CLAIMS

1. A polypeptide comprising a heavy chain variable domain (VH) variant, such as a single domain antibody (sdAb) variant, comprising a plurality of antigen-binding regions and a framework comprising framework region 1 (FWR1), framework region 2 (FWR2) and framework region 3 (FWR3), wherein the VH variant has an amino acid sequence comprising a plurality of mutations compared to i) a native camelid framework as defined in any one of SEQ ID NO: 152, SEQ ID NO: 155 or SEQ ID NO: 157, or ii) a synthetic framework as defined in any one of SEQ ID NO: 72 or SEQ ID NO:73, in which at least eight of the following criteria i) to x) are fulfilled:

i) the residue in Kabat position 19 is R, S, K or T;
ii) the residue in Kabat position 23 is T, V, A or S, preferably T;
iii) the residue in Kabat position 24 is I, V or S;
iv) the residue in Kabat position 40 is selected from R, I, T or K, preferably R;
v) the residue in Kabat position 43 is R, K, Q, E or G, preferably R or K;
vi) the residue in Kabat position 44 is E, Q, A, D or G;
vii) the residue in Kabat position 45 is R, I or L, preferably R;
viii) the residue in Kabat position 76 is N or Q;
ix) the residue in Kabat position 79 is Y, W or F; and
x) the residue in Kabat position 89 V or I.

2. The polypeptide according to claim 1, wherein the VH variant has an amino acid sequence in which at least five of the following criteria i) to vi) are fulfilled:

i) the residue in Kabat position 24 is I, V or S;
ii) the residue in Kabat position 40 is selected from R, I, K, or T, preferably R;
iii) the residue in Kabat position 45 is R, I or L, preferably R;
iv) the residue in Kabat position 76 is N or Q;
v) the residue in Kabat position 79 is Y, W or F; and
vi) the residue in Kabat position 89 is V or I.

3. The polypeptide according to any one of the preceding claims, wherein the VH variant has a framework region 1 (FWR1) comprising an amino acid sequence according to

$X_1X_2QLX_5X_6SGGGX_{11}VQX_{14}GGSLX_{19}LSCX_{23}X_{24}SG$ (SEQ ID NO: 1)

wherein, independently,

X_1 is Q, V, D or E, preferably Q or E;

X_2 is V or D, preferably V;

X_5 is Q, V or E, preferably Q;

X_6 is Q or E, preferably Q;

X_{11} is S or L, preferably S;

X_{14} is A, S, or T, preferably A;

X_{19} is R, S, K or T, preferably R or T;

X_{23} is T, V, A or S, preferably T; and

X_{24} is I, V or S, preferably I;

a framework region 2 (FWR2) comprising an amino acid sequence according to

$WFRQX_5PGX_8X_9X_{10}EX_{12}VA$ (SEQ ID NO: 2)

wherein, independently,

X_5 is R, I, T or K, preferably R;

X_8 is R, K, Q, E or G, preferably K or R;

X_9 is E, Q, A, D or G;

X_{10} is R, I or L, preferably R; and

X_{12} is F or L, preferably F; and

a framework region 3 (FWR3) comprising an amino acid sequence according to

$YX_2X_3X_4VX_6GRFTISRDNAKX_{18}TX_{20}X_{21}LQMNX_{26}LKPEDTAX_{34}YYCAA$ (SEQ ID NO: 3)

wherein, independently,

X_2 is A, T, N, Q or S, preferably A;

X_3 is D or S, preferably D;

X_4 is S or A, preferably S;

X_6 is K or A, preferably K;

X_{18} is N or Q, preferably N;

X_{20} is V or A, preferably V;

X_{21} is Y, F or W;

X_{26} is N, S, T or Q, preferably S; and

X_{34} is V or I.

4. The polypeptide according to any one of any one of the preceding claims, wherein the VH variant comprises a framework region 4 (FWR4) comprising the amino acid sequence

WGQGTQVTVSS (SEQ ID NO: 71).

5. The polypeptide according to any one of the claims 1-3, wherein each of FWR1, FWR2 and FWR3 has at least 80 %, such as at least 85%, 90% or 95%, identity to the amino acid sequence of a corresponding framework region of SEQ ID NO: 80, wherein for the purpose of determining sequence identity, the Kabat positions 14, 19, 23, 24, 40, 43, 44, 45,47, 60, 76, 79, 82b and 89, and optionally position 6, are omitted.

