

CLAIMS

1. An antigen-binding molecule (ABM) comprising a cognate light chain/ heavy chain (LC/HC) dimer of an antibody light chain (LC) composed of a VL and a CL antibody domain, associated to an antibody heavy chain (HC) comprising at least a VH and a CH1 antibody domain, which association is through pairing the VL and VH domains and the CL and CH1 domains, wherein the amino acids at the position 18 in the CL domain and at the position 26 in the CH1 domain are polar and of opposite polarity and defined according to any one of embodiments A-C, wherein

A:

a) the CL domain is Ckappa comprising an amino acid sequence of SEQ ID NO:1 which contains the point mutation T18X, wherein X is any of R, H, or K; and

b) the CH1 domain comprises an amino acid sequence of SEQ ID 3 which contains the point mutation K26X, wherein X is any of D, or E;

or B:

a) the CL domain is Clambda comprising an amino acid sequence of SEQ ID NO:2 wherein K at position 18 is not substituted by any other amino acid, or which contains the point mutation K18X, wherein X is any of R, or H; and

b) the CH1 domain comprises an amino acid sequence of SEQ ID NO:3 which contains the point mutation K26X, wherein X is any of D, or E;

or C:

the CL and CH1 domains are a) and b), respectively in said embodiment A, or are a) and b), respectively in said embodiment B,

wherein the CL domain further comprises the point mutation F7X, wherein X is any of S, A, or V and the CH1 domain further comprises the point mutation A20L,

wherein numbering is according to the IMGT.

2. The ABM of claim 1, which cognate LC/HC dimer comprises at least one interdomain disulfide bridge between the CL and CH1 domains.

3. The ABM of claim 1 or 2, wherein the VL and VH domains do not contain any point mutation changing the polarity of an amino acid in the interface region.

4. The ABM of any of claims 1 to 3, wherein the HC further comprises at least one CH2 and at least one CH3 domain.

5. The ABM of any of claims 1 to 3, which is any of an antibody Fab or (Fab)₂ fragment, or a full-length antibody comprising an Fc part, preferably wherein the ABM is a full-length IgG antibody.

6. The ABM of any of claims 1 to 4, which is a heterodimeric antibody comprising a first and a second Fab arm recognizing different antigens or epitopes, wherein only one of the first and second Fab arms comprises the cognate LC/HC dimer.

7. The ABM of claim 6, wherein only one of the first and second Fab arms comprises

- a) the point mutation F7X in the CL domain, wherein X is any of S, A, or V; and
 - b) the point mutation A20L in the CH1 domain;
- wherein numbering is according to the IMGT.

8. The ABM of claim 6 or 7, wherein

A

a) said first Fab arm comprises the cognate LC/HC dimer which is characterized by the point mutations identified in embodiment C ; and

- b) said second Fab arm does not comprise any of the point mutations of a)

B

a) said first Fab arm comprises the cognate LC/HC dimer which is characterized by the point mutations identified in embodiments A or B; and

b) said second Fab arm which CL domain further comprises the point mutation F7X, wherein X is any of S, A, or V and the CH1 domain further comprises the point mutation A20L.

9. The ABM of any of claims 6 to 8, which further comprises two HCs each comprising a CH2 and a CH3 domain which HCs dimerize into an Fc region, wherein the CH3 domains are engineered to introduce one or more of the following:

a) strand-exchange engineered domain (SEED) CH3 heterodimers that are composed of alternating segments of human IgA and IgG CH3 sequences;

b) one or more knob or hole mutations, preferably any of T366Y/Y407'T, F405A/T394'W, T366Y:F405A/T394'W:Y407'T, T366W/Y407'A and S354C:T366W/Y349'C:T366'S:L368'A:Y407'V;

c) a cysteine residue in the first CH3 domain that is covalently linked to a cysteine residue in the second CH3 domain, thereby introducing an interdomain disulfide bridge, preferably linking the C-terminus of both CH3 domains;

d) one or more mutations where repulsive charge suppresses heterodimer formation, preferably any of: K409D/D399'K, K409D/D399'R, K409E/D399'K, K409E/D399'R, K409D:K392D/D399'K:E356'K or K409D:K392D:K370D/D399'K:E356'K:E357'K; and/or

e) one or more mutations selected for heterodimer formation and/or thermostability, preferably any of:

T350V:L351Y:F405A:Y407V/T350V:T366L:K392L:T394W,
T350V:L351Y:F405A:Y407V/T350V:T366L:K392M:T394W,
L351Y:F405A:Y407V/T366L:K392M:T394W,
F405A:Y407V/T366L:K392M:T394W, or
F405A:Y407V/T366L:T394W,

wherein numbering is according to the EU index of Kabat.

10. An isolated nucleic acid encoding the ABM of any of claims 1 to 9.

11. An expression cassette or vector incorporating the nucleic acid of claim 10.

12. A host cell comprising the nucleic acid of claim 10, or the expression cassette or vector of claim 11.

13. A method of producing the ABM of any of claims 1 to 9, by cultivating a host cell of claim 12 under conditions to express said ABM.