Polypeptides with enhanced clipping profile

[1] The invention relates to a polypeptide or polypeptide construct comprising a first target antigen binding domain, wherein said first target antigen binding domain comprises a VH and a VL variable region linked by a peptide linker, wherein the peptide linker comprises or consists of S(G4X)n or (G4X)n, wherein X is selected from the group consisting of Q, T, N, C, G, A, V, I, L, and M, and wherein n is an integer selected from integers 1 to 20. The invention also relates to a method for improving stability of a polypeptide or polypeptide construct. Moreover, the invention relates to a polynucleotide encoding the polypeptide or polypeptide construct of the invention, a vector comprising said polynucleotide and a host cell transformed or transfected with said polynucleotide or with said vector. Moreover, the invention also provides for a process for the production of said polypeptide or polypeptide construct and a pharmaceutical composition comprising said polypeptide or polypeptide construct of the invention. Furthermore, the invention relates to medical uses of said polypeptide or polypeptide construct and kits comprising said polypeptide or polypeptide construct.

[2] Clipping, also known as fragmentation, of formulated, purified biomolecules e.g. antibodies, T cell engager antibodies, etc. during liquid storage is a widely observed liability. Depending on the rate in which clipping occurs, a liquid formulation may not be feasible without compromises regarding the stability and therefore composition of the biomolecule. Such compromises are not always an option, in particular in highly regulated environments such as, e.g., in the pharmaceutical industry where it is of utmost importance to provide functional, uniform and stable medicines. Since biomolecules come in a variety of shapes, structures and functions, there is an ongoing need to improve their stability by way of decreasing the clipping rate, i.e. the rate at which clipping occurs. One way of trying to reduce clipping is, for example, the lyophilization of the biomolecule. However, a need for lyophilized formulation may significantly impact the commercial production flexibility and thus may result in higher cost of goods for manufacturing (COGM). Another option is to manipulate the biomolecule itself so that it becomes less prone to clipping and, inter alia, allow a liquid formulation of the biomolecule. A liquid formulation of biomolecules versus lyophilization eliminates, for example, the need for the error-prone reconstitution process of lyophilized material, thereby increasing safety and handling comfort. Clipping occurs in particular also in polypeptides or polypeptide constructs comprising antibody-derived binding domains. As such, there is an ongoing need to reduce the clipping rate of corresponding biomolecules.

Summary

[2a] In one aspect, the present disclosure provides a polypeptide or polypeptide construct comprising a first target antigen binding domain, wherein said first target antigen binding domain comprises a VH and a VL variable region linked by a peptide linker, wherein the peptide linker comprises or consists of S(G4X)n or (G4X)n, wherein X is selected from the group consisting of Q, T, N, C, G, A, V, I, L, and M, and wherein n is an integer selected from integers 1 to 20.

[2b] In another aspect, the present disclosure provides a polynucleotide encoding a polypeptide or polypeptide construct as defined herein.

[2c] In another aspect, the present disclosure provides a vector comprising a polynucleotide as defined herein.

[2d] In another aspect, the present disclosure provides a host cell transformed or transfected with the polynucleotide as defined herein or with the vector as defined herein.

[2e] In another aspect, the present disclosure provides a process for the production of a polypeptide or polypeptide construct as disclosed herein, said process comprising culturing a host cell as defined in claim under conditions allowing the expression of the polypeptide or polypeptide construct as defined herein and recovering the produced polypeptide or polypeptide construct from the culture.

[2f] In another aspect, the present disclosure provides a pharmaceutical composition comprising a polypeptide or polypeptide construct as disclosed herein, or produced according to the process as disclosed herein.

[2g] In another aspect, the present disclosure provides the polypeptide or polypeptide construct as disclosed herein, or the polypeptide or polypeptide construct produced according to the process as disclosed herein, for use in therapy.

[2h] In another aspect, the present disclosure provides the use of the polypeptide or polypeptide construct as disclosed herein, or the polypeptide or polypeptide construct produced according to the process as disclosed herein in the manufacture of a medicament for the prevention, treatment or amelioration of a tumorous disease.

[2i] In another aspect, the present disclosure provides a kit comprising a polypeptide or polypeptide construct as disclosed herein, or produced according to the process as disclosed herein, a polynucleotide as defined herein, a vector as defined herein, and/or a host cell as defined herein.

