

In another example, in the multi-valent binding protein the native amino acid residue at the first position of FR4 is substituted by a positively charged amino acid residue, such as a lysine (K), arginine (R) or histidine (H) residue, preferably an arginine (R) residue.

In a further example, the multi-valent binding protein comprises a tryptophan (W) to arginine (R) substitution at the first amino acid residue in the fourth framework (FR4) region.

In all examples, the multi-valent binding protein may comprise one or more further mutations in one or more framework regions.

In a further example, the disclosure concerns a recombinant heavy chain-only immunoglobulin (Ig) locus comprising one or more human V gene segments, one or more human D gene segments, and one or more human J gene segments, which when recombined with each other in the genome of a non-human animal, and following affinity maturation, encode a heavy chain variable (VH) region comprising complementarity determining regions (CDRs) and framework (FR) regions, wherein at least one of said human J segments comprises a codon encoding a non-native amino acid residue at the first position of the fourth framework region (FR4) that is capable of disrupting a surface-exposed hydrophobic patch comprising or associated with the native amino acid residue at that position.

In one aspect, there is provided a recombinant heavy chain-only immunoglobulin (Ig) locus comprising: (i) one or more human V gene segments, one or more human D gene segments, and one or more human J gene segments, which when recombined with each other in the genome of a non-human animal, and following affinity maturation, encode a heavy chain variable (VH) region comprising complementarity determining regions (CDRs) and framework (FR) regions, wherein at least one of said human J segments comprises a codon encoding a different amino acid residue at the first position of the fourth framework region (FR4) that is charged or polar, and is capable of disrupting a surface-exposed hydrophobic patch comprising or associated with the native amino acid residue at that position; and (ii) a constant (C) region gene segment, ~~encoding an immunoglobulin constant effector region~~ lacking a C μ coding region, and comprising a plurality of C γ coding regions, each lacking a CH1 exon, and further comprising a C ϵ or a C α coding region that comprises a CH1 exon ~~CH1 functionality~~.

In one example, the recombinant heavy chain-only Ig locus further comprises a constant (C) region gene segment, encoding an immunoglobulin constant effector region lacking CH1 functionality.

In various examples, the recombinant heavy chain-only Ig locus comprises two to 40 D gene segments, and/or two to 20 J gene segments.

In another example, more than one of the human J segments comprise a codon encoding a non-native amino acid residue at the first position of the fourth framework region (FR4) that is capable of disrupting a surface-exposed hydrophobic patch comprising or associated with the native amino acid residue at that position.

In yet another example, in the recombinant heavy chain-only Ig locus of all of the human J segments comprise a codon encoding a non-native amino acid residue at the first position of the fourth framework region (FR4) that is capable of disrupting a surface-exposed hydrophobic patch comprising or associated with the native amino acid residue at that position.

In a further example, in the encoded heavy VH region the native amino acid residue at the first position of FR4 is substituted by a polar amino acid residue, such as a lysine (K), arginine (R) or histidine (H), preferably an arginine (R) residue.

In a still further example, the encoded VH region comprises a tryptophan (W) to arginine (R) substitution at the first amino acid residue in the fourth framework (FR4) region.

In yet another example, the recombinant heavy chain-only Ig locus comprises a J4 gene segment in which the codon for W is replaced by R.

In all examples, the recombinant heavy chain-only Ig locus encodes a VH region which comprises one or more further mutations in one or more framework regions.

In a further example, the recombinant heavy chain-only Ig locus encodes a human or humanized heavy chain-only antibody comprising a VH region as hereinabove described.

In another aspect, there is provided a transgenic non-human animal comprising a recombinant heavy chain-only Ig locus as hereinabove described.

In various example, the transgenic non-human animal is a non-human mammal, as non-human vertebrate, a rodent, a mouse, or a rat, such as a UniRat™.

In another example, there is provided a transgenic non-human animal that does not express any functional immunoglobulin light chain genes and comprises a heterologous heavy chain-only Ig locus comprising one or more V gene segments, one or more D gene segments, and one or more J gene segments, which when recombined with each other and following affinity maturation encode a VH domain comprising complementarity determining regions (CDRs) and framework regions (FRs), in which the native amino acid residue at the first position of the fourth framework region (FR4) of said VH domain is substituted by a different amino acid residue that is capable of disrupting a surface-exposed hydrophobic patch comprising or associated with the native amino acid residue at that position, and one or more constant effector region gene segments, each of which encodes an antibody constant effector region including CH1 functionality, wherein the gene segments are arranged such that a V, a D and a J gene segment and a constant region gene segment recombine to produce a rearranged affinity matured heavy chain-only gene locus encoding a heavy chain-only antibody (HCAb).

