDR residues are identified.

Figure 11: BLI analysis of C03 (as a VH) binding to LEN (A), SMA (B) and REC (C). Various concentrations of C03 were used. Binding traces are provided for each ligand with 1:1 binding model.

Description of the sequences:

SEQ ID NO	Sequence	Name
1	GFNFGSSDMN	C03-CDR1
2	SIERGAGGTDYADSVQG	C03-CDR2
3	SDNSGYYKY	C03-CDR3
4	GFTFSNYYMF	C06-CDR1
5	GINSGGGSTNYADSVKG	C06-CDR2
6	PRRGPWKY	C06-CDR3 B04-CDR3 H02-CDR3
7	GFSFGAHAMS	A03-CDR1
8	TIIGGGGSTSYADSVKG	A03-CDR2
9	SQGNYWYVFDY	A03-CDR3 D04-CDR3
10	GFALSSYGMS	D07-CDR1
11	GINSGGATISYGDSVKG	D07-CDR2
12	YVGSSWWRGYLEV	D07-CDR3
13	GFSYREYSMA	D09-CDR1
14	AITPSGFKTAYADSVKG	D09-CDR2
15	HRRDAWFSEYDV	D09-CDR3
16	EVQLVESGGGLVLPGGSLKLSCAASGFNFGSSDMNWVRQAAGKGPE WVASIERGAGGTDYADSVQGRFTVSRDNAKSTLWLQMNSLKAEDTAV YYCVVSDNSGYYKYWGQGTQVTVSS	C03-VH
17	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYYMFWVRQAPGKGLE WVSGINSGGGSTNYADSVKGRFTISRDNAKNTLYLQMNNLKPEDTAV YYCVNPRRGPWKYWGQGTQVTVSS	C06-VH
18	EVQLVESGGGVVQPGESLRLSCAASGFSFGAHAMSWVRQAPGKGLE WVSTIIGGGGSTSYADSVKGRFTISKDNAKNTLYLQMDGLKPEDTAVY YCTKSQGNYWYVFDYWGQGTQVTVSS	A03-VH
19	EVQLVESGGGLVQPGGSLRLSCAASGFALSSYGMSWVRQAPGKGLE WVSGINSGGATISYGDSVKGRFTISRDNAKNTLFLQMNSLTPEDTAVY YCAKYVGSSWWRGYLEVWGRGTLVTVSS	D07-VH
20	EVQLVESGGDLVQPGGSLRVTCTASGFSYREYSMAWVRLLPGKGLE WVAAITPSGFKTAYADSVKGRFTISRDNPKNTLHLEMNNLKPEDTALY YCARHRRDAWFSEYDVWGQGTQVTVSS	D09-VH

21	EVQLVESGGGLVQPGGSLRVSCAVSGFPFSNYGMSWVRQAPGKGLE WVSAIDHGGGETSYADSVKGRFTISRDNAKNTLYLQMNNLKPEDTAVY YCVNPRRGPWKYWGQGTQVTVSS	B04-VH
22	EVQLVESGGGLVQAGGSLRLSCAASGRTFSGSAMSWVRQAPGKGLE WVSTISSGGGNSAYADSVKGRFTISRDSAKNTLYLQMNNLKPEDTAVY YCVNPRRGPWKYWGQGTQVTVSS	H02-VH
23	QVQLVESGGGFAQPGGSLRLSCATSGFTFDDYAMSWVRQAPGKGLE WVAGISWSGDSAYYSQTIKGRFTVSRDNAKNTMYLQMNNLKPEDSAR YYCTKSQGNYWYVFDYWGQGTQVTVSS	D04-VH
24	EVQLVESGGGVVQPGGSLRLSCAASGFTFDDYDMSWVRQAPGKGLE WVSAISWNGGNTYYAESLKGRFTISRDNAKNTVYLQMNSLKPEDTAV YYCARSLYTVERGGYDYWGQGTQVTVSS	G02-VH
25	QVQLVESGGGLVQPGGSLRLSCAAFGFRFDDYAMSWVRQVPGKGLE WVAAITWDGSSQFYAESIKGRFSISRDNTNNTLSLQMNNLKPEDTAVY YCAKNLGDVITTAFGSWGQGTLVTVSS	C10-VH
26	QVQLVESGGGLVQPGGSLRLSCSASGFTFSNQIMGWVRQAPGKGLE WVSTINTGDSTYYADSVKGRFTISRDNAKNTLYLQMNSLKAEDTAVYY CAKDVGHGYDYWGQGTQVTVSS	D01-VH
27	EVQLVESGGX1X2VX3PGX4SLX5X6X7CX8AS With; X1=G or D; X2=L or V; X3=Q or L; X4=G or E; X5=R or K; X6=L or V; X7=S or T; X8=A or T	FR1-consensus
28	WVRX1X2X3GKGX4EWVX5 With X1=Q or L; X2=A or L; X3=P or A; X4=L or P; X5=A or S	FR2 consensus
29	RFTX1SX2DNX3KX4TLX5LX6MX7X8LX9X10EDTAX11YYCX12X13 With X1=V or I; X2=R or K; X3=A or P; X4=N or S; X5=W,Y,F or H; X6=Q or E; X7=N or D; X8=S,G or N; X9=K or T; X10=P or A; X11=V or L; X12=V, T or A; X13=V, N, K or R	FR3-consensus
30	WGX1GTX2VTVSS With X1=Q or R and X2=Q or L	FR-4 consensus
31	EVQLVESGGGLVLPGGSLKLSCAAS	FR1-C03
32	WVRQAAGKGPEWVA	FR2-C03
33	WVRQAPGKGLEWVS	FR2-C06, A03, D07
34	RFTVSRDNAKSTLWLQMNSLKAEDTAVYYCVV	FR3-C03
35	WGQGTQVTVSS	FR4-C03, C06,A03,D09
36	EVQLVESGGGLVQPGGSLRLSCAAS	FR1-C06,D07

