

CLAIMS

1. An isolated antibody fragment, wherein the fragment is the knob domain of a bovine ultralong CDR-H3 or a portion thereof which binds to an antigen of interest.
2. An isolated antibody fragment according to claim 1, which comprises at least two, or at least four, or at least six, or at least eight, or at least ten cysteine residues.
3. An isolated antibody fragment according to claim 1 or 2, which comprises at least one, or at least two, or at least three, or at least four, or at least five disulphide bonds.
4. An isolated antibody fragment according to any one of claims 1 to 3, which comprises a (Z₁) X₁ C X₂ motif at its N-terminal extremity, wherein:
 - a. Z₁ is present or absent, and when Z₁ is present, Z₁ represents 1 amino acid or 2, 3, 4, or 5 independently selected amino acids; and,
 - b. X₁ is any amino acid residue, preferably selected from the list consisting of Serine, Threonine, Asparagine, Alanine, Glycine, Proline, Histidine, Lysine, Valine, Arginine, Isoleucine, Leucine, Phenylalanine and Aspartic acid; and,
 - c. C is cysteine; and,
 - d. X₂ is an amino acid selected from the list consisting of Proline, Arginine, Histidine, Lysine, Glycine and Serine.
5. An isolated antibody fragment according to any one of claims 1 to 4, which comprises a (AB)_n and/or (BA)_n motif, wherein A is any amino acid residue, B is an aromatic amino acid selected from the group consisting of: tyrosine (Y), phenylalanine (F), tryptophan (W), and histidine (H), and wherein n is 1, 2, 3 or 4.
6. An isolated antibody fragment according to any one of the preceding claims, which is 5 amino acids in length or more, 10 amino acids in length or more, 15 amino acids in length or more, 20 amino acids in length or more, 25 amino acids in length or more, 30 amino acids in length or more, 35 amino acids in length or more, 40 amino acids in length or more, 45 amino acids in length or more, and which is up to 55 amino acids in length.

7. An isolated antibody fragment according to any one of the preceding claims, which is between 5 and 55, or between 15 and 50, or between 20 and 45 or between 25 and 40 amino acids in length.
8. An isolated antibody fragment according to any one of the preceding claims, which comprises a sequence which is a variant of a naturally occurring sequence.
9. An isolated antibody fragment according to any one of the preceding claims, which further comprises a bridging moiety between two amino acids.
10. An isolated antibody fragment according to claim 9, wherein the bridging moiety comprises a feature selected from the group consisting of a disulphide bond, an amide bond (lactam), a thioether bond, an aromatic ring, an unsaturated aliphatic hydrocarbon chain, a saturated aliphatic hydrocarbon chain and a triazole ring.
11. An isolated antibody fragment according to any one of the preceding claims, which is fully bovine.
12. An isolated antibody fragment according to any one of claims 1 to 10, which is chimeric.
13. An isolated antibody fragment according to any one of claims 1 to 10, which is synthetic.
14. An isolated antibody fragment according to any one of the preceding claims, wherein the antigen of interest is the component C5 of the Complement.
15. An isolated antibody fragment according to claim 14, which has a sequence selected from the list consisting of SEQ ID NO: 157 to SEQ ID NO: 310, SEQ ID NO: 313, SEQ ID NO: 315, SEQ ID NO: 317, SEQ ID NO: 318, SEQ ID NO: 320, SEQ ID NO: 322, SEQ ID NO: 324, SEQ ID NO: 326 to SEQ ID NO: 331, SEQ ID NO: 334, SEQ ID NO: 336, SEQ ID NO: 339, SEQ ID NO: 341 to SEQ ID NO: 350, SEQ ID NO: 352, and SEQ ID NO: 572 to SEQ ID NO: 609 or any one of the same with at least 95%, 96%, 97%, 98% or 99% similarity or identity.
16. An isolated antibody fragment according to any one of claims 1 to 13, wherein the antigen of interest is human serum albumin.
17. An isolated antibody fragment according to claim 16, which has the sequence SEQ ID NO: 510.

