compositions that provide for enhanced stabilization of antibodies or therapeutic proteins and reduce aggregation and denaturation or degradation during formulation, filling, shipping, storage and administration, thereby preventing loss-of-function and adverse immunogenic reactions.

SUMMARY

[0010] The present disclosure provides pharmaceutical compositions and/or formulations useful to minimize or reduce aggregation and/or high-molecular weight species of masked antigen binding proteins. In one aspect, the present disclosure provides a pharmaceutical composition or formulation comprising: (A) a masked antigen binding protein (including, e.g., a Probody or a ProTIA prodrug) comprising: (i) an antibody or antigen binding fragment thereof (AB1) that binds to an antigen; and (ii) a masking domain (MD1) coupled to AB1, wherein the masking domain comprises: (a) a masking peptide or masking polypeptide (MP1) that inhibits or reduces the binding of AB1 to the antigen; and (b) a protein recognition site (PR1), wherein binding to or cleavage of the PR1 by a protein or a protease increases AB1 binding to the antigen; (B) at least one buffer agent; and (C) at least one polyol, wherein the pH of the pharmaceutical composition or formulation is below the isoelectric point (pI) of the AB1 and MD1 regions of the masked antigen binding protein. In another aspect, the present disclosure provides use of the composition as described herein in the manufacture of a medicament for treating cancer in a subject.

[0011] In some other examples, the present disclosure also provides a pharmaceutical composition or formulation comprising: (A) a masked antigen binding protein (including, e.g., a Probody or a ProTIA prodrug) comprising: (i) a first antibody or antigen binding fragment thereof (AB1) that binds to an antigen; and (ii) a first masking domain (MD1) coupled to AB1, wherein the masking domain comprises: (a) a masking peptide or masking polypeptide (MP1) that inhibits or reduces the binding of AB1 to the antigen; and (b) a protein recognition site (PR1), wherein binding to or cleavage of the PR1 by a protein or a protease increases AB1 binding to the antigen; (iii) a second antibody or antigen binding fragment thereof (AB2) that binds to a second antigen; and (vi) a second masking domain (MD2) coupled to AB2, wherein the second masking domain comprises: (a) a masking peptide or masking polypeptide (MP2) that inhibits or reduces the binding of AB2 to the antigen; and (b) a second protein recognition site (PR2), wherein binding to or cleavage of the PR2 by a protein or a protease

increases AB2 binding to the second antigen, (B) at least one buffer agent; and (C) at least one polyol, wherein the pH of the pharmaceutical composition or formulation is below the isoelectric points (pI) of the AB1, AB2, MD1 and MD2 regions of the masked antigen binding protein.

[0012] In some examples, the AB1 and/or AB2 regions of the masked antigen binding protein (including, e.g., a Probody or a ProTIA prodrug) is an scFv or a Fab, and/or binds an antigen expressed on the surface of a cell (including, e.g., CD3).

[0013] The pharmaceutical compositions or formulations of the disclosure comprises at least one buffer agent. In some examples, the buffer agent is selected from the group consisting of acetate, glutamate, citrate, succinate, tartrate, fumarate, maleate, histidine, phosphate, and 2-(N-morpholino)ethanesulfonate or a combination thereof. In some examples, the at least one buffer agent is present in the composition or formulation at a concentration ranging from about 5 mM to about 200 mM (e.g., 5 mM to 200 mM) or about 10 mM to about 50 mM (e.g., 10 mM to 50 mM).

[0014] The pharmaceutical compositions or formulations of the disclosure also comprises at least one polyol. In some examples, the polyol is a saccharide. In some examples, the saccharide is selected from the group consisting of a monosaccharide, a disaccharide, a cyclic polysaccharide, a sugar alcohol, a linear branched dextran, and a linear non-branched dextran, or combinations thereof. In some examples, the polyol is a sugar alcohol (e.g., sorbitol). In some examples, the polyol is sucrose, trehalose, mannitol, sorbitol, xylitol, erythritol, isomalt, glycerol, a cyclodextrin or captisol. In some examples, the at least one polyol is present in the composition or formulation at a concentration ranging from about 1 to about 20% (w/V) (or about 9 to about 12% (w/V)).