37	RFTISRDNAKNTLYLQMNNLKPEDTAVYYCVN	FR3-C06
38	EVQLVESGGGVVQPGESLRLSCAAS	FR1-A03
39	RFTISKDNAKNTLYLQMDGLKPEDTAVYYCTK	FR3-A03
40	RFTISRDNAKNTLFLQMNSLTPEDTAVYYCAK	FR3-D07
41	WGRGTLVTVSS	FR4-D07
42	EVQLVESGGDLVQPGGSLRVTCTAS	FR1-D09
43	WVRLLPGKGLEWVA	FR2-D09
44	RFTISRDNPKNTLHLEMNNLKPEDTALYYCAR	FR3-D09
45	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNSKNYLAWYQQKPG QPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQ QYYSTPYSFGQGTKLEIKR	LEN
46	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNRNYLAWYQQKLG QPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCH QYYSHPQTFGQGTKLELKR	SMA
47	DIVMTQSPDSLAVSPGERATINCKSSQNLLDASFDTNTLAWYQQKPGQ PPKLLIYWASSRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQ YYSTPPTFGGGTKVEIKR	REC
48	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	Hu Fc (IgG1)
49	SKPTCPPPELLGGPSVFIFPPKPKDTLMISRTPEVTCVVVDVSQDDPEV QFTWYINNEQVRTARPPLREQQFNSTIRVVSTLPIAHQDWLRGKEFKC KVHNKALPAPIEKTISKARGQPLEPKVYTMGPPREELSSRSVSLTCMIN GFYPSDISVEWEKNGKAEDNYKTTPAVLDSDGSYFLYSKLSVPTSEW QRGDVFTCSVMHEALHNHYTQKSISRSPGK	RbFc (IgG)
50	GCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDPEVQF SWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCR VNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDF FPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLNVQKSNWE AGNTFTCSVLHEGLHNHHTEKSLSHSPGK	MsFc (IgG1)
51	ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS RLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK	Hu Fc (IgG4)
52	ESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQD	Hu Fc (IgG4P)

	WLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS RLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK	

Note: in the above Table, right column, "VH" stands for variable heavy chain, "CDR" for complementarity determining regions and "FR" for Framework region.

Examples

Methods

Expression and Purification of Light Chain Variable Domains (i.e. the target): The coding region for the three light chains SMA, LEN and REC were cloned into pET11a(+) with a C-terminal Avi-tag and an N-terminal hexa-histidine tag with a SUMO cleavage site, alongside the pOPINS vector with an N-terminal hexa-histidine tag with a SUMO cleavage site. Light chain (LC) constructs were transformed into SHuffle cells (New England Bioscience) and grown at 37°C, with protein expression induced by the addition of 0.5 mM IPTG for 16h at 16°C. After harvesting, cells were lysed using a cell disruptor (Constant Systems) and the clarified lysate (in 20 mM Na2HPO4, 500 mM NaCl, 20 mM Imidazole, pH 7.4) was loaded onto a 5 mL His-Trap FF column (GE Healthcare) and the bound protein eluted over 5 column volumes in 250 mM imidazole. The His6-SUMO tag was removed by SUMO protease cleavage at 4°C whilst dialysing into PBS (Sigma), before re-applying to the His-Trap column and collecting the flow-through. Purified LCs were dialysed into 10% v/v PBS:ddH2O, lyophilised and reconstituted in 10% of the original volume ddH2O.

Protein biotinylation: Avi-tagged LEN, SMA and REC were site specifically biotinylated using the BirA-500 kit (Avidity), as per manufacturer's instruction.

Phage library enrichment against LCs: Enrichment of a naïve llama VHH phage library against monomeric/dimeric LCs was performed as follows. 500 nM biotinylated LC was pre-immobilized on Dynabeads M-280 Streptavidin- (Invitrogen) and Sera-Mag SpeedBeads Neutravidin-coated beads (Cytiva), for round 1 and 2/3 respectively. The beads were then incubated with the phage population. Following incubation, unbound phage were removed by 5 or 10 washes with PBS-T buffer (PBS, pH 7.4, and 0.1% (v/v) Tween-20) for round 1/2 and 3, respectively. Bound phages were eluted from the beads by the addition of Tris-buffered saline/calcium chloride (TBSC) buffer containing 100 µg/ml trypsin and incubated for 30 minutes at room temperature. Phages were allowed to infect *E. coli* and rescued as described previously (Wilkes et al., 2020)

Isolation of anti-LC Nanobodies: Individual colonies of phage from rounds 2 and 3 of the panning were subjected to monoclonal rescue, as previously described (Wilkes et al., 2020). An ELISA assay was used to assess binding to/pairing with three LC, here LEN, SMA and REC (using standard protocol).

VH Fc-Fusion Protein Construct Design, Mammalian Expression and Purification: Selected VH sequences were synthesized and cloned into a mammalian Fc-fusion protein expression plasmid (Twist Bioscience) and expressed using the Expi-293 Expression System Kit, as by manufacturer's

instruction. Purification of the VH-fusion proteins was achieved using the PhyNexus MEA2 system, utilising protein A tips. Eluted fractions were pooled, and buffer exchanged into PBS using Zeba Spin 7K MWCO columns (Thermofisher).

VH Antibody Fragment Expression and Purification: Selected VH coding regions were cloned into pET11a(+) with an N-terminal His6-tag with a TEV cleavage site. The VH constructs were transformed into SHuffle cells (New England Bioscience) and grown at 37 °C, with protein expression induced by the addition of 0.5 mM IPTG for 16h at 16°C. After harvesting, cells were lysed using a cell disruptor (Constant Systems) and the clarified lysate (in 20 mM Na2HPO4, 500 mM NaCl, 20 mM Imidazole, pH 7.4) was loaded onto a 5 mL His-Trap FF column (GE Healthcare) and eluted over 5 column volumes in 250 mM imidazole. C03 was further purified using a HiLoad 16/600 Superdex 200pg gel filtration column (Cytiva) equilibrated in PBS.

VH-LC Interactions - Analytical Gel Filtration: 1:1 LC-VH complex, at a total concentration of 1 mg/mL of (i.e. 0.5 mg/mL of each protein) was applied, via a superloop, to a HiLoad 10/300 Superdex 75 pg size exclusion column. The column had been pre-equilibrated with PBS and the chromatographic profile recorded at a flow rate of 0.5 mL/min. LCs only and VH only controls (500 µL of 0.5 mg/mL) were also recorded.