In one aspect, there is provided a transgenic non-human animal that does not express any functional immunoglobulin light chain genes and comprises a heterologous heavy chain-only Ig locus comprising one or more V gene segments, one or more D gene segments, and one or more J gene segments, which when recombined with each other and following affinity maturation encode a VH domain comprising complementarity determining regions (CDRs) and framework regions (FRs), in which the native amino acid residue at the first position of the fourth framework region (FR4) of said VH domain is substituted by a different amino acid residue that is charged or polar, and is capable of disrupting a surface-exposed hydrophobic patch comprising or associated with the native amino acid residue at that position, and one or more constant effector region gene segments, encoding an antibody constant effector region lacking a C μ coding region, and comprising a plurality of C γ coding regions, each lacking a CH1 exon, and further comprising a C ϵ or a C α coding region that comprises a CH1 exon~~CH1 functionality~~, wherein the gene segments are arranged such that a V, a D and a J gene segment and a constant region gene segment recombine to produce a rearranged affinity matured heavy chain-only gene locus encoding a heavy chain-only antibody (HCAb).

In one example, in the VH domain of said transgenic non-human animal the native amino acid residue at the first position of FR4 is substituted by a polar amino acid residue, or a positively charged amino acid residues, such as lysine (K), arginine (R) or histidine (H) residue, preferably an arginine (R) residue.

In another example, in the transgenic non-human animal the VH domain comprises a tryptophan (W) to arginine (R) substitution at the first amino acid residue in the fourth framework (FR4) region.

In yet another example, in the transgenic non-human animal the heterologous heavy chain-only locus comprises a J4 segment in which a codon for W is replaced by a codon for R.

CLAIMS:

1. A recombinant heavy chain-only immunoglobulin (Ig) locus comprising:
 - (i) one or more human V gene segments, one or more human D gene segments, and one or more human J gene segments, which when recombined with each other in the genome of a non-human animal, and following affinity maturation, encode a heavy chain variable (VH) region comprising complementarity determining regions (CDRs) and framework (FR) regions, wherein at least one of said human J segments comprises a codon encoding a different amino acid residue at the first position of the fourth framework region (FR4) that is charged or polar, and is capable of disrupting a surface-exposed hydrophobic patch comprising or associated with the native amino acid residue at that position; and
 - (ii) a constant (C) region gene segment, ~~encoding an immunoglobulin constant effector region~~ lacking ~~CH1 functionality~~ a C μ coding region, and comprising a plurality of C γ coding regions, each lacking a CH1 exon, and further comprising a C ϵ or a C α coding region that comprises a CH1 exon.
2. The recombinant heavy chain-only Ig locus of claim 1, comprising 2 to 40 D gene segments, and 2 to 20 J gene segments.
3. The recombinant heavy chain-only Ig locus of claim 2, wherein more than one of said human J gene segments comprise a codon encoding a different amino acid residue at the first position of the fourth framework region (FR4) that is capable of disrupting a surface-exposed hydrophobic patch comprising or associated with the native amino acid residue at that position.
4. The recombinant heavy chain-only Ig locus of claim 3, wherein all of said human J gene segments comprise a codon encoding a different amino acid residue at the first position of the fourth framework region (FR4) that is capable of disrupting a surface-exposed hydrophobic patch comprising or associated with the native amino acid residue at that position.
5. The recombinant heavy chain-only Ig locus of claim 4, wherein in the encoded VH region, the native amino acid residue at the first position of FR4 is substituted by a polar amino acid residue.
6. The recombinant heavy chain-only Ig locus of claim 4, wherein in the encoded VH region, the native amino acid residue at the first position of FR4 is substituted by a positively charged amino acid residue.
7. The recombinant heavy chain-only Ig locus of claim 6, wherein in the encoded VH region, the positively charged amino acid residue is selected from the group consisting of lysine (K), arginine (R) and histidine (H).

8. The recombinant heavy chain-only Ig locus of claim 7, wherein in the encoded VH region, the positively charged amino acid residue is arginine (R).