[0015] In some instances, the pharmaceutical compositions or formulations of the disclosure may optionally further comprise at least one surfactant. In some examples, the surfactant is selected from the group consisting of polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, poloxamer 188, pluronic F68, triton X-100, polyoxyethylen3, and PEG 3350, PEG 4000, PEG 8000 or a combination thereof. In some examples, the at least one surfactant is present in the composition at a concentration ranging from 0.004 to about 0.5% (w/V) (or about 0.001 to about 0.01% (w/V)).

[0016] In some examples, the pH of the composition or formulation is greater than 3.5, and/or the pH is about 2 pH units lower than the pI of AB1, AB2, MD1, and/or MD2 region(s)

(described further below) of the masked antigen binding protein (including, e.g., a Probody or a ProTIA prodrug). In some examples, the pH of the composition or formulation is between 7.0 and 3.6. In some examples, the pH of the composition or formulation ranges from about 3.5 to about 5.5 or ranges from 4.0 to 5.0. In some examples, the pH of the composition or formulation is about 4.2 or about 3.6. In some examples, the pH of the composition or formulation is 4.2 or 3.6.

[0017] The pharmaceutical compositions or formulations of the disclosure may optionally further comprise an amino acid excipient. In some examples, the amino acid excipient is present at a concentration ranging from about 0.1 to about 20% (mM).

[0018] The pharmaceutical composition or formulations of the disclosure, in some examples, comprises 10 mM glutamate, 9% (w/V) sucrose and 0.01% (w/V) polysorbate 80, and wherein the pH of the liquid pharmaceutical composition is 4.2 or 3.6. In some examples, the masked antigen binding protein is present in the composition at a concentration ranging from about 0.1 mg/mL to about 17 mg/mL or from about 0.1 mg/mL to about 30 mg/mL. In some examples, the masked antigen binding protein is present in the composition in an amount ranging from about 50 μg to about 200 mg.

[0019] The pharmaceutical compositions of the disclosure can be a lyophilized composition or a liquid composition. In some examples, the pharmaceutical composition is a reconstituted lyophilized composition.

[0020] In some examples, the pharmaceutical composition is a liquid composition that is stable after storage at 2-8°C, 4°C, and/or 25°C for 0, 1, 2 and/or 4 weeks. In some examples, the pharmaceutical composition or formulation has reduced antibody aggregation and/or reduced high-molecular weight antibody species as measured by ultra-high performance liquid chromatography (SE-UHPLC) of about 1-fold, about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold or about 10-fold compared to a reference pharmaceutical composition consisting of 1 mg/ml masked antigen binding protein, 100 mM glycine at pH 4.9.

[0021] In another example, described herein is a method of treating cancer in a subject in need thereof comprising administering a composition or formulation of the disclosure to the subject.

[0022] The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to,” and permit the presence of

one or more features or components) unless otherwise noted. It should be understood that while various examples in the specification are presented using "comprising" language, under various circumstances, a related example may also be described using "consisting of" or "consisting essentially of" language. It is to be noted that the term "a" or "an" refers to one or more, for example, "an immunoglobulin molecule," is understood to represent one or more immunoglobulin molecules, unless context dictates otherwise. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein. Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following examples: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0023] It should also be understood that when describing a range of values, the characteristic being described could be an individual value found within the range. For example, "a pH from about pH 4 to about pH 6," could be, but is not limited to, pH 4, 4.2, 4.6, 5.1, 5.5, etc. and any value in between such values. Additionally, "a pH from about pH 4 to about pH 6," should not be construed to mean that the pH of a formulation in question varies 2 pH units in the range from pH 4 to pH 6 during storage, but rather a value may be picked in that range for the pH of the solution, and the pH remains buffered at about that pH. In some examples, when the term "about" is used, it means the recited number plus or minus 10% of that recited number.

[0024] In any of the ranges described herein, the endpoints of the range are included in the range. However, the description also contemplates the same ranges in which the lower and/or the higher endpoint is excluded. Additional features and variations of the disclosure will be apparent to those skilled in the art from the entirety of this application, including the drawing and detailed description, and all such features are intended as examples of the disclosure. Likewise, features of the disclosure described herein can be re-combined into additional examples that also are intended as examples of the disclosure, irrespective of whether the combination of features is specifically mentioned above as an example or example of the disclosure. Also, only such limitations which are described herein as critical to the disclosure should be viewed as such; variations of the disclosure lacking limitations which have not been described herein as critical are intended as examples of the disclosure.