VH-LC Binding Kinetics - Surface Plasmon Resonance (SPR)(heavy chain immobilisation): Kinetics experiments were performed using Biacore systems (Cytiva) in PBS pH 7.4 and were automated. A Series S Sensor Chip NTA (GE healthcare) was prepared by a capture coupling method, whereby an initial nickel injection and activation using a mixture of N-hydroxyl-succinimide (NHS) and N-ethyl-N-(3-diethylaminopropyl) carbodiimide (EDC) was performed, followed by the immobilisation of 0.2 µM His-tagged VH (flow rate 30 µL/minute; contact time 180 s) (as by manufacturer's instructions). A control flow cell was prepared without ligand immobilization and used to correct the experimental data for refractive index changes and non-specific interactions. Each sensor chip contained 1 reference flow cell channel and 3 sample flow cells. To derive the binding kinetics of LEN, SMA and REC, five point, three-fold serial dilutions (range of 1–0.012 µM) were prepared and injected sequentially over the functional and reference flow cells (flow rate 5 µL/minute: contact time 120 s). This was followed by a dissociation step with running buffer (flow rate 5 µL/minute: contact time 80 s). A total of n=3 biological repeats (9 technical) were recorded for SMA, n=2 (6 technical) were recorded for LEN and REC. The light chains were injected over the captured C03 at varying concentrations; 0.012 µM, 0.037 µM, 0.11 µM, 0.33 µM and 1 µM.

VH-LC Binding Kinetics via Biolayer Interferometry (light chain immobilisation): Streptavidin-coated biosensor tips (ForteBio, Inc.) were pre-wetted with PBS 0.05% tween20 buffer for 1 minute to establish a baseline prior to LC immobilisation. The biotinylated LCs at a concentration of 500 nM, were loaded, for 5 minutes, before allowing to associate with concentrations of VH1 (4 µM-31.3 nm for LEN and SMA, 8 µM-250 nm for REC) until saturation of the highest concentration was reached. Sensors were placed back into the baseline buffer for 5 minutes to detect dissociation.

Differential Scanning Fluorimetry Assays: Thermal stability assays were performed using a StepOnePlus Real-Time PCR System (Applied Biosystems) over a temperature range of 25-95 °C using Sypro-Orange protein gel stain (Invitrogen). 10 µM SMA and VH alone and 20 µM of the SMA:VH complex were prepared in PBS (20 mM Na2HPO4, 150 mM NaCl, pH 7.4). Data were processed using the Boltzmann equation to generate thermal transition curves. The melting temperature (Tm), corresponding to the equilibrium point of unfolding, was recorded as the V50 value of the sigmoidal melt curve.

Circular Dichroism Spectroscopy: Protein samples were dialysed into 20 mM Na2HPO4, 150 mM KF pH 7.4 to overcome interference from salt ions at lower wavelengths. Proteins were analysed at 0.05 mg/mL using a 1 mm pathlength cuvette on a J-100 Series CD spectropolarimeter (JASCO) over a range of 180-260 nm. Scan speed was set at 50 nm/minute, with a digital integration time (DIT) of 1 second and a total of 3 accumulations. Spectra were recorded at 2-degree intervals between 20-80 °C. Each temperature increment was held for 120 seconds before the spectra was recorded.

Light Chain Amyloid Fibril Formation and Thioflavin T detection: 1 mg/mL (~80 µM) of each LC protein was incubated at 37°C with agitation for 18 days. Samples were taken at specific time points (2-day intervals), flash frozen in liquid nitrogen and stored at -80ºC. To analyse, these samples were diluted to 20 µM, to occupy 50 µL per well in clear bottom/black-walled microplates (Greiner) and 1 µL ThT solution added at a final concentration of 20 µM. Three repeat wells were used for each LC at each time point. The contents of the plates were mixed and ThT fluorescence recorded using an excitation at 440nm and emission at 490nm (Flexstation 3, Molecular devices). There were three repeats per experimentation.

Transmission Electron Microscopy: Endpoint samples from ThT experiments were analysed by transmission electron microscopy. 5 µL were mounted onto a carbon-coated copper grid for 2 minutes, blotted and stained with 4% (w/v) uranyl acetate for 30 seconds before being allowed to dry. Images were collected on a 120kV Tecnai G2 Spirit BioTWIN electron microscope (FEI) with a SIS Megaview III camera.

Crystallisation: Crystals for SMA:VH complex were grown by hanging drop method in 48 well Hampton research tray facilitated by seeding, 1.5 µL of protein at 8 mg/mL concentration was mixed with 1.5 µL of reservoir solution composed from Bis-Tris buffer at pH 5.5 and 20% PEG3350 and equilibrated over the well containing 100 µL of the reservoir solution. For pure SMA crystals were grown from similar concentration by sitting-drop vapour diffusion in 96 well tray in JCSG+ F11 condition (0.1M HEPES buffer pH 7.0, 1M Succinic acid and 1% w/v PEG2000 MME). For SMA:VH complex crystals were transferred in reservoir solution supplemented with 20% Ethylene glycol, while for pure SMA 20% glycerol supplement was used before flash freezing in liquid nitrogen.

Data Collection, Refinement and Model Building: Data were collected at IO4 beamline (wavelength = 0.95373 Å) at the Diamond light source synchrotron using an Eiger2 XE 16M detector. Data were

processed with DIALS (Winter et al., 2018) and scaled by AIMLESS (Evans and Murshudov, 2013). Both structures were solved using MOLREP (Vagin and Teplyakov, 1997) with LEN structure (PDB ID:1LVE) as a starting model and refined with REFMAC5 (Murshudov et al., 1997) in the CCP4i2 suite. Model building between cycles of refinement was performed with COOT (Emsley and Cowtan, 2004). The protein structure images were created using Pymol. The crystal structures were analysed with the PISA (Protein Interfaces, Surfaces, and Assemblies) server (https://www.ebi.ac.uk/msd-srv/prot_int/cgi-bin/piserver)(Krissinel and Henrick, 2007). Crystallographic data collection and refinement statistics are shown in Table 2.