18. A polypeptide comprising at least one isolated antibody fragment as defined in any one of claims 1 to 17.
19. A polypeptide comprising at least two isolated antibody fragments as defined in any one of claims 1 to 17, wherein the antibody fragments are linked together, optionally via a linker, for example a cleavable linker.
20. A polypeptide according to claim 19, wherein the at least two isolated antibody fragments bind to a same antigen.
21. A polypeptide according to claim 19, wherein the at least two isolated antibody fragments bind to different antigens.
22. A polypeptide according to any one of claims 18 to 21, wherein the polypeptide comprises at least one bridging moiety between two amino acids.
23. An isolated antibody fragment according to any one of claims 1 to 17, or a polypeptide according to any one of claims 18 to 22, wherein said fragment or polypeptide is fused to one or more effector molecules, optionally via a linker, for example a cleavable linker.
24. An isolated antibody fragment or a polypeptide according to claim 23, wherein the effector molecule is an antibody.
25. An isolated antibody fragment or a polypeptide according to claim 24, wherein the effector molecule is a full IgG.
26. An isolated antibody fragment or a polypeptide according to claim 24, wherein the effector molecule is selected from the list consisting of a Fab, a VHH, a VH, a VL, a scFv, and a dsscFv.
27. An isolated antibody fragment or a polypeptide according to any one of claims 24 to 26, wherein the effector molecule comprises an albumin binding domain.
28. An isolated antibody fragment or a polypeptide according to claim 23, wherein the effector molecule is albumin or a protein comprising an albumin binding domain.
29. An isolated antibody fragment or a polypeptide according to claim 27 or 28, wherein the albumin binding domain comprises SEQ ID NO: 435 for CDR-H1, SEQ ID NO: 436 for CDR-H2, SEQ ID NO: 437 for CDR-H3, SEQ ID NO: 430 for CDR-L1, SEQ

ID NO: 431 for CDR-L2 and SEQ ID NO: 432 for CDR-L3; or a heavy chain variable domain selected from SEQ ID NO: 434 and SEQ ID NO: 444 and a light chain variable domain selected from SEQ ID NO: 429 and SEQ ID NO: 443.

30. A pharmaceutical composition comprising an isolated antibody fragment as defined in any one of claims 1 to 17 or any one of claims 23 to 2829, or a polypeptide as defined in any one of claims 18 to 29, in combination with one or more of a pharmaceutically acceptable excipient, diluent or carrier.
31. An isolated antibody fragment as defined in any one of claims 1 to 17 or any one of claims 23 to 29, or a polypeptide as defined in any one of claims 18 to 29, for use in therapy.
32. A polynucleotide encoding an isolated antibody fragment as defined in any one of claims 1 to 17 or any one of claims 23 to 29, or a polypeptide as defined in any one of claims 18 to 29.
33. A vector comprising a polynucleotide according to claim 32.
34. A host cell comprising the polynucleotide or vector of claim 32 or 33 respectively.
35. A process for producing an isolated antibody fragment as defined in any one of claims 1 to 17 or any one of claims 23 to 29, or a polypeptide as defined in any one of claims 18 to 29, said process comprising expressing an isolated antibody fragment as defined in any one of claims 1 to 17 or any one of claims 23 to 29, or a polypeptide as defined in any one of claims 18 to 29, from a host cell defined in claim 34.
36. A method of producing an isolated antibody fragment as defined in any one of claims 1 to 17 or any one of claims 23 to 29, or a polypeptide as defined in any one of claims 18 to 29, said method comprising a step of chemical synthesis.
37. A method according to claim 36, wherein the chemical synthesis comprises a step of incorporating a coupling reagent with a radioisotope.
38. A method according to claim 37, wherein the radioisotope is an alpha emitting radioisotope, preferably Astatine 211.