Detailed Description

[3] The invention relates a polypeptide or polypeptide construct comprising a first target antigen binding domain, wherein said first target antigen binding domain comprises a VH and

a VL variable region linked by a peptide linker, wherein the peptide linker comprises or consists of S(G4X)n or (G4X)n, wherein X is selected from the group consisting of Q, T, N, C, G, A, V, I, L, and M, and wherein n is an integer selected from integers 1 to 20.

[4] The term “polypeptide construct” (alternatively referred to also as “compound” herein) refers to an antigen-binding (or epitope-binding) molecule comprising binding domains themselves comprising paratopes. In the context of the present invention, a polypeptide construct is understood as an organic polymer which comprises at least one continuous, unbranched amino acid chain that naturally is not existing, but was engineered. An example of a polypeptide construct that is a single polypeptide is a BiTE® molecule that comprises a core structure comprising at least one functional target antigen binding domain together with at least one complete functional CD3 binding domain on a single polypeptide chain, wherein these domains are linked directly by flexible peptide (a “linker”) without any further inserted domain unlike, for example, Xmabs that comprise the target binder and the CD3 binder on different polypeptide chains. In the context of the present invention, such a polypeptide construct comprising more than one amino acid chain is likewise envisaged. It is preferred that the term “polypeptide” is used in connection with single chain forms of the compounds of the present invention, whereas “polypeptide construct” may preferably be more adequate to describe also polypeptides that comprise more than one polypeptide chain, for example two, three or four polypeptide chains. Preferably, the polypeptide or polypeptide construct of the invention is a single chain polypeptide or polypeptide construct. Additionally, the term “polypeptide construct” is also suitable to describe compounds of the invention that comprise one or more non-amino acid-based constituents, e.g. human serum albumin, etc. (HSA). An amino acid chain of a polypeptide typically comprises at least 50 amino acids, preferably at least 100, 200, 300, 400 or 500 amino acids. It is also envisaged

Claims

1. A polypeptide or polypeptide construct comprising a first target antigen binding domain, wherein said first target antigen binding domain comprises a VH and a VL variable region linked by a peptide linker, wherein the peptide linker comprises or consists of S(G4X)n or (G4X)n, wherein X is selected from the group consisting of Q, T, N, C, G, A, V, I, L, and M, and wherein n is an integer selected from integers 1 to 20.

2. The polypeptide or polypeptide construct of claim 1, wherein n is 1, 2, 3, 4 ,5 or 6.

3. The polypeptide or polypeptide construct of claim 1 or 2, wherein X is Q.

4. The polypeptide or polypeptide construct of any one of claims 1 to 3, wherein the peptide linker is (G4X)n, n is 3, and X is Q.

5. The polypeptide or polypeptide construct of any one of claims 1 to 4, comprising at least one further binding domain binding to a target antigen.

6. The polypeptide or polypeptide construct of claim 5, wherein the at least one further target antigen binding domain comprises the same components as the first target antigen binding domain.

7. The polypeptide or polypeptide construct of claim 5 or 6, wherein each target antigen binding domain binds to one target antigen.

8. The polypeptide or polypeptide construct of any one of claims 5 to 7, wherein said polypeptide or polypeptide comprises:
   Binding Domain 1 (VH/VL – Peptide Linker – VH/VL) – Linker – Binding Domain 2 (VH/VL – Peptide Linker – VH/VL)

9. The polypeptide or polypeptide construct of claim 8, wherein said polypeptide or polypeptide construct comprises:
   Binding Domain 1 (VH/VL – Peptide Linker – VH/VL) – Linker – Binding Domain 2 (VH/VL – Peptide Linker – VH/VL) – Binding Domain 3 (VH/VL – Peptide Linker – VH/VL)

10. The polypeptide or polypeptide construct of claims 8 to 9, wherein the C-terminal binding domain is binding to CD3, and wherein said remaining N-terminal binding domain(s) is/are binding to a cell surface antigen.

11. The polypeptide or polypeptide construct of any one of claims 8 to 10, wherein the linker linking the binding domains comprises or consists of S(G4X)n or (G4X)n, wherein X is selected from the group consisting of Q, T, N, C, G, A, V, I, L, and M, and wherein n is an integer selected from integers 1 to 20.