Table 2 - SMA homodimer and SMA:C03 heterodimer data collection and refinement statistics (note: Vh1 is an alternative name for C03)

Crystal	***SMA:SMA***	***SMA:VH1***
Space Group	P31 1 2	P 21 21 21
Unit-cell parameters		
a (Å)	56.816	38.613
b (Å)	56.816	53.544
c (Å)	59.780	99.070
α (deg)	90	90
β (deg)	90	90
γ (deg)	120	90
Resolution Range (Å) (Outer Shell)	49.17-2.20 (2.27-2.20)	49.54-1.18 (1.20-1.18)
Rmerge (Outer Shell)	0.228 (1.688)	0.036 (0.179)
R p.i.m.	0.110 (0.842)	0.022 (0.141)
<I/σ(I)>	5.6 (1.4)	21.5 (4.3)
CC1/2	0.995 (0.702)	0.999 (0.948)
Total Number Reflections Measured	59405	404898
Unique Reflections	5926	86233
Redundancy	10.2	8.1
Completeness (%) (Outer Shell)	100 (100)	96.5 (71.1)
Wilson B-factor (Å²)	33.24	9.04
Refinement		
No. of monomers in A.U.	1	2
Resolution (Å)	49.22- 2.20	49.58-1.18
Rwork/Rfree (%)	18.0/24.5	10.8/ 12.4
No. of atoms		
Protein	1726	3750
Waters	66	368
B-factors (Å²)		
Protein	30.6	12.3
Waters	41.4	28.4
r.m.s deviations		
Bond length (Å)	0.017	0.014
Bond angles (deg)	2.722	1.864

Example 1 – Identification of five promising candidates

Identification of promising binders: Surprisingly, although representing less than 10% of the initial naïve llama- library, only conventional VH-like sequences were identified during the panning process. After three rounds of screening there was >90% hit rate with >0.15 ELISA absorbance reading. 141 clones (47 clones from each of the individual LEN, SMA and RCE titration plates)

were selected, sequenced and outputs analysed. From those, only 11 binders were more promising (see Table 3; each name is based of the original panning target and the plate-well number (e.g. LEN A01), however for simplicity reason they will herein generally be referred to only by their well numbers (e.g. A01)). Some of the CDR3 sequences in these eleven hits were recurring. This convergence indicated an advantage for binding, highlighting them as hits of particular interest. Selection criteria included choosing representative sequences from each convergent CDR3 group, as well as selecting multiple sequences from those groups where differential binding patterns were observed.

Table 3 – CDR3 of the 11 VH proteins selected after screening

Name	CDR3	Name	CDR3
C03	SDNSGYYKY	G02	SLYTVERGGYDY
B04	PRRGPWKY	D07	YVGSSWWRGYLEV
C06		C10	NLGDVITTAFGS
H02		D09	HRRDAWFSEYDV
A03	SQGNYWYVFDY	D01	DVGHGYDY
D04			

The selected VHs form a stable complex with LCs: Analytical gel filtration was performed to determine if the interactions identified during the initial screening between VH candidates and LCs were transient or resulted in the formation of stable complexes. For C03 for instance, an elution peak was observed at 15.8 mL (Figure 1). Although very similar in size, LEN eluted at roughly 18 mL (Figure 1A) and SMA 16.9mL (Figure 1B). When LEN or SMA and C03 were combined in a 1:1 ratio, a strong elution peak was observed at 13.4 mL for both (see Figures 1). These shifts in elution volume confirmed the formation of stable C03:LC complexes, with little/no visible C03 or LC peaks remaining in the samples, suggesting they were both incorporated into a stable complex at a 1:1 ratio. LEN and SMA appeared to be monomeric at the 0.5mg/mL concentration used throughout this experiment, corresponding with their respective reported KD values of ~40 and ~70 µM (Stevens et al., 1995). In contrast, REC eluted at a volume of 13.4 mL (Figure 1C), indicating its molecular weight was comparable with that of dimeric complexes, consistent with the lower reported KD of ~0.2 µM, for REC (Stevens et al., 1995). Despite being dimeric at this concentration, REC was still able to form a stable complex with C03, as shown by an increased peak size at 13.4 mL, and reduction in peak at 15.7 mL (Figure 1C).

The binding of the eleven hits toward not only their original target but also the two others was assessed (Table 4). The ELISA results indicated that C03, B04, H02, C10, D09 and D01 were able to bind to (or pair with) all three light LCs. A03 and D04 also did so but appeared to bind SMA less efficiently. The same goes for G02, although REC was bound less in this case. C06 was shown not to bind REC and D07 was specific to SMA. C03 had a particular interest, as its level of protein

expression was considerably lower than the other VHs (data not shown) but still appeared to be a very strong binder. The binding of some of the candidates for the three LCs suggests that the LCs share similar configuration, allowing the pairing with various VHs.

Table 4 - Binding profiles of the 11 VH proteins determined by ELISA - Table of the A630 readings for each well with the streptavidin controls subtracted, alongside the assigned binding category (Y = yes, N = no, Y(-) = weaker binding). Criteria for assignment were values <0.5 taken as no binding, 0.5-2 as weaker binding and >2 as strong binding.

	C03	B04	C06	H02	A03	D04	G02	D07	C10	D09	D01	Control
LEN	2.985	3.568	3.098	2.977	3.282	2.974	3.378	0.372	3.423	3.327	3.496	0.309
SMA	3.525	3.600	3.177	2.444	1.425	1.123	3.438	2.918	3.478	3.354	3.456	0.219
REC	3.053	2.679	0.037	1.537	3.07	2.641	1.324	0.192	2.876	2.867	2.528	0.078
	C03	B04	C06	H02	A03	D04	G02	D07	C10	D09	D01	Control
LEN	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	N
SMA	Y	Y	Y	Y	Y(-)	Y(-)	Y	Y	Y	Y	Y	N
REC	Y	Y	N	Y(-)	Y	Y	Y(-)	N	Y	Y	Y	N

SPR was employed to evaluate binding kinetics.C03 was chosen for this experiment. Sensorgrams showed a proportional increase in SPR signal with injection of LC suggesting specific interactions of increasing amounts of LC with C03 (Figure 2). C03 seems to bind LEN and SMA with greater affinity than with REC. To determine approximate LC:C03 binding kinetics, SPR data was analysed by two different kinetic models: i) the 1:1 model and ii) the heterogeneous ligand model (Figure 2i, ii, respectively, black lines). Based on this analysis, it became clear that the heterogeneous ligand model fitted better to the experimental data compared to the 1:1 model. Gel filtration data suggested that the LC and C03 interaction occurred by 1:1 binding, the ligand site heterogeneity could be explained due to lack of controlled amine coupling of C03.