6. The polypeptide according to any one of claims 2-5, wherein all criteria i)-x) of claim 1 and all criteria i)-vi) of claim 2 are fulfilled.

7. The polypeptide according to any one of the claims 1-6, wherein the amino acid sequence of the VH variant further fulfils any one, such as more than one, such as all, of the following:

the residue in Kabat position 6 is Q or E;
the residue in Kabat position 14 is A, S or T;
the residue in Kabat position 47 is F or L, preferably F;
the residue in Kabat position 60 is A, T, N, Q or S, preferably A or S;
the residue in Kabat position 82b is N, S, T or Q.

8. The polypeptide according to any one of the preceding claims, wherein framework region 2 (FWR2) has an amino acid sequence having at least 70 %, such as at least 75%, such as at least 80 %, such as at least 85 % identity to a sequence selected from the group consisting of SEQ ID NOs:23-24 and 26-41.

9. A multimeric polypeptide comprising at least two moieties, each moiety being a VH variant as defined in any one of the preceding claims, said moieties optionally being joined by a peptide linker.

10. A fusion protein comprising at least one polypeptide according to any one of claims 1-8 or a multimer according to claim 9, and at least one further polypeptide moiety.

11. The fusion protein according to claim 10, wherein the further polypeptide moiety comprises a sequence having least 80 % identity such as at least 85 % or at least 90 % identity to SEQ ID NO: 159 or to SEQ ID NO: 160.

12. The fusion protein according to any one of claim 10-11, comprising the following structure

$([A\text{-}L1]_m\text{-}[Z\text{-}L2]_n)_p$

wherein

A represents a polypeptide as defined in any one of claims 1-8,

L1 for each occurrence may be present or absent, and where present, represents a linker or spacer,

Z represents a further polypeptide moiety according to claim 11,

L2 for each occurrence may be present or absent, and where present, represents a linker or spacer,

m represents an integer of from 1 to 4,

n represents an integer of from 1 to 4, or when $m \geq 2$, n represents 0 or an integer of from 1 to 4,

and

p represents an integer of from 1 to 4.

13. The fusion protein according to any one of claims 10-12, comprising a C-terminal tag comprising a plurality of histidine residues, preferably at least 6 histidine residues.

14. Use of the polypeptide according to any one of claims 1-8, the multimer of claim 9 or the fusion protein of claims 10-13, as affinity ligand for capturing a target entity.

15. An adsorbent material comprising the polypeptide according to any one of claims 1-8, the multimer of claim 9 or the fusion protein of claims 10-13 coupled to a solid support.

16. The adsorbent material according to claim 15, wherein the support material is selected from a particle, a bead, a fiber, a fibrous membrane, a filter, a sheet, a porous monolith, a chip, a plate, and a well.

17. The adsorbent material according to any one of claims 15-16, wherein the support material is a fibrous matrix, such as a nonwoven fibrous matrix.

18. The adsorbent material according to any one of claim 15-16, wherein the support comprises agar or agarose or a derivative thereof, such as crosslinked agarose.

19. The adsorbent material according to any one of claim 15-18, wherein the support is a chromatography matrix.

20. A method of separation, comprising the steps of

(a) Providing an adsorbent material according to any one of claims 15-19 wherein the VH variant of the polypeptide has a binding affinity for a target entity,
(b) Contacting the adsorbent material with a liquid sample comprising said target entity under conditions allowing the target entity to bind to the polypeptide,
(c) Optionally washing the adsorbent material,
(d) Eluting the target entity from the adsorbent material, and
(e) Cleaning the adsorbent material with a cleaning liquid, such as an alkaline cleaning liquid.

21. The method of claim 20, wherein the cleaning liquid is alkaline, and preferably comprises from 0.05 to 0.5 M NaOH, such as 0.1-0.5 M NaOH.

22. The method of any one of claim 20-21, wherein steps (a)-(e) are repeated at least 10 times.

23. The method of claim 22, wherein after 12 cycles of contact with alkaline cleaning liquid, the polypeptide or fusion protein retains at least 50 % such as at least 60 %, such as at least 80 %, such as at least 90 %, of the initial target entity binding capacity.

24. The method of any one of claims 22-23, wherein, after 12 cycles of contact with alkaline cleaning liquid, the polypeptide or the fusion protein retains at least 50 % such as at least 60 %, such as at least 80 %, such as at least 90 %, such as at least 95 %, such as at least 98 %, of the target entity binding capacity of the 2nd cycle.