12. The polypeptide or polypeptide construct of claim 11, wherein the linker is S(G4X)n, n is 1 and X is Q.

13. The polypeptide or polypeptide construct of any one of claims 1 to 12, comprising a half-life extending domain.

14. The polypeptide or polypeptide construct of claim 13, wherein said half-life extending domain (HLE domain) comprising, or consisting of, two polypeptide monomers comprises a hinge, a CH2 domain and a CH3 domain, wherein said two polypeptide monomers are fused to each other via a peptide linker, comprising in an amino to carboxyl order: hinge-CH2-CH3-peptide linker-hinge-CH2-CH3.

15. The polypeptide or polypeptide construct of any one of claims 8 and 10 to 14, wherein said polypeptide or polypeptide construct comprises in an amino to carboxyl order: Binding Domain 1 (VH/VL – Peptide Linker – VH/VL) – Linker – Binding Domain 2 (VH/VL – Peptide Linker – VH/VL) – HLE domain.

16. The polypeptide or polypeptide construct of any one of claims 9 and 10 to 14, wherein said polypeptide or polypeptide construct comprises in an amino to carboxyl order: Binding Domain 1 (VH/VL – Peptide Linker – VH/VL) – Linker – Binding Domain 2 (VH/VL – Peptide Linker – VH/VL) – Linker – Binding Domain 3 (VH/VL – Peptide Linker – VH/VL) – HLE domain.

17. The polypeptide or polypeptide construct of claim 15, wherein said polypeptide or polypeptide construct comprises in an amino to carboxyl order: Binding Domain 1 (VH/VL – Peptide Linker – VH/VL) – Linker – Binding Domain 2 (VH/VL – Peptide Linker – VH/VL) – Linker – HLE domain – Linker – Binding Domain 3 (VH/VL – Peptide Linker – VH/VL) – Linker – Binding Domain 4 (VH/VL – Peptide Linker – VH/VL),
wherein Binding Domain 1 binds to a first cell surface antigen, Binding domains 2 and 3 bind to CD3, wherein Binding Domain 4 binds to a second cell surface antigen.

18. The polypeptide or polypeptide construct of claim 17, wherein the peptide linkers within the binding domains is (G4Q)3 and the peptide linker within the HLE domain is (G4Q)6,

the linker linking said binding domains is S(G4Q), and wherein the linkers linking the HLE domain to the binding domains are G4 linkers.

19. The polypeptide or polypeptide construct of any one of claims 14 to 16, wherein the linkers linking the HLE domain to the binding domains are G4 linkers.

20. The polypeptide or polypeptide construct of any one of claims 10 to 19, wherein said cell surface antigen is a tumor antigen.

21. The polypeptide or polypeptide construct of claim 20, wherein the tumor antigen is selected from the group consisting of BCMA, CD123, CD19, CD20, CD22, CD33, CD70, CDH19, CDH3, CLL1, CS1, CLDN6, CLDN18.2, DLL3, EGFRvIII, FLT3, MAGEB2, MART1, MSLN, MUC17, PSMA, and STEAP1.

22. A polynucleotide encoding a polypeptide or polypeptide construct as defined in any one of claims 1 to 21.

23. A vector comprising a polynucleotide as defined in claim 22.

24. A host cell transformed or transfected with the polynucleotide as defined in claim 22 or with the vector as defined in claim 23.

25. A process for the production of a polypeptide or polypeptide construct according to any one of claims 1 to 21, said process comprising culturing a host cell as defined in claim under conditions allowing the expression of the polypeptide or polypeptide construct as defined in any one of claims 1 to 21 and recovering the produced polypeptide or polypeptide construct from the culture.

26. A pharmaceutical composition comprising a polypeptide or polypeptide construct according to any one of claims 1 to 21, or produced according to the process of claim 25.

27. The polypeptide or polypeptide construct of claim 20 or 21, or the polypeptide or polypeptide construct produced according to the process of claim 25, for use in ~~the prevention, treatment or amelioration of a tumorous disease~~therapy.

28. Use of the polypeptide or polypeptide construct according to any one of claims 20 to 21, or the polypeptide or polypeptide construct produced according to the process of claim 25 in the manufacture of a medicament for ~~A method for~~ the prevention, treatment or amelioration of a tumorous disease~~, comprising the step of administering to a subject in~~

~~need thereof the polypeptide or polypeptide construct according to any one of claims 20 to 21, or produced according to the process of claim 25~~.

29. A kit comprising a polypeptide or polypeptide construct according to any one of claims 1 to 21, or produced according to the process of claim 25, a polynucleotide as defined in claim 22, a vector as defined in claim 23, and/or a host cell as defined in claim 24.