Calculated equilibrium constants (KD), given by Kd/Ka, for the 1:1 model of LEN, SMA and REC were 5.6 nM, 16 nM and 499 nM respectively. These values, although approximate due to the poorly fitting model, suggested high affinity binding of C03 to LEN and SMA, and significantly lower binding to REC. This was further supported by the average Rmax values, where REC gave a value of 55.05 RU, compared to 195.62 RU for LEN and 145.13 RU for SMA. Equilibrium constants for the heterogeneous model were LEN: K_D1 = 1.19 nM and K_D2 = 26.6 nM, SMA: K_D1 = 131 nM, K_D2= 17.8 nM and REC: K_D1 = 14.2 µM, K_D2: 382 nM. The equilibrium constants recorded for each LC described two distinct K_Ds, suggesting that in each case the binding site was not uniformly available. This supported the hypothesis that the random orientation of C03 was responsible for the apparent heterogeneous ligand model, caused by partial obstruction of the binding site. Alternatively, there may be different pools of LC species with variable aggregation states/orientations present with different affinities for C03.

Table 5 - Binding kinetics parameters recorded by SPR for LEN, SMA and REC to C03, using a 1:1 and heterogeneous ligand model.

		LEN*	SMA**	REC*
1:1 Model	**Ka (1/Ms) [1]**	**2.63×10^{6}**	**2.98×10^{7}**	**9.24×10^{6}**
	SEM	1.22×10^{6}	1.91×10^{7}	9.13×10^{6}
	Kd (1/s) [2]	**2.57×10^{-2}**	**9.52×10^{-1}**	**3.28**
	SEM	1.21×10^{-2}	6.11×10^{-1}	3.22
	KD (M) [3]	**5.61×10^{-9}**	**1.60×10^{-8}**	**4.99×10^{-7}**
	SEM	1.88×10^{-9}	3.90×10^{-8}	3.43×10^{-8}
	Rmax (RU) [4]	**195.62**	**145.13**	**55.05**
	SEM	43.14	9.56	7.14
Heterogeneous Ligand	**Ka1 (1/Ms)**	**4.02×10^{5}**	**3.71×10^{5}**	**1.20×10^{5}**
	SEM	2.28×10^{5}	1.82×10^{5}	5.63×10^{4}
	Ka2 (1/Ms)	**8.22×10^{5}**	**5.53×10^{5}**	**5.06×10^{4}**
	SEM	6.60×10^{5}	3.22×10^{5}	2.65×10^{4}
	Kd1 (1/s)	**2.32×10^{-4}**	**1.07×10^{-2}**	**7.98×10^{-2}**
	SEM	1.34×10^{-4}	3.09×10^{-3}	4.40×10^{-2}
	Kd2 (1/s)	**1.12×10^{-2}**	**9.05×10^{-3}**	**2.56×10^{-2}**
	SEM	6.29×10^{-3}	4.54×10^{-3}	1.59×10^{-2}
	KD1 (M)	**1.19×10^{-9}**	**1.31×10^{-7}**	**1.42×10^{-6}**
	SEM	3.23×10^{-10}	6.81×10^{-8}	1.33×10^{-5}
	KD2 (M)	**2.66×10^{-8}**	**1.78×10^{-8}**	**3.82×10^{-7}**
	SEM	1.51×10^{-3}	7.56×10^{-8}	1.13×10^{-7}

[1] Rate of association (Ka) ; [2] Rate of dissociation (Kd) ; [3] Equilibrium dissociation constant (KD)

[4] Maximum overall response (Rmax) ; * n=6 repeat ; ** n=9 repeat

Final selection: In view of the data cumulated about the eleven candidates, five binders were finally selected to progress further (C03, C06, A03, D07 and D09). This decision was based not only on their specific binding profiles, but also whether they consistently gave reliable binding by both ELISA and BLI. For instance C03, A03 and D09 bound all three LCs to a high affinity, whereas C06 and D07 appeared to show specificity to LEN/SMA and SMA respectively. Those pan-LC binders suggest the presence of a common conformation/similar conformation between the LEN, SMA and REC. This is potentially beneficial, as if this is proven to be a stabilising interaction, binding may slow progression of SMA and REC into amyloid fibrils and show promise therapeutically.

VHs vs VHHs: As mentioned above, only VHs candidates were identified in the VHH library that has been used. This specific type of selection may be explained by the innate framework natural interactions that can occur between the heavy and light chains. The typical hydrophobic interface within the FR2 region of VH proteins, enables the native pairing to VLs (van der Linden et al., 1999). This region, between amino acids ~36-49, often comprises of the sequence 'WVRQAAGKGLEWV' that may differ slightly due to conservative mutations (See Figure 10). This sequence feature was observed in the eleven selected VHs. The conserved residues V37, G44, L45 and W47 tend to form a tight association with VL via hydrophobic bonds in the full IgG

structure. This bonding was therefore likely of higher affinity than that possible by the VHH clones present in the panning library. VHH proteins lack these hydrophobic residues and have a longer CDR3 loop that packs against the framework. Whilst these additional characteristics do give beneficial properties, such as improved thermal stability and solubility, they would prevent this type of framework interaction, between the VH and VL, from occurring and gives further evidence as to why they were not selected for during panning.

Conclusion of example 1: 141 clones were screened for binding and 115 binders were identified and sequenced. Eleven of them have shown promising binding results. Five binders, were then further selected, based on a combination of their specific binding profiles, and the consistent and reliable binding by both ELISA and BLI.

Example 2 – The VHs are able to stabilise LC

Results: Stabilisation studies have been performed on one of the VHs candidates, C03. Differential scanning fluorimetry and circular dichroism were utilised to try and understand how binding of the VHs candidates to LCs affected their thermal stability. The acquisition of thermal transition curves enabled melting temperatures to be determined using the equilibrium point between folded and unfolded states. CD spectra at the start (20ºC) and end (80ºC) temperatures confirmed a shift from β -sheet structure to random coil, indicating complete unfolding for all conditions. All LCs showed an increase in stability following complex formation with C03 (Figure 3). The most substantial shift in thermal stability >5.5°C was observed for SMA (Figure 3A, B, Table 6). SMA and REC exhibited a single transition, occurring between 40-60ºC (Figure 3A-D). However, LEN appeared to undergo a two-phase unfolding step observed by DSF (Figure 3E, i), evidenced by multiple transitions. This bi-phasic unfolding was also observed in the LEN-C03 complex sample. To more effectively assess the effects of C03 on LEN's thermal stability, the first and second transitions for LEN and the LEN:C03 complex were analysed separately (Figure 3E, ii and iii). Comparable Tm values showing an increase in LC stability in the presence of C03 were obtained from both methods (Table 6). CD analysis of LEN displayed a single transition in contrast to the double transition observed by DSF. CD measures change in secondary structure upon unfolding, whereas DSF monitors exposure of hydrophobic surfaces suggesting that DSF may be more sensitive to local conformational changes which may differ in LEN compared to SMA and REC.