[0025] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

[0026] Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, amino acid sequences are written left to right in amino to carboxy orientation. The headings provided herein are not limitations of the various examples or examples of the disclosure, which can be had by reference to the specification as a whole.

[0027] All references cited herein are hereby incorporated by reference in their entireties.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] **Figures 1A-1D** are bar graphs providing the results of size exclusion ultra-high-performance liquid chromatography (SE-UHPLC) analysis, showing the % Main Peak (left panels) and % high molecular weight species (right panels) of the bispecific masked antigen binding protein in Formulation B, when stored at either 2-8°C or 25°C at 0, 2 weeks and 4 weeks.

[0029] **Figures 2A-2D** are bar graphs providing the results of SE-UHPLC analysis, showing the % Main Peak (left panels) and % high molecular weight species (right panels) of the bispecific masked antigen binding protein in Formulation C, when stored at either 2-8°C or 25°C at 0, 1 week, 2 weeks and 4 weeks.

[0030] **Figures 3A-3D** are bar graphs providing the results of SE-UHPLC analysis, showing the % Main Peak (left panels) and % high molecular weight species (right panels) of the bispecific masked antigen binding protein in Formulation D, when stored at either 2-8°C or 25°C at 0, 2 weeks and 4 weeks.

[0031] **Figures 4A-4D** are bar graphs providing the results of SE-UHPLC analysis, showing the % Main Peak (left panels) and % high molecular weight species (right panels) of the bispecific masked antigen binding protein in Formulation E, when stored at either 2-8°C or 25°C at 0, 2 weeks and 4 weeks.

[0032] **Figures 5A-5D** are bar graphs providing the results of SE-UHPLC analysis, showing the % Main Peak (left panels) and % high molecular weight species (right panels) of the bispecific masked antigen binding protein in Formulation F, when stored at either 2-8°C or 25°C at 0, 2 weeks and 4 weeks.

CLAIMS

1. A pharmaceutical composition comprising:

(a) a masked antigen binding protein comprising:

(i) an antibody or antigen binding fragment thereof (AB1) that binds to an antigen; and

(ii) a masking domain (MD1) coupled to AB1, wherein the masking domain comprises:

a. a masking peptide or masking polypeptide (MP1) that inhibits or reduces the binding of AB1 to the antigen; and

b. a protein recognition site (PR1), wherein binding to or cleavage of the PR1 by a protein or a protease increases AB1 binding to the antigen;

(b) at least one buffer agent; and

(c) at least one polyol,

wherein the pH of the pharmaceutical composition is below the isoelectric point (pI) of the AB1 and MD1 regions of the masked antigen binding protein.

2. The pharmaceutical composition of claim 1, wherein the masked antigen binding protein further comprises:

(iii) a second antibody or antigen binding fragment thereof (AB2) that binds to a second antigen; and

(iv) a second masking domain (MD2) coupled to AB2, wherein the second masking domain comprises:

a. a masking peptide or masking polypeptide (MP2) that inhibits or reduces the binding of AB2 to the antigen; and

b. a protein recognition site (PR2), wherein binding to or cleavage of the PR2 by a protein or a protease increases AB2 binding to the second antigen,

wherein the pH of the pharmaceutical composition is below the isoelectric points (pI) of the AB1, AB2, MD1 and MD2 regions of the masked antigen binding protein.

3. The pharmaceutical composition of claim 1 or 2, wherein the masked antigen binding protein is a Probody or a ProTIA prodrug.

4. The pharmaceutical composition of any one of claims 1-3, wherein AB1 and/or AB2 is an scFv or a Fab.

5. The pharmaceutical composition of any one of claims 1-4, wherein AB1 and/or AB2 binds an antigen expressed on the surface of a cell.

6. The pharmaceutical composition of any one of claims 1-5, wherein the at least one buffer agent is selected from the group consisting of acetate, glutamate, citrate, succinate, tartrate, fumarate, maleate, histidine, phosphate, and 2-(N-morpholino) ethanesulfonate or a combination thereof.

7. The pharmaceutical composition of claim 6, wherein the at least one buffer agent is present at a concentration range of 5 mM to 200 mM, optionally, 10 mM to 50 mM.