39. A method of producing an isolated antibody fragment as defined in any one of claims 1 to 17 or any one of claims 23 to 29, or a polypeptide as defined in any one of claims 18 to 29, said method comprising:
- a) immunising a bovine with an immunogenic composition, and;
 - b) isolating antigen-specific memory B-cells, and;
 - c) sequencing the cDNA of CDR-H3 or portions thereof, and;
 - d) expressing or synthesising the knob domain of the ultralong CDR-H3 or portion thereof,
- wherein the immunogenic composition comprises an antigen of interest or immunogenic portion thereof, or DNA encoding the same.
40. A method according to claim 39, wherein said method further comprises a step of screening, for example for binding to said antigen of interest, and wherein, optionally, the screening step is preceded by a step of reformatting the ultralong CDR-H3 or the knob domain of the ultralong CDR-H3 or portion thereof into a screening format.
41. A method according to claim 40, wherein the step of reformatting the ultralong CDR-H3 or the knob domain of the ultralong CDR-H3 or portion thereof into a screening format comprises fusing the ultralong CDR-H3 or the knob domain of the ultralong CDR-H3 or portion thereof, to a carrier, optionally via a linker, for example a cleavable linker.
42. A method according to claim 41, wherein the carrier is an Fc polypeptide.
43. A method according to claim 42, wherein the Fc polypeptide is a scFc.
44. A library comprising at least one isolated antibody fragment as defined in any one of claims 1 to 17.
45. A library according to claim 44, which is a synthetic library.
46. A library according to claim 44, which is a phage library.
47. A library according to claim 46, which is a naive library.
48. A library according to claim 46, which is an immune library.

49. A library according to claim 47 or 48, wherein the library is prepared from cattle.
50. A phage display library, comprising a plurality of recombinant phages;
each of the plurality of recombinant phages comprising an M13-derived expression vector, wherein the M13-derived expression vector comprises a polynucleotide sequence encoding an isolated antibody fragment as defined in any one of claims 1 to 17, optionally displayed within the full sequence of ultralong CDR-H3.
51. A phage display library according to claim 50, wherein said polynucleotide sequence encoding said isolated antibody fragment optionally displayed within the full sequence of ultralong CDR-H3, is fused to the sequence encoding the pIII coat protein of the M13 phage, directly or via a spacer.
52. A method for generating a phage display library of ultralong CDR-H3 sequences, said method comprising:
- immunising a bovine with an immunogenic composition, and;
 - isolating total RNA from PBMC or secondary lymphoid organ, and;
 - amplifying the cDNA sequences of the ultralong CDR-H3, and;
 - fusing the sequences obtained in c) to the sequence coding for the pIII protein of a M13 phage within a phagemid vector, and;
 - transforming host bacteria with the phagemid vector obtained at step d) in combination with a helper phage co-infection, and;
 - culturing the bacteria obtained at step e), and;
 - recovering the phages from the culture medium of the bacteria,
- wherein the immunogenic composition comprises an antigen of interest or immunogenic portion thereof, or DNA encoding the same.
53. A method for generating a phage display library of ultralong CDR-H3 sequences according to claim 52, wherein step c) comprises:
- a primary PCR with primers flanking CDR-H3, annealing to the conserved framework 3 and framework 4 of the VH, to amplify all CDR-H3 sequences, and
 - a second round of PCR using stalk primers to specifically amplify ultralong sequences from the primary PCR.

54. A method for generating a phage display library of ultralong CDR-H3 sequences according to claim 53, wherein the primers used at step a) comprise or consist of SEQ ID NO:446 and SEQ ID NO: 447 and/or the primers used at step b) are selected from the group consisting of SEQ ID NO:482 to SEQ ID NO:494.
55. A method for producing an isolated antibody fragment which binds to an antigen of interest as defined in any one of claims 1 to 17, said method comprising:
- a) generating a phage display library of ultralong CDR-H3 sequences, for example according to any one of claims 52 to 54; and,
 - b) enriching the phage display library against the antigen of interest to produce an enriched population of phage which bind the antigen of interest; and,
 - c) sequencing an ultralong CDR-H3 from the enriched population of phage obtained in step b); and,
 - d) expressing or synthesising an isolated antibody fragment derived from the ultralong CDR-H3 obtained in step c).
56. A method for producing an isolated antibody fragment which binds to an antigen of interest as defined in any one of claims 1 to 17, said method comprising:
- a) generating a phage display library of isolated antibody fragments as defined in any one of claims 1 to 17; and,
 - b) enriching the phage display library against the antigen of interest to produce an enriched population of phage which bind the antigen of interest; and,
 - c) sequencing an isolated antibody fragment from the enriched population of phage obtained in step b); and,
 - d) expressing or synthesising an isolated antibody fragment obtained in step c).