Conclusion of example 2: the VHs candidates, as shown with C03, are able to increase the stability of LEN, SMA and REC. The most substantial shift was seen for the binding of C03 to SMA. An increase in Tm of 8 ºC was observed, which appeared to confer a level of resistance to fibril formation, detected by ThT assay (see example 3).

Table 6 - Calculated Tm values from DSF and CD data. Values presented for LC alone and in complex with C03 in °C with standard error in brackets (note: VH1 is an alternative name for C03)

	DSF		CD	
	LC alone	Complex with VH1	LC alone	Complex with VH1
SMA	49.8 (0.025)	55.4 (0.076)	50.3 (0.34)	59.0 (0.15)
REC	48.1 (0.044)	51.0 (0.096)	48.4 (0.82)	55.3 (0.45)
LEN	44.0 (0.028)	49.2 (0.035)	57.4 (0.29)	61.7 (0.12)
	57.5 (0.019)	60.7 (0.037)		

Example 3 – The VHs inhibit SMA Fibril Formation

Results: Investigations were further performed on the VHs candidates, using C03 as a model. SMA is known to aggregate into fibrils upon incubation at 37ºC with agitation (Raffen et al., 1999). To investigate how C03's interactions with SMA affected its propensity to aggregate, a Thioflavin-T assay was performed. Here, SMA was incubated with C03 in a 1:1, 2:1 and 10:1 ratio for the duration of 18 days, with timepoints taken at two-day intervals. SMA displayed a large increase in ThT fluorescence signal after day 12 which is accompanies by observation of fibrils by transmission electron microscopy (Figure 4A-E). However, in the presence of C03 at 1:1, 2:1 and 10:1 SMA:C03 ratios no increase in fluorescence was observed suggesting a reduction in fibril formation (Figure 4A-C), confirmed by electron microscopy (Figure 4F-H). Interestingly, C03 incubated alone at 1 mg/mL displayed an increase in ThT fluorescence after day 10 (Figure 4A), suggesting that C03 also aggregates at these concentrations but not at lower concentrations (Figure 4B and C). Fibrils were observed at day 18 for 1mg/mL C03 incubated alone (Figure 4I), but not at lower concentrations (data not shown).

Conclusion of example 3: A substantial reduction in fibril formation was observed by C03 binding at a 1:1, 2:1 and 10:1 SMA:VH1 concentration. The very few fibrils observed by TEM following incubation in the presence of VH1 had visibly altered morphologies compared to SMA alone, such as what appeared to be 'broken' fibrils

Example 4 – Further characterisation

Crystallography: C03 was confirmed to bind to/pair with SMA and form a complex with analytical GF, DSF and CD revealing this interaction to be both stable and of high affinity and promoting an increase in thermal stability. This data formed the hypothesis that C03 was binding in a 1:1 ratio, disrupting the LC dimer and causing stabilisation of the monomeric subunit. Utilising X-ray protein crystallography we identified the structure of C03 binding to SMA and were able to compare this to the newly solved SMA dimer structure.

Structural characterisation of the SMA homodimer: The crystal structure of SMA alone was solved to a resolution of 2.2 Å (Figure 5). Crystallographic data collection and refinement statistics are

shown in Table 2. As expected, SMA forms a dimeric structure, where both subunits are orientated in the same direction. This, termed a canonical dimer, resembles that of the dimeric LEN structure (PDB ID:1LVE). Like other LCs, the SMA monomeric subunit is composed of nine β-strands forming a 4:5 Greek key β-sandwich motif. This motif is stabilised by the disulphide bond formed between the Cys23 and Cys94 residues (data not shown) which immobilises both β-sheets in this conformation.

PISA analysis of the SMA crystal structure identified 18 residues at the SMA dimer interface, forming 12 hydrogen bonds and 4 salt bridges. Interacting SMA residues were mapped onto the dimeric structure, alongside the identified hydrogen bonds and salt bridges, in Figure 5. Here, the residues involved in binding appear to locate primarily on loop regions, with interactions grouping towards the top and bottom of the interface. Gln44, Gln48 and Tyr93 on each SMA chain form a network of hydrogen bonds at the base of the interface (Figure 5A). Bond distances range from 2.85 Å, (between Gln44:Gln44), to 3.67 Å (between Tyr93:Gln44). The top region of the dimer interface is stabilised by a total of four salt bridges formed between residues Glu61 and His100 on each chain (Figure 5B) and two hydrogen bonds between Glu61 and Gln102 (Figure 5B).

Structural Characterisation of the SMA:C03 Complex: The crystal structure of the SMA:C03 complex was solved to a resolution of 1.18 Å (Figure 6). As predicted, the SMA:C03 complex was formed in a 1:1 ratio and adopted a dimeric structure comparable with that determined for the SMA homodimer, whereby both the SMA and C03 subunits were orientated in the same direction. C03 appeared to bind a very similar position to that observed between subunits of the native SMA dimer; the difference being a slight rotation of the C03 compared to SMA. As in the homodimer, the heterodimeric SMA monomer formed a β-sandwich motif, stabilised by an intramolecular disulphide bond formed between residues Cys23 and Cys94 (picture not shown). Like SMA, the C03 monomeric structure was also composed of nine β-strands arranged into a 4:5 β-sandwich motif. In this case, the inter-β-sheet disulphide bond was formed between the Cys22 and Cys96 residues (picture not shown).

PISA analysis of the SMA:C03 crystal structure identified 24 SMA and 23 C03 residues at the SMA:C03 heterodimer interface, forming 9 hydrogen bonds and 3 salt bridges. Interacting SMA and C03 residues were mapped onto the dimeric structure, alongside the identified hydrogen bonds and salt bridges (Figure 6). Like the SMA dimer, the residues involved in binding appear to group towards the top and bottom of the interface. SMA residues Gln44 and Tyr93 are again involved in hydrogen bonding towards the base of the interface, forming a network of bonds with C03 residues Gln39/Tyr95 and Gln39/Lys43 respectively (Figure 6A). The identified hydrogen bond distances ranged from 2.91 Å, (between Gln44:Gln39), to 3.71 Å (between Gln44:Tyr95). The top region of the dimer interface was stabilised by a total of three salt bridges formed between the Glu61 and His100 of SMA and the Lys106 and Asp59 of C03 respectively (Figure 6B).