8. The pharmaceutical composition of any one of claims 1-7, wherein the at least one polyol is selected from the group consisting of a saccharide, a cyclic polysaccharide, a sugar alcohol, a linear branched dextran, and a linear non-branched dextran, or combinations thereof, optionally, wherein the saccharide is a monosaccharide or a disaccharide.

9. The pharmaceutical composition of any one claims of 1-8, wherein the polyol is selected from the group consisting of sucrose, trehalose, mannitol, sorbitol, xylitol, erythitol, isomalt, glycerol, a cyclodextrin, captisol or a combination thereof.

10. The pharmaceutical composition of any one of claims 1-9, wherein the at least one polyol is present at a concentration in the range of 1 to 20% (w/V), optionally, 9 to 12% (w/V).

11. The pharmaceutical composition of any one of claims 1-10, further comprising at least one surfactant, optionally, wherein the at least one surfactant is selected from the group consisting of polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, poloxamer 188, pluronic F68, triton X-100, polyoxyethylen3, and PEG 3350, PEG 4000, and PEG 8000 or a combination thereof.

12. The pharmaceutical composition of claim 11, wherein the at least one surfactant is present at a concentration in the range of 0.001 to 0.5 % (w/V), optionally, 0.001 to 0.01% (w/V).

13 The pharmaceutical composition of any one of claims 1-12, wherein the pH of the composition is about 2 pH units lower than the pI of AB1, AB2, MD1, and/or MD2.

14. The pharmaceutical composition of any one of claims 1-13, wherein the pH of the composition is (a) greater than about 3.5; (b) between 7.0 and 3.6;(c) between 4.0 and 5.0;(d) between 3.5 and 5.5; (e) about 4.2 or 3.6; or (f) a combination thereof.

15. The pharmaceutical composition of any one of claims 1-14, having an osmolarity in the range of 150 to 500 mOsm.

16. The pharmaceutical composition of any one of claims 1-15, further comprising an amino acid excipient.

17. The pharmaceutical composition of claim 16, wherein the amino acid excipient is present in the concentration range of 0.1 to 20 mM.

18. The pharmaceutical composition of any one of claims 1-17, wherein the composition comprises 10 mM glutamate, 9% (w/V) sucrose and 0.01% (w/V) polysorbate 80, and wherein the pH of the pharmaceutical composition is 4.2 or 3.6.

19. The pharmaceutical composition of any one of claims 1-18, wherein the masked antigen binding protein is present in a concentration range of 0.1 to 30 mg/ml.

20. The pharmaceutical composition of any one of claims 1-19, wherein the masked antigen binding protein is present in an amount ranging from 50 µg to 200 mg.

21. The pharmaceutical composition of any one of claims 1-20, wherein the pharmaceutical composition is a lyophilized composition.

22. The pharmaceutical composition of any one of claims 1-21, wherein the pharmaceutical composition is a liquid composition.

23. The pharmaceutical composition of claim 22, wherein the pharmaceutical composition is a reconstituted lyophilized composition.

24. The pharmaceutical composition of any one of claims 1-23, wherein the composition is a liquid composition and is stable after storage at 2-8°C, 4°C and/or 25°C for 0 weeks, 1 week, 2,

weeks, 4 weeks, 2 months, 6 months, 1 year and/or 2 years; or wherein the composition is a lyophilized composition and is stable after storage at 2-8°C, 4°C and/or 25°C for 0 weeks, 1 week, 2, weeks, 4 weeks, 2 months, 6 months, 1 year, 2 years, 3 years, 4 years and/or 5 years.

25. The pharmaceutical composition of any one of claims 1-24, wherein (a) the amount of antibody aggregation is reduced;(b) the amount of high-molecular weight antibody species is reduced as measured by ultra-high performance liquid chromatography (SE-UHPLC); (c) the amount of antibody aggregation and/or high-molecular weight antibody species is reduced by about 1-fold, about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold or about 10-fold compared to a reference pharmaceutical composition at a higher pH than the pharmaceutical composition;(d) the pI is measured or determined using the Bjellqvist algorithm; or (e) a combination thereof.

26. Use of the composition of any one of claims 1-25 in the manufacture of a medicament for treating cancer in a subject.