9. The recombinant heavy chain-only Ig locus of claim 8, wherein the encoded VH region comprises a tryptophan (W) to arginine (R) substitution at the first amino acid residue in the fourth framework (FR4) region.

10. The recombinant heavy chain-only Ig locus of claim 9, comprising a J4 gene segment in which the codon for W is replaced by R.

~~11. The recombinant heavy chain-only Ig locus of any one of claims 1 to 10, wherein the encoded VH region comprises one or more further mutations in one or more framework regions.~~

1~~1~~2. The recombinant heavy chain-only Ig locus of any one of claims 1 to ~~11~~10, encoding a human or humanized heavy chain-only antibody comprising said VH region.

1~~2~~3. A transgenic non-human animal comprising a recombinant heavy chain-only Ig locus of any one of claims 1 to ~~12~~11.

1~~3~~4. The transgenic non-human animal of claim 1~~2~~3, which is a non-human mammal.

1~~5~~4. The transgenic non-human animal of claim 1~~3~~4, which is a rodent.

1~~5~~6. The transgenic non-human animal of claim 1~~4~~5, which is a rat or a mouse.

1~~7~~6. The transgenic non-human animal of claim 1~~5~~6, which is a rat.

1~~7~~8. The transgenic non-human animal of claim 1~~6~~7, wherein the rat is capable of producing a human or chimeric heavy chain-only antibody.

1~~8~~9. A transgenic non-human animal that does not express any functional immunoglobulin light chain genes and comprises a heterologous heavy chain-only Ig locus comprising one or more V gene segments, one or more D gene segments, and one or more J gene segments, which when recombined with each other and following affinity maturation encode a VH domain comprising complementarity determining regions (CDRs) and framework regions (FRs), in which the native amino acid residue at the first position of the fourth framework region (FR4) of said VH domain is substituted by a different amino acid residue that is charged or polar, and is capable of disrupting a surface-exposed hydrophobic patch comprising or associated with the native amino acid residue at that position, and one or more constant effector region gene segments, encoding an antibody constant effector region lacking ~~CHI-functionality~~ a C_μ coding region, and comprising a plurality of C_γ coding regions, each

lacking a CH1 exon, and further comprising a C ϵ or a C α coding region that comprises a CH1 exon,

wherein the gene segments are arranged such that a V, a D and a J gene segment and a constant region gene segment recombine to produce a rearranged affinity matured heavy chain-only gene locus encoding a heavy chain-only antibody (HCAb).

19~~20~~. The transgenic non-human animal of claim 18~~9~~, wherein in said VH domain, the native amino acid residue at the first position of FR4 is substituted by a polar amino acid residue.

24~~0~~. The transgenic non-human animal of claim 18~~9~~, wherein in said VH domain, the native amino acid residue at the first position of FR4 is substituted by a positively charged amino acid residue.

22~~1~~. The transgenic non-human animal of claim 20~~1~~, wherein in said VH domain, the positively charged amino acid residue is selected from the group consisting of lysine (K), arginine (R) and histidine (H).

23~~2~~. The transgenic non-human animal of claim 22~~1~~, wherein in said VH domain, the positively charged amino acid residue is arginine (R).

24~~3~~. The transgenic non-human animal of claim 23~~2~~, wherein said VH domain comprises a tryptophan (W) to arginine (R) substitution at the first amino acid residue in the fourth framework (FR4) region.

24~~5~~. The transgenic non-human animal of claim 24~~3~~, wherein said heterologous heavy chain-only locus comprises a J4 segment in which a codon for W is replaced by a codon for R.

~~26. — The transgenic non-human animal of any one of claims 19 to 25, wherein the encoded heavy chain-only antibody comprises one or more further mutations in one or more framework regions.~~

25~~7~~. The transgenic non-human animal of any one of claims 19 to 26~~25~~, which is a mammal.

26~~8~~. The transgenic non-human animal of claim 27~~5~~, which is a rodent.

27~~9~~. The transgenic non-human animal of claim 26~~8~~, which is a rat or a mouse.

28~~30~~. The transgenic non-human animal of claim 29~~7~~, which is a rat.

29~~31~~. The transgenic non-human animal of claim 28~~30~~, wherein the rat is capable of producing a human or chimeric heavy chain-only antibody.