Comparison of the SMA Homodimer and SMA:C03 Heterodimer Interfaces: The previous sections have described the crystal structures of the SMA homodimer and SMA:C03 heterodimer and

provided insight into the residues responsible for stabilising the interface interactions. PISA analysis suggests that the heterodimeric complex interface is more favourable than the homodimer, with respective complex formation significance score (CSS) values of 1.000 compared with 0.829. The increased likelihood of SMA to form a complex with C03 is further supported by the calculated free energy gain (ΔiG) of both complexes (data not shown).

Whilst the heterodimer had an increased number of residues at the interface (24 SMA residues) compared to the homodimer (18 SMA residues), the homodimer was predicted to have more hydrogen bonds and salt bridges at the interface. Interestingly, this did not correlate with the obtained binding and stability data. The binding affinity of SMA:C03 was estimated to be in the low nanomolar range by SPR, an order of magnitude lower than the KD of the SMA dimer (~70 μM), and a >5.5ºC increase in thermal stability of SMA was observed upon C03 binding. Alongside the PISA results described above, the increased affinity and stability of the SMA:C03 complex could be further explained by the increased packing of side chains observed at the interface, compared to the SMA homodimer. A surface view of the homodimer highlights the clear separation of subunits and the pocket of space between the two SMA subunits. In comparison, the heterodimer shows clear merging of the interface, suggesting that the side chains are in close proximity and packed efficiently. The bond distances of the homo- and heterodimer respectively, do not provide an explanation as to why the increased distance between the homodimer subunits is observed. Therefore, it is likely that additional residues present at, or near, the interface are causing a certain level of repulsion or steric hindrance.

VH1 LC Binding Kinetics: In order to further evaluate binding kinetics, Biolayer Interferometry (BLI) was used. As shown in Figure 11, a proportional increase in signal is observed with injection of C03 confirming specific interactions of increasing amounts of C03 with LC. C03 appeared to bind LEN and SMA with greater affinity than REC, evidenced by requiring higher concentration of C03 when REC was immobilised to reach the same level of response and increased dissociation observed. The results from Figure 11 confirmed the 1:1 model (see Example 1). The kinetic parameters are reported in Table 7. In this setting of experiment, the KD, given by Kd/Ka, for the 1:1 model of LEN, SMA and REC were 0.64 μM, 1.69 μM and 43.5 μM respectively. These values, confirm high affinity binding of C03 to LEN and SMA, with REC being lower. However, as shown in Example 1/Figure 1C, REC seems to be present as a dimer, implying that direct comparison of Kd values obtained with REC should be treated with caution given the difference in monomer-dimer state of REC compared with SMA and LEN.

Table 7: Binding kinetics parameters with standard error of the mean (SEM) recorded by BLI for VH1 binding to LEN, SMA and REC, using a 1:1 model.

	LEN	SMA	REC
K_a (1/Ms) [1]	**8.58 x10^3**	**6.53 x10^3**	**1.36 x10^3**
SEM	1.45 x10^1	3.14 x10^1	3.97 x10^1

	LEN	SMA	REC
K_d (1/s) [2]	**5.50 x10^{-3}**	**1.10 x10^{-2}**	**5.93 x10^{-2}**
SEM	2.49 x10^{-6}	1.05 x10^{-5}	1.40 x10^{-4}
K_D (M) [3]	**6.41 x10^{-7}**	**1.69 x10^{-6}**	**4.35 x10^{-5}**
SEM	1.12 x10^{-9}	8.29 x10^{-9}	1.27 x10^{-6}

[1] Rate of association (Ka); [2] Rate of dissociation (Kd); [3] Equilibrium dissociation constant (KD)

Overall conclusion:

The inhibition of amyloid formation by stabilisation of the LC monomer, as described here, is promising as a potential therapeutic mechanism. Despite there being uncertainty in the exact non-native LC structures causing organ toxicity, stabilisation of the native and correctly folded LC had previously been hypothesised to cause a reduction in misfolding and aggregation (Morgan et al., 2021). This mode of kinetic stabilisation has proven successful in the treatment of a different type of amyloidosis TTR amyloidosis, whereby a stabilising interaction between the drug tafamadis and the TTR tetramer has proven particularly effective at reducing aggregation and slowing disease progression (Bulawa et al., 2012). In the examples presented here, the selective binding of C03 in particular to SMA's and LEN's native state likely increased the free energy barrier to unfolding, causing the observed slowing in its unfolding and aggregation. The pharmacological kinetic stabilisation described herein could prove beneficial in AL amyloidosis patients as it would enable increased excretion of native LCs by the kidneys. The inventors showed that sub-stoichiometric concentrations of C03 were sufficient to reduce LC aggregation. This strongly suggests it is sufficient to remove a subspecies of LCs that are prone to enhancing seeding or elongation of LC fibrils in order to prevent fibril formation. This explanation is consistent with heterogeneous binding observed during SPR suggesting the presence of multiple LC species with different binding preferences for C03.

In summary, this present invention describes novel VH-based antibodies and active fragments thereof that are able to bind/pair and disrupt the formation of light chains-based fibrils. It has been shown that stabilising the native structure of light chains with VHs, it was possible to prevent the misfolding of the LC, in turn stopping initiation of the aggregation pathway, characterised by formation of fibrils. This interaction was confirmed, by SPR analysis, to be of high affinity with the equilibrium constants of the LC:C03 complex estimated to be in the low nanomolar range, in comparison with the native VH:VL interaction estimated to be ~200 nM (Jäger and Plückthun, 1999), confirming an effective panning process.

These VH- based antibodies and active fragments thereof could therefore credibly be used for the treatment of disorders involving light chains-based fibrils, such as those described in the present description as a whole.

References

1) Gertz, M.A., 2018. Blood Cancer Journal; 8(5), p.44.
2) Fotiou, D. et al., 2020. HemaSphere; 4(4), p.12.
3) Merlini et al, 2013, haematologica, 98(10):1492-1495
4) Gurung and Tingting, 2022, The American Journal of Medicine, 135:S38−S43
5) Weiss, B.M. et al., 2016. Blood; 128(22), p.4448.
6) Edwards, C.V. et al., 2021. Blood; 138(25), pp.2632–2641.
7) Gertz, M.A. et al., 2022a. Blood; 140(Supplement 1), pp.1839–1840
8) Broggini, L.et al., 2023. Journal of Molecular Biology; 435(24), p.168320
9) Solomon, A., 1985. Methods Enzymol.: 116: 101-121
10) Pras et al., 1968. J Clin Invest 47(4): 924-933.
11) Stevens et al., 1995. Protein Sci 4(3):421-432.
12) Rajkumar et al., 2005, Blood. 106(3):812-817
13) Kabat et al., 1991. NIH publication No. 91-3242
14) Chothia, C. and Lesk, A.M., 1987, Journal of Molecular Biology; 196:901-917
15) Angal et al., 1993, Mol Immunol, 30:105-108
16) WO9109967
17) Wilkes, J. et al., 2020. ACS Synthetic Biology; 9(10):2828–2839
18) Winter, G. et al., 2018. Research papers Acta Cryst; 74:85–97.
19) Evans, P.R. and Murshudov, G.N, 2013. urn:issn:0907-4449. 69(7):1204–1214]
20) Vagin, A. and Teplyakov, A, 1997. J. Appl. Cryst.; 30:1022–1025
21) Murshudov, G. et al., 1997. Acta Crystallogr D Biol Crystallogr.; 53(3):240–255.
22) Emsley, P. and Cowtan, K., 2004. Acta Crystallogr D Biol Crystallogr.; 60(12):2126–2132
23) Krissinel, E. and Henrick, K., 2007. Journal of Molecular Biology; 372(3):774–797
24) Raffen, R. et al., 1999. Protein Science. 8(3):509–517.
25) van der Linden et al., 1999

CLAIMS

1. An antibody that specifically binds to free light chains, such as free kappa light chains and/or (kappa) amyloidogenic light chains, wherein the antibody comprises a heavy chain variable region comprising:

   i. a CDR-H1 consisting of SEQ ID NO: 1, 4, 7, 10 or 13, a CDR-H2 consisting of SEQ ID NO: 2, 5, 8, 11 or 14 and a CDR-H3 consisting of SEQ ID NO: 3, 6, 9, 12 or 15; or

   ii. a combination of CDR-H1, CDR-H2 and CDR-H3 sequences having at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity or similarity to any of the sequences listed in i.

2. The antibody according to claim 1, wherein said antibody comprises a heavy chain variable region comprising:

   i. CDR-H1 consisting of SEQ ID NO: 1; a CDR-H2 consisting of SEQ ID NO: 2; and a CDR-H3 consisting of SEQ ID NO: 3;

   ii. a CDR-H1 consisting of SEQ ID NO: 4; a CDR-H2 consisting of SEQ ID NO: 5; and a CDR-H3 consisting of SEQ ID NO: 6;

   iii. a CDR-H1 consisting of SEQ ID NO: 7; a CDR-H2 consisting of SEQ ID NO: 8; and a CDR-H3 consisting of SEQ ID NO: 9;

   iv. a CDR-H1 consisting of SEQ ID NO: 10; a CDR-H2 consisting of SEQ ID NO: 11; and a CDR-H3 consisting of SEQ ID NO: 12;

   v. a CDR-H1 consisting of SEQ ID NO: 13; a CDR-H2 consisting of SEQ ID NO: 14; and a CDR-H3 consisting of SEQ ID NO: 15; or

   vi. CDR-H1, CDR-H2 and CDR-H3 sequences having at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity or similarity to any of the sequences listed in any one of i. to v.

3. An antibody that specifically binds to light chains, such as kappa light chains and/or (kappa) amyloidogenic light chains, wherein the antibody has a heavy chain variable region comprising any one of SEQ ID NO: 16 to 26 or a sequence having at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereof .

4. The antibody according to any one of the preceding claims, wherein the antibody is selected from the group consisting of: i) an antibody moiety comprising a VH domain but no VL domain or ii) a nanobody.

5. The antibody according to any one of the preceding claims wherein the antibody is a nanobody.

6. The antibody according to any one of the preceding claims wherein the antibody is chimeric or humanized.

7. The antibody according to any one of the preceding claims, wherein the antibody comprises a Fc domain selected from the group consisting of any one of SEQ ID NO: 48 to 52 or

a sequence having at least 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity thereof.

8. The antibody according to any one of the preceding claims, wherein said antibody neutralises or inhibits the formation of light chains dimers and/or light chains fibrils.

9. The antibody according to any one of the preceding claims, wherein said antibody has an equilibrium dissociation constant (K_D) of less than 500 nM for light chains.

10. An isolated polynucleotide encoding the antibody according to any one of claims 1 to 9.

11. A cloning or expression vector comprising the polynucleotide according to claim 10.

12. A host cell comprising the polynucleotide according to claim 10 or the expression vector according to claim 11.

13. A process for the production of an antibody according to any one of claims 1 to 9, comprising culturing the host cell according to claim 12 under suitable conditions for producing the antibody and isolating the antibody.

14. A pharmaceutical composition comprising the antibody according to any one of claims 1 to 9, or a polynucleotide according to claim 10, and one or more pharmaceutically acceptable carriers, excipients and/or diluents.

15. The antibody according to any one of claims 1 to 9, the polynucleotide according to claim 10 or the pharmaceutical composition according to claim 14 for use in therapy and/or diagnosis.

16. The antibody according to any one of claims 1 to 9 and 15, the polynucleotide according to claims 10 or 15 or the pharmaceutical composition according to claims 14 and 15 for use in the treatment of a disorder or condition selected from the group consisting of light chain (AL) amyloidosis, Light chain deposition disease (LCDD), light- and heavy-chain deposition disease (LHCDD), Crystal-storing histiocytosis, asthma, an inflammatory and autoimmune disease (such as rheumatoid arthritis, Sjogren's disease, idiopathic urticaria, multiple sclerosis, diabetes mellitus, systemic lupus erythematosus, lupus nephritis and inflammatory bowel disease) and some categories of cancers such as multiple myeloma (including lambda myeloma and kappa